

From Contaminated Soil to Ecological Restoration: The Key Role of Bioremediation and Ecotoxicology

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ABSTRACT

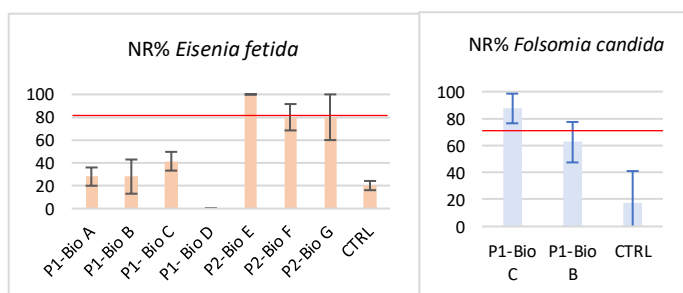
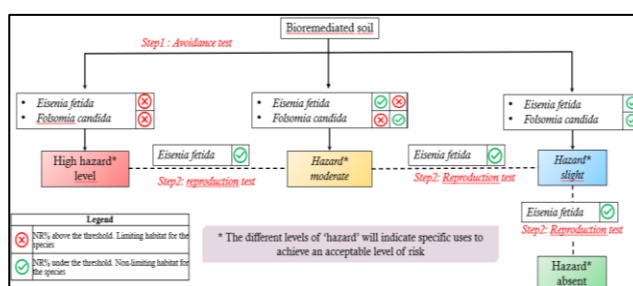
Background information

Contaminated soil is a major type of hazardous waste in Europe, amounting to millions of tonnes per year. Bioremediation, which uses microorganisms to reduce the concentration of organic contaminants, is a sustainable treatment method that reduces environmental impact while maintaining or even enhancing both the biological and agronomic qualities of the soil, ensuring that its characteristics and functions remain intact. Because of these properties, bioremediated soils can be safely reused in landscape restoration and natural engineering projects, providing a sustainable solution for their management. Standardised assessment methods are needed to support this. A risk-based approach incorporating ecotoxicological analysis should replace rigid thresholds. Ecotoxicological tests are particularly valuable in assessing overall soil quality, as they provide insight into the real impact of treated soil on living organisms. The aim is to develop a structured ecotoxicological assessment workflow to determine the hazard classification of remediated soils to ensure their safe and sustainable reuse.

Main results

In this study, the biological quality of the soil after dynamic biopile bioremediation is evaluated through ecotoxicological assessments. We have developed a workflow (Figure 1) based on naturally occurring soil species such as *E. fetida* and *F. candida* to provide ecologically relevant results that guide sustainable soil management and support its application in land restoration.

The workflow is divided into steps: Step 1: Avoidance tests¹⁻² to verify the behaviour of target species in the presence of bioremediated soils compared to control soils. These tests help to identify sub-lethal effects that may not be detectable by chemical analysis alone. Step 2 (optional): Reproduction test³ on *E. fetida* to assess chronic soil toxicity. Based on the results of the avoidance and reproduction tests, an ecotoxicological hazard level of the material between "high", "moderate", "low" or "none" will be returned. The ecotoxicological assessment will contribute to the definition of the most appropriate use of the bioremediated soil. Step 1 of this protocol has already been tested with samples collected from two sites subjected to dynamic biopile remediation. Each sample was tested in replicates, together with a negative control. Transparent glass vessels (15x8 cm) were used with a Plexiglas partition dividing them into two sections: one filled with the soil sample and the other with reference soil (LUFA 2.2), both sieved to 2 mm. For negative controls, both sections contained LUFA 2.2. After filling, the partition was removed, and the test organisms were placed in the centre (light exposure: 16:8 h light-dark). Results were expressed as Net Response Percentage (NR%). Avoidance values varied widely: some samples showed 80-100%



avoidance, indicating unfavourable conditions or potential toxicity, although no contaminants were detected, possibly due to soil texture, pH, moisture, organic matter or microbial imbalances. Others had lower avoidance values of 0-40%, indicating more suitable conditions. Hazard classes were assigned based on both tests: P1-Bio C was classified as moderate, as it was unfavourable to Collembola but not to earthworms, while P1-Bio B was low, with no critical responses from any species.

Conclusions

The integration of ecotoxicological approaches will be essential for a comprehensive assessment of remediated soils to ensure informed environmental decisions. The testing phases will be completed, the sample size will be increased, and a weighted criterion will be defined for the interpretation and integration of soil characterisation data with ecotoxicological results.

References:

UNI EN ISO 17512-2 - Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 2: Tests with collembola (*Folsomia candida*)

UNI EN ISO 17512-1 - Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*)

UNI EN ISO 11268-2 - Effects of pollutants on earthworms - Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia andrei* and other earthworm species