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Toxicity assays applied to wastewater treatment

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Abstract

The utility and validity of toxicity tests for monitoring of wastewater treatment have been assessed. The evaluated acute toxicity tests have been *Vibrio fischeri*, *Selenastrum capricornotum* and *Daphnia magna* tests. The validation studies indicated that the acute toxicity tests can be considered as high sensitivity analytical tools to detect common environmental concentrations of the pollutants at concentration levels as low as ng l^{-1}. The toxicity tests showed to have discriminatory ability to distinguish between different degrees of toxicity, and the toxic specificity of the compounds on target organisms. Synergistic, additive or antagonistic effects were evaluated indicating the capacity of the toxicity test to assess the combined effects of chemicals in wastewaters. The reproducibility of these tests, calculated as relative standard deviation, is acceptable in the range of 5–22.3%. The application of multivariate date analysis proved that toxicity and chemical measures are complementary analytical tools for monitoring of wastewaters quality. The toxicity tests are useful analytical tools for screening of chemical analysis and as an early warning system to monitor the treatment of WWTPs. The use of single toxicity test or battery of tests is the best approach to evaluate the risk because they are reliable indices of the toxic impact of effluents in the aquatic environment. The toxicity tests were applied in the quality control of different European WWTPs.

Keywords: Toxicity; Wastewater; Validation; *D. magna*; *V. fischeri*; *S. capricornotum*

1. Introduction

The standard chemical quality of effluents, has traditionally been based on the control of global parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD) or total suspended solid (TSS) according to the Urban Waste Water Treatment Directive 91/271/EEC [1]. More recently, the current guidelines (Directive 2000/60/EC and Decision 2455/2001/EEC) have been based in the detection of specific pollutants included in a list of priority organic pollutants [2,3]. The use of antifouling agents such as diuron or TBT, which are used for controlling growth of marine organisms, have been regulated because toxicity studies have demonstrated their potential negative impact on aquatic ecosystems. Pesticides, which are created to affect weeds, fungi or invertebrate organisms, are other chemicals included in this list because of their known toxic effects.

For monitoring the priority organic pollutants, several analytical methods such as gas or liquid chromatography coupled to mass spectrometry have been developed to assess and maintain the quality of surface waters, which is directly influenced by the wastewater treatment plants (WWTPs) [4–6].

In addition to priority organic pollutants, in the last few years, reports about residues of pharmaceuticals in surface wasters have increased, however there is still an almost complete lack of data concerning their effects on aquatic fauna. Environmental problems may arise when drugs enter via sewage effluent from domestic dwellings or hospitals because conventional biological treatments of WWTPs are insufficient to removal polar compounds. The occurrence of pharmaceuticals has generated a growing demand for analytical methods to monitor the quality of wastewater [7,8].

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The quality control of wastewater based on global chemical measures of total organic pollution load such as total organic carbon (TOC) or the detection of specific pollutants, is not sufficient to assess the environmental risk. They are not real measurements of the toxicity effects on the aquatic ecosystem because toxicity is a biological response. Therefore, effective tools for the evaluation of the negative effects on living organisms are need. The use of biological assays can provide a direct and appropriate measure of toxicity to complement the physicochemical measures of quality of wastewaters [9–11].

The acceptance of a toxicity test as an effective analytical tool requires guarantees of standardization and validation of the experimental procedure to evaluate its sensitivity, accuracy or precision.

In this sense, the main objectives of this work have been to assess the utility and validity of toxicity tests and to apply the toxicity tests for monitoring of wastewater treatment. The acute toxicity tests subject of study have been Biotox based on bacteria organism, Algaltoxkit based on microalgae and Daphtoxkit based on crustaceans. The utility and validity of these toxicity tests have been evaluated using the groups of chemicals mentioned above, including antifouling agents, pesticides and pharmaceuticals.

2. Experimental

2.1. Chemicals

The following group of chemicals were used for validation studies of acute toxicity tests. Pesticides: formetanate, carbofuran, cyromazine and fenamiphos. Antifouling agents: chlorothalonil, Sea nine 211 (4,5-dichloro-2-*n*-octyl-4-*iso*-thiazolin-3-one), irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-s-triazine), diuron, dichlofluanid, TCMTB (2-thiocyanomethyl-*thio*-benthiazole) and tributlytin (TBT). Pharmaceuticals: fenofibrate, gemfibrocil, clofibric acid, sotalol, betaxolol, metoprolol, bezafibrate and atenolol. Pesticides and antifouling agents were purchased from Ciba–Geigy (Barcelona, Spain), Rhom & Hass (Philadelphia, USA), Chemservice (West Chester, USA) and Riedel-de-Haën (Seelze, Germany). Pharmaceuticals were purchased from Sigma–Aldrich, Spain.

Individual stock solutions of the standard compounds were prepared in the specific culturing medium for the three toxicity tests. Mixtures of antifouling agents were prepared by combining individual stock solutions in a composition ratio 1:1. All working solutions were adjusted to a neutral pH.

2.2. Wastewater samples

Samples were colleted from nine different European wastewater treatment plants (WWTPs) designated in this work as follow: from WWTP1 to WWTP9. A pilot survey study was performed in WWTP1 over 5 months, with monthly sampling. Wastewaters were collected in Pyrex borosilicate amber glass containers previously rinsed with tap water and high-purity water and kept in the dark at 4 °C. Toxicity analyses were performed within 24 h of sampling. Before toxicity evaluations, the water samples were adjusted to a neutral pH.

2.3. Bacterial bioluminescence test

The experimental procedure for conducting the bacterial bioluminescence assay was based on the ISO 11348 standard protocol [12]. The bacterial assay used the commercially available Biotox test (Bio-Orbit Oy, Turku, Finland). The freeze – dried *V. fischeri* 1500 Reagent (*Vibrio fischeri* NRRL-B 11177) was reconstituted with 12.5 ml of 2% NaCl, and incubating at +3 °C for 10 min and at 15 °C for 15 min before use. The concentration of toxicants in the test which caused a 50% reduction in light (Inhibition = 50%) after exposure for 15 or 30 min was designed as the 15 or 30 min EC_{50} (effective concentration) value. Tests were performed at 15 °C. The measurements of light were made using a luminometer.

2.4. Daphnia acute immobilisation test

Daphnia tests were conducted following the European Guideline: "methods for determination of ecotoxicity; Annex V, C.2, *Daphnia* acute immbolisation test" (Commission of the European Communities, 1992) [13]. The *D. magna* bioassay used a commercially available text kit ((Daphtoxkit F^{TM} magna, Creasel, Belgium). Daphnids were bred in culture medium imitating natural fresh water. Test plates with *D. magna* neonates were incubated for 24–48 h at 20 °C in the dark. Acute toxicity was assessed by noting the effects of the test compounds on the mobility of *D. magna*. The neonates were considered immobile, if after 24 or 48 h of incubation with the toxicant they remained settled at the bottom of the test container and did not resume swimming within the 15 s observation period. The toxicity end-point (EC_{50}) was determined as the concentration of the toxicant required to immobilize 50% of the daphnids after exposure time.

2.5. Algae growth inhibition test

Algae tests were conducted following the European Guideline: "methods for determination of ecotoxicity; Annex V, C.3, Algal inhibition test" (Commission of the European Communities, 1993) [14]. The commercially available Algaltoxkit (Creasel, Belgium) was used. In the Algaltoxkit F^{TM}, the inhibition of the growth rate of algae *Selenastrum capricornotum* was measured. The initial algal culture was prepared from the immobilized algal beads as described in the instructions and the immobilized cells were pregrown in the sterile growth medium at 25 °C. The initial number of algal cells was adjusted to 10^6 cells ml^{-1} and the test tubes were