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ORIGINAL ARTICLE

Geographical and ecological outline of metal(loid) accumulating plants in Italian vascular flora

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Abstract – The decontamination of heavy metal polluted soils is one of the major challenges that our industrialized world has to face. Remediation technologies are being developed and employed in order to reduce the potential hazards of metal and metalloid contamination. Plants capable of uptaking metals and metalloids in their tissues can be an effective tool to remove such pollutants from contaminated soils. The use of this plant-driven process (Phytoremediation) requires the knowledge of the right phytoextractors to use when facing different types of contamination. The aim of this paper is to provide an inventory of phytoextractors that can be used in Phytoremediation procedures in Italy. The checklist includes 172 native or non-invasive alien accumulating and hyperaccumulating plants. An ecological outline of the accumulating flora was done by using the Ellenberg indicator values (EIVs). The high ecological plasticity of these species in different environmental conditions offers a wide spectrum of phytoextractors to choose from for any phytoremediation procedure.

Keywords – Accumulating plants, Ellenberg indicator values, heavy metals, metal uptake, phytoextractors.

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Introduction

Heavy metal soil contamination is a major environmental issue in both developed and developing countries (Panagos et al., 2013; Su et al., 2014).

In the European Union, heavy metals (HMs) are by far the most frequent soil contaminants, accounting for more than 34% of cases of soil contamination, followed by mineral oil (23,8%), polycyclic aromatic hydrocarbons (10,9%) and others (EEA, 2011). Even though the European Environment Agency indicators reveal that during the past 15 years heavy metal (HM) emissions have been decreasing in most of the member states, the "European Commission In-depht Report on soil contamination" (2013) shows that HM contamination is still a widespread problem. Moreover, in developing world, HM pollution has dramatically increased over the past decades (Hu et al., 2014; Zhai et al., 2013).

Intensive farming, wastewater irrigation, mining, heavy industry, smelting procedures and improper waste disposal are some of the human activities that contribute to HM soil contamination (Sharma et al., 2007; Khan et al., 2008). As heavy metals cannot be degraded by microorganisms and tend to remain in polluted sites for long periods of time, soil contamination by HMs represents a serious threat to human health (Roberts and Goodman, 1973; Järup, 2003).

Conventional soil remediation techniques, such as soil washing, excavation, in situ vitrification, solidification and stabilization, can be effectively employed in order to achieve remediation of HM contaminated soils. Anyway, in the last decades other approaches have been developed. Some plants are known to be able to uptake and accumulate metals in their above-ground tissues from the soil in which they grow. The exploitation of this ability is known as "phytoextraction" (Salt et al., 1995). Phytoextraction is a well known technology that can be used to remove heavy metals and/or metalloids from contaminated soils for bioremediation purposes (Phytoremediation) or to extract valuable metals for monetary return (Phytomining).

Some plants can also uptake organic pollutants and degrade them enzymatically, a process called phytodegradation. Phytoextraction and phytodegradation are green, non-invasive and feasible methods to clean up contaminated lands. Other phytoremediation techniques include phytostabilization and phytovolatilization (Salt et al., 1998).

Over the last decades, there has been a growing interest in phytoremediation technologies. In developing countries, there is urgent need for cheap and viable remediation techniques to reclaim HM contaminated soils. Therefore, in developing world, Phytoextraction has gained increasing consideration as a reliable, sustainable and lowcost alternative to conventional soil remediation technologies (Rajakaruna et al., 2006).

The idea that plants could be used to extract metals from soil has been fascinating scientists and businessmen for over one century. Lungwitz (1900) was the first to suggest the inspection of plant tissues to locate gold deposits. In the late 1940s, Minguzzi and Vergnano (1948) identified the first nickel accumulating plant (*Alyssum bertolonii* Desv.). Brooks et al. (1977) first used the term hyperaccumulators to describe plants that contained 1000 mg kg^{-1} (0.1%) Ni in their dried tissues. Later, Warren and Delavault (1950) found gold and silver in some trees and horsetails. Further investigations on gold uptake by plants were carried out in the Soviet Union by Kitayev and Zhukova (1980). In the last 20 years, researchers (especially in China, India and Australia) have discovered a considerable number of plant species that accumulate or hyperaccumulate metals and/or metalloids. To date, more than 400 metal hyperaccumulating plant species are known. (Sheoran et al., 2009).

Publication data on phytoextracting plants have been collected and stored in several databases, such as "Phytoremediation" (Famulari and Witz., 2015) and PHYTOREM Database (McIntyre, 2003), which are designed for ease of use even for non-scientific users.

In Italy, research on phytoextraction is still in the early stages. Nevertheless, some studies have been carried out in Italy as well. For instance, Marchiol et al. (2007) found that *Helianthus annuus* L. and *Sorghum bicolor* L. were able to remove Cu and Zn from a polluted soil. Massa et al. (2010) did a screening among some autochthonous plants of North West Italy and discovered two HM hyperaccumulators. In an abandoned Sardinian mining site, among native species, Barbafieri et al. (2011) identified three new phytoextractors. Brunetti et al. (2011) investigated the uptake of Cr, Cu, Pb and Zn by *Brassica napus* L. Moreover, extremely high concentrations of thallium were detected in the tissues of *Biscutella laevigata* L. (Fellet et al., 2012). Malagoli et al (2014) evaluated the phytoextraction potential of *Sinapis alba* L. and *Festuca rubra* L. The following year, Concas et al. (2015) determined the metal content of epigean and hypogean tissues of *Pistacia lentiscus* L.

More recently, Roccotiello et al. (2016) studied the effects of nickel on the physiology of a Mediterranean plant (*Alyssoides utriculata* (L.) Medik.).

To date, the literature lacks a systematic overview of phytoextracting plants growing in Italy. In this paper, we present a first checklist of phytoextrators, either native or naturalized to Italy. To define the habitat niches and their occurrence along environmental gradients, we characterized the ecology of the accumulating plants by using the Ellenberg indicator values (EIVs). The Ellenberg indicator values are bioindicators that describe the most important abiotic environmental traits (L = light; T = temperature; K= continentality; F= moisture; R = soil reaction= N nitrogen) for each plant species. (Ellenberg et al., 1991). The high ecological diversity of these species offers a huge number of phytoextractors that can be used in different scenarios of contaminated soils.

Materials and methods

The data were gathered from published materials. The existing literature was screened to find metal and/or metalloid (hyper)accumulating plants.

According to Baker et al. (2000), the hyperaccumulation threshold for most of the elements is 1000 mg kg^{-1} dry mass, except for Gold (mg kg^{-1}), Hg (10 mg kg^{-1}), Cd (100 mg kg^{-1}) Tl (500 mg kg^{-1}), Zn and Mn (10000 mg kg^{-1}). Thus, plants were considered as hyperaccumulators if their aerial tissues contained metal(loid)s at concentrations exceeding these thresholds. Instead, plants failing to exceed the hyperaccumulation threshold, but still capable of concentrating metal(loid)s to levels close to the hyperaccumulation limit were considered as "accumulators".

We selected the plants that strictly belong to the Italian flora from the available published resources on accumulating species. To ascertain the actual presence of the selected plant species in the Italian flora, we used Conti et al. (2005) annotated checklist of Italian vascular flora. It should be noted that even if allochtonous invasive species are all naturalized plants, such plants pose a potential threat to biodiversity (Sala et al., 2000). To keep these species out of the list, an evaluation of invasiveness was performed using checklist of allochthonous vascular plants of Italy by Celesti-Grapow et al. (2010). Hence, all the species listed in this work are either native or non-invasive allochtonous plants growing in Italy. The list is sorted in alphabetical order and nomenclature follows the standards set by The Plant List (2013). The geographical distribution of Metallophytes within the Italian regions is based on the data provided by Conti et al. (2005). For each taxon, Raunkiaer's life forms (Raunkiaer, 1934) were recorded according to Pignatti (1982).

The ecological relevance in phytoremediation procedures was also taken into account. The ecological characterization of plants was carried out using Ellenberg's indicator values (Eivs) (Ellenberg et al., 1991) as published by Pignatti et al. (2005) and updated by Guarino et al. (2012) and Domina et al. (2018). For light and temperature, EIVs have a wider range (up to 12), as modified by Pignatti et al. (2005) to better fit the characteristics of the Italian geographical region.

The statistical analysis was performed using the R-Studio software and map was designed under ESRI ArcGis environment.

Results

Metal(loid) accumulation

In our screening, 172 infraspecific and specific taxa of accumulators were found. As shown in table 1, these species accumulate 18 metals and 2 metalloids.

Table 1 List of Phytoextractors of Italian Flora (hyperaccumulated elements in bold; L.F.= Life Forms).

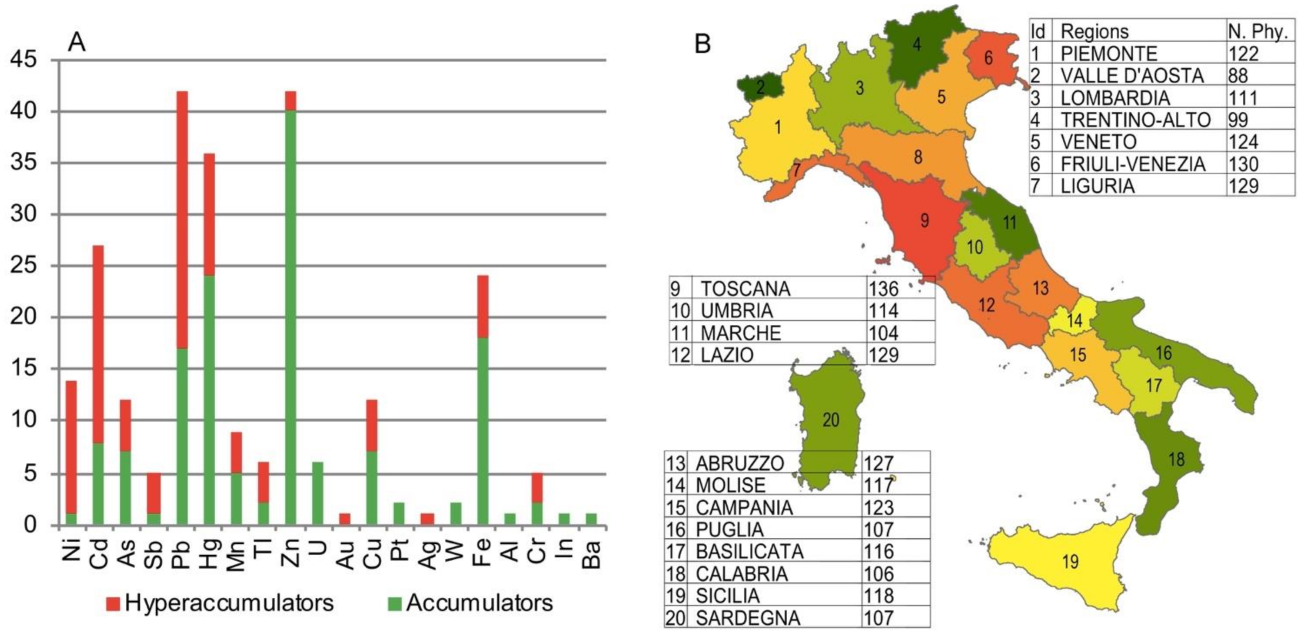
Taxa	L. F.	Metal(loid)s	References
Achillea ageratum L.	H	**Sb**	(Baroni et al. 2000)
Alternanthera philoxeroides (Mart.) Griseb.	I	**Cd,** Mn, **Zn**	(Nan et al. 2013)
Alyssoides utriculata (L.) Medik.	CH	**Ni**	(Roccotiello et al. 2016)
Alyssum argenteum All.	CH	**Ni**	(Vergnano Gambi et al. 1979)
Alyssum bertolonii Desv.	CH	**Ni**	(Álvarez-López et al. 2016)
Alyssum wulfenianum Benth. ex Willd.	CH	Pb, **Tl**	(Fellet et al. 2012)
Amaranthus caudatus L.	T	Cd	(Bosiacki et al. 2013)
Anarrhinum bellidifolium (L.) Willd.	H	Hg	(Fernández et al. 2017)
Anthemis cretica L.	H	W	(Erdemir et al. 2017)
Arabidopsis halleri (L.) O'Kane & Al-Shehbaz	H	**Cd**, Zn	(Küpper et al. 2000; Wenzel and Jockwer 1999)
Arabis alpina var. *parviflora* Franch	H	**Pb**	(Yanqun et al. 2005)
Arabis sagittata (Bertol.) DC.	H	Ni, Cu	(Alekseeva-Popova et al. 2015)
Arthrocnemum macrostachyum (Moric.) K.Koch	CH	As, Pb, Zn, **Fe**	(Martínez-Sánchez et al. 2012)
Asplenium adiantum-nigrum L.	H	Hg	(Fernández et al. 2017)
Atriplex halimus L.	P	**Cd**, Zn	(Amer et al. 2013; Lutts et al. 2004)
Atriplex prostrata Boucher ex DC.	T	Zn	(Moreira et al. 2011)
Avena strigosa Schreb.	T	**Cd**	(Uraguchi et al. 2006)
Beta vulgaris L.	H	Cd	(Song et al. 2012)
Betula pubescens Ehrh.	P	**Cd**	(Fernández et al. 2008)
Bidens pilosa L.	T	**Cd**	(Sun et al. 2009)
Biscutella laevigata L.	H	**Pb**, **Tl**	(Fellet et al. 2012; Wenzel and Jockwer 1999)
Blackstonia perfoliata (L.) Huds.	T	**Hg**	(Fernández et al. 2017)
Boehmeria nivea (L.) Gaudich.	H	Zn	(Nan et al. 2013)
Brachypodium pinnatum (L.) P.Beauv.	H	**Hg**	(Fernández et al. 2017)
Brassica juncea (L.) Czern.	T	Cd, U*, **Au***	(Chang et al. 2005; Ghosh and Singh 2005; Lamb et al. 2001)
Brassica napus L.	T	Cr	(Brunetti et al. 2011)
Brassica oleracea L.	CH	Mn, Fe, Tl	(Radulescu et al. 2013; Ning et al. 2015)
Brassica rapa L.	T	Cd, Fe, Zn	(Ghosh and Singh 2005; Parveen et al. 2015; Purakayastha et al. 2008)
Callitriche hamulata Kütz. ex W.D.J.Koch	I	U	(Favas et al. 2014)
Callitriche stagnalis Scop.	I	U	(Favas et al. 2014)
Cannabis sativa L.	T	**Pb***	(Kos et al. 2003)
Carthamus tinctorius L.	T	**Pb**	(Al Chami et al. 2015)
Celosia argentea L.	T	**Mn**	(Liu et al. 2014)
Centaurea nigra L.	H	Hg	(Fernández et al. 2017)
Centaurium erythraea Rafn	H	**Hg**	(Fernández et al. 2017)
Centaurium pulchellum (Sw.) Druce	T	**Hg**	(Fernández et al. 2017)
Chenopodium giganteum D.Don	T	Cu	(Bhargava et al. 2008)
Cistus salvifolius L.	NP	Cd	(Barbafieri et al. 2011)
Coincya monensis (L.) Greuter & Burdet	H	Zn	(Fernández et al. 2017)
Convolvulus arvensis L.	G	**Cu**, **Fe**, **Cr**	(Gardea-Torresdey et al. 2004; Massa et al. 2010)
Cornus sanguinea L.	P	Hg	(Fernández et al. 2017)
Cucumis sativus L.	T	As	(Hong et al. 2011)
Cucurbita moschata Duchesne	T	Zn	(Nan et al. 2013)
Dactylis glomerata L.	H	**Hg**	(Fernández et al. 2017)
Daucus carota L.	H	**Hg**	(Fernández et al. 2017; Massa et al. 2010)
Dittrichia viscosa (L.) Greuter	H	**Cd**, Pb, **Fe**	(Barbafieri et al. 2011; Buscaroli et al. 2017; Fernández et al. 2008)
Dysphania botrys (L.) Mosyakin & Clemants	T	**Fe**	(Malayeri et al. 2013)
Eleocharis acicularis (L.) Roem. & Schult.	G	**Pb**, Cu, **Ag** In	(Ha et al. 2011)
Elymus elongatus (Host) Runemark	H	Zn	(Sipos et al. 2013)
Equisetum ramosissimum Desf	G	Hg	(Fernández et al. 2017)
Equisetum telmateia Ehrh.	G	**Hg**	(Fernández et al. 2017)
Erica arborea L.	P	Hg	(Fernández et al. 2017)
Erica cinerea L.	CH	**Hg**	(Fernández et al. 2017)
Erica scoparia L.	P	Fe	(De La Fuente et al. 2010)
Erodium malacoides (L.) L'Hér.	T	Fe	(Baycu et al. 2015)
Euphorbia dendroides L.	NP	Pb, Zn	(Barbafieri et al. 2011)
Euphrasia stricta D. Wolff	T	Hg	(Fernández et al. 2017)

Taxa	L. F.	Metal(loid)s	References
Festuca ovina L.	H	**Pb**	(Yanqun et al. 2005)
Festuca rubra L.	H	Cu	(Lago-Vila et al. 2015; Malagoli et al. 2014)
Fraxinus excelsior L.	P	Hg	(Fernández et al. 2017)
Galinsoga parviflora Cav.	T	**Cd**	(Lin et al. 2014)
Galium aparine L.	T	Hg	(Massa et al. 2010)
Glaucium flavum Crantz	H	Pb, Zn	(Martínez-Sánchez et al. 2012)
Glycine max (L.) Merr.	P	As, Zn, Cu	(Fellet et al. 2007)
Helianthus annuus L.	T	**Ni**, **Cd**, **As**, Pb, U*, **Cr**	(Cutright et al. 2010; Mihalík et al. 2010; Solhi et al. 2005)
Helichrysum italicum (Roth) G.Don	CH	Pb	(Barbafieri et al. 2011)
Heliotropium europaeum L.	T	Fe	(Nematian and Kazemeini 2013)
Hirschfeldia incana (L.) Lagr.-Foss.	H	**Pb**	(Fahr et al. 2015)
Holcus lanatus L.	H	As	(Karczewska et al. 2013)
Hordeum distichon L.	H	Zn	(Soriano and Fereres 2003)
Hypericum perforatum L.	H	Hg, Zn	(Fernández et al. 2017)
Iberis carnosa Willd.	H	Zn	(Fernández et al. 2017)
Iberis linifolia L.	T	**Tl**	(Anderson et al. 1999)
Imperata cylindrica (L.) Raeusch.	G	Al, **Fe**	(Rodríguez et al. 2005)
Koeleria vallesiana (Honck.) Bertol. ex Schult.	H	Zn	(Fernández et al. 2017)
Lactuca serriola L.	H	Sb	(Hajiani et al. 2015)
Leersia oryzoides (L.) Sw.	G	As	(Ampiah-Bonney et al. 2007)
Lemna gibba L.	I	**As**	(Mkandawire and Dudel 2005)
Leontodon taraxacoides Hoppe & Hornsch.	H	**Hg**	(Fernández et al. 2017)
Lepidium sativum L.	T	Pt	(Asztemborska et al. 2015)
Linaria alpina (L.) Mill.	H	Ni	(Vergnano Gambi and Gabrielli 1981)
Lolium perenne L.	H	As, **Pb***	(Karczewska et al. 2013; Perry et al. 2012)
Lotus corniculatus L.	H	Zn, Fe, Hg	(Fernández et al. 2017; Massa et al. 2010)
Luzula lutea (All.) DC.	H	**Ni**	(Vergnano Gambi and Gabrielli 1981)
Lythrum salicaria L.	H	Hg, Zn	(Fernández et al. 2017)
Malva neglecta Wallr.	T	Zn, **Cu**	(Malayeri et al. 2013; Nematian and Kazemeini 2013)
Malva nicaeensis All.	T	Fe	(Baycu et al. 2015)
Medicago lupulina L.	T	**Pb**	(Amer et al. 2013)
Medicago sativa L.	H	**Pb***	(Pajuelo et al. 2007)
Melilotus albus Medik.	T	**Pb**	(Fernández et al. 2012)
Melilotus officinalis (L.) Pall.	H	**Pb**	(Fernández et al. 2012)
Minuartia laricifolia (L.) Schinz & Thell	CH	**Ni**	(Vergnano Gambi and Gabrielli 1981)
Minuartia verna (L.) Hiern	CH	**Pb**, Zn	(Fernández et al. 2017; Wenzel and Jockwer 1999)
Mirabilis jalapa L.	G	**Cd***	(Wang and Liu 2014)
Moricandia arvensis (L.) DC	T	**Cd**, Pb	(Karimi et al. 2012)
Musa basjoo Siebold & Zucc. ex Iinuma	G	Pb, Zn	(Nan et al. 2013)
Nicotiana glauca Graham	NP	Zn	(Barazani et al. 2004)
Nicotiana tabacum L.	T	U	(Stojanović et al. 2012)
Persicaria hydropiper (L.) Delarbre	T	**Mn**	(Liu et al. 2010)
Persicaria lapathifolia (L.) Delarbre	T	**Mn**	(Liu et 2016)
Phagnalon rupestre (L.) DC.	CH	Fe	(Baycu et al. 2015)
Phaseolus vulgaris L.	T	**Cd**, **Pb***, Zn, **Cu**	(Luo et al. 2005; Luo et al. 2008)
Phillyrea angustifolia L.	P	**Cd**	(Tapia et al. 2011)
Phragmites australis (Cav.) Trin. ex Steud.	G	Hg, Mn	(Bonanno and Giudice 2010)

Taxa	L. F.	Metal(loid)s	References
Phytolacca americana L.	G	**Mn**	(Peng et al. 2008)
Picris hieracioides Sibth. & Sm.	H	Hg	(Fernández et al. 2017)
Piptatherum miliaceum (L.) Coss.	H	Hg	(Fernández et al. 2017)
Pistacia lentiscus L.	P	Pb, Zn	(Concas et al. 2015)
Pistacia terebinthus L.	P	Fe	(Baycu et al. 2015)
Pisum sativum L.	T	**Pb***	(Chen et al. 2004; Huang et al. 1997)
Plantago lanceolata L.	H	Hg	(Fernández et al. 2017)
Poa alpina L.	H	Zn	(Fernández et al. 2017)
Poa annua L.	T	**Cd**, Pb, Zn	(Barbafieri et al. 2011)
Polygonum amphibium L.	G	Zn	(Nan et al. 2013)
Polygonum aviculare L.	T	**Hg**, Zn	(González and González-Chávez 2006; Massa et al. 2010)
Polypogon monspeliensis (L.) Desf.	T	**Hg**	(Su et al. 2008)
Populus × canadensis Moench	P	Zn	(Nan et al. 2013)
Potentilla micrantha Ramond ex DC.	H	Hg	(Fernández et al. 2017)
Pteridium aquilinum (L.) Kuhn	G	Pb, Zn, Hg	(Fernández et al. 2017)
Pteris cretica L.	H	**Sb**	(Feng et al. 2015)
Pteris multifida Poir.	H	**As**	(Wang et al. 2007)
Pteris vittata L.	H	**As**, **Sb**, **Hg**	(Su et al. 2008; Tisarum et al. 2014; Tu et al. 2002)
Quercus robur L.	P	Hg	(Fernández et al. 2017)
Ranunculus peltatus subsp. *fucoides* (Freyn) Muñoz Garm	I	U	(Favas et al. 2014)
Raphanus raphanistrum subsp. *sativus* (L.) Domin	T	Zn	(Marchiol et al. 2004)
Rapistrum rugosum (L.) All.	T	Pb	(Saghi et al. 2016)
Reseda lutea L.	H	**Fe**	(Nematian and Kazemeini 2013)
Rhamnus alaternus L.	P	**Cd**	(Tapia et al. 2011)
Ricinus communis L.	T	Cd, As	(Huang et al. 2011; Melo et al. 2009)
Rosmarinus officinalis L.	NP	Cd	(Tapia et al. 2011)
Rubus ulmifolius Schott	NP	**As**, **Pb**, Hg	(Fernández et al. 2017; Marques et al. 2009)
Salix atrocinerea Brot.	P	Hg, Zn	(Fernández et al. 2017)
Salix caprea L.	P	Hg, Zn	(Fernández et al. 2017)
Salsola kali L.	T	**Cr**	(Gardea-Torresdey et al. 2005)
Saxifraga exarata Vill.	H	Ni	(Vergnano Gambi and Gabrielli 1981)
Saxifraga paniculata Mill	H	Ni	(Vergnano Gambi and Gabrielli 1981)
Senecio vulgaris L.	T	Fe	(Baycu et al. 2015)
Serapias vomeracea (Burm.f.) Briq.	G	Fe	(Baycu et al. 2015)
Silene ciliata Pourr.	H	Zn	(Fernández et al. 2017)
Silene latifolia Poir.	H	**Tl**	(Escarré et al. 2011)
Silene nutans L.	H	Hg	(Fernández et al. 2017)
Silene vulgaris (Moench) Garcke	H	**Sb**	(Baroni et al. 2000)
Sinapis alba L.	T	Tl, Cu, Pt	(Asztemborska et al. 2015; Malagoli et al. 2014; Vaněk et al. 2010)
Sinapis arvensis L.	T	**Pb**	(Saghi et al. 2016)
Smilax aspera L.	NP	Pb, Ba	(Poschenrieder et al. 2012)
Solanum americanum Mill.	T	**Cd**	(Wei et al. 2005)
Sonchus asper (L.) Hill	T	**Pb**	(Yanqun et al. 2005)
Sonchus oleraceus (L.) L.	T	Fe	(Baycu et al. 2015)
Sorghum bicolor (L.) Moench	T	Pb*, Zn, Cu	(Al Chami et al. 2015; Marchiol et al. 2007; Zhuang et al. 2009)
Spinacia oleracea L.	T	Fe	(Pathak et al. 2013)
Stachys recta L.	H	Fe	(Dudić et al. 2007)
Stipa barbata Desf.	H	**Cu**	(Malayeri et al. 2013)
Tagetes erecta L.	T	**Cd**	(Uraguchi et al. 2006)
Taraxacum campylodes G.E.Haglund	H	Fe, **Pb**, Zn	(Bech et al. 2016; Giacomino et al. 2016; Keane et al. 2001)

Taxa	L. F.	Metal(loid)s	References
Teucrium flavum subsp. *glaucum* (Jord. & Fourr.) Ronniger	CH	Pb	(Cao et al. 2009)
Teucrium polium L.	CH	**Ni**	(Yaman 2014)
Teucrium scorodonia L.	H	Hg	(Fernández et al. 2017)
Thlaspi caerulescens J.Presl & C.Presl	CH	**Ni**, **Cd**, **Pb**, **Zn**	(Reeves et al. 2001)
Thlaspi rotundifolium subsp. *cepaeifolium* (Wulfen) Rouy & Foucaud	H	**Cd**, **Pb**, Zn	(Wenzel and Jockwer 1999)
Thlaspi sylvium Gaudin	CH	**Ni**, Zn	(Taylor and Macnair 2006)
Tragopogon porrifolius L.	H	Fe	(Baycu et al. 2015)
Trifolium pallescens Schreb.	H	Ni	(Reeves and Brooks 1983)
Trifolium repens L.	CH	**Pb**, Zn	(Bech et al. 2016)
Trifolium subterraneum L.	T	**Pb***	(Pajuelo et al. 2007)
Trifolium thalii Vill.	H	Zn	(Fernández et al. 2017)
Trisetum flavescens (L.) P.Beauv.	H	W	(Erdemir et al. 2017)
Typha angustifolia L.	G	**Pb**	(Panich-Pat et al. 2010)
Typha latifolia L.	G	Mn	(Salem et al. 2017)
Vicia faba L.	T	Pb	(Karimi et al. 2012)
Xanthium strumarium L.	T	Fe	(Nematian and Kazemeini 2013)
Zea mays L.	T	**Pb***, **Cu***	(Huang et al. 1997; Luo et al. 2005)
Zygophyllum fabago L.	NP	Fe	(Martínez-Sánchez et al. 2012)

* Chelating agents added.



Id	Regions	N. Phy.
1	PIEMONTE	122
2	VALLE D'AOSTA	88
3	LOMBARDIA	111
4	TRENTINO-ALTO	99
5	VENETO	124
6	FRIULI-VENEZIA	130
7	LIGURIA	129
9	TOSCANA	136
10	UMBRIA	114
11	MARCHE	104
12	LAZIO	129
13	ABRUZZO	127
14	MOLISE	117
15	CAMPANIA	123
16	PUGLIA	107
17	BASILICATA	116
18	CALABRIA	106
19	SICILIA	118
20	SARDEGNA	107

Figure 1. Number of accumulators and hyperaccumulators for each metal(loid) (A) and regional distribution of Phytoextractors in Italy (B).

Eighty-eight plant species are hyperaccumulators, while the other phytoaccumulators cannot reach or exceed the hyperaccumulation threshold.

Lead and zinc are the best accumulated metals, followed by mercury, cadmium and iron. (Fig. 1A).

For lead, we report that 25 taxa are hyperaccumulators while 17 are accumulators.

The highest lead concentration is reported in the roots of *Medicago lupulina* L. (63500 mg kg^{-1}).A wetland species, *Typha angustifolia* L. (20173 mg kg^{-1}), and two cultivated plants, *Pisum sativum* L. (11000 mg kg^{-1}) and *Zea mays* L. (10000 mg kg^{-1}), are among the most efficient (and therefore health-threatening) lead hyperaccumulators (Table 1).

For zinc, only 2 of the 42 listed accumulators are hyperaccumulators, with *Thlaspi caerulescens* J. & C. Presl (43710 mg kg^{-1}) being the best extractor, followed by *Alternanthera philoxeroides* (Mart.) Griseb. (43254 mg kg^{-1}). Of the 36 mercury accumulators, 12 are hyperacccumulators. Among these, metal concentration values are very high in two species (43254 mg kg^{-1} in *Polypogon monspeliensis* (L.) Desf. and 43254 mg kg^{-1} in *Pteris vittata* L.), with other values dropping to values close to the threshold.

A substantial part of cadmium phytoextractors (19 of 27) are hyperaccumulators, with values ranging from 5722 mg kg^{-1} in *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz to 107 mg kg^{-1} in *Phyllirea angustifolia* L. Iron is another element with a considerable number of phytoaccumulators (> 20). For this metal, six species, such as *Reseda lutea* (48116 mg kg^{-1}) are hyperaccumulators, while 18 cannot reach the threshold.

Worldwide, most of the known Hyperaccumulating plants are nickel accumulators (Boyd, 2009; Sheoran, 2009). In our list, given that the hyperaccumulation threshold for nickel has been set at 1000 mg kg^{-1} (Baker et al., 2000), *Arabis sagittata* (Bertol.) DC. (120 mg kg^{-1}) is the only plant species that does not reach the specified hyperaccumulation limit. Instead, *Teucrium polium* L. (14110 mg kg^{-1}), *Thlaspi caerulescens* (12880 mg kg^{-1}), *Alyssum bertolonii* (8727 mg kg^{-1}), *Alyssoides utriculata* (L.) Medik (1100 mg kg^{-1}), *Thlaspi sylvium* Gaudin. (1064 mg kg^{-1}) and *Helianthus annuus* L. (2944 mg kg^{-1}), all exceed the threshold and are therefore considered as hyperaccumulators (Tab. 1). Astonishingly, the concentration of nickel in *Teucrium polium* L. is fourteen times higher than the hyperaccumulation limit.

Uncommon metals, such as barium, indium and gold can be extracted by *Smilax aspera* (180 mg kg^{-1}), *Eleocharis acicularis* (L.) Roem. & Schult. (353 mg kg^{-1}) and *Brassica juncea* (L.) Czern. (326 mg kg^{-1}), respectively (see Supplementary file Table S01 for details).

Taxonomical, geographical and ecological characterization

The floristic list reports 167 species, 4 subspecies and 1 variety, for a total of 172 taxa belonging to 52 families. The most represented family is *Brassicaceae* with 25 plant species (14.53%), followed by *Poaceae* (12.21%), *Asteraceae* (11.63%), *Leguminosae* (7.56%), *Chenopodiaceae* (4.65%), *Caryophyllaceae* (3.49%), *Lamiaceae* (2.91), *Polygonaceae* (2.33%) and others (< 2%).

Considering life forms, most of the phytoextractors are hemicryptophytes (35.47%) followed by therophytes (31.40%), chamaephytes (8.72%), geophytes (8.72%), phanerophytes (8.72%), nanophanerophytes (4.07%) and idrophytes (2.91%).

The phytoextractors were found to be spread in all the regions of Italy. Toscana has the highest number of Phytoextractors (136), followed by Friuli-Venezia Giulia (130), Lazio (129) and Liguria (129), while Marche (104), Trentino-Alto Adige (99) and Valle d'Aosta (88) are regions with a “low” number of taxa (Fig. 1B). Of all the taxa, 139 are native to Italy, while 33 are alien species.

Ellenberg’s indicator values range very widely. As shown in Table 2 and Fig. 2, moisture (F) and nitrogen (N) values have the highest Standard Deviations (2.38 respectively). Mean values (Fig. 2) are: 8.02 (N), 6.78 (T), 4.72 (K), 4.50 (F), 5.63 (R), 3.99 (N), 0.17 (SAL). Compared to the theoretical value (6), light (L) and temperature values (T) have a higher median value, while continentality values (K), moisture values (F) and nitrogen values (N) have lower median values (Fig. 2).

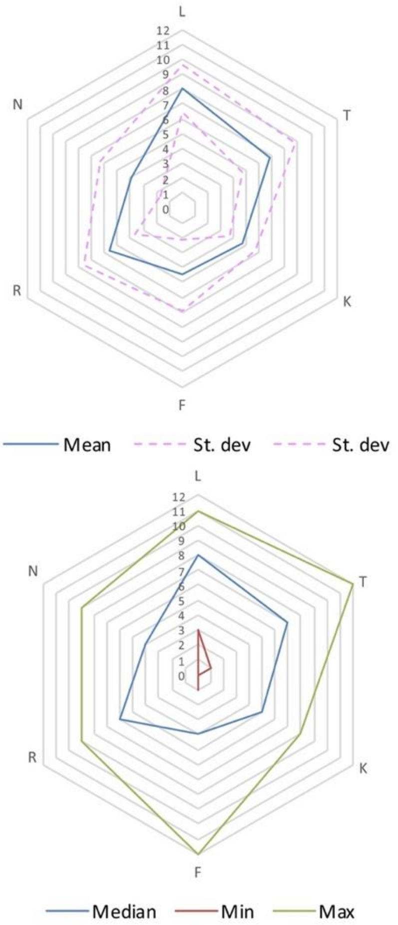


Figure 2. Ecograms of Ellenberg Environmental values for the Phytoextractors of Italy. (L = light; T = temperature; K= continentality; F= moisture; R = Soil reaction= N nitrogen).

Table 2. Ellenberg values[1] statistics for Italian Phytoextractors.

	L	T	K	F	R	N	S
Minimum	3	1	0	1	0	0	0
Maximum	11	12	8	12	9	9	9
Mean	8.02	6.78	4.72	4.50	5.63	3.99	0.17
Median	8	7	5	4	6	4	0
Standard Deviation	1.63	2.05	1.01	2.38	2.02	2.38	0.93
Variance	2.63	4.16	1.02	5.62	4.04	5.61	0.86

[1] L = Light value; T = Temperature value; K = Continentality value; F = Moisture value; R= Reaction of soil value (PH); N = Nitrogen value; S= Salinity value.

Discussion

Geographical distribution of metal(loid) accumulating plants in Italy

In Italy, heavy metal soil contamination has a quite heterogeneous and diversified geographical pattern. For instance, it is not uncommon to find different concentrations of various heavy metals in the same land areas. According to Tóth et al. (2016), many countries of Europe have regions with a high percentage of topsoil samples which have concentrations of inorganic pollutants well above the normal values. Many Regions of Italy are affected by heavy metal soil pollution, as well (Tóth et al., 2016). Anyway, all the Italian Regions, have a fairly large number of phytoextractors in their flora to be used for soil remediation needs. In fact, almost all the regions host more than 100 taxa of phytoextractors (Fig. 1B).

A comparison between the whole flora and the number of phytoextractors for each region gave a 0.6 Pearson Index. This indicates a significant correlation between the two variables and also suggests that the number of phytoextractors might be primarily dependent on the floristic richness of regions. Worldwide, the metal(loid) accumulating plant species are usually abundant in areas with a significant presence of serpentine soils, such as Cuba, South Africa and New Caledonia (Callahan et al., 2012; Reeves et al., 2018). In Italy, serpentine geological islands range from the central-western Alps to the northern Apennines, mainly appearing in the region of Toscana, where several outcrops are scattered in different biogeographic sectors (Selvi, 2006; Bini and Maleci, 2014; Bini et al., 2017). As expected, in our analysis the majority of the phytoaccumulators are plants of the Tuscan flora, where serpentine soils are somewhat common. However, of the 87 serpentinophytes observed by Selvi (2006), only *Alyssum bertolonii*, *Cistus salvifolius* L., *Helichrysum italicum* (Roth) G. Don and *Erica arborea* L. are known to be phytoaccumulators (Table 1). Therefore, further investigations might lead to the discovery of more metal accumulating plant species within the Italian serpentinophytes.

The regional distribution of phytoaccumulators for any given metal is shown in figure 3. The phytoextractors of indium, barium, aluminium, silver and gold have the most limited geographical distribution.

Taxonomical considerations on metal(loid) accumulating plants

Many different families have large numbers of species that tolerate high levels of HMs. The family with the largest number of phytoextractors is *Brassicaceae*, followed by *Asteraceacae, Caryophyllaceae, Poaceae* and *Leguminosae*. Many authors have pointed out that most of the known accumulators belong to the *Brassicaceae* family (Krämer, 2010; Szczygłowska et al., 2011). As already mentioned above, this is consistent with our data. From the placement of the 52 families of phytoextractors into the phylogenetic tree (Angiosperm Phylogeny Group III, 2009) it can be noted that the phytoextractors tend to distribute in different clades. According to Broadley et al. (2001), the phenomenon of hyperaccumulation is more common in certain orders than in others. For instance, Ni hyperaccumulators are very common in Brassicales, Asterales and Malphigiales.

As noted by Baker and Brooks (1989), even if the (hyper)accumulators of Nickel seem to have some phylogenetic relationships at the family level, the phenomenon of Metal hyperaccumulation has evolved in a wide range of apparently unrelated taxa.

The phylogenetic distribution of the accumulators in our list suggests that the ability to accumulate metals may be independent of phylogeny.

Ecological behavior of metal(loid) accumulating plants

The ecological relationship amongst accumulating plants, climate and soil conditions is described using the Ellenberg's indicator values (Eiv). Such indicators are an empirical tool used to describe the ecological response of plants to environment (Marcenò et Guarino 2015). However, because EIVs are ordinal scales, EIV values are generally not considered mathematically rigorous enough. Still, since it has been proved that all kinds of statistical tests based on mean and variance can be used with acceptable confidence levels (Domina et al., 2018), we decided to use the EIVs to gather informations on the ecology of metal(loid) accumulating plants.

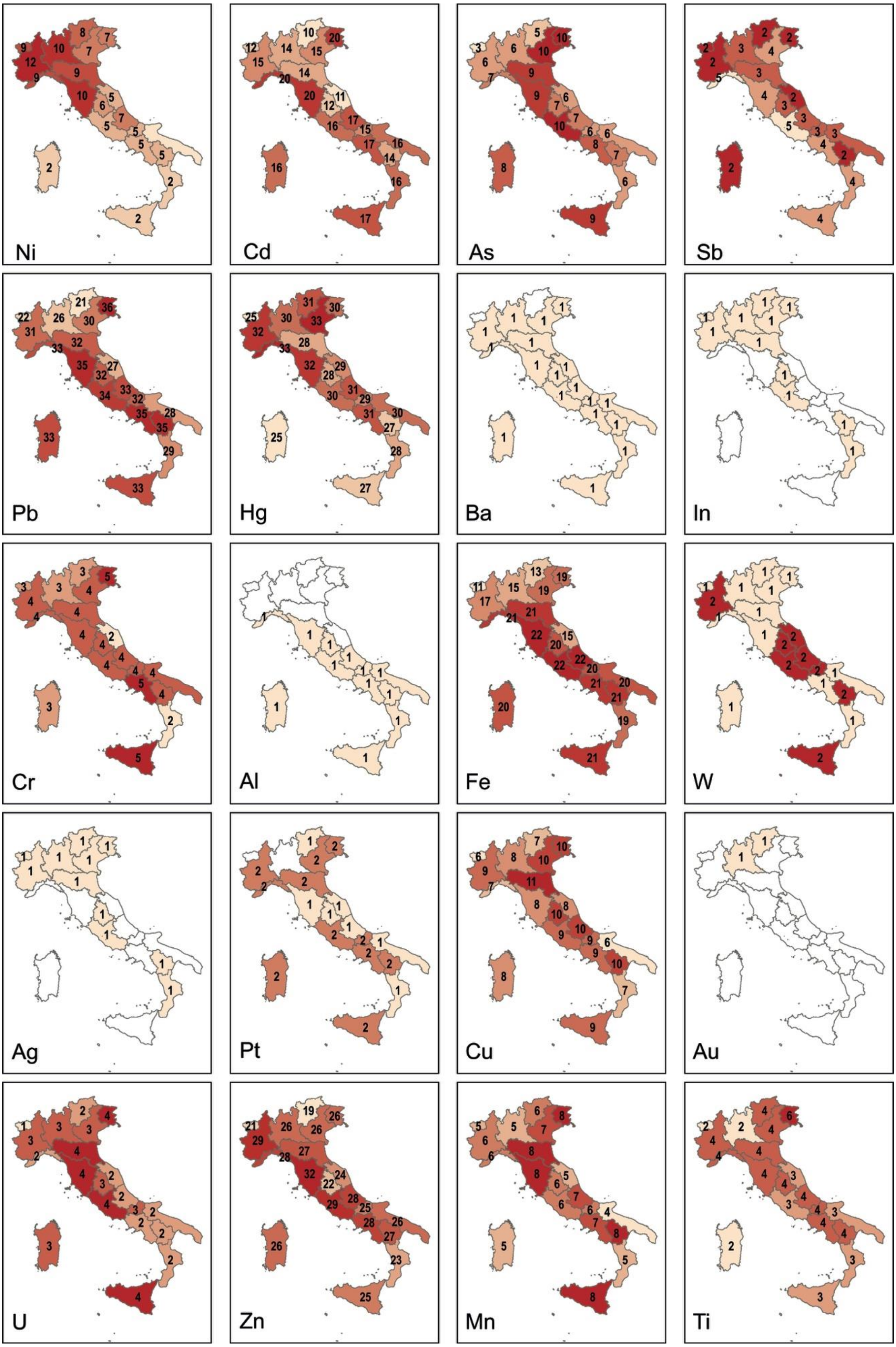


Figure 3. Regional distribution of phytoaccumulators for any given metal.

Pignatti's EIVs assessment for the whole Italian native flora includes 5774 taxa (Pignatti 2005; Domina et al., 2018). In our checklist, the native accumulating plants (139 taxa) represent the 2,41% of these taxa. The alien species have a similar percentage, being the 2.07% of the whole assessed alien taxa (1597), which were listed by Domina et al (2018).

The EIVs mean comparison between the accumulating native flora and the whole flora of Italy (Pignatti et al., 2005) indicates that the native phytoextractors have higher nutrient requirements (N= 3.71 vs 3.26), higher soil reaction requirements (R= 5.75 vs 5.45) and higher edaphic humidity requirements (F= 4.48 vs 4.17), while having similar ecological requirements for Light (L= 7.94 vs7.84), Temperature (T= 6.50 vs 6.36) and Continentality (K= 4.67 vs 4.60). Despite the low number of alien taxa, the EIVs for the alien phytoextractors are in line with the national EIVs distribution for the alien plants. Within the accumulating plants, the alien species are more thermophilous than the native species and this is consistent with the considerations of Domina et al. (2018) for the whole Italian flora. Mean EIVs for the metal(loid) accumulating flora clearly shows that this subset of flora is ecologically representative of the entire Italian flora.

The distribution of R values (Fig. 4A) for the best accumulated metal(loid)s shows that most of the phytoextractors naturally grow in neutral to basic soils. For example, species with high R-numbers have higher Fe-solubilizing capacity than most species with low values (Bartelheimer and Poschlod, 2016). On the contrary, *Erica arborea* (an acidophilus species with an R value of 1) contains very low levels of Fe (202 mg kg^{-1}) (Fernández et al., 2017). For Zn and Pb, a basophile plant such as *Thlaspi rotundifolium* subsp. *cepaeifolium* (Wulfen) Rouy (R=9) has a very good accumulation capability and its ecological requirements are in line with the observations of Bartelheimer and Poschlod (2016). *Trifolium repens* L. (R=5), a plant growing in soils with a near-neutral pH, is also capable of accumulating high concentrations of metal(loid)s in substrates with a great calcium carbonate percentage (Bech et al., 2016). Consistently with these considerations, we did not find any correlation between the R values and accumulation capability (Pearson r = 0.145 for Pb; Pearson r = -0.001 for Zn). For nitrogen/nutrients (Fig. 4B), values range from N = 2 in species growing on poor soils to N= 7 in plants often found in richly fertile places. The box plot (Fig. 4B) shows that the data have a broad distribution, with low median values (except for As and Cu). Such variability of the ecological requirements offers a wide spectrum of phytoextractors to choose from for any phytoremediation procedure in Italy.

Outcomes and implications

Over the last decades, the phenomenon of hyperaccumulation of metal(loid)s in plants aroused great interest within the scientific community, both from a biological point of view (understanding the mechanisms of metal absorption and translocation) and for the potential use of metal accumulating plants in bioremediation procedures. The research on this topic produced thousands of publications, resulting in the discovery of a huge number of metal(loid) accumulating taxa, some of which accumulate metal(loid)s at exceptional concentrations. The bibliographic screening in this work allowed us to identify a fairly large number of metal(loid) accumulating species that belong to the Italian flora. Interestingly, the EIVs ecological outline revealed a high ecological plasticity of the entire accumulating flora.

In conclusion, this work wants to provide a first synthesis of what is known about the Italian phytoaccumulating species. Also, we think that it can be useful to have a list of phytoaccumulators to use in various contexts of soil contamination for phytoremediation purposes.

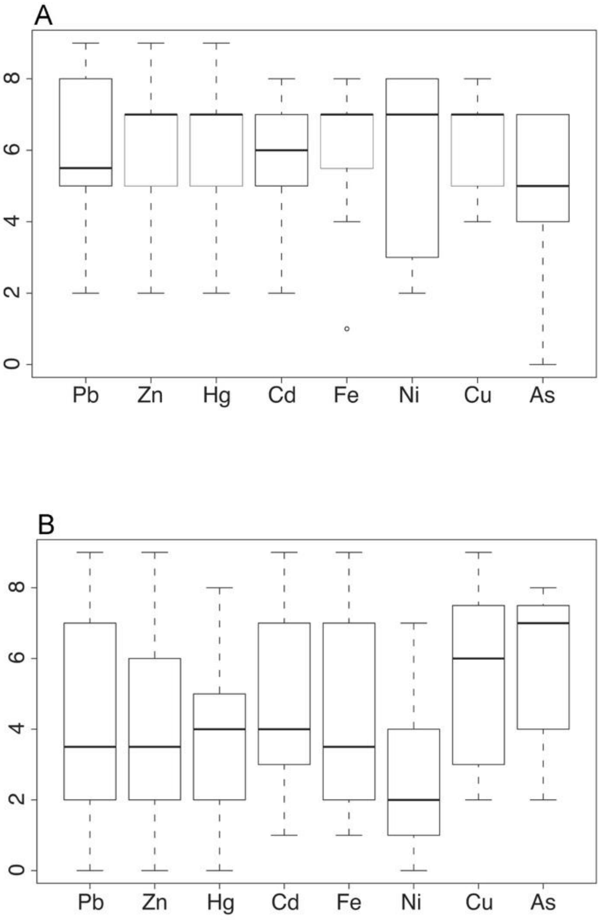


Figure 4. Box plots of the distribution of the Soil Reaction values (A) and Nitrogen/Nutrient values (B) for the most important metals.

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