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Original article

# Integrated approach of metal removal and bioprecipitation followed by fungal degradation of organic pollutants from contaminated soils

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**Abstract**

This study reports the assessment of a novel process combining sequential treatments of a historically contaminated soil from the ACNA site (Cengio, Savona, Italy), a decommissioned industrial area. The soil was leached with 0.5 M citric acid leading to a removal of metals in the following order: Pb (74.2%) > Cu (72.6%) > Zn (40.2%) > Ni (55.7%) > Cd (41.5%) > Cr > (21.7%) Co > (19%) Fe (8.2%) with a concomitant low removal of organic contaminants (12%). The leachate was then incubated with the metal-resistant *Klebsiella oxytoca* strain BAS-10, capable of using residual citrate to produce an iron gel that co-precipitated metals. Concomitantly, the leached solid waste was bioaugmented with the autochthonous isolate of *Allescheriella* sp. DABAC 1 leading to a complete degradation of several organic contaminants, including trichlorobenzene, naphtalene, dichloroaniline and pentachloroaniline. The overall removals of organic contaminants by the fungus were 44.3% and 63.8% in non-leached and leached soil.


*Keywords:* Metal leaching; Bioprecipitation; *Klebsiella oxytoca*; Fungus; Hydrocarbon biodegradation

## 1. Introduction

Several contaminated areas, dismissed industrial sites in particular, are often characterized by the concomitant presence of both organic contaminants and heavy metals. In this scenario, attempts at recovering the area are often unsuccessful [21]. For this reason, in the past a common approach to the problem was to bury the waste in safety without any physical, chemical or biological treatment. Such inertization of contaminated sediments is the most common procedure in Italy. This procedure is of regular use in Marghera (Venice, Italy), one of the most polluted industrial sites in Italy [12]. However, the inertization solution causes a significant subtraction of lands, with consequent losses in land value.

An increasing awareness has been thus developed for the need of developing *in situ* or *on site* decontamination processes, thereby allowing the recycling of treated waste for civil purposes.

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The ACNA (Aziende Chimiche Nazionali Associate) di Cengio (SV), is a decommissioned industrial area where a large-scale production of a wide array of organic chemicals had taken place for a century. It represents a paradigmatic example of simultaneous contamination by organic xenobiotics and heavy metals. In 1999 ACNA was included in a list of national priorities for environmental reclamation (see: http://www.bonificare.it/). The ACNA contaminated sediments had been previously investigated for an *in situ* biostimulation approach with autochthonous microflora without any evidence of pollutant degradation [7]. More successful was the use of humic acids as natural surfactants [8] in soil washing techniques to remove organic pollutants from polluted sediments.

The objective of the present work was to investigate the technical feasibility of an integrated approach of bioremediation on aged contaminated soil of the ACNA site. The technique consists of a preliminary metal removal followed by a precipitation recovery with the metal-resistant strain BAS 10 of *K. oxytoca*, and by fungal bioaugmentation in the leached solid waste. The employed bacterial strain isolated from pyrite mines was found to differ from other *Klebsiella* species because it thrived on high ferric citrate concentrations and produced an iron gel capable of sequestering other heavy metals from the solution [5]. The subsequent bioaugmentation step made use of *Allescheriella* sp. (strain DABAC 1) that had been previously isolated from the same contaminated area and was found to efficiently degrade aromatic hydrocarbons [9]. In order to investigate the impact of the preliminary leaching treatment on fungal activity, the bioaugmentation step was also performed on the unleached waste.

## 2. Materials and methods

### 2.1. Contaminated solid waste and microorganisms

The contaminated solid waste, from the large decommissioned chemical industry (ACNA, Cengio, Savona, Italy), contains a high concentration of heavy metals (Table 1) and aromatic hydrocarbons (Table 2). One kg of sample was air dried and sieved (<2 mm) before the analyses. The initial pH of solid waste was 8.10. The sample was constituted by 12.8% coarse sand, 38.8% fine sand, 43.6% silt and 4.8% clay. The total carbon content was 2.7% and total nitrogen 0.13% [8].

### 2.2. Microorganism culture conditions

*Klebsiella oxytoca* strain BAS-10 was isolated from sediments from pyrite mine tailings [5]. The strain was maintained in cryovials at −80 °C and the culture thawed at the moment of inoculation. BAS 10 was routinely grown under anaerobic conditions on FeC medium containing: 2.5 g $l^{-1}$ $NaHCO_3$, 1.5 g $l^{-1}$ $NH_4Cl$, 0.6 g $l^{-1}$ $NaH_2PO_4$, 0.1 g $l^{-1}$ KCl, a solution of vitamin mix and micro-elements and 50 mM Fe(III)-citrate [5].

Table 1
Initial concentration of heavy metals determined in solid waste before and after citric acid leaching

| | Initial concentration mg $kg^{-1}$ | After leaching procedure | |
|---|---|---|---|
| | | Residual concentration mg $kg^{-1}$ | Metal removal percentage (%) |
| Cd | 2.48 ± 0.26 | 1.45 ± 0.15 | 41.5 |
| Co | 33.6 ± 2.54 | 27.2 ± 2.14 | 19.0 |
| Cr | 156 ± 10.5 | 119 ± 6.85 | 21.7 |
| Cu | 427 ± 14.8 | 117 ± 3.75 | 72.6 |
| Fe | 103823 ± 10153 | 95301 ± 8558 | 8.21 |
| Ni | 274 ± 10.2 | 121 ± 3.37 | 55.7 |
| Pb | 278 ± 24.1 | 71.5 ± 6.4 | 74.2 |
| Zn | 150 ± 10.7 | 60.1 ± 3.78 | 40.2 |

Data are mean ± standard deviations of three replicates.

*Allescheriella* sp DABAC 1 was isolated, identified and characterized for its hydrocarbon-degrading ability as reported elsewhere [9]. Fungal inocula were prepared in shaken culture as previously reported [10] and the final fungal suspensions had a biomass concentration of approximately 10 g $l^{-1}$.

### 2.3. Leaching procedure and heavy metal removal with K. oxytoca

Optimal composition of the leaching solution was set-up on the basis of preliminary experiments (data not shown). A 5 g aliquot of sample was leached with 20 ml non-buffered 0.5 M citric acid solution (25% final bulk density) to remove metals from the solid waste. In particular, triplicate samples were incubated under orbital shaking (250 rpm) for 11 days in 200 ml flasks containing the slurry (v/w 4:1). Samples of leachate were withdrawn on a daily basis in order to follow the extraction kinetics of metals.

After three days of leaching, the solution containing metals was brought to pH 7.5 by adding few drops of a 1 N NaOH solution and then twenty-fold diluted with FeC medium. The bacterial strain BAS 10 was inoculated (6.7 $10^7$ cell $ml^{-1}$ culture broth) in a 100 ml solution. The vials were sealed in order to switch from aerobic to anaerobic conditions in 2 days. The citrate was consumed quickly by BAS 10 in Krebs cycle allowing a fast cell growth. When oxygen was depleted in the vial, citrate initiated to be fermented. In this way