Synergic action of organic matter-microorganism-plant in soil bioremediation

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Introduction

Bioremediation is a natural process, which relies on bacteria, fungi, and plants to degrade, break down, transform, and/or essentially remove contaminants, ensuring the conservation of the ecosystem biophysical properties. Since microorganisms are the former agents for the degradation of organic contaminants in soil, the application of organic matter (such as compost, sewage sludge, etc.), which increases microbial density and also provides nutrients and readily degradable organic matter (bioenhancement-bioaugmentation) can be considered useful to accelerate the contaminant degradation. Moreover, the organic matter addition, by means of the increase of cation exchange capacity, soil porosity and water-holding capacity, enhances the soil health and provides a medium satisfactory for microorganism activity. Plants have been also recently used in soil reclamation strategy both for their ability to uptake, transform, and store the contaminants (Atagana et al., 2011), and to promote the degradation of contaminants by microbes at rhizosphere level. It is widely recognized that plant, through organic materials, nutrients and oxygen supply, produces a rich microenvironment capable of promoting microbial proliferation and activity.

Case studies

Experiments at different levels, micro-scale, meso-scale, field-scale, were carried out in order to investigate the effectiveness of organic matter-microorganism-plant interaction in soil bioremediation. Organic matter was used alone or in combination with plant and/or earthworms.

Micro-scale experiment

In the micro-scale *(laboratory)* experiment, 1 kg of polluted soil (hydrocarbons) was placed in plastic containers (microcosms), that were supplied by air and maintained under controlled temperature and humidity for three months. The bioremediation treatments were the following: i) a mixture of microorganisms-enzymes and nutrients; ii) compost alone; iii) compost with earthworms (*Eisenia fetida*) and iv) control soil (without treatment).

Meso-scale experiment

In the meso-scale experiment, 90 kg of polluted soil (hydrocarbon and heavy metals) were placed in pots, which were planted with *Paulownia tomentosa* or *Cytisus scoparius* or *Populus nigra* (var.italica). Three different treatments were performed for each plant: i) untreated soil as control; ii) soil + horse manure; iii) soil + horse manure + earthworms. The pots were kept for six months in the open field conditions.

Field-scale experiment

In the field-scale experiment, a soil (about 0.5 ha) historically contaminated by heavy metals, hydrocarbons and polychlorobiphenyls located in an industrial area in San Giuliano Terme Municipality (Pisa, Italy) was subjected, for 1 year, to bioremediation treatment with organic matter and *Populus nigra* (var.italica).

Monitoring parameters

In the attempts to assess both effectiveness and evolution of the remediation system towards a natural soil ecosystem, besides the pollution parameters (total petroleum hydrocarbon (TPH) and heavy metals) also parameters describing the nutritional content of soil (total and soluble forms of C, N, and P) and the efficiency of the microbiological components (enzyme activities), were investigated in all the experiments.

Enzyme activities have often been used as indicators of microbial activity (such as, dehydrogenase enzyme, Masciandaro et al., 2000) and can also be useful to interpret the intensity of microbial metabolism in soil (van Beelen and Doelman, 1997). Nannipieri (1990) showed that the measurement of the activity of several enzymes in soil may be a good method for estimating the overall microbial activity and its response to widespread pollution. Enzymes, in fact, are the catalysts of important metabolic processes, including the decomposition and the detoxification of contaminants (Nannipieri and Bollag, 1991).

Results and discussion

In the micro-scale experiment, the greatest stimulation of soil microbial activity was observed in the compost treatments, especially in presence of earthworms, achieving the best result for hydrocarbon degradation, with a reduction higher than 50%. This action could be a direct degradation of the organic substrates by earthworms, and/or an indirect effect through the stimulation of soil microbial metabolism by available substrate from compost and earthworm casting.

In the meso-scale experiment, the synergic action of plant, organic matter and earthworms, was particularly able to stimulate the processes involved in the decontamination of organic pollutants thus improving the soil biochemical and agronomical properties. In this triple-ecological approach, enzyme activities were 2-4 fold higher than in the treatments with only plant, and TPH were reduced until 70%. Surprisingly, this system was, also, the most efficient in the removal of heavy metals from soil; probably, earthworms acting at rhizosphere level,feed on the microbial biomass of roots, ingest soil and release heavy metals and nutrients available for plant uptake.

In the one year field experiment, organic matter and *Populus nigra* were effectiveness in the reclamation of polluted soil, as showed by the reduction in both inorganic (60%) and organic (80% TPH and 60% PCBs) contaminants. The treatment with only organic matter, showed a lower reduction in organic contaminant (30% TPH), and as expected, did not show significant decrease in the total metal concentration. However, in each treatment, the increase of biological parameters (dehydrogenase, β–glucosidase and phosphatase activities) over the time indicated the activation of microbial metabolism favoured by the organic matter application and the plant rootsmicroorganisms interaction. A preliminary metaproteomic analysis has permitted the purification of a greater number of proteins (SDS-page bands) in planted with respect to unplanted and initial soil, thus confirming the improvement of the soil functional status. This methodological approach may become a basic tool for the detection of the biogeochemical evolution of soil during decontamination (Doni et al., 2012).

Conclusions

The synergic action of organic matter and microorganisms was effectiveness in the reclamation of polluted soil at each scale-level, showing a great reduction in organic contaminants and heavy metals, especially when appropriate plant species were used. Moreover, the application of organic matter and plants stimulated metabolic processes (high enzyme activities) and improved soil quality, thus making this natural technology appropriate for the functional recovery of compromised soil.

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