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ORIGINAL PAPER

Treatment of mixed domestic–industrial wastewater using cyanobacteria

Ebtesam El-Bestawy

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Abstract Alexandria Sanitary Drainage Company (ASDCO), Alexandria, Egypt has two primary treatment plants, the eastern and the western wastewater treatment plants (EWTP and WWTP) that receive mixed domestic–industrial influents and discharge into L. Mariut. The lake is subjected therefore to severe levels of pollution and dominated by members of cyanobacteria that can cope with the high pollution load in the lake water. Isolation and utilization of the locally generated cyanobacterial biomass for remediation processes of highly toxic pollutants offers a very efficient and cheap tool for governmental or private industrial activities in Alexandria and will generate a source of revenue in Egyptian localities. The main objective of the present study was to investigate the biodegradation and biosorption capacity of some potential cyanobacterial species dominating the lake ecosystem toward organic and inorganic contaminants polluting the primary-treated effluents of the EWTP and WWTP. The primary effluents were subjected to biological treatment using three axenic cyanobacterial strains (*Anabaena oryzae, Anabaena variabilis* and *Tolypothrix ceytonica*) as batch system for 7 days. Removal efficiencies (RE) of the different contaminants were evaluated and compared. Results confirmed the high efficiencies of the investigated species for the removal of the target contaminants which were species and contaminant-dependent. BOD_5 and COD recorded 89.29 and 73.68% as maximum RE(s) achieved by *Anabaena variabilis* and *Anabaena oryzae*, respectively. The highest RE of the TSS recorded 64.37% achieved by *Tolypothrix ceytonica*, while 38.84% was recorded as the highest TSD RE achieved by *Anabaena variabilis*. *Tolypothrix ceytonica* also exhibited the highest RE for FOG recorded 93.75%. Concerning the contaminant metals, *Tolypothrix ceytonica* showed the highest biosorption capacity where 86.12 and 94.63% RE were achieved for Zn and Cu, respectively. In conclusion, results of the present study confirmed the advantageous potential of using the tested cyanobacterial species for the treatment of contaminated wastewater. Results also clearly showed the quality improvement of the discharged wastewater which in turn will eliminate or at least minimize the expected deterioration of the receiving environment.

Keywords Biodegradation · Bioaccumulation · Cyanobacteria · Domestic · Organic matter · Heavy metals · Industrial · Wastewater treatment

E. El-Bestawy (✉)
Department of Environmental Studies,
Institute of Graduate Studies and Research,
Alexandria University, 163 Horria Ave. El-Shatby,
P.O. Box 832, Alexandria, Egypt
e-mail: ebtesamelbestawy@yahoo.com

Introduction

The domestic–industrial, primary-treated effluents of EWTP and WWTP, Alexandria discharge directly into L. Mariut, the close brackish water lake located south of Alexandria. Lake Mariut is classified as a hypertrophic lake according to nitrogen and phosphorus levels (N and P). Beside nutrients, the lake water and sediments showed terrible levels of biodegradable organic matter, pathogenic organisms, fertilizers, pesticides and heavy metals exceeding those reported in the highly polluted lakes worldwide. This is mainly due to continuous discharge of huge quantities of the primary-treated effluents as well as untreated agricultural wastewater which lead to massive deterioration in the water quality of this lake [18–20, 37].

The consequences manifested by amplification of the levels of such pollutants with time and the shift of bacterial and algal populations toward more resistant species such as the planktonic cyanobacteria that dominate the lake water especially in the warm seasons. These species characterized by great ability to tolerate such high levels of pollution and proved high efficiency for degrading highly persistent organic contaminants [20, 37] as well as accumulating heavy metals [1]. Therefore, they could be efficiently used in advanced technologies for bioremediation of the EWTP and WWTP effluents and recreation of L. Mariut.

Being the base for all food chains and primary production, aquatic phytoplankton play a key and indispensable role in the cycling of elements and pollutants in any ecosystem. Cyanobacteria (blue-green algae) have a great deal of potential as a source of fine chemicals, as a bio-fertilizer, and as a source of renewable fuel [35]. Recently, there has been increasing inertest about using cyanobacteria as pollution control agents since they possess many advantages over other microorganisms isolated from soil. Their photoautotrophic nature and the ability of some species to fix atmospheric nitrogen enable them to be producers, as opposed to consumers, and make their growth and maintenance inexpensive [7]. Cyanobacteria are also known to inhabit various aquatic polluted and heavily polluted environments where they widely distributed and dominate the micro floral populations [25, 27, 57]. They acquired natural resistance and selectivity against environmental pollutants due to their presence in polluted systems while their viability and metabolic activity are not affected by the decrease in the levels of the biodegradable pollutants that they may break down. Cyanobacteria have been shown to be highly effective as accumulators and degraders of different kinds of environmental pollutants, including pesticides [20, 37, 41], crude oil [2, 3, 57] naphthalene [9, 10], phenanthrene [44], phenol and catechol [22, 54] and xenobiotics [42]. Cyanobacteria have been used efficiently as a low-cost method for remediating dairy wastewater [36], dissolved inorganic nutrients from fish farms [17] and nutrients (N and P) [14, 15, 30, 45, 48]. They were also reported as efficient agents for the assimilation of organic matter from contaminated media [4, 43, 60] as well as transformation and removal of heavy metals [4, 34, 49]. Not only the polluted wastewater, but also in natural aquatic environments and soil cyanobacteria developed as mats have been successfully used in bioremediation of oil spills in different parts of the world [12, 50, 51, 56].

Remediation capabilities of cyanobacteria toward environmental pollutants can be improved and enhanced through genetic engineering technologies [31] to allow them to be used as economic and low-maintenance technology for contaminated systems. However, the beneficial application of cyanobacteria in remediation of contaminated waters, either natural aquatic environment, domestic or industrial effluents, is still not optimally manipulated. Application of bioremediation using indigenous microorganisms such as cyanobacteria for decontamination of the domestic and industrial effluents polluted with organic contaminants and heavy metals provides a viable and sustainable approach for environmental resources. The main objective of the present study was to investigate the remediation capacity of some potential cyanobacterial species isolated from L. Mariut toward different contaminants polluting the primary-treated domestic and industrial effluents of the EWTP and WWTP.

Materials and methods

Microorganisms

In the present study, three different cyanobacterial species; two *Anabaena spp. (Anabaena oryzae, Anabaena variabilis)* and *Tolypothrix ceytonica* (Fig. 1); were investigated as free-living cells for their ability for organic matter biodegradation and heavy metal removal from the primary-treated effluents of the EWTP and WWTP. They were selected based on their dominance and survival in the highly polluted water of L. Mariut where they acquired high resistance and acclimatized to deal with high loads of different contaminants. They were also proven high ability for degradation of the highly persistent chlorinated hydrocarbon Lindane [20] and heavy metals [1]. Therefore, the selected species were considered promising candidates for biological treatment of the mixed domestic and industrial effluents. They were kindly provided as axenic strains by the algal culture collection at the Mansoura University where they were identified using the classical methods.

Axenity (purification) of the strains

Cyanobacteria are mostly associated with bacteria that live in symbiotic relations. Since bacterial contamination in the miscellaneous sheath of cyanobacteria can affect the results leading to artifacts, it is very important to use axenic strains. During the present study, the selected species were provided as axenic cultures. However, before using these strains in the bioremediation of the contaminated wastewater, their axenity was checked using agar phototactic response method. Semi solid standard agar medium was prepared and aliquoted into test tubes and sterilized. Each tube was inoculated with 100 µl of cyanobacterial culture (two replicates per culture). Light was prevented to reach the top 10 cm of the tube using aluminum foil. All the tubes were incubated in optimal conditions (25–35 °C) in an illuminated incubator. Based on the phototactic response

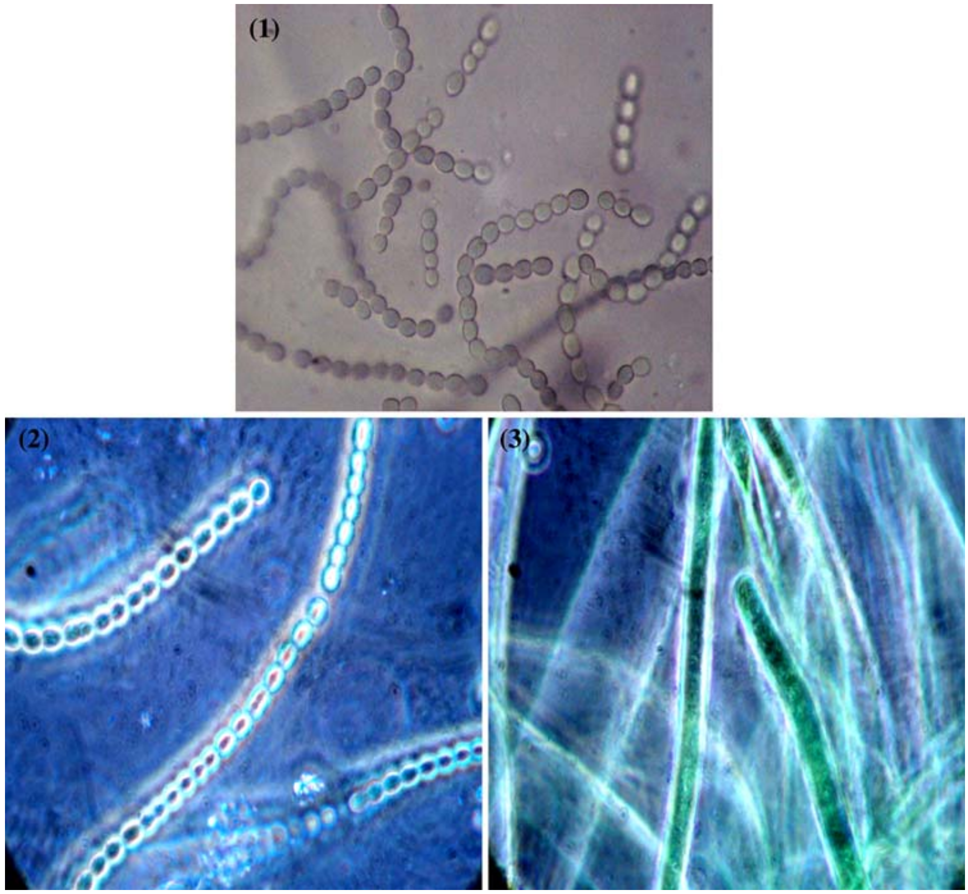

Fig. 1 Selected cyanobacteria used for the treatment of domestic and industrial effluents. **1** *Anabaena variabilis,* **2** *Anabaena oryzae* and **3** *Tolypthrix ceytonica*

phenomena the cyanobacterial filaments were grown toward light direction through the semi solid agar, but bacteria did not. After incubation of 7 days the agar column was dragged out the tube on sterilized Petri dish. The agar column was sliced into ten slices 1 cm per each. Each slice was stranded longitudinally and transversally cut under common sterilized conditions to separate the algal filaments surrounded with a small piece of agar. Each agar piece involving cyanobacterial growth was inoculated into standard selective liquid medium. After incubation, each inoculated culture was tested for contamination using general bacterial medium (nutrient agar). If the culture was contaminated the same procedure should be repeated again (in most cases only one repeat is efficient).

Growth medium and culturing conditions

The selected cyanobacterial species were grown in MBL selective medium. It consists of solutions A (macronutrients) and B (micronutrients). Solution A contained (in g/l): $CaCl_2.H_2O$, 36.76; $MgSO_4.7H_2O$, 36.97; $NaHCO_3$, 12.60; K_2HPO_4, 8.71; $NaNO_3$, 85.01 and $Na_2SiO_3.9H_2O$, 28.42. Solution B contained (in g/l): $Na_2.EDTA$, 4.36; $FeCl_3$. 6H2O, 3.15; $CuSO_4.5H_2O$, 0.01; $ZnSO_4.7H_2O$, 0.022; $CoCl_2.6H_2O$, 0.01; MnCl2.4H_2O, 0.18; $NaMoO_3.2H_2O$, 0.006 and the pH was adjusted to 7.2 with HCl. Each component of solution A was separately prepared as stock solution while all the components of solution B were prepared as a mixture. Solution A components were sterilized by autoclaving separately at 121 °C for 20 min. Micronutrient solution was sterilized by filtration through 0.22 μm polycarbonate membrane to avoid interaction and precipitation of heavy metals. MBL medium was freshly prepared from A and B where 1.0 ml of each component of solution A and 1.0 ml of Solution B were combined and diluted to 1.0 l, sterilized as mentioned and used for selective culturing of the selected species. After inoculation, all the selected species were incubated at room temperature (25–35 °C) and day light with manual shaking every 24 h to avoid adhesion of the algae on the walls of the glass vessels until heavy growth appeared within 2 weeks.

Sampling of wastewater

Samples were collected from the primary treated, mixed domestic–industrial effluents of the EWTP and WWTP, Alexandria. Thus, it is expected that their final effluents contain industrial pollutants such as heavy metals which are not likely to be removed by that kind of treatment. Grab samples representing all wastes entering the plant during

24 h were collected from both plants to avoid the fluctuation in the flow and the strength of the influent.

Characterization of wastewater

Wastewater quality parameters included biochemical oxygen demand (BOD_5); chemical oxygen demand (COD); total suspended solids (TSS); total dissolved solids (TDS); fat, oil and grease (FOG) and two heavy metals (Zn, Cu) were characterized before and after treatment to determine the effectiveness of the remediation process. Among the heavy metals, Cu and Zn were selected because their average levels in the primary-treated effluents were higher than the maximum permissible limits (MPL) stated by the Egyptian Environmental Laws (48/1982 and 4/1994) for the safe discharge into water courses. All the investigated parameters were determined using the standard techniques described by Celesseri et al. [8] in the standard methods for the examination of water and wastewater. Natural microbial growth in the tested wastewater composed of enterobacteria (Coliform bacteria) which was routinely determined at both the treatment plants. Both total and fecal coliform annual average levels for both plants were supplied by the central lab of the company and are mentioned in the results and discussion section.

Remediation bioassay

As mentioned before, prior to bioremediation bioassay, the tested species were checked for their axenity, and the liquid cultures were tested for the presence of heterotrophic bacteria microscopically and by plating on bacterial nutrient medium (nutrient agar, Difco, UK) and incubating at 30 °C for 1 week. Only axenic cultures were involved in the assays. The selected species were inoculated individually in 100 ml culturing medium (three replicates) and incubated for 2 weeks till heavy growth was obtained. Wastewater from both plants (EWTP and WWTP) was dispensed (900 ml each) in 18 sterilized conical flasks (2 l), nine flasks for each effluent. Each culture (100 ml) was separately seeded into the wastewater from both plants (EWTP and WWTP) at a final volume of 1 l each (three replicates/strain/effluent) and incubated under the previously mentioned conditions for 7 days. Another six flasks (three flasks for each plant) were supplied by 1.0 l each of the wastewater of both plants without seeding with cyanobacteria to serve as control for the bioassay. They were incubated under the same conditions, sampled and characterized at the same intervals as the treated wastewater. Bioremediation bioassay was performed exactly as a simulation to the conditions applied to large scale wastewater treatment process (day light, no agitation and ambient temperature). For the determination of heavy metals and other parameters residues, samples were collected at 24 h interval. At each sampling time, 130 ml from each flask were aseptically drawn, where all the investigated parameters were determined and their removal efficiencies using the selected species were calculated.

Results and discussion

Wastewater characteristics

Primary-treated wastewater produced by the two plants was characterized (Table 1). BOD_5, COD, TSS, TDS, FOG, Zn and Cu recorded averages of 155, 380, 184, 1250, 22, 0.1779 and 0.0577 mg/l, respectively, in the primary-treated wastewater of the EWTP. Significantly higher levels for almost all the tested parameters were detected in the WWTP effluent especially organic content, solids and oil and grease where 280, 519, 435, 1609, 32 mg/l were recorded as average levels for BOD_5, COD, TSS, TDS and FOG, respectively. This is mainly attributed to the fact that WWTP serves wider sectors of Alexandria and overloaded with much more quantities of industrial effluents from Alexandria West where more than 80% of industrial activities centralized. However, Zn recorded much lower average in the WWTP effluent (0.0564 mg/l) compared to that of the EWTP (0.1779 mg/l) while no significant differences were recorded in the Cu levels among the two plants (0.0528 and 0.0577 mg/l in the WWTP and EWTP effluents). Dilution with the huge quantities if wastewater is the main reason behind the low metal levels recorded in the WWTP effluent.

Since they are mainly domestic wastewater mixed with some untreated industrial effluents with different proportions, both effluents are dominated by coliform bacteria which are monitored daily at both plants (total and fecal coilform TC and FC). Also the high nitrogen and phosphorus content in both effluents [21] along with the toxic industrial contaminants suppressed the growth of cyanobacteria or any other algae (as no growth was obtained when inoculated in selective medium). These results are consistent with Ernst et al. [24] where high concentration of nitrate and phosphate in the mixed domestic–industrial effluents found attributed to the disappearance of cyanobacterial members which require nitrate and phosphate concentrations similar to those found in the natural environment. Therefore, only members of enterobacteria are considered partially responsible for the removal of some contaminants in the present study. The annual 2007 averages of TC and FC (supplied by the monitoring and data record unit, central lab of the drainage company) recorded 33×10^{10} and 30×10^{10} cell/100 ml, respectively, in the

Table 1 Residue concentrations (RC) of the quality parameters from the contaminated EWTP and WWTP effluents using the selected cyanobacteria at different exposure time

Time (days)	Control							*Anabaena oryzae*						
	BOD	COD	TSS	TDS	FOG	Zn	Cu	BOD	COD	TSS	TDS	FOG	Zn	Cu
I. EWTP effluent														
Raw water	155	380	184	1,250	22	0.18	0.06	155	380	184	1,250	22	0.18	0.06
1	99.6	241	122	794	12.4	0.11	0.04	134	360	180	1,243	21	0.16	0.05
2	81.9	200	93.2	659	10.5	0.09	0.03	124	337	176	1,225	19	0.10	0.04
3	77.5	195	90.8	646	8.6	0.09	0.03	118	281	156	1,203	14	0.04	0.02
5	69.7	171	83.5	562	8.6	0.08	0.03	97	180	147	1,189	13	0.04	0.01
6	138	341	167	1,135	15.3	0.12	0.04	42	120	137	1,123	12	0.04	0.01
7	150	360	176	1,210	19.3	0.15	0.05	30	100	125	1,119	9	0.04	0.01
II. WWTP effluent														
Raw water	280	519	435	1,609	32	0.06	0.05	280	519	435	1,609	32	0.06	0.05
1	239	444	375	1,372	27	0.04	0.05	224	440	391	1,509	30	0.05	0.05
2	204	379	333	1,176	23	0.04	0.04	144	360	362	1,493	27	0.05	0.04
3	175	333	275	1,029	18	0.04	0.03	120	320	347	1,480	24	0.04	0.03
5	188	349	293	1,077	19	0.04	0.04	96	280	289	1,369	22	0.04	0.02
6	185	346	306	1,056	21	0.04	0.03	96	240	267	1,249	17	0.02	0.01
7	193	354	324	1,104	26	0.04	0.03	84	220	166	1,141	14	0.02	0.01

Time (days)	*Anabaena variabilis*							*Tolypothrix ceytonica*						
	BOD	COD	TSS	TDS	FOG	Zn	Cu	BOD	COD	TSS	TDS	FOG	Zn	Cu
I. EWTP effluent														
Raw water	155	380	184	1,250	22	0.18	0.06	155	380	184	1,250	22	0.18	0.06
1	130	380	173	1,225	22	0.13	0.06	142	340	183	1,207	19	0.12	0.05
2	118	380	159	1,201	20	0.10	0.04	124	340	178	1,193	16	0.07	0.02
3	84	380	148	1,184	19	0.04	0.01	96	340	164	1,193	13	0.05	0.01
5	78	363	141	1,163	15	0.04	0.01	54	320	158	1,181	11	0.04	0.01
6	48	213	109	1,118	11	0.04	0.01	54	279	136	1,147	10	0.03	0.01
7	24	180	102	1,109	7	0.03	0.01	42	246	133	1,078	8	0.02	0.00
II. WWTP effluent														
Raw water	280	519	435	1,609	32	0.06	0.05	280	519	435	1,609	32	0.06	0.05
1	234	400	409	1,590	29	0.05	0.05	174	380	425	1,590	29	0.06	0.04
2	150	380	397	1,417	25	0.04	0.03	132	355	405	1,537	28	0.05	0.03
3	72	380	369	1,351	17	0.04	0.03	102	280	355	1,461	26	0.04	0.03
5	72	220	329	1,299	16	0.03	0.02	90	280	335	1,381	17	0.02	0.01
6	42	200	210	1,059	15	0.03	0.01	72	240	258	1,108	9	0.02	0.01
7	30	180	199	984	14	0.02	0.01	36	180	155	1,089	2	0.01	0.00

influent of the WWTP which were reduced to 24×10^8 and 24×10^8 due to the preliminary and primary treatment. Again because due to the fact that EWTP effluent is less strong than that of the WWTP, lower averages of TC and FC were recorded. The annual 2007 averages of TC and FC in the EWTP effluent recorded 46×10^7 and 42×10^7 cell/100 ml, respectively, in the influent reduced to 26×10^7 and 24×10^7 due to the preliminary and primary treatment.

Treatability and removal efficiency of wastewater contaminants

Residue levels of the selected quality parameters were determined (Table 1) and the removal efficiencies (RE%) as a result of the biological treatment using the selected species were calculated (Figs. 2–8). As a general trend, the three tested species exhibited positive correlation between their RE% of the all the tested parameters and the exposure

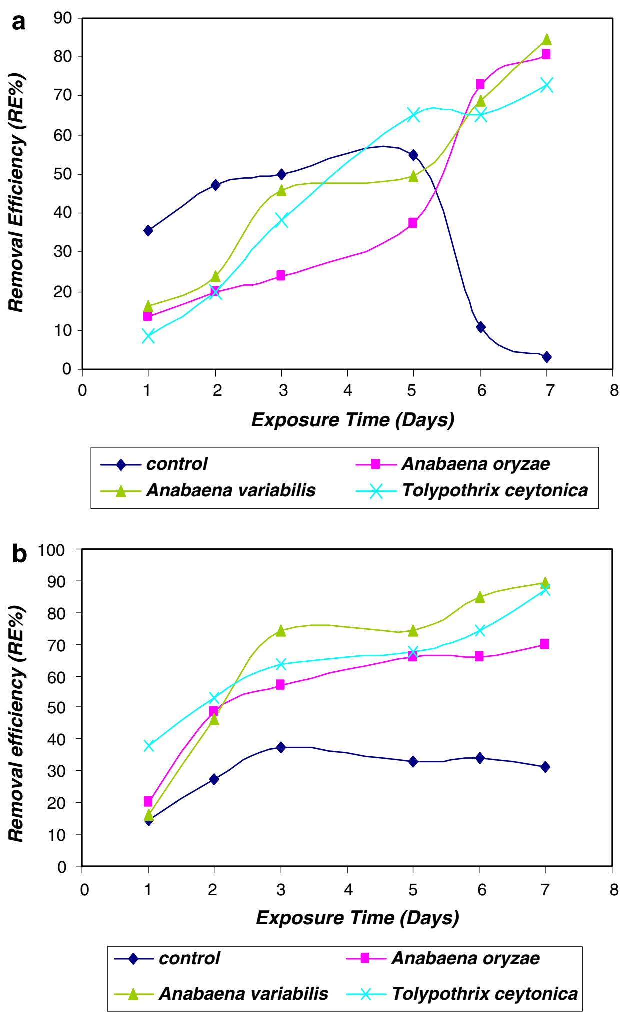

Fig. 2 Removal efficiency (RE%) of BOD_5 from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

time up to the last exposure day for both types of effluents (EWTP and WWTP).

Organic matter removal

Biochemical oxygen demand Removal of BOD_5 from the primary-treated effluents of both plants (Fig. 2a, b) using the selected algae revealed the following points:

1. High REs% were obtained for BOD_5 removal from EWTP effluent by the selected species with *A. variabilis*, considered as the most effective, achieving the highest RE (84.52%) followed by *A. oryzae* (80.65%) and finally *T. ceytonica* (72.9%). *A. variabilis* exhibited higher BOD_5 RE% at the shorter exposures (up to 3rd day) compared to *A. oryzae* and *T. ceytonica*.
2. Despite the RE variations of BOD_5 achieved by the tested species, RC(s) of the BOD_5 in the EWTP final effluent reached acceptable limits (24, 30 and 42 mg/l by *A. variabilis*, *A. oryzae* and *T. ceytonica*, respectively) after 7 exposure of days which is much lower than the maximum permissible limit (MPL) of 60 mg/l stated by the Egyptian Environmental Laws for safe discharge into surface water courses. When these figures compared with those obtained by the control it was showed that the natural microbial population of the effluent achieved a maximum removal of 55% after 5 days equivalent to 69.7 mg/l (> MPL of the BOD) after which there was a sharp decline in the efficiency associated by increasing the RC reaching 150 mg/l and 3.22% RE after 7 exposure of days (Fig. 2a).
3. WWTP effluent is generally much stronger than that of the EWTP. Again *A. variabilis* considered the most effective achieving the maximum BOD RE (89.29%) from WWTP primary effluent followed by *T. ceytonica* (87.14%) compared to (70%) achieved by *A. oryzae*. *T. ceytonica* exhibited higher BOD RE% at the shorter exposures (up to 2nd day) compared to *A. oryzae* and *A. variabilis.*
4. On the 7th day, *A. variabilis* and *T. ceytonica* efficiently brought BOD_5 below the MPL where they achieved residue levels of only 30 and 36 mg/l, respectively, which considered very good quality. On the other hand, *A. oryzae* could reduce the BOD_5 levels from 280 to 84 mg/l (7th exposure day) which is slightly higher than the MPL of the BOD_5 indicating that it require longer time to be compile with the law.
5. As expected lower REs% were achieved by the control culture compared to that of the EWTP due to the high strength of the WWTP. It achieved removal of 37.44 and 31.01% after 3 and 7 days, respectively, equivalent to 175.15 and 193.16 mg/l both of which are much higher than 60 mg/l the MPL (Fig. 2b). This indicates the high efficiency of the tested cyanobacteria for organic matter removal which is attributed to their photoautotrophic nature that allow them to survive and perform their metabolic activities opposed to the heterotrophic bacteria.
6. Comparing BOD_5 removal by the selected cyanobacteria from the two plants revealed very high efficiency for all of them in the degradation of biodegradable organic matter which is stimulated by increasing the levels of the pollutant in the wastewater. This was shown by a maximum BOD RE% of 84.52 achieved by *A. variabilis* from EWTP effluent with initial BOD of 155 mg/l which increased to 89.29% by the same species in the WWTP effluent with initial BOD of 280 mg/l.

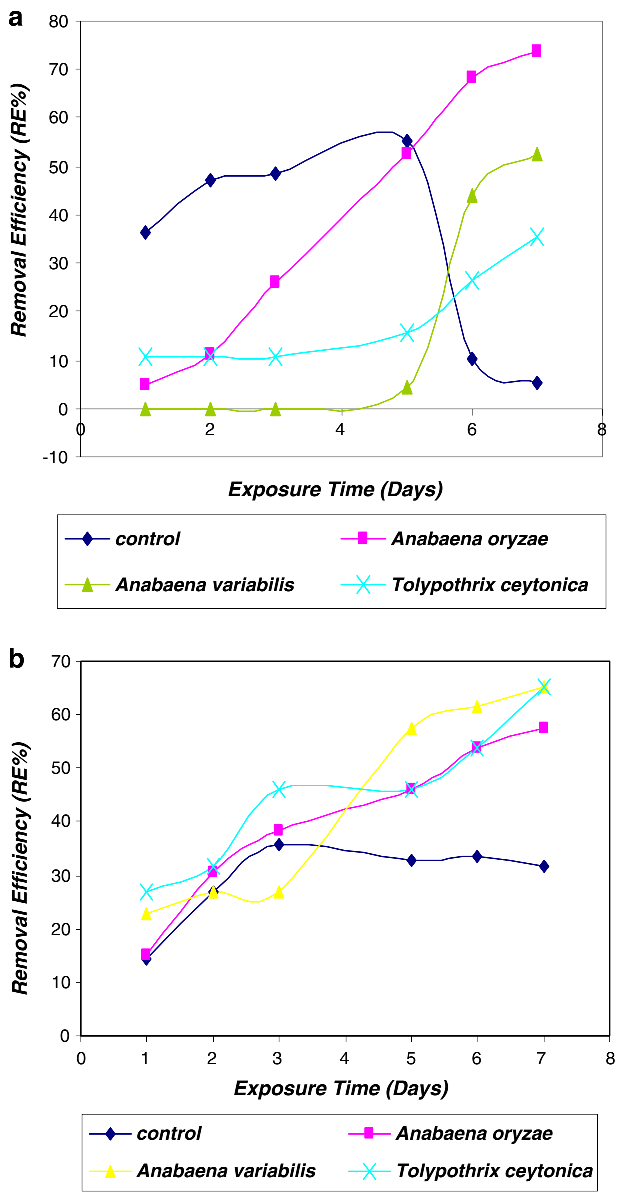

Fig. 3 Removal efficiency (RE%) of COD from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

Chemical oxygen demand Removal of COD from the primary-treated effluents of both plants (Fig. 3a, b) using the selected species revealed the following points:

1. *A. oryzae* considered the most effective for removing COD from the EWTP effluent achieving the maximum RE of 73.68% followed by *A. variabilis* (52.63%) compared to the RE achieved by *T. ceytonica* (35.26%) after 7 exposure days. However, *T. ceytonica* exhibited higher COD RE% within the first 24 h compared to *A. oryzae* and *A. variabilis*.
2. The lowest residue concentration of 100 mg/l was achieved by *A. oryzae* which is the maximum acceptable limit stated by the law (MPL for COD = 100 mg/l) while *A. variabilis* and *T. ceytonica* could not bring the COD levels of the EWTP effluent to better quality. They recorded 180 and 246 mg/l, respectively, and required longer exposures. The highest RE% achieved by the control culture recorded 55.11% (170.59 mg/l) after 5 exposure days declined to 5.25% (360.06 mg/l) after seven days recording unsafe quality for discharging without treatment with the efficient agents(Fig. 3a).
3. Concerning WWTP effluent, higher COD average level was detected (519 mg/l) compared to 380 mg/l in the EWTP effluent. *A. variabilis* and *T. ceytonica* considered the most effective achieving higher RE of 65.32% for both effluents after 7th day compared to 57.61% obtained by *A. oryzae*. *T. ceytonica* exhibited higher COD RE% at the shorter exposures (up to 3rd day) compared to *A. oryzae* and *A. variabilis.*
4. Similar to BOD removal, the natural microorganisms in the control culture were inhibited by the high strength of the WWTP effluent leading to reduction in the COD removal. It achieved 35.89% as a maximum RE after 3 days (332.7 mg/l) followed by regular decline reaching 31.77% after 7 days (354.11 mg/l) indicating more than threefolds increase than the MPL for COD (Fig. 3b).
5. Although high REs% of the COD were achieved by the selected species, none of them could bring the COD levels in the WWTP final effluent below the MPL during the investigated exposure time (1 week). This may be attributed to the need for longer time for achieving the proper quality. It could also result from the inhibition in cyanobacterial growth due to the higher COD levels in the WWTP effluent compared to that of the EWTP.

Solids removal

Total suspended solids (TSS) Removal of TSS from the primary-treated effluents of both plants (Fig. 4a, b) using the selected species revealed the following points:

1. The highest recorded TSS REs% in the EWTP effluent recorded 44.57, 32.1 and 27.72% achieved by *A. variabilis*, *A. oryzae*, and *T. ceytonica* (102, 125 and 133 mg/l RC), respectively, after 7 days. *A. variabilis* also exhibited significantly higher TSS RE% all over the experiment compared to *A. oryzae* and *T. ceytonica* (Fig. 4a).
2. For the strong WWTP effluent, much higher TSS removals were achieved by the selected species (initial TSS = 435 mg/l) compared to those obtained by the same species for the effluent of EWTP (initial

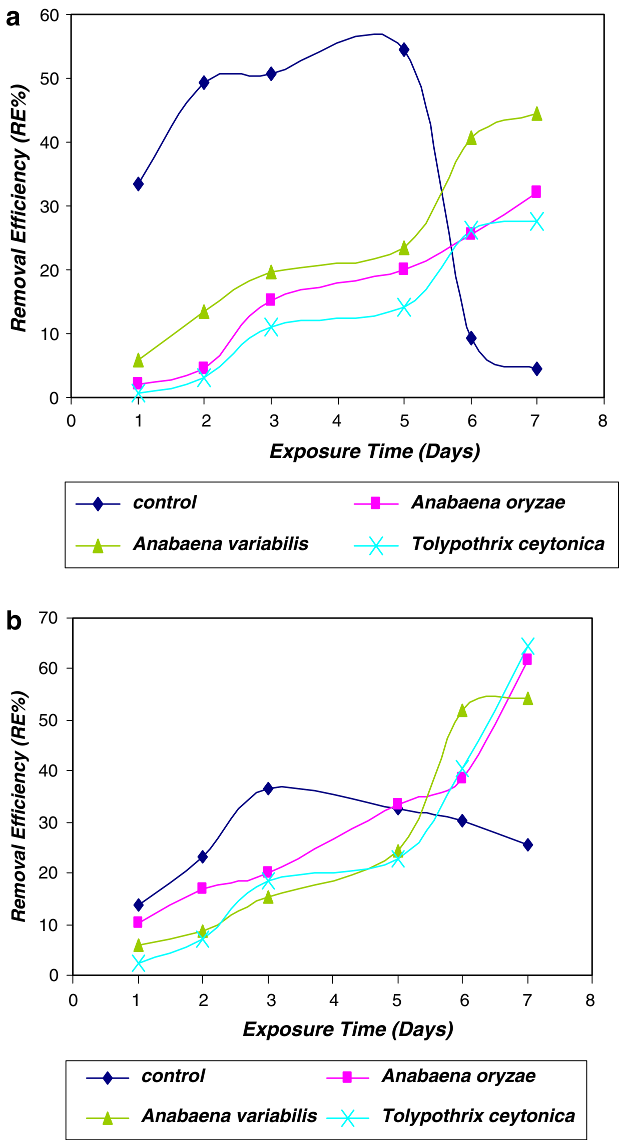

Fig. 4 Removal efficiency (RE%) of TSS from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

TSS = 184 mg/l) indicating stimulation of the enzymatic activity with increasing pollutants levels.

3. TSS REs% recorded 64.37, 61.87 and 54.25 achieved by *T. ceytonica, A. oryzae* and *A. variabilis* (155, 166 and 199 mg/l RC), respectively, after 7 days. For that effluent, *A. oryzae* exhibited higher TSS RE% at the shorter exposures (up to 4th day) compared to *A. variabilis* and *T. ceytonica* (Fig. 4b).
4. According to the law, 60 mg/l is stated as the MPL of the TSS; therefore none of the tested species reached the required efficiency to bring the TSS in the EWTP and WWTP effluents below the MPL during the tested exposure time. This indicates that they required longer time, heavier biomass or different application using the same species to achieve that quality.
5. In contrast to cyanobateria, the indigenous bacteria of the control culture achieved higher TSS removal form the EWTP effluent compared to that of the WWTP effluent. This was shown by maximum REs of 54.61% (after 5 days) and 36.69 (after 3 days) for the EWTP and WWTP effluents, respectively, equivalent to 83.53 and 275.38 mg/l, respectively, both of which are not compile with the law. This was followed by remarkable decline especially for the EWTP effluent reaching 4.36% after 7 days compared to 25.52% for the WWTP effluent (Fig. 3a, b). Therefore, cyanobacterial seeding considered an efficient tool for minimizing contaminant level that can be improved by adopting different application to achieve the desired quality.

Total dissolved solids (TDS) Removal of TDS from the primary-treated effluents of both plants (Fig. 5a, b) using the selected species revealed the following points:

1. The maximum TDS REs% obtained for the EWTP effluent by the tested species ranged between a maximum of 13.76% (1,078 mg/l) achieved by *T. ceytonica* and a minimum of 10.48% (1,119 mg/l) obtained by *A. oryzae* after 7 exposure days. *T. ceytonica* exhibited higher TDS RE% at the shorter exposures (up to 2nd day) compared to *A. variabilis* and *A. oryzae* (Fig. 5a).
2. As expected higher TDS levels were recorded in the effluent of the WWTP (1,609 mg/l) compared to that of the EWTP. Similar to TSS removal, the activity of the tested species was stimulated by the increase in the TDS levels in the WWTP leading to higher REs% by all the species compared to those for the EWTP. *A. variabilis* considered the most effective achieving the highest RE of 38.84% (984 mg/l) followed by *T. ceytonica* recording 32.32% (1089 mg/l) and finally *A. oryzae* with the lowest RE of 29.09% (1141 mg/l) after 7 treatment days (Fig. 5b).
3. Similar behavior for TSS removal was shown by bacteria of the control culture where higher TDS removal was achieved form the EWTP effluent compared to that of the WWTP effluent. 55.01% (561.66 mg/l) after 5 days and 36.0% (1029.76 mg/l) after 3 days were achieved as maximum RE of TDS from EWTP and WWTP effluents, respectively. Then the control cultures showed significant decline in their RE of the TDS from both effluents reaching 3.14% (1210.7 mg/l) and 31.35% (1104.5 mg/l) from EWTP and WWTP effluents after 7 days (Fig. 5a, b).
4. Since the TDS content in the EWTP and WWTP effluents were lower than the MPL of the TDS

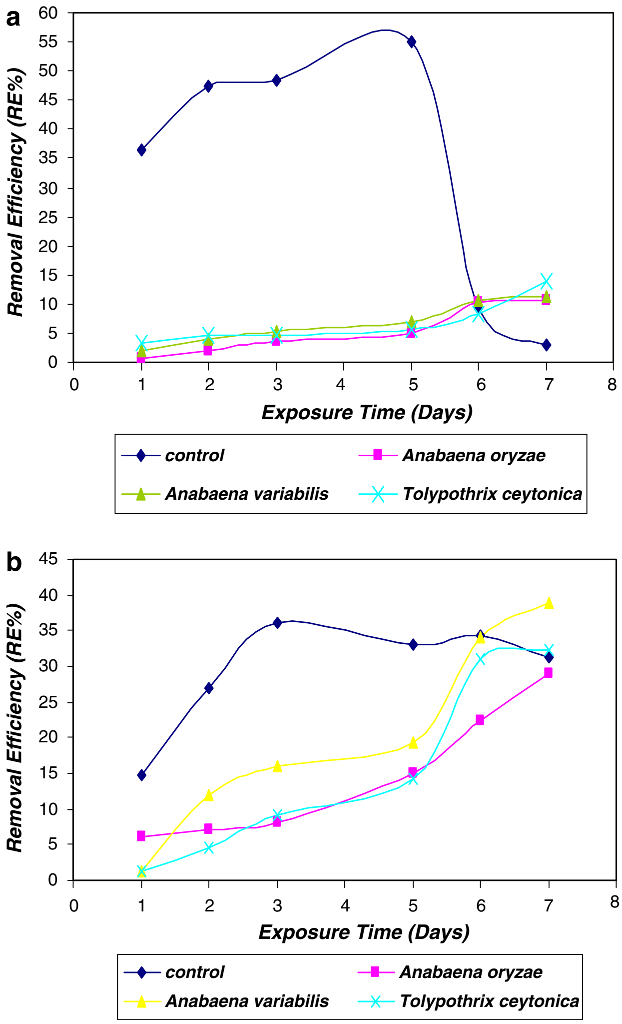

Fig. 5 Removal efficiency (RE%) of TDS from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

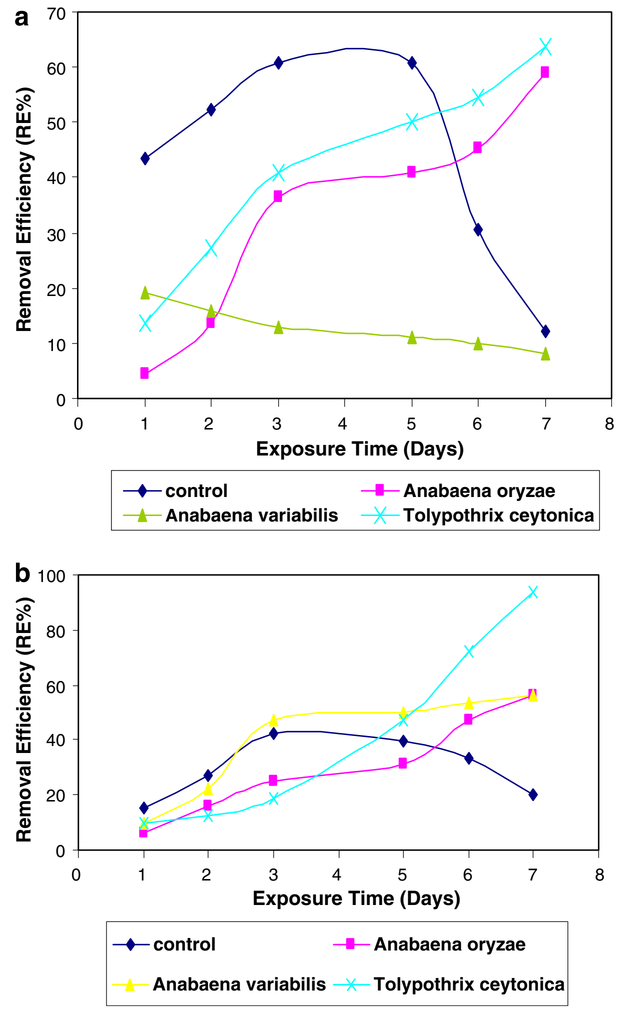

Fig. 6 Removal efficiency (RE%) of FOG from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

(2,000 mg/l), the residual concentrations of the TDS produced in the final effluents by all the tested species as well as the two controls improved and still within the safe range for discharging.

Fat, oil and grease (FOG) removal

Removal of FOG from the primary-treated effluents of both plants (Fig. 6a, b) using the selected species revealed the following points:

1. *A. variabilis* achieved the highest FOG RE of 68.18% from the EWTP effluent (7 mg/l) followed by *T. ceytonica* achieving 63.63% (8 mg/l) and finally *A. oryzae* with 59.1% (9 mg/l) after 7 treatment days. *T. ceytonica* exhibited higher FOG RE% at the shorter exposures (up to 5th day) compared to *A. oryzae* and *A. variabilis.*
2. *A. variabilis, T. ceytonica* and *A. oryzae* efficiently brought FOG below the MPL (15 mg/l) in 6 treatment days recording 11.0, 10.0 and 12.0 mg/l, respectively, which considered very good quality (Fig. 6a).
3. *T. ceytonica* considered the most effective species for WWTP effluent achieving 93.75% FOG RE (2 mg/l), followed by *A. variabilis* and *A. oryzae* both achieved 56.25% RE (14 mg/l) after 7 treatment days. However, both *T. ceytonica* and *A. variabilis* exhibited higher FOG RE% at the shorter exposures (up to 1st and 4th day, respectively) compared to *A. variabilis* (Fig. 6b).
4. Control culture could achieve high FOG RE reaching 60.87% (8.61 mg/l) for the EWTP effluent within 5 exposure days which was reduced to 12.1% after the 7th day (19.34 mg/l). However, the control culture of the WWTP effluent recorded lower efficiencies for

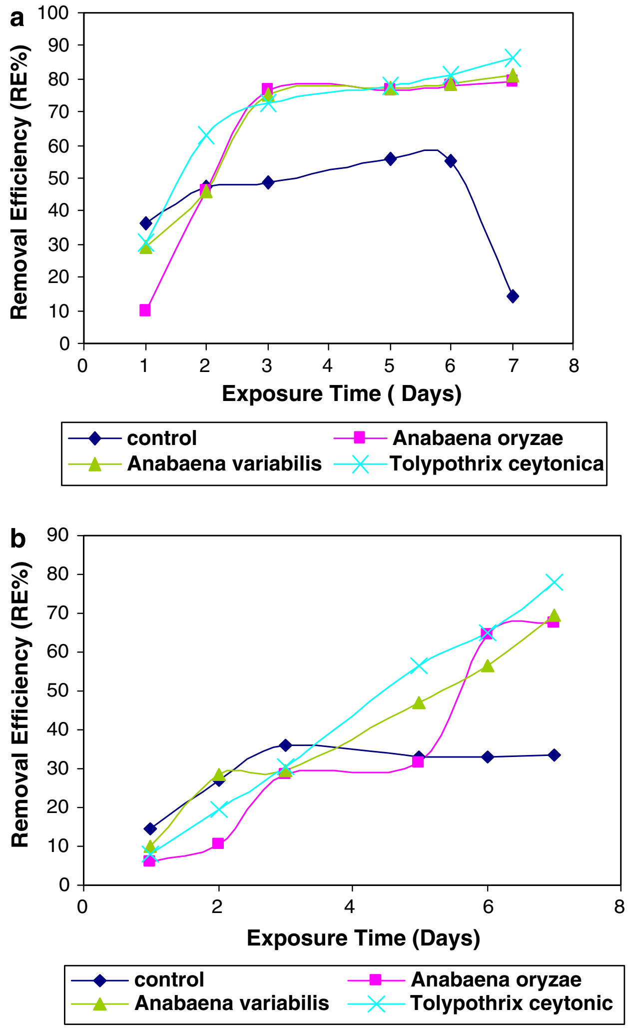

Fig. 7 Removal efficiency (RE%) of Zn from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

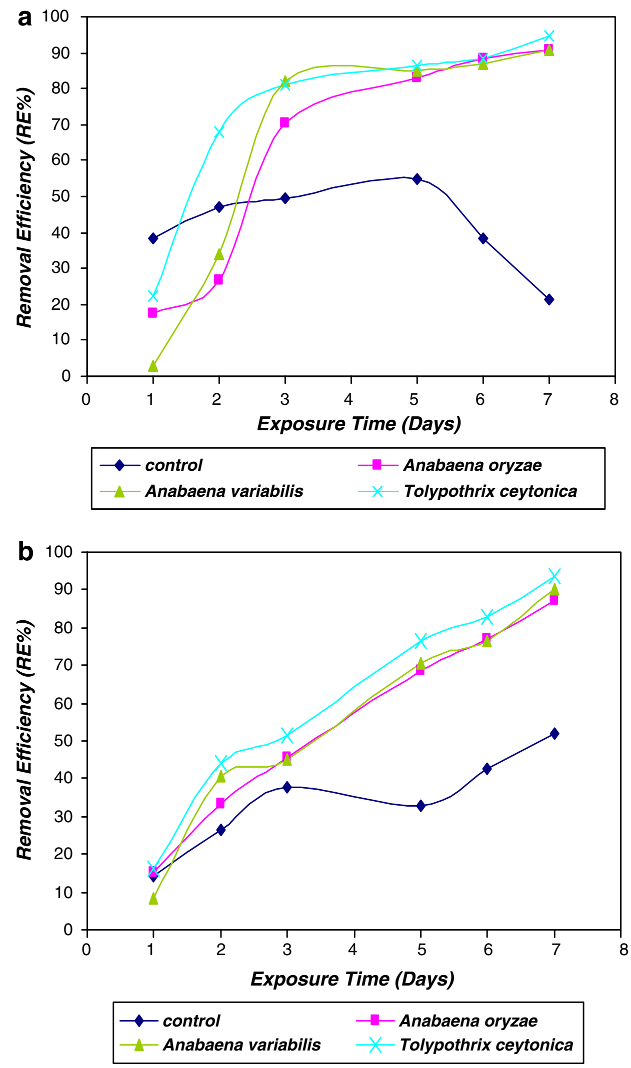

Fig. 8 Removal efficiency (RE%) of Cu from EWTP (**a**) and WWTP (**b**) effluents using the selected cyanobacteria at different exposure times

FOG removal reaching a maximum of 42.42% after 3 days (18.42 mg/l) which was declined to 20.0% (25.6 mg/l) after 7 days both of which are higher than the MPL of the FOG (Fig. 6a, b).

Heavy metals removal

Figures 7 and 8a, b represent REs% of Zn and Cu in the primary-treated effluents of the EWTP and WWTP using the selected cyanobateria along the tested exposure times. Results revealed the following points:

1. *T. ceytonica* recorded the highest REs% for Zn from EWTP (86.12) followed by *A. variabilis* (81.23) and finally *A. oryzae* (78.98%) recording RCs of 0.0247, 0.0334 and 0.0374 mg/l by the three species, respectively, after 7 days (Fig. 7a).
2. Although low Zn levels were detected in the WWTP effluent, lower Zn REs were achieved compared to those obtained for the EWTP effluent. Zn removal recorded 78.2, 69.33 and 67.73% achieved as the highest Zn REs% by *T. ceytonica, A. variabilis* and *A. oryzae*, respectively (0.0123, 0.0173 and 0.0182 mg/l, respectively) after 7 days. *A. variabilis* exhibited higher Zn RE% at the shorter exposures (up to 2nd day) compared to *T. ceytonica* and *A. oryzae* (Fig. 7b).
3. Higher removal of Zn was achieved by the control culture of the EWTP wastewater reaching a maximum of 55.8% (0.0787 mg/l) after 5 days, while only 36.0% (0.0361 mg/l) was recorded as the highest Zn removal from WWTP effluent after 3 days (Fig. 7a, b) both of which still lower than those obtained by the cyanobacterial cultures.

4. Although all the average levels of Zn for both effluents were below the MPL of 5 mg/l before the treatment, Zn levels were reduced producing much better effluent quality. Zinc removal was stimulated by increasing its level in the wastewater.
5. Concerning Cu (Fig. 8a, b), much higher REs% were recorded for EWTP effluent compared to those obtained for Zn removal regardless it's high toxicity. This may be attributed to the high resistance of the selected members which was stimulated by increasing Cu levels in the wastewater. 94.63, 90.99 and 90.64% RE of Cu were achieved by *T. ceytonica, A. variabilis* and *A. oryzae* (0.0031, 0.0052 and 0.0054 mg/l RC), respectively, after 7 days (Fig. 8a). *T. ceytonica* exhibited higher Cu RE% at the shorter exposures (up to 2nd day and then from 4th day till last day) compared to *A. oryzae* and *A. variabilis.*
6. For WWTP effluent, Cu removal recorded 93.75, 90.34 and 87.12% (0.0033, 0.0051 and 0.0068 mg/l, respectively) as the highest Cu RE by *T. ceytonica, A. variabilis* and *A. oryzae* respectively (Fig. 8b).
7. Again higher removal of Cu was achieved by the control culture of the EWTP wastewater reaching a maximum of 54.94% (0.0260 mg/l) after 5 days, while 52.08% (0.0253 mg/l) was recorded as the highest Zn removal from WWTP effluent after 7 days (Fig. 8a, b) both of which still lower than those obtained by the cyanobacterial cultures confirming their role in the treatment.
8. All the average levels of Cu in the primary and final effluents of both treatment plants before and after the treatment were below the MPL of 1.5 mg/l.
9. In conclusion, results confirmed that the most effective species for Cu and Zn removal from the primary effluents of the two wastewater treatment plants are in the following order *T. ceytonica* > *A. variabilis* > *A. oryzae* which may be attributed to the selective uptake of the investigated metals by the tested cyanobacterial species.

Discussion

A large proportion of sewage effluent is dumped without treatment into the aquatic environment. Unfortunately, sewage effluent often contains waste from industry which contaminates the sludges with pollutants, particularly heavy metals that are toxic if present in large concentrations. The danger is serious only where domestic and industrial wastes are mixed. The main aim of sewage treatment is to remove the solids, nutrients, biodegradable and toxic contaminants from the liquid, which can then be safely released into watercourses.

Untreated sewage not only causes eutrophication, but is also a major health hazard because of the likely presence of various kinds of pathogens. Detoxification of untreated waste is a particularly important service Although very little is known about how many species are necessary to provide detoxification services or how changes in species composition affect detoxification processes and rates, these services are likely to critically depend on a small number of specialized species. Plants and microbes in inland water systems are particularly important. Some waste contaminants (including heavy metals and salts) cannot be converted to harmless materials and will remain in the environment permanently, but many other contaminants (such as organic chemicals and pathogens) can be degraded to harmless components, at varying rates [4].

Cyanobacteria are involved in many biotechnological applications since they are well known for producing bioactive compounds under unfavorable conditions such as cyanotoxins. At least one secondary metabolite, cyanovirin, has shown to possess anti-HIV activity. Some cyanobacteria are sold as food, notably *Aphanizomenon flos-aquae* and *Arthrospira platensis* (*Spirulina*). Moreover, some hydrogen producing cyanobacteria are being considered as an alternative energy source in the US supported by the U.S. Department of Energy as well as in Sweden. Therefore, recently many cyanobacterial members were fully sequenced and molecularly characterized in order to optimize their beneficial use [16, 23, 29, 40, 52, 58]. Since the early 1950s, more than 100 cyanobacterial strains, belonging to 20 different genera, have been investigated with regard to the production of exocellular polysaccharides (RPS) into the culture medium which could be an effective cation-chelating material. These facts suggest the possibility to manipulate RPS-producing cyanobacteria, a multiproduct strategy to produce a wide range of biopolymers suited to various industrial applications, in addition to the residual biomass effective in the recovery of heavy metals from polluted waters [13].

Implementation of passive treatment using the naturally occurring geochemical and biological processes represents an advantageous trend to improve water quality with minimal operation and maintenance requirements [26]. Primary productivity in natural systems considered an effective and a low-cost method for removing chemical and biological contaminants from wastewater [38]. During the present study, the selected environmental cyanobacterial species performed high efficiencies as suspended growth application toward the removal of both organic (BOD, COD and FOG), inorganic (Zn and Cu) as well as physical contaminants (solids; suspended and dissolved) from mixed domestic–industrial wastewater in a relatively short duration. Many authors supported the findings of these results and attributed these abilities to the nature of cyanobacteria, the extreme environments and conditions that they easily acclimatized with and to the products they release under these stressed conditions. Cyanobacteria efficiently used in wastewater decontamination. Many studies were performed at different scales confirming the high ability for cyanobacterial species for nutrient uptake (N and P) from contaminated media and system. This was shown by *Phormidium bohneri* [33], the thermophilic cyanobacterium *P. laminosum* [53], *P. tenue* and *Oscillatoria O-210*, isolated from Arctic and Antarctic environments [11, 15] and other species [14, 15, 30, 45, 48]. Removal of nutrients using cyanobacteria offers, in addition to the high efficiency and low cost, a very simple technology compared to other complicated methodology, in which integration between chemical (coagulation) and biological (activated sludge) was performed to achieve higher efficiencies for nitrogen and phosphorus removal [21].

Cyanobacteria were also reported as efficient agents for the assimilation of organic matter from contaminated media [4, 43, 60]. These studies supported the present findings where BOD, COD and FOG were efficiently minimized, in almost both types of wastewater tested except in the case of COD of the WWTP effluent, to safe limits acceptable for the discharge into surface water. They required 7 days when applied as suspended growth system. These capabilities for the multi-contaminants removal can be greatly enhanced using such species in fixed applications as biofilm or immobilized cells [53]. They also can be used as a consortium instead of the individual application which would significantly reduce the treatment time required for best removal.

Metals affect the growth of plants by depriving them of nitrogen. In the soil with a high concentration of metals, the biomass of free-living microbes is halved, and blue-green algae (cyanobacteria) are effectively eliminated. Cadmium, zinc, copper and nickel are probably the most toxic elements normally found in large amounts in sewage sludge. Heavy metals in sewage become bound to the organic matter which settles out in sedimentation tanks. Although the initial concentration of metals may be very small, the removal of water concentrates them in the sludge. There are chemical treatments for recovering metals from effluents before they leave the factory. However, the costs of such systems rise steeply as they become more efficient [28]. During the present study, Zn and Cu contaminating the primary effluents of the EWTP and WWTP were efficiently removed even at their low levels which normally complicate the removal process. Removal by the three selected species based on selective uptake among them. Removal of heavy metals using microorganisms could be achieved through the production of extra-cellular components, such as chelatins and polysaccharides, which are capable of complexing metal ions [13, 39].

Alkaline precipitation of heavy metals from acidic water streams is a popular and long standing treatment process as well as another application where cyanobacteria are involved in heavy metals removal. The ability of certain algal species to increase the alkalinity of the surrounding medium as a by-product of their inorganic carbon accumulating mechanism has been documented [55] but the potential use of this alkalinity in the precipitation of metals has, to date, not been widely reported. The cyanobacterium *Spirulina* sp. was utilized in a continuous treatment system of tannery effluent to precipitate heavy metals (Fe and Zn) using the alkalinity generated by this alga. Using *Spirulina* sp. reduced the treatment running costs which considered additional benefit of treating either a tannery or sewage effluent using cyanobacteria [39, 46]. Moreover, the increased efficiency, coupled with the reduced operating costs involved in the algal treatment system for saline wastes produced from Leathers Tannery suggests that the system has considerable potential for the treatment of metal polluted acid waste waters. Also, *Spirulina* was effectively and directly used to treat acid mine drainage (AMD) over a short time period [5]. To overcome the problem of heavy metal toxicity on *Spirulina*, a system combines the principles of passive treatment with some simple, low-cost reactors associated with active treatment methods has been developed. In this system, the growing culture was separated from the untreated effluent resulted in a system with the potential to be as effective as active treatment processes, but with the cost and sustainability advantages of passive treatment systems [47].

Bio-sorption of heavy metals is becoming an important component in the integrated approach to the treatment of aqueous effluents using either microbial biomass or plant (including micro-algae) [32, 59].

The selective uptake and/or degradation capabilities of the selected species are attributed to the changes composition of the cell wall on which metal ions binding takes place as well as variation in their growth under different conditions [6].

Conclusion

Results of the present study proved the biotechnological importance and advantages of using the tested microalgae for wastewater treatment where promising removal of the investigated contaminants were achieved in a short time not exceeding a week. For organic matter removal, the highest achieved BOD_5 and COD RE recorded 89.29 and 73.68% achieved by *A. variabilis* and *A. oryzae,* respectively. For solids, the highest achieved RE for the TSS and TSD recorded 64.37 and 38.84% achieved by *T. ceytonica* and *A. variabilis*, respectively. For fats, oil and grease the highest achieved FOG RE recorded 93.75% and was achieved by *T. ceytonica*. High efficiencies were obtained for the removal of the two tested metals reaching 86.12 and 94.63% for Zn and Cu achieved by *T. ceytonica* and *T. ceytonica,* respectively. Therefore it is clear that the proposed treatment approach offers a low-cost, efficient and environmentally friendly technology for the treatment of either domestic or industrial or both kinds of wastewater. Further research can be done to emphasize the application on larger scale especially with fixed cyanobacterial forms.

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