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# Estimation of the Hazard Concentration of Industrial Wastewaters Using Algal Bioassay

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Toxic heavy metals are available to biota from various sources of industrial effluents (Prasad 1997). In the past, dilution of wastewaters discharged to lakes and rivers was an adequate method of treatment (Harlin and Darley 1988). In recent years several requests have been made to adopt the toxicity inhibition of microalgae as part of effluent control schemes or as part of testing schemes prescribed for draining industrial and agricultural wastes in water surface. The test has been used to assess toxicity of both pure chemicals and complex samples (Kusk and Nyhlom 1991). Algal toxicity are frequently required for notification of chemicals, environmental monitoring assessment of pollution (Fargasová 1996; Fernandez-Leborans and Novillo 1996; Wang and Freemark 1995) and also increasingly being used to manage chemical discharge (Lewis 1993, Peterson and Nyholm 1993). Previous studies, using such bioassays have shown that toxic effects of chemicals on test organisms vary considerably and it seems that the species-depend (Wanberg *et al.* 1995).

The objectives of this study were to develop an algal toxicity test system based on the EPA Algal Assay Procedure Bottle Test (EPA 1971) for determining the median effective concentration ($EC_{50}$) values of industrial wastewaters containing heavy metals as potential pollutants.

## MATERIALS AND METHODS

Wastewater samples were collected from the outlet of two industries. The first waste is the outlet of metallic industries and the second is the outlet of ceramic industries. Effluent samples were collected in 5L polyethylene container previously acid washed. A part of both wastewaters was filtered through 0.45 µm membrane filter to omit the suspended particles. Physico-chemical parameters of filtered and unfiltered wastewaters were anlyzed according to APHA (1995). Also, metals content of filtered and unfiltered wastewaters were determined using the flame technique for metal analysis. The preservation and analysis were carried out according to APHA (1995).

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Two species of Chlorophyta group, *Scenedesmus obliquus* and *Ankistrodesmus acicularis* were isolated from River Nile water. The tested organisms were grown in the stock culture, transferred into a fresh algal nutrient medium approximately seven days prior to the experiment, so that the organisms were in the logarithmic phase when introduced to the standard algal nutrient medium. The macroelements of the algal nutrient medium were composed of : (mg/l in culuture medium) $NaNO_3$-25.5; $K_2HPO_4$-1.044; $MgCl_2$-5.7; $MgSO_4.7H_2O$-14.7; $CaCl_2 2H_2O$-4.41 and $NaHCO_3$- 15 (EPA 1971). Both organisms were treated with the filtered and unfiltered raw wastewater which were diluted with distilled water enriched with nutrient media suitable for algal growth (EPA 1971). The dilution ratios were: 100% waste + 0.0 media, 75% waste + 25% media, 50% waste + 50% media, 25% waste + 75% media and 0.0 waste + 100% media (control).

Bioassay flasks were incubated at 24±2°C, and exposed to a continuous white florescent light (≅ 2500 Lux). Flasks were shaken once per day to prevent clumping of the cells. Each experiment was run for 10 days, to allow good growth but without causing nutrients shortages. Growth in the cultures was determined by daily measurements of chlorophyll "a" content (Fitzgerald 1971). Also, after the $10^{th}$ day of the experiments, algal mass was collected to determine carbohydrate (Dubois *et al.* 1956) and protein (Lowary *et al.* 1951) content.

## RESULTS AND DISCUSSION

Analysis of physico-chemical characteristics and metals composition of both unfiltered and filtered industrial wastewaters were carried out and presented in Table (1) and (2).

**Table 1.** Physico-chemical analysis of unfiltered &filtered industrial wastewaters

| Parameter | Unit | Metallic Wastewater | | Ceramic Wastewater | |
|---|---|---|---|---|---|
| | | Unfiltered | Filtered | Unfiltered | Filtered |
| - PH | | 7.9 | 7.8 | 7.8 | 7.5 |
| - Turbidity | NTU | 2.2 | 1.2 | 190 | 2.5 |
| - Electric Conductivity | µmhos $cm^{-1}$ | 410 | 390 | 1300 | 1300 |
| - Total Alkalinity (as $CaCO_3$) | mg/L | 136 | 136 | 210 | 190 |
| - Total Hardness (as $CaCO_3$) | mg/L | 124 | 124 | 400 | 380 |
| - Calcium hardness (as $CaCO_3$) | mg/L | 76 | 76 | 268 | 264 |
| - Magnesium hardness(as $CaCO_3$) | mg/L | 48 | 48 | 132 | 116 |
| - Chloride | mg/L Cl | 20 | 20 | 68 | 68 |
| - Sulphate | mg/L $SO_4$ | 12.6 | 12.6 | 128.8 | 128.8 |
| - Silica | mg/L $SiO_2$ | 2.5 | 2.5 | 9.8 | 9.8 |
| - Ammonia | mg/L N | 0.0 | 0.0 | 0.0 | 0.0 |
| - Nitrite | mg/L N | 0.02 | 0.02 | 0.02 | 0.02 |
| - Nitrate | mg/L N | 0.18 | 0.17 | 0.32 | 0.13 |
| - Total organic nitrogen | mg/L N | 0.9 | 0.7 | 2.4 | 1.0 |
| - Total phosphorus | mg/L P | 0.21 | 0.11 | 0.49 | 0.46 |
| - Iron | mg/L Fe | 3.9 | 0.35 | 1.54 | 0.36 |
| - Manganese | mg/L Mn | 1.41 | 0.97 | 0.9 | 0.9 |
| - Chemical oxygen demand | mg/L $O_2$ | 15.5 | 4.6 | 39.9 | 13.9 |

**Table 2.** Metals composition of unfiltered and filtered industrial wastes.

| Wastewaters Type | Metal Concentration (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cd | Ni | Pb | Cr | Cu | Zn | Na | K |
| - Unfiltered Metallic Waste | 0.08 | 0.16 | 0.15 | 0.17 | 0.09 | 10.0 | 77.6 | 18.1 |
| - Filtered Metallic Waste | 0.003 | 0.05 | 0.0 | 0.003 | 0.06 | 0.34 | 11.2 | 13.6 |
| - Unfiltered Ceramic Waste | 0.07 | 0.38 | 0.29 | 0.056 | 17.2 | 210 | 35.2 | 38.9 |
| - Filtered Ceramic Waste | 0.005 | 0.01 | 0.22 | 0.05 | 0.12 | 0.09 | 7.1 | 10.4 |

The physico-chemical parameters of both raw wastewaters were within the expected range with two exceptions. The first one was the high turbidity value of unfiltered ceramic wastewater (190 NTU) which may be due to the high concentration of suspended solids. The second exception was the high concentration of Cu and Zn in unfiltered ceramic wastewaters. It is mainly due to the nature of raw wastewaters, which contain metal pigments, used in the glazed tile.

Although soluble forms of contaminants are known to be more toxic, they are not recommended for surveillance programmes because it is too time-consuming to do both total and soluble concentrations, Moreover, toxicity guidelines for contaminants are given for total chemical concentration. From many soluble forms of metals, free metal ions are the most bioavailable and most toxic, but in fact it is impossible to determine from the test water which metals will enter the algal cells, though the presence of free metal ions is a good indication of probable metal toxicity (Wong *et al.*, 1997). So, from the growth curves of tested algae growing in different effluents concentrations (Fig. 1 and 2), variations in growth response were not only found between both effluents in various concentrations but also among the filtered and unfiltered effluents of the same wastewaters. Compared to control, the effects of metallic effluent were found to differ with respect to the both test organisms. Severe toxic effect was observed with 100% of unfiltered and filtered metallic waste on the growth of *S. obliquus* (Fig. 1). Growth of *A. acicularis* was decreased with the increase percentage in filtered and unfiltered wastewater concentrations (Fig. 1). So, the $EC_{50}$ of metallic wastewater concentration to *S. obliquus* reached to 80% (Unfiltered wastewater) and 40% (Filtered wastewater), while it reached to 110% (Unfiltered wastewater) and 82% (Filtered wastewater) for *A. acicularis.*

Changes in both carbohydrate and protein contents, compared to control reflect the stress of algal metabolic activities. Protein content of both organisms (Fig. 3A) was highly affected by the unfiltered and filtered metallic wastewater concentrations. At 100% wastewater (unfiltered and filtered), percentage inhibition of protein content was 100% of control. Carbohydrate content of both organisms (Fig. 3B) showed a response differed from that obtained with protein. *A. acicularis* treated with unfiltered metallic wastewater reflected percentage increase of carbohydrate decreased with the increase in wastewater concentration. According to Wong *et al.* (1997) inorganic Pb and organometallic compounds in industrial wastewaters altered the fine structure of *Chlorella* cells. Also, Priha

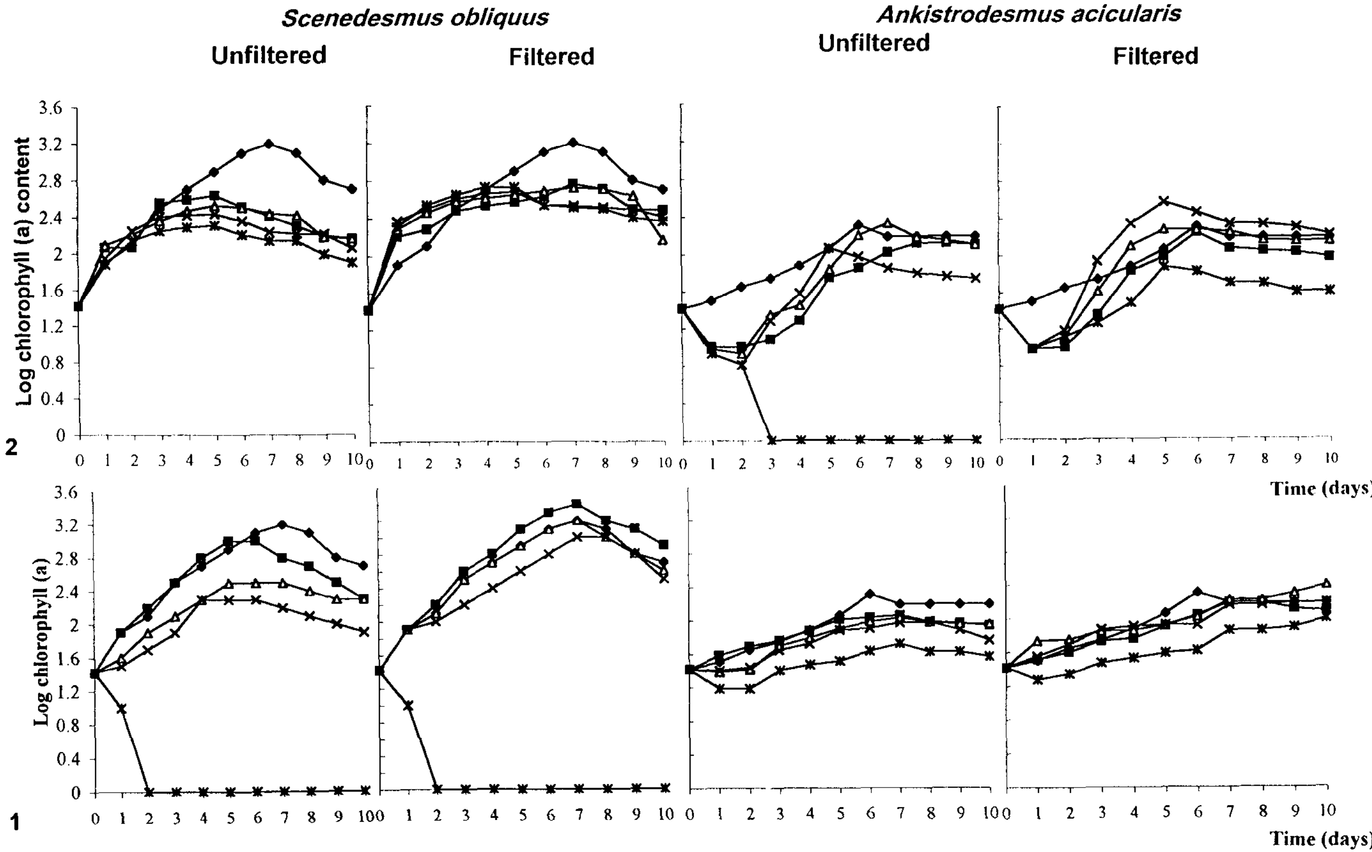


**Figure 1. Effect of different metallic wastewater concentrations on chlorophyll "a" content of tested algae**

**Figure 2. Effect of different ceramic wastewater concentrations on chlorophyll "a" content of tested algae**

**—◆— 100% nutrient media —■— 75% media + 25% wastewater —△— 50% media + 50% wastewater**
**—×— 25% media + 75 wastewater —✱— 100% wastewater**

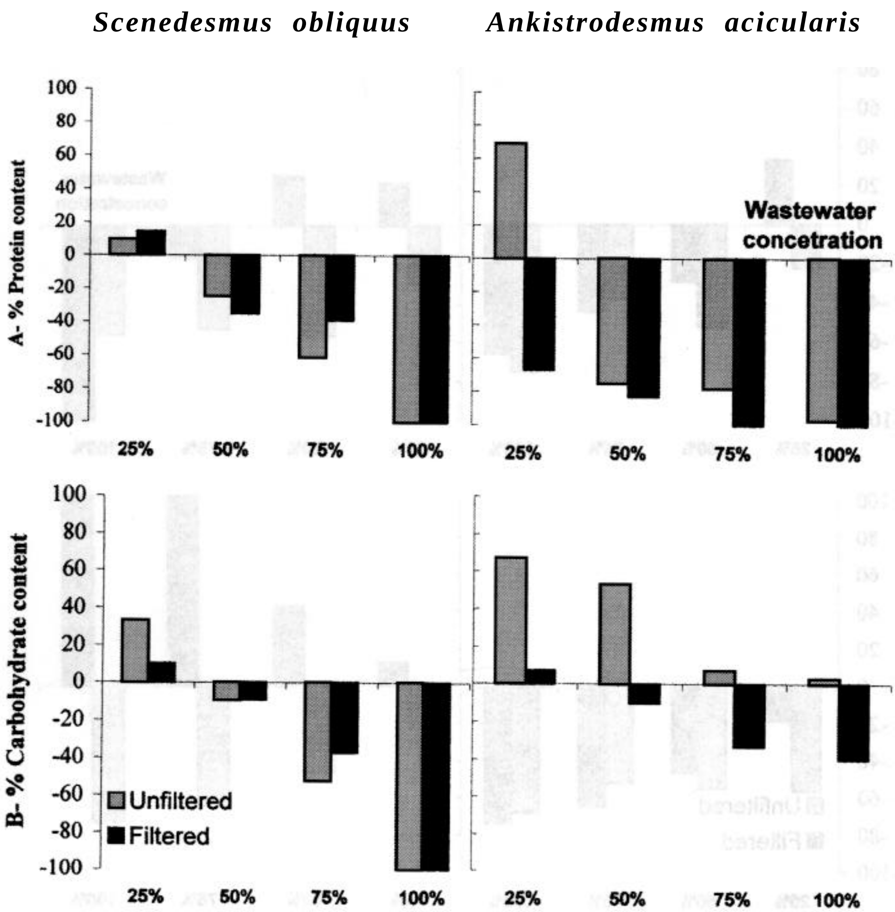


**Figure 3. Percentage inhibition and/or activation of different metallic wastewater concentrations on: A-Protein content, B-Carbohydrate content**

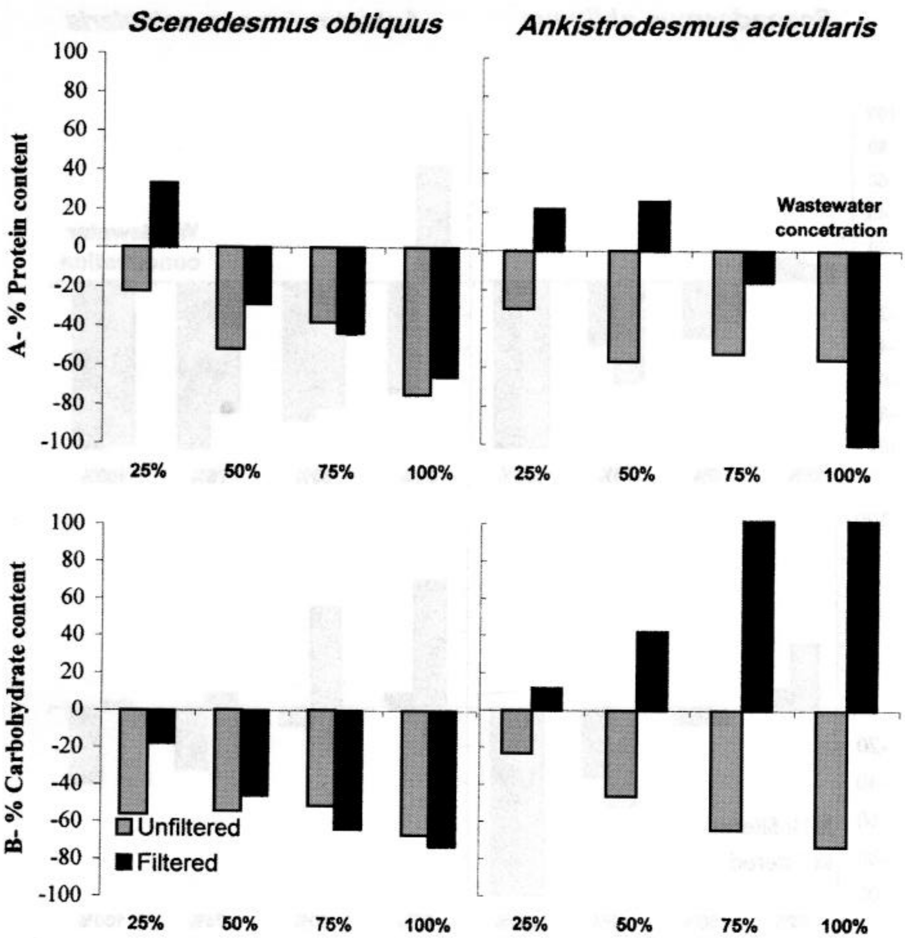


**Figure 4. Percentage inhibition and/or activation of different ceramic wastewater concentrations on: A-Protein content, B-Carbohydrate content**

(1996) stated that effluent from 11.5 Finnish pulp mills stimulated the growth of *Selenastrum capricornutum* in some of the test concentration.

Ceramic wastewater concentrations have diverse effect on chlorophyll “a” content of the tested organisms. Unfiltered and filtered ceramic waste concentrations lead to growth inhibition of *S. obliquus* (Fig. 2). The percentage inhibition ranged between 64% to 87% expressed by chlorophyll “a” content respectively. Concentrations of 50% unfiltered and 75% filtered effluents of the same wastewaters resulted in growth enhancement to *A. acicularis* while other concentrations revealed inhibitory effect (Fig. 2). Only, a concentration of 100% unfiltered ceramic waste have a toxic effect to *A. acicularis* growth. Referring to the $EC_{50}$ calculated from the chlorophyll “a” content revealed that, the $EC_{50}$ ceramic waste concentration to *S. obliquus* was 20% (Unfiltered wastewater) and 17% (Filtered wastewater) while it was 80% (Unfiltered wastewater) and 40% (Filtered wastewater) for *A. acicularis.*

Protein and carbohydrate content of the tested species were highly affected by the different concentrations of filtered and unfiltered ceramic wastewaters (Fig. 4). Protein content of *A. acicularis* in presence of unfiltered ceramic waste revealed percentage inhibition which increased with the increase in waste concentrations (Fig. 4A). *S. obliquus* in presence of unfiltered and filtered ceramic wastewater revealed percentage inhibition increased with the increase in ceramic wastewater concentration (Fig. 4A). Carbohydrate content of *S. obliquus* in whatever unfiltered and filtered wastewater concentrations was inhibited and the percentage increased in the increase in wastewater concentrations (Fig. 4B). Although carbohydrated content of *A. acicularis* in filtered ceramic wastewater concentrations was increased with increase in wastewater concentrations, it was inhibited by all unfiltered ceramic wastewater concentrations (Fig. 4B). Abdel-Hamid *et al.* (1993) found that, from six different industrial effluents with *Selenastrum capricornutum*, two of the effluents were highly inhibitory with $EC_{50}$ values ranging between 1 and 10% (v/v) effluents concentrations. Also, they stated that other effluents showed how growth inhibitory effects values derived from the growth parameters, algal dry weight, cell count, growth rate, and area under the growth curve were almost similar for those two effluents exhibiting highly inhibitory effects.

This study reaffirmed that, the variation in sensitivity of the tested species to the tested substances in this study indicates that more than one algal species should be utilized when assessing the potential impact of toxic pollutants on an ecosystem. However, dependence on a single species can provide erroneous results concerning the potential toxicity of toxic element in a given ecosystem. Moreover, the highly toxic nature of potential pollutants from different industrial sources to algae emphasize the need for minimizing stock effluent pollutants

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