CHEMICAL ELEMENTS VARIATION IN LEAVES WITH DIFFERENT DEVELOPMENT STAGES OF *CISTUS* PLANTS FROM S. DOMINGOS MINE AREA, SOUTH PORTUGAL

M. J. Batista[1], O. Gonzalez-Fernandez[2], M. M. Abreu[3], L. Carvalho[4]

[1]LNEG, Estrada da Portela, Ap. 7586, 2721-866 Alfragide, Portugal, joao.batista@ineti.pt;

[2]X-Ray Analytical Applications Laboratory, Institute of Earth Sciences "Jaume Almera"-CSIC, Luís Solé i Sabarís, s/n, 08032 Barcelona, Espanha, ogonzalez@ija.csic.es;

[3]Unidade de Investigação de Química Ambiental, Instituto Superior de Agronomia, Universidade Técnica de Lisboa (TULisbon), 1349-017 Tapada da Ajuda, Portugal, manuelaabreu@isa.utl.pt;

[4]CFA-UL, Av. Prof. Gama Pinto, 21649-003 Lisboa, Portugal

Abstract

Soils and leaves of *Cistus ladanifer L.* and *Cistus salviifolius L.* in different stages of development (young and mature) were sampled in different sites of the S. Domingos mine. The soils are thin and were developed on heterogeneous materials of metallurgical slags, gossanous materials and weathered host rocks. In general, mature leaves have higher concentrations of As, Cu, Fe, Mn, Pb and Zn than young leaves. Nevertheless, in Moitinhos site the young leaves have higher concentrations of As, Cu and Mn than mature leaves. Near the mine buildings *C. ladanifer* leaves have higher concentrations of As in mature leaves than *C. salviifolius.* The Pb concentrations in mature leaves are significantly higher and *C. ladanifer* contain two fold more than *C. salviifolius* leaves. *Cistus* plants show different behaviour on the trace elements uptake and translocation. This knowledge is useful in order to implement remediation programs in mine areas of the Mediterranean region using these pioneer plants.

Keywords: Pyrite abandoned mine; *Cistus ladanifer*; *Cistus salviifolius*; Phytoremediation.

Resumo

Solos e folhas novas e maduras de *Cistus ladanifer L.* e de *Cistus salviifolius L.* foram amostrados em diferentes locais da mina de S. Domingos. As plantas cresciam em solos esqueléticos desenvolvidos sobre escórias, materiais de *gossan* e rochas encaixantes alteradas. Geralmente, as folhas maduras apresentavam concentrações mais altas de As, Cu, Fe, Mn, Pb e Zn, mas no monte de Moitinhos as folhas jovens apresentavam concentrações mais elevadas de As, Cu e Mn. Junto às oficinas os valores de As nas folhas maduras de *C. ladanifer* são mais elevados do que os do *C. salviifolius.* Também o Pb apresentava concentrações mais altas (cerca do dobro) nas folhas maduras do *C. Ladanifer.* As duas espécies do género *Cistus* apresentam comportamento diferente relativamente à absorção e translocação dos elementos vestigiais. Este conhecimento é fundamental para o uso destas plantas pioneiras em programas de recuperação de áreas mineiras na região mediterrânica.

Palavras chave: Mina abandonada de pirite; *Cistus ladanifer*; *Cistus salviifolius*; Fitorremediação

Introduction

The EU Strategy for Soil Protection (COM 2002 179) and the Water Framework Directive (2000/60/EC) combined with the Convention on Biological Diversity from the United Nations Environmental Programme 2010 show the importance that the European Community presently addresses to the preservation of soils, waters and local ecosystems. Mining sites through Mine

Waste Directive addressed to the management of wastes from the extractive industries due to old practices of mining brought the attention of the Environmental Authorities to the abandoned mines of European countries, a matter that was mostly handled by Geological Surveys and Universities. Presently, more and more, is pointed out that certain soil remediation practices result in significant impacts on the environment, especially the *ex situ* remediation processes applied in certain contexts. Eco-efficient remediation techniques, such as phytostabilization, with or without specific amendments, are a new tendency as a remediation approach of the mine sites (Mendez and Maier, 2008). However, a detailed knowledge of the mine site is a need for the correct improvement of these remediation strategies. Exploiting the soil-plant relation potentialities could be used to immobilize the potential contaminants at low cost, but the knowledge of site specific conditions (soil characteristics and the native plant species) are essential for the efficiency of the process (Adriano et al., 2004, Mendez and Maier, 2008).

Abandoned mine sites frequently are extreme environments where soils/spoils present low pH, organic matter and nutrients content, high concentrations of hazardous trace elements and in the majority of the cases there also acid mine drainage generation. Some of these soils/spoils are barren. However several examples of spontaneous and even new species that grow on these degraded and contaminated soils are known (Abreu et al., 2008; Batista et al., 2007; Mendez et al., 2007; Santos, 2007). These species that survive in such contaminated environments without any symptoms of toxicity can play an important role on the stabilization of soils and spoils of the mine areas.

The plant species, degree of leaves maturity, climatic conditions of the region, and the transpiration rate facilitate metal translocation to plant top. In some plants the metals tends to be concentrated in the senescent leaves (Dinelli and Lombini, 1996) or be immobilized on roots (Adriano et al., 2004).

The objective of this study was to compare the behaviour of two species of the genius *Cistus* (*C. ladanifer*L. and *C. salviifolius* L.), growing on a polymetallic mine area (S. Domingos), related to the trace elements uptake and translocation from young to mature leaves. This knowledge can be useful in order to implement remediation programs in mine areas of the Mediterranean region using these pioneer plants.

Site Description

S. Domingos Mine, located in the Southeast part of Portugal in the Baixo Alentejo Province, approximately 60 km SE from Beja, is one historical mining centre that date from pre- and roman times. Its particular features lies in the unusual characteristics of the area, showing a unique landscape. S. Domingos Mine belongs to the metallogenetic province of Iberian Pyrite Belt (IPB), which extends from Spain along the south area of Portugal, in Baixo Alentejo Province. The general geology of the mine consists of the Volcano-Sedimentary Complex with acid and basic rocks from Tournaisian age. Webb (1958) described the area as being underlain by Palaeozoic sediments, "comprised mainly of clay-slates with interbedded grits, quartzites and occasional tuffaceous horizons". More recently, mapping identified these Palaeozoic sediments as follows: in the northern part, older formations from Pulo do Lobo Antiform [Gafo Fm. (schists, silts, greywackes acid and basic volcanism) and Represa Fm. (schists, silts, greywackes and quartzwackes)] from the Upper Devonian age, to south in the mine area Phylito-Quartzitic

(PQ) Fm. (phyllites, silts, quartzites and quartzwackes) and Barranco do Homem Fm. (phyllites, silts and greywackes) of same age (Oliveira and Brandão Silva, 1990).

All the recent mining past (in the last 150 years) is an important part of the Portuguese cultural assets. The large area impacted by the mine, was a result of extraction ore techniques during mining operation and makes it one of the most interesting abandoned mines in Portugal (Gaspar, 1998). The main activity of the mine was copper concentrate production. Aside from this, 9.9 Mt of pyrite were processed as an elementary source of sulphur used in the national market (Rego, 1996).

The climate of the region is Mediterranean type with Atlantic and/or Continental influences, characterised by long dry summers and short winters. The annual average air temperature is 17.6 °C, and annual precipitation is 559 mm.

Materials and methods

Leaves samples of *Cistus ladanifer L.* (sites 5, 6, 7, 12 and 16) and *Cistus salviifolius L.* (site 12), with different development stages (young and mature) were collected in S. Domingos mine in different areas located between the open pit and some 500 m south to the two pyrite burning factories (Achada do Gamo) (Fig. 1).

Soils where these plants were developed were also sampled and analyzed for the total concentrations of As, Cu, Fe, Mn, Pb, S and Zn using two different partial extractions. Soils were developed on the following materials: shales and quartzwackes of PQ formation with metallurgical slag materials spread above (sites 5 and 12); Phylito-Quartzite Complex (site 6); heterogeneous materials composed of metallurgical slags, gossanous materials and weathered host rock (Site 7); river bank sediments of the S. Domingos stream (site 16).

Plant leaves were washed several times with tap water followed by distilled water, air dried and finely ground. Three sub-samples of each powdered plant sample were pressed into pellets of 2.0 cm in diameter without any chemical treatment. Each pellet was glued on a Mylar film, attached to a sample holder and placed directly on the X-ray beam of an energy dispersive X-ray fluorescence spectrometer for chemical elemental determination. The spectrometer used for the multi-element measurements of vegetation consists of experimental equipment that has an X-ray tube with W as primary excitation target and equipped with a changeable secondary target in molybdenum. This is a self-constructed system, using a Philips X-ray generator (PW 1140/00/60 3 kV). To reduce the background and improve the detection limits, some special details were taken into account. An absorber in silver was placed between the primary X-ray beam and the secondary target to absorb the low energy side of the bremsstrahlung, which does not contribute to ionise the secondary target, rather contributing for diffusion radiation, increasing the background. Quality control was obtained using the quantitative calculations made through the fundamental parameters method (Rindby, 1989) where the means of three Certified Reference Materials (CRMs) of bush, branches and leaves (GBW07603, GBW07604 and GBW07605 from the National Research Centre for Certified Reference Materials of China) were used. Each sample was measured three times and the mean value and the standard deviation were calculated.

The soil samples were air dried and passed through a 2 mm sieve. Pellets of soil samples (fraction < 2 mm) were elementally analysed using a commercial WDXRF spectrometer (Bruker S4

Explorer), equipped with a Rh anticathode X-ray tube (1 kW power: 50 kV maximum voltage, 20 mA maximum intensity); four analyser crystals (OVO-B, OVO-55, LiF 200 and PET) and a flow proportional counter for light element detection and a scintillation counter for heavy elements.

The method DIN 38414-S4 (Official German DIN method, 1984) was used for leaching chemical elements simulation from soils and analysed by ICP.

Soil available fraction of chemical elements was extracted by DTPA 0.005 M, adjusted at pH 7.6 (Lindsay and Norvell, 1978) and analysed by ICP.

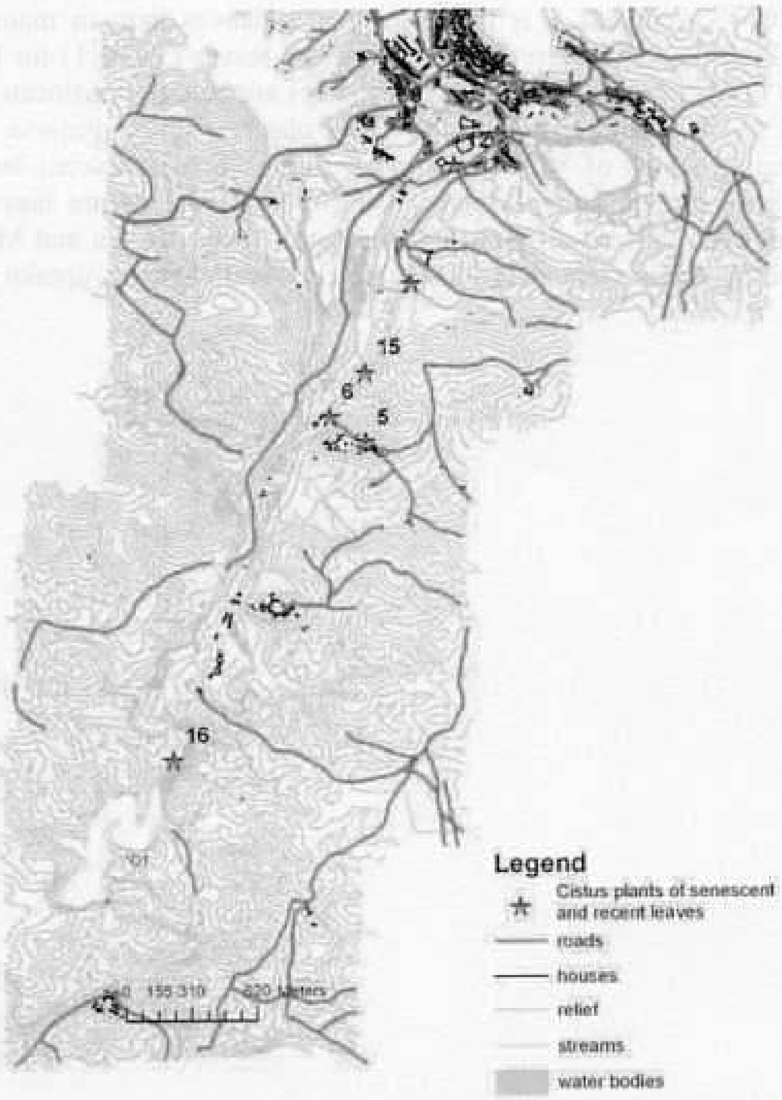

Figure 1 – Soils and *Cistus* plants location at S. Domingos mine

Results and discussion

Total concentrations of chemical elements in soils presented high values, especially for As and

Pb that can attain 6.2 g kg^{-1} and 14.5 g kg^{-1} respectively (Table 1). However, only a small percentage (<1%) of the total concentration were water soluble or extracted with the DTPA solution, meaning that the available fraction of these elements for the plants, and determined by this methodology, was in general low. This can be a consequence of soil characteristics as pH, organic matter content or clay mineralogy that can induce biogeochemical processes such as adsorption, precipitation and redox reactions that may decrease the chemical elements available fraction (Adriano *et al.*, 2004) or even the presence of solid phases with low water solubility and containing trace elements as As, Fe and Pb (Abreu et al., 2009a) which contribute to its immobilization in soil.

The obtained results showed that S is higher in young leaves than in mature leaves for *C. ladanifer*, whereas Fe, Zn and Pb were highest in mature leaves (Table 1) for both species with the exception of Zn in *C. salviifolius*. Both *Cistus* species are able to translocate to mature leaves and accumulate Fe and Pb. Similar results were also observed in *S. armeria, Salix spp* and *P nigra* species in mining spoils of Vigonzano mine, Italy, where senescent leaves have higher concentrations of those elements than young leaves. Young and mature leaves of *C. ladnifer* collected in different soils showed different trends regarding the As, Cu and Mn concentrations. In fact, different soil materials seem to influence the chemical elements uptake and translocation by plants.

Table 1 – Total elemental concentrations of S, Mn, Fe, Cu, Zn, As and Pb in young and mature leaves of *Cistus ladanifer L.* (Cil) and *Cistus Salviifolius L.* (CiV) mg kg^{-1}

Sample name	S	Mn	Fe	Cu	Zn	As	Pb
Soil 5 total concentrations	3324.2	379.5	65809	367.5	265.1	234.8	603.4
Soil 5 DIN	4.0	160.0	1.8	52.0	0.2	11.0	26.0
Soil 5 DTPA	6.39	0.4153	87.23	0.3825	0.2380	0.0820	0.2152
1SD-5Cil young leaves	1996.7	1171.8	90.75	11.84	65.66	6.88	7.92
1SD-5Cil mature leaves	1486.2	2048.0	449.59	15.32	253.03	9.19	19.72
Soil 6 total concentrations	29597	542.1	74210	471.3	249.1	908.9	7463.7
Soil 6 DIN	5.3	763.0	0.0	86.0	0.2	BDL	320.0
Soil 6 DTPA	78.78	1.2550	105.24	0.9214	0.3372	0.1397	5.3930
1SD-6Cil young leaves	1805.2	2859.2	126.03	11.98	109.70	6.46	46.00
1SD-6Cil mature leaves	1783.2	2010.7	509.17	10.00	149.18	4.88	276.10
Soil 7 total concentrations	16821	511.1	83092	1422.0	305.3	1590.5	3156.3
Soil 7 DIN	4.5	33.0	1.2	34.0	0.1	22.0	33.0
Soil 7 DTPA	4.94	0.1635	9.60	0.9409	0.1550	0.6284	0.0888
1SD-7Cil young leaves	2171.4	309.94	63.79	14.34	152.41	6.59	5.55
1SD-7Cil mature leaves	1756.4	124.45	233.92	20.17	221.07	9.45	8.09
Soil 12 total concentrations	15700	387.2	165415	1358.1	696.5	6256.1	14482
Soil 12 DIN	14.9	224.3	0.8	33.7	0.4	63.2	112.7
Soil 12 DTPA	19.01	0.1057	16.25	0.7544	0.3777	0.1453	0.0958
1SD-12Cil young leaves	n a	212	113	13	191	8	3
1SD-12Cil mature leaves	n a	285	716	18	391	16	54
1SD-12CiV young leaves	n a	551	83	28	377	10	2
1SD-12CiV mature leaves	n a	390	212	34	344	9	19
Soil 16 total concentrations	18744	216.8	117924	447.4	160.7	2870.5	4725.1
Soil 16 DIN	43.3	78.7	0.1	261.2	0.2	8.9	1238.0
Soil 16 DTPA	112.20	0.0112	205.85	0.2900	0.0363	0.7543	2.7570
1SD-16Cil young leaves	1986.4	84.58	90.28	7.58	56.35	7.95	7.36
1SD-16Cil mature leaves	1244.2	243.66	386.90	4.12	151.48	8.98	23.94

BDL – below detection limit; na – not analysed; DIN – water soluble fraction; DTPA – available fraction extracted by 0.005 M DTPA solution

Comparing the elements concentration in both leaves type of the two species growing in the same soil (site 12, located near the mechanical facilities of S. Domingos mining buildings, where different materials were deposited) Cu, Fe and Pb presented similar behaviour, but As, Mn and Zn are different (Table 1). This different tendency observed for chemical elements concentration between young and mature leaves may be attributed to the specificity of each species for elements translocation and accumulation due to different plant physiological processes or to the heterogeneity of the substratum where these plants were grown.

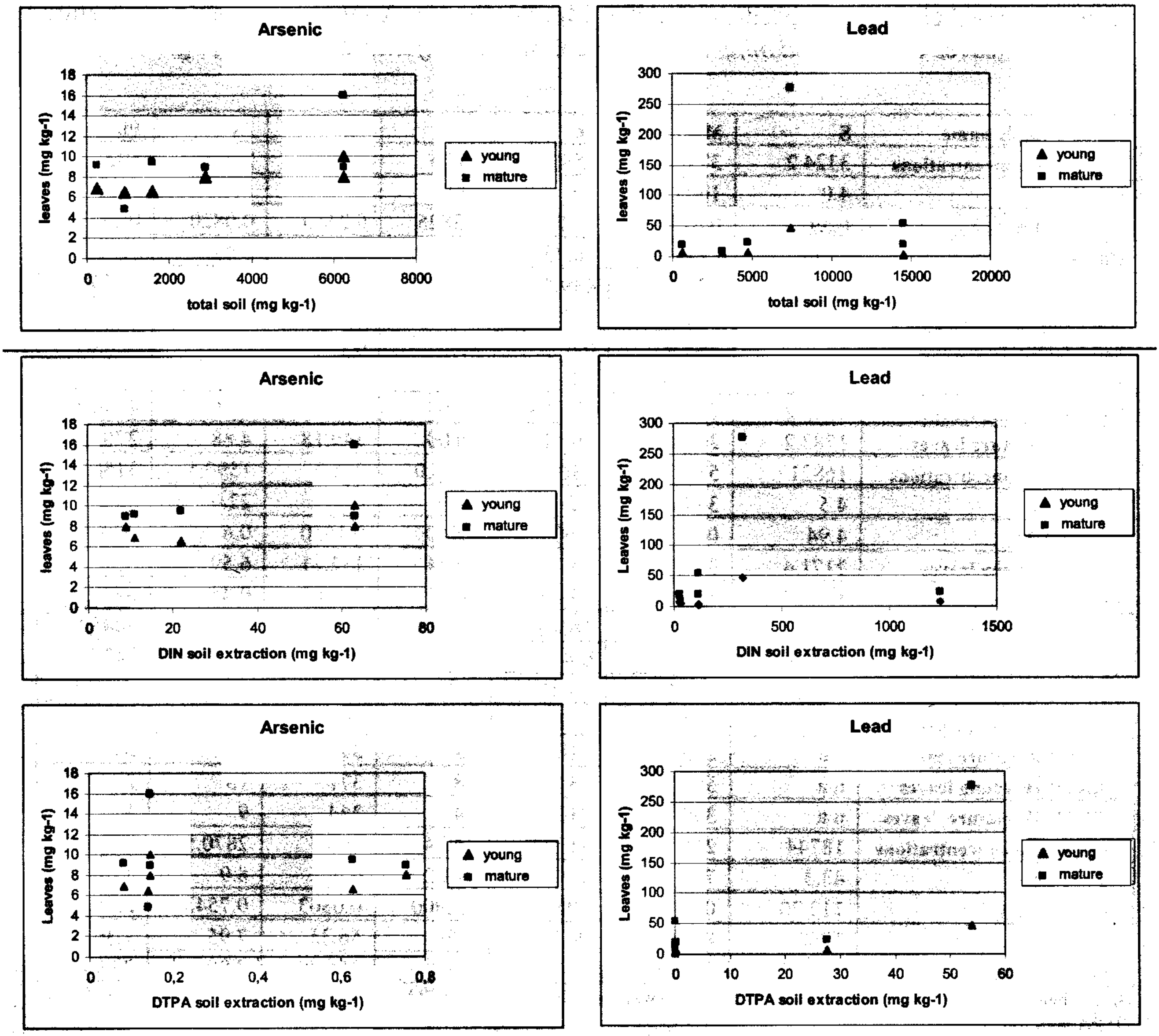

Figure 2 – Scatter plot diagrams of soil-plant relationship of As and Pb concentrations in total soil content, water soluble and DTPA extraction, and young and mature leaves of *C. ladanifer* and in site 12 also *C. salviifolius*.

Significant correlations between chemical elements concentration in leaves and soil (total and available fractions) were not found, however the scatter plots in figure 2 allows to evaluate soil-plant behaviour. The chemical elements content in *C. ladanifer* leaves does not reflect either the total or the available fraction (water soluble and DTPA extracted) of the same element in soil. As and Pb uptake by this plant species seems to be independent on soil content (Fig. 2); mature leaves from *C. ladanifer* plants growing on soil 6 contain five times more Pb than the same leaves collected on soil 12 which contain the double of the total soil Pb concentration (Table 1). The elements absorption by plants involves complex soil-plant-microbes mechanisms and can not only be ascribed to the available soil fraction of the elements determined by chemical methods.

So, other soil processes must be involved in the elements uptake by these plants. In addition, high total concentrations of the elements in soils, in the majority of the cases did not indicate toxicity for plants, because plants growing on the more contaminated soils showed the same vegetative development than the plants growing on soils containing the lowest trace elements concentrations and did not present any symptoms of toxicity. Similar results were obtained by Abreu et al. (2009b), Santos (2007) and Santos et al. (2009) for the same plant species growing on mine areas.

The highest concentrations of As in soil, total (6256 mg kg^{-1}) and water soluble (63 mg kg^{-1}) fractions, were found in site 12 where the two species of *Cistus* were sampled. *C. ladanifer* from this site presented higher concentrations of As in mature leaves than *C. salviifolius*, but in contrast young leaves presented the lower concentrations. Also Pb concentrations in mature leaves were significantly higher than in young leaves, being two fold more in *C. ladanifer* than in *C. salviifolius*. The plant responses to trace elements in the soil vary from species to species and should always be investigated for the particular soil–plant system (Adriano et al., 2004; Kabata-Pendias, 2004). In the present study it is obvious that some of the obtained results need further investigation.

Conclusions

Sampled soils contained high total concentrations of chemical elements, but the available fractions (water soluble and DTPA extracted) only represent a very small fraction of the total (<1%). Mature leaves showed, in general, higher concentrations for the majority of the studied chemical elements than young leaves. Sulphur presented an opposite behaviour. Some elements, like Mn, Zn and As are also substrata and/or species dependent.

The study of other plant species that spontaneously grow in the mine area seems to be necessary and very important, as an intervention for the site phytoremediation can be more efficient if more than one species was used for natural attenuation of mining areas. This knowledge is useful in order to implement remediation programs in mine areas of the Mediterranean region using these pioneer plants.

References

Abreu, M.M., Tavares, T., Batista, M. J. (2008). Potential use of Erica andevalensis and Erica australis in phytoremediation of sulphide mine environments: São Domingos, Portugal, *Journal of Geochemical Exploration* 96, 210-222.

Abreu, M.M., Santos, E.S., Magalhães, M.C.F., Nabais, C. (2009a). Fases portadoras do arsénio em solos da área mineira de São Domingos e em solos não contaminados do Pomarão e Serra do Caldeirão, Revista de Ciências Agrárias XXXI nº.1, 155-169.

Abreu, M.M., Santos, E.S., Anjos, C., Magalhães, M.C.F., Nabais, C. (2009b). Capacidade de absorção do chumbo por plantas do género *Cistus* espontâneas em ambientes mineiros. Revista de Ciências Agrárias XXXI nº.1, 170-181.

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S. (2004). Role of assisted natural attenuation in environmental cleanup. *Geoderma* 122, 121-142.

Batista, M. J., Abreu, M. M., Serrano Pinto, M. (2007) Biogeochemistry in Neves Corvo mining region, Iberian Pyrite Belt, Portugal. *Journal of Geochemical Exploration* 92, 159–176

DIN 38414-S4. (1984). Schlamm und Sedimente, Bestimmung der Eluierbarkeit mit Wasser. DIN Deutsches Institut für Normung, Berlin, 1984.

Dinelli, E., Lombini, A. (1996). Metal distributions in plants growing on copper mine spoils in Northern Apennines. Italy: evaluation of seasonal variations. *Applied Geochemistry* 11, 375-385.

Gaspar, O (1998) História da Mineração dos Depósitos de Sulfuretos Maciços Vulcanogénicos da Faixa Piritosa Portuguesa. *Boletim de Minas Vol.35 N°4 Lisboa.*

Kabata-Pendias, A. (2004). Soil-plant transfer of trace elements-an environmental issue. *Geoderma* **122**, 143-149.

Lindsay, W.L., Norvell, W.A. (1978). Development of a DTPA soil test for zinc. iron. manganese. and copper. *Soil Science Society of America Journal* 42, 421–428.

Mendez, M.O., Glenn, E.P., Maier, R.M. (2007). Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. *Journal Environmental Quality* 36, 245-253.

Mendez, M.O., Maier, R.M. (2008). Phytostabilization of mine tailings in arid and semiarid environments – An emerging remediation technology. *Environmental Health Perspectives* 116, 278-283.

Oliveira, J. T., Brandão Silva. J. (1990). Notícia Explicativa da Carta Geológica à escala 1:50000. Folha 46-D-Mértola. *Serviços Geológicos de Portugal.*

Rego, M. (1996). *Mineração no Baixo Alentejo.* Câmara Municipal de Castro Verde.

Rindby, A. (1989). Software for energy-dispersive X-ray fluorescence. *X-ray spectrometry* 18, 113-118.

Santos, E. (2007). *Potencial de utilização de Cistus ladanifer na vegetalização de áreas mineiras.* Tese de Mestrado em Gestão e Conservação da Natureza, Universidade do Algarve.

Santos, E.S., Ferreira, M.B., Abreu, M.M., Nabais, C. (2009). Contribuição das plantas de *Cistus ladanifer* e de *Cistus salviifolius* para a recuperação ambiental de áreas mineiras abandonadas da Faixa Piritosa. *Revista de Ciências Agrárias*. (*Submitted*)

Webb, J. (1958). Observations on the geology and origin of the S. Domingos Pyrite Deposits. Portugal. *Com. Serv. Geol. Portugal.* Lisboa. T. 42. pp.129-143.