



## Research article

# Long-term investigation of constructed wetland wastewater treatment and reuse: Selection of adapted plant species for metaremediation



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## ABSTRACT

A highly diverse plant community in a constructed wetland was used to investigate an ecological treatment system for human wastewater in an arid climate. The eight-year operation of the system has allowed the identification of a highly adapted and effective plant consortium that is convenient for plant-assisted metaremediation of wastewater. This constructed wetland pilot station demonstrated effective performance over this extended period. Originally, there were twenty-five plant species. However, because of environmental constraints and pressure from interspecific competition, only seven species persisted. Interestingly, the molecular phylogenetic analyses and an investigation of the photosynthetic physiology showed that the naturally selected plants are predominately monocot species with C4 or C4-like photosynthetic pathways. Despite the loss of 72% of initially used species in the constructed wetland, the removal efficiencies of BOD, COD, TSS, total phosphorus, ammonia and nitrate were maintained at high levels, approximately 90%, 80%, 94%, 60% and 50%, respectively. Concomitantly, the microbiological water tests showed an extremely high reduction of total coliform bacteria and streptococci, about 99%, even without a specific disinfection step. Hence, the constructed wetland system produced water of high quality that can be used for agricultural purposes. In the present investigation, we provide a comprehensive set of plant species that might be used for long-term and large-scale wastewater treatment.

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## 1. Introduction

Because of increasing anthropogenic activity and poor wastewater management policies, humanity is now facing water shortages and water quality crises in many areas of the world. Several investigations have reported that only 20% of the wastewater worldwide receives adequate treatment (United Nations Educational, Scientific and Cultural Organization (UNESCO) UN-WATER, n.d.), and the treatment capacity is as low as 8% in low-income countries (Sato

et al., 2013). Wastewater from human dwellings and activities has been a primary target of many treatment technologies. In parallel, many strategies have been used in the attempt to solve this concern. Ecological and green strategies have emerged as low-cost, effective and socially accepted approaches. Concomitantly, recent 'omic' approaches, when combined with other methods, such as stable-isotope probing (SIP), yield information on community members that metabolize a particular substrate in complex ecosystems. Hence, it is possible to link taxonomic groups with organic pollutant degradation (Singleton et al., 2007, 2005). These approaches have highlighted the idea that complex organic pollutants are metabolized by microbial consortia and assemblages of green plants in the field (Terrence et al., 2013). These innovative approaches bring new insight on how organisms can function as a meta-organismic entity to both remediate polluted ecosystems and, by effectively treating

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human and industrial wastewater, prevent the contamination of water resources (El Amrani et al., 2015).

Indeed, plants have been shown to be the main ecological engineer in polluted environments, as plant roots release exudates that contain various nutritional and signalling molecules that influence bacterial and fungal populations. In wetlands, water-tolerant plants also pump air into their root systems, creating microzones where aerobic microbes can thrive. The complex interactions of these populations play a pivotal role in the biodegradation of complex organic xenobiotics. Studies on emerging integrative approaches, such as (meta-) genomics, (meta-) transcriptomics, (meta-) metabolomics, and (meta-) proteomics, illustrate how “omics” approaches can bring new insight to deciphering the molecular mechanisms of pollutants at both the single species and the community levels (El Amrani et al., 2015).

Accumulating data, considering the functioning of the whole biome involved in decontamination, show that plants that have adapted to contaminated ecosystems deeply influence the microbiome associated with the rhizosphere (defined as the area located in the vicinity of roots) and reprogram the microbiome in this area. Thereby, as distinct from phytoremediation, bioremediation or mycoremediation, which separately consider plants, bacteria or fungus, respectively, metaremediation was recently proposed as a new strategy to remediate polluted environments (El Amrani et al., 2015). Recent data have suggested that the joint action of plant-fungal-bacterial consortia results in the rapid and effective degradation of complex molecules (Boonchan et al., 2000). Consequently, in natural ecosystems, remediation must be evaluated at the meta-organismic level (Bell et al., 2014). Therefore, to reach a high catabolic potential, it is necessary to integrate the global meta-organism/holobiont scale to increase the efficiency of biologically based remediation.

The constructed Temacine wetland (Algeria) traces its origins to Biosphere 2 project systems based on high-biodiversity, subsurface-flow wetlands treatment/recycling systems for wastewater, which were developed by NASA and then further evolved into “Wastewater Gardens” (Nelson and Wolverton, 2011; Nelson, n.d.). The system studied was implemented by Dr. Mark Nelson and Florence Cattin of Wastewater Gardens International ([www.wastewatergardens.com](http://www.wastewatergardens.com)). The project was financed by the Algerian Government Ministry of Water Resources, Department of Sanitation and Environmental Protection (Ministère des Ressources en Eau (MRE) - Direction de l'Assainissement et de la Protection de l'Environnement (DAPE/MRE), and the town of Temacine with the support of the Belgian Technical Cooperation for the study and training part of the project. Covering 400 m<sup>2</sup> and shaped like a crescent moon, the Wastewater Gardens system was designed to handle 15 m<sup>3</sup> of daily wastewater effluent from 100–150 people (Nelson and Wolverton, 2011).

Constructed wetlands are engineered systems designed to use natural wetland processes, associated with wetland hydrology, soils, microbes and plants, to treat wastewater (Vymazal, 2009). Recent investigations have confirmed that the rhizosphere in wetlands is especially effective at reducing contaminants and improving water quality (Kaplan et al., 2016).

This technological approach has been spreading around the world as thousands of wastewater-constructed wetlands were implemented in many countries in varying climates and ecological conditions over the last few decades (Kadlec RH and Wallace SD, n.d.; United Nations Educational, Scientific and Cultural Organization (UNESCO) UN-WATER, n.d.). This strategy has been shown to produce high water quality in a myriad of conditions; thus, the concept of using constructed wetlands has gained support in Africa. Firstly in South Africa then in the late 1990s, wetlands were piloted in Egypt in Alexandria, Abbu Attwa, Ismailia (Williams et al., 1995), and several

systems were implemented in Morocco (Salama et al., 2014). However, there is no information concerning the longevity of the functioning of these particular wetland ecosystems and how the initial high biodiversity evolved in this wetland and climatic environment.

In this work, we present the data collected during eight years, from 2008 to 2015, of a pilot station of a national program aiming to use biological treatment of wastewater in small agricultural communities and towns. The constructed wetland studied in this paper was installed in the town of Temacine located in Wilaya de Ouargla (Algeria) in 2007. Our results show that the wastewater treatment of the pilot station accomplished a significant reduction of the organic load in an arid climate over the course of the study and produced an effluent of high-quality water, which meets the requirements and is being used for agricultural/horticultural irrigation. At the same time, from the initially higher biodiversity of plant species, only the plants that are well-adapted to these wetland and environmental conditions were maintained. Hence, in the present investigation we provide a comprehensive set of plant species which might be used for a long treatment program and for large-scale wastewater treatment in arid areas.

## 2. Materials and methods

### 2.1. Pilot station and samplings

The trial was conducted in a subsurface, horizontal flow, constructed wetland in Temacine designed (Lat. 31° 36' N, Long. 5° 54' 6" N and 32° 18' E, alt. 49 m) by Wastewater Gardens International (Fig. 1). The climate is Saharan and arid with an average annual rainfall of approximately 60 mm. The mean temperature ranges between 10 °C and 35.8 °C. The experimental design contained a wastewater sedimentation and primary treatment tank in the form of a 2-chamber septic tank with a final filter followed by a 400 m<sup>2</sup> treatment basin with 3% bottom slope, which had a 60 cm gravel bed (8–12 mm particle size, with an average porosity of 29%) with 5 cm of dry gravel covering the wastewater in the plant-assisted metaremediation treatment basin, thus insuring no bad odour or human contact occurs. The sample-collecting pipes are located as indicated in Fig. 1. These collecting points were used to collect samples for the biochemical and microbiological analyses.

A large plant biodiversity was established at the installation of the pilot station to optimize the absorption and biodegradation of organic and inorganic molecules contained in wastewater. Special attention was given to the inclusion of plants with high added values. The choice of plants was made according three main criteria: (i) plants adapted to arid environments and developing different root systems: deep, medium and shallow, (ii) plants with high utility and productivity and/or pleasing aesthetics, and (iii) plants which were to be tested (some of which were previously used in Wastewater Garden systems but not yet tested in this particular arid climate). The plants were initially planted from April to May 2007. Several supplementary species were added the following year to increase plant biodiversity.

Wastewater was discharged in the pilot station with a gravity-led horizontal flow of 15 m<sup>3</sup>/day. This corresponds to a retention time of 2.7 days in the sedimentation tank and 5 days in the metaremediation constructed wetland basin. The water flows reached the wet zone by gravity at an average speed of 4 L per second. The treated water was conducted through a drainage network for drip irrigation of an adjacent planted field.

### 2.2. Biochemical and bacteriological analysis

Samples were collected twice a month in the morning and then analysed in the laboratory of Saharan Bioresources at Ouargla

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