



**Brazilian Sanitary
Surveillance Agency**

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**Brazilian Sanitary
Surveillance Agency**

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Studies carried out in several countries have demonstrated that many species of bacteria, plants and animals are able to survive, even after long trips, in the ballast water and sediments carried by ships. Later discharge of the ballast water and sediments in the waters of ports can result in the establishment of harmful aquatic organisms and pathogenic agents that may pose a threat to human life, the environment and the balance of ecosystems.

The Marine Environment Protection Committee (MEPC), of the International Maritime Organization (IMO), has been working since 1993 in order to develop legal devices pertaining ballast water management, as well as guidelines for their effective implementation. The 1997 IMO Assembly adopted, through Resolution A.868 (20), the “Guidelines for the Control and Management of Ships Ballast Water to Minimize the Transfer of Harmful Aquatic Organisms and Pathogens” with a view to decreasing the risk of invasion of undesirable organisms through ballast water, while preserving the security of the ships.

In view of the commitments undertaken by the Brazilian delegation during its participation in the 46th MECP Meeting, and on the basis of the potential impact of the introduction through ballast water of microorganisms that are pathogenic for humans, the Brazilian Sanitary Surveillance Agency/Ministry of Health - ANVISA, proposed to develop strategies and design an Exploratory Study on the sanitary quality of the ballast water discharged on the Brazilian shores. The aim of the Study was to support the Brazilian position at the 47th MEPC Meeting and, therefore, to support the IMO Convention (draft in annex 3 of Doc/IMO 48/2) to be held in 2003.

ANVISA concluded, in 2002, the Exploratory Study for Identification and Characterization of Pathogenic Agents in Ballast Water, having carried out a total of 99 collections at 9 Brazilian ports. The results confirmed the initial suspicion that ballast water is a carrier of pathogenic organisms that can pose a threat to public health. The partial results of this study were presented in March 2002, at the 47th MEPC Meeting.

During the 47^a MEPC, the Brazilian delegation managed to include, in the scientific discussion group of the IMO and of the Correspondence Official Group, the issue of pathogenic organisms carried in ballast water, with a view to broad discussion of the topic

among member-countries and their support to the inclusion of the topic in the next Convention, leading to the definition of future guidelines that also include control of transfer of pathogenic organisms, as well as of exotic organisms. This achievement is the result of the research work presented by Brazil and its commitment to pursue these studies.



Ballast collections, in ellipses, carried out in the Exploratory Study

In the study undertaken by ANVISA, all the researched microbiological indicators were detected, and the results proved the presence of cultivable marine bacteria in 71% of the ballast water samples analyzed, varying from 1,000 up to 5.4 million bacteria per liter of sample. The results revealed transportation of vibrios (31%), fecal coliforms (13%), *Escherichia coli* (5%), enterococci fecal (22%), *Clostridium perfringens* (15%), coliphages (29%), *V. cholerae* O1 (7%) and of *V. C.* non-O1 (23%) in ballast water samples and (21%) in plankton samples. 12 strains had been found in 7 samples of ballast water, being identified as *V. C.* O1 EL TOR, 2 of them were toxicogenic.

It was also found that 62% of the ships whose captains claimed to have carried out deep-sea ballast water exchange, in compliance with IMO guidelines, had probably not done so, or done so partially, since they had ballast water with salinity lower than 35.

Currently, ANVISA is pursuing the research work on ballast water through the following projects:

- 1) Molecular characterization of strains of *Vibrio cholerae* O1 and non-O1 isolated from ballast waters at Brazilian ports, with the following objectives: (i) to characterize, regarding factors associated to virulence, strains of *Vibrio cholerae* O1 and non-O1 isolated from ballast waters of ships that moor at the ports of Belém (PA); Fortaleza (CE);

Santos (SP); Rio de Janeiro (RJ); and Recife (PE); (ii) to determine the genetic diversity and the cloning relation of the isolates of *Vibrio cholerae* O1 and non-O1 from ballast waters of moored ships from Belém (PA); Fortaleza (CE); Santos (SP); Rio de Janeiro (RJ); and Recife (PE), employing the ribo-typing method; (iii) to analyze the ribo-types obtained and verify their association with clinical and environmental isolates from Brazil and other continents; and (iv) to determine the levels of similarity between the strains of *V. cholerae* O1 isolated from ballast water.

Cholera appeared in Latin America in 1991 and until today has caused over 1.2 million cases and 12 thousand deaths. Brazil reached the highest number of cholera cases of the continent in 1993 and 1994 and, more recently, in 1999, on the coast of Paraná (Paranaguá) where 467 cases were confirmed. There is scientific evidence that the first cases of cholera occurred in the coastal region of ports, which suggests that the outbreaks or epidemics may have been provoked by the ballast water of ships coming from endemic areas.

Currently, there are at least four distinct clones of toxigenic *V. cholerae* O1 in the world: from the seventh pandemic (eastern hemisphere), from Gulf Coast USA, the Australian and the Latin American.

The present study intends to characterize, at the molecular level, the origin of strains isolated from ballast water collected at five Brazilian ports, using the ribo-typing method.

Ribo-typing has been used successfully to characterize the clones of *V. cholerae* and other species of microorganisms. Ribo-typing is based on the analysis of the DNA of the bacterium using a highly conserved genetic probe specific for bacterial species.

2) Evaluation of the efficiency of ballast water exchange by means of microbiological indicators, with the following objectives: (i) to compare the efficiency of deep-sea ballast water exchange, carried out through the three methods approved by the IMO: dilution, sequential and flow through; (ii) to evaluate the most appropriate physical, chemical and microbiological variables to check the exchange, comparing concentrations at the port, in the ocean and in the ballast tanks before and after the exchange; (iii) to analyze the sediment of the ballast tanks in order to explain possible influences in the ballast water before and after the exchange.

The dilution method was developed and recommended for Brazil (Petrobras). The sequential and flow through methods are included in IMO Resolution A.868 (20).

The project for evaluation of the efficiency of the open-sea exchange has already been executed on board an oil ship, NT Itaituba, of Petrobras, which left the Port of Itaqui, in São Luís, carried out the exchange at open-sea, at a region more than 2.000 meters deep, as recommended by the IMO, and moved on to the Port of Macapá, in the Amazon River, without interfering with the normal procedures of the ship.



Over Flow Method



Dilution Method

In this study, 13 samples were collected and analyzed: 4 from the sediment in the ballast water tanks before ballasting; 1 from the water of the Port of Itaqui/São Luís/MA; 4 from ballast water collected at the port, and stored in the 4 tanks; 1 from ocean water and 3 from ballast water after exchange using the three exchange methods: dilution, empty/full and over flow.

Considering the physical-chemical and microbiological parameters analyzed, it was found, preliminarily, that:

a) Despite the low concentrations of microorganisms in the Port of Itaqui, deep-sea ballast water exchange proved very efficient with the three exchange methods used, presenting close to 100% reduction of the organisms resulting from antropic activities.

b) The water sample from the Port of Itaqui contained fecal coliforms (CF), *E. coli* (EC) and fecal enterocci (EF), proving that the port receives microorganisms from human or animal fecal biota.

c) The levels of phosphate, nitrate, and ammonia were significantly reduced from Port to ocean, proving to be a powerful tool for the verification of deep-sea exchange.



Fecal coliforms present in ballast water



Laboratory installed on board

d) There was a reduction in the concentration of zooplankton after ocean exchange in all the ballast tanks. The taxonomic composition in the tanks was completely different before and after the ocean exchange, that is, before the exchange, zooplankton was characterized by estuary species and after the exchange by ocean species. The tainting species might be used as indicators of ocean waters and the larvae would be indicators of coastal waters.

The concentration of phytoplankton organisms and the number of particles will also be used to evaluate the efficiency of the exchange; however, these data are presently unavailable.

The data on granulometry and organic matter of the sediment will also be used to evaluate the influence of the sediment in the exchange.

3) Microbiological diagnosis of areas of risk at six selected Brazilian ports (Belém, Fortaleza, Recife, Sepetiba, Santos and Rio Grande), aimed at: (i) evaluating the microbiological quality of marine environments of ports by analyzing sea water samples and bivalves (marine products); (ii) verifying the diversity of zooplankton in the marine environment of ports; (iii) searching for the presence of *V. cholerae* O1, *Escherichia coli* and *Salmonella spp* in the marine environment of ports; and (iv) identifying the areas of microbiological risk at ports, based on the results obtained for the aims mentioned above.

Human activities are primarily causing, directly and/or indirectly, changes in the biodiversity of marine environments. On the other hand, pathogenic microorganisms present in domestic, hospital or industrial sewage, when discharged into the marine environment without appropriate treatment, can reach areas of bathers or of cultivation of marine products, posing a risk to the human and animal population living in port and/or coastal regions.

The present project will allow us to know and to determine the critical contamination spots of marine environments, to know the typical microbiota of ports and finally to evaluate the impact that can result from discharge of ballast waters from ships.

This project had been developed since September 9 and the collection stage was concluded by October 4, 2002.

4) Pilot-training of technicians from seven port offices, in the Rapid Analysis of Plankton present in ballast water, to enable them to identify the environmental origin of the water, with the final objective of determining the technical feasibility of the implementation of the Rapid Analysis of Plankton as a routine tool for sanitary monitoring of ballast water at the other Brazilian ports.

The first stage of the training course in Rapid Analysis of Plankton took place at the State University of Santa Cruz (UESC, Ilhéus, BA), from August 27 to 29 of 2002. During a brief theoretical exposition, the ANVISA technicians received basic information on oceanographic characteristics of interest to the understanding of environmental problems related to ballast water. In this context, focus was placed on coast-ocean variations in parameters such as depth, water temperature, salinity, nutrients, concentration and composition of plankton, etc.



Training in Rapid Analysis of Zooplankton

The remainder of the course was dedicated to practical lessons in identification of the main taxonomic groups of marine zooplankton, in samples obtained from the coastal environment and ship ballast tanks analyzed during ANVISA's Exploratory Study. On the last day of the training at UESC, the technicians observed selected samples, listing the

zooplankton groups found in appropriate files. The information was discussed and compared with that obtained by the course instructor for the same samples. In this exercise, it was found that the results of the first stage of training had been very positive and promising, since the percentage of correct identifications carried out by the ANVISA technicians was always close to 100%. Along the month of September, the ANVISA technicians had carried out salinity and zooplankton samplings in the ballast tanks of vessels and in the environment next to the docks of ports. The technicians themselves analyzed the zooplankton samples, with the logistic support of universities located close to the port offices of ANVISA, with a view to the development of a list of the taxonomic groups studied in the first stage of the training. The course instructor analyzed the same samples, in order to determine the degree of correctness of the analyses executed by the ANVISA technicians. On the basis of his comparative study, it will be possible to evaluate the feasibility of the implementation of the Rapid Analysis of Plankton as one of the tools for identification of the environmental origin of ballast water at Brazilian ports.

In MEPC-48 meeting, held from October 7 to 11, ANVISA has forwarded a document proposal (attached) containing the conclusions and the final results of the analyses carried out. The representative of ANVISA had also presented, for discussion at meetings, the conclusions of the new projects carried out in 2002 and the preliminary evaluation of the staff training in the rapid identification of zooplankton present in ballast water.