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# Application of Microbiotests and Activated Sludge Respirometry for the Evaluation of Industrial Wastewater Toxicity

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A lot of different bioassays have been used in order to investigate the effect of toxicants on aquatic ecosystems. Among the bioassays that have been used for the estimation of the toxic effects of chemicals on aquatic organisms, microscale procedures, now commonly referred to as microbiotests, have emerged as cost-effective and simple to operate methods (Blaise, 2000). On the other hand, different techniques have been used in order to monitor toxicants present in industrial sewage that reduce the efficiency of biological wastewater treatment due to intoxication phenomena. Most of toxicity directed approaches for the evaluation of industrial effluent toxicity mainly utilize a respirometric biosensor (Vanrolleghem et al., 1994). Moreover, the majority of studies correlating respirometry with bioassays deal with pure substances (Kong et al., 1993). There has not been an indication whether the respirometric techniques can be correlated with microbiotests in order to monitor industrial effluents. It is worthwhile to investigate such a correlation, since respirometric techniques involve great costs both for the acquisition of the equipment and for its maintenance and operation, while microbiotests are inexpensive. In this study, three microbiotests, the crustacean *Daphnia magna* test, the marine bacterium *Vibrio fischeri* test and protozoan *Tetrahymena thermophila* test were used in order to investigate their potential correlation with a respirometric technique (Vanrolleghem et al., 1994).

Another issue related to the toxicity effects of industrial effluents is that, in many cases, pretreated industrial effluents end up to natural ecosystems and consequently affect the biota. Living organisms have the ability to respond to actual disruptions in a range of effects and bioassays have become important tools for environmental management (Persoone et al., 2003). Toxicity tests using *Daphnia magna*, *Vibrio fischeri* and protozoa are widely used for industrial effluent toxicity monitoring (Reemtsma, 1999; Okamura et al., 1999; Scheers et al., 2002). In the present study, three different bioassays and respirometry were used for the toxicity evaluation of pretreated industrial effluents. The primary aim of this study was the investigation of a suitable bioassay that could potentially be correlated to respirometry. Additionally, the three different microbiotests used, *Daphnia magna* test, *Vibrio fischeri* test and *Tetrahymena thermophila* test, were compared in terms of their relative sensitivity, for three different groups of industries, metal coating, food and dyeing industries.

## MATERIALS AND METHODS

Wastewater samples were collected from 16 pretreated industrial effluents of Thessaloniki industrial area. The samples obtained were categorized according to the industrial activity to food industries, metal coating and dyeing industries. The toxicity of wastewater was assessed by applying a battery of microbiotests that included the bacterium *Vibrio fischeri* (Microtox test), the crustacean *Daphnia magna* (Daphtoxkit F test) and the protozoan *Tetrahymena thermophila* (Protoxkit F test). There was a random selection of 9 out of 16 samples, representative of all the industrial categories that were tested by the respirometric biosensor. The results of the respiration technique were compared with the microbiotests in order to evaluate their in-between correlations.

The determination of acute toxicity of wastewater samples was performed on non-diluted samples with the battery of microbiotests. The pH of wastewater samples was adjusted to 7 ± 0.2 before conducting the toxicity tests, in order to eliminate any toxic disturbances due to unsuitable pH levels for the viability of the organism.

Microtox test is based on the measurement of bioluminescence inhibition of the marine bacteria *Vibrio fischeri* within a short exposure time (15 min). The toxicity of the samples was measured after 15 min exposure of the bacteria in one sample containing 82 % of the effluent (Microbics, 1992). The toxicity tests on *D. magna* were carried out using the Daphtoxkit F magna. The experiments were based on the immobilization of the crustacean *D. magna* that was caused after 24 h exposure of the test organism in undiluted wastewater sample. In the microbiotest with the ciliate protozoan *Tetrahymena thermophila,* the growth inhibition of the ciliate protozoan was evaluated, after 24 h exposure in the undiluted wastewater sample, at 30°C in darkness. The test is based on the optical density measurement of the food substrate provided to the ciliates, in 1 cm disposable spectrophotometric cells. For all three bioassays used in this study, two replicates were used and the average toxicity was estimated and used in further calculations.

A biosensor called RODTOX (Kelma NV, Belgium) was used in this study for the evaluation of toxicity properties of industrial effluents. The biosensor consisted of a reactor vessel, filled with 10 L of activated sludge taken from the aeration basin of an industrial wastewater treatment plant. The activated sludge was subjected to constant aeration (about 15 L/min); it was continuously stirred and thermostated at 25 ± 0.1°C. Dissolved oxygen probe (DO) was installed in the cover of the bioreactor and data were analyzed by a microprocessor. Toxicity measurement commenced by introduction of a known volume of a calibration substrate into the bioreactor and continuous monitoring of the DO profile (respirogram). The calibration substrate used was a multi-substrate solution ($CH_3COOH$-$CH_3COONa$) corresponding to about 20000 mg/L $BOD_5$. Acetic acid and sodium acetate were provided by Panreac Quimica S.A. and were of 99.7 % purity. The instrument was calibrated prior to each measurement with the calibration

substrate. Afterwards, 500 mL effluent sample were injected to the bioreactor. Then, calibration substrate was injected. The percentage of inhibition is calculated by comparing the respirographic parameters (peak height, peak area and peak slope) of the calibration substrate before and after the introduction of the sample (Vanrolleghem et al., 1994). The activated sludge in the bioreactor was renewed after each measurement, in order to avoid degradation of the sludge quality, due to possible toxic effects caused by previous samples.

## RESULTS AND DISCUSSION

In Thessaloniki industrial wastewater treatment plant, the respirometer RODTOX has been used in order to monitor the toxicity of influents towards activated sludge microorganisms. The respirometer is a heavy piece of equipment and it is permanently installed in the industrial wastewater treatment plant. On the other hand, the wastewater treatment plant operators would occasionally like to evaluate the toxicities of effluents from specific industries towards activated sludge microorganisms in order to find out whether these effluents could be treated by the wastewater treatment plant without causing problems to the plant microfauna.

In an attempt to assess the suitability of microbiotests to replace respirometry in a case of emergency, three bioassays were compared in terms of result correlation to respirometry. The correlations between the bioassays of *D. magna* and respirometry as well as *V. fischeri* and respirometry were calculated and found to be very low, 0.05 and 0.2 respectively. Therefore, it was assumed that toxicity of industrial effluents towards activated sludge microorganisms could not be predicted using the *D. magna* or *V. fischeri* bioassays. On the contrary, the industrial effluent toxicities towards *T. thermophila* and the activated sludge microfauna were found to be comparable (Table 1). Moreover, the correlation coefficient between toxicity of industrial effluents towards *T. thermophila* and towards activated sludge respirometry is high (see Figure 1), which suggests that the very expensive respirometric technique can be substituted by the protozoan bioassay in wastewater toxicity screening applications. Ciliated protozoa and especially those of *Tetrahymena* species not only play an important ecological role in the self-purification and matter cycling of natural aquatic ecosystems, but also in the artificial system of sewage treatment plants. Their feeding on bacteria improves the treatment, resulting in higher transparency, i.e. lower organic loads in the output water of the treated wastes. This status of ciliates as an important functional group, improves the process in municipal sewage treatment. As such they can be ideal early-warning indicators. It should also be kept in mind that protozoa constitute the majority of the activated sludge microfauna and thus similar physiological responses are expected. On the other hand, it is very difficult to establish relationships between susceptibilities of various species tested, since the toxicities of chemicals depend on test organisms.

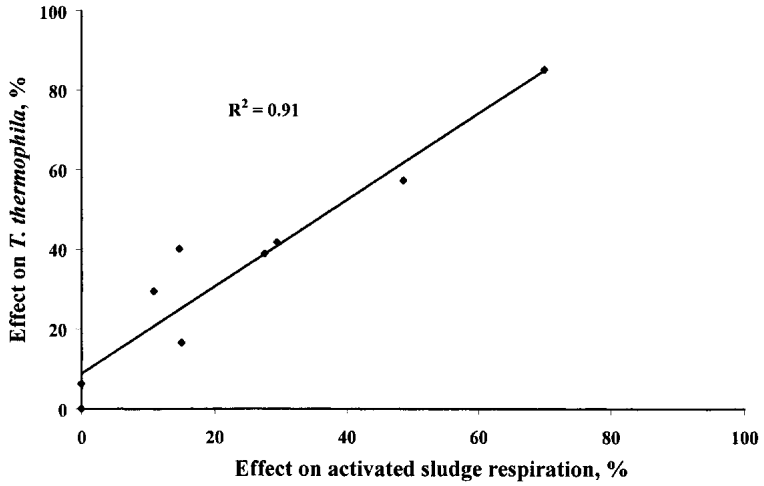


**Figure 1.** Correlation between toxicity of industrial effluents towards *T. thermophila* and towards activated sludge respirometry ($p < 0.05$).

**Table 1**. Toxic effects of the industrial effluents on the respiration of activated sludge microfauna and on the protozoan density.

| Bioassay | Number of samples | Range, % | Average, % |
|---|---|---|---|
| *T. thermophila* | 9 | 0- 85 | 35 |
| Respirometry | 9 | 0 - 70 | 24 |

In the second part of this study the relative sensitivity of *V. fischeri* and *D. magna* for evaluating industrial effluent toxicity was tested. The *T. thermophila* bioassay was not used in this part of the study, since it is generally less sensitive than the other two bioassays. After all, it was found in the first part of this study that the *T. thermophila* test is most appropriate for evaluating influent toxicity in a wastewater treatment plant. For the evaluation of the effluent toxicity, more sensitive bioassays are needed. The results from the toxicity testing of industrial effluents with *V. fischeri* and *D. magna* are summarized in Table 2, while the comparison between the toxic effect on *V. fischeri* and *D. magna* for each industrial activity is depicted in Figure 2.

**Table 2.** Toxic effects of the industrial effluents on the bioluminescence of *V. fischeri* and on the mobility of *D. magna*.

| Industries | Number of samples | Average toxic effect, % | |
|---|---|---|---|
| | | *V. fischeri* | *D. magna* |
| Food | 7 | 52 | 57 |
| Dyeing | 5 | 63 | 58 |
| Metal coating | 4 | 64 | 83 |

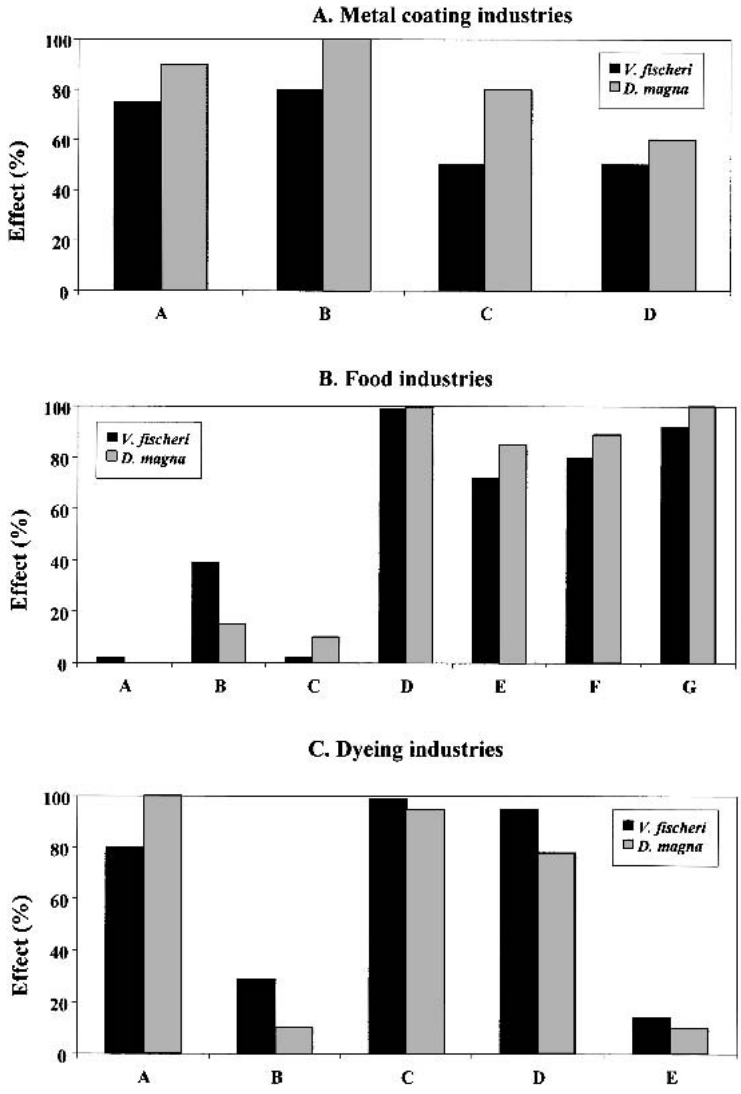


**Figure 2.** Comparative toxic effect of three groups of industrial effluents on *V. fischeri* and *D. magna* (the Latin letters A, B etc. on x-axis refer to individual factories).

It can be seen in Figure 2 that the toxic effects on *V. fischeri* and *D. magna* show similar patterns for food and dyeing industries. For these two groups of industries, the average toxic effect was also comparable (see Table 2), while for metal coating industries the average toxic effect was 83 % towards *D. magna* and 64 % towards *V. fischeri*. It can be assumed that the sensitivities of *V. fischeri* and *D. magna* in detecting toxic substances present in food and dyestuff industries are quite comparable.

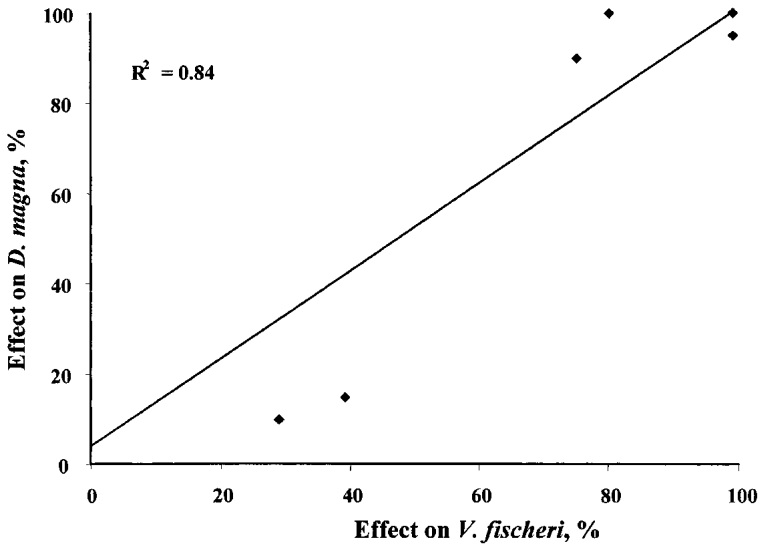


**Figure 3.** Correlation between the sensitivities of *V. fischeri* and *D. magna* bioassays for assessing toxicities of industrial effluents ($p < 0.05$).

However, in the case of metal coating industries, *D. magna* presented distinctively higher sensitivity. That aspect can be due to the potential presence of metal compounds in such effluents. It has been stated that *D. magna* is more sensitive than *V. fischeri* in detecting metal toxicity (Kungolos et al., 2002; Sorvari and Silanpaa, 1996).

In the third part of this study, statistical analysis was performed in order to compare two bioassays for assessing effluent toxicity (see Figure 3). The luminescent marine bacterial test (Microtox test) has been compared with the *Daphnia* test (Daphtoxkit F test) in order to assess the toxicity of industrial effluents coming from different industries. The testing was performed in undiluted samples in order to qualitatively assess the most suitable organism in terms of sensitivity. The correlation between the two microbiotests was generally good ($R^2 = 0.84$ for $p < 0.05$), therefore the two bioassays can be used interchangeably for testing whole effluent toxicity. In the past, various studies have been conducted comparing the sensitivities of the two bioassays either for pure compounds (Sweet and Meier, 1997) or for effluents (Choi and Meier, 2001; Manusadzianas et al., 2003). Other researchers, who have tried to evaluate correlations for toxicities of effluents towards *D. magna* and *V. fischeri*, have found similar results. Choi and Meier (2001) have found correlation coefficients ranging between 0.458 and 0.865 for the effect of metal plating wastewater on the two test organisms, the correlation depending on the duration of Microtox test. Manusadzianas et al. (2003) have found a correlation coefficient of 0.56 for the effect of industrial and urban

wastewaters in Lithuania and Estonia on the two test organisms. The correlation for the effect of industrial effluents on *D. magna* and *V. fischeri* cannot be expected to have a high value, since industrial effluents contain a mixture of substances out of which some may be more toxic towards one organism and some to the other. A recent study conducted by Hsieh et al. (2004) assesses the toxic effects of priority pollutant metals with *V. fischeri*. It is noted in that study that the use of *V. fischeri* bioassay exhibits greater sensitivity when the concentrations of metals are relatively high (ppm level), whereas at ppb concentrations (typical of treated industrial and domestic effluents) a more sensitive bioassay is most appropriate. As such, an effective toxicity-monitoring scheme for the protection of aquatic ecosystems should include both bioassays, since the organisms involved belong to different organism levels, and thus exhibit different responses to various pollutants. Therefore, it would be more appropriate that both *V. fischeri* and *D. magna* bioassays be incorporated in a battery of tests in order to evaluate the toxicity of effluents towards aquatic organisms.

The most important result originating from this study was the relatively high correlation between the effect exhibited by industrial effluents on RODTOX respirometer and on the protozoan *T. thermophila* (Protoxkit F test). This result was based on experiments done in Thessaloniki industrial area. It should be kept in mind that industrial effluents have a variable composition, so it is necessary to perform more experiments with effluents of different compositions, probably in different industrial areas, in order to check the validity of this result. It is worthy to further investigate the potential correlation between toxicity of industrial effluents to RODTOX activated sludge microorganisms and protozoan *T. thermophila*, because not every industry can afford a respirometer while the protozoan *T. thermophila* Protoxkit bioassay is an inexpensive and easy-to perform test. On the other hand, it is worthwhile mentioning that the correlation between RODTOX respirometer and *D. magna* or *V. fischeri* tests was very low. This finding may reflect the fact that protozoan organisms are essential in the fauna of activated sludge, while organisms such as *D. magna* (cladoceran) and *V. fischeri* (marine bacterium) are not present in wastewater treatment plants. Furthermore, in the present study the two biotests (*D. magna* test and *V. fischeri* test) were extended to practical use in industrial waste. It was found that *D. magna* was more sensitive than *V. fischeri* in detecting toxicity coming from metal plating industries. In other industries, such as food or dyeing industries, the two bioassays showed comparable sensitivities. The correlation found for the toxicities of industrial effluents on *D. magna* and *V. fischeri* was relatively good and comparable with the results found by other scientists.

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