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Phytoremediation potential of *Arundo donax* (Giant Reed) in contaminated soil by heavy metals

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ABSTRACT

Soil pollution from heavy metals poses a serious risk for environment and public health. Phytoremediation is an eco-friendly and cheaper alternative compared to chemical-physical techniques.

We carried out *in vitro* tests where three microorganisms *Trichoderma harzianum*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* were exposed to eight different heavy metals (one metal at a time) in order to evaluate resistance, growth and bioaccumulation capability for each metal (Ni, Cd, Cu, V, Zn, As, Pb, Hg).

Taking into account the natural characteristics of *T. harzianum*, (resistance to environmental stress, resistance to pathogenic fungi, ability to establish symbiotic relationships with superior green plants) and the good bioaccumulation capacity for V, As, Cd, Hg, Pb shown after *in vitro* tests, it was chosen as a microorganism to be used in greenhouse tests. Controlled exposure tests were performed in greenhouse, where *Arundo donax* and mycorrhized *Arundo donax* with *T. harzianum* were exposed for 7 months at two different doses (L1 and L2) of a heavy metal mix, so as to assess whether the symbiotic association could improve the bioaccumulation capability of the superior green plant *A. donax*.

Heavy metals were determined with ICP-MS. The average bioaccumulation percentage values of *A. donax* for L1 and L2 were, respectively: Ni (31%, 26%); Cd (35%, 50%); Cu (30%, 35%); As (19%, 27%); Pb (18%, 14%); Hg (42%, 45%); V (39%, 26%); Zn (23%, 9%). The average bioaccumulation percentage values of mycorrhized *A. donax* with *T. harzianum* for L1 and L2 were, respectively: Ni (27%, 38%); Cd (44%, 42%); Cu (36%, 29%); As (17%, 23%); Pb (37%, 54%); Hg (44%, 60%); V (16%, 20%); Zn (14%, 7%).

A. donax showed the highest BAF (bioaccumulation factor) for Cd (0.50), Cu (0.35), As (0.27) and Hg (0.45) after exposure to L2; mycorrhized *A. donax* with *T. harzianum* showed the highest BAF for Ni (0.38), Cd (0.42), Pb (0.54) and Hg (0.60) after exposure to L2. *A. donax* showed the highest TF (translocation factor) values for Cd (0.28) and Hg (0.26) after exposition at L1 and L2 respectively; *A. donax* mycorrhized with *T. harzianum* showed the highest TF values for Cd (0.70), As (0.56), V (0.24), Pb (0.18) after exposition at L2, and Zn (0.30) after exposition at L1.

Our study showed a good growth capability in contaminated soils and a good bioaccumulation capability of heavy metals, both for *A. donax* and mycorrhized *A. donax* with *T. harzianum*. Furthermore, for three metals (Ni, Pb and Hg) the bioaccumulation capability was improved by the symbiosis of *T. harzianum* with *A. donax*. So, these results proved the suitability both for *A. donax* and mycorrhized *A. donax* with *T. harzianum* for phytoremediation processes.

1. Introduction

Industrialization, agriculture, urbanization and other anthropogenic activities increased in the last century (Dehghani et al., 2017) with large releases into the environment of inorganic and organic compounds (e.g. heavy metals, hydrocarbons, organic solvents, radioactive waste, etc.) [Copat et al., 2012; Ali et al., 2017; Ferrante et al., 2018; Saleh et al., 2019]. Environmental pollution is an urgent issue for the

Abbreviations: Ni, nickel; Cd, cadmium; Cu, copper; As, arsenic; Zn, zinc; Pb, lead; V, vanadium; Hg, mercury

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public health, because the presence of pollutants in environmental matrices represent a serious risk for the onset of several human diseases. Soil, air and water pollution are an actual challenge for the public health policy due to the high remediation costs of the environment and for the technological limitations [Ali et al., 2017; Cristaldi et al., 2017; Ashraf et al., 2019] still current, which do not make possible to reduce completely the human exposure to contaminants [Cristaldi et al., 2017; Bahrami et al., 2018; Dehghani et al., 2017; Zuccarello et al., 2018; Daiber et al., 2019; Fiore et al., 2019; Zuccarello et al., 2019a; Zuccarello et al., 2019b].

Soil represent the final acceptor for various pollutants [Jiang et al., 2015; Tahir et al., 2015; Cristaldi et al., 2017]. Soil contamination is due to direct oil and wastewaters spills, fertilizers and pesticides use, accidental or natural introduction of organic and inorganic compounds (fires, volcanic eruptions, etc.), air pollutants transferred through the dry and wet deposition [Conte et al., 2016; Copat et al., 2018; Ferrante et al., 2018; Fakhry et al., 2018; Wu et al., 2019]. To safeguard the public health need to recovery contaminated sites to ensure a better health for the current population and the environmental preservation for the future generations.

Today it is possible to use some less expensive remediation methodologies that involve the use of living organisms such as plants, bacteria, fungi for the recovery of environmental matrices, in particular soil and water [Nsanganwimana et al., 2014; Cristaldi et al., 2017; Sarma et al., 2019].

Heavy metals are persistent, non-biodegradable and high density elements (> 5 g cm^{-3}). They can enter into the body through ingestion [Conte et al., 2005; Copat et al., 2012; Dadar et al., 2016; Copat et al., 2018; Fakhry et al., 2018] and inhalation [Ferrante et al., 2018; Oguri et al., 2018; Dehghani, 2017], so they can pose a serious threat to human life [Kos et al., 2003; Jing et al., 2007; Chauhan et al., 2015; Bahrami et al., 2018]. Although heavy metals are natural constituents of the earth's crust, their presence (e.g. Hg, Cd, Pb, As) can cause toxic effects on living organisms also at trace levels [Ferrante and Oliveri Conti, 2015; Copat et al., 2013; Tchounwou et al., 2012; Ali et al., 2017; Copat et al., 2018].

A valid eco-friendly method for soil remediation against heavy metals could be the bioremediation. Bioremediation technique provides the biological removal or degradation of pollutants under controlled conditions to get concentrations below regulatory limits [Kumar et al., 2011; Cristaldi et al., 2017; Ashraf et al., 2019]. Bioremediation can be performed either by microorganisms such as bacteria and fungi, which are able to bind and degrade various pollutants, either by terrestrial and aquatic plants able to remove pollutants through root system translocating and accumulating these in shoots and leaves [Matsubara et al., 2006; Nsanganwimana et al., 2014; Cristaldi et al., 2017; Ashraf et al., 2019]. Plants and microorganisms can interact among themselves through symbiotic relationships, improving the process of bioremediation, and thus constituting an assisted phytoremediation process [McCutcheon and Jørgensen, 2008; Fiorentino et al., 2013; Fiorentino et al., 2016; Sreelali and Jayanthi, 2017; Ashraf et al., 2019]. Among the bioremediation techniques, phytoremediation is a valuable opportunity for possible application in soil remediation, because it was proposed as an alternative eco-friendly and low cost. Plants more efficient in phytoremediation processes are the hyperaccumulator plants, because they exhibit some characteristics that allow to tolerate and accumulate metals present into the soil; however these plants show a low production of biomass [Barcelò and Poschenrieder, 2003; Fiorentino et al., 2013; Van Oosten and Maggio, 2015; Fiorentino et al., 2016]. So, it is possible to use fast-growing species, and among these, *Arundo donax* (known as Giant reed) seems to be one of the most interesting. *A. donax* extends from the Mediterranean basin to the Middle East to India, but it can be found both cultivated and naturalized in temperate and subtropical regions. *A. donax* is a species that requires few soil treatments, has a low demand for nutrients, high resistance to pathogens/parasites, water and thermal stresses; thanks to these characteristics it can adapt to inhospitable and marginal areas as sites with strong saline concentration or heavily polluted [Fiorentino et al., 2013; Fiorentino et al., 2016; Nsanganwimana et al., 2014]. *A. donax* is not appetite by the animals, thus avoiding the spread of toxic and persistent substances within the food chain [Fiorentino et al., 2013].

An important disadvantage of the phytoremediation is the slowness of the process, and therefore techniques have been developed to increase the accumulation of potentially toxic elements (PTE) in the plants. Therefore, the employment of symbiotic microorganisms permit to establish a positive relationship with the plant improving the uptake efficiency of roots of PTE [Fiorentino et al., 2013; Cristaldi et al., 2017].

Several species of filamentous fungi, but also some yeasts or bacterial species, can be coupled with the plants in the phytoremediation process because they have shown the remarkable ability to survive in extreme conditions of pH, temperature and variability of nutrients. Furthermore, they showed a good tolerance towards organic and inorganic contaminants, and they are able to degrade, sequester or transfer the contaminants from the soil to the plants [Matsubara et al., 2006; Cristaldi et al., 2017; Oladipo et al., 2018].

Aims of our study were:

1. Test the ability of *Trichoderma harzianum*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* to accumulate eight heavy metals (Ni, Cd, Cu, As, Zn, Pb, V, Hg) through an *in vitro* test.
2. Select the better microorganism to couple to *A. donax* to carry out a phytoremediation of fortified soils with Ni, Cd, Cu, As, Zn, Pb, V, Hg at two different concentrations in a controlled growth test carried out in greenhouse.
3. Evaluate the capability of phytoremediation of *A. donax* and mycorrized *A. donax* with *T. harzianum* through metals analysis by ICP-MS both in soil and plant, and the bioaccumulation factor (BAF) and translocation factor (TF).

2. Materials and methods

2.1. Chemicals

Suprapur nitric acid (65%), hydrogen peroxide (30%), nickel nitrate hexahydrate, cadmium chloride, copper chloride dihydrate, sodium metavanadate, arsenic trioxide, lead nitrate, zinc chloride, mercury acetate, and nitrocellulose filters (0.45 μm) were purchased by Sigma Aldrich© (Saint Louis, Missouri, USA). Ultrapure water by Milli-Q© system by Merck.

2.2.

Eppendorf© 5702 Series Centrifuge Model 5702 R, Autoclave Fedegari© FVS, FALC© Heating model STF-N 120, Incubator Labcold© RLCG 01502 (Labcold, UK), Multiflask Horizontal Shaking Shaker VKS 75 B control VWR, Mettler Toledo Analytical Balance XS64, Milli-Q ultrapure water system (Merck© KGaA, Darmstadt, Germany), Microwave Digestion System Start D (Milestone© Inc., USA), DigiPrep Jr (SCP Science©, USA), ICP-MS Elan DRC-e (PerkinElmer© Inc. USA).

2.3. Selected microorganisms

Trianum-P, constituted by spores of fungus *T. harzianum* strain T22, was purchased by Koppert BV Holland. Trianum-P not contain harmful chemicals listed in Article I of the CE Directive 67/548, then it was selected for our experiment. *T. harzianum* T22 strain growth and develop to different conditions, both in acidic and basic soils, sandy and clay, rich in nutrients and poor [Harman et al., 2004].

T. harzianum has already been used in several bioremediation processes and it has shown promising results [Fiorentino et al., 2013; Zafra and Cortès-Espinosa, 2015]. *T. harzianum* shows anti-pathogenic activity towards parasitic fungi [Benitez et al., 2004; Zafra and Cortès-

Espinosa, 2015], thus it contributes to the stability of the microbial communities that lives in the rhizosphere [Benitez et al., 2004] and also stimulates the growth of other fungal groups, naturally present in the soil, capable of tolerating a wide range of pollutants [Matsubara et al., 2006]. *T. harzianum* can to establish mutual symbiosis with a superior green plant [Fiorentino et al., 2013; Fiorentino et al., 2016], so as to improve health and development of the root, and therefore of the whole plant [Fiorentino et al., 2013].

The yeasts used in our study (*S. cerevisiae* BCA 61 and *W. anomalus* BS 91) were kindly provided by Di3A controlled culture (Department of Agriculture, Food and Environment), University of Catania, Italy, and were isolated from naturally fermented olive brine and pomegranate [Parafati et al., 2015]. The yeast cultures in sterile Petri dishes of Di3A were stored at 4 °C in Malt Extract Agar (MEA, Oxoid, Basingstoke, UK) until the use.

Malt Extract Broth (MEB, CM0057, Oxoid, Basingstoke, UK) was used for culture of *S. cerevisiae* and *W. anomalus*; Potato Dextrose Broth (PDB, CM0139, Oxoid, Basingstoke, UK) was used for broth culture of *T. harzianum*.

S. cerevisiae is naturally present into the soil and it is considered a safe microorganism [Wang and Chen, 2006; Wang and Chen, 2009], therefore, its use in bioremediation actions would be easily accepted by the authorities and public opinion. It is used in food and beverage industries, and furthermore, it is cheap and it is used as by-product from industrial fermentation processes [Wang and Chen, 2009]. Some studies [Kapoor and Viraraghavan, 1997; Wang and Chen 2009; Machado et al., 2010] highlighted the ability of *S. cerevisae* to absorb and remove various heavy metals present in aqueous solutions. Furthermore, Wang and Chen [2006] reported that *S. cerevisiae* is an excellent model for identifying the mechanisms involved in the metal ion bioabsorption process.

W. anomalus, previously identified as *Hansenula anomala* and also *Pichia anomala*, is a yeast of Saccharomycetes family. It is present in nature and it is traditionally used in the food industries as a preservative agent. *W. anomalus* is highly tolerant to environmental stress, in fact it is able to develop in variable conditions of temperature (3–37 °C), pH (2–12) and osmolarity. This robustness makes this yeast highly competitive in many different environments. Furthermore, *W. anomalus* shows a wide spectrum of antimicrobial activity (unusual feature in yeasts), versus a variety of microorganisms including other yeasts and bacteria [Cappelli et al., 2014]. Although there is little data in literature regarding the use of *W. anomalus* in bioremendation processes [Wang and Chen, 2009], given its characteristics it may be interesting to test it.

In addition to the characteristics described, the three microorganisms used for microbiological tests were considered suitable for experimentation because they are not considered sources of biological risk e they are naturally present in the environment.

2.4. Arundo donax

The rhizomes of *A. donax* (Fiumefreddo clone) used for our study were kindly provided by the Experimental Agricultural Company of the University of Catania.

2.5. Topsoil used for experimentation in greenhouse

The topsoil was taken from the Experimental Agricultural Company of the University of Catania and preliminary analysis was carried out to assess the possible original presence of heavy metals (see paragraph 2.7).

2.6. Test on microorganisms uptake

2.6.1. In vitro tests

10^8 CFU of *S. cerevisiae*, *W. anomalus* and *T. harzianum* were inoculated in 20 ml of culture broth (according producer instructions) in a tube of 50 ml respectively. Before the inoculate, each culture broths were spiked with heavy metal. Concentration values of individual heavy metals were calculated according to the legal limits imposed by Italian Legislative Decree 152/06 (Table 1, columns A and B, Annex 5, Title V, Part IV Legislative Decree No. 152/2006). Then, we prepared four different levels of increasing concentrations named L1, L2, L3, L4 (see Table 1) for each metal in triplicate, for a final number of 108 fortified samples. For each level of each metal we prepared also 1 blank for a final number of 36 blanks. In Table 1 were reported the percentage of metals lower, equal, higher 50 and 75% higher, compared to LD 152/2006 legal limits. These preparation batch were performed for each microorganism with a total of 432 samples and then sterilized in autoclave at the 121 °C for 20 min and the pH was measured. After inoculation of microorganisms, all samples were incubated at 26 °C for 96 h. So, the tubes were centrifuged at 4400 rpm for 15 min. The supernatant was removed, and the pellet was rinsed with Milli-Q ultrapure distilled water and discharged in order to eliminate the possible unabsorbed heavy metal by the microorganisms. Then, the pellet contained in each tube was placed in a heater at 95 °C for 6 h and the dry sample was weighted.

Table 1
Increasing levels of heavy metal concentrations added to the culture broth.

Heavy Metals	L1 [a]	L2 [a]	L3 [a]	L4 [a]
Ni	8.00	10.0	15.0	17.5
Cd	0.24	0.30	0.45	0.52
Cu	9.60	12.0	18.0	21.0
As	0.80	1.00	1.50	1.70
V	24.0	30.0	45.0	52.5
Pb	4.00	5.00	7.50	8.75
Zn	16.0	20.0	30.0	35.0
Hg	0.07	0.10	0.15	0.17

L1 = Exposure level 1 < 20% of LL.
L2 = Exposure level 2 = LL.
L3 = Exposure level 3 > 50% of LL.
L4 = Exposure level 4 > 75% LL.
LL: legal limit of Legislative Decree 152/06.
[a] (mg/20 ml).

2.6.2. Chemical analyses

Five ml of HNO_3 (65%) and 1 ml of H_2O_2 (30%) were added into each tube with the sample and so, the pellet was digested (80 °C for 30 min) using a DigiPrep Digestion System. Only samples for Hg determination were digested in a Milestone Microwave Digestion System Start D at 200 °C for 20 min to avoid the loss of Hg vapors.

All digested pellets were diluted with MilliQ® water until a final volume of 20 ml and filtered using nitrocellulose filters (0.45 μm). Detection and quantification of heavy metals were carried out through the ICP-MS according to EPA method 6020 B, using Yittrium and Renium as Internal Standard. The recovery test for each element was verified, obtaining recovery values between 83 and 114% for all metals analysed. The method detection limits (MDL - mg/g d. w.) were calculated for: As 0.0009, Cd 0.0003, Cu 0.0002, Pb 0.0007, Hg 0.0002, Ni 0.0003, V 0.001, and Zn 0.003.

2.7. Topsoil preparation

Three sampling points were made at the collection site, so as to verify eventual amount of heavy metals already present and to evaluate if the site was adapt to be used for the collection of topsoil for our experiments.

Soil samples were oven dried (70 °C until constant weight) and shattered with a mortar for the chemical analysis performing. Microwave Digestion was performed for each soil samples: 1 g of soil was placed in a PTFE vessel with 7 ml of HNO_3 65% and 1 ml of H_2O_2

Table 2
Values of heavy metals found during preliminary analyzes of the topsoil used for our experimentation.

Heavy metal	Average values (mg/kg) 1st point	Average values (mg/kg) 2nd point	Average values (mg/kg) 3rd point
Ni	30	30	26
Cd	0.80	0.67	0.82
Cu	21	28	22
V	63	65	61
As	5	5	4
Zn	63	61	66
Pb	16	15	18
Hg	0.48	0.42	0.51

*The values reported for each sampling point are given by the average of three replicates of the sample under analysis.

30% at 200 °C for 30 min, and finally the samples were detected using an ICP-MS DRC-e PerkinElmer according to EPA 6020 B. Results (Table 2) showed that all metals in topsoil samples were all below the legal limit prescribed by Legislative Decree 152/06, and although there were no particular differences between the various sampling points (1, 2, and 3) of the site, the topsoil was taken from site 2 because it showed lower average values for Cd, Pb, and Hg. So, this topsoil was suitable for the fill (20 kg of topsoil each) of pots (ø 33 cm, h 40 cm) for a total of 24 pots samples aimed to the planting of *A. donax*.

Experimental tests in greenhouse were carried out at the Experimental Agricultural Company of the University of Catania situated in the locality Primosole, Reitana district, Catania (Italy).

The topsoil was spiked with a mix of eight heavy metal at two different concentrations, so as to have two hypothetical conditions of pollution: the first concentration (L1) was the threshold of LD 152/06 limit value, and the second concentration (L2) was the threshold LD 152/06 limit value increased of 50% (Table 3).

The implant has been realized in greenhouse as follow:

- 3 replicates for exposure level 1 for each rhizome of *A. donax* and 3 respective control.
- 3 replicates for exposure level 2 for each rhizome of *A. donax* and 3 respective control.
- 3 replicates for exposure level 1 for each rhizome of mycorrhized *A. donax* with *T. harzianum* and 3 respective control.
- 3 replicates for exposure level 2 for each rhizome of mycorrhized *A. donax* with *T. harzianum* and 3 respective control.

Control samples were prepared (without the spiked metals) so as to subtract the heavy metals already present in the topsoil used (topsoil site 2).

The trial lasted seven months, from the beginning of December 2018 to the end of June 2019. Irrigation has been done regularly once a week.

Table 3
Threshold limit value (mg/Kg) for each heavy metal, reported by the D.Lgs 152/06 (Table 1, columns A and B, Annex 5, Title V, Part IV, Legislative Decree 152/2006) for commercial and industrial sites. L1: threshold limit value; L2: threshold limit value increased by 50%.

Heavy metal	L1 (mg/kg)	L2 (mg/kg)
Ni	500	750
Cd	15	22.5
Cu	600	900
V	250	375
As	50	75
Pb	1000	1500
Zn	1500	2250
Hg	5	7.5

2.8. A. donax heavy metal detection

At the 7th month of growth both *A. donax* and mycorrhized *A. donax* were collected from each pot and were divided into rhizomes, culms and leaves; these have been oven dried (60 °C until constant weight) and shredded for chemical analysis. Topsoil samples from each pot sample were taken also. Microwave Digestion was performed for each component of the plant and for the respective soil, adopting a specific thermal program for each type of sample. Each sample of rhizomes (1 g), culms (1 g) and leaves (1 g) was placed in a respective PTFE vessel with 7 ml of HNO_3 65% and 1 ml of H_2O_2 30% at 200 °C for 20 min; each soil sample (1 g) was placed in a PTFE vessel with 7 ml of HNO_3 65% and 1 ml of H_2O_2 30% at 200 °C for 30 min. After mineralization each digested sample was brought to volume to 20 ml with MilliQ® ultra-pure water and filtered with 0.45 μm nitrocellulose filters. Analyses were carried out using ICP-MS DRC-e PerkinElmer according to EPA 6020 B, using Yittrium and Renium as Internal Standard. The recovery test for each element was verified, obtaining recovery values between 83 and 112%. MDL for each metal was in a range of values included between 0.2 and 1 μg/g. The results will be expressed in micrograms of metal accumulated per grams (μg/g) of plant (rhizome, culm, leave, and total plants), and in BAF and TF (both dimensionless). The bioaccumulation factor (BAF), i.e. the capability of the plant to accumulate metals contained in the soil, it is given by the ratio between the concentration of metals in the plant (referred to the dry weight) and in the soil. If the BAF > 1 the plant is considered a hyper accumulator [Paterson et al., 1990].

$$BAF = C_{plant}/C_{soil}$$

where C represent the metal concentration [Fiorentino et al., 2013].

Translocation factor (TF) indicates the ability of the plant to transfer metals from the roots to the upper parts (stem and leaves); it is measured by the ratio between the metal concentration in the shoots (leaves) and in the roots.

$$TF = C_{leaves}/C_{roots}$$

where C represent the metal concentration [Fiorentino et al., 2013].

A species can be considered as a translocator when it has TF > 1; if the translocation factor is lower, the species can be considered a candidate for phytostabilization techniques [Fitz and Wenzel, 2002; Rizzi et al., 2004].

2.9. Statistical analysis

Results obtained for the three microorganisms were performed with the software IBM SPSS Statistics 20.0, and the data obtained were expressed as mean ± Standard Deviation (SD). Significant differences were assessed by univariate analysis of variance (ANOVA) followed by the Tukey's test.

Results obtained for *A. donax* experiments were expressed as mean ± Deviation (SD) and were examined with two-tailed Student's *t*-tests. P-value of less than 0.05 or 0.01 was considered statistically significant or very significant, respectively.

3. Results and discussions

3.1. In vitro analysis

For each exposure level (L1, L2, L3, L4) three replicates were made, and the average percentage obtained by the accumulated metal/available metal ratio by each microorganism exposed to each metal was calculated.

3.1.1. T. harzianum

After 96 h, *T. harzianum* showed a good growth rate, taking into account the biomass produced (see Table 4), and a good

Table 4
Dry weight (mean ± standard deviation) of the mycelium of *T. harzianum*, *S. cerevisiae*, *W. anomalus*, respectively, after 96th hour of exposure to the four different levels of each individual heavy metal.

Heavy metals	*T. harzianum* weight after exposure to level 1 (mg)	*T. harzianum* weight after exposure to level 2 (mg)	*T. harzianum* weight after exposure to level 3 (mg)	*T. harzianum* weight after exposure to level 4 (mg)
Ni	66.7 ± 4.51	70.3 ± 5.13	67.0 ± 2.00	68.0 ± 7.00
Cd	75.3 ± 11.9	73.3 ± 1.53	79.6 ± 7.09	76.0 ± 3.61
Cu	57.7 ± 2.68	60.0 ± 2.65	55.0 ± 3.46	58.6 ± 4.62
V	81.4 ± 3.21	84.0 ± 6.08	89.2 ± 16.4	83.0 ± 3.61
As	45.0 ± 5.20	74.2 ± 3.21	72.5 ± 11.0	72.0 ± 3.61
Zn	90.2 ± 2.52	73.8 ± 5.51	82.6 ± 6.03	85.7 ± 3.51
Pb	91.6 ± 7.02	90.0 ± 6.08	92.0 ± 6.56	92.7 ± 3.06
Hg	77.0 ± 4.58	69.7 ± 4.16	81.3 ± 11.4	87.3 ± 27.5
Control	77.0 ± 6.65	83.3 ± 8.14	79.5 ± 4.67	83.0 ± 7.24
Heavy metals	***S. cerevisiae* weight after exposure to level 1 (mg)**	***S. cerevisiae* weight after exposure to level 2 (mg)**	***S. cerevisiae* weight after exposure to level 3 (mg)**	***S. cerevisiae* weight after exposure to level 4 (mg)**
Ni	73.3 ± 2.52	72.7 ± 4.16	72.0 ± 6.00	74.0 ± 4.00
Cd	73.7 ± 17.4	85.7 ± 11.6	63.3 ± 16.3	49.3 ± 6.66
Cu	71.0 ± 2.65	72.3 ± 1.53	75.7 ± 4.93	75.0 ± 4.36
V	61.7 ± 3.06	56.7 ± 3.06	53.3 ± 1.53	47.3 ± 3.79
As	62.3 ± 0.58	61.7 ± 2.08	64.3 ± 1.15	52.7 ± 2.52
Zn	57.3 ± 5.69	61.7 ± 4.63	67.3 ± 3.51	57.6 ± 0.58
Pb	50.0 ± 6.24	52.0 ± 5.00	54.0 ± 4.58	55.7 ± 4.73
Hg	55.0 ± 4.36	56.3 ± 0.58	53.0 ± 3.00	51.2 ± 1.53
Control	80.2 ± 2.22	80.1 ± 4.82	80.9 ± 3.51	77.3 ± 5.56
Heavy metals	***W. anomalus* weight after exposure to level 1 (mg)**	***W. anomalus* weight after exposure to level 2 (mg)**	***W. anomalus* weight after exposure to level 3 (mg)**	***W. anomalus* weight after exposure to level 4 (mg)**
Ni	41.3 ± 0.58	41.7 ± 1.53	40.0 ± 1.00	44.0 ± 1.00
Cd	59.7 ± 1.53	62.3 ± 1.15	56.0 ± 2.65	58.3 ± 2.31
Cu	49.0 ± 2.65	48.7 ± 5.51	47.0 ± 4.36	47.6 ± 4.73
V	71.6 ± 13.4	62.0 ± 2.65	33.7 ± 2.08	37.0 ± 2.65
As	64.3 ± 1.53	60.2 ± 1.15	58.0 ± 2.65	51.7 ± 1.53
Zn	71.3 ± 5.03	74.0 ± 1.00	76.3 ± 2.31	69.0 ± 2.65
Pb	45.0 ± 6.24	47.7 ± 6.51	48.6 ± 5.51	54.0 ± 13.2
Hg	43.0 ± 1.73	37.0 ± 2.00	44.3 ± 2.08	42.0 ± 3.00
Control	81.6 ± 5.58	78.1 ± 4.68	80.4 ± 3.95	81.4 ± 3.10

bioaccumulation capability for the heavy metals used in this study (Fig. 1). We have evaluated the bioaccumulation capability of *T. harzianum* for each individual metal studied. Our results showed the trend was V > As > Cd > Hg > Pb > Cu > Zn. The highest bioaccumulation values, expressed as percentage average obtained from the four exposure levels, were for V (77%), As (73%), Cd (72%), Hg (68%), Pb (63%).

T. harzianum showed variable bioaccumulation values depending on the level of exposures dose for each metal to which it has been exposed (Fig. 1).

Ni exposures: *T. harzianum* showed an increasing bioaccumulation capability with increasing exposure levels (L1, L2, L3, L4), but the average absorption percentage, obtained from the available metal/absorbed metal ratio, decreases (78%; 66%; 47%; 41%); this is not attributable to the fungal biomass which remained more or less constant in all four exposure levels (Table 4).

Cd exposures: *T. harzianum* showed a good bioaccumulation values. These values were directly proportional to the exposure doses increase (L1 = 67%; L2 = 70%; L4 = 88.5%) except in L3 (62%). The mycelium weight was steady for L2, L3 and L4 levels (Table 4).

Cu exposures: *T. harzianum* showed low bioaccumulation values of Cu (L1 = 58%; L2 = 39%; L4 = 50%) compared to other metals, with exception for L3 = 73%, although the mycelium showed a steady weight in all exposure levels (Table 4). This trend could be due to the lower quantity of fungal biomass, in fact, Hajieghrari [2010] reports that Cu can inhibits the growth of *T. harzianum* mycelium.

V exposures: *T. harzianum* showed good growth and bioaccumulation versus V. A steady weight of mycelium was recorded in all four levels of exposure (Table 4), nevertheless, the bioaccumulation percentages showed an optimal values, particularly at L2 (L1 = 62%; L2 = 95%; L3 = 75%; L4 = 76%).

As exposures: *T. harzianum* showed a good bioaccumulation capability (L1 = 61%, L2 = 87%, L3 = 74%, L4 = 70.5%). At L1 we recorded both the smaller accumulation value and the smaller mycelium weight (Table 4). In all other levels, the mycelium weight was steady (Table 4), and the percentages of bioaccumulation decrease with the increasing of exposure dose. This effect, probably, is due to a saturation effect of the higher levels of arsenic. Results shows a good tolerance of *T. harzianum* against the As, as already described by several authors [Caporale et al., 2014; Tripathi et al., 2017].

Zn exposures: *T. harzianum* showed a poor Zn accumulation capability (L1 = 42%, L2 = 41%, L3 = 19.5%, L4 = 39) despite the mycelium well grown (Table 4). Thus, our results is in line of others data reported in scientific literature where *T. harzianum* showed a good rate of growth but a lower bioaccumulation than other heavy metals [Mohammadian et al., 2017].

Pb exposures: *T. harzianum* showed for Pb the highest values of mycelium weight compared to others metals evaluated (Table 4) but did not show a steady accumulation for the four levels of exposure, in fact, a fluctuating trend (L1 = 91.5%; L2 = 44%; L3 = 74%; L4 = 43%) was recorded. Our results partially confirm what reported by other authors, in fact Adebisi et al. [2014], Mohammadian et al., [2017] and Govarthanan et al., [2018], report a high accumulation capability of the Pb by the fungus, with a strong correlation between increased availability and accumulation capability.

Hg exposures: *T. harzianum* shows good accumulation values (L1 = 71%, L3 = 73%, L4 = 76%), except for L2 (50%), this low value is probably due to the lower fungal biomass compared to the other three levels (Table 4).

Several international studies [Hajieghrari, 2010; Adebisi et al., 2014; Ali et al., 2017; Awasthi et al., 2017; Govarthanan et al., 2018] highlighted the applicability of *T. harzianum* in bioremediation processes versus heavy metals such as Cd, V, As, Pb, Hg, and also our results confirm this trend. Hence, *T. harzianum* can be a good candidate for improving the phytoremediation process in symbiotic association with a superior green plant.

3.1.2. *S. cerevisiae*

Also the yeast *S. cerevisiae* showed both a good mycelium growth rate (see Table 4) and a good accumulation capability for the studied heavy metals (Fig. 2).

We have evaluated the bioaccumulation capability of *S. cerevisiae* for each individual metal studied and our results showed the trend was Hg > Ni > Cu > Cd > Zn > V > Pb > As. The highest bioaccumulation values, expressed as a percentage average obtained from the four exposure levels, were found for Hg (76%), Ni (74%), Cu (74%) Cd (71%).

Ni exposures: Although yeast biomass showed a steady weight for all exposure levels (Table 4), *S. cerevisiae* showed an increasing bioaccumulation capability with increasing exposure levels (L1, L2, L3, L4), but the average absorption percentage, obtained from the available metal/absorbed metal ratio, decreases (L1 = 91%; L2 = 78%; L3 = 66%; L4 = 62%).

Cd exposures: *S. cerevisiae* shows good accumulation values for Cd (L1 = 75%, L2 = 60%, L3 = 60%, L4 = 90%), and the highest values were found at L4, despite the biomass produced tends to decrease (Table 4). This indicates a good tolerance versus Cd and our results confirm the data of other previous studies [Park et al., 2003; Vasudevan et al., 2003; Wang and Chen, 2009].

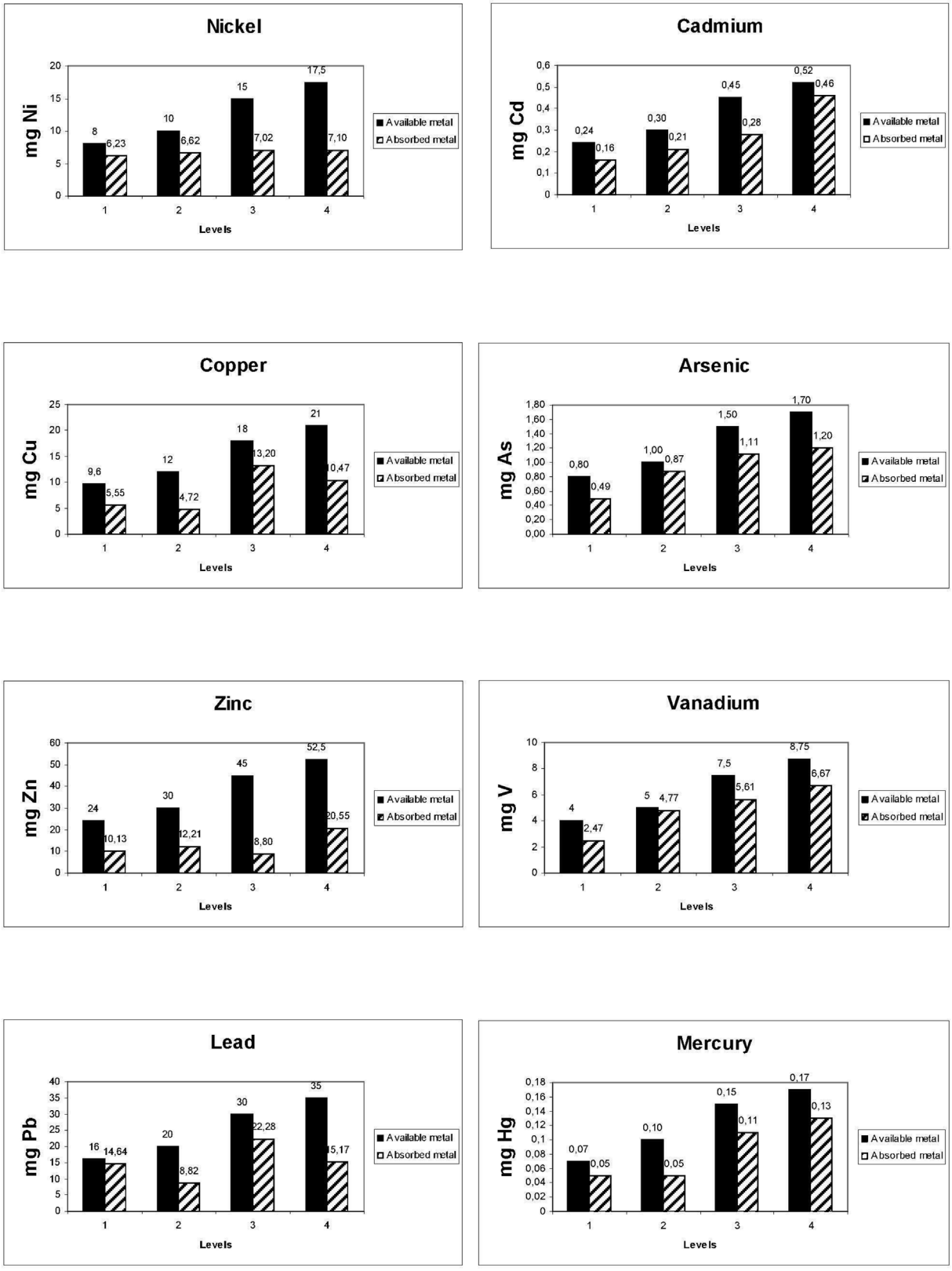

Fig. 1. Heavy metal bioaccumulation of *T harzianum* after *in vitro* exposure to the four levels (L1, L2, L3, L4) of each heavy metals (Ni, Cd, Cu, As, Zn, V, Pb, Hg).

Cu exposures: *S. cerevisiae* provided good results versus Cu. The yeast biomass weight was steady in all levels, as well as the percentages of accumulation (L1 = 72%, L2 = 71%, L3 = 79%, L4 = 74%), after exposure to the four levels. This confirms, as reported in other studies [Bag et al., 1999; Donmez and Aksu, 1999; Machado et al., 2010], the good ability to bioaccumulate Cu by *S. cerevisiae*.

V exposures: *S. cerevisiae* showed a good level of accumulation only at L1 and L2 (L1 = 75%; L2 = 60%; L3 = 28% L4 = 25.5%). Also, the yeast growth tends to decrease with increasing exposure doses (Table 4). Thus, the results obtained show that exposure to higher levels

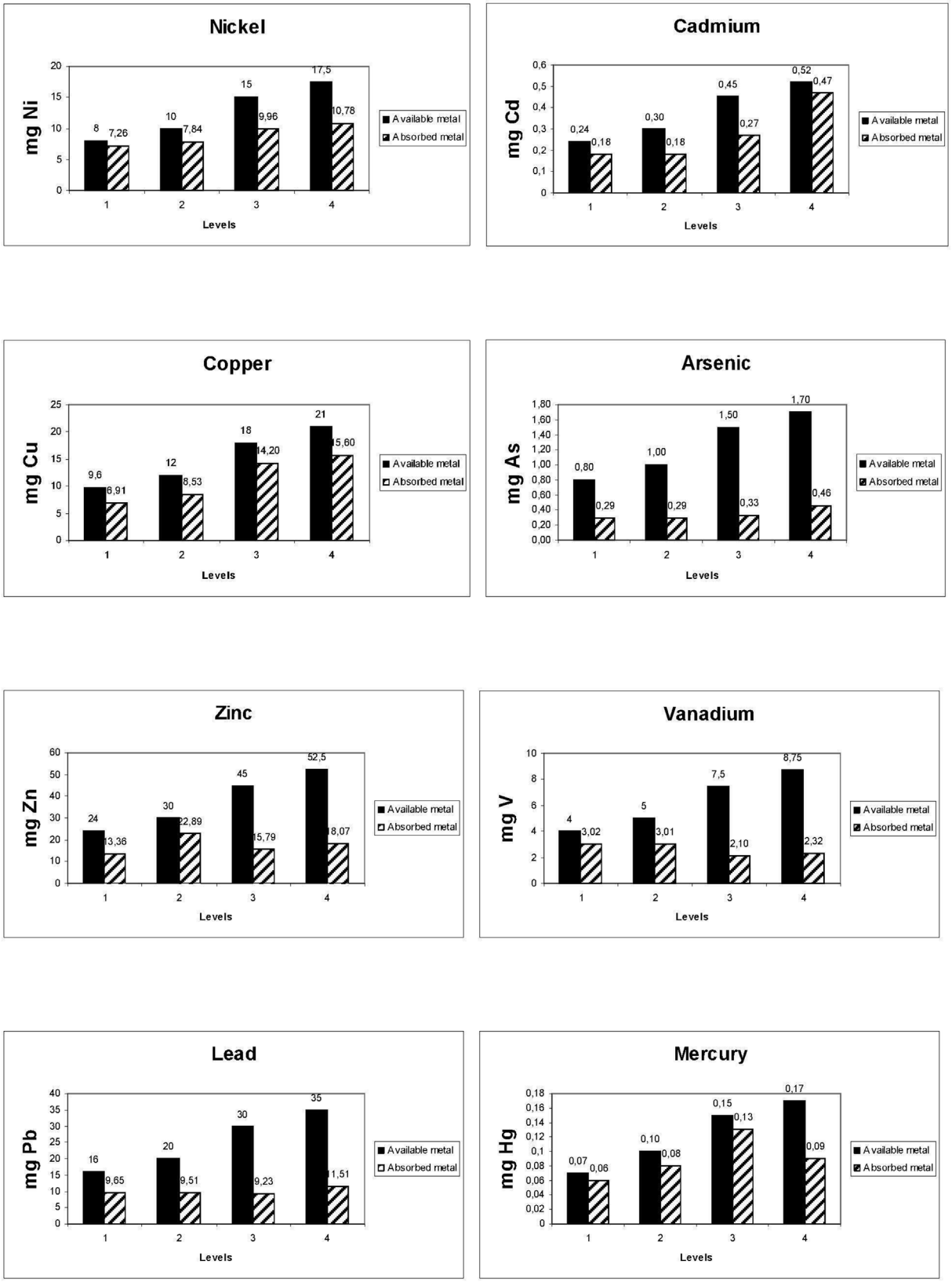

Fig. 2. Heavy metal bioaccumulation of *S. cerevisiae* after *in vitro* exposure to the four levels (L1, L2, L3, L4) of each heavy metals (Ni, Cd, Cu, As, Zn, V, Pb, Hg).

of V affects the growth of the yeast and the bioaccumulation of this metal.

As, Zn, and Pb exposures: *S. cerevisiae* not showed a good accumulation capability for As (L1 = 36%; L2 = 29%; L3 = 22%; L4 = 27%), Zn (L1 = 56%, L2 = 76%, L3 = 35%, L4 = 34%) and Pb (L1 = 60%, L2 = 48%, L3 = 31%, L4 = 33%); with only two exceptions for Zn in L2 and Pb in L1. Yeast growth for all three heavy metals was not particularly high (Table 4). Our results are similar to

other data reported on the topic, in fact, *S. cerevisiae* does not showed a good accumulation capability for As, Zn and Pb; however, other authors reported for Pb a good accumulation capability [Al-Saraj et al., 1999; Ozer and Ozer, 2003; Wang and Chen, 2009; Cabuk et al., 2007].

Hg exposures: *S. cerevisiae* showed the very good accumulation values (L1 = 86%, L2 = 80%, L3 = 87% L4 = 53%) and this capability has already been reported by other studies [Al-Saraj et al., 1999; Wang and Chen, 2006; Zhu et al., 2004]. The yeast biomass weight was steady in all exposure levels (Table 4). The lower value obtained at L4 could be explained by the minor growth of yeast (Fig. 2).

Several international studies [Wang and Chen, 2006; Wang and Chen, 2009; Machado et al., 2010] have highlighted the applicability of *S. cerevisiae* in bioremediation processes versus Ni, Cd, Cu and Hg heavy metals. Our results confirm this trend, however we not obtained satisfactory results for other heavy metals like V, As, Zn, Pb. Furthermore, *S. cerevisiae* showed less biomass production than *T. harzianum*.

3.1.3. W. anomalus

The yeast *W. anomalus* showed a good growth rate, although lower than the biomass values obtained for *T. harzianum* and *S. cerevisiae* (Table 4), and the yeast has also showed a good accumulation capability for the studied heavy metals (Fig. 3). We have evaluated the bioaccumulation capability of *W. anomalus* for each individual metal studied, and our results showed the trend was Zn > Ni > V > Cd > Cu > Hg > Pb > As. The highest bioaccumulation values between all exposure levels were detected for Zn (70%), Ni (69%), V (65%) and Cd (60%).

Ni exposure: *W. anomalus* showed the highest bioaccumulation value at L1 (99%) and a steady weight of biomass for all levels of exposure (Table 4); the amount of Ni bioaccumulated by *W. anomalus* was inversely proportional to the increase of exposure levels (L1 = 99%; L2 = 82%; L3 = 53%; L4 = 44%).

Cd exposure: although yeast biomass showed a steady weight for all exposure levels (Table 4), the amount of Cd accumulated by *W. anomalus* shows the best average percentage values at L1 (75%) and L2 (67%), whereas decrease at L3 (49%) and L4 (50%).

Cu exposure: although yeast biomass showed a steady weight for all exposure levels (Table 4), the amount of Cu accumulated by *W. anomalus* shows the best average absorption percentage values at L1 (80%) and L3 (57%), whereas decrease at L2 (55%) and L4 (38,5%).

V exposure: *W. anomalus* showed good average percentage values for V (L1 = 75%; L2 = 78%; L3 = 78%), except in L4 (29%); this low absorption at L4 is probably due to the lower fungal biomass (Table 4) than the other three levels. Therefore, as for the other yeast, *S. cerevisiae*, the results obtained show that exposure to higher levels of V affects the growth of the yeast and the accumulation of this metal.

As and Pb exposure: *W. anomalus* not showed a good accumulation values for As (L1 = 32.5%, L2 = 33%, L3 = 35%, L4 = 38%) and Pb (L1 = 57%, L2 = 45%, L3 = 29%, L4 = 28%). Yeast growth for either heavy metals was not high, particularly after exposure to Pb (Table 4) So, *W. anomalus* is not particularly efficient for the bioaccumulation of As and Pb.

Zn exposure: *W. anomalus* showed the best bioaccumulation values, after exposure to Zn (L1 = 89%; L2 = 90%; L3 = 60%); only at L4 (41%) the growth rate is lower, although yeast biomass showed a steady weight for all levels of exposure (Table 4). From the results obtained, *W. anomalus* appears to be the best of the three microorganisms as regards zinc bioaccumulation.

Hg exposure: *W. anomalus* showed the highest value at L3 (80%). Yeast biomass showed a steady weight for all exposure levels (Table 4), and the average percentage of Hg accumulated by the yeast is steady for the L2 (50%) and L4 (53%), instead, at L1 (43%) the growth rate is lower. As we have seen for V, the results obtained show that higher Hg exposure levels can affect the growth of yeast and the accumulation of this metal.

Nowadays, there are not many studies and applications [Podgorskii et al., 2004; Wang and Chen, 2009; Souza et al., 2018] of *W. anomalus* in bioremediation processes, and through our study we evaluated the potential ability of this microorganism in bioremediation processes. *W. anomalus* shows a lower biomass production compared to the other two microorganisms used in our study, and shows a lower capability to bioaccumulate heavy metals, except for Zn, Ni, V and Cd. Although it is not the best of the three microorganisms tested, it could be a good alternative, perhaps to be used in association with other microorganisms that have not shown good bioaccumulation capacity of Zn, Ni, V, Cd.

3.1.4. ANOVA test

Each test was conducted in triplicate. One-Way ANOVA test shows that:

Ni: Ni bioaccumulation levels are significantly different at L3 ($p < 0.05$) and L4 ($p < 0.01$); no significant differences were found between L1 and L2. Furthermore, Tukey's test shows that *S. cerevisiae* is able to accumulate Ni significantly higher than *T. harzianum* ($p < 0.05$) after exposure at L3, and *S. cerevisiae* accumulates significantly higher doses compared to *T. harzianum* and *W. anomalus* ($p < 0.01$) after exposure at L4.

Pb: significantly different concentrations were found at L1 ($p < 0.01$) and L3 ($p < 0.05$). *T. harzianum* showed a better bioaccumulation capability and the post hoc Tukey's test revealed that this species accumulates higher concentrations of *S. cerevisiae* and *W. anomalus* with $p < 0.01$ at L1 and $p < 0.05$ at L3.

Zn: significantly different concentrations were found at L1 ($p < 0.05$) and L3 ($p < 0.01$). Results from the Tukey's test showed that *W. anomalus* accumulates significantly higher concentrations than *T. harzianum* after the exposure at L1 ($p < 0.05$); *W. anomalus* accumulates significantly higher concentrations than the *S. cerevisiae* ($p < 0.05$) and *T. harzianum* ($p < 0.01$) after the exposure at L3.

As: significantly different accumulation were found in L1, L2 and L4. Post hoc Tukey's test showed that *T. harzianum* accumulates significantly higher concentrations compared to the other two microorganisms after exposure at L1 ($p < 0.05$ for *S. cerevisiae* and $p < 0.01$ for *W. anomalus*), L2 ($p < 0.001$ for *S. cerevisiae* and *W. anomalus*) and L4 ($p < 0.001$ for *S. cerevisiae* and $p < 0.05$ for *W. anomalus*).

Cu: significantly different accumulation were found at L1 ($p < 0.01$), L2 ($p < 0.05$) and L4 ($p < 0.01$). Post hoc Tukey's test shows at L1 that *S. cerevisiae* and *W. anomalus* accumulate significantly higher concentrations compared to *T. harzianum* ($p < 0.01$), and at L4 *S. cerevisiae* accumulate significantly higher concentrations than the *T. harzianum* ($p < 0.01$) and *W. anomalus* ($p < 0.01$).

V: significantly different accumulation were found at L2, L3 and L4. Post hoc Tukey's test shows that *T. harzianum* has a higher accumulation capacity than the other two species; the bioaccumulation at the L2 was significantly higher than *S. cerevisiae* ($p < 0.05$); at L3, both *T. harzianum* and *W. anomalus* have accumulated V doses significantly greater than *S. cerevisiae* ($p < 0.05$); at L4 level, *T. harzianum* has accumulated significantly higher doses respect to the others two species ($p < 0.001$).

Hg: a significant difference was observed for the accumulation of Hg at L1, L2 and L4. Tukey's test showed that *S. cerevisiae* accumulates significantly higher concentrations than *W. anomalus* after the exposure at L1 ($p < 0.01$); *S. cerevisiae* accumulates Hg concentrations significantly higher than the other two species after the exposure at L2 ($p < 0.05$); at L4, *T. harzianum* showed a better accumulation capability than *S. cerevisiae* and *W. anomalus* after the exposure at L4 ($p < 0.05$).

Cd: There was no significant difference between the various exposure levels (L1, L2, L3, L4) for accumulation of Cd between the three species.

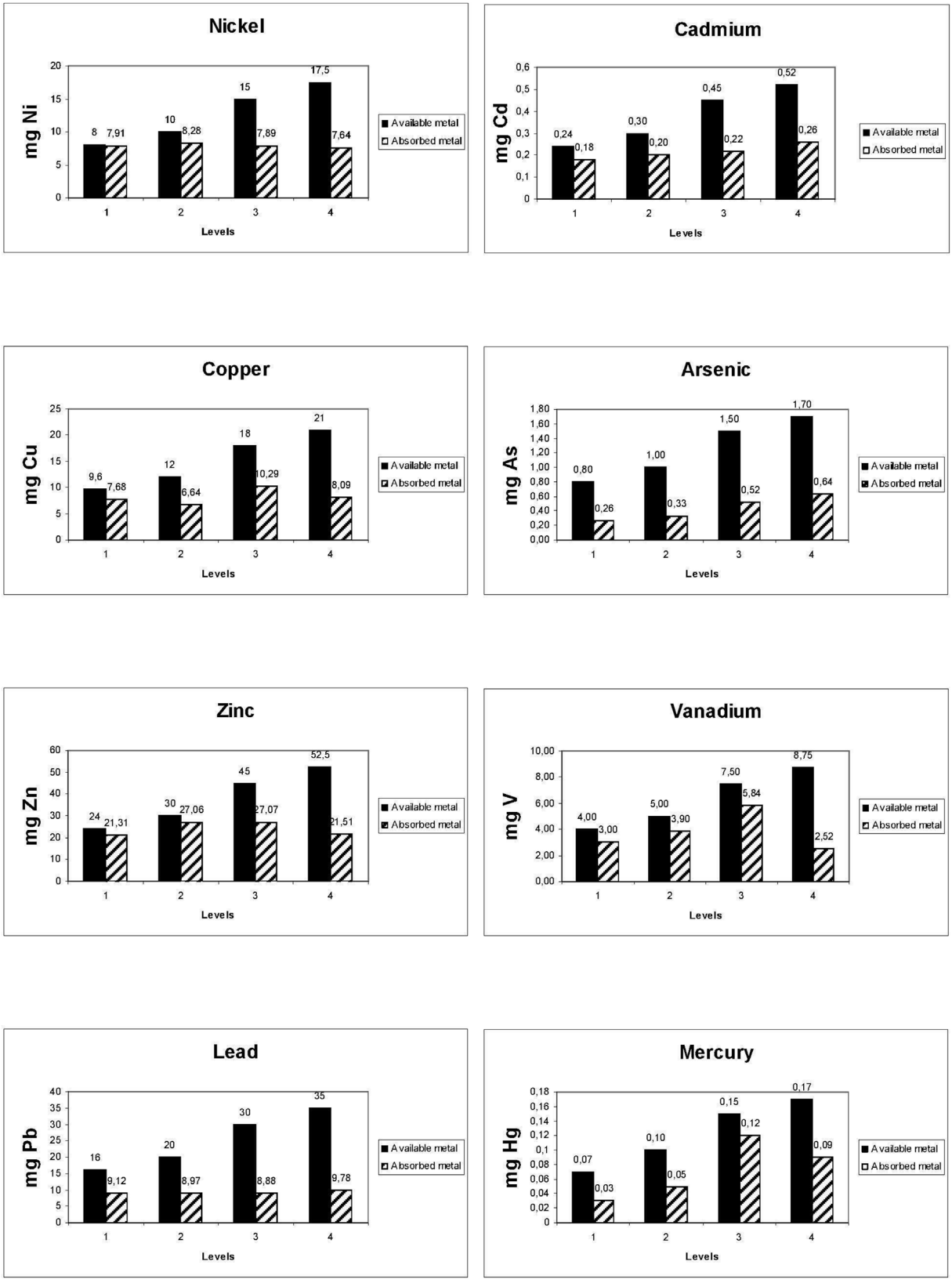

Fig. 3. Heavy metal bioaccumulation of *W. anomalus* after *in vitro* exposure to the four levels (L1, L2, L3, L4) of each heavy metals (Ni, Cd, Cu, As, Zn, V, Pb, Hg).

3.2. Arundo donax and mycorrhized A. donax with T. harzianum

The three microorganisms showed good growth and bioaccumulation capability towards the heavy metals to which they were exposed. However, for the second part of our study we chose to use *T. harzianum*, both for the ability to accumulate some of the metals to which it was exposed (V, As, Cd, Hg, Pb), and for its natural characteristics (resistance to pathogens, environmental factors, ability to establish

Table 5
Descriptive statistic expressed in μg/g for each sample of *A. donax* and mycorrhized *A. donax* with *T. harzianum*.

A. donax		Ni	Cd	Cu	As	Zn	V	Pb	Hg
L1	**Mean**	153.2	5.3	181.9	9.6	349.9	97.9	177.8	2.1
	S.D	73.4	0.5	33.9	4.9	169.3	26.0	88.6	0.5
	Min.	71.3	4.8	148.2	6.0	157.9	68.4	121.9	1.6
	Max.	213.2	5.8	216.0	15.2	478.1	117.5	280.0	2.6
L2	**Mean**	193.3	11.3	312.3	20.0	201.0	98.2	214.8	3.3
	S.D	47.4	1.6	69.0	3.7	66.6	21.2	117.7	1.2
	Min.	153.6	9.4	252.8	16.5	131.7	79.2	137.7	2.3
	Max.	245.8	12.3	387.9	23.8	264.5	121.0	350.2	4.6
A. donax* with *T. harzianum		**Ni**	**Cd**	**Cu**	**As**	**Zn**	**V**	**Pb**	**Hg**
L1	**Mean**	136.9	6.6	218.8	8.3	205.8	41.3	372.4	2.2
	S.D	91.4	0.1	28.0	1.4	47.6	4.6	116.4	0.5
	Min.	47.8	6.5	189.0	7.5	169.2	37.1	291.3	1.6
	Max.	230.5	6.7	244.6	9.9	259.7	46.3	505.8	2.6
L2	**Mean**	288.2	9.4	261.3	17.0	165.1	75.9	807.5	4.5
	S.D	18.3	3.9	37.9	8.3	10.7	23.6	148.1	0.0
	Min.	267.3	4.9	218.2	9.3	156.8	56.6	638.8	4.5
	Max.	301.7	11.9	289.5	25.7	177.1	102.3	915.8	4.6

SD: Standard Deviation; Min: Minimum; Max: Maximum.

symbiosis with the root system of the plant).

Results of the descriptive statistic expressed in μg/g for each sample of *A. donax* and mycorrhized *A. donax* with *T. harzianum* are reported in Table 5. The means of heavy metals analysed have been obtained from the three independent replicates for each of the two exposure doses.

All plants were grown under semi controlled conditions in greenhouse. Although *A. donax* is not a hyper accumulator plant, it showed bioaccumulation capability after exposure to the mix of heavy metals (both exposure levels, L1 and L2). We report the Bioaccumulation Factor (BAF), also called Bioconcentration Factor (BCF); as reported in the materials and methods section, this factor (dimensionless) is given by the ratio between the concentration of metals in the plant (referred to the dry weight) and in the soil.

Then, *A. donax* shows the highest BAF for Cd (0.50), Cu (0.35), As (0.27) and Hg (0.45) after exposure at L2 (Table 6). The two-tailed t-Student test shows significant differences of bioaccumulation between L1 and L2 for Cd ($p < 0.01$), Cu ($p < 0.05$) and As ($p < 0.05$); no statistically significant differences has been revealed for other heavy metals (see Table 1 in supplementary files).

The mycorrhized *A. donax* with *T. harzianum* shows the highest BAF for Ni (0.38), Cd (0.42), Pb (0.54) and Hg (0.60) after exposure at L2 (Table 7). The two-tailed t-Student test shows significant differences of bioaccumulation between L1 and L2 for Ni ($p < 0.05$), Pb ($p < 0.05$) and Hg ($p < 0.01$); no statistically significant differences has been revealed for other heavy metals (see Table 2 in supplementary files).

After the plant exposure at L1, we highlight that mycorrhized *A. donax* with *T. harzianum* shows a higher BAF than *A. donax* for Cd (0.44 vs. 0.35), Cu (0.36 vs 0.30), Pb (0.37 vs 0.18), but for Hg, BAF is almost constant for both exposure levels (0.44 vs 0.42); the two-tailed t-Student test shows significant difference of bioaccumulation for Cd ($p < 0.05$) (see Table 3 in supplementary files). Only for V at L1, *A. donax* shows a higher bioaccumulation factor than mycorrhized *A. donax* with *T. harzianum* (0.39 vs 0.16), and the two-tailed t-Student test shows significant difference of bioaccumulation (p-value < 0.05); no statistically significant differences has been revealed for other heavy metals (see Table 3 in supplementary files).

After exposure at L2, we highlighted that the mycorrhized *A. donax* with *T. harzianum* shows a higher BAF than *A. donax* only for Ni (0.38 vs 0.26), Pb (0.54 vs. 0.14) and Hg (0.60 vs 0.45). The two-tailed t-Student test shows significant differences of bioaccumulation for Ni ($p < 0.05$) and Pb ($p < 0.01$); no statistically significant differences has been revealed for other heavy metals (see Table 4 in supplementary files).

Furthermore, we report the Translocation Factor (TF), and as described in the materials and methods section, this factor (dimensionless) is given by the ratio between the metal concentration in the shoots (leaves) and in the roots.

A. donax shows the highest TF values for Cd (0.28) and Hg (0.26) after exposition at L1 and L2 respectively; mycorrhized *A. donax* with *T. harzianum* shows the highest TF values for Cd (0.70), As (0.56) V (0.24), Pb (0.18) after exposition at L2, and Zn (0.30) after exposition at L1. Although the TF values found are not greater than 1, it is possible to highlight that there has been a transfer of metals by roots to leaves, after only seven months of growth; but that could certainly increase with a longer development and growth time, as was reported by other similar studies [Guo and Miao, 2010; Fiorentino et al., 2013; Fiorentino et al., 2016].

So, after the exposure test in greenhouse, *A. donax* has proved to be a plant species that is well suited for use in the recovery processes of soils contaminated by heavy metals, in particular because it produces a good amount of biomass, is not desirable for animals, it resists pests and water stresses and, therefore, it is able to adapt in inhospitable environments [Guo and Miao, 2010; Mirza et al., 2011; Cristaldi et al., 2017; Ashraf et al., 2019]. Furthermore, *A. donax* not showed signs of stress on the foliar apparatus, which was well developed and of an intense green color, as well as culms were of excellent appearance with an average length of 86 cm and a maximum of 103 cm.

In general, *A. donax* showed good bioaccumulation capability after exposure to heavy metals, for both L1 and L2. The highest BAF values were found for Ni (0.31), Cd (0.35), Cu (0.30), V (0.39) and Hg (0.42), after exposure to L1; after exposure to L2, the BAF values higher than L1 were found only for Cd (0.50), Cu (0.35), As (0.27) and Hg (0.45). Instead, for the other metals (Ni, Zn, V and Pb) the results obtained show that the exposure to L2 negatively influences their accumulation by *A. donax* since the BAF value is lower than the BAF obtained after exposure to L1 (Table 6).

Mycorrhized *A. donax* with *T. harzianum* also showed good bioaccumulation capability. The data obtained show an increase in the BAF values from L1 to L2 for Ni, As, V, Pb and Hg (Table 7). We evaluated whether the plant-fungus symbiotic association could improve BAF. This only occurred for three of the eight metals tested (Ni, Pb, Hg). In fact, the BAF values obtained by Mycorrhized *A. donax* with *T. harzianum* after exposure to these metals have increased than to the BAF values obtained by *A. donax* (Tables 6 and 7). A better BAF value is given by the fungus interacts with the plant and promotes the growth of roots and culms [Harman et al., 2004], and also limits the growth of pathogenic fungi that could compromise the health of the plant [Fiorentino et al., 2013]. Furthermore, from our *in vitro* exposure tests, *T. harzianum* had shown a good growth rate in the presence of Pb and Hg.

A. donax has proven to be a plant more suitable for the phytostabilization process rather than for the phytoextraction process. In fact, the TF values found were not greater than 1 for both *A. donax* and Mycorrhized *A. donax* with *T. harzianum*. The only exceptions were found for mycorrhized *A. donax* with *T. harzianum* after exposure at L2 of Cd (TF = 0.70) and As (TF 0.56). Also in this case the TF value is not equal to or greater than 1 and therefore we cannot consider the process as a phytoextraction process, but with a longer time available the TF value could increase as it has been described in other studies [Fiorentino et al., 2013]. However, it is clear that the ability to transfer contaminants from the soil to the upper parts of the plant (shoots and leaves) depends on the characteristics of the plant itself, despite the support of *T. harzianum* which has increased the TF value for Cd and As. Therefore, in the short term, *A. donax* remains a plant suitable for the phytostabilization processes for metals that we have tested in our study.

Table 6
BAF and TF for *A. donax*.

Ni	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	500	202.336	155.584	7.301	12.111	**174.996**	**35**	**0.35**	**0.08**
L1 R2	500	243.946	180.342	5.549	27.354	**213.245**	**42.6**	**0.43**	**0.15**
L1 R3	500	117.587	53.379	12.153	5.786	**71.318**	**14.3**	**0.14**	**0.11**
						Mean	**31**	**0.31**	**0.11**
L2 R1	750	389.033	156.520	82.097	7.176	**245.793**	**32.8**	**0.33**	**0.05**
L2 R2	750	274.105	104.996	37.555	11.084	**153.635**	**20.5**	**0.20**	**0.11**
L2 R3	750	435.695	144.601	30.278	5.720	**180.599**	**24.1**	**0.24**	**0.04**
						Mean	**26**	**0.26**	**0.07**

Cd	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	15	10.662	2.512	1.258	0.996	**4.766**	**31.8**	**0.32**	**0.40**
L1 R2	15	4.611	3.972	0.960	0.902	**5.834**	**38.9**	**0.39**	**0.23**
L1 R3	15	8.096	3.016	1.673	0.604	**5.293**	**35.3**	**0.35**	**0.20**
						Mean	**35**	**0.35**	**0.28**
L2 R1	22.5	2.189	5.873	3.410	0.115	**9.399**	**41.8**	**0.42**	**0.02**
L2 R2	22.5	3.448	9.048	1.721	1.558	**12.327**	**54.8**	**0.55**	**0.17**
L2 R3	22.5	8.981	6.881	3.256	1.902	**12.039**	**53.5**	**0.54**	**0.28**
						Mean	**50**	**0.50**	**0.16**

Cu	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	600	289.332	200.170	8.814	7.005	**215.989**	**36**	**0.36**	**0.03**
L1 R2	600	151.849	166.280	6.635	8.683	**181.599**	**30.3**	**0.30**	**0.05**
L1 R3	600	131.545	125.513	15.790	6.945	**148.248**	**24.7**	**0.25**	**0.06**
						Mean	**30**	**0.30**	**0.05**
L2 R1	900	176.495	221.679	159.06	7.152	**387.893**	**43.1**	**0.43**	**0.03**
L2 R2	900	302.664	190.880	51.742	10.210	**252.832**	**28.1**	**0.28**	**0.05**
L2 R3	900	344.057	255.671	33.675	6.855	**296.201**	**32.9**	**0.33**	**0.03**
						Mean	**35**	**0.35**	**0.04**

V	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	250	87.146	94.568	11.229	1.854	**107.651**	**43.1**	**0.43**	**0.02**
L1 R2	250	100.560	61.777	4.624	2.047	**68.448**	**27.4**	**0.27**	**0.03**
L1 R3	250	125.623	100.129	16.900	0.495	**117.524**	**47**	**0.47**	**0.005**
						Mean	**39**	**0.47**	**0.005**
L2 R1	375	103.852	38.675	38.776	1.706	**79.157**	**21.1**	**0.21**	**0.04**
L2 R2	375	78.266	65.334	27.997	1.085	**94.416**	**25.2**	**0.25**	**0.02**
L2 R3	375	99.661	68.442	51.607	0.928	**120.976**	**32.3**	**0.32**	**0.01**
						Mean	**26**	**0.26**	**0.02**

As	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	50	18.934	5.228	1.996	0.415	**7.639**	**15.3**	**0.15**	**0.08**
L1 R2	50	3.959	4.812	0.737	0.482	**6.031**	**12.1**	**0.12**	**0.10**
L1 R3	50	32.202	11.692	3.208	0.277	**15.177**	**30.4**	**0.30**	**0.02**
						Mean	**19**	**0.19**	**0.07**
L2 R1	75	38.651	10.876	12.611	0.331	**23.818**	**31.8**	**0.32**	**0.03**
L2 R2	75	31.883	8.310	10.882	0.572	**19.764**	**26.4**	**0.26**	**0.07**
L2 R3	75	39.447	5.637	10.640	0.240	**16.518**	**22**	**0.22**	**0.04**
						Mean	**27**	**0.27**	**0.05**

(*continued on next page*)

Table 6 (*continued*)

Ni	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
Zn	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	1500	310.663	402.335	35.774	40.008	**478.117**	**31.9**	**0.32**	**0.10**
L1 R2	1500	299.743	359.696	22.396	31.473	**413.565**	**27.6**	**0.28**	**0.09**
L1 R3	1500	194.374	105.739	28.243	23.913	**157.895**	**10.5**	**0.11**	**0.23**
						Mean	**23**	**0.24**	**0.14**
L2 R1	2250	622.249	142.119	43.809	20.801	**206.729**	**9.2**	**0.09**	**0.15**
L2 R2	2250	742.851	202.665	40.223	21.596	**264.484**	**11.8**	**0.12**	**0.11**
L2 R3	2250	903.309	90.572	23.356	17.749	**131.676**	**5.8**	**0.06**	**0.20**
						Mean	**9**	**0.09**	**0.15**
Pb	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	1000	734.487	90.025	32.663	8.883	**131.571**	**13.2**	**0.13**	**0.10**
L1 R2	1000	842.996	83.891	27.932	10.044	**121.867**	**12.2**	**0.12**	**0.12**
L1 R3	1000	408.596	111.553	165.90	2.539	**279.995**	**27.9**	**0.28**	**0.02**
						Mean	**18**	**0.18**	**0.08**
L2 R1	1500	743.945	198.791	144.03	7.428	**350.249**	**23.3**	**0.23**	**0.04**
L2 R2	1500	807.264	91.556	54.002	10.887	**156.445**	**10.4**	**0.10**	**0.12**
L2 R3	1500	1087.293	85.806	48.503	3.414	**137.723**	**9.2**	**0.09**	**0.04**
						Mean	**14**	**0.14**	**0.07**
Hg	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	5	1.316	1.294	0.425	0.366	**2.085**	**41.7**	**0.42**	**0.28**
L1 R2	5	1.852	1.860	0.500	0.272	**2.632**	**52.6**	**0.53**	**0.15**
L1 R3	5	0.386	1.434	0.096	0.039	**1.569**	**31.4**	**0.31**	**0.03**
						Mean	**42**	**0.42**	**0.15**
L2 R1	7.5	3.672	1.903	0.183	0.199	**2.284**	**30.4**	**0.30**	**0.10**
L2 R2	7.5	2.611	1.852	0.477	0.803	**3.132**	**41.8**	**0.42**	**0.43**
L2 R3	7.5	2.238	3.204	0.650	0.770	**4.623**	**61.6**	**0.62**	**0.24**
						Mean	**45**	**0.45**	**0.26**

rh = rhizone, cu = culm, le = leave.
L1R1 = Level 1 Replicate 1.
L1R2 = Level 1 Replicate 2.
L1R3 = Level 1 Replicate 3.
L2R1 = Level 2 Replicate 1.
L2R2 = Level 2 Replicate 2.
L2R3 = Level 2 Replicate 3.

[a] The metal concentration found in topsoil 2 was subtracted (e.g. Ni: 30 μg/g in topsoil 2, spiked with 500 μg/g, total 530 μg/g, but 530–30 = 500 μg/g, this is the initial concentration in soil).

Our results is in line of others data reported in scientific literature, where Fiorentino et al., [2016] showed excellent bioaccumulation capability of *A. donax* for Pb; Chary et al., [2008], Guo e Miao, [2010], Fiorentino et al., [2016] have highlighted how *A. donax* is able to grow in a soil with high concentrations of Ni and Cd, and the obtained values of BAF and TF were major of 1 for both metals [Fiorentino et al., 2013]. Fiorentino et al., [2013] showed excellent bioaccumulation capability of *A. donax* for Ni and Cd in open field conditions with compost fertilization and inoculations of *T. harzianum*. In these studies, the exposure time of the plant to metals was greater than ours and in fact both the BAF and TF were greater than 1. Therefore, it is possible to assert that *A. donax* shows good phytostabilization capability in the short-term, while in the long-term can be used in phytoextraction processes.

Finally, our results proved the suitability of the *A. donax* for assisted-phytoremediation with the use of *T. harzianum*, after the exposures at two levels of the heavy metals mixtures, and so, this technique could be use for a future application of the soil bioremediation in open field.

4. Conclusion

In the first part of our study we verified the growth (reporting the weight of the biomass) and the bioaccumulation (reporting the amount of metal per dry mass) of each of the three microorganisms used in our study. After our *in vitro* tests *T. harzianum* was chosen, both for the bioaccumulation capability of some of the metals to which it was exposed, and for its natural characteristics (resistance to pathogens, environmental factors, ability to establish symbiosis with the root system of the plant). In the second part, the ability of *A. donax* and mycorrhized *A. donax* with *T. harzianum* to be used successfully in

Table 7
BAF and TF for mycorrhized *A. donax* with *T. harzianum*.

Ni	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	500	311.037	22.793	15.895	9.117	**47.805**	**9.6**	**0.10**	**0.40**
L1 R2	500	316.494	123.433	7.020	2.087	**132.539**	**26.5**	**0.27**	**0.02**
L1 R3	500	245.670	184.785	19.511	26.188	**230.485**	**46.1**	**0.46**	**0.14**
						Mean	**27**	**0.28**	**0.19**
L2 R1	750	185.230	192.269	61.103	13.958	**267.330**	**35.6**	**0.36**	**0.07**
L2 R2	750	110.142	196.816	79.895	18.709	**295.420**	**39.4**	**0.39**	**0.10**
L2 R3	750	134.677	197.331	80.140	24.239	**301.710**	**40.2**	**0.40**	**0.12**
						Mean	**38**	**0.38**	**0.10**

Cd	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	15	5.543	3.048	2.456	1.223	**6.727**	**44.8**	**0.45**	**0.40**
L1 R2	15	6.833	5.021	1.496	0.173	**6.690**	**44.6**	**0.45**	**0.03**
L1 R3	15	8.134	3.435	1.323	1.723	**6.481**	**43.2**	**0.43**	**0.50**
						Mean	**44**	**0.44**	**0.31**
L2 R1	22.5	8.795	8.135	2.691	1.076	**11.902**	**52.9**	**0.53**	**0.13**
L2 R2	22.5	11.236	4.047	3.659	3.836	**11.542**	**51.3**	**0.51**	**0.95**
L2 R3	22.5	16.916	1.867	1.152	1.884	**4.903**	**21.8**	**0.22**	**1.01**
						Mean	**42**	**0.42**	**0.70**

Cu	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	600	227.295	208.90	7.966	5.862	**222.726**	**37.1**	**0.37**	**0.03**
L1 R2	600	313.946	174.53	10.170	4.262	**188.964**	**31.5**	**0.31**	**0.02**
L1 R3	600	208.249	204.55	32.762	7.262	**244.571**	**40.8**	**0.41**	**0.04**
						Mean	**36**	**0.36**	**0.03**
L2 R1	900	223.071	201.09	64.867	23.588	**289.548**	**32.2**	**0.32**	**0.12**
L2 R2	900	208.390	131.25	72.632	14.350	**218.229**	**24.2**	**0.24**	**0.11**
L2 R3	900	229.012	211.12	54.367	10.648	**276.132**	**30.7**	**0.31**	**0.05**
						Mean	**29**	**0.29**	**0.09**

V	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	250	159.075	32.198	7.501	0.759	**40.458**	**16.2**	**0.16**	**0.02**
L1 R2	250	184.855	38.032	7.873	0.360	**46.266**	**18.5**	**0.19**	**0.01**
L1 R3	250	194.730	23.448	7.385	6.721	**37.103**	**14.8**	**0.15**	**0.29**
						Mean	**16**	**0.17**	**0.11**
L2 R1	375	96.832	81.210	12.683	8.393	**102.286**	**27.3**	**0.27**	**0.10**
L2 R2	375	82.283	30.244	15.789	10.551	**56.584**	**15.1**	**0.15**	**0.35**
L2 R3	375	83.071	37.816	21.383	9.753	**68.952**	**18.4**	**0.18**	**0.26**
						Mean	**20**	**0.20**	**0.24**

As	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	50	20.313	8.155	1.452	0.260	**9.866**	**19.7**	**0.20**	**0.03**
L1 R2	50	20.574	5.591	1.751	0.157	**7.499**	**15**	**0.15**	**0.03**
L1 R3	50	39.066	3.281	3.252	0.946	**7.480**	**15**	**0.15**	**0.29**
						Mean	**17**	**0.17**	**0.12**
L2 R1	75	17.174	3.201	1.862	4.199	**9.262**	**12.3**	**0.12**	**1.31**
L2 R2	75	9.968	9.452	14.332	1.900	**25.684**	**34.2**	**0.34**	**0.20**
L2 R3	75	18.106	9.707	4.721	1.578	**16.006**	**21.3**	**0.21**	**0.16**
						Mean	**23**	**0.22**	**0.56**

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Table 7 (*continued*)

Ni	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
Zn	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	1500	401.597	113.32	33.715	22.125	**169.155**	**11.3**	**0.11**	**0.20**
L1 R2	1500	663.730	216.18	25.237	18.263	**259.675**	**17.3**	**0.17**	**0.08**
L1 R3	1500	689.343	82.672	54.981	51	**188.653**	**12.6**	**0.13**	**0.62**
						Mean	**14**	**0.14**	**0.30**
L2 R1	2250	112.072	102.41	27.090	27.275	**156.773**	**7**	**0.07**	**0.27**
L2 R2	2250	126.545	102.46	32.916	25.910	**161.287**	**7.2**	**0.07**	**0.25**
L2 R3	2250	116.715	98.575	54.795	23.737	**177.107**	**7.9**	**0.08**	**0.24**
						Mean	**7**	**0.07**	**0.25**
Pb	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	1000	241.393	236.55	49.392	5.326	**291.267**	**29.1**	**0.29**	**0.02**
L1 R2	1000	295.393	258.55	59.303	2.187	**320.039**	**32**	**0.32**	**0.01**
L1 R3	1000	301.393	376.55	85.55	43.672	**505.772**	**50.6**	**0.51**	**0.12**
						Mean	**37**	**0.37**	**0.05**
L2 R1	1500	444.632	610.21	81.362	176.49	**868.064**	**57.9**	**0.58**	**0.29**
L2 R2	1500	416.806	741.06	95.038	79.701	**915.795**	**61**	**0.61**	**0.11**
L2 R3	1500	465.632	455.21	124.36	59.204	**638.777**	**42.6**	**0.43**	**0.13**
						Mean	**54**	**0.54**	**0.18**
Hg	Initial concentration in soil (μg/g)[a]	Final concentration in soil (μg/g)	Rhizome uptake (μg/g)	Culm uptake (μg/g)	Leave uptake (μg/g)	Total uptake rh + cu + le (μg/g)	Total uptake rh + cu + le (%)	Bioaccumulation factor (BAF)	Translocation factor (TF)
L1 R1	5	1.694	1.139	0.993	0.422	**2.554**	**51.1**	**0.51**	**0.37**
L1 R2	5	1.806	0.949	0.524	0.123	**1.597**	**31.9**	**0.32**	**0.13**
L1 R3	5	2.258	1.312	0.923	0.166	**2.401**	**48**	**0.48**	**0.13**
						Mean	**44**	**0.44**	**0.21**
L2 R1	7.5	1.376	3.169	0.885	0.431	**4.486**	**59.8**	**0.60**	**0.14**
L2 R2	7.5	1.019	3.489	0.801	0.263	**4.552**	**60.7**	**0.61**	**0.08**
L2 R3	7.5	2.087	3.758	0.380	0.382	**4.521**	**60.3**	**0.60**	**0.10**
						Mean	**60**	**0.60**	**0.11**

rh = rhizone, cu = culm, le = leave.
L1R1 = Level 1 Replicate 1.
L1R2 = Level 1 Replicate 2.
L1R3 = Level 1 Replicate 3.
L2R1 = Level 2 Replicate 1.
L2R2 = Level 2 Replicate 2.
L2R3 = Level 2 Replicate 3.

[a] The metal concentration found in topsoil 2 was subtracted (e.g. Ni: 30 μg/g in topsoil 2, spiked with 500 μg/g, total 530 μg/g, but 530–30 = 500 μg/g, this is the initial concentration in soil).

phytoremediation processes was evaluated. Plants did not die after exposure to metal and showed no visible signs of stress. We evaluated the BAF and TF values for each metal to which they were exposed and whether mycorrhization brought an advantage or not.

At the end of the period of exposure to heavy metals, *A. donax* showed good phytostabilization abilities, but from the comparison with other international studies it is possible to assert that *A. donax* and mycorrhized *A. donax* with *T. harzianum* may also be suitable for the phytoextraction process with a longer exposure time. The phytoextraction process has a fundamental advantage, because the contaminants are transported from the soil to the upper parts of the plant (shoots and leaves), unlike the phytostabilization process which only involves the immobilization of contaminants in the soil.

Today, phytoremediation represent a good alternative respect to the chemical-physical soil remediation technologies. So, the future development of phytoremediation strategies will be directed towards the use of eco-friendly technologies with the aim of restituting a commercial and economic value to the degraded areas.

Credit author statement

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Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Appendix A. Supplementary data

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