Water Quality Assessment Using *Daphnia*: A Brief Review

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Due to the need to discover methods that can measure the toxicity level of fresh and marine water quality, this study aimed to present a systematic review of the literature, indicating the culture conditions and toxicological tests used for different *Daphnia* species in the evaluation of water quality. Thus, the applied methodology was a systematic review that identified studies that addressed the application of *Daphnia* in the assessment of water quality. The results obtained from this research consisted of a compilation of articles, which presented the parameters most analyzed in Daphnia species, such as mobility and mortality, which can change when exposed to different chemical substances.
Keywords: *Daphnia*. Water Quality. Toxicity.

Introduction

Unfortunately, the environment has been suffering the consequences of the impact of anthropogenic activities over the years, especially the aquatic environment, where numerous chemical compounds are found in it, which in addition to promoting ocean contamination, leaves this environment toxic and harmful to beings that live in it [1]. Therefore, research has been developed in search of methods that can measure the toxicology of these compounds on water quality, and their impact on the aquatic ecosystem, due to the lack of analytical tests that can make such information viable [2].

In this scenario of pollution in the oceans, there are aquatic beings that present a sensitivity to the chemicals that are dumped into the water, in a way that it is possible to detect the level of toxicology of these compounds and how they can affect these beings [2,3]. These organisms are microcrustaceans belonging to zooplankton species, called *Daphnia*, which is considered the oldest model used in bioassays aimed at the toxicology of water quality [4]. It is associated with their behavioral and physiological characteristics, which respond to environmental bioindicators or chemical compounds [3].

Daphnia is a microcrustaceans belonging to the *Cladocera* order, which has species that can be found both in the sea and in freshwater, in the way that, due to their high sensitivity to different compounds, are widely applied in ecotoxicological tests [5]. Thus, the species most used for toxicological tests are *Daphnia magna*, *Daphnia smilis*, and *Daphnia pulex*. They are usually found in freshwater, besides being easily cultivated in laboratories. They respond when in contact with different chemical compounds [5,6].

However, although *Daphnia* species are similar to each other, they have a different level of sensitivity: one species may be more sensitive to some compounds than another [6]. So, in this point, toxicological studies are developed to compare the impact of contaminants in more than one species of *Daphnia* [6]. Therefore, the most observed effect in toxicological tests is the mobility of organisms exposed to chemical substances soluble or dispersed in water [7].

In this context, it is necessary to emphasize that the toxicity tests involving *Daphnia* follow criteria for carrying out the tests of these species, since, according to the CONAMA Nº 430 DE 13/05/2011 resolution, the ecotoxicological tests realized in effluent must use aquatic organisms of at least two different trophic levels [8]. Thus, for the manipulation of *Daphnia* in the laboratory, one of the most used guidelines is the OECD Guidelines for the Testing of Chemicals, whose main objective is to guide the best way in the

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acute toxicity tests of *Daphnia*, providing the necessary information for the cultivation of these beings (culture medium, solutions, test medium, etc), in addition to the ideal time of exposure of the organism to the analyzed substance [9]. In the case of the OECD, the two standards used are OECD 211, which is specifically aimed at *Daphnia magna*, and OECD 202, which can be used for different species of *Daphnia* [9, 10].

Although standards are essential to contribute to effective analyzes in the use of *Daphnia* in the laboratory, it is necessary that the country where the study is performed, has this type of guideline, to ensure that the tests are in the appropriate conditions for analysis [6]. Thus, NBR12713, established by the Brazilian Association of Technical Standards (ABNT) has as its main guideline, specifying a method for evaluating the acute toxicity of liquid samples and water-soluble or dispersed chemical substances, using the species of *Daphnia similis* and *Daphnia magna* [7].

Therefore, this article aims to present a systematic review of literature, indicating the culture conditions and toxicological tests used for the analysis of different *Daphnia* species.

Materials and Methods

We did a systematic review of the literature, focused on studies of the application of *Daphnia* in toxicological tests for the assessment of water quality. The flow of this systematic review is described in Figure 1.

As previously presented, the systematic review of this study was performed in the following steps:

i) Choice of keywords with Boolean operators (and, or): "water quality", "toxicity", "*daphnia* test", "*daphnia*", "characteristics".
ii) Use of databases: Scopus (www.scopus.com), science direct (www.sciencedirect.com), capes (www-periodicos-capes-gov-br.ez68.periodicos.capes.gov.br), web of science (www. webofknowledge.com) and google of scholar (https://scholar.google.com.br).
iii) Search period: 2011 to 2021.
iv) Application of filters, aimed to synthesize the articles found during the search.

The use of Boolean operators (and, or) was important for a more refined result of articles that approached studies of *Daphnia* in toxicological tests, in addition to the use of filters, that served as a facilitator for the convergence of articles that attend the purpose of this study. However, although this method has allowed the acquisition of valid articles for this study, it is necessary to remember that in the current pandemic scenario, the remote access to these databases was not so simple, in a way that, the path used for the access of the databases mentioned previously, was through Google Scholar and Cape's Periodic.

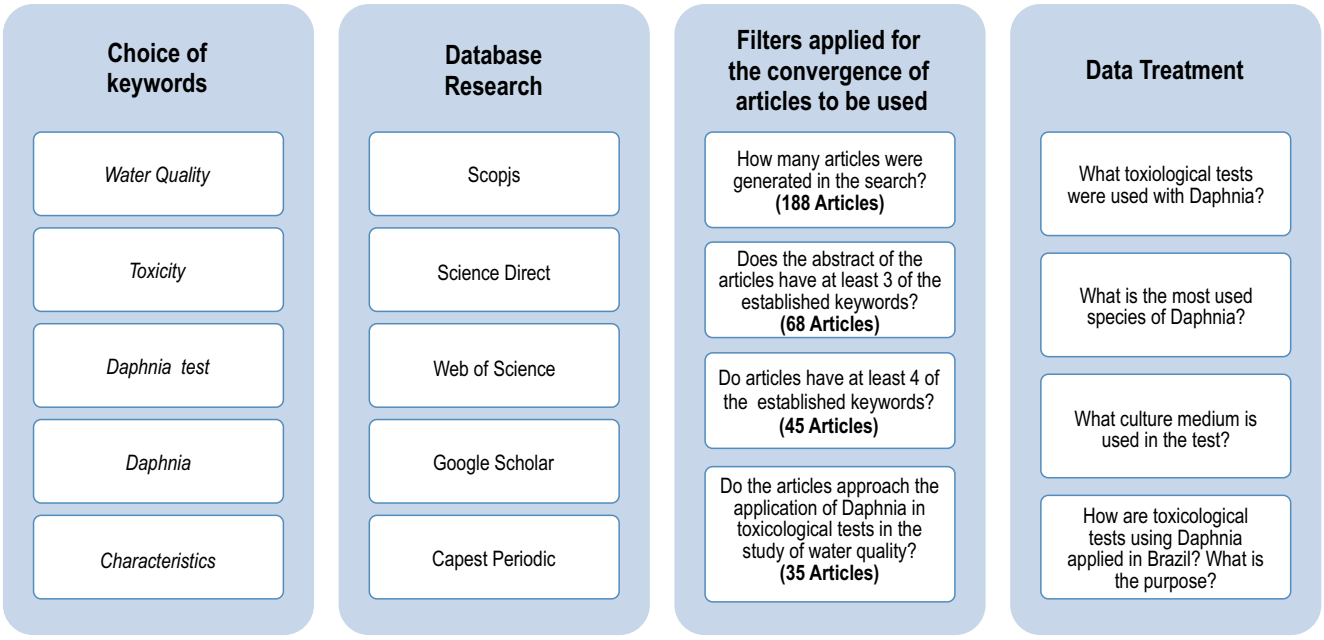
Results and Discussion

From the systematic review, it was possible to compile some articles, comparing the toxicological tests with the use of *Daphnia*, the most used *Daphnia* species, the culture medium used, and the toxicological parameters analyzed (Table 1).

In this study, we could not possible present all the articles found since the number of them was too high for this information space. Thus, six articles are presented that correlate the methods and standards used for it.

From the comparative analysis presented in Table 1, it was possible to detect that most of the explained articles used the *Daphnia magna* species for toxicological bioassays. This data is linked to the fact that *D. magna* is widely used as a bioindicator for the analysis of water quality since it has a short doubling time and high sensitivity [11]. However, *Daphnia similis* also has a significant level of importance when referred to ecotoxicological assessments, for being broadly distributed in the world, as in Europe, North America, and South America [6, 12]. In Brazil, it is frequently used for tests and considered a standard species, due to

Figure 1. Systematic review flow.



its accessibility in temporary, shallow, and turbid lakes [12].

The toxicological tests, in all the studies presented, used the mobility of organisms as an essential parameter for analysis. The immobilization test of these beings indicated for how long of an exposure to the analyzed compound, these organisms tend to lose their mobility [12, 14]. It was analyzed how long these beings exposed to chemical substances survive and what is the necessary age of *Daphnia* to be used for acute toxicity tests [12-14]. In addition, some articles approached the study of *Daphnia* species compared to other trophic levels, such as algae and zebrafish, to measure the sensitivity of these organisms in the presence of thallium and it can be observed that *Daphnia* species are less sensitive than the microalgae analyzed [14].

Due to the use of different species in the articles presented, a table was made with the studied *Daphnia* species, indicating its applicability in toxicological tests and the parameters analyzed for these organisms (Table 2).

We point out that these indicators are associated with the physiological system of *Daphnia*, such as mortality, which is associated with feeding activities determined by filtration and ingestion rates (Figure 1). The filtration rate consists of the volume of medium released per unit of time, in which the filtration process takes place in the thoracic limbs (the activity of the thoracic limbs is measured by calculating their beats per unit of time), generating a stream of water throughout the which food particles are directed to the mouth for ingestion [15]. The ingestion rate corresponds to the number of cells consumed by *Daphnia* in a specific period. It is noteworthy that these two rates can be quantified by measuring the decrease in particle concentration in water over time and by analyzing the material accumulated in the viscera [15]. In this context, the indicators of toxicity to chemical substances were analyzed (Table 2), for different species of *Daphnia* (*magna*, *similis*, and *pulex*). It was observed that all articles analyzed the same parameters, however, mortality was one of the most analyzed indicators, showing a degree

Table 1. Comparison of toxicological studies using *Daphnia* species.

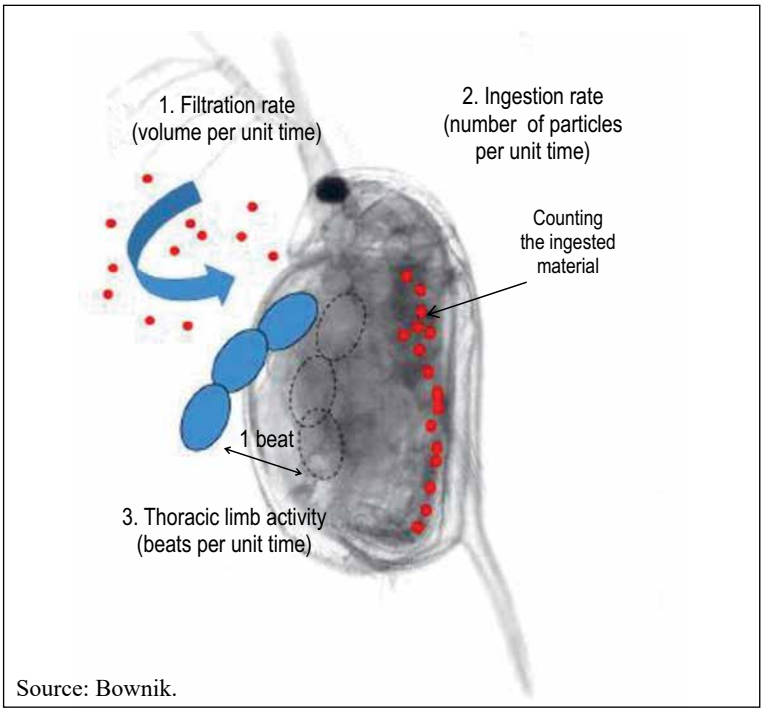
Article Title	Country	Evaluation Site	Year	Article Purpose	Tested Daphnia	Toxicity Test
Advanceents in effect-based surface water quality assessment [1]	Netherlands	Laboratory of the University of Amsterdam	2020	Evaluate the water quality and get analytical answers about specific sources of contamination	*Daphnia magna*	Bioassays performed on dilution series of extracts from all three passive samplers, resulting in nine *in vivo* responses which was immobilized for 48 hours of exposure
Assessing domestic wastewater effluent with a battery of bioassays after treatment with a specific consortium of microalgae and different flocculation methods [11]	South Africa	University of Stellenbosch	2020	To determine the toxicity of supernatants resulting from alum coagulation and chitosan flocculation of algae biomass from wastewater effluent using the bioindicator species	*Daphnia magna*	Five newborns of similar size were selected for each tray compartment. A series of five samples were selected for each solution tested. Mortality was defined as a lack of movement after a gentle nudge after 24 and 48 h
Toxicity of lead and mancozeb differs in two monophyletic *Daphnia* species [6]	Brazil and Portugal	Department of Biology & CESAM, University of Aveiro, 3810-193, Portugal	2019	To compare the sensitivity of two monophyletic *Daphnia* from different climatic areas to two different contaminants (inorganic and organic) to induce negative effects on the ecosystem	*Daphnia magna* and *Daphnia similis*	Newborns of both species between 6 and 24 h were exposed to a range of chemical concentrations for 21 days. For each species, the concentration range was based on its sensitivity to the test chemicals as well as the results of acute toxicity tests
Saxitoxi n-producing *Raphidiopsis raciborskii* (cyanobacteria) inhibits swimming and physiological parameters in *Daphnia similis* [12]	Brazil	Laboratory of Evaluation and Promotion of Environmental Health, Instituto Oswaldo Cruz, FIOCRUZ	2019	To test the effects of a neurotoxic strain of cyanobacterium *Raphidiopsis raciborskii* (CYRF-01) on swimming, activity and physiological parameters of *Daphnia similis*	Tested *Daphnia*	Acute tests were performed to detect the immobilization of *D. similis* during an exposure period of 48 h. The number of dead and immobilized individuals were counted after 30 min, 1, 2, 24 and 48 h after exposure
Protein profiling as early detection biomarkers for TiO_2 nanoparticle toxicity in *Daphnia magna* [13]	Portugal	Nova Lisboa University	2018	Evaluate the toxicity and effects of *Daphnia magna* exposed to TiO_2- NPs, through the response of the protein profile	*Daphnia similis*	The acute toxicity of TiO_2-NPs in *Daphnia magna* was evaluated according to ISO 6341, in which the inhibition of mobility of juveniles *D.magna*, aged between 6-24 h and exposed in 48 h
The acute toxicity of thallium to freshwater organisms: Implications for risk assessment [14]	United Kingdom	University of Plymouth	2015	To assess the acute toxicity of TI(1) to three key trophic species according standardized OECD methods	*Daphnia magna*	Newborns were exposed in triplicate (with 30 animals per treatment) and for 48 h to TI (I) concentrations ranging from 60 to 1200 pg I -1

Table 2. Comparison of applicability of different *Daphnia* species.

Daphnia Species	Application in toxicity	Parameter Analyzed
Daphnia magna [1, 6, 11, 13, 14]	Metal Exposure [1] Exposure to metal lead(Pb) and the fungicide mancozeb [6] Exposure to supernatants resulting from alum coagulation and chitosan flocculation of algae biomass from wastewater effluent [11] Exposure to TiO_2^- [13] Exposure to TI [14]	Mortality [1, 11, 13, 14] Immobilization [6, 11] Reproduction [6, 13]
Daphnia similis [6, 12]	Exposure to metal lead(Pb) and the fungicide mancozeb [6] Exposure to effects of a neurotoxic strain of cyanobacterium *Raphidiopsis raciborskii* [12]	Immobilization [6] Antennae movements, thoracic limbs, post- abdominal claw and heartrate [12]
Daphnia pulex [14]	Exposure to TI [14]	Mortality [14] Immobilization [14]

of sensitivity of species to exposure of these compounds: *Daphnia pulex* > *Daphnia similis* > *Daphnia magna*. These parameters were expressed in different ways in the studies, where the lethal concentration (LC) indicates the value range of a certain compound that can cause the death of *Daphnia*, as well as the effect concentration (EC), that indicates the responses that a determinate compound can induce in these organisms after a time of exposure (Figure 2).

Figure 2. *Daphnia* feeding activity.



Source: Bownik.

It was also identified that among the compounds analyzed in the studies, *Daphnia* species are more sensitive to metals. For example, the study that addresses the exposure of *Daphnia* to lead (Pb), showed that *Daphnia magna* tends to decrease in size compared to *Daphnia similis* [6]. Each *Daphnia* species reacts differently to a certain chemical compound, to the detriment of that, it is necessary that more studies are developed, to mitigate the toxicity of different compounds found in the aquatic environment.

Conclusion

Through the systematic review applied to this study, it was possible to generate a compilation of articles, which provided data for the use of the *Daphnia* species in the assessment of water quality toxicity, from the type of *Daphnia* to be used, to the ideal medium of culture and parameters to be analyzed. Therefore, from these studies, it was possible to detect that the species of *Daphnia magna* and *Daphnia similis* are the most used bioindicators in toxicological tests. This happens since they have a high sensitivity to chemical substances and physiology that allows analyzing the behavior of compounds in these beings. Also, it was possible to identify parameters (mortality, mobility, reproduction) in *Daphnia* that can change in the presence of chemical substances, such as mobility and mortality of these beings, that can indicate the level of toxicity of these chemical compounds.

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