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Daphniatox – Online monitoring of aquatic pollution and toxic substances

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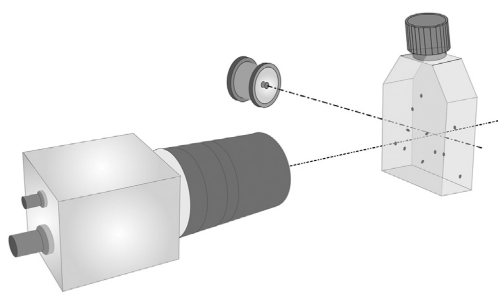
HIGHLIGHTS

- *Daphnia* is an accepted bioassay organism and the biomonitoring protocol is standardized and established in many countries.
- The fully automatic Daphniatox warrants rapid standardized monitoring and provides high statistical significance of the data.
- Using 14 independent endpoints of motility, orientation, size and form indicate different classes of chemical toxicants and pollutants.
- The system is suitable for short- and long-term monitoring of fresh water and industrial, household and agricultural waste water.
- High sensitivity of the micro-crustacean *Daphnia* and low initial costs and negligible running costs characterize the system.

GRAPHICAL ABSTRACT



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ABSTRACT

The microcrustacean *Daphnia* is sensitive to many toxic substances and can be cultured easily. The Daphniatox instrument is based on computerized image analysis tracking swimming organisms in real time. The software evaluates 14 endpoints including motility, swimming velocity, orientation with respect to light and gravity as well as cell form and size. The system determines movement vectors of a large number of organisms to warrant high statistical significance and calculates mean values as well as standard deviation. Tests with K dichromate show that the toxin inhibits motility (EC_{50} 0.75 mg/L), swimming velocity (EC_{50} 0.70 mg/L) and even causes a significant decrease in length (16% at 4 mg/L) and changes the form of the animals, This bioassay can be used to monitor the toxicity of a large number of dissolved pollutants and toxic substances such as arsenic, dichromate and persistent organic pollutants.

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1. Introduction

Most of the Earth's water is not accessible for human consumption because it is either too salty or bound in the form of glaciers and snow. The small fraction of available fresh water compromises less than 1% of the global water reservoir (Gleick, 2014). But the need for fresh water for households, industry and agriculture has increased exponentially over the past few decades and further increases are predicted (Kasei et al., 2014).

At the same time the limited resources are increasingly diminished by pollution from domestic, agricultural and industrial wastes (Jones, 2014). The main components of the growing number of pollutants include heavy metals, persistent organic pollutants (POP) as well as pharmaceuticals (Gleick, 2014). In addition, the excessive use of fertilizers in agriculture results in accumulation of nitrogen and phosphorous compounds which reach surface waters and ground water reservoirs by the runoff from the fields (Wu et al., 2014). Global climate change, which causes increasing temperatures, changing precipitation patterns and draught episodes as well as dropping ground water tables, is expected to deteriorate the conditions for fresh water availability (Elliott et al., 2014). In addition, the limited resources of fresh water for irrigation are bound to decrease agricultural productivity (Cotrufo, 2014; Kurukulasuriya and Rosenthal, 2013; Müller et al., 2014).

About 780 million people in developing countries lack access to clean fresh water (WHO/UNICEF, 2012) and 2.2 billion fail to have safe sanitation (WHO, 2014). Each year an estimated 5 million people die from diseases related to polluted water resources such as diarrhoea and infections transmitted by water-borne parasites (http://www.who.int/topics/mortality/en/); many hundreds of thousands of premature deaths could be avoided by simple measures to improve fresh water quality for human consumption (Apte et al., 2015).

High concentrations of heavy metals were found in rivers in Pakistan and India (Muhammad et al., 2011). In addition, organic pollutants as well as inorganic toxicants are accumulated in sediments and pose considerable long-term risks for the biota and humans (Kukučka et al., 2015). Chlorophenol compounds derived from degradation of pesticides and chlorinated hydrocarbons (Karci, 2014) are considered to be among the most toxic pollutants in aquatic ecosystems because of their high toxicity, chemical stability and low degradability (Lindholm-Lehto et al., 2015; Wei et al., 2015).

Personal care products and pharmaceuticals introduced into surface waters by household wastes are recycled and concentrated in rivers. The water of some rivers in heavily populated areas is recycled several times in the form of drinking water and household effluents so that substances like estrogens create problems such as feminization in fish and amphibian species (Valdés et al., 2014). In addition, chemicals such as antibiotics accumulate in crop plants when these waters are used for irrigation (Pan et al., 2014).

Because of the increasing demand for clean fresh water, monitoring and assessment of both ground water and surface water reservoirs has a high priority. Chemical analyses are of limited value because they are time consuming, expensive and usually are restricted to a few classes of chemicals while the number of potentially toxic chemicals is excessive (Ginebreda et al., 2014). Often the toxicity of a certain chemical is underestimated or it may operate synergistically with other substances which is not detected in routine chemical analyses (Chen et al., 2015). Regulations vary between countries and upper limits for toxins may be altered over time and may not represent the effective threat for the biota or human consumers of fresh water (Häder, 2013), Alternatively, water quality can be monitored using bioassys. Early examples for this procedure include the deployment of fish in potentially polluted water. When they died or showed abnormal swimming behavior this was considered as a sign for the presence of a pollutant in the water. Many different organisms have been employed in bioassays such as bacteria, microorganisms, lower and higher plants as well as invertebrates and vertebrates (de Castro-Català et al., 2015; Hafner et al., 2015; Kottuparambil et al., 2014; Ma et al., 2014). Depending on the time scale for the evaluation different endpoints can be considered including mortality, motility and behavior, growth and reproduction as well as physiological parameters such as photosynthesis, protein biosynthesis and genetic alteration of aquatic organisms (Davies and Mazurek, 2014).

While chemical analysis can identify a potentially toxic substance or at least a class of chemicals, bioassays do not provide this information. They indicate the presence of a toxin in the water which - above a certain concentration - can present a potential health hazard for the biota or for human consumption (Häder and Erzinger, 2015).

The toxicity of a pollutant is often determined by the EC_{50} value which causes a 50% inhibition (Sebaugh, 2011) but any other value can be used instead.

There are a number of commercially established bioassays. The Microtox test is based on the diminuation of the bioluminescence generated by genetically modified bacteria in the presence of toxic materials (Fernández-Piñas et al., 2014). The biotest Lemnatox analyzes the growth of an aquatic higher plant under the influence of pollutants (Newman and van Valkenburg, 2013). A recently developed bioassay for municipal and industrial wastewater effluents is based on the delay of *zoo*spore release from marginal disks cut from the thallus of the marine green alga *Ulva pertusa* under the influence of a toxin during a 96-h period (Han and Choi, 2005; Y.-J. Kim et al., 2014). Disadvantages of some commercial bioassays are the low sensitivity toward toxic chemicals and/or the long times required for analysis which can be on the order of several days. One approach is based on the on-line image analysis of motile photosynthetic microorganisms monitoring motility, orienation and cell shape fully automatically (Azizullah et al., 2013; Azizullah et al., 2011). This unicellular flagellate (*Euglena*) has also been employed to indicate the toxicity of phenolic substances (Kottuparambil et al., 2014). The microcrustacean *Daphnia* has been used as a sensitive organism to indicate the potential toxicity in water samples (Chen et al., 2012). There are a number of automatic bioassays on the market. However, many have serious limitations, such has high price tag, high costs of consumables, low sensitivity, long analysis time spans, complicated performance and large size. The aim of the current work is to describe a recently developed on-line, fully automatic image analysis system to monitor motility, organism shape and orientation of the water flea *Daphnia*. In contrast to the Ecotox bioassay, which uses a unicellular flagellate, Daphniatox employs a multicellular crustacean. The test is certified in many countries. The organism is as sensitive as the Ecotox, but differs with respect to the chemical nature of the toxicant. The Daphniatox test is also faster than the Ecotox test. The advantages of this test include high sensitivity of the organisms (Struewing et al., 2014) and the fast analysis of the selected 14 end points. While a bioassay by definition cannot identify the pollutant or toxic substance, the different endpoints show different sensitivities to varous groups of chemicals, which gives some indication to the nature of pollution.

2. Materials and methods

2.1. Organisms for the bioassay

The water flea *Daphnia* is a genus of small (0.5–5 mm length) Cladoceran crustaceans found in fresh water bodies like streams, ponds and lakes. Culture conditions are simple, and they grow and

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