Toxicity assessment of eight azo-dyes using *Tetrahymena pyriformis*

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Increasing environmental pollution and the continuous development of new chemicals and drugs has led to ever growing concern about the potential effects of these compounds directly or indirectly on human health and, more recently, on the health of ecosystems. Aquatic environments have become major receptors of toxicants originating mainly from industrial sewage. On the other hand, these contaminants reduce the efficiency of biological wastewater treatments due to intoxicant phenomena.

Ciliated protozoa often reach densities of about 10^7 cells L^{-1} in the aeration tank of the activated sludge wastewater treatment plant. They play an essential role in the purification process by removing, through grazing, the majority of dispersed bacteria, which would cause high turbidity in the final effluent. Ciliated protozoa are very sensitive to environmental variations and, furthermore, it is recognised that changes in this community may affect the whole food web of these artificial ecosystems.

Azo-dyes are frequently found in industrial sewage because they are intensely used in chemical and textile industry which are respectively their main manufacturers and users. Tons of azo-dyes are produced worldwide per year due to the low cost of production and because they have broad range applications, and it is estimated that 10 to 15% of this amount is released in the environment.

This emphasizes the need of determining, in a safe way, the concentrations of azo-dyes that can be introduced in the wastewater treatment systems without significant harm to the inhabiting communities. Over recent years, much research has been carried out on the toxicity of various relevant toxic compounds in a series of biotests using several test organisms and different parameters to evaluate the toxicological response. Nevertheless, azo-dyes have not been intensively studied in this aspect.

The ciliated protozoa *Tetrahymena pyriformis* was for more than four decades the organism of choice in analyses, evaluation of protein quality and determination of effects of several toxic substances. Moreover, it was the first protozoon to be cultivated axenically, i.e., in a standard medium, free from bacteria or other organisms, making it a suitable model cell system, since the addition of a compound is, in principle, the only change in culture conditions.

In the present study, *T. pyriformis* was used to study the responses to the exposure to eight synthetic azo-dyes with the ultimate goal of inferring about the influence that these compounds could have upon the aquatic communities, and namely, to the microfauna of activated-sludge wastewater treatment systems. Eight azo dyes were synthesized by diazotation of *meta*- or *para*-aminobenzoic or aminosulphonic acids, as diazo components and coupling with 2-methoxyphenol (guaiacol) or 2,6-dimethoxyphenol (syringol) as coupling components.

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Three toxicological responses were evaluated: growth, grazing and morphometry. The assays were accomplished in a set of miniaturized tests using axenic cultures of *T. pyriformis*, exposed to four concentrations of the eight azo-dyes (from 5 ppm to 100 ppm) for 48 hours.

Toxicity was not relevant for most of the azo-dyes in the range tested and for all the parameters evaluated. Higher concentrations can be tested in the future but its relevance is questionable, if the influence of these compounds is to be accessed for the microfauna of wastewater treatment plants, as the usual concentrations of azo-dyes in these systems stay below 100 ppm.
Keywords: azo-dyes; toxicity; Tetrahymena pyriformis; protozoa; wastewater treatment plants.