ECOLOGICAL RISK ASSESSMENT (ERA) APPLICATION FOR THE EVALUATION OF IMPACTS ON NATURAL AQUATIC ECOSYSTEMS

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1 Introduction

Several qualitative and quantitative methods can be used to assess the impacts of anthropogenic activities on water courses and they make use of different parameters to characterize the adverse effects on the biotic and abiotic environments in the respective areas of interest. However, environmental assessment is commonly carried out parameter by parameter. Methods integrating separate analyses, which could contribute towards

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obtaining a better understanding of the effects on the ecosystem as a whole, are scarce.

Ecological Risk Assessment (ERA) is a process that meets the need to integrate the analyses of various effects on the ecosystem. One of the methods the ERA adopts to achieve such integration is the TRIAD approach. The TRIAD was the first method proposed by Long and Chapman (1985) to evaluate the quality of sediments, which has been improved along time. In their in-depth analysis of ERA, Jensen and Mesman (2006) define it as a technique for calculating the probability of occurrence of adverse effects on ecosystems. To evaluate this method, environmental data are gathered, organized and analyzed to enable the assessment of the impact caused by several anthropic activities.

A TRIAD-based characterization of risks is achieved by integrating three lines of evidence (LoEs) such as chemical, ecotoxicological and ecological. The first describes the chemical substances and respective concentrations to which the environment is exposed; the second uses standardized tests to analyze the effects of the chemicals on the living organisms in different trophic levels while the third makes a qualitative and quantitative assessment of the ecosystem's limnological/ecological characteristics.

Risk characterization models are widely used internationally, mainly due to their regulation undertaken by American and European environmental organizations (GHEJ; BOGATU 2009). Internationally, the ERA process has been very well structured due to the several studies, which applied that approach. However, most of them are focused on temperate climate environments (WARD et al., 2007; GHEJU and BOGATU, 2009; RAND et al., 2010).

In the Brazilian context, the Mineral Technology Center (*Centro de Tecnologia Mineral* - CETEM) published, in 2011, a document characterizing Ecological Risk Assessment and presenting different methodologies. The document's text follows the ERA characterization as indicated by the United States Environmental Protection Agency (USEPA) and, among other methodologies, it refers to the TRIAD approach, integrating chemical, ecotoxicological and ecological assessments. Despite that characterization from 2011, there are still no official ERA standards in Brazil and those described by the USEPA are generally used. However, the first steps have been taken in Brazil towards standardizing and regulating ERA with the Brazilian Technical Norms Association (*Associação Brasileira de Normas Técnicas* - ABNT) by means of the Technical Group studying Ecological Risk.

Among the research studies involving the TRIAD approach in Brazil, there are some of which may be cited here. Ferraz (2008) analyzed and compared risks for the Monjolinho River, São Carlos, Sao Paulo state, during the dry and the wet season. Sanchez (2012) assessed the quality of aquatic ecosystems in the Lobo drainage basin (Itirapina/Brotas, Sao Paulo state). Niemeyer et al. (2010), conducted a study in a metal contaminated area in the city of Santo Amaro, Bahia state. Moreover, Tallini and co-authors (2012) has been used the USEPA guideline during a monitoring program at the Jacui River in the municipality of Sao Jeronimo in the state of Rio Grande do Sul. Results has demonstrated the importance of using ERA as an instrument in decision-making on aquatic environment management.

Thus, it is important to note the need of other studies verifying the viability of using the integrating analysis in Ecological Risk Assessment applications for ecosystems located in Brazil's tropical and sub-tropical regions.

Accordingly, to explore the application of a TRIAD approach for the Ecological Risk Assessment in tropical natural environments, a study was conducted in the municipality of Bom Repouso in the southern part of the state of Minas Gerais (MG). The main economic activity in this region is strawberry and potato crop farming. The conventional models for planting (intensive use of pesticides and synthetic fertilizers) are common, which makes the surrounding environment prone to contamination (BRIGANTE et al., 2007). The application of the ERA for the water systems in Bom Repouso is made feasible by the abundant information in the literature describing physical, chemical, biological and ecotoxicological parameters for the region.

2 Methodology

2.1 Study location

The municipality of Bom Repouso, MG, is part of the Mogi-Guaçu river drainage basin and 993 water springs/sources, which contributes to the entire basin, has been registered, making it extremely important for the system in terms of water resources (CUNHA, 2009). It is located in a region of high altitude and has many steep slopes with angles of 45º or more, most of which are devoid of any natural vegetation (BRIGANTE et al., 2007).

Located in one of the main Brazilian's region for strawberry production, Bom Repouso has been the target of several studies, most of them endeavoring to assess the toxicity of pesticides, commonly used in Brazil for that kind of crop, like abacmectin-based pesticides, and also to evaluate any possible alterations they make to the quality of the region's waters and soils (CASALI-PEREIRA et al., 2015; VASCONCELOS et al., 2016; MENEZES-OLIVEIRA et al., 2018; NUNES et al., 2016).

2.2 Experimental design

The application of the Environmental Risk Assessment in Bom Repouso, Minas Gerais was divided into two steps, as follows:

Step 1 – Determination of the risk for 2008. Water sampling occurred from January 23 to 26 and July 9 to 11. Results obtained are described in the thesis entitled Assessment of Water Supply Sources in the Rural Area of the Municipality of Bom Repouso, MG (*Avaliação das Águas de Abastecimento da Área Rural do Município de Bom Repouso,* MG) (REZENDE, 2009). Those results enabled the comparison of the water characteristics between the rainy season and the dry season for that region, in 2008. During this step, 34 sampling points were assessed in order to cover the several types of land use as well as their water springs uses.

Step 2 – Ecological Risk Assessment for the year 2014. Sampling were performed from April 26 to 27 in order to compare both sampling years (2008 and 2014) and observe possible changes in ecological risk in Bom Repouso. Five of the 34 sampling points selected by Rezende (2009) were used for comparison in 2014, based on the following criteria: I) Sampling carried out in both periods selected by Rezende (2009); II) characteristics

of the surrounding areas; III) Different types of water use and, IV) Ease of access. The characteristics of the five sampling points selected were as follow: P1 – diffuse water spring located in an area with well preserved gallery vegetation; P2 – water dammed up on the top of a hill; P3 – water supply in the central area of the city; P4 – water tank, placed in a well-preserved area, for the farmers water supply, and P5 – water supply to a private dwelling, coming from a nearby water spring.

Samples were collected using polyethylene recipients and preserved at a temperature of 4ºC as recommended by the respective Brazilian Standards specification NBR 9898.

2.3 Ecological Risk Assessment (ERA)

The Ecological Risk Assessment performed in this research was adapted from the procedures described by Jensen and Mesman (2006). Originally, the procedure consisted on defining the risk value for any given sampling point based on chemical, ecological and ecotoxicological variables in order to obtain a final value on a scale from 0 to 1. Values in the range of 0.000 to 0.250 represent low risk, from 0.251 to 0.500 means moderate risk, from 0.501 to 0.750, high risk, and from 0.751 to 1.000, very high risk.

The Ecological Line of Evidence was not used in this work because it was not possible to sampling ecological data *in situ* for analysis. Instead, a new Line of Evidence was adopted using physical-chemical parameters, considering their importance to any environmental characterization. Risk was calculated based on the formulae described for the chemical line of evidence but instead of using the data together with those of the chemical substances it was used as a distinct Line of Evidence as described by Crévecoeur et al. (2011).

To define the reference values for the three lines of evidence, it was necessary to define a sampling point to be used as background. P1 and P4 sampling points showed the most well preserved surroundings. P1 was selected due to the results obtained, especially regarding the chemical analyses. In addition, obtained values were adjusted, based on the quality-standardized values established for each contaminant in CONAMA Resolution N°357, dated March 17, 2005, for rivers denominated as class 2, as the Mogi-Guaçu River.

The parameters evaluated for each line of evidence at the different sampling points and in the different seasons were as follows:

- **Chemical LoE:** Iron, Manganese, Lead, Copper, Zinc, Cadmium and Chromium;
- **Physical-chemical LoE:** pH, Conductivity and Dissolved Oxygen;
- **Ecotoxicological LoE:** Acute test - *D. similis* and Chronic test - *C. dúbia*.

2.3.1 Chemical Line of Evidence (Chemical *LoE*)

As mentioned above, the analysis for the Chemical Line of Evidence consisted in the evaluation of the total concentrations of the metals Iron, Manganese, Lead, Copper, Zinc, Cadmium and Chromium

To ensure maximum data reliability, an analysis of relevance for the different substances was performed based on a ranking, where variables with higher degree of uncertainty have lower weight (JENSEN and MESMAN, 2006). The maximum weight (1.0) was attributed to the metals lead, copper, cadmium and chromium and a weight of 0.5 was attributed to iron, manganese and zinc as it is expected to have a lower impact of the last three chemical substances to the aquatic environment (PIVELLI AND KATO, 2005).

Based on the values obtained in this research and the reference values specified in CONAMA Resolution Nº 357/05, the following steps were performed to quantify the risks for the Chemical LoE:

1) Calculation of R3 for each contaminant as the ratio between R1 and R2.
2) Calculation of the risk for each contaminant:

$$R4 = 1 - \frac{1}{1 + R3}$$

3) Adjustment of the values in relation to the values from the reference sampling point:

$$R5 = \frac{R4 - R4_{REF}}{1 - R4_{REF}}$$

4) Calculation of the combined risk of all contaminants using the adjusted values with n being the number of variables, based on the formula:

$$R6 = 1 - ((1 - R5)_1 x (1 - R5)_2 x (1 - R5)_3 x \ldots x (1 - R5)_n)$$

In which, R3 = the toxic pressure of each chemical substance; R2 = reference value for each chemical substance and R1 = concentration of each chemical substance measured at the different sampling points.

Once the risk values were defined for each variable, a single risk value could be calculated for the chemical line of evidence, as described in the following steps:

1) Calculation of the values for *R1 = log (1-X)*, where X is the risk value (associated, in this case, to the toxic pressure and the toxic concentration);
2) Calculation of the mean (R2) of the values obtained in step 1;
3) Transformation of the values obtained in step 2 using the formula:

R3 = 1 – (10^{R2}), where R2 are the values obtained in step 2 and R3 represents the integrated risk value.

2.3.2 Physical-chemical Line of Evidence (Physical-chemical *LoE*)

The parameters used in the Physical-chemical Line of Evidence (pH, conductivity and dissolved Oxygen) were measured for all samples collected at the several sampling points. The reference values were based on those set out in CONAMA Resolution Nᵒ 357/05. For pH, the reference values ranged from 5 to 9. Thus, all measured values within this range were considered as 1 (100%) and for those out of the range, a percentage of variation from that was calculated, both for values below and above the range.

The attribution of weights for this LoE was also necessary and obeyed the same criteria as those adopted for the Chemical LoE. However, in this case, the maximum weight (1) was attributed for all measured variables. The risk value for the range of physical-chemical parameters was calculated using the same steps taken for the Chemical LoE (See item 2.3.1).

2.3.3 Ecotoxicological Line of Evidence (Ecotoxicological *LoE*)

Ecotoxicological tests are carried out in order to evaluate possible toxicity of the substances present in the environment to test organisms. Tests can be acute (short duration), in which mortality or immobility of the test organisms are assessed, or they can be chronic (long duration), embracing the organism's entire life cycle and evaluating sub-lethal parameters such as reproduction, growth and deformities (ROMANELLI, 2004).

In the present research, acute toxicity tests were conducted using the species *Daphnia similis* as test organism. The endpoint used was mortality and/or immobility of the organisms, according to the methodology defined in the guideline ABNT NBR 12.713. For the chronic test, survival and reproduction of the species *Ceriodaphnia dubia* were assessed, over a period of eight days, in accordance with the procedure described in the guideline ABNT NBR 13.373.

The calculation of the risk associated to this line of evidence were performed in accordance to the procedure described by Jensen e Mesman (2006), wherein the toxicity values are scaled by the following steps:

1) Mortality and/or immobility values expressed as percentages are divided by 100, obtaining R1 values;
2) Risk values (R2) are obtained using the following formula: $R2 = \dfrac{(R1 - R_{REF})}{(1 - R_{REF})}$

Once the risk values for the acute and chronic tests were defined, the overall risk for this LoE could be calculated as follows:

1) Calculation of the values for $R1 = \log (1\text{-}X)$, where X is the risk value;
2) Calculation of the mean (R2) of the values obtained in step 1;
3) Transformation of the values obtained in step 2 using the formula:

$R3 = 1 - (10^{R2})$, where R2 are the values obtained in step 2 and R3 represents the integrated risk.

2.3.4. Integration of the Lines of Evidence

The final result of a TRIAD-based Ecological Risk Analysis is an integrated analysis of the chemical, physical-chemical and ecotoxicological lines of evidence. This integration provides a single value for the possible risks to the ecosystems under study, based on the assessed parameters. Integration is carried out as described in the following steps:

1) Calculation of the values for $R1 = \log(1 - X)$, , where X is the value of the risk associated to each line of evidence;

2) Calculation of the weighted arithmetical means (R2) of the values obtained for R1;

3) Transformation of the values obtained for R2 by means of the formula $R3 = 1 - 10^{R2}$, where R2 represents the final integrated risk value for each sampling point.

3 Results

Results obtained for each parameter used to assess the ecological risk for the three lines of evidence are described in Table 1. For the chemical analyses, any values found below the Quantification Limit (QL) were reported as zero (0).

Table 1: Results obtained for the parameters used in the three Lines of Evidence

Point:	P1			P2			P3			P4			P5			VMP*
	Jan/08	Jul/08	Apr/14	Jan/08	Jul/08	Apr/14	Jan/08	Jul/08	Apr/14	Jan/08	jul/08	Apr/14	Jan/08	Jul/08	Apr/14	
Chemical LoE																
Iron (mg/L Fe)	0	0	**0.422**	0	0	0.214	0	0	0.165	**11.09**	0	**0.553**	0	0	0.200	0.300
Manganese (mg/L Mn)	0	0	0.004	0	0	0	0	0	0	0	0	0.027	0	0	0	0.100
Lead (mg/L Pb)	0	0	**0.450**	0	0	**0.190**	0	0	**0.430**	0	0	**0.190**	0	0	**0.140**	0.010
Copper (mg/L Cu)	0	0	0	0	0	0	0	0	0.006	0	0	0	0	0	0	0.009
Zinc (mg/L Zn)	0	0	0.076	0	0.002	0.072	0	0	0.071	0	0.002	0.179	0	0.006	0.064	0.180
Cadmium (mg/L Cd)	0	0	**0.085**	0	0	**0.092**	0	0	0.067	0	0	**0.101**	0	0	**0.960**	0.001
Chromium (mg/L Cr)	0.019	0	0	0.023	0	0	0.018	0	0	0.024	0	0	0.018	0	0	0.050
Physical-chemical LoE																
pH	6.420	7.790	**5.830**	6.700	7.700	**5.680**	6.520	6.520	6.455	**5.700**	**5.380**	**4.740**	6.230	6.410	**5.500**	6.0 a 9.0
Conductivity (µS/cm)	**12.00**	**20.00**	**10.00**	21.00	25.33	17.00	62.67	55.00	35.00	13.00	15.33	11.00	21.00	22.00	23.00	6.3
DO (mg/L)	7.780	7.140	10.100	9.420	8.070	10.300	**3.070**	5.640	8.360	**4.820**	6.390	9.610	5.650	7.950	8.470	> 5.0
Ecotoxicological LoE (mortality %)																
D. similis	25	5	0	0	100	0	0	80	20	0	10	5	35	5	10	-
C. dubia	90	100	60	70	0	0	N.R.**	100	50	N.R.**	100	40	60	100	0	-

*Maximum value allowed by CONAMA Resolution Nº 357/2005 for class 2 rivers – Freshwater** Tests not carried out*
*** Values in bold are non-compliant with CONAMA Resolution Nº 357/2005specifictions*

The results of the risk associated to the Chemical Line of Evidence for the five sampling points and in the three different periods can be observed in Figure 1. Only in January 2008, at P4, the risk was considered as very high, being the iron, with a concentration of 11.09 mg.L⁻¹ the chemical substance responsible for this scenario, since the measured concentration is far higher than the maximum tolerable value established by CONAMA Resolution Nº 357/2005, which is 0.300 mg.L⁻¹.

In July 2014, the measured concentrations for zinc in P2 and P4 (0.002 mg.L^{-1}) and P5 (0.006 mg.L^{-1}) were responsible for the low risk values attributed to those sampling points. In April 2014, none of the concentrations were above the limit established by the legislation for the metals manganese, copper, zinc, or chromium. However, lead and cadmium, showed to be above the acceptable level of 0.01 mg.L^{-1} in several sampling point.

The values for lead ranged from 140 mg.L^{-1} (P5) to 0.450 (P1) and for cadmium, from 0.067 (P3) to 0.101 mg.L^{-1} (P4). The CONAMA reference value for iron (0.300 mg.L^{-1}) was only exceeded at P4 where the concentration registered was 0.553 mg.L^{-1}. At sampling points P2 and P5 the risk values determined were low: 0.075 and 0.113 respectively. Risk values for P4 and P3 were considered moderate: 0.318 and 0.400 respectively. It must be remembered that the risk value for P1 was defined as 0 (zero) given that it was defined as the reference sampling point.

Figure 1 – Risk associated to the Chemical Line of Evidence for the five sampling points in the municipality of Bom Repouso for the three periods of analysis
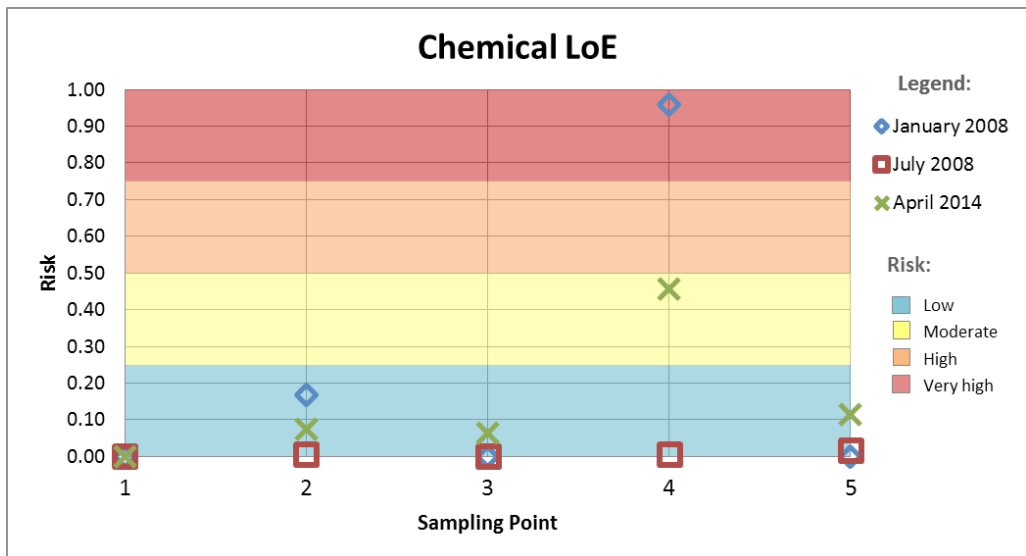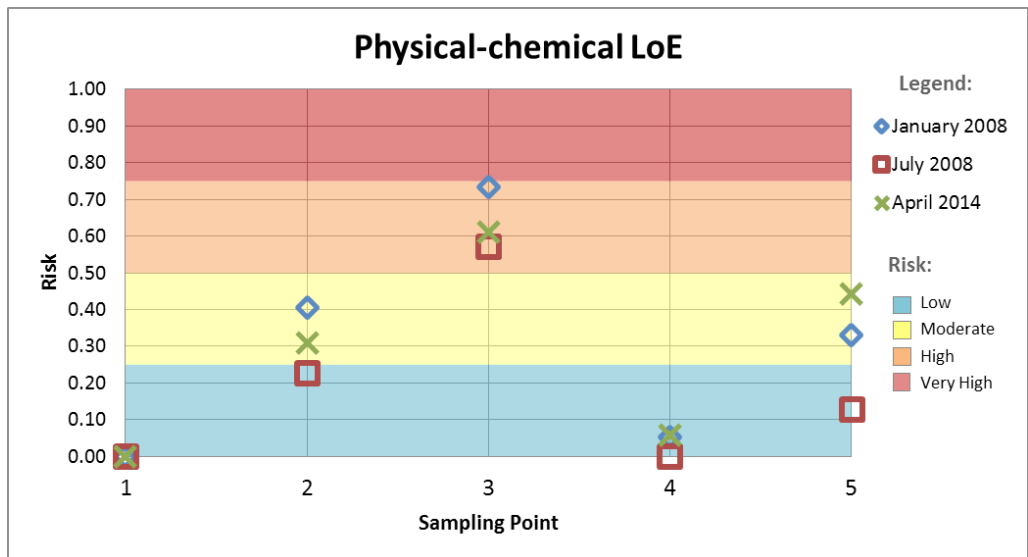

Source: Self-elaboration

The values obtained for the three variables of the Physical-chemical LoE were out of the range established as a limit by the legislation. The lowest value registered for pH was 4.74 (P4 in April 2014). For conductivity, the highest value was 62.67 μS.cm^{-1} (P3 in January 2008) and the lowest value for dissolved Oxygen was 3.07 mg.L^{-1} (P3 in January 2008).

The risk associated to the Physical-chemical LoE varied from low to high, being four of the fifteen analyses considered as moderate risk with values, ranging from 0.31 (P2 in April 2014) to 0.44 (P5 in April 2014).The highest risk was registered for P3 in all three periods: 0.735 for January 2008, 0.571 for July 2008 and 0.611 for April 2014.

Once again, it must be remembered that the risk value was not determined for P1 because this sampling point was used as reference. The risks associated to the Physical-chemical Line of Evidence are displayed in Figure 2.

Figure 2 - Risk associated to the Physical-chemical Line of Evidence for the five sampling points in the municipality of Bom Repouso for the three periods of analysis
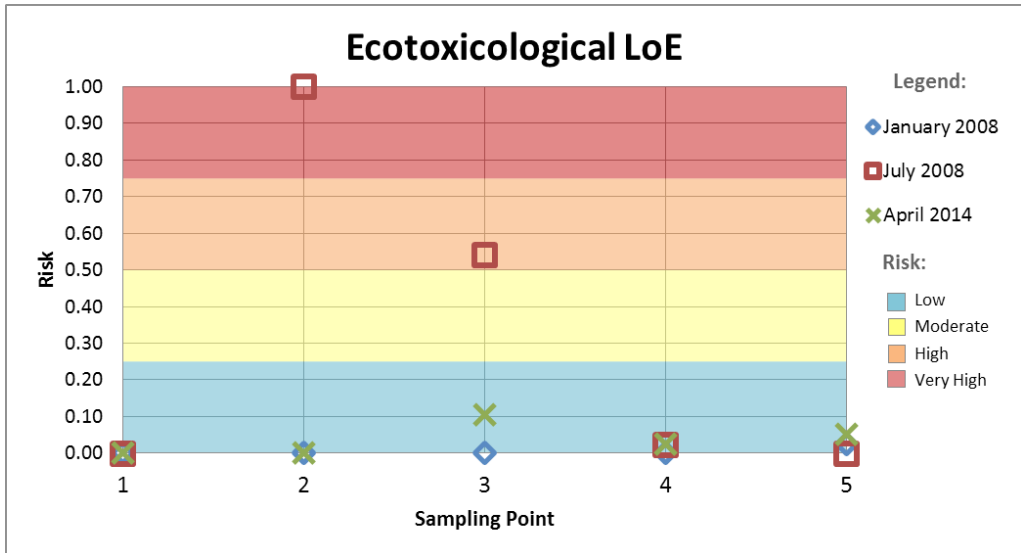

Source: Self-elaboration

The results obtained for P1 were also defined as the reference values for the Eco-toxicological Line of Evidence. However, the scenario differed considerably from those found for the preceding LoEs since a high level of toxicity was observed for that sampling point in both acute test with *Daphnia similis,* and chronic test with *Ceriodaphnia dubia.* Although that high toxicity level at the reference sampling point does not invalidate the method, it does show that not even the reference point is totally free from contamination.

Assessing the toxicity of the samples from January 2008, it was found immobility in 25% of the organisms in the acute test and 90% in the chronic test at the sampling point P1. In July 2008, those toxicity values for P1 were 5% in the acute test and 100% in the chronic test, thereby making it unfeasible to equate the risk for any of the other sampling points for that period. During April 2014, immobility effects were only registered at the chronic test, recording immobilization or death for 60% of the test organisms, a value higher than the ones found to any the other sampling points.

In accordance with the methodological proposal, even though toxicity was found at the reference sampling point, the risk in this point has to be considered as zero. Thus, for all the other points, the risk in January 2008 was null due to the lower toxicity when compared to the reference. The only exception was P5 where the risk attributed was 0.069 due to the mortality that occurred in the acute test (35%).

For the sampling from July 2008, results found in the acute toxicity tests were the responsible for the high and very high risks assessed in this line of evidence and their values varied from 0 (zero) for P5 to 1.000 for P2. In April 2014, the risk associated to this line of evidence was low and varied from 0 (zero) for P2 to 0.106 for P3, as can be seen in Figure 3.

Figure 3 - Risk associated to the Physical-chemical Line of Evidence for the five sampling points in the municipality of Bom Repouso for the three periods of analysis
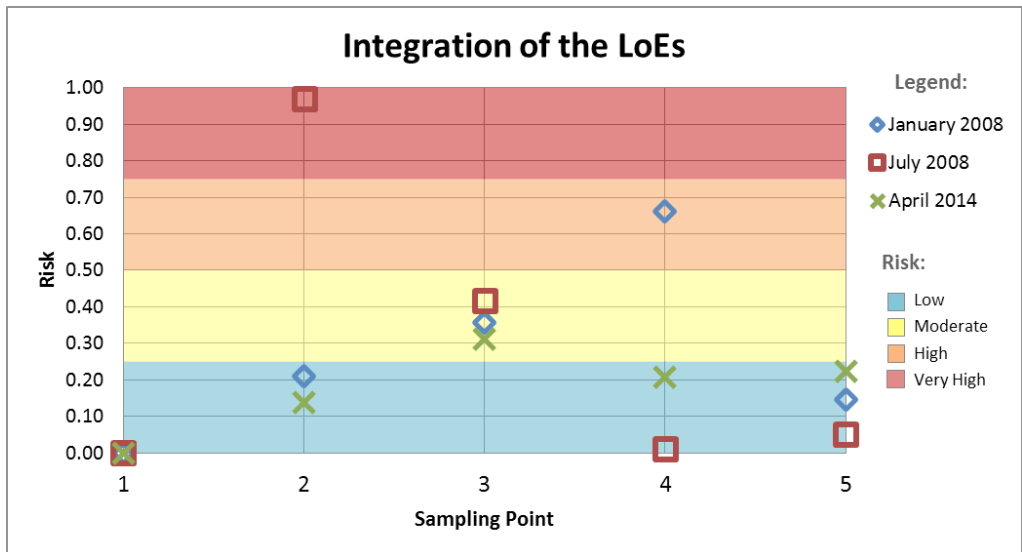

Source: Self-elaboration

When integrating the chemical, physical-chemical and ecotoxicological lines of evidence, it was observed that the highest risk at P2 and P3 was registered in July 2008, which means that the ecotoxicological line was the one dominating the analysis with the results from the acute tests. In January 2008, the highest risk was obtained for P4 due to the high concentration of iron detected for the chemical LoE and in April 2014 the greatest risk was associated to P5 due to the high risk found to the physical-chemical LoE.

The single analysis of each sampling revealed a high risk value for P4 in January 2008 and a very high risk value for P2 in July 2008. Figure 4 displays the risk values for each point and for the three sampling periods.

Figura 4 – Integrated risk for the five sampling points and the three periods of analysis in the municipality of Bom Repouso

Source: Self-elaboration

4 Discussion

At all sampling points, in April 2014, the concentrations of the metals lead and cadmium were above the legal limits determined by CONAMA resolution Nº 357/05. Despite of that, however, the ecological risk values for the respective sampling points were not altered. This response probably occurred due to the presence of high concentration of metals at the sampling point adopted as the reference one (P1).

Even in very low concentrations, heavy metals such as lead and cadmium may cause adverse effects to the water systems and Human health since the conventional systems used of water treatment do not involve any efficient processes able to remove such metals, as Piveli and Kato (2005) remarked. Those authors have also pointed out that the main sources of such metals in water are the effluents of extractive and chemical industries.

Iron was by far the most abundant metal (11.09 mg.L^{-1}) at P2 in July 2008 with a concentration 37 times higher than the value specified by legislation (0.300 mg.L^{-1}). Although iron is a trace metal commonly found in tropical waters (BRIGANTE et al., 2003), when present in high concentrations it can affect the equilibrium of the ecosystem. Its presence may favor excessive proliferation of planktonic organisms (GUIMARÃES, 2016) and even cause problems for the public water supply system due to, for example, the deposition of iron-oxidizing bacterias, which gives color and taste to the water, and can even stain clothes and domestic utensils (PIVELI and KATO, 2005).

The attribution of weights for the chemical LoE variables followed the recommendations of Jensen and Mesman (2006), which emphasizes the importance of the attribution

of weights to the obtained values, in order to give more visibility to parameters of specific environmental interest and diminish the uncertainties present in the tests. However, it was observed that great care and good knowledge of the study area are needed to adequately attribute weights, given that they may vary according to the authors' perceptions, perhaps introducing conflicting factors.

The physical-chemical line of evidence was the only line not presenting very high levels of risk to any of the sampling points studied. The risk varied from low to high, although the measured parameters were found to be out of the acceptable range defined by CONAMA resolution Nº 357/2005. However, the use of physical-chemical parameters as chemical parameters is not a very clear issue. It is possible to find studies in which the physical-chemical parameters are not included in the calculation. In this case, these parameters are used only for discussion purposes, both for chemical and ecological aspects (GUTIÉRREZ et al., 2015; RIBÉ et al., 2012). Other studies, in turn, uses the physical-chemical parameters as being chemical parameters, pooling them together into the Chemical Line of Evidence (MENDES et al., 2017). In the present study, we have chosen to calculate the physical-chemical parameters as chemical parameters but data was presented separately as one other line of evidence into ERA (CRÉVECOEUR et al., 2011).

The ecotoxicological LoE presented the greatest degree of variation compared to the other lines. There were registrations of very high and high risk levels for P2 and P3 sampling points, respectively, in July 2008, and a low risk level for all other samples. In both samplings from July 2008, the highest risk values were caused by the immobility found in the acute test using with *D. similis* (100% for P2 and 80% for P3), which were much than that found at the reference sampling point (25% of immobility registered for in P1). The high levels of toxicity associated to the samples collected during the winter in July 2008 at P2 and P3, where the water courses are dammed up to ensure water supply, may be related to low rainfall and temperatures typical of that time of year in the region under study. They result in reduced water flows and may increase metal concentrations and their bioavailability for aquatic organisms.

It is important to note that, no place free of contamination was found at the study site, which could have been used as a better reference site. That situation is increasingly common in agricultural regions. The well preserved conditions of P1 surrounding areas, which motivated the choice of P1 as the reference, proved to be insufficient to guarantee the water quality. The impact of anthropic activities on the water springs was also discussed by Rezende (2009), which showed that all 34 sampling points in 2008 presented at least one parameters in discordance with the environmental legislation.

The integration of the three lines of evidence showed low ecological risk for 10 (ten) of the 15 (fifteen) samples analyzed and a moderate risk for 3 (three) other samples. In January 2008, a high risk level was founded for P4, mainly due to the results of the Chemical Line of Evidence, and a very high risk level for P2 in July 2008 due to the high immobility of the test organisms in the ecotoxicological tests.

The high risk detected for P4 in January 2008 is a matter of concern since this place is mainly used for the human water supply and so its contamination may be harmful to human health. However, it can be seen that the subsequent analyses in July 2008 and

April 2014 did not detect such risk, so the contamination registered in the previous analysis may have been just a sporadic occurrence at the time.

Regarding the differences between seasons, Rezende (2009) reported that variables such as dissolved oxygen for which the lower temperatures during the dry season favored the solubilization of Oxygen and the concentration of metals. Although the risk value obtained for the chemical LoE in the rainy season (January 2008) was higher than at the other periods, the integrated risk analysis at the different sampling points did not identify any pattern which could allow comparisons of the two seasons analyzed.

Similarly, changes in the risk scenarios from 2008 to 2014 did not occur in a uniform way for all sampling points, given that the risk in July 2008 was greater than in April 2014 at points P2 and P3. In the case of P5, however, an evolution of the ecological risk in the course of time was identified: the risk value registered for April 2014 (0.260) was higher than those for January and July 2008 (0.129 and 0.228, respectively) and that was mainly due to the concentrations of the metal cadmium in 2014, at levels much higher than the reference values.

Besides the presence of metals and physical-chemical parameters in discordance with the legislation, the high toxicity levels observed at al sampling points may also be associated with the excessive use of pesticides and chemical fertilizers (no determined in the chemical analyses), which are routinely applied in the cultivation of strawberries and potatoes, the main local plantations in Bom Repouso. Pesticides may enter the environment by several ways such as spray drift, direct application, surface runoff, erosion, leaching or volatilization after the initial contact with the soil (DORES and DE-LAMONICA, 1999).

One of the pesticides widely used in the region is the insecticide Vertimec® 18 EC whose active ingredient is abamectin. Vanderlei (2015) analyzed the effects and final destination of abamectin in the rural aquatic ecosystem of Bom Repouso using ecotoxicological tests with organisms from different trophic levels, in association with mathematical models, to calculate pesticide dispersion in the environment. The author concluded that the concentration of contaminants in the water courses could be as much as 800 times higher than the level considered safe for 95% of the species.

The effects of the insecticide Vertimec® 18 EC to the cladocera *Ceriodaphnia silvestrii* have also been evaluated using both acute and chronic tests (CASALI-PEREIRA et al., 2015). The acute tests revealed that a concentration of 1.47 μg a.i./L immobilized 50% of the organisms within 48 hours. The chronic test, however, did not reveal any effects on survival or reproduction of the test organism in concentrations of 169 and 84 ng a.i./L, respectively. In addition to the aquatic organisms, this insecticide effects were also assessed to the amphibian species *Lithobates catesbeianus* using both avoidance and chronic tests (VANCONCELOS et al. 2016) and to terrestrial organisms *Eisenia andrei, Folsomia candida, Hypoaspis aculeifer* and *Enchytraeus crypticus* (NUNES et al., 2016; MENEZES-OLIVEIRA et al., 2018) using acute and chronic tests and demonstrating the effects to the aquatic and terrestrial biota, respectively.

5 Conclusion

The procedure used in the present study to assess ecological risks proved to be efficient in attributing a value to the degree of degradation. Thus, the research achieved its objective of promoting an integrated analysis of the water resource based on the ERA descriptive method and generate risk values which were consistent with the reality observed in the municipality of Bom Repouso

The three lines of evidence are complementary and important to verify the water quality, especially considering that in this case study, the classification of the risk was different for each line of evidence. In that sense, it must be emphasized that the physical-chemical parameters showed themselves to be important in composing the TRIAD, inserted here as a distinct LoE.

The ecological risk assessment obtained by the present method quantifies, but does not qualify the origin of the environmental degradation, so it does not enable to identify the causes of the contamination of the system. This response emphasizes the importance of the characterization in the field, conducting research based on primary and secondary data concerning the region and of conducting additional analyses. Each variable can be analyzed individually providing support for the adoption of prevention or mitigation measures the water bodies management.

The main uncertainties present in the method proposed by Jensen and Mesman (2006) are the choice of the reference sampling point and the weight to be attributed to each variable. Further studies are required to diminish errors associated to the calculations.

Despite the known uncertainties, this research has shown the advanced stage of degradation of the spring waters from Bom Repouso and results may serve as an incentive for the elaboration of standardized Ecological Risk Analysis methodologies specifically for tropical environments, which would allow the ERAs to be used in a legal ambit, validating risks determined for those kind of ecosystems.

References

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT). NBR 9898. **Preservação e técnicas de amostragem de efluentes líquidos e corpos receptores**. 1987.

_____. NBR 12713. **Ecotoxicologia aquática - Toxicidade crônica - Método de ensaio com *Daphnia spp* (Cladocera, Crustacea)**. 2ª ed. 2004.

_____. NBR 13373. **Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com *Cerioaphnia spp* (Cladocera, Crustacea)**. 2ª ed. 2005.

BRIGANTE, J.; DA COSTA, J. N. M. N.; MENDONÇA, A. H.; ESPÍNDOLA, E. L. G. Ações de Restauração Florestal: Valorização dos Recursos Florestais da Bacia Hidrográfica de Montante do Rio Mogi-Guaçu. In: ESPÍNDOLA, E. L. G.; BRIGANTE, J. (Org). **Projeto Mogi-Guaçu:** Desenvolvendo Ações Ambientais. São Carlos. RiMa. 2007.

BRIGANTE, J.; ESPÍNDOLA, E. L. G. O Projeto Mogi-Guaçu. In: ESPÍNDOLA, E. L. G.; BRIGANTE, J. (Org). **Projeto Mogi-Guaçu:** Desenvolvendo Ações Ambientais. São Carlos. RiMa. 2007.

BRIGANTE, J.; SILVA, M.R.C.; QUEIROZ, L.A.; COPPI, E. Quantificação de metais na água e no sedimento do rio Mogi-Guaçu. In: BRIGANTE, J. & ESPÍNDOLA, E.L.G. **Limnologia Fluvial:** um estudo no rio Mogi-Guaçu. São Carlos. RiMa. 2003. 255p.

CASALI-PEREIRA, M. P; DAAM, M. A.; RESENDE, J. C.; VASCONCELOS, A. M.; ESPÍNDOLA, E. L. G.; BOTTA, C. M. R. Toxicity of Vertimec® 18 EC (active ingrediente abamectin) to the neotropical cladoceran Ceriodaphnia silvestrii. **Chemosphere**. Elsevier. Ed. 139. 2015.

CENTRO DE TECNOLOGIA MINERAL (CETEM). **Avaliação de risco ecológico:** conceitos básicos, metodologia e estudo de caso. Ministério da Ciência e Tecnologia (MCT). Rio de Janeiro. 126p. 2011.

CONSELHO NACIONAL DE MEIO AMBIENTE (CONAMA). **Resolução nº 357**, de 17 de Março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências.

CRÉVECOEUR, S.; DEBACKER, V.; JOAQUIM-JUSTO, C.; DOBERT, S.; SCIPPO, M.; DEJOGHE, W. et al. Groundwater quality assessment of one former industrial site in Belgium using a TRIAD-like approach. **Environmental Pollution** 159. Elsevier. 2011.

CUNHA, G. P. Q. **Caracterização ambiental da região de montante do rio Mogi-Guaçu (Bom Repouso - MG):** estratégias para replicabilidade e diretrizes para elaboração do plano de adequação ambiental**.** Escola de Engenharia de São Carlos (EESC-USP). São Carlos. 2009.

DORES, E. F. G. C.; DE-LAMONICA-FREIRE, E. M. **Contaminação do Ambiente Aquático por pesticidas:** Vias de Contaminação e Dinâmica dos Pesticidas no Ambiente Aquático. Pesticidas: Ecotoxicologia e Meio Ambiente. Curitiba. 1999.

ESTEVES, F.A. **Fundamentos de Limnologia**. 2a Edição, Rio de Janeiro. Interciência Ltda. 1998. 602p.

FERRAZ, A. P. **Avaliação de risco ecológico para o rio Monjolinho (São Carlos/SP).** Monografia (Graduação em Engenharia Ambiental). Escola de Engenharia de São Carlos (EESC-USP), São Carlos. 2008.

GHEJU, M.; BOGATU, C. **Preliminary Ecological Risk Assessment to Aquatic Environment of Bega River due to Presence of Ammonia in Treated Sewage Effluent.** Chem. Bull. "POLITEHNICA" Univ. 2009.

GUIMARÃES, M. Os danos escondidos na lama: Argila fina e alto teor de metais no material liberado pelo rompimento das barragens em Minas Gerais podem alterar dinâmica ecológica e de sedimentos da foz do rio Doce. **Revista Pesquisa FAPESP**. Ed. 239, January

2016. In: <http://revistapesquisa.fapesp.br/2016/01/12/os-danos-escondidos-na-lama/>. Accessed in June, 2016.

GUTIÉRREZ, L.; GARBISUB, C.; CIPRIÁNA, E.; BECERRILC, J. M.; SOTOD, M.; ETXEBARRIAE, J. et al. Application of ecological risk assessment based on a novel TRIAD-tiered approach to contaminated soil surrounding a closed non-sealed land ll. **Science of the Total Environment** 514. Elsevier. 2015.

HANAI, F. Y. **Sistema de indicadores de sustentabilidade**: uma aplicação ao contexto de desenvolvimento do turismo da região de Bueno Brandão, Estado de Minas Gerais, Brasil. Tese (doutorado) - Escola de Engenharia de São Carlos (EESC - USP). São Carlos. 2009.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). **Censo Demográfico de 2010.** Dados referentes ao município de Bom Repouso, MG, fornecidos em meio eletrônico. 2010.

JENSEN, J.; MESMAN, M. (eds). **Ecological risk assessment of contaminated land Decision support for site specific investigations**. 2006. 136 p.

LONG, E. R.; CHAPMAN, P. M. A Sediment Quality Triad: Measures of Sediment Contamination, Toxicity and Infaunal Community Composition in Puget Sound. **Mantle Pollution Bulletin**. Volume 16. Number 10. pp 405-415. 1985.

MENDES, M. P.; SALOMÃO, A. L. S.; NIEMEYER, J. C.; MARQUE, M. Ecological Risk Assessment in a Tropical Wetland Contaminated with Gasoline: Tier 1. **Human and Ecological Risk Assessment:** An International Journal. 2017.

MENEZES-OLIVEIRA, V.B.; BIANCHI, M. O.; ESPÍNDOLA, E. L. G. Hazard Assessment of the Pesticides Kraft 36 EC and Score in a Tropical Natural Soil Using an Ecotoxicological Test Battery. **Environmental Toxicology and Chemistry**. 2018.

NIEMEYER, J. C.; MOREIRA-SANTOS, M.; NOGUEIRA, M. A.; CARVALHO, G. M.; RIBEIRO, R.; SILVA, et al. Environmental risk assessment of metal-contaminated area in the Tropics. Tier I: screening phase. **Ecotoxicology**. 2010.

NIVA, C. C.; NIEMEYER, J. C.; DA SILVA JÚNIOR; F. M. R.; NUNES, M. E. T.; DE SOUSA, D. L.; ARAGÃO, C. W. S. et al. Soil ecotoxicology in Brazil is taking its course. **Environ Sci Pollut Res**. 2016.

NUNES, M. E. T.; DAAM, M. A.; ESPÍNDOLA, E. L. G. Survival, morphology and reproduction of Eisenia andrei (Annelida, Oligochaeta) as affected by Vertimec® 18 EC (a.i. abamectin) in tests performed under tropical conditions. **Applied Soil Ecology**. ed. 100. Elsevier. 2016.

PIVELI, R. P.; KATO, M. T. **Qualidade das Águas e Poluição:** Aspectos Físico-Químicos. 01. ed. São Paulo/SP: ABES - Associação Brasileira de Engenharia Sanitária e Ambiental. 2005.

RAND, G. M.; CARRINGER, J. F.; GARDINALLI, P. R.; J. CASTRO. **Endosulfan and its metabolite, endosulfan sulfate, in freshwater ecosystems of South Florida:** a probabilistic aquatic ecological risk assessment. 22p. 2010.

REZENDE, J. C.; **Avaliação das Águas de Abastecimento da Área Rural do Município de Bom Repouso, MG.** Monografia de Graduação, Escola de Engenharia de São Carlos (EESC). Universidade de São Paulo (USP). São Carlos. 2009.

RIBÉA, V., AULENIUSA, E.; NEHRENHEIMA, E.; MARTELLB, U.; ODLAR, M. Applying the Triad method in a risk assessment of a former surface treatment and metal industry site. **Journal of Hazardous Materials** 207–208. Elsevier. 2012.

ROMANELLI, M. F. **Avaliação da toxicidade aguda e crônica dos surfactantes DSS e LAS submetidos à irradiação com feixes de elétrons**, p. 10. IPEN, São Paulo. 2004.

SANCHEZ, A. L. **Análise de risco ecológico para o diagnóstico de impactos ambientais em ecossistemas aquáticos continentais tropicais.** Dissertação (Mestrado) – Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos, 2012.

SANCHEZ, A. L. **Avaliação de risco ecológico de pesticidas em ambientes aquáticos continentais tropicais com ênfase na biodiversidade e nas funções e serviços ecossistêmicos.** Qualificação (Pós-graduação em Ciências da Engenharia Ambiental). EESC-USP, São Carlos. 2013.

TALLINI, K.; GUIMARÃES, L. S. P.; FACHEL, J. M. G.; RODRIGUEZ, M. T. R. **Estabelecim**ento de Protocolo de Avaliação de Risco Ecológico em Ambiente Aquático tendo o Programa de Monitoramento do Rio Jacuí, São Jerônimo (RS). J. **Braz. Soc. Ecotoxicol**. v. 7, n 1, 55-63. 2012.

U. S. ENVIRONMENTAL PROTECTION AGENCY (USEPA). **Framework for ecological risk assessment.** Risk Assessment Forum, EPA/630/R-92/001. 1992.

VANDERLEI, M. R. **Efeitos dos agrotóxicos Kraft®36EC e Score®250EC (e seus princípios ativos) em ecossistemas aquáticos**: análises comparativas e ecossistêmicas. 2015. Dissertação (Mestrado) – Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos, 2015.

VASCONCELOS, A. M.; DAAM, M. A.; DOS SANTOS, L. R. A.; SANCHES, A. L. M.; ARAÚJO, C. V. M.; ESPÍNDOLA, E. L. G. **Acute and chronic sensitivity, avoidance behavior and sensitive life stages of bullfrog tadpoles exposed to the biopesticide abamectin. Ecotoxicology**. Springer Science+Business Media New York. 2016.

WARD, S.; AUGSPUGER, T.; DWYER, F. J.; KANE, C. & C. G. INGERSOLL. **Risk assessment of water quality in three North Carolina, USA, streams supporting federally endangered freshwater mussels (Unionidae).** Environmental Toxicology and Chemistry. 2007.

ECOLOGICAL RISK ASSESSMENT (ERA) APPLICATION FOR THE EVALUATION OF IMPACTS ON NATURAL AQUATIC ECOSYSTEMS

Abstract: The present research employs the Ecological Risk Assessment (ERA) method to evaluate the probability of adverse effects in the water supply of Bom Repouso (MG), a city where the agriculture has caused an advanced degree of degradation of its sources. The methodology is based on the integration of different variables, divided into three Lines of Evidence (chemical, physical-chemical and ecotoxicological), and allows for the evaluation of risk assessment between 0.0 and 1.0 in the sampled environment. Five sampling points were evaluated in three periods, with the results varying between the four possible ratings (from low to very high), and it was not possible to identify a pattern of risk evolution between them. Thus, the method used proved to be efficient in assessing the degree of degradation of the environment, however, additional studies are required to improve this type of systemic impact assessment, based on the evaluation of the environmental degradation.

Keywords: Ecological Risk Assessment (ERA), Toxicological triad, Bom Repouso City.

Resumo: A presente pesquisa emprega o método de Avaliação de Risco Ecológico (ARE) para a valoração da probabilidade de ocorrência de efeitos adversos em águas de abastecimento de Bom Repouso (MG), município onde a agricultura causou um avançado grau de degradação de suas nascentes. A metodologia utilizada se baseia na integração de diferentes variáveis, divididas em três linhas de evidência (química, físico-química e ecotoxicológica), e permite a valoração do risco (entre 0,0 e 1,0) no ambiente amostrado. Foram avaliados cinco pontos de amostragem, em três períodos, tendo os resultados variado entre as quatro possíveis classificações (de baixo a altíssimo), não sendo possível identificar um padrão de evolução de risco entre eles. Deste modo, o método utilizado demonstrou-se eficiente na valoração do grau de degradação do ambiente, porém, estudos adicionais são requeridos para aprimoramento deste tipo de avaliação de impacto sistêmico com base na valoração de degradação do ambiente.

Palavras-chave: Avaliação de Risco Ecológico (ARE), Tríade Toxicológica, Município de Bom Repouso.

Resumen: El presente estudio emplea el método de Evaluación de Riesgo Ambiental (ERA) para valoración de la probabilidad de ocurrencia de efectos adversos en aguas de abastecimiento de Bom Repouso (MG), municipio que la agricultura ha provocado un

avanzado grado de degradación de sus nacientes. La metodología utilizada tiene como base la integración de diferentes variables, divididas en tres líneas de evidencia (química, fisicoquímica y ecotoxicológica) y permite la valoración del riesgo (entre 0,0 y 1,0) en el ambiente de muestra. Fueron evaluados cinco puntos de muestreo en tres períodos, con resultados variables entre las cuatro clasificaciones posibles (de bajo a altísimo), no identificando un estándar de evolución entre ellos. De este modo, el método utilizado se ha demostrado eficiente en la valoración del grado de degradación del ambiente. Todavía, se requieren estudios adicionales para mejorar este tipo de análisis sistémica, basado en la valoración de la degradación del ambiente.

Palabras clave: Evaluación de Riesgo Ambiental (ERA), Tríade Toxicológica, Ciudad de Bom Repouso.