ARSENIC ACCUMULATION, STRESS RESPONSES AND TOLERANCE IN AGROSTIS CASTELLANA: PHYTOREMEDIATION POTENTIAL OF NATIVE FLORA

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ABSTRACT

Significant concentrations of arsenic (As) in mine tailings and surrounding soils suggest that As-contamination is a matter of great concern in the studied mining area. This study aims to evaluate the As contamination, stress responses, tolerance and phytoremediation potential of native flora of As-contaminated tailings in an abandoned mine. The 'Vale das Gatas' mine tailings (Northern Portugal) contain high concentrations of As (541–5,770 mg/kg) and heavy metals such as lead, zinc and copper. Average As concentration in colonizing plants, such as *Agrostis castellana* Boiss. & Reut., range from 13.2 mg/kg in the stems to 30.9 mg/kg in the leaves. Considering the high concentrations of As in the tailings, *A. castellana* may have developed resistance to these metalloid. In this study, two plant populations of *A. castellana* were tested in relation to As(V) ion as a possible damaging agent of root membranes *in vivo*. Stress responses and tolerance to As(V) was tested by a "root growth test" on specimens from the tailings ('tolerant'), with reference to a population collected outside of the mine influence ('sensitive'). The tests were performed in hydroponics, for several As(V) concentrations: 10, 20, 40, 80, 160, 320, 640, 1,280 μM. The tolerance index was determined, as the ratio between the average "maximum roots growth" in the presence of As(V) and the average "maximum roots growth" in the absence of As(V). The results allow to verify tolerance indices significantly higher in specimens that grow on the tailings (59.3% maximum), compared with specimens from non-contaminated areas (29.5% maximum). Arsenic concentrations higher than toxic level in *A. castellana* species, with significant tolerance indices, indicate that internal detoxification tolerance mechanisms might exist. Therefore, this species growing on tailings would be a great advantage in the revegetation of mine areas and can fulfill the objectives of phytostabilization, phytoattenuation, and visual improvement.

Keywords: *arsenate, mine tailings, phytostabilization, root growth test, Vale das Gatas mine*

INTRODUCTION

It is possible to find a wide variety of plant species that can colonize areas highly polluted with heavy metals and metalloids, such as mine tailings or soils degraded and contaminated by mining/industrial activities. Therefore, many studies have been conducted in contaminated mining and industrial areas and in natural metalliferous soils in order to inventory and screen the indigenous species and evaluate their potential for phytoremediation of contaminated soils [1].

The aim of this study is to evaluate the arsenic (As) contamination, stress responses, tolerance and phytoremediation potential of native flora of As-contaminated tailings in the abandoned ‘Vale das Gatas’ mine (Northern Portugal).

MATERIALS AND METHODS

Study area

The abandoned ‘Vale das Gatas’ mine is located in northern Portugal (Figure 1). The principal types of rocks of the region are Neoproterozoic – Lower Cambrian metasedimentary rocks of Douro Group (a subdivision of the Dúrico-Beirão supergroup, formerly known as the Schist-Greywacke Complex), Hercynian granites, and mineralized and non-mineralized vein rocks [2], [3]. Tin-tungsten (Sn-W) mineralization is dominant in the study area. The ore mineralogy consists mainly of wolframite. Cassiterite, scheelite, several sulfides (pyrite, chalcopyrite, sphalerite, galena, arsenopyrite, pyrrhotite, stannite, covellite, marcasite), silver (Ag), lead (Pb), and bismuth (Bi) sulfo-salts and native Bi are also presented [2], [3]. The gangue minerals that host mineralization are essentially quartz, fluorite and muscovite.

The deposit was exploited for tungsten and tin by underground mining from 1883 till 1992. The ores were concentrated at the site, and the tailings (which contain high levels of sulfides) were deposited on the ground. The mines were abandoned without any environmental remediation. Exposure of these sulfides to air and water produces acidic water, facilitating the release of contaminants into the surrounding environment [4], [5], [6], [7], [8].

Sampling and sample preparation

Soils, tailings and plants were collected from 69 random sites in the study area. On the selected sites, samples of soil, tailings and plant specimens were collected in an area of approximately 1 m^2. At each location, four random partial soil samples weighing 0.5 kg each were collected from a 0 to 20 cm depth and mixed to obtain a single composite sample [7]. Plant sampling was focused on the whole plant, considering plants of similar maturity and the separation of the different tissue types (leaves, stems and roots). In the laboratory, the soil samples were oven-dried at a constant temperature of 40 ºC, manually homogenized and quartered. Two equivalent fractions were obtained from each quartered sample. One was used for the determination of physico-chemical properties, and the other for chemical analysis. Samples for chemical analysis were sieved using a 2 mm mesh sieve to remove plant matter and were subsequently screened to pass through a 180 µm screen [7]. The vegetal material was washed thoroughly, first in running water followed by distilled water and then oven-dried at a temperature of 50 ºC. When dry, the material was milled into a homogenous powder [7].

Analytical procedures

The determination of total element content in soil samples was performed using a tri-acid digestion (HNO_3 + $HClO_4$ + HF) in open Teflon vessels, followed by atomic absorption spectrophotometry (AAS, Perkin Elmer 2380) for heavy metal, and a hydride generation system (HGS) linked to an AAS device for arsenic [7]. The analytical processes for the plant samples involved digestion with HCl and HNO_3 in open vessels. The analysis was performed in the same way as that for soil samples. Data were collected in triplicate. Certified reference materials were also used [7].

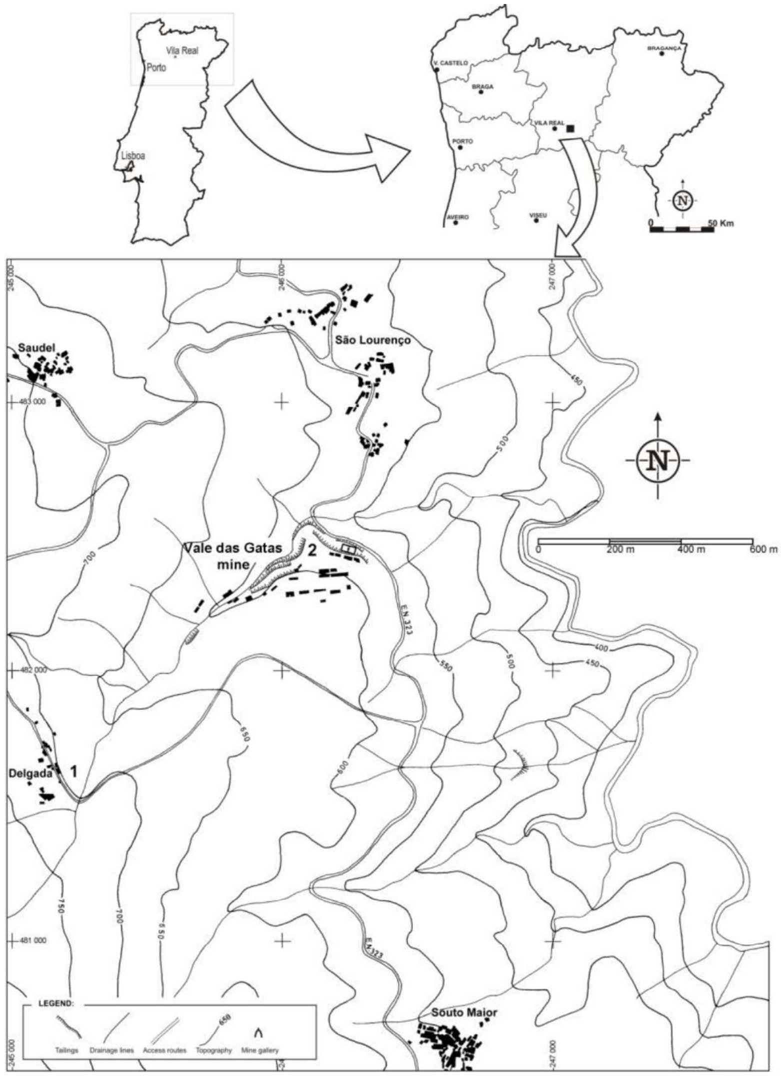

Figure 1. Location of the sampling sites of Agrostis castellana plant 'populations': 1- sensitive population; 2- tolerant population (tailings).

Root growth test

Two plant 'populations' of *A. castellana* were tested in relation to As(V) ion. Responses to As(V) was tested by a "root growth test" on specimens from the tailings ('tolerant'), with reference to a population collected outside of the mine influence ('sensitive'). The tests were performed in hydroponics (Hoagland solution, without phosphate), for several As(V) concentrations: 0, 10, 20, 40, 80, 160, 320, 640, 1,280 μM. The tolerance index was determined, as the ratio between the average "maximum root length" in the presence of As(V) and the average "maximum root length" in the absence of As(V).

RESULTS AND DISCUSSION

As expected, the 'Vale das Gatas' mine tailings contain high concentrations of As (541–5,770 mg/kg) and heavy metals such as lead, zinc and copper (Table 1). Significant concentrations of As in mine tailings and surrounding soils suggests that As-contamination is a matter of great concern in the studied mining area.

Table 1. Total metal(loid)s concentrations in the tailings and surrounding soils from the 'Vale das Gatas' mine.

	Tailings (mg/kg; n = 12)				Soil (mg/kg; n = 57)			
	As	Cu	Pb	Zn	As	Cu	Pb	Zn
Minimum	541	164	584	187	26.7	11.7	55.4	63.1
Maximum	5,770	352	6,299	469	163	330	412	467
Mean	1,963	259	2,152	302	73.4	76.5	129	173
Median	1,322	253	1,455	280	51.8	27.9	94.3	124
S.D.	2,175	75.2	2,372	103	46.6	107	115	130

In contaminated soils and in the tailings the metal(loid)s concentration in plant tissues are high due to the high metal concentrations in the substratum. In general, the content variations in the plants were strongly related to the content variations in the soil samples. *Holcus lanatus*, *Pteridium aquilinum* and *Agrostis castellana* were the main accumulators of As (Figure 2).

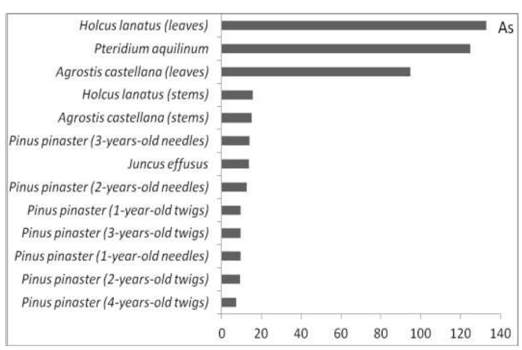

Figure 2. Accumulation of As (mg/kg DW) in plant species of the 'Vale das Gatas' mining area.

The *Pinus pinaster* trees growing on the tailings and contaminated soils also accumulated high quantities of As [7]. In the *P. pinaster* samples from tailings and contaminated soils, the older needles (2- and 3-years-old) show a tendency to accumulate higher concentrations of As [7]. This allowed to conclude that the As concentrations in plants depend as much on the plant organ as on its age and. However, the species showed a great variability in the As accumulation behaviour with the age of the organ.

Considering the high concentrations of As in the tailings, colonizing plants such as *A. castellana* may have developed resistance to these metalloid. In this context, two plant 'populations' of *A. castellana* were tested in relation to As(V) ion as a possible damaging agent of root membranes *in vivo*, for several As(V) concentrations in hydroponic medium with a phosphate-free Hoagland solution. Due to the chemical similarity, phosphate and arsenate can compete either for the same adsorption sites on soil particles and for the same absorption mechanisms by the roots. Stress responses and tolerance to As(V) was tested by a "root growth test" on specimens from the tailings ('tolerant'), with reference to specimens collected outside of the mine influence ('sensitive'). The results (Table 2) allow to verify tolerance indices significantly higher in the 'tolerant population' (59.3% maximum), compared with the 'sensitive population' (29.5% maximum).

Table 2. Root growth test (RGT) results considering the mean values of the maximum root length (MRL) and the tolerance index (TI).

	Sensitive		Tolerant	
Medium	Mean MRL (mm)	TI (%)	Mean MRL (mm)	TI (%)
-P, -As	13.2	—	13.4	—
-P, -As	13.6	—	14.0	—
-P, +As (10 μM)	3.96	29.5	5.90	43.0
-P, +As (20 μM)	3.23	24.1	8.00	59.3
-P, +As (40 μM)	1.47	10.9	5.73	41.7
-P, +As (80 μM)	1.06	7.90	3.37	24.5
-P, +As (160 μM)	1.15	8.50	4.00	29.2
-P, +As (320 μM)	0.59	4.40	3.11	22.6
-P, +As (640 μM)	0.00	0.00	2.59	18.9
-P, +As (1,280 μM)	0.00	0.00	1.60	11.7

-P = phosphate-free; -As = arsenic-free; +As = with arsenic

CONCLUSION

Significant accumulation of As in both soils and native wild flora suggests that As-contamination is a matter of great concern in the studied mining areas. Arsenic concentrations higher than toxic level in *A. castellana* species, with significant tolerance indices, indicate that internal detoxification tolerance mechanisms might exist. Therefore, this species growing on tailings would be a great advantage in the revegetation of mine areas and can fulfill the objectives of phytostabilization, phytoattenuation, and visual improvement. Also, high As concentrations in the other studied species, *H. lanatus*, *P. pinaster*, *P. aquilinum*, indicate their utility for phytoremediation purposes. Furthermore, these plants could grow and propagate in substrata with low nutrient conditions which would be a great advantage in the revegetation of mine tailings. A vegetative cover is one of the most effective and inexpensive way to minimize the erosion of tailings and contaminated soils and attenuate the contaminants dispersion. Therefore should be taken advantage of the potential of colonizing plant species, which are adapted to the existing conditions and are representative of the local flora.

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REFERENCES

[1] Favas P.J.C., Pratas J., Chaturvedi R., Paul M.S., Prasad M.N.V., Tree crops on abandoned mines for environmental remediation and industrial feedstock, Bioremediation and bioeconomy, Amsterdam, Elsevier, pp 219-249, 2016.

[2] Ribeiro A., Ramos J.F., Structural recognition of the tungsten deposit of Vale das Gatas (Vila Real), Technical Report, Serviços Geológicos de Portugal, Lisbon, 1979 (in Portuguese).

[3] Pereira E., Cruz J., Surface geological reconnaissance and prospecting work state in the Vale das Gatas mine, Technical Report, Direcção Geral de Geologia e Minas, Porto 1980 (in Portuguese).

[4] Favas P., Conde L., The impact of an abandoned mine on the quality of surface waters: the example of Vale das Gatas mines (Sabrosa - Vila Real), Recursos Hídricos, vol. 21, pp 31-45, 2000 (in Portuguese).

[5] Favas P., Restrictions to the farming use of the soils in the surroundings of an abandoned mine (Vale das Gatas Mines, Sabrosa, Vila Real), Revista de Ciências Agrárias, vol. 25, pp 280-292, 2002 (in Portuguese).

[6] Favas P.J.C., Pratas J.A.M.S., Conde L.E.N., Bioaccumulation of heavy metals in plants that colonize tailings (Vale das Gatas mine – Vila Real – North of Portugal), Studies on Environmental Contamination in Iberian Peninsula, Lisbon, Instituto Piaget, pp 259-272, 2002 (in Portuguese).

[7] Favas P.J C., Pratas J., Prasad M.N.V., Temporal variation in the arsenic and metal accumulation in the maritime pine tree grown on contaminated soils, Int. J. Environ. Sci. Technol., vol. 10, pp 809-826, 2013.

[8] Favas P.J.C., Sarkar S.K., Rakshit D., Geochemical fractionation of trace elements in stream sediments contaminated by Mining activity, Clean – Soil, Air, Water, vol. 43 (3), pp 446-455, 2015.