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Bacteriophages in Engineered Wetland for Domestic Wastewater Treatment

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Abstract: The use of alternative domestic wastewater treatment technologies such as constructed wetlands has gradually developed over the past 20 years. The present study aims at investigating a type of short-deep treatment constructed wetland beds BIOWATSYST to treat wastewater. Pathogenic bacteria and viruses are the most serious elements that contaminate domestic wastewater. The present study reveals that the BIOWATSYST system had a moderate efficiency to remove the load of pathogenic bacteria from the influent as *Salmonella* sp. (48%), *Shigella* sp. (52%), *Vibrio* sp. (49%) and *Pseudomonas* sp. (49%). Coliphages against *Escherichia coli* (RRL-3704) were isolated from the inlet and outlet water collected from the BIOWATSYST. The mean counts of these coliphages were 1357.5 pfu mL^{-1} in the influent, while their mean counts in effluents were 628.7 pfu mL^{-1}. It was noticed that the mean bacteriophage counts of the influent against *E. coli*, *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *Pseudomonas* sp. isolates were in the range of (7-75 pfu mL^{-1}). Also, the mean bacteriophage counts of the influent against *Salmonella typhimurium* (NCMB 74), *Shigella boydii* (ATCC 9207), *Vibrio* sp. and *Pseudomonas aeruginosa* (NCMB 8295) were in the range of (7-60 pfu mL^{-1}). Very low counts of bacteriophages against bacterial isolates and bacterial test strains in effluents of all treatment beds were observed. Bacteriophages as a component of engineered wetlands received attention in the current study as indicators of pollution. When comparing somatic coliphages with classic bacterial indicators, it was noticed that there was a highly significant positive correlation between coliphages and all these groups of bacteria. These results may present confidant in the usage of coliphages as pollution indicator for secondary treated domestic wastewater. Furthermore the Addition of mixture of bacteriophages isolated from raw sewage resulted in the removal of 37% of fecal coliforms, while addition of high titer of coliphages resulted in the removal of 34%. Accordingly the ability these phages to eliminate their host pathogens from such systems is discussed.

Key words: Bacteriophages, constructed wetlands, wastewater treatment, pathogenic bacteria, faecal coliform

INTRODUCTION

Usage of non-conventional water resources such as treated industrial, agricultural or domestic wastewater are in part responsible for the re-emergence of waterborne pathogenic diseases, because partially-treated water can act as a vector for the spread of many potentially pathogenic microorganisms (Baggi *et al*., 2001). Therefore, highly efficient wastewater treatment systems are required to control the pathogens present in the wastewater, then treated effluents can be safely discharged to inland or coastal waters or reused for irrigation (Senzia *et al*., 2003). Constructed wetlands are popular alternatives for wastewater treatment in developing areas (Williams *et al*., 1997; Vymazal, 2005). They are becoming acceptable worldwide as they save money, energy and need the

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minimum maintenance requirements (Senzia *et al*., 2003). Applied research indicated that constructed wetlands significantly reduce suspended solids, oxygen depleting substances, organic matter, nutrients and most chemical and biological pollutants (Ojo and Mashauri, 1996; Mashauri *et al*., 2000; Nokes *et al*., 2003; Xia *et al*., 2006). The efficiency of the system in a semi-arid climate like Egypt has been reported (Butler and Dewedar, 1991; Abdulla, 1994; Elshatoury, 2001). The main weakness of this system was the required large land area. Therefore a Biological Wastewater Treatment System (BIOWATSYST) with short, deep treatment beds was developed as alternative design to assess the effectiveness of constructed wetlands as a low-technology and low-cost solution to domestic wastewater treatment.

Pathogenic bacteria present in the wastewater are difficult to detect and/or quantify. Current detection procedures are complex, time consuming and involve concentration processes and subsequent selective enrichment or amplification by molecular biology methods (Borrego and Figueras, 1997). Because of these limitations, certain groups of bacteria have been used as indicators for the possible presence of enteric pathogens in wastewaters. The most widely used groups are total coliforms, fecal coliforms and fecal enterococci (Polo *et al*., 1998; Baggi *et al*., 2001; Hodgson *et al*., 2004; Sleytr *et al*., 2007).

Several research groups supported the usage of bacteriophages as indicators of enteric viruses (Borrego *et al*., 1987; Thurston *et al*., 2001; Yousefi *et al*., 2004). Bacteriophages are similar to human viruses in composition, structure and morphology (Gantzer *et al*., 1998). In addition, bacteriophages are generally present in water environments whenever enteric viruses are present, they are present in more or less the same numbers of other viruses, they are highly specific for fecal pollution, they are generally as resistant as viruses to unfavourable conditions in the environment, they can not multiply in water environments without their bacterial hosts and they are non-pathogenic to human (Grabow, 2001). Since the presence of phages reflects survival of enteric viruses as well as sewage contamination, hence, phages could be considered as good indicators of microbiological water quality (Pepper *et al*., 1995).

Bacteriophages were recognized as epizootic infections of bacteria and were used to treat a bacterial infection, which is known as phage therapy (Summers, 2001). Phage therapy might be effective against broad range of human infections caused by members of the genera *Staphylococcus*, *Salmonella*, *Klebsiella*, *Escherichia*, *Proteus* or *Pseudomonas* (Alisky *et al*., 1998). Generally, the lethality and specificity of phages for particular bacteria and the ability of phages to replicate within infected animal/human hosts make them effective antibacterial agents (Duckworth and Gulig, 2002).

The aim of the present study is to assess the possible use of bacteriophages as a reliable indicator for the treatment process and investigating the capability of specific bacteriophages in the elimination of pathogenic enteric bacteria, that were previously isolated from the system, under laboratory conditions.

MATERIALS AND METHODS

The constructed wetland system (Fig. 1) was established and operated during 1998-2005 at an experimental station belonged to Suez Canal University at Ismailia, Egypt.

It consists of six-parallel treatment beds, three sterilization ponds and a disinfection pond. The detailed design of the system is published elsewhere (Dewedar *et al*., 2006). Six water samples were collected from the BIOWATSYST on monthly basis. The samples represent the primary treated wastewater (influent) sample and effluents of the five treatment beds. Water samples were collected in two replicates of clean, wide-mouthed, plastic bottles. One bottle was used for physicochemical analyses in which turbulence was carefully avoided. The second bottle was used for microbiological analyses. Samples were stored in an ice box while transported to the Laboratory. The water quality

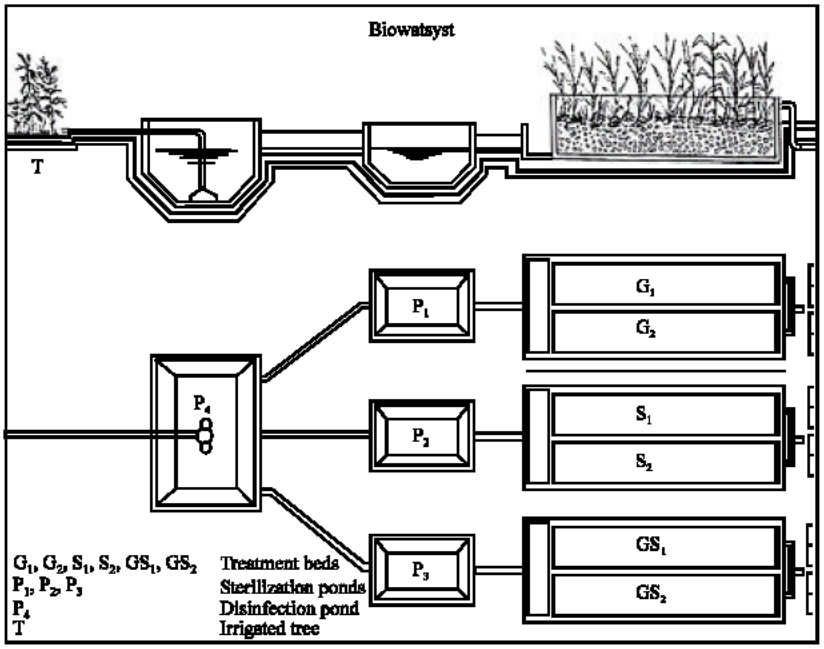

Fig. 1: Schematic representation of the BIOWATSYST that illustrates the arrangement of six-parallel treatment beds (G_1, G_2, S_1, S_2, GS_1 and GS_2), three sterilization ponds (P_1, P_2 and P_3) and final disinfection pond (P_4)

of both influent and effluents of the treatment beds was monitored according to the Greenberg *et al*. (1985 and 1992). This was carried out through determination of various physicochemical and microbiological parameters the latter including fecal coliforms (FC) on m-Endo agar, *Pseudomonas* sp. on *Pseudomonas* selective agar base, *Salmonella* sp., *Shigella* sp. on SS agar (Biolife)) and *Vibrio* sp. on TCBS agar (Difico).

Bacteriophages Isolation

In the present study different techniques of bacteriophage concentration, various enrichment media and different dilutions of bacteriophage suspension were examined to select the most suitable method for coliphage count (pfu mL^{-1}) as described in the following section. Experiments were done in triplicates.

Concentration of Bacteriophages

Two different techniques were used for bacteriophage concentration from wastewater samples.

- **Filtration:** Disposable filter holders with two different diameters (0.2 and 0.45 μm) were used to concentrate bacteriophages from 10 mL water samples and the filtrates containing somatic coliphages were stored at 4°C until used. Counts (pfu mL^{-1}) of bacteriophages were compared in order to select the best filter diameter.
- **Centrifugation:** Ten milliliter of wastewater was centrifuged (Jouan E96) at 4000 x g for 10 min 0.5 mL chloroform was added to the supernatant. The sample was then shaken by a mechanical shaker for 30 sec and then left to settle at 4°C until phase separation was completed. Tubes containing phages were stored at 4°C.

The agar-overlay technique has been used for the determination of the coliphage count according to Beishir (1996), Stukus (1997) and Wistreich (1997).

Isolation and Purification of Specific Bacteriophages

Agar-overlay technique was proceeded to isolate specific phages by using overnight broth culture of its own species of host bacteria as follows: coliphages were isolated by using an *Escherichia coli* (NRRL B-3704) or wild *E. coli* isolate, *Pseudomonas* phages were isolated by using a *Pseudomonas aeruginosa* (NCMB 8295) and a *Pseudomonas* sp. isolate, *Salmonella* phages were isolated by using a *Salmonella Typhimurium* (NCMB 74) or a *Salmonella* sp. isolate, *Shigella* phages were isolated by using a *Shigella boydii* (ATCC 9207) or a *Shigella* sp. isolate and vibriophages were isolated by using *Vibrio* sp. isolates. The isolated plaques have been purified according to Carlson and Miller (1994).

Role of Coliphages in the Elimination of *E. coli* under Laboratory Condition

A bacteriological method was used to demonstrate the role of coliphage in the elimination of an *E. coli* isolate in raw sewage. The steps were as follows:

- A set of 12 flasks each containing 50 mL of wastewater sample was divided into 6 groups in this experiment.
- The first group was inoculated with 1 mL of mixed bacteriophage suspension (~ 3×10^4 pfu mL^{-1}).
- The second group was inoculated with 1 mL of purified coliphage suspension (P_1) previously isolated from the studied system (~ 2.4×10^4 pfu mL^{-1}).
- The third group was inoculated with 1 mL of another purified coliphage suspension P_2 (~ 1.2×10^4 pfu mL^{-1}).
- The fourth group was inoculated with 1 mL of a mixture of the above two purified coliphage suspension $P_1 + P_2$ (~ 7.5×10^4 pfu mL^{-1}).
- The fifth group was inoculated with 1 mL of a suspension of high coliphage titre against *E. coli* isolate (100×10^2 pfu mL^{-1}).
- The last group was left without any addition as control.
- The flasks were incubated at 37°C with shaking.
- Counts of faecal coliforms were determined using pour plate method on m-Endo agar in samples taken at intervals of 24, 48 and 72 h.

Antibacteriophage Activity of Treatment Plants

In order to test the effect of plant materials on the counts of bacteriophages, an anti-bacteriophage assay was developed (Khafagi *et al*., 2003). Aqueous extracts prepared from both shoot and root systems of actively grown *Phragmites australis* plants (common reed) that were collected from the BIOWATSYST were prepared. Onto three small rectangular carpets of the *E. coli* (NRRL B-3704), three drops of bacteriophage suspension were added; one with a drop of an aqueous extract of common reed shoots, the second with drop of aqueous extract of the rhizome of common reed and the last drop of phage suspension was added as a control. Plates were incubated overnight at 37°C and then examined.

RESULTS

The present study was carried out on five only of the six BIOWATSYST treatment beds. A number of physicochemical and microbiological parameters were studied in four seasons over one year to evaluate the performance of the system for treating domestic wastewater these results are published elsewhere (Dewedar *et al*., 2006). Counts of coliphages, specific bacteriophages and pathogenic bacteria were traced over one year in influent and effluents of various treatment beds of the system.

Table 1: Mean Counts of different bacterial groups in the inlet and outlet water of the BIOWATSYST over one year of the study

Bacterial groups (cfu mL^{-1})	Fecal coliform	*Salmonella*	*Shigella*	*Vibrio*	*Pseudomonas*
In	6805	230	603	1445	1852
Out	3200	117	301	590	1071

Table 2: Mean Counts of bacteriophages in the inlet and outlet water of the BIOWATSYST over one year of the study using wild strains previously isolated from the system

Bacterial group (cfu mL^{-1})	*E. coli*	*Salmonella*	*Shigella*	*Vibrio*	*Pseudomonas*
In	51	15	30	35.0	28
Out	31	1	12	11.5	15

Faecal Coliforms (FC)

Mean FC counts of the influent throughout the study period were 6.8×10^3 cfu mL^{-1} while the effluents mean counts were 3.2×10^3 cfu mL^{-1} (Table 1).

Counts of Pathogenic Bacteria (cfu mL^{-1})

***Salmonella* sp.**

Counts of *Salmonella* sp. in the influent ranged from 70 to 5.5×10^2 cfu mL^{-1} throughout the study period. The results obtained show that mean counts were 2.3×10^2 and 1.2×10^2 cfu mL^{-1} for influents and effluent, respectively (Table 1).

***Shigella* sp.**

Counts of *Shigella* sp. in the influent ranged from 2.5×10^2 to 1.2×10^2 cfu mL^{-1} throughout the study period. *Shigella* mean counts mean counts were 6×10^2 and 3×10^2 cfu mL^{-1} for influents and effluent, respectively (Table 1).

***Vibrio* sp.**

Counts (cfu mL^{-1}) of *Vibrio* sp. present in the influent ranged from 4.1×10^2 to 2.5×10^2 cfu mL^{-1} throughout the study period. Mean counts of the influent was 1.4×10^3 while mean counts of the effluents were 6×10^2 cfu mL^{-1}.

***Pseudomonas* sp.**

Mean counts of the *Pseudomonas* sp. in the influent were 1.8×10^3 cfu mL^{-1} while mean counts of the effluents were 1×10^3 cfu mL^{-1}.

Counts of Bacteriophages

Bacteriophages Against Some Pathogenic Bacterial Isolated from the System

Two different techniques (filtration and centrifugation) were used for bacteriophage concentration from wastewater samples. Lower counts were obtained with centrifugation method. Results of the total counts (pfu mL^{-1}) of coliphages showed that the 0.2 μm filter could be used for bacteriophage concentration in all subsequent phage assays. The mean bacteriophage counts of the influent against different wild hosts (previously isolated from the system) *E. coli*, *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *Pseudomonas* sp. were 35, 15, 30, 35 and 28 cfu mL^{-1}, respectively. Generally, counts (pfu mL^{-1}) of specific bacteriophages against *E. coli*, *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *Pseudomonas* sp. isolates were decreased in all the effluents of the treatment beds compared to that of the influent as counts were 31, 1, 12, 11.5 and 15, respectively (Table 2).

Bacteriophages Against Bacterial Type Cultures

The mean bacteriophage count of the influent against *E. coli* (NRRL B-3704) was 1.4×10^3 cfu mL^{-1} and the effluent mean count was 5.6×10^2 pfu mL^{-1}. The mean bacteriophage counts

Table 3: Mean Counts of bacteriophages in the inlet and outlet water of the BIOWATSYST over one year of the study using type strains *Escherichia coli* (NRRL B-3704) *Salmonella Typhimurium* (NCMB 74), *Shigella boydii* (ATCC 9207), *Vibrio* sp, *Pseudomonas aeruginosa* (NCMB 8295)

Bacterial group (pfu mL^{-1})	*E. coli*	*Salmonella*	*Shigella*	*Vibrio*	*Pseudomonas*
In	1360	18.0	35	45	35
Out	556	12.4	17	18	20

Table 4: Counts of Coliphage (pfu mL^{-1}) after addition of *Phragmites australis* shoot and rhizome aqueous extracts compared with control (without addition)

Bacterial counts	Control	Extract of shoot	Extract of rhizome
Coliphage (pfu mL^{-1})	788	146	153

Table 5: Correlation matrices between somatic coliphage counts, bacterial indicators and pathogenic bacteria monitored in water samples collected from the system throughout the study (n = 24)

Bacterial counts	Coliphage	TC	FC	FE	*Pseudomonas*	*Salmonella*	*Shigella*	*Vibrio*
Coliphage	1.000	0.950	0.818	0.982	0.974	0.824	0.892	0.826
TC	0.950	1.000	0.763	0.950	0.943	0.838	0.892	0.823
FC	0.818	0.763	1.000	0.805	0.816	0.807	0.800	0.823
FE	0.982	0.950	0.805	1.000	0.990	0.837	0.883	0.847
Pseudomonas	0.974	0.943	0.816	0.990	1.000	0.894	0.928	0.904
Salmonella	0.824	0.838	0.807	0.837	0.894	1.000	0.963	0.984
Shigella	0.892	0.892	0.800	0.883	0.928	0.963	1.000	0.960
Vibrio	0.826	0.823	0.823	0.847	0.904	0.984	0.960	1.000

TC: Total Coliform, FC: Faecal Colifrom, FE: Faecal Enterococci

of the influent against *Salmonella typhimurium* (NCMB 74), *Shigella boydii* (ATCC 9207), *Vibrio* sp. and *Pseudomonas aeruginosa* (NCMB 8295) were 18, 35, 45 and 35 pfu mL^{-1}, respectively (Table 3).

In all treatment beds, the counts of bacteriophages against bacterial type cultures decreased in the effluents of the treatment beds than that in the influent entered the treatment system where counts of 12.4, 17, 18 and 20 pfu mL^{-1} were recorded using *Salmonella typhimurium* (NCMB 74), *Shigella boydii* (ATCC 9207), *Vibrio* sp. and *Pseudomonas aeruginosa* (NCMB 8295), respectively as bacterial hosts (Table 3).

Antibacteriophage Activity

The antibacteriophage bioassay was performed using coliphage against *E. coli* (NRRL B-3704) in the direct plaque assay (Ackermann and DuBow, 1987) which may also indicates the antiviral activity of aqueous extracts of *Phragmites australis* shoot and rhizome. Results in Table 4 showed the antibacteriophage activity of both shoot and rhizome aqueous extracts of *P. australis*. About 8×10^2 pfu mL^{-1} phage counts were recorded against *E. coli* (NRRL B-3704) strain. However, the addition of aqueous extracts of both shoot and rhizome of *P. australis* decreased the plaque counts to 146 (shoots) and 153 (rhizome) pfu mL^{-1}.

Correlation Between Coliphages and Bacterial Indicators

When comparing coliphages with bacterial indicators, it was noticed that there was a high positive correlation between coliphages and fecal coliforms (0.818) (Table 5).

Correlation Between Coliphages and Pathogenic Bacteria

Coliphages showed a high positive correlation when compared with some pathogenic bacteria: *Salmonella* sp. (0.824), *Shigella* sp. (0.892), *Vibrio* sp. (0.826) and *Pseudomonas* sp. (0.974) (Table 5).

Role of Coliphages in the Elimination of *E. coli*

The percentage removal of fecal coliforms by bacteriophage suspension, previously filtered from raw sewage, ranged from 31.86 to 36.89% (Fig. 2). While, the percentage removal of fecal

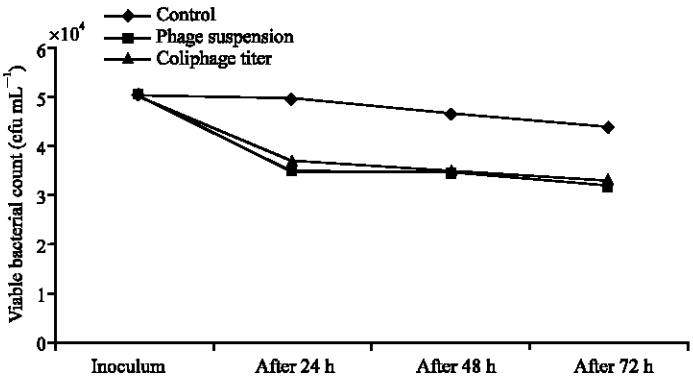

Fig. 2: Counts of *E. coli* colonies (cfu mL^{-1}) after 24, 48 and 72 h incubation from the addition of bacteriophage suspension and high titre of coliphage against *E. coli* wild isolate in 50 mL of the influent

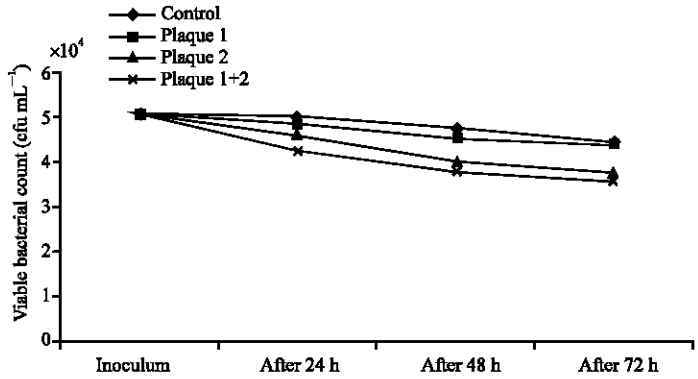

Fig. 3: Counts of *E. coli* colonies (cfu mL^{-1}) after 24, 48 and 72 h incubation from the addition of enriched coliphage plaque P1 against *E. coli* (NRRL B-3704), another enriched coliphage plaque P2 and the mixture of the two enriched coliphage plaques P1+P2 to 50 mL of the influent

coliforms by high titre of coliphages prepared using *E. coli*, isolated from the system, as a host was 26.97-34.25% (Fig. 2).

Synergistic Effect of Mixed Coliphages

High titre of coliphages (P1 and P2) against *E. coli* (NRRL B-3704) were used separately and in combination to investigate the role of coliphages coassociation in the elimination of *E. coli* present in the raw sewage (Fig. 3).

It was noticed that the enriched coliphage P1 reduced counts of fecal coliforms to 3.84 % after 24 h reached to 14.0% after 72 h. The another coliphage P2 reduced counts of fecal coliforms to 9.04% after 24 h reached to 25.44% after 72 h. On the other hand the mixture of P1+P2 reduced counts of faecal coliforms to 16.59% after 24 h reached to 30.29% after 72 h.

DISCUSSION

Biological treatment systems using constructed wetland are by far natural alternative to conventional treatment systems that needs no sophisticated operation or maintenance facilities. Higher

plants, microbial biofilm built around plant roots and gravel particles as well as bacteriophages are the main possible contributors to the treatment processes.

The main scope of the present study was to evaluate the reliability of the bacteriophages as pathogen eliminator and as indicator for the water quality and consequently for the treatment efficiency of the system under investigation.

Pathogenic bacteria and viruses are the most serious elements that contaminate domestic wastewater (Borrego and Figueras, 1997). In the present study, the results showed that the BIOWATSYST has ability to reduce pathogenic bacteria (e.g., *Salmonella*, *Shigella*, *Vibrio* and *Pseudomonas*) with a removal efficiency of about 50%. Similar results were obtained in the study of Abdulla (1994) who showed that *Salmonella* counts highly decreased down the first 18 m of the GBH treatment bed from 965 to 30 cfu mL^{-1} (96% reduction). The role of bacteriophages in the elimination of pathogenic bacteria present in wastewater received very little attention (Müller, 1980), though they are direct enemies of their host bacteria (Kudva *et al*., 1999; Summers, 2001). Bacteriophages were used extensively in the 1950's and 1960's for the control of human and animal infections produced from bacterial diseases (Smith *et al*., 1987; Berchieri *et al*., 1991; Park *et al*., 2000; Huff *et al*., 2002).

As bacteriophages are usually associated with their host bacteria, phages were isolated from water samples collected from the BIOWATSYST treatment beds in variable numbers. Very low counts of bacteriophages against host bacteria isolated from the system and bacterial type strains in effluents of all treatment beds were observed. Reduction percentages only reached up to 54%.

Several studies traced the presence of bacteriophages in constructed wetlands for wastewater treatment systems. Thurston *et al*. (2001) reported that the influent of the wetland system contained 2.5×10^2 pfu mL^{-1} and the effluent contained 4.7 pfu mL^{-1} with reduction efficiency of 95.2%. Furthermore, Hench *et al*. (2003) reported that the influent of the wetland mesocosm contained 16×10^2 pfu mL^{-1} and the effluent contained 31.6 pfu mL^{-1} with reduction efficiency of 98%. However, in the study of Abdulla (1994), results revealed that the coliphages mean counts decreased from 400 to 207 pfu mL^{-1} after 18 m distance in the GBH treatment bed with reduction 40%. These low counts of bacteriophages may be due to the antibacteriophage activity of common reed shoots and rhizomes (Khafagi *et al*., 2003). In the present study, the results revealed that about 8×10^2 pfu mL^{-1} coliphage counts were recorded against *E. coli* (NRRL B-3704) strain. However, addition of aqueous extracts of either shoot or rhizome of *Phragmites australis* decreased the plaque counts to 146 and 153 pfu mL^{-1} for shoots and rhizomes, respectively. This may reflect the effect of *in situ* secondary metabolites produced from macrophytes on the bacteriophage load found in the treatment system or may be the effect on the host itself.

The macrophyte *Phragmites* is not only known for its phytoremediation potential of pollutants in wastewater but also proved to have antibacteriophage properties associated with its polyphenolic compounds (Tsitsa-Tzardi *et al*., 1990). This activity was also detected in the marine plant Avicennia marina (Khafagi *et al*., 2003), which is proven to have high phenolic metabolism.

Enrichment of bacteriophages and its usage in the elimination of their corresponding host bacteria was examined in this study. Somatic coliphage plaques against *E. coli* (NRRL B-3704) appeared to have various morphological distinctions. One of the main differences is the variation in the plaque diameters. Generally, it was noticed that various phages in a heterogeneous load in a wastewater sample (mixed bacteriophage suspension) influence the pathogenic bacterial counts more efficiently than through using pure phage. Bacteriophage specific activities that may be associated with morphologically different distinctions were interesting behaviour to examine in the laboratory. Similarly, Kudva *et al*. (1999) found that mixture of three specific phages was more efficient in the lysis of host bacteria than the pure phage.

On the other hand, bacteriophages as a component of engineered wetlands received some attention as indicators of pollution (Borrego and Figueras, 1997; Abid and Samwuel, 1999; Grabow, 2001;

Thurston *et al*., 2001; Tanji *et al*., 2002). When comparing somatic coliphages of the BIOWATSYST with some classic bacterial indicators, it was noticed that there was a highly significant positive correlation between coliphages and those groups. Furthermore, coliphages showed a highly significant positive correlation when compared with some pathogenic bacteria: *Salmonella* sp. (0.824), *Shigella* sp. (0.892), *Vibrio* sp. (0.826) and *Pseudomonas* sp. (0.974). These results may present confidant in the usage of coliphages as pollution indicator for secondary treated domestic wastewater. This finding supported by the work of Borrego *et al*. (1987) who studied the relationship between coliphage and both their bacterial hosts and pathogenic bacteria in three different aqueous ecosystems (seawater in the vicinity of sewage outfalls, river water contaminated by domestic and industrial sewage discharges and estuarine water) in Spain. They found that coliphages are highly correlated to both their bacterial host and pathogenic bacteria and suggested that coliphages are better indicators of faecal pollution than the classic indicators. Also, Tanji *et al*. (2002) studied the fate of coliphage in wastewater treatment system in the central part of Japan. They concluded that coliphages could be used as wastewater treatment indicators. In conclusion bacteriophages are seems to be reliable indicators for the efficiency of the treatment process in the constructed wetlands and they may have a noticed ability in the pathogenic removal process.

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