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Characterization of Wastewater Generated from Pharmaceutical Industry

M.I. Badawy*, Hussein I. Abdel-Shafy, A.S. El-Kalliny
* National Research Centre, Water Pollution Research Department

Abstract
The wastewater generated from El-Nasr pharmaceutical and chemical company was studied through extensive sampling program. It was found that it is characterized by high loads of organic pollutants represented by COD, BOD5 and TOC. The mean concentration at these parameters are 7408, 2341 and 2250 mg/l respectively. Some of these pollutants are refractory or toxic which are poorly biodegraded and the industrial wastewater required chemical pretreatment before discharge into sewer system.

XAD resins. DPA-6S and GAC (Carbopack B) were used as sorbent material for solid phase extraction of organic compounds from wastewater. It was found that XAD2 sorbent proved to be the most suitable sorbent as it provided the investigation compounds. The recovery percentage was ranged from 63 to 87.

Keywords
Pharmaceutical Wastewater, Organic Pollutants, Refractory Organic Compounds, Solid Phase Extraction.

Introduction

The availability of water at an acceptable quality in Egypt is limited and getting even more restricted. Meanwhile, the needs for water increase as a result of population growth, industrial development and of cultivation desert land. Egypt depends for more than 90 percent of its water supply on the River Nile. Nile water has been changed dramatically in the past few years due to pollution arises from the increase in industrial activities and the discharge of inadequate treated wastewater into the Nile.

It was estimated that the industrial activities in Egypt produce about 639 million m^3/year of wastewater. About 312 million m^3/year is discharged into the River Nile and its associations. The organic synthesis industry comprises different sectors such as polymers and resins, paints, oil and petrochemicals, textile and pharmaceutical chemistry. It was estimated that more than 200 million m^3/year of wastewater are generated from such sectors in Egypt. These wastewater are characterized by high loads of hazardous chemicals, TSS, TOC, COD, strong colors, with highly fluctuating

Corresponding Author
* M.I. Badawy, E- mail: Badawy46@hotmail.com

pH and temperatures and thus particularly objectionable if discharged into open water without treatment.

Previous study revealed that pharmaceutical substances are used extensively in human and veterinary medicine and can reach the aquatic environmental following manufacture application or ingestion/excretion (Hilton and Thomas 2003). The majority of human pharmaceutical compounds discharged into aquatic systems in the form of the non-metabolised parent compounds or as metabolites via the sewage treatment network. Inputs of pharmaceutical substances into aquatic systems via this route have led to their occurrence in sewage treatment effluent, river and marine surface water, ground water (Cahill et al., 2004; Kummerer, 2001; Zuccato et al., 2001; Ternes, 1998; Poiger et al., 2001) and in soil (Hamscher et al., 2000).

El-Nasr pharmaceutical and chemical company is located in Abo-Zabal City, 25Km away from Cairo (south-east of Cairo). El-Nasr Company produces drugs and other chemicals. The company discharges both industrial (4000 m^3/d) and human wastewater (128 m^3/d) without treatment into a near-by evaporation bond. The physico-chemical analysis of the wastes indicated that it is acidic and contains very high concentration of organic compounds, suspended particulate, sulfate, phenolic compounds, oil and grease (El-Gohary et al., 1995). Therefore, the mixed effluent from the company can be posed several environmental problems involving an impact on water ecology and potential effect on human health. To insure the proper selection of wastewater treatment operations, it was necessary to characterize the wastewater generated by the company. Therefore, the aim of the present study is to characterize the wastewater generated from this pharmaceutical company.

Materials and Methods

Materials

Chloroamphenicol, diclofenac, salicylic acid, paracetamol, p-aminophenol, nitrobenzene, benzoic acid and phenol are used as standard compounds. Chloroamphenicol, diclofenac, salicylic acid and paracetamol are widely produced as pharmaceutical agents. However, nitrobenzene, benzoic acid and phenolic compounds represent intermediate byproducts of pharmaceuticals producing companies. Amberlite XAD2, XAD4 and XAD7 nonionic polymeric, C18-Strata cartridge and DPA-6S cartridge (from Supelco Co.) were used in solid phase extraction processes (SPE).

Methods

-Sampling

Composite samples were collected over the working hours from the end of pipe effluent. Physico-chemical parameters were analysed according to APHA (1998) for assessment of the effluent quality inside the company.

-Sample extraction for organic analysis

Several Solid Phase Extraction (SPE) stationary phases were tested under a range of elution conditions for optimum recovery. Samples (200ml) were passed through a glass fiber filter (GFC, 0.45 μm) and the pH adjusted to 3 by addition of 50% H_2SO_4. Upon selection, the investigated compounds were isolated from wastewater samples using Lichrolut vacuum manifold (from Merck Co.), XAD2 (1g) 6ml SPE column with a positive-displacement pump. A performance surrogate containing 1.0 μg of $[C^{13}]$ 1-ethoxy-phenacetin in 100 μl of methanol was added to each filtered sample. The SPE cartridges were conditioned with 6ml of methanol, followed by vacuum drying, followed by 6ml of HPLC-grade water. Samples were processed through the SPE cartridge at 5ml/min. Prior to extraction, 1ml of 5% methanol in HPLC-grade water was passed through the cartridge using vacuum manifold, and the water discarded. The cartridge then was sequentially eluted with 3ml aliquots of methanol followed by 2ml aliquots methanol acidified to pH 3. The combined elutes were evaporated to ~100 μl under a stream of pure nitrogen. The extract was filtered through 0.45 polypropylene syringe filters.

Chromatographic analysis

HPLC (Model Agilent 1100 Series), equipped with micro vacuum degasser, quaternary pump and Diode Array Detector (DAD) and Zorbax SB-C18 analytical column (4.6mm x 250mm, 5μm) was used. A mixture of methanol, HPLC water and 25% acetic acid was used as a mobile phase at a flow rate of 1 ml/min. The elution gradient used for separation is shown in Table (1).

Table (1) *The elution gradient used for HPLC separation*

Time (min)	Volume Percent (%)	
	Methanol	H_2O + 25% acetic acid (96:4)
0	50	50
7	50	50
8	80	20
15	80	20
16	100	0

Site Descriptions

El-Nasr Pharmaceutical Company produces drugs, diuretics, Laboratory chemicals, etc. The company is located 30km north of Cairo. It discharges both industrial ($4000m^3/d$) and domestic wastewater ($128m^3/d$), without treatment into a nearby evaporation pond. Industrial wastewater from the company is collected through a gravity sewerage network. The network discharges its flow into the sump of a pump station where the acidic wastewater is neutralized by the addition of lime. The neutralized waste is then pumped to another sump of a main pump station.

The domestic wastewater is also collected by a separate gravity sewer network which finally discharges its flow into the sump of the main pump station. The combined wastewater (domestic and industrial) is pumped for a distance of 600m through two force mains to the evaporation pond. A small portion of the accumulated wastewater is being used for irrigation purposes. Percolation of wastewater from the pond to groundwater may result in pollution problems.

Results and Discussion

Source of wastewater

The industrial wastewater is mainly produced from the water formed during the chemical reaction or through a separation stage, such as centrifugation, decantation process, steam washes, product washes, equipment and floor washes. Wastewater is originated from spent acid and caustic streams may be discharged during the separation process. This step follows the reaction in which acids and basic reagents are used to facilitate, catalyze, or participate. Other, wastewater may be originated from condensed steam and from packing oil lubricate pumps. Therefore, the characteristics of reach wastewater are then depending mainly on the source of pollution.

Industrial wastewater characterization

Physico-Chemical Parameters

Due to the great variation in the quantity and quality of wastewater generated from El-Nasr pharmaceutical and chemical company, a continuous monitoring program was designed and implemented for one full year to characterize waste-streams up to the final discharge point.

Information gathered from the present study indicates that wastewaters generated from pharmaceutical company contain high loads of organic pollutants represented by COD, BOD_5 values, suspended solids, oil and grease, phenol, sulfate, sulfide, TKN, NH_3 and TP.

Table (2) *Physico-chemical characterization for real wastewater of El-Nasr Co. (mg/l)*

Parameter	13/01/2003	27/01/2003	05/03/2003	27.04.2004	14 06 2004	18 10 2004	Mean
pH	9.8	3.33	4.17	7.7	3.2	11.9	6.68
TRS	27396	1749	949	532	4413	37604	12107.17
TSS	56	20	27.5	134	16	50	50.58
TDS	2734	1729	921.5	398	4397	37554	7955.58
COD	10545	13023	2470	378	6048	11987	7408.50
BOD	3550	3900	1100	97	2400	3000	2341.17
TOC	2615	3417	773	105	1916	4679	2250.83
Oil&grease	31.4	600	312	112.2	112.2	27.3	199.18
Phenols	47	40	35	36	53	69	46.67
TKN	2212	588	31.5	29.12	56	1156.4	678.84
Nitrates	0.24	0.7	0.25	0.69	0.0092	1.41	0.55
Nitrites	0.025	0.02	0.02	0.0044	N.D.	0.78	0.14
K-Ammonia	448	N.D.	N.D.	1.68	12.88	585.2	174.63
TP	17.6	6.4	2.8	1.73	3.7	4.4	6.11
Sulphides	7.17	8.69	7.8	0.4	0.08	120	24.02
Sulphates	140	94	54	26.4	42.6	788	190.83

The BOD/COD ratio is normally used to express the biodegradability of the wastewater (Adams et al., 1997). The obtained results Table (2) showed that the BOD/COD values ranged from 0.25 to 0.45 with an average of 0.33 indicating the presence of refractory organic compounds which are poorly biodegraded. Therefore, such industrial wastewater requires chemical treatment before discharged into sewer system.

A significant variation of the concentrations of the studied physicochemical parameters was observed during the period of the study. These variations may be due to the changes in production schedules as well as the reasonable variation. Schroder (1999) reported that wastewater produced from pharmaceutical industries pose several problems for successful biological treatment due to the presence of high levels of suspended solids and soluble organics that are recalcitrant.

Generally, wastewaters produced from the company do not comply with the Egyptian standard regulation (Law 93/1962 and the Ministry of Housing Decree No. 44/200). Therefore, an effective waste minimization program includes end of pipe treatment has to be conducted to reduce the amount of hazardous wastewater.

Organic Analysis

The most widely used polymeric sorbents are the styrene- divinylbenzene copolymers (XAD resins) (Masque et al., 1998). XAD2, XAD4, XAD7, DPA-6S and GAC (Carbopack B) have been used in the Solid Phase Extraction (SPE) of some organic compounds from the studied wastewater. It was found that the XAD resins have more efficient than C18 bonded silica. This result indicates that XAD resins overcome many of the limitations of bonded silica C18. The XAD resins have greater analyte retention, mainly for polar compounds, than bonded silicas due to their hydrophobic surface contains a relatively large number of active aromatic sites which allow π-π interaction with unsaturated analytes (Masque et al., 1998).

To obtain optimum recovery percent, several factors have been investigated such as type of adsorbents, sampling flow rate and the effect of pH values (Fig 1-4). The obtained results indicated that XAD2 and DPA-6S sorbents proved to be the most suitable sorbents as they provided these compounds. Meanwhile, the recovery percent of salicylic acid in the case of XAD2 sorbent is better than in that of DPA-6S sorbent. Also, the results show that there is no significant change in recovery percent when the weight of adsorbent increased from 1g to 4g. The optimum values of the sample flow rate and the pH value are 5ml/min and pH 3, respectively. The pH adjustment of the sample is another factor that can be modified. Polar acidic analytes can be analyzed in contaminated surface-water samples, can only be provided at low pH value (acidic media). When a C18 cartridge is used for the extraction and concentration of acidic herbicides, the sample has to be acidified at pH2 or 3 (Hennion, 1999).

HPLC chromatograms (Fig. 5) show that the response of the studied compounds varied substantially. Such variation is due to the diversity of chemical classes among the selected pharmaceutical compounds. However, overall response for all compounds was sufficient for analysis at the expected ambient environmental concentrations.

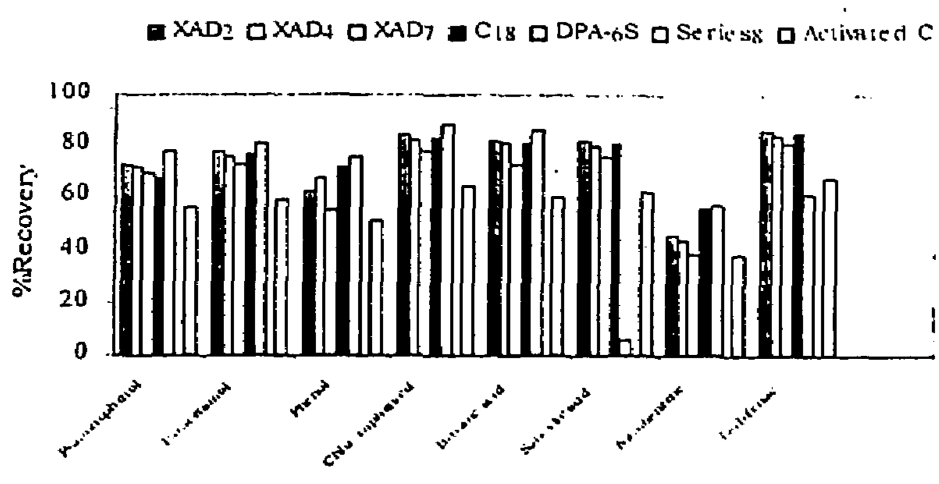

Fig (1) *Recovery percent by using various sorbents*

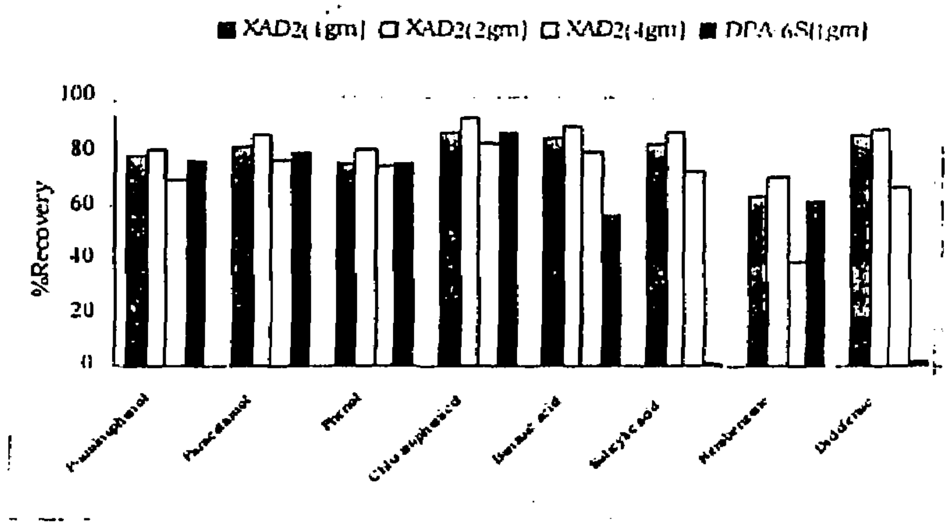

Fig (2) *Effect of sorbent weight on the efficiency of the recovery*

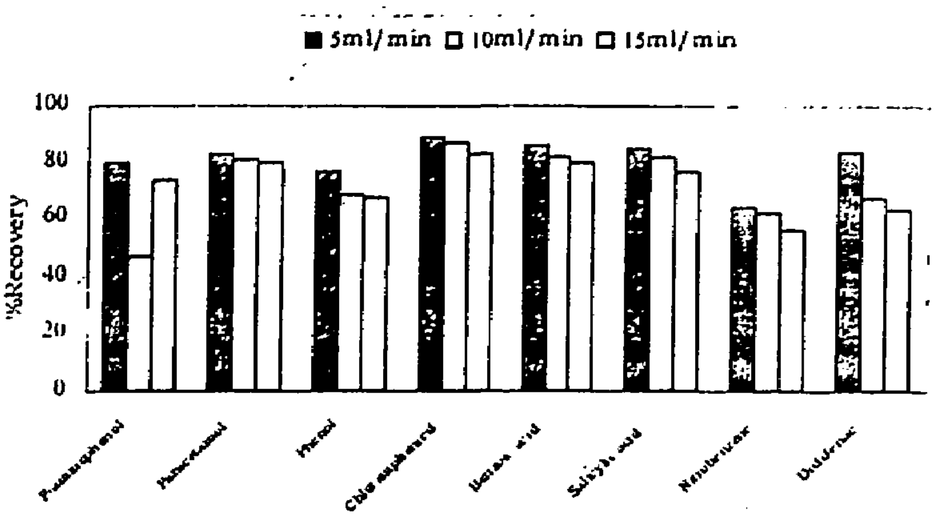

Fig (3) *Effect of sample flow rate on the efficiency of the recovery*

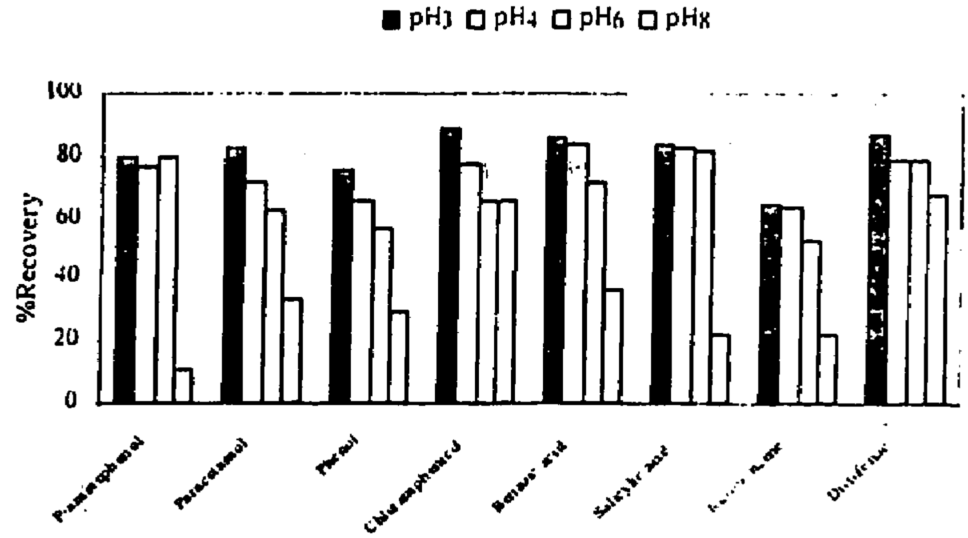

Fig (4) *Effect of the sample pH on the efficiency of the recovery*

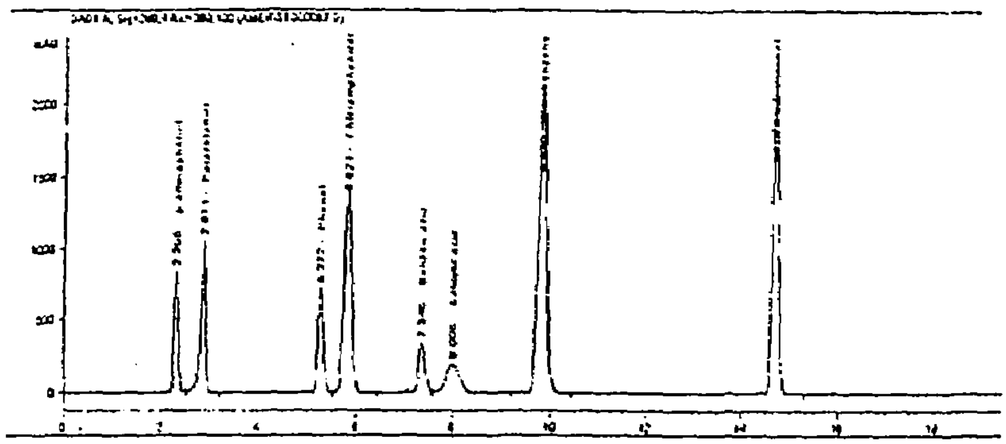

Fig (5) *HPLC chromatogram for standard mixture of 8 compounds*

Table (3) *Recovery percent by using various sorbents*

Compound	XAD2	XAD4	XAD7	C18	DPA-6S	Activated C
P-aminophenol	72.8	71.65	70.19	67.8	77.9	56.09
Paracetamol	77.99	76.15	73.22	77.21	81.04	59.47
Phenol	62.88	67.27	55.53	72.1	76.12	50.67
Chloramphenicol	84.3	82.67	77.72	82.94	88.26	64.77
Benzoic acid	82.16	80.84	73.34	81.04	86	60.66
Salicylic acid	81.86	80.61	76.33	81.74	6.33	62.63
Nitrobenzene	46.12	44.21	38.48	56.53	56.98	38.11
Diclofenac	86.66	83.91	81.3	85.73	61.85	68.18

The variety of chemical classes of the investigated compounds provided varying results among the solid-phase media tested. The best overall solid phase extraction recoveries were achieved using XAD copylmer. The average recoveries for the studied pharmaceutical compounds are listed in Table (3). All the compounds tested were recovered at greater than 70% except nitrobenzene. Low recovery of polar compounds can attributed to the poor retention on the polymeric sorbent. Cahill et al. (2004) reported that highly polar compounds such as the histamine (H_2) receptor antagonist's ranitidine and cimetidine, were recovered at less than 50%. It is important to notice that the extremely polar compounds metformin oil amoxicillin were not recovered at all.

The analysis of wastewater samples collected from the pharmaceutical company was carried out at the optimum operating conditions and the results presented in Table (4). Diclofenac, Chloramphenicol, Paracetamol drugs and their byproducts p-aminophenol, phenol, benzoic acid, nitrobenzene and salicylic acid were detected in all wastewater samples and their mean concentrations was 2.274, 15.76, 27.757, 23.965, 50.365, 27.908, 12.638 and 129.418 mg/l, respectively. Phenylacetic acid was not detected in any wastewater samples. The majority of these compounds enter aquatic environment and most of current wastewater treatment plants WWTP were not designed to deal with such compounds. Consequently they may reach the sewage effluent which is a matter of potential risk in the production of drinking water (Cahill et al. 2004).

Recent data from Europe indicate the conventional aerobic operations of sewage treatment plants resulted in the incomplete removal of pharmaceutical, hence as much as 80% of the total load of pharmaceuticals that reaching the sewage treatment plant and eventually discharged into surface water (Cahill et al. 2004). On the other hand, biodegradation, at best, can only be effective as partial removal of some pharmaceutical residues.

Table (4) *HPLC analysis of real wastewater*

compound	13/1/2003 (mg/l)	27/1/2003 (mg/l)	5/3/2003 (mg/l)	27/4/2004 (mg/l)	14/6/2004 (mg/l)	18/10/2004 (mg/l)	Mean
P-aminophenol	0.655	0.135	0.009	0.050	0.000	142.938	23.965
Paracetamol	2.907	5.612	2.030	0.330	1.549	154.112	27.757
Phenol	2.278	0.506	0.081	0.064	3.768	295.491	50.365
Chloramphenicol	5.197	0.408	0.110	0.861	0.028	87.958	15.760
Benzoic acid	4.414	2.506	0.098	7.929	0.056	152.443	27.908
Salicylic acid	39.570	1.029	1.424	0.000	20.075	714.407	129.418
Nitrobenzene	1.162	0.000	0.011	0.011	0.000	74.642	12.638
Diclofenac	0.531	0.477	0.205	0.053	0.008	12.368	2.274

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 (6 and 7). The toxic effect varied with the pH value of the waste which characterized by the acidic pH. Wastewater of pH value 3.8 showed high toxicity to the alga growth since, the EC50 after 96h was detected at waste dilution about 2% (Fig. 6). Other waste dilutions lead to acute toxic effect to the alga growth (Fig. 6). 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, the toxicity effect of pharmaceutical wastewater on phytoplankton community was investigated. The high toxicity effect was established in pharmaceuticals. The results in (Fig. 6) showed that the highest toxicity effect was found in the case of pharmaceutical wastewater at pH3.9. The acute toxic effects to algal growth where the EC_{50} (calculated) after 96h was 7% (Fig. 7). When, the pH raised to 7.2, the EC_{50} (calculated) of waste dilution was changed to 14.5% (Fig. 7).

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. The effect of pH values of the wastewater on the algae was studied. Algal community after exposure to pharmaceutical waste at pH 3.8 showed major changes in algal predominance, where Scenedesmus qudricauda 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, certain 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 discusses 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., (2003) recorded that phytoplankton communities were characterized by decrease in species composition but with increase in numbers of some species on treatment with 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.

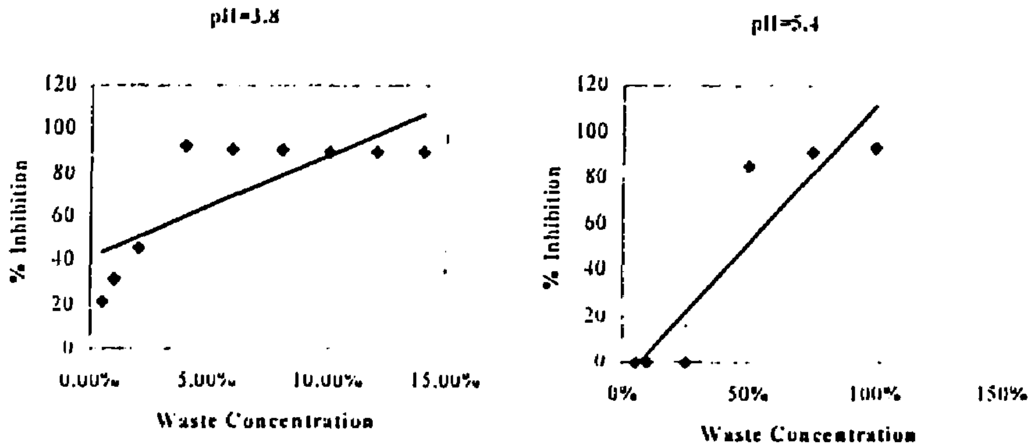

Fig (6) *EC_{50} of pharmaceutical wastewater to Scenedesmus obliquus (after 96 hrs incubation period).*

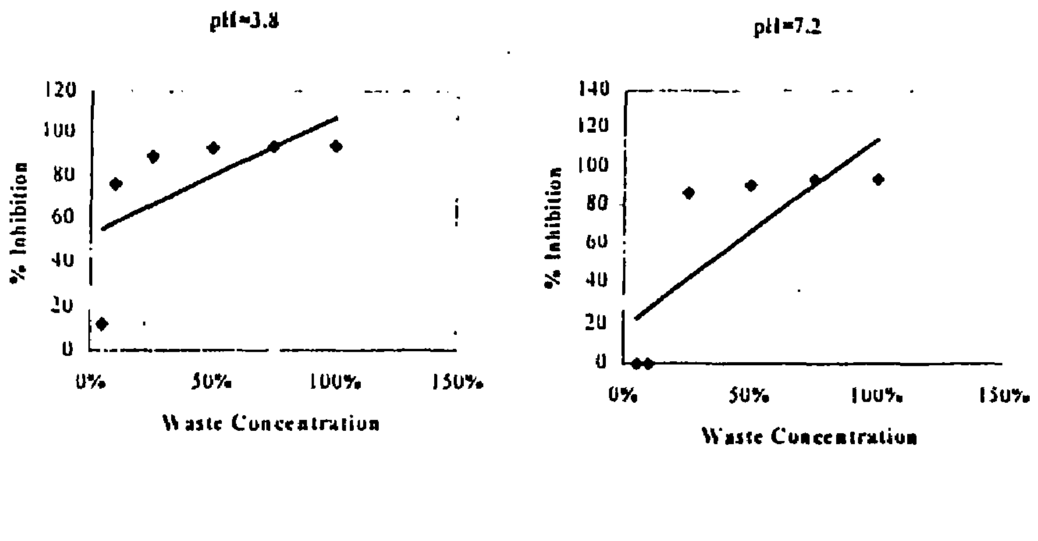

Fig (7) ***EC50 of pharmaceutical wastewater to natural phytoplankton assemblages before and after pH adjustment (after 96 hrs incubation period).***

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