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RANKING WASTEWATER SAMPLES WITH *PHAEODACTYLUM TRICORNUTUM* (BOHLIN)

CLASSIFICAZIONE DELLE ACQUE DI SCARICO CON PHAEODACTYLUM TRICORNUTUM *(BOHLIN)*

Abstract - *Marine phytoplankton is highly productive in coastal ecosystems where it generally occurs the major exposure and uptake of contaminants associated with anthropogenic pollution discharges. Phytoplankton presents very high surface-to-volume ratio and may respond rapidly to suspended contaminants showing either bloom events or inhibition effects. The diatom* Phaeodactylum tricornutum *Bohlin is commonly used in toxicity testing with standardised protocols even if a complete inventory of tested substances and matrices has not been compiled yet. The purpose of this study is setting up a wastewater effect score based on* P. tricornutum *considering samples originated by domestic, municipal and industrial sources. Results showed the existence of two main effect scenarios with a prevalence of biostimulation rather than toxicity.*

Key-words: *wastewater, marine ecotoxicology, microalgae, stimulation, inhibition.*

Introduction - According to EINECS, point-source wastewaters can contain more than 100,000 substances (EINECS, 2013). Therefore, the whole characterization of a wastewater sample could be uneasy and costly just on a chemical basis, thus ecotoxicology can give a great contribution (*i.e.* a battery of marine organisms for effluents discharged to seawater) (Libralato *et al*., 2010a,b; Libralato, 2013). To date, the assessment of wastewater toxicity has used different types of algal assays, but gaps still remain about how to use, interpret and integrate their results. Some toxicity tools already exist to assess and rank the potential ecotoxicity of wastewater samples (Libralato *et al*., 2010a), but none makes explicit reference to phytotoxicity and phytostimulation. Indeed, phytostimulation could adversely affect environmental quality as well. Only few saltwater phytoplanktonic biological models are really widespread and standardized for toxicity testing such as *Phaeodactylum tricornutum* Bohlin (Muller *et al*., 2007). It would be interesting classifying wastewater samples by an integrated approach not only according to Whole Effluent Toxicity (WET) (USEPA, 2004), but on a Whole Effluent Assessment (WEA) perspective (OSPAR, 2007) thus including not only toxicity (growth inhibition), but also stimulation effects. The aim of this paper is to evaluate the ability of *P. tricornutum* to discriminate the quality of domestic, municipal and industrial wastewater samples ranking potential hazards for receiving water bodies.

Materials and methods - Experimental activities focused on 93 samples collected from various wastewater treatment plants located in Venice (Italy). The full physico-chemical characterization of samples was provided. Toxicity tests with *P. tricornutum* were carried on according to the ISO 10253:2006 method in triplicate. The algal culture was kept at 20±2 °C and 6000-10000 lux, obtaining a cellular density $>10^6$ cells mL^{-1}. The initial algal density in the test was obtained by dilution of algal culture and ranged between 2×10^3-10^4 cells mL^{-1}. Negative and positive ($K_2Cr_2O_7$) controls were included. Cellular density was evaluated by microscopy using a Bürker counting chamber. Toxicity and stimulation effect data normalised on negative controls were determined as percentages of inhibition/stimulation effect inducing a 50% growth inhibition (inhibition concentration, IC50) or 50% growth stimulation (biostimulation concentration, BC50) in the observed population. The hypothesis test was verified using Analysis of Variance and Tukey's test.

Results - Domestic, municipal and industrial wastewater presented an average stimulation effect of 74% (n=25), 40% (n=47) and 36% (n=3) and an average inhibition effect of 66% (n=2), 43% (n=10) and 67% (n=5), in that order. On the basis of Libralato *et al*. (2010a), a 5-class effect score was defined ranking stimulation and toxicity data separately, but in a symmetrical way. The effect score was centred on the "no effect" rank; low, medium, high and very high toxicity classes were organized to classify all effects. Reducing to a minimum the expert judgment, the choice was addressed to the 10^{th}-, 50^{th}- and 90^{th}-percentile values of effect data providing information about the probability of effects rarely, likely and more likely to occur (Libralato *et al*., 2010a). The assessment of wastewater samples via the effect score showed the following classification within the 75 samples inducing algae growth stimulation: 7, 30, 31 and 7 samples with low, medium, high and very high effects, in that order. Besides, the 17 samples showing algae growth inhibition were ranked as follows: 1, 8, 7 and 1 with low, medium, high and very high effects, accordingly. Only one sample presented no effect at all compared to the negative control ($p<0.05$). In general, most part of wastewater samples (90%) showed from medium to very high stimulation/toxicity effects indicating the need to further increase their management to support the full application of Nitrates (91/676/EEC) and Water Framework Directives (2000/60/EC). Uni- and multivariate statistical analysis showed no significant correlations between physico-chemical and ecotoxicological data suggesting the presence of undetected agents or interactive effects within wastewater components.

Conclusions - In the case of *P. tricornutum*, domestic, municipal and industrial wastewaters showed more stimulation than inhibitory effects. Stimulation effects have to be identified, ranked and used to increase wastewater hazard management and thus the quality of the final receiving water body due to the fact that inhibitory effects represented only a minor occurrence.

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