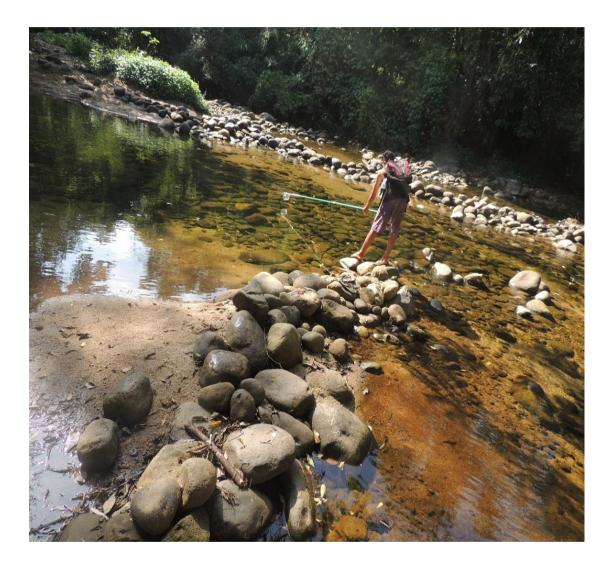
# DIAGNOSIS OF THE ENVIRONMENTAL CONDITIONS OF RIVERS AND RIACHES

Script for field class and laboratory class



Elias Gonçalves Dorino Denise Celeste Godoy de Andrade Rodrigues Rosana Aparecida Ravaglia Soares Elias Gonçalves Dorino Denise Celeste Godoy de Andrade Rodrigues Rosana Aparecida Ravaglia Soares

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Volta Redonda 2019

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### 1. INTRODUCTION

Considering that in primary schools there are few perspectives of field classes or laboratories with a focus on environmental education focused on water resources preservation, the present work seeks to insert students in the problems related to this theme.

The lack of courses and training that provides a better preparation of education professionals, opens a great opportunity for the development of works that present conditions of improvement, to propose educational solutions with a focus on environmental education, especially with a view to preserving water resources. increasingly scarce.

Evaluate and diagnose the ecological conditions of certain aquatic environments, identifying problems and presenting proposals and solutions that minimize damage to the environment.

The approach needs to be adapted for problems related to the nature of the professions. The basic idea remains the same: confront the student with situations close to those he will find at work and build knowledge from these situations, which emphasize the relevance and lack of some resources (PERRENOUD, 2002).

Thus, it is hoped that by confronting students with the problems caused to water resources near their homes, they will be able to give students better conditions to minimize the environmental damage suffered by the rivers in the region.

#### 2. LESSON PLANNING

#### 2.1. EDUCATIONAL GOALS.

The main goal of monitoring river quality is to analyze the health of the river, and to register the increase of anthropogenic influences in the environment. So that students can assimilate and understand their importance, involving them is a priority condition so that in the future, they can intervene and mitigate the harmful effects caused by the environmental degradation of the fountainheads of the places where they live.

# 2.2. FIELD CLASSES PLANNING.

Prioritize the safety of students when executing the work, and use the personal protective equipment required, while the collecting samples for that specific water body, and for analyzes that will be performed in the laboratory of the school. The teacher mustn't lose the main focus that is the students' safety.

The teacher should guide and do previous training with the students who will collect the samples of water in the river. The notions of sampling to perform water analysis are to avoid possible contamination of the samples and of the students during the task. Choosing the place where the sampling will be performed, having the proper planning that involves obtaining preliminary information about the area of influence of the body of water to be sampled, of the studies that have already been done on the site, which can contribute with information about the studied area.

Making a sketch with the location of possible sample collection points is very important, and for this to happen the teacher should provide a map of the region with the sampling sites already identified.

For the development of this work, 7 sampling points were defined, taking as a premise that the sites were easily accessible and safe along the entire way of the river for the entire fieldwork team. Keeping the data of the geographical coordinates of the sampling points, mentioning any characteristics that would be relevant to the work.

#### 2.3. TRANSPORTATION AND SAFETY

Within the various aspects that can make the implementation of field classes harder, the ones that draw my attention, are the planning, especially those related to transportation. It is very difficult to raise financial resources to hire transportation for students these days. As in the case of this work, the Perequê and Mambucaba rivers pass nearly the school. It was suggested by the students themselves to reduce the number of members in the field research group, limited to who wanted and had the availability to ride a bicycle. Another relevant point in the choice of sampling sites was the safe access to the riverside and without the risk or possibility of falling students in the river.

Another important point was to inform the direction of the school and obtain permission to perform field activities such as students. To ask them for logistical support such as water, snacks, first aid kit and supplement with school board guidelines.

Integrating the field class with the classroom. In this case the integration will be made with the laboratory classes. Students should be informed about how the field activity will be developed, both in the collection of samples and in the visual analysis to complete the rapid assessment protocol in the field.

Review the safety of all staff. Check for transportation (bicycle), footwear, clothing, rubber gloves, water trap (with extended shaft measuring 2.85 m), to prevent students from getting too close to the water (FIGURE 1).

Establish the best form of group communication. Create for example a group on WhatsApp with named Fieldwork, in which all students willing to participate in the field activity are entered and informed about the meeting place and time for the activity. Any unforeseen events must be reported by the group created.

#### 2.4. IMPORTANT NOTES

Take into account the characteristics of the students and the school level, being considered three highly relevant moments: the preparation, the accomplishment and the result / evaluation of the field class.

The first step, the teacher should visit the study area to analyze the georeferencing of the collection sites, through GPS (Global Position System), and there can use an app from the phone itself. Fieldwork should never be done without the teacher having previously made a data collecting of the place explored, what means that, he / she should have a complete knowledge of the work to be coordinated.

Verify the access roads, as well as their status and the time required to perform the work.

To problematize the field classes, proposing problematic situations so that the students can solve them or suggest a solution, this way they won't be dispersed. If this is not possible, guide students who need to see or pay attention. In field class the students can't be sparse, each one of them needs to be focused.

Encourage the conscious use of natural resources, enquire them about the results of the analyzes that should be performed at the water sampling sites, such as DO (dissolved oxygen), turbidity, PH and temperatures, which may present divergence in results among students.

Distribute the activities to each student group member according to the class schedule. The person responsible for photographing, taking notes, collecting the waste generated, which will handle the sample collector, the person responsible for identifying and transporting the sample vials, which will measure temperatures, turbidity, analysis of the dissolved oxygen and PH. The teacher will be the drone operator and the coordinator of the entire work team.

#### 2.5. LABORATORY CLASSES PLANNING

Accidents of any kind should be reported to the teacher. The tools used, especially those which are made of glass, should be perfect, without edges or cracked borders or ruptures.

While staying in the laboratory, avoid touching your mouth, eyes, or nose. When leaving, wash your hands. Never experience substances or vacuum gases or vapors without ensuring that they are not toxic. Do not heat substances in closed containers.

Carefully close gas taps in order to prevent leaks. To heat a test tube with liquid, contact only the side of the tube, not the bottom, with fire. When handling with test tube, never turn the top towards a person.

If acid or any other chemical spills, immediately wash the area with plenty of water. Do not leave hot pieces of glass (or other material) where anyone can pick them up. Do not place hot glass on cold surfaces, as thermal shock may break it.

Before using a reagent, carefully read the vial label. Always hold a reagent bottle with the label facing the palm of your hand. Work with method, attention and calmness. Before performing a lab exercise, carefully read the procedures provided in the book and the teacher's guidance.

#### 3. WATER SAMPLE COLLECTION AND PRESERVATION

Specimen collection and preservation is a fundamental part of the analytical process of measuring physicochemical, biological and microbiological parameters. This step consists of removing a representative portion of the study site, in other words, a photograph and time. Collecting 1000 ml of river water usually represents 0.004% of its volume in just one second (TOMASI, 2018).

Specimen collection is probably the most important step for the study area assessment and, as such as it, sampling should be performed with a lot of care and technique to avoid possible contamination and loss and also, to represent the sampled body of water. The technique to be used for sampling depends on the matrix to be sampled (surface water, deep water, groundwater, treated water, wastewater, sediment, aquatic biota, among others), the type of sampling (single, composite or integrated sample) and, of the requested tests (physicochemical, microbiological, biological and taxological tests).

Simple samples (punctual or instantaneous) are those taken from a single sample, at a certain time, to the realization of determination and testing. The total sample volume will depend on the parameters chosen. It is indicated for cases where the flow and composition of the liquid (water or effluent) do not present significant variations. It is a mandatory for parameters in which characteristics change rapidly or do not allow the transfer of recipient (sulfides, dissolved oxygen, halogenated solvents, oils and greases, microbiological).

Composite sample consists in a serie of simple samples, collected within a period of time and mixed to form a single homogenized sample. Sampling in surface waters such as rivers, ponds and seas is generally performed with the help of a vessel. Thus, it is possible to collect the sample at the indicated location through geographical coordinates. This sampling is performed on the surface and also at varying depths. Or using the Van Dorn bottle that allows the sample to be collected while preserving the movement and natural flow of the current (TOMASI, 2018). This procedure is adopted to enable the reduction of the amount of samples to be analyzed, especially when there

is a great variation of flow and / or liquid composition. It is especially recommended when the tests are far away so as not to exceed the sample shelf life.

Integrated sampling is the one made with samplers that allow simultaneous or in a period of time as close as possible of the aliquots to be collected in a single sample. For better representativeness of the sampled site, sampling with replicas (duplicates or triplicate) can also be performed when the sample is sequentially and independently collected over a given period of time or space (ANA, 2011).

Quality control in the sample is considered the weakest point of the process and needs special care. The concern has to be increased in order not to compromise all the study. Preservation techniques, proper selection of the recipient and the form of storage aim to retard biological action and alteration of chemical compounds; reduce volatility or precipitation of constituents and adsorption effects; and / or preserving organisms, avoiding or minimizing morphological, physiological and population density changes, at all stages of sampling collection, packaging, transport, storage, until the time of testing (ANA, 2011).

For specimen storage, the specimen bottle should be kept in a cold environment. In this study the vials were placed in a Styrofoam box with ice. Freezing is an acceptable technique for some tests and serves to increase the interval between collection and assay of the fresh sample without compromising the latest (ANA, 2011).

So we used a 500 ml mineral water bottle to ensure that these bottles are clean without contamination. The empty vials came from my own personal use, as long as they were emptied, they were kept in the refrigerator until we reached the necessary amount to start the fieldwork.

To collect the water samples, it was developed from a 1000 ml PET bottle, a collector approximately 400 ml, with a retractable rod of 2.80 m (cable used for painting) (FIGURES 1 and 2). Thus the Students may be at a safe distance from the river borders, minimizing the risk of falling.



Figure 1 - Water sample collector.

Figure 2 - Collector with retractable arm



The following precautions should be taken:

Check the cleanliness of the bottles and other materials and equipment that will be used. To do this field experiment 500 ml plastic bottles of mineral water were used. The inner parts of the bottles mustn't be touched by the hand or exposed to dust, smoke and other impurities.

Always set the collection equipment with the local water. Ensure that liquid samples do not contain large particles, debris, leaves or other accidental material during collection.

Collect an amount of sample enough to avoid any need to repeat the test. Place samples in the shadow immediately after collection and preservation.

To package in ice-cold boxes the samples that require refrigeration for their preservation.

Note: samples for DO (dissolved oxygen) analysis should not be kept refrigerated, this assay should be done at its own sampling site.

#### 4. MATERIAL RESOURCES

The management of material resources contributes to a satisfactory result of the research. In order to realize this study, the items mentioned below were used.

GPS (Mobile App - My GPS Coordinates Pro), Camera, DRONE (dji MAVIC PRO), Thermometer (0 to 100 ° C), ice for specimen conservation. For packing the samples we used a Styrofoam box, a bottle to collect water (500 ml mineral water), noncarbonated mineral water washer flask to wash the analysis vials and a 70% ethyl alcohol washer flask to wash the analysis vials. We manipulated a water collector (2.80 m), rubber gloves, clipboard, A4 sheet of paper for notes and pens. Toilet paper to dry materials during sampling, 70% alcohol for cleaning hands and plastic buckets, trash bags, batteries or batteries for electronic equipment.

The environmental education kit used for the development of this work was acquired the environmental education kit of the company ALFAKIT, for its low cost and because it is very easy to understand its analytical method.

It is important to point the need to carry a map with the identification of the sample water sites water and also to bring a snack for all participants.

#### 5 HOW TO EVALUATE A FIELD CLASS

Field classes are a great tool to schools. It is precisely through these methodologies that students learn to harmonize theory and practice. Classes in parks, squares, gardens, zoos stimulate students' creativity, imagination and understanding. The educator should expand the perception of the environment under study, giving positive and negative examples of the impacts that preservation (or lack of it) can have on the environment and affect living beings. He should also talk about the results which affects the environment and that come from anthropic actions. Moreover, it's necessary to understand the consequences of what can influence ecosystems, mainly, what has been practiced in the past.

The teacher should propose during the field classes problematization of the environmental conditions found for students to discuss and identify the main causes that may have caused the environmental damage. Also ask students to present solutions that can minimize the observed damage and generated condition.

The solutions they present, served as a way of evaluation, or if the teacher does not propose a problem, the simplest way to test the acquired knowledge is to apply an activity or questionnaire on the subject studied and the contents of the subject. Another way is to apply an assessment which can be the exposure, impressions and conclusions that students have had or a discussion wheel as in the form of a report.

The teacher should pay attention to his students, and take actions that keep them all highly motivated, challenging them in a constructive and positive way.

#### 5.1. CARE TO BE OBSERVED DURING CLASS

Wear long trousers, closed shoes and rubber gloves for field class activities, prepare the Styrofoam ice box to transport and storage of samples. Bring the 500 ml plastic bottles (mineral water) in a number compatible with the amount of samples to be collected. The collector sampler with retractable cable should be well washed previously with mineral water and then with 70% ethyl alcohol. Before taking the samples, realize the adaptation, first of the sample collector, after of the bottles where the samples will be stored.

The procedures for cleaning the collection and storage containers should be done with mineral water and then with 70% ethyl alcohol, as well as the test tubes for analysis.

The analysis of temperature, turbidity, and dissolved oxygen should be done at the sampling sites and always in an airy place. The other analyzes will be performed in the laboratory of the school.

Reagent containers, vials as well as test tubes and passages, which are part of the environmental education kit, should be washed with mineral water, followed by 70% ethyl alcohol. Dry well with toilet paper to avoid moisture and compromise further reagent removal from their respective bottles.

It is necessary to avoid contact of the reagents with skin. If this happens, wash the affected area with plenty of mineral water. In case of eye contact or reagent ingestion, look for medical advice immediately. Store the waste and neutralize it with a baking soda solution prepared with 3 tbsp of baking soda in 300 ml of water, before discarding or send to a specialist company. Avoid exposing reagents to sunlight.

#### 6. PARAMETERS TO BE ANALYZED

The first analysis to be performed should always be of dissolved oxygen, and this sample cannot be filtered and must be done at the sampling site itself. Collection will be performed with the syringe and should be slowly aspirated to prevent bubbles. Thus ensure that there is no influence of oxygen concentration in the sample by aeration during sampling.

Place the sampling syringe hose float (FIGURE 3) to the desired collection depth. Depending on the depth of the river to be sampled, put a weight of 20 g (fishing bait) at the top of the small hose. Pull the syringe plunger until it locks. Initially transfer to the identified test tube the sample for analysis of dissolved oxygen avoiding bubbles.



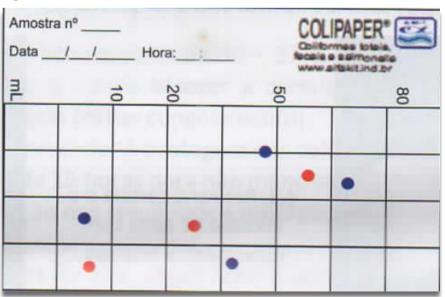
Figure 3 - Water sampling syringe for dissolved oxygen analysis.

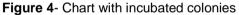
Between the samples, wash the test tube with clean water (mineral water) and then with 70% ethyl alcohol. Dissolved oxygen has the analysis process with measuring capacity from 0.5 to 9.0 mg/l, ranging from 0.5; 1.0; 3.0; 5.0; 6.0; 7.0; 8.0 and 9.0 mg/l of the possible measurement values.

The temperature of water and air will be measured by using an alcohol thermometer from 0 to 100 °C, which minimum graduation is 1°C. The acidity or basicity of the water will be determined by reagents that identify the pH (hydrogen potential) when changing color, ranging from 0 to 7 (acidic pH), from 7 to 14 (basic pH) and neutral. PH 7. Orthophosphate has its measurement values from 0.0 to 3.0 mg/l, ranging from 0.0; 0.75; 1.0; 1.5; 1.75; 2.0; 2.5 and 3.0 mg/l. This methodology is for low  $PO4^{-3}$  concentration.

In nitrate analysis, the card has a measuring capacity of 0.10 to 2.5 mg/l, ranging from 0.10; 0.30; 0.50; 0.70; 1.0; 2.50 mg/l. For nitrite analysis, the chart has a measuring capacity of 0.01 to 0.50 mg/l: 0.01; 0.03; 0.05; 0.10; 0.20; 0.30; 0.50 mg/l. For the analysis of ammonia, the card has a measuring capacity of 0,0 to 3,0 mg/l: 0,0; 0.10; 0.25; 0.50; 1.0; 2.0; 3.0 mg/l. The determination of turbidity has in the rod scale the measuring capacity from 20 to 100 NTU: 20; 25; 30; 40; 50; 60; 80; 100; 150; 200; 300; 500; 700; 1ML; 2ML and 4ML.

Microbiological analysis of total and fecal coliforms depends on the amount of colonies formed and the dilution of the sample, and on the colony formation capacity that will be identified on the gel pack (FIGURE 4).





Source: Instruction manual of ALFAKIT, 2018

#### 6.1. PHYSICAL CHEMICAL ANALYSIS

To ensure better reliability in visual comparisons with color charts, some care should be taken when performing physicochemical analysis using the ALFAKIT environmental education kit. Place the test tube in the middle of the pack in an inclined position approximately 45 °. The comparison should not be done in sunlight, but the place should have good lighting. Strictly follow the reaction time. Variations in temperature may influence the reaction time.

#### 6.1.1. Turbidity Analysis - (using the turbidity minidisk)

With a 1000 ml plastic beaker. Fill it with the water sample in order to analyze the turbidity. Read the gauge when the rod base is within the limit of identifying the black and white of the turbidimeter rod base (FIGURE 5). Hold the top of the metal rod, as shown in (FIGURE 6), in a position where the student can see the turbidity scale numbers (transparency on the NTU scale). The mathematical unit used to measure turbidity is NTU, which comes from the English Nephelometric Turbidity Unit. The minidisk measures approximately 35 cm, ranging in scale from 20 to 4000 NTU. So in this minidisk the smallest detectable value is 20 NTU. This type of test is currently performed on sophisticated and accurate equipment with the lowest detection level of 0.01 NTU. Students should be trained in turbidity reading procedures using the turbidimeter.



Figure 5.a - Turbidity meter read until you cannot distinguish black from white.

Figure 5.b - Turbidity reading at water level, directly on the of marks.



#### 6.1.2. Ammonia analysis

Measure in the 10 ml beaker, 5 ml of the sample, and transfer to the test tube. Perform the beaker with the sample. The beaker must be well washed with distilled water and, in the lack of this, wash with mineral water and then 70% ethyl alcohol. Washing with 70% ethyl alcohol is not a primary condition, but it does increase the assurance of asepsis, especially when performing multiple assays with the same test container.

Keep hands and bench cleaned to avoid sample contamination during analysis. Add 03 drops of reagent 1, shake carefully so as not to breathe this mixture on the student or another person. Hold the vial in one hand, move and tap the palm of the other open hand without splashing the specimen. Add 03 drops of reagent 2, shake carefully so as not to breathe this mixture. Repeat the previous homogenization process.

Add 03 drops of reagent 3. Repeat the same homogenization process as above. Wait 10 minutes. Position the test tube slightly inclined and make the color comparisons of the test tube with those of the chart (FIGURE 6).

#### 6.1.2.1. Result Calculations

Mg/I N-NH3 = Result read from chart. To express the result in NH3, multiply the value read by 1.214. MW (molecular weight) of NH3 equal to the sum of the atomic weights of N -14 and the atomic weight of H - 1 x 3 amount of H in the formula NH3. Thus PM = 17. The relationship between NH3 and N is 14 and 17:14 = 1.214.

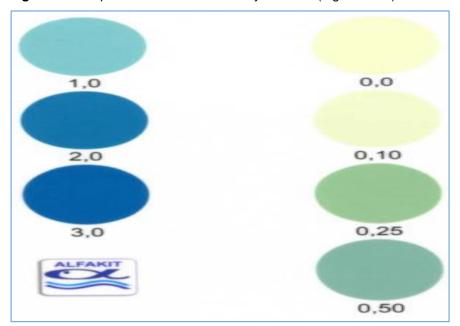


Figure 6 - Interpretation of ammonia analysis result (mg/I N-NH3)

Source: Instruction manual of ALFAKIT, 2018

## 6.1.3. Dissolved Oxygen (DO) Analysis

Measure in the 10 ml beaker 5 ml of the sample spilling into the vessel walls without generating bubbles and transfer to the test tube. Perform the beaker with the sample. The beaker must be thoroughly washed with mineral water and 70% ethyl alcohol beforehand.

Washing with 70% ethyl alcohol is not a primary condition, but it does increase the assurance of asepsis, especially when performing multiple assays with the same test container. Keep hands and bench clean and avoid sample contamination during analysis.

Add 1 drop of Reagent 1, avoiding bubbles, and shake gently so that you do not breathe the mixture on the student or his / her classmates. Hold the vial in one hand and move, and tapping the palm of the other open hand without splashing sample. Add 2 drops of reagent 2, repeat the stirring process carefully. Add 01 level measure (slurry # 02) of reagent 3 and shake carefully to homogenize so that the mixture does not breathe. Wait 5 minutes. Position the test tube slightly inclined, and make the color comparisons of the test tube with those on the chart (FIGURE 7).

## 6.1.3.1 Calculations of Results

Mg/I O2 = Result read from chart as in (FIGURE 8). Analyze the dissolved oxygen at the time of sample collection or add Reagent 1 at the time of collection if it is to be analyzed later in the laboratory.

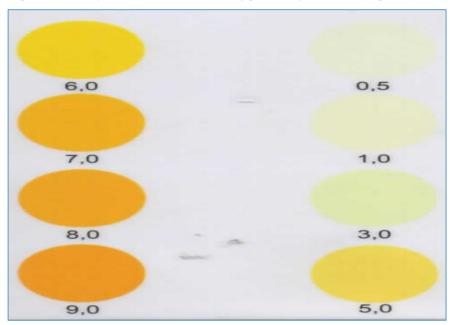


Figure 7 - Interpretation of dissolved oxygen analysis result (mg/I O2)

Source: Instruction manual of ALFAKIT, 2018.

# 6.1.4. Nitrate Analysis

Measure in the 10 ml beaker 5 ml of the sample by carefully pouring it into the container walls and transfer to the 10 ml test tube. Perform the beaker with the sample. The beaker must be previously washed with mineral water and 70% ethyl alcohol. Washing with 70% ethyl alcohol is not a primary condition, but it does increase the assurance of asepsis especially when performing multiple assays with the same test container.

Keep hands and bench clean to avoid sample contamination during analysis. Add 1 shallow measure of Reagent 1, with # 1 and stir until it dissolves for 2 minutes, carefully so as not to breathe the mixture on the student or his colleagues. Hold the vial in one hand and move by tapping the palm of the other open hand without splashing sample.

Add 01 shallow measure (sliver # 01) of reagent 2 and shake until dissolved carefully to homogenize as described above. Add 02 drops of reagent No. 03 and shake until dissolved, carefully homogenize. Wait for 15 minutes. Position the test tube slightly inclined and make the color comparisons of the test tube with those of the chart (FIGURE 8).

# 6.1.4.1 Calculations of Results

mg/I N-NO3 = result read from chart. If the sample contains nitrite, perform the calculation mg / I N-NO3 = (N-NO3) - (N-NO2), where N-NO3 = Nitrate chart result N-NO2 = Nitrite result.

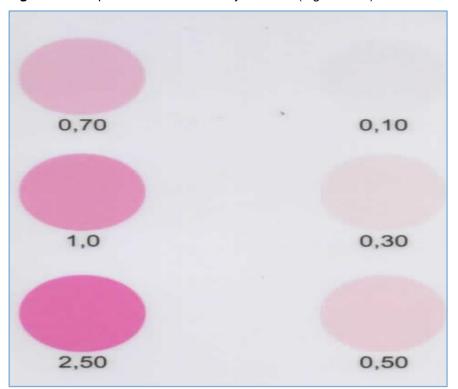


Figure 8 - Interpretation of nitrate analysis result (mg/l N-NO<sub>3</sub>)

Source: Instruction manual of ALFAKIT, 2018

# 6.1.5. Nitrite Analysis

Measure in the 10 ml beaker 5 ml of the sample by carefully pouring it into the container walls and transfer to the 10 ml test tube. Perform the beaker with the sample.

The beaker must be thoroughly washed with mineral water and 70% ethyl alcohol beforehand. Washing with 70% ethyl alcohol is not a primary condition, but it does increase the assurance of asepsis especially when performing multiple assays with the same test container. Maintain clean hands and workbench to avoid contaminating the sample during analysis.

Add 1 shallow measure of Reagent 1, with # 1 and shake gently for 2 minutes until dissolved, so as not to breathe the mixture into the student or his / her peers. Add 01 level measure (sliver # 01) of reagent 2 and shake carefully to homogenize until dissolved. Add 02 drops of reagent No. 03 and shake carefully to homogenize so that the mixture does not spill. Wait 15 minutes.

Position the test tube slightly inclined and make the color comparisons of the test tube with those of the chart.

If the sample contains nitrite, the calculation will be: mg / I N-NO2 = result read from the chart (FIGURE 9).

6.1.5.1. Result Calculations

To express the result in NO2 multiply the value read by the factor 3,280. MW (molecular weight NO2 / N = 46/14 = 3.280.



Figure 9 - Interpretation of nitrite analysis result (mg/I N-NO<sub>2</sub>)

Source: Instruction manual of ALFAKIT, 2018

#### 6.1.6. Orthophosphate Analysis

Measure in the 10 ml beaker 5 ml of the sample by carefully pouring it into the container walls and transfer to the 10 ml test tube. Perform the beaker with the sample. The beaker must be thoroughly washed with mineral water and 70% ethyl alcohol beforehand. Washing with 70% ethyl alcohol is not a primary condition, but it does increase the assurance of asepsis especially when performing multiple assays with the same test container. Maintain clean hands and workbench to avoid contaminating the sample during analysis.

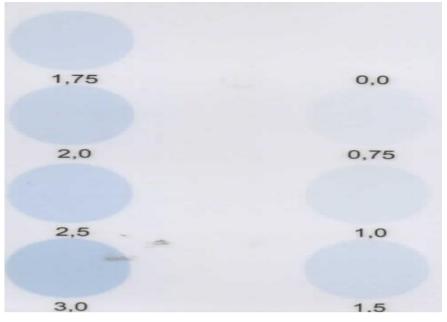
Add 05 drops of Reagent 1, and shake carefully so as not to splash the mixture. Add 01 shallow measure (sieve # 01) of reagent 2.

Wait 10 minutes. Position the test tube slightly inclined and make the color comparisons of the test tube with those of the chart (FIGURE 10).

#### 6.1.6.1 Calculations of Results

To express the result in P2O5, multiply the result read by 1.494, MW (molecular weight) P2O5 / PO4 = 142/95 = 1.494. To express the result in P (phosphorus), multiply the result read by 0,3263, PA (atomic weight) of P / PM (molecular weight of PO4) = P / PO4 = 31/95 = 0,3263.

If the color intensity is greater than can be read on the card, repeat the analysis using 2.5 ml of sample and 2.5 ml of mineral water. Add the reagents according to the technique and multiply the final result by 2 (because the sample was diluted 2 times (instead of taking 5 ml in a test tube was placed 2.5 ml).



**Figure 10** - Interpretation of the result of low concentration orthophosphate analysis (mg/l PO<sub>4</sub>)

Source: Instruction manual of ALFAKIT, 2018

## 6.1.7. pH analysis.

Measure in the 10 ml beaker 5 ml of the sample by carefully pouring it into the container walls and transfer to the 10 ml test tube. Perform the beaker with the sample. The beaker must be thoroughly washed with mineral water and 70% ethyl alcohol beforehand. Washing with 70% ethyl alcohol is not a primary condition, but it does increase the assurance of asepsis especially when performing multiple assays with the same test container. Maintain clean hands and workbench to avoid contaminating the sample during analysis.

Add 1 drop of Reagent 1, and shake carefully so that you do not spill the mixture on yourself or your classmates. Hold the vial in one hand and move and tap the open palm without splashing sample. Position the test tube slightly inclined and make the color comparisons of the test tube with those of the chart (FIGURE 11).

### 6.1.7.1. Result Calculations

To calculate the result, pH = Result read from the chart. Make the comparison in a place with good lighting, but never in the sun.

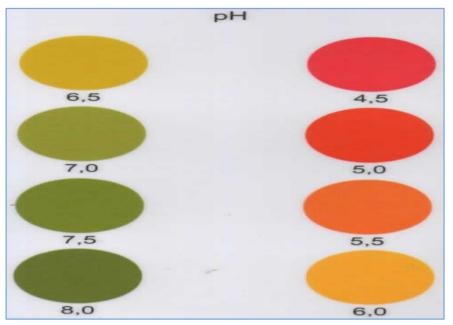


Figure 11 - Interpretation of the result of the analysis of pH

Source: Instruction manual of ALFAKIT, 2018

#### 6.2. MICROBIOLOGICAL ANALYSIS OF E.COLI AND TOTAL COLIFORM

Use the hydrated gel culture medium from the environmental education kit for microbiological analysis to identify the presence of E. coli and total coliforms. This methodology is a traditional method and one of the most used to characterize the microbiological quality of water known as standard plate count (CPP). It is suitable for analysis of water, domestic and industrial effluents, rivers, bathing, ponds, swimming pool, surfaces, vegetables and milk.

In the standard plate count (CPP), an aliquot of water is plated with the culture way and incubated at 36 ° C for 15 hours. Bacteria in water, which are viable, grow to such an extent that they are visible to the naked eye. These are called colonies. With this it is possible to count how many colonies grew as a function of sample volume, the

standard plate count (CPP) expressed in colony forming units per 100 ml of water (CFU /100 ml) is determined.

Wash hands thoroughly before handling the microbiological chart (FIGURE 12) to avoid contamination that could interfere with results. Never touch the test card below the perforation.

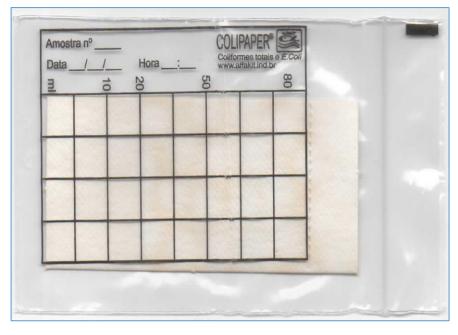
Take the sample vial and place approximately 100 ml of the sample into a 200 ml disposable beaker. Remove the microbiological card by tapping just above the perforation. Immerse the sample pack in the sample to be analyzed until it is perforated in the 200 ml disposable plastic beaker with approximately 100 ml of the sample and allow it to moisten.

Remove the sample card and remove excess water by touching the bottom of the moistened incubation card on a piece of toilet paper to absorb excess water. In this way avoid sudden movements and splashing of water on yourself or others and not contaminating bench or materials.

Replace the cartouche in the plastic packaging and remove the part of the perforation without touching the rest, throw the perforation in the garbage bag. Bring the oven to room temperature for 15 hours at 36-37 °C. For field collection, take some precautions to take the pack to the greenhouse. Keep the pack in a plastic bag in a styrofoam package with little ice (avoid freezing the sample).

After 15 hours of incubation, count the colonies. Consider both sides of the pack. Do not exceed 15:00 hours so as not to stain the incubation card.

Figure 12 - Incubation Chart



Source: Instruction manual of ALFAKIT, 2018

#### 6.2.1. Result Calculations

Multiply the number of colonies by the factor of correction 80, and the result expressed in CFU / 100 ml. Interpretation of the results: *E.Coli*: violet to blue dots. Total coliforms: violet to blue and rosy to red points. Count both sides of the pack.

If the coliform count is high, dilute the sample. If there are too many colonies, squares are used to count (FIGURE 13). *E. Coli*: violet to blue dots and total coliforms: violet to blue and rosy to red dots. Select two or three squares. Count the colonies within it. Calculate the average. Multiply for 6400 to have the result in CFU / 100 ml.

The sample may be diluted using mineral water and a 1 ml disposable syringe and a 50 ml disposable syringe. Measure 50 ml of water in a 20 ml disposable cup, then measure 1 ml of the sample, and transfer to the same cup containing the 50 ml of water. The sample will be diluted 50 times. Homogenize and moisten the incubation card, removing the water excess immediately. Follow the same process for incubation in a greenhouse at 36 to 37 ° C. Multiply the result obtained by 50 times the dilution.

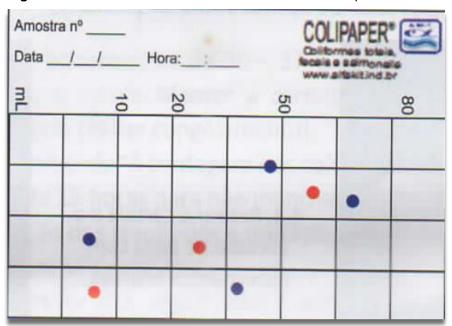


Figure 13 - Cardboard colonies incubated with diluted samples

Source: Instruction manual of ALFAKIT, 2018

Other forms of dilution are also recommended, depending on the results found in the first analysis to improve visualization and counting of incubation cards. The purpose is to avoid highly stained or practically unable to count colonies for a highly contaminated sample, being analyzed by a disproportionate dilution of readability and interpretation. That is, if the dilution is made 50 times (chart without marks and views of coliform colonies).

During the preparation of samples for analysis, the best result was obtained with the samples performing the dilutions 2 times and in 10 times.

Distilled water is very difficult to obtain, I suggest using residential filter water. To ensure the neutrality of this water that will be used to perform the dilution, and subsequently humidify the incubation card, quality control should be provided for comparison purposes.

The white term refers to controls performed to analyze the presence of contamination in specific parts of the collection procedures. Usually it is used deionized or distilled water to serve as white, so as to proof guarantee the relief of the compounds to be analyzed. The deionized or distilled water is not easy to achieve, besides access

is difficult and expensive. Thus we use mineral water for this purpose or even filtered tap water, which passed through the same process of sample analysis. There is the standard governing quality control that governs the rules in sampling the NBR / IEC 17025.

To perform the 2-fold dilution, measure 50 ml of filter water in a 100 ml beaker, place in the 200 ml disposable beaker, and then measure 50 ml of the water sample to be analyzed.

To perform the dilution 10 times - measure 90 ml of filtered water in a 100 ml beaker and place in the 200 ml disposable beaker, then measure 10 ml with a sample pipette and place in the same disposable beaker containing the 90 ml of water.

## 7. PROTOCOL FOR QUICK ASSESSMENT - A VISUAL ANALYSIS RIVER TRECHES

The results of visual analyzes performed on the river stretches should be recorded in the form of (FIGURE 14). Students should be guided so that everything they observe has to be related to the characteristics described in the RAP form, and that relevant remarks should be recorded separately for later discussion and if important may be on the form for future RAP modifications. After completing the form, the sum and scores of the PAR will highlight these characteristics.

In order to begin the evaluations, the teacher must take into account the scores that determined the level of disturbance at the sampling points in each environment, and the least impacted place will serve as a reference for the other points becoming a natural / preserved environment. Scores between 0 - 26 => is considered an impacted environment; Variations between 27 - 45 => altered environment and scores greater than 45 => should be considered a natural environment.

Other environments will always be compared with the reference environment (natural environment). This evaluation will allow students to have a better understanding of the process of environmental degradation by comparing common but highly different environments due to modifications derivated anthropic action.

The scoring quantification system of the most preserved environments will have their values related to the level of environmental disturbance. The reference sampling point will show the disturbance level classified as natural (having higher scores), because it is close to a more preserved forest region.

Students will be able to observe during the walks for diagnosis, the sampling sites in the river, thus becoming familiar with the local environment. The completion of the entire form is a mandatory and then added to obtain the disturbance level.

#### FIGURE 14 - QUICK EVALUATION PROTOCOL - A VISUAL ANALYSIS OF RIVER TRECHES

ENVIRONMENT DESCRIPTION							
Location (sampling point):		Geographic coordinat	Geographic coordinates S:				
			W:				
Date of Collection:							
Collection Time:							
Climate:							
Winds: absent ( ) light ( ) medium ( ) strong ( )							
Rain: Thunderstorms ( ) Cloudy ( ) Partly Cloudy ( ) Rain Showers ( ) Sunny ( )							
Collection Mode (collector) Setting Type: stream ( ) River ( ) - NAME:							
Setting Type: stream ( ) Riv	ver () - NAME:	DUNCTU					
PARAMETERS	PUNCTUATION PARAMETERS 4 points 2 points 0 points		0 points	Result			
1 - Type of occupation of	4 points	Pasture field /	0 points	Occupation			
margins - VISUAL		agriculture /	Residential /	Occupation			
Left	Natural	monoculture /	Commercial / Industri	al L			
Right	vegetation	Reforestation		R			
2 - Near Erosion and / or	Absent			Erosion			
Riverbanks - VISUAL	Absent	accentuate					
Left	]	Moderate		L			
Right				R			
3 - Anthropic Changes	Absent	Changes of domestic	Industrial / urban	Alt. Antrop.			
VISUAL		origin (sewage,	changes (factories,	L			
		garbage)	plumbing)	R			
4- Bed cover - VISUAL	<b>-</b>			Vegetal			
	Total	Partial	Absent	cover			
				R			
5- Oiliness of water	411	Moderate	Abundant	ĸ			
VISUAL	Absent	Moderate	Abundani				
6- Bottom oiliness	Absent	Moderate	Abundant				
7- Water odor	Neither	Sewer (rotten egg)	Oil / Industrial				
8 - Water Transparency	Transparent	Blurred strong tea	Opaque or colored	1			
9 - Deep sediment odor	Neither	Sewer (rotten egg)	Oil / Industrial				
Summation							

Source: DORINO, 2018.

Punctuation	Disturbance level		
0 - 26	impacted		
26 <b>-</b> 45	changed		
> 45	native		

OBS: Rapid Habitat Diversity Assessment Protocol modified from the CAWTHRON INSTITUTE (National Rapid Habitat Assement Protocol Development For Streams And Rivers) Environmental Protection Agency Ohio (EUA) (EPA, 1987).

#### 8. FINAL CONSIDERATIONS

This study proposes to fill a small gap in environmental education classes in basic education, with the inclusion of the monitoring of river water quality. This script guides teachers in the field class activities, allied with the laboratory practical classes, in which students will perform playful activities of environmental education, where they will learn through records and photographs the local environmental conditions on the borders of the rivers. Then the students will have to take the samples of the water in question, and will be able to perform the physicochemical and microbiological analyzes, that proof through the analytical results, what the visual data could not inform.

However, in order for the environmental education classes to be educationally effective, the teacher must raise with the students the possible causes that have caused the environmental damage and propose a proposal that may be effective to mitigate the degradation of the studied environment. In addition, it will be necessary to create a means to disseminate the studies carried out, as well as possible solutions.

All this interaction will cause students to understand the necessary environmental awareness so that they can be the multipliers among their families and the community of the gravity and degradation that is occurring in the neighborhood water resources, which is only seen and understood by the massive means of communication, where we often can't feel what's happening around us.