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Full Length Research Paper

Pilot study of constructed wetlands for tertiary wastewater treatment using duckweed and immobilized microalgae

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Duckweed-microalgae constructed wetland (DM-CW) was a continuous flow pilot wetland used in wastewater phytoremediation assays. This study investigated the microbial and chemical wastewater quality before and after treatment in order to evaluate the DM-CW efficiency. Results indicated that with 3 days as hydraulic residence time, the hydraulic efficiency was high and allowed significant removal for chemical and microbial parameters. COD, BOD and ammoniacal nitrogen removals were the highest with a percentage of 67.5±8.2, 70.6±9.5 and 65.9%, respectively. Total phosphorus removal was acceptable (21.5%) but was closely related to the algal growth. Total and faecal coliforms percentage removals were respectively 68.5 and 47.16%. The quality of the treated wastewater issued from the DM-CW was approved and can be reused in the agriculture domain.

Key words: Duckweed based pond, microalgae based pond, wastewater treatment, phytoremediation.

INTRODUCTION

Recently, emerging technology using aquatic macrophytes and microalgae for wastewater treatment has gained great interest because of its cost-effective and environmentally sound approach (Vacca et al., 2005). Scientists and engineers from several countries have paid attention to the potential of aquatic macrophytes to treat and recycle pollutants from municipal and industrial wastewater (Brix and Schierup, 1989; Rao 1986). These plants have the capacity to assimilate nutrients and to convert them directly into valuable biomass (Reed et al., 1995). Effluents from the secondary stage treatment still contain various pollutants (organic, bacterial, fungal, nutrients…). Nevertheless, treated wastewater must be adequately treated before being discharged into the environment. Nutrients (NH_4^+, NO_3^- and PO_4^{3-}) were identified as the main causes of natural water eutrophication (Rebi and Piet, 2004). On the other hand, microbial water pollution has become a growing concern for environmental safety and public health. The role of fecal indicator organisms is central in any attempt to overcome this worldwide concern. Fecal coliforms remain the best overall indicators of water fecal pollution (Edberg et al., 2000). Several processes exist for the removal of chemical and microbial pollutants from wastewater but they are expensive and produce high sludge content (Alejandro et al., 2010).

Wastewater phytoremediation approach using micro-algae, macrophytes and different other water plants, floating or submerged (Noemi et al., 2004) is based on natural processes to remove different wastewater pollutants. Among macrophytes, duckweeds are very small floating aquatic macrophytes belonging to the Lemnaceae family which grow on the nutrient rich surface and in fresh waters and they are known for their efficiency in nutrient uptake (Bal-Krishna and Polprasert, 2008). Likewise, Lemnaceae have the greatest capacity in organic matter removal and in absorbing the micro-elements such as potassium, calcium, sodium and magnesium among others. However, duckweed plants

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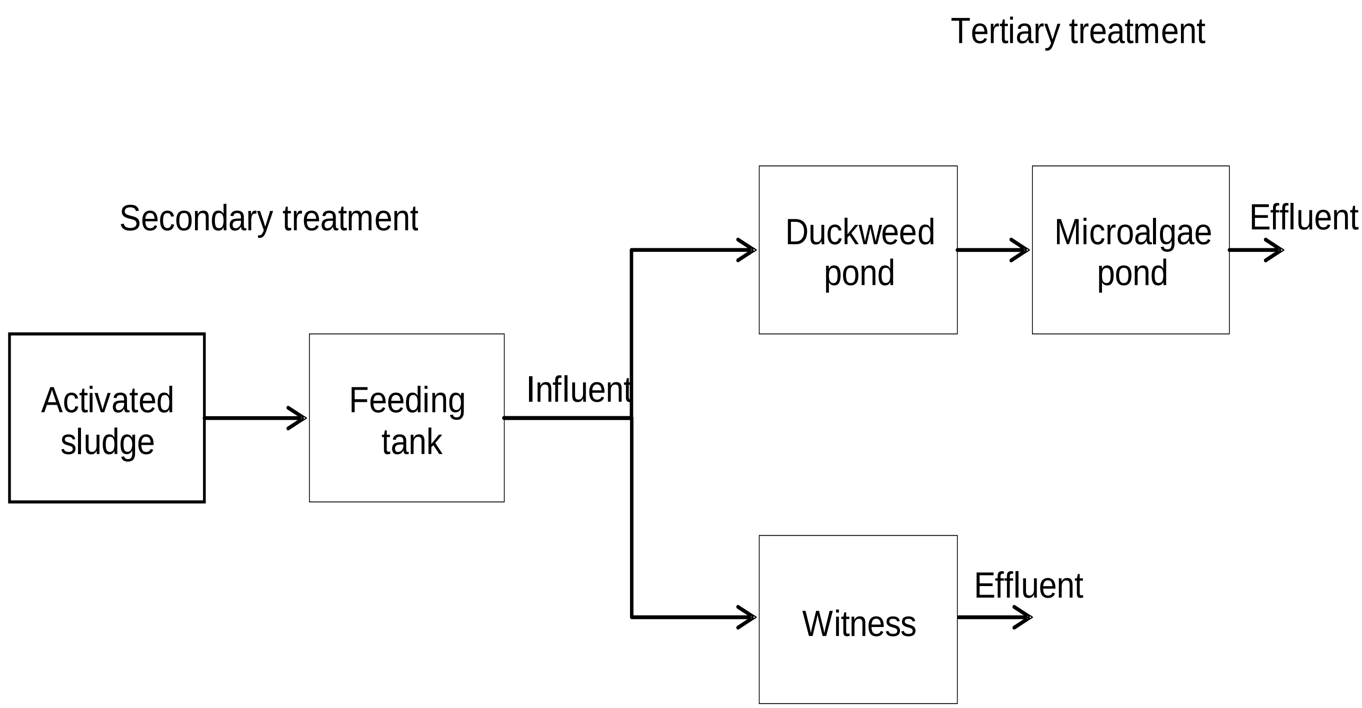


Figure 1. Schematic representation of the Duckweed-Microalgae constructed wetland, Witness: control system.

grow only in the upper water surface layer where mainly pollutant removal takes place (Dalu and Ndamba, 2003). As well as duckweeds, different microalgae species have been used in many wastewater treatment plants particularly those used for small communities due to its low cost and high efficiency (Oswald, 1988; De la Noue et al., 1992; Laliberte et al., 1994). Among the unicellular algal species, *Chlorella* is a common and effective species for the bioremediation purposes (Sing-Lai et al., 2010; Tam et al., 1994; Lau et al., 1997). However, one of the major problems in using microalgae for wastewater treatment is their recovery from the treated effluent (De la Noue et al., 1992; Laliberte et al., 1994). Immobilization technology, which fixes the microalgae cells into a mineral support, solves the harvest problem (Chevalier and De La Noue, 1985). The success in employing immobilized microalgae for wastewater treatment depends upon many factors, including algal species, immobilization matrix, cell and bead concentration, bead morphology, aeration, retention time… (Tam and Wong, 1999). Higher bioremediation efficiency has been recorded more in the immobilized algal biomass than in the freely suspended cells within the same algal species (Gonzalez et al., 2008). It is also recommended to use a combination of several types of plants floating and submerged to increase effluent quality.

More than 70% of the Tunisian wastewater treatment plant use activated sludge process. The quality of the treated wastewater supplied from this process was in most cases insufficient and can damage the environment where it was discharged. A complementary treatment was indispensable for improving the quality of the treated water.

This study investigated the *in situ* phytoremediation assays using the association of duckweed plant (*L. minor*) and three microalgae species (*Chlorella* sp.*, Senedesmus* sp.*, Euglena* sp.) during a tertiary proposed treatment in order to improve treated wastewater quality. Understanding this tertiary treatment will be of the utmost interest, as it will provide a better understanding of the phytoremediation process and can help us understand the possible links between the wastewater composition, the temperature variation and the different actors in the DM-CW.

MATERIALS AND METHODS

DM-CW plant design

The DM-CW is located in Mahdia city, Tunisia (35°30'0''N-11°3'36''E) where domestic wastewaters are biologically treated. The system was set in an open field exposed to weather conditions: with a summer average water temperature of 20.9 ± 3°C, and an average winter temperature of 11.7 ± 1°C. After primary clarification, wastewaters are treated by activated sludge process then routed towards the DM-CW which acts as a tertiary treatment plant (Figure 1).

Three microalgae small basins were set in the laboratory under a monochromatic light, at ambient temperature and with sterilized wastewater. The first contains 10^5 cell/ml of *Chlorella* sp., the second contains 10^5 cell/ml of *Scenedesmus quadricauda* and the third contains 10^5 cell/ml of *Euglena* sp. Pebbles were chosen as

Table 1. Description of physic-chemical and microbial analyses used in this study.

Parameter	Analysis frequency	Method description
Physical parameters	Daily	pH and Temperature : NF T 90-008 Conductivity : NF EN-27 888 Dissolved Dissolved oxygen: oxy-meter: air calibration Beaker oxi cal-Sl
Chemical parameters	Weekly	BOD : NF EN 1899-1 COD : NF T 90-101 TSS : Afnor T 90-105 Ammoniacal Nitrogen : NF T 90-015-2 PO_4 : ISO 6878
Microbial parameters	Once in 3 weeks	Total coliforms Faecal coliforms NPP method for Faecal streptococcus wastewater

mineral support for fixing micro-algae. The latter appear to be relatively indifferent to the chemical composition of the substratum of the mineral support. Pebbles may provide a rough surface for algal attachment. The stability and hardness of pebbles as substrata is obviously important to form stable habitats.

Pebbles used in this study as mineral support are collected from the beach of Mahdia and are composed from calcite and tiny pieces of shells of dead sea animals that have been cemented together and we usually observed pores between pieces. As a consequence, used pebbles have a high porosity and algae can colonize this habitat with a high level.

Pebbles are transferred from the three small basins above mentioned to DM-CW algal pond. Pebbles were washed every fifth day using 10 L of freshwater in order to evaluate their concentration. To evaluate the effectiveness of the immobilization method a filtration of the treated water was performed. Filtration experiments reveal that the algae effectively bind to the pebbles particles.

Regarding the used macrophyte, they belong to the family of *Lamnacea,* habitually called duckweed and were collected from water pond near the Mahdia sanitation company where they grow naturally. The duckweed was subjected to a harvesting regime every 15 days by collecting between 50 and 70% of the surface of basin.

The treatment system received secondary effluents into a feeding tank of 1000 L. The heart of the treatment system was composed of two ponds (Figure 1), located consecutively and with a continuous flow configuration, operating at the depth of 0.4 m and total surface area of 8.5 m^2. The DM-CW was built in a continuous flow configuration to increase the hydraulic efficiency and improve the organic matter removal. Hydraulic residence time was fixed to 72 h. A witness system with the same characteristics was constructed (without duckweed and microalgae) and received the same influent quality.

Physico-chemical and microbiological studied parameters

To assess wastewater quality, analyses were achieved using sampled treated water from the DM-CW inlet and outlet. Water quality parameters were assessed in accordance with the French standard methods (www.afnor.org) for the examination of water and wastewater (Table 1). Samples were stored at 4°C for a maximum of 4 h before being analysed. On the other hand, to study the contribution of each basin to the global pollutants removal, a specific sampling was performed from the following sampling points: DM-CW influent, duckweed effluent and final effluent. Therefore, 12 samples were taken from the described sample points (Figure 1) and are analysed.

Algal identification and quantification

Algal identification was performed by optic microscope. The identification was performed based on the mobility and the morphology of the microalgae. The algal growth was monitored by cell count performed on the water collected from the washed pebbles (improved double-Neubauer aemocytometer). The number of cells by millilitre was calculated using the following formula: N = average × (d/v) × 10^5 (Average: is the number of the cells by rectangle of the aemocytometer; d: the dilution of the algal cheek; v: volume of the sample).

Statistical analyses

The mean and standard deviation values of the triplicates for each treatment were calculated. The correlation between different parameters (physical, chemical and microbiological) was evaluated by Pearson coefficient at a confidence intervals of 95%. All statistical calculations were performed on an IBM-compatible PC with statistical package called SPSS 16.

RESULTS AND DISCUSSION

System efficiency and effluent quality

Table 2 showed the average parameters based on 12 samples collected from the DM-CW inlet, the duckweed pond outlet, the final outlet and the witness system outlet (with standard deviations of the different analyzed parameters). DM-CW results compared with those achieved by the witness system showed that for all conditions, significant organic matter, nutrients and microbial removals were achieved by the studied system. Whereas, in the witness system the removal for all parameters was negligible (Table 2). The highest removal

Table 2. Average influent and effluent (with standard deviation) quality of the different parameters analysed within the treatment system based on 12 samples.

Parameter	DM-T inlet	Duckweed pond effluent	DM-T outlet	control system outlet
Hydraulic residence time		3 day		
Temperature (°C)		18.5±2		
pH	8.4±0.1	8.04±0.1	8.9±0.1	8.2±0.1
conductivity (mS/cm)	7.8	7.8	7.8	7.8
Cl- (mg/L)	3	3	2.5	3.1
TSS (mg/L)	60±5	20±4	21±2	23±2
Total COD (mg/L)	310±15	238±12	71±18	286±8
Total BOD5 (mg/L)	69.5±9	49±6	20±7	71±2
NH_4-N (mg/L)	41.7±6	37±0.9	14±2	33±2
PO_4-P (mg/L)	6.7±0.9	6.7±01	5.1±0.6	6.7±0.6
NO_3 (mg/L)	0.3	-	0.23	0.29
Dissolved oxygen (mg/L)	1.1± 0.33	1.8±0.5	8±0.87	2.3±0.3
Fecal coliform (NPP/100 m/L)	40	11	Absence	63
Fecal streptococcus (NPP/100 ml)	11	Absence	Absence	Absence

rate was observed for the TSS with 77.7±16.3%. Organic matters were measured as COD and BOD and showed an average removal of 67.57±8.2% and 70.67±9.5%, respectively. Table 2 showed a good ammoniacal nitrogen removal (65.19%). Regarding the phosphorus removal, it was lower than 30%. Likewise a high removal was observed for the turbidity (50%). The pH values showed an increase between influent and effluent that matches the increases in alkalinity. On the other hand, the electrical conductivity still unchanged from the inlet to outlet, despite a decrease in the concentration of chlorides (Table 2). Concerning the pathogens removal, Faecal coliform concentration showed a decrease of approximately 67%.

The pH variation

The pH-values in the system were higher than in the influent at any given time. In the algae based pond, the microalgal photosynthesis activity caused a pH increase particularly in the warm season when the pH values exceed 8. Nevertheless, in the cold season when microalgae density decreased, the pH was about 7.4. Zimmo et al. (2004) showed that in the algal based pond, the pH value was generally above 8.0 compared with the duckweed pond pH, the duckweed mat prevented substantial algae photosynthesis and as a result lower pH values were observed.

The average pH value in the duckweed based pond ranged between 6.9 and 7.3 during the experiment period. Christian et al. (2003), in their constructed wetland have recorded a pH that ranged between 8 and 11.3. Romero and Brix (1999) found that the pH >8.5 can hamper or even damage the plankton biocenosis and macrophyte root. The performance of the DM-CW plant was affected by low or high pH levels. pH has an effect on the living micro-organisms found in the ponds. Dalu and Ndamba (2003) suggested that the pH in the ponds is expected to rise to around 9 for an effective fecal bacterial removal.

Algal dominance and dissolved oxygen

Figure 2a showed that dissolved oxygen increased proportionally with the temperature. The highest oxygen concentration was recorded during the month of May (8.15 mg/l). The bivariant correlation coefficient value calculated (Pearson coefficient) for the algal concentration and the dissolved oxygen was 0.835, the correlation is significant to the level 0.01. This correlation proved that algal cells during the hot season produce a great deal of oxygen through the photosynthesis process. Ignacio et al. (2010) proved that piggery wastewater biodegradation performance was significantly influenced by microalgae species supporting oxygenation process. In this study the temperature and with less significance organic matter as COD and BOD are the key factors that modify the dominance among the three algal species. Figure 2b showed that *Chlorella* and *Scenedesmus* was dominant with respectively 5.10^5 and 3.10^3 cell ml^{-1} when temperature ranged between 11 and 19° Celsius and with medium organic load (COD and BOD). *Euglena* sp was dominant in the hot season when temperatures are >20 °C.

On the other hand, acting as a tertiary treatment process the DM-CW did not receive a high organic load. Nevertheless during March, April and May when the highest COD and BOD values are recorded, a relatively high algal concentration was reached. Consequently we can conclude that the organic matter increase can stimulate the algal growth and may change the dominance between the three microalgal species.

These findings are in agreement with previous studies carried out in microalgae based sewage treatment processes showing that *Euglena* and *Chlorella* was often dominant at high organic loads (Ignacio et al., 2010; Gonzalez et al., 2008).

COD and BOD variation

The average COD and BOD removal recorded by the combined duckweed-microalgae system was respectively, 55.55 and 59.34%. As showed by Figure 2c the maximum COD and BOD removal was reached during the month of May when the dominant microalgae *Euglena* sp concentration was around 6.10^5 cell ml^{-1} (Figure 2b). At the DM-CW outlet the average COD values were 44± 6 mg/l. Figure 2b showed that *Euglena* resists better to the organic load increase than *Chlorella* and has an important growth rate. Likewise, a recent study has reported that a strain of *Euglena* exhibited higher growth rates in diluted animal waste than *Chlorella* and *Microcystis* (cyanobacterium) strains (Park et al., 2009). As showed by Figure 2c and d, the COD and BOD removal was acceptable in the cold season when microalgae are less abundant. At the DM-CW outlet the average COD and BOD values were respectively, 48.16± 7 and 24± 4 mgL^{-1}. As a consequence, we can confirm that the quality of the treated wastewater generated by the DM-CW was below the discharge consent limit recommended by the Tunisian water authority (NT 106-002: 90 mg/l for the COD and 30 mg/l for the BOD).

In order to study the contribution of each basin to the global pollutants removal, 12 samples are analysed (Table 2). The percentage of removal for each basin compared to global removal was calculated. Thus for the duckweed pond, the recorded removal percentage was 19% for the COD and 23% for the BOD. Likewise, the algal based pond removal percentage was 77% for the COD and 70% for the BOD. These results proved that the organic matters removed by the DM-CW are mostly achieved by the immobilized microalgae. The duckweed has no effect on COD removal and a slight effect on BOD. These findings are in agreement with previous studies focused on duckweed or microalgae based sewage treatment processes. In this context, Dilek et al. (1999) found that for all conditions significant COD removal was achieved in the algal based pond in the range of 55-60% after 42 days operation period for a paper industry wastewater. Noemi et al. (2004) found a removal percentage of 67.5 ±8.2 and 70.6 ± 9.5% for respectively, COD and BOD was achieved in a duckweed based pond used for domestic wastewater treatment. Statistical result confirmed that COD and BOD removal rate was positively correlated with the algal growth increase (Pearson coefficient = 0.801) observed during March, April and May. This positive correlation is significant at the level 0.01.

Ammoniacal nitrogen and phosphorus removal

Figure 2e and f showed that both ammoniacal nitrogen and phosphorus removals were reasonable in the algal based pond during the warm period when algae are abundant. Jinsoo et al. (2010) demonstrated that *Chlorella vulgaris* has potential to remove nitrogen (ammonia and ammonium ion) at a reasonable uptake rate from wastewater.

Result showed a decreasing concentration of both parameters from the influent to the effluent. A low removal of the NH_4-N and the PO_4-p was registered by the duckweed based pond. About 20% of the dissolved nitrogen entering the duckweed system was immobilized and extracted with the harvest. The nutrients removal was mainly caused by the incorporation into algal biomass. This was confirmed by Zimmo et al. (2004) who demonstrated that ammoniacal nitrogen and phosphorus removal are low in the duckweed treatment processes.

Shen et al. (2006) reported that duckweed uptake, ammonia volatilization, and nitrification were the major pathways for NH_4-N removal in a duckweed pond. Nevertheless, bacterial nitrification does not represent an important pathway for NH_4-N removal in this study because we did not observe an increase of NO_3-N concentration which decreases from 0.3 in the inlet to 0.23 (Table 2) in the inlet.

The average reduction performed by the DM-CW was 65.1% for the ammoniacal nitrogen and 21.5% for the PO_4-P. The pH in the DM-CW ranged between 7.4 and 8.7 thereby confirming that ammonia was abundant in the water as NH_4 which is the favourable form of nitrogen uptake by the duckweed and microalgae without the need to metabolize it. Al-Nozaily et al. (2000) showed that the reduction of the NH_4-N concentration in the algal pond were significantly better than those without algae, suggesting that the uptake of ammonia and assimilation into algal biomass are the essential processes. The NH_4-N is generally preferred as a nitrogen source by algae, and ammonium uptake has been shown low energy demand to be assimilated by microalgae than nitrate or urea (McCarthy et al., 1977; Mohamed et al., 2007).

Duckweed and microalgae uptake was the dominant pathway for phosphorus and ammonium removal especially when PO_4-P was the principal form of phosphorus in this study, while nitrogen could also be removed through ammonia volatilization.

Removal of faecal coliforms and faecal streptococcus

The variation of faecal coliforms and faecal streptococcus is showed in the Figure 3a and b. The system generated

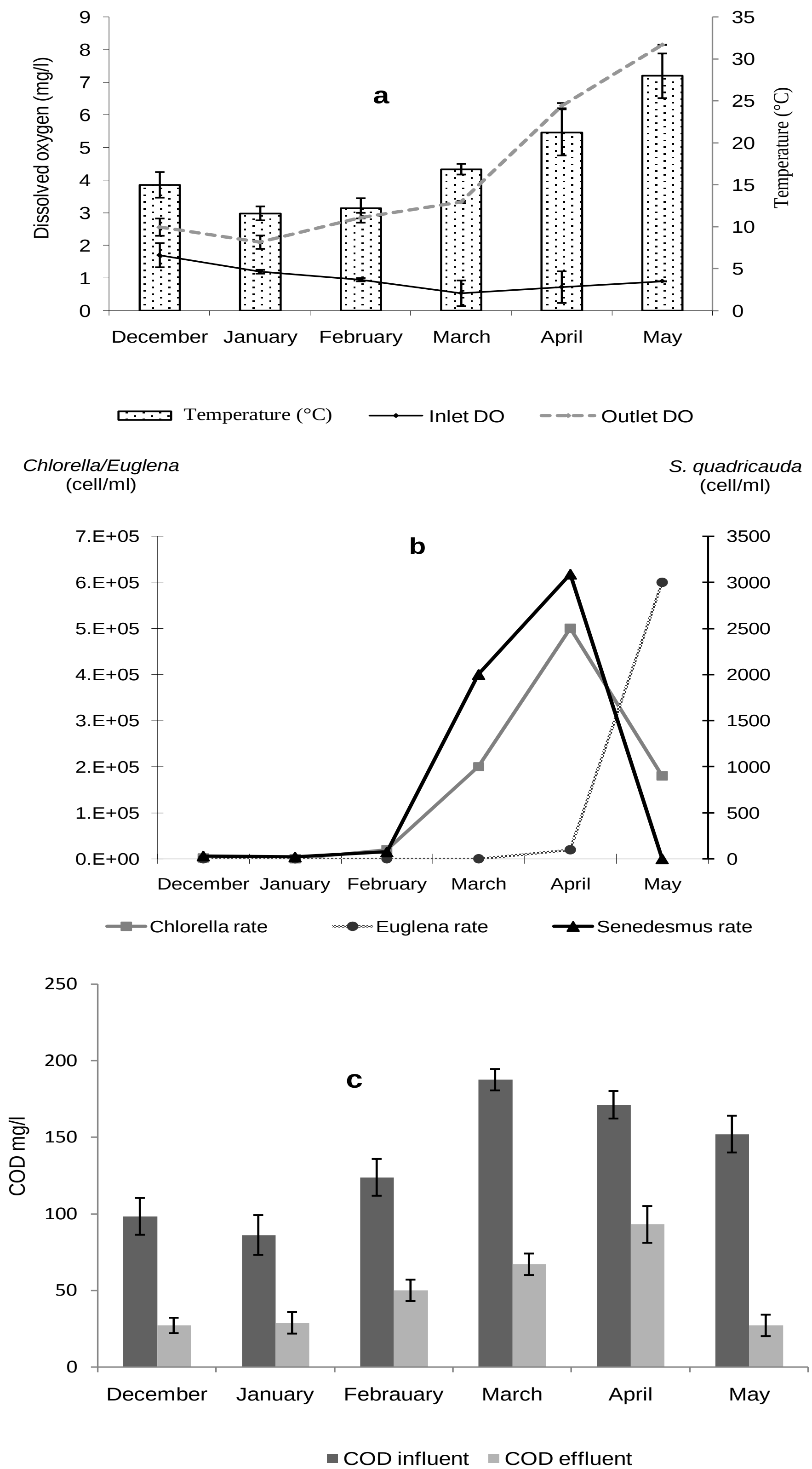
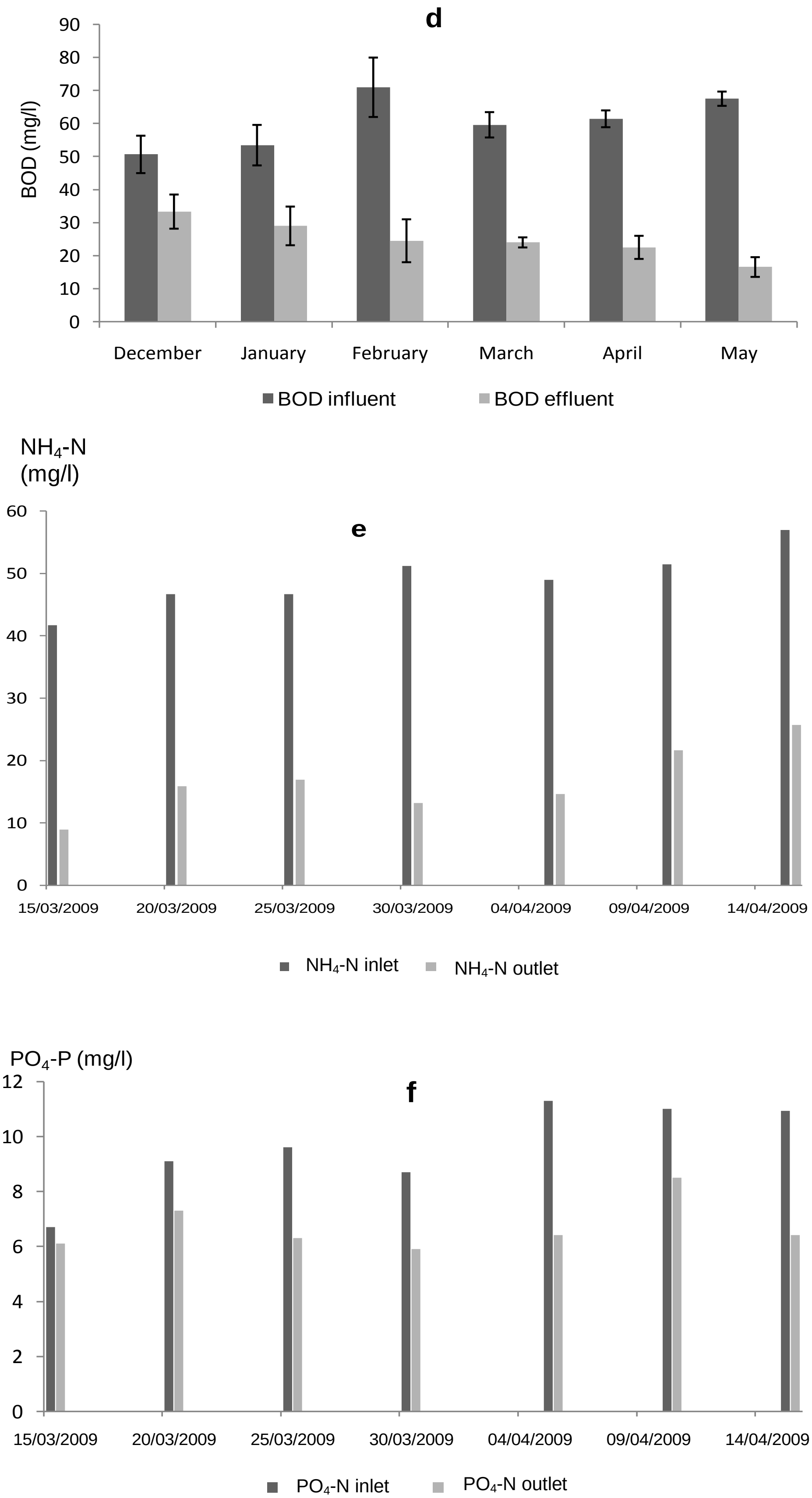


Figure 2. Chemical analysis of wastewater before and after treatment.a: dissolved oxygen (influent and effluent) and temperature variation; b: water concentration of the threemicroalgae species; c: chemical oxygen demand (COD) removal; d: Biological Oxygen Demand (BOD) removal; e: Ammoniacal nitrogen (NH_4-N) removal; f : PO_4-Premoval.



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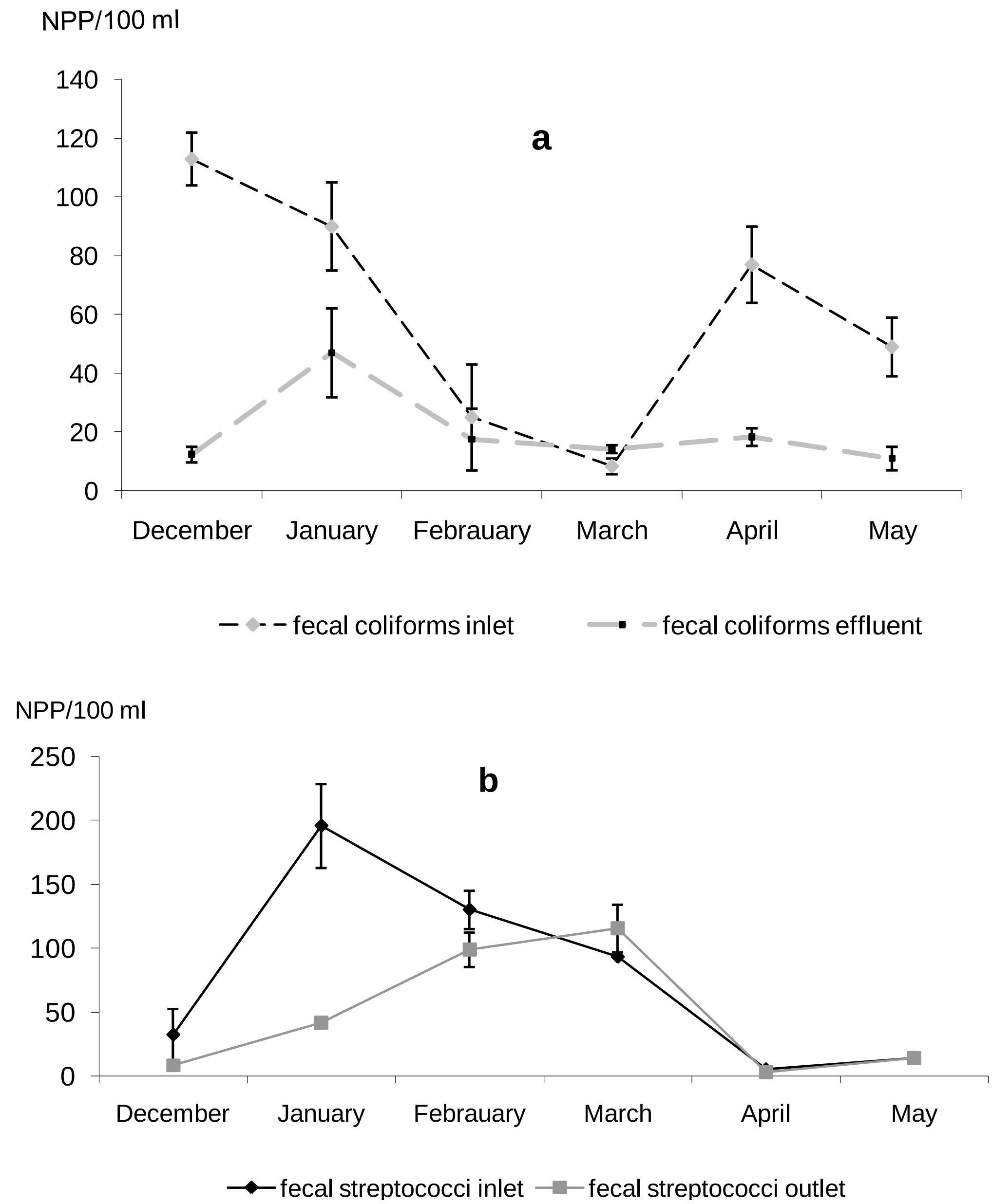

Figure 3. Fecal coliform and streptococcus removal. a: concentration of fecal coliform in influent and effluent wastewater; b: concentration of fecal streptococcus in influent (fecal strep inf) and effluent wastewater (fecal strep eff).

a fecal coliforms and fecal streptococcus removals of 68.5 and 47.16%, Respectively. Noemi et al. (2004) reported a high removal for fecal coliform (95%) using a duckweed based pond.

As can be seen in Figure 3a and b, a continuous reduction was observed during the experiment period except the month of March. Alejandro et al. (2010) in a semi-continuous bioreactor for wastewater treatment showed that the high pH values observed caused 95% of fecal coliforms removals. Alejandro et al. (2010) also reported that at pH 10, 99% removal of fecal coliforms and *Escherichia coli* was achieved in batch and semi-continuous cultures. This confirms that the high pH produced as a result of microalgae activity is unfavorable for the survival of fecal coliforms. The average efficiency of pathogen removal is satisfactory during the whole period and the effluent content of fecal coliform and streptococci is meeting the Tunisian norm of 1000 CFU/100 ml. The principal way of pathogens removal is the sun radiation and the basic pH in the microalgae based pond. The diminution of pathogens in the duckweed pond cannot be explained by the duckweed harvesting, suggestion a specific mechanism for pathogen removal in the duckweed ponds.

Conclusion

To conclude, the present study demonstrates that the duckweed-microalgae constructed wetland was effective in removing organic matter, ammoniacal nitrogen and phosphorus from wastewater. The registered result indicated that the system is efficient and effective and specific conclusion can be made: (i) A higher removal of COD and BOD was achieved within 3 days of treatment. (ii) Removal mechanism is mainly performed by microalgae and duckweed assimilation (iii) The high efficiency observed in warm season can be explained by the increase of the algae concentration in the DM-CW. The combined system has many advantages: (i) Minimal operation and maintenance, (ii) Higher dissolved oxygen concentration in the water without the need of mechanical aerator (iii) Higher effluent quality that can be used in irrigation.

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