



Microbial enzymes as an early warning management tool for monitoring mining site soils



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ABSTRACT

Soil contamination may influence negatively soil health, which often limits and sometimes disqualifies soil biodiversity and decreases plant growth. However, the increased concentration and distribution of potentially toxic elements (PTEs) in soils by mismanagement of industrial activities, overuse of agrochemicals, and waste disposal are causing worldwide concern. This study focused at developing an early warning tool, based on a consortium of different microbial enzymes, for the assessment and monitoring of soil health in response to heavy metal pollution. Soils were collected from an abandoned mine area in northeast Italy, and the concentration of different heavy metals (Cr, Cu, Fe, Mn, Ni, Pb, Zn) were measured and analyzed. The results showed that soils affected by mining activities presented total Zn, Cu, Pb, and Fe concentrations above the internationally recommended permissible limits. A highly significant correlation occurred between the metal concentrations in soils (Fe, Pb, Zn, and Cu). Enzymatic activities varied proportionally with the level of heavy metals in soils and were negatively correlated: arylsulfatase (−0.55), leucine aminopeptidase (−0.12), β-glucosidase (−0.40), alkaline phosphatase (−0.35), and chitinase (−0.58). Conversely, microbial activities were positively correlated with organic matter content and microbial biomass. This study clearly highlights in situ interactions between different patterns of PTEs, represented by different combinations of heavy metals, and enzymatic activity of soil microbial communities, and demonstrate the type of interactions taking place between heavy metals, soil properties, and enzymatic activities. The assessment of changes in the activities of microbial community can precede some detectable changes in soil chemo-physical properties, and strongly support the utility of using enzymatic activity of soil microbial communities as an early warning tool for monitoring soil pollution with heavy metals.

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1. Introduction

Potentially toxic elements (PTEs) in soils derived from activities of urban centers, rural properties, and industries are constantly released into the terrestrial environment and therefore they are of increasingly growing concern worldwide (Wahsha et al., 2015; Fontanetti et al., 2001). Soil contamination by PTEs may influence negatively soil health, which often limits and sometimes disqualifies soil biodiversity and decreases plant growth (Wahsha et al., 2012a). Soil health is the continued capacity of the soil to function as a vital living system, providing essential ecosystem services. Within soils, all bio-geo-chemical processes of the different ecosystem components are combined. These processes are able to sustain biological productivity of soil, to maintain the quality of surrounding air and water environments, as well as to promote plant, animal, and human health (Wahsha et al., 2014a). A common criterion

to evaluate long-term sustainability of ecosystems is to assess the quality of soil.

Recently, several bioindicators and biomarkers of soil quality have been reviewed (Parisi et al., 2005; Wahsha et al., 2012b; Ferrarini et al., 2014). Biological factors may better indicate the environmental status through the biotic indexes, derived from the observation of bioindicator species (Fontanetti et al., 2001). Although all members of the soil biota respond relatively to soil pollution, microbial communities are considered to be the first and most swift responders to such environmental pollutants; due to their high sensitivity to respond to environmental changes, they play a fundamental role in the dynamics of organic matter and in the fragmentation of soils, at different scales of time and space (Nannipieri et al., 2002; Loranger-Merciris et al., 2007). Thus, they can also contribute to metal translocation through the ecosystem in polluted environments (Bini and Wahsha, 2014).

Soil enzymes are a group of enzymes that are usual inhabitants in the soil and are continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility, and soil health

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(Zornoza et al., 2009). Soil enzyme activities are recently used as indices of microbial growth and activity in soils (Fornasier et al., 2014). These enzymes have been suggested as suitable indicators of soil quality because they are strictly related to the nutrient cycles and transformations, and they have high sensitivity to the changes caused by both natural and anthropogenic factors (Gianfreda et al., 2005). The soil organic matter is associated to several biochemical functions of soil microbial enzymes, including several metabolisms related to the life processes of soil structure (Nannipieri et al., 2002). Numerous studies, in particular, have found positive correlations between soil enzymes activities and organic matter content (Wang et al., 2011; Bastida et al., 2012; Liang et al., 2014). The rapid response of soil enzymes to changes in soil management can effectively contribute to the assessment of soil biological health state. The activity of these enzymes in soil undergoes numerous biochemical processes that consist of ecologically connected synthetic processes, immobilization, and stability (Ferrarini et al., 2014; Nannipieri et al., 2002). Moreover, many studies have indicated that enzyme persistence in the soil ranges from a few days to several years depending on the location and soil conditions such as soil depth, temperature, acidity, particle size distribution and organic matter (Ekenler and Tabatabai, 2003; Bastida and Moreno, 2006; Singh et al., 2007; Singh and Ghoshal, 2013; Liang et al., 2014). Nutrient availability also influences the microorganisms' enzyme since they control their enzyme production in response to nutrient availability (Chrost, 1991).

Soil contamination by PTEs can cause acute and long-term toxic effects on both the ecosystems and human health (Bini and Wahsha, 2014). Yet toxic effects of PTEs in soil affect the microbial community biomass and metabolic activities related to such communities. Although all members of the soil biota respond relatively to soil pollution, microbial communities are considered to be the first and most swift responders to such environmental pollutants (Wahsha and Al-Rshaidat, 2014; Wahsha et al., 2013a,b; Bohme et al., 2005; Yao and Huang, 2003). For example, Haanstra and Doelman (1991) demonstrated that copper could reduce the β -glucosidase activity more than cellulose activity. Donderski and Swiontek Brzezinska (2005) explained that Cu, Zn, Ni, Pb, Cd, and Cr could inhibit the activity of alkaline phosphatase. Wyszowska et al. (2006) reported the inhibitory effects of heavy metals on the activity of chitinases. Shukla and Varma (2010) reported

that As can significantly influence the arylsulfatase activity but not alkaline phosphatase. Renella et al. (2011) found that different concentrations of trace elements in soils decreased arylesterase enzyme activity at increased contamination.

This study focused at developing an early warning tool, based on a consortium of different microbial enzymes, for the assessment and monitoring of soil health in response to heavy metal pollution.

In order to accomplish this objective, we carried out several field observations and laboratory measurements on soil samples from Imperina Valley, an abandoned mining site in Italy. Field observations concerned vegetation cover, humus, and soil morphology. Laboratory measurements covered heavy metal concentrations, soil physicochemical parameters, biomass of soil's microbial community, and enzymatic activity.

2. Materials and methods

2.1. Study area

The Imperina Valley is located in the mountain district of Belluno (northeast Italy, Fig. 1), with an altitude ranging between 543 m MSL and 990 m MSL, and oriented in the SW–NE direction (Bini, 2011). The geological substrate consists of dolomite rocks (Dolomia Principale, Upper Triassic) on the right side and the predominantly metamorphic basement (pre-Permian) on the left side, while at the bottom the calcareous-arenaceous complex of Werfen (upper Permian–lower Triassic) outcrops (Fontana et al., 2010). The vegetation cover is consistent with the climatic conditions of the region and is composed mostly of mixed forests (*Picea abies*, *Abies alba*, *Fagus sylvatica*, and *Ostrya carpinifolia*). The Imperina stream crosses the valley; even if no settlements can be found in this area, many buildings and tunnel outlets still bear witness to the past mining activity. Part of the area (right side and a portion of the bottom) lies within the National Park of the Belluno Dolomites. The mineralized area of Valle Imperina, which is located along the contact between the metamorphic basement and the dolomite rocks, is a deposit of mixed sulfides, composed primarily of cupriferous pyrite, pyrite, and chalcopyrite, with minor amounts of other metallic minerals (Wahsha et al., 2012c). Its exploitation has continued almost uninterruptedly from the XV century until the year

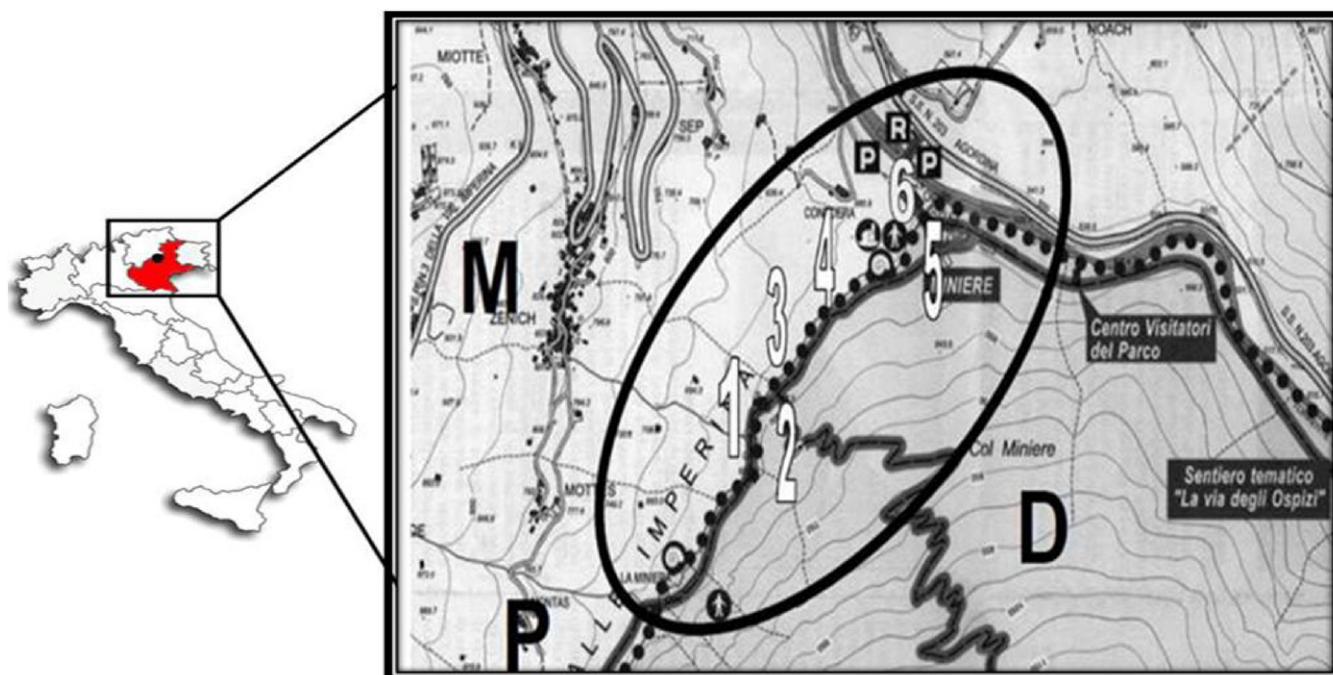


Fig. 1. Location of the studied area and sampling sites of Imperina Valley. M = metamorphic basement, P = phyllite, D = dolomite. Modified after Spaziopadova, 2011.

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