Phytodepuration of Pyroligneous Liquor: A Case Study

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Abstract: Wastewaters generated by the pyrolytic process require treatments to reduce the risks of contamination in rivers, lakes, and coastal waters. Utilizing constructed wetlands is one of the possible approaches according to a Circular Economy System. Plant Growth-Promoting Bacteria (PGPB) and Arbuscular Mycorrhizal Fungi (AMF) can improve plant growth and enhance the bioremediation of wastewater. Two experiments were set up: in the first, a pilot mesocosm was designed to evaluate the effects of a consortium of AM fungi and a PGPB strain on Phragmites australis. After 60 days, the highest plant growth was obtained after inoculation with the combination of microorganisms. In the second experiment, a constructed wetland was built to remediate wastewaters from gasification plant. The plants were efficient in scavenging biological oxygen demand (BOD₅), chemical oxygen demand (COD), total fat and oils, hydrocarbons, phenols, aldehydes, surfactants, fluorides, sulfites, sulfates, nitrate, and phosphorus. These data suggest that inoculation of P. australis with AMF and PGPB strains significantly improve the depuration process of wastewaters from gasification plants via constructed wetlands.

Keywords: constructed wetlands AMF; PGPB; Phragmites australis; bioremediation

1. Introduction

Pyrolysis is a degradative technique for producing energy from biomasses, in the absence of oxygen [1], and represents an example of a Circular Economy System. Pyrolysis process generates two types of wastes: (i) a carbon-based material that could be used in different pathways (such as production of carbon activated materials) and (ii) a “pyroligneous liquor” (PL) composed by water, alcohols, organic acids, phenols, aldehydes, ketones, esters, furan and pyran derivatives, hydrocarbons, and nitrogen compounds [2]. According to the Italian Law, PL represents an environmental risk due to its composition so remediation of the waste is required. Among other clean-up technologies, phytoremediation entails a set of natural processes of polluted water treatment based on how the soil-vegetation system can behave as a natural filter for water purification. Compared to traditional practices, phytoremediation requires larger surfaces, but leads to lower employment of energy and technology, and is more sustainable under an economic point of view [3].

Moreover, plants used for remediation of wastewater produce a relevant biomass that is of great interest for energy production and heavy metal recovery for industrial purposes [4–8]. Phytoremediation of PL can be achieved using constructed wetlands (CWs) that are based on the interactions of plants and the associated microflora living in the rhizosphere [9]. For improving the efficiency of CWs, bioaugmentation could be a possibility to accelerate the establishment of microflora to remediate hard degradable industrial pollutants, such as PL [10]. Among the microflora in direct contact with a root system, Arbuscular Mycorrhizae (AM) are an example of important symbiosis involving fungi and...
plants, resulting in a nutritional exchange in which fungi improve the mineral nutrition of plants and receive photosynthates [11]. AM fungi (AMF) can act as biofilters, reducing abiotic stresses in plants due to organic compounds such as insecticides [12], aromatic hydrocarbons and polycyclic aromatic hydrocarbons [13–16]. Moreover, the interaction between plants and AMF plays an active role in heavy metals stresses and accumulation [17–20]. Despite the wide range of research applications of AMF in CWs [21], little is known about the remediation of PL.

Bioaugmentation can also be performed by adding bacteria that directly or indirectly interact with plants: as previously reported, specific bacterial strains can promote symbiosis between plant roots and AMF [22,23]. Moreover, selected rhizobacteria can degrade total petroleum hydrocarbon effluent [24], organic chemicals [25], heavy metals [26], and contribute to nitrification [27] and denitrification [28].

Bacteria living in rhizosphere with beneficial effects on the plant development and health (the so-called Plant Growth- Promoting Bacteria or PGPB) can promote plant growth by producing plant hormones, solubilizing phosphate, producing enzymes such as the enzyme 1–aminocyclopropane–1-carboxylate (ACC) deaminase to alleviate biotic and abiotic stresses [29,30].

Here, we report on the results obtained in a CW, using Phragmites australis inoculated with a pool of AMF and a PGPB strain, to remediate PL polluted waters.

2. Materials and Methods

2.1. Biological Materials

Rhizomes of reed plants [Phragmites australis (Cav.) Trin ex Steud.] provided by Mybasol Srl (Alessandria, Italy) from a local natural field, were transferred in pots filled with 100 mL gravel (12–15 mm) on bottom and 600 mL substrate constituted of unsterile 1:2 loam/quartz sand (diameter 1–2 mm) (Punto Elle, Turin, Italy) and grown in a greenhouse. Two experiments were set up: the first experiment on a mesocosm (see below), and the second one in a CW. AMF inoculum included sporocarps, spores, hyphae, and root fragments colonized by a consortium of AMF belonging to the genus Rhizophagus, collected from an agricultural soil, produced and provided by Mybasol S.r.l. Bacteria were inoculated as 20 mL of a bacterial suspension (10^8 cfu ml^-1) around the plant bases. Pseudomonas protegens PF7 was previously isolated from forest soil in Sassello (SV, Italy) by Dr. Elisa Gamalero (Università del Piemonte Orientale, Alessandria, and Mybasol S.r.l.) [27].

2.2. Mesocosm Experiment

In this experiment, 75 rhizomes were inoculated with a mycorrhizal inoculum (AMF), 25 with the PGPB strain P. protegens PF7 (PF7), 25 with AM fungi and the PGPB (AMF+PF7), and 25 were uninoculated and then used as a control (C). The mesocosm experiment was set up in 3 polycarbonate tanks, each containing 25 plants prepared as described above. P. australis plants were maintained in semi-submersion conditions and provided a Long Ashton nutrient solution containing 32 µM phosphate [31]. The level of the nutrient solution was constantly maintained at 7 cm from the ridge of the pot. The plants were grown in a protected environment (greenhouse with 30% shading) from March to April 2012. Then, 15 plants for each treatment were harvested to investigate the effects of AMF and/or PGPB on the above-ground shoot fresh and dry weights and the fresh weights and dry weights of roots. The root systems of ten plants from the treatments AMF and AMF+PF7 were used for the assessment of AMF colonization as described below.

2.3. Mycorrhizal Colonization

Root portions were fixed in 70% ethanol, stored at 4 °C and cut in forty 1 cm long pieces. Root pieces were cleared in 10% KOH for 45 min at 60 °C, stained with 1% methyl blue in lactic acid and mounted on a slide. Mycorrhizal colonization was estimated according to [32].
2.4. Field Experiment: Study Site and Wetland Design

The gasification plant was located in Ivrea, Turin, North-West Italy (Syntechnology Srl, TO), equipped with a fixed bed type Imbert downdraft reactor. The biomass used was chestnut wood chips dried by augers at a temperature of 600 °C up to a humidity level of 15%. The treatment capacity was 250 kg/hr with a production of wastewater was around 80–120 lt/hr (depending on whether the gasification plant was at 75% of the rated power or full capacity). The retention time was 2.5 min and the composition of syngas was 27% H₂, 7% CH₄ with a production of char of 8.5%. The CW was built into the horizontal subsurface flow (HSF) wetland, with dimensions of 6 m × 20 m × 1 m and a slope of 1%, under supervision of a qualified engineer. After a covertecur of polyethylene with a thickness of 0.5 cm, the CW was filled from bottom to top with gravels (size) of 20–40 mm, 15–25 mm, 5–15 mm, forming depths of 25 cm, 25 cm and 30 cm (Figure 1). The CW was filled with local agricultural soil forming a depth of 20 cm. The hydraulic retention time of the CW was 4 days. Two points of monitoring were constructed at the beginning (IN) and the end (OUT) of CW. The CW was planted with 300 P. australis at a density of 2.5 plants (approximately 50 cm in height) per square meter, with a rhombic shape (Figure 2).

![Design of the constructed wetland.](image1)

![Figure 1. Design of the constructed wetland.](image2)

(a) (b)

**Figure 2.** (a) CW before planting *P. australis* (b) CW after 1 month

2.5. Chemical Analysis of Wastewater from the Gasification Plant

From June 2012 to November 2012, wastewaters from IN and OUT of CW were sampled approximately every 2 weeks (n = 14 for each point). Wastewater samples were analyzed to measure chemical oxygen demand (COD), biological oxygen demand (BOD₅), ammonium–nitrogen (NH₄–N), nitrate–nitrogen (NO₃–N), nitrite–nitrogen (NO₂–N), total phosphorus (TP), total suspended solids (TSS), pH, surfactants (total, anionic, cationic and nonionic), total phenols, total aldehydes, total hydrocarbons (THs). The amounts of 8 metals (Ba, Cd, Cr, Cu, Fe, Ni, Pb and Zn) were measured. BOD₅ was determined with OxiTop Control Measurement System (WTW, Xylem Analytics, Letchworth UK). Determination of COD, NH₄–N, NO₃–N, NO₂–N, TP, surfactants, and total phenols were determined spectrophotometrically using a HACH-DR 2800 spectrophotometer (Hach-Lange Gmbh,
Berlin, Germany). Organic pollutants were analyzed by gas or liquid chromatography coupled with a mass spectrometer (GC–MS and LC–MS). Metals were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and atomic fluorescence spectroscopy (AFS). All the parameters mentioned above were determined according to Italian decree 152/06. The water pH was measured with an Orion Portable pH Meter (Model 250Aplus, USA). Odor testing was conducted in accordance to APAT 29/2003 within 24 h from the time of sampling (1 lt of wastewater collected), with human assessors and defined on a scale of values [33] (p. 143).

2.6. Ecotoxicological Risk Assessment

Ecotoxicity was assessed by measuring the inhibition of *Vibrio fischeri*, *Pseudokirchneleriella subcapitata* and *Daphnia magna* exposed to PL fractions from IN and OUT. The bioassays with *V. fischeri* were carried out, according to ISO 11348-3:2007, to assess the reduction of bioluminescence after 15 min in a Microtox M500 luminometer (UK). Bioassays with *P. subcapitata* were performed using the commercially available kit and referring to the ISO 8692:2012. The algal cultures were performed in triplicate. The inhibition of algal growth was determined using a Beckman Coulter cell counter, with an incubation temperature of 24 ± 2 °C for 72 h. The biotest with *D. magna* was applied according to the UNI EN ISO 6341:2012. Organisms of the same age were exposed to PL for 24 h in triplicate. At the end of the test, the immobilized organisms were counted in comparison with the control (Milli-Q water). The percentage of mobile *D. magna* in the sample resulting higher than 50% of the control was determined, according to the Italian Decree 152/06.

2.7. Statistical Analysis

According to the different analysis, data were statistically analyzed either by one-way ANOVA. The significance of the comparisons was estimated by a Fisher’s probable least-squares difference test with cutoff significance at *p*-value < 0.05.

3. Results

3.1. Mesocosm Experiment

During the 60 days of the experiment in the mesocosms, AMF alone or in combination with PGPB *P. protegens* PF7 significantly improved plant growth: more precisely, by inoculating with AMF the dry weight of the aerial and sub-aerial parts increased by 51% and 23%, respectively, compared to the control. The shoot dry weight of plants co-inoculated with AMF and the bacterial strain was significantly higher than that of control plants (+72%) and the dry weight of sub-aerial parts increased by 23% (Figures 3 and 4). Considering the effects of beneficial microorganisms on dry weight, the enhanced growth was significantly marked in the double treatment.
Mycorrhizal Colonization

The mycorrhizal colonization in plants inoculated or not with the bacterial strain was similar except for the frequency of mycorrhiza (F %) that was lower in plants inoculated with *P. protegens* PF7 (Figure 5). Root colonization was not extensive, but sufficient to produce an effect on plant growth (Figure 4). The co-inoculation with PF7 did not increase mycorrhizal colonization degree. Double treatment AMF + PF7 was chosen for field experiment.

![Figure 3](image3.png)

**Figure 3.** Average fresh weight of shoots (a) and roots (b) in the mesocosm experiment. Different letters indicated that the differences among the means were significant (*p*-value < 0.05).

![Figure 4](image4.png)

**Figure 4.** Average dry weight of shoots (a) and roots (b) in the mesocosm experiment. Different letters indicated that the differences among the means were significant (*p*-value < 0.05).

![Figure 5](image5.png)

**Figure 5.** Mycorrhizal colonization in absence/presence of PF7 on mesocosms. Different letters indicated that the differences among the means were significant (*p*-value < 0.05).
3.2. Field Experiment

3.2.1. Chemical Analysis

During the 180 days of the experiment in the CW, the pH values of the wastewater from the gasification plant increased from 6.2 to 7.6 after the treatment with *P. australis* (Table 1). The color of PL before the phytodepuration process was not detectable at a dilution of 1/40; after the biological treatment, the color was not detectable at a dilution of 1/20, and the unpleasant smell was also completely removed. These results are in agreement with the decreases of TSS, phenols, and hydrocarbons: at the exit of CW, the decrease of TSS was around 57% and 99.9% for phenols and total hydrocarbons, respectively (Tables 1 and 2).

### Table 1. Physical and chemical characteristics of PL in inflow and outflow. Different letters indicated that the differences were significant (*p*-value < 0.05).

| Parameter                  | IN       | OUT      | u.m.   |
|----------------------------|----------|----------|--------|
| pH                         | 6.2 ± 0.1 a | 7.6 ± 0.2 b | pH     |
| Color                      | N.p. 1/40 | N.p. 1/20 | -      |
| Odor                       | unpleasant | not perceptible | -      |
| Total suspended solids     | 60.4 ± 1.4 a | 34.5 ± 2.7 b | mg/L   |
| BOD₅                       | 800.6 ± 1.4 a | 4.8 ± 0.3 b  | mg/L O₂ |
| COD                        | 1413.4 ± 3.8 a | 29.5 ± 1 b   | mg/L O₂ |

### Table 2. Organic contaminants previous and after phytodepuration. The values after the CW treatment were significantly different (*p*-value < 0.05).

| Contaminants               | IN        | OUT      | u.m.   |
|----------------------------|-----------|----------|--------|
| Fats and oils              | 9.5 ± 0.8 a | <0.01 b  | mg/L   |
| Total hydrocarbons         | 4.5 ± 0.7 a | <0.01 b  | mg/L   |
| Total phenols              | 27 ± 3 a   | <0.01 b  | mg/L   |
| Aldehydes                  | 8.2 ± 0.8 a | <0.01 b  | mg/L   |
| Total surfactants          | 1.38 ± 0.45 a | <0.01 b | mg/L   |
| Fats and oils              | 9.5 ± 0.8 a | <0.01 b  | mg/L   |

The determination of BOD₅ and COD showed a very high bio-treatment efficiency (~99.4 and ~98%, respectively) (Table 1). The high removal of BOD₅ and COD is related to the deposition and filtration of settleable organics and biodegradation of organic compounds mediated by the heterotrophic microorganisms. In fact, the reduction of fats and oils was significantly high; the inflow concentrations were 9.5 ppm, and the outflow did not contain any recordable trace of this fraction. The degradation/removal of total surfactants after CW depuration was assessed to be around 99.9% (Table 2).

Regarding the nitrogen sources, a different trend was observed: nitrite was not recordable both in inflow and in outflow, while nitrate decreased by 88%, and ammonium increased from 0.65 ppm to 3.8 ppm. The results depicted in Figure 6 reported that denitrification process took place: this process is related to organic matter content and it is positively correlated with pH at 7–8. Total phosphorus decreased by around 94% (Figure 6). The high removal rate of phosphorus may be attributed to the long contact time within the wetland (4 days). Sulfites decreased in the inflow around 85% and sulfates around 40%. The level of detected chlorides was similar in inflow and outflow of CW; on the contrary, fluorides were not registered in outflow (Table 3). The chemical analysis of heavy metals showed traces of Fe, Ni and Zn with a significant reduction only for Zn (85%) (Table 4).
V. fischeri showed low toxicity in the inflow (13%), but not in the outflow (78%), who suggested that change of pH to alkaline condition was probably due to the addition of oxygen. This result is in agreement with [34], who suggested that change of pH to alkaline condition was probably due to the addition of oxygen. 

In our experiment, the pH of PL wastewater was initially acid (Table 1) in the inflow; the difference among the means were significant (p-value < 0.05). The determination of BOD5 and COD showed a very high bio-treatment efficiency (-99.4 and -99.5%, respectively) (Table 1). The high removal of BOD5 and COD is related to the deposition and filtration of settleable organics and biodegradation of organic compounds mediated by the heterotrophic microorganisms. In fact, the reduction of fats and oils was significantly high; the inflow concentrations were 9.5 ppm, and the outflow did not contain any recordable trace of this fraction.

Contents of sulfites, sulfates and halogenates in wastewater previous and after the CW treatment. Different letters indicate that the differences among the means were significant (p-value < 0.05).

| Contaminant | IN         | OUT         | u.m. |
|------------|------------|-------------|------|
| Sulfites   | 11.72 ± 0.1 a | 1.66 ± 0.2 a | mg/L |
| Sulfates   | 55.14 ± 2.1 a | 33.41 ± 2.7 b | mg/L |
| Chlorides  | 10.2 ± 1.3 a  | 5.46 ± 2.4 a  | mg/L |
| Fluorides  | 0.45 ± 0.08 a | <0.01 b     | mg/L |

Physical and chemical characteristics of PL in inflow and outflow. Different letters indicate that the differences were significant (p-value < 0.05).

3.2.2. Ecotoxicological Risk Assessment

No acute toxicity was detected by the ecotoxicological tests performed with the selected organisms: V. fischeri showed low toxicity in the inflow (13%), but not in the outflow (<0.1%); similarly, the bioluminescence rate decreased. P. subcapitata showed a positive growth in the exit, +78%, and +63% for IN and OUT, respectively. D. magna test indicated a low immobilization in the inlet (7%), not occurring after the CW treatment (<0.1%) (Table 5).

Ecotoxicological risk assessment of wastewaters before and after the CW treatment. Different letters indicated that the differences among the means were significant (p-value < 0.05).

| V. fischeri | P. subcapitata | D. magna |
|------------|----------------|----------|
| IN         | 13 ± 2% a      | 7% ± 1% a |          |
| OUT        | <0.1% b        | 63 ± 1% a | <0.1% b  |

4. Discussion

In this paper, we show how a Constructed Wetland, connected to a pyrolysis plant, was able to remediate the wastewater with the aim of contributing to the water reuse in the context of a Circular Economy. Moreover, our results confirm the possible use of CWs, not only for urban wastewater up to 2000 equivalent people, as suggested by the Italian D.lgs 152/06, but also for industrial applications.

In our experiment, the pH of PL wastewater was initially acid (Table 1) in the inflow; the effect of the CW treatment provided a pH variation to slightly alkaline. This result is in agreement with [34], who suggested that change of pH to alkaline condition was probably due to the addition of oxygen.
through dissolution of atmospheric oxygen and photosynthetic activity by the plant. COD and BOD$_5$ produced by the pyrolysis plant were higher than the limit of the Italian Law; the results obtained after the CW treatment indicate that the CW was able to oxygenate the wastewater to a level that supports the aerobic degradation of the contaminants produced by pyrolysis, as previously observed by [35]. The CW treatment also improved the physical characteristics of the wastewaters, such as color and odor. These results are supported by the chemical analyses that showed a decrease of the evaluated parameters, except for the ammonium source of nitrogen, whose value significantly increased within the limit of the Italian Law. The observed increase could be ascribed to bacterial dissimilatory nitrate reduction to ammonium (DNRA), also known as nitrate/nitrite ammonification, a not very common process that is the result of anaerobic respiration by chemo-organo-heterotrophic microbes. This process could been performed by naturally occurring bacteria [36]. The nitrate source of nitrogen, significantly decreased, according with previous results obtained by using the same biological system on nitrate rich waters [27]. The ecotoxicological risk showed a positive impact of CW due to the efficient removal of almost all the targeted contaminants [37,38]. These results can be partly explained by the effects induced by AMF on the host plants, as shown by the mesocosm experiment: while improving the mineral nutrition of the plants, AMF can also increase their tolerance towards some pollutants, either organic or inorganic [39]. Here we show that plants inoculated with AMF were characterized by higher biomass than uninoculated ones and were also tolerant of PL pollutants. AMF generally increase plant biomass [39] and induce changes in root morphology, among which a higher degree of root branching [40]. An increment in root biomass was also found in the mesocosm experiment (Figures 3 and 4), meaning a higher absorption surface and, therefore, a higher depuration capability. The combination of AMF with the PGPB used in this experiment, led to a synergistic action, as already observed in many other plant species [19,41,42], furtherly improving the efficiency of the system: it has previously been shown that co-inoculation of AMF + PGPB promotes plant growth, with a synergistic effect either on plant growth or on root architecture [41]. Moreover, P. protegens PF7 showed to promote plant growth and not mycorrhiza establishment. Finally, the bacteria could themselves have colonized the large surface of the gravels filling the CW, acting as an active biofilm.

In conclusion, our data show that the remediation of pyroligneous wastewaters is possible by using constructed wetland technology: the phytodepuration allowed to reach the limits prescribed by the Italian Law concerning the quality of surface/sewerage water.

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**References**

1. Ioannidou, O.; Zabaniotou, A. Agricultural residues as precursors for activated carbon production—A review. *Renew. Sustain. Energy Rev.* 2007, 11, 1966–2005. [CrossRef]
2. Guerrero, M.; Ruiz, M.P.; Alzueta, M.U.; Bilbao, R.; Millera, A. Pyrolysis of eucalyptus at different heating rates: Studies of char characterization and oxidative reactivity. *J. Anal. Appl. Pyrolysis* 2005, 74, 307–314. [CrossRef]
3. Elizabeth Pilon-Smits, P. Phytoremediation. *Annu. Rev. Plant Biol.* 2005, 56, 15–39. [CrossRef]
4. Kabata-Pendias, A.H.P. *Trace Metals in Soils and Plants*; CRC Press: Boca Raton, FL, USA, 2001; Volume 140.
5. Ebbs, S.D.; Lasat, M.M.; Brady, D.J.; Cornish, J.; Gordon, R.; Kochian, L.V. Phytoextraction of Cadmium and Zinc from a Contaminated Soil. *J. Environ. Qual.* 1997, 26, 1424–1430. [CrossRef]
6. Paulson, M.; Bardos, P.; Harmsen, J.; Wilczek, J.; Barton, M.; Edwards, D. The practical use of short rotation coppice in land restoration. Land Contam. Reclam. 2003, 11, 323–338. [CrossRef]

7. Pulford, I.D.; Dickinson, N.M. Phytoremediation Technologies Using Trees. In Trace Elements in the Environment: Biogeochemistry, Biotechnology, and Bioremediation; CRC Press: Boca Raton, FL, USA, 2005; pp. 383–403.

8. Lone, M.I.; He, Z.L.; Stoffella, P.J.; Yang, X.E. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. J. Zhejiang Univ. Sci. B 2008, 9, 210–220. [CrossRef]

9. Vymazal, J. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. 2007, 380, 48–65. [CrossRef] [PubMed]

10. Merlin, G.; Cottin, N. Lab-Scale Performance Evaluation of Vertical Flow Reed Beds for the Treatment of Chlorobenzene Contaminated Groundwater. J. Environ. Prot. 2012, 3, 875–885. [CrossRef]

11. Smith, S.E.; Read, D. Mineral nutrition, toxic element accumulation and water relations of arbuscular mycorrhizal plants. In Mycorrhizal Symbiosis; Academic Press: Cambridge, MA, USA, 2008; pp. 145–187.

12. Korade, D.L.; Fulekar, M.H. Rhizosphere remediation of chlorpyrifos in mycorrhizospheric soil using ryegrass. J. Hazard. Mater. 2009, 172, 1344–1350. [CrossRef]

13. Volante, A.; Lingua, G.; Cesaro, P.; Cresta, A.; Puppo, M.; Ariati, L.; Berta, G. Influence of three species of arbuscular mycorrhizal fungi on the persistence of aromatic hydrocarbons in contaminated substrates. Mycorrhiza 2005, 16, 43–50. [CrossRef]

14. Wu, N.; Zhang, S.; Huang, H.; Christie, P. Enhanced dissipation of phenanthrene in spiked soil by arbuscular mycorrhizal alfalfa combined with a non-ionic surfactant amendment. Sci. Total Environ. 2008, 394, 230–236. [CrossRef] [PubMed]

15. Gao, Y.; Cheng, Z.; Ling, W.; Huang, J. Arbuscular mycorrhizal fungal hyphae contribute to the uptake of polycyclic aromatic hydrocarbons by plant roots. Bioresour. Technol. 2010, 101, 6895–6901. [CrossRef] [PubMed]

16. Rajtor, M.; Piotrowska-SEget, Z. Prospects for arbuscular mycorrhizal fungi (AMF) to assist in phytoremediation of soil hydrocarbon contaminants. Chemosphere 2016, 162, 105–116. [CrossRef] [PubMed]

17. Citterio, S.; Prato, N.; Fumagalli, P.; Aina, R.; Massa, N.; Santagostino, A.; Sgorbati, S.; Berta, G. The arbuscular mycorrhizal fungus Glomus mosseae induces growth and metal accumulation changes in Cannabis sativa L. Chemosphere 2005, 59, 21–29. [CrossRef] [PubMed]

18. Todeschini, V.; Burlando, B.; Berta, G.; Lingua, G.; Franchin, C.; Biondi, S.N.; Torrigiani, P.; Castiglione, S. Responses to copper of two registered poplar clones inoculated or not with arbuscular mycorrhizal fungi. Caryologia 2007, 60, 146–155.

19. Gamalero, E.; Lingua, G.; Berta, G.; Glick, B.R. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can. J. Microbiol. 2009, 55, 501–514. [CrossRef]

20. Miransari, M. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol. Adv. 2011, 29, 645–653. [CrossRef]

21. Xu, Z.; Ban, Y.; Jiang, Y.; Zhang, X.; Liu, X. Arbuscular Mycorrhizal Fungi in Wetland Habitats and Their Application in Constructed Wetland: A Review. Pedosphere 2016, 26, 592–617. [CrossRef]

22. Frey-Klett, P.; Garbaye, J.; Tarkka, M. The mycorrhiza helper bacteria revisited. New Phytol. 2007, 176, 22–36. [CrossRef]

23. Lingua, G.; Gamalero, E.; Fusconi, A.; Lemanceau, P.; Berta, G. Colonization of Plant Roots by Pseudomonads and AM Fungi: A Dynamic Phenomenon, Affecting Plant Growth and Health. In Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics, 3rd ed.; Springer: Berlin/Heidelberg, Germany, 2008; pp. 601–626.

24. Al-Baldawi, I.A.; Abdullah, S.R.S.; Anuar, N.; Mushrifah, I. Bioaugmentation for the enhancement of hydrocarbon phytoremediation by rhizobacteria consortium in pilot horizontal subsurface flow constructed wetlands. Int. J. Environ. Sci. Technol. 2017, 14, 75–84. [CrossRef]

25. Simon, M.A.; Bonner, J.S.; Page, C.A.; Townsend, R.T.; Mueller, D.C.; Fuller, C.B.; Autenrieth, R.L. Evaluation of two commercial bioaugmentation products for enhanced removal of petroleum from a wetland. Ecol. Eng. 2004, 22, 263–277. [CrossRef]

26. Park, Y.J.; Ko, J.J.; Yun, S.L.; Lee, E.Y.; Kim, S.J.; Kang, S.W.; Lee, B.C.; Kim, S.K. Enhancement of bioremediation by Ralstonia sp. HM-1 in sediment polluted by Cd and Zn. Bioresour. Technol. 2008, 99, 7458–7463. [CrossRef]
27. Lingua, G.; Copetta, A.; Musso, D.; Aimo, S.; Ranzenigo, A.; Buico, A.; Gianotti, V.; Osella, D.; Berta, G. Effect of arbuscular mycorrhizal and bacterial inocula on nitrate concentration in mesocosms simulating a wastewater treatment system relying on phytodepuration. *Environ. Sci. Pollut. Res.* 2015, 22, 18616–18625. [CrossRef] [PubMed]

28. Pii, Y.; Mimmo, T.; Tomasi, N.; Terzano, R.; Cesco, S.; Crecchio, C. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol. Fertil. Soils* 2015, 51, 403–415. [CrossRef]

29. Gamalero, E.; Glick, B.R. Bacterial ACC Deaminase and IAA: Interactions and Consequences for Plant Growth in Polluted Environments. In *Handbook of Phytoremediation*; Nova Science Publishers: New York, NY, USA, 2011; pp. 763–773.

30. Goswami, D.; Thakker, J.N.; Dhandhukia, P.C. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food Agric.* 2016, 2, 1127500. [CrossRef]

31. Trotta, A.; Varese, G.C.; Gnavi, E.; Fusconi, A.; Sampò, S.; Berta, G. Interactions between the soilborne root pathogen Phytophthora nicotianae var. parasitica and the arbuscular mycorrhizal fungus Glomus mosseae in tomato plants. In *Plant and Soil*; Springer: Berlin/Heidelberg, Germany, 1996; Volume 185, pp. 199–209.

32. Trouvelot, A.; Kough, J.L.; Gianinazzi-Pearson, V. Mesure du Taux de Mycorhization VA d’un Système Radiculaire. Recherche de Méthodes D’estimation Ayant une Signification Fonctionnelle. In *Physiological and Genetical Aspects of Mycorrhizae*; Gianinazzi-Pearson, V., Gianinazzi, S., Eds.; INRA: Paris, France, 1986; pp. 217–221.

33. IRSA-CN, APAT. Metodi Analitici per le Acque. In *Manuali e Linee Guida*; APAT: Roma, Italy, 2003; p. 143.

34. Kumari, M.; Tripathi, B.D. Efficiency of *Phragmites australis* and Typha latifolia for heavy metal removal from wastewater. *Ecotoxicol. Environ. Saf.* 2015, 112, 80–86. [CrossRef] [PubMed]

35. Abdelhakeem, S.G.; Aboulroos, S.A.; Kamel, M.M. Performance of a vertical subsurface flow constructed wetland under different operational conditions. *J. Adv. Res.* 2016, 7, 803–814. [CrossRef]

36. Dahiya, S.; Venkata Mohan, S. Strategic design of synthetic consortium with embedded wastewater treatment potential: Deciphering the competence of isolates from diverse microbiome. *Front. Environ. Sci.* 2016, 4, 30. [CrossRef]

37. Arslan, M.; Ullah, I.; Müller, J.A.; Shahid, N.; Afzal, M. Organic Micropollutants in the Environment: Ecotoxicity Potential and Methods for Remediation. In *Enhancing Cleanup of Environmental Pollutants*; Springer International Publishing: Cham, Switzerland, 2017; Volume 1, pp. 65–99.

38. Matamoros, V.; Rodríguez, Y.; Bayona, J.M. Mitigation of emerging contaminants by full-scale horizontal flow constructed wetlands fed with secondary treated wastewater. *Ecol. Eng.* 2017, 99, 222–227. [CrossRef]

39. Hodge, A.; Berta, G.; Doussan, C.; Merchan, F.; Crespi, M. Plant root growth, architecture and function. *Plant Soil* 2009, 321, 153–187. [CrossRef]

40. Berta, G.; Fusconi, A.; Hooker, J.E. Arbuscular Mycorrhizal Modifications to Plant Root Systems: Scale, Mechanisms and Consequences. In *Mycorrhizal Technology in Agriculture*; Birkhäuser: Basel, Switzerland, 2002; pp. 71–85.

41. Gamalero, E.; Trotta, A.; Massa, N.; Copetta, A.; Martinotti, M.G.; Berta, G. Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza* 2004, 14, 185–192. [CrossRef] [PubMed]

42. Bona, E.; Lingua, G.; Manassero, P.; Cantamessa, S.; Marsano, F.; Todeschini, V.; Copetta, A.; D’Agostino, G.; Massa, N.; Avidano, L.; et al. AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. *Mycorrhiza* 2015, 25, 181–193. [CrossRef] [PubMed]