

Methods for Sanitary Inspection of Microbiological and Parasitary Quality of Water and Sand of Recreation Areas

Adriana Sotero-Martins¹, Natasha Berendonk Handam³, Priscila Gonçalves Moura³, Ludimila Santos Amaral³, Luana Vilela Lopes Caldas², Elvira Carvajal⁴

¹(Department of Sanitation and Environment Health, Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation - FIOCRUZ, Brazil)

²(Program of Doctorate's Degree in Public Health and Environment/ Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation - FIOCRUZ, Brazil)

³(Program of Doctorate's Degree in Public Health and Environment/ Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation - FIOCRUZ, Brazil)

⁴(Department of Cell Biology, Institute of Biology Roberto Alcabara Gomes, Rio de Janeiro State University - UERJ, Brazil)

ABSTRACT: Recreation areas have primary environmental contact matrices (water and sand) with the population and therefore need to be monitored periodically, due to the risks to human and animal health posed by the potential presence of pathogenic microorganisms. The purpose of this work was to recommend methods which can be used in the investigation of bioindicators of importance in the area of environmental sanitation, and discussed the standards used in the legal regulations to support the monitoring, which ensure the actions of the environmental and health systems. Protocols used in scientific research and by sanitation companies and bibliographic reviews were described. In Brazil, the National Council for the Environment has determined the acceptable standard limits for coliforms found in the water matrix used for bathing only. There are simply recommendations for the environmental agencies the evaluation of the parasitological and microbiological quality of the sand for future standardizations. Isolated initiatives of municipal environmental agencies established classification limits for sand matrix, considering only the bacteriological parameters. We recommend incorporating new protocols for evaluation of environmental sanitary conditions. The protocols proposed for evaluation of the presence of fungi and parasites can be used to determine the sanitary quality of the recreation areas.

Keywords: Coliforms, Parasites, Fungi, Recreation Areas, Sanitary evaluation.

I. INTRODUCTION

Deficiencies in health infrastructure in developing countries result in concentrated sources of contaminants that lead to water and soil deterioration. The interface between sanitation and water resources is narrow. The vulnerability of water sources to contamination can pose health problems for the population. The risk to human health is higher where degraded areas are used for recreation.

Environmental sanitation needs to be understood as a strategy to improve the quality of life and health of the population. Good practices and methodologies monitoring water resources and sanitation contribute to an integrated environmental management (Philippi Junior and Galvão Junior, 2012). Public policies play a vital role in the sustainable use of environmental resources, with hydrographic basins as units of socio-environmental analysis where passive agents (natural resources) and assets (anthropic action) are associated (Brazil, 2009).

Considering the need for actions to control and monitor the sanitary quality of water and soil used by the population in recreation areas such as beaches, squares and fields. Public authorities should provide safe information about the risks of using these environments, whether for bathing or recreation (Sotero-Martins et al., 2014).

The objective of this work was to discuss methods that can be used in the construction of environmental bioindicators (coliforms, parasites and fungi), and the legal standard limits to ensure monitoring and support actions of environmental and health control agencies. The search of the described methods included the publications of reviews, legislation, indexed articles, using the descriptors: sanitary quality analysis methods,

recreation areas, microorganisms, parasites and fungi. Efforts have been concentrated in the publications describing simple methodologies to be executed, involving a low cost within the ten last years.

II. SOIL COLIMETRY

To evaluate the soil sanitary quality of recreational areas, the total and thermotolerant coliforms, which includes *Escherichia coli*, are the preferred bioindicators of environmental contamination used by sanitation agencies worldwide (Sotero-Martins et al., 2014), because the microorganisms eliminated in the human feces are of different types, however the coliforms (*Escherichia coli*, *Aerobacter aerogenes* and *Aerobacter cloacae*) are present in large quantity, being able to reach one billion per gram of feces (FUNASA, 2007). The coliforms are defined as bacteria that are in the form of gram-negative bacilli, aerobic or facultative anaerobic, non-spore forming, oxidase-negative. They have the ability to grow in the presence of bile salts or other surface active compounds. They ferment lactose with production of acid, aldehyde and gas at 35-37 °C in 24-48 hours, and may exhibit β -galactosidase enzyme activity. Most bacteria of the total coliforms belong to the genres *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. They are present in the feces of homeotherms animals, so when present indirectly indicate the presence of human feces. The definition for thermotolerant coliforms is the same as that of the total coliform group, but they are capable of fermenting lactose with acid and gas production in 24 hours at 42 °C. Therefore, thermotolerant coliforms are a subgroup of total coliforms and the main representative species is *Escherichia coli*, being the most found among coliforms, and is also considered the most specific indicator of recent fecal contamination and pathogenic organisms (APHA, 2012). This is abundant in human and animal feces found in sewage, effluent, natural waters and contaminated soils (Pinto and Oliveira, 2011).

In the analysis of different environmental matrices, including soil, coliforms are one of the parameters most used in the world by environmental agencies. The main detection methodology is still the multiple tube technique (Sotero-Martins et al., 2014), expressing the results in Most Probable Numbers (MPN), calculated according to the American Public Health Association (APHA). However, more accurate techniques with results expressed in Colony Forming Units (CFU) have been described by other authors (Gronewold and Wolpert, 2008; Bonilla et al., 2007). The technique that can be used to investigate coliforms in soil with more accurate results expressed in CFU is the filter membrane method (APHA, 2012), using the chromogenic culture medium indicator Chromocult® Coliform Agar (Cat. No. Merck), and Millipore® sterile cellulose ester membrane with porosity and diameter of 0.45 μ m and 47 mm, respectively (Handam, 2016). The soil samples collected should be processed within 24 hours (APHA, 2012). To perform this method, samples of 50 g of soil must undergo an initial dilution in 200 mL buffer solution, consisting of: 1.25 mL/L stock A solution (monopotassium phosphate 34g/L) and 5.0 mL/L stock B solution (magnesium chloride 81 g/L), final pH 7,5 \pm 0,25; sterilized at 121°C. After vigorous shaking of samples in buffer solution, they should remain decanting for 30 minutes (Handam, 2016). Then is necessary to carry out a serial dilution. The first dilution consists of 5 mL of sample in a falcon containing 45 mL of phosphate water. Following, an 1 mL aliquot of this first dilution is transferred to another falcon containing 49 mL of phosphate water, composing the second serial dilution; this is transferred to another 1 mL falcon containing 49 mL of phosphate water, composing the third serial dilution (Handam, 2016). After the dilutions proceeds to the filter membrane method (APHA, 2012), in which 10 mL of the diluted soil samples are filtered with the aid of a vacuum pump. Subsequently, the membranes is placed on the culture medium chromogenic indicator Chromocult® Coliform agar in a petri dish and are transferred to a bacteriological incubator at 37 °C and remain there for 24 hours. This method allows the direct count of bacteria based on the appearance of colonies on the membrane surface. At the time of bacterial reading, Merck's Chromocult® chromogenic culture medium differentiates the colonies, being total coliforms with salmon/reddish color, and *Escherichia coli* with blue/violet coloration. In this technique colimetric quantification is performed by CFU per soil grams. Therefore, in order to compare the values of the standards described in the soil quality regulations that may be described in MPN, it is necessary to perform the conversion to CFU values, considering that MPN values are 2,167 times greater than in CFU (Handam, 2016), according to data observed in a published study (Gronewold and Wolpert, 2008).

In Brazil there is still no federal regulation on the sanitary quality of the soil. The Resolution of the National Environmental Council - CONAMA No. 274/00 (Brazil, 2001), which defines the criteria for bathing in Brazilian waters, recommended to the environmental agencies the evaluation of the parasitological and microbiological conditions of the sands for future standardization. In view of this recommendation, there are legislations in some states, such as the Resolution of the Municipal Secretary of Rio de Janeiro - SMAC No. 468/2010, which determines the quality standards of the sands of Rio de Janeiro (Rio de Janeiro, 2010). However, the regulations only have the standards regarding the bacteriological conditions of the sand, do not have the standards for the presence of fungi, parasites and virus.

III. WATER COLIMETRY

Several water resources have the quality of their waters compromised by the growing disposition of sewage and garbage considered as biological pollution. This type of pollution exposes the environmental matrices to microorganisms of human and animal origin, like diverse bacteria of the groups of the fecal and total coliforms. Some of these bacteria are described as pollution bioindicators in particular *E. coli* because they indicate the presence of recent sewage in the water, which endanger public and environmental health (Sotero-Martins *et al.*, 2014; Moura *et al.*, 2016). For colimetric analyzes in water, it is important to make sure that the flasks used for collection are commercially sterile vials or vials previously sterilized by autoclaving (121 °C/ 1 atm) or by continuous exposure to UV radiation in laminar flow hood during 40 minutes. The vials containing the samples must be packed in styrofoam box and kept under refrigeration until arrival in the laboratory. The main methodologies used for the microbiological evaluation of water are the Colilert enzymatic assays (commercial kit) and the filter membrane method associated with the Chromocult chromogenic assay. The colilert enzymatic assay uses as a principle the reagent mixture defined as "Defined Substrate Technology" (DST) in 100 mL of the sample followed by incubation for 18 h for a presence/absence (P/A) or most probable number (MPN). The sample acquires yellow color when the coliform bacteria metabolize orthonitrophenyl- β -D-galactopyranoside (ONPG), through the action of β -galactosidase that serves as nutrient and indicator and fluoresces under UV light when *E. coli* metabolizes the 4-methylumbelliferyl- β -D-glucuronide (MUG), another nutrient and indicator due to the action of β -glucuronidase. The reagent-sample mixture is added to a Quanti-Tray plate, which is then sealed with the Quanti-Tray Sealer sealer and then incubated. Then, the positive wells are counted and the MPNs of coliform bacteria and / or *E. coli* are determined from a table (Idexx laboratories, 2008).

The filter membrane method associated with the Chromocult chromogenic assay also uses enzymatic metabolism where the colonies are differentiated by staining. The standard methods for the examination of the water and wastewater (Eaton *et al.*, 2005), describe the analysis of the total coliform and *E. coli* levels by the filter membrane method, which, after different dilutions, allows colony counting. Dilutions should take into account the degree of pollution impact and pre-evaluate the water course to better determine which dilution range is most appropriate. The methodology described in the Merck Manual (2000) uses Chromocult® Coliform Agar chromogenic culture medium (Cat. No. 1.10426.0100 / 500), where the colonies are differentiated by colorimetric process, which is also based on the an enzymatic apparatus present in the coliform groups, in which total coliforms present as salmon / red colonies by metabolizing the ONPG chromogenic substrate and fecal (thermotolerant) coliforms as dark blue to violet colonies by metabolizing the MUG substrate consuming the glucuronide carbons and releasing four methyl a beliferone (360 nm) (Sotero-Martins, 2014). A 10 mL volume of the diluent is filtered through a 47 mm sterol cell membrane where the microorganisms are retained. After filtration the membrane is placed on Chromocult medium, and colony growth is observed after 24h at 37 °C. The result of the number of colonies counted must be multiplied by the dilution factor, obtaining the expected Colony Forming Unit (CFU) per mL of water. The data generated in CFU can be correlated to the unit values of "most probable numbers" (MPN) (Rego, 2008), this correlation is assumed based on the data of Gronewold and Wolpert (2008). The colony count data, presumed to be total or fecal (thermotolerant) coliforms, can thus be used to assess the sanitary conditions of the waters based on the Technical Manual on Water Analyzes for Human Consumption (1999) and on specific resolutions or legislations. In addition to the Brazilian laws cited, international laws also use these methods to assess the quality of their waters, such as the Blue Flag Association of Europe (BFAE) (2008), European legislation that defines the tolerable limits for water quality assessment and the Environmental Protection Agency (EPA) (2000), the North American agency responsible for the definition of reliable parameters in the evaluation of sanitary conditions of water.

VI. SOIL PARASITOLOGY

Contamination of the soil verified by the presence of eggs and larvae of helminths confers a risk of infection to the population and is characterized as an improper soil, since there must be no parasites, according to the law project No. 585/2015 (Rio de Janeiro, 2015). As described by Vasconcelos *et al.* (2011) soil fecal contamination is the main mechanism for the diffusion of diseases caused by helminths. The soil provides favorable conditions for nutrition and development of parasitic forms, besides, it allows keeping the infecting parasite in the environment until it meets the host, thus increasing the chance of infection (Saito and Rodrigues, 2012). Contamination of public spaces such as beach sand, public parks and playground by excreta of dogs and cats, contributes to the increase of the occurrence of helminthiasis in humans, since, many of these organisms present stage of development in the soil, resulting in the formation of (Amaral *et al.*, 2006). The diagnosis of certainty of a parasitological process is given by demonstrating the presence of the parasite or its products in the host organism (Rey, 2008).

Protocols to determine the presence of parasites in environmental samples were developed, among them the following: the analytical technique used for the research of eggs, cysts and larvae in the dry sand

samples by Lutz (1919), due to its simplicity and low cost. And for the research of nematode larvae the technique of Baermann-Moraes (1917) is appropriated. Both techniques were adapted by Amaral (2012), to better use the environmental matrices.

For identification of parasites by the Lutz adapted method take 100 grams of sand should be used and transferred to a glass container with 100 mL of 0.5% commercial neutral detergent. Subsequently, the solution is vigorously shaken for 5 minutes and allowed to stand for complete decanting of the substrate. Then the supernatant is filtered in four gauzes folded over a polypropylene sieve into a settling cup, having completed its volume up to 250 mL with distilled water. After this, the contents should remain at rest for 2 hours for spontaneous sedimentation. Then, the supernatant liquid is discarded to a next volume of the sediment, and the material deposited at the bottom of the cup recovered and transferred to a conical tube type Falcon 15 mL for new sedimentation. After 10 minutes, 50 μ L of the material should be pipetted into the blade, one drop of the Lugol Reagent being covered with a cover slip. The Adapted Baermann-Moraes method is similar to the Adapted Lutz technique, only what differs is that the 100 grams of the soil sample are homogenized with 180 mL of distilled water heated to 45 °C with the aid of an electric boiler and thermometer, so as to cover the sample (Amaral, 2012).

For each sample three replicates of the slides were performed, being examined under a microscope using increases of 100 and 400X. The obtained sediment was divided into aliquots of 1.5mL and preserved one in SAF for later microscopic reading and for analysis in molecular biology, and another frozen without preservatives to be used in the ELISA, in order to increase the detection capacity of the cysts of *G. lamblia* and *E. histolytica / dispar* and oocysts of *Cryptosporidium* sp. By means of commercial kits destined to the research of parasitic antigens described in Amaral et al (2015). Another methodology that can be used in the investigation of soil parasites is molecular biology. In which an aliquot of the supernatant is obtained by conventional Lutz and Baermann-Moraes techniques and subjected to DNA extraction by commercial QIAamp DNA Stool Mini kit (QIAGEN, Germany). For the use of this commercial QIAGEN kit, the manufacturer's instructions were followed, but some adaptations were made for use with environmental samples. Where, 0.5 mL of the sample is pipetted. The extracted DNA was maintained at -20 °C until use.

V. WATER PARASITOLOGY

According to article number 2 of CONAMA Resolution 274 of November 29 (2000) from Brazil, fresh, brackish and saline waters destined for bathing (primary contact recreation) will have their condition evaluated in the proper and inappropriate categories for analysis of fecal coliforms or *Escherichia coli* or Enterococcus, but does not establish limits for parasitological analysis. But it is possible to do parasitological tests in water. Caldas (2015) carried out the research of parasites in water using a commercially available Micro Wind® polypropylene blanket with 1 μ m porosity rewound wire (micrometer), coupled to a suction pump with filtration flow that allows to pass at the end of the 20 thousand liters, when the water source was above the level to realize the passage through the system, it is calculated the flow that will be necessary to leave the system running. At the end the filters must be packed in thermal boxes until the arrival in the laboratory. This methodology allows the detection of oocysts and cysts of protozoa in water (Anonymous, 1999; Aldom and Chagla, 1995; EPA, 1999; Vesey et al., 1993; Barbosa et al., 2013). The MicroWind® Blanket Commercial Filter should be stripped and washed with 1 L of 0.05% Tween-80 Solution (non-ionic emulsifiers) with the aid of hand rubbing to undo the weft. Part of the wash deposited in the wash basin (300 mL) is transferred to the settling cup, containing folded gauzes and attached sieve holder. The remaining pellet is preserved in SAF (Sodium Acetate Solution Formalin: sodium acetate - 1.5 g, acetic acid: 20 mL, formalin: 40%: 40 mL, distilled water: 92.5 mL). The filtrate rinse eluate that was filtered into gels folded into a settling cup at the end has its volume completed to 250 mL with distilled water. This suspension is allowed to stand for 2 hours, and then the supernatant is discarded to the volume next to the pellet. It is then transferred to a conical tube with a capacity of 15 mL for a new sedimentation. The precipitate is monitored with 50 μ L (microliters) of the slide material, plus one drop of Lugol Reagent 2% (Metallic Iodine Solution and Potassium Iodide), covered with cover slip. Three blades were made for each sample. Microscope preparation is examined at 100 X and 400 X increments. Measurements of eggs and larvae are taken with the aid of micrometer eyepieces (Caldas, 2015).

In Brazil, the inclusion of pathogenic protozoan research in the monitoring of environmental samples of water is beginning. Although resolution No. 2914/201, of the Ministry of Health (Brazil, 2011) recommends the evaluation of the presence of protozoa *Cryptosporidium* spp. and *Giardia* spp. In water intended for human consumption, and stresses the obligation of monitoring the sources according to the concentration of *Escherichia coli* present in the water body. However, several factors hinder the short-term treatment of this recommendation, among them the inherent complexity of detection methods, the shortage of qualified human resources for work and the scarcity of available information on the occurrence of these pathogens in national water resources (Franco, 2007).

VI. FUNGUS IN SOIL AND WATER

The environmental health of soil and water resources can be evaluated by the presence of fungi. The suggestion for the use of the presence of fungi as a bioindicator is not recent (Sarin and Oliveira, 1996; Heinen et al., 2009; Mancini et al., 2011; Viana, 2011). Among the evaluation protocols on fungi identification and determination of levels of colony forming units per gram of soil or milliliter of water compared to levels of coliform contamination is described by Rego (2010). This protocol has the following:

Stage of Isolation and Analysis of Microorganisms - Count and isolate colonies of yeasts in 24 hours, classifying them into 2 phenotype categories: CSF - round orange yeast; L24h- white or yellow yeast grown with 24 hours, this is reinoculated in selective chromogenic medium CHROMagar Candida®, specific for the genus *Candida*. The inoculated plate should be kept at 29°C to allow the appearance of filamentous fungi (FF) present in the sample. These should be isolated after 5-7 days of incubation and identified phenotypically in one of 10 categories: AR- yellow round; LEA- cottony orange spat; NBB- black with white border; BE-white fumed; VBB-blue-green filamentous white border; NFA-black cotton filamentous; BPeq- small white; BEC-mirrored white; MA- cottony brown; BEA- white spat cottony. After the isolation and culture of the FF in SAB medium, samples are observed directly under the optical microscope aiming the classification by the genus. Thus, after 7 days of cultivation on Sabouraud Dextrose Agar slant, leaf culture is performed for the observation of structures and identification and, finally, the preservation of filamentous fungi in a specific culture medium. About 30 - 40% of the FF identified with the VBB phenotype belong to the genus *Penicillium* sp and 70-80% of the FF identified with the NBB phenotype are of the genus *Aspergillus* sp.

Definition of limit values for fungi - Limit values for FF phenotypes are suggested according to Rego (2010). The unit for the filamentous fungus is defined as Biomass Growth Unit (UCB), as suggested by Espósito and Azevedo (2004), whereas for the fungus phenotype the unit was Colony Forming Unit (CFU). Only those campaigns where correlation with the data for *E. coli* and for the NBB phenotype should be observed when there is a correlation between the MNP unit and *E. coli* CFU, as indicated by the data presented by Gronewold and Wolpert (2008). The limit of the EPA (1986) in CFU was set to 2.35 per milliliter. The indication of this limit value for water resulted from the analysis of epidemiological data, with a correlation between 1 gram and 1 milliliter. Consider the mean of colimetric analysis data for *E. coli* (in CFU/g) only from the correlated campaigns, with the significant Pearson (r) correlation value between *E. coli* and NBB in dry sand, and thus the number of times the mean *E. coli* was greater than the limit value of 2.35 CFU/g. For the definition of the limits presented, the mean values of fungi values divided by the number of times the average of *E. coli* was higher than the limit value of 2.35 CFU/g were considered. Four classification ranges: the first from zero to the value as the lowest standard deviation (own for recreation = excellent); The second with value up to the value of the defined limit (own for recreation, with low risk = good); The third to the highest standard deviation value (not recommended for recreation, medium risk = inappropriate); And the fourth above this maximum value of the previous track (not recommended for recreation, high risk = improper).

According to ABAE (2008) the species of the genus *Aspergillus* found in the environment can cause aspergillosis, in the form invasive, allergic or toxic, and opportunistic *Aspergillus* species can infect various organs in immunocompromised individuals such as children and the elderly. The species of *Aspergillus*, *Cladosporium* and *Penicillium*, found on the beaches, may be a source of infection for superficial and deep mycoses, and species of the genus *Aspergillus* the most responsible for respiratory infections in immunocompromised patients.

VII. CONCLUSION

It is possible to incorporate multiple bioindicators of pathogenicity to monitor the risk related to exposure to contaminated sand and water from recreation areas. Our group used most of protocols described in this articles. The monitoring of different bioindicators by health and environmental surveillance agencies, comparing with the appropriate parameters, may lead to a reduction in the difficulty of associating the occurrence of pathogens with accurate data on the appearance of diseases in the population and greater control the environment. The proposed protocols for evaluation of the presence of fungi and parasites can be used to determine the sanitary quality of the recreation áreas.

ACKNOWLEDGEMENTS

We thank the Brazilian National Research Council (CNPq) for the financial support to carry out the project (Project No. 577432/2008-7).

REFERENCES

- [1] A.Philippi Junior A, A.C. Galvão Junior, Gestão do saneamento básico: abastecimento de água e esgotamento sanitário, *Coleção ambiental* (Editora Manole Ltda, Barueri, 2012).
- [2] Brasil. Ministério das Cidades. Secretaria Nacional de Saneamento Ambiental. Programa de Modernização do Setor Saneamento (PMSS). Cordeiro BS (coord). Prestação dos Serviços Públicos de Saneamento Básico. Lei Nacional de Saneamento Básico: perspectivas para as políticas e gestão dos serviços públicos. Volume 3. Brasília; 2009.
- [3] A. Sotero-Martins, A.N. Duarte, E. Carvajal, M.I.M. Sarquis, O.C.C. Fernandes, Microbiological and Parasitological Quality Control of Recreation Areas, *Revista Eletrônica Gestão & Saúde*, 05(03), 2014, 2059-2078.
- [4] FUNASA, Fundação Nacional de Saúde, Ministério da Saúde, Brasil, Manual de Saneamento, Brasília, DF, 2007.
- [5] APHA, American Public Health Association, Standard methods for examination of water and waste water. 22th ed. Washington: 2012.
- [6] A.B. Pinto and A.J.F.C. Oliveira, Diversity of indicator microorganisms in the evaluation of sea recreational beach sand fecal contamination: current knowledge and perspectives, *O Mundo da Saúde*, 35(1), 2011, 105-114.
- [7] A.D. Gronewold and R.L. Wolpert, Modeling the relationship between most probable number (MNP) and colony-forming unit (CFU) estimates of fecal coliform concentration, *Water Research*, 42, 2008, 3327-3334.
- [8] T.D. Bonilla, K. Nowosielski, M. Cuvelier, A. Hartz, M. Green, N. Esiobu, D.S. McCorquodale, J.M. Fleisher, A. Rogerson, Prevalence and distribution of fecal indicator organisms in South Florida beach sand and preliminary assessment of health effects associated with beach sand exposure, *Marine Pollution Bulletin*, 54, 2007, 1472-1482.
- [9] N.B. Handam, *Condições Sanitárias da Água Residencial, do Solo Peridomiciliar e dos Rios das Comunidades do Território de Mangueiros, RJ*, master diss., Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, BR, 2016.
- [10] Brasil, Ministério do Meio Ambiente, Conselho Nacional de Meio Ambiente. Resolução n.274, de 29 de novembro de 2000. Diário Oficial da República Federativa do Brasil, Brasília, 25 jan. Seção 1, p. 70-71. 2001.
- [11] Rio de Janeiro (Município), Secretaria Municipal do Meio Ambiente. Resolução SMAC n.468 de 28 de janeiro de 2010. Dispõe sobre a análise e informações das condições das areias das praias no Município do Rio de Janeiro, 2010.
- [12] Rio de Janeiro (Estado), Projeto de Lei nº 585/2015, Normatiza o monitoramento da qualidade da areia de parques, praças, tanques, creches e escolas destinados ao lazer, recreação e atividades educativas, esportivas e culturais de áreas públicas e privadas do estado do rio de janeiro, bem como dispõe sobre a obrigatoriedade de tratamento, limpeza e conservação da areia visando prevenir e/ou combater os agentes transmissores de doenças. Assembleia Legislativa do Estado do Rio de Janeiro. 29 de Junho de 2015
- [13] R.K. Saito and A.F.S.F. Rodrigues, A importância da análise do solo nos estudos epidemiológicos sobre parasitoses intestinais. *Revista de Atenção Primária à Saúde*, 15(2), 2012, 158-163.
- [14] EPA. Improved Enumeration Methods for the Recreational Water Quality Indicators: *Enterococci and Escherichia coli*. Test, 49, 2000.
- [15] Brasil, Portaria nº 2914 de Ministério da Saúde de 12 de dezembro de 2011, Diário Oficial da República Federativa do Brasil, Brasília, DF, 2011.
- [16] E.P. Sauer, J.L. Vandewalle, M.J. Bootsma, S.L. McLellan, Detection of the human specific *Bacteroides* genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment, *Water Res. Elsevier Ltd*, 45(14), 2011, 4081-91.
- [17] P.G. Moura, A.N. Duarte, L.H. Silva, A. Sotero-Martins, Environmental Monitoring Model of Health, Parasitological and Colorimetric Conditions of Rivers Bacaxá and Capivari in Rio De Janeiro Lakes Region, *Int J Eng Sci*, 5(11), 2016, 15-21.
- [18] Idexx Laboratories, Validation of the Colilert-18/Quanti-Tray method for counting *E. coli* and coliform bacteria in water, *DRIVE IOI, Westbrook maine*, 2008.
- [19] A.D. Eaton, L.S. Clescerl, E.W. Rice, A.E. Greenberg, M.A. Franson, Standard Methods for the Examination of Water and Wastewater, 21st Edition, American Water Works Association, New York, 2005.
- [20] Microbiology Manual, *Merck Laboratory*, 2000.
- [21] J.C.V. Rego, Qualidade Sanitária de Água e Areia de Praias da Baía de Guanabara, master diss., Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, BR, 2008.
- [22] Ministério da Saúde, Manual técnico de análises de água para o consumo humano *Fundação Nacional de Saúde*, 1999.
- [23] Blue Flag Association of Europe, Microbiologic quality of sands of coast beaches, *Portuguese Agency of the Environment, Lisbon*, 2008.
- [24] Environmental Protection Agency, (United States), Improved enumeration methods for the recreational water quality indicators: *Enterococci and Escherichia coli*, Washington, DC, 2000.
- [25] L.S.Amaral, A.N.Duarte,V. Laurentino-Silva, L.C.L. Pinna, A. Sotero-Martins, Parasitological indicators of contamination at sand of beaches and monitoring by Traditional methods and immunoenzymatic assay, *Brazilian Journal Biosystems Engineering*, 09(04), 2015, 304-311.
- [26] L. Rey, *Parasitologia: Parasitos e doenças parasitárias do homem nas Américas e na África* (Guanabara Koogan, RJ, 2008).
- [27] A.O. Lutz, *Schistosoma mansoni* e a schistosomatose segundo observações feitas no Brasil, *Memórias do Instituto Oswaldo Cruz*, 11, 1919, 121-155.
- [28] G. Baermann, Simple method for the detection of *Ankylostomum* (Nematode) larvae in soil tests, *Mededelingen uit het Geneeskundig Laboratorium te Weltevreden*, (Batavia: Javasche Boekhandel & Drukkerij, 1917) 41-47.
- [29] L.S. Amaral, *Monitoramento de parasito e coliformes como parâmetro de avaliação sanitária de areia e água de praias da Baía de Guanabara/ RJ*, Master Science diss.; Programa de Saúde Pública e Meio Ambiente, Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, RJ, 2012.
- [30] Anonymous. Standard operating protocol for the monitoring of *Cryptosporidium* oocysts in treated water supplies to satisfy water supply (water quality) (amendment) regulations. SI N°1524, 1999.
- [31] L.V.L. Caldas, *Salubridade Ambiental Da Água E Do Solo Peridomiciliar Da Região Sul E Central Da Ilha Da Marambaia/RJ*, master diss., Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, BR, 2015.
- [32] J.E. Aldom, A.M. Chagla, Recovery of *Cryptosporidium* oocysts from water by a membrane filter dissolution method. *Letters Appl Microbiol* 20: 186-187, 1995.
- [33] EPA - Environmental Protection Agency. Office of Water. Method 1622: *Cryptosporidium* in water by filtration/IMS/FA. EPA 821-R-99-001. US Environmental Protection Agency, Washington DC, EEUU, 1999.
- [34] G. Vesey, J.S. Slade, M. Byrne, K. Shepherd, C.R. Fricker. A new method for the concentration of *Cryptosporidium* oocysts from water. *J Appl Bacteriol* 75: 82-86, 1993.

- [35] A.S. Barbosa, C.M.A. Uchôa, V.L. Silva, A.N. Duarte, N.F. Conceição, M.B. Vianna. Avaliação parasitológica da água de abastecimento e do solo peridomiciliar de Aldeias Guarani. *Rev Inst Adolfo Lutz*. São Paulo, 2013; 72(1):72-8.
- [36] R.M.B. Franco, Protozoários de veiculação hídrica: relevância em Saúde Pública. *Rev Pan Infectol* 9: 36-43, 2007.
- [37] M.I.M. Sarquis and P. C. Oliveira, Diversity of microfungi in the sandy soil of Ipanema Beach, Rio de Janeiro, Brazil. *Journal of Basic Microbiology*, 36(1), 1996, 51-58.
- [38] R.C. Heinen, J.C.V. Rego, M.I.M. Sarquis, E. Carvajal, A. Sotero-Martins, Controle Ambiental de Leveduras e Fungos Filamentosos nas Praias da Baía de Guanabara, in: De Araujo, C. E. M. *Revista UNIABEU - Educação e Saúde*. V. 2. N. 4 (2009): 84. p. 71–76. Available from: <http://revista.uniabeu.edu.br/index.php/RU/article/view/75/132>
- [39] L. Mancini, A.M. D'Angelo, E. Pierdominici, C. Ferrari, A. Anselmo, L. Venturi, L. Fazzo, P. Formichetti, M. Iaconelli, B. Pennelli, Microbiological quality of Italian beach sand. *Microchemical Journal*, 79, 2005, 257-261.
- [40] M.O. Viana, *Bioprospecting of bacteria with biotechnological potential for bioremediation and monitoring of impacted beaches of Guanabara Bay, RJ*, master diss., Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, BR, 2011.
- [41] J.C.V. Rego, *Qualidade sanitária de água e areia de praias da Baía de Guanabara. Rio de Janeiro*, máster diss., Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, BR, 2010.
- [42] E.W. Koneman and S.D. Allen, *Color Atlas and Textbook of Diagnostic Microbiology*, 6th Edition (J. B. Lincpott Company, Philadelphia, 2006.
- [43] E. Esposito and J.L. Azevedo, *Fungos: uma introdução à biologia, bioquímica e biotecnologia* (Editora da Universidade de Caxias do Sul, Rio Grande do Sul: EDUCS, 2004).
- [44] J.J.C. Sidrim and J.L.B. Moreira, *Fundamentos clínicos e laboratoriais da micologia médica* (Guanabara-Koogan, 1999).
- [45] R. Sabino, C. Verissimo, M.A. Cunha, B. Wergikoski, F.C. Ferreira, R. Rodrigues, H. Parada, L. Falcão, L. Rosado, C. Pinheiro, E. Paixão, J. Brandão, Pathogenic fungi: an unacknowledged risk at coastal resorts? New insights on microbiological sand quality in Portugal. *Marine Pollution Bulletin*, 62(7), 2011, 1506-1511.
- [46] R. Sabino, R. Rodrigues, I. Costa, C. Carneiro, M. Cunha, A. Duarte, N. Faria, F.C. Ferreira, M.J. Gargaté, C. Júlio, M.L. Martins, M.B. Nevers, M. Oleastro, H. Solo-Gabriele, C. Veríssimo, C. Viegas, R.L. Whitman, Routine screening of harmful microorganisms in beach sands: implications to public health, *Science of The Total Environment*, 472, 2014, 1062-1069.