REVISTA DE CIÊNCIAS AMBIENTAIS - RCA (ISSN 1981-8858)
http://www.revistas.unilasalle.edu.br/index.php/Rbca
Canoas, vol. 8, n. 2, 2014

PRESENCE AND ABUNDANCE OF PUBLIC INTEREST BACTERIA IN SEDIMENTS FROM THE UPPER COMPARTMENTS OF BARRA BONITAS RESERVOIRS, SÃO PAULO - BRAZIL (TIETÊ AND PIRACICABA RIVERS)

Julieta Bramorski[1]
Swami Marcondes Villela[2]

ABSTRACT

Barra Bonita's reservoiris in a highly dense demographic region, and receives high loads of sewage daily *in natura*which are transported mainly by the Piracicaba and Tietê rivers. This fact has provoked the available hydric resources commitmentfor both human consumption and plantation irrigations thus increasing considerably the risk of waterborne diseases. It is known that high densities of fecal originated microorganisms accumulate among the sediments in the deeper parts of aquatic ecosystems. With the purpose of determining the presence and abundance of public interest microorganism in recent sediment samples, analyzing samples were taken from the reservoirs major entrance compartments (Tietê and Piracicaba rivers)and the region of confluence between both. Samples were analyzed by Chromocult kit, determining the presence and abundance of coliforms, *Escherichia coli* e *Salmonella* sp. The results indicate that there is a high *Salmonella* concentration in all collection stationsshowing poor sanitary and environmental conditions and considering the inter-relations between sediment and water column there is the possibility of compromising recreational activities and vegetable irrigation.

Keywords: sediments quality; initial compartments; public interest microorganisms

RESUMO

Presença e abundância de bactérias de interesse público em sedimentos a partir dos compartimentos superiores dos reservatórios de Barra Bonitas, São Paulo - Brasil (Tietê e rios Piracicaba). O reservatório de Barra Bonita está em uma região demográfica altamente densa e recebe altas cargas de esgoto diariamente i*n natura* que são transportados principalmente pelos rios Piracicaba e Tietê. Este fato provocou o comprometimento de recursos hídricos disponíveis para consumo humano e irrigação de plantação, aumentando assim consideravelmente o risco de doenças transmitidas pela água. É sabido que altas densidades de microorganismos de origem fecais se acumulam entre os sedimentos nas partes mais profundas dos ecossistemas aquáticos.Com o objetivo de determinar a presença e abundância de microorganismos de interesse público em amostras de sedimentos recentes, amostras de análise foram retiradas dos comportamentos de entrada principal dos reservatórios (rios Tietê e Piracicaba) e na região de confluência entre ambos.As amostras foram analisadas pelo kit Chromocult, determinando a presença e abundância de coliformes, Escherichia coli e Salmonellasp.Os resultados indicam que há uma alta concentração de Salmonela em todas as estações de coleta mostrando más condições sanitárias e ambientais e considerando as inter-relações entre a coluna de água e sedimentos, há a possibilidade de comprometer as atividades recreativas e a irrigação de vegetal.

Palavras-chave: qualidade de sedimentos; compartimentos iniciais; microorganismos de interesse público

[1] Centro de Recursos Hídricos e Ecologia Aplicada, Universidade de São Paulo – USP, São Carlos, SP, Brasil. Email: bramorski@gmail.com
[2] Professor do Depto. de Hidráulica e Saneamento – Universidade de São Paulo – USP, São Carlos, SP, Brasil

Introduction

Barra Bonita's Reservoir is located in a highly developed region and is a targetof countless anthropic activities. All studies carried out at this point of the reservoir where high loads of domestic sewage is directly discharged or not along its main tributaries. The accelerated demographic growth and the consequent raw domestic loads have lowered water quality for human consumption thus considerably increasing the risk of water-borne diseases (Naidoo and Olaniran, 2014).

The areas that have a direct influence on the reservoir include the Piracicaba, Capivari and Jundiaí basin via the Piracicaba River and the Sorocaba/Middle Tietê Basin via the Tietê River. Contamination of the aquatic ecosystems that has been occurring for some time in this region is widely known to be caused by industrial development, demographic growth and an intense and accelerated soil occupation.

Drinking water for the majority of the population is obtained from the rivers and lakes which can possibly be contaminated by the domestic and industrial sewage loads and also by the surfaces inflow. As a mean of transportation for pathogenic germsthe water can cause health and lifelong risks (Naidoo and Olaniran, 2014).

It is clear that the microbial population (including pathogens) can be associated with the sediment fraction both in the suspended and bed sediment phase (Krometis et al., 2007; Rehmann and Soupir, 2009). Bacteria and pathogens have been shown to be the result of a higher magnitude in associationwith sediments (both bed and suspended) with the bed sedimentrepresenting a significant reservoir with possible pathogenic organisms (Droppo et al., 2001; Droppo et al., 2009).

Droppo et al. (2011) conclude that monitoring programs intended to assess risk to human health which currently neglect the sediment phase of pathogen existence and the energy regime they preside in may provide management decisions which are erroneous with possible detrimental impacts. As such, it is critical that management protocols and policy development for the protection of aquatic and human health take into account both the SS and of bed sediment indicator organism population.

The heterotrophic bacteria has been studied because in this group all the pathogenic bacteriafor humans have been found including other animals and plantsand also in a great part of the microbial population of the human environment (Allen et al., 2004).

Fecal bacteria such as fecal coliform, *Escherichia coli* are widely used to monitor the fecal contamination of water bodies around the world (Bai and Lungo, 2005). Fecal bacteria can survive much longer in sediment bed than in the water column (Burton et al., 1987; Davies et al., 1995).

According to An et al. (2002) the coliforms present in the sediment may in determined conditions resuspend in the water column thus representing a continuous source of environmental quality degradation. Nagels et al. (2002) compared the *E. coli* concentrations during a natural flood and an artificial flood and found that around 30% of the bacteria were resuspended from the bed sediments.

In this context, data on the presence and quantity of these public-interest microorganisms in the top sediment compartments of this reservoir constitute relevant information about the environmental quality of the system and taking into consideration two of its many uses: irrigation and public water supply.The objective of this study wasto characterize the sediments of the two tributaries of the Barra Bonita reservoir in terms of presence and the density of public-interest microorganisms.

MATERIAL AND METHODS

Field Work

The collection stations were chosen from topographic maps at sites located along each tributary arm and their meeting point. A map of Barra Bonita reservoir, showing its main tributaries and the collection stations, are presented in figure 1. The geographic coordinates of each collection station are showed in table 1 (GPS Trimble Navigation).

In each collection station 3 sediment samples were taken. These samples were mixed and then a representative sample of about 1 kg was selected. The samples were taken using an Ekman-Birge grab and stored in sterile flasks avoiding direct contact with the sample. On the same day as the collection thermal, boxes at approximately 4°C were used for transportation to the laboratory. The collections were made on a single occasion in the dry season in 2002.

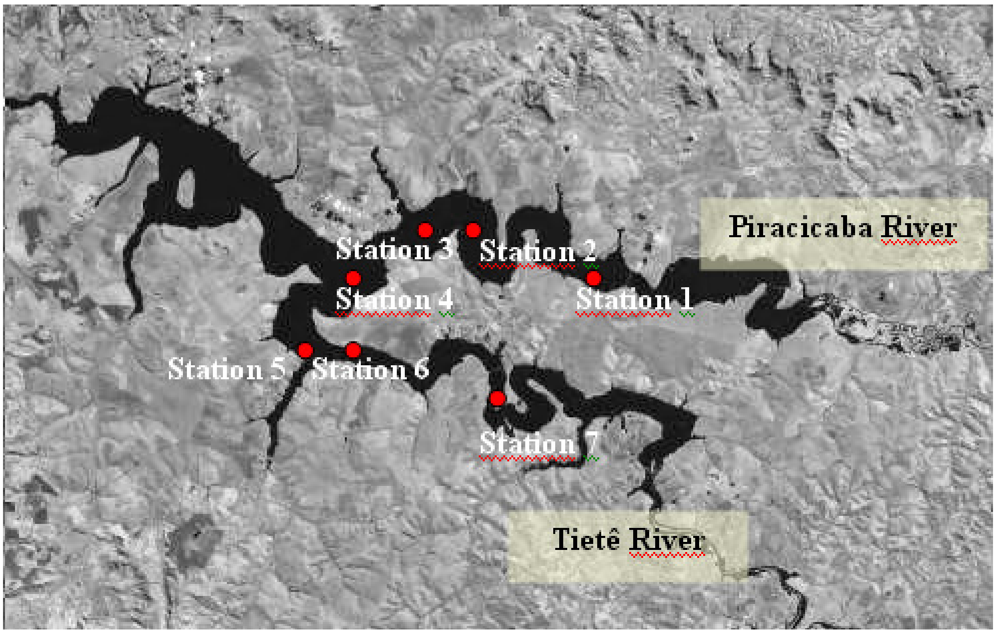

Figure 1. Location of collection sites in the Barra Bonita reservoir.

Table 1. Geographic coordinates of the sampling stations.

Collection Station	Tributary	Geographic Coordinates	
Station 1	Piracicaba river	22° 37'10.51" S	48°12' 24.41" W
Station 2	Piracicaba river	22° 35' 38.26" S	48° 14' 50.48" W
Station 3	Piracicaba river	22° 35' 59.17" S	48° 17' 14.07" W
Station 4	Confluence region	22° 36' 55.76" S	48° 20' 13.01" W
Station 5	Tietê river	22° 40' 2.75" S	48° 20' 57.14" W
Station 6	Tietê river	22° 40' 10.70" S	48° 19' 26.41" W

Laboratory Research

For the isolation counting and colony culture of bacteria from the sediment samples 5 mL samples of sediment were taken (sediment + interstitial water) and transferred to 500 mL measuring cylinders and then filled with distilled water and inverted for 1 minute. Aliquots were taken (100 mL) from the cylinder and diluted serially 4 times in distilled water. Thus eachsample provided 4 dilutions which were subjected to a membrane filter technique, according to “Standard of Methods for the Examination of Water and Wastewater”, for the bacteriological diagnosis.

This technique consists of the following steps:

- a known volume of diluted sediment is passed through a sterile filter disc (47 mm across; 0.2 μm pores) and the bacteria are held on the membrane surface;
- the membrane is then removed and placed on an absorbent cushion which has already been saturated with the appropriate medium (Chromocult kit, by SARTORIUS BIOTEC - Germany) and placed in a Petri disk which isthen incubated for 24 hours at 37°C;
- after incubation, are observed to develop colonies on the filter membrane at the points where the bacteria are retained on the filtering;
- the colonies are manually counted by observing the different colorations, included the heterotrophic bacteria;
- the result is given by the number of colonies observed multiplied by the dilution factor and expressed as colony-forming units per 100 mL of sediment suspension (CFU/100 mL).

Statistical Analysis

A cluster analysis was applied to the acquired data. The grouping and linking steps of this process results in a cophenetic matrix and by comparing this with the similaritymatrix of the row data the cophenetic correlation coefficient can be derivedwhich provides an estimate of the degree of distortion in the original cluster. According to Legendre and Legendre (1983), indexes over 0.80 are acceptable. The cophenetic correlation coefficient obtained from this study was 0.9476, indicating very little distortionof the original data. Clustering by unweighted linkage was chosen, attributing similarity between pairs of clusters in a less extreme way.

RESULTS AND DISCUSSION

Density and relative abundance total of coliform (*Escherichia coli* and *Salmonella*) in the main upper compartments of Barra Bonita´s Reservoir.

Thedensities oftotal coliform, *Escherichia coli* and *Salmonella* sp. are shown in figure 2. As these microorganisms are present in human feces they can reach water bodies through the disposal of sewage and the pathogenic bacteria can present an epidemic risk through the consumption of contaminated water or food. It can be seen that the pathogenic enterobacteria *Salmonella* sp. are dominant in all collection stations with high densities varying from $16x10^5$ CFU/100 mL to $62x10^5$ CFU /100 mL.

As for the spacial distribution of the *Salmonella* sp. among the collection stations, it was observed that the greater densities occurred at uppermost stations (Station 1 in the Piracicaba river arm and Stations 6 and 7 in the Tietê river arm).

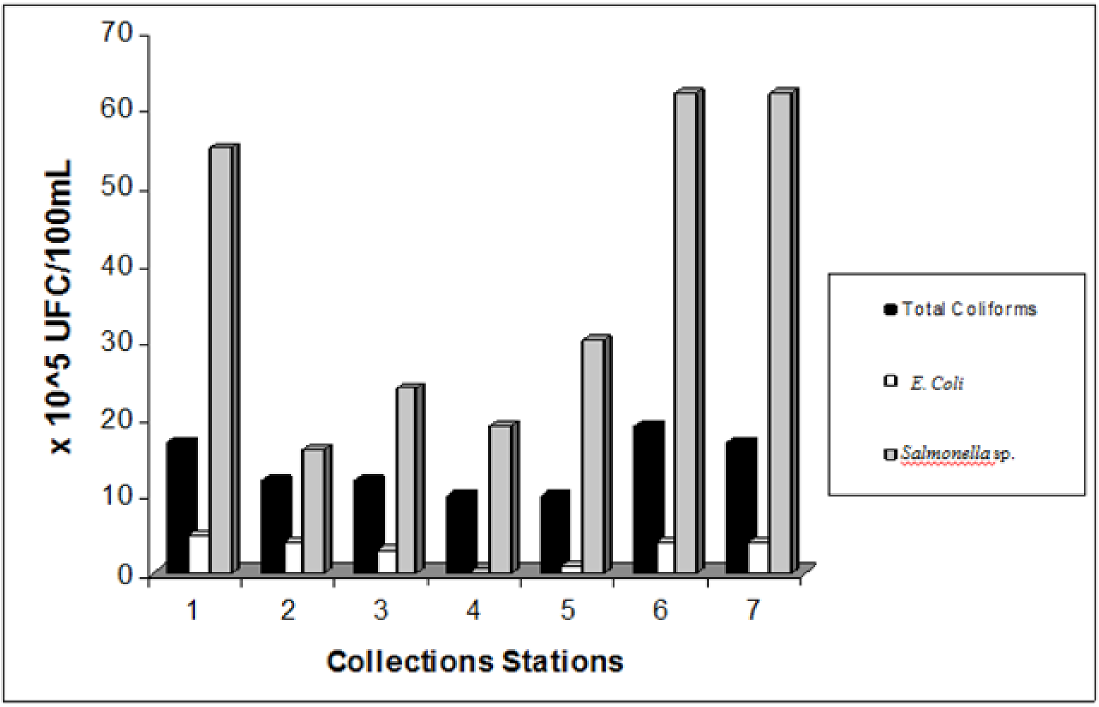

Figure 2. Bacterial density in sediment at each collection stations.

In relation to the *Escherichia coli* it can be seen that its density was lower than that of other microorganisms, with a maximum of $5x10^5$ CFU/100 mL, in station 1 (Piracicaba river) and the microorganism was absence at the confluence region of the Piracicaba and Tietê rivers. An et al. (2002) also found a maximum of $5x10^5$ UFC/100 mL in the sediments of the Texoma Lake –USA, and demonstrated that the density of this microorganism in the sediment was very much higher than in the water, showing evidence of re-suspension of the bacteria from the sediment in the summer months owing to recreational activities such as boating.

Bacteria and pathogens have been shown to be orders of magnitude higher in association with sediments (both bed and suspended) with the bed sediment representing a significant reservoir of possible pathogenic organisms (Obiri-Danso and Jones, 2000; Hirotani and Yoshino, 2010). Hence, in places used for recreational purposes the presence of *Escherichia coli* does not always indicate recent fecal contamination but may reflect a simple resuspension of the bacteria from the sediments caused by the motion of the boats and other sources of disturbance.

The maximum total coliforms density found in the sediment samples was $19x10^5$ CFU/100 mL, at station 6 (Tietê river). The sediment from the Texoma Lake, USA, was subjected to a highly anthropic influence. An et al. (2002), analyzing the sediments from the Texoma Lake-USA, a highly anthropic influenced place detected a maximum density as $28x10^5$ CFU/100 mL.

The relative abundance of bacteria at each collection station is show in figure 3. It must be mentioned that while all salmonellae are pathogenic, not all kinds are able to cause infirmities in humans. The abundant presence of this microorganism gives an indication of low environmental quality, since besides being evidence of fecal material, they can seriously damage the water quality. It is known that the lifetime of the *Salmonella* sp., in aquatic environments is approximately one week which shows that the pattern found in this study may reflecta momentary situation, although even if that is thecase human contact and consequent damage to health would still be possible.

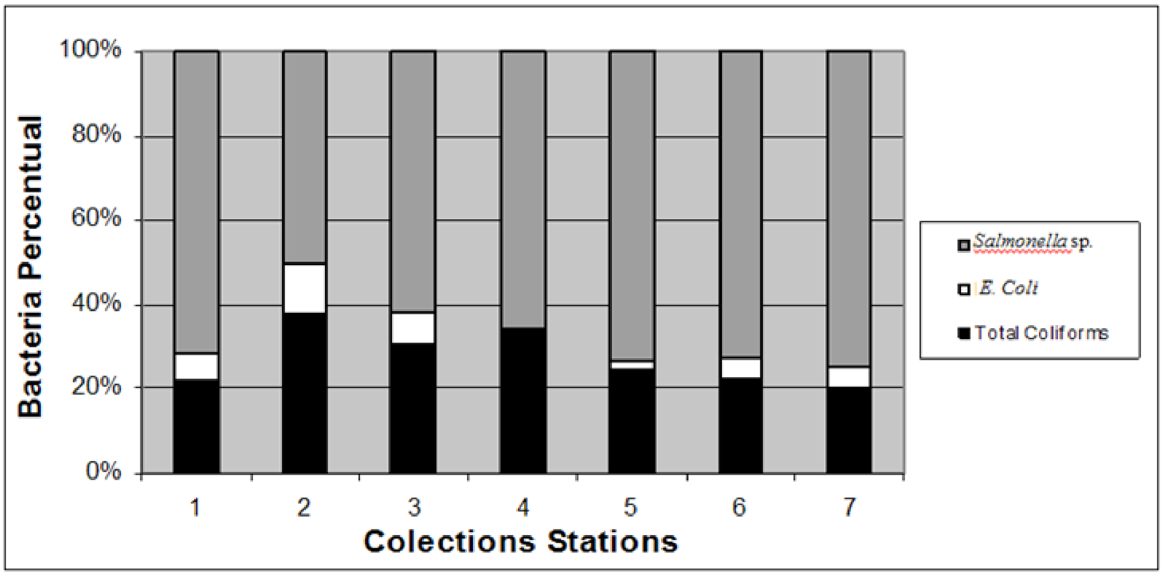

Figure 3. Relative abundance of microorganisms in different collection stations sediments.

Although there are no established standard limits for coliforms in sediments the organisms densities found can be considered as high and similar to the values found by An et al. (2002) in a highly impacted lake (Texoma Lake-USA). However, the focus must be not onlybe the total microorganisms density but on the pathogenic typespresentat all collection stations. The highest densities of all analyzed microorganisms were observed at stations 1 (Piracicaba river), 6 and 7 (Tietê river). These stations are located at the points that are upstream from the confluence region and thus at a point transition between the lotic and lentic environments. In the area influenced by the damming the flow speed decreases and the microorganisms are carried over shorter distances.

Jones and Obiri-Danso (1999) said that the coliforms densities of fecal origin are influenced by diverse environmental factors. High temperatures, pH, dissolved oxygen levelsand intense solar radiation can increase the mortality rates of these microorganisms.

The sediment environment is characterized by lower temperatures than the water column, anoxia and low (or no) solar radiation and promoting good conditions for these microorganisms to remain alive. Gough and Sthahl (2002) studied bacterial density in sediments which found greater densities in sediments contaminated by metals than in uncontaminated sediments. This is a significant indicator that the bacteria can persist in highly contaminated environments, although other factorssuch as nutrient loads, physical properties of the sediment and rates of predation by other organisms may strongly influence the observed pattern.

Recreational practices, fishing and crops irrigationconstitute to activities that can promote direct human contact with these pathogenic microorganisms. In the case of, the main risks relate to vegetables that are eaten raw. Hence it is not recommended to irrigate these plants with water that contains high levels of coliforms, indicating high concentrations of fecal matter, since this water carries parasites, parasite eggs and some protozoans, such as amoeba and Giardia, usually transmitted vegetables.

In figure 4 a similar dendrogram which is formed by clustering the data so that they are more uniform in the same ensemble. At the “cut” in 100,000 (average euclidian distance), two distinct groups are observed: the first group consist of stations 2, 3 (Piracicaba river), 4 (confluence) and 5 (Tietê river); the second of stations 1 (Piracicaba river), 6 and 7 (Tietê river).

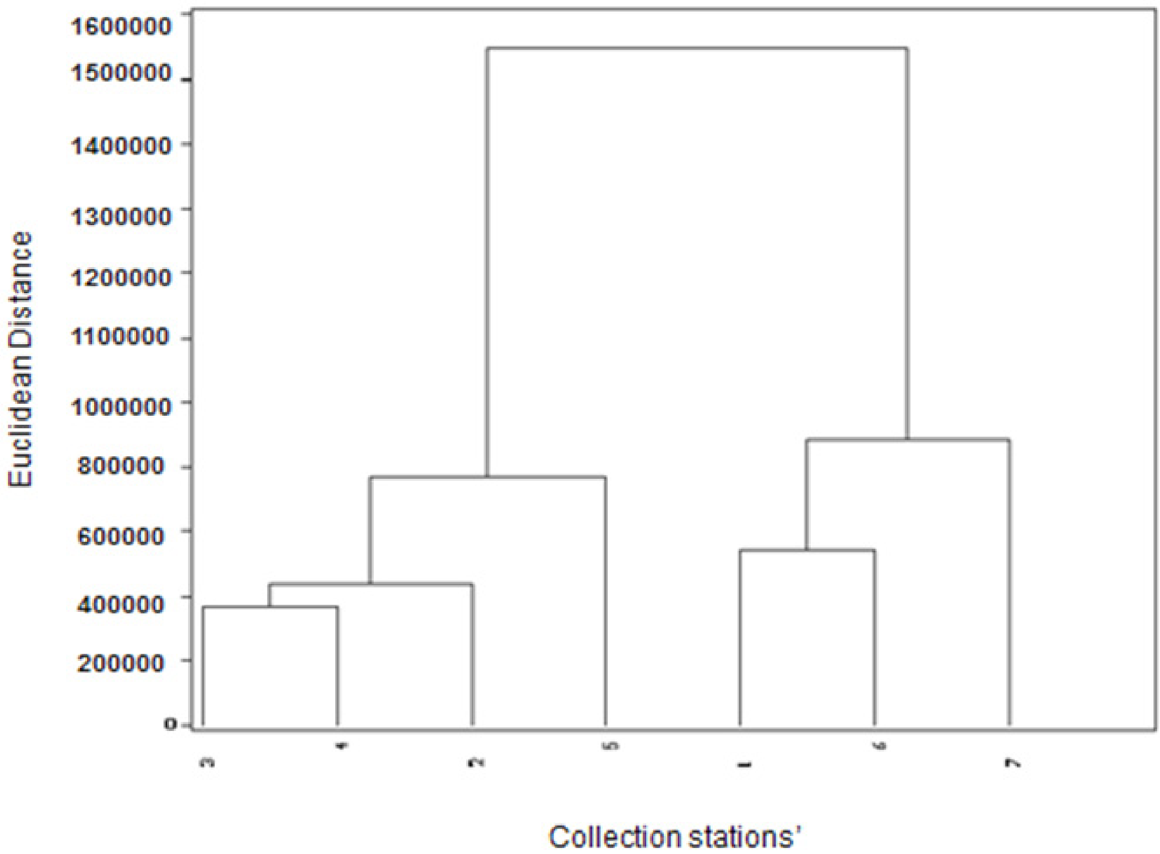

Figure 4. Dendrogram of microbial densities found at each sampling points.

It is shown through cluster analysis that the observed grouping represents two distinct conditions, one group being formed by the stations located in the area influenced by the damming of the waters and the other by the areas in the transition zone between the lotic and lentic environments.

The lotic environment is mainly governed by water flow and is characterized by high rates of allochthonous material transportation. The lentic environment has less turbulence and a weakerforce flow and consequentlymore deposition of particulate material. While a considerable amount of transport in the transition region still occurs it is when a continuous deposition process begins.

It must be pointed out that stations 6 and 7 located on the Tietê River show bacterial densities and are also the point that is most affected by the accumulation of nutrients, metal contamination and toxic effects to the test-organisms, according to Bramorski (2004) .

The hydrodynamics of the sedimentation processes and the physical and chemical conditions at these stationscan possibly provide adequate conditions for the maintenance and viability of the microorganisms analyzed in this study.

CONCLUSIONS

The high sewage loads discharged *in natura* on the Piracicaba and Tietê Rivers produce considerable densities of public interest microorganisms which are deposited in the sediments.

The high density and predominance of pathogenic bacteria in all sampled stations reveal critical sanitary conditions with reflexes onthe water quality of this system.

In this system the sediments constitute for a continuous source of the quality of the water quality degradation and some activities (boating, recreationetc) may promote the resuspension of the microorganisms to the water column.

The presented scenario compromisesof several uses to which this body of water is put to use such as a source of irrigation used mainly for raw vegetables which is general ingested without boiling.

Public water supplies systems that make their captation in this area should be alert to the initial disinfection (chlorine or other product) thereof, whose presence of pathogens of interest in public health proved to be very significant in recent layer sediment and can easily be re-suspended in the water column. In this case, the simple chlorination is not indicated, being necessary to invest in safer methods due to the condition observed.

The stations located on the transition zone between the lotic and lentic environments exhibited the highest densities of microorganisms thus constituting a distinct cluster. It is believed that the coliforms are extremely resistant in sediments at impacted sites, representinga good indicator of environmental quality.

The conditions that promote microorganisms development were not tested in this study, but it is known that the sediment region is characterized by anoxia, low (or no) solar radiation and lower temperatures than the water column, factors that benefit the growth survival of this bacteria. Nutrient storage is another factor that may contribute to the development of these microorganisms;

Knowledge about the interactions between the sediment region and the water column brings substantial changes in water quality assessment for consumption and for irrigation and for sport especially swimming. The presence of *Escherichia coli* (fecal coliformdoesn't always indicate recent fecal contamination,which reflects many times in the re-suspension of bacteria deposited in sediments caused by turbulence in the water column.

ACKNOWLEDGEMENTS

The authors are grateful to CNPq for the scholarship, to FAPESP for the research financing, (Process 2003/01301-2) and the Studies and Projects Financing (FINEP) through the CT-HIDRO 2001 Project, which financed part of the data-collecting activities (FINEP's agreement with FIPAI - Research and Industrial Development Support Foundation, process 23.01.0606.00).

REFERENCES

ALLEN, M. J. ; EDBERGB, S. C.; REASONERCD. J. 2004. Heterotrophic plate count bacteria - what is their significance in drinking water? **International Journal of Food Microbiology**, **92**:265–274.

AN, Y.; KAMPBELL, D. H.; BREIDENBACH, G. P. 2002. *Escherichia coli* and total coliforms in water and sediment at lake marinas. **Environmental Pollution**, **120**:771-778.

BAI, S.; LUNG, W. S. 2005. Modeling sediment impact on thetransport of fecal bacteria. **Water Research**, **39**:5232–5240.

BRAMORSKI, J. 2004. Avaliação da qualidade de sedimentos dos rios Tietê e Piracicaba nos seus compartimentos de entrada no reservatório de Barra Bonita, SP. Dissertação (Mestrado em Ciências da Engenharia Ambiental) - Universidade de São Paulo, 114 p.

BURTON, G. A.; GUNNISON, D.; LANZA, G. R. 1987. Survival ofpathogenic bacteria in various freshwater sediments. **Appl.Environ. Microbiol**., **53**(4):633–638.

DAVIES, C. M et al. 1995. Survival of fecal microorganisms in marine and freshwatersediments. **Appl. Environ. Microbiol**., **61**(5):1888-1896.

DROPPO, I. G.; LAU, Y. L.; MITCHELL, C . 2001. The effect of depositional history on contaminated bed sediment stability. **Set. Total Environ.**, **266**:7-13 .

DROPPO, I. G. et al. 2009. The dynamic existence of waterborne pathogens within river sediment compartments e Implications for water quality regulatory affairs. **Env. Sci. Technol**., **43**(6):1737-1743.

_____. 2011. Modelling sediment-microbial dynamics in the South NationRiver, Ontario, Canada: Towards the prediction of aquatic and human health risk. **Water Research**, **45**:3797-3809.

GOUGH, H. L; STHAL, D. A. 2002. Optimization of direct cell counting in sediment. **Journal of Microbiological Methods**, **52**:39-46.

HIROTANI, H.; YOSHINO, M. 2010. Microbial indicators in natural biofilms developed in the riverbed. **Wat. Sci. Technol**., **62**:1149-1153.

JONES, K.; OBIRI-DANSO, K. 1999. Non-compliance of beaches with the EU directives of bathing water quality: evidence of non-point sources of pollution in Morecambe Bay. **Journal of Applied Microbiology**, **85**:101-107.

KROMETIS, L. H. et al. 2007. Intra-storm variability in microbial loading rates. **Water Research.**, **41**:506–516.

LEGENDRE, P.; LEGENDRE, L. 1983. **Numerical Ecology**: developments in environmental modelling. Amsterdam: Elsevier Science, 317p.

NAGELS, J. et al. 2002. Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. **Water Sci. Technol.**, **45**:45-52.

NAIDOO, S.; OLANIRAN, A. O. 2014. Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. **Int. J. Environ. Res. Public Health**, **11**(1):249-270.

OBIRI-DANSO, K.; JONES, K. 2000. Intertidal sediments as reservoirsfor hippurate negative campylobacters, salmonellae andfaecal indicators in three EU recognised bathing waters inNorth West England. **Water Res**., **34**:519-527.

REHMANN, C .R.; SOUPIR, M. L. 2009. Importance of interactions between the water column and the sediment for microbial concentrations in streams, **Water Research, 43**:4579-4589.