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Using native species and perennial grasses for characterization, remediation/securing and monitoring of contaminated sites

Dottorando	**Tutor**
Dott. Donato Visconti	*Prof. Massimo Fagnano*
	Co-Tutor
	Dott. Nunzio Fiorentino

Coordinatore
Prof. Guido D'urso

Summary

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1. ABSTRACT

Potential Toxic Elements (PTEs) soil contamination is one of the most serious environmental problems threatening human health and ecosystems functioning for their high persistence and possible accumulation in different organisms with the transference to other systems. Phytoremediation uses plants to remove or immobilize bioavailable PTEs in contaminated sites, but the effectiveness of this technique requires to identify plant species efficient in extracting or immobilizing PTEs and tolerant to high levels of contamination (Kumar et al., 1995). An effective identification of the most suitable species for natural phytoremediation can be done assessing the composition of natural vegetation already grown in contaminated sites. The assisted phytoextraction uses plant species for PTE removal enhancing root efficiency and increasing PTE bioavailability with organic amendments (Meers et al., 2005; Saifullah et al., 2009) or biopromoters like microorganism. Composted municipal wastes may be applied to cropland as a source of nutrients and for improving the physical properties of the soil, leading to an increase in plant growth (Fagnano et al., 2011),while biompromoters like Trichoderma, soil bacteria, endomycorrhiza, can improve plant yield, resistance to environmental stresses and increase root PTE uptake. In this thesis we report the results of three experiments aimed at: i) studying the potential of phytoscreening to identify the most suitable plant species for phytoremediation and the characterization of the environmental quality of potentially contaminated sites; ii) verify the growth performance of grass species on contaminated industrial soils in order to evaluate their possible use in environmental securing.

In the first experiment phytoscreening has been combined with soil analysis in two potentially contaminated sites (an agricultural and an industrial site) in order to: identify species that can tolerate highest concentrations of PTEs by using plant community study; identify metal pollution and their possible sources by using PCA and HCA; evaluate the bioaccumulation potential of plant species; investigate the relationships between the total EPTs in the plants, the total EPTs content of soils and extract EPTs concentrations to know the extraction method that better represents the plants availability and to know the relation between specific plant species and soil, in particular to know if some plant can be used as a *bioindicator* of PTEs. Among the plant species screened in the industrial site, *S. latifolia* was identified as a hyperaccumulator of Tl. In both sites, majority of PTEs concentrations

in natural plants exceeded the upper limits of the normal range for terrestrial plants grown in uncontaminated soils, demonstrating that the plants accumulate higher PTEs levels when grown in contaminated soils. In the industrial site, the concentration in the plants was too low to consider them for phytoremediation purpose except *A. annua* and *C. arvense* for Cd and *S. latifolia* for Tl.

Furthermore a linear relationship between Zn concentrations in shoots and soil was recorded for *E. repens* grown in the industrial site, meaning that this species can be used as indicator of Zn soil contamination.

A good correlation between As and Cd content in roots and soil was recorded for *L. perenne* in the agricultural site, suggesting the use of this species for biomonitoring programmes. Principal component analysis and hierarchical cluster analysis performed on soil PTE content of the industrial site highlighted that Pb, Cd, As, Cu and Zn come from different anthropogenic pollution sources while Sb and Tl seems mainly of geogenical origins. In the agricultural site, PCA and HCA confirmed the attribution of Cr and Cd to anthropogenic origins while Pb, As and Zn were considered from geogenical origins.

In the second experiment they have been evaluated the effects of an organic amendment (green waste compost) and two commercial biopromoters (TB: Trianum-P containing *Trichoderma harzianum* - strain T22 – Koppert b.v. ®; TA: consortium called "Panoramix" – Koppert b.v. ® containing *Endomycorrhiza* and *Trichoderma* species along with humic and fulvic acids) in two substrates (soil of an industrial site and the sludge derived from the soil-washing) on the growth, EPTs phytoextraction/phytostabilization of a grass commercial mix (*Festuca arundinacea, Poa pratensis* and *Lolium perenne*). The plants resulted well adapted to the contamination of soils and sludges showing a good growth during the year of experimentation. The application of compost and TA increased plant growth, nutrient uptake and Zn uptake. TB treatment showed lower plant growth alone and in combination with compost. The PTEs accumulation in the aerial part of plants was low except for Zn. Therefore, the plant species used in this experiment, were suitable for a phytostabilization purpose reducing the uplift and dispersion of the contaminated soil particles and limiting the leaching of PTEs in the soil profile.

In the third experiment they have been evaluated the effects of two doses of compost from RSU (C1=25 mg FW ha^{-1} and C2=50 mg FW ha^{-1}) and two commercial biopromoters (TB: Trianum-P containing *Trichoderma harzianum* - strain T22 – Koppert b.v. ®; TA: consortium called "Panoramix" – Koppert b.v. ® containing *Endomycorrhiza* and *Trichoderma*

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species along with humic and fulvic acids) on growth, EPTs phytoextraction/phytostabilization of natural grass species (*Dactylis glomerata*) and a grass commercial mix (*Festuca arundinacea, Poa pratensis and Lolium perenne*) grown in a highly contaminated site (Pb and Cd). The application of the lower compost dose and biopromoters increased growth, N uptake and Cd uptake. The application of TA reduced the bioavailable Pb as compared to control and to TB and reduced the bioavailable Pb fraction with respect to the initial values and to a bare soil control. The combination of compost and biopromoters also reduced Cd soluble fraction as compared to the bare soil control highlighting the importance of a vegetal soil cover for avoiding PTEs leaching in the soil profile.

2. INTRODUCTION

2.1 Anthropogenic contamination

Anthropic activities, through as industrial processes (Kaitantzian et al., 2013), extractive activities, energy production (Rodriguez Martin et al., 2013), vehicular traffic (Argyraki and Kelepertzis 2014) and agriculture with the application of pesticides and fertilizers (Koch and Rotard 2001) have resulted in a significant in-crease in the concentration of organic and inorganic contaminants in the environment. Among these contaminants an important role is covered by inorganic contaminants, potentially toxic elements (PTEs) that are dumped into soils and water at toxic level or very close to them (Bai et al 2009, 2011). Inorganic PTEs include different metals and metalloids de-fined by the US State Environmental Protection Organization as "metal elements with high atomic weights that even at low concentrations can damage animal and plant kingdom, the biota. The same ones do not degrade and tend to accumulate in plants, animals and people causing health problems (U.S.EPA, 2012). This scenario led to a progressive damage to of natural resources, territorial compromise and dangers to the human health of populations. These dangers are related due to the strong persistence of PTEs in the environment, their bioaccumulation capability (Bai et al., 2012) and to produce teratogenic, mutagenic and carcinogenic effects in living organisms (Vithanage et al., 2012). In particular, lead is the second most dangerous agent (Agency for Toxic Sub-stances & Disease Registry - ATSDR) for the high risk of human health related risk to trough ingestion, dermal contact and inhalation especially for children (Jennings, 2013). Potentially contaminated and potentially contaminated Italian areas include distribution facilities and fuel depots, handicraft and industrial areas, dismantled or decommissioning facilities, abusive landfills and waste storage areas. These areas are often a source of pollution for the surrounding environment due to the diffusion of pollutants such as dust dispersion and contaminant leaching by atmospheric agents. This entails a considerable complexity of soil contamination and groundwater contamination, which must be taken into account during the reclamation phase (Venturi, 2002). Contaminated sites can damage represent a risk not only for individual organisms of an ecosystem but also the functionality and structure of the ecosystem itself by modifying biogeochemical

cycles or causing loss of reducing bio-diversity. PTE-related contamination falls within the broader framework of degradation of the physical, chemical and bio-logic soils shown in Fig. 1.
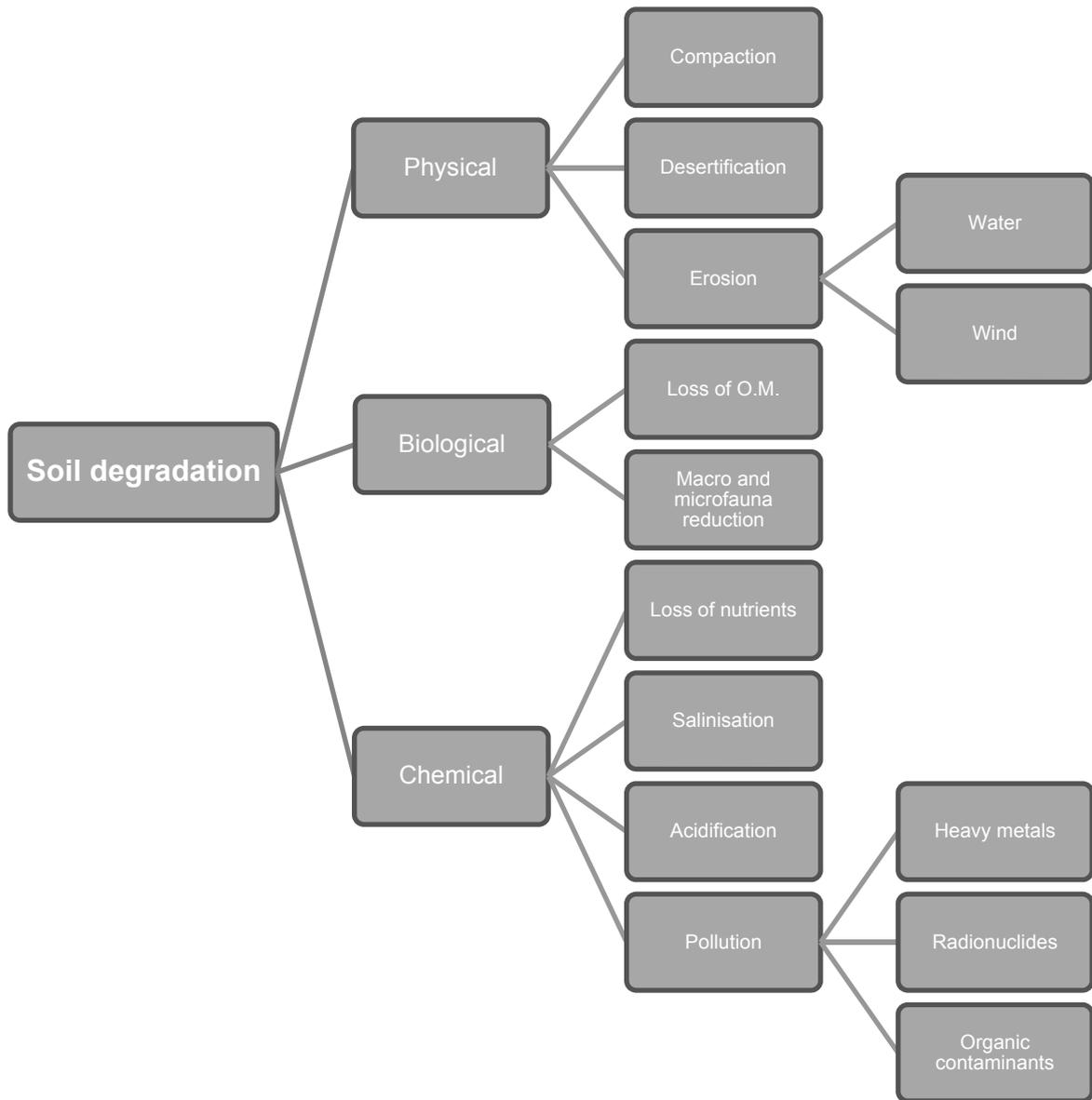
Fig. 1. Processes that influence the physical, chemical and biological degradation of the soil (Modified from Lai, 1998.)

2.2 National legislation on contaminated and potentially contaminated sites

Currently the national legislation on the environment is represented by the Decree n. 152 of April 3, 2006 and m.i., known as the Environment Code (EC), which provides for the

repeal of the Decree Ronchi and D.M. 471/99 (Article 264, paragraph 1) by collecting, re-arranging and modifying the previous environmental legislation; the decree is made of six sections, as below reported:

- Common provisions and general principles;
- EIA (Environmental Impact Assessment), SEA (Strategic Environmental Assessment) and IEA (Integrated Environmental Authorization);
- Soil protection, water conservation and water management;
- Waste management and reclamation of contaminated sites;
- Air protection and emissions reductions in the atmosphere;
- Protect against damage to the environment.

Legislation on remediation and environmental restoration of contaminated sites is contained in Part Four of Legislative Decree 152/06 defining the procedures, criteria and procedures for carrying out the operations necessary for the elimination of pollution sources and to reduce concentrations of pollutants.

In Legislative Decree 152/06 pursuant to art. 240 defines the definition of a contaminated site as follows: *"a geographically defined area or part of territory, intended in the various environmental matrices (soil, subsoil and groundwater) and comprehensible of any existing building and plant structures, in which the values of Concentration Risk Threshold (CRT) ... are exceeded "*. The concepts of Concentration Threshold of Contamination (CTC) are thus introduced, namely: "contamination levels of environmental matrices that constitute values above which site characterization and specific site risk analysis are required" and the concept of Concentration Risk Threshold (CRT), namely: *"contamination levels of environmental matrices, to be determined on a case by case basis with the application of the site specific risk analysis ... and based on the results of the characterization plan"* .

As reported in Fig. 2, according to Legislative Decree 152/06, a site is considered potentially contaminated if there is the overrun of CTCs whose values, in terms of soil and subsoil, are in the Annex 5 of L.D. and specifically in Tab. 1, as already in Annex 1 of DM. n. 471/1999. CTCs values for each pollutant vary depending on whether the site is for public, private or residential use (column A) or commercial and industrial use (column B), taking into account the baseline values.

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In the case of CTC overriding values, the L. D. 152/2006 provides for emergency securing, site characterization according to the conceptual model that includes the following 3 components:

1. Secondary source of pollutants through the exploration of shallow, deep, and deep ground);
2. Delivery Mechanisms;
3. Contamination targets (human receptor).

After this phase, the calculation of CRT steams from a site specific risk analysis according to the intended use of soils (urban or industrial) and to the type and duration of site attendance by the population. For urban and industrial areas, account is taken of the number of hours spent on the site being this factor correlated to the toxicity risk due to ingestion, inhalation of particulate matter as well as to dermal contact. Being defined the output of a site specific risk analysis, two scenarios are possible: for concentrations of soil contaminants lower than CRTs, monitoring of their fate must be performed and no other intervention occurs; otherwise, if CRT is exceeded, securing or site reclamation must be carried out together with monitoring of pollutants. Legislative Decree 152/06 specifies as **remediate** the removal or reduction of contaminants below CRTs. The **securing** specified in the U. T. includes a set of measures to ensure the interruption of the exposure path for living organisms, reducing the particulates produced by wind erosion and leaching in underlying or adjacent aquifers. Depending on the use of the site, there is a permanent or operational securing. The operative securing provides remediation of sites where human activity is still in use and therefore aims to reduce the risk of contamination. It provides permanent safety in disposed sites unsuitable for agriculture (e. g. industrial areas and related road) through a complete and definitive isolation of contaminants from other environmental compartments. In addition, environmental monitoring (air, surface and deep water, and vegetation) must be carried out. In addition to remediation and securing, environmental and landscaping techniques are included in **environmental restoration** and serve to ensure that the site is used for various purposes (sports, recreation, etc.).
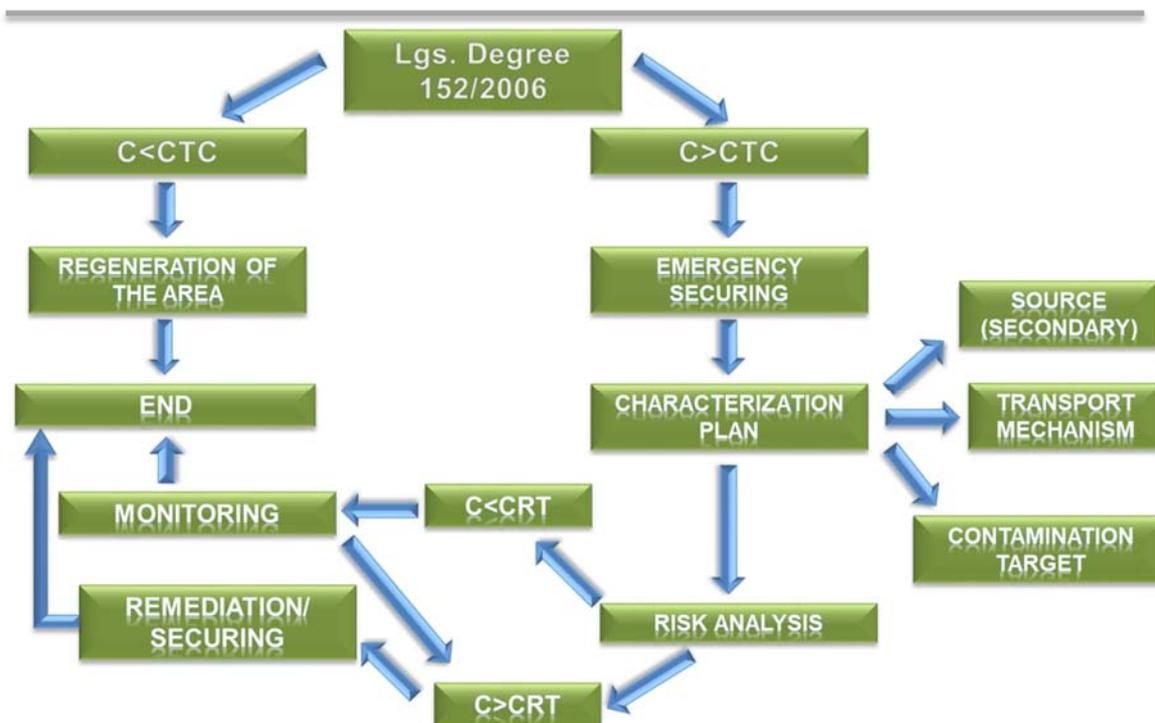
Fig.2. Phases provided by L. D. 152/06 for potentially contaminated and contaminated sites

There is no specific legislation for agricultural land, as the regulation provided by art. 241 has never been emanated. The interministerial working group referred to L.D. 136 of 10/12/2013 analyzed the suitability of soils for agricultural use. They drawn up a specific regulation for agricultural areas submitted to the Government for the approval on 2015. This regulation was advocated by art. 241 of the Single Environmental Act (Legislative Decree 152/06), which provides for the classification of the environmental quality of agricultural soils. The classification can be carried on the basis of the bioavailability of PTEs and above all the quality and health of agricultural products. This because, the risk associated with the contamination of an agricultural land is not only the exposure of agricultural workers (direct risks), but also the potential accumulation in the food crops depending on their bioavailability (root uptake). So analyzing PTEs content in tissues of natural vegetation and the edible part of vulnerable crops standing on a potentially contaminated site, can allow to asses the indirect risk for food consumers (Carlon C., 2007) (Ecoremed, 2017) .

2.3   Characteristics and origin of PTEs present in the soil

PTEs include several heavy metals and metalloids that the US Environmental Protection Agency (U.S. EPA) has included in the Priority Pollutants List. Among the top 250 in the

list we can list: As, Pb, Hg, Cd, Cr, Be, Co, Ni, Zn, Cu, Mn, Al, V, Ag, Sb and Tl. Some of these are essential for plants nutrition (Cu, Mn and Zn) while others are essential for animals' nutrition: As, Cu, Co, Mn, Zn, Cr, Ni, V and Zn (Adriano, 2001). The other elements do not have a known function in living organisms. The top 5 places in the rankings are filled by As, Pb, Hg, Cd and Cr (hexavalent) which are considered by EPA as the most dangerous inorganic PTEs for human health, a scenario shared by the National Agency for Cancer Research as well considered carcinogenic and causes multiple organ damage even at low concentrations (Tchounwou et al., 2014). As for the category of heavy metals, they usually act as cations, have low solubility of their hydrates, are characterized by different oxidation states based on pH and Eh, tend to form complex bonds, have low solubility of their oxides and have great affinity for the sulphides in which they tend to concentrate. The origins of PTEs can be both natural and anthropic. While parental rocks and metal minerals are the main natural sources, anthropogenic sources include: agriculture (fertilizers, animal manure, pesticides, etc.), metallurgy (mines, foundries, metalworking, etc.), energy production (lead fuels, battery manufacturing, power plants, etc.), and wastes. Pollutants can be released in gaseous form (aerosol), particulate matter, water, or solid form depending on the industry from which it derives. They may also have a point or diffuse origin (Adriano, 2001).

Natural origin of PTEs

Under natural conditions, the PTE content of soils depends largely on the composition of the parental rock and the degradation processes it was subjected. The PTE content varies greatly among the different rocks, in fact it is very low in sedimentary rocks and greater in igneous ones. Sedimentary rocks are the most abundant emerging rocks, consisting largely of silicates and aluminosilicates in which low concentrations of PTE are present due to isomorphic substitutions between ions with similar ionic radius, electronegativity and charge. Generally clay and schists are among the sedimentary rocks with higher PTE content. Two phases are responsible for soil formation and are difficult to differentiate as they occur simultaneously: the first phase is the alteration of primary minerals constituting the parental rock by chemical-physical rock degradation processes in which the organic substance and living organisms play a key role by releasing carbonic acid and organic chelates; the second phase of pedogenesis leads to the formation of the mature soil profile by the degradation of rock material (Kabata-Pendias, 2011). However, the concentration of PTEs in the soil also comes from other natural inputs such as atmospheric deposition of dusts from other soils, rocks and volcanic ashes.

Anthropogenic origin of PTEs

The main anthropogenic sources of PTE are:

- Agricultural activity: mineral fertilizers are an important source of contamination of agricultural soils together with sewage sludge, waste water and bio-solid (Mortvedt, 1996). Phosphatic fertilizers in particular, contain not negligible concentrations of Cd resulting from the phosphate rocks from which they are obtained. Other PTEs detected are As, Cr, Pb, Hg, Ni and V which can accumulate in the soil after repeated applications. In addition to fertilizers, several pesticides can also be another PTE source. The extent of contamination is much dependent on the composition of the product applied to the soil/crop (Garcia et al., 1996);

- Industrial activities: refineries and industrial activities related to the processing of metals may lead to the release of different PTEs in the environment such as Hg, Cd, Zn and Pb, either through the emission of aerosols and fumes that can also be deposited at long distances from the source of emission;

- Mining and Extraction Activities: generates both point-to-point and diffused pollution due to processes of leaching or atmospheric dispersion of contaminants;

- Waste disposal: both waste disposal in not secure old landfills (ante D.P.R. 915/82) and unauthorized disposal of waste as exhausted batteries may lead to contamination of the soil and subsoil. In addition, when waste is combusted, it is possible to have a diffuse contamination with the release of both organic and inorganic PTEs in the atmosphere.

- Energy production: the majority of power generation plants use fossil fuels such as coal or oil. Combustion can release into the atmosphere fumes containing Cr, Mn, Pb, Zn, Cu, Ni and Co (Sushil et al., 2006);

- Transportation: conventional transports use internal combustion engines fueled by petroleum fuels (oil, diesel, etc.) that can release PTEs such as Pb, Cu, Mn, Zn, Cd and Ni (Sezgin et al., 2004).

2.4 Biogeochemical processes regulating the mobility of PTEs

The soil has a solid phase consisting of primary and secondary minerals, humus (also called organic matter derived from the decomposition of organic residues), soil biomass (plants, animals and microorganisms), precipitated (carbonates, phosphates and sulphites)

and colloids. The soil also has a liquid phase known as circulating solution in which various organic, inorganic (complex, free and other ligands) and some gaseous compounds are dissolved; The liquid phase is the site of the microbial and radical processes, in addition to the speciation of the different biogeochemical forms of metals. The main biotic and abiotic activities occur in the interface between the solid and liquid phase of the soil. Major phenomena include ion exchange (**adsorption and desorption**), solubilization (**precipitation and dissolution**) by living biomass. Microorganisms and roots interact with dissolved species and microbial and radical exudates can also influence the solubility and eventually the transport of dissolved chemical species. These processes affect the biogeochemical pathways of PTEs and affect their solubility, mobility, bioavailability and toxicity, but predominance of processes is influenced by a number of geobiochemical factors, among which the most important are adsorption and desorption processes. Adsorption is the exchange of ions (including PTEs) between the soil solution and the organic and inorganic constituents of the soil. The latter comprise mainly: Fe and Mn oxides and, in the least, Al and Si oxides; clays; organic matter; carbonates, phosphates, sulphides and basic salts. Among them, clay, oxides and hydroxides as well as organic matter are considered the most important factors for adsorption of PTEs. According to Blume and Brummer (1987), the organic substance adsorbs strongly Cr, Fe, Pb and Hg, moderately Cd, Ni and Co and weakly Mn, Zn and other elements; the clays strongly adsorb only Fe, with moderate force Cd, Co and Ni and moderately the other elements; Oxides and hydroxides adsorb strongly Cr, Hg and Pb. pH, CEC and OM influence the adsorption/desorption processes and therefore the PTE mobility in the soil.

2.5 Factors that influence the mobility and bioavailability of PTEs

The factors most influencing the biogeochemical processes and therefore the mobility of the PTEs are:

1   pH: soil pH acts on surface charges of clays, of OM and of Fe and Al oxides indirectly affecting PTEs mobility. In general, the more pH decreases, the greater is the ability of the soil components to hold PTEs and other elements. An exception is represented by As, Mo, Se, V and Cr being more mobile under alkaline conditions as shown in Tab. 1;

2   Redox Potential: Some PTEs such as As, Se, Cr, Mn are susceptible to the potential change of redox potential (Eh) of the soil and can be mobilized as a result of the change of the latter as well as other PTEs in different soil conditions and pH (Tab. 1).

Tab. 1. Bioavailability of PTE in function of pH and Eh (modified from Kabata Pendias, 2014)

Soil properties			Bioavailability	
Redox potential	pH		High	Moderate
Oxidant	<3		Cd, Zn, Co, Cu, Ni	Mn, Hg, V
Oxidant	>5		Cd, Zn	Mo, Se, Sr, Te, V
Oxidant rich in Fe	>5			Cd, Zn
Reducing	>5		Se, Mo	Cd, Zn, Cu, Mn, Pb, Sr
Reducing in presence of H2S	>5			Mn, Sr

1.   Cation Exchange Capability (CEC): CEC represents the amount of exchangeable cations that a material, said exchanger, with adsorption properties can retain by ion exchange and depends on the amount and type of clay, OM and Fe oxides, Al and Mn present in the soil. In general, the greater the CEC the greater the amount of metals the soil can consider to be thus limiting the solubility and mobility of PTEs;

2.   Clay content: Clay in the soil can be divided into four groups: Kaolinites (Kaolinite, Halloysite, Dickite, and Nacrite), Smectites (Montmorillonite, Nontronite) Illite, Chlorite. These minerals have a high surface area electrically charged that influences the chemical-physical characteristics of the soil in the adsorption and release reactions of cations influenced above all by pH and Eh. Clay can contain a certain amount of PTE. This property varies mainly depending on CEC of different clays as reported in the following sequence: montmorillonite, imogolite> vermiculite> illite, chlorite> halloysite> kaolinite.

3.   Organic Matter: soil OM content and composition depend on degradation and humification of organic residues. The final products of these processes are: humic substances, low molecular weight organic acids, carbohydrates, proteins, amino acids, lipids, waxes, polycyclic aromatic hydrocarbons and lignin, in addition to the exudates emitted by roots consisting of simple organic acids. However, the final composition of the OM depends heavily on climatic conditions, soil type and agronomic practices. OM affects different soil chemical properties, in fact increases CEC, caus-

es PTE immobilization due to the high adsorption capacity of free cations even though the intensity of this process depends on the composition of the OM. The most stable compounds of the OM are the humic compounds with chain structure containing different functional groups (CO_2, OH, C = C, COOH, SH, CO_2H) with high affinity with metallic ions such as Cd, Cu, Ni and Pb. Humic substances consist of: humic acids, fulvic acids and humina with similar structure but different behavior in soil chemical reactions. In general fulvic acids have high acidity and mobility, and are present in acid soils with low biological activity; humic acids have average and medium mobility acidity, and are present in low acid and neutral soils with high biological activity; humina is characterized by low acidity and is widespread in all soil types. The reactions between humic substances and metals include: ion exchange, surface adsorption, chelation, coagulation and peptification leading to the formation of soluble and/or insoluble complexes in water.

4. Fe and Mn oxides and hydroxides: Fe and Mn oxides and hydroxides are present in the soil as soil particle coatings, as splitting in splits and veins, or as concretions or nodules. Hydroxides have a high PTE adsorption capacity especially in nodules or particularly rich Fe and Mn points. The adsorption mechanisms involve isomorphic substitution of bivalent or trivalent cations with Fe and Mn ions, cationic exchange reactions and oxidation processes. However, the above reactions are influenced by pH and Eh. Fe and Mn oxides and hydroxides exhibit different ion affinity, including PTEs of similar size, such as: Mn^{2+}, Mn^{3+}, Fe^{2+}, Fe^{3+}, Co^+, Co^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{4+} and Ag^+;

5. Microbial activity: includes fauna and flora of different sizes (macro, meso and microorganisms) in the soil. The usefulness of soil organisms in PTEs translates into the use of these as indicators of soil contamination, but also because such organisms are involved in processes such as mobilization and accumulation of PTEs also by their ability to adapt at high concentrations of contaminants. In addition, the same soil organisms may accumulate PTEs (microbial accumulation) and may be symbiotic (mycorrhizae) of higher plants also hyperaccumulators that are used in phytoextraction (Skinner et al., 2005). Arbuscular Mycorrhizal Fungi (AMF) have several mechanisms for controlling the absorption of PTEs by plants (Biró et al., 2006) or may be involved in bioremediation of multiple contaminated soils (Lebeau et al., 2008).

2.6 Plants and PTEs: bioavailability, biological role, phytotoxicity, tolerance and bioaccumulation

As soil PTEs become mobile according to the above described processes, their **bioavailability** needs to be considered to know the real danger of PTEs for living organisms. This concept has several definitions, including the most suitable for PTEs: "biologically releasable chemicals that can be absorbed by an organism from the environment" (Adriano, 2001). The readily soluble fraction of PTEs is generally considered bioavailable but the latter and the remaining bioavailable fraction varies widely between sites and over time based on climatic fluctuations and soil management practices. For example, in agricultural soils, the various agronomic practices including fertilization and irrigation may modify soil characteristics. Several methodologies have been developed to evaluate the bioavailable fraction of PTEs. To sample the soil solution, lysimeters and centrifugation of soil samples have been used, while several extraction methods have been developed to evaluate the bioavailable fraction with single extracts as well as multiple extracts by sequential extraction. All these procedures are based on the concept that the chemical species of PTEs present in the soil are the following:

1   Soluble in water (for example in soil solution);
2   Exchangeable;
3   Linked to OM;
4   Linked to Fe and Mn oxides;
5   In defined compounds (carbonates, phosphates, sulphides);
6   Structurally bound to silicates and / or other primary minerals (residual fraction).

PTEs aboundance in the above mentioned compartments depends on the physico-chemical characteristics of the metal and the soil considered. The combination of soluble and exchangeable fraction represents the mobile fraction of PTEs in the soil while the other fractions are much less mobile and the mobilization of these fractions is a slow process (Jalali et al., 2008).

Soil extracts used to evaluate bioavailable PTEs can be divided into four groups: acids (e. g. HCl, NHO$_3$, aqua regia); chelating acids (e. g. EDTA, DTPA, TEA); saline buffer solutions (e.g. AAAc buffer), saline solutions (e. g. CaCl$_2$, MgCl$_2$, NaNO$_3$, NH$_4$NO$_3$). Acids solutions can extract almost all of the PTEs from the soil based on the mineralogical char-

acteristics of the latter. Chelating agents and buffer solutions can extract the potentially mobile fraction of PTEs while neutral salt solutions that simulate the soil solutions characteristics allow to extract the mobile fraction of PTEs (soluble + exchangeable). The suitability of one or other method in evaluating the bioavailable fraction of PTEs depends on several factors as reported for Cd by Feng et al. (2005) and Menzies et al. (2007). There is therefore no universal extractant to estimate the bioavailability of PTEs due to the complexity of the dynamics of metal ions in the soil and their interaction with plants and environmental factors throughout the process (Adriano, 2001). For this reason alternative methods are being developed to evaluate the bioavailability of PTEs as the biological tests discussed later.

Several PTEs play a biological role in higher plants being essential for plant growth, development or reproduction processes (Barbafieri, 2005). Essential elements are generally classified as macronutrients or micronutrients, depending on their relative concentrations in plant tissue. The main macroelements are: S, P, Mg, Ca, K, N, O, C and H. While the main micronutrients for plants are Mo, Cu, Zn, Mn, Fe, B, and Cl. For Cd, Hg, Pb and As there is a not a recognized biological function are Cd, Hg, Pb and As. Essential elements can also be classified for the biochemical role and for their physiological function that is linked to enzymes that allow the transport of electrons or act as cofactors (e.g. Cu and Zn). The effects on plants of PTEs concentration in the substrate vary depending on whether they are essential or not essential as shown in Fig. 3.
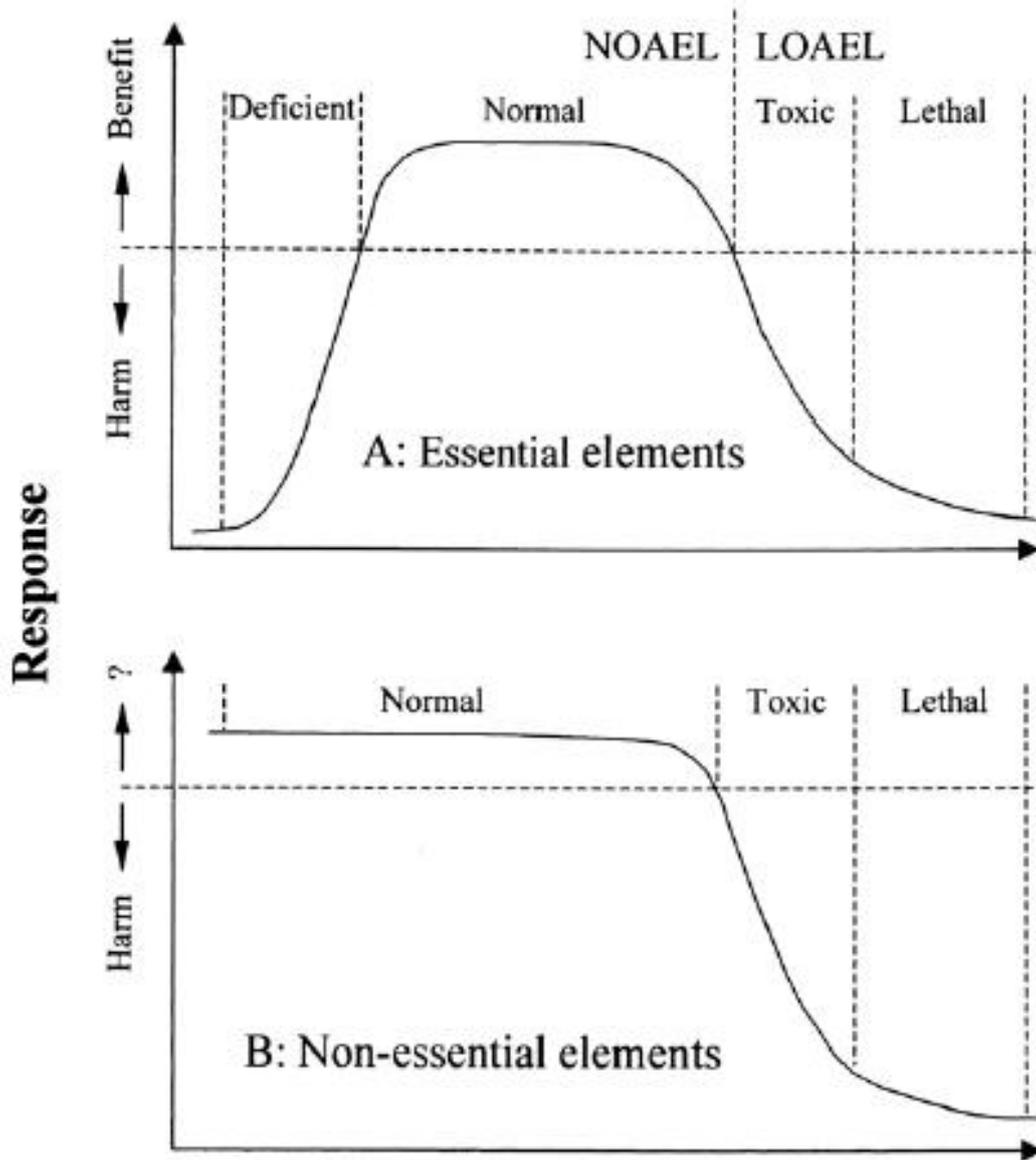
Fig. 3. Report of dose - plant response to Essential (A) and Non-Essential Elements (B) (modified from Adriano, 2001)

For the essential elements there are three parts in the curve: the deficiency level in which there is an increase in biological activity as the concentration of the essential element increases, the optimum range and the range of toxicity in which as the concentration of essential elements increases there is the loss of metabolic functions without observable adverse effects (NOAEL - no observable adverse effect level threshold) until it is lethal beyond a certain concentration threshold called LOAEL (lowest observable adverse effect Level). The curve pattern may vary depending on the chemical-physical characteristics of the element, the sensitivity or tolerance of the plant, and depending on the soil characteristics. PTEs excesses and the resulting toxic effects include:

- Change in cell membrane permeability (Ag, Br, Cd, Cu, Hg, Pb);
- Reactions of thiol groups with cations (Ag, Hg and Pb);

- Competition for sites with essential metabolites (As, Sb);
- Reaction affinity with phosphate groups and active ADP or ATP groups (Al, Be);
- Replacement of essential ions (Cs, Li, Rb);
- Occupation of sites for essential groups such as phosphate and nitrate (As, Br).

The overall effect of these mechanisms usually manifests in plant function alterations such as photosynthesis, respiration, absorption of mineral nutrients, or alterations in membrane structure and gene expression. The most deleterious effect to plants due to excess of PTEs is definitely the damage of the photosynthetic apparatus, even tough plants can withstand high concentrations of PTEs in their environment. Lower plants such as mosses and lichens show such behavior (Boquete et al., 2013; et al., 2009) as well as higher plants. PTEs tolerance can characterize the whole plant population colonizing a heavily contaminated area and individuals/species capable of tolerating high concentrations of contaminants compared to others. Plant species showing tolerance to PTEs commonly belong to the following families: *Caryophyllaceae, Cruciferae, Cyperaceae, Gramineae, Leguminosae and Chenopodiaceae* (Kabata-Pendias, 2011). The tolerance mechanisms adopted by the plants include:

- Association with mycorrhiza;
- Selective ion absorption;
- Reduction of cell wall permeability or other structural differences in the cell membrane;
- Immobilization of PTEs in different organs (especially roots) through the synthesis of specific compounds;
- Release of the ions by percolation from the leaves, guttation, precipitation through radical exudates;
- Alteration of Metabolic Flows;
- Chelation by different peptides and compartmentation of PTEs in vacuoles (Pavliková et al., 2008).

Plant species tolerant to the high concentration of PTEs can be assimilated to three different groups depending on their behavior in the presence of such contaminants (Baker, 1981):

A. "Excluders", where the PTEs content in the aerial biomass is kept below the LOAEL at different concentrations of PTE bioavailable in the soil up to a critical level in which there is the plant dead;

B. "Accumulators" in which PTEs concentrate in plant tissues both in the presence of low and high concentrations of PTEs in the soil;

C. "Indicators" where PTE concentrations in plant tissues reflect PTEs concentrations in soil, used in antiquity for mineral exploration (Tab. 2). For example, the research of the alum (Al and K sulphate) was carried out with indicating plants, since ancient times. Giovanni Da Castro noticed in Tolfa Mountains (Lazio Region - Italy) the holly and other plants also present in Turkey mines and found the alunite. Georg Bauer (Det. Agricola, 1494-1555) in his work *"De Re Metallica"* argued that for the purpose of identifying new metal deposits, it was necessary to reject the "magic" practices and read the "signs of nature" because where metal deposits are present, plants and mushrooms grow in the surrounding areas. Thalius in 1588, described *Minuartia verna*, indicative species of Pb and Zn deposits in Mount Harz (Germany); *Viscaria (Lychnis) alpine* (Cu flower) was used in Scandinavian countries to locate mineral deposits and Viscaria is the name of a Cu mine in Lapland (Sweden). *Alyssum bertolonii* is one of the typical species of serpentines (e. g. Mt. Ferrato). Other indigenous species in seleniferous soils and mixed sulphides. *Viola calaminaria* and some species of *Thlaspi* are typical indicators of substrates rich in Zn.

Tab. 2. Bioaccumulators and/or indicators species of PTE**s**

Species	PTE
Ailantus glandulosa, *Cynodon dactylon*, *Achyrocline alata*, *Senecio leptolobus*, *Taraxacum officinale*, *Trifolium repens* (Bech et al., 2016)	Pb, Zn
Agrostis gigiantea, *Minuartia verna*, *Myriophyllum alterniflorum*, *Potamogeton crispus*, *Potamogeton perfloiatus*, *Rumex acetosa*, *Viola dubyana*	Pb, Zn, Cu
Agrostis tenuis, *Arundo donax* (Barbosa et al., 2016), *Hordeum vulgare*, *Lolium multiflorum* (Lambrechts et al. 2011) *Miscanthus spp.* (Barbosa et al., 2016), *Thlaspi rotundifolium* ssp. *Cepaefolium*, *Viola calaminaria*	Zn
Alyssum argenteum	Ni
Artemisia artemisiifolia, *Helianthus annuus* (Tassi et al., 2008), *Phaseolus vulgari*, *Verbascum thapsus*	Pb, Cd, Zn
Artemisia vulgaris	Cd, Zn
Baccharis trimera	Cd
Brassica juncea, *Brassica carinata*, *Brassica napus*, *Tagetes patula* (Marchiol et al. 2004; Mahbuboor et al., 2016)	Cr, Cu, Zn, Pb
Cerastium latifolium	Cr
Festuca spp. (Alvarez et al., 2003)	Cu, Zn
Glycine max (L.) Merr. (Fellet et al., 2007)	As, Cu, Pb, Zn
Lolium perenne (Alvarenga et al., 2009), *Senecio brasiliensis*	Pb
Populus alba, *Populus nigra*	Pb, Cd
Phragmites australis (Bonanno et al., 2010)	Mn, Zn, Pb, Cu
Pteris vittata	As, Zn, Sb
Robinia pseudoacacia	Zn, Cu
Viscaria alpina	Cu
*Zea mays (*Murakami et al., 2009)	Cd, Ni, Cu, Pb, Zn

A particular subgroup consists of hyperaccumulators species (Adriano, 2001), which are capable of completing their life cycle on soils contaminated by PTEs without evidence of phytotoxicity (Baker et al., 1981) and accumulate high PTEs concentrations in the aerial part (especially in the leaves). PTEs threshold concentrations in the aerial part to define a natural hyperaccumulator species are given in Tab. 3 with their PTEs.

Hyperaccumulators species may be distinct in obliged, endemic for some metallic soils which always show PTEs absorption and optional, where only a few individuals in the population behave like hyperaccumulators (Pollard et al., 2002). Differences between individuals of optional hyperaccumulators species

genetic differences and soil related differences (e. g., different bioavailability).

The bioavailability of PTEs can in fact result from variations in the total concentration of PTEs and their chemical forms, pH differences, organic matter, clay, and soil limestone.

Tab. 3. PTEs threshold value in the aerial part to define super-accumulative species (Van der Ent et al., 2013)

PTE	Threshold value
Cd	100 mg kg^{-1}
Tl	100 mg kg^{-1}
Se	100 mg kg^{-1}
Co	300 mg kg^{-1}
Cu	300 mg kg^{-1}
Cr	300 mg kg^{-1}
Ni	1,000 mg kg^{-1}
Pb	1,000 mg kg^{-1}
As	1,000 mg kg^{-1}
Zn	3,000 mg kg^{-1}
Mn	10,000 mg kg^{-1}

Optional hyperaccumulators species have the inherent propensity to PTEs accumulation and local factors guarantee bioavailability. As shown in Fig. 4, "normal" species do not tolerate the presence of PTEs and therefore die at low concentrations of soil bioavailable PTEs; tolerant species ("indicators" and "excluders") will continue to survive to certain concentrations of bioavailable PTEs in the soil, while "hyperaccumulators" will survive at even higher concentrations of bioavailable PTEs in the soil. Thus, in theory, from the vegetation survey of a contaminated site, as the concentration of contaminants in the soil in a specific area increases, there will be a reduction in the number of species (in favour of more tolerant and hence competitive species) in the following order: "normal" plants > "indicators"> "excluders" plants> "hyperaccumulators" plants.
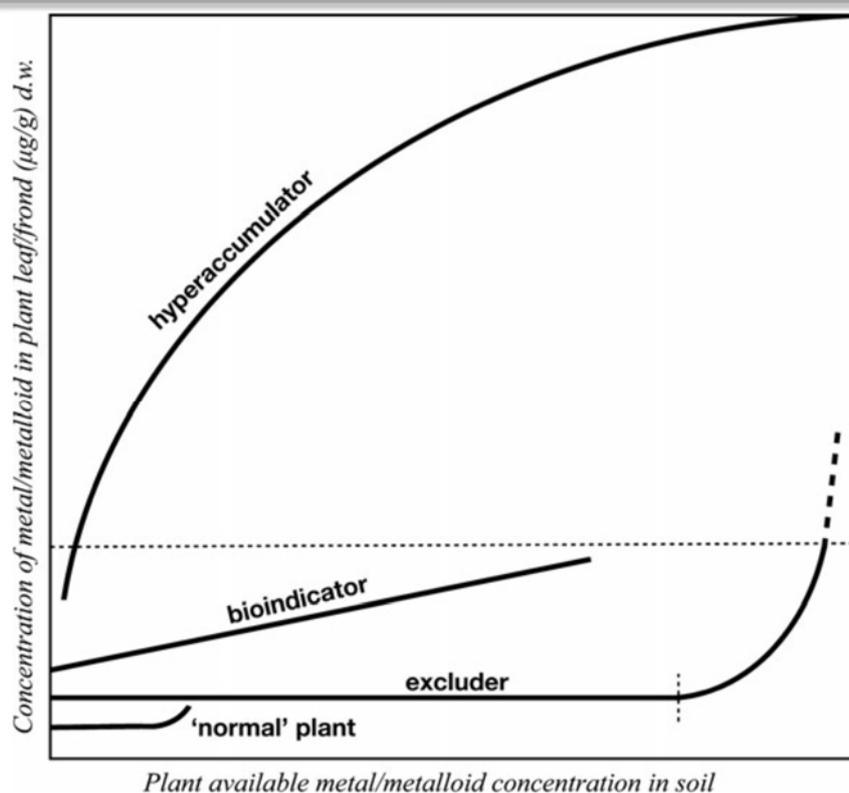
Fig. 4. Graph of the absorption response of PTEs in the different plant species to the concentration of bioavailable PTEs in soil (Baker, 1981; McGrath et al., 1999)

Currently over 500 plant species have been cited in the literature as hyperaccumulators of one or more elements (As, Cd, Co, Cu, Mn, Ni, Pb, Se, Tl, Zn), belonging to 101 families including: *Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cumouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae* and *Euphorbiaceae* (Sarma, 2011). Hence the presence of these species can indicate the co-presence of highly contaminated soils (Tab.4).

Tab. 4. Hyperaccumulator species of PTEs (Van der Ent et al. 2013)

Species	PTE
Gomphrena canescens, Thlaspi ochroleucum	Zn
Arabis paniculata	Cd, Pb Zn
Ageratina sp. (Bech et al., 2016)	Pb, Zn
Carpobrotus rossii (Liu et al., 2016), *Arabidopsis halleri, Rorippa globulosa, Solanum nigrum, Sedum alfredii, Viola baoshanensis, Viola principis*	Cd
Thlaspi (Noccaea) caerulescens, Cortaderia hapalotricha (Bech et al., 2016)	Pb
Rinorea bengalensis, Pimelea leptospermoides	Ni
Biscutella laevigata, Iberis intermedia, Silene latifolia	Tl
Elsholtzia splendens, Commelina communis, Geniosporum tenuiflorum, Laportea ruderalis	Cu
Nyssa sylvatica	Co
Leersia hexandra, Spartina argentinensis	Cr

2.7 Characterization of contaminated sites with biological tests

In recent years methods have been developed for measuring concentrations of many types of pollutants directly in different matrices such as soil, water and air, using very burdensome and costly analytical methods. The information obtained trough these approaches are often not particularly suitable to depict the current (and also future) quality of the environment. Only in recent decades attention has been paid to the possible use of biological indicators for environmental monitoring (biomonitoring). Compared to traditional methods, the use of a biological system has the enormous advantage of being able to evaluate the actual toxicity of pollutants on living organisms even in very complex matrices, and hence of being a good indicator of the hazard potential for human health. Among the various biological systems, plant species have been widely studied in recent years to find bioindicators and accumulators suitable for green-soil remediation. As shown in the previous paragraph, plants can accumulate in their PTE tissues acting like indicators, excluders and accumulators, depending on their adaptability to site specific conditions, and therefore their analysis can give information about environmental conditions of a particular area. To

achieve these objectives, particular attention has been paid to natural species present in contaminated sites, as soil contamination and human activities can lead to the emergence of a patchwork of secondary plant communities. The presence of specific natural plant communities depends on the potential of these species to germinate, survive and reproduce in contaminated/degrades sites; hence the presence of these species means that they are well adapted to contaminants and can remove or accumulate them, thereby reducing their toxicity (Chaney et al., 2000). Analysis of the quantity and quality of wild plant species present in a contaminated site may therefore indicate the level of its contamination (bio-monitoring) and suggest possible phytoremediation strategies. With regard to plant indica-tors, biomonitoring is based on the greater or lesser degree of survival or growth of each plant species. Although much can be learnt from the use of plant indicators, the main in-formation concerns:

a) the detection of pollutants: the first sign of an abnormal situation to be examined in greater detail; signals of distress due to the synergistic action of more than one pol-lutant which, individually, may also be present in non-hazardous doses.

b) indication of a specific pollutant: characteristic reaction to the action of certain pol-lutants (e.g. chlorotic spots on tobacco leaves or due to tropospheric ozone air pol-lution);

c) the ability to assess the level or intensity of pollution.

A different analysis must be carried out for biomonitoring, when using indicators or accu-mulators of potentially toxic elements (PTEs) and generally species that tolerate the pres-ence of PTEs in the soil without presenting specific symptoms. In this case it is useful to carry out an analysis of the vegetation (floristic survey) naturally occurring on a contami-nated site, evaluate the response of plants to environmental factors and their changes in terms of biodiversity (at various levels), and evaluate the accumulation of pollutants (both in different plant organs and in their respective rhizospheric soils). This assessment is based on the fact that each plant species has its own specific range of tolerance to different environmental stresses, within which there is its ecological and physiological optimum. Hence for each environment the characteristic species can be defined, allowing the quality of a given area to be defined simply by observing the vegetation.

A floristic survey is based on the recognition of individual plant species that make up an association, considered a more or less stable plant grouping and in equilibrium with the environment, characterized by a floristic composition, where certain plant species detect with their presence a particular and independent ecology. The floristic survey considers the individual plant species as a bioindicator. The census of plant species is performed within

homogeneous areas and provides the quantitative assessment of the abundance of each species (visual estimation) or precise count of the number of species. Visual estimation, that is, the percentage of the plot covered by individual species, highlights the relative importance of the individual components of the vegetation. This is a method linked to the subjectivity of the detector and is much coarser compared with the precise method of individual species identification at specific points. Whatever method of operation, the census should be carried out in at least three zones and for a minimum of three repetitions (years, seasons etc.), given that each year the results may undergo changes. Of course, for an overall picture of the vegetation, there must be a sufficient number of surveys, according to the variability of existing micro-environments in the area under study.

The following interesting parameters may be obtained from the floristic survey:

- species richness or density, understood as the number of species found with the floristic survey;
- species diversity, i.e. the relative abundance of each species, generally expressed as the number of individuals / number of total species;
- dominance, or the degree of prevalence of the most representative species.

These parameters are used to calculate indices such as the **Shannon-Weaver Diversity Index (H)**, used to quantify species diversity in a community. This index is calculated by the following formula (Shannon and Weaver, 1963):

$$H = -\sum_{i=1}^{\%} (P_i * log\, P_i)$$

where Pi is the presence of each species in relation to the total (%); relative abundance is given by the ratio of the number of individuals of each "i^{th}" species (n_i) to the total number of individuals in the community (N), that is: $P_i = n_i/N$. The higher the H index, the greater the biodiversity in the community. From this index the *evenness* or *equitability* index of Shannon can then be calculated (E_H):

$$E_H = \frac{H}{H_{max}} = \frac{H}{log\, N}$$

This index varies from 0 to 1 and quantifies the equipartition (same or similar number of individuals for the various species belonging to a specific area or ecosystem). The closer

the index is to 1, the greater the evenness of species in a community or ecosystem, with complete evenness corresponding to 1.

2.8   Remediation technologies

Soil recovery refers to practices addressed to: remove contaminants (as in the case of PTEs that can not be degraded); convert them into simpler intermediates (as in the case of organic contaminants) or immobilize them (i.e. convert contaminants into less mobile and therefore less bioavailable) to avoid spreading contamination.

The choice of technology to be adopted is generally based on the nature of the contaminant and the soil type. Remediation technologies can be divided into two categories: in situ, if the reclamation takes place without the handling of contaminated and ex-situ if take place with the handling of contaminated matrix. The latter category may be further divided into: on-site if the handling of the material takes place in the site or in the immediate vicinity or off-site if the contaminated material is processed in specific facilities away from the contaminated site. In the past, ex situ technology was preferred, albeit more costly, but in recent years with the change of remediation targets addressed to minimize the environmental and health risks instead of the total content of pollutants, cheaper on site techniques are more considered. Such technologies are slower but allow to reduce the impact of remediation on the environment.

The main reclamation technologies (Tab. 5) are based on:

- Physical processes, which reduce access to the contaminant by removal or containment;
- Chemical processes that alter the chemical speciation of the contaminants by increasing their mobility with different chemical extracts or in turn reduce the mobility of the contaminant by reducing exposure to it and spread to other environmental compartments;
- Biological processes, exploiting living organisms enhancing biochemical pathways to degrade, accumulate (then remove) or reduce the mobility of contaminants.

Tab. 5. Main reclamation technologies **(modified from Adriano et al. 2001)**

Physical	Thermal desorption (*ex situ*)	process involving the heating of the soil at a temperature between 90 and 650°C causing volatilization and pyrolysis of volatile and semi-flammable organic compounds
	Incineration (*ex situ*)	volatilization and thermal oxidation of organic contaminants, operating at temperatures between 600 and 2000°C
	Venting (*in situ*)	This is a land remediation technology that consists in extracting contaminants from the ground of a site, in the form of vapors, through suction devices that are usually made up of wells. The technology is mainly used for the remediation of volatile organic compounds and light hydrocarbons
	Vitrification (*ex situ*, o *in situ*)	Heating the contaminated medium to produce a glassy, non-porous, non-leachable material
	Encapsulation (*in situ*)	Polluted site coverage with waterproof layer
	Washing (*ex situ*)	Extraction of contaminants with acid or chelating solution after particle separation
	Flushing (*in situ*)	Leaching of contaminants with an acid or chelating solution
	electrokinetic (*in situ*)	Migration of contaminants (ions) to electrodes induced by electric current
	Underground freezing	Temporary artificial barrier formation induced by cryogenic application
Chemical	Neutralization	Neutralization of contaminated soil acidity or alkalinity
	Solidification	Add cementing agents to the contaminated medium to produce hardened, non-porous and non-leachable material
	Stabilization	Immobilization of constituents by the addition of materials to induce absorption/precipitation
Biological	Phytoremediation	Using plants to accumulate, stabilize or degrade contaminants
	Revegetation (Rehabilitation)	Site stabilization with plant cover against wind and water erosion

2.8.1 Physical-chemical technologies

Chemical technologies allow the degradation of pollutants incompatible with biological systems, nevertheless they present some limitations. It may happen that secondary com-

pounds deriving from the partial degradation of target pollutants are more toxic than the starting ones, or that the reagents applied to the soil have an high toxic profile. Physical non-thermal technologies, on the other hand, are addressed to separate contaminants from the polluted matrix and to concentrate them in a new matrix, then destined to subsequent treatment or disposal. Physical-thermal treatments can induce the separation of the pollutant by desorption/volatilization with pyrolysis or incineration or causing immobilization by fusion of the solid matrix in which they are found (vitrification). Compared to other physical methods, it can achieve total degradation of contaminants but requires large amounts of energy, it can generate new pollutants and has devastating effects on the structure and organic matter of the soil. To overcome the drawbacks of these technologies and chemicals a combination of the two methods can be adopted, in order to exploit the benefits of both and reduce the negative externalities, as in soil washing. This is one of the most popular reclamation techniques and is divided into 3 phases: pretreatment (soil sieving based on granulometry); washing and extraction of contaminants by intensively mixing the soil with the extracting agent and thus transferring the pollutants to the liquid phase; separation of the extraction solution from the sediment. Soil may eventually be treated again to increase the extraction efficiency. The solutions used are: acid solutions (hydrochloric acid, sulfuric acid and nitric acid), complexed agents such as NTA, EDTA, EDDS, organic acids (acetic acid, citric acid) that are less impacted on the environment and have lower cost of management and excellent extraction capabilities. The aim of this process is to separate fractions with larger granulometry (such as sand and gravel) from silt and clay and concentrate contaminants in the fine fraction. Larger particles after washing can be reused and returned to the site of origin. The soil washing technique can be used in combination with the solvent extraction technique in the case of mixed contamination even by organic contaminants for which they leave the soil washing plant, these being boned by solvent extraction treatment.

2.8.2 Biological technologies

Biological techniques use living organisms, as microorganisms (bioremediation) or plants (phytoremediation), for degradation, accumulation or attenuation of pollutants. Microorganisms can be used for the treatment of organic compounds but can also be used for PTEs management by exploiting bio-leaching or red-ox reactions.

Some bacteria such as *Thiobacillus* and *Aspergillus niger* may increase PTE mobility. The first by the production of sulfuric acid (Tichy et al., 1992; Mulligan et al., 2001) and the second by the production of citric and gluconic acid (Mulligan et al., 2001). Conversely, some microorganisms can oxidize (Hg and Cd) or reduce (As) PTEs leading to bioprecipitation of these. Another process performed by microorganisms is biomethylation, which implies the attack of a methyl group at PTEs such as As, Hg, Cd or Pb, transforming them into more mobile and sometimes volatile forms (Mulligan et al., 2001). To increase the efficiency of bioremediation it is recommended to use autochthonous microorganisms from contaminated sites to be reclaimed. This because autochthonous species are well adapted to site specific conditions and can alter the valence of metals and cause their soil desorption, or instead of immobilizing them in the same particles.

The main advantages of biological methods consist in the ability to intervene on a large number of organic pollutants, their potential beneficial effect on the structure and fertility of the soil, and the fact that they are absolutely non-invasive. The main limits are the reclamation time which is influenced by environmental conditions, implementation times are difficult to estimate, as well as the fact that not all contaminants can be treated biologically.

In addition to microorganisms, plants can also be used to remove counting, this process is known as phytoremediation. The phytoremediation consists of a series of technologies used for environmental remediation based on the ability of certain plant species to assimilate, accumulate and degrade the contaminants. This method of rehabilitation exploits the complex interaction between root, plant and soil organisms, and can be a solution for environmental recovery. This technique will be widely discussed in the following paragraph.

2.9 Phytoremediation

The limits of chemical-physical remediation technologies lie in the high cost of such interventions and the high environmental impact consisting of profound chemical, physical and biological alterations of the treated substrates. Such technologies often return soil which is no longer suitable for cultivation because during the decontamination process, any biological activity involving microorganisms (fungi, nitrogen fixators, mycorrhiza) and terrestrial fauna is drastically compromised (Mancuso et al., 2004). To address these issues, research has been geared towards the development of cheaper and environmentally friendly alterna-

tives. An innovative, reliable, eco-sustainable, and widely applicable technology is represented by phytoremediation.

Phytoremediation is a remediation technique that basically consists in the use of plants for the treatment of polluted matrices. The technique is based on some natural processes that are carried out by plants, among which:

- direct absorption of metals and some organic compounds;
- accumulation or transformation of the same chemicals by ligating, metabolizing, volatilizing;
- use of enzymes released from plants to catalyze degradation of pollutant organic compounds;
- release of exudates in the rhizosphere, which bring carbon to the soil, modify pH and stimulate microbial activity for the degradation of contaminants.

Based on the various mechanisms of action shown in Fig. 5, different techniques of phytoremediation can be classified into: phytoextraction, phytodegradation (or phytotransformation), phytostabilization, phytostimulation (or rhizodegradation), phytovolatilization and rhizofiltration (Pulford and Watson, 2003, Wong, 2003; Zerbi et Marchiol, 2004; Mertens et al., 2004; Rizzi et al., 2004; Kramer, 2005).
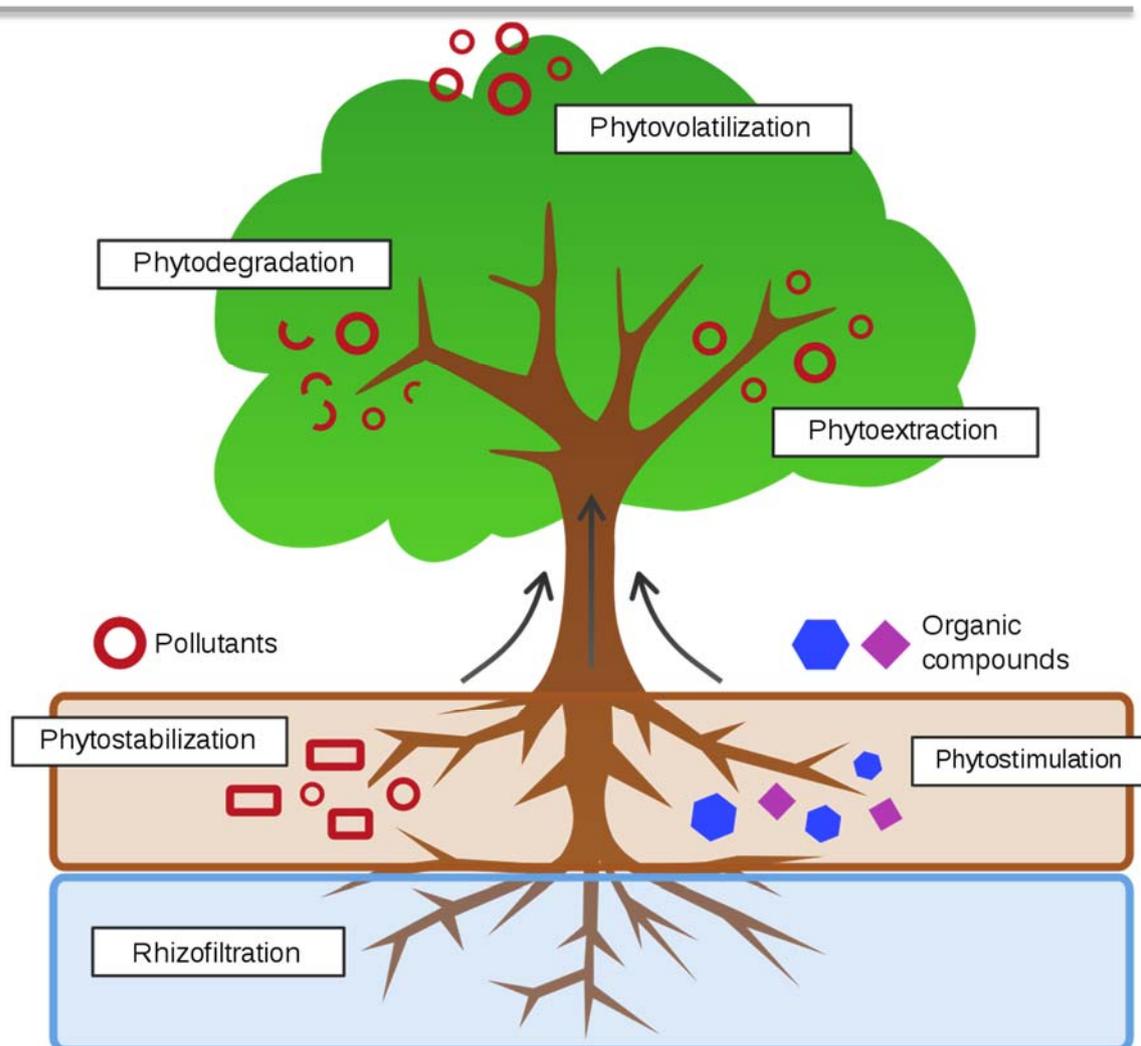
Fig.5. Schematic representation of the phytoremediation process

Phytoextraction (also known as phytoaccumulation, phytoabsorption or phytoabduction): it is the mechanism by which plants assimilate the pollutant through the roots and dislocate and accumulate it in the above ground biomass.

Phytodegradation (or phytotransformation): it is the general term to indicate the degradation of contaminants that occur within the plants through metabolic processes and enzymatic action. It is very effective in soil decontamination by organic pollutants through the transformation of complex organic molecules into simple molecules and in the possible accumulation of non-toxic catabolites in plant tissues.

Phytostabilization: this technique aims at immobilizing the pollutants: on the walls of the radical cells thanks to proteins associated directly to the wall; in radical cells thanks to transport proteins present on the membranes that facilitate the transport of the contaminant within the cell where it is immobilized in the vacuole; in the rhizosphere thanks also to the

production of radical exudates. This is the main process in highly PTEs contaminated sites where reclamation time of phytoextraction are too long and where it is essential to avoid PTEs dispersion as volatile particulate or leachate.

Rhizodegradation: consists in the degradation of organic contaminants by exploiting the microbial activity that originates in the rhizosphere, thanks to the compounds exuded by the roots of the plants.

Phytovolatilization: consists in the absorption of organic pollutants and some PTEs (Se and Hg) by plant and the successive release into the atmosphere through respiration. The chemical species of contaminants can be transformed into rhizosphere before being absorbed, or in the plant after absorption. The use is restricted by the fact that the process does not fix the contaminant completely but transfers it only from the ground to the atmosphere from where it can precipitate and re-enter the food cycle.

Rhizofiltration: this technology involves the capture by plants of pollutants present in dissolved form in groundwater. It occurs in the root zone, through adsorption, concentration or precipitation of contaminants. This technique is water-specific, but may include soil leachates, since it requires contaminants to be in solution and free to come into contact with the root plant.

2.9.1 Phytoextraction and phytostabilization: reference parameters for species selection

The success of phyto-remediation/securing of a contaminated site, starts from the choice of species suitable for the predetermined objective. Several authors believe that the best plant species to be used in a project of deforestation are the natural ones present on the site for their survival, growth, reproduction under strong environmental stress, compared to plants introduced by other environments (Adriano, 2001).

Phytoextraction
The species to be used for phytoextraction must meet the following characteristics:
- Tolerance at high concentrations of PTE;
- Translocation and accumulation in tissues that will be removed;

- Rapid growth and high production of biomass;
- Well developed radical structure;
- Easy management, resistance to phytopathies, low cropping inputs;
- Low palability for grazing animals.

The phases involved in phytoextraction are the radical absorption and the consequent translocation and accumulation of contaminants in the tissues that can be harvested (generally the aerial parts); this process depends on the increased solubility of contaminants favoured by root exudation of chelating agents and/or modifications of soil reaction in the rhizosphere. In addition microbial and mycorrhizal activity can be enhanced to improve the mobilization and absorption of contaminants. PTEs are usually absorbed at low concentrations in plant tissues except for hyperaccumulator plant species which however exhibit low biomass production. For this reason, several genetic studies are underway to transfer the hyperaccumulation genetic information to other biomass high-yield plant species through somatic hybridization or genetic engineering. Meanwhile, high-yield biomass species (poplar, robinia, willow) are used in phytoextraction programs. Phytoextraction has become very attractive as a technology for the remediation of contaminated sites by Pb since traditional techniques only lead to the physical stabilization of the contaminant (mixing the soil with cementitious substances or other solidifying agents) while remediation can only be carried out with soil washing with strong acids.

Phytostabilization

Species suitable for phyto-stabilization must have the following characteristics:
- Tolerance at high concentrations of PTEs;
- Low translocation of contaminants from the roots to the aerial part and strong accumulation in the roots relative to the aerial part;
- Rapid growth;
- Well developed radical structure;
- Easy management, resistance to phytopathies, low edaphic requirements;
- Low interest from grazing animals.

In species suitable for phyto-stabilization, the largest accumulation of PTEs occurs in the roots or in the rhizosphere thus reducing the further dispersion of the contaminant. Hence species not suitable for phytoextraction can be used to stabilize soil contaminants if they have good adaptability to site contamination and a good PTEs accumulation in the roots.

The parameters that are normally used to select the species and evaluate the efficiency of PTEs extraction or stabilization are: the bioaccumulation factor for shoots (BACs); the bi-

oaccumulation factor for roots (BAC$_R$) and the translocation factor (TF). The BACs and the BAC$_R$ are calculated, respectively, as the ratio between the PTEs concentrations in the aerial part and plant roots and the concentration of PTEs in the respective rhizosphere soil while the TF as the ratio between PTEs concentration in the aerial part and roots (Putwattana et al., 2015).

$$BAC_s = \frac{concentration\ PTEs\ aerial\ part}{concentration\ PTEs\ soil}$$

$$BAC_R = \frac{concentration\ PTEs\ roots}{concentration\ PTEs\ soil}$$

$$TF = \frac{concentration\ PTEs\ aerial\ part}{concentration\ PTEs\ roots}$$

The plants can be divided into four categories according to the value assumed by the BAC:

•No accumulators of PTEs if BAC <0.01;

• Limited PTEs accumulators if 0.01 <BAC <0.1;

• Moderate PTEs accumulators if 0.1 <BAC <1.0;

• Remarkable PTEs accumulators if 1.0 <BAC <10.0 (Sekabira et al., 2011).

Moreover, if TF> 1 means that the plants actually transpose PTEs from roots to the aerial part (Baker and Brooks, 1989). In general, a species with a BCA$_S$> 1 and a TF> 1 is suitable for phytoextraction, whereas a species with a BCA$_R$> 1 and a TF <1 is suitable for phytostabilization, but in reality, very few species have strictly these characteristics, so the choice of the species must also take into account:

1. adaptability of the species to the contaminated site;

2. objectives of remediation/securing;

3. concentration of contaminants in different organs of plant species.

Many authors (Hamon and McLaughlin 1999; Barbafieri et al., 2011; Petruzzelli et al., 2011) argue that the most realistic approach to phytoextraction is the strategy known as "Bioavailable Contaminants Stripping (BCS)", which is only the removal of the bioavailable fraction of the PTEs. To assess the ability of plants in this sense, can be used the bioaccumulation indexes modified (mBAC$_S$ and mBAC$_R$) as follows:

$$mBAC_S = \frac{concentration\ PTEs\ aerial\ part}{concentration\ PTEs\ bioavailable\ in\ soil}$$

$$mBAC_R = \frac{concentration\ PTEs\ roots}{concentration\ PTEs\ bioavailable\ in\ soil}$$

The bioavailable PTEs that can be used for calculating the indices can be those extracted in EDTA, DTPA or the sum of fractions extracted with sequential extractions.

2.9.2 Role of amendments in assisted phytoremediation techniques

The effectiveness of phytoremediation techniques is strongly influenced by the characteristics of contaminants in the site, the soil characteristics and the plant used as described above. Various techniques have been tested in assisted phytoremediation. For example, chelators can be applied to the soil to increase PTEs mobility while amendment are used to adjust soil pH in order to increase or decrease the mobility of PTEs or improve the soil physical and biological fertility.

Use of chelators

Synthetic chelators, used most in the past, can be added to the soil as phytoextraction modifiers in order to increase the absorption and translocation of PTEs (Meagher, 2000; Kim et al., 2010) or radionucleotides (Duquene et al. 2009) by plants. The chelating break the balance between the solid and liquid phase of the soil by favoring the desorption of the ions into the liquid phase where they can be absorbed by the roots of the plants. Various synthetic chelating are reported in the literature and include ethylenediaminetetraacetic acid (EDTA), ethylen diamine disuccinic acid (EDDS), hydroxyethyl iminodiacetic acid (HEIDA), diethylentriamine pentacetate (DTPA), which allowed higher accumulation of PTEs by plants (Halim et al 2003, Luo et al 2005). The problem with the use of synthetic chelating is their low selectivity against soil ions, low degradability, the risk of leaching of soil contaminants and the toxicity for plants (Evangelou et al., 2004). For the above-mentioned problems, as an alternative to synthetic chelating, are proposed various easily digestible and low phytotoxicity chelating agents such as nitrilotriacetic acid (NTA) and low molecular weight (LMWOA) acids such as acetic acid, citric acid and oxalic acid (Chen et al 2003, Wenger et al 2003) that are produced in nature in plant exudates.

Agronomic practices

Phytoremediation utilizes plants for the remediation of contaminated sites, so the development of agronomic best practices for their growth will allow to increase biomass production by reducing the phytotoxic effect of contaminants. Moreover, some agronomic practices affecting soil pH allow to increase/decrease the bioavailability of contaminants. Agronomic practices include the use of inorganic fertilizers (N, P, K), various organic or inorganic amendments. Inorganic acids and elemental sulfur (in calcareous soils) are used to reduce pH by favoring the mobility of PTEs for phytoextraction (Kayser et al., 1999); Calcium carbonate or lime hydroxide, on the other hand, allows pH to be increased in acidic soils (often mine soils) by promoting plant growth and reducing the mobility of contaminants. Organic amendments used over the years include waste products from agricultural, civil and industrial production including:

- Compost: obtained through an aerobic process of OM stabilization from urban, agricultural or agro-food waste. Compost is rich in stable humified organic matter that improves the physical and biological structure of the soil by slowly releasing nutrients that favor plant growth. It also permits immobilization of PTEs through the formation of bonds between these and humic substances, such as for Cu and Pb;

- Biochar: represents the solid product obtained from the pyrolysis of residual biomass from agricultural and forestry (Zhang et al., 2013). It has a high specific surface, high cationic exchange capacity and high pH. Its application can lead to the immobilization of PTEs by limiting their bioavailability and toxicity as well as benefiting crops through the release of nutrients and soil fertility improvement (Park J H et al., 2011).

- Sewage Sludge: Water Purification Residue. It improves soil fertility and bring nutrients to plants;

- Farm residues: cellulose and lignin rich crop residues that improve plant growth by supplying nutrients such as N, P and K, and soil fertility. Studies on residues of sugar extraction from sugar cane, for example, showed an improvement in Fe, Zn, B, Cd, Cr and Ni Phytoextraction using *Trifolium repens L.* (Medina et al., 2006; Azcon et al 2009), *Tetraclinis articulata* and *Crithmum maritimum L.* (Fernandez et al., 2012);

- Biostimulants: include substances and/or microorganisms whose function is to stimulate natural processes to increase/improve nutrient absorption and efficacy, tolerance to abiotic stress and crop quality (EBIC, 2011). Most used include:

- Hydrolysed proteins and amino acids: improve plant growth by promoting the development of the radical apparatus, absorbing nutrients and stimulating the response of plants to stress (thermic, salinity, water, alkalinity, nutrient deficiency);

- Humic substances: improve soil fertility and promote crop growth and can be very useful for phytoremediation, as they can promote or inhibit PTEs mobility. In particular, the presence of the soluble fraction of humic substances (e.g. fulvic acids) promotes the mobility of As and Zn, whereas high concentrations of the insoluble fraction of humic substances promote immobilization of PTEs such as Cu and Pb (Hattab et al., 2014);

- Seaweed extracts: used as fertilizer additives or foliar treatments, they stimulate plant growth and development, while algal and derivatives polysaccharides stimulate the physiological response of plants to stress;

- Microorganisms: also called plant growth promoting rhizobacteria (PGPR) or plant growth promoting bacteria (PGPB) include naturally occurring microorganisms in the soil or in the rhizosphere (free or symbiont) but can also be inoculated into the soil by benefiting plants with different mechanisms of action: synthesis of compounds, facilitating absorption of nutrients, prevention of pathogens.

The microorganisms naturally present in soil or rhizosphere include:
- Free nitrogen fixing bacteria (*Azospirillum spp.*, *Pseudomonas spp.*, *Azotobacter spp.*, *Bacillus spp.*);
- Symbiotic nitrogen fixing (*Rhizobium spp.*) Cyanobacteria;
- Phosphate solubilizing bacteria (*Bacillus spp.*);
- Fungi (*Ascomycota, Basidiomycota*);
- Mycorrhizal fungi.

The interaction between plant and microorganisms in the rhizosphere can promote plant growth and nutrient uptake due to:
- Increased absorption of nutrients;
- Solubilization of nutrients;
- Chelation of microelements due to siderofors;

- Organic acids production;
- Production of phytohormones such as auxins, cytokinins, gibberellins.
- Resistance to abiotic stress.

Different fungi like mycorrhizal fungi and *Trichoderma spp.*, can improve the efficiency of the Phytoextraction process by diluting contaminants in the plant due to the increased biomass produced; or improvements in the phytostabilization process through the precipitation or chelation of PTEs in the rhizosphere or the direct immobilization of these in the mycorrhizal fungus (Christie et al., 2004; Harman et al., 2004). *Trichoderma* genus which is very competitive in soil with other organisms and very suitable for different environmental conditions tolerating a wide range of contaminants, favoring the development of roots and shoots (Harman et al., 2004) by controlling the harmful microorganisms present in the rhizosphere.

For these reasons, mycorrhizal fungi and *Trichoderma spp.* can be a valuable tool for assisted phytoremediation.

2.10 Thesis objectives

Contamination of PTEs deriving from illegal waste disposal and dumping represents one of the most pressing threats to water and soil resources as well as to human health. Phytoremediation can be potentially used for risk management, phytostabilization and remediation of PTEs-contaminated sites.

The main objectives of the first part of the research (Chapter 3) were:

1. To evaluate the potential of native vegetation for analysis of environmental risks due to soil contamination in industrial and agricultural contexts.
2. To study the potential of native plant species in extracting and accumulating soil PTEs from two contaminated sites.
3. To identify the extraction method that better represents the PTEs availability for plants by studying the relations between PTEs in the plants and total and bioavailable PTEs content of soils.
4. To evaluate if some plant can be used as a *bioindicator* of PTEs bioavailability by studying the relationships between PTEs upake of different plant species and different analytical methods for assessing PTEs bioavailability..

5. To identify the probable origin of contaminants by analizing the soils of each plot using Hierarchical Cluster Analysis and Principle Component Analysis.

In the second part of the research, two pots experiments (Chapter 4 and 5) were carried out by using industrial site sediments with the aim to evaluate the efficacy of different agronomical techniques of assisted phytoremediation (i.e. compost fertilization, biopromoters). The main objective of these pot experiment were the evaluation of the effects of organic amendment and commercial biopromoters on resistance of grass species to PTEs contamination and on PTEs phytoextraction/phytostabilization in a context aimed to reduce the environmental and sanitary risks of industrial contaminated sites by avoiding dispersion of contaminated soil particles.

3. CHARACTERIZATION AND RISK EVALUATION OF TWO POTENTIALLY CONTAMINATED SITES

3.1 Materials and Methods

3.1.1 Studied area and Risk Analysis

The studied areas included an industrial area (site A) south of Marcianise (Campania Region, Italy- 41°00'48.9"N - 14°17'49.7"E), close to a company operating in the recycling of automotive electric batteries and a metal waste recycling plant and a farmland (site B), potentially contaminated Cr and Zn by tannery sludges dumping (Giugliano – Campania Region, Italy - 40°56'46.57"N - 14° 6'7.42"E). For both sites PTEs concentrations in soils were above Contamination Concentration Thresholds (CCT) so risk analysis was performed according to art. 240 of Italian Legislative decree 152/06. Results of risk analysis showed that only site A was contaminated while site B was only potentially contaminated. For site A the risk for workers who frequent the site was linked to Pb and Cd. The route exposition for site A was inhalation or dermal contact of contaminated soil particles.

3.1.2 Soil and plants: Sampling and Analysis

11 plots (3x3 m) representative of the vegetation types were set up in June 2015 for site A and July 2015 for site B (Fig.6). Within each plot a variable number of plant samples (>3 when available) with the higher soil coverage were collected and identified, rhizo-soil was sampled together with vegetation. The abundance of each species was calculated in accordance with Braun-Blanquet (1964).

Fig. 6. Territorial framework of studied areas: administrative territory of Giugliano – Campania Region – Italy (A) and administrative territory of Marcianise – Campania Region – Italy (B) with indication of the sampling plots

Plants samples were separated in shoots and roots and then where washed with tap water, rinsed with deionized water, oven dried at 60°C until constant weight and ground prior to analysis. A composite sample representative of plants of each plot was analysed (acid digestion with HNO_3 and aqua regia followed by ICP-MS) for Potential Toxic Elements (PTEs) total content. Recorded values were compared to legal PTEs thresholds in plants and soils (REG UE N. 1275/2013 modified in d.w. (dry weight) content according to an average water content and D.Lgs 152/2006, respectively). For metals not included in the

current legislation mean values found in grasses grown on polluted sites were used (Kabata-Pendias, 2011) as reference.

Rhizo-soil was dried at 50°C until constant weight, homogenized and sieved through a 2 mm sieve. The following determinations were made on rhizo-soils: Texture (Normalized Methods for soil analysis, ISS, 1985), pH-H$_2$O (1:2.5 soil:water solution ratio), Electric conductibility (1:2.5 soil:water solution ratio-(Conductimeter basic 30, Crison), organic carbon (Walkley and Black method, 1934), nitrogen (Kjeldahl method) and carbonate content (Dietrich–Frühling calcimeter method, Loeppert and Suarez, 1996) and PTEs concentrations (acid digestion with aqua regia followed by ICP-MS).

PTEs bioavailability was estimated by two different single extractions: 1M NH$_4$NO$_3$ extractant was used to assess the readily soluble fraction (DIN 19730, 1995), whilst the PTE potentially bioavailable fraction was determined by 0.05 mol L^{-1} DTPA solution (Lindsay e Norwell, 1978). PTE concentration in the solution was determined respectively by inductively coupled plasma-atomic emission spectrometry (Perkin Elmer ICP-AES Optima 7300DV) for DTPA extracts and by ICP-MS for NH$_4$NO$_3$ extracts

3.1.3 Phytoextraction efficiency and hyperaccumulators

The following indices were tested to assess the ability of the plants to tolerate and accumulate PTEs: the bioaccumulation coefficient for shoots (BAC$_S$), bioaccumulation factor for roots (BAC$_R$) and translocation factor (TF). The BAC$_S$ and BAC$_R$ were calculated, respectively, as the ratio between the concentration of PTEs in the aerial part and the roots of plants and the concentration of PTEs in the respective rhizospheric soil (Putwattana et al., 2015).

$$BAC_s = \frac{PTEs\ concentration\ in\ aerial\ part\ (mg\ kg^{-1})}{PTEs\ concentration\ in\ soil\ (mg\ kg^{-1})}$$

$$BAC_R = \frac{PTEs\ concentration\ in\ roots\ (mg\ kg^{-1})}{PTEs\ concentration\ in\ soil\ (mg\ kg^{-1})}$$

$$TF = \frac{PTEs\ concentration\ in\ aerial\ part\ (mg\ kg^{-1})}{PTEs\ concentration\ in\ roots\ (mg\ kg^{-1})}$$

The plants where divided into four categories of accumulation of PTEs based on the value assumed by the BAC:

- no accumulators of PTEs if BAC <0.01;
- limited accumulators of PTEs if 0.01 <BAC <0.1;
- moderate accumulators of PTEs if 0.1 <BAC <1.0;
- high accumulators of PTEs if 1.0 <BAC <10.0 (Sekabira et al., 2011).

Furthermore if TF > 1 it means that the plant effectively translocate heavy metals from roots to the shoots (Baker and Brooks, 1989).

Many authors (Hamon and McLaughlin 1999; Barbafieri et al. 2011; Petruzzelli et al. 2011) report that the more realistic approach of phytoextraction is the Bioavailable Contaminants Stripping (BCS) technology aimed to eliminate only the bioavailable fractions of contaminants. To evaluate the capacity of plants for that purpose, a modified bioaccumulation coefficient (mBAC) was calculated for shots and roots as follow:

$$mBAC_S = \frac{PTEs\ concentration\ in\ aerial\ part\ (mg\ kg^{-1})}{bioavailable\ PTEs\ concentration\ in\ soil\ (mg\ kg^{-1})}$$

$$mBAC_R = \frac{PTEs\ concentration\ in\ roots\ (mg\ kg^{-1})}{bioavailable\ PTEs\ concentration\ in\ soil\ (mg\ kg^{-1})}$$

The metal bioavailable content in the soil, determined by DTPA extraction, represents the amount of contaminant potentially bioavailable for plant uptake.

To evaluate the presence of hyperaccumulator plants we also compared PTEs concentration values in shoots with reference values given by Van der Ent et al. (2013).

3.1.4 Assessment Index

The potential ecological risk index (ERI) represents the sensitivity of the biological community to toxic substance and illustrates the potential ecological risk caused by the overall contamination (Zhao and Li 2013). It was selected for evaluating the potential risk for biological community and also for human health from combined pollution of multiple PTEs. It can also be used for assessing the tolerance of different plants for different ecological risks. The equation used for calculating ERI is as follow (Hakanson 1980; Rahman et al. 2014):

$$ERI = \sum_{i=1}^{n} E_r^i = \sum_{i=1}^{n} T_r^i \times C_f^i = \sum_{i=1}^{n} \left(T_r^i \times \frac{C^i}{C_n^i} \right)$$

where: E_r^i is the potential ecological risk index of the PTE i; T_r^i is the toxic response factor for a specific PTE i (e.g. As=10, Cd=30, Cr=2, Cu=5, Pb=5, and Zn=1); C_f^i is the contamination factor of PTE i; C^i is the content of PTE i in the samples (mg kg^{-1}), and C_n^i is the background value of PTE i in the study area (mg kg^{-1}).

In this study, soil background values of Campania Region were used as C_n^i (As: 22.50; Cd: 0.95; Cr: 8; Cu: 98; Pb: 88.0; Zn: 90.5 mg kg^{-1}). The contamination degrees and the potential ecological risk of a PTE (E_r^i) were classified as low degree (E_r^i <30), moderate degree (30≤E_r^i <60), considerable degree (60≤E_r^i <120), high degree (120≤E_r^i <240), and very high degree (E_r^i ≥240). The ERI were classified as low risk (ERI<50), moderate risk (50≤ERI<100), high risk (100≤ERI<200), and very high risk (ERI≥220) (Hakanson 1980; Rahman et al. 2014).

3.1.5 Statistical Analysis

The statistical analyses were all carried out by using Ms Excel 2007 and SPSS 21 (SPSS Inc. Chicago, USA). Pearson correlation analyses were made to investigate the relationships between total PTEs in the plants, total PTEs content of soils and extracts PTEs concentrations, with the aim to study element phytoavailability among the different plots and to know the extraction method that better represents the availability for plants. Statistical

significance in this analysis was defined at $p < 0.05$ and $p < 0.01$. For plants that were more frequent on the site (at least in 3 plots) a correlation analysis was made to know the relation between specific plant species and soil, in particular with the aim to know if some plant can be used as a *bioindicator* of PTEs on the basis of the interaction plant-soil (Albornoz et al. 2016) and to study phytoavailability for specific plant species. Further correlation analysis were made between PTEs content in plants to know their interactions (Andra et al. 2011, Yao et al. 2016, Marrugo-Negrete et al. 2016, Soriano-Disla et al. 2010, Garcia-Salgado et al. 2012). Hierarchical Cluster Analysis was implemented on rhizo-soils PTEs to group similar clusters using Pearson coefficient as distance and between groups by linkage methods to obtain groups of similar metals. PCA was realized on PTEs concentration in rhizo-soils to obtain groups of similar PTEs (Rahman et al. 2014). Normalized variables (original variables) were transformed into the rotated components to extract significant principal components (PC) by suppressing the contribution of variables with minor significance, after Kaiser–Meyer–Olken (KMO) test that determines the appropriateness of data reduction through PCA analysis. Furthermore, these PC's were subjected to varimax orthogonal rotation with loading coefficients (> 0.1) to generate PC factors/groups. The number of components to keep was based on the Kaiser normalization, for which only components with eigenvalues greater than unity are retained. Contribution of a component can be considered significant when the corresponding eigenvalue is greater than unity.

3.2 Results and Discussions

3.2.1 Abundance and floral composition

The floristic composition of the site A site is shown in Tab. 6 while the floristic composition of the site B is shown in Tab. 7.

Tab. 6. Botanical characteristics of the species collected from the site A, analysis of frequency and abundance

Plant Species	Family	Frequency (%)	Total cover (%)	Average cover (%)
Artemisia annua L.	Plantaginaceae	18.1	22	2.0
Artemisia vulgaris L.	Asteraceae	18.1	31	2.8
Cirsium arvense (L.) Scop.	Asteraceae	18.1	57	5.2
Dittrichia viscosa (L.) Greuter subsp. viscosa	Asteraceae	18.1	50	4.5
Erigeron sumatrensis Retz.	Asteraceae	9.1	7	0.6
Ballota nigra L. subsp. meridionalis (Bég.) Bég.	Lamiaceae	9.1	45	4.1
Elymus repens (L.) Gould subsp. repens	Poaceae	27.2	185	16.8
Dactylis glomerata L. subsp. glomerata	Poaceae	18.1	25	2.2
Sorghum halepense (L.) Pers.	Poaceae	9.1	25	2.2
Holcus lanatus L.	Poaceae	18.1	45	4.1
Epilobium tetragonum L. subsp. tetragonum	Onagraceae	18.1	70	6.3
Rubus ulmifolius Schott	Rosaceae	18.1	198	18.1
Sambucus ebulus L.	Caprifoliaceae	18.1	160	14.5
Silene latifolia Poir. s.l.	Caryophyllaceae	18.1	20	1.8

Tab. 7. Botanical characteristics of the species collected from the site B, analysis of frequency and abundance

Plant Species	Family	Frequency (%)	Total cover (%)	Average cover (%)
Amaranthus retroflexus	Amaranthaceae	9.1	20	1.8
Aster tripolium	Asteraceae	18.1	30	2.7
Cirsium arvense	Asteraceae	18.1	80	7.2
Erigeron sumatrensis	Asteraceae	54.5	175	15.9
Hordeum leporinum	Poaceae	18.1	45	4.0
Lolium perenne	Poaceae	27.2	120	10.9
Piptatherum thomasii	Poaceae	27.2	25	2.2
Cynodon dactylon	Poaceae	72.7	266	24.1
Cyperus rotundus	Cyperaceae	27.2	16	1.4
Mercurialis annua	Euphorbiaceae	9.1	30	2.7
Rumex sp.	Polygonaceae	18.1	20	1.8
Echium vulgare	Boraginaceae	18.1	15	1.3

Fourteen species were observed and grouped into 8 families for the site A while twelve species and 7 families were reported for site B. The most abundant families for the 2 sites were Poaceae and Asteraceae. The most frequent species was *E. repens* for the site A and

C. dactylon for site B. The species with the highest mean cover were *R. ulmifolius* and *E. repens* for site A and *C. dactylon* and *E. sumatrensis* for site B. Most of the inventoried species of Asteraceae family are common for areas contaminated by industrial wastes like *A. vulgaris* (Wójcik et al.,2014), *C. arvense* (Desjardins D. et al., 2014*), E. sumatrensis* (Moreirta et al., 2011) and species from Poaceae family like *E. repens* and *D. glomerata* (Dygus H. K., 2013), *C. Dactylon* (Albornoz et al., 2016), *H. lanatus* (Cullaj A. et al., 2004). *R. ulmifolius* was also reported by Massa et al. (2010) in an industrial area in the north of Italy.

3.2.2 PTEs in plants and soils

The general characteristics of the soil samples of site A are given in Tab. 8 and soil characteristics of site B are showed in Tab. 9.

Tab. 8. Selected properties of soils sampled in site A.

	pH	Electrical conductivity (μS cm^{-1})	CaCO$_3$ (g kg^{-1})	Organic Carbon (g kg^{-1})	Sand (%)	Clay (%)
Plot 1	7.1	196	15.0	26.9	55.7	17.5
Plot 2	7.1	174	21.3	42.4	60.2	15.2
Plot 3	7.1	689	130	113	62.5	15.0
Plot 4	7.1	284	13.0	42.5	59.2	14.7
Plot 5	6.7	201	87.4	68.3	67.0	12.5
Plot 6	6.8	296	68.8	73.6	66.5	10.0
Plot 7	7.1	627	46.4	48.9	60.5	17.0
Plot 8	7.3	359	40.9	55.9	66.5	15.0
Plot 9	7.4	264	64.0	31.3	61.2	16.5
Plot 10	7.4	306	90.6	16.5	62.5	16.5
Plot 11	7.5	532	31.1	46.5	63.5	15
Mean	7.1	357	55.3	51.5	62.3	15.0
Standard deviation	0.2	178	36.9	26.6	3.5	2.1

Tab. 9. Selected properties of soils sampled in site B.

	pH	Electrical conductivity (μS cm^{-1})	CaCO$_3$ (g kg^{-1})	Organic Carbon (g kg^{-1})	Sand (%)	Clay (%)
Plot 1	7.2	180	9.0	15.3	68.5	6.7
Plot 2	7.2	265	4.5	18.9	61.7	12.2
Plot 3	7.4	229	6.5	18.7	55.5	14.5
Plot 4	7.4	201	12.7	19.5	65.2	11.2
Plot 5	7.1	201	3.1	19.4	59.2	18.7
Plot 6	7.5	188	6.7	23.5	62.0	17.0
Plot 7	7.5	157	11.4	21.3	71.2	13.7
Plot 8	7.5	219	11.5	22.6	65.7	13.2
Plot 9	7.4	163	9.1	23.0	59.5	17.5
Plot 10	7.5	224	11.4	19.5	63.5	11.7
Plot 11	7.4	191	15.0	23.6	59.7	15.0
Mean	7.4	202	9.2	20.5	62.9	13.8
Standard deviation	0.1	31.4	3.7	2.6	4.5	3.4

Most samples in site A showed sub-alkaline pH values, with an average value of 7.2. Majority of soils presented a good organic matter content except for plot 10 – site A and Plot 1 – site B that have a low organic matter content; salinity was low for all the examined soils and calcium carbonate was high for site A (mean value of 55 g kg^{-1}). Soils sampled in Site B showed a low carbonate content (mean value of 9.21 g kg^{-1}).

The total concentration of Pb, Cd, As, Cu, Zn, Tl and Sb in rhizo-soils sampled in site A are presented in Tab. 10.

Tab. 10. PTEs concentrations of soils sampled in different plots of site A.

	Cu	Pb	Sb	Zn	As	Cd	Tl	Cr
	(mg kg^{-1})							
Plot 1	291	818	9	434	28	3	3.6	44
Plot 2	436	21009	20	502	68	83	3.5	48
Plot 3	349	6231	491	242	25	19	3.7	47
Plot 4	679	5995	131	419	38	27	4.5	47
Plot 5	266	3134	86	356	32	10	3.2	48
Plot 6	639	100000	23	450	861	298	1.6	69
Plot 7	1412	53076	7260	802	174	183	17.2	61
Plot 8	504	53201	856	501	185	195	11.4	62
Plot 9	363	35017	743	344	189	126	4.3	44
Plot 10	340	39263	803	429	97	153	3.8	55
Plot 11	41	100	787	314	18	0.6	1.3	326

The concentrations in the soil widely varied with higher concentrations of all PTEs in Plots 6, 7, 8 and 10 indicating a large proportion of anthropogenic input. On the basis of Italian screening values (SV) for industrial soils (D.Lgs 152/2006), we found that all the PTEs contents examined were high. Soil Pb concentration ranged from 100 to over 100000 mg kg^{-1} and was often above SV for industrial sites (1000 mg kg^{-1}) in all the Plots. Soil Cd concentration ranged from 0.60 to 298.60 mg kg^{-1} and was above legal threshold (15 mg kg^{-1}) except for Plots 1, 5 and 11. Soil As concentration ranged from 18 to 861 mg kg^{-1} with maximum value in in Plot 6 and above SV (50 mg kg^{-1}) for all the plots except for Plots 1, 3, 4, 5 and 11. Soil Cu concentration was also above the SV (600 mg kg^{-1}) in some plots with values between 42 and 1413 mg kg^{-1}. Concentration of Zn in soils was lower than SV (1500 mg kg^{-1}) in all the soils whit max values of 450 mg kg^{-1} in plot 6. Thallium concentration in soils was above SV (10 mg kg^{-1}) only in two plots while Sb soil concentration was above SV (30 mg kg^{-1}) for all the Plots except for Plots 1, 2 and 6. Chromium concentration in soils was lower than SV (800 mg kg^{-1}) in all the plots.

The total concentration of Pb, Cd, As, Cu, Zn, Tl and Sb in rhizo-soils sampled in site B are presented in Tab. 11.

Tab. 11. PTEs concentrations of soils sampled in different plots of site B.

	Cu	Pb	Zn	Cd	Cr	As
	(mg kg^{-1})					
Plot 1	66	101	280	0.4	347	22
Plot 2	68	100	223	0.3	221	27
Plot 3	67	160	267	0.8	325	21
Plot 4	68	113	272	0.5	326	23
Plot 5	68	122	464	6.0	759	25
Plot 6	56	88	474	4.8	954	18
Plot 7	54	83	498	0.3	851	18
Plot 8	66	117	514	1.0	764	21
Plot 9	53	89	291	0.7	332	23
Plot 10	53	82	270	0.3	317	22
Plot 11	64	180	768	1.4	266	34

On the basis of Italian Legislation for agricultural soils that are assimilated to the residential sites (D.Lgs 152/2006), we found that Pb, Zn, Cd, Cr and As contents were high. Soil Pb concentration ranged from 82 to 160 mg kg^{-1} and was above SV for residential sites (100 mg kg^{-1}) in all the Plots except for Plots 6, 7, 9 and 10. Soil Cd concentration ranged from 0.31 to 6.01 mg kg^{-1} and was above SV (2 mg kg^{-1}) only for Plot 5 and Plot 6. Concentration of Zn in soils was above SV (150 mg kg^{-1}) for all the soils whit max values of 769 mg kg^{-1} in plot 11. Chromium concentration in soils was above legal thresholds (150 mg kg^{-1}) for all the examined Plots. Soil As concentration ranged from 18 to 34 mg kg^{-1} with maximum value in in Plot 11 and above legal threshold (20 mg kg^{-1}) for all the plots except for Plot 6 and 7.

Thirteen species were found as representative of the investigated plots in the industrial site (site A) and twelve in the agricultural soil (site B). Plant species are listed in Tab. 12 for the site A and in Tab. 13 for the site B, together with the label code and the PTEs concentrations in plant tissues and rhizo-soil.

Tab. 12. PTEs concentrations in different plants part and in rhizo-soils of the native species found in the industrial site (site A).

Plant Species	Label		Cu	Pb	Sb	Zn	As	Cd	Cr	Tl
			mg kg^{-1} (d.w.)							
Artemisia vulgaris	AV	Soils	67	208	6.3	121	15.0	0.7	37.0	1.7
		Shoots	9	33	0.9	23	0.3	0.4	1.6	0.0
		Roots	15	17	0.3	18	0.4	0.4	1.8	0.3
Dittrichia viscosa	DV	Soils	224	609	3.0	313	13.0	2.5	52.0	1.9
		Shoots	15	47	1.0	33	0.1	0.8	2.0	0.1
		Roots	20	16	0.6	13	0.9	0.3	10.2	0.4
Epilobium tetragonum	ET	Soils	160	561	10.5	235	14.0	2.1	49.0	1.7
		Shoots	12	32	0.7	28	0.1	0.2	1.6	0.0
		Roots	50	101	1.4	97	0.0	1.8	2.5	0.2
Sorghum halepense	SH	Soils	276	20449	9.8	267	54.0	81.7	47.0	1.8
		Shoots	16	74	1.4	50	0.4	1.0	2.4	0.2
		Roots	36	76	1.1	72	0.1	2.2	2.8	0.3
Sambucus ebulus	SE	Soils	196	3166	639.0	278	21.5	9.9	186.0	2.6
		Shoots	8	66	1.5	22	0.1	0.3	2.1	0.1
		Roots	19	159	2.0	18	0.0	3.7	1.6	0.7
Dactylis Glomerata	DG	Soils	155	11795	192.0	164	54.5	50.7	47.0	2.3
		Shoots	44	323	5.8	69	0.3	3.8	7.0	0.3
		Roots	47	590	8.7	53	2.6	17.9	3.1	0.9
Cirsium arvense	CA	Soils	572	4663	24.9	281	22.0	21.9	47.0	2.1
		Shoots	24	213	3.8	54	0.1	3.8	3.8	0.2
		Roots	39	197	23	47	0.3	7.6	1.8	0.6
Artemisia annua	AA	Soils	107	1428	65.7	148	17.0	4.9	51.0	1.7
		Shoots	14	106	2.7	40	0.4	9.4	2.1	0.1
		Roots	33	321	3.9	38	2.1	8.5	3.9	0.6
Holcus lanatus	HL	Soils	159	1707	21.1	208	15.0	5.8	46.0	1.5
		Shoots	11	70	1.5	33	0.3	1.3	2.5	0.1
		Roots	46	358	4.1	71	1.0	4.9	3.3	0.4
Rubus ulmifolius	RU	Soils	490	69631	413.0	439	479.0	225.0	62.0	2.7
		Shoots	14	106	2.2	29	0.1	2.0	2.5	0.2
		Roots	32	174	2.3	48	0.2	3.6	2.6	0.6
Silene latifolia	SL	Soils	460	49647	6089.6	437	159.0	175.0	67.0	9.4
		Shoots	56	217	4.9	41	0.9	7.7	2.9	102.5
		Roots	70	3404	87.1	81	20.7	41.2	3.1	44.0
Elymus repens	ER	Soils	477	16085	606.0	278	70.0	59.7	52.0	5.2
		Shoots	28	283	4.2	44	0.2	5.2	2.7	1.0
		Roots	38	1407	13.8	67	1.8	26.2	2.3	1.8
Ballota nigra	BN	Soils	187	21135	671.0	184	86.0	55.0	65.0	5.9
		Shoots	35	176	4.2	31	0.6	5.9	2.5	1.7
		Roots	28	671	3.8	34	0.1	20.0	1.7	4.1

Tab. 13. PTEs concentrations in different plants part and in rhizo-soils of the native species found on the farmland (site B).

Plant Species	Label		Cu	Pb	Zn	Cd	Cr	As
			mg kg^{-1} (d.w.)					
Cirsium arvense	CA	Soils	47.7	106.0	267.0	0.60	357.0	23.00
		Shoots	9.0	0.4	35.1	0.08	2.5	0.15
		Roots	10.4	1.0	29.4	0.93	5.6	0.40
Cynodon dactylon	CD	Soils	66.0	89.4	389.0	0.68	524.0	22.40
		Shoots	7.2	1.1	56.9	0.05	6.5	0.20
		Roots	10.1	2.5	67.1	0.52	26.2	0.62
Lolium perenne	LP	Soils	59.7	152.0	659.0	4.90	472.0	32.00
		Shoots	5.1	0.8	52.1	0.08	4.7	0.11
		Roots	14.1	5.3	64.6	0.11	9.6	0.47
Rumex sp.	RS	Soils	88.9	109.0	217.0	0.60	158.0	28.00
		Shoots	6.2	0.7	19.8	0.03	2.5	0.20
		Roots	10.8	2.6	54.3	0.20	8.4	0.30
Erigeron sumatrensis	ES	Soils	55.8	135.0	401.0	3.57	674.0	17.00
		Shoots	12.6	0.5	54.5	1.33	2.2	0.13
		Roots	8.8	1.3	25.2	0.14	11.1	0.30
Hordeum leporinum	HL	Soils	83.5	99.6	206.0	0.60	142.0	25.00
		Shoots	2.1	0.1	43.6	0.03	3.1	0.40
		Roots	9.6	3.8	54.2	0.10	26.4	0.10
Piptatherum thomasii	PT	Soils	72.3	156.0	351.0	12.0	415.0	25.00
		Shoots	4.7	0.7	22.2	0.05	2.6	0.20
		Roots	19.2	20.4	145.2	0.21	6.9	0.50
Amaranthus retroflexus	AR	Soils	65.7	83.8	696.0	9.60	1414.0	23.00
		Shoots	5.0	2.2	88.1	2.65	2.2	0.10
		Roots	3.6	0.7	58.7	0.04	4.2	0.20
Echium vulgare	EV	Soils	44.3	77.2	251.0	0.60	488.0	14.00
		Shoots	10.2	0.3	54.2	0.02	1.9	0.10
		Roots	9.1	1.6	62.8	0.29	3.9	0.20
Mercurialis annua	MA	Soils	64.5	140.0	404.0	1.50	509.0	23.00
		Shoots	4.5	0.7	69.7	0.03	3.8	0.20
		Roots	5.2	2.4	34.5	0.10	9.8	0.90
Aster tripolium	AT	Soils	44.5	81.4	175.0	0.50	137.0	23.00
		Shoots	9.7	0.3	29.9	0.07	3.2	0.30
		Roots	11.1	0.5	36.6	0.31	48.4	2.00
Cyperus rotundus	CR	Soils	62.2	83.3	366.0	0.80	504.0	23.00
		Shoots	8.8	1.1	96.0	0.27	25.2	0.02
		Roots	11.4	4.1	143.0	0.13	173.2	6.10

Cu is an essential element for plants. There is an increasing evidence of the active absorption of Cu; however, passive absorption is likely to occur, especially in the toxic range of this metal in solutions. In root tissue, Cu is almost entirely in complexed forms; however, it is most likely that the metal enters root cells in dissociated forms (Kabata-Pendias, 2011). In site A, only DG, CA, SL, ER and BN reported concentrations above values recorded from Kabata-Pendias (2011) (21 mg kg^{-1}). Total Cu concentrations in the roots of collected plants ranged from 15.5 to 69.8 mg kg^{-1} with the maximum concentration was found in *S. Latifolia*. The Cu shoots concentration ranged from 7.8 to 56.0 mg kg^{-1} with the maximum accumulation in the shoots of *S. Latifolia*. In site B no plant species presented concentrations above values recorded from Kabata-Pendias (2011) in shoots. Total Cu concentrations in the roots of collected plants ranged from 3.6 to 19.3 mg kg^{-1} (*P. thomasii)*. The Cu shoots concentration ranged from 2.1 to 12.6 mg kg^{-1} with the maximum accumulation in the shoots of *E. sumatrensis*.

Zn is also an essential element for plants. However, hyperaccumulation of Zn by plants is not very usual. It is very mobile during weathering processes, and its easily soluble compounds are readily precipitated by reactions with carbonates, or it is absorbed by minerals and organic compounds, especially in the presence of sulphur anions, hence minimizing uptake and transport from roots to the aerial parts of plants (Kabata-Pendias, 2011). In site A, total Zn concentration in the roots ranged from 13.2 to 97.5 mg kg^{-1}, this being the maximum concentration observed for *E. tetragonum*. Moreover, the maximum Zn concentration in the shoots was in *D. glomerata*. Concentrations above values recorded from Kabata-Pendias (2011) (31.5 mg kg^{-1}) were reported only for DV, SH, DG, CA, AA, HL, SR and ER. In site B, total Zn concentration in the roots ranged from 25.2 to 145.2 mg kg^{-1}, this being the maximum concentration observed for *P. thomasii* like for Zn. Zn shoot concentration ranged from 19.8 to 96.0 mg kg^{-1} with the maximum Zn concentration in *C. rotundus*. Concentrations above values recorded from Kabata-Pendias (2011) (31.5 mg kg^{-1}) were reported for all the plant species except for RS, PT and AT.

For site A, total Cd concentrations in the roots ranged from 0.3 to 41.2 mg kg^{-1}, the maximum occurring in *S. latifolia* as already reported for Cu. Cd concentration in shoots ranged from 0.2 to 9.4 mg kg^{-1} with maximum concentration observed in *A. annua*. All plant species except for AV, DV, ET and SE accumulated Cd above legal PTEs thresholds in plants (REG UE N. 1275/2013 (1.0 mg kg^{-1}). For site B, total Cd concentrations in the roots ranged from 0.04 to 0.93 mg kg^{-1}, the maximum value occurring in *C.arvense* like for Cu. Shoots concentration values ranged from 0.02 to 2.65 mg kg^{-1} with maximum concentra-

tion in *A. retroflexus*. Only ES and AR accumulated Cd above legal PTEs thresholds in plants (REG UE N. 1275/2013 - 1.0 mg kg^{-1}). According to Kabata- Pendias (2011), soil pH is listed as the major soil factor controlling both total and relative uptake of Cd, as well as its concentration in growth media.

For site A, total root Pb concentration ranged from 16 to as high as 3404 mg kg^{-1}, the maximum being in the roots of *S. latifolia* while shoots concentration values ranged from 31.6 to 326.3 mg kg^{-1} with maximum concentration observed in *S. latifolia*. All plant species accumulated Pb above legal PTEs thresholds in plants (REG UE N. 1275/2013 (30 mg kg^{-1}). For site B, total root Pb concentration ranged from 0.48 to as high as 20.44 mg kg^{-1}, the maximum being in the roots of *P. thomasii* while shoots concentration values ranged from 0.32 to 2.22 mg kg^{-1} with maximum concentration observed in *A. retroflexus*. No one of the plant species accumulated Pb above legal PTEs thresholds in plants (REG UE N. 1275/2013 -30 mg kg^{-1}). As reported by Kabata- Pendias (2011), the Pb uptake by plants depends on several soils properties, such as OMM, granulometric composition, CEC, pH, as well as genetic plant factors, root surface area, and root exudates.

For site A, total As concentrations in the plants roots ranged from 0.05 to 20.70 mg kg^{-1}, while in the shoots, As concentrations ranged from 0.05 to 0.90 mg kg^{-1}. The maximum As concentration in shoots and roots was found in *S latifolia*. No plant species accumulated As above legal PTEs thresholds in plants (REG UE N. 1275/2013 (2 mg kg^{-1}). For site B, total As concentrations in the plants roots ranged from 0.10 to 6.10 mg kg^{-1}, while in the shoots, As concentrations ranged from 0.02 to 0.40 mg kg^{-1}. The maximum As concentration in shoots was found in *H. leporinum* while roots maximum concentration was reported for *C. rotundus*. Despite from high concentrations of As in soils above legal threshold, no plant species accumulated As above legal PTEs thresholds in plants (REG UE N. 1275/2013 - 2.00 mg kg^{-1}). Report on the linear relationship between As contents of vegetation and concentrations in soils of both total and soluble species suggest that plants take up As passively with the water flow (Kabata- Pendias, 2011).

For site A, total Sb concentration in the roots ranged from 0.3 to 87.0 mg kg^{-1} and, in the shoots, from 0.75 to 5.84 mg kg^{-1}. The maximum accumulation there was for *S. latifolia*. The mean concentration of terrestrial plants according to Kabata- Pendias (2011) was 0.06 mg kg^{-1} and all plants species accumulated above the values reported by the author.

For site A, total root Cr concentration ranged from 1.65 to as high as 10.20 mg kg^{-1}, the maximum being in the roots of *D. Viscosa* while shoots concentration values ranged from 1.60 to 7.00 mg kg^{-1} with maximum concentration observed in *D. glomerata*. Only AV,

DV, ET, SE and AA not accumulated Cr above concentration of terrestrial plants according to Kabata- Pendias (2011) (2.20 mg kg^{-1}). For site B, total root Cr concentration ranged from 3.90 to as high as 173.20 mg kg^{-1}, the maximum being in the roots of *C. rotundus* while shoot values ranged from 1.90 to 25.20 mg kg^{-1} with maximum concentration observed in *C. rotundus*. All sampled plants accumulated Cr above concentration of terrestrial plants according to Kabata- Pendias (2011) (2.20 mg kg^{-1}) except for EV. As reported by Kabata- Pendias (2011), Chromium is slightly available to plants and not easily translocated, but it is concentrated mainly in roots. The most available to plants is Cr^{6+}, which is the very unstable form under normal soil conditions and its availability depends on soils properties, and especially on soil texture and pH.

For site A, total Tl concentrations in the roots ranged from 0.21 to 43.99 mg kg^{-1}, the maximum occurring in *S. latifolia* like for other PTEs. However, for shoots, concentration values ranged from 0.03 to 102.54 mg kg^{-1} with maximum concentration also observed in *S. latifolia*. Only SL, ER and BN accumulated Tl above concentration of terrestrial plants according to Kabata- Pendias (2011) (0.51 mg kg^{-1}). According to Kabata- Pendias (2011), the Tl content of plants seems to be a function of Tl concentrations in soils and plants from Zn–Pb industrial area contain Tl concentrations higher than other sites. Almost all collected plant species showed higher PTE concentration than the normal levels. These results indicated that the species were tolerant to these metals with varying degrees. None of the plant species showed metal concentrations that allow to define them as hyperaccumulators according to Van der Ent et al. (2013) concentration criteria (100 mg kg^{-1} for Cd, Se, and Tl; 300 mg kg^{-1} for Co, Cu, and Cr; 1000 mg kg^{-1} for Ni, Pb, and As; 3000 mg kg^{-1} for Zn; and 10,000 mg kg^{-1} for Mn) except for *Silene latifolia* that accumulated in the aerial part Tl in concentration above 100 mg kg^{-1}. This result is in according with Escarrè et al. (2011).

For site A, the trend for PTEs accumulation (mg kg^{-1} dry weight) in the shoots was:

- Pb > Zn > Cr > Sb > Cd > As > Tl in AV, ET, SH, DG, RU and HL;
- Pb > Zn > Cu > Cr > Sb > Cd > Tl > As in DV and SE;
- Pb > Zn > Cu > Sb > Cr > Cd > Tl > As in CA;
- Pb > Zn > Cu > Cd > Sb > Cr > Tl > As in ER and BN;
- Pb > Tl > Cu > Zn > Cd > Sb > Cr > As in SL;
- Pb > Zn > Cu > Cd > Sb > Cr > As > Tl in AA.

For site A, the trend for PTEs accumulation (mg kg^{-1} dry weight) in the roots was:

- Zn > Pb > Cu > Cr > Cd > As > Sb > Tl in AV;

- Pb > Zn > Cu > Cr > Cd > Sb > Tl > As in ET and SH;

- Pb > Zn > Cu > Sb > Cr > Cd > As > Tl in DG and HL;

- Pb > Zn > Cu > Cd > Cr > Sb > Tl > As in RU, SE, CA and ER;

- Cu >Pb > Zn > Cr > As > Sb > Cd > Tl in DV;

- Pb > Zn > Cu > Cd > Cr > Sb > As > Tl in AA;

- Pb > Sb > Zn > Cu > Tl > Cd > As > Cr in SL;

- Pb > Zn > Cu > Cd > Sb > Tl > Cr >As in BN.

For site B, the trend for PTEs accumulation (mg kg^{-1} dry weight) in shoots was:

- Zn > Cu > Cr > Pb > Cd>As in CA, CD, LP, RS, PT, EV, MA, AT;

- Zn > Cu > Cr > Cd >Pb>As in ES;

- Zn > Cr > Cu > Pb > Cd>As in HL and CR;

- Zn > Cu > Cd > Pb > Cr>As in AR.

For site B, the trend for PTEs accumulation (mg kg^{-1} dry weight) in the roots was:

- Zn > Cu > Cr > Pb > Cd>As in CA, LP, RS, EV;

- Zn> Cr > Cu >Pb > Cd>As in CD, ES, HL, AR and MA;

- Zn > Pb > Cu > Cr > Cd>As in PT;

- Cr > Zn > Cu > Pb > Cd>As in AT and CR.

In the majority of the collected species Zn and Pb were the most absorbed PTE, while Cd and As the least absorbed. As reported by Mengel and Kirkby (2001) and Pandey (2012), Zn inhibits the Cd uptake due to its competitive behaviour, because both metals are transported by a common carrier at the root plasma membrane, which has more affinity for Zn than Cd (Hart et al. 2005).

BACs, BACR and TF were calculated for the species retrieved from the two sites. Results for site A are showed in Tab. 14.

Tab. 14. BAC$_S$, BAC$_R$ and TF of the native plants found on the industrial site (site A).

Plant species	Label		Cu	Pb	Sb	Zn	Cd	Cr	Tl
Artemisia vulgaris	AV	TF	0.59	**1.95**	**2.85**	**1.28**	0.89	0.89	0.12
		BAC$_S$	0.14	0.16	0.15	0.19	0.57	0.04	0.02
		BAC$_R$	0.23	0.08	0.05	0.15	0.64	0.05	0.19
Dittrichia viscosa	DV	TF	0.72	**2.84**	**1.63**	**2.52**	**2.52**	0.20	0.15
		BAC$_S$	0.07	0.08	0.35	0.11	0.33	0.04	0.03
		BAC$_R$	0.09	0.03	0.22	0.04	0.13	0.20	0.22
Epilobium tetragonum	ET	TF	0.24	0.31	0.52	0.29	0.10	0.64	0.14
		BAC$_S$	0.07	0.06	0.07	0.12	0.08	0.03	0.02
		BAC$_R$	0.31	0.18	0.14	0.41	0.84	0.05	0.12
Sorghum halepense	SH	TF	0.44	0.97	**1.27**	0.70	0.47	0.86	0.65
		BAC$_S$	0.06	0.00	0.14	0.19	0.01	0.05	0.12
		BAC$_R$	0.13	0.00	0.11	0.27	0.03	0.06	0.19
Sambucus ebulus	SE	TF	0.42	0.42	0.72	**1.22**	0.07	**1.30**	0.12
		BAC$_S$	0.04	0.02	0.00	0.08	0.03	0.01	0.03
		BAC$_R$	0.09	0.05	0.00	0.07	0.37	0.01	0.26
Dactylis Glomerata	DG	TF	0.95	0.55	0.67	**1.30**	0.21	**2.26**	0.29
		BAC$_S$	0.29	0.03	0.03	0.42	0.07	0.15	0.12
		BAC$_R$	0.30	0.05	0.04	0.32	0.35	0.07	0.42
Cirsium arvense	CA	TF	0.62	**1.08**	**1.66**	**1.16**	0.50	**2.11**	0.33
		BAC$_S$	0.04	0.05	0.15	0.19	0.17	0.08	0.09
		BAC$_R$	0.07	0.04	0.09	0.17	0.35	0.04	0.28
Artemisia annua	AA	TF	0.42	0.33	0.68	**1.04**	**1.10**	0.54	0.23
		BAC$_S$	0.13	0.07	0.04	0.27	**1.92**	0.04	0.08
		BAC$_R$	0.30	0.22	0.06	0.26	**1.74**	0.08	0.36
Holcus lanatus	HL	TF	0.23	0.20	0.38	0.47	0.26	0.76	0.24
		BAC$_S$	0.07	0.04	0.07	0.16	0.22	0.05	0.07
		BAC$_R$	0.29	0.21	0.19	0.34	0.85	0.07	0.29
Rubus ulmifolius	RU	TF	0.42	0.61	0.97	0.61	0.56	0.98	0.40
		BAC$_S$	0.03	0.00	0.01	0.07	0.01	0.04	0.08
		BAC$_R$	0.07	0.00	0.01	0.11	0.02	0.04	0.21
Silene latifolia	SL	TF	0.80	0.06	0.06	0.50	0.19	0.94	**2.33**
		BAC$_S$	0.12	0.00	0.00	0.09	0.04	0.04	**10.9**
		BAC$_R$	0.15	0.07	0.01	0.19	0.23	0.05	**4.68**
Elymus repens	ER	TF	0.73	0.20	0.30	0.65	0.20	**1.14**	0.54
		BAC$_S$	0.06	0.02	0.01	0.16	0.09	0.05	0.19
		BAC$_R$	0.08	0.09	0.02	0.24	0.44	0.05	0.35
Ballota nigra	BN	TF	**1.24**	0.26	**1.11**	0.91	0.30	**1.47**	0.41
		BAC$_S$	0.19	0.01	0.01	0.17	0.11	0.04	0.28
		BAC$_R$	0.15	0.03	0.01	0.19	0.36	0.03	0.69

BAC$_S$, BAC$_R$ and TF can be all used for estimating a plant potential for phytoremediation purposes. Among the selected 13 plants samples, bioaccumulation factor of shoots and roots of plants growing in the contaminated site ranged between:

- 0.03 and 0.29 (shoots) and 0.07 and 0.31 (roots) for Cu;
- 0.002 and 0.16 (shoots) and 0.02 and 0.22 (roots) for Pb;
- 0.001 and 0.35 (shoots) and 0.003 and 0.22 (roots) for Sb;
- 0.007 and 0.42 (shoots) and 0.04 and 0.41 (roots) for Zn;

- 0.001 and 1.92 (shoots) and 0.02 and 1.74 (roots) for Cd;

- 0.01 and 0.15 (shoots) and 0.01 and 0.20 (roots) for Cr;

- 0.02 and 10.92 (shoots) and 0.12 and 4.68 (roots) for Tl.

For Cu, Pb, Sb and Cr BAC_S, and BAC_R were lower than 1 for all the species while TF was higher than 1 for different species, but the concentrations in the plants was too low for considering that species for phytoremediation purpose.

For Cu only BN was effective in translocation with a TF of 1.24.

For Pb AV, DV and CA were effective in translocation with max value in DV (2.84).

For Sb, AV, DV, SH, CA and BN were effective in translocation with max value in AV (2.85).

For Zn, AV, DV, SE, DG, CA and AA were effective in translocation with max value in DV (2.52).

For Cd, AV, DV, SE, DG, CA and AA were effective in translocation with max value in DV (2.52).

For Cr, SE, DG, CA, ER and BN were effective in translocation with max value in DG (2.26).

For Tl only SL was effective in translocate with a TF value of 2.33.

For Cd *A. annua* showed BAC_S, BAC_R and TF higher than 1 with a high concentration in the aerial part. For Tl *S. latifolia* reported high concentration both in shoot than roots at a hyperaccumulator levels (Van Der Ent et al., 2013) as also reported by Escarrè et al. (2011) with BAC_S, BAC_R and TF higher than 1 confirming the hyperaccumulator hypothesis of this plant species. According to our results, *S. latifolia* and *A. annua* have the potential to be used for phytoextraction of Cd and Tl respectively.

BAC_S, BAC_R and TF for site B are showed in Tab. 15.

Tab. 15. Soil DTPA extractable PTEs and BAC_S, BAC_R and TF of the native plants found on farmland (site B).

Plant species	Label		Cu	Pb	Zn	Cd	Cr	As
Cirsium arvense	CA	TF	0.87	0.39	**1.20**	0.08	0.44	0.38
		BAC_S	0.19	0.00	0.13	0.13	0.01	0.01
		BAC_R	0.22	0.01	0.11	**1.55**	0.02	0.02
Cynodon dactylon	CD	TF	0.71	0.44	0.85	0.10	0.25	0.32
		BAC_S	0.11	0.01	0.15	0.08	0.01	0.01
		BAC_R	0.15	0.03	0.17	0.76	0.05	0.03
Lolium perenne	LP	TF	0.36	0.16	0.81	0.71	0.48	0.23
		BAC_S	0.09	0.01	0.08	0.02	0.01	0.00
		BAC_R	0.24	0.03	0.10	0.02	0.02	0.01
Rumex sp.	RS	TF	0.57	0.25	0.36	0.15	0.30	0.67
		BAC_S	0.07	0.01	0.09	0.05	0.02	0.01
		BAC_R	0.12	0.02	0.25	0.33	0.05	0.01
Erigeron sumatrensis	ES	TF	**1.43**	0.39	**2.16**	**9.52**	0.20	0.44
		BAC_S	0.23	0.00	0.14	0.37	0.00	0.01
		BAC_R	0.16	0.01	0.06	0.04	0.02	0.02
Hordeum leporinum	HL	TF	0.22	0.13	0.80	0.30	0.12	**4.00**
		BAC_S	0.02	0.01	0.21	0.05	0.02	0.02
		BAC_R	0.11	0.04	0.26	0.17	0.19	0.00
Piptatherum thomasii	PT	TF	0.25	0.04	0.15	0.24	0.38	0.40
		BAC_S	0.07	0.00	0.06	0.00	0.01	0.01
		BAC_R	0.27	0.13	0.41	0.02	0.02	0.02
Amaranthus retroflexus	AR	TF	**1.38**	**3.00**	**1.50**	66.25	0.52	0.50
		BAC_S	0.08	0.03	0.13	0.28	0.00	0.00
		BAC_R	0.06	0.01	0.08	0.00	0.00	0.01
Echium vulgare	EV	TF	**1.13**	0.21	0.86	0.07	0.49	0.50
		BAC_S	0.23	0.00	0.22	0.03	0.00	0.01
		BAC_R	0.20	0.02	0.25	0.48	0.01	0.01
Mercurialis annua	MA	TF	0.87	0.27	2.02	0.30	0.39	0.22
		BAC_S	0.07	0.00	0.17	0.02	0.01	0.01
		BAC_R	0.08	0.02	0.09	0.07	0.02	0.04
Aster tripolium	AT	TF	0.87	0.67	0.82	0.23	0.07	0.15
		BAC_S	0.22	0.00	0.17	0.14	0.02	0.01
		BAC_R	0.25	0.01	0.21	0.62	0.35	0.09
Cyperus rotundus	CR	TF	0.77	0.26	0.67	**2.08**	0.15	0.00
		BAC_S	0.14	0.01	0.26	0.34	0.05	0.00
		BAC_R	0.18	0.05	0.39	0.16	0.34	0.27

Among the selected 11 plants samples, BAC_S, of plants growing in the contaminated site ranged between:

- 0.02 and 0.23 for Cu;

- 0.004 and 0.03 for Pb;

- 0.06 and 0.26 for Zn;

- 0.004 and 0.37 for Cd;

- 0.002 and 0.05 for Cr;

- 0.001 and 0.02 for As.

While BAC_R of plants growing in the contaminated site ranged between:

- 0.06 and 0.23 for Cu;

- 0.01 and 0.13 for Pb;

- 0.06 and 0.41 for Zn;

- 0.004 and 1.55 for Cd;

- 0.003 and 0.35 for Cr;

- 0.004 and 0.27 for As.

BAC_S, and BAC_R were lower than 1 for Cu, Pb, Zn, Cr and As for all the species except for *C. arvense* that showed a BAC_R higher than 1 for Cd. For that reason, according to Yoon et al., 2006 and Lorestani et al., 2011, *C. arvense* has the potential for phytostabilization. TF was higher than 1 for: CA for Zn; ES for Cu, Zn and Cd; AR for Cu, Zn, Pb and Cd; MA for Zn; CR for Cd; HL for As. Like for site A, even if TF was higher than 1 for some species, the concentration in the plants was too lower to consider that plant for phytoremediation purpose.

The modified bioaccumulation coefficient for the shoots ($mBAC_S$) and the modified bioaccumulation coefficient for the roots ($mBAC_R$) for each species were also calculated as showed in Tab. 16 for site A and in Tab. 17 for site B using the bioavailable metal concentrations from the rhizo-soils (Barbafieri et al. 2011).

Tab. 16. Soil DTPA extracted Pb and mBAC$_S$ and mBAC$_R$ of the native plants found on the industrial site (site A).

Plant species	Label		Pb
Artemisia vulgaris	AV	DTPA	23.6 (mg kg^{-1})
		mBAC$_S$	**1.42**
		mBAC$_R$	0.73
Dittrichia viscosa	DV	DTPA	31.5 (mg kg^{-1})
		mBAC$_S$	**1.50**
		mBAC$_R$	0.53
Epilobium tetragonum	ET	DTPA	26.6 (mg kg^{-1})
		mBAC$_S$	**1.19**
		mBAC$_R$	**3.79**
Sorghum halepense	SH	DTPA	117.6 (mg kg^{-1})
		mBAC$_S$	0.63
		mBAC$_R$	0.65
Sambucus ebulus	SE	DTPA	1873.0 (mg kg^{-1})
		mBAC$_S$	0.04
		mBAC$_R$	0.08
Dactylis Glomerata	DG	DTPA	3103.4 (mg kg^{-1})
		mBAC$_S$	0.10
		mBAC$_R$	0.19
Cirsium arvense	CA	DTPA	852.0 (mg kg^{-1})
		mBAC$_S$	0.25
		mBAC$_R$	0.23
Artemisia annua	AA	DTPA	304.6 (mg kg^{-1})
		mBAC$_S$	0.35
		mBAC$_R$	**1.05**
Holcus lanatus	HL	DTPA	382.2 (mg kg^{-1})
		mBAC$_S$	0.18
		mBAC$_R$	0.94
Rubus ulmifolius	RU	DTPA	2576.0 (mg kg^{-1})
		mBAC$_S$	0.04
		mBAC$_R$	0.07
Silene latifolia	SL	DTPA	1754.0 (mg kg^{-1})
		mBAC$_S$	0.12
		mBAC$_R$	**1.94**
Elymus repens	ER	DTPA	6598.0 (mg kg^{-1})
		mBAC$_S$	0.04
		mBAC$_R$	0.21
Ballota nigra	BN	DTPA	1660.0 (mg kg^{-1})
		mBAC$_S$	0.11
		mBAC$_R$	0.40

The PTEs extracted with DTPA may include elements from the water-soluble and exchangeable phases plus organically-bound elements so representing the metals potentially bioavailable for plant uptake, and thus mBAC$_S$ and mBAC$_R$ can more realistically represent the capacity of metal transfer to plants.

According to the values listed in Tab. 16, AV and DV showed the higher ability to transfer the potential bioavailable fraction of Pb to the aerial part thus they could be considered interesting candidates for phytoextraction, even if not favoured by the low biomass produced. ET, AA and SL reported a high mBAC$_R$ so they could be suitable for Pb phytosta-

bilization in revegetating the area, stabilizing the soil by their root apparatus, while their shrubs can act as a barrier to weathering (rain and wind erosion). However $mBAC_S$ and $mBAC_R$ values were lower of BAC_S and BAC_R of corresponding plants. Indicate that these species had different adaptations to hostile environment and specific environmental conditions (water availability, matrix characteristics, temperature, etc.) of each site. These results suggested that the performance of native plants is site-specific as also reported by other authors like Barbafieri et al. (2011).

Tab. 17. Soil DTPA exctracted Cd and $mBAC_S$ and $mBAC_R$ of the native plants found on the agricoltural site (site B).

Plant species	Label		Cd
Cirsium arvense	CA	DTPA	0.13 (mg kg^{-1})
		$mBAC_S$	0.59
		$mBAC_R$	**7.27**
Cynodon dactylon	CD	DTPA	0.21 (mg kg^{-1})
		$mBAC_S$	0.26
		$mBAC_R$	**2.46**
Lolium perenne	LP	DTPA	0.16 (mg kg^{-1})
		$mBAC_S$	0.49
		$mBAC_R$	0.69
Rumex sp.	RS	DTPA	0.13 (mg kg^{-1})
		$mBAC_S$	0.24
		$mBAC_R$	**1.59**
Erigeron sumatrensis	ES	DTPA	1.36 (mg kg^{-1})
		$mBAC_S$	0.98
		$mBAC_R$	0.10
Hordeum leporinum	HL	DTPA	0.00 (mg kg^{-1})
		$mBAC_S$	ND
		$mBAC_R$	ND
Piptatherum thomasii	PT	DTPA	0.16 (mg kg^{-1})
		$mBAC_S$	0.32
		$mBAC_R$	**1.33**
Amaranthus retroflexus	AR	DTPA	5.44 (mg kg^{-1})
		$mBAC_S$	0.49
		$mBAC_R$	0.01
Echium vulgare	EV	DTPA	0.13 (mg kg^{-1})
		$mBAC_S$	0.15
		$mBAC_R$	**2.18**
Mercurialis annua	MA	DTPA	0.174 (mg kg^{-1})
		$mBAC_S$	0.17
		$mBAC_R$	0.58
Aster tripolium	AT	DTPA	0.336 (mg kg^{-1})
		$mBAC_S$	0.21
		$mBAC_R$	0.92
Cyperus rotundus	CR	DTPA	0.236 (mg kg^{-1})
		$mBAC_S$	**1.14**
		$mBAC_R$	0.55

According to the values listed in Tab. 17, CR showed the ability to transfer the potential bioavailable fraction of Cd to the aerial part, so it could be considered interesting candidate

for phytoextraction, even if not favoured by the low biomass produced. On the other hand, CA, CD, RS, PT and EV reported the ability to transfer Cd to the roots with a high $mBAC_R$ so they could be suitable for Cd phytostabilization. As for site A, also for site B $mBAC_S$ values were lower than BAC_S values described before. On the other hand, $mBAC_R$ values were higher than one in according with BAC_R values described before, so *C. arvense* can be effective used for phytostabilization purpose.

3.3 PTEs extractability

Concentrations determined by chemical analysis by using extractant agents and single extractions are more suitable than total element concentration to assess element transfer from soils to plants, animals, or water (Menzies et al. 2007). The extractable As, Cd, Cr, Cu, Pb, Sb, Tl and Zn in soils, calculated as the percentage of extracted concentration (DTPA and NH_4NO_3) with compared to the total present in the soil below each plant species in site A, are shown in Fig. 7 and Fig. 8.

Fig. 7. Extractable Tl, Cr, As, Zn, Sb, Pb and Cu in soils using DTPA, calculated as extracted concentration/total concentration ratio, in percentage, for the different plant species growing in the site A
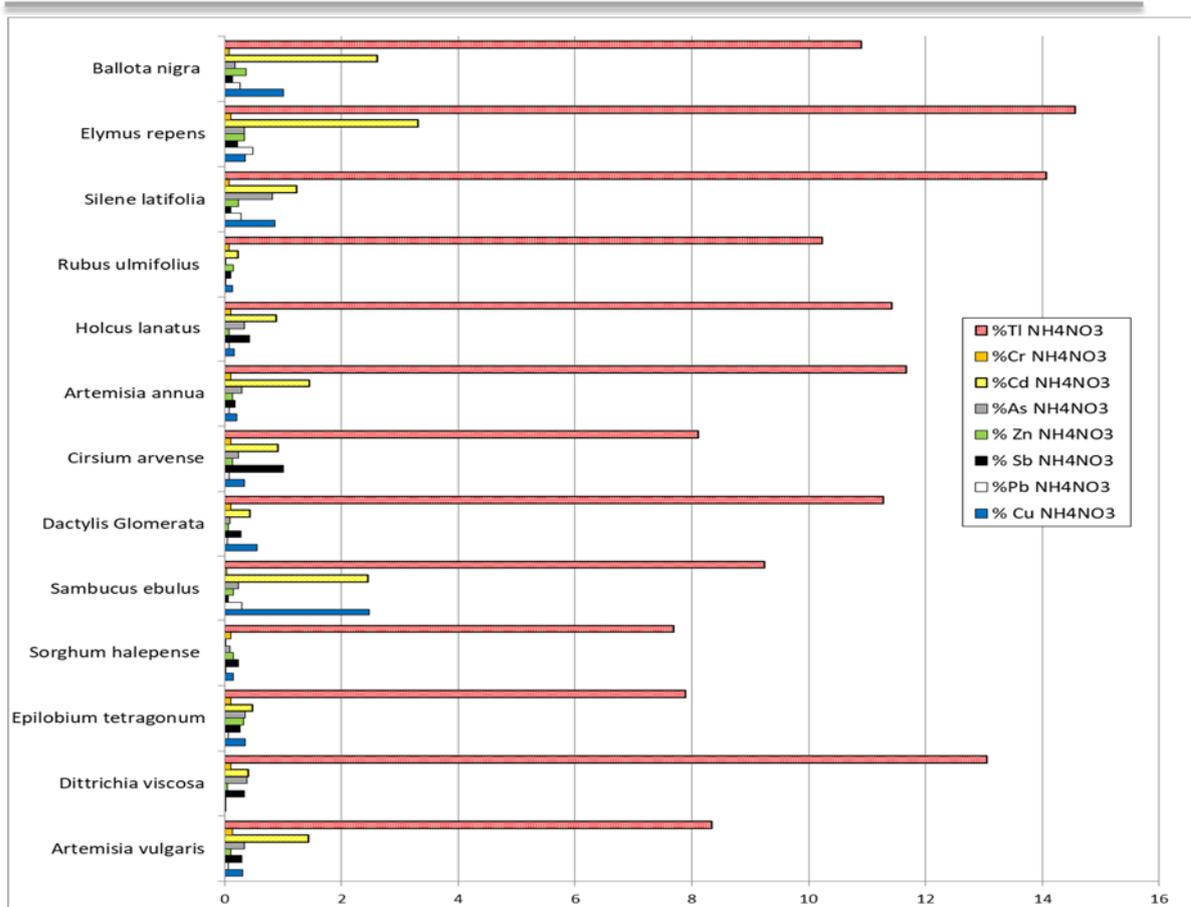
Fig. 8. Extractable Tl, Cr, As, Zn, Sb, Pb and Cu in soils using ammonium nitrate, calculated as extracted concentration/total concentration ratio, in percentage, for the different plant species growing in the site A

The extractable Cd, Cr, Cu, Pb, and Zn in soils, calculated as the percentage of extracted concentration (DTPA and NH_4NO_3) with compared to the total present in the soil below each plant species in site B, are shown in Fig. 9 and Fig. 10.
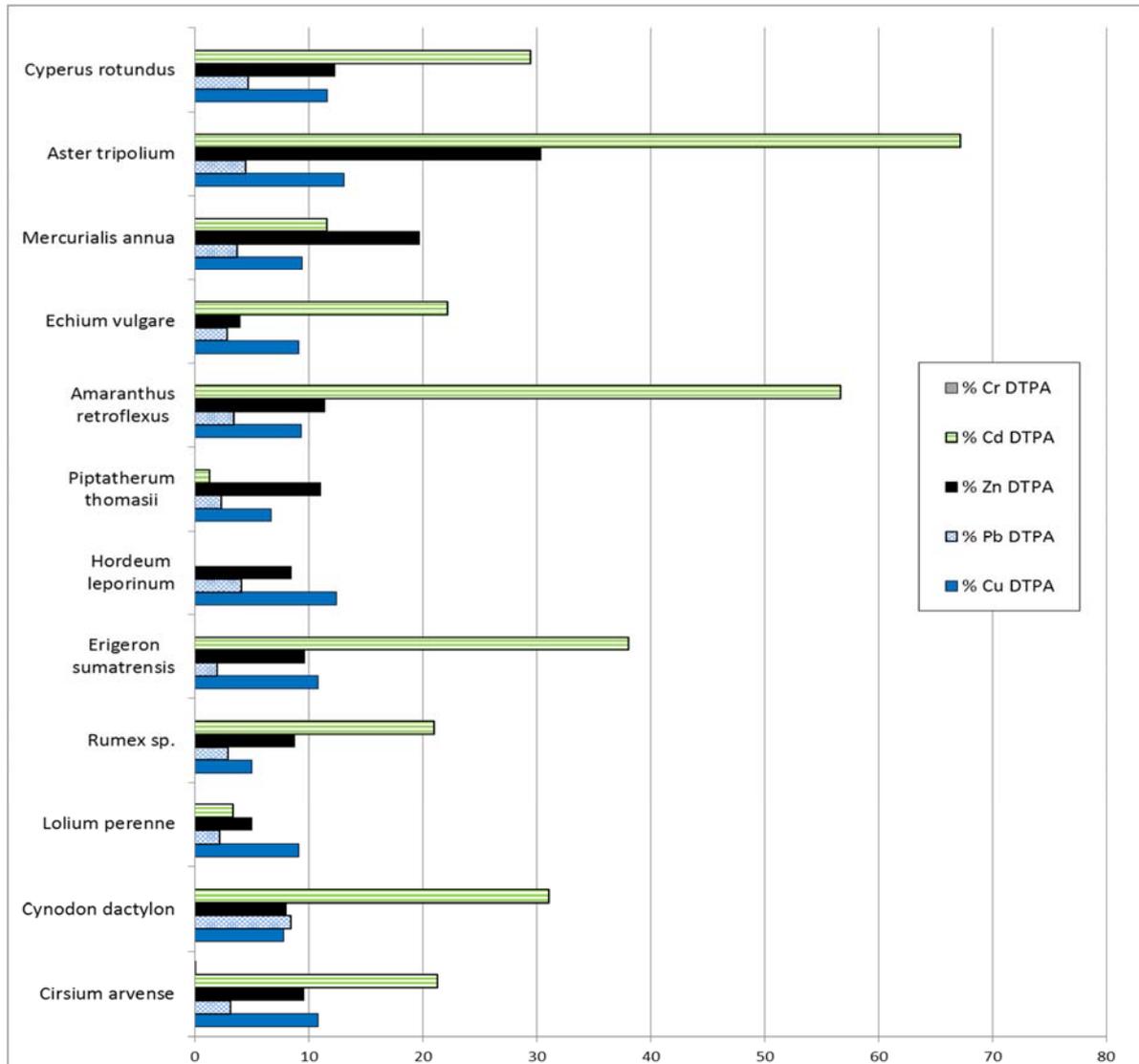
Fig. 9. Extractable Cr, Zn, Pb, Cd and Cu in soils using DTPA, calculated as extracted concentration/total concentration ratio, in percentage, for the different plant species growing in the site B
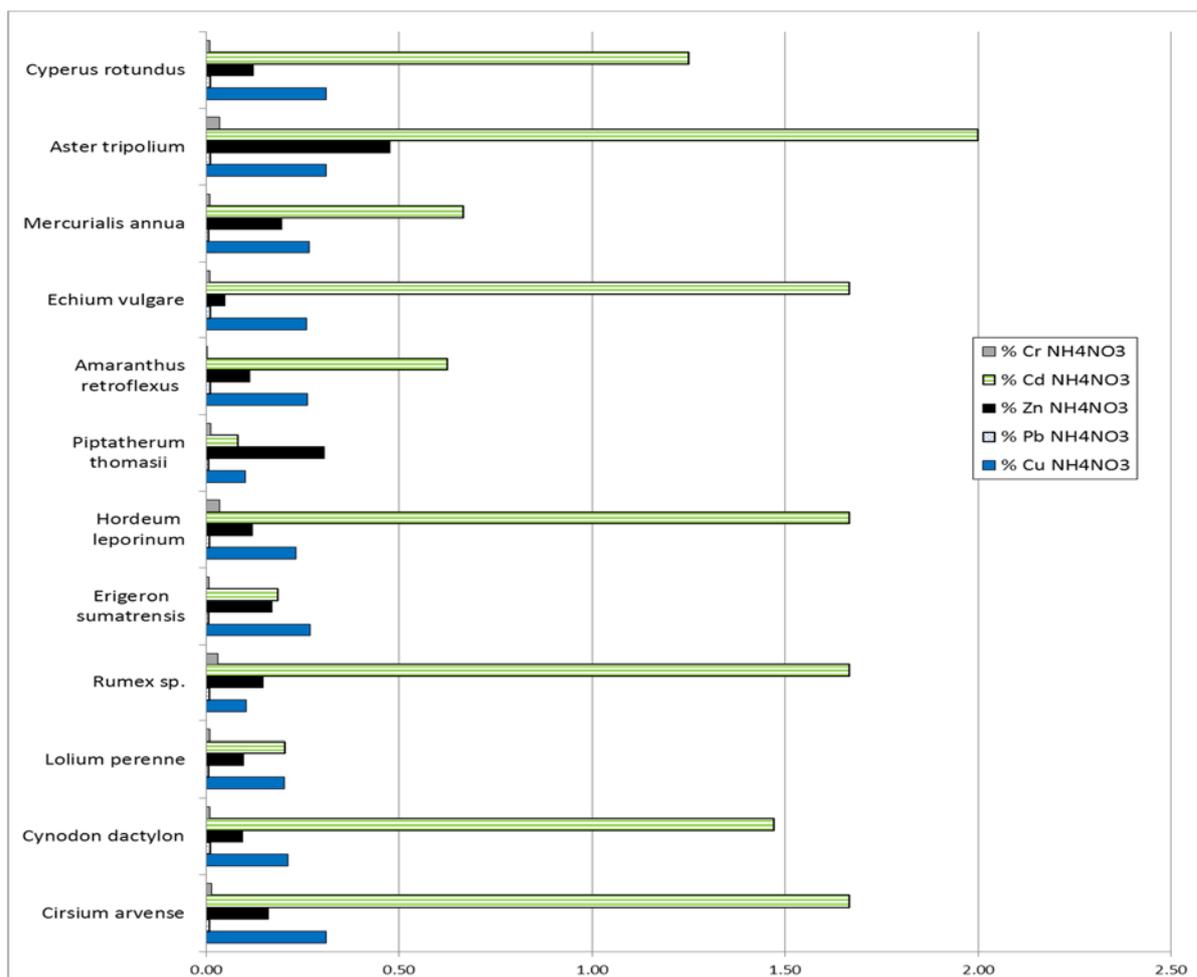
Fig. 10. Extractable Tl, Cr, As, Zn, Sb, Pb and Cu in soils using ammonium nitrate, calculated as extracted concentration/total concentration ratio, in percentage, for the rhizo-soils corresponding to different plant species growing in the site B

In general, the results showed PTE extraction percentages with ammonium nitrate lower than 10% (except for Tl and Cd) in site A and lower than 2% in site B. In both sites, very high PTE extraction was obtained with DTPA for Cd, with values above 70% of total content, showing the higher environmental risk represented by Cd, despite its lower total concentration in soils, in agreement with Moreno-Jiménez et al. (2009). In site A, the lowest percentage of extractable elements was found for Sb, As, Cr and Tl using DTPA while in site B the lowest values were reported for Pb and Cr. On the other hand, in site A, high percentage of thallium was extracted with ammonium nitrate (max: 14%, min: 7%, mean: 10%) while other PTEs showed a low extractability with lowest values for As and Cr. In site B, low extractability was found for Cr, Zn, Pb and Cu while the highest values were

reported for Cd (max: 2%, min: 0.8%, mean: 1.10%). According to Rao et al. (2008), diluted salt solutions such as NH_4NO_3 can only extract elements from the water- soluble and exchangeable phases. The DTPA extractant additionally attacks organically-bound elements. For this reason DTPA extract percentage was higher than ammonium nitrate in both sites except for DTPA-extractable thallium in site A that was very low compared with ammonium nitrate. This result is in according with Zbiral et al. (2002) and Rao et al. (2008). The last author reported the highest thallium extractability with ammonium salt and the lowest with chelating reagent DTPA in a thallium-contaminated soil. The explanation of the higher extraction percentage in ammonium nitrate can be related to the ionic radius (1.49 Å) of thallium (Tl^+), which is similar to the radius of ammonium (NH_4^+, 1.43 Å) and potassium (K^+, 1.33 Å) ions (Rao et al. 2008). Thus, certain quantities of NH_4^+ or K^+ in soils potentially compete with thallium ions at the exchangeable and/or specific adsorption sites in soil minerals and organic colloids (Lee et al. 2015). The percentage of extracted concentration for Cr, As and Sb resulted very low in DTPA and ammonium nitrate and similar to values reported by Pinto et al. (2015) while for Pb, Cd and Sb they resulted higher in our soils. Moreover, Cr has been reported to be strongly retained by soil components (McLaughlin et al. 2000), which was evident in our study due to the low solubility of this metal and the small amount extracted by DTPA.

Total PTEs concentration, pH and organic matter are soils parameters that generally controlled the PTEs availability (Adriano 2001). In our study, considering that pH and organic matter values were similar, a correlation analysis between extracted and total PTEs concentrations in soils was performed.

Correlation coefficients for total and extractable concentrations of Cu, Pb, Sb, Zn, As, Cd and Tl in site A, showed considerable variation between total and extractable concentrations (Tab. 18). No significant correlations were reported for total and extractable concentrations of PTEs for site B (data not reported).

Tab. 18. Pearson correlation (r) between extractable levels and total soil concentration of copper, lead, antimony, zinc, arsenic, cadmium and thallium for site A

PTEs	DTPA	Ammonium nitrate
Copper	-0.29	0.16
Lead	0.65[**]	0.59[**]
Antimony	0.85[**]	0.92[**]
Zinc	-0.08	0.53[*]
Arsenic	ND	0.15
Cadmium	0.46	0.60[**]
Thallium	-0.33	0.96[**]

*, **Indicate significant relationship at probability level, $P<0.05$, $P<0.01$, respectively (n= 18).

Soil extractable concentrations for site A were well correlated with total soil concentrations for lead and antimony for both the extractants. There were significant correlations ($P<0.01$) between total and extractable concentrations for cadmium (r=0.60) and thallium (r=0.96) with ammonium nitrate. A significant correlation was observed between total and extractable concentrations in soils ($P<0.05$) for zinc in ammonium nitrate. In our study, correlation between total and extractable metal concentrations by different extraction procedures (phytoavailable metals) was element specific. The strong correlation between total soil and extractable concentrations for thallium obtained with ammonium salts reflects the fact that monovalent thallium and ammonium ions are of similar ionic sizes; hence ammonium ions displace sorbed thallium ions (Lee et al. 2015). Although total soil concentrations of metals are not generally considered a predictor for metal phytoavailability (Tack and Verloo 1995; Peijnenburg et al. 1997; Song et al. 2004), significant positive correlation between total and extractable PTEs concentrations in soils was reported. This result is in agreement with Abedin et al. (2012) for ammonium nitrate extractant and with Soriano-Disla et al. (2010) for DTPA extractant showing that total soil concentration is a possible phytoavailability indicator for some polluted soils and PTEs.

In order to confirm previous correlations and to better study element phytoavailability, total and extracted metal concentrations (as obtained by the different extraction methods) were correlated with metal concentrations in shoots and roots for both sites (Tab. 19 and Tab. 20).

Tab. 19. Linear correlations (r value) between the total PTE in soil (TPTEs), DTPA-extractable PTEs and ammonium nitrate extractable PTEs and concentrations of PTEs in shoots/roots for site A

	TPTEs		DTPA		Ammonium nitrate	
	shoots	roots	shoots	roots	shoots	roots
Copper	0.40	-0.45	-0.13	0.24	0.28	-0.26
Lead	0,51*	0.05	0,79**	-0.20	0,74**	-0.38
Antimony	0,59**	-0.26	0,70**	-0.23	0,80**	-0.32
Zinc	-0.02	-0.23	0.13	-0.09	-0.03	-0.44
Arsenic	0.26	0.21	---	---	0,50	-0.54
Cadmium	0.35	0.11	0.45	-0.26	0,59**	-0.32
Thallium	0,86**	-0.42	0.21	-0.16	0,82**	-0.50

*, ** Indicate significant relationship at probability level, $P<0.05$, $P<0.01$, respectively (n= 18).

Tab. 20. Linear correlations (r value) between the total heavy metals in soil (TPTEs), DTPA-extractable PTEs and ammonium nitrate extractable PTEs and PTEs concentrations in shoots/roots for site B

	TPTEs		DTPA		Ammonium nitrate	
	shoots	roots	shoots	roots	shoots	roots
Copper	0.46	0.13	-0.26	0.14	0.14	-0.46
Lead	-0.05	0.27	-0,04	0.15	ND	ND
Zinc	0.44*	0.13	0.37	0.17	0.07	0.07
Cadmium	0.05	-0.18	0.54**	-0.26	0,85**	-0.43

*, ** Indicate significant relationship at probability level, $P<0.05$, $P<0.01$, respectively (n= 22).

For both sites, additional comparisons were done after eliminating the outliers (rhizo-soil samples with corresponding plants with levels of PTEs higher than 2 times soil average content).

In particular, in site A for Pb, Sb, Cd and Tl (Tab. 21) they were eliminated: 3 samples for Pb, 1 for Sb, 4 4 for Cd and 2 4 for Tl (roots concentration for one of the soils corresponding to Plot7 – *Silene latifolia* was not detectable).

In site B for Zn and Cd (Tab. 22), they were eliminated 3 soils for Zn and 3 for Cd.

Tab. 21. Linear correlations (r value) between the total heavy metals in soil (TPTEs), DTPA-extractable PTEs and ammonium nitrate extractable PTEs and PTEs concentrations in shoots/roots in site A when the soils containing high levels of Pb, Sb, Cd and Tl were not considered

	TPTEs		DTPA		Ammonium nitrate	
	shoots	roots	shoots	roots	shoots	roots
Lead	0,62*	-0.08	0,81**	-0.24	0,74**	-0.46
Antimony	0,55*	-0.33	0,70**	-0.31	0,80**	-0.40
Cadmium	0.43	0.03	0.32	-0.35	0,50	-0.46
Thallium	0,82**	0.90	0.07	0.15	0,76**	0.91

*, ** Indicate significant relationship at probability level, $P<0.05$, $P<0.01$, respectively (n= 18).

Tab. 22. Linear correlations (r value) between the total PTEs in soil (TPTEs), DTPA-extractable PTEs and ammonium nitrate extractable PTEs and PTEs concentrations in shoots/roots in site B when the soils containing high levels of Zn and Cd were not considered

	TPTEs		DTPA		Ammonium nitrate	
	shoots	roots	shoots	roots	shoots	roots
Zinc	0.36	0.10	0.33	-0.35	-0,006	0.11
Cadmium	-0,44	-0.27	-0.25	0.12	0,30	-0.56

*, ** Indicate significant relationship at probability level, $P<0.05$, $P<0.01$, respectively (n= 18).

The best results were found for the correlations of PTE concentrations in shoots compared with roots. In site A, the TPTEs in soil was satisfactory correlated with Sb, Tl and Pb shoot concentrations as correlation coefficients were higher that in the correlation made considering all the samples. TPTEs also correlate with the Tl root concentration when the soils with high levels Tl were removed. In this case it must be considered that soil removed corresponded to a probably hyperaccumulator plant as described before on and, as reported by Van der Ent et al. (2013), hyperaccumulator plants don't have a linear correlation between soils and plant tissues, so concentration values for this plant can influenced the r value for Tl. In site B TPTEs correlated only the Zn shoot concentration, but this method could only be used for distinguishing between low and high values of Zn in shoots as the significance of correlations for Zn decreased when the soils with high PTEs concentrations were removed. In the same site, DTPA and ammonium nitrate correlated only with Cd shoot concentrations but also in this case, these methods can only be used for distinguishing between low and high values of Cd in shoots as the significance of correlations for Cd decreased when the soils with high PTEs concentrations were removed. In site A, the Pb and Sb shoot concentrations were satisfactorily correlated with DTPA extracts. In the same site,

the ammonium nitrate was satisfactorily correlated with the shoot content of Pb, Sb, and Tl but this method could only be used for distinguishing between low and high values of Cd in shoots as the significance of correlations for Cd decreased when the soils with high PTEs concentrations were removed. Ammonium nitrate was also correlated with the Tl root concentration in site A when the soils with high levels Tl were removed, probably for the same reasons explained for the TPTEs. Best results of Tl with TPTEs and DTPA were in agreement with some authors that described better results for the assessment of PTEs accumulation by the roots instead of the shoots for the direct contact of roots with the soil solution and the transfer of heavy metals to shoots is controlled by plant physiology (Soriano-Disla et al. 2010). DTPA extraction reported lower results probably related to the method which was originally developed for quantifying deficiencies of essential metals (Fe, Zn, Mn, and Cu) in near neutral and calcareous soils (Lindsay and Norvell 1978). In particular, Pb plant concentrations were successfully estimated by DTPA like reported by different authors (Hooda et al. 1997; Brun et al. 2001; Meers et al. 2007). Similar results were reported by ammonium nitrate extraction and, in minor part by TPTEs in soil, indicating that Pb phytoavailability is better predicted by the extractable fraction than by the total metal concentrations, in agreement with Menzies et al. (2007) that reported good results for the bioavailability of Cd using DTPA, although he also reported that the method did not perform well when used across a wide range of soil types. However, considerable amounts of PTE were released in the DTPA method and were not related to plant availability. These results are in agreement with reports from Feng et al. (2005) and Menzies et al. (2007) indicating that DTPA is generally a poor estimator of these metal concentrations. However, as expected, phytoavailability of PTEs depends on the plant species and not only on the element mobility in soils (Garcia-Salgado et al. 2012). Among all the species, the *Elymus repens* was the more frequent (present in 3 plots) in site A while *C. dactylon* (present in 5 plots), *L. perenne* (present in 3 plots) and *E. sumatrensis* (present in 3 plots) where the plant species more frequent in site B.

From the correlation analysis for *E. repens* in site A there was a good correlation between the Zn content in aerial plant part and total soil content (r = 0.99, p < 0.01). In site B only *L. perenne* reported a good correlation between the As content in roots and total soil content (r = 0.99, p < 0.05) and between the Cd content in roots and total soil content (r = 0.99, p < 0.05). Similar positive correlations were also reported in *Cynodon dactylon* by Madejon et al. (2002) in samples of soil (EDTA values) and C. *dactylon* (r = 0.63 and r = 0.84) by Albornoz et al. (2016) that reported a positive correlation between total PTEs con-

tent, roots = 0.94 and leaves r = 0.91. As reported for *Cynodon dactylon*, also *Elymus repens* for Zn and *L. perenne* for Cd and As have higher tolerance and accumulation when the PTEs concentrations in soils were increased (Shu et al. 2002; Leung 2013). These significant correlation well underline the potential of *Elymus repens* and *L. perenne* for biomonitoring of PTEs pollution in contaminated sites. The use of biomonitors living and growing in a given area can give important information on the presence of a stressor like pollutants, but it can also give information on the impact that the stress factors could have on the environment (Wang et al., 1997; Chang et al., 2009; Ngayila et al., 2009). The use of biomonitors has many advantages: reduces the need to make assumptions regarding bio-availability of a substance (Chaphekar, 1991); could help to identify the origin of stressors (anthropogenic vs natural. Mhatre, 1991) by allowing for the separation of covarying parameters (Sprenger and McIntosh, 1989; Outridge and Noller, 1991) and can also provide historical information regarding past environmental conditions (Bargagli, 1998; Franzle, 2006). According to different authors (Raskin and Ensley 2000; Clemens 2006 and Verbruggen et al. 2009), PTEs uptake by plants can be explained by competitive interactions between different PTEs due to a similarity in their chemistry. This is in accordance with the significant correlation between Cu and Zn concentrations in the shoots that we observed in site A for *Elymus repens* (r= 0.998, p < 0.05) and in site B for *L. perenne* (r= 0.999, p < 0.05). These interactions are commonly observed because they are apparently absorbed by the same mechanism and therefore each may competitively inhibit root absorption of the other (Kabate-Pendias, 2013).

3.4   Relation between physiochemical characteristics and identification of the source of PTEs of the rhizospheric soils

In order to identify the relationships between different metals and its corresponding origins, principal component analysis (PCA) and cluster analysis (CA) have been conducted on rhizo-soils for site A. PCA and CA could provide some indications for association of PTE and give some information about the origins of contamination like proved by many previous studies in which it was reported that good associations of different metals indicated similar sources of pollution (Ogwueleka 2014).

The results of the PCA for site A are reported in Tab. 23. According to the results of the initial eigenvalues, two principal components are considered, which accounted for 77.83% of the total variance. As shown in Tab. 23, all the elements are consequently well represented by these two principal components. Pb, As and Cd showed the higher values in the first component followed by Cu and Zn, whereas Tl and Sb are greater in the second component. Both before and after the rotation.

Tab. 23. Total variance explained and component matrixes (two factors selected) for site A

Total Variance Explained

Com-ponent	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative (%)	Total	% of Variance	Cumulative (%)	Total	% of Variance	Cumulative (%)
1	4.87	60.84	60.84	4.87	60.84	60.84	4.27	53.34	53.34
2	1.36	16.99	77.83	1.36	16.99	77.83	1.96	24.49	77.83
3	0.89	11.19	89.01						
4	0.51	6.41	95.42						
5	0.22	2.69	98.12						
6	0.10	1.19	99.31						
7	0.05	0.66	99.97						
8	0.00	0.03	100.00						

Element	Component Matrix			Rotated Component Matrix	
	Component			Component	
	1	2		1	2
Cu	0.79	0.01		0.71	0.33
Pb	0.94	-0.11		0.91	0.29
Sb	0.47	0.78		0.10	0.90
Zn	0.66	0.02		0.59	0.29
As	0.84	-0.29		0.89	0.09
Cd	0.94	-0.13		0.91	0.27
Tl	0.62	0.70		0.27	0.89

Extraction method: Principal Component Analysis. Rotation method: Varimax with Kaiser normalisation. Rotation converged in three iterations.

The first component are showing a contamination origin while the second component another origin. In summary, it is considered that a PCA analysis using two factors is suitable for examining the data set as showed in Fig. 11.
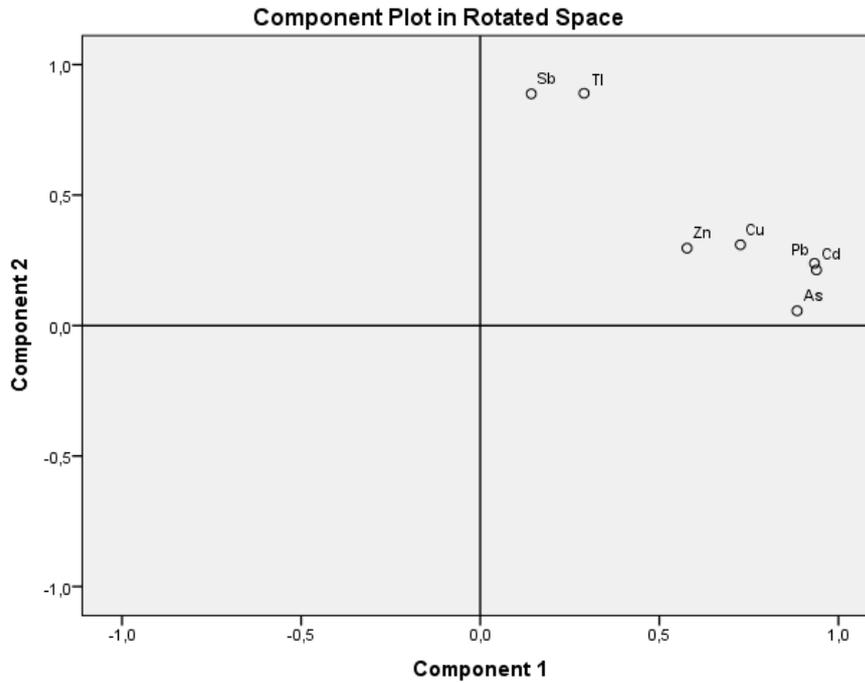
Fig. 11. Principal Components Analysis loading plots (initial eigenvalues) for site A. Two components are extracted.

To confirm results, HCA was performed on chemical parameters using Pearson coefficient as distance and between groups by linkage methods. Unlike PCA that normally uses only two or three PCs for display purposes, cluster analysis uses all the variance or information contained in the original data set (Vega et al. 1998). The results are shown in Fig. 12.

Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	2	7	,993	0	0	2
2	2	6	,857	1	0	3
3	2	5	,815	2	0	6
4	3	8	,706	0	0	7
5	1	4	,651	0	0	6
6	1	2	,538	5	3	7
7	1	3	,337	6	4	0
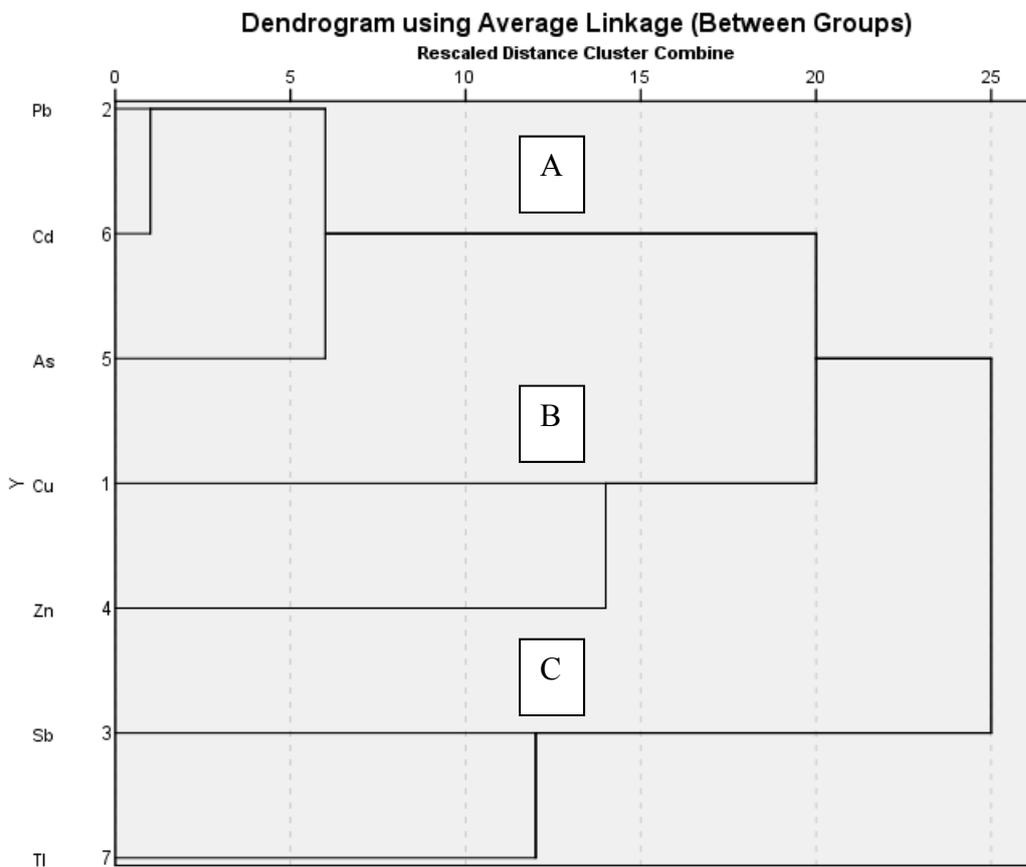
Fig. 12. Agglomeration schedule of the Cluster Analysis, based on the correlation coefficients for site A.

The distance axis represents the degree of association between groups of variables, i.e. the lower the value on the axis, the more significant the association. Pb and Cd are very well correlated with each other and form another cluster with As. Cu and Zn are correlated each other and at a later stage are associated with Pb, Cd and Zn. Sb and Tl are only correlated each other forming another group. The results did not confirm the attribution of the PTEs in the two factors as defined with the PCA. In fact factors of the first component divided into two clusters with a total of three clusters like showed in Fig. 12. Probably this is caused by an origin of factor B by different sources. (Facchinelli et al. 2000). The factor A

probably derived from lead-battery disposal in the soil. Pb is used for the plates of car batteries while As and Cd are often used like doping agents in batteries. The Factor B can derive from disposal of car parts, electric apparatus or other metal wastes; by aerial deposition from metal recycling plants or other factories in the area. In fact the principal uses of Cu is in the production of electrical wires and other electrical apparatus but is also used in containers such as boilers, steam pipes, automobile radiators, and kitchenware. Zn is used as a protective coating for iron and steel but is also used like Cu in automobile industry, electrical apparatus (Adriano, 2001). The Factor C has geogenical origins, perhaps in some plots Sb showed high values related to high values of Pb. This can be explained by the presence of Sb as an alloy with lead in plates of lead–acid batteries.

The results of the PCA for site B are reported in Tab. 24. According to the results of the initial eigenvalues, two principal components are considered, which accounted for 81.61% of the total variance. PTEs in the soil are well represented by these two principal components. Pb, As and Zn formed a group with high values in the first component; Cr and Cd formed a second group on the second component. Both before and after the rotation.

Tab. 24. Total variance explained and component matrixes (two factors selected) for site B

Total Variance Explained

Compo-nent	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumula-tive (%)	Total	% of Variance	Cumula-tive (%)	Total	% of Variance	Cumula-tive (%)
1	2,199	43,981	43,981	2,199	43,981	43,981	2,127	42,539	42,539
2	1,882	37,631	81,612	1,882	37,631	81,612	1,954	39,073	81,612
3	,507	10,146	91,758						
4	,336	6,713	98,471						
5	,076	1,529	100,000						

Element	Component Matrix		Rotated Component Matrix	
	Component		Component	
	1	2	1	2
Pb	0,699	-0,534	0,869	-0,136
Zn	0,910	0,185	0,712	0,596
As	0,768	-0,457	0,893	-0,035
Cd	0,493	0,709	0,096	0,858
Cr	0,223	0,923	-0,244	0,917

Extraction method: Principal Component Analysis. Rotation method: Varimax with Kaiser normalisation. Rotation converged in three iterations.

The second component are showing a contamination origin while the first component another origin. In this site, it is considered that a PCA analysis using two factors is suitable for examining the data set as showed in Fig. 13.
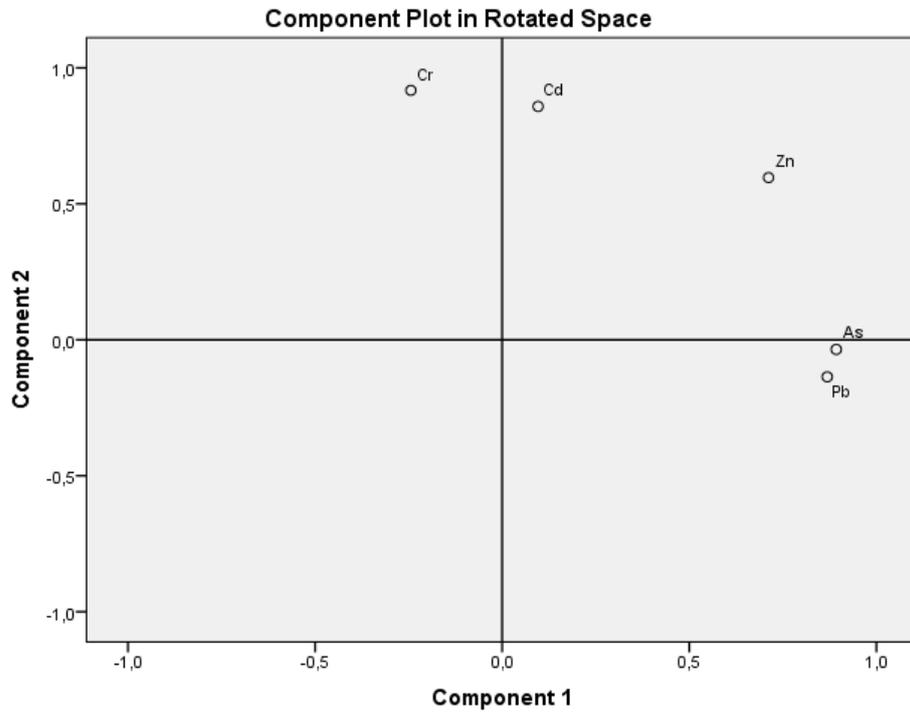
Fig. 13. Principal Components Analysis loading plots (initial eigenvalues) for site B. Two components are extracted.

To confirm these results, as for site A, HCA was performed on chemical parameters using Pearson coefficient as distance and between groups by linkage methods. The results are shown in Fig. 14.

Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	4	5	0,629	0	0	4
2	1	3	0,595	0	0	3
3	1	2	0,536	2	0	4
4	1	4	0,057	3	1	0
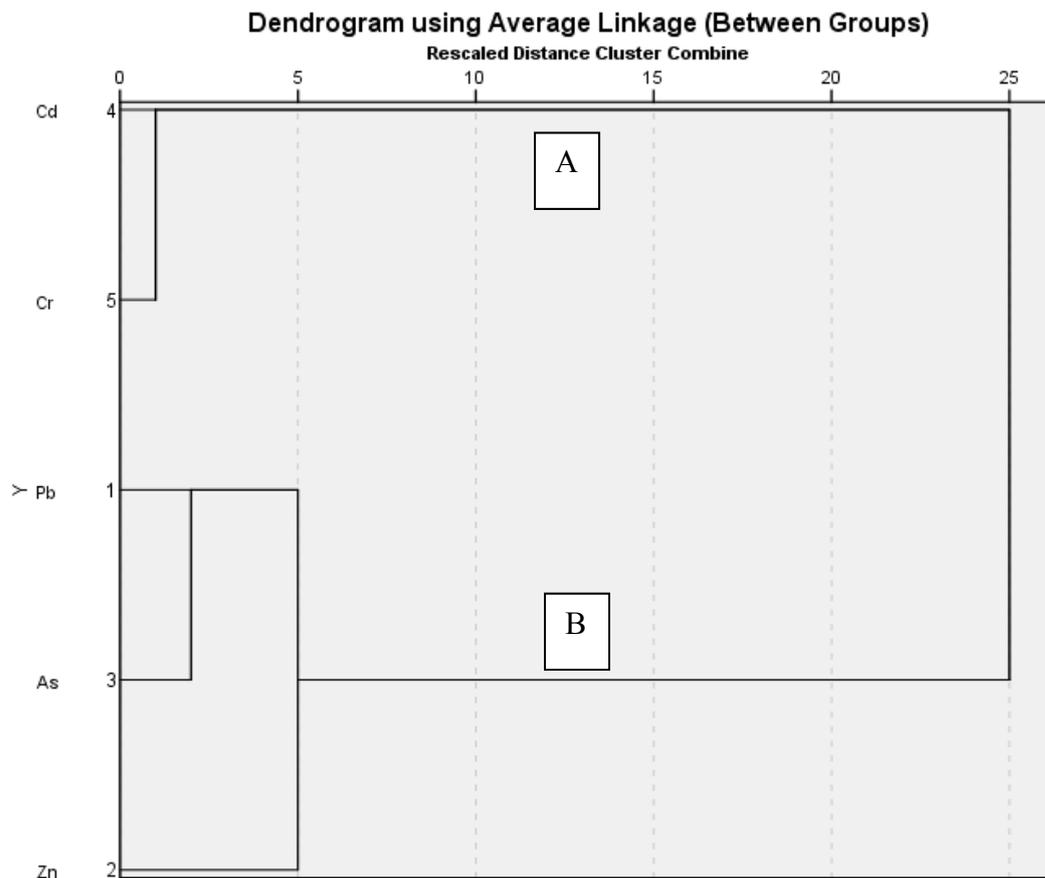
Fig. 14. Agglomeration schedule of the Cluster Analysis, based on the correlation coefficients for site B.

Pb and As are very well correlated with each other and at a later stage associated with Zn forming a cluster with this PTE. Cr and Cd form another group. The results confirmed the attribution of the PTEs in the two factors as defined with the PCA. The factor A has anthropogenic origins. In fact Cr and Cd are used in tannery industry for tanning hides and tannery effluent are rich oh this PTEs (Mwinyihija 2010). Factor B derived mostly from geogenical origins but also probably from pesticides fertilizers and amendments applications (Haar, Bayard 1971, Kim, Fergusson 1994, Das et al. 1997).

As was shown in Tab. 25 for site A, all rhizo soils presented a high o very high ecological risk. Only the RI values of *A. vulgaris*, *D. viscosa* and *E tetragonum* presented high ecological risk (RI from 100 to 200), while other soils reported a RI higher than 200 implying a very high ecological risk. By comparison of RI values between different rhizo soils, *R. ulmifolius* displayed the highest RI value (11348.26) while A. *vulgaris* showed the lowest RI value (54.65).

Tab. 25. Mean values of ecological risk factor (E_r^i) and potential ecological risk index (RI) for different PTEs in different rhizo soils of site A

	E_r^i						RI
	Cu	Pb	Zn	As	Cd	Cr	
Artemisia vulgaris	3.4	12	1.34	6.7	22	9.2	55
Dittrichia viscosa	11.4	35	3.46	5.8	79	13.0	147
Epilobium tetragonum	8.2	32	2.60	6.2	66	12.2	127
Sorghum halepense	14.1	1162	2.95	24.0	2580	11.7	3795
Sambucus ebulus	10.0	180	3.07	9.6	314	46.6	563
Dactylis Glomerata	7.9	670	1.82	24.2	1603	11.7	2319
Cirsium arvense	29.2	265	3.10	9.8	692	11.7	1010
Artemisia annua	5.4	81	1.64	7.6	155	12.7	263
Holcus lanatus	8.1	97	2.30	6.7	183	11.5	309
Rubus ulmifolius	25.0	3956	4.86	212	7134	15.5	11348
Silene latifolia	23.5	2821	4.83	70.7	5545	16.7	8482
Elymus repens	24.3	914	3.08	31.1	1881	13.0	2866
Ballota nigra	9.5	1201	2.03	38.2	1737	16.2	3004

The risk degree of Cu, Zn and Cr was low for all rhizo soils except for Cr in *S. ebulus* that presented the higher tolerance to this PTE. Cd exhibited a contribution to RI values higher than other PTEs and species more tolerant to this PTE were *R. ulmifolius* and *S. latifolia* with an ecological risk for this PTE very high. The same tolerance was shown by the same species for Pb (with a very high potential ecological risk for both the species for this PTE) and As (respectively high potential ecological risk and considerable ecological risk). *R. ulmifolius* presented the greatest tolerance to multi PTEs contamination according to Marques et al., 2011 that reported the same tolerance in a site contaminated by high levels of Pb, As and Ni.

Assessment Index for site B was shown in Tab. 26. Also in site B, all rhizo soils presented a high o very high ecological risk. Only the RI values of *Rumex sp.*, *H. Leporinum* and *A. tripolium* presented high ecological risk (RI from 100 to 200), while other soils reported a RI higher than 200 implying a very high ecological risk. The higher RI was reported by *P. thomasii* (1931.31), the lowest by *A.tripolium*.

Tab. 26. Mean values of ecological risk factor (E_r^i) and potential ecological risk index (RI) for different PTEs in different rhizo soils of site B

	E_r^i						RI
	Cu	Pb	Zn	As	Cd	Cr	
Cirsium arvense	2.43	6.03	2.96	10.2	90	89	200
Cynodon dactylon	3.37	5.08	4.30	9.9	102	131	255
Lolium perenne	3.05	8.65	7.29	14.2	735	118	886
Rumex sp.	4.54	6.19	2.40	12.4	90	39	155
Erigeron sumatrensis	2.85	7.72	4.44	7.6	535	168	726
Hordeum leporinum	4.26	5.66	2.28	11.1	90	35	148
Piptatherum thomasii	3.69	8.89	3.88	11.1	1800	103	1931
Amaranthus retroflexus	3.35	4.76	7.69	10.2	1440	353	1819
Echium vulgare	2.68	4.71	3.37	8.4	90	123	232
Mercurialis annua	3.29	8.01	4.46	10.2	225	127	378
Aster tripolium	2.27	4.63	1.93	10.2	75	34	128
Cyperus rotundus	3.17	4.73	4.04	10.2	120	126	268

The risk degree of Cu, Pb, Zn and As was low for all rhizo soils. Also in this site, Cd exhibited the highest contribution to RI values than other PTEs and species more tolerant to this PTE were *P. thomasii* and *A. retroflexus*. The ecological risk for this PTE was very high for all the rhizo soils, except for *C. arvense*, *C. dactylon*, *Rumex sp.*, *H. leporinum*, *E. vulgare*, *A. tripolium* and *C. rotundus* that reported moderate or high risk. For Cr that showed a high concentration in soil, actually, showed a very high ecological risk only in rhizo soils of *A. retroflexus* that also was the more tolerant to this PTE together with *E. sumatrensis*. In the other rhizo soils the risk degree for Cr was moderate or high.

3.5 Conclusion

Among the plant species screened in site A, *S. latifolia* was identified as a hyperaccumulator of Tl. In both sites, majority of PTEs concentrations in natural plants exceeded the upper limits of the normal range of terrestrial plants grown in uncontaminated soils, demonstrating that the plants accumulate higher PTEs levels when grown in contaminated soils. Many plant species in site A were most effective in translocating Cu, Pb, Sb, Zn, Cd and Tl with TF higher than 1, but the concentration in the plants was too lower to consider that plant for phytoremediation purpose except as regards Cd for *A. annua* that showed BAC$_S$, BAC$_R$ and TF higher than 1 with a high concentration of Cd in the aerial part and for Tl for *S. latifolia* that reported high concentration in both shoot and roots at a hyperaccumulator levels and also BAC$_S$, BAC$_R$ and TF higher than 1 confirmed the hyperaccumulator hypothesis of this plant species.

In site B, BAC$_S$, and BAC$_R$ were lower than 1 for Cu, Pb, Zn, Cr and As for all the species except for *C. arvense* that showed a BAC$_R$ higher than 1 for Cd. TF was higher than 1 for many plant species but the concentration in the plants was too lower to consider those plants for phytoremediation purpose.

Considering modified BAC for shoot and roots calculated by using the bioavailable metal concentrations (DTPA) from the rhizo-soils that can represent the metals potentially bioavailable, *Artemisia vulgaris* and *Dittrichia viscosa* in site A showed the ability to transfer the potential bioavailable fraction of Pb to the aerial part and thus they could be considered interesting candidates for phytoextraction while *Epilobium tetragonum*, *Artemisia Annua* and *Silene latifolia* reported a high mBAC$_R$ so they could be suitable for Pb phytostabilization. In site B *Cyperus rotundus* showed the ability to transfer the potential bioavailable fraction of Cd to the aerial part, so it could be interesting for phytoextraction purpose while *Cirsium arvense*, *Cynodon dactylon*, *Rumex sp.*, *Piptatherum thomasii* and *Echium vulgare* reported the ability to transfer Cd to the roots with a high mBAC$_R$ so they could be suitable for Cd phytostabilization. For site A, mBAC$_R$ values were higher than one in according with BAC$_R$ values described before, so *C. arvense* can be effective used for phytostabilization purpose.

One of the aims of this study also was to assess the ability of various techniques for determining the PTEs bioavailability for the natural species. In site A, only the Pb and Sb shoot concentrations could be satisfactorily predicted by DTPA extraction. The ammonium nitrate satisfactorily predicted the shoot content of Pb, Sb, and Tl but this method could only

be used for distinguishing between low and high values of Cd in shoots. Based on the results obtained in site A, the TPTEs in soil were satisfactorily related to Sb and Tl shoot concentrations and in minor part to Pb.

All the methods used in this study failed to find a relationship with the Cu, Zn and As concentrations in the plants.

In site B TPTEs could predict only the Zn shoot concentration, but this method could only be used for distinguishing between low and high values of Zn in shoots while DTPA and ammonium nitrate predicted only Cd shoot concentrations but also in this case, these methods can only be used for distinguishing between low and high values of Cd in shoots. However, as expected, phytoavailability of PTEs depends on the plant species and not only on the element mobility in soils. Furthermore this study pointed out that *E. repens* in site A presented a linear behaviour between concentrations of Zn in shoots and soil so it can be used as indicators of metal contamination within soil while *L. perenne* in site B reported a good correlation between the As content in roots and total soil content and between the Cd content in roots and total soil content. *E. repens* and *L. perenne* can be applied for biomonitoring programmes aiming at providing the quantitative assessment of environmental quality regarding contaminated soils. Principal component analysis could suggest that Tl and Sb in the industrial site have a geogenic origin while Zn, Cu, Cd, Pb and As a contamination origin. This contamination origin is confirmed in particular for Pb deriving from the vehicles battery dumping in the site. Nevertheless the results of HCA did not confirm the attribution of the PTEs to two factors but to three factors A, B and C (A: Pb, Cd, As; B: Cu and Zn; C: Sb and Tl). The first two factors seems of anthropogenic origins, maybe belonging to two different pollution sources, while the third factor seems mainly of geogenic origins.

In the agricultural site, PCA suggested and HCA confirmed the attribution of the PTEs in the two factors: factor A includes Cr and Cd with anthropogenic origins and Factor B includes Pb, As and Zn mostly from geogenical origins but also probably from other sources such as pesticides, fertilizers and amendment applications.

4. GRASS SPECIES FOR THE SECURING AND THE ASSISTED PHYTOEXTRACTION OF SOILS AND SEDIMENTS POTENTIALLY CONTAMINATED DERIVED FROM SOIL WASHING TREATMENT

4.1 Materials and Methods

4.1.1 Study area

Substrates used in this experiment were collected from a brownfield site named ex-ILVA (40°48.570′N, 14°10.557′E, 2–10 m a.s.l.), included in the Napoli-Bagnoli Coroglio NIPS (national interest priority site) located in Naples (Campania Region – Italy). This industrial site (120 ha) was one of the largest Italian steel production plants since 1905, then abandoned in 1992 and classified as a NIPS by the Italian Parliament in 2000. Soils from this area are characterized by PTEs concentration above Italian screening values for residential soil use (Carlon, 2007), derived from both the industrial and volcanic activity (Adamo et al., 2002; Buondonno et al., 1998; De Vivo and Lima, 2008). In 1994, a remediation project mainly based on excavation and soil-washing techniques funded by the Italian government started in this site (CIPE, 1994).

4.1.2 Experimental setup

A year experiment from November 2015 to November 2016 was performed in 36 pots (V: 0.15 m³ – D: 0.65 m – H:0.51 m) in open air in the experimental facilities of the Department of Agricultural Sciences of Naples University Federico II (Portici, Campania Region, Italy - 40°49′N, 14°21′E) by using the ILVA brownfield soil (S) and the sludge derived from the soil-washing treatment (F). A preliminary soil characterization (Tab. 27) showed: sandy loam texture in S, silt loam in F; a sub-alkaline pH; a low content of carbonates; high levels of organic matter and a medium content of total nitrogen. Both the substrates resulted potentially contaminated by As, Pb and Zn as regards the residential use, with val-

ues in F higher than S. Values of Be and V were lower than the background values of the area (De Vivo and Lima, 2008).

Tab. 27. Initial characteristics (mean ± standard error) of soil (S) and sludge (F)

		Sludge (F)	Soil (S)	CTC (L.D. 152/06)	
				residential site	industrial site
Sand	%	30	65		
Silt	%	62	33		
Clay	%	8	2		
pH		7.6	7.5		
EC	$\mu S\ cm^{-1}$	358.3	322.0		
Carbonates	%	3.7	5.3		
OM	$g\ kg^{-1}$	24.8	19.5		
OC	$g\ kg^{-1}$	14.4	11.3		
TN	$g\ kg^{-1}$	0.9	0.7		
Ca	$g\ kg^{-1}$	40.5	59.4		
P	$g\ kg^{-1}$	1.0	1.7		
Mg	$g\ kg^{-1}$	7.1	9.3		
K	$g\ kg^{-1}$	46.0	43.3		
Cu	$mg\ kg^{-1}$	69 ± 1.9	69 ± 5.4	120	600
Pb	$mg\ kg^{-1}$	**281.2 ± 0.9**	**171 ± 7.6**	100	1000
Zn	$mg\ kg^{-1}$	**1208 ± 9.8**	**362 ± 10.0**	150	1500
Ni	$mg\ kg^{-1}$	21.5 ± 0.2	72.3 ± 3.1	120	500
Co	$mg\ kg^{-1}$	11.7 ± 0.3	14.3 ± 0.3	20	250
As	$mg\ kg^{-1}$	**47.7 ± 2.0**	**40.7 ± 1.4**	20	50
Cd	$mg\ kg^{-1}$	1.9 ± 0.0	1.1 ± 0.0	2	15
Sb	$mg\ kg^{-1}$	9.9 ± 0.1	6.7 ± 0.5	10	30
V	$mg\ kg^{-1}$	74 ± 0.6	**119 ± 3.8**	90	250
Cr	$mg\ kg^{-1}$	28.3 ± 0.3	143 ± 13.6	150	800
Be	$mg\ kg^{-1}$	**10 ± 1.1**	**8.7 ± 0.9**	2	10

A total of 36 experimental units consisting in 0.15 m³ lysimeters (D: 0.65 m – H:0.51 m) cropped with a mix of microthermal grass species (*F. arundinacea, P . pratensis, L. perenne*) were arranged in completely randomized design with three replicates to test the following factors: i) 2 Substrates: soil (S) vs sludge (F); ii) 2 Fertilisation levels: fertilization with commercial green waste compost (OC: 230 g kg⁻¹; TN: 8 g kg⁻¹ - C) vs non-fertilised control (NOC); iii) 3 Biopromoter levels: TB (Trianum-P containing *Trichoderma harzianum* - strain T22 – Koppert b.v. ®), TA (consortium called "Panoramix" – Kop-

pert b.v. ® containing Endomycorrhiza and Trichoderma species along with humic and fulvic acids) and a control without biopromoters (NOT).

Compost was carefully mixed with the soil (0.5% w/w) prior to be added to lysimeters; during the 4 weeks before grass sowing experimental units were watered to keep soil moisture close to field capacity to stabilize soils and avoid subsidence during the experiment. Biopromoter treatment (TA and TB) was made directly on seeds and sowing was made on November 2015 with 20 g of seeds per lysimeter. Soil moisture was kept close to field capacity during the whole experiment.

4.1.3 Soil and plants: Sampling and Analysis

Soil samples were collected before sowing (October 2015) for chemical-physical characterisation. Grass biomass and soils were collected within a standardized sampling area (1600 cm^2) from each experimental unit in May, July and November 2016 respectively. Plant samples were washed with tap water, rinsed with deionized water, oven dried at 60°C until constant weight and ground prior to analysis. A composite sample representative of plants of each pot was analysed (acid digestion with aqua regia followed by ICP-MS) for Potential Toxic Elements (PTEs) total content. Pb, Cd, As, values were compared to legal PTEs thresholds for forages (REG UE N. 1275/2013). For metals not included in the current legislation, values reported by (Kabata-Pendias, 2011) were used as reference.

Rhizo-soil was dried at 50°C until constant weight, homogenized and sieved through a 2 mm sieve. The following determinations were made: Texture (Normalized Methods for soil analysis, ISS, 1985), pH-H$_2$O (1:2.5 soil:water solution ratio), Electric conductibility (1:2.5 soil:water solution ratio-Conductimeter basic 30, Crison), organic carbon (Walkley and Black method, 1934), nitrogen (Kjeldahl method) and carbonate content (Dietrich–Frühling calcimeter method, Loeppert and Suarez, 1996) and PTEs concentrations (acid digestion with aqua regia followed by ICP-MS).

PTEs mobility was estimated by single extractions at the begin of the experiment and in the third cut: 1M NH$_4$NO$_3$ extractant was used to assess the readily soluble fraction (DIN 19730, 1995) and PTE concentration in the solution was determined by ICP-MS.

4.1.4 Statistical Analysis

The statistical analyses were all carried out by using Ms Excel 2013 and SPSS 21 (SPSS Inc. Chicago, USA). All data were subjected to analysis of variance (ANOVA) using a general linear model and means were separated according to LSD Sidak test with $p<0.05$. Normality of distribution and homogeneity of variance were verified by using the Kolmogorov–Smirnov and Levene tests, respectively. Logarithmic transformation was applied to variables when necessary.

4.2 Results and discussions

4.2.1 Plant biomass production and nutrient status

The analysis of variance for biomass production and nutrient status of grass species for the three cuts is showed in Tab. 28.

Tab. 28. Analysis of variance for the biomass production and nutrient status for the three cuts.

	Factors	Plant biomass (D.W.g pot^{-1})	N (%)	N (mg pot^{-1})	P (%)	P (mg pot^{-1})
FIRST CUT	S (substrate)	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**
	C (compost)	**0.000**	0.350	**0.000**	**0.003**	**0.000**
	T (biopromoter)	**0.000**	**0.035**	**0.000**	0.872	**0.000**
	SxC	0.100	0.069	**0.030**	**0.036**	**0.023**
	SxT	**0.000**	**0.000**	**0.000**	0.106	**0.000**
	CxT	**0.046**	**0.001**	0.059	0.948	0.085
	SxCxT	**0.030**	**0.002**	**0.001**	0.420	**0.002**
SECOND CUT		Plant biomass (D.W.g pot^{-1})	N (%)	N (mg pot^{-1})	P (%)	P (mg pot^{-1})
	S (substrate)	0.068	**0.017**	**0.022**	**0.000**	**0.001**
	C (compost)	**0.013**	0.064	**0.010**	**0.002**	**0.000**
	T (biopromoter)	**0.000**	0.693	**0.000**	0.134	**0.000**
	SxC	0.154	0.375	0.150	0.863	0.248
	SxT	**0.005**	0.166	**0.001**	**0.015**	**0.002**
	CxT	**0.000**	0.400	**0.018**	0.132	**0.004**
	SxCxT	0.117	0.776	0.212	0.819	0.133
THIRD CUT		Plant biomass (D.W.g pot^{-1})	N (%)	N (mg pot^{-1})	P (%)	P (mg pot^{-1})
	S (substrate)	**0.000**	0.907	**0.003**	0.332	**0.005**
	C (compost)	**0.000**	0.511	**0.000**	0.239	**0.000**
	T (biopromoter)	**0.000**	0.301	**0.000**	0.252	**0.000**
	SxC	**0.000**	0.437	**0.000**	0.154	**0.000**
	SxT	**0.000**	0.155	**0.000**	**0.011**	**0.000**
	CxT	**0.000**	0.919	**0.000**	0.559	**0.000**
	SxCxT	0.819	0.092	0.163	0.328	0.532

Bold values indicates significance with p<0.05.

Main factors effects on biomass production and nutrient status are shown in Tab. 29

Tab. 29. Main factors effects on biomass production and nutrient status in the three cuts (mean values ± standard error).

FIRST CUT					
Principal Factors	Plant biomass (Dry weight – g pot^{-1})	N (%)	N (mg pot^{-1})	P (%)	P (mg pot^{-1})
Substrate					
F	17.4 ± 2.8	1.39 ± 0.02	245 ± 41	0.22 ± 0.01	40.3 ± 7.2
S	27.9 ± 2.5	1.52 ± 0.06	423 ± 37	0.25 ± 0.01	68.8 ± 6.2
Compost					
C	28.4 ± 2.8	1.43 ± 0.02	410 ± 43	0.24 ± 0.01	69.9 ± 7.3
NoC	16.9 ± 2.3	1.48 ± 0.06	258 ± 39	0.23 ± 0.01	39.3 ± 5.8
Biopromoters					
TA	32.0 ± 2.7	1.44 ± 0.03	461 ± 40	0.24 ± 0.01	76.4 ± 7.26
TB	14.2 ± 2.1	1.40 ± 0.03	202 ± 33	0.23 ± 0.01	34.3 ± 5.85
NoT	21.8 ± 3.7	1.53 ± 0.08	340 ± 60	0.23 ± 0.01	53.0 ± 10.0
Mean	22.6	1.45	334	0.23	54.6

SECOND CUT					
Principal Factors	Plant biomass (Dry weight – g pot^{-1})	N (%)	N (mg pot^{-1})	P (%)	P (mg pot^{-1})
Substrate					
F	11.4 ± 0.64	1.91 ± 0.04	219 ± 15	0.31 ± 0.01	35.9 ± 2.6
S	14.2 ± 1.85	2.08 ± 0.05	300 ± 41	0.37 ± 0.01	52.9 ± 7.4
Compost					
C	14.3 ± 1.77	2.05 ± 0.04	298 ± 40	0.36 ± 0.01	53.0 ± 7.3
NoC	11.3 ± 0.80	1.93 ± 0.05	222 ± 19	0.32 ± 0.01	35.8 ± 2.7
Biopromoters					
TA	17.1 ± 2.37	2.03 ± 0.05	355 ± 55	0.35 ± 0.01	62.0 ± 9.9
TB	11.3 ± 0.91	1.98 ± 0.07	225 ± 21	0.34 ± 0.01	38.4 ± 3.1
NoT	10.0 ± 0.60	1.98 ± 0.06	199 ± 15	0.32 ± 0.02	32.8 ± 3.1
Mean	12.8	1.99	260	0.34	44.4

			THIRD CUT		
Principal Factors	Plant biomass (Dry weight – g pot^{-1})	N (%)	N (mg pot^{-1})	P (%)	P (mg pot^{-1})
Substrate					
F	28.7 ± 3.51	3.31 ± 0.11	953 ± 119	0.44 ± 0.01	123 ± 14
S	30.9 ± 2.66	3.31 ± 0.08	1000 ± 73	0.42 ± 0.01	128 ± 9
Compost					
C	38.8 ± 2.24	3.25 ± 0.05	1259 ± 74	0.42 ± 0.01	161 ± 8
NoC	21.3 ± 2.43	3.37 ± 0.12	694 ± 70	0.44 ± 0.01	90 ± 9
Biopromoters					
TA	36.0 ± 3.55	3.17 ± 0.12	1238 ± 101	0.40 ± 0.01	152 ± 9
TB	20.7 ± 2.75	3.24 ± 0.08	670 ± 86	0.43 ± 0.01	90 ± 12
NoT	34.6 ± 3.32	3.51 ± 0.12	1021 ± 114	0.46 ± 0.01	135 ± 16
Mean	30.1	3.31	977	0.43	125

C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments respectively.

Grass species in sludge (F) showed higher growth than in soil (S) probably due to the different fertility and different contamination. Indeed F reported a higher contamination of Pb and Zn. On the average compost application increased biomass production and nutrient uptake in all the cuts. Biopromoters application showed different behaviour belong the different cuts. TA increased biomass production and nutrient uptake compared to the control and TB in all the cuts; TB reported lower biomass compared to control in the first and third cut while showed no differences compared to the control in the second cut.

The analysis of the interaction SxCxT in the first cut and the analysis of the interaction CxT in second and third cut on biomass production are shown in Fig. 15
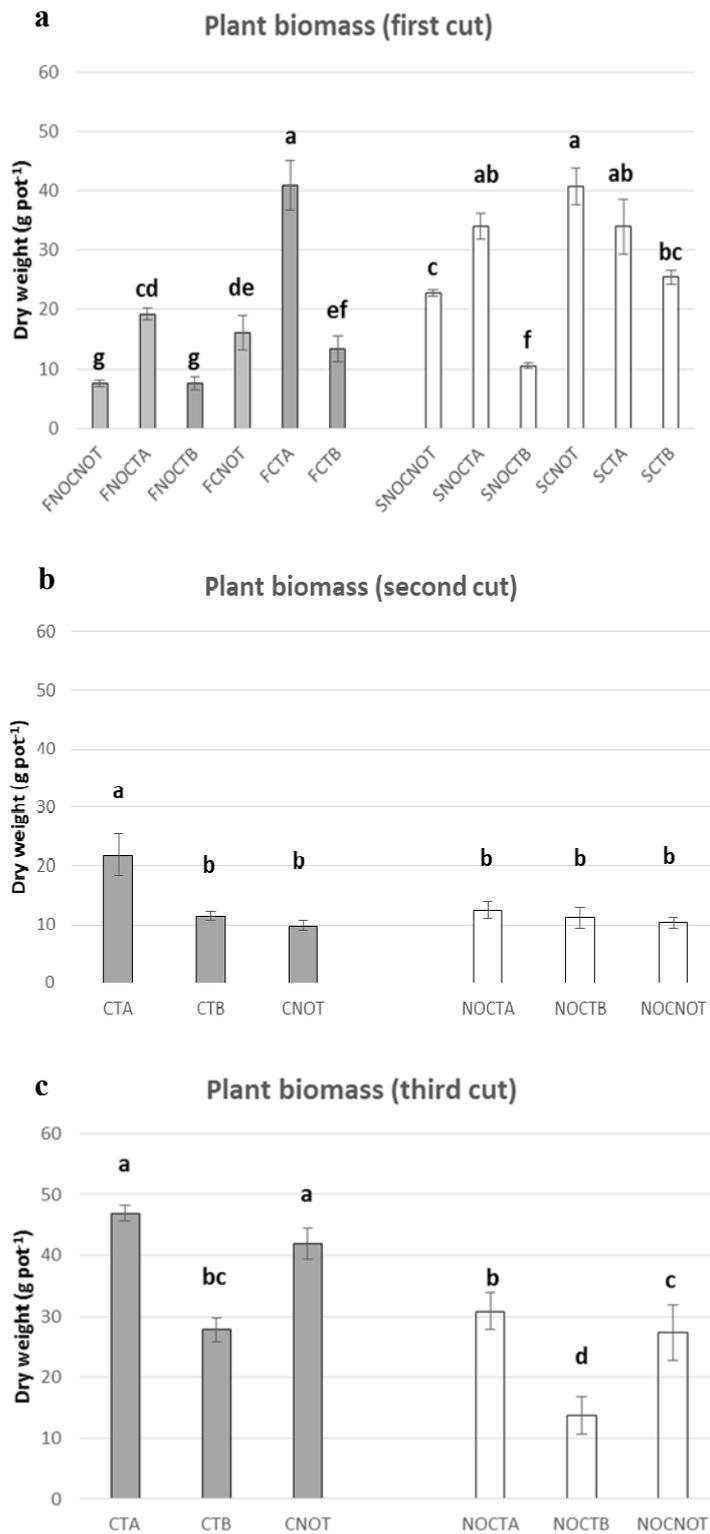
Fig. 15. Compost and biopromoters effect on biomass production in the first (a – interaction SxCxT), second (b – interaction CxT) and third (c – interaction CxT) cut respectively. C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments respectively. F and S are sludge and soil substrates respectively. Bars indicate ± standard errors. Mean values with the same letter do not differ according to the LSD test (p<0.05).

The analysis of the effect of the interaction SxCxT in the first cut and the analysis of the interaction CxT in second and third cut on nutrient status are reported in Tab. 30.

Tab. 30. Compost and biopromoters effect (means ± standard errors) on nutrient uptake (N and P) in the first, second and third cut. C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments respectively. F and S are sludge and soil substrates respectively. Mean values with the same letter do not differ according to the LSD test (p<0.05).

		P (mg pot^{-1})	N (mg pot^{-1})
FIRST CUT	FNOCNOT	15.0±0.8 **f**	101±8.9 **f**
	FNOCTA	40.7±2.1 **d**	261±6.1 **c**
	FNOCTB	16.2±2.5 **f**	109±17.0 **f**
	FCNOT	37.2±7.8 **d**	214±36.6 **cd**
	FCTA	101.0±5.3 **a**	598±20.3 **a**
	FCTB	31.1±5.8 **de**	190±37.8 **de**
	SNOCNOT	57.6±2.9 **c**	452±13.7 **ab**
	SNOCTA	81.3±5.0 **ab**	491±24.6 **ab**
	SNOCTB	24.9±0.9 **e**	136±10.5 **ef**
	SCNOT	102±7.0 **a**	592±49.0 **a**
	SCTA	81.7±10.6 **ab**	49±67.2 **ab**
	SCTB	64.9±5.7 **bc**	373±32.9 **b**
	Mean	54.6	334
		P (mg pot^{-1})	N (mg pot^{-1})
SECOND CUT	CTA	84.2±14.9 **a**	463±85 **a**
	CTB	43.1±3.2 **b**	236±15 **b**
	CNOT	31.8±3.3 **c**	194±21 **b**
	NOCTA	39.8±4.0 **bc**	246±34 **b**
	NOCTB	33.8±4.7 **c**	215±40 **b**
	NOCNOT	33.9±5.61 **c**	204±24 **b**
	Mean	44.4	260
		P (mg pot^{-1})	N (mg pot^{-1})
THIRD CUT	CTA	177±5.09 **a**	1518±40 **a**
	CTB	122±7.07 **b**	894±53 **b**
	CNOT	184±12.1 **a**	1366±93 **a**
	NOCTA	126±10.3 **b**	959±110 **b**
	NOCTB	58±12.8 **d**	446±99 **d**
	NOCNOT	86±5.10 **c**	677±43 **c**
	Mean	125	977

The application of compost increased the biomass production and nutrient uptake in the first (SCNOT, FCNOT) and third cut (CNOT) compared to FNOCNOT and SNOCNOT in the first cut and to NOCNOT in the third cut, while biopromoters application showed an higher biomass production and nutrient uptake only for TA in the first (FNOCTA, SNOC-TA) and third cut (NOCTA) compared to FNOCNOT and SNOCNOT in the first cut and to NOCNOT in the third cut. In the first and second cut the combination of compost and TA reported higher biomass production as well as N and P uptake. The application of TB reported a lower biomass production and nutrient uptake compared to SNOCNOT in the first cut while showed a lower biomass production and lower nutrient uptake compared to

NOCNOT in the third cut. The combination of compost with TB reported a lower biomass production and nutrient uptake in the first and third cut wile in the second cut don't showed differences compared to the others treatments.

The differences between plants biomass yield in the three cuts can be related to differences in meteorological conditions. For that reason in the second cut there was the lowest mean biomass yield (high air temperature and only two months from the previous cut) and in the third there was the highest biomass yield (moderate temperature). The effect of compost on plant biomass supports previous studies, which indicated that the addition of organic fertilizers promoted biomass production (Singh et al. 2010; Liu et al. 2016; Moreno-Jimenez et al. 2016). The compost can improve soil microbial activity, provides nutrients and organic matter to the soil (Fagnano et al., 2011; Alluvione et al., 2013) and improves soil physical characteristics, including porosity and water-holding capacity (Álvarez-López et al. 2016; Buddha and Singh 2015). Moreover, compost acts as a long term reserve and slow-release sources of major nutrient like N, P and K (Sullivan et al., 2002) that can allow a higher nutrient plant uptake. Nevertheless, the amount of nutrient contents for plant growth provided by organic manure depends on the mineralization rate (Rosen and Allan 2007). For this reason compost increased biomass production in the first and third cut compared to the second cut (Fig. 15) as well as N and P uptake (Tab. 30). Also the biopromoters like *Trichoderma* strains can increase plant growth by changing the microbial composition rhizosphere, enhancing nutrient uptake and solubilisation of soil nutrients by enhancing root development, hair formation and deep of roots (Harman, 2000; Harman et al. 2004). Results are compatible with beneficial effects of *Trichoderma* in the experiment, but only for TA that increased the mean biomass production as compared to TB by 113%, 52% and 57% respectively in the first, second and third cut (Fig. 15) as well as N and P uptake (Tab. 30). TA also increased the mean biomass production as compared to NOCNOT by 68%, 58% and 4% in the three cuts respectively. TB showed a reduction of biomass yield in the first and third cut compared to control and a similar biomass production in the second cut. This behaviour is not common for strain T22, in fact Strain T22 of *T. harzianum* generally increases plant growth and development and control diseases but the effects of its application vary between plant species and between individuals of same species. For this reason, strain T22 also can have a negative effect on plants growth as reported in another plant of *Poaceae* genre by Harman (2006).

Compost and TA application also had a synergic effect on plant growth and nutrient uptake (Fig. 15, Tab. 30), in fact in all the cuts, the interaction between compost and TA reported the higher biomass production. Increased C availability for the fungus due to root exudation and compost enhanced biostimulation of the host plant increasing water and nutrient uptake from the soil (Agarwal et al., 2017). The same interaction between compost and TB showed a lower biomass production compared to FCNOT and SCONOT in the first cut and compared to CNOT in the third. The N and P concentrations don't showed differences between the treatments in the three cuts also when compost and biopromoters were applied. This phenomenon can be attributed to a dilution effect caused by the much higher plants biomass (Tab. 30) as reported by Houben et al., 2013 and Taub et al., 2008.

4.2.2 PTEs in plants

The analysis of variance for PTEs content and uptake of grass species for the three cuts is showed in Tab. 31.

Tab. 31. Analysis of variance for the PTEs content and uptake of plants for the three cuts.

	Factors	Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Zn content (mg kg^{-1})	Zn uptake (mg pot^{-1})
FIRST CUT	S (substrate)	0.493	**0.000**	**0.000**	0.091
	C (compost)	0.239	**0.000**	**0.000**	**0.000**
	T (biopromoter)	0.079	**0.000**	0.776	**0.000**
	SxC	**0.005**	0.159	0.072	0.515
	SxT	0.733	**0.004**	0.926	**0.000**
	CxT	0.063	**0.014**	0.316	**0.008**
	SxCxT	0.157	**0.030**	0.564	**0.001**
		Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Zn content (mg kg^{-1})	Zn uptake (mg pot^{-1})
SECOND CUT	S (substrate)	0.293	0.057	**0.000**	**0.024**
	C (compost)	**0.005**	0.974	0.163	0.456
	T (biopromoter)	**0.008**	**0.000**	0.182	**0.000**
	SxC	**0.000**	**0.002**	0.803	0.383
	SxT	**0.007**	**0.000**	0.846	**0.001**
	CxT	**0.019**	0.411	0.579	**0.007**
	SxCxT	0.105	0.124	0.071	0.522
		Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Zn content (mg kg^{-1})	Zn uptake (mg pot^{-1})
THIRD CUT	S (substrate)	0.125	**0.001**	**0.000**	**0.000**
	C (compost)	**0.000**	**0.000**	**0.014**	**0.000**
	T (biopromoter)	0.607	**0.001**	0.182	**0.000**
	SxC	**0.000**	**0.040**	0.182	**0.000**
	SxT	**0.005**	**0.002**	**0.044**	**0.000**
	CxT	**0.032**	**0.000**	**0.031**	**0.000**
	SxCxT	0.094	0.146	**0.035**	**0.028**

Bold values indicates significance with p<0.05.

Main factors effects on PTEs content in plants and PTEs plants uptake in the three cuts are shown in Tab. 32

Tab. 32. Main factors effects on PTEs content in plants and PTEs plants uptake in the three cuts (mean values ± standard error).

FIRST CUT				
Principal Factors	Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Zn content (mg kg^{-1})	Zn uptake (mg pot^{-1})
Substrate				
F	0.41 ± 0.03	0.007 ± 0.001	44.9 ± 1.30	0.75 ± 0.11
S	0.43 ± 0.03	0.013 ± 0.002	27.9 ± 0.82	0.78 ± 0.07
Compost				
C	0.40 ± 0.03	0.012 ± 0.002	33.4 ± 1.74	0.91 ± 0.09
NoC	0.44 ± 0.03	0.007 ± 0.001	39.3 ± 2.59	0.62 ± 0.07
Biopromoters				
TA	0.40 ± 0.04	0.0123 ± 0.001	36.5 ± 2.89	1.12 ± 0.10
TB	0.38 ± 0.03	0.0055 ± 0.001	35.8 ± 2.79	0.47 ± 0.05
NoT	0.48 ± 0.04	0.0114 ± 0.002	36.9 ± 2.96	0.70 ± 0.08
Mean	0.42	0.0097	36.4	0.76

SECOND CUT				
Principal Factors	Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Zn content (mg kg^{-1})	Zn uptake (mg pot^{-1})
Substrate				
F	0.47 ± 0.03	0.005 ± 0.001	76.0 ± 4.11	0.87 ± 0.07
S	0.49 ± 0.03	0.007 ± 0.001	53.2 ± 2.88	0.75 ± 0.10
Compost				
C	0.43 ± 0.02	0.0064 ± 0.001	61.4 ± 4.64	0.86 ± 0.10
NoC	0.53 ± 0.04	0.0063 ± 0.001	67.8 ± 4.21	0.76 ± 0.07
Biopromoters				
TA	0.55 ± 0.05	0.0094 ± 0.0014	70.1 ± 6.54	1.10 ± 0.11
TB	0.44 ± 0.05	0.0051 ± 0.0007	65.3 ± 4.38	0.75 ± 0.09
NoT	0.45 ± 0.03	0.0045 ± 0.0004	58.4 ± 5.07	0.58 ± 0.06
Mean	0.48	0.006	64.6	0.81

	THIRD CUT			
Principal Factors	Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Zn content (mg kg^{-1})	Zn uptake (mg pot^{-1})
Substrate				
F	0.92 ± 0.07	0.03 ± 0.003	86.08 ± 3.79	2.35 ± 0.28
S	1.13 ± 0.13	0.04 ± 0.006	41.11 ± 1.32	1.24 ± 0.09
Compost				
C	1.26 ± 0.12	0.05 ± 0.004	59.52 ± 5.13	2.36 ± 0.27
NoC	0.80 ± 0.07	0.02 ± 0.002	67.67 ± 6.88	1.23 ± 0.11
Biopromoters				
TA	0.91 ± 0.13	0.036 ± 0.007	60.41 ± 7.12	2.28 ± 0.31
TB	1.14 ± 0.17	0.026 ± 0.005	68.13 ± 8.99	1.26 ± 0.20
NoT	1.03 ± 0.10	0.030 ± 0.005	62.24 ± 6.39	1.84 ± 0.32
Mean	1.03	0.03	63.59	1.79

C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments, respectively. F and S are sludge and soil substrates respectively

The compost application increased Pb uptake in the first and third cut while biopromoters effect on Pb uptake was greater for TA in all the cuts compared to control and TB. TB effect on the Pb uptake by plants was similar to the control in the first and third cut while was lower in the second cut. The compost application decreased the Zn content of plants in the first and third cut while increased Zn uptake in the same cuts probably for a dilution effect related with higher biomass production. TA increased Zn uptake in all the cuts compared to control and TB.

Pb concentration in the aerial part (Tab. 32) reported a mean in the three harvests of 0.64 mg kg^{-1}, lower than values reported by Kabata-Pendias, 2011 in plants growing in non-contaminated sites (2.09 mg kg^{-1}). Zn concentration in plants (Tab. 32) was higher than values reported by Kabata-Pendias, 2011 in plants growing in non-contaminated sites (31.5 mg kg^{-1}). Zn concentration were higher in plants grown on sludges in all the harvests with mean values of 80 mg kg^{-1} similar to the results reported by Zhao et al., 2013 in *Festuca arundinacea*.

Effect of the interaction of main factors on Pb and Zn concentration in plants in the third cuts are shown, respectively, in Fig. 16 and Fig. 17.
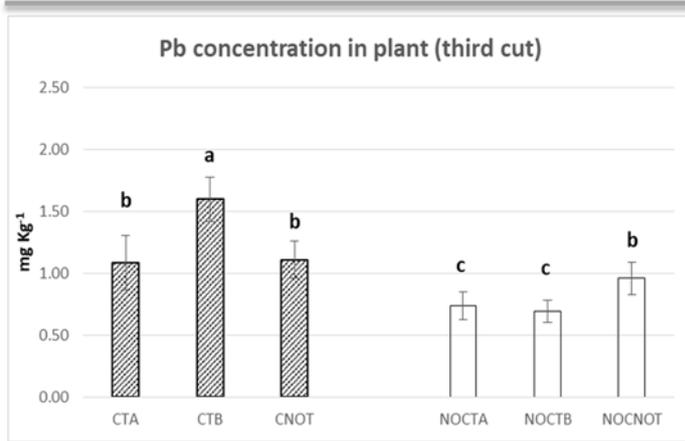
Fig. 16. Compost and biopromoters effect on Pb concentrations in third cut (effect of the interaction CxT). C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments respectively. F and S are sludge and soil substrates respectively. Bars indicate ± standard errors. Mean values with the same letter do not differ according to the LSD test (p<0.05). Bars without letters indicate no significance at p<0.05

Fig. 17. Compost and biopromoters effect on Zn concentrations in the third cut (effect of the interaction SxCxT).
C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments respectively. F and S are sludge and soil substrates respectively. Bars indicate ± standard errors (n = 3). Mean values with the same letter do not differ according to the LSD test (p<0.05). Bars without letters indicate no significance at p<0.05

Effect of the interaction of main factors on Pb and Zn uptake were also evaluated and results are showed respectively in Fig. 18 and 19.
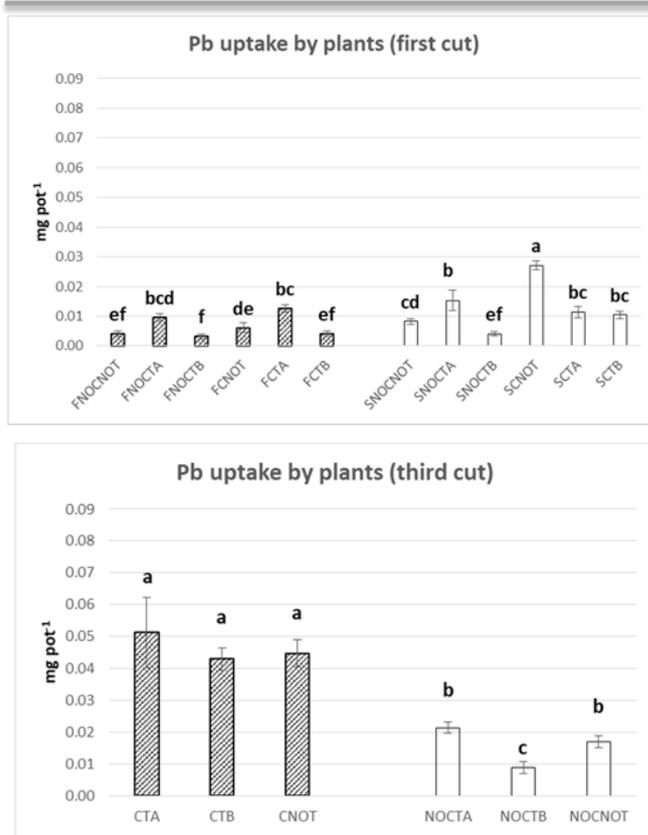
Fig. 18. Compost and biopromoters effect on Pb uptake in the first and third cut (effect of the interaction SxCxT in the first cut and CxT in the third).

C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Tria-num-P and no biopromoters treatments respectively. F and S are sludge and soil substrates respectively. Bars indicate ± standard errors (n = 3). Mean values with the same letter do not differ according to the LSD test (p<0.05). Bars without letters indicate no significance at p<0.05

The compost application in the first cut in S increased Pb uptake while only TA application was effective in increasing Pb uptake, with better results in combination with compost. In the third cut the interaction amendment x biopromoter reported a higher Pb concentration in C-TB compared to other treatments, with lower Pb uptake in TB without compost probably for a dilution effect related with higher biomass production. Pb uptake reported an increase in the third cut compared to the first one.
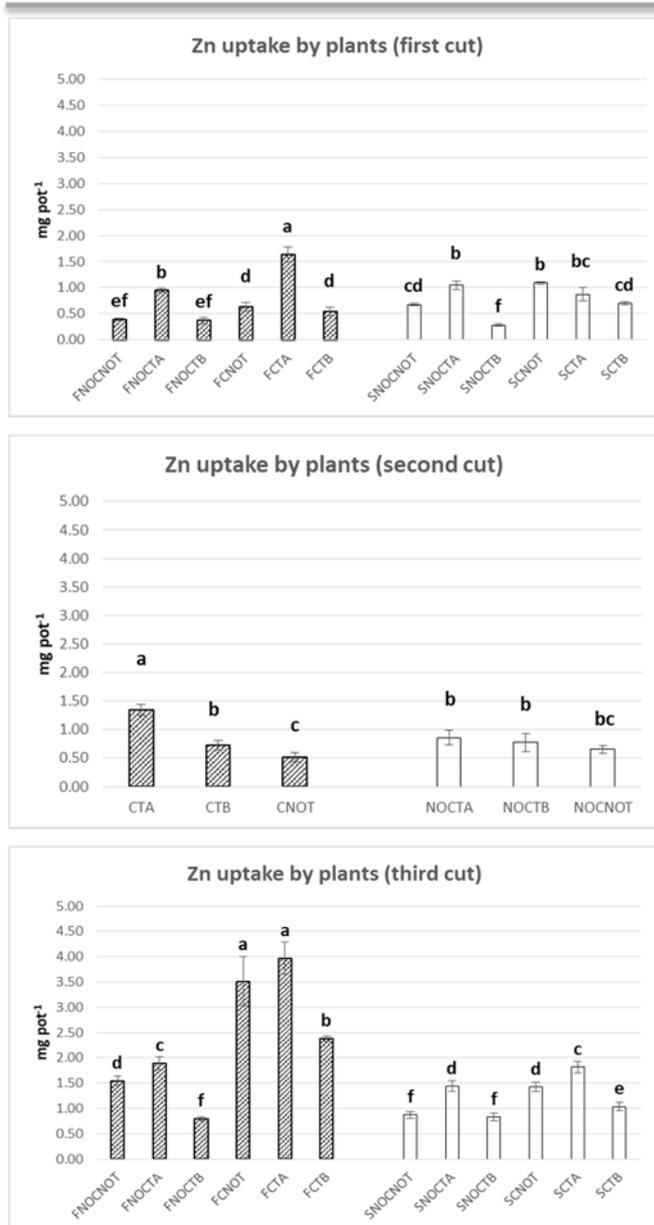
Fig. 19. Compost and biopromoters effect on Zn uptake in the first, second and third cut. C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no bi-opromoters treatments respectively. F and S are sludge and soil substrates respectively. Bars indicate ± standard errors (n = 3). Mean values with the same letter do not differ according to the LSD test (p<0.05). Bars with no letter indicate no significance at p<0.05

Compost application in first and third cut increased Zn uptake by plants only with TA application. TB application showed a similar or lower Zn uptake compared to the control. The compost application also increased Zn concentration in the third cut compared to treatments without compost with higher concentration in the combination of compost and TB, but the uptake of Zn when the combination of compost and TB was applied reported a lower uptake probably for a dilution effect related with higher biomass production as also seen for Pb uptake. In the second cut the amendment by biopromoter interaction reported a

significant effect of CTA compared to the other treatments. The interaction between compost and TA reported the higher Zn uptake probably because the increased C availability for the fungus due to root exudation and compost fertilization enhanced biostimulation of the host plant increasing water and nutrient uptake from the soil (Agarwal et al. 2017)

4.2.3 Available soil PTEs concentrations

The analysis of variance for the extractable Pb and Zn concentrations in soil respectively a month after sowing (T0) and in correspondence of the third cut (T3), are shown in Tab. 33. The extractable concentrations of Pb and Zn in soil substrates were very low and under the detection values (Pb<0.02 mg kg^{-1} and Zn<0.1 mg kg^{-1}), therefore only Pb and Zn extractable concentration in sludge are shown.

Tab. 33. Analysis of variance for the extractable Pb and Zn at time T0 (1 month after sowing) and T3 (12 month after sowing).

	Factors	Extractable Pb (mg kg^{-1})	Extractable Zn (mg kg^{-1})
T0	C (compost)	**0.03**	0.19
	T (biopromoter)	**0.02**	0.34
	CxT	0.62	0.18
	Factors	Extractable Pb (mg kg^{-1})	Extractable Zn (mg kg^{-1})
T3	C (compost)	0.74	0.90
	T (biopromoter)	0.62	0.96
	CxT	0.29	0.96

Bold values indicates significance with p<0.05.

Main factors effects on extractable Pb and Zn concentrations in soil respectively a month after sowing (T0) and in correspondence of the third cut (T3), are shown in Tab. 34.

Tab. 34. Main factors effects on extractable Pb and Zn at time (1 month after sowing) and T3 (12 month after sowing). (mean values ± standard error). C and NOC are compost and no compost treatments respectively; TA, TB and NOT are "Panoramix", Trianum-P and no biopromoters treatments respectively.

T0		
Principal Factors	Extractable Pb (mg kg^{-1})	Extractable Zn (mg kg^{-1})
Compost		
C	0.06 ± 0.01	0.57 ± 0.03
NoC	0.04 ± 0.01	0.52 ± 0.03
Biopromoters		
TA	0.04 ± 0.01	0.53 ± 0.03
TB	0.07 ± 0.01	0.59 ± 0.04
NoT	0.05 ± 0.01	0.52 ± 0.03
Mean	0.05	0.55
T3		
Principal Factors	Extractable Pb (mg kg^{-1})	Extractable Zn (mg kg^{-1})
Compost		
C	0.05 ± 0.01	0.58 ± 0.02
NoC	0.06 ± 0.02	0.55 ± 0.07
Biopromoters		
TA	0.07 ± 0.02	0.66 ± 0.07
TB	0.04 ± 0.01	0.48 ± 0.05
NoT	0.05 ± 0.01	0.54± 0.04
Mean	0.05	0.56

Phytoavailability of Pb and Zn (Tab.35) was close to 0 both in T0 and T3, highlighting that PTEs were in a stable form not easily assimilated by plants, even if total concentrations were higher than CTC. This confirms that the total content of PTE is not a good indicator of the environmental risks due to their presence into soils that is instead related to their mobility (Adamo et al., 2002).

Pb and Zn extractable concentrations reported no difference between T0 and T3 except Pb extractable content in T0 where TB increased and TA decreased Pb phytoavailability. This behaviour can be linked to the different composition of two biopromoters: TB including only *Trichoderma* can be able to increase bioavailability through the release of chelating compounds of organic acids (Kacprzak et al. 2014). TA including also mycorrhizae can have the opposite effect contributing to the immobilization of PTEs in the soil with the immobilization of metals by compounds secreted by the fungus, precipitation in polyphos-

phate granules in the soil, adsorption to fungal cell walls, and chelation of PTEs inside the fungus as reported by Agarwal et al. (2017) and Gaur and Adholeya (2004).

Compost fertilization increased bioavailability as already reported by Fagnano et al., (2011) who related this effect to the combined effect of compost amendment and roots exudates probably by the formation of metal chelates by humic acids or low molecular weight organic compounds.

4.3 Conclusions

The study was made with aim to evaluate the effects of organic amendment and biopromoters on the phytoextraction/phytostabilization potential of a commercial grass mix. Plant species were well adapted to the contamination of soils and sludges showing a good growth during the year of experimentation. The application of compost and biostimulant containing Endomycorrhiza and Trichoderma species along with humic and fulvic acids (TA) increased the plant growth, nutrients uptake and Zn uptake especially when combined with compost amendment. Biostimulants containing *Trichoderma harzianum* strain T22 (TB) showed lower results alone and in combination with compost with lower biomass production and PTEs uptake compared not inoculated control. The accumulation of PTEs in the aerial part of plants was low for the considered PTEs except for Zn as well as the soil bioavailable fraction. So the plant species tested with this experiment can be considered for phytostabilization purpose reducing the leaching of PTEs in the soil profile, preventing with the complete soil coverage the dispersion of the contaminated soil particles and minimizing the risk for human health. Furthermore the harvested biomass can be used in no food chains for the production of compost, biopolymers and bioenergy.

5. EVALUATION OF GRASS SPECIES FOR THE SECURING OF AN INDUSTRIAL CONTAMINATED SOIL

5.1 Materials and Methods

5.1.1 Study area

The study area was the brownfield site described in the chapter 4 located in Marcianise (Campania Region, Italy- 41°00'48.9"N - 14°17'49.7"E), adjacent to a plant for Pb recycling from exhausted batteries, used for several years as a temporary landfill. Almost 1 year before the start of experimental activities an environmental characterization has been carried out by the owner company of the site according to the actual environmental legislation in order to complete the risk evaluation. Results of risk analysis showed that the site was contaminated and the risk for workers who frequent the site was linked to Pb and Cd. The routes of exposition were inhalation and dermal contact of contaminated soil particles. Successively the site has been secured for limiting dust lift due to wind erosion and PTE (potentially toxic elements) leaching in the water table. From 2016 the site is managed according to the LIFE ECOREMED protocol in order to limit dispersion of contaminated soil particles by using permanent meadows and high density plant of poplar for reducing wind speed at ground.

5.1.2 Experimental setup

Soil used for this experiment was collected in a hot spot (Cu= 343 mg Kg^{-1}; Pb=36439 mg Kg^{-1}; Zn=283 mg Kg^{-1}; As=80 mg Kg^{-1}; Cd=132 mg Kg^{-1}) of the site, and carefully homogenized before the preparation of experimental units in order to homogenize chemical-physical characteristics of the whole soil mass. The preliminary soil characterization is reported in Tab. 35 and can be resumed as follows: sandy texture; a neutral pH; a low con-

tent of carbonates; high levels of organic matter and total nitrogen. Potential Toxic Elements (PTEs) were also analysed (Tab.35). Values of Pb, As and Cd were higher than the Contamination Threshold Concentration (CTC) established for industrial use by the Italian Ministry of the Environment (Italian Parliament, 2006).

Tab. 35. Initial characteristics (mean ± standard error) of brownfield soil

		Soil	CTC (L.D. 152/06)	
			residential use	industrial use
Sand	%	63	-	
Silt	%	21	-	
Clay	%	16	-	
pH		6.7	-	
EC	$\mu S\ cm^{-1}$	767	-	
Carbonates	%	1.6	-	
OM	$g\ kg^{-1}$	48.5	-	
OC	$g\ kg^{-1}$	28.1	-	
TN	$g\ kg^{-1}$	2.5	-	
Cu	$mg\ kg^{-1}$	343 ± 81	120	600
Pb	$mg\ kg^{-1}$	**36439 ± 665**	100	1000
Zn	$mg\ kg^{-1}$	283 ± 3.6	150	1500
As	$mg\ kg^{-1}$	**80 ± 5.8**	20	50
Cd	$mg\ kg^{-1}$	**132 ± 1.6**	2	15

A growth chamber experiment (T ranging from 22 to 28°C; UR=85%) was carried out with 56 pots (D: 0.15 m – H:0.15 m) in order to test the following factors:

i) 2 grasses: D = *Dactylis glomerata* (8 g seed m^{-2}), that is an autochthonous species of the site; M= commercial grass mixture (40 g seed m^{-2}) including *Lolium perenne L.* (10%) *Poa pratensis L.* (10%) and *Festuca arundinacea L.* (80%);

ii) 3 doses of Compost from MSW: C0 = non-fertilized control; C1=25 mg FW ha^{-1} and C2=50 mg FW ha^{-1};

iii) 3 levels of commercial biopromoters: TB (Trianum-P containing *Trichoderma harzianum* - strain T22 – Koppert b.v. ®) and TA (consortium called "Panoramix" – Koppert b.v. ® containing Endomycorrhiza and Trichoderma species along with humic and fulvic acids) compared with a control (NoT).

Biopromoters were added to seeds prior of sowing. A total of 18 treatments deriving from the combination of the above mentioned factors were arranged in a completely randomized scheme with 3 replicates. Fertilizers were mixed to the soil before its distribution in the

pots. Soil was kept at Field Capacity in order to allow the better traspirative conditions to the crop.

Harvest of meadows was made 60 days sowing, aboveground fresh biomass was washed with tap water, rinsed with deionized water, oven dried at 60°C until constant weight and ground. A composite sample representative of plants of each pot was analysed (acid digestion with aqua regia followed by ICP-MS) for Potential Toxic Elements (PTEs) total content. Values were compared to legal PTEs thresholds in plants and soils (REG UE N. 1275/2013 and L. D. 152/2006, respectively). For metals not included in the current legislation mean values found in grasses grown on polluted sites (Kabata-Pendias, 2011) were used as reference.

Rhizo-soil was dried at 50°C until constant weight, homogenized and sieved through a 2 mm sieve. The following determinations were made on rhizo-soils and on initial soil: Texture (Normalized Methods for soil analysis, ISS, 1985); pH-H_2O (1:2.5 soil:water solution ratio); Electric conductibility on a 1:2.5 soil:water solution (Conductimeter basic 30, Crison); organic carbon (Walkley and Black method, 1934); nitrogen (Kjeldahl method), carbonate content (Dietrich–Frühling calcimeter method, Loeppert and Suarez, 1996) and PTEs concentrations (acid digestion with aqua regia followed by ICP-MS).

PTEs mobility was estimated:

- By a single extraction with 1M NH_4NO_3 in correspondence at experiment start and at harvest to assess the readily soluble fraction (DIN 19730, 1995). PTE concentration in the solution was determined by ICP-MS.

- By the analyses of pore water sampled with rhizo samplers (Rhizosphere Research Products, The Netherlands) inserted with a 45° angle in each pot at harvest. Pore water samples were analyzed for pH, EC and PTEs concentrations with AAS.

5.1.3 Statistical Analysis

The statistical analyses were all carried out by using Ms Excel 2013 and SPSS 21 (SPSS Inc. Chicago, USA). All data were subjected to an analysis of variance (ANOVA) using a general linear model and between means were separated by using LSD Sidak test with $p<0.05$. Normality of distribution and homogeneity of variance were verified using the Kolmogorov–Smirnov and Levene tests, respectively. Logarithmic transformation was applied to dependent variables when necessary.

5.2   Results and discussions

5.2.1 Plant biomass production and nutrient status

The analysis of variance for biomass production and nutrient status of grass species is showed in Tab. 36.

Tab. 36. Analysis of variance for the biomass production and nutrient status.

Factors	Plant biomass (Dry weight – g pot^{-1})	N (%)	N (mg pot^{-1})
S (species)	0.133	**0.000**	0.084
C (compost)	0.739	0.796	0.979
T (biopromoter)	**0.005**	0.654	**0.006**
SxC	0.15	**0.000**	0.378
SxT	0.381	0.124	0.730
CxT	**0.000**	0.150	**0.004**
SxCxT	0.126	0.108	0.485

Bold values indicates significance with p<0.05.

Main factors effects on biomass production and nutrient status are shown in Tab. 37

Tab. 37. Main factors effects on biomass production and nutrient status in the three cuts (mean values ± standard error). C1=25 mg FW ha^{-1}, C2=50 mg FW ha^{-1}, C0=no Compost; D= *Dactlys glomerata*, M=mix of microthermal species. TA=Panoramix, TB=Trianum-P, NoT=no biopromoters.

Principal Factors	Plant biomass (Dry weight – g pot^{-1})	N (%)	N (mg pot^{-1})
Plant species			
D	0.50 ± 0.03	4.64 ± 0.28	22.0 ± 1.4
M	0.44 ± 0.02	5.74 ± 0.20	24.0 ± 1.4
Compost			
C0	0.46 ± 0.03	5.28 ± 0.35	23.1 ± 1.7
C1	0.47 ± 0.04	5.29 ± 0.38	24.1 ± 2.2
C2	0.47 ± 0.03	5.01 ± 0.35	22.8 ± 1.6
Biopromoters			
TA	0.52 ± 0.03	5.37 ± 0.32	27.2 ± 1.6
TB	0.46 ± 0.04	5.17 ± 0.38	22.4 ± 1.7
NoT	0.42 ± 0.04	5.05 ± 0.29	20.3 ± 1.4
Mean	0.47	5.19	23.3

The biomass production of grass species is showed in Fig.20.
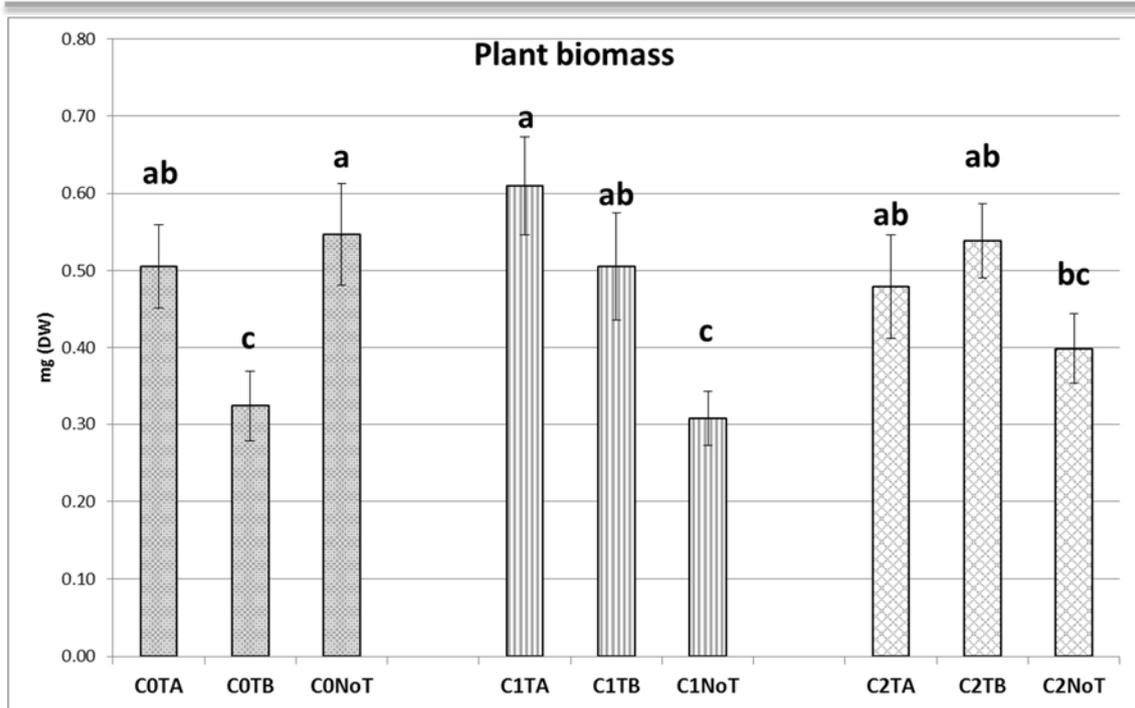
Fig. 20. Plants biomass (dry weight) yield (**effect of the interaction fertilization x biopromoters**) using two doses of composts and two biopromoters.
C1=25 mg FW ha^{-1}, C2=50 mg FW ha^{-1}, C0=no Compost; TA=Panoramix, TB=Trianum-P, NoT=no bio-promoters. Bars indicate ± standard errors (n = 3). Mean values with the same letter do not differ according to the LSD test (p<0.01).

The N uptake of grass species is showed in Fig.21.
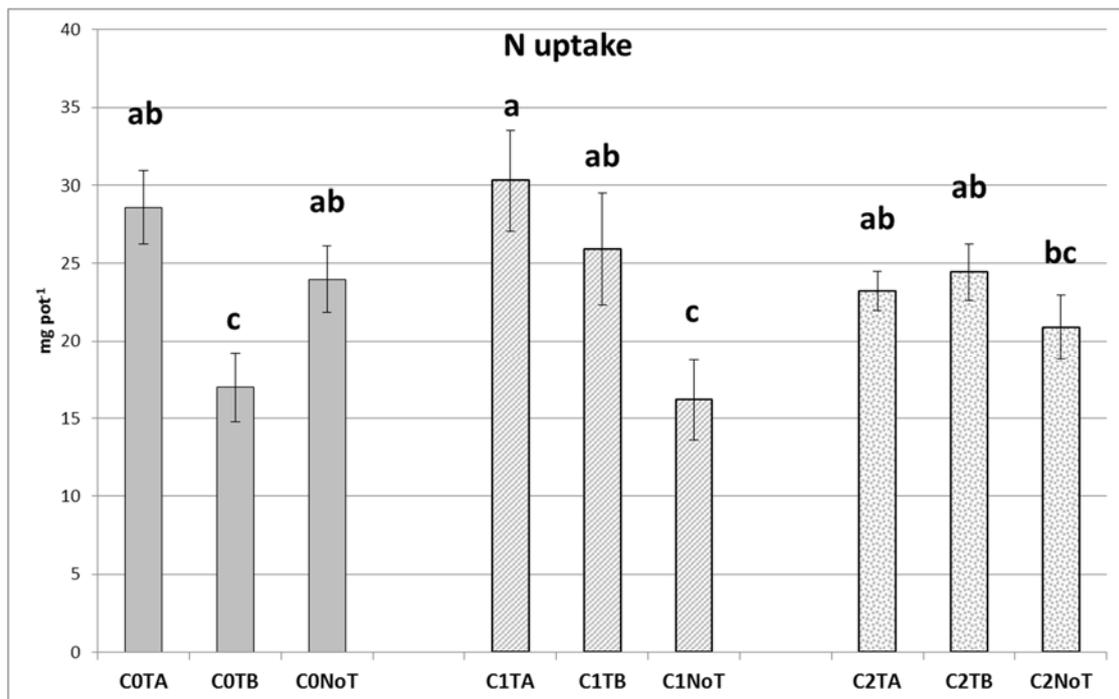
Fig. 21. N uptake by plants (**effect of the interaction fertilization x biopromoters**) using two doses of composts and two biopromoters.
C1=25 mg FW ha^{-1}, C2=50 mg FW ha^{-1}, C0=no Compost; TA=Panoramix, TB=Trianum-P, NoT=no bio-promoters. Bars indicate ± standard errors (n = 3). Mean values with the same letter do not differ according to the LSD test (p<0.01).

The average effect of biopromoters was significant ($p<0.01$) on plant biomass production reporting a higher biomass production and N uptake with TA as compared to the control without biopromoters, but the interactions showed a different behaviour linked to the fertilization (Fig 20, Fig. 21). The biopromoters effect was moderate without differences with TA and with a biomass reduction and N uptake with TB application as compared to control. The lower production of TB is not common for strain T22 of *T. harzianum* generally associated to increased plant growth, N uptake and controls diseases but are coherent with results reported by Harman (2006) for T22 on growth of plants belonging to *Poaceae* family.

A synergic effect of the lower compost dose and biopromoters was recorded with an increased plant biomass and N uptake in TA and TB compared to NOT that showed a biomass reduction when compost was applied. The highest compost dose had no effect on crop performance compared with C0NoT. Increased C availability for the fungus due to root exudation and compost enhanced biostimulation of the host plant increasing water and nutrient uptake from the soil (Agarwal et al., 2017) while the growth and N uptake reduction in C1NoT can be due to the immobilization of N by the compost as reported by Alluvione et al. (2013).

5.2.2 PTEs in plants and soil bioavailable PTEs concentrations

The analysis of variance for PTEs concentration and uptake of grass species is showed in Tab. 38.

Tab. 38. Analysis of variance for the PTEs content and uptake by plants.

Factors	Pb content (mg kg⁻¹)	Pb uptake (mg pot⁻¹)	Cd content (mg kg⁻¹)	Cd uptake (mg pot⁻¹)
S (species)	0.860	**0.039**	**0.002**	0.217
C (compost)	0.832	0.949	**0.007**	0.221
T (biopromoter)	0.114	0.340	0.702	**0.025**
SxC	0.588	0.374	0.696	0.051
SxT	0.348	0.237	0.999	0.618
CxT	0.113	0.907	0.315	**0.002**
SxCxT	0.107	0.151	0.176	0.846

Bold values indicates significance per $P<0.05$.

PTEs concentrations in plants are shown in Tab. 39

Tab. 39. Main factors effects on PTEs content and uptake by plants (mean values ± standard error).

Principal Factors	Pb content (mg kg^{-1})	Pb uptake (mg pot^{-1})	Cd content (mg kg^{-1})	Cd uptake (mg pot^{-1})
Plant species				
D	591 ± 79	0.27 ± 0.03 **a**	67 ± 3.1 **b**	0.03 ± 0.002
M	422 ± 50	0.18 ± 0.02 **b**	83 ± 3.9 **a**	0.04 ± 0.002
Compost				
C0	495 ± 64	0.21 ± 0.03	85 ± 5.2 **a**	0.04 ± 0.003
C1	566 ± 113	0.24 ± 0.04	76 ± 3.8 **ab**	0.04 ± 0.003
C2	458 ± 67	0.22 ± 0.03	65 ± 5.1 **b**	0.03 ± 0.003
Biopromoters				
NoT	665 ± 11	0.27 ± 0.05	75 ± 4.8	0.03 ± 0.003
TA	440 ± 60	0.23 ± 0.03	77 ± 4.4	0.04 ± 0.003
TB	411 ± 51	0.17 ± 0.01	73 ± 5.0	0.03 ± 0.003
Mean	506	0.22	75	0.03

C1=25 mg FW ha^{-1}, C2=50 mg FW ha^{-1}, C0=no Compost; D= *Dactlys glomerata*, M=mix of microthermal species. TA=Panoramix, TB=Trianum-P, NoT=no biopromoters. Mean values with the same letter do not differ according to the LSD test (p<0.05). Mean values without letters indicates no significance at p<0.05

Both plant species showed a high concentration of PTEs in aerial part.

Dactylis glomerata reported a higher concentration of Pb than the mix and a higher uptake while the mix of microthermal plants showed the higher concentration of Cd as compared to *Dactylis glomerata*. Both plant species accumulated Pb above above legal PTEs thresholds of forage crops (REG UE N. 1275/2013 - 1.0 mg kg^{-1}) and were similar to the values reported by Kabata-Pendias (2011) for plants growed in battery manufacturers sites (931 mg kg^{-1}). The two plant species accumulated Cd above legal PTEs thresholds for forage (REG UE N. 1275/2013 - 1.0 mg kg^{-1}) and were higher than the values reported by Kabata-Pendias (2011) for grass plants growing in metal processing sites (8.2 mg kg^{-1}). Fertilization treatments did not affect Pb concentrations in plants, but the biopromoters TA and TB reduced the Pb concentration. This result may reflect a dilution effect as a result of the increased plant biomass accumulation (Houben et al. 2013). The mean effect of compost fertilization reported a reduction of Cd concentration in plants while Cd uptake was not affected by the same treatments.
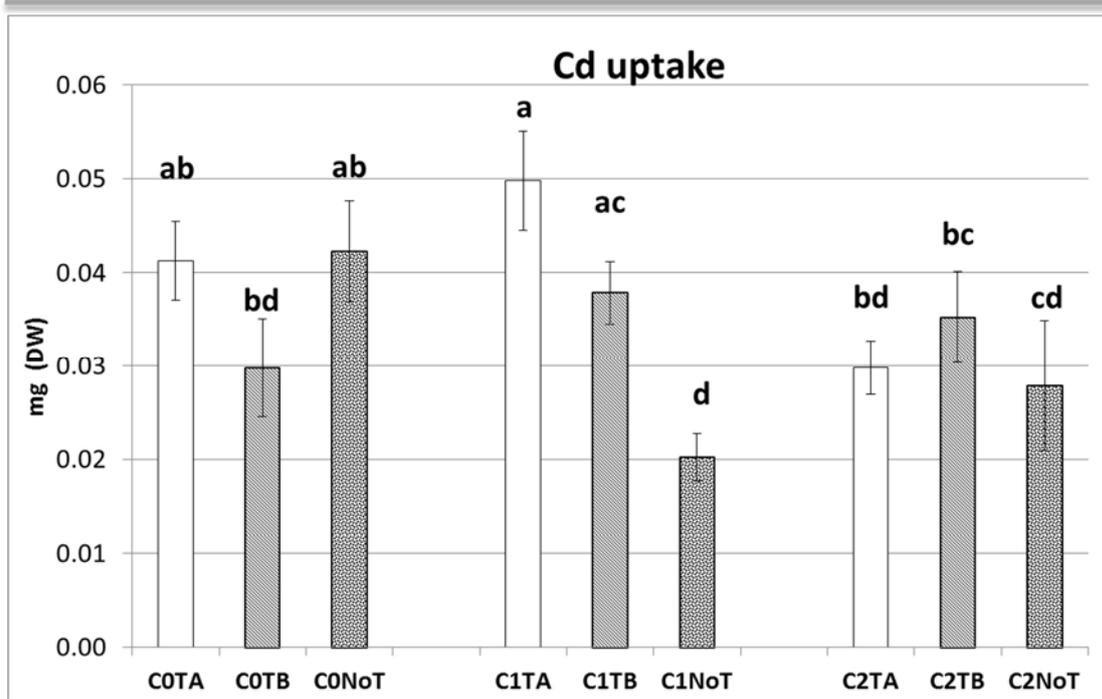
Fig. 22. Cd uptake (mg pot^{-1}) of plants **(effects of the interaction fertilization x biopromoters)**.
C1=25 mg FW ha^{-1}, C2=50 mg FW ha^{-1}, C0=no Compost; TA=Panoramix, TB=Trianum-P, NoT=no bio-promoters. Bars indicate ± standard errors. Mean values with the same letter do not differ according to the LSD test (p<0.01).

The compost by biopromoter interaction (Fig. 22) highlights that the decrease in Cd uptake due to compost fertilization was significant only in plants grown without biopromoters. This result are probably be linked to the release of chelating compounds (e.g. organic ac-ids) by *Trichoderma* that can increase PTEs bioavailability as well Cd uptake (Kacprzak et al. 2014); on the contrary compost application can exert an opposite effect by formation of insoluble organometallic complexes in the soil that reduced the mobility of Cd and in-crease Cd uptake (Achiba et al., 2009 and Pardo et al., 2014).

The analysis of variance for PTEs concentration and uptake of grass species is showed in Tab. 40.

Tab. 40. Analysis of variance for bioavailable PTEs extracted in ammonium nitrate and water.

Factors	Ammonium nitrate soluble (mg kg^{-1})		Water soluble (mg l^{-1})	
	Pb	Cd	Pb	Cd
S (species)	0.263	**0.039**	0.206	0.780
C (compost)	0.323	0.949	0.606	0.103
T (biopromoter)	**0.048**	0.340	0.931	0.692
SxC	0.263	0.374	0.297	**0.005**
SxT	0.515	0.237	0.529	0.171
CxT	0.357	0.907	0.230	**0.012**
SxCxT	0.822	0.151	0.376	0.068

Bold values indicates significance per P<0.05.

The average values of main factors of bioavailable Cd and Pb in water are reported in Tab. 41

Tab. 41. Main factors effects on bioavailable PTEs extracted in ammonium nitrate and water (mean values ± standard error).

Principal Factors	Ammonium nitrate soluble (mg kg^{-1})		Water soluble (mg l^{-1})	
	Pb	Cd	Pb	Cd
Plant species				
D	31.9 ± 1.4	2.24 ± 0.07 **a**	0.89 ± 0.07	0.13 ± 0.01
M	33.6 ± 1.2	2.19 ± 0.04 **b**	0.76 ± 0.07	0.13 ± 0.01
Compost				
C0	34.8 ± 1.8	2.29 ± 0.05	0.70 ± 0.06	0.13 ± 0.01
C1	31.7 ± 0.8	2.18 ± 0.07	0.84 ± 0.08	0.14 ± 0.01
C2	31.8 ± 2.4	2.17 ± 0.14	0.94 ± 0.12	0.11 ± 0.02
Biopromoters				
NoT	33.3 ± 2.3 **ab**	2.27 ± 1.42	0.83 ± 0.11	0.13 ± 0.02
TA	29.8 ± 2.1 **b**	2.10 ± 1.31	0.82 ± 0.09	0.12 ± 0.02
TB	35.1 ± 2.3 **a**	2.27 ± 1.74	0.80 ± 0.08	0.13 ± 0.02
Mean	32.8	2.21	0.83	0.13

C1=25 Mg FW ha^{-1}, C2=50 Mg FW ha^{-1}, C0=no Compost; D= *Dactlys glomerata*, M=mix of microthermal species. TA=Panoramix, TB=Trianum-P, NoT=no biopromoters. Mean values with the same letter do not differ according to the LSD test (p<0.05). Mean values without letters indicates no significance at p<0.05

On the average TB application increased Pb phytoavailability as compared to TA. This behaviour can be linked to the different composition of two biopromoters: TB including only *Trichoderma* can be able to increase bioavailability through the release of chelating compounds or organic acids (Kacprzak et al. 2014). TA including also mycorrhizae can have the opposite effect contributing to the immobilization of PTEs in the soil with the immobilization of metals by compounds secreted by the fungus, precipitation in polyphosphate granules in the soil, adsorption to fungal cell walls, and chelation of PTEs inside the fungus as reported by Agarwal et al (2017) and Gaur and Adholeya (2004).

Compost limiting effect on plant Cd uptake (Fig. 22) matches with the concentration of bioavailable Cd in water (Fig.23) since the first compost dose reduced the concentration of bioavailable Cd probably due the formation of insoluble organometallic complexes in the soil.
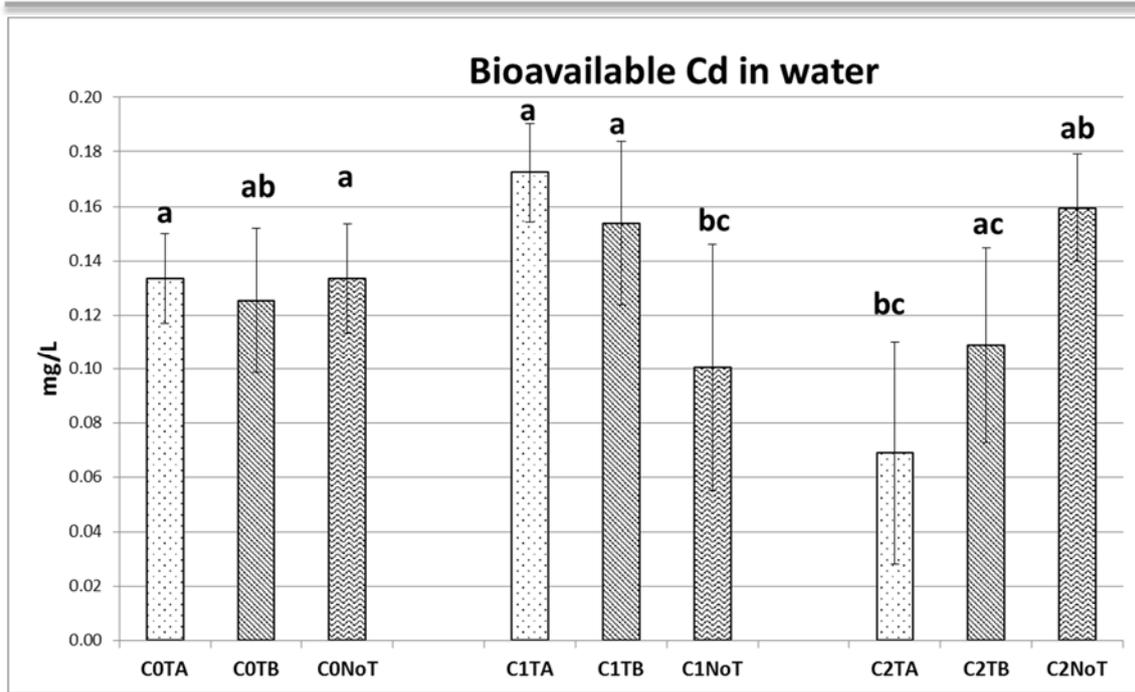
Fig. 23. Extractable Cd (mg l^{-1}) in water **(effects of the interaction fertilization x biopromoters)**. C1=25 mg FW ha^{-1}, C2=50 mg FW ha^{-1}, C0=no Compost; TA=Panoramix, TB=Trianum-P, NoT=no bio-promoters. Bars indicate ± standard errors. Mean values with the same letter do not differ according to the LSD test (p<0.01).

Pb and Cd soluble concentration in ammonium nitrate was compared to the bare soil and to the initial soil samples while Pb and Cd soluble concentration in water was only compared to the bare soil.

The analysis of variance for PTEs concentration and uptake of grass species is showed in Tab. 42.

Tab. 42. Analysis of variance for percentage change of Pb and Cd concentrations in ammonium nitrate from the bare soil and from initial soil samples

	Percentage change from bare soil		Percentage change from initial bioavailability	
Factors	Pb	Cd	Pb	Cd
S (species)	0.336	0.636	0.336	0.636
C (compost)	0.263	0.518	0.263	0.518
T (biopromoter)	**0.048**	0.266	**0.046**	0.266
SxC	0.281	0.622	0.281	0.622
SxT	0.540	0.967	0.540	0.967
CxT	0.242	0.964	0.242	0.964
SxCxT	0.775	0.732	0.775	0.732

Bold values indicates significance per P<0.05.

The average values of main factors of the percentage change of Pb and Cd concentrations in ammonium nitrate are reported in Tab. 43.

Tab. 43. Main factors effects on percentage change of Pb and Cd concentrations in ammonium nitrate from the bare soil and from initial soil samples (mean values ± standard error).

Main Factors	Percentage change from bare soil		Percentage change from initial bioavailability	
	Pb	Cd	Pb	Cd
Plant species				
D	17.0 ± 5.0	-14.4 ± 2.9	-3.6 ± 4.1	-3.2 ± 3.3
M	23.1 ± 4.3	-16.1 ± 1.7	1.5 ± 3.5	-5.2 ± 1.9
Compost				
C0	27.5 ± 6.7	-12.3 ± 2.0	5.1 ± 5.5	-0.8 ± 2.2
C1	16.3 ± 3.0	-16.7 ± 2.9	-4.2 ± 2.5	-5.8 ± 3.3
C2	16.4 ± 6.2	-16.8 ± 3.6	-4.1 ± 5.2	-5.9 ± 3.9
Biopromoters				
NoT	22.1 ± 2.6 **a**	-13.1 ± 4.3	0.6 ± 4.3 **a**	-1.7 ± 3.0
TA	9.1 ± 2.9 **b**	-19.8 ± 3.9	-10.1 ± 3.9 **b**	-9.2 ± 3.1
TB	28.8 ± 6.4 **ab**	-13.2 ± 3.0	6.1 ± 5.3 **a**	-1.8 ± 3.3
Mean	20.04	-15.30	-1.09	-4.20

C1=25 Mg FW ha^{-1}, C2=50 Mg FW ha^{-1}, C0=no Compost; D= *Dactlys glomerata*, M=mix of microthermal species. TA=Panoramix, TB=Trianum-P, NoT=no biopromoters. Mean values with the same letter do not differ according to the LSD test ($p<0.05$). Mean values without letters indicates no significance at $p<0.05$

Percentage change of Pb and Cd concentrations in ammonium nitrate from the bare soil and from initial soil samples showed no differences, but the average effect of biopromoters application showed significant differences in Pb concentration both from the initial and the no plants control. Bioavailability of Pb was higher than bare soil probably for the processes that happened in the rhizosphere but there was an effective reduction of the soluble fraction of Pb with TA application as compared to control and the same biopromoters reported an average reduction of 10% from the initial soluble Pb while TB was similar to control. This phenomenon can be related, as said before, to the different composition of two biopromoters: TB can be able to increase bioavailability through the release of chelating compounds of organic acids (Kacprzak et al. 2014). TA including also mycorrhizae can have the opposite effect contributing to the immobilization of PTEs in the soil with the immobilization of metals by compounds secreted by the fungus, precipitation in polyphosphate granules in the soil, adsorption to fungal cell walls, and chelation of PTEs inside the fungus as reported by Agarwal et al (2017) and Gaur and Adholeya (2004). Cd soluble fraction in ammonium nitrate did not show differences between the treatments, but the main factors reduced the Cd soluble fraction up to 15% from the bare soil and 4% from the initial values.

5.3 Conclusions

Autochthonous and commercial plant species were well adapted to the high contamination of this industrial soil showing a good growth during the experimental period.

The combined application of the lowest compost dose (25 mg FW ha^{-1}) and biopromoters increased plant growth, N uptake and Cd uptake. The application of TA reduced the bioavailable Pb as compared to bare soil and to TB and reduced the bioavailable Pb from the initial conditions and the bare soil. The combination of compost and biopromoters also reduced the Cd soluble fraction as compared to bare soil highlighting the importance of a vegetal soil cover for limiting PTEs leaching in the soil profile.

6. GENERAL CONCLUSIONS

From the results of the three experiments described in this thesis, it's possible to achieve the following conclusions.

From the first experiment regarding the analysis of natural plants and soils for the characterization of two potentially contaminated sites, we concluded that:

1. Phytoscreening of native plants of polluted sites can be carried out to identify plant species that can tolerate very high PTEs concentrations and can be used for phytoremediation.

2. Bioaccumulation coefficient of shoots (BACs), Bioaccumulation coefficient of roots (BAC$_R$) and a modified bioaccumulation coefficient (mBAC) that considers only the bioavailable fractions of contaminants (DTPA), can be used for assessing the relationships between weeds and the contaminants into the soil.

3. *Artemisia vulgaris* and *Dittrichia viscosa* in industrial site were able to transfer the potential bioavailable fraction of Pb to the aerial part and thus they could be considered interesting candidates for phytoextraction; *Epilobium tetragonum*, *Artemisia annua* and *Silene latifolia* reported a high mBAC$_R$ so they could be suitable for Pb phytostabilization. In the agricultural site, *Cyperus rotundus* showed the ability to transfer the potential bioavailable fraction of Cd to the aerial part, so it could be interesting for phytoextraction purpose while *Cirsium arvense*, *Cynodon dactylon*, *Rumex sp.*, *Piptatherum thomasii* and *Echium vulgare* reported the ability to transfer Cd to the roots with a high mBAC$_R$ so they could be suitable for Cd phytostabilization;

4. PCA and HCA can be used to identify the different PTE pollution sources. Principal component analysis and hierarchical cluster analysis suggest that Pb, Cd, As, Cu and Zn seems form different pollution sources while Sb and Tl seems mainly of geogenical origins. In the agricultural site, Cr and Cd are considered of anthropogenic origins while Pb, As and Zn mostly from geogenical origins;

5. It is possible to identify the extraction method that better represents the metal bioavailability and to know the relation between specific plant species and soil contamination. In the industrial site, only the Pb and Sb shoot concentrations well predicted by DTPA extraction. The ammonium nitrate extraction well predicted the shoot content of Pb, Sb, and Tl. The total soil PTEs content (TPTEs) were satisfac-

torily related to Sb and Tl shoot concentrations and in minor part to Pb. In agricultural site, TPTEs well predicted only the Zn shoot concentration.

6. *Elymus. repens* in industrial site can be used as indicator of soil Zn contamination while *Lolium perenne* in the agricultural site, can be used as indicator of As and Cd contamination.

The second experiment, made on a potentially contaminated soil (Pb, Zn) allowed to get the following conclusions:

1. Application of compost and Biopromoters increased the plant growth, nutrient and Zn uptake;

2. The plant species used in this experiment resulted suitable for a phytostabilization purpose reducing the leaching of PTEs in the soil profile. Furthermore the biomass taken from the harvest can be used in no food chains and the complete coverage of the soil prevents the dispersion of the contaminated soil particles, thus allowing to reduce the risk for human health.

The third experiment, made on a highly contaminated soil (Pb, Cd), allowed to get the following conclusions:

1. The application of first dose of compost and biopromoters increased the growth, N uptake and Cd uptake;

2. The application of TA (consortium called "Panoramix" – Koppert b.v. ® containing Endomycorrhiza and Trichoderma species along with humic and fulvic acids) reduced the bioavailable Pb as compared to control and to TB and reduced the bioavailable Pb from the initial conditions and the bare soil.

3. The combination of compost and biopromoters reduced the Cd soluble fraction as compared highlighting the importance of a vegetal soil cover for avoiding PTEs leaching in the soil profile.

4. The combination grass species-organic amendments-biopromoters can be successfully used in a phytoremediation project increasing the biomass production and PTEs uptake. The grass species can ensure the soil capping necessary for securing contaminated sites, but also for improving soil structural aggregation that also prevents the dispersion of the contaminated soil particles.

5. The differences in the results between Pb and Zn in the second experiment and Pb and Cd in the third experiment highlighted the need to make preliminary studies to found the most suitable technique in relation to the specific contamination of a site.

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