



## ENVIRONMENTAL IMPACT OF HOSPITAL SEWAGE IN SUB-AVERAGE SÃO FRANCISCO VALLEY

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### ABSTRACT

This study aims to map the environmental impact in the valley lower basin of the São Francisco river, caused by liquid waste from the sewage and wastewater from two hospitals located in the cities of Juazeiro / BA and Petrolina / PE, identifying the composition of the waste and its potential risk to health and environment and can affect local population and quality of water supplied population and irrigation. This research aims General: to map the environmental impact of waste liquids from sewage and wastewater from health facility in the sub-medium São Francisco Valley, through the analysis of effluents produced by public hospitals in the municipalities of Petrolina / PE and Juazeiro / BA. In order to enable the achievement of the ultimate goal was traced to some specific objectives are: to identify the main points of discharge of effluents and their potential for contamination; to determine the physical chemical and biological effluents and wastewater hospitals in these health services; to evaluate the water quality at the point of hospital discharge of effluents; to evaluate the environmental impact of waste, its pathogenicity and risks to community health. It will be collecting samples of waste water and five points, two on the river, and three others located at the headquarters of municipalities. After a comparative analysis will be made to the parameters established with current legislation and culminated in drafting proposals to compose the local waste management of health services-PGRSS. So, opted for a procedure comparative methodological character, alternating with reflections moments order space-social-environmental in order to base the research giving you tools to formulations thoughts, using several authors that, in principle, are in different areas of science, but whose thoughts when articulated produce knowledge necessary to understand the question posed in an effort multi-and transdisciplinary.

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### INTRODUCTION

In urban settlements the production of liquid waste is a problem that reaches gigantic proportions. The treatment of this by-product of human consumption has become an important environmental problem (Brasileiro and Matos, 2015; Queiroz, 2005). Problem that has many concerns in the search of how to minimize this impact on springs, streams, streams, rivers and seas.

This problem transcends the individual or institutional character, happens to be of the daily life of each one. This collective role reveals us how individual actions can affect the balance of the environment also from the point of view of solutions (Hamel, Grubba, and Hamel, 2016; Tilman and Lehman, 2001) Returning to the focus of the issue, it is known that pollution directly affects the supply of drinking water, and in regions such as the semi-arid Northeastern region where this supply is even smaller, everyone suffers this consequence. In

addition to the common sewage, thousands of liters of liquid waste from hospitals, clinics and other health services are dumped, loaded with bacteria, viruses, fungi, body fluids, antibiotics and many other chemicals from exams (Erdtmann, 2004; Tadesse and Kumie, 2014). This highly contaminating and infectious liquid becomes an effluent and is dumped many times without any treatment in the rivers that supply the cities. In research conducted by Vecchia *et al* (2009), through an electronic questionnaire that it tried to identify the control and treatment of hospital liquid waste in 200 hospitals in all regions of Brazil. They found, in relation to the answers posted by the hospitals, that in two regions the North and Northeast, there was no return of the response of the questions; which makes us even more intrigued to know what environmental impact these effluents bring to the environment, and to the basins such as São Francisco River. In the Brazilian semi-arid region on the border of two states Pernambuco and Bahia, between the cities of Petrolina and Juazeiro, the estimated population together is almost 500 thousand inhabitants. This is an important economic and financial pole of irrigated agriculture; the largest producer of fruits in the country, all this wealth revolves around the benefits brought by the largest genuinely Brazilian river, the São Francisco River responsible for supplying water to the cities that are bathed in five Brazilian states, a source of "drinking water" that affords many homes located in its surroundings. This is the scenario that is in addition to raw water source for irrigation, this also serves as feeder of the systems of local water give.

So, what is the responsibility of our family and society in general in maintenance of health of São Francisco River? And the range of wastes deposited in it are actually treated? Is the public sewer system ready and adequate to control and treat waste from health services? What is the impact thereof on the environment and the drinking water supply to coastal communities and for agriculture dependent? In this sense, thinking about the environmental impact of this spring, which gives sustenance to this region and which brings it so much wealth and improvement, has not only a purely environmental relevance, but also a set of values that permeate cultural, social and sustainability questions (Fong, Catagnus, Brodhead, Quigley, and Field, 2016; Pyhälä *et al.*, 2016). This broader dimension, the proportions and questions, raised about how and what is being done to preserve and ensure the permanence and quality of its raw material, that is, "water", and that leads us to this restlessness and search for answers (Axelsson *et al.*, 2013; Fielding and Hornsey, 2016). The aim of this study was to identify the environmental impact of wastewater from sewage and sanitary wastewater in the São Francisco river basin, through the analysis of effluents produced by public hospitals in the municipalities of Petrolina / PE and Juazeiro / BA. In order to enable the achievement of the larger aim were outlined some specific objectives: to identify the main points of disposal of waste and its potential contamination; to determine the physical chemical and biological parameters of hospital effluents and wastewater in these health services; to evaluate the water quality at the outflow points of hospital effluents, environmental impacts, their pathogenicity and risks to the health of community.

## MATERIAL AND METHODS

**Method:** The research had as its focus the positivist paradigm, with bases concretized and systematized in sixteenth, seventeenth and eighteenth centuries with Bacon, Robbes and

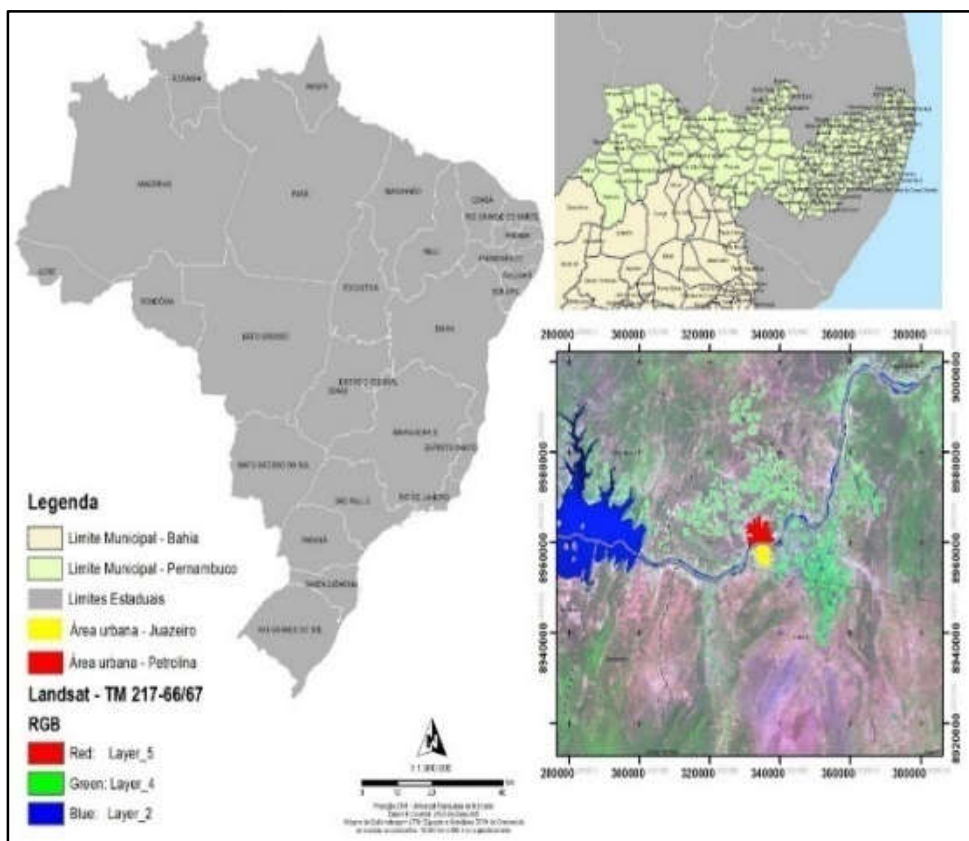
Hume, especially, but it was with Augusto Comte that in nineteenth century there was consolidation, known as its founder (Germano, 2011; Santos, 1988). It was used as method to approach this research, the hypothetical deductive (Marconi e Lakatos, 2010), begins with perception of knowledge gap, a hypothesis is formulated and, through the deductive process, the prediction of occurrence of phenomena is tested (Prado, 2013; Vieira, Clemente, Dias, and Filho, 2017).

**Study Area:** The study was developed in area corresponding to sub-region of São Francisco River, between the states of Pernambuco and Bahia, in border of the municipalities of Petrolina and Juazeiro respectively, these being with part of their territorial limits established by Rio itself.

**Study Region:** The study was conducted in municipalities of Juazeiro / BA and Petrolina / PE, where it made an analysis of local water supply and sewage treatment systems, with emphasis on hospital liquid waste, Regional Juazeiro hospitals - HRJ, located in Juazeiro. This hospital is characterized as being of medium size, providing general care, performing regional care, being a reference for municipalities of the northern region of state of Bahia, with a population estimated at 500 thousand inhabitants, having Juazeiro a quantitative of 197,965 inhabitants (IBGE, 2010), at its headquarters. The mentioned municipality is located to right margin of São Francisco river, important pole of irrigated national fruit tree, it has in river its great source of wealth and well worth. Juazeiro has a border with municipalities of Jaguarari, Sobradinho, Curaçá and Campo Formoso in Bahia, as well as making border with Petrolina in Pernambuco State. On the other side of river is Petrolina, located on left bank of São Francisco River, in the southwest region of Pernambuco State, which the hospital studied was Emergency and Trauma Hospital - ETH, that performs general care, is a reference in traumatology for the municipalities that make up this region of Pernambuco, beyond the Integrated Region of Economic Development - RIDE. This is composed of municipalities that are part of region known as for São Francisco Valley, with municipalities of Pernambuco and Bahia states. In the case of Petrolina, the city currently has a population estimated at 294,081 inhabitants (IBGE, 2010), its economy is based on irrigated fruit trees, which together with Juazeiro constitute one of largest fruit import. centers in country (Figure 1).

**Collection Sites:** The research had its development through collection of data obtained through these analyzes that were collected in five defined points and delimited by georeferencing (Table 01), denominated: P1 (Captation River), P2 (Local Supply Network), P3 (Hospital Level), P4 (Treatment substation Level) and P5 (River Effluent Level) (Figures 2; 3; 4; 5; 6; 7; 8), with a test sample and control, duly identified and conditioned; the control samples were stored for a period of 5 years.

The collection occurred at one time in Juazeiro / BA and another in Petrolina / PE during the dry season. Results of these were compared with the rates acceptable by the current legislation. After analysis, a map was drawn up in which capture points and degree of pollution and contamination were identified. The samples collected from Hospital Regional de Juazeiro - Juazeiro / BA (P3) and Emergency and Trauma Hospital of Petrolina / PE (P3) were analyzed in the biochemistry laboratory of University of Pernambuco - UPE, Campus Petrolina.



Source: Acquired 2012

Figure 1. Geographical location of municipalities of Juazeiro - BA and Petrolina – PE

Table 1. Collection points Georeferenced and date of collection

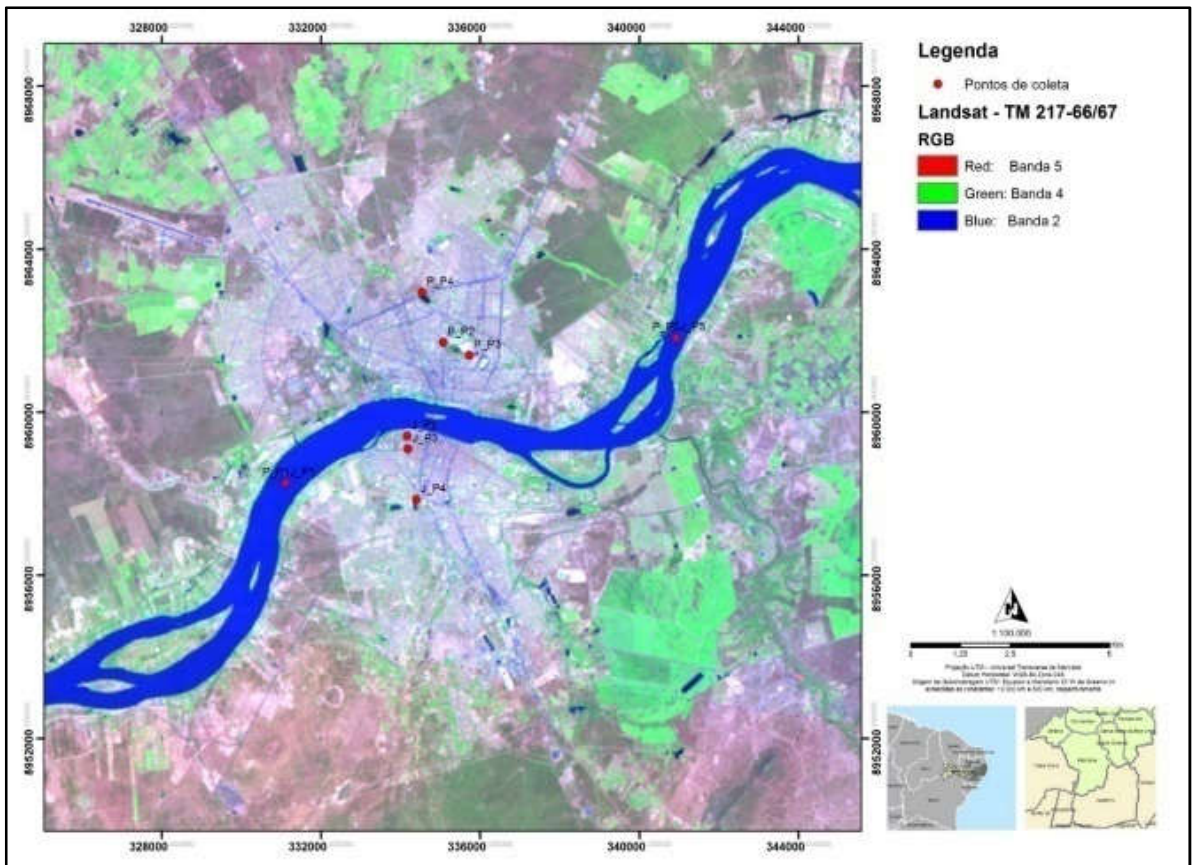
	(P1) Captação (R.S.F.)	(P2) Compesa E.T.A	(P3) HUT	(P4) Lagoa Est. KM 2 (Gar. Progresso)	(P5) Descarte (R.S.F.)	(P1) Captação (R.S.F.)	(P2) SAAE E.T.A	(P3) HRJ	(P4) Lagoa Est. Jard. Florida (Arm. Café)	(P5) Descarte (R.S.F.)
Lat. /Long.	9°25'14 S 40°32'15 O	9°23'22 S 40°30'07 O	9°23'33 S 40°29'46 O	9°22'43 S 40°30'24 O	9°23'19 S 40°26'55 O	9°25'14 S 40°32'15 O	9°24'37 S 40°30'37 O	9°24'48 S 40°30'37 O	9°25'28 S 40°30'30 O	9°23'19 S 40°26'55 O
UTM	8958269 N 24 331116 E	8961718 N 24 335072 E	8961401 N 24 335725 E	8962939 N 24 334545 E	8961831 N 24 340910 E	8958269 N 24 331116 E	8959421 N 24 334167 E	8959101 N 24 334182 E	8957865 N 24 334396 E	8961831 N 24 340910 E
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**Experimental Design**

Within methodological proposal, following components were analyzed: pH, electrical conductivity, dehydrogenase activity, FDA hydrolysis activity, basal microbial respiration and fecal coliforms. Total carbon was determined by oxidation with potassium dichromate in the medium with sulfuric and phosphoric acid, followed by titration with ammoniacal ferrous sulfate using diphenylamine as a pH indicator. Values were expressed g L<sup>-1</sup>(Embrapa, 2006). The pH of water was measured in water / distilled water solution (1: 2.5 v / v) in potentiometer (Analyzer, pH / Ion 450 M) after vigorous agitation and rest for one hour (Embrapa, 2006).

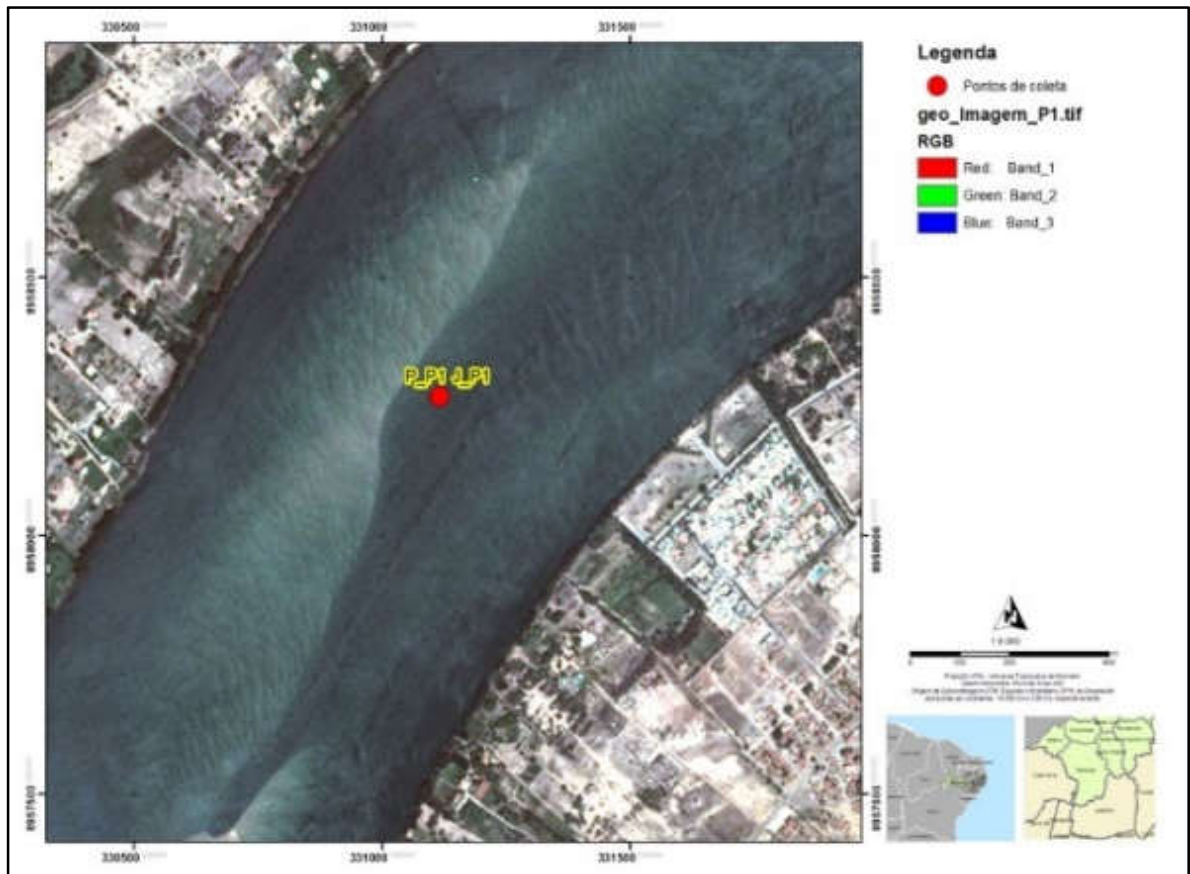
Microbial respiration: 100 mL aliquots of water were incubated in a screw-flask with 10 mL KOH (0.5 N) for 15 days, protected from light. CO<sub>2</sub> released and captured by KOH solution was quantified by titration with 0.1N HCl using phenolphthalein (0.1% in ethanol) and methyl orange (1%) as pH indicators. The carbon CO<sub>2</sub> emitted by respiration of microorganisms was expressed in g C-CO<sub>2</sub> g<sup>-1</sup> dry soil day<sup>-1</sup>(Grisi, 1978; Souto, Bakke, Souto, and Oliveira, 2009). For dehydrogenase activity: 5 ml of water was incubated with 5 ml of 1% TTC (2,3,5-triphenyltetrazolium chloride) in a water bath (37°C) for 24 hours. After that period the reaction was interrupted with 10 mL of methanol and TTF (triphenylformazan) formed by action of dehydrogenase in reduction of TTC was measured in a spectrophotometer (485 nm).





Source: Acquired 2012

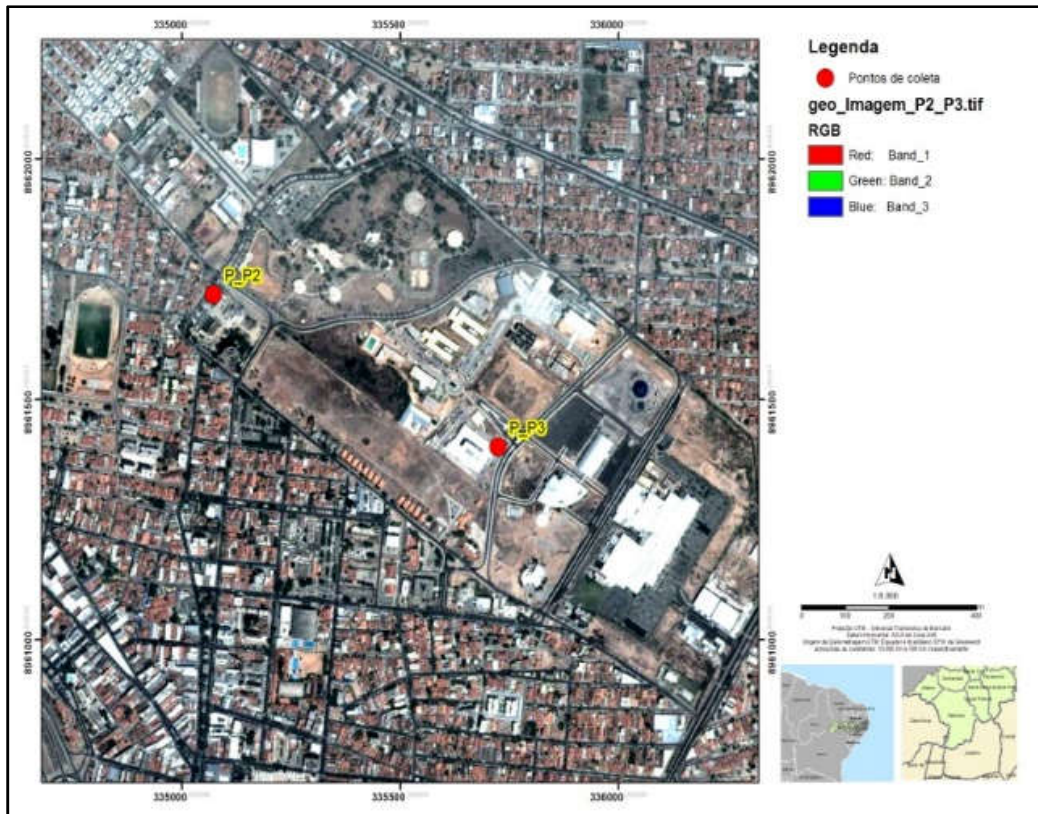
Figure 2. Collection locations points



Source: Acquired 2012

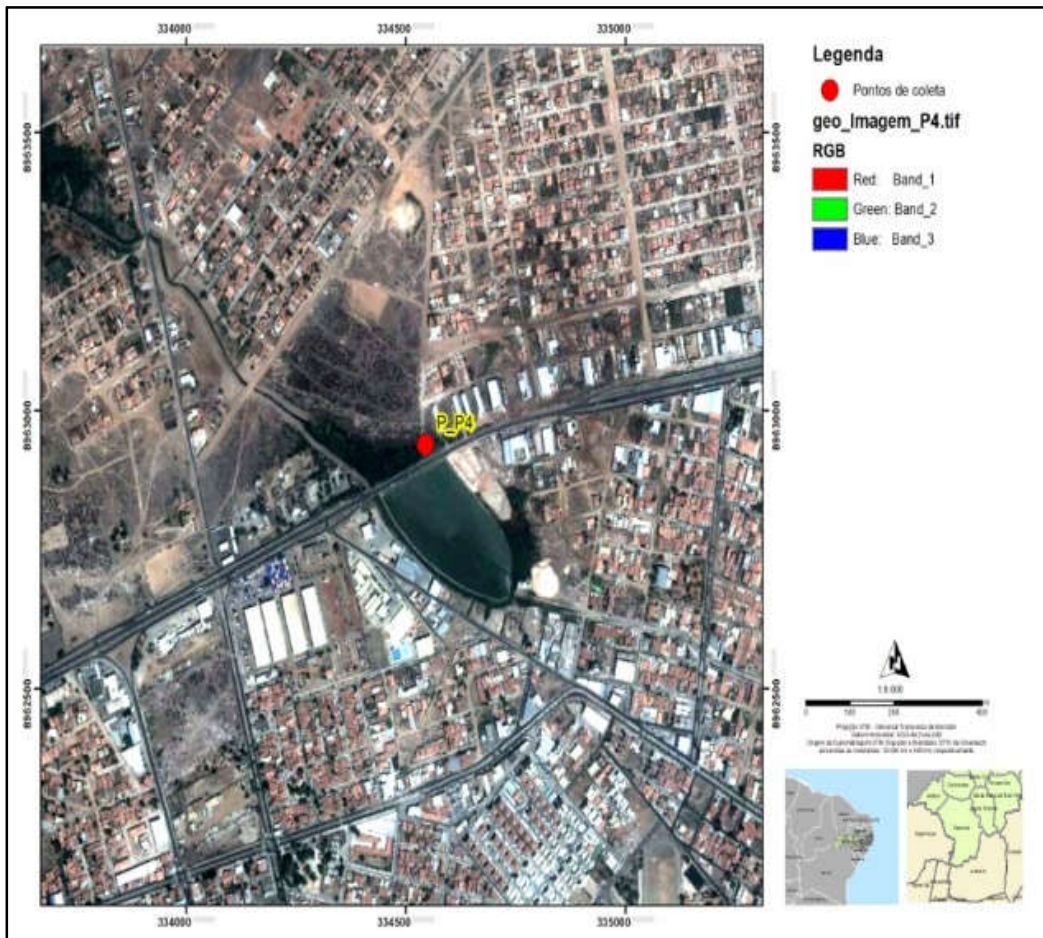
Figure 3. Collection points, samples P.P1 and J.P1 (river)





Source: Acquired 2012

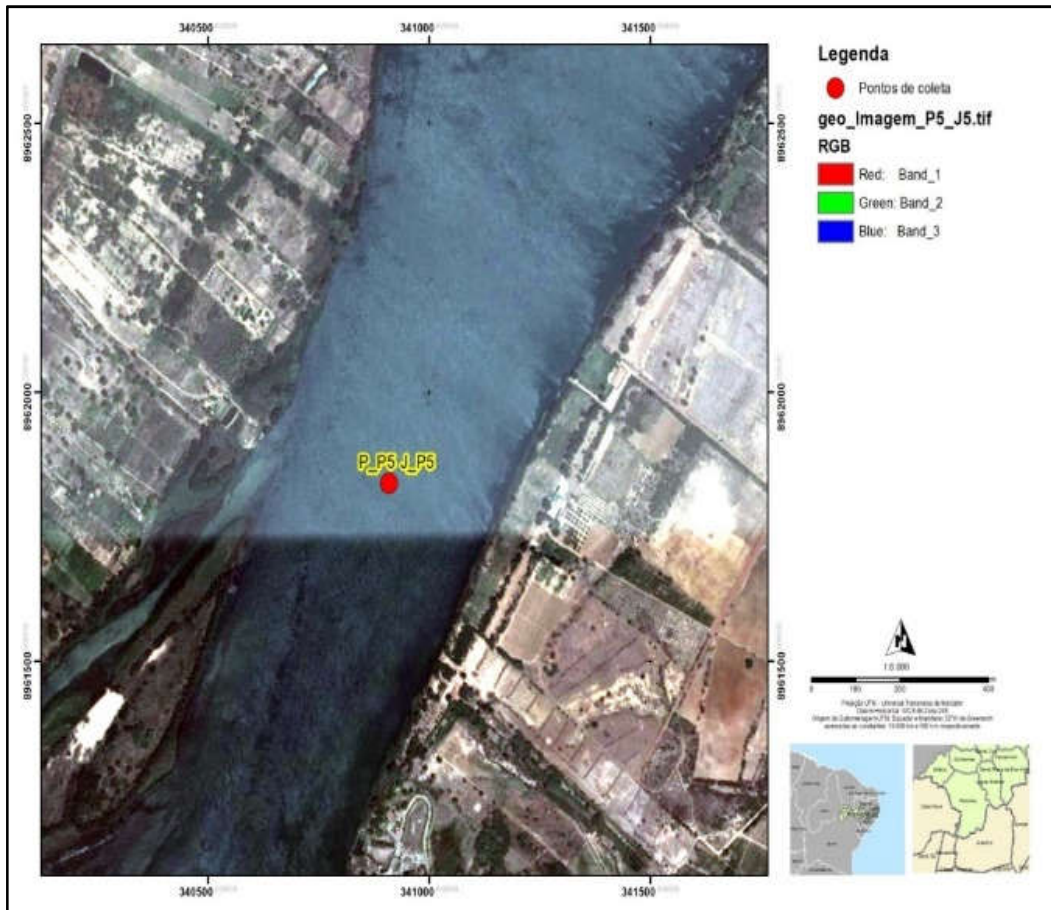
Figure 4. Collection locations, samples P.P2 (COMPESA) and P.P3 (ETH)



Source: Acquired 2012

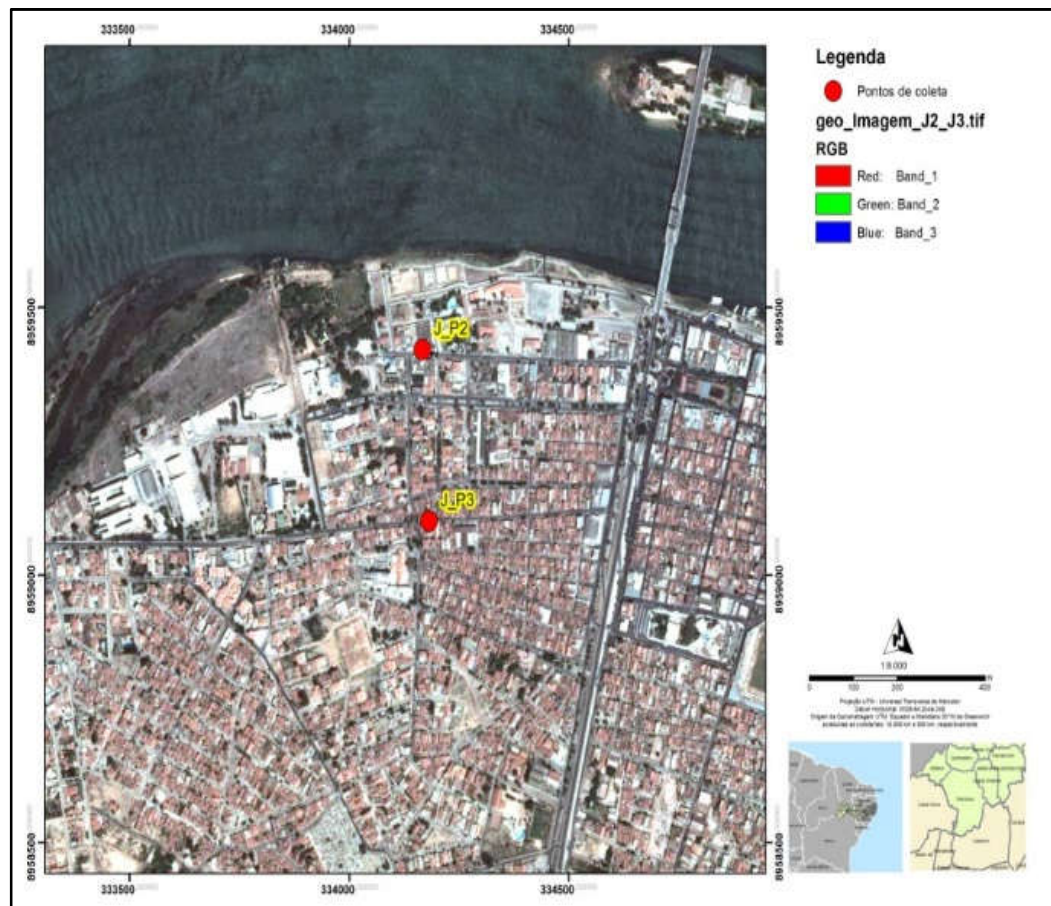
Figure 5. Collection points, samples P.P4 (Stabilization Pond)





Source: Acquired 2012

Figure 6. Collection locations, samples P.P5 and J.P5 (river)



Source: Acquired 2012

Figure 7. Collection locations, samples J.P2 (SAAE) and J.P3 (HRJ)

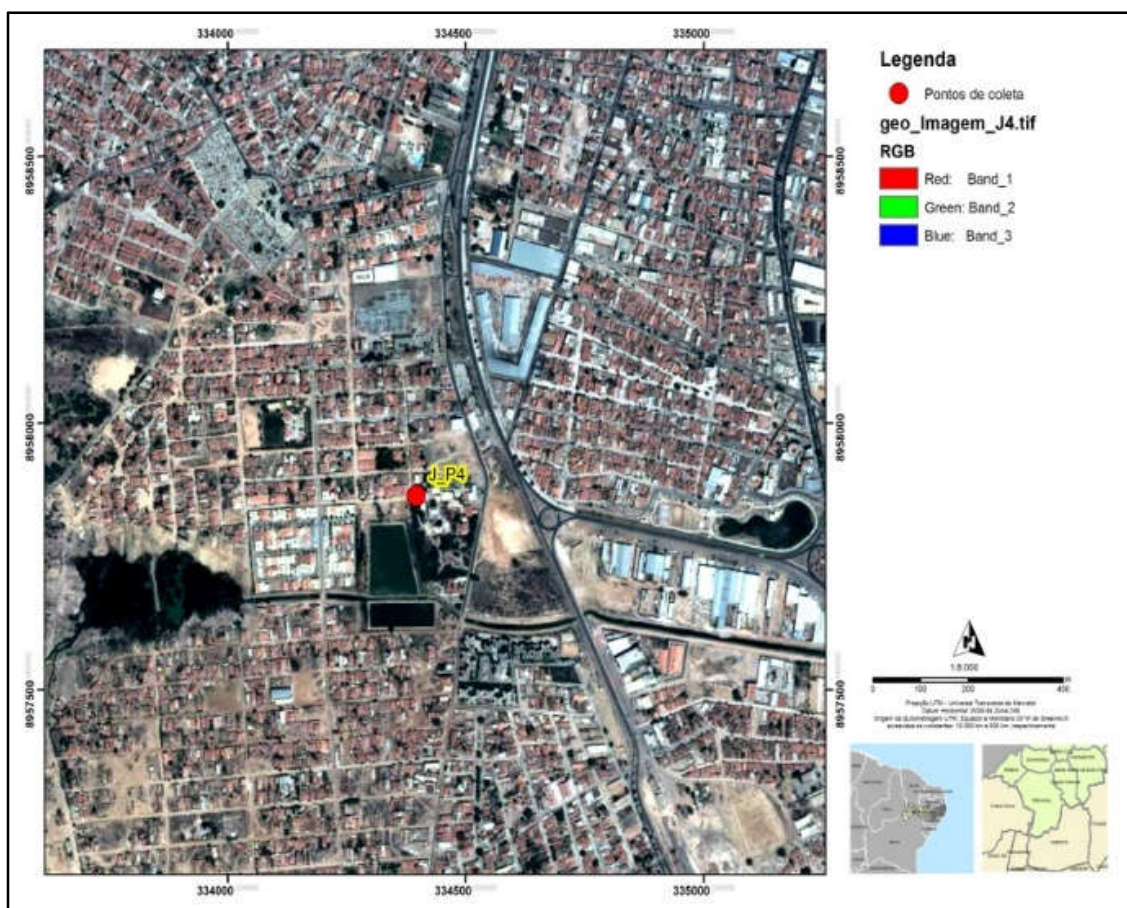


Figure 8. Collection locations, samples J.P4 (Stabilization Pond)

For standard curve of TTF solution (1% in methanol) was used and the values of enzymatic activity expressed in  $\mu\text{g TTF mL}^{-1}$  of water (Benfield, Howard, and Howard, 1977; Wolinska and Stepniwski, 2012). Fluorescein diacetate (FDA) hydrolysis activity: 5 mL water samples were incubated in Erlenmeyer flask with 20 mL of potassium phosphate buffer (66 mM, pH 7.6) and 200  $\mu\text{L}$  of FDA solution (0.02 g / 10 mL acetone) for 20 minutes. After this period, the reaction was stopped by adding 20 mL of acetone and the readings were performed in a spectrophotometer (490 nm). For standard curve, increasing concentrations of FDA previously hydrolyzed by heat ( $100^\circ\text{C}$ ) were used. Enzymatic activity was expressed as  $\mu\text{g}$  of hydrolysed fluorescein  $\text{g}^{-1}$  dry soil  $\text{h}^{-1}$  (Green, Stott, and Diack, 2005a; Swisher and Carroll, 1980). Experimental design was random comparative type with five treatments: in 5 replications, totaling 50 experimental plots. Data were subjected to ANOVA and means were compared by Tukey test ( $P < 0.05$ ). Pearson's (r) simple correlation analyzes were also performed between the variables studied. It used cluster analysis, considering all parameters evaluated and using the Euclidean Distance method and UPGMA algorithm (unweighted pair-group method). The analyzes were performed using STATISTICA 6.0 program (F. de A. S. Silva and Azevedo, 2009). Analysis was performed of liquid waste, determining the biochemical composition and comparing the same with parameters established in literature and / or legislation. It conducted the analysis of samples collected in points: P1, P2, P4 and P5, which allowed partially describe water path used for consumption from pickup point into the river until its disposal after use in urban area of municipalities studied and these were analyzed by technique described above in biochemistry laboratory of University of Pernambuco - UPE

Campus Petrolina, determining the biochemical composition and comparing the same with parameters set current literature (CONAMA, 2005), which comprises on classification of bodies of water and environmental guidelines for their classification. To collect these samples was made employing vessel that was allocated for this purpose, this served to carry out the sampling of P1 and P5 samples of two cities in different days and cars which served to shift within the surveyed municipalities to collect the samples at points P2, P3 and P4, and these were performed together with the samples of municipalities, respectively. To perform the analysis of the results, these were described in a quantitative and comparative way (Minayo, 2010). To develop the research actions were drafted the terms of consent (letter of consent), and confidentiality, which served as the entry tool for data collection after the statements of those entities.

## RESULT AND DISCUSSION

Embodiments of samples were collected on one day and for each point were collected 5 samples, as described in experimental design, totaling 50 samples, these were analyzed in biochemistry laboratory of University of Pernambuco - UPE Campus Petrolina and laboratory wastewater analysis of the Company's water treatment substation Pernambuco Sanitation - COMPESA, both located in the city of Petrolina.

### Hydrogen potential – pH

The letters pH are hydrogen ion potential abbreviation. The pH is a dimensionless parameter and calculated value is the negative logarithm of activity or concentration of hydrogen



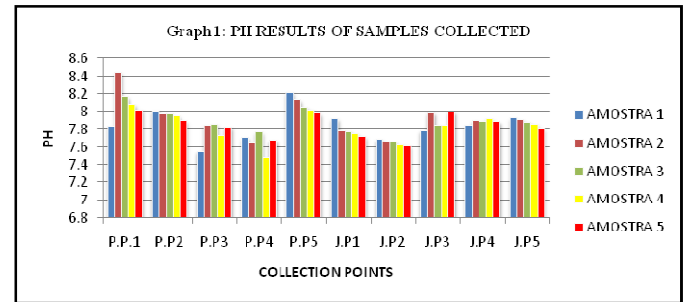
ions (H<sup>+</sup>). Not counting on exceptional factors, pH value of natural waters ranges from 6.5 to 8.5. PH values in range of 6 to 9 are considered compatible in long run for most organisms. PH values above or below these limits are harmful or lethal to most aquatic organisms, especially to fish. Some rivers such as Negro and others of naturally dark coloration can naturally present pH between 4.0 and 6.0 due to the presence of humic substances (Monteiro, Crepaldi, Avelar, Peterlini, and Pedreira, 2012; Pinto, Camargo, Almeida, Alves, and Botrel, 2011). PH values of rivers above 10.0 or below 4.0 indicate contamination by industrial effluents or the occurrence of accidents with chemical leakage. In lakes and reservoirs with a high density of phytoplankton, pH can naturally reach values above 9.0 during the period of maximum sunshine, due to the photosynthetic activity of algae, which remove CO<sub>2</sub> by altering the entire carbonate system. In these situations, increasing the pH (> 8.0) intensifies the eutrophication process, because absorbed phosphate hydroxides of iron (III) and aluminum and sediment deposited on bottom of lakes and reservoirs are released again, enriching waters with nutrients (ANA, 2012). The toxicity of pH is related, among other things, to its influence on chemical composition of water. PH influences in solubility of the substances (metal salts), in predominance of certain species more or less toxic and in processes of adsorption / sedimentation of metals and other substances in water. PH values outside the range of 6.0 to 9.0 may result in partial or complete inhibition of the metabolic processes (natural) microorganisms involved in stabilization of organic matter, especially by anaerobic process (ANA, 2012). Below (Table 2), mean of results obtained after the sample collection. Means followed by the same letter are not statistically different from each other. It was applied Tukey's test at 5% probability.

**Table 2. pH average of samples collected after application of Tukey's test**

Treatment	average	
P.P1	8,11200	a
P.P2	7,96000	abc
P.P3	7,76400	cd
P.P4	7,65800	d
P.P5	8,08200	ab
J.P1	7,77200	cd
J.P2	7,65200	d
J.P3	7,89600	abc
J.P4	7,89000	bc
J.P5	7,87800	bc

It can be observed that in results obtained (Table 2 and Figure 1), it is evident that the collected samples behaved within acceptable limits with regard to pH, even though the degree of pollution at points P.P3, P. P4, J.P3 and JP4; because they were sewage effluents from hospital and the stabilization ponds that were used to collect the samples. Thus it can be concluded that this parameter does not become sensitive or reliable for the measurement of environmental impacts in some specific situations, since during the collection (annexed photos) the large scale fish mortality in one of the studied lagoons was observed. However, if we measure the impacts taking into account the change in pH and the rates recommended by law we see that the values extracted from the samples are within the standards of normality recommended (ANA, 2012; CONAMA, 2005). The pH is an important parameter that, along with others, can provide evidence of the degree of pollution, communities metabolism or impact on an aquatic ecosystem.

Natural water presents a pH between 4 and 9, which is influenced by the dissolution of CO<sub>2</sub>, which causes low pH values, and by the reactions of HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>=</sup> with water, resulting in higher pH values. In general, when the pH approaches 9, is the removal of carbon dioxide in water by algae photosynthesis. It notes that the dominant chemical species depend on the final pH of the water body (also determined by the existence of other acids and bases), and the respective equilibrium constants of the reactions (Fowler *et al.*, 2013).



### Electrical Conductivity - EC

Electrical conductivity is ability of water to conduct electric current. This parameter is related to presence of dissolved ions in water, which are electrically charged particles. The greater the amount of dissolved ions, the greater electrical conductivity of water. In continental waters, the ions directly responsible for conductivity values are, among others, calcium, magnesium, potassium, sodium, carbonates, carbides, sulphates and chlorides. Electrical conductivity parameter does not specifically determine which ions are present in a given water sample, but may contribute to possible environmental impacts that occur in drainage basin caused by releases of industrial waste, mining, sewage, etc. (Hayashi, 2004; Wang, Mookherjee, Xu, and Karato, 2006). The electric conductivity can vary with temperature and total concentration of dissolved ionized substances. In water whose pH values are located at extreme ranges (pH > 9 or pH < 5), conductivity values are due only to high concentrations of a few ions in solution, among which most common are H<sup>+</sup> and OH<sup>-</sup> (Hayashi, 2004; Wang *et al.*, 2006). The conductivity determination can be done through the electrometric method, using a digital conductivity meter. Electrical conductivity is a measure of the ability of an aqueous solution to conduct an electric current due to presence of ions. This property varies with the total concentration of ionized substances dissolved in water, with temperature, mobility of ions, with valence of the ions and with actual and relative concentrations of each ion (Costa, Queiroz, Pinto, Reis, and Santos, 2014). The electrical conductivity can be expressed by different units and, mainly, by their multiples. In the International System of Units (S.I.) is reported as Siemens per meter (S / m). However, in measurements performed on samples of water, is preferably used microSiemens (S/cm) or milliSiemens per centimeter (mS/cm). To report electrical conductivity data on S.I. units, following is the ratio 1mS / cm = 10 μS / cm. The United States adopts the mho/cm unit, maintaining the ratio mho / cm = S / cm (Costa *et al.*, 2014). Electrical conductivity is a property that depends expressively on temperature. Electric conductivity data should be monitored in which temperature was measured. For comparative purposes of electrical conductivity data, one of reference temperatures (20 ° C or 25 ° C) is defined. In operation of the Hydrometeorological Network adopts the reference



temperature 20 ° C (Wang *et al.*, 2006). The equipment currently available are able to provide the electrical conductivity has been already converted to one of the reference temperatures. However, data in which they do not refer to these temperatures can be converted using the following equations: Electrical conductivity at 20° C = where:  $\chi_{\text{measured}} = \text{electrical conductivity}_{\text{measured}}; T = \text{Temperature measurement of electrical conductivity or according (APHA : Electrical conductivity at 25 ° C = (2510 Conductivity-B) where: } \chi_{\text{measured}} = \text{measured electrical conductivity T = temperature measurement of electrical conductivity (Hayashi, 2004). The electrical conductivity of water is determined by the presence of dissolved substances that dissociate into anions, cations and temperature. The main sources of salts naturally contained in the flowing waters and anthropogenic origin are industrial discharges salts, salt consumption in households and commerce, excretions salts by humans and animals. Specific conductance provides a good indication of changes in water composition, especially in its mineral concentration, but gives no indication of the relative amounts of the various components. As more dissolved solids are added, the specific conductivity of the water increases. High values may indicate corrosive characteristics of water (ANA, 2003). Within the collected samples it was observed (table 3) that the samples follow within tolerable limits, except for point J.P4, which shows above the established. Emmanuel, Pierre and Perrodin (2009) report that values between 297 and 324 $\mu\text{S}/\text{cm}$  are characteristic of a high concentration of mineral, which indicates that the sample has corrosive attributes. Averages followed by the same letter do not differ statistically from each other. It was applied Tukey's test at 5% probability.$

**Table 3. Electrical Conductivity Means pH of samples collected after application of Tukey's test**

Treatment	average	
P.P1	45,12000	b
P.P2	32,42000	b
P.P3	66,94000	b
P.P4	65,30000	b
P.P5	31,66000	b
J.P1	33,98000	b
J.P2	41,00000	b
J.P3	93,78340	b
J.P4	438,40000	a
J.P5	44,10000	b

These evictions, mostly from residences, basically consist of urine, faeces, food waste, soap, detergents and washing waters, containing a high amount of organic matter, which contribute to the entrance, in the body of water, of the ionic species such as calcium, magnesium, potassium, sodium, phosphates, carbonates, sulfates, chlorides, nitrates, nitrites and ammonia, among others (Marques, 2013, Guimarães and Nour, 2001). From this assumption we can show that the quality of water bodies is directly linked to the amount of organic matter in it. Thus, in the analyzes of the samples P.P3, P.P4 and J.P3 the levels differ from those ratified in the raw or treated water samples, even though within the parameters.

### Thermotolerant Coliform

The preservation of water quality is a universal need, which requires attention from health authorities and consumers in general, particularly with regard to water from wells, mines, springs, lakes and others, for human consumption, since their contamination by excreta of human and animal origin can

make them a vehicle for transmission of infectious and parasitic disease agents (Amaral *et al.*, 2003), which directly influence the health of the population. The assessment of the microbiological quality of water can be carried out through identification of fecal contamination and biological indicators, when appropriate, using aquatic organisms and / or communities. Diseases of water origin usually come from living organisms and not from dead organic matter or dissolved mineral salts such as lead salts, zinc, nitrates, among others. It is universally known that man and animal excreta contain coliform bacteria in large numbers (100 to 1000 million g) (Straub and Chandler, 2003). Coliform bacteria are Gram-negative rod-shaped, non-spore forming bacteria capable of developing in the presence of bile salts, lactose fermenters with gas formation, easily cultivable, either aerobic or anaerobic. Because of their ability to ferment lactose producing gas and acid, they are distinguished from a large group that include pathogenic organisms such as typhoid and paratyphoid bacteria. Pathogenic bacteria do not multiply and develop in water, they weaken and tend to die because they have lost their food and ideal conditions of life.

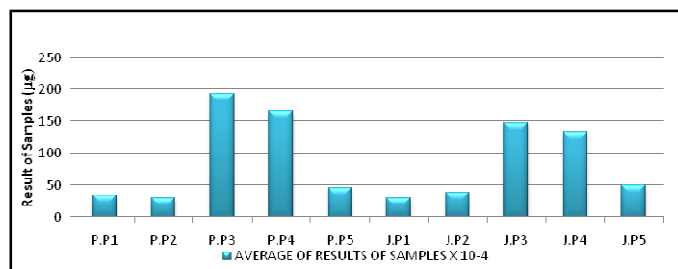
For this reason, it is difficult to isolate and identify directly any disease organism present in drinking water, then a method is used to identify the presence of organisms belonging to the "Coliform Group" of intestinal origin (Silva *et al.*, 2011). When the coliform group is present in the water, it is seen as an indication of pollution with sewage and likely presence of pathogenic bacteria. A watercourse can be polluted with sewage and does not have pathogenic organisms, but the presence of coliforms indicates a potentially dangerous water that needs disinfection treatment (SILVA *et al.*, 2011). The presence of coliforms in water does not in itself pose a health hazard but indicates the possible presence of other organisms causing health problems. The main indicators of fecal contamination are concentrations of total coliforms and fecal coliforms, expressed in number of organisms per 100 ml of water. Most of bacteria of coliform group belong to genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. In addition to being found in faeces, they may occur in environment, in waters with high levels of organic material, soil or decaying vegetation. Some species can multiply in waters of human consumption, for example, *Serratiafonticola*. (CETESB, 2002). Fecal coliforms (*Escherichia coli*) are found in sewage, treated effluents and natural waters and soils subject to recent fecal contamination of humans, domestic animals, wild and birds, and their presence requires immediate action. Rapid tests are now available for their identification, some of them subject to international standardization and accepted for routine use (CETESB, 2002). When analyzing the samples and coliforms (Figure9), we verified that according to the CONAMA resolution n° 274 of 2000, samples P.P2 and J.P2 follow the established standards.

When we look at other points of analysis and their samples, we see that indices are above what is recommended, also showing a slight change in indices between the points of abstraction and disposal, leading to conclusion that, with regard to treatment and return for river of treated waste, COMPESA and SAAE services are in disagreement with legislation. When we relate the indices between the hospital waste and stabilization pond, we can see that there is a higher concentration of Thermotolerant Coliforms at points P.P3 and J.P3, which leads us to believe that composition of domestic waste differs from that collected in hospitals.

**Hydrolysis of Fluorescein Diacetate–FDA:** Fluorescein is a xanthine, a class of compounds widely used as colorants. It was synthesized for first time by German chemist Johann Friedrich Adolf von Bayer. It is named after yellow-green fluorescent coloration that it presents in alkaline solution, also known as uranine. Its melting point is 314-316 ° C. It appears as dark orange solid. Even in the sodium form it is only slightly soluble in water. Soluble in alcohol. Fluorescence of this molecule is very high, its excitation occurs at 494 nm and emission at 521 nm(Alves *et al.*, 2016; Ghini, Mendes, and Bettiol, 1997). Fluorescein has a pKa of 6.4 and its ionization equilibrium leads to pH-dependent absorption and emission in range of 5 to 9. Thus, lifetimes of protonated and deprotonated fluorescent forms are approximately 3 and 4 ns, which allow the determination of pH of non-intensely basic measurements. Lifetimes can be retrieved using either single photon counting or phase modulation fluorometry (Alves *et al.*, 2016; Ghini *et al.*, 1997; M. Silva, Siqueira, and Luis Da Silva Costa, 2004).

COLLECTION POINTS	SAMPLES COLLECTED				
	01	02	03	04	05
River catchment point Petrolina	10x10 <sup>5</sup>	00	00	10x10 <sup>5</sup>	40x10 <sup>5</sup>
Substation treatment COMPESA	00	00	00	00	00
Emergency and Trauma Hospital - ETH	2,20x10 <sup>6</sup>	1,70x10 <sup>6</sup>	5,00x10 <sup>5</sup>	1,00x10 <sup>5</sup>	1,60x10 <sup>6</sup>
Stabilization pond	7,00x10 <sup>4</sup>	2,40x10 <sup>5</sup>	4,90x10 <sup>5</sup>	1,03x10 <sup>6</sup>	9,30x10 <sup>4</sup>
River discard point Petrolina	00	00	00	00	1,00x10 <sup>2</sup>
River catchment point Petrolina	00	20x10 <sup>5</sup>	30x10 <sup>5</sup>	10x10 <sup>5</sup>	30x10 <sup>5</sup>
Treatment substation SAAE	00	00	00	00	00
Regional Hospital of Juazeiro	3,00x10 <sup>5</sup>	5,00x10 <sup>5</sup>	5,00x10 <sup>5</sup>	00	3,00x10 <sup>5</sup>
Stabilization pond	1,00x10 <sup>5</sup>	1,00x10 <sup>4</sup>	1,40x10 <sup>3</sup>	2,00x10 <sup>4</sup>	1,00x10 <sup>3</sup>
River discard point Petrolina	00	00	10x10 <sup>5</sup>	30x10 <sup>5</sup>	70x10 <sup>5</sup>

FDA hydrolysis is a method that evaluates the indiscriminate hydrolytic activity of bacteria and fungi. Hydrolysis of FDA has been positively correlated with soil respiration (Green *et al.*, 2005a; Schnurer and Rosswall, 1982), in particular the enzyme activity in soil provides catalyzing various reactions that are necessary for life cycle of microorganisms, in decomposition of organic residues during the nutrient cycle and in formation of organic matter and soil structure (Green, Stott, and Diack, 2005b). Several investigations using FDA to determine the enzymatic activity of soil have already been evidenced. Enzymatic activities (FDA, urea and  $\beta$  - glycosidase) served as indicators of potential of ecosystem functionality and, together with other biological attributes, become good indicators of soil quality(Green *et al.*, 2005a; Schnurer and Rosswall, 1982). In this work we used as one of indicators for analysis of samples the FDA hydrolysis, efficiency of this method for determination of organic matter and its enzymatic activity was evidenced by Green *et al.* (2005b). In their study this new bioindicator for analysis of water, arriving at end of its experiment proving that the same one is more sensitive alterations, originating of antropic activity, being able to replace or to collaborate in the Analyzes of Biochemical Demand of Oxygen - BOD and Chemical Demand of Oxygen - CDO. Making the FDA hydrolysis another option in the analysis to determine the microbial activity in liquid effluents with presence of organic matter.



Graph 2. Average of fda samples

FDA hydrolysis method can provide us with an indication of intrinsic biodegradability of sample which may already contain microorganisms adapted to oxidize specific organic matter of each type of environmental / industrial sample(Schnurer and Rosswall, 1982). We can observe (Graph 2) that the most sensitive samples are those collected near sites with the highest concentration of organic matter (P.P.3, P.P.4, J.P.3 and J.P.4), which shows a high degree of biodegradability due to degradation activity of the waste from hospital and domestic sewage respectively. As enzymatic activity indexes are more active (P.P.3 and J.P.3), in these points which wastewater and sewage from hospital services were collected, that allows us to point out that there is a difference between this effluent and domestic, but that is minimized when in stabilization ponds these are put together and receive the same treatment, decanting. We also know that this is an average (Table 4), which shows that we can still have higher values, which allows us to conclude that the degree of pollution in these points is relevant, and that type of treatment submitted to them must be adequate and rigorous, in order to preserve quality of bodies of water where they will be dumped. Averages followed by the same letter do not differ statistically from each other. It was applied Tukey's test at 5% probability.

### Basal Microbial Respiration

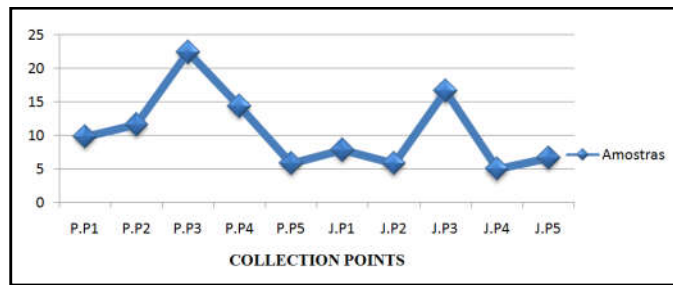
We measure Biochemical Oxygen Demand (BOD), which is used to express the value of pollution produced by biologically oxidizable organic matter corresponding to amount of oxygen that is spent by microorganisms of sewage or polluted waters in biological oxidation, when maintained at a given temperature for agreed period of time. Demand may be large enough to consume all oxygen dissolved in water, which conditions the death of all aerobic organisms underwater breathing(Oliveira, Deniculi, Itaborahy, and Cecon, 2000). The CDO is based on fact that all organic compounds, with few exceptions, can be oxidized by action of a strong acid oxidizing agent. A barrier, however, is the fact that test does not differentiate between biodegradable organic matter and non-biodegradable organic matter, the first one determined by BOD test. Advantage is test time, performed in a few hours, while BOD test requires at least 05 days (incubation period) (Hsieh and Chung, 2014; Oliveira *et al.*, 2000). Microbial respiration is used as a method to evaluate the quality of water bodies. However, it should be associated with other factors and bioindicators. Anthropogenic actions of any stable balanced system tend to cause more losses than carbon gains, leading a decrease in quality of these systems over time (Dadalto, Fernandes, Teixeira, Cecon, and Matos, 2015). These losses are due to release of CO<sub>2</sub> in respiration, resulting from decomposition of soil organic matter by microbial hydrolysis, leaching and losses of compounds by water erosion, these last two paths being of lower magnitude in subtropical soils



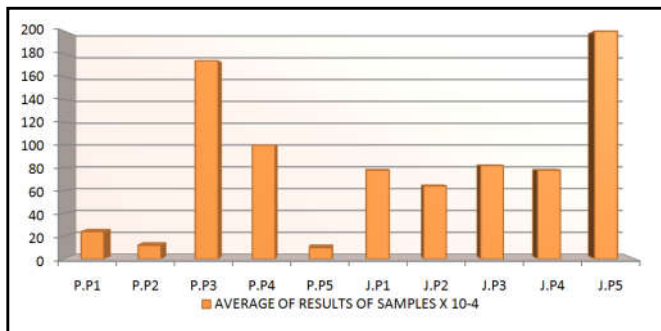
(Kallenbach, Frey, and Grandy, 2016; Pereira, Baretta, Bini, Vasconcellos, and Cardoso, 2013). Basal respiration represents oxidation of organic matter to CO<sub>2</sub> by aerobic microorganisms, occupying an important position in carbon cycle in ecosystems (Kallenbach *et al.*, 2016; Moreira and Malavolta, 2004). Measurement of microbial respiration is a way to measure the level of activity of microorganisms, which is suggestive of rate of decomposition of organic matter present in medium to be analyzed.

**Table 4. FDA Hydrolysis Averages of Samples Collected After Application of Tukey's Test**

Treatmentaverage		
P.P1	0.00340	b
P.P2	0.00300	b
P.P3	0.01940	a
P.P4	0.01660	a
P.P5	0.00460	b
J.P1	0.00300	b
J.P2	0.00380	b
J.P3	0.01480	a
J.P4	0.01320	a
J.P5	0.00500	b



**Graph 3. Average microbial respiration basal (µg C-CO<sub>2</sub> g<sup>-1</sup>)**



**Graph 4. Average desidrogenase activity samples (µg TTF mL<sup>-1</sup>)**

Edaphic respiration or microbial activity is the production of carbon dioxide through of medium, due in large part to activity of decomposing organisms present that degrade organic matter, and to lesser extent, respiratory activity of subterranean systems of plants (Ferreira, Stone, and Martin-Didonet, 2017). Microbial biomass plays leading role in environmental sustainability scenario and can be estimated by relatively simple methods (Capuani, Rigon, Beltrão and Neto, 2012; Ferreira *et al.*, 2017). Measurement of respiratory rate or microbial activity (Graph 3), determined by evolution of CbO<sub>2</sub> from respiration of aerobic heterotrophic microorganisms during oxidation of organic compounds (Capuani *et al.*, 2012). Several factors including temperature, humidity, depth, aeration and microbial populations determine CO<sub>2</sub> flow rate to surface (G. A. and Silva, Souto, and Araujo, 2006). Knowing that many references on respiration are closely linked to soil

analyzes, soil preparation being a combination with other management practices and with the action of temperature and humidity influence emission rate of C-CO<sub>2</sub> to atmosphere. Averages followed by the same letter do not differ statistically from each other. It was applied Tukey's test at 5% probability.

**Table 5. Basal Microbial Breath Averages of samples collected after Tukey's test**

Treatmentaverage		
P.P1	9.85000	ab
P.P2	11.61400	ab
P.P3	22.40600	a
P.P4	14.37000	ab
P.P5	5.86800	b
J.P1	7.86200	ab
J.P2	5.86800	b
J.P3	16.65600	ab
J.P4	5.04600	b
J.P5	6.68600	b

Biological activity of soil depends on abiotic factors, such as temperature, water and nutrients, and biotic factors such as, mainly, addition of carbon to its activity and development (Cunha, Stone, Ferreira, Didonet, and Moreira, 2012).

**Dehydrogenase Activity**

When we talk about bioindicators we have to elucidate, because these are defined as organisms or communities that respond to environmental pollution, changing their vital functions or accumulating toxins. This meaning can be amplified when it is appreciated that bioindicators are organisms or communities that react to environmental changes transforming their vital functions and / or their chemical composition and with this they provide information about environmental situation. Bioindicators are organisms or communities whose vital functions correlate so closely with certain environmental factors which can be used as indicators in evaluation a given area (DeBerardinis and Thompson, 2012; Jirova *et al.*, 2016). This definition consciously includes indication of natural behaviors, for example in agriculture, where we can infer about characteristics of a region only by presence or absence of certain plant species. Enzymatic action of dehydrogenase is involved in oxidation reactions of cells of microorganisms, having a close connection with microbial respiration (Aguar and Ferraz, 2011; O'Connell, Zhao, Ellington, and Marcotte, 2012). Its activity reflects the redox potential of soil and, as this is an intracellular enzyme of low activity when in free state in soil, reflects the bioactivity of a large part of active microbial population (Aguar and Ferraz, 2011; Jirova *et al.*, 2016; Resende, Resende, Pardal, Almeida and Azeiteiro, 2010). Dehydrogenase activity is commonly estimated by conversion of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,3,5-triphenyltetrazolium formazan (TTF-formazan) by action of enzyme (Liwarska-Bizukojc, 2011; Longo, Ribeir and Melo, 2011). TTF formazan product is a compound of pink color in presence of acetone, which quantification can be done by spectrophotometry. Because it is an extremely correlated process with metabolism of edaphic microorganisms, it is possible to evaluate if there is a short or long term influence of the pesticides on the microbial activity of the soil (Capuani *et al.*, 2012; G. A. and Silva *et al.*, 2006). The observation of activity of enzymes dehydrogenase, phosphatase, urease, arylsulfatase and β-glucosidase in soil

treated with metalaxyl allowed to conclude that dissipation of fungicide in soil occurred mainly because of microbial activity, where as total amount of nitrogen and carbon were altered, as well as activities of different enzymes (Sukul, 2006). These changes may have as a direct impact on nutrient cycling and soil energy flow. Pandey and Singh (2006) monitored for one year the activity of dehydrogenase and arginine deaminase in soils under peanut cultivation, evaluating the effects of applications of insecticides chlorpyrifos and quinalphos used in seed treatment. They observed that dehydrogenase activity was inhibited by 37% 15days after treatment with quinalphos, and 17.2% after treatment with chlorpyrifos. Activity of arginine deaminase was stimulated after treatment with chlorpyrifos and inhibited after treatment with quinalphos. Therefore, it has been found that different insecticides can have different effects on activity of different soil enzymes, and therefore, non-critical use of these substances can alter soil fertility over time(Pandey and Singh, 2006).

Dehydrogenases are a group of enzymes included in class of oxidoreductases. Its activity is evaluated by incubation of soil samples with 2, 3, 5 triphenyltetrazolium and measure production of triphenyl formazan (Liwarska-Bizukojc, 2011) Toluol is not used in method whereas it destroys the enzymatic activity. Thus, the contribution to activity observed by proliferation of microorganisms is unknown, but may be important in trials where soils are altered with organic substrates (such as sewage sludge and tannery sludge) and incubated for 24 hours(Holik, Kučera, Rejšek, and Vranová, 2017; Pandey and Singh, 2006). Active dehydrogenases are considered in existing in soils as integral part of intact cells, and dehydrogenase activities are used to reflect full range of oxidative activities of soil microflora (Pereira *et al.*, 2013). Biological monitoring is performed mainly through the application of different assessment protocols, biological and multimetric indexes, based on use of bioindicators of water quality and habitat. The main methods involved include survey and evaluation of changes in species richness and diversity indexes; abundance of resistant organisms; loss of sensitive species; primary and secondary productivity measures; sensitivity to concentrations of toxic substances (ecotoxicological tests), etc.(Barbour, Gerritsen, Snyder, and Stribling, 2013).

## Conclusions

After the analyzes have been ratified that in some indicators is no difference between domestic and hospital waste, which leads us to see that there is an urgent and emerging need for proper treatment of this waste. During the research it was found that local sewage treatment systems are not prepared to process and treat this type of effluent. Therefore these systems do not achieve adequate treatment of this by-product. This was evidenced in some samples that, as recommended by CONAMA for the return of these effluents, the degree of pollution is above that established in the legislation. In some samples the quality of the water bodies was verified, whereas after discard the points are altered, which shows ineffectiveness of the actions and treatment measures. Controversy arises is that the disposal of medical waste produced by health centers, their impact on environment and which may cause possible damage to perpetuation of human species. Although there is evidence of an awareness of environmental problems, global movements for protection of

nature and environment are uncoordinated and confused and suffer interference of dominant political-economic-capitalist system, whose hegemony goes beyond limits of Western world and begins to reach whole world. It is perceived that despite the scope of theme "environment", all converge towards a common and present cause directed to the future concern of planet, in perspective guided by reduction of degradation of environment and gradual reversal of actions that affect the climate, vegetation, seas, fauna, flora, air, mankind and other aspects of environment. Epidemiological risks attributed to medical waste currently generate numerous discussions between experts in infectious and parasitic diseases, epidemiologists, sanitary engineers, bacteriologists, businessmen of national and international industry, members of the journalistic community(Cafure and Patriarcha-Gracioli, 2014; Weir, 2002). These discussions, very controversial, result in a number of suggestions for hospital waste problem solution(Hu and Shy, 2001; Porta, Milani, Lazzarino, Perucci, and Forastiere, 2009; Tadesse and Kumie, 2014).

Objective discussion has been hampered by cultural prejudices, medical-sanitarian disinformation, and financial interests(André, Santos, Veiga, Mendes and Takayanagui, 2013; Zanon, 1990). In controversy over issue of mandatory sterilization of solid waste generated in health units "(...) align themselves with those who claim that these wastes are hazardous to health, (...)" (Zanon, 1990) and claim at federal, state and municipal level, legal protection that requires special collection and treatment (incineration, pyrolysis, autoclaving and other) for them(Edmunds *et al.*, 2016; Weir, 2002; Zanon, 1990). After taking possession of these diverse forms of approach and understanding of environments and their nuances, elucidated by various authors where there was an opportunity to reflect on readings, which raised questions, one can perceive fragility and dependence of built systems society. This environment considered habitable, suffering several metamorphoses of natural and man-made order, the latter in order to adapt to the momentary or later needs to be gregarious. And this, reveals its exhaustion and overload, leaving a reflection for all that question: how long will it meet its necessities and what will be left for future generations?

Thus, it is necessary to think of each environment individually to seek balance and sustainability so that there can be harmony in systems of production and service delivery with least wear and degradation of nature, it is an individual and collective responsibility. To provide plans for management and control of waste emission and treatment, whether in hospital environment or any other, is a virtue and an object that must be incorporated into manager and management. It is unacceptable that because of environmental problems caused by certain waste, public managers, in general, let society by inexperience and non-compliance with standards for packaging, disposal and final and proper disposal of hospital waste. It has been already known how much harm these can bring their degree of contamination and existing biological load. In this proposal, at end the search, the results will support development and implementation of programs and projects in order to ensure the lowest degree of pollution of these by-products of human consumption, is the most effective and feasible way to minimize these damaging consequences that they subject our populations, and compromise the balance between man and nature, between natural environment and built. This work also served to build new knowledge about theme and water quality of São Francisco River, opening up a range of opportunities to



deepen theme. Many other indicators can be researched in order to better real diagnose situation and commitment of this source.

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