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Co, Cr and Ni contents in soils and plants from a serpentinite quarry

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SED

6, 3361–3391, 2014

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Abstract

Several soils developed on the former serpentinite quarry of Penas Albas (Moeche, Galicia, NW Spain) were studied, together with the vegetation growing spontaneously over them. The aim of this work was to identify the bioavailability of heavy metals and to evaluate the potential of spontaneous vegetation for the phytoremediation and/or phytostabilisation of these areas. The pH of the soils ranges from neutral to basic, with very low organic matter and nitrogen contents. There are imbalances between exchangeable cations that can strongly limit plant production. Moreover, in all of the soils there are high levels of Co, Cr and Ni (> 70, > 1500, and $> 1325\,\mathrm{mg\,kg^{-1}}$, respectively). They exceed the intervention limits indicated in different guides. Different soil extractions were performed in order to evaluate bioavailability. $CaCl_2$ $0.01\,M$ is the most effective extraction reagent, although the reagent that best predicts plant availability is the mixture of low molecular weight organic acids. *Festuca rubra*, L. is the spontaneous plant growing in the soils that accumulates the highest amount of the metals, both in shoot and roots. *Festuca* also has the highest translocation factor values, although they are only > 1 for Cr. The bioconcentration factor is > 1 in all of the cases, except in the shoot of *Juncus* sp. for Co and Ni. The results indicate that *Festuca* is a phytostabilizer of Co and Ni and an accumulator of Cr, while *Juncus* sp. is suitable for phytostabilization. Both of the studied species contribute towards the phytostabilisation of the soils and their recovery, improving their characteristics and making it possible to start planting other species.

1 Introduction

Environmental pollution is a global threat of increasing severity due to urban growth, industrialisation and changing lifestyles. The thesis defended in the "EC Guidance on Undertaking Non-Energy Extractive Activities in Accordance with Natura 2000 Requirements" is to make extractive activities compatible with the natural environment.

Land Degradation is taking place in the world due to soil erosion, deforestation, soil degradation (Biro et al., 2013; de Souza et al., 2013; Mandal and Sharda, 2013; Milder et al., 2013; Zhao et al., 2013) and due to soil pollution (Fernández Calviño et al., 2013; Vacca et al., 2012; Yang et al., 2012). Serpentinite soils offer a stressful environment with low calcium to magnesium ratio, deficiencies of essential macronutrients, high concentrations of heavy metals and low water-holding capacity (Doubková et al., 2012).

5 % of Galicia (Spain) is covered by serpentinitic areas; these sites were formerly quarries from which materials for roads, ballast for railway, and ornamental rock were extracted. The tailings left behind are often a source of contamination. The soils formed on these tailings (Spolic Technosols) must be rehabilitated, as they provide an unsuitable environment for plant growth, are susceptible to weathering and can cause environmental degradation mainly due to their high content of heavy metals and low organic matter and nutrient content.

The ultramafic complex of Ortegal Cape is a group of dominant, acidic and intermediate basic rocks with sedimentary insertions and discontinuous serpentinite bodies, separated from other geological units (Castroviejo et al., 2004).

The Penas Albas quarry is located at Moeche (43° 31′ 42.46″ N, 8° 0′ 35.61″ W), in A Coruña (Galicia, NW Spain). This serpentinite quarry (formed by the metamorphism of ultrabasic rocks) operated between the 1960s and mid-1990s. It left behind a large amount of waste material scattered over the surrounding area, as well as tailing areas.

The quarry produced around $50\,000\,\text{Mg year}^{-1}$ of serpentinite needed in the steel and construction industries (Pereira et al., 2007). The quarry is now abandoned, and hardly any rehabilitation work has been carried out.

Spolic Technosols from this quarry are very young soils that form over unstable materials with low cohesion and physical, chemical and biological deficiencies; this is due to their low nutrient and organic matter content, and a high content of heavy metals, which limits the development of bacteria, plants and animals (Deng et al., 2006; Ali et al., 2013).

The lack of nutrients and anomalous physicochemical properties means that the establishment of plant cover is strongly limited in these areas, favouring the accelerated weathering of the soil (Mendez and Maier, 2008). In addition, the limited plant cover contributes to the migration of heavy metals that contaminate surface and underground waters.

The depositing of waste materials from quarries, where ultrabasic rocks such as serpentinite are mined, leads to environmental degradation and soil contamination, because large amounts of material are extracted. This affects the environment by altering or destroying the topography, soil, plant cover, hydrology, fauna, microclimate and landscape, making it either extremely difficult or impossible to recover previously existing conditions (Mendez and Maier, 2008).

In the soils from these types of tailings, the levels of Cu, Co, Cr and Ni are usually high, and there is also a deficiency of essential nutrients for plants, such as nitrogen, phosphorus and potassium. Therefore, the recovery of serpentinite quarry soils must not only consist of eliminating or immobilising the contaminants, but also of improving the quality and fertility of the soils.

It is well known that using the total content to evaluate heavy metal contamination and to determine the toxicity level is not enough, as the total concentration includes all of the chemical forms that exist in the soil; neither does it provide any information on the mobility, availability and toxicity of the metals (Adamo et al., 2002; Pueyo et al., 2004).

This means that it is essential to know the available content which can interact with an organism and become incorporated into its structure. This content depends on a large number of factors, which include the properties of the contaminating element and the soil (Naidu et al., 2008).

Physicochemical and biological methods, such as precipitation–flocculation coupled with pre/post-oxidation, reduction and concentration, have all been studied in order to decontaminate soils with a high content of heavy metals and to preserve the environment; they are also often employed to control environmental pollution. These

techniques, known as "removal-disposal", have numerous drawbacks, such as their high cost, low efficiency, lengthy and complex treatments for a wide variety of metals, and the formation of large amounts of toxic sub-products (Adki et al., 2013). Consequently, processes based on "recovery–reuse" are now being increasingly projected and used (Agrawal et al., 2006). Phytoremediation could avoid some of the problems of the aforementioned treatments, as it is a harmless procedure that respects the environment (Adki et al., 2013; Ali et al., 2013; Paz-Ferreiro et al., 2014).

Therefore, it is of great interest to study and analyse the plants that grow spontaneously in these zones; due to their adaptation to the high concentrations of certain metals present in these soils, together with other limiting factors for plant growth, they may provide an indication of the procedure to apply in the restoration process.

Hyperaccumulator plants are able to grow in these soils, as they have an extraordinary ability to absorb metals; but their efficiency may be limited due to the low bioavailability of the metals in the soils (Knight et al., 1997; Ali et al., 2013). These plants have unique characteristics, such as the ability to absorb and translocate metals from their roots to their shoot, and a high tolerance. Hyperaccumulators normally have little biomass, because they need a great deal of energy for the mechanisms required to adapt to the high concentrations of metals in their tissues (Garbisu and Alkorta, 2001).

The ideal plant for phytoextraction should be capable of growing in soils with large amounts of metals; it should also have a large radicular system and high levels of biomass production based on optimum growth and development, and be able to accumulate high concentrations of metals in its shoot, store several different metals at the same time and be resistant to pests and diseases (Garbisu and Alkorta, 2001).

Phytoextraction reduces the metal content of the bioavailable fraction of soils, and so this technique is used to reduce the damage caused to the environment (Martin and Ruby, 2004; Ali et al., 2013). When phytoextraction is not possible, phytostabilisation should be carried out. This consists of fixing the metals in the soil, stabilising contaminated soils and reducing the flow of contaminants into the environment. Plant

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cover also protects against weathering, thus reducing the risk of water infiltration and metals reaching aquifers. In the phytostabilisation process, plants do not accumulate metals in their shoots, limiting the risk in terms of food safety (Garbisu and Alkorta, 2001; Ali et al., 2013).

5   In light of these issues, the aims of this study were: (a) to verify which is the ideal extractant to determine the phytoavailability of the heavy metals contained in soils from a former serpentine mine and (b) to evaluate the phytoremediation/phytostabilisation capacity of the spontaneous vegetation growing in these soils.

2   Material and methods

10   ### 2.1   Material

The study area is located in the Penas Albas serpentinite quarry (43° 31′ 42.46″ N, 8° 0′ 35.61″ W) (Moeche, Coruña, Spain).

Four zones were selected (Fig. 1): three in different quarry spoils (S1, S2 and S3) and one (S4) in the cut zone (natural soils, whose parent matter is the living rock: serpentinite). The control soil (CS) was sampled outside the quarry, in an area which has been reforested and treated with fertilizer and animal manure (Fig. 1, Table 1).

In each selected area, three sub-areas were selected with different degrees of plant cover and diversity, as well as different degrees of slope, so that they represent the whole of the study area. *Festuca rubra* L. and *Juncus* sp. L. were chosen for this study because they are the most abundant species.

20   In each of the sub-areas, three surface soil (20 cm) samples were collected using an Eijkelkamp sampler and then stored in polyethylene bags. The soil samples from each subarea were pooled, air dried, sieved (2 mm), and homogenized in a Fritsch Laborette rotary sample divider, thus obtaining a composite sample of each sub-area. Each one of these composite samples was divided into three sub-samples to perform different analyses.

The soil profiles were described according to the FAO (2006) guidelines and the descriptions are shown in Table 1. The soil colours were determined using revised standard soil colour charts.

In each zone, several specimens of *Festuca rubra* and *Juncus* sp. were sampled. In the laboratory, the specimens were washed several times with bidistilled water to remove the remaining soil particles adhered to their surface. Subsequently, the roots and shoots were separated and dried in an oven at 60 °C until reaching constant weight. Afterwards they were crushed and stored in hermetically sealed polythene bags, ready for use.

Three sub-samples per soil and plant were finally used for all of the analytical measurements, meaning that all of the analyses were performed in triplicate.

2.2 Methods

Soil pH, Kjeldahl-N, and organic C (TOC) were determined, respectively, with a pH electrode in 2 : 1 water/soil extracts, according to Bremner and Mulvaney (1982) and following the Walkley and Black (1934) procedure. The Fe, Mn and Al oxide contents were determined using the dithionite-citrate method. The concentration in the extract was determined by ICP-OES in a Perkin Elmer Optima 4300 DV apparatus. The effective cation exchange capacity (ECEC) and exchangeable cation content were determined according to Hendershot and Duquette (1986). Al, Ca, K, Mg and Na were extracted with 0.1 M $BaCl_2$, and their concentrations were determined by ICP-OES as above.

Particle size distribution was determined after oxidising the organic matter with H_2O_2, separating the upper fraction (50 mm) by sieving and using the lower fraction in the internationally endorsed procedure.

The total metal content was analysed by the fusion method with $Li_2B_4O_7$-$LiBO_2$, mixing 0.5 gr of the sample with 3.5 of $Li_2B_4O_7$-$LiBO_2$ flux (50/50 w/w) and 0.1 g of LiI in a platinum crucible. The mixture was fused in a propane-beader Claisse (Perl induction heating machine) for 20 min. The content of the crucibles was hot-poured

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into Teflon precipitate flasks containing 100 mL of HNO_3 and then magnetically shaken to help dissolve the fused mixture, which was then transferred to a 500 mL flask and made up to volume with 5 % HCl. The final solution was analysed by ICP-OES and the control was a standard aqueous multi-element dissolution.

In order to determine the bioavailable Co, Cr and Ni content in soils, five extractants were selected – specifically, the most widely used by numerous authors. In accordance with Houba et al. (2000), soil samples were extracted with 0.01 M $CaCl_2$; EDTA (0.01 M Na_2-EDTA + 1 M CH_3COONH_4) was used following AFNOR (1994). Extractions with DTPA (0.005 M DTPA + 0.1 M TEA + 0.01 M $CaCl_2$) were done in accordance with Lindsay and Norvell (1978). Bidistilled water (BDW) was used following Pueyo et al. (2004) and a 10 mM mixture of five different low molecular weight organic acids (LMWOA) was used according to instructions by Feng et al. (2005). The LMWOA composition was acetic, lactic, citric, malic, and formic acids with a molar concentration ratio of 4 : 2 : 1 : 1 : 1, respectively. In all the extractions the concentration of Co, Cr and Zn was determined by ICP-OES.

The selected plants (shoot and root) were also analysed for total Co, Cr and Ni contents after being extracted with H_2O_2 and HNO_3 in a microwave oven. As above, the Co, Cr and Ni concentrations in the supernatant were analysed by ICP-OES.

The extraction efficiency (EF) of each of the extractants was estimated by the proportion of the total content extracted by each one as given below:

$$EF = 100 C_e / C_t$$

where C_e and C_t are the metal extracted and total metal content ($mg\,kg^{-1}$).

The translocation factor (TF) was estimated as the ratio between the trace metal content ($mg\,kg^{-1}$) in shoot (C_s) and the one in the roots (C_r):

$$TF = C_s / C_r$$

TF > 1 indicates that the plant translocates metals effectively from the root to the shoot (Baker and Brooks, 1989).

The ratio of metal concentration in the plant to soil was used to determine the bioconcentration factor (BF):

$$BF = C_p/C_{so}$$

where C_p and C_{so} are metal concentrations in the plant (shoot and root) and in the soil, respectively. Hyperaccumulator plants are used to show BF values greater than 1 and sometimes even ranging from 50 to 100 (McGrath, 1998).

2.3 Statistical analysis

The data obtained in the analytical determinations were analysed with the statistical program IBM-SPSS Statistics 19 (SPSS, Inc., Chicago, IL). The results obtained in all the determinations were the average with the standard deviation of three analyses and were expressed on a dry material basis. Different analyses of variance (ANOVA) were carried out, together with homogeneity of variance tests for the variables found. In case of homogeneity of variance, the minimum significant distance test among soil properties was carried out as a post-hoc test, or otherwise Dunnett's T3 test. A bivariate correlation analysis between extracted Co, Cr and Ni and their content in plants was also carried out, calculating Pearson's correlation coefficient.

3 Results and discussion

3.1 Characterisation of the soils

The characteristics of the soils differ significantly, especially those with a high influence on the retention of trace elements (Table 2).

The pH_{H_2O} (Table 2) was between 8.05 (S3) and 5.99 (CS), while the pH_{KCl} varied between 7.92 (S3) and 4.76 (CS). The soils are basic, which affects the retention of trace elements positively. However, CS was slightly acid, probably because of the vegetation and its organic matter content, as discussed below.

The organic carbon (OC) content is very low in the quarry soils (Table 2), and it is directly related to the presence of vegetation (Table 1), as the serpentinite barely contributes to the C and N pools of the soils (Corti et al., 2002). S2 and CS have the highest OC content.

The N content in the soils is very low: it was not detected in S1, S3 and S4, the content in S2 is moderately low and in CS, slightly high. These results agree with the levels found in soils developed over tailings from extractive activities (Mendez and Maier, 2008). The higher content in CS is due to the received treatments and to the plants growing there.

The oxide contents are low in all of the soils except for CS (Table 2). The highest levels are of iron oxides. The lack of Mn and Al oxides, especially in S3 and S4, is directly related to the parent matter, where the mineral chrysotile is in high proportion and lacks Fe, Mn and Al. In the rest of the soils, serpentine $(Mg, Al, Fe, Mn, Ni, Zn)_{2-3}$ $(Si, Al, Fe)_2O_5 (OH)_4$ predominates in the parent matter and their oxide content is higher, especially of iron oxides.

The effective cation exchange capacity is high in S2 and CS (19.59 and 16.76 $cmol_{(+)}\,kg^{-1}$, respectively) and low in the rest of the soils. All of the soils are saturated in bases and Ca^{2+} and Mg^{2+} predominate. The latter is the highest in S1 (59.69 %), S2 (58.70 %) and CS (72.37 %). Ca^{2+} predominates in S3 (62.44 %) and S4 (68.20 %). All soils are hypermagnesic, which strongly limits plant production, as a deficit of Ca may occur, despite the fact that there is a high content in the soils. The exchangeable K^+ varies between 0.50 $cmol_{(+)}\,kg^{-1}$ (S2) and 0.07 $cmol_{(+)}\,kg^{-1}$ (S4), and Na^+ between 0.40 $cmol_{(+)}\,kg^{-1}$ (CS) and 0.12 $cmol_{(+)}\,kg^{-1}$ (S4). The soils have virtually no exchangeable Al^{3+}, except CS (0.12 $cmol_{(+)}\,kg^{-1}$), which contributes to the moderately acidic character of this soil.

3.2 Total metal content

The levels of Co, Cr and Ni are high compared to the contents in soils developed over other materials in the region (Macías et al., 1993). Ni and Cr are potentially toxic elements and the content in the soils is very high (Table 3). The levels of Co are also

5 high but in this case there are few toxicity data for higher plants (Li et al., 2009). Studies have been carried out into how Co toxicity affects soil microbes and invertebrates (Chatterjee and Chatterjee, 2000; Lock et al., 2006) and they have revealed that Co is relatively toxic to plants when given in high doses, but there is still little information regarding the toxicity of Co to higher plants.

10 Cr is the most abundant in all the studied soils, followed by Ni and Co, except in S1 where the most abundant is Ni. S2 has the highest amount of all three metals. The Co values are between $147\,\text{mg kg}^{-1}$ (S2) and $76\,\text{mg kg}^{-1}$ (S3), Cr contents range from $2689\,\text{mg kg}^{-1}$ (CS) to $1566\,\text{mg kg}^{-1}$ (S3), and the Ni contents between $2039\,\text{mg kg}^{-1}$ (S1) and $1342\,\text{mg kg}^{-1}$ (S3). Most of these contents exceed the intervention limits

15 stipulated in different guides (DEFRA and Environmental Agency, 2006; RIVM, 2001).

None of the total content of the metals analyzed (Table 3), except those of Co, Cr and Ni, reached values that suggest toxic effects. Co, Cr and Ni seem to be responsible for the soil contamination and their availability and absorption by plants was studied.

3.3 Soil extractions

20 The soil extractions were carried out using different reagents. The results (Table 4) show they pose different extraction capacities for each of the three metals studied. The reagent that extracts the most Co, Ni and Cr is $0.01\,\text{M CaCl}_2$, but this fact does not mean that it is ideal for estimating the availability, as this depends on different aspects of the soil and metal in question. The available content must be related more to the

25 content in plant than to the total content of the soil.

In general, the sequences of greater to lesser extraction capacity differ depending on the metal and in the case of Ni, this sequence depends on the organic matter content

of the soils:

Co: CaCl$_2$ > EDTA > LMWOA > DTPA > BDW
Cr: CaCl$_2$ > LMWOA > EDTA ≈ DTPA ≈ BDW
Ni (OM): CaCl$_2$ > EDTA > DTPA > LMWOA > BDW
Ni (no OM): CaCl$_2$ > EDTA > LMWOA > DTPA > BDW

3.4 Extraction efficiency

It was found that the extraction efficiency (Fig. 2) does not only depend on the reagent used, but also on the characteristics of both the soil and the metal.

The reagent with the best extraction efficiency for all of the soils and all of the metals studied is CaCl$_2$. The highest efficiency with this reagent is for Co, reaching 27.6 % of the total content in S1 but it does not reach 4 % of the total content of the others studied metals. Besides, all the other extractants are more effective for Co than for Cr and Ni.

The extraction efficiency for Cr is very low (< 0.5 % in all cases). CaCl$_2$ is also the most efficient, followed by LMWOA, EDTA, BDW and DTPA, although there are hardly any significant differences between the last three.

Ni is also more efficiently extracted with CaCl$_2$, followed by EDTA, LMWOA, DTPA and BDW. In S2 and CS, DTPA shows higher extraction efficiency than LMWOA (these soils have lower pH, higher OC content, higher ECEC, and higher exchangeable Ca and Mg contents).

In general, the lowest extraction efficiency for all of the metals in the study, was detected in the soils with the highest pH (S1, S3 and S4) and the more basic the soil, the stronger the retention of the metal cations.

3.5 Metal content in the plants

The extraction capacity and efficiency of the reagents does not predict the availability of trace-elements for the plants. This study looked into the relationship between the content in the plants and the amount extracted using the different techniques.

5 The plants studied were chosen because they grow spontaneously and are the most abundant in the soils (except in two areas where there was practically no vegetation). They are *Festuca rubra* and *Juncus* sp. and the content in both the shoot and roots was determined (Table 4).

In general, *Festuca* absorbs the largest amounts of the three metals, both in its shoot
10 and roots, except in soil S1, where the roots of *Juncus* sp. accumulated a larger amount of Co and Ni. Ni is accumulated in the highest amounts by both plants, followed by Cr and Co.

Both species accumulate more Ni, Cr and Co in the roots than in the shoot, except in S2, where *Festuca* accumulated more Cr in the shoot than in its roots.

15 Several authors indicated (Li et al., 2009) that plants can accumulate small amounts of Co, and that their absorption and distribution depends on the species being controlled by different mechanisms. The absorption of Co^{2+} by the roots involves active transportation through the cell membranes, although the molecular mechanisms involved are still unknown (Li et al., 2009). Its distribution may involve organic
20 complexes, although the low mobility of Co^{2+} in the plants restricts its transportation from the roots to the shoot, as seen in this study (Table 4).

In turn, soil properties also influence heavy metal availability for plants (Li et al., 2009). There is very little useful information available to quantify the effect of soil properties on the toxicity of Co in different plant species. On the whole, the baseline
25 information is insufficient to support the adoption of new guidelines in the European Union in order to evaluate the risks posed by Co (European Commission, 2003). It has been suggested that threshold toxicity levels should be standardised using the exchangeable Ca content of the soil, as this content is correlated with the CECe; this

means that it is indicative of the sorption capacity of the soil, which influences the solubility of Co.

Calcium can reduce the toxicity of Co for plants by competing for binding sites in the root cells and Li et al. (2009) suggested that the Ca^{2+} ion competes with different metallic ions for the binding sites, thus reducing their toxicity. The exchangeable Ca content in the studied soils can influence the low Co content found in the plants, nevertheless, there is a good relationship between the amount of Co extracted (with the reagent that best represents the availability) and its concentration in the plants.

Chromium is accumulated in higher amounts in the roots than in the shoot. These results agree with those of other authors (Adki et al., 2013; Rafati et al., 2011). They indicated that the lowest amounts are always in the vegetative and reproductive organs.

As mentioned above, Nickel was absorbed in greater amounts (except in CS, Table 4) and this is probably because of the high pH of the soils from the quarry area. In general, the uptake of Ni usually declines at high soil solution pH values due to the formation of less soluble complexes (Yusuf et al., 2011). These complexes can remain on the soil surfaces in available forms. CS is the soil with the highest content of exchangeable Ca, which affects the decrease of Ni absorption, as demonstrated by Yusuf et al. (2011).

A plant growing in a soil containing heavy metals can be considered a hyperaccumulator if it concentrates in its shoot without suffering from toxicity problems, up to 1 % of Mn or Zn, 0.1 % of As, Co, Cr, Cu, Ni, Pb, Sb, Se and Tl or 0.01 % of Cd (Verbruggen et al., 2009). Also, according to Mongkhonsin et al. (2011), Reeves and Baker (2000), and Tappero et al. (2007), considering a plant a hyperaccumulator of Cr is based on three criteria: that the Cr concentration in the shoot $> 50\,mg\,kg^{-1}$, that the concentration of Cr in the aerial biomass is 10 to 500 times greater than in the non-metallophytes (0.2–$5\,mg\,kg^{-1}$ of Cr) and that the Cr concentration in the shoot is greater than in the roots.

Therefore, none of the plant species we evaluated behave like hyperaccumulators, as the amounts of metals absorbed by the plants are less than those previously indicated. Only the *Festuca* growing in S2 contains more Cr in the shoot than in the root (>

50 mg kg^{-1}); therefore it could be considered as having a certain hyperaccumulator capacity (Reeves and Baker, 2000).

It is well known that the total content is not suitable for establishing the mobility, availability and therefore the possible toxicity on trace elements.

Some authors, like Roy and McDonald (2014), indicated that the combined soluble and exchangeable fractions from the Tessier (1979) method could be correlated with Cd and Zn uptake in plants more so than the total soil concentration, but did not find any correlation to other elements, such as Pb and Cu. In this paper, in order to determine the extractant that best predicts bioavailability, a correlation analysis was carried out between the amount accumulated by the plant (root or shoot), and the amount extracted with the different reagents used (Table 5).

A positive and highly significant correlation was established ($P < 0.01$) between the amount of Co extracted by practically all of the extractants used and the amount accumulated by *Festuca* and *Juncus*, except between the Co extracted with DTPA and the content in the roots of *Juncus*.

A positive and highly significant correlation was also found between the amount of Cr accumulated in both the shoot and roots of *Festuca* and the amount extracted by CaCl$_2$, LMWOA and BDW. The Cr content in *Juncus* is only correlated with the content extracted with LMWOA.

In the case of Ni, the correlation is between the content in the shoot of *Festuca* and the amount extracted by BDW, as well as between the concentration in the root of the plant and the amount extracted by CaCl$_2$, LMWOA and BDW. In the case of *Juncus* the correlation is between the content in both the shoot and the root and the amount extracted by CaCl$_2$ and LMWOA.

It can therefore be deduced from these results that above all, LMWOA is the extractant that best predicts the bioavailability of Cr, Ni and Co for these plants in the soils from the Moeche quarry.

3.6 Translocation and bioconcentration factors

The highest TF values correspond to *Festuca* (Table 6), although they are not > 1 except for Cr (1.08) in the plants growing in S2. Out of the three metals in this study, Cr is translocated the best in *Festuca*, followed by Co and Ni.

⁵   The TF values in *Juncus* are very low, no higher than 0.11, and Ni is the metal that is translocated the best.

Based on these results, *Juncus* acts as a phytostabilizer, because it fixes the metals in its roots, reducing their mobility within the plant and soil. Due to the small amount of metal that accumulates in its shoot, it reduces the risk of metals transferring to other

¹⁰ compartments of the ecosystem.

The bioconcentration factor (Table 6) links the available content in the soils with the amount absorbed by the plants. The bioconcentration factor (BF) in the studied plants was determined by calculating the ratio of metal concentration in the plant (C_p; root and shoot) to soil (C_{LMWOA}).

¹⁵ The BF (Table 6) in the shoot of *Juncus* is generally very low and < 1, except for Cr, due to the low or inexistent translocation of Ni and Co from the root. The BF in its root is > 1, with the highest value corresponding to Cr, while the values for Co and Ni confirm that, as previously indicated, the plant accumulates both metals in the root and does not transfer them to the shoot, behaving as a phytostabilizer.

²⁰ Moreover, these results indicate that *Festuca* is a phytostabiliser of Co and Ni, which is consistent with Simon (2005) for Cd, Cu, Pb and Zn; however we have confirmed that it is an accumulator of Cr.

In addition, the BF for the shoot and root of *Festuca* is > 1 in all of the cases, which means that *Festuca* behaves as an accumulator, especially of Cr.

4   Conclusions

The levels of Co, Cr and Ni in the studied soils exceed the intervention values indicated in different reference guides. Although $CaCl_2$ is the reagent with the highest extraction efficiency for all of the soils and metals studied, the extractant that best predicts the bioavailability of the metals is the mixture of low molecular weight organic acids.

Festuca generally accumulates the largest amount of Co, Cr and Ni and also has the highest translocation factor, although it is only > 1 in the case of Cr. Furthermore, the bioconcentration factor is > 1 for Cr in the shoot and root of *Festuca* and *Juncus*, and is also > 1 for Co and Ni in *Festuca*. It is < 1 for these two elements in the shoot of *Juncus*.

Juncus seems to be a suitable plant for phytostabilisation, while *Festuca* is a phytostabiliser of Co and Ni, and an accumulator of Cr. Both species contribute towards the phytostabilisation of the soils and their recovery, improving their characteristics and making it possible to start planting other species.

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Table 1. Profile description.

S1	Sampling site information: Moeche (A Coruña, Spain). 43° 31.769′ N 8° 00.506′ W. Altitude: 149 m. Quarry spoil. Undulating, slope: class 2. Vegetation: *Festuca rubra* L. and *Juncus* sp. L.
	Soil information: Spolic technosol. Quarry tailing. Wet, drainage: class 0. Stony (class 4) with serpentinite stones and gravel on the surface. No signs of erosion. Descriptive: AC: 0–15 cm. Gley 2′4/10 BG. Sandy texture. Unstructured. Without consistency in wet and dry, slightly sticky and without plasticity. Few roots. C: +15 cm. Mixture of spolic materials (serpentinite fragments).
S2	Sampling site information: Moeche (A Coruña, Spain). 43° 31.718′ N 8° 00.571′ W. Altitude: 149 m. Quarry spoil. Located on a flat or nearly flat area, slope: class 1. Vegetation: *Festuca rubra* L, *Salix atrocinerea* Brot and *Juncus* sp. L.
	Soil information: Spolic technosol. Quarry tailing. Wet, drainage: class 1.Stony (class 4) with serpentinite stones and gravel on the surface. No signs of erosion. Descriptive: AC: 0–15 cm. 10 YR 4/3. Sandy loam texture. Moderate crumbly structure. Slightly hard, very friable, slightly sticky and plastic. Abundant roots. C: +15 cm. Mixture of spolic materials (serpentinite fragments).
S3	Sampling site information: Moeche (A Coruña, Spain). 43° 31.700′ N 8° 00.598′ W. Altitude: 140 m. Quarry spoil. Hilly, slope: class 5. No vegetation.
	Soil information: Spolic technosol. Quarry tailing. Wet, drainage: class 2. Stony (class 4) with gravel, stones and boulders of serpentinite with chrysotile abundance. Evidence of water erosion. Descriptive: AC: 0–25 cm. Gley 1/5 GY. Sandy loam texture. Unstructured. Without consistency in wet and dry, sticky and without plasticity. C: +25 cm. Mixture of spolic materials (serpentinite fragments, with chrysotile abundance).

Table 1. Continued.

S4	Sampling site information: Moeche (A Coruña, Spain). 43° 31.674′ N 8°00.608′ W. Altitude: 148 m. Cut zone. Hilly, slope: class 5. No vegetation (coverage < 1 %). Soil information: Lithic leptosol. Wet, drainage: class 2. Stony (class 4) with stones and boulders of serpentinite with chrysotile abundance. Evidence of water erosion. Descriptive: A: 0–10 cm. Gley 5/5 GY. Loamy sand texture. Unstructured. Without consistency in wet and dry, sticky and without plasticity. R: +10 cm. Serpentinite (mainly composed of serpentine and chrysotile)
CS	Sampling site information: Moeche (A Coruña, Spain). 43° 31.690′ N 8° 00.687′ W. Altitude: 132 m. Reforested area outside the quarry. Sloping: class 2. Vegetation: *Pinus pinaster* Ait, *Festuca rubra* L. (the most abundant) and very few of *Ulex europaeus* L. and *Rubus ulmifolius* Schot. Soil information: Mollic leptosol (Control soil). Wet, drainage: class 2. Stoniness: class 1 (with gravels and stones of serpentinite). Slight evidence of water erosion. Descriptive: A: 0–30 cm. 7′5 YR 3/3. Loam texture. Moderate crumb structure. Slightly hard, highly friable, slightly sticky and slightly plastic. Abundance of roots of various sizes. R: (+30 cm). Serpentinite.

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Table 2. Some physicochemical characteristics of the soils (mean values).

	Unit	S1	S2	S3	S4	CS
$pH_{(H_2O)}$		7.98 ± 0.06 ab	7.81 ± 0.06 b	8.05 ± 0.03 a	7.87 ± 0.08 b	5.99 ± 0.16 c
$pH_{(KCl)}$		7.11 ± 0.01 c	6.96 ± 0.03 d	7.92 ± 0.02 a	7.72 ± 0.03 b	4.76 ± 0.03 e
Total N	$g\,kg^{-1}$	ul	0.42 ± 0.00 b	ul	ul	2.75 ± 0.05 a
OC		0.30 ± 0.07 c	3.58 ± 0.45 b	ul	0.38 ± 0.071 b	11.29 ± 0.97 a
Fe oxides		2.24 ± 0.07 c	15.65 ± 0.15 b	1.61 ± 0.02 d	1.30 ± 0.03 e	23.98 ± 0.16 a
Mn oxides		0.12 ± 0.00 d	0.63 ± 0.01 b	0.16 ± 0.00 c	0.11 ± 0.00 d	0.89 ± 0.01 a
Al oxides		0.09 ± 0.01 e	1.71 ± 0.00 b	0.21 ± 0.00 c	0.18 ± 0.01 d	4.31 ± 0.06 a
Exchangeable cation and ECEC						
ECEC	$cmol_{(+)}\,kg^{-1}$	5.21 ± 0.15 d	19.59 ± 0.89 a	6.15 ± 0.41 c	5.66 ± 0.25 cd	16.76 ± 0.46 b
Ca^{2+}		1.54 ± 0.07 c	7.20 ± 0.36 a	3.84 ± 0.25 b	3.86 ± 0.18 b	3.83 ± 0.11 b
K^+		0.36 ± 0.09 b	0.50 ± 0.03 a	0.10 ± 0.01 d	0.07 ± 0.01 d	0.28 ± 0.02 c
Mg^{2+}		3.11 ± 0.13 c	11.50 ± 0.51 b	2.05 ± 0.15 d	1.61 ± 0.05 d	12.13 ± 0.31 a
Na^{2+}		0.19 ± 0.06 b	0.38 ± 0.02 a	0.16 ± 0.03 bc	0.12 ± 0.01 c	0.40 ± 0.02 a
Al^{3+}		0.01 ± 0.01 b	0.01 ± 0.01 b	ul	ul	0.12 ± 0.00 a
Particle size distribution						
Sand	%	89.39 ± 0.75 a	59.83 ± 0.95 d	74.68 ± 0.80 c	82.16 ± 0.71 b	26.73 ± 0.56 e
Silt		6.56 ± 0.54 e	26.06 ± 0.72 b	13.75 ± 0.38 c	10.76 ± 0.50 d	46.56 ± 1.05 a
Clay		4.05 ± 0.28 e	14.11 ± 0.24 b	11.56 ± 0.73 c	7.08 ± 0.24 d	26.71 ± 0.51 a

ul: undetectable level (detection limit $0.1\,mg\,kg^{-1}$). OC: organic carbon. ECEC: eEffective cationic exchange capacity. For each parameter, values followed by different letters differ significantly with $P < 0.05$.

Table 3. Total content of metals ($mg\,kg^{-1}$) (mean values).

C	S1	S2	S3	S4	CS
Al	6623 ± 112 e	22 675 ± 374 b	13 775 ± 273 d	18 728 ± 354 c	42 890 ± 678 a
Ba	8 ± 2 c	41 ± 5 b	8 ± 2 c	6 ± 2 c	87 ± 7 a
Ca	6589 ± 167 e	10 044 ± 184 d	28 579 ± 476 a	23 416 ± 345 b	22 008 ± 253 c
Co	110 ± 11 b	147 ± 10 a	80 ± 6 cd	76 ± 2 d	97 ± 14 bc
Cr	1672 ± 110 b	2605 ± 37 a	1366 ± 49 c	1472 ± 116 c	2689 ± 82 a
Cu	145 ± 7 d	150 ± 8 d	327 ± 11 a	209 ± 12 c	291 ± 12 b
Fe	52 808 ± 235 c	77 775 ± 346 a	39 747 ± 232 e	43 449 ± 227 d	74 310 ± 354 b
K	2722 ± 97 b	4074 ± 110 a	661 ± 39 c	733 ± 44 c	4156 ± 87 a
Mg	303 045 ± 890 a	205 696 ± 742 c	186 262 ± 635 d	207 809 ± 958 b	102 653 ± 386 e
Mn	900 ± 85 c	1602 ± 88 b	751 ± 30 d	802 ± 52 cd	1850 ± 122 a
Na	283 ± 25 c	3264 ± 287 b	569 ± 96 c	358 ± 71 c	10 579 ± 312 a
Ni	2039 ± 107 a	1861 ± 62 b	1342 ± 32 d	1499 ± 89 c	1470 ± 82 cd
Sr	19 ± 2 b	21 ± 3 b	15 ± 1 c	13 ± 1 c	41 ± 3 a
V	1 ± 0 e	52 ± 5 b	16 ± 4 d	39 ± 6 c	68 ± 5 a
Zn	34 ± 2 c	63 ± 5 b	58 ± 5 b	32 ± 2 c	115 ± 8 a

For each element, values followed by different letters differ significantly with $P < 0.05$.

Table 4. Mean values of metal extracted from soils, and content in plants ($mg\,kg^{-1}$).

Extractant	Metal extracted from the soils				
	CaCl$_2$	EDTA	DTPA	LMWOA	BDW
			Co		
S1	25.59 ± 0.81 A,a	3.47 ± 0.07 B,a	0.98 ± 0.06 D,b	1.71 ± 0.19 C,a	0.07 ± 0.01 E,a
S2	25.27 ± 0.40 A,a	2.49 ± 0.25 B,b	1.16 ± 0.05 D,a	1.65 ± 0.17 C,a	0.07 ± 0.02 E,a
S3	17.67 ± 0.27 A,b	0.89 ± 0.05 B,d	0.39 ± 0.02 C,d	0.51 ± 0.26 BC,b	0.02 ± 0.01 D,b
S4	16.81 ± 0.44 A,b	1.17 ± 0.09 B,c	0.47 ± 0.03 C,c	0.46 ± 0.01 C,b	0.01 ± 0.01 D,b
CS	3.29 ± 0.18 A,c	0.84 ± 0.06 B,d	0.17 ± 0.03 D,e	0.38 ± 0.01 C,b	0.02 ± 0.01 E,b
			Cr		
S1	4.02 ± 0.05 A,c	0.28 ± 0.01 C,b	0.07 ± 0.00 D,b	0.68 ± 0.05 B,c	0.11 ± 0.02 D,bc
S2	7.65 ± 0.17 A,a	0.44 ± 0.04 C,b	0.10 ± 0.01 D,b	0.84 ± 0.02 B,ab	0.38 ± 0.11 C,a
S3	5.74 ± 0.27 A,b	0.33 ± 0.02 C,b	0.09 ± 0.02 C,b	0.72 ± 0.17 B,bc	0.09 ± 0.02 C,c
S4	7.13 ± 0.24 A,a	0.36 ± 0.02 C,b	0.07 ± 0.01 D,b	0.88 ± 0.01 B,a	0.04 ± 0.01 D,c
CS	4.83 ± 0.24 A,bc	1.11 ± 0.07 B,a	0.42 ± 0.04 C,a	0.58 ± 0.02 C,c	0.20 ± 0.04 D,b
			Ni		
S1	273.86 ± 6.71 A,a	51.25 ± 0.80 B,b	24.83 ± 1.04 D,b	42.58 ± 2.41 C,a	1.14 ± 0.12 E,a
S2	153.00 ± 3.23 A,b	32.22 ± 1.11 B,c	19.99 ± 0.13 C,c	12.15 ± 0.57 D,b	1.31 ± 0.21 E,a
S3	99.05 ± 2.66 A,d	12.03 ± 0.60 B,e	4.98 ± 0.26 C,e	12.50 ± 1.09 B,b	0.31 ± 0.12 D,b
S4	111.35 ± 2.21 A,c	15.56 ± 0.93 B,d	6.37 ± 0.10 D,d	12.69 ± 0.22 C,b	0.14 ± 0.03 E,b
CS	96.01 ± 2.69 A,d	67.34 ± 1.14 B,a	66.08 ± 1.40 B,a	6.68 ± 0.07 C,c	0.44 ± 0.14 D,b

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Table 4. Continued.

Soil	Plant	Plant metal content					
		Co	Cr	Ni	Co	Cr	Ni
		Shoot			Root		
S1	*Festuca*	5.17 ± 0.64 b	15.63 ± 2.03 b	71.19 ± 2.94 b	15.37 ± 1.60 b	19.63 ± 1.79 cd	212.59 ± 18.97 b
S1	*Juncus*	1.10 ± 0.00 c	0.73 ± 0.32 c	21.01 ± 2.81 c	46.83 ± 5.91 a	25.53 ± 2.02 bc	290.38 ± 19.13 a
S2	*Festuca*	8.17 ± 1.70 a	82.84 ± 6.09a	109.22 ± 9.61 a	13.74 ± 0.63 b	77.04 ± 8.00 a	170.72 ± 8.73 c
S2	*Juncus*	0.92 ± 0.32 c	1.29 ± 0.32c	9.40 ± 3.14 d	11.21 ± 1.02 b	29.49 ± 3.40 b	84.34 ± 11.07 d
CS	*Festuca*	0.73 ± 0.32 c	15.51 ± 2.28b	26.46 ± 1.14 c	1.14 ± 0.00 c	16.85 ± 1.43 d	66.64 ± 5.15 d

In each row, for metal concentration in the extracts, values followed by the different capital letter differ significantly ($P < 0.05$); in each column, values followed by the different lowercase letter differ significantly ($P < 0.05$).
LMWOA: low-molecular-weight organic acids. BDW: Bidistilled water.
In each column (for shoot or root) values followed by different letters differ significantly ($P < 0.05$).

Table 5. Pearson's correlation between extracted Co, Cr, and Ni and its content in plants.

Plant	Extractant				
	CaCl$_2$	EDTA	DTPA	LMWOA	BDW
Co					
Festuca shoot	0.876[b]	Nc	0.925[b]	0.943[b]	0.875[b]
Festuca root	0.957[b]	0.944[b]	0.962[b]	0.991[b]	0.987[b]
Juncus shoot	0.904[b]	0.913[b]	0.916[b]	0.949[b]	0.943[b]
Juncus root	0.688[a]	0.890[b]	Nc	0.694[a]	0.678[a]
Cr					
Festuca shoot	0.862[b]	Nc	Nc	0.896[b]	0.794[a]
Festuca root	0.858[b]	Nc	Nc	0.903[b]	0.751[a]
Juncus shoot	Nc	Nc	Nc	0.906[b]	Nc
Juncus root	Nc	Nc	Nc	0.803[b]	Nc
Ni					
Festuca shoot	Nc	Nc	Nc	0.692[a]	0.899[b]
Festuca root	0.875[b]	Nc	Nc	0.895[a]	0.837[b]
Juncus shoot	0.909[b]	Nc	Nc	0.969[b]	Nc
Juncus root	0.906[b]	Nc	Nc	0.996[b]	Nc

[a] Correlation is significant at level 0.05 (bilateral).
[b] Correlation is significant at level 0.01 (bilateral).
Nc: No correlation.

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Table 6. Translocation and bioconcentration factors (mean values).

Translocation factor Soil	*Festuca*	*Juncus*	
Co			
S1	0.34 ± 0.04 a	0.02 ± 0.00 b	
S2	0.59 ± 0.12 a	0.08 ± 0.02 a	
CS	0.64 ± 0.28 a	NP	
Cr			
S1	0.80 ± 0.11 b	0.03 ± 0.01 a	
S2	1.08 ± 0.09 a	0.04 ± 0.01 a	
CS	0.92 ± 0.07 ab	NP	
Ni			
S1	0.33 ± 0.04 b	0.07 ± 0.01 a	
S2	0.64 ± 0.03 a	0.11 ± 0.02 a	
CS	0.40 ± 0.01 b	NP	

Bioconcentration factor Soil	Plant		Co	Cr	Ni
S1	*Festuca*	Shoot	3.02 ± 0.38 b	22.99 ± 2.99 b	1.67 ± 0.07 c
S1	*Juncus*		0.64 ± 0.00 c	1.07 ± 0.46 c	0.49 ± 0.07 d
S2	*Festuca*		4.95 ± 1.03 a	98.62 ± 9.64 a	8.99 ± 0.79 a
S2	*Juncus*		0.56 ± 0.19 c	1.54 ± 0.38 c	0.77 ± 0.26 d
CS	*Festuca*		1.92 ± 0.83 b	26.74 ± 3.93 b	3.96 ± 0.17 b
S1	*Festuca*	Root	8.99 ± 0.94 b	28.87 ± 2.62 c	4.99 ± 0.44 d
S1	*Juncus*		27.39 ± 1.02 a	37.54 ± 2.98 b	6.82 ± 0.45 c
S2	*Festuca*		8.33 ± 0.38 b	91.71 ± 2.05 a	14.05 ± 0.72 a
S2	*Juncus*		6.79 ± 0.62 c	35.11 ± 4.06 b	6.94 ± 0.91 c
CS	*Festuca*		3.00 ± 0.00 d	29.05 ± 2.47 c	9.98 ± 0.77 b

In each column (for TF) values followed by different letters differ significantly ($P < 0.05$).
NP: No plant.
In each column (for BF, and for shoot or root) values followed by different letters differ significantly ($P < 0.05$).
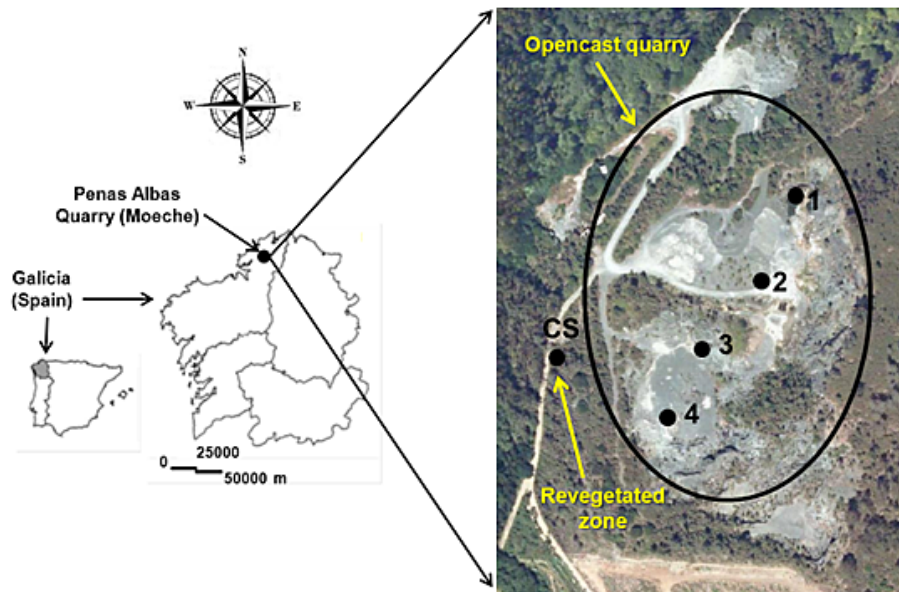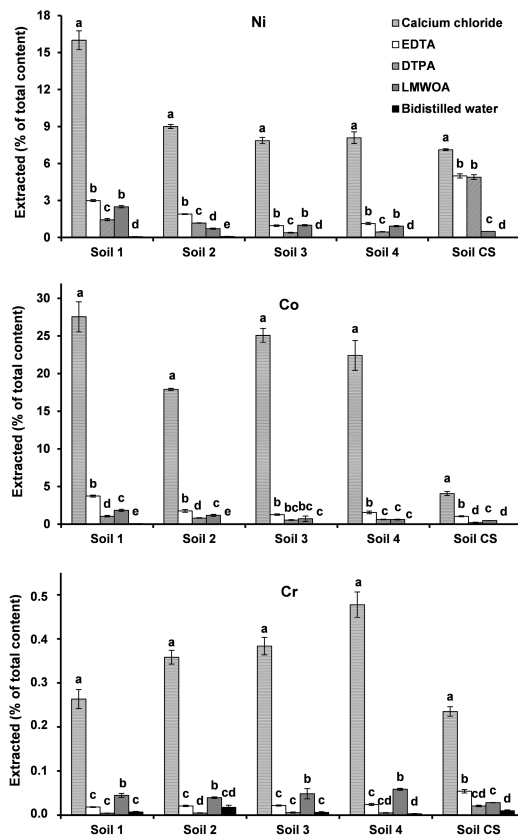
Figure 1. Study zone.

Figure 2. Extraction efficiency. In each soil, bars with different letters indicate significantly different EF values ($P < 0.05$) for each metal.