https://doi.org/10.1590/S1413-415220230054

Literature Review

Use of ecotoxicological bioindicators in effluent monitoring — legal implications in Brazil

Use of ecotoxicological bioindicators in effluent monitoring — legal implications in Brazil

Daniela Pereira da Silva Alves[1]*, Flaviane Borges Teixeira[1], Monatha Nayara Guimarães Teófilo[1], Carlos Filipe Camilo Cotrim[1], Junilson Augusto de Paula Silva[1], Luciane Madureira Almeida[1], Elisa Flávia Luiz Cardoso Bailão[1]

ABSTRACT

Environmental changes directly influence quality of life, and environmental protection laws arise to ensure the maintenance of ecological balance. Aquatic environments have been contaminated by substances of anthropic origin, which are called effluents. Improper disposal of effluents has adverse effects on the environment and human health. In Brazilian legislation, the resolutions of the National Council for the Environment (CONAMA) RE 357/2005 and RE 430/2011 address the use of indicator species to evaluate the effluents' toxicity. Despite these, much still needs to be done to control and monitor the emission of effluents in Brazilian water resources. The objective of this work was to carry out a historical survey of the regulations on the disposal of effluents in Brazil and to show the differences in each state's regulations, highlighting the indicator species accepted in each regulation and their advantages and disadvantages. In this way, this review summarizes and organizes the information of the Brazilian legislation on the disposal of effluents and helps the researcher in the area to choose between the methodologies adopted for the analysis of their environmental samples. Despite the insertion of bioassays, it is evident that the current Brazilian legislation is permissive, requiring adaptations and definitions to increase adherence to the ecotoxicological monitoring of water resources by the States.

Keywords: bioassays; CONAMA; ecotoxicological assays; Brazilian legislation.

RESUMO

As mudanças ambientais influenciam diretamente a qualidade de vida, e leis de proteção ambiental surgem para garantir a manutenção do equilíbrio ecológico. Os ambientes aquáticos têm sido contaminados por substâncias de origem antrópica, denominadas efluentes. O descarte inadequado de efluentes traz efeitos adversos ao meio ambiente e à saúde humana. Na legislação brasileira, as resoluções do Conselho Nacional do Meio Ambiente (CONAMA) RE 357/2005 e RE 430/2011 abordam o uso de espécies indicadoras para avaliar a toxicidade dos efluentes. Apesar destas, muito ainda precisa ser feito para controlar e monitorar a emissão de efluentes nos recursos hídricos brasileiros. O objetivo deste trabalho foi realizar um levantamento histórico das regulamentações sobre o descarte de efluentes no Brasil e mostrar as diferenças nas regulamentações de cada estado, destacando as espécies indicadoras aceitas em cada regulamentação e suas vantagens e desvantagens. Dessa forma, esta revisão resume e organiza as informações da legislação brasileira sobre o descarte de efluentes e auxilia o pesquisador da área a escolher entre as metodologias adotadas para a análise de suas amostras ambientais. Apesar da inserção de bioensaios, fica evidente que a legislação brasileira atual é permissiva, exigindo adaptações e definições para aumentar a adesão ao monitoramento ecotoxicológico dos recursos hídricos pelos estados.

Palavras-chave: bioensaios; CONAMA; ensaios ecotoxicológicos; legislação brasileira.

1. INTRODUCTION

Aquatic environments have been contaminated by substances of anthropic origin, which reach rivers, seas, and lakes intentionally or accidentally due to poor planning, lack of treatment and/or resources, or environmental irresponsibility (PALANIAPPAN *et al.*, 2010). These residues, called effluents, can come from the metallurgical, pharmaceutical, food, and agricultural industries, hospital, and domestic environments. The combination of these discards can cause over-potentialized contaminants (LOUREIRO *et al.*, 2006; LYUBENOVA, 2011).

Once these toxic compounds are carried to water bodies, they interact with the biota (DE PAIVA MAGALHÃES and FERRÃO FILHO, 2008) and may

[1]Universidade Estadual de Goiás, *Campus* Anápolis de Ciências Exatas e Tecnológicas Henrique Santillo – Anápolis (GO), Brazil.
***Corresponding author:** luciane.almeida@ueg.br; almeidalm@hotmail.com
Conflicts of interest: the authors declare no conflicts of interest.
Funding: none.
Received on: 04/20/2023 – **Accepted on:** 12/12/2023.

cause the mechanism known as biomagnification, which is the accumulation of toxic substances along the food chain (PINTO *et al.*, 2014). This cycle can start with consuming contaminated producing organisms and, through trophic levels, reach humans (GAVRILESCU, 2004; FORSTNER and WITTMANN, 2012). The toxic agent in the organism can alter the metabolism, and physiological and genetic characteristics of the host, being able to cause changes in growth, reproduction, birth, and survival rates (SILVA, POMPÊO and PAIVA, 2015). Thus, the contaminants found in water resources are a worldwide concern, as they negatively impact the ecosystem and can affect all trophic levels of the food chain.

Although the visibility and proof of environmental damage are common knowledge, until recently, there was no concern with characterizing effluents and assessing their environmental impacts. However, the growing level of impairment to human health, and the reports of diseases caused by these wastes, led to the elaboration of a legislation to regulate the disposal of effluents. CONAMA Resolution No. 430/2011 aimed to increase environmental awareness and prompt industries to implement projects to quantify effluents and define their composition. In this context, ecotoxicological tests were inserted in the industrial scope to evaluate the toxicity of the effluent and analyze the pollutants in their isolated form or associated with other components (ARENZON, NETO and GERBER, 2011).

Ecotoxicological tests consist of using indicator species to assess the toxicity of environmental contaminants. Thus, the living organisms used in toxicity tests function as biosensors/bioindicators, organisms that respond to pollutants (DE PAIVA MAGALHÃES and FERÃO FILHO, 2008). The advantage of using living organisms is that they react to concentrations of substances below the limits of detection by chemical analysis methods, which allows a safe assessment of the toxic potential of substances or contaminated media (MANAHAN, 2010). However, it is necessary to use acute and chronic exposure bioassays in different species. This is because toxic agents act differently in organisms, and not all life forms are equally susceptible to the same xenobiotic (FERNÁNDEZ-ALBA *et al.*, 2001). For this reason, evaluating one or more biological parameters (biomarkers), which can be behavioral, morphological, physiological, biochemical, or molecular, is suggested.

Improper disposal of effluents has negative impacts on the environment and human health. In Brazilian legislation, the resolutions of the National Council for the Environment (CONAMA) RE 357/2005 and RE 430/2011 address the use of indicator species to evaluate the effluents' toxicity. Despite the existence of these resolutions, much still needs to be done to control and monitor the emission of effluents in Brazilian water resources. This work reviews Brazilian legislation regarding the disposal of effluents, focusing on the use of legally accepted bioassays and highlighting the advantages and disadvantages of each model.

2. LEGISLATION: THE HISTORICAL ASPECT

Humanity has directly or indirectly consumed natural resources rampant in pursuit of its social and financial well-being. To minimize the impacts generated, the federal and some state governments have been implementing measures since 1970 to preserve the environment. **Figure 1** summarizes the main events and resolutions implemented in Brazil.

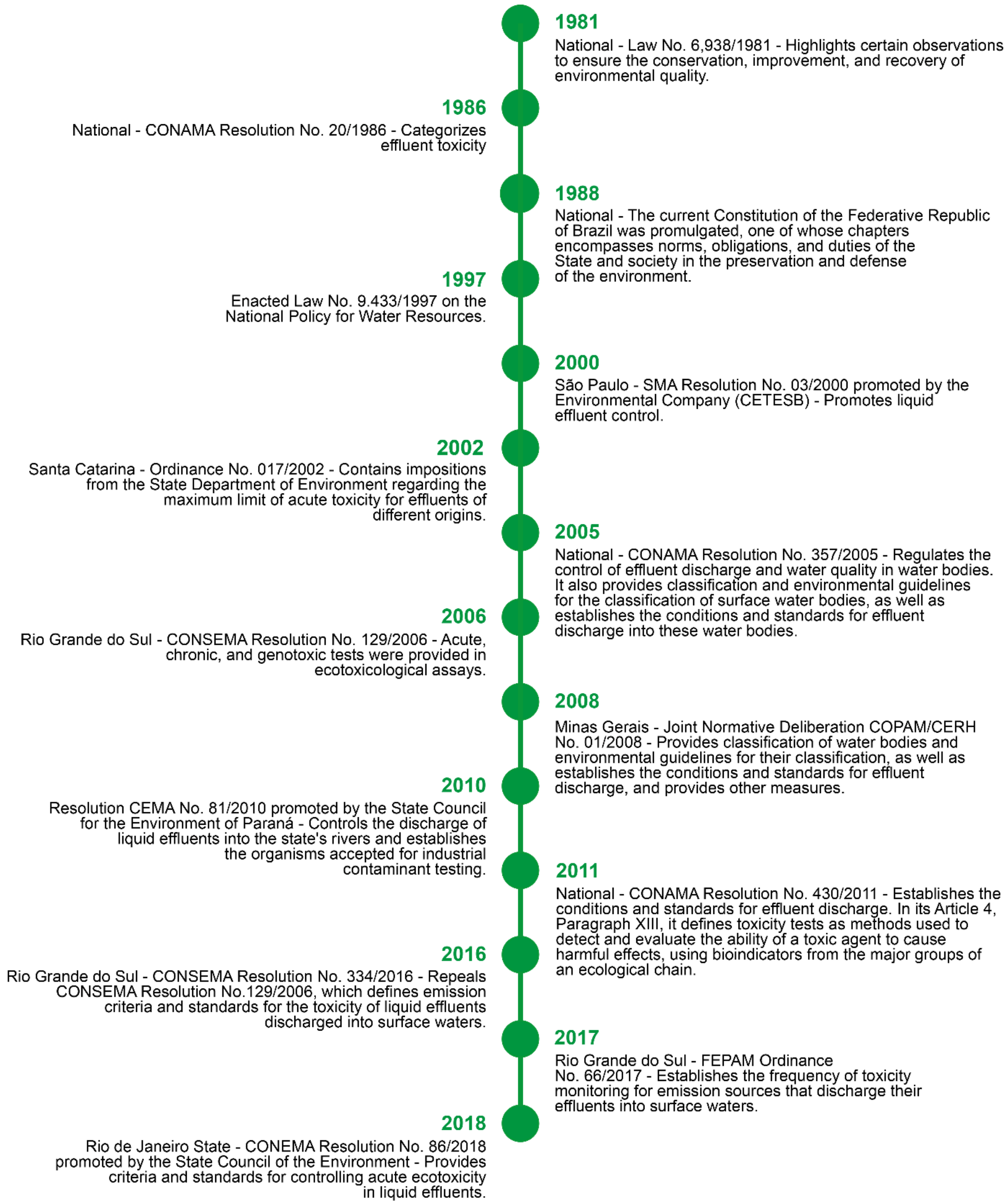
In the 1970s, the Federal Instance was implemented in Brazil, defined by Law No. 6.938/1981, which mentions the importance of conservation, improvement, and recovery of environmental quality. However, it was only in 1988 that the Brazilian government promulgated the Federal Constitution, with a chapter in which it declares the norms, obligations, and duties of the State and society in the preservation and defense of the environment. After almost a decade, in 1997, the National Water Resources Policy was decreed in Brazil with Law No. 9.433, which makes explicit, in the 9th and 10th articles, the responsibility of the environmental legislation in establishing the classes of water bodies, followed by the framework of water resources (NIVA and BROWN, 2019).

Bodies of water are classified into four classes according to the treatment carried out and the purpose for human consumption. Therefore, water quality must be based not only on its momentary evaluation state but also on the categorization of its use and social consumption. In this sense, the classifications aid water reuse through the degree of quality (CONAMA, 2005). Moreover, waters are classified by CONAMA No. 357 according to the salinity percentage presented in freshwater (salinity $\leq 0.5\%$), brackish (salinity $> 0.5\%$ and $< 30\%$), and saline water ($\geq 30\%$).

After the classification of water bodies in 1986, a concern arose in Brazilian legislation to categorize the toxicity of effluents. However, approaches were vague, as the government did not mention how the pollutants would be evaluated, under what conditions, and the possible standards of acceptability (SANCHEZ-GALAN *et al.*, 1999). Thus, even after CONAMA Resolution No. 20, effluents continued to be discharged into water resources (CESAR, SILVA and SANTOS, 1997). In recognition of this failure, in 2005, CONAMA Resolution No. 357 was published, which established that effluents from any polluting source could not be released into water flows if they presented toxic effects to aquatic organisms. Thus, the legislation gave the states the responsibility to stipulate the ecotoxicological criteria. In this way, the federal environmental agency was responsible for outlining the tests according to the characteristics of the effluents and the organisms receiving the toxic agent (CONAMA, 2005). On the other hand, the states' environmental agencies were responsible for designating the methodology, the bioindicators, the periodicity of the analyzes, and the acceptance levels to be used in their territory. However, the flexibilization built into CONAMA Resolution 375/2005 generated a low integration of the states, with no homogeneity in requirements and therefore low credibility of the legislation.

For this reason, CONAMA Resolution No. 357 was changed to complement the instructions, it order to adapt and stipulate assertive definitions of the conditions and standards for effluents to be released into water bodies, resulting in CONAMA Resolution No. 430/2011. From then on, it was decided that the environmental agencies of all Brazilian states should indicate the ecotoxicity tests, their reference standards, and the other analysis scenarios (GAZOLA, 2020).

The applicability of CONAMA No. 430 was to help the states that did not support the monitoring proposals for toxic components, requiring them to carry out tests of acute and chronic natures in at least two test organisms (SILVA, POMPÊO and PAIVA, 2015). Therefore, according to Arenzon (2017), freedom in choosing the method ends up allowing the choice of species that are more convenient according to the interests of those evaluated. Currently, despite the existence of legislation, adherence by the states is still low. Only São Paulo, Santa Catarina, Paraná, Rio de Janeiro, Minas Gerais, and Rio Grande do Sul provide toxicological tests for effluents to be released into water bodies (RUBINGER, 2009).



Source: elaborated by the authors (2023).

Figure 1 – Timeline with the main events and resolutions implemented in Brazil.

3. GUIDELINES ADOPTED IN THE STATES

Figure 2 shows the states that have created their guidelines and the species accepted in testing.

In the State of São Paulo, implementation of the Resolution of the State Secretariat for the Environment (SMA No. 03/2000) was effected by the Environmental Company (CETESB) in the 1990s, with the control of liquid effluents. The development of regulations led to improved environmental control, and toxicity tests began to help investigate fish mortality in environmental accidents. In addition, the state created the Manual for the Ecotoxicological Control of Effluents in São Paulo, with fundamental instructions for the ecotoxicological

Source: elaborated by the authors (2023).

Figure 2 – States and species accepted in their guidelines. Green: States that have their own legislation; Red: states that do not have their own legislation; 1 to 12: indicator species accepted in the legislation of each state.

control of liquid effluents. According to it, bioassays should use the species *Daphnia similis* and *Ceriodaphnia dubia* to assess the acute and chronic toxicity, respectively, of freshwater effluents. For effluents in marine environments, a simultaneous test of the acute assays with mysids or *Vibrio fischeri* and the chronic test with sea urchins should be standardized (BERTOLETTI, 2009).

In Santa Catarina, Ordinance No. 017/2002 was instituted, containing impositions from the Environment Secretariat regarding the maximum limit of acute toxicity for effluents from different sources unable to present any signs of toxic effects and morphological/physiological alterations. The decree also defined the methods of acute toxicity for *Daphnia magna* and *Vibrio fischeri* from industrial effluents (GAZOLA, 2020).

In Paraná, the State Council for the Environment (CEMA) created CEMA Resolution No. 81/2010 in 2010. It controls the disposal of liquid effluents in the state's rivers and establishes the organisms accepted for testing industrial contaminants to analyze toxic agents from various sources. For freshwater effluent assessments, *Daphnia magna* and *Vibrio fischeri* were the species included for acute toxicological assessment. For chronic tests, the legislation requests verification of the origin of the effluent to designate the most viable organism, usually *Ceriodaphnia dubia* or *Scenedesmus subspicatus*. In brackish waters, acute tests should use *Vibrio fischeri* or mysids (*Mysidopsis juniae*/*Mysidopsis gracile*). In chronic tests, the recommended species are *Lythechinus variegatus* or *Echinometra lacunter* and *Skeletonema costatum* (RUBINGER, 2009).

CONEMA Resolution No. 86/2018 was established for the State of Rio de Janeiro. When compared to the other states, this state has an environmental legislation that is supported by other legal determinations, as its implementation is combined with the Criteria and Standards for Control of Acute Ecotoxicity in Liquid Effluents (NOP-INEA-08-REV00), which establishes the use of ecotoxicological tests as essential for the Environmental Licensing System. For the acute tests, assays with *Danio rerio* and *Pimephales promelas* fish, microcrustaceans of the genus *Daphnia* and the luminescent bacteria *Vibrio fischeri* are included. In this resolution, there are no evaluation criteria for chronic trials. Aiming at companies' commitment to the treatment and ecotoxicological testing of the effluent, as well as compliance with monitoring by CONEMA, the resolution outlines environmental goals to improve the current scenario, including a progressive evaluation after two years of the proposal's approval. An interesting observation regards the description of the duties of businesses. Entrepreneurs can request the replacement of test organisms, and these tests can be carried out by third-party and accredited laboratories which make use of the methods recommended by the Brazilian Association of Technical Standards — ABNT (GAZOLA, 2020).

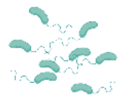
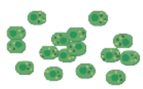
In Minas Gerais, the bodies responsible for inspecting environmental activities are the State Environmental Policy Council (COPAM) and the State Water Resources Council (CERH). These sectors combined their knowledge, made theoretical revisions, and developed the Joint Normative Deliberation COPAM/CERH No. 01/2008. The document presents all crucial information for effluent treatment, the classification of water bodies, as well as principles and guidelines for carrying out bioassays. Although the state took the first step, the regulation does not include toxicity criteria. Therefore, it is essential to be guided by CONAMA Resolution No. 430/2011.

The State Council for the Environment (CONSEMA) Resolutions 357/2005 and 430/2011 sought, in 2006, to publish the first resolution on ecotoxicological tests in Rio Grande do Sul. Resolution No. 129 provided for acute, chronic, and genotoxicity tests. However, it was revoked by Resolution No. 334/2016. In 2017, State Environmental Protection Foundation (FEPAM) Ordinance No. 66 was published, which establishes the frequency of toxicity monitoring for emission sources that discharge their effluents into surface waters in the territory of Rio Grande do Sul. This monitoring frequency is based on the flow of effluent released into water bodies by companies. In addition, this Ordinance requires that the ecotoxicity analysis be carried out in at least two trophic levels following the criteria of § 3, article 18 of CONAMA Resolution No. 430/2011 (RUBINGER, 2009).

The other Brazilian states have not yet presented their own regulations following the CONAMA Federal Resolution No. 430/2011. This resolution, however, gives the states the responsibility to establish their choices regarding the methodology of acute and chronic toxicity tests, using at least two test organisms from different trophic levels. These definitions are paramount, as they establish standards in regulating tests and releasing industrial effluents into water resources. Ideally, the states should regulate the use of organisms representing three different trophic levels to achieve effective results and eliminate the possibility of contradictory results (SILVA, POMPÊO and PAIVA, 2015).

4. CHARACTERISTICS OF THE MAIN ECOTOXICOLOGICAL BIOASSAYS ACCEPTED IN BRAZIL

In Brazil, the ABNT defines the main methodologies used in effluent toxicity assessments. Specific guidelines from all Brazilian states must comply with these standards. We gathered the main advantages, limitations, and endpoints of bioassays accepted in Brazil (**Figure 3**).

BIOASSAYS	ENDPOINTS	BENEFITS	LIMITATIONS
Vibrio fischeri 	Luminescence	Acute, rapid, low test cost, high sensitivity, substance analysis solid and liquid	Low sensitivity to antibiotics
Microcrustaceans 	Mobility, number of heartbeats, feeding and intake rate, offspring and enzyme/protein evaluation	Fast, high sensitivity, acute and chronic test, low cost, multigenerational and transparent body	Complex and costly reagents/solutions, light and temperature control
Scenedesmus subspicatus 	Growth rate of the species, production of photosynthetic pigments, determination of extracellular proteins and carbohydrates	Acute and chronic test sensible, economical, quick answer	Difficulty in reproducing the natural conditions of the aquatic environment (e.g. pH)
Fish 	Hatching rate, mobility, morphometric, physiological, biochemical and genetic parameters	Acute and chronic test, multigenerational, high similarity to biological systems, fast development, high fecundity and adaptability, easy maintenance	Use of pools of embryos and larvae, via of dermal exposure, sexual dimorphism only three weeks after fertilization

Source: elaborated by the authors (2023).

Figure 3 – Description of the main bioassays in legislation, their advantages, and limitations.

3.1 *Vibrio fischeri*

Among the methodologies developed by ABNT (2012a, 2012b, 2012c), the Brazilian Norm — NBR 15411 (1,2 and 3) offers the possibility of using the bioluminescent marine bacterium *Vibrio fischeri* for monitoring the chronic toxicity of different types of samples. This bioassay is widely used for acute ecotoxicological tests (**Figure 3**), evaluating the toxic potential of substances released into aquatic ecosystems. It is a species of Gram-negative bacteria with a rod shape and flagellum, which allows it to move. It can live in a mutualistic relationship, colonizing some species, such as squid and fish, which emit light from *V. fischeri* (ABBAS *et al*., 2018; DUNN *et al*., 2012).

The bioassay evaluation mechanism occurs by monitoring the luminescence emitted by *V. fischeri* and its changes in contact with the tested substance (DUNN *et al*., 2012). The *V. fischeri* bioluminescence mechanism is regulated by luxCDABEG genes that encode all components for light production. The α and β subunits produce the enzyme luciferase, with light emissions during oxidation of a long-chain aldehyde and reduced flavin (MIYASHIRO and RUBY, 2012). In this way, it is possible to evaluate possible toxic effects of altering the metabolic pathway of bioluminescence, such as the inhibition of light emitted by *V. fischeri* (ABBAS *et al*., 2018).

There is a commercial version of this assay called Microtox®. The basic procedure evaluates toxicological changes that can cause alterations in bacterial growth and inhibition of enzymatic activity, consequently decreasing luminescence (ABBAS *et al*., 2018). This bioassay is characterized as acute, as the observation of toxicological effects is quick. In addition, the bioassay has high sensitivity, good reproducibility, and low cost, and can be applied to liquid and solid samples (MONTALBÁN *et al*., 2016; ABBAS *et al*., 2018).

The use of *V. fischeri* as a bioindicator is widely known. The bioassay is used to assess the toxicological risks of residential and industrial effluents and to verify whether the treatments carried out for the disposal of effluents are effective. If the effluents are discarded without prior treatment, they can generate toxicological contamination in water bodies, depending on the disposal site (FLOHR, CASTILHOS JÚNIOR and MATIAS, 2012). The *V. fischeri* bioassay has been used to evaluate the ecotoxicity of different compounds, such as antibiotics (FROEHNER, BACKHAUS and GRIMME, 2000; IOELE, DE LUCA and RAGNO, 2016), ionic liquids (MONTALBÁN *et al*., 2016), and pesticides (POLEZA *et al*., 2008), making their use appropriate for ecotoxicological assessments.

3.2 Bioassays with Microcrustaceans

3.2.1 Daphnia magna *and* Ceriodaphnia dubia

The ABNT also suggests the use of microcrustaceans as another possibility to monitor chronic toxicity in effluents. NBR 12713 (ABNT, 2009) regulates the use of *Daphnia spp* (Cladocera, Crustacea) and NBR 13373 (ABNT, 2010) the use of *Ceriodaphnia ssp*.

Popularly known as "water fleas", *Daphnia magna* and *Ceriodaphnia dubia* are small, freshwater microcrustaceans with short life cycles, parthenogenetic reproduction, large litter sizes, and high population growth rates (SARMA and NANDINI, 2006). Differences between species consist in the fact that *C. dubia* has a wide aquatic distribution, is morphologically smaller, more rounded, and does not have a prominent rostral projection. *D. magna*, on the other hand, is larger, oval, has a prominent rostral projection and is typical of temperate regions

(TKACZYK *et al.*, 2020). Both are highly sensitive to different toxic substances and are used as bioindicators in the toxicological assessment of various environmental contaminants (VERSTEEG *et al.*, 1997) (**Figure 3**).

In addition to high sensitivity, the main advantages of using these organisms are fast, low-cost, easy-to-maintain assays, transparent bodies, and multigenerational analyses (SARMA and NANDINI, 2006). Furthermore, both aspects of acute toxicity (≅ 24 h), such as the occurrence of immobilization damage and mortality, and those related to chronic toxicity (≅ 21 days) can be evaluated in more than one generation of descendants, allowing the observation of the impact of environmental stressors in individuals of different ages and generations (TKACZYK *et al.*, 2020).

In contrast, the use of microcrustaceans is limited by lack of culture methods for each species. For many species, the specialized ecological requirements are often impracticable. Moreover, it is essential to control temperature and luminosity in this test since changes in these variables can lead to the death of organisms. In addition, there is sensitivity variation to toxicants during the life cycle (MACIOROWSKI and CLARKE, 1980).

Toxic effects can be observed from: physiological parameters, according to the number of heartbeats, the rate of feeding and ingestion, which can be evaluated non-invasively due to the transparent body; behavioral parameters, such as the quantification of changes in feeding, jumping frequency, and swimming behavior; reproductive parameters, such as quantification of neonates and assessment of the effect on multigenerational exposure; and by enzymatic and non-enzymatic biochemical activities, allowing the analysis of metabolic changes related to other biomarkers as a way to understand the changes in the life habit of the microcrustacean (TKACZYK *et al.*, 2020). These different markers expand the use of these species, as they allow the analysis of the toxic potential of different chemical compounds present in residential/industrial effluents that can be released into aquatic ecosystems during disposal. In addition, it is possible to predict the effects on the biota since some compounds can be bioaccumulated, causing biomagnification (KIM *et al.*, 2018).

Several scientific studies have demonstrated the potential of these organisms (*D. magna* and *C. dubia*) in the assessment of acute and/or chronic toxicity in the aquatic environment after episodes of punctual pollution (MEBANE *et al.*, 2021), exposure to metals (OKAMOTO, MASUNAGA and TATARAZAKO, 2021) and other contaminants (GOMES *et al.*, 2018), nanoparticles (ISWARYA *et al.*, 2018), insecticides (RABY *et al.*, 2018), various drugs, such as antibiotics and anti-inflammatories (TKACZYK *et al.*, 2020), and effluents from industry (BRIX, GERDES and GROSELL, 2010; RAPTIS, JURASKE and HELLWEG, 2014), proving to be standardized and reliable models in the analysis of ecotoxicological agents.

3.3 *Scenedesmus subspicatus*

Scenedesmus subspicatus is a freshwater green microalgae rich in lipids, lutein, and proteins. It contributes to primary production in most habitats and responds rapidly when exposed to contaminants, thus predicting the first impacts on the ecosystem (FAWAZ, KAMAREDDINE and SALAM, 2019). The advantages of using this species in bioassays are its high sensitivity to different toxic substances and relatively short life cycle, allowing the observation of probable effects in several generations. Therefore, this bioassay is considered sensitive, economical, and fast-response (**Figure 3**). The NBR 12648 (ABNT, 2011b) defines the methodologies used in toxicity assessments using *Scenedesmus subspicatus*.

However, this bioassay also exhibits some limitations, such as difficulty in mimicking the favorable conditions (salt and mineral concentration, temperature, and pH) of the species' natural environment (LIN *et al.*, 2005). The pH is the most critical among these conditions due to its sensitivity to variation during the test. It is influenced by the metabolism of algae (photosynthesis) and may occur outside the range typically found in aquatic environments (between 6 and 9), causing damage to the development and welfare of the species, thus decreasing fidelity in the effects of the compounds tested (PETERSON,1994).

In toxicological tests, effects on the development of the species are evaluated, such as growth rate (inhibition or stimulation) and biochemical characteristics, such as the production of photosynthetic pigments, determination of proteins, and extracellular carbohydrates. In addition, *S. subspicatus* can be used as a study model both directly, being the research organism, or indirectly, in biomagnification research, serving as food for other organisms, such as *Daphnia magna* (DAI *et al.*, 2013). For tests with microalgae, chronic tests require a time of 96 hours (ABNT, 2021).

Due to its characteristics, several studies have shown that *S. subspicatus* has a high potential for use in evaluation tests of industrial effluents, whether from logging (KACZALA *et al.*, 2011) or food by-products (REGINATTO *et al.*, 2009). In addition, this species has also been applied in tests to determine the impacts of surface water contaminants (HYBSKÁ *et al.*, 2018) and evaluation of the toxicity promoted by herbicides (VENDRELL *et al.*, 2009). Therefore, *S. subspicatus* can identify the ecotoxicological risks of substances, which can be used in the future as tools for environmental management.

3.4 Bioassays with fish

In the ecotoxicological evaluation of contaminants present in water, Brazilian legislation suggests using species such as *Danio rerio* and *Pimephales promelas*. These species have protocols and guidelines standardized by the Organization for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO) for acute and chronic assays and by the ABNT (2007, 2011a). Considering that the effluent may suffer dilution when released into water resources, it is important to evaluate acute and chronic toxicities. In this way, it is possible to verify sublethal effects resulting from this exposure using bioaccumulation markers and studying the behavior of environmental contaminants at low levels (VAN DER OOST, BEYER and VERMEULEN, 2003).

In the case of *Danio rerio*, also known as zebrafish, knowledge of the morphological, physiological, biochemical, and genetic parameters in all its stages of development, as well as in both sexes, makes this fish an ideal model system to be used in toxicology research to identify the adverse effects of xenobiotics (CANEDO and ROCHA, 2021). In addition, zebrafish are small (about 4–5 cm), have rapid development, high fecundity, high adaptability to the laboratory environment, and easy maintenance (RIBEIRO *et al.*, 2022). Rapid development is an excellent feature in genotoxicity studies, as it allows monitoring of all toxic effects in a short period and even transgenerational implications (DAI *et al.*, 2014). Another advantage of the species is the transparency of the embryos, which allows for monitoring and identifying the effect of xenobiotics on the development of the individual (GIANNACCINI *et al.*, 2014; SIEBER *et al.*, 2019). Regarding the ethics of using animals, tests with zebrafish fit the 3 Rs concept (CANEDO *et al.*, 2022), in addition to being cheap, fast, and requiring fewer chemicals in exposure, as they can use many embryos in a single reproduction (DAI *et al.*, 2014). Regarding similarity, 70% of zebrafish genes are orthologous to humans (HOWE *et al*., 2013). These characteristics make zebrafish ideal models for research on gene functions (VARSHNEY *et al*., 2013).

However, this model also has limitations, including the need to use pools of embryos and larvae due to their small blood volume and the number of cells/biological material (SIEBER *et al*., 2019). Another limitation is the route of exposure. Using the test substance diluted in water, it will generally be absorbed by the fish via the dermal route. These results may differ in humans, who are mainly exposed orally, which affects the mode of pollutant absorption, distribution, metabolism, and excretion (BAMBINO and CHU, 2017). Another issue is that some pollutants behave differently depending on gender. However, zebrafish have no genetically discernible sex and only exhibit sexual dimorphism three weeks after fertilization. Despite the limitations, interest in this species has grown immensely in recent decades, which is easily verified by the number of published scientific articles, and the species is currently a reference in toxicology (RIBEIRO *et al*., 2022).

Another species commonly used in ecotoxicological tests is *Pimephales promelas*, belonging to the Cypriniformes fish group. This species is tolerant to environmental conditions and adapts well to poorly oxygenated bodies of water (CASARES *et al*., 2013). Thus, it is an important tool for detecting toxic effects in wastewater, and is also considered a model sensitive to variations in industrial and agricultural chemicals and mainly urban effluents (CARNIKIAN, MIGUEZ and VIZZIANO-CANTONNET, 2011). The most used parameters are biochemical ones, through the evaluation of metabolic processes using enzymatic activities, considered important biomarkers for oxidative stress and neurotoxicity with acute and chronic responses of effluents (AICH *et al*., 2015). It is also possible to perform histopathological analyses to assess potential tissue damage (MARINS *et al*., 2020).

CONCLUSIONS

The quality of the environment directly influences One Health, and environmental protection laws arise to ensure the maintenance of the ecological balance of human and animal health (ANTUNES, 2001). In this context, a survey was carried out of the methodologies used to detect the toxicity of effluents and their advantages and limitations.

In Brazil, toxicological contaminants are not identified by the analyses commonly performed by sewage treatment plants (SILVA, POMPÊO and PAIVA, 2015). This is because the treatment of Brazilian effluents only aims to eliminate microbiological loads and guarantee the physicochemical aspects of the water, seeking the quality of the specified parameters for its return to rivers and lakes. The chemical and physical protocols commonly used to determine water quality, established by environmental regulations, are not satisfactorily acceptable for assessing the potential environmental risk of contaminants, because the methods applied do not have the necessary robustness to distinguish the substances that affect the biological system from those that are inert in the environment (COSTA *et al*., 2008). On the other hand, bioassays allow the detection of the toxic effects of substances, demonstrating the importance of this type of analysis in verifying the presence or absence of risks to the environment where the object of study was found (DE PAIVA MAGALHÃES and FILHO, 2008). The combined use of physicochemical and ecotoxicological tools in environmental monitoring increases the potential for detecting environmental pollutants (SILVA, POMPÊO and PAIVA, 2015) and helps in the construction of concepts and definition of criteria for the safe disposal of effluents (LOUREIRO *et al*., 2006; LYUBENOVA, 2011).

Although a few states of the Brazilian Federation have sought environmental adequacy, including ecotoxicological tests, these resolutions still present the same problem: a flexibility in the choice of species and the performance of tests on indicator species of two trophic levels. This makes evaluations worrying and inconclusive since the choice of species may be related to convenience and greater tolerance of the organisms in relation to the test substance. This demonstrates that the current legislation is permissive, requiring modifications to minimize gaps, such as increasing the testing requirement to three or even four different trophic levels, increasing the probability of detecting total toxicity in the samples. Without a critical analysis by the environmental agency, many results provided in the monitoring stage can be falsely considered non-toxic. Given the above, it is noticeable that the Brazilian states have difficulties adhering to ecotoxicological monitoring of water resources.

ACKNOWLEDGEMENTS

We would like to thank the Brazilian funding agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG). LMA and EFLCB thank Universidade Estadual de Goiás (UEG) for the financial support (grants PrP 04/2021, PrP 05/2021, PrP 01/2023).

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