

FIRST CHARACTERIZATION OF THE ECOTOXICOLOGIC EFFECTS OF WATERS FROM THE NORTH OF PORTUGAL, USING MORTALITY OF THE CRUSTACEAN *DAPHNIA MAGNA* AS EFFECT CRITERION

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ABSTRACT

Water contamination can result in the deterioration of its ecological quality and value for human consumption. The present work assessed the ecotoxicologic effects of potential contaminants present in several water samples from varied provenience (aquifer, municipal spring, rivers, public distribution system and treated effluents from a car garage). The adopted end-point was immobilization of *Daphnia magna* juveniles. The obtained results show high toxicity for samples collected from the aquifer, municipal spring and public distribution system. Intermediate toxicity was attributed to the treated garage effluents. No toxicity was observed for river water.

KEYWORDS

Ecotoxicology; Freshwater; *Daphnia magna*; Acute lethality

RESUMO

A contaminação da água pode resultar na deterioração da sua qualidade tanto ecológica como para consumo humano. Este trabalho avaliou os efeitos ecotoxicológicos de contaminantes em amostras de água de diversa proveniência (aquífero, fonte municipal, rios, água de distribuição pública e efluentes tratados de uma oficina). O parâmetro avaliado foi a imobilização de juvenis de *Daphnia magna*. Os resultados obtidos demonstram alta toxicidade para amostras recolhidas no aquífero, fonte municipal e água de rede pública. Toxicidade intermédia foi observada para os efluentes tratados da oficina. Nenhuma toxicidade foi observada para a água dos rios.

PALAVRAS-CHAVE

Ecotoxicologia; Água doce; *Daphnia magna*; Letalidade aguda

1. INTRODUCTION

Water for human consumption comes primarily from rivers, lakes, dams (and are generically designated as surface waters) and aquifers (underground water; Mendes and Oliveira). These two main types of water sources possess varied quality characteristics, which derive from the environments by which they circulate and accumulate (Silveira). Besides this natural influence, different waters can also contact with anthropogenic residues that can alter their properties and also contaminate them with several substances with ecologic and toxicological relevance (Mendes and Oliveira).

Considering the growing importance of anthropogenic influence on water quality, it is mandatory to distinguish natural characteristics from those deriving from human activity (Silveira). Water quality is not only important for the protection of human health, but also to assure the integrity and functionality of the diverse aquatic ecosystems (Mendes and Oliveira). For the assessment of deterioration processes, one can have two approaches: quantification of physical-chemical characteristics or the evaluation of biological effects caused by exposure of aquatic organisms to the water. Chemical analysis is an important step to identify and determine the concentration of a given substance in water; however, it does not inform about its bioavailability, and gives no further indication concerning the toxic effects that it might cause. It is thus extremely important to study the potential adverse effect that it may exert on exposed aquatic organisms. With this objective, modern Ecotoxicology provides several examples of suitable bioassays, which may be used to assess effects caused by a single substance, but also by a complex mixture. These bioassays usually require exposure of selected aquatic species, under laboratory-controlled conditions, to different concentrations or dilutions of the test substance or mixture (Barroso; Odum). Furthermore, it is also possible to use a similar approach to analyze biologic effects of real samples (e.g.: river water, effluents), from water bodies to be characterized (Mendes and Oliveira). The results that are obtained from these bioassays are important for the identification of contaminated areas, helping to determine adequate measures to minimize the anthropogenic impact associated with the contamination and also to improve the conservation of affected communities (Barroso).

Classic ecotoxicologic bioassays are usually acute or chronic, and follow detailed testing protocols, usually standardized by institutions such as Organization for the Cooperation and Economic Development (OECD). Among these, acute toxicity bioassays are the most used for the evaluation of toxicity of chemicals and water samples. These types of tests are developed in short periods (in relation with the life span of the test species), and toxicity is indicated by means of lethality, expressed by the value of "lethal concentration 50", LC50: concentration of the test substance that is responsible for the death of 50% of the exposed population (Odum). For this purpose, several crustacean species have been indicated as suitable organisms for the development of ecotoxicological bioassays. For the particular case of freshwater testing, several species of the genera *Daphnia* have been postulated to be good candidates for toxicity assessment: *D. pulex*, *D. pulicaria*, *D. magna* and *D. similis* were successfully reared under laboratory conditions and subsequently used in testing (Adema). Nowadays, the genera *Daphnia* is the organism mentioned in both national and international legislation, for the ecotoxicological evaluation of chemical agents, urban and industrial effluents and freshwater ecosystems (Beatrice; Adema).

Daphnia magna is by far, the most cultivated species to be used in freshwater toxicity analyses. It presents several obvious advantages, justifying its use under laboratory controlled

conditions in toxicity assessment (Beatrice). *Daphnia magna* is a freshwater microcrustacean, commonly known as water flea, due to the specific movement of its appendices that give it the appearance of moving by leaps. It has a carapace that is shed on a daily basis. Its presence is common in freshwater systems, feeding on microalgae and constitutes a prey for numerous fish species (Barroso; Stringhini and Salomon). The reproductive traits of this species involve cyclic parthenogenesis, with numerous broods, and descendants are genetically identical to progenitor females. This is highly important in testing, since it permits to eliminate genetic variability from bioassays, and facilitates obtaining large number of individuals (Barroso). This fact represents the elimination of one of the major components responsible for the lack of reproducibility of bioassays and consequent results: sexuate reproduction inherently introduces an additional source of bias due to genetic variations. Due to its assexuate reproduction, it is possible to obtain and rear clones of *D. magna*, with a common genetic patrimony and common life traits that will respond equally when subjected to chemical stress (Barroso; Beatrice). The life cycle of these organisms can be of 40 days (at a temperature of 25 °C) or 56 days (at 20 °C). When reared under laboratory conditions, this species has broods every 2 days, and needs a period of 6 to 7 days to have its first brood. Eggs are liberated from the organisms when the carapaces are shed (Stringhini and Salomon). Its size is approximately 1.5 mm; this is an advantage, since it reduces the biological material and the water volume required for the development of the tests. This reduced body size allows its easy handling and rearing, and also the direct observance of various parameters (Adema). In toxicological terms, this is an interesting species, since it is sensitive to toxic substances, and also to abiotic factors. However, the laboratory use of *D. magna* as a standard test species is not without objections. This is not a common species inhabiting most of freshwater ecosystems. In fact, it is not possible to find *D. magna* in Portugal, which is critical information to consider when performing biomonitoring programs with the use of this species. It is thus not representative of all freshwater habitats; nevertheless, its massive and standardized use, and the amount of knowledge already in use by ecotoxicologists, compensates its natural drawbacks, such as lack of ecological representativeness.

Due to all the mentioned reasons, *D. magna* was the selected test organism for the development of the present work, which aimed at determining the ecotoxicologic effects (in terms of lethality) of water samples from the North of Portugal: water from an aquifer, from a municipal spring and from the River Uíma, in Santa Maria da Feira; water for public distribution in Vila do Prado (Vila Verde), water from the River Cávado and treated effluents from a car garage.

2. MATERIAL AND METHODS

2.1. LABORATORY REARING OF A DAPHNIA MAGNA CLONE

For the development of ecotoxicologic bioassays, we established a clone of *Daphnia magna* under laboratory conditions. In observance of the testing protocol defined by OECD 202 guideline, toxicological assays must be performed using *D. magna* juveniles, with less than 24 hours of age, obtained from monoparental cultures kept under controlled conditions. Other abiotic conditions are as follows: 16 hours of day/ 8 hours of darkness, light intensity not exceeding $15 - 20 \mu\text{E.m}^{-2}\text{s}^{-1}$, and temperature between 18 and 22 °C. During bioassays, temperature must be kept at $20 \pm 1^\circ \text{C}$. Animals are fed three times a week, with a diet constituted by a suspension of live cells of the microalgae *Pseudokirchneriella subcapitata*. In order to complete the feeding regime of the animals, an extract of the algal species *Asco-*

phyllum nodosum was also added to the cultured organisms (Baird et al.). Culture media was composed by dechlorinated tap water, which does not possess chemicals and microorganisms that may compromise the animal behavior and development (EPA).

2.2. ECOTOXICITY BIOASSAYS

According to the adopted guideline, organisms to be used in tests must be less than 24 hours of age, and must have been previously subjected to conditions similar to those already referred. Animals were not fed during the duration of the test (48 hours). The adopted effect criterion was immobilization of the organisms, after gentle shaking of the test flask and a period of 15 seconds (OECD). The testing process involved exposure of test organisms to several dilutions of samples: 25%, 50%, 75% e 100%; dilutions were made with dechlorinated tap water. A control, composed by dechlorinated tap water (similar to the culture media) was used. Treatment and control flasks were prepared in triplicate. Each test flask contained a group of 10 juveniles, per 100 ml of test media. All alterations were registered, as stated in the adopted testing guideline.

2.3. STATISTICAL ANALYSIS

We proceeded with an analysis of variance, followed by a Dunnet test, to discriminate significant differences in comparison with the control. The adopted level of significance was $p < 0.05$.

3. RESULTS

The water coming from the aquifer in Santa Maria da Feira, was shown to be effective in terms of lethality of *D. magna* juveniles, since it caused significant mortality for all tested dilutions ($F=110.4$; d.f. = 4, 10; $p < 0.05$). (figure 1).

The water from the municipal spring was also toxic, at least in the higher concentrations (75 and 100%; figure 2) ($F = 42.8$; d. f. = 4, 10; $p < 0.05$).

No effects were observed for the water from the River Uíma ($F = 1.81$; d.f. = 4, 10; $p < 0.05$) (figure 3).

Tap water, from the public distribution system, was shown to be toxic to *D. magna*, in all tested concentrations (with the exception of the 25% dilution) ($F = 169.4$; d.f. = 4, 10; $p < 0.05$) (figure 4).

The higher tested dilutions (75 and 100%) of the garage effluent were toxic to *D. magna* juveniles ($F = 19.6$; d.f. = 4, 10; $p < 0.05$) (figure 5).

No toxicity was observed for the water of the River Cávado ($F = 1$; d.f. = 4, 10; $p < 0.05$) (figure 6).

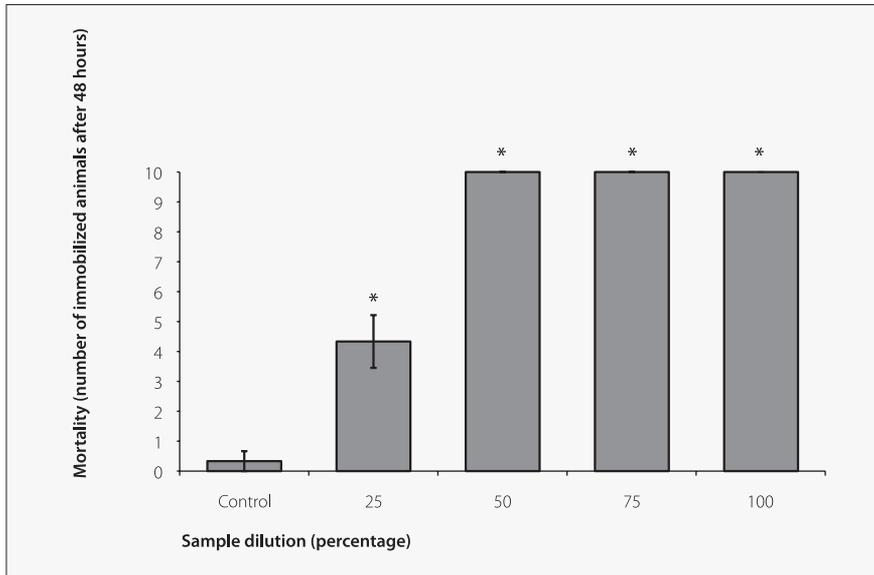


FIGURE 1 - toxicity of water from the aquifer, in Santa Maria da Feira; values correspond to the mean mortality values, after 48 hours of exposure, and standard error bars; * - significant differences, $p < 0.05$.

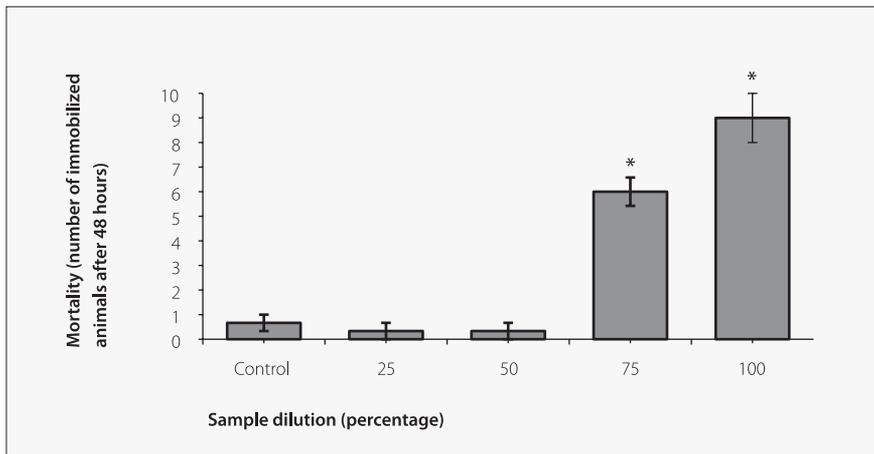


FIGURE 2 - toxicity of water from a municipal spring, in Santa Maria da Feira; values correspond to the mean mortality values, after 48 hours of exposure, and standard error bars; * - significant differences, $p < 0.05$.

4. DISCUSSION

The use of biological analytical methodologies for the assessment of aquatic contamination is an important alternative for the specific physical-chemical analyses (Martins et al.). For this purpose, we selected the organisms *Daphnia magna* to serve as a test organism for the evaluation of the ecotoxicologic effects of contaminants present in water samples, from the North of Portugal. Water samples coming from the aquifer and from the municipal spring

in Santa Maria da Feira were shown to be toxic to *D. magna*. These two particular samples were obtained in rural areas, with strong potential agricultural impact. It is thus expectable that contamination by pesticides and fertilizers may occur, after leaching through the soil layers and reaching the aquifer. Due to the already reported sensitivity of *D. magna* to these compounds, it is thus justifiable that the observed toxicity was at least partly derived from the eventual presence of such contaminants in water samples (Martins et al.).

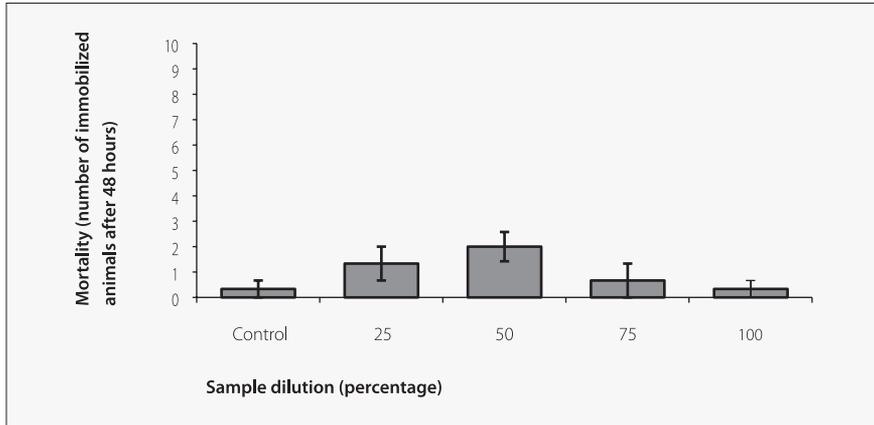


FIGURE 3 - toxicity of water from the River Uíma, in Santa Maria da Feira; values correspond to the mean mortality values, after 48 hours of exposure, and standard error bars.

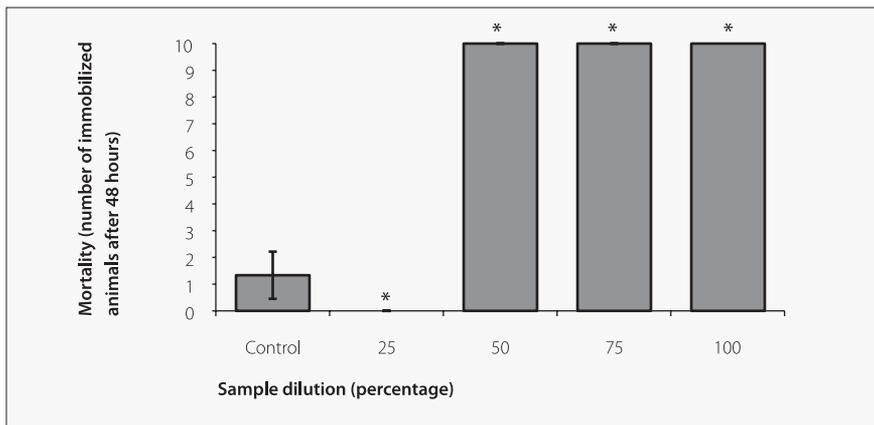


FIGURE 4 - toxicity of tap water from public distribution system, in Vila do Prado (Vila Verde); values correspond to the mean mortality values, after 48 hours of exposure, and standard error bars; * - significant differences, $p < 0.05$.

The sample coming from the River Uíma did not present significant differences when compared to the control treatment. It is noteworthy that the sample was collected in a specific geographic area that favors the movement of contaminants to the river: very steep margins tend to lixiviate contaminants from the agricultural areas, forcing them to enter the river basin. In spite of these considerations, values of toxicity were almost negligible, which may

be due to the high precipitation that occurred previously to sampling, a factor that might have diluted the contamination. Indeed, the period of the year can affect and modulate the biologic response of *D. magna* caused by toxicants; it is thus important to assess the toxicological effect during Summer and Winter months, as shown by Hosokawa et al. and Damásio et al. Previous works state that the feasibility of using *Daphnia magna* as test organism to assess the effects of chemical contamination in rivers may not be adequate, as shown by Hosokawa et al.

Tap water, from the public distribution system in Vila do Prado (Vila Verde) had a prominent effect on *D. magna* mortality. In the three higher tested concentrations (50%, 75% and 100%), it caused significant mortality in exposed animals. This constitutes unexpected results, since this tap water was dechlorinated prior to the performance of the test. Chloride can be highly toxic to other crustacean species, such as, *Ceriodaphnia dubia* which is another freshwater standard species for ecotoxicity testing (Silveira).

The analysis of the water sample from the car garage effluent revealed toxicity, especially for the two highest concentrations (75% and 100%). In spite of being initially treated, this effluent was toxic. The treatment is thus not effective, and is only effective for the separation of sand and insoluble fractions, such as oils; in fact, the major portion of hydrocarbon compounds is eliminated, since the effluent is filtrated through a specific hydrocarbon filter. However, the here obtained results point to the overall inefficacy of the process, since the final effluents eliminate toxic compounds. The toxicity of hydrocarbons to *D. magna* was already studied by Damato et al., and these authors showed that effluents from oil refinery (producing lubricant oils, gasoline and other petroleum derivatives) were toxic to this crustacean species. Among the most toxic compounds, authors identified several volatile chemicals that were responsible for the highest toxicity reported. Due to the treatment process to which our samples were subjected, it is not expectable that volatile chemicals may be present; however, high toxicity values were reported, in good agreement with previous published data.

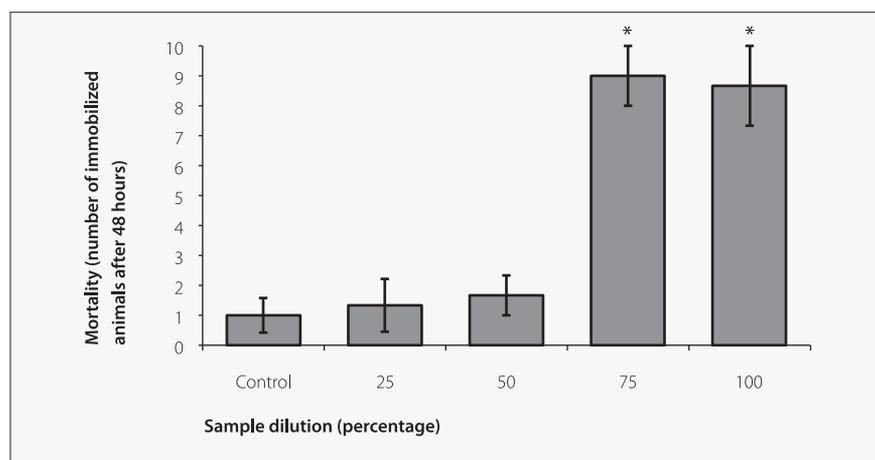


FIGURE 5 - toxicity of effluents of a garage, after treatment; values correspond to the mean mortality values, after 48 hours of exposure, and standard error bars; * - significant differences, $p < 0.05$.

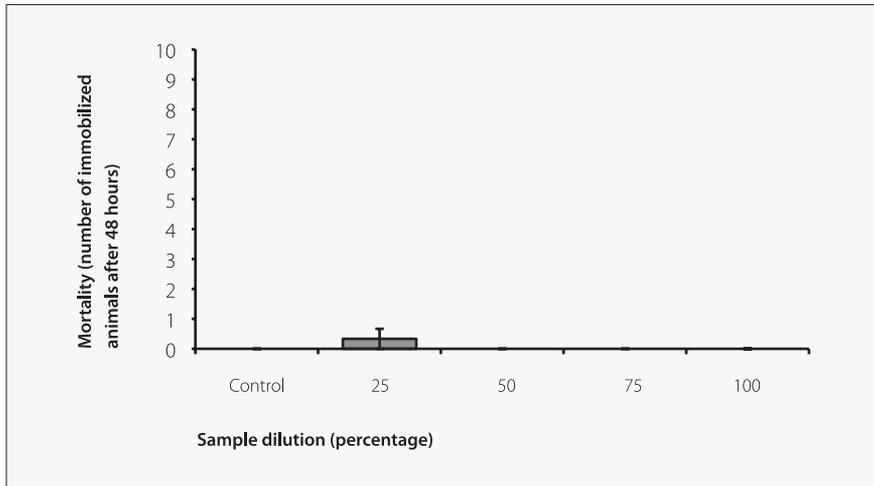


FIGURE 6 - toxicity of water from the River Cávado, in the fluvial beach at Vila do Prado; values correspond to the mean mortality values, after 48 hours of exposure, and standard error bars.

Water from the River Cávado was not toxic, when compared to the control treatment. Again, the issue of the suitability of *D. magna* to be used in toxicological assessment of river water quality must be raised. According to Sakai, the levels of toxic compounds in river water are usually too low to be causative of adverse effects on this crustacean species. In spite of being collected in an urban/rural area, with potential contamination of both sources and decreased water quality (Damásio et al.), no effects were observed.

From the obtained data, it was possible to have a first characterization of the ecotoxicological effects that one may expect from the selected samples. In spite of being a generic and preliminary tool, lethality of the microcrustacean species *Daphnia magna* was shown to be a highly responsive criterion. However, further analyses are necessary to understand ecological deleterious modifications that may occur at higher organizational and trophic levels, following exposure to the aquatic matrices.

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