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Note to readers

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Résumé

La réhabilitation des friches industrielles dans les zones urbaines est un enjeu majeur pour le développement durable des villes. La gestion et la reconversion de ces sites, imposées par la réglementation, nécessitent toutefois le développement d'outils d'évaluation des risques environnementaux et sanitaires, et de techniques de remédiation durables.

Cette thèse a porté sur la mise en place d'outils multidisciplinaires pour la gestion durable des sites pollués, avec le cas particulier d’un site de recyclage de batteries au plomb caractérisé par une pollution historique en plomb principalement mais également d’autres polluants inorganiques (Cd, Sb, As, Cu et Zn), couramment définis par le terme d’Eléments Traces Métalliques (ETM). Ce travail a cherché à renseigner les mécanismes impliqués dans les systèmes polluants-sol-plantes pour renforcer la prise en compte de la qualité globale des sols dans la gestion des sites industriels, tout en essayant de répondre à des questions de la recherche appliquée.

En plus des outils et procédures classiquement utilisés pour évaluer, contrôler et réduire les risques environnementaux et sanitaires causés par les sols pollués, le développement des mesures de biodisponibilité (plantes et les humains) et d'écotoxicité (différents tests biologiques: inhibition de la mobilité de daphnies, Microtox® et induction de bactéries bioluminescentes, microbiologie) permet d'affiner la classification des sols contaminés en termes de dangerosité. De plus, des plantes engrais verts (bourrache, phacélie et moutarde), communément utilisées en agriculture ou par les jardiniers (car elles améliorent les propriétés bio-physico-chimiques des sols avec un système racinaire et une grande production d'exsudats racinaires), ont été testées pour la re-fonctionnalisation des sols pollués. Enfin, les mécanismes impliqués dans le devenir des polluants dans la rhizosphère et les micro-organismes associés ont été étudiés.

L’ensemble des résultats fourni des éléments de réponse et des moyens d'améliorer la gestion des sols contaminés par des métaux et métalloïdes. (1) Tout d'abord, la séparation par taille des différentes fractions de sol permet une réduction significative des tonnages de matériaux contaminés et donc une économie réelle lors de la mise en décharge des sols excavés avec un gain certain en termes d'empreinte écologique. (2) Ensuite, le calcul d'écoscores pour les differents échantillons de sols pollués, sur la base des résultats des essais d'écotoxicité, ont permis d’affiner plus précisemment les risques par rapport aux paramètres physico-chimiques « classiques » requis par la réglementation. Des différences de sensibilité ont été observées en fonction de la nature de l'essai biologique, l'origine de l'échantillon, les propriétés physico-chimiques et les concentrations totales de polluants. (3) Contrairement à la phacélie, la bourrache et la moutarde ont amélioré la respiration du sol, réduit l'écotoxicité et la quantité de plomb bioaccessible et totale dans le sol, respectivement par phytostabilisation et stockage dans les racines (Pb, Sb) ou par phytoextraction et donc stockage dans les parties aériennes. En outre, ces plantes pourraient être testées sur le terrain pour une utilisation en phytoremédiation des friches industrielles et des jardins modérément pollués. Selon la nature du métal, du type de sol et des plantes, la compartimentation et la spéciation du polluant diffèrent, ainsi qu’en fonction des caractéristiques agronomiques du sol et l'activité microbienne de la rhizosphère. Un criblage moléculaire et une méta-analyse de la génomique microbienne ont permis de mettre en évidence les différences dans les communautés bactériennes étudiées en fonction du niveau de concentration métallique, des espèces végétales et des caractéristiques des sols étudiés.

Mots clés: Management durable des sites industriels – Biogéochimie – (Eco)Toxicité – Environnement-Santé-Société.

Abstract

Rehabilitation of brownfields in urban areas is a major challenge for the sustainable development of cities. Management and conversion of these sites, imposed by regulation, however, require the development of tools for environmental risk assessment and health and sustainable remediation techniques. This thesis focused on the establishment of multidisciplinary tools for the sustainable management of polluted site, with the particular case of rehabilitation recycling of lead batteries with a mainly historical lead pollution and other inorganic pollutants (Cd, Sb, As, Cu and Zn), currently defined as Metal Trace Elements (MTE). While trying to answer questions of applied research, this work has sought to investigate the mechanisms involved in the soil-plant pollutants to strengthen the consideration of the overall quality of soil management for industrial sites. In addition to the tools and procedures conventionally used to assess, control and reduce environmental and health risks caused by polluted soils; measures of bioavailability (plants and humans) and ecotoxicity (different bioassays: inhibition of the mobility of *Daphnia magna*, Microtox® and induction of bioluminescent bacteria and microbiology) have been developed with the aim to refine the classification of contaminated soils in terms of dangerousness. Moreover, green manure plants (borage, phacelia and mustard), commonly used in agriculture or by gardeners because they improve the bio-physico-chemical properties of soils with a root system and a large production of root exudates were tested for re-functionalization of polluted soils. Finally, the mechanisms involved in the fate of pollutants in the rhizosphere and their microorganisms in the plant were studied.

The main results provide some answers and ways of improving the management of soils contaminated by metals and metalloids. (1) First, the size separation for soil fractions allows a significant reduction in tonnages of contaminated material and therefore costs for the landfill excavated soil with a gain result in terms of ecological footprint. (2) Then, calculation for the differents polluted soil samples of eco-scores based on the results of ecotoxicity tests can discriminate more accurately compared to physicochemical parameters required by the regulations. Differences in sensitivity were observed depending on the nature of the bioassay, the origin of the sample, physico-chemical properties and total concentrations of pollutants. (3) Unlike phacelia, borage and mustard improve soil respiration, ecotoxicity and reduce the amount of bioaccessible and total lead in soil, respectively by phytostabilisation and storage in roots (Pb, Sb) or phytoextraction and storage in aerial parts. Further, these plants could be field tested for use in phytoremediation of brownfields and gardens moderately polluted. Depending on the nature of the metal, the type of soil and plant, compartmentalization and speciation of the pollutant differ, and in conjunction with agronomic characteristics of soil and rhizosphere microbial activity. Molecular screening and meta-analysis of microbial genomics have enabled highlight differences in bacterial communities studied by species and growing conditions.

Keywords: Sustainable management of industrial sites – Biogeochemistry– (Eco)Toxicity – Health-Environment-Society

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Fundamental Principles in Sciences

« Si c'est vert ou si ça remue, c'est de la biologie.
Si ça pue, c'est de la chimie.
Si ça ne fonctionne pas, c'est de la physique.
Si ça occupe des tableaux entiers de formules, c'est des mathématiques. »

Révision de la Classification des Sciences selon Murphy

« Le monde réel est un cas particulier. »

Théorème d'admissibilité

« Tout peut être admis avec suffisamment de bonne volonté. »

Généralisation de la Loi de Horngren

« La logique est une méthode systématique d'arriver en confiance à la mauvaise conclusion. »

Maxime de Manly

« En théorie, la pratique égale la théorie.
En pratique, la théorie diffère de la pratique. »

Théorie pratique de Moine

« La théorie c'est quand ça ne marche pas mais que l'on sait pourquoi.
La pratique, c'est quand ça marche mais qu'on ne sait pas pourquoi.
Quand la théorie rejoint la pratique, ça ne marche pas et on ne sait pas pourquoi. »

Lemme Éternel du CNRS

« Les expériences doivent être reproductibles. Elles doivent toute échouer de la même manière. »

Règle de Finagle

« Dans des conditions parfaitement contrôlées de pression, température, humidité, volume, taux d'oxygène, débit et autres variables, un organisme fera absolument et uniquement tout ce qu'il lui plaira. »

Principe d'Incertitude biologique de Harvard

« Il suffit de qualifier une boîte de témoin pour que toutes sortes de phénomènes étranges et indésirables s'y produisent.
Corollaire : Une bonne boîte témoin est une boîte vide. »

Contre-Pétri de Bernadat

Glossary

APSM: Affinerie de Pont Sainte Maxence
BARGE: BioAccessibility Research Group of Europe
BASIAS: Base données des Anciens Sites Industriels et Activités de Services
BASOL: Base de données sur les sites et Sols pollués ou potentiellement pollués
BCF: Bio-Concentration Factor
BRGM: Bureau de Recherches Géologiques et Minières
EQRS: Evaluation Quantitative des Risques Sanitaires
ICP-MS: Induced Coupled Plasma-Mass Spectrometry
ICP-OES: Induced Coupled Plasma-Optical Emission Spectrometry
ICPE: Installation Classée pour la Protection de l’Environnement
IED: Indutrial Emission Directive
IEM: Interprétation de l’Etat des Milieux
IPPC: Integrated Prevention and Pollution Control
ISDD: Installation de Stockage de Déchets Dangeureux
ISDI: Installation de Stockage de Déchets Inertes
ISDND: Installation de Stockage de Déchets Non Dangeureux
NGB: National Geochemical Background
µFX: X-Ray micro-fluorescence
MTE: Metal Trace Element (Elément Trace Métallique – MTE)
PCR: Polymerase Chain Reaction
PP: Poly-Propylène
PPRT: Plan de Prévention des Risques Technologiques
REACH: Registration, Evaluation and Authorisation of CHemicals
STCM: Société de Traitements Chimiques des Métaux
SVHC: Substance of Very High Concern
TF: Translocation Factor
TOC: Total Organic Carbon
T-RFLP: Terminal Restriction Fragment Length Polymorphism
UBM: Unified Barge Method
UPDS: Union des Professionnels de la Dépollution des Sols
VTR: Valeur Toxicologique de Référence

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Chapter 1

Scientific context and objectives

► In this chapter, a brief description of the scientific context in which these research works were performed, is firstly presented. Then, a bibliographic synthesis presents the state of the art of metal(loid)s behaviour in soils and a summary of the techniques usually used for their remediation. This part includes a scientific article published in 2012 in "Environnement, Risques & Santé".

Finally, the main scientific objectives are detailed.

1.1. Introduction

Because of their potential toxicity and their high persistence, metals (Pb, Cd, Zn, etc.) and metalloids (As, Sb, etc.), when their concentrations increase in the environment and in function of their chemical speciation and compartmentation induce deleterious effects on ecosystems *(Peralta-Videa et al., 2009; Uzu et al., 2010)*. Many lead compounds in particular are regarded as substances of very high concern (SVHC) in the REACH Regulation (EC No. 1907/2006), which require industries to follow their evolution throughout their life cycle (from manufacturing to disposal).

Recyclers of lead-acid batteries are currently the main source of particulate air emissions rich in lead and other metal(loids) (i.e. metal trace elements – MTE – as As, Sb, Cu, Zn, Sb, etc.) *(Dumat et al., 2006)*. In France, they are classified as installations for environmental protection (ICPE) and managers of these companies are responsible for implementing management strategies to minimize impacts on the environment and human health. Recovery of these sites is prepared well in advance especially because sorting contaminated land for their management (*in situ* remediation, excavation and landfill, etc.) can be long and expensive. Management of excavated soils can be complex and a major a financial worry *(Actu-Environnement, 2010)*. Indeed, a site can be identified as compatible with the intended uses thanks to the health risk assessment. But soils which are excavated may have traces of pollution and " transformed" them in waste as soon as they leave the site. In France, the Ministerial Decree of 15 March 2006 transcribed into French law the European directive on landfilling, sets the criteria establishing the inert / non-inert character of excavated soil and therefore the possible pathways for reuse, recovery or disposal. According to the professionals of this sector, the thresholds in this decree are too restrictive and generaly lead soils in landfills. In practice, soil parameters (Total Organic Carbon – TOC), soluble sulphate, total pollutant concentrations, etc.) very often exceed the acceptance criteria *(HPC Envirotec, Intersol 2010)* even in “clean” soils and correspond to geochemical background noise of “natural” soils. These criteria also determine the type of storage facilities: inert (class III), non-hazardous (class II) and hazardous waste (class I). This context provides a significant additional cost to developers of sites knowing that the storage costs as inert waste are twice lower than for non-hazardous waste. Asked about these issues together professionals in the Union of Professional Business Remediation Sites (UPDS, *Union des Professionnels de la Dépollution des Sols*), the French Ministry of Sustainable Development has begun, in 2008, to deal with the problem. The European Framework Directive No. 2008/98/CE on waste of 19 November 2008, transposed into French law in December 2010, aims to protect the environment and human health by preventing the harmful effects of the production and management of waste, which include contaminated soils when they are excavated, and even more when they leave the site. The ordinance defines waste product and opens the possibility that some substances, such as soils, can not be considered as waste after undergoing appropriate treatment. It outlines the main stages of waste management in the ranking order of priority: prevention, reuse, recycling, including energy recovery and finally disposal. Professionals are hoping that this will be enough to upset the current practices in France which favours the use of landfill. In the meantime, it is advisable to limit the excavations and focus as much as possible on reuse of soils on the site to avoid their classification within the waste regulatory. In the case that the excavation of soils and exit are inevitable, management of excavated soils and associated costs must be considered.

With 77% of urban population, France must control its urban sprawl, a recurring problem for both developed countries (84% in the USA, 90% in Germany, etc.) and for countries in development (from 11% to 50% between 1950 and 2000 in China, from 26% to over 75% between 1940 and 2000 for Brazil, etc.). Management and redevelopment of brownfields to optimize the density of cities is a real challenge for Politics. Management of these sites to minimize impacts and master their environment and health is imposed by regulation. At the heart of the tools used in France for the management of contaminated sites and soils, the conceptual scheme with the description of the sources of pollution, potential targets and pathways. Several criteria can indeed influence the hazard of a substance in soil: its quantity, its bioavailability and inherent toxicity or ability to meet the target (due to solubility, size, volatility, chemical speciation, etc.). So, the complete inventory of the state of the environment and possible transfers is a necessary first step to take. A comprehensive cost-benefit analysis incorporating trio's pillars of sustainable development and a strategy for communication with the competent authorities, local communities and citizens must also be implemented in order to carry out this type of project.

The overall objective of this thesis was to develop a global sustainable strategy to rehabilitate the polluted sites, in particular for a rational land management which aimed at a limited landfilling. The industrial site of the Société de Traitements Chimiques des Métaux (STCM) in Toulouse (ICPE, whose activity relates to the treatment of lead-acid batteries) was chosen as one example of experimental site.

En raison de leurs toxicité potentielle et persistance élevée, les métaux (Pb, Cd, Zn, etc) et métalloïdes (As, Sb, etc), induisent des effets délétères sur les écosystèmes lorsque leur concentration augmente dans l'environnement et selon leurs compartimentation et spéciation chimique, (Peralta-Videa et al, 2009; Uzu et al, 2010). De nombreux composés du plomb en particulier sont considérés comme substances extrêmement préoccupantes (SVHC) dans le règlement REACH (CE n ° 1907/2006), qui impose aux industries de suivre leur évolution tout au long du cycle de vie (de la fabrication à l'élimination).

Les recycleurs de batteries plomb-acide sont actuellement la principale source d'émissions atmosphériques de particules riches en plomb et autres métaux et métalloïdes (c'est-à-dire les éléments traces métalliques - ETM – tells que As, Sb, Cu, Zn, Sb, etc) (Dumat et al., 2006). En France, elles sont définies comme étant des installations classées pour la protection de l'environnement (ICPE) et les gestionnaires de ces entreprises sont responsables de la mise en œuvre de stratégies de gestion visant à minimiser les impacts sur l'environnement et la santé humaine.

La réhabilitation de ces sites (lorsque des pollutions historiques existent) doit être anticipée en particulier parce que le tri puis la gestion des terres contaminées (dépollution in situ, excavation et enfouissement, etc) peut être longue et coûteuse. La gestion des terres excavées peut être complexe et générer des problèmes financiers (Actu-Environnement, 2010). En effet, un site peut être considéré comme compatible avec les usages prévus suite à l'évaluation des risques sanitaires, mais les terres qui en sont évacuées sont considérées comme desdéchets plus ou moins dangereux. En France, l'arrêté ministériel du 15 mars 2006 qui transcrit en droit français la directive européenne sur la mise en décharge, définit les critères établissant le caractère inerte / non-inerte des terres excavées et donc les voies possibles de réutilisation, valorisation ou élimination. Selon les professionnels de ce secteur, les seuils dans le présent décret sont trop restrictifs et conduisent presque systématiquement les sols contaminés dans les décharges. Dans la pratique, certains paramètres du sol (carbone organique total (COT), sulfates solubles, concentrations totales de polluants, etc.) dépassent très souvent les critères d'acceptation de mse en décharge (HPC Envirotec, Intersol 2010), même pour les sols qui présentent des concentrations en ETM proches du bruit de fond géochimique des « sols naturels ». Ces critères déterminent également le type d'installations de stockage: déchets inertes (classe III), déchets non dangereux (classe II) et dangereux (classe I). Ce contexte donne un surcoût important pour les développeurs de sites sachant que les coûts de stockage pour les déchets inertes sont 2 fois plus faibles que pour les déchets non dangereux. Interrogés sur ces questions, les professionnels de l'Union des Professionnels de la Dépollution des Sols (UDPS) ou le Ministère français du Développement Durable a commencé, en 2008, à faire face à ce problème. Des possibilités de ré-utilisation des terres excavées très encadrées exitent désormais comme la réutilisation en sous-bassement de routes (Colombano et al., 2010). La directive européenne n° 2008/98/CE du 19 novembre 2008, transposée en droit français en décembre 2010, vise à protéger l'environnement et la santé humaine par la prévention des effets nocifs de la production et de la gestion des déchets, parmis lesquels figurent les terres contaminées dès lors qu'elles sont excavées, et plus encore lorsqu'elles sortent du site. L'ordonnance définit la notion de déchet et ouvre la possibilité que certaines substances, telles que les sols, puissent ne plus être considérées comme des déchets après avoir subi les traitements appropriés. Elle précise les grandes étapes de la gestion des déchets en les classant par ordre de priorité : la prévention, la réutilisation, le recyclage, la valorisation notamment énergétique et enfin l'élimination. Les professionnels espèrent que cela sera suffisant pour bouleverser les pratiques actuelles en

France qui favorisent l'utilisation des sites d'enfouissement. En attendant, il est conseillé de limiter les excavations et de se concentrer autant que possible sur la réutilisation des sols sur le site pour éviter leur transfert vers les centres de stockage. Dans le cas où l'excavation des sols et leur sortie sont inévitables, la gestion des sols excavés et les coûts associés doivent être considérés.

Avec 77% de population urbaine, la France doit maîtriser son expansion urbaine, une problématique récurrente aussi bien pour les pays développés (84% aux Etats-Unis, 90% en Allemagne, etc.), que pour les pays en voie de développement (de 11% à 50% entre 1950 et 2000 en Chine, de 26% à plus de 75% entre 1940 et 2000 pour le Brésil, etc.). La gestion et le réaménagement des friches industrielles pour optimiser la densité des villes est un réel défi politique. La règlementation impose également de minimiser et de maîtriser les impacts sur l'environnement et la santé. Au cœur des outils utilisés en France pour la gestion des sites et sols, le schéma conceptuel décrit les sources de pollution, les cibles potentielles et les voies de transferts des contaminants vers les cibles. Plusieurs critères peuvent en effet influer sur le danger d'une substance dans le sol: sa quantité, sa biodisponibilité et la toxicité intrinsèque ou la capacité à atteindre la cible (en fonction de la solubilité, la taille, la spéciation chimique, etc.). Ainsi, l'inventaire complet des possibles transferts vers l'environnement et les personnes (travailleurs et habitants des environs proches) est une première étape nécessaire à réaliser. Une analyse coûts-avantages complète intégrant les trois piliers du développement durable et une stratégie de communication avec les autorités compétentes, les collectivités locales et les citoyens, doivent également être mis en œuvre afin de mener à bien ce type de projet.

L'objectif général de cette thèse est de développer une stratégie durable pour la réhabilitation des sites pollués, en particulier pour une gestion rationnelle des terres qui vise à une mise en décharge limitée. *Le site industriel de la Société de Traitements Chimiques des Métaux (STCM) située à Toulouse (ICPE, dont l'activité est le recyclage des batteries plomb-acide) a été choisi comme site expérimental pour ce travail de thèse.*

1.2. Soil pollution by metal trace elements

1.2.1. Origins

1.2.1.1. Natural sources of MTE

All soils naturally contain MTE. Thus, their presence alone is not indicative of pollution. MTE concentration in soil uncontaminated by human activity is largely related to the geology of the parent material from which the soil was formed. According to the local geology, MTE concentrations in soil exceed the variation ranges widely accepted.

Soil natural geochemical background (NGB) is the concentration of a chemical in soil (either a major or MTE) resulting from natural evolution, geological and soil, without any contribution of human origin *(Baize, 1997)*. This level depends on original rock constituting the soil (parent material or bedrock) and biogeochemical processes, alteration, leaching, migration, redistribution, occurred during geological periods of soil formation, which could leach or concentrate the element. NGB can widely vary accordingly to geographic area. The soil geochemical bottom is defined by a mean value and variability of the dependent spatial scale (area, reference horizon etc.). State of knowledge on the national French soil geochemical background was prepared by Darmendrail et al. *(2000)*. They are based in particular on the work of Baize *(1997)* and describe the different databases developed in France. The NGB may be the cause of relatively high levels of MTE in the surface horizons which are natural anomalies (*Table I-1*)

Element	***Cd***	***Co***	***Cr***	***Cu***	***Ni***	***Pb***	***Zn***	***Mn***	***Fe***
Average concentrations in French soils (mg kg^{-1} DW)	0.4	17.1	75.0	14.9	41.3	64.8	149	1585	3.6

Table I-1: MTE national geochemical backgrounds (Baize, 1997)

1.2.1.2. Anthropogenic origins

The anthropogenic pollution of soils can be of different kinds (industries, agriculture, accidents, etc.). Diffuse contamination of soils is generally associated with atmospheric deposition (emissions from industrial, automotive, heating) (Uzu *et al.*, 2009). Contamination of soil distinguished by the presence of hazardous substances from industrial activity producing organic matter and fat (food industry), various chemicals (chemical industry), radioactive materials (nuclear power, radioactive waste) and metallurgy (Ali *et al.*, 2013). Mining waste and industrial waste dumps are a particularly important source of pollution by zinc, lead and cadmium (Khan *et al.*, 2007). Over the last decades, the contribution of metal(loid)s in soil in the world has grown; currently is estimated at 22,000 tons of cadmium, 939,000 t of copper, 783,000 t of lead, and 1,350,000 t zinc (Singh *et al.*, 2003). These substances, unconfined and at high concentrations, rise to "contaminated sites locally" (NF ISO 11074-1, ISO, 2005). Mining and industrial sites fit into this framework. Certain agricultural practices are responsible for the introduction of heavy metals in the soil. Products to improve the physico-chemical properties of the soil are often richer in metals than the soil itself e.g. fertilizers, composts and sewage sludge (Luo et al., 2011) (*Figure I-1*).

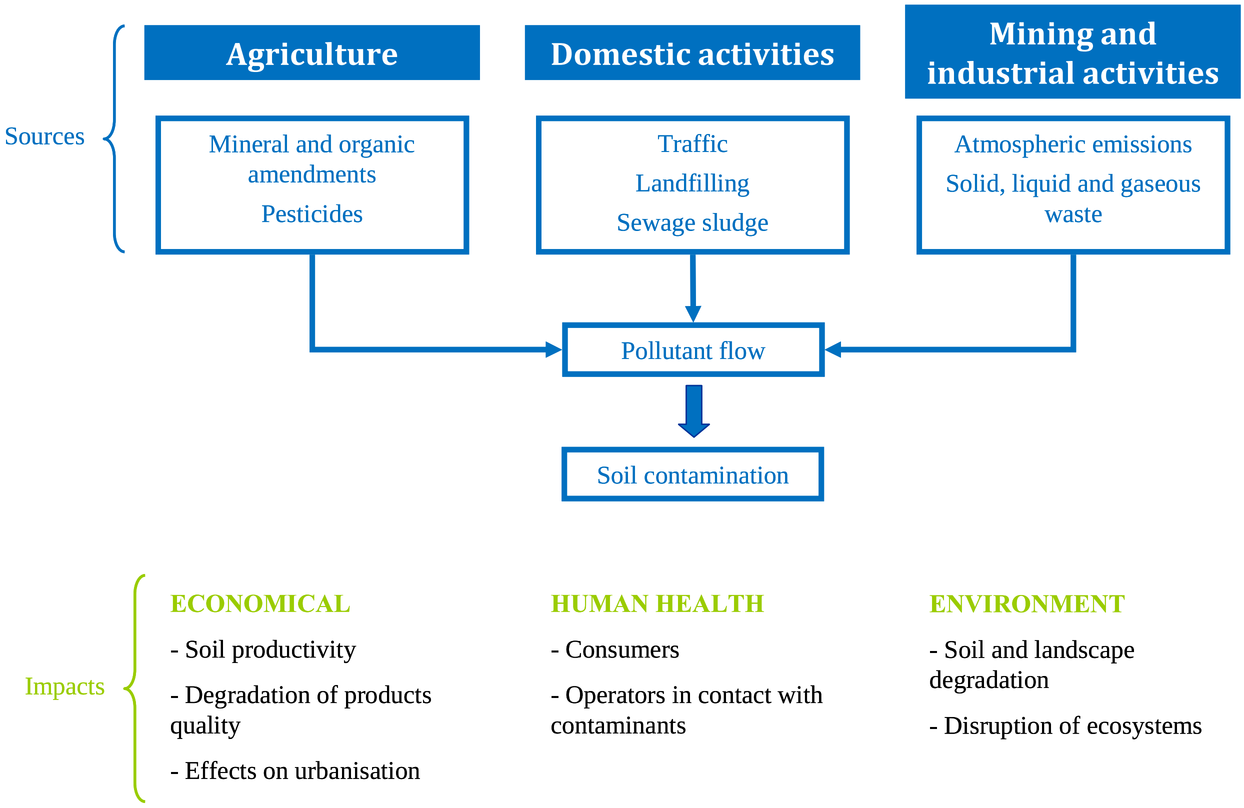

Figure I-1: Sources of MTE release in environment and impacts

The *Figure I-1* describes the different sources of soil pollution by agricultural, domestical and industrial activities, and their impacts on human health, environment and economy.

A soil is considered polluted when it contains one or more pollutant(s) or contaminant(s) that may cause biological, physical and chemical alterations of the soil ecosystem *(AFNOR, 1994)*. The soil is at the interface of water and air. The concept of soil pollution is therefore generally called the contamination of one or more components of the ecosystem (atmosphere, aquatic environments) or the accumulation and transfer of pollutants into the food chain through organisms in direct or indirect contact with the soil (plants, mesofauna, fungi, bacteria) *(Schreck et al., 2013)*. Beyond a certain threshold, depending on their chemical nature, MTE contents can become toxic to living organisms, accumulate in food chains and affect soil biodiversity.

1.2.1.3. Processes of soil degradation

Soil formation is the result of complex biogeochemical processes *(Ramade, 1993)*. Living environment, dynamic, responsive and constantly evolving *(Lavelle & Spain, 2001)*, its position at the interface of the lithosphere, atmosphere and biosphere, influenced the formation and functioning *(Robert, 1996)* and gives it a key role in a number of biogeochemical cycles (C, N, P, etc.), and in the regulation of gas exchange and water cycle *(Robert, 1996; Gobat et al., 2003)*. But, soil contamination can disturb these balances and processes thus leading to their degradation.

Degradation of soil quality can be defined as the reduction or loss of ability to perform one or more of its functions *(Citeau et al., 2008)*. Any damage to its structure also damages other environmental media and ecosystems. Various degradation processes or threats may be cited: erosion, decline in organic matter, local and diffuse contamination, acidification, sealing, compaction, soil biodiversity reduction, salinization, floods and landslides *(Jones et al., 2005)*. Soil erosion is mainly caused by water and, to a lesser extent, by the wind. This process is, with lower organic matter content, responsible for a decline in soil productivity and a more general framework leads to a reduction of soil functions. The protection of biodiversity is to preserve soil organisms that perform essential ecological functions including soil formation (improving the structure, degradation of complex organic matter such as plant debris), and in maintaining soil fertility. Compaction, sealing and landslides affect the physical dynamics of the soil. They have a significant impact on the permeability of the medium which implies strong perturbations of its ecosystems. Finally, pollution is a major problem, it involves risks for ecosystems, food chains, and thus for human and animal health *(Leveque et al., 2013)*. It was distinguished diffuse contamination very intense but chronic and widespread contamination point when the source of pollution is clearly localized *(Chassin et al., 1996)*.

1.2.1.4. French regulations for the management of contaminated sites

Although the soil is, in many ways, subject to regulations as property and land ownership, European legislation hardly provides a specific framework for soil protection. However, the European Commission proposed a framework directive on soil protection. This draft Directive of the European Parliament and of the Council dated 22nd September 2006. In the meantime, to define the legal framework for the protection of soil, it is also necessary to refer to different texts governing the protection of the environment and that by multiple approaches.

The problem of "soil pollution" appears in texts related to water, such as those concerning the use of sewage sludge in agriculture (Council Directive 86/278/EEC of 12 June 1986), the quality of surface waters for the production of drinking water (Council Directive 75/440/EEC of 16 June 1975) or the protection of groundwater against pollution caused by certain dangerous substances (Council Directive 80/68/EEC of 17 December 1979).

Soil protection is also discussed in the legislation relating to waste management such as those concerning the disposal of waste oils (Council Directive 75/439/EEC of 16 June 1975), the landfill of waste (Directive 1999/31/EC of 26 April 1999), or air pollution legislation to reduce volatile organic compounds (Council Directive 1999/13/EC of 11 March 1999), on the restriction emissions of certain pollutants into the air from large combustion plants (Directive 2001/80/EC of the European Parliament and of the Council of 23 October 2001) or the law on the prevention and reduction integrated pollution (Council Directive 96/61/EC of 24 September 1996 concerning integrated prevention and reduction of pollution, IPPC), which is now abrogated by the Industrial Emission Directive (IED).

IED is an evolution of the Directive on IPPC. It retains the guidelines while the strengthening and more closely supervising the implementation in order to avoid distortions of application between Member States. It completes the legislative framework applicable to industrial and agricultural activities can be the source of pollution (energy activities, production and processing of metals, mineral industry, chemical industry, waste management, intensive farming, etc.) of some 6,500 installations classified for the protection of the environment present in France. The specificitiess of the new Directive in relation to the existing legislation affecting both the use of best available techniques, the periodic review of permits, restoration of the site at the end of activity, and public participation.

Finally, upon cessation of activity, IED required in addition to the principle of "rehabilitation of the site in view of its future use", already present in the ICPE legislation, taking into account the state ground when the request for authorization of new installations or during the first review of existing facilities

Under French law, the protection of soil does not benefit of a specific legal framework. Consequently, the thematic soil does not exist; it should also refer to various regulations, including regulations relating to classified installations for the protection of the environment and the specific regulations on the themes of water, air, waste, fertilizers and pesticides.

More recently, the result of the work carried out in 2006, in consultation with all stakeholders in the field of polluted sites and soils, the Ministry for the Environment has developed new methods of management based on back experience of existing methodological tools and taking into account best practices. Up to this period, risks assessment do not take into account the concepts of availability and speciation of the element in the soil, or the vapour / particulate inhalation may contaminate humans or animals, or contamination of food or synergies between pollutants or between pollutant and communities. This feedback led to the ministerial note of 8 February 2007 accompanied by three appendices (policy and management of contaminated sites in France, the terms management and redevelopment of contaminated sites and tools to support approaches management) with management approaches (the process of interpretation of the state of the environment and the management plan), who have been recognized as state of the art in the field.

In 2008, a circular involved passing a simple sanitary risk assessment to a process of **Interpretation of the State Environments** (IEM, *Interprétation de l'Etat des Milieux*) and **management plans** (*Figure I-2*). This circular now asks for a risk modelling taking into account the best media (water, air, soil, and ecosystems) and all routes of exposure. This is to ensure that the state of the environment is compatible with uses already established or recognized. A management plan can be set up to act both on the state of the site (by development or pollution control measures) on the uses that may be defined.

The *Figure I-2* details the two ways for the management of contaminated sites according to the type of situation:

- ***The approach of interpretation of the state of environment: ensure compatibility between the state of the environment and uses***
- ***The management plan: rehabilitation project: build healthy homes***

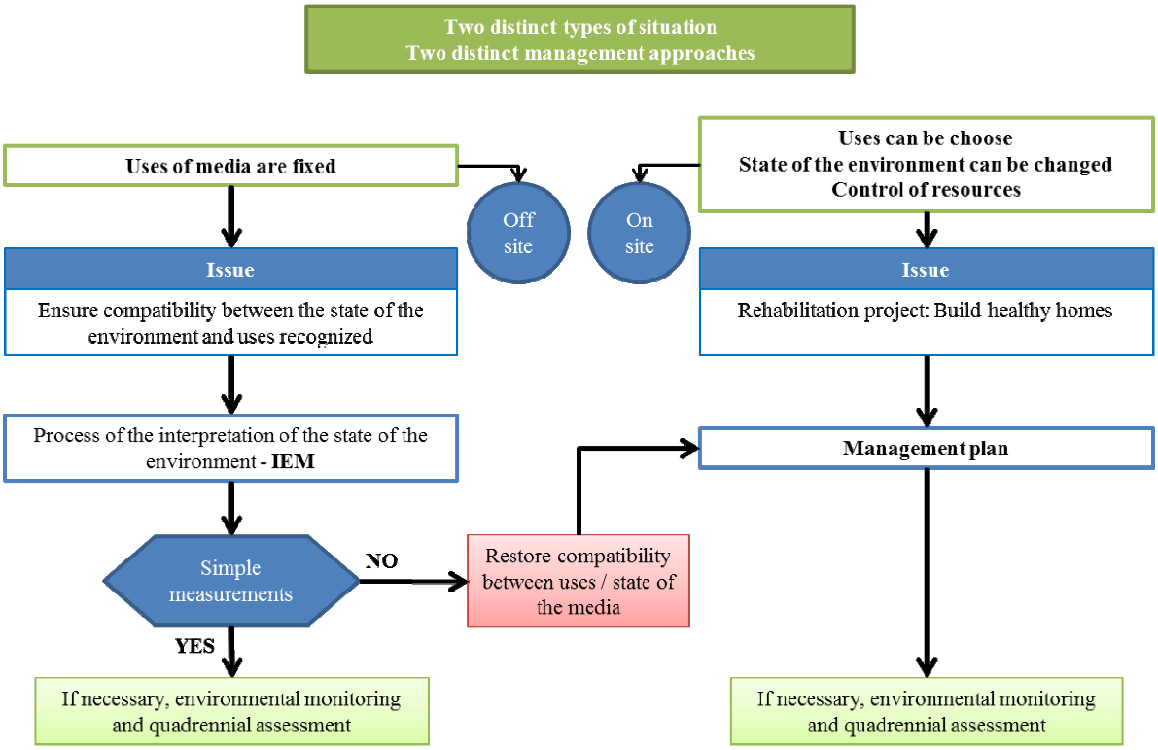

Figure I-2: The two ways of management of contaminated sites (MEDD, 2007).

1.2.2. Physico-chemical properties of MTE

1.2.2.1. Speciation

Originally, the term speciation defines the valence state of a compound (Pb^{2+}, Cd^{2+}, As^{3+}, As^{5+}, Sb^{3+}, etc.) and aims to define associations between anions and cations in solution. This concept has been extended to the study of patterns of associations and localization of cations or anions on or in the matrix *(Conil & Cozel, 1999)*. Chemical speciation is defined as the study of the different chemical forms *(Shahid et al., 2011)*. Compartimentalization refers to the mode of attachment of the MTE to the solid phases of the environment, called bearing phases: clays, OM, carbonates, iron oxides, manganese, aluminium, etc. *(Quenea et al., 2009; Shahid et al., 2013)*. To estimate the distribution of MTE throughout the bearing phases, different physical (XRD, EXAFS, SEM-EDS, etc.) *(Uzu et al., 2010, 2011; Schreck et al., 2012a)* and chemical (extractions with EDTA, H_2O_2, etc.) methods can be used *(Cecchi et al., 2008)*. The dynamics of dissolution, precipitation, fixation, desorption, resulting from abiotic and biotic mechanisms that directly affect the elements or phases carriers. The MTE in soils will therefore present in a complex mixture of solid phases and different morphologies.

The variation of the distribution of the MTE bearing phases depends on several physico-chemical parameters: pH, OM, concentration of inorganic colloids, oxides of iron, manganese, aluminium. In the aqueous phase of soil, metals are free, complexed with inorganic ligands (hydroxide or chloride) or organic molecules *(Shahid et al., 2012; Schreck et al., 2011)*. As with all chemical elements, the speciation in aqueous phase (soil solution in particular) is strongly controlled by pH and redox potential, as well as the type and concentration of complexing agents in solution *(Shahid et al., 2013)*.

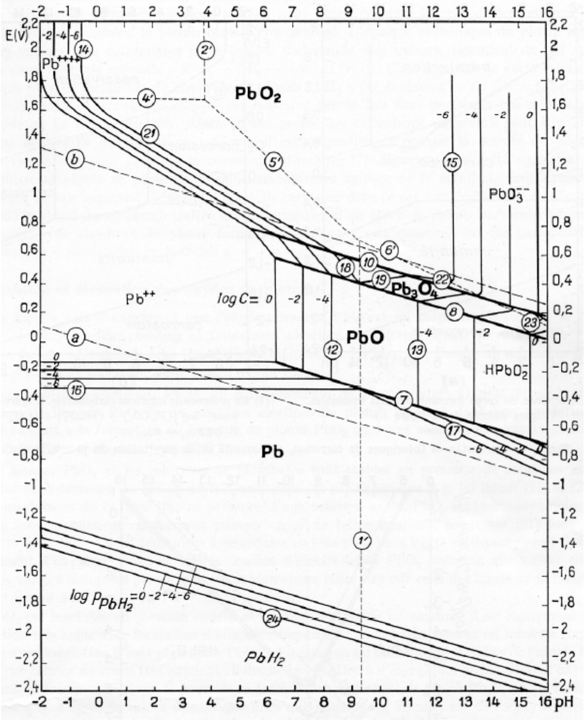

Figure I-3: pH-Eh diagramm of lead (Pourbaix, 1963)

The *Figure I-3* describes the different chemical forms of lead according to pH and Eh of water solution. The major valences for this element are II and IV. With the exception of $Pb(NO_3)_2$ and $PbCl_2$, the inorganic lead compounds are virtually insoluble in water. At acidic or neutral pH, the ions Pb^{2+} and $Pb(OH)^+$ predominate in the solution. As the pH decreases, the amount of Pb^{2+} in solution down two orders of magnitude when the pH decrease by one. When the pH increases, it precipitates as $Pb(OH)_2$ and $Pb(OH)_3^-$ ions and $Pb(OH)_4^{2-}$ are again found in solution for pH values above 8. The complexes formed with organic acids are quite stable *(Bourrelier et al., 1998; Shahid et al., 2012)*.

1.2.2.2. Mobility

MTE mobility is mainly controlled by their speciation in the aqueous phase and the adsorption / desorption and dissolution / precipitation of insoluble compounds *(Glasauer et al., 2004)* (*Figure I-4*).

The role of certain parameters such as pH, redox potential, the mineralogical composition of soil or sediment and the presence of organic ligands or colloids in the phase will be crucial *(Violante et al., 2005)*. Indeed, these parameters influence several bio-physico-chemical mechanisms which can intervene to solubilise, precipitate or concentrate MTE present in the soil *(Manceau et al., 2002)*.

In addition, the temporal and spatial variations in soil composition are accompanied by changes in chemical speciation of metals, making the prediction of the mobility and availability extremely difficult *(Staunton, 2004)*. Indeed, Nunan et al. *(2002, 2003)* showed that bacterial communities are not randomly distributed in the soil and the spatial organization and microbial functions are organized in the ground a few mm² to several m². This element is not affected by redox processes, but under the conditions of pH and Eh, it may undergo different mechanisms of control of solubility by reduction, oxidation and methylation *(Gadd, 2000; Lovley, 2001)*.

The *Figure I-IV* presents the different sorption processes controlling MTE mobility at the soil interface.

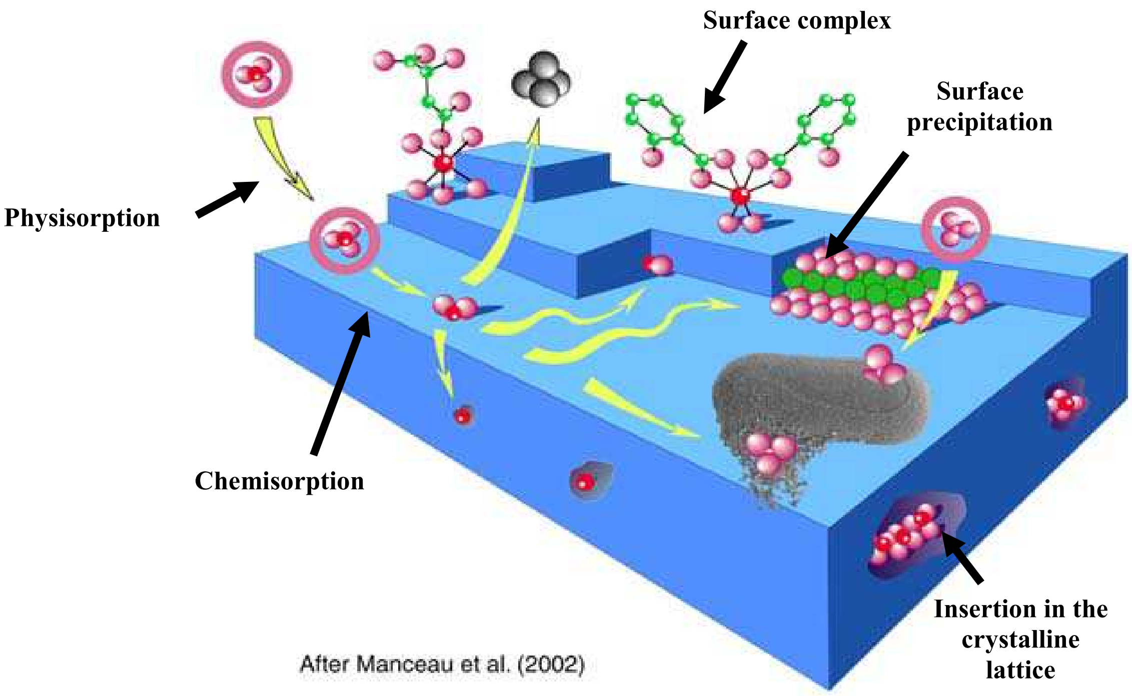

Figure I-4: Sorption process of elements at the soil interface (Manceau et al., 2002).

Soil pH is one factor that most influences the mobility and bioavailability of MTE (Bourrelier et al., 1998; Shahid et al., 2012). Organic matter and clay are the main bearing phases for lead (Cecchi, 2008). The low mobility of lead in soils is largely due to its complexation with humic substances (Johnson & Petras, 1998). In calcareous soils and soil minerals, low in OM, the presence of minerals containing calcium, magnesium, iron, causes the precipitation of lead (Hernandez-Soriano & Jimenez-Lopez, 2012). Bio-sorption, another mechanism of immobilization of metals, is the microbial removal of soluble and insoluble metal species by physico-chemical mechanisms such as adsorption, and living cells, metabolic activity, can influence this process because of changes pH, Eh, nutrients and excretion of metabolites (Compant et al., 2010). The nature of micro-organisms (fungi, bacteria, protozoa) (Rajapaksha et al., 2004) and their diversity and quantity greatly affect the sorption properties of micro-organisms (Ginn et al., 2008).

It is also dependent on its interaction with mineral phases. The reactions of adsorption / desorption, precipitation / dissolution control the mobility of MTE in the environment. These reactions are influenced by:

- *characteristic of the solid phases (surface area, surface charge, structural changes of the solid phase);*

- *chemistry of the aqueous phase (pH, redox potential, presence of anions competitors);*
- *presence of living organisms (mainly microorganisms);*
- *different mineral phases such as sulphides, oxides and hydroxides of Fe, aluminium (Al) and manganese (Mn), clays and / or calcium carbonate (calcite) in soils, sediments and aquifers are likely to trap the As.*

► **In conclusion, according to the physico-chemical properties and soil conditions, different mechanisms are implemented. Depending on the distribution of MTE bearing phases, the mobility of elements will vary** *(Cecchi et al., 2008)*.

1.2.3. Risks for human health and ecosystems

1.2.3.1. MTE toxicity and relation with compartmentalization and speciation

Unlike organic contaminants, metals in soils do not undergo chemical or biological degradation and, in fact, the total concentrations and ecotoxicity of these metals in soils can persist very long term after their introduction *(Guo et al., 2006)*. The determination of the total concentration of a potentially toxic element gives only limited information about its toxicity. Indeed, the toxicity of an element is directly related to the chemical form in which the element is present (and thus speciation). Indeed, the rate of absorption by the human body or plants is often directly related to the particular chemical form and the solubility of the chemical species considered *(Caboche, 2009)*. Therefore, it is important to know the speciation of potentially toxic elements in airborne dust to better assess their toxicity.

In addition, a metallic element retained on the surface of a material (by physisorption or chemisorption) (*Figure I-4*) will be dissolved more quickly and therefore may present a higher risk of toxicity if it is inserted in the network crystallographic material.

Each element exists in the environment in many chemical forms, and only some of them are toxic according to the historic of the site, the soil nature, soil physico-chemical parameters, etc. (*Table I-2*). Thus, the presence of the element in a sample is not necessarily dangerous. This highlights the need to go beyond the simple determination of the total concentration of an element in a sample in order to understand its overall toxicity.

	Low	Average	High
Geochemical form			
Sulphides	x		
Elementar (metal)	x		
Sulfates		X	
Carbonates			x
Oxydes			x
Particle size (bearing phases)			
Little			x
Large	x		
Age of contamination			
Sulphides	x →		
Elementar (metal)	x →		
Carbonates			← x
Oxydes			← x
Soil chemistry			
Acid pH		X	
Basic pH			x
Alcalin soils			x
High OM content	x		
High complexing agent content (Fe, Mn, Si)		X	

Table I-2: Impact of historical contamination and some physco-chemical parameters on mobility and toxicity of MTE (Caboche, 2009)

1.2.3.2. Quantitative sanitary risks assessment

The approach to quantification of health risks (EQRS, *Evaluation Quantitative des Risques Sanitaires*) as practiced in France for the rehabilitation of polluted sites and soils is an estimate of the occurrence of adverse effects on human health, taking into account the toxicity of and different routes of human exposure to these substances. Humans can absorb contaminants in soil by three major routes of exposure: inhalation, skin absorption and ingestion (Pautenbach, 2000). Ingestion of soil and dust is the predominant route of exposure with respect to the non-volatile MTE (Kwon et al., 2004; Carrizales et al., 2005; Yamamoto et al., 2006; Schreck et al., 2012b). For physiological and behavioural, the population most sensitive to soil intake includes children from 0-6 years (Wang et al., 2003). This is mainly due to the behaviour of carrying the hand to mouth which is common in children aged 12 months to 2 years (Ellis & Schnoes, 2005; Ljung, 2006). Irrespective of the type of behaviour geophagia, it is estimated that a child will ingest 100 to 150 mg soil / day (US EPA, 1997; Calabaresse et al., 1999). In addition, this population is more sensitive to the toxicity of metal pollutants such as Pb (wall more permeable blood-brain) (WHO, 1995; ATSDR, 2007) and children also have a gastrointestinal absorption greater than the adults. It is currently considered that the absorption of Pb by the digestive system is 10 to 20% in adults and from 30 to 50% for children (Laquatra, 2001).

In a process of risk assessment for ingestion of contaminants and pollutants from soil, it is considered that the total concentration of a substance in soil that is likely to produce a toxic effect on the humans (Oomen et al., 2006). In fact the estimated level of risk based on direct comparison of the dose ingested with soil to the toxicological reference value (VTR, *Valeur Toxicologique de Référence*) for the oral route. This method does not take into account the difference in bioavailability of the element in contaminated soil and the matrix used for the preparation of the VTR. Yet numerous studies show that the bioavailability of an element in soil is lower than in a different matrix such as food and water (Casteel et al., 1997; Schroder et al., 2004). Thus, neglecting the difference in bioavailability amounts to overestimate the risks associated with ingestion of contaminated soil. It is therefore necessary to consider this parameter to improve the calculation of risk (Pelfrêne et al., 2013). To approach, the bioavailability of a simplified manner, we will look at measuring the bioaccessibility, main objective of this course, which according to Denys *et al*. (2007) is an appropriate step to estimate the bioavailability of metals. To understand this topic it is necessary to define these notions.

1.2.3.3. The different concepts of bioavailability and bioaccessibility

The oral bioavailability is the fraction of compounds ingested that is absorbed and reaches the systemic circulation (Casteel et al., 2006) (*Figure I-5*). The absolute bioavailability can be defined as the ratio of the absorbed dose of the administered dose (Ruby et al., 1996; Basta et al., 2000).

The notion of relative bioavailability is to compare the absorption of a compound in two different speciations, such as Pb carbonate vs Pb acetate, or the same compound administered by different media such as food, soil and water (Kelley et al., 2002). This is important because a calculation of risk returns to link exposure doses and VTR; they are based on a specific matrix such as food or water, and the substance in the matrix question to a specific bioavailability. So if the bioavailability of the offending substance in soil is greater than the bioavailability in the matrix used for study leading to the award of the VTR, the relative bioavailability is greater than 1 and the level of risk will be higher than the calculated level not incorporate this setting (Pelfrêne et al., 2013).

The bioavailability is measured through *in vivo* experiments in animal models including the digestive system is similar to that of man. The models most commonly used are the juvenile pigs (US EPA, 2007). But these methods are costly and require expertise in animal experiments, which may in some cases, raise ethical questions, particularly in the context of REACH, where a decrease in *in vivo* experiments is required.

The absolute bioavailability is measured by comparing the concentrations of the element measured in the blood after intravenous injection and after oral administration. The bioavailability can also be measured from the concentration of the substance in a target organ after an exposure period of weeks (Casteel et al., 2006; US EPA, 2007). To avoid passing through animal testing to measure the bioavailability, one must include another parameter, the bioaccessibility.

Bioaccessibility is the fraction of pollutant that is extracted by the digestive fluids in saliva of the system and the gastrointestinal tract. It can be expressed as the ratio:

Dose extracted from the soil matrix / dose administered × 100

Then to find the extent of bioavailability, the following equation is used:

$$B = Ba \times Fa \times Fh$$

With:

- *B: absolute bioavailability*
- *Ba: absolute bioaccessibility*
- *Fa: absorption through the digestive system of the bioaccessible fraction*
- *Fh: transmission rate to the systemic circulation of the absorbed fraction.*

The bioavailable fraction of a substance is less than the bioaccessible fraction of this substance, which allows us to say that bioaccessibility is a limiting parameter of bioavailability. The relationship between bioaccessibility and absolute bioavailability, for plants and human, is schematically illustrated in the *Figure I-5*.

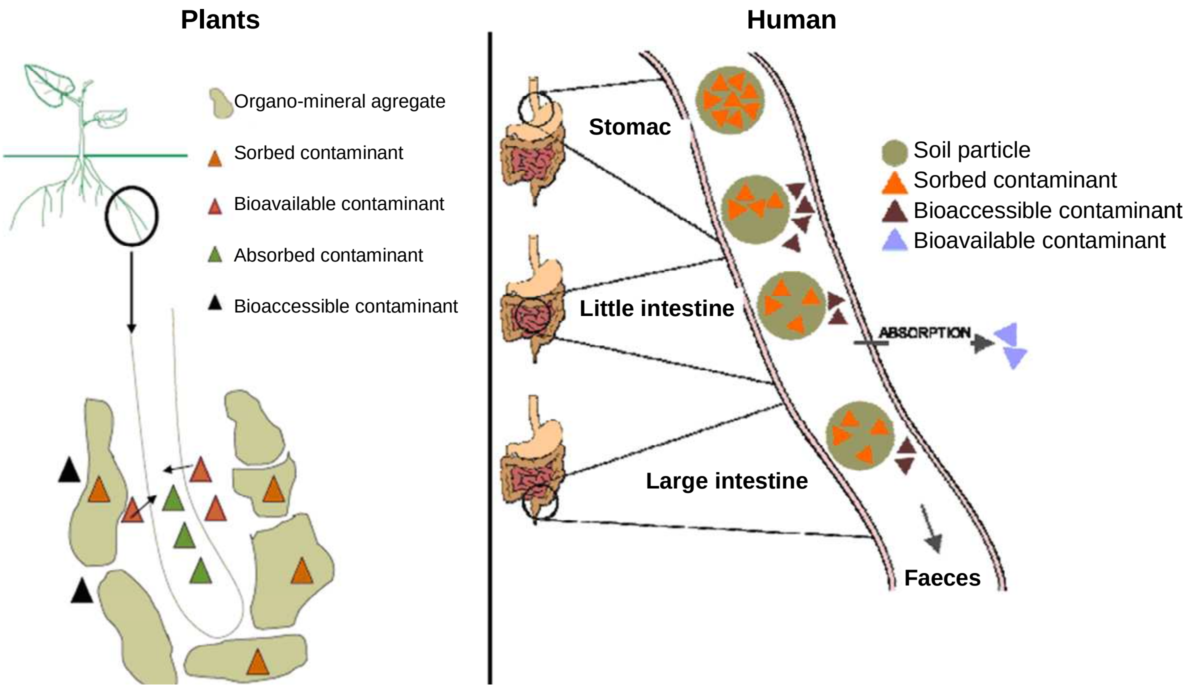

Figure I-5: Diagram showing the concept of bioavailable and bioaccessible fractions in higher plants and humans.

As the relative bioavailability, the notion of relative bioaccessibility is to compare the extraction of a compound in two different speciations (Pb carbonate vs. Pb acetate) or the same compound extracted from different matrices (food, soil or water) *(Caboche, 2009)*.

► **To calculate the risk, in which it is necessary to distinguish the difference in bioaccessibility between the ground and the matrix used for the establishment of the VTR, the notion of relative bioaccessibility is very important.**

1.2.4. Soil-plants transfers

Kabata-Pendias *(2004)* explains that the soil-plant transfer of substances depends on many factors that interact, which explains the high variability of pollutant concentrations measured in plants. The concentrations of lead (metal pollutants) measured in lettuce range from 0.06 to 87µg of lead per kg of dry matter of lettuce (or a variation factor of 1500) depending on the characteristics of soils, plant variety and nature the source of lead *(Dumat et al., 2006)*. In more detail, these factors are in particular: total concentrations, the nature and speciation (chemical form) of the elements in soils, soil characteristics (Dumat et al., 2001, 2006; Twining et al., 2004; Wang et al., 2006; Gandois et al., 2010), factors related to the plant: species, variety / cultivar, etc. (Alexander et al., 2006).

The *Figure I-6* below shows the different phenomena that may occur in the case of MTE behaviour in the soil-plant-atmosphere: adsorption, complexation, leaf interception, root absorption, etc.

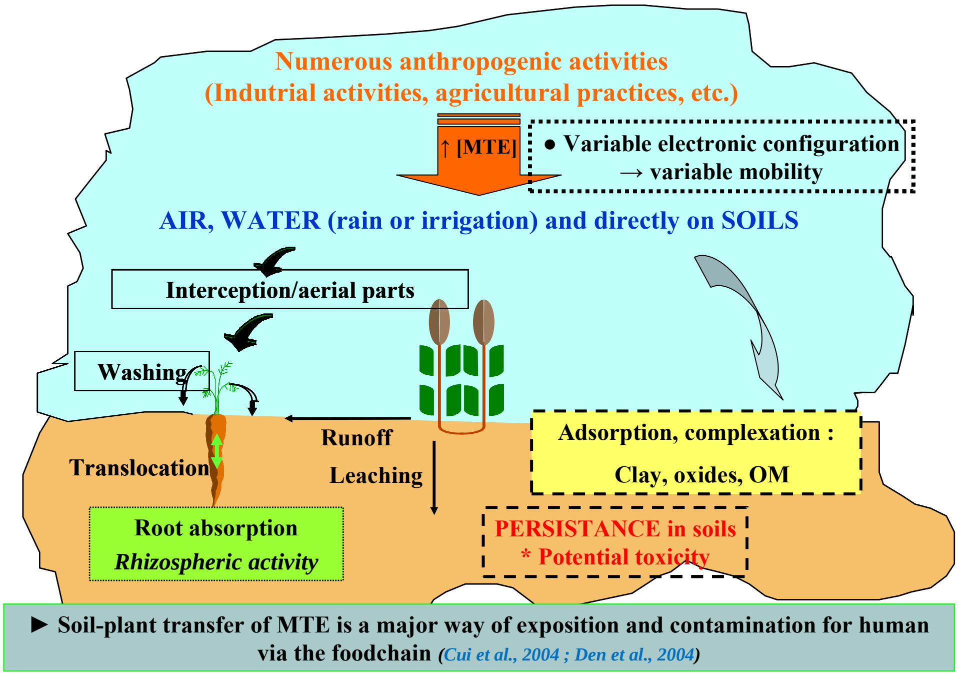

Figure I-6: Soil-plant interactions (accordingly to Dumat et al., 2006)

1.2.4.1. Root absorption

The plant absorbs water and the elements necessary for growth with its roots. The mineral absorption is mainly provided by the non-suberized roots (root hairs). Most items come through systems of passive transport (diffusion through the plasma membrane) or active (transport controlled by metabolism). Exchanges between different cellular compartments and accumulation of substances in the plant take place at the plasma membrane where different mechanisms are involved:

- *The protons pump H^+, which determines the pH and the transport of anions and cations. More generally, the mechanisms related to concentration gradients promote international exchange membrane* (Salisbury & Ross, 1992).

- *Complex formation MTE-chelate: in case of deficiency of one or more metals, the plant produces a chelating agent that captures the substance at the root system. The complex formed is then transported through the plant with the help of transport proteins. These mechanisms involve problems of selectivity because the chelating agents are specific to each substance and were not produced by all plants* (Shahid et al., 2012; 2013).

- *Passive diffusion of ions is in the direction of the concentration gradient across the plasma membrane, the ions from the middle the most concentrated to least concentrated. The diffusion rate depends on electrochemical gradient and the permeability of the walls and cell membranes to the ion. Passive diffusion is a phenomenon not saturable. Facilitated diffusion allows a faster passage of ions or molecules through a transport protein present in the membrane. This system also acts in the direction of the electrochemical gradient without energy supply but accelerates the process. Active transport requires energy supply and occurs via a transport protein. This type of transport is specific but the mono or divalent cations such as cesium-toxic, lead, nickel, etc., are competitors vis-à-vis other essential cations such as K^+, Ca^{2+}, Mn^{2+} and Zn^{2+}. For high concentrations, diffusion is the major process of absorption, the active transport of ions in low concentrations* (Mitter et al., 2013).

Physiologically, soluble ions can enter the root by two paths: a so-called extracellular apoplasm (wall and meatus), a known intracellular symplasmic (cytoplasm and plasmodesmata) *(Barber, 1995)*. Through the apoplasm, the items will be transported to the endoderm but cannot cross the band Caspary. Symplasmic transport of ions is in turn, through the plasmodesmata, a continuous pathway from cell to cell. However, the xylem cells have an important and CEC can fix many of the ions and thus limit their transfer.

The absorption of elements depends, in addition, on plant species and those requiring large amounts of an essential element can absorb its chemical analogues.

1.2.4.2. MTE translocation

The elements translocation process towards the top includes the transport of elements within the plant and its distribution in different plant organs. For Marschner (1995), translocation of an MTE to the aerial parts of the plant via the xylem occurs via the symplastic but preferably by apoplastic. This is a discontinuous way, controlled by the endoderm, which has hydrophobic surface sites and blocks the passive transfer of solutes in the steme, and indeed to the xylem. The transport of elements in the xylem of the plant involves various organic molecules (mainly citric acid), amino acids (such as histidine) and various peptides (Shahid et al., 2012). The *Figure I-7* below shows these different ways of transport in plants.

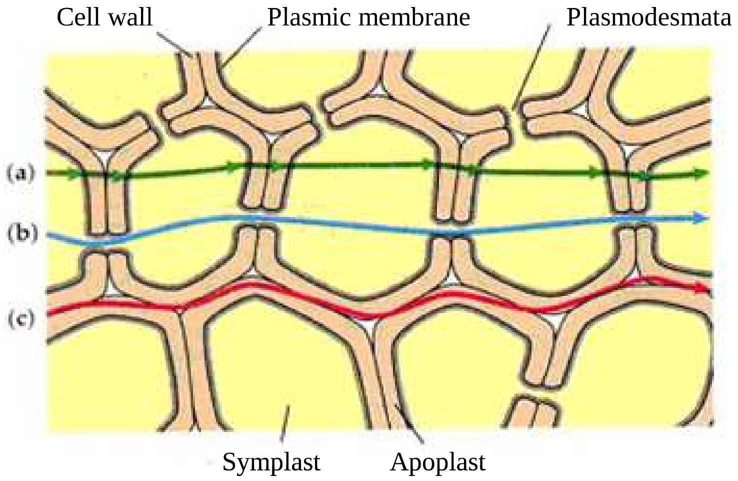

Figure I-7: The different modes of membrane transport of water: (a) transcellular route, (b) symplastic pathway, (c) apoplastic pathway

Once transferred, the distribution of elements in different plant organs varies among species. For the majority of them, the roots retain much of the absorbed elements and element concentration following order: roots> stems> leaves (Prasad & Freitas, 2000). Some species, however, have the particularity to transfer large amounts of items to the aerial parts. This is the case for tobacco that accumulates 80% of the absorbed cadmium in its leaves (Mench et al., 1989), and many leafy vegetables (lettuce, etc.) for many items.

Mobility and transfer of elements in the soil–plant system are controlled by many physico-chemical parameters of soil and the plant itself. In addition, in the system soil and rhizosphere microorganisms can change the status of chemical elements and therefore also act on their availability and their transfer to the plant. Transport through the membrane of the MTE is an important area of investigation (Chaney et al., 2007).

1.2.4.3. Influence of microorganisms

Other parameters, such as the biological activity of a soil can change the phytoavailability of MTE. The mobility of elements in the rhizosphere can be modified directly or indirectly by root activity. Direct processes are related to physicochemical processes (pH, Eh, presence of complexing agents, etc.), while indirect processes are due to the activity of microorganisms by mechanisms that can more or less specific, increase or decrease considerable mobility or availability of components and ultimately the removal of the plant workforce (Compant et al., 2010).

The physico-chemical parameters of soil strongly influence the biological activity (micro- and meso-biological), which in turn modifies soil parameters (pH, organic matter, structure, etc.), and plays a role in the bioavailability MTE process by biosorption, bioaccumulation and solubilisation. When considering the mobility of MTE in the rhizosphere, the rhizosphere pH is not the most important factor (Lasat, 2002; Tao et al., 2005). In the rhizosphere, very special area of soil, organic material (plant or microbial) present and microbiological activity appear to play a more important role than acidification. Indeed, organic acids such as citric acid, malic acid or oxalic acid are able to desorb lead and facilitate its solubilisation in the soil (Wu et al., 2003; Yang et al., 2006). For example, lead reacts with organic acids in the soil solution according to the following reactions (Cecchi, 2008):

$$\mathbf{R\text{-}COOH + Pb^{2+} \rightarrow R\text{-}COOPb^{+} + H^{+}} \qquad \text{or} \qquad \mathbf{R\text{-}OH^{+}\ Pb^{2+} \rightarrow R\text{-}OPb^{+} + H^{+}}$$

With:

- *R-COOH: carboxyle acid;*
- *R-OH: alcohol or phenols (organic ligands).*

The degradation of molecules takes place in the rhizosphere by the combined action of three closely related: plant - soil – microorganisms (Compant et al., 2010). Their combination allows an enzymatic detoxification of the environment by increasing the bioavailability of the molecules in plants *(Qiu et al., 1997; Anhalt et al., 2000)*. For that roots secrete exudates that acidify the environment and alter the chemical behaviour of molecules *(Robinson et al., 2009)*. Currently, research focuses on understanding the metabolic reactions or the degradation potential of fungi and bacteria. The effectiveness of this method is also dependent on many parameters (soil temperature, pH, concentration of organic matter, % O_2, etc.) that condition the development of microbial species. This technique suffers from the moment of inefficiencies but also reproducibility due to the different interactions. This is certainly a very effective technique for remediation of a site but gaps remain in terms of molecular reactions and products formed by micro-organisms *(Neumann et al., 2003; Devars et al., 1998)*. The analytical techniques normally used to conduct this type of analysis are not enough to learn about the composition of metabolites and intermediate complexes *(LeDuc & Terry, 2005)* of current research on the role of chloroplasts in the process of phytoremediation.

1.3. Management of brownfields and contaminated sites

1.3.1. Foreword

Remediation of soils contaminated by metals in urban areas is a challenge *(Wong et al., 2006)*. The techniques of "remediation" based on the excavation, transportation and burial of wastes and soils contaminated by metals pose little risk but can cause damage to the ecosystem in place and are costly to implement *(Raicevic et al., 2009)*. Moreover, the recent increase in land costs "clean" has affected the rates of excavation *(Tandy et al., 2004)*. This technique must be accompanied by complementary actions to address and / or storage of excavated soil *(Colombano et al., 2010)*. Remediation techniques, describe in the *Figure I-8*, are numerous and involve a variety of physical reactions, chemical and biological pollutant targeted by heavy metals, radionuclides, nitrate, phosphate, inorganic acids and organic molecules *(Arthur et al., 2005)*. Overall, these techniques are called *in situ* or *ex situ* according to the decontamination is performed directly on the area to be decontaminated or outside of this area (e.g. in a specialized processing unit that can be located at several kilometres from the site) *(Saadoun & Al-Ghzawi, 2005)*.

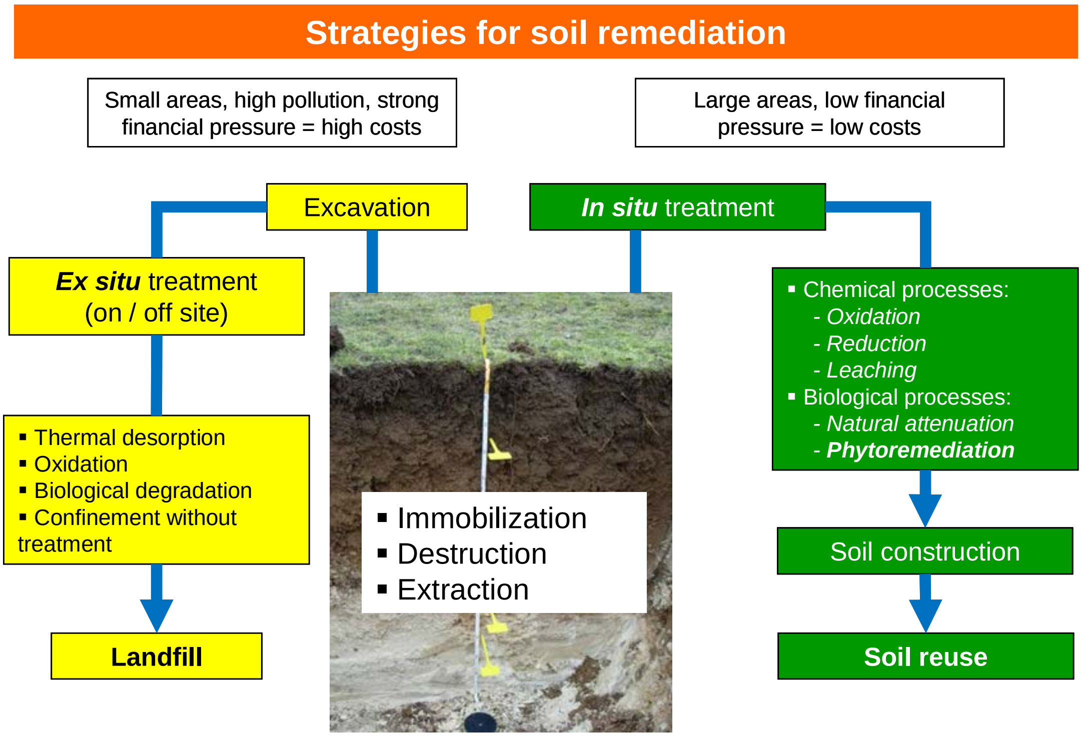

Figure I-8: The different techniques of soil treatments

The technique chosen should, above all to control the pollution source and the transfer of pollution, treating the maximum substances, and reduce the maximum residual risks (thus residual exposures) (Colombano et al., 2010). If it is impossible to completely remove the source of pollution (after taking into account the best techniques to an economically acceptable cost), it is imperative to ensure that impacts from residual sources are controlled and acceptable to the people and the environment.

► The next part concerning the sustainable management of the polluted soils is presented in a form of an accepted article published in 2012 in the journal Environnement, Risques et Santé (11, 61–66).

1.3.2. Vers une gestion raisonnée des terres excavées contaminées par des Eléments Traces Métalliques

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1.3.2.1. Résumé

En France, l’industrie métallurgique est encore souvent à l’origine de contaminations des sols par les éléments traces métalliques et métalloïdes (Pb, As, Cd, Cu, Zn, Sb, Se, etc.), notés ETM, issus principalement des émissions atmosphériques ou des lieux de stockage de déchets. Initialement situés en périphérie des villes, certains sites, parfois à l’abandon, se *trouvent désormais en zone urbaine et sont alors susceptibles de présenter des risques environnementaux et sanitaires significatifs pour les populations avoisinantes. Les pouvoirs publics et la société en général, prennent donc conscience de la nécessité de réhabiliter ces friches industrielles, qui représentent de plus un fort enjeu en termes d'urbanisme durable.*

Les techniques de remédiation des sols contaminés par les ETM font appel à de nombreux procédés: stabilisation, lavage, etc. Toutefois, lorsque ces sites sont situés en zone urbaine, l'excavation est très fréquemment mise en œuvre. Les terres excavées peuvent alors suivre deux filières : (i) la mise en décharge, coûteuse (jusqu'à 200€/tonne) et énergivore ou (ii) la valorisation s'inscrivant dans une logique de développement durable. Le choix d'une de ces voies dépend en particulier de la nature de la concentration et forme chimique des ETM. Plus les terres sont contaminées et ont un fort potentiel de lixiviation, plus leur « degré de dangerosité » est élevé. Leur mise en décharge devient alors inévitable, avec des moyens de mise en sécurité contraignants. Dans ce contexte, optimiser le tri des terres excavées constitue un moyen efficace pour les entreprises de réduire le montant de la dépollution (transports et stockage) et de maitriser les risques sanitaires et environnementaux. L'exemple du criblage granulométrique mis en œuvre dans le cadre de la réhabilitation des sols pollués par les ETM d'un site de recyclage de batteries en zone urbaine est décrit dans cet article afin d'apporter des éléments d'information quantifiés sur l'efficacité de cette pratique.

Mots clés : valorisation des terres excavées, tri granulométrique, remédiation des sols pollués, friches industrielles, zone urbaine, développement durable.

1.3.2.2. Enjeux de la réhabilitation des friches industrielles contaminées par les ETM en zone urbaine et gestion des terres excavées

En France, plus de 4000 sites, parmi 3 million à travers le monde, se retrouvent aujourd'hui à l'état de friches polluées par d'anciennes activités industrielles [1]. La question de leur valorisation se pose, parfois après de longues périodes d'abandon. En effet, avec 77% de population urbaine, la France doit maîtriser l'étalement urbain et ses besoins de transports, participant ainsi activement aux démarches de développement durable des villes [2]. Les pressions engendrées par l'expansion des zones urbaines, aussi bien environnementales, sanitaires, sociales et foncières, conduisent aujourd'hui les pouvoirs publics à réhabiliter ces anciens sites, devenus une préoccupation majeure pour la société [3].

Les critères de sélection d'une technique de remédiation, dont l'efficacité est conditionnée par la nature du polluant, l'étendue de la pollution, les caractéristiques naturelles du sol, etc., dépendent eux-aussi de paramètres socio-économiques et environnementaux [4,5]. Néanmoins, la reconversion des sites pollués se traduit le plus souvent par l'excavation des terres contaminées [6]. En fonction des usages futurs de ces terrains, l'évacuation des terres polluées peut s'avérer nécessaire pour réduire les risques sanitaires et environnementaux [7]. Toutefois, ces terres excavées sont généralement considérées comme des déchets. Aussi, la cessation d'activité d'une entreprise doit se préparer très en amont, car le tri des terres contaminées, en vue de leur gestion, peut s'avérer long (de quelques semaines à plusieurs mois) et coûteux (de quelques dizaines à plusieurs centaines d'euros la tonne) [8]. Une analyse coût-bénéfice globale intégrant les trois piliers du développement durable et une stratégie de communication avec les autorités compétentes, les collectivités locales et les citoyens doivent également être mises en place [9]. Dans ce contexte, le criblage des terres excavées en fonction de leur degré de contamination apparait comme une étape indispensable pour réduire les quantités des terres fortement contaminées destinées aux décharges de classe I [10]. En raison des enjeux environnementaux, sanitaires et économiques, les organismes techniques en collaboration avec les organismes industriels et de l'état élaborent actuellement des guides concernant la réhabilitation des friches en zones urbaines et la problématique des terres excavées. Dans ce contexte, le présent article a pour objectif de présenter un retour d'expérience sur un site en zone urbaine en cours de réhabilitation visant plus particulièrement le sujet du criblage des terres. En effet, si le criblage des terres excavées est largement utilisé dans la gestion des sols pollués, peu d'informations quantifiées sur l'efficacité de cette pratique sont disponibles.

1.3.2.3. Gestion courante des sols pollués par les ETM

1.3.2.3.1. Criblage des terres et optimisation des coûts

La faisabilité technico-économique d'un procédé de dépollution, est fortement influencée par la nature de la pollution métallique (nature minéralogique et taille des phases porteuses des ETM) et les propriétés physico-chimiques du sol (texture, matières organiques, pH, etc.) [11,12].

En règle générale, la première étape de traitement consiste donc à réaliser un tri granulométrique des terres contaminées, étape cruciale pour réduire la masse de matériaux contaminés en séparant les particules contaminées des particules « propres » [6]. Le sol initial est ainsi décomposé en deux fractions : (i) la première correspondant à une part importante des solides du sol, faiblement concentrée en polluant et permettant une réutilisation de ce matériau et (ii) la seconde, de volume réduit, concentrant la quasi-totalité des polluants. En effet, la littérature et les retours d'expériences montrent que les fractions fines des sols (< 63µm et argiles (< 2µm)) concentrent la grande majorité de la pollution [13] en raison des grandes surfaces spécifiques développées et des nombreux sites d'adsorption qui y sont présents.

Compte-tenu des caractéristiques des sols, des coûts de transport et des remblais, les terres les plus polluées sont dirigées vers des installations de stockage de déchets, tandis que les moins contaminées peuvent éventuellement être réutilisées sur le site [7]. Cette technique de tri permet donc de concentrer les phases porteuses (matières organiques, colloïdes, carbonates, phases hydroxylées, etc.) des ETM tout en réduisant les quantités de terres excavées [7]. Elle a donc un impact positif sur l'empreinte écologique de la réhabilitation des sites pollués en réduisant ses besoins d'absorption de déchets.

1.3.2.3.2. Mise en décharge

La règlementation française (arrêté ministériel du 15 mars 2006 fixant la liste de déchets inertes admissibles dans des installations de stockage de déchets inertes, décision du Conseil du 19 décembre 2002 établissant les critères et procédures d’admission des déchets dans les décharges, et annexe 1 de l'arrêté du 31 décembre 2002 relatif au stockage de déchets dangereux) fixe le caractère inerte / non inerte des terres excavées, et donc les filières possibles de réutilisation, valorisation ou évacuation. Les terres contaminées qui ne peuvent être valorisées sont considérées comme des déchets et vont suivre différentes filières de stockage.

Les critères sont basés sur la quantité de métaux et métalloïdes libérés par lixiviation des terres suivant plusieurs procédures de lixiviation (essai de lixiviation NF EN 12457 (*Tableau I-4*) ou essai de percolation à écoulement ascendant NF CEN/TS 14405 : injection d’eau à la base d’une colonne de sol (L/S = 0,1) et récupération des solutions de percolation pour analyse). Selon la directive n° 1999/31/CE du 26 avril 1999 concernant la mise en décharge des déchets, on distingue trois catégories de décharges : (1) installation de stockage de déchets dangereux (ISDD, classe I) ; (2) installation de stockage de déchets non dangereux (ISDND, classe II) ;(3) installation de stockage de déchets inertes (ISDI, classe III).

Paramètres	**Valeurs limites**		
	Déchets inertes *(Annexe 2 de l'arrêté du 15-03-2006)*	**Déchets non dangereux** *(Décision du Conseil du 19-12-2002)*	**Déchets dangereux** *(Annexe 1 de l'arrêté du 31-12-2002)*
Classe	III	II	I
Coûts	30-50 € / T	70-100 € / T	100-150 € / T
Plomb	0,5	10	50
Arsenic	0,5	2	25
Cadmium	0,04	1	5
Antimoine	0,06	0,7	5
Chrome	0,5	10	70
Cuivre	2	50	100
Baryum	20	100	300
Mercure	0,01	0,2	2
Molybdène	0,5	10	30
Nickel	0,4	10	40
Zinc	4	50	200
Sélénium	0,1	0,5	7
COT*	500	800	1000
Fraction soluble	4000	60000	100000
Indice phénol	1		

*Tableau I-3: Valeurs limites en matière de lixiviation sous NF EN 12457 applicables aux déchets admissibles dans les décharges pour déchets inertes, non dangereux et dangereux et coûts associés (*COT = Carbone Organique Total) : mise en contact d’un sol (granulométrie < 4mm) avec de l’eau déminéralisée selon un ratio L/S=10 en masse, agitation par retournement pendant 24h, filtration des solutions à 0,45 µm et analyses des lixiviats par ICP-OES/MS.*

Le *Tableau I-3* indique les valeurs limites de concentrations en ETM solubilisées lors du protocole normalisé de lixiviation (NF 12457-2) ainsi que le carbone organique total (COT), les anions (sulfates, chlorures, fluorures, déterminé selon NF EN ISO 10304-2) et l'indice phénol (NF T 90-109) qui caractérise la présence de composé phénols. Ces trois critères sont demandés en routine. Dans le cas des sols fortement pollués par les métaux et métalloïdes, le critère ETM est donc prépondérant.

Les professionnels du secteur considèrent les seuils fixés dans l'arrêté pour les ETM comme trop contraignants. Les concentrations mesurées dans une grande partie des terres excavées mises en décharge sont souvent comparables à celles du fond géochimique naturel [14]. Un surcoût significatif est alors supporté par les aménageurs de sites (sachant que les coûts varient du simple au triple entre les classes de décharges) et ceci participe également à la multiplication des décharges de classe I.

1.3.2.3.3. Réutilisation sur site

Un projet de réhabilitation peut consister à définir des usages ou des configurations d'aménagement qui, combinés à des actions sur les voies de transfert, conduisent à réutiliser les terres peu ou pas contaminées comme remblais, tout en les confinant, notamment par la mise en place d'une alvéole de stockage. Néanmoins, de telles mesures doivent être pérennes et adaptées aux usages futurs du site [10].

La technique consiste à disposer les terres dans une alvéole dont la perméabilité est très faible et dont les eaux d'infiltration sont collectées et traitées. Les techniques de confinement de sites ont fortement évolué ces dernières années en bénéficiant, d'une part, d'améliorations effectives dans le cadre de la prédiction du comportement et de la migration des polluants, d'autre part, d'une meilleure compréhension du comportement à long terme des matériaux de confinement [6]. La technique de confinement s'inscrit donc pleinement dans une démarche de développement durable car: i) le risque environnemental est maitrisé; ii) le site est mis en sécurité et une surveillance à moyen terme est instaurée; iii) les surfaces réhabilitées peuvent être réutilisées.

L'analyse des risques résiduels (liés à la présence du contaminant qui demeure dans le sol après la décontamination) et la conservation de la mémoire sont deux éléments essentiels de la démarche de gestion des sites en France. Pour respecter les objectifs du plan de gestion et atteindre la concentration résiduelle fixée en lien avec les futurs usages du site, tant que les objectifs de réhabilitation ne sont pas atteints, une nouvelle démarche de gestion est conduite. Après un chantier de dépollution, la conservation de la mémoire de ce site et l'information des opérateurs et aménageurs sont nécessaires pour éviter qu'un site, actuellement sans impact, ne le devienne par suite de travaux ou de nouveaux usages inappropriés. Des outils comme BASIAS (Inventaire historique de Sites Industriels et Activités de Service) et BASOL (Base de données sur les sites et sols pollués ou potentiellement pollués appelant une action des pouvoirs publics, à titre préventif ou curatif) permettent effectivement de répondre à ces exigences de traçabilité.

1.3.2.4. Retour d'expérience : réhabilitation du site STCM de Toulouse

En France, la législation (loi ICPE n° 76-663 du 19/10/1976) sur les installations classées pour la protection de l'environnement (ICPE) impose à ces entreprises de mettre en œuvre des stratégies de gestion pour limiter leurs impacts sur l'environnement et la santé et remettre en état les terrains occupés après cessation d'activité (loi du 30/07/2003).

La Société de Traitements Chimiques des Métaux (STCM), dont l'activité de recyclage de batteries plomb-acide est soumise à cette règlementation, prévoit de stopper ses activités dans le cadre d'une réhabilitation potentielle et constitue un site pilote d'intérêt pour la mise en place d'une stratégie de réhabilitation et gestion durable d'un site industriel en zone urbaine présentant une pollution historique en plomb et arsenic. Crée en 1952, la société était à l'origine située dans une zone éloignée de toute habitation. Au cours du temps, s'est développé un pôle industriel important de la ville de Toulouse. De nos jours, une laiterie et des lotissements sont situés à proximité du site (*Figure I-9*). Parmi les entreprises qui bordent la STCM, le dépôt de carburant (classé Seveso seuil haut) est soumis à un Plan de Prévention des Risques Technologiques (PPRT) depuis 2010 délimitant un périmètre d'exposition aux risques (dans lequel sont inclues la STCM et une la laiterie.), et tenant compte de la nature et de l'intensité des risques technologiques et des mesures de prévention mises en œuvre. De fait, la STCM a fait le choix de l'expropriation d'une partie de ses installations et de son terrain, et doit donc engager une démarche de réhabilitation pour cette zone.

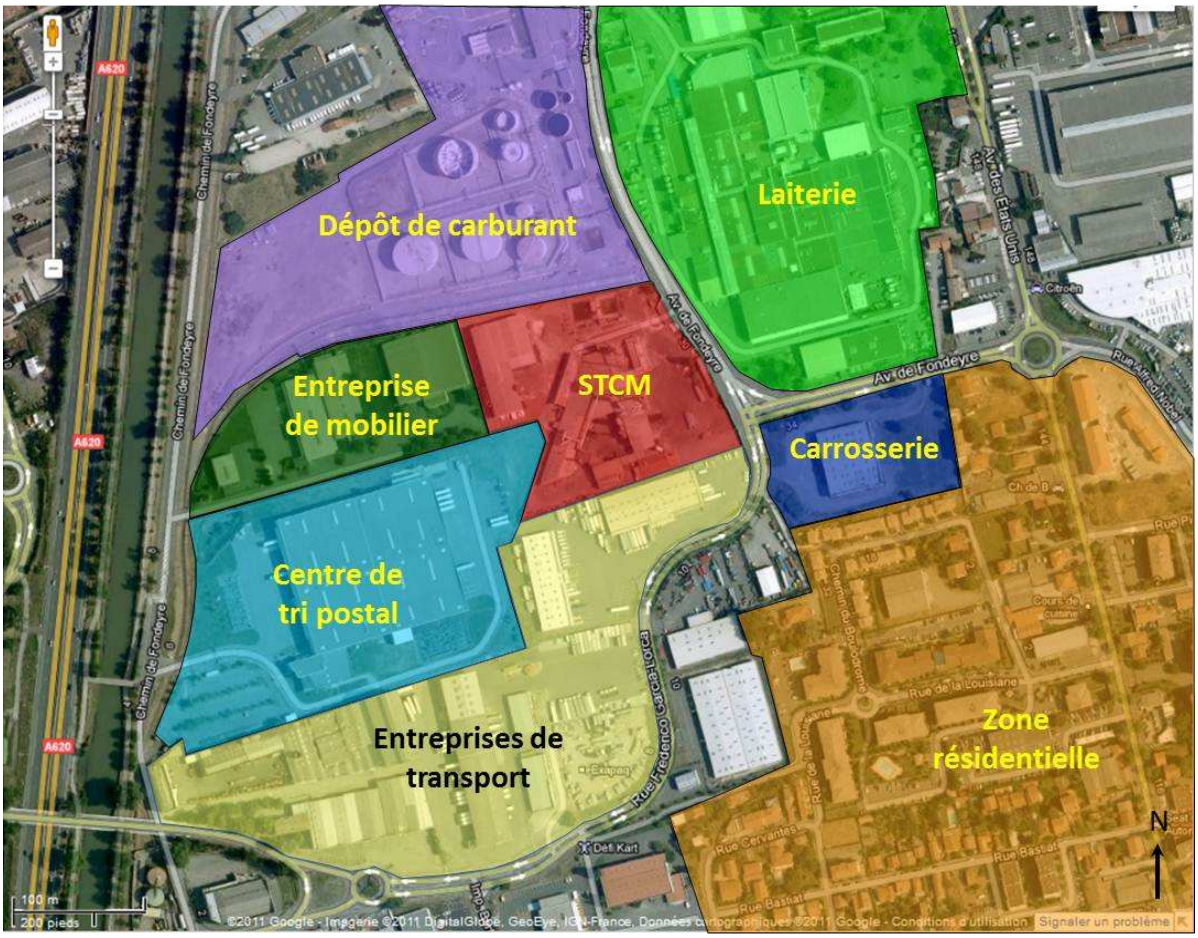

Figure I-9: Plan de situation du site STCM de Toulouse et des infrastructures situées à proximité. (Source : Google Map pour le fond de carte)

Pour choisir la démarche de réhabilitation la plus pertinente, un diagnostic de l'état initial de contamination du site a été réalisé. Sur ce site de 24 000

m^2, plus de 60 forages, d'une profondeur de 0 à 3-4m et un maillage de 10 m, ont été effectués: pour chaque colonne de sol récupérée, les teneurs en Pb, As et autres ETM ont été mesurée tous les 25 cm en surface par fluorescence X de terrain (calibration avec des mesures de concentrations totales effectuées sur minéralisas HF par ICP-OES), soit 450 mesures. Ces résultats ont permis d'obtenir une cartographie des niveaux de contamination et donc des zones les plus sensibles. L'analyse des données obtenues permet d'effectuer les constats suivants : i) une surface importante de l'usine est impactée par une pollution en plomb des sols superficiels ([Pb]>1000 mg.kg^{-1} en moyenne), ii) les zones concernées par la présence actuelle ou ancienne d'acide, présentent des pollutions plus intenses et plus profondes (ancienne zone de casse des batteries, zones de fissures, etc.) ([Pb] = de 10 000 à 7 000 mg kg^{-1} entre 0 et 4m), iii) les zones non couvertes sont systématiquement fortement impactées par la présence de plomb ([Pb]>35 000 mg kg^{-1} entre 0 et 25 cm), iv) malgré des teneurs élevées dans les sols, le plomb est proportionnellement peu mobile.

Les conclusions de ce diagnostic révèlent également que la réhabilitation concerne 18 300 tonnes de terres contaminées. Compte-tenu du contexte environnemental et sociétal du site, l'excavation des terres semble inévitable. De plus, au vu des usages futurs du site (servitude d'utilité publique dans le contexte du PPRT interdisant toute activité), la valeur de 500 mg$_{Pb}$ kg^{-1} de sol a été retenue comme seuil de dépollution.

Pour optimiser les coûts de dépollution, un criblage des terres s'impose. Des tests de tri granulométrique par tamisage (voie sèche ou humide en fonction de la taille) suivis d'une analyse des concentrations totales ont permis de montrer que la fraction granulométrique la plus polluée est inférieure à 4 mm. Néanmoins, comme un criblage de ce diamètre nécessite d'être réalisé sous eau, augmentant les coûts de traitement (prix de l'eau et du traitement des eaux usées), le seuil de séparation 10 mm qui permet d'obtenir 30% de refus semble le plus pertinent. Toutefois, l'intérêt du site est de posséder une installation de traitement des eaux et des métaux. Par ailleurs, si la concentration en plomb libéré par lixiviation est supérieure à 0.5 mg$_{Pb}$ kg^{-1}, alors les terres concernées sont considérées comme des déchets. Dans le cas contraire, et si la concentration totale en plomb sur matériau brut est inférieure à 500 mg$_{Pb}$ kg^{-1}, alors elles peuvent être utilisées comme remblais sur le site.

Concrètement, le projet de réhabilitation de ce site se déroule en trois phases:

i) Démontage des installations, déconstruction des bâtiments et démolition des bétons,

ii) Phases d'excavation et de criblage des terres à 10 mm,

iii) Remise en état du site, remblais et couverture du sol.

Grâce à une gestion raisonnée des terres contaminées passant par un criblage efficace, la STCM espère ainsi réduire de 10 à 25% le montant initial prévu dans le cas d'une mise en décharge généralisée.

1.3.2.5. Conclusions et perspectives

Le tri granulométrique est donc une étape indispensable à la gestion raisonnée des terres excavées issues de sites pollués par les ETM. Dans un contexte de développement durable, la valorisation des terres excavées lors de la réhabilitation des sites industriels contaminés par les ETM est en effet une filière à privilégier.

Par ailleurs, en France, les critères de mise en décharge s'appuient actuellement sur des tests chimiques de lixiviation qui permettent d'évaluer la fraction mobilisable des ETM susceptible de présenter un risque pour les écosystèmes.

► La mise en décharge n'est pas orientée par des tests écotoxicologiques. Pourtant, l'utilisation de biotests (Daphnies, Microtox®, etc.), apparait pertinente car elle permettrait d'intégrer l'impact des ETM sur les écosystèmes et de mieux cerner le lien environnement et santé.

1.3.2.6. Remerciements et autres mentions

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1.3.3. Phytoremediation

Soil treatment includes a set of techniques whose reliability depends on the nature and extent of pollution but also the performance of the method used and its cost. The importance of environmental issues (groundwater pollution and health risks) and economic development has different technologies for the remediation of contaminated sites. In this context, the future use of the site strongly influences the choice of treatment. In order to overcome the main limitations of currently available methods in control strategies (*ex-situ* treatment by biological degradation of organic pollutants, thermal desorption, incineration or washing the floor after extraction), the research focuses in recent years to the use of higher plants. Today, the term phytoremediation is widely accepted and includes a set of methods based on the use of higher plants with the objective of rehabilitation of polluted sites *(Ali et al., 2013)*.

Phytoremediation is the use of plants and associated micro-organisms with them to contain, inactivate, degrade, or remove soil contaminants. It applies especially to chemical contaminants, organic molecules and MTE, harmful to human health and ecosystems. The term "phytoremediation" covers different methods which are rhizo-degradation, phyto-degradation, phyto-volatilization, phyto-stabilization and phyto-extraction. Only the two latter processes are adaptable to MTE, which, by definition, cannot be degraded (*Figure I-10*).

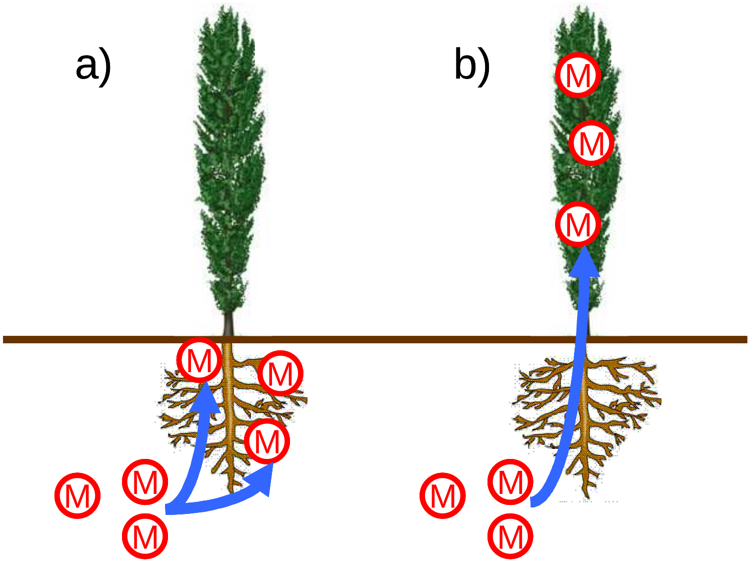

Figure I-10: Principles of phytoremediation: a) Phytostabilization; b) Phytoextraction *(Foucault, 2013)*

The *Figure I-10* above shows the two techniques applicable to MTE phytoremediation: **phytostabilisation is their fixing to the roots and phytoextraction when they are extracted by plants and migrate to the aerial parts**.

1.3.3.1. Phytostabilisation

It reduces chemically or biologically available fraction of contaminants in the rhizosphere soil, storing them in the root system or promoting its insolubility in the rhizosphere. The establishment of perennials on the ground to limit the spread and transfer of pollutants to other media *(Alvarenga et al., 2008; Kumpiene et al., 2009)*, indeed, the contribution of vegetation on polluted soil reduces the risk of erosion and leaching. In this case, it is not desired extraction of pollutants but keeping them in the root environment. Plants with an extensive root system are preferred to increase the contact surfaces of the soil-plant. This approach aims to reduce the risk of transfer of pollution outside the contaminated soil *(Bolan et al., 2011)* and at the same time enables a better landscape integration for sites with large volumes impossible to store in Class I landfill. Plant candidates for a process phytostabilisation must have several properties, including an ability to colonize the site easy, rapid growth, be tolerant to contaminants, low metal translocation to the upper parts, a long life cycle, high levels reproduction and commercial benefits / economic profitability contaminated site *(Mench et al., 2005)*. This technique can be improved by making amendments to soils can reduce the mobility and availability of MTE or fertilize the soil. The purpose of adding amendments is to increase the sorption of contaminants on solid ground bearing phases or promote physico-chemical reactions (eg pH increase) or biological that decrease the labile pool of contaminants including the soil solution *(Gupta & Sinha, 2006)*. Several reaction mechanisms are possible, the sorption - adsorption, reaction acid - base, precipitation, ion exchange, complexation or redox *(Ademe, 2012)*. Many materials are known for these properties, such as beringite *(Mench et al., 2003, 2006)*, fly ash *(Gupta and Sinha, 2006; Pourrut et al., 2011)*, montmorillonite *(Huang et al. 2010)*, zeolite *(Shi et al., 2009)*, iron shot *(Mench et al., 2003, 2006; Kumpiene et al., 2011)*, organic matter *(Mench et al., 2003; Shi et al ., 2009)*, carbonate or phosphate products *(McGowen et al., 2001; Hettiarachchi & Pierzynski, 2002; Shahid et al., 2013)*, oxides and hydroxides of aluminium, iron and manganese *(Hettiarachchi & Pierzynski, 2002)*. The use of higher plants in the control strategies is considered today a growing interest. On the North American continent, phytoremediation techniques are particularly useful, twenty patents filed during the past 7 years. Some of them protect a specific methodology and have international coverage *(Raskin et al., 1994, Chaney et al., 1998)*, while others relate to technical developments or the use of plant species.

1.3.3.2. Phytoextraction

It is based on the use of accumulator plants which absorb MTE present in soils and selectively accumulate in the aerial organs easy to harvest *(Arshad et al., 2008; Zhao & McGrath, 2009)*. These plants must have a large biomass, be competitive and tolerant to contamination and adverse agronomic conditions *(Pilon-Smits, 2005)*. This technology is effective for a wide variety of MTE (Pb, Cd, Ni, Zn, etc.) and is now offered by many companies with a potential market for Europe and the U.S. from 1.2 to 1.4 billion U.S. $ for the treatment of polluted sites by MTE *(Lewandowski et al., 2006)*. This technique is complemented by the concept of induced phytoextraction. It is based on the use of plants with high biomass and rapid growth. The accumulation of trace elements is stimulated by the addition of a natural organic chelating agent (amino acids, organic acids, etc.) or synthetic (ethylene diamine tetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), etc.) to bioavailability increase the targeted element *(Evangelou et al., 2007; Komárek et al., 2010)*. New approaches are moving towards the use of genetically modified plants, such as *Nicotiana tabacum*, *Brassica oleracea*, *Populus tremula P. alba*, etc., stimulation with several functions such as the activity of membrane transporters, the production of enzymes of sulfur metabolism and the synthesis of intracellular chelators such as metallothioneins or phytochelatins *(Sappin Didier et al., 2005; Kotrba et al., 2009)*. One disadvantage of this technique is the creation of a new source of exposure with risk of entry of contaminants into the food chain and potential return to the ground with the falling leaves and the formation of the litter *(Stoltz & Greger, 2002)*. Thus, Blaylock et al. *(1997)* have shown that the use of a method in *Brassica juncea* assisted phytoextraction results in the production of 6 t.ha^{-1} organic waste which may contain from 1 to 1.5 g of Pb per kg dry matter. One of the first steps of treatment of these wastes is the reduction in volume by composting or compaction necessarily with the anticipation of a leachate collection system *(Raskin et al., 1997)*. The biomass is then treated mainly by thermo-chemical *(Ghosh & Singh, 2005)*. The final product can be stored in a landfill or undergo liquid extraction of MTE in ash *(Sas-Nowosielska et al., 2004)*.

1.3.3.3. Hyperaccumulator plants

To establish, develop and reproduce on soils rich in metal(loid)s, metallophytes species have developed different strategies to tolerate, accumulate and carry excess trace elements in the soil *(Remon, 2006)*. According to the physic-chemical soil parameters, the nature of the metallic elements, their contents and their speciation, plant species, varieties and processes involved in the rhizosphere, the mechanisms involved are different in plants *(Shahid et al., 2012)*.

The accumulating plants are characterized by high biomass and large roots to increase their contact area with the ground and storage of trace elements. Metals and metalloids are absorbed preferentially concentrated in the aerial parts of the plant *(Faucon, 2009)*. The accumulation is then accompanied by MTE detoxification of many molecular processes that often direct to the complexation of pollutants with organic molecules to facilitate their transfer or storage. Different mechanisms are involved in the accumulation of metals and metalloids in plants, such as the ability to mobilize MTE soil to absorb into the root system, the sequestration part in the vacuoles of root cells, transferring them to aerial parts *via* the xylem to allow storage in the vacuoles of leaf cells *(Clemens et al., 2002; Verbruggen et al., 2009)*. The use of this type of plant phytoextraction has the advantage of producing a greater amount of biomass for better absorption of trace elements and in the case of recovery, a higher production. A study was conducted on different cultivars of *Salix* for phytoextraction of Cd, Cu, Hg, Pb and Zn in a contaminated site and showed a specific absorption capacity metal and the variety of Salix used, but production relatively high biomass *(Mleczek et al., 2010)*.

The hyperaccumulator plants have two essential characteristics. On the one hand, a translocation factor (TF), the concentration ratio of MTE in leaves concentration in the roots, which is greater than 1, and secondly, they concentrate pollutants metal concentrations at least 10 times more important than accumulating plants and often greater than 0.1% of dry matter excepted for Cd and Hg *(Zhao, 2009)*. Most hyperaccumulator plants do not accumulate the MTE similarly. Thus, *A. halleri* species is a hyperaccumulator of Cd and Zn in the leaves but accumulates mainly Cu and Pb in the roots *(Dahmani-Muller et al., 2001)*. The term hyperaccumulator species has also been defined as a plant capable of accumulating in the leaves of higher levels MTE *(Baker et al., 2000; Reeves & Baker, 2000)*.

To date, approximately 400 plant species have been identified as hyperaccumulators of a given metal or metalloid, representing only 0.2% of all flowering plants *(Brooks, 1998; Baker et al., 2000)*. In fact, few plant species are recognized today as hyperaccumulators of As *(Gonzaga et al., 2006)*. The majority of known species are ferns of the family and *Pteridaceae* first *Pteris vittata* L. *(Ma et al., 2001, Wang et al., 2007)*, followed by L. *Pityrogramma calomelanos* *(Francesconi et al., 2002)* and many other species of the genus *Pteris*, as *P. cretica* L. *(Zhao et al., 2002, Wang et al., 2007)*, *P. longifolia* L., *P. umbrosa* L.

Most hyperaccumulator plants are characterized by a low rooting and slow growth, which makes them less effective for large-scale use in relation to the species accumulating high biomass *(Prasad & Hagemeyer, 1999)*.

At present, the main obstacle to the development of phytoremediation processes lies in the limited number of species usable on an industrial scale. Indeed, the selection of species metallophytes depends on the type of pollutant, but also culture conditions (climate and soil) and the characteristics of the selected species (perennial or perennial, non-invasive, etc.). All these parameters are difficult to control and represent a further limitation of the process.

► Therefore, it is necessary to continue research to select new plants tolerant and / or accumulating and understanding their behaviour on contaminated site. Moreover, in addition to the study of metal(loid)s behaviour during the process of phytoremediation it appears important to also study the global impoving of soil characteristics and environmental and sanitary risks.

1.4. Objectives of the thesis

Accordingly to the global scientific context precedently described, the overall objective of this thesis is the development of a rational strategy for the rehabilitation of the industrial polluted sites, especially for the management of lands for a landfill limited in particular in class I site (hazardous materials). Actually, the rehabilitation of brownfields located in urban areas is a key issue in terms of sustainable development. Industrial practices lead to the sometimes radical transformation of the original ground and cause a wide variety of situations depending on the multiplicity of practices. In addition, due to the complexity of the phenomena involved in the transfer of substances into the environment an extensive characterization of soils and a multidisciplinary (physical chemistry, ecotoxicology and toxicology) polluted areas are essential. The knowledge gained so far in the rehabilitation of sites are often scattered from one-off case studies. The data are of variable quality and often reflect partial knowledge transfer in soils and the toxicity of substances.

As part of this thesis, this work aims to identify the sustainable management strategy that best suits to the polluted sites in urban areas through: (i) a synthesis of literature remediation techniques and management of contaminated soils, (ii) scientific experiments both on laboratory and field measurements of total concentrations, measurement of bioavailable fractions of metals and parameter measurements of soil quality (soil respiration, microorganisms characterization, etc.) before and after phytoremediation treatment.

► In this scientific, regulatory and industrial contexts, the phD aims to:

1) Establish a rational management of excavated polluted soils

2) Assess risks for environment by using (eco)toxicological tests and develop a methodology to classify the polluted soils more finely than chemical tests.

3) Study the use of new “green manures crops” for the phyto-restoration of global soil quality from environmental, agronomical and sanitary points of view.

4) Understand the role of rhizospheric microorganisms on MTE mobility.

The following figure presents the context in which the PhD was performed and the different questions which are discussed in this work. The objectives for these different questions are detailed in the following paragraph, the first having been treated in § 1.3.2.

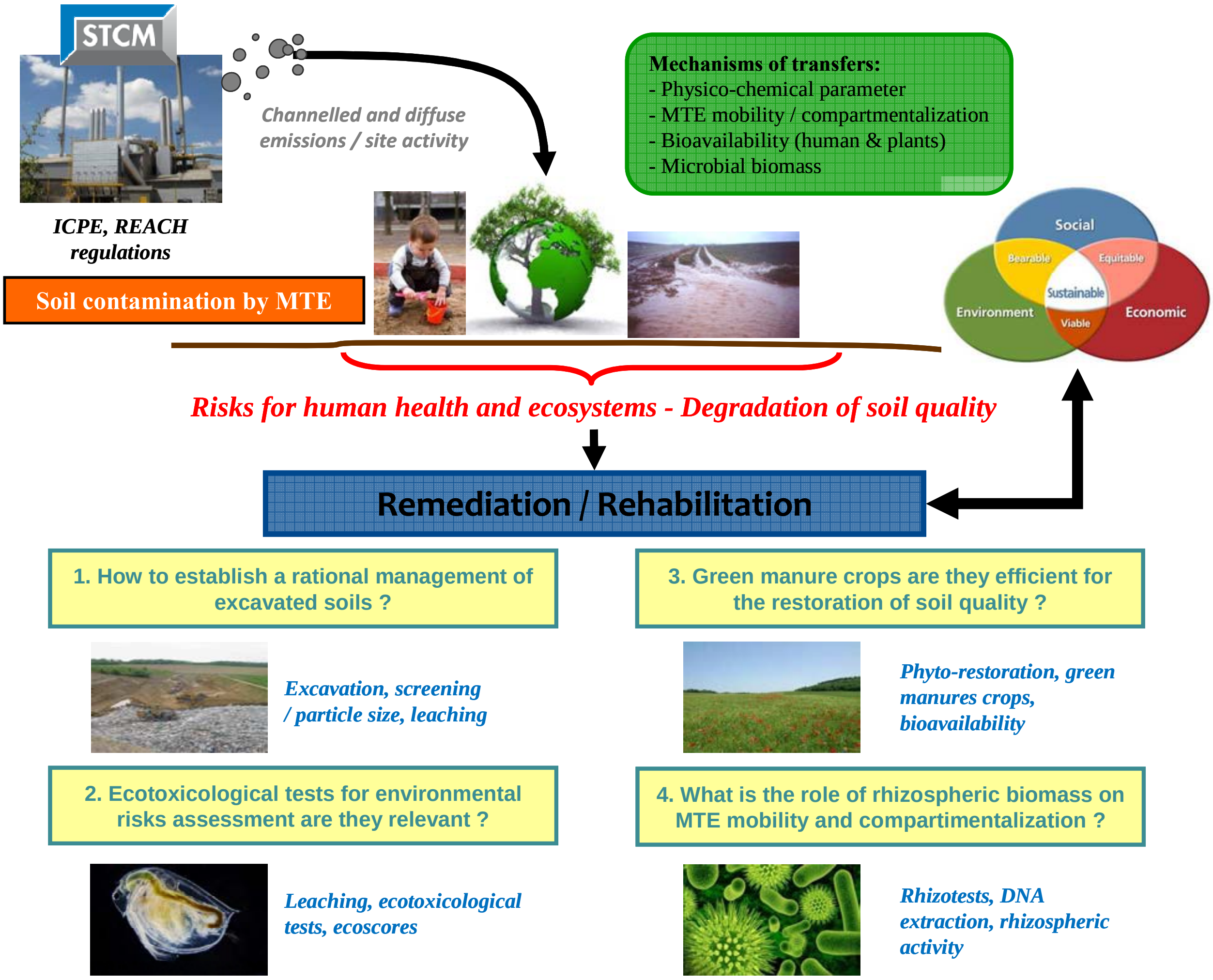

Figure I-11: Representation of scientific objectives (1, 2, 3, 4) covered in this thesis

1.4.1. Use of ecotoxicity tests to improve landfilling

Given the specific absorption systems organizations and the ability of some organizations to change their immediate environment, the total chemical analyses of contaminants in soil and soil materials do not provide information on the bioavailable fraction. Biological tests can be used to evaluate the effect of the bioavailable fraction of all pollutants in soil or soil material.

For information on the potential effects of pollutants on specific organisms and subsequent effects on the biota or habitat function of soils, it is necessary to conduct studies with representative organisms.

Scientific objective n°1

Risk assessments are based on total concentrations and on a description of the physico-chemical parameters of soils, but do not inform on bioavailability and ecotoxicity of MTE in relation with their compartmentalization.

The first objective was to test the efficiency of ecotoxicity tests to improve the classification of polluted soil samples in terms of environmental risk assessment.

In addition to current physicochemical tests, the actual leachate ecotoxicity of 10 soils sampled from the industrial polluted site was therefore evaluated using different complementary tools.

Ecotoxicity tests on micro-organisms have been carried out in collaboration with different laboratories.

1.4.2. Restoration of soil quality

Many studies and experiments show that among the techniques of remediation of soil polluted with metals, physico-chemical processes (physical sorting, washing, stabilization, etc.) are widely used *(Colombano et al., 2010)*. However, due to high processing costs, and a rational approach to managing soil biological techniques have been developed in recent years

Thus, phytoremediation techniques are an excellent alternative to physico-chemical processes generally used for the remediation of contaminated land as financially more attractive and "green" *(Kavamura & Esposito, 2010)*. Two techniques are particularly suitable for metals: (i) phytoextraction, which uses a plant's ability to remove pollutants from soil *(Wei et al., 2008)*, mainly as bioavailable *(Colombano et al., 2010)*, using biomass of the soil and store them in the aerial parts *(January et al., 2008; Butcher, 2009)*, (ii) the phytostabilization, which is to capture and stabilize contaminants in the rhizosphere through root systems of plants *(Velasquez* et al., 2006). These techniques reduce the toxicity of metals (Gwodz & Kopyra, 2003), and therefore the risk of human exposure by reducing the bioavailability (Butcher, 2009; Scheckel et al., 2009).

The amount of contaminants absorbed or stabilized and accumulated by plants is generally based on the physicochemical characteristics of these substances, soil type (and its parameters), the climatic conditions and plant species chosen (which can change the properties rhizospheric soil) (Arthur et al., 2005; Arshad et al., 2008; Robinson et al., 2009).

Usually used in agriculture or gardeners, green manures improve soil biological activity and some of its properties (structure, pH). Moreover, the mechanisms involved are very similar detoxification mechanisms of plants commonly used (concentration of polyphenols in the roots and high biomass) (Chin et al., 2009), and can potentially reduce the bioavailability and bioaccessibility of metals. Adapted to a temperate climate, low maintenance requires that these cultures and their ability to grow easily, now lead us to consider their use as adjunctive therapy for the remediation of soils contaminated with metals Unlike, for example, maize (*Zea mays*) and sunflower (*Helianthus annus*) that need to make the most of chemical fertilizers, pesticides, and especially large amounts of water for their development (Evangelou et al., 2007).

The second scientific goal of the PhD was to use three species of green manure (borage, *Borago officinalis*, phacelia, *Phacelia stala* and white mustard, *Sinapis alba* L.) to modify some physico-chemical properties of soils impacted by the benefits derived from activities second merger in order to restore and improve quality. Individuals in the vicinity of these sites, and therefore subject to these benefits, can also enjoy this invention to enable them to reduce the health risk in their own gardens by reducing the bioavailability while improving soil quality also.

Today, costs of treating land contaminated by heavy metals range from 50 to over 200 €/t. In contrast, with costs ranging from 2 to 10 €/m², it is clear that phytoremediation techniques are financially more attractive.Besides the financial aspect, these decontamination techniques also allows the industry to identify a good brand image and shows its commitment to integrate the environment in the heart of its concerns.

In addition, the cultivation of the plants has already been developed in many gardeners for their properties (improved soil quality). The ease and speed of culture is not foreign and is very suitable, in fact, both the gardens exposed to heavy metal pollution, as industrial sites themselves, while requiring little maintenance. On the other hand, such a cover can limit re-flying of dust, leaching and soil erosion, and reduces the risk of exposure. The benefits of these crops on soil quality are numerous: improved soil structure, organic enrichment, protection of crops against weeds, nitrogen fixation, etc. Finally, these plants are also interesting for their medicinal properties or taste, and make pleasant gardens at the time of flowering.

In a society where the concept of sustainable development is unavoidable, this technique seems to be made a place among the techniques more expensive and less "green".

Scientific objective n°2

The purpose of this part is to test the efficiency of different green manure plants *(borage, Borago officinalis, phacelia, Phacelia stala and comfrey, Symphytum officinale) to modify some bio-physic-chemical properties of soils affected by spillovers from the activities of a second fusion, in order* ***to restore and improve soil quality****. Moreover, in addition to environmental risks, sanitary risk were also assess throughout the measure of pollutants bioaccessibility.*

Neighbours of these sites, and therefore subject to these benefits, could also benefit from these cultures to reduce the health risk in their own gardens by reducing the bioavailability while improving soil quality also.

1.4.3. Role of rhizospheric micro-organisms

Understand the interactions between pollutants and soil components, describe the flow of pollutants into plant organs as well as the fate of pollutants in the rhizosphere could permit to improve the use of plants for polluted soils refunctionalization. Actually, if this phD research aims application in phytoremediation, it’s also a way to better understand the dynamics of pollutants in all cultivated ecosystem.

Control of phytoextraction and phyto-stabilisation requires a description of the processes involved in bioavailability of soil pollutants. These processes take place in the soil under the influence of roots, the rhizosphere. The physical, chemical and biochemical differ from the rhizosphere soil by the roots not colonized because of the growth of these, their removal of water and solutes, respiration and rhizo-deposition. The rhizosphere is thus characterized by a high concentration of organic carbon, high biological activity and pronounced variations in pH and moisture.

Scientific objective n°3

Plant use can improve soil quality by producing roots exudates which influence the rhizospheric living biomass. This living microorganism biomass can in turn secrete chemical compounds which can modify MTE speciation and thus their mobility.

In this part, ***investigations were conducted to determine effects on the rhizospheric microbial biomass and MTE behavior****.*

Chapter 2

Materials and Methods

► During the thesis works, experiments of soil characterization, (eco)toxicology tests, as well as measurement of soil-plant transfers were conducted. This chapter describes all the techniques and protocols used. Further details are provided in the various chapters targeted.

2.1. Presentation of the study site and its environment

During the present phD research scientific approach was developed with the aim to adquiere new data to improve the management of polluted soils. As a application of the scientific questions the work had beneficied of a partenariat with the STCM company. This part first aims to provide information on the activities of the company partner of the thesis. Why this company should conduct studies on its impact on the environment? Several elements in this chapter answer this question: regulatory aspects, the situation of the plant in urban areas, etc.

2.1.1. The Société de Traitements Chimiques des Métaux (STCM)

2.1.1.1. Presentation

STCM is specialized in the manufacturing and trading of secondary lead, developed in its processing by the recycling of lead batteries and other lead materials (scraps, waste, oxides, etc.).

STCM produces soft lead and lead alloys (calcium - antimony - tin). With 65,000 tons of lead per year, STCM is the leading producer of recycled lead in France. The main markets are the batteries, ballistics, ionizing protections building.

Established in 1952, STCM began operations in Toulouse. A second factory was established in Bazoches-Les-Gallerandes (Loiret) in 1967. Initially, family-oriented company, STCM was bought in 1993 by the German group Metallgesellschaft then in 1996 by the American group Quexco, through its UK subsidiary ECO-BAT Technologies. In June 2001, STCM integrated Gast plant located Pont Sainte-Maxence in Oise belonged to Exide Group, a manufacturer of batteries. Since June 2001, the plant continues to operate under the name APSM (Affinerie de Pont-Sainte-Maxence). Toulouse and Bazoches plants operate under the name of STCM, Pont Sainte-Maxence plant operates under the name of APSM (ingots keeping their brand Gast).

The headquarters Holding STCM includes activities of STCM and APSM. It is located in Bazoches and includes human resources activities, sales, purchasing and finance.

2.1.1.2. Group synergy

ECO-BAT Technologies PLC Headquarters is located in Darley Dale England, near Manchester. ECO-BAT Technologies depends on its parent company, a holding company headquartered in DALLAS, United States.

The ECO-BAT Group has 14 production lead: one in South Africa, three in the U.S. and seven others in various European countries (Italy 2, Germany 3, Austria 1, England 1) and three French factories. Apart from a German factory that produces primary lead, other factories are specialized in the secondary lead.

The society also produces laminated lead in France through the factory Le Plomb Français located in Estrees-Saint-Denis, but also in the United Kingdom and South Africa. ECO-BAT has also diversified its business around the life cycle of the battery with its collector companies in the UK and Germany, but also through its subsidiaries distributing new batteries in the UK and Benelux.

STCM is integrated into the group and benefits from research and technical resources provided by RSR Technologies, a subsidiary of ECO-BAT dedicated to problematic processes / products lead.

2.1.1.3. Quality – Safety – Environmental politic

Political Environment - Health - Safety of ECOBAT group work is coordinated by a manager in the field of Health, Environment, and Safety.

The STCM is certified ISO 9001 and ISO / TS 16949 (at the request of cars customers) since August 2003 for its management system of quality (SMQ, *Système de Management de la Qualité*). The ISO 14001 certification was obtained in September 1999 and re-certified together with the OHSAS 18001 certification since December 2002 for its environmental, health and safety management systems (respectively SME, *Système de Management de l’Environnement,* and SMS, *Système de Management de la Santé et de la Sécurité au travail*). All these certifications are grouped in an integrated management system (*SMI*, *Système de Management Intégré*).

2.1.1.4. Manufacturing process and generated products

The process employed by the STCM in the factory in Toulouse is a complete process of development of lead from lead-acid batteries and materials coulters (*Figure II-1*).

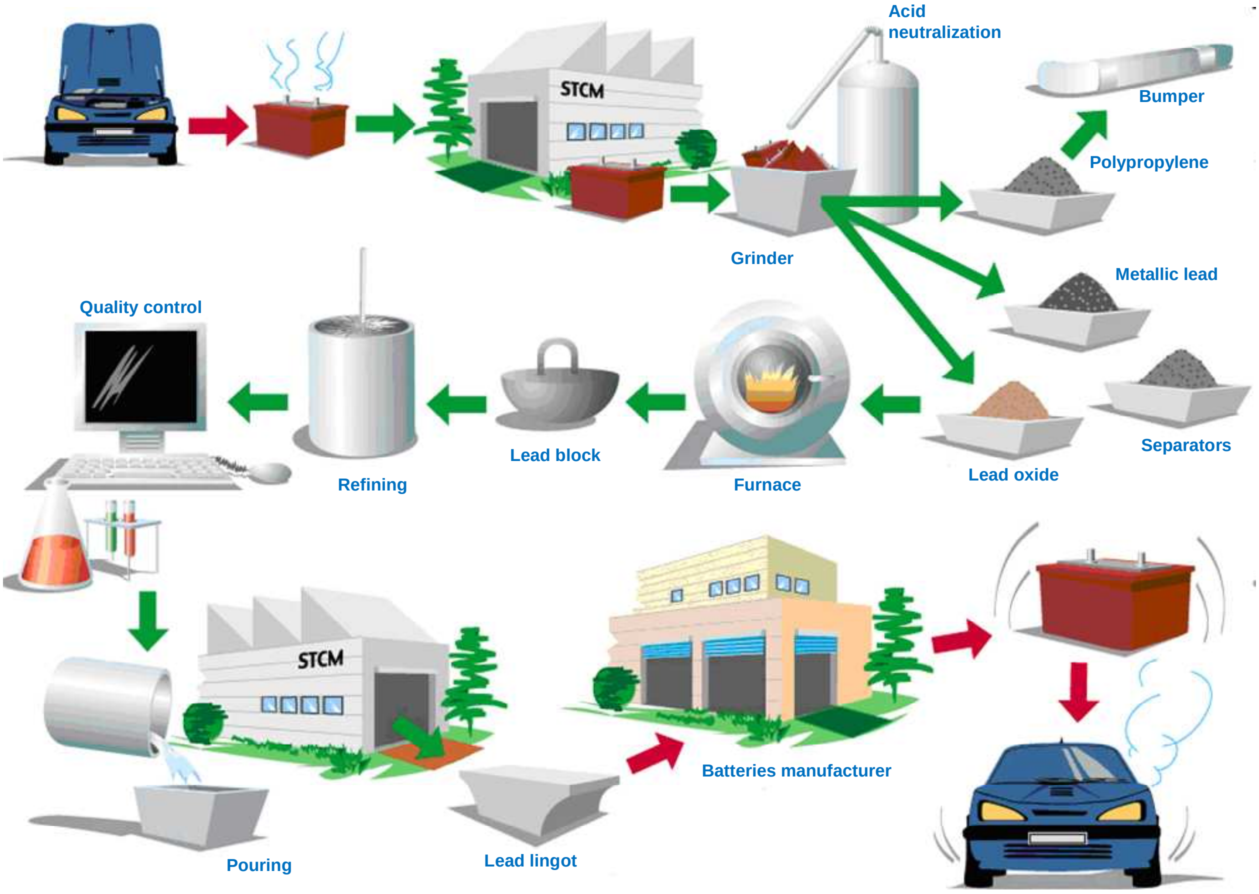

Figure II-1: Life cycle and recycling of lead-acid batteries

Actually, the emissions of lead in the atmosphere with possible fallouts on the soils are strictelly surveyed. However historical pollution needs to be managed nowadays due to old emissions in particular during the steps of battery recycling (during grinding, reduction in furnaces or refining) or with re-flying.

2.1.1.4.1. Reception of raw materials

The batteries are stored in bulk on a covered area with slopes and ground treatment used to collect all the runoff (water and sludge laden with metals and acid) which are then processed in a water treatment plant at the factory internal (capacity of 300 m^3/day). The raw materials are mainly car batteries works with an electrode lead wire and an electrode lead dioxide (PbO_2), and old pipes, ducts son, etc., this lead does not undergo the first stage of grinding, but is directly introduced into the furnace.

2.1.1.4.2. Grinding of batteries.

The batteries are loaded into the hopper of the crusher; after verification of the absence of foreign conflict (including nickel-cadmium batteries), the shreds pass on a grid where the materials are separated by density and recovered:

- *Lead paste from the active material of the battery: it is sulphate and lead oxides (PbO, $PbSO_4$, etc.) and metal(loid)s (Ca, Cu, Sb, As, Sn, etc.) very wetlands that are decanted and dried;*
- *Metallic lead grids, terminals and connections, mainly Pb and Pb alloys;*
- *Polypropylene (PP) of the casing of the battery;*
- *Separators, that is to say the strings, boxes, etc., in the batteries, which are non-recyclable waste;*
- *Fines: it is non-metallic parts which lead is still connected. These parts are from a screening of the pulp and plastic particles;*
- *Acid electrolyte, not valued as highly diluted, neutralized locally by the following reaction:*

$$H_2SO_4 + Ca(OH)_2 \rightarrow CaSO_4 + 2H_2O$$

The Pb metal and oxidized Pb are stored in the lobby of composition of the charges, while the PP is kept in a box before being sent to the recycling plant. Other plastic waste is evacuated to a landfill.

2.1.1.4.3. Fusion and reduction

Since December 2011, this operation is not realised any more in Toulouse, but still exists in the other plants (see § 2.1.3.).

Fusion and load reduction are carried out in rotary furnaces, heated by gas burners (oxygen in sur-oxygenation, temperature 800 °C). Each oven is completely overturned by a hood connected to a purification filter that avoids the emission of fumes in the workshop. The treatment of these two materials will be made in part because the mixture grids and clusters containing about 10 % of sulphates and oxides must be reduced. Addition of different elements in furnaces is also used for these treatments:

- *Carbon (C): use as carbon source for burning;*
- *Iron (Fe): use to neutralize S;*
- *Na_2CO_3: use to decrease the fusion point and to neutralize S too.*

Finally, this process takes place along the two following reactions:

- *Reduction of oxides*

$$2PbOx + xC \rightarrow 2Pb + x/2\ CO_2 \text{ with } 0.5<x<2$$

- *Reduction of sulphates*

$$PbSO_4 + Fe \rightarrow FeS + Pb + O_2$$

$$2C + 2O_2 \rightarrow 2\ CO_2$$

$$PbSO_4 + Fe + 2C \rightarrow FeS + Pb + 2CO_2$$

and

$$PbSO_4 + 2Na \rightarrow Na_2S + Pb + 2O_2$$

$$Na_2CO_3 \rightarrow 2Na + CO_2 + \tfrac{1}{2}O_2$$

$$3C + 3O_2 \rightarrow 3CO_2$$

$$PbSO_4 + Na_2CO_3 + 3C + \tfrac{1}{2}O_2 \rightarrow Na_2S + Pb + 4CO_2$$

Sulphur is determined by the iron oxides which are reduced by carbon to lower the reaction temperature and ensure good separation by melting metal; the two reactions are carried out in presence of sodium carbonate.

Lead to "work" obtained is 98.5% pure. The next step in refining lead provides "soft" commercial 99.97% pure or different alloys.

2.1.1.4.4. Refining and ingot casting

This activity was stopped in 2009 in Toulouse. This operation was done in four tanks of 50 tons and 3 tanks of 100 tons capacity. Each is equipped with a suction ring designed to capture fumes and dust. The refining operations are in the liquid phase of Pb, between 380 and 650 °C. They are designed to remove impurities from raw Pb, adding other elements (Sn, Cu, Sb, etc.) needed to obtain an alloy in accordance with the technical specifications required for the manufacture of new batteries (or for radioprotection, bullets production, etc.). The entire process is continuously monitored by the control laboratory of the plant that, when the alloy is within specification, triggers the casting or ingot casting. This is from the refining kettle by pumping the liquid metal (450 °C), an automatic chain of beds. The molds are cooled by water in a closed circuit. The obtained ingots weighing 35 kg are numbered and automatically deposited in bundles of 35 ingots.

2.1.2. Site environment

2.1.2.1. Industrial environment

At its inception in 1952, the plant in Toulouse was in an area remote from any dwelling. Over time, an industrial area has developed, making the north of Toulouse the industrial centre of the city where the plant is nowadays located. The site, which covers an area of 23,650 m², is now surrounded by a fuel depot classified as a Seveso site, a dairy and subdivisions among the buildings more sensitive (See *Figure I-9* in § 1.3.2.3). This status implies prevention and risk management and security based on hazardous materials stored on site. These measures embody the "best practices" in risk management: introduction of provisions on land use to reduce the consequences of major accidents, taking into account the organizational aspects of safety, improving the content of the safety report, strengthening the participation and consultation of the public. This status Seveso also includes the development of a safety report (hazard study in French law) which can then serve as a basis for emergency plans, control of urbanization, etc.

2.1.2.2. Environmental vulnerability

2.1.2.2.1. Soils diagnostic

In 2007, the consulting firm Aquila made a diagnosis of the soil pollution. This study includes key information such as site history, a study of vulnerability, etc. To make this work, approximately 400 soil samples were collected, geo-referenced and analysed, thus constituting an excellent initial database as part of the thesis.

2.1.2.2.2. Sampling program

Based on a mesh of drilling every 20 m, the sampling required no fewer than 66 wells. During this work, several surveys have characterized the best level of pollution:

- *A detailed description of horizons met (concrete slabs or asphalt, silt, etc.) was conducted to determine soil characteristics considering a possible transfer of pollutants, and so to anticipate a possible excavation for pollution.*
- *Observations of organoleptic indices of pollution (colour, suspicious odours, evidence of pollution, etc.) were noted and correlated with the facies encountered.*
- *A profile of the metal content in the different levels was performed using a portable X-ray fluorescence apparatus (µFX). The depth distribution of content allowed approaching the distribution of pollutants or anthropogenic fill in laterally or vertically by gravity.*

Complementary samples were chosen for laboratory analysis: (i) to obtain correlations with measures of fluorescence-X; (ii) characterize any organic pollution detected by sensory cues; (iii) verify the characteristics of soils impacted according to the acceptability criteria in the elimination channels.

2.1.2.2.3. Measurements

The portable analyser X-ray fluorescence used for this study allows obtaining a large range of measures in a short time. The measurement is not longer than 2 min. The apparatus is calibrated to measure the levels of 20 different metals. The threshold measurement for each of these metals can vary and depend on the one hand the intrinsic sensitivity of the unit opposite of each metal, and, on the other hand, the overall composition of the sample (humidity for instance). Thus, the system of the device may have varying degrees of difficulties in measuring energy peaks neighbours and tainting the extent of uncertainty varies according to circumstances. In addition, the direct detection camera of various metals in the sample is a measurement with a window of 1 cm² and it is necessary to ensure the representativeness of the sample to overcome the effect of "nugget". However, this disadvantage is offset by the possibility of a large number of instantaneous measurements and thus to "calibrate" the measurements with field observations. This mode of action differs from the one-time laboratory test in which a soil sample is homogenized and sieved prior to chemical attack for the dissolution of the elements.

2.1.2.2.4. Results

The study of environmental vulnerability of the site identified important points:

- *The site is located above a water table. Pollution that would cross the first three meters can thus directly reach the groundwater. The presence on the entire site of a waterproof cover is a significant factor in preventing the containment of contaminated soil leaching through the soil profile.*
- *In terms of uses, groundwater under the site is not used for drinking water. However the presence of individual dwellings around the site is suspected for watering private, as it is usual in the region.*
- *Although the settlement area of the plant is primarily oriented towards an industrial and commercial occupation, there is the existence of land occupied and cultivated a few hundred meters far from the plant, thus potentially vulnerable to past pollution by air.*
- *In terms of surface water, the site is fully waterproof, storm water are collected and treated before being discharged into the city network. These facilities are subject to regular monitoring by ISO 14001 procedures and are regularly checked by the inspection of ICPE.*

Although the presence of lead in soils is proven, the sealing of the site limits the potential for mobilization to the water but requires monitoring of groundwater and soil environments. It is therefore evident that the effectiveness of this protection is critical in controlling the risks of pollution, either to prevent direct contact with the soil or to limit the training lead to groundwater.

2.1.3. Rehabilitation project

Plans for prevention of technological risks (PPRT) were established by law "Bachelot" of the 30th July 2003 to prevent the risks to safety and industrial sites classified as Seveso II.

The realization of the PPRT of Esso (a gasoline storage site) neighbouring the plant, showed that in case of explosion, a part of infrastructure facilities and buildings of the STCM would not survive to the blast. In this context, and for strategic and economic reasons, STCM made the option of expropriation of the building containing the furnaces which are threatened, and thus, stopped this activity at the end of 2011.

According to the legislation on the cessation of activity of ICPE, and to respect the polluter pays principle, the company must now rehabilitate these soils heavily contaminated with Pb, As, and other metal(loid)s (Cd, Cu, Zn, Sb, etc.).

2.2. Soil sampling and preparation

2.2.1. Selection of sampling points

Thanks to the initial diagnostic realised by Aquila, 10 sample points were defined according to their position on the site relating to past and present activities (furnaces, storages, etc.) or properties (area not covered, infiltration zone) (See *Figure III-2* in § 3.2.2.2.).

Samples were performed using a drill with a diameter of 400 mm. A soil sample was collected between 0 and 25 cm for each drilling, dried, freed of roots and other foreign and sieved at 2 mm. The amount of soil remaining, about 15 tons of soil, were stored individually in containers for further analysis. In the context of the implementation of technical and applied rehabilitation project, and to optimize the sorting of excavated soil, a prototype table for screening soil was prepared with grids of 5 cm, 2 cm and 1 cm, followed by a manual sieving to 2 mm.

Figure II-2: Phase of soil sampling: drillings, prototype of screening table and storage.

2.2.2. Soils preparation and physico-chemical characterisation

For sampled soils, a complete characterization of physico-chemical parameters was realised by Galys, an independent laboratory in agronomy and environment, approved by Franch state under COFRAC accreditation. This characterisation involved measures of pH, CEC, organic matter and carbonates contents, granulometry and determination of total concentrations of Pb, Sb, As, Cu, Cd, and Zn. The *Table II-X* below presents the technique used for each measure the results are available § 3.2.3.1.

Parameter	**Technique**
pH	ISO 10390
CEC (Metson)	NF X 31-310
OM	ISO 10694
$CaCO_3$	ISO 10693
Granulometry	ISO 11277
MTE (Pb, Cd, As, Sb, Cu, Zn)	ISO 11466

Table II-1: Measured parameters and corresponding normalized technique

2.3. Presentation of plant species

2.3.1. Interest of green manures plants

Green manure is a special cultivation technique often used by gardeners to restore the land once fees nutritious.

The culture of green manure has the advantage of fixing nitrogen in the soil and improves drainage on site while retaining the land by limiting erosion due to the dense root system of selected species. Soil structure is improved both physical and chemical and biological. The other benefit of green manure is to prevent the invasion of land by weeds. Sowing is usually done before the first spring planting or falls after harvest.

Several plants are used in the context of green manure. The choice depends on the nature of the ground and the needs of crops. Generally, the most practiced seedlings are made with forage legumes such as clover, alfalfa, or non-legumes such as mustard, phacelia and vetch. Other plants such as rapeseed, buckwheat, rye, cabbage, ryegrass, white lupine, etc., are also used.

In this context, three plant species were selected: borage (*Borago officinalis*), phacelia (*Phacelia stala*) and white mustard (*Sinapis alba* L.) (*Figure II-3*).

Figure II-3: Studied plant species: a) Borage; b) Phacelia; c) White mustard.

2.3.2. Borage

2.3.2.1. Origins

Thought to originate in Syria, borage is now naturalized throughout most of Europe and the United States. Some sources ascribe a North African origin, but it would have reached America from Spain and Morocco, where she was already cultivated in the Middle Ages. It is a common plant found growing in the wild areas of most European and Mediterranean countries, where the climate suits the herb *(El Hafid et al., 2002)*, because it is sturdy, undemanding and easily reproduced. It flourishes as a weed near houses and on rubbish heaps. While many modern gardeners consider it a nuisance, it was traditionally grown in gardens to use as an herb, for its edible flowers, and for its ability to increase the yields of honey. Its name comes from the Arabic *abou rach* (father of sweat), which refers to its properties sweat. In Latin *burra* meaning "long pile fabric" and refers to the hairy appearance of the plant. Its botanical name *Boragocomes* from the Latin *cor ago* which means "I stimulates the heart".

2.3.2.2. Description

Borage is an annual plant measuring between 30 and 80 cm in height. The stem is hollow and hairy. The large leaves are alternate long bristly hairs over their entire surface, which is specific to the family *Boraginaceae*. The green parts of the plant have a strong smell of cucumber *(Haughton, 2001)*. It produces an abundance of small star-shaped flowers from purple to blue, to pink when they begin to decline. They are edible, but must be parts of the calyx hairy sepals and five achenes grouped into four seeds in the center of the flower. Flowering ephemeral renewed daily and usually lasts for two months from mid-July to mid-September. Blackish brown seeds without endosperm mature gradually. The flowers are self-sterile and must be pollinated by insects *(Filière des Plantes Médicinales Biologiques du Québec, 2010)*.

2.3.2.3. Culture

Borage grows naturally on sunny sites and part shade and poor soil. However, she prefers deep soils and well drained. Under suitable conditions, at a cash crop, the plants can reach up to 80 cm in height. At the time of germination, the soil should be kept moist and warm. Borage is not very competitive at the time of germination but proliferates in soil well amended. It can also be propagated by division of rootstocks *(Hoffman 1995)*. During the growth period, the plant can adapt to relatively dry conditions. A water supply of 35 mm per week is adequate for normal soil and can double in sandy soil. A pH between 4.8 and 8.3 is favourable, 6.6 being ideal. Borage appreciates the freshness and has a high resistance to cold. It takes about 8 weeks to flower and flowering may continue beyond the first frost *(Filière des Plantes Médicinales Biologiques du Québec, 2010)*.

2.3.2.4. Uses

Traditionally, a medicinal borage flower is recognized, which is attributed diuretic, febrifuge and sudorific. They are also used to relieve respiratory and skin irritations. Herbalists use the aerial parts, that is to say, the leaves, stems and fresh flowers, infusion, alone or mixed with other plants. However, in 1991, the Commission E has found that there was insufficient evidence to recognize the aerial parts therapeutic efficacy *(Hälvä & Craker, 1996)*. The borage seed oil is also a source of omega-6 *(Health Canada, 2006)*. Some studies have shown the benefits of oil to relieve the symptoms of rheumatoid arthritis. The industry proposes borage oil in two forms: virgin olive oil bottle and capsules for internal use. Found on the different market-based cosmetics borage oils, which are also attributed virtues to tone and soften the skin, reduce blemishes and participate in the hydration process. They are also used as food, so that the leaves can be added to salads and sandwiches, or used as herbs to meet the sauces and soups. As for the flowers, they are assessed confectionery, syrup or as a topping on baked goods. In agriculture, borage plant is very attractive to honey bees. It also has anti-fungal properties, among others, against gray mold, which would make an interesting companion plant for strawberries. For Animal Production, it is used as a tonic *(Filière des Plantes Médicinales Biologiques du Québec, 2010)*.

2.3.3. Phacelia

2.3.3.1. Origins

Phacelia is an herb native to northern Mexico and southern USA (Arizona, California, Nevada). This plant has been introduced very recently in Europe for its quality honey. First introduced back in 1832, its genus name (*Phacelia*) comes from the Greek "*fakelos*" meaning the beam or bundle alluding to the shape of his stick (inflorescence) shaped beam. The variety *stala* was developed in the countries of Eastern Europe to improve the properties of the honey variety *tanacetifolia*.

2.3.3.2. Description

Stem solid and rigid, covered with stiff hairs and thick enough, which can reach a meter, is tinged with red. Its root system is dense. The leaves are alternate, deeply divided and reminiscent of tansy (*Tanecetum*). The beautiful flowers are very fragrant and very interesting special. They are grouped in inflorescences scorpioid (shaped butt that takes place gradually as and for the development of small flowers starting 1 cm from the top) of such tight cyme. Floral elements have shades blue lavender are the five stamens and two styles that emerge clearly from the corolla with five petals. Close to the calyx sepals hairy. The flowering period is summer and may extend fall. The fruits are small capsules dehiscent containing four small black seeds (3 mm long).

2.3.3.3. Culture

Phacelia just needs a little digging and possibly a quick weeding. Its powerful roots are able to unpack the soil and suppress weeds. The earth needs to be scratched on 2/3 inches for the seeds have a seed bed well crumbled. Phacelia is sown on the fly, from March to June. The seeds should be covered through the rake then compacting the soil so that they are in contact with the ground. Watering is recommended if the weather is dry. The plant germs when the soil temperature reaches about 10 °C. Flowering occurs in summer, when the plant is covered with purple flowers. Phacelia is a long-day plant that shows its many beautiful lavender blue flowers 12 mm wide when the days were longer than 13 hours. Typically, flowering begins about eight weeks after emergence and lasts four to six weeks. Then, the flowers give way to four capsules containing seeds each *(Cloutier et al., 1992)*.

2.3.3.4. Uses

Phacelia is now increasingly grown as it has many interests in agriculture:

- *Its flowers attract many insects (the beetle bombyle, or syrphide) who are officers of culture. By feeding on aphids and scale insects, they limit the development of these pests of cultivated plants;*
- *Phacelia is an excellent honey plant that attracts bees needed to pollinate neighboring crops. Honey phacelia is known to be very fragrant;*
- *This plant is very aggressive vis-à-vis weeds. It is capable of removing weeds such as quack grass;*
- *Autumn phacelia is used as green manure rich in nitrogen.*

Before the flowers go to seed, the plant is cut, crushed and buried. In addition, its long roots penetrate the soil depth and to aerate the soil.

2.3.4. White mustard

2.3.4.1. Origins

Like that of many foods, spices or condiments, the origin and the different uses of the mustard is lost in the mists of time. The Greeks attributed its discovery to Aesculapius. The Egyptians claimed to have identified the virtues since ancient times. But it is absolutely known that the Romans were great lovers and the whole Empire was in a usual accompaniment of food. This species is native to North Africa, Europe (excluding the Arctic) and West Asia (Middle East). It is a common plant in France, in the fields and roadsides.

2.3.4.2. Description

White mustard is an annual plant of 20-80 cm with an erect stem and numerous branches springing from the main stem. Leaves are alternately arranged, petiolate and serrated, with short, white bristles along the veins. All its leaves are stalked, terminal division developed, usually much larger than the lateral. The flowers are yellowish with long sepals (7-10 mm) and petals filiform tab. The fruit is a silique spread, 2 to 4 cm long, bumpy, stiff bristle persistent with beak strongly compressed, as long as the valves. Valvaire part contains at most 8 seeds arranged in a row in the silique, pale yellow, brown or purple (Haugton, 2013).

2.3.4.3. Culture

Mustard grows in any soil, even very poor. As green manure, it even has the particularity to fertilize the soil by providing nitrogen. Mustard does not really fear the weed because it grows very fast (and thus occupies the land before the other plants are installed). A quick digging is enough. Sowing is done on the fly, from May to September. The mustard seeds and grows very quickly. It can reach 50 to 80 cm, its adult size in just one month if conditions are favourable. Rather, it puts two months to mature. According to the future use, mustard will not be harvested at the same time *(Pousset, 2002)*.

2.3.4.4. Uses

White mustard is cultivated for four possible applications:

- *The seeds are rich in lipids (about 35%) and produce oil for industrial or food use. They are the basis for the preparation of condiment with the same name, mustard.*
- *White mustard is a forage and honey plant.*
- *It is sometimes planted as a green manure or trap nitrates, that is to say intercropping avoiding leaving fields bare to limit leaching of soluble nitrates. In this case, for example sown after a cereal, it must be destroyed before bolting to prevent it naturally reseed and become a weed, especially in oilseed rape.*
- *It is also useful to kill nematodes, which is not overlooked at the time of the return to using natural techniques. In addition, its powerful roots can break the clods of clay with a very heavy soil, and thus to facilitate the incorporation of humus and soil improvement.*

2.4. Study of transfers and (eco)toxicity of MTE

2.4.1. Use of specific bacterial strains

2.4.1.1. Principe

There are bacterial sensors dedicated to the specific detection of pollutants or pollutant family. For this, bio-elements bacterial strains used are genetically modified. In all cases, these reporter genes are cloned downstream of a promoter allowing to highlight the specific presence or semi-specific for certain compounds in a sample *(D'Souza, 2001; Lei et al., 2006)*. *Figure II-4* the general principle of detection of bacteria changed: in the absence of inducer (chemical specific), the controller inhibits transcription and translation of reporter genes. In the presence of the inductor, it complexes with the regulatory protein causing a lifting of the inhibition of transcription/translation of reporter genes: there is an emission of a detectable signal.

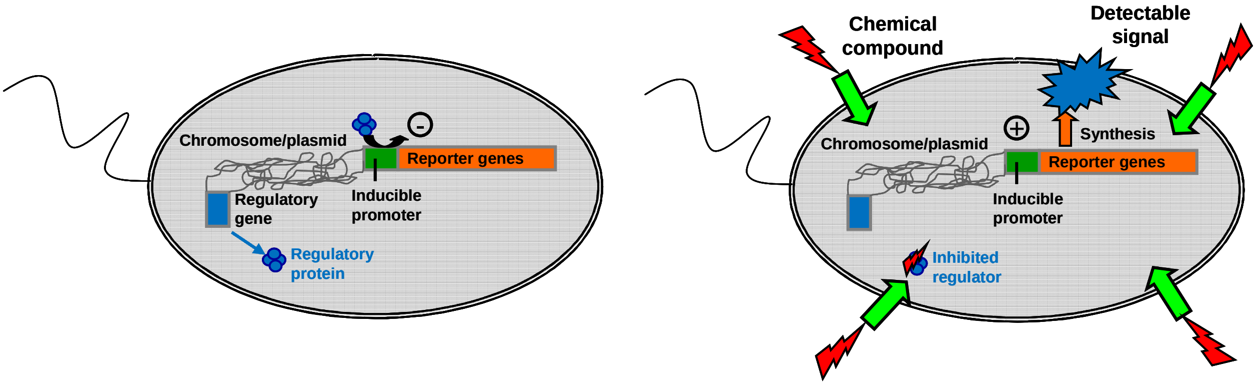

Figure II-4: Mechanism for specific detection of bacteria modified by: a) Repression of reporter genes by the regulatory protein in the absence of specific chemical compound, b) Raising the inhibition of transcription of reporter genes linked to the intracellular penetration of the chemical compound specific, resulting in the appearance of a detectable signal.

In the case of metal detection, the promoters used are mostly developers involved in the mechanisms of bacterial resistance to heavy metals *(Grass et al., 2000; Brown et al., 2002; Verma & Singh, 2005; Charrier et al., 2010)*.

2.4.1.2. Strategy for construction of plasmid pBMerlux

The construction strategy was to clone the promoter and regulatory gene pMerT Merr upstream of luxCDABE genes. And in the presence of induction, the regulatory protein merR complexes with the inductor and the inhibition of the promoter pMerT allows transcription and translation of genes luxCDABE. This building was constructed in a plasmid vector and is included in the following diagram (*Figure II-5*).

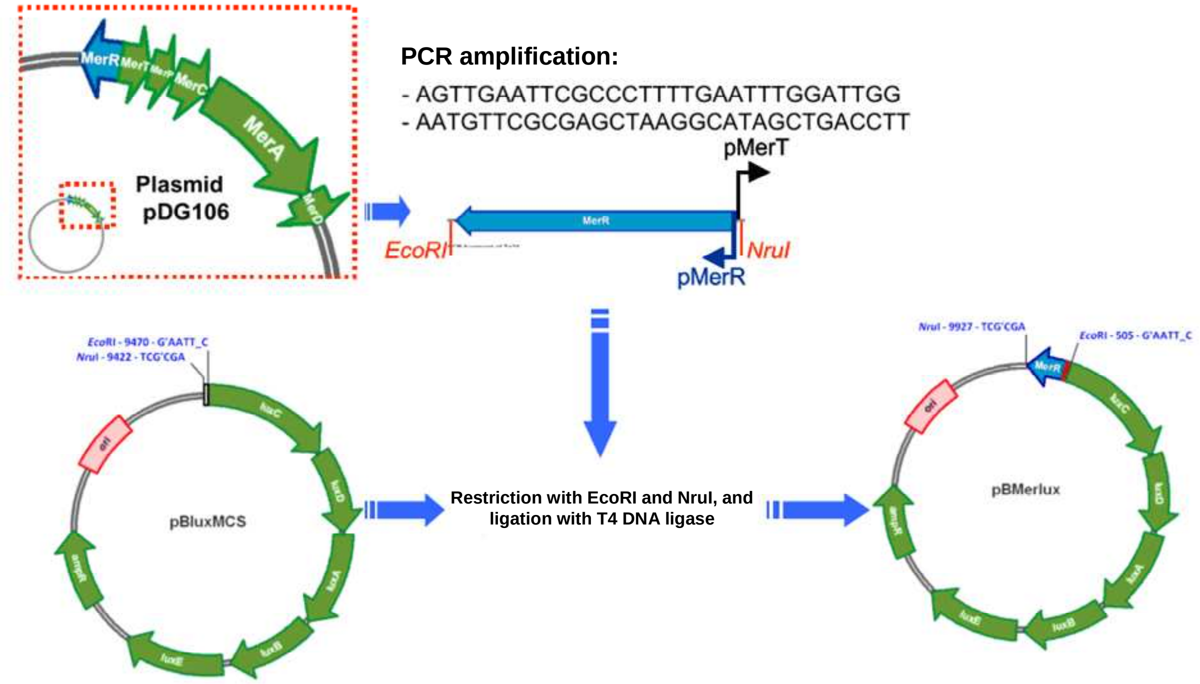

Figure II-5: Strategy for cloning of the plasmid pBMerlux

Plasmids used are summerized in the following *Table II-2*:

Plasmid name	Plasmid host	Reporter system	Promoter	Regulator	Bibliography
pBTaclux	pBtac2	Operon luxfischeria	Ptac	/	*Charrier, 2006*
pBZntlux	pBTacluxb		pzntA	/	*Charrier, 2006*
pBCoplux	pBTacluxb		pcopA	/	*Charrier, 2006*
pBArslux	pBTacluxb		parsR	ArsR	*Charrier, 2006*
pBMerlux	pBlux MCS		pmerT	MerR	*Gambill & Summers, 1985; Gueuné, 2007; Jouanneau et al., 2011*

Table II-2: Used plasmids for recombined bacteria: a)After the lux operon marine bacterial strain Vibrio fischeri and consists of five genes luxC, luxD, luxA, luxB genes and luxury; b) the promoter ptac was replaced by the developer wanted; /: Not made to the regulatory gene plasmid construction

2.4.1.3. Trial of induction.

In the case of freeze-dried bacteria, a preliminary step of rehydration is necessary. 100µl of distilled water are added to each well of the microplate containing bacteria. This is then incubated at 30°C for 30 minutes. 25 µl of solution to be tested are introduced per well. Monitoring of bioluminescence is produced using a microplate luminometer (Berthold ® Plus Microlumat Lb96V). Measurement parameters are:

- *Integration time: 1 second per well*
- *Temperature setpoint imposed on the Peltier element 30°C*
- *The measurement is carried out after 60 minutes of contact bacteria / sample during which they were incubated at 30°C.*

The raw data are converted into bioluminescence induction factor or inhibition rate depending on the bacterial strains used. Bacterial strains with inducible, the data is converted into induction factor using the following formula:

$$\mathbf{IF = (BL_{measured}) / (BL_{control})}$$

- *IF: induction factor*
- $BL_{measured}$: *bioluminescence emitted by the bacterial strain in contact with the test sample* ($RLU.s^{-1}$)
- $BL_{control}$: *bioluminescence emitted by the bacterial strain in contact with the control (distilled water)* ($RLU.s^{-1}$)

In some cases, the induction factors were transformed into logarithms to reduce the distances between some arithmetic values and thus amplify the differences.

With the bacterial strain constitutive data are converted into bioluminescence inhibition rate using the following equation:

$$\mathbf{IT = 1 - (BL_{measured}) / (BL_{control})}$$

- *IT: Rate of inhibition of induction*

► As a conclusion, the coupling between freeze-dried bacteria in microplate and statistical analysis for decision trees from biological data bioluminescence greatly facilitates the interpretation of data. This model also allows a semi-quantitative concentration of metal present in the sample analyzed.

2.4.2. Soil–plant transfers: the Rhizotest

2.4.2.1. Principe

Created by Niebes *et al.* *(1993)* and adapted by Guivarch *et al.* *(1999)* and Chaignon & Hinsinger *(2003)*, the technique of micro-culture hydroponics allows the growth of plants whose roots are in contact (but separate) with the ground, to analyze more easily the different compartments of plant in the soil–root system. Given the small amount of soil used and considering the intimate contact between soil and roots, soil studied can be considered as rhizospheric soil. This test is also the subject of a draft standard *(ISO, 2011)* entitled “Soil quality - A plant bioassay for assessing the environmental bioavailability of trace elements for plants” (*Figure II-6*).

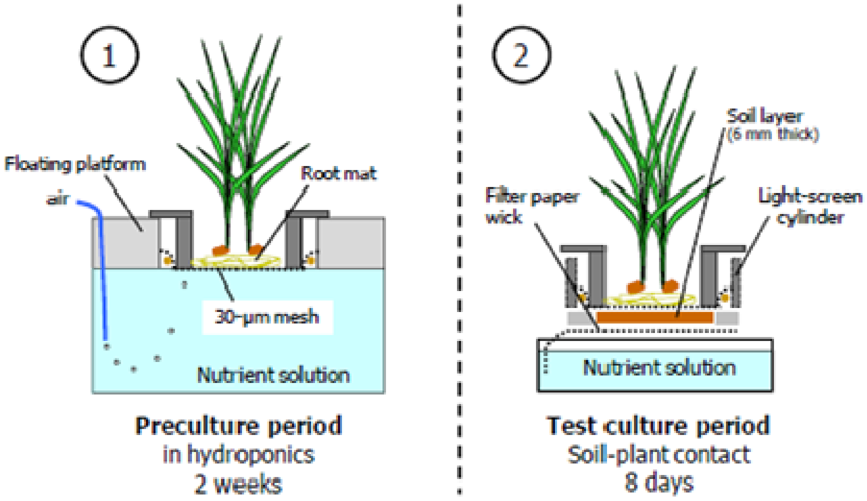

Figure II-6: The two steps procedure of experimental device of the plant biotest for the assessment of the environmental bioavailability of trace elements to plants (ISO, 2011).

Many studies were conducted using hydroponic cultures (in nutrient solution) *(Cecchi et al., 2008; Uzu et al., 2009)* well suited for routine use. The bioassay of plant consists of two stages for plant growth. In the first stage (pre-culture), planting of the plants are grown hydroponically for two weeks to reach an adequate plant biomass and a dense root system. In the second stage (culture test), the material culture of pre-root plants are brought into contact for 8 days with a thin layer of about 6 mm from a soil sample sieved to 2 mm.

A set of control plants (leaves and roots) was harvested at the end of the pre-culture in hydroponics in order to have control of their element concentrations before exposure to the ground. Whole plants (leaves and roots) are then harvested at the end of the culture period of the test. The concentrations of trace elements in biomass and roots were determined. The primary endpoints of this bioassay are (i) the concentration of trace elements in leaves and roots at the end of the culture period, and (ii) the absorption flux elements by plants during this period.

2.4.2.2. Procedure

The seeds are first disinfected with hydrogen peroxide (H_2O_2) 6% (v / v) for 10 minutes and rinsed with deionized water. Each seed is then deposited on the grid at the base of each cylinder inside to get a plant through the device.

Receiving the pot the plant is designed to hold it at the beginning of the period of pre-culture at the end of the period of the test crop. The pot should allow the plant to develop a dense root system and planar, while maintaining physical separation with the soil samples analysed. The pot consists of a cylinder (PVC, Ø = 25 mm) equipped with a top plate and the bottom closed with a nylon mesh with a pore size of 30 μm using rubber bands (*Figure II-7*).

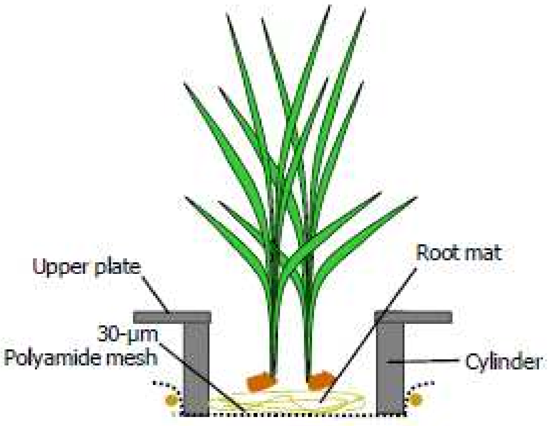

Figure II-7: Device used for the plant-receiving pot (ISO, 2011).

The device used for the pre-culture is designed to allow seed germination and seedling development plan and a dense mat of roots (*Figure II-8*). This should allow a close contact or partial immersion of the seeds and roots in the nutrient solution contained in a tray. This device consists of a perforated floating platform on the surface of the nutrient solution. Perforations on the platform used to keep the pots on the surface. A system for continuously bubbling aerated nutrient solution. The buoyancy of the platform is essential to ensure a uniform contact with the pots of different nutrient solution in spite of continuous aeration. This device should also avoid excessive exposure to the nutrient solution to light radiation and the consequent development of algae.

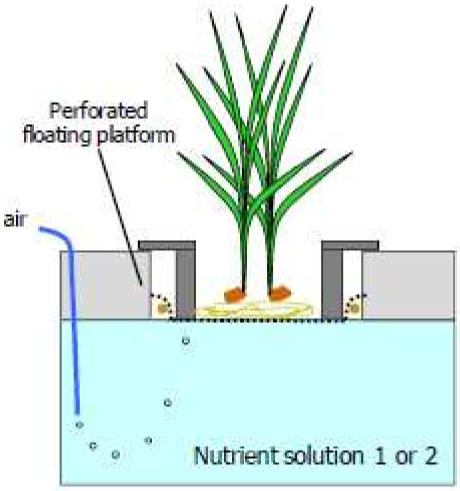

Figure II-8: Device used for the pre-culture period (ISO, 2011).

The device used for the cultivation period is designed to allow close contact between the mat of roots and the soil layer (*Figure II-9*). It consists of two rooms and a wick of filter paper sandwiched between: (i) a contact system that firmly press the pot on the layer of soil with fixings and (ii) a bucket filled with the solution 3 nutritious. This feature allows you to maintain (i) the root system in contact with the surface of the soil layer and (ii) the wick of filter paper moistened throughout the test period.

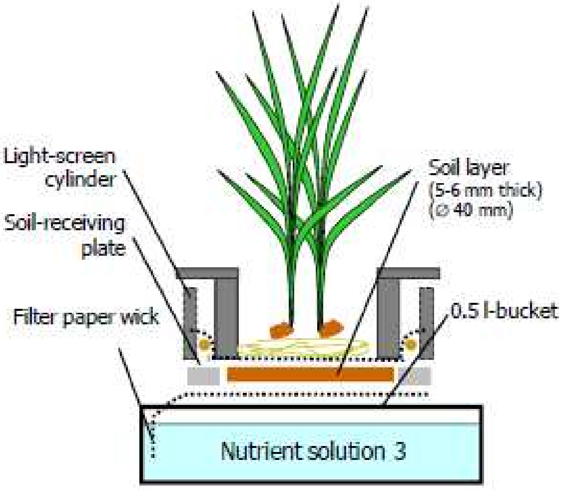

Figure II-9: Device used for the test culture period (ISO, 2011).

2.4.3. Transfers to human: Bioaccessibility measurement

2.4.3.1. Presentation

The *in vitro* test UBM has been developed since 2005 by various members of the European Group BARGE, with a view to obtaining a standardized *in vitro* test by setting each of the test parameters are most representative of the physiological reality of a child from 0 to 6 years. Approach and the development of UBM test based on several principles:

- *It must be based on the physiology of the child (0-6 years), mimicking the physico-chemical environment of the gastrointestinal tract in the stomach and small intestine;*
- *It must represent the most conservative case possible to not underestimate the level of risk associated with the ingestion of contaminated land, where the development of a representative test conditions a child fasting;*
- *It should be applicable for all trace elements.*

The method developed by the group BARGE is based on a method previously published and developed by the RIVM *(Oomen, 2000; Oomen et al., 2002, 2003)*. **This method was considered the most representative physiologic conditions in the gastrointestinal tract of the child** *(Oomen et al., 2002)*. However, some changes were made to this method thanks to the work of Caboche *(2009)*, to ensure it is in the case the most conservative possible that the *in vitro* assay is robust and applicable to soil characteristics varied (historical contamination, physico-chemical properties).

	Saliva (pH = 6.5 ± 0.5)	Gastric solution (pH = 1.0 ± 0.2)	Intestinal solution (pH = 7.4 ± 0.2)	Bile solution (pH = 8.0 ± 0.2)
Inorganic	448 mg KCl 444 mg NaH_2PO_4 100 mg KSCN 285 mg Na_2SO_4 149 mg NaCl 0.9 ml NaOH 1M	1376 mg NaCl 133 mg Na_2PO_4 412 mg KCl 200 mg $CaCl_2$ 153 mg NH_4Cl 4.15 ml HCl (37% g g^{-1})	3506 mg NaCl 2803 mg $NaHCO_3$ 40 mg KH_2PO_4 282 mg KCl 25 mg $MgCl_2$ 90 µl HCl (37% g g^{-1})	2630 mg NaCl 2893 mg $NaHCO_3$ 188 mg KCl 90 µl HCl (37% g g^{-1})
Organic	100 mg urea	325 mg glucose 10 mg glucuronic acid 42.5 mg urea 165 mg glucosamine hydrochloride	50 mg urea	125 mg urea
Added compounds	72.5 mg α-amylase 25 mg mucin 7.5 mg uric acid	500 mg bovine albumin 1500 mg mucin 500 mg pepsin	100 mg $CaCl_2$ 500 mg bovine albumin 1500 mg pancreatin 250 mg lipase	111 mg $CaCl_2$ 900 mg bovine albumin 3000 mg porcine bile

Table II-3: Composition of digestive fluids used for the UBM test

2.4.3.2. Experimental procedure

The UBM test is based on successive extractions with synthetic solutions digestive stimulating physical and chemical processes in the mouth, stomach and small intestine. The protocol takes place in vitro, therefore several stages of preparation and extraction.

2.4.3.2.1. Preparation of digestive solutions

The solutions are prepared before the completion of the *in vitro* test UBM. The composition of each solution is digestive presented in *Table II-3*. For each solution digestive solution "inorganic" and then a solution "organic" are prepared in 250 mL volumetric flasks, the volume of each is adjusted with UHQ water. Then, after complete dissolution of the compounds, reagents (including enzymes) were added to one or other of the solutions previously prepared. Then, the two solutions (organic and inorganic) are mixed to obtain 500 mL of digestion. Finally, the four solutions obtained are placed in digestive stirring (with a magnetic stirrer) overnight at room temperature. The solutions are prepared each time a test is scheduled in vitro. On the test day, the fluids are maintained and allowed to warm to 37 °C ± 2 °C at least 2 hours before use. The pH of each solution is measured and must be in the range specified *Table II*-3 Otherwise, the pH is adjusted with hydrochloric acid (37%) or sodium hydroxide (10 M).

2.4.3.2.2. Sample preparation

For each soil sample (dried and ground <250 m), 0.6 g twice (± 0.05) are weighed and introduced into centrifuge tubes Nalgene ® polycarbonate to obtain two sub-samples. One of the samples represent the phase "gastro-salivary" known as gastric phase, and the other sample represent the phase "gastrointestinal" called intestinal phase.

2.4.3.2.3. Procedure of the test

Figure II-10 shows the characteristics and conduct of *in vitro* test, realized under aerobic conditions. In a first step, the pH of the sample white "stomach" (9 ml saliva solution + 13.5 ml gastric solution) is measured to verify that it is in the range 1.2-1.7. Otherwise, it is adjusted with HCl or NaOH. Then, the test begins by introducing digestion of 9 ml of saliva (pH = 6.5) for each soil sample. The mixture is stirred manually for 5 min. Then, 13.5 ml of gastric (pH 1.0) are added. The pH of the mixture is controlled and if necessary fixed between 1.0 and 1.2 using the reagents cited above. Then, the mixture is agitated for 1 h at 37 °C (rotating end-over-end). At the end of the gastric phase, the pH of the solution was again checked and should be between 1.2 and 1.7. If the pH is not in this range, the experiment stops and two new sub-samples of the same soil are used for a new series (with the addition of HCl from the beginning). pH values out of range are explained by very high levels of carbonates. This step is very important not to underestimate the values of bioaccessibility of MTE which can precipitate with increasing pH in the gastric phase. If the pH at the end of the gastric phase is within the desired range, a sub-sample of each soil was centrifuged at 3,000 g for 5 min to obtain a representative sample of the gastric phase.

The second sub-sample is used for the remainder of the test corresponding to the intestinal phase. Following step stomach, duodenal solutions (27 ml, pH = 7.8) and bladder (9 ml, pH = 8.0) are added. The pH of the solution is checked and must be in the range 6.3 ± 0.5. Otherwise, it is adjusted with HCl or NaOH. The mixture is then stirred for 4 h at 37 °C. The pH is again measured and adjusted to 6.3 ± 0.5 if necessary. Finally, after centrifugation at 3,000 g for 5 min, the supernatant was collected and acidified (1 ml HNO_3 65%) and stored at 4 ° C before being analysed by ICP-OES.

As bioavailability, bioaccessibility may be described in absolute or relative, the latter being used in the correlations *in vitro* / *in vivo*.

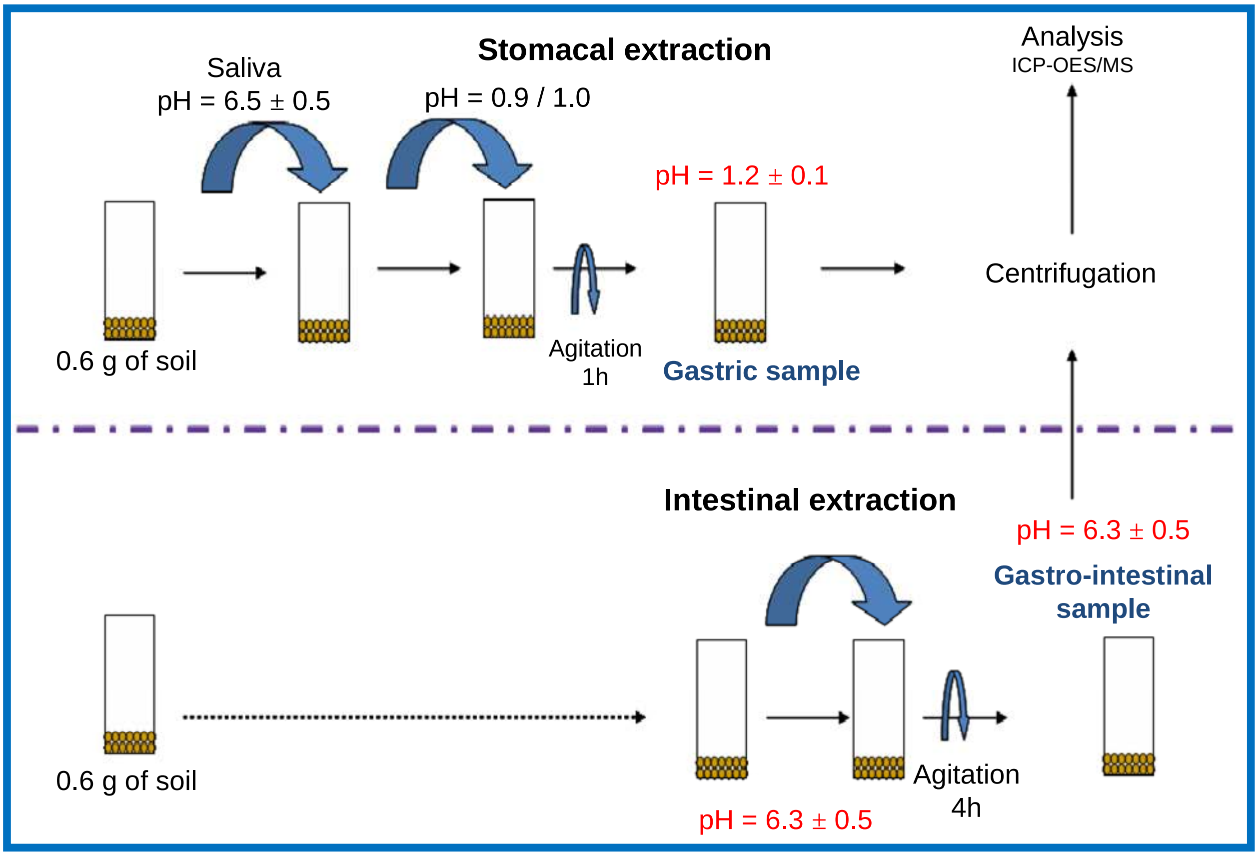

Figure II-10: Procedure of the BARGE protocol (Caboche, 2009)

2.4.4. Terminal Restriction Fragment Length Polymorphism – T-RFLP

In 1980, Botstein & Coll have published a genetic map of the human genome which, for the first time, used the technique from restriction fragment length polymorphism genetic markers. This technique was immediately a big hit with geneticists because it gave access to a very large number of markers distributed throughout the genome.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) is a molecular biology technique for profiling of microbial communities based on the position of a restriction site closest to a labeled end of an amplified gene. The method is based on digesting a mixture of Polymerase Chain Reaction (PCR) amplified variants of a single gene using one or more restriction enzymes and detecting the size of each of the individual resulting terminal fragments using a DNA sequencer. The result is a graph image where the X axis represents the sizes of the fragment and the Y axis represents their fluorescence intensity.

T-RFLP analysis is a method of comparative community analysis. It is based on the restriction endonuclease digestion of fluorescently end-labelled PCR products (in our case the 16S rRNA gene). The digested products are separated by gel electrophoresis and detected on an automated sequence analyser. **The method provides distinct profiles (fingerprints) dependent on the species composition of the communities of the samples**. The *Figure II-11* below illustrates the procedure of the method.

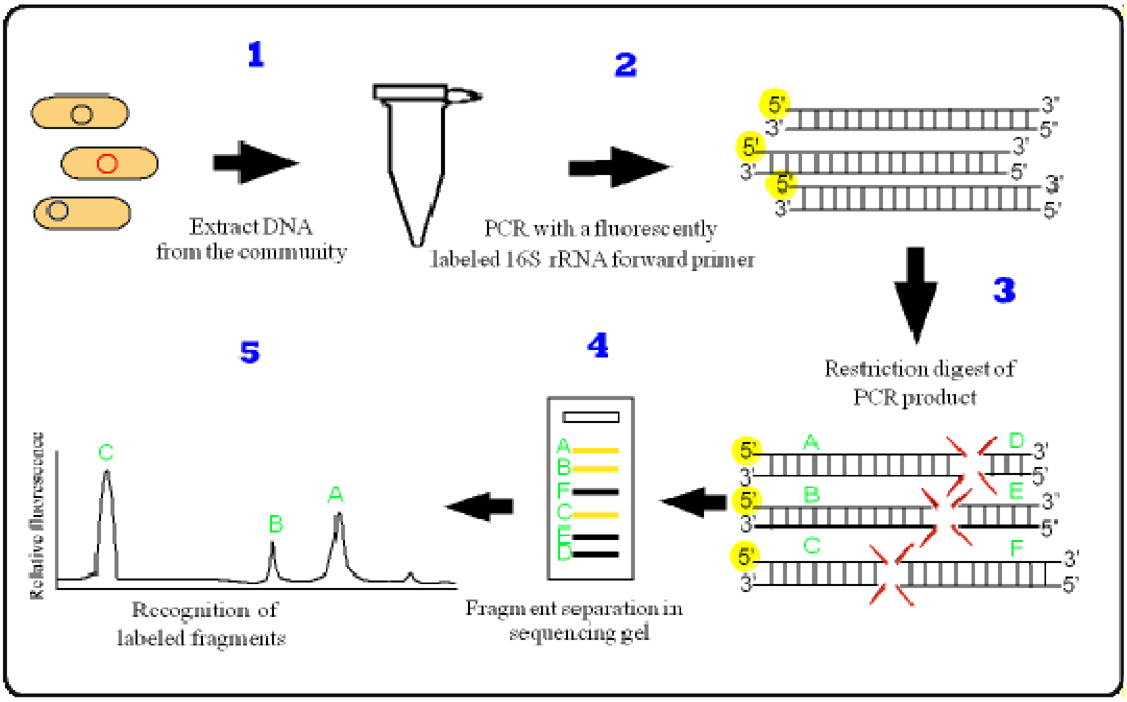

Figure II-11: Procedure of the T-RFLP method

The DNA is harvested from the analyzed sample (1). The gene of interest is amplified using the PCR with a fluorescently labeled primer (2). This yields a mixture of amplicons of the same or similar sizes with a fluorescent label at one end. After purification, the amplicon mixture is digested with a restriction enzyme, which generates fragments of different sizes (A-F) (3). These are separated through gel or capillary electrophoresis (4). A laser reader detects the labeled fragments and generates a profile based on fragment lengths (5).

► ***The following chapters represent the R&D part of the thesis work and are presented in the form of scientific publications published or submitted.***

Chapter 3

Screening of soil pollution using ecotoxicological tests

► Nowadays, only total and leached MTE-concentrations are used for risks assessment, but they do not reflect the real effects on living organisms. In this chapter, we investigated the relevance of ecotoxicity tests to improve the management of excavated contaminated soils. Ecoscores were calculated from the various data adquired to rank the dangerousness of soils samples from STCM according the results of a battery of biotests performed.

3.1. Foreword

Rehabilitation of brownfield sites located in urban areas has become a major concern. Management of contaminated soils in relation with environmental and sanitary risk concerns is therefore a strong aim needing the development of both useful tools for risk assessment and sustainable remediation techniques. For soils polluted by metals and metalloids (MTE), the criteria for landfilling are currently not based on ecotoxicological tests but on total MTE concentrations and leaching tests. In this study, the ecotoxicity of leachates from MTE polluted soils sampled from an industrial site recycling lead-acid batteries were evaluated by using both modified *E. coli* strains with luminescence modulated by metals and normalized *Daphnia magna* and *Alivibrio fischeri* bioassays. The results were clearly related to the type of microorganisms (crustacean, different strains of bacteria) whose sensitivity varied. Ecotoxicity was also different according to sample location on the site, total concentrations and physico-chemical properties of each soil. For comparison, standard leaching tests were also performed. Potentially phytoavailable fraction of MTE in soils and physicochemical measures were finally performed in order to highlight the mechanisms. The results demonstrated that the use of a panel of microorganisms is suitable for hazard classification of polluted soils. In addition, calculated eco-scores permit to rank the polluted soils according to their potentially of dangerousness. Influence of soil and MTE characteristics on MTE mobility and ecotoxicity was also highlighted.

This chapter is presented in a form of an accepted article published in 2013 in the Journal of Hazardous Materials (246–247, 291–299):

Ecotoxicity test and ecoscores to improve polluted soils management: case of a secondary lead smelter plant.

Foucault, Y., Durand, M.J., Tack, K., Schreck, E., Geret, F., Lévêque, T., Pradère, P., Goix, S. & Dumat, C.

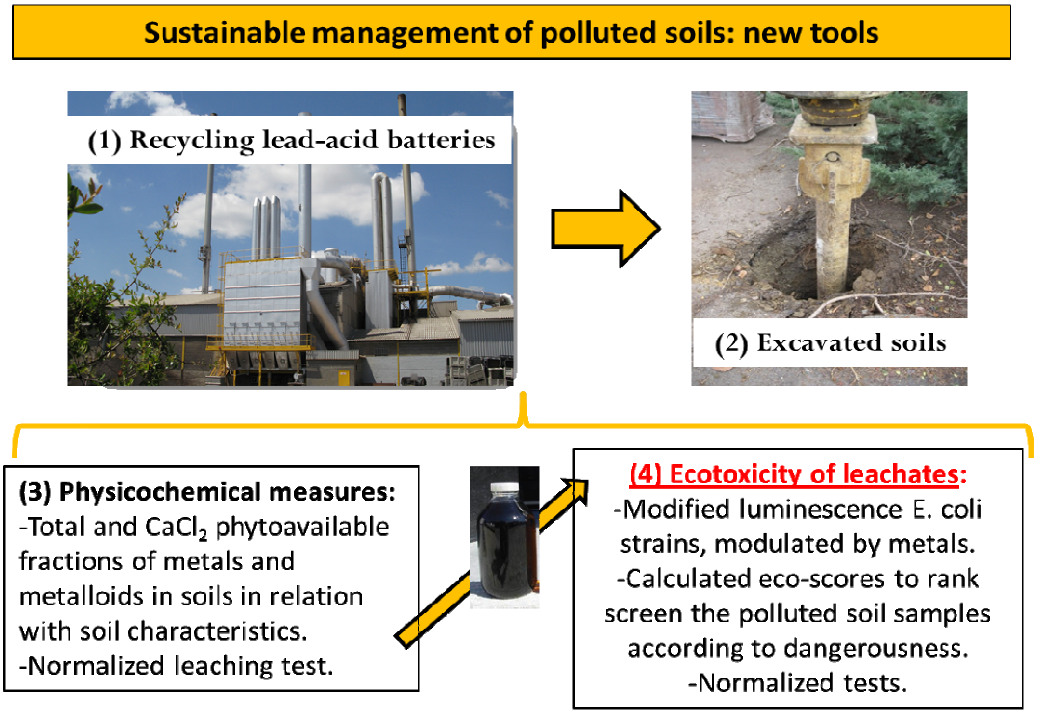

Figure III-1: Schematic abstract of the used approach

3.2. Ecotoxicity tests and ecoscores to improve polluted soils management: case of secondary lead smelter plant.

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Keywords: sustainable management of polluted soils, metal trace elements, ecotoxicity, landfilling, leaching.

3.2.1. Introduction

Originally located on the outskirts of cities, numerous industrial sites, sometimes abandoned, are now in urban areas and are therefore likely to have environmental and health risks to surrounding populations [1, 2]. Currently, rehabilitation of the sites frequently entails excavation of polluted soils [3]. Excavated soils can thus follow two different ways: landfilling, expensive and energy intensive, or reuse/recycling, integrated to sustainable development. The choice of a specific track mainly depends on total and leachable concentrations of the pollutant in the soil [2]. Among the numerous pollutants observed in urban and peri-urban areas, trace metals are often present in soils [4]; atmosphere emissions by smelters being one of the main anthropogenic source [5, 6]. MTE speciation and compartmentalization in soils can modify their impact on living organisms [5]. Now, numerous publications concluded that these two parameters are strongly influenced by soil organic matter (OM) content, pH and texture [7, 8, 9]. According to Matejczyk et al. [10], chemical weathering of soil minerals favours MTE solubilisation and leachates production. Then, these leachates can pollute surrounding soils and waters. According to the council directive n°1999/31/CE, leaching tests with chemical analysis are therefore currently used for the assessment of environmental hazards of polluted soils. But, landfilling is often inevitable for strongly polluted soils, with high “hazard level” (assessed by leached and total MTE concentrations). Moreover, according to Foucault et al. [1], professionals consider the threshold set as too restrictive and they regret that excavated soils are almost always managed as waste.

In addition to the measure of total and leached MTE concentrations, it appears therefore that knowledge of MTE availability [11] and ecotoxicity may carry useful information [12, 13, 14] to improve environmental risk assessment [10]. Actually, the accurate estimation of metal phytoavailability in polluted soils and solid wastes, using single chemical extraction [15] carry interesting data to perform pertinent risk assessment and remediation efforts [16, 17]. Soil quality integrates both physicochemical and biological characteristics [18]. Moreover, according to Plaza et al. [14] microorganisms play important roles in numerous soil functions. Soils are often polluted with a large variety of compounds leading to possible interactions [19], thus as reviewed by Kim and Owens [20] study of leachates ecotoxicity provides a direct functional characterization of various pollutant mixtures. But, only few studies concern the use of ecotoxicological tests to monitor contamination and bioremediation efficiency of polluted soils [21] and new tests are required by industrial sites managers to assess environmental risks. Among them, microbial bioassays offer quick, cheap and easy ecotoxicity (toxicity and mutagenicity) and bioavailability measurements on bacteria [22, 23]. However, in many cases, microbial bioassays cannot be directly used for the identification and quantification of compounds due to the lack of specificity of the engineered microorganisms [24] and further studies are needed to improve these biotests.

The aim of this study was therefore to assess the ecotoxicity of leachates for landfilling of MTE contaminated soils by various complementary biotests, in addition to usual physicochemical measures. **More precisely, the following two scientific objectives were aimed: (1) what is the pertinence of ecotoxicity tests to assess a more realistic human exposition to contaminated soil leachates? (2) What is the influence of soil physicochemical parameters on MTE mobility and**

leachates ecotoxicity? Several studies use specific bacteria strain to sense the presence of metals in soils [25, 26, 27, 28], nevertheless, the development of statistical model to understand the link between chemical concentration of compound and bacteria sensors is still on-going work [23]. So, the originality of this study was to combine the use of new bacterial strains never tested in a context of the remediation of an industrial polluted site and calculation of eco-scores which facilitates the comparisons between different soils.

3.2.2. Materials and methods

3.2.2.1. Soil sampling and preparation

According to Wong et al. [29], the most relevant soil layer to study the environmental and sanitary impacts of MTE in urban areas is between 0 and 25 cm. Ten top soil samples (*Figure III-2*) were therefore collected in the courtyard of the Chemical Metal Treatments Society (STCM), a secondary lead smelter which currently recycles batteries located in the urban area of Toulouse (43°38’12’’N, 01°25’34’’E). This plant was chosen because of its activity and urban location, and many data are already available [1, 4, 5, 6, 30]. These data allowed defining different areas in terms of environmental and sanitary risks that can vary according to past and present activities. Moreover, previous studies of the particles released in the atmosphere by Uzu et al. [5] and Schreck et al. [4] revealed the presence of several MTE (Pb, As, Cu, Cd, Zn and Sb) and gave information on the main lead speciation: PbS, $PbSO_4$, $PbO–PbSO_4$, α–PbO and Pb (by order of abundance). All soil sampling points are presented in *Figure III-2*; they were dried, sieved under 2 mm and treated in triplicate.

3.2.2.2. Physico-chemical analysis

pH, organic matter and limestone contents, cation exchange capacity (CEC Metson) and texture, were determined for all soil samples respectively according to the norms ISO 10390 [31], ISO 10694 [32], ISO 10693 [33], NF X31-310 [34] and ISO 11277 [35]. Pb, As, Cu, Cd, Zn and Sb total concentrations were measured by ICP-OES (IRIS Intrepid II XXDL) after mineralization in aqua regia according to ISO 11466 [36] (HNO_3 65 %, HCl 37 %, ratio 1:3 v/v). The detection limits of Pb, Cd, Sb, As, Cu and Zn were 0.3, 0.2, 0.2, 0.2, 1.3 and 2.2 µg L^{-1}, respectively, whereas the limits of quantification were about 0.4, 0.3, 0.4, 0.3, 2 and 3 µg L^{-1}, respectively. The accuracy of measurements was checked using a certified reference material 141R (BCR, Brussels). The concentrations found were within 95–102% of the certified values for all measured elements.

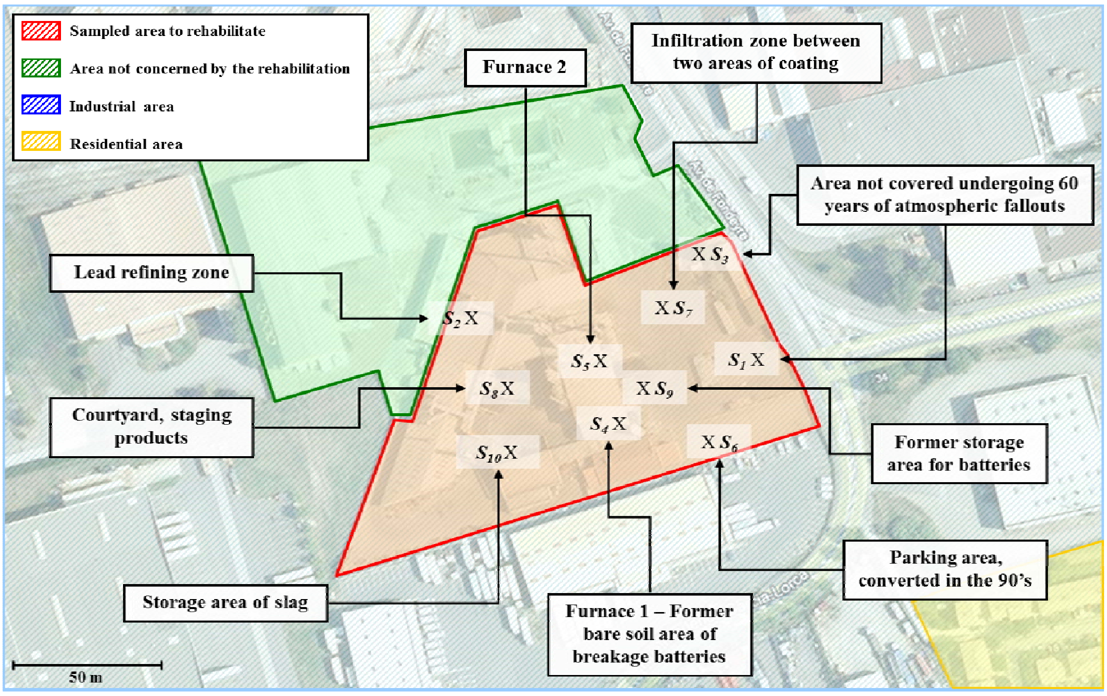


Figure III-2 : Situation of the industrial study site, location and characteristics of the 10 sampling points

3.2.2.3. Leaching tests

Normalized leaching test [37] was applied to all soil samples. This procedure consisted of a single extraction with deionised water, using a solid-to-liquid ratio of 1/10. 10 g of soil (granulometry at least < 4 mm according to the norm) was mixed with 100 mL deionised water during 24h with end-over-end agitation at 5 rpm. After centrifugation at 3,000 g during 15 min, the leachates were filtered with cellulose 0.45 µm (Millipore®) filters. 10 mL of each leachates were then acidified with HNO_3 65 % prior to analysis by ICP-OES (IRIS Intrepid II XXDL, analytical errors < 5%). The other part of leachates was not acidified so as not to disturb microorganisms used for further ecotoxicological tests.

3.2.2.4. MTE phytoavailability estimate

Potentially phytoavailable MTE concentrations were estimated by $CaCl_2$ extractions according to Uzu et al. [5]. In 25 mL polypropylene centrifugation tubes, 10 mL of 10^{-2} M $CaCl_2$ were added to 1.0 g of soil. The liquid to solid ratio of 10 is high enough to avoid samples heterogeneities [38]. After agitation end-over-end during two hours at 5 rpm at 20 °C, samples were then centrifuged during 30 min at 10,000 g. Supernatant was sieved through a 0.22 µm mesh and acidified at 2% with HNO_3 (15 N, suprapur 99.9 %). MTE concentrations were finally measured by ICP-OES (IRIS Intrepid II XXDL, analytical errors < 5 %). A house reference soil was used to quality control of $CaCl_2$ extraction: this soil described by Schreck et al. [4] has the advantage of presenting the same type of contamination that the soils studied in this work. Actually, it's a soil historically polluted by battery recycling emissions ([Pb] = 1650 ± 20 mg kg^{-1}). Using that reference soil, the detection limits of Pb, Cd, Sb, As, Cu and Zn were 0.3, 0.2, 0.2, 0.2, 1.3 and 2.2 µg L^{-1}, respectively, whereas the limits of quantification were about 0.4, 0.3, 0.4, 0.3, 2 and 3 µg L^{-1}, respectively. The concentrations found

]

were within 95–102 % of the reference values for all measured elements.

3.2.2.5. Ecotoxicity assessment

3.2.2.5.1. Daphnia magna

A first acute toxicity of leachates was performed on the water flea *Daphnia magna* (Origin, less than 24 h old) according to [39]. Four replicates were tested for each soil solution and five neonates were used in each replicate, with 10 mL of test solution. Organisms were fed two hours before but not during the experiment. A parafilm strip was then put on the multiwall plate placed in the incubator at 20 °C in darkness. The mobility of Daphnia magna was recorded after 24 h and 48 h, and inhibition rate was calculated.

3.2.2.5.2. Microtox®

First used to assess acute ecotoxicity of metals in aquatic media [40] and normalized since 2007 [41], solid-phase Microtox® test is now currently used to evaluate the toxicity of contaminated soils or sediments [4, 42, 43]. The Microtox test measures the decrease in light emitted by the bioluminescent bacteria *Vibrio fischeri* after 5, 15 and 30 min of exposure [43]. In view of evaluating toxicity of collected soils, the Microtox 81.9 % Basic Test with the instrument MICROTOX M 500 purchased from R-Biopharm (France) was used. 100 µL of revitalized bacteria (Lot 10J1010A) were added to each Microtox® tube, gently mixed with a pipette. 900 µL of each leachate was transferred into the glass cuvette in the Microtox® analyzer and allowed to equilibrate for 5 min before reading [44]. Light emission was recorded and the output data analysed using Microtox® Omni software Version 1.18 [45]. All samples were tested in triplicate. Bacteria validity and the set-up of the measurement procedures were verified by reference toxin ($ZnSO_4$, $7H_2O$) according to the ISO 11348 regulation specifications [41].

3.2.2.5.3. Induction of bioluminescent bacteria

Many bacterial sensors are dedicated to the specific detection of pollutants or pollutant family. These used bio-elements are bacterial strains (*Escherichia coli*) genetically modified. In all cases, reporter genes lux CDABE are cloned downstream of a promoter allowing to highlight the specific or semi-specific presence for certain compounds in a sample [46, 47]. In the case of metal detection, the promoters used are mostly involved in the mechanisms of bacterial resistance to heavy metals [48, 49, 50]. A set of five bioluminescent bacteria namely *E. coli* Taclux, *E. coli* Zntlux, *E. coli* Arslux, *E. coli* Coplux and *E. coli* Merlux was used in this study (*Table III-1*). Bacterial growth and lyophilisation were realized according to Jouanneau et al. [24]. At the beginning of the bioassay, the lyophilized bacteria were reconstituted with 100 µL per well of distilled water for 30 min at + 30 °C. 25 µL of leachate was added to each well, and afterward the microplate was incubated for 60 min at + 30 °C. Monitoring of bioluminescence was recorded using a microplate luminometer (Microlumat Plus Lb96V). The results were expressed by the logarithm of the induction ratio or the inhibition rate for the inducible strains and the constitutive strain respectively. The induction ratio (IR) was calculated as follows:

$$\mathbf{IR = (RLU.s^{-1})_{i\text{-}IR}/(RLU.s^{-1})_{0\text{-}IR}.}$$

- *$(RLU.s^{-1})_{i\text{-}IR}$ is the bioluminescence after induction with a sample, and $(RLU.s^{-1})_{0\text{-}IR}$ is the background luminescence.*

The inhibition rate (InR) was calculated as follows:

$$\mathbf{InR = 1 - (RLU.s^{-1})_{i\text{-}InR}/(RLU.s^{-1})_{0\text{-}InR}.}$$

Where:

- *(RLU.s-1)i-InR is the bioluminescence after exposure with a sample,*
- *(RLU.s-1)0-InR is the background luminescence.*

MTE	**Detection limit of bacterial strains (standard deviation) (µM)**			
	Zntlux	**Arslux**	**Merlux**	**Coplux**
Cd	0.0045 (0.0003)	5.9 (2.3)	0.011 (0.002)	-
Hg	0.01 (0.005)	-	1.2×10^{-7} (1×10^{-7})	-
As $^{(III)}$	28.52 (7.1)	0.256 (0.0014)	15.6 (4.3)	-
Cu	16.92 (2.9)	-	-	90.5 (11.7)
Pb	2.2 (0.6)	4.16 (0.8)	-	-
Sn	12.95 (4.24)	-	-	-
As $^{(V)}$	9.32 (1.24)	0.3 (0.06)	12.65 (5.4)	
Zn	1.7 (0.62)	-	2.3 (0.14)	-
Ni	4.4 (1.6)	-	-	-
Co	0.22 (0.014)	-	-	-
Cr $^{(VI)}$	597.2 (121.3)	-	-	-
Ag	-	-	-	2.75 (0.11)
Fe	4.34 (0.48)	-	16.1 (7.6)	-
Mn	-	-	-	-

Table III-1: Observed MTE and detection limits for the different E. coli strains [24].

Decision trees were designed from the learning set of bacterial bioluminescence data using the software “Metalsoft”. They are specific to only one compound and organized in several binary branches. Each branch splits data according to the values of only one variable: induction ration or inhibition rate obtained from one strain. The process continues until the target value is obtained [24].

3.2.2.6. Statistical analysis

All tests were performed in triplicate and results are presented as mean ± SD (standard deviation). The statistical significance of values was checked using an analysis of variance (ANOVA) using the Statistica 9.0 package software. Each MTE-extracted concentration (both by water and $CaCl_2$ procedures) was compared to respective total concentration. Significant differences (p-value < 0.05) were measured by the LSD Fisher test.

3.2.3. Results

3.2.3.1. Physico-chemical characteristics

Soil properties are reported in *Table III-2*. These physicochemical characteristics significantly differ in function of sample origin: it means localization on the industrial site in relation with process. pH value varied between 6.9 to 9.2, CEC value varies between 2.6 to 10.5 cmol(+) kg^{-1} and amounts of soil organic matter and carbonates ($CaCO_3$) were highly variable: respectively from 0.9 to 46.7 g kg^{-1} and from 0 to 15.0 g kg^{-1}. MTE concentrations in polluted soil samples (*Table III-3*) were also very heterogeneous: maximum lead concentration is 42,400 $mg_{Pb}.kg^{-1}$ and other elements are also present at high levels (up to 2095 mg_{Sb} kg^{-1}, 288 mg_{As} kg^{-1}, 286 mg_{Cu} kg^{-1}, 294 mg_{Zn} kg^{-1} and 80.9 mg_{Cd} kg^{-1}). All these concentrations were clearly above the national geochemical background as shown in *Table III-3*.

Sample	pH water	Organic Matter (g kg^{-1})	CEC (cmol(+) kg^{-1})	Limestone $CaCO_3$ (g kg^{-1})	Granulometry (%)				
					Clay	*Thin Silt*	*Rough Silt*	*Thin Sand*	*Rough Sand*
S_1	7.0	31.4	8.9	8.0	13.3	18.3	14.2	15.7	38.5
S_2	8.7	9.6	3.7	7.0	3.6	10.7	10.9	19.7	55.1
S_3	6.7	14.0	6.0	0.0	9.2	13.7	12.6	19.7	44.8
S_4	8.7	12.3	8.9	15.0	7.8	12.1	11.4	21.3	47.3
S_5	9.2	0.9	2.6	7.0	2.7	4.3	4.8	2.9	85.3
S_6	9.0	1.6	3.3	4.0	4.3	8.1	6.7	15.5	65.4
S_7	6.9	46.7	10.5	0.0	6.7	9.4	8.7	21.6	53.5
S_8	7.5	3.4	3.3	4.0	3.3	5.9	4.1	8.6	78.0
S_9	8.5	6.0	6.9	4.0	10.4	17.3	11.2	19.5	41.6
S_{10}	8.9	1.3	3.3	8.0	1.5	5.6	4.4	7.3	81.2

Table III-2: Main physicochemical characteristics of the ten soil samples

3.2.3.2. Chemical extractions

MTE (Pb, As, Cu, Cd, Zn and Sb) concentrations were measured for all the solutions obtained by performing the three extractions (*aqua regia*, water and $CaCl_2$) on the studied polluted soil samples (*Tables III-3* and *III-4; Figures III-2* and *III-3*). Leached MTE amounts in water and corresponding ratios (in comparison with aqua regia extraction, considered as "total") were significantly depending on element nature (*Table III-4-a* and *Figure III-2*). The highest extracted concentrations were recorded for lead, antimony and zinc (MTE with high total concentrations): respectively 152.5, 158 and 9 mg kg^{-1}, i.e. 8.7 %, 7.3 % and 7.9 %. In comparison, copper (at equivalent content) was significantly less extracted than zinc. Although quantitatively low extracted (≤ 5.4 mg$_{Cd}$ kg^{-1}), Cd was proportionally one of the most water-soluble element (up to 15.9 % for S6). Arsenic was the less extracted MTE with a maximum concentration reached of 2.2 mg$_{As}$ kg^{-1} (7.5 % of the total for S_4).

MTE	*NGB**	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
Pb	9 - 50	39800	1425	42400	37250	1020	297	35700	1445	1750	1065
As	1 - 25	288	28.7	51.5	52.5	5.8	9.3	34.3	8.65	13.3	10.2
Cu	2 - 20	286	14.7	143.5	116	13.6	16.1	249	19.4	59.5	60.5
Cd	0.05 - 0.45	18.4	2.24	34.3	4.15	3.39	0.69	80.9	3.39	4.7	11.3
Zn	10 - 100	294	37.1	216	218	42.8	41.9	545	55	116	94
Sb	0.2 - 10	2095	53.5	1555	2175	23.5	13.1	1955	15.9	44.5	9.15

**NGB:National Geochemical Background in France*

Table III-3: Aqua regia MTE concentrations (mg kg^{-1}) for the 10 soil samples (mineralization according to [36]).

$CaCl_2$ extractions results (*Table III-4-b* and *Figure III-3*) showed several contrasted behaviours depending both on chemical element and soil properties. The highest lead quantities extracted by $CaCl_2$ were observed for S_1, S_3 and S_7 (up to 178.5 mg kg^{-1}). However, for S_4 sample with high total lead concentration, the extracted fraction is low (1.3 mg kg^{-1}). Conversely, antimony extracted concentration reached 306.6 mg_{Sb} kg^{-1}. Other MTE showed a low extractability (in terms of quantity and ratio) whatever the sample, except for S_7 which registered pronounced pools of Cd and Zn associated with high total concentrations. *Figures III-3-a* and *b* show the fraction of the extracted element in relation to total concentration. Cadmium appeared as the most potentially phytoavailable element. Sb and Zn also represented high extracted fractions respectively for S_4 and S_7. Moreover, compared to the *aqua regia* fraction, the $CaCl_2$ fraction remained lower, except for the most potentially phytoavailable Cd element (2 – 32 %).

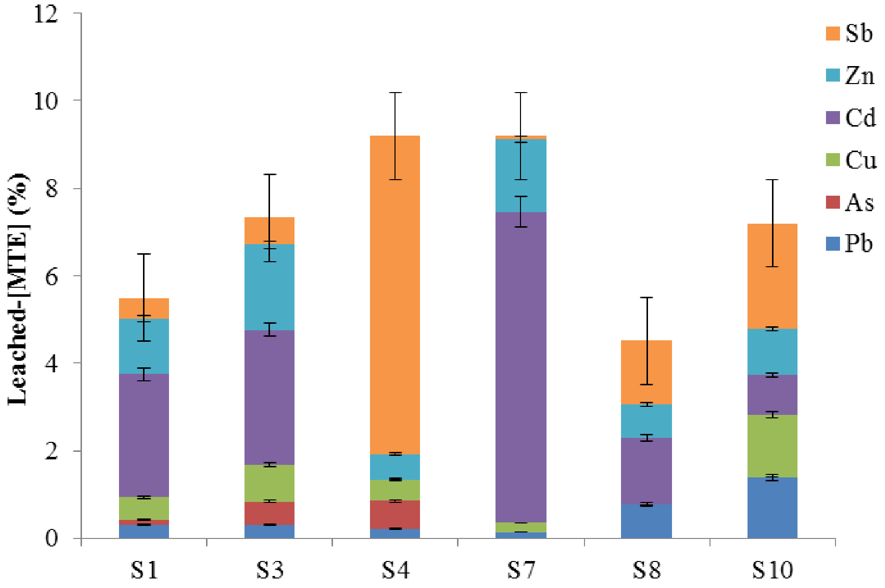
Leached MTE	***S_1***	***S_2***	***S_3***	***S_4***	***S_5***	***S_6***	***S_7***	***S_8***	***S_9***	***S_{10}***
Pb	127.8	63.0	126.2	85.6	53.4	51.8	51.7	11.1	152.5	14,9
As	0.29	2.17	0.28	0.33	*nd**	0.38	*nd*	*nd*	0.54	*nd*
Cu	1.50	0.71	1.20	0.57	1.44	1.67	0.52	*nd*	5.04	0.87
Cd	0.52	0.21	1.06	*nd*	0.21	0.11	5.75	0.05	0.39	0.10
Zn	3.71	1.90	4.19	1.26	3.32	3.86	9.04	0.41	9.13	1.00
Sb	10.0	3.69	9.63	158.2	0.81	2.30	1.64	0.23	3.13	0.22

a)

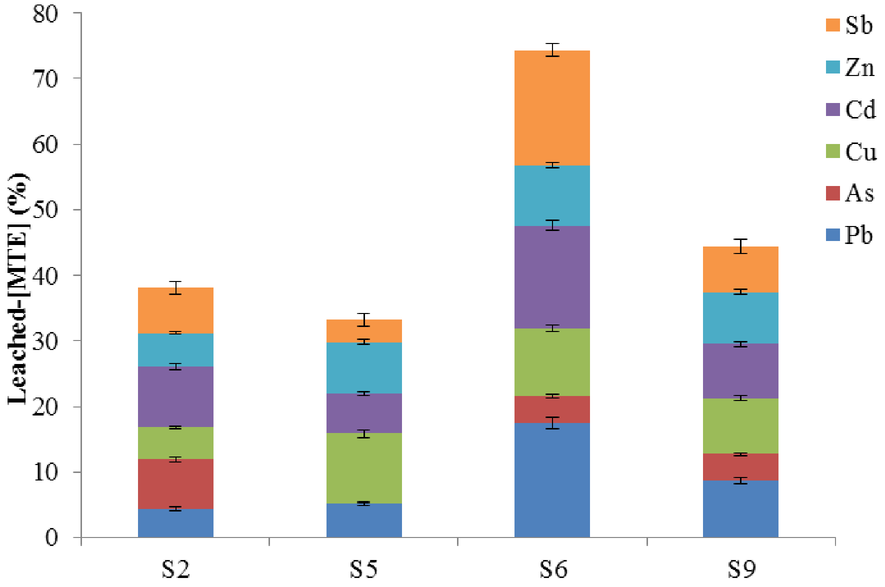
$CaCl_2$-extracted MTE	***S_1***	***S_2***	***S_3***	***S_4***	***S_5***	***S_6***	***S_7***	***S_8***	***S_9***	***S_{10}***
Pb	178.5	0.71	162.9	1.30	0.52	0.60	89.4	14.7	0.31	3.64
As	*nd*	*nd*	*nd*	0.17	*nd*	*nd*	*nd*	*nd*	0.04	0.01
Cu	0.43	0.20	0.37	0.38	0.19	0.18	0,32	0.21	0.29	0.64
Cd	3.16	0.20	4.16	0.22	0.31	0.22	20.1	0.68	0.22	0.24
Zn	2.77	*nd*	3.51	0.06	0.20	*nd*	25.6	0.44	0.08	0.10
Sb	1.54	0.48	1.56	306.6	0.16	0.28	0.77	0.08	0.35	0.11

b)

Table III-4: a) Leached MTE concentrations (mg.kg^{-1}); b) Phyto-available MTE concentrations (mg kg^{-1}) assessed by the $CaCl_2$ procedure.

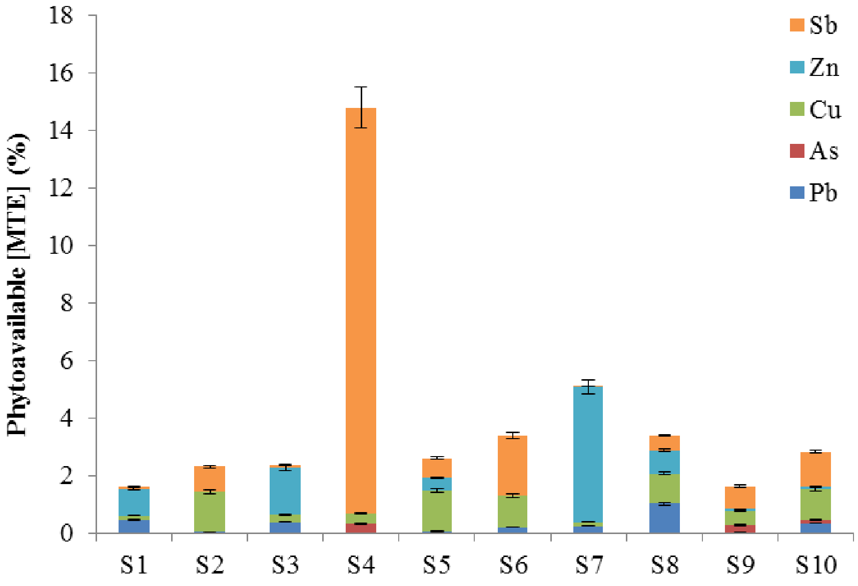


a)

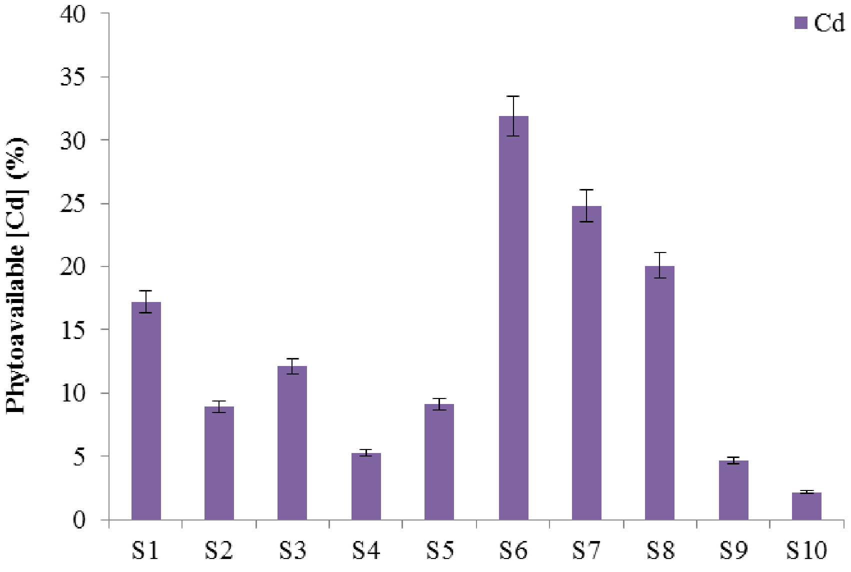


b)

Figure III-3: Ratios (%) between MTE leached according to the EN 12457–2 procedure and aqua regia extraction, for the 10 soil samples: a) sum of the ratios of leached-concentrations (in %) < 10%; b) sum of the ratios of leached-concentrations (in %) > 30%



a)



b)

Figures III-4: Ratios (%) between MTE extracted by $CaCl_2$ and aqua regia, for the 10 soil samples: a) Pb, As, Cu, Zn, Sb; b) Cd.

3.2.3.3. Ecotoxicity tests

Ecotoxicity of leachates measured by the inhibition of *Daphnia magna* mobility was highly variable (*Table III-5*). Whatever the sample tested, the inhibition of daphnia mobility increased between 24 h and 48 h, except for S_3 whose inhibition was near 100 % after only 24 h. Ecotoxicity was also maximal (i.e. 100 %) for S_1, S_2, S_7 and S_8 after 48 h; while the lower inhibition was observed for S_5 (15 %). Ecotoxicity was not simply dependant of MTE concentration: (i) the most MTE-enriched leachates were not always the more toxicant; (ii) leachate of S_8 had low MTE concentrations while the inhibition of daphnia mobility was 100 %.

Ecotoxicity test		**Sample**									
		S_1	***S_2***	***S_3***	***S_4***	***S_5***	***S_6***	***S_7***	***S_8***	***S_9***	***S_{10}***
D. magna [a]	*24 h*	90	35	100	40	10	25	80	65	45	5
	48 h	100	100	100	65	15	80	100	100	65	70
Microtox® [b]	*5 min*	29	*nt**	*nt*	*nt*	*nt*	*nt*	63	*nt*	*nt*	*nt*
	15 min	78.5	*nt*	*nt*	12	*nt*	*nt*	92	*nt*	*nt*	*nt*
	30 min	93	*nt*	34	34	*nt*	*nt*	96	*nt*	*nt*	*nt*

[a]Inhibition of mobility (%); bInhibition of bioluminescence (%).
**nt: not toxic*

Table III-5: Results of the Daphnia magna and Microtox® tests for the 10 leachates

The mean EC_{50}-30 min value obtained for zinc sulphate heptahydrate (expressed as Zn^{2+}) was 2.38 mg L^{-1}, allowing concluding that the invertebrates lot fulfilled the validation specifications according to [34]. Microtox® test results (*Table III-5*) showed an increase in the number of toxic samples with the contact time: inhibition of bioluminescence was detected in two samples at the beginning of the experiment and for four of them at the end (S_1 and S_7 to 5 min; S_1, S_4 and S_7 to 15 min; S_1, S_7, S_4 and S_3 at 30 min). The measured ecotoxicity also increased over time and was above 90 % for S_1 and S_7 after 30 min of contact. The bacteria were most affected by S_7 with an inhibition of the luminescence of 63 % (5 min). Unlike the test on Daphnia, S_2, S_6, S_8, S_9 and S_{10} showed no toxicity, as S5 in both bioassays. As described above, S_1, S_3, S_4 and S_7 are among the most contaminated leachates ([Pb] > 80 mg kg^{-1}; [Sb] > 10 mg kg^{-1} (except S_7)) (*Table III-4-a*).

The sensitivity and specificity of inducible bacteria were measured after 60 min in contact with leachates. None of the sample induced the luminescence of Coplux strain. Only two samples, S1 and S_7, showed a slight toxicity as demonstrated by the inhibition of luminescence of the constitutive strain pBtaclux (*Table III-6-a*).

Sample	Bacterial strain			
	ZntLux [a]	*Arslux* [a]	*Merlux* [a]	*pBtaclux* [b]
S_1	4.8	99.6	9.9	6.5
S_2	0.9	324.9	0.8	-
S_3	4.0	1.2	12.9	-
S_4	1.4	0.8	0.9	-
S_5	0.7	0.9	1.0	-
S_6	0.6	0.8	0.9	-
S_7	5.4	79.8	138.1	18.4
S_8	1,2	0.9	0.9	-
S_9	1,3	2.1	0.8	-
S_{10}	1.2	1.2	0.8	-

[a] *IF: Induction Factor.*
[b] *Inhibition rate (%).*

a)

Sample	Chemical analysis				Biological analysis (prediction with decision trees)			
	Cd	*As*	*Cu*	*Hg*	*Cd*	*As*	*Cu*	*Hg*
S_1	$4.5\ 10^{-7}$	$3.7\ 10^{-7}$	$2.3\ 10^{-6}$	-	10^{-8} - 10^{-7}	10^{-6} - 10^{-5}	-	-
S_2	$1.8\ 10^{-7}$	$29.7\ 10^{-7}$	$1.1\ 10^{-6}$	-	-	10^{-6} - 10^{-5}	-	-
S_3	$9.1\ 10^{-7}$	$3.6\ 10^{-7}$	$1.8\ 10^{-6}$	-	10^{-8} - 10^{-7}	$< 10^{-6}$	-	-
S_7	$48.9\ 10^{-7}$	$3.3\ 10^{-7}$	$0.8\ 10^{-6}$	-	10^{-8} - 10^{-7}	10^{-6} - 10^{-5}	10^{-4} - 10^{-5}	10^{-9} - 10^{-5}

b)

Table III-6: Ecotoxicity results of bioluminescence emitted by the bacterial strains: a) Induction factor and inhibition rate calculated from the bioluminescence; b) Comparison between MTE leached water-soluble concentrations and range "biologically" detected by the bacterial strains (unit: M)

These samples also induced the luminescence of Zntlux, Arslux and Merlux strains. For S_1 and S_7 the maximum IR was recorded for Arslux (IR = 99.6) and Merlux (IR = 138.1) respectively. S_3 increased moderately the luminescence of Merlux (IR = 12.9) and Zntlux (IR = 4.0) while S_2 induced only Arslux (IR = 324.9). The analysis with decision trees was then used to determine the elements potentially responsible for ecotoxicity of S_1, S_2, S_3 and S_7. Crosses between results suggested the presence of arsenic in these four leachates (up to 10^{-5} M, i.e. more than by chemical analysis), cadmium for S_1, S_3 and S_7, biologically at levels lower than those measured chemically (*Table III-6-b*). Analysis of S_7 also showed the presence of copper and mercury (from 10^{-4} to 10^{-5} M and 10^{-9} to 10^{-5} M). According to the previous tests, these results also concluded to the ecotoxicity of S_1 and S_7, and, to a minor extent, the ecotoxicity of S_3 and S_4.

3.2.4. Discussion

3.2.4.1. Mobility and phytoavailability of MTE

In this study, soil pH were basic or close to neutral conditions and leaching procedure slightly reduced the pH by water addition. Conversely, $CaCl_2$ is already known to not modify soil pH and give results closer from field reality [15]. Thus, hazard proposed classification of polluted soils differs between water leaching and $CaCl_2$ procedures. Several studies already showed that MTE extractability is strongly influenced by the nature of the extracting agent which can control element mobility [15, 16]. Moreover, according to Dumat et al. [51] or Ferrari et al. [52], solid-liquid MTE transfers during chemical extractions are complex reactions involving numerous factors that can influence MTE speciation and release. Contact times chosen for chemical extractions were 24 h for water and only 2h for $CaCl_2$ in accordance with the commonly used protocols: these two procedures carry complementary information but the results are not directly comparable.

All MTE were extracted in substantially equal proportions with water (from 0 to 18 %); MTE concentrations in $CaCl_2$ extracts and corresponding ratios, varied in the range of those reported in the literature [5, 58, 61], and Cd was the most available element (up to 32 %). Extracted concentrations were strongly correlated to total concentrations: $R^2 = 0.92$ and 0.95 ($p < 0.0001$) respectively for water and $CaCl_2$. At the reverse side, for all the other elements no relevant correlation was found between total and extracted fractions. In agreement with previous publications [5, 15], these results highlighted the influence of soil properties and MTE nature on its behaviour. Differences observed in function of MTE nature can be explained by different OM or $CaCO_3$ soil contents, CEC or soil pH. In soils, cadmium is generally easy to dissolve which explain its relatively high extractability [53]. High correlation factors were observed between exchangeable Cu and Zn fractions and soil organic matter amount: $R^2 = 0.82$ for Cu ($p < 0.05$) and $R^2 = 0.91$ for Zn ($p < 000.1$). These elements were thus less mobile because of their affinity for this soil fraction [11, 54]. Concerning lead behaviour, no relationship was found between extracted and total concentrations, and the influence of even one soil parameter was difficult to highlight. Nevertheless, low extraction ratios compared to the most concentrated samples (S_1, S_3, S_4 and S_7) can be explained by stronger bounds on soil phases as mineral fraction [29]. Finally, sorption of metalloids as As and Sb, is mainly controlled by mineral phases [55]. The high Sb amount extracted from S_4 could be explained not only by a higher total concentration but also by the highest $CaCO_3$ content [56]. Sb could be solubilised under the influence of soil bio-physico-chemical parameters controlling its sorption [57, 58, 59]. pH and CEC were already described as influent parameters of element extractability [60], retention and mobility in soils [11]. Thus, according to the origin of soil sampling, differences in soil parameters were observed (*Figure 1*): in the industrial site, areas not covered or infiltration zones were the most impacted by MTE. Their organic matter content and CEC were also the higher, thus confirming their role in sorption / desorption mechanisms. The choice of the extractant is thereby an important step to be relevant in risk assessment and to avoid an under- or over-estimation of phytotoxicity. Finally, the data obtained by chemical tests are difficult to interpret because of the many parameters interact. The realization of ecotoxicity tests to measure the impact of pollution on ecosystems seems therefore particularly appropriate in this type of study.

3.2.4.2. Relevance of ecotoxicity tests for risk assessment posed by landfilling

The *D. magna* ecotoxicity test was more sensitive to MTE impact than the Microtox® test. But, unlike tests on the different bacterial strains, they do not both provide information on MTE quantification. Ecotoxicity differences were measured for some samples, especially S_2 and S_8. These differences can be firstly explained by water flea sensitivity. Detection capabilities of the ecotoxicity of the leachate are actually dependent on the test used [62, 63] and it has been already shown that V. fischeri was generally less sensitive than *D. magna* [10, 64]. Instead of these tests, experiments by using bacterial strains allowed to determine and quantify the element which was potentially bioavailable and / or toxic for bacteria [50]. Then, response in ecotoxicity tests was not always directly correlated with total or water-soluble concentrations [65]. These results are in agreement with data previously obtained by Plaza et al. [14] concerning the influence of pH and CEC on MTE behaviour in soils.

Results of this study have shown that this new bioassay enables the screening of samples in terms of environmental risk during remediation process [24]. However, the drawback of the lack of specificity of one strain and the effect of a mixture of MTE (synergistic or antagonistic effects) could be overcome by using a panel of bacterial strains coupled with a predictive model [24]. Due to the lack of specific bacteria for lead, the introduction of other strains induced by lead like *Rastonia Metallidurans* AE 1433 [66] could improve the interpretation of the data.

3.2.4.3. Hazard classification according to ecotoxicity

According to Persoone et al. [67] and Matejczyk et al. [10], the samples were ranked into one of five classes on the basis of the percentage effect (PE) found in Daphnia and Microtox® tests. Ranking was based on induction/inhibition rates for bacterial strains. A weight score was calculated for each hazard class to indicate the quantitative importance (weight) of the ecotoxicity in that class. The weight score was expressed as percentage.

Class weight score = (∑ all test scores) / number of tests performed (= 6)

Class weight score (%) = (class weight score) / maximum class weight score × 100

That classification system aimed at the integration of ecotoxicity data obtained in a battery of bioassays as describe by Lors et al. [68]. The classification system is based on two values: a ranking in five acute toxicity classes and a weight score for each toxicity class. The classification of the samples tested in the investigation is reported in *Table III-7*. Samples were classified as slightly and highly toxic in 10 %, toxic in 30 %, and very highly toxic in 50 %. The percentage of class weight class was above 5 % for only S_7 and S_1 (75 % and 62.5 % respectively). These samples were definitely considered as the most hazardous and acutely toxic to the microfauna. The final classification of ecotoxicity risks was $S_7 > S_1 > S_3 > S_2 = S_4 = S_9 = S_{10} > S_8 > S_5 = S_6$. Although the toxicity of some samples (S_2 and S_8, for instance) could be different depending on the test used, the ranking based on total concentrations and leachable contents of MTE was almost the same; samples S_1, S_3, S_4 and S_7 presenting the greatest risks while the less contaminated areas were generally the less hazardous. However, as our results demonstrated that only a small fraction of total MTE soil concentrations can be solubilised and phytoavailable; ecotoxicity measures complete therefore efficiently standard performed tests for a realistic risks assessment of MTE-contaminated soils accordingly to [10, 14]. Moreover, the use of eco-scores improves the comparisons between bioassays and suggests the use of a restricted battery of tests to perform a cost-effective risk assessment of MTE-contaminated soils.

Sample	Score						Class[a]	MCW[b]	W[c]	PW[d]
	D. magna	*Microtox*	*Zntlux*	*Arslux*	*Merlux*	*pBtaclux*				
S_1	4	3	1	3	2	2	V	4	2.5	62.5
S_2	4	0	0	4	0	0	V	4	1.3	33.3
S_3	4	1	1	1	2	0	V	4	1.5	37.5
S_4	2	1	1	0	0	0	III	2	0.7	33.3
S_5	0	0	0	0	1	0	II	1	0.2	16.7
S_6	3	0	0	0	0	0	IV	3	0.5	16.7
S_7	4	3	2	3	4	2	V	4	3.0	75.0
S_8	4	0	1	0	0	0	V	4	0.8	20.8
S_9	2	0	1	1	0	0	III	2	0.7	33.3
S_{10}	2	0	1	1	0	0	III	2	0.7	33.3

[a]Class				*MCW*
I	*no acute toxicity*	$PE < 20$ %	$IR / InR < 1$	*0*
II	*slight acute toxicity*	$20 \% \leq PE < 50 \%$	$1 \leq IR / InR < 5$	*1*
III	*acute toxicity*	$50 \% \leq PE < 75 \%$	$5 \leq IR / InR < 50$	*2*
IV	*high acute toxicity*	$75 \% \leq PE < 100 \%$	$50 \leq IR / InR < 100$	*3*
V	*very high acute toxicity*	$PE \geq 100 \%$	$IR / InR \geq 100$	*4*

[b]MCW: maximum class weight score.
[c]W: class weight score.
[d]PW: class weight score in percent.

Table III-7: Hazard classification, adapted from [10, 67].

3.2.5. Conclusion.

Biotests and eco-scores improve standard tests performed to assess risk on ecosystems induced by polluted soils. In particular, modified bacteria strains sensitive to metals are useful tools highlighting the presence of different MTE and the influence of soil parameters that lead to synergistic or antagonistic effects.

► **Moreover, eco-scores calculation allows an easy and cheap screening of a large number of polluted soil samples and suggests a restricted battery of bioassays to perform a cost-effective risk assessment**.

Nevertheless, further researches are required to develop a panel of bacterial strains specific of each MTE. Moreover, the chemical characterization of leachates could provide additional relevant insight with the use of modelling software for determination of MTE speciation and thus to evaluate the predominant parameter influencing soil availability and ecotoxicity.

► **Finally, the procedure developed in the present work could be used in the context of bioremediation techniques like phytoremediation performed on polluted soils, to assessment their efficiency and favour their use in a context of sustainable development**.

3.2.6. Acknowledgments.

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3.2.7. References

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Chapter 4

Phyto-restoration of polluted soils and re-functionalization of soil quality

► After a rehabilitation project, soils on the treated site are generally "tired" and need to be re-functionalized in order to increase biological activity and to recover the ecosystem services. Several green manures crops were therefore used to improve the bio-physic-chemical soil properties, leading to potential modifications of lead compartmentalization and thus mobility. In this context, three plants were tested: borage, phacelia (two new plants for phytoremediation uses) and white mustard (already known for phytoremediation use). Their effects on MTE behaviour was assessed using ecotoxicological test and measurements of bioavailability for human and plants in order to perform a global study of treatment efficiency.

4.1. Foreword

Borage, white mustard and phacelia, green manure plants currently used in agriculture to improve soil properties were cultivated for 10 wk on various polluted soils with metal(loid) concentrations representative of urban brownfields or polluted kitchen gardens. Metal(loid) bioavailability and ecotoxicity were measured in relation to soil characteristics before and after treatment. All the plants efficiently grow on the various polluted soils. But borage and mustard only are able to modify the soil characteristics and metal(loid) impact: soil respiration increased while ecotoxicity, bioaccessible lead and total metal(loid) quantities in soils can be decreased respectively by phytostabilization and phytoextraction mechanisms. These two plants could therefore be used for urban polluted soil refunctionalization. However, plant efficiency to improve soil quality strongly depends on soil characteristics.

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Green manure plants for remediation of soils polluted by metals and metalloids: ecotoxicity and human bioavailability assessment.

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4.2. Green manure plants for remediation of soils polluted by metals and metalloids: ecotoxicity and human bioavailability assessment.

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Keywords: Green manure plants, metal(loid)s, polluted soil, ecotoxicity, bioaccessibility, phytoremediation.

4.2.1. Introduction

In many countries, the regulation was recently reinforced to improve the management of (eco)toxicity due to chemicals uses *(Schreck et al., 2013)*. Total quantity of lead emitted into the environment strongly decreased last years *(Cecchi et al., 2008)*. But in the world numerous brownfields and kitchen gardens are polluted *(Bacigalupo and Hale, 2012)*. However, the recovery of urban brownfields is required and the possibility of healthy soil gardening becomes an important issue *(Foucault et al., 2012)*. Phytoremediation techniques modify total and/or bioavailable soil metal(loid) concentrations in relation with compartmentalization and/or speciation *(Butcher, 2009;Vamerali et al., 2010)*. Soil (micro)biology is improved *(Kidd et al., 2009)*, large machinery and excavation equipment are not needed and soil erosion is reduced *(Wong et al., 2006)*. Green manure undemanding plants, usually used in agriculture to improve soil fertility thanks to a high rhizosphere activity *(Zotarelli et al., 2012)* appear as good candidates for phytoremediation, as certain mustard species *(Kim et al., 2010)*. Otherwise, in addition to the measure of total metal(loid) soil concentrations, ecotoxicity *(Plaza et al., 2005)* and availability measures *(Denys et al., 2007)* are needed. As these parameters depend on physicochemical soil properties *(Foucault et al., 2013)*, due to their rhizosphere activity *(Vamerali et al., 2010; Shahid et al., 2011; Shahid et al., 2013)*, green manure plants could change metal(loid) impact. **For the first time to our knowledge, green manure plants were therefore tested for phytoremediation on various polluted soils, with both metal(loid) human bioavailability and ecotoxicity assessment in relation with soil characteristics.**

4.2.2. Materials & methods

4.2.2.1. Soil sampling, preparation and characterization

Various polluted soils were prepared from a highly contaminated sandy top soil (call T) collected from a secondary lead smelter located in Toulouse with pH = 7, CEC (cation exchange capacity) = 8.9 cmol(+)kg^{-1}, CaCO$_3$ = 8 g kg^{-1}, metal(loid) concentrations (mg kg-1): Pb 39,800 ± 796, As 288 ± 6, Cu 286 ± 6, Cd 18 ± 1, Zn 294 ± 6 and Sb 2095 ± 42. Soils were cleaned of roots and visible plant materials, dried and sieved under 2 mm. According to Leveque et al. *(2013)*, soil T was mixed with two unpolluted loamy calcic top cambisol profiles, with metal(loid) concentrations close to the natural geochemical background and noticed C$_1$, in order to prepare the metal(loid) concentrations ranges: 400 mgPb kg^{-1} (C$_2$) and 825 mg kg^{-1} (C$_3$). Soil$_1$ has a high OM (Organic Matter) content (44.7 g kg^{-1}), pH = 6.5, CEC= 12.3 cmol(+)kg^{-1} and a low CaCO$_3$ content (16.1 g kg^{-1}); while soil$_2$ is basic (pH = 8.3), carbonated (98 g kg^{-1}) with low OM content (12.5 g kg^{-1}). Before and after treatment, CEC, OM (organic matter) and CaCO$_3$ contents and pH, were measured respectively according to NF X 31-130, ISO 10694, ISO 10693 and ISO 10390, as the total metal(loid) concentrations were determined by ICP-OES IRIS Intrepid II XXDL, after mineralization with aqua regia (HNO$_3$, HCl, ratio 1:3 v/v) according to ISO 11466. The detection limits of Pb, Cd, Sb, Cu and Zn were 0.3, 0.2, 0.2, 1.3 and 2.2 µg L^{-1}. The accuracy of measurements was checked using a certified reference material 141R (BCR, Brussels).

4.2.2.2. Ecotoxicity tests

4.2.2.2.1. Germination tests

For each soil condition, plants of borage (*Borago officinalis*), phacelia

(*Phacelia stala*) and white mustard (*Sinapis alba* L.) were cultivated in pots in five replicates. Germination tests and growth assays were performed to investigate soil phytotoxicity *(Ma et al., 2010)*. Seeds were immersed in a 10% sodium hypochloride solution for 10 min to ensure surface sterility *(Lin and Xing, 2007)* and rinsed with deionised water. Then, 200 g dry weight of soils were placed in plastic pots: 8 cm (top) in diameter and 7 cm in height with some drain holes on the bottom. Germination was determined by visual seedling emergence and recorded after 8 d in exposed seeds and controls *(Vila et al., 2007)*. After germination recording, only three seedlings of the most uniform plants were kept in each pot to perform growth assays *(Gong et al., 2001)*. Root and shoot lengths were measured after 17 d of growth. Shoot height was measured from the shoot base to the top of the longest leaf and root length was measured from the root–shoot junction to the top of the longest root *(Liu et al., 2005)*. As described by Barrena et al. *(2009)*, phytotoxicity was then expressed by germination index and relative root elongation:

GI = (Relative seed germination × E)/100

RRE = (Mean root length in contaminated soils/mean root length with control) × 100.

With

RRE = (seeds germinated in contaminated soils/seeds germinated in control) × 100.

4.2.2.2.2. Daphnia magna tests on leachates

Normalized CEN 12457-2 leaching test was applied to all soil samples. Ecotoxicity of leachates was then assessed with the water flea *Daphnia magna* (less than 24 h old) according to ISO 6341. Four replicates were tested for each soil solution and five neonates were used in each replicate, with 10 mL of test solution. Organisms were fed two hours before the experiment. The multiwall plate placed in the incubator at 20 °C in darkness. The mobility of *Daphnia magna* was recorded after 24 and 48 h, and inhibition rate was calculated. Microorganisms' validity was verified by reference toxin ($K_2Cr_2O_7$) according to the norm specifications.

4.2.2.3. Soil respiration measurement

Soil respiration (CO_2 efflux) was measured in situ before and after treatment with a LICOR 6400 portable photosynthesis system (infrared gaz analyzer, IRGA) fitted with a LICOR 6000-9 soil respiration chamber (LICOR, Lincoln NE). To minimize soil surface disturbances, a 10 cm diameter soil PVC collar (about 81 cm² area) was installed (1-2 cm deep) one day before the measurements, in each pot in a cleared area *(Han et al., 2007)*. CO_2 flux is computed based on a running average of change in CO_2 concentration with time as CO_2 refills the chamber to a described concentration above ambient concentration (Yim et al., 2002). The process is repeated through three cycles and the intermediate flux data are fit with a regression, which is then used to calculate soil respiration (μmol CO_2 m^{-2} s^{-1}) at ambient CO_2 *(Ramsey et al., 2005)*. The average measurement taken at each pot was used to report soil respiration.

4.2.2.4. Plant experiments

For each experimental condition, 5 kg of soil were placed in pots in a greenhouse. 10 seeds of each species were sown per pot after 10 min immersion in H_2O_2 (10%) to ensure surface sterility *(Lin and Xing, 2007)*. After 10 d of germination, only 3 seedlings of the most uniform plants were kept in each pot to perform crops assays for 10 wk *(Gong et al., 2001)*. Roots and shoots were then separated. Samples were washed with deionised water to remove potentially surface contamination *(Evangelou et al., 2007)* and oven-dried 48 h at 40 °C. The dry weight was determined and the plant parts were grinded to homogenize particle size. Then, they were mineralised 4 h in a 1:1 mixture of HNO_3 and H_2O_2 at 80 °C (Schreck et al., 2011). Metal(loid) concentrations in plant samples were finally measured by ICP-OES (IRIS Intrepid II XXDL) The accuracy of acidic digestion and analytical procedures was checked using Virginia tobacco leaves (CTA-VTL-2, ICHTJ) as reference. All analyses were realised in triplicate

4.2.2.5. Evaluation of metal(loid)s phytoavailability with CaCl2 extraction

Experiments were performed according to Schreck et al. (2011).

4.2.2.6. Lead bioaccessibility

The *in vitro* test consists of two parallel three step extraction procedure and simulates the chemical processes occurring in the mouth, stomach and intestine compartments using synthetic digestive solutions included both gastric and the gastro-intestinal extractions according to physiological transit times (Denys et al., 2007). According to Caboche (2009), only the gastric phase was carried out. Lead bioaccessibility was expressed as the ratio between extracted and total concentrations.

4.2.2.7. Statistical analysis

All tests were performed in five replicates and the results were presented as mean standard deviation. The statistical significance of values was checked using an analysis of variance ANOVA with the Least Significant Difference Fisher post-hoc test using the Statistica 9.0 package software (StatSoft, Tulsa, OK, USA). Each effect was compared to its corresponding control (with an uncontaminated soil). Statistical difference was accepted when the probability of the result assuming the null hypothesis (p) was less than 0.05.

4.2.3. Results

4.2.3.1. Ecotoxicity of soil samples

The three plants grow on the polluted soils without observed phytotoxicity symptoms, butthe GI and root length of borage decreased when soil Pb concentration increased. However, root length was different according to the type of soil: roots were between 2 and 3 cm longer for $soil_2$ than in $soil_1$. Concerning the others species, root length decreased from 12 to 10 cm in $soil_1$ and from 12.7 to 8.7 cm in $soil_2$ for mustard; and for phacelia, from 11 to 7 cm and from 13.5 to 8.6 cm respectively in $soil_1$ and $soil_2$. RRE also decreased with lead concentrations and ratios varied between 55 and 100% for the two species. Daphnia test for borage showed a higher ecotoxicity after 48h than after 24h of contact with the polluted soils. Mobility inhibition was comprised between 10 and 25% for $soil_1$ and between 15 and 33% for $soil_2$ before the experiment. After the culture-period, a decrease was registered for both soils and final ecotoxicity varied from 7.5 to 10% for $soil_1$ and from 5 to 15% for $soil_2$. The same trend was observed for mustard and no influence of phacelia culture was observed on daphnia mobility.

4.2.3.2. Metal(loid)s concentrations and soil parameters before and after cultures

*Figures IV-1*a and *1b* concerning borage experiments respectively for $soil_1$ and $soil_2$, shows total soil metal(loid) concentrations reduction during plant-soil contact function of the nature and initial metal(loid) concentration. In the $soil_2$, the decrease was similar for the conditions C_2 and C_3 with concentrations of lead and antimony reduced by 55 to 60% respectively, from 33 to 49% for Cd and more modestly for Zn (from 23 to 27%) and Cu (from 9 to 19%). Decrease of metal(loid) concentrations was less pronounced in $soil_1$, except for the condition C_3. The variation of Sb was 17%, 21% for Cd and was almost zero for lead in $soil_1$-C_2. These values increased up to 92% for Sb, 53% for Cd and 42% for Pb in $soil_1$-C_3. Except for antimony, recorded variations for $soil_1$ were lower than those in the $soil_2$. Concerning mustard, variations of metal(loid) concentrations were close to those of borage, except for lead where the registered decrease was on average twice lower (maximum was 51% in $soil_1$ and 44% in $soil_2$). Finally, no changes were observed with phacelia. Borage and mustard induced changes of CEC, pH, and $CaCO_3$ in soil1. At the beginning of the experiment, differences in soil parameters were explained by the dilution step. $CaCO_3$ content increased from 16 to 71, from 12 to 31 and from 46 to 148 g g^{-1} respectively for C_1, C_2 and C_3. CEC varied from 7.8 to 10.4 cmol kg^{-1} and pH increased from 5.7 to 8.1. Under mustard crop, same trends were registered and values were also within the same range.

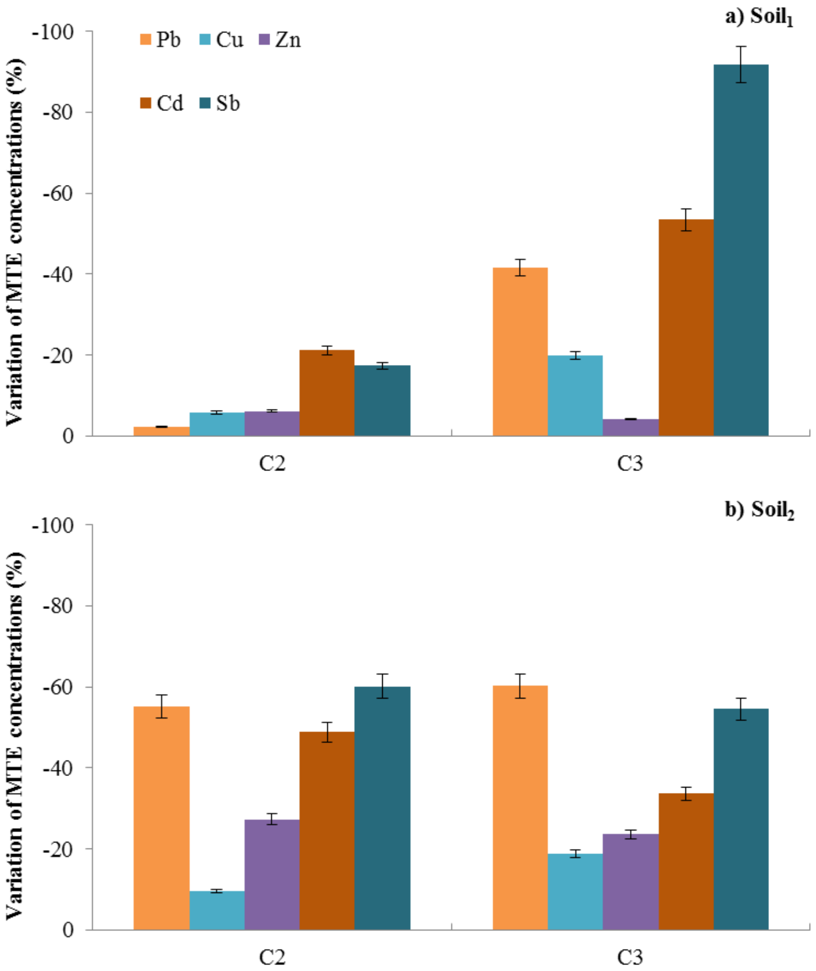

Figure IV-1: Variations of metal(loid)s concentrations (in %) in $Soil_1$ and $Soil_2$ during the culture-period

4.2.3.3. Metal(loid)s concentrations in roots and shoots

Table IV-1 presented metal(loid) concentrations in borage shoots and roots. Cu and Zn were mostly present in shoots, between 26 and 188 mg kg^{-1} and between 70 and 196 mg kg^{-1} respectively, and were not detected in roots except for soil$_2$-C$_3$ with concentrations close to 2 mg kg^{-1}. The same trend was recorded for Cd with concentrations up to 16 mg kg^{-1}. Conversely, lead and antimony were up to 20 times more concentrated in the roots. Cu and Zn were principally found in mustard shoots (up to 69 mg Cu kg^{-1} and 260 mg Zn kg^{-1}). A same trend was found for cadmium, with maximum concentrations in shoots of 11.1 mg kg^{-1} in soil$_1$. Conversely to borage, lead concentrations were higher in mustard shoots: up to 1131 mg kg^{-1} in soil$_2$. Only Sb followed the same trend for borage and mustard whose concentrations in roots reached 229 and 218 mg kg^{-1} for soil$_1$ and soil$_2$ respectively. Amounts of metal(loid)s recorded for phacelia both in shoots and roots were very low accordingly to low variation of metal(loid)s in soils.

1a	**Pb**		**Cu**		**Zn**		**Cd**		**Sb**	
	Sh.	***Ro.***	***Sh.***	***Ro.***	***Sh.***	***Ro.***	***Sh.***	***Ro.***	***Sh.***	***Ro.***
Soil$_1$-C$_1$	1.2	17.1	188	< DL*	196	< DL	< DL	0.5	< DL	< DL
Soil$_1$-C$_2$	239	210.3	71.8	< DL	152.9	< DL	16.2	< DL	12.6	1.8
Soil$_1$-C$_3$	160.5	589.9	49.0	< DL	88.5	< DL	2.0	0.3	10.4	91.5
Soil$_2$-C$_1$	0.9	9.9	99.5	< DL	102.1	< DL	< DL	< DL	< DL	< DL
Soil$_2$-C$_2$	111.6	463.7	56.2	< DL	84.2	< DL	1.4	< DL	7.9	40.3
Soil$_2$-C$_3$	90.0	936.8	26.3	2.0	69.9	1.9	2.8	0.6	6.1	139
1b	**Pb**		**Cu**		**Zn**		**Cd**		**Sb**	
	Sh.	***Ro.***	***Sh.***	***Ro.***	***Sh.***	***Ro.***	***Sh.***	***Ro.***	***Sh.***	***Ro.***
Soil$_1$-C$_1$	1.3	< DL	69.0	< DL	122.9	< DL	0.9	< DL	7.3	< DL
Soil$_1$-C$_2$	253.0	2.6	31.9	< DL	165.4	< DL	5.5	< DL	18.9	4.4
Soil$_1$-C$_3$	250.4	5.9	12.7	2.2	260.3	9.6	11.1	< DL	12.1	229.3
Soil$_2$-C$_1$	15.3	< DL	54.4	< DL	78.7	< DL	< DL	< DL	17.1	< DL
Soil$_2$-C$_2$	89.6	20.4	18.4	<DL	61.4	< DL	1.9	< DL	5.8	12.2
Soil$_2$-C$_3$	1131	363.6	58.8	3.5	91.1	4.5	3.3	1.1	79.3	218.5

*Table IV-1: Metal(loid)s concentrations in shoots and roots (mg kg^{-1} DW): a) borage; b) mustard. Values are given as mean of five replicates with three seedlings each. *DL: Detection Limit (= 0.1 mg kg^{-1})*

4.2.3.4. Soil respiration

Depending of soil characteristics and initial metal(loid) concentrations, soil respiration only increased with borage (see *Figure IV-2*) and mustard. Concerning borage, CO_2 flux initially ranged from 0.84 to 1.57 µmol CO_2 m^{-2} s^{-1} between soil$_1$-C$_1$ and C$_3$, but only from 0.64 to 0.85 µmol CO_2 m^{-2} s^{-1} between soil$_2$-C$_1$ and C$_3$. Similarly, after 10 weeks of culture, the amplitude was higher for soil$_1$. Moreover, soil respiration increased with metal(loid) concentrations. For soil$_1$ (µmol CO_2 m^{-2} s^{-1}): 1.7 (C$_3$), 0.5 (C$_2$) and 0.26 (C$_1$), and for soil$_2$ (µmol CO_2 m^{-2} s^{-1}): 1.6 (C$_3$), 1.5 (C$_2$) and 1 (C$_1$).

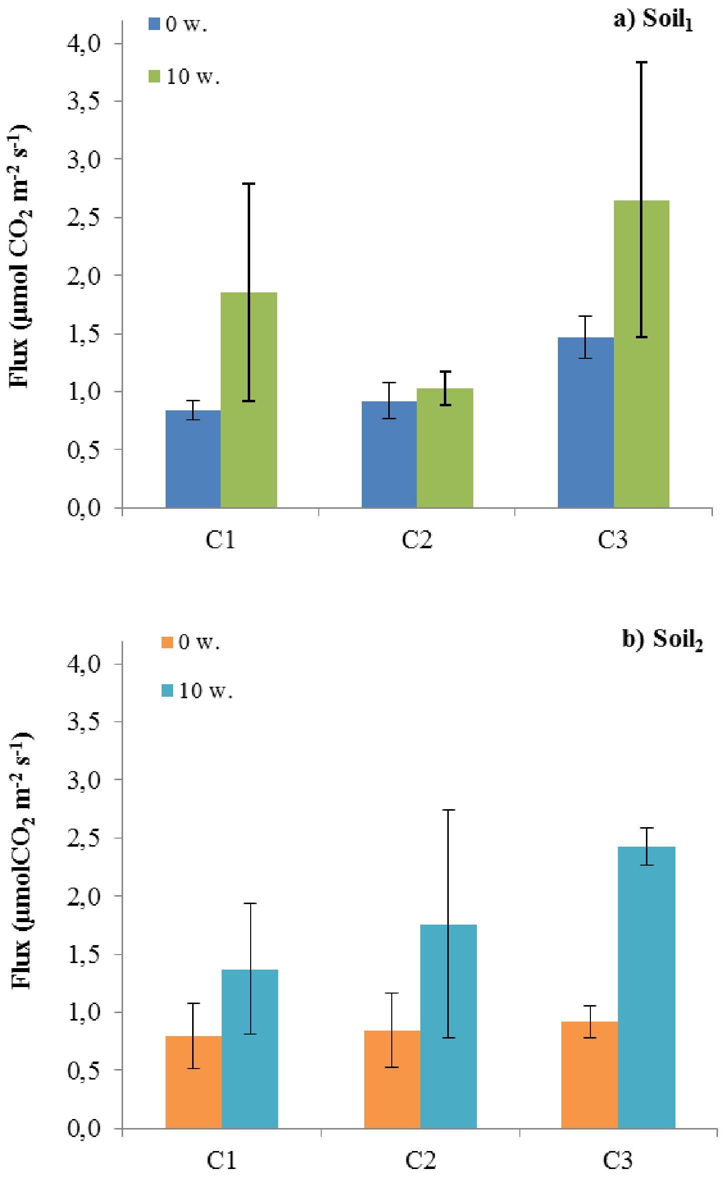

Figure IV-2: Comparison of mean soil respiration rates between the beginning and the end of the experimentation for soil$_1$ (a) and soil$_2$ (b) (in µmolCO$_2$ m^{-2} s^{-1}).

4.2.3.5. Metal(loid)s bioavailability

Experiments focused on lead: phytoavailability varied from 0 to 8 or 10 mg kg^{-1} respectively for soil$_2$ and soil$_1$. Concerning soil$_1$, even if the condition C$_2$ initially presented the highest extracted fraction (66 mg kg^{-1}), after treatment, lead concentration was 0.3, 6.7 and 10.4 mg kg^{-1}, respectively for C$_1$, C$_2$ and C$_3$. In soil$_2$, Pb-contents were initially below the limit detection for C$_1$ and C$_2$, and reached 8.6 mg kg^{-1} for C$_3$. Then, concentrations for C$_1$ and C$_2$ are around 4.8 mg kg^{-1}, while it was 1.8 mg kg^{-1} in C$_3$. Pb quantities extracted under mustard were in the same range, but not detected for phacelia.

Amounts potentially accessible for human were higher than those available for plants. In the soil$_1$, lead bioaccessibility was initially 39, 63 and 35% respectively for C_1, C_2 and C_3. These ratios increased after treatment with borage to reach 46, 92 and 75% respectively for C_1, C_2 and C_3. A linear trend was observed for the soil$_2$. Lead bioaccessibility increased with soil concentration: from 8 to 15% (C_1), 27 to 50% (C_2) and 54 to 98% (C_3). However, when results are expressed as quantities, taking into account the modification of lead concentrations in soils, two behaviours were distinguished in function of soil nature. For soil$_1$, the fraction of extracted lead grown with the treatment: from 251 to 359 mg kg^{-1} (C_2) and from 291 to 362 mg kg^{-1} (C_3); conversely, for soil$_2$ bioaccessible fraction decreased from 110 to 89 mg kg^{-1} (C_2) and from 444 to 323 mg kg^{-1} (C_3).

4.2.4. Discussion

4.2.4.1. Improvement of soil quality

Borage, mustard and phacelia were able to grow on various polluted soils. A trend for germination reduction was however observed as roots are directly in contact with pollutants *(Shreck et al., 2011)*. Root length was influenced by lead concentration ($R^2 = 0.56$ and 0.59 for soil$_1$ and soil$_2$ respectively), as RRE was too (R^2-soil$_1$ = 0.74; R^2-soil$_2$ = 0.62). Soil characteristics also affected root length as showed by correlations: (i) in the soil$_1$, R^2 = 0.73 for pH and OM content and 0.63 for CEC; in soil$_2$, R^2 = 0.83, 0.66 and 0.69 with pH, OM and $CaCO_3$ content respectively. Root toxicity was lower in soil$_2$ because, as noted by Birkefield et al. *(2007)*, lead oxide particles incubated in calcareous soil can be covered by a crust of lead carbonate. Soil respiration significantly increased after phytoremediation, certainly in relation with both changes of abiotic *(Han et al., 2007)* and biotic factors as microbial activity *(Nwachukwu et al., 2011)*.

4.2.4.2. Evolution of environmental and sanitary risks

In soil$_2$, the concentration of phytoavailable lead is related to the soil concentration ($R^2 = 0.95$ and 0.94 at 0 and 10 wk respectively). Moreover, as for soil respiration and root toxicity, $CaCO_3$ amount is influent: R^2-soil$_1$ = 0.89 and R^2-soil$_2$ = 0.95 at the end of experiment. Soil properties can strongly modify metal bioavailability *(Kidd et al., 2009)*. Soil$_1$ showed an $CaCO_3$ content increase and a more basic pH which can lead to a reduction of metal(loid) availability. Lead bioaccessibility (in %) increased during the culture-period and was statistically correlated with total lead-concentrations (R^2-soil$_1$ = 0.61 and 0.95 at 0 and 10 wk respectively; R^2-soil$_2$ = 0.75 initially and 0.98 after treatment), and accordingly to Caboche *(2009)*, with the soil characteristics: pH and CEC for soil$_1$; carbonate and OM content for soil$_2$. However, a lower lead quantity was bioaccessible after 10 wk in soil$_2$ conversely to soil$_1$. Moreover, soil ecotoxicity measured by sensitive daphnia test was reduced by the treatment, certainly due to rhizosphere activity.

4.2.4.3. Suitability of green manures crops for phytorestoration of polluted soils

Metal(loid) compartmentalisations in the plants depend both on pollutant and plant type: copper and zinc were mainly concentrated in shoots; lead and antimony were mainly concentrated in roots of borage but in shoots of mustards. According to Fan et al. *(2011)*, bio-concentration and translocation factors are respectively defined as BCF (Bio-Concentration Factor)

= $(Q_2 + Q_3)/Q_1$ and TF (Translocation Factor) = Q_3/Q_2; where Q_1, Q_2, Q_3 are average metal(loid) quantities (in mg kg^{-1}) respectively in soil, roots and shoots. BCF > 1 indicates that the plants accumulate the pollutants and BCF < 1 indicates excluder plants *(Arshad et al., 2008)*. Accordingly to Evangelou et al. *(2007)*, using $PbNO_3$ spiked soils, calculated BCF for borage was below 1 for Pb and Sb. BCF was far above 1 for Cu and Zn (and Cd in a lesser extent) (*Table IV-2*), which are essential elements for plants *(Zheng et al., 2011)*. TF calculated for borage were below 1 for Pb, Sb and Cd (except for soil$_1$-C$_2$) and above 1 for Cu and Zn. Borage stabilises lead and antimony into its roots *(McGrath and Zhao, 2003)*. Zou et al. *(2011)* showed that metal(loid)s are unevenly distributed in roots, where different root tissues act as barriers to apoplastic and symplastic transport, thereby restricting transport to the shoots. Borage extracts and translocates Cu and Zn and could therefore be used for remediation of polluted vineyards *(Banas et al., 2010)*. Calculated TF confirmed that mustard was relevant for phytoextraction *(Bareen and Tahira, 2010)*. Finally, for all the performed experiments, the soil-plant metal(loid) transfer was influenced by the soil type, as metal(loid) availability may change according to the interactions with the soil matrix *(Niemeyer et al., 2012; Shahid et al., 2012)*.

2a	**Pb**		**Cu**		**Zn**		**Cd**		**Sb**	
	BCF	***TF***	***BCF***	***TF***	***BCF***	***TF***	***BCF***	***TF***	***BCF***	***TF***
Soil$_1$-C$_1$	1.0	0.1	10	> 1884	3.7	> 1962	0.6	0.2	0.1	< 0.5
Soil$_1$-C$_2$	0.4	0.2	3.7	> 718	2.1	> 1528	0.6	17	0.2	0.2
Soil$_1$-C$_3$	0.4	0.3	2.1	> 489	1.2	> 885	0.5	6.7	0.1	0.1
Soil$_2$-C$_1$	0.9	0.1	6.0	> 994	2.9	> 1020	1.0	1.5	0.1	< 1
Soil$_2$-C$_2$	0.4	0.2	1.8	> 562	1.4	> 842	0.6	13	0.6	0.3
Soil$_2$-C$_3$	0.2	0.1	0.8	13	0.7	37	0.7	4.6	0.5	0.1

2b	**Pb**		**Cu**		**Zn**		**Cd**		**Sb**	
	BCF	***TF***	***BCF***	***TF***	***BCF***	***TF***	***BCF***	***TF***	***BCF***	***TF***
Soil$_1$-C$_1$	0.1	> 13	3.9	> 690	2.3	> 1228	1	> 8.9	2.9	> 73
Soil$_1$-C$_2$	0.2	96	1.7	> 319	2.3	> 1653	2.6	> 54	0.3	4.3
Soil$_1$-C$_3$	0.1	423	0.6	> 5.7	3.7	27	2.6	> 111	1.0	<0.1
Soil$_2$-C$_1$	1.4	> 153	3.3	> 544	2.2	> 786	0.2	> 1.0	6.9	> 1701
Soil$_2$-C$_2$	0.2	7.2	0.6	> 184	1.1	> 614	0.9	> 19	0.2	0.5
Soil$_2$-C$_3$	0.3	3.1	1.8	16.8	1.0	20	0.8	2.9	0.9	0.4

Table IV-2: Bioaccumulation factors (BCF) and translocation factors (TF) calculated for a) borage; b) mustard.

4.2.5. Conclusion

Unlike to phacelia, **borage and mustard can improve soil respiration, reduce total and bioaccessible metal(loid) quantities and their ecotoxicity**. For lead and antimony, **contrasted mechanisms were developed: phytoextraction with storage in shoots and phytostabilization with storage in roots respectively for mustard and borage**. Thanks to these treatments, metal(loid)s entering in the food chain *via* water, wind erosion and re-flying can be reduced and a global analysis of the remediation techniques is performed. Further, as borage plants are not perennial, they should be harvested to avoid the release of metal(loid)s in soil, and treated in a waste treatment unit. Moreover, a better understanding of the complex interactions between plants and rhizosphere microorganisms is required to improve the treatment efficiency.

4.2.6. Acknowledgement

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4.2.7. References

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Chapter 5

Role of the rhizospheric biomass on MTE mobility

► The chapter before demonstrated borage and mustard are good candidates for phytoremediation. This technique is influenced by rhizospheric microorganisms which can modify MTE compartmentalization, and, thus, ways of transfer to plants. In the next part, we investigated changes of microbial communities under these crops by a recent biotest, rhizostest, which allowed obtaining rhizospheric soil, area where mechanisms of phytoextraction and phytostabilisation occur.

5.1. Foreword

Phytoremediation techniques use the plant's capabilities with its associated microorganisms to decrease the environmental impact of metal(loid)s as well as improve the soil's properties. Use and development of metallophytes for rehabilitation of contaminated urban brownfields is crucial for the preservation of soil ecosystem services. A better understanding of the microflora associated with these plants is however of special interest.

In this study impacts of metalloids on the rhizosphere microflora of two green manure cultures (*Sinapis alba* L. and *Borago officinalis* L.) were evaluated under different polluted soils (with up to 825 mg kg^{-1} Pb and presence of Sb, Cd, Cu and Zn) as well as soil types (pH, texture, $CaCO_3$ and organic matter contents) conditions. A biotest rhizotest was used before taxonomic changes and bacterial communities were determined using T-RFLP analyses.

This chapter is presented in a form of an article in preparation for the journal Plant Soil:

Activity and evolution of rhizospheric microorganisms under green manures crops influenced by different soil properties and MTE contaminations.

Foucault, Y., Compant, S., Mitter, B., Polt, M., Xiong, T., Austruy, A., Sessitsch, A. & Dumat, C.

5.2. Activity and evolution of rhizospheric microorganisms under green manures crops influenced by different soil properties and MTE contaminations.

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Keywords: Rhizotest, MTE-contaminated soils, green manures, microbial communities, T-RFLP.

5.2.1. Introduction

Pressures generated by the expansion of urban areas, e.g. environmental, health, social and land pressures, now lead governments to rehabilitate metal(loid)s-contaminated sites as well as brownfields (Wong et al., 2006; Foucault et al., 2013a). Mainly applicable for the treatment of polluted soils with media metal(loid)s concentrations, plant based technologies such as the use of metallophytes (Baker et al., 2010; Alford et al., 2010) allow the biological restoration of degraded soils with a reasonable cost (Khan, 2005). This is of special interest therefore to remove contamination from polluted sites and to rehabilitate sites even with high metalloids contaminations.

The efficiency of new green manures crops on global soil quality improvement and restoration was demonstrated in a context of a lead-contaminated site near a batteries-recycling plant (Foucault et al., 2013b). Several questions related to this contaminated site need however to be answered. Especially, how microbes living in close association with this green manure crops and enabling phytoremediation of metal trace elements should be impacted need to be studied. In both natural and managed soils, bacteria inhabiting the rhizosphere play indeed a key role in host plant adaptation to a changing environment (Sturz & Nowak, 2000; Mitter et al., 2013). The release by the roots and the microorganisms in the rhizosphere of various compounds can modify metal(loid)s compartmentalization as well as speciation with consequences on their solubilisation and bioavailability (Lin et al., 2004; Mitter et al., 2013). It is well known that rhizobacteria help the plant to remediate polluted soils. However under some conditions, there is still unsuffisant indication on how metalloid can impact their communities. Due to the compartmentalization of the metal(loid)s between various fractions, each with different bioavailability, there is moreover usually a poor relationship between the plant uptake and total content of metal(loid)s in soils (Lin et al., 2010). A better understanding of the physical, chemical and biological rhizospheric processes in the soil-plant systems (Renella et al., 2006; Schwab et al., 2008) and the interactions between hyperaccumulator and non-accumulator plant species is needed to enhance phytoremediation (Khan, 2005; Kuffner et al., 2008; Mitter et al., 2013).

The aim of this present study was therefore to study the potential changes in bacterial communities induced by lead pollution (0 or 825 mgPb kg^{-1}), under different soils conditions as well as with different plant species (borage or mustard) having phytoremediation abilities. An analyze of soil properties was combined with deciphering the **rhizobacterial communities using Terminal restriction length polymorphism (T-RFLP) analysis to know better how the plant rhizospheric communities of green manures plants can be impacted by metalloids**.

5.2.2. Materials & methods

5.2.2.1. Soil sampling and preparation

To study the influence of soil physico-chemical properties and green manures crops on soil quality, two ranges of soils were firstly prepared from a highly lead-contaminated soil with Pb (and other Metal Trace Elements; MTE, as Cd, Sb, Cu, Zn, etc.). This soil, called T, was collected from a secondary lead smelter (STCM, Société de Traitements Chimiques des Métaux, Toulouse, France), between 0 and 25 cm. Physico-chemical properties and MTE concentrations (Pb, As, Cd, Cu, Zn, and Sb) of this soil are given *Tables V-1* and *V-2*.

Soils	**Texture (%)**					**pH**	**OM (g kg^{-1})**	**CEC (cmol(+)kg^{-1})**	**CaCO$_3$ (g kg^{-1})**
	Clay	***Thin silt***	***Rough Silt***	***Thin Sand***	***Rough Sand***				
Soil T	13.3	18.3	14.2	15.7	38.5	7.0	31.4	8.9	8.0
S_1	13.3	24.8	23.5	22.5	15.9	6.5	44.7	12.3	16.1
S_2	19.5	15.4	23.6	23.8	17.7	8.4	12.5	8.8	98.0

Table V-1: Physico-chemical properties of the three sampled soils. Soil T was collected from a secondary lead smelter (STCM, Toulouse, France). Two top soils of two calcic cambisol profiles (called Soil-1 and Soil-2 and designed S_1 and S_2) were collected around several battery recycling plants as the study site.

Pb	***As***	***Cu***	***Cd***	***Zn***	***Sb***
39,800 ± 796	288 ± 5.76	286 ± 5.72	18.4 ± 0.37	294 ± 5.88	2095 ± 41.9

Table V-2: MTE concentrations in soil-T (in mg kg^{-1}).

Top soils of two calcic cambisol profiles (FAO, 1998) produced by quaternary alluvial deposits, were sampled to prepare the ranges of MTE contaminations. These soils were chosen because this kind of soil has been observed around several battery recycling plants as the study site (Uzu et al., 2009). These two soils (called Soil-1 and Soil-2 and designed S_1 and S_2) differed by texture, soil cultivation, OM content and CEC as shown in *Table V-1*. All soils were roots and visible plant materials cleaned with deionised water, dried and then sieved under 2 mm. The contaminated soil was diluted and mixed in each soils to obtain lead concentrations in soils of 825 mg kg^{-1} of MTE (designed C_2) and two controls soils (designed C_1) were designed to corresponding concentrations closed to the natural geochemical background (between 30 and 50 mg kg^{-1} for these soils).

5.2.2.2. Plant species used for remediation

For each soil condition, two plant species were cultivated in pots. The first species was white mustard (*Sinapis Alba* L.) (called M in this study) whose hyper-accumulator capacities have been well documented (Meyers et al., 2008; Kim et al., 2010). The second one was borage (*Borago officinalis* L.) (called B), already currently used by farmers and gardeners because of its ease of culture and impact on soil quality improvement through a high production of root exudates (Foucault et al., 2013b).

5.2.2.3. Rhizotest

A biotest was used in this study to assess MTE bioavailability for plants. This biotest, also known as rhizotest, was firstly described by Niebes et al. (1993), then adapted by Chaignon and Hinsinger (2003). It is now the subject of a draft standard for studying trace elements for soil quality and plants growing on these soils (ISO 16198) (ISO, 2011). This test presented two main advantages: (i) the roots are physically separated from the soil which enables total recovery of the shoots, roots and soil; (ii) the thickness of the soil layer used enables it to be considered it as rhizosphere soil and provides enough rhizosphere material to evaluate root-induced changes in metal speciation.

Biotest was used for two stages for plant growth (*Figure V-1*). In the first stage (pre-culture), plants were grown hydroponically for two weeks to reach an adequate plant biomass and a dense root system. A small PVC cylinder (25 mm inner diameter) was closed by a polyamide net (900 µm mesh) inserted into a larger cylinder, itself closed by a finer polyamide mesh (30 µm, Fyltis/Nytel, Sefar filtration). A space of 3 mm was left between the net and the finer mesh, where the roots could develop as a mat. Commercial seeds of green manure plants (Botanic, France) were surface sterilized 6% hydrogen peroxide for 10 min before to be rinsed with deionised water. Three seeds were sown in each container on the surface of the coarser mesh and five replicates were carried out. The devices were placed on top of troughs containing an aerated complete nutrient solution with macro-elements (5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM KH_2PO_4 and 1.5 mM $MgSO_4$), and oligo-elements (9.11 µM $MnSO_4$, 1.53 µM $ZnSO_4$, 0.235 µM $CuSO_4$, 24.05 µM H_3BO_3, 0.1 µM Na_2MoO_4 and 268.6 µM Fe/EDTA) (all compounds were from VWR International SAS, France). The height of the nutrient solution was adjusted daily to keep the fine mesh wet during the whole pre-culture period.

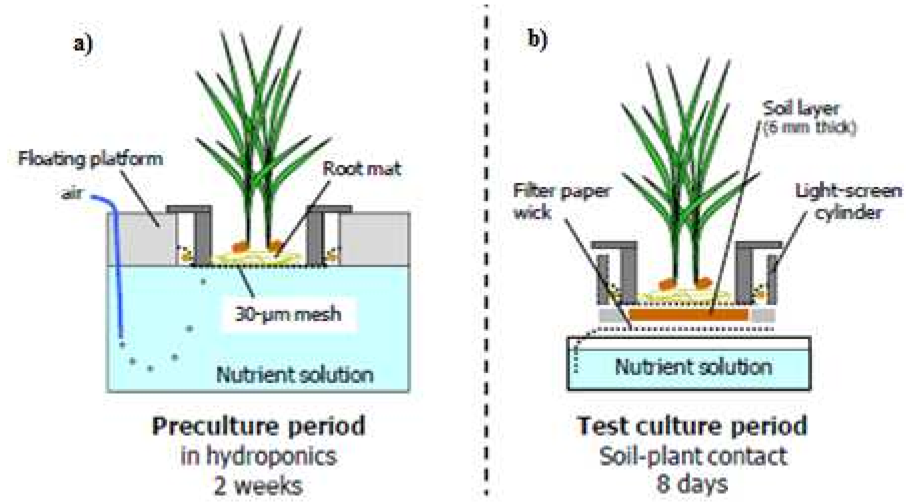


Figure V-1: The two steps procedure of experimental device of the plant biotest for the assessment of the environmental bioavailability of trace elements to plants (ISO, 2011): a) Pre-culture period in hydroponic conditions; b) Test culture period, i.e. soil-plant contact.

In the second stage (culture test), the material culture of pre-rooted plants were brought into contact to soil for 8 days with a thin layer of about 6 mm from a soil sample sieved to 2 mm. All the experiments were conducted in a growth chamber (with temperature of 24 ± 0.5 °C / 18 ± 0.5 °C day/night; a photoperiod of 16 h under daylight fluorescent lamps and 8 h darkness, and a 70% relative humidity). After the soil-phase contact, i.e. 21 days after the beginning of the test, soils were collected, dried and sieved under 2 mm.

5.2.2.4. Plant sampling and analyses of MTE concentrations

At the end of the Rhizotest experiment, plants were cut short above the ground to separate aerial plant parts and roots. Both shoots and roots were washed with deionised water to remove potentially surface contamination according to Evangelou et al. (2007), and were then oven-dried at 40 °C for 48 h. Samples were mineralised in a 1:1 mixture of HNO_3 and H_2O_2 at 80°C for 4 h according to Uzu et al. (2009) and Schreck et al. (2011). MTE concentrations in roots were finally measured by ICP-OES (IRIS Intrepid II XXDL; Thermo Scientific, USA), and the accuracy of acidic digestion and analytical procedures was checked using Virginia tobacco leaves (CTA-VTL-2, ICHT, Poland) as a reference.

5.2.2.5. Bacterial DNA extraction and 16S rDNA gene amplification

DNA from rhizospheric soils obtained from rhizotest was extracted using FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA) according to the manufacturer's instructions before to be purified with a silica-based GENECLEAN® procedure using spin filters and concentrations quantified using a nanodrop (Nanodrop ND-1000; Nanodrop Technologies Wilmington, Germany). DNA were then diluted as 1:100 to avoid effect of inhibitors such as humic acids on PCR (according to Williamson et al., 2011). 16S rDNA sequences were then amplified with a thermocycler (Biometra, Goettingen, Germany) using an initial denaturing step of 5 min at 95 °C followed by 34 cycles of 30 s at 95 °C, 1 min annealing at 52 °C and 1.5 min extension at 72 °C. The PCR reaction mixtures (25 µL) contained 2.5 µL of 10X reaction buffer (Gibco, BRL), $MgCl_2$ (25 mM), dNTPs (2 mM µL-1), 5 µL of primers 8f FAM (1.5 µM) (5'-AGA GTT TGA TCC TGG CTC AG-3') and 518rev (1.5 µM) (5'-GTA TTA CCG CGG CTG CTG G-3') (Edwards et al., 1989), 0.3 µL of Taq DNA polymerase (Gibco, BRL), 4.2 µL of ultra-pure water and 3 µL of extracted DNA. To confirm PCR amplification products, electrophoresis was then performed on a 1% agarose gel stained with ethidium bromide at 120 volts for 45 min with 5 µL of each sample. After electrophoresis, the DNA bands were visualised with a Biospectrum® Imaging System. Purification of DNA bands was then performed using a QIAquick PCR purification kit (QIAgen, Germany) according to the manufacturer's instructions. DNA concentration was then checked again using a NanoDrop spectrophotometer and adjusted to a final concentration of 100 ng µL^{-1}.

5.2.2.6. PCR products digestion and T-RFLP analysis

Purified PCR amplification products were digested with restriction enzymes *Alu*I and *Hae*III (Gibco, BRL). For this, aliquots of 100 ng µL^{-1} were mixed with 0.5 µL of enzyme, 1.5 µL of 1 × buffer and ultra-pure water to obtain a mixture of 15 µL, and incubated at 37 °C during 3h. Digestion assays were then purified by passage through a DNA grade Sephadex G50 (GE Healthcare) columns before using HiDi-formamide (Applied Biosystems), and samples were denatured at 95 °C for 2 min and immediately chilled on ice prior to T-RFLP. Analyses were then performed with a 3130xl Genetic Analyzer (Applied Biosystems) for T-RFLP and representative sample profiles were analysed with Peak Scanner® software 1.0.

Considering that a difference of ± 2 bp in the sizes of T-RFs is possible to occur due to the nature of the gel separation with the automated DNA sequencer, T-RFs that differed by less than 2 bp were clustered.

Additionally, to avoid detection of primers and uncertainties of size determination, terminal fragments smaller than 35 bp and higher than 500 bp were excluded from the analysis.

Three replicates samples of all treatments were analyzed individually and T-RFLP profiles were normalized according to a procedure suggested by Dunbar et al. (2000). The sum of peak heights in each replicate profile was calculated, indicating the total DNA quantity. Total fluorescence was then adjusted to the medium DNA quantity using a correction factor. With three replicate profiles having total fluorescence values of 1,700, 2,000 and 2,400, each peak in the latter profile was for instance multiplied with a factor of 0.83 (i.e., the quotient of 2,000/2,400), and peaks in the first profile were multiplied with a factor of 1.18 (i.e., a quotient of 2,000/1,700). After adjustment, only peaks of ≥ 50 fluorescence units were considered. T-RFs were scored additionally as positives only when they were present at least in two of three replicates.

5.2.2.7. Statistical analysis

All experiments have been realized in triplicate using each time 5 pots for each plant species. T-RFLP profiles were compared using multivariate statistical method. Principal component analyses were used using CANOCO software for windows with the aim of identifying samples generating similar patterns.

5.2.3. Results

5.2.3.1. MTE concentrations

For lead, high concentrations were measured for both plants on contaminated soils (whatever the origin), between 541 and 651 mg kg^{-1}, while it was between 11.6 and 18.7 mg kg^{-1} for clean soils (*Tables V-3* and *V-4*).

Borage	***OM rich soil***	Not contaminated	BS_1C_1
		Contaminated	BS_1C_2
	Carbonated soil	Not contaminated	BS_2C_1
		Contaminated	BS_2C_2
Mustard	***OM rich soil***	Not contaminated	MS_1C_1
		Contaminated	MS_1C_2
	Carbonated soil	Not contaminated	MS_2C_1
		Contaminated	MS_2C_2

Table V-3: Abbreviations of the systems used. B. Borage; Borago officinalis L., M. Mustard; Sinapis Alba L., C_1 *not contaminated,* C_2 *contaminated,* S_1 *soil rich in organic material,* S_2 *carbonated soil*

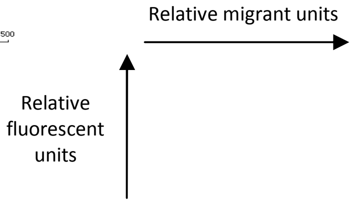
Sample	[Pb]	[As]	[Cd]	[Sb]	[Cu]	[Zn]
BS_1C_1	18.7	nd*	nd	1.1	16.6	49.9
BS_1C_2	627	2.2	22.3	54.9	21.6	76.2
BS_2C_1	11.6	2.7	0.6	1.8	11.2	52.4
BS_2C_2	541	3.7	7.5	64.5	21.5	77.1
MS_1C_1	12.2	0.2	0.9	0.9	10.5	33.9
MS_1C_2	549	2.5	10.4	55.3	14.4	47.2
MS_2C_1	16.7	4.8	0.4	1.3	8.9	30.2
MS_2C_2	651	7.4	5.2	60.2	15.4	33.0

Table V-4. Total metal(loid)s concentrations in roots (mg kg^{-1} DW) of plants species used in the study.

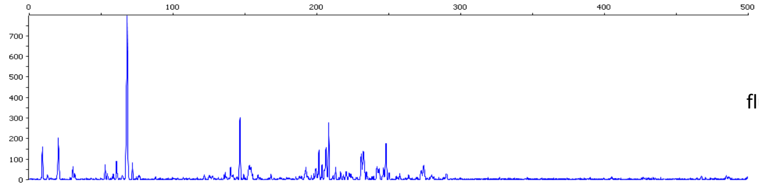
These results were very homogenous independently of the plant species used (borage or mustard). Same trend was recorded for Sb with homogeneous values between 0.9 and 1.8 mg kg^{-1} on uncontaminated soils, and concentrations between 54.9 and 64.5 mg kg^{-1} for contaminated soils (*Table V-4*). For Cu and Zn, concentrations were different according to the level of contamination, and to the plant species (*Table V-4*). For borage, Cu concentrations varied between 11.2 and 16.6 mg kg^{-1} on non-contaminated soil, and was around 21.5 mg kg^{-1} on contaminated soil; Zn concentrations were close to 50 mg kg^{-1} for C_1 and 76-77 mg kg^{-1} for C_2. Conversely, under mustard crops, mean Cu values were around 9 mg kg^{-1} for C_1 and 15 mg kg^{-1} for C_2, and, for Zn, around 32 mg kg^{-1} and 48 mg kg^{-1} for C_1 and C_2 respectively (*Table V-4*). Maximum concentrations in roots depended on the type of soil for the two species: from below the limit detection to 2.5 mg kg^{-1} for soil$_1$, and from 2.6 to 7.4 mg kg^{-1} for soil$_2$. Moreover, for soil$_2$, values were higher for mustard than for borage: 4.8 mg kg^{-1} for MS_2C_1 and 7.4 mg kg^{-1} for MS_2C_2, and 2.7 mg kg^{-1} for BS_2C_1 and 3.7 mg kg^{-1} for BS_2C_2. Finally, Cd concentrations also varied according to the type of soil and plant species. Under borage, concentrations reach 22.3 mg kg^{-1} for soil$_1$ and 7.5 mg kg^{-1} for soil$_2$, while for mustard these concentrations were 10.4 and 5.2 mg kg^{-1} for soil$_1$ and soil$_2$ respectively.

5.2.3.2. T-RFLP analysis

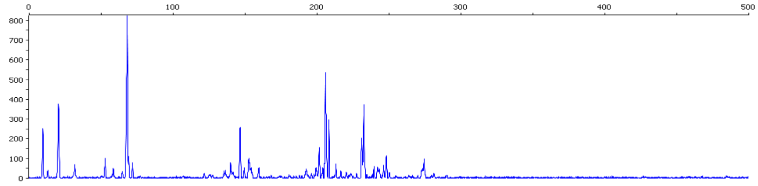
Bacterial population structures in the different soils were determined by T-RFLP analyses. *Figure V-2* represents the different profiles obtained for each condition.



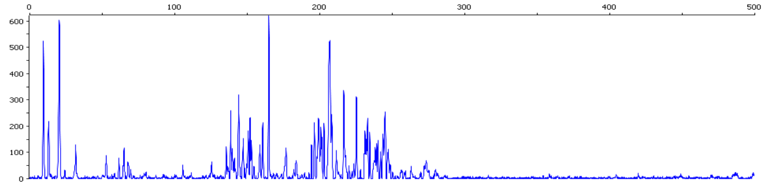
BS$_1$C$_1$



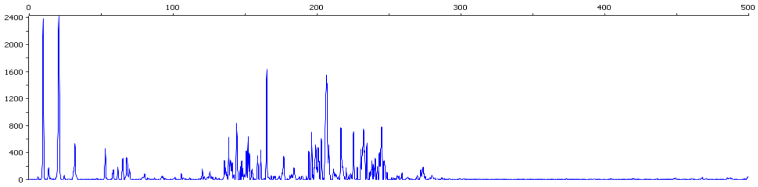
BS$_1$C$_2$



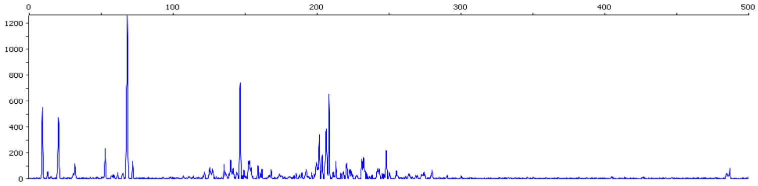
BS$_2$C$_1$



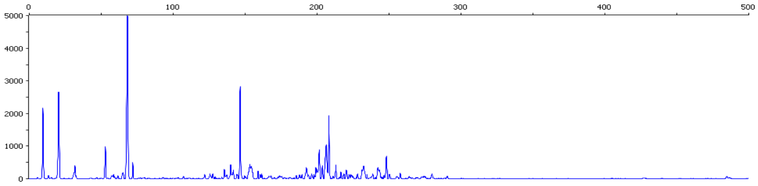
BS$_2$C$_2$



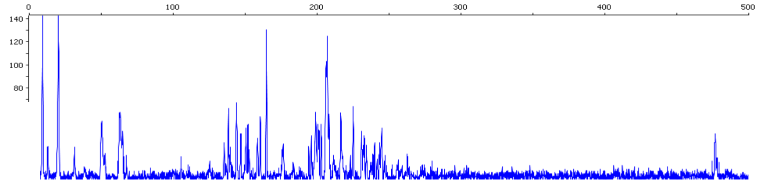
MS$_1$C$_1$



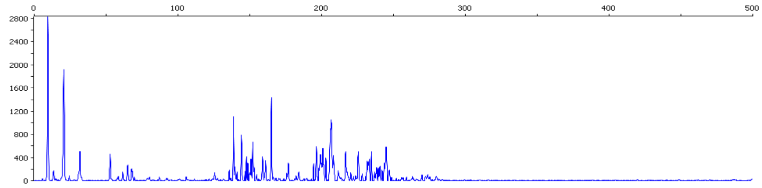
MS$_1$C$_2$



MS$_2$C$_1$

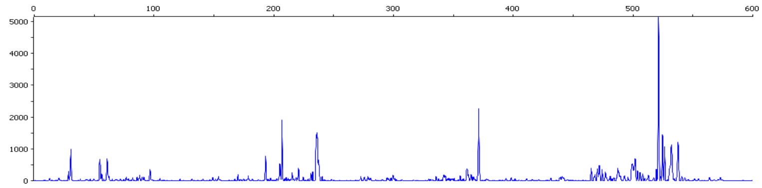


MS$_2$C$_2$

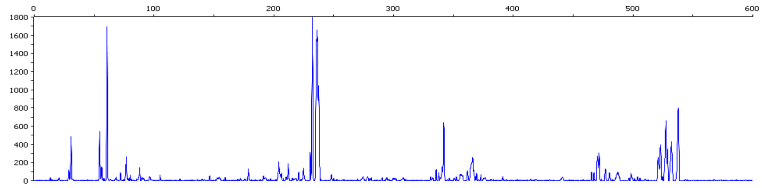


a) AluI

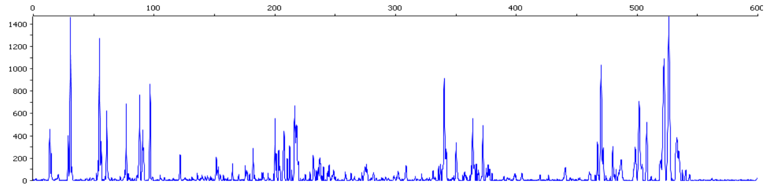
BS$_1$C$_1$



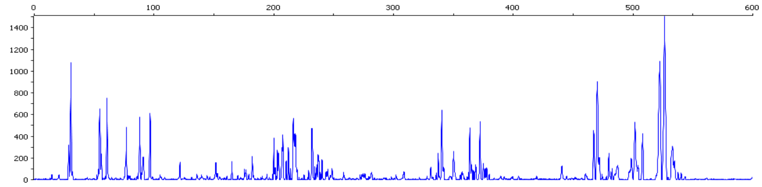
BS$_1$C$_2$



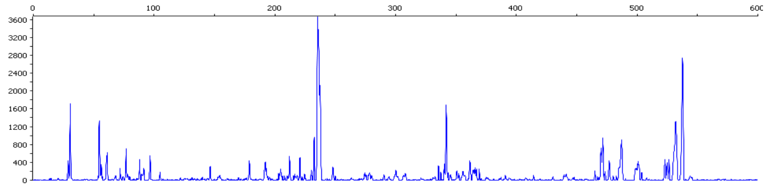
BS$_2$C$_1$



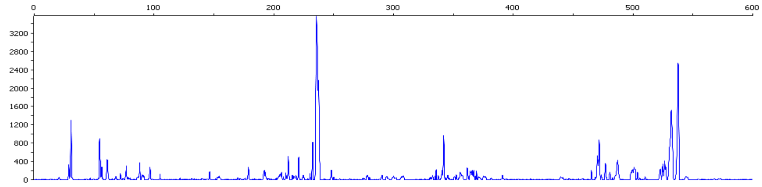
BS$_2$C$_2$

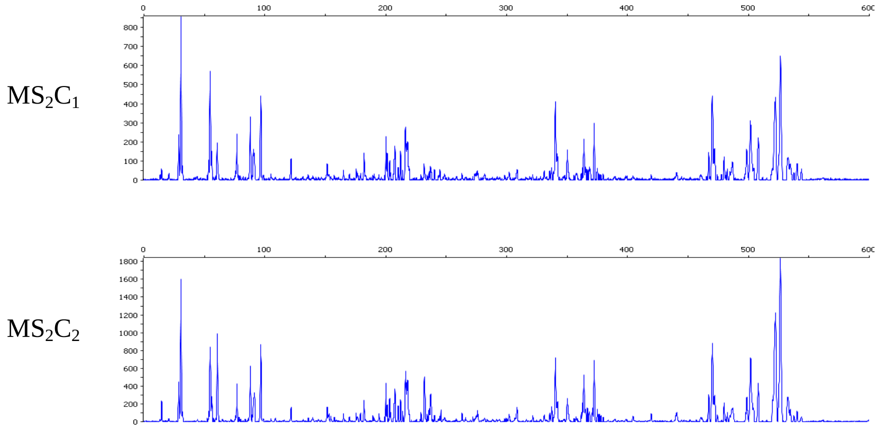


MS$_1$C$_1$



MS$_1$C$_2$





b) HhaeIII

Figure V-2: T-RFLP profiles using restriction enzyme a) AluI, b) HaeIII.

Replicates showed a certain degree of variation; they were however grouped together when all profiles were compared. *Tables V-5a* and *b* presents the main characteristics of each T-RFLP profiles for the two enzymes.

AluI					
Sample	**Global range bp**	**$n_{tot.}$ peaks**	**Distribution**		
			50<FU<100	***100<FU<150***	***FU>150***
BS_1C_1	55-276	15	10	2	3
BS_1C_2		13	6	2	5
BS_2C_1		19	8	6	5
BS_2C_2		26	8	5	13
MS_1C_1		6	3	1	2
MS_1C_2		32	9	4	19
MS_2C_1		9	8	1	0
MS_2C_2		31	9	4	18

a) AluI

***Hae*III**					
Sample	**Global range bp**	**$n_{tot.}$ peaks**	**Distribution**		
			50<FU<100	***100<FU<150***	***FU>150***
BS_1C_1	55-499	34	7	5	22
BS_1C_2		30	12	9	9
BS_2C_1		41	14	5	22
BS_2C_2		37	17	7	13
MS_1C_1		27	9	6	12
MS_1C_2		38	11	5	21
MS_2C_1		35	15	6	14
MS_2C_2		38	12	8	18

b) HhaeIII

Table V-5. Comparison of peaks for each plant species obtained by T-RFLP.

A total of 36 different T-RFs were found for *Alu*I assay, from 55 to 276 bp, and 52 T-RFs for *Hae*III, from 55 to 499 bp. Differences were recorded according to samples analysed. Concerning *Alu*I, the number of T-RFs in individual samples profiles ranged from 13 (BS_1C_2) to 26 (BS_2C_2) peaks for borage, and peak intensity was majority comprised between 50 and 100 FU, except for BS_2C_2 with more peaks with FU > 150. For mustard, number of peaks ranged from 6 (MS_1C_1) to 32 (MS_1C_2). With this species, the gap between C_1 and C_2 was higher (from 6 peaks to 32 in $soil_1$ and from 9 to 31 in $soil_2$) compared to borage. Moreover, more peaks were above 150 FU for condition C_2, while a majority were comprised between 50 and 100 FU for C_1. Results with *Hae*III showed a more homogeneous range with a number of peaks between 30 and 41 for borage and between 27 and 38 for mustard. Most of them were higher than 150 FU for both plants, except for BS_1C_2 and BS_2C_2 with more peaks between 50 and 100 FU (12 peaks and 17 peaks respectively) (Table V-4).

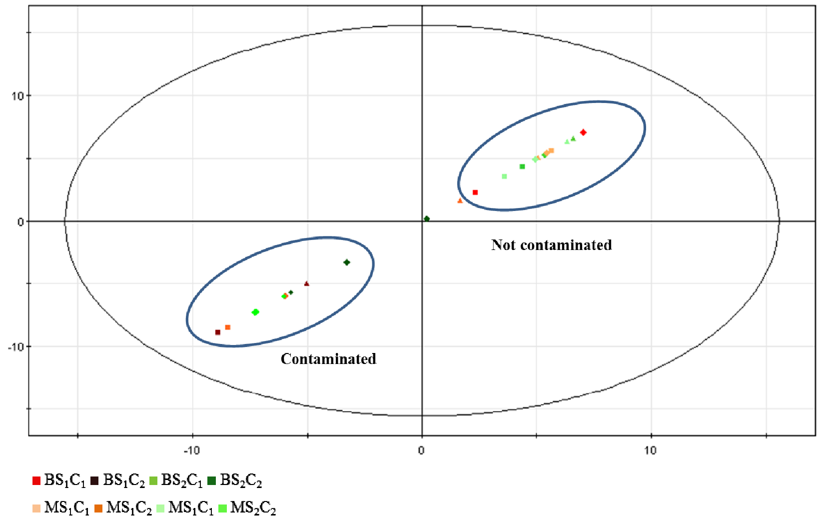
The *Table V-6* presents the most frequent T-RFs (≥ 6/8 samples) that were detected and allowed a more detail description of differences between samples. With *Alu*I, intensive fluorescence signals were recorded and comprised fragment sizes of 55, 67, 140, 146, 152, 200, 205, 208, and 248. Peaks at 105 ($MS_{1/2}C_2$), 184 (B/MS_2C_2), 188 (MS_1C_2), 193 (BS_1C_1/MS_1C_2), 256 ($MS_{1/2}C_2$), 264 (MS_1C_2), and 270 (MS_2C_2) bp were exclusive (≤ 2/8 samples). Only three sizes were common to *Hae*III (i.e. 55, 205 and 248), but 18 peaks more were revealed: 61, 77, 88, 97, 193, 212, 217, 232, 235, 336, 341, 362, 366, 368, 467, 471, 480, 487, and 499. Exclusive peaks were situated at 291 (B/MS_1C_2), 372 (BS_2C_2/MS_1C_2), 378 and 460 (BS_2C_1/MS_2C_2) bp.

bp	AluI										Hae1II								
	BS_1C_1	BS_1C_2	BS_2C_1	BS_2C_2		MS_1C_1	MS_1C_2	MS_2C_1	MS_2C_2		BS_1C_1	BS_1C_2	BS_2C_1	BS_2C_2		MS_1C_1	MS_1C_2	MS_2C_1	MS2C2
55	□	❍	□	◆		□	◆		◆		◆	◆	◆	◆		◆	◆	◆	□
61				□			□		□		◆	◆	◆	◆		◆	◆	◆	□
67	◆	◆	◆	❍		◆	◆		◆										
71	□	□	□				◆				❍	□				□	□		
77											◆	◆	◆	◆		◆	◆	◆	□
88											◆	◆	◆	◆				◆	□
90											□					❍	◆	❍	□
97											◆	□	◆	◆		◆	◆	◆	□
105							□		□		□	□				□	□		
122													◆	□				❍	□
126				□			□		□										
136				□			◆		❍										
140	□	□	❍	◆			◆	□	◆										
146	◆	◆	◆	◆		◆	◆	□	◆		◆	□				❍	❍		
149			□	❍			◆		◆										
152	□	□	❍	◆			◆	□					❍	□			□	□	□
160			□	◆			◆	□	◆										
165			◆	◆				❍	◆				❍	□				□	□
177				□			□		◆		◆	❍	❍	□		❍	◆	□	□
181													◆	❍				❍	□
184				□					❍										
193	□						◆				◆	□	□			❍	◆		□
196			□	◆			❍		◆										
200	□	❍	□	◆			◆	□	◆		□		◆	❍				◆	□
205	□	◆	□			□	◆		◆		◆	❍	◆	❍		□	◆	□	□
208	◆	◆	◆	◆		❍	◆	□	◆		□		◆	◆				◆	□
212	□	□		□			◆		□		◆	◆	◆	❍		◆	◆	□	□
217			❍	◆			❍	□	◆				◆	◆			□	◆	◆
221							❍		□		◆	❍				◆	◆		
225			❍	◆			□	□	◆		❍	❍					❍		
228				❍			□		□										
232	❍	◆	❍				◆		◆		◆	◆	◆	◆		◆	◆	□	◆
235				◆			◆		◆		◆	◆	❍	□		◆	◆	□	❍
238			❍	❍			◆		❍										
240	□		□	❍			◆		◆				□	□				□	□
244			◆	◆					◆				□	□				□	
248	❍	□		□		□	◆		❍		◆	□	□	□		❍	◆		
256							□		□										
276	□	□		□			□		□				□	□				□	
280											□		□	□			□		
291												□					□		
309													□	□			□	□	□
331													□	□			□	□	□
336											❍	❍	□			□	❍	□	□
341											◆	◆	◆	◆		◆	◆	◆	◆
351													◆	❍			□	◆	◆
356											❍	□	□			□	❍		
362											◆	❍	◆	◆		❍	◆		❍
366											◆	❍	❍	□		□	◆	◆	◆
368											❍	□				□	❍	□	□
372														◆			◆		
375													◆	□			□	◆	◆
378													□						□
440											□		□	□					□
460													□						□
467											◆	□	◆	◆		□	◆	❍	◆
471											◆	◆	◆	◆		◆		◆	◆
477											◆	❍				◆	◆		
480											□	□	◆	❍			□	❍	❍
483													□	□				□	□
487											◆	❍	◆	□		◆	◆	❍	❍
499	·	·	·	·	·	·	·	·	·	·	◆	□	◆	❍	·	□	◆	◆	◆

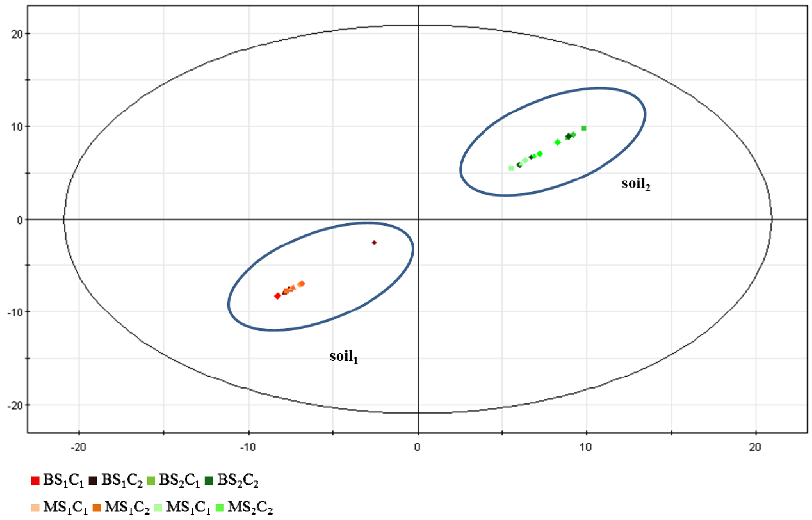
□ 50 to 99 fluorescence units; ❍ 100 to 149 fluorescence units; ◆> 149 fluorescence units.

Table V-6. Representative T-RFLP profiles of soil samples according to the enzyme restriction, plant genotype, type of soil and lead concentration.

PCA analyses showed that results from samples from contaminated soils were separated from non contaminated using *Alu*I both for mustard and borage as well as the two types of soils. As a difference PCA analyses revealed that soil type differences were recorded using *Hae*III but not contaminated vs. non contaminated as well as no differences between the plant species (*Figure V-3*).



a) AluI



b) HhaeIII

Figure V-3: Principal component analyses of the T-RFLP profiles using restriction enzyme a) AluI, b) HaeIII showing differences of contaminated vs. non contaminated using AluI, and soil type differences using HaeIII.

5.2.4. Discussion

Microorganisms can affect plant growth and nutrient uptake by release of growth-stimulating or inhibiting substances that influence root physiology and root system architecture (Ryu et al., 2005; Govindasamy et al., 2009). Plant beneficial microorganisms are of interest for application in agriculture either as for bio-fertilisers as well as for phytoremediation applications (Berg, 2009; Weyens et al., 2009). Moreover, the rhizosphere selects not only a specific plant interaction, but also the interactions with other rhizospheric microorganisms can help the plant to absorb elements improving plant performance and consequently, soil quality (Yang et al., 2009; Kavamura and Esposito, 2010).

In this study high concentrations of Pb were measured in roots for both plants on contaminated soils wit MTE coming from a pollute site. These results were very homogenous independently of the plant species used (borage or mustard). Same trend was recorded for Sb. For Cu and Zn, concentrations were different according to the level of contamination, and to the plant species. Maximum concentrations in roots depended also on the type of soil for the two species. Cd concentrations also varied according to the type of soil and plant species. The efficiency of new green manures crops on global soil quality improvement and restoration was previously demonstrated in a context of a lead-contaminated site near a batteries-recycling plant (Foucault et al., 2013b). However how MTE contamination of this site could affect bacteria communities associated with plants was still unknown.

We used T-RFLP techniques to determine how MTE can affect rhizobacterial communities of two manure crops. Our results showed that differences of bacterial communities structures of the rhizosphere of borage and mustard occurred in case of MTE contamination and differences according to soil types can be also recorded. We were able to show to identify TRFs which were only changing to soil type or contaminations. Although the main limits of T-RFLP is the difficulty to obtain taxonomic information of the microorganisms for a particular TRFs (Frey et al., 2006), and that combined approaches with techniques such as for instance 16S rDNA library can help to determine TRFs, we showed that MTE contamination can lead to a switch in rhizobacterial communities structures of the two manure crops. Additionally, intensities of peaks differ according to the level of contamination showing that differences occurred due to MTE contamination. A general increase of some peaks was recorded. Some new peaks also appeared in case of contamination reflecting the abilities of some rhizobacterial groups to take advantages of niches in the rhizosphere in case of MTE contamination. This was however plant species dependent as described with *Hae*III. More strong peaks (FU >150) were recorded in case of mustard growing on MTE contamination soil while for borage a reduction was recorded. This was inversely correlated to peaks inferior to 100 of FU. In case of *Alu*I, a general increase has been described in case of contamination and both for borage or mustard. Several studies have shown that metal stress results in change in microbial community structures, and activity. Particularly, bacterial biomass has been reported to decrease, or that some new dominant group took place in the soil niches as in the case of Hg (Rasmussen and Sørensen, 2001), As, Cr, Cu and that bacteria compensate for loss of diversity (Turpeinene et al., 2004) through maintaining their metabolic activities. Shift in rhizobacterial communities of hyperaccumulator due to MTE contamination were previously demonstrated (Frey et al., 2006). Also the Ni hyperaccumulator Thlaspi goesingense can have its rhizosphere and shoot endosphere communities structures changing under heavy metal contamination (Idris et al., 2004). Several other examples can be found regarding how heavy metals can affect bacterial population structures. It is well known that bacteria inhabiting the rhizosphere can modify metal(loid)s compartmentalization as well as speciation with consequences on their solubilisation and bioavailability *(Lin et al., 2004; Mitter et al., 2013)*, and therefore help the plant to remediate polluted soils. In this study we used soil contaminated from a specific site for high contamination for which it will need to be rehabilitated due to human expansion area, adding therefore further information on heavy metals and rhizobacterial communities' adaptation.

5.2.5. Conclusions and perspectives

In this study impacts of metal(loid)s on the rhizosphere bacteria of two manure crops used for rehabilitation of a polluted site were evaluated.

► T-RFLP analyses show that rhizobacterial communities's structures are impacted and changes occur for adaptation to the relevant contaminating metalloids.

These results open perspectives to modulate the soil-plant transfers of metal(loid)s with applications in the management of contaminated soils.

5.2.6. Acknowledgement

This work has received support from the National Research Agency under reference ANR-12-0011-VBDU. ANRT (National Agency for Research and Technology), STCM (Société de Traitements Chimiques des Métaux) and INPT (Institut National Polytechnique de Toulouse) are acknowledged for their financial support and technical help.

5.2.7. References

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Chapter 6

General conclusions & Perspectives

6.1. Scientif results & perspectives.

Since several decades, second smelter metal industry contributes significantly to emissions of MTE (*Donisa et al., 2000*) and can cause soil contamination at the global scale. In European countries, the last decades, regulation was renforced to avoid these strong soil pollutions. But both historical pollutions in Europe and actual pollution in several countries in the world where the regulation is less exigent need to develop sustainable remediation techniques for polluted soils. Therefore, this thesis was interested in the sustainable management of soil pollution due to particles emitted by the process of recycling lead. Metal(loid)s ransfers in soil and plants and human bioavailability in relation to physic-chemical properties of soil was investigated.

► **Several scientific questions were at the origin of this scientific study with structuration of the phD in two main aspects: sustainable management of excavated soil and developpement of ecological remediation technique to improve the polluted soil remaining in the field.**

Excavated soil:

1) **First, the relevance of a size screening of excavated soils to improve their landfilling and to reduce the costs was assessed.**

2) **Secondly, the pertinence of ecotoxicity tests to assess a more realistic human exposition to contaminated soil leachates, and the influence of soil physic-chemical parameters on MTE mobility and leachates ecotoxicity was investigated.**

Management of the polluted soil remaining in the field:

3) **Then, green manure plants were tested for phytoremediation on various polluted soils, with both metal(loid)s human bioavailability and ecotoxicity assessment in relation with soil characteristics.**

4) **Finally, changes in bacterial communities influenced by different crops, lead concentrations and soil properties were studied.**

A gradual experimental approach has allowed to answer our scientific questions, and to develop a comprehensive, practical and transversal approach to understand environmental impacts of metal(loid)s emitted by a battery recycling plant.

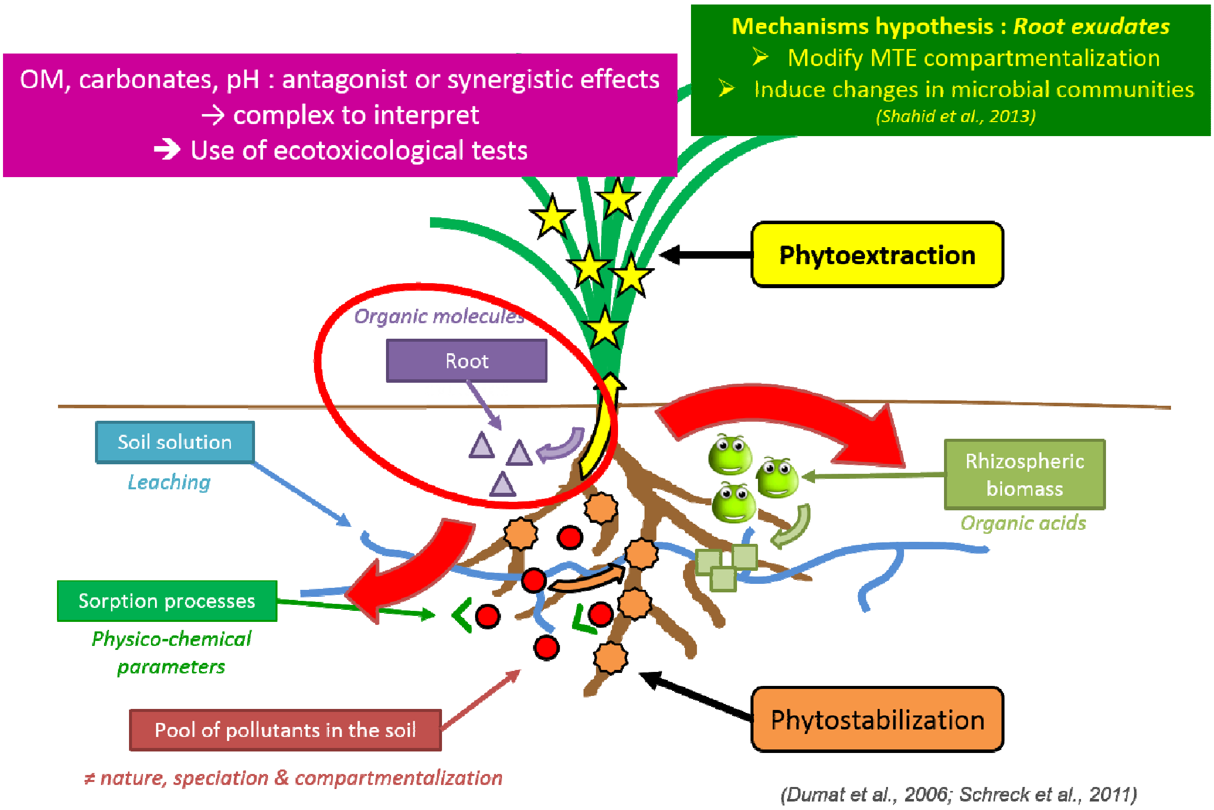
1) Field soil screening in the industrial site according to different particle sizes was highly relevant to improve landfilling because the finer fraction is generally the most contaminated. The granulometric separation is a necessary condition for rational management of excavated soil from contaminated sites by step. Using this technique, the initial quantities of soils and costs could be reduced by 10 to 25% in the case of a systematic landfilling.

2) Nowadays, soil management is mainly based on total and leached MTE – concentrations but it do not reflect the interactions and effects on microorganisms. Ecotoxicity tests were performed on *Daphnia*, *Alivibrio fisheri* and, in this context of industrial polluted soils with various metal(loid)s, for the first time, on new bacterial strains which bioluminence is specific of different MTE and concentrations. Then, ecoscores were calculated to rank the soil samples according to the risk they present. Results demonstrated a restricted battery of ecotoxicity tests allows an easy and cheap screening of a large number of polluted soil samples and performs a cost-effective risk assessment.

3) Soil remediation with adapted plants offers several advantages (cheap, light treatments, less erosion) for a use in urban, peri-urban area or in gardens. In this context, we tested "green manures" plants, Borage – Phacelia – White Mustard. These plants arecommonly used in agriculture to improve soil fertility with a developed root system and the production of exudates which enhance the (micro)biological activity in the rhizosphere. But except for mustard, these plants were never used with these ecological/agronomical objectives. Their efficiency to improve soil quality was assessed by measurements, before and after a 10 weeks culture-period, of agronomic parameters, soil respiration, total concentrations in soils and plants, and MTE bioavailability both for human and plants. Our results demonstrated that borage and mustard can significantly improve soil respiration, reduce total and bioaccessible metal(loid)s quantities and their ecotoxicity. For lead and antimony, contrasted phytoremediation mechanisms were developedin function of plant specie: phytoextraction with storage in shoots and phytostabilization with storage in roots respectively for mustard and borage. So, these plants appear to be good candidates for the re-functionalization of MTE-contaminated soils.

4) The final part of this thesis was to investigate the potential changes of rhizospheric microorganisms biomass under different green manure crops and level of soil contamination as these microorganisms potentially favoured by rhizospheric exsudates could induce changes in pollutants behaviour. Borage and mustard cultures were performed with different polluted soils (in terms of agronomy and pollution gradient). A biotest rhizotest was used in order to focus on the rhizosphere soil. Then, taxonomic changes and bacterial communities were determined using T-RFLP analyses. Results demonstrated that rhizobacterial communities's structures are influenced by metal(loid)s and changes occur for adaptation to the stress induced by pollution.

According to the various results obtain in the phD, hypothesis for the mechanisms occurring in the **polluted soils-green manure plants-rhizosphere microorganisms systems**, were sum up on the *Figure VI-1* below.



(Dumat et al., 2006; Schreck et al., 2011)

Figure VI-1: Parameters, interactions and potential mechanisms that influence MTE behaviours in soils

So, in the soil, MTE are found under different speciations and compartmentalizations andtheir behaviour is influenced by numerous parameters.

Thus, sorption processes can modify MTE mobility through physico-chemical properties like pH, OM or carbonate contents, and, according to their compartmentalization, MTE can potentially be leached by soil solution. Among these parameters, soil organic matters and carbonate contents or pH can have antagonist or synergistic effects but these interactions are difficult to highlight, that's why ecotoxicological tests were implemented.

Most of that, in the presence of certain plants as borage or mustard (as green manure plants are known to their ability to modify soil properties by farmers and gardeners), two other mechanisms can influence MTE behaviour. First, excretion of root exudates composed of organic molecules with complexant capacities can also change MTE compartmentalization and so their bioavailbility. Then, this type of plant can improve the development of rhizospheric biomass which is favoured by the nutrients present in the rhizosphere and can then produce microbial organic acids, themselves able to change MTE mobility.

So, all these parameters can influence MTE mobility and, most of, their bioavailability which is a predominant indicator for risk assessment of human health and environment.

Finally, for soil pollution by MTE, two mechanisms can occur for phytoremediation:

- *Phytostabilisation, when MTE concentrate in or on roots,*
- *Phytoextraction when MTE concentrate and accumulate in aerial parts.*

The main mechanisms hypothesis is the influence of root exudates which can change phytoavailability by direct process or/and by influencing microorganisms activity.

But these results raised others scientific questions. For example, for a better understanding of MTE mobility, modelling of interactions in leachates could provide information on MTE speciation: that speciation analysiswill highlight the pollutants behaviour and ecotoxicity. .

Moreover, we saw that bacterial strains used here were specific of arsenic, zinc, but the development of a strain specific of lead could improve soil screening as that inorganic pollutant is widely found at the global scale due to its numerous past and actual uses in a lot of industrial areas.

Then, concerning the phyto-restoration of soil quality, further experiments both in the field and in controlled experiments could be implemented for a better characterization of mechanisms which occur in the rhizosphere, and, especially, on the role and effects of microbial biomass on MTE mobility.

6.2. Applied results & perspectives.

From an engineering point of view, these works provide new interesting perspectives for the management of contaminated site by the responsibles of regulated sites.

Industrial processes can lead to soil contamination by the emission of particles enriched in metals and metalloids. But, when they are submitted to the legislation on regulated activities, companies have to work out on management strategies while considering economical, societal and environmental context.

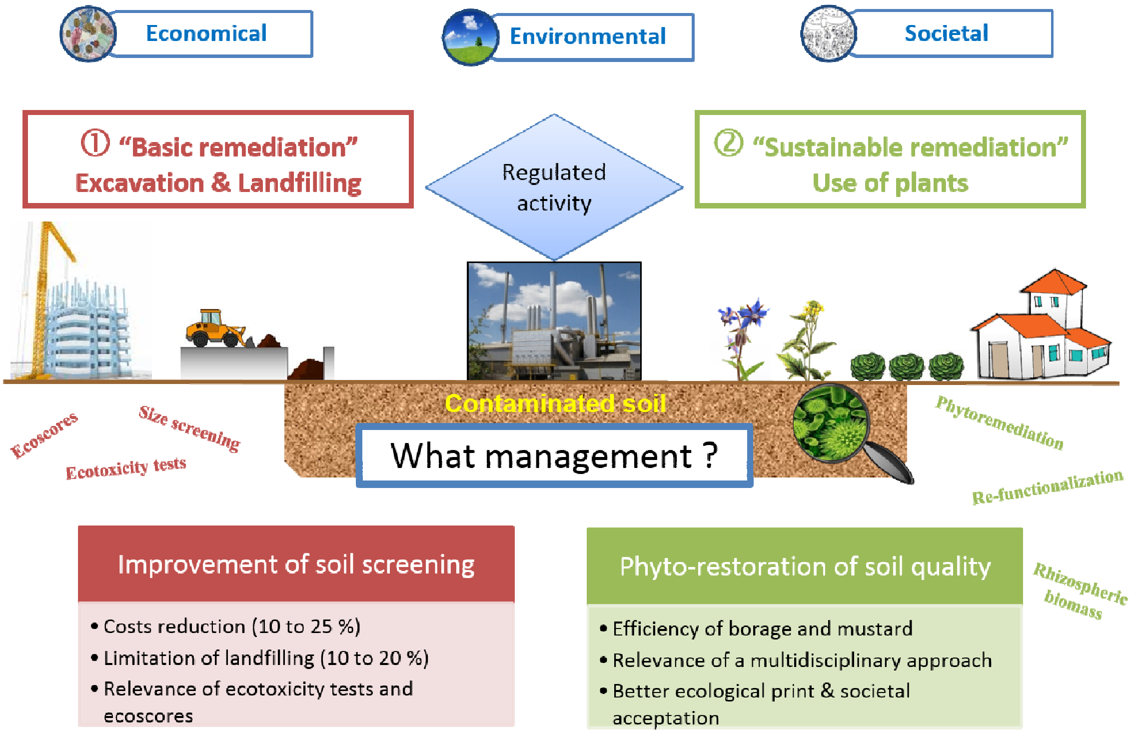


Figure VI-2: Balance of carried works and achieved objectives

Two solutions can be implemented as shown in the *Figure IV-2*:

- *First, a "basic" remediation consisting in excavation and landfilling. Today, this approach is based on MTE total concentrations and physico-chemical parameters of soil, but, size screening, ecotoxicological tests and ecoscores can improve this way of management for a more realistic approach. These relevance tools reduce landfilling and costs from 10 to 25 %.*

- *The second way for soil management consists in the use of plants. Indeed, we demonstrated phytoremediation is efficient for the re-functionalization of soil quality. Borage and mustard were very suitable in this context. That solution provides a better ecological print and societal acceptation because it is less destructive and cheaper than excavation and landfilling.Moreover as we demonstrated in the case of medium polluted soils (representative of polluted soils remaining in the industrial sites after excavation or numerous urban anthropogenic soils as old gardens or soils near roads), the use of green manure plants in addition of the reduction of risks induced by the metal(loid)s are able to improve the global agronomic soil quality and therefore permit a thrue refunctionnalization of these initially degradated soils. These techniques could therefore be used in the case of industrial soils during the functionnement of factories and not only at the end of the activity when the soil must absolutly to be remediated*

To continue this research, eco-scores should be democratized with a larger use by professionals of soil remediation and the creation of database in order to allow an easier interpretation of the results and better understanding of the relationship between the characteristics of the polluted soil samples, ecotoxicity and human bioavailability.

Moreover, additional tests on various contaminated field by metal(loid)s need to be done, particularly with regard to the effectiveness of different “green manure” plants for the re-functionalization of polluted soils and to reduce environmental and health risks related to pollutants transfers. Further crops of borage, mustard, comfrey, etc., are required during severals years on polluted soils with a gradient of metal(loid)s pollution and measurements before and after treatment of the following set of soil parameters:

- *Agricultural (conventional physico-chemical, biological experiments, soil microbiology);*
- *Bioavailability for plants, soil biota and humans by measuring the bioavailability of pollutants;*
- *(Eco)Toxicology, biomarker validation.*

Observations and field measurements will be performed for the "applied part", and targeted experiments in the laboratory for the "basic science" aspect. Complementary experiments on microorganisms should also be performed for a better understanding of their role on MTE compartmentalization at the soil-root interface. Moreover, the overall feasibility of the use of "green manure plants" for the remediation of soil could be achieved through projects submitted to ADEME or ANR (GESSIPOL APR: EcoSol project 2013). Finally, the establishment of a chain of composting and heat treatment in the furnaces of the STCM could permit to develop the phytoremediation techniques.

Further details can be find in the various publications and congress acts listed p. 177.

Conclusions et perspectives

Depuis plusieurs décennies, l’industrie de seconde fusion contribue de manière significative aux émissions d’ETM (Donisa et al., 2000) et peut entraîner la contamination des sols à l'échelle mondiale. Dans les pays européens, la réglementation a été renforcée pour éviter ces fortes pollutions de sols. Mais les deux types de pollutions, historiques en Europe et la pollution réelle dans plusieurs pays dans le monde où la réglementation est moins exigeante, nécessitent de développer des techniques de remédiation durables pour les sols pollués. Par conséquent, cette thèse s'est intéressée à la gestion durable de la pollution des sols due aux particules émises par le processus de recyclage du plomb. Les transferts des ETM dans le sol et les plantes et la biodisponibilité humaine en relation avec les propriétés physico-chimiques du sol ont été étudiés.

► Plusieurs questions scientifiques ont été investiguées au cours de cette thèse, structurée en deux aspects principaux: la gestion durable des terres excavées et le developpement d’une technique de remédiation écologique pour améliorer la qualité des sols:

Gestion des terres excavées :

1) Tout d'abord, la pertinence d'un tri granulométrique des terres excavées afin d'améliorer leur mise en décharge et de réduire les coûts a été évaluée.

2) Deuxièmement, la pertinence des tests d'écotoxicité pour évaluer une exposition humaine plus réaliste de lixiviats de sols contaminés, et l'influence des paramètres physico-chimiques du sol sur la mobilité des ETM et l’écotoxicité des lixiviats ont été étudiées.

Phyto-restoration de la qualité des sols :

3) Ensuite, des plantes engrais verts ont été testées pour la phytoremédiation de différents sols pollués par les ETM, en lien avec la biodisponibilité humaine et l'évaluation de l'écotoxicité selon les caractéristiques du sol.

4) Enfin, les changements de communautés bactériennes de la rhizosphère ont été évalués sur des micro-cultures de bourrache et moutarde en fonction des concentrations en plomb et des propriétés du sol.

Une approche expérimentale progressive a permis de répondre aux questions scientifiques visées par la thèse et de développer une approche globale, pratique et transversale pour comprendre les incidences environnementales de plomb émis par une usine de recyclage de batteries.

1) Le criblage de sols contaminés issus du site d’étude en fonction de la taille des particules est pertinent pour améliorer la mise en décharge. Le tri des terres est une condition nécessaire pour une gestion rationnelle des déblais provenant de sites contaminés. Grâce à *cette technique, les coûts pourraientt être réduits de 10 à 25% dans le cas d'une décharge systématique.*

2) *De nos jours, la gestion des sols est principalement basée sur les concentrations totales et lixiviables en ETM, mais elles ne reflètent pas les interactions et les effets sur les micro-organismes. Des tests d'écotoxicité ont été effectués sur Daphnia, Alivibrio fisheri et, dans ce contexte d'un site industriel contaminé par les ETM, pour la première fois, sur de nouvelles souches bactériennes dont la bioluminence est spécifique des différents ETM et de leurs concentrations. Ensuite, le calcul des écoscores a permis de classer les échantillons de sol en fonction du risque qu'ils présentent. Les résultats ont démontré qu'une batterie de tests d'écotoxicité permet un dépistage facile et bon marché d'un grand nombre d'échantillons de sols pollués et d'effectuer une évaluation des risques rentable.*

3) *La remédiation du sol avec des plantes adaptées offre plusieurs avantages (traitements relativement bon marché, moins d'érosion, etc.) pour une utilisation en milieu urbain, péri-urbain ou dans les jardins. Dans ce contexte, nous avons testé des plantes «engrais verts», bourrache - phacélie - moutarde blanche, communément utilisées en agriculture pour améliorer la fertilité du sol grâce à un système racinaire développé et à la production d'exsudats qui améliorent l'activité (micro)biologique de la rhizosphère. Mais à l'exception de la moutarde, ces plantes n'ont jamais été utilisés avec ces objectifs écologiques / agronomique. Leur efficacité pour améliorer la qualité du sol a été évaluée par des mesures, avant et après 10 semaines de culture, des paramètres agronomiques, de respiration du sol, des concentrations totales dans les sols et les plantes, et de la biodisponibilité des ETM pour les plantes et l'homme. Nos résultats ont démontré que la bourrache et la moutarde peuvent considérablement améliorer la respiration du sol, réduire les quantités totales et bioaccessibles des ETM et leur écotoxicité. Pour le plomb et l'antimoine, des mécanismes de phytoremédiation contrastés ont été relevés en fonction de l'espèce cultivée: phyto-extraction avec le stockage dans les pousses et phytostabilisation avec le stockage dans les racines pour, respectivement, la moutarde et la bourrache. Ainsi, ces plantes semblent être de bons candidats pour la re-fonctionnalisation des sols contaminés par les ETM.*

4) *La dernière partie de cette thèse fut l'étude des changements potentiels de la biomasse microbienne de la rhizosphère sous différentes cultures d'engrais verts et différents niveau de contamination du sol, car ces micro-organismes, potentiellement favorisés par les exsudats rhizosphériques, pourraient induire des changements dans le comportement des polluants. Des cultures de bourrache et de moutarde ont été réalisées avec différents types de sols pollués (en termes d'agronomie et de gradient de pollution). Un biotest, le RHIZOtest, a été utilisé afin de se concentrer sur le sol de la rhizosphère. Ensuite, les changements taxonomiques et les communautés bactériennes ont été déterminés en utilisant des analyses de T-RFLP. Les résultats ont montré que les structures des communautés de rhizobactéries sont influencées par les ETM et des changements se produisent pour s'adapter au stress induit par la pollution.*

Grâce aux différentes conclusions de ces recherches, les paramètres, interactions et hypothèses de mécanismes se produisant dans le sol, ont été réumé sur la Figure VI-1.

Ainsi, dans le sol, les ETM sont présents sous différentes spéciations et compartimentations, et leur mobilité est influencée par de nombreux paramètres.

Les processus de sorption peuvent modifier la mobilité des ETM selon les propriétés physico-chimiques des sols comme le pH, la teneur en matière organique du sol, carbonates, et, en fonction de leur compartimentation, ces éléments peuvent potentiellement être lixiviés *par la solution du sol. Ces paramètres peuvent avoir des effets antagonistes ou synergiques, d'où la difficulté de mettre en évidence l'influence d'un de ces paramètres, c'est pourquoi des tests écotoxicologiques ont été mis en œuvre.*

De plus, en présence de certaines plantes comme la bourrache ou la moutarde (car les plantes engrais verts sont bien connues pour leur capacité à modifier les propriétés des sols par les agriculteurs et les jardiniers), deux autres mécanismes peuvent influencer le comportement des ETM. Tout d'abord, l'excrétion d'exsudats racinaires composés de molécules organiques avec des capacités complexantes pouvant modifier la compartimentation des ETM et donc leur mobilité. Ensuite, ce type de cultures peut améliorer le développement de la biomasse rhizospheric qui est favorisé par les nutriments présents dans la rhizosphère et peut donc produire des acides organiques microbiens, eux-mêmes en mesure de changer la mobilité des ETM.

Donc, tous ces paramètres peuvent influencer la mobilité des ETM et, surtout, leur biodisponibilité qui est un indicateur prépondérant pour l'évaluation des risques pour la santé humaine et l'environnement.

Enfin, les résultats ont montré que la phytoremédiation peut prendre deux formes :

o *Phytostabilisation des ETM lorsqu'ils se concentrent dans ou sur les racines,*

o *Phytoextraction des ETM lorsqu'ils s'accumuler dans les parties aériennes.*

L'hypothèse principale des mécanismes en jeu est l'influence des exsudats racinaires susceptibles de changer les processus de phytodisponibilité et/ou d'influencer l'activité des microorganismes.

Mais ces résultats ont soulevé d'autres questions scientifiques. Par exemple, pour une meilleure compréhension de la mobilité des ETM, la modélisation des interactions dans les lixiviats pourrait fournir des informations sur la spéciation des ETM et mettrait en avant leur comportement et leur écotoxicité.

Par ailleurs, les souches bactériennes utilisées ici sont spécifiques de l'arsenic, du zinc, etc., mais le développement d'une souche spécifique du plomb serait pertinent pour améliorer le tri des terres car ce polluant inorganique est largement répandu à l'échelle mondiale en raison de ses nombreuses utilisations passées et présentes dans beaucoup de zones industrielles.

Enfin, concernant la phyto- restauration de la qualité des sols, de nouvelles expériences, en conditions contrôllées et de terrain. pourraient être mises en œuvre pour une meilleure caractérisation des mécanismes qui se produisent dans la rhizosphère, et, en particulier, sur le rôle et les effets de la biomasse microbienne sur la mobilité des ETM.

D'un point de vue technique, ces travaux ouvrent de nouvelles perspectives intéressantes pour la gestion des sites contaminés par les responsables de sites ICPE.

Certaines activités industrielles peuvent entraîner une contamination des sols par les émissions de particules enrichies en métaux et métalloïdes. Mais, quand elles sont soumises à la législation sur les installations classées pour la protection de l'environnement (ICPE), les entreprises doivent élaborer des stratégies de gestion tout en considérant le contexte économique, sociétal et environnemental.

Deux solutions peuvent être mises en œuvre comme indiqué dans la Figure IV-2 :

o *Tout d'abord, une réhabilitation « classique » consistant à excaver et mettre en décharge les terres contaminées. Aujourd'hui, cette approche est basée sur les concentrations totales en ETM et les paramètres physico -chimiques du sol, mais, la séparation granulométrique, les tests écotoxicologiques et les écoscores peuvent améliorer ce mode de gestion pour une approche plus réaliste. Ces outils permettent réduire la mise en décharge et les coûts de 10 à 25%.*

o *La deuxième voie concernant la gestion des sols consiste en l'utilisation de plantes. En effet, la phytoremédiation des est efficace pour la re- fonctionnalisation de la qualité du sol. L'utilisation de la bourrache et la moutarde sont pertinentes dans ce contexte. Cette solution fournit une meilleure empreinte écologique et l'acceptation sociétale car moins destructive et moins cher que l'excavation et la mise en décharge. En outre, comme nous l'avons démontré dans le cas de sols moyennement pollués (représentatifs des sols pollués restant dans les sites industriels après excavation ou de nombreux sols anthropiques urbains tels que d'anciens jardins ou les sols à proximité des routes), l'utilisation de plantes engrais verts, en plus réduire les risques induits par les ETM, sont en mesure d'améliorer la qualité agronomique globale du sol et donc permet une réelle re-fonctionnalisation de ces sols initialement dégradés. Ces techniques pourraient donc être utilisées dans le cas de sols industriels d'usines en fonctionnement et pas seulement à la fin de l'activité lorsque le sol doit absolument être assaini.*

Afin de poursuivre ces recherches, les éco-scores devraient être démocratisés par une utilisation plus large par les professionnels de la dépollution des sols et la création de base de données doit permettre une interprétation plus facile des résultats et une meilleure compréhension de la relation entre les caractéristiques des échantillons de sols pollués, l'écotoxicité et la biodisponibilité humaine

De plus, des essais supplémentaires sur des terrains variés contaminés par des métaux et métalloïdes doivent être menés, en particulier pour tester à l'échelle de la parcelle, l'efficacité des différentes plantes «engrais vert» pour la re-fonctionnalisation des sols et à réduire les risques environnementaux et sanitaires liés aux transferts des polluants. D'autres cultures de bourrache, moutarde, consoude, etc, seront réalisées sur un sol contaminé selon un gradient de pollution par des métaux et métalloïdes avec des mesures avant et après traitement sur un ensemble de paramètres du sol:

o *Agronomiques (physico-chimiques, biologique et microbiologie des sols);*

o *Biodisponibilité pour les plantes et l'homme par la mesure de la bioaccessibilité des polluants;*

o *(Eco)Toxicologie, validation de biomarqueurs.*

Les observations et mesures de terrain seront effectuées pour la partie «appliquée», et des expériences ciblées au laboratoire pour l'aspect «recherche fondamentale». Des expériences complémentaires sur les micro-organismes doivent également être réalisées pour une meilleure compréhension de leur rôle sur la compartimentation ETM à l'interface sol-racine.

En outre, la faisabilité globale de l'utilisation des «plantes d'engrais verts» pour l'assainissement des sols pourrait être atteinte grâce à la mise en place d'une chaîne de compostage et de traitement thermique dans les fours de la STCM.

Pour plus de détails, la liste des travaux publiés est présentée p. 177.

Benefits for STCM

Beyond management in accordance with the regulations, STCM initiated a proactive approach to develop a methodology for assessing the environmental risks associated with emissions of metal particles. The objective of this global project is to study the consequences of rejecting lead recyclers of lead-acid batteries and develop innovative solutions to significantly reduce the negative impact on humans and the environment.

The society has supported several research projects in close partnership with INERIS, INP-ENSAT, ADEME or BRGM. These collaborations have resulted in the funding of research work (experiments, publications, research theses, etc.) whose main objective is the understanding of the transfer mechanisms and effects of lead in the environment and, more generally, some MTE such as cadmium (Cd), arsenic (As), zinc (Zn), antimony (Sb), tin (Sn), copper (Cu), etc.

Work on the various themes helped to increase knowledge of the impact of lead on humans and the environment and therefore to develop appropriate strategy to reduce these effects.

Through works presented in this manuscript, the main objective for STCM was to develop a strategy to rehabilitate the industrial site of Toulouse, especially for the rational management of land for a landfill limited.

Alongside this research work, I was also asked to participate in various activities of the company, partularly with the rehabilitation project. Indeed, the authorities require a number of information and records, and the company must be prepared to deal with this type of work: IEM, choice of providers, file deconstruction (including asbestos), preliminary characterization studies soils, etc. In these tasks, also occasionally add some actions in the context of QSE monitoring for the company.

Beyond the scientific interest, phytoremediation is a real concrete and applicable for future business solutions. STCM also sees opportunities for technical applications in a medium term horizon.

Valuation of works

- **Published articles :**

- **Foucault, Y.**, Lévêque, T., Xiong, T., Schreck, E., Austruy, A., Shahid, M. & Dumat, C. 2013. Green manure plants for remediation of soils polluted by metals and metalloids: ecotoxicity and human bioavailability assessment. Chemosphere. DOI: http://dx.doi.org/10.1016/j.chemosphere.2013.07.040.

- **Foucault, Y.**, Durand, M.J., Tack, K., Schreck, E., Geret, F., Lévêque, T., Pradère, P., Goix, S. & Dumat, C. 2013. Ecotoxicity test and ecoscores to improve polluted soils management: case of a secondary lead smelter plant. *Journal of Hazardous Materials* 246–247, 291–299.

- **Foucault, Y.**, Schreck, E., Lévêque, T., Pradère, P. & Dumat, C. 2012. Vers une gestion raisonnée des terres excavées contaminées par des éléments traces métalliques (ETM). *Environnement Risques & Santé* 11, 61–66.

- Schreck, E., **Foucault, Y.**, Geret, F., Pradère, P. & Dumat, C. 2011. Influence of soil ageing on bioavailability and ecotoxicity of lead carried by process waste metallic ultrafine particles. *Chemosphere* 85(10), 1555–1562.

- Schreck, E., **Foucault, Y.**, Sarret, G., Sobanska, S., Cécillon, L., Castrec-Rouelle, M., Uzu, G. & Dumat, C. 2012. Metal and metalloid foliar uptake by various plant species exposed to atmospheric industrial fallout: Mechanisms involved for lead. *Science of the Total Environment* 427 – 428, 253–262.

- Schreck, E., Bonnard, R., Laplanche, C., Lévêque, T., **Foucault, Y.** & Dumat, C. 2012. DECA: A new model for assessing the foliar uptake of atmospheric lead by vegetation, using Lactuca sativa as an exemple. *Journal of Environmental Management* 112, 233–239.

- Schreck, E., Laplanche, C., Le Guédard, M., Bessoule, J.J., Austruy, A., Xiong, T., **Foucault, Y.** & Dumat, C. 2013. Influence of fine process particles enriched with metals and metalloids on *Lactuca sativa* L. leaf fatty acid composition following air and/or soil-plant field exposure. *Environmental Pollution* 179, 242–249.

- Lévêque, T., Capowiez, Y., Schreck, E., Mazzia, C., Auffan, M., **Foucault, Y.**, Austruy, A. & Dumat, C. 2013. Assessing ecotoxicity and uptake of metals and metalloids in relation to two different earthworm species (*Eiseina hortensis* and *Lumbricus terrestris*). *Environmental Pollution* 179, 232–241.

- Shahid, M., Xiong, T., Masood, N., Leveque, T., Quenea, K., Austruy, A., **Foucault, Y.** & Dumat C. 2013. Influence of plant species and phosphorus amendments on metal speciation and bioavailability in a smelter impacted soil: a case study of food chain contamination. *Journal of Soils and Sediments*. DOI: 10.1007/s11368-013-0745-8

- **<u>Submitted articles :</u>**

- Xiong, T., Lévêque, T., Austruy, A., Goix, S., Schreck, E., Dappe, V., Sobanska, S., **Foucault, Y.** & Dumat, C. 2013. Foliar uptake and bioaccessibility of metal(loid)s in vegetables polluted by ultrafine particles. *Ecotoxicology and Environmental Safety*.

- **<u>Oral communications :</u>**

- **Y. Foucault**, S. Compant, B. Mitter, T. Lévêque, A. Sessitsch & C. Dumat. Activity and evolution of rhizospheric microorganisms under green manures crops influenced by different soil properties and pte contaminations. 7th SUITMA International Conference, 16-20 September 2013, Torun, Poland.

- **Y. Foucault,** M.J. Durand, K. Tack, E. Schreck, F. Geret, T. Levêque, P. Pradère & C. Dumat. Use of ecotoxicity tests and ecoscores to improve management of polluted soils. 12th O+International UFZ-Deltares Conference on Groundwater-Soil-Systems and Water Resource Management – AquaConSoil. 16-19 April 2013, Barcelona, Spain.

- **Y. Foucault**, K. Tack, E. Schreck, T. Levêque, Y. Capowiez, P. Pradère & C. Dumat. Phyto-restoration of lead-contaminated soils using green manures crops. 6th SUITMA International Conference, 3-7 October 2011, Marrakech, Marocco.

- T. Leveque, **Y. Foucault**, E. Schreck, P. Pradère, Y. Capowiez & C. Dumat. Earthworms bioturbation influence on metals bioavailability in polluted soils. 4th International Congress EUROSOIL 2012, 2-6 July 2012, Bari, Italy.

- E. Schreck, **Y. Foucault**, S. Sobanska, G. Sarret, R. Bonnard & C. Dumat. Comparison of foliar lead uptake mechanisms for various plant species. 12th International Conference on Environmental Science and Technology (CEST Conference), 8-10 September 2011, Rhodes, Greece.

- T. Leveque, Y. Capowiez, **Y. Foucault**, E. Schreck, M. Couveignes & C. Dumat. Earthworm: a multifunction tool for biomonitoring and refunctionalization of polluted soils. International Conference on Environmental Geochemistry and Health, 8-12 July 2013, Toulouse, France.

- T. Xiong, A. Austruy, E. Schreck, G. Uzu, **Y. Foucault** & C. Dumat. Study of the impact of classified installations on the quality of productions in kitchen gardens. Colloque International et Interdisciplinaire Dynamiques Environnementales, Politiques Publiques, pratiques locales : quelles interactions ? GEODE, 4-6 June 2013, Toulouse, France.

- **Posters :**

- **Y. Foucault**, T. Leveque, T. Xiong, E. Schreck, A. Austruy, M. Shahid & C. Dumat. Green manure plants for polluted soil re-functionalization in the case of urban brownfields or kitchen gardens contaminated by metals and metalloids. 7th SUITMA International Conference, 16-20 September 2013, Torun, Poland.

- **Y. Foucault**, T. Leveque, M. Couveignes, S. Goix, Muhammad Shahid & C. Dumat. Study of bioavailability and ecotoxicity of metals and metalloids in polluted soils to assess the efficiency of phytoremediation techniques. International Conference on Environmental Geochemistry and Health, 8-12 July 2013, Toulouse, France.

- **Y. Foucault**, T. Leveque, K. Tack, A. Austruy, T. Xiong & C. Dumat. Refunctionalization of polluted soils using green manures. Environmental Health 2013 – Science and Policy to Protect Future Generations, 3-6 March 2013, Boston, USA.

- **Y. Foucault**, T. Levêque, M.J. Durand, E. Schreck, A. Paul, K. Tack, P. Pradere & C. Dumat. Ecotoxicity tests to assess landfilling of lead-contaminated soils. 4th International Congress EUROSOIL 2012, 2-6 July 2012, Bari, Italy.

- **Y. Foucault**, M.J. Durand, E. Schreck, K. Tack, T. Levêque, A. Paul, P. Pradere & C. Dumat. Risk assessment around a lead-batteries recycling company using ecotoxicity tests. 6th SETAC World Congress 2012, 20-24 May 2012, Berlin, Germany.

- **Y. Foucault**, K. Tack, E. Schreck, T. Levêque, P. Pradere & C. Dumat. Evaluation des risques sanitaires et écotoxicologiques d'une usine de recyclage de batteries. Colloque Adebiotech Ineris, 15-16 November 2011, Paris, France.

- **Y. Foucault**, K. Tack, E. Schreck, T. Levêque, P. Pradere & C. Dumat. Environmental and sanitary risks assessment for lead-battery recycling activities. 6th SUITMA International Conference, 3-7 October 2011, Marrakech, Marocco.

- E. Schreck, **Y. Foucault**, F. Geret, T. Leveque & C. Dumat. Ageing influence on ecotoxicityof contaminated soils by process metallic ultra-fine particles. 6th SUITMA International Conference, 3-7 October 2011, Marrakech, Marocco.

- E. Schreck, **Y. Foucault**, S. Sobanska, T. Leveque, P. Pradère & C. Dumat. Transfert foliaire de particules atmosphériques fines et ultra-fines vers différentes plantes – Exposition par ingestion. Colloque Adebiotech Ineris, 15-16 November 2011, Paris, France

- E. Schreck, **Y. Foucault**, S. Sobanska, G. Sarret, F. Geret, R. Bonnard, P. Pradere & C. Dumat. Influence of metal process micronic and submicronic particles (PM) on vegetables quality and ecosystems. 11th International Conference on the Biogeochemistry of Trace Elements (ICOBTE), 3-8 July 2011, Florence, Italy.

- T. Leveque, **Y. Foucault**, E. Schreck, Y. Capowiez, A. Austruy, C. Laplanche & C. Dumat. Earthworms as a new tool to assess human bioaccessibility and bioavailability of metals. Environmental Health 2013 – Science and Policy to Protect Future Generations, 3-6 March 2013, Boston, USA.

- Austruy, **Y. Foucault,** T. Xiong, T. Leveque, C. Moussard, A. Hitmi & C. Dumat. Adaptative strategy of S. vulgaris metallophyte plant to soils polluted by metals. International Conference on Environmental Geochemistry and Health, 8-12 July 2013, Toulouse, France.

- S. Goix, T. Xiong, **Y. Foucault**, T. Leveque & C. Dumat. Soil pollution by atmospheric fine particles enriches with metal(loid)s: biodisponibility and (eco)toxicity.7th SUITMA International Conference, 16-20 September 2013, Torun, Poland.

- T. Xiong, A. Austruy, **Y. Foucault**, T. Leveque, V. Dappe, S. Sobanska & C. Dumat. Human health risk due to ingestion of vegetables cultivated on polluted soils and/or exposed to atmospheric fallouts of fine particles enriched with metal(loid)s. 7th SUITMA International Conference, 16-20 September 2013, Torun, Poland

- T. Leveque, Y. Capowiez, E. Schreck, **Y. Foucault**, C. Ricard, R.G. Rakotomanana & C. Dumat. Earthworm influence on metals bioavailability in relation with metal speciation and ecotoxicity. 6th SETAC World Congress 2012, 20-24 May 2012, Berlin, Germany.

- T. Leveque, Y. Capowiez, E. Schreck, **Y. Foucault** & C. Dumat. Influence de la Bioturbation des Vers de Terre sur la Bioaccessibilité et la Biodisponibilité des Métaux dans les sols pollués. Colloque Adebiotech Ineris, 15-16 November 2011, Paris, France

- Austruy, C. Gers, T. Leveque, E. Schreck, **Y. Foucault** & C. Dumat. Effects and accumulation of trace metals on the microarthropods communities in contaminated soil. International Conference on Environmental Geochemistry and Health, 8-12 July 2013, Toulouse, France.

- T. Xiong, A. Austruy, T. Leveque, G. Uzu, E. Schreck, **Y. Foucault**, C. Laplanche & C. Dumat. Impact of atmosphere fine particles enriched with metals on vegetables in kitchen gardens. Environmental Health 2013 – Science and Policy to Protect Future Generations, 3-6 March 2013, Boston, USA.

- **<u>Awards :</u>**

- Grant to assist with attendance at the 13th European Lead Battery Conference, 25-28 September 2012, Paris, France.

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