Journal of Plant Biology, October 2006, 49(5) : 398-402 **SHORT COMMUNICATION**

Effect of Sewage Wastewater Treated by *Nostoc muscorum* and *Anabaena subcylindrica* on the Growth of *Zea mays* and *Phaseolus vulgaris*

Mohamed El-Anwar Hosain Osman, Wagih Abd EL-Fatah El-Shouny*, Mostafa Mohamed El-Sheekh, and Eman Wahba El Sayed El-Gammal

Botany Department, Faculty of Science, Tanta University, Tanta, Egypt

***Zea mays* (Sakha 321) and *Phaseolus vulagris* (Giza 6) were irrigated with sewage wastewater treated with *Nostoc muscorum* and *Anabaena subcylindrica*. The results showed that irrigation of plants by untreated or treated wastewater caused stimulation in the measured growth parameters of both plants. In addition pigmentation as well as protein and carbohydrate contents were stimulated. This stimulation was attributed to the presence of high levels of essential nutrients such as nitrogen, phosphorus, and organic matters in wastewater. On the other side, there was a slight inhibitory effect of wastewater on some measured growth parameters of the plants. This effect may be due to the presence of some heavy metals in wastewater.**

Keywords: carbohydrate, cyanobacteria, irrigation, pigmentation, protein, sewage

Irrigation is an excellent use for sewage effluent because it is mostly water with nutrients. Treated wastewater is a valued resource for irrigation, particularly in arid and semi-arid regions. The nutrients in treated wastewater may be considered as fertilizers capable of improving the crop yield (Tsadilas and Chatzoulakis, 1997). The use of untreated wastewater may, however, present serious sanitary and toxicity problems. Therefore, the use of treated wastewater for irrigation was recommended by several investigators (Asha et al., 1990; Ranalli et al., 1996; Jimenez and Chavez, 1997).

The maximum permissible limits of the effluent of sewage to be discharged into the drain are as follows: BOD 60 mg L^{-1}, COD 80 mg L^{-1}, PV 40 mg L^{-1}, pH 6-9, dissolved materials 2 g L^{-1}, PO_4^{3-} zero, and NO_3^{1-} 50 mg L^{-1}, total heavy metals 1 mg L^{-1} according to Ministry of Health and Population and World Health Organization (1982). Discharge of untreated sewage and organic wastes possessing much higher contents cause serious pollution problems, so wastewater and sewage must be treated (Pescod, 1988).

The methods of treatment used for altering the characteristics of liquid wastes fall into the following classifications: (1) Preliminary treatments, (2) Primary treatments, (3) Secondary treatments, and (4) Tertiary treatments. Secondary treatment processes may be applied for further reduction of pollutants present in the water component, this is a biological treatment in which the waste or pollutants serve as a food source for the microorganisms which can flourish and remove suspended and dissolved pollutants.

Through the reduction of the number of pathogenic microorganisms and the oxidation of organic materials, the treated effluents and sludges can be used for irrigation purposes and as plant fertilizers (Wu et al., l993; Butorac et al., 1995; Al-Nakshabandi et al., 1996; Tsadilas and Chatzoulakis, 1997). The physical, chemical and biochemical properties of soil were improved following the sewage water addition but a limiting factor to their continual and generalized agronomic utilization would be the problem of heavy metal accumulation, thus semi-polluted water can be used for irrigation because the heavy metal contents is below the dangerous limit (Abou El-Hawa et al., 1998).

Sartori and Rizzetto (1999) found that the recycled purified water had higher levels of nitrogen, phosphorus and sulfur compounds, while canal water contained more chlorine components. Over the course of maize irrigation, there was no overall difference between the canal and purified water irrigation treatments. Ammar et al. (2000) confirmed that the effect of the microbiologically desalinated sea water did not markedly vary from that of the tap water in the irrigation of *Zea mays*.

Present study aimed at the use of sewage water treated by cyanobacteria for irrigation of two plants (*Z. mays* and *Phaseolus vulgaris*) and determination of the growth and productivity of such plants after irrigation by the treated water.

MATERIALS AND METHODS

Wastewater Sampling

Wastewater effluent of sewage station at Kafr El-Zayaat City, Egypt was used. The water samples were collected before treatment in clean and well stopered polyethylene bottles. The samples were stored, refrigerated and analyzed within few hours after arrival to the laboratory (El-Gammal, 2003).

Microorganisms

Cyanobacteria (*Anabaena subcylindrica* and *Nostoc muscorum*) were isolated from drain channels in the delta region in Egypt and identified according to Prescott (1978). The two organisms were confirmed for identification by comparison with the same those from Trebon Culture Collection, Czech Republic. The nutritive medium of Allen and Stanier (1968) was used for the growth of cyanobacteria at pH 7.8. The inoculum was prepared by dispensing 250 mL of Allen's medium in 500 mL conical flasks and then autoclaved. Inoculation was carried out by one loop of 10 d old cultures followed by incubation at 30°C under continuous light

*Corresponding author; fax +20-20-3350804
e-mail elshounyw@yahoo.com

(2000 lux) for 10 d. The cultures were gased with dry air which contained about 0.3% (v/v) CO_2. Before bubbling into cultures, air was allowed to pass through a series of "Wolf" bottles containing disinfecting solution of 0.1% (w/v) $CuSO_4$, 0.1% (w/v) $HgCl_2$ and ended with sterilized water. The rate of gas was regulated by means of plastic valves (Lorenzen, 1964). Growth of the cyanobacterial cultures or inoculum was assessed at 750 nm to adjust the number of cells. The cultures were centrifuged at 3000g for 10 min under aseptic conditions and the supernatant was discarded. The pellets were resuspended in 1500 mL of sterile wastewater in 2000 mL conical flask. The relative volume of inoculum was 10% (v/v). After cultivation of cyanobacteria on wastewater, all flasks were incubated at 30°C using sterile bubbling for 10 d. At the end of each experiment, the cyanobacterial masses were separated from wastewater by filtration, then the filtered water was taken for further analysis against a control without cyanobacterial cultivation (El-Gammal, 2003). Another set of wastewater was filtered and kept in a refrigerator for irrigation of plants. The cyanobacteria were taken for analysis of chlorophyll, carotenoids and proteins (El-Sheekh et al., 2005).

Cultivation of *Z. mays* Sakha 321 and *P. vulgaris* Giza 6

The clay type of soil, 350 g of dry soil per plastic pot, was used for *Z. mays* and *P. vulgaris* cultivation. All pots were seeded with five seeds per pot, and the plants were grown in a chamber with a day length of 12 h with fluorescent lights (4000 lux) at 30 ± 2°C. The pots were irrigated with the three types of treatments according to the type of water; 1) Tap water as a negative control, 2) Sterilized untreated wastewater as a positive control, and 3) Wastewater treated by cyanobacteria. Ages of plants were from 20 to 22 d after germination. The plants were irrigated every day. All plants were measured for their shoot length, root length, number of lateral roots and percentage of germination, but three plants from each treatment were measured for fresh weight, dry weight, chlorophyll a and b and carotenoid contents, total proteins and carbohydrates.

Physiological Measurements

At the time of sampling, plants were carefully removed from the growth medium and washed thoroughly. Root depth, shoot height, number of lateral roots and percentage of germination were measured. Roots and shoots were separated and weighed immediately, then dried in an oven at 45°C to constant weight. The different photosynthetic pigment fractions of leaves were extracted in 85% (v/v) acetone and measured as described by Metzner et al. (1965). Total soluble proteins of dry shoots and roots were extracted by borate buffer (pH 8.0) and estimated quantitatively using the method of Lowry et al. (1951). Total carbohydrates were determined by the method of Dubios et al. (1956).

Statistical Analysis

One way analysis of variance (ANOVA) was used according to SPSS (1999).

RESULTS AND DISCUSSION

Changes in chlorophyll (chl) a and b and carotenoid contents of *Z. mays* and *P. vulgaris* are presented in Table 1. Statistical analysis showed that irrigation with wastewater treated by *N. muscorum* caused low significant reduction in the pigments content of *Z. mays*, except chl a/b ratio which was significantly increased as compared with the tap water control. Irrigation of *P. vulgaris* by the treated wastewater induced significant increase in the pigments content except chl b that was only slightly increased. Chl a/b ratio of the plants irrigated with the treated wastewater showed significant reduction.

The results shown in Table 2 revealed that irrigation of both plants by the treated wastewater caused insignificant increase in the pigments content, except chl a/b ratio of *Z. mays* and chl a as well as chl a + b of *P. vulgaris* that showed significant increase.

Table 3 showed that application of the untreated wastewater and the treated wastewater with *N. muscorum* induced a pronounced reduction in protein content of root and carbohydrate content of shoot and root of *Z. mays*, with the exception of shoot protein content that showed significant increase after the treated water application. Irrigation of *P. vulgaris* with the untreated and the treated wastewater caused increases in the measured parameters, except protein content of shoot which decreased in the untreated application. Thus, the treated wastewater appeared to exert more stimulatory effect on most parameters in both plants.

It was obvious that untreated wastewater negatively affected shoot and root protein as well as shoot and root carbohydrate in both plants (Table 4). However, general stimulation in most parameters was observed from the wastewater treated with *A. subcylindrica*. Thus, the recorded values were very close or higher than that of the tap water control. There was insignificant relation between the irrigation of the treated wastewater and the protein and carbohydrate contents of shoot and root of both plants. However, in case of root protein and shoot carbohydrate of *Z. mays* and root carbohydrate of *P. vulgaris,* the relation was highly significant.

Land application is often the most economic means of disposing sewage effluents and sludges. Shortage of water could be partially overcome by identifying new water sources such as treated sewage effluent (Al–Nakshabandi et al., 1996). Furthermore, the chemical composition of soil provides useful information on the feasibility of using sewage water or semi-purified one in the irrigation because the soil solution is the medium for mineral absorption by plant roots (Abou El-Hawa et al., 1998).

The results showed that the measured growth parameters (percentage of germination, seedling length, fresh and dry weights, pigments, protein and carbohydrate of shoot and root) of *Z. mays* and *P. vulgaris* under the irrigation by untreated or treated sewage wastewater were significantly greater than or similar to the ones obtained with tap water. Similar observation was previously recorded by Nelisen et al. (1989), i.e., yields of *P. vulgaris* with secondary effluent irrigation were greater than or similar to yields obtained with well water. Effluent irrigation resulted in variable results for nutrients in plant tissue. Al-Nakshabandi et al. (1996) reported that eggplant yield under the application with

Table 1. Effect of sewage wastewater treated by *N. muscorum* on the pigmentation (mg/g fresh weight) of *Z. mays* (A) and *P. vulgaris* (B) after 21 d of sowing.

Treatment		Chl a	Chl b	Chl a+b	Chl a/b	Carotenoid	Total
Tap water-control	A	1.17 ± 0.03	0.49 ± 0.01	1.66 ± 0.05	2.4 ± 0.01	0.34 ± 0.06	2.0 ± 0.11
	B	1.28 ± 0.11	0.5 ± 0.07	1.78 ± 0.17	2.56 ± 0.15	0.46 ± 0.05	2.24 ± 0.21
Untreated wastewater	A	1.1 ± 0.03	0.40 ± 0.04	1.5 ± 0.07	2.8 ± 0.20	0.30 ± 0.03	1.8 ± 0.10
	B	1.47 ± 0.04	0.69 ± 0.02	2.16 ± 0.05	2.13 ± 0.01	0.45 ± 0.06	2.61 ± 0.11
Treated wastewater	A	1.1 ± 0.03	0.40 ± 0.02	1.5 ± 0.05	2.8 ± 0.04	0.30 ± 0.01	1.8 ± 0.06
	B	1.61 ± 0.25	0.66 ± 0.09	2.27 ± 0.31	2.44 ± 0.03	0.46 ± 0.07	2.73 ± 0.38
Change of unt/tap (%)	A	-5.90	-18.40	-9.60	16.70	-11.70	-10.0
	B	14.80	38.00	21.40	-16.80	-2.20	16.50
Change of tr/tap (%)	A	-5.90	-18.40	-9.60	16.70	-11.70	-10.0
	B	25.80	32.00	27.50	-4.70	0.00	21.90
Change of tr/unt (%)	A	0.00	0.00	0.00	0.00	0.00	0.00
	B	9.50	-4.30	5.10	14.60	2.20	4.60
F-value	A	5.72*	12.04**	8.72*	10.76**	1.04 (N.S)	5.09*
	B	3.67 (N.S)	7.82*	4.69 (N.S)	19.38**	0.01 (N.S)	3.03 (N.S)
LSD at 0.05	A	0.025	0.021	0.045	0.094	0.032	0.073
	B	0.129	0.052	0.167	0.072	0.049	0.210

Minus data indicate the decrease in the percentage of change.
Each value is the mean of three readings ± standard deviation.
N.S, insignificant; tr, treated; unt, untreated. *P ≤0.05, **P ≤0.01, ***P ≤0.001.

Table 2. Effect of sewage wastewater treated by *A. subcylindrica* on the pigmentation (mg/g fresh weight) of *Z. mays* (A) and *P. vulgaris* (B) after 21 d of sowing.

Treatment		Chl a	Chl b	Chl a+b	Chl a/b	Carotenoid	Total
Tap water-control	A	1.13 ± 0.12	0.42 ± 0.05	1.55 ± 0.11	2.69 ± 0.18	0.39 ± 0.04	1.94 ± 0.20
	B	1.32 ± 0.10	0.51 ± 0.09	1.83 ± 0.19	2.59 ± 0.22	0.44 ± 0.04	2.27 ± 0.23
Untreated wastewater	A	1.09 ± 0.26	0.33 ± 0.12	1.42 ± 0.34	3.36 ± 0.35	0.34 ± 0.09	1.76 ± 0.47
	B	1.49 ± 0.01	0.64 ± 0.06	2.13 ± 0.07	2.33 ± 0.23	0.50 ± 0.04	2.63 ± 0.11
Treated wastewater	A	1.36 ± 0.07	0.47 ± 0.05	1.83 ± 0.12	2.89 ± 0.19	0.42 ± 0.04	2.25 ± 0.15
	B	1.60 ± 0.11	0.62 ± 0.04	2.22 ± 0.14	2.59 ± 0.12	0.48 ± 0.04	2.70 ± 0.18
Change of unt/tap (%)	A	-3.50	-21.40	-8.40	24.90	-12.80	-9.30
	B	12.90	25.50	16.40	-10.00	13.60	15.90
Change of tr/tap (%)	A	20.40	11.90	18.90	7.40	7.70	15.90
	B	21.20	21.60	21.30	0.00	9.10	18.90
Change of tr/unt (%)	A	24.80	42.40	28.90	-13.90	23.50	27.80
	B	7.40	-3.10	4.20	11.20	-4.00	2.70
F-value	A	0.77 (N.S)	2.49 (N.S)	2.37 (N.S)	5.44*	1.39 (N.S)	2.02 (N.S)
	B	7.71*	3.47 (N.S)	6.27*	1.74 (N.S)	1.75 (N.S)	4.95 (N.S)
LSD at 0.05	A	0.256	0.066	0.198	0.206	0.049	0.251
	B	0.070	0.052	0.114	0.158	0.033	0.145

Minus data indicate the decrease in the percentage of change.
Each value is the mean of three readings ± standard deviation.
N.S, insignificant; tr, treated; unt, untreated. *P ≤0.05, **P ≤0.01, ***P ≤0.001.

treated effluent of As-Samra wastewater stabilization ponds in Jordan was twice the average eggplant production with fresh water irrigation using conventional fertilizer.

The data further indicate that the increase in plant growth and productivity as a result of sewage wastewater irrigation may be due to the presence of high levels of essential nutrients such as nitrogen, phosphorus and potassium in wastewater (El-Gammal, 2003). This observation is in agreement with Pescod (1992), Al-Nakshabandi et al. (1996) and Sekiranda and Kiwanuka (1997) who mentioned that increase in yield and plant growth was attributed to the presence of high levels of essential nutrient elements in wastewater. Investigators showed that the application of sewage enhanced crop yield (Al-Nahidh and Gomah, 1991; Butorac et al., 1995) and improved the soil structure (Pagliai and Vittori, 1993; Abou El-Hawa et al., 1998), because waste products are rich in nitrogen, potassium and organic matter.

On the other hand, Ramirez-Saad et al. (1992) found that during the first months of growth of *Casuarina*, wastewater induced dwarf seedlings. The depressed growth parameters, in some cases, of the two plants under the effect of irrigation by wastewater may be attributed to the presence of heavy metals in wastewater (El-Sheakh et al.,

Table 3. Effect of sewage wastewater treated by *N. muscorum* on the protein and carbohydrate contents (mg/g dry weight), of *Z. mays* (A) and *P. vulgaris* (B) after 21 d of sowing.

Treatment	Shoot protein		Root protein		Shoot carbohydrate		Root carbohydrate	
	A	B	A	B	A	B	A	B
Tap water-control	28.0 ± 0.2	22.6 ± 2.4	22.2 ± 1.6	12.1 ± 0.1	82 ± 1.3	64.9 ± 2.1	131 ± 0.4	101 ± 3.0
Untreated wastewater	28.2 ± 0.0	18.1 ± 1.9	19.1 ± 0.2	13.7 ± 1.6	65 ± 2.3	67.3 ± 1.9	108 ± 4.0	120 ± 2.0
Treated wastewater	28.0 ± 3.8	23.4 ± 1.5	19.6 ± 0.3	14.9 ± 1.2	79 ± 1.7	69.7 ± 2.4	139 ± 1.0	139 ± 5.3
Change of unt/tap (%)	0.7	-19.9	-13.9	13.2	-20.7	3.7	-17.6	18.8
Change of tr/tap (%)	0.0	3.5	-11.7	23.1	-3.7	7.4	6.1	37.6
Change of tr/unt (%)	-0.7	29.3	2.6	8.8	21.5	3.6	28.7	15.8
F-value	0.01 (N.S)	6.19*	8.71*	4.43 (N.S)	75.08***	3.76 (N.S)	135.84***	80.33***
LSD at 0.05	1.79	1.60	0.78	0.94	1.48	1.75	1.95	3.02

Minus data indicate the decrease in the percentage of change.
Each value is the mean of three readings ± standard deviation.
N.S, insignificant; tr, treated; unt, untreated. *P ≤0.05, **P ≤0.01, ***P ≤0.001.

Table 4. Effect of sewage wastewater treated by *A. subcylindrica* on the protein and carbohydrate contents (mg/g dry weight), of *Z. mays* (A) and *P. vulgaris* (B) after 21 d of sowing.

Treatment	Shoot protein		Root protein		Shoot carbohydrate		Root carbohydrate	
	A	B	A	B	A	B	A	B
Tap water-control	27.1 ± 0.3	23.4 ± 1.3	16.9 ± 0.1	16.1 ± 0.8	79.3 ± 7.5	63.7 ± 3.3	128 ± 17.0	96.1 ± 0.3
Untreated wastewater	27.0 ± 0.8	20.2 ± 1.0	16.5 ± 0.3	15.3 ± 0.4	54.0 ± 2.0	61.3 ± 1.8	131 ± 5.0	80.5 ± 3.8
Treated wastewater	26.2 ± 0.1	23.0 ± 2.7	21.2 ± 0.1	16.0 ± 0.1	67.7 ± 0.6	63.7 ± 2.4	128 ± 14.0	106.9 ± 6.5
Change of unt/tap (%)	-0.4	-13.7	-2.4	-4.9	-31.9	-3.8	2.3	-16.2
Change of tr/tap (%)	-3.3	-1.7	25.4	-0.6	-14.6	0.0	0.0	11.2
Change of tr/unt (%)	-2.9	13.9	28.5	4.6	25.4	3.9	-2.3	32.8
F-value	2.96 (N.S)	2.80 (N.S)	571.80***	2.13 (N.S)	23.85***	0.85 (N.S)	0.05 (N.S)	27.92***
LSD at 0.05	0.41	1.49	0.15	0.43	3.67	2.11	10.65	3.55

Minus data indicate the decrease in the percentage of change.
Each value is the mean of three readings ± standard deviation.
N.S, insignificant; tr, treated; unt, untreated. *P ≤0.05, **P ≤0.01, ***P ≤0.001.

2005). Toxicity by heavy metals depresses photosynthesis, causes inhibition of growth and decreases nutrient uptake and transport (Fernands and Henriqes, 1991; Greger and Bertell, 1992).

Plants can evolve several tolerance mechanisms to avoid metal toxicity. For example, copper may be trapped outside of the plant by organic acids exudated by the plant (Macnair, 1981). Cadmium and copper may be also trapped by intracellular metal-binding compounds, such as phytochelatines or metallothionein-like cysteine-rich polypeptides (Steffens, 1990). Al-Nakshabandi et al. (1996) reported that there was no appreciable difference in heavy metal concentration in plant tissues under fresh or effluent irrigation water. These observations were in accordance with the findings of El-Gammal (2003) and El-Sheekh et al. (2005) who recorded high concentrations of heavy metals in wastewater treated with *N. muscorum*, but in the same time, internal concentration of heavy metals in the plants did not vary.

ACKNOWLEDGMENT

We thank Prof. Dr. Yasein El-Sudany, Faculty of Education, Kafr El-Shaeck branch, Tanta University, Egypt, for the statistical analysis of the data.

Received October 10, 2005; revised Arpil 5, 2001; accepted May 2, 2006.

LITERATURE CITED

Abou El-Hawa M, Abd El-Ghany W, Daif H (1998) Biological and chemical changes of the soil irrigated with different water qualities. Bull Fac Sci Zagazig Univ 20: 1-12

Allen MM, Stanier ST (1968) Selective isolation of blue green algae from water and soil. J Gen Microbiol 51: 203

Al-Nahidh SI, Gomah AHM (1991) Response of wheat to dual inoculation with VA-*mycorrhiza* and *Azospirillum*, fertilized with NPK and irrigated with sewage effluent. Arid Soil Res Rehabilitation 5: 83-96

Al-Nakshabandi GA, Saqqar MM, Shatanawi MR, Fayyad M, Al-Horani H (1996) Some environmental problems associated with the use of treated wastewater for irrigation in Jordan. Ag Water Manag 34: 81-94

Ammar MS, Azab MS, Muharram MM (2000) The possible application of microbiologically desalinated sea water in crop irrigation in Egypt. Egypt J Biotechnol 8: 88-97

Asha J, Dutta SA, Juwarkar A (1990) Impact of distillery effluent application to land on soil microflora. Environ Monitoring Assessment 15: 201-210

Dubois M, Gilles KA, Hamilton JK, Repers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 18: 350-356

El-Gammal EWE (2003) Role of some bacteria and cyanobacteria in treatment of polluted water. M.Sc. Thesis. Botany Department, Faculty of Science, Tanta University, Tanta

El-Sheekh MM, EL-Shouny WA, Osman MEH, El-Gammal EWE (2005) Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. Environ Toxicol Pharmacol 19: 357-365

Fernandes JC, Henriques FS (1991) Biochemical, physiological, and structural effects of excess copper in plants. Bot Rev 57: 246-273

Greger M, Bertell G (1992) Effects of Ca^{2+} and Cd^{2+} on the carbohydrate metabolism in sugar beet (*Beta vulgaris*). J Exp Bot 43: 167-173

Jimenez CB, Chavez MA (1997) Treatment of Mexico city wastewater for irrigation purposes. Environ Tech 18: 721- 730

Koltukova NV, Mendzhul MI, Zabolotny GM, Koshe MI (l995) Choice of cyanobacteria cultures for tertiary treatment of sewage of alcohol-yeast production. Microbiol ZH 57: 73-82

Lorenzen H (1964) Synchronization of *Chlorella* with light dark changes and periodical dilution to a standard cell number, *In* E Zeiten, ed, Synchrony in Cell Division and Growth. Wiley Inerscience, New York, pp 571-578

Lowry OH, Rosebrough NJ, Farr LA, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275

Macnair MR (1981) The uptake of copper by plants of *Mimulus guttatus* differing in genotype primarily at a single major copper tolerance locus. New Phytol 88: 723-730

Ministry of Health and Population, World Health Organization (1982) Law 48 to protect the River Nile and water canals from Pollution part 6, Table 61 and Table 66

Nelisen GH, Stevenson DS, Fitzpatrick JJ, Brownlee CH (1989) Yield and plant nutrient content of vegetables trickle-irrigated with municipal wastewater. Hortsci 24: 249-252

Pagliai M, Vittori AL (1993) Influence of waste organic matter on soil micro and macrostructure. Bioresource Technol 43: 205-213

Pescod MB (1992) Wastewater Treatment and Use in Agriculture. FAO Irrig Drain Paper 47, FAO, Rome, pp 125

Prescott GW (1978) How to Know Freshwater Algae. Ed 3, Wm C Brown Company Publishers, Dubuque

Ramirez-Saad H, Valdes-Ramires M, Cruz-Cisneros R (1992) Inoculation and irrigation with wastewater of Casuarina in nurseries. Turrialba 42: 476- 481

Ranalli A, Mattia G, de-Mattia G (1996) Role of piloted biological processes in the purification of oil mill wastewater. Rivista Italiana delle Sostanze Grasse 73: 61-66

Sekiranda SBK, Kiwanuka S (1997) A study of nutrient removal efficiency of *Phragmites mauritianus* in experimental reactors in Uganda. Hydrobiologia 364: 83-91

SPSS (1999) SPSS Base of 10 User's Guide. SPSS, Chicago

Steffens JC (1990) The heavy metal-binding peptides of plants. Ann Rev Plant Physiol Plant Mol Biol 41: 553-575

Tsadilas CD, Chatzoulakis KS (1997) Irrigation of corn with municipal wastewater. Acta Horticulturae 449: 699-705

Wu ZB, Xia YC, Zhang YY, Deng TQ, Chen XT, Zhan FC, Wang DM, Bhamidimarri R, Li X, Liu S (1993) Studies on the purification and reclamation of wastewater from a medium-sized city by an integrated biological pond system. Water Sci Technol 28: 209-216