ALTERATIONS IN SOIL FERTILITY AFTER USED LUBRICATING OIL BIOREMEDIATION

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Abstract
Regardless of the outcome of any environmental rehabilitation technique applied, subsequent monitoring is indispensable to assess information about soil toxicity after the treatment. Various bioremediation methods (natural attenuation, biostimulation, bioaugmentation and the usage of an organic additive) were previously performed to decontaminate soil samples taken from a railway station area polluted with used lubricating oils. In this study, ecotoxicological responses revealed that seed germination and primary root length of Indian mustard (*Brassica juncea*) were decreased in each remediated soil presumably by inhibiting breakdown products due to the biodegradation of used lubricants, while viability of root tips increased significantly indicating more vital mustard seedlings grown in remediated soils.

Introduction
Lubricating oils (LOs) are mostly produced for reducing friction in engines of motorized vehicles such as cars, motorcycles or locomotives. Therefore, used lubricating oils (ULOs) containing long chain hydrocarbons, additives and heavy metals are considered as widespread, hazardous pollutants and hence potential targets of different rehabilitation processes [1-3]. Several physicochemical and biological waste management techniques are available for neutralizing oil-related pollutants in the environment [4-6]. Bioremediation, using plants and/or microorganisms for this purpose [7-8], is one of the most promising approach, since it is an environmentally friendly and cost effective technology [9-11]. Remediation treatments always need to be followed by further monitoring including ecotoxicology assays, since reduction in the concentration of the original pollutants alone does not necessarily decrease toxicity as well [12-14].

Experimental
Soil samples from a railway station area long-term contaminated with ULOs were previously rehabilitated applying different bioremediation methods such as natural attenuation (NA, soil moisture was set with water only), biostimulation (BS, soil moisture was set with the addition of water and inorganic nutrients contaning nitrogen and phosphorus) and bioaugmentation combined with biostimulation (BAS, in addition to stimulation, oil-degrader *Rhodococcus erythropolis* PR4 and *Rhodococcus* sp. C strains were introduced into the polluted soil). In addition to BS and BAS treatments, samples named BS+OA and BAS+OA were supplied with an organic additive, which stimulates cellular activity of certain bacteria.

Indian mustard (*Brassica juncea* L. Czern. Var. 'Negro Caballo') was used as a model organism for soil ecotoxicology assays. In order to assess soil condition after different bioremediation treatments, seed germination [13], primary root length [14-15] and cell viability in root tips [16-17] were measured at the beginning and at the end of the 60 days long remediation expreriments. Mustard seeds germinated for 4 days at 25 ^{o}C in darkness before processing [13]. Data are expressed as mean ± SE (standard error). Statistical significance was analyzed using one way analysis of variance (ANOVA) and Duncan's test.

Results and discussion

Even in the non contaminated soil (taken from the vicinity of the polluted site) reduced seed germination (80%) could be observed confirming our preliminary data regarding its poor quality. Results were normalized using this soil as control. Seeds germinated similarly in untreated, ULO-contaminated soil (t_0), and each treatment (t_{60}) further caused significant decreasement (Fig. 1.), especially in the case of BS and BS+OA samples.

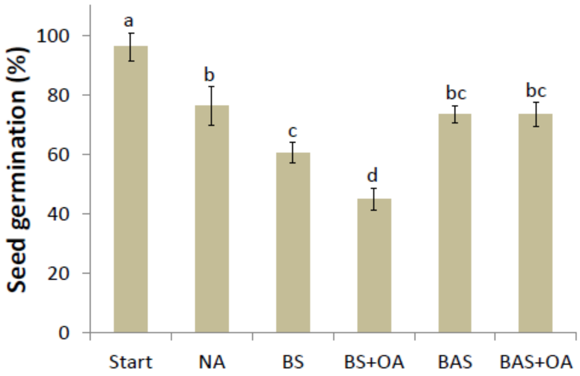

Figure 1. Seed germination at the onset and after 60 days for each bioremediation treatment. Different letters indicate statistical differences among treatments (n≥4, p≤0.05).

Significant decrease in primary root lengths was also observed in treated samples coinciding with seed germination results (Table 1.).

Table 1. Root length of Indian mustard seedlings grown in non contaminated soil and soils at the onset and after 60 days for each bioremediation treatment. Different letters indicate statistical differences among treatments (n≥20, p≤0.05).

Soil sample	Root length (mm)
Non contaminated (NC)	22.99 ± 0.80a
Start (t_0)	10.23 ± 0.32bc
Natural attenuation (NA, t_{60})	10.75 ± 0.49b
Biostimulation (BS, t_{60})	9.04 ± 0.48cd
Biostimulation supplied with organic additive (BS+OA, t_{60})	6.50 ± 0.42f
Bioaugmentation combined with biostimulation (BAS, t_{60})	7.98 ± 0.33de
Bioaugmentation combined with biostimulation supplied with organic additive (BAS+OA, t_{60})	7.43 ± 0.38ef

The greatest degrees of growth inhibition were measured in BS+OA and BAS+OA samples. Based on root length and seed germination, we suppose that inhibition might be caused by

increased quantity or bioavailability of intermediers and breakdown products due to ULO-biodegradation in treated samples.
Fluorescent staining (fluorescein diacetate for viable root meristem cells [15] and propidium iodide for dead cells [16]) revealed that each bioremediation treatment caused increased vitality of mustard seedlings (Fig. 2.).

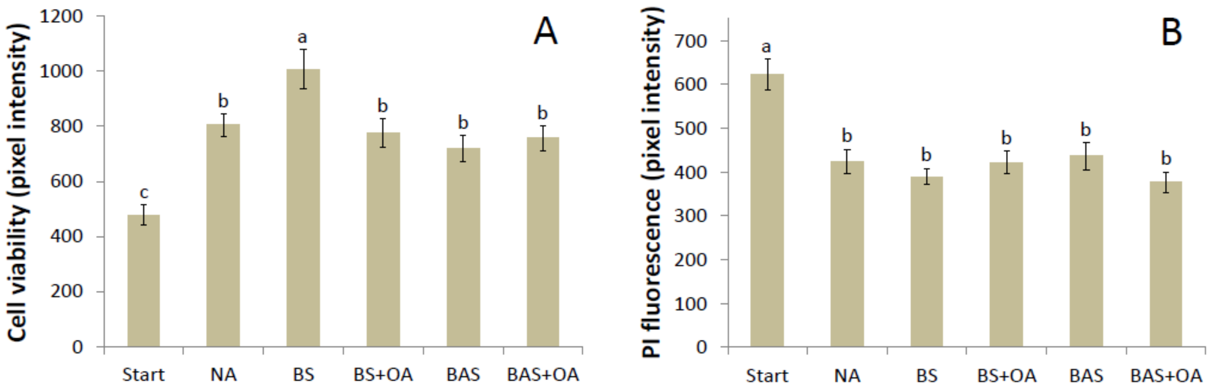

Figure 2. Cell viability (A) and propidium iodide (PI) fluorescence (B) of root meristem at the onset and after 60 days for each bioremediation treatment. Different letters indicate statistical differences among treatments ($n \geq 14$, $p \leq 0.05$).

Conclusion

Although intermediers or breakdown products from ULO-biodegradation can inhibit seed germination and root growth, we found that seedlings become more viable and vital grown in remediated soils. Our results further support previously reported suggestions by other authors [13] that decreased hydrocarbon concentration and reduced soil toxicity not necessarily have direct correlation.

Acknowledgements

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