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Efficiency of the Biological Waste Water Treatment System in Pollution Control and Wastewater Management

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ABSTRACT

Water, gravel, sand, rhizosphere and rhizoplane samples, were collected seasonally for over one year from treatment beds in the BIOWATSYST treatment station. Samples were, subjected to microbiological and physicochemical analyses. Results showed that the maximum counts of total viable bacteria (TVB), actinomycetes (ACTI), total coliform (TC), fecal coliforms (FC) and fecal streptococci (FS) were 11.2 x 10^6, 37.5 x 10, 25.6 x 10^3, 14.6 x 10^3 and 14.9 x 10^3 cfu/mL, respectively. The efficiency of the BIOWATSYST beds to remove some heterotrophic bacterial indicators such as total coliforms, fecal coliforms and fecal streptococci through the treatment process was reached to reduction percentages of 91.3%, 52%, 48.7%, 65.2% for TVB, TC, FC and FS, respectively during the period of study. Also, the values of some physico-chemical parameters were decreased in the ratio of biochemical oxygen demand 70%, chemical oxygen demand 61.8%, ammonia 21.2%, total nitrogen 8.7%, total suspended solids 62.7%, total dissolved solids 26.2% and organic matter 56.8%. However, there was an increase in dissolved oxygen in ratio of 79.4% and oxidized nitrogen 67.6%. Little variations in values of calcium and orthophosphate were recorded between influent and effluents water of the treatment beds during the studied seasons. Coarse sand was recorded as the most efficient filling material.

Key Words: Wastewater treatment; Bacteria; Actinomycetes; Physico-chemical

INTRODUCTION

Constructed wetland is defined as a wetland specifically constructed for the purpose of pollution control and waste management, at a location other than existing natural wetlands. Subsurface Flow (SF) wetlands are the commonly used type of constructed wetland that are characterized by the growth of emergent plants using soil, sand, gravel, or rock as a growth substrate in a lined channel or bed. Within the bed, facultative microorganisms were attached to the media and plant roots by contacting the wastewater that flows horizontally or vertically through the bed. The treatment in constructed wetlands depends primarily on the activity of microorganisms to decompose organic substances (Butler *et al*., 1993; Hatano *et al*., 1994).

Hatano *et al*. (1993) showed that vegetation in submerged constructed wetland beds, which received domestic wastewater, played an important role in the propagation of aerobic bacteria especially actinomycetes, which have diverse decomposition activities on organic substances. Heterotrophic bacteria play an active role in the sewage water treatment systems, which decompose chitin, pectin, lignin, starches, cellulose, hemi-cellulose, proteins and organic matter containing biosolids. They have the ability to reduce phosphate, nitrogen, sulfur, organic matter, chemical oxygen demand (COD) and biological oxygen demand (BOD). Some bacteria also have the ability to convert nitrogen gas (N_2), into ammonia (NH_3), a form to be absorbed by plant roots (Kutzner & Mahro, 1996; Doddamani & Ninnekar, 2000). Cooper and Findlater (1990) stated that aerobic and anaerobic microbial degradation as well as sedimentation are known to contribute to BOD removal. The coarse media beds act primarily as filters with most BOD in settling the suspended solids.

The present study aimed to assess the efficiency of the biological and physico-chemical treatment processes and to evaluate the interactions between bacterial populations and other variables in that system including the main Physico-chemical parameters.

MATERIALS AND METHODS

A- Sampling. The biological wastewater treatment system (BIOWATSYST) consists of 3 pairs treatment planted beds each of them have dimensions of 20.0 m x 2.5 m x 1.0 m and filled with different filling materials. Three beds were, selected for the present study, each of them representing a different filling material, gravel bed (B.2), sand bed (B.4) and gravel/sand bed (B.6). The beds were, planted with *Phragmites australis*. Water, gravel, sand and root samples were collected seasonally from the selected beds (B.2, B.4, & B.6) for over one year. Sewage influent was, adjusted to feed into planted beds at a flow rate of 10 L/minute for 16 h/day. The samples were, collected according to the method described by Clark (1965) and Wollum (1982).

B- Microbiological analyses. Microbiological analysis was,

carried out on Influent and effluents water, gravel, sand, rhizosphere and rhizoplane samples according to methods described by Wollum (1982). A decimal dilution series was prepared up to 10^{-3}. Culture media for enumeration of the bacterial groups have been developed from the methods described in American Public Health Association, APHA (1985). 1 mL of the suitable dilutions was, plated on to triplicates of appropriate agar medium. Spread plate technique was used for enumeration of actinomycetes on Starch Casein Agar (Kuster & Williams, 1964) amended with cyclohexamide (0.05 g/l), to inhibit fungal growth. Plates were, incubated at 28 and 55°C for the mesophilic and thermophilic actinomycetes, respectively for 3 - 7 days. Pour plate technique was used for enumeration of the heterotrophic bacteria. Total viable bacterial count was carried out on plate count agar medium (APHA, 1985) at 30°C for 24 - 36 h, total and fecal coliforms on m-endo agar medium (APHA, 1985) at 37 and 44.5°C, respectively for 24 - 36 h. Fecal streptococci was enumerated using m-enterococcus agar medium (APHA, 1985) at 28°C for 24 - 36 h. All bacterial counts were enumerated using electrode plate counter (Fisher M 133 - 8002A).

C- Physico-chemical analyses. Physico-chemical analyses included temperature (°C), hydrogen ion concentration (pH), dissolved Oxygen (DO) mg L^{-1}, biochemical oxygen demand (BOD) mg L^{-1}, chemical oxygen demand (COD) mg L^{-1}, ammonia (NH_3) mg L^{-1}, oxidized nitrogen (Ox.N) mg L^{-1}, total nitrogen (TN) mg L^{-1}, orthophosphate (PO_4) mg L^{-1}, calcium (Ca^{+2}) mg L^{-1}, total suspended solids dried at 103 - 105°C (TSS) mg L^{-1}, total dissolved solids dried at 180°C (TDS) mg L^{-1}, fixed and volatile solids (organic matter); ignited at 550°C (OM) mg L^{-1}; were carried out according to the methods described by APHA (1985).

E- Statistical analyses of data. The data were performed using data analysis tool of Microsoft Excel XP and were, presented in terms of frequency, percentage, mean and standard errors using data analysis tool of SPSS version 11. Analysis of variance (ANOVA) was used to test the significance between variables at $p < 0.05$. These statistics were, also performed using data tool of SPSS version 11. The interactions between studied bacterial groups and physico-chemical parameters in the BIOWATSYST system were, further studied using Canonical variate analysis (CVA) using SPSS version 11. This test calculates the variable weightings that maximize the differences between the groups of data. These weightings (Canonical coefficients) allow the identification of variables that are more correlated (Randerson, 1993). P-value was set at < 0.05 for significant results and < 0.01 for highly significant results. Pearson's correlation coefficient (r) value was, applied to evaluate the strength of the relation between the studied variables.

RESULTS

Distribution of heterotrophic bacteria. The differences in distribution of total viable bacteria (TVB), actinomycetes (ACTI), total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) are given in Table I. The highest count was reported for TVB (39.7×10^5 cfu/g) from rhizosphere sample in sand bed (B.4) and the lowest count was (3.3 cfu/g) for FC from rhizoplane sample in sand bed (B.4). Result also, proved the complete absence of some bacterial groups; TC, FC and FS in some gravel, sand, rhizosphere and rhizoplane samples. These differences in counts were of high statistical significance ($p < 0.01$).

Seasonal variation of heterotrophic bacteria. The seasonal variation of total viable bacteria (TVB), actinomycetes (ACTI), total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) are given in Table II. For TVB, counts in influent water were always higher than that of effluent water of beds no. 2, 4 and 6 during the four seasons, which ranged from 11.2×10^6 cfu/mL in influent water during autumn to 11.7×10^4 cfu/mL in effluent water during summer. These differences were statistically significant ($p < 0.05$).

Counts of TC in influent water were, ranged from 25.6×10^3 cfu/mL in spring to 37.1×10^2 cfu/mL in autumn. These counts decreased in effluent water of beds no. 2, 4 and 6. The highest count (14.3×10^3 cfu/mL) was isolated from effluent water of bed no.2 during winter, while the minimum one (24.7 x 10 cfu/mL), was detected during autumn from effluent water of bed no.4. There were significant differences in counts of TC between influent and effluent water of beds no.2, 4 and 6 during the studied seasons ($p < 0.05$).

Counts of FC in influent water were, ranged from 14.6×10^3 in winter to 25.9×10^2 in autumn. These counts decreased in effluent water of the treatment beds. The highest count (12.7×10^3 cfu/mL) of FC was, recorded in effluent water of bed no.2 during spring, while the lowest one was (61.8 x 10 cfu/mL) in bed no.4 during summer.

Counts of FS in influent water were higher than that detected in effluent water of beds no. 2, 4 and 6 ($p < 0.05$), where the maximum count was, detected in winter (14.9×10^3 cfu/mL) and the minimum one was, detected in autumn (23.8 x 10 cfu/mL). The differences were highly significant ($p < 0.01$). In effluent water, the highest count was 80×10^2 in bed no. 2 during winter, while the lowest one was 30.8 cfu/mL in bed no.4 during autumn.

In general, results showed that the removal of the bacterial indicators was, done in sand bed (B.4) at rate higher than that of gravel/sand bed (B.6) and gravel bed (B.2).

Physico-chemical characters of the treatment beds in the BIOWATSYST. The seasonal variation of Physico-chemical parameters are given in Table III. Result showed that there was, significant differences between values of temperature in influent and effluent water of beds no.2, 4 and 6 during the studied seasons. The values ranged from 29.67°C in summer to 17.17°C in winter. There are no significant differences of temperature values within the same season between influent and effluent water of beds no.

Table I. Mean count (cfu/ml; g) of heterotrophic bacteria isolated from influent water, effluent water, gravel, sand, rhizosphere and rhizoplane samples

Sample	TVB	ACTI.	TC	FC	FS
Influent	18.8×10^5	28.4 x 10	57.8×10^2	37.8×10^2	15.5×10^2
Effluent B.2	35.7×10^4	20.1 x 10	46.2×10^2	22.5×10^2	10.9×10^2
Effluent B.4	15.7×10^4	83.8	13.3×10^2	61.8 x 10	30.3 x 10
Effluent B.6	11.7×10^4	70.5	10.0×10^2	48.1 x 10	27.1 x 10
Gravel B.2	41.7×10^4	52.2	NIL	NIL	30.0 x 10
Gravel B.6	91.0×10^4	10.3×10^2	NIL	NIL	66.0
Sand B.4	15.5×10^5	11.7×10^3	NIL	NIL	28.7×10^2
Sand B.6	56.6×10^3	30×10^2	33.0	NIL	10.0 x 10
Rhizosphere B.2	70.0×10^4	16.3×10^3	NIL	NIL	27.7×10^2
Rhizosphere B.4	39.7×10^5	50×10^2	23.3 x 10	NIL	60.0×10^2
Rhizosphere B.6	27.0×10^4	51.3×10^3	NIL	NIL	NIL
Rhizoplane B.2	48.0×10^3	11.3×10^2	NIL	NIL	NIL
Rhizoplane B.4	37.0×10^4	80.1 x 10	10.0 x 10	3.3	13.3 x 10
Rhizoplane B.6	19.7×10^3	37×10^2	NIL	NIL	NIL

Table II. Mean count (cfu/ml) of heterotrophic bacteria isolated from influent water and effluent water of beds no.2, 4 and 6 in the BIOWATSYST during four seasons

Season	Sample	TVB	ACT.	TC	FC	FS
Autumn	Influent	11.2×10^6	37.5 x 10	37.1×10^2	25.9×10^2	23.8 x 10
	Effluent B.2	27.3×10^4	12.5 x 10	10.7×10^2	75.9 x 10	48.1
	Effluent B.4	33.7×10^4	69 x 10	24.7 x 10	66.6 x 10	30.8
	Effluent B.6	12.8×10^4	36.8	97.5 x 10	74.8 x 10	63.1
Winter	Influent	21.2×10^5	39.4	21.7×10^3	14.6×10^3	14.9×10^3
	Effluent B.2	47.7×10^4	7.72	14.3×10^3	92.0×10^2	80.0×10^2
	Effluent B.4	31.4×10^4	5.7	72.2×10^2	54.7×10^2	16.6×10^2
	Effluent B.6	30.2×10^4	6.6	13.1×10^3	71.0×10^2	73.1×10^2
Spring	Influent	14.2×10^5	10.9	25.6×10^3	12.0×10^3	93.5×10^2
	Effluent B.2	61.4×10^4	8.4	13.2×10^3	12.7×10^3	54.5×10^2
	Effluent B.4	33.0×10^4	7.8	11.9×10^3	57.2×10^2	57.5 x 10
	Effluent B.6	90.5×10^4	10.7	13.1×10^3	50.7×10^2	23.7×10^2
Summer	Influent	18.8×10^5	28.4 x 10	57.8×10^2	37.8×10^2	15.5×10^2
	Effluent B.2	35.7×10^4	20.1 x 10	46.2×10^2	22.5×10^2	10.9×10^2
	Effluent B.4	15.7×10^4	83.8	13.3×10^2	61.8 x 10	30.3 x 10
	Effluent B.6	11.7×10^4	70.5	10.0×10^2	48.1 x 10	27.1 x 10

2, 4 and 6 ($p > 0.05$). Values of pH in influent water were in the neutral range all over the study period (6.91 - 7.4). In effluent water the values ranged from 8.00 in bed no.2 during winter to 7.24 in bed no.6 during summer. There were no significant differences of pH levels between influent and effluent water of beds no.2, 4 and 6 ($p > 0.05$). Also there were no statistically significant differences between values of phosphorus in influent and effluent water of beds no.2, 4 and 6 within the same season ($p > 0.05$). In influent water the values of phosphorus ranged from 1.31 mg L^{-1} in summer to 0.57 mg L^{-1} in winter ($p < 0.01$). In effluent water the values ranged from 1.55 mg L^{-1} in bed no.6 during summer to 0.52 mg L^{-1} in bed no.2 during winter ($p < 0.01$). There were no significant differences of calcium values in influent water during the studied seasons ($p > 0.05$) and the values ranged from 48.36 to 41.12 mg L^{-1}. In effluent water of beds no.2, 4 and 6 the maximum values ranged from 53.88 mg L^{-1} in bed no.2 during autumn to 38.92 mg L^{-1} in bed no.2 during summer. There were no statistically significant differences between values of calcium in influent and effluent water of beds no. 2, 4 and 6 during the studied seasons ($p > 0.05$).

Values of BOD in influent water ranged from 158.59 to 104.17 mg L^{-1}. These values were, reduced in effluent water of beds 2, 4 and 6. The values detected in effluent water ranged from 55.94 mg L^{-1} in bed no.2 during winter to 21.0 mg L^{-1} in bed no.4 during autumn. The maximum value of COD in influent water (783.33 mg L^{-1}) was detected in winter and the minimum one (520 mg L^{-1}) was detected in spring. These values were decreased in effluent water of beds no.2, 4 and 6 in which the maximum value (502.22 mg L^{-1}) was detected in bed no.2 during winter and the minimum value (85.11 mg L^{-1}), was detected in bed no.4 during spring ($p < 0.01$). In influent water, the maximum values of DO were (0.59 mg L^{-1}) in summer and the minimum value (0.06 mg L^{-1}) was in spring and these values increased in the effluent water of the treatment beds. The highest value (2.59 mg L^{-1}) was, detected in bed no.4 during summer to 0.33 mg L^{-1} during winter. Values detected from bed no.4 were higher than that detected from bed no.6 ($p = 0.022$).

Values of NH_3 in the influent water ranged from 31.48 mg L^{-1} in winter to 25.39 mg L^{-1} in autumn. These values decreased in the treatment beds, where the maximum

Table III. The physico-chemical analyses of water samples collected from the influent and effluent water during four seasons

Season	Sample	T °C		pH		PO_4 mg/l		Ca mg/l		BOD mg/l		COD mg/l		DO mg/l		NH_3 mg/l		Ox.N mg/l		TN mg/l		OM mg/l		TDS mg/l		TSS mg/l	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Autumn	Influent	24.00	1.95	7.40	0.24	1.07	0.28	45.15	2.98	104.17	16.97	582.22	20.72	0.50	0.2	25.39	0.39	0.88	0.13	29.14	0.67	36.20	3.88	1230.6	240.94	49.91	6.64
	Effluent B.2	23.50	1.88	7.48	0.28	0.75	0.2	47.47	2.08	29.67	4.7	166.78	28.6	2.13	0.27	19.98	2.23	1.56	0.36	25.12	0.86	20.50	2.66	639.67	46	22.00	2.09
	Effluent B.4	22.78	1.78	7.43	0.24	0.80	0.21	53.88	3.19	21.00	3.32	155.56	41	1.96	0.31	14.87	2.37	2.62	0.6	21.53	1.9	18.76	1.95	701.33	52	19.37	1.8
	Effluent B.6	22.67	1.92	7.51	0.22	0.83	0.21	44.19	3.25	30.28	6.31	252.00	26.4	1.73	0.32	19.58	1.55	1.76	0.3	24.99	1.72	17.09	1.97	726.22	96.46	17.18	1.85
Winter	Influent	18.26	0.72	7.60	0.16	0.57	0.15	48.36	3.05	129.83	14.96	783.33	40.28	0.50	0	31.48	1.34	0.27	0.12	32.09	1.21	38.11	5.02	597.58	105.48	49.00	7.24
	Effluent B.2	17.17	0.61	8.00	0.12	0.52	0.15	49.47	1.8	55.94	5.79	502.22	30.7	0.61	0.19	28.02	1.32	0.84	0.26	32.30	1.12	23.33	2.06	543.83	90	28.44	2.32
	Effluent B.4	17.17	0.69	7.78	0.13	0.55	0.17	48.92	2.3	52.12	7.29	426.67	36.1	0.33	0.14	26.84	1.26	0.93	0.21	30.05	1.23	18.11	2.15	540.52	91	21.78	2.22
	Effluent B.6	17.29	0.48	8.00	0.13	0.53	0.16	51.62	3.75	47.71	6.09	398.89	55.6	0.47	0.13	29.21	1.02	0.68	0.23	32.26	0.83	14.89	2.04	505.00	85.76	18.78	2.37
Spring	Influent	26.00	0.67	7.49	0.08	1.30	0.12	48.15	1.98	158.59	12.52	520.00	70.76	0.06	0.06	30.97	1.29	0.62	0.22	33.34	1.18	68.11	14	693.44	57.184	96.33	18.4
	Effluent B.2	25.00	0.47	7.58	0.1	1.44	0.06	42.81	2.18	53.01	5.54	202.62	40.1	0.56	0.13	24.80	1.45	2.53	0.85	36.80	1.34	22.22	1.99	628.11	41	26.33	2.16
	Effluent B.4	25.33	0.6	7.47	0.06	1.38	0.09	48.86	2.39	24.18	6.65	85.11	31.2	2.24	0.75	18.23	1.53	3.36	1.09	29.88	2.63	20.33	1.63	602.44	43	22.67	1.82
	Effluent B.6	25.56	0.69	7.54	0.05	1.31	0.08	47.43	1.79	44.66	4.79	124.56	34.9	1.16	0.38	26.79	1.5	1.62	0.43	33.16	2.22	20.44	2.23	652.22	49.42	23.44	2.32
Summer	Influent	29.39	0.26	6.91	0.06	1.37	0.05	41.12	2.55	130.93	8.66	680.56	30.03	0.59	0.18	27.84	1.02	0.47	0.12	30.43	0.89	44.33	1.35	817.78	142.45	54.22	2.49
	Effluent B.2	29.00	1.09	7.28	0.11	1.30	0.1	38.92	2.6	39.46	4.75	217.78	45.1	1.87	0.48	22.99	0.86	1.45	0.15	26.58	0.81	25.67	1.46	600.33	33	30.56	1.48
	Effluent B.4	28.78	0.15	6.94	0.12	1.43	0.08	41.15	2.62	28.80	4.59	193.89	62.8	2.59	0.4	16.42	0.58	2.61	0.62	21.46	0.87	21.44	0.85	638.00	37	25.33	1.13
	Effluent B.6	29.67	0.28	7.24	0.13	1.55	0.05	39.99	2.11	46.00	4.86	211.67	33.5	1.06	0.25	25.60	0.87	0.81	0.13	28.33	0.89	19.11	0.56	615.11	19.87	23.11	0.7

removal occurred in effluent water of bed no. 4 (14.87 mg L^{-1}) in autumn and the minimum removal was 29.21 mg L^{-1} in bed no.6 during winter. The differences were statistically significant ($p < 0.01$). The values of Ox.N in influent water ranged from 0.88 mg L^{-1} in autumn to 0.27 mg L^{-1} in winter. These values increased in beds 2, 4 and 6. This increase in effluent water of bed no.4 was higher than that occurred in effluent water of beds no. 2 and 6 ($p < 0.01$) during the studied season. Values of TN in influent water ranged from 33.34 mg L^{-1} in spring to 29.14 mg L^{-1} in autumn. These values reduced in the effluents water, where the reduction occurred in bed no.4 was higher than that occurred in beds no.2 and 6 during the studied season ($p < 0.01$).

In influent water values of organic matter (OM) ranged from 68.11 mg L^{-1} in spring to 36.20 mg L^{-1} in autumn. Values of OM in the influent water were higher than that of effluent water of beds no.2, 4 and 6 during all the studied seasons ($p < 0.01$). The values in effluent water ranged from 25.67 mg L^{-1} in bed no. 2 during summer to 14.89 mg L^{-1} in bed no.6 during winter. The value of total dissolved solids (TDS) in influent water ranged from 1230.56 mg L^{-1} during autumn to 597.58 mg L^{-1} during winter. The values in influent water during autumn were higher than that of effluent water of beds no.2, 4 and 6 ($p = 0.004, 0.009, 0.012$). In effluents water the maximum value (726.22 mg L^{-1}) of TDS was in bed no.6 during autumn and the minimum one (505 mg L^{-1}) was in bed no.6 during winter. In influent water, the values of total suspended solids (TSS) ranged from 96.33 mg L^{-1} in spring to 49.00 mg L^{-1} in winter. These values were, decreased in treatment beds no.2, 4 and 6, where the maximum value was 30.56 mg L^{-1} in bed no.2 during summer and the minimum one was 17.18 mg L^{-1} in bed no.6 during autumn.

Interactions between bacterial groups and other variables in the BIOWATSYST. The influence of physico-chemical parameters on distribution of bacterial populations was exploited by Canonical variate analysis (CVA). It is a generalized multiple regression that find linear combinations within each set of variables, that best explain the relation between them (Randerson, 1993). The analysis gave canonical correlations (Table VI; Fig. 1). The correlations suggest how the conditions in the wastewater may influence the distributed populations in the biofilm throughout the bed. From the test it could be concluded that there was positive associations between counts of TVB, TC, FC, FS and BOD, COD, NH3, TN, OM, TSS; and negative associations between these bacterial groups and DO, OX.N. In contrast actinomycetes showed negative associations with TVB, TC, FC, FS, BOD, COD, NH3, TN, OM, TSS and relatively high positive associations with DO, OX.N. and Ca

DISCUSSION

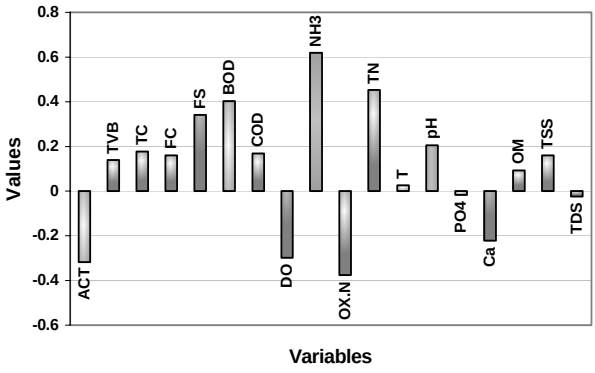
In many systems utilizing subsurface wetlands (SF), a single treatment bed is expected to accomplish many treatment goals. The removals of organic matter, solids, nitrogen, heavy metals and pathogens are all considered when designing a particular system. The removal of each constituent is governed by different biological, physical and chemical processes (Brix, 1993). There is a direct proportional relation between the number and generic diversity of heterotrophic bacteria and the physical, chemical and biological features in the studied system (Jiang & Xu Appl, 1995).

The root biofilms represented the more favorable conditions for the growth of TVB and actinomycetes than gravel and sand biofilms. Yet, they were less dominant in water samples. The high counts of TVB and actinomycetes in the root zone more than other samples could be attached to plant roots exudates such as amino acids, simple sugars and organic acids that providing a continuous energy supply

Table VI. Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions, Variables ordered by absolute size of correlation within function

Variable	Function
Act.	-0.32
TVB	0.14
TC	0.18
FC	0.16
FS	0.34
BOD	0.40
COD	0.17
DO	-0.30
NH_3	0.62
OX.N	-0.38
TN	0.45
T	0.03
pH	0.21
PO_4	-0.02
Ca	-0.22
OM	0.09
TSS	0.16
TDS	-0.02

Fig. 1. Interaction between different variables in the BIOWATSYST with bacterial population counts in influent and effluent water of beds (2), (4) and (6) using canonical variant analysis



to bacteria living in the rhizosphere (Zenova & Zvyagintsev, 2003). The observed increase in counts on root biofilms could be related to the more aerobic conditions created by roots and rhizomes of *Phragmites*, which is known to pump oxygen across the bed, as indicated by DO values, which are favorable for growth and multiplication (Goodfellow & Williams, 1983).

In the present study, the more favorable growth of TVB and actinomycetes were in sand bed (B.4) rather than that in gravel bed (B.2) and gravel/sand bed (B.6). This pattern indicates that the design and conditions created in sand bed (B.4) were better for the growth of these organisms than those of the other beds. This is probably due to the nature of the sand particles, which exhibit higher filtration capacity to the flowing sewage and also provide more surface area for microbial colonization.

The presence of bacterial indicators TC, FC and FS and other pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio* spp., coagulase positive *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* indicated the polluted conditions of the water. Some of these pathogens have dangerous extracellular products, which were well known to be virulence linked factors (Mansour *et al*., 2006).

The efficiency of the BIOWATSYST beds to remove such bacterial indicators from the water effluent reaching to values > 91% during the periods of study, where the reduction percentages were 91.3%, 52%, 48.7%, 65.2% for TVB, TC, FC and FS, respectively. Similar reductions were, reported in previous studies on gravel bed hydroponic (GBH) system beds (May *et al*., 1990; Dewedar *et al*., 1993; Williams *et al*., 1995). The removal of these bacteria may be achieved by a combination of physical factors, including filtration and ultraviolet radiation as well as biological factors, such as toxic effects of root exudates and predation by other microbial populations (Duncan & Groffman, 1994).

Sand bed (B.4) was observed to achieve maximum removal percentages in comparing with the other beds, where the reduction percentage reaching to > 93% for all studied heterotrophic bacteria. This is probably due to the plant-sand matrix, which serves to filter the sewage effluent more efficiently than gravel matrix (May *et al.*, 1990).

Fecal coliforms and fecal streptococci were also declined in the gravel, sand and root biofilm in a ratio of 74.6%, while these ratio in effluents water of the treatment beds decreased to 56%. The explanations for their reduction on these surfaces may be due to the physical filtration through the plant, gravel and sand matrix. The counts of TC, FC and FS in wastewater samples were higher than that of actinomycetes due to the absence of the actual actinomycetes competence for nutrients and the lack of active metabolism of actinomycetes in water samples as they are still either sporulated or germinated (Tadashi, 1976; Lechevalier & Lechevalier, 1976). It is also possible that actinomycetes left unused simple nutritive materials for the bacteria to grow and flourish (Lemmer & Baumann, 1988). In contrast, counts of TC, FC and FS were lower than actinomycetes on gravel, sand and root biofilms and this pattern may be due to its inability to compete with actinomycetes like those recorded to have antagonistic activity in the present study.

Groth and Saiz-Jimenez (1999) had suggested that the environmental conditions, together with the availability of nutrients and the nature of the organic matter are major factors controlling the activity of different bacterial groups. The present study indicated that, temperature changes during the four seasons may have affected the counts of different microbial groups during the course of the study. The pH values showed little variations in effluent water of the studied beds during the study seasons. Values of pH in effluent water of gravel beds (B.2) and gravel/sand bed (B.6) were higher than that of effluent water of sand bed (B.4). This pattern was explained by Dazzo (1980) and Corpe (1980), who revealed that the high pH values may affect the

bed performance. It is expected that, the increased pH values may affect the *Phragmites* root uptake of ions and consequently affected its ability to percolate oxygen into the rhizosphere.

In the present study the efficiency of the BIOWATSYST beds was assessed as reduction of some physico-chemical parameters; biochemical oxygen demand (BOD) 70%, chemical oxygen demand (COD) 61.8%, ammonia (NH_3) 21.2%, total nitrogen (TN) 8.7%, total suspended solids (TSS) 62.7%, total dissolved solids (TDS) 26.2% and organic matter (OM) 56.8%. However, there was an increase in dissolved oxygen (DO) in a ratio of 79.4% and oxidized nitrogen (Ox.N) 67.6%. Little variations in values of calcium and orthophosphate (PO_4) were, recorded between influent and effluents water of the treatment beds during the study seasons, where PO_4 values just reduced in a ratio of 4.2%.

The dissolved oxygen (DO) was, increased during the study period, consequently increasing aerobic activities and mineralization processes in the system. May *et al*. (1990) revealed that treatment process can occur in the presence or absence of oxygen. However, in the absence of oxygen, decomposition of organic waste occurs at a much slower rate and can create severe odor problems. As such, oxygen is an essential ingredient in efficient treatment.

Aerobic and anaerobic microbial degradation as well as sedimentation are known, to contribute to BOD removal. The coarse media beds act primarily as filters with most BOD being removed in association with the filtration and settling of suspended solids (Cooper & Findlater, 1990). A major promise of the root-zone method is that the wetland plants are able to provide oxygen to the actinomycetes and heterotrophic bacteria in the rhizosphere thereby, allowing aerobic degradation of organic matter and nitrification to occur (Brix, 1987).

Nitrogen removal within wetland systems is thought to take place mainly through nitrification, denitrification, plant up-take, and volatilization. Nitrification and denitrification are the predominant removal mechanisms (White, 1995). Amm-N is released during the biological transformation of nitrogen combined in organic matter such as, humic substances, proteins and nucleic acids. The process is mediated by a variety of heterotrophic microorganisms, including actinomycetes, under both aerobic and anaerobic conditions; although the later provide greater release of Amm-N (Herbert, 1999).

The removal of suspended solids is accomplished through sedimentation and filtration by the media and plant roots (Cooper & Findlater, 1990). Organic pollutants are degraded aerobically by bacteria attached to plant and sediment surfaces (Wood, 1995). Most heterotrophic bacteria are well known, for their ability to degrade complex and recalcitrant molecules, especially cellulose, lignocellulose and lignin, which make them particularly important in sewage water treatment system. The possibility of a biodegradation of organic micro-pollutants with a wide class actinomycetes often present in the sewage water (Kutzner & Mahro, 1996; Doddamani & Ninnekar, 2000). Loveridge *et al.,* (1997) found that the presence of actinomycetes in the aerobic regions of the sewage water treatment beds may be significant in relation to the accumulation and stabilization of humic materials. This might prove of benefit in the breakdown of complex aromatic chemicals. Removal of some metals has occurred through bioaccumulation in microbial cells, direct uptake by the plant and adsorption to organic particles in the system. Phosphorous and calcium removal occurs mainly as a consequence of adsorption, complexation and precipitation in the sediment (Nuttall *et al*., 1997).

To sum up, The treatment process resulted in a decrease of TC, FC, FS, BOD, COD, NH_3, TN, OM, TSS and an increase of DO and OX.N. These occurred mainly in sand bed (B.4) more than gravel/sand bed (B.6), while the least figure was observed in gravel bed (B.2). The differences in design of these beds revealed that good water treatment was achieved in sand bed (B.4).

Focused light on why the treatment processes occur in sand bed (B.4) better than gravel/sand bed (B.6) and the lowest efficiency for gravel bed (B.2)? This can be explained as, the first role of the filling materials is physical treatment of the wastewater.Filtration and sedimentation of suspended solids and pathogens occurs along with the absorption of phosphorus, and dissolved organics (Lance *et al.,* 1976). The root zone is generally used as the basis for the design of SF wetland system. Wastewater is distributed uniformly across the wetland bed by the influent zone, which is composed of fine gravels or coarse sand or both of them. Wastewater then flows vertically through the media filled beds where it is treated by physical, biological, and chemical processes. These processes are said to take place in the rhizosphere, which is composed of the media, the plant roots, the plant rhizomes, and the associated microbial communities (Conley *et al*., 1991). Sand, are more effective in absorption and filtration of the wastewater than gravel or rocks because the smaller media contain smaller pore sizes and larger surface areas (Conley *et al*., 1991). Sand and gravel also provides a stable surface area for the attachment of microbial biofilms, which perform biological treatment of the wastewater passing through the root zone bed. SF wetlands are commonly referred to as attached growth biofilters (Wood, 1995).

From the previous explanation for the importance of the filling materials of the treatment beds and from the description of our treatment beds one could note that sand bed (B.4), which was filled with a layer of 0.20 m fine gravel and another layer of coarse sand over it measured 0.60 m in depth, has shown a relatively good treatment efficiency, this may due to the high retention time of water in the bed, as sand layers allows higher retardation of the water than the gravel layers. Also, these sand layers gave a large surface area for biological, physical and chemical treatment as well as mechanical treatment. Gravel bed (B.2),

which was filled with 0.60 m fine gravels, had a less retention time of water in the bed and a small area for the mentioned treatment processes. In case of gravel/sand bed (B.6), which was filled with 0.20 m fine gravels and a second layer of 0.20 m coarse sand as well as a third layer of 0.60 m fine gravels was placed to sandwich the water between it and the first two layers at the bottom, showed an intermediate stage between sand bed and gravel bed.

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