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Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation

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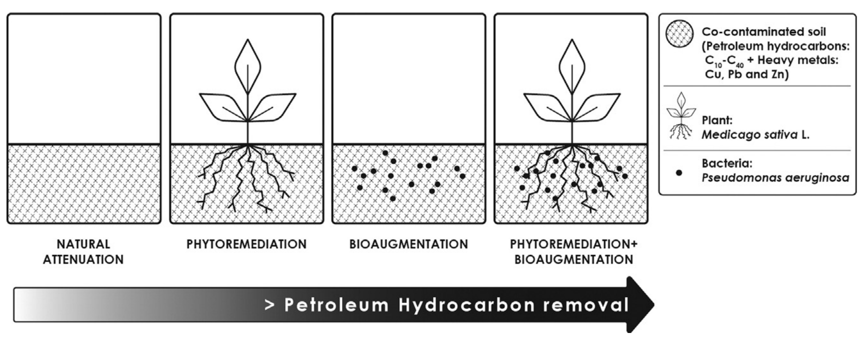
HIGHLIGHTS

- Knowledge on biological remediation of co-contaminated soils is not sufficient.
- Bioremediation strategies were compared through a pot experiment in growth-chamber.
- Alfalfa was able to tolerate and grow in a moderately co-contaminated soil.
- *Pseudomonas aeruginosa* promoted plant growth and alleviated plant stress.
- Alfalfa-*Pseudomonas aeruginosa* association enhanced petroleum hydrocarbon removal.

GRAPHICAL ABSTRACT



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ABSTRACT

Biological remediation technologies are an environmentally friendly approach for the treatment of polluted soils. This study evaluated through a pot experiment four bioremediation strategies: a) natural attenuation, b) phytoremediation with alfalfa (*Medicago sativa* L.), c) bioaugmentation with *Pseudomonas aeruginosa* and d) bioaugmentation-assisted phytoremediation, for the treatment of a co-contaminated soil presenting moderate levels of heavy metals (Cu, Pb and Zn at 87, 100 and 110 mg kg^{-1} DW, respectively) and petroleum hydrocarbons (3800 mg kg^{-1} DW). As demonstrated by plant biomass and selected physiological parameters alfalfa plants were able to tolerate and grow in the co-contaminated soil, especially when soil was inoculated with *P. aeruginosa*, which promoted plant growth (56% and 105% increase for shoots and roots, respectively) and appeared to alleviate plant stress. The content of heavy metals in alfalfa plants was limited and followed the order: Zn > Cu > Pb. Heavy metals were mainly concentrated in plant roots and were poorly translocated, favouring their stabilization in the root zone. Bioaugmentation of planted soil with *P. aeruginosa* generally led to a decrease of plant metal concentration and translocation. The highest degree of total petroleum hydrocarbon removal was obtained for bioaugmentation-assisted phytoremediation treatment (68%), followed by bioaugmentation (59%), phytoremediation (47%) and natural attenuation (37%). The results of this study demonstrated that the

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combined use of plant and bacteria was the most advantageous option for the treatment of the present co-contaminated soil, as compared to natural attenuation, bioaugmentation or phytoremediation applied alone.

1. Introduction

A recent European report estimates a total number of 2.5 million potentially contaminated sites in Europe and it is expected that 340,000 of these sites are contaminated and likely to require remediation, showing the significance of this problem (Van Liedekerke et al., 2014). The most frequent contaminants are heavy metals and mineral oils, affecting 35% and 24% of European soils, respectively (Van Liedekerke et al., 2014). Moreover, it is not uncommon that these pollutants are present together in polluted soils, rendering their remediation more difficult. These pollutants arise in the environment from various sources deriving from anthropogenic activities. Heavy metals originate mainly from human activities related to energy and mineral consumption (Kabata-Pendias, 2011), while petroleum hydrocarbons usually come from accidental spills of petroleum-based products commonly-used as fuels for transportation (Chartered Institute of Environmental Health, 2009). Both types of pollutants entail a danger for the environment and living organisms (Tchounwou et al., 2012; Todd et al., 1999).

In contrast to "traditional" soil remediation technologies (e.g. excavation, off-site disposal), biological methods are environmentally friendly and particularly attractive because of their low cost and relatively simple maintenance (Mirsal, 2008). Natural attenuation, bioaugmentation and phytoremediation are examples of biological remediation strategies and can be used for the remediation of soils affected by different types of pollutants. Natural attenuation consists of the use of natural processes (e.g. biodegradation, dispersion, sorption, volatilization, (bio)chemical stabilization) to contain and/or reduce the concentration of pollutants at contaminated sites (EPA, 1999; Mulligan and Yong, 2004). Biodegradation of target compounds by indigenous microbial communities is frequently considered to be the primary mechanism for attenuation of contaminants (Declercq et al., 2012). Bioaugmentation enables an increase of biodegradative capacities of contaminated sites by the introduction of single strains or consortia of microorganisms with the desired catalytic capabilities (Lebeau, 2011; Mrozik and Piotrowska-Seget, 2010). Finally, phytoremediation comprises a group of technologies that use plants and their associated microorganisms to remove pollutants from the environment or to make them harmless (Salt et al., 1998). Plant uptake, translocation and accumulation of heavy metals (phytoextraction), their stabilization in the root zone (phytostabilization) and the metabolism of organic pollutants by rhizosphere microorganisms (rhizodegradation) are examples of phytoremediation processes. Natural attenuation, bioaugmentation and phytoremediation approaches can be used not only as remediation technologies in themselves but also in combination. For instance, bioaugmentation can be coupled with phytoremediation to intensify clean-up processes (Glick, 2003; White, 2001). In particular, bioaugmentation-assisted phytoextraction optimizes the synergistic effect of plants and microorganisms and has been used for the cleaning-up of soils contaminated by metals (Huguenot et al., 2015; Lebeau et al., 2008). This enhanced trace element uptake by plants can be ascribed to an increase in root absorption ability and/or to an enhancement of trace metal bioavailability in the rhizosphere, mediated by microorganisms (Sessitsch et al., 2013).

Moreover, plant-microorganism associations can also be used to facilitate the removal of organic contaminants (Glick, 2010). In particular, some studies have addressed the combined use of plants and biodegradative bacteria with the aim to remove petroleum products (Lin et al., 2008b), which seems to be a promising remediation strategy.

A key aspect in biological remediation methods is the selection of appropriate plant-bacteria partnerships for the remediation of polluted soils (Khan et al., 2013). Among plants used in phytoremediation, alfalfa (*Medicago sativa* L.) is of particular relevance. It is a fast growing species (Hallam et al., 2001) that develops an extensive tap root system favourable for the establishment of rhizosphere microorganisms (Kirk et al., 2005) and can associate with symbiotic nitrogen fixing bacteria (Truchet et al., 1991). Alfalfa has been assessed for the remediation of several types of pollutants: heavy metals like Cd, Cr, Cu, Ni and Zn (Bonfranceschi et al., 2009; Peralta-Videa et al., 2002, 2004), petroleum hydrocarbons (Kirk et al., 2002; Wiltse et al., 1998), polycyclic aromatic hydrocarbons (PAHs) (Fan et al., 2008) or organochlorines (Li and Yang, 2013). Moreover, recent findings have shown promising results for alfalfa phytoremediation of co-contaminated soils (Ding and Luo, 2005; Ouvrard et al., 2011; Zhang et al., 2013).

Among bacterial strains used for bioremediation, *Pseudomonas aeruginosa* is especially interesting because it can improve pollutant remediation through various mechanisms. Firstly, *P. aeruginosa* has been described to produce metal chelating siderophores, which could improve metal bioavailability (Visca et al., 2007). Secondly it produces biosurfactants (rhamnolipids) that can enhance the solubility of poor water-soluble organic compounds and the mobility of heavy metals (Mulligan, 2005; Zhang et al., 2012), improving their bioavailability. As a result, *P. aeruginosa* has been tested for bioremediation of metals (Singh et al., 2013) and hydrocarbons (Das and Mukherjee, 2007). Finally, a role as plant growth promoting rhizobacteria (PGPR) has been described for *P. aeruginosa*, which leads to improved plant growth and enhanced phytoremediation rates (Wang et al., 2011).

The aim of this study was to perform a comparative assessment of four bioremediation strategies: a) natural attenuation, b) phytoremediation with alfalfa, c) bioaugmentation with *P. aeruginosa* and d) bioaugmentation-assisted phytoremediation, for the treatment of a soil co-contaminated by moderate levels of heavy metals and petroleum hydrocarbons.

2. Materials and methods

2.1. Soil samples

Soil samples were collected from an urban area close to a fuel station with a history of contamination by heavy metals and petroleum hydrocarbons, mostly diesel. Samples were taken with a drill auger, which allowed collecting soil from different depths between 0 and 100 cm. This soil was sieved to pass through a 6 mm mesh and homogenized. To limit the level of pollutants, the contaminated soil was mixed (1:1 *w*/*w*) with soil from the same site but characterized by negligible hydrocarbon contamination. Before mixing, this soil was sieved through a 2 mm mesh. Selected chemical and physical properties of this unique composite sample (1:1 *w*/*w* mix of both soils) are presented in Table 1. Initial physicochemical characterization of soil samples was performed by a certified laboratory: ALcontrol Laboratories (The Netherlands). ALcontrol is accredited by the Cofrac (Comité français d'accréditation) and by the RvA (Raad voor Accreditatie) under number L028, in accordance with the criteria of laboratory analysis: ISO/IEC 17025:2005.

2.2. Plants

Alfalfa seeds (*M. sativa* L. v. La Bella Campagnola, purity: 99%, germinability: 85%) were surface disinfected by immersion in 2% (*v*/*v*) hydrogen peroxide for 8 min (Qu et al., 2011), in order to avoid the addition of non-indigenous microorganisms to the system. Then, seeds were thoroughly rinsed three times with sterile water and used for the pot experiment.

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