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Microalgae as Biomarkers for Toxicity of Pharmaceutical Wastewater

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WASTEWATERS of pharmaceutical industries are characterized by high loads of hazardous chemicals, high organic load, strong colors and highly fluctuating pH and temperature. The green alga *Scenedesmus obliquus* and natural phytoplankton assemblages of Nile river were used for evaluating the toxicity of wastewater from pharmaceutical factory. Wastewater samples were collected from El-Nasr Pharmaceutical Company for the production of drugs and other chemicals. The effluent from the company is mainly characterized by low pH value. The results of toxicity bioassay found that the toxic effect of pharmaceutical wastewater is due to the acid pH rather than to the presence of toxic substances in the waste. The EC_{50} was changed by the change in pH of the waste. Bioassay experiments with natural phytoplankton community of Nile River showed population shifts in the algal community treated with pharmaceutical wastewater. *Oscillatoria limnetica* and *Nitzschia linearis* were observed in low number in the initial samples of the Nile River, in culture treated with low wastewater concentration it became the most abundant species. High dominance of *Micractinium pusilium*, *Ulothrix subtilissima* and *Actinastrum hantzschii* were observed at high waste concentration. This study reaffirmed that algal toxicity and algae as bioindicator are frequently required for notification of chemicals, environmental monitoring assessment of pollution and also increasingly being used to manage chemicals discharge.

Keywords: Bioindicator, Green Algae, Pharmaceutical, Phytoplankton, Toxicity, Wastewater.

The discharge of pharmaceutical compounds from wastewater treatment plants into surface waters is an emerging issue. The occurrence of pharmaceutical compounds in surface water and groundwater has drawn the attention from the drinking water producers where they pose a risk to the environment (Mons *et al.*, 2000).

Although these compounds have been found at very low concentrations, damages to aquatic biota can not be excluded. Little is known about the extent of environmental occurrence, transport, ultimate fate, effects and risks associated with the release of pharmaceuticals to surface water (Andreozzi *et al.*, 2002).

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A toxicity evaluation is an important parameter in wastewater quality monitoring, as it provides the response of test organisms to all the compounds in the wastewater and are required to ensure that the discharge will not have adverse effects on the aquatic organisms in the receiving water. Among the few studies that have examined pharmaceutical effects, single-species, acute laboratory toxicity tests were primarily used (Honkoop *et al.*, 1999; Uhler *et al.*, 2000). Toxicity of algae is frequently used for notification of chemicals, environmental monitoring assessment of pollution (Wang & Freemark 1995) and also increasingl being used to manage chemical discharge (Lewis, 1993).

The current research aimed to evaluate the potential aquatic toxicity of pharmaceutical wastewater. Single species laboratory toxicity tests as well as phytoplankton assemblages were performed to assess hazard to aquatic biota.

Material and Methods

Pharmaceutical wastewater samples were collected from the outlet of El-Nasr Pharmaceuticals (South east of Cairo). It produces drugs and other chemicals. The company discharges both industrial and human wastewater without treatment into a near-by evaporation pond. The physico-chemical analysis of the wastewater indicated that it is acidic and contains very high concentration of organic compounds. Mixed effluent samples were collected in 5 L-polyethylene container previously acid washed. Physico-chemical characters of the wastewater were analyzed according to APHA (1998).

The green alga *Scenedesmus obliquus* was isolated from River Nile water. The tested organism was grown in stock culture, transferred into a fresh algal nutrient medium BG_{II} (Carmichael, 1986) approximately seven days prior to the experiment. So that the organism was in the logarithmic phase when introduced to the standard nutrient medium. The natural phytoplankton assemblage was collected from El-Gezira site (Cairo district) and concentrated using phytoplankton net (80μ mesh). Three main algal groups namely, green algae, blue-green algae and diatoms were present in the phytoplankton assemblages. The isolated test organism as well as natural phytoplankton assemblages were treated with pharmaceutical raw wastewater which was diluted in proportion with distilled water enriched with nutrient media suitable for algal growth (BG_{II} for *Scenedesmus* and EPA, 1972 for phytoplankton community). Since the pharmaceutical wastewater is highly acidic, the experimental design was conducted through two series with and without pH (of wastewater) adjustment to the value of nutrient media (pH =7.2).

Bioassay flasks were incubated at 24 ± 2°C, and exposed to continuous white florescent light (≈ 2500Lux). 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. Each experiment was repeated three times, so, the results are the average of three experiments. Growth of

Scenedesmus obliquus in the cultures was determined by daily measurements of chlorophyll "a" content (Fitzgerald, 1971). For the phytoplankton experimental study, daily samples from each waste treatment were collected and fixed with Lugol's Iodine solution. Algal dominance and chlorophyll "a" contents were determined according to APHA (1998) and algal taxonomic keys (Starmach, 1966; Streble & Krauter, 1978).

Results and Discussion

The results of chemical analyses of the wastewater from the pharmaceutical industry are given in Table (1). The data show that, the effluent was polluted with organic compounds, as COD, BOD are higher than permissible levels according to our regulations for discharging wastewater into surface stream waters. Permissible levels from our regulations were also exceeded in the case of total phosphorus, ammonia, nitrite, and nitrate (Table 1).

TABLE 1. Characteristics of wastewater of pharmaceutical industry at El-Nasr.

Characteristics		Values	Permissible Level
pH		3.8 - 5.4	6 – 10
Total Dissolved Solids	mg / L	1730 - 10000	2000
Total Suspended Solids	mg / L	20 - 36	500
Chemical Oxygen Demand	mg / L	13023 - 8679	700
Biochemical Oxygen Demand	mg / L	3900 - 2850	400
Ammonia	mg N / L	0.0 - 448	100
Nitrite	mg N/ L	0.0 - 0.02	*
Nitrate	mg N/ L	0.4 - 0.7	30
Total Kjeldahl Nitrogen	mg N/L	588 - 943	*
Total Phosphorus	mg P/L	6.4 - 8.9	5

* Not found in the regulation.

Toxicity of pharmaceutical wastewater to Scenedesmus obliquus

The results of toxicity tests with green alga *Scenedesmus obliquus* exposed to wastewater from the pharmaceutical industry are given in Figs. (1,2). The toxic effect varied with the pH value of the waste and was related to acidic pH. Wastewater of pH value 3.8 revealed high toxicity to the alga growth since, the EC_{50} after 96h was detected at waste dilution about 2% (Fig. 3). Other waste dilutions lead to acute toxic effect to the alga growth (Fig. 1). Therefore, the

percentage inhibition was 93%, 91, 91, 90, 90, and 90% for waste concentrations of 4%, 6, 8, 10, 12 and 14%, respectively. Brooks *et al.*, (2003) observed that pharmaceutical fluoxetine adversely reduce growth of green alga *Pseudokirchneriella subcapitata.* In addition, pH adjustment to 7.2 caused a pronounced alga growth promotion (Fig. 1). It resulted in growth activation equivalent or increased over than that of control by percentage are 100%, 102, 100, 107, 115, 117, 126, 132 and 145% for waste dilutions of 0.5%, 1, 2, 4, 6, 8, 10, 12 and 14%, respectively.

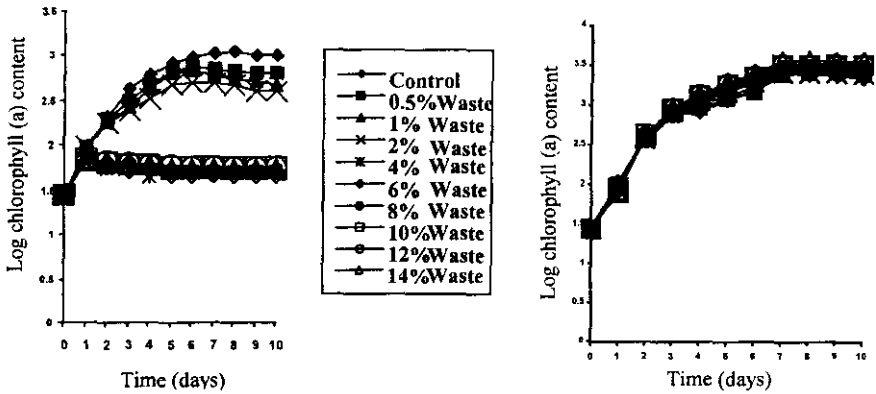

Fig. 1. Growth response of *Scenedesmus obliquus* exposed to pharmaceutical wastewater with initial pH=3.8.

The same is true with the second pH of pharmaceutical wastewater (pH=5.4) where raising the pH to 7.2 could reverse the toxic effect of acidic pharmaceutical wastewater. So that, all wastewater dilutions tested (initial pH=5.4) showed a promoting growth effect to *Scenedesmus* as the pH changed to pH=7.2 (Fig. 2). On the other hand, low waste dilutions at pH 5.4 have a promoting effect to the alga growth while higher concentrations of waste had acute toxic effect (Fig. 2). The waste dilution caused EC_{50} to the alga growth after 96h was 38% (Fig. 3).

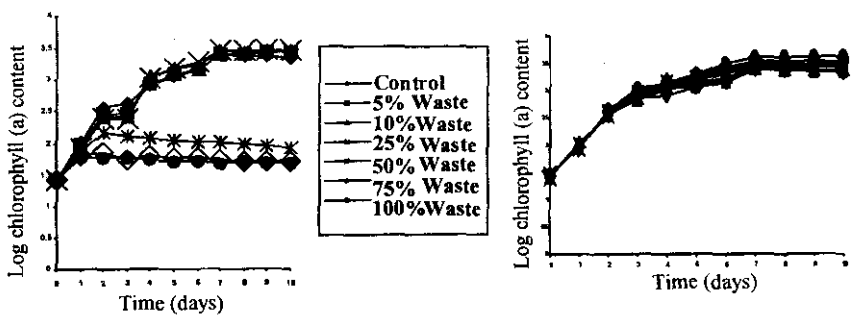

Fig. 2. Growth response of *Scenedesmus obliquus* exposed to pharmaceutical wastewater with initial pH=5.4.

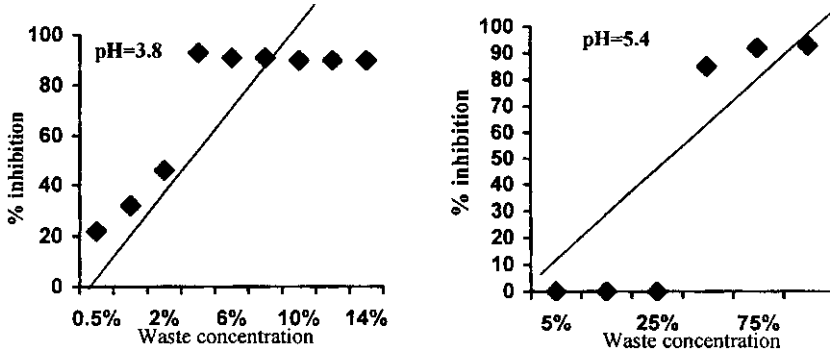

Fig. 3. EC_{50} of pharmaceutical wastewater to *Scenedesmus obliquus* (after 96 hrs incubation period).

Therefore, it may be concluded that the toxicity effect of pharmaceutical wastewater against isolated algal strain is due to the high acidity of the waste not due to the presence of toxic substances.

Toxicity of pharmaceutical to natural phytoplankton assemblages and the indicators

The results of toxicity tests showed that the pharmaceutical wastewater have a distinguish effect on natural phytoplankton community. The highest toxicity effect was established in pharmaceutical wastewater with pH value 3.8 (Fig. 4). Without pH adjustment, waste dilution 5% has a pronounced good algal growth approximately equivalent to that of control. Other waste dilutions had acute toxic effect to algal growth where the EC_{50} (calculated) after 96h was 7% (Fig. 5). As the pH of waste raised to 7.2, the EC_{50} (calculated) of waste dilution was changed to 14.5% (Fig. 5).

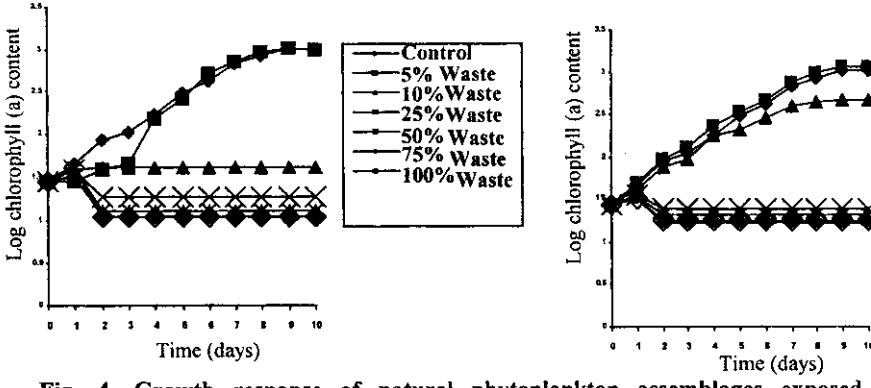

Fig. 4. Growth response of natural phytoplankton assemblages exposed to pharmaceutical wastewater initial pH=3.8.

Pharmaceutical wastewater with initial pH 5.4 has a pronounced effect on the growth of phytoplankton community, it was completely different from that of pH 3.89. Natural phytoplankton assemblages exposed to pharmaceutical wastewater with pH value 5.4, before and after pH adjustment revealed high algal biomass approximately equivalent to that of control (Fig. 6).

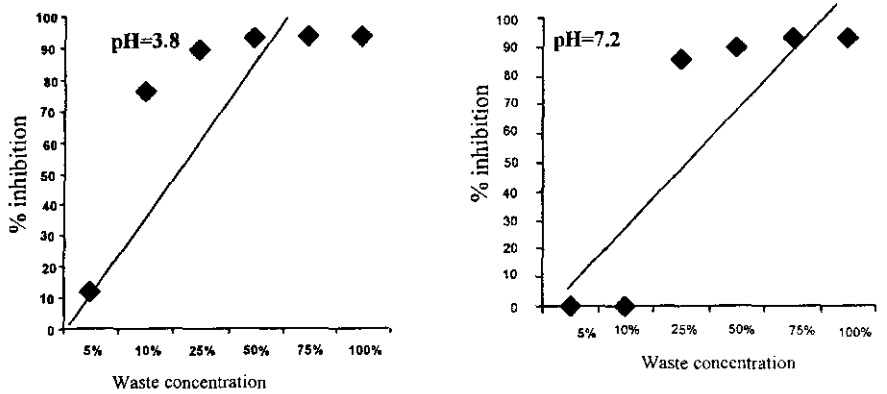

Fig. 5. EC_{50} of pharmaceutical wastewater to natural phytoplankton assemblages before and after pH adjustment (after 96 hrs incubation period).

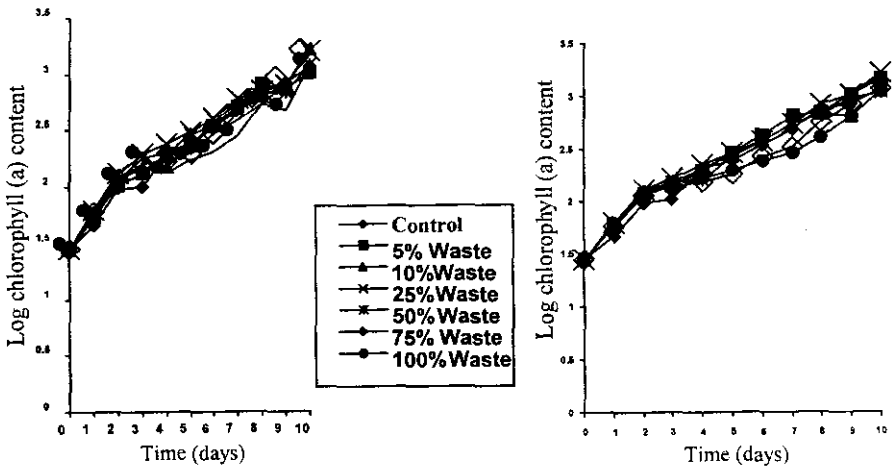

Fig. 6. Growth response of natural phytoplankton assemblages exposed to pharmaceutical wastewater with initial pH=5.4.

In another point of view, studying the community structure of phytoplankton assemblages revealed the dominance of each: *Oscillatoria limnetica, Coelosphaerium kuetzingaianum* (blue-green algae), *Staurastrum paradoxum, Scenedesmus obliquus, Oocystis parva, Ankistrodesmus acicularis, Sphaerocystis schroeteri, Pediastrum clathratum, Coelastrum microporum* (green algae), *Melosira granulata and Cyclotella comta* (diatoms) in control culture. Algal community after exposure to pharmaceutical waste at pH 3.8 showed major change in algal predominance, where *Scenedesmus quadricauda* and *Microactinium pusilium* are the most abundant species. As the pH changed to 7.2, the algal community changed and revealed the predominance of *Nitzschia linearis* and *Actinastrum hantzschii*. In addition, high dominance of *Oscillatoria limnetica, Oscillatoria agardhii, Scenedesmus quadricauda, Stephanodiscus astraea, Pediastrum clathratum, Merismopedia glauca* and *Botryococcus braunii* was detected over phytoplankton culture treated with waste of pH 5.4. Then after the pH raised from 5.4 to 7.2, high dominance of *Ulothrix subtilissima, Microactinium pusilium, Merismopedia glauca* and *Actinastrum hantzschii* was observed.

Although algae have been used successfully as bioindicators of industrial wastewater pollution, there are a large number of environmental and chemical factors interacting to determine community structure, and hence, which species may be recognized as biological markers of pollution status. Therefore, it is clear that the pH value of pharmaceutical wastewater lead to a pronounced change in the dominance and population shifts of natural phytoplankton assemblages. Many authors discussed the role of waste type, environmental conditions, nutrient availability and the source of waste in the abundance and the shift in the dominance of phytoplankton (Whitton, 1984; Singh & Gaur, 1989; Bernard *et al.*, 1996; Ali & Abd El-Salam, 1999). So, the toxicity of wastes varied substantially by test species, sampling site and discharge source. Brooks *et al.*, (2003a) recorded that phytoplankton communities were characterized by decrease in species composition but with increase in numbers of some species on treatment with the pharmaceutical fluoxetine. Wilson *et al.*, (2003) suggested that three pharmaceutical and personal care products might potentially influence both the structure and the function of algal communities in stream ecosystems receiving wastewater. These changes could result in shifts in both the nutrient processing capacity and the natural food web structure of these streams.

This study confirmed that, the assessment of potential impact of toxic pollutants on an ecosystem depend on many factors affecting the response of organisms used as a test organism. The different degree of sensitivity of the organism is explained by the varying composition of the pharmaceutical wastewater. Moreover, the highly toxic nature of pharmaceuticals clearly indicates a need for greater understanding of the pharmaceutical waste interactions and effects on multiple levels of biological organization. Such information that is required before more definitive assessments of pharmaceutical wastewater in the environment may be performed.

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استخدام الطحالب كمؤثر بيولوجى للدلالة على سمية المخلفات السائلة الدوائية

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تحتوى المخلفات السائلة الناتجة من مصانع الادوية على كميات كبيرة من المواد الكيماوية الضارة بالاضافة الى احتوائها على احمال عضوية عالية ومواد ملونة. كذلك تتميز بالتفاوت الكبير فى الاس الهيدروجينى و درجات الحرارة . ولتقييم مدى سمية المخلفات السائلة الناتجة من مصانع الادوية على الكائنات الدقيقة المائية و خاصة الطحالب تم استخدام المخلف الناتج من شركة النصر للصناعات الدوائية و الكيماوية لدراسة تاثيرة على نمو كل من طحلب السنيدسمس اوبليكس *Scenedesmus o bliquus* (من مجموعة الطحالب الخضراء) و مجاميع الطحالب الموجودة بمياه النيل و التى تتميز بوجود ثلاث مجاميع رئيسية هى مجموعة الطحالب الخضراء – مجموعة الطحالب الخضراء المزرقة – مجموعة الدياتومات. هذا و قد لوحظ ان المخلف الناتج من شركة النصر للادوية يتميز بانخفاض الاس الهيدروجينى بدرجة كبيرة.

اثبتت النتائج ان سمية المخلفات الناتجة من الصناعات الدوائية ترجع الى انخفاض الاس الهيدروجينى لهذه المخلفات اكثر من احتوائها على مواد ضارة. حيث اظهرت النتائج ان التركيز الذى يحدث تثبيط لنمو طحلب السنيدسمس بمقدار ٥٠% (EC50) يتغير بالتغير فى الاس الهيدروجينى. كذلك اوضحت النتائج ان المخلفات السائلة الدوائية لها تاثير على التنوع فى اعداد و انواع الطحالب السائدة لمجاميع طحالب مياه النيل. فقد ادت التركيزات المختلفة المستخدمة من المخلفات السائلة الدوائية الى اختفاء انواع من الطحالب و سيادة بعض الانواع الاخرى.