

## Performance Evaluation of a Waste Stabilization Pond in a Rural Area in Egypt

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**Abstract:** The performance evaluation of the waste stabilization pond (WSP) as a model of domestic wastewater treatment unit in rural area was carried out. The unit comprised of anaerobic, facultative and maturation ponds in two series. The effluents of WSP which are discharged in the drain had the BOD reduced to 109-245 mg L<sup>-1</sup> (Mean = 145.3 mg L<sup>-1</sup>, 50.65% removal), while the COD was reduced to 221-400 mg L<sup>-1</sup> (Mean = 289 mg L<sup>-1</sup>, 48.95% removal) and the total suspended solids (TSS) were reduced to 118-190 mg L<sup>-1</sup> (Mean = 157.8 mg L<sup>-1</sup>, 44.3% removal). The reduction percentages of total coliform (TC), faecal coliform (FC), *E.coli*, faecal streptococci (FS), salmonellae and Listeria were 98.8, 95.6, 79.4, 96.8, 97.9 and 89.5% respectively. Also, the removal percentages of coliphage and infectious rotaviruses were 49.03 and 99.66% respectively. Identical sequences of rotaviruses VP-6 detected in the final effluent of the pond and the drain were observed. *Euglena* variables and *Chlamydomonas reinhardtii* were predominant in anaerobic, facultative and maturation effluents. It has been noticed that pollution affected species diversity of zooplankton; the number of species in facultative pond was 8 species because of high pollution level, whereas in maturation pond increased to 21 species. Also, pollution in anaerobic pond increased density of ciliates (Protozoa) which are known to be bio-indicators of organic pollution. The percent removal of ciliates in the maturation pond was 70%. It is recommended to make some modifications in the design to increase the efficiency of WSP.

**Key words:** zooplankton- protozoa- algae- bacteria- viruses

### INTRODUCTION

The most appropriate wastewater treatment is that which will produce an effluent meeting the recommended microbiological and chemical quality guidelines both at low cost and with minimal operational and maintenance requirements. Different systems are used worldwide for wastewater treatment such as activated sludge, trickling filter and waste stabilization pond systems. Pond systems are commonly employed for municipal sewage purification, especially in developing countries, due to its cost-effectiveness and high potential of removing different pollutants<sup>[3, 6]</sup>.

WSPs are designed to achieve different forms of treatment up to three stages in series, depending on the organic strength of the input waste and effluent quality objectives. Usually, classical WSPs consist of an anaerobic pond, followed by primary or secondary facultative ponds. If further pathogen reduction is necessary, maturation ponds will be introduced to provide tertiary treatment. WSPs are very widely used for small rural communities but large systems exist in Mediterranean basin, France and also in Spain and

Portugal. However, in warmer climates (the Middle East, Africa, Asia and Latin America) ponds are commonly used for large populations<sup>[15]</sup>.

In developing countries and especially in the tropical and equatorial regions like Egypt, a shortage of wastewater treatment systems is observed in rural communities. There is a great need to wastewater treatment systems to avoid the health risk problems in these communities. Wastewater treatment by WSPs has been considered an ideal way of using natural processes to improve wastewater effluents. In natural treatment systems such as WSP, the pathogens are progressively removed along the pond series with the highest removal efficiency taking place in the maturation ponds<sup>[21]</sup>.

The aim of this study was to evaluate the performance of WSP in rural area in Egypt and to determine its role in the contamination of the drain.

### MATERIALS AND METHODS

Wastewater treatment system in El-Mofti (Kafr El-Sheikh, Egypt) was designed to serve 3000 persons. Wastewater flow is about 225 m<sup>3</sup>/day mainly of domestic origin. This system consists of 500 primary

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septic tanks (each septic tank has approximately volume 1.8 m<sup>3</sup> with area 1.12 m<sup>2</sup> and depth 1.6 m) which used as primary treatment, a pumping station and wastewater stabilization pond which has two lines in parallel. Each line of pond consists of an anaerobic pond with volume 1400 m<sup>3</sup> (depth 3 m and area 475 m<sup>2</sup>), a facultative pond with volume 1500 m<sup>3</sup> (depth 1.5 m and area 1050 m<sup>2</sup>) and a maturation pond with volume 850 m<sup>3</sup> (depth 1.4 m and area 635 m<sup>2</sup>).

Effluents of 500 septic tanks are collected and discharged to pump station which in turn is discharged to WSP. The final effluents of WSP are discharged into El-Sabahi agricultural drain.

**Sampling sites:** Wastewater and water samples were collected monthly during the period from may 2005 until February 2006 at seven sites from each stages of WSP and agricultural drain which receives the final effluents of WSP. Samples from 1-4 represent: 1-influent (effluent of all septic tanks), 2-anaerobic effluents, 3-facultative effluents and 4-maturation effluents. Samples no.5-7 represent: 5-drain before mixing with treated effluents, 6-mixing point and 7-after 700 m from mixing point in El-Sabahi drain which receives the final effluent of WSP.

All samples were collected and transported within ice box and analyzed within 6 h of collection for chemical and biological examinations.

### Samples analysis

**Physico-chemical analysis:** Some physicochemical parameters such as temperature, pH, total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD) were determined according to APHA<sup>[2]</sup> and phosphate according to Gales *et al.*<sup>[13]</sup>. Additionally, nitrate was analyzed according to DEV<sup>[9]</sup>.

### Biological examination

**Algae:** Algal growth was determined by measuring Chlorophyll a content Chl(a) spectrophotometrically and calculated according to APHA<sup>[2]</sup>. Identification of algal community structure was examined by identification keys<sup>[31,32]</sup>.

**Zooplankton:** For zooplankton identification, few samples were filtered through a net of 55 µm pore size to concentrate zooplankton in 100 ml of water but other samples containing great numbers of organisms were taken without filtration. Concentrated samples and nonfiltered samples were then preserved by Lugol's solution<sup>[20]</sup>.

Zooplankton organisms were identified according to Edmondson<sup>[10]</sup> and were counted microscopically in 1.5 ml sub-samples in a Hawksley cell until attaining at least 60 individuals<sup>[23]</sup>

**Bacteriological examination:** Total bacterial count was determined using poured plate method while classical bacterial indicator (total coliform TC, faecal coliform FC, *Escherishia coli* (*E.coli*) and Faecal streptococci FC) were determined using MPN method. All parameters were carried out according to APHA<sup>[2]</sup> except FC and *E.coli*. They were carried out according to Kamel<sup>[16]</sup>. Additionally, salmonellae and Listeria determination were carried out according to El-Taweel *et al.*<sup>[12]</sup>.

### Virological examination:

**Concentration of water and wastewater samples:** All samples were concentrated by filtration through negatively charged nitrocellulose membranes according to Smith and Gerba<sup>[30]</sup> and Rose *et al.*<sup>[28]</sup>. Then all samples were reconcentrated using an organic flocculation method according to Katzenelson *et al.*<sup>[18]</sup>.

**Nucleic acid extraction:** Nucleic acids were extracted using RNA viral extraction Kit (Qiagen) according to manufacturer's instructions.

**Rotavirus detection using RT-PCR:** The primers VP6-3 5-GCTTTAAAACGAAGTCTTCAAC-3 and VP6-4 5-GGTAAATTACCAATTCCTCCAG-3 were used for amplification of a fragment of the VP6-coding gene corresponding to nucleotides 2-187 for rotavirus with a predicted product size of 190 bp<sup>[33]</sup>.

**Sequencing of amplified products:** RT-PCR products of selected samples were sequenced. Fifty to one hundred µl of the RT-PCR products were purified using a high pure PCR products purification kit (Qiagen) following the manufacturer's instructions. Cycle sequencing was performed on 1 to 7 ml of the purified products with an ABI prism Big dye termination cycle sequencing ready reaction kit (applied biosystem) using the same primers as in the PCR and following the manufacturer's instructions. The DNA was sequenced with an ABI prism 310 automated DNA sequencer.

Sequence data from both strands of the PCR products were aligned and compared by using the clustalw and blast programs (European bioinformatics institute).

**Infection of CaCo-2 cells:** Infection of CaCo-2 cells was performed as previously described<sup>[26]</sup>. Briefly, after 30-min of preactivation with 10 µg of trypsin/ml

(grade IX; Sigma) at 37°C, samples were 10-fold diluted in PBS. Then, 100 µl of direct samples or dilutions were inoculated into CaCo-2 monolayers grown in multiwell-plates (6 wells). After a 1h adsorption, a serum free overlay medium (3 ml) containing trypsin (5 µg/ml) was added and the cells were placed at 37°C. Four days postinfection, cells were collected by centrifugation at 800 g, resuspended in 300 µl of PBS and frozen and thawed three times.

**Detection of rotavirus infectious units using CC-RT-PCR:** Rotavirus cell culture RT-PCR (CC-RT-PCR) assay was performed on suspensions of infected CaCo-2 cells. Primers VP6-3 and VP6-4 were used. RT-PCR method was the same as described previously. The detection limit in this tissue culture assay using 100 µl of inoculation is 1X10<sup>1</sup> CC-RT-PCR units/ml, (where CC-RT-PCR units is the reciprocal end point dilution detectable by CC-RT-PCR)<sup>[1]</sup>.

### RESULTS

In this study, septic tanks were used as a pretreatment of house holds wastewater. The overall flow of wastewater to WSP which is the effluent of the septic tanks was 225 m<sup>3</sup>/day. The water temperature records were between 18 °C and 29°C, the average water temperature in anaerobic and facultative was 23.4°C while in maturation pond was 21.2°C. The average removal efficiencies of organic load in WSP measured as COD were 28.9, 20.24 and 9.9% after anaerobic, facultative and maturation ponds respectively. The anaerobic effluent indicated a BOD average value of 229 mg L<sup>-1</sup>, the facultative effluents 180.7 mg L<sup>-1</sup> and maturation effluents 145.3 mg L<sup>-1</sup>. The removal efficiencies of this parameter were 22,

21.1 and 19.6% in anaerobic, facultative and maturation effluents respectively Table 1. The mean values of TSS in this system were 283.3 mg L<sup>-1</sup>, 214.3 mg L<sup>-1</sup>, 176.3 mg L<sup>-1</sup> and 157.8 mg L<sup>-1</sup> in influent , anaerobic , facultative , maturation effluents and the reduction of TSS was 24.4, 17.7 and 10.5% in anaerobic, facultative and maturation ponds respectively. The overall reduction of dissolved phosphorus and nitrate were 51.4 and 55.5% respectively. The characteristic properties of drain before discharge revealed that pH was 7.8 while, COD, BOD and TSS were 199.3 mg L<sup>-1</sup>, 101.5 mg L<sup>-1</sup> and 152 mg L<sup>-1</sup> respectively. The concentration of these parameters were increased after discharge the WSP effluents to the drain where, pH was 7.9 while COD, BOD and TSS were 276 mg L<sup>-1</sup>, 143 mg L<sup>-1</sup> and 161 mg L<sup>-1</sup> respectively. After 700 m of discharge point pH was 7.5 and COD, BOD and TSS were 101 mg L<sup>-1</sup>, 49 mg L<sup>-1</sup> and 41 mg L<sup>-1</sup>.

The mean and removal percentages of microbial indicators and bacterial pathogens for each stage of WSP and each of the three points of the drain receiving the WSP effluent are presented in Table (2). The results showed that the load of bacterial-indicators with influent of WSP samples were 10<sup>12</sup>-10<sup>13</sup> Cfu/ml for total bacterial count, 10<sup>12</sup> for TC, 10<sup>10</sup> for FC, 10<sup>8</sup> for E. coli and 10<sup>8</sup> for FS as Mpn/100ml. Also, coliphage count was 1.49x10<sup>2</sup>Cfu/100ml and the bacterial pathogens of influent sample were 4.3x10<sup>4</sup> and 1.3 x10<sup>6</sup> Cfu/100ml for salmonellae and Listeria respectively. The average removal efficiencies of bacterial loads with anaerobic pond were 1-2 log<sub>10</sub> unit (88.4-94.4%) for total bacterial counts, one log<sub>10</sub> unit for both TC (96.2%) and FC (94.3%) and 2 log<sub>10</sub> units for both E. coli (98.5%) and FS (98.9%).

Table 1: Some physico-chemical characteristics of wastewater samples (standard deviation is in between brackets)

Sampling sites	Parameters						pH
	Nitrate NO <sup>3</sup> -N mg L <sup>-1</sup>	Dissolved phosphate PO <sub>4</sub> -P mg L <sup>-1</sup>	Total phosphate PO <sub>4</sub> -P mg L <sup>-1</sup>	Biological oxygen demand mg O <sub>2</sub> L <sup>-1</sup>	Chemical oxygen demand mg O <sub>2</sub> L <sup>-1</sup>	Total suspended solids mg L <sup>-1</sup>	
<b>WSP influents</b>							
Range	0.81-1.01	11-18	14-26	204-420	445-782	196-370	7.7-8.2
Mean	0.91 (0.15)	14.4 (2.6)	20.1 (4.5)	294.4 (85.9)	566.1 (117.2)	283.3 (65.7)	7.89 (0.184)
<b>Anaerobic effluents</b>							
Range	0.34-0.69	9-15	12-25	165-425	239-750	116-360	7.74-8.2
Mean	0.51 (0.15)	11.6 (2.4)	16.7 (4.2)	229 (94.5)	402.5 (178.0)	214.3 (92.3)	8.019 (0.182)
<b>Facultative effluents</b>							
Range	0.25-0.65	5.5-12	8-13	138-267	207-507	80-300	7.7-8.6
Mean	0.384 (0.163)	7.9 (2.2)	11.1 (1.7)	180.7 (48.4)	321 (114.9)	176.3 (90.2)	8.26 (0.23)
<b>Maturation effluents</b>							
Range	0.17-0.67	4-11	7-13	109-245	221-400	118-190	7.9-9.0
Mean	0.39 (0.15)	7 (2.4)	10.5 (2.3)	145.3 (46.3)	289 (69.2)	157.8 (30.9)	8.34 (0.40)

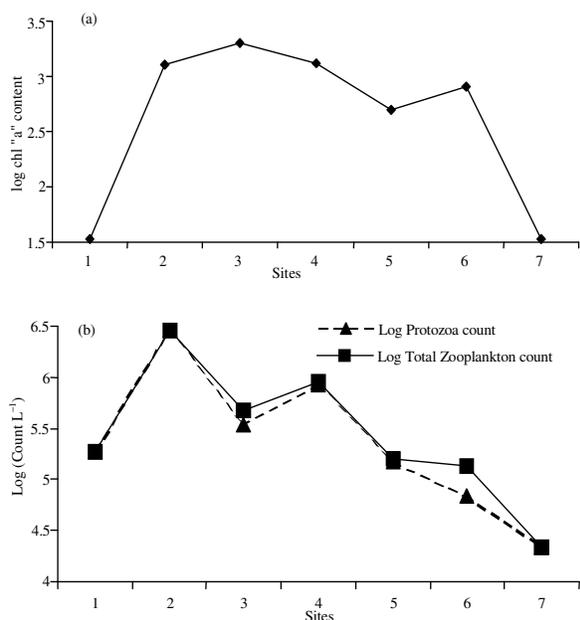


Fig. 1: Correlation between Chlorophyll "a" Concentrations (a) and Zooplankton Counts (b) at Different Sites of Operational Steps in El-Mofti Stabilization Pond and Drain

Removal efficiency of coliphage was 34.7%. The removal efficiencies in facultative and maturation ponds were more than 95% for classical bacterial indicators except total bacterial counts at 37°C (90%) and *E. coli* (79.4%) in maturation pond. The removal of coliphage was 36.9% in facultative pond and 49.03% in maturation pond. Bacterial pathogens reductions were 85.3, 96.2, 97.7% for salmonellae and 62.3, 84.5 and 89.5% for *Listeria* in anaerobic, facultative and maturation ponds respectively (Table 2). The microbial loads of water drain at mixing point were higher than the microbial loads before mixing point and considerable decrease in the loads was observed at 700 m from mixing point (Table 2). The mean count of *Listeria* was 59.11 Cfu/100ml but salmonellae was absent at 700 m from the mixing point in the drain.

Change in Chl (a) content of the studied wastewater during the various treatment stages is presented in Fig. 1. Available data revealed an increase in Chl (a) content of the raw wastewater (33.8 µg L<sup>-1</sup>) as it passes from the anaerobic to the facultative ponds which amounted to 1261.6 µg L<sup>-1</sup> and 1833 µg L<sup>-1</sup> respectively, in maturation pond Chl(a) was decreased. The successive changes in algal community as the wastewater flow from the anaerobic to the

Table 2: Microbiological characteristics of wastewater stabilization pond and drain water receiving final effluent of pond

Sampling site	Total bacterial count Cfu/ml		MPN-index/100ml				Coliphage Cfu/100 ml	Pathogenic bacteria Cfu/100 ml	
	At 37°C	At 22°C	TC	FC	<i>E.coli</i>	FS		Salmonellae	<i>Listeria</i>
<b>WSP influents</b>									
Mean	1.4×10 <sup>13</sup>	4.9×10 <sup>12</sup>	3.4×10 <sup>12</sup>	6.9×10 <sup>10</sup>	5.2×10 <sup>8</sup>	2.3×10 <sup>9</sup>	149.4	4.3×10 <sup>4</sup>	1.3×10 <sup>4</sup>
S.D	36×10 <sup>13</sup>	6.7×10 <sup>12</sup>	5.6×10 <sup>12</sup>	6.6×10 <sup>10</sup>	7.6×10 <sup>8</sup>	3.9×10 <sup>9</sup>	38.04	6.1×10 <sup>4</sup>	1.5×10 <sup>4</sup>
<b>Anaerobic effluents</b>									
Mean	7.9×10 <sup>11</sup>	5.7×10 <sup>11</sup>	1.3×10 <sup>11</sup>	3.9×10 <sup>9</sup>	7.9×10 <sup>6</sup>	2.4×10 <sup>7</sup>	97.6	6.3×10 <sup>3</sup>	4.9×10 <sup>3</sup>
R%	94.4	88.4	96.2	94.3	98.5	98.9	34.7	85.3	62.3
S.D	2.4×10 <sup>12</sup>	1.4×10 <sup>12</sup>	3.6×10 <sup>11</sup>	7×10 <sup>10</sup>	1.5×10 <sup>7</sup>	7.3×10 <sup>7</sup>	16.9	77×10 <sup>3</sup>	6.8×10 <sup>3</sup>
<b>Facultative effluents</b>									
Mean	7.9×10 <sup>9</sup>	1.1×10 <sup>10</sup>	3.6×10 <sup>8</sup>	3.6×10 <sup>7</sup>	3.6×10 <sup>5</sup>	8.1×10 <sup>5</sup>	61.6	2.4×10 <sup>2</sup>	7.6×10 <sup>2</sup>
R%	99	98.1	99.7	99.1	95.4	96.6	36.9	96.2	84.5
S.D	1.9×10 <sup>10</sup>	2.8×10 <sup>10</sup>	9.2×10 <sup>8</sup>	3.6×10 <sup>7</sup>	4.1×10 <sup>5</sup>	1.4×10 <sup>6</sup>	8.8	4.2×10 <sup>2</sup>	1.1×10 <sup>3</sup>
<b>Maturation effluents</b>									
Mean	7.9×10 <sup>8</sup>	2×10 <sup>7</sup>	3.8×10 <sup>6</sup>	1.6×10 <sup>6</sup>	7.4×10 <sup>4</sup>	2.6×10 <sup>4</sup>	31.4	4.9	1.1×10 <sup>2</sup>
R%	90	99.8	98.8	95.6	79.4	96.8	49.03	97.9	89.5
S.D	7.3×10 <sup>9</sup>	2.8×10 <sup>7</sup>	7.3×10 <sup>6</sup>	3.9×10 <sup>6</sup>	1.4×10 <sup>5</sup>	4.9×10 <sup>4</sup>	6.5	14.7	91.3
<b>Drain water before mixing</b>									
Mean	2.9×10 <sup>6</sup>	9.9×10 <sup>6</sup>	2.5×10 <sup>6</sup>	3×10 <sup>4</sup>	3.2×10 <sup>3</sup>	1.2×10 <sup>4</sup>	11.8	2	81.8
S.D	4.8×10 <sup>6</sup>	2.4×10 <sup>7</sup>	4.8×10 <sup>6</sup>	3.9×10 <sup>4</sup>	7.1×10 <sup>3</sup>	3.1×10 <sup>4</sup>	2.9	6	87.4
<b>Mixing point</b>									
Mean	4×10 <sup>8</sup>	3.6×10 <sup>8</sup>	6.4×10 <sup>7</sup>	9.8×10 <sup>6</sup>	7.6×10 <sup>4</sup>	7.1×10 <sup>5</sup>	20.8	18.9	2.7×10 <sup>2</sup>
S.D	8.1×10 <sup>8</sup>	6.1×10 <sup>8</sup>	1.7×10 <sup>8</sup>	2.2×10 <sup>7</sup>	1.6×10 <sup>5</sup>	1.5×10 <sup>6</sup>	2.3	53.01	2.4×10 <sup>2</sup>
<b>700m after mixing point</b>									
Mean	1.1×10 <sup>7</sup>	4.4×10 <sup>6</sup>	6.7×10 <sup>5</sup>	2.7×10 <sup>5</sup>	6×10 <sup>2</sup>	1×10 <sup>4</sup>	9.4	0	59.11
S.D	2.3×10 <sup>7</sup>	6.3×10 <sup>6</sup>	1.5×10 <sup>6</sup>	7.9×10 <sup>5</sup>	1.5×10 <sup>3</sup>	2.2×10 <sup>4</sup>	2.9	0	82.11

Table 3: Change in community structure of algae in El-Mofti WSP

Algal taxa	Sampling site						
	Raw	Anaerobic		Facultative		Maturation	
		Start	End	Start	End	Start	End
Green algae							
<i>Euglena variabilis</i>	±	++++	+	+++	++	+	++
<i>Chlamydomonas reinhardii</i>	±	++++	+++	+++	+++	++	+++
<i>Cryptomonas erosa</i>	±	++++	+	+	-	+	-
<i>Pandorina morium</i>	±	±	-	±	-	±	-
<i>Phacus triquetre</i>	±	±	+	++	++	+	++
<i>Haematococcus pluviialis</i>	±	±	+	±	±	±	±
<i>Microactinum pusillum</i>	±	±	++	+	++	±	+
<i>Siderocelis elegans</i>	±	-	-	±	±	-	-
<i>Pediastrum clathratum</i>	±	-	-	±	±	-	-
Blue-green algae							
<i>Oscillatoria limnetica</i>	±	±	±	±	±	±	±
<i>Oscillatoria chlorine</i>	±	±	±	±	±	±	±
++++Dominant	+++ High	++ Low	+Detectable	± Rare			

maturity ponds are given in Table 3. In influent 9 green algal species found in rare numbers, also 2 species from blue green algae and diatoms group are not represented. The anaerobic pond was almost dominated by *Euglena variabilis*, *Chlamydomonas reinhardii*, *Cryptomonas erosa*, however in the facultative and maturation pond, algal community was represented by *Euglena variabilis*, *Chlamydomonas reinhardii*, *Phacus triquetre*. A pronounced change in Chl (a) value had occurred, 503 µg L<sup>-1</sup> before discharge and 816 µg L<sup>-1</sup> at mixing point. As a result of dilution factor Chl (a) content become 33.5 µg L<sup>-1</sup> in the drain after 700 meters. Available results revealed that *Phacus triquetre*, *Euglena variabilis*, *Chlamydomonas reinhardii* and *Microactinum pusillum* were detectable at the discharge point. After discharge by 700 meters, where the organic load diluted and the previous species were found in rare count beside the following species which represented the three algal groups: Diatoms, green algae and blue green algae. Diatoms were represented by *Diatoma elongatum*, *Gryosigma attenuatum*, *Fragillaria capunica* and *Synedra ulna*, green algae were represented by *Scenedesmus quadricauda* and and blue green algae were represented by *Oscillatoria limnetica*, *Oscillatoria chlorine*.

Zooplankton communities of this study are presented in Table 4a and 4b. Zooplankton groups were usually identified to the genus level except Copepoda that was identified to the suborder level (namely, Calanoida, Cyclopoida and Harpacticoida). In influent, protozoans especially ciliates dominated zooplankton biomass (count/liter) of which *Didinium* (1380x10<sup>2</sup>/liter) was the most abundant genus followed by less numbers of *Astylozoon*. Other zooplankton groups detected in influent were rotifers represented by

*Philodina* at 280/liter, crustaceans represented by the ostracod *Cyprinotus* at 140/liter, larval stages of insects at 540/liter and larval stages of Nematoda at 400/liter. In the anaerobic pond, the total zooplankton count was at its maximum (28736x10<sup>2</sup>/liter). In that site the phylum Protozoa was also the predominant group because of the dominancy of the genus *Stombidium* (20400x10<sup>2</sup>/liter) which was followed by *Aristerostoma* at 6030x10<sup>2</sup>/liter. The phylum Rotatoria represented by *Brachionus* showed mean count 140/liter and there were no representatives of the two groups Arthropoda and Nematoda. The facultative pond was dominated by phylum Protozoa followed by rotifers and as in the anaerobic pond, representatives of arthropods and nematodes were not detected. The ciliate *Aristerostoma* (2254x10<sup>2</sup>/liter) was the dominant genus followed by *Astylozoon* at 673x10<sup>2</sup>/liter and by less number of *Paramecium* at 460x10<sup>2</sup>/liter. As we proceed from the facultative pond to the maturation pond, the mean count of zooplankton increased from 4720x10<sup>2</sup>/liter to 8810x10<sup>2</sup>/liter. The ciliates *Dysteria*, *Tintinnopsis*, *Astylozoon* and *Mesodinium* appeared in the wastewater samples taken from the maturation pond at mean counts of 1028x10<sup>2</sup>, 760x10<sup>2</sup>, 208x10<sup>2</sup> and 178x10<sup>2</sup> organisms/liter respectively and were preceded by the abundance of *Aristerostoma* at 6000x10<sup>2</sup>/liter. The rotifer *Asplanchna* was the dominant genus in the phylum Rotatoria and its count decreased from 1140x10<sup>2</sup>/liter in the facultative pond to 217x10<sup>2</sup>/liter in the maturation pond. The Zooplankton counts decreased gradually in the water samples as passing from maturation pond to the discharge point, then after mixing with the agricultural drain until reached to their minimum (220x10<sup>2</sup>/liter) at 700 m from the mixing point with the drain.

Table 4a: Zooplankton counts (Mean± SD) per liter in the wastewater samples taken from the treatment steps of the oxidation pond.

Zooplankton	Sites			
	Influents	Anaerobic effluents	Facultative effluents	Maturation effluents
<b>Phylum: Protozoa</b>				
<b>Class: Rhizopoda and Actinopoda</b>				
<i>Centropyxis</i>	5.4±7.79	497.4±951.9	82±164	105.4±139.47
<i>Plagiopyxis</i>	-	-	-	-
<i>Amoeba</i>	-	-	-	-
<i>Arcella</i>	-	-	-	-
<b>Class: Ciliata</b>				
<i>Vorticella</i>	-	1.4±2.8	-	-
<i>Paramecium</i>	-	-	460±920	17.4±23.73
<i>Mesodinium</i>	-	5.4±10.8	-	2.6±5.2 178.356
<i>Opercularia</i>	21.4±39.39	480. ±845.13	-	4±8
<i>Aristerostoma</i>	45.4±90.8	6030±11890.7	2254±4374.25	6000±12000
<i>Astylozoon</i>	384±768	1204±1497.22	673.4±831.14	208±255.45
<i>Acropisthium</i>	-	122±178.71	-	-
<i>Eschaneustyla</i>	-	-	-	-
<i>Podophrya</i>	-	-	-	-
<i>Aspidisca</i>	-	-	-	-
<i>Tintinnopsis</i>	-	-	-	760±1520
<i>Coleps</i>	-	-	-	-
<i>Didinium</i>	1380±2760	-	-	-
<i>Chilodonella</i>	-	-	-	-
<i>Condylostoma</i>	-	-	-	40±80
<i>Thecacinata</i>	-	-	-	-
<i>Stombidium.</i>	-	204000±40800	-	-
<i>Dysteria</i>	-	-	-	1028±2056
<i>Frontonia</i>	-	-	-	-
<i>Tetrahymena</i>	-	-	-	-
<b>Class: Zooflagellata</b>				
<i>Paramastix</i>	-	-	1.4±2.8	-
<b>Phylum: Rotatoria Class: Rotifera</b>				
<i>Brachionus</i>	-	1.4±2.8	38.6±21.2	23.8±20.76
<i>Keratella</i>	-	-	-	1.4±2.8
<i>Lepadella</i>	-	-	-	-
<i>Philodina</i>	2.8±3.43	-	10.6±2.12	10.8±13.23
<i>Asplanchna</i>	-	-	1140±2280	216.6±426.73
<i>Asplanchnopus</i>	-	-	57.4±114.8	-
<i>Enteroplea</i>	-	-	-	1.4±2.8
<i>Manfredium</i>	-	-	-	1.4±2.8
<i>Polyarthra</i>	-	-	-	1.4±2.8
<i>Platyias</i>	-	-	-	-
<i>Trichotria</i>	-	-	-	-
<i>Diplois</i>	-	-	-	-
<b>Phylum: Arthropoda Class: Crustacea</b>				
<b>Subclass: Branchiopoda</b>				
<b>Order: Cladocera</b>				
<i>Bosmina</i>	-	-	-	-
<i>Alona</i>	-	-	-	2.6±5.2
<i>Moinodaphnia</i>	-	-	-	-
<b>Subclass: Copepoda</b>				
Cyclopoids	-	-	-	1.4±2.8
Harpacticoids	-	-	-	-
Nauplius larva	-	-	-	1.4±2.8
<b>Subclass: Ostracoda</b>				
<i>Cyprinotus</i>	1.4±2.80	-	-	4±8
<b>Class: Insecta Larval Stages</b>				
<i>Larval stages</i>	5.4±10.8	-	-	-
<b>Phylum: Nematoda Larval stages</b>				
<i>Larval stages</i>	4±5.25	-	-	2.6±5.2
<b>Total Count /L</b>	<b>1855.2± 2616.04</b>	<b>28736.2± 40883.76</b>	<b>4720± 5206.43</b>	<b>8809.6± 11324.37</b>

N.B.: All zooplankton counts are divided by 10<sup>2</sup>

Table 4b: Zooplankton counts (Mean± SD) per liter in the water samples taken from El Sabahi Drain

Zooplankton	Sites		
	Drain water before mixing	Mixing point	After 700meters of mixing point
<b>Phylum: Protozoa</b>			
<b>Class: Rhizopoda and Actinopoda</b>			
<i>Centropyxis</i>	10.6±13.62	16.28±28.63	-
<i>Plagiopyxis</i>	5.4±10.8	16±32	30±51.96
<i>Amoeba</i>	-	4±8	-
<i>Arcella</i>	-	1.4±2.8	-
<b>Class: Ciliata</b>			
<i>Vorticella</i>	-	14.62±29.19	-
<i>Paramecium</i>	-	-	1.75±3.03
<i>Mesodinium</i>	245.4±477.41	0.146±0.292	120.78±201.69
<i>Opercularia</i>	-	-	-
<i>Aristerostoma</i>	-	-	-
<i>Astylozoon</i>	18±36	-	-
<i>Acropisthium</i>	-	0.146±0.168	0.08±0.14
<i>Eschaneustyla</i>	-	0.084±0.168	1.75±3.03
<i>Podophrya</i>	1.4±2.8	-	-
<i>Aspidisca</i>	4±8	102±2.4	52.5±9093
<i>Tintinnopsis</i>	-	-	-
<i>Coleps</i>	5.4±10.8	5.4±10.8	3.25±5.63
<i>Didinium</i>	-	-	-
<i>Chilodonella</i>	-	0.124±0.248	-
<i>Condylostoma</i>	-	-	-
<i>Thecacinata</i>	1180±2360	-	-
<i>Stombidium</i>	-	-	-
<i>Dysteria</i>	-	-	-
<i>Frontonia</i>	-	9.4±18.8	-
<i>Tetrahymena</i>	-	506±1012	-
<b>Class: Zooflagellata</b>			
<i>Paramastix</i>	12±24	20±40	5±8.66
<b>Phylum: Rotatoria Class: Rotifera</b>			
<i>Brachionus</i>	62.6±118.81	62.62±97.98	-
<i>Keratella</i>	0.6±1.2	-	0.08±0.14
<i>Lepadella</i>	2±4	-	-
<i>Philodina</i>	34.6±69.2	18.7±27.56	1.75±3.03
<i>Asplanchna</i>	-	5.4±10.8	-
<i>Asplanchnopus</i>	-	568±1136	-
<i>Enteroplea</i>	-	-	-
<i>Manfredium</i>	-	-	-
<i>Polyarthra</i>	-	-	-
<i>Platylas</i>	1.4±2.8	-	-
<i>Trichotria</i>	1.4±2.8	-	-
<i>Diplois</i>	-	0.02±0.04	-
<b>Phylum: Arthropoda Class: Crustacea</b>			
<b>Subclass: Branchiopoda</b>			
<b>Order: Cladocera</b>			
<i>Bomina</i>	-	0.02±0.04	-
<i>Alona</i>	-	-	-
<i>Moinodaphnia</i>	-	0.02±0.04	-
<b>Subclass: Copepoda</b>			
Cyclopoids	0.6±1.2	-	-
Harpacticoids	-	-	-
Nauplius larva	4.8±6.68	0.02±0.04	1.75±3.03
<b>Subclass: Ostracoda</b>			
<i>Cyprinotus</i>	-	-	-
<b>Class: Insecta Larval Stages</b>	0.6±1.2	-	-
<b>Phylum: Nematoda Larval stages</b>	6±12	1.4±2.8	-
<b>Total Count /L</b>	1596.8± 2756.10	1351.54±2056.64	220.5±212.55

N.B.: All zooplankton counts are divided by 10<sup>2</sup>

Table 5: Number of infectious particles of rotaviruses per liter in the WSP treatment steps and drain water samples

Samples	No. of rotavirus infectious particles (CC-RT-PCR units/liter)							
	May	June	July	August	September	December	January	February
	2005	2005	2005	2005	2005	2005	2006	2006
WSP influents	---	---	---	0	0	1x10 <sup>4</sup>	1x10 <sup>6</sup>	1x10 <sup>4</sup>
Anaerobic effluents	---	---	---	---	---	1x10 <sup>3</sup>	1x10 <sup>4</sup>	1x10 <sup>3</sup>
Facultative effluents	---	---	---	---	---	1x10 <sup>2</sup>	1x10 <sup>3</sup>	1x10
Maturation effluents	---	---	---	---	---	1x10 <sup>2</sup>	1x10 <sup>2</sup>	1
Drain water before mixing	---	---	---	---	---	1x10	0	---
Mixing point	---	---	---	---	---	---	1x10	---
After 700 meters of mixing point	---	---	---	---	---	---	1	---

(---) negative VP-6 samples in the screening using RT-PCR

Rotaviruses were detected in 5 influent, 3 anaerobic, 3 facultative and 3 maturation effluent samples out of 8 samples for each stage using RT-PCR. Also, rotaviruses were detected two times before mixing and one time at both mixing point and after 700 m from mixing point of drain water samples. Sequence analysis of RT-PCR products revealed that they were belonged to rotavirus VP6. On the other hand, Table (5) showed the number of rotaviral infectious particles in the positive rotavirus VP6 samples. It can be observed that the number of infectious units ranged from 0 to 10<sup>6</sup>, 10<sup>2</sup> to 10<sup>4</sup> and 10 to 10<sup>3</sup> and from 1 to 10<sup>2</sup> CC-RT-PCR units/liter in influent, anaerobic effluent, facultative effluent and maturation effluent samples respectively. The count of infectious particles in water drain samples were 10, 10 and 1 CC-RT-PCR units/liter before mixing point, at mixing point and after 700 m from mixing point samples respectively. Sequence analysis revealed that identical sequences of rotavirus between influent, after anaerobic pond, after facultative pond, after maturation pond, at mixing point in the drain and after 700 m from the mixing point samples of January was observed.

## DISCUSSION

Waste stabilization pond systems are a widely used technique for the treatment of wastewater for rural areas [27]. In this study, settling of solid particulates of wastewater in the septic tanks is one of the main processes to remove organic material from liquid phase [17]. The effluents of the septic tanks are used as influent in WSP. Our results showed that the overall reductions were 44.3% (TSS), 48.9% (COD), 50.6% (BOD), 51.4% (DP) and 52% NO<sub>3</sub>-N. The performance of WSP attained a lower efficiency than expected. The highest efficiency was recorded with anaerobic than facultative and maturation ponds for TSS, COD and BOD reduction.

From the obtained results, it can be observed that the facultative pond was more efficient in the reduction of classical bacterial indicators such as TC, FC and FS. The reduction of classical bacterial indicators was 6 log units for TC, 4 log<sub>10</sub> units for both FC and *E.coli*, 5 log<sub>10</sub> units for FS and one log unit for coliphage. Additionally, the reduction of salmonellae and *Listeria* were two log units. The final effluent of maturation was

still high in microbial load FC (10<sup>6</sup>), *E.coli* (10<sup>4</sup>) Mpn/100ml and Coliphage (3.1X10) Pfu/100 ml. The final effluent complied with *E.coli* WHO guidelines for restricted irrigation [34].

Generally Barjenbrach and Erler [5] reported that, there are several causes for deterioration of the purification performance; such as unsuitable design of the pond; incomplete mixing of aerated pond; type of preliminary treatment; insufficient maintenance and increased organic influent loads.

In our study, although, the retention time is sufficient in the ponds, bad removal of BOD, COD and pathogens was observed. The poor removal in maturation pond may be due to some defects in the design of the ponds. The entrance of wastewater to different ponds was from one point. It means bad distribution of the wastewater and bad mixing with the microorganisms in the pond. Also, the increase in the detention time more than recommended may lead to the death of some bacteria and then decrease of the efficiency of the ponds. Modifications of the design of the pond by adding some additional points for entrance of wastewater to the ponds to make complete mix in the different ponds are needed.

The treatment occurring in WSP results from the complex symbiosis of bacteria and algal species which results in an ecological pattern different from that of these organisms grown in pure culture. Changes of pH, temperature and light intensity control the abundance and activity of specific groups of microorganisms in the multi-species microbial communities' characteristic of facultative ponds [24, 35].

From the results, pH values increased from 7.79 to 8.34. It was associated with the increasing algal activity which is expressed as Chl (a). The increasing in pH value is due to CO<sub>2</sub> consumed during photosynthesis of the algae. The obtained chlorophyll (a) values were as mentioned in literature from 500 - 2000 µg L<sup>-1</sup> to be occurring in facultative pond [21]. Nitrate and phosphate had an inverse relation with Chl (a), this can be explained by the fact that extensive algal growth exhausts available nutrients. Yan and Jameson, [36] reported that the amount of nitrogen and phosphorus removed from maturation pond depend on algal biomass. *Euglena variabilis*, *Chlamydomonas reinhardtii* were the most dominant types of green algae that were indication for high organic load [21].

In this study, the zooplankton community comprises three main classes of phylum Protozoa, phylum Rotatoria, Crustacea (Cladocera, Copepoda and Ostracoda) and larval stages of both Insecta and Nematoda. Protozoa specially Ciliata was the predominant group in all samples taken from the nominated sites and this group was followed by less numbers of rotifers (phylum: Rotatoria). The other two groups of zooplankton, namely, arthropods and larval stages of nematodes were present in few numbers. The dominance of protozoans indicates the presence of organic pollution. In this respect Ghazy<sup>[14]</sup> on a study on wastewater of Starch and Glucose Factory showed a positive effect on the majority of protozoan species, more specifically on some ciliates like Paramecium. Also, other species of zooplankton especially amongst cladocerans and rotifers may be used as indicators of organic and chemical pollution<sup>[22]</sup>. These types of pollution were noticed in the studied WSP from the influent and reached maximum in the anaerobic pond as a result of coinciding with protozoans (especially ciliates) peak in this pond and decreased gradually from maturation pond until reached minimum at 700 m of the effluent mixing point with the agricultural drain water.

On the other hand, it was noticed that zooplankton count and species diversity in influent were less than those in anaerobic pond, facultative pond or maturation pond and this may be due to the presence of other pollutants in this site. These pollutants may be chemicals, pesticides, or toxin-producing strains of fecal bacteria *E. coli*<sup>[8]</sup>.

Chlorophyll "a" measures are included as an approximation of total phytoplankton abundance and also as an indicator of energy inputs into the system through primary productivity. Chlorophyll "a" levels in all sampling sites peak in the facultative pond where the mean zooplankton count /liter ( $4720 \times 10^2 \pm 520 \times 10^2$ ) in this site decreased compared with those in the anaerobic pond ( $28736 \times 10^2 \pm 40884 \times 10^2$ ) and the maturation pond ( $8810 \times 10^2 \pm 11324 \times 10^2$ ). Chlorophyll "a" content in anaerobic and maturation ponds were high ( $1261.6$  and  $1333.4 \mu\text{g L}^{-1}$ ) but decreased than that in facultative pond as a result of grazing of phytoplankton by zooplankton in these two sites. In influent, the chlorophyll "a" content was at its minimum because of high content of suspended solids which obscure light responsible for photosynthesis and the zooplankton count was relatively high in this site in accordance with high counts on ciliates that feed mainly on bacteria and organic matter available in this type of wastewater as indicated from total suspended solids ( $283 \text{ mg L}^{-1}$ ).

Thus, zooplankton peak in the anaerobic pond is coincided either with increased green- algal abundance of *Clamydomonas*, *Euglena* and *Cryptomonas* (hence chlorophyll "a" peak), or abundance of bacteria; Muylaert *et al.*<sup>[25]</sup> stated that in aquatic ecosystems, bacteria play a key role in the breakdown of organic matter and the remineralization of nutrients. They are grazed upon by protozoa and some metazoans and, as such, from the base of heterotrophic aquatic food chain. Exudates produced by phytoplankton are an important

organic substrate for bacteria in many aquatic ecosystems<sup>[4]</sup>. Under oligotrophic conditions, inorganic nutrients may limit bacterial growth<sup>[7]</sup>. Heterotrophic nanoflagellates are often the dominant grazers on bacteria in aquatic ecosystems<sup>[29]</sup>, but specially in eutrophic environments, ciliates can be an important grazers too<sup>[19]</sup>.

In this study, it has been noticed that pollution affected species diversity of zooplankton, the number of species in both influent and facultative pond was 10 and 8 species because of high pollution level, whereas in maturation pond increased to 21 species, due to the presence of low pollution levels. Also, pollution affected zooplankton density; pollution in anaerobic pond increased density of ciliates (Protozoa) which are known to be bio-indicators of organic pollution.

Rotaviruses were detected in 62.5% of influent of WSP. Villena *et al.*<sup>[33]</sup> found that rotaviruses were detected in 85.7% of raw sewage samples in wastewater treatment plants in Cairo. The variation in rotavirus percentage of frequency may be due to increasing of population in Cairo than the population in El-Moffi village. Another reason is that the influent of WSP in this study was the effluent of septic tanks.

The reductions of infectious rotaviruses during WSP processes in this study were 1-2  $\log_{10}$  units after anaerobic pond, one  $\log_{10}$  after facultative pond and 0-1  $\log_{10}$  after maturation pond. From these results, it can be observed that WSP failed to realize complete removal of infectious rotaviruses. It may be due to the resistancy of rotaviruses to treatment processes. El-Senousy *et al.*<sup>[11]</sup> reported that rotaviruses were the most resistant RNA enteric viruses to an activated sludge treatment processes. In this study, the identical sequences of rotaviral VP6 detected in final maturation effluent and in water drain samples after discharge point and after 700 meters of discharge point in January 2006 showed the role of WSP in contaminating drain water with infectious rotaviruses. The absence of infectious rotaviruses before discharge point confirmed this conclusion. Sequencing of amplified products of viral (pathogen) genome may be an evidence to prove the source of contamination of drain water which receives effluents of wastewater treatment plants.

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