Phytoremediation potential of the arsenic hyperaccumulator Pteris vittata: preliminary results from a field study

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Abstract: Phytoextraction is a promising technique for the remediation of soils contaminated by metals and metalloids and is proposed as a *green* alternative to conventional remediation methods. This paper reports the preliminary results of a field study carried out to evaluate the potential of the fern *Pteris vittata* for the phytoremediation of arsenic polluted sites. *P. vittata* is a known arsenic hyperaccumulator and its properties have been assessed in a number of studies, mainly at laboratory or glasshouse scale, while few field investigations are reported in the literature. The experimental activity was planned to compare and evaluate the effect of different conditions on plant growth and on as uptake by *P. vittata*. The study area is located in northeastern Italy. During the experimental period, pedoclimatic conditions were shown to affect strongly plant growth as well as As bioaccumulation. The results of two consecutive field trials confirm the phytoextraction ability of *P. vittata* under field conditions, but indicate also that the optimization of agronomic practices is crucial for the success of a phytoextraction application at fullscale. The inoculation of ferns with arbuscular mycorrhizal (AM) fungi seems to have a positive influence on plant growth, while its role on phytoextraction efficiency still remains unclear.

Keywords: Phytoremediation, Phytoextraction, Arsenic, *Pteris vittata*, Soil, Field study

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1. Introduction

Phytoremediation is an emerging green technology for the remediation of contaminated sites.

It is based on the use of plants to remove pollutants from the environment and is proposed as a cost-effective and environmental friendly alternative to conventional methods.

Among the various phytoremediation techniques, phytoextraction is receiving increasing attention, primarily for the *in situ* cleanup of soils and waters contaminated by metals and metalloids. Phytoextraction makes use of plants that have the ability to take up metals through the root system and concentrate them in the shoots; the metal-enriched aboveground biomass can then be harvested and disposed of safely [1-3]

Phytoextraction has several advantages over other remediation techniques, such for example soil excavation and disposal to landfill: relatively low cost, low energy requirements, soil erosion control, preservation or even improvement of soil quality; it is particularly suitable for the remediation of large areas with soils characterized by a diffuse pollution of low-medium level, and can be potentially applied in remote sites. In addition, it is supposed to have a higher public acceptance, due to its positive impact on the landscape in the course of longterm applications [1-3]. One of the limitation of this technique lies just in the long times required for remediation, related to the growth cycles of the plants and dependent on their extraction efficiency and biomass production.

A candidate plant for phytoextraction should be tolerant to the target metal/metalloid, have a high

capacity to accumulate it in the shoots, have an extensive root system and a high biomass yield. Depending on their ability to take up metals, plants that can be used for phytoextraction are defined as accumulators or hyperaccumulators [1-3].

The rising interest in phytoremediation has encouraged the search for novel hyperaccumulating plants, mostly by assessing the accumulation capability of natural populations growing in sites with high metal contamination.

Recently, the Chinese brake fern *Pteris vittata* has been identified as a very efficient arsenic hyperaccumulator [4-5]. Since then, an extensive research has been carried out to assess the potential for phytoextraction of this species, by investigating the extent of As uptake and translocation from roots to fronds, the mechanisms involved and the optimal conditions to improve the efficiency of the process [6-9]. These studies produced mainly comparable results and, as a common conclusion, they suggest that *P. vittata* possesses the features of a very promising plant species for the phytoremediation of As polluted soils: high As tolerance, efficient As extraction from soil into fronds, fast growth rate.

Most investigations however were conducted under laboratory or greenhouse conditions, with plants grown in hydroponic solution or in pots, often with artificially contaminated soils; this means that plants are grown under strictly controlled conditions and that As bioavailability in soil could not reflect the actual bioavailability under field conditions. On the other hand, only limited information is available on the behaviour of *P. vittata* in field-scale applications [10-13].

In this study we report the preliminary results of a small-scale phytoextraction field trial with *P. vittata* conducted in a moderately contaminated site located near Venice (North-eastern Italy), aimed at estimating the feasibility of a full-scale phytoremediation intervention in this site and in similar areas.

Some critical parameters that may affect As uptake were evaluated, such as As levels and bioavailability in soil, physico-chemical characteristics of the soil, fern growth and survival under the local climatic and pedological conditions. The optimization of agronomic practices is just a key point for a successful phytoremediation intervention, since *P. vittata*, actually native to Asia and naturalized in the Mediterranean, is widespread in Italy along the Tyrrhenian coast from Sicily to Liguria [14-15], but is not present in the study area and nearby.

The effect of mycorrhizal symbiosis on both plant growth and phytoextraction efficiency was also investigated. Arbuscular mycorrhizal (AM) fungi are root symbionts present in most plant species, and are known to enhance nutrient absorption and tolerance to biotic and abiotic stress. This has suggested a possible positive role of AM fungi in metal tolerance and uptake, but literature results are not always consistent [16]. Only few studies have investigated the influence of AM fungi on As phytoextraction by *P. vittata* [14, 17-19] and, to our knowledge, no information is available on their effect under field conditions.

2. Materials and methods

2.1. Study area

The study area (S. Giuliano) is located in the mainland of the city of Venice and is included in the contaminated site of national interest (SIN) of Porto-Marghera (Figure 1). Formerly a marshy land, it was reclaimed in the past by filling with both controlled and waste material. As commonly found in the watershed of the Venice lagoon, natural background values of arsenic concentration in soil of this area are higher than the regulatory threshold (20 mg/kg), but the contribution of anthropogenic enrichment is still an open question [20,21], since highly contaminated hot spots have been often found.

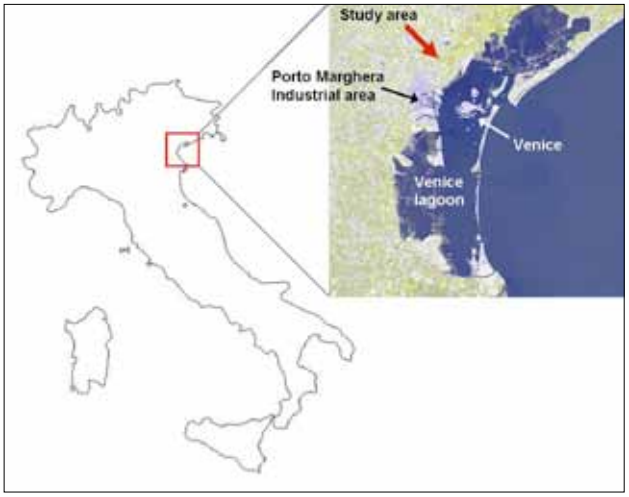


Fig. 1. Location of the study area.

The site is characterized by a silty-clayey soil, with a neutral pH, medium-low cation exchange capacity

and medium-high percent base saturation. Though the high percentage of clay allows for the maintenance of chemical fertility, the poor organic carbon content (about 0.9 %) could affect physical fertility. Details on site characterization are reported elsewhere [22].

2.2. Phytoextraction trial setup

Ferns were propagate from spores as detailed in Trotta et al. [14]. Prior to planting, the experimental site was treated with Glyphosate to remove the present vegetation and tilled.

For the first trial (first year), two plots were prepared at the site, one for mycorrhized plants and one for non-mycorrhized plants, and were covered with a black plastic sheet (Figure 2) After planting, ferns were watered daily with tap water and periodically with a Hoagland nutrient solution. The plots were handweeded when necessary. Ferns were transplanted in late spring and grown at the site until autumn.

Fig. 2. *Pteris vittata* planted at the experimental site.

In the second trial (second year), manure addition was used to increase the organic carbon content of soil and older ferns were used. Four different plots were used for fern transplantation, with respectively: 1) no manure addition, nonmycorrhized plants, 2) no manure addition, mycorrhized plants, 3) manure addition, non-mycorrhized plants 4) manure addition, mycorrhized plants. Ferns were transplanted in spring and grown at the site until winter; they were watered, fertilized and sampled as in the first trial.

2.3. Arsenic analysis in fern samples

The bioaccumulation of arsenic by *P. vittata* was assessed by periodical sampling of fronds for the determination of arsenic concentration.

Samples were washed carefully with deionized water, freeze-dried and homogenized.

Milli-Q (Millipore) water with a resistivity of 18.2 MΩ cm was used for the preparation of reagents and standards. High purity reagents were used for sample digestion. Pyrex, polyethylene and Teflon containers were treated before use with 0.1 M HNO_3 over 48 h and then washed thoroughly with Milli-Q water.

Aliquots of about 0.2 g of plant tissue samples, accurately weighed, were digested completely with a 1:1 mixture of H_2SO_4/HNO_3 in closed Teflon PFA vessels for 30 min at T = 170 °C, using a microwave sample preparation system (CEM MDS 2000). Arsenic determination was performed by hydride generation-atomic absorption spectrometry (HG-AAS) using a Varian SpectrAA-250 Plus spectrometer equipped with a Varian VGA-77 vapor generation accessory.

Quality control was carried out by analyzing the certified reference material BCR-62 (olive leaves).

2.4. Arsenic and metal determination in soil: total concentration and partitioning among soil component

Surface soil samples were collected at planting time, freeze-dried, homogeneized and analyzed for As and metal content.

For total concentration analysis, 0.1 g-aliquots of soil samples were microwave digested with an *aqua regia*/HF mixture for 60 min at T = 170 °C.

Potential bioavailability of metals and arsenic was estimated by means of a sequential extraction procedure (Scheme 1), developed on the basis of the original Tessier procedure [23], modified according to previous studies [24] and to literature procedures [25].

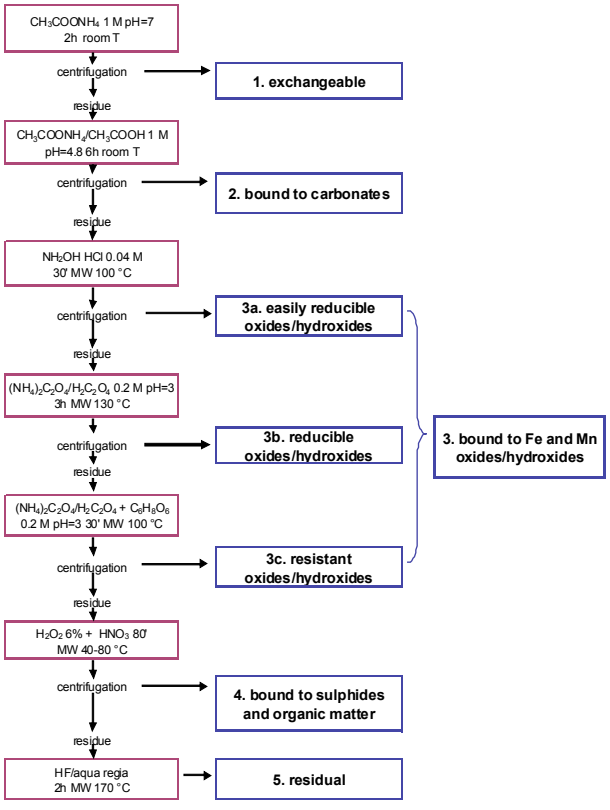
The sequential extraction procedure is designed to partition elements among five operationally defined fractions, according to their association with

soil components: 1) exchangeable, 2) bound to carbonates, 3) bound to Fe and Mn oxides/hydroxides, 4) bound to organic matter and sulphides, 5) residual.

The exchangeable fraction is the most available one for plant uptake and can be released when the ionic strength of the medium changes.

The metal fraction bound to carbonates can be mobilized when the pH is lowered. Changes in the redox state of the soil can cause the release of the metal fraction bound to Fe–Mn oxides/hydroxides (change to reducing conditions) or of the fraction bound to organic matter and sulphides (change to oxidising conditions). Thus, all these fractions are potentially bioavailable as a consequence of changes in environmental conditions. The residual fraction, on the other hand, is not bioavailable and can only be solubilised as a result of weathering.

Analytical determination of As and metals in the solutions obtained after each extraction and after total digestion was carried out by AAS, ICP-MS or ICP-OES, depending on the concentration levels.



Scheme 1. Sequential extraction scheme.

3. Results and discussion

3.1. Arsenic and metals in soil

Metal and As partitioning among the operationally-defined fractions of the adopted sequential extraction scheme was determined for soils collected at two sampling points. Percent distribution among the various fractions is shown in Figure 3 for one sampling point (data for the second one were comparable) , while Table 1 reports total concentrations of metals and arsenic at both sampling points.

The results in Table 1, which reports also the regulatory limits set by the Italian law for the presence of some elements in soils (legislative Decree 152/06), show that the spatial distribution of metal contamination at the experimental site is rather homogenous. The measured Arsenic concentration is higher than the regulatory limit of 20 mg/kg, as expected on the basis of the background values that characterize this area [20, 21].

As can be seen in Figure 3, arsenic is found almost completely in association with oxides / hydroxides of Fe and Mn and is thus potentially bioavailable under reducing conditions. Except for Cr, the other metals are also present mainly in potentially available forms, while only a minor percentage of the total content is found in the residual fraction (not bioavailable).

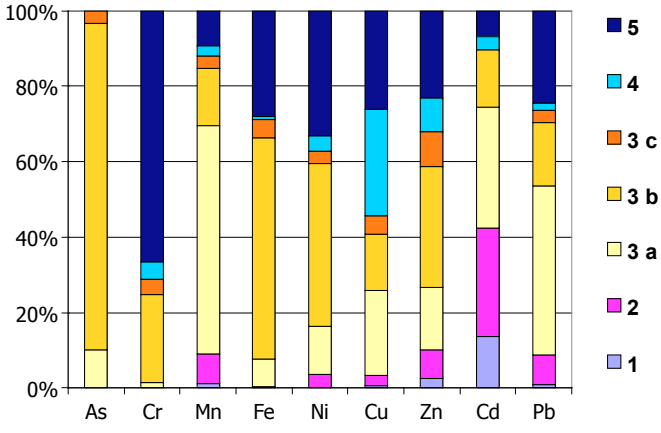


Fig. 3. Percent distribution of As and metals among the operationally defined fractions of the sequential extraction scheme for one sampling point in the study area.

Table 1. Average concentration of metals and arsenic in soil at two sampling points in the study area, and regulatory limits set by the Italian law for the residential (column A) and commercial/ industrial (column B) use of soil.

		mean ± SD [a]	column A	column B
		mg/kg d.w.	*mg/kg d.w.*	*mg/kg d.w.*
As	*1*	49 ± 6	20	50
	2	42 ± 5		
Cr	*1*	55 ± 4	150	800
	2	60 ± 5		
Mn	*1*	670 ± 30		
	2	543 ± 15		
Fe	*1*	37600 ± 1300		
	2	36800 ± 1300		
Ni	*1*	26.0 ± 0.9	120	500
	2	25 ± 1		
Cu	*1*	35 ± 3	120	600
	2	35 ± 2		
Zn	*1*	230 ± 10	150	1500
	2	220 ± 10		
Cd	*1*	0.65 ± 0.05	2	15
	2	0.70 ± 0.05		
Pb	*1*	32 ± 2	100	1000
	2	29 ± 3		

[a] n=3

Before the second phytoextraction trial, manure was added in part of the experimental site to increase organic carbon content of the soil; the resulting arsenic concentration was 35 mg/kg d.w., and As partitioning among sediment components did not show significant differences (results not shown).

3.2. Arsenic bioaccumulation in P. vittata

In the first trial young plants of *P. vittata* (about 6 months old) were used. Though supplying regularly the necessary amounts and water and nutrients, plants did not grow well at the experimental site; during summer in particular, growth was drastically reduced and some plants died. This was ascribed to the adverse pedo-climatic characteristics of the site and not to soil contamination, since in another field study carried out in a nearby location, we found that *P. vittata* was resistant to significantly higher concentrations of arsenic and metals [26]. The high clay and the low organic carbon content of the soil, together with the significant changes in climatic conditions over the experimental period, established unfavourable conditions for plant growth. The high temperature and strong solar radiation in summer produced soil crust formation, which restricted root growth and penetration, while heavy rainfalls in autumn caused excessive water retention, which limited gas exchange and consequently resulted in root asphyxia.

Despite the reduced growth, *P. vittata* showed rather high levels of arsenic bioaccumulation, as can be seen in figure 4; about 100 days after transplantation, concentrations in the fronds were in the range 446-887 mg/kg d.w. (average value 658) for non mycorrhized ferns and in the range 214-882 mg/kg d.w. (average value 503) for the mycorrhized ones. The high variability within each group of plants, however, does not allow to evaluate if mycorrhizzation had a positive effect on arsenic uptake.

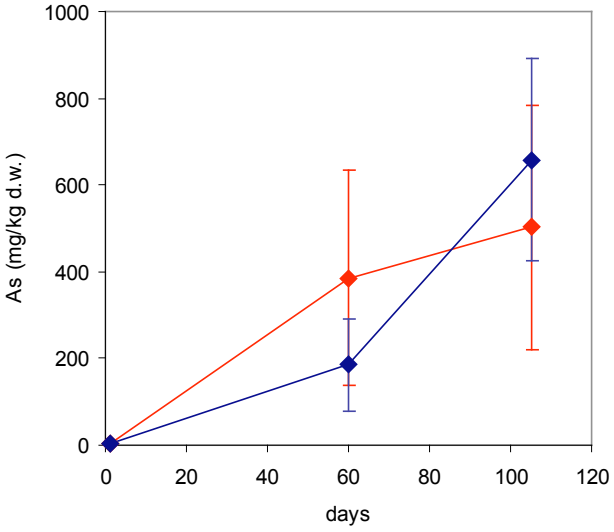


Fig. 4. Average arsenic concentration determined in frond samples collected from mycorrhized plants (red) and non-mycorrhized plants (blue) during the first field trial. Error bars indicate standard deviation (n=4).

In order to improve the efficiency of the phytoextraction process, in the second trial older plants were used (about one-year old), which proved to be more resistant to transplantation and to field environmental conditions.

In the plots where manure was added, ferns grew better and, as can be seen in Figure 5, they also accumulated higher arsenic concentrations in the aerial biomass; after about 5 months of growth in the field, concentrations in the fronds were in the range 249-298 mg/kg^{-1} d.w. (average value 270) for non mycorrhized ferns and in the range 143-378 mg/kg d.w. (average value 298) for the mycorrhized ones, which showed a higher variability.

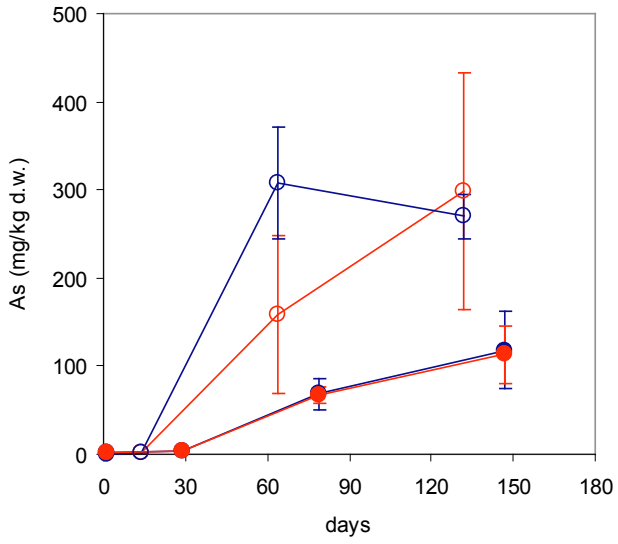


Fig. 5. Average arsenic concentration determined in frond samples collected during the second field trial from mycorrhized plants (red) and non-mycorrhized plants (blue) in the experimental plot with (empty circles) and without (full circles) manure addition. Error bars indicate standard deviation (n=3).

Arsenic uptake was significantly lower in the plots without the addition of manure, where non mycorrhized and mycorrhized ferns behaved quite similarly, with concentrations in the range 90.8-170 mg/kg d.w. (average value 118) and 92.3-151 mg/kg d.w. (average value 113), respectively.

For the second trial as well, it was not possible to point out any effect of fern inoculation with AM fungi on arsenic bioaccumulation, while it seemed to facilitate fern growth.

Samples of senescent fronds, collected at the end of autumn, showed drastically reduced concentrations, down to 20-40 mg/kg, thus giving a useful information about the planning of harvest time in field phytoextraction applications.

The lower concentrations found in the second trial are likely to be associated to a dilution effect due to the increased plant growth and, for the purpose of phytoextraction, they can be counterbalanced by the highest biomass yield.

The levels of bioaccumulated arsenic are in accordance with literature values for moderately contaminated soils [13, 27], and yielded bioaccumulation factors (BF), defined as the ratio of arsenic concentration in the plant tissue to that in the soil) from 2.5 to 14, significantly greater than one, and thus typical of hyperaccumulators. These BF values are far higher than those reported for *P. vittata* (0.45-0.80) by Niazi et al. [12] for an Australian site contaminated by As-containing pesticides, but lower than those found by Kertulis-Tartar et al. (29-45) in a site in Florida contaminated by chromated copper arsenate (CCA), used in the USA as a wood preservative [11]. Gonzaga et al. [27] on the other hand found BF varying in a wide range (4.7-48) when using in a greenhouse experiment six soils with different sources of contamination. This points out the dependence of As phytoextraction efficiency by *P. vittata* on soil properties, among which the most important is As bioavailability.

4. Conclusions

The preliminary results reported in this study confirm that *P. vittata* is an efficient hyperaccumulator under field conditions, but at the same time point out the importance of agronomic practices for the success of a phytoremediation intervention.

The use of older plants is proper, because they show a greater resistance when transplanted in the field; the inoculation with AM fungi seems to promote plant growth, but its effect on arsenic uptake by ferns still remains unclear.

Further research is needed for a thorough evaluation of the phytoremediation potential of *P. vittata* under field conditions, taking into account additional parameters such for example the optimal plant density in the field, the number of harvests, the As phytoextraction efficiency after repeated growth cycles.

5. Acknowledgements

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6. References

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