

The Role of Different Macrophytes Groups in Water Quality, Sediment Chemistry and Microbial Flora of Both Irrigation and Drainage Canals

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Abstract: This study was carried out to determine the influence of aquatic macrophytes, *Eichhornia crassipes*, *Nymphaea lotus*, *Echinochloa stagnina* and *Pistia stratiotes* on water quality and sediment chemistry in samples collected (in June 2008) from both irrigation and drainage canals, with emphasis on the effect of different plant groups on distribution of microbial flora at the studied areas. It was found that, aquatic macrophytes have pronounced effects on the aquatic habitats resulting in the reduction amount of some elements Ca^{2+} , Na^+ , NH_4 , NO_3 , N, P and K^+ from both water and sediment. Besides, a considerable variations in elements contents between water, sediment and macrophytes with the greatest amounts of micro-elements in plants tissues was observed. At the same time the microbial flora varied with plant species and site of sample collection where, the rhizosphere of *Eichhornia crassipes* (from drainage canal) attended the highest frequencies of total viable bacterial count and the number of heterotrophic bacteria on the surface of *Echinochloa stagnina* recorded the highest value (22×10^6 CFU/g) while, yeast mould count associated with macrophytes recorded the highest counts. It could be concluded that, the higher tissue concentration of both macro and micro-elements, high rate of microbial removal and greater rate of biomass production by *Eichhornia crassipes* and *Echinochloa stagnina* may contribute to greater rate of water purification in irrigation and drainage canal system so, the obtained results could be useful in the practical use of the tested plant species in wastewater treatment

Key words: Water • Sediment • Macrophytes • Microbial flora

INTRODUCTION

Macrophytes are an important component of wetland because they supply organic matter for aquatic and terrestrial organisms and they increase the physical and biological complexity of aquatic habitats that provide high diversity of fish and invertebrates [1]. Aquatic macrophyte system can be used to reduce pollutant level in water bodies and reducing the level of nitrogen and phosphorus N and P in wastewater [2, 3]. The mineral absorbing potential from the aquatic environment could possibly be harnessed to reduce the nutrient content of the eutrophic waters and thus circumvent the potential for excessive plant growth [4].

The plant serve as a substrate for microbial activity that removes nutrients such as nitrates, ammonia and phosphates [5-7].

The microbial community in constructed wetlands consists of autochthonous (indigenous) and allochthonous (foreign) microorganisms. Autochthonous microbes exhibit adaptive features, they are able to possess metabolic activity, survive and grow in wetland systems, while allochthonous microbes (including pathogens entering with wastewater) [8]. The formation of biofilms on plant surfaces seems to be linked to active processes of bacterial attachment and production of exopolymers substances [9]. Bacterial communities associated with the roots of aquatic macrophytes have been considered important in energy transfer [10] and nutrient recycling [11]. A key role in curbing the rate and progress of eutrophication in water body is played by heterotrophic bacteria including epiphytic bacteria growing on the surface of various hydromacrophytes [12].

This investigation was carried out to understand the role of some macrophytes in the surrounding media through, studying the effects of *Eichhornia crassipes* (C. Mart. Solm), *Nymphaea lotus* Linn, *Echinochloa stagnina* (Retz.) and *Pistia stratiotes* Linn in water quality, sediment chemistry as well as, on distribution and community structure of their rhizosphere microbial flora.

MATERIALS AND METHODS

Study Area: The study was conducted at two water sources (irrigation and drainage canals) lies at El-Serw Village, South of Manzalah Lake, at the eastern north part of Egypt.

Water and Sediment Collection: Water and sediment samples were collected in July 2008 from seven sites; four sites at irrigation canal. 1) un-vegetated area, 2) area dominated by *Eichhornia crassipes*, 3) area dominated by *Nymphaea lotus*. 4) area dominated by *Echinochloa stagnina* and; three sites at drainage canal 5) area dominated by *Pistia stratiotes*, site 6) area dominated by *Eichhornia crassipes* and 7) un-vegetated area.

Water Analysis: Water samples at a depth of 5-20 cm were taken from stands of plants collection. Water temperature, pH and electrical conductivity (EC) using a combined pH meter digital model 5986 were measured in the field. Total soluble salts (T.S.S.) were determined as described by Jackson [13]. Total nitrogen content (N) was determined by Kjeldahl method described by Black [14] and total phosphorus (P) was determined by a colorimetric molybdenum method, outlined by Allen [15].

Sediment Analysis: From each stand of plant and water collection, sediment samples were collected. Two types of extractant were used depending on the type of element to be determined. Olsen's reagent (0.5 M sodium bicarbonate at pH 8.5) 1: 20 (extractant/soil) was used in phosphate determination. The rest of elements were extracted using 1:5, soil/water [16].

Plant Material: Four macrophyte species, which formed distinct, largely monospecific stands in irrigation and drainage canals were chosen for this study. They were classified and identified as: free floating macrophyte water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), floating leaves water-lily (*Nymphaea lotus*) and the emergent species *Echinochloa stagnina* [17, 18]. Their roots were

separated, stored in canal water till the laboratory where, they were cleaned and rinsed in distilled water, dried at 60°C to constant weight. The dry samples were ground to fine powder and preserved in well stopper sample vessels.

Plant Analysis: The plant extract was prepared using sulphuric and perchloric acids [19]. Total nitrogen content was determined by Kjeldahl method [14]. Total phosphorus was estimated colorimetry using the hydroquinone method [20]. Total potassium was measured photometrically [21]. For determination of metals, dried plant tissue was digested at 80°C for two hours, using nitric acid [15]. Metals were determined by (Perkin Elmer 2100 Flam Atomic Absorption Spectrophotometer with an Autosampler).

Microbiological Determinations: Samples were taken manually under possible aseptic condition in 150 ml glass bottles and stored in the dark inside a cool box during transport to the laboratory [22]. Selective differential and non selective media were used to cultivate and confirm the presence of specific bacteria from water, sediment and macrophytes samples. The plate method was used for the determination of aerobic heterotrophic bacteria, using nutrient agar medium (NA; 1-140, Scharlau Chemie, S.A.), bacto-actinomycete isolation agar and starch casein agar supplemented with antibacterial compounds [23] for analysis of actinomycetes. Yeast mould count was detected on potato dextrose agar medium (PDA; 1-483, Scharlau Chemie, S. A.). Total coliform and faecal coliform on McConkey broth (2-118, Scharlau Chemie, S.A.) *Pseudomonas aeruginosa* using asparagine broth (2-271, Scharlau Chemie, S. A) expressed as colony forming unit (CFU) 100 mL⁻¹ based on Most probable number (MPN) method [24]. *Escherichia coli* counts were recorded on EMB agar plate (1-068, Scharlau Chemie, S. A.) and *Salmonella/Shigella* was detected on S/S agar (1-171, Scharlau Chemie, S.A.).

The samples were serially diluted in sterile physiological saline and 100 µl aliquots from the appropriate dilution were spread on the surface of agar media to determine the microbial flora of water and sediment. In order to determine the number of microbial biomass inhabiting the surface of the studied plants, 10 g of roots were collected, added 90ml sterile buffer water to samples and they were homogenized in a homogenizer for 2 min. at 40,000 rpm. The resulting homogenates were serially diluted, three replicates were used. Inoculated plates at 28°C ±2. Microorganisms recovered on the plates were counted 10 days after inoculation.

Identification of Bacteria: Bacterial strains were identified on the bases of the diagnosis schema proposed by Bergey's Manual of Determinative Bacteriology [25]. The isolates were identified parallel with the use of API20E, API20NE and APICH50 systems (bioMérieux).

Statistical Analysis: The data were analyzed using one way ANOVA by SPSS10.00 for Windows.

RESULTS

Quality of Water: As shown in Table 1, during the period of samples collection the values of water temperature ranged from 26°C at vegetated areas to 28°C at un-vegetated areas. The pH values ranged from 7.62 at site (5) to 8.75 at site (3) with, the highest value in area around *Nymphaea lotus* indicating the existence of almost highly alkaline water at this site. The highest T.S.S. was found in drainage water samples (940.8 mg L⁻¹), while, the lowest values (838.4 mg L⁻¹) were found in irrigation water. Further, the values varied from site to site depending on the presence or absence of vegetation as well as on plant species.

The values of CO₃²⁻ increased with the presence of macrophytes in both irrigated and drainage canal, while HCO₃⁻ increased with *Nymphaea lotus* and *Echinochloa stagnina* but, decreased with the rest of plants used. The sulphate level was raised with the presence of macrophytes, recorded the maximum value in area around *Eichhornia crassipes* (137.76 mg L⁻¹) but, as un-expected the highest values were recorded for irrigation canal samples and the lowest for drainage canal. In contrast to sulphate, calcium content was higher at site (7) compared with site (1) with a considerable increase in drainage canal water corresponding with the presence of vegetation. However, Mg²⁺ content was higher at site (7) compared with site (1) its values increased in irrigation canal with the presence of vegetation while, it decreased in drainage canal. At the same time Na⁺ concentration was very high at site (7) compared with other sites and its values decreased with the presence of macrophytes. NH₄⁺ content in water can be observed at Table 1 where, the highest values was recorded at un-vegetated areas reaching 7.12 mg L⁻¹ at (site 7) and the lowest value 3.46 mg L⁻¹ in area around *Eichhornia crassipes* (site 2). The highest value of NO₃⁻ (6.2 mg L⁻¹) was recorded at site 7 and the lowest (1.24 mg L⁻¹) at site 4.

As shown in Table 2 different values of macro and micro-elements were recorded in the studied water samples. In general the values of N, P and K⁺ were higher at site 1 compared with site 7. Comparing the values

recorded for water samples from irrigation canal, the lowest N value was recorded for sample from the area around the floating species *N. lotus* (0.9 ppm) while, the highest was found in area around the free floating species *E. crassipes* (1.57 ppm). At the same time in samples from drainage canal, the highest N value was found in area around *P. stratiotes* (1.57 ppm) followed by, *E. crassipes* (1.12 ppm). A considerable decrease in K⁺ and P values corresponding with the presence of different plant groups can be observed in samples from irrigation canal while, in drainage canal the value increased with the presence of *P. stratiotes*.

The Fe²⁺ values showed a tendency to decrease with the presence of macrophytes. In irrigation canal the emergent species *Echinochloa stagnina* and the floating leaves *Nymphaea lotus* decreased its value from 0.03 to 0.01 ppm while, in drainage canal the minimum value 0.27 ppm was found for the area of *Pistia stratiote*. The values of Zn²⁺ and Mn²⁺ were very low and fluctuated in a very narrow range. At the same time Cu²⁺ can not be detected in all samples.

Table 3 shows a considerable variation between all studied samples, the highest values of T.S.S., EC, HCO₃⁻, Cl⁻, Mg²⁺, Na⁺ and available K⁺ in samples from drainage canal with, a considerable decrease below the vegetative areas. In contrast to this the highest values of SO₄²⁻ were recorded for irrigation canal samples with a tendency of all values to increase with the presence of plants. At irrigation canal the value of SO₄²⁻ increased from 73.92 ppm to 137.76 ppm with the presence of *E. crassipes* while, in drainage canal the highest value was recorded for the area below *Pistia stratiote* (54.24 ppm).

In irrigation canal the values of Ca²⁺ decreased to 33.00 ppm, while in drainage canal the values increased to 107.80 ppm. In contrast to the way of changing in Ca²⁺ content the values of Mg²⁺ increased in irrigation canal, while decreased in drainage canal reaching to the minimum value (4.44 ppm).

As shown in Table 4, the plants were more efficient in reducing nutrient level in all studied sites with the lowest level of N in area below *E. crassipes* for both irrigation and drainage canal (81.00 and 45.00 ppm, respectively). However, in irrigation canal the lowest values of P and K⁺ was found in areas below *Eichnocolia staganiana* (20.00 and 900.00 ppm for the two elements, respectively).

In both irrigation and drainage canals the values of Fe²⁺ and Mn²⁺ increased with the presence of vegetation, while the values of Zn²⁺ decreased except in areas vegetated by *E. crassipes*. The values of Cu²⁺ decreased with the presence of vegetation with a minimum values

Table1: Physico-chemical charactereries of water samples collected from different sources (mg L⁻¹).

Samples	Irrigation Canal				Drainage Canal			Min.	Mean	±SD	Max
	Site 1	Site 2	Site 3	Site 4	Site 5	site 6	Site 7				
Elements	Un-vegetated area	Area of <i>E. crassipes</i>	Area of <i>N. lotus</i>	Area of <i>Echi. stagnina</i>	Area of <i>P. stratiotes</i>	Area of <i>E. crassipes</i>	Un-Vegetated Area				
Temp. °C	28.00	26.00	26.00	26.00	26.00	27.00	28.00	26.00	26.70	0.95	28.00
pH	8.40	8.30	8.75	7.99	7.62	7.86	7.84	7.62	8.11	0.40	8.80
EC mhos/cm	1.39	1.40	1.30	1.30	1.18	1.19	1.47	1.18	1.32	0.11	1.50
T.S.S.	889.60	876.80	838.40	844.80	755.20	768.00	940.80	755.20	845.00	66.10	940.80
CO ₃ ²⁻	24.00	42.00	36.00	30.00	36.00	12.00	18.00	12.00	28.30	10.80	42.00
H CO ₃ ⁻	203.10	216.70	230.20	243.80	243.80	270.90	379.20	203.10	255.00	58.70	379.20
Cl ⁻	340.40	300.30	267.00	283.70	233.60	250.30	277.00	233.60	279.00	34.80	340.40
SO ₄ ²⁻	8.80	13.80	29.10	10.30	1.15	5.30	3.94	1.15	10.30	9.30	29.10
Ca ⁺⁺	101.20	110.00	92.40	101.20	66.00	66.00	176.00	66.00	102.00	37.00	176.00
Mg ⁺⁺	70.60	67.60	71.30	59.10	59.50	80.00	32.60	32.60	61.70	13.80	71.30
Na ⁺	63.10	57.20	56.60	72.20	79.30	61.80	71.50	56.60	65.90	8.50	79.30
NH ₄	4.30	3.50	3.80	4.00	5.80	5.80	7.10	3.50	4.88	1.40	7.10
NO ₃	1.90	3.00	5.00	1.30	4.34	4.30	6.20	1.20	3.69	1.80	6.20

Table 2: Values of macro and micro-elements (ppm) in water samples from un-vegetated areas and areas around the selected plants

Samples	Irrigation Canal				Drainage Canal			Min.	Mean	±SD	Max
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7				
Elements	Un-vegetated	<i>E. crassipes</i>	<i>N. lotus</i>	<i>Echi. stagnina</i>	<i>P. stratiotes</i>	<i>E. crassipes</i>	Un-Vegetated				
N	1.34	1.57	0.90	1.12	1.57	1.12	1.12	0.90	1.24	0.25	1.57
K ⁺	8.00	3.50	3.50	3.50	4.00	3.50	3.50	3.50	4.21	1.67	8.00
P	0.39	0.31	0.31	0.12	0.12	0.08	0.08	0.08	0.20	0.13	0.39
Fe ⁺⁺	0.03	0.04	0.01	0.01	0.27	0.32	0.35	0.01	0.14	0.15	0.35
Zn ⁺⁺	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01
Mn ⁺⁺	0.03	0.01	0.03	0.02	0.01	0.02	0.01	0.01	0.02	0.01	0.03
Cu ⁺⁺	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3: Values of cations and anions (ppm) in sediment extracts

Samples	Irrigation Canal				Drainage Canal			Min.	Mean	±SD	Max
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7				
Elements	Un-vegetated area	Area of <i>E. crassipes</i>	Area of <i>N. lotus</i>	Area of <i>Echi. stagnina</i>	Area of <i>P. stratiotes</i>	Area of <i>E. crassipes</i>	Un-Vegetated area				
T.S.S.%	0.40	0.32	0.33	0.22	2.07	1.85	2.70	1.127	1.00	0.22	2.70
EC mhos/cm	1.24	1.00	1.04	0.70	6.46	5.77	8.44	3.521	3.30	0.70	8.44
CO ₃ ²⁻	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HCO ₃ ⁻	48.80	42.70	61.00	54.90	85.40	85.40	97.60	67.97	21.00	42.70	97.60
Cl ⁻	142.00	55.03	56.32	33.37	1084.50	967.73	1344.20	526.20	579.00	33.40	1344.00
SO ₄ ²⁻	73.92	137.76	130.08	83.52	54.24	42.24	12.00	76.25	46.00	12.00	137.80
Ca ⁺⁺	61.60	48.40	44.00	33.00	92.40	107.80	88.00	67.89	28.00	33.00	107.80
Mg ⁺⁺	3.36	12.48	8.16	14.76	13.68	4.44	16.32	10.46	5.20	3.36	16.32
Na ⁺	61.41	31.74	49.45	10.35	612.26	529.46	841.11	305.10	346.00	10.40	841.10
K ⁺ available	12.09	10.92	10.92	9.36	26.91	30.03	34.32	19.22	11.00	9.36	34.32

Table 4: Macro and micro-elements values (ppm) in sediment samples

Samples	Irrigation Canal				Drainage Canal			Min.	Mean	±SD	Max
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7				
Elements	Un-vegetated area	Area of <i>E. crassipes</i>	Area of <i>N. lotus</i>	Area of <i>Echi. stagnina</i>	Area of <i>P. stratiotes</i>	Area of <i>E. crassipes</i>	Un-Vegetated area				
N	103.00	81.00	94.00	94.00	94.00	45.00	90.00	45.00	85.90	19.20	103.00
P	110.00	50.00	21.00	20.00	30.00	10.00	20.00	10.00	37.30	34.40	110.00
K	1280.00	960.00	1120.00	900.00	900.00	1300.00	1000.00	900.00	1066.00	170.00	1300.00
Fe ⁺⁺	4.70	4.50	5.12	5.22	5.82	5.76	5.68	4.50	5.26	0.52	5.82
Mn ⁺⁺	2.88	8.64	3.84	2.72	2.74	2.92	2.48	2.48	3.75	2.20	8.64
Zn ⁺⁺	0.52	0.62	0.42	0.48	0.53	0.76	0.72	0.42	0.58	0.13	0.76
Cu ⁺⁺	1.08	0.086	1.02	1.08	0.72	0.98	1.02	0.086	0.86	0.36	1.08
Pb ⁺⁺	0.146	0.172	0.184	0.142	0.15	0.173	0.162	0.142	0.16	0.02	0.184

Table 5: Values of macro and micro-elements expressed as % of DW and ppm in roots of the studied plants

Samples Elements	Irrigation canal			Drainage canal	
	<i>E. crassipes</i>	<i>N. lotus</i>	<i>Echi. stagnina</i>	<i>E. crassipes</i>	<i>P. stratiote</i>
N	2.21	3.43	3.02	2.01	2.81
P	0.67	0.94	0.82	0.57	0.87
K	2.30	3.15	2.70	2.12	2.90
Fe ⁺⁺	750.00	1075.00	1190.00	795.00	1110.00
Mn ⁺⁺	116.00	102.00	105.50	138.00	113.00
Zn ⁺⁺	146.00	105.00	116.50	197.50	108.50
Cu ⁺⁺	195.00	225.00	205.00	185.00	195.00
Pb ⁺⁺	58.00	46.00	49.00	49.00	51.00

in area vegetated by *E. crassipes* 0.086 ppm (in irrigation canal) while, in drainage canal the minimum value was recorded for sediment samples below *Pistia stratiote* (0.72ppm). The values of Pb⁺⁺ was also affected by the presence of macrophytes, it was increased with the presence of *E. crassipes* and *Nymphaea lotus*, while decreased with the presence *Echinochloa stagnina* and *Pistia stratiote*.

The results in Table 5 show the variations in concentrations of all studied elements with plant species and site of sample collection. The highest levels of N, P and K⁺ were recorded for dry tissue roots of the floating species *Nymphaea lotus* (3.43, 0.942 and 3.15% of Dw, respectively) followed by the emergent species *Echinochloa stagnina* and the free floating *Pistia stratiote* while, the lowest values were recorded for the free floating *E. crassipes*. The results also showed the variations within the same species from different water sources where, the contents of N, P and K⁺ were higher in *E. crassipes* from irrigation canal while, the values of Fe⁺⁺, Mn⁺⁺ and Zn⁺⁺ were higher in samples of the same species collected from drainage canal. The variation in micro-elements concentrations among different plant groups was also observed (Table 5).

Recovery of Microbes from Water, Sediment and Macrophytes: Data obtained from direct dilution plating of samples are presented in Fig. 1. According to the microbial diversity, there were association between the medium and the sample type, The highest total viable bacterial count was recorded with water sample obtained from site 6 (1x10⁷ CFU mL⁻¹), while sediment sample collected from sites, 2 recorded the highest value (20x10⁶ CFU g⁻¹), the data revealed no significant differences (P<0.05) between bacterial count obtained from sediment samples 1, 6 and 5 (3x10⁶ CFU g⁻¹) (Fig. 1,A). The majority of actinomycetes were isolated from the sediment samples

and very few of them were recovered from macrophytes; but it was not detectable in water samples (Fig. 1,B). No significant differences (P<0.05) were detected for yeast/mould counts (1x10² CFU g⁻¹) in water samples 3, 4 and 5 (Fig. 1,C). The MBN of total coliforms, in the water and sediment ranged from 1.5-21.0x10² CFU/100 ml and 23.0-240.0x10² CFU/100 g respectively, summarized in Fig. 1,D. The population of faecal coliforms in the tested samples (Fig. 1,E) ranged from; 0.5-3.0x10² CFU/100 ml in water samples, without significant differences between samples obtained from stations 5 and 7. The results cleared that *Salmonella* and *Shigella* were not detected in all tested water sample except 7W (35x10² CFU/ml). Similar trend was noted in population densities in the sediment samples where all sediment samples were *Salmonella* and *Shigella* free, except those of 6S and 7S where 7S recorded the highest population (6.2x10² CFU/ml) (Fig. 1,F). *Pseudomonas aeruginosa* were rare, the highest numbers of *P. aeruginosa* were found in the sediment samples, it ranged from 7-29 CFUg⁻¹ (Fig. 1, G).

The quantitative analysis of epiphytic bacteria inhabiting the surface of the studied macrophytes revealed that, the number of heterotrophic bacteria on the surface of *Echinochloa stagnina* recorded the highest value (Log₁₀) 7.322 CFU g⁻¹, while heterotrophic bacteria on the surface of *Pistia stratiote* and *E. crassipes* did not exhibited any significant differences. The highest values (Log₁₀) of yeast mould count were associated with *Nymphaea lotus* 3.9 CFU g⁻¹. The population of total coliform ranged from 3.6-4.0 CFU/100g, while faecal coliforms showed that, macrophytes samples contain faecal coliforms values ranged from (log₁₀) 2.7-3.4 CFU/100g. *Salmonella* and *Shigella* population associated with *P. stratiote* and *E. crassipes* did not show significant differences (3.8 CFU g⁻¹). *Pseudomonas aeruginosa* associated with macrophytes ranged from (Log₁₀) 0.95-1.4 CFU/100g (Fig. 2).

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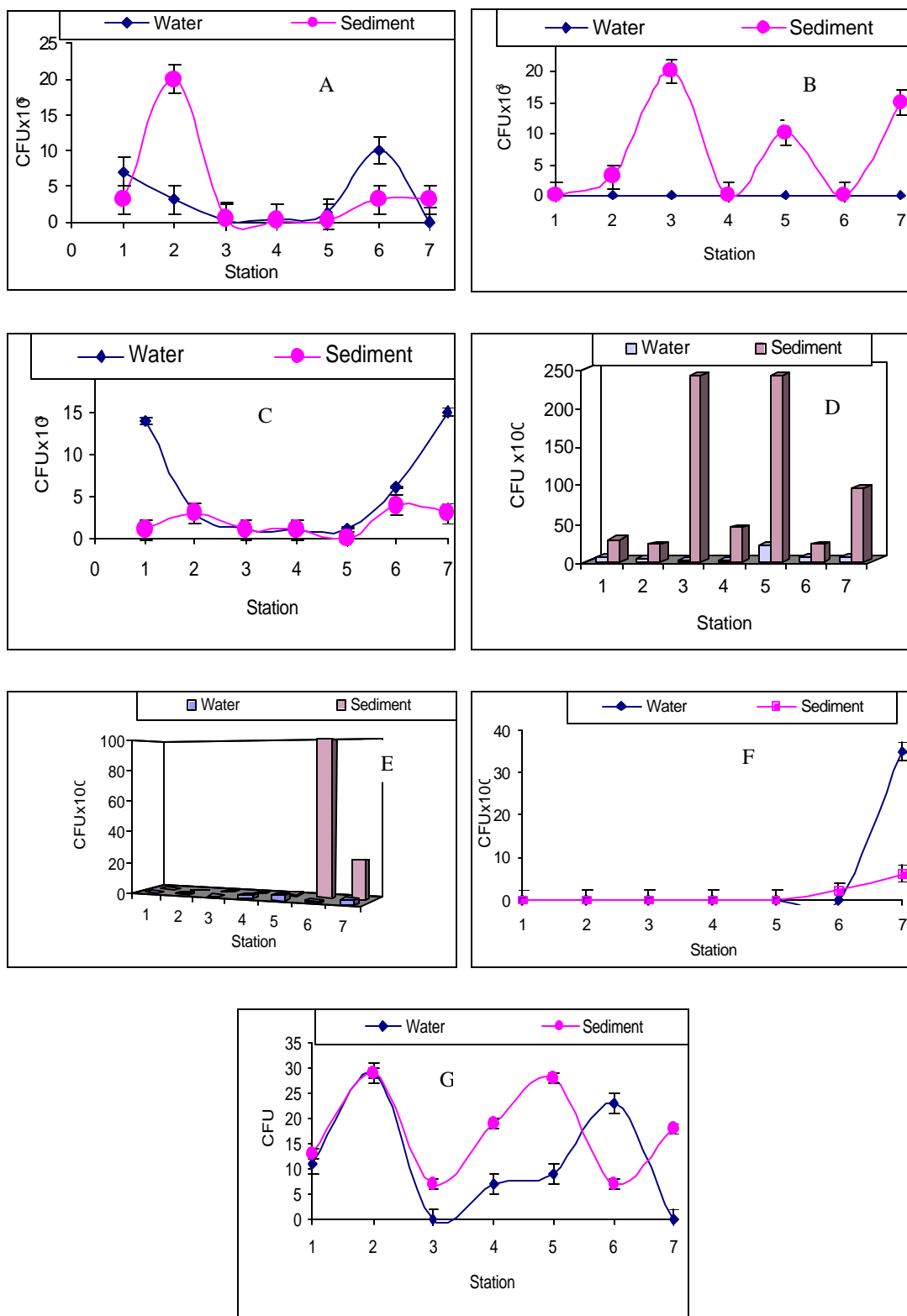


Fig. 1: Microbial flora of water and sediment samples; A) total viable bacterial count, B) Actinomycetes count, C) yeast and mould count, D) Total coliform, E) faecal coliform, F) Salmonella and Shigella count and G) *Pseudomonas aeruginosa*

Table 6: The epiphytic bacteria isolated from the root surface of the selecte macrophytes.

Bacteria	Irrigation Canal			Drainage Canal	
	<i>E. crassipes</i>	<i>N. lotus</i>	<i>Echi. stagnina</i>	<i>P. stratiotes</i>	<i>E. crassipes</i>
<i>Bacillus</i> sp.					
<i>B. cereus</i>	15	8	9	20	11
<i>B. subtilis</i>	25	0	3	5	3
<i>B. firmus</i>	20	11	8	1	5
<i>B. megaterium</i>	12	0	3	5	3
<i>B. fastidious</i>	35	3	4	18	26
<i>B. leicheniformis</i>	15	0	15	10	12
<i>B. pasteurii</i>	11	0	6	3	0
<i>P. aeruginosa</i>	35	21	18	28	30
<i>Escherichia coli</i>	14	18	22	35	26
<i>Staphylococcus</i> sp.	8	6	3	8	10
<i>Streptococcus</i> sp.	38	28	18	25	15
<i>Salmonella</i> sp.	11	6	0	8	7
<i>Shigella</i> sp.	9	8	0	6	5

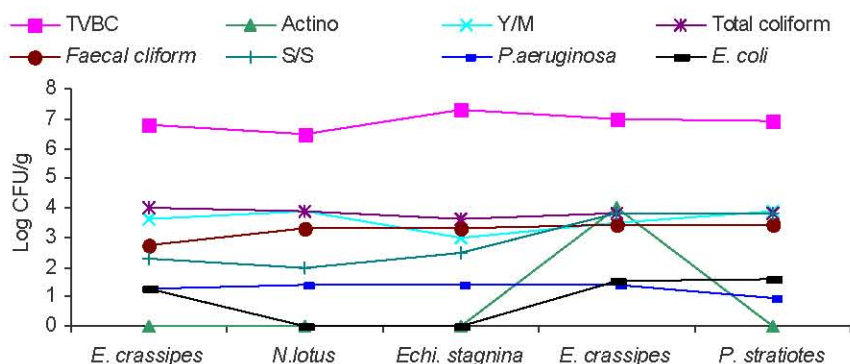


Fig. 2: Microbial flora associated with macrophytes samples

The generic composition of the isolated microorganisms displayed distinct variability, linked to the species of the plant. Bacteria from the genus *Bacillus* were dominant constituting about 83.26% of all the isolated species,

Bacillus species on the surface of *E. crassipes* constituting about 53.63% of all the isolated species, where *Bacillus* sp. showed the most dominated species in all samples (Table 6). Most of those species constituted between 2.6 and 11.2% of all the strains. The following species were dominant in total isolates: *Pseudomonas aeruginosa* (13.8%) and *Escherichia coli* (15.0%), on the other hand *Staphylococcus* sp., *Salmonella* and *Shigella* sp. accounted for the lowest percentage (4.6, 4.2 and 3.7%, respectively).

DISCUSSION

Macrophytes play either direct or indirect an important role in nutrient removal and storage. The removal of soluble inorganic nitrogen and phosphorus

via absorption from either the water column or the sediment, assimilation and storage in plant tissue is a direct mechanism of nutrient sequestration [26]. The removal of naturally growing aquatic macrophytes is used to remove nitrogen, phosphorus, ammonia, potassium and heavy metals by consuming them in the form of plant nutrients [27-29]. It follows from the obtained results that, the concentrations of most analyzed metals decreased with the presence of vegetation, but with different degrees depending on plant species, site of sampling collection as well as the microbial flora associated. The pH values lie on the alkaline side and increased with the presence of vegetation, related to the effect of photosynthesis that involves the uptake of free carbon dioxide from the water and the precipitation of calcium carbonate [30, 31].

In the purification process, a complex variety of physical, chemical and biological processes is involved in the transformation and consumption of organic matter and plant nutrients [32-35]. It is well-known that oxygen photosynthetically generated by water hyacinth played

an important role in the biodegradation of organic matter of wastewater by aerobic and facultative bacteria [36]. In this study and as a result of the decay of phytoplankton, macrophytes or oxidation of sulphide to sulphate in the presence of photosynthetic sulphur bacteria associated with macrophytes rhizosphere the values of SO_4^{2-} increased in both water and sediment samples with the presence of vegetation.

The interaction between macrophytes and microbes is essential for nitrogen removal. In aerobic microenvironments around the rhizosphere, nitrification of ammonium occurs. These nitrates can then be taken up directly by the roots [26]. The results of the present study showed low levels of NH_4 and NO_3 in vegetated areas, except in area vegetated by *Eichhornia crassipes* and *Nymphaea lotus* (irrigation canals) where, the level of NO_3 raised. This could be explained by the observation of Reddy *et al.* [37] they recorded that, the overall nutrient requirement of water hyacinth is very low. However, it is being difficult to detect the effect of macrophytes in reducing micro-elements level in water, where the recorded values were very low and sometimes can not be detected. In sediment samples a considerable variation depending on the plant species and site of sample collection can be observed. Furthermore, the selectivity towards elements absorption by different macrophytes groups was observed where, the free floating *Eichhornia crassipes* attended the highest concentrations of Zn^{++} and Pb^{++} , the emergent plant proved to be effective in absorbing Fe^{++} (1190 ppm) and the floating leaves plant *Nymphaea lotus* has the highest content of Cu^{++} (225 ppm).

Another aim of this study was to assess the microbial flora in water, sediment and macrophytes, with emphasis on characterizing the most frequent macrophytes-associated microorganisms. One of the most significant groups of microorganisms in water bodies are bacteria inhabiting permanent abiotic and living substrates. They abundantly inhabit the surface of plants as well as sea and fresh water [38, 39]. In comparison with planktonic or benthic bacteria, these bacteria are more metabolically active [40]. Their occurrence and distribution depends on many physical, chemical and biological factors. From the data obtained in the present study, it follows that the number of heterotrophic bacteria was characterized by the sample type variability. The relatively high number of bacteria in water samples linked with macrophytes may be due to the organic substances produced by macrophytes which increase the total amount of carbon involved in the process of

microbial metabolism [41]. As un-expected the total bacterial count in sediment samples at sites 4, 5 and 6 was lower than that recorded in water samples, this may be related to the difference in roots morphological features of the selected plants where, *Echinochloa stagnina*, *Pistia stratiotes* and *Eichhornia crassipes* were characterized by their numerous hairy roots that provide a large surface area suitable for microbial attachment resulting in decreasing bacterial precipitation in sediment. While, *N. lotus* was characterized by its submerged rhizome with long spongy roots that firmly anchor the plant to the sediment, but not suitable for microbial attachment. Besides, the roots protect themselves and control their rhizosphere organisms by bioactive chemicals which often also have antimicrobial properties [42] and these active substances was varied with plant species. At the same time, the leaves surface areas which affect the penetration of light as well as the environmental variables associated was also of a prime importance in the process of microbial attachment.

The presence of total coliforms, faecal coliform and *E. coli* on the water samples indicated faecal pollution of the water. The high densities of these faecal indicator organisms observed at all the samples indicative of faecal pollution. Agriculture organic pollutants deposited in the soil (in these potentially pathogenic bacteria and pathogens as such) may migrate with precipitation waters into water bearing layers [43, 44], thereby deteriorating the bacteriological state of waters and increasing the risk of spreading various diseases [45]. *P. aeruginosa* was sporadic and low counts in water similar to *E. coli*, according to Wheeler *et al.* [46] these were the species commonly present in aquatic environments. Salmonella contamination is usually associated with contaminated food and animal feed and its presence in water signals fecal contamination of both human and animal origin [47]. On the basis of colony characteristics and morphological properties, organisms were classified into different isolates. Chand *et al.* [48], found that the bacterial cell shape and biochemical properties confirm that colonies with identical physical properties (Color, size and morphology) belong to same bacterial group.

The findings of this study suggest that, the morphological feature and the greater potential for biomass production of the free floating plant *Eichhornia crassipes* and emergent plant *Echinochloa stagnina* favored it over *Nymphaea lotus* and *Pistia stratiotes* as the most suitable plants for microbial removal and water purifications.

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