Chemical and bacteriological evaluation of the water and mussels from Santos bay, São Paulo, Brazil

Avaliação química e bacteriológica na água e mexilhões da baía de Santos, São Paulo, Brasil

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ABSTRACT
The seawater and *Perna perna* mussels samples were bimonthly collected from Santos bay from December 2006 to April 2008, and the occurrence of heavy metals, organic compounds and bacteria were investigated. Water and mussel meat quality were assessed following the Brazilian legislation. The analyses on heavy metals mercury, lead, zinc and cadmium contents were performed using atomic absorption spectrometry. The mussel meat was investigated by analytical methodologies following the international recommendations. Among the analyzed seawater samples, at least one sample showed chlorine, phosphorus, sulfide, total fluoride, ammoniac nitrogen, aluminum, lead and iron contents out of the established limits. In one mussel meat sample only a high peak of zinc concentration was found. The bacteriological agents found in water and mussel meat samples were sporadically above the recommended values.
Key words. *Perna perna*, mussel natural beds, bacteriological contamination, heavy metals, mussel breeding, tropical waters.

RESUMO
Amostras de água do mar e de mexilhão *Perna perna,* coletadas bimestralmente da baía de Santos, no período de dezembro de 2006 a abril de 2008, foram analisadas quanto à presença de metais pesados, compostos orgânicos e bactérias. A qualidade da água e da carne de mexilhão foi avaliada seguindo-se a legislação brasileira. As análises dos metais pesados mercúrio, chumbo, zinco e cádmio foram realizadas pela técnica de espectrometria de absorção atômica. As amostras de carne de mexilhão foram analisadas de acordo com as recomendações internacionais. Entre as amostras de água do mar, pelo menos uma apresentou teor de o cloro, fósforo, sulfeto, fluoreto total, nitrogênio amoniacal, alumínio, chumbo e ferro fora dos limites estabelecidos. Apenas uma amostra de carne do mexilhão apresentou pico elevado de concentração de zinco. Os agentes bacteriológicos analisados nas amostras de água e de carne estavam esporadicamente acima dos valores recomendados.
Palavras-chave. *Perna perna*, banco de mexilhões, contaminação bacteriológica, metais pesados, cultivo mexilhões, águas tropicais.

INTRODUCTION

Bivalve mollusks of the Mytilidae family, commonly called mussels, are widely used in the human diet as a source of animal protein. They present low cost and high nutritional value, and are represented in Santos bay and estuary by the species *Perna perna*, *Mytella falcata* and *M. guyanensis*[1].

The bay-estuary complex of Santos continually gives rise to concern regarding its biological and social characteristics, since it is not only considered a nursery and mussel producer, but also shelters human communities that survive through extracting these resources. This environment is intensely impacted by many anthropic activities that may have implications for public health due to human consumption of mussels[2].

At certain concentrations, organic compounds and heavy metals present in the water can make consumption of extracted bivalves impossible. Disease outbreaks relating to eating these organisms have been reported on several continents, especially associated with gastrointestinal problems, with some reports of fatal cases[3].

High concentrations of metals in the water directly affect these organism's physiology, causing valve closure for long periods, thus damaging growth[4]. Pessatti et al[5] reported that the effect of lead on *P. perna* mussels decreases food absorption, thereby negatively altering the energy flow of the animal's metabolism.

Considering the importance of *Perna perna* mussel consumption within the local scenario and the possibility of implementation of future commercial cultivation in the region, the chemical and bacteriological aspects of the seawater and soft tissue (meat) of individuals of this species, extracted from the bay of Santos, were evaluated.

MATERIALS AND METHODS

Samples of *P. perna* mussels and seawater were gathered every two months (December 2006, February 2007, April 2007, June 2007, August 2007 and November 2007) in Santos bay (Figure 1). This area is located on the south coast of the state of São Paulo and is a part of the estuarine complex of Santos. It is geographically delimited by the island of São Vicente to the north and by two rocky promontories to the east and west, and three different municipalities border the bay: Guarujá, Santos and São Vicente.

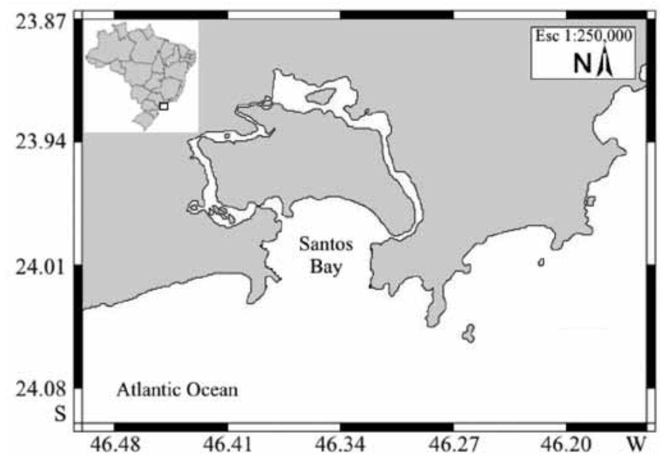

Figure 1. Map of Santos bay with the latitude (S) and longitude (W) coordinates in degrees
(Fonte: NOAA/NGDC Marine Geology and Geophysics Division)

Each sample consisted of 3.0 kg of the bivalves, extracted from the natural mussel beds. The samples were washed with seawater at the location and were stored in an isothermal box for transportation to the laboratory, where all the encrusted organisms were removed.

The water analyses followed the recommendations of Article 18 of the National Environmental Council (Conselho Nacional de Meio Ambiente – CONAMA) Resolution No. 357 of 2005[6], regarding Class 1 saline waters, i.e. waters ideal for breeding aquatic organisms.

The following parameters were evaluated: thermotolerant coliforms; chemical elements/inorganic substances (aluminum, arsenic, barium, beryllium, boron, cadmium, lead, cyanide, residual chlorine, copper, chromium, iron, fluoride, phosphorus, manganese, mercury, nickel, nitrate, nitrite, ammoniacal nitrogen, silver, selenium, sulfides, thallium, uranium and zinc) and organic pesticide substances: aldrin/dieldrin, benzene, benzidine, benzo(a) anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k) fluoranthene, carbaryl, chlordane, 2-chlorophenol, chrysene, DDT, demeton, dibenzo(a,h)anthracene, dichlorobenzidine, dichloroethane, dichloroethene, dodecachloride, endosulfan, endrin, ethylbenzene, total phenols, gution, heptachlorine, hexachlorobenzene, indeno(1,2,3-cd)pyrene, lindane, malathion, methoxychlor, monochlorobenzene, PCBs (polychlorinated biphenyls), pentachlorophenol, tetrachloroethene, toluene, toxaphene, tributyltin, trichlorobenzene, trichloroethene and trichlorophenol.

The recovery standards were carried out with dibromofluoromethane, toluene-d8 and bromofluorobenzene, selected as representatives of the different classes of volatile organic compounds (VOC). The validation parameters of the method were the estimated precision, based on the coefficient of variation between repetitions, and the accuracy of the mean recovery between repetitions, detection limits and quantification[7].

The analytical methods followed the recommendations of the Compendium of Methods for the Microbiological Examination of Foods[8] and the Bacteriological Analytical Manual[9].

The analysis of bacteriological contamination followed Directive RDC No. 12, 7a of Jan 2, 2001, from the National Agency of Sanitary Surveillance (Agência Nacional de Vigilância Sanitária – ANVISA), Ministry of Health, which establishes the following maximum values: coliform bacteria of fecal origin must not surpass $10^2.25g^{-1}$ of meat; absence of *Salmonella* sp in 25 g of meat; most probable number (MPN) of $10^3.g^{-1}$ for *Staphylococcus aureus* and does not provide values for *Vibrio parahaemolyticus*.

Contamination by heavy metals (mercury, lead, zinc and cadmium) was evaluated by means of atomic absorption spectrometry in accordance with EPA 3050/6010B (USA). To prepare the samples, the method described by the Association of Official Analytical Chemists was used[10].

The concentrations of zinc in the mussel meat were compared with the maximum allowed value (MAV) as stated by CONAMA Resolution 357, by means of the two-tailed t-test. The values of the inorganic and organic parameters given by this Resolution for seawater and the parameters for mussel meat were subjected to principal component analysis (PCA) to determine the structure of the data and to reduce the number of variables. To minimize the scale differences, the variables were standardized based on a correlation matrix[11].

The MPN values for total and fecal coliforms obtained in the bacteriological assays were analyzed using the generalized linear model (GLM) protocol, with a model of the form: $Y_{ijk} = \mu + \alpha_j + \beta_k + \gamma_l + \text{interactions} + \varepsilon_{ijk}$, where Y_{ijk} = dependent variable (MPN) of the coliforms i, in the medium j, during the month k; μ = constant (mean population); and the independent variables are α_j = type of coliforms, β_k = medium (water or meat), γ_l = month and ε_{ijk} = random error component[12].

RESULTS

The analytical methods were efficient (recovery between 81 and 126 % and CV < 25% of the matrix spike), considering the acceptance rate between 45 and 135%. The quantification of the heavy metals analyzed in the mussel meat was 0.05 $mg.kg^{-1}$ (Pb), 0.02 $mg.kg^{-1}$ (Cd) and 0.05 $mg.kg^{-1}$ (Hg).

Among the various parameters established in Article 18 of CONAMA Resolution No. 357/2005 for water quality during the study period, chlorine, phosphorus, total fluoride, ammoniacal nitrogen, aluminum, lead and iron presented samples that were slightly outside of the established limits. However, nitrate and sulfide presented greater variations in relation to the MAV, with peaks in December 2006 for sulfide and in February 2007 for nitrate (Table 1).

Table 1. Concentrations ($mg.L^{-1}$) of the parameters detected above the MAV in seawater gathered in Santos bay, from December 2006 to April 2008

Parameters	MAV*	Month-Year					
		Dec 06	Feb 07	Apr 07	Jun 07	Aug 07	Nov 07
Dissolved aluminum	1.500	0.047	0.174	0.000	0.000	0.000	2.188
Total lead	0.010	0.000	0.000	0.015	0.000	0.000	0.000
Free chlorine	0.010	0.050	0.000	0.000	0.090	0.000	0.000
Dissolved iron	0.300	0.111	0.168	0.137	0.000	0.140	0.900
Total fluoride	1.400	1.350	0.000	2.760	0.000	0.000	0.683
Total phosphorus	0.062	0.225	0.335	0.000	0.000	0.000	0.000
Nitrate	0.400	1.460	21.180	0.000	10.930	13.030	4.660
Total ammoniacal nitrogen	0.400	0.170	0.025	1.090	1.070	0.000	0.290
Sulfide	0.002	16.000	0.003	0.000	0.000	0.007	0.000

**MAV – Maximum Allowed Values ($mg.L^{-1}$) according to Article 18 of CONAMA Resolution 357/2005*

Most of the contaminants detected in non-permitted concentrations cause eutrophication in water. Lead is the only contaminant that can trigger problems due to bioaccumulation. However, the values for this metal in water were only slightly beyond the limit (0.01 mg.L^{-1}), and the bivalves did not present contamination. Zinc was the only metal found in high concentrations in these organisms, even if only in April 2007, with no statistical difference (t: P = 0.629) when testing the mean of all the concentrations against the MAV (Figure 2). As well as lead and zinc, mercury and cadmium were also investigated in the bivalve meat, and were within the limits established by the legislation.

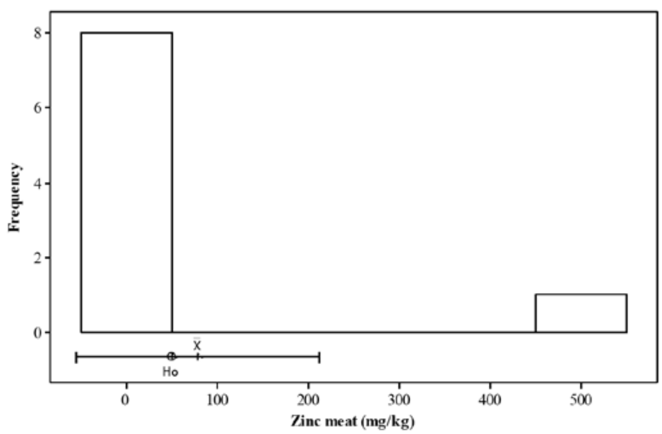

Figure 2. Distribution of zinc concentration frequencies in *Perna perna* meat, gathered in Santos bay, from December 2006 to April 2008. H_0 = MAV = 50.0 mg.kg^{-1} and sample mean = 79.0 mg.kg^{-1}

The variation (eigenvalue) of the first principal component was 6.24 and explained 31.2% of the total variation. The coefficients were estimated based on the following values: 0.37 nitrites; 0.33 fluorides; 0.33 zinc in the meat; 0.32 lead in the water and also in the meat, influenced by the levels of nitrite, fluoride and zinc effects, since all the coefficients of these terms presented the same sign and are not close to zero.

The variation of the second principal component was 5.12 and explained 25.6% of the data variability. This component can be interpreted as the level of cadmium contrast in the meat and of phosphorus contrast in the water. The most important eigenvectors forming the third principal component were chlorine, anionic surfactants, copper and barium in water.

Together, the first two and the first three principal components represented 56.8% and 76.0% of the total variability, respectively. Thus, most of the data structure was captured within these three underlying dimensions. The remaining principal components explained a very small proportion of the variability and were considered as being of no statistical significance (Figure 3).

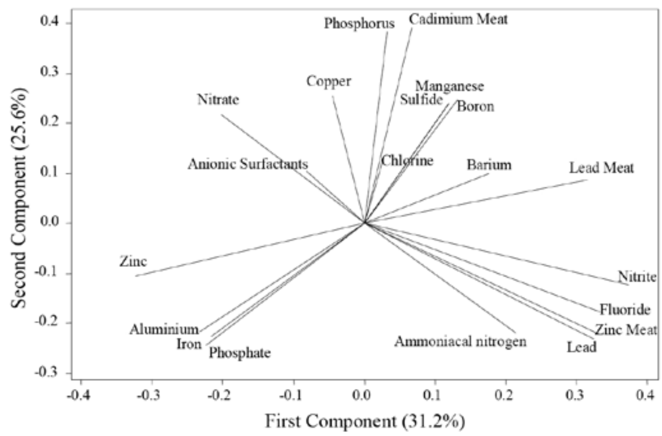

Figure 3. Principal component analysis (PC-1 and PC-2) at the factor level of organic and inorganic parameters in seawater and meat from Perna perna extracted from natural mussel beds in Santos bay

In the microbiological analysis for *Vibrio parahaemolyticus*, the values remained below of MPN_{max}= 10^3.mL^{-1}, except in August 2007 with MPN = 1.3 x 10^5.mL^{-1}. Contamination above the MPN for coliforms in mussel meat was only found in the sample from October/November 2007. The GLM model detected a significant variation of the MPN values, with n = 24 and r^2 = 0.64, only for the factor "medium" (P = 0.003). The factors "type of coliform" (P = 0.148) and "month" (P = 0.064) were not statistically important in the total variation (Figure 4).

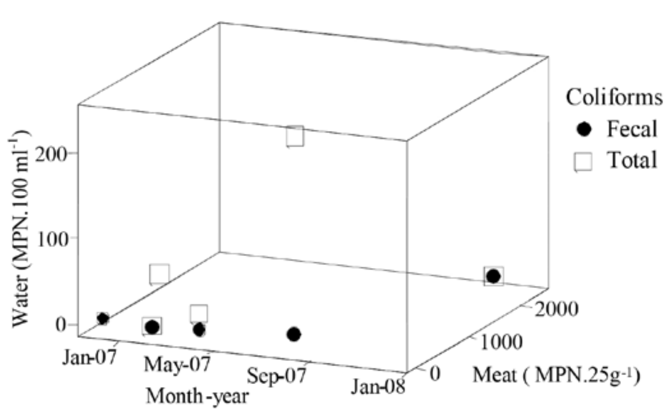

Figure 4. MPN values of coliforms in seawater and meat from Perna perna mussels extracted from natural mussel beds in Santos bay

DISCUSSION

In the seawater, nitrate and sulfide presented, respectively, concentrations that were 53 and 8000 times above the MAV. In the case of nitrate, this occurrence could be related to the load of organic matter coming from the estuary. This condition was observed by Araujo et al[13], when studying the dynamics of zooplankton in urbanized estuaries.

Compounds at concentrations above the MAV can cause eutrophication of the environment, thereby making it easier for mussels to incorporate heavy metals. According to Rainbow[14], marine bivalves are more exposed to contamination by particles in suspension in the water than by the dissolved fraction that needs to be incorporated into the plankton in order to transfer the contamination load to the bivalve.

The peak of zinc found in the mussel meat in April 2007 may indicate the occurrence of positive tropism. Bat et al[15] reported that this metal participates in the metabolism of different species of mollusks capable of concentrating it, even if the presence of this element in water is not permanently above the MAV established by legislation. Sokolowski et al[16] concluded that zinc is regulated by physiological mechanisms that are capable of increasing bioaccumulation, especially in the sexual products of females during spawning periods, regardless of the concentration levels found in seawater. The zinc concentration values observed in the meat from mussels of Santos bay may be related to reproduction. However, Galvao et al[17] registered reproductive peaks for *P. perna* in January and September.

For Nolan e Dahlgaard[18], zinc is an essential element for mussels and is necessary for forming various biological molecules, among them structural proteins and enzymes. Jorge et al[19] showed in laboratory assays that *P. perna* larvae react negatively to the presence of zinc sulfate, by reducing their aerobic metabolism.

Zinc is related not only to the life cycle of mussels, but also to that of other bivalves. Studying bioaccumulation of pollutants[20] among different groups of mollusks in the Todos os Santos bay (Bahia, Brazil), observed concentrations of zinc above the MAV for the oyster *Crassostrea rhyzophorae*.

Avelar et al[21] detected relatively high values of lead and chromium among *P. perna* bivalves in January and July at the Itaguá beach, Ubatuba bay, SP. Baraj et al[22] evaluated the contamination by lead, cadmium, copper, zinc, chromium, manganese and iron for the same bivalve along 800 km of the southern coast of Brazil and found that the concentrations were below the MAV.

Sidoumou[23] analyzed bioaccumulations of cadmium, copper and zinc in *P. perna* from the coast of Senegal and observed cadmium in mean concentrations of 2.37 $mg.kg^{-1}$. In this study, the highest concentration of this heavy metal found in mussel meat was 0.25 $mg.kg^{-1}$, i.e. one fourth of the MAV.

In another study[24], levels of Pb, Cd, Hg, Cu and Zn were analyzed in samples of natural beds of mussels from the coast of Sao Paulo State, Brazil. In all samples of *P. perna* and *M. falcata* analyzed metals were below the limit. While in oysters *C. brasiliana*, Zn concentration was above the legal limit.

The bacteriological contamination in the water and meat of *P. perna* did not present any kind of pattern, only occasional peaks. Previous studies carried out at the same place showed oscillations in the levels of these contaminations, with a possible association with periods of intense rain and greater population density[25, 26]. According to current legislation in Brazil, the occurrence of only a single case of meat contamination condemns the product with regard to human consumption[27]. This contamination is detected in analyses of the water from where the animals are extracted, unlike the norms used by the European Union, which are based on analyses of the meat[27].

It was observed that in the analyses subsequent to those with bacteriological contamination, the mussels presented approved quality, thus demonstrating that there was natural cleansing. For this reason, establishing rejection criteria based on maximum occurrence frequency of contaminated samples, as is done in the European Union, could also be adopted in Brazil.

CONCLUSIONS

The evaluation on the chemical and bacteriological quality of *P. perna* meat showed that extraction of these mussels from Santos bay for consumption needs constant sanitary quality monitoring, due to the lack of predictability of the contaminating agents. These data, when gathered periodically and systematically, especially from batches prior to extraction, may serve to monitor the environment, so as to offer a product with certified quality to consumers.

Considering the low incidence of contaminated meat and seawater samples, it is proposed that an additional criterion based on the maximum percentage occurrence of contaminated samples from the exploitation or cultivation site should be applied before condemning the suspected batch.

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REFERENCES

1. Fagundes L, Henriques MB, Ostini S, Gelli VC. Custos e Benefícios da mitilicultura em espinhel no sistema empresarial e familiar. Informações Econômicas. 1997; 27(2): 33-54.
2. Silva NJR, Reno SF, Henriques MB. Atividade extrativa do mexilhão *Perna perna* em bancos naturais da baía de Santos, estado de São Paulo: uma abordagem sócio-econômica. Informações Econômicas. 2009; 39(9): 62-73.
3. Potasman I, Paz A, Odeh M. Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. Clin Infect Dis. 2002; 35: 921-8.
4. Kraak MHS, Ainscough C. Fernández A, Vlaardingen P, Voogt P, Admiraal WA. Short-term and chronic exposure of the zebra mussel (*Dreissenia polymorpha*) to acridine: effects and metabolism. Aquat Toxicol. 1997; 37: 9-20.
5. Pessatti ML, Resgalla JRC, Reis Filho RW, Kuehn J, Salomão LC, Fontana JD. Variability of filtration and food assimilation rates, respiratory activity and multixenobiotic resistance (MXR) mechanism in the mussel *Perna perna* under lead influence. Braz J Biol. 2002; 62(4): 651-6.
6. Brasil. Resolução CONAMA (Conselho Nacional de Meio Ambiente) nº. 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União. Brasília, DF, 18 mar. 2005. Seção 1, nº 53. p. 58-63.
7. Ramsey MH, Ellison SLR, editors. Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide. Measurement uncertainty arising from sampling: a guide to methods and approaches. Eurachem; 2007.
8. Vanderzant C, Splittstocsser DF. Compendium of Methods for the Microbiological Examination of Foods. 3ª. ed. Washington (U.S.A.): American Public Health Association; 1992.
9. United States Food and Drug Administration. Bacteriological Analytical Manual. 7ª. ed. Arlington (U.S.A.): A.O.A.C. International; 1992.
10. AOAC. Association of Official Analytical Chemists. Official methods of analysis. 15ª. ed. Washington (D.C.); 1995.
11. Santiago-Rivas S, Moreda-Piñeiro A, Barciela-Alonso MC, Bermejo-Barrera P. Characterization of raft mussels according to total trace elements and trace elements bound to metallothionein-like proteins. J Environ Monit. 2009; 11: 1389-96.
12. Sonier R, Mayrand E, Ouellette M, Boghen AD, Mallet V. Spatial and temporal faecal coliform variations in surface water, sediments and cultivated oyster's meat, *Crassostrea virginica*, from Richibucto estuary, New Brunswick. Can Tech Fish Aquat Sci. 2006; 2658: 28.
13. Araujo HMP, Nascimento-Vieira DAB, Neumann-Leitão SB, Schwamborn RC, Lucas APOA, Alves JPHD. Zooplankton community dynamics in relation to the seasonal cycle and nutrient inputs in an urban tropical estuary in Brazil. Braz J Biol. 2008; 68(4): 751-62.
14. Rainbow PS. Biomonitoring of Heavy Metal Availability in the Marine Environment. Mar Poll Bull. 1995; 31(4-12): 183-92.
15. Bat L, Gündogdu A, Öztürk M. Copper, Zinc, Lead and Cadmium Concentrations in the Mediterranean Mussel *Mytilus galloprovincialis* Lamarck, 1819 from the Sinop Coast of the Black Sea. Tr J of Zoology. 1999; 23: 321-6.
16. Sokolowski A, Bawazir AS, Wolowicz M. Trace metals in the brown mussel *Perna perna* from the coastal waters off Yemen (Gulf of Aden): How concentrations are affected by weight, sex, and seasonal cycle. Arch Environ Contam Toxicol. 2004; 46(1): 67-80.
17. Galvão MSN, Henriques MB, Pereira OM, Marques HLA. Ciclo reprodutivo e infestação parasitária de mexilhões *Perna perna* (Linnaeus, 1758). B Inst Pesca. 2006; 32(1): 59-71.
18. Nolan C, Dahlgaard H. Accumulation of metal radiotracers by *Mytilus edulis*. Mar Ecol Prog Ser. 1991; 70: 165-74.
19. Jorge R, Lemos D, Moreira GS. Effect of zinc and benzene on respiration and excretion of mussel larvae (*Perna perna*) (Linnaeus, 1758) (Mollusca; Bivalvia). Braz J Biol. 2007; 67(1): 111-5.
20. Amado-Filho GM, Salgado LT, Rebelo MF, Rezende CE, Karez CS, Pfeiffer WC. Metais pesados em organismos bentônicos da Baía de Todos os Santos, Brasil. Braz J Biol. 2008; 68(1): 95-100.
21. Avelar WE, Mantelatto FL, Tomazelli AC, Silva DM, Shuhama T, Lopes JL. The marine mussel *Perna perna* (mollusca, bivalvia, mytilidae) as an indicator of contamination by heavy metals in the Ubatuba Bay, São Paulo, Brazil. Water, Air, & Soil Pollution. 2000; 118(1-2): 65-72.
22. Baraj B, Niencheski LF, Corradi C. Trace metal content trend of mussel *Perna perna* (Linnaeus, 1758) from the Atlantic Coast of Southern Brazil. Water, Air, & Soil Pollution. 2003; 145(1-4): 205-14.
23. Sidoumou Z, Gnassia-Barelli M, Siau Y, Morton V, Romeo M. Heavy metal concentrations in molluscs from the Senegal coast. Environ. International. 2006; 32: 384-7.
24. Pereira OM, Henriques MB, Zenebon O, Sakuma A, Kira CS. Determinação dos teores de Hg, Pb, Cd, Cu e Zn em moluscos (*Crassostrea brasiliana, Perna perna* e *Mytella falcata*). Rev Inst Adolfo Lutz. 2002; 61(1): 19-25.

25. Henriques MB, Marques HLA, Lombardi JV, Pereira OM, Brossi-Garcia A. Influência da contaminação bacteriológica sobre a resistência do mexilhão *Perna perna* (Linnaeus, 1758) à exposição ao ar. Arq Cienc Mar. 2003; 36: 95-9.

26. Henriques MB, Zamarioli LA, Pereira OM, Faustino JS. Contaminação bacteriológica no tecido mole do mexilhão *Perna perna* (Linnaeus, 1758) nos bancos naturais do litoral da Baixada Santista, Estado de São Paulo. Arq Cienc Mar. 2003; 33: 69-76.

27. Brasil. Ministério da Saúde. Resolução RDC nº. 33, de 02 de janeiro de 2001. Aprova o Regulamento Técnico sobre Padrões Microbiológicos para Alimentos. Diário Oficial [da] União. Brasília, DF, 10 jan. 2001. Disponível em: [http://www.anvisa.gov.br/legis/resol/12_01rdc.htm].

28. Alzieu C. Water. The medium of culture. *In*: Munday B, Eleftheriou A, Kentouri M, Divanach P. The Interactions of Aquaculture and the Environment: a Bibliographical Review. Belgium: Commission of the European Communities, DG Fisheries; 1989.