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Efficiency Evaluation of a wastewater Treatment Plant by Activated Sludge

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Abstract: Performance of El-Agamyeen Plant for wastewater treatment (WWTP) by activated sludge was studied over a period of 20 weeks. Results showed that chemical oxygen demand; COD, biochemical oxygen demand; BOD, total suspended solids; TSS and ammonia decreased through stages of treatment process. The percentage removal of these parameters during studied period ranged 86-95, 91-96, 90-94 and 71-85 % respectively. Dissolved oxygen; DO in aeration tank was not less than 1.2 mg/L during study. Of microfauna, amoebae and flagellates appeared at low DO and high organic load, dominancy of crawling and stalked ciliates affected efficiency of treatment. Rotifers count ranged from $1.3x10^3$ to $9x10^4$ indicating good sludge quality.High toxicity could be obtained with the inlet wastewater and decreased with mixed liquor; the outlet water has been shown the least average toxic effects (<10% mortality) on the test organism. The results indicated that wastewater treatment in this Plant during the study period reduced pollution significantly and effluent water can be left for discharge into water bodies.

Key words: Wastewater Treatment, Activated sludge, Microfauna, Toxicity testing, *Daphnia magna*

INTRODUCTION

Activated sludge, a microbial community, consists of free, flocculated and filamentous bacteria, protozoa, rotifers and a few other higher invertebrates. Protozoa and metazoa are said to be an important factor in shaping the morphological and taxonomical compositions of communities in the activated sludge process (Richard, 1991).

More than 80% of biological wastewater plants are based on the principle of activated sludge process, in which suspended bacteria oxidize the carbonaceous and nitrogen compounds to produce an effluent that is in accordance with legal standards, and that corresponds to a minimal environmental impact (Tizghadam *et al*., 2008).

Treatment of domestic and industrial wastewater is crucial for protection of receiving waters. Parameters such as pH, dissolved oxygen, BOD, COD, TOC, TDS, and TSS are generally used for evaluation of effluent quality. However, these parameters cannot be used for evaluation of toxicity effect on receiving waters due to some specific defects. The best way to evaluate effluent toxicity effect is to use biotoxicity test (Davis and Ford, 1992; Metcalf and Eddy, 2003). The water flea *Daphnia magna straus* is the most commonly used zooplankton in toxicological tests.

Protozoa and other higher life forms constitute approximately 5% of the activated sludge biomass. These organisms perform several important functions in activated sludge. The most important of which is their removal of non-flocculated bacteria from wastewater through their feeding activities, yielding a clarified effluent. Studies have shown species of protozoa that excrete specific materials to cause flocculation of bacteria and suspended solids in the wastewater. This suggests that protozoa play a role in effluent quality (Esteban *et al*., 1991). Protozoa by predation indirectly increases bacterial activity by preventing bacteria from reaching self-limiting numbers. Bacteria are thus kept in a state of prolonged youth and their rate of assimilation of organic materials is greatly increased. They also contribute to biomass flocculation through production of faecal pellets and mucus. Protozoa may also function to break up large floc masses and encourage a more active biomass through their motility. They perform beneficial roles in the wastewater system, including the clarification of the secondary effluent and act as bio-indicators of the health of the sludge (Curds and Cockburn, 1970).

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Apparently, aquatic toxicity tests are used in detecting and evaluating the potential toxicological effects of chemicals (Okpokwasili and Odokuma, 1994) on aquatic organisms (Franklin, 1973). Toxicity tests became desirable in chemical quality evaluations as a consequence of the inability of the physical and chemical tests alone to sufficiently assess the potential effect on aquatic biota (Powell *et al.,* 1985). Furthermore, chemicals which by itselves would have been harmless may cause deleterious/synergistic effects by interacting in the general milieu of contaminated waters (Kingham, 1981).

The aim of this study was to evaluate the operational efficiency of El-Agamyeen Plant for Wastewater Treatment (WWTP) by activated sludge using: (a) physico-chemical parameters, (b) microscopical examination of microfauna compositions; Protozoa and Metazoa, and (c) toxicity testing using *Daphnia magna* as a test organism.

MATERIALS AND METHODS

Sampling Sites:

Twelve wastewater samples were collected weekly during the period from the start of December, 2009 through March, 2010 from inlet, mixed liquor and outlet of El-Agamyeen WWTP for physico-chemical Analyses, microscopical examination and toxicity testing. Totally, 48 samples were tested during the study period of the four months.

1-Physico-chemical Analyses:

The parameters used for the determination of the efficiency of the WWTP were total dissolved solids; TDS, total suspended solids; TSS, chemical oxygen demand; COD, biochemical oxygen demand; BOD, nitrate; NO_3-N, ammonia; NH_3-N, total phosphate; PO_4, oil and grease, sulfide, pH, temperature and dissolved oxygen. The characteristic parameters were measured according to Standard Methods of Analysis (APHA, 2005).

2-Microscopial Examination:

In the collected wastewater samples, microfauna were identified according to Edmondson (1963), Kudo (1977) and Streble & Krauter (1978) and were counted in 1.5 ml subsamples after preservation with Lugol's solution (Lewis,1979) in a Hawksley cell under the microscope at 150X magnification until attaining at least 60 individuals, as recommended by McCauley (1984).

3-Toxicity Testing

Experimental animals and food:

A freshwater *D. magna* strain that has been successfully grown in the laboratory in synthetic freshwater media (Fayed and Ghazy, 2000), was used as the test organism for this study. Gravid females were transferred at regular intervals to 1-L glass beakers, in which the culture medium; synthetic freshwater medium (pH; 7.9 – 8.3, total hardness; 90 mg/L as $CaCO_3$, alkalinity; 34 mg/L as $CaCO_3$, conductivity; 260 µmhos/cm) was renewed 3 times a week and were checked daily for the release of neonates to be used in starting experiments. In these beakers, the animals were fed 3 times a week with the green micro alga *Scenedesmus obliquus*.

The algal culture was renewed once a week to maintain the algae solution in good condition. The algae and daphnids were kept at a temperature 22± 2°C with a light period of 16 L: 8 D both during culturing and experimental periods.

Acute Toxicity tests:

Acute toxicity testing were conducted in triplicates, where groups of 10 < 24 h-old daphnids were placed in 250-ml beakers, each containing 100 ml test wastewater and subjected to test conditions for 48 h. Tests were run without food addition. The number of live organisms after the elapse of 48h was recorded. Control test was run in parallel in triplicates; each control container containing 100 ml synthetic freshwater medium. Temperature was maintained at 22± 2º C by automatic heater. A mercury thermometer was used to measure temperature in test containers.

The samples were tested as it without dilution. Ten daphnids were added to each test and control container and the results of daphnid mortality were recorded after 48 h. The results of experiments were acceptable only in cases where daphnids in the control containers were observed to have a mortality rate of less than 10%.

RESULTS AND DISCUSSION

1-Physico-chemical Analyses:

During the study period, it was found that the pH of wastewater samples taken from the three sampling sites; treatment steps of the plant, was slightly alkaline and temperature was in the range from 19 to 23.4 ºC, which is generally the temperature trend in water bodies in the area. In inlet wastewater, the average TDS was in the range 1041-1287 mg/L, TSS in the range258-326 mg/L,COD 334-500 mg/L, BOD 246-310 mg/L, nitrate as N 1.3-8.7 mg/L, ammonia as N 31-67 mg/L, total phosphate 21.4-32.2 mg/L, oil & grease, 14-25 mg/L, sulfide 7-14 mg/L. In outlet water, the range of percent removal of TSS, COD, BOD, and ammonia was 90 – 94%, 86 -95%, 91- 96%, and 71 - 85%, respectively. Dissolved oxygen in outlet water was in the range 4 to 5.2 mg/L during the investigated period (Table 1 and Fig. 1).

Table 1: Physico-chemical parameters of water samples taken from 1-Inlet 2-Mixed Liquor 3- outlet of the wastewater treatment plant between Dec.,2009 and Mar., 2010

Parameters	Months					
	Dec.,2009			Jan., 2010		
	1	2	3	1	2	3
PH	7.4 (7.3-7.5)	7.53 (7.4-7.6)	7.4 (7.2-7.6)	7.6 (7.3-7.8)	7.5 (7.3-7.7)	7.5 (7.2-7.7)
Temp °c	21.2 (21.2-23.4)	22 (21-23.4)	22.5 (20.8-23)	20.7(20.5-21)	21 (20.2-22.6)	20.5 (19.9-21)
TDS mg/l	1041(958-1114)	1191(1102-1344)	1188(1158-1193)	1161(1048-1318)	1326.5(814-1800)	1310(1236-1387)
TSS mg/l	310 (245-375)	3158(1900-4860)	20.9 (18.5-25.5)	258 (186-300)	4017 (3200-4592)	26.3 (18.5-34)
COD mg/l	500 (390-580)	8500(6830-9889)	35.4 (28.3-40.6)	334 (230-385)	9510(8780-9920)	40.8 (30-58)
BOD_5 mg/l	253 (142-260)	1470(802-2020)	14.1 (11.6-16.1)	246 (180-298)	1653(1240-2000)	21.4 (15.8-32)
NO_3 mg/l	8.7 (4.3-13)	150 (1.45-402)	26.7 (16.8-45.2)	3.8 (4-5)	74.8(48-108)	10 (4.6-15.8)
NH_3 mg/l	62 (33-71)	22.6 (15.6-31.7)	10.4 (6.5-15.2)	31 (28-45)	12.9 (8.7-17.9)	5.2 (5.4-8.6)
$T.PO_4$ mg/l	23.6 (8.12-47.8)	50.5 (19-108)	4.6 (2.6-6.67)	32.2 (9.3-56.4)	36.5 (22-52)	6.1 (3.1-12)
O&G mg/l	24 (12-30)	70(51-89)	10.5 (3.2-16)	14 (12-19.2)	90(68 -112)	6 (4-8)
S^{-2}mg/l	12.5 (8.8-15)	23(17-28)	0.8 (0.6-1)	7 (6 -10)	21(13-27)	0.7 (0.4-0.8)
DO mg/l	0.3(0.17-0.46)	1.83 (1-2.3)	4(3.4-4.8)	0.22(0.15-0.32)	2.18(1.8-2.5)	5.2 (5-5.8)
Parameters	Months					
	Feb.,2010			Mar., 2010		
	1	2	3	1	2	3
PH	7.3 (7.2-7.4)	7.5 (7.4-7.6)	7.7 (7.4-7.8)	7.3 (7-7.5)	7.3 (7.1-7.7)	7.3 (7.1-7.4)
Temp °c	22 (20.8-23)	21.8 (21-23.3)	21.08(19-23)	22 (21.7-22.7)	22.4 (22-23.8)	22.2 (22-24)
TDS mg/l	1119 (986-1272)	1583(1190-1900)	1190 (1158-1193)	1287(1243-1340)	1426(1196-1780)	1196(1067-1235)
TSS mg/l	326.5(278-375)	4018(3790-4210)	29.2 (22.7-35)	273 (216-330)	3418(2180-4180)	41 (21-53)
COD mg/l	388 (354-421)	9473(8560-9920)	44.4 (35.8-64.4)	387 (320-560)	8663(7120-9950)	56.5 (44-78)
BOD5 mg/l	246(142-260)	1267(1010-1560)	16.7 (11.6-28)	271(192-350)	2480(1998-2980)	23.4 (15-40.5)
NO3 mg/l	2.2 (1-8)	53.8 (15-78)	22.4 (18-25)	0.6(0.2-1.3)	30.2 (18-60)	6 (4-12)
NH4 mg/l	59 (39-63)	36 (29-41.2)	9.3(8-12.3)	67 (48-86)	20.4 (10-38)	10.8 (4-25)
T.PO4 mg/l l	21.4(12-32)	34(21.3-52.3)	5(8.2-7.2)	17.8(15-24)	71.6(32-108)	8.5(1-15)
O&G mg/l	25 (18-30)	68(58-78)	8.5 (3.8-9)	20.5 (10-28)	53(46-60)	5 (4-6)
S-2mg/l	14 (10-18)	18(15-22)	0.6 (0.5-0.8)	11.1 (5-12.5)	20(18-24)	0.5 (0.4-0.6)
DO mg/l	0.2(0.11-0.26)	2 (1.6-2.6)	4.7 (3.4-5.5)	0.16(0.05-0.23)	1.78 (1-2.4)	4.6 (3.8-5.4)

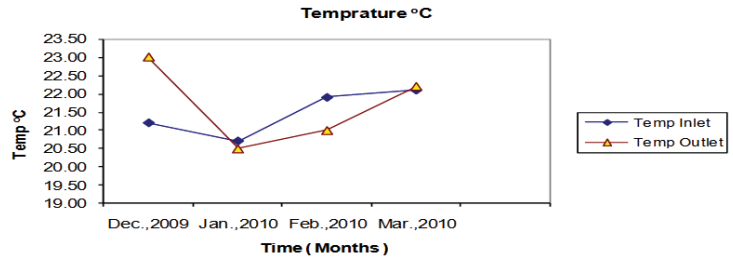
Average and range in parentheses are shown

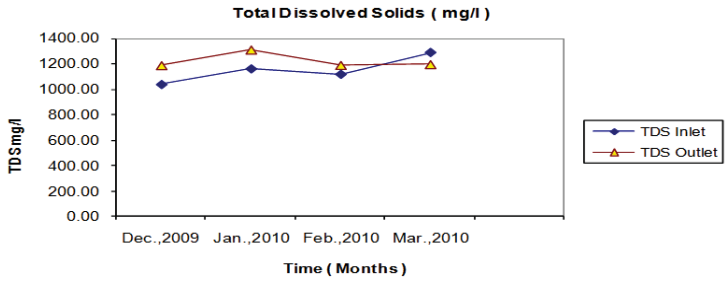
2-Microscopial Examination of Microfauna composition:

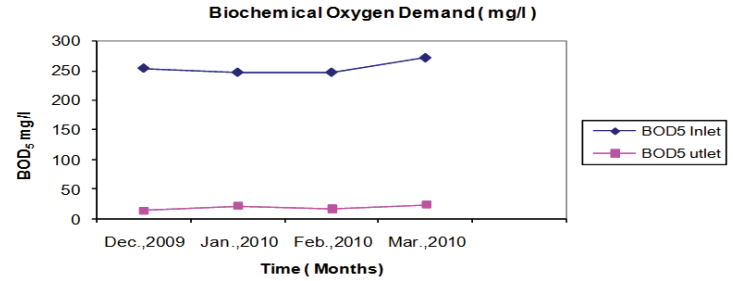
Microscopical examinations of Protozoa and metazoa during the study period are given in Table 2 and Figure 2. In mixed liquor Protozoa are $1.2x10^6$ ind /L in Mar., 2010, to $2.1x10^6$ ind /L in Jan., 2010, and Metazoa are $4.3x10^3$ ind /L in Jan., 2010, to $7.2x10^4$ ind./L in Feb., 2010, respectively.

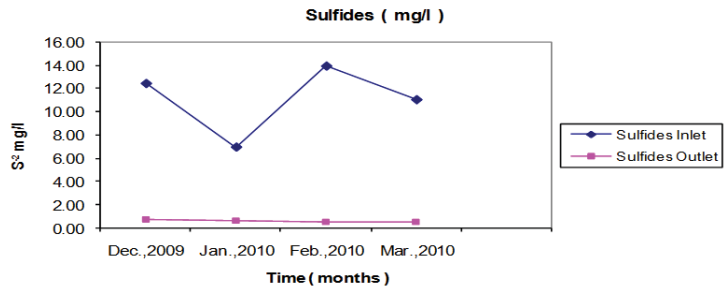
Three main groups of protozoa identified in the wastewater treatment plant were amoebae, flagellates and ciliates, while ciliates are the most abundant forms. Free swimming and stalked (sessile) ciliates were the most frequent genera identified in wastewater samples of mixed liquor followed by that of outlet. In mixed liquor, total free swimming ciliates ranged from $5.2x10^5$ ind./L in Feb., 2010 to $3.1x\ 10^6$ ind /L in Jan., 2010, and in outlet from $7x10^3$ ind /L in Feb., 2010 to 10^5 ind /L in Dec., 2009. These ciliates were represented by the genera *Paramecium*, *Chilodonella*, *Euplotes*, *Lionotus*, *Aspidisca*, *Tetrahymena*, *Spathidium*, *Trachelophyllum*, *Pseudoprorodon*, *Amphiliptus*, *Spirostomum*, *Glaucoma*, *Colpoda*, *Stentor* and *Colpidium*. Of these, *Aspidisca* was the most abundant genus followed by *Lionotus* during the investigated period.

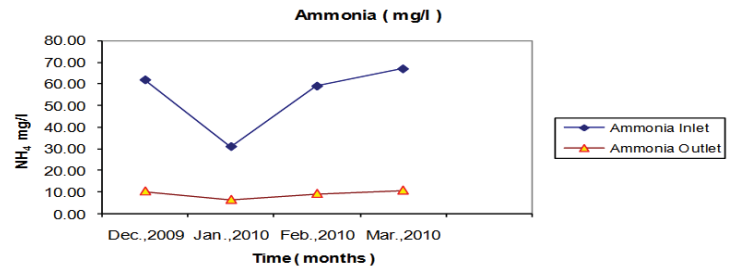
On the other hand, total stalked (sessile) ciliates in wastewater samples taken from mixed liquor $3.5x10^5$ ind /L in Dec., 2009 to $7.5x10^5$ ind /L in Feb., 2010. These ciliates were represented by the dominant genera *Vorticella*, *Opercularia*, *Carchesium* and *Epistylis* and also by fewer counts of *Vaginicola*, *Tokophrya*, *Podophrya*, *Acineta*, and *Zoothamnium*.

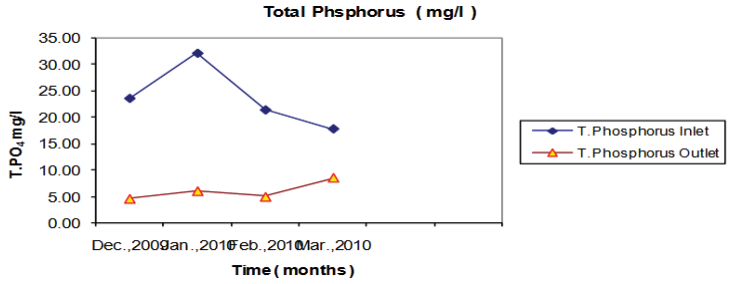

Temprature °C
Temp °C
23.50
23.00
22.50
22.00
21.50
21.00
20.50
20.00
19.50
19.00
Dec.,2009 Jan.,2010 Feb.,2010 Mar.,2010
Time (Months)
Temp Inlet
Temp Outlet

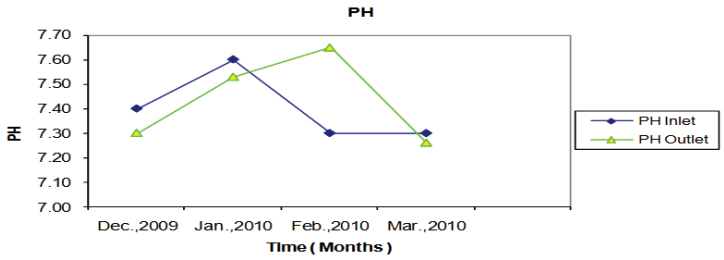

Total Dissolved Solids (mg/l)
TDS mg/l
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
Dec.,2009 Jan.,2010 Feb.,2010 Mar.,2010
Time (Months)
TDS Inlet
TDS Outlet

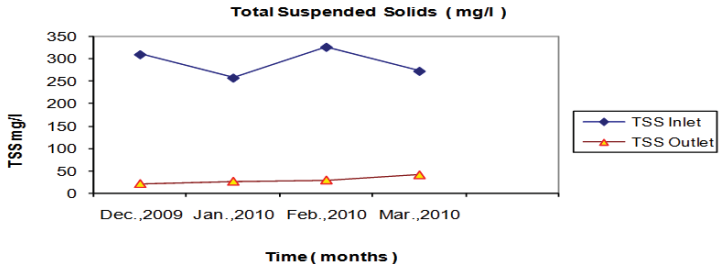

Biochemical Oxygen Demand (mg/l)
BOD5 mg/l
300
250
200
150
100
50
0
Dec.,2009 Jan.,2010 Feb.,2010 Mar.,2010
Time (Months)
BOD5 Inlet
BOD5 utlet

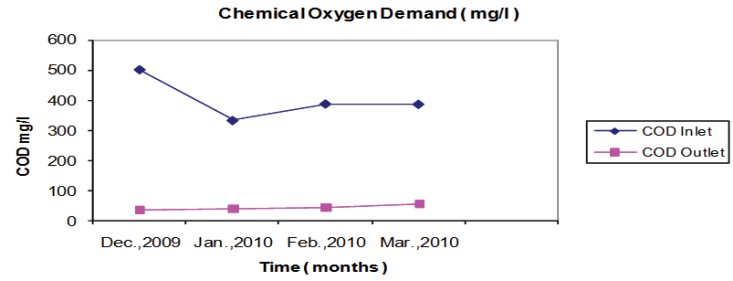

Sulfides (mg/l)
S2- mg/l
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Dec.,2009 Jan.,2010 Feb.,2010 Mar.,2010
Time (months)
Sulfides Inlet
Sulfides Outlet

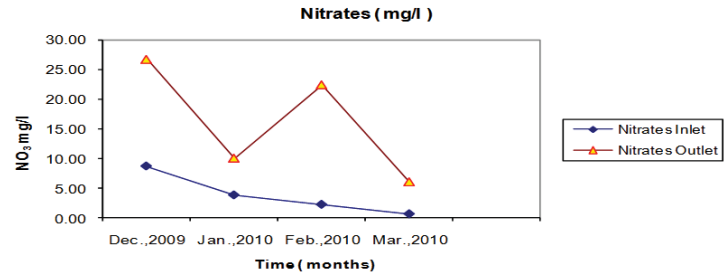

Ammonia (mg/l)
NH4 mg/l
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Dec.,2009 Jan.,2010 Feb.,2010 Mar.,2010
Time (months)
Ammonia Inlet
Ammonia Outlet


Total Phsphorus (mg/l)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
T.PO4 mg/l
Dec.,2009
Jan.,2010
Feb.,2010
Mar.,2010
Time (months)
T.Phosphorus Inlet
T.Phosphorus Outlet


PH
7.70
7.60
7.50
7.40
7.30
7.20
7.10
7.00
PH
Dec.,2009
Jan.,2010
Feb.,2010
Mar.,2010
Time (Months)
PH Inlet
PH Outlet


Total Suspended Solids (mg/l)
350
300
250
200
150
100
50
0
TSS mg/l
Dec.,2009
Jan.,2010
Feb.,2010
Mar.,2010
Time (months)
TSS Inlet
TSS Outlet


Chemical Oxygen Demand (mg/l)
600
500
400
300
200
100
0
COD mg/l
Dec.,2009
Jan.,2010
Feb.,2010
Mar.,2010
Time (months)
COD Inlet
COD Outlet


Nitrates (mg/l)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
NO3 mg/l
Dec.,2009
Jan.,2010
Feb.,2010
Mar.,2010
Time (months)
Nitrates Inlet
Nitrates Outlet

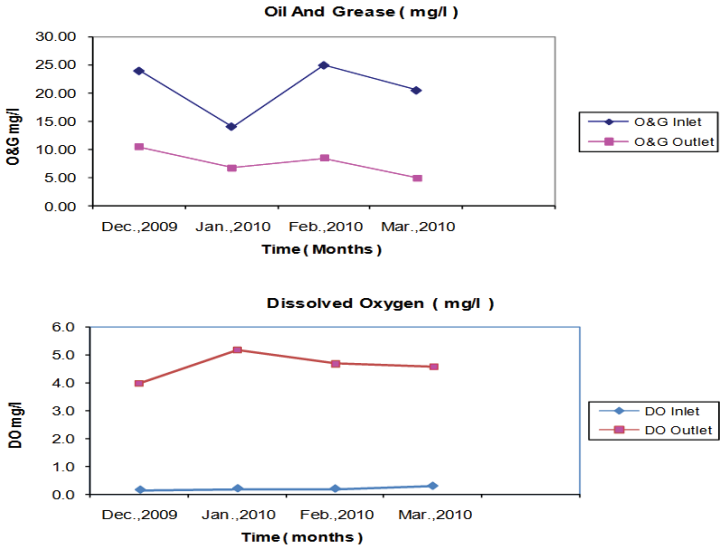


Fig. 1: Variation of wastewater pH, temperature, total dissolved solids, total suspended solids, BOD, COD, sulfides, total phosphorus, nitrates, ammonia, oil & grease and dissolved oxygen in the inlet and outlet of the treatment plant during the study period.

Table 2: Microfauna densities (ind./L) as genera and groups in the 1-Inlet, 2-Mixed Liquor and 3- Outlet of the wastewater treatment plant between December, 2009 and March, 2010.

Microfauna	Months					
	Dec.,2009			Jan., 2010		
	1	2	3	1	2	3
(A) Protozoa						
-*Ameoboids*	3.8 $x10^3$(0-6.6 $x10^3$)	2$x10^4$(1$x10^4$- 3.3$x10^4$)	1.7 $x10^2$(0-6.7 $x10^2$)	1.1 $x10^3$(0-1.5 $x10^3$)	1.7$x10^4$(1.7$x10^4$-1.7$x10^4$)	1.7 $x10^2$(0-6.7 $x10^2$)
-Flagellates						
Peranema sp.	3 $x10^3$(0-1.3 $x10^3$)	5 $x10^3$(0-1 $x10^4$)	0	0	8.3 $x10^2$(0-3.3 $x10^3$)	0
*Total *Flagellates/L*	3 $x10^3$	5 $x10^3$	0	0	8.3 $x10^2$	0
-Free swimming ciliates						
Paramecium sp	0	2.8 $x10^4$(2.7 $x10^4$-3 $x10^4$)	1.7 $x10^2$(0-6.7 $x10^2$)	1 $x10^3$(0-2.7 $x10^3$)	1.8 $x10^4$(1.7 $x10^4$-2.3 $x10^3$)	3.3 $x10^2$(0-1.3 $x10^3$)
Chilodonella Sp	0	1.8 $x10^4$(6.7 $x10^3$-3.3 $x10^4$)	3.3 $x10^2$(0-1.3 $x10^3$)	0	2.1 $x10^4$(2 $x10^4$-2.3 $x10^4$)	3 .3$x10^2$(0-6.7 $x10^2$)
Euplotes Sp	0	1.8 $x10^4$(0-4 $x10^4$)	1.7 $x10^2$(0-6.7 $x10^3$)	0	2.4 $x10^4$(1.7 $x10^4$– 3.3 $x10^4$)	5 $x10^2$(0-6.7 $x10^2$)
Lionotus Sp	0	9.2 $x10^4$(3.3 $x10^4$-2.3 $x10^5$)	1.7 $x10^3$(6.7$x10^2$-3.3$x10^3$)	0	3.3 $x10^4$(5 $x10^3$-6 $x10^4$)	8.7 $x10^2$(0-1.5 $x10^3$)
Aspidisca Sp	0	5.9 $x10^5$(2.5 $x10^5$-1.3 $x10^6$)	7.5 $x10^3$(5.3 $x10^3$ -1 $x10^4$)	0	1.3 $x10^6$(1.1 $x10^6$-1.6 $x10^6$)	3 $x10^3$(8 $x10^3$-1.1 $x10^4$)
Tetrahymena Sp	0	4.4X10^4(0-1.3X10^5)	0	0	3.3X10^3(0-6.7X10^3)	0
Spathidium SP	0	1.7X10^3(0-6.7X10^3)	0	0	9.2X10^3(0-2.3X10^4)	1.7X10^2(0-6.7X10^2)
Trachelophyllum Sp	0	8.3X10^2(0-3.3X10^3)	0	0	1.3X10^4(3.3X10^3-2.3X10^4)	1.7X10^2(0-6.7X10^2)
Pseudoprorodon Sp	0	6.7X10^3(0-1.6X10^4)	0	0	0	0
Amphileptus Sp	0	3.4X10^4(0-1.1X10^5)	1.7X10^2 (0-6.7X10^2)	0	0	0
Spirostomum Sp	0	1.7X10^4(0-6.7X10^4)	0	0	0	0
Glaucoma Sp	0	4.2X10^3(0-1.7X10^4)	0	0	0	0
Colpoda Sp	0	0	0	0	0	0
Colpidium Sp	0	0	0	0	0	0
Stentor SP	0	1.7X10^3(0-3.3X10^3)	0	0	1.74X10^3(0-3.3X10^4)	6.7X10^2(0-2.7X10^3)
-Very small un-known ciliates	6.8x10^3	0	0	1.7X10^3	0	0
*Total Free *Swimming Ciliates/L*	6.8x10^3	1x10^6	1X10^5	2.7 $x10^3$	3.1x10^6	1.2x10^4
-Stalked ciliates						
Vorticella sp	2.7 $x10^3$(0-3.3 $x10^3$)	1 $x10^5$(6.7 $x10^4$-1.7 $x10^5$)	2.4 $x10^3$(1.3 $x10^3$ -4 $x10^3$)	1.7 $x10^2$(0-6.7 $x10^2$)	1.1 $x10^5$(8.3 $x10^4$-1.3 $x10^5$)	1.7 $x10^3$(1.3x10^3-2.7x10^3)
Opercularia SP	0	9.6 $x10^4$(1.7 $x10^4$-1.5 $x10^5$)	1.5 $x10^3$(0-3.3 $x10^3$)	0	1.6 $x10^5$(1.2 $x10^5$-1.9 $x10^5$)	2.2 $x10^3$(6.7 $x10^2$ -4 $x10^3$)
Carchesium sp	0	1.4 $x10^5$(7.3 $x10^4$-1.8 $x10^5$)	2.3 $x10^3$(6.7 $x10^2$- 4 $x10^3$)	0	1.1 $x10^5$(5.3 $x10^4$-1.5 $x10^5$)	1.7 $x10^2$(0-4 $x10^3$)
Epistylis SP	0	1 $x10^5$(6 $x10^4$ -1.4 $x10^5$)	4.4 $x10^3$(0-1.5 $x10^4$)	0	1.7 $x10^5$(1.5 $x10^5$-2 $x10^5$)	1.1 $x10^3$(0-1.5 $x10^3$)
Vaginicola sp	0	1.5 $x10^4$(1 $x10^4$ -4 $x10^4$)	1.7 $x10^2$(0-6.6 $x10^2$)	0	3.5 $x10^4$(2.3 $x10^4$-4.7 $x10^4$)	1.7 $x10^2$(0-6.7 $x10^2$)
Tokophrya SP	0	1.2 $x10^4$(1 $x10^4$ -1.7 $x10^4$)	1.7 $x10^2$(0-6.6 $x10^2$)	0	1 $x10^4$(6.7 $x10^3$-1.6 $x10^4$)	0
Podophrya sp	0	2.5 $x10^3$(0-6.7 $x10^3$)	0	0	3.3 $x10^3$(0-6.7 $x10^3$)	0
Acineta SP	0	8.3 $x10^2$(0-3.3 $x10^3$)	0	0	2.5 $x10^3$(0-6.7 $x10^3$)	0
Zoothamnium Sp	0	2.1X10^4(0-6.6X10^4)	0	0	2.8X10^4(0-6X10^4)	0
* *Total Stalked Ciliates/L*	2.7 $x10^3$	3.5x10^5	1.1x10^4	1.7 $x10^2$	4.5x10^5	7.5x10^3
(B)Metazoa						
-*Rotifers*	0	4$x10^4$(2 $x10^4$- 6.3 $x10^4$)	1.7 $x10^3$(0-2 $x10^3$)	0	3.7 $x10^4$(3 $x10^4$-4 $x10^4$)	0
- *Nematodes*	3.3 $x10^2$(0-1.3 $x10^3$)	1.8x10^3(6.7 $x10^2$-3.3 $x10^3$)	1.7 $x10^2$(0-6.7 $x10^2$)	0	3.3 $x10^3$(3.3 $x10^3$-3.3 $x10^3$)	0
- *Oligochaetes*	0	1 $x10^3$(0-3.3 $x10^4$)	1.7 $x10^2$(0-6.7 $x10^2$)	0	2.5x10^3(0-6.710^3)	0
(C) Total count/L	1.4 $x10^4$	1.4X10^6	2.3 $x10^4$	4.2 $x10^3$	2.1 $x10^6$	2.1 $x10^4$

Table 2: Continue

Microfauna	Months					
	Feb., 2010			Mar.,2010		
	1	2	3	1	2	3
(A) Protozoa						
-*Ameoboids*	$1x10^3$(0-1.3 $x10^3$)	1.8$x10^4$(1$x10^4$-3.3$x10^4$)	1.7 $x10^2$(0-6.7 $x10^2$)	9.3 $x10^2$(0-1.3 $x10^3$)	2$x10^3$(0-2$x10^4$)	6.7 $x10^2$(0-1.5 $x10^3$)
-Flagellates						
Peranema sp.	3.3 $x10^2$(0-1.3 $x10^3$)	1.7 $x10^3$(0-6.7 $x10^3$)	0	0	1.3 $x10^3$(0-3.3 $x10^3$)	0
*Total *Flagellates/L*	3.3 $x10^2$	1.7 $x10^3$	0	0	1.3 $x10^3$	0
-Free swimming						
Paramecium sp	3.3 $x10^2$(0-1.3 $x10^3$)	1 $x10^4$(0-1.7 $x10^4$)	0	9.3 $x10^2$(0-3.3 $x10^3$)	5.7 $x10^4$(3.3 $x10^3$-1.5 $x10^5$)	1.3 $x10^4$(0-4 $x10^4$)
Chilodonella Sp	0	2.6 $x10^4$(1.7 $x10^4$ -6 $x10^4$)	0	1.3 $x10^2$(0-6.7 $x10^2$)	1.7 $x10^4$(0-4$x10^4$)	1.3 $x10^2$(0-6.7 $x10^2$)
Euplotes Sp	0	2.8 $x10^4$(1.3 $x10^4$-6.3 $x10^4$)	0	0	2.1 $x10^4$(6.7 $x10^3$-5 $x10^4$)	1.3 $x10^3$(0-4.7 $x10^3$)
Lionotus Sp	0	1 $x10^5$(5 $x10^4$ -2.3 $x10^5$)	0	0	3.8 $x10^4$(0-1 $x10^5$)	8$X10^2$(0-2.7$X10^3$)
Aspidisca Sp	0	7.5 $x10^5$(2.2 $x10^4$ -1.2 $x10^6$)	7$x10^3$(2.7$x10^3$-1$x10^4$)	0	3.7 $x10^5$(1.5 $x10^5$-8 $x10^5$)	3.8 $x10^3$(6.7 $x10^2$ -8 $x10^3$)
Tetrahymena Sp	0	3.3$X10^3$(0-1.3$X10^4$)	0	0	8$X10^3$(0-2$X10^4$)	0
Spathidium SP	0	4.2$X10^3$(0-1$X10^4$)	0	0	6.7$X10^2$(0-3.3$X10^3$)	0
Trachelophyllum Sp	0	1.7$X10^3$(0-6.7$X10^3$)	0	0	1.1$X10^3$(0-3.3$X10^3$)	0
Pseudoporodon Sp	0	0	0	0	8.7$X10^3$(0-4$X10^4$)	0
Amphileptus Sp	0	5.7$X10^4$(0-1.1$X10^5$)	5$X10^2$(0-1.3$X10^3$)	0	1$X10^3$(0-1.3$X10^4$)	0
Spirostomum Sp	0	2.5$X10^3$(0-1$X10^4$)	0	0	0	0
Glaucoma Sp	0	1.7$X10^3$(0-6.7$X10^3$)	0	0	0	0
Colpoda Sp	0	0	0	0	6.7$X10^2$(0-3.3$X10^3$)	0
Colpidium Sp	0	0	0	0	3.3$X10^3$(0-1.7$X10^4$)	0
Stentor SP	0	4.2$X10^3$(0-1$X10^4$)	0	0	0	0
-Very small un-known ciliates	1.3 $x10^3$(0-4 $x10^3$)	0	0	5.3 $x10^3$(0-1.7$x10^4$)	0	0
*Total Free *Swimming Ciliates/L*	4.6 $x10^2$	5.2$x10^5$	7$x10^3$	5.8$x10^3$	5.3$x10^5$	2$x10^4$
-Stalked ciliates						
Vorticella sp	3.3 $x10^2$(0-6.7 $x10^2$)	1.2 $x10^5$(1.6 $x10^4$ -1.5 $x10^5$)	1.3 $x10^3$(0-2.7 $x10^3$)	5.7 $x10^2$(0-1.5 $x10^3$)	2.7 $x10^5$(2.5 $x10^5$-3.5 $x10^5$)	5.5 $x10^3$(6.7 $x10^2$-1.7 $x10^4$)
Opercularia SP	4.9 $x10^2$(0-1.3 $x10^3$)	2 $x10^5$(1.7 $x10^4$ -3 $x10^5$)	1.4 $x10^3$(0-1.5 $x10^3$)	1.3 $x10^2$(0-6.7 $x10^2$)	1.6 $x10^5$(9.3 $x10^4$-3.2 $x10^5$)	1.1 $x10^3$(0-1.5 $x10^3$)
Carchesium sp	0	1.4 $x10^5$(1.1 $x10^5$ -1.8 $x10^5$)	1.5 $x10^3$(1.3 $x10^3$ -1.5 $x10^3$)	1.3 $x10^2$(0-6.7 $x10^2$)	9.4 $x10^4$(3 $x10^4$-1.8 $x10^5$)	0
Epistylis SP	0	2.2 $x10^5$(1 $x10^5$ -2.8 $x10^5$)	2.3 $x10^3$(6.7 $x10^2$ -3.3 $x10^3$)	0	1.7 $x10^5$(1 $x10^5$-2.7 $x10^5$)	0
Vaginicola sp	0	5.1 $x10^4$(4 $x10^4$ -6 $x10^4$)	3.3 $x10^2$(0-6.7 $x10^2$)	0	1.4 $x10^4$(0-9.3 $x10^4$)	0
Tokophrya SP	0	1.2 $x10^4$(0-2 $x10^4$)	0	0	5.3 $x10^3$(0-1.3 $x10^4$)	0
Podophrya sp	0	0	0	0	5.3 $x10^3$(0-2.3 $x10^4$)	0
Acineta SP	0	1.1 $x10^4$(0-3.3 $x10^4$)	0	0	1.3 $x10^4$(0-6.7$x10^4$)	0
Zoothamnium Sp	0	5.4$X10^4$(0-1$X10^5$)	3.3$X10^2$(0-6.7$X10^2$)	0	1.8$X10^4$(0-5$X10^4$)	0
* *Total Stalked Ciliates/L*	8.3$x10^2$	3.9$x10^5$	7.1$x10^3$	8.3$x10^2$	7.5$x10^5$	7$x10^3$
(B)Metazoa						
- *Rotifers*	0	6.7 $x10^4$(5.3$x10^4$ -8.7 $x10^4$)	1.4$x10^3$(0-2.1 $x10^3$)	0	3.1$x10^4$(1.3 $x10^3$ -9.6 $x10^4$)	8 $x10^2$(0-1.3 $x10^3$)
- *Nematodes*	0	2.5 $x10^3$(0-3.3 $x10^3$)	0	3 $x10^2$(0-1.5 $x10^3$)	2.7 $x10^3$(0-6.7 $x10^3$)	2.7 $x10^2$(0-6.7 $x10^2$)
- *Oligochaetes*	0	2.5 $x10^3$(0-6.7 $x10^3$)	0	0	1.3 $x10^3$(0-6.7 $x10^3$)	0
(C) Total count/L	3.8 $x10^3$	2 $x10^6$	1.6$X10^4$	8.4 $x10^3$	1. 3 $x10^6$	2.7 $x10^4$

Ameoboids were dominant in mixed liquor ranging from $2x10^3$ ind /L in Mar., 2010 to $2x10^4$ ind /L in Dec., 2009 and upon comparing amoeboids of inlet and outlet, it was found that percent removal ranged from 28% in Mar., 2010 to maximum removal of 96% in Dec., 2009.

Flagellates represented by *Peranema* dominated in inlet wastewater in Feb., 2010 followed by less counts in Dec., 2009 and through treatment processes were removed at 100% from inlet water.

The groups of metazoa that have been identified in wastewater samples were rotifers, nematodes and oligochaetes. Rotifera was the dominant group ranging from $3.1x10^4$ ind /L in Mar., 2010 to $6.7x10^4$ ind /L in Feb., 2010 in mixed liquor. In outlet, rotifers were removed in Jan., 2010 but ranged from $8x10^2$ ind /L in March 2010 to $1.7x10^3$ ind /L in Dec., 2009.

Nematodes in mixed liquor ranged from $1.8x10^3$ ind /L in Dec., 2009 to $3.3x10^3$ ind /L in Jan. Nematodes removed from outlet water 100% at Jan., 2010 and Feb., 2010 but at Dec., 2009 and Mar., 2010 the removal was only 90%.

Oligochaetes in mixed liquor ranged from 10^3 ind /L in Dec., 2009 to $2.5x10^3$ ind /L in Jan. and Feb., 2010 and removed from outlet water at 100% during study period except in Dec., 2009 removal was only 83%.

3-Toxicity Testing:

From Table (3) and Figure (3), it was found that percent mortality of *Daphnia magna* decreased in wastewater samples of mixed liquor than that of inlet to reach to its minimum in samples of outlet of the wastewater treatment plant during the study period. The average mortality varied from 17 to 77.5% in inlet wastewater, from 9 to 42.5% in mixed liquor and from zero to 10% in outlet water. In inlet wastewater, daphnids mortality showed its maximum (77.5%) in Dec.2009 and the minimum mortality (17%) was in Feb.2010, whereas in outlet water decreased to 10 and zero%, in these two months, respectively. It was noticed that average mortality of water samples taken from mixed liquor in Jan. 2010 increased to 42.5% than that of inlet water (25%) and this was due to the presence of oil film on water sample surface that affected daphnids respiration during tests; thus causing partial mortality to the test organism due to physical effect and not to chemical toxicity. The presence of oil film occurred also in some water samples taken from mixed liquor during Feb. and March 2010.

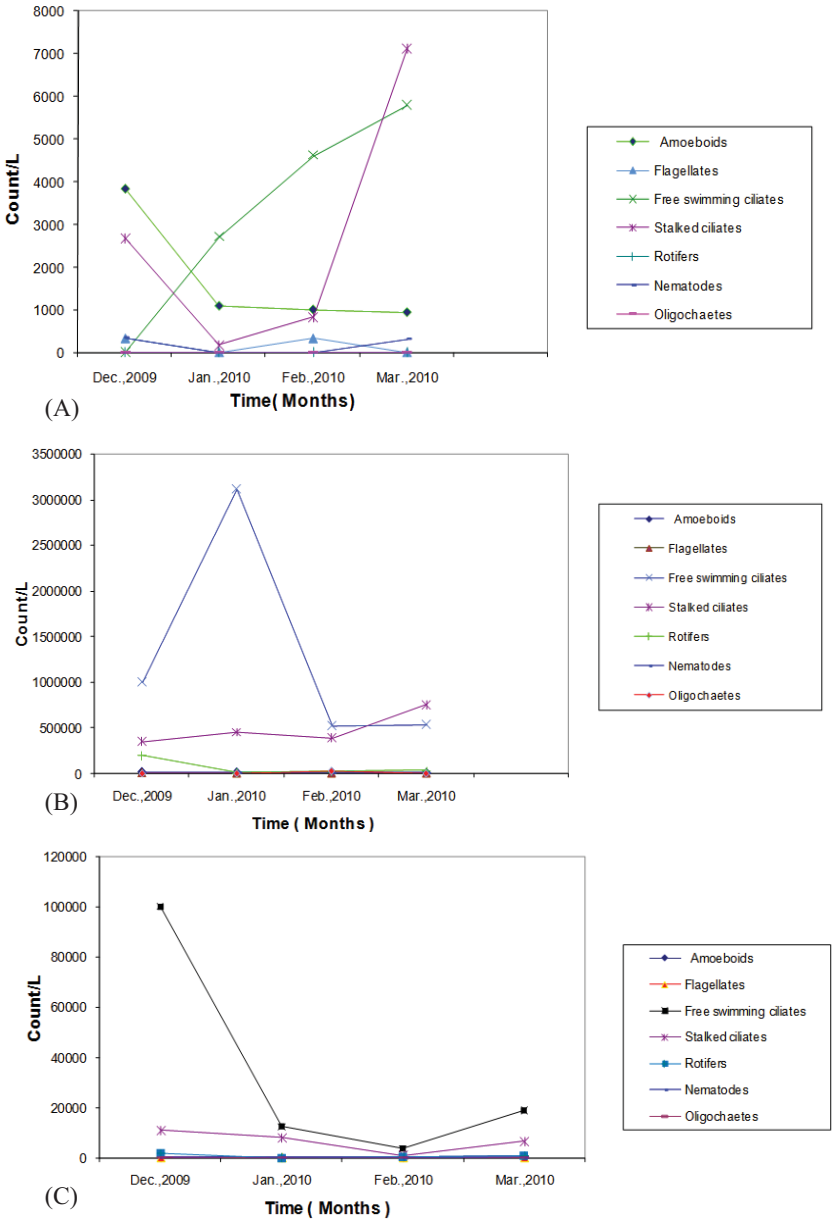


Fig. 2: Density of microfaunal groups examined in the three sampling sites of the treatment plant during the study period (A) Inlet, (B) Mixed Liquor, and (C) Outlet

Table 3: Percent mortality of *Daphnia magna* as a test organism in acute tests for 48h in water samples of the wastewater treatment plant

Sampling Sites	% Mortality											
	Dec., 2009			Jan., 2010			Feb., 2010			Mar., 2010		
	Min.	Max.	Aver.	Min.	Max.	Aver.	Min.	Max.	Aver.	Min.	Max.	Aver.
-Inlet	60	100	77.5	0	80	25	10	30	17	0	100	30
-Mixed Liquor	0	70	32.5	20	50	42.5*	0	20*	12	0	35*	9
-Outlet	0	36	9	0	10	2.5	0	0	0	0	0	0

Min.: Minimum Max.: Maximum Aver. : Average

*Part of % Mortality of Daphnia magna in assigned samples was attributed to Physical effect of oil film on water surface and not to toxic effects.

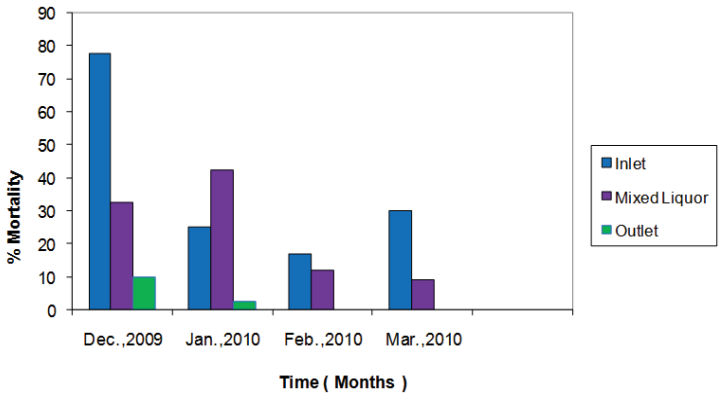


Fig. 3: Percent Mortality of *Daphnia magna* in wastewater samples taken From inlet, mixed liquor and outlet of the plant during the study period

Discussion:

Outlet water temperature and pH data obtained from this study were in the accepted range to be drained to the water bodies. The percentage removal of COD, BOD, TSS and ammonia increased through stages of treatment process ranging in effluent water 86-95, 85-96, 90-94 and 71-85 % respectively.

BOD is a measure of biological oxygen demand needed by bacteria to decompose organic matter in water bodies. High BOD as reported by Boyd and Lichikoppler (1979) has undesirable consequences on aquatic life. The obtained BOD (Table 1) in inlet wastewater was in the range 246-310 mg/L and removed through treatment by 91-96% in outlet water to meet the accepted limit.

The percent removal of total suspended solids, TSS in outlet water was at a range 90-94%. This lower content of suspended solids may be connected to specific protozoa population. Papadimitriou *et al*. (2004) reported that *Aspidisca cicada* and *Vorticella microstsoma* was correlated to BOD_5 removal capacity, while *Podophrya fixa* could be used as an indicator of low effluent SS content.

Of the free-swimming ciliates, *Aspidisca* was the most abundant genus (Table 2) followed by fewer counts of *Lionotus*. In this respect, Martin- Cereceda *et al*., (1996) stated that the presence of *Aspidisca* sp which even in low densities may induce good flocculation capacity.

The composition of protozoa community may be affected by several parameters such as the type of biological treatment and the influent characteristics, sludge loading and sludge age, organic loading rate (Curds and Cockburn, 1970 a&b; Salvado and Gracia ,1993; Madoni ,1994).

These protozoa have an important role for the good balance of the biological ecosystem: they eliminate the bacteria in excess and stimulate their growth and they promote flocculation (Gerardi and Horsfall., 1995). By consuming the free bacteria they help to decrease the effluent turbidity as well as its BOD and its suspended matter content (Curds *et al*., 1968).

Common genera were recognized in all samples like the amoeboids and the sessile ciliate *Vorticella sp*. These genera have been reported as typical populations developed in activated sludge aeration tanks, associated with the organic flocs (Macek, 1991). In addition, Abraham *et al*. (1996) stated that some genera like *Vorticella* and *Epistylis* might be tolerant in conditions of treating both low and high strength wastewaters.

A number of genera of protozoa have been identified in activated sludge. Protozoa are single-celled organisms that can consume food such as bacteria and particulate matter. Ciliated protozoa are numerically the most common genera in the activated sludge, but flagellated protozoa like *Peranema* dominated in inlet wastewater and amoeboids were present in all samples. Free-swimming ciliates were represented by the genera *Paramecium*, *Chilodonella*, *Euplotes*, *Lionotus*, *Aspidisca*, *Tetrahymena*, *Spathidium*, *Trachelophyllum*, *Pseudoprorodon*, *Amphiliptus*, *Spirostomum*, *Glaucoma*, *Colpoda, Stentor* and *Colpidium*. On the other hand, total stalked (sessile) ciliates in wastewater samples taken from mixed liquor were represented by the dominant genera *Vorticella*, *Opercularia*, *Carchesium* and *Epistylis* and also by fewer counts of *Vaginicola*, *Tokophrya*, *Podophrya*, *Acineta*, and *Zoothamnium*. Curds and Cockburn, (1970) investigated that the species of ciliated protozoa most commonly observed in wastewater treatment processes include *Aspidisca costata*, *Carchesium polypinum*, *Chilodonella uncinata*, *Opercularia coarcta* and *O. microdiscum*, *Trachelophyllum pusillum*, *Vorticella convallaria* and *V. microstoma*.

Sudo and Aiba, (1972) reported that free-swimming ciliates usually occur under conditions of good floc formation and generally indicate good activated sludge operation. They added that *Euplotes* and *Aspidisca* are common in activated sludge and their presence is desired as they indicate a well operating works. If the free-swimming ciliates are the dominant protozoan group, this indicates that the bacterial population and DO concentration are high. It also indicates a wastewater environment that is not yet stabilized and a sludge that is intermediate in health.

Jenkins *et. al*. (1993) investigated that attached ciliates are generally a sign of stable, healthy activated sludge operation. An example of an attached ciliate is the *Vorticella*. If treatment conditions are bad, for example, low DO levels or toxicity, *Vorticella* will leave their stalks. They observed during microscopic examination a bunch of empty stalks indicating a poor condition. *Vorticella* grow best in rapidly flowing water and seems to enhance nitrification.

Further, Curds and Fey (1969) stated that ciliated protozoa play the dominant role in the removal of *Escherichia coli* from wastewater by predation or flocculation, and added that *E. coli* population is generally reduced by 91 to 99 percent in the activated-sludge process.

Amoebae grow well on particulate organic matter and are able to tolerate low DO environments. They are present in high numbers during start-up of a treatment plant that has recovered from a toxic discharge (Esteban *et al*., 1991).

Jenkins *et. al*. (1993) reported that many of flagellates feed on soluble organic matter and are usually found in high numbers during recovery from a toxic discharge to the treatment plant or at low DO levels. If flagellates are present as the dominant protozoan group, this could indicate an unstable wastewater environment and a sludge that is in poor health. However, high population densities of flagellates have been observed in a municipal treatment plant operating under high organic loading conditions (Curds and Cockburn,1970, Mudrack and Kunst ,1986).

Pásztor and Szentgyörgyi,(2004) stated that the protozoa and metazoa community of the wastewater do not take part in the basic pollutant removal processes, but they live in close relationship with the bacteria and influence flock formation. Because of this interaction the protozoa species can be used as bioindicators of wastewater treatment plant performance.

Ginoris *et al*. (2007) investigated that optimum activated sludge performance occurs with a balance among free-swimming and attached ciliates and rotifers. An overabundance of flagellates, amoebae or free-swimming ciliates is an indication of high organic loading while an overabundance of attached ciliates, rotifers and other higher life forms indicates the opposite.

Rotifers are multicellular aquatic microorganisms that look like rapidly revolving wheels when they are in motion. This is due to the fact that the anterior end of the animal is modified into a retractable disc, or corona, bearing circles of strong cilia. Rotifers are able to consume both microbes and particulate matter. Like protozoa, these microorganisms are strict aerobes and are more sensitive to toxic conditions than bacteria. Rotifers are found only in a very stable activated-sludge environment. (Sládeček, 1983).

The efficiency of wastewater treatment plants by activated sludge is linked to the bacterial population but also to the protozoa (Nicolau *et al*., 1997). Different species can be found and have been listed by various authors: Curds and Cockburn (1970a), Martin-Cereceda *et al*. (1996), Richard (1991), Sasahara et Ogawa (1983), etc. In normal conditions their concentrations are larger than 10^6 protozoa/L. 10^7 protozoa/L corresponds to a very good pollution abatement. On the contrary concentrations lower than 10^5 protozoa /L is indicative of a poor efficiency of the plant (Drakides, 1978).

Protozoa are a useful biological indicator of the condition of the activated sludge. Being strict aerobes, these microorganisms prove to be excellent indicators of an aerobic environment (though some protozoa are capable of surviving up to 12 hours in the absence of oxygen). Protozoa also act as indicators of a toxic environment, as they exhibit a greater sensitivity to toxicity than bacteria. A clue that toxicity may be a problem in a system is the absence of or a lack of mobility of these organisms in activated sludge. The hallmark of a well-operated, stable activated-sludge system is the existence of large numbers of highly evolved protozoa in the biological mass. (Arregui *et al*., 2008).

Toxicity evaluation is an important parameter in wastewater quality monitoring as it provides the complete response of test organisms to all compounds in wastewater. Percent mortality of *Daphnia magna* decreased in wastewater samples of mixed liquor than that of inlet to reach to its minimum in samples of outlet of the wastewater treatment plant during the study period. The average mortality varied from 17 to 77.5% in inlet wastewater, from 9 to 42.5% in mixed liquor and from zero to 10% in outlet water (Table 3 and Fig. 3). These results indicated good efficiency of the studied treatment plant. Villegas-Navaro *et al*. (1999) reported that the use of *D. magna* as a toxicity indicator for wastewater effluents to show that the toxicity tests combined with physico-chemical analysis are essential in the evaluation of effluent quality and also in the assessment of the wastewater treatment plant efficiency

Conclusions:

The conclusion that the percentage removal of COD, BOD, TSS and ammonia increasing through stages of treatment process, indicated the efficiency of the studied WWTP.

It was also concluded that every wastewater treatment plant has a specific microorganism community, which is not directly determined by the characteristics of the wastewater or the treatment technology. Therefore long term microscopic tracking of activated sludge flock structure and microorganisms at a particular plant can provide more information about treatment efficiency, then the comparison of microbial fauna of different plants. The relative abundance of a certain species in a wastewater treatment plant may be considered as an indication of the importance of this species for the successful performance of the specific biological system concerned. Since the efficiency of wastewater treatment plants by activated sludge is linked to the protozoa population which is more than 10^6 protozoa/L in the present study, this count was corresponded to good pollution abatement.

Toxicity evaluation is an important parameter in wastewater quality monitoring as it provides the complete response of test organisms to all compounds in wastewater. Mortality of *Daphnia magna* decreased in outlet water; the maximum percentage reached 10%. These results are accepted in toxicity testing confirming good efficiency of the studied treatment plant.

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