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Toxicity of Pesticide Industrial Wastewater to the Green Alga *Scenedesmus obliquus*: A Case Study

A.M. Abdel-Aty, M.A. El-Dib and M.I. Badawy
Department of Water Pollution Research, National Research Centre, Dokki, Cairo, Egypt

Abstract: Algal bioassay using *Scenedesmus obliquus* was an efficient procedure to evaluate the toxicity of wastewaters generated by pesticide industry. Toxicity of raw and wastewaters treated by advanced oxidation processes namely Fenton and Photo-fenton reactions were tested. The study revealed the toxic effect of wastewater in terms of Chl (a) content, EC_{50} value, carbohydrate and protein contents of algae. The toxic effect was increased by increasing the ratio of the wastewater in the algal culture. The EC_{50} values at 96 h were 1.8, 18 and 28% for the raw, Fenton and Photo-fenton treated wastewater, respectively; indicating the improvement in wastewater quality. This trend matches that indicated by the high percentage removal of pesticides residues (92-97%) and increased BOD/COD ratio for the wastewater treated by the advanced oxidation processes.

Key words: *Scenedesmus obliquus*, chlorophyll (a), EC_{50}, advanced oxidation processes (AOPs), carbohydrates, proteins

INTRODUCTION

The pesticide industry generates wastewater containing toxic and non-biodegradable compounds that remain in the aquatic environment even after the wastewater is subjected to conventional treatment (Jannsens *et al*., 1997; Bertanza *et al*., 2001). Due to the complex mix of compounds that might occur in such industrial wastewater, toxicity detection is crucial in assessing environmental contamination (EPA, 1993; Bertanza *et al*., 2001). A single unicellular algal assay is useful for use in water pollution control and leads to the development of specific standards for the permissible concentration of pollutants (OECD, 1984; Rhee, 1988).

This study was run to investigate the toxicity of raw wastewater discharged by the HELB pesticide Co. as well as effluent toxicity after treatment by advanced oxidation processes, namely the Fenton and Photo-fenton reactions.

MATERIALS AND METHODS

Wastewater samples were collected from HELB Co. for the production of pesticides and several chemicals. The company is located at new Damietta City, in the delta region of Egypt. Both industrial and municipal wastewaters are discharged into the sewer system, without treatment.

COD, BOD, TOC, other physico-chemical analyses, individual organic pesticides (organophosphorus) and heavy metals were investigated according to APHA (1998). Total organic carbon (TOC) was measured using a PHENOX TOC analyzer. GC (Model HP 6890) was used for total organophosphorus pesticides analysis. Atomic absorption (model Varian Spectra 220) was used for heavy metal measurements. Advanced oxidation processes (AOPs) namely the Fenton reaction (Fe^{2+}/H_2O_2) and Photo-fenton ($Fe^{2+}/UV/H_2O_2$) reaction were run according to Kang *et al*. (2002) and Faust (1994). The Fenton system is an oxidative technique for the treatment of refractory organic pollutants. By to this method strong free radical OH° is produced:

$$H_2O_2 + Fe^{2+} \longrightarrow OH^\circ + HO + Fe^{3+}$$

The photo-fenton reaction is highly effective due to irradiation with UV/ visible light where ferrous ions react with H_2O_2 generating a second HO° radical:

$$Fe^{3+} + H_2O \xrightarrow{U.V} Fe^{2+} + HO^\circ$$

The EPA (1971) Algal Assay Bottle test was conducted. The green alga *Scenedesmus obliquus* was isolated from Nile river water, purified and recultivated in modified BG_{11}. The composition of BG_{11} medium includes the following macro elements $NaNO_3$, K_2HPO_4, $MgSO_4$, $CaCl_2$, citric acid, Na_2CO_3, Na_2EDTA and ferric ammonium citrate (Carmichael, 1986). The modification was done by diluting the concentration of sodium nitrate ($NaNO_3$) to 1/5th the original concentration according to Gamila

Corresponding Author: Dr. A.M. Abdel-Aty, Department of Water Pollution Research, National Research Centre, Dokki, Cairo, Egypt

(2004). The test organism was in the logarithmic phase of growth when introduced to the standard algal culture medium. Bioassay flasks were incubated at 24±2°C under continuous illumination (≈2500 Lux). Prior to conducting the bioassay, the organism was separated from the medium by centrifugation and washed with distilled water (Epstein and Schultz, 1965). The applied raw wastewater concentrations ranged between 0.5 and 8% (v/v). Wastewaters treated via the Fenton process, were applied at concentration of 16 to 28% (v/v), whereas, in case of the photo-fenton process the applied concentrations ranged between 20 to 50% (v/v). To all assay flasks, suitable volumes of distilled water enriched with nutrient medium (BG_{11}) were added to complete the net volume to 100%. Each concentration was represented by three flasks. A control sample, containing the algal inoculum and the medium, was run for each test to represent the normal order of growth. All tests were run for 10 days to allow good growth without causing nutrient shortages.

Algal growth was determined by measuring chlorophyll (a) content Chl (a). Chl (a) was extracted with hot 100% methanol (Sartory and Grobbelar, 1984) and measured spectrophotometrically at 630, 647 and 664 nm. Chl (a) was calculated according to APHA (1998) and the effective concentration (EC_{50}) which leads to 50% growth inhibition was determined according to Finney (1971). At maximum growth of algae, algal mass of each treatment was collected and used for carbohydrate determination according to DuBois *et al.* (1956) and protein was determined according to Lowry *et al.* (1951) as modified by Quian and Wang (1989). Results of carbohydrate and protein were subjected to statistical analysis according to Duncan's Mltiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The present study revealed the relative toxicity of the wastewater discharged by the HELB pesticide Co., when measured in terms of changes of Chl (a), EC_{50}, carbohydrate and protein content.

A concentration of 0.5% raw wastewater (v/v) showed a progressive increase in Chl (a) content of *Scenedesmus obliquus* culture up to the 6th day of incubation where by it approached the value of the control indicating no toxic effect of the wastewater at that concentration. However, as the dose of the raw wastewater was increased in the range of 1 to 2% (v/v) a progressive decrease in the Chl (a) content was attained which amounted to 35 and 66%, respectively, compared to the control (Fig. 1). Increasing the concentration of the raw wastewater to 8% (v/v) resulted in the decay of algal cells. Results presented in Fig. 1 for wastewater treated with the Fenton process, revealed the improvement in the quality of the wastewater where its inhibitory effect on algae was greatly reduced. Consequently, at a concentration of 16% (v/v) of the treated wastewater, the percentage inhibition of algal growth, in terms of Chl (a) amounted to 13%, compared to the control at the 8th day of incubation. Treatment of the HELB wastewater with the Photo-fenton process resulted in further improvement in the quality of the wastewater (Fig. 1). Under such conditions, algal medium dosed with 20% (v/v) of the treated wastewater, showed slight decrease in Chl (a) content up to the 4th day of incubation, whereafter the algal culture showed progressive increase in Chl (a) content which approached the level of the control (Fig. 1). Addition of a low concentration of the treated pesticide wastewater tends to stimulate algal growth and an increase in Chl (a) content was recorded. Such a result is in agreement with previous studies where addition of low concentration of the phenylamide herbicide patoran, increased Chl (a) content of *Scendesmus sp.* culture (El-Dib *et al.*, 1991). The stimulative effect of low herbicide concentration on photosynthetic pigment and growth may be a result of algal cell adaptation (Koening, 1990; El-Dib *et al.*, 2000).

EC_{50} values attained by probit analysis, in terms of Chl (a) content were 2.3 and 1.8% for the raw wastewater, at 48 and 96 h, respectively. After treatment with the Fenton process the EC_{50} values amounted to 19.5 and 18.5% at the previously given time intervals, respectively. In the case of Photo-fenton treatment, the EC_{50} of wastewater amounted to 28.5 and 28% at 48 and 96 h, respectively. The results of decreasing toxicity of HELB wastewater as revealed by the increase in EC_{50} values are a good indication of the effectiveness of the Fenton and Photo-fenton reactions as advanced oxidation procedures (Hornstorm, 1990) According to Abdel-Hamid *et al.* (1993), two of the industrial effluents, out of six, were highly inhibitory to *Selenastrum capricornutum* and showed EC_{50} values ranging between 1 and 10% (v/v) at 96 h.

Build up of carbohydrate and protein by higher plants and algae is a good indicator of normal photosynthesis activity and metabolic functions. The stress imposed by toxic pollutants in the raw and treated wastewater was reflected on carbohydrate and protein contents of *Scenedesmus* algal culture (Fig. 2). In general, algal contents of carbohydrate and protein significantly decreased as the applied doses of wastewater were increased. El-Dib *et al.* (2000) indicated that metabolic build up of carbohydrate by *Selenastrum capricornutum* was only inhibited in the presence of high doses from pentachlorophenol (0.669 to 1.84 mg L^{-1}). Protein

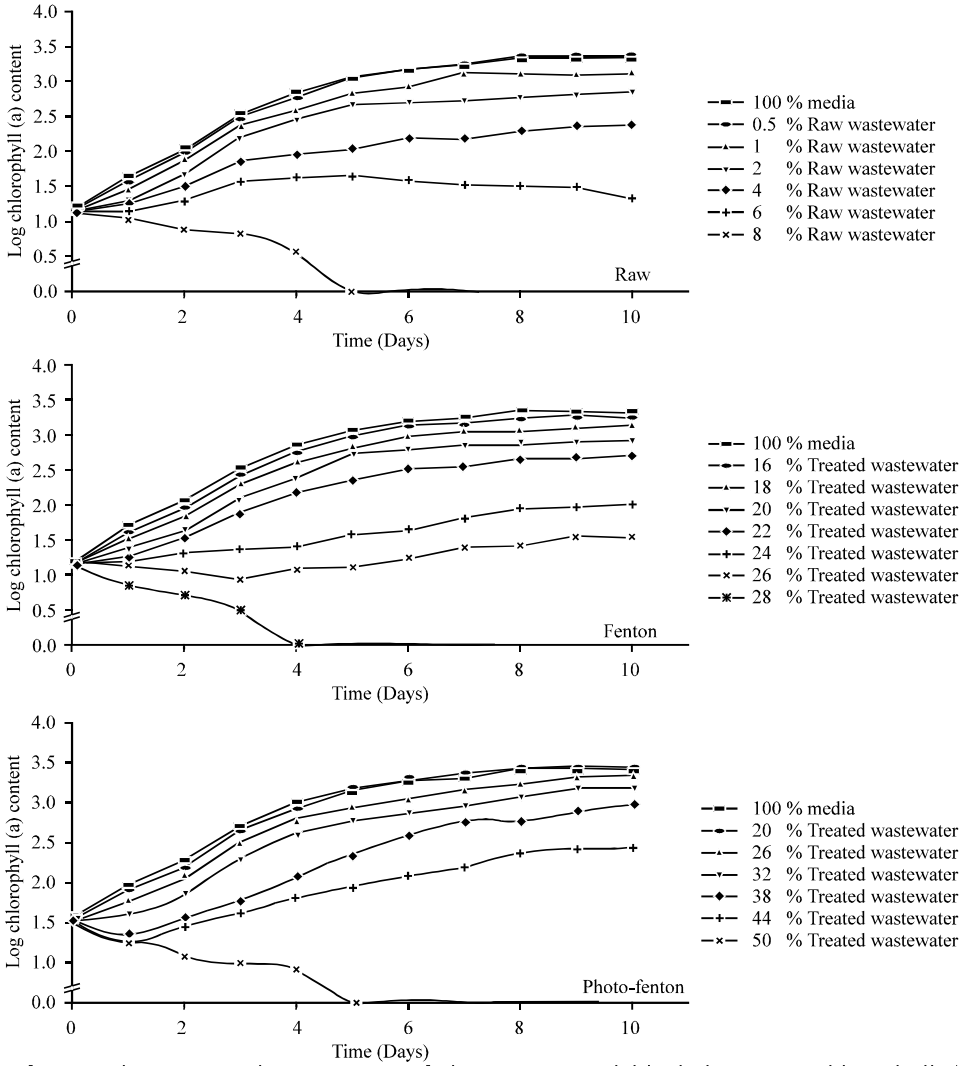

Fig. 1: Effect of pre-and post-treated wastewater of the HELB pesticide industry on chlorophyll (a) content of *Scenedesmus obliquus*

Table 1: Physico-chemical characteristics of pre-and post-treated wastewater from the pesticide industry (HELB)

Parameter	Raw wastewater	Treated effluent	
		Fenton	Photo-fenton
pH value	7.1	7.7	8.0
Total suspended solids (mg L^{-1})	517	3.0	2.0
Settlable matter 10 min (cm^3)	0.0	0.0	0.0
Settlable matter 30 min (cm^3)	0.0	0.0	0.0
Chemical oxygen demand (mg O_2 L^{-1})	2130	216	95
Biological oxygen demand (mg O_2 L^{-1})	495	83	52
BOD/COD	0.23	0.38	0.55
TOC (mg L^{-1})	249	45	45
Total phosphorus (mg P L^{-1})	32	24	39
TKN as N_2 (mg L^{-1})	21.8	8.4	ND*
Total extractable matter (mg L^{-1})	464.7	18.4	9.3
Total phenols (mg L^{-1})	0.25	<0.005	<0.005
Total organophosphorus pesticides (mg L^{-1})	21.8	1.6	0.6
Heavy metal:			
Cr^{+6} (mg L^{-1})	<0.02	ND	ND
Ni (mg L^{-1})	<0.02	ND	ND
Zn (mg L^{-1})	<0.01	ND	ND

* = Not Detected

synthesis, on the other hand, was inhibited by all concentration levels. Also, El-Sheekh *et al.* (1994) reported that a sublethal concentration of atrazine herbicide to the green algae *Chlorella* sp. induced a general inhibition of growth, photosynthesis and reduction in protein synthesis with increasing herbicide concentration. This trend matches that shown by the decrease in Chl (a) content. However, the toxic and/or inhibitory effects of HELB wastewater, on algal metabolic activity and build up of photosynthesis, tended to decrease after treatment with the advanced oxidative procedures. The concentration of heavy metals in the HELB wastewater, either raw or treated was relatively low and not expected to exert toxic effects on *Scenedesmus* (Gamila and Naglaa, 1999). Consequently, toxicity of the wastewater is mainly due to organic and pesticide residues, which are oxidized by the applied Fenton and Photo-fenton processes (Table 1).

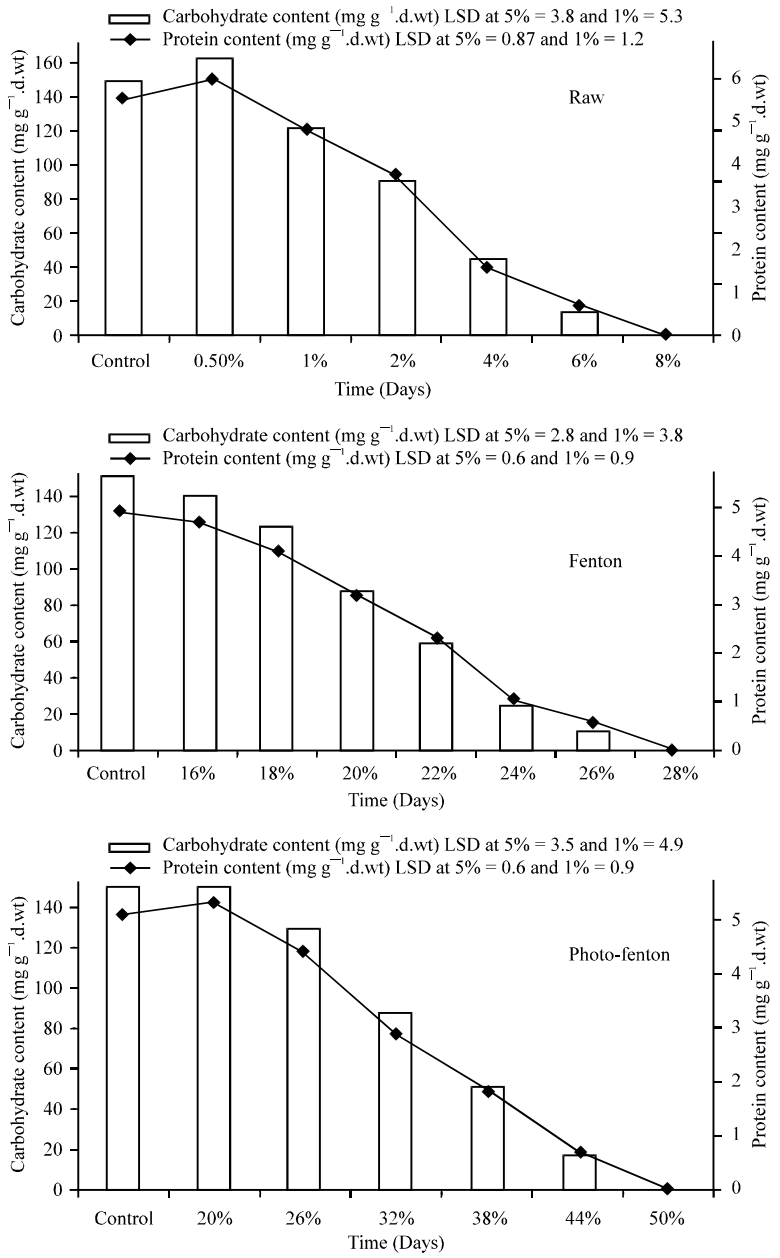

Fig. 2: Effect of pre-and post-treated wastewater from the HELB pesticide industry on carbohydrate and protein content of *Scenedesmus obliquus* at maximum growth

The BOD/COD ratio is used to express the biodegradability of a wastewater. A BOD/COD ratio more than 0.3 is an indication of a good biodegradability (Chun and Wang 1999; Sarria *et al*., 2001). Results given in Table 1 indicated a BOD/COD ratio of less than 0.3 for the raw wastewater whereas for the treated wastewater the ratio was 0.38 and 0.55 for the Fenton and Photo-fenton processes, respectively. Such results indicate degradation of the refractory and toxic pesticide compounds.

The present study showed that an algal bioassay using *Scenedesmus obliquus* presents an appropriate method for evaluating the toxicity of industrial wastewater and measuring the efficiency of wastewater treatment in the elimination of toxic chemicals.

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