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Etude de l'adaptation de *Miscanthus* x *giganteus* pour la revalorisation d'un ancien site minier fortement contaminé

Potential adaptation of *Miscanthus* x *giganteus* for the phytoremediation of a former mine site highly contaminated

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Liste des abréviations

Al : Aluminium
As : Arsenic
Bi : Bismuth
Ca : Calcium
Cd : Cadmium
Co : Cobalt
Cu : Cuivre - Copper
Fe : Fer - Iron
In : Indium
K : Potassium
Mg : Magnesium
Mn : Manganese
Na : Sodium
Ni : Nickel
PAH : Hydrocarbures Aromatiques Polycliques - Polycylic Aromatic Hydrocarbon
PCB : Polychlorobyphényles - PolyChlorinated Biphenyl
Pb : Plomb - Lead
Si : Silicium
Sb : Antimoine - Antimony
Zn : Zinc
DW : Dry weight

INTRODUCTION GENERALE

A l’interface avec l’atmosphère, la biosphère, l’hydrosphère et la lithosphère, le sol possède un rôle majeur pour la faune et la flore dans les écosystèmes tout en étant le support des activités humaines. Il joue un rôle irremplaçable à la surface du globe (concept de « critical zone », Brantley *et al.*, 2006) et, tout en évoluant, garde aussi en mémoire l’Histoire des civilisations et des cultures (Gobat *et al.*, 2010). Depuis le début de l’industrialisation au 19^e siècle, le développement des activités anthropiques s’est accompagné de contaminations de sols à des niveaux et de natures variables, dont les risques sanitaires, aussi bien pour l’Homme que pour l’ensemble des espèces vivantes, ne sont pas encore toujours bien maîtrisés.

De toutes les sources de contamination que sont les activités industrielles, incinérateurs, trafic routier, l’extraction minière, par la production inéluctable de déchets souvent fortement contaminés en éléments métalliques et/ou métalloïdes, est l’une de celles étant à l’origine de contaminations environnementales et en particulier des sols (soit par retombées atmosphériques, soit par dépôts de déchets). De ce fait, la plupart des pays ont mis en place une législation de réhabilitation des sites miniers plus ou moins précise et exigeante (Le Roux, 2002). Quelle que soit la législation, la nature de la réhabilitation doit être adaptée à la configuration du site en tenant compte de la nature et du niveau de la contamination.

Les sites pollués ne peuvent être ignorés et sont à décontaminer pour plusieurs raisons. Ils représentent une source de contamination diffuse pour les eaux superficielles et souterraines par lessivage ainsi que pour l’atmosphère sous forme de particules éoliennes. L’être humain en sera la cible, soit par la filière alimentaire, soit par la voie pulmonaire. De plus, une pénurie de sols de qualité pour la production alimentaire d’une population mondiale en croissance s’annonce, occasionnée, entre autres, par la « bétonisation » de nos sols, également en Europe, et par la production, au niveau mondial, de plantes à vocation bioénergétiques, suite à des décisions politiques. Il est essentiel de réhabiliter les sites pollués vers la production, soit alimentaire lorsque la qualité des sols le permet, soit bioénergétique sur les sols de moindre qualité. Pour cela, il est donc nécessaire de développer des connaissances sur les pollutions, leurs effets, leur maîtrise.

Contrairement aux pollutions organiques, il n'existe pas de voie de dégradation biologique possible (notamment par les micro-organismes) pour les éléments métalliques et métalloïdes et les techniques classiques de dépollution (l'excavation par exemple, …) sont souvent très chères et peu respectueuses de l'environnement. Actuellement, les solutions de dépollution passent de plus en plus par l'utilisation de plantes ; c'est ce qu'on appelle la phytoremédiation. Ce concept fut proposé au milieu des années 90 et présenté dans une revue par Salt *et al.* (1998). Il s'agit d'une technique peu coûteuse, utilisant les plantes et visant à limiter la dispersion de la contamination aux différents compartiments environnementaux ainsi qu'à la chaîne alimentaire. Parmi les différents types de phytoremédiation (phytoextraction, phytodégradation, phytostabilisation, phytovolatilisation et rhyzofiltration), on distingue deux principes majeurs impliquant les végétaux : la phytoextraction et la phytostabilisation des polluants inorganisques.

La phytoextraction consiste en l'accumulation et la concentration des polluants dans la partie aérienne récoltable de plantes à croissance rapide qui tolèrent bien la contamination (Salt *et al.,* 1998). Les plantes les plus efficaces pour la phytoextraction sont les plantes hyperaccumulatrices comme *Thlaspi caerulescens,* hyperaccumulatrice du nickel, du zinc et du cadmium, mais qui produit très peu de biomasse et ne sera, par conséquent, pas une solution satisfaisante dans la pratique. D'autre part, cette technique n'est envisageable que pour les situations de contamination modérée et sur une profondeur n'excédant pas 50 cm (Vangronsveld *et al.*, 2009).

La phytostabilisation, par la culture de plantes tolérantes aux contaminations, n'est pas une technique de nettoyage et de décontamination à proprement parler, mais plus une stratégie de management des sites contaminés visant à stabiliser les éléments potentiellement toxiques (EPTs – Eléments Potentiellement Toxiques) en vue de limiter les phénomènes de transferts vers les eaux souterraines, de diminuer leur biodisponibilité pour les végétaux (Vangronsveld *et al.,* 2009) et par conséquent de limiter la contamination de la chaine alimentaire.

L'extraction minière est l'une des principales activités productrice de déchets souvent à haute teneur en EPTs. En effet, le procédé d'extraction d'un minerai s'accompagne de l’extraction de la roche encaissante susceptible d’être composée de phases minérales riches en éléments métalliques et métalloïdes formant ensuite le déchet minier. Ce dernier est alors stocké et constitue le stérile. Dans le cas de l’extraction d’or, le métal extrait est souvent associé à des arsénopyrites riches en arsenic devenant par conséquent l’un des éléments potentiellement toxiques du déchet minier. L’altération des matériaux résultant de cette extraction et par conséquent des phases primaires conduit à la formation de phases secondaires potentiellement plus stables dans les conditions supergènes.

Dans les milieux contaminés, l’activité des micro-organismes, une condidtion *sine quanone* du fonctionnement des sols, est susceptible d’être altérée. Ces micro-organismes jouent notamment un rôle fondamental dans le cycle des nutriments et dans la stabilisation des sols, par conséquent, leur protection est assimilable à celle de la qualité des sols. Ils interviennent dans le cycle de l’azote et en particulier dans la nitrification. La nitrification étant la formation des nitrates (NO_3^-, macro-nutriments des végétaux) par oxydation de l’ammonium et avec comme étape intermédiaire la formation de nitrite (NO_2^-), elle présente donc un rôle essentiel pour le fonctionnement des sols. L’activité des bactéries impliquées dans la nitrification est connue depuis longtemps mais récemment, le rôle des archées (Leininger et *al.*, 2006) a également été mis en évidence par l’étude du gène *amoA*, présent chez ces deux populations.

Dans un contexte de réductions des gaz à effet de serre et d’un besoin croissant de trouver des énergies alternatives au pétrole, les biomasses végétales comme matériel de combustion pour la transformation d’énergie sont de plus en plus répandues en Europe. Ainsi, pour la phytoremédiation de notre site d’étude non apte à la production de plantes alimentaires, le choix s’est porté sur la graminée *Miscanthus* x *giganteus* originaire d’Asie du Sud Est et s’adaptant bien au climat des latitudes européenne en produisant 15 à 25 t de biomasse à l’hectare (selon le taux d’humidité à la récolte). La décontamination des polluants inorganiques serait associée à une utilisation économiquement et écologiquement raisonnée de la plante.

L’ensemble de cette étude portera sur les sols développés à partir des déchets miniers d’une ancienne mine d’or, entreposés sur un site naturel, fortement contaminés en métaux et métalloïdes, en particulier en arsenic (As) mais également en plomb (Pb) et en antimoine (Sb).

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CHAPITRE I

Literature review

The risks of food chain and ground water contamination besides the biological functioning of the soil itself are the main consequence of soil contamination. Anthropogenization of soils may lead to various degradations such as contamination by inorganic elements and organic molecules. Thus, these types of soils have composition and properties which differ from natural soils. Since several years, soil classifications include their description which will be detailed here.

The following paragraphs describe the mining environments and soil contamination by metallic elements and metalloids. The focus is given on metals and metalloids contamination of the soil-plant system with the aim of assessing the transfer of these elements to plants. After giving an overview on the methods of remediation of contaminated soils, another paragraph outlines the recent development of science on this topic. The phytoremediation of soils and the use of *Miscanthus* x *giganteus* are described in more details as a large part of the thesis focuses on this topic.

I. Mining environment

I.1 Mining exploitation

Mining exploitation is basically the extraction of ores and mineral raw materials which are used in metallurgy and other technologies. Important ores that are extracted are Zn, Pb, Cu, Cr, Ni, Fe, U, Au, Ag and Sn. These elements are included in rocks, mainly in the form of veins in the bedrock. For example, in Portugal, Silurian phyllites host Sb and Pb-Sb-quartz veins (Bragança district); in the region of Dúrico-beirã, Sb-Au-quartz are mainly hosted by schist metagraywacke complex as well as Ordovician phyllites and quartzites (Neiva *et al.*, 2008). Besides metals, coal is also another resource which is extracted in mines.

Depending on the extraction process, solid wastes are usually accumulated in tailings near the mine. Moreover, some extraction processes also use water and other liquids leading to wastes in the form of liquid muds. In this case, mining wastes are generally evacuated in a settling basin. A settling basin allows the collection of all these muddy wastes to prevent their spreading out in the environment. In some cases, the extraction process generates tailings as well as muds stored in a settling basin. All these wastes (tailings, settling basin, etc) induce environmental risks. For example, in Hungary, at the end of 2009 in the aluminum plant, about 700 000 m^3 of red mud rich in metallic elements, As, as well as cyanide have been spread out causing serious contamination of all the surrounding ecosystems.

I.2 Contamination sources

Mining activities produce huge amounts of wastes which are usually contaminated at different levels and might represent a local source of contamination. Indeed, the bearing phases forming the bedrock potentially containing metallic elements or metalloids might be weathered, leading to the formation of secondary phases which might be particularly harmful for ecosystems and human health. For example, at a mine site in NovaScotia, Canada, the gold deposit, naturally rich in arsenopyrites (FeAsS). In the near-surface of the tailing, sulphide minerals been have completely oxidized in scorodite ($FeAsO_4$, $2H_2O$) and Ca-Fe arsenate as well as iron oxyhydroxydes. Therefore, toxic elements remains in the secondary bearing phases present in the tailing (Corriveau *et al.,* 2011). Moreover, depending on the extraction process, elements supposed to be extracted can remain in wastes increasing their toxicity by oxidation leading acid mine drainage. The fact that elements remain in the wastes happened especially in the past when the extraction process was not optimal. Pollutants may also remain in tailings because they were not the targeted elements. Indeed, in the gold extraction, As is usually associated to the extracted rock and then remain in the wastes although gold is the only targeted ore. The management of contaminated wastes is a key point for the environment protection in order to prevent any pollution spreading. Even if solutions of wastes management exist, pollutions can spread out by different ways: tailings, waters, air, sediments, soils, risking contaminating the food chain and hence to affect human health.

I.3 Regulations of mining sites

National and international laws classify soil pollution by defining threshold values of elements or pollutants concentrations. Sites that are classified as polluted sites have to be managed according to the laws and directives (for example the Directive 2006/12/CE, European Parlement and Consil). In France, the laws regulating polluted sites are rather well established and describe all the steps required in the management of these kinds of sites. Moreover, the law entitled 'circulaire du 08/02/2007' suggests performing an analysis of the initial state of the site in order to adapt the risks prevention. During the exploitation, the prevention aims at anticipating pollutions of the environment in the area of the exploited site. The owner has to set up a survey of emissions impacts on the environment. In case of pollution, it is of major concern to be able to act in order to limit serious contaminations and their spread out.

In case of the activity cessation, a specific regulation exists as well, and is the object of another law, 'circulaire du 03/12/93'. It implies a realistic treatment of contaminated soils and sites by the evaluation of risks and of the vulnerability of each site.

II. Soil contamination with metallic elements and metalloids

II.1 Origin of elements in soil

II.11 Natural origin

Under natural conditions soil evolution results from the alteration of bedrock and degradation of organic matter which is interacting with the mineral fraction. Soil is the interface of dynamic exchanges between hydrosphere, biosphere, atmosphere and geosphere (Gobat *et al.*, 2010). All these interactions provide soils a key role in the dynamic of elements. Indeed, the pedogenetic processes lead to formation of components such as clays, Fe-, Al- and/or Mn oxides and hydroxides as well as soil organic matter, which regulate inorganic pollutants mobility and availability.

The natural pedogeo-chemical concentrations can be defined as the concentration of elements resulting only from geological and pedological processes, excluding all anthropogenic inputs.

As a result, according to the local bedrock, theses concentrations markedly differ according to the nature of the parental material and the type of developed soil (Baize, 1997). The natural pedo-geochemical concentrations may also vary from a horizon to another one according to processes of alteration, lixiviation, migrations, redistributions.

II.12 Anthropogenic origin

Human activities did not change the global amount of metallic elements and metalloids on Earth but mainly modified their distribution as well as their chemical form and their concentrations.

The main contamination sources are:

- Atmospheric deposits in the vicinity of industries or linked to cars circulation. These deposits reach soils, waters and plants. Concentrations are the highest near the emissions source.
- Chemical and organic fertilization
- Pesticides
- Sewage sludge and waste waters
- Mining activities.

The contamination of soils caused by atmospheric depositions in the form of dusts or aerosols is often associated to acid rains (Probst *et al.,* 1992). Persistent organic pollutants such as PAH and PCB may also be introduced under the form of atmospheric deposits (Motelay-Massei *et al.,* 2004). It was shown that the atmospheric pollutants can spread over very large distances as well (for example, from areas in Europe to Southern Norway) (Steinnes *et al.,* 1997) and therefore contaminate soils which are not directly influenced by human activities. Another example is illustrated by Douay *et al.* (2008) describing the urban soil contamination generated by 2 smelters in Northern France (Mateleurop Nord and Umicore) in Cd, In, Pb, Sb and Sb and in lower concentrations in Ag, Bi, Cu and Hg.

Under the direct influence of humans**,** the situation can be different with respect to the quality and quantity of contamination. Since Neolithic, mining and metallurgical activities led to soils contamination by metallic elements and metalloids. More recently, paleo metallurgical activities in medieval period produced contaminated slags which still represent a contamination source for soils in the Mont-Lozère (France) (Baron *et al.*, 2006).

Today, many other human activities such as for example agriculture are also a source of contamination. Use of soils is generally connected to the supply of the soil with other materials, for example by fertilization and the use of pesticides which can persist or degrade in more harmful metabolites (Arias-Estévez *et al.*, 2007). The use of inorganic fungicides Cu-based represent high contamination of vineyard soils (Komárek *et al.*, 2010). Indeed, Cu is usually immobilized by soil organic matter and/or Fe/Mn (hydr)-oxides and hence superficial horizons easily reach 200 mg Cu.kg^{-1}. Many pesticides also contain metallic elements or metalloids such as As, Hg, Cu, Mn, Cd or Pb (Girard *et al.*, 2005, Atafar *et al.*, 2010).

Sewage sludges are also a major contamination source as they often contain metallic elements and metalloids. Thus, Denaix *et al.* (2011) demonstrated a clear increase in total elements concentrations 2 years after sewage sludge application in a forest soils, especially in topsoil (0-20 cm).

The only regulation existing about elements concentrations in soils is related to sewage sludge application. Limit concentrations in sewage sludge are fixed for the next elements: Cd, Cr, Cu, Hg, Ni, Pb and Zn. Table I.1 summarizes these concentrations.

Table I.1. Limit concentrations of few elements in sewage sludges for application in agriculture

Elements	threshold concentration (mg.kg^{-1}, DW)	Cumulated concentration in 10 years (g.m^{-2})
Cd	10	0.015
Cr	1 000	1.5
Cu	1 000	1.5
Hg	10	0.015
Ni	200	0.3
Pb	800	1.5
Zn	3 000	4.5

Another major source of soil contamination is caused by industrial emissions. Mining activities lead to soils heavily contaminated owing to dissemination of contaminated particles by wind and/or by runoff from tailings. Usually, this type of soil get a low organic matter content as well as low total N and available P levels (Boularbah *et al.*, 2006; Boussen *et al.*, 2010). Another example is the emissions of contaminated dusts by smelters which contaminate strongly the topsoils in the surrounding area. Thus, in the Zambian Copperbelt region, concentration in Co, Cu, Pb, Zn and As are 2 to 7 times higher in the smelting area. (Ettler *et al.*, 2011).

Overall, the main sectors emitting metallic elements are mining and metallurgical activities, production of paints, pigments and inks, batteries, electronic compounds. Wastes of manufactured products and incineration are also emission sources of metallic pollutions which can possibly contaminate soils by deposition. Hunting, military activities and war also spread out considerable amount of pollutants in soils.

We distinguish different kinds of pollutions: accidental or chronic pollutions, local or diffusive pollutions. Thus, accidental pollutions typically happen by accident and hence spread out pollutants. It can be for example a truck transporting dangerous substances after an accident happened. Chronic pollutions correspond to an outflow which can be low but for a long time leading finally to a serious pollution, *i.e.* a storage cistern leaking. The local pollutions are related to a localized and identified source, for example to an industrial plant. The diffusive pollutions result of pulverization on large surfaces, like in agriculture for example (www.brgm.fr). In this case, the contamination level can be low but over large surfaces.

II.2 Distribution of elements in soils

Soils harbor different kinds of compounds which can be in interaction with the pollutants. These interactions also depend on the physical-chemical properties of soils. Overall, the speciation of the pollutants is the first criteria which influence interactions with soil compounds and their mobility (Figure I.1).

II.21 Physical-chemical properties

pH and redox potential are the two main parameters which may regulate interaction between metallic and metalloid elements, and constituents of soils (Figure I.1). They are of prime importance in determining the mobility of elements.

pH

pH mainly results of the dissociation of CO_2 providing protons (H^+) arising from microbial and roots respiration. pH is probably the feature which influence the most metals mobility in soils as well as metals retention by soil components (Hooda, 2010; McLean and Bledsoe, 1992). Overall, low pHs (2-3) favor metals mobility by increasing their solubility and changing the balance between precipitation and dissolution and the redox reactions. But conversely, if elements are stable under anionic form (*i.e.* AsO_4^{3-}…), their solubility increases with the pH (Bourg and Loch, 1996).

Moreover, pH influences the surface charges of clays, organic matter, Fe-, Al-, and Mn-oxides by protonating and deprotonating the functional groups of the surface particles (Adriano, 2001).

Cappuyns and Swennen (2008) investigated the effect of pH changes on the metal release in soils. Thus, leaching of Cd, Zn, Cu, Ni, Mn, Ca and Fe increased clearly with a decreasing pH (from 6 to 2) but Cu, Ni and Fe were also leached under alkaline conditions (pH = 8-10). As a result, adsorption site in soils are pH-dependent: the number of negative sites for cations sorption declines with a decreasing pH. Furthermore, under alkaline conditions, metals might precipitate by inclusion in oxides, hydroxides, carbonates or phosphates. These authors expected a higher As solubility at high pH, as it occurs for arseniate (AsO_4^{3-}) but they also recorded high solubility at low pH. They attributed this likely to the clays dissolution at pH 2.

The redox potential

The redox potential, *Eh*, represents the airing state and moisture content of soils, and depends on agricultural practices and weather conditions. Redox reactions are due to electrons transfer between an electron donor and an electron acceptor. The redox potential represents the electrochemical potential or the availability of electron within a system (McLean and Bledsoe, 1992). Thus, low *Eh* values represent a low O_2 content and by decreasing values other electron transfers occur instead of oxygen, using nitrate, sulfur oxides or CO_2. pH and temperature are the two main parameters influencing redox potential (Bradl *et al.,* 2005) under given aeration and moisture.

Three phenomenon influence metallic elements or metalloids mobility in soils: (i) a change of the oxidation state of the element itself, (ii) a change of the oxidation state of an element linked to the inorganic element forming a soluble complex and (iii) formation or dissolution of bearing phases containing the inorganic pollutant(s) (Bourrelier and Berthelin, 1998).

An Eh change influences directly solubility and mobility of elements, *i.e.* the reduction of AsV into the more mobile form, AsIII (Wilson *et al.,* 2010). Thus, a change in the oxidation state leads to precipitation or dissolution of metals. The components which are the most sensitive to *Eh* changes are Fe-, Al- and Mn-oxi-hydroxides and phases fixing metals, capable to release these elements. Under oxidative conditions, inorganic pollutants might be released by oxidation. *Eh* conditions also influence seriously the sulfur status. Thus, under reducing conditions, metals are removed from solution as sulfides minerals (Bradl *et al.,* 2005). At mining sites this is one process that contributes to the pollution of mine drainage waters (EPA, 2011).

Chuan *et al.,* (1995) demonstrated that, at constant pH values, metal solubility increased with a decrease in redox potential. Thus, acidic and reducing conditions favored metal solubilization. Moreover, Frohne *et al.*, (2011) showed that reducing conditions enhanced methyl form of As and Sb in a floodplain soil. They attribute the higher mobility of Cd, Cu, Mn, Ni and Zn to the dissolution of sulfides releasing metals under oxidizing conditions.

Temperature

The temperature influences the mobility of metals in soils rather indirectly by modifying O_2 and CO_2 solubility, changing respectively *Eh* and pH status in soils. In general, the temperature is positively correlated to the reaction kinetic, particularly biological kinetic by influencing microbial growth and activities. Besides the influence on biological processes, temperature influences O_2 and CO_2 solubility and hence modifies respectively *Eh* and pH. As a result, temperature also regulates stability of the mineralogical bearing phases which are directly linked to the behavior and mobility of the inorganic pollutants in soils.

II.22 Soil constituents

Oxides and Oxy-hydroxides

Oxy-hydroxides (*e.g.* ferrihydrite) and oxides (*e.g.* hematite) are amorphous or crystallized components. Moreover, new synthesized forms result from alteration. They are mainly composed of Fe, Al, or Mn with OH groups and are known to bear metallic elements and metalloids (Bourrelier and Berthelin, 1998). Especially hydroxides and also stable oxides are able to fix metals and metalloids by exchange of OH_2 or OH^- groups. For example, Vaněk *et al.* (2008) demonstrated the affinity of Zn and Pb for Fe- and Mn-oxides whereas As had mainly affinity for Fe oxides only.

Thus, under reducing conditions and acidic pH, inorganic pollutants are released owing to the dissolution of oxides and oxy-hydroxides. As a result, this process mainly occurs in hydromorphic soils and represents a potential pollution risk (Bourrelier and Berthelin, 1998). They play a major role in the dynamic of elements depending on the physical-chemical conditions.

Organic matter

Soil organic matter consists of (i) living organisms, (ii) soluble biochemical (amino-acids, proteins, carbohydrates, organic acids, polysaccharides, lignin…) and (iii) rather insoluble humic substances. Organic matter plays a major role in metal(loid) dynamic in soils (McLean and Bledsoe, 1992). Humic acids, molecules of high molecular weight, may interact with elements in soils through: (i) formation of soluble complexes with ion metals, (ii) precipitation of metal ions by reduction or sorption processes, and (iii) modification of the sorption behavior of mineral surfaces or colloidal particles (Wood, 1996). Biochemical interactions form water soluble complexes with metals increasing their mobility (McLean and Bledsoe, 1992). On the other hand, Clemente *et al.*, (2010) demonstrated that addition of green waste compost (GWC) that supports biological activity increased solubility and mobility of both metals and metalloids increasing their plant uptake, phenomena recorded 2 years after the application of GWC.

Clays - phyllosilicates

Phyllosilicates are composed of succession of tetrahedral and octaedral sheets. Tetrahedral sheets are formed of tetrahedrons (SiO_4) whereas octaedra sheets are formed of octahedron ($Al_2(O,OH)_6$). Substitutions may occur and lead to charges deficit. Therefore, phyllosilicates are negatively charge and are able to fix metals and metalloids; *e.g.* they are strong As binding (example with bentonite) (Kumpiene *et al.*, 2008). pH do not influence the surface charges of clays. Violante and Pigna (2002) demonstrated that phyllosilicates particularly rich in Fe (*i.e.* ferruginous smectites or nontronite) were very effective in sorbing AsO_4. Overall, Dynamic between clays and elements is also very complex due to the large amount of clay structure.

Sulfur, sulfate

Acid sulfate soils usually developed from sulfur sediments. The exposition to ambient air of sulfurs bearing deposits oxides sulfur and releases acid. Here, an example is given with pyrite (FeS_2):

$FeS_2\ (s) + 15/4O_2 + 7/2H_2O \rightarrow Fe(OH)_3\ (s) + 2SO_4^{2-} + 4H^+$

Therefore, if the acid release exceeds the soil capacity to neutralize the acidity, pH may strongly decrease (between 2 and 4). Gröger *et al.*, (2011) demonstrated that As and Pb were sequestered during jarosite [$KFe^{3+}{}_3(SO_4)_2(OH)_6$] precipitation forming Pb-As-jarosite corresponding to a sink for these elements in acid sulfate soil. Therefore, elements such as As and Pb are trapped into secondary bearing phases which are thermodynamically more stable under oxidative conditions (Roussel *et al.*, 2000).

II.23 Interactions between elements and solid phase

According to physical-chemical properties and the type of constituents, many different interactions between elements and solid phase occur. The main interactions are precipitation / dissolution, and adsorption/desorption (Figure I.1).

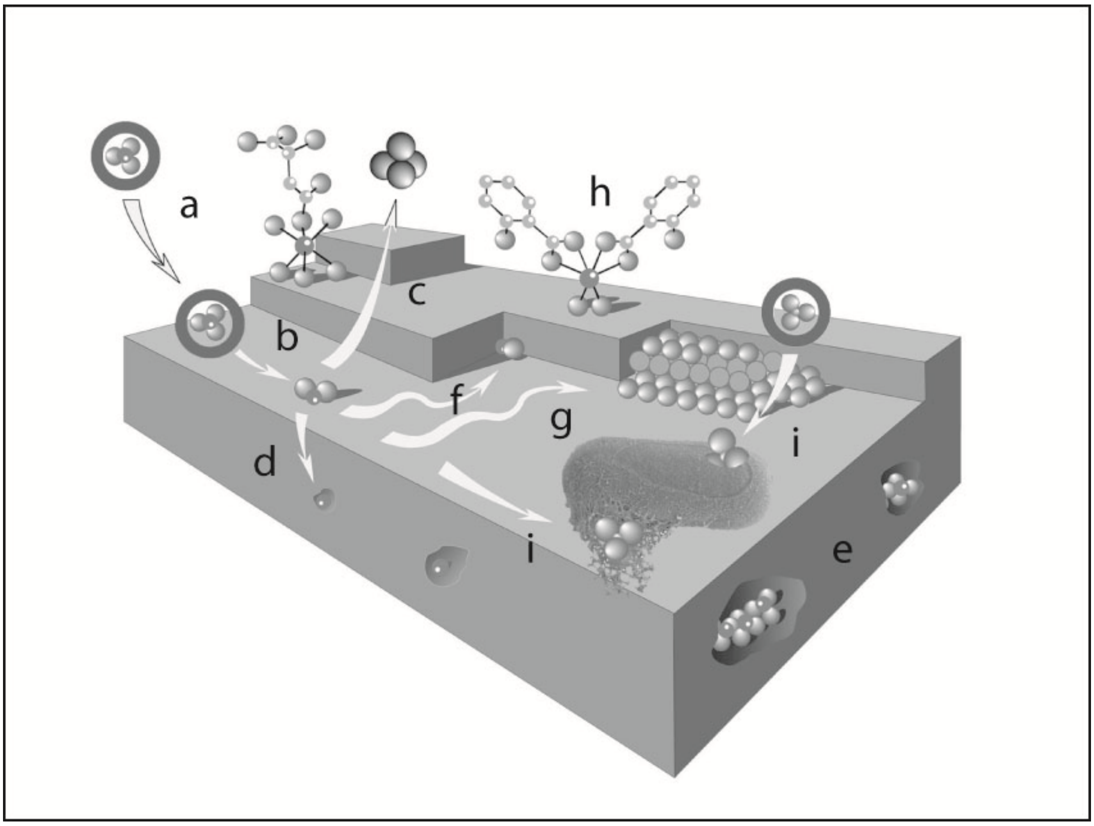


Figure I.1. Basic processes of adsorbate molecules and atoms at mineral-water interface, after Manceau *et al.*, 2002. Letters refers to: a) physisorption; b) chemisorption; c) detachment; d) absorption or inclusion (impurity ion that has a size and charge similar to those of one of the ions in the crystal); e) occlusion (pockets of impurity that are literally trapped inside the growing crystal); f) attachment; g) hetero-nucleation (epitaxial growth); h) organo-mineral complexation; i) complexation to bacterial exopolymer.

Adsorption / desorption

Adsorption of an element is a specific form of sorption and is defined by the fixation of an element at the surface of a soil constituent. Basically, it is ‘an accumulation of sorbate at the interface between an aqueous solution and a solid adsorbent phase’ (Bradl *et al.*, 2005). The physical-chemical parameters which mainly governs metals adsorption are pH, redox potential, clay, soil organic matter, Fe and Mn oxides, and calcium carbonates. Anion adsorption is correlated to Fe and Mn oxides, pH and redox potential (McLean and Bledsoe, 1992). Desorption is the opposite phenomenon and hence corresponds to the release of the fixed element. The main components able to adsorb metals or metalloids are organic matter, oxi-hydroxides, clays, carbonates and sulfates.

Dissolution / precipitation

These phenomena are mainly sensitive to changes of the redox potential as well as acidification. Acidification, attacking phases with H^+ leads to their dissolution and hence to the release of metals and metalloids. Dissolution releases elements which may be into a crystal network. Precipitation is the formation of a solid phase and may include pollutants.

II.3 The main studied elements: As, Sb and Pb

II.31 Arsenic

Arsenic (noted As) is a metalloid having chemical properties of metallic elements as well as a non-metallic elements. It has 3 different oxidation states: As(O), As(III) and As (V), with As(III) dominating in reduced environmental conditions and As(V) more stable in oxidizing conditions. As(III) is the most toxic form (Webb, 1966; Apte *et al.*, 1986; Pokrovski *et al.*, 1996; Hirano *et al.*, 2004). The methylation of As is also particularly dangerous (Dopp *et al.*, 2004). Thus As can lead to serious diseases such as cancers, skin disorders, blood circulation troubles or stiffness.

Overall, As pedogeo-chemical concentration in undisturbed soils ranges between 1 and 25 $mg.kg^{-1}$ (Baize, 1997) and occurs naturally in soils. The main As bearing phases in rocks are arsenopyrite (FeAsS), orpiment (As_2S_3) and realgar (AsS). In soils, As mobility is mainly controlled by adsorption/desorption processes and co-precipitation with metal oxides, particularly Fe oxi-hydroxides. Adsorption is possible by exchange of As for OH_2 or OH^- groups on Fe oxi-hydroxides surface (Kumpiene *et al.*, 2008). Besides adsorption, As may also move in soils under paticles forms.

Since the industrial revolution, large amounts of wastes with high As concentrations resulted from mining and metallurgy (North *et al.*, 1997). The exposition to precipitations generated acid solution (acid mine drainage). As a result, after 85 years old of inactivity, the wastes of a former Sb mine are drained and waters show concentrations in As and Sb respectively of 78 $\mu g.L^{-1}$ and 32 $\mu g.L^{-1}$. The bedrock was rich in As- and Sb-bearing phases such as stibnite

(Sb_2S_3), arsenopyrite (FeAsS), galena (PbS), sphalerite ((Zn-Fe)S) and pyrite (FeS) (Casiot *et al.,* 2007). These drainage waters have high influence on human health and are recognized to be high carcinogenic (Nodstrom, 2002). Nowadays, arsenic is used industrially in the manufacture of numerous products including glass, ceramics, electronics, cosmetics, and fireworks (Smith *et al.*, 1998). In the latter half of the 20th century, As was also widely used in pesticides and herbicides formulations and in wood preserving, although such use is now declining (Azcue and Nriagu, 1994).

Arsenic is not an essential element for plants, but low concentrations sometimes enhance plant growth (results published for *Spartina alterniflora,* Carbonell *et al.,* 1998). Some species are even able to hyperaccumulate As, *i.e. Pteris vittata* and *Pteris calomelanos (*Francesconi *et al.*, 2002; Xiao *et al.,* 2008). Due to the chemical similarity of arseniate and phosphate, arsenate can be uptaken by plant by the same transporters.

II.32 Antimony

Antimony (noted Sb) is also a metalloid. It occurs naturally in soil at concentrations ranged between 0.3 and 8.4 mg.kg^{-1} (Murciego *et al.,* 2007). It has a large range of use: manufacture of semiconductors, diodes, flameproof retardants, lead hardener, batteries, small arms, tracer bullets, automobile brake linings, and pigments (Filella *et al.,* 2002).

Sb occurs naturally in soils originating by weathering of soil parent material. Stibine (Sb_2S_3) and valentine (Sb_2O_3) are the 2 main Sb bearing phases (Filella *et al.,* 2002). In the environment, Sb occurs mainly as Sb(III) and Sb(V) (Filella *et al.,* 2001). As arsenic, Sb (III) is the most toxic form and overall, Sb biochemical behavior is comparable with As (Babula *et al.,* 2008).

There is little literature reporting uptake, toxicity transport and mechanisms of Sb toxic effects toward plants. Bebula *et al.*, (2008) assume that after uptake, toxic Sb (III) form is converted to less toxic Sb(V) form, and consequently complexed with proteins (phytochelatins?) or carbohydrates and stored in vacuoles of plant cells.

Hammel *et al.,* (2000) described soils from a mining area with high Sb concentrations, reaching 500 mg.kg^{-1}, although with Sb content in plants similar to plants from uncontaminated areas. As a result, the hazard to health is not expected in this case. Flynn *et al.,* (2003) also recorded a very low mobility for this element despite total content of up to

700 mg.kg^{-1} and did the same conclusion as Hammel *et al*., (2000). Thus, Ettler *et al.*, (2010) studied Sb speciation in agricultural and forest soils close to a former Pb Smelter emitting Sb. They found that Sb is less mobile than As, as it is mainly linked to amorphous or less crystalline Fe-oxides and to residual fraction of soil. But, Sb can have a high affinity for organic matter (Wilson *et al.,* 2010): humic acids could have the capacity to low the mobility of Sb and could participate to the transformation of Sb (III) into the less dangerous form Sb (V) in contaminated soils (Steely *et al.,* 2007). Overall, interactions between Sb and organic matter are quite complex. Filella (2011) concludes his review by explaining that there is no satisfactory explanation for the different Sb behavior in waters and soils, at the moment. Indeed, in waters, Sb is mainly under dissolved form namely very mobile whereas in soils, Sb is strongly bound either to Fe oxides or the natural organic matter. Thus, he suggests to proceed investigations especially with respect to the strong role of pH in Sb binding by organic matter and Fe.

Few plants have been described as potential Sb bioaccumulators: *Dittrichia viscosa* (Murciego *et al.,* 2007), *Digitalis purpurea*, *Erica umbellata*, *Calluna vulgaris* and *Cistus ladanifer* (Pratas *et al.,* 2005). *Agrostis capillaris* is the only species able to grow at a highly Sb, As polluted site close to a former Sb mine (in Pyrenees) with acidic pH. *A. cappilaris* shoots accumulated 240 mg As.kg^{-1} and 68 mg Sb.kg^{-1}. The authors attribute the Sb tolerance of this plant to efficient exclusion. However, they tried to perform a pot culture under controlled conditions and *A. capillaris* (commercially seeds) was unable to germinate on the severely contaminated soil. The commercial variety uptook much higher concentrations than the metal tolerant ecotype from the field. This is probably due to better water availability leading to higher transpiration rate and maybe favoring higher metal uptake.

II.33 Lead

Natural Pb concentrations in soils range between 9 and 50 mg.kg^{-1} (Baize, 1997). About 240 minerals contain Pb: galena (PbS), cerrusite ($PbCO_3$) and Anglesite ($PbSO_4$) are the most important economically. Pb has been used since Antiquity, therefore, Pb contaminations are probably as old as civilization (Shotyk and Le Roux, 2005). Among the different oxidation state, Pb (II) is the most common at Earth surface. The others are Pb (IV) and Pb (0). This element is implied in many industrial fields and under different forms:

metallic Pb (lead-acid battery components, welding (Pb-Sn)), PbO (batteries, incinerators ashes). It was also added in paints, petrol-gas, pesticides… (Ruby *et al.*, 1999). Soil Pb contamination is mainly due to atmospheric deposits. However, Pb has been banned or in process to be banned from many products (paint, gasoline, water pipes…) because of its toxicity.

In their review of soil contamination by lead, Markus and McBratney (2001) summarize and analyze a large number lead surveys of agricultural, urban and industrial areas in different countries. Next to the spatial distribution of lead concentrations, the authors review a large number of studies that show that the exposure to lead may cause adverse effects to human health and the environment. A more recent study (Saby *et al.*, 2006) uses the data of the French soil monitoring network to analyze the soil lead contamination around Paris and states that there is a strong gradient of anthropogenic stock of Pb around the urban Paris area corresponding to an average accumulation of 5.9 $t.km^{-2}$.

Pb is known for his affinity with organic matter (Strawn and Sparks 2000) but, due to a complex set of parameters including soil texture, mineralogy, pH and redox potential, hydraulic conductivity, abundance of organic matter and oxyhydroxides of Fe, Al and Mn, it is very difficult to propose any general conclusions regarding the fate of anthropogenic Pb in soils. Besides soil parameters, external factors such climate, site and nature of the parental material can influence Pb evolution in soils. A recent modelling study (Caboche *et al.*, 2010) used 25 soil samples from sites that were contaminated by Pb by mining and smelting, states that bioavailability of Pb was highly determined by the Pb-bearing phase distribution.

Few plant species are known to accumulate Pb: *Brassica juncea* (Kumar *et al.*, 1995), *Helianthus annus*, *Nicotiana tabaccum*, *Vetivera zizanioide*s (Boonyapookana *et al.*, 2005). Pb phytoextraction usually needs to be coupled to chelatant addition like EDTA improving the accumulation in plants. Nevertheless, Liu *et al.*, (2008) reported the list of the 5 Pb hyperaccumulators: *Armeria martima*, *Thlaspi rotundifolium*, *Thlaspi alpestre*, *Alyssum wulfenianum*, and *Polycarpaea synandra*.

II.4 Micro-organisms in contaminated soils

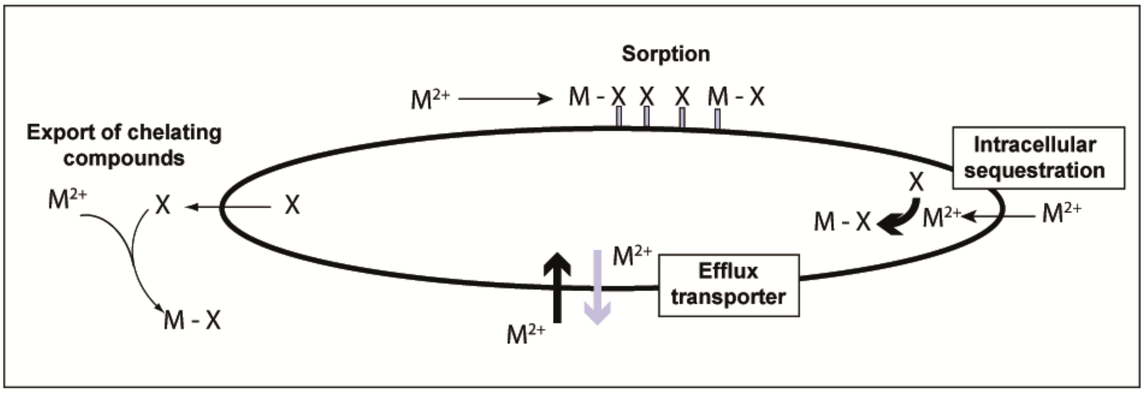
Microbes play a decisive role in the cycling of nitrogen and organic matter in soils and thus have an important function in soil ecosystems. This function can be degraded by the contamination of the soil by heavy metals as reviewed by Giller *et al.* (1998). The authors of this review focus on agricultural soils contaminated by sewage but the threshold concentrations of pollutants that reduce the microbial function may be also valid for mining sites and other Anthroposols.

In contaminated soils, micro-organisms have to cope with high concentrations of different heavy metals. The microbial community is affected by high concentrations in heavy metals which expresses in disruption of their growth, their structure, their diversity and their enzymatic activity (Haferburg and Kothe, 2007).

For example, they are known to be implied into acid mine drainage. In mining area where sulfides ores are exposed to air and water, communities of autotrophic and heterotrophic archaea and bacteria catalyze iron and sulfur oxidation which generate highly acidic toxic solutions. This phenomena corresponds to acid mine drainage (AMD) (Baker and Banfiel, 2003). AMD implies very important microbes-minerals interactions leading to dissolution of sulfide minerals. Microbes are metabolically active even below pH 2. Fe also plays a major role as micro-organisms use Fe^{2+} as electron donor to satisfy their energetic demands. Pyrite (FeS_2) is the typical dissolved mineral (Haferburg and Kothe, 2007).

Some micro-organisms can adapt to toxic environments, particularly to As toxicity (Duker *et al.,* 2005). Thus, As can be oxidized, reduced biomethyled according to redox conditions of the environment. Depending on its speciation, As mobility and subsequently toxicity will be different. The resistance mechanism is due to a reduced arsenate uptake whereas the concentration of phosphate transport into bacteria cells is increased (Willsky and Malamy, 1980). However metallic elements are rather known to reduce microbial activity (Šmejkalová *et al.,* 2003). Arsenic is toxic to most micro-organisms as well as antimony, especially their As(III) and Sb(III) forms (Dopson *et al.,* 2003, Wang *et al.,* 2011).

Haferburg and Kothe (2007) summarized the main microbial strategies of metals resistance (Figure I.2). Thus, they relate metal resistance to (1) intra- and extra-cellular mechanisms, (2) metal excretion via efflux transport systems, (3) binding and detoxifying metals by sequestering compounds of the cytosol, (4) bounding and fixation of metals by the release of chelators into the extracellular milieu and (5) sorption to the cell wall which is prone to bind large amount of metal, preventing the influx.



FigureI.2. Overview of microbial resistance mechanisms. (X) – Cell constituents interacting with metal cations, (M) – Metal Cation (Haferburg and Kothe, 2007**)**

III. Technosols

III.1 Definition and nomenclature

In the case of mining soils, as well as in urban and suburban areas, the soils are often so seriously influenced by humans that their understanding is a growing concern (De Kimpe *et al.*, 2000). Special nomenclatures have been elaborated.

Thus, soils strongly influenced by human activities contain artefacts. Rossiter (2007) defines artefacts as ‘material created or substantially modified by humans as part of an industrial or artisanal manufacturing process and with more or less their original properties’. Artefacts also include ‘material excavated from a depth where they were not influenced by surface processes, with properties substantially different from the environment where they are placed’. Examples of artefacts are bricks, pottery, glass, crushed or dressed stone, industrial waste, garbage, processed oil products, mine spoil and crude oil.

Both World Reference Base (FAO, 2006) and Référentiel Pédologique (2008) (French nomenclature) named these types of soil respectively as Anthrosol, Technosol (WRB) and Anthroposol (Référentiel Pédologique).

In WRB, Anthrosols are soils that have been profoundly modified through human activities, such as addition of organic materials or household wastes, irrigation and cultivation. Technosol, from Greek *technikos*: “skillfully made”, are soils dominated or strongly influenced by human-made materials and correspond to soils whose properties and pedogenesis are dominated by technical origin. Their parental material is made of all kind of materials ‘made or exposed by human activity that otherwise would not occur at the Earth’s surface’. They can also be sealed by technical hard rock. Artefacts are of different natures: wastes (landfills, sludge, cinders, mine spoils and ashes), pavements with their underlying unconsolidated materials, soils with geomembranes and constructed soils in human-made materials.

In Référentiel pédologique, Anthroposols are defined as soils strongly modified or made by humans. In this nomenclature, many different types are distinguished: Anthroposol Transformé, Antroposol Construit or Antroposol Reconstitué, Anthroposol Artificiel and Anthroposol Archéologique (Figure I.3).

- Anthroposols Transformés result of anthropogenic modifications occurring in country areas to improve soil fertility and to harvest sufficient food to feed populations. These modifications are usually former modifications (paddy fields, terraces, oasis).
- Antroposols Construits or Antroposols Reconstitués result of operations of “pedological engineering”: it aims at getting a soil as fertile as possible for example, in case of revegetalization.
- Anthroposols Artificiels entirely results of human inputs of various materials. It usually corresponds to areas with high human activities such as urban, industrial, mine, artisanal and road areas.
- Anthroposols Archéologiques are soils containing former modifications namely soils developed from materials entirely impacted by ancient (archeo-anthropogenic) activities resulting in the parental material of the current soil (representing more than 50 cm thick).

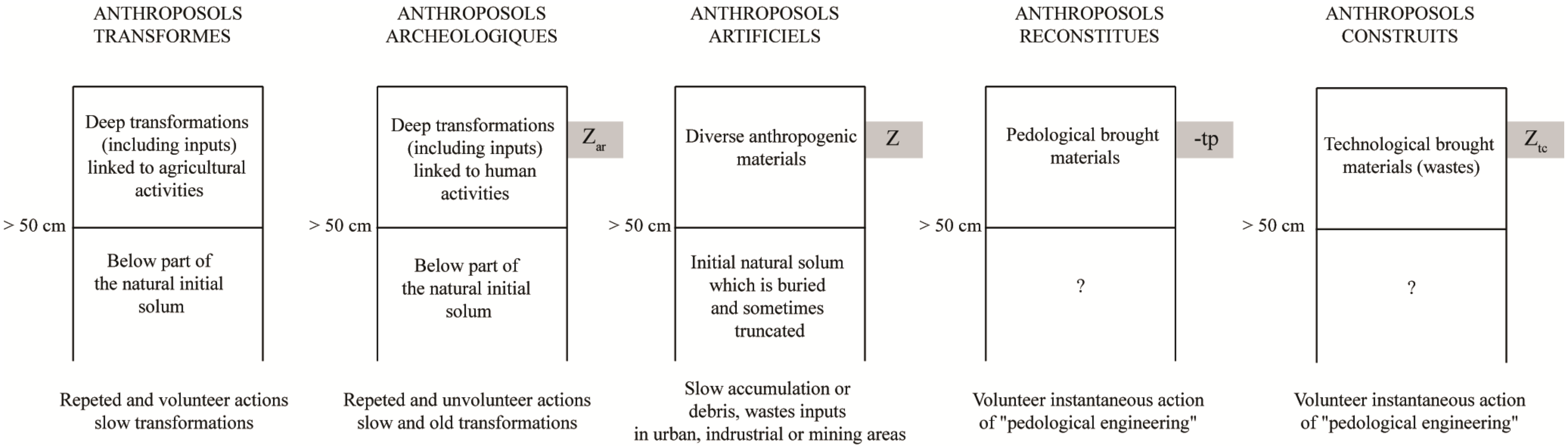


Figure I.3. The different types of Anthroposols adapted from the Référentiel Pédologique (2008), French nomenclature.

III.2 Examples of Technosol / Anthroposols

Technosols / Anthroposols are soils including artefacts (technogenic materials) and resulting from incorporation by humans. For example, an amount of fertile soil rich in humus can be added to a soil made of materials without any organic content. In urban areas, most surfaces are sealed preventing all energy exchanges (mainly water and gas) between soils and atmosphere, and disturbing biotas as well by affecting all organisms (Scalenghe and Ajmone Marsan, 2009). Indeed, the sealed soil is totally impermeable to water inflow. One of the consequences is the increase of the amount and speed of runoff, hence, increasing the risk of erosion of unsealed areas. Scalenghe and Ajmone Marsan (2009) reported that the runoff waters are also charged of pollutants, especially in the case of highways and pavements adjacent to roads. Theses authors also explained that sealing soils lead to a decrease of biodiversity.

However, despite of the presence of external elements such as paper-mill sludges, green waste compost combined to thermally treated soil, some authors recorded pedogenesis phenomena (Séré *et al.,* 2010): physical and chemical changes of the parent material in a first phase. Indeed, for example, soil compaction is recorded after 30 months as well as a decrease of available water content. Finally, an intense weathering of soluble minor minerals is also measured. The second phase is rather pedogenic changes which occur in natural soils under similar pedoclimatic conditions, *i.e.* decarbonatisation or aggregates formation. Therefore, constructed soils can behave like natural soils (Séré *et al.,* 2008). Furthermore, constructed soils are a nice way to reclaim these surfaces as well as to recycle wastes and by-products.

IV. Metals and metalloids in the soil-plant system

IV.1 Bioavailability and phytoavailability

The toxicity of an element is governed by its concentration in a system, its speciation and therefore its capacity to be mobile. Thus, the bioavailability of an element is the fraction of a total element which is in the interstitial water and soil particles which is available to the receptor organisms (Vig *et al.,* 2003). Basically, it is defined as the fraction which can potentially be absorbed by plants or other living beings (Lebourg *et al.,* 1996). As a result, bioavailability depends on species, age of the plant, cultural practices, type of soil, depth… we can also define the bioavailability as the capacity of an element to be transferred from the soil fraction to a living organism (Baize, 1997). Therefore, when the organism is a plant the term phytoavailability can be used.

The need to assess the bioavailability toward organisms or plants is of major concern. Indeed, the most an element is available the most organisms (fauna or flora) will have the possibility to uptake it or even accumulate it. The availability of elements for plant is defined as the phytoavailability. Moreover, this method allows assessing the phytotoxicity risk and as a consequence the potential risk of contamination of food chain.

The most suitable tool to assess bioavailability is to study the organisms itself (*i.e.* the plant, Figure I.4. by measuring elements concentrations in plants. But, this type of measurement is laborious and expensive.

Chemical reagents, by realizing selective chemical extraction, can substitute to direct measurements on the studied organism. The chemical reagent is supposed to target a determined fraction *i.e.* the bioavailable fraction. However, the term partial extraction appears to be more suitable as far as we are never entirely sure to extract only the required fraction: the reactant might also extract element linked to other soil fractions. Finally, we are also never sure to extract the entire fraction.

As a result, many chemical reagents are known to represent the availability of an element but no agreement has been found about the most accurate method for the estimation of bioavailability.

The different chemical reagents are the next one:

- Chelating agents such as EDTA (ethylene diamine tetraacetic acid) or DTPA (diethylene diamine pentaacetic acid). For example DTPA is commonly used to determine the fraction of elements bounded to organic complexes and hydroxides (Beckett, 1989).
- Neutral salts such as $CaCl_2$, $MgCl_2$, $NaNO_3$,
- Acid extractants such as acetic acid, chloric acid
- A-RHIZO method which is a mix of organic acids (lactic, acetic, citric, malic and formic acids to a final concentration of 0.01*M*) reflecting the root exudates and their action on the solubilisation of elements being potentially phytoavailable (Fang *et al.*, 2007).

Menzies *et al.*, (2007) demonstrated that neutral salts ($CaCl_2$ and $NaNO_3$ 0.01 M) provide the most useful phytoavailability estimation of Cd, Zn, Ni, Cu and Pb.

Given the difference in ionic speciation between metals and metalloids, the suitable extractants for metals are not the same as for metalloids. Wenzel *et al.* (2001) adapted a sequential extraction procedure to arsenic. The first step uses ($(NH_4)_2(SO_4)$ 0.05*M*) to extract the As mobile fraction; this reactant might be more suitable to assess arsenic availability.

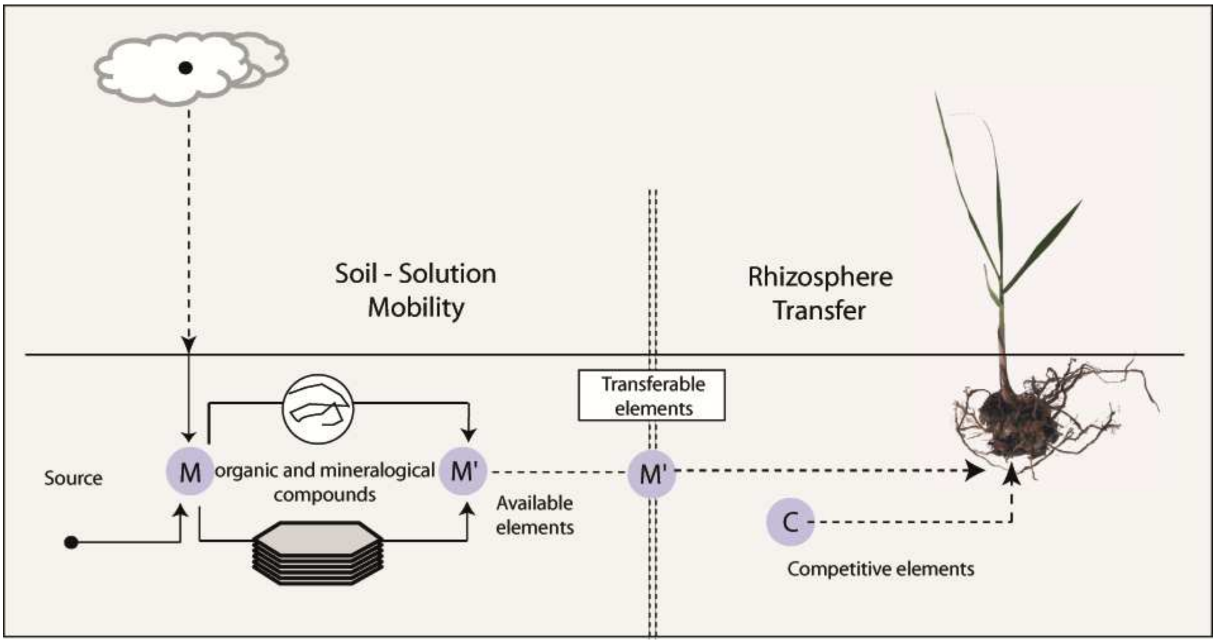


Figure I.4. General pattern of mobility and transfer of elements in the soil-plant system, after Deneux-Mustin *et al.*, (2003). M represents metal. M' represents the the metal under a bioavailable form. C is competitive elements which can be uptaken by the plant instead of the metal.

If phytoavailability is determined by measuring elements concentrations in tissues of the plant, different ratios can describe the plant behavior toward its ability to accumulate elements or concentrate the element and translocate the element to aerial parts. Thus, the two main ratios commonly used are (i) the bioaccumulation factor (BCF) defined as the ratio between the concentration in roots and the concentration in the soil and (ii) the translocation factor (TF) which is the concentration in aerial parts divided by concentration in roots. If TF is higher than 1, the plant can be considered as a hyperaccumulator namely a plant which accumulate the pollutants in its tissues (Bradl *et al.*, 2005). Recently, Li *et al.* (2011) proposed a new ratio to assess the potential of a plant to be used in phytoremediation: the Phytoremediation Factor (PF). It corresponds to: the target element concentration in the plant shoot x biomass of the plant shoot / target element concentration in the tailings. Thus, in their study, *Miscanthus floridulus* had the highest PF for Ni (25) among few species, naturally developed on tailings.

IV.3 Plant uptake and elements translocation

Contrary to organics for which there are no specific transporters in membrane to be uptaken by plants, inorganic elements are uptaken by biological processes via membrane transporters proteins (Pilon-Smits, 2005). Indeed, inorganic elements are nutrients themselves or chemically similar to nutrients (*i.e.* arsenate chemically similar to phosphate and using the same transporters).

Plant uptake of inorganic pollutants follows different steps: chelation and compartmentation in roots, translocation, chelation and compartmentation in leaves (Figure I.5).

Plants can exudate from their roots compounds affecting the solubility and the uptake by plant of inorganic pollutants. Pilon-Smits (2005) reviewed the most common of these chelators: phytosiderophore (mainly involved in Fe uptake), nicotianamine, organic acids (citrate, malate, histidine...), glutathione, phytochelatins, Cys-rich methallothionein protein. When metals are chelated in roots, they might be stored in the vacuole (Figure I.5) or translocated to shoots via the xylem. Root sequestration is the principle of phytostabilization whereas export to xylem is the one of phytoextraction.

Translocation of pollutants to aerial parts implies a membrane transport step from roots symplast into xylem apoplast. Indeed, the Casparian strip is an impermeable suberin layer in the cell wall of the roots endodermis preventing the flow of solutes from soil solution or roots apoplast to the root xylem. Organic acids sometimes chelate metals during xylem transport but there is still a need for better understanding via which transporters proteins and to which chelators – if any- they are bound when metals are exported to roots xylem (Pilon-Smits, 2005). The solute flow from roots to shoots is driven by transpiration. The transpiration rate differs according to photosynthetic pathway (C3, C4 and CAM), anatomical features as well as environmental conditions.

Transfer into leave cell also requires transport membrane proteins. Once into the leave cell, many chelators can be involved in metals sequestration.

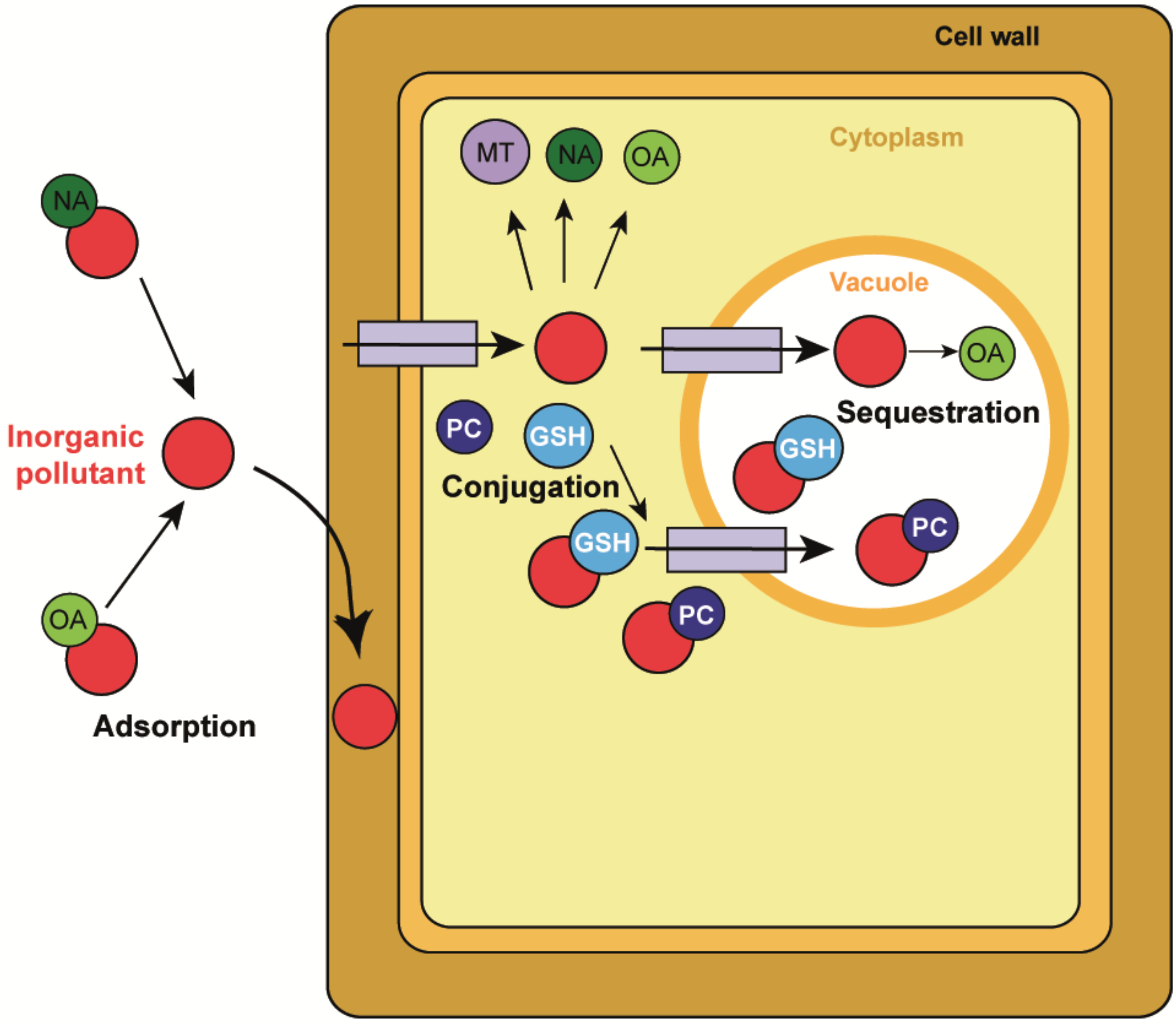


Figure I.5 Tolerance mechanisms for inorganic pollutants in plant cell, adapted from Pilon-Smits (2005). Detoxification generally involves conjugation followed by active sequestration in the vacuole and apoplast, where the pollutant can do the least harm. Chelators are GSH: Glutathione, MT: Metallothioneins, NA: Nicotianamine, OA: Organic Acid, PC: Phytochelatins**.**

IV.4 Impact of inorganic pollutant on plant physiology

A soil contaminated by heavy metals can clearly affect the photosynthesis activity and nutrients absorption of plants causing growth reduction (Sharma and Agrawal, 2005). For example, photosynthesis activity is one of the physiological processes which is reduced by contaminants. Thus, the assessment of gas exchanges can reflect the adaptability and the capacity of a plant to tolerate a contamination or the stress of the plant owing to the contamination (Lage-Pinto *et al.*, 2008, Vernay *et al.*, 2008). If the contamination has an effect on the plant physiology, it is usually materialized by a decrease of the photosystem II efficiency, recorded as a decrease of the Fv/Fm ratio.

V. Soil remediation

V.1 Different techniques

V.11 Physical and chemical treatments

Due to the non-degradability of metallic elements and metalloids, these elements persist in soils and represent a risk for water resource and contamination of food chain.

As detailed by Dermont *et al.*, (2008), physical treatments consist of a physical separation to extract the metal-bearing particles from mining ores (*i.e.* by gravity differences, froth flotation, magnetic or electrostatic separation). Theses authors also reviewed the chemical treatments using an extracting fluid containing a chemical reagent to transfer metal from the solid fraction into an aqueous solution. For example, redox reactions create valence change, enhancing metal solubilization. Moreover, acids induce ion exchange and dissolution of soil components which will be easily extracted. Chelating agents lead to metal complexation in order to solubilize them. Surfactants realize metal desorption from soil interface. The main leaching solution types are (1) acids, (2) salts and high concentration chloride solutions, (3) chelating agents, (4) surfactants and (5) reduction or oxidizing (redox) agents. The main advantage of chemical treatments is the possibility to extract sorbed metal forms. The choice of the extracting reagent depends on the type of elements, their concentration, their speciation and the soil characteristics. Overall, a combination of physical and chemical treatments is usually performed.

But the physical-chemical technologies (chemical washing, thermal treatments…) to remove metallic elements from soils are expensive and alter soil biological functions (Glass *et al.,* 2000).

V.12 Phytoremediation

Phytoremediation is based on the use of green plants to remove pollutants from the environment or render them harmless (Cunningham and Berti, 1993; Raskin *et al*., 1994). Pilon-Smits (2005) summarized the phytoremediation as “the use of plants and their associated microbes for environmental clean-up”. The main advantage of this technique is the low cost compared to physical and chemical treatments as well as the harmless toward environment. Suresh and Ravishankar 2004 pointed out the economical advantages of this cheap method to remediate soils and the increasing number of companies researching on the field of phytoremediation. This technique is considered as a cheap technique as this is an *in situ* treatment, only using plants without any other chemicals or tools. Plants are really the main actor and just have to grow and therefore act. However, this approach need long time laps, as one vegetation period leads to the extraction of a minor part of the metals.

In the last decade the research on phytoremediation and the engineering of new species that are able to remediate soils has strongly developed. In a review of the year 2004, Krämer concludes that 'Phytoremediation technologies are currently available for only a small subset of pollution problems, such as arsenic' and that plants and technologies (genetic engineering) at this time are not efficient enough for a wide-range application. Since then, a number of studies could extend the applications to other environmental problems and introduce new species that can be possible phytoremediators.

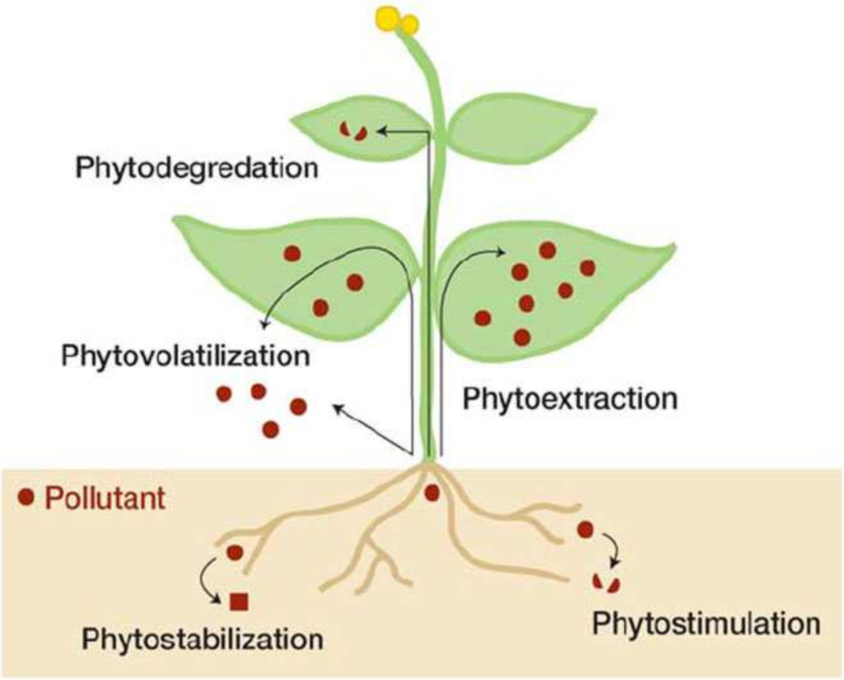


Figure I.6 Possible fates of pollutants (represented by red circles) during phytoremediation after Pilon-Smits (2005).

Phytoremediation techniques

Phytoremediation appears to be a suitable technique to remediate contaminated soils as it is a cheap and *in situ* technique. This technique uses plants which are tolerant to metallic elements and metalloids.

In their review of phytoremediation, Salt *et al.*, (1998) could distinguish 5 major techniques which differ according to the next definitions (Figure I.6):

(i) Phytoextraction – removal and concentration of metals into harvestable plant parts.

(ii) Phytodegradation – degradation of contaminants by plants and their associated microbes.

(iii) Rhizofilteration – absorption of metals by plant roots from contaminated waters.

(iv) Phytovolatilization – volatilization of contaminants by plants from the soil into the atmosphere.

(v) Phytostabilization – immobilization and reduction in the mobility and bioavailability of contaminants by plant roots and their associated microbes.

(i) Phytoextraction

Schwartz *et al.*, (2001) studied a former zinc-smelter site with particularly high Zn concentrations covered by metallophyte plants for 30 years. Thus, *Arabidopsis halleri* and *Arrhenatherum elatius* could together phytoextract till 10 kg Zn.ha^{-1} without any agricultural practices. To enhance the phytoextraction, Jiang *et al.*, (2010) investigated the effect of co-cropping of a hyperaccumulator plant (*Thlaspi caerulescens* named now *Noccaea caerulescens*) with ryegrass (*Lolium perenne*). They demonstrated that co-cropping improved the growth of the hyperaccumulator and its capacity to accumulate Cd and Zn. Moreover, the metal from the ryegrass roots could be transported to the hyperaccumulator. An abandoned mine in Sardinia (Italy) contaminated by Pb, Zn and Cd could be remediated by the native flora of the site, *Inula viscosa*, *Euphorbia dendroides*, and *Poa annua* according to the concentrations measured in their aerial parts (Barbafieri *et al.*, 2011). Thus, tailings had concentrations in Zn, Pb and Cd reaching respectively 7 400, 1 800 and 56 mg.kg^{-1}. *I. viscosa* could accumulate till 1 680 mg Zn.kg^{-1}, *E. dendroides* till 240 mg Pb.kg^{-1} and *P. annua* till 19 mg Cd.kg^{-1}. The biomass can be subsequently used for non food purpose (*e.g.* wood, cardboard) or ashed followed by disposal in landfill (Pilon-Smith, 2005).

Besides native plants which can phytoremediate a site, some other species are specifically cropped to hyperaccumulate pollutants. We define as hyperaccumulators plants as plants able to accumulate > 1 000 mg.kg^{-1} Pb, Cu, Co, Cr or Ni and > 10 000 Mn or Zn (Baker and Brooks, 1989). Thus, the Cretan brake fern, *Pteris vittata L.* is able to hyperaccumulate simultaneously As and Sb, and uptaken Sb was enhanced with increasing As concentrations in the hydroponic solution (Feng *et al.*, 2011).

Harvested hyperaccumulating plants might not be suitable for certain uses. After burning in view of energy supply, the ash might be highly concentrated in pollutants and should be proceeded.

(ii) Phytodegradation

The phytodegradation can occur inside the plant or in the rhizosphere. This technique is suitable for organic compounds such as solvents, petroleum, aromatic compounds and volatile compounds in the air (Newman and Reynolds, 2004). But, it remains rather hard to measure plant effect on PAH (Polycyclic Aromatic Hydrocarbon) degradation, for example, because of all the interactions between soil, micro-organisms, and plant in the rhizosphere.

(iii) Rhizofiltration

Rhizofiltration is the absorption of metals by plant roots from contaminated waters. An ideal plant for rhizofiltration should have rapidely growing roots with the ability to remove toxic metals from solution over extended period (Raskin *et al.*, 1997). Ghassemzadeh *et al.* (2008) examine the possibility of As and Sb removal by rhizofiltration by common reed (*Phregmites australis*) and state that the plant could be used for rhizofiltration of these elements.

(iv) Phytovolatilization

Phytovolatilization is probably not an efficient remediation method as it leads to the transfer of the pollution to an unpolluted environment by volatilization (Ernst, 20005). Only few studies report this process where plants can convert a pollutant to a less toxic form: in association with micro-organisms, plant transforms selenium into dimethyl selenide, a less toxic form. The possibility to reduce mercury into Hg(0) results in the metal volatilization (Watanabe, 1997). This technic may be used in a closed system allowing the capture and treatment of the polluted atmosphere, as a filtration being more simple than a soil treatment.

(v) Phytostabilization

Phytostabilization aims at immobilizing the pollutants in the root system of metal tolerant plants. Different mechanisms can induce the immobilization: as reviewed by Mendez and Maier (2008), metal can precipitate to less soluble form, complex with organic compounds, or be sorbed onto roots surface. Furthermore, these plants do not transfer and accumulate much the contaminants in their aerial part as they have a $TF < 1$ (Translocation Factor which represents the ratio between the contaminant concentration in stem and in roots).

Overall, organic compounds, depending on their properties, can be degraded close to roots or taken up and then be degraded, sequestrated or volatilized. Because of their non degradability, inorganic pollutants can be phytoremediated by phytostabilization or sequestration in harvestable plant parts (Pilon-Smits, 2005). One of the limitations of the phytoremediation technique is the time required to clean-up, usually taking years. Another limiting factor is the bioavailability of elements. Phytoremediation will not be applicable if only a part of elements is bioavailable. However, amendments might enhance their bioavailability.

The fate of the contaminated biomass is the next step after the accumulation. It can be used as non food purposes (wood, cardboard) or be ashed. We can also try to recycle the elements form the biomass which is called phytomining (Chaney *et al.*, 2000).

V.2 Bioenergetic plant for phytoremediation

V.21 Short overview of plants with high biomass

The main interest of the crop of plant with high biomass is to substitute the fossil energy in way to reduce green house gas emissions particularly CO_2. Indeed, wooden and non-wooden plants can be used as biofuel. All trees residues can be transformed in pellets in order to supply boilers. Indeed, the wood industry produces a huge amount of residues. However, poplar (many species of the *Populus* genus) and willow (many species of the *Salix* genus) are the main trees which are cropped for their biomass.

The other plants are Switchgrass (*Panicum virgatum*), Miscanthus (*Miscanthus* x *giganteus*), both with a C4 metabolism and producing respectively 10-15 and 20-25 $t.ha^{-1}$ dry matter. Colza (*Brassica napus*) is more used as bioethanol (Damien, 2008).

In this thesis, the choice has been made for *M*. x *giganteus*, given its economical interests developed below. This plant also has the capacity to tolerate metals contamination and hence could lead to a valuable revalorization of contaminated soils.

Thus, the main stakes of the possibility to crop a bioenergetic plant on contaminated sites is the preservation of arable soils for the food and feed production. Indeed, currently, bioenergetics plants occupy large arable surfaces. Furthermore, the culture of bioenergetic plants on contaminated surfaces implies the necessity that the transfer of pollutants to aerial parts must be as low as possible.

V.22 *Miscanthus* x *giganteus*

Miscanthus x *giganteus* is an industrial sterile clone which is triploid (n=57) resulting from the hybridation of *Miscanthus sinensis* (diploid, n=36) and *Miscanthus sacchariflorus* (tetraploid, n=76) (Linde-Laursen, 1993; Greef and Deuter, 1993). It belongs to the Poaceae family (Gramineae). It is a perennial C4 plant originating from eastern Asia and known as a promising biomass crop (Heaton *et al.*, 2010). The main advantage of perennial crop is their capacity to produce huge biomass yield rich in lingo-cellulose with 20 to 50% less nitrogen inputs than other type of plants (Karp and Shield, 2008). Thus, this plant has a very high biomass yield, reaching $25t.ha^{-1}$ after 2 or 3 years of culture, making it one of the most productive terrestrial plant in temperate climate. The biomass has many industrial applications: currently, it is mainly a biofuel and it is implied in the electricity production (co-generation). Moreover, the use of *M.* x *giganteus* biomass takes part to the reduction of greenhouse gas emission as a substitute to fossil fuel energy (Heaton *et al.*, 2008). The biomass could also be used as animal litter, biomaterial, or bioplastic.

The culture of this plant requires low input as most of the key nutrients are translocated to the rhizome at the end of the growing season in order to support the next germination. Therefore, the harvest occurs at the end of winter, as the biomass content in mineral and water is low, a positive factor in order to improve the biomass combustion.

Besides the high biomass yield, *Miscanthus* x *giganteus* is a metal tolerant plant which does not transfer much the pollutants to aerial parts. Many authors demonstrated this feature, but mainly in hydroponic conditions and with respect to the pollutants Cd, Cr, Cu, Ni and Pb (Arduini *et al.*, 2004, 2006a, 2006b; Fernando *et al.*, 2004a; 2004b). However, Leung *et al.* (2007) recorded low mycorrhizal colonization in *Miscanthus* sp., growing on contaminated mining sites from Southern China. Moreover, Qin *et al*. (2010) investigated the physiological response of *Miscanthus floridulus* growing in a mine site. Under Cd stress, they observed decreases in net photosynthetic rate, transpiration rate, stomatal conductance and chlorophyll content as well as a damage in the chloroplast ultrastructure. Fluorescence of photosynthem II reaction center showed a small decrease under Cd stress but the authors found that the capacity to use light remained higher for the plant from the mine. *Miscanthus floridulus* is also a species recommended for the remediation of Cr contaminated soils (Zhong *et al.*, 2010).

The aims of this work are (i) the study of soils developed from mining wastes particularly with the characterization of the geochemical behavior of As, Sb and Pb, (ii) the assessment of the elements transfer and the micro-organisms (bacteria and archaea) response to the contamination in the soil / plant continuum, (iii) the assessment of the transfer of these 3 inorganic pollutants to the native vegetation compared to their bioavailability chemically assessed and (iv) the potentiality of remediation with *Miscanthus* x *giganteus* of the studied soils in order to use the biomass as an energetic resource.

Thus, the thesis is composed of 4 manuscripts to be published and related to the 4 aims and a general discussion which will summarize the main original results of the work as well as the contribution of this study to contaminated soils phytoremediation.

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CHAPITRE II

Matériels et méthodes

I. Site d'étude et échantillonnage

I.1 Description du site

L'ensemble des travaux de cette thèse a été réalisé sur des sols développés à partir des déchets miniers d'une ancienne mine d'or. Cette ancienne mine, située à 60 km au Nord de Limoges dans le département de la Creuse (France) n'est plus en activité depuis le milieu des années 60. Ses coordonnées GPS sont 01°34'23''E de longitude pour 46°08'37''N de latitude (Figure II.1). L'extraction de l'or a conduit à la formation de déchets miniers stockés dans un bassin de décantation dont la superficie est d'environ 12 000 m². On estime la production de déchets à 34 000 t pendant l'activité de la mine. Lors du processus d'extraction de l'or, la roche a été broyée finement selon une granulométrie de 75 µm. Des études précédentes sur ce site ont déjà été menées par le laboratoire d'Analyse structurale de Hydrothermalisme actuellement GRESE, Groupement de Recherche Eau Sol et Environement de l'Université de Limoges (Roussel et *al.*, 2000 ; Courtin-Nomade et *al.*, 2002 ; Neel, et *al.*, 2003). Ces études ont été le point de départ de notre réflexion et de notre travail.

Notre étude a porté sur des échantillons provenant principalement de deux profils établis dans deux zones différentes de part le type de végétation qui s'y développe (zone 1 et 2, Figure II.1) dont la description rapide est établie ci-dessous :

Le profil 1, d'une profondeur de 15 cm environ est un Anthroposol Artificiel (Référentiel Pédologique, 2008). Il possède un horizon organique de 2 cm d'épaisseur (horizon O), de couleur 10YR 2/3 (very dark brown, marron très foncé), une litière dont la structure est aérée. La transition avec l'horizon sous-jacent (horizon A) est nette. Cet horizon (2-5 cm), à la structure particulaire, possède une forte densité racinaire et une pierrosité nulle. Sa couleur est 10YR 2/2 (very dark brown, marron très foncé). La transition avec l'horizon C est également nette. Cet horizon C (5-11 cm), de couleur 10YR 5/6 (yellowish brown, marron jaunâtre) possède une texture limono-sableuse et une structure polyédrique. Quelques racines fines persistent.

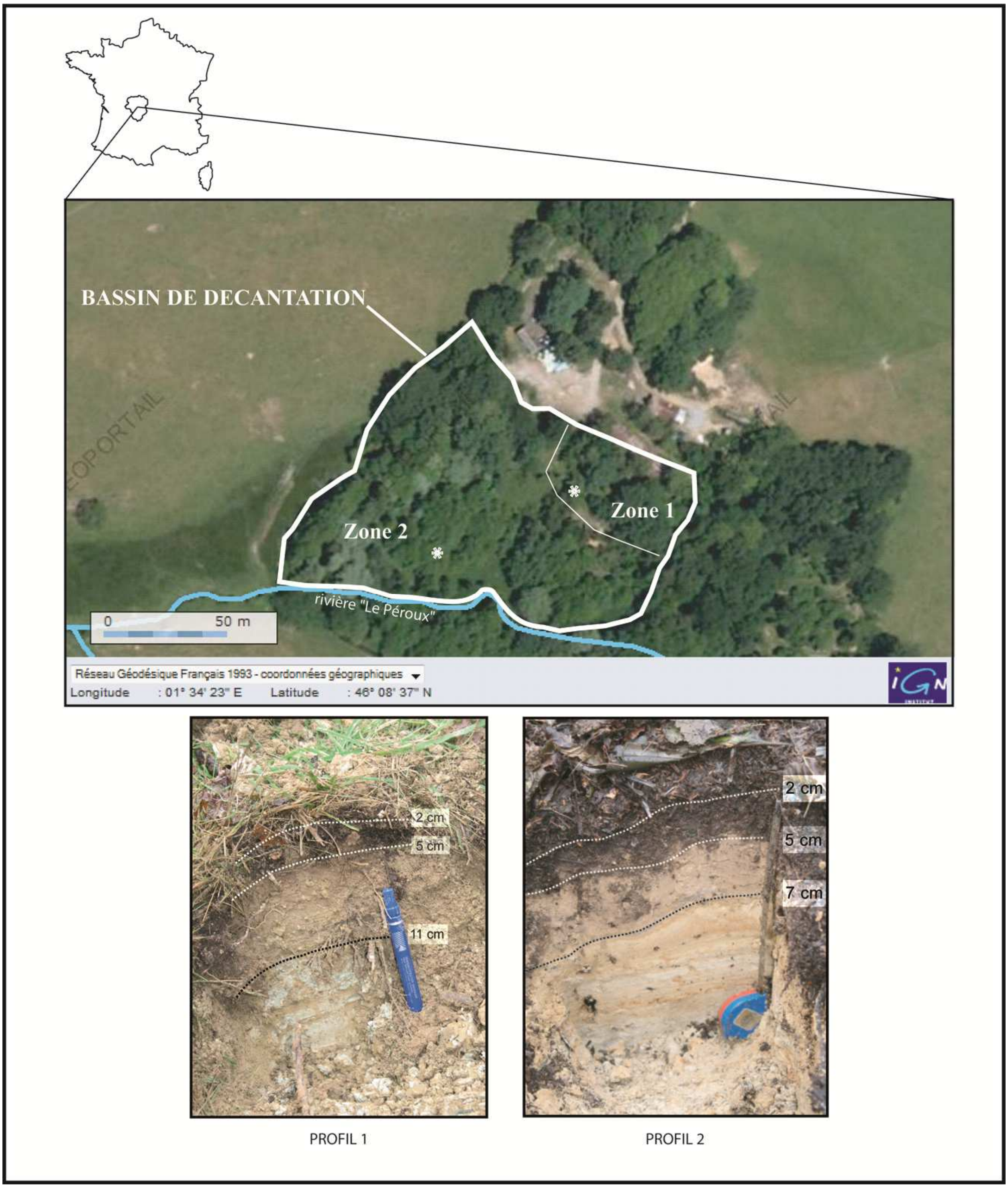

Figure II.1. Localisation du site de La Petite Faye et des profils 1 et 2 décrits respectivement dans les zones 1 et 2 (photographie aérienne téléchargée sur http://www.geoportail.com, juin 2010).

L'horizon Z_{tc}, (désignation pour un matériau anthropique technologique), > 11 cm, est de couleur 10YR 6/4 (light yellowish brown, marron jaunâtre clair) et 5Y 6/2(pinkish gray, gris rosé). Sa texture est argilo-limoneuse et décrit une structure lamellaire compacte.

Le profil 2 d'une profondeur de 10 cm est un également un Anthroposol Artificiel. Il possède un horizon organique (OL+OF) épais et dense de 3 cm de litière non décomposée, de couleur 10YR 2/1 (black, noir) en transition nette avec l'horizon OH (3-5cm) dont la litière reflète un état de décomposition plus avancé. La couleur de cet horizon est 10YR 2/2 (very dark brown, marron très foncé). La transition avec l'horizon A (5-7 cm), dont la couleur est 10YR 2/3 (very dark brown, marron très foncé), est également nette. Sa texture est argilo-limono-sableuse alors que sa structure est particulaire et peu cohésive. La pierrosité est nulle sur tout le profil et la densité racinaire est assez élevée. La transition avec l'horizon Z_{tc} (> 7 cm) est nette et ondulée. Cet horizon, représentant le matériau parental, a une couleur 10YR 4/4 (dark yellowish brown, marron jaunâtre foncé), une texture limoneuse et une structure polyédrique.

I.2 Données climatiques

Les données climatiques indiquent des précipitations moyennes, au cours des 10 dernières années, de 1018 mm par an pour une température moyenne annuelle de 11.3°C. En hiver, la température des mois de décembre, janvier et février est en moyenne de 4.2°C alors qu'en période estivale, (mai à septembre) la température moyenne est de 17.1°C.

II. Caractérisation des échantillons

II.1 Préparation des échantillons

L'ensemble des échantillons caractérisés a été prélevé dans chacun des horizons des 2 profils étudiés, c'est-à-dire des horizons O à Z_{tc} pour le profil 1 et des horizons OL-OF à Z_{tc} pour le profil 2. Le prélèvement a été fait au moyen d'outils en plastiques. Chaque échantillon, stocké dans des sachets en plastique, a été séché à l'air puis tamisé à 2 mm. La plupart des expérimentations ont été réalisées sur la fraction < 2 mm ; dans le cas contraire, il sera précisé sur quelle fraction ont été réalisées les mesures.

II.2 Caractérisation pédologique

Les mesures du pH eau (eau déminéralisée) et pH KCl (1*M*) ont été réalisées selon un ratio solide:liquide de 2:5. Après destruction de la matière organique des différents échantillons, la granulométrie a été estimée à la pipette Robinson pour les fractions inférieures à 50 µm selon trois prélèvements (< 2µm, argiles, < 20 µm, limons fins et < 50 µm, limons grossiers). Les fractions > 200 µm et > 50 µm ont été obtenues par tamisage humide (NF X 31-107, Afnor, 2003). La densité apparente a été estimée, en trois répliquats par horizon, selon la méthode des anneaux de densité, autrement dit par différence de masse avant et après séchage à 105°C. La capacité d'échange cationique (CEC) a été déterminée par la méthode des chlorures de cobaltihexamine, sans modification du pH du sol, selon la norme NF X 31-130 (Afnor, 1999). Dans le surnageant, la quantité des cations échangeables Ca^{2+}, Mg^{2+}, Na^+ et K^+ ainsi que de Al^{3+} et des protons a été analysée. La mesure du carbone organique et de l'azote totale a été faite par combustion sèche selon les normes NF ISO 10694 (Afnor, 1995a) et NF ISO 13878 (Afnor, 1995b) respectivement.

L'ensemble de ces analyses ont été réalisées au laboratoire INRA de Arras, à l'exception des mesures de densité réalisées au laboratoire.

II.3 Caractérisation minéralogique

La minéralogie a été caractérisée, après broyage des échantillons < 2 mm, par diffraction des rayons X (DRX), par des mesures réalisées sur un Brucker D8 Advance (CuKα, 40 kV, 40mA) entre 2 to 65°2θ selon un pas de temps de 0.17°2θ/min (Université de Poitiers). Des analyses DRX ont également été menées sur la fraction < 2µm extraite selon la loi de Stokes. Celles-ci ont été complétées par des observations de la fraction < 2 mm au microscope électronique à balayage (MEB) couplées à des analyses chimiques semi-quantitatives (EDS – Energy Dispersive X ray Spectroscopy) avec l'appareil PHILIPS XL30 à 20kV (SERMIEL, Université de Limoges, France). Ces observations ont été faites sur des lames minces sur lesquelles les échantillons bruts (sans broyage) ont été fixés et indurés dans une résine avant d'avoir été polis.

III. Analyses chimiques

III.1 Analyses chimiques totales des échantillons de sol

La composition chimique des échantillons a été déterminée par fusion alcaline (métaborate et tétraborate de lithium) et par minéralisation à l'acide nitrique. Les éléments mineurs ont été dosés par ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) alors que les éléments majeurs l'ont été par ICP-AES (Inductively Coupled Plasma – Atomic Emission Spectrometry). L'ensemble de cette caractérisation a été réalisée par le laboratoire ACME Analytical Laboratories (Vancouver, Canada).

III.2 Détermination de la spéciation chimique des éléments

La spéciation chimique des éléments a été déterminée par l'extraction séquentielle type BCR (Bureau Community of Reference) en quatre étapes et accélérée par des ultrasons (Perez Cid *et al.*, 1998). Pour cela, 0,5 g de sol ont été placés dans un tube à centrifuger. Chaque étape était séparée par une étape de rinçage à l'eau ultrapure avec agitation pendant 20 min et centrifugation afin d'éliminer l'eau de rinçage et poursuivre l'extraction.

La première étape qui a estimé la fraction échangeable et faiblement liée aux constituants du sol a été extraite avec 20 mL d'acide acétique à 0,11 mol.L^{-1} et 7 min d'ultrason à 20W. L'ensemble a ensuite été centrifugé à 5 000 tr.min^{-1} pendant 15 min.

La seconde étape, estimant la fraction réductible des éléments, autrement dit les éléments liés aux oxy-hydroxydes de fer et aluminium a été extraite avec 20 mL de chlorure d'hydroxylammonium (0,5 mol.L^{-1}) ajusté à un pH de 2. Le temps de sonication à 20W fut également de 7 min.

La troisième étape reflète la part des éléments contenus dans la fraction oxydable et donc transformable en CO_2 et notamment liée à la matière organique. Cette fraction a été extraite en deux temps. Pour commencer, 10 mL de péroxyde d'hydrogène (300 mg.g^{-1} – 8 mol.L^{-1}) et 2 min de sonication à 20W. Ensuite, 25 mL d'acétate d'ammonium à 1 mol.L^{-1}- pH 2 sont ajoutés avec un temps de sonication de 6 min.

La dernière étape consistait à minéraliser la fraction résiduelle par une minéralisation à l'eau régale (2/3 HCl et 1/3 HNO_3) et une digestion aux micro-ondes (Anton Paar Multiwave 3000) s'effectuant par augmentation de la température pendant 5 min pour atteindre 1400°C, température maintenue pendant 25 min, suivi d'un refroidissement pendant 20 min.

III.3 Oxy-hydroxydes de fer amorphes et cristallisés

L'extraction au citrate bicarbonate dithionite attaque les oxy-hydroxydes de fer amorphes et cristallisés. A 2g d'échantillon, nous avons ajouté 75 mL d'extractant (pour 1L de solution, 78g de tricitrate de sodium, 9g de bicarbonate de sodium et 23g de chlorure de sodium). L'ensemble a été chauffé au bain-marie à 75-80°C. Lorsque cette température a été atteinte, 2g de dithionite de sodium ont été ajoutés en laissant agir pendant 30 min avant centrifugation afin d'extraire le surnageant à analyser.

Pour extraire les oxy-hydroxydes de fer amorphes, une extraction à l'oxalate d'ammonium 0,1134 mol.L^{-1} et l'acide oxalique 0,0866 mol.L^{-1} a été réalisée à l'obscurité pendant 4h ajustée à un pH de 3. Le surnageant a également été prélevé après centrifugation.

III.4 Estimation de la biodisponibilité des éléments As et Pb

Les trois extractions suivantes ont été mises en œuvre pour l'analyse de la fraction théoriquement biodisponible des éléments étudiés :

(i) une extraction au $CaCl_2$ à une concentration de 0,01 mol.L^{-1} selon un ratio solide:liquide de 1g:10mL avec une agitation pendant 2h (Novozamsky *et al.*, 1993).

(ii) une extraction selon la méthode A-Rhizo correspondant à un mélange d'acides organiques mimant l'action des exsudats racinaires. Ce mélange se composait de l'acide acétique, l'acide lactique, l'acide citrique, l'acide malique et l'acide formique. Leur concentration finale était de 0,01 mol.L^{-1}. Le ratio solide:liquide est de 1g:10mL avec un temps de contact de 16h (Fang *et al.*, 2007).

(iii) une extraction au DTPA (Acide Diéthylène Triamine Penta-Acétique), selon un ratio solide:liquide de 1g:2mL. La solution extractante se composait de 0.005*M* de DTPA, 0,01*M* de $CaCl_2$, 0,01*M* de triéthanolamine, le tout à un pH ajusté à 7,3 et un temps de contact de 2h.

III.5 Dosage des éléments par spectrométrie d'absorption atomique

Les solutions issues des extractions décrites précédemment en III.2, III.3 et III.4 ont systématiquement été filtrées à 0,2 µm (filtre en acétate de cellulose, Sartorius). En fonction de la concentration de l'élément à doser, celui-ci a été dosé après que l'échantillon ait été dilué de façon à se trouver dans la gamme d'étalonnage pour une mesure avec l'appareil muni d'un four ou d'une flamme. Les fortes concentrations, de l'ordre du mg.L^{-1} ont été analysées en flamme (Varian SpectrAA 220) dont l'appareil est équipé d'une correction du signal de fond au deutérium. A l'inverse, les échantillons de plus faibles concentrations (µg.L^{-1}) ont été analysés en four graphite avec une correction Zeeman du signal de fond (Varian SpectrAA 880 Z).

IV. Culture de *Miscanthus* x *giganteus*

IV.1 Les conditions de culture

Pour toutes les cultures de *Miscanthus* x *giganteus*, nous avons effectué un prélèvement grossier sur le site d'étude, au moyen d'une pelle en acier, des 20 premiers cm de sol, dans les zones des deux profils d'étude, après avoir enlevé la litière. Ainsi, les horizons A-Z_{tc} et A-Z_{tc} respectifs des profils 1 et 2 constitueront les échantillons globaux dénommés composites dans lesquels la plante se développera et sera étudiée. Pour les mesures de biologie moléculaire effectuées dans la rhizosphère de *M.* x *giganteus* et détaillées au paragraphe V, la plante a également été cultivée sur un sol composite supplémentaire prélevé comme décrit précédemment, dans la zone 2, juste au sud de la zone 1. Ce sol composite présentait des concentrations en As et Sb intermédiaires aux deux autres points de prélèvement des zones 1 et 2.

La culture de miscanthus a été réalisée en phytotron pendant 3 mois selon un cycle jour/nuit de 8h/16h à une température jour de 23°C et à 18°C pendant la nuit. L'intensité lumineuse était de 350 µmol.m^{-2}.s^{-1}.

Quatre litres de sol tamisés à 2 mm et séché à l'air ont été placés dans des sachets en plastique à l'intérieur de pots en plastique afin d'éviter la perte d'éléments par lessivage. Après une semaine d'équilibrage de l'humidité du sol à 60% de leur capacité au champ, un rhizome de *M.* x *giganteus* par pot a été ajouté et introduit dans le sol à environ 5-10 cm de la surface du pot. Un rhizome est une tige souterraine qui forme de jeunes pousses chaque année. Les pots ont été arrosés deux fois par semaine à la capacité au champ des sols de façon à ne pas submerger les sols. La durée de culture a été de 3 mois. Huit répliquats par type de sol ont été mis en culture : quatre étaient destinés à être récoltés après un mois et demi de culture (t_1) pour des analyses relatives aux micro-organismes (voir V), quatre autres répliquats, récoltés après 3 mois de culture (t_2) ont servis à l'analyse des paramètres photosynthétiques (IV.2), de la mesure de l'accumulation en As, Sb et Pb (IV.3) et à nouveau aux mesures de biologie moléculaire (V).

IV.2 Mesures des paramètres photosynthétiques

Avant la récolte finale, la mesure des paramètres photosynthétiques (photosynthèse nette, conductance stomatique, concentration en CO_2 substomatale et transpiration) a été réalisée avec l'appareil Li-Cor Model 6400, Lincoln, NE, USA. Nous avons fait une mesure par pied sur une jeune feuille.

Des mesures de la fluorescence de la chlorophylle *a* ont également été effectuées (PAM FMS1, Hansatech Instruments Ltd., Norfolk, UK). Pour cela, l'évaluation des paramètres F_O/F_m et F_v/F_m a été réalisée (avec F_O, la fluorescence initiale, F_m la fluorescence maximale et F_v, la différence entre F_m et F_O). F_m a été obtenue après un flash saturateur de 1s à 13000 $\mu mol.m^{-2}.s^{-1}$.

A la récolte, les différentes parties de la plante (racines, rhizome, tige et feuilles) ont été soigneusement séparées, nettoyées à l'eau ultrapure, pesées et séchées à 60°C jusqu'à stabilisation du poids sec. La masse de ces différents tissus sera mesurée avec précision (Precisa, Swiss Quality).

IV.3 Analyses chimiques des différents tissus de la plante

La composition chimique des différentes parties de *M.* x *giganteus* a été déterminée sur la biomasse sèche après broyage par une attaque acide à HNO_3 puis à l'eau régale. Les éléments As, Sb et Pb ont été dosés par ICP-MS par le laboratoire Acme Lab, Vancouver, Canada.

V. Evaluation de l'abondance des bactéries et archées oxydantes de l'ammonium

V.1 Extraction d'ADN et quantification du gène amoA dans les sols composites

L'ADN (Acide DésoxyriboNucléique) du sol de chaque pot de culture (décrit en IV.1), avant l'ajout du rhizome de miscanthus, a été extrait à partir d'échantillons de 0,5 g. Cette extraction a été réalisée au moyen d'un kit d'extraction spécial pour les sols (FastDNA SPIN kit, MP Biomedical). La quantité d'ADN a été vérifiée avec l'appareil NANODROP 1000 spectrophotometer (Institut fur Bodenökologie, HelmholtzZentrum, Munich).

La PCR quantitative (Polymerase Chain Reaction, ou, en français, Réaction en Chaine par Polymérase) du gène *amoA* (ammonium monooxygénase) présent chez les bactéries oxydantes de l'ammonium (AOB) et les archées oxydantes de l'ammonium (AOA) a été effectuée en triplicat pour chaque échantillon de sol prélevé après que les sols aient été humidifiés pendant une semaine et avant la mise en place des rhizomes. Ces mesures ont été réalisées sur le ABI Prism 7300 CYCLER (Institut fur Bodenökologie, HelmholtzZentrum, Munich). Chaque échantillon d'ADN a été mélangé à du sérum de bovin, des amorces et du Power SYBR Green PCR master mix dont les volumes sont détaillés dans le Tableau II.1. Le primer sens utilisé (*amoA*-1F ; 5'GGGGTTTCTACTGGTGGT) visait la partie correspondant aux positions 332 à 349 et le primer anti-sens (*amoA*-2R ; 5'CCCTCKGSAAAGCCTTCTTC, avec K=G ou T et S=G ou C) visait la partie correspondant aux positions 802 à 822 de la fenêtre de lecture publiée pour la séquence du gène *amoA* chez *Nitrosomas europaea* (McTavish *et al.*, 1993).

Tableau II.1. Composition des 25 µL de Master Mix pour la quantification du gène *amoA* de AOB et AOA.

Groupe étudié	Gène ciblé	2x Power SYBR green	3% BSA	Amorce sens	Amorce anti-sens	ADN	Eau
		µL					
AOB	*amoA*	12,5	0,5	0,75	0,75	2	8,5
AOA	*amoA*	12,5	0,50	0,5	0,50	2	9,0

Chaque cycle d'analyse PCR commençait par une étape d'activation de l'enzyme à 95°C pendant 10 min. Le profil thermique du cycle du gène *amoA* de AOB était 94°C/60s, 60°C/60s, 72°C/60s sur 39 cycles. Pour le gène *amoA* de AOA le profile était : 94°C/45s, 50°C/45s, 72°C/45s sur 40 cycles.

V.2 Evaluation de l'abondance du gène amoA *chez AOB et AOA dans le sol rhizosphérique de* Miscanthus x *giganteus*

Les modalités de culture de *Miscanthus* x *giganteus* étaient celles décrites au paragraphe IV1. L'évolution de l'abondance du gène *amoA* de AOB et AOA au cours de la culture de *M.* x *giganteus* a été effectuées dans la rhizosphère de la plante à 2 temps différents de croissance (t_1 et t_2). De manière pratique, les racines entourées de la fine couche de rhizosphère constituent les échantillons d'analyse.

Après un mois et demi de culture (t_1), quatre plantes par sol ont été récoltées. Les racines et leur rhizosphère ont été congelées dans l'azote liquide et stockées à -20°C jusqu'aux analyses. Le reste des plantes a été récolté comme décrit en V.2. Le même échantillonnage de quatre autres plantes a été effectué au bout de 3 mois de culture (t_2). Ainsi, l'abondance du gène *amoA* chez AOA et AOB a été mesurée dans chacune des rhizosphères des plantes ayant poussé sur 3 sols composites, aux 2 temps de récoltes et comme décrit en V.1).

V.3 Analyses chimiques du sol rhizosphérique

Des mesures de pH, de l'estimation de la biodisponibilité de As et Pb (extraction $CaCl_2$, comme décrit en IV.4) ainsi que la mesure des teneurs en carbone organique extractible à l'eau (WEOC pour Water Extractible Organic Carbon, c'est-à-dire carbone organique extractible à l'eau), en ammonium (NH_4^+-N) et en nitrate (NO_3^--N) ont également été effectuées sur le sol de culture avant la présence de la plante ainsi que sur les sols rhizosphériques. Cette dernière extraction a été faite selon un ratio solide:liquide de 1:4 au $CaCl_2$ 0,01*M* avec une agitation pendant 45 min. L'ensemble des solutions extraites a été filtré à 0,45µm. Les teneurs en NH_4^+-N et NO_3^--N ont été analysées en flux continu (SA 20/40, Skalar Analytical, The Netherlands). Le carbone organique a été mesuré sur l'appareil TOC-5050A, Shimadzu.

Pour cet ensemble d'expérimentations de biologie moléculaire, le pH des sols a été mesuré au $CaCl_2$ et non au KCl afin d'être dans les mêmes conditions que les extractions pour la biodisponibilité et la mesure de l'ammonium, des nitrates et du carbone organique dissout.

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CHAPITRE III

Comportement géochimique de arsenic, antimoine et plomb dans les sols étudiés

Les activités anthropiques sont à l’origine de nombreux phénomènes de contaminations des sols, des eaux, et de l’air. Dans le cas des sols contaminés, les principaux risques sont liés à la contamination de la chaîne alimentaire par le biais de la végétation ou de la ressource en eau. Il est donc impératif d’éviter toute dispersion de ces contaminants. Pour cette étude, les travaux se sont focalisés sur un ancien bassin de décantation stockant les déchets provenant de l’industrie extractive de l’or et de son traitement, terminé depuis 1964. De ce fait, ces matériaux sont très fortement contaminés en divers éléments potentiellement toxiques (EPTs) comme l’arsenic, l’antimoine ou encore le plomb et présentent donc un risque environnemental marqué. Par ailleurs, suite à l’arrêt de l’activité minière dans les années 60, des sols se sont progressivement développés à partir de ces déchets laissés à l’abandon.

L’évolution de ces matériaux et le développement progressif de sols conditionnent le comportement environnemental des EPTs. C’est pour cette raison qu’avant de mettre en place une gestion appropriée et s’appliquant aux sites et sols pollués, il était fondamental de réaliser une étude pédologique, géochimique et minéralogique des matériaux en question afin de mieux appréhender le devenir et la mobilité des contaminants. Ainsi, leur éventuel transfert à la végétation ou d’éventuelles dispersions dans l’environnement pourraient être mieux gérés et limités.

L’ensemble de ces travaux est détaillé sous la forme d’un article intitulé “ Fate and geochemical behaviour of arsenic, antimony and lead in two anthroposols developed from mining wastes ” dans le but d’être soumis à la revue Geoderma.

Fate and geochemical behaviour of arsenic, antimony and lead in two anthroposols developed from mining wastes

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Abstract: The mining wastes deriving from gold extraction in Massif Central (France) have been stored and abandoned in a settling basin since 1964. Their contents in PTEs (Potentially Toxic Elements) such as As, Sb and Pb reaching several thousand mg.kg^{-1} might represent a strong potential hazards for soils, groundwater or even the atmosphere. Formation of soils concomitant to vegetation colonization of this site impacted fate and geochemical behaviour of the studied PTEs. Theses soils were characterized as Technosols / Anthroposols Artificiels according to respectively WRB and French nomenclature. BCR sequential extraction procedure indicated that As and Pb were mainly content in residual fraction corresponding to beudantite and scorodite (the main secondary mineralogical phases) but not preventing PTEs interaction with other soil constituents *i.e.* organic matter. Furthermore, available fraction of As and Pb seemed to be low as it constituted a very low percent of total concentrations. However, in absolute value, it might be a risk for vegetation or groundwater in case of leaching.

Key words: anthroposol, mining wastes, soil mineralogy, geochemical behaviour, metalloids.

I. Introduction

Anthropogenic activities such as mining activities are responsible for the increase in inorganic pollutants occurrence in the environment. One of the main concerns of mining activities is the production of a huge amount of contaminated wastes which are usually stored in the vicinity of mines. Waste materials usually contain potential toxic elements (PTEs) in various amounts (depending of industrial process) representing contamination hazards for groundwater and food chain especially through transfer to vegetation. Consequently and due to the no degradability of inorganic pollutants in the environment, it is necessary to understand and assess their behaviour and fate to prevent their releasing and mobility in the environment. According to the type of climate, a soil may develop from wastes and vegetation gradually may colonize the site inducing changes in PTEs behaviour.

The remediation of contaminated sites is of key importance, but remediation solutions need to be adapted to the site configuration and to the nature of wastes deposits. *In situ* treatments, combining the use of vegetation, are currently mostly proposed with the aim of phytostabilizing (roots system preventing transfer to groundwater) or phytoextracting (PTEs extraction through biomass accumulation reducing pollution rate of soil) (Salt *et al.*, 1995).

Released elements may migrate toward soil profile, precipitate or also induce neoformation of secondary minerals (Valente and Gomes, 2009). Furthermore, PTEs behaviour is controlled by elements speciation. For example, if they are weakly linked to soils constituents, their mobility will be higher than if they belong to a mineral structure. Moreover, in case of wastes rich in sulfides/sulfates, oxidation can lead to elements release increasing their mobility. As a result, PTEs behaviour can be well understood by their chemical speciation assessed by a sequential extraction procedure (SEP) which provides semi-quantitative information for their mobility. Community Bureau of Reference (BCR) initialized by the European Community, is commonly performed on sediments and soils. This SEP leads to determine four different fractions: the fraction easily exchangeable and weakly bound to soil constituents, the reducible fraction, the oxidable fraction and then the residual fraction. Accordingly, it offers a qualitative repartition of contaminants in soils and therefore allows assuming their behaviour to short term (Anju *et al.*, 2010; Pueyo *et al.*, 2008). As this kind of situation is more and more common, processes implied in pedogenesis are specific and has to be characterized. Indeed, soil evolution in contaminated situations has to be better apprehended.

The aim of this study was to determine the geochemical behaviour of As, Sb and Pb in relation to their chemical speciation in young soils developed from mining wastes while local vegetation succeeded in growing on such a contaminated material. Hence, a former gold mine abandoned for about 45 years where wastes have been stored in settling basin was investigated (Néel *et al.*, 2003). The main contaminants or PTEs were arsenic (As), lead (Pb) and antimony (Sb) given their concentrations reaching several percentages. Additionally, mobility, bioavailability and relation to soil properties were discussed in order to assess As, Sb and Pb pollution and their environmental risk, in case of a potential future phytoremediation.

II. Materials and methods

II.1 Description of the sampling site

The studied site was a settling basin located at La Petite Faye (Limoges, French Massif Central) where about 35000 t wastes from a former gold mine are stored since 1964, year of the end of mining activities. Consequently, these wastes materials were characterized by high total concentrations in PTEs reaching sometimes several ten percentages (*e.g.* up to 11% As, 21% Pb, 1400 mg.kg^{-1} Sb). Despite high concentrations in PTEs, native vegetation has colonized the site following a zonation leading to delimitation of 2 zones: whereas the vegetation in zone 1 was sparse and essentially dominated by *Graminea*, zone 2 had a vegetal cover very dense and vigorous composed by giant horsetail (*Equisetum telmateia*). Two soil profiles have been described and sampled in each zone and were respectively named profile 1 and profile 2. Sampling followed differentiated layers between 0 and more than 11 cm in profile 1 and from 0 to more than 7 cm in profile 2. Samples have been air dried and sieved to 2 mm prior to analysis.

II.2 Pedological parameters

pH was measured in a solid:liquid ratio of 2:5 with ultra pure water and KCl 1*M* to consider exchange acidity. Particles size distribution of <2 mm was determined on samples without organic matter (destruction by H_2O_2) using Robinson pipette for fraction <50 µm and by sieving under water for sands. The cation exchange capacity (CEC) was determined by the 0.05*N* cobalthexamine method performed at the soil pH (Orsini and Remy, 1976) since actual soil pHs were lower than 6. Organic carbon was determined by dry combustion with a C/H/N elemental analyzer. Bulk densities of soil horizons were measured by the core cylinder method, with three replicates per horizon.

Dithionite citrate bicarbonate (DCB) soluble element reflects the poorly ordered inorganic, organic and crystalline components commonly named amorphous and crystalline oxy-hydroxydes. Elements were extracted according to the method of Mehra and Jackson (1960). Moreover, acid-ammonium oxalate extraction, related to elements fixed on poorly ordered inorganic and organic components (amorphous oxy-hydroxydes), was also realized according to the protocol of Mc Keague (1966). Measurements of extracted Fe were determined by flame atomic absorption spectrometry (Varian SpectrAA 220) or graphite furnace atomic absorption spectrometry (Varian SpectrAA 880 Z), according to its concentration.

II.3 Total chemical composition

Total chemical analysis of bulk soil < 2 mm in order to determine major elements were achieved after a lithium metaborate / tetraborate fusion and nitric acid digestion. Major elements were measured by ICP-AES whereas inorganic pollutants (As, Sb, Pb) were determined after an *aqua regia* digestion and measured by ICP-MS both at ACME Analytical Laboratories (Vancouver) ltd, Canada.

II.4 Enrichment factors

To evaluate the vertical distribution of these elements in studied soils, enrichment factors (EF) were calculated using Zr as the invariant element and according to the next equation (Soubrand-Colin *et al.*, 2007):

$$EF_{(X)} = [(X/i)/(X_0/i_0)]$$

where i and i_0 were respectively invariant element concentrations in soil horizon and corresponding parental material and X and X_0 were concentrations of a given element respectively in soil horizon and in parental material.

II.5 BCR sequential extraction procedure

The modified sequential extraction procedure BCR (Community Bureau of Reference) accelerated by ultrasounds was performed on 0.5 g of soil (< 2 mm fraction; Pérez-Cid, *et al.*, 1998). Each extract was separated by centrifugation (5000 rpm, 15 min) and a water rinse (10 mL) with ultrapure water was achieved between each step. The first step F1, the exchangeable fraction, was extracted by 20 mL of acetic acid (0.11 mol.L^{-1} - 7 min ultrasound, 20W). To the residue of step 1, 20 mL of hydroxylammonium chloride (0.5 mol.L^{-1}, pH 2 - 7 min of ultrasound, 20W) was added, corresponding to the extracted fraction F2 (the reducible faction). Then, 10 mL of hydrogen peroxide (300 mg.g^{-1}, 8 mol/L - 2 min of ultrasound, 20W) followed by 25 mL of ammonium acetate (1 mol.L^{-1}, pH 2 - 6 min of ultrasound, 20W) corresponding to the oxidizable step, F3, was added to the residue of step 2. The residual fraction (F4) was mineralized with *aqua regia* and a microwave (Anton Paar Multiwave 3000) digestion during 5min up to 1400°C, 25min hold and 20min cooling. Fe, As and Pb concentrations were determined (see before).

II.6 Mineralogical characterization

Powder X-ray diffraction was used for mineralogical characterization in bulk (< 2 mm) and clay (< 2 µm) fractions from each horizons using a diffractometer Philips

PANalytical (CuKα, 40 kV, 30mA) between 5 to 65°2θ at 0.12° 2θ/min. The < 2 µm fraction was obtained by sedimentation according to Stockes law after dispersion with NaCl 1*N*.
Local analyses of minerals were carried out on thin section of the bulk sample using Philips XL30 Scanning Electron Microscope (SEM) at 20kV equipped with energy dispersive X-Ray spectrometer (SerMiEl, Université de Limoges, France). Electron microprobe analysis (EMPA, Cameca SX-50) was also used for *in-situ* analysis of the major elements (ISTO-BRGM, France).

III. Results and discussion

III.1 Soil characteristics

The studied soils located in Limousin (France) have developed in 45 years on the highly contaminated tailing which present a large vegetation cover leading to a zonation. Whatever the zone, soils developed from acidic material were primarily very thin (only till 15 cm depth), and without any B horizon differentiation (Figure II.1). This is in accordance with findings of Remon *et al.* (2005) who reported same properties for soils developed under alkaline substrate, as well as with the minimum profile development as a consequence of young age (Scalenghe and Ferraris, 2009). As expected, soils presented low pHs (zone 1: 3.4 to 4.4; zone 2: 4.2 to 5.7; Table III.1). One of the major differences between both zones was related to the textural class of soils (sandy loam in the zone 1 vs. clay in the zone 2) which was attributed to the period where wastes were thrown into the basin. Indeed, the lateral grain size distribution has been explained by the localisation of the alimentation pipe situated near the zone 1. Finest particles were naturally transported at the opposite of the alimentation namely “far” from the alimentation (corresponding to zone 2) whereas heaviest minerals stayed close to this one (zone 1). As a result, this textural differentiation might contribute to changes between the two zones soil properties as well as probably the various vegetation cover. Moreover, the CEC of the soils was relatively low given the mineralogy of the parental material.

The high organic carbon (Corg) and nitrogen contents were clear but despite any input

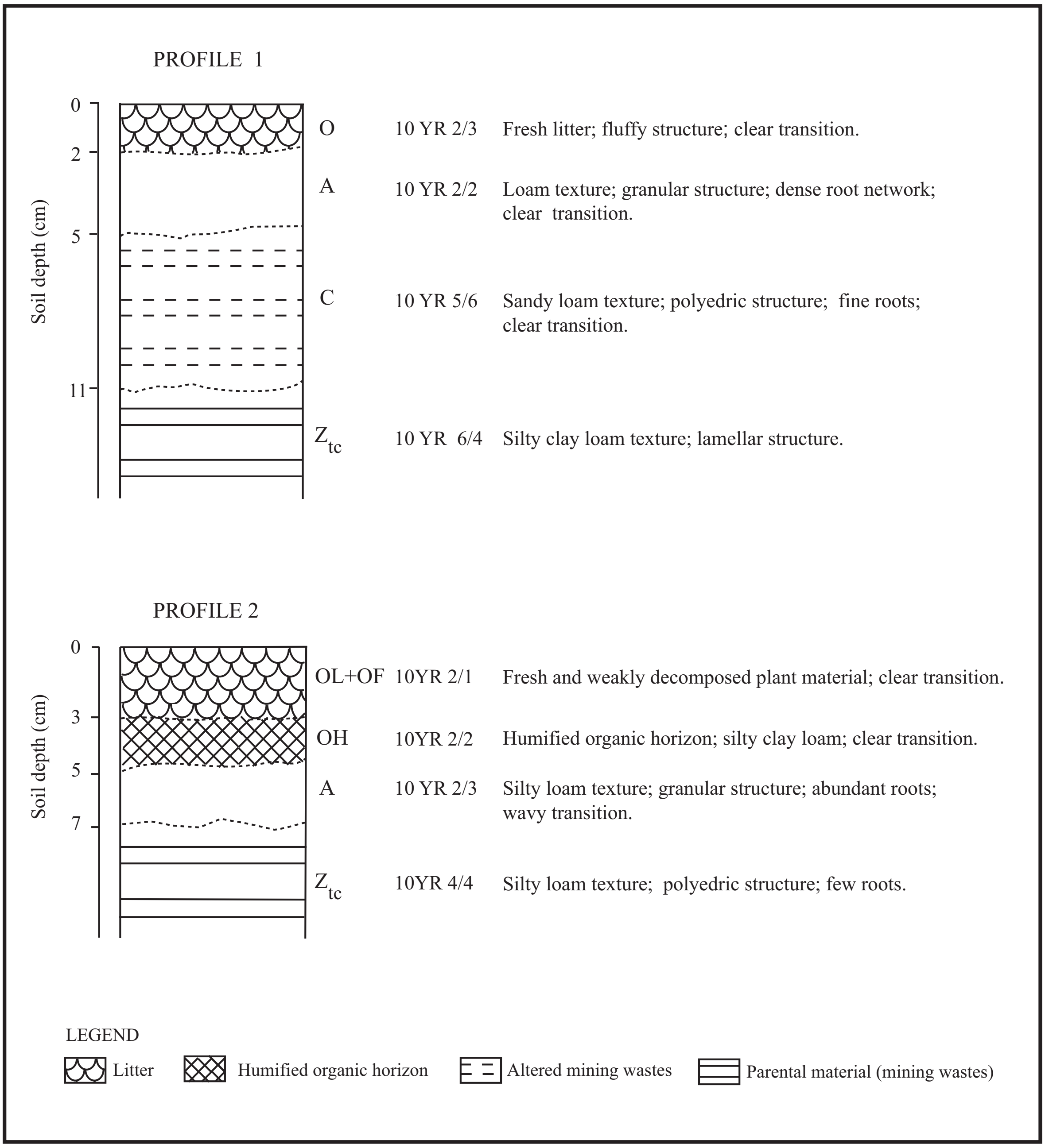

Figure III.1. Macroscopic soil morphology and main pedological features of both studied profiles

(till 426 C_{org} g.kg^{-1} decreasing with depth, Table III.1) contrary to most mining soils presenting usually lower levels (*e.g.* 50 to 70 g.kg^{-1}; Franco-Hernández *et al.*, 2010; Lei and Duan, 2008; Moreno-Jiménez *et al.*, 2009; Remon *et al.*, 2005; Wang *et al.*, 2008). As expected, their amounts were strongly higher in the top horizons, particularly in the profile 2 (*e.g.* up to two times higher than in profile 1; till 426 and 28 g.kg^{-1} respectively FOR Corg and Total N), which is in accordance with the larger vegetal cover compared to profile 1. The amount of Corg, and the C/N ratio (from 16.3 to 10.4 in profile 1 and 12.1 to 15.2 in profile 2;

Table III.2), which were related to stages of soil development were associated to plant cover density, and could be perceived as evidence of soil evolution.

Indeed, previous works on this site by Néel *et al.* (2003) have shown that only algaes and fungi recovered a large part of the site, the soil being classified as Entisol with incomplete A-C horizon differentiation. Ten years later, dense natural vegetation cover was marked as well as soil development. Since parental material had an anthropogenic origin, these soils were either classified as Technosols (‘dominated by human-made materials’) as it is described in WRB (World Base Reference, 2006) or ‘Anthroposols Artificiels’ (entirely resulting of anthropogenic materials inputs after *i.e.* mining activities) in the French nomenclature (Référentiel Pédologique, 2008). Given their depth, and their development since 45 years old, it corresponds to average rates of about 0.3 cm per year. This rate is in accordance with the time required for generating soil horizons in mining or waste deposits (Ciolkosz *et al.*, 1985; Varela *et al.*, 1993). As in all pedogenesis, the soil development lead to the redistribution of elements toward soil profiles which might modify their speciation.

III.2 Distribution of metal and metalloids in soils

The total chemical compositions of the two studied soil profiles are reported in the Table III.2. Chemical composition for the major elements seemed to be relatively stable along both profiles. Briefly, Si was the major element with concentration reaching about 20% in weight, whereas Fe content was up to 10% and less than 5% for Al. The basic element concentrations (Ca, Na, Mg, K) as well as S were very low and lower than 1%. The chemical composition of the soils derived from parental material (Table III.2), which matched directly with the initial acidic gold ores (Nénert *et al.*, 1997). The special interest of this site was related to the gold extraction process which concentrated contaminants in the settling basin. For example, As, Sb and Pb concentrations were measured till 119000, 1406 and 21300 mg.kg^{-1} respectively, concentrations increasing with depth and being lower in zone 2. These measured amounts of metals and metalloids were exceptionally high for soil, even compared to other soils developed from mining waste (Flynn *et al.*, 2003; Razo *et al.*, 2004; Chopin et *al.*, 2007; Murciego Murciego *et al.*, 2007; Navarro *et al.*, 2008; Rodriguez *et al.*, 2009; Boussen *et al.*, 2010).

Table III.1. Physico-chemical properties of the two Technosols / Anthroposols studied from La Petite Faye.

	Depth	Horizon	pH		Bulk Density	C_{Org}	Tot N	C/N	Sand	Silt	Clay	CEC	Fe_O	Fe_d	Fe_O/Fe_d
			H_20	KCl											
	cm					g.kg^{-1}			%			cmol(+).kg^{-1}	mg.kg^{-1} (extracted %)		
Profile 1	0-2	**O**	4.4	3.9	0.44	181.0	11.1	16.3	32.1	48.6	19.3	19.1	na	na	na
	2-5	**A**	3.8	3.3	0.68	96.6	6.5	14.8	41.7	44.9	13.4	8.8	na	na	na
	5-11	**C**	4.3	3.4	1.03	8.2	0.6	13.8	49.9	45.5	4.6	2.1	48425 (64)	71437 (94)	0.68
	> 11	**Z_{tc}**	3.4	3.2	1.04	4.9	0.5	10.4	12.9	58.0	29.1	2.7	82716 (68)	100441 (82)	0.82
Profile 2	0-3	**OF-OL**	5.0	4.6	0.36	426.0	28	15.2	4.1	44.5	51.4	63.6	na	na	na
	3-5	**OH**	4.5	3.9	0.36	350.0	24.5	14.3	10.5	52.3	37.2	53.7	na	na	na
	5-7	**A**	4.2	3.6	1.22	188.0	15.5	12.1	19.3	56.1	24.6	29.2	19842 (46)	32707 (75)	0.61
	> 7	**Z_{tc}**	4.5	3.4	1.22	13.7	1	13.6	13.8	69.7	16.5	5.8	71584 (79)	87885 (97)	0.81

Fe_O = acid-oxalate extractable Fe; Fe_d = dithionite extractable Fe; na = not analyzed

Table III.2. Bulk chemical composition of each horizon from the two profiles developed since the end of gold extraction.

Samples	Depth	Horizons	Si	Al	Fe	S	Ca	Mg	Na	K	Pb	As	Sb
	cm						%					mg.kg^{-1}	
Profile 1	0-2	**O**	18.9	2.6	5.0	0.3	0.50	0.31	0.43	1.29	6939	32200	432
	2-5	**A**	25.2	3.6	6.8	0.1	0.33	0.34	0.58	1.64	9304	41900	530
	5-11	**C**	27.7	3.5	7.6	0.2	0.16	0.21	0.66	1.63	14100	64200	808
	> 11	**Z_{tc}**	17.6	4.5	12.2	1.5	0.08	0.19	0.47	1.74	21300	119900	1406
Profile 2	0-3	**OF-OL**	na	na	na	0.1	0.60	0.06	0.01	0.12	1299	6712	108
	3-5	**OH**	8.5	0.9	1.4	0.3	1.09	0.13	0.14	0.54	2537	5465	103
	5-7	**A**	19.5	2.3	4.3	0.2	0.69	0.17	0.39	1.10	5761	22000	295
	> 7	**Z_{tc}**	24.2	3.8	9.0	0.2	0.19	0.24	0.56	1.20	14500	68100	930

In both profiles, EF (enrichment factor) of Si, Al and Fe elements (Figure III.2) described different patterns: EF(Al) and EF(Fe) decreased from depth to surface (from 1 to about 0.50) in profile 1 whereas EF(Al) was relatively stable in profile 2 and a depletion of EF(Fe) was noted. Si was rather constant along profile for both zones. However, a slight enrichment was recorded in the O horizon of the zone 2 which might be the result of an intense biocycling due to the presence of *Equisetum telmateia*, a Si-rich plant (Fraysse *et al.*, 2006). In the cases of studied PTEs, EF of As, Sb and Pb presented the same trends in both pedons, *i.e.* a large decrease from parental material to topsoil (Figure III.2). The behavior of As, Sb and Pb could be explained by an intense profiles leaching within 30 to 70 % of loss, compared to parental material. However, in more detailed, figure III.2 highlighted the next findings: (i) no significant differences were noted between EF(As), EF(Sb) and EF(Pb) in

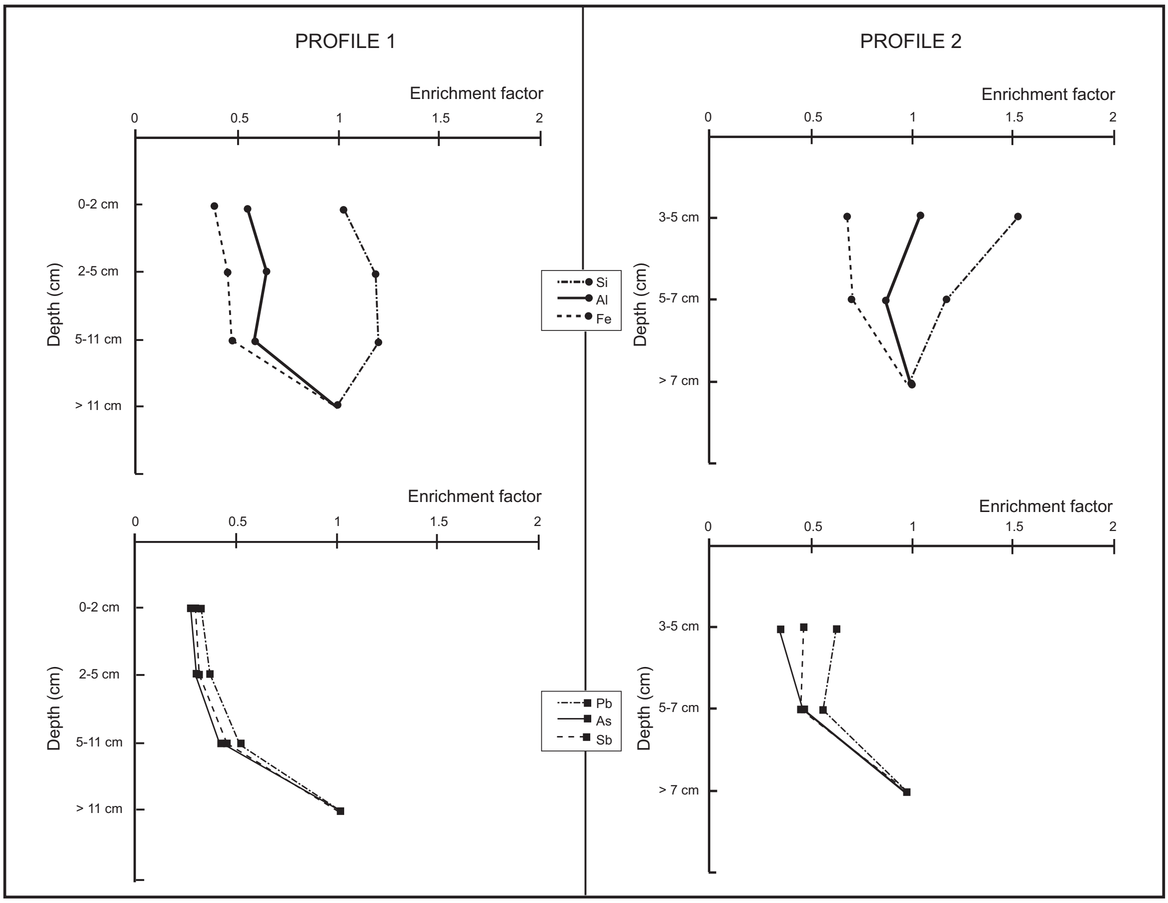

Figure III.2. Enrichment factors (EF) of major elements (Si, Al and Fe) and studied PTEs (As, Sb and Pb) in the two investigated soil profiles developed from mining wastes.

profile 1 whereas different proportions was clear in profile 2; (ii) Pb was less leached in profile 2 than in profile 1, and (iii) enrichment factors emphasized the following general sequence of mobility in profile 2: Pb< Sb <As. Furthermore, the EF(Fe) seemed to be similar to the EF(As, Sb or Pb) reflecting the close relation between these elements, probably toward the mineralogical phases (see after).

Whatever the zones, the EF of investigated profiles reflected a “natural” variability with EF values ranging from 0.5 to 1.5 (Hernandez *et al.*, 2003). Therefore, in this highly contaminated soils context and given EFs values, no additional contamination was underlined, PTEs being inherited from parental material. The geochemical composition of the various horizons was to a great extent similar between them but quite different to that of parental material. It indicated a low evolution degree of these young Anthroposols, as already shown in young volcanic soils (*e.g*. andosols; Martinez Cortizas *et al.*, 2003).

III.3 Metal and metalloids partitioning: relationship between mobility and mineralogy

The metal and metalloids speciation in soil samples horizons was assessed by the BCR (Bureau Community of Reference) sequential extraction procedure, which is suitable and usually perform in contaminated contexts (Figure III.3). The F1 fraction, namely the exchangeable fraction, was always lower than 1% for all horizons for Pb and 3% for As. Even though these percents were small, absolute values in concentrations were not negligible inducing a large potential amount of bioavailable As and Pb. Arsenic held by reducible phases (F2) followed the same evolution (from 1.98 to 0.31% with depth, profile 2) while Pb was rather constant in the profile 2. The respective proportions of As and Pb (Fe also) of oxidable fractions (F3) decreased with depth. Briefly, the proportion of Pb in F3 fraction was higher than As, and decreased with depth (for example from 9.75% in OH horizon to 1.11% in horizon Z_{tc} for As in profile 1). Since (i) As and Pb affinity for OM is well known (Lee *et al.*, 1998; Bauer and Blodau, 2009) and (ii) organic matter level was very high in these horizons, this latter might play a very important role in PTEs behavior.

In both profiles, studied elements were predominantly concentrated in the residual fractions (F4), representing 95% in the profile 1. This fraction corresponded to a stable fraction with very low risks of contaminants release in the environment. These results were in accordance with the works of Chopin *et al.* (2007) from soils in the vicinity of mine and smelters in

Spain. In term of elements mobility, the first three fractions of BCR extraction procedures corresponded to the potentially mobile fractions, while the immobile was represented by F4 fraction to short time as well as under natural conditions (Ure *et al.,* 1995). In this study, the major contaminants As, Sb et Pb were concentrated in the residual fractions (Figure III.4). In order to understand their potential mobility over the time, the describtion of the mineralogical composition of this residual fraction was necessary to complete the understanding of this phenomenon.

Powder XRD patterns of bulk (< 2mm) and fine fractions (< 2µm), SEM investigations and EMPA analysis lead to characterize soils mineralogy. Major results are reported in the Figures III.4 and III.5. Concisely, XRD of soils samples evidenced the presence of silicate species such as quartz, muscovite, clinoclore, feldspars and/or plagioclases in all samples. Even though quartz seemed to be relatively stable along soil profiles, the other minerals decreased in proportion from depth to surface. As expected from total chemical analyses, large amounts of As- and Pb-bearing phases were characterized. Thus, beudantite and scorodite ($(PbFe_3(AsO_4)SO_4(OH)_6$ and $FeAsO_4$ $2H_2O$ respectively), were evidenced in all profiles and their respective horizons. However, differences were clear toward XRD data in term of (i) fractions sizes, (ii) phases concentrations and, (iii) presence of amorphous components. Indeed, fine fraction was richer in clay minerals and As- and Pb-bearing phases compared to bulk fractions. Chemical analyses were in accordance with this fact since concentration in As were twice enriched than bulk composition (data not shown), and EDS analyses indicated that Sb appeared to be born by beudantite (Figure III.5).

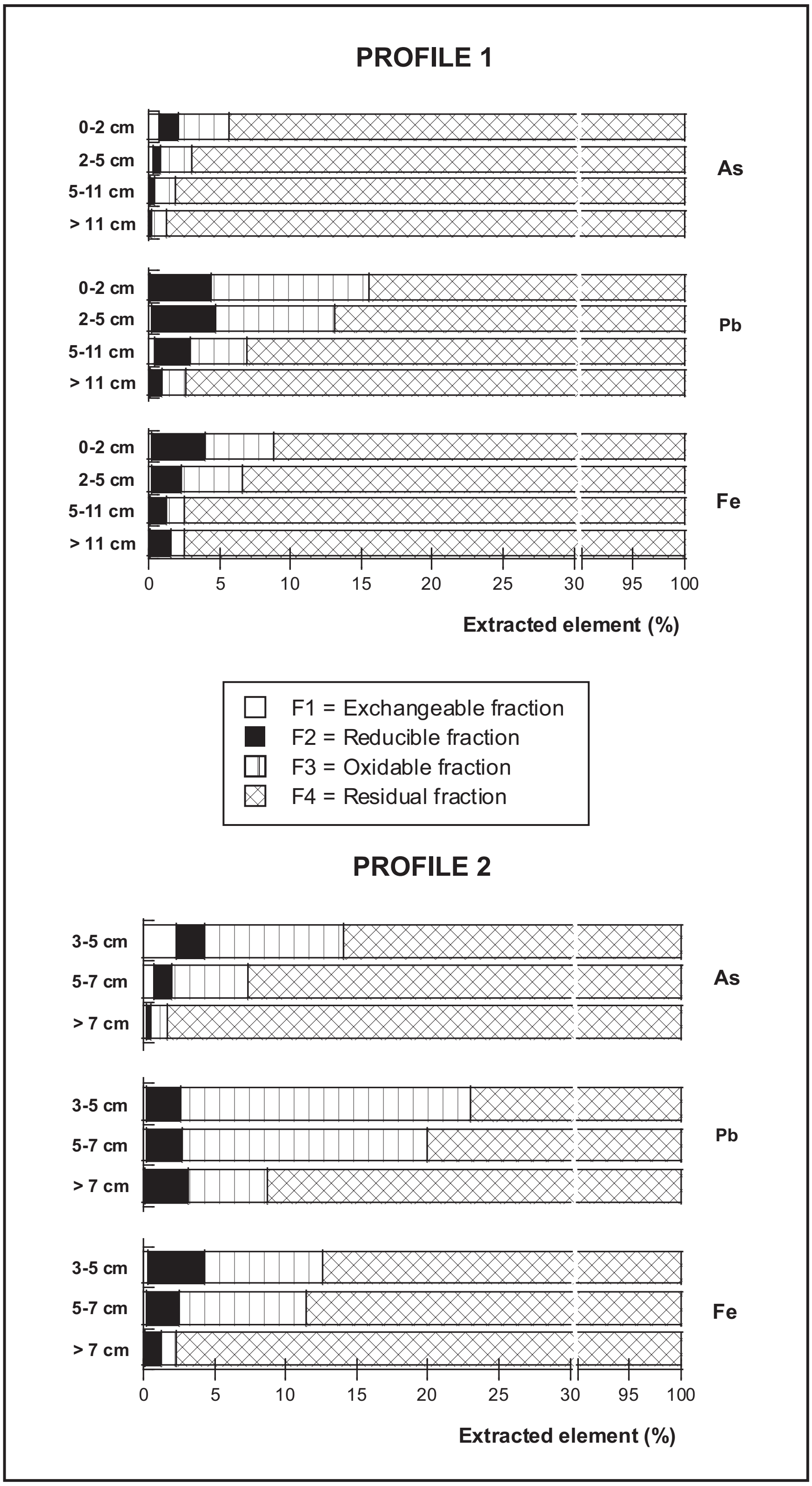

Figure III.3. BCR extraction procedure of < 2mm soil samples from each horizons of the studied anthroposols.

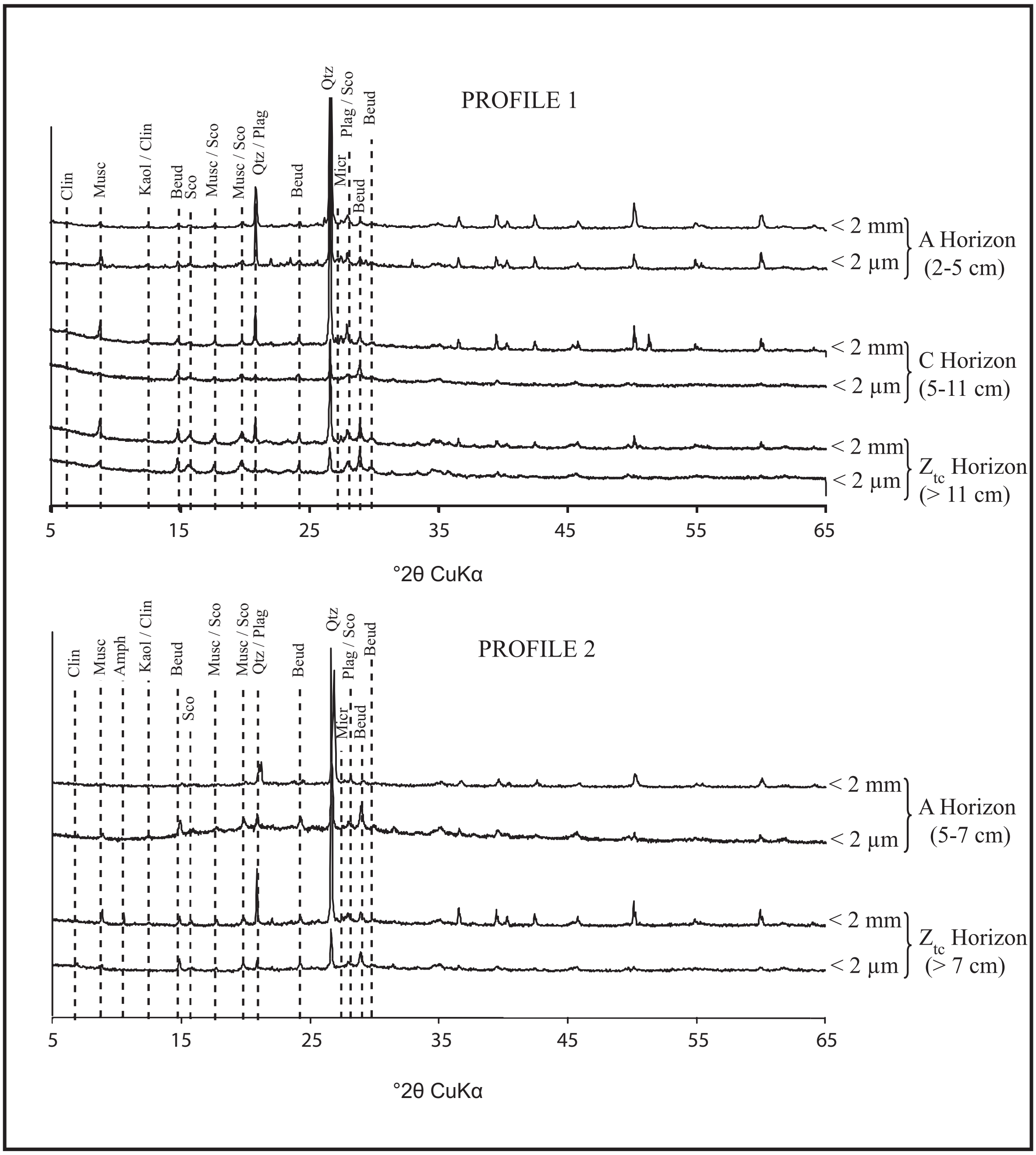

Figure III.4. XRD patterns of < 2mm and < 2µm fractions from the studied Technosols / Anthroposols; clin, musc, kaol, beud, sco, qtz, plag, micr refer respectively to cilnochlore, muscovite, kaolinite, beudantite, scorodite, quartz, plagioclase, microcline.

Moreover, XRD patterns of fine fractions were characterized by a dome between 20 and 30°2theta. This bump, which was more pronounced in the topsoil compared to parental material, was due to the presence of amorphous components such as As-rich iron oxy-hydroxydes or Fe-rich arseniate (such as symplesite; Roussel *et al.*, 1999) associate to organic matter effect. The Fe speciation obtained from DCB and acid oxalate extractions (Table III.1) clearly showed the same behaviour in both zones with a Feo/Fed > 0.6 (Table III.1) which is quite common in natural soils that have been temporally water-saturated, and with a high degree of Fe oxide activity (Schwertmann, 1985). The decrease of this ratio was probably due to a reorganisation of Fe status.

As shown before, the role of F4 fraction as a PTEs immobilisation was effective. Figure III.6 presents an example of XRD patterns performed on extractions of samples residues from profile 1 (Z_{tc} horizon) after the first three steps of BCR or after DCB extraction. Thus, scorodite and beudantite as well as micas, clinochlore and kaolinite have been affected by the first three steps of BCR being, nevertheless, still detectable. They have not been dissolved by reagents but a probable alteration might begin explaining the very weak releasing of As and Pb. Besides, Frentiu *et al.* (2009) have already shown that beudantite is not sensitive to BCR extractions.

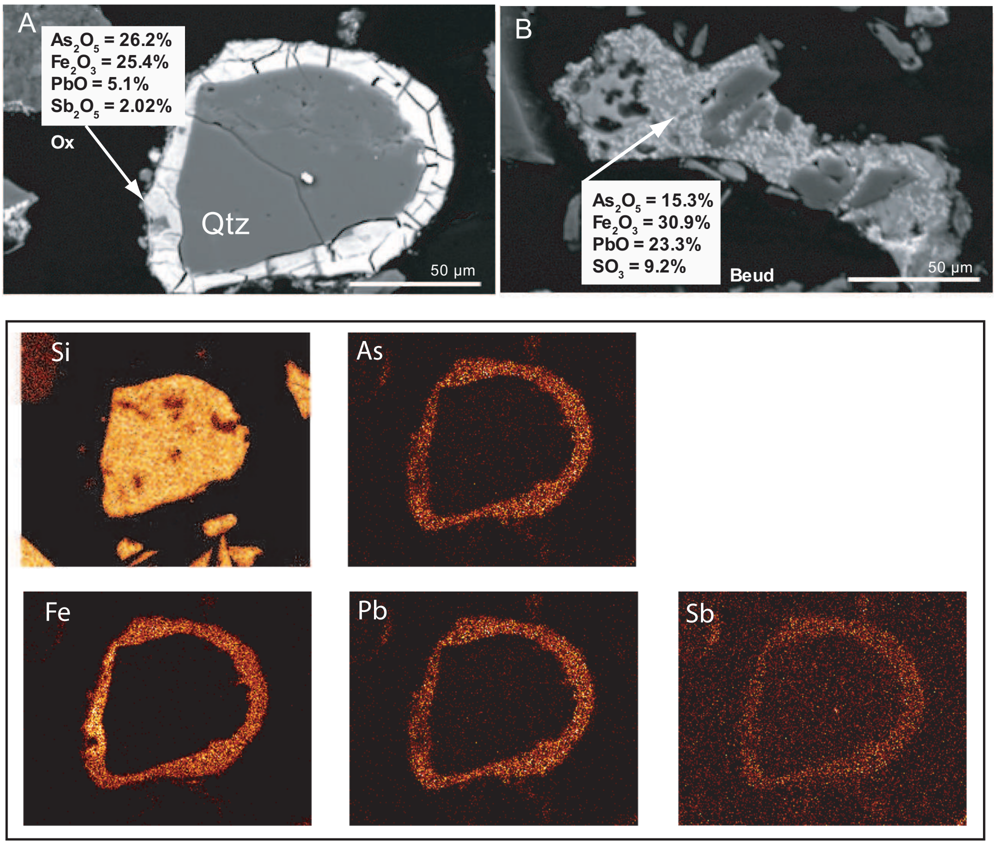

Figure III.5. SEM-EDX images in BSE mode of A horizon (A) from profile 1 and from profile 2 (B), with chemical map of (A) samples.

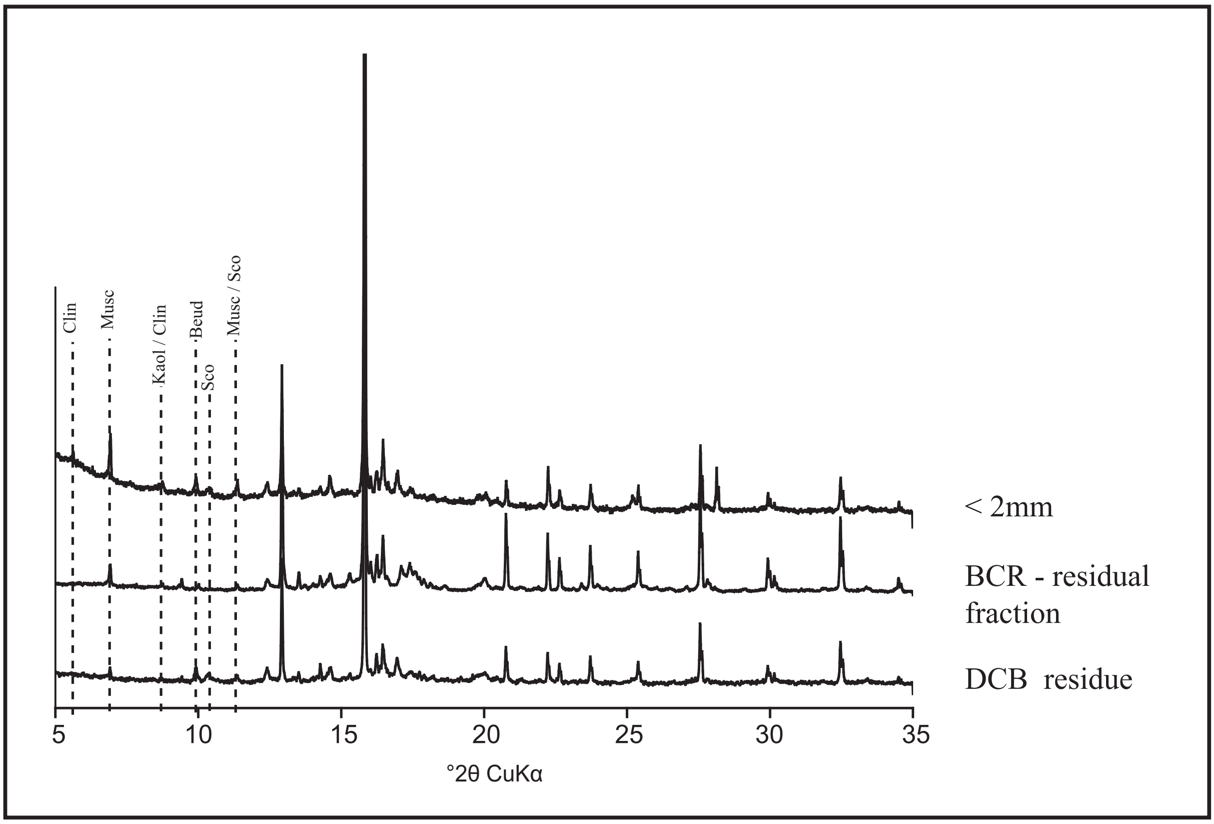

Figure III.6. Example of XRD patterns performed on residual fractions after BCR treatment or after DCB extraction (<2mm fractions from Z_{tc} horizon, profile 1); clin, musc, kaol, beud, sco, qtz refer respectively to clinochlore, muscovite, kaolinite, beudantite, scorodite, and quartz.

IV. Conclusion

The studied Technosols / Anthroposols have developed directly from highly contaminated waste materials (i) in a short time, and (ii) with development of vegetation cover, currently rather dense. Contrary to the large number of studies, there were no surface contaminants inputs. Furthermore, this study gave evidence for the influence of inheritance on the origin and distribution of metals and metalloids in soils. A depletion at the profile scale of As, Sb and Pb was observed in both profiles during the short pedogenesis rate. BCR extraction procedure indicated that As and Pb were trapped in the solid phases (among beudantite and scorodite) which were low weathered phases. During weathering and pedogenesis, As, Sb and Pb have been remobilized and (i) leached toward soil profile and/or then (ii) associated to other soil components like organic matter. However, even if the amount of mobile fraction (*i.e.* F1, exchangeable fractions) was little in term of extracted elements percent (up to 3%), the element content in concentration was very high (several hundreds of $mg.kg^{-1}$) and might induce a potential risk of ground water and food chain contamination. A further study would be interesting in the way to understand the mobility and bioavailability of contaminants on the studied site toward native plants.

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CE QU’IL FAUT RETENIR…

… Dans ce chapitre, une caractérisation détaillée des sols a été réalisée afin de bien comprendre le comportement géochimique et la spéciation chimique des Eléments Potentiellement Toxiques (EPTs) As, Sb et Pb.

Nous avons étudié deux profils de sols situés dans deux différentes zones du site d’étude. Les principales différences entre ces deux profils sont leur teneur en EPTs ainsi que leurs caractéristiques pédologiques (CEC, teneur en carbone organique). Ainsi, le profile 1 possède une CEC et une teneur en carbone organique inférieures à celui de la zone 2 mais présente de plus fortes concentrations en As, Pb et Sb même si les concentrations en EPTs du profile 2 restent très fortes.

Les EPTs, apparaissent comme étant remobilisés. On peut supposer, premièrement, que les éléments peuvent être immobilisés par exemple par la formation de minéraux secondaires ou par des phénomènes d’adsorption aux différents constituants du sol. Deuxièmement, les éléments peuvent aussi être exportés hors du profil de sol (phénomènes de lixiviation ou lessivage). Par conséquent, la spéciation chimique détermine fortement la mobilité des EPTs dans les sols de ce site d’étude.

Ces sols se sont donc formés en l’espace d’une quarantaine d’années ce qui est un temps court et malgré un taux de contamination élevé, la végétation a pu se développer progressivement. La contamination est susceptible d’être à l’origine de nombreuses perturbations au sein de l’écosystème et notamment au niveau de l’activité microbienne. De ce fait, notre étude a également portée sur la caractérisation des populations bactériennes et archéennes impliquées dans le cycle de l’azote et en particulier dans la nitrification, formant les nitrates, éléments essentiels à la croissance des plantes.

CHAPITRE IV

Evaluation de la qualité écologique des sols contaminés
Exemple de la nitrification autotrophique

Le cycle de l’azote dans les sols est d’une importance considérable dans la mesure où il est par exemple à l’origine de la formation des nitrates NO_3, l’un des nutriments majeurs des végétaux et des organismes du sol (Paul and Clark, 1989). Les nitrates peuvent être issus de l’oxydation de l’ammonium se réalisant par voie microbienne conduisant à la formation de nitrites (NO_2^-) puis de nitrates (NO_3^-). Cependant, les produits formés au cours du cycle de l’azote peuvent être à l’origine de problèmes environnementaux tels que la pollution des eaux souterraines par les nitrates ou la production du gaz à effet de serre N_2O (protoxyde d’azote) émis par les réactions de dénitrification. En effet, ce dernier a un potentiel d’action pour le réchauffement climatique 310 fois supérieur à celui du CO_2 (IPCC, 1995). De même, dans un contexte de changement climatique clairement identifié par la communauté scientifique, il est fondamental de bien comprendre les communautés microbiennes afin de prévenir les incidences de ces changements sur leur structure et les processus dans lesquels elles sont impliquées.

L’azote est un constituant majeur du vivant, en particulier de par sa prévalence dans les protéines ou dans la molécule d’ADN. Ainsi, y compris dans les sols, le statut de l’azote organique est majoritairement sous forme protéique, généralement selon une répartition hétérogène. A l’échelle d’une parcelle, on distingue des microsites riches et d’autres pauvres en azote. Cette répartition affecte les interactions entre plantes et micro-organismes. Depuis environ 70 ans, les recherches ont été particulièrement orientées vers la prédiction de la minéralisation de l’azote, de sa biodisponibilité et des besoins fertilisant sans que les résultats soient, pour autant, entièrement pertinents et permettent des apports d’intrants bien adaptés aux types de cultures, à la nature des parcelles et de leur topographie, aux types de climats etc (Nannipieri et Eldor, 2009).

Toute culture de plantes requiert des éléments minéraux essentiels à son développement, sa croissance, sa santé et sa production. L’azote, sous forme de nitrates NO_3^-, assure aux plantes la principale ressource en N nécessaire à leur métabolisme azoté. Ces besoins en N sont régulés par des facteurs externes tels que le pH du milieu, l'éclairement, la température, la disponibilité en éléments nutritifs… et par des facteurs internes comme le pH cellulaire, la teneur en glucides, l'état de croissance et de développement de la plante… (Morot-Gaudry, 1997).

La nutrition minérale, sous forme d'apports en ammonium et nitrate, s'accompagne de transformations microbiennes. Il y a encore peu de temps, on pensait que l'oxydation de l'ammonium était entièrement réalisée par des bactéries oxydantes de l'ammonium appartenant à des groupes spécifiques des β- et γ-proteobacteries. Ces bactéries possèdent le gène *amoA* codant pour la sous unité α de l'enzyme clef ammonium mono-oxygénase ; *amoB* et *amoC* codant pour les deux autres sous unités (Nicol et Schleper, 2006). Mais récemment, Treusch *et al.*, (2005) et Leininger *et al.*, (2006) ont suggéré l'implication des archées dans le processus global de nitrification dont l'efficacité pourrait même être, dans certains contextes, supérieure à celle des bactéries (Adair et Schwartz, 2008 ; Hermann *et al.*, 2008). Cependant, dans une situation de contamination des sols comme ceux de ce travail, le développement de ces micro-organismes est susceptible d'être affecté, en particulier l'abondance relative des bactéries par rapport aux archées, procaryotes, communautés toutes deux actives dans la nitrification. Leur comportement dans le sol et au niveau rhizosphérique est susceptible de changer aussi en fonction des conditions physico-chimiques du milieu, des espèces végétales et du climat.

La rhizosphère est définie comme le sol sous influence directe de l'exsudation racinaire. De façon pratique, il s'agit de la fine pellicule de sol restant attachée aux racines après avoir arraché une plante. Cette zone, de par les substances organiques libérées par les racines, est le siège d'une activité microbienne intense et particulièrement active d'un point de vue biochimique, chimique et physique (Hinsinger *et al.,* 2005).

L'un des objectifs de cette thèse est d'évaluer la capacité potentielle de la plante *Miscanthus* x *giganteus* à revaloriser les terrains miniers de La Petite Faye. Dans la mesure où cette plante a été choisie pour sa forte production de biomasse, il était primordial de connaitre la capacité des sols contaminés de ce site à transformer l'azote de façon à ce qu'il soit assimilable par la plante. Ceci a été évalué par l'étude du rôle des bactéries et archées impliquées dans l'oxydation de l'ammonium. L'outil utilisé pour cette caractérisation du gène *amoA* présent chez ces deux communautés est la PCR quantitative (qPCR).

Ainsi, les principaux objectifs de ce chapitre sont :

(i) de déterminer l'abondance du gène *amoA* des bactéries oxydantes de l'ammonium (AOB) et archées oxydantes de l'ammonium (AOA) dans trois sols composites, aux taux de contamination différents, prélevés à La Petite Faye.

(ii) d'évaluer l'évolution de ces communautés dans le complexe racines – rhizosphère de *Miscanthus* x *giganteus* cultivé en conditions contrôlées pendant 3 mois.

L'article présenté dans ce chapitre, intitulé « Abundance and diversity of ammonia oxidizing prokaryotes in the root rhizosphere complex of *Miscanthus* x *giganteus* grown in differently metal and metalloid contaminated soils » présente les premiers résultats de cette étude menée en collaboration directe avec Julien OLLIVIER, doctorant de l'équipe TEG (Terrestrial Ecogenetics) de l'institut IBOE (Institute for Soil Ecology, HelmholtzZentrum, Munich) et certains résultats (tRFLP) sont encore en cours d'acquisition. L'article sera soumis au journal Applied Soil Ecology.

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Abundance and diversity of ammonia oxidizing prokaryotes in the root rhizosphere complex of *Miscanthus* x *giganteus* grown in differently metal and metalloid contaminated soils.

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Running Title: Ammonia oxidizing microbes in the rhizosphere of *Miscanthus*

Keywords: Ammonia oxidizing bacteria; ammonia oxidizing archaea; Miscanthus; metal contaminated soils

I. Introduction

The mining industry produces large amounts of wastes contaminated by metals and metalloids (Alloway, 1990) leading to the accumulation of toxic elements in the environment. While classical decontamination techniques are costly and not aesthetic (*e.g.* excavation of contaminated material and physical-chemical treatments), the use of plant to restore polluted sites, known as phytoremediation, appears to a better option (Vangronsveld *et al.*, 2009). In addition to decreasing or stabilizing contamination, this technique allows commercial exploitation of soils unsuitable for food production in growing energy crops.

However, macronutrients deficiency is one of the growth-limiting factors of plants culture on contaminated soils (Tordoff *et al.*, 2000). It is therefore of key importance to investigate the response of the functional microbial biomass involved in nutrient turnover to the contamination. In this respect, nitrogen cycling is of major concern as contaminated sites are usually not fertilized and plant available nitrogen is closely linked to mineralization of nitrogen from dead biomass and subsequent transformation.

Nitrification has been considered as the pinhole of the whole nitrogen cycle (Leininger *et al.*, 2006). It is a two-step process, consisting of: (i) the oxidation of NH_4^+ to NO_2^- by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Kowalchuk and Stephen, 2001; Leininger *et al.*, 2006), and (ii) the oxidation of NO_2^- to NO_3^- by nitrite-oxidizing bacteria (NOB), the first step being assumed as rate-limiting (Prosser, 1989). While metallic contamination can affect AOB community structure (Stephen *et al.*, 1999; Gremion *et al.*, 2004; Mertens *et al.*, 2006), little is known about how AOA diversity is affected by contamination. Although AOA are thought to be more tolerant to chronic stress conditions than bacteria (Schleper *et al.*, 2005; Valentine, 2007), mainly the influence of metals on AOA is discussed controversy in literature. Mertens *et al.* (2009) could demonstrate that AOA were more sensitive to Zn contamination than AOB in a bulk soil. In contrast, Xia *et al.*, 2007 postulated that AOA are more tolerant than AOB to Cu contamination.

Therefore the aim of this study was to investigate the abundance, diversity and performance of the ammonia oxidizing microbes in soils with low pH, contaminated to different degrees with arsenic (As) and lead (Pb) from the mining area of La Petite Faye (Limousin, France) in response to the planting of the energy crop *Miscanthus* x *giganteus* in a greenhouse experiment.

II. Material and Methods

II.1 Experimental design

Soil samples were taken from the former gold mining area at La Petite Faye (Limousin, France). This site, which was abandoned since 1964, presented zones with different As and Pb concentrations and has been colonized by the local vegetation. Three sites with different As and Pb concentrations have been selected according to the next designation: S, Severe contamination, M, Medium contamination and L, Low contamination. At the site with severe contamination, gramineae, ferns (*Pteridium aquilinum*), birches (*Betula pendula*), jennets (*Cytisus scoparius*), brambles (*Rubus fructicosus*) were mostly found. In zone with medium contamination, soils were covered by gramineae and jennets, as well as trees such as alders (*Alnus serrulata*), oaks (*Quercus robur*) and brambles. Soils with low contamination were mainly characterized by giant horsetails (*Equisetum telmateia*). These soils, recently developed, have been classified as Anthroposol Artificiel according to the French classification (Référentiel, 2008) or Technosol (WRB nomenclature, FAO 2006). Major physical and chemical parameters of the soils, measured according to ISO standard normative protocols (pH, CEC, available P), are given in table IV.1. Briefly, pH values ($CaCl_2$) of the soil samples ranged from 3.4 (S) to 5.3 (L) and low CEC as well as reduced available P were measured in the three soil samples.

Soils were sampled in January 2010 up to 20 cm soil depth after removing the organic horizon. 4L of sieved mixed soil samples (2mm) (corresponding to soil composites) were transferred into plastic bags and put into pots sized of 21x21x21 cm. Bulk density (determined by the core cylinder method) was respectively of 0.9, 1.1 and 1.0 for S, M and L soil (Table IV.1). After an equilibration phase of 7 days at 50 to 60% of field water capacity at 20 ^{o}C, 1 pre-grown *Miscanthus* x *giganteus* rhizome was introduced per pot. Rhizomes were provided by the company Novabiom (Champhol, France). *Miscanthus* x *giganteus* were grown in a phytotron for 3 months, without any fertilization. Plants were subjected to a photoperiod of 16 h light at 350 µmol.m^{-2}.s^{-1} and 8 h of darkness, at temperatures of 23°C and 18°C, respectively, under constant soil water content (field capacity). Soil and rhizosphere samplings were performed before planting the rhizome (t_0) and after 6 (t_1) and 12 (t_2) weeks. The experiment was conducted in a randomized block design with four independent replicates per soil and per sampling time point.

II.2 Soil and plant analysis

A composite sample of the roots with the attached soil was taken from each pot. After shaking the roots vigorously, the roots and attached soil were treated as one compartment called the root-rhizosphere complex (RRC). One part of the RRC was immediately shock-frozen in liquid nitrogen and stored at -80°C for nucleic acid extraction; the other part was directly extracted with 0.01 *M* $CaCl_2$ for the determination of pH, $CaCl_2$-extractable As, Pb (according to a soil:liquid ratio of 1:10) and water extractable organic carbon (WEOC), ammonium (NH_4^+-N), nitrate (NO_3^--N) (with 1:4 as the soil:solution ratio). Bulk soil samples at t_0 were additionally taken and treated the same way like described above.

Pb and As were measured by graphite furnace atomic absorption spectrometry (SpectrAA 880 Z, Varian, California, US) equipped with a Zeeman background correction. Ammonium-N and nitrate-N were measured using a scalar analyzer (SA 20/40, Skalar Analytical, The Netherlands). WEOC was determined using a TOC analyser (TOC-5050A, Shimadzu Corporation, Japan).

In addition, their total As and Pb contents have been measured by ICP-MS after a lithium metaborate / tetraborate fusion and dilute nitric acid digestion by ACME Analytical Laboratories ltd (Vancouver, Canada).

The dry weight of roots, rhizome, stem and leaves of Miscanthus plants was measured after 6 (t_1) and 12 (t_2) weeks Measurements of As and Pb accumulation in each organ were performed by Acme Lab (Vancouver, Canada) after a digestion first into HNO_3 and then by *aqua regia*. Elements concentration was measured by ICP-MS.

II.3 DNA extraction and molecular analysis of ammonia oxidizing microbes

DNA was extracted from 0.5 g of bulk soil and RRC, respectively, after a bead beater lysis step (Bertin Technologie, France), using the FastDNA SPIN kit for soil (MP biomedicals, Germany). DNA yield was measured by using a microvolume fluorospectrometer (NanoDrop Technologies, Delaware).

Quantitative PCR (qPCR) was used to determine the abundance of functional communities involved in ammonium-oxidation by targeting *amoA* genes (encoding the ammonia monooxygenase) in both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). Absolute quantification of all investigated target genes was carried out in 25 µL samples in triplicate on the ABI Prism 7300 Cycler (Applied Biosystems, Germany). PCR conditions are given in table IV.2. The specificity of the amplification products was confirmed by melting-curve analysis. No template controls served as null value. Samples were diluted 10 fold as no inhibitory effects of environmental DNA extracts on the PCR amplification were detected when known amounts of standard (AOB and AOA) were spiked with 10 fold diluted environmental DNA samples (data not shown). Dilution series of plasmid DNA with cloned *amoA* for both AOB and AOA gene fragments were used to generate standard curves ranging from 10^1 to 10^6 gene copies.μL^{-1} with efficiencies ranging from 90 to 95%.

II.4 Statistic analysis

Prior to analysis, gene abundance data were ln-transformed to achieve normal distribution. Data were analysed by one-way ANOVA with soil (L, M, S) as factor at a given time-point. Homogeneity of the variances was checked by the Leven test. For pairwise comparison of means, the Tukey HSD test was applied, and if homogeneity of variances was not given, the Dunnett-T3 test was used. Significance level was set to $\alpha = 0.05$ for all tests. Statistical tests were calculated in SPSS 11.5 (SPSS, Inc., Illinois, USA).

Table IV.1. Physio-chemical properties of the soils

	pH		**Bulk Density**	**CEC**	**Available P**	**$[As]_{total}$**	**$[Pb]_{total}$**	**Available As**			**Available Pb**		
	H_20	**$CaCl_2$**											
				cmol(+).kg^{-1}	**g.kg^{-1}**	**mg/kg**							
								t_0 (bulk soil)	**t_1 (RRC)**	**t_2 (RRC)**	**t_0 (bulk soil)**	**t_1 (RRC)**	**t_2 (RRC)**
S	3.5	3.4	0.9	1.95	0.582	83 000	15 200	2.1 ± 0.9	1.8 ± 0.2	1.7 ± 0.3	134.2 ± 4.6	62.1 ± 6.6	63.9 ± 31.3
M	4.0	3.6	1.1	nd	nd	9 308	178	7.7 ± 0.7	7.0 ± 0.2	5.8 ± 0.2	1.6 ± 0.1	1.5 ± 1	1.6 ± 0.3
L	6.1	5.3	1.0	8.9	0.075	1 727	325	10.4 ± 0.6	11.3 ± 0.9	9.9 ± 0.3	18.3 ± 3.4	0.0 ± 0.0	0.0 ± 0.0

S = severe contamination, M= medium contamination, L = Low contamination
t_1 = 6 weeks, t_2 = 12 weeks. RRC = Roots Rhizosphere Complex

Table IV.2. Parameters of analysis of target gene involved in ammonia oxidation

Amount (µL) of assay component									
	Target gene	2x Power SYBR green	3% BSA	Primer (10pmol/µL) Forward	Primer (10pmol/µL) Reverse	DNA	PCR water	Thermal cycling profile	Nb of cycles
AOB	*amoA*	12.5	0.5	0.75	0.75	2	8.5	94°C/60s, 58°C/60s, 72°C/60s	39
AOA	*amoA*	12.5	0.5	0.50	0.50	2	9	94°C/45s, 55°C/45s, 72°C/45s	40

III. Results

III.1 Metal and metalloid concentrations in the bulk soil, RRC and plant samples

As expected the severely impacted soil S showed the highest total and $CaCl_2$ extractable concentrations for Pb (Table IV.1). Although for total As, the highest concentrations were also found in this soil, the bioavailable fraction of As was surprisingly low. In the medium and low contaminated soils (M and L) total concentrations of As and Pb followed the expected trend. The same was true for $CaCl_2$ extractable Pb. However, both soils had higher available As contents compared to soil S.

Whereas available As concentrations did not change over time in all three soils, the planting of *Miscanthus* x *giganteus* resulted in a clearly reduced amount of available Pb already at t_1 in soil S and L. Surprisingly in the soil with the intermediate contamination type (soil M) no decrease of available Pb could be found (Table IV.1). These results clearly showed that even if the soils were highly contaminated, only a little amount of As and Pb was available given the stability of As- and Pb-bearing phases in supergene context.

After three months cultivation, the total *M.* x *giganteus* biomass was in the range of 2 g dry weight (dw) per plant independent from the soil used (data not shown). As expected, metals and metalloids were mainly concentrated in the roots system while rhizome concentrations were markedly lower than in roots (Table IV.3). In stems, concentrations were again lower compared to roots and rhizomes. For both elements, a clear correlation between the degree of contamination and the amount of contaminants in the plant parts could be found, with the only exception of *M.* X *giganteus* plants grown in soil with the intermediate contamination (soil M): due to the small amount of roots, it was impossible to measure their As and Pb concentrations, but we assume that roots mainly concentrated contaminants as for S and L soils even though the highest As accumulation rates in the plants from soils M was found in all plant tissues (Table IV.3).

Table IV.3. As and Pb accumulation in the different parts of *M.* x *giganteus* (dry weight) growing in a phytotron on 3 contaminated composite soils for 3 months.

		As	Pb
		mg.kg^{-1}	
Soil S	Roots	1 284.5	326.7
	Rhizome	49.5	30.6
	Stem	5.4	29.5
	Leaves	17.1	43.1
Soil M	Roots	nd	nd
	Rhizome	195.7	70.0
	Stem	66.9	28.6
	Leaves	20.1	12.9
Soil L	Roots	602.4	38.2
	Rhizome	48.3	3.8
	Stem	3.6	0.6
	Leaves	4.3	1.1

nd = not determined because too less samples

III.2 Nitrate, ammonium and water extractable carbon concentrations

Nitrate and ammonium concentrations decreased in all soils over the culture but their initial rates were independent from their degree of metal and metalloid contamination. Ammonium concentrations were the highest in the severely contaminated bulk soil reaching 119 mg.kg^{-1}. In contrast, nitrate concentrations were highest in the bulk soil with the lowest heavy metal concentrations (6.1 mg.kg^{-1}), indicating a reduced rate of *in situ* nitrification in soil S.

Overall WEOC concentrations doubled over time with the culture in each soil, *i.e.* from 16.8 to 30.1 mg.kg^{-1} (soil M), as an effect of plant growth and exudation in all soils (Table IV.4).

Table IV.4. Nitrate, amonium and water extractable organic carbon in the three studied soils (t_0: bulk soil; t_1 and t_2: RRC) at the three time sampling points

	NO_3^--N (mg.kg^{-1})			NH_4^+-N (mg.kg^{-1})			WEOC (mg.kg^{-1})		
	t_0 (bulk soil)	t_1 (RRC)	t_2 (RRC)	t_0 (bulk soil)	t_1 (RRC)	t_2 (RRC)	t_0 (bulk soil)	t_1 (RRC)	t_2 (RRC)
Soil S	3.9 ± 2.6^a	0.6 ± 0.2^a	0.5 ± 0.4^a	119.7 ± 63.4^a	55.7 ± 6.7^a	47.1 ± 7.3^a	16.3 ± 5.3^a	29.3 ± 2.4^a	30.6 ± 2.8^a
Soil M	4.4 ± 1.4^a	1.4 ± 0.6^a	0.9 ± 0.2^a	82.6 ± 25.3^a	37.1 ± 11.1^b	33.3 ± 5.4^a	16.8 ± 4.5^a	25.0 ± 5.2^a	30.1 ± 4.5^a
Soil L	6.1 ± 1.4^a	4.72 ± 0.6^b	2.4 ± 1.9^a	85.9 ± 22.5^a	28.0 ± 9.4^b	33.1 ± 7.1^a	20.1 ± 3.1^a	33.4 ± 8.2^a	44.1 ± 16.1^a

Significant differences between treatment are indicated by different superscript letters (n=5)

III.3 amoA gene copy numbers

In the soil and RRC samples of the severely contaminated soil (soil S), at all sampling time points, the abundance of ammonia oxidizers (both for AOA and AOB) was clearly reduced (up to two orders of magnitude) compared to soil M and soil L (within 5.23×10^4 (t_0) and 4.38×10^3 (t2) *amoA* copies.g^{-1} of soil and RRC respectively) (Figure IV.1). Gene copy numbers for ammonium oxidizers (AOA and AOB) in soil M (medium contamination) and soil L (low contamination) were in a comparable range. However, whereas in soil M the abundance of AOA was significantly higher compared to AOB, in soil L the abundance of AOB outmatched the gene copy numbers of the archaeal counterpart at all sampling time points, reaching 4.81×10^5 copies.g^{-1} of soil at t_0. Although the cultivation of *Miscanthus* x *giganteus* on soil S caused a reduction of mainly archaeal ammonia oxidizers and a clear shift in the AOA:AOB ratio over time, in soil L both groups of ammonia oxidizers were negatively affected by plant cultivation. Only in soil M, the cultivation of *M.* x *giganteus* caused a slight increase of AOA and AOB over time, *i.e.* for AOA, from 2.88×10^5 to 5.63×10^5 *amoA* copies.g^{-1} of soil and RRC respectively between t_0 and t_2.

IV. Discussion

IV.1 Influence of soil properties on studied prokaryotes.

In this study soils particularly heavily contaminated with As and Pb have been investigated (Table IV.1). However the correlation between total content of Pb and As did not follow the same trend compared to the amounts of $CaCl_2$ extractable metal and metalloid, which might be related to (i) the different pH values of the different soils and (ii) the difference in their speciation related to mineralogy (*e.g.* respective amount of As- and Pb-bearing phases). This was also demonstrated in a study by (Cui *et al.*, 2010)), where the availability of As increased with increasing pH in soil whereas Pb availability was higher in soils with low soil pH. Even though overall As and Pb availability was relatively low according to total concentration, their potentially mobile fractions reached levels which could reduce microbial activity involved in key processes like nitrification.

Archaea have been recognized as separate domain of life from Eukarya and Bacteria and are now considered as the early evolution of cellular life forms. They previously have been characterised in marine plankton and as extremophiles organisms while they are now also recognized as mesophilic in marine plankton (clade of *Crenarchaeota* representing over 20% of the picoplankton – 0.2 -2 µM cell size, in the world ocean) (Karner *et al.*, 2001). Literature counts studies of archaea in many kinds of environments such as soils, estuaries and marine plankton as well as hot springs and hydrothermal vent systems, as reported by Spang *et al.* (2010). The phylogenetic placement of major lineages forming Archaea is still under debate. Thus, phylogenetic analyses of Archaea indicate three lineages namely Crenarchaeota, Euryarchaeota and Thaumarchaeota. AOA from terrestrial environments belong to the latter one and might also contain a specific membrane ascribing them a resistance to hostile environments (Valentine, 2007; Spang *et al.*, 2010).

IV.2 AOA and AOB amoA gene copy numbers in Root-Rhizosphere Complex (RRC)

Only few studies investigated the response of AOA and AOB to a heavy metal contamination (Xia *et al.*, 2007; Li *et al.*, 2009; Mertens *et al.*, 2009; Mertens *et al.*, 2010; Ruyters *et al.*, 2010). However, all studies, so far, have been performed using soils spiked with heavy metals and no plant effects have been included. Therefore, only little is known about the behaviour of ammonia-oxidizing microbes in contaminated soils during bioremediation.

In this study, soils were chosen with a different degree of contamination of As and Pb due to mining wastes deposit. As the contamination has been present since about 45 years, a specific microflora, which is able to tolerate metals stress might have developed. Haferburg and Kothe (2007) summarized the main microbes strategies of metals resistance. Thus, they explained metal resistance due to (1) intra- and extra-cellular mechanisms, (2) metal excretion via efflux transport systems, (3) binding and detoxifying metals by sequestering compounds of the cytosol, (4) bounding and fixation of metals by the release of chelators into the extracellular milieu and (5) sorption to the cell wall which is prone to bind large amount of metal, preventing the influx. However, up to now, no specific resistance mechanism against metals for AOB or AOA has been reported.

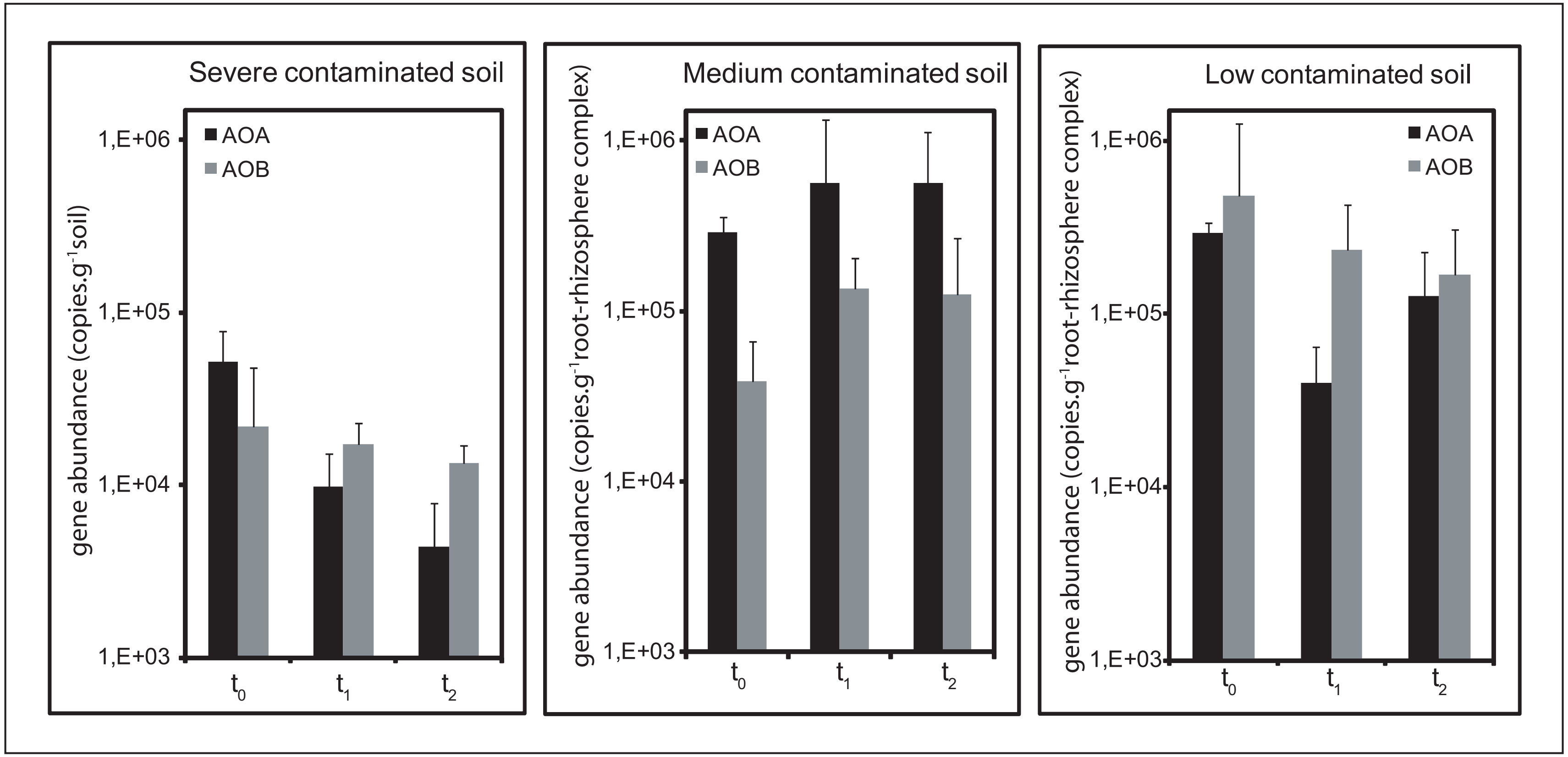

Figure IV.1. Evolution of *amoA* from AOB and AOA gene abundance in soil and in *M.* x *giganteus* grown on 3 contaminated RCC for 3 months in a phytotron.

Lower gene copy numbers (below 5.6×10^5 and 4.8×10^5 respectively for AOA and AOB in our study) were found in this study as compared to other studies (Xia *et al.,* 2007; Li *et al.,* 2009; Mertens *et al.,* 2009; Mertens *et al.,* 2010; Ruyters *et al.,* 2010). However, the investigated soil was not of agricultural provenance but of mine-wastes origin, presumably hostile for microbial populations and low in organic matter content.

In soil S (severely contaminated), gene copy numbers for both groups of ammonia oxidizers were clearly reduced indicating a negative impact of the high metal concentrations for AOA and AOB. Another reason for the low copy numbers of archaeal and bacterial *amoA* gene might be the low pH of the soil, which inhibit nitrification and the corresponding microbes (Sauve *et al.,* 1999). Overall, highest ammonium concentrations and low nitrate values were found in soil S at all sampling dates indicating low *in situ* turnover rates of ammonium in response to the high heavy metal contamination.

Conversely, in the soil with the intermediate concentration (soil M) AOA abundance was clearly increased, up to two orders of magnitude, compared to soil S, indicating that AOA are able to tolerate heavy metal contamination, at least to a certain extend. In contrast, at t_0 (bulk soil), no differences were found for AOB between soil S and soil M, indicating a high sensitivity of AOB towards heavy metals. With increasing abundance of AOA, the amount of ammonium in the soil samples was clearly reduced, indicating that the AOA present in soil M are also actively involved in ammonium oxidation.

In soil L (the soil with the lowest concentration of heavy metals) AOB outcompeted AOA mainly in the RRC. This confirms results from another study where AOB was more frequent in the rhizosphere, although abundance of AOA was higher in bulk soil (Ollivier *et al.,* 2010). Surprisingly ammonia concentrations did not change compared to soil M, which could be related to a faster mineralization of nitrogen in soil L (Fuka *et al.*, 2008) and therefore *per se* higher ammonium concentrations present compared to soil M, where mineralization might be influenced by the presence of the metals. It can be assumed that turnover rates in soil L of ammonium should be faster due to the dominance of AOB (Schauss *et al.*, 2009).

However, the presence of nitrification inhibitors produced by the plant may also impact the *in situ* turnover of ammonium. For example Subbarao *et al.* (2009) could prove the inhibition of nitrification in roots of *Brachiaria humidicola,* a pasture grass native to tropical Africa. They highlighted the capacity of this plant to produce brachialactone, a molecule exudated by roots and responsible for deletion of soil nitrifier populations. As ammonium concentrations were low in soil L and highest nitrate values have been observed, the role of nitrification inhibitors in our experiment can be neglected.

IV.3 Soil-Miscanthus-prokaryotes interactions

In the RRC of soils S and L, abundance of AOA and AOB were both significantly reduced. This might be a result of two different mechanisms. On one hand, in the rhizosphere of soil S, there might be a remobilization of metals taking place and resulting in higher bioavailable concentrations, which may negatively interfere with both groups of ammonia oxidizers: this is confirmed by the high concentrations of both elements mainly in the roots and rhizomes (Table IV.3).

In contrast, in soil L, a typical rhizosphere effect might be the reason for the reduced copy numbers of AOA and AOB. Better plant performance and improved plant – microbe interactions (*e.g.* with nitrogen fixing bacteria (Davis *et al.,* 2010)) might result in improved exudation rates. This hypothesis has been confirmed by the increased DOC values in the RRC of soil L (Table IV.4). The high nutrient contents in the rhizosphere might outcompete autotrophs like AOA and AOB.

Surprisingly in soil M, the rhizosphere effect was not as pronounced as in soil L. This might be related to overall plant performance in the soil which was still high in As and Pb and therefore reduced exudation rates.

V. Conclusion

This study, under controlled conditions, indicated that functional microbial biomass implied in nitrification as well as ammonium turnover were impacted by the very high level of contamination in As and Pb and low pH especially for AOB community. However, a lower contamination rate allowed AOB to outcompete AOA. The presence of *Miscanthus* x *giganteus* induced a modification of AOA community in its RRC by reducing the gene abundance particularly in severe and low contaminated soils. The plant induced either a potential contaminants remobilization or higher root exudation. However, the collected data were based on constant climatic conditions without taking into account other environmental factors like drought, freeze or farming practices. As a result, a field study could confirm these results or not.

Besides the response of functional nitrification gene copies numbers to the contamination and the culture of the bioenergetic plant *Miscanthus* x *giganteus*, it would be interesting to investigate the microbial diversity patterns in these soils and in the RRC of the plant. Indeed, hostile environments such as these heavily contaminated soils might reduce microbe adaptation. Moreover, denitrification should also be studied in order to assess N_2O (nitrous oxide, a major greenhouse gas) emissions risks especially in the case of *M.* x *giganteus* crop.

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Ce qu'il faut retenir…

… Dans ce chapitre, nous avons observé les effets de différentes teneurs en métaux et métalloïdes de sols présentant des propriétés similaires, sur les communautés des bactéries et des archées impliquées dans la nitrification au travers de la quantification du gène *amoA* présent chez ces deux communautés. L'influence de la culture de *Miscanthus* x *giganteus* sur celles-ci, pendant 3 mois et en conditions contrôlées a également été évaluée dans le complexe racines-rhizosphère de cette plante. En effet, cette plante représente un fort potentiel en termes de bioressource (cf chapitre VI), il était par conséquent important d'évaluer l'évolution de la réponse bactérienne et archéenne dans sa rhizosphère.

Bien que les milieux de culture possèdent un pH très acide (sol S) à légèrement acide (sol L), les concentrations en ammonium ne constituaient pas un facteur limitant pour que la nitrification par voie autotrophique soit effective.

Globalement, l'abondance de ce gène était plus faible que dans des sols agricoles, même contaminés artificiellement. La contamination en As et Pb ainsi que l'acidité des sols sont donc relativement hostiles à ces 2 communautés. L'abondance du gène *amoA* était la plus faible dans le sol S, le plus fortement contaminé, et diminuait même dans la rhizosphère de la plante à cause d'une possible remobilisation des éléments contaminants. Dans le sol M, moins contaminé que le sol S, les abondances de AOB étaient plus importantes. Ces abondances augmentaient dans la rhizosphère et au cours de la culture. Pour le sol le moins contaminé, le sol L, les abondances du gène *amoA* étaient les plus fortes mais l'abondance des AOB semblaient entrer en compétition avec les archées. L'abondance des archées était par conséquent plus faible que celle des bactéries.

Le complexe racines – rhizosphère est également l'interface d'échanges et donc d'absorption par la plante des éléments nutritionnels voire des contaminants. Ainsi, une étude du transfert des contaminants à la végétation colonisatrice du site permettra de comprendre quels sont les paramètres controllant leur accessibilité aux plantes. De ce fait, il sera possible ensuite d'estimer la faculté d'adaptation de *Miscanthus* x *giganteus* sur sites et sols pollués afin d'envisager son utilisation potentielle comme ressource bioénergétique.

CHAPITRE V

Transfert sol-plante d'élément métallique et métalloïdes des Antroposols à la végétation naturelle du site

La présence d'une végétation spécifique développée sur le site d'étude nous a conduit à étudier les mécanismes de transfert des métaux et métalloïdes vers cette végétation. En effet, si les espèces végétales sont parvenues à s'implanter est-ce (i) parce qu'elles ont la capacité à sélectionner les éléments qu'elles absorbent ou (ii) au contraire sont-elles métallo-tolérantes et donc capables de se développer tout en absorbant ces contaminants ?

La biodisponibilité est définie comme la fraction disponible d'un métal qui est prélevée par la plante au travers de processus physiologiques d'absorption. La biodisponiblité peut aussi être définie comme étant le flux de métal vers la surface du système racinaire d'une plante cible en tenant compte de la durée d'exposition (Thornton, 1999, ISO 2006). Généralement, la biodisponibilité est estimée de manière chimique, mais face à la diversité de ces tests chimiques, cette étude a également intégré l'évaluation du transfert potentiel des métaux et métalloïdes vers les différents organes des espèces végétales colonisatrices du site d'étude.

L'objectif de ce chapitre était donc d'étudier le transfert des métaux et métalloïdes du sol vers les 4 plantes majoritaires ayant colonisé le site d'étude. Pour cela, une approche intégrée pédologique, minéralogique et chimique (extractions sélectives partielles) a été réalisée. Les résultats des extractions sélectives ont ensuite été comparés à l'accumulation réelle dans les plantes représentatives du site d'étude.

L'ensemble de ces travaux ont fait l'objet d'un article soumis à Environmental Geochemistry and Health : "Metal and metalloids availability from mining anthropols : transfer to natural vegetation cover in temperate climate".

Metal and metalloids availability from mining anthroposols: transfer to natural vegetation cover in temperate climate

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Keywords: mining anthroposol, bioavailability, arsenic, antimony, lead.

Abstract

Pedological and physico-chemical properties of soils developed from old mine tailing (France) were investigated in relation to the natural vegetation cover, the aims being to evaluate the risk of metal and metalloids (Pb, As, Sb) mobility and their potential transfer to native plants. The soils were classified as Anthroposols/Technosols with high contamination levels in As, Sb and Pb. The single selective extractions tested ($CaCl_2$, acetic acid, A-Rhizo and DTPA) showed low labile fractions (< 5 % of bulk soil contents) but important in absolutes values, related to the highly contaminated levels of soils. Even over phytoxic levels, native plants were characterized by small amounts of As, Sb and Pb in their tissues given the low translocation factors. This works evidenced the important role of (i) mineralogical phases by controlling the solubility of metal and metalloids, and (ii) of the native plants cover in term of phytostabilization feasibility.

Capsule: mineralogy controls the solubility of metals and metalloids in highly contaminated mining anthroposols inducing their low transfer to native plant.

I. Introduction

After extraction of economic elements, abandoned mine sites contain large amounts of waste materials characterized by high concentrations of metals and metalloids such as As, Sb, Pb, Ni, Cr, Zn, etc, increasing their prevalence and occurrence at the Earth's surface (*e.g.* Chopin and Alloway, 2007; He *et al.,* 2005; Margui *et al.,* 2007; Rodriguez *et al.,* 2009; Wang *et al.,* 2008; Yang *et al.,* 2009; Yao *et al.,* 2009). The dispersion of these pollutants throughout wind or water erosion in the environment around mine tailing and smelter is an important anthropogenic way of contamination. Due to the potential hazards of water resource contamination, pollution control, remediation and revegetation of abandoned mine sites is of major concern (e.g. Archer and Caldwell, 2004; Ettler *et al.,* 2007; Freitas *et al.,* 2004; Lei and Duan, 2008; Maiz *et al.,* 2000; Remon *et al.,* 2005; Wang *et al.,* 2008; Wong, 2003; Zhang *et al.,* 2001).

A huge literature describes studies of metal and metalloids contaminated soils around mine sites (e.g. Boussen *et al.,* 2010; Dudka and Adriano, 1997; Dudka *et al.,* 1995, 1996; Moutsatsou *et al.,* 2006; Sterckeman *et al.,* 2000, Yang *et al.,* 2009) with the aim of assessing factors controlling their vertical distribution, their behaviour and their environmental impact throughout vegetation or human health. However, there are only few datas about "naturally" developed soils on mine tailings probably owing to the little time needed for soil development since the end of the industrial activity. Given the large amounts of contaminated areas which may extent over several hectares, economic interests appeared for these soils in the way to allow crop or forest land use. Studies are needed to understand the potential re-use of these soils and their biogeochemical cycles (Yao *et al.,* 2009).

Due to the special chemical and physical properties of contaminated soils (*e.g.* extreme pH (Conesa *et al.,* 2006); nutrients and biological deficiency (Chiu *et al.,* 2006; Vega *et al.,* 2006; Wang *et al.,* 2008)), adapted plants to specific environments evolving through natural selection can colonize such sites (Lei and Duan, 2008; Rao and Tarafdar, 1998). Indeed, remediation of sites by revegetation of appropriate plants offers the most effective method to achieve sustainable restoration and visual improvement (Remon *et al.,* 2005; Vangronsveld *et al.,* 1995; Wang *et al.,* 2008). Natural site vegetation can be considered as the first step since it represents appropriate plant species (Lei and Duan, 2008; Madejon *et al.,* 2003; Wang *et* *al.*, 2008). Indeed, natural vegetation plays a considerable role by tolerating inorganic contamination through many mechanisms to grow and reproduce on contaminated lands. Indeed, recent studies with commercial species clearly show failure in land reclamation (*e.g.* Andres *et al.*, 1996; Andres and Jorba, 2000; Martinez-Ruiz *et al.*, 2007) probably due to their lack of adaptation on contaminated soils (Wang *et al.*, 2008) and potentially to the prevailing climate (Singh *et al.*, 2002; Li, 2006).

This study was carried out on highly contaminated gold mine tailing (As, Sb, Pb, Zn…) in Limousin (France). This site is abandoned since 1964, inducing its colonisation by pioneer plants. The main objectives of this work were (i) to evaluate soil pedogenesis evolution since the end of mining activity concerning their physical chemical properties and metal concentrations, (ii) to assess bioavailability of main inorganic pollutants (As, Sb and Pb) through various single extraction procedures ($CaCl_2$, acetic acid, A-Rhizo and DTPA) compared to natural vegetation cover. Moreover, the influence of soil mineralogy as well as feasibility of site reclamation will be discussed.

II. Materiels and methods

II.1 Studied site

The site, La Petite Faye, is located at 60 km North East from Limoges (French Massif Central). The annual average rainfalls and temperature are about 1020 mm and 11.3°C respectively. The former mining activity led to an accumulation of about 35 000 t of contaminated wastes by metallic elements and metalloids. All wastes were stored in a settling basin (150 x 80 m), delimited by loamy dams on sides. Since mining activity stopped (45 years ago), vegetation began slowly to colonize the site and lead to the development of a soil from these wastes. Differences in parental material (pH, particles size distribution, metals contents and mineralogy, see previous study by Néel *et al.*, 2003) have been clearly identified inducing (previously) the delimitation of distinct zones (Figure V.1).

Type of vegetation was different in both zones (Figure V.1): the first zone had a vegetation cover very scarce with mainly grass (*Graminea*), some birch trees (*Betula pendula*), jennets (*Cytisus decumbens*), and ferns (*Pteridium aquilinum*). On the contrary, the vegetation was very dense and vigorous in the second zone but with less diversity: giant horsetails

(*Equisetum telmateia*) and also birch trees (*Betula pendula*), brambles (*Rubus fructicosus*), nettles (*Urticae*).

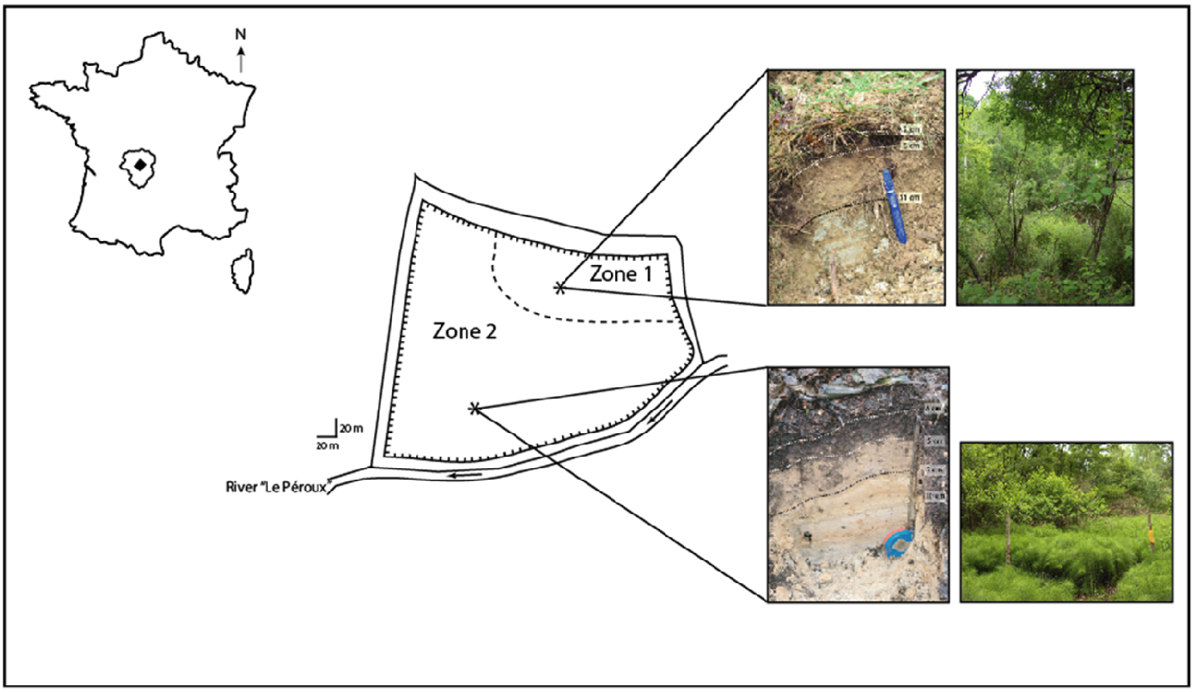

Figure V.1. Location and plant cover of the studied site adapted from Néel et al. (2003). * indicates sampling points as profile 1 and 2 in the zone 1 and 2, respectively.

II.2 Soil sampling and pedological parameters

Analyses were focused on two soil profiles located in each zone 1 and 2 (Figure V.1). Each horizon, as well as parental material were sampled, air-dried and sieved at 2 mm before analysis. Colour was determined using Munsell chart, pH was measured in a solid:liquid ratio of 2:5 with ultra pure water and KCl 1*M*. Particles size distribution of < 2 mm was determined after organic matter oxidation by H_2O_2, using Robinson pipette for fraction < 50 µm and then by sieving under water (LAS, Arras). The cation exchange capacity (CEC) was determined by the 0.05*N* cobalthexamine method at the soil pH (Orsini and Remy, 1976). This method was chosen as the current soil pH values were lower than 6. Exchangeable cations were measured by atomic absorption spectroscopy and exchangeable acidity by potentiometry. Organic C and N contents were determined by dry combustion with a C/H/N elemental analyzer. Bulk densities of soil horizons were measured by the core cylinder method, with three replicates per horizon.

The mineralogy of bulk soil samples (< 2mm) was determined by X-Ray Diffraction (XRD) using a diffractometer Siemens D 5000 (CuKα, 40 kV, 30mA) between 5 to 65°2θ at

0.12°2θ/min. Chemical composition of the mineral and their assemblages occurring in the soil horizons were identified by scanning electron microscopy (SEM-EDS) using a SEM Philips XL30 at 20 kV (SERMIEL, Université de Limoges, France).

II.3 Soils and plants chemical analysis

The total chemical composition of each soil horizon (<2mm) was performed by ACME Analytical Laboratories ltd (Vancouver, Canada). Major elements were determined by ICP-AES after a lithium metaborate / tetraborate fusion and nitric acid digestion. Trace elements were measured by ICP-MS after an *aqua regia* digestion.

The zone 1 was essentially composed by *Graminea*, *Betula pendula* and *Pteridium aquilinum*, whereas *Equisetum telmateia* were taken in zone 2. Each representative plant was sampled in triplicate. Roots have been carefully rinsed with ultrapure water and analysed in total metal contents (as described upper). Plants were dried at 60°C until a constant weight. Roots, shoots and leaves were separated and heavy metals contents were determined after a mineralization of 0.5g of split (digestion by HNO_3 and *aqua regia*) and analysed by ICP-MS (ACME Analytical Laboratories ltd; Vancouver, Canada). Each analyse was realized in triplicate, data shown are the mean of three plots.

II.4 Mobility and bioavailability assessment of chemical elements

Availability of metallic and metalloid elements was assessed through different selective extractions currently used in the literature: $CaCl_2$, A-Rhizo method, acetic acid and DTPA (diethylene triamine pentaacetic acid) according to protocols expressed in Table V.1. Extracted lead (Pb) was determined by flame atomic absorption spectrometry (Varian SpectrAA 220) equipped with a deuterium background correction and Arsenic (As) by graphite furnace atomic absorption spectrometry (Varian SpectrAA 880 Z) equipped with a Zeeman background correction. All the measurements were realized in triplicates pooled samples for each of the two locations site.

II.5 Statistical data treatment

Microsoft Excel 2004 and SPSS 19.0 for windows were used for calculation of descriptive statistics and for statistical analysis of datas. Data was subjected to Pearson correlation coefficients with a statistical significance was set at the $p < 0.05$ confidence level.

Table V.1. Protocols of bioavailability assessment performed according to different standard tests from literature.

Reagent	Expected extracted fraction	Procedure	References
$CaCl_2$	Immediately available fraction	0.01*M* $CaCl_2$, shake for 2h (solid-solution ratio 1:10)	Novozamsky *et al.*, (1993)
Acetic acid	Exchangeable fraction	0.11*M* acetic acid with 7 min of ultrasonication (solid:solution ratio 1:40)	Pérez-Cid *et al.*, (1998)
A-Rhyzo	Phytoavailable fraction	Mix of LMWOAs (Low Molecular Weight Organic Acids) acetic, lactic, citric, malic, formic acids shake for 16h. Total concentration of 0.01*M* (solid-solution ratio 1:10)	Fang *et al.*, (2007)
DTPA	Mobilisable fraction	0.005*M* DTPA + 0.01*M* $CaCl_2$ + 0.01*M* triethanolamine ajusted to pH 7.3. Shake for 2h. (solid:solution ratio: 1:2)	Lindsay and Norvell (1978)

III. Results and Discussion

III.1 Physical-chemical properties and mineralogical assemblage of soils

The studied soils located in Limousin (France) developed on the mining waste presented a large vegetation cover as shown in Figure V.1. The physical chemical properties of soils are reported in the Table V.2. Briefly, soils presented low pHs whatever the studied zones (zone 1: 3.4 to 4; zone 2: 4.2 to 5.7; Table V.2) and were very thin without any B horizon. However, high organic carbon and azote contents, therefore high C/N ratio, were effective, up to 426 $g.kg^{-1}$ Corg in the top horizons, especially in the zone 2 probably due to the large vegetal cover. Many studies reported nutrients deficiency of soils developed from mining wastes as observed in this study contributing to low agronomical properties (lower CEC or P content; Table V.2). For example, total phosphorus content was rather low (only up to 1.3 $g.kg^{-1}$ in organic layer) and in a comparable range as soils close to mining dumps (0.06-0.8 $g.kg^{-1}$, Moreno-Jiménez *et al.*, 2009) or 0.7 $g.kg^{-1}$ in tailings studied by Ye *et al.* (2000).

Table V.2. Physical-chemical properties of both studied profiles. CEC refers to cation exchange capacity.

	Depth	Hrz	pH		Bulk Density	Org C	Tot N	Tot P	C/N	CEC	H^+	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	Na^+
			H_2O	**KCl**												
	cm		-	-	**$g.cm^{-3}$**	**$g.kg^{-1}$**			-	**$cmol(+).kg^{-1}$**						
Profile 1	0-2	**O**	4.4	3.9	0.44	181.0	11.1	0.9	16.3	19.1	1.0	0.2	16.2	5.7	2.3	0.1
	2-5	**A**	3.8	3.3	0.68	96.6	6.5	0.8	14.8	8.8	0.7	0.1	7.2	2.2	1.1	0.06
	5-11	**C**	4.3	3.4	1.03	8.2	0.6	0.4	13.8	2.1	0.9	0.7	0.3	0.2	0.1	0.01
	>11	**Z**	3.4	3.2	1.04	4.9	0.5	0.4	10.4	2.7	2.0	1.7	0.05	0.03	0.1	0.03
Profile 2	0-3	**OF-OL**	5.0	4.6	0.36	426.0	28	0.6	15.2	63.6	2.2	0.06	67.9	9.5	6.4	0.2
	3-5	**OH**	4.5	3.9	0.36	350.0	24.5	1.1	14.3	53.7	1.1	0.1	47.2	5.0	3.2	0.1
	5-7	**A**	4.2	3.6	1.22	188.0	15.5	1.3	12.1	29.2	1.1	0.2	26.2	2.8	1.4	0.05
	>70	**Z**	4.5	3.4	1.22	13.7	1	0.5	13.6	5.8	0.8	0.2	3.9	0.5	0.6	0.03

Table V.3. Major elements, metal and metalloids concentrations of each horizon from the studied profiles.

	Depth	**Hrz**	**Si**	**Al**	**Fe**	**S**	**Ca**	**Mg**	**Na**	**K**	**Pb**	**As**	**Sb**
	cm		**$g.100g^{-1}$**								**$mg.kg^{-1}$**		
Profile 1	0-2	**O**	18.9	2.6	5.0	0.3	0.50	0.31	0.43	1.29	6939	32200	432
	2-5	**A**	25.2	3.6	6.8	0.1	0.33	0.34	0.58	1.64	9304	41900	530
	5-11	**C**	27.7	3.5	7.6	0.2	0.16	0.21	0.66	1.63	14100	64200	808
	> 11	**Z**	17.6	4.5	12.2	1.5	0.08	0.19	0.47	1.74	21300	119900	1406
Profile 2	0-3	**OF-OL**	nd	nd	nd	0.1	0.61	0.06	0.01	0.12	1299	6712	108
	3-5	**OH**	8.5	0.9	1.4	0.3	1.09	0.13	0.14	0.54	2537	5465	103
	5-7	**A**	19.5	2.3	4.3	0.2	0.69	0.17	0.39	1.10	5761	22000	295
	> 7	**Z**	24.2	3.8	9.0	0.2	0.19	0.24	0.56	1.20	14500	68100	930

nd= not determined

Since parental material has an anthropogenic origin, these soils were either classified as Technosols ('dominated by human-made materials') as it is described in WRB (FAO, 2006) or "Anthroposols Artificiels" in the French nomenclature (Référentiel Pédologique 2008). For example, Remon *et al.* (2005) reported same properties for Technosols developed under alkaline substrate, as well as the minimum profile development as a consequence of young age (Scalenghe and Ferraris, 2009).

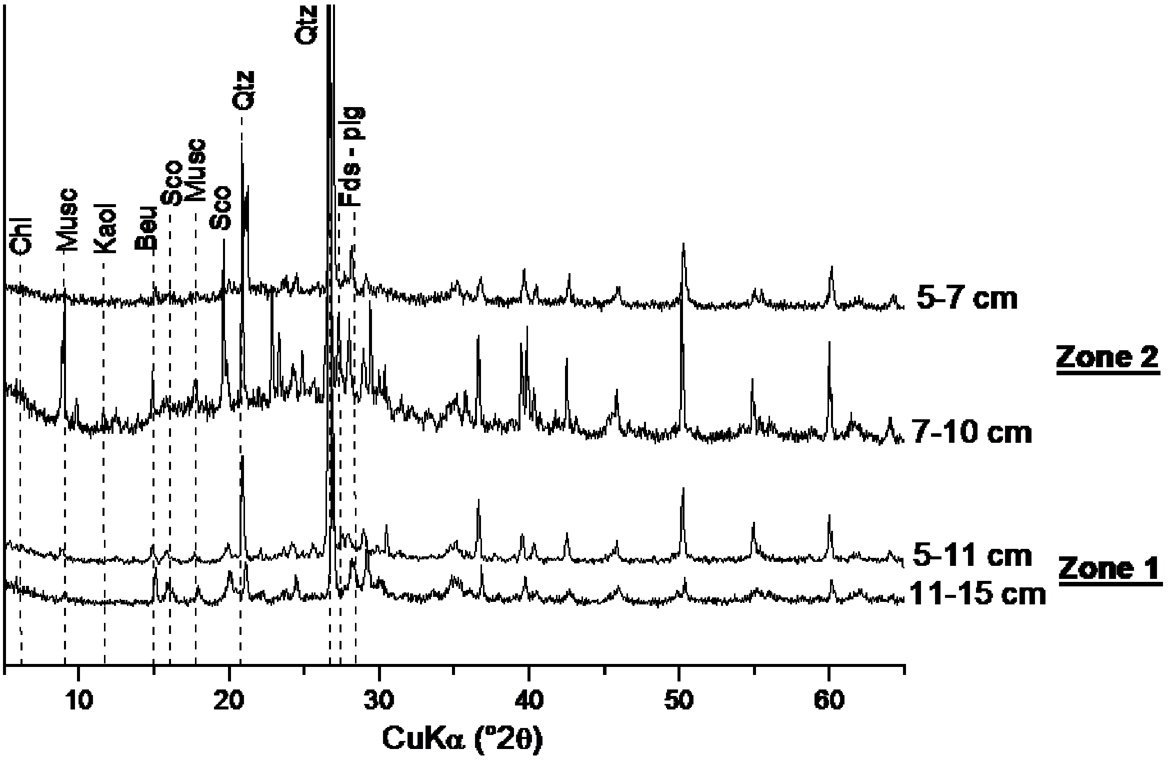

Figure V.2 Powder XRD patterns of the bulk soil (< 2 mm fractions) from each inorganic horizon of the two studied profiles. Only the majors reflexions peaks are referenced: Chl, Musc, Kaol, Beu, Sco, Qtz and Fds-plg refers to chlorite, muscovite, kaolinite, beudantite, scorodite, quartz and feldspaths-plagioclases respectively.

The nutrients deficiency as well as the low CEC in soils may be related to the mineralogical assemblage, high values of CEC observed in the top horizons being attributed to the contribution of organic matter. As expected, from industrial waste originating from acidic rocks mining activities, XRD and SEM-EDS data (Figures V.2 and V.3) evidenced the presence of quartz, feldspars in each studied horizon, as well as phyllosilicates (clinoclore and muscovite). These silicates mineral phases corresponded essentially to primary minerals inherited from the known hydrothermal processes involved in gold ore genesis (Nénert *et al.*, 1997). Weathering and pedogenesis induced the formation of secondary mineral phases (clays and clay minerals): kaolinite, illite and As- and Pb-bearing phases such as scorodite, anglesite, beudantite and As-rich iron oxyhydroxides pointed out with their main reference peaks (see XRD patterns and SEM results, Figures V.2 and V.3; see also Roussel *et al.*, 2000 and Néel *et al.*, 2003). These observed mineralogical phases were clearly associated to weathering processes of sulphide oxidation, as already shown by numerous authors (e.g. Mihaljevič *et al.*, 2009; Roussel *et al.*, 2000).

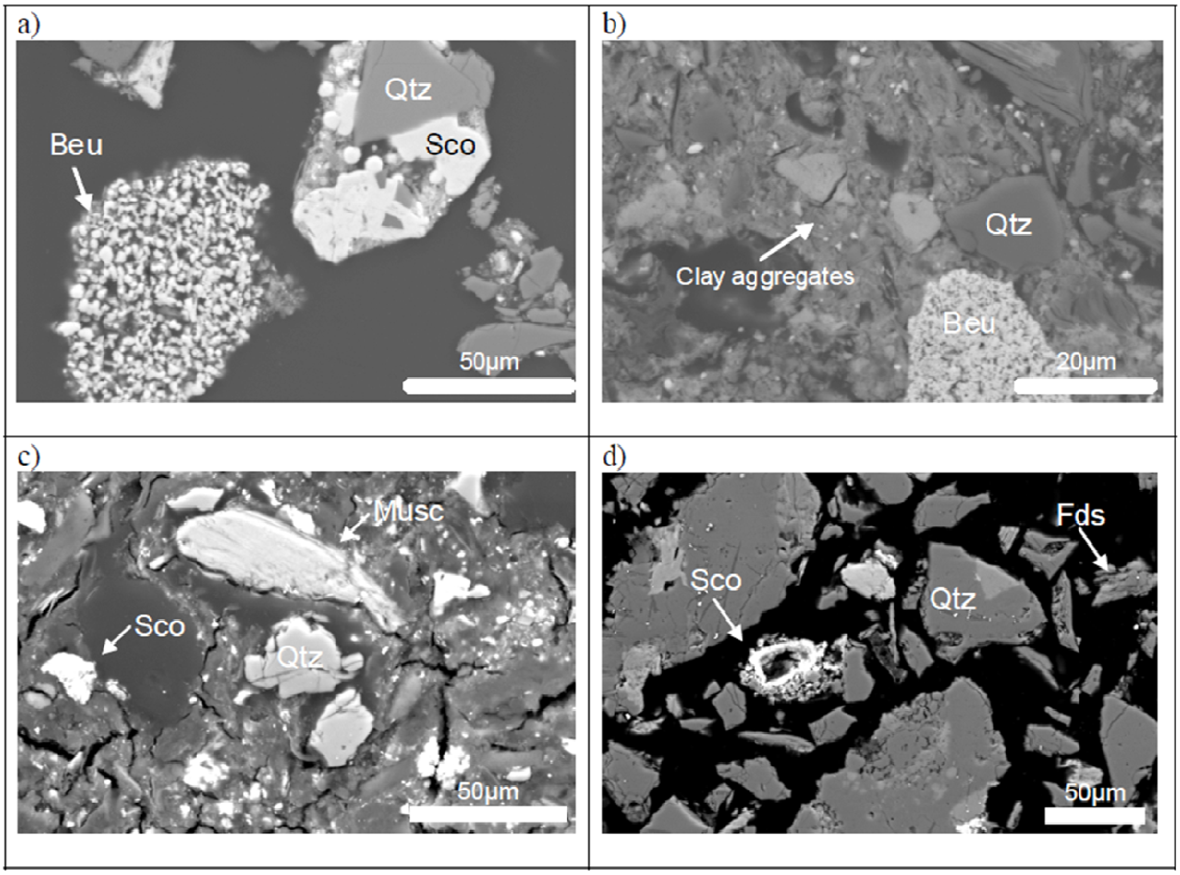

Figure V.3. SEM photographs in backscattered electron mode of the bulk soil fraction (< 2 mm). a) and b) refers respectively to the upper (2-5 cm) and deeper (11-15 cm) horizons from profile 1, c) and d) to the upper (3-5 cm) and deeper (7-10 cm) horizons from profile 2. Beu, Musc, Sco, Fds and Qtz refers to beudantite, muscovite, scorodite, feldspaths and quartz respectively.

Basically, scorodite and beudantite were present in both profiles either in organic horizons or in parental material but their amount seemed to decrease with depth, and obviously in organic horizons (Tables V.2 and V.3), but to increase with granulometric fractionation (see Figure V.3; Roussel *et al.,* 2000) suggesting high reactivity. As a result, soil properties (*e.g.* pH, CEC, mineralogy) directly reflected those of waste materials and its weathering, thus the waste material seemed to control the soil properties development as well as probably the behavior of chemical elements.

III.2 Soil chemical concentrations

The chemical analyses of the studied soils were presented in the Table V.3. As expected, the major elements in both studied horizons were Si, Al and Fe and increased with depth, Si being the most important till 36.7% in zone 2 whereas the amount of Al and Fe were up to 4.5% and 12.2 % respectively. The chemical composition of the parental material (deeper horizons) matched with the initial gold ores (Nénert *et al.*, 1997) and with the mineralogical assemblages (Figures V.2 and V.3).

As, Sb and Pb were the most abundant toxic elements, their concentrations being very high (Table V.3). Overall, whatever the elements, they increased with depth: As, Sb and Pb had exactly the same behavior in both profiles but (i) in different proportions (Chapter 3 in the thesis), and (ii) in accordance with the relative amounts of mineralogical phases. Arsenic content were within 32200 and 119900 mg.kg^{-1} in zone 1 and between 853 and 68100 mg.kg^{-1} in zone 2. Sb concentrations were higher in zone 1 from 432 to 1406 mg.kg^{-1} than in zone 2 in which they extended from 108 to 930 mg.kg^{-1}. Pb concentrations increased with depth from 6939 to 21300 mg.kg^{-1} in zone 1 and from 1299 to 14500 mg.kg^{-1} in zone 2. These contents in As, Sb and Pb were largely greater than (i) the “possible” toxicity values (5-20, 150 and 30-300 mg.kg^{-1} respectively; Kabata-Pendias, 2001) and (ii) the normal background levels given by several authors (*e.g.* Baize and Sterckeman, 2001; Boyle and Johanson, 1973; Chéry and Gateau, 1998; Norish, 1975; Tsai and Chen, 1999) for soils of diverse origin and lithology throughout the world. Due to poor literature about soils directly developed from highly polluted tailings, this study shows one of the most polluted young Technosol in the world. Indeed, the literature reports generally very high levels of inorganic pollutants in mine tailings but not in soils developed on them. For example, Saunders et al. (2010) described tailings with 42000 As mg.kg^{-1}, Rodríguez *et al.* (2009) showed Pb concentrations reaching about 94000 mg.kg^{-1} in mine tailings. Moreover, others studies about adjacent soil were due to aerial contamination or by waste deposit in mixture with soils (Boussen *et al.*, 2010; Rizzi *et al.*, 2004; Shu *et al.*, 2005; Zheng *et al.*, 2003). However, total chemical analysis of studied soils was not sufficient to predict the potential contamination risk of water resource or the accumulation in native plants.

III.3 Solubility and extractability of metal and metalloids

Assessment of metal and metalloids soil phytoavailability was determined by 4 singles extractions (SE) (Table V.1), the results being reported in the Figure V.4. As explained by Kabata-Pendias (1993), soluble, exchangeable and chelated fractions were quite labile and more available for plant as well as food chain. These single extraction procedures have been performed to study metal and metalloids mobility and as a result their potential bioavailability.

$CaCl_2$ and acetic acid reflect exchangeable fraction, weakly linked to particles in soils (mobile fraction, Houba *et al.* 1990; Lebourg *et al.* 1996; Maiz *et al.,* 2000; Rauret et al. 1999; Table V.1). Both SE procedures should represent the easily "immediate" mobile and short-term available fraction in soils. For the purpose of better assessing the mobilisable fraction, the use of DTPA and A-Rhizo methods was performed in this study. DTPA is a metal complexant currently used in literature, it theoretically extracts metallic elements bounded to organic complexes and hydroxides (Beckett, 1989) (Table V.1). A-Rhizo method extracts metal and metalloids fraction supposed to be mobilized in rhizosphere under the action of exudates roots such as the Low Molecular Weight Organic Acids group (LMWOAs) (Table V.1, Fang *et al.,* 2007; Feng *et al.,* 2005). As and Pb results are reported in the Figure V.4 as extracted amounts and as percentages of the different fraction with respect to the total amount of soils. Whatever the considered element, the phytoavailability is higher (i) in the soil horizons compared to waste material, and (ii) in zone 1 than in zone 2.

$CaCl_2$ extractable As was included between 3.6 and 42.9 $mg.kg^{-1}$ in zone 1 and 2.7 and 55.2 $mg.kg^{-1}$ for the second zone. With DTPA, extractable As was never higher than 2 $mg.kg^{-1}$. The variation range of A-Rhizo As extractable was within 24 to 269 $mg.kg^{-1}$ in zone 1 and 76 to 578 $mg.kg^{-1}$ in zone 2. Overall, behaviour of phytoavailable As along profiles had the same trend in both zones: As phytoavailable decreased from surface to depth. In zone 2, the trend was the same except for A-Rhizo with a higher phytoavailability in deeper horizons.

Variations in Pb phytoavailability evolution along profiles were clearly evidenced (Figure V.4). DTPA was the strongest extractant after acetic acid, A-Rhizo and $CaCl_2$. DTPA extractable Pb was higher in zone 1 than in zone 2 reaching 391.9 $mg.kg^{-1}$ and only 37.0 $mg.kg^{-1}$ respectively. No more than 1.5 $mg.kg^{-1}$ of Pb are extracted by $CaCl_2$ in both zones.

Acetic acid extracted only 20.2 to 51.8 mg.kg^{-1} of Pb in zone 1 and 0.4 to 17.0 mg.kg^{-1} in zone 2. A-Rhizo extracted maximum 19.0 mg.kg^{-1} of Pb in zone 1 and 15.5 mg.kg^{-1} of Pb.

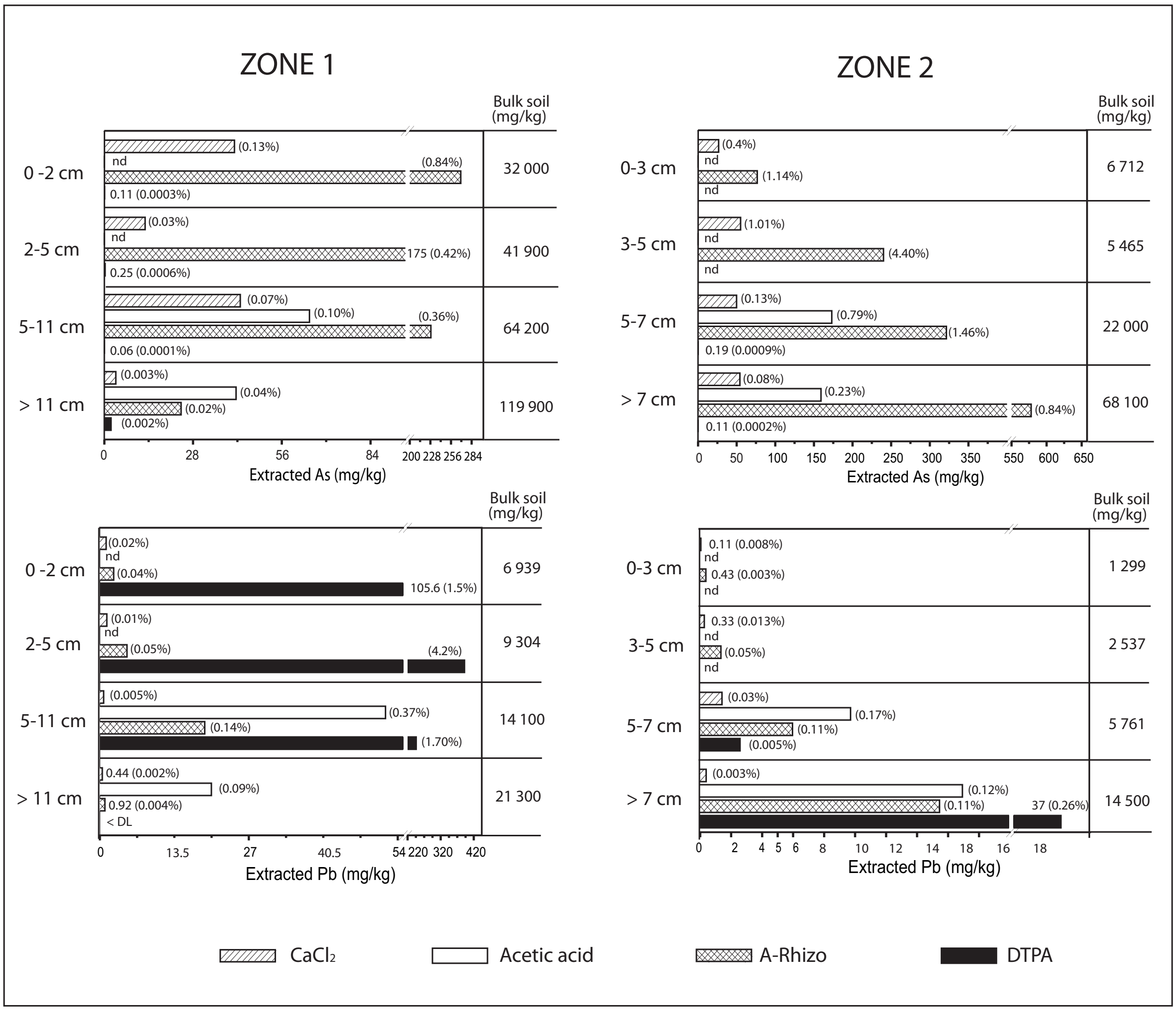

Figure V.4. As and Pb extracted by the 4 selective extraction procedures ($CaCl_2$, acetic acid, A-rhizo and DTPA, detailed in Table V.1) with the extracted percentages corresponding (into brakets) and the total concentrations in soils (right hand).

As shown in the Figure V.4, extracted fractions expressed in percentage of total element concentration in bulk soil could lead to the findings that inorganic pollutants were slightly available to vegetation as values were very low: mainly lower than 1% for extractable Pb by $CaCl_2$, A-Rhizo method or acetic acid but up to 5% by DTPA. Extracted As was lower than 4.4% of total As concentration by A-Rhizo method or even lower than 0.001% on average by DTPA.

The percentages of labile fractions (mobile + mobilisable fractions; Maiz *et al.*, 2000) of both pollutants represented less than 5% of bulk soil contents. Margui *et al.* (2007) and Remon *et* *al.* (2005) observed the same trend for heavy metals in contaminated environment near industrial activities or in mining contexts. Our results highlight the fact that the solubility of As and Pb seemed to be controlled by mineralogical phases. These results are in line with the fact that As- and Pb-bearing phases in these soils seemed to be relatively thermodynamicaly stable (beudantite and scorodite) maintaining low As and Pb solubility (Frentiu *et al.*, 2009; Paktunc and Bruggeman, 2010; Roussel *et al.*, 2000). However, considering the very high pollutants contents in bulk soil, these values represented a considerable amount of As and Pb which was potentially available in short and medium term.

III.4 Accumulation and translocation of metals in native plants tissues

Concentrations of inorganic pollutants (As, Sb and Pb) in each part of the representative plants studied are shown in Table V.4. As already explained, sampled plants have been chosen as they were the representative species growing and being well established in each zone. Zone 1 showed high plant diversity with three main representative species (*Graminea*, *Betula pendula* and *Pteridium aquilinum*) contrary to zone 2 essentially recovered by *Equisetum telmateia*. As, Sb and Pb can be considered as toxic element owing to their total concentrations in soils according to maximum allowable concentrations of trace elements in agricultural soils proposed or given by directives in various countries (Kabata-Pendias, 2001; see before). Even if uptaken inorganic pollutants by plant depends on their speciation and plant species, whatever the element and the plants studied, metal concentrations in roots were higher than those in aerial parts (shoots and leaves). As and Pb were elements the most accumulated in roots: up to 2198 mg.kg^{-1} As DW (dry weight) and 667 mg.kg^{-1} Pb DW of *Graminea* respectively (Table V.4). These values were largely upper than the excessive levels in plants grown in contaminated sites (Davies and Ginnever, 1979; Kabata-Pendias, 2001). For example, Chang *et al.* (2009) reported As amount up to 17.6 mg.kg^{-1} in *Pteridium aquilinum* rhizome growing on contaminated abandoned mines in Korea. For Sb, *Betula pendula* roots accumulated more with 83 mg.kg^{-1} DW, *Graminea* and *Pteridium aquilinum* roots having quite the same Sb concentrations (around 40 mg.kg^{-1} DW). Despite the high Sb concentration in the soils, relatively low concentrations were observed in plants as already shown by Hammel *et al.* (2000).

If we consider aerial parts, As and Pb were the most accumulated elements. *Graminea* concentrated the most amount of As, Sb and Pb (554, 9 and 155 mg.kg^{-1} DW respectively).

These values were very high, particularly As compared to concentrations in plants grown on uncontaminated soils which varied from 0.009 to 1.5 mg.kg^{-1} DW (Kabata-Pendias, 2001; Kabata-Pendias and Pendias, 1979; Kitagishi and Yamane, 1981; Laul *et al.*, 1979; Porter and Peterson, 1975; Shacklette, 1980) but in the average of As in plants grown in contaminated sites (e.g. Kabata-Pendias, 2001; Porter and Peterson, 1975).

Table V.4. As, Sb and Pb accumulation in various organs of plants from the natural vegetation cover (in mg.kg^{-1} DW) representative of the site. TF represents the translocation factor as the ratio of elements concentration in aerial part-taking into account organs weight, to elements concentration in roots.

	As	**Sb**	**Pb**
Graminea			
roots	2198.0	43.8	667.3
arial part	554.4	8.7	155.2
TF	0.25	0.19	0.23
Betula pendula			
roots	1141.0	83.5	306.2
branches	84.1	1.7	81.0
leaves	47.6	0.6	15.2
$TF_{branches}$	0.07	0.02	0.26
TF_{leaves}	0.04	0.007	0.05
Pteridium aquilinum			
rhizome	893.3	38.6	270.0
stem	7.6	0.1	56.7
frond	7.3	0.1	9.2
TF_{stem}	0.009	0.004	0.21
TF_{frond}	0.008	0.003	0.03
Equisetum telmateia			
rhizome	147.9	12.1	44.4
stem	9.9	0.3	2.9
branches	23.5	0.7	6.3
TF_{stem}	0.07	0.02	0.06
$TF_{branches}$	0.16	0.06	0.14

Several plant species are known to tolerate a high level of As in tissues. As toxicity is commonly noted in plants growing on mine wastes, on soils treated with arsenical pesticides, and on soils with As added by sewage sludge treatment. Moreover, although Pb occurs naturally in all plants, it has not been shown to play any essential roles in their metabolism (Kabata-Pendias, 2001). Kabata-Pendias (2001) reported that Pb levels in soils which are toxic to plants are not easy to evaluate but several authors gave quite similar concentrations, ranging from 100 to 500 mg.kg^{-1} (Davies, 1977, 1980; Troyer *et al.*, 1980; Roberts *et al.*, 1979). Broyer et al. (1972) explained that Pb is necessary for plants but a level of 2 to 6 µg.kg^{-1} DW should be sufficient, Pb being an element toxic to plants at upper values, which is the case in this study. However, Unterbrunner *et al.* (2007) described notably more elevated Pb concentrations in *Betula pendula* leaves compared to our results, within 74.9 to 263 mg.kg^{-1} DW. Whatever the extraction used and the pollutant content in various tissues of plants, no significant correlation was obtained at $p < 0.05$ (data not shown). An hypothesis may be realized since Rao *et al.* (2008), through a study published by Basta *et al.* (2005) pointed out that accumulation in different organs is species dependent, according to own rhizosphere biochemistry and plant physiology : two different crops growing on the same soil presenting the same contamination will not transfer inorganic pollutants in the same proportions.

It is well known that exclusion and accumulation are the two basic tolerance strategies evolved by plant with highly contaminated soils (e.g. Baker, 1981, 1987; Baker and Walker, 1990). Despite very high As, Sb and Pb concentrations in roots, translocation to aerial parts, which was evaluated by TF (translocation factor) calculated for each species and elements as the ratio of PTE concentration in aerial part-taking into account organs weight, to inorganic pollutants concentration in roots was low. Whatever the plants samples studied, as well as the different organs used, the TFs were very low < 1 (Table V.4). Some authors define as excluders plants with TF < 1 (Baker and Whiting, 2002). Briefly, angiosperms accumulated overall more than pteridophytes (namely *Pteridium aquilinum* and *Equisetum telmateia*). Large literature reports this behavior, e.g. Müller *et al.* (2009) for Sb and As with *Pteris vittata*, the well known As hyperaccumulator. These results indicated that plants were considered as playing a phytostabilization role (Mendez and Maier, 2008) but with concentrations over phytoxic level (Chaney, 1989). In order to assess potential spreading in trophic levels, bioconcentration factor (BCF) reflecting plant's ability to accumulate metals and metalloids from contaminated soils have been determined (expressed as PTE concentration in aerial above ground part divided by total metal concentration in soil, data not shown). BCF values of each studied species relevant to As, Sb and Pb were very low, between 0.001 and 0.18. In other words, these low BCF values were other features ascribing a phytostabilization role to all studied species preventing metals to enter the ecosystem through the food chain. Overall, native plants growing at La Petite Faye were able to tolerate As, Sb and Pb contamination with reduced transfer to their aerial parts leading to phytostabilize elements preventing transfer to ground water and aeolian dispersion.

IV. Conclusion

The investigated soils located in Limousin (France) quickly developed in 40 years on the highly contaminated waste material presenting a large natural vegetation cover. This work highlighted:

1) The short term development of soil (up to 40 years) in acidic conditions, pedogenesis being strongly related to (i) the parental material which was heavily contaminated in Pb, As and Sb controlling the soil properties development, and (ii) differences in native vegetation cover. These soils were either classified as Technosols or Anthroposols Artificiels,
2) The natural colonisation of various vegetation species (e.g. *Graminea*, *Betula pendula*, *Equisetum telmateia* and *Pteridium aquilinum*) even if soils were highly contaminated in metals and metalloids sometimes until several percent. The translocation and/or the bioaccumulation factors for the studied plants were very low (TF and BCF << 1),
3) Labile fractions of As and Pb obtained by single extractions were low (< 5 % of bulk soil contents), and may explain the small amount of pollutants measured in the plants tissues,
4) The major role of the mineralogical phases (*e.g.* clay minerals, As- and Pb-bearing phases as beudantite and scorodite) thermodynamically stable in this context, therefore controlling the solubility of As and Pb. In this study, the mineralogical control contributed to the fact that no used extraction methods seemed to be able to approximately and entirely predict phytoavailability of the studied elements. A fine geochemical and mineralogical pathway may be a good tool to better understand the behavior of the elements in soils,
5) The natural vegetation cover played an important role in term of phytostabilization, even with concentrations over phytoxic level, by its adaptability to these contaminated soils.

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CE QU’IL FAUT RETENIR…

Depuis la fin de l’activité minière, soit une cinquantaine d’années, et malgré une très forte contamination en métaux et métalloïdes, un couvert végétal s’est développé sur l’ensemble du bassin de décantation, cette végétation pouvant être relativement très dense dans le cas des prêles de la zone 2. Les résultats montrent clairement la capacité d’adaptation des plantes colonisatrices malgré la teneur élevée en contaminants et le faible potentiel agronomique de ces sols (pH très acides, faibles CEC et faibles teneurs en éléments nutritifs entre autres). De plus, quelles que soient les espèces végétales étudiées, les facteurs de transfert ou de bioaccumulation sont très faibles. Ceci montre (i) le rôle de la stabilité thermodynamique des phases porteuses (cf. chapitre 2) dans ce contexte et par conséquent le contrôle de la solubilité de As, Sb et Pb et donc de leur biodisponibilité/bioaccessibilité potentielle pour les plantes; et (ii) laisse supposer que les plantes bloqueraient le passage des contaminants étudiés vers leurs divers organes ou, au moins, vers les parties aériennes.

La présence de la végétation sur cet ancien site minier joue clairement un rôle de phytostabilisation naturelle. Ceci limite de manière efficace, sans impliquer un management du site couteux, le transfert de métaux et métalloïdes dans l’environnement.

Pour le cas de sites et sols pollués de grande superficie, il est fondamental de réfléchir à des solutions (i) de stabilisation de la contamination dans le but de limiter voire de supprimer les phénomènes de dispersion à d’autres compartiments environnementaux (chaîne alimentaire, ressource en eau et/ou air atmosphérique), et (ii) de valorisation potentielle de ce type de terrains. Dans ce cas, la culture d’une plante présentant un (des) intérêt(s) économique(s), à faible coût énergétique et agronomique, ainsi qu’une bonne adaptabilité à ce genre de support serait capitale.

CHAPITRE VI

Réponses physiologiques de *Miscanthus* x *giganteus* cultivé sur des sols contaminés

Les situations de contamination des sols représentent de très larges surfaces. En effet, l'Union Européenne a recensé près de 3.5 millions de sites qui seraient potentiellement pollués (CCE, 2006). Il est donc incontestable qu'il faille trouver des solutions visant à dépolluer ces sites ou au moins à stabiliser les pollutions afin de prévenir ou de limiter leurs transferts aux nappes souterraines ou vers la chaîne trophique.

Les pollutions métalliques se distinguent des pollutions organiques dans la mesure où aucune dégradation biologique n'est envisageable. L'une des possibilités de réhabilitation des sites contaminés par des pollutions métalliques est la phytoremédiation, une technique utilisant les végétaux pour leur revalorisation. Deux procédés sont distingués (i) la phytoextraction qui vise à extraire les EPTs (Eléments Potentiellement Pollués) d'un sol par le système racinaire des plantes puis à les accumuler dans leur partie aérienne (ii) La phytostabilisation qui a pour but l'immobilisation des EPTs par le système racinaire afin de limiter les phénomènes de lixiviation dans les sols, de transferts dans les eaux souterraines et/ou de dispersion de la contamination par voie éolienne. L'immobilisation des EPTs est réalisée au niveau du sol rhizosphérique, par adsorption sur les racines ou par accumulation dans les racines avec un faible transfert vers les tiges et les feuilles.

Chez les espèces végétales, les contaminations en éléments métalliques et métalloïdes peuvent être à l'origine de phytotoxicité se manifestant par l'apparition de phénomènes de chlorose (Baryla *et al*., 2001), par des perturbations des mécanismes de photosynthèse ou encore par une réduction de la croissance (Vernay *et al*., 2008).

Dans cette étude, le choix s'est porté sur la plante *Miscanthus* x *giganteus* (Figure VI.a), une poaceae (graminée) au métabolisme en C4, originaire d'Asie du sud est et qui s'adapte bien au climat tempéré (Zub et Brancourt-Hulmel, 2011).

I. *Miscanthus* x *giganteus*, une plante aux intérêts multiples

I.1 La plante

Miscanthus s.l est un groupe d'environ 20 espèces. *Miscanthus* x *giganteus* (Figure VI.a) est une plante triploïde stérile, utilisée dans l'industrie et qui est issue de l'hybridation naturelle entre *M. sinensis* (un diploïde) et *M. sacchariflorus* (un tétraploïde) (Linde-Laursen, 1993). Cette plante herbacée est pérenne et se développe à partir d'un rhizome assurant une repousse naturelle chaque année pendant plus de 15 ans. Comme Cadoux *et al.*, (2011) l'ont résumé, après les deux premières années de culture, réalisées sans apports d'intrants sur sols agricoles, les apports en N, P et K ne doivent pas dépasser les quantités exportées dans la biomasse récoltée soient respectivement 4.9, 0.45 et 7.0 $g.kg^{-1}$ de matière sèche. Globalement, les besoins en nutriments pour la culture de *M.* x *giganteus* sont faibles par rapport aux autres cultures du fait (i) de son système racinaire très développé assurant une forte absorption de nutriments, (ii) d'un cycle des nutriments important au sein de la plante entre rhizome et biomasse aérienne, (iii) d'un apport de matières organiques par la chute naturelle des feuilles sous forme de mulch et (iv) d'une potentielle fixation de l'azote par les bactéries de la rhizosphère. Par ailleurs, la culture de cette plante requiert également une bonne ressource en eau (Cadoux *et al*., 2011). Le pH optimum de culture se situe entre 5.5 et 7.5 (Damien, 2008).

Cette plante a été choisie pour ses intérêts industriels dans la mesure où sa biomasse est lignocellulosique c'est-à-dire qu'elle contient de la cellulose, de l'hémicellulose et de la lignine, des molécules intéressantes à plusieurs points de vue. Les cultures de *M*. x *giganteus* produisent une forte biomasse (jusqu'à 3-4 m de hauteur pour une plante mature) et présentent un rendement annuel de 20 à 25 $t.ha^{-1}$ après trois années de culture (Collura *et al.*, 2006). La bonne combustion de la biomasse dépend de sa teneur en eau, de sa teneur en éléments minéraux et en particulier de ses concentrations en N (azote), S (soufre) et Cl (chlorure). Dès 1997, Lewandowski et Kicherer exprimaient les qualités de combustion de *M*. x *giganteus* comme étant similaires à celles du saule.

Figure VI.a. Photos de *Miscanthus* x *giganteus* cultivé sur sol agricole à différents stades de développement d'un cycle annuel de la plante, Novabiom 2011.

L'analyse thermogravimétrique de combustion d'une biomasse permet d'étudier le comportement thermique d'une biomasse. Les caractéristiques relatives à *M.* x *giganteus* sont exprimées dans le tableau VI.a d'après l'étude de Jeguirim *et al.* (2010). Ainsi, lors de sa combustion, il y a une montée en température avec : une première phase de déshydratation où l'eau s'échappe en vapeur puis une phase de dévolatilisation où les composés organiques les plus volatils s'échappent de la matière et brûlent (c'est-à-dire qu'il y a rupture des liaisons c-c par oxydation) puis une seconde phase de combustion où les composés organiques restants du combustible brûlent en oxydant leurs molécules et en fournissant de l'énergie. Ensuite, s'opère une phase de combustion résiduelle de matière, alors principalement non organique, dans laquelle on peut distinguer trois parties : une partie détectable dans leurs conditions d'expérimentation par leur analyse, une part non détectable (déduite par bilan de masse) et la fraction résiduelle des cendres.

Tableau VI.a. Caractéristiques de dégradation thermique de *Miscanthus* x *giganteus* à pression atmosphérique, d'après Jeguirim *et al.* (2010)

	Gamme de température (°C)	Taux d'humidité (%)	T_{max} (°C)	$(dx/dt)_{max}$ (s^{-1})
Devolatilisation	180	94.13	289	$1.4x10^{-3}$
	340	32.72		
Combustion (fraction détectable)	340	32.72	401	$3.0x10^{-3}$
	410	05.96		
Combustion (fraction non détectable) + fraction résiduelle (cendres)	410	05.96		
	450	02.04		

Il existe d'autres applications industrielles pour l'utilisation de la biomasse de Miscanthus, par exemple en tant que litière pour les volailles ou les chevaux, ou encore pour la fabrication de bio-béton ou panneaux utilisés comme matériaux de construction et pour l'isolement des bâtiments.

I.2 Réponse aux contaminations métalliques.

Les premières études impliquant des cultures de *Miscanthus* x *giganteus* en situation de contamination ont été menées aussi bien en hydroponie qu'en culture sur sol.

Actuellement, de plus en plus de projets dans des contextes de sols contaminés sont à l'étude (Barbu *et al.,* 2010, Hartley *et al.,* 2009), parfois en partenariat avec l'Union Européenne. C'est le cas du projet Phytner « phytostabilisation sur des sols contaminés par des métaux à des fins énergétiques » coordonné par Francis Douay à Lille, et du projet FEDER Compétitivité régionale 2008-2013 intitulé « Bioremediation de la qualité des sols au moyen de l'association de *Miscanthus* x *giganteus* et des bactéries du sol ; valorisation des déchets associés » de Reims. Un troisième projet en cours, le projet ANR ECS RESACOR, coordonné par Isabelle Lamy (Pessac, Versailles) porte principalement sur l'étude de l'impact d'une culture de Miscanthus sur la dynamique des contaminants dans les sols pollués.

Le comportement de *Miscanthus* x *giganteux* vis-à-vis de contamination en cadmium (Cd) ou chrome (Cr) a été étudié par Arduini et al., (2004, 2006a, 2006b). L'inhibition de l'élongation des plantes est observée à partir de 0.75 mg.L^{-1} de Cd. La formation de nouvelles feuilles est inhibée à partir de 1.5 mg.L^{-1} Cd et celle des nouvelles tiges à partir de 3 mg.L^{-1} Cd (Arduini *et al.,* 2006a). De plus faibles concentrations de cet élément (0.5 mg.L^{-1}) stimulent significativement la croissance des racines après un mois de culture (Arduini *et al.,* 2004). Globalement, les parties aériennes accumulent moins le Cd que les parties racinaires *i.e.* après 3 mois de culture et 0.75 mg.L^{-1} Cd, les racines ont accumulé jusqu'à 332 mg.kg^{-1} de Cd contre 40 mg.kg^{-1} de Cd dans la tige principale, cette concentration n'étant pas significativement différente de l'accumulation en Cd dans la tige principale pour une concentration de 0.25 mg.L^{-1} (Arduini *et al.*, 2004). Ainsi le facteur net de translocation du Cd (du rhizome aux parties aériennes) est d'environ 60 et 18 mg.g^{-1}.jr^{-1} pour des concentrations respectives en Cd de 0.5 et 0.75 mg.L^{-1}. Par conséquent, *M.* x *giganteus* apparait comme étant une plante non hyperaccumulatrice du Cd.

Pour le Cr, les mécanismes d'absorption et d'accumulation sont similaires. Ainsi, les parties racinaires de *M.* x *giganteus* sont celles qui l'accumulent le plus. Entre 0 et 150 mg.L^{-1} Cr, le volume racinaire double en formant de fines racines mais au-delà, plus aucune racine ne se forme. La concentration en chrome dans les feuilles vertes atteint 70 mg.kg^{-1} pour une concentration de Cr en solution de 200 mg.L^{-1}. De plus le poids sec de la plante diminue de 17, 37 et 59% pour des concentrations en nitrate de Cr respectivement de 50, 100 et 150-200 mg.L^{-1}. Pour résumer, le facteur net de translocation du Cr des parties souterraines aux parties aériennes est à son maximum pour la plus forte des concentrations en Cr (Arduini *et al.*, 2006b).

Fernando et Oliveira (2004) ont démontré que *M.* x *giganteus* présente une grande tolérance à la toxicité de Pb en terme de paramètres de croissance et de production de biomasse (résultats obtenus après une culture en pots, sur sols artificiellement contaminés par des solutions salines). Les concentrations testées pour le plomb étaient de 0.75 et 1 g.m^{-2}. Cependant, toujours selon ces auteurs, le Pb pourrait être à l'origine de carence minérale au niveau de la plante et notamment en P (phosphore). En effet, ils ont mesuré une réduction de 15% de la teneur en P dans les tiges de *M.* x *giganteus* sur sol contaminé en Pb.

Une contamination en cuivre (Cu) de 0.6 et 0.8 g.m^{-2} provoque une réduction de moitié de la longueur de la plante. Ainsi, Fernando et Oliveira (2004) en concluent que la tolérance de *M.* x *giganteus* à la toxicité de Cu est faible. Cependant, la plante apparaitrait comme étant capable d'exclure le nickel (Ni) et le zinc (Zn) (Fernando et Oliveira, 2004).

Fernando *et al.* (2004) ont également fait des essais de cultures avec ajouts de boues de station d'épuration lorsque des plants matures avaient atteint 70 cm de hauteur. Les boues étaient contaminées en Cu, Cd, Ni, Pb, Zn et Cr. Il apparait qu'après trois années de culture, les concentrations mesurées dans la biomasse sont bien plus faibles au bout de trois ans qu'au bout d'un an.

Miscanthus sinensis, une espèce sauvage, peut accumuler jusqu'à 730 mg.kg^{-1} d'As dans ses tiges en poussant sur le stérile minier d'une ancienne mine d'arsenic présentant des teneurs pouvant atteindre 24 153 mg.kg^{-1} d'arsenic (Wei et Chen, 2002).

Globalement, *Miscanthus* x *giganteus* apparait comme étant une plante non hyperaccumulatrice de Cd, Cr, Ni, Cu, Pb et As (ce dernier pour *Miscanthus sinensis*). Cependant, les résultats de l'ensemble de ces études ont été obtenus dans des conditions de cultures différentes. Par conséquent, des résultats similaires ou au contraire bien distincts sont susceptibles d'être acquis, à niveau de contamination égal, du fait des propriétés pédologiques et agronomiques des sols de culture. De ce fait, nous avons évalué la capacité de *M.* x *giganteus* à s'adapter à des sols développés à partir de déchets miniers présentant une contamination multi-élémentaire.

L'objectif principal de ce chapitre sera donc d'évaluer les potentialités d'adaptation de *Miscanthus* x *gigantus* ainsi que d'éventuelles perturbations induites par les sols de notre site d'étude sur son développement. Ainsi, un suivi de la biomasse et une évaluation du fonctionnement des systèmes photosynthétiques ont été établis sur la plante cultivée pendant 3 mois en conditions contrôlées. Pour cela, des mélanges de sol ont été prélevés dans les zone 1 et 2 du site, dénommés respectivement S et L, et correspondant à des composites de sol. Par ailleurs, la photosynthèse nette, la conductance stomatique, la concentration en CO_2 ainsi que la transpiration ont été mesurées et comparées aux plantes se développant sur un terreau. Certains paramètres de la fluorescence de la chlorophylle *a* ont évalués avec les résulats de F_v/F_m et F_v/F_O. Une attention particulière a été portée sur les effets de la contamination sur la croissance de la plante et sur l'accumulation des EPTs (Eléments Potentiellement Toxiques) As, Sb et Pb dans les différents organes.

L'ensemble des résultats de cette étude sont présentés dans le manuscrit « Potential use of *Miscanthus* x *giganteus* on highly contaminated soils: physiological response and adaptability» dont la soumission est prévue dans la revue Bioresource and Technology.

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Potential use of *Miscanthus* x *giganteus* on highly contaminated soils: physiological response and adaptability

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Keywords: anthroposol, *Miscanthus* x *giganteus*, metallic elements, metalloids, photosynthetic activity

Abstract: Mining activities produce a huge amount of contaminated wastes, particularly concentrated in metals and metalloids. These elements present serious hazards for environment namely for groundwater, food chain contamination or atmosphere. As classical decontamination solutions are very expensive, the necessity to find alternative rehabilitation is fundamental. The aim of this study was to assess the potential adaptation of *Miscanthus* x *giganteus* (high ligniocellulosic biomass mainly used as biofuel) on highly contaminated site. The chosen studied site was a former gold mine. Mining wastes were highly contaminated by As, Pb and Sb with concentrations reaching sometimes several ten percents. Soils developed from these mining wastes showed, after several years, acidic pH, low CEC and nutrients content. During three months and in controlled conditions (phytotron), miscanthus was grown in highly contaminated composite soils from two areas of this site and in compost. Physiological parameters such as chlorophyll *a* fluorescence, gas exchanges (particularly Pn, gs, Ci, Tr and WUE) were assessed to determine whether the plant was stressed by the contamination. Results gave evidence of a clear aerial biomass reduction in contaminated soils. Values of Fv/Fm (the maximum photochemical efficiency) and leaves gas exchanges indicate an impact on physiological activity through a slight inhibition of its activity and physiological development. Metals and metalloids accumulation in biomass (translocation factor to aerial parts) was very low. The major conclusions are (i) the real capacity of growth of *Miscanthus* x *giganteus* on highly contaminated soils, and (ii) its short-term use to phytostabilize soils by cropping the plant without any inputs since contaminants content in biomass was above the threshold for industrial use as energy cropping.

Keywords: anthroposol, *Miscanthus* x *giganteus,* metals, metalloids, photosynthetic activity, biomass.

I. Introduction

Mining activities produce a huge amount of wastes usually containing a large amount of various contaminants. In order to discharge arable soils of energy crops and to prevent food chain and ground water contaminations, it is more and more necessary to rehabilitate contaminated sites. Classical decontamination processes are usually very expensive and not well adapted to metallic contamination. Thus, more and more, we turn forward using plants to either phytoextracte or phytostabilize the contamination. The necessity to decontaminate polluted sites is well recognised, both socially and politically due to the increasing importance place on environmental protection and human health. In case of mining wastes, contamination level being very high, phytoextraction is not suitable. Phytoextraction has been defined by Salt *et al.* (1998) as the extraction and accumulation of pollutants by plants in harvestable aerial parts. Phytostabilization seems to be one of the suitable short-term solutions to prevent pollution scattering by cropping metal tolerant plants which immobilize inorganic contaminants and reduce their mobility by (i) accumulating pollutants in roots, (ii) adsorbing them on roots surface, and (iii) decreasing metal mobility through changes in soil chemistry (Alford *et al.,* 2010). Furthermore, the main problem of contaminated areas is their still absence of economical value.

Another alternative can be to use energy crops which have the potential (i) to revalorise old industrial sites and municipal wastes and (ii) either to stabilise the pollutants in soils in order to avoid the contamination spreading, or to clean up contaminated land. This solution could prevent contaminants to enter human food chain combining long-term breakdown of organic matter in soils toward soil formation and large amount production of bioenergy. *Miscanthus* x *giganteus* is one of the most potential candidates (Cadoux *et al.,* 2011, Jeguirim *et al.,* 2010, Hartley *et al.*, 2009). *M.* x *giganteus* is a C_4, perennial and rhizomatous Poaceae originated from South Asia producing a biomass yield reaching 17.7 $t.ha.^{-1}.y^{-1}$ (Christian *et al.,* 2008) or even 25 t of dry matter per hectare after three years of cultivation (Collura *et al.,* 2006, Zub and Brancourt-Hulmel 2011) in order to produce energetic basic material. This plant has a high photosynthetic potential among C_4 plants when it grows under temperate field conditions (Naidu and Long, 2004). Therefore, *M.* x *giganteus* represents a high potential for biofuel materiel and constitutes a real alternative to fossil fuel

(Chou, 2009). Moreover, the adaptation and the tolerance to contaminated ecosystems have already been investigated mostly in hydropony or on contaminated soils by Cd- and Pb-rich sewage sludge (*e.g*. Arduini *et al.*, 2004 and 2006b; Fernando and Oliveira, 2004). Usually, it tolerates rather well contaminations as the transfer to its aerial parts is limited (Barbu *et al.*, 2010). We can assume a physiological tolerance mechanism in *M.* x *giganteus* as the antioxidant capacity of *Miscanthus sinensis* has also already been established (García *et al.*, 2010). In term of economical and industrial point of view, *M.* x *giganteus* thermal decomposition begins at a lower temperature and occurs at a higher rate than that of wood species. Decomposition products of biomass are mainly water, carbon monoxide and carbon dioxide, smaller amounts of methane and hydrogen (Meszaros *et al.*, 2007). Thanks to its low water, Cl, K, N, S contents, low ash concentration and large percentages of volatile matter, *M.* x *giganteus* has a higher thermal degradability compared to *Arundo donax*, anoter lingo-cellulosic plants (Jeguirim *et al.*, 2010). A delayed harvest, by reducing the water content to less than 20%, enhances the combustion (Lewandowski and Kicherer, 1997). Miscanthus biomass is also used in paper production particularly writing and printing papers (Ververis *et al.*, 2004), and as animal litter or biomaterials.

The aim of this study was to assess (i) the feasibility of a potential adaptation of *M.* x *giganteus*, a plant economically and energetically interesting, under highly contaminated soils by inorganic pollutants such as As, Sb and Pb, and (ii) the potential use of biomass production as energy. To assess whether this plant would be a sustainable candidate to be used on contaminated site as economical resource, we will follow the possible changes in photosynthetic activity induced by the contamination and the potential contaminants transfer to aerial parts of the plant.

II. Materials and methods

II.1 Studied site

The investigated site situated in Limousin (Creuse, French Massif Central, with GPS coordinates 01°34’23’’E and 46°80’37’’N) was a former gold mine (La Petite Faye) which its activity stopped in 1964. About 34 000 t of wastes have been stored in a settling basin on a surface of about 10 000 m^2. Since around 10 years, dense local vegetation has colonized the site leading to the development of soil classified as Technosol (WRB nomenclature, FAO 2006) or Anthroposol Artificiel (Référentiel Pédologique, 2008). The climate was oceanic temperate with a weather rather wet (altitude = 380 m / annual rainfall of 1018 mm / annual mean air temperature of 11.3°C).

II.2 Sampling and composite soils characterization

Soils developed from mining wastes have been sampled between 2-3 cm and 20 cm (fresh litters were removed) in two different areas of the site according to a decreasing As gradient (Table VI.1). These composites, named here soil S (severe contaminated) and soil L (low contaminated) represented soils as if they have been homogenized by ploughing. To assess the effect of the contamination on the growth of *Miscanthus* x *giganteus*, plants were also grown on classical compost corresponding to an optimal medium crop. Indeed, the content in N, P and K was 0.8 $kg.m^{-3}$ according to a proportion of 14, 16 and 18 respectively, and organic matter represented 60% of dry matter.

The composite soil properties have been characterized according to ISO standards methods. Both composites sampled in the former gold mine were very acidic to slightly acid from 3.5 to 6.1 (pH_{H2O}, Table VI.1). Bulk density was within 0.9 (soil S) and 1.0 (soil L). CEC was lower (1.9 $cmol(+).kg^{-1}$ and 8.9 $cmol(+).kg^{-1}$ for soil S and L respectively) with a low exchangeable bases content (Table VI.1) as well as available P (0.58 and 0.07 $g.kg^{-1}$ for soil S and soil L). Compared to contaminated soils, compost showed a high CEC (50.6 $cmol(+).kg^{-1}$), large contents in exchangeable bases, and a high available P (1030 $g.kg^{-1}$; Table VI.1). Despite the very high contamination level, bioavailability chemically assessed by 0.01*M* $CaCl_2$ showed low concentrations (Table VI.1). Indeed, bioavailable As was within 2.1 (soil S) and 10.4 $mg.kg^{-1}$ (soil L) and bioavailable Pb was within 1.6 and 134.1 $mg.kg^{-1}$ respectively for soil L and soil S. Therefore, the highest contaminated soil had the lowest bioavailable As.

Total concentrations in metals and metalloids (As, Sb, Pb) were measured after a lithium metaborate / tetraborate fusion and nitric acid digestion. Trace elements were measured by ICP-MS after an *aqua regia* digestion and performed by ACME Analytical Laboratories

(Vancouver, Canada). These studied composite soils were highly contaminated by metals and metalloids such as As, Sb and Pb reaching till several percent (Table VI.1).

II.3 Plant culture

4 dm^3 of soil were placed in plastic bags in pots to prevent runoff and one rhizome per pot has been placed at a few centimeters depth (within 5 and 10 cm). During 3 months plants were placed in a phytotron delivering light of 350 $\mu mol.m^{-2}.s^{-1}$ during 16h at 23 ± 1°C according to a humidity of 55 ± 5% on average. Night lasted 8h at 18°C. Plants were watered every 3 days to field capacity with distillated water. Five plants per condition have been grown.

II.4 Measurements of gas exchanges, chlorophyll a florescence and harvest

Before harvesting each plant, leaves gas exchanges such as net photosynthesis (P_n), stomatal conductance (*gs*), substomatal CO_2 concentration (C_i) and transpiration rate (*E*) were measured with a portable infrared gas analyzer (Li-Cor Model 6400, Lincoln, NE, USA). The measures were performed on 6 cm² leaf area with a clamp-on leaf cuvette. Inside, light, temperature and humidity were 350 $\mu mol.m^{-2}.s^{-1}$, 23°C and 30% respectively. CO_2 was maintained at a constant level of 360 $\mu mol.L^{-1}$ using a LI-Cor 6400-01 CO_2 injector (LI-Cor Lincoln, NE, USA) with a high pressure liquefied CO_2 cartridge source. Water use efficiency (WUE) measured the above-ground biomass produced per unit amount of transpired water at the whole-system level. It was calculated as the ratio of net CO_2 uptake to transpiration (Sinclair *et al.*, 1984).

Chlorophyll *a* fluorescence was also measured. It was determined by pulse amplitude modulation (PAM FMS1, Hansatech Instruments Ltd.,Norfolk, UK). Measurements were performed on young leaves. The initial fluorescence (F_o) was measured after a darkness of at least 30 min. The maximum fluorescence (F_m) was obtained after a saturating flash (1s, 13 000 $\mu mol.m^{-2}.s^{-1}$). F_v/F_m represents the variable to maximal fluorescence namely the maximum quantum yield of photosystem II (PS II) with F_v which is the difference between F_m and F_o. These values were strongly correlated to the quantum yield of net photosynthesis.

At the harvest, each part of the plant was separated into roots, rhizome, shoot(s) and leaves, weighted and then dried to 60°C till a stable weight.

II.5 Potential phytoavailability assessment

As and Pb phytoavailability in soils was determined by a $CaCl_2$ 0.01*M* extraction according to a solid:liquid ratio of 1:10 by shaking during 2 hours (Novozamsky *et al.*, 1993), then filtered to 0.2 µm prior analysis. As and Pb concentrations were measured by graphite furnace atomic absorption spectrometry (Varian SpectrAA 880 Z) equipped with a Zeeman background correction.

II.6 Metals and metalloids accumulation in plant

Measurements of metal and metalloids accumulation in each part of the plant (roots, rhizome, stem and leaves) were performed by ICP-MS (Acme Lab, Vancouver, Canada) after a digestion first in HNO_3 and then with *aqua regia*.

The stocks of metal and metalloids in different parts of *Miscanthus* x *giganteus* were calculated by multiplying metals and metalloids concentrations in each compartment by its biomass. Stocks of elements in soils were calculated by multiplying element concentrations by the apparent density and the depth of soil. Stocks in vegetation and soil were expressed in g or $mg.m^{-2}$.

Table VI.1. Main physical-chemical properties of both composite soils and compost.

	pH		Bulk Density	CEC	Ca^{2+}	Mg^{2+}	Na^+	K^+	Available PO_4^{3-}	$[As]_{bulk\ soil}$	Available As	$[Pb]_{bulk\ soil}$	Available Pb	$[Sb]_{bulk\ soil}$
	H_20	$CaCl_2$												
				cmol(+).kg^{-1}					mg.kg^{-1}					
Soil S	3.5	3.4	0.9	1.9	0.5	0.2	0.025	0.2	580	83 000	2.1	15 200	134.1	1 113
Soil L	6.1	5.3	1.0	8.9	8.7	1.5	0.016	0.5	70	1 727	10.4	325	19.1	76
Compost	6.5	nd	0.2	50.6	47.6	6.4	0.850	4.3	1 030	19	nd	22	nd	1.7

nd = not determined

III. Results and discussion

III.1 Plant yield

Whatever the composite soils or compost replications, all the *M.* x *giganteus* plants presented slight signs of wilting at leaves extremity after 3 months of growth. Nevertheless, Miscanthus appeared to be able to develop in multi contaminated soils. As expected, aerial biomass (stems + leaves) was strongly higher when *M.* x *giganteus* grew on compost (11.3 g dw) compared to both contaminated composite soils (below to 2 g dw; Figure VI.1). Our results were in accordance with findings of Hartley *et al.* (2009) who clearly observed reduced biomass production of *M.* x *giganteus* on multi-contaminated soils. In this study, results gave evidences of an inhibitory effect on biomass production in the contaminated medium growth. Aerial part biomass from the compost medium represented about 50% of total biomass compared to about 11% and 8% for the soil composites S and L respectively. Underground and aerial parts growth reacted differently with contamination. Underground biomass (roots + rhizome) of plants growing on both contaminated composite soils and compost was in the same range for all conditions of culture within 9.8 g dw per pot (soil L) and 13.1g dw per pot (soil S; Figure VI.1). Root systems appeared to be more developed with many secondary fine roots whether plant was growing in soil S. On the contrary, only few big roots were growing in soil L. Wong and Bradshaw (1982) demonstrated that root biomass can be highly reduced whereas stems continue to elongate. Moreover, with the highest contamination, reduction of root growth leads to a decrease of water and nutrients absorption, hence, the growth of the whole plant was affected and reduced as already shown by Fernando and Oliveira (2004). We could also wonder if the soils sieving did not induce a real porosity reduction preventing roots of air access. In zone from soil S, soil had a slightly higher granularity size than in zone where soil M was sampled (Néel *et al.*, 2003). Therefore, after sieving, soil S might benefit of a better air exchange than soil L. Moreover, *M.* x *giganteus* is a plant sensitive to pH, therefore it is recommended to grow the plant in soil with a pH value higher than 5.8. But, this property may not be directly link to the low biomass development as soil L had a pH value of 6.1 (Table VI.1). From the climatic conditions point of view, at La Petite Faye, *M.* x *giganteus* culture was compatible for such a climate even if frost episodes below -3.5°C can happen and could lead to plant losses (Zub and Brancourt-Hulmel, 2011).

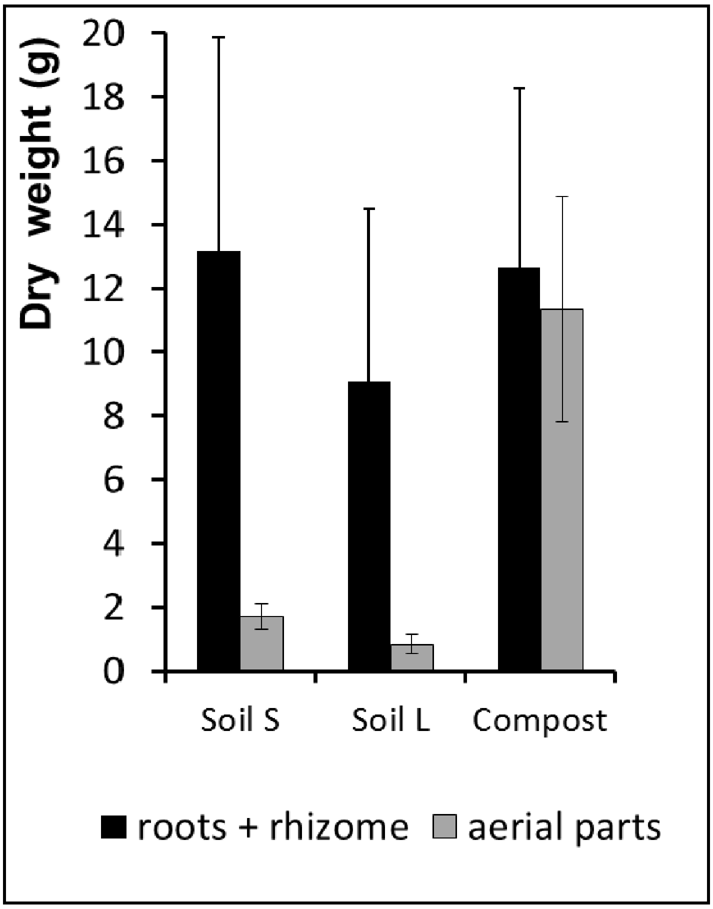

Figure VI.1. Biomass yield of *Miscanthus* x *giganteus* after a 3 months culture on 3 substrates in phytotron.

III.2 Photosynthetic activities of <u>Miscanthus x giganteus</u>

Measurements of photosynthetic activity can indicate whether a plant is stressed by a soil contamination (Vernay *et al.,* 2008). The results are represented in the Figures VI.2 and VI.3. Plants from composite soils S and L pointed out net photosynthetic rate close to compost with mean values respectively of 4.28 (significantly different of compost), 5.49 and 6.15 µmol $CO_2.m^{-2}.s^{-1}$. These results were in accordance with the stomatal conductance measurements which were close to 0.05 mol $H_2O.m^{-2}.s^{-1}$ for all plants. But, even in compost, these values were lower than those measured by Naidu and Long (2004) and Beale *et al.*

(1996) for *M.* x *giganteus.* The decrease of photosynthetic activity could be explained by the fact that measurements of Beale *et al.* (1996) have been done on plants of three years old under field conditions. However, our results appeared to be independent of the high contamination in metals and metalloids of soils since compost and composites responded in the same way. Substomatal CO_2 concentration described the same trend (Figure VI.2). Nevertheless, plants in compost were significantly different from composites soil S and L with 149.8 µmol $CO_2.m^{-2}.s^{-1}$ vs. 65 µmol $CO_2.m^{-2}.s^{-1}$ for soils. These values were also particularly lower than those measured by Beale *et al.* (1996). Transpiration rate described a different pattern with the compost which was significantly higher (1.5 mol $H_2O.m^{-2}.s^{-1}$) than all contaminated soils with values close to 0.7 and 0.9 $H_2O.m^{-2}.s^{-1}$ (Figure VI.2).

The water use efficiency (WUE) represents the capacity to assimilate carbon and produce biomass according to consumed water (Polley, 2002). Plants from contaminated composites soil S and L had a WUE close to 6, slightly higher than compost. It has already been reported that plant stress, induced by metals in soil, did not influence WUE but reduce plant growth (Becerril *et al.,* 1989). However, this is not in agreement with the studies of Menon *et al.* (2007) and Moustakas *et al.* (1997) who found that plants growing on multipolluted soils exhibited considerable less WUE than plants growing on fertile soil. As a consequence, our results clearly indicated that photosynthesis activity of plants were not likely due to soil contamination. Overall, variability in values of leave gas exchanges implied in photosynthesis was markedly higher for plants grown in contaminated composite soils than in compost (Figure VI.2).

According to mean values, variable to maximal fluorescence Fv/Fm ratio (Figure VI.3) were almost similar whatever the culture medium (0.7-0.8, on average) and in agreement with measurements made by Naidu and Long on the same plant (2004). Björkman and Demmig (1987) determined that whether Fv/Fm is higher than 0.8, the potential efficiency of PSII (photosystem II) is not affected. Thus, Fv/Fm values of *M.* x *giganteus* were slightly lower or equal to this threshold. As a result, we assume that potential efficiency of PSII was not strongly affected but was not optimal. Furthermore, the plants maturity grown in compost being more in advance, the PSII efficiency had already started to decrease. Therefore this later was not due to the contamination level (data not shown) as leaves were already light green.

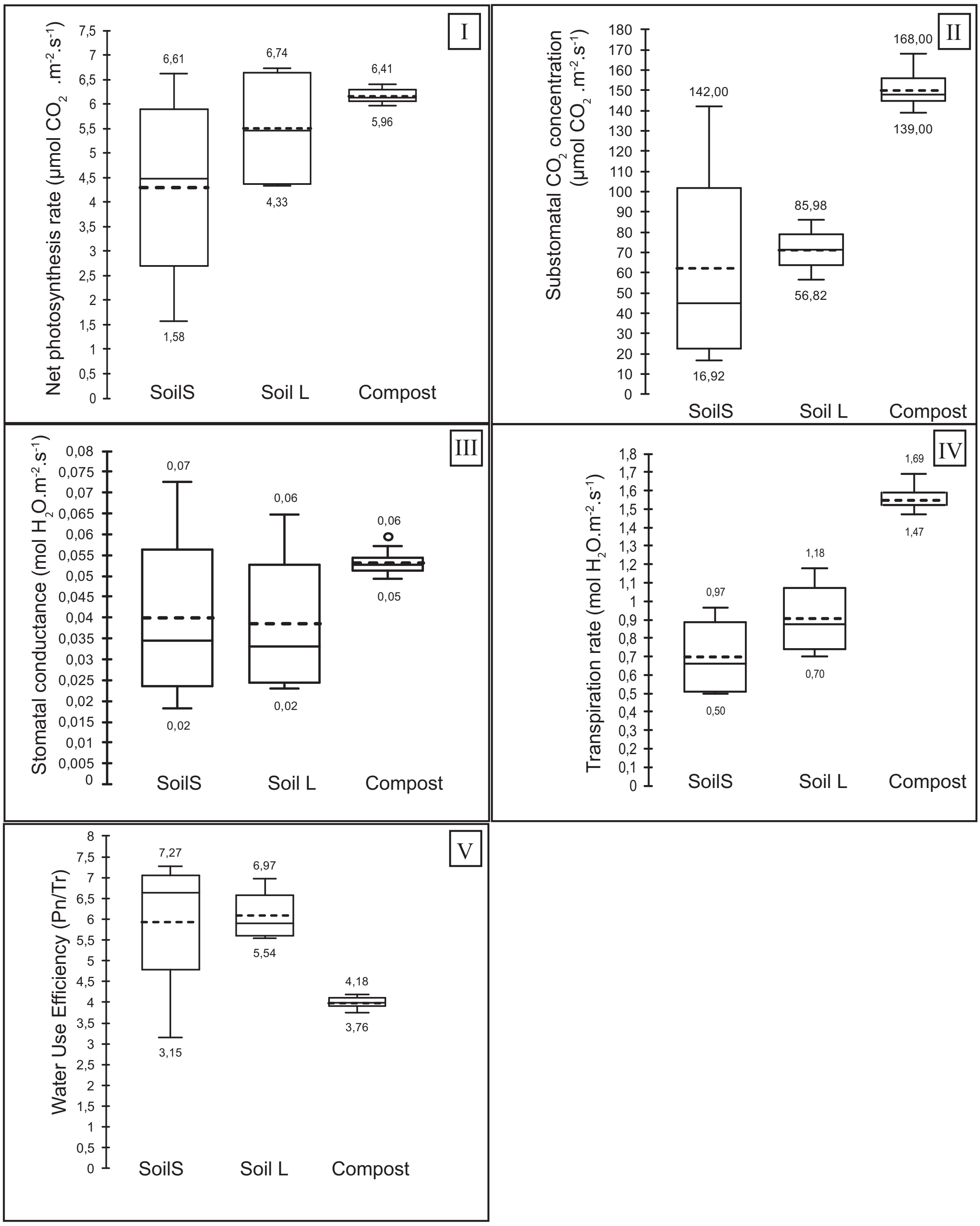

Figure VI.2. Photosynthesis activity of *Miscanthus* x *giganteus*, grown in phytotron on 3 substrates, through measurements of I) net photosynthesis rate, II) substomatal CO_2 concentration, III) stomatal conductance, IV) transpiration rate and V) water use efficiency (ratio between net photosynthesis rate and transpiration).

Dotted lines materialize mean values and median is represented by full lines.

The variable chlorophyll fluorescence ratio F_v/F_o reflects changes in the rates of photosynthetic quantum conversion (Maxwell and Johnson, 2000). In this study, this ratio was more sensitive to soil contamination than F_v/F_m one. Values were about 2.8 whatever the medium growth. F_O which represents the minimal fluorescence level in dark adapted state is sensitive to environmental stresses such as soil pollution. F_O values of plants from contaminated composite soils were slightly lower than plants grown in compost (data not shown). Therefore, contamination either might induce little alteration in the pigment-protein complexes of PSII or hamper the transfer from antennae to reaction centers. Thus, the ability of photon-harvesting assemblages to trap photons and transfer the energy to the acceptor of PSII seemed to be affected (Ouzounidou, 1993). Besides, variability of Chlorophyll *a* fluorescence values was once again higher for plants grown on contaminated composite soils than on compost.

III.3 Metals and metalloids soil to plant transfer

The metals and metalloids accumulation in *M.* x *giganteus* as well as the associated Translocation Factors (TF) are reported in the Table VI.2. As expected, compost grown plants displayed the lowest concentration in metals and metalloids compared to contaminated composites soils whatever the studied plant tissue. In contaminated composite soil studied here, roots were organs which accumulated the most metals and metalloids: from 602.4 to 1284.5 mg As.kg^{-1}, from 17.9 to 26.6 mg Sb.kg^{-1} and from 38.2 to 326.7 mg Pb.kg^{-1} respectively for soil S and L compared to 5.5 mg As.kg^{-1}, 0.2 mg Sb.kg^{-1} and 2.9 mg Pb.kg^{-1} in compost (Table VI.2). Hartley *et al.* (2009) studied the growth of *M.* x *giganteus* in contaminated soils (up to 71.9 mg.kg^{-1} of As in soils), but As accumulation was never higher than 8 mg.kg^{-1} in roots. On the contrary, another species from *Miscanthus* genus, *Miscanthus floridulus*, growing naturally in a former mine of China (mainly contaminated by As, Pb and Zn) recorded till 659 mg.kg-1 dw in roots (Leung *et al.*, 2007). Rhizome concentrations of metals and metalloids were at least ten fold below roots one, except for compost which did not react in the same way. This is probably due to the difference in (i) the small amount of inorganic pollutants in compost as well as (ii) the totally different physical-chemical and mineralogical status of compost.

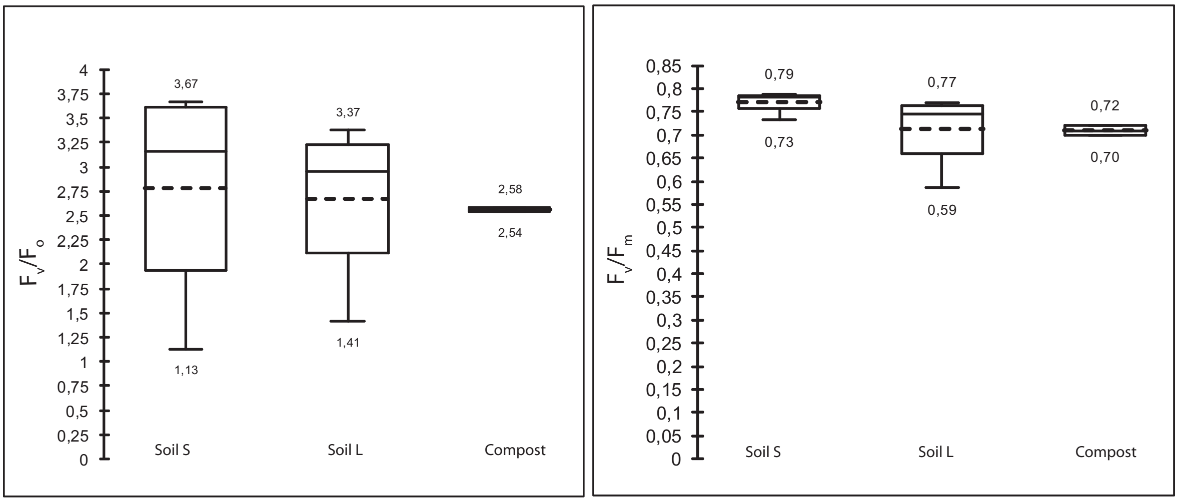

Figure VI.3. Chlorophyll *a* fluorescence implied in the photosynthesis activity of Miscanthus grown in phytotron on 3 substrates and assessed by F_V/F_O and F_V/F_m values. Dotted lines materialize mean values and median is represented by full lines.

Aerial parts (shoots and leaves) accumulated less: from 3.6 to 17 mg As.kg^{-1}, from 0.2 to 1.1 mg Sb.kg^{-1} and from 0.6 to 43 mg Pb.kg^{-1} in soil S and L compared to 7.1 mg As.kg^{-1}, 1.4 mg Sb.kg^{-1} and 3 mg Pb.kg^{-1} in leaves from compost (Table VI.2). These results clearly demonstrated that metals and metalloids were physiologically blocked in the roots of *M.* x *giganteus* during the first three months culture. It can be concluded that metals and metalloids transfer to aerial parts was low. Whatever the medium growth, leaves accumulated more metals and metalloids than stems. Fernando and Oliveira (2004) measured low Pb concentrations in aerial parts (stems) of *M.* x *giganteus* reaching 2.3 mg.kg^{-1} in a spiked soil with Pb. These authors explained that higher Pb rate led to a lower Pb concentration in stems. Arsenic concentrations in aerial parts of the studied plant never reached concentrations measured by Wei and Chen (2002) in *Miscanthus sinensis.* Growing on mine tailings with total As concentration reaching 24153 mg.kg^{-1}, they measured till 730 mg As.kg^{-1} in stems.

Arsenic and PO_4^{3-} are well known to have the same chemical shape and therefore compete for the same uptake sites (Adriano, 2001). Thus, As uptake generally increases when available P is low (Fayiga *et al.*, 2008). But in this study, soil S presented a high available P, and a low As availability (Table VI.1) although, As roots concentrations were the highest. Therefore, we suppose that As uptake was not only governed by the P available fraction. The roots system of *M.* x *giganteus* grown on soil S was well developed; consequently, exudation might be higher and remove more As from soil particles (Gonzaga *et al.*, 2009). Furthermore, an increase of P concentration through fertilization can lead to an increase of As uptake (Jiang and Singh, 1994). The Sb transfer was also light (up to 1.4 mg.kg^{-1}) but lower in contaminated soils S and L (exhibiting highest Sb contamination) than in compost. This result strengthens the idea of the major role of physical elements speciation as well as texture in their mobility in soils. Indeed, As, Sb and Pb in soils S and L were mainly born by mineralogical phases characterized in Chapter 3.

Translocation factors (TF), defined as the ratio between concentration in leaves and concentration in roots were always lower than 0.1 (Table VI.2). Therefore, these low TF confirmed that *M.* x *giganteus* did not transfer metals and metaloids such as As, Pb and Sb to aerial biomass. Compost TFs were much higher than 1 but lower whether we considered values of metals and metalloids concentrations in this substrate.

The C_4 plant sugarcane transferred easily metals and metalloids (Cd, Cr, Cu, Hg, Mn, Pb, Zn,) to stems but the concentrations in leaves were always lower (Sugaro-Muñoz *et al.*, 2006). Besides, Viisoottiviseth *et al.*, (2002) measured 40 mg.kg^{-1} dw in *Panicum repens*, another Poacaea also able to develop on mine tailing contaminated by As. Arsenic accumulation in *Paspalum tuberosum* shoot, a rhizomatous Poacea reached 1 130 mg.kg-1 growing in soil with a total As concentration of 7 670 mg.kg-1 (Fitz and Wezel, 2002).

Table VI.2. As, Sb and Pb accumulation in *Miscanthus* x *giganteus* grown in phytotron on 3 substrates, and respective Translocation Factors (TF).

		As	Sb	Pb
		(mg.kg^{-1} dry wt)		
Soil S	Roots	1 284.5	17.9	326.7
	Rhizomes	49.5	1.0	30.6
	Stem	5.4	0.8	29.5
	Leaves	17.1	1.1	43.1
	TF (no unit)	0.013	0.063	0.132
Soil L	Roots	602.4	26.6	38.2
	Rhizomes	48.3	3.1	3.8
	Stem	3.6	1	0.6
	Leaves	4.3	0.2	1.1
	TF (no unit)	0.007	0.007	0.03
Compost	Roots	5.5	0.2	2.9
	Rhizomes	1.5	0.05	0.9
	Stem	0.7	0.7	0.3
	Leaves	7.1	1.4	3.0
	TF (no unit)	1.291	6.95	1.038

If results of metals and metalloids concentrations are expressed in mass per unit of surface area as employed by Néel *et al*. (2007) and Gandois *et al.* (2010) taking into account apparent density of soil and biomass yield (Table VI.3), comparison of elements pools, also designated as stock, is more reliable. Metal and metalloids stocks in vegetation were in the following order (on a weight basis): Sb< Pb< As. On the one hand, As pools contained in *M.* x *giganteus* were within 89.6 and 22.60 mg.m^{-2} in soil S and soil L respectively, and of 2.20 mg.m^{-2} in compost. On the other hand, Sb stocks in vegetation growing in composites soils S (1.46 mg.m^{-2}) and L (1.36 mg.m^{-2}) were similar whereas their bulk soil contents were of 1113 mg.kg^{-1} and of 28 mg.kg^{-1} in soil S and L. Metal and metalloids stocks were calculated in the different *M.* x *giganteus* compartments in order to assess their partitioning and their dynamics in the plant. Their distribution was very different between compost and contaminated composites (Table VI.3 and Figure VI.4). Thus, underground parts (roots and rhizomes) were the main reservoirs of As, Sb and Pb in plants growing in soil S and soil L whereas foliage (stems and leaves) contained less important stocks for these elements. Indeed, underground parts accounted for 92 to 99 % of the total As, Sb and Pb stocks in *M.* x *giganteus* growing in contaminated composites soils. Metal and metalloids distributions in the plant were markedly different in compost from soil S and soil L. Indeed, elements were strongly transferred to aerial parts (Figure VI.4) even if metal and metalloids concentrations in compost were clearly smaller. Again, this result clearly showed the key role of physical-chemical and mineralogical properties of culture soil in the dynamic of metals and metalloids elements. Overall, *Miscanthus* x *giganteus* did not transfer intensely metals and metalloids such as As, Sb and Pb to aerial biomass, which could allow cropping this plant in soil contaminated zones.

Table VI.3. Biomass and stocks in total vegetation, in soils and different parts of *Miscanthus* x *giganteus* grown 3 months in phytotron and on 3 substrates.

		Biomass (t.ha^{-1})	Stocks (g. ha^{-1})		
			As	Sb	Pb
Soil S	Underground parts	5.4	888.5	140.0	308.1
	Foliage	0.7	7.50	60.0	24.1
	Total biomass	6.0	896.0	146.0	332.2
	Soil (kg. ha^{-1})		11 54950	154790	2 11254
Soil L	Underground parts	3.8	22.6	13.6	17.1
	Foliage	32.1	0.1	0.4	0.3
	Total biomass	4.1	22.7	14.0	17.4
	Soil		2667	1175	5030
Compost	Underground parts	4.9	11.6	6.5	0.4
	Foliage	4.4	10.3	3.9	4.3
	Total biomass	9.3	21.9	10.4	4.7
	Soil		57	5	68

III.4 Potential use of Miscanthus x *giganteus from highly contaminated sites*

Miscanthus x *giganteus* biomass is currently proposed for fossil fuel substitution (Heaton *et al.,* 2010) and might be a promising energy crop satisfying climate and energy policies (Cadoux *et al.,* 2011). The need to crop bioenergy plant in degraded – contaminated lands is of major concern and could avoid the lack of arable soils to produce food. (Tilman *et al.,* 2006; Cadoux *et al.,* 2011). The biofuel expansion and the associated direct and indirect land clearing as well as the fate of the soil quality are also of key importance. In this study, results evidenced that the amount of metals and metalloids uptaken by Miscanthus and as a result the transfer of these elements from soil to aerial parts (foliage) was very low. This finding makes the plant potentially suitable for use as green energy. However, even if metals and metalloids concentrations in aerial parts of *M.* x *giganteus* were not too high given the soil contamination rate, an additional question is how to consider this biomass: as a classical biomass or on the contrary as a waste? Moreover, ashes produced by combustion might also be a contaminated waste.

The potential use of biomass as bioenergy also implies to take into account gas, pollutants and particles emissions (SO_2, NO_x, dust, CO, and the total hydrocarbon) by combustion. In France, an effective regulation for concentrations of gas, particles and pollutant emissions is function of the boiler power (above 20 MWth namely classified plants, submissive to an official authorization) and the emitted amount per hour. Measurements must be done continuously and in the area where the impact is supposed to be the highest. These one must be performed by the industry which emits the pollution (Article 12 from the text dating of July, 23th 2010 and according to potential local restriction). Thus, arsenic, selenium and tellurium concentrations should not exceed 50 $g.h^{-1}$ in emissions as well as antimony, chromium, cobalt, copper, tin, manganese, nickel, lead, vanadium, zinc and their compounds 500 $g.h^{-1}$. Concentration in lead and its compounds should be below 100 $g.h^{-1}$. However, *M.* x *giganteus* biomass is sometimes mixed to wood biomass. Therefore, detailed analysis of biomass should be achieved likewise the follow of these measurements results in the channel should be facilitated in order to prevent polluted emissions. Thus, hazardous emissions would be well managed and controlled. Therefore, aerial parts of plants grown on contaminated soils S and L exceeded this limit. As a result, it would be impossible to use *Miscanthus* x *giganteus* biomass for industrial application or process in this case.

On the other hand, *Miscanthus* x *giganteus* could be appropriate to mine tailings or highly contaminated soils for phytostabilizing. Indeed, focuses on short-term stabilization and containment of the pollutant, *M.* x *giganteus* could be able to (i) reduce wind erosion, (ii) immobilize the pollutants by adsorption or accumulation in roots, (ii) limit the transfer to aerial parts since contaminants were sequestered in soil near the roots but not in plant tissues. As a result, pollutants would become less bioavailable, therefore livestock, wildlife, and human exposure would be reduced. However, the inorganic pollutant fate in soils when roots, and later, rhizomes will be either naturally decomposed in soils or removed, has to be investigated in order to expect what could be the future cultivable crop. In addition, their decomposition toward soil organic matter would support soil formation and stabilization as well as physical and chemical soil properties improvement.

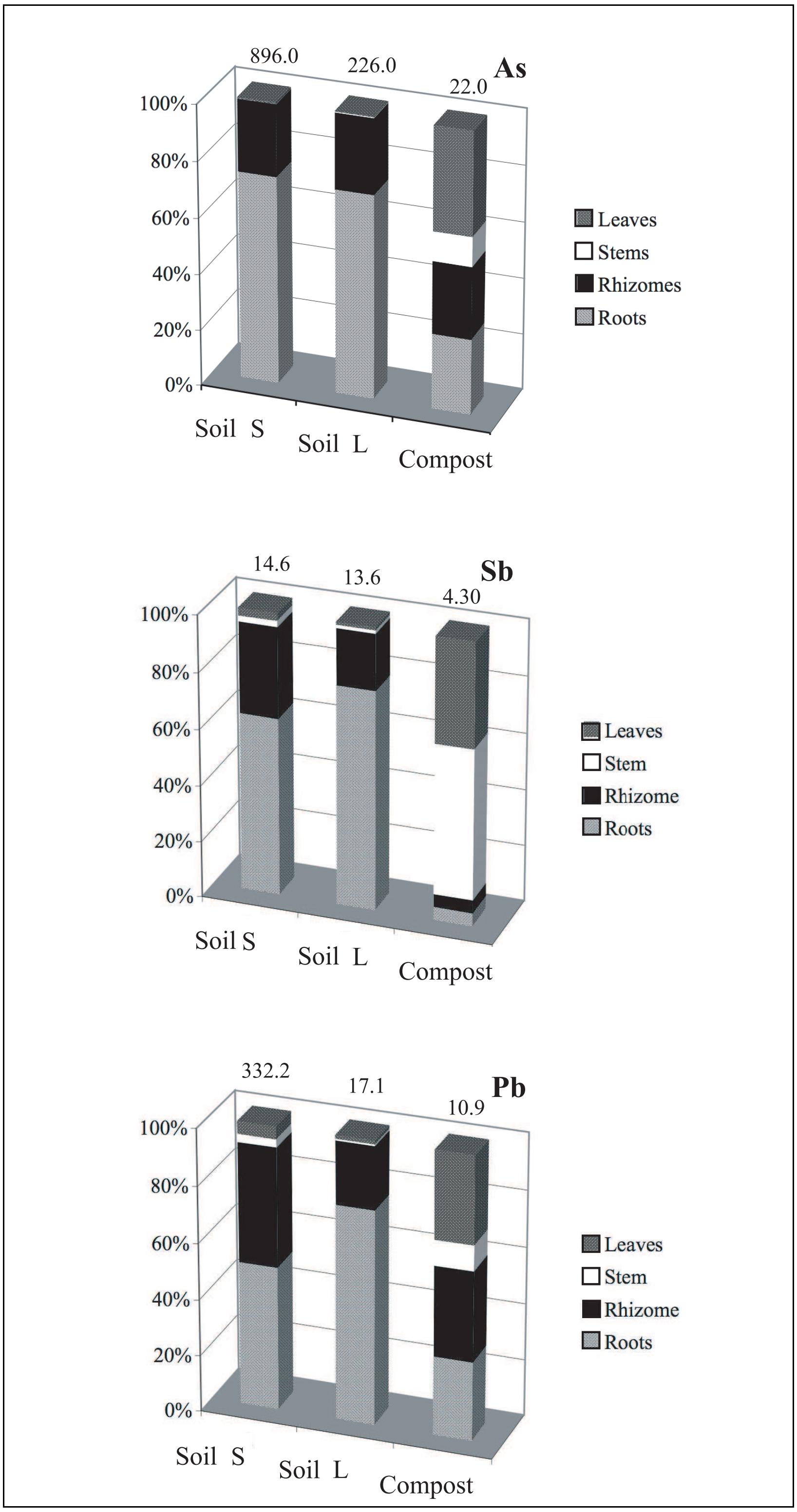

Figure VI.4. Stocks of metal and metalloids and their distribution among Miscanthus tissues after 3 months growth in phytotron on 3 substrates.

IV. Conclusion

This study presents the first results of potential adaptability of *Miscanthus* x *giganteus*, a valuable energy plant, to strong contaminated soils (As, Sb and Pb for example) as well as the transfer of these elements to aerial biomass. The main findings are:

- results from pot studies clearly indicated that *Miscanthus* x *giganteus* crops could be successfully grown in highly contaminated soils/lands although high levels of inorganic contaminants (metals and metalloids) or lack of nutrients and too low pH may reduce crop productivity (reduced biomass production). The physiological responses clearly showed a reduction of stomatal conductance and substomatal CO_2 concentration but a net photosynthesis and a WUE higher being similar within contaminated soils and compost.
- metal and metalloids amounts in *Miscanthus* x *giganteus* cropped in highly contaminated soils reminded very low. The metal accumulation was recorded in roots and rhizomes rather than in harvested aerial parts. As a result this herbaceous plant could be considered as phytoexcluder at least in first months growth, and might be suitable to grow in old industrial sites as well as arable surfaces.
- in terms of biomass productivity, there were however significant differences among the plants according to the different levels of contamination and nutrients pools (*e.g*. compost). Productivity could be increased by nutrients amendments (N for example), or using waste sewage from WWTP (Waste Water Treatment Plants). This last hypothesis might induce no additional environmental problems in case of metals contamination, while the site is already highly contaminated. In other contaminated situations, this suggestion should be well assessed.
- due to the high level of contaminated soils studied here, harvest has been done earlier than the harvest of plants destined to combustion.

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CE QU'IL FAUT RETENIR…

Ce chapitre a permis de mettre en évidence (i) la réponse physiologique de *Miscanthus* x *giganteus* cultivé sur les sols contaminés du site d'étude de La Petite Faye sans apports fertilisants et en conditions contrôlées, et (ii) d'évaluer les potentialités d'adaptation et d'éventuelle valorisation de *Miscanthus* x *giganteux* sur site fortement contaminé.

A partir des résultats obtenus en laboratoire, des points positifs se dégagent nettement comme l'adaptation du *Miscanthus* x *giganteus* sur des sols fortement impactés par les activités anthropiques à l'origine de contamination, une réponse physiologique intéressante ou encore le faible transfert des contaminants vers les parties aériennes de la plante. Cependant, la très nette différence de production de biomasse entre culture sur sols contaminés et sur terreau est indéniable. De plus, bien que le transfert des contaminants soit faible, celui-ci reste encore trop important dans le cas de cette étude pour une potentielle valorisation de la plante en tant que biomasse. Par conséquent, il parait encore difficile de pouvoir envisager l'utilisation de la biomasse de *M*. x *giganteus* en tant que biocombustible à part entière compte tenu des faibles rendements et du taux d'accumulation des éléments potentiellement toxiques. Par ailleurs, des améliorations agronomiques pourraient très probablement pallier ces inconvénients actuels (apport de fertilisants naturels et/ou de boues de station d'épuration des eaux usées).

Pour conclure, le Miscanthus apparait comme étant cultivable sur des sols présentant différents degrés de contamination, mais son utilisation ne sera pas la même selon qu'il sera une plante phytostabilisatrice ou une production pour sa biomasse aux intérêts énergétiques. Une étude *in situ* devra vérifier ces résultats.

CONCLUSIONS GENERALES

L'exploitation minière est à la base de la production des minerais sur lesquels le développement technologique sociétal s'appuie, en particulier de nos jours pour les composés électroniques. Cependant, les activités minières s'accompagnent inévitablement de la production de déchets bien souvent riches en éléments métalliques potentiellement toxiques (EPTs) et dont le stockage se fait, en général, à proximité des exploitations. Ainsi, la visée de cette thèse était l'étude de l'utilisation potentielle des anciens sites miniers pour la production de plantes bioénergétiques. En effet, il y a nécessité de réduction globale de consommation des énergies fossiles émettrices de gaz à effet de serre impliqués dans le changement climatique, et un besoin grandissant en ressources alimentaires. De ce fait, le maintien des terres agricoles devient indispensable compte tenu également de l'accroissement de la population mondiale et du désir de mettre fin à la malnutrition.

Dans le cadre de cette étude, l'extraction d'or de l'ancienne mine de la Petite Faye (Limousin, France) fut productrice d'un matériau hautement contaminé en divers éléments chimiques dont l'arsenic (As), l'antimoine (Sb) et le plomb (Pb), stocké dans un bassin de décantation depuis les années 1960 et depuis laissé à l'abandon. Ce site est l'exemple type d'un site où une réhabilitation n'a pas été opérée. C'est pourquoi l'objectif final de ces travaux de thèse était d'évaluer une possibilité de revalorisation de ce site par des plantes, en étudiant la capacité d'adaptation d'une plante bioénergétique telle que *Miscanthus* x *giganteus* sur ces sols. Après avoir caractérisé pédologiquement, géochimiquement et microbiologiquement les sols évoluant sur ce site, nous avons testé l'hypothèse, jusqu'à présent principalement démontrée en hydroponie, selon laquelle *M.* x *giganteus* est une plante métalotolérante, s'adaptant facilement sur un support peu fertile, explicable par la translocation des nutriments vers le rhizome en fin de saison, tout en ayant un faible transfert des éléments minéraux (en particulier Cl, N et K) vers les parties aériennes (Lewandowski *et al.,* 2000 ; Strullu *et al.,* 2011) facilitant leur revalorisation industrielle. Ce terrain, pauvre en nutriments (Chapitre 5) et présentant un important taux de contamination (Chapitre 3), était autrement dit *a priori* hostile au développement de cette plante et susceptible d'engendrer un éventuel transfert des polluants issus du sol aux parties aériennes. En effet, dans le cas de certaines espèces se développant naturellement sur le site, le bouleau ou les graminées prairiales sont les végétaux accumulant le plus les contaminants (Chapitre 5). En outre, la contamination affecte bactéries et archées autotrophes impliquées dans la nitrification (Chapitre 4).

I. Discussion générale

I.1 Caractérisation des Anthroposols du site d'étude pour une revalorisation adaptée

Les déchets miniers de la Petite Faye, stockés depuis les années 60, issus d'un filon porteur d'or ont été directement soumis aux conditions climatiques tempérées de la région Limousin pendant presque 30 ans. L'altération, c'est-à-dire le processus induisant entre autre la déstabilisation des sulfures initialement présents dans le matériau parental a induit une forte acidité (jusqu'à un pH de 3.4 mesuré en zone 1) ayant probablement ralenti la colonisation de la végétation locale, a laissé place à la formation de phases minéralogiques secondaires (« recombinaison » minéralogique). Ainsi, par une identification nette d'horizons de sol et par leur caractérisation physico-chimique, une pédogenèse (de type Anthroposol Artificiel) a été clairement mise en évidence (Chapitre 3). Le stade d'évolution des 2 profils de sol étudiés est principalement lié à la nature du matériau parental (ici des déchets accumulés sous forme de strates « sédimentaires ») ainsi qu'à la nature du couvert végétal et aux conditions climatiques. Par ailleurs, les EPTs As, Sb et Pb, impliqués dans des phases minéralogiques cristallisées (beudantite, scorodite), mal cristallisées ou amorphes (de types oxy-hydroxydes de fer riche en As ou arséniates riche en fer – symplesite) et la présence de phénomènes de pédogenèse, confèrent un comportement géochimique particulier à ces éléments (Chapitre 3). Comme l'indiquent les résultats de l'extraction séquentielle BCR, les phases porteuses des EPTs sont relativement stables puisqu'elles sont constitutives de l'étape 4 (phase résiduelle) et encore détectables en Diffraction de Rayons X. Toutefois, les réactifs extractant les 3 premières fractions de l'extraction séquentielle entrainent la libération des EPTs, suggérant une possible altération de leurs phases porteuses.

Les extractions sélectives chimiques visant à estimer la fraction théoriquement biodisponible des EPTs, c'est-à-dire accessible aux espèces végétales du site, (Chapitre 5) ont indiqué qu'elle représentait moins de 5% (pour la plus importante) voire moins de 1% de la fraction totale des éléments As et Pb. Cependant, compte tenu des valeurs des concentrations totales en As et Pb dans les horizons de sols, les valeurs de leur fraction biodisponible atteignent des teneurs très élevées représentant un réel risque de contamination des espèces végétales et de la chaîne alimentaire. Par conséquent, il était difficile de prédire la capacité d'adaptation de *M.* x *giganteus* sur le site d'étude. Certaines espèces végétales, dites métallophytes, sont néammoins connues pour se développer sur des sols riches en éléments métalliques. En Europe centrale : *Minuartina verna*, *Armeria maritima* ssp. *halleri*, *Arabidopsis halleri*, *Thlaspi calaminare* et *Viola lutea* ssp. *calamnaria* (endémique de l'Allemagne de l'Ouest et de la Belgique) sont des exemples typiques. En Slovénie, *Silene vulgaris* var. *humilis*, *Viola tricolor* ou *Thlaspi praecox* sont des espèces particulièrement abondantes sur sols pollués en éléments métalliques (Hildebrandt *et al.*, 2007). Ces plantes possèdent généralement des champignons mycorhiziens arbusculaires qui les protègent en atténuant les effets des contaminations (Redon *et al.*, 2009, Zarei *et al.*, 2010). Cependant, *M.* x *giganteus,* appartenant à une famille distincte de ces espèces, méritait d'être étudié.

I.2 <u>Miscanthus</u> <u>x</u> *<u>giganteus</u>, véritable solution de revalorisation ?*

Le site étudié était intéressant pour envisager une revalorisation par phytoremédiation compte tenu d'un niveau de contamination en As, Sb et Pb particulièrement élevé. Dans cette thèse, le choix s'est donc porté sur l'adaptabilité de *Miscanthus* x *giganteus* (Chapitre 6), plante bioénergétique c'est-à-dire productrice de biomasse en vue d'une revalorisation industrielle (sous forme de biocombustible, papier, biomatériaux, litière pour animaux, bioéthanol, bioplastique). Cette plante peut aussi participer à la production d'électricité (par co-génération) (Heaton *et al.*, 2010).

A l'issue d'une culture pendant 3 mois en conditions contrôlées sur 2 sols composites prélevés à la Petite Faye, nous avons donc démontré que cette plante a la véritable capacité de se développer sur ce type de terrain particulièrement contaminé, présentant des pH acides et une faible teneur en nutriments.

Elle semble tolérante au vue de ses paramètres photosynthétiques indiquant une bonne adaptation du métabolisme de la plante. La réduction de biomasse serait reliée à la faible valeur agronomique des sols privant la plante des nutriments nécessaires à son développement même si cette espèce est peu exigeante en éléments nutritionnels.

Bien que les paramètres photosynthétiques soient globalement peu altérés par rapport à des Miscanthus cultivés sur terreau, la production de biomasse et les concentrations en As, Sb et

Pb transférées aux parties aériennes empêcheraient toute valorisation industrielle, au vu des résultats obtenus. Par conséquent, pour le moment, la culture de cette plante sur le site de La Petite Faye ne serait envisageable qu'au titre d'une phytostabilisation à l'image des plantes pionnières, actuellement naturellement développées sur le site et qui présentent une tolérance à la contamination (Chapitre 4). Néanmoins, d'après les concentrations en As, Sb et Pb mesurées (Chapitres 4 et 5), à l'issue des 3 mois de cultures en phytotron, les parties aériennes de *M.* x *giganteus* accumulent beaucoup moins ces éléments que les plantes colonisatrices du site échantillonnées en fin de saison estivale. Alors que bouleaux et prêles géantes accumulent de manière égale Sb par rapport à *M.* x *giganteus*, fougères et prêles accumulent respectivement moins Sb et Pb. Cependant, ces mesures ont été effectuées sur différentes espèces et à des stades de développement différents. Il convient donc de comparer ces valeurs avec prudence. Toutefois, le taux de contamination des anciens sites miniers tel que celui de La Petite Faye est relativement rare. Par conséquent, l'utilisation de cette plante sur des sites contaminés acides mais moins riches en EPTs n'en reste pas moins intéressante afin de pouvoir mieux encadrer la conversion de ce genre de sols à la culture de plantes bioénergétiques.

Du point de vue agronomique, les rhizomes doivent être plantés sur un sol préalablement travaillé et dépourvu de plantes adventices. Il est possible d'utiliser une planteuse spécifique ou manuelle comme celle utilisée pour la pomme de terre (Novabiom). De ce fait, cette première étape, particulièrement cruciale étant donné la pérennité de la plante repoussant spontanément chaque année pendant environ 15 ans pourrait, dans notre cas, conduire, par exemple, à une dispersion par transport éolien de particules de sol, alors dépourvus de végétaux. Par conséquent, à l'amélioration agronomique à faible coût de ce type de terrains (rehausse du pH par chaulage, amélioration de la fertilité des sols par apports - mais qui resteront faibles pour *M.* x *giganteus,…*), s'ajoute une réflexion nécessaire et fondamentale sur les contraintes pratiques liées à l'itinéraire technique des cultures de plantes bioénergétiques du type *M.* x *giganteus* dans un contexte contaminé.

En outre, au-delà des 3 premières années de culture, le système racinaire de *M.* x *giganteus* se développe considérablement, avec des racines pouvant se déployer jusqu'à 2.5 m de profondeur et représentant 1 à 2 t de matière sèche par hectare (Neukirchen *et al.,* 1999, Amougou *et al.,* 2011). D'après l'étude de Néel *et al.,* (2003) menée sur le même site, les concentrations en EPTs à 2 ou 3 m de profondeur, bien que plus faibles en surface ne seraient donc, a priori, pas un frein au développement des racines sur ce site. Cependant, en profondeur, le déchet minier est stratifié en des niveaux particulièrement fortement indurés (surtout en zone 1) et de ce fait très faiblement perméable, limitant mécaniquement le développement racinaire, la ressource en eau et en oxygène. Par conséquent, le développement des racines en profondeur serait donc susceptible d'être difficile.

L'important taux de ligno-cellulose et les forts rendements sont 2 des atouts de *M.* x *giganteus* lui conférant des qualités énergétiques économiquement intéressantes. Sur sols agricoles, la culture de cette plante requiert également très peu d'intrants grâce au retour au sol des éléments minéraux essentiels par la chute des feuilles en automne (Khale *et al.,* 2001). Ainsi, ce mulch, après décomposition, représente un retour de carbone atteignant 1.5 t $C.ha^{-1}.an^{-1}$, ce qui conduirait à la formation de matière organique stabilisant les sols. Dans les parties souterraines, le rhizome est moins riche en lignine mais plus riches en sucres et composés azotés que les racines. En tant que résidu cultural, ils possèdent donc un taux de décomposition plus rapide (Amougou *et al.,* 2011).
Le métabolisme en C4, c'est-à-dire impliquant une faible perte de composés carbonés nécessaires à la photorespiration, assure un meilleur rendement photosynthétique et donc une forte production de biomasse. Ainsi, environ 27% du carbone redistribué aux parties souterraines constitue la rhizodéposition, soit 11 % du carbone fixé, équivalant grossièrement à 400-600 kg $C.ha^{-1}$ pour les céréales et les herbacées (Jones *et al.,* 2009). Par conséquent, la culture d'une plante en C4 comme *M.* x *giganteus* engendrerait un important relarguage de carbone organique susceptible de se minéraliser et d'entrer en interaction avec les polluants du site d'étude dont la labilité serait affectée. Dans un contexte comme celui de la Petite Faye, l'activité biologique apparait réduite puisque la litière est accumulée sur les 5 premiers cm (profile 2, chapitre 2). De plus, la nature siliceuse de cette litière issue des prêles géantes induit probablement une dégradation d'autant plus faible (Fraysse *et al.*, 2010). De ce fait, la présence de *M.* x *giganteus* sur ce terrain pourrait également, à plus long terme, modifier le comportement géochimique des EPTs et leur interaction à la matière organique, pouvant aussi bien réduire qu'augmenter leur labilité et donc leur capacité à être transférés à la plante.

I.3 Transformation de l'azote sous l'action des micro-organismes

L'étude des communautés bactériennes et archéennes autotrophes permettant l'oxydation de l'ammonium a démontré que le taux de contamination induit une réduction du potentiel génétique de la nitrification (évaluée par l'abondance du gène *amoA* présent chez ces 2 communautés, Chapitre 3) par rapport à des sols agricoles, mais sans l'empêcher complètement. Par ailleurs, *M.* x *giganteus* a une influence sur les communautés microbiennes au sein même de sa rhizosphère. Globalement, la réduction de l'abondance des communautés fonctionnelles assurant la première étape de la nitrification résulte du taux de contamination élevé, de l'acidité des sols ainsi que d'une possible compétition entre organismes autotrophes et hétérotrophes pour l'azote du fait de l'augmentation du carbone organique dissous dans la rhizosphère.

M. x *giganteus* étant une plante rhizomateuse, ses besoins en nutriments et en particulier en azote du sol sont réduits, du fait d'une remobilisation des éléments des parties aériennes vers le rhizome en fin de saison, éléments utilisés lors de la reprise de la plante au printemps. L'enjeu majeur de la fertilisation est le risque de lessivage. Par conséquent il est indispensable d'ajuster les quantités apportées aux besoins d'une culture. A la Petite Faye, les teneurs en azote extractible au $CaCl_2$ mesurées ne représentent pas plus de 7, 9 et 12 kg $N.ha^{-1}$ respectivement pour les sols S, M et L (au taux de contamination décroissant). Cadoux *et al.,* (2011) suggèrent que 60kg $N.ha^{-1}$ serait un bon compromis entre besoin de la plante, maintien des réserves et risque limité de lessivage. Pour les sols agricoles, 30 kg $N.ha^{-1}$ sont également recommandés. Par conséquent, les sols du site d'étude nécessiteraient des apports adaptés et raisonnés pour envisager la culture de *M.* x *giganteus*.

En dépit d'une capacité de *M.* x *giganteus* à se développer sur les sols contaminés en conditions contrôlées et au vu des résultats obtenus, il n'est pas possible de certifier une entière adaptation sur un site d'une telle contamination et pendant plusieurs années. En effet, les résultats de la culture ne nous indiquent pas si la plante peut concrètement évoluer dans de telles conditions sur au moins un cycle végétatif, ni si la repousse spontanée de la plante est vraiment possible, compte tenu de sa pérennité. Pourtant, nos résultats sont effectivement encourageants et laissent présager chez cette plante un réel potentiel de lutte contre l'érosion et une phytostabilisation des contaminants. Quant à une revalorisation industrielle en tant que telle, bien qu'inenvisageable à l'issue d'une culture comme celle que nous avons menée sans apports et amélioration(s) agronomique(s) des sols étudiés, nous avons démontré et confirmé le faible transfert des contaminants aux parties aériennes : les racines restent des organes particulièrement accumulateurs risquant aussi d'être fragilisées par la contamination. Ceci laisse supposer de véritables potentialités, du moins sur des sites de moins forte contamination.

I.4 Hypothétiques réponses des Anthroposols au changement climatique

Sans être encore vraiment capables de prédire de manière exacte l'évolution de la vitesse à laquelle les changements climatiques s'opèreront, les climatologues admettent que nous sommes dans une phase de changement (Solomon *et al.*, 2007). Ces changements, selon les endroits, s'accompagnent/eront de phénomènes plus contrastés (plus sec, plus froid, plus chaud, plus de précipitations, …) qui sont renforcés par l'augmentation de la concentration en gaz à effet de serre (principalement CO_2, N_2O, CH_4, vapeur d'eau… issus des activités anthropiques). Globalement, l'augmentation de la température moyenne induira, par exemple, une augmentation générale de l'activité biologique. En France, il y aurait plus de précipitations en hiver mais moins en été et une augmentation générale des températures annuelles. Par conséquent, les éléments comme l'arsenic ou le plomb, connus comme ayant de l'affinité pour la matière organique, seront potentiellement plus complexés à cette fraction, déjà particulièrement importante sur le site d'étude (plus de 400 mg.kg de Corg dans l'horizon de surface, profile 2).

II. Perspectives

Ce travail de thèse, par une approche pluri-disciplinaire, constitue l'une des premières études ayant permis d'obtenir des résultats afin de comprendre la capacité d'adaptation de la plante pérenne et bioenergétique *Miscanthus* x *giganteus* sur des Anthroposols Artificiels fortement contaminés en As, Sb et Pb. Comme toute étude, celle-ci a également soulevé un certain nombre d'interrogations auxquelles il sera nécessaire de répondre avant de pouvoir envisager la culture de cette plante dans les conditions réelles du terrain, en vue d'une commercialisation de cette biomasse et de l'adaptation de la plante sur d'autres sites de ce type.

Du point de vue de la culture de *Miscanthus* x *giganteus*, dans la mesure où nos résultats n'ont été obtenus qu'en conditions contrôlées, il faudrait envisager la mise en place de cultures sur site afin d'évaluer la véritable adaptation de la plante sur des durées supérieures à

3 ans, et ainsi, bien comprendre le comportement de cette plante. Afin de pallier le problème du rendement de biomasse, il serait nécessaire de faire des améliorations agronomiques des sols, par exemple en chaulant dans le but de tamponner l'acidité du milieu, mais également en apportant de matières organiques en vue de rehausser la fertilité des sols. L'apport d'éléments minéraux permettrait d'entretenir la capacité de transformation de l'ammonium en nitrate des sols, assurant l'accès à l'un des nutriments majeurs aux végétaux. La dénitrification devrait également être étudiée, en particulier dans le but d'adapter la fertilisation azotée et réduire ainsi les risques d'émission de N_2O (gaz hautement à effet de serre) produit lorsque la dénitrification n'est pas complète. Cependant, ce type d'apports est susceptible de modifier la spéciation des polluants, par conséquent leur mobilité et donc leur disponibilité vis-à-vis de la plante (Hartley *et al.* 2009; Padmavathiamma et Li 2010). Cette modification pourrait d'ailleurs être en faveur d'une immobilisation des polluants du fait de pH moins faibles par exemple.

Bien sûr, il serait donc pertinent de faire des essais de culture sur des terrains présentant des taux de contamination plus faibles, mais de même origine, pour déterminer les teneurs seuil dans les sols permettant une production de biomasse moins contaminée, afin d'envisager une revalorisation industrielle.

Alors que nous avons seulement évalué la réponse des échanges gazeux impliqués dans la photosynthèse comme indicateur de stress en réponse à la contamination du site, il serait important de compléter ces résultats par l'étude du métabolisme anti-oxydant ou des mécanismes de séquestration impliqués dans la résistance aux éléments métalliques et métalloïdes de *M.* x *giganteus*.

Outre une potentielle modification de la mobilité des polluants suite à des apports d'intrants, il pourrait également y avoir une modification de la communauté microbienne, en particulier des bactéries et archées impliquées dans la nitrification. Il serait donc très intéressant d'étudier la diversité bactérienne lors d'une culture de *M.* x *giganteus* sans apports et avec différentes combinaison d'amendements, et d'évaluer les éventuels changements au niveau de ces communautés, ainsi que les impacts sur la production de biomasse. Dans un tout premier temps, en lien direct avec l'étude du Chapitre 3, les résultats de tRFLP (terminal Restriction Fragment Lenght Polymorphism) du gène *amoA* des archées (AOA) permettraient d'estimer leur diversité dans les sols de la Petite Faye et une potentielle évolution de la structure de la communauté des archées pendant la culture de *M.* x *giganteus* sur 3 mois, en fonction des propriétés physico-chimiques des sols.

Par ailleurs, il pourrait être intéressant de mettre en évidence le rôle des exsudats racinaires ou encore des micro-organismes dans le relarguage des EPTs et par conséquent l'altération potentielle des phases minérales porteuses. Ceci contribuerait ou pourrait participer à l'augmentation de la mobilité des EPTs susceptibles de devenir disponibles pour les espèces végétales.

Du point de vue minéralogique et de la réactivité des phases porteuses des éléments polluants, une caractérisation des phases les plus fines et notamment des colloïdes (actuellement en cours dans le cadre d'une thèse débutée en 2009), phases les plus mobiles du sol, permettra de mieux appréhender les risques potentiels de transfert à la végétation ou aux eaux souterraines. Cela pourrait aussi être évalué par la technique DGT (Diffusive Gradients in Thin films) qui permettrait d'estimer la capacité du sol à réalimenter la solution du sol en EPTs. L'interaction de ces phases avec les matières organiques pourrait également être caractérisée dans la mesure où le taux de dégradation des matières organiques semble être lent, en particulier dans la zone 2, principalement couverte de prêles géantes aux structures siliceuses et difficilement dégradables.

L'étude des fractions fines (entre autres les oxydes, oxyhydroxides et phyllosilicates), fractions susceptibles de jouer un rôle de piégeur des EPTs, permettrait de compléter la compréhension du comportement de As, Sb et Pb du site étudié mais aussi de la pédogenèse.

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Résumé

Dans le contexte actuel, il devient nécessaire de trouver des énergies alternatives et de préserver les sols agricoles pour la production de ressources alimentaires. Ainsi, ce travail avait pour objectif majeur d'étudier la potentielle adaptation et utilisation d'une plante bioénergétique, le *Miscanthus* x *giganteus*, sur sites et sols fortement contaminés en métaux et métalloïdes, donc impropres à toute activité alimentaire. Le site d'étude choisi est un ancien site minier situé dans le Limousin sur lequel le traitement de l'or dans les années 60 a induit des dépôts fortement contaminés, et où un couvert végétal est actuellement en développement.

Les résultats de culture mettent clairement en évidence les capacités d'adaptation de cette plante, avec un faible transfert d'éléments potentiellement toxiques, même sur des sols acides fortement contaminés (As jusqu'à 83000 mg.kg^{-1}, Pb - 15000 mg.kg^{-1} et Sb - 1100 mg.kg^{-1}) et ayant de faibles propriétés agronomiques. De plus, la biodisponibilité des métaux ne représente que 1 à 5% des teneurs totales en fonction de l'extractant utilisé, ce qui représente de très importantes valeurs, étant donné les teneurs totales. Ceci est directement lié à la minéralogie du site.

La réponse du *M.* x *giganteus* au stress métallique met en évidence une forte réduction du rendement de biomasse, une faible perturbation de la photosynthèse nette, une réduction de la conductance stomatique et de la concentration intracellulaire en CO_2. De plus, la plante modifie la réponse potentielle des bactéries et archées rhizosphériques oxydatrices de l'ammonium en nitrite dans la réaction de nitrification. Comme attendu, plus la contamination est importante, et plus la réponse de *M.* x *giganteus* est marquée.

En dépit d'un transfert d'As, Sb et Pb très réduit vers les parties aériennes de la plante, les concentrations mesurées ne permettraient pas une revalorisation industrielle rapide du site mais la culture de la plante serait plutôt stabilisatrice du sol. Des amendements de type agronomiques seraient susceptibles d'améliorer les rendements en vue d'une revalorisation industrielle.

Abstract

The use of alternative energies as fuel and the protection of arable soils as resource for food crop are of major concern in the current context of deficiency of fertile soils. Thus, the main aim of this thesis was to study the potential adaptation of the bioenergetic plant *Miscanthus* x *giganteus* on soils highly contaminated by metals and metalloids, and unsuitable for food crops. The experimental site is a former mine located in Limousin (France) where gold treatment induced heavily contaminated tailings, in the sixties. The vegetation cover is nowadays developing.

The culture clearly highlighted adaptation capacities of the plant with a low transfer of potentially toxic elements even though soils were acidic, exceptionally contaminated (till 83000 mg As.kg^{-1}, 15000 mg Pb.kg^{-1} and 1100 mg Sb.kg^{-1}) and with low agronomic performances. Moreover, bioavailability represented only 1 to 5% of total concentrations according to the used reactant which corresponded to very high amounts, given values of total concentrations. These findings are directly link to the mineralogy of the site.

M. x *giganteus* responded to metallic stress with a strong reduction of biomass yield, a slight disturbance of net photosynthesis rate and a reduction of stomatal conductance and substomatal CO_2 concentration. Furthermore, the plant modified the potential activity of rhizospheric ammonia oxidizing bacteria and archaea involved in nitrification. As expected, the more the contamination was high the more *M.* x *giganteus* response was marked.

Despite a clear reduced As, Sb and Pb transfer to aerial parts, values of measured concentrations could not allow an industrial revalorization. However, this plant could stabilize the soil. Agronomic improvements of the soil might enhance yields for an industrial revalorization.