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Zinc tolerance and accumulation capability by *Penicillium* strains isolated from Neutral Mine Drainage (NDM) colloidal precipitates

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Abstract

Zinc, at low concentration, is one of the oligo elements essential for life. As well as many others metals, high environmental Zn concentrations are toxic for several organisms and can compromise their lifecycle. Neutral (or alkaline) Mine Drainage (NMD) are metal-rich solutions with near-neutral pH values, often generated by weathering of sulphide minerals (mainly Zn and Pb sulphides), circulating in mining areas. As this phenomenon favours the dispersion of metals and contributes to the rising of concentration of toxic elements in the groundwaters, NMD threatens the surrounding environment. The effectiveness of autochthonous fungal strains in bioremediation process of ecotoxic metal is today well founded. We investigated the Zn tolerance and accumulation capability of four *Penicillium* strains (two *P. janthinellum*, one *P. olsonii*, and one *P. waksmanii*) isolated from an NMD environment. The experimental results showed a positive growth response of all strains at 750 ppm of Zn, while only two strains (*P. olsonii* and *P. waksmanii*) were able to grow at 1500 ppm of Zn. Further tests showed a bioaccumulation capability by *P. olsonii* strains up to 8600 ppm. These data, reporting the bioaccumulation capabilities of fungal species that have not been studied previously, are a further confirmation of the potential usefulness of autochthonous microfungi in bioremediation of heavy metals, and highlight the need of increase the number of tested strains.

Keywords: extreme environments; woodwardite; microfungi; mycoextraction; Penicillium olsonii

Riassunto

Lo zinco, in basse concentrazioni, è uno degli oligoelementi essenziali per la vita. Così come molti altri metalli, alte concentrazioni di Zn nell'ambiente risultano tossiche per diversi organismi, compromettendone talvolta il ciclo vitale. Il drenaggio Neutro (o alcalino) di miniera (NMD), caratterizzato da alte concentrazioni di metalli e valori di pH prossimi alla neutralità, è un particolare processo generato dall'alterazione di minerali, spesso di solfuri, (in molti casi Zn e Pb), in ambiente di miniera. Questo fenomeno favorisce la dispersione dei metalli e contribuisce all'aumento della concentrazione di questi elementi tossici nelle acque circolanti minacciando l'ambiente circostante. Oggi è ormai assodata l'efficacia dei funghi (in particolare di ceppi autoctoni) nei processi di biorimedio di metalli ecotossici. Nel nostro lavoro abbiamo studiato la capacità di tolleranza e accumulo di Zn in quattro ceppi di *Penicillium* (due *P. janthinellum*, un *P. olsonii* e un *P. waksmanii*) isolati da un ambiente soggetto a NMD. I risultati ottenuti hanno mostrato una risposta di crescita positiva di tutti i ceppi in presenza di 750 ppm di Zn, mentre solo due ceppi (*P. olsonii* e *P. waksmanii*) sono stati in grado di crescere a concentrazioni pari a 1500 ppm di Zn. Inoltre, ulteriori prove hanno dimostrato una capacità di bioaccumulo del ceppo di *P. olsonii* fino a 8600 ppm. Questi dati, evidenziando la capacità di bioaccumulo di specie poco studiate in precedenza, sono un'ulteriore conferma della potenziale utilità dei microfunghi autoctoni nel biorisanamento di terreni contenenti metalli pesanti.

Parole chiave: ambienti estremi; woodwardite; microfunghi; micoestrazione; Penicillium olsonii

1. Introduction

The weathering of sulphide minerals can generate solutions with low pH values, and a high amount of dissolved sulphates and metals of environmental concern. The formation of these acid solutions is typical of sulphide ore mining environments, and it is known as Acid Mine Drainage (AMD) (Blowes *et al.*, 2003). When mine waters have near neutral pH, the definition of Neutral (or alkaline) Mine Drainage (NMD) is applied (Scharer *et al.*, 2000). Although high concentrations of dissolved metals, especially Zn and Pb, can be reached in these waters, this phenomenon has received less attention than AMD. Nonetheless, these waters represent a threat to the quality of the surrounding aquatic system (Iribar, 2004; Frau *et al.*, 2015). In NMD from Cu mines one of the most important secondary mineral in the precipitates is woodwardite, $(Cu_{1-x}Al_x)(OH)_2(SO_4)_{x/2}{\cdot}nH_2O$, a member of the hydrotalcites group (Dinelli *et al.*, 1998; Tumiati *et al.*, 2008; Carbone *et al.*, 2013). This mineral forms a complete isomorphic series with zincowoodwardite (Witzke and Raade, 2000), thus Cu can be substituted by Zn, making woodwardite a good sink for this element. The precipitation of colloidal particles generally produces a positive effect on the water quality (Sracek *et al.*, 2011), although colloids can be transported for long distances (Schemel *et al.*, 2000), or become a secondary source of contaminants (Consani *et al.*, 2017). For these reasons, mine waters and related precipitates have a major role in increasing the concentrations of ecotoxic elements in surrounding freshwaters and soils, and therefore are a potential risk for the environment and consequently for human health.
Investigating the capability of some organisms to survive in these inhospitable conditions is an intriguing task, since the same organisms could constitute possible agents able to remediate the environment. Recently, among the organisms that can survive in polluted areas, fungi have shown a huge potential in bioremediation of organic and inorganic pollutants (Gadd, 2010; Aytar *et al.*, 2014; Jakubiak *et al.*, 2014; Zotti *et al.*, 2014; Pozdnyakova *et al.*, 2006). In particular, as concern heavy-metal mycoremediation (bioremediation using fungi), several fungal strains were tested with various metals, such as V (Ceci *et al.*, 2012), Cu (Yap *et al.*, 2011; Wang and Wang, 2013), Ni (Cecchi *et al.*, 2017a), Ag (Cecchi *et al.*, 2017b), Pb (Zucconi *et al.*, 2003), and Zn (Fan *et al.*, 2008). Surely, among fungi, *Penicillium* is one of the main and more common genera. This genus includes more than 300 widespread species, characterised by a wide ecological range. *Penicillium* species have been isolated from various environments and substrata such as soil, freshwater and seawater, food stocks, etc (Domsch *et al.*, 2007; Pitt and Hocking, 2009). Owing to this ecophysiological flexibility, *Penicillium* species have been often exploited for industrial and biotechnological purposes (Adrio and Demain, 2003). For the same reasons, as shown by Leitão (2009), several studies in the last few years have highlighted how *Penicillium* species could be fruitfully exploited for mycoremediation purposes.
In this context, several studies were carried out in order to assess the microbial flora occurring in AMD environments (Colmer and Hinkle, 1947; Das *et al.*, 2009; Bond *et al.*, 2000; Zotti *et al.*, 2014);

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conversely, only recently few authors started to study the microbial communities (mainly bacterial) in NMD environments (Lindsday *et al.*, 2009; Majzlan *et al.*, 2011; Dockrey *et al.*, 2014).
Our work investigated the Zn tolerance and accumulation capability of four *Penicillium* strains (belonging to three different species) isolated from a NMD environment. These strains were mostly recurrently isolated during a survey (unpublished data) carried out in the Libiola Cu-Fe sulphide mine located near the Sestri Levante town (Liguria, North-Western Italy). In this precipitate, Zn is one of the major constituents (Carbone *et al.*, 2013), and reaches high concentrations between 0.5% to 2%: for this reason we focused our study on this element.
More specifically, the goal of our work was to verify the *in vitro* tolerance and bioaccumulation capability of native *Penicillium* strains on two different substrata: greenish-blue colloidal precipitate and $ZnSO_4 \cdot 7H_2O$ enriched media (MUD-MEA and Zn-MEA). The achieved results propel us to deepen our research in order to find the best conditions for exploiting the capability of these strains.

2. Materials and methods

2.1. Study area

The study area was located at the Libiola mine site (lat. 44°17’18’’ N, long. 9° 26’42’’ E), located about 8 km NE of Sestri Levante (Eastern Liguria, Italy). The mine area extends over an area of about 4 km^2 within the basin of the Gromolo torrent (Fig. 1).

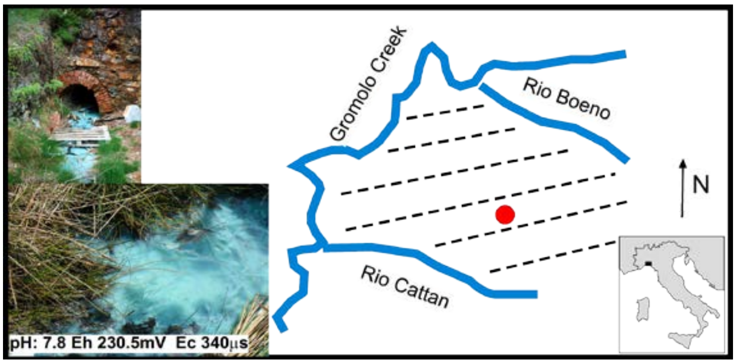

Fig. 1. Geographical location of the mining area in Italy (bottom right); position of mining area (red dot), nearest streams (blue lines), area subject to NMD (dotted area), pictures of two out flow streams and related parameters (pH, Eh, Ec).
Fig. 1. Localizzazione geografica dell’area di miniera rispetto al territorio italiano (in basso a destra); posizione dell’area di miniera (punto rosso), dei corsi d’acqua circostanti e dell’area soggetta NMD (area tratteggiata). Nelle immagini sono presenti due tratti dei corsi d’acqua con i relativi parametri (pH, Eh, Ec).

The climate of the area is classified as Mediterranean, is characterised by an average temperature during the year of 15 °C (seasonal average 5 °C in Winter, 10.5 °C in Spring, 19 °C in Summer, 13 °C in Autumn) and rainfall during the year range between 1100 and 1600 mm/year. The mineralisation is a Volcanogenic Massive Sulphide (VMS) deposit occurring, as massive lenses and stockwork mineralised veins, within the basalts associated with the Internal Ligurian Ophiolites. Pyrite and chalcopyrite are the dominant sulphide minerals in the site, with minor amounts of sphalerite and pyrrhotite, and gangue minerals are primarily composed by quartz and chlorite with minor amounts of calcite (Zaccarini and Garuti, 2008). The host rocks are mainly represented by basalts, serpentinites, and to a lesser extent by ophiolitic breccias (Ferrario and Garuti, 1980). At one site inside the mining area, the Margherita adit, the precipitation of greenish-blue precipitates takes place (Dinelli et al., 1998; Carbone et al., 2013). Seasonal variations led to pH oscillations from 6.3 to 7.7, and do not affect the chemical composition of the precipitates, which is always composed mainly by Al, Si, and Cu. The amount of Zn varies from 0.5% to 2% (Carbone et al., 2013).

2.2 Colloidal precipitates sampling

On the whole five samples of colloidal precipitates by NMD were collected at the Margherita mine adit in April 2015. More precisely one sample was used for mineralogical characterization, and the remaining four were processed for microfungal isolation. The sampling was performed with a plastic syringe, then the solid + liquid suspension was stored in polypropylene bottles, filtered through 16 µm filters and air-dried. The pH, Eh and Electrical Conductivity (EC) of related waters were measured in the field using a portable pH-metre "PH330i" (WTW, Germany), equipped with "SenTix41" and "SenTix ORP" electrodes and a "Eutech XS COND6+" conductivity metre.

2.3 Isolation of native fungal strains

Mycological analysis was performed in August 2013. Four samples were collected sucking 50 ml of precipitates with a sterile syringe, the samples were put in falcon tubes and stored in a thermal bag at 6°C until the arrival to the laboratory; then the samples were stored in a refrigerator at 6°C.
In order to select vial strains, the mycological analysis was performed following a dilution plate method modified from Gams (1984), as follows, within 2 days from the sampling.
The isolation of the strains was performed plating 1 ml of the precipitates both pure and diluted (1:10, $1:10^{-2}$, $1:10^{-3}$) on two different media (Rose Bengal Sigma-Aldrich® and Malt Extract Agar added with chloramphenicol Sigma-Aldrich®). A total of 16 Petri dishes (9 cm Ø) were inoculated for each sample. The dishes were incubated at 24°C for 7 days. Given the high level of toxicity of the matrix, the dishes were monitored daily for three more weeks, in order to isolate also the strains whose growth was slowed down by the high heavy metal concentrations.
The strains isolated were identified at genus level by conventional mycological methods by observing their micro- and macromorphological features and considering the different trophic and physiological requirements. In order to identify species belonging to genus *Penicillium*, molecular analyses ß-tubulin gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson, 1995). The isolated strains were conserved in the culture collection of the Mycological Laboratory of DISTAV (University of Genoa, Italy).

2.4 Tolerance and bioaccumulation tests

Firstly, the ability of the strains to survive and germinate at high Zn concentration was tested on 750 and 1500 ppm Zn enriched Malt Extract Agar (Zn-MEA750 and Zn-MEA1500). The media were prepared by adding 3.706 and 7.412 g of $ZnSO_4{\cdot}7H_2O$, respectively, to a standard MEA (1 L of distilled water, 20 g glucose, 20 g malt extract, 1 g peptone, 20 g agar), and autoclaving for 10 minutes at 120°C. In order to separate mycelial biomass (essential for subsequent analysis) from the Zn-MEA, a 9 cm diam. Cellophane Membrane Backing Biorad® disc was placed in each Petri dish. A conidial suspension ($5x10^8$ conidia/mL) was obtained by adding 20 ml of deionised water + 10 µL of tween 80 to 14-d-old cultures and by scraping the surface, The conidial suspension was then inoculated on Zn-MEA 9 cm diam. Petri dishes, and incubated at 24°C for 21 days. The colonies were then separated from the media, and analysed with a Tescan Vega scanning electron microscope equipped with an EDS (Energy Dispersive Spectrometry) probe (EDAX manufacturer; see paragraph 2.5) to obtain a first evaluation of the presence and distribution of Zn in the mycelial biomass.
The bioaccumulation capability of the strain that showed the higher Zn signal during the EDS analysis was tested *in vitro*. The bioaccumulation test was performed on Zn-MEA750 (see above) and MUD-

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MEA (1 L greenish-blue precipitates and water suspension, 20 g glucose, 20 g malt extract, 1 g peptone 20 agar). As done during the tolerance test, a 9 cm Cellophane Membrane Backing Biorad disc was placed in each Petri dish. A $5x10^8$ per ml conidia suspension was prepared as described above. The conidial suspension was inoculated on MEA (control), MEA enriched with $ZnSO_4{\cdot}7H_2O$ and MEA-Mud dishes. The mycelium of 14-days culture was analysed by Tescan Vega scanning electron microscope equipped with an EDS (Energy Dispersive Spectrometry) probe in order to verify the heavy metal uptake capacity of the mycelium.

2.5 Instrumental analyses

The mineralogical characterisation of the natural colloidal precipitate samples was determined by X-Ray Diffraction (XRD) with Co Kα radiation (current 20 mA, voltage 40 kV). The sample of colloidal precipitates was ground with an agate mortar and pestle, and mounted on zero-background silicon plates. The analysis was performed scanning the sample between 5 and 80° 2θ at a scan rate of 5°/min.

The major element composition was investigated with Inductively Coupled Plasma-Emission Spectrometry ICP-ES on 1 g of sample digested in hot Aqua Regia, while minor and trace elements were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis of a 0.5 g samples after modified Aqua Regia digestion at Bureau Veritas Mineral Laboratories (Canada).

3. Results and discussion

3.1 Mineralogical characterisation

The XRD results (Fig. 2) show that the greenish-blue precipitates consist mainly of allophane [(Al2O3)(SiO2)1.3-2·2.5-3H2O], an amorphous hydrated Si-Al aluminosilicate, and woodwardite [(Cu1-xAlx)(OH)2(SO4)x/2·nH2O] or hydrowodwardite, which differs from woodwardite only for its higher water content (Witzke, 1999).

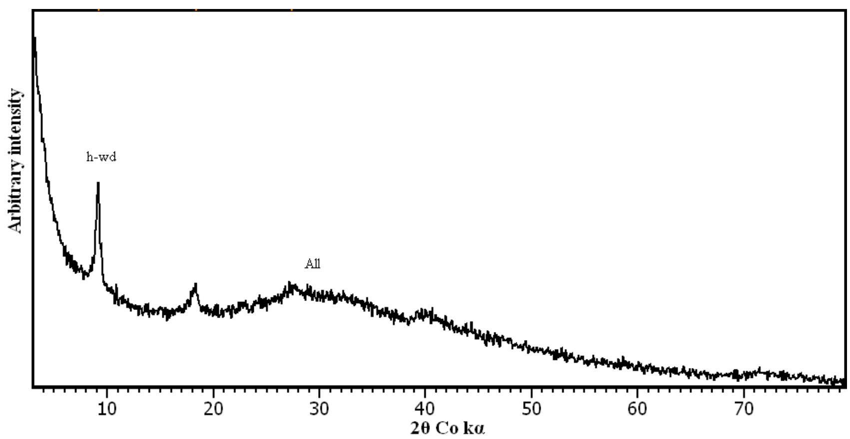

Fig. 2. XRD diagram of the sample (h-wd= hydrowoodwardite; All= allophane).
Fig. 2. Diagramma XRD del campione (h-wd= hydrowoodwardite; All= allofane).

From the bulk chemistry of the sample (Fig. 3) it is evident that the major constituents of the precipitates are Al and Si (11.84% and 9.57%, respectively), followed by Cu (8.14%), S (1.37%), and Fe (1.16%). The concentration of Zn in the sample is 5.729 mg kg^{-1}.

The colloidal precipitate composition shows a high concentration of several toxic metals (see Fig. 3), which are detrimental to living organisms; therefore, this colloidal precipitate can be considered an extreme environment.

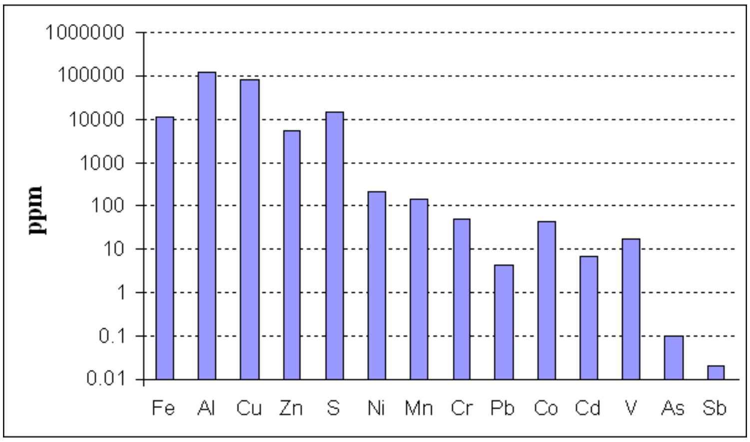

Fig. 3. Main chemical compounds of the sample.
Fig. 3. Principali componenti chimici presenti nel campione.

3.2 Tolerant/bioaccumulant strains

As previously reported, we opted to use the native strains to test the Zn tolerance and accumulation capability, since these strains are more likely to survive to the high metal concentrations. In previous works we displayed that autochthonous microfungi evolved adaptation strategies against limiting factors and/or pollutants (Zotti et al., 2014; Cecchi et al., 2017 a,b), and for this reasons are the best candidate to show a positive growth response on contaminated media in laboratory test. However, in our case, as shown in Table 1, the growth response test (G) performed using the 4 most frequent strains present in this NMD area highlight different threshold for different species. Observing the Table 1, it is clear that all strains are able to germinate at 750 ppm of Zn, suggesting that the propagules of these two species have surely adapted to this environment without completely loosing the germination capability. However, only two strains (P. olsonii and P. waksmanii) are able to germinate with Zn concentration of 1500 ppm.

Tab. 1. Positive (+) or negative (-) growth response (G) of the selected strains at 750 and 1500 ppm.
Tab. 1. Risposta di crescita (G) positiva (+) o negativa (-) dei ceppi saggiati a concentrazioni di 750 e 1500 ppm.

Strain	Closest match (accession)	Identities	G 750	G 1500
1	*Penicillium janthinellum* Biourge (KJ608359.1)	99%	+	-
2	*Penicillium janthinellum* Biourge (DQ486649.1)	99%	+	-
3	*Penicillium olsonii* Bainier & Sartory (AY674443.1)	99%	+	+
4	*Penicillium waksmanii* K.M. Zalessky (JQ965078.1)	99%	+	+

In spite of the fact that 4 tolerant strains were positive at the 750 ppm G test, and 2 at the 1500 ppm G test, the following accumulation screening performed by SEM and EDS probe on the fungal biomass samples shows that only *P. olsonii* biomass grown on Zn-MEA750 is able to accumulate Zn (see Fig. 4

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and 5). The SEM image in figure 4 shows a well developped conidiophores of the P. olsonii strain on Zn-MEA750; while the results of EDS analysis on the mycelium of the same strain are reported in the figure 5 showing a peak of Zn in the right part of the graphic. We must take into account that this EDS analysis is a semi quantitative measure, therefore it confirms the presence of at least 1% of Zn in the fungal biomass, but cannot provide the exact amount.

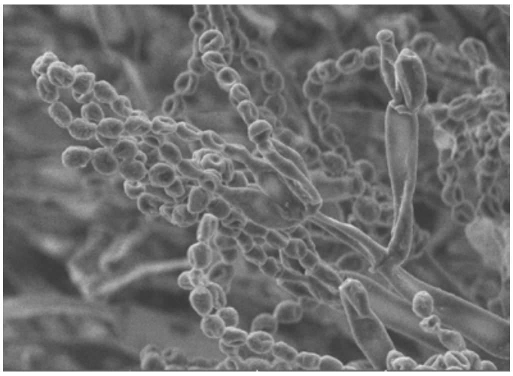

Fig. 4. Scanning Electron Microscope image of a conidiophore of *P. olsonii* grown on Zn-MEA750.
Fig. 4. Immagine al microscopio elettronico a scansione di un conidioforo di *P. olsonii* cresciuto su Zn-MEA750.

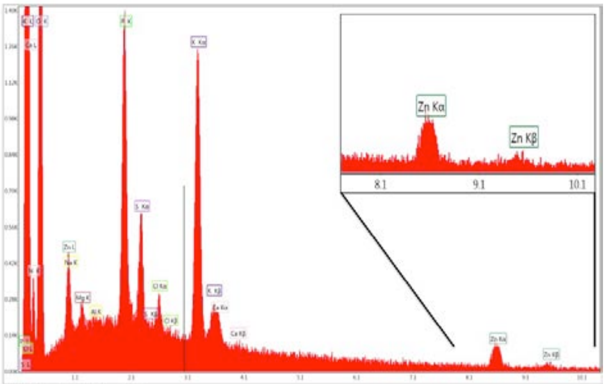

Fig. 5. EDS analysis of conidiophores of *P. olsonii* grown on Zn-MEA750, in the frame is present the enlargment of the Zn peaks.
Fig. 5. Immagine relativa all'analisi EDS dei conidiofori di *P. olsonii* cresciti su Zn-MEA750, nel riquadro è presente un ingrandimento relativo ai picchi di rilevazione di Zn.

For this reason the following bioaccumulation tests with a P. olsonii strain on Zn-MEA750 and MUD-MEA were carried out to quantify the exact amount of Zn bioabsorbed in the biomass. After the incubation period, the ICP-MS analysis on mycelial biomass performed at the ASL Scandinavia AB Lab (Luleå, Sweden) confirms, as shown in table 2, the Zn uptake capability of this P. olsonii strain, with a concentration of 1520 ppm (from MEA-Mud) and 8630 ppm (from Zn-MEA750).

Tab. 2. ICP-MS analysis on *P. olsonii* biomass and related medium on MEA, MEA+$ZnSO_4 \cdot 7H_2O$, and MEA+Mud
Tab. 2. Analisi ICP-MS del contenuto in Zn nella biomassa di *P. olsonii* crescito sui differenti terreni (MEA, MEA+ZnSO4·7H2O, and MEA+Mud).

Medium	Sample	Zn (mg/kg)
MEA	*P. olsonii* biomass	b.d.l.
Zn-MEA1500	*P. olsonii* biomass	8630
MEA-Mud	*P. olsonii* biomass	1520

Penicillium olsonii is well known as agent of food spoilage, and in the past it has been tested as a potential antagonist of the plant pathogen *Rhizoctonia solani* (Demirci *et al.*, 2011), but till now has not been reported in literature to accumulate heavy metals. Our results represent the first report of *P. olsonii* metal tolerance and bioaccumulation capability, and demonstrate that harsh environmental conditions make a selection which can favour the evolution of strain-specific strategies in fungi.
These results invite us to deepen this investigation, in order to increase ì) the number of heavy metal tolerant strains ìì) the knowledge of the heavy metals uptake patterns of native filamentous fungi.

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References

Adrio J.L., Demain A.L. (2003). Fungal biotechnology. International Microbiology 6(3):191-199.

Aytar P., Gedikli S., Buruk Y., Cabuk A., Burnak N. (2014). Lead and nickel biosorption with a fungal biomass isolated from metal mine drainage: Box–Behnken experimental design. International Journal of Environmental Science and Technology 11:1631-1640.

Blowes D.W., Ptacek C.J., Jambor J.L., Weisener C.G. (2003) The geochemistry of Acid Mine Drainage. Treatise on Geochemistry. Vol. 9 p.149-204. Eds. H.D. Holland and K.K. Turekian. pp. 612. ISBN 0-08-043751-6. Elsevier.

Bond P.L., Druschel G.K., Banfield J.F. (2000). Comparison of Acid Mine Drainage Microbial Communities in Physically and Geochemically Distinct Ecosystems. Applied and Environmental Microbiology 66 (11): 4962-4971.

Carbone C., Dinelli E., Marescotti P., Gasparotto G., Lucchetti G. (2013). The role of AMD secondary minerals in controlling environmental pollution: indications from bulk leaching tests. Journal of Geochemical Exploration 132:188–200.

Cecchi G., Roccotiello E., Di Piazza S., Riggi A., Mariotti M.G., Zotti M. (2017a). Assessment of Ni accumulation capability by fungi for a possible approach to remove metals from soils and waters. Journal of Environmental Science and Health Part B. 52:1–5.

Cecchi G., Marescotti P., Di Piazza S., Zotti M., (2017b). Native fungi as metal remediators: silver myco-accumulation from metal contaminated waste-rock dumps (Libiola Mine, Italy). Journal of Environmental Science and Health B?. 52: 191–195.

Ceci, A., Maggi, O., Pinzari, F., Persiani, A.M. (2012). Growth responses to and accumulation of vanadium in agricultural soil fungi. Applied Soil and Ecology 58: 1-11.

Colmer A.R. and Hinkle M.E. (1947) The role of Microorganisms in Acid Mine Drainage. Science 106 (2751): 253-256.

Consani S., Carbone C., Dinelli E., Balić-ŽunićT., Cutroneo L., Capello M., Salviulo G., Lucchetti G. (2017). Metal transport and remobilisation in a basin affected by acid mine drainage: the role of ochreous amorphous precipitates. Environmental Science and Pollution Research 24: 15735-15747.

Das B.K., Roy A., Koschorreck M., Mandal S.M., Wendt-Potthoff K., Bhattacharya J. (2009) Occurrence and role of algae and fungi in acid mine drainage environment with special reference to metals and sulphate immobilization. Water Research 43: 883-894.

Demirci, E., Dane, E., & Eken, C. (2011). In vitro antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. Turkish Journal of Biology 35(4): 457-462.

Dinelli E., Morandi N., Tateo F. (1998). Fine grained weathering products in waste disposal from two sulphide mines in the northern Appennines, Italy. Clay Mineral 33: 423-433.

DOI: https://doi.org/10.6092/issn.2531-7342/8651

Dockrey J.W., Lindsay M.B.J., Mayer K.U., Beckie R.D., Norlund K.L.I., Warren L.A., Southam G. (2014). Acidic Microenvironments in Waste Rock Characterized by Neutral Drainage: Bacteria–Mineral Interactions at Sulfide Surfaces. Minerals 4 (1): 170-190.

Domsch K.H., Gams W., Anderson T.-H. (2007). Compendium of soil fungi, Second Edition. 1-672 IHW-Verlag, Eching

Fan T., Liu Y., Feng B., Zeng G., Yang C., Zhou M., Zhou M., Tan Z., Wang X. (2008). Biosorption of cadmium(II), zinc(II) and lead(II) by *Penicillium simplicissimum*: Isotherms, kinetics and thermodynamics. Journal of Hazardous Materials, 160 (2–3): 655–661.

Ferrario A. and Garuti G. (1980). Copper deposit in the basal breccias and volcano sedimentary

sequences of the Eastern Ligurianophiolites (Italy). Mineralium Deposita 15: 291-303.

Frau F., Medas D., Da Pelo S., Wanty R., Cidu R. (2015). Environmental effects on the aquatic system and metal discharge to the Mediterranean Sea from a Near-Neutral zinc-ferrous sulfate Mine Drainage. Water, Air and Soil Pollution 226: 55.

Gadd G.M. (2010). Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156: 609-643.

Glass N.L., Donaldson G.C. (1995) Development of Primer Sets Designed for Use with the PCR To Amplify Conserved Genes from Filamentous Ascomycetes. Applied and Environmental Microbiology 61:1323– 1330.

Iribar V. (2004). Origin of neutral mine water in flooded underground mines: an appraisal using geochemical and hydrogeological methodologies. In Mine Water 2004 – Process, Policy, and Progress, Proceedings of International Mine Water Association Symposium 2004, Newcastle upon Tyne, United Kingdom 20–25 September 2004. (pp. 260–274) ISBN: 978-1-62276-985-8.

Jakubiak M., Giska I., Asztemborska M., Bystrzejewska-Piotrowska G. (2014). Bioaccumulation and biosorption of inorganic nanoparticles: factors affecting the efficiency of nanoparticle mycoextraction by liquid-grown mycelia of *Pleurotus eryngii* and *Trametes versicolor*. Mycological Progress 13: 525-532.

Leitão A.L. (2009). Potential of *Penicillium* Species in the Bioremediation Field. International Jourenal of Environmental Research and Public Health 6(4): 1393-1417.

Lindsay M.B.J., Condon P.D., Jambor J.L., Lear K.G., Blowes D.W., Ptacek C.J. (2009). Mineralogical, geochemical, and microbial investigation of a sulfide-rich tailings deposit characterized by neutral drainage. Applied Geochemistry 24 (12): 2212-2221.

Majzlan J., Lalinská B., Chovan M., Bläß U., Brecht B., Göttlicher J., Steininger R., Hug K., Ziegler S., Gescher J. (2011). A mineralogical, geochemical, and microbiogical assessment of the antimony- and arsenic-rich neutral mine drainage tailings near Pezinok, Slovakia. American Mineralogist 96: 1-13.

Pitt J.I. and Hocking A.D. (2009). Fungi and food spoilage. Vol. XV. Springer, New York, pag. 519. ISBN: 978-0-387-92206-5

Pozdnyakova N.N., Rodakiewicz-Nowak J., Turkovskaya O.V., Haber J. (2006). Oxidative degradation of polyaromatic hydrocarbons catalyzed by blue laccase from *Pleurotus ostreatus* D1 in the presence of synthetic mediators. Enzyme and Microbial Technology 39: 1242-1249.

Scharer J.M., Pettit C.M., Kirkaldy J.L., Bolduc L., Halbert B.E., Chambers D.B. (2000). Leaching of metals from sulphide mines wastes at neutral pH. In: ICARD 2000 Proc from the Fifth Intl Conf on Acid Rock Drainage, Society for Mining, Metallurgy, and Exploration, Inc. vol. 1. pp 191-201

Schemel L.E., Kimball B.A., Bencala K.E. (2000). Colloid formation and metal transport through two mixing zones affected by acid mine drainage near Silverton, Colorado. Applied Geochemistry 15: 1003-1018.

Sracek O., Filip J., Mihaljevič M., Křibek B., Majer V., Veselovsky F. (2011). Attenuation of dissolved metals in neutral mine drainage in the Zambian Copperbelt. Environmental Monitoring and Assessment 172: 287-299.

Tumiati S., Godard G., Masciocchi N., Martin S., Monticelli D. (2008). Environmental factors controlling the precipitation of Cu-bearing hydrotalcite-like compounds from mine waters. The case of the "*Eve verda*" spring (Aosta Valley, Italy). European Journal of Mineralogy 20: 73-94.

DOI: https://doi.org/10.6092/issn.2531-7342/8651

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

Witzke T. (1999). Hydrowoodwardite, a new mineral of the hydrotalcite group from Königswalde near Annaberg, Saxony/Germany and other localities. Neues Jahrbuch für Minerralogie – Monatshefte 2(2):75-86

Witzke T. and Raade G. (2000) Zincowoodwardite, $[Zn_{1-x}Al_x(OH)_2][(SO_4)_{X/2}(H_2O)_n]$, a new mineral of the hydrotalcite group. Neues Jahrbuch für Minerralogie – Monatshefte, 10: 455-465.

Yap C.K., Yazdani M., Abdullah F., Tan S.G. (2011). Is the high Cu tolerance of *Trichoderma* atroviride isolated from the Cu-polluted sediment due to adaptation? an *in vitro* toxicological study. Sains Malaysiana 40 (2): 119-124.

Zaccarini F. and Garuti G. (2008). Mineralogy and chemical composition of VMS deposits of northern Apennine ophiolites, Italy: evidence for the influence of country rock type on ore composition. Mineralogy and Petrology 94: 61-83.

Zotti M., Di Piazza S., Roccotiello E., Lucchetti G., Mariotti M.G., Marescotti P. (2014). Microfungi in highly copper-contaminated soils from an abandoned Fe– Cu sulphidemine. growth responses, tolerance and bioaccumulation. Chemosphere 117: 471-476.

Zucconi, L., Ripa, C., Alianiello, F., Benedetti, A., Onofri, S., (2003). Lead resistance, sorption and accumulation in a *Paecilomyces lilacinus* strain. Biology and Fertility of Soils 37: 17-22.