



# Enhanced remediation of sewage effluent by endophyte-assisted floating treatment wetlands



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## ABSTRACT

The use of floating treatment wetlands (FTWs) is a promising approach for the remediation of sewage effluent. The efficiency of FTWs can be improved by the combined use of plants and pollutant-degrading bacteria. The aim of this study was to evaluate the influence of inoculation of endophytic bacteria on the detoxification of sewage effluent in FTWs. A terrestrial plant, *Brachiaria mutica*, was vegetated on a floating mat and inoculated with three endophytic bacterial strains, *Acinetobacter sp.* strain BRSI56, *Bacillus cereus* strain BRSI57, and *Bacillus licheniformis* strain BRSI58, to develop FTWs for the remediation of sewage effluent of Faisalabad city (Pakistan). Results indicated that *B. mutica* has the potential to remove both organic and inorganic contaminants from sewage effluent. However, endophytic inoculation in FTWs further enhanced the removal efficiency. Maximum reduction in chemical oxygen demand (COD), biochemical oxygen demand (BOD<sub>5</sub>), total nitrogen (N), and phosphate (PO<sub>4</sub>) was achieved by the combined use of plants and bacteria. Moreover, the inoculated bacteria showed persistence in water as well as colonization in the root and shoot of the plant. Treated effluent met the national wastewater discharge standards of Pakistan and can be discharged in the environment without any environmental risks. This study provides useful evidence of endophyte-assisted FTWs to be the most sustainable and affordable approach for *in situ* remediation of sewage effluent.

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## 1. Introduction

Worldwide urbanization has increased the release of sewage effluent in surface water resources. Sewage effluent contains different types of organic and inorganic contaminants, which deteriorate the quality of water resources (Bueno et al., 2012). The use of FTWs is a relatively new approach whereby plants are vegetated on soil-less buoyant mats in a manner that underground biomass hangs freely in the water column flowing underneath the mat (De Stefani et al., 2011; Hwang and LePage, 2011). Despite the simple setup and ease of establishment of FTWs, researchers have reported them to be a highly effective approach toward improving the quality of sewage effluent (Borne et al., 2013; Lynch et al., 2015; Zhang et al., 2014).

In FTWs, plants, in combination with microbial partners, employ natural physical, chemical, and biological processes to remove pollutants from contaminated water (Chang et al., 2013; Zhang et al., 2014). In plant–bacteria association, bacteria are capable of

contributing toward overall pollutant removal by degrading complex organic pollutants and assimilating N and phosphorous (P) (Afzal et al., 2014a; Khan et al., 2013a; Li et al., 2010). Bacteria are also reported to reduce concentration of nitrate (NO<sub>3</sub>) (Nagadomi et al., 2000), PO<sub>4</sub>, and heavy metals in wastewater (El-Sheekh et al., 2005), through metabolism-dependent and independent methods (da Costa and de França, 2003). Although microorganisms play an important role in the mineralization of organic pollutants and the biogeochemical transformation of nutrients in wetlands, nothing is known about the persistence and activity of the inoculated bacteria in different components (water, root and shoot) of FTWs.

Several microorganisms from water can colonize on the root or rhizome surface and establish the so-called biofilm through a repeating proliferation process (Zhang et al., 2014). Some of the root colonizing bacteria penetrate the root, colonize within it, and/or migrate to the aerial parts; these are known as endophytes (Afzal et al., 2014b; Compant et al., 2010). Microbial population on the root surface and inside the plant tissues enhances the removal of pollutants from water (Newman and Reynolds, 2005; Weyens et al., 2013; Shehzadi et al., 2014).

Different aquatic plants, such as *Pontederia cordata*, *Schoenoplectus tabernaemontani* (Wang et al., 2015), *Cyperus ustulatus*, *Juncus edgariae* (Tanner and Headley, 2011), *Juncus effusus*, *Pontederia cordata* (Chang et al., 2013), *Typha angustifolia* (Keizer-Vlek

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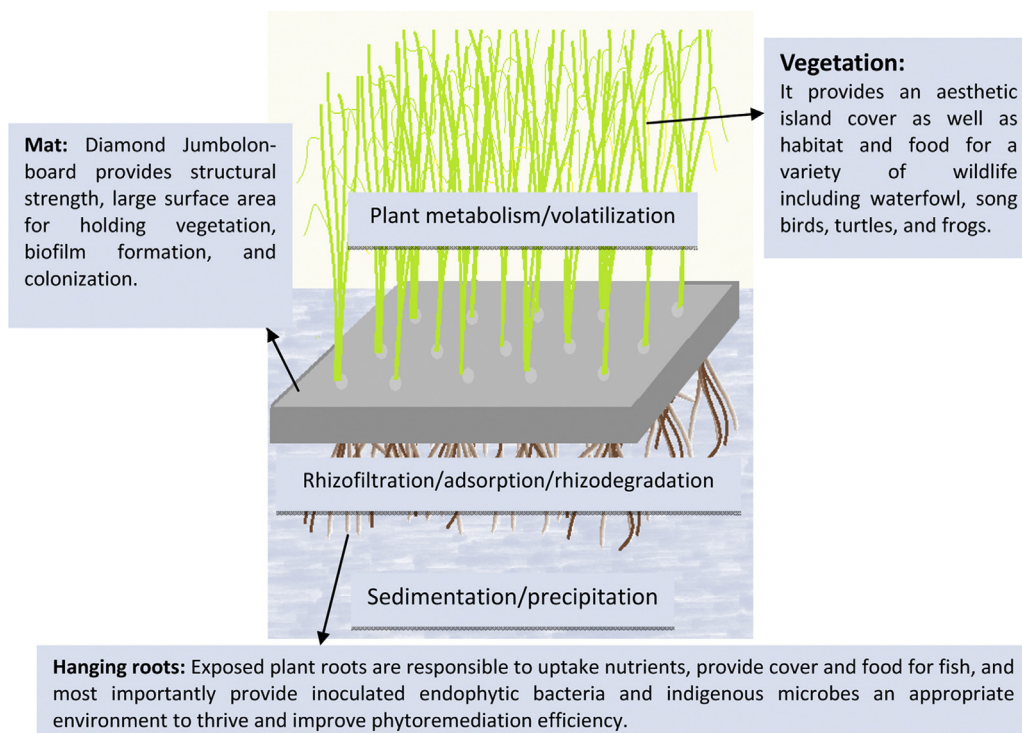


Fig. 1. Schematic diagram showing the development of floating treatment wetlands for the treatment of sewage effluent.

et al., 2014), *Oenanthe javanica* (Zhou and Wang, 2010), *Gardenia jasminoides* (Zhu et al., 2011), *Canna indica* (Sun et al., 2009) and *Carex virgate* (Borne et al., 2013) have been used to develop FTWs. However, *Brachiaria mutica*, commonly known as para grass, has not been tested to develop FTWs to date. Moreover, the combined use of plants and endophytic bacteria has not been evaluated in FTWs for the remediation of sewage effluent. Therefore, the aim of this study is to evaluate the potential of *B. mutica* on its own and in combination with endophytic bacteria in FTWs for the remediation of sewage effluent. Removal of organic and inorganic pollutants as well as toxicity reduction of the treated effluent has been observed. Moreover, the persistence of the inoculated bacteria has been determined in different components (root, shoot and water) of the FTWs as well.

## 2. Methodology

### 2.1. Sewage effluent collection and characterization

Faisalabad city is the industrial hub of Pakistan with a population of more than 2.7 million and sewage effluent generation of nearly 435 million gallons per day that is discharged in the Chenab and Ravi rivers through Paharang and Madhuana drains, respectively (Kahlowan et al., 2006). Many small and large scale industries are located in the city area and discharge their wastewater in these sewage effluent drains. Sewage effluent was collected in September and October 2014 from Paharang and Madhuana drains, respectively, and analyzed for various physicochemical parameters such as pH, electrical conductivity (EC), COD, BOD<sub>5</sub>, total dissolved solids

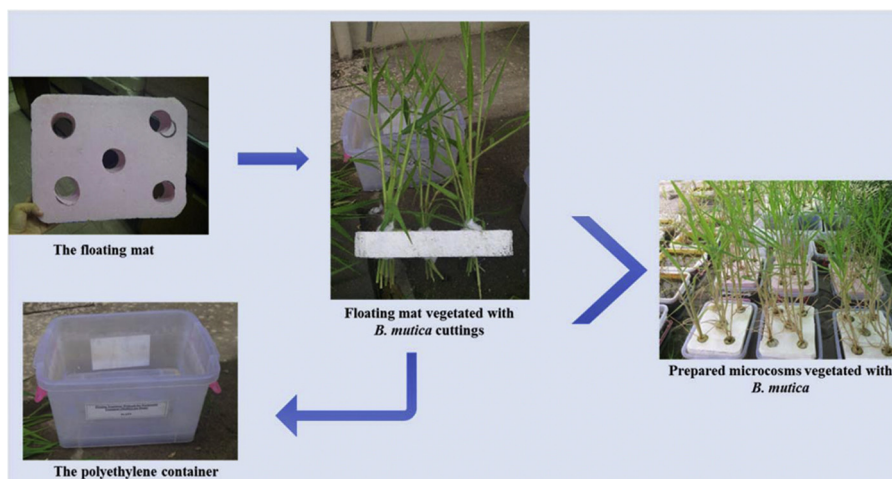


Fig. 2. Different components of floating treatment wetland microcosm.

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