

Green Remediation for the Sustainable Management of Oil Spills in Agricultural Areas

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Spills of petroleum products resulting from illegal pipeline extraction also affect agricultural areas. These areas must be subject to remediation interventions to bring the concentrations of contaminants below the alarm levels and avoid further damage to the environment and living beings. In these cases, green technologies such as bioremediation and phytoremediation are an excellent approach to reduce impacts on agriculture. This contribution evaluates the effectiveness of combining some green techniques in managing soil contaminated by oil spills. A feasibility test of phytoremediation at a microcosm scale with three plant species (corn, lupine and alfalfa) was conducted, combining the approach with plant growth-promoting rhizobacteria (PGPR). Particular attention was given to the reclamation from polycyclic aromatic hydrocarbons (PAHs). At the end of the experiments, biomass production and PAHs concentration in the soil and plants (roots and aerial parts) were determined. The remediation strategy was aimed at two concurrent objectives: the need to remove the maximum amount of contaminants from the soils affected by oil spills and the restoration of the agricultural activity to be carried out in absolute safety. The results show a decrease in the concentration of hydrocarbons in the soil favored by the presence of tested plants, which manage to grow satisfactorily on the soil under examination, albeit with an inevitable decrease in yield compared to uncontaminated soil. Looking at the concentration of pyrene, which is usually considered as indicator of PAHs contamination, the removal reaches values higher than 50 % in vegetated soils. The addition of the selected PGPR counteract the negative effect of contamination, favoring the growth of plants and allowing the production of fresh biomass comparable to that obtained on the uncontaminated control soil. This results in a further reduction of the contaminant in question up to an additional 20 %. Therefore, the presence of organic contaminants can be concretely reduced in a sustainable and cost-effective way by the joint action of plants and microorganisms that promote the processes of rizodegradation.

1. Introduction

Contamination by petroleum derivatives can affect soil quality due to relevant changes in several chemical-physical and biological characteristics. Petroleum hydrocarbons include alkanes, monocyclic aromatic hydrocarbons such as BTEX, and polycyclic aromatic hydrocarbons (PAHs) and asphaltenes. The negative effects in the soil can also be observed at very low concentrations since petroleum hydrocarbons can both damage soil microorganisms, with a consequent decrease in their activity, and saturate the soil matrix to avoid the functionality of the root compromising the plants' life (Wyszkowski and Sivitskaya, 2012). Among the various constituents, PAHs are of relevant environmental and human health concerns due to their toxicity and possible bioaccumulation through food chain (Zhang et al., 2017). Some of them have been recognized as environmental carcinogens and identified as "priority pollutants" by the United States Environmental Protection Agency. PAHs do not degrade easily under natural conditions, showing a high persistence in soil, which increases with an increase in molecular weight. Since the highest quantity of PAHs remains in the superficial soil strata explored by plants roots (Patowary et al., 2017), these compounds represent a potential threat to human health. The

main pathways for PAHs entry into plants comprise soil-to-root uptake and atmospheric deposition through stomata leaves (Marchal et al., 2014). Plants uptake depends on PAHs properties such as octanol-water partition coefficient (Kow), soil characteristics and plant species (Li and Ma, 2016). High hydrophobic compounds with $\log Kow > 5$ may strongly adsorb onto the root surfaces, while hydrophilic compounds with $\log Kow < 5$ can potentially be taken up by roots and translocated to the aerial parts of plants. In particular, the polar and water-soluble compounds, such as pyrene ($\log Kow = 4.88$), may be absorbed by plant roots and translocated to shoot (Dettenmaier et al., 2009).

Contamination of agricultural areas is of particular concern because contaminants can enter the food chain with direct consequences on human health. In this context, the need to use non-invasive technologies emerges to minimize potential negative consequences on this ecosystem. A wide range of remediation technologies are available on the market and they differ substantially in terms of the environmental impact (Wan et al., 2020). However, contaminated sites management issue is moving towards a synergy among environmental, economic and social aspects (Grifoni et al., 2022). In accordance with this new vision of remediation (Green Remediation), it is necessary to select technologies able to achieve the desired security levels, while considering sustainable recovery concepts of contaminated sites. Phytoremediation technologies that are based on processes of extraction, degradation and stabilization of organic contaminants are particularly suitable for recovering soils destined for agricultural production (Song et al., 2020). This technology also offers the advantage of not particularly interfering with the activities in progress in the areas not affected by the oil spill. Moreover, phytotechnologies offer attractive remediation features, being characterized by low cost and only a small environmental footprint (Vocciante et al., 2021). While the presence of plants is essential to decrease the concentration of PAHs in contaminated soil due to the increase in hydrocarbon-oxidizing microorganisms following the release of roots exudates, the biodegradation of petroleum hydrocarbons in vegetated soils can become effective only by exploiting suitable rhizodegradation processes (Allamin et al., 2020). However, the specificity of the action of the PGPR and their effectiveness, which depends on numerous factors related to the complex interactions with the soil and plants, makes it non-trivial to select potentially effective consortia (Vocciante et al., 2022). The aim of this work was to evaluate, at a laboratory scale, the feasibility of phytoremediation by exploiting the addition of ad hoc PGPR to highly promote the biodegradation of PAHs by plants, thus providing an economic and eco-friendly solution in line with the adoption of "green methods" and the replacement of processes and materials with sustainable alternatives (Reverberi et al., 2017).

2. Materials and Methods

The relevant information on the modalities adopted for the experimentation is shown below.

2.1 Site description and soil characterization

The area under investigation is a cultivated field where a break-in in the adjacent oil pipeline caused a fuel spill into the soil. The chemical analysis of soil samples taken from 0 to 2 meters deep from the impacted area revealed significant quantities of hydrocarbons $> C_{12}$ with maximum concentrations around $5,000 \text{ mg kg}^{-1}$. Four composite soils were prepared by grouping the most superficial samples (0-1m): soil A (about $4,000 \text{ mg kg}^{-1}$) and soil B (about $2,000 \text{ mg kg}^{-1}$), and the deeper ones (1-2 m): soil C (about $3,500 \text{ mg kg}^{-1}$) and soil D (about $5,000 \text{ mg kg}^{-1}$).

2.2 Microcosm experiments

A phytoremediation trial was performed following a fully randomized strategy with about 500 g of each contaminated soil and 0.8 g of *Medicago Sativa* (alfalfa) seeds, 5 seeds of *Zea Mais* (corn) and 6 seeds of *Lupinus Albus* (lupine). This choice is derived from previous experiments in comparable conditions (Franchi et al., 2019). Two control microcosms for each species were run simultaneously using uncontaminated agricultural soil (control sample). The same number of pots was set up for the experimentation with PGPR. PGPR used as inoculum belong to the family of Bacillaceae and Micrococcaceae. They were isolated from the contaminated soil and selected for the presence of at least three growth-promoting properties (production of 3-indole acetic acid, siderophores and ammonia). For each microcosm, five replicas were prepared by adding inocula with a concentration of 10^8 CFU (Colony Forming Units), and the growths were performed in a climatic chamber (CCL300BH-AS S.p.A., Perugia, Italy) with the following conditions: photoperiod of 14 h light at 24°C and 10 h dark at 19°C , photosynthetic photon flux density of $130 \mu\text{mol m}^{-2} \text{ s}^{-1}$. All the microcosms were irrigated daily with tap water. The experiment lasted 30 days; then, the plants were harvested and collected, separating the roots and shoots. The fresh biomass (FW) was determined gravimetrically (Franchi et al., 2019). In soil and plants, PAHs were determined according to EPA 3545A:2007 and EPA 8270E:2017 by GC-MS analysis. Due to the meagre amount, it was impossible to analyze the root portions of plants. The content of PAHs in soil samples was determined before starting the experiment and after plant harvesting.

3. Results and Discussion

Plants grown in the contaminated soil did not show any outward signs of stress or phytotoxicity, except that root and shoot biomass were somewhat lower than those grown in control soils (Figure 1).

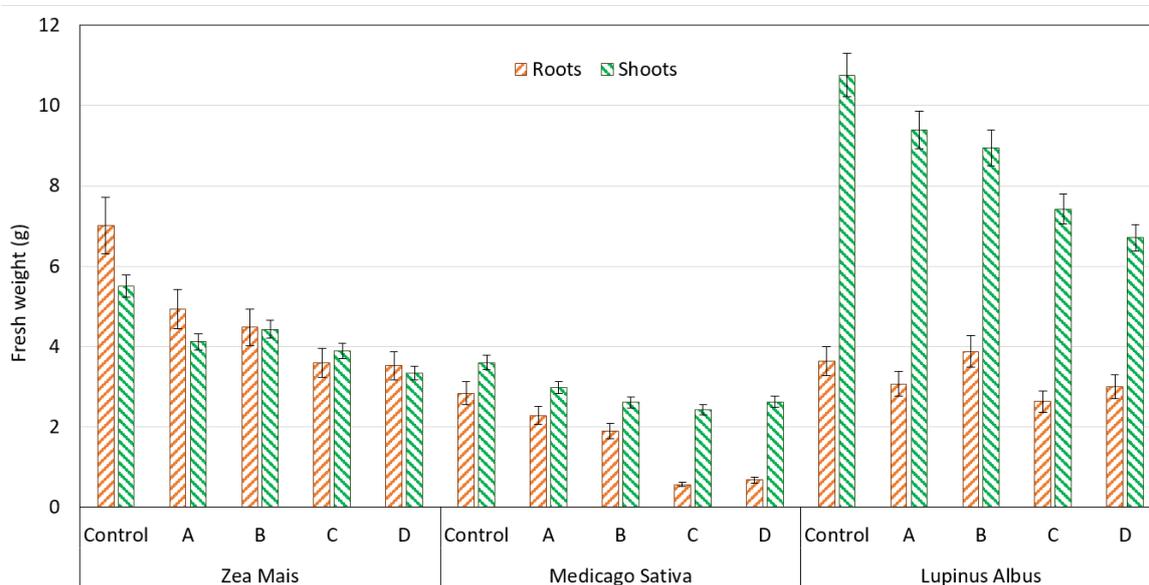


Figure 1: Fresh weight (g) of plants (shoots and roots) grown on different soils. The reported value is the mean of the replicas with the standard deviation

Similar results have been reported for different kind of vegetables with increasing negative effects on plant growing at increasing PAHs concentrations in soil (Esmaeli et al., 2021). The reduced biomass yield did not match a significant increase in PAH concentration in the used plants. Indeed, it should be noted that the concentration of PAHs in plants is usually lower than the quantification limit. For this reason, the detailed data are not reported. Only in some sporadic cases concentrations of around $5 \mu\text{g kg}^{-1}$ have been found for benzo (g, h, i) perylene and pyrene in the aerial part of Alfalfa. A concentration of around $2 \mu\text{g kg}^{-1}$ of pyrene was also found in one sample of the aerial part of the corn. However, the concentrations in plants of these PAH were not directly linked to their concentrations in soils. The very low uptake of pyrene by plants used in this experiment is in agreement with previous studies that reported the uptake from the soil is often negligible since pyrene is generally scarcely dissolved in the liquid phase, i.e. it is present in not available forms (Jia et al., 2017).

As regards the efficiency of phytoremediation, the most important aspect is the evaluation of the decrease in the concentration of PAHs in the soil following the growth of plants. The data obtained are shown in Table 1. Although, as mentioned, an expected decrease in biomass yield with the polluted soil was noted compared to the control soil, the tested plants were able to grow satisfactorily on soils, and at the end of the experimentation a decrease in the concentration of PAHs in soil was observed, more significant in the presence of plants with respect to non-vegetated soil. The addition of PGPR further promoted plant growing on contaminated soils, and the production of fresh biomass became similar to that in the control soil. Plant roots release a broad range of root exudates, which can provide nutrients leading to an increase in the number of microorganisms in the rhizosphere. This, in turn, promotes the rate of microbial degradation of PAHs in soil.

In the case of PAHs present in the soils studied in reduced concentrations, e.g. benzo (a) pyrene, the concentration in the soil is lower than the detection limit after plant growth. Benzo (b) fluoranthene and Benzo (k) fluoranthene degradation seems to occur even in the absence of plants, while for Benzo (g, h, i) perylene, a certain degradation seems to occur only in the soils where the plants have grown. More specifically, in the case of benzo (g, h, i) perylene a decrease in the average concentration value occurred only after the addition of PGPR. Unfortunately, there are no strains more effective in general, since all are potentially effective depending on the soil conditions and the type of contamination. However, the strains isolated with the indicated procedure proved to be suitable for the purpose. Clearer results were obtained with chrysene, which shows a tendency to decrease in vegetated soils, and above all for pyrene, which is the hydrocarbon present in higher concentration: for all soils and all plant species, there are significant decreases in vegetated soils, in particular in the case of adding PGPR.

Of particular interest is the assessment of the decrease in the concentration of pyrene, which is considered an indicator both of PAHs contamination in soils and of the origin of contamination (Gabriele et al., 2021). The data after the growth of the plants in the case of soils sown with corn is shown in Figure 2.

Table 1: Mean values concentration ($\mu\text{g kg}^{-1}$) of PAHs in the soils at the beginning (Ti) and end (Tf) of the test after the growth of the different plant species *Z. Mais* (corn), *L. Albus* (lupine) and *M. Sativa* (alfalfa). Addition of PGPR is indicated with (*), nd = not detected. For each soil, different superscript letters in each row indicate significant differences using one-way ANOVA ($p < 0.05$). Note: Benzo(a)anthracene, dibenzo(a,e)pyrene, dibenzo(a,l)pyrene, dibenzo(a,h)pyrene and dibenzo(a,h)anthracene are below the detection limit.

	A				B											
	Corn		Lupine		Alfalfa		Corn		Lupine		Alfalfa					
	Ti	Tf	Tf	Tf *	Tf	Tf *	Tf	Tf *	Ti	Tf	Tf	Tf *	Tf	Tf *	Tf	Tf *
Benzo(a)pyrene	0.74 ^c	0.70 ^b	nd	nd	nd	nd	0.65 ^{ab}	0.58 ^a	0.38 ^a	0.74 ^b	nd	nd	nd	nd	nd	nd
Benzo(b)fluoranthene	3.1	nd	nd	nd	nd	nd	nd	nd	2.1	nd						
Benzo(k)fluoranthene	1.7	nd	nd	nd	nd	nd	nd	nd	1.0	nd						
Benzo(g,h,i)perylene	8.6 ^e	7.2 ^d	6.2 ^c	5.7 ^c	4.2 ^b	3.1 ^a	6.5 ^c	5.5 ^c	5.4 ^b	6.8 ^c	5.2 ^b	5.1 ^b	3.4 ^a	2.9 ^a	5.2 ^b	5.0 ^b
Dibenzo(a,i)pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chrysene	28.0 ^c	21.0 ^b	17.8 ^a	16.4 ^a	16.5 ^a	15.4 ^a	18.5 ^a	16.8 ^a	26 ^d	22 ^c	18.1 ^b	14.8 ^a	15.4 ^a	13.9 ^a	20.0 ^b	15.0 ^a
Indeno(1,2,3-c,d)pyrene	0.81 ^a	0.82 ^a	nd	nd	nd	nd	nd	nd	0.45 ^a	0.43 ^a	nd	nd	nd	nd	nd	nd
Pyrene	1,500 ^e	890 ^d	725 ^c	650 ^b	683 ^b	536 ^a	720 ^b	564 ^a	1,100 ^e	800 ^d	681 ^c	610 ^b	630 ^b	546 ^a	632 ^b	535 ^a

	C				D											
	Corn		Lupine		Alfalfa		Corn		Lupine		Alfalfa					
	Ti	Tf	Tf	Tf *	Tf	Tf *	Tf	Tf *	Ti	Tf	Tf	Tf *	Tf	Tf *	Tf	Tf *
Benzo(a)pyrene	0.66 ^a	1.2 ^b	nd	nd	nd	nd	0.81 ^b	0.5 ^a	0.78 ^a	0.81 ^a	nd	nd	nd	nd	0.68	0.55
Benzo(b)fluoranthene	3.3	nd	2.7	nd												
Benzo(k)fluoranthene	1.6	nd	1.7	nd												
Benzo(g,h,i)perylene	10.0 ^b	13.0 ^d	9.5 ^b	8.5 ^{ab}	8.1 ^a	7.6 ^a	9.4 ^b	7.8 ^a	11.1 ^b	10 ^b	8.3 ^a	7.1 ^a	8.0 ^a	6.8 ^a	10.1 ^b	8.1 ^a
Dibenzo(a,i)pyrene	0.62	nd	0.66	nd												
Chrysene	52.0 ^d	29.1 ^c	23.4 ^b	20.4 ^a	20.1 ^a	17.2 ^a	30.5 ^c	20.4 ^a	53.2 ^e	42 ^d	35.1 ^c	23.8 ^a	30.4 ^b	18.6 ^a	39.6 ^c	21.4 ^a
Indeno(1,2,3-c,d)pyrene	0.81 ^a	0.92 ^a	nd	nd	nd	nd	nd	nd	0.84 ^a	0.79 ^a	nd	nd	nd	nd	nd	nd
Pyrene	1,700 ^e	780 ^d	598 ^b	530 ^a	562 ^b	509 ^a	694 ^c	588 ^b	1,600 ^e	633 ^b	511 ^a	500 ^a	495 ^a	464 ^a	651 ^b	573 ^a

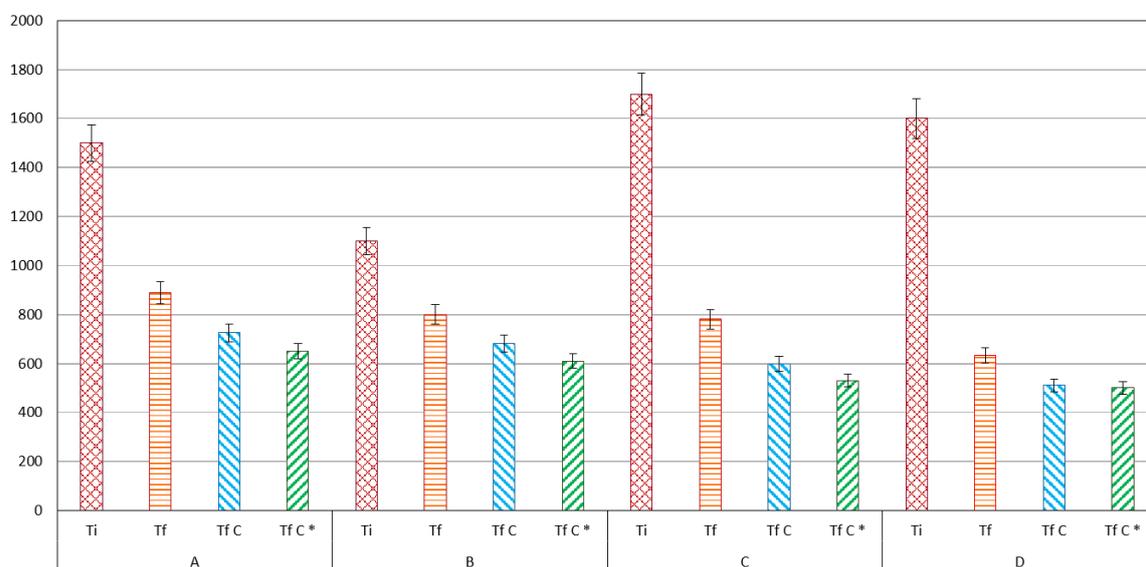


Figure 2: Concentration ($\mu\text{g kg}^{-1}$) of pyrene in the soil at the beginning and at the end of the test after the growth of corn plants. Ti: concentration of the pyrene at the initial time; Tf = concentration of pyrene at the final time in the non-vegetated soil; Tf C = concentration of pyrene at the final time after corn growth; Tf C* = concentration of pyrene at the final time after corn growth and addition of PGPR. The reported value is the mean of the replicas with the standard deviation

From obtained results, it is possible to infer that the rhizodegradation processes favor the degradation of PAHs with significant reductions in their concentrations in soil. Further, there is a significant degradation of PAHs after the addition of the ad hoc inocula isolated within the presented trial. The percentage of PAHs concentration reductions ranged from 42 to 68 % in soils planted with corn, from 48 to 71 % in those planted with lupine, and from 50 to 64 % in those with Alfalfa. Therefore, the presence of organic contaminants can be concretely reduced by the joint action of plants and specifically selected microorganisms that can use organic compounds as a primary source of carbon. However, it must be taken into account that these very high reductions are partly due to the specificity of the microcosm test, in which the roots have the opportunity to explore all the contaminated soil in which the plants grow, a condition that is difficult to replicate in the field.

Given the very low concentration in plants, rhizodegradation can be considered as the prevailing process for the removal of pyrene from the soil under consideration. The process is based on the transformation of pyrene into simpler products by microbial activities, which become even more intense in the presence of plants, in the areas explored by the roots. This hypothesis is also confirmed by the results obtained with the addition of PGPR, which increases the effectiveness of microbial activity in the rhizosphere by promoting the development of plants. This confirms the possibility of using the illustrated approach to treat PAHs contamination, even in the case of soils intended for agricultural use. Relying exclusively on the synergistic biological activity of selected plants and PGPRs, this solution is intrinsically cost-effective and with a low environmental impact, resulting in CO₂ equivalent emissions tens of times lower than conventional approaches widely used such as landfill disposal (Vocciante et al., 2021). These positive aspects can be further improved by disposing of the biomass produced through waste-to-energy solutions such as incineration with electricity production or pyrolysis with bio-oil production, increasing overall efficiency and reducing the environmental impact of the treatment by approximately an additional 30 % (Vocciante et al., 2021).

4. Conclusions

In agricultural areas contaminated by oil spills, it is necessary to utilize soft remediation technologies in order not to create further environmental problems due to invasive interventions maintaining in the meantime the normal agricultural activities in the surrounding areas. Phytotechnologies offer considerable potential, especially in relation to recalcitrant organic compounds, such as PAHs.

Looking at the results presented, e.g. for the pyrene in soil A, at the end of the experiment the concentration value decreased from the initial 1,500 µg kg⁻¹ to 890 µg kg⁻¹ in the non-vegetated soil. In vegetated soils, the reduction in the concentration of pyrene decreased to values of 725, 683 and 720 µg kg⁻¹ in soils planted with corn, lupine and alfalfa, respectively. These values are further lowered in the presence of PGPR by 10 % in the case of soil vegetated with corn and by over 20 % for the soils vegetated with the other two species.

These findings confirm the potential utilization of phytoremediation for agricultural soils contaminated by petroleum PAHs. Obviously, the efficiency of the technology is dependent on plant species and the ability to promote degrading microbial activity in the rhizosphere and then in the bulk soil. However, the results obtained confirm how the use of selected PGPR can enhance the remediation efficiency by promoting the production of roots exudates, which in turn increasingly stimulate the activity of the rhizospheric microbial population. This offers the possibility of making the most of the characteristics of phytotechnologies, such as high social acceptability, and environmental and economic sustainability, to successfully treat large areas affected by PAHs contamination. In addition, among the various possible solutions, after harvest the plants can be disposed of by incineration, possibly exploiting the biomass produced by waste-to-energy solutions with a further advantage for the environment. Based on the promising results obtained, a field trial has been planned and is in the implementation stage.

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