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Removal of faecal coliforms in facultative lagoon in the state of São Paulo

Remoção de coliformes fecais em lagoa facultativa no estado de São Paulo

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Ariston da Silva Melo Júnior
Lattes: http://lattes.cnpq.br/0010807076892082

Kleber Aristides Ribeiro
Lattes: http://lattes.cnpq.br/8299301338155638

Eriston Da Silva Melo

ABSTRACT
The pollution of water sources on the planet is a constant concern of society and should be widely discussed in the media in general. Faced with the reality of the precariousness of some countries in stimulating the conservation of water sources in their territory and the problem caused by the irregularity of the distribution of surface water on the surface of the planet, it is important to study technologies for the treatment and conservation of water resources. Scientists, biologists, and engineers around the world are creating new ways to treat sewage to ensure water preservation. Among the technologies employed, there is the adoption of the system of stabilization ponds that create an improvement in water quality by the anaerobic and aerobic action present in the effluent itself when passing through large reservoirs sized for this purpose. The present study monitored and analyzed the removal of fecal coliforms present in the waters of the Capanema stream by a stabilization pond. The study lasted nine weeks and showed that from the Colilert methodology for coliforms, the pond system promoted a percentage clearance of 16.42% in relation to the number of fecal coliforms before treatment.

Keywords: Contamination, Water, Alternative treatment, Environment.

1 INTRODUCTION

The growing need for water to maintain life on the planet has been an important topic of debate in the academic-scientific environment.

The water irregularity in the distribution of drinking water sources on the planet causes the need for conservation and maintenance of water reserves in order to allow the viability of the existence of life on planet Earth.

The problem faced is so challenging that the United Nations (UN) in 2012 found that no region of the world is free from the pressures on the lack of water resources.

According to Albano (2014) in Europe, for example, 120 million citizens do not have access to drinking water. In certain parts of the continent, waterways can lose up to 80% of their volume in the summer.

When one thinks of the African continent, the observed values tend to worsen, since the average demographic rate has an annual increase of 2.6%. While the world average is only 1.2%.

The increase in water needs to ensure manufacturing processes and in agriculture, among other activities, causes the demand for water to accelerate the deterioration of its water resources.

The non-uniformity and homogeneity in relation to water reserves in relation to the growing population have considered that continents such as Asia and the Pacific that are home to 60% of the world's population, but only 36% of water resources have serious water supply problems.

According to the UN report, some 480 million people did not have access to a quality water source in 2008, and 1.9 billion did not have adequate sanitation infrastructure.

In Latin America while the rate of extraction of water sources was doubled at the end of the twentieth century due to the growing needs for public and industrial supply. In the Middle East, at least twelve countries suffer from complete water scarcity, with no adequate water sources for the public.

The UN report also reports that around 80% of wastewater worldwide is not collected or treated but goes straight to other water bodies or infiltrates underground, which ends up causing health problems in the population and deterioration of the environment. Considering this situation, the need for treatment and disinfection of sanitary effluents becomes accentuated.

Currently there is a great diversity of technical alternatives for sanitation treatment near the sewage source.

They are simple systems, of proven efficiency, cheaper and with greater efficiency compared to traditional alternatives.

Some of these alternatives can even generate savings with the use of the gases released in digestion as a source of energy for the domestic stove and the liquid part as a bio-fertilizer rich in natural nutrients that do not harm the environment.

Among the alternatives pointed out: wetlands, slow filtration, modified septic tank, aerobic and anaerobic reactor, infiltration ditches and stabilization ponds or also known as stabilization ponds.

Stabilization ponds have been the most widely used domestic sewage treatment technique in developing countries (YÁNEZ, 2000).

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According to Perígolo (2004), in Brasília, of the 16 Sewage Treatment Plants (ETEs) operated by the Companhia de Saneamento do Distrito Federal – CAESB, 10 of them have, in part or in whole, the process of stabilization ponds.

The ponds are widely used because it is a natural process of sewage treatment, have low maintenance and installation costs (when the value of the land is low). In addition, it can achieve excellent removal efficiencies of organic matter, nutrients and pathogens.

There are several types of stabilization ponds, the anaerobic, where the organic material is decomposed below the surface, without contact with the air; aerates which, in turn, require an electromechanical device to help maintain a concentration of oxygen in the liquid part of the mass, causing biological reactions to separate the organic material from the water; stabilization, in which the deposit of sludge at the bottom facilitates anaerobic decomposition, but photosynthetic reduction and aerobic oxidation also occur; finally, those of maturation, which assist in the removal of disease-causing microorganisms with the help of ultraviolet rays emitted by the Sun.

After receiving the treatment in one of the types of ponds, the water is reused, with satisfactory sanitary conditions.

The water from the sewage treated by the stabilization pond system is used for various activities, among them the most common is irrigation. The reuse of water helps to avoid environmental pollution and helps in cultivation, through the use of nutrients present in the effluents.

The study proposal of this research was to monitor and evaluate the degree of efficiency regarding the removal of fecal coliforms, using the Colilert method, in a stabilization pond present in the municipality of Itirapuã that treats the waters of the Capanema River.

2 WATER RESOURCES: ENVIRONMENTAL MANAGEMENT

The last decades have brought the awareness that water, even in regions where there is great water availability, must be managed as an exhaustible resource. Water management should be a concern of the whole society and not only of those directly involved in the sectors responsible for water supply (PORTO, 1999).

In 1990 the World Health Organization (WHO) estimated that 1.23 billion people did not have access to water in good drinking conditions. In 2000, this number increased by more than 900 million people (CAMPOS, 2014). Added to this is an ever-increasing *per capita* demand for water. Only in England and Wales is water use expected to increase by 10% to 20% between 1990 and 2021, disregarding the aggravating factor of global warming (UN, 2012).

The world population grows at a rate of approximately 80 million people per year, generating a demand for drinking water of 64 billion cubic meters (UN, 2012).

As an aggravating factor, of the three billion people who will be added to the world population by 2050, 90% will be in developing countries, which have serious problems with the supply of drinking water (CAMPOS, 2014).

Despite holding 12% of global freshwater sources, Brazil has a non-uniform distribution of these reserves within its territory, with 70% of the country's water reserves in regions of low demographics, such as the northern region of the country. While of its 5 territorial regions, the Northeast region is the one with the worst water availability in the country and its population in the sertaneja zone lives in very precarious conditions (ALBANO, 2014).

As Silva (2011) points out, the way water is used does not depend only on the professionals directly involved in water resources, but on the whole society. Where decisions made by politicians, businesses, and society as a whole affect water use. Water resource managers decide the use that is given to water, meeting the demands. However, often the performance of these professionals does not achieve the required objectives, due to the scarcity of water, financial or human resources. This makes it necessary a multidisciplinary approach to water supply (CAMPOS, 2014).

The growing demand for water resources and the degradation of the water sources that constitute the main supply of these resources, make water a limiting factor for agricultural, urban and industrial development. Therefore, conservation practices, water reuse and loss reduction are essential for the management of water resources and pollution reduction. In addition of course new technologies for the treatment of polluted water (ALBANO, 2014).

2.1 WATER REUSE – APPLICATION

As a way of preserving the water resource, the domestic sewage collected should be destined to the sewage treatment plants (ETE), for later final disposal. Exist numerous forms of sewage disposal, being the dilution in streams, rivers, and lakes, one of the most frequent in Brazil.

In most cases, the dilution that occurred is insufficient to maintain the quality standard of the water body within the limits established by Conama Resolution 357/05, which classifies water bodies by variable categories from 1 to 4, and the quality tends to decrease increasingly.

Another form of sewage disposal is reuse, until some years seen as an exotic option is today an alternative that can not be ignored (CAMPOS, 2014), because in addition to disposing of sewage, it helps to combat water scarcity.

In industry, the reuse of sewage can be as cooling water in boilers, washing systems and transportation of materials, in the irrigation of gardens and processing of products (CAMPOS, 2014). For irrigation of green areas, parks and municipal gardens, cleaning of courtyards and streets and in sanitary discharge facilities treated sewage can also be used. However, one of the most promising ways of reusing sewage is through application to the soil for the purpose of irrigating agricultural crops.

In addition to providing water, sewage is also rich in nutrients and organic matter, and when conveniently applied to the soil improves its fertility, reflecting in higher crop productivity, as already proven in several scientific works (CAMPOS, 2014).

The application of sewage for irrigation is conditioned to aspects of public health, choice of crops and environmental impacts (ARAÚJO, 2000).

The safety of this type of reuse depends on the reliability of sewage disinfection (CORAUCCI FILHO *et al*., 2003). Thus, minimum microbiological quality requirements for sewage were established by the World Health Organization (WHO) in 1989, based on the presence of Helminth eggs and fecal coliforms (CAMPOS, 2014).

2.2 STABILIZATION PONDS – CONCEPTS

The application of stabilization ponds has grown as a treatment of effluent sewage from anaerobic processes. But, currently, ponds that treat raw sewage (with or without preliminary treatment) are still preserved and installed (PERÍGOLO, 2004).

As mentioned, the term facultative refers to a mixture of aerobic and anaerobic conditions. In the upper layer of the inner volume of the pond is the aerobic medium, and in the lower layer, the anaerobic.

Most of the oxygen required to keep the top layer in aerobic conditions comes from the photosynthetic activity of algae. The other part, considered negligible, comes from the reaeration resulting from contact with air and wind on the surface of the lagoon (VON SPERLING, 1996).

The growth of algae in the ponds is favored by the nutrient-rich environment and exposure to sunlight, the main factors of their metabolism. In fact, due to the large concentration of algae, the stabilization ponds have a greenish color.

According to Perígolo (2004), in the aerobic zone, microorganisms use the oxygen produced by the algae through photosynthesis, and the algae in turn use the CO2, resulting from the respiration of these microorganisms, to perform photosynthesis. This characterizes a process of symbiosis between algae and bacteria.

According to Perígolo (2004) the organic material to be degraded is basically formed by particles of smaller size, which tend not to sediment.

Algae also use other products resulting from the metabolism of microorganisms, such as ammonium (NH4+) and phosphate (PO4-3) to perform photosynthesis. There is also a gas exchange between oxygen (O2) and carbon dioxide (CO2) present in the lagoon with the gases present in the atmosphere.

For Perígolo (2004) the transition position from the aerobic to the anaerobic layer (oxypause) oscillates according to the production/consumption of oxygen, which varies between night and day, morning and afternoon, cloudy weather and radiant sun. The region characterized by intermittency in the presence of oxygen is called the facultative zone, where microorganisms called facultative survive, because they adapt to both the presence and absence of oxygen.

According to Von Sperling (1996) for the degradation of organic matter, these organisms use oxygen or nitrates ($NO3^-$) (when under anaerobic conditions) as electron acceptors.

The in-depth studies of Von Sperling (1996) lead to conclusions that in the anaerobic zone, microorganisms are adapted to survive in the absence of oxygen. For the degradation of organic matter, they use sulfates (SO4-2) and CO2 as electron acceptors. This zone is mainly composed of bottom sludge, which is formed by the sedimentation of particulate matter in the process of deposition by gravitational action.

The material that forms the bottom sludge, anaeroically degraded, slowly converts into carbon dioxide (CO2), hydrogen sulfide gas (H2S), water (H2O), methane gas (CH4) and others.

The process of gas conversion by microbial action in the sludge leaves only the mineralized inert material (non-biodegradable) at the bottom.

The gases resulting from the degradation reactions tend to rise, and can be absorbed into the liquid mass or release into the atmosphere. H2S gas that has an unpleasant odor, similar to the "rotten egg" smell, when passing through the upper aerobic layer, is oxidized by chemical and biochemical processes, and so does not cause bad smell problems.

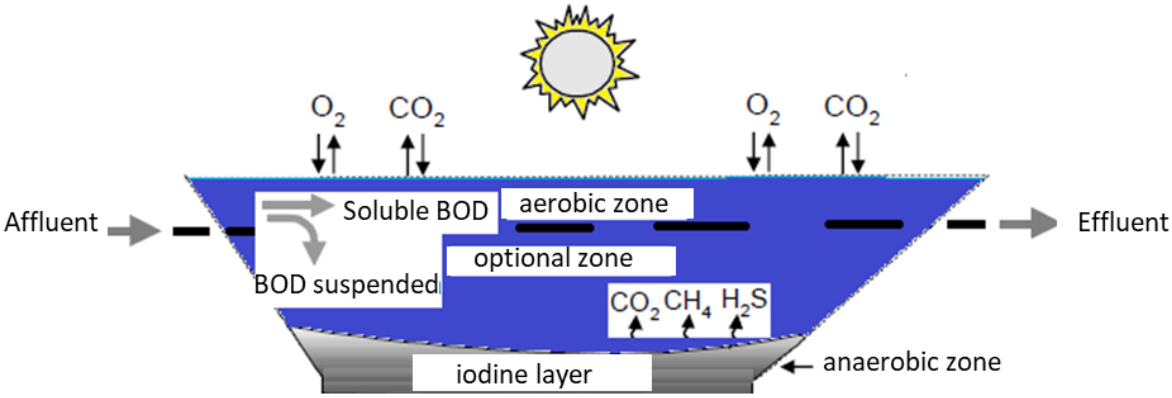
Thus, based on the conclusions of Von Sperling (1996), the stabilization pond degrades sewage into three zones: aerobic, facultative, and anaerobic.

The dissolved organic matter (soluble) and the suspended matter of small dimensions (finely particulate) is dispersed in the sewage, being oxidized aerobically in the most superficial layer and by facultative organisms in the intermediate layer (PERÍGOLO, 2004).

Particulate organic matter, on the other hand, tends to sediment, forming the bottom sludge, which degrades anaerobically (PERÍGOLO, 2004).

The treatment process of the stabilization ponds can be seen in Figure 1, below.

Figure 1 - Simplified schematic of a stabilization pond (VON SPERLING, 1996)



According to Perígolo (2004) the factors that interfere in the treatment process of stabilization ponds can be divided into external and internal.

The external factors and their influence on the treatment are presented in table 1, below, based on the notes raised by Perígolo (2004).

Table 1 - External environmental factors (PERÍGOLO, 2004).

Factor	**Influence**
Solar radiation	- Speed of photosynthesis
Temperature	- Speed of photosynthesis - Bacterial decomposition rate - Solubility and gas transfer - Mixing conditions
Wind	- Mixing conditions - Atmospheric reaeration (*****)

(*****) mechanism of minor importance in the balance of Dissolved Oxygen (DO)

The internal factors interfering in the process of treatment of domestic sewage by stabilization ponds are the algae (photosynthesis) and the applied organic load (PERÍGOLO, 2004).

If the organic load applied to the pond is appropriate, we would have only the algae as an internal factor of influence in the treatment. Since external and internal factors interfere in the efficiency of treatment, they should be analyzed together (PERÍGOLO, 2004).

2.3 ACTION OF FECAL COLIFORMS IN WASTEWATER

According to Bitton (2005) and Cabral (2010) fecal coliforms or also called thermotolerant are exclusive bacteria of fecal origin, among them: *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Proteus*.

Fecal coliforms are Gram-negative bacteria, in the form of bacilli, oxidase negative, which can ferment lactose at 44.5°C and form colonies when exposed to Agar medium which is a gelatinous medium used to form granules of bacterial colonies (BITTON, 2005; CABRAL, 2010).

According to Buma (2017) among the microorganisms belonging to the *Enterobacteriaceae family*, E. coli is the most reliable indicator in the identification of contamination of human and animal fecal origin because it is the only one of the coliform group that is exclusively of fecal origin. Unlike the species belonging to the genera *Klebsiella*, *Enterobacter* and *Citobacter* which are not of exclusively fecal origin. However, they can be easily isolated in soil, nutrient-rich environmental waters, plants, decaying organic matter and other environmental matrices. Thus, the presence of thermotolerant coliforms in environmental waters does not categorically mean that it came from faecal origin.

E. coli is characterized by laboratory techniques by presenting an expression of the enzyme ß-glucuronidase when exposed in media of specific substrates, produces indole from the amino acid tryptophan, being the only species of the thermotolerant coliform group whose exclusive and primary habitat is the intestine of mammals and birds (CERQUEIRA *et al*., 1999; CABRAL, 2010; COSTA *et al*., 2011).

Thus, thermotolerant E.coli is the enterobacterium used worldwide as the most accurate indicator of fecal contamination in ambient waters and, therefore, is present in water quality analysis standards (BUMA, 2017).

3 METHODOLOGY

3.1 CASE STUDY

The study was conducted in the municipality of Itirapuã in a stabilization pond system used for water treatment of the Capanema stream, during the period from January 2, 2023 to March 2, 2023, with sampling before (tributary) and after (effluent) of the lagoon system. The collections during the period were weekly, totaling 18 samples in the nine weeks of the study.

The system operates with the help of the Basic Sanitation Company of the State of São Paulo (SABESP).

Figure 2 shows the location of the region of scientific interest.

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Figure 2 – Municipality of Itirapuã with the treatment system. Source: Google maps.

The system adopted receives the effluent from the Ribeirão Capanema, classified according to the norm of CONAMA (National Council of Environment) no 357 – for class of rivers, as class 4.

The treatment system has a maximum operational capacity for flows of up to 1,149.12 cubic meters per day, consisting of three chambers that form the stabilization pond system (source: PREFEITURA MUNICIPAL de Itirapuã 2011).

Figure 3 shows a photo of the sewage treatment set. For a better use of the surface area donated by the municipality of Itirapuã, the assembled set has a triangular profile if you observe the peripheral part of the site.

Figure 3 – Stabilization Lagoon System (ALBANO, 2014)

The system works by gravitational runoff and each reservoir assists in the purification of sewage.

The set consists of: anaerobic pond that assists in the anoxic process with elimination of aerobic bacteria and then two stabilization ponds called – primary and secondary.

For the set, a period of sludge stabilization was used, called hydraulic detention time (θ_h) of 12.5 days.

Although the operating system has a maximum flow capacity of 1,149.12 m3.d-1, SABESP in conjunction with the city of Itirapuã recommends the use of 846.72 m $^{3.d-1}$, that is, 73.68% of its capacity to avoid risks of overloading the system.

3.2 SYSTEM CHARACTERIZATION

The characteristics of the stabilization pond system complex are observed from table 2 (below), provided by the Itirapuã prefecture.

Table 2 – Characterization of the Stabilization Lagoon complex

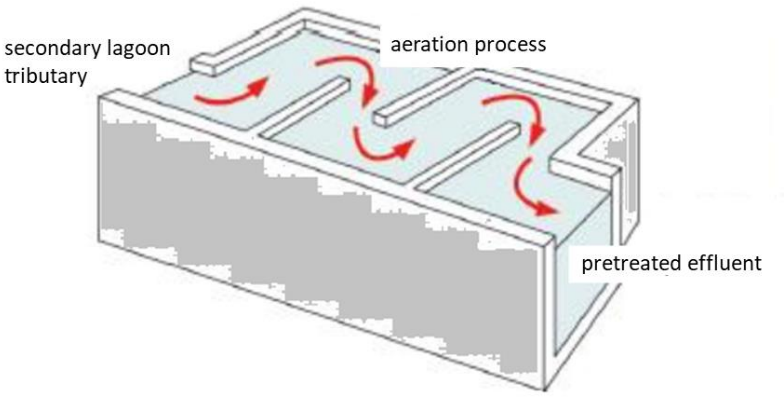
	Anaerobic Lagoon	**Primary stabilization pond**	**Secondary stabilization pond**
Surface area (A)	3.000 m2	2.560 m2	3.160 m2
Effective volume (V)	5.580 m3	3.830 m3	4.960 m3
Depth (h)	2,80 m	1,80 m	1,80 m
Hydraulic detention time (θ_h)	4,9 d	3,3 d	4,3 d
Design flow rate (Q_p)	1.149,12 m3.d^{-1}	1.149,12 m3.d^{-1}	1.149,12 m3.d^{-1}
Demand flow (Q_d)	846.72 m3.d-1	846.72 m3.d-1	846.72 m3.d-1

Source: Sabesp data (2011)

After the sewage passes through the second stabilization pond, the depollution process proceeds from an aeration tank, called a square profile contact tank and volume 28.73 m3.

The contact tank is compartmentalized and inside it is divided by horizontal chicanes, as shown in Figure 4.

Figure 4 – Schematic of the contact tank adopted



Having the volume of the contact tank (V_{tc}) and the demand flow (Q_d), through the hydraulic equation observed by researchers such as PORTO (1999) it is possible to determine hydraulic detention time (θ_h), presented from equation 1:

$$\theta_h = \frac{V_{tc}}{Q_d}$$

Equation 1 (PORTO, 1999)

With the help of the information of V_{tc} and Q d introduced in equation 1, the hydraulic detention time calculated was 0.034 d, that is, a value of approximately 49 minutes, from the conversion of time by the process of dimensional analysis.

3.3 METHOD – COLILERT PACK FOR DETECTION OF FECAL COLIFORMS

The Colilert method consists of the quantification of the total and fecal coliforms present in a given sample, through the mixture between the sample and the patented Colilert reagent, with subsequent transfer of the solution to a sterile carton (100 ml), which is sealed and kept incubated at 35±2ºC for 24 hours (1st reading) and 48 hours (2nd reading-confirmation).

The results are obtained by the relationship of positive values between the largest and smallest squares of the card, with those verified in the standard table for the Colilert test.

For the application of the method were used:

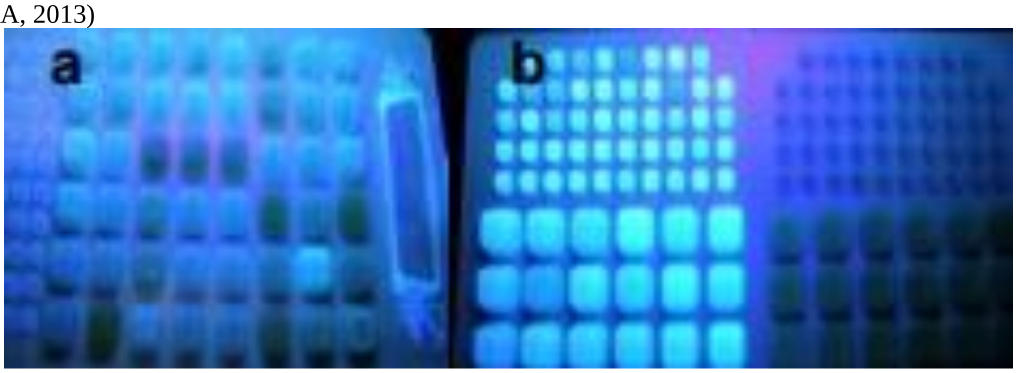
- Sealer for Colilert cards;
- Vertical autoclave;
- Darkroom equipped with UV radiation;
- Thermo-adjustable incubator (35±2ºC);
- Flat-bottomed balloon (sterilized*); and
- Cylinder 100 ml.

The time of use in autoclave was 15 minutes, where the bottles were totally sealed with tampons (prepared with gases), aluminum foil and kraft paper (double layer).

During the fecal coliform analysis procedure by the Colilert method, 50 ml of wastewater sample were used, which went through the following steps:

- The sample volume was transferred in a 100 ml sterile flat-bottomed volumetric flask for the intended dilution so that the final volume was 100 ml;
- In each sample, a pack of the Colilert reagent was added and stirred until complete dissolution;
- The final 100 ml were transferred to a sterile Colilert pack, placed on the sealer holder and sealed it;
- The pack was kept in a thermo-adjustable incubator at 35±2ºC;
- After 24 hours in the incubator, positive values were recorded in the large (49 spaces) and small (48 spaces) squares. The positive values were those in which a strong yellow coloration developed;
- The same procedure was performed by observing the cards in a darkroom equipped with UV light, so that, for this case, the large and small squares to be noted were those that developed a characteristic blue luminescence (figure 4);
- The values recorded were recorded; and the procedure was repeated after 48 hours to confirm the results;

Figure 4 – Characteristic colonies of E. coli in 4a and 4b difference of positive and negative wells of E. coli. (OLIVEIRA, 2013)



3.4 CALCULATION OF THE MOST LIKELY NUMBER

The results are obtained from the standard chart of the method that correlates the values observed in the large squares with those observed in the small squares. For example, in an analysis that used 50 ml of sample were observed 10 large positive squares with 15 small positive squares for total coliforms and 5 large positive squares with 3 small positive squares for fecal coliforms (OLIVEIRA, 2013).

4 RESULTS

The values obtained by the Colilert method were analyzed in the decimal base logarithmic relationship with the number of microorganisms (N/g) detected for the sample collection period that was from January 2, 2023 to March 2, 2023.

Table 3 presents the values obtained by the procedure for the nine weeks of study of samples of inlet (tributary) and outlet (effluent) of the stabilization pond.

Table 3 – Results by the Colilert method (log N/g) for the lagoon samples

Date	**Entrance**	**Output**
02/01/23	3,47	2,80
09/01/23	3,42	2,82
16/01/23	3,40	2,79
23/01/23	3,42	2,81
30/01/23	3,29	2,82
06/02/23	3,30	2,80
13/02/23	3,32	2,80
20/02/23	3,31	2,81
27/02/23	3,30	2,79
02/03/23	3,33	2,80

Table 3 shows that the stabilization pond system played a relevant role in the removal of fecal coliforms present in wastewater.

This finding is more remarkable if the percentage values of the pond's performance are analyzed.

Table 4 presents the percentage analysis of efficiency that can be noted.

Table 4 – Percentage of fecal coliform removal efficiency

Data	Efficiency (%)
02/01/23	19,31
09/01/23	17,54
16/01/23	17,94
23/01/23	17,84
30/01/23	14,29
06/02/23	15,15
13/02/23	15,66
20/02/23	15,11
27/02/23	15,45
02/03/23	15,92
MEDIA	16,42

Table 4 shows that the average performance for the nine weeks of study obtained an average of 16.42% of fecal coliform removal from wastewater by the treatment of the stabilization pond.

The highest efficiency was found on January 2, 2023, which was 19.31%.

For January 30, the performance was 14.29% of fecal coliform removal, such variability is due to climatic factors that also interfere in the purification process such as temperature, wind and relative humidity.

5 CONCLUSION

The performance noted for the pre-treatment of sewage by the stabilization pond system had a significant importance for the process of purification and removal of fecal coliforms present in the Capanema stream.

The levels of fecal coliform removal allow us to estimate that the water quality of the stream would obtain an improvement even in its classification for water bodies, stabilized by the CONAMA standard 357.

The improvement of water quality for the presence of fecal coliforms makes pre-treatment important since it allows eliminating a pathogen of great relevance in the proliferation of diseases and water pollution.

The continuous study of sewage treatment methods and technologies is important and should be encouraged by public agents and society in general. A future study of the effluent treatment relationships and their interrelationship with the climatic aspects linked to the site should be taken as a suggestion.

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