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Constructed wetlands as a sustainable solution for domestic wastewater treatment in Egypt

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ABSTRACT

Water scarcity and limited access to effective wastewater treatment represent critical challenges in arid and semi-arid regions, where water reuse is increasingly necessary to support agriculture and local communities. To address these challenges, a Nature-Based Solution (NbS) was implemented in El-Banger village near Alexandria of Egypt, where a sand biofilter system for civil wastewater treatment was previously unable to meet reuse standards. This study presents the design and evaluation of a new bench-scale Constructed Wetland (CW) that operated with a hydraulic retention time of approximately 20 h over a two-week experimental period, and integrated locally available macrophytes (*Phragmites australis*, etc.) with a multilayer filtering substrate. The bench-scale CW efficiently remediated the influent wastewater, achieving over 93 % Chemical Oxygen Demand (COD) removal, contributing to meet Egyptian standards for agricultural and gardening reuse, when considered together with the overall physicochemical and microbiological assessment. Moreover, pathogen counts, including *Escherichia coli* and *Staphylococcus aureus*, were reduced by more than three orders of magnitude, and solar-powered UV disinfection eliminated residual contamination. Toxicity and phytotoxicity tests using *Daphnia magna* and *Solanum lycopersicum* seeds confirmed the absence of adverse effects. Metagenomic analyses revealed functional bacterial taxa (*e.g.*, Actinobacteria, Proteobacteria, Bacteroidetes) contributing to pollutant degradation, along with reduced fungal diversity post-treatment. These results confirm that our bench-scale CW, based on local materials and macrophyte consortia, can replace conventional wastewater treatments in the context of limited resources. However, future research should focus on system upscaling, long-term performance assessment under real operating conditions and the optimization of the CW plant efficiency for an optimal wastewater reuse in arid and Mediterranean regions.

1. Introduction

Worldwide more than 1.5 billion people are threatened by soil degradation and loss or limitation of food production, and approximately 400 million hectares of agricultural lands are saline, mostly in arid or semi-arid regions [1]. The Mediterranean basin is experiencing the effects of global warming more rapidly than rest of the world, and this phenomenon is increasing the risk of soil salinization. In Egypt, the desert climate dominates, but an arid climate characterizes its Mediterranean coast with rainfall ranging from 80 to 200 mm per year. The imbalance between land productivity and food needs will increase since Egypt population is expected to grow [2,3]. The management of freshwater resources is therefore a critical issue in the development and adaptation to climate change of the Egyptian population living along the Mediterranean coast [4]. The current water crisis is due not only to its scarcity, but also to the lack of adequate infrastructures and water resource mismanagement, especially in all the Mediterranean internal and marginalized areas [5]. According to data from the Holding Company for Water Supply and Wastewater, in Egypt, the annual collected wastewater averaged 6.5 billion Cubic Meters (BCM) between 2009 and

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2014, constituting approximately 81 % of the total domestically produced water. Notably, about 44 % of the nationally generated wastewater remained untreated, representing a substantial volume equivalent to 5 % of Egypt annual share from the Nile River [6]. On the other hand, only 20 % of the annually treated wastewater underwent primary treatment, while the remaining 80 % underwent secondary treatment, with tertiary treatment being the least utilized option due to its high cost [7].

Unfortunately, there is no institutional form for sanitary drainage in these regions, due to financial constraints and the availability of low-cost treatment plants, depending mainly on the behavior of the society. Therefore, the only practical solution to address the current situation is to explore alternative water resources such as benefiting from more accessible wastewater treatment processes to reuse the remediated water [8,9].

In this context, the use of phytoremediation (PR) technology could represent one of the most exploitable alternatives for local communities, thanks to their environmental and economic sustainability [10–12]. PR is a Nature-Based Solution (NbS) exploiting the ecological relationship through biotic (*e.g.*, plants, microorganisms, etc.) and abiotic components (*e.g.*, sand) to reclaim wastewater [13]. This relationship can be artificially designed and realized through the implementation of Constructed Wetlands (CWs). CWs are based on natural and spontaneous biochemical, physical and physiological reactions, reproducing the removal processes of pollutants typical of natural aquatic systems or wetlands [14]. About that, several studies have demonstrated the effectiveness of CWs and PR as sustainable alternatives to conventional wastewater treatments, particularly in areas where the resource are limited [15,16]. Specifically, the applications in arid and semi-arid regions (*e.g*, Egypt) have shown that CWs plants can ensure reliable pollutant removal at the same time minimizing energy demand, operational and management costs [17,18]. In this context, the selection of appropriate macrophyte species plays a key role in CW performance, resilience and long-term stability. The vegetation typically used in CW beds is typically selected based on their functional traits. Species such as *Phragmites australis* (Cav.) Trin. ex Steud., *Typha* spp. or *Arundo donax* L., widely diffusing both in Mediterranean and arid regions, are known for their high tolerance to salinity and organic loads, extensive root systems, rapid and huge biomass production, hence, for all these reasons they are widely used [19,20].

Despite the proven effectiveness of CWs in Mediterranean regions (*e.g.*, Egypt), several practical aspects remain insufficiently addressed. Limited attention has been dedicated to CWs oriented to wastewater reuse, combining reduced land requirements with integrated physicochemical, microbiological and ecotoxicological assessments.

In this context, we realized a bench-scale CW based on Horizontal Subsurface Flow (HSSF) [21] for the treatment of civil wastewater, with the aim to evaluate its capability to achieve wastewater quality suitable for agriculture reuse, under the hypothesis that the combined effect of an optimized CW configuration and macrophyte diversity could improve treatment efficiency.

2. Materials and methods

The CW bench-scale apparatus was realized as a finishing process downstream of a NbS system already implemented in an agricultural area of Banger El Village (Alexandria of Egypt suburb). Since this area was affected by high salt concentration in irrigation water, and the domestic sewage (grey and black) was discharged in unsealed soak pits with approximate dimensions infiltrating it directly into the subsurface layers contaminating the environmental matrices also with pathogenic microorganisms.

2.1. Sizing and multi-stratification of a pilot HSSF CW

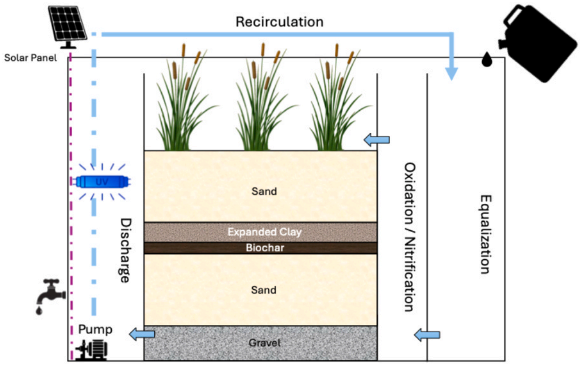

Fig. 1. HSSF bench-scale CW design. Light blue arrows indicate the water flow.

The HHSF CW bench-scale apparatus (Fig. 1), was built up and an aquarium pump with a flow of 350 liters per hour for wastewater recycling was used, and gravity flow was exploited to limit energy consumption. The bench-scale CW was designed to include sequential treatment phases in different compartments (Fig. S1): i) equalization (0.10 × 0.40 × 0.50 m, 20 L volume, 20 min minimum retention time); ii) oxidation-nitrification (0.06 × 0.40 × 0.50 m, 12 L volume, 2.5 h retention time); iii) CW bed (0.35 × 0.40 × 0.50 m, 70 L volume, 20 h retention time); iv) discharge-recirculation (0.74 × 0.40 × 0.50 m). The bench-scale CW (Fig. S2) went operative at the laboratories of SRTA-City (Borg El Arab, Alexandria of Egypt), the temperature was in the range 20–23 °C and light for the macrophyte were irradiated by two LED lamps with 12 h of light and 12 of dark. In order to reduce the ratio between the surface area required for optimal water remediation and the number of people equivalent (p.e.), natural high specific surface was used as part of the filling media of the bench-scale CW bed. Specifically, the CW bed was multi-layer stratified with mainly inert filling materials (*e.g.*, sands of different grain sizes). Although the wetland bed was stratified using substrates of different granulometry, the system operated under a horizontal subsurface flow regime. Substrate layering was adopted to improve filtration, root anchorage, and microbial activity, and was not intended to generate vertical flow conditions.

The CW bed was therefore stratified, starting from the bottom, with: 8–12 cm gravel (10 L); 15–20 cm sand (20 L); biochar (1 L in small pieces and enough to cover the bed surface); expanded clay (2 L, as designed, $S_s = 1000\ m^2\ m^{-3}$); 20–25 cm sand (30 L) in which plants were allocated (one individual for each of the macrophyte species, placed at the four corners of the CW bed), obtaining a 63 L reactor volume. Different macrophyte species were selected and used as consortium, because the presence of different macrophytes in the bench-scale CW, enhancing its ecosystemic biodiversity, favored the bio-phyto-remediation processes respect to a macrophyte monoculture (Spiniello et al. [21]; Tuttolomondo et al. [22]). Therefore, specimens of *Arundo donax* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Typha* spp. and *Nerium oleander* L. were chosen [see Supplementary Materials (SM) for more details)].

All the calculations elaborated for the multilayer bed stratification of the bench-scale CW are also given in SM.

2.2. Experimental plan

The experiment was carried out with the bench-scale CW for the treatment of effluents from a sand biofilter operating in the village El Banger, Borg El Arab in Alexandria. This sand biofilter system (consisting of a septic tank completed by a sand filter) worked for the treatment of civil wastewater, but it produced an effluent that still did not meet all the quality standards required for water reuse in agriculture and gardening.

Specifically, 60 L of the wastewater, collected after the passage through the biofilter, was used as influent and recirculated continuously for 14 days. In addition, small wastewater additions were performed to compensate for evapotranspiration losses, using volumes adjusted according to the composition of the latest recirculated effluent.

The biofilter wastewater, collected as effluent, before being loaded in the bench-scale CW, was filtered three times on a cotton sieve to avoid clogging problems during the treatment/recirculation due to the presence of coarse solids. Chemical analyses [*e.g.*, COD, N compounds, pH, Electrical Conductivity (EC), TDS, etc.] were performed on water samples at difference time points: before and after the initial filtration step (*pre-f* and *post-f* samples); at zero time (t_0) or a brief recirculation after loading the filtered wastewater into bench-scale CW (recirculation rate = 60 L in about 1.0 min). During the wastewater recirculation in the bench-scale CW samples at: 24, 48, 72, 142 h, and the final effluent, at the end of the treatment were collected. TSS analysis was performed on samples: pre- and post-filtration, t_0 and at 142 h (t_{142}). Microbiological counts of pathogenic microorganisms were performed at the beginning of the treatment, after about one week, and at the end of the experiment (14 days) after UV treatment. The UV disinfection was performed by immersing a UV lamp (15 W) in the final compartment of the bench-scale CW for 30 min. Having established that a single week of recirculation in the bench-scale CW was sufficient to achieve the limits laid down in Egyptian legislation for reuse in agriculture, other kinds of additional analysis [*e.g.*, Heavy Metals (HMs) / elements] were carried out only on the starting sample and after a week of treatment, that is, the time necessary to consider for possible practical applications.

Finally, we repeated the experiment twice to confirm the obtained data. About that, also the second experiment showed similar results.

2.3. Chemical analyses

The methods of chemical analyses of influents and effluents are described in detail in SM.

2.4. Microbiological analyses

The *3M™ Petrifilm™* plates (Neogen Corporation - Lansing, MI 48912 USA) were used for the count of total aerobic microorganisms, *Escherichia coli*, *Staphylococcus aureus*, yeast and molds. The samples were serially diluted up to 10^{-4} and 1.0 mL of each dilution was spread on the plates in duplicate; then the plates were incubated for the time and at the temperature suggested by the manufacturer. The counts of Colony Forming Units (CFU) mL^{-1} were finally calculated.

2.5. Toxicity test on Daphnia magna

In order to carry out a toxicity screening of the influents and effluents of the bench-scale CW, acute 24–48 h mobility inhibition tests on those water samples with the freshwater crustacean *Daphnia magna* was performed in accordance with ISO 6341 and OECD Guidelines 202. Parthenogenetic females of *D. magna*, aged no more than 24 h, from long-lasting forms (efippi), were exposed for 24–48 h to the water collected samples (at different dilution and in six biological replicates for each sample dilution) at 25 °C with a 16 h light - 8 h dark photoperiod. The measured endpoint was represented by immobilization. This standard method is compatible with the use of the commercial *Daphtoxkit F* (MicroBio Tests Inc. - Kleimoer 15 9030 Gent, Belgium). Finally, for each sample, the "*lethal concentration 50%*" (LC_{50}) was calculated as shown in the equation below, that is the % sample concentration at which half the members of the *D. magna* population are killed after a certain period of exposure, by means of the *AAT Bioquest LC50* calculator online.

$$\boldsymbol{Y = Min + \frac{Max - Min}{1 + \left(\frac{X}{LC_{50}}\right)^{Hill\ coefficient}}} \tag{1}$$

2.6. Germination test on tomato seeds

Phytotoxicity test, that is germination/elongation test, on seeds of tomato, irrigated either with the influents or effluents, was also carried out.

Seeds were sterilized immersing them in 70 % ethanol for 2 min under shaking at 100 rpm (rotation per minute), followed by a series of one-minute washes in sterile water, then immersed in 1.5 % hypochlorite for 15 min under shaking at 100 rpm, and, finally, again undergone a series of one-minute washes in sterile water. Seeds, in duplicate, were then lying on three sterile sheets of blotting paper in 90*15 mm Petri dishes (10 seeds per plate). An experiment consisting of three groups: control (irrigated with Hoagland solution), influent and effluent (the latter collected after 1 week of recirculation in the bench-scale CW) was set up. Petri dishes were kept in dark for the first three days and then exposed at a natural light photoperiod for the following eight days, the number of germinated seeds was recorded every day. At the end of the phytotoxicity test, seedlings were removed from the plate and, for each one, the length of stem and roots was measured by taking a picture on graph paper and then the data analyzed with the open-source ImageJ software. Different indices (*e.g.*, Relative Seed Germination, Coefficient of Velocity of Germination, *etc.*) were calculated to estimate the phytotoxicity on tomato seeds of the influent or effluent respect with the control.

2.7. Data and metagenomic analyses

Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using PAST software v. 4.13. Differences between multiple groups were assessed by one-way ANOVA followed by Tukey's post-hoc test for pairwise comparisons. A $p\text{-value} < 0.05$ was considered statistically significant. The methods for data and metagenomic analyses are described in detail in SM.

3. Results

After two weeks of civil wastewater (60 L) recirculation in the bench-scale CW, its remediation capability was determined by analyzing several chemical, physical and biological parameters either of the influent or of the effluents collected at different time points.

3.1. HSSF CW performance (chemical physical parameters)

The COD-BOD_5 and N compound concentrations at different time points are reported in Fig. 2A,B.

The COD values decreased according to a hyperbolic trend. The average biodegradability index (BOD_5/COD ratio) was equal to 0.26, which complies with the standards reported for civil wastewater, but it fluctuated during the different recirculation times. In fact, initially it was equal to 0.34; it decreased significantly during the initial recirculation time reaching its minimum at t_0 (0.002), then it increased again (maximum value equal to 0.52 at 48 h), and finally it lowered at the end of the recirculation (0.06 at two weeks). Either the filtration process (upstream of the bench-scale CW) or the first wastewater recirculation in the bench-scale CW have contributed significantly to the reduction of COD and BOD_5 values. Then, their values reached the regulatory limits provided by Egyptian legislation (Table 1) for water reuse already within 24 h of recirculation. The same notable abatement during the initial recirculation time occurred for suspended solids, in fact, from a starting value of 140 ± 4 ppm, an almost halved (80.0 ± 1.5) and an almost zero value (less than 1.0) were reached, respectively, in the *post-f* and t_0 samples, remaining low until the end of the first week of recirculation (value equal to 5.0 ± 0.1 in sample t_{142}).

In the case of N compounds, the only available results (mg L^{-1}) for sample *pre-f* and *post-f*, due to technical limitations related to the turbidity of the samples, are total N (sample *pre-f* = 107.81 ± 2.78 and

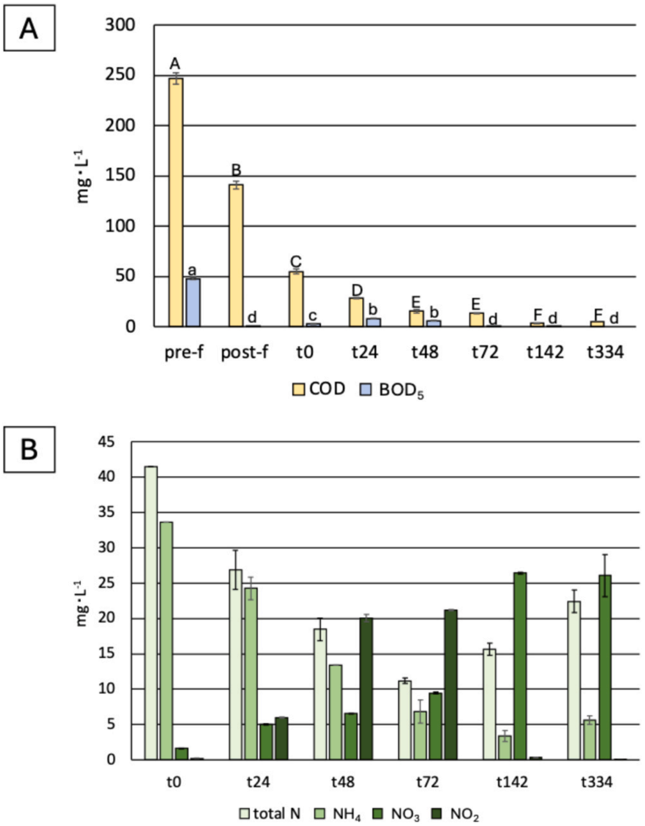

Fig. 2. Different time frames analysis for COD-BOD$_5$ (A-upper panel) and N compounds (B-lower panel). Legend of samples: pre-f = pre-filtration and pre-treatment in bench-scale CW; post-f = post-filtration and pre-treatment in bench-scale CW; t0 = time zero (t_0) recirculation in bench-scale CW; t24 = time 24 h (t_{24}) recirculation in bench-scale CW; t48 = time 48 h (t_{48}) recirculation in bench-scale CW; t72 = time 72 h (t_{72}) recirculation in bench-scale CW; t142 = time 142 h (t_{142}) recirculation in bench-scale CW; t334 = time after two weeks of recirculation in bench-scale CW. Different uppercase letters indicate statistically significant differences ($p < 0.05$) among sampling times for COD, while different lowercase letters indicate statistically significant differences for BOD₅. Nitrogen species (NH_4, NO_3, NO_2) are shown as temporal profiles without statistical lettering, as they represent transformation dynamics rather than monotonic removal trends.

Table 1
Chemical-physical characteristics of the initial wastewater after a week of treatment in HSSF CW, compared with the Egyptian regulatory limits for reuse in agriculture. Legend of samples: pre-f = pre-filtration and pre-treatment in HSSF CW; t142 = time 142 h (t_{142}) treatment in HSSF. *Statistically significant differences ($p < 0.05$) between sample collected at t_0 and t_{142}. ND = not detectable.

Parameter	Sample *pre-f*	Sample *post-f*	Sample t_0	Sample t_{142}	Egyptian limit on the reservoirs and Nile branches and canals DM 82/48
COD (mg L^{-1})	246.76 ± 3.75	141.0 ± 5.6	55.0 ± 3.8	*3.50 ± 0.17	30
BOD$_5$ (mg L^{-1})	84.00 ± 1.61	48 ± 0.9	0.2 ± 0.02	*1.01 ± 0.03	20
Total N (mg L^{-1})	107.81 ± 2.78	61.6 ± 1.6	41.44 ± 2.78	*25.68 ± 1.47	/
NH_4 (mg L^{-1})	ND	45.17 ± 2.78	33.60 ± 0.65	*1.36 ± 0.08	/
NO_3 (mg L^{-1})	ND	ND	1.67 ± 0.05	*26.40 ± 1.39	30
NO_2 (mg L^{-1})	ND	ND	0.23 ± 0.01	*0.40 ± 0.01	/
Chlorides (mg L^{-1})	183.5 ± 1.1	104.85 ± 2.10	51.0 ± 1.1	*70.00 ± 0.43	400
Mg (mg L^{-1})	29.85 ± 0.95	ND	14.51 ± 0.74	*11.33 ± 0.46	100
Ca (mg L^{-1})	361.51 ± 2.01	ND	175.4 ± 0.9	*136.49 ± 1.06	230
Na (mg L^{-1})	221.63 ± 1.53	ND	107.8 ± 1.1	107.62 ± 0.80	230
SAR	3.01 ± 0.01	ND	ND	2.37 ± 0.02	6–9
Ir (mg L^{-1})	0.75 ± 0.05	0.43 ± 0.05	0.21 ± 0.04	*0.098 ± 0.010	1
Cu (mg L^{-1})	< 0.08	< 0.08	< 0.08	< 0.08	1
pH	7.30 ± 0.05	7.52 ± 0.04	7.76 ± 0.05	*8.11 ± 0.01	9
EC uS cm^{-1}	1241 ± 19	1286 ± 27	1078 ± 21	*849 ± 15	/
TDS ppm	859 ± 10	777 ± 16	579 ± 11	*480 ± 8	800
TSS (mg L^{-1})	140.0 ± 1.4	80.0 ± 2.1	< 1.0	*5.0 ± 0.1	30

sample *post-f* = 61.6 ± 1.61) and NH_4 (sample *post-f* = 45.17 ± 2.78). A decrease in the total N concentration was detected since the beginning until the end of the recirculation time. The most abundant N fraction on the total was initially represented by NH_4, then by NO_2^- (from 48 to 72 h) and finally by NO_3^- (from 142 h until the end of the process); it is noteworthy the gradual completion of the nitrification process during the wastewater recirculation ($NH_4 \rightarrow NO_2^- \rightarrow NO_3^-$). Anyway, despite the peak of NO_3^- at 142 h, they still felt within the limits required by Egyptian legislation. The chloride values, pH, EC and TDS at all collection times are reported in the graphs of Fig. S3. The chloride concentrations showed trend quite similar to both COD and TSS, namely a significant reduction between the *pre-f* and *post-f* samples and then at t_0, keeping around the one of t_0 value until the end of the wastewater recirculation; pH values were slightly alkaline throughout the process, with a peak of 8.15 ± 0.01 at t_{72}; both the EC and TDS values, likewise, decreased over time following a gently sloping line.

The values of all the analyzed parameters, after one week of bench-scale CW recirculation, comply with local regulatory settings. For almost all the analyzed parameters there was a significant reduction, with the only exception of the increase of NO_3^-. As regards SAR parameter, it decreased over time although not in a significant way. As for HMs, Fe and Cu concentrations were already below the limits at the beginning of the wastewater recirculation; however, Fe had a considerable reduction during the first week of recirculation. Regarding data processing and thus the calculation of Remediation Efficiencies (RE) and Rates (RR) (at t_{142} versus t_0), the highest values were achieved for COD (RE = 93 % and RR = 0.36 mg h^{-1}). In contrast, BOD$_5$, and NO_3^- experienced an increase from the start of recirculation in the bench-scale CW until the end of the first recirculation week. The same experiment was repeated another time and data showed the same trends.

Finally, macroscopic and morphological effects on macrophytes (attributable to the contact time with the wastewater) were assessed. However, neither toxic effect, plant growth reduction, nor leaf chlorosis or browning phenomena were observed at the end of the experiment (data not shown).

3.2. HSSF CW disinfection capabilities

As regards the bench-scale CW disinfection capabilities, the number of CFU per mL (resulting from aerobic microorganisms, *E. coli* and *S. aureus*, *etc.*) were reduced by 2 or more orders of magnitude after one and two weeks of the wastewater recirculation in the bench-scale CW. In addition, the final UV lamp treatment eliminated all the *E. coli*, and it reduced, after two weeks, by an additional order of magnitude the colonies count of the recirculated wastewater (Fig. 3).

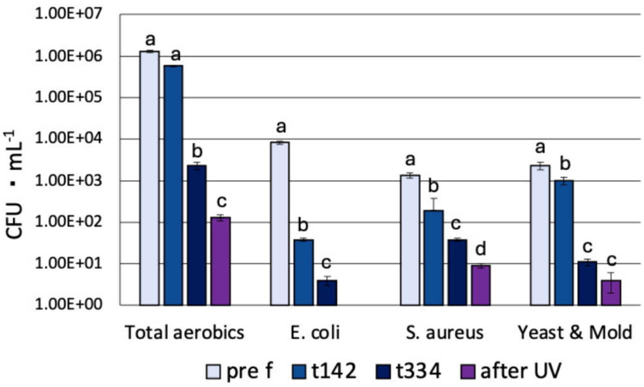

Fig. 3. Variation of microbial counts (expressed as CFU·mL^{-1}) in the wastewater samples collected at different treatment times: before filtration (pre f), after 142 h of recirculation (t142), after 334 h (t334), and after UV disinfection (after UV). The monitored microbial groups include total aerobics, *Escherichia coli*, *Staphylococcus aureus*, and yeasts & molds. Data are presented as mean values ± standard deviation. Different letters (a–d) indicate statistically significant differences among treatment times for each microbial group ($p < 0.05$).

3.3. *Wastewater toxicity test*

The results of the toxicity test, after 48 h of *D. magna* exposition to the *pre-f* sample (Table S1), are reported in Fig. 4, where graph was obtained using the *AAT Bioquest LC50* software and in compliance with the formula reported in the 2.5 section.

The resulting LC50 was equal to 74.95, therefore a *pre-f* sample concentration of 75 % was sufficient to immobilize half of the *D. magna* individuals. On the contrary, all the individuals, treated with the wastewater recirculated at *t142*, were vital at the end of the test, therefore LC50 was not estimated because no toxicity was observed.

Table 2 shows the results of phytotoxicity (germination) tests on tomato seeds: several indices were calculated for *pre-f* and *t142* samples, in relation to the control group where the seeds were irrigated only with Hoagland solution. The data matrix collected for each experimental group (number of seeds germinated every day, final lengths of roots and shoots are reported in Table S2.

Compared to the control, a slight decrease was observed in the RSG index for seeds irrigated with the influent; while the RGS and shoot GI indices slightly decreased using both samples (influent or effluent), but the differences were not statistically significant. Differences were detected for the RGR and root GI indices for seeds irrigated with the influent, which were significantly higher with respect to the control.

3.4. *Metagenomics results*

The results of NGS analyses on bacterial 16S rDNA and fungal ITS relative to the influent, and effluent (after wastewater recirculation) made it possible to understand the microbial dynamics occurring over time in the associated ecosystem with the bench-scale CW. Fig. 5 displays bar diagrams illustrating the relative frequencies of Operational Taxonomic Units (at Order level for bacteria and at Class level for fungi) identified by means of bioinformatic analysis for the analyzed samples. For each OTU, the sum of the relative frequencies between the samples is equal to 1. Fig. 6 shows the Shannon diversity index for the bacterial and fungal community. The bacterial diversity showed a slight decrease from influent to effluent samples, although this difference is not statistically significant. In contrast, the fungal community exhibited a marked and significant reduction in α-diversity ($p < 0.05$).

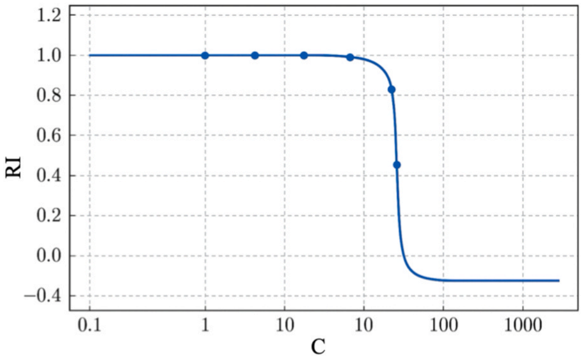

Fig. 4. Results of the *D. magna* toxicity test for sample pre-filtration (*pre-f*). C = sample concentrations [%]; RI = Response Index.

Table 2
Germination test results for sample *pre-f* and t_{142} relative to the control. *Statistically significant differences compared to the control (Kruskal-Wallis test, $p < 0.05$). The Coefficient of Velocity of Germination value for the control is equal to 44.26; for all other indices the control data coincided with the value 100.

Index (%)	Sample *pre-f*	Sample t_{142}
Relative Seed Germination (RSG)	94.7	105.3
Relative Growth of Root (RGR)	164.9*	111.1
root Germination Index (root GI)	156.2*	117.0
Relative Growth of Shoot (RGS)	79.1	80.4
shoot Germination Index (shoot GI)	74.9	84.6
Coefficient of Velocity of Germination (CVG)	43.25	41.05

4. Discussion

The effectiveness of CW has been already demonstrated at pilot scale, as a sustainable solution for the treatment of civil wastewater, also aimed at targeted reuse of treated water [21,23,24]. In our case, the wastewater treatment was based on the use of a HSSF CW built *ad hoc* and filled only with natural and very inexpensive materials, with emphasis on sustainability and, at the same time, maintaining the goal of the effluent reuse. Despite this being a crucial challenge, significant removal of all contaminants, from the treated wastewater, was achieved even from a microbiological point of view. In fact, after only a few hours/days of wastewater recirculation in the implemented bench-scale CW the microorganisms present in it were greatly reduced. In addition, it is noteworthy that the surface area of the implemented bench-scale CW was considerably reduced when compared with those currently employed worldwide (ratio m^2 EI^{-1} reduced from 4 to 10 m^2 per EI down to almost 1:1), as well as the contact times necessary for optimal influent remediation. This improvement is due to the use of a combination of technical innovations including: the hydraulic configuration adopted (HSSF with wastewater recirculation), the substrate materials used (*e.g.,* expanded clay and biochar) and the selection of the most appropriate native macrophytes to be used as a consortium [25].

A comparative analysis with recent studies on phytoremediation of wastewater, particularly urban wastewater, highlighted the efficacy in contaminant removal of the bench-scale CW employed in our study [26–34]. In fact, compared to previous studies adopting different configurations (including hybrid flow CW plants) and different plant species (*e.g.*, *P. australis*), the designed and built bench-scale CW showed either comparable or even better removal efficiencies despite its low surface and short contact time [22,34,35].

4.1. *Contribution of the selected plant species*

The results showed that the four selected macrophytes (*P. australis, A. donax*, *N. oleander* and *Typha spp.*) operated successfully thanks to their physiological and ecological characteristics essential in the phytoremediation processes (*e.g.*, fast growth, high biomass and phyto-

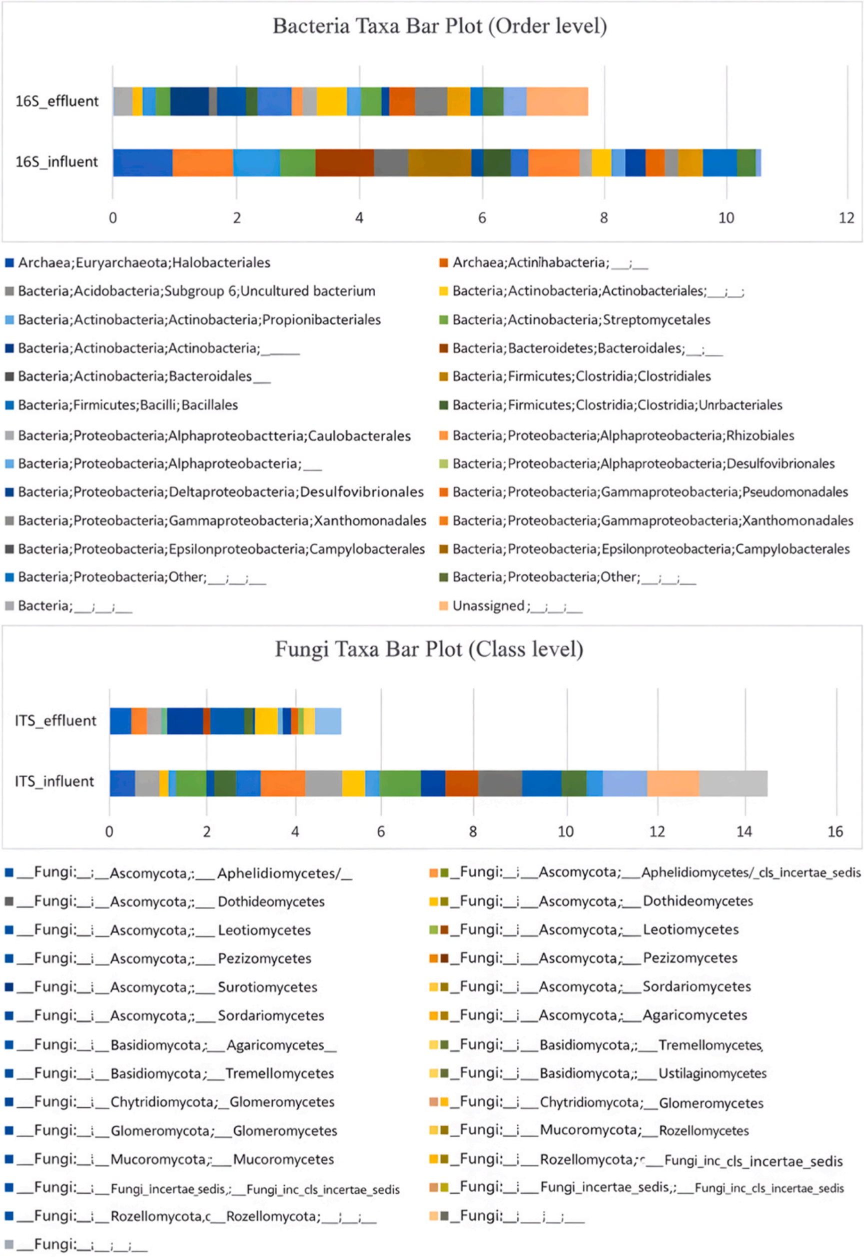

Fig. 5. Taxa bar plot of the relative frequencies of different bacterial orders (upper) and fungal classes (lower) identified in the various samples.

transpiration rate, resistance and tolerance, etc.) [36]. Furthermore, these plants were able to tolerate stress conditions, such as the presence of pathogenic microorganism and HMs (*e.g.*, Fe), or high concentration of ammonia and chlorides. The use of these species as a consortium in the bench-scale CW confirmed their high-rate performances (previously reported with the use of a single plant species) despite the very short contact times of wastewater treatment and without any evident plant growth limitation or competition among them [37]. The same result was observed in other studies at pilot-scale, confirming the synergistic effect due to the increased ecosystem biodiversity in terms of different plant species and microorganism communities [21].

4.2. Contribution of the CW configuration and stratification

Currently, the CW design, the most widely used in Europe, adopts a (sub)-submerged flow. In this context, HSSF plants have been mainly built because, although providing lower remediation yields, they don't cause management problems compared to Vertical Flow (VF) systems. In recent years, however, the need to reach more stringent effluent quality standards has led to a growing interest in vertical and combined/hybrid flow systems [34]. In fact, the use of combined ones allows to overcome the limitations related to the one-stage systems and at the same time exploits their advantages [22]. In the present study, the limitation of large areas employed in the remediation process has been overcome thanks to several technical innovations, mainly aimed at improving the efficiency of the bench-scale CW. Furthermore, the use of several inert materials (sand of different granulometry) adopted in the stratification did not affect the functioning dynamics of the bench-scale CW, but, instead, provided a support to the semi-hydroponic growth of both macrophyte plants and microorganisms, allowing at the same time a rapid and constant recirculation of the wastewater without clogging problems. All these strategies enabled to reduce the Hydraulic Retention Time (HRT), that is the time during which pollutants needs to be in contact with the plant rhizosphere, microbial biofilms and substrates, so

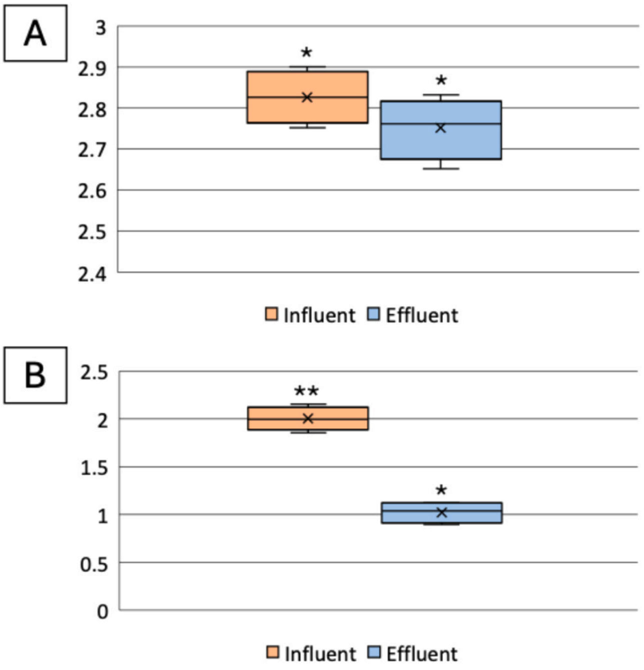

Fig. 6. Shannon index for bacterial (A) and fungal (B) community. * Indicates statistical differences ($p < 0.05$).

as to be degraded or accumulated, this is definitely a crucial factor controlling the removal mechanisms of contaminants and, ultimately, the use of the CWs for wastewater treatment. Therefore, although no hybrid configuration was adopted in our study, the HSSF CW allowed us to achieve the set objectives of wastewater reuse either in agriculture or gardening. Finally, the use of natural materials with high ratio Surface/Volume as expanded clay, instead of plastic carriers, commonly used for suspended biomass activated sludge treatment systems, which can also be relatively expensive, avoids the risk of plastic release into the environment, especially if not properly disposed of after their use.

4.3. Mechanisms of contaminant removal

The mechanisms for reducing BOD_5 and COD in CWs are known to be affected by the biodegradability of compounds and process conditions [38]. In our study, COD and BOD_5 values were found to comply with Egyptian reuse regulatory limits after only 24 h of treatment in the bench-scale CW. The same notable abatement in the initial stages occurred for the suspended solids. However, the experiment lasted about a week to ensure the adequate reduction of other pollutant concentrations (*e.g.*, N compounds) and the presence of pathogenic microorganisms, allowing the effluent reuse as purified water in agriculture. This positive outcome can be attributed to the significant contribution of both pre-filtrations upstream of the bench-scale CW recirculation and the adsorption capacity of the CW physical substrate at the beginning of the wastewater treatment. The BOD_5 value at t_0 was indeed almost zero, and the slight increase recorded subsequently can be attributed to the usual metabolic and physiological processes carried out by the biotic component of the CW. Anyway, the biodegradability index of the water from the beginning to the end of the process experienced a substantial decrease, reaching 0.06 at the end of the treatment, confirming the depletion of biodegradable organic matter. Usually, the use of a hybrid-flow CW, combining HSSF and VF, ensures good removal of the suspended solids and organic matter [39]. However, the final COD RE (93 %) obtained in our experiment, with the exclusive use of an HSSF configuration, highlights the importance of the technical innovations adopted, including the careful selection of mainly inert filling materials, macrophyte species and their use as a consortium. In accordance with other authors, indeed, the use of diverse macrophytes favored a more efficient root system distribution filtration bed of the bench-scale CW and the development of a diversified microbiome/microbiota both in the rhizosphere and on the substrate particles of the different matrices employed for the multilayer stratification [40].

In the present study, the increase in NO_3^- content observed after CW treatment, together with the decrease in NO_2^- and NH_4^+, indicates the progression and near completion of the nitrification process. This suggests that nitrification was the dominant nitrogen pathway under the prevailing operational conditions, likely favored by wastewater recirculation, high oxygen availability in the rhizosphere, and the presence of high-specific surface substrates supporting active microbial biofilms. On the other hand, the accumulation of NO_3^- in the effluent may also be partially attributed to a limited nitrate uptake by macrophytes and to reduced denitrification activity, potentially due to reduced availability of anoxic zones [24]. Although denitrification and anammox processes cannot be excluded in localize micro-anoxic niches within the matrices or biofilms, no direct evidence was collected in our study to confirm their occurrence.

A strategy to further reduce the presence of NO_3^- could be to increase the depth of the phytoremediation bed, especially in the HSSF one, to improve anoxic conditions and also enhance the completion of the denitrification processes. In any case, the final concentration of the NO_3^- did not pose any problem since it fell within the Egyptian limits suitable for reuse. Therefore, good N removal efficiencies was obtained with our bench-scale CW, in line with reuse objectives, and this result can also be explained by the dynamic interaction that took place among plants and microorganisms [21]. In this context, also the richness and biodiversity of macrophytes had positive effects on the N removal capabilities of the HSSF c, in agreement with other authors, some of them estimated that species richness could explain more than 25 % of the removal in the N concentration of the effluent [41–45].

A high content of chlorides in the influents can greatly impact the performance of CWs and inhibit the activities of macrophytes and microorganisms, as widely documented in the literature [46,47]. The mechanisms of chloride removal in CWs are mainly due to plant physiological activities such as photosynthesis and/or transpiration, thus underlining once more the importance of choosing the right macrophytes for realization of the CWs. In our case, the bench-scale CW, excluding the initial pre-filtration, achieved a chloride RE of 33 % (t_{142}), ensuring compliance with limits for agricultural water reuse at the end of the treatment. This result, in the case of CW remediation in real applications, would also avoid the risks related to the irrigation of crops with saline water, leading, in the long run, to soil deterioration, which is what's happening nowadays in Egypt. This result might probably be due also to the presence of a great number of halophilic microbial species deriving from the local context [48]. In accordance with the above, the measured values of EC and TDS likewise decreased over time along a gently sloping line. As regard metal content present in the influents, removal mechanisms in CWs include both chemical-physical processes as substrate adsorption, complexation, binding to organic matter, (co-) precipitation and biological processes as root absorption, bacterial oxide-reduction, etc. [49]. In our study, HMs concentrations were below the legal limits even before treatment. Anyway, remediation processes carried out by the bench-scale CW during wastewater recirculation reduced significantly iron and other metal concentration.

4.4. Disinfection capacities of the CW and toxicity tests

The modelling and design of our bench-scale CW ensured a good disinfection of the reclaimed wastewater, significantly reducing or eliminating the pathogenic bacteria present in the influent. Vymazal reviewed, in 2005, 60 CWs around the world with emergent vegetation, revealing that the rate of removal of fecal coliforms was usually from 95 up to 99 %, as also confirmed by subsequent studies [50–54]. Our results show, once more, how the CWs for wastewater disinfection, in comparison to other finishing treatments, are less expensive and highly sustainable. Many mechanisms contribute to the successful disinfection treatment performed by CWs, widely described in the literature, including physical processes of filtration and sedimentation, adsorption to the substrates, action of antibiotics present in root depositions/extrusions, or of antibiotic-active rhizosphere bacteria [55,56].

Moreover, one week of treatment was sufficient to significantly reduce (by two or more orders of magnitude) the number of CFU per mL in aerobic microorganisms, *E. coli*, *S. aureus* and yeasts and molds. Additionally, considering the ambitious goal of using remediated wastewater for irrigation of crops and vegetables for human consumption, a subsequent disinfection with UV lamps, immersed in the tank collecting the outlet, was achieved. The UV treatment allowed the elimination of *E. coli* and resulted in an additional order of magnitude reduction in the CFU. Therefore, this additional step, in the optic of greater sustainability (*e.g.*, the use of solar panels to power UV lamps), should be assessed also in the real case of CW application to ensure greater public health and safety.

The results of toxicity/germinability tests, conducted on *Daphnia magna* larvae or tomato seeds using either the inlet or outlet of the bench-scale CW, revealed that the effluents were not toxic to both living organisms. On the other hand, the absence of toxicity of the influent towards tomato seeds highlighted how organisms, belonging to different kingdoms, animal versus plant, exhibit different adaptive capabilities and of response to wastewater contaminants.

4.5. Microbial communities

The metagenomic analyses conducted on the influents and effluents shaded light on the dynamics of microbial communities, forming a complex system as that of the CW, underlying the wastewater treatment process.

The main bacterial phyla belonging to functional microorganisms in CWs were *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes*. These microorganisms play a crucial role in removing pollutants from CWs through catalyzing chemical reactions, biodegradation, biosorption, and supporting plant growth, among other processes [57]. The order *Halobacteriales* was also present; the bacteria belonging to this order are common in environments with high quantities of salts and organic matter and, although aerobic, can thrive in salty environments due to their ability of creating energy through photosynthesis [58].

Similarly, the presence of fungal taxa (mainly at the class level) that significantly decreased between the influent and the effluent communities were compared.

In a study concerning the structure of the fungal communities present in different wastewater, specifically in the activated sludge from treatment plants, analyzed in the Gauteng province of South Africa, *Basidiomycota* and *Ascomycota* were identified as the two dominant and characteristic phyla (their relative abundances were 48.38 % and 38.36 %, respectively, in accordance with previous studies) [59]. As they were found to be dominant in the co-occurrence network, this suggested that these two phyla can adapt to different environments. Many of the dominant fungal genera, identified in the above cited study, belonging to the class *Eurotiomycetes* (such as *Aspergillus*, *Penicillium*, *Talaromyces*, *Paecilomyces*, *Cladophialophora*), have the capability to transform/degrade organic contaminants such as toluene, polycyclic aromatic hydrocarbons (PAHs), dioxins, and many others. Calabon et al. reported that members of the genus *Penicillium* may confer better drainage and filtration to activated sludge [60]. In addition, the dominance of *Ascomycota* (2968 species belonging to 1018 genera) in freshwater fungal taxa, including the *Eurotiomycetes* characterized by 276 species and 49 genera, is well-documented in the literature. Therefore, it is relevant that these two phyla were largely present in the fungal community of our bench-scale CW and probably they have contributed to pollutant removal.

The analyses on the microbial diversity of the different analyzed samples revealed how the contact of wastewater with the biotic and abiotic components of the bench-scale CW may have impacted the richness of the microbial taxa present in the effluent [57]. In fact, although the effluent recorded the highest number of identifications of gene sequences related to fungi, its relative Shannon diversity index, calculated from the fungal taxa in the effluent sample, was significantly lower compared to the influent samples. Furthermore, the biodiversity indices calculated for bacteria are consistently higher, in each analyzed sample, than those observed for the fungi. However, this result could be influenced by the quality of databases used for taxonomic identification since the fungal databases are less annotated and limited to class-level (rather than order-level as for the bacteria).

5. Conclusion

This study addressed key challenges in civil wastewater treatment and disposal, proposing phytoremediation as a more sustainable alternative, both environmentally and economically, when compared to conventional ones. This innovative NbS approach has proved particularly useful for rural and marginal areas and especially for the desertic or semi-desertic ones. The investigation at bench-scale demonstrated the viability of phytoremediation process as secondary treatments following primary sedimentation, with an emphasis on compliance with regulatory standards for both surface water discharge and agricultural reuse. Moreover, the effluent being rich in NO$_3^-$, it can be considered a resource for crop fertilization without any negative impact on the agricultural soils. Therefore, our HSSF CW can be a sustainable alternative for addressing the challenges of wastewater management, particularly in sites where land is a quite limited resource. In fact, the relevant reduction of the sizes (1/4) of the areas, that need to be allocated for phytoremediation, must be assumed as a considerable innovation respect with the same plants previously used. Finally, future research should focus on long-term performance under real operating conditions, deeper investigation of nitrogen transformation pathways, and quantitative assessment of macrophyte growth and biomass to further optimize the system for wastewater reuse applications.

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CRediT authorship contribution statement

Angela Cicatelli: Writing – review & editing, Data curation. **Gianmaria Oliva:** Writing – review & editing, Visualization. **Stefano Castiglione:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Carmen Tammone:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ranya A. Amer:** Writing – review & editing, Validation, Supervision, Data curation. **Tarek H. Taha:** Writing – review & editing, Validation, Methodology. **Reham M. Elkout:** Investigation, Formal analysis. **Francesco Guarino:** Writing – review & editing, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stefano Castiglione, Angela Cicatelli, Francesco Guarino has patent #102020000005737 licensed to Ministero delle Imprese e del Made in Italy. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2026.121793.

Data availability

Data will be made available on request.

References

[1] S. Navarro-Torre, P. Garcia-Caparrós, A. Nogales, M.M. Abreu, E. Santos, A. L. Cortinhas, A.D. Caperta, Sustainable agricultural management of saline soils in arid and semi-arid Mediterranean regions through halophytes, microbial and soil-based technologies, Environ. Exp. Bot. 212 (2023) 105397, https://doi.org/10.1016/j.envexpbot.2023.105397.

[2] K. Ben Hamed, A. Castagna, A. Ranieri, P. García-Caparrós, M. Santin, J. A. Hernandez, G.B. Espin, Halophyte based Mediterranean agriculture in the contexts of food insecurity and global climate change, Environ. Exp. Bot. 191 (2021) 104601, https://doi.org/10.1016/j.envexpbot.2021.104601.

[3] A. Goujon, H. Alkitkat, Population et capital humain en Egypte à l'horizon 2050, Conflu. Méditerranée N°75 (2010) 33–47, https://doi.org/10.3917/come.075.0033.

[4] M. Khedr, Challenges and issues in water, climate change, and food security in Egypt, in: A.M. Negm (Ed.), Conventional Water Resources and Agriculture in Egypt, Springer International Publishing, Cham, 2019, pp. 229–243, https://doi.org/10.1007/698_2017_67.

[5] R. El Bedawy, Water resources management: alarming crisis for Egypt, J. Manag. Sustain. 4 (2014) p108, https://doi.org/10.5539/jms.v4n3p108.

[6] S.S. Fouad, E. Heggy, M. Ramah, A.Z. Abotalib, E.M. Palmer, S. Jomaa, U. Weilacher, Egypt's waterways conservation campaigns under growing intrinsic demand and Nile upstream damming, J. Hydrol. Reg. Stud. 50 (2023) 101537, https://doi.org/10.1016/j.ejrh.2023.101537.

[7] M. El-Liethy, G. Hassan, A. Al-Aneny, M. Hellal, Technologies for wastewater treatment and reuse in Egypt: Prospectives and future challenges, in: 2021: pp. 275–307. https://doi.org/10.1016/B978-0-12-822134-1.00010-5.

[8] M.D. Nguyen, M. Thomas, A. Surapaneni, E.M. Moon, N.A. Milne, Beneficial reuse of water treatment sludge in the context of circular economy, Environ. Technol. Innov. 28 (2022) 102651, https://doi.org/10.1016/j.eti.2022.102651.

[9] J.A. Silva, Wastewater treatment and reuse for sustainable water resources management: a systematic literature review, Sustainability 15 (2023) 10940, https://doi.org/10.3390/su151410940.

[10] U. Riaz, T. Athar, U. Mustafa, R. Iqbal, Chapter 23 - Economic feasibility of phytoremediation, in: R.A. Bhat, F.M.P. Tonelli, G.H. Dar, K. Hakeem (Eds.), Phytoremediation, Academic Press, 2022, pp. 481–502, https://doi.org/10.1016/B978-0-323-89874-4.00025-X.

[11] M.M. Waly, T. Ahmed, Z. Abunada, S.B. Mickovski, C. Thomson, M.M. Waly, T. Ahmed, Z. Abunada, S.B. Mickovski, C. Thomson, Constructed wetland for sustainable and low-cost wastewater treatment: review article, Land 11 (2022), https://doi.org/10.3390/land11091388.

[12] J. Santos, S. Rodrigues, M. Magalhães, K. Rodrigues, L. Pereira, G. Marinho, A state-of-the-art review (2019–2023) on constructed wetlands for greywater treatment and reuse, Environ. Chall. 16 (2024) 100973, https://doi.org/10.1016/j.envc.2024.100973.

[13] A.M.N. Masoud, A. Alfarra, S. Sorlini, Constructed wetlands as a solution for sustainable sanitation: a comprehensive review on integrating climate change resilience and circular economy, Water 14 (2022) 3232, https://doi.org/10.3390/w14203232.

[14] H. Zhang, W. Tang, W. Wang, W. Yin, H. Liu, X. Ma, Y. Zhou, P. Lei, D. Wei, L. Zhang, C. Liu, J. Zha, A review on China's constructed wetlands in recent three decades: application and practice, J. Environ. Sci. 104 (2021) 53–68, https://doi.org/10.1016/j.jes.2020.11.032.

[15] A.I. Stefanakis, Constructed wetlands for sustainable wastewater treatment in hot and arid climates: opportunities, challenges and case studies in the middle East, Water 12 (2020), https://doi.org/10.3390/w12061665.

[16] T. Saeed, M.J. Miah, T. Khan, Intensified constructed wetlands for the treatment of municipal wastewater: experimental investigation and kinetic modelling, Environ. Sci. Pollut. Res 28 (2021) 30908–30928, https://doi.org/10.1007/s11356-021-12700-8.

[17] F. Licciardello, O. Mahjoub, D. Ventura, H. Kallali, A. Mohamed, S. Barbagallo, G. L. Cirelli, Nature-based treatment systems for reclaimed water use in agriculture in Mediterranean countries, in: M. Nasr, A.M. Negm (Eds.), Cost-Efficient Wastewater Treatment Technologies: Natural Systems, Springer International Publishing, Cham, 2023, pp. 19–44, https://doi.org/10.1007/698_2021_783.

[18] M.A. El-Khateeb, S. El-Abeid, M.S.A. Haridy, H.M. Ahmed, H.I. Abdel-Shafy, Phytoremediation of different types of domestic wastewater using constructed wetlands: case studies in Egypt, in: A.A.H. Abdel Latef, E.M. Zayed, A.A. Omar (Eds.), Sustainable Remediation for Pollution and Climate Resilience, Springer Nature, Singapore, 2025, pp. 27–39, https://doi.org/10.1007/978-981-96-5674-5_2.

[19] S. Zamora, M. Hernández-Orduña, J. Moreno-Seceña, G. Martínez Escalante, J. Lomeli, I. Zitácuaro-Contreras, J. Marín-Muñiz, Maximizing value in constructed wetlands: a review of ornamental plants for wastewater treatment and artisanal applications, Earth 6 (2025) 1–19, https://doi.org/10.3390/earth6040126.

[20] E.A. Betanzo-Torres, G. Ballut-Dajud, G. Aguilar-Cortés, E. Delfín-Portela, L.C. S. Herazo, E.A. Betanzo-Torres, G. Ballut-Dajud, G. Aguilar-Cortés, E. Delfín-Portela, L.C.S. Herazo, Plants used in constructed wetlands for aquaculture: a systematic review, Sustainability 17 (2025), https://doi.org/10.3390/su17146298.

[21] I. Spiniello, M. De Carluccio, S. Castiglione, E. Amineva, N. Kostryukova, A. Cicatelli, L. Rizzo, F. Guarino, Landfill leachate treatment by a combination of a multiple plant hybrid constructed wetland system with a solar photoFenton process in a raceway pond reactor, J. Environ. Manag. 331 (2023) 117211, https://doi.org/10.1016/j.jenvman.2022.117211.

[22] T. Tuttolomondo, G. Virga, M. Licata, C. Leto, S. La Bella, Constructed wetlands as sustainable technology for the treatment and reuse of the first-flush stormwater in agriculture—a case study in Sicily (Italy), Water 12 (2020) 2542, https://doi.org/10.3390/w12092542.

[23] A. Faggiano, M. Ricciardi, A. Fiorentino, R. Cucciniello, O. Motta, L. Rizzo, A. Proto, Combination of foam fractionation and photo-Fenton like processes for greywater treatment, Sep. Purif. Technol. 293 (2022) 121114, https://doi.org/10.1016/j.seppur.2022.121114.

[24] C. Tammone, D.N. Tangredi, A. Cicatelli, F. Giuliano, I. Spiniello, F. Guarino, S. Castiglione, Efficient phytoremediation of first-flush storm water using an innovative constructed wetland: a pilot-scale study towards sustainable water reuse, Plant Biosyst. Int. J. Deal. All Asp. Plant Biol. 159 (2025) 66–78, https://doi.org/10.1080/11263504.2024.2446782.

[25] Z. Ji, W. Tang, Y. Pei, Constructed wetland substrates: a review on development, function mechanisms, and application in contaminants removal, Chemosphere 286 (2022) 131564, https://doi.org/10.1016/j.chemosphere.2021.131564.

[26] H.I. Abdel-Shafy, M.A. El-Khateeb, M.S.M. Mansour, Constructed wetlands for wastewater management in Egypt: an overview of 30-years experiences in small/medium-size treatment plants, in: A. Stefanakis (Ed.), Constructed Wetlands for Wastewater Treatment in Hot and Arid Climates, Springer International Publishing, Cham, 2022, pp. 71–99, https://doi.org/10.1007/978-3-031-03600-2_5.

[27] Q.-L. Chen, X.-L. An, B.-X. Zheng, M. Gillings, J. Peñuelas, L. Cui, J.-Q. Su, Y.-G. Zhu, Loss of soil microbial diversity exacerbates spread of antibiotic resistance, Soil Ecol. Lett. 1 (2019) 3–13, https://doi.org/10.1007/s42832-019-0011-0.

[28] M. Hazra, H. Joshi, J.B. Williams, J.E.M. Watts, Antibiotics and antibiotic resistant bacteria/genes in urban wastewater: a comparison of their fate in conventional treatment systems and constructed wetlands, Chemosphere 303 (2022) 135148, https://doi.org/10.1016/j.chemosphere.2022.135148.

[29] M. Hdidou, M.C. Necibi, J. Labille, S. El Hajjaji, D. Dhiba, A. Chehbouni, N. Roche, Potential use of constructed wetland systems for rural sanitation and wastewater reuse in agriculture in the moroccan context, Energies 15 (2021) 156, https://doi.org/10.3390/en15010156.

[30] X. Liu, Y. Liu, S. Lu, W. Guo, B. Xi, Performance and mechanism into TiO2/Zeolite composites for sulfadiazine adsorption and photodegradation, Chem. Eng. J. 350 (2018) 131–147, https://doi.org/10.1016/j.cej.2018.05.141.

[31] R.K. Manoharan, F. Ishaque, Y.-H. Ahn, Fate of antibiotic resistant genes in wastewater environments and treatment strategies - a review, Chemosphere 298 (2022) 134671, https://doi.org/10.1016/j.chemosphere.2022.134671.

[32] A. Stefanakis, The role of constructed wetlands as green infrastructure for sustainable urban water management, Sustainability 11 (2019) 6981, https://doi.org/10.3390/su11246981.

[33] Y. Yang, W. Shi, S.-Y. Lu, J. Liu, H. Liang, Y. Yang, G. Duan, Y. Li, H. Wang, A. Zhang, Prevalence of antibiotic resistance genes in bacteriophage DNA fraction from Funan River water in Sichuan, China, Sci. Total Environ. 626 (2018) 835–841, https://doi.org/10.1016/j.scitotenv.2018.01.148.

[34] D.Q. Zhang, K.B.S.N. Jinadasa, R.M. Gersberg, Y. Liu, W.J. Ng, S.K. Tan, Application of constructed wetlands for wastewater treatment in developing countries – a review of recent developments (2000–2013), J. Environ. Manag. 141 (2014) 116–131, https://doi.org/10.1016/j.jenvman.2014.03.015.

[35] I. Kabenge, G. Ouma, D. Aboagye, N. Banadda, Performance of a constructed wetland as an upstream intervention for stormwater runoff quality management, Environ. Sci. Pollut. Res. 25 (2018) 36765–36774, https://doi.org/10.1007/s11356-018-3580-z.

[36] A. Cristaldi, G. Oliveri Conti, S.L. Cosentino, G. Mauromicale, C. Copat, A. Grasso, P. Zuccarello, M. Fiore, C. Restuccia, M. Ferrante, Phytoremediation potential of Arundo donax (Giant Reed) in contaminated soil by heavy metals, Environ. Res. 185 (2020) 109427, https://doi.org/10.1016/j.envres.2020.109427.

[37] G. Bonanno, Trace element accumulation and distribution in the organs of Phragmites australis (Common Reed) and biomonitoring applications, Ecotoxicol. Environ. Saf. 74 (2011) 1057–1064, https://doi.org/10.1016/j.ecoenv.2011.01.018.

[38] A. Luis, E.-G. Ana Lorena, L. Amaya, T. Iñaki, Unplanted wetland-type filter for co-treatment of landfill leachate and septic tank wastewater: analysing gravel replacement by plastic and passive (filling-emptied) aeration effects at pilot scale, J. Environ. Manag. 294 (2021) 112940, https://doi.org/10.1016/j.jenvman.2021.112940.

[39] C.-H. Chen, I.-J. Chiou, Remediation of heavy metal-contaminated farm soil using turnover and attenuation method guided with a sustainable management framework, Environ. Eng. Sci. 25 (2008) 11–32, https://doi.org/10.1089/ees.2006.0183.

[40] F. Zurita, J. De Anda, M.A. Belmont, Treatment of domestic wastewater and production of commercial flowers in vertical and horizontal subsurface-flow

constructed wetlands, Ecol. Eng. 35 (2009) 861–869, https://doi.org/10.1016/j.ecoleng.2008.12.026.

[41] Y. Ge, W. Han, C. Huang, H. Wang, D. Liu, S.X. Chang, B. Gu, C. Zhang, B. Gu, X. Fan, Y. Du, J. Chang, Positive effects of plant diversity on nitrogen removal in microcosms of constructed wetlands with high ammonium loading, Ecol. Eng. 82 (2015) 614–623, https://doi.org/10.1016/j.ecoleng.2015.05.030.

[42] Y. Kamiji, J. Pang, S.P. Milroy, J.A. Palta, Shoot biomass in wheat is the driver for nitrogen uptake under low nitrogen supply, but not under high nitrogen supply, Field Crops Res. 165 (2014) 92–98, https://doi.org/10.1016/j.fcr.2014.04.009.

[43] H. Sun, C. Zhang, C. Song, S.X. Chang, B. Gu, Z. Chen, C. Peng, J. Chang, Y. Ge, The effects of plant diversity on nitrous oxide emissions in hydroponic microcosms, Atmos. Environ. 77 (2013) 544–547, https://doi.org/10.1016/j.atmosenv.2013.05.058.

[44] C.-B. Zhang, J. Wang, W.-L. Liu, S.-X. Zhu, D. Liu, S.X. Chang, J. Chang, Y. Ge, Effects of plant diversity on nutrient retention and enzyme activities in a full-scale constructed wetland, Bioresour. Technol. 101 (2010) 1686–1692, https://doi.org/10.1016/j.biortech.2009.10.001.

[45] X. Zuo, H. Zhang, J. Yu, Microbial diversity for the improvement of nitrogen removal in stormwater bioretention cells with three aquatic plants, Chemosphere 244 (2020) 125626, https://doi.org/10.1016/j.chemosphere.2019.125626.

[46] A. Srivastava, V.K. Parida, A. Majumder, B. Gupta, A.K. Gupta, Treatment of saline wastewater using physicochemical, biological, and hybrid processes: Insights into inhibition mechanisms, treatment efficiencies and performance enhancement, J. Environ. Chem. Eng. 9 (2021) 105775, https://doi.org/10.1016/j.jece.2021.105775.

[47] C. Yang, T. Fu, H. Wang, R. Chen, B. Wang, T. He, Y. Pi, J. Zhou, T. Liang, M. Chen, Removal of organic pollutants by effluent recirculation constructed wetlands system treating landfill leachate, Environ. Technol. Innov. 24 (2021) 101843, https://doi.org/10.1016/j.eti.2021.101843.

[48] A.E. Turcios, R. Miglio, R. Vela, G. Sánchez, T. Bergier, A. Włodyka-Bergier, J. I. Cifuentes, G. Pignataro, T. Avellan, J. Papenbrock, From natural habitats to successful application - role of halophytes in the treatment of saline wastewater in constructed wetlands with a focus on Latin America, Environ. Exp. Bot. 190 (2021) 104583, https://doi.org/10.1016/j.envexpbot.2021.104583.

[49] R. Bakhshoodeh, N. Alavi, C. Oldham, R.M. Santos, A.A. Babaei, J. Vymazal, P. Paydary, Constructed wetlands for landfill leachate treatment: a review, Ecol. Eng. 146 (2020) 105725, https://doi.org/10.1016/j.ecoleng.2020.105725.

[50] M.P. Ciria, M.L. Solano, P. Soriano, Role of macrophyte Typha latifolia in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel, Biosyst. Eng. 92 (2005) 535–544, https://doi.org/10.1016/j.biosystemseng.2005.08.007.

[51] S. Patel, Threats, management and envisaged utilizations of aquatic weed Eichhornia crassipes: an overview, Rev. Environ. Sci. Bio/Technol. 11 (2012) 249–259, https://doi.org/10.1007/s11157-012-9289-4.

[52] Z.W. Song, L. Wu, G. Yang, M. Xu, S.P. Wen, Indicator microorganisms and pathogens removal function performed by copepods in constructed wetlands, Bull. Environ. Contam. Toxicol. 81 (2008) 459–463, https://doi.org/10.1007/s00128-008-9527-1.

[53] J. Vymazal, Constructed wetlands for wastewater treatment, Ecol. Eng. 25 (2005) 475–477, https://doi.org/10.1016/j.ecoleng.2005.07.002.

[54] H. Wand, G. Vacca, P. Kuschk, M. Krüger, M. Kästner, Removal of bacteria by filtration in planted and non-planted sand columns, Water Res. 41 (2007) 159–167, https://doi.org/10.1016/j.watres.2006.08.024.

[55] L. Boutilier, R. Jamieson, R. Gordon, C. Lake, W. Hart, Adsorption, sedimentation, and inactivation of E. coli within wastewater treatment wetlands, Water Res. 43 (2009) 4370–4380, https://doi.org/10.1016/j.watres.2009.06.039.

[56] J. Vymazal, Constructed wetlands for treatment of industrial wastewaters: a review, Ecol. Eng. 73 (2014) 724–751, https://doi.org/10.1016/j.ecoleng.2014.09.034.

[57] J. Wang, Y. Long, G. Yu, G. Wang, Z. Zhou, P. Li, Y. Zhang, K. Yang, S. Wang, A review on microorganisms in constructed wetlands for typical pollutant removal: species, function, and diversity, Front. Microbiol. 13 (2022) 845725, https://doi.org/10.3389/fmicb.2022.845725.

[58] N.H. Youssef, K.N. Ashlock-Savage, M.S. Elshahed, Phylogenetic diversities and community structure of members of the extremely halophilic archaea (Order halobacteriales) in multiple saline sediment habitats, Appl. Environ. Microbiol. 78 (2012) 1332–1344, https://doi.org/10.1128/AEM.07420-11.

[59] H.A. Assress, R. Selvarajan, H. Nyoni, K. Ntushelo, B.B. Mamba, T.A.M. Msagati, Diversity, co-occurrence and implications of fungal communities in wastewater treatment plants, Sci. Rep. 9 (2019) 14056, https://doi.org/10.1038/s41598-019-50624-z.

[60] M.S. Calabon, K.D. Hyde, E.B.G. Jones, Z.-L. Luo, W. Dong, V.G. Hurdeal, E. Gentekaki, W. Rossi, M. Leonardi, V. Thiyagaraja, A.S. Lestari, H.-W. Shen, D.-F. Bao, N. Boonyuen, M. Zeng, Freshwater fungal numbers, Fungal Divers. 114 (2022) 3–235, https://doi.org/10.1007/s13225-022-00503-2.