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Biodiversity in Metal-Contaminated Sites – Problem and Perspective – A Case Study

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1. Introduction

Metal contamination is one of the major environmental problems in the world, posing significant risks to ecosystems and human population in abiotic environmental compartments (soil, water, air) and in the related biota (e.g., uptake by fungi and plants).

The primary causes of soil contamination are intensive industrial activities and inadequate waste disposal and treatment (although these categories vary widely across Europe) [1]. Good knowledge of the content and variability of metals in soils linked to both the contribution of parent rock (lithogenic sources) and human activities (anthropogenic sources) is also necessary for evaluating metal pollution. These tasks are particularly difficult to achieve in ancient populated areas, such as the European Mediterranean region, where unpolluted soils are almost impossible to find [2, 3]. Among anthropogenic sources, mining activities are the fourth largest source of land pollution (e.g., 7% of the National Priority (Superfund) Sites in the USA; [4]).

About 2.5 million sites in Europe produce potentially polluting activities [5]. With the help of improved data collection, the number of recorded polluted sites is expected to increase, as are research studies on the topic. Considering present tendencies and laws, contaminated sites will presumably rise by up to 50% by 2025 [5]. Although still at a low rate, there has nonetheless been progress in the remediation of contaminated sites.

Around 59,000 sites have already been remediated. Nonetheless, it should be noted that around 340,000 sites may need urgent remediation [5]. However, conventional technologies for metal-contaminated soil remediation have often been expensive and disruptive [6].

A good tool for evaluating environmental damage might be the Ecological Risk Assessment (ERA), which is already used in the US [7, 8] and in some countries in the European Union (e.g., Netherlands, [9] and the UK [10]), while in Italy, it still remains largely unknown. Consequently, threshold contaminant concentration, according to Italian law [11], is a human-based risk that lacks consideration in terms of biodiversity and ecosystem services.

Before starting to plan restoration of polluted industrial environments, the 'target condition' should first be assessed. However, the remediation of a contaminated site with all its particular biotic and abiotic characteristics seems unrealistic. Several authors have indicated the lack of data providing detailed characterization of the biotic and abiotic components of environments in a pre-industrial condition and have highlighted that communities did not return to their previous states [12-15].

The best approach for preserving or restoring the biodiversity of metal-contaminated environments should be site-specific characterization. For this purpose, the evaluation of the surrounding area's biodiversity, a choice of best reasonable target condition for biotic components and the selection of tolerant organisms occurring on the polluted sites are essential. Among the biotic components of soil, fungi and plants are the pioneer organisms that play a key role in the colonization of contaminated sites.

1.1. Biodiversity in metal-contaminated sites

The physicochemical properties of metal-contaminated environments tend to inhibit soil-forming processes and affect the area's biodiversity by exerting a strong selective pressure on fungi and plants [16-19]. Specifically, the bulk metal content of soils and its metal releasability are among the most important edaphic factors determining vegetation composition. Other than metal toxicity, vegetation successions are also retarded by low nutrient status, poorly developed soil structure and water-restricted conditions [20].

In addition, microorganism communities play a significant part in the detoxification of noxious chemicals and in the control of plant growth [21], and also provide pivotal information about soil metal bioavailability [22]. Metalliferous biota is increasingly exploited for the stabilization or active remediation of the metal-contaminated ecosystems and represents an important research topic in the contemporary field of green technology [23, 24].

It is well-known that a number of plants and fungi are able to survive and actively grow in metal-contaminated soils. For instance, recent studies [25] have shown that some arbuscular mycorrhizal fungi from Cu-contaminated soils [*Claroideoglomus claroideum* (N.C. Schenck and G.S. Sm.) C. Walker and A. Schüßler in association with *Imperata condensata* Steud. and *Rhizophagus irregularis* (Błaszk., Wubet, Renker and Buscot) C. Walker and A. Schüßler in association with carrot roots] are able to compartmentalize Cu in spores as a survival strategy in polluted environments. Additionally, microfungi are essential in colonizing and detoxifying metal-contaminated soil ecosystems and consequently have environmental and economic significance [16, 26-28].

Mine dumps cause high selective pressure, enabling bacteria and microfungi to be the first organisms able to re-colonize mine soils [29]. Under this pressure, microfungal communities change their composition and several resistant strains are selected [28]. In addition, plant communities have established a primary succession on mine wastes [30] and can be exploited for biogeochemical prospecting and mine stabilization (e.g., abandoned mines contaminated with arsenic, antimony and tungsten [17]).

1.2. Selecting native fungi and plants for bioremediation

Critical environmental conditions related to high metal concentration are present either in natural soils (e.g., serpentinitic and ultramafic soils; [31, 32 and references therein]) and in anthropogenic contaminated sites (such as industrial, agricultural and mining sites [33, 1 and references therein]).

Fungi and plants from metal-rich soils develop specific strategies to cope with metals via avoidance, accumulation or hyperaccumulation [34]. Care should be taken in choosing the right species for the application of bioremediation techniques, because the introduction of alien fungi and plants may alter and disrupt indigenous ecosystems [35], or may be unsuitable for local climate conditions [36]. Therefore, a more appropriate option is to find native hyperaccumulator fungi and plants that have adapted to growing on metalliferous sites, and use them for soil bioremediation in the same region [37, 38].

Bioremediation process consists of two main approaches: 1) myco- and phytostabilization; 2) myco- or phyto-extraction. Myco- and phyto-stabilization are mechanisms that immobilize pollutants – mainly metals – within the root zone, by adsorption, chelation and metal ion precipitation, thus preventing migration of contaminants by erosion, leaching and runoff [39,40].

Myco- and phyto-extraction are typically used to uptake metals, metalloids and radionuclides. The metals accumulate in the fruit-bodies or in the plant's aboveground biomass and can be removed or recovered by harvesting and burning the biomass.

Several organisms, including microbes, micro- and macro-fungi, agricultural and vegetable crops, ornamentals and wild metal hyperaccumulating plants have been tested both in laboratory and field conditions for selecting and providing organisms able to clean-up metalliferous substrates [24].

In fact, recent studies have shown how macrofungi such as *Trametes versicolor* (L.) Lloyd [41] and microfungi such as *Aspergillus niger* Tiegh. [42], *A. terreus* Thom [43], *A. versicolor* (Vuill.) Tirab. [44], *Penicillium notatum* Westling [45], *Rhizopus arrhizus* A. Fisch. [46], *Trichoderma atroviride* P. Karst. [47], *T. viride* Pers. [48] are able to absorb Cu from contaminated liquid and solid matrices.

Similarly, plant taxa naturally occurring in metalliferous soils have been selected, tested and confirmed as hyperaccumulators under experimental conditions for different metals like Ni (e.g., *Alyssum bertolonii* Desv., *A. murale* Waldst. & Kit., *A. lesbiacum* (Candargy) Rech.f., *A.*

corsicum Duby) and Cd (*Thlaspi caerulescens* J.Presl & C.Presl and *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz) [49-54].

Little information is available about the processes occurring at the soil-rhizosphere level. Though roots are the only organ directly interacting with soil trace elements, most of the studies on hyperaccumulation by plants have focused on above-ground organs. Less than 10% of the known hyperaccumulator species have been investigated at the rhizosphere level [55].

Bacteria and fungi in the hyperaccumulator rhizosphere may exhibit increased metal tolerance by i) acting as a plant growth promoting microorganisms; ii) modifying metal speciation and solubility; iii) influencing plant trace element concentrations [55, 56-58].

Fungal species not identified as mycorrhizae have also been found in the hyperaccumulator rhizosphere [59, 60]. The role of these organisms still needs to be established, but some have been identified as able to concentrate and volatilize pollutants [61]. The knowledge about hyperaccumulators and associated microorganisms continuously increases, thus suggesting the significant roles of bacteria and fungi in hyperaccumulation.

2. Case study – Multidisciplinary investigations on biodiversity into a sulphide-rich waste-rock dump

The present review illustrates the results of a six-year multidisciplinary study aimed at understanding the relationships among the mineralogy and chemistry of the Libiola mine (eastern Liguria, Italy, Fig. 1) and the metal uptake by fungi and plants spontaneously growing in the mine-waste dump. The mine is located in a moderately steep mountainous terrain at an altitude between 40 and 400 m asl, close to the Liguria sea coast.

This mine had already been known during the Bronze Age [63] and was industrially exploited from 1864 until 1962. During this period, it produced over 1 Mt of Fe-Cu sulphides with an average grade ranging from 7 to 14 Cu wt%, thus representing one of the most important Fe-Cu sulphide mines [64, 65] in Italy. Sulphide mineralization occurs within the Jurassic ophiolites of the Northern Apennines (Vara supergroup; [66]) and is geologically characterized primarily by pillow basalts with minor serpentinites, gabbros and ophiolitic breccias. During exploitation, five major waste-rock dumps were built up through the progressive accumulation of heterogeneous sterile rocks (derived from galleries and open-pit excavations) and non-valuable ore-fragments, with metal concentrations below the economic cut-off produced during beneficiation processes [67]. The soils of the dumps are characterized by severe edaphic conditions due to their peculiar physical (steep slopes, low moisture retainability, impermeabilization due to cementification and hardpan formation; [68]) and chemical (high Cr-, Cu-, Co-, Ni- and Zn-concentrations, low pH values and the low availability of essential macronutrients) properties. This site presents several environmental problems as a result of active acid mine drainage (AMD) processes, which determines the water acidification and metal pollution of soils and waters.

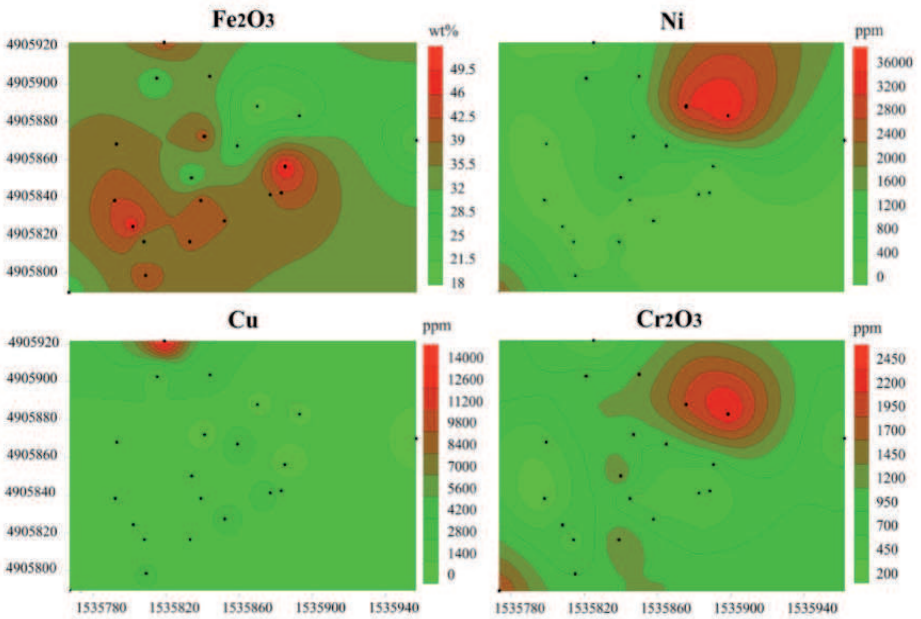**Figure 1.** Location of Libiola mine site (from [62], modified).

Figure 2. Contour maps of selected elements (Fe2O3, Cr2O3, Cu and Ni) in the main waste-rock dump of the Libiola mine (from [67], modified).

We evaluated the plant and fungal diversity in these contaminated soils in order to 1) identify factors that influenced the pioneer fungi and plants colonizing this stressed environment; 2) identify and select tolerant and hyperaccumulating plants and fungal strains suitable for mine remediation.

The waste-rock materials deposited in the mining area are mainly gravely-sandy sediments with a relatively uniform particle size distribution in the range 2-64 mm; the silt and clay fractions are subordinate components and vary from 5% to 26%. Most of the dumps evidence strong superficial cementation induced by Fe-oxides precipitating from acid sulphate water seepage, which determines the formation of centimetre-thick impermeable hardpans on several parts of the exposed surface of the dumps [67, 68].

Due to active and widespread AMD processes, the pH of waste-dump soils is generally acidic (3.5-4.3) and significantly low, compared to the surrounding serpentinitic and basaltic soils (6.2-6.8) [30, 69, 68].

The mineralogical and lithological composition of the waste-rock materials is quite homogeneous throughout the mine area, though the relative proportions of the detected lithotypes and mineral species significantly vary from site to site. The studied samples are mainly composed of polycrystalline rock fragments, which can be grouped into the following lithological classes [65, 67, 68]: 1) serpentinites 20%-50%; 2) basalts 5%-10%; 3) sulphide mineralizations (2%-10%); 4) massive Fe-oxyhydroxides and -oxides clasts (35%-65%). Other subordinate components (3%-6%) are represented by garnet- and epidote-rich rodingites, polygenic ophiolitic breccias and brecciated basalts.

The mineral species occurring within the waste-rock dump [65, 67, 68] can be divided into three major groups on the basis of their origin and/or origin: 1) host rocks and gangue minerals; 2) ore minerals with different degrees of alteration; 3) authigenic secondary minerals. Serpentine minerals (60%-70% of the recognized mineral species) and Fe-oxyhydroxides (mainly goethite) are by far the most abundant species, respectively representing the main rock-forming minerals of the lithotypes of the surrounding area and the main authigenic minerals forming as a result of the ongoing AMD processes. Sulphides (mainly pyrite and chalcopyrite) are subordinate but important components from an environmental point of view, either for their role in triggering the AMD processes and/or for the release of metals (particularly Fe, Cu and Zn). Magnetite, Cr-bearing magnetite and chromian spinels are the only Cr-bearing minerals, whereas serpentines are the main Ni-bearing minerals. Nevertheless, they are stable mineral species, even in this highly reactive environment, that likely do not contribute significantly to the bioavailable fraction of toxic metals.

According to the mineralogical and lithological composition, the waste rock dumps of the Libiola mine are invariably characterized by very high concentrations of several potentially toxic metals (such as Ti, Mn, Co, Ni, V, Cr, Cu, Zn and Cd). Although a notable variability is always present and several hot spots have been found (see, for example, Fig. 2), most of the detected metals (in particular Cr, Cu, Co, Ni and Zn) strongly exceed the Italian limits for residential and industrial sites [11].

Xero-acidophilous plant communities characterize the areas surrounding the mine site. These are different aspects of sclerophyllous evergreen maquis and mixed sclerophyllous evergreen and deciduous shrub thickets (pseudomaquis) formed by *B. sempervirens* L. and/or *Genista desoleana* Valsecchi, *Erica arborea* L., *Calicotome spinosa* (L.) Link., *Juniperus oxycedrus* L. subsp. *oxycedrus* and *Arbutus unedo* L. Chamaephytic and sub-shrubby layers are well represented by *Euphorbia spinosa* L. subsp. *ligustica* (Fiori) Pignatti, *Helichrysum italicum* (Roth) G. Don, *Minuartia laricifolia* (L.) Schinz. and Thell subsp. *ophiolitica* Pignatti, *Thymus* sp.pl., and *Satureja montana* L.; Maritime pine (*Pinus pinaster* Aiton) old reforestations are also present. Less frequent are relics of holm oak (*Quercus ilex* L.), pubescent oak (*Quercus pubescens* Willd.) copses and thermophile mixed woods [3, 70, 71].

The study area is characterized by bare soils or by different successions of plant communities ranging from herbaceous to arboreal layers (Fig. 3). In all the plots, species richness and vegetation cover were extremely low and the flora showed acidophilous traits [30]. The bare soil is a substrate with an almost complete absence of vegetation (Fig. 3B). The herbaceous layer has pioneer vegetation dominated by discontinuous communities of low-sized grasses and herbs such as *Deschampsia flexuosa* (L.) Trin., *M. laricifolia* subsp. *ophiolitica*, *Sesamoides interrupta* (Boreau) G.López, *Festuca robustifolia* Markgr.-Dann. and, in sites with more developed soil, also by *Cerastium ligusticum* Viv. and *Asplenium adiantum-nigrum* L.. The subsequent layers are colonized by *Thymus vulgaris* L., *S. montana*, *E. spinosa* subsp. *ligustica*.

The shrub layer is mainly located on serpentine debris on the edge of the landfill areas and colonized by semi-natural plant communities dominated by *E. spinosa* subsp. *ligustica*, *Alyssoides utriculata* (L.) Medik., *T. caerulescens*, *Silene paradoxa* L., *F. robustifolia* and *H. italicum* (Fig. 3C).

Despite harsh environmental conditions, the waste-rock dump has been progressively colonized since 2008 by several plants of *Pinus pinaster* Aiton, already found naturally on metal-rich sites, thereby establishing ectomycorrhizal symbiosis (ECM) [72]. The maritime pine populations constituting the tree layer derive from seeds dispersed by the surrounding plants and employed for the revegetation of fired areas (Fig. 3D). A few herbaceous species such qw *D. flexuosa*, *M. laricifolia* subsp. *ophiolitica* and *F. robustifolia* are associated with *P. pinaster*. The absence of the shrub layer in the mine dump and the presence of the tree layer strictly composed of pine is particularly uncommon, and the success of *P. pinaster* colonization is mainly due to the presence of *Scleroderma polyrhizum* (J.F. Gmel.) Pers (Fig. 4A) and *Telephora terrestris* Ehrh. (Fig. 4B) ectomycorrhizic with pine (see Fig. 4C-D) [69]. Maritime pine is known to be able to cope with some limiting factors such as a low level of macronutrients, a lack of organic matter and water stress, typical of dismissed mining areas [73] such as the one in our study. In addition, we found that *P. pinaster* is able to completely exclude toxic metals from its tissue (Fig. 5), thereby acting as a phytostabilizer, as demonstrated by bioaccumulation (i.e., BF = shoot:soil metal concentration) and translocation (i.e., TF = shoot:root metal concentration) factors (BFs>1 and TFs<1, respectively [68]). Where metal concentrations decrease [30, 68], plants constitute semi-natural Mediterranean serpentine vegetation, typical to NW Italy [70, 71]
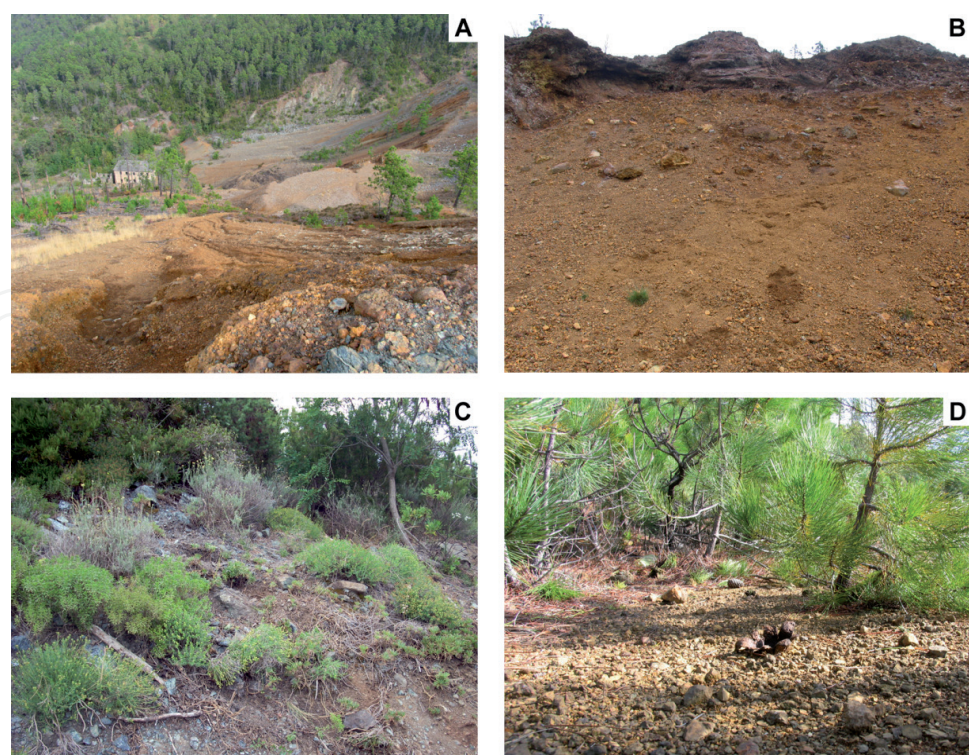
Figure 3. Libiola mine site **A**) View of the mine waste rock dump; **B - D**) sampling sites; **B)** bare soil with no vegetation; **C**) shrub layer; **D**) tree layer with macrofungi.
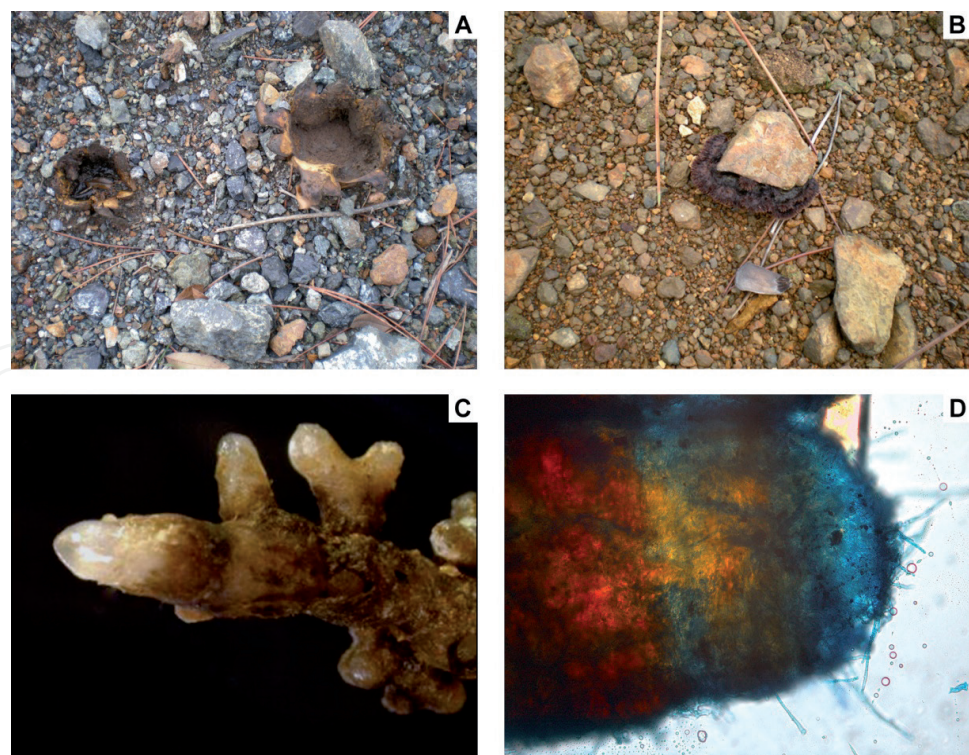

Figure 4. Macrofungi at the Libiola mine and their ECM symbiosis. **A)** *S. polyrhizum*; **B)** *T. terrestris*; **C)** *P. pinaster* roots with ECM fungi; **D)** details of root apex with ECM fungi; blue cotton staining.
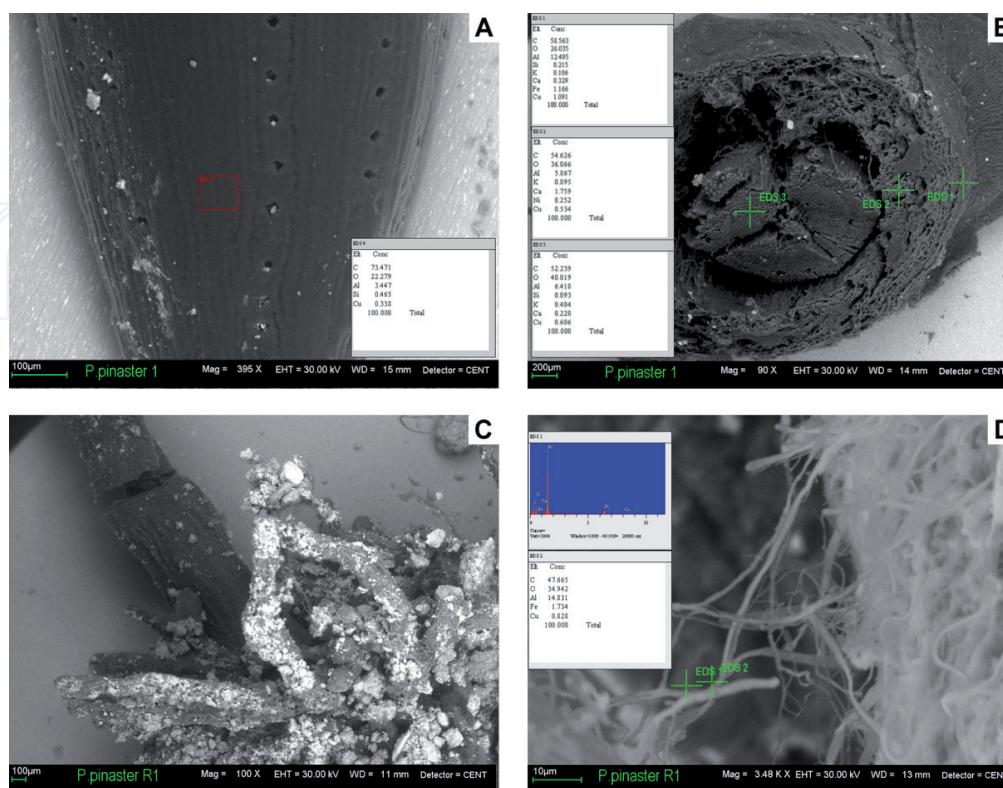
Figure 5. ESEM micrographs of *P. pinaster* with EDS spectra. **A**) *P. pinaster* leaves showed soil particles on stomata, but no metals were detected inside tissues; Bar = 20 μm. **B**) *P. pinaster* stems; Bar = 200 μm **C**). *P. pinaster* roots with ECM fungi; Bar = 100 μm. **D**) details of root hairs; Bar = 10 μm. EHT: 30 KV, WD: 14 mm, detector: Centaurus.

Among the mine plants screened for Ni accumulation in plant tissue, only the well-known metal hyperaccumulator *Thlaspi caerulescens* J. & C. Presl and *A. utriculata* yielded a positive response (Fig. 6). The latter was recently confirmed as a new Ni facultative hyperaccumulator, able to concentrate more than 1000 mg kg^{-1} Ni DW in leaves [74]. Plant efficiency tests were carried out on native soils to evaluate the growing ability and the ecophysiology of this promising species, and recent experiments have confirmed its suitability for phytoextraction (data not shown).

Soil samples collected from the *A. utriculata* rhizosphere and barren mine soils were examined to determine microfungal flora. On the whole, the majority of isolated colonies belonged to the genus *Aspergillus*, *Botrytis*, *Clonostachys*, *Penicillium* and *Trichoderma* (Fig. 7). Regarding *A. utriculata*, the rhizosphere were isolated species such as *Eurotium amstelodami* L. Mangin, *Aspergillus carbonarius* (Bainier) Thom, *A. tubingensis* Mosseray, *Penicillium waksmanii* K.M. Zalessky and *Rhodotorula* spp. not found in other mine soil samples. We can hypothesize that these microfungi, growing in the hyperaccumulator rhizosphere, may function as plant growth promoting factors altering element solubility and increasing plant metal uptake.
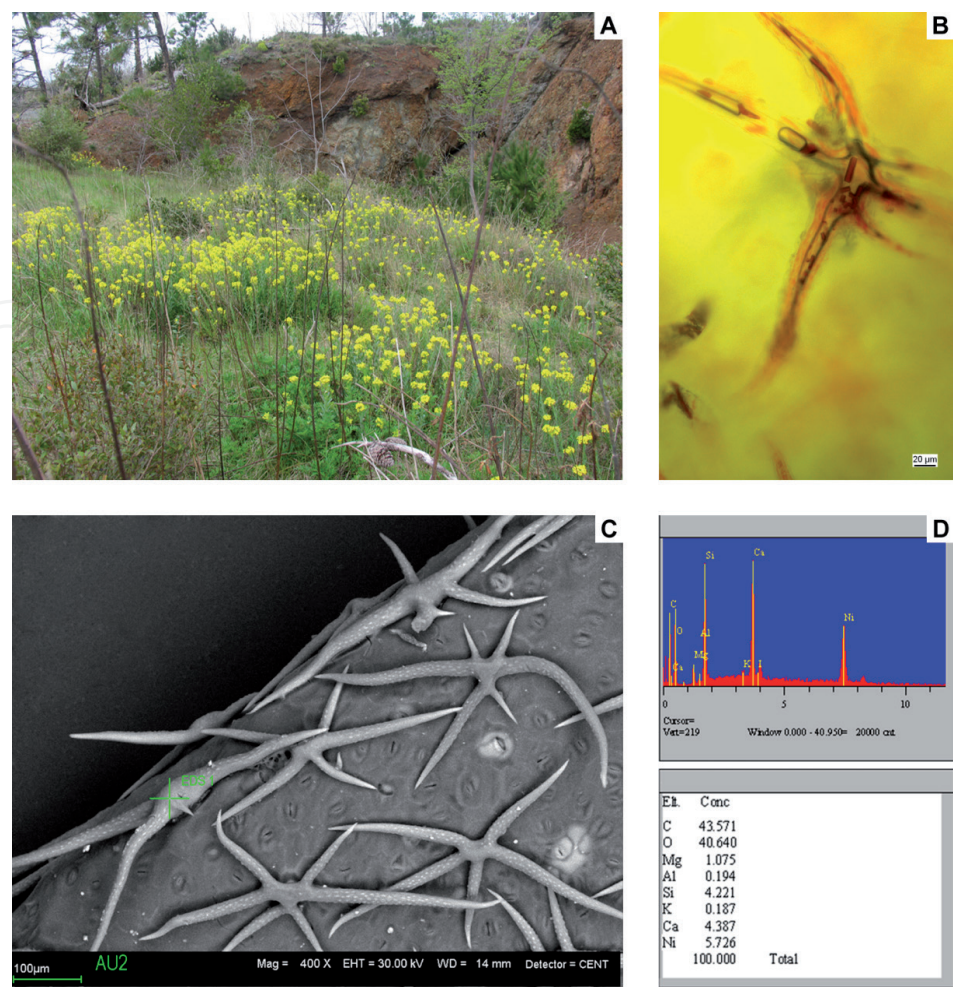
Figure 6. *Alyssoides utriculata* **A**) in the mine dump; **B**) positive leaf trichome DMG test, light microscopy micrographs; **C, D**) ESEM micrographs and EDS spectrum of leaf trichomes storing Ni, up to 8%; Bar = 100 μm. EHT: 30 KV, WD: 14 mm, detector: Centaurus.

The ectomychorrizal macrofungi collected reveal a highly significant metal accumulation, in particular Cu>1000 mg kg^{-1} in *Telephora terrestris* and Ag>50000 μg kg^{-1} in *Scleroderma polyrhizum*. When present in mine sites, these fungi are able to actively absorb most of the potential toxic elements in the sites' basidiomata. The absorption sequence Cu>Zn>Cr>Ni>Co obtained for these macrofungi overlaps well with the sequences obtained using EDTA extractions and water leaching tests [69, 68]. Both species also established ECM symbiosis with pine and we could not exclude that they played a role in the phytostabilization process at the root level.

Finally, we studied soil microfungi to test the growth responses of culturable isolated microfungal strains in copper enriched media and to evaluate their potential use in mycoremediation. The species most recurrent were filamentous microfungi: *Trichoderma harzianum*, *Clonostachys rosea* and *Aspergillus alliaceus*. We hypothesized that these fungi were particularly tolerant/resistant to copper. The Cu tolerance level of *T. harzianum* and *C. rosea* were tested *in vitro* at increasing Cu(II) concentrations. The tests showed a Cu(II)-tolerance capability ranging from 100 to 400 mg L^{-1} [75]. These preliminary analyses proved that several fungal species were able to grow in Cu-contaminated media, thereby underlying the importance of selecting new
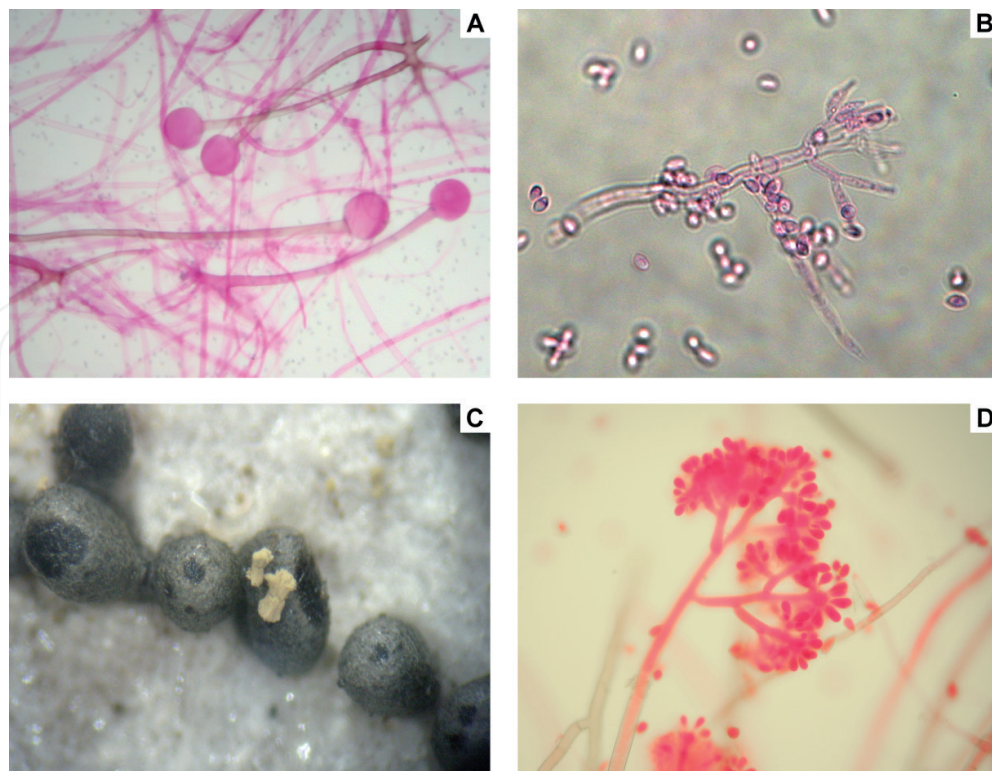
Figure 7. Microfungi isolated from mine site: **A**) *Rhizopus oryzae* Went & Prins. Geerl., acid fuchsine staining, light microscopy; **B**) *Clonostachys rosea* (Link Schroers, Samuels, Seifert & W. Gams, acid fuchsine staining, light microscopy; **C**) sclerotia of *Aspergillus alliaceus* Thom & Church, stereomicroscopy; **D**) *Botrytis cinerea* Pers. ex Nocca & Balb., acid fuchsine staining, light microscopy.

tolerant strains and testing their potential metal uptake capabilities for application to mycoremediation protocols.

Silver, a noble metal of historical and economic importance, also indicated a high concentration in the Libiola mine soil. Even if silver is usually not considered an environmental contaminant, Ag^+ represents one of the most toxic metals to bacteria, algae and fish [76], and can damage cellular components and reduce enzymatic activities. Some studies have shown that bacteria, yeasts and macrofungi can accumulate silver; however, the chemical form of Ag in the macrofungal fruit-bodies was not investigated in detail [77, 78, 76]. In this context, we have tested the potential Ag^+ accumulation by microfungi isolated from the Libiola mine soils. First, we tested the *in vitro* growth capabilities on Ag^+-enriched media by *Aspergillus alliaceus*, *Trichoderma* sp., *C. rosea* in order to select the most tolerant microfungal strain. *Trichoderma* sp. showed the best and speediest capabilities for growing *in vitro* on media spiked with 400 mg kg^{-1} of Ag^+, uptaking 150 mg kg^{-1} dry weight (Fig. 8), as confirmed by ICP-MS analysis.

The considered contaminated environment chiefly affected the biodiversity of the area and exerted a strong selective pressure on the local flora and mycoflora. These results suggest the use of *P. pinaster*, *A. utriculata*, *T. terrestris*, *S. polyrhizum*, *T. harzianum* and *C. rosea* for developing experimental protocols of bioremediation and habitat restoration for avoiding ecosystem disruption.
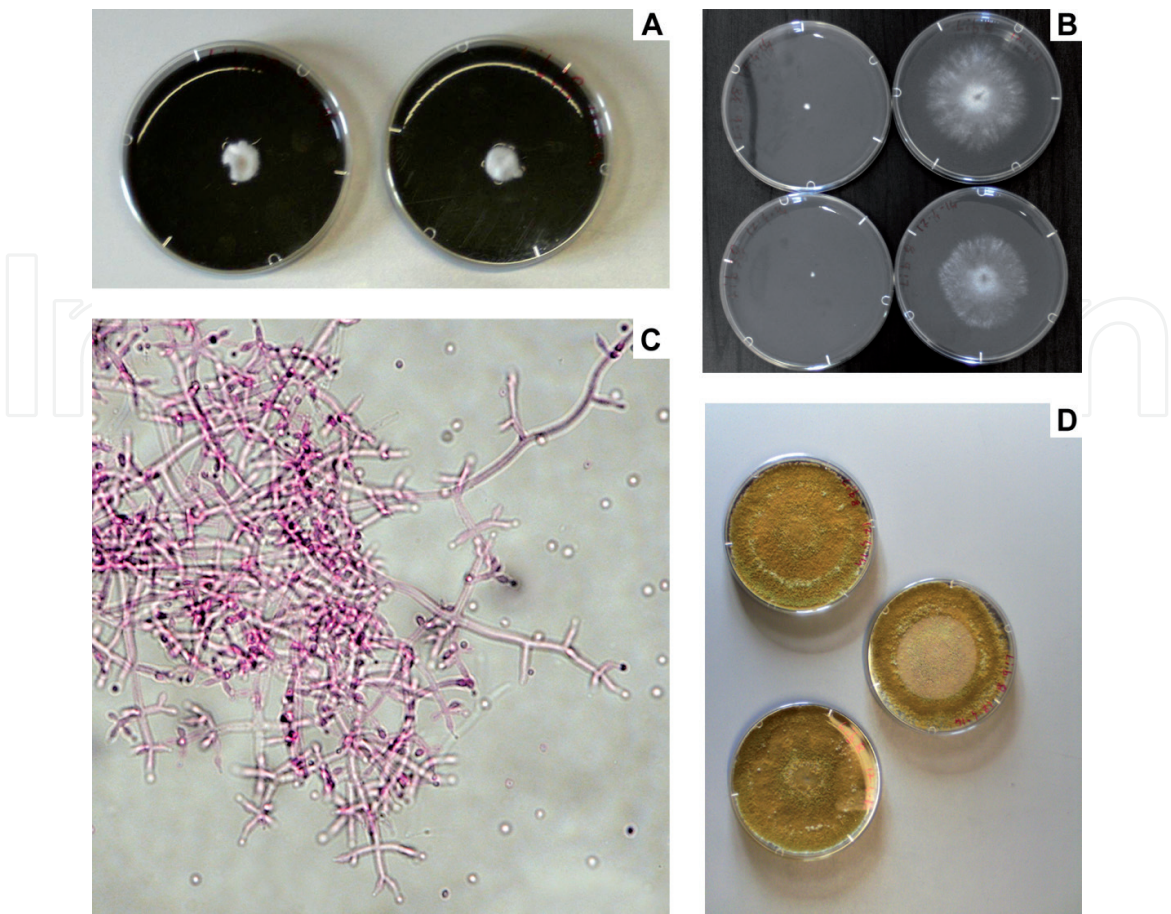
Figure 8. Ag accumulation tests. **A-B**) Screening test of microfungal growth capability on silver enriched media; **C**) microscopic detail of the selected *Trichoderma* sp. strain **D**) *Trichoderma* sp. growth.

The study highlighted differences between mineralogy, geochemistry, flora and mycology among strongly polluted selected sites of the Libiola mine, which are key points for future reclamation of the area. In particular, our results evidenced the significant control of soil mineralogy and chemistry on the biodiversity of the mining area, as well as on the capacity of mycoflora and flora to accumulate specific metals. Knowing what the factors are influencing the first colonization by plants and the interaction among plants, fungi and soils, allow us to develop a method for the land restoration of metal polluted sites in a manner that minimizes interventions and costs.

3. Future perspectives – How to apply what we have learned

Due to the complexity of soil and *in-situ* conditions, each contaminated site requires its own strategy and site-specific designs for decontamination, especially in Mediterranean areas. Multi-element contaminated soils contain several pollutants; consequently, it is necessary to screen out fungi and plants that can survive on different pollutants simultaneously and to accumulate or stabilize some of them.

The use of metal tolerant species adapted to native conditions can assist in balancing the ecological pressure generated by soil pollution. Consequently, it is necessary to evaluate the potential bioremediation of native fungi and plants from contaminated sites before choosing other species suitable for bioremediation.

Phytostabilization may be employed as a temporary solution until new techniques are available. However, for large contaminated sites (e.g., mining or industrial sites), phytostabilization likely represents the best option for ecosystem restoration [79]. Moreover, below-ground restoration success involves the employment of native microfungi for developing and improving the soil microbial biomass [80].

In the joint use of plants and soil microorganisms, plants provide a C source for microorganisms, which absorb, degrade or release elements for plant absorption [81]. The plant allocates most of the metals to their roots so that plant shoots can be more efficient under metal stress [82]. Rhizospheric fungi are able to alleviate the stress of metals on plant growth through soil bioremediation (bioaugmentation) and can sometimes alleviate the unfavourable effects of metal on plant growth by the process of phytostabilization [83]. With the employment of the right soil, microfungi and ECM fungi, we can avoid amendments to soil, thereby improving organic matter and soil-forming processes that are essential for the colonization of pioneer plant species. These species will guarantee the durable, sustainable and ecological restoration of polluted mine sites, thereby increasing soil fertility.

The enormous potential of native fungi and plants that are able to colonize metal-contaminated soils need to be studied in-depth in order to preserve the natural genetic resources of metal-liferous habitats and to increase our basic knowledge about the natural adaptation mechanisms of hyperaccumulators in order to employ them in phytoremediation purposes.

Acknowledgements

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References

[1] Alloway BJ. Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability. Dordrecht: Springer; 2013.

[2] Facchinelli A, Sacchi E, Mallen L. Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environmental Pollution 2001;114: 313-324.

[3] Marsili S, Roccotiello E, Carbone C, Marescotti P, Cornara L, Mariotti MG. Plant colonization on a contaminated serpentine site. Northeastern Naturalist. Special issue: Soil Biota Serpentine: A World View 2009;16: 297-308.

[4] Allen HE, Huang CP, Bailey GW, Bowers AR. Metal Speciation and Contamination of Soil. Boca Raton: Lewis Publishers; 1995.

[5] European Environment Agency, (EEA) Progress in management of contaminated sites (LSI003) Assessment May 2014. http://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment

[6] European Environment Agency state of the environment report (SOER). The European environment - state and outlook – SOIL. 2010. http://www.eea.europa.eu/soer/europe/soil/download

[7] U.S. Environmental Protection Agency Guidelines for Ecological Risk Assessment, EPA/630/R-95/002F 1998. http://www.epa.gov/raf/publications/guidelines-ecological-risk-assessment.htm

[8] ASTM Standard Guide for Risk-Based Corrective Action for Protection of Ecological Resource, ASTM E 2205/E2205M – 02 (Reapproved 2009); 2009. http://www.astm.org/Standards/E2205.htm

[9] Honders T. New risk-based soil quality standards in the Netherlands: conference proceedings, seventh International conference on the Environmental and Technical Implications of Construction with Alternative Materials (WASCON, 2009). Lyon, France; 2009.

[10] Environment Agency An ecological risk assessment framework for contaminants in soil Science report SC070009/SR1 2008. https://www.gov.uk/government/publications/contaminants-in-soil-assessing-the-ecological-risk (accessed 1 October 2008).

[11] Legislative Decree n. 152 del 3 Aprile (2006) Norme in materia ambientale. Gazzetta Ufficiale della Repubblica Italiana, n. 88 del 14/4/2006, Supplemento ordinario, n. 96. http://www.camera.it/parlam/leggi/deleghe/06152dl.htm

[12] Batty LC, Hallberg KB. Consequences of living in an industrial world. In: Batty LC, Hallberg KB. (eds.) Ecology of Industrial Pollution. Cambridge: Cambridge University Press; 2010. p1-6.

[13] Langford TEL, Shaw PJ, Howard SR, Ferguson AJD, Ottewell D, Eley R. Ecological recovery in a river polluted to its sources: the River Tame in the English Midlands.

In: Batty LC, Hallberg KB. (eds.) Ecology of Industrial Pollution. Cambridge: Cambridge University Press; 2010. p255-275.

[14] Tibbett M. Large-scale mine site restoration of Australian eucalypt forests after bauxite mining: soil management and ecosystem development. In: Batty LC, Hallberg KB. (eds.) Ecology of Industrial Pollution. Cambridge: Cambridge University Press; 2010. p309-326.

[15] Williams AE, Waterfall RJ, White KN, Hendry K. Manchester Ship Canal and Salford Quays: industrial legacy and ecological restoration. In: Batty LC, Hallberg KB. (eds.) Ecology of Industrial Pollution. Cambridge: Cambridge University Press; 2010. p276-308.

[16] Gadd GM. Interactions of fungi with toxic metals. New Phytologist 1993;124: 25-60.

[17] Pratas J, Prasad MNV, Freitas H, Conde L. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. Journal of Geochemical Exploration 2005;85: 99-107.

[18] Crane S, Dighton J, Barkay T. Growth responses to and accumulation of mercury by ectomycorrhizal fungi. Fungal Biology 2010;114: 873-880.

[19] Ceci A, Maggi O, Pinzari F, Persiani AM. Growth responses to and accumulation of vanadium in agricultural soil fungi. Applied Soil Ecology 2012;58 :1-11.

[20] Baker AJM, Ernst WHO, van der Ent A, Malaisse F, Ginocchio R. Metallophytes: the unique biological resource, its ecology and conservational status in Europe, central Africa and Latin America In: Batty LC, Hallberg KB. (eds.) Ecology of Industrial Pollution. Cambridge: Cambridge University Press; 2010. p7-40.

[21] Filip Z. International approach to assessing soil quality by ecologically related biological parameters. Agriculture Ecosystems & Environment 2002;88(2): 169-174.

[22] Saeki K, Kunito T, Oyazu H, Matsumoto S. Relationships between Bacterial tolerance Levels and Forms of Copper and Zinc in Soils. Journal of Environmental Quality 2002;32: 1570-1575.

[23] Adriano DC. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals. New York: Springer; 2001.

[24] Prasad MNV, Freitas H. Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 2003;6: 275-321. http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v6n3-6/617 (accessed 15 December 2003)

[25] Cornejo P, Pérez-Tienda J, Meier S, Valderas A, Borie F, Azcón-Aguilar C, Ferrol N. Copper compartmentalization in spores as a survival strategy of arbuscular mycorrhizal fungi in Cu-polluted environments. Soil Biology and Biochemistry 2013;57: 925-928.

[26] Fomina MA, Alexander IJ, Colpaert JV, Gadd G.M. Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biology & Biochemistry 2005;37: 851-866.

[27] Fomina MA, Gadd GM, Burford EP. Toxic Metals and Fungal Communities. In: Dighton J, White JF, Oudemans P. (eds.) The fungal community: its organization and role in the ecosystem, 3rd edition. Boca Raton: CRC Press; 2005. p733-758.

[28] Gadd GM. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycological Research 2007;111: 3-49.

[29] Novàkovà A. Soil microfungi in two post-mining chronosequences with different vegetation types. Restoration Ecology 2001;9: 351-358.

[30] Micò C, Recatalà M, Peris M, Sánchez J. Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. Chemosphere 2006;65: 863-872.

[31] Oze C, Fendorf S, Bird DK, Coleman RG. Chromium geochemistry of serpentine soils. International Geology Review 2004;46: 97-126.

[32] Alexander EB, Coleman RG, Keeler-Wolf T, Harrison SP. Serpentine geoecology of western North America: Geology, Soils, and Vegetation. New York: Oxford University Press; 2007.

[33] Jambor JL, Blowes DW. Short course handbook on environmental geochemistry of sulfide mine-waste. Mineralogical Association of Canada, Waterloo, Ontario; 1994.

[34] Van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant and Soil 2013;362: 319-334.

[35] Angle J, Chaney R, Li YM, Baker A. The risk associated with the introduction of native and hyperaccumulators plants. Abstract. USDA, Agricultural Research Service, USA. 2001.

[36] Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nevajova E, van der Lelie D, Mench M. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environmental Science and Pollution Research 2009;67: 765-794.

[37] Pilon-Smits EAH, Freeman JL. Environmental cleanup using plants: biotechnological advances and ecological considerations. Frontiers in Ecology and the Environment 2006;44: 203-210.

[38] Carrillo Gonzalez R, Gonzalez-Chavez MCA. Metal accumulation in wild plants surrounding mining wastes. Environmental Pollution 2005;144: 84-92.

[39] Pivetz BE. Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites. Ground Water Issue, U.S. Environmental Protection Agency, Office of

Research and Development and Office of Solid Waste and Emergency Response. 2001.

[40] Bystrzejewska-Piotrowska G, Pianka D, Bazała MA, Stęborowski R, Manjon JL, Urban PL. Pilot study of bioaccumulation and distribution of cesium, potassium, sodium and calcium in king oyster mushroom (*Pleurotus eryngii*) grown under controlled conditions. International Journal of Phytoremediation 2008;10(6):503-514.

[41] Şahan T, Ceylan H, Şahiner N, Aktaş N. Optimization of removal conditions of copper ions from aqueous solutions by *Trametes versicolor*. Bioresource Technology 2010;101: 4520-4526.

[42] Dursun AY, Uslua G, Cucia Y, Aksub Z. Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing *Aspergillus niger*. Process Biochemistry 2003;38: 1647-1651.

[43] Cerino-Córdova FJ, García-León AM, Soto-Regalado E, Sánchez-González MN, Lozano-Ramírez T, García-Avalos BC, Loredo-Medrano JA. Experimental design for the optimization of copper biosorption from aqueous solution by *Aspergillus terreus*. Journal of Environmental Management 2012;95 Suppl.: S77-82.

[44] Taştan BE, Ertuğrul S, Dönmez G. Effective bioremoval of reactive dye and heavy metals by *Aspergillus versicolor*. Bioresource Technology 2010;101: 870-876.

[45] Anjum F, Bhatti HN, Ghauri MA, Bhatti IA, Asgher M, Asi MR. Bioleaching of copper, cobalt and zinc from black shale by *Penicillium notatum*. African Journal of Biotechnology 2009;8: 5038-5045.

[46] Sağ Y, Akçael B, Kutsal T. Ternary biosorption equilibria of chromium(VI), copper(II), and cadmium(II) on *Rhizopus arrhizus*. Separation Science and Technology 2002;37: 279-309.

[47] Yap CK, Yazdani M, Abdullah F, Tan SG. Is the High Cu Tolerance of *Trichoderma atroviride* Isolated from the Cu-Polluted Sediment Due to Adaptation? An *In Vitro* Toxicological Study. Sains Malaysiana 2011;40(2): 119-124.

[48] Wang B, Wang K. Removal of copper from acid wastewater of bioleaching by adsorption onto ramie residue and uptake by *Trichoderma viride*. Bioresource Technology 2013;136: 244-250.

[49] Robinson BH, Chiarucci A, Brooks RR, Petit D, Kirkman JH, Gregg PEH, De Dominicis V. The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. Journal of Geochemical Exploration 1997;59: 75-86.

[50] Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PEH. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant and Soil 1998;203: 47-56.

[51] Reeves RD, Schwartz C, Morel JL, Edmondson J. Distribution and metal-accumulating behavior of *Thlaspi caerulescens* and associated metallophytes in France. International Journal of Phytoremediation 2001;3: 145-172.

[52] Bert V, Bonnin I, Saumitou-Laprade P, De Laguérie P, Petit D. Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? New Phytologist 2002;155: 47-57

[53] Singer AC, Bell T, Heywood CA, Smith JAC, Thompson IP. Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant *Alyssum lesbiacum*: evidence of histidine as a measure of phytoextractable nickel. Environmental Pollution 2007;147: 74-82.

[54] Broadhurst CL, Tappero RV, Maugel TK, Erbe EF, Sparks DL, Chaney RL. Nickel and manganese accumulation, interaction and localization in leaves of the Ni hyperaccumulators *Alyssum murale* and *Alyssum corsicum*. Plant and Soil 2008;314: 35-48.

[55] Alford ER, Pilon-Smits EAH, Paschke MW. Metallophytes – a view from the rhizosphere. Plant and Soil 2010;337: 33-50.

[56] Turnau K, Mesjasz-Przybylowicz J. Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. Mycorrhiza 2003;13: 185-190.

[57] Abou-Shanab RAI, Angle JS, Chaney RL. Bacterial inoculants affecting nickel uptake by *Alyssum murale* from low, moderate and high Ni soils. Soil Biology & Biochemistry 2006;38: 2882-2889.

[58] Farinati S, Dal Corso G, Bona E, Corbella M, Lampis S, Cecconi D, Polati R, Berta G, Vallini G, Furini A. Proteomic analysis of *Arabidopsis halleri* shoots in response to the heavy metals cadmium and zinc and rhizosphere microorganisms. Proteomics 2009;9: 4837-4850.

[59] Jankong P, Visoottiviseth P, Khokiattiwong S. Enhanced phytoremediation of arsenic contaminated land. Chemosphere 2007;68: 1906-1912.

[60] Wangeline AL, Reeves FB. Two new *Alternaria* species from selenium-rich habitats in the Rocky Mountain Front Range. Mycotaxon 2007;99: 83-89.

[61] Wangeline AL. Fungi from seleniferous habitats and the relationship of selenium to fungal oxidative stress. PhD thesis. Colorado State University, Fort Collins; 2007.

[62] Brigo L, Ferraio A. Le mineralizzazioni della Liguria Orientale. Rendiconti S.I.M.P. 1974;30: 305-316.

[63] Campana N, Maggi R, Pearce M. Ricerche archeologiche nelle miniere di Libiola e di M. Loreto (Genova). Paleoexpress 1999;3: 9-10.

[64] Carbone C, Di Benedetto F, Marescotti P, Martinelli A, Sangregorio C, Cipriani C, Lucchetti G, Romanelli M. Genetic evolution of nanocrystalline Fe oxide and oxy-

hydroxide assemblages from the Libiola mine (Eastern Liguria, Italy): structural and microstructural investigations. European Journal of Mineralogy 2005;17:785-795.

[65] Marescotti P, Carbone C, De Capitani L, Grieco G, Lucchetti G, Servida D. Mineralogical and geochemical characterisation of open-air tailing and waste-rock dumps from the Libiola Fe-Cu sulphide mine (Eastern Liguria, Italy). Environmental Geology 2008;53: 1613-1626.

[66] Abbate E, Bortolotti V, Principi G. Appennine Ophiolites: a peculiar oceanic crust. Ofioliti Special Issue 'Thethian Ophiolites: 1, western area' 1980;1: 59-96.

[67] Marescotti P, Azzali E, Servida D, Carbone C, Grieco G, De Capitani L, Lucchetti G. Mineralogical and geochemical spatial analyses of a waste-rock dump at the Libiola Fe-Cu sulphide mine (Eastern Liguria, Italy). Environmental Earth Sciences 2010;61: 187-199.

[68] Marescotti P, Roccotiello E, Zotti M, De Capitani L, Carbone C, Azzali E, Mariotti MG, Lucchetti G. Influence of soil mineralogy and chemistry on fungi and plants in a waste-rock dump from the Libiola mine (eastern Liguria, Italy). Periodico di Mineralogia 2013;82: 141-162.

[69] Roccotiello E, Zotti M, Mesiti S, Marescotti P, Carbone C, Cornara L, Mariotti MG. Biodiversity in metal-polluted soils. Fresenius Environmental Bulletin 2010;19(10b): 2420-2425.

[70] Mariotti MG. Osservazioni sulle formazioni a *Buxus sempervirens* e a *Genista salzmannii* della Liguria orientale. Memorie della Accademia Lunigianese di Scienze 1994;59: 77-125.

[71] Vagge I. Le garighe a *Genista desoleana* Valsecchi ed *Euphorbia spinosa* L. subsp. *ligustica* (Fiori) Pign. della Liguria orientale (Italia NW). Fitosociologia 1997;32: 239-243.

[72] Zotti M, Di Piazza S, Ambrosio E, Mariotti MG, Roccotiello E, Vizzini A. Macrofungal diversity in *Pinus nigra* plantations in Northwest Italy (Liguria) Sydowia 2013;65(2): 223-243.

[73] Bradshaw AD, Chadwick MJ. The restoration of land. Berkeley: University of California Press; 1980.

[74] Roccotiello E, Serrano HC, Mariotti MG, Branquinho C. Nickel phytoremediation potential of the Mediterranean *Alyssoides utriculata* (L.) Medik. Chemosphere 2015;119: 1372–1378.

[75] Zotti M, Di Piazza S, Roccotiello E, Lucchetti G, Mariotti MG, Marescotti P. Microfungi in highly copper-contaminated soils from an abandoned Fe-Cu sulphide mine: growth responses, tolerance and bioaccumulation. Chemosphere 2014;117: 471-476.

[76] Borovička J, Kotrba P, Gryndler M, Mihaljevič M, Řanda Z, Rohovec J, Cajthaml T, Stijve T, Dunn CE. Bioaccumulation of silver in ectomycorrhizal and saprobic macro-

fungi from pristine and polluted areas. Science of the Total Environment 2010;408: 2733-2744.

[77] Gomes NCM, Rosa CA, Pimentel PF, Mendonça-Hagler LCS. Uptake of free and complexed silver ions by different strains of *Rhodotorula mucilaginosa*. Brazilian Journal of Microbiology. 2002;33: 62-66.

[78] Borovička J, Randa Z, Jelinek E, Kotrba P, Dunn CE. Hyperaccumulation of silver by *Amanita strobiliformis* and related species of the section *Lepidella*. Mycological research 2007;III: 1339-1344.

[79] Schwitzguébel JP, Kumpiene J, Comino E, Vanek T. From green to clean: a promising and sustainable approach towards environmental remediation and human health for the 21st century. Agrochimica 2009;53: 209-237.

[80] Harris J. Microbiological tools for monitoring and managing restoration progress. In: Moore HM, Fox HR, Elliot S. (eds.) Land Reclamation. Lisse: A.A. Balkema Publishers; 2003. p201-206.

[81] Miransari M. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnology Advances 2011;29: 645-653.

[82] Audet P, Charest C. Allocation plasticity and plant-metal partitioning: Meta-analytical perspectives in phytoremediation. Environmental Pollution 2008;156: 290-296.

[83] Janouskova M, Pavlıkova D, Vosatka M. Potential contribution of arbuscular mycorrhiza to cadmium immobilisation in soil. Chemosphere 2006;65: 1959-1965.