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Impact de l'occupation des sols agricoles contaminés sur la disponibilité des éléments trace : Mise en évidence du rôle des matières organiques dans le cas de cultures annuelles et cultures pérennes à vocation énergétique

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Abstract

The changes in land use of agricultural soils are susceptible to modify quality and quantity of soil organic matter. Soil organic matter is known to have a role of trophic resource for soil organisms as well as the role of ligand for trace metals in contaminated soils. Changes in the organic matter quality and quantity in soils can lead towards modification of trace metal speciation in soils. But the role of soil organic matter in the determinism of trace metal availabilities in contaminated soils is not well documented. The objective of the thesis work was to assess the role of soil organic matter in determinism of trace metal availabilities in contaminated agricultural soils under annual crop and under perennial energy crop miscanthus. A strategy of studying Cu, Pb, Zn and Cd availabilities of two sites differing in the soil texture and origin of pollution was adopted i.e. the site of Metaleurop (North of France) with silt loam soils contaminated by atmospheric metal fallout and the site of Pierrelaye (Paris Region) with sandy soils contaminated through long term untreated waste water input from city of Paris. Role of organic matter in trace metal availability was assessed through studying; 1) the soils under with two different land uses i.e. under annual crop and under perennial energy crop miscanthus, 2) the soil samples before and after soil organic mineralization through in vitro soil incubations, 3) the soil samples with and without the particulate organic matter (POM) fractions i.e. the most labile organic matter fractions in order to highlight the specific role of this organic matter fraction. A combined approach of metal availability assessment through chemical metal extractions and of studying metal localization in different soil size fractions was used. The results of the trace metal availabilities of the soil under miscanthus compared to annual crop soil showed that the influence of changes in organic matter through land use was different at both sites. For the Metaleurop site, the availability of Cu and Pb which are the metals known to be preferentially bound to organic matter was decreased with an increase in their localization in soil finer fractions. No change in the availability and localization of Zn and Cd was observed for this site. Contrarily, in the Pierrelaye site with organic matter rich sandy soils the availability of metals which are known to be bound to soil mineral phases i.e. Zn and Cd was changed without a change in their localization while no change was observed for Cu and Pb. The results of soil incubation study revealed that the effect of soil organic mineralization on trace metal availability was site dependent for Pb and Cd while effect on Cu and Zn was similar in all soils whatever the site. Our work on the metal enriched particulate organic matter fractions showed that POM being a free organic matter fraction participated in the metal availability and that the POM associated copper was bioavailable for an organism in our conditions. These results concerning the trace metal availabilities in the soils with varying nature and quantity of organic matter are discussed.

Key words: Trace metals, Cu, Pb, Zn, Cd, availability, contaminated agricultural soils, organic matter, chemical extraction, granulometric fractionation, POM

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INTRODUCTION

Context of the study

As we enter the 21st century with more than 6.5 billion world population, the world's soils need to be sustainably managed to meet increasing demands including: increased food production, biomass production, restoration of degraded lands, C sequestration, increased resource use efficiency in agriculture, and preservation of biodiversity (Lal, 2009). But at the same time increased industrialization and urbanization to meet the basic necessities of human being have created a challenge through strong anthropogenic perturbations of soil and environment. One type of these perturbations is the elevated levels of trace metals in the soils. Trace metals are defined as metals in natural materials at a concentration lower than 1000 mg kg^{-1} (Kabata-Pendias and Pendias, 2001). Some of the trace metals are essential for biological life but can be toxic at elevated levels; others are non-essential and can also be toxic. The major challenge in case of soil trace metal contamination is that the trace metals are non-degradable and persist in soils. Elevated levels of trace metals not only cause toxicity to the soil organisms but can also cause serious human health hazards by entering the food chain via plant uptake in agricultural soils (Ramade, 2007).

Despite advancements in science and technologies, removal of metal pollution from soils is neither effective nor easy, particularly for agricultural soils where metal pollution levels are lower than in former industrial sites (Dickinson, 2000). Sustainable use of contaminated agricultural soils should thus require management in a way that the potential risks of trace metals are minimized in particular through plant uptake if alimentary crops are to be maintained, or in general through soil functioning. Assessment of total soil metal contents is the initial step towards risk assessment but main concern is the determination of metal fractions which are available to interact with soil ecological receptors (Harmsen, 2007). These metal fractions concern the "soil metal supply" or the "trace metal availability" as named in the recent norm ISO 17402 aiming to define the bioavailability concept (ISO, 2008). It is well known that metal availability in soils is related to soil metal speciation which depends on many factors like the type of soil or the soil physico-chemical conditions (Bermond et al., 1998). Soil being a complex medium the assessment of metal speciation is difficult and measurement of trace metal availability as well as knowledge of its determinism is still a challenge. Different chemical methods are used to assess metal availability in soils but there is still no one generally accepted method allowing comparisons between studies.

Among the different soil constituents soil organic matter is a labile constituent and remains under continuous change due to different turnover of its fractions varying from plant debris to dissolved organic molecules. In case of non-contaminated soil conditions the role of soil organic matter on different soil properties and on soil functioning has been an important focus of soil researchers. It is thus well established the way the soil organic matter affects soil physico-chemical and biological characteristics (Ekwue, 1990; Hussain et al., 1999; Lal, 2009). In case of contaminated soils the soil organic matter still has a role as a trophic resource for organisms but furthermore acts as a ligand for metals. So the nature and quantity of soil organic matter can play an important role in the determinism of trace metal availability in contaminated agricultural soils. The reactivity and affinity for trace metals of different individual soil organic matter fractions has been reported in literature (Sauvé et al., 2000; Kalis et al., 2006; Sebastia et al., 2008). But the existence of a large number of varieties of organic materials in the soils and the inability to completely characterize soil organic matter make difficult to assess its role in trace metal availability. Such a role has been often described in the case of new exogenous organic matter introduced into contaminated soils as amendments like sewage sludge or composts, then assessing the impact on trace metal availability (Udom et al., 2004; Torri and Lavado, 2008; Smith, 2009; Ingelmo et al., 2011). But rarer are studies concerning changes in indigenous soil organic matter status, due to a change in land use or management practices rather than addition of exogenous organic matter, on trace matter availabilities. Such studies will be useful, however, in the next future to bring elements of answer to the changes of cultural practices, particularly in the case of metal contaminated agricultural soils the vocation of which to produce food cultures is questioned.

More generally, effects of changes in land use or management practices on main soil properties like pH, soil organic matter quality and quantity, or cation exchange capacity are well reported in the literature (Ross et al., 1999; Six et al., 2000; Dilly et al., 2003). In contaminated agricultural soils such modifications in soil physico-chemical conditions are expected to induce changes in metal speciation, but the way these changes will occur are not already known. The consequent effects on the soil trace metal availabilities are then difficult to predict. This will be particularly true in the case of changes from annual alimentary crops to perennial non-alimentary crops like bioenergy crops as suggested for marginal lands (Campbell, 2008; Gopalakrishnan et al., 2009), where the effects due to non-tillage will add.

In this work the need to assess the impact of the adopted practices for the sustainable management of contaminated agricultural soils on the soil organic matter and the resulting effect on trace metal dynamics in soils had been applied to the knowledge of the changes in trace metal availabilities.

The work presented here took benefits from two scientific programs developed in the Pessac research unit: an ANR CES research project (Agence National de la Recherche "Contaminants, Environnement et Santé" named "RESACOR" (REconversion des Sols Agricoles Contaminés : impact des cultures à vocation énergétiques sur la biodisponibilité des éléments traces et la relation avec la réponse des ORganismes du sol) and an INSU EC2CO (Institut National des Sciences de l'Univers,) research project named "MOBIPO-Cu" (Rôle de la matière organique et de la biocénose dans le devenir d'une pollution chronique et diffuse au cuivre dans un sol viticole). In these projects concerning metal contaminated soils, the objectives were to assess the impacts on soil properties of changes from annual alimentary crops to perennial miscanthus non-alimentary crop, and the specific role of some soil organic fractions in trace metal bioavailability. In the present work focus was made on the availability part of the bioavailability, i.e. the characterization and the knowledge of the determinism of the trace metal soil supply, as well as the role of a specific fraction of the soil organic matter: the particulate organic matters, applied in the cases chosen in accordance with these different projects.

Aims of the thesis research

In this context the main objective of the work presented in this thesis was to assess the effects of metal contaminated land uses on the soil trace metals availability using in situ contaminated soil samples and a combination of laboratory experiments. We chose to compare two land uses in the specific case of soils cultivated either under annual alimentary crops or under perennial miscanthus crop. We also chose to compare two sites which mainly differ in their soil texture but also in the origin of metal pollution. But for a given contaminated site we made comparison inside the same cultivated parcel where the changes in land use occurred (annual versus miscanthus crops). The main hypothesis we made concern the role of both the soil texture and origin of the pollution (mainly when sites are compared) and of the soil organic matter (for a given site, between soil under annual or perennial crops, but also in our case when sites are compared). Thus the experimental design of soil sampling was used in order to assess both the determinism of trace metal availabilities and to highlight the specific effect of soil organic matter when coupled with laboratory experiments.

The thesis work thus focuses on three parts:

1) In a first part (chapter 3) we aimed at assessing in situ for a given type of soil the effect to cultivate a contaminated parcel in two different ways (annual *vs* perennial miscanthus crop) on trace metal availabilities. We hypothesized i) that changes in soil organic matter status due to changes in soil use as well as the absence or presence of tillage will affect the trace metal availabilities, and ii) that this impact will depend on the soil texture.

2) In a second part (chapter 4) we made complementary laboratory investigations to assess the potential role of organic matter apart of the effect of presence of absence of tillage. For that, we hypothesized that an evolution in the soil organic matter status between soil samples (under annual *vs* miscanthus perennial crop and between soils of different textures) will affect differently their trace metal availabilities. For that we enhanced the mineralization of the soil organic matter of each soil sample through in vitro soil incubations and assessed the resulting effect on soil trace metal availabilities

3) In a third part (chapter 5) we focused on the most labile soil organic matter fraction, the particulate organic matters, in order to assess their potential role in the trace metal availabilities. We aimed to assess i) whether particulate organic matter plays a role of source or sink of trace metals in soils, linked to the trace metal availabilities and ii) to what extent the metals associated with this organic matter fraction are bioavailable, as can be seen using a biotest.

Finally, this thesis study is articulated into five chapters. The first chapter presents a review of our present knowledge on the fate of trace metals in the soils, the soil organic matter and its affinity towards trace metals and the various concepts of trace metal availability and the ways of its assessment. In the second chapter we present all the material and methods used in this work. The three following chapters are based on the results obtained during the thesis work corresponding to the three specific aims of the study.

Chapter 1

Literature Review

1.1. Trace metals and agricultural soils

Trace metals (TMs) are naturally present in soils, the so-called pedo-geochemical background levels reflecting the occurrence of trace metals in the soil parent material. With the increased industrialization and urbanization, a tremendous amount of these metals is added to soils and water environment annually from different sources (Figure 1.1). In France e.g. according to an assessment published in 2007 a total of 4869 tons year $^{-1}$ of Cu, 696 tons year $^{-1}$ of Pb, 15190 tons year $^{-1}$ of Zn and 54 tons year $^{-1}$ of Cd (Figure 1.2) are added to the soils from different anthropogenic sources (Sorgreah-Ademe, 2007). Because of their non biodegradation and persistence in the soils, the trace metals are considered to be potential environmental contaminants for the biological life. The pollutant activities lead to an irreversible and diffusive contamination (contamination from a remote e.g vehicles exhausts, heating plants, waste water irrigation etc) in the long term, being likely to compromise the fertility and the quality of the agricultural soils (Chassin et al., 1996; Ramade, 2007). In addition to these diffusive contaminations, local industrial and urban exploitations can also cause soil contamination (e.g. case of Metaleurop in the north of France which has contaminated the nearby agricultural soils).

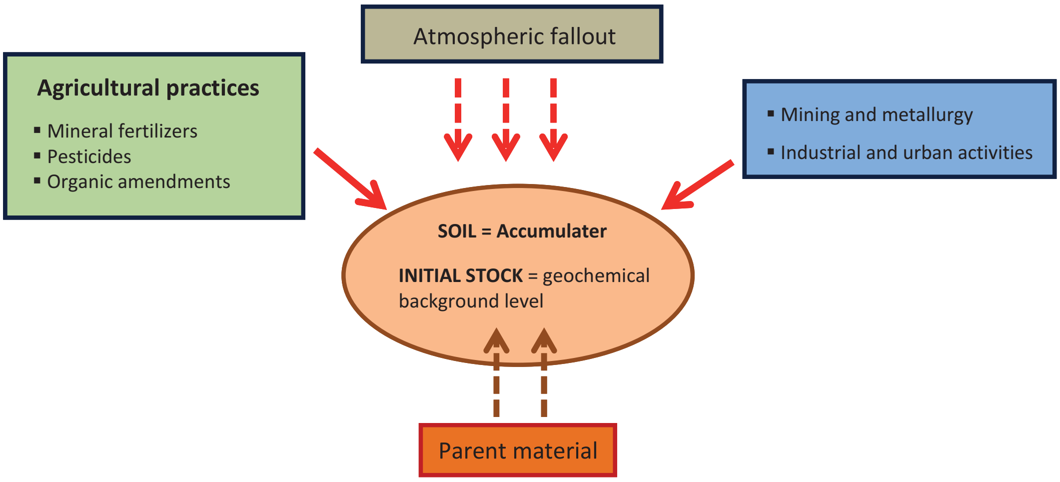


Figure 1.1: *Major input fluxes of trace metals into agricultural soils.*

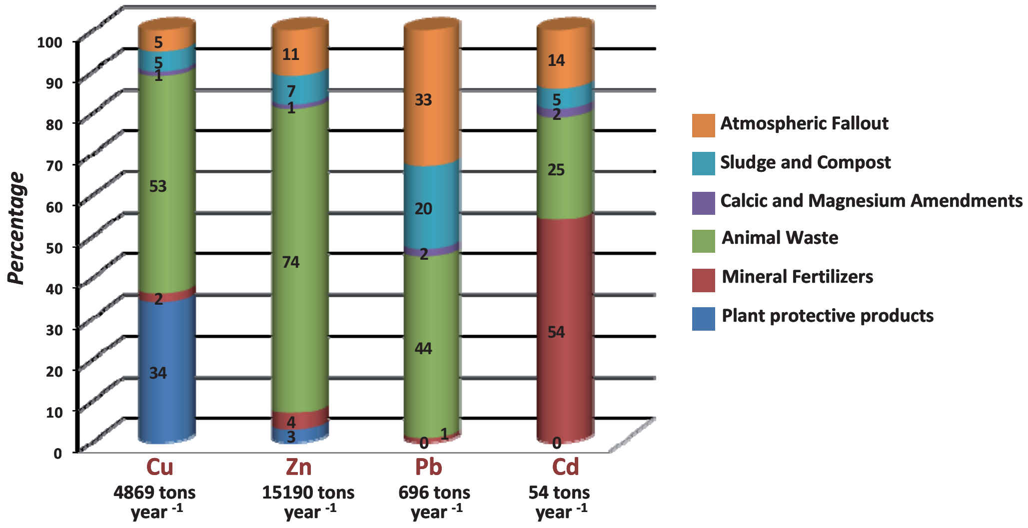


Figure 1.2: *Estimation of annual input of trace metals into agricultural soils of France with their principle sources. (Values taken from Sorgreah-Ademe, 2007)*

In this thesis work, we will be interested more particularly in Cu, Zn, Pb and Cd, which are the major trace metals found in the agricultural soils (Ramade, 2007; Zheljazkov et al., 2008). Cu and Zn are essential trace elements and are necessary for the enzymatic reactions of the biological functions of the living organisms (Tyler, 1981). Contrary, they become toxic for soil organisms at excessive concentrations. Agricultural and urban wastes and plant protective products are the major sources of soil contamination by these essential elements. In France Cu has been used in the products applied to vineyards since several decades to fight against the parasites or the “mildew”. Thus the vineyards soils, nearly a million hectare in France, have high Cu percentages (beyond 500 mg/kg of soil), limiting in certain cases the microbial activity and then decreasing the fertility of the soil for any other crops.

Pb and Cd do not have a known biological role (Maestri et al., 2010). Considered as harmful, they cause noxious biological effects even at weak concentrations (Tyler, 1981). Pb contamination of the soils is mainly caused by atmospheric fallout due to combustions. The mineral fertilizers, especially phosphorus fertilizers, contain considerable contents of metals. Besides these are major inputs for Cd (Nziguheba and Smolders, 2008).

1.2. Factors affecting fate of trace metals in the soils

It should be stressed that the dynamics of the trace metals in the environment depend on their speciation (their chemical form in the soil) and on the intrinsic physico-chemical characteristics of the soils. The soil organic matter is known for its reactivity with respect to metals such as Cu and Pb. The trace metals like Zn are adsorbed to clay or to iron hydroxides in the soils (Tyler, 1981; Labanowski et al., 2008). Due to the retention of metals in the surface horizons of soils the quantities of metals transferred vertically towards underground water are low in the majority of soils (Chassin et al., 1996).

The speciation of trace metal, their mobility and bioavailability are the characteristics linked to each other and are defined by the interaction of trace metals with their environment i.e. the soil, climate and biotope etc.

Various physicochemical parameters of the soil influence the mobility and the bioavailability of the trace metals in soils. Among which are pH, redox potential, nature of the components of soil, cation exchange capacity, porosity of the soil matrix, available phosphate contents, soil organic matter contents and soil biological activities (Juste, 1988; Alloway, 1995). The availability of soil trace metals depends as a whole on these parameters and not only on a single mechanism. The age and the nature of the contamination, the soil moisture or the interactions between different major elements in the soils and trace metals can also influence the trace metal mobility and bioavailability.

1.2.a. pH and redox potential

Soil pH and redox potential directly influence all chemical processes and consequently also determine the behavior of metals in soils. Many authors underline the major influence of pH on the dynamics of trace metals in soils (Alloway, 1995; Rieuwerts et al., 2006). Increase in pH increases the number of sorption sites of clays, of hydroxides or of organic matter (Ure and Davidson, 2002). This can increase the quantity of trace metal adsorbed to the soil components, provided that these components have an important affinity for a given metal and that quantity of sorption sites is sufficient (Ure and Davidson, 2002). Opposite to this, it was shown that the solubility of metal cations increases with the reduction in pH (Pueyo et al., 2004), while anions become less soluble (Hesterberg, 1998).

The redox potential (Eh) makes it possible to characterize the exchanges of electrons between the chemical species. Thus low values of Eh support the dissolution of hydroxides and involve an increase in the concentration of metals associated with soil components (Chaignon, 2001). Moreover, modification of the oxidation state of the ligands or elements with which the metals are bound in soil influences the solubility of trace metals indirectly. For example, in reducing conditions, sulphates are reduced to sulphides which readily trap the metal elements such as Pb, Cd, Zn (Deneux-Mustin et al., 2003). The influence of oxydoreduction conditions of the soil seems very important for the mobility of the metal elements, but it is secondary compared to the soil pH. For a given soil, the variation in Eh is opposite to the change in pH, it increases when the pH decreases (Deneux-Mustin et al., 2003).

1.2.b. Inorganic soil constituents

Inorganic soil constituents like clay minerals, oxy (hydroxides), carbonates and phosphates, because of their chemical properties, play an important role in the behavior of trace metals in the soils.

Li and Li, (2000) showed that trace metals can be adsorbed and immobilized by clay minerals or also be complexed to soil organic matter by forming organometallic complex. Metals can adsorb between the layers or on the surface of the clay (permanent charge), on the edges of the hydroxyl groups (variable charge) and on the bridges formed because of the irregularity of the matrix structure (Carrillo-González et al., 2006; Sajidu et al., 2008). The sorption force of cations on clay minerals varies with the type of clay (Carrillo-González et al., 2006). Thus, clays made up of two tetrahedral layers and one octahedral layer (e.g the montmorillonite) have a higher cation exchange capacity, and thus a better capacity of fixing cations, than the clays made up of one tetrahedral layer and one octahedral layer (McBride, 1994; Dube et al., 2001; Brigatti et al., 2005).

The presence of different types of oxides and oxy-hydroxides in the soils can alter the trace metal availability in the soils because of the degree affinity of the metals to them. Fe oxides (hematite, maghemite, magnetite) and oxy-hydroxides (goethite, ferrihydrite, feroxyhite, akaganeite, lepidocrocite), Al oxides (corundum), hydroxides (gibbsite) and oxy-hydroxides (boehmite), as well as Mn oxides (birnessite) are common and naturally present in soils (Sparks, 2003). These oxides are very reactive for the trace metals and are able to fix them to differing degree. Trivedi and Axe (2001) classified oxides according to their capacity of adsorption: Mn oxides > Fe oxides > Al oxides. The co-precipitation of trace metals with oxides reduces their solubility and their availability. This capacity was used in the works of remediation of strongly contaminated soils, in particular by the addition of shots of Fe (Boisson et al., 1998; Mench et al., 2000). This product, composed mainly of Fe (97% FeO), oxidizes naturally in soil and generates oxides of Fe and Mn reacting with Cd and As present in the soil solution (Mench et al., 2003).

The precipitation or the co-precipitation of certain trace metals with the carbonates and sulphates is also responsible for their retention in the soils (Bataillard et al., 2003; Miretzky and Fernandez-Cirelli, 2008). Zachara et al., (1991) showed that the sorption of divalent metal ions was dependent on the aqueous concentration of Ca^{2+} ions and the following selectivity sequence was observed: Cd > Zn ≥ Mn > Co > Ni > Ba = Sr. The influence of phosphates on the mobility of trace metals in the soils was especially studied for the use of these products in remediation, because of their strong affinity for trace metals and in particular for Pb. The effectiveness of phosphates to immobilize Pb in soils (Chen et al., 2007; Miretzky and Fernandez-Cirelli, 2008) depends on the formation of pyromorphite and chloropyromorphite, the latter being the most insoluble mineral phase of Pb in soils (Davies, 1992). It should be noted that Pb immobilization by the hydroxyapatite can be restricted by the presence of other elements (like Al, Cu, Fe, Cd, Zn, Ni), because of competitions for exchange sites. The influence of these elements on Pb immobilization decreases according to the following order: Al > Cu > Fe (II) > Cd > Zn > Ni when Pb concentration is high and Cu > Fe (L) > Cd > Zn > Al > Ni when Pb concentrations are lower (Ma et al., 1994).

1.2.c. Biological activities

Biological activities of the soils also affect the trace metal availability. The activities of soil living organisms can induce different processes in the soils modifying the mobility and availability of the trace metals directly or indirectly. Among micro-organisms bacteria and fungi are the main groups whose metabolic activities influence the mobility of trace metals. However, much of these mechanisms are also common to the plants. Fungi and plant roots excrete acids to facilitate nutrient absorption from soils, or simply as metabolic wastes (Foy et al., 1978; Chaignon, 2001; Deneux-Mustin et al., 2003). This acidification supports also the mobility of other elements which are not essential for the metabolism of soil organisms. In addition several other organic molecules, able to complex specifically main essential elements in soluble forms, can be also released in case of nutritive deficiency.

Among these complexing/chelating agents, siderophores as organic molecule of lower molecular weight are produced by bacteria and fungi and also by plants. These molecules support metal mobility in soils and a diffusive transfer occurs towards plants in which they accumulate (Chaignon, 2001). Although the external detoxification of trace metals by root exudates was never assessed (Baker and Walker, 1990), organic acids of low molecular weight, like oxalic, citric or fumaric acids which are involved in the intracellular complexation of nutritive elements can be secreted in the external medium by plants. These organic acids would thus limit the element transfers towards plants by mechanisms of complexation. Volatilization of elements like mercury, arsenic and selenium is another process induced by certain microbial activities, thereby changing their availability in the soils.

1.3. Soil organic matter as a labile soil constituent

Soil organic matter (SOM) is a soil constituent which is under constant renewal especially in case of agricultural soils. Once added to the soil the plant and animal wastes continuously undergo a series of biological, chemical and physical changes and therefore remain changing from one form to another. The term "Soil organic matter" (SOM) has been used in different ways to describe the organic constituents of soil. For the present, the word soil organic matter will be used as defined by Baldock and Skjemstad (1999) as "all organic materials found in soils irrespective of their origin or state of decomposition". Since SOM consists of C, H, O, N, P and S, it is difficult to actually measure the SOM content and the widely accepted analytical method is to determine the soil organic carbon (SOC) content and estimate SOM through a conversion factor.

1.3.a. Fractions of soil organic matter and their affinity to trace metals in the soils

Soil organic matter can mainly be divided into two categories, living and non living soil organic matter. Living organic matter is reported to represent about 15% of SOM and it includes micro-organisms (bacteria, fungi, algae and actinomycetes), soil fauna (protozoa, mites, earthworms, nematodes and insects) and plant roots (Oláh et al., 1978). The non living soil organic matter can further be divided into three fractions on the basis of their properties and turnover i.e. dissolved organic matter, particulate organic matter and humic substances.

Dissolved organic matter (DOM):

DOM constitutes the <0.45µm diameter organic materials in solution. It is a mixture of soluble organic compounds. A small part of DOM is mainly composed of organic molecules of low molecular weight which can be identified chemically like organic acids, sugars and amino acids etc. But a larger part of DOM consists of complex macromolecules of high molecular weight substances like fulvic and humic acids (Harter and Naidu, 1995; Kalbitz et al., 2000; Strobel et al., 2001).

Sources of DOM in soils include litter, organic matter degradation, root exudates and microbial activities especially the decomposers which include a wide variety of bacteria, fungi, protists and invertebrates (Oláh et al., 1978). Microorganisms have a dual role, both as consumers and producers of DOM (Lundquist et al., 1999). Among the microorganisms, fungi are considered to be most important in DOM production process as they partially degrade organic matter (Moller et al., 1999).

There is often a close correspondence of metal concentration to DOM in ground water, with a large fraction of the dissolved metals (particularly Cd, Cu, and Pb) in organically complexed form (McBride et al., 1997; Römkens and Dolfing, 1998; Temminghoff et al., 1998). At long-term sewage sludge application sites, DOM in shallow groundwater may remain elevated for decades, presumably due to continued microbial activity, resulting in higher dissolved concentrations of a wide range of metals. Despite the fact that metal speciation models have predicted a low tendency for Cd^{2+} and Zn^{2+} to complex with fulvic acids, experimental measurements in Cd contaminated soils have shown a large fraction of Cd and Zn in many cases to be complexed (Almås et al., 2000; Sauvé et al., 2000). The addition of organic matter such as manure to soils can therefore increase Cd and Zn solubility by increasing the DOM in soil water (Almås et al., 2000). In naturally high-Zn, high-Cd peat soils, it was found that a large fraction of the dissolved Zn (but less of the Cd) was in a non labile (strongly complexed) form (MartÍnez et al., 2002).

DOM stays dissolved in the soil solution under natural conditions and it has been found that it may be responsible for the dissolution equilibria of metals in the soil solution especially at neutral pH values (Harter and Naidu, 1995). DOM also has a unique role in the chemistry of heavy metals in soils; it reduces metal adsorption onto soil surfaces by either by competing more effectively for the free metal ion and forming soluble organo-metallic complexes or by its preferential adsorption onto the surfaces instead of the metals it is competing with (Giusquiani et al., 1998). DOM can increase metal availability to plants which are then taken up by plant roots (Hamon et al., 1995). Krishnamurti et al., (1997) maintained that this could establish a diffusion gradient to transport more chelated metals towards the root surface. The effect of DOM on the chemistry of heavy metals in sludge-treated soils is of great environmental importance. The soil pH conditions in which DOM of sewage sludge is more effective in competing with heavy metals are the same as those found in agricultural soils (pH 5–7) and this implies risks for increased heavy metal accumulation in crops and possible movement of heavy metals down the soil profile (Lamy et al., 1993). Baziramakenga and Simard (1998) suggested that it is not only the quantity of DOM that changes over time, but the quality as well. They reported that in fresh sludge aliphatic acids with high pK organo-metallic association values are predominant, while in mature sludge aliphatic acids with lower pK values become more predominant. Lower pK values indicate more stable associations between DOM and metals. This means that over time DOM can become more effective in controlling metal solubility highlighting the importance of the role that DOM can have on heavy metal availability. The additions of DOC probably affected the chemical reactions of Cd, Ni and Zn in the soil system and it is likely that organo-metallic complexes became predominant in controlling metal solubility, especially in the higher sludge application rate and in the higher DOC additions.

The quality as well as quantity of dissolved organic matter organic matter can change from one soil to another and at different time scales within a soil, thereby changing its reactivity to the metals.

Humic substances

Widely studied by scientists, humic substances (HS) are the major organic fraction in soils. The first attempts to extract humic substances were carried out by Achard in 1786 (Schnitzer, 1991). He extracted a black solution by applying to peat an alkaline solution which precipitated after acidification. These initial extractions made by Achard opened a large area of work on humic substances, which continues today.

The HS were defined as "a series of high molecular weight substances relatively brown to black in colour, a by-product of secondary synthetic reactions" (Stevenson, 1994) or as "a set of naturally occurring organic substances in all terrestrial and aquatic environments resulted from degradation of animal and plant residues" (Aiken et al., 1985).

The molecular structure of HS has not been defined and, therefore, their fate and functions in soils are difficult to assess (Aiken et al., 1985; Balesdent, 1996). They are, however, operationally defined and classified according to their solubility into three fractions (Schnitzer, 1991):

Humin: Insoluble fraction of humic substances at any pH.

Humic acids (HA): Insoluble fraction of humic substances at acidic pH (<1).

Fulvic acids (FA): Fraction of humic substances soluble at any pH.

Major fraction of the SOM, HS has been much studied for their acid-base properties and affinity with metals because of their ease of extraction and purification. These are materials have been used to characterize the reactivity of SOM (Fiol et al., 1999; Cances et al., 2003; Lumsdon, 2004). Functional groups in the humic substances have an affinity for metal ions in the following order (Chaberek and Martell, 1959; Stevenson, 1994):

$-O-$	>	$-NH_2$	>	$-N=N-$	>	$=N$	>	$-COO^-$	>	$-O-$	>	$C=0$
enolate		amine		azo		ring N		caiboxylate		ether		carbonyl

Carboxyl and phenolic groups are the most significant for metal binding (Sparks, 2003). Conditional stability constants for metal-humic substance complexes generally increase with increasing pH as organic functional groups become more negatively charged (due to deprotonation). Metal-humic interactions may be generally predicted by "hard" and "soft" acid or base principals (Buffle and Stumm, 1994). Essentially, hard acids (Group I elements i.e. H, Li, Na, etc.) of high positive charge and small size react with hard bases (highly electronegative, low polarity), and soft acids (smaller positive charge, large size Group III elements Cd, Cu, Hg, Pb, etc.) with soft bases (low electronegativity, high polarity). For humic substances, hard sites include the carboxyl and phenolic groups and soft sites include N or S containing functional groups (Sparks, 2003).

Solid phases of humic materials, such as colloidal aggregates of humic substances can sorb or chelate metals. Metals sequestered in the structure of organic molecules may not readily desorb, suggesting that this may be a mechanism for attenuation. For example, Bunzl et al., (1976) showed that the degree of desorption of Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, and Ca^{2+} from peat was much less than the amount sorbed. Desorption of metals from organic matter will, to a certain extent, be pH dependent as the main functional groups (carboxylic and phenolic) on SOM exhibit pH-dependent charge (Spark et al., 1997).

Particulate organic matter (POM):

Particulate organic matter mainly consists of plant residues under degradation by micro-organisms such as fungal hyphae and bacteria but still recognizable and which can be separated from soil as free organic particles. Moreover this organic matter fraction also includes the proportion of coal present in the soil owing to the history of the site like burning of plant stubbles, forest fire (Spycher et al., 1983; Wander, 2004).

Balesdent et al. (1991) defined particulate organic matter as the organic residues of size > 50µm which can be separated from the soil by densimetric fractionation. But in some other studies POM between 20 and 50µm have also been separated (Ducaroir and Lamy, 1995). Free organic particle were seen in the clay size (<2µm) fractions (Chenu and Plante, 2002). All these studies suggest that POM of all size scales exist in soils. Christensen (2001) defined POM as "non-complexed organic matter" as a transition between the litter pool on one hand and the mineral associated organic matter on the other hand. This fraction of SOM however, does not include particles of litter (> 2 mm) and the organic particles incorporated into organo-mineral complexes. Due to the existence of organic particles of different sizes, POM is considered to be a group of further smaller organic matter fractions and is considered as plural. In this manuscript we have used word “POM Fractions” indifferently for all the free particulate organic fractions. Where the precision was needed POM fractions were called as coarse POM (200-2000µm) of fine POM (50-200µm) fraction.

POM can represent about 15-40% of the SOM in the surface horizons of soils under permanent vegetation, while in cultivated soils, POM are generally less than 10% of SOM. These organic fractions were studied in particular for their lability and their turnover in soil (Balesdent, 1996). Their turnover is less than the litter but more than the organic matter associated to clay or silt (Christensen, 2001). Because of their lability, POM fractions have been proposed as an index of the quality of SOM (Haynes, 2000; Wander, 2004). The proportion of POM fractions in the soil is reported to be affected by the type of vegetation, agricultural practices and other factors such as climate, soil type and micro-faunal activities (Balesdent et al., 1998; Christensen, 2001).

The trace metal contents of POM fractions of contaminated and non contaminated soils have been studied. The reported work shows that metal content of POM fractions is more than the soils so there is an enrichment (Ducaroir and Lamy, 1995; Besnard et al., 2001; Balabane and van Oort, 2002). The metal enrichment factors for POM fractions have been reported to vary from 1 to 12 depending on the size of POM studied (Besnard et al., 2001; Balabane and van Oort, 2002) and this enrichment was found irrespective of the source of metal pollution and the total metal contents of the soils (Table 1.1).

Table 1.1: *Metal enrichment factors of the particulate organic fractions of some soils.*

soil	Origine of Pollution	Metal	Enrichment factor of POM	Reference
Loamy sand	Fungicide (Bordeaux mixture)	Cu	29.7 (50-100 µm)	Flores-Vélez *et al.,* 1996
Sandy loam	Atmospheric fallout	Pb Zn Cd	6.3 (50-100 µm) 7.4 8.8	Balabane et van oort, 2002
Clay loam	Atmospheric fallout	Cu Pb Cd Zn	7.9 (50-100µm) 2.8 3.7 2.1	Lamy *et al.,* 2002
Sandy loam	Atmospheric fallout	Cu Pb Cd Zn	4.6 (50-100 µm) 3.7 5.2 3.2	Labanowski *et al.,*2007
Sandy loam	Non polluted	Cu	3.4 (50-200 µm)	Sebastia *et al.,* 2008

These studies showing the metal enrichments of POM fractions have further reported that the enrichment of fine POM fractions was more than the coarse POM fractions. On the other hand, in a range of total soil metal content from 10 to 5000 mg/kg of metals in soil, Lamy et al., (2002) observed that the higher the metal content of soil, the higher the metal contents of POM without showing any signs of metal saturation of POM.

The nature of metal-POM association, however, is not yet known. So Lamy et al., (2002) hypothesized that in agricultural soils, the accumulation of metals in POM is the result of both abiotic (adsorption, complexation) and biotic (microbial activities) processes. In the case of metallophyte grassland Balabane et al., (1999), explained that the higher metal contents of fine POM was due to the selective microbial decomposition of the parts of coarse POM with low metal contents, thereby leaving behind the metal rich portions. A comparative study on the biodegradation of Zn and Cd contaminated and non contaminated metallophyte leaves seemed to confirm this hypothesis (Boucher et al., 2005a; 2005b). Boucher et al. (2005a) showed that at a first time the biodegradation of contaminated and uncontaminated leaves was identical, which could correspond to the degradation of readily biodegradable carbon. In a second step, they observed that uncontaminated leaves were biodegraded prior to the metal rich leaves. In addition, it was shown that in contaminated soils, about 10 - 30% of the total metal soil metal contents could be located in POM fractions (> 50µm) which is a non negligible proportion of total soil metal contents (Flores-VéLez et al., 1996; Besnard et al., 2001). The mechanisms underlined between POM fractions and metals were assessed by the study of POM extracted from a former smelter impacted soil contaminated with Zn and Pb (Labanowski et al., 2007). They suggest that POM fractions have high surface reactivity towards metals due to the creation of reactive sites during their microbial biodegradation in soils.

The metal enrichment of the POM fractions and their short turn-over in soils question the fate of metals linked to these organic matter fractions. So the role of POM fractions as source or sink of trace metals in the soils is a question of great scientific interest.

1.3.b. Soil organic matter and its effect on different soil properties

Soil structure and aggregate stability

The relationships between soil organic matter and soil structure have been extensively studied and are already presented in the form of reviews (Tisdall and Oades, 1982; Carter and Stewart, 1996). Soil organic matter plays a role of binding agent for the structural stability of the soils. The increase in soil organic matter reduces bulk density, increases water holding capacity and the soil aggregate stability. Kay and Angers, (1999) suggested that most of soil organic carbon (SOC) fractions were involved to different degrees in aggregate formation and their stabilization. The studies carried out on loam or clay soils have reported positive effects of carbohydrate extracts on aggregate stability. For example Haynes et al., (1991), Carter (1992) and ShepherdB et al. (2001) found that polysaccharides were more implicated in aggregate formation than the humic substances. Haynes and Naidu, (1998), observed an increase in the microbial activity, fungal growth and extracellular polysaccharides after addition of easily decomposable organic matter resulting in a rapid increase in aggregate stability. An important role of hydrophobic materials on aggregate stability was observed by Capriel et al. (1990) who found a high correlation coefficient between aggregate stability and the aliphatic (hydrophobic) fraction (extracted with supercritical hexane) and soil microbial biomass.

Water-Holding Capacity (WHC)

Soil organic carbon is generally considered to have positive effect on the WHC of soil but the types of carbon responsible for this effect and the synergistic behavior with other soil properties are not well understood. De Jong (1983) and Haynes and Naidu (1998) found an increase in water content with increasing SOC content and noted that an increase of 1% SOM can add 1.5% additional moisture by volume at field capacity (Wolf and Snyder, 2003). However, opposite relationships between WHC and SOC content has also been reported. Loveland and Webb (2003) reported that SOC contributed to less than 15% to the variance in soil water content held in agricultural topsoils but Danalatos et al. (1994) failed to prove any effects of SOM on water retention. The effect of SOC on WHC tended to be greater in coarse textured compared with fine textured soils; in fact, WHC in heavy clay soils decreased with increasing SOC content (Bauer and Black, 1992). There is a strong relationship between clay content, SOC content and WHC and it is likely that these factors influence each other synergistically (Rawls et al., 2003).

Cation Exchange Capacity (CEC)

The charge development in SOM is generally negative, as it is provided by functional groups (mainly carboxylic and phenolic acids), but a relatively small positive charge can also occur because of the protonation of amino groups (Duxbury et al., 1989). Most soils carry both permanent and variable charges which are contributed by clays and organic matter or organo-mineral complexes respectively. The contribution of SOM to the CEC can vary depending on soil type between 25-90% (Stevenson, 1994), but most studies observed a contribution between 30-60% (Loveland and Webb, 2003), or 40-50% (Thomasson and Carter, 1989; Haynes and Naidu, 1998). Eshetu et al. (2004) reported that in forest soils of the Philippines a strong linear correlation exists between total CEC, SOC content and exchangeable and total Ca and that SOC content accounted for most of the variability. Parfitt et al. (1995) found in their studies that most of the soil CEC was due to organic matter (carboxyl groups) and that the presence of SOC reduced the CEC of smectite. Fertilizers and manure application can both increase the CEC of the soil (Haynes and Naidu, 1998). Charcoal has been shown to be a potentially important contributor to increasing CEC (Glaser et al., 2002).

Buffer Capacity and pH

Different functional groups (e.g. carboxylic, phenolic, acidic alcoholic, amine, amide and others) allow SOM to act as a buffer at a wide range of soil pH values. Buffer capacity is usually greater in the surface of the soils which are organic matter rich compared with the deeper mineral horizons (James and Riha, 1986). Application of organic residues to soils can result in an increase in soil pH (Hue, 1992; Lungu et al., 1993; Noble et al., 1996). Generally, initially there is an increase in pH for the first 1 or 2 months of residue decomposition followed by a decline to above or below the initial pH level (Hue, 1992; Wong et al., 2000). The increase in soil pH varies depending on the type of residue, its rate of application and the buffering capacity of the soil. A good correlation between buffer capacity and organic matter content has been reported in several studies (Curtin et al., 1996; Starr et al., 1996) and the importance of SOM to maintain stable pH values, despite acidifying factors, was documented (Cayley et al., 2002).

Biological Functions

Soil organic matter plays an important role in the soil biological systems as a reservoir of metabolic energy that drives biological processes, as a supply of macro-and micro-nutrients and by ensuring that both energy and nutrients are stored and released in a sustainable way. Importantly, biological processes in turn influence both soil chemical and soil structural properties as they greatly affect soil structure and soil redox reactions.

SOM as a Source of Energy

One of the most fundamental functions of SOM is the provision of metabolic energy which drives soil biological processes. Anderson (1995) suggested that the transformation from relatively labile SOM into increasingly complex, stabilized SOM can be viewed as a form of energy conservation. Indeed, the largest pool of SOM consists of the humus pool which is recalcitrant enough to endure in an edaphic environment for longer periods of time but still allows for decomposition and nutrient release to take place. The energy released from decomposition processes in the soil is mainly in the form of heat and Russell (2008) calculated that the annual heat loss from a hectare of an untreated, low producing soil was equivalent to the heat value of nearly a metric ton of coal: By comparison, a hectare of a productive soil, receiving farm yard manure, would release the equivalent of about 12 Mg of coal annually.

Microorganisms play a particularly important role in the transformation of organic matter and cycling of nutrients as 80-90% of the total soil metabolism is due to microbial processes (Brady and Weil, 1999). It has been reported that 1-5% of C and N in soil is stored in living microbial tissue (Duxbury et al., 1989) and microbial biomass in temperate grasslands is estimated to be 1-2 t ha^{-1} (Nannipieri et al., 2003).

SOM as a source of nutrients

Most of the nutrients in soils are derived from the mineralization of SOM and become available for plant uptake during decomposition and for this reason the particulate organic matter fraction is often considered the most important proportion of SOM in providing nutrients to plants (Wolf and Snyder, 2003). While a decrease in total SOC content may not be in proportion to the decrease in release of nutrients, 1% SOC is considered a threshold value (in relation to N supply from plant residues), below which an effective N supply is reduced (Loveland and Webb, 2003). It was noted that particularly with respect to N, the ‘active’ or POM pool of SOM was most important for providing N for crop growth and to increase the availability of micronutrients (Matus and Rodriguez, 1994). Nutrients like C, N and P and SOM seemed to be most stable in the fine silt and coarse clay fraction, whereas the nutrients associated with the fine clay fraction were easily mineralizable (Duxbury et al., 1989). The ‘active’ fraction was implicated as being the most important component in providing nutrients and regulating nutrient supply (Loveland and Webb, 2003). Several studies found that SOM and nutrient mineralization increased with decreasing aggregate size, (Elliott, 1986) found greater mineralization of both C and N in macro-aggregates compared with micro-aggregates. He postulated that the mineralization of inter-aggregate SOM was the main source of nutrient release.

While conversion of soils under native vegetation to agriculture usually results in a decline in nutrients and C, N, P levels, Zhang and He (2004) reported that the conversion of upland red soils to irrigated flooded rice fields in China increased C, N, P, K and POM contents and raised pH, CEC and aggregate stability in the plough layer (0-15 cm). The study was conducted on 66 rice fields where conversion had taken place from 2 to 100 years ago. The increase in C and N levels was observed not only for the surface but also for the subsurface soil layers and lasted for 30-40 years after conversion and then stabilized, whereas total K and clay content decreased after that time period.

1.3.c. Soil organic matter and changes in land use

The changes of soil organic matter quantity as well as its quality as a response of changes in land use are widely discussed in literature (Hussain et al., 1999; Halvorson et al., 2002; Zibilske et al., 2002). Soil management practices can modify SOM levels by affecting organic matter inputs and turnover. Studies have shown that increasing C inputs (with additions of manure and fertilizer, inclusion of legumes and cover crops, and reduction in fallow frequency) can cause gradual SOM accumulation (Kuo et al., 1997).

Land use is a broader term and includes countless options on the basis of type of plant being cultivated, crop rotation, type of tillage, irrigation, type of amendment etc. In this work we were in two generalized scenarios i.e the annual vs perennial crop systems and tillage and no tillage systems. Reviews of the comparison of many bio-fuel production systems have revealed that perennial plant systems, such as prairie grasses, store higher levels of soil organic carbon. Such systems offer the most carbon negative land-use changes options among all studied systems for the atmospheric carbon sequestration and in terms of carbon release to atmosphere (Fargione et al., 2008). A study based on the meta-analysis of documented studies involving changes in soil organic carbon after land use changes revealed that converting agricultural soils to perennial grasslands and to secondary forests were the most C negative land use options (Guo and Gifford, 2002). During studies conducted at the Biological Station in Michigan, it was observed that more soil organic carbon was accumulated in the plots under perennial alfalfa and young secondary forest than in the plots under annual crops (Grandy and Robertson, 2007). Glover et al. (2010) reported that the annually harvested prairie meadows in Kansas yielded higher biomass and similar nitrogen yields as conventionally cultivated wheat fields on the same soils while their energy input was only 8%, over the last 80 years.

Tillage is a soil management practice which affects soil organic matter levels. No-tillage usually increases the SOM content and aggregation compared to plow tillage (Havlin et al., 1990; Paustian et al., 1992; Smith et al., 1998; Diaz-Zorita and Grove, 2002; Amado et al., 2006). This has been explained by decreased aggregate turnover in no-tillage, favoring the formation of stable micro-aggregates that stabilize C for a longer period (Six et al., 2000). But the magnitude of these soil organic matter changes due to no-tillage varies among soils and is dependant to other management practices being used. Paustian et al. (1992) estimated that no-tillage caused an increase of 285 g m^{-2} in soil organic carbon compared to the conventional tillage. Smith et al. (1998) observed that with a conversion from conventional tillage to no-tillage the average increase in soil organic matter was 0.7 ± 0.4% yr^{-1}, and they estimated that this increase may continue up to 50-100 years before the soil organic matter level reaches equilibrium. Similarly a global data analysis of soil organic carbon sequestration rate involving the study of crop rotation and tillage practices indicated that the conversion from conventional tillage to no tillage may cause as much as 57 ± 14 g C m^{-2} yr^{-1} (West and Wilfred, 2002). Land management practices can affect the nutrient status and nutrient release from SOM. While reduction in tillage or no till usually increases the SOC content, it tends to reduce the availability of nutrients to crops and results in gradual accumulation of nutrient reserves in SOM (Duxbury et al., 1989). In short all these studies report that soil organic matter change with the changes in land use.

1.3.d. Soil organic matter a source or sink of trace metals?

As discussed above on one hand soil organic matter is a reactive soil constituent and the metals in the soils are retained by its different fractions. But on the other hand it is under continuous change in soil being the labile constituent of soil and changes as a result of different soil management practices. Most of the existing literature on the effect of organic matter on the availability and mobility of trace metals in soil is conducted by employing different organic amendments like raw sewage sludge or partially humified compost material as source of organic carbon. Besides the strict legislation for the quality control of these amendments, they still contain a minimum quantity of contaminants which are added to the soils and remain accumulating in the soils. In soils irrigated with waste water or amended with organic amendments like sewage sludge trace metal are mostly bound to soil organic matter fractions (Udom et al., 2004; Al-Najar et al., 2005; Dère et al., 2007). The mineralization of organic matter with the passage of time may release the trace metals into the soils thereby causing an increase in their availabilities in the soils, the aspect termed as time bomb hypothesis (McGrath et al., 2000; Dère et al., 2007). Similarly the metal enrichment of particulate organic matter fractions was discussed above. But these are the organic matter fractions which have short turn-over in soils. The fate of trace metals linked to these soil organic matter fractions still needs to be discussed. So there is a need to know how the changes in soil organic matter due to different management strategies of contaminated soils will change the trace metal availability of the soils. In other words the role of soil organic matter as source or sink of metals need to be addressed.

1.4. <u>Bioavailability of trace metals-concepts and definitions</u>

Soil solid phases frequently have high concentrations of metals compared to the concentrations of metals in the dissolved phase or in (terrestrial) organisms. Total concentrations of contaminants, however, are not always directly related to effects since exposure of biota may be limited by processes that render the metals unavailable for uptake. Nevertheless, due to the relative ease of analysis, it is tempting to establish environmental quality criteria based solely on the total concentration of metals in the environmental matrix (soil, sediment, air, or water). This is a common practice in many countries (Peijnenburg et al., 1997).

But now with the advancement of the technologies it is emphasized that the assessment of total metal concentrations is not an efficient way to assess their potential risks. The interaction of soil biological receptors to the effective metals fractions to which they are exposed in soils should be taken into account for environmental management. But soil being a complex medium, the varieties of metal species in the soils is great and difficult to be assessed. In addition the interactions of trace metals and soil matrix itself and with and soil organisms are dependent of the soil physicochemical factors. Bioavailability is a concept for which no simple generic definition can be formulated, although a large number of definitions can be found in literature. The definition given in ISO 11074 (ISO, 2008) that "Bioavailability is the degree to which chemicals present in the soil may be absorbed or metabolized by human or ecological receptors or are available for interaction with biological systems" is comprehensive but is not explicit enough as a definition of bioavailability as a measurable quantity. Bioavailability depends on a specific target organism and on a specific contaminant and includes the following aspects: exposure time, transfer of contaminants from soil to organisms, their accumulation in the target organisms, and the subsequent effects (Harmsen, 2007).

For practical purposes, more specific conceptual definitions have been derived, for example: (i) Bioavailability is the flux of contaminants to biota, (ii) bioavailability is the amount of chemicals in the soil that are present in forms and amounts that plants or other organisms can take up during the time they are living (Warrington and Skogley, 1997), and (iii) bioavailability is the rate at which a chemical compound can be transported to the specified biological population (Shor and Kosson, 2000). These definitions can be grouped into two categories: (i) bioavailability defined in terms of flux or rate (mol m^{-2} s^{-1}) and (ii) bioavailability defined in terms of content (mol kg^{-1}). Theoretically, the content-based bioavailability is obtained by integrating the rate- or flux-based bioavailability over a period of exposition. In practice, it is difficult to directly measure the rate of processes, and the flux- or rate-based bioavailability is estimated from content- based bioavailability. Therefore, the content-based bioavailability is of primary importance from an evaluation point of view. It is better to present bioavailability as a concept and relate it to specific situations or to values measured by specific chemical extractions or bioassays (Harmsen, 2007; Hooda, 2011). Bioavailability has to be defined conceptually and operationally.

To understand the bioavailability, an integrated understanding of the system soil-metal-organism is necessary. Figure 1.3 presents a schematic diagram of the bioavailability processes in the soil or sediment. The processes in Figure 1.3 can be thought of as barriers that must be overcome for a contaminant in soil or sediment to become bioavailable (Ehlers and Luthy, 2003). Typically, a few steps will be most restrictive, and these are expected to control bioavailability.

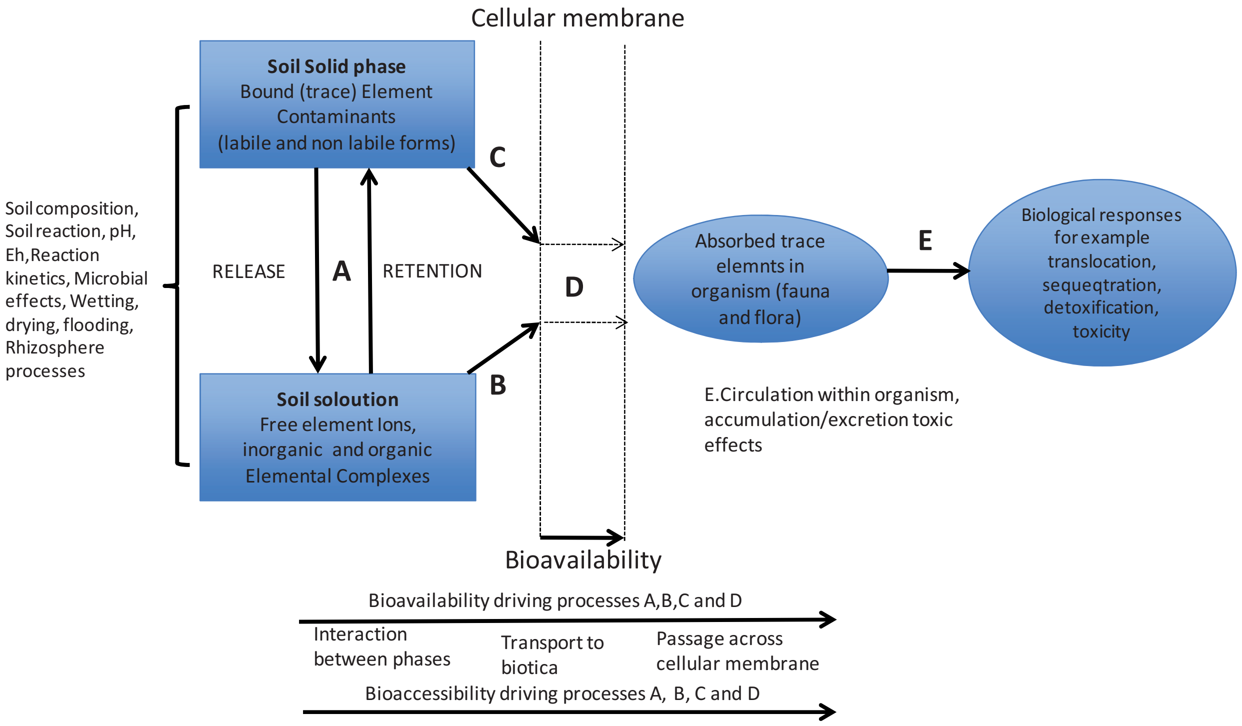


Figure 1.3: *Bioavailability processes of soil trace metal partitioning between soil solid and solution phases including speciation in soil solution an how they can be influenced by soil properties and environmental conditions. (Reproduced from Hooda, 2011)*

A refers to the physical, chemical, and biochemical phenomena that bind, unbind, expose, or solubilize a contaminant associated with soil or sediment. Contaminant–solid binding may occur by adsorption on solid surfaces, by absorption within a phase like natural organic matter, or by precipitation, whereas contaminant release to fluids in contact with the soil or sediment occurs in response to changes in water saturation, in water and gas chemistry, and in solid surface properties. The exchanges between solid-solution phases are dependent on the physicochemical parameters. Time is an important aspect governing contaminant–solid interactions.

B involves the movement of a released contaminant to the membrane of an organism, whereas **C** involves the movement of contaminants still bound to the solid phase. Although not often associated with bioavailability, processes B and C are integral fate and-transport processes that can control an organism’s overall exposure.

D entails movement from the external environment through a physiological barrier and into a living system. Because of the great diversity of organisms, the actual process of contaminant uptake into a cell—or factors that may impede or facilitate uptake—varies depending on receptor type. Human uptake mechanisms include absorption across the gut wall, the skin, and the lining of the lungs. One common factor among all organisms is the presence of a cellular membrane that separates the cell interior from the external environment, through which most contaminants must pass before deleterious effects on the cell or organism occur.

E refers to paths taken by the chemical following uptake across a membrane, for example, metabolic processing or exerting a toxic effect within a particular tissue. Of particular importance is the bioaccumulation of contaminants within tissues that are often inaccessible to normal elimination mechanisms, such as metabolism and excretion. Slow release of the chemicals from these storage sites can cause protracted “exposure” within the body even when the external exposure has been reduced. In addition, bioaccumulated contaminants (e.g., polychlorinated biphenyls) may become available at some point to higher-order organisms that eat the plant or animal in which the contaminants are stored.

All the compartments (boxes/circles) and processes (arrow signs) involved in trace metal availability are presented above in Figure 1.3. In a single study it is difficult to assess all the processes involved. Moreover, the mechanistic understanding of these processes is not easy and quantitative descriptive models of bioavailability processes are lacking in most cases. There is a need to study these compartments separately and to assess the mechanisms in a way that it leads to understand the relations between at least two compartments.

Usually in the literature the studies addressing these compartments use the term of bioavailability in a general manner irrespective of the method used to assess and the studied compartment. A number of definitions of bioavailability, bioaccessibility and bioaccumulation exist in the literature. This has created a considerable degree of ambiguity within scientific and regulatory communities, as often these terms have been used interchangeably. Other studies similarly express the concept of bioavailability including availability, plant availability or extractability. There is a connection between these terms; for example, free ionic species of trace metals influence their bioavailability and perhaps their uptake of plants or other soil biota. The bioavailability, availability and extractability, are however, different concepts.

Recently an international norm (Harmsen 2007; ISO, 2008) and the identical French norm (AFNOR, 2008) have emphasized that these different compartments should be addressed separately and have proposed the complementary concepts of trace metal bioavailability by using the same schematic diagram but in a simplified way (Figure 1.4). These definitions of the bioavailability are given as under:

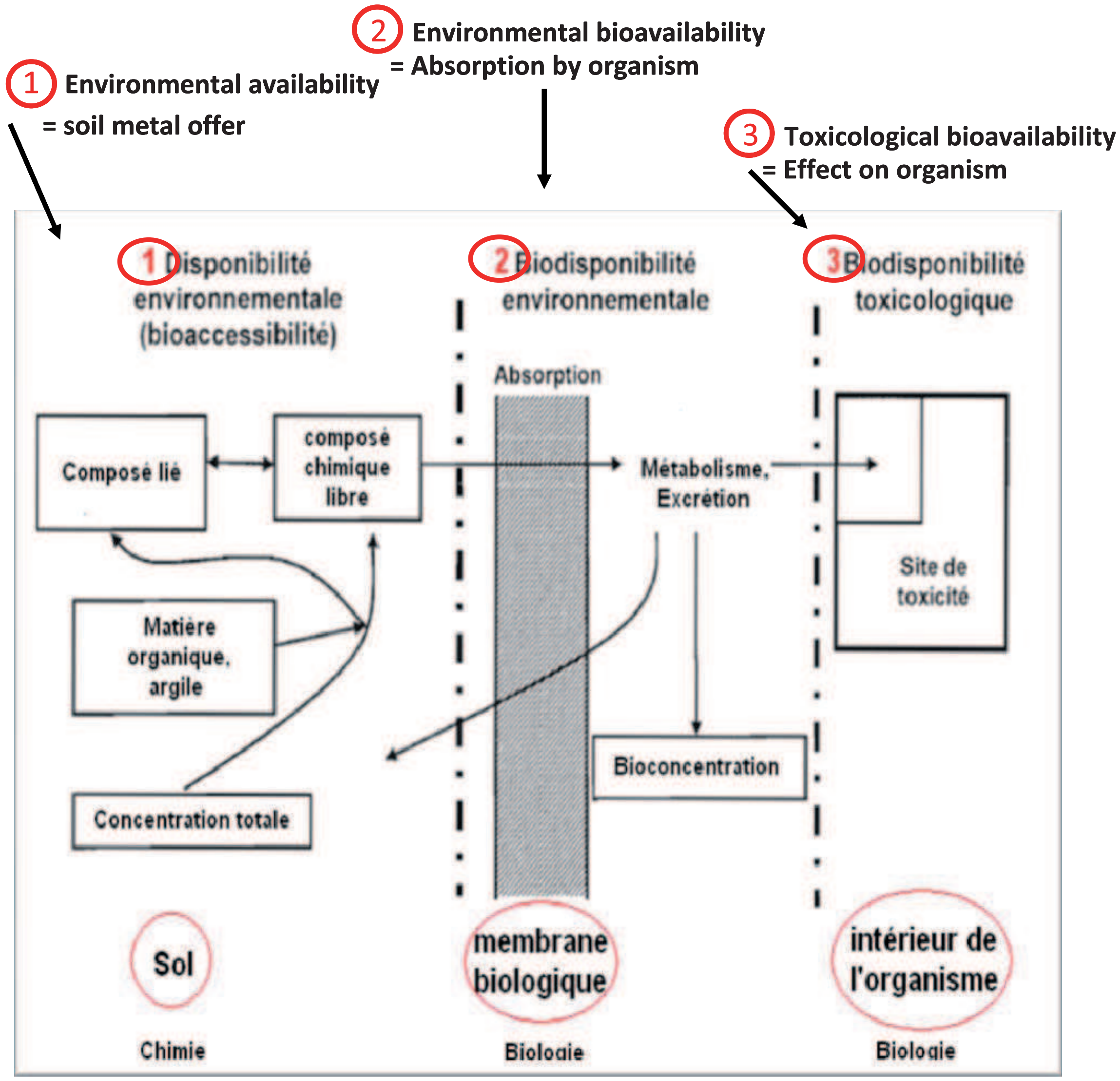


Figure 1.4: *Trace metal bioavailability in the soils as described by norm ISO 17402 (ISO , 2008).*

Environmental availability: This refers to the ability of a contaminant in a soil to be available for interaction with biological targets of the soils. Environmental availability can be assessed by chemical ways, e.g. chemical extractions. The biological target is not necessarily involved in the aspect of environmental availability of contaminants. Soil environmental availability also corresponds to the soil metal offer. In this manuscript term “trace metal availability” corresponds to this aspect of environmental availability.

Environmental bioavailability: This refers to the uptake of the contaminant by the soil organism i.e. adsorption or absorption. But this can be without causing any effect on soil organism

Toxicological bioavailability: This refers to the fact that accumulation or uptake of the trace metals into soil organisms caused an impact on the functioning of soil organisms.

This norm only proposed the concepts of trace metal bioavailability and some prevailing methods for the assessment of each these aspects of bioavailability. This emphasizes that there is not a single generally accepted method to assess/quantify the contaminant bioavailability.

1.5. Methods of trace metal availability assessment

1.5.a. Single extraction

Variety of single extraction tests have been used as surrogate measures for the availability of soil trace metals. The underpinning principle is that such extractions are capable of mobilizing and isolating certain ‘species’ or forms of trace metals that quantify their mobility in soil. But trace metals extracted by such chemical leaching have also been functionally defined as exchangeable, mobile, plant-available or bio-available. In fact these fractions are operationally defined and the extractions correspond to a fraction of trace metals that is potentially plant-available, mobile or bioavailability. An ideal test should extract only the available metal fraction and should not alter soil-specific element partitioning controls between the phases. The commonly used single extraction tests can be grouped as follows:

1. Strong chelating solutions, mainly with ethylenediamine tetraacetic acid (EDTA) diethylenetriamine pentaacetic acid (DTPA);

2. Unbuffered neutral salt solutions, for example $CaCl_2$, $MgCl_2$, NH_4NO_3, $NaNO_3$, KNO_3;
3. Organic (weak) acids alone or combined acid-salt solutions, for example acetic acid (CH_3COOH), oxalic acid (H_2C_2O4), citric acid ($C_6H_8O_7$), ammonium acetate CH_3COONH_4), sodium acetate (CH_3COONa).
4. Diluted mineral acid solutions, for example HCl, HNO_3, HCl + HNO_3 or HCl + H_2SO_4.

General approach has been to establish correlations between trace metals extracted either by a single extractant solution (for example EDTA, DTPA or $CaCl_2$) or extracted by a group of such extractants with the elements accumulation in the whole or parts of plants or other fauna. The extent of correlation between the organism element content and that extracted by the chemical extraction test is used to assess the suitability of the test for bioavailability assessment purposes.

The DTPA and EDTA tests, originally designed to assess micronutrient deficiency/availability in soils, were preferred for estimating availability of metals in contaminated soils for plant uptake. DTPA extraction test was first designed to study the availability of micronutrients in calcareous soils especially for Cu, Fe, Mn and Zn (Lindsay and Norvell, 1978). It was an aqueous mixture of 0.005 M DTPA, 0.1 M TEA (triethanolamine) and 0.01 M $CaCl_2$, buffered to pH 7.3. The efficiency of extractants such as DTPA in contaminated soils was questioned by O'Connor (1988), nevertheless, the test continues to be widely used on contaminated soils and regardless of soil type, for example acidic, calcareous, non calcareous (Korcak and Fanning, 1978; Merry et al., 1986; Hooda and Alloway, 1994b). Similarly, the use of EDTA was initially proposed for assessing soil micronutrient supply (Viro, 1955). Subsequently, it was adapted for trace metals extraction in contaminated soils (Clayton and Tiller, 1979). Despite this early adaptation work, several variants of EDTA (for example Na or NH_4 EDTA, 0.005-1.0 M) have been used extensively for assessing plant availability of soil trace metals (Hooda and Alloway, 1994b; Hammer and Keller, 2002; Chaignon et al., 2003; Feng et al., 2005). EDTA was twice an effective extractant as DTPA for nearly all elements. The former solubilises carbonate-occluded metals, whereas the latter is designed to minimize dissolution of calcium carbonate (McGrath, 1996). Both DTPA and EDTA as strong chelating agents are known to extract labile and non labile element fractions (Payà-Pérez et al., 1993; Bermond et al., 1998; Peijnenburg et al., 2007) and while they may correlate with plant metal uptake (Hooda and Alloway, 1994b), they are likely to overestimate bioavailability in contaminated soils.

Despite reports of good correlations between elements extracted by chelating agents (for example DPTA and EDTA) and their uptake by plants, there is a general acceptance that milder chemical extractants, particularly unbuffered neutral salt solutions can provide a more realistic estimation of potential bioavailability of trace metals in contaminated soils (Houba et al., 1990; Aten and Gupta, 1996; Peijnenburg et al., 1999). Examples of such aqueous chemical solutions used for bioavailability assessment purposes include $CaCl_2$, NH_4NO_3, $Ca(NO_3)_2$, $NaNO_3$, KNO_3, $MgCl_2$ (Houba et al., 1990; Lebourg et al., 1998; Rauret, 1998; Pueyo et al., 2004; Gupta and Sinha, 2007; Peijnenburg et al., 2007). Lebourg et al., (1998), while studying the speciation of Cd, Cu, Pb and Zn in different neutral salt extracts observed similarities between the composition of these extracts and the soil solution. These unbuffered neutral salt solutions are therefore not expected to alter soil conditions/processes and thus can provide a reasonable estimate of the labile trace metal pool. But under strong contaminated soil conditions these extractants can underestimate the trace metal availability by extracting only a small fraction of the total extractable metals and were placed in a category of poor extractants (Gupta and Sinha, 2007).

The use of low molecular weight organic acids e.g. acetic acid is also a method used in bioavailability research because of the presence of Low Molecular Weight Organic Acids (LMWOA) in the rhizosphere of many plants. An interesting approach in this regard is to mimic soil solution levels in the rhizosphere in a specifically designed extraction procedure for phytoavailability assessment (Feng et al., 2005). This possibility is also investigated by Wang et al. (2003), who proposed a $10mMl^{-1}$ LMWOA extraction solution, consisting of acetic acid, formic acid, citric acid and malic acid at a ratio of 2:2:1:1 to estimate metal phytoavailability. These authors observed better correlations between plant uptake and extractable levels for the $10mMl^{-1}$ LMWOA extractant in comparison to $0.11Ml^{-1}$ acetic acid. The use of 1M ammonium acetate as extractant (L:S 20:1, shaken for 1h) has been adopted in French legislation French standard NF X 31-108 (AFNOR, 1996) for assessment of exchangeable bases.

Besides their success in specific case studies, there is still not any singly accepted method to study the metal availability in the soils. Moreover, most of these extraction methods involve the study at equilibrium conditions and therefore can predict only the final states while the soil biological systems are never at equilibrium.

1.5.b. Sequential Extraction Procedures

The characterization of trace metal species present in the solid phases of soil or sediments has been the focus of much research during the past 40 years. Within the soil solid phase trace metals exist in different forms; some solid forms are relatively stable (for example bound to carbonates or Fe and Mn oxides) while other forms (for example water-soluble and easily exchangeable) can significantly increase the risk of leaching and plant uptake. As a result, information about the forms of trace metals has been considered potentially valuable for predicting their availability to biological targets, their movement in the soil profile and transformation between different forms in soils (McBride et al., 1981; Soon and Bates, 1982; Hooda and Alloway, 1994a).

Sequential extraction procedures (SEPs) involve the extractions by using different extractants considered to react with specific soil compartments and extract metals from these compartments. Among the numerous SEPs, most widely used are those proposed by Tessier et al. (1979) and Stover et al. (1976) or certain variants. The sequential extraction procedure proposed by Tessier et al. (1979) fractionates trace metals into the following forms: (a) exchangeable, (b) bound to carbonates, (c) bound to Fe and Mn oxides, bound to organic matter, and (e) residual, that is bound to primary mineral structure. SEPs have been designed to study the trace metal speciation in the soils but the metal fractions studied through SEPs are used to predict the trace metal availability by correlating these fractions with the uptake of metals to plant and other ecological receptors (Sims and Kline, 1991; Cajuste et al., 2000). All such schemes, however, are fraught with a number of problems, for example: lack of Specificity of the extractants as a result overlapping of the fractions (Sheppard and Stephenson, 1997), incomplete dissolution or solubilization of multiple phases in one extract (Miller et al., 1986), extractants changing pH during the extraction, which may result in re-adsorption (Bermond, 2001) or redistribution as well as re-adsorption (Bermond and Yousfi, 1997) and lack of a uniform sample pretreatment method (Gleyzes et al., 2002).

The extent of such problems or at least the possibility of their occurrence generally increases with the number of stages in the sequential extraction procedure. These fractionation procedures, however, are hugely labour-intensive. Another added problem is that plethora of procedures exist for these so-called functionally defined schemes, making difficult to compare the findings or the validity of the procedures used (Quevauviller et al., 1993).

1.5.c. Isotopic Dilution Techniques

Initially, isotopic dilution (ID) techniques were used to study the reactions, fixation and residual availability of fertilizer nutrients, particularly phosphorus and potassium, using radioisotopes of P and K. Subsequently these techniques have been used for trace metals, for example Ni, Zn, Cd, Cd and As (Tiller et al., 1972; Hamon et al., 1997; Hutchinson et al., 2000; Donner et al., 2010). Isotopic dilution techniques using radioisotopes of trace metals allow examination of their partitioning into isotopically and non isotopically exchangeable pools, which are described as labile and non labile, respectively. Trace metals in the non labile pool are considered as 'fixed', while those in the labile pool are potentially available for plant uptake or soil reactions/processes such as precipitation, co-precipitation with secondary soil minerals or attenuation/fixation. As any other form or fraction of trace metals in soils, exchange between labile and non labile pools occurs. In other words, non labile pool can be mobilized, for example following a change in soil pH and/or Eh and reaction with plant root exudates organic compounds and their metabolites (Hamon et al., 1997; Young et al., 2000).

The partitioning of trace metals using isotopic dilution methods can be seen as similar to SEPs but there are two major differences: (a) instead of fractionating trace metals in several pools as in SEPs (for example exchangeable, bound to carbonates, organic matter and metal oxides), ID fractionates them into two distinct pools, labile and non labile; and (b) being less intrusive and more or less direct measurements the ID techniques do not suffer from procedural artifacts as is common in SEPs. Also, these techniques have similarities with chemical extraction-based soil tests used for assessing bioavailability of trace metals in soils, as they are intended to provide an estimate of their labile pool, although some strong chemical reagents (for example EDTA) are known to interact with non labile trace metal fractions as well. So an isotopic dilution approach in essence is a combined solution and solid-phase fractionation technique. The labile pool is the isotopically exchangeable trace metals in the solution and those associated with the solid phase, which like chemical extraction-based tests reflects their potential bioavailability. The isotopic dilution technique gives an idea of labile and non labile pools of metals but the measurements involve equilibrated soil solutions and the dynamic aspect of the bioavailability is ignored.

1.5.d. Diffusive Gradient in Thin Films (DGT)

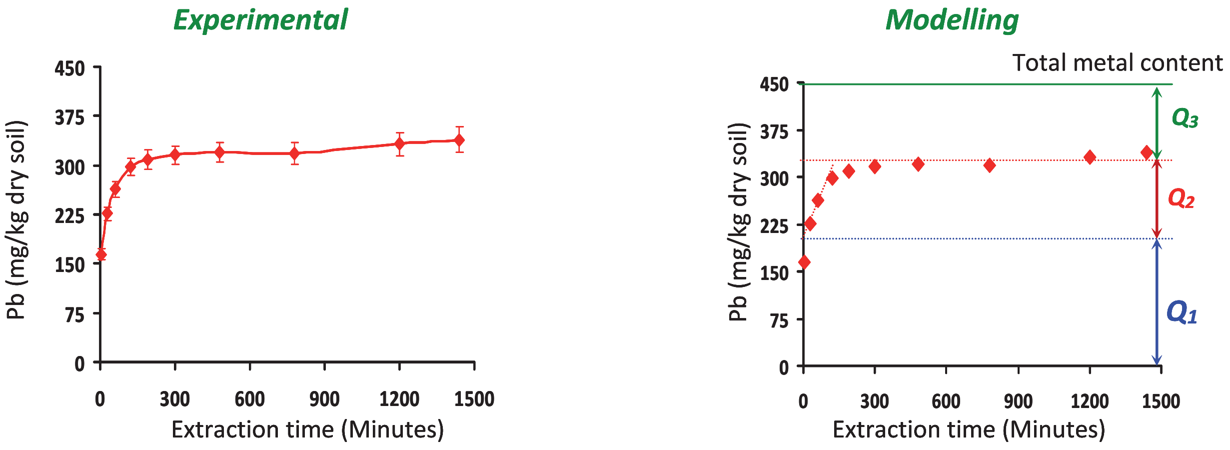
It has been suggested that measurement of concentrations of trace metals present in soil solution can be a more rational and sensitive approach for evaluating their bioavailability compared with the chemical extraction-based procedures (McGrath et al., 1999). The bioavailability of trace metals is related to their flux into the plant roots or cell membrane of soil-dwelling (non-soil-ingesting) organisms, which is dependent on both their concentration in soil solution and their transport rate through the soil. Where there is active removal of trace metals, the local soil solution equilibrium concentration is further dependent on the resupply from the solid phase. Quantitative interpretation of this flux of trace metals from the solid phase is central to considerations of soil testing for bioavailability purposes, yet it is not assessed by conventional soil testing procedures. An in situ technique capable of quantitatively measuring labile metal species in waters was developed (Davison and Zhang, 1994). Further work on this technique, known as DGT, has shown its application for the measurement of labile metal species in soils (Zhang et al., 1998; Hooda et al., 1999).

The application of DGT for the purpose of assessing metal bioavailability has been developed on the premise that in conventional methods of testing soil solution (a) metal speciation may change during sampling and extraction, and (b) the kinetics of metal re-supply from solid phase to solution are not considered. The theoretical background to DGT as well as the protocols of its application in soil and the interpretation of the measurements has been provided in literature in detail (Zhang et al., 1998; Hooda et al., 1999; Degryse et al., 2009). A DGT unit with ion exchange resin embedded in a hydrogel and deployed in a soil mixture is supposed to mimic the real situation of plant uptake, similar to plant roots. It locally lowers metal concentrations in the soil solution. This depletion induces metal resupply from labile species in the solution and in the kinetically labile metal pool in the soil solid phase. The free ions in the solid phase represent the real potentially (bio) available concentration of metals (Cattani et al., 2006). The DGT technique mimics this buffer capacity of the soil, diffusive transport effects, and chemical forms of metals. Diffusive gradients in thin films has been used to determine an effective concentration (C_E), that expresses the equivalent soil solution concentration experienced by the DGT device as it is supplied from both solid phase and solution (Zhang et al., 1998). The C_E concentration represents the concentration of metal species potentially available to plant roots and other soil organisms. Depletion of metals in soil solution, as a consequence of plant uptake or leaching, results in the re-supply from the solid phase. DGT can provide information about this solid phase to solution re-supply of metals. DGT devices with different diffusive gel thickness can be deployed to create various diffusion gradients and hence different rates of demand for metal ions (Hooda et al., 1999).

Various studies have reported the efficiency of DGT measured Effective concentrations of metals though its correlations with the concentrations of metals found in plants and soil organisms (Koster et al., 2005; Cattani et al., 2006; Lehto et al., 2006; Zhao et al., 2006). But most of these studies have been conducted on previously well homogenized soils and did not provide a real picture of field conditions. Another requirement of DGT application is the maintenance of high soil moisture because the DGT response is dependent on the soil moisture.

1.5.e. Kinetic extractions

In most of these methods seen before, the metal concentration is measured at equilibrium whereas natural systems are generally subject to changing conditions and are practically never at the equilibrium. Indeed, for these dynamic systems, the metal availability may be believed to be controlled by kinetic factors. Apart from the metal extractions using single extractants at equilibrium, methodologies involving trace metal kinetic fractionation in soils and sediments are proposed (Bermond et al., 1998; Manouchehri et al., 2006; Labanowski et al., 2008; Santos et al., 2010). The kinetic approach is based on the study of the desorption fluxes of the metals from the soils caused by the action of one specific added chemical reagent i.e. EDTA. This time-based fractionation highlights the differences in the lability of complexes with similar stability constants. Indeed, mobilization and bioavailability of metal ions depend not only on their concentrations in soil but also on the soil capacity to release those metal ions. These kinetic approaches involve the study of extraction rate of metal ions from soils (Bermond et al., 1998; Fangueiro et al., 2005; Labanowski et al., 2008; Santos et al., 2010). The kinetic extraction data can then be modelled to define various metal pools based on their lability in the soils. Among various kinetic models the two first order reaction models was proposed to fit efficiently Cu, Cd and Pb data obtained in a wide range of concentrations in sediments (Fangueiro et al., 2005). This model has then been applied successfully for the kinetic extraction data in soils (Labanowski et al., 2008; Santos et al., 2010). The two reactions model can distinguishes two types of soil compartments from where the metals can be extracted with the reagent: one from which the metals are quickly extracted, and the other from which extraction is slower (Figure 1.5). However, it must be considered that the two compartments from which the metals are extracted are operationally defined, in terms of the kinetics and do not correspond to classical constituents such as organic matter or iron oxides.



Q_1 = labile fraction ; Q_2 = slowly labile fraction ; Q_3= non extractable metal fraction

Figure 1.5: *Time metal extraction experimental curve (Right) and a curve obtained by data modeling (left) by two first order reaction model***.**

These operationally defined labile and slowly labile pools are considered to be the potentially bioavailable metal pools in the soils. They represent the metal pools based on the strength of their association to the soil compartments with labile pool being easily extractable can be considered to be bioavailable at shorter terms and the slowly labile pool being less easily extractable will be the metal pool bioavailable at longer terms. Along with the quantification of these metals pools, the kinetic extractions give us also the values of the extractions rate constants of these metal pools. So at the place of one value of available metal pools in the soils in case of single extraction methods, the kinetic extraction approach further defines the available metals into labile and slowly labile pools and their extractions rates. Thus it gives us more insights into the trace metal availability by showing the possibility for example that where the single value given by metal extraction at equilibrium seems to remain unchanged, the proportion of labile and slowly labile pools of the metal may have changed.

Most of the environmental studies involve a single method of studying the trace metal availability and dynamics into the soils. Soils being a complex solid medium, a lot of mechanisms are occurring simultaneously and at different scales changing from a single chemical compound to bulk soil levels. But employing a single method to highlight the changes in trace metal dynamics in soils remains unable to assess these changes at different scales and their interaction with each other. A better strategy can be to couple more than one of these methods to assess the trace metal availability in the soils.

1.6. Potential of Trametes versicolor as a biomarker of trace metal availability assessment

Since fungi represent one of the largest biomass in terrestrial ecosystems, the response of their extracellular enzymatic systems to metals offers promising perspectives for ecotoxicological assessment, providing the mechanisms of metal action are well known (Lebrun et al., 2010). In particular the potential of *Trametes versicolor* has been studied in many studies because of its morphological and physiological response to different contaminants (Lebourg et al., 1998; Mougin et al., 2003). By laboratory inoculations the use of *Trametes versicolor* has been studied successfully in bioassays ecotoxicity of various organic as well as inorganic contaminants (Mougin et al., 2002; Kahkonen et al., 2008; Lebrun et al., 2010). At the morphological level, the changes in permeabilization and pigment composition of the fungi have been observed after metal exposure. Similarly the reproduction of fungi (maturation of spores, germination etc) as well as the fungal mycelium growth have been reported to be affected by metals (Baldrian, 2003). Saprophytic fungi, considered the predominant degraders of lignin, have been particularly studied for their ligninolytic oxidoreductases, such as laccases or peroxidases (Bollag and Leonowicz, 1984; Orth et al., 1991).

Environmental interest in this fungal set of enzymes has increased because of their ability to degrade a variety of organic xenobiotics (Mougin et al., 2003). Although fungi have mechanisms for metal tolerance (Baldrian, 2003), the activity of their extracellular enzymes has been shown to be modulated during the metal exposures in liquid cultures. Copper increases the extracellular laccase in different filamentous fungi (Crowe and Olsson, 2001; Baldrian et al., 2005; Levin et al., 2010). This oxidase response to metals has been explained at transcriptional level. It is also known that Cu induces the laccase transcription in *Trametes versicolor*. Collins and Dobson, (1995) showed that the stimulation of laccase activity by Cu corresponds to an increase in its mRNA in *Trametes versicolor*. It has also been observed that laccase can be induced as different glycosylated isoforms after exposure to 2,5-xylidine, a well known inducer of fungal laccases (Bertrand et al., 2002; Kollmann et al., 2005). Furthermore, fungi secrete isoenzymes of oxidases, i.e. enzymes catalyzing the same reactions but exhibiting different sequences in amino acids (Cassland and Jönsson, 1999; Necochea et al., 2005). Thus, differential expressions of enzymes or isoenzymes under a metal stress could provide fungal biomarkers of exposure. Moreover a recent study has explored the potential of *Trametes versicolor* to respond differently to different metal speciation conditions (Lebrun et al., 2011) which further endorses the potentiality of laboratory cultures of *Trametes versicolor* to be used as biomarkers in trace metal availability studies.

Chapter 2

Material and methods

2.1 Choice of sites and situations

2.1.a Metaleurop

The study was carried out on the contaminated site belonging to Courcelles-lès-Lens town in the region Nord-Pas de Calais of France. This site is situated near a large metallurgical complex called Metaleurop situated in Noyelles-Godault (Pas de Calais) in the region which has been working for the whole 20th century and stopped its working in 2003 (Figure 2.1). Hundreds of hectares of land around this complex are severely contaminated by the atmospheric fallout of trace metals because of the activity of this metallurgical complex (Sterckeman et al., 2006; Douay et al., 2008). In 2002, the annual emissions of trace metals from the complex were around 16.9t of Pb, 32t of Zn and 977kg of Cd (DRIRE, 2003). The major pollution in the area is of Cd, Pb and Zn but to a lesser extent As, Bi, Cu, Hg, In, Ni, Sb, Se, Sn and Tl contamination are also found (Sterckeman et al., 2002).

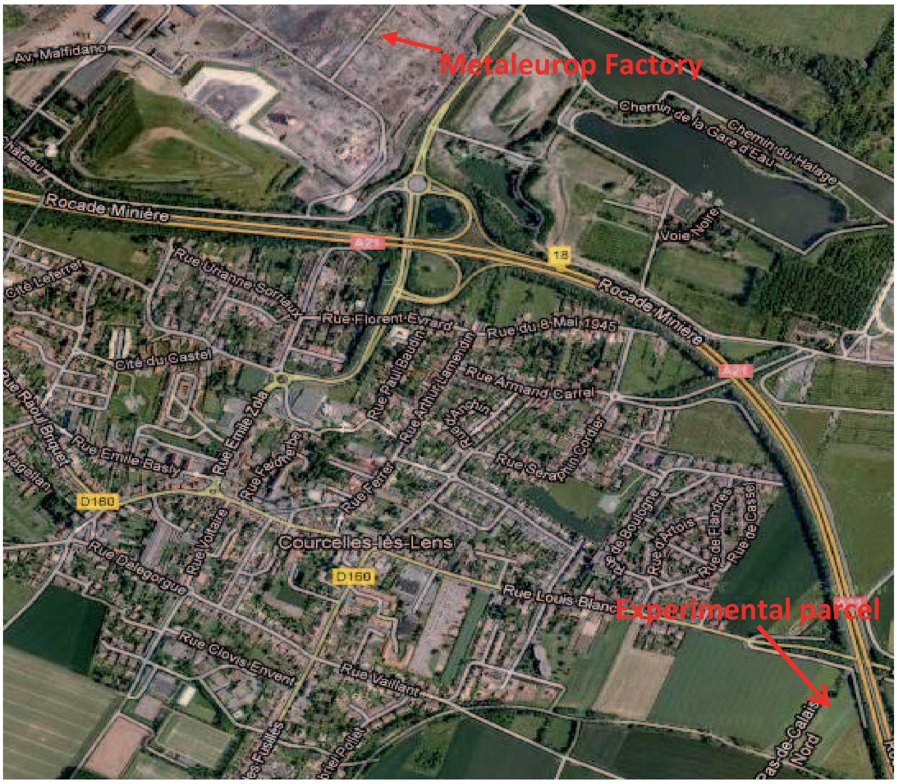


Figure 2.1: *Map of experimental site Metaleurop.*

2.1.b Pierrelaye

The other site selected for this study is the plain of Pierrelaye-Bessancourt (Figure 2.2). This plain is a part of the large irrigation perimeter located approximately 6km from the Seine Aval wastewater treatment, the plant treating most of the Paris city and close suburbs waste water, estimated at about 300 x 10^6 m^3/day at the end of 1990s (Tamtam et al., 2010). Between 1899 and 2000, the soils of this plain were irrigated with untreated waste water from Paris and its suburbs. This was done not only considering the high infiltration rates of these coarse textured sandy soils but also because these irrigation practices enhanced agricultural use of these soils for market gardening thereby helping to fulfill the food requirements of Paris (Baize et al., 2002). Irrigation of these soils by untreated waste water for a century has caused severe anthropogenic perturbation at this site by keeping the soils immerged in waste water for longer periods many times and through accumulation of several organic and inorganic pollutants notably trace metals along with the increase in soil organic matter (Dère et al., 2006; Lamy et al., 2006; van Oort et al., 2008) which may cause severe environmental concerns. After the detection of strong accumulation of trace metals in the crop plants grown on these soils, food crop production was banned in this site in 2000.

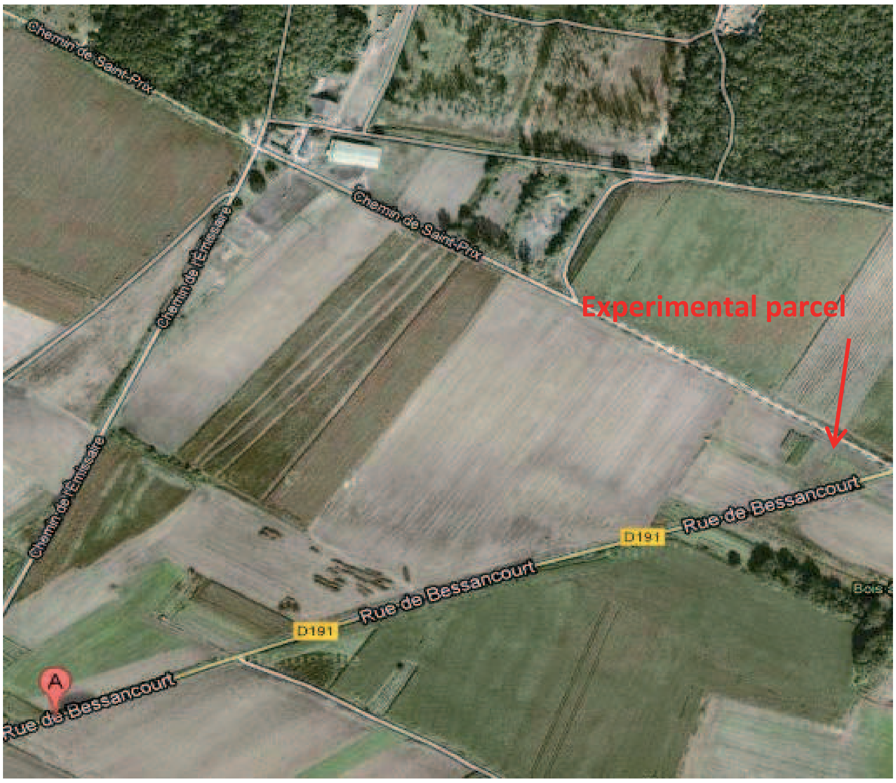


Figure 2.2: *Map of experimental site Pierrelaye.*

The application of waste water at the sandy soils of Pierrelaye have caused not only many fold increase in the total trace metal contents of the soils compared to the geochemical background values of the metal contents of the soils but also the major elements and organic matter have been added to the soil (Baize et al., 2002; Lamy et al., 2006). But the unequal distribution of waste water depending upon the topographic conditions of the soils, the distance of soils from the discharge point of waste water have caused severe spatial heterogeneity in the distribution of metals and organic matter in the soils of this plain. But as the trace metals and organic matter have been brought together to the soils so despite of this heterogeneity in the waste water distribution in this area the trace metals and organic carbon contents of the soils are correlated in these soils (Bourennane et al., 2006; van Oort, 2011).

2.1.c Annual crop vs miscanthus

In order to study the effect of land use of polluted soils on the trace metal availability, we selected the case of soils under annual crop system versus perennial crop system. For this at both sites we sampled the soils of the land parcels where one part of the field continue to be used for growing annual crops while on the other part of the field, a heavy biomass perennial crop, miscanthus is cultivated since 2007 (3 years before sampling for our study). Miscanthus cultivar grown at both sites was Miscanthus *giganteus.* At Metaleurop site, the annual crops grown since 2007 were wheat, broad beans and sugar beet. At Pierrelaye site, the part of parcel under annual crop has been cultivated with oat, sorghum and maize since 2007. Two non-contaminated soils corresponding to each site were also sampled for using as reference in particular for soil incubation experiments. For Pierrelaye site this non contaminated soil was with pedo-geochemical background similar to the soil under annual crop and miscanthus but was situated out of the zone of waste water irrigation. The soils in the wide area around Metaleurop site are all contaminated because of the wide dispersal of pollution through wind so it was difficult to find a non contaminated agricultural soil. For this we sampled a soil of similar texture from the field experimental site belonging to INRA Versailles-Grignon.

In order to get representative samples we sampled soils of the surface horizons (0-30cm) from multiple points in each parcel and prepared a composite soil sample for each parcel under annual and perennial crop at both sites. After soil sampling, the soils were sorted for the larger roots, plant debris and soil macro fauna and were sieved through a stainless sieve of 5mm. A part of the samples was kept in refrigerator at 4°C. While the other part of bulk soil samples was air dried at room temperature and sieved at 2mm before storage in plastic bags for further studies. For each further analysis, soil samples were mixed well and quartered to obtain maximum homogeneity in sub-samples.

Table 2.1: *Selected physico-chemical characteristics of the soil samples. Data presented is expressed on soil dry weight basis.*

	Metaleurop			*Pierrelaye*		
Parameter	*Reference*	*Annual crop*	*Miscanthus*	*Reference*	*Annual crop*	*Miscanthus*
Particle size distribution						
Clay (%)	19	17	18.1	8.6	8.4	8
Silt (%)	57	54.3	53	7.5	16.3	12.3
Sand (%)	24	28.7	28.9	84.7	75.3	79.7
Organic matter						
Organic C (g kg $^{-1}$)	9.9	16.9	17.7	9.6	62.2	52.3
Total N (g kg $^{-1}$)	1	1.3	1.3	0.8	2.4	2.1
C/N	9.9	12.9	13.8	12.0	26.2	24.9
pH	7.4	7.6	7.4	8.5	7.4	7.6
CEC (cmol $^{+}$ kg $^{-1}$)	11.5	13.3	13.8	5.6	10.1	8.6
P (g kg $^{-1}$)	nd	1.6	1.5	12.5	4.9	4.3
S (g kg $^{-1}$)	nd	243.5	257.2	182.5	709.3	635.5
$CaCO_3$ (g kg $^{-1}$)	traces	< 1	2.1	24.9	37.0	45.4
Ca (g kg $^{-1}$)	nd	5.6	6.1	11.3	19.6	21.3
Al (g kg $^{-1}$)	nd	36.6	38.0	10.8	19.9	17.8
Fe (g kg $^{-1}$)	nd	21.2	22.6	10.0	18.3	15.8
Trace elements						
Cu (mg kg $^{-1}$)	nd	18.9	20.4	11.4	245.0	148.5
Pb (mg kg $^{-1}$)	45	201.6	216.8	20.9	455.0	400.0
Zn (mg kg $^{-1}$)	54	318.8	338.4	34.6	711.6	530.2
Cd (mg kg $^{-1}$)	0.2	4.4	4.2	0.3	3.3	2.2

nd = not determined

2.2 Soil physico-chemical characteristics

Main physico-chemical characteristics of the soil samples are presented in table 2.1. The soils of Metaleurop are silt loam Cambisols according to USDA particle sizes whereas the soils of Pierrelaye are sandy Luvisols. Contaminated samples of Pierrelaye were more enriched in organic carbon and trace metals than the contaminated samples of Metaleurop site except Cd which was more in the samples of Metaleurop. Organic carbon contents of the contaminated samples of Metaleurop site were two times more than the reference sample. The trace metal contents were also high in these contaminated samples than reference soil sample. For Pierrelaye site organic carbon contents of the contaminated samples were 5-6 times more than that of the reference sample and similarly their trace metal contents were many fold high than the reference soil. C/N ratios of the contaminated samples of Pierrelaye were more double to all other soils. pH of all the soil samples was slightly basic and was similar for all soil except of the non contaminated sample of Pierrelaye. CEC of Metaleurop soil samples was slightly higher than the Pierrelaye samples. Samples of Metaleurop site were non calcareous whereas samples of the Pierrelaye site were calcareous. Both contaminated samples of Metaleurop have similar characteristics like organic carbon, total N, pH, CEC and $CaCO_3$ contents. The annual crop soil sample of Pierrelaye was more contaminated and organic carbon enriched than the soil sample under miscanthus soil linked to the unequal distribution of waste water at the site as reported earlier.

2.3 <u>Choice of methods</u>

2.3.a Soil analysis

Main soil physico-chemical parameters of the soil samples (particle size distribution, CEC $_{(Metson)}$, organic carbon, nitrogen, $CaCO_{3,}$ phosphorus, sulfur, Ca, Al and Fe contents) were determined using standard methods (AFNOR, 1996) by French national soil analysis laboratory, INRA Arras. pH $_{(water)}$ was measured by the method proposed in international norms (ISO, 1994). Total metal contents of the soil samples were measured by using atomic absorption spectrophotometry (AAS), either flame or furnace (Varian SpectrAA 220) depending upon the metal quantification limit of the apparatus and the metal content of the samples. For this 0.5g test portions of soil samples sieved at 200µm were weighed in Teflon containers and acid digestion was carried out by adding HF/HNO_3 (1:3, v:v) followed by microwave heating (CEM MarsX). HF and HNO_3 used were of analytical grade and water of high purity obtained from Millipore system (Water resistivity=18mΩ) was used for making final volumes and dilution when necessary. Quality control was assured by using triplicate samples, running blanks, using standard additions and certified reference materials (SRM-1640; Natural water rich in trace metals, National Institute of standard and Technology, USA and TMDA-54.4; Trace elements standards made in Lake Ontario water, Environment Canada). Measurements of metal concentrations were validated only when the measured concentrations of elements of the reference materials were close (± 10%) to the certified values.

2.3.b Metal extraction at equilibrium

DTPA and NH_4NO_3 extractable metal contents of the soils were measured by the French national standard soil laboratory INRA Arras by following the procedures described in French norms. Metals extraction with DTPA was performed by this laboratory according to the procedure described by French norm NF 31-121 (AFNOR, 1993) by using a buffered solution of DTPA and $CaCl_2$ with a soil solution ratio of 1:2 and time of shaking of 2hours. Metal extraction with NH_4NO_3 was performed by following the procedure described in norm ISO 19730 (ISO, 2008) by using 1M solution of ammonium nitrate with a soil to solution ratio of 1:5 and time of shaking of 2hours. EDTA extractable metal contents were determined at our laboratory. Different assays were performed at the laboratory to achieve optimal extraction conditions (data not presented). Finally an EDTA concentration of 0.05M, pH 7.5 (near to soil pH) and a soil: solution ratio of 1:20 was used for the trace metal extractions. The schematic diagram of procedure of metal extraction is given in figure 2.3.

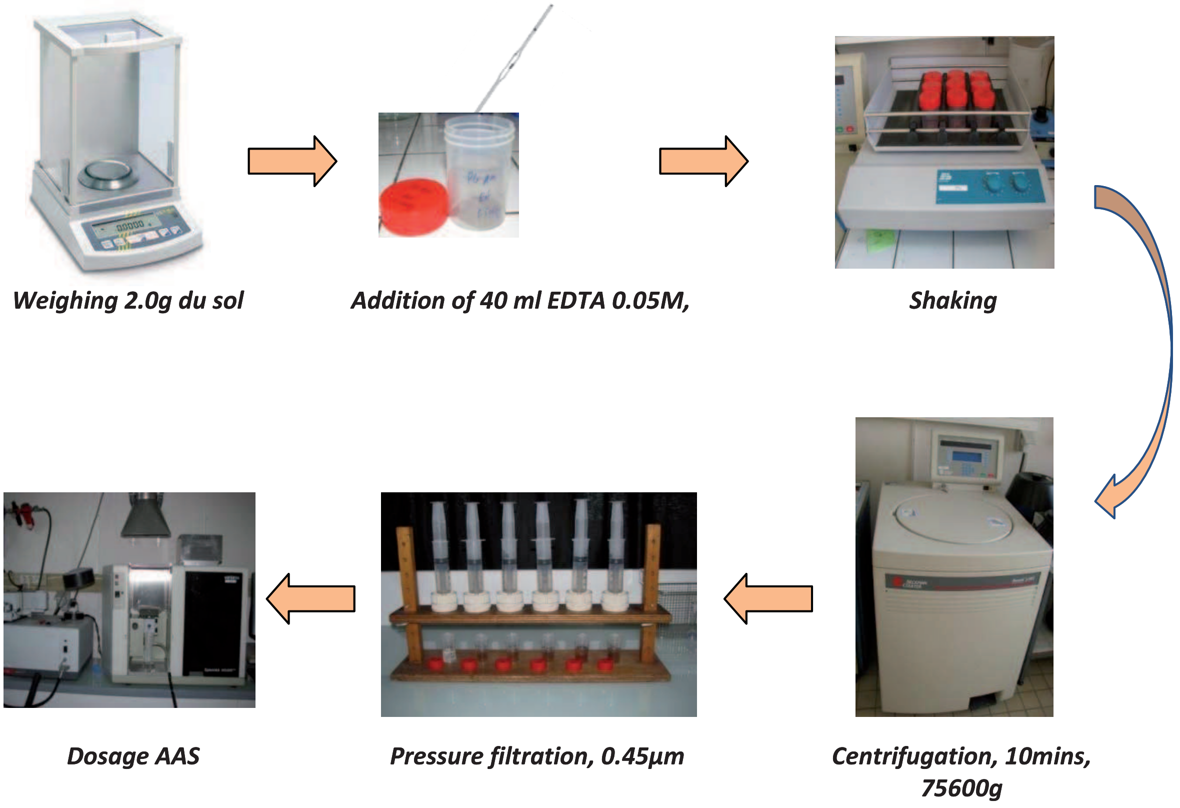


Figure 2.3: *Schematic diagram of various steps of trace metal extraction.*

Test portions of 2g soil sieved at 200µm were taken in polyethylene bottles and were shaken with 40ml of EDTA solution at 250 rpm on a mechanical shaker (Edmund Bülher) at ambient temperature for 24 hours. Immediately after shaking, suspensions were centrifuged at 75600g for 5-8 min and were then filtered on 0.45µm cellulose membranes and stored at 4°C before measurement of extracted metals. Trace metal contents of these solutions were then determined by following the procedure as described in section soil analysis.

2.3.c Kinetic extraction of metals

Kinetic extractions (at 10 time lags between 5min and 24h) were also carried out by using EDTA. Test portions of 2g soil sieved at 200µm were taken in polyethylene bottles and were shaken with 40ml of EDTA solution at 250 rpm on a mechanical shaker (Edmund Bülher) at ambient temperature for different periods (5, 30 min; 1, 2, 3, 5, 8, 12, 20 and 24h) and then same procedure was followed as described for extraction at equilibrium except for 5mins for which the centrifugation step was omitted.

As suggested by several studies (Bermond et al., 1998; Fangueiro et al., 2005; Labanowski et al., 2008) kinetic extraction data were modeled by using a "two first order reaction model" (Eq. (1)) to quantify two metal pools, labile (easily extractable) and slowly labile (less easily extractable) metal fractions.

$$Q = Q_1\ (1\text{-}\exp^{-K_1 t}) + Q_2\ (1\text{-}\exp^{-K_2 t}) \qquad (1)$$

Where Q_1 = labile metal fraction, Q_2 = slowly labile metal fraction, K_1 = extraction rate constant of the labile metal fraction and K_2 = Extraction rate constant of the slowly labile metal fraction.

2.3.d Physical and granulo-densimetric size fractionation of the soils

Physical size fractionation of the soil samples was carried out by following the methods described by (Balesdent et al., 1991) and according to recent French norm (AFNOR, 2007) after some modifications. The schematic diagram of fractionation procedure followed is presented in figure 2.4. Fractionation was performed in 3 replicates for all soil samples. For this, 50 g air dried soil samples (sieved at 2mm) were taken in polyethylene bottles. 125 ml of de-ionized water and 10 glass beads of 5mm diameter were added to the soil samples. Samples were shaken on a rotator shaker for 16h. The different sand and silt size fractions

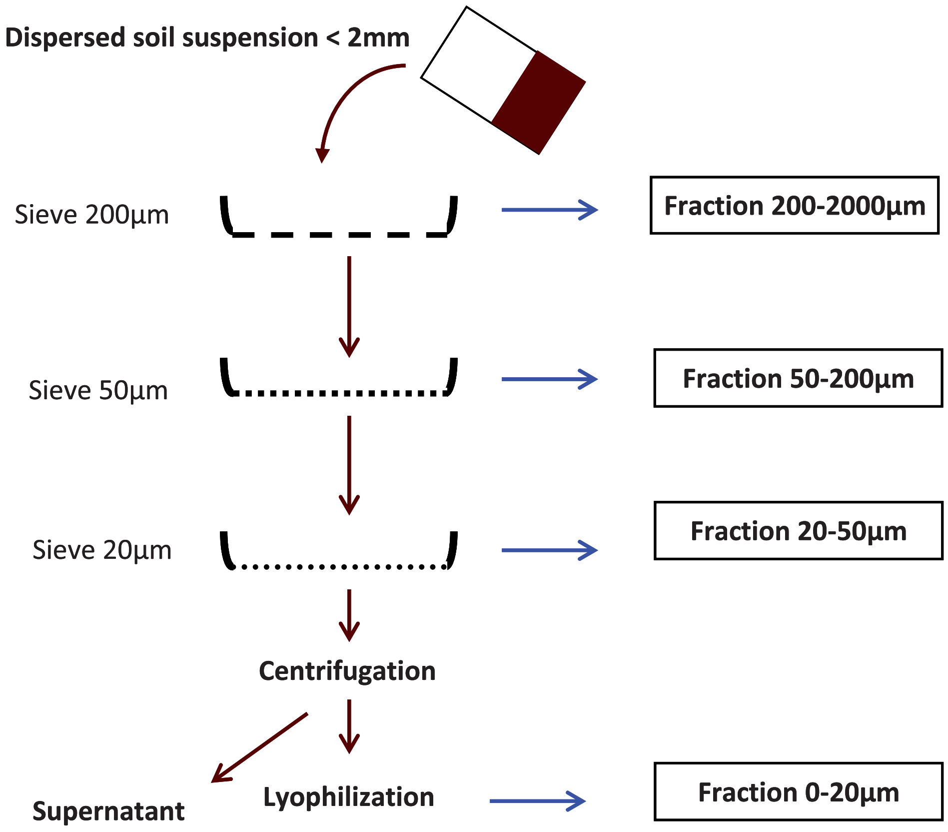


Figure 2.4: *Schematic diagram of various steps of physical fractionation of soils.*

were separated by performing wet sieving of the soil suspension using stainless steel sieves of 200µm, 50µm and 20µm mesh size. Thus the particle size fractions obtained were 200-2000µm, 50-200µm, 20-50µm and <20µm. The first three fractions i.e. 200-2000µm, 50-200µm and 20-50µm were obtained simply by wet sieving while the <20µm fraction was separated from the soil suspension by centrifugation and was then lyophilized after freezing. The other fractions were dried at 50°C and stocked in air tight polyethylene containers. Cu, Pb, Zn and Cd contents of these fractions were then measured by using the method described in section 2.3.a. For the study of the role of the particulate organic matter fractions in trace metal availability, the particulate organic matter of the soils was separated. For this in addition to the above mentioned procedure of fractionation, the two coarser fractions i.e. 200-2000µm, 50-200µm were further divided into mineral and organic fractions. Particulate organic matter fractions (free vegetative debris) being lighter in weight float in water while the mineral fractions being heavier in weight settle down in water. So these fractions could be separated using floatation in water.

2.3.e Soil incubation

a) Preparation and maintenance of soil microcosms in the laboratory

After sampling the soils to be used for incubation were kept in refrigerator at 4°C before the start of soil incubation. An equivalent of 400g dry soil was taken in airtight glass jars of 1L and a measured quantity of distilled water was added to the soils to obtain a soil humidity equivalent to 60-70% of their water holding capacity. The soils within the glass jars were then weighed to obtain the initial weight of the microcosms. The soils in the airtight jars were incubated in the incubator in dark at 28°C for a period of 200days. A series of 5 jars for each soil was incubated where three jars were dedicated for capturing the CO_2 produced from soil during incubation to measure the mineralization of soil organic matter during incubation. To avoid anaerobic environment within the jars during incubation, all jars were opened periodically to allow the exchange of air. At the start the jars were opened at day 1, 3 and 7 days of incubation, then increasing this period to once in a week until day 70 and afterwards the jars were opened every ten days until end of the experiment. Each time when the jars were opened, they were weighed to measure water lost through evaporation during incubation and the corresponding amount of water was added to maintain the soil humidity till the end of incubation. At the same time the vials of NaOH were also replaced with new vials and the old vials were kept in airtight environment before the dosage of CO_2 captured (Figure 2.5).

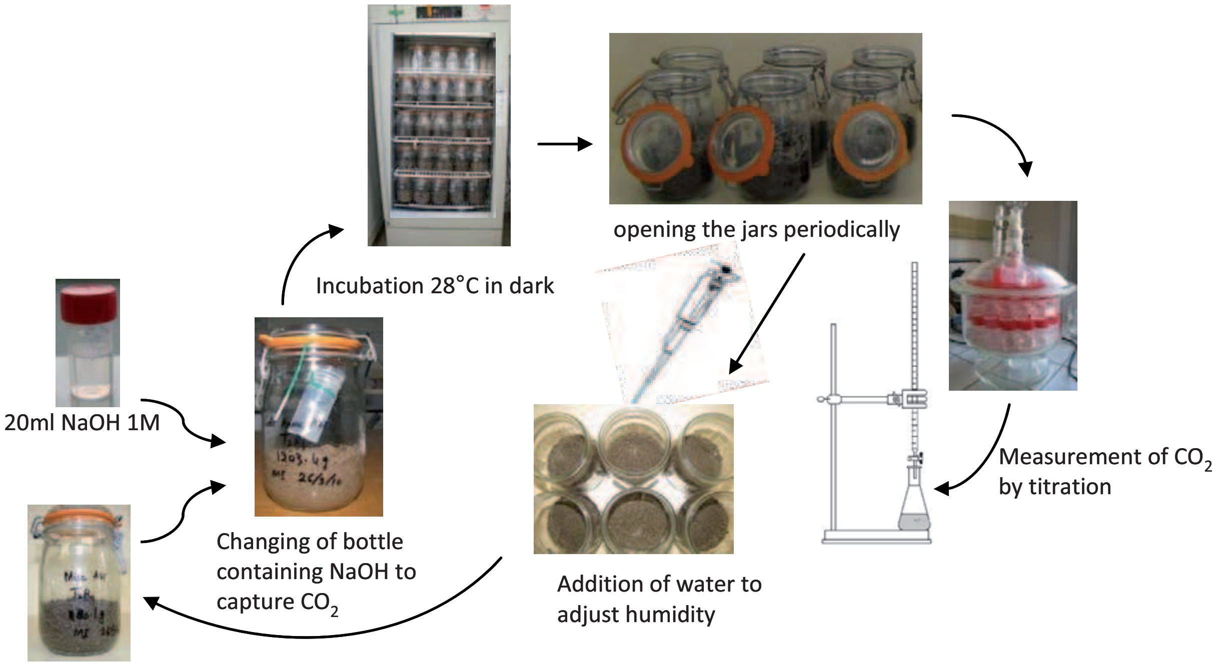


Figure 2.5: *Schematic diagram of various steps of CO_2 measurement during soil incubation and maintenance of soil microcosms.*

b) *Measurement of soil organic matter mineralization*

Mineralization of the organic matter of the soils during incubation in the laboratory was measured by capturing the CO_2 produced. The head space CO_2 produced from organic matter mineralization was captured by keeping 10ml of 1M NaOH solutions in plastic vials which were hanged in the incubation jars. A series of three jars of each soil was incubated to measure organic matter mineralization. Along with this series of jars with soils, a series of three empty jars without soil but with vials of NaOH was also incubated to be served as control. The amount of captured CO_2 was measured by titration of NaOH with 1M HCl in the presence of $BaCl_2$ in excess and Thymolphthalein was used as acid-base indicator. The mineralization of soil organic matter was calculated by the method described by Tiessen and Moir (1993) as a difference between CO_2 –C produced in the jars with the soils and that captured in the control jars and was expressed as the percentage of initial total organic carbon of the soils.

c) *Measurement of water soluble organic carbon*

Water soluble organic carbon from the soils was extracted by using a protocol developed in the laboratory. The schematic diagram of the procedure followed is shown in figure 2.6. An aliquot of 5g of air dried soil sample sieved through 5mm mesh size sieve was taken in polypropylene tubes of 40ml. 25ml of de-ionized water were added and the tubes were closed firmly. The samples were then shaken for 30 minutes at 250rpm at a mechanical shaker (Edmund Bülher). The samples were then centrifuged for 10 minutes at 75 600g in a centrifuge machine (BECKMAN COULTER Avanti centrifuge J-30I). The supernatants were then filtered (under pressure) through already washed 0.45µm nitrocellulose filters (Millipore) to separate the dissolved organic carbon from the particulate organic carbon. The extractions were performed in triplicate. Quality control was assured by running the controls (samples without soil but with de-ionized water). A part of the filtered supernatants was used to measure the contents of extracted organic carbon while a small aliquot of the same supernatants was used to measure the SUVA i.e. the Specific Ultra Violet Absorption. The extracted water soluble organic carbon contents of the soils were measured immediately after extraction by using a total organic carbon analyzer (SCHIMADZU TOC-V).

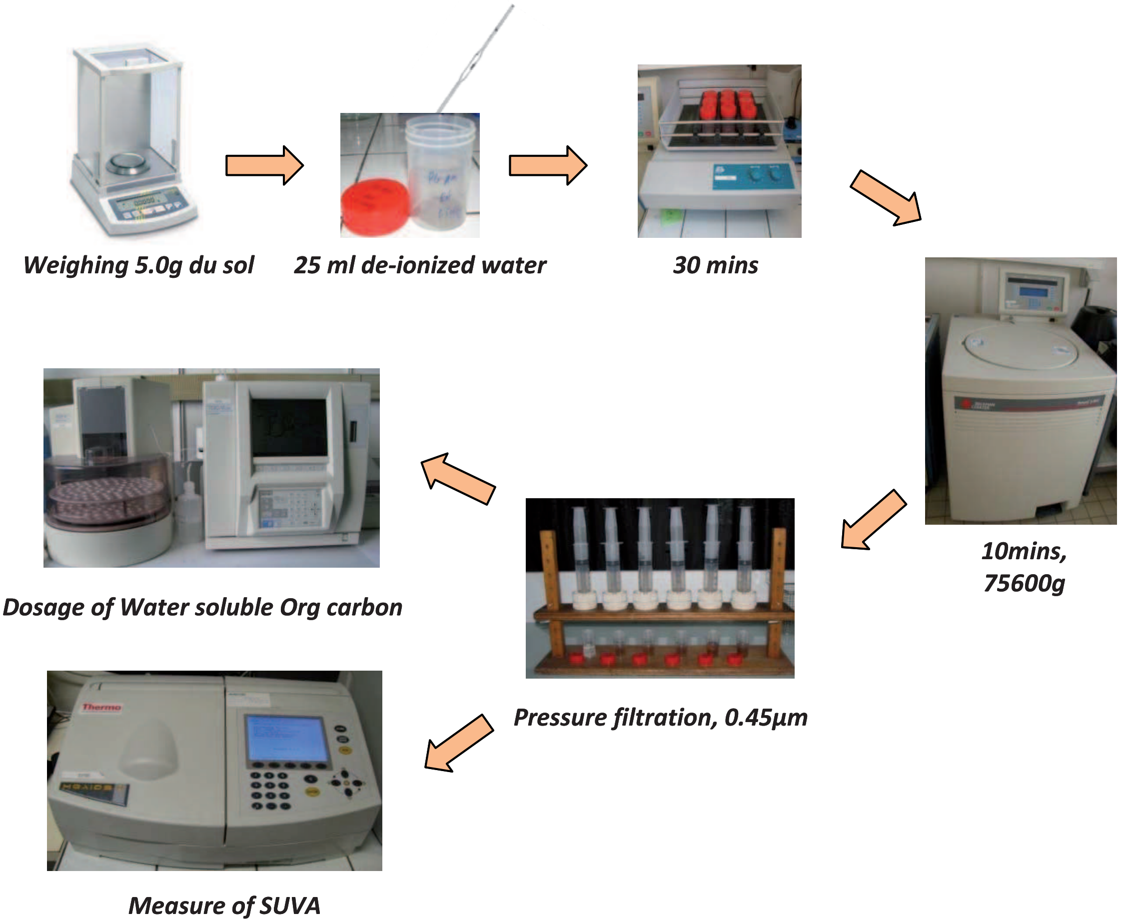


Figure 2.6: *Schematic diagram of various steps of soluble organic carbon extraction.*

d) Specific UV-absorbance (SUVA) of water soluble organic carbon of the soils

SUVA (UV-absorption A_{254nm} (cm)/g C L^{-1}) a simple index of the nature of dissolved organic matter, is defined as the UV absorbance of a given sample at 254nm divided by the dissolved organic carbon concentration of the sample. This test is suggested to be a simple test for the measure of the nature of dissolved soil organic matter in terms of the aromaticity of its molecules when no complicated methods like ^{13}C NMR could be used (Weishaar et al., 2003). In this study a small aliquot of the filtered supernatants obtained for measure of water soluble organic carbon as described above, was used to measure the UV absorbance of the samples at a fixed wavelength of 254nm in a quartz cell of 1cm diameter. A spectrophotometer (Hitachi U-2000) was used for absorption measurements. SUVA of each sample was then calculated by dividing this absorbance with its concentration of total organic carbon.

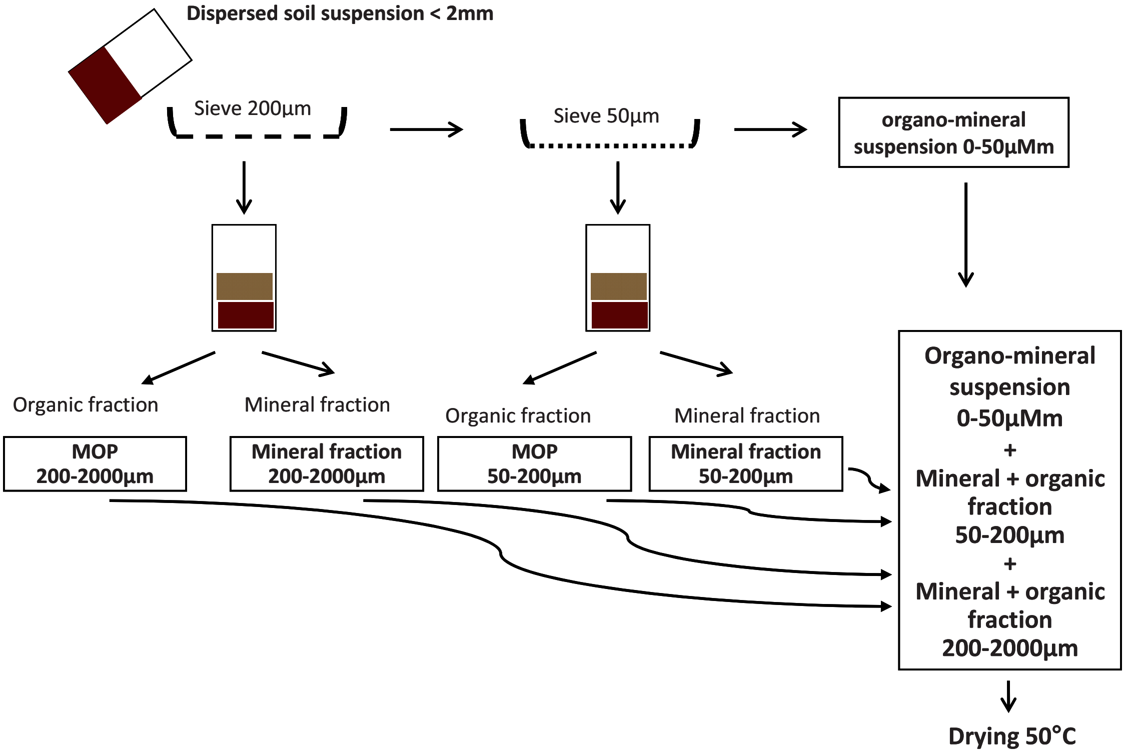


Figure 2.7a: *Schematic diagram of various steps followed to prepare soil samples with particulate organic matter.*

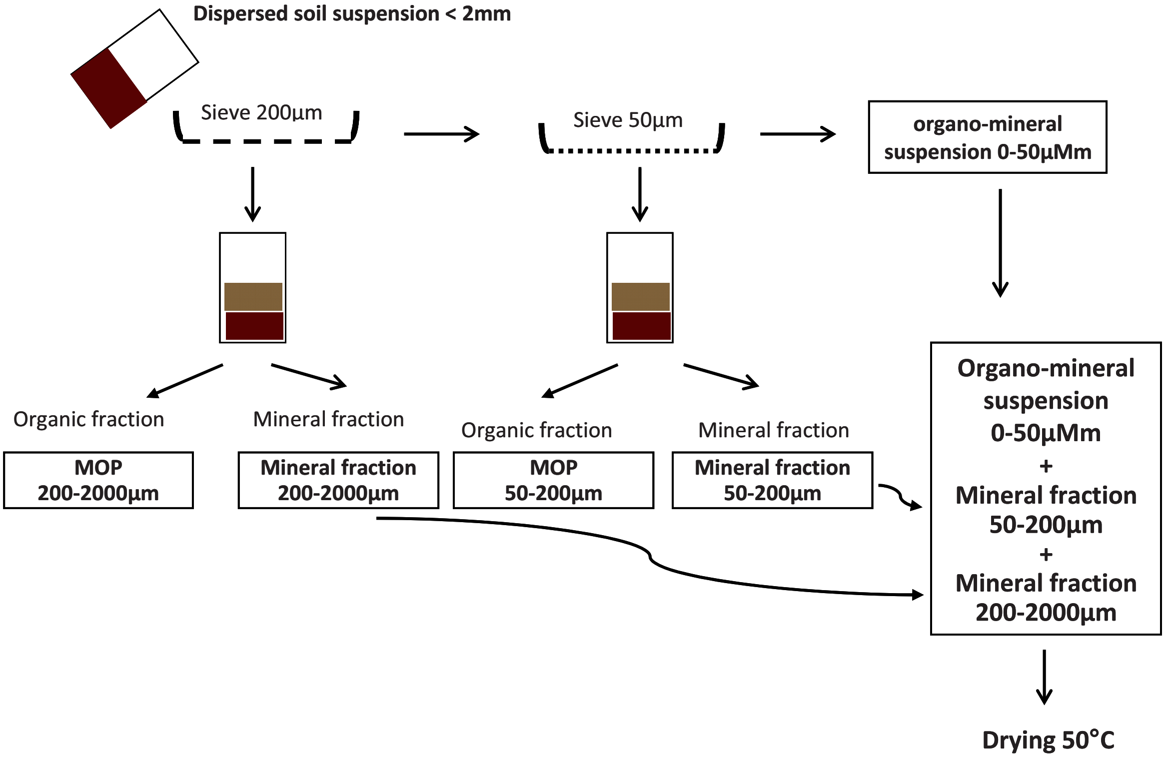


Figure 2.7b: *Schematic diagram of various steps followed to prepare soil samples without particulate organic matter.*

2.4 Preparation of soil samples with and without particulate organic matter

The preparation of soil samples with and without particulate organic matter same procedure of the separation of particulate organic matter from soil was followed as described previously in the section 2.3. The schematic diagrams of the steps followed to prepare soil samples with and without particulate organic matter are presented in figure 2.7a and 2.7b. 100g soil was dispersed in 250ml water through overnight shaking with 20 glass beads in a polyethylene bottle. The soil suspension was then divided mainly into 200-2000µm, 50-200µm fractions and a soil suspension of <50µm through wet sieving. From 200-2000µm, 50-200µm fractions, particulate organic matter was separated through floatation in water. The mineral fractions obtained in this step and the water used for separating particulate organic fractions were then again mixed with the <50µm suspension. This suspension was mixed thoroughly and was subdivided into glass plates which were kept in an oven with air circulating system at 45°C to allow the water evaporation. Particulate organic matter fractions were dried separately. After drying the soil was collected carefully to avoid minimum loss of soil. To avoid the artifacts of experiment, instead of using non-fractionated soil samples as samples with particulate organic matter fractions, the soil samples were treated in the same way for the soils with and without particulate organic matter fractions. But in case of soil without particulate organic matter fractions, the POM fractions were dried separately and were not mixed with other suspension. For soil with POM, after POM separation we again mixed the POM and mineral fractions with the <50µm suspension which were then dried together.

2.5 Preparation of fungal cultures for the trace metal bioavailability study

Bioavailability of the trace metals linked to particulate organic matter fractions was assessed by a biotest involving the growth of fungi *Trametes versicolor* in a liquid medium. These fungi have been reported as potential biomarkers of exposition to organic or inorganic contaminants through certain indices like the enhanced production of extra-cellular oxydases e.g laccase in the presence of contamination (Lebrun et al., 2011). In this biotest we assessed the bioavailability of copper per linked to particulate organic matter by using these fractions as a source of copper in the liquid medium where no copper was added (except through yeast extract) and by comparing the bioavailability of copper added in the form of metal salt ($CuSO_4$) in the same conditions. For this biotest we used the POM fractions of the soils of a 15-year old field experiment involving the assessment of effect of different organic amendments on soil properties, located in the vineyard site of Mâcon Clessé (Burgundy, France). The parcel sampled for our study has been amended with conifer compost laid at 100 m^3 ha^{-1} every 3-4 years. But this addition of amendment was stopped five years before our sampling. This soil was not contaminated and had metal contents in the range of the pedological background except the slight contamination of Cu (Sebastia, 2007). In this study, about 700g of the soil of the parcel amended with conifer composts for several years was fractionated to extract a stock of about 4.5g of coarse POM fractions (200-2000µm) and 10g of fine POM fractions (50-200µm).

The schematic diagram of this test is given in the figure 2.8 and the main steps are given in the following:

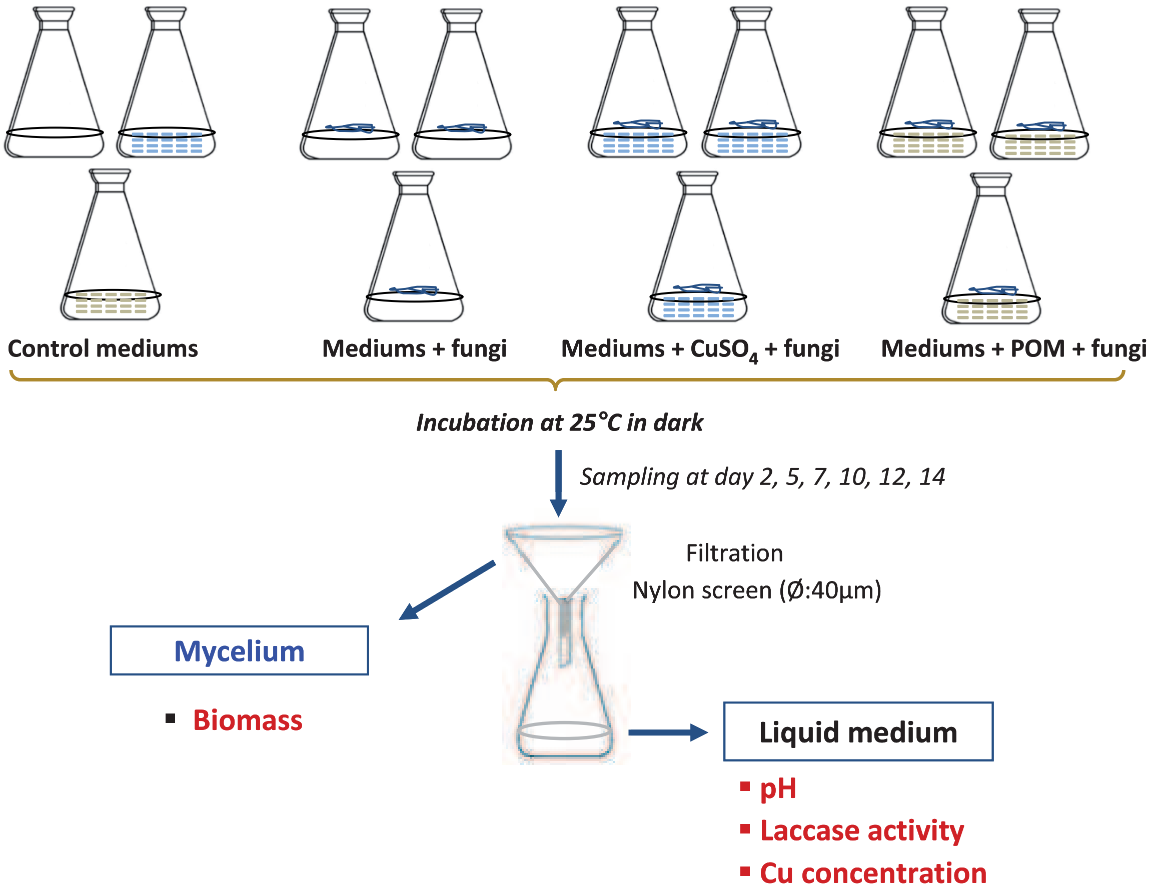


Figure 2.8: *Schematic diagram of the biotest for studying trace metal availability by using fungal cultures.*

2.5.a Culture medium composition and conditions

Trametes versicolor ATCC 32745 was grown in liquid culture media herein called Abadulla. This medium was adapted from (Abadulla et al., 2000) and is already used in our laboratory for the cultures of fungi for toxicity assessment assays because of the presence of less complexing agents in it compared to other liquid mediums (Lebrun et al., 2011). The composition of this medium is given in table 2.2. The liquid culture medium was heat sterilized in an autoclave at 121°C. Cultures were incubated in 150mL Erlenmeyer flasks which were also heat sterilized before use. Copper as essential trace metal was added to the liquid medium in the form of salt solution or POM fraction (50-200µm) before the start of culture incubations. In case of addition as salt solution, 50µl of 20mg/L $CuSO_4$ solution already sterilized by filtration (0.2µm pore size membrane) was added in 10mL liquid medium (for having a final concentration of 100µg/L Cu in liquid medium). After certain initial assays in the laboratory we found that some copper is released in liquid medium when we add POM fractions. A quantity of 5mg of fine POM fraction (50-200µm) containing 500mg Cu/kg was required to be added in the 10mL of liquid medium for having 100µg/L Cu in liquid medium. So in case of Cu supply to be added in form of Cu linked to POM fractions, 5mg of POM were directly weighed in the Erlenmeyer flasks before their sterilization and POM fractions and Erlenmeyer flasks were thus heat sterilized together. Mycelium mats of 10mm diameter were taken from outer circumference of a fungal colony grown in agar-plate (of 8-10 days age) and were inoculated in the 10mL of liquid medium in the Erlenmeyer flasks. The cultures were incubated statically at 25°C in dark for 14 days. Biological controls (culture mediums without fungi) as well as chemical controls (culture mediums without copper but with fungi) were also incubated. The experiment was conducted with three replicates (3 fungal cultures) per treatment (3 conditions: without Cu, with $CuSO_4$, with POM) and per sampling day (6 dates).

Table 2.2: *Composition of the liquid culture medium used for the biotest.*

Ingredients	g/L
KCl	0.5
$KH2PO_4$	2.5
$MgSO_4$	0.5
$CaCl_2$	0.1
Yeast extract	2.5
Glucose	10.0
NH_4Cl	2.5

2.5.b Measure of fungal biomass and pH of liquid mediums

At day 2, 5, 7, 10, 12 and 14 of culture incubation, the specified Erlenmeyer flasks were taken out of incubator and the fungal mycelia were separated from the liquid medium by filtration through a nylon screen (40µm). The mycelium was collected in already weighed Eppendorf tubes and was dried for 48 hours at 80°C. The growth of fungal biomass was determined weighing the mycelium after drying. Three aliquots of the liquid mediums were separated into different containers each specified for separate measurements. One of these aliquots was immediately used for measuring pH of liquid mediums by using a combined electrode ($AgCl_2$/KCl) and a pH meter (TT Processor Radiometer). The other aliquots were frozen at -18°C before the analysis of laccase activities and copper contents of liquid mediums.

2.5.c Measurement of laccase activities of the liquid mediums

Laccase (EC 1.10.3.2) activity of the liquid mediums was measured by monitoring the oxidation of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) at 420nm in a citrate buffer (CPB; 0.1M, pH 3.0) at 30°C (Wolfenden and Willson, 1982). 20µL of the liquid medium, 50µL of ABTS and 930µL of citrate buffer were taken for a total reactive volume of 1mL and the kinetics of substrate catalysis were observed in already regulated spectrophotometer during 1min. One unit of enzyme activity was defined as the amount of enzyme that catalyzed 1µmol substrate in 1 min. The activities were measured in triplicate from liquid mediums and were expressed in U per g dry weight of fungal biomass (U g^{-1}).

2.5.d Measurement of copper concentrations of the liquid mediums after fungal growth

The copper concentrations of the control liquid mediums were measured by using graphite furnace electro-thermal automization technique of atomic absorption spectrophotometery (Varian SpectrAA 220) while the concentrations of mediums with added Cu were measured through flame atomic absorption spectrophotometery by using an atomic concentrator tube (ACT 820 © Agilent Technologies, Inc., 1989). To avoid interference from the organic matrix of the samples the procedure was adopted by regulating apparatus as well as by using the matrix modifying materials. Quality control was assured by running controls and by measuring recovery of standard additions in the samples and of the certified reference materials (SRM-1640; Natural water rich in trace metals, National Institute of standard and Technology, USA).

2.6 Statistical analysis and data modeling

Statistical analyses ANOVA and Pearson correlation test were carried out with the software XLSTAT version 2010 (Addinsoft^{TM}). The data were subjected to ANOVA and statistical differences between means were determined according to Tukey's test. The normality of data was checked by performing Shapiro-Wilk Test by using the software OriginPro 8 SRP (Origin Lab Corporation USA). When needed the data were transformed into logarithmic values before performing ANOVA. Pearson correlation test was performed to study the relation between metal availability parameters i.e. total EDTA extractable contents (Ex-EDTA), kinetically defined labile metal fraction (Q_1), slowly labile fractions (Q_2), extraction constant of labile and slowly labile metal fractions (K_1 and K_2 respectively) and the metal distribution among different physical size fractions of the soils. Data concerning kinetic extractions were fitted to a "two first order kinetic reaction model" using SigmaPlot 9.0 software (SPSS Inc.). Validity of the modeling was tested by statistical parameters like the coefficient of fitting (R^2) and the standard error of estimate (SEE).

Chapter 3

Impact of land use on trace metal availabilities in contaminated agricultural soils

3.1. Introduction

The land use of contaminated agricultural soils is suspected to influence the main soil physico-chemical properties directly or indirectly via the changes in soil organic matter status and then trace metal dynamics and their availabilities in the soils. In this context the work presented in this chapter was designed to test the hypothesis that;

1. Trace metal availabilities of the contaminated agricultural soils are influenced by their land use
2. The influence of land use on trace metal availabilities of the contaminated soils is dependent of the soil texture and organic matter contents
3. The trace metal localization in the physical size fractions of the soils is linked to the trace metal availabilities of the soils

In this work we tested furthermore 3 ways of studying availability in order to show their complementarity. For this we compared the results of trace metal availabilities studied by performing metal extractions at equilibrium by using three different extractants (EDTA, DTPA and NH_4NO_3) as well as by performing kinetic metal extractions using EDTA. The strategy of studying the trace metal availabilities of the contaminated soils of two sites differing in their texture and organic matter content i.e. Metaleurop and Pierrelaye was followed. The influence of land use on trace metal availabilities was studied by comparing the metal availabilities of the soils under annual crop and under perennial crop miscanthus. The pertinence of using different methods was checked between both sites and for assessing the influence of the soil land use on their trace metal availabilities. In the end the relations of soil trace metal availabilities with their localization in different physical size fractions of the soils were examined via Pearson correlations, and discussed.

3.2. Results:

3.2.1. Influence of land use on trace metal availabilities

3.2.1.a. EDTA extractable metal contents at equilibrium

Metaleurop

Table 3.1 presents the percentages of EDTA extractable metal contents in the soil samples. For the Metaleurop soil samples it was observed that whatever the land use, in our soils EDTA extractable percentages were more for Pb and Cd always >50% than Cu and Zn always <50%. Comparing miscanthus and annual crop soil we observed that the percentages of EDTA extractable Cu and Pb were lower in miscanthus soil (47% and 98% respectively) than the annual crop soil (44% and 93% respectively). A slight decrease in Cu and Pb extractability occurred in the soil under miscanthus as compared to the annual crop soil. On the contrary, no significant differences were observed between the miscanthus and annual crop soil for EDTA extractable Zn and Cd percentages.

Pierrelaye

For Pierrelaye soil samples it was observed that the percentages of extraction of all studied metals were high always 50% (Table 3.1). However, as for Metaleurop soil samples; the percentages of extraction of Pb and Cd were higher than that of Cu and Zn with Zn being the least extractable. Comparing the results of soil samples of two land uses with a one-way analysis of variance (ANOVA) showed no significant differences for the percentage extractability of Cu and Pb whereas the extractable percentages of Zn and Cd were found significantly different at a high significance ($p < 0.01$).

Table 3.1: *EDTA extractable metal contents (at equilibrium) of the soil samples. Mean values of 3 replicates with standard deviation among these replicates. Different letters indicate statistically significant difference (Tukey's test, $p < 0.01$) between annual crop and miscanthus soil of each site.*

	Soil	Metaleurop (%)	Pierrelaye (%)
Cu	***Annual crop***	46.6 ± 0.4 ^{a}	77.6 ± 5.0 ^{a}
	Miscanthus	43.7 ± 0.9 ^{b}	73.4 ± 2.5 ^{a}
Pb	***Annual crop***	98.2 ± 2.5 ^{a}	88.4 ± 3.7 ^{a}
	Miscanthus	93.7 ± 1.7 ^{b}	85.9 ± 4.0 ^{a}
Zn	***Annual crop***	28.4 ± 0.6 ^{a}	61.0 ± 3.1 ^{a}
	Miscanthus	28.6 ± 0.4 ^{a}	55.8 ± 1.0 ^{b}
Cd	***Annual crop***	84.6 ± 1.2 ^{a}	80.1 ± 0.7 ^{b}
	Miscanthus	82.2 ± 1.0 ^{a}	94.2 ± 1.0 ^{b}

It was found however, that Zn and Cd behave differently: the extractable zinc percentage was lowered in the miscanthus soil compare to the annual crop soil whereas the percentage of extractable Cd was higher in the miscanthus soil as compared to the annual crop soil.

Comparing the results of both sites found that the EDTA extractabilities of Zn and Cu were high (2 and 5 times, respectively) in the soils of Pierrelaye than the Metaleurop soils. Contrary to this the Pb and Cd extractabilities were higher in the soils of Metaleurop than the Pierrelaye.

In short:
The influence of land use on EDTA extractable metals was found site and metal specific: only Cu and Pb were implicated at one site and only Zn and Cd at the other site.

- In the silty clay sandy soil only Cu and Pb were concerned by the change in land use, while in the sandy soil only Zn and Cd but in opposite side of change

3.2.1.b. DTPA extractable metal contents at equilibrium

Table 3.2 presents the percentages of DTPA extractable metal contents in the soils. As for EDTA, it was observed that whatever the land use, in our soils the percentages of DTPA extractable Cu and Zn were more in the soil samples of Pierrelaye than Metaleurop while percentages of extractable Pb and Cd were more in the Metaleurop soil than Pierrelaye but the percentages of DTPA extractability of all metals were lower than EDTA extractable metal percentages in all the soils.

Table 3.2: *DTPA extractable metal contents of the soils before and after soil incubation. Mean values of 3 replicates with standard deviation among these replicates. Different letters indicate statistically significant difference (Tukey's test, $p < 0.01$) of metal extractability between annual crop and miscanthus soil for each site.*

	Soil	Metaleurop (%)	Pierrelaye (%)
Cu	***Annual crop***	15.1 ± 0.8 ^{a}	24.6 ± 1.2 ^{a}
	Miscanthus	14.7 ± 0.7 ^{a}	20.7 ± 1.0 ^{a}
Pb	***Annual crop***	34.6 ± 1.7 ^{a}	11.9 ± 0.6 ^{a}
	Miscanthus	29.7 ± 1.5 ^{a}	9.2 ± 0.5 ^{b}
Zn	***Annual crop***	14.9 ± 0.7 ^{a}	20.8 ± 1.0 ^{a}
	Miscanthus	13.4 ± 0.7 ^{a}	18.4 ± 0.9 ^{a}
Cd	***Annual crop***	57.9 ± 2.9 ^{a}	39.7 ± 2.0 ^{a}
	Miscanthus	50.8 ± 2.5 ^{a}	36.2 ± 1.8 ^{a}

Whatever the site Metaleurop or Pierrelaye, no statistically difference was found between the annual crop and miscanthus soils for the percentages of DTPA extractable contents of Cu, Zn and Cd. Only for Pb the results were different: percentages of DTPA extractable Pb contents were similar in both the annual crop and miscanthus soil of Metaleurop while at Pierrelaye the percentage of Pb extraction was more in the annual crop soil than the miscanthus soil.

In short:

Contrarily to EDTA extractions, no influence of land use on DTPA extractable metal contents except for Pb at Pierrelaye site where availability decreased under miscanthus.

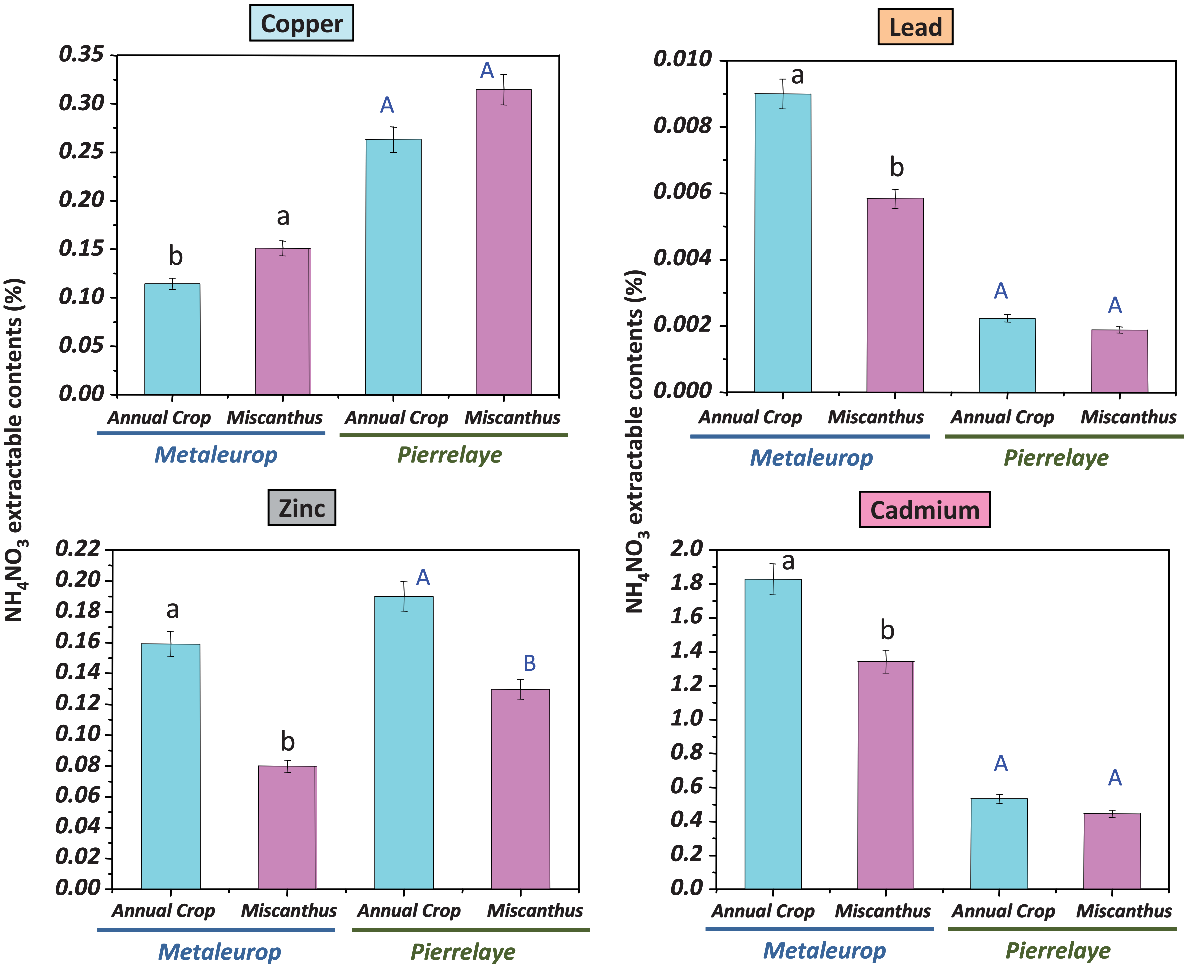


Figure 3.1: *NH_4NO_3 extractable metal contents of the soils before and after soil incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Different letters indicate statistically significant difference (Tukey's test, $p < 0.01$) of metal extractability between annual crop and miscanthus soil for each site.*

3.2.1.c. NH_4NO_3 extractable metal contents at equilibrium

The results of NH_4NO_3 extractable metal contents are presented in the figure 3.1. As expected NH_4NO_3 extractable metals present a much poor metal fraction and the metal extractions were low i.e. < 0.01% of total soil Pb contents, <0.3 % for total Cu and Zn contents of the soils, and <2% of soil cadmium contents. Metal wise NH_4NO_3 extraction order in all the soils was Cd > Cu > Zn > Pb. Comparing the results of both sites it was found that for Cu and Zn the percentages of NH_4NO_3 extractable contents were more for the soils of Pierrelaye while for Pb and Cd the percentages of NH_4NO_3 extractable contents were more for the soils of Metaleurop than Pierrelaye. In terms of the influence of land use on NH_4NO_3 extractable metal contents, we observed that for Metaleurop site the miscanthus soil had lower percentages of Pb, Zn and Cd than the annual crop soil, but had higher Cu percentage. At Pierrelaye, percentages of NH_4NO_3 extractable contents of Cu, Pb and Cd were found similar in both miscanthus and annual crop soils while Zn percentage was lower in the miscanthus soil than the annual crop soil.

In short:
The influence of land use on NH_4NO_3 extractable contents as exchangeable metals was different for both sites:

- Metaleurop:
  - Finally Cu is more exchangeable but less available under miscanthus
  - Pb is less exchangeable and less available
  - Cd and Zn less exchangeable but no change in availability
- Pierrelaye:
  - Whereas Cu and Pb do not change at Pierrelaye
  - Zn less exchangeable and less available, Cd is more available

❖ These results are much variable and cannot be explain our hypothesis (i.e. effect of change in organic matter or presence or absence of tillage). Therefore other techniques of trace metal availabilities studies involving metal extraction are needed in complement to get insights

3.2.1.d. Kinetically defined labile and slowly labile metal fractions and their associated extraction rate constants

Metaleurop

The labile and slowly labile metal fractions (Q_1 and Q_2, respectively) of Cu, Pb, Cd and Zn for both miscanthus and annual crop soils expressed as percentages of the total trace metal contents of the soils along with the extraction rates of labile and slowly labile fractions (K_1 and K_2, respectively) are presented in table 3.3. Whatever the land use and the metals, we found that the percentages of labile metal fractions were more than the slowly labile metal fractions. It was observed that the miscanthus and annual crop soils differ in their labile and slowly labile metal fractions of Cu and Pb, while no significant difference was observed between both soils for the labile and slowly labile fractions of Zn and Cd. These results are consistent with the extractions at equilibrium with EDTA and not with those with DTPA and NH_4NO_3. Labile fractions of Cu and Pb were lower in the soil under miscanthus than in the annual crop soil with a lowering of 12% for labile Cu and 17% for labile Pb. In the same time, slowly labile fractions of Cu and Pb were higher in miscanthus soil than the annual crop soil with a more noticeable difference for Pb than Cu (an increase of 10% in slowly labile Cu and 60% in slowly labile Pb). The results of extraction rate constants K_1 of the labile fractions of all metals were higher in the soil under miscanthus soil than in the annual crop soil except for Zn. The extraction rate constants K_2 of the slowly labile fractions of all the metals were also higher in the soil under miscanthus than in the annual crop soil except for Cd for which extraction rate constants are similar in both soils.

Table 3.3: *Percentages of labile metal fraction (Q_1), slowly labile metal fraction (Q_2) of the soil samples and the corresponding extraction rate constants of these fractions K_1 and K_2 respectively for the soils of Metaleurop. Mean values of 5 replicates ± standard deviation. Different letters within each column indicate statistically significant difference (Tukey's test, $p < 0.01$) between annual crop and miscanthus soil for the metal fractions ($Q_1$and Q_2) and the extraction rate constants these metal fractions (K_1 and K_2) for each metal.*

Metal	Soil	Q_1 (%)	Q_2 (%)	K_1 (min^{-1})	K_2 (min^{-1}, $X 10^{-3}$)
Cu	*Annual Crop*	33.7 ± 0.4^{a}	12.9 ± 0.1^{b}	0.34 ± 0.01^{b}	4.34 ± 0.13^{b}
	Miscanthus	29.6 ± 0.5^{b}	14.1 ± 0.2^{a}	0.47 ± 0.02^{a}	7.46 ± 0.43^{a}
Pb	*Annual Crop*	78.8 ± 0.6^{a}	18.2 ± 1.9^{b}	0.34 ± 0.01^{b}	3.52 ± 0.64^{b}
	Miscanthus	65.2 ± 2.2^{b}	29.3 ± 0.8^{a}	0.70 ± 0.12^{a}	8.98 ± 1.40^{a}
Zn	*Annual Crop*	20.6 ± 0.6^{a}	7.8 ± 0.4^{a}	0.42 ± 0.01^{a}	4.66 ± 0.76^{b}
	Miscanthus	21.4 ± 0.5^{a}	7.7 ± 0.2^{a}	0.38 ± 0.01^{b}	8.72 ± 0.90^{a}
Cd	*Annual Crop*	70.8 ± 1.7^{a}	13.3 ± 0.8^{a}	0.42 ± 0.02^{b}	2.10 ± 0.37^{a}
	Miscanthus	67.8 ± 0.4^{a}	13.7 ± 0.5^{a}	0.59 ± 0.03^{a}	2.24 ± 0.28^{a}

Pierrelaye

Figure 3.2 presents the percentages of the labile (Q_1) and slowly labile metal fractions (Q_2) of the studied metals for the both soil samples and the corresponding extraction rate constants (K_1 and K_2) are presented in table 3.4. It was observed that for all the studied metals, percentages of labile metal fractions were more than double than their slowly labile fractions in both soils except for Pb in miscanthus soil for which the percentages of labile and slowly labile metal fractions were almost equal. Comparing the results for annual crop and miscanthus soils, it was observed that both soils did not differ in the percentages of labile Cu fraction nor in that of slowly labile Cu fraction. For Pb the labile metal fraction was lower in the miscanthus soil than in the annual crop soil whereas the percentage of slowly labile Pb was higher in the miscanthus soil than in the annual crop soil. For Zn and Cd both soil samples have similar percentages of slowly labile fractions but the labile Zn percentages was lower in the miscanthus soil than in the annual crop soil while the labile Cd percentage was higher in the miscanthus soil than in the annual crop soil.

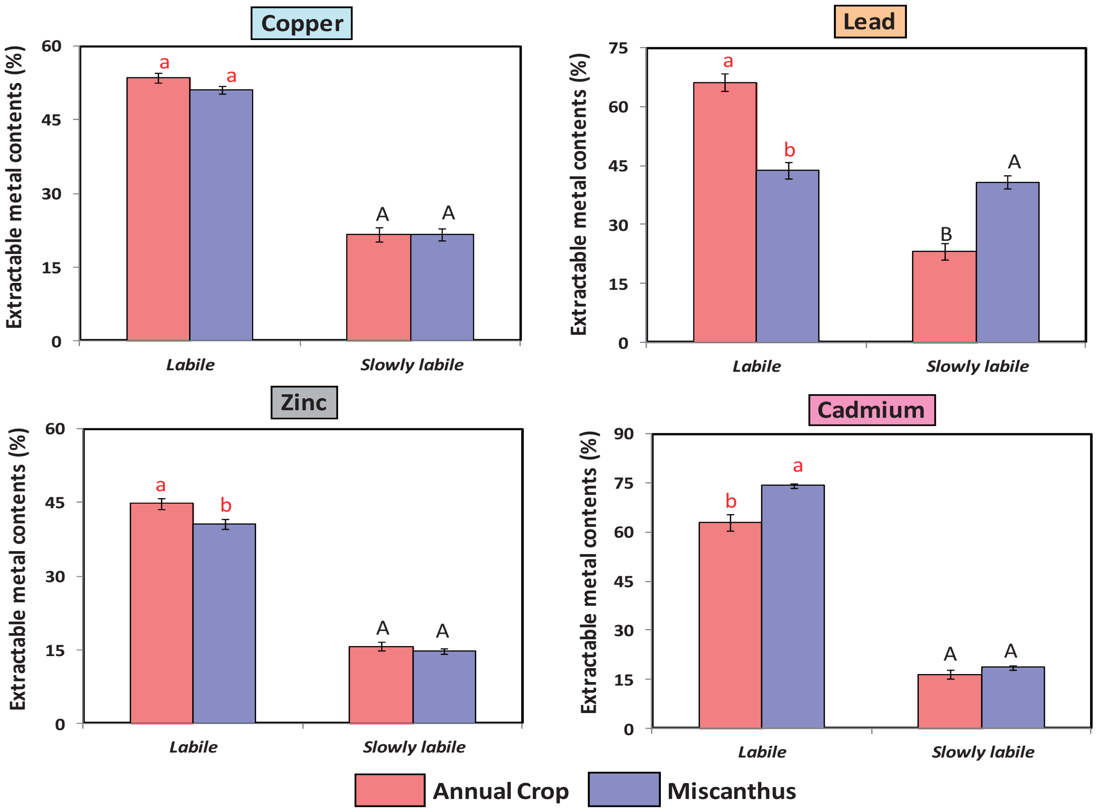


Figure 3.2: *Percentages of labile metal fraction (Q_1), slowly labile metal fraction (Q_2) of the soil samples of Pierrelaye. Bar graphs represent mean values of 5 replicates while the error bars represent standard deviation among these replicates. Different letters indicate statistically significant difference (Tukey's test, $p < 0.01$) between annual crop and miscanthus soil for each fraction.*

The extraction rate constants of the labile metal fractions of Cu and Pb were found no significantly different in both soil samples. The extraction rate constants of the slowly labile fraction of Cu and Pb were found higher in the miscanthus soil than in the annual crop soil. The extraction rate constants associated to labile fractions of Cd and Zn were lower in the miscanthus soil than in the annual crop soil whereas both soil samples were similar for the extraction rate constants of the slowly labile fractions of these metals.

Table 3.4: *Extraction rate constants of labile metal fraction (K_1) and of slowly labile metal fraction (K_2) of the soil samples of Pierrelaye. Mean values ± Standard Deviation (n=5). Different letters in the columns indicate statistically significant difference (Tukey's test, $p < 0.01$) between annual crop and miscanthus soil for the rate constant of each fraction for each metal.*

Metal	**Soil**	***K_1 (min^{-1})***	***K_2 (min^{-1}, X 10^3)***
Cu	***Annual Crop***	0.48 ± 0.02^{a}	4.38 ± 0.65^{b}
	Miscanthus	0.44 ± 0.02^{a}	8.22 ± 01.64^{a}
Pb	***Annual Crop***	0.27 ± 0.01^{a}	2.56 ± 0.43^{b}
	Miscanthus	0.40 ± 0.16^{a}	10.66 ± 4.63^{a}
Zn	***Annual Crop***	0.52 ± 0.03^{a}	6.60 ± 1.03^{a}
	Miscanthus	0.42 ± 0.04^{b}	6.62 ± 0.58^{a}
Cd	***Annual Crop***	0.53 ± 0.06^{a}	8.70 ± 2.20^{a}
	Miscanthus	0.41 ± 0.04^{b}	7.62 ± 0.63^{a}

In short:

- In one case (Metaleurop), results of kinetics extractions were found consistent with those at equilibrium, and brought a complementary insight. In particular, the fact that the extraction rates changed for Zn and Cd while no other value of NH_4NO_3 exchangeability, nor EDTA or DTPA changed do signifies that a slight change occurred in the bindings of metals with soil phases or soil constituents, but not enough to enhance or decrease the relative maximum value extracted.

- Even less straightforward, the same reasoning could be applied from the results of the other site studied (Pierrelaye) while the case of Pb seems to clearly show that some links with the soil constituents were also perturbed

    - But globally, what seems to be said by the extraction rates is that something had changed without the fact that the absolute value of extraction can indicated such change. Thus, another way of viewing potential changes is required to assess possible impact. The other way we chose is linked to physical approach rather than chemical which seem to fail to show eventual changes.

3.2.2. Influence of land uses on trace metal localization in physical size fractions:

3.2.2.a. Mass distribution of the soils in the physical size fractions

Metaleurop

Figure 3.3a shows the mass distribution of the soils into the different physical size fractions. It was observed that miscanthus and annual crop soil samples differed significantly in their mass distribution in the 20-50µm and 50-200µm fraction. The 20-50µm fraction of the miscanthus soil is 12% higher as compared to the same fraction in the annual crop soil, while the 50-200µm fraction of the miscanthus soil has 5% less mass than in the corresponding fraction of annual crop soil. Both miscanthus and annual crop soil have similar mass of 200-2000µm and < 20µm fraction.

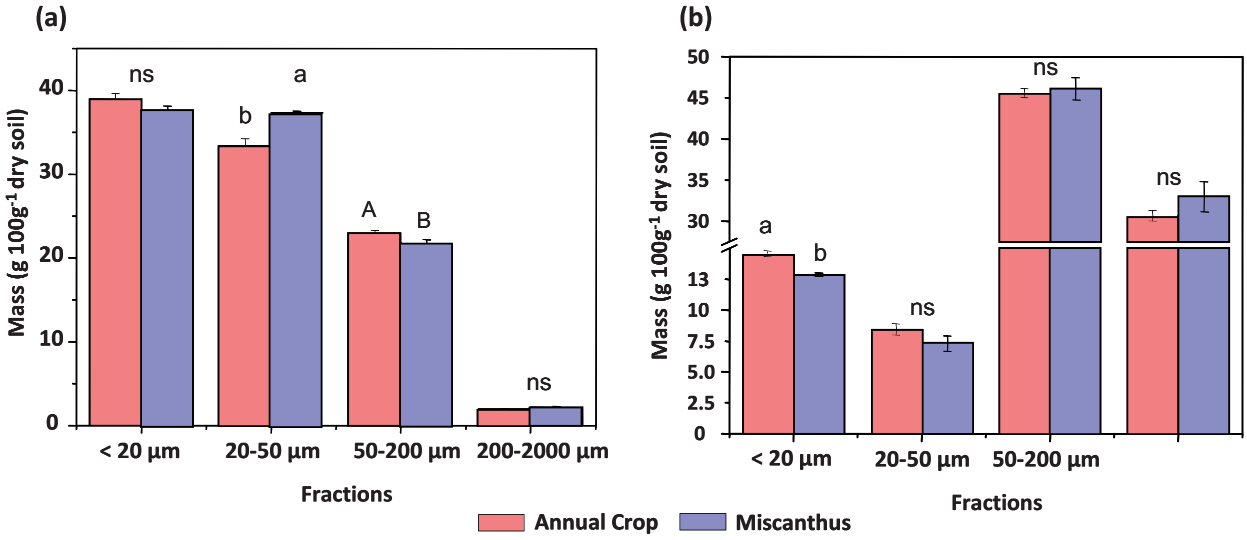


Figure 3.3: *Distribution of the soil mass into different physical size fractions of the soils of Metaleurop (a) and Pierrelaye (b). Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.05$) between annual crop and miscanthus soil for a given fraction is showed by different letters while "ns" represent non significant differences.*

Pierrelaye

Figure 3.3b shows the mass distribution of the soil samples into the different physical size fractions. As expected more than 70% of the soil mass are in the sand size fractions (> 50µm) owing to the sandy texture. Both soil samples have low mass percentages in the 20-50µm fraction whereas the <20µm fractions contribute as 13% and 15% of the total soil mass. Pair-wise comparisons show that both soil samples have similar mass distribution except for the <20µm fraction for which the miscanthus soil has slight but statistically significant lower mass than the annual crop soil.

In short:

The influence of land use on the mass distribution in the physical size fractions was different for both sites:

- Metaleurop: Increase in mass in the 20-50µm fractions and decrease in mass in the 50-200µm fraction in the miscanthus soil compared to the annual crop
- Pierrelaye: Decrease in mass in the <20µm fraction in the miscanthus soil compared to the annual crop soil
- ❖ Both hypothesize we made could explained these differences: an influence of the absence/presence of tillage and/or a difference in the organic status in the favor of slightly more aggregation in the absence of tillage

3.2.2.b. Distribution of organic carbon and trace metal contents in the physical size fractions of the soils

Metaleurop

Figure 3.4 presents the data regarding distribution of the metal and organic carbon contents of the soil samples into the different physical size fractions. It was found that whatever the land use, the percentage allocation of the trace metals as well as organic carbon decreased with increasing size of physical fractions with < 20µm fraction having highest metals and organic carbon allocation. Results of trace metal distribution show that the main differences between miscanthus and annual crop soil samples were found for the localization in the < 20µm and 20-50µm fractions for Cu, Pb and Cd and in 50-200µm fraction for Cd. Miscanthus soil has higher Cu percentage in the <20µm and 20-50µm fractions than the annual crop soil sample while both soil samples have similar Cu distribution in the coarse size fractions i.e. 50-200µm and 200-2000µm fractions. For Pb, miscanthus soil sample has higher metal percentage in the 20-50µm fraction than for the annual crop soil but the distribution is similar for both samples in other physical fractions. For Cd distribution, miscanthus soil sample have high metal percentage in 20-50µm fraction and less percentage in 50-200µm fractions than the corresponding fractions of the annual crop soil sample. For organic carbon distribution miscanthus soil sample has lower percentages in <20µm and 50-200µm fractions than in the corresponding fractions of the annual crop soil sample. In the

20-50µm and 200-2000µm fractions organic carbon percentages are higher in the miscanthus soil sample than in the annual crop soil sample.

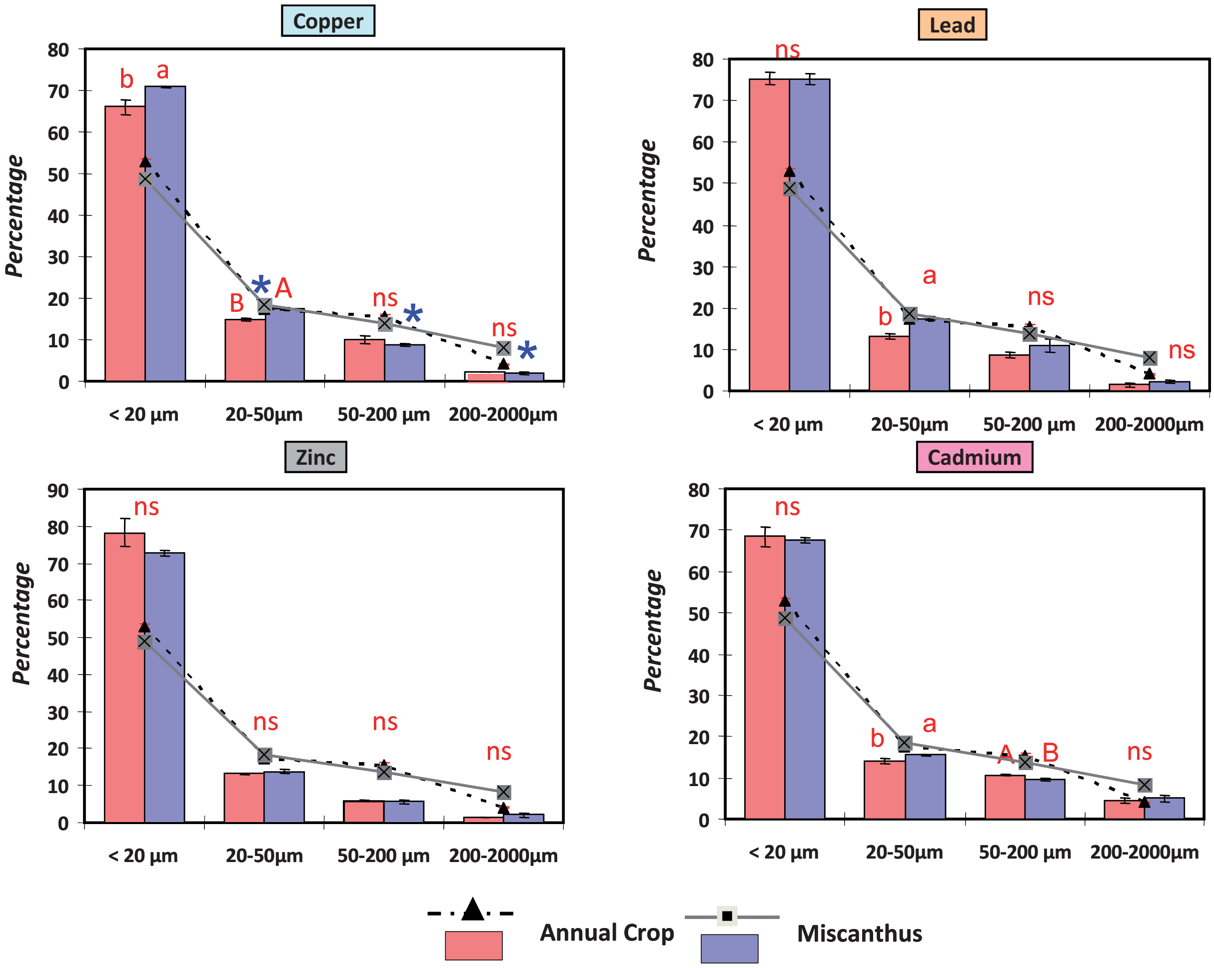


Figure 3.4: *Distribution of trace metals and organic carbon contents into different size fractions of the soils of Metaleurop site. Curves represent the organic carbon distribution in different fractions. Bar graphs represent mean values of 3 replicates for metal distribution while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.05$) of metal distribution between annual crop and miscanthus soil for a given fraction is showed by different letters while "ns" represent non significant differences. For organic carbon distribution the statistically significant difference is shown by the sign (*).*

Pierrelaye

Figure 3.5 shows the percentage allocation of the organic carbon and of the trace metal of into the physical size fractions of the soil samples. Contrary to soil samples of Metaleurop, it was observed that in Pierrelaye soil samples the organic carbon and metal distribution in physical size fractions. In both soil samples of Pierrelaye, for metals the highest percentage localization was in the <20µm fraction, whereas the highest organic carbon distribution was found in the coarse 200-2000µm fraction. Metal distribution among the physical size fractions revealed that both soil samples have the highest percentage (>45%) of all metals in the < 20µm fractions. Both soil samples did not differ significantly in the Zn and Cd distribution in their physical size fractions. Contrarily Cu and Pb distribution in the different physical size fractions differed between miscanthus and annual crop soil samples: for Cu in the 50-200µm and for Pb in the <20µm fractions. Miscanthus soil sample has more percentage of Cu in the 50-200µm fraction than the corresponding fraction in the annual crop soil sample, and the Pb percentage in the <20µm fraction of the miscanthus soil sample was lower than the corresponding fraction in the annual crop soil sample. Results for the soil organic carbon distribution show that both soil samples have similar distribution of organic carbon into the physical size fractions. The highest allocation of organic carbon contents (44 and 48%) was in the 200-2000µm fraction whereas the 20-50µm fractions have lowest percentage allocation of organic carbon.

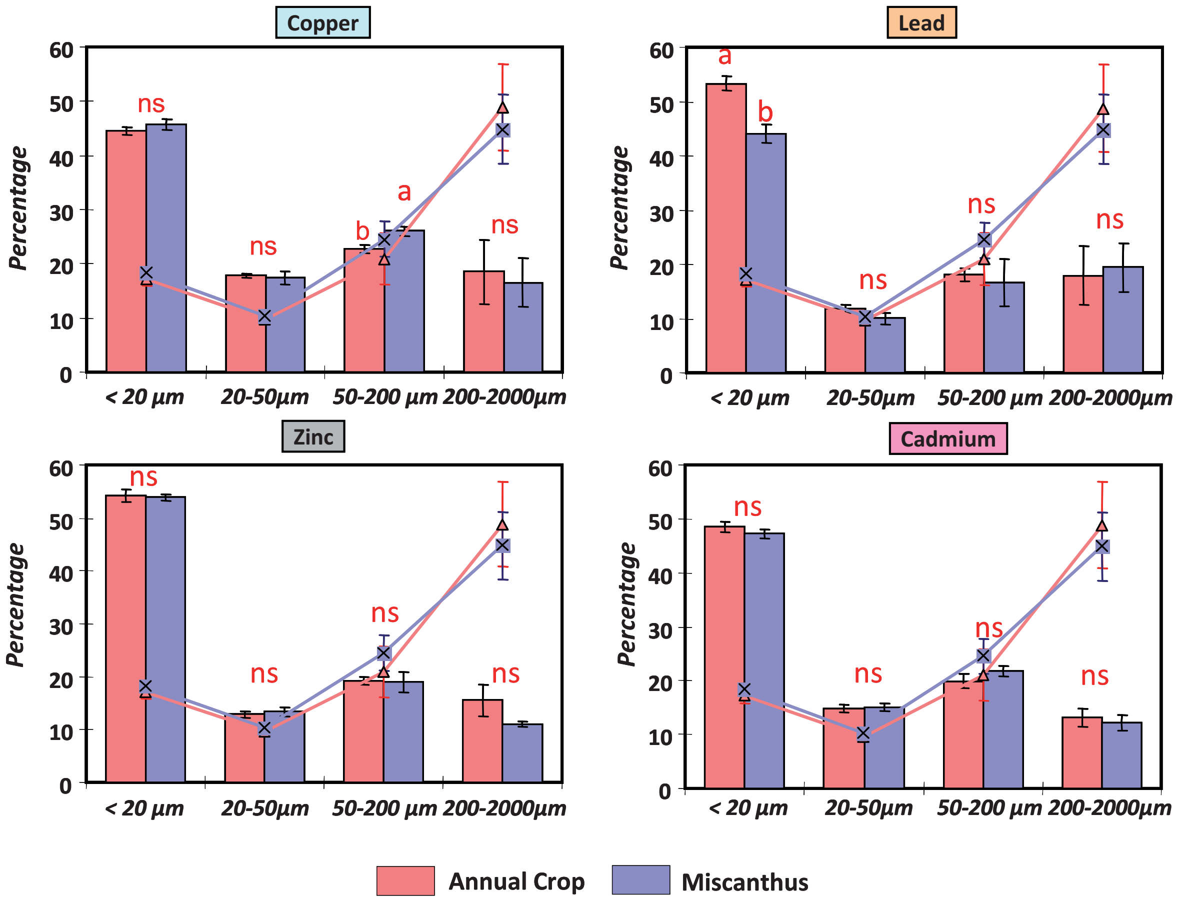


Figure 3.5: *Distribution of the trace metals and organic carbon contents into different size fractions of the soils of Pierrelaye site. Bar graphs represent mean values of 3 replicates for metal distribution while curves present the organic carbon distribution these fractions. The error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, p < 0.05) between annual crop and miscanthus soil for a given fraction is showed different letters while "ns" represent non significant differences.*

In short:
The influence of land use on trace metal distribution in the physical size fractions of the soils is site specific and metal dependant where:

- The different results obtained can be explained in relation with the initial contents of the soil in organic carbon. In the soil richer in C org (Pierrelaye), no change could be observed because differences are too low compared to the high values in the fractions, while for the soil with a lower organic carbon content (Metaleurop) the differences could be assessed in C org localization. It must be noted, however, that this could also be due to the fact that no change in organic carbon could be assessed in the Pierrelaye soils, either because of a lower growth of miscanthus in Pierrelaye site compared to Metaleurop and/or the absence or low rate of organisms which can help to incorporate organic carbon from miscanthus into the soil (results of the RESACOR project, personal communication). But the fact that the metals Cu and Pb known to be linked to organic matter do change in Pierrelaye is also in favor to the fact that some re-organization of organic carbon could have been done with a net final localization identical to the initial one.

3.2.3. Relations between soil metal availabilities and soil metal localization

In order to study the relations between trace metal availabilities in the soils and the metal localization in the physical size fractions, we performed Pearson correlation tests among these variables. The correlations obtained were different for the soil samples of the two sites and the details for each site are presented in the following two subsections.

i) Metaleurop

Table 3.5 presents the relations for the Metaleurop soil samples. For Cu and Pb, EDTA and DTPA extractable fractions were not correlated to their physical localization in size fractions of the soil samples whereas other available fractions i.e. labile pool, slowly labile pool and NH_4NO_3 extractable fractions of these metals were correlated to their localization in the fine fractions. For Zn only EDTA and NH_4NO_3 extractable fractions were correlated to its physical localization in the 20-50µm fraction while not any correlation was observed between the other Zn available fractions and its localization in the physical size fractions of

Table 3.5: *Correlation matrix (Pearson correlation coefficients) among metal availability parameters i.e. total EDTA extractable contents (Ex-EDTA), kinetically defined labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3 and DTPA extractable metal contents and the metal and organic carbon distribution among different physical size fractions of the soils of Metaleurop site (n=6; Values in bold are different from 0 with a significance level $p< 0.05$.*

Variables	Cu EDTA	Cu Lab	Cu Slab	Cu NH_4NO_3	Cu DTPA	Cu 0-20	Cu 20-50	Cu 50-200	Cu 200-2000
Cu EDTA	**1.00**								
Cu Lab	-0.30	**1.00**							
Cu Slab	0.12	**-0.93**	**1.00**						
Cu NH_4NO_3	0.06	**-0.89**	**0.98**	**1.00**					
Cu DTPA	-0.26	-0.37	0.57	0.55	**1.00**				
Cu 0-20	0.31	**-0.86**	**0.96**	**0.94**	0.57	**1.00**			
Cu 20-50	0.34	**-0.92**	**0.97**	**0.95**	0.46	**0.98**	**1.00**		
Cu 50-200	-0.48	0.51	-0.54	-0.46	-0.71	-0.67	-0.59	**1.00**	
Cu 200-2000	-0.36	0.45	-0.38	-0.21	0.14	-0.36	-0.37	0.18	**1.00**

Variables	Pb EDTA	Pb Lab	Pb Slab	Pb NH4NO3	Pb DTPA	Pb 0-20	Pb 20-50	Pb 50-200	Pb 200-2000
Pb EDTA	**1.00**								
Pb Lab	0.04	**1.00**							
Pb Slab	-0.26	**-0.96**	**1.00**						
Pb NH4NO3	0.05	**0.99**	**-0.93**	**1.00**					
Pb DTPA	-0.05	**0.86**	-0.73	**0.90**	**1.00**				
Pb 0-20	-0.26	0.48	-0.24	0.51	0.68	**1.00**			
Pb 20-50	-0.11	**-0.97**	**0.98**	**-0.95**	-0.80	-0.33	**1.00**		
Pb 50-200	-0.51	-0.69	0.69	-0.70	-0.57	-0.51	0.60	**1.00**	
Pb 200-2000	0.27	-0.74	0.70	-0.67	-0.45	-0.36	0.77	0.31	**1.00**

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000
Zn EDTA	**1.00**								
Zn Lab	0.71	**1.00**							
Zn Slab	0.37	**0.82**	**1.00**						
Zn NH4NO3	**-0.86**	**-0.82**	-0.62	**1.00**					
Zn DTPA	-0.31	-0.17	-0.33	0.62	**1.00**				
Zn 0-20	0.28	0.17	0.07	0.13	0.59	**1.00**			
Zn 20-50	**0.94**	0.69	0.52	**-0.89**	-0.53	0.22	**1.00**		
Zn 50-200	-0.02	0.32	0.09	-0.29	-0.17	-0.48	-0.03	**1.00**	
Zn 200-2000	**0.82**	0.51	0.37	**-0.83**	-0.67	0.09	**0.95**	0.09	**1.00**

Variables	Cd EDTA	Cd Lab	Cd Slab	Cd NH4NO3	Cd DTPA	Cd 0-20	Cd 20-50	Cd 50-200	Cd 200-2000
Cd EDTA	**1.00**								
Cd Lab	0.78	**1.00**							
Cd Slab	0.39	-0.24	**1.00**						
Cd NH4NO3	**0.96**	**0.92**	0.11	**1.00**					
Cd DTPA	0.79	**0.96**	-0.12	**0.89**	**1.00**				
Cd 0-20	0.65	**0.97**	-0.42	**0.84**	**0.88**	**1.00**			
Cd 20-50	**-0.86**	-0.39	-0.76	-0.70	-0.49	-0.20	**1.00**		
Cd 50-200	**0.95**	**0.84**	0.29	**0.94**	**0.90**	0.72	-0.80	**1.00**	
Cd 200-2000	-0.03	-0.50	**0.83**	-0.28	-0.44	-0.62	-0.31	-0.12	**1.00**

[*Ex-EDTA*], [Ex-DTPA] = mg kg^{-1} dry soil; [Ex-NH_4NO_3] = µg kg^{-1} dry soil; [Q_i] = mg kg^{-1} dry soil; data of metal and organic carbon distribution was taken as stocks in each fraction (mg of metal or carbon present in each fraction/100g of the dry soil), the soil samples. For Cd all available metal fractions except slowly labile fraction were correlated to its localization in the <20µm and 50-200µm fractions of the soil samples.

The nature of these correlations between available metal fractions and the metal localization i.e. either these were positive or negative, is as following:

Case of Cu: The slowly labile Cu and the NH_4NO_3 extractable Cu were positively correlated with the Cu localization in fine fractions i.e. < 20µm and 20-50µm fractions while the labile Cu pool was negatively correlated with the Cu localization in these fractions.

Case of Pb: The labile Pb pool and the NH_4NO_3 extractable Pb were negatively correlated with the Pb localization in 20-50µm fraction while the slowly labile Pb was positively correlated with Pb localization in this fraction.

Case of Zn: The EDTA extractable Zn was positively correlated while the NH_4NO_3 extractable Zn was negatively correlated with Zn localization in the < 20µm and 200-2000µm fractions.

Case of Cd: The EDTA extractable Cd was negatively correlated with Cd localization in the 20-50µm and was positively correlated with Cd localization in the 50-200µm fraction. While the Labile Cd pool, NH_4NO_3 extractable Cd and the DTPA extractable Cd were positively correlated with Cd in <20µm and 50-200µm fractions.

ii) Pierrelaye

Table 3.6 presents the relations for the Pierrelaye soil samples. DTPA extractability of all metals except Cd was not correlated to the metal localization in physical size fractions of the soil samples. For Zn and Cd all the available metal fractions i.e. EDTA extractable, the labile and slowly labile pools, the NH_4NO_3 extractable and the DTPA extractable fractions were correlated to the localization of these metals in all physical size fractions of the samples. For Cu and Pb, all the metal availability except the DTPA extractable fractions was correlated to the localization of these metals in the <20µm and the 20-50µm fractions while in case of Cu, correlation of its available fractions with 50-200µm fraction were also found.

The nature of these correlations (positive or negative) is given in the following:

Case of Cu: The EDTA extractable Cu, the labile Cu pool and the slowly labile Cu were positively correlated with Cu localization in the <20µm, 20-50µm and 50-200 µm fractions while the NH_4NO_3 extractable Cu was negatively correlated with Cu localization in these fractions

Table 3.6: *Correlation matrix (Pearson correlation coefficients) among metal availability parameters i.e. total EDTA extractable contents (Ex-EDTA), kinetically defined labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3 and DTPA extractable metal contents and the metal and organic carbon distribution among different physical size fractions of the soils of Pierrelaye site (n=6; Values in bold are different from 0 with a significance level p< 0.05.*

Variables	Cu EDTA	Cu Lab	Cu Slab	Cu NH4NO3	Cu DTPA	Cu 0-20	Cu 20-50	Cu 50-200	Cu 200-2000
Cu EDTA	**1.00**								
Cu Lab	**0.97**	**1.00**							
Cu Slab	**0.85**	**0.93**	**1.00**						
Cu NH4NO3	**-0.94**	**-0.96**	**-0.95**	**1.00**					
Cu DTPA	-0.54	-0.52	-0.69	0.75	**1.00**				
Cu 0-20	**0.97**	**1.00**	**0.92**	**-0.96**	-0.52	**1.00**			
Cu 20-50	**0.97**	**1.00**	**0.92**	**-0.94**	-0.49	**1.00**	**1.00**		
Cu 50-200	**0.96**	**0.98**	**0.96**	**-0.98**	-0.64	**0.98**	**0.98**	**1.00**	

Variables	Pb EDTA	Pb Lab	Pb Slab	Pb NH4NO3	Pb DTPA	Pb 0-20	Pb 20-50	Pb 50-200	Pb 200-2000
Pb EDTA	**1.00**								
Pb Lab	**0.91**	**1.00**							
Pb Slab	-0.80	**-0.89**	**1.00**						
Pb NH4NO3	**0.94**	**0.94**	**-0.83**	**1.00**					
Pb DTPA	0.28	0.39	-0.45	0.56	**1.00**				
Pb 0-20	**0.98**	**0.97**	**-0.89**	**0.97**	0.40	**1.00**			
Pb 20-50	**0.87**	**0.87**	**-0.85**	**0.96**	0.71	**0.92**	**1.00**		
Pb 50-200	0.56	0.56	-0.48	0.70	**0.86**	0.60	**0.82**	**1.00**	
Pb 200-2000	0.03	0.09	0.32	0.13	0.22	0.01	0.05	0.42	**1.00**

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000
Zn EDTA	**1.00**								
Zn Lab	**0.98**	**1.00**							
Zn Slab	**0.95**	**0.97**	**1.00**						
Zn NH4NO3	**0.98**	**0.98**	**0.93**	**1.00**					
Zn DTPA	0.04	0.06	-0.01	0.22	**1.00**				
Zn 0-20	**1.00**	**0.98**	**0.95**	**0.98**	0.06	**1.00**			
Zn 20-50	**0.94**	**0.92**	**0.87**	**0.98**	0.38	**0.94**	**1.00**		
Zn 50-200	**0.95**	**0.95**	**0.94**	**0.94**	-0.01	**0.95**	**0.89**	**1.00**	
Zn 200-2000	**0.90**	**0.95**	**0.98**	**0.88**	-0.07	**0.90**	0.79	**0.90**	**1.00**

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000
Cd EDTA	**1.00**								
Cd Lab	**0.97**	**1.00**							
Cd Slab	**0.87**	0.75	**1.00**						
Cd NH4NO3	**0.97**	**0.93**	**0.84**	**1.00**					
Cd DTPA	**0.86**	0.81	0.72	**0.96**	**1.00**				
Cd 0-20	**0.99**	**0.95**	**0.91**	**0.97**	**0.86**	**1.00**			
Cd 20-50	**0.97**	**0.91**	**0.87**	**1.00**	**0.95**	**0.97**	**1.00**		
Cd 50-200	**0.95**	**0.96**	0.70	**0.95**	**0.88**	**0.93**	**0.94**	**1.00**	
Cd 200-2000	**0.92**	**0.94**	**0.81**	**0.82**	0.63	**0.91**	0.81	**0.82**	**1.00**

[*Ex-EDTA*], [Ex-DTPA] = mg kg^{-1} dry soil; [Ex-NH_4NO_3] = µg kg^{-1} dry soil; [Q_i] = mg kg^{-1} dry soil; data of metal and organic carbon distribution was taken as stocks in each fraction (mg of metal or carbon present in each fraction/100g of the dry soil),

Case of Pb: The EDTA extractable, the labile Pb pool and the NH_4NO_3 extractable Pb were positively correlated with Pb localization in the <20µm and the 20-50µm fractions while the slowly labile Pb was negatively correlated with Pb localization in these fractions.

Case of Zn: All the Zn available fractions except the DTPA extractable Zn were positively correlated with Zn localization in all the physical size fractions of the soil samples.

Case of Cd: All the Cd available fractions were positively correlated with Cd localization in all the physical size fractions of the soil samples.

In short:

When some relations were found, they were found consistent with already known reactivity of soil fractions: the finer the fractions, the more reactive towards metals they are, so the least the extractability of the metal are. The complexity of the Pierrelaye mainly due to the nature of the inputs is here put forward.

3.3. Discussion:

3.3.1. Study of soil trace metal availabilities by the metal extraction at equilibrium and kinetic metal extractions

The results of the trace metal availabilities of the soils show that for all the extractants used, the percentages of extractability of Cu and Zn were higher for the soils of Pierrelaye than for the soils of Metaleurop. On the contrary the Pb and Cd percentages of extractability were higher in the soil of Metaleurop than the Pierrelaye soils. This trend between two sites was found for all the three methods used of metal extraction at equilibrium i.e. EDTA, DTPA and NH_4NO_3. However whatever the site and land use, as expected the order showed by the extractants in terms of the quantity of metal extracted was EDTA > DTPA > NH_4NO_3. Results of kinetic metal extractions showed that for both labile metal pools are higher than the slowly labile pools .The ratios of the labile to slowly labile metal pools for the soils of both sites were also comparable. These results show that the assessment of the difference of metal availabilities of the soil samples of both sites is independent of the method used for the assessment and similar trend of difference between both sites is observed whatever the method used.

However, results of trace metal availabilities of the soils under different land use at both sites showed that the trend of difference between the metal availabilities of annual crop and miscanthus soils was found to be different depending on the method used for assessment. Summary of these results is presented in the following table (Table 3.7).

Table 3.7: *Summary of the results of comparison of trace metal availabilities between annual crop and miscanthus soils studied by using different methods and extractants. The signs after metal symbols present the trend of change in metal availabilities in the soils under miscanthus compared to annual crop soils (increase ↗, decrease ↘, no change ≈).*

	Extractant/ metal pools	Metaleurop	Pierrelaye
Equilibrium	EDTA	**Cu↘, Pb↘,** Zn≈, Cd≈	Cu≈, Pb≈, **Zn↘, Cd↗**
	DTPA	Cu≈, Pb≈, Zn≈, Cd≈	Cu≈, **Pb↘**, Zn≈, Cd≈
	NH_4NO_3	**Cu↗, Pb↘, Zn↘, Cd↘**	Cu≈, Pb≈, **Zn↘**, Cd≈
Kinetic	Labile pool	**Cu↘, Pb↘,** Zn≈, Cd≈	Cu≈, **Pb↗, Zn↘, Cd↗**
	Slowly labile pool	**Cu↗, Pb↗,** Zn≈, Cd≈	Cu≈, **Pb↘**, Zn≈, Cd≈
	K_1	**Cu↗, Pb↗, Zn↗, Cd↘**	**Cu↗, Pb↗**, Zn≈, Cd≈
	K_2	**Cu↘, Pb↘,** Zn≈, Cd≈	Cu≈, Pb≈, **Zn↗, Cd↗**

In our conditions, when we studied the trace metal availabilities in the soils belonging to two sites, the magnitude of availability (percentage of extraction) was different according to the extractant used but the trend of difference observed between both sites was same for all the methods (Cu and Zn high at Pierrelaye and Pb and Cd were high at Metaleurop). But in the case of study of difference of trace metal availabilities between annual crop and miscanthus soils, the results obtained for both sites showed that DTPA extraction method did not highlighted the influence of land use while in the case of two other extractants we observed the difference of metal availabilities between annual crop and miscanthus soil samples.

Comparison of results obtained by metal extraction at equilibrium and kinetic metal extractions by using EDTA gave more insight into the difference of trace metal availabilities between annual crop and miscanthus soil samples. It was observed that at the Metaleurop site when the metal availabilities at equilibrium were different for annual crop and miscanthus soils (case of Cu and Pb but not Zn and Cd), the results of kinetic extraction showed that this change was because of the change in the labile and slowly labile pools of these metals. At the opposite at the Pierrelaye site, Zn and Cd availabilities studied at equilibrium were found different between the annual crop and the miscanthus soils but not

Cu and Pb. But results of kinetic extraction of these metals showed that the difference in Zn and Cd availabilities are due to differences in the labile pools and not the slowly labile pools. An interesting result concerns the Pb availability, which was similar in both soil samples of Pierrelaye when studied at equilibrium, but the results of kinetic extractions showed that the labile pool increased and slowly labile pool decreased keeping net Pb availability quantification similar in both soil samples. Moreover, the kinetic extraction method highlighted also the impact of land use on the extraction rate constants of the labile and slowly labile pools of metals also.

These results support the need to use complementary approaches for the assessment of trace metal availabilities of contaminated soils. Moreover rather than using two extraction methods at equilibrium, a combined approach of using equilibrium and kinetic extractions can b a promising methods to assess the impact of a perturbation (change in land use in our case) on the trace metal availabilities of the soils.

3.3.2. Effect of land use on trace metal availabilities and metal localization in physical fractions

Changes in trace metal distributions into physical size fractions between miscanthus soil sample and the annual crop soil samples at Metaleurop were more than these changes in the soil samples of Pierrelaye. Moreover it can be noticed that at Metaleurop the changes in trace metal distribution occurs for the same couple of metals for which the trace availabilities are also changed, i.e. Cu and Pb and not Zn and Cd. But for the Pierrelaye soil samples the metals for which the both types of changes occur are different i.e. trace metal availabilities of Zn and Cd were changed but metal localization changed for Cu and Pb. So the influence of land use on the soil trace metal availability and localization was more in the soils of Metaleurop than at Pierrelaye. The main difference in both sites was for the texture and the organic matter contents with Pierrelaye soils being richer in organic matter than Metaleurop, so the effect of perturbation created by change in land use from annual to miscanthus influenced the metal dynamics more in Metaleurop soils than Pierrelaye soils. In the Metaleurop site, little disturbance in soil system equilibrium created by the miscanthus cultivation caused the changes of availabilities and dynamics of metals known to be more bound to organic matter in the soils (i.e. Cu and Pb) and the trace metal availabilities of other metals remain unchanged. While in the Pierrelaye soils the changes occurred for the metals less selective to be bound to organic matter (Cd and Zn) and the changes for organic matter bound metals (Cu and Pb) were either absent or little. During the study of speciation of the metals in the soils of the plain of Pierrelaye, Dère et al., (2007) used the sequential extraction scheme proposed by Tessier et al., (1979), and found that 94% of Pb, 82% of Cu and 78% of the total soil Zn was extracted during step 3 (bound to iron and manganese oxyhydroxides and organic matter) which shows that the soil organic matter was the main metal bearing phase in these soils.

Contradictory results are reported in the studies involving trace metal availabilities and dynamics in the soils under various land use. As for as the studies related to trace metal availability in the agricultural soils under different land uses are concerned, most of these studies rely upon the sequential or single extraction methods. The only study involving kinetic extractions methods is of Fernandez-Cornudet, (2006) which reported the Zn and Pb availability in arable and permanent grasslands. But contrary to our results they found higher Pb and Zn availabilities of grassland soil compared to conventional arable soil. However in that case the metal pollutants were laid on soils by atmospheric deposits while the soil was functioning as perennial or annual cultures on the long term. In our case we are looking for characterizing the impact in management system on a pollution already established. These authors attributed the increase in metal availability under perennial grass to the earthworm activities for which the increase in metal availabilities in the casts is well known. In our case earthworms are not yet present in much quantities in our soils in miscanthus part of parcels (Hedde, 2011, personal communication).

In a study of the depth distribution and bioavailability of pollutants in long-term differently tilled soils, During et al., (2002) reported that sorption rates of metals were higher in the soil under no tillage especially for Cd and Zn suggesting reduced transport of these metals in the soil under zero or reduced tillage. Moreover they suggested that the enrichment of organic carbon in the soils under reduced or zero tillage soils may emphasize the role of soils as a sink of pollutants, reducing their leaching or transfer into crops. But in our conditions a small input of organic carbon of from the miscanthus crop has been reported in case of Metaleurop soils and not for Pierrelaye site (Beaumelle, 2011, personal communication). But our results are contrary to this study and we found the difference of availability for Cu and Pb in Metaleurop soils and not for Cd and Zn. The difference of trace metal availabilities in the soils under miscanthus and annual crop soils can be linked to the nature of plant roots exudates in both crops cultures. Researchers have found that plant root exudates can play an important role in increasing the trace metal availability in soils by dissolving the trace metals linked to various soil solid phases through low molecular organic acids often found in root exudates (Mench and Martin, 1991). Moreover, different root exudates excreted by plant roots have different binding strength with metals (Mench et al., 1987; Mench et al., 1988; Cieslinski et al., 1998), so metal speciation and availabilities in the soils under the constraint of different plant species may differ. In an *in vitro* study of the biostimulation of PAH degradation by miscanthus root exudates Técher et al., (2011) identified polyphenolics a major class of secondary plant metabolites in the miscanthus root exudates e.g. quercetin, rutin, apigenin, isovitexin, and gallic acids. It has been reported that formation of soil aggregates because of the increased release of dissolved soil organic matter influence the sorption processes and oxidation of soil functional groups changing the structure of compounds and their metal binding capacity (Huang, 1997; Wenzel and Blum, 1997). In our conditions a start of change in soil structure was observed in case of miscanthus soil at both sites because it was found that the mass percentage the fine fractions was different between miscanthus and annual crop soil (decrease in mass in the <20µm fraction in case of Pierrelaye and increase in the 20-50µm fraction in Metaleurop site). So the difference of trace metal availabilities in miscanthus and annual crops can be linked to these changes in soil structure and metal distribution too.

3.3.3. Relations between trace metal availabilities and metal localization

It is well known that trace metal availability depends on the chemical form of metals in soils but another aspect to talk about trace metal bioavailability studies, is the physical exposure of biological targets to the contaminant. This last aspect put forward the importance of the soil physical size fractions in the process of trace metal bioavailability. Studies with the aim to assess relationships between trace metal availability in bulk soils and metal distribution in physical size fractions are scare. In this study we aimed at a better understanding using a combined approach of equilibrium and kinetic trace metal extraction together with the distribution of the metals among different soil physical fractions in the soils. A summary of the relations among metal availabilities as highlighted by chemical extractions and the metal localization as determined by metal contents in physical size fractions found in our study is given in table 3.8. For the soils of Metaleurop relationships can be observed between the metal availability and the metal localization in fine fractions and the positive and negative correlations were found among them. For Pierrelaye soils most of the metal available fractions are correlated to the metal localization in more than two physical size fractions and no to a specific size fraction.

Table 3.8: *Summary of the correlations between trace metal availabilities and their distribution in physical size fractions. The signs associated with chemically extracted metal fractions give the type of correlations (Positive correlation +, negative correlation -) while the fractions under line are the soil physical size fractions with which the metal availabilities are correlated.*

Metal	Metaleurop	Pierrelaye
Cu	**NH_4NO_3 +, Q_1 -, Q_2 +** 0-20µm, 20-50µm	**EDTA +, NH_4NO_3 -, Q_1 +, Q_2 +** 0-20µm, 20-50µm, 50-200µm
Pb	**NH_4NO_3 -, Q_1 -, Q_2 +** 20-50µm	**EDTA +, NH_4NO_3 +, Q_1 +, Q_2 -** 0-20µm, 20-50µm
Zn	**EDTA +, NH_4NO_3 -** 20-50µm, 200-2000µm	**EDTA +, NH_4NO_3 +, Q_1 +, Q_2 +** 0-20µm, 20-50µm, 50-200µm, 200-2000µm
Cd	**EDTA -,DTPA +, NH_4NO_3 +, Q_1 +,** 0-20µm, 20-50µm, 50-200µm	**EDTA +,DTPA +, NH_4NO_3 +, Q_1 +, Q_2 +** 0-20µm, 20-50µm, 50-200µm, 200-2000µm

There are very few studies reporting the dynamics of trace metals in the soils in terms of their distribution among different physical size fractions of the soils. (Qian et al., 1996) studied the metal distribution in different physical size fractions and plant availability of heavy metals in 10 Chinese soils. With regard to the relative contribution of different size fractions, they reported that silt was the major fraction responsible for metal availability, primarily because of its abundance in all the soil samples. For Ni, Cu, and Pb, clay and fine sand fractions were found to have a significant influence on metal availability. In a study of the interaction of metals and organic matter in different physical size fractions Quenea et al., (2009) revealed that the quantities of the metal extracted from the size fractions increased with decreasing size but the percentage of extractability extractability of almost all metals studied (Cu, Pb, Zn and Cd) were lower in the <2µm and 20-50µm fractions than all other fractions. Similarly in a study of copper distribution among physical and chemical fractions in a vineyard soil Lagomarsino et al., (2010) reported that Cu concentration were high in silt and clay fractions and that the soil organic matter played an important role in decreasing Cu toxicity through the adsorption of the metal on humified SOM and clay-SOM complexes. In another study it was reported that the mobility, bioavailability and bioaccessibility of Pb and Zn in bulk urban soils determined by extraction methods by acetic acid, DTPA and physiologically based extraction tests correlated significantly with metal concentrations in fine silt and/or very fine sand fractions (Luo et al., 2011). Similarly some studies have also discussed the dynamics of trace metal in the soils in terms of their distribution in physical size fractions and the extraction of metals from individual fractions (Orro et al., 2009). Most of these studies either discuss the metal extractability from the individual fractions or the retention of the metals in these fractions. In such cases the aspect of soil structure and the competition among different fractions for reaction with extracting agent is not taken into account.

To our knowledge relationships of changes in trace metal localization in different fractions with changes in trace metal availability in the bulk soil samples are rarely discussed. Our results show that coupling a chemical extraction method with the metal localization in physical fractions can be a useful approach in trace metal availabilities assessments. In our conditions for example, we observed that for the soils of Metaleurop the changes in metal availabilities were accompanied by the changes in localization of these metals in physical size fractions. With the increase in Cu and Pb localization in fine size fractions, the chemically kinetically defined labile pools of these metals decreased while the slowly labile pools of these metals increased. While such trend was not observed in the soils of Pierrelaye site where the changes in metal localization in different size fractions occurred for Cu and Pb, the couple of metals for which the changes in metal availability were not observed.

3.4. ***<u>Conclusion:</u>***

In our conditions the trends of difference the of trace metal availability of the soils belonging to two different sites were found similar whatever the method of assessment used. Cu and Zn availabilities were more in the organic matter rich sandy soils and Pb and Zn in the silty clayed sandy soils belonging to the other site. Moreover the trace metal availabilities of these contaminated agricultural soils were found to be influenced by their land use, but this influence was different for the both sites studied. It was found that changing the land use from annual crop to miscanthus decreased the Cu and Pb availabilities the site with silty clayed sandy while at the other site Zn availability was decreased and Cd availability increased. Trace metal distribution in the physical size fractions of the soils was found influenced by the land use. Relationships between chemical metal availability and physical metal distribution in the physical size fractions of the soils show that in the silty clayed sandy soils metal availabilities are correlated to the metal localization in fine fractions (< 20µm and 20-50µm) but for sandy soils where fine fractions are poor trace metal availabilities are correlated to their distribution in all physical fractions. These results suggest that at the site with silty clayed sandy the changes in trace metal availabilities in the soils are accompanied by the changes in metal localization but this trend cannot be identified for the site with sandy soils. The mechanisms through which the trace metal availabilities of soils of both sites are influenced by their land uses are different. As the soils of both sites differ much in their organic matter, with sandy soils being much richer in organic matter than the silty clayed sandy soils, and the changes in land use are also known to modify soil organic matter. Further work involving the study of effect of soil organic matter on the soil trace metal availability was thus done to verify if the changes in trace metal availabilities of the soils under different land use occur mainly due to the changes in soil organic matter or not.

Chapter 4

Soil organic matter as a main parameter involved in changes in trace metal availabilities of contaminated agricultural soils

4.1. Introduction

In the previous chapter we have found that the land use of contaminated soils influences their trace metal availabilities but also that this influence differed for the soils of two sites. Furthermore we know both that 1) from the literature changes in land use of agricultural soils modify their soil organic matter and 2) the soils of the studied sites were different in their organic matter contents and C/N with Pierrelaye soil samples being 3-4 times more rich in organic matter than that of Metaleurop and the C/N of Pierrelaye soils is double than that of the Metaleurop soil samples. Our hypothesis for the work presented in the present chapter was that soil organic matter is the main factor governing the changes in trace metal availabilities in the contaminated soils and that:

1) Changing the organic matter of the soils with different organic matter contents will change the trace metal availabilities in the soils differently.

2) These changes in trace metal availabilities will be different for the soils under different land use i.e. differing in the nature of soil organic matter and its quantity in the soils

Most of the in-situ as well as in vitro studies of the assessment of role of soil organic matter in trace metal availability of contaminated soils involve adding exogenous organic matter with the input of organic amendments like sewage sludge, composts, plant parts etc in the soils. But these studies have certain discrepancies like the creation of further perturbation in soil equilibrium and in addition the results of these types of studies cannot be applied to the case of contaminated agricultural soils in which no exogenous organic matter is brought. To avoid these problems, we assessed the role of soil organic matter in the trace metal availabilities of our contaminated agricultural soils, by adopting a strategy involving a change in the soil organic matter status in-vitro by enhancing organic matter mineralization during soil incubations. For testing the above hypothesis, the changes in the trace metal availabilities after incubation were studied in the soils belonging to the two sites and with both land uses. The same combined approach of chemical fractionation of metals through extraction at equilibrium as well as kinetic metal extractions and of physical fractionation of the soils was used to assess the effect of incubation on; (1) trace metal availabilities of the bulk soils, (2) trace metal and organic carbon distribution in the physical size fractions of the soils and (3) on the evolution in the relationships between the trace metal availability in the bulk soils and trace metal and organic carbon distribution in physical size fractions of the soils.

For this the organic matter mineralization during soil incubation was measured by trapping the CO_2 produced from organic matter mineralization. Certain soil organic matter quality indicators like water soluble organic carbon and specific UV absorbance of dissolved organic matter and total organic carbon contents were also measured to study change in soil organic matter during incubation. The details of these analysis and techniques are given in material and method section.

4.2. Results and discussion:

4.2.1. Effect of soil incubation on soil organic matter and selected soil chemical properties

4.2.1.a. Mineralization of soil organic matter during incubation

Figure 4.1 presents the results of organic matter mineralization in function of time during the process of incubation. Results in percentage of the initial soil organic carbon contents of the soil samples mineralized during incubation. The results of organic matter mineralization in the four polluted soil samples belonging to two study sites are compared with the results of two non-polluted soil samples similar in their texture incubated in the same conditions what we call the reference curves.

The results show that after the 200 days of incubation at laboratory conditions the percentage of mineralized soil organic carbon was 5.9% in the annual crop soil and 6.6% in the miscanthus soil of Metaleurop site. For the soils of Pierrelaye it was found that 10.12% of the organic carbon mineralized during incubation of the non-polluted reference soil, 2.61% in annual crop soil and 3.6% in miscanthus soil.

It is apparent from the results that although the soil samples of Pierrelaye were richer in soil organic carbon contents than the soil samples of Metaleurop, their percentage of soil organic carbon mineralization was more in the soils of Metaleurop than the soils of Pierrelaye. For the reference soils, the organic carbon mineralization was more in the

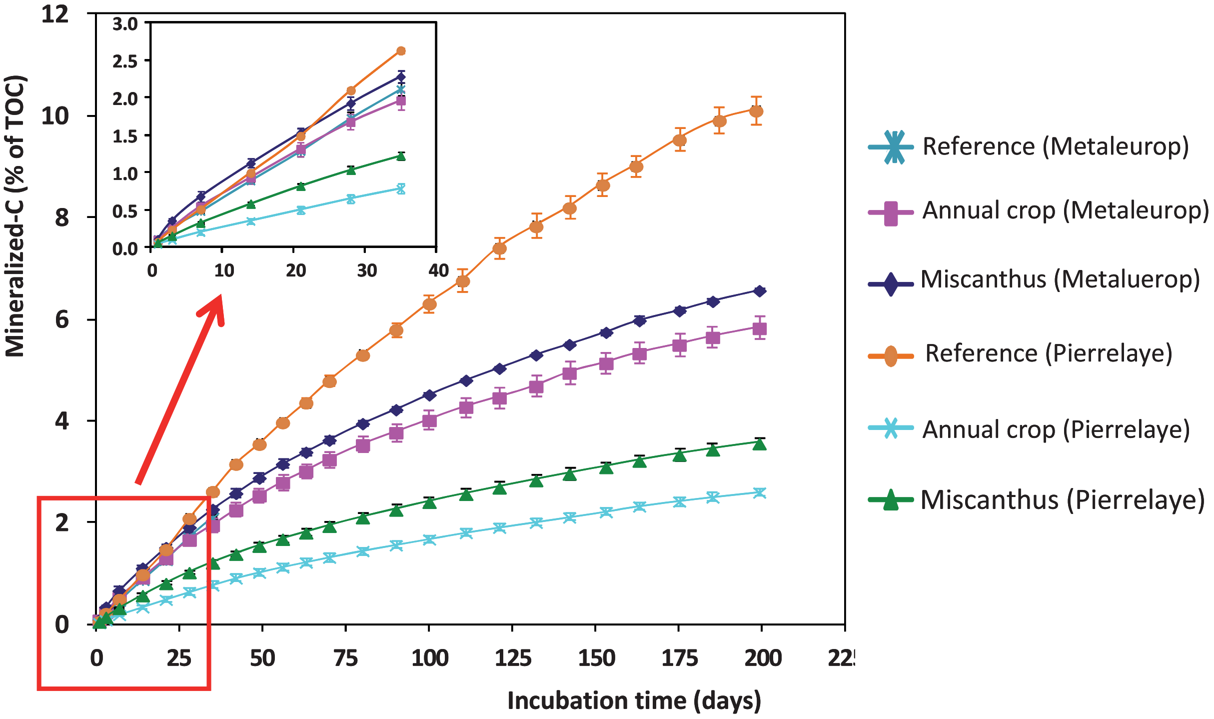


Figure 4.1: *Evolution in the mineralization of organic matter of the soils with the time of incubation (expressed as percentage of initial soil organic carbon contents). Curves represent mean values of three replicates while error bars represent standard deviations.*

reference soil of Pierrelaye than the polluted soils of this site. Unfortunately because of a problem in experiment we could not continue the incubation of reference soil of Metaleurop from day 35 onwards but until day 35, it was observed that the organic matter mineralization of reference soil was not different from the polluted soils of this site. Comparing the soils under different land use it was observed that the organic matter of the soils under miscanthus mineralized more than the soil under annual crop at both sites.

Table 4.1 : *Selected chemical characteristics of the soil samples before and after soil incubation. Data is presented on soil dry weight basis. Where present the different letters in superscript show the statistically significant difference (Tukey's test, $p < 0.01$) of different characteristics for each soil before and after incubation.*

Site	Soil		*pH*	*CEC*	C_{org} (g kg $^{-1}$)	N_{tot} (g kg $^{-1}$)	*C/N*	EC_w (mS m $^{-1}$)
Metaleurop	**Annual Crop**	***Before incubation***	7.59	13.3	16.9	1.31	12.87	9.0 ± 0.4^{b}
		After incubation	7.31	12.93	15.17	1.33	11.4	21.1 ± 0.5^{a}
	Miscanthus	***Before incubation***	7.41	13.8	17.7	1.28	13.84	7.6 ± 0.2^{b}
		After incubation	7.29	12.94	16.09	1.35	11.9	22.1 ± 1.1^{a}
Pierrelaye	**Annual Crop**	***Before incubation***	7.42	10.1	62.2	2.38	26.15	10.4 ± 0.2^{b}
		After incubation	7.03	9.87	62.68	2.42	25.9	26.0 ± 0.8^{a}
	Miscanthus	***Before incubation***	7.63	8.6	52.3	2.10	24.93	8.1 ± 0.8^{b}
		After incubation	7.11	9.09	54.06	2.15	25.2	22.7 ± 0.4^{a}

4.2.1.b. Effect of soil incubation on soil properties

Table 4.1 presents the main soil chemical properties of the soils before and after incubation. It was observed that the pH of the soils was very slightly lowered after incubation. But values of CEC, organic carbon, total nitrogen remained in the same range. The electrical conductivity of the water extracted solution, however, increased after soil incubation, indicating release of more ions in solution.

4.2.1.c. Effect of soil incubation on Water soluble organic carbon contents

Figure 4.2a presents the water soluble organic carbon contents of the soils before and after incubation. It was found that the water soluble organic carbon contents of all the soils decreased after incubation. Before incubation the water soluble organic contents were slightly higher in the soil samples of miscanthus than in the annual crop soil sample at both sites. Although the total organic carbon contents of the soil samples of Pierrelaye were 3-4 times higher than that of the soil samples of Metaleurop, but the percentage of water soluble organic carbon was less in the soils of Pierrelaye than in the Metaleurop soils indicating that the nature of organic matter were not the same and/or that the links with the solid phases were not the same.

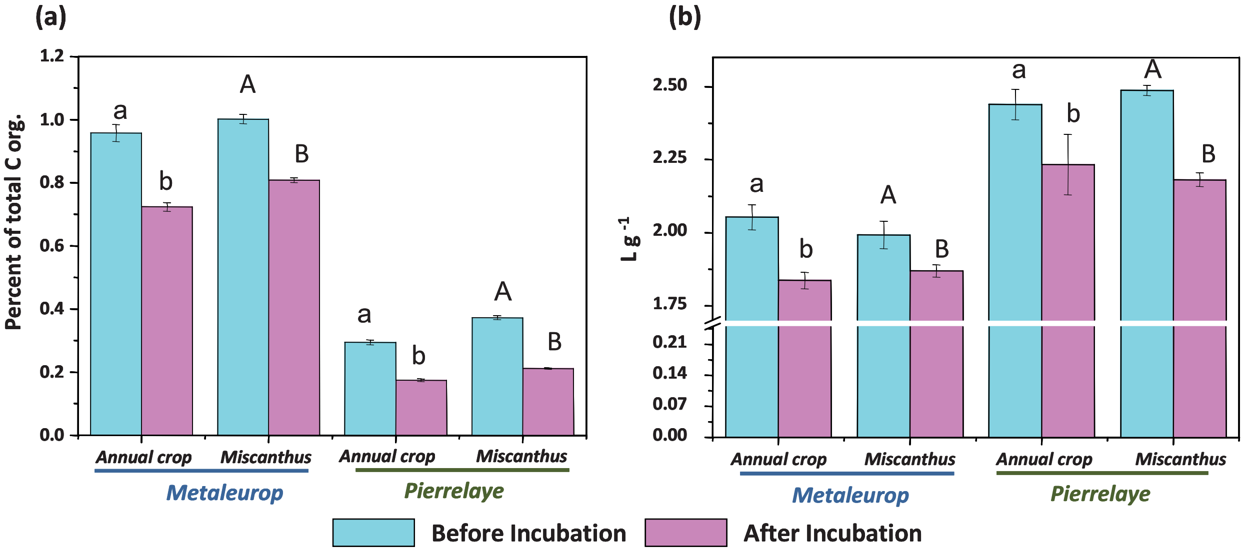


Figure 4.2 : *Water soluble organic carbon contents (a) and SUVA $_{420}$ (b) of the soil samples before and after soil incubation. Data is presented on soil dry weight basis. The statistically significant difference (Tukey's test, $p < 0.01$) for water soluble organic carbon contents and SUVA$_{420}$ of each soil before and after incubation is presented by different letters.*

4.2.1.d. Effect of soil incubation on specific UV absorbance at 420nm (SUVA 420)

Changes in the specific ultraviolet absorbance at 420nm of the soluble organic matter, an indicator of the soluble organic matter quality of the soils were measured. Results obtained are presented in the figure 4.2b. SUVA $_{420}$ values of the Pierrelaye soils were slightly higher than for the soils of Metaleurop. Before incubation the SUVA index for annual crop and miscanthus soils were similar for one given site. After incubation the SUVA $_{420}$ values were decreased for all the soils, showing that the quality of soluble organic carbon has changed as the result of processes occurring during soil incubation.

In short:
As a result of soil incubation in laboratory conditions:

- Soil organic matter mineralized and the percentage of mineralization was more for Metaleurop soils than Pierrelaye soils.
- Organic matter of miscanthus soil mineralized more than annual crop soil for both sites
- pH, CEC, C org, C/N were found similar before and after incubation
- Electrical conductivity of water extracted solution increased
- Water soluble organic carbon contents and specific UV absorbance decreased

The soils of Pierrelaye site were 3-4 fold rich in organic matter than the soils of Metaleurop. Along with the texture i.e. sandy in case of Pierrelaye soils and silty clay in case of Metaleurop soils, the soils of both sites were also different for the nature of their organic matter with Pierrelaye soils with 2 times more C/N ratios than the Metaleurop soils (Table 2.1, Chapter 2). The results of organic matter mineralization of the soils during incubation showed that the percentage of organic carbon mineralization was more in the soils of Metaleurop than the soils Pierrelaye. Taking into account the texture of soils it was likely that the organic matter of the sandy soils mineralized more than the silty clay soils because of the protection of organic matter in clayey soils through organic matter protection in aggregates or adsorption of organic complexes on clay fractions (Van Veen and Kuikman, 1990; Thomsen et al., 1999). But our results were not consistent with this mainly because of the difference of the nature of soil organic matter. In case of Pierrelaye soils the organic matter is brought with metallic and organic pollution through waste water input while in Metaleurop the contamination was through atmospheric fallout of metals. For the soils of Pierrelaye it has been reported that the soils contain many types organic molecules like pesticides, antibiotics, petroleum wastes brought through waste water input (Lamy et al., 2006; Tamtam et al., 2010) which may persist in soils. It was observed that during incubation the organic matter mineralization was more in the miscanthus soil than the annual crop soils for the soils of both sites. Several reasons can explain this result. The soils under annual crop system are continuously tilled thereby favoring the soil organic matter in field conditions whereas in the soils under miscanthus the soils are not tilled so the organic matter continues accumulating in the soils. So when the soils are incubated in laboratory the organic matter of miscanthus soils is mineralized more than the organic matter of annual crops. In case of miscanthus it is also known that fresh organic matter is added into the soils through its root system as well as by leaf shedding (Neukirchen et al., 1999; Beuch et al., 2000). Studies in the literature have reported that the fresh organic matter in the soils is degraded more rapidly than the old organic matter of the soils (Dumat et al., 2006). So during soil incubation this freshly added organic matter in the miscanthus soils is degraded more rapidly than the organic matter of the soils under annual crops. Other than this organic matter mineralization measurement, we also measured the water soluble organic carbon contents and the specific UV absorbance (SUVA) of the dissolved organic matter in the soils before and after incubation. Water soluble organic carbon is reported to be a small organic carbon fraction (McGill et al., 1986; Rochette and Gregorich, 1998). In our soils this organic carbon fractions was <1% of total organic carbon of the soils. Despite the much high organic carbon contents of Pierrelaye soils than the Metaleurop soils, the percentage of water soluble organic carbon was more in the Metaleurop soils (Figure 4.2). Water soluble organic carbon contents and SUVA of all the soils decreased after incubation. Water soluble organic carbon is the most bioavailable organic matter fraction because all microbial uptake requires water environment (Marschner and Kalbitz, 2003) so it is easily approached by soil microorganisms and degraded during soil incubation.

4.2.2. ***Influence of soil incubation on availability of the trace metals***

4.2.2.a. EDTA extractable metal contents

Metaleurop

Left sides in the graphs of figure 4.3 presents the results of the trace metal availability of the soil samples of Metaleurop before and after incubation studied by the metal extraction at equilibrium by using EDTA. The main result is that the availability of Cu increased after incubation in both annual crop and miscanthus soil samples whereas the availability of Zn decreased. An increase of 3.5% in the Cu availability of the annual crop soil sample and of 11% for the miscanthus soil sample was observed after incubation. The decrease in Zn availability after incubation was 6.6% and 3.4 % in annual crop and miscanthus soils, respectively. No change in the availability of Pb and Cd was found after incubation when comparing both annual crop and miscanthus soil samples.

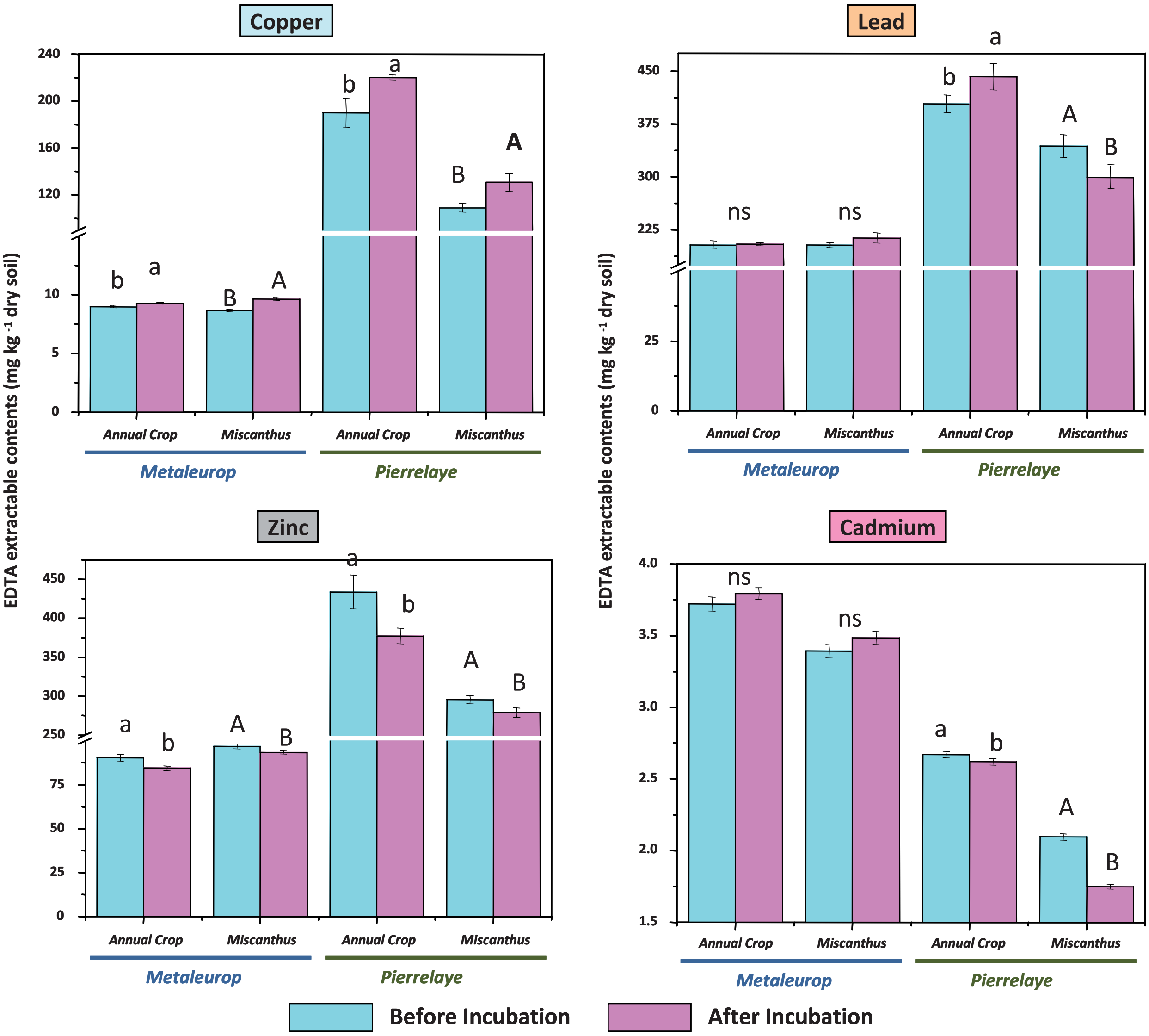


Figure 4.3: *EDTA extractable metal contents (at equilibrium) of the soils before and after incubation. Bar graphs represent mean values of 5 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, p < 0.01) for the metal contents for each soil before and after incubation is represented by different letters while "ns" represent non significant differences.*

Pierrelaye

For the soils of Pierrelaye the main result was also that in both annual crop and miscanthus soils after incubation the availability of Cu increased while the availabilities of Zn abut also that of Cd was decreased (right sides of graphs of figure 4.3). Contrasting results, however, were obtained for the availability of Pb which increased in the annual crop soil sample but decreased in miscanthus soil after incubation. Compared to the trace metal availabilities in the soils before incubation a larger increase in Cu availability than for Metaleurop was found of 16% for in annual crop soil sample and of 20% in miscanthus soil sample. The reduction in Zn availability after incubation was always higher than for Metaleurop and was 13% in the annual crop soil sample and 6% in the miscanthus soil sample. Cd availability decreased after incubation but compared to Zn the percentage of decrease was more important in the miscanthus soil (a decrease of 16%) and was less important in the annual crop soil (a decrease of 2.5%). The increase in the Pb availability of annual crop soil after incubation was by 10% while the decrease in miscanthus soil was 13% compared to its availabilities in the soils before incubation (Figure 4.3).

In short:
The influence of soil incubation on EDTA extractable metals is site specific for Pb and Cd whereas the changes in Cu and Zn were similar for the soils of both sites:

- Incubation induced an increase in Cu availability and a decrease in Zn availability
- Incubation induced a decrease in Cd only for soils of Pierrelaye site
- Only metal for which the influence of incubation was dependent of soil land use is Pb

4.2.2.b. DTPA extractable and NH4NO3 extractable (exchangeable) metal contents in the soils

Metaleurop

DTPA extractable metal contents are presented in figure 4.4 while figure 4.5 shows the NH_4NO_3 extractable metal contents. Left sides of the graphs in the figures show results from the soil samples the soils of Metaleurop. Contrary to EDTA extractions, it was observed that in all the soil samples studied, the DTPA extractable contents remained unchanged in both annual crop and miscanthus soils after soil incubation. For the NH_4NO_3 extractable metal contents from the soils, it was found that for all metals except Cd the extractability increased in the both soils after soil incubation.

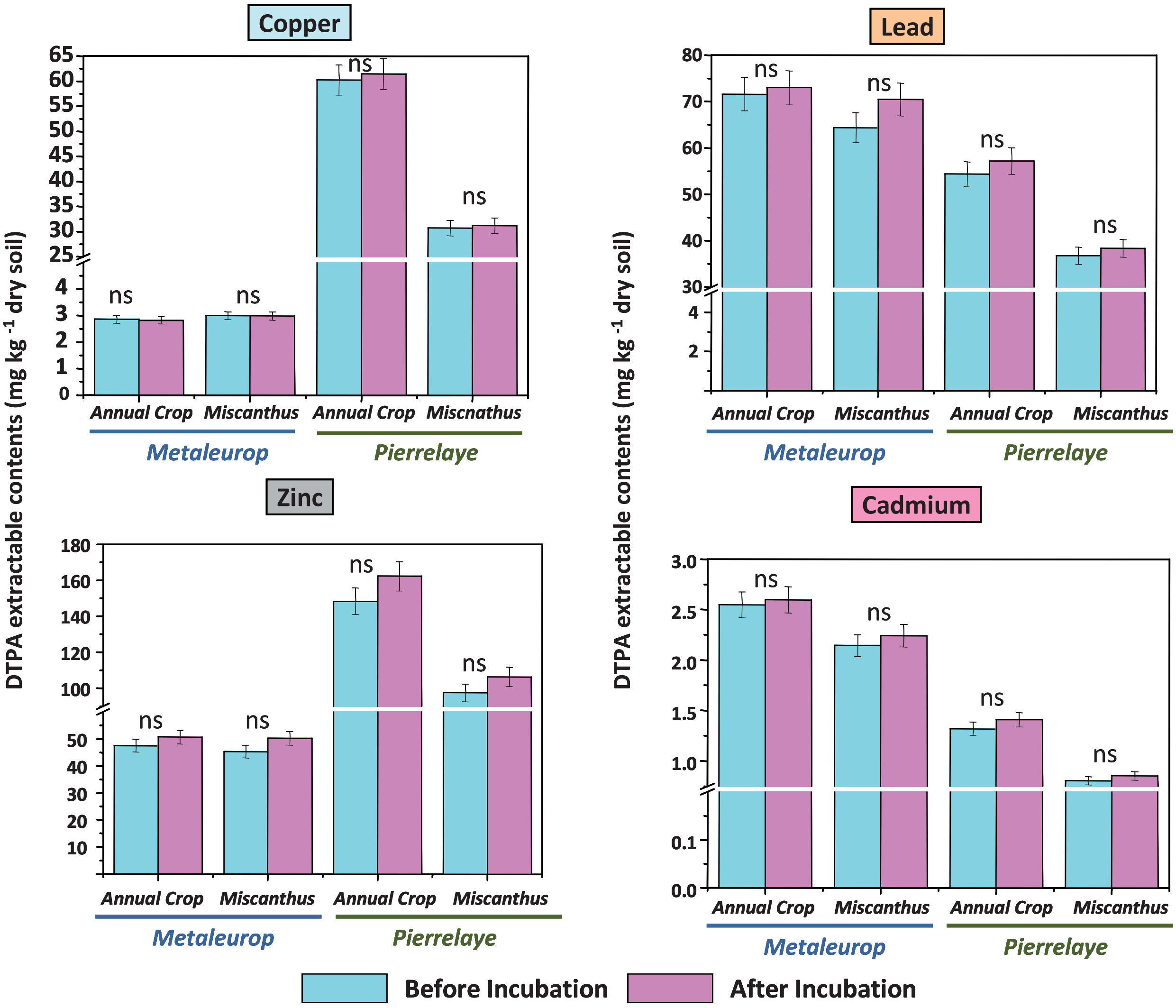


Figure 4.4: *DTPA extractable metal contents of the soils before and after soil incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.01$) for extractable metal contents for each soil before and after incubation is represented by different letters while "ns" represent non significant differences.*

Pierrelaye

DTPA extractable metal contents are presented in figure 4.4 while figure 4.5 shows the NH_4NO_3 extractable metal contents. Right sides of the graphs in the figures show results from the soil samples the soils of Pierrelaye. Similar to the soils of Metaleurop site for the soils of Pierrelaye, it was also observed that the DTPA extractable contents of all the studied metals did not changed significantly in both annual crop and miscanthus soils after soil incubation. For the NH_4NO_3 extractable metal contents of the soils, it was found that for Pb and Zn the extractability increased in both the soils after soil incubation while Cu and Cd extractability did not changed significantly in case of both soils after incubation.

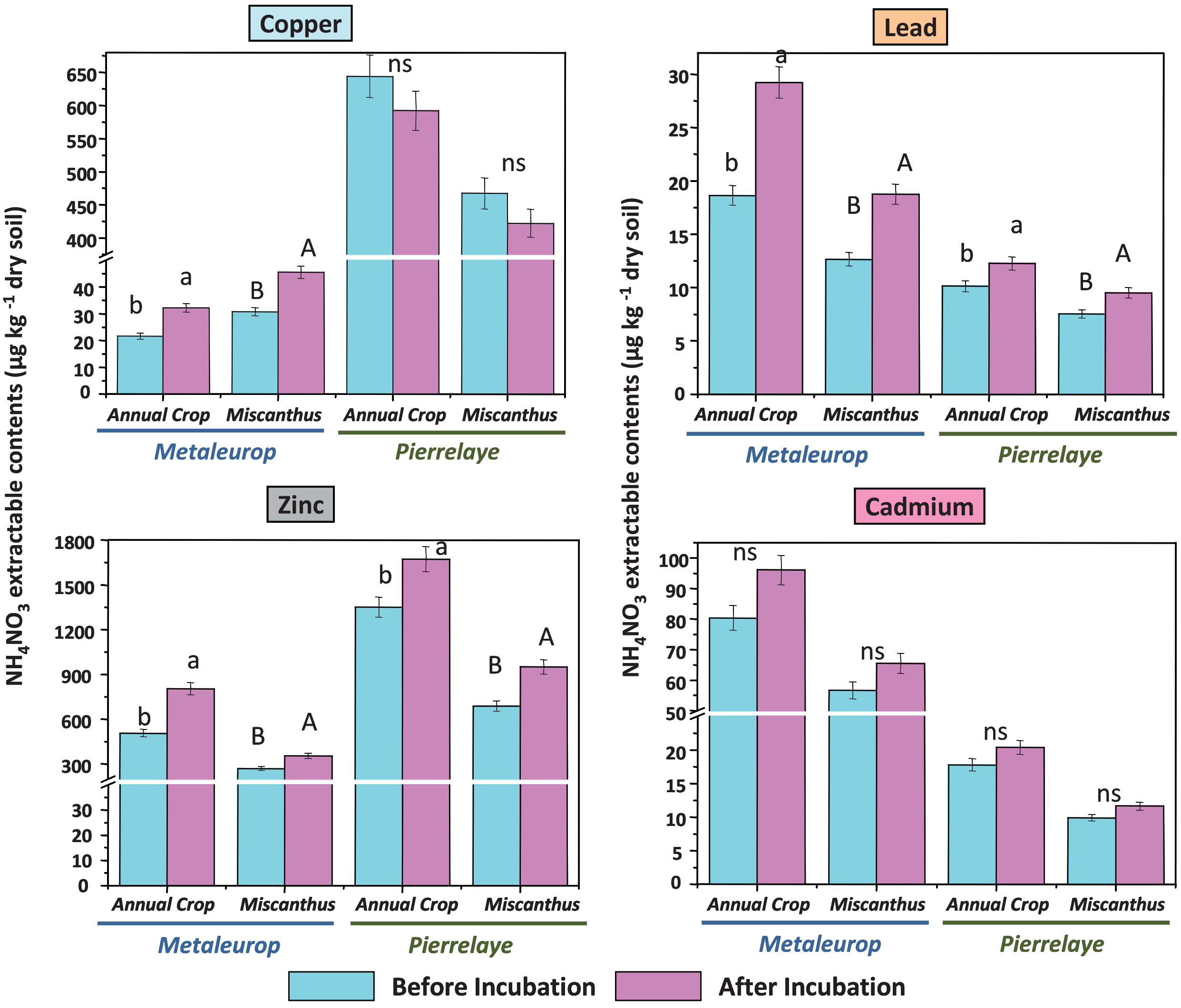


Figure 4.5: *NH_4NO_3 extractable metal contents of the soils before and after soil incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.01$) for extractable metal contents for each soil before and after incubation is represented by different letters while "ns" represent non significant differences.*

In short:

No influence of soil on DTPA extractable metal contents. The influence of soil incubation on NH_4NO_3 extractable contents was metal and site dependent:

- Incubation induced increase in NH_4NO_3 extractable Pb, Zn in all soils
- Incubation induced increase in NH_4NO_3 extractable Cu in Metaleurop soils
- No influence of incubation on NH_4NO_3 extractable Cd in all soils

4.2.2.c. Labile and slowly labile metal pools and their extraction rates constants

The results of labile and slowly labile metal pools of the soils before and after incubation are presented in figure 4.6. While table 4.2 shows the results of extraction rate constants of the labile and slowly labile pools of these soils.

<u>Case of Cu and Pb:</u>

Metaleurop

For Cu, in the annual crop soil sample the labile pool remained similar while the slowly labile pool Cu increased after incubation and in miscanthus soil the labile pool increased and the slowly labile Cu pool decreased after incubation. For Pb, both the labile and slowly labile pools were similar in the annual crop soil samples before and after incubation but in miscanthus soil as for Cu, the labile Pb pool increased and the slowly labile Pb pool decreased in the soil sample after incubation. The extraction rate constants (K_1 and K_2) of the labile and the slowly labile Cu pools of annual crop soil sample increased after incubation while no change was observed for the extraction rate constants of labile and slowly labile Pb pools of this soil sample after incubation (table 4.2). For the miscanthus soil sample it was observed that the extraction rate constants of the labile and the slowly labile pools of both Cu and Pb increased after incubation.

Pierrelaye

The results of labile and slowly labile pools showed that for the soil samples of Pierrelaye site, both the labile and the slowly labile Cu pools of the annual crop soil sample increased after incubation while for miscanthus soil sample the labile Cu pool remained similar and the slowly labile pool increased after incubation. For Pb, the labile pool of the annual crop soil sample remained similar and the slowly labile pool increased after incubation. While in the miscanthus soil sample the labile Pb pool increased and the slowly labile pool increased after soil incubation. In the annual crop soil sample the extraction rate constants (K_1 and K_2) of the labile and the slowly labile pools of Cu and extraction rate of labile pool of Pb remained similar while the extraction rate constant of the slowly labile pool decreased after incubation. In the miscanthus soil sample it was found that the extraction rate constants of labile pools of Cu and Pb remained similar while the extraction rates of slowly labile pools of Cu and Pb decreased after incubation.

<u>Case of Zn and Cd:</u>

Metaleurop

For the labile and slowly labile pools of Zn it was found that the labile pool decreased after incubation in the annual crop and in the miscanthus soil samples of Metaleurop while the slowly labile pools remained similar. For Cd, it was found that both labile and slowly

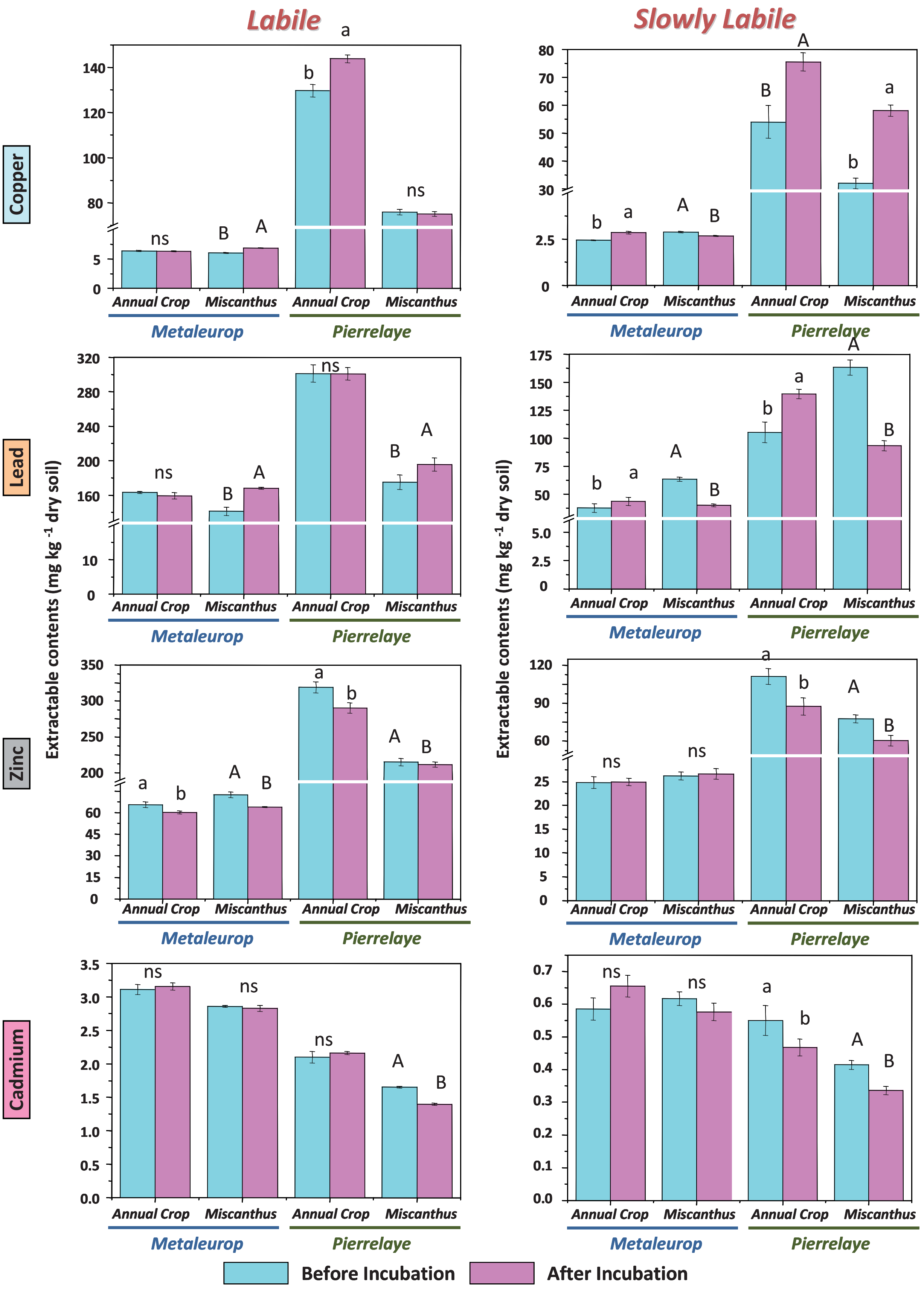


Figure 4.6: *Labile metal fraction (Q_1) and slowly labile metal fraction (Q_2) of the soil samples before and after incubation. Bar graphs represent mean values of 5 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.01$) for the metal contents for each soil before and after incubation is represented by different letters while "ns" represent non significant differences.*

labile pools remained similar after incubation in both soil samples. The extraction rate constants of the labile and the slowly labile Zn pools shows that for the annual crop soil sample the extraction rate constant of the labile Zn pool remained similar and of the slowly labile increased after soil incubation. Contrary to as found for Zn, the extraction rate constant of the labile Cd pool increase in the annual crop soil sample after incubation and the extraction rate of slowly labile Cd remained similar. For the miscanthus soil sample the extraction rate constants of labile pools of Zn and Cd remained similar while the extraction rate of slowly labile pools of both metals decreased after incubation (Table 4.2).

Table 4.2 : *Extraction rate constants of labile metal fraction (K_1) and of slowly labile metal fraction (K_2) of the soil samples before and after incubation. Mean values ± Standard Deviation (n=5). Different letters in the columns indicate pair-wise statistically significant difference (Tukey's test, $p < 0.01$) for the rate constants of the metals for each soil before and after incubation.*

Metal			***Metaleurop***		***Pierrelaye***	
			Annual Crop	*Miscanthus*	*Annual Crop*	*Miscanthus*
Cu	K_1 (min^{-1})	*Before incubation*	0.34 ± 0.004 ^{b}	0.47 ± 0.02 ^{a}	0.48 ± 0.02 ^{a}	0.44 ± 0.06 ^{a}
		After incubation	0.42 ± 0.01 ^{a}	0.37 ± 0.02 ^{b}	0.41 ± 0.06 ^{a}	0.34 ± 0.04 ^{a}
	K_2 (min^{-1}, x10^{3})	*Before incubation*	4.34 ± 0.13 ^{b}	7.46 ± 0.43 ^{a}	4.38 ± 0.65 ^{a}	8.22 ± 1.64 ^{a}
		After incubation	5.98 ± 0.30 ^{a}	4.50 ± 0.07 ^{b}	4.46 ± 0.56 ^{a}	2.42 ± 0.60 ^{b}
Pb	K_1 (min^{-1})	*Before incubation*	0.34 ± 0.01 ^{a}	0.70 ± 0.12 ^{a}	0.27 ± 0.01 ^{a}	0.40 ± 0.16 ^{a}
		After incubation	0.38 ± 0.03 ^{a}	0.35 ± 0.02 ^{b}	0.28 ± 0.02 ^{a}	0.22 ± 0.02 ^{a}
	K_2 (min^{-1}, x10^{3})	*Before incubation*	3.52 ± 0.64 ^{a}	8.98 ± 1.40 ^{a}	2.56 ± 0.43 ^{b}	10.7 ± 4.63 ^{a}
		After incubation	6.08 ± 2.34 ^{a}	4.34 ± 1.68 ^{b}	9.80 ± 1.37 ^{a}	2.86 ± 0.25 ^{b}
Zn	K_1 (min^{-1})	*Before incubation*	0.42 ± 0.02 ^{a}	0.59 ± 0.03 ^{a}	0.52 ± 0.03 ^{a}	0.42 ± 0.04 ^{a}
		After incubation	0.42 ± 0.01 ^{a}	0.47 ± 0.04 ^{b}	0.57 ± 0.06 ^{a}	0.40 ± 0.05 ^{a}
	K_2 (min^{-1}, x10^{3})	*Before incubation*	2.10 ± 0.37 ^{b}	2.24 ± 0.28 ^{b}	6.60 ± 1.03 ^{a}	6.62 ± 0.58 ^{a}
		After incubation	3.26 ± 0.29 ^{a}	3.70 ± 0.35 ^{a}	6.50 ± 1.35 ^{a}	4.12 ± 0.93 ^{b}
Cd	K_1 (min^{-1})	*Before incubation*	0.42 ± 0.02 ^{b}	0.38 ± 0.01 ^{b}	0.53 ± 0.05 ^{a}	0.41 ± 0.04 ^{a}
		After incubation	0.48 ± 0.03 ^{a}	0.50 ± 0.03 ^{a}	0.42 ± 0.03 ^{b}	0.38 ± 0.05 ^{a}
	K_2 (min^{-1}, x10^{3})	*Before incubation*	4.70 ± 0.76 ^{a}	8.72 ± 0.90 ^{a}	8.70 ± 2.20 ^{a}	7.62 ± 0.63 ^{a}
		After incubation	4.60 ± 0.46 ^{a}	8.86 ± 1.74 ^{a}	4.64 ± 0.73 ^{b}	4.58 ± 0.76 ^{b}

Pierrelaye

For the soils at Pierrelaye it was found that both labile and slowly labile Zn pools decreased in the annual crop soil after incubation while in miscanthus soil the labile Zn pool remained similar but the slowly labile pool decreased after incubation. Similarly for Cd the labile pool of the annual crop soil remains similar but the slowly labile Cd pool decreased after incubation. While for the miscanthus soil, both the labile and the slowly labile Cd pools decreased after soil incubation (Figure 4.6). For the annual crop soil sample, no change in the extraction rate constants (K_1 and K_2) of the labile and slowly labile Zn pools was observed after incubation whereas the extraction rate constants of both labile and slowly labile pools of Cd were decreased after incubation. For the miscanthus soil sample the extraction rate constants of the labile pools of Zn and Cd did not changed after incubation whereas the extractions rate constants of the slowly labile pools of both metals decreased after incubation (Table 4.2).

In short:
The influence of soil incubation on labile and slowly labile metal pools and the extraction rates of these metal pools was site specific and was metal dependant. Contrary to extractions at equilibrium, the influence of soil incubation studied through kinetic extractions was different for the annual crop and the miscanthus soils of both sites.

Table 4.3 : *Summary of the results of changes in trace metal availabilities after soil incubation studied by using metal extraction at equilibrium and kinetic metal extractions. Where for extractions at equilibrium Ext 1= EDTA, Ext 2= DTPA, Ext 3= NH_4NO_3 and for kinetic extractions Q_1=labile pool, Q_2= slowly labile pool, K_1= Extraction rate of labile pool and K_2= Extraction rate of slowly labile pool. The signs in front of metals present the trend of change in metal availabilities in the soils after incubation compared to the soil before incubation (increase ↗, decrease ↘, no change ≈).*

	Metal	Metaleurop		Pierrelaye	
		Annual crop	*Miscanthus*	*Annual crop*	*Miscanthus*
Equilibrium	Cu	Ext 1↗, Ext 2≈, Ext 3↗	Ext 1↗, Ext 2≈, Ext 3↗	Ext 1↗, Ext 2≈, Ext 3≈	Ext 1↗, Ext 2≈, Ext 3≈
	Pb	Ext 1≈, Ext 2≈, Ext 3↗	Ext 1≈, Ext 2≈, Ext 3↗	Ext 1↗, Ext 2≈, Ext 3↗	Ext 1↘, Ext 2≈, Ext 3↗
	Zn	Ext 1↘, Ext 2≈, Ext 3↗	Ext 1↘, Ext 2≈, Ext 3↗	Ext 1↘, Ext 2≈, Ext 3↗	Ext 1↘, Ext 2≈, Ext 3↗
	Cd	Ext 1≈, Ext 2≈, Ext 3≈	Ext 1≈, Ext 2≈, Ext 3≈	Ext 1↘, Ext 2≈, Ext 3≈	Ext 1↘, Ext 2≈, Ext 3≈
Kinetic	Cu	Q_1≈, Q_2↗, K_1↗, K_2↗	Q_1↗, Q_2↘, K_1↘, K_2↘	Q_1↗, Q_2↗, K_1≈, K_2≈	Q_1≈, Q_2↗, K_1≈, K_2↘
	Pb	Q_1≈, Q_2≈, K_1≈, K_2≈	Q_1↗, Q_2↘, K_1↘, K_2↘	Q_1≈, Q_2↗, K_1≈, K_2↗	Q_1↗, Q_2↘, K_1≈, K_2↘
	Zn	Q_1↘, Q_2≈, K_1≈, K_2↗	Q_1↘, Q_2≈, K_1↘, K_2↗	Q_1↘, Q_2↘, K_1≈, K_2≈	Q_1≈, Q_2↘, K_1≈, K_2↘
	Cd	Q_1≈, Q_2≈,,K_1↗ ,K_2≈	Q_1≈, Q_2≈, K_1↗, K_2≈	Q_1≈, Q_2↘, K_1↘, K_2↘	Q_1↘, Q_2↘, K_1≈, K_2↘

The summary of results is presented in table 4.3. The results of the extractions at equilibrium showed that the changes in availabilities were dependent on the metal studied and were similar for the soils belonging to the same site i.e. similar changes were observed in the trace metal availabilities of annual crop and miscanthus soils of the same site after soil incubation. On the contrary the metal extraction with DTPA failed to highlight changes in trace metal availabilities in the soils after incubation in our conditions while the changes in metal availabilities highlighted through NH_4NO_3 extractions were found as EDTA, site and metal specific. The results of metal availabilities studied through EDTA extraction at equilibrium showed that Cu availability was increased in all the soils after incubation and Zn availability was decreased in all the soils. Whereas Cd and Pb availabilities studied with EDTA extraction did not change for the soils of Metaleurop. For Pierrelaye soils Cd availabilities decreased for both annual crop and miscanthus soils, while Pb availability in miscanthus soil decreased and increased in annual crop soil after incubation. For NH_4NO_3 extractable metals, it was found that in Metaleurop soils the extraction of all metals except Cd was increased while in Pierrelaye soils Pb and Zn extraction increased after soil incubation.

The measurement of labile and slowly labile pools and their extraction rates through kinetic extractions instead of studying a single available metal fraction at equilibrium have given us more detailed insights into the changes occurring in the trace metal availabilities of the soils after incubation. The results of kinetic extractions have highlighted that the changes occurred in the annual crop and miscanthus soils of the same site after incubation were not always similar contrary to what we observed through studying equilibrium extraction. It was found either that the change (increase or decrease) in metal availability through extraction at equilibrium was because of change in a single pool (labile or slowly labile metal pool) or that the change was the additive effect of change in both pools. For example the results of EDTA extraction of Cu showed that Cu availability increased in all the soils of both sites after incubation. Although this increase in Cu availability of Pierrelaye soils was more than the soils of Metaleurop the increase in Cu availability of annual crop compared to miscanthus soil of a given site was similar. But the results of kinetic extraction showed that the changes occurring in annual crop and miscanthus soils of a given site were not similar. In the annual crop soil of Metaleurop only the labile pool increased after soil incubation while in miscanthus soil labile pool increased and slowly labile pool decreased but the increase in labile pool was more than the slowly labile pool. Similarly for the soils of Pierrelaye it was observed that both labile and slowly labile Cu pools increased in the annual crop soil while in miscanthus soil only the slowly labile pool increased. The changes in extraction rate constants of labile and slowly labile Cu pools were also not similar in both soils of a site and it was found that in annual crop soil of Metaleurop the extraction rates of both pools are increased while in miscanthus soil the extraction rates of both pools is decreased. In case of Pierrelaye soils the only change in extraction rate constants was of slowly labile pool in miscanthus soil which increased. Similar cases were observed for all the other metals studied and it was found that in one soil only one pool changed while in the other soil both labile and slowly labile pool changed.

Several studies have reported the changes in trace metal availabilities of the soils during incubation but main of these studies were conducted on artificially polluted soils, so conclusions cannot be strictly compared because of the processes of ageing etc. Studies where metals were laid on soils with organic amendments in the form plant leafs, sewage sludge or composts are more in the case of our study with the Pierrelaye site (Leita et al., 1995; Boucher et al., 2005b; Donner et al., 2010; Santos et al., 2010). The changes in trace metal availabilities during soil incubation in our study can be explained by several reasons. The fact that NH_4NO_3 extractable contents of the metals were increased after soil incubation could be due to a release during the organic matter mineralization in the soils. NH_4NO_3 is known to extract metals loosely bound to the exchange sites of the soils (Schramel et al., 2000; Gupta and Sinha, 2007). Among the four metals studied, Cu and Pb are known to be more bound to soil organic matter than the Cd and Zn (Harter and Naidu, 1995; Stalikas et al., 1999). So there is a possibility that during soil incubation the Cu and Pb are transformed to more available forms after organic matter mineralization. Zn availability during soil incubation is reported to be decreased through the immobilization of Zn by microbial biomass (Leita et al., 1995).

4.2.3. *Changes in mass, organic carbon and trace metal localization in different size fractions during incubation*

4.2.3.a. Mass distribution in different size fractions of the soils

Physical size fractionation of the soils before and after incubation was performed to study the effect of soil incubation. Figure 4.7 and figure 4.8 present the mass distribution in the soils before and after incubation while the percentage recoveries of the soil mass after physical fractionation are given in table 4.4. The percentage recoveries of the mass for all soils were 97-99%.

Metaleurop

It was found that in the annual crop soil of Metaleurop the soil mass in the <20µm fraction was lower and in the 20-50µm fraction was high after incubation than the corresponding fractions of the sample before incubation. For the miscanthus soil it was observed that the mass in the 20-50µm fraction was lower after incubation than the corresponding fraction of the soil sample before incubation while the mass of the other fractions remained similar.

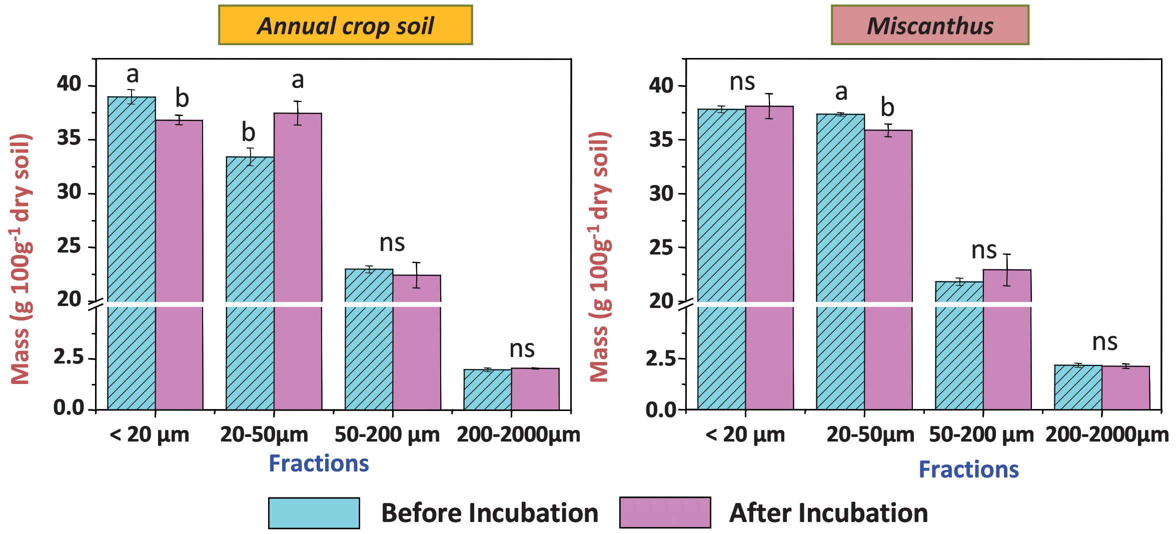


Figure 4.7: *Distribution of the soil mass into different physical size fractions of the soils of Metaleurop site before and after incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.05$) for the mass of a given fraction before and after incubation of the soils is showed by different letters while "ns" represent non significant differences.*

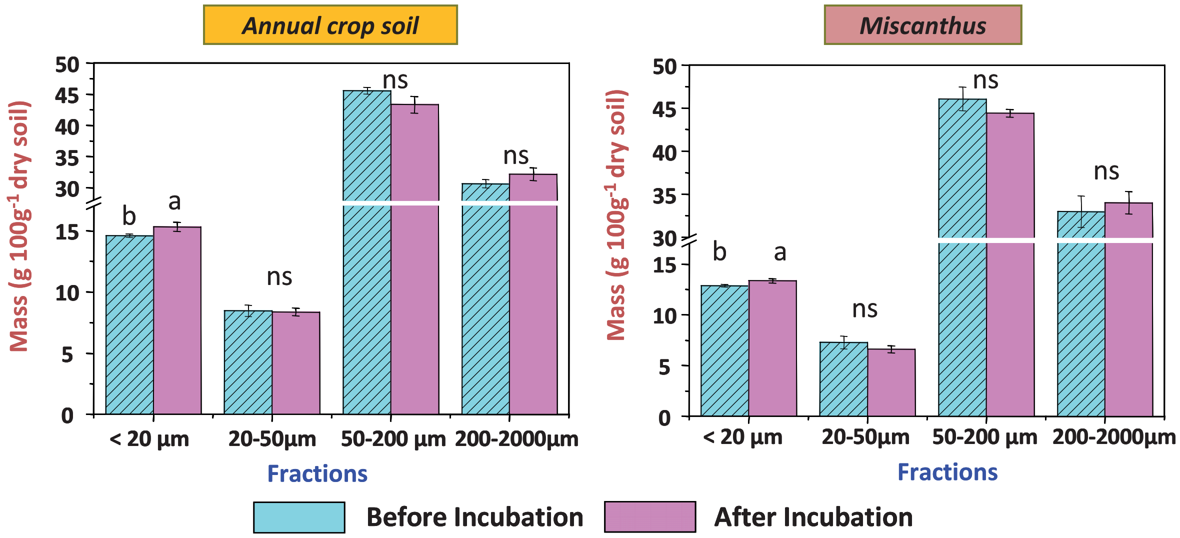


Figure 4.8: *Distribution of the soil mass into different physical size fractions of the soils of Pierrelaye site before and after incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.05$) for the mass of a given fraction before and after incubation of the soils is showed by different letters while "ns" represent non significant differences.*

Table 4.4: *Percentage of mass recovery after physical fractionation of the soils before and after soil incubation.*

Site	Soils	Before Incubation	After Incubation
Metaleurop	***Annual crop***	97.4 ± 1.3	98.8 ± 0.3
	Miscanthus	99.2 ± 0.2	99.2 ± 0.3
Pierrelaye	***Annual crop***	99.3 ± 0.1	99.2 ± 0.1
	Miscanthus	99.2 ± 0.2	98.4 ± 1.2

Pierrelaye

On the contrary for both the annual crop and miscanthus soil samples of the Pierrelaye site it was observed that the mass in the <20µm fraction was higher after incubation than the corresponding fractions of the samples before incubation. While the mass distribution in the other fractions of the samples remained similar in both the soil samples.

In short:

The influence of soil incubation on mass distribution of the soils in the physical size fractions was site dependent:

- Land use impacts the result of incubation for soils of Metaleurop site but not for Pierrelaye where same trends were observed:

4.2.3.b. Trace metals distribution in different size fractions of the soils

The results of the distribution of trace metals in different physical fractions of the soils of Metaleurop site before and after incubation are presented in the figure 4.9. While figure 4.10 shows the trace metal distribution in the physical size fractions for the soils of Pierrelaye before and after incubation. The percentages of metals recoveries of the soils are presented in table 4.6. The changes in metal localization were site specific and generally for Pierrelaye soils metal localization increased in <20µm fractions and for Metaleurop soils increased localization were observed in <20 and 20-50µm in most cases. Decrease in metal localizations was observed for coarse fractions especially for Zn at both sites and Cd in Metaleurop soils. The detailed results of the metal localization are presented in the following:

Metaleurop

The results of the copper distribution among the physical size fractions of the soil samples of Metaleurop site before and after incubation are presented in figure 4.9. It was observed that in the both annual crop and miscanthus soil samples the Cu and Pb localization changed only in one size fraction after incubation but this fraction was different for both metals. Contrarily, the changes in Cd and Zn localization occurred in more than one size fractions for both the annual and miscanthus soils samples after incubation. The type of these changes i.e. increases or decreases are described for each metal in the following:

Case of copper:

In the annual crop soil sample, the Cu percentage was higher in the 20-50µm fraction after incubation than the corresponding fractions of the sample before incubation. For the miscanthus soil sample the Cu percentage in the <20µm fraction was higher after incubation compared to the corresponding fraction in the sample before incubation. In the other size fractions of both samples Cu distribution remained unchanged during incubation.

Case of lead:

Concerning Pb distribution in different size fractions in both the annual crop and the miscanthus soils of Metaleurop site the Pb percentage was higher in the coarse fraction 200-2000µm fractions after incubation but in all other size fractions of these soil samples Pb distribution remained similar in the samples before and after incubation .

Case of zinc:

For the annual crop soil sample, the Zn localization was higher in the 20-50µm fraction and was lower in the 200-2000µm fraction in the sample after soil incubation than the corresponding fractions of sample before incubation. Similarly for the miscanthus soil sample, Zn localization was higher in the < 20µm fraction and was lower in the 20-50µm fractions of the sample after incubation than the corresponding fractions before incubation. While Zn distribution remained similar in the other fractions of the samples before and after incubation.

Case of cadmium:

It was observed that the Cd percentages in the coarse 200-2000µm fraction and in 50-200µm fraction were lower in the annual crop soil sample after soil incubation while in

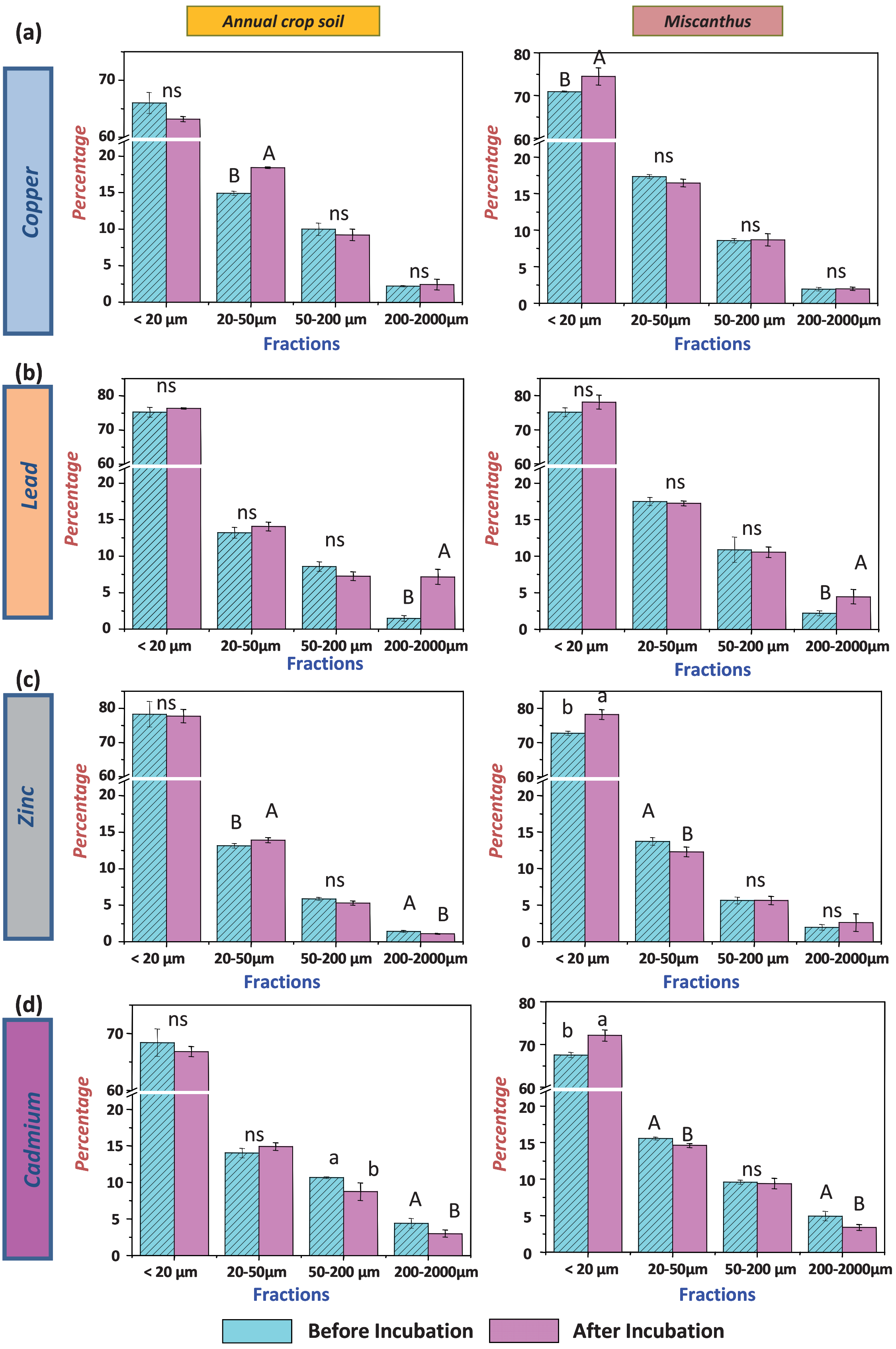


Figure 4.9: *Distribution of trace metals in the different physical size fractions of the soils of Metaleurop before and after incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistical difference (Tukey's test, p < 0.05) for the metal percentage in the fractions before and after incubation of the soils is showed by different letters while "ns" represent non significant differences.*

the rest of the fractions the Cd distribution did not changed significantly. In the miscanthus soil of Metaleurop site Cd distribution in 200-2000µm fraction and in 20-50µm fractions was lower and was higher in the <20µm fraction after incubation than the corresponding fractions of the sample before incubation.

Pierrelaye

Figure 4.10 shows the trace metal distribution in the physical size fractions in the samples before and after incubation for the soil samples of the Pierrelaye site. It was found that in the both annual crop and miscanthus soil samples and for all metals, the localization of metals was higher in the <20µm fractions after soil incubation than the corresponding fractions of the samples before incubation.

Case of copper:

The Cu percentages in the <20µm fractions of both the annual crop and the miscanthus soil samples was higher in the samples after incubation than the corresponding fractions of the samples before incubation. Cu distribution in other size fractions of the samples of both the annual crop and the miscanthus soils remained unchanged during incubation.

Case of lead:

As for Cu the Pb percentages in the <20µm fractions of the annual crop soil sample was higher in the sample after incubation than the corresponding fraction of the sample before incubation. Pb distribution in other size fractions of the annual crop soil sample and in all the size fractions of the miscanthus soil samples remained unchanged during incubation.

Case of zinc:

For the soils of Pierrelaye site, the Zn percentage was higher in the <20µm fraction of both the annual crop and the miscanthus soil samples after incubation than the corresponding fractions before incubation. In all the other fractions of annual crop soil sample and in 20-50µm fraction of the miscanthus soil sample Zn percentages were lower in the samples after incubation than the corresponding fractions of the samples before incubation.

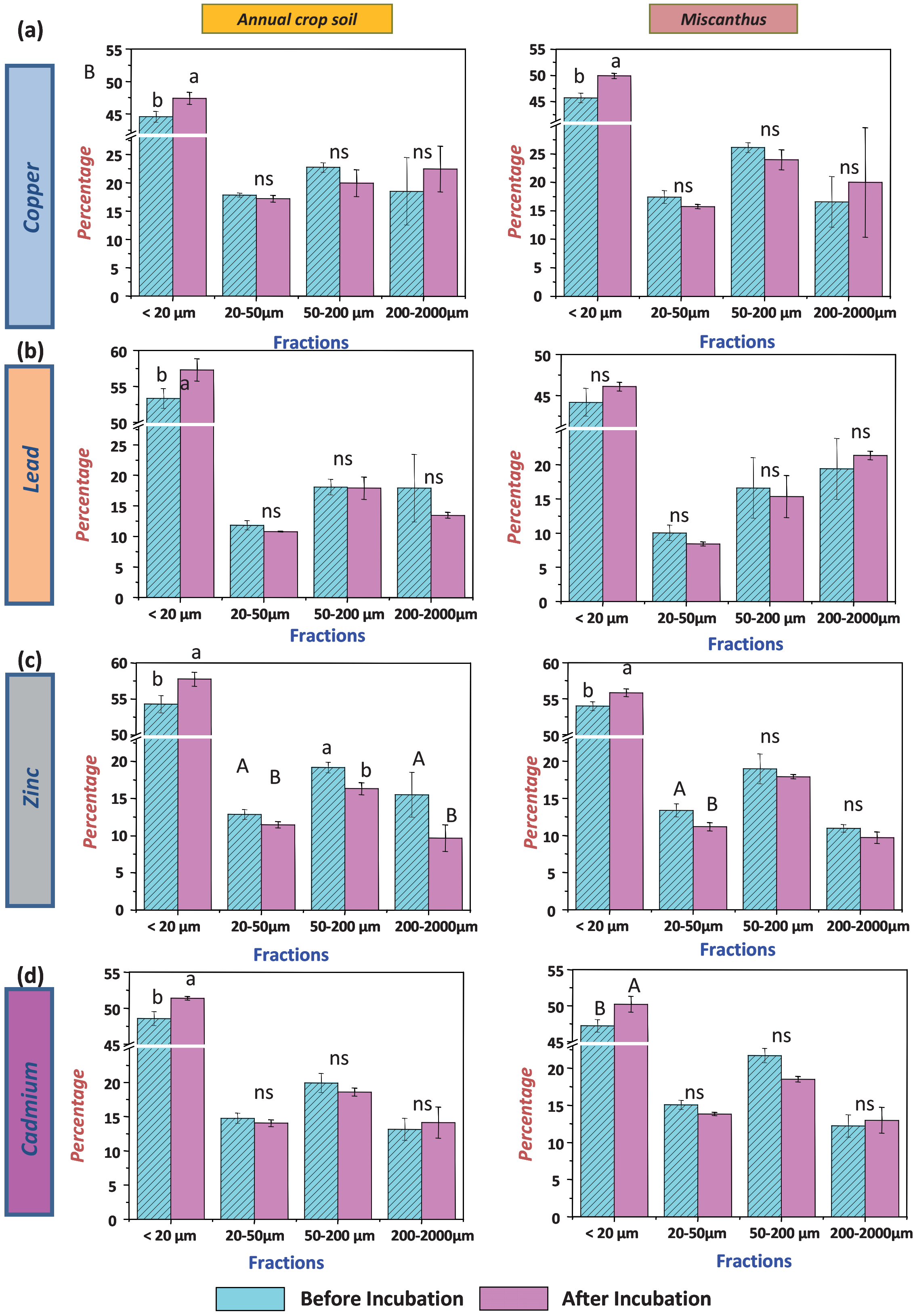


Figure 4.10: *Distribution of trace metals in the different physical size fractions of the soils of Pierrelaye before and after incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistical difference (Tukey's test, p < 0.05) for the metal percentage in the fractions before and after incubation of the soils is showed by different letters while "ns" represent non significant differences.*

Case of cadmium:

As for Cu and Pb, the Cd percentage was higher in the <20µm fractions of both the annual crop and the miscanthus soil samples after incubation than that of the corresponding fractions in samples before incubation. Cd distribution in the other size fractions of both the annual crop and the miscanthus soil samples before and after incubation remained similar.

In short:
The changes in metal localization were site specific and generally for Pierrelaye soils soil incubation induced increase in metal localization in <20µm fractions and for Metaleurop soils increased localization were observed in <20 and 20-50µm in most cases. Decrease in metal localizations was observed for coarse fractions especially for Zn at both sites and Cd in Metaleurop soils.

4.2.3.c. Distribution of total organic carbon contents into different physical size fractions of the soils

The results of the organic carbon distribution in the physical size fractions of the soils before and after incubation are presented in the figure 4.11 while the percentage recoveries are given in table 4.5.

Metaleurop

For annual crop soil the organic carbon percentage of was higher in the <20µm fraction and in the 20-50µm fractions and was lower in the 50-200µm fraction of the sample after incubation than in the corresponding fractions of the sample before incubation. For miscanthus soil the organic carbon percentage was higher in the <20µm fraction of the sample after incubation than the corresponding fractions in the sample before incubation. In the other fractions of miscanthus soil the organic carbon distribution remained unchanged during incubation.

Pierrelaye

Before and after incubation the annual crop soil have similar distribution of organic carbon in all the physical size fractions. By contrast in the miscanthus soil sample the organic carbon percentage was higher in the <20µm fraction after soil incubation than in the sample before incubation while the organic carbon distribution remain unchanged in the other size fractions of the soil samples.

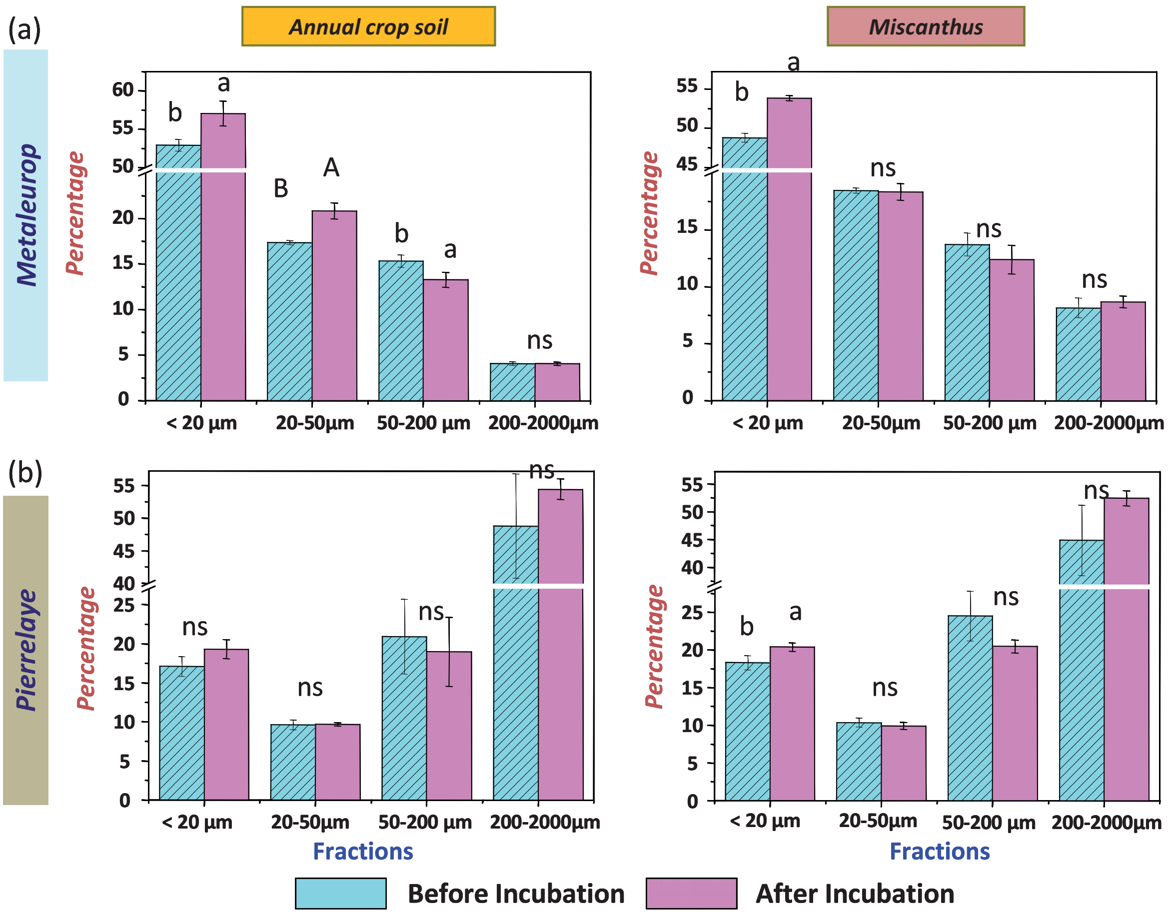


Figure 4.11: *Distribution of organic carbon in the different physical size fractions. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistical difference (Tukey's test, $p < 0.05$) for organic carbon percentage in the fractions before and after incubation of the soils is showed by different letters while "ns" represent non significant differences.*

Table 4.5: *Percentage of metal recovery after physical fractionation of the soils before and incubation.*

Elements	Site	Soils	Before Incubation	After Incubation
Cu	**Metaleurop**	***Annual crop***	93.2 ± 1.8	93.2 ± 0.4
		Miscanthus	98.8 ± 0.1	101.6 ± 1.0
	Pierrelaye	***Annual crop***	103.6 ± 6.2	107.0 ± 2.8
		Miscanthus	105.8 ± 3.0	109.6 ± 9.3
Pb	**Metaleurop**	***Annual crop***	98.5 ± 1.6	104.8 ± 2.2
		Miscanthus	105.7 ± 1.2	110.4 ± 0.9
	Pierrelaye	***Annual crop***	101.2 ± 4.2	99.5 ± 3.6
		Miscanthus	90.3 ± 9.4	91.3 ± 3.9
Zn	**Metaleurop**	***Annual crop***	98.8 ± 3.9	98.1 ± 1.5
		Miscanthus	94.1 ± 0.9	98.8 ± 1.2
	Pierrelaye	***Annual crop***	101.9 ± 2.9	95.4 ± 1.7
		Miscanthus	97.3 ± 2.2	94.6 ± 0.8
Cd	**Metaleurop**	***Annual crop***	97.5 ± 2.3	93.5 ± 1.0
		Miscanthus	97.7 ± 0.7	99.5 ± 1.1
	Pierrelaye	***Annual crop***	96.4 ± 0.8	98.2 ± 2.1
		Miscanthus	96.2 ± 0.8	95.5 ± 3.2
C_{org}	**Metaleurop**	***Annual crop***	89.7 ± 0.7	98.1 ± 1.5
		Miscanthus	89.2 ± 1.6	93.3 ± 1.0
	Pierrelaye	***Annual crop***	96.4 ± 5.4	102.4 ± 2.2
		Miscanthus	98.0 ± 3.9	103.2 ± 1.3

In short:

The influence of soil incubation on organic carbon in the physical size fractions of the soils was site specific and it was different according to land use

- Metaleurop:
  - Increased organic carbon localization in the <20µm fractions of both soils but a decrease in the 20-50µm fractions in annual crop soils
- Pierrelaye:
  - No change in organic carbon distribution in size fractions of the annual crop soil while increased organic carbon localization in the <20µm fraction of the miscanthus soil

The comparison of the changes in trace metals and organic carbon distribution in physical size fractions of the soils after incubation are summarized in table 4.6. As the soils were incubated in closed jars with no metal loss, the changes in metal localization between fractions were thus redistributed in other fractions. Indeed we verified the similar total metal contents of the soils before and after incubation. In addition the percentage recoveries of the metal distribution in the physical fractions before and after incubation were also comparable (Table 4.5). It can be observed that in most of the cases the metals as well as carbon localization increased in 0-20µm or 20-50µm fractions (F1 in Table 4.6) with few exceptions. For Cu and Pb in all soils and Cd in the Pierrelaye soils the decrease in other fractions was not statistically significant showing that the increase in metal localization in a single fraction (0-20µm fraction except Pb in Metaleurop soils) is contributed by the decrease in metal localization from all other fractions.

Table 4.6: *Summary of the results of changes in trace metal and organic carbon distribution in physical size fractions of the soils after incubation. Where F_1 =0-20µm, F_2 =20-50µm, F_3 =50-200µm, and F_4 =200-2000µm fraction. The signs in front of fractions show the trend of change in distribution in the soils after incubation compared to the soil before incubation (increase ↗, decrease ↘, no change ≈).*

	Metaleurop		**Pierrelaye**	
	Annual crop	***Miscanthus***	***Annual crop***	***Miscanthus***
Cu	F_1≈, F_2 ↗, F_3 ≈, F_4 ≈	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈
Pb	F_1≈, F_2 ≈, F_3 ≈, F_4 ↗	F_1≈, F_2 ≈, F_3 ≈, F_4 ↗	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈	F_1≈, F_2 ≈, F_3 ≈, F_4 ≈
Zn	F_1≈, F_2 ↗, F_3 ≈, F_4 ↘	F_1↗, F_2 ↘, F_3 ≈, F_4 ≈	F_1↗, F_2 ↘, F_3 ↘, F_4 ↘	F_1↗, F_2 ↘, F_3 ≈, F_4 ≈
Cd	F_1≈, F_2 ≈, F_3 ↘, F_4 ↘	F_1↗, F_2 ↘, F_3 ≈, F_4↘	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈
C org.	F_1↗, F_2 ↘, F_3 ↘, F_4 ≈	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈	F_1≈, F_2 ≈, F_3 ≈, F_4 ≈	F_1↗, F_2 ≈, F_3 ≈, F_4 ≈

Whereas in case of Zn in all soils and Cd in miscanthus soil of Metaleurop can be observed that the increase in the metal localization in fine fractions (0- 20µm or 20-50µm) was contributed by decrease in metal localization of coarse fractions (50-20µm and 200-2000µm). For organic carbon, keeping in view the changes in total organic carbon contents of soils due to organic matter mineralization during incubation, the distribution in physical size fractions of the soils before and after incubation was calculated by using the total organic carbon contents of the soils before and after incubation. For the annual crop soil of Pierrelaye there was no change in the organic carbon localization in its physical fractions while in all other soils organic carbon localization was increased in the 0-20µm fraction. This increase in organic carbon localization in the 0-20µm fractions of miscanthus soils was contributed by overall change in all other fractions because no significant change in any other particular fraction was found. Concerning the annual crop soil of Metaleurop, the increase of organic carbon in 0-20µm fractions was contributed by 20-50µm and 50-200µm fractions for which a significant decrease in the localization was observed.

At this stage of study we did not separated particulate organic matter fractions and global organo-mineral fractions were studied. But it is evident from literature and from the results of studies already conducted in our laboratory that particulate organic matter (POM) is the labile fraction of organic matter and is vulnerable to more rapid degradation than the organic matter in the fine fractions (Cambardella and Elliott, 1992; Besnard et al., 2001). In case of contaminated soils these are fractions of soil which are many fold enriched in trace metals (Ducaroir and Lamy, 1995; Balabane et al., 1999; Balabane and van Oort, 2002). During a study combining fate of Zn and Cd from plant materials and the degradation of metallophyte *Arabidopsis halleri* plants in microcosms, it was reported that the metals are released during POM degradation and a part of these metals is adsorbed by the reactive colloidal organic matter in the clay fractions (Boucher et al., 2005b). The turnover of different soil organic matter fractions is reported to be impacted by trace metal contents of these fractions (Boucher et al., 2005a; Dumat et al., 2006) and it was reported that the organic matter of the fine metal rich fractions was more resistant to microbial biodegradation. So in our case it is possible that the organic matter of the coarser fractions undergoes more biodegradation during incubation than organic matter in fine fractions and thus the metals associated to these coarser fractions are released into the soils. The fine clay and silt soil fractions are reported to have greater reactivity towards metals and increased capacity to adsorb metals (Murray et al., 2004; Carrillo-González et al., 2006; Sipos et al., 2008). Moreover it is also known that the humification of the soil organic matter increases with the decrease in the size of soil fractions. So in our case the organic matter in fine fractions was not degraded during incubation and the metals in these fractions were not released. In addition the adsorption of metals and fine organic matter released during incubation was adsorbed in these fine fractions, increasing the metal as well as organic carbon localization. Another reason in the increase of the metal localization in finer fractions in our case may be the increase of mass in these fractions thereby presenting more surface area for the metals to be absorbed. In our case the results of mass distribution showed that in Pierrelaye soils the soil mass in 0-20µm fraction was increased after soil incubation (Figure 4.8). This increase in soil mass may have occurred either because of the addition of more finer organic matter particles in this fraction from the biodegradation of coarser organic matter fractions or because of the loss of soil structure (macro and micro aggregates) during incubation and thereby increasing mass in fine fractions.

4.2.4. <u>Relationships among trace metal availability, metal localization and organic carbon distribution in physical size fractions</u>

Relationships among trace metal availability of the soils, trace metal distribution and the distribution of soil organic carbon in the physical fractions of the soils samples were studied by performing correlation tests among these parameters. The changes in these relations due to the evolution of soil organic matter after soil incubation were studied by comparing the results of soils before and after incubation.

Metaleurop

It was observed that in the soils of Metaleurop most of the correlations found in metal availability, carbon and metal distribution in physical fractions which were found in the soils before incubation disappeared in the soils after incubation. The details of these results are given in the following:

<u>Case of copper:</u>

Before soil incubation the parameters of Cu availability i.e. labile Cu, slowly labile Cu and NH_4NO_3 extractable Cu were correlated with copper and organic carbon localization in fine fractions i.e. 0-20µm and 20-50µm fractions and organic carbon distribution in coarse

Table 4.7: *Correlation matrix (Pearson correlation coefficients) among Cu availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Cu and organic carbon distribution among different physical size fractions of the soils of Metaleurop site (n=6; bold numbers are for $p < 0.05$).*

(a)

Before Incubation

Variables	Cu EDTA	Cu Lab	Cu Slab	Cu NH4NO3	Cu DTPA	Cu 0-20	Cu 20-50	Cu 50-200	Cu 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cu EDTA	**1.00**												
Cu Lab	-0.30	**1.00**											
Cu Slab	0.12	**-0.93**	**1.00**										
Cu NH4NO3	0.06	**-0.89**	**0.98**	**1.00**									
Cu DTPA	-0.26	-0.37	0.57	0.55	**1.00**								
Cu 0-20	0.31	**-0.86**	**0.96**	**0.94**	0.57	**1.00**							
Cu 20-50	0.34	**-0.92**	**0.97**	**0.95**	0.46	**0.98**	**1.00**						
Cu 50-200	-0.48	0.51	-0.54	-0.46	-0.71	-0.67	-0.59	**1.00**					
Cu 200-2000	-0.36	0.45	-0.38	-0.21	0.14	-0.36	-0.37	0.18	**1.00**				
C 0-20	-0.37	**0.97**	**-0.84**	-0.77	-0.20	-0.75	**-0.83**	0.44	0.60	**1.00**			
C 20-50	0.41	**-0.91**	**0.95**	**0.92**	0.47	**0.99**	**0.99**	-0.65	-0.41	**-0.83**	**1.00**		
C 50-200	-0.12	0.47	-0.52	-0.66	-0.33	-0.55	-0.59	0.26	-0.49	0.28	-0.55	**1.00**	
C 200-2000	0.03	**-0.88**	**0.99**	**0.98**	0.57	**0.95**	**0.95**	-0.47	-0.35	-0.77	**0.92**	-0.51	**1.00**

(b)

After Incubation

Variables	Cu EDTA	Cu Lab	Cu Slab	Cu NH4NO3	Cu DTPA	Cu 0-20	Cu 20-50	Cu 50-200	Cu 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cu EDTA	**1.00**												
Cu Lab	**0.87**	**1.00**											
Cu Slab	-0.74	**-0.93**	**1.00**										
Cu NH4NO3	**0.86**	**0.93**	**-0.92**	**1.00**									
Cu DTPA	0.46	0.52	-0.68	0.75	**1.00**								
Cu 0-20	**0.87**	**0.95**	**-0.93**	**0.96**	0.57	**1.00**							
Cu 20-50	-0.47	-0.60	0.77	-0.80	-0.75	-0.78	**1.00**						
Cu 50-200	0.47	0.06	0.16	0.11	0.09	-0.01	0.32	**1.00**					
Cu 200-2000	-0.58	-0.23	0.23	-0.34	-0.44	-0.26	0.11	-0.70	**1.00**				
C 0-20	-0.17	-0.03	0.17	-0.09	-0.45	0.04	-0.06	-0.38	0.78	**1.00**			
C 20-50	-0.50	-0.63	0.54	-0.72	-0.40	-0.70	0.67	0.10	-0.26	-0.58	**1.00**		
C 50-200	-0.16	-0.02	0.19	-0.04	0.00	-0.17	0.22	0.14	0.50	0.43	-0.42	**1.00**	
C 200-2000	**0.87**	**0.96**	**-0.92**	**0.99**	0.64	**0.99**	-0.77	0.06	-0.25	0.03	-0.76	-0.03	**1.00**

Table 4.8: *Correlation matrix (Pearson correlation coefficients) among Pb availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Pb and organic carbon distribution among different physical size fractions of the soils of Metaleurop site (n=6; bold numbers are for $p < 0.05$).*

(a)

Before Incubation

Variables	Pb EDTA	Pb Lab	Pb Slab	Pb NH4NO3	Pb DTPA	Pb 0-20	Pb 20-50	Pb 50-200	Pb 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Pb EDTA	**1.00**												
Pb Lab	0.04	**1.00**											
Pb Slab	-0.26	**-0.96**	**1.00**										
Pb NH4NO3	0.05	**0.99**	**-0.93**	**1.00**									
Pb DTPA	-0.05	**0.86**	-0.73	**0.90**	**1.00**								
Pb 0-20	-0.26	0.48	-0.24	0.51	0.68	**1.00**							
Pb 20-50	-0.11	**-0.97**	**0.98**	**-0.95**	-0.80	-0.33	**1.00**						
Pb 50-200	-0.51	-0.69	0.69	-0.70	-0.57	-0.51	0.60	**1.00**					
Pb 200-2000	0.27	-0.74	0.70	-0.67	-0.45	-0.36	0.77	0.31	**1.00**				
C 0-20	-0.17	**0.89**	-0.73	**0.92**	**0.95**	0.79	-0.80	-0.64	-0.60	**1.00**			
C 20-50	-0.09	**-0.98**	**0.98**	**-0.96**	-0.80	-0.39	**1.00**	0.64	0.79	**-0.83**	**1.00**		
C 50-200	-0.41	0.51	-0.51	0.43	0.10	-0.07	-0.53	-0.06	**-0.81**	0.28	-0.55	**1.00**	
C 200-2000	-0.24	**-0.94**	**0.95**	**-0.93**	-0.70	-0.35	**0.90**	**0.82**	0.65	-0.77	**0.92**	-0.51	**1.00**

(b)

After Incubation

Variables	Pb EDTA	Pb Lab	Pb Slab	Pb NH4NO3	Pb DTPA	Pb 0-20	Pb 20-50	Pb 50-200	Pb 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Pb EDTA	**1.00**												
Pb Lab	0.53	**1.00**											
Pb Slab	-0.24	**-0.90**	**1.00**										
Pb NH4NO3	-0.59	**-0.96**	0.76	**1.00**									
Pb DTPA	-0.37	-0.70	**0.85**	0.56	**1.00**								
Pb 0-20	-0.46	0.16	-0.10	-0.24	0.36	**1.00**							
Pb 20-50	0.48	**0.85**	-0.59	**-0.93**	-0.31	0.36	**1.00**						
Pb 50-200	0.67	**0.93**	-0.69	**-0.94**	-0.42	0.21	**0.91**	**1.00**					
Pb 200-2000	-0.32	-0.66	0.41	**0.82**	0.17	-0.60	-0.77	-0.71	**1.00**				
C 0-20	-0.31	0.34	-0.66	-0.16	-0.56	0.25	-0.09	0.07	-0.12	**1.00**			
C 20-50	-0.15	-0.76	0.71	0.74	0.32	-0.64	-0.63	-0.74	0.75	-0.58	**1.00**		
C 50-200	0.17	0.01	-0.03	0.06	0.05	0.08	-0.27	0.10	-0.06	0.43	-0.42	**1.00**	
C 200-2000	0.53	**0.86**	-0.59	**-0.96**	-0.30	0.43	**0.95**	**0.93**	**-0.91**	0.03	-0.76	-0.03	**1.00**

fraction 200-2000µm (table 4.7). After soil incubation the correlations of metal availability parameters with Cu and C_{org} localization in 20-50µm fraction and C_{org} localization in 0-20µm fraction were not found (table 4.7).

Case of lead:

Before soil incubation the parameters of Pb availability i.e. EDTA extractable Pb, labile Pb and NH_4NO_3 extractable Pb were correlated with Pb and organic carbon (C_{org}) distribution in fine fractions i.e. 0-20µm and organic carbon distribution in fine 20-50µm fraction and coarse fraction 200-2000µm (table 4.8). After soil incubation the correlations of these Pb availability parameters with C_{org} localization in 0-20 and 20-50µm fractions were not found. But it was found that in the soils after incubation labile and NH_4NO_3 extractable Pb became correlated with Pb localization in 50-200µm fraction (table 4.8).

Table 4.9: *Correlation matrix (Pearson correlation coefficients) among Zn availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Zn and organic carbon distribution among different physical size fractions of the soils of Metaleurop site (n=6; bold numbers are for $p < 0.05$).*

(a) **Before Incubation**

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Zn EDTA	**1.00**												
Zn Lab	0.71	**1.00**											
Zn Slab	0.37	**0.82**	**1.00**										
Zn NH4NO3	**-0.86**	**-0.82**	-0.62	**1.00**									
Zn DTPA	-0.31	-0.17	-0.33	0.62	**1.00**								
Zn 0-20	0.28	0.17	0.07	0.13	0.59	**1.00**							
Zn 20-50	**0.94**	0.69	0.52	**-0.89**	-0.53	0.22	**1.00**						
Zn 50-200	-0.02	0.32	0.09	-0.29	-0.17	-0.48	-0.03	**1.00**					
Zn 200-2000	**0.82**	0.51	0.37	**-0.83**	-0.67	0.09	**0.95**	0.09	**1.00**				
C 0-20	-0.75	-0.49	-0.32	**0.90**	**0.83**	0.34	**-0.83**	-0.24	**-0.88**	**1.00**			
C 20-50	**0.90**	**0.86**	0.69	**-0.97**	-0.54	0.08	**0.95**	0.15	**0.86**	**-0.83**	**1.00**		
C 50-200	-0.78	-0.53	-0.20	0.48	-0.20	-0.47	-0.57	0.36	-0.32	0.28	-0.55	**1.00**	
C 200-2000	**0.84**	**0.90**	0.58	**-0.95**	-0.40	-0.04	0.81	0.46	0.73	-0.77	**0.92**	-0.51	**1.00**

(b) **After Incubation**

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Zn EDTA	**1.00**												
Zn Lab	**0.91**	**1.00**											
Zn Slab	0.66	0.67	**1.00**										
Zn NH4NO3	**-0.98**	**-0.90**	-0.57	**1.00**									
Zn DTPA	-0.21	-0.21	-0.64	0.22	**1.00**								
Zn 0-20	**0.92**	**0.94**	0.60	**-0.93**	-0.35	**1.00**							
Zn 20-50	-0.54	-0.56	-0.14	0.66	0.44	-0.76	**1.00**						
Zn 50-200	0.76	0.54	0.74	-0.70	-0.26	0.48	-0.04	**1.00**					
Zn 200-2000	0.75	0.71	0.45	-0.73	0.23	0.52	-0.08	0.73	**1.00**				
C 0-20	0.00	0.15	0.23	-0.11	-0.58	0.14	-0.44	-0.02	0.02	**1.00**			
C 20-50	-0.66	-0.69	-0.35	0.74	0.11	-0.60	0.53	-0.49	-0.77	-0.58	**1.00**		
C 50-200	-0.02	0.16	0.36	0.05	0.04	-0.16	0.42	0.26	0.50	0.43	-0.42	**1.00**	
C 200-2000	**0.96**	**0.89**	0.44	**-0.98**	-0.02	**0.89**	-0.60	0.63	0.79	0.03	-0.76	-0.03	**1.00**

Case of zinc:

Before soil incubation the parameters of Zn availability i.e. EDTA extractable Zn and NH_4NO_3 extractable Zn were correlated with Zn distribution in 20-50 and 200-2000µm fractions and also EDTA extractable Zn, labile Zn and NH_4NO_3 extractable Zn were correlated with organic carbon (C_{org}) distribution in 20-50 and 200-2000µm fractions (table 4.9). After soil incubation the correlations of these Zn availability parameters with Zn localization in 20-50 and 200-2000µm and with C_{org} localization in 20-50µm fractions were not found. But these availability parameters became correlated with Zn in 0-20µm fraction (table 4.9).

Case of cadmium:

Before soil incubation the parameters of Cd availability i.e. labile Cd, NH_4NO_3 extractable Cd and DTPA extractable Cd were correlated with Cd and C_{org} localization in 0-20µm and 50-200µm fractions and also EDTA extractable Cd was correlated with Cd localization in 20-50µm and 50-200µm and with organic carbon (C_{org}) distribution in 20-50 and 200-2000µm fractions (table 4.10). After soil incubation the correlations of the Cd availability parameters with Cd localization in 0-20, 50-200µm fractions and with C_{org} localization in 0-20, 20-50µm fractions were not found. But the availability parameters EDTA extractable Cd and labile Cd became correlated with Cd in 20-50µm fraction (table 4.10).

Table 4.10: *Correlation matrix (Pearson correlation coefficients) among Cd availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Cd and organic carbon distribution among different physical size fractions of the soils of Metaleurop site (n=6; bold numbers are for $p < 0.05$).*

(a) **Before Incubation**

Variables	Cd EDTA	Cd Lab	Cd Slab	Cd NH4NO3	Cd DTPA	Cd 0-20	Cd 20-50	Cd 50-200	Cd 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cd EDTA	**1.00**												
Cd Lab	0.78	**1.00**											
Cd Slab	0.39	-0.24	**1.00**										
Cd NH4NO3	**0.96**	**0.92**	0.11	**1.00**									
Cd DTPA	0.79	**0.96**	-0.12	**0.89**	**1.00**								
Cd 0-20	0.65	**0.97**	-0.42	**0.84**	**0.88**	**1.00**							
Cd 20-50	**-0.86**	-0.39	-0.76	-0.70	-0.49	-0.20	**1.00**						
Cd 50-200	**0.95**	**0.84**	0.29	**0.94**	**0.90**	0.72	-0.80	**1.00**					
Cd 200-2000	-0.03	-0.50	**0.83**	-0.28	-0.44	-0.62	-0.31	-0.12	**1.00**				
C 0-20	0.76	**0.96**	-0.17	**0.88**	**0.86**	**0.94**	-0.35	0.79	-0.32	**1.00**			
C 20-50	**-0.97**	**-0.84**	-0.29	**-0.96**	**-0.84**	-0.74	0.79	**-0.98**	0.09	**-0.83**	**1.00**		
C 50-200	0.64	0.45	0.36	0.56	0.67	0.26	-0.69	0.69	-0.03	0.28	-0.55	**1.00**	
C 200-2000	**-0.87**	**-0.87**	-0.02	**-0.94**	**-0.86**	**-0.82**	0.65	**-0.91**	0.43	-0.77	**0.92**	-0.51	**1.00**

(b) **After Incubation**

Variables	Cd EDTA	Cd Lab	Cd Slab	Cd NH4NO3	Cd DTPA	Cd 0-20	Cd 20-50	Cd 50-200	Cd 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cd EDTA	**1.00**												
Cd Lab	**0.94**	**1.00**											
Cd Slab	0.64	0.40	**1.00**										
Cd NH4NO3	**0.89**	**0.92**	0.59	**1.00**									
Cd DTPA	0.75	0.81	0.48	**0.96**	**1.00**								
Cd 0-20	-0.79	-0.71	-0.66	-0.74	-0.68	**1.00**							
Cd 20-50	**0.85**	**0.91**	0.20	0.76	0.64	-0.42	**1.00**						
Cd 50-200	0.04	-0.25	0.50	-0.15	-0.16	-0.39	-0.34	**1.00**					
Cd 200-2000	-0.47	-0.17	**-0.83**	-0.21	-0.04	0.61	-0.01	-0.76	**1.00**				
C 0-20	0.11	0.00	-0.06	-0.17	-0.33	0.42	0.37	-0.06	-0.04	**1.00**			
C 20-50	0.62	0.67	0.55	0.73	0.66	-0.76	0.31	-0.12	-0.34	-0.58	**1.00**		
C 50-200	0.25	0.27	-0.34	0.06	0.06	-0.09	0.52	0.09	0.16	0.43	-0.42	**1.00**	
C 200-2000	**-0.97**	**-0.94**	-0.68	**-0.95**	**-0.82**	0.77	-0.79	0.08	0.41	0.03	-0.76	-0.03	**1.00**

Pierrelaye

For the soils before incubation, most of the metal availability parameters were correlated to the metal localization in all physical size fractions and were rarely correlated with organic carbon localization in the physical fractions of the soils. In the soils after incubation new correlation among the parameters of metal availability and organic carbon localization in physical fractions appeared in addition to the already found correlations among metal availability parameters and metal distribution in physical fractions in the soils. The details of these results are given in the following:

<u>Case of copper:</u>

Before soil incubation the parameters of Cu availability i.e. EDTA extractable Cu, labile Cu, slowly labile Cu and NH_4NO_3 extractable Cu were correlated with copper distribution in 0-20µm, 20-50µm and 50-200 µm fractions (table 4.11). After soil incubation in addition to these above mentioned correlations of Cu availability parameters with Cu localization, the availability parameters become correlated with C_{org} localization in 0-20µm, 20-50 and 200-2000µm fraction (table 4.11).

Table 4.11: *Correlation matrix (Pearson correlation coefficients) among Cu availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Cu and organic carbon distribution among different physical size fractions of the soils of Pierrelaye site (n=6; bold numbers are for $p < 0.05$).*

(a) **Before Incubation**

Variables	Cu EDTA	Cu Lab	Cu Slab	Cu NH4NO3	Cu DTPA	Cu 0-20	Cu 20-50	Cu 50-200	Cu 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cu EDTA	**1.00**												
Cu Lab	**0.97**	**1.00**											
Cu Slab	**0.85**	**0.93**	**1.00**										
Cu NH4NO3	**-0.94**	**-0.96**	**-0.95**	**1.00**									
Cu DTPA	-0.54	-0.52	-0.69	0.75	**1.00**								
Cu 0-20	**0.97**	**1.00**	**0.92**	**-0.96**	-0.52	**1.00**							
Cu 20-50	**0.97**	**1.00**	**0.92**	**-0.94**	-0.49	**1.00**	**1.00**						
Cu 50-200	**0.96**	**0.98**	**0.96**	**-0.98**	-0.64	**0.98**	**0.98**	**1.00**					
Cu 200-2000	0.62	0.77	**0.89**	-0.78	-0.56	0.76	0.75	0.77	**1.00**				
C 0-20	0.61	0.69	**0.86**	-0.73	-0.60	0.69	0.69	0.74	0.81	**1.00**			
C 20-50	0.78	0.72	0.45	-0.54	0.08	0.73	0.75	0.64	0.20	0.22	**1.00**		
C 50-200	-0.05	0.09	0.03	0.01	0.23	0.08	0.08	0.02	0.23	-0.33	0.01	**1.00**	
C 200-2000	0.69	0.67	0.79	-0.77	-0.74	0.67	0.66	0.74	0.63	**0.91**	0.26	-0.57	**1.00**

(b) **After Incubation**

Variables	Cu EDTA	Cu Lab	Cu Slab	Cu NH4NO3	Cu DTPA	Cu 0-20	Cu 20-50	Cu 50-200	Cu 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cu EDTA	**1.00**												
Cu Lab	**0.99**	**1.00**											
Cu Slab	**0.95**	**0.96**	**1.00**										
Cu NH4NO3	**0.97**	**0.97**	**0.95**	**1.00**									
Cu DTPA	**0.99**	**0.99**	**0.97**	**0.99**	**1.00**								
Cu 0-20	**0.99**	**1.00**	**0.97**	**0.96**	**0.99**	**1.00**							
Cu 20-50	**0.99**	**1.00**	**0.95**	**0.96**	**0.98**	**0.99**	**1.00**						
Cu 50-200	**0.91**	**0.89**	**0.90**	**0.95**	**0.94**	**0.90**	**0.87**	**1.00**					
Cu 200-2000	0.76	**0.82**	**0.81**	0.68	0.75	**0.83**	**0.84**	0.56	**1.00**				
C 0-20	**0.82**	**0.84**	**0.82**	0.71	0.76	**0.84**	**0.85**	0.55	**0.91**	**1.00**			
C 20-50	**0.96**	**0.95**	**0.86**	**0.94**	**0.94**	**0.92**	**0.95**	0.81	0.67	0.78	**1.00**		
C 50-200	0.29	0.33	0.21	0.40	0.38	0.28	0.34	0.43	0.11	-0.12	0.35	**1.00**	
C 200-2000	**0.98**	**0.98**	**0.99**	**0.96**	**0.97**	**0.98**	**0.97**	**0.88**	0.81	**0.86**	**0.91**	0.18	**1.00**

<u>*Case of lead:*</u>

Before soil incubation the parameters of Pb availability i.e. EDTA extractable Pb, labile Pb, slowly labile Pb and NH_4NO_3 extractable Pb were correlated with Pb distribution in 0-20µm and 20-50µm fractions (table 4.12). After soil incubation in addition to these above mentioned correlations of Pb availability parameters with Pb localization, the availability parameters were also correlated with Pb localization in 200-2000 and with C_{org} localization in 0-20µm, 20-50 and 200-2000µm fraction (table 4.12).

Table 4.12: *Correlation matrix (Pearson correlation coefficients) among Pb availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Pb and organic carbon distribution among different physical size fractions of the soils of Pierrelaye site (n=6; bold numbers are for $p < 0.05$).*

(a) **Before Incubation**

Variables	Pb EDTA	Pb Lab	Pb Slab	Pb NH4NO3	Pb DTPA	Pb 0-20	Pb 20-50	Pb 50-200	Pb 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Pb EDTA	**1.00**												
Pb Lab	**0.91**	**1.00**											
Pb Slab	-0.80	**-0.89**	**1.00**										
Pb NH4NO3	**0.94**	**0.94**	**-0.83**	**1.00**									
Pb DTPA	0.28	0.39	-0.45	0.56	**1.00**								
Pb 0-20	**0.98**	**0.97**	**-0.89**	**0.97**	0.40	**1.00**							
Pb 20-50	**0.87**	**0.87**	**-0.85**	**0.96**	0.71	**0.92**	**1.00**						
Pb 50-200	0.56	0.56	-0.48	0.70	**0.86**	0.60	**0.82**	**1.00**					
Pb 200-2000	0.03	0.09	0.32	0.13	0.22	0.01	0.05	0.42	**1.00**				
C 0-20	0.76	0.68	-0.37	0.63	0.04	0.69	0.53	0.51	0.51	**1.00**			
C 20-50	0.67	0.66	-0.81	0.79	0.81	0.75	**0.92**	0.78	-0.19	0.22	**1.00**		
C 50-200	-0.16	0.17	-0.11	0.13	0.33	-0.01	0.05	-0.04	0.20	-0.33	0.01	**1.00**	
C 200-2000	**0.82**	0.60	-0.39	0.60	-0.12	0.70	0.51	0.36	0.19	**0.91**	0.26	-0.57	**1.00**

(b) **After Incubation**

Variables	Pb EDTA	Pb Lab	Pb Slab	Pb NH4NO3	Pb DTPA	Pb 0-20	Pb 20-50	Pb 50-200	Pb 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Pb EDTA	**1.00**												
Pb Lab	**0.97**	**1.00**											
Pb Slab	**1.00**	**0.97**	**1.00**										
Pb NH4NO3	**0.91**	**0.91**	**0.93**	**1.00**									
Pb DTPA	**0.95**	**0.94**	**0.96**	**0.99**	**1.00**								
Pb 0-20	**0.98**	**0.98**	**0.99**	**0.95**	**0.98**	**1.00**							
Pb 20-50	**0.96**	**0.98**	**0.97**	**0.96**	**0.98**	**0.99**	**1.00**						
Pb 50-200	0.75	0.75	0.77	0.76	0.76	0.80	0.80	**1.00**					
Pb 200-2000	**-0.93**	**-0.97**	**-0.94**	**-0.95**	**-0.97**	**-0.97**	**-0.99**	-0.72	**1.00**				
C 0-20	**0.83**	**0.87**	**0.83**	0.67	0.73	**0.84**	**0.83**	**0.84**	-0.78	**1.00**			
C 20-50	**0.84**	**0.92**	**0.87**	**0.93**	**0.94**	**0.93**	**0.96**	**0.81**	**-0.97**	0.78	**1.00**		
C 50-200	0.24	0.32	0.26	0.41	0.39	0.25	0.33	-0.16	-0.41	-0.12	0.35	**1.00**	
C 200-2000	**0.98**	**0.96**	**0.99**	**0.94**	**0.97**	**0.99**	**0.98**	**0.86**	**-0.94**	**0.86**	**0.91**	0.18	**1.00**

<u>*Case of zinc:*</u>

Before soil incubation the parameters of Zn availability i.e. EDTA extractable Zn, labile Zn, slowly labile Zn and NH_4NO_3 extractable Zn were correlated with Zn distribution in al four physical size fractions of the soils (table 4.13). After soil incubation in addition to these above mentioned correlations of Zn availability parameters with Zn localization, the availability parameters were also correlated with C_{org} localization in 0-20µm, 20-50 and 200-2000µm fraction (table 4.13).

Table 4.13: *Correlation matrix (Pearson correlation coefficients) among Zn availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Zn and organic carbon distribution among different physical size fractions of the soils of Pierrelaye site (n=6; bold numbers are for $p < 0.05$).*

(a)

Before Incubation

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Zn EDTA	**1.00**												
Zn Lab	**0.98**	**1.00**											
Zn Slab	**0.95**	**0.97**	**1.00**										
Zn NH4NO3	**0.98**	**0.98**	**0.93**	**1.00**									
Zn DTPA	0.04	0.06	-0.01	0.22	**1.00**								
Zn 0-20	**1.00**	**0.98**	**0.95**	**0.98**	0.06	**1.00**							
Zn 20-50	**0.94**	**0.92**	**0.87**	**0.98**	0.38	**0.94**	**1.00**						
Zn 50-200	**0.95**	**0.95**	**0.94**	**0.94**	-0.01	**0.95**	**0.89**	**1.00**					
Zn 200-2000	**0.90**	**0.95**	**0.98**	**0.88**	-0.07	**0.90**	0.79	**0.90**	**1.00**				
C 0-20	0.71	0.77	0.81	0.66	-0.17	0.72	0.55	0.60	**0.87**	**1.00**			
C 20-50	0.69	0.61	0.53	0.75	0.65	0.69	**0.87**	0.56	0.40	0.22	**1.00**		
C 50-200	-0.02	0.05	0.09	0.11	0.34	-0.01	0.14	0.27	0.07	-0.33	0.01	**1.00**	
C 200-2000	0.73	0.74	0.73	0.64	-0.35	0.74	0.52	0.57	0.76	**0.91**	0.26	-0.57	**1.00**

(b)

After Incubation

Variables	Zn EDTA	Zn Lab	Zn Slab	Zn NH4NO3	Zn DTPA	Zn 0-20	Zn 20-50	Zn 50-200	Zn 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Zn EDTA	**1.00**												
Zn Lab	**0.95**	**1.00**											
Zn Slab	**0.96**	**0.91**	**1.00**										
Zn NH4NO3	**0.95**	**0.99**	**0.86**	**1.00**									
Zn DTPA	**0.93**	**0.98**	**0.84**	**1.00**	**1.00**								
Zn 0-20	**0.98**	**0.99**	**0.93**	**0.98**	**0.98**	**1.00**							
Zn 20-50	**0.97**	**0.95**	**0.87**	**0.98**	**0.97**	**0.97**	**1.00**						
Zn 50-200	**0.95**	**0.93**	**0.83**	**0.97**	**0.96**	**0.95**	**0.99**	**1.00**					
Zn 200-2000	0.71	**0.86**	0.77	0.79	0.78	**0.82**	0.67	0.62	**1.00**				
C 0-20	**0.88**	**0.82**	**0.96**	0.75	0.73	**0.85**	0.79	0.72	0.68	**1.00**			
C 20-50	**0.92**	**0.91**	**0.81**	**0.94**	**0.94**	**0.92**	**0.98**	**0.95**	0.59	0.78	**1.00**		
C 50-200	0.32	0.26	0.09	0.38	0.39	0.28	0.41	0.54	-0.07	-0.12	0.35	**1.00**	
C 200-2000	**0.95**	**0.99**	**0.93**	**0.97**	**0.97**	**0.99**	**0.94**	**0.91**	**0.86**	**0.86**	**0.91**	0.18	**1.00**

Table 4.14: *Correlation matrix (Pearson correlation coefficients) among Cd availability parameters i.e. total EDTA extractable contents (Ex-EDTA), labile metal fraction (Q_1), slowly labile fractions (Q_2), NH_4NO_3, DTPA extractable and the Cd and organic carbon distribution among different physical size fractions of the soils of Pierrelaye site (n=6; bold numbers are for $p < 0.05$).*

(a)

Before Incubation

Variables	Cd EDTA	Cd Lab	Cd Slab	Cd NH4NO3	Cd DTPA	Cd 0-20	Cd 20-50	Cd 50-200	Cd 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cd EDTA	**1.00**												
Cd Lab	**0.97**	**1.00**											
Cd Slab	**0.87**	0.75	**1.00**										
Cd NH4NO3	**0.97**	**0.93**	**0.84**	**1.00**									
Cd DTPA	**0.86**	0.81	0.72	**0.96**	**1.00**								
Cd 0-20	**0.99**	**0.95**	**0.91**	**0.97**	**0.86**	**1.00**							
Cd 20-50	**0.97**	**0.91**	**0.87**	**1.00**	**0.95**	**0.97**	**1.00**						
Cd 50-200	**0.95**	**0.96**	0.70	**0.95**	**0.88**	**0.93**	**0.94**	**1.00**					
Cd 200-2000	**0.92**	**0.94**	**0.81**	**0.82**	0.63	**0.91**	0.81	**0.82**	**1.00**				
C 0-20	0.73	0.81	0.55	0.62	0.48	0.69	0.59	0.63	**0.89**	**1.00**			
C 20-50	0.67	0.54	0.73	0.80	**0.89**	0.71	**0.82**	0.63	0.40	0.22	**1.00**		
C 50-200	0.06	0.11	-0.29	0.14	0.22	0.02	0.13	0.34	-0.18	-0.33	0.01	**1.00**	
C 200-2000	0.71	0.72	0.74	0.58	0.39	0.71	0.58	0.52	**0.90**	**0.91**	0.26	-0.57	**1.00**

(b)

After Incubation

Variables	Cd EDTA	Cd Lab	Cd Slab	Cd NH4NO3	Cd DTPA	Cd 0-20	Cd 20-50	Cd 50-200	Cd 200-2000	C 0-20	C 20-50	C 50-200	C 200-2000
Cd EDTA	**1.00**												
Cd Lab	**1.00**	**1.00**											
Cd Slab	**0.95**	**0.94**	**1.00**										
Cd NH4NO3	**0.98**	**0.99**	**0.91**	**1.00**									
Cd DTPA	**0.98**	**0.99**	**0.90**	**1.00**	**1.00**								
Cd 0-20	**1.00**	**1.00**	**0.95**	**0.98**	**0.98**	**1.00**							
Cd 20-50	**0.99**	**0.99**	**0.92**	**0.97**	**0.97**	**0.99**	**1.00**						
Cd 50-200	**0.99**	**0.99**	**0.94**	**1.00**	**0.99**	**0.99**	**0.98**	**1.00**					
Cd 200-2000	**0.88**	**0.87**	**0.94**	**0.87**	**0.87**	**0.89**	**0.83**	**0.91**	**1.00**				
C 0-20	**0.84**	**0.83**	**0.93**	0.75	0.75	**0.85**	**0.83**	0.79	0.80	**1.00**			
C 20-50	**0.93**	**0.94**	**0.83**	**0.94**	**0.94**	**0.94**	**0.95**	**0.92**	0.71	0.78	**1.00**		
C 50-200	0.33	0.35	0.05	0.38	0.39	0.32	0.40	0.34	0.01	-0.12	0.35	**1.00**	
C 200-2000	**0.98**	**0.98**	**0.97**	**0.97**	**0.97**	**0.98**	**0.95**	**0.98**	**0.94**	**0.86**	**0.91**	0.18	**1.00**

Case of cadmium:

Before soil incubation the parameters of Cd availability i.e. EDTA extractable Cd, labile Cd, slowly labile Cd and NH_4NO_3 extractable Cd were correlated with Cd distribution in al four physical size fractions of the soils (table 4.14). After soil incubation in addition to these above mentioned correlations of Cd availability parameters with Cd localization, the availability parameters were also correlated with C_{org} localization in 0-20µm, 20-50 and 200-2000µm fraction (table 4.14).

In short:
The influence of soil incubation on the relations among trace metal availability in the soils and the metal and organic carbon distribution in physical size fractions was different for the soils of both sites.

- Metaleurop: It was found that the some of the relations which were present in the soils before incubation was lost after soil incubation
- Pierrelaye: In the Pierrelaye soils it was found that in addition to the already present correlations in the soil before incubation, some new correlations were created after soil incubation

A contrasting effect of soil incubation on these relations in the soils of both sites was found. These relations depend upon the three types of variables studied i.e. metal availability in the bulk soils, metal localization in physical size fractions and organic carbon localization in physical size fractions. As described in previous sections, not always but in most of cases at least one of these three types of variables was changed during soil incubation. So the relationships between these variables are also changed. For Metaleurop soils it was found that certain relationships which were observed in these three variables before soil incubation were lost after soil incubation. But in case of Pierrelaye soils this change was opposite and more relationships were observed after soil incubation in addition to the already found relationships before soil incubation. Moreover, it was observed that in case of Metaleurop soils the loss of correlations was between metal availability and metal as well as organic carbon localization. But in case of Pierrelaye soils, the new relationships observed after soil incubation were between metal availability and organic carbon localization in the physical fractions of the soils. So the study of the relationships among these variables is an interesting factor to see how the trace metals and organic carbon dynamics are evolved during soil incubation. It has given us more insight into the changes of trace metal and organic matter dynamics during soil incubation.

4.3. Conclusion

The role of soil organic matter status in the availability of trace metals and their dynamics in soils was studied by in vitro soil incubation and by relating the trace metal availabilities of the bulk soils and the trace metal and carbon distribution in physical size fractions before and after incubation. Results of chemical extraction at equilibrium showed that in our conditions, two groups of metals behaved differently i.e. the behavior of Cu and Zn was similar for the both sites studied while Pb and Cd behavior was site specific. Changing the organic matter status through soil incubation induced increase in the Cu availabilities and decrease in the Zn availabilities whatever the site and land use. Pb and Cd availabilities in all the soils poorer in organic matter remained unchanged after soil incubation. In the soils of the site rich in organic matter, Cd availability decreased in both soils while changes in Pb availabilities were land use dependent i.e. increased in annual crop soil and decreased in miscanthus soil of the site. Results of kinetic extractions highlighted that the changes in trace metal availabilities of the soils were land use dependent and depending upon the metal studied either only labile or both labile and slowly labile pools were changed in the annual crop and the miscanthus soils of both sites. It was observed that the metal and organic carbon localization in physical size fractions of the soils also changed during soil incubation and in most of the cases the localization in fine soil fractions was increased. Changes in soil organic matter status via soil incubation also impacted the relationships among trace metal availability in bulk soils and metal and organic carbon localization in physical size fractions. But the changes in these relationships were different for the soils of both sites studied with increased correlations in the soils rich in organic matter whereas for the soils relatively poor in organic matter the correlations were lost. This show that the influence of in-vitro changes in soil organic matter on the trace metal dynamics of the soils was different for both sites.

Chapter 5

Role of particulate organic matter (POM) fractions in the trace metal availability in soils

5.1. Introduction

In the previous chapter we have found that enhancing an evolution in the soil organic matter status may change the trace metal availabilities of soils. But the question that still remains is which fraction of organic matter is mainly involved in these changes? As discussed earlier (in chapter 1; section 1.3) soil organic matter is a blend of various organic materials varying from tiny plant debris and dead animal parts, which are easy to isolate from the other soil constituents (herein called particulate organic matter), to the humified fractions of soil organic matter linked to the other soil constituents in particular clay fraction. These different materials have different turn over in soils depending upon their nature and the soil conditions. Particulate organic matter (POM) fractions were reported to have the most rapid turnover compared to the organic matter linked to soil constituents. Keeping in view the rapid turnover of the POM fractions we hypothesized that the changes in trace metal availabilities of the soils observed at short time scales are mainly linked with the POM fractions. Moreover the role of POM fractions as source or sink of trace metals and their contribution to the trace metal availability is poorly addressed. The work presented in this chapter therefore was designed to answer to the following questions:

1. Does the metal enrichment of the POM fractions vary as a result of change in soil organic matter at short term?
2. Do the particulate organic matter fractions contribute to the trace metal availability of the soils?
3. Are the trace metals linked to particulate organic matter bioavailable?

The answers to these questions were searched by studying: 1) the changes in trace metal enrichment in POM fractions after six months of soil incubation using the samples of the preceding experiments, 2) the trace metal availabilities of the soils with and without particulate organic matter fractions to study the role of POM fractions and 3) the bioavailability of POM associated copper for a living organism. In this last case the strategy was to use the POM fractions of a non-polluted soil in a biological test comprising the fungal growth in a liquid medium where Cu in the form of salt had been replaced by Cu in the form linked to POM fractions. With this test we could have an approach of the environmental and the toxicological bioavailability of copper as described by the recent norm ISO 17402 (ISO, 2008) by studying respectively the copper contents in the fungi and the response of the fungi to the copper exposure with the determination of laccase activities.

5.2. Results and Discussion

5.2.1. Effect of soil incubation on particulate organic matter fractions of the contaminated soil samples

5.2.1.a. Quantity of particulate organic matter (POM) fractions in the soils

The quantities of fine (50-200µm) and coarse POM (200-2000µm) fractions in the soils before and after incubation are presented in the figure 5.1. Whatever the soil sample or the site, the quantity of fine POM fractions was more than the coarse POM fractions.

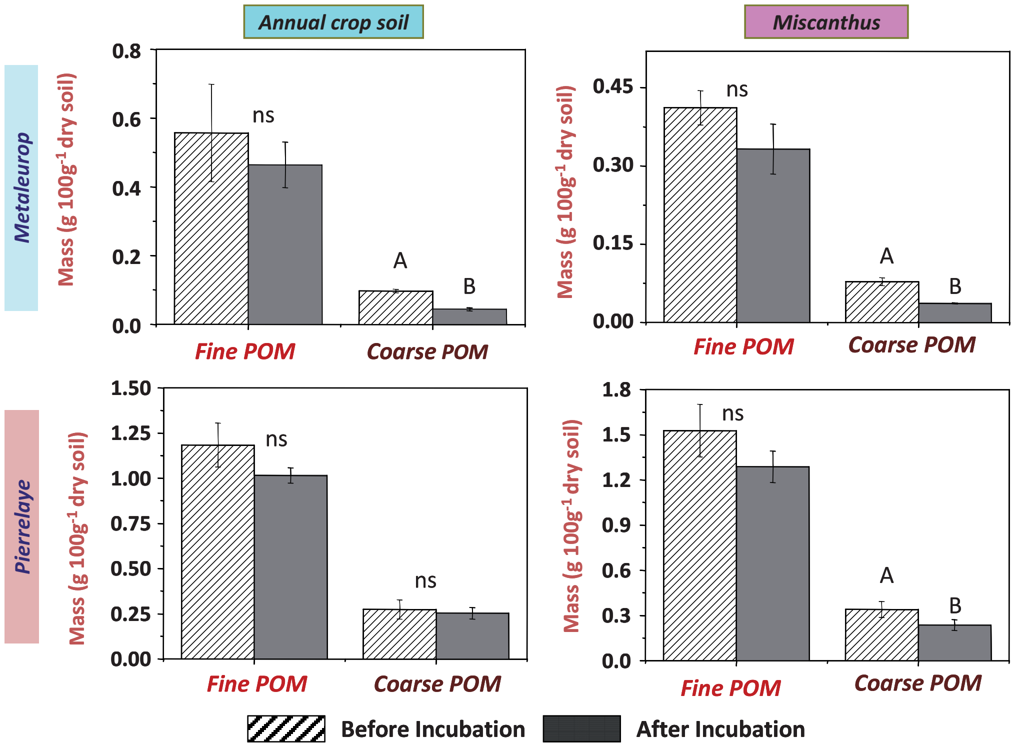


Figure 5.1: *Mass of fine and coarse particulate organic matter fractions of the soils before and after incubation. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.05$) for the mass of a given fraction before and after incubation of the soils is showed by different letters while "ns" represent non significant differences.*

Comparing the results between both sites, it was observed that the soils of Pierrelaye have more quantities of both fine and coarse POM fractions than the soils of Metaleurop. Results after incubation showed that the quantity of coarse POM fractions significantly decreased in all soil samples except for the annual crop soil of Pierrelaye. The decrease was not statistically significant for the fine POM fractions. Our results are consistent with several reported studies who have discussed the rapid turnover of coarse sized POM compared to the fine POM fractions in both contaminated and non contaminated soils (Balesdent, 1996; Boucher et al., 2005a). One explanation is that fine POM fractions are derived from the degradation of coarse POM fractions in the soils and therefore consist of more stabilized organic matter. In our case, during incubation the degradation of coarse POM fractions may have resulted either in CO_2 evolved or in the production of fine sized organic matter, thereby more or less increasing the mass of fine POM fractions. Another reason behind the less degradation of fine POM fractions during the time of incubation may be their increased trace metal contents and because of these elevated metal contents they are not preferred by the microorganisms for degradation (Boucher et al., 2005b).

5.2.1.b. Effect of soil incubation on metal enrichment factors of particulate organic matter fractions

Metal enrichment factors of the POM fractions were calculated by dividing the metal contents of the POM fractions by the metal contents of the corresponding bulk soil samples. Table 5.1 presents the metal enrichment factors for the fine and coarse POM fractions of the soil samples of the two sites before and after incubation. As expected before incubation both coarse and fine POM fractions were found to be enriched with enrichment factors >1. In our soil samples whatever the soil samples or the site, the POM fractions were more enriched in Cu and Cd than in Zn and Pb with enrichment factors varying from 5 to 9 for Cu, from 3.4 to 13 for Cd and from 1.5 to 3.5 for Pb and Zn. After incubation, it was found that whatever the sample or site both the fine and coarse POM fractions were more enriched in Cu except for the annual crop soil of Pierrelaye (non significant difference before and after incubation). Contrary to Cu, Pb enrichment factors of the POM fractions in the soils before and after incubation were found similar except for the coarse POM fraction the miscanthus soil of Metaleurop (Pb enrichment increased after incubation). Zn and Cd enrichment factors showed same trends as Pb for both fine and coarse POM fractions with no change after incubation except for the miscanthus soil sample of Metaleurop. In that case, the Zn enrichment factors of the coarse POM fraction increased after soil incubation whereas for Cd a decrease in the enrichment factors for both fine and coarse POM fractions of this soil sample was found after the incubation.

Table 5.1: *Metal enrichment factors of the fine and coarse particulate organic matter fractions of the soils. Mean values ± standard deviation of 3 replicates. Different letters indicate the statistically significant difference (Tukey's test, $p < 0.05$) for the metal enrichment factors in the POM fractions before and after soil incubation.*

Site	Soil	Time	Cu		Pb		Zn		Cd	
			POM Fine	POM Coarse	POM Fine	POM Coarse	POM Fine	POM Coarse	POM Fine	POM Coarse
Metaleurop	**Annual Crop**	***Before incubation***	6.4 ± 0.2^{b}	4.9 ± 0.3^{b}	2.3 ± 0.02^{a}	2.0 ± 0.3^{a}	2.5 ± 0.2^{a}	2.3 ± 0.1^{a}	5.2 ± 0.2^{a}	6.6 ± 0.2^{a}
		After incubation	7.7 ± 0.3^{a}	9.8 ± 1.1^{a}	2.3 ± 0.03^{a}	3.3 ± 1.2^{a}	2.6 ± 0.04^{a}	2.6 ± 0.2^{a}	4.0 ± 1.4^{a}	7.8 ± 1.0^{a}
	Miscanthus	***Before incubation***	6.4 ± 0.2^{b}	6.0 ± 0.2^{b}	2.5 ± 0.02^{a}	1.4 ± 0.1^{b}	2.3 ± 0.04^{a}	1.4 ± 0.1^{b}	6.4 ± 0.1^{a}	12.7 ± 0.8^{a}
		After incubation	7.2 ± 0.3^{a}	7.4 ± 0.5^{a}	2.6 ± 0.1^{a}	2.3 ± 0.1^{a}	2.2 ± 0.01^{b}	2.1 ± 0.2^{a}	5.1 ± 0.2^{b}	5.9 ± 0.2^{b}
Pierrelaye	**Annual Crop**	***Before incubation***	5.0 ± 1.0^{a}	4.7 ± 0.1^{a}	2.7 ± 0.6^{a}	1.5 ± 0.2^{a}	3.1 ± 0.04^{a}	2.5 ± 0.1^{a}	3.9 ± 0.1^{a}	3.4 ± 0.4^{a}
		After incubation	6.8 ± 1.5^{a}	5.1 ± 1.0^{a}	2.4 ± 0.1^{a}	1.2 ± 0.2^{a}	3.1 ± 0.04^{a}	2.1 ± 0.4^{a}	4.1 ± 0.1^{a}	3.4 ± 0.4^{a}
	Miscanthus	***Before incubation***	7.4 ± 0.2^{b}	5.3 ± 0.1^{b}	2.2 ± 0.1^{a}	1.2 ± 0.0^{a}	3.6 ± 0.1^{a}	2.4 ± 0.2^{a}	4.9 ± 0.2^{a}	4.2 ± 0.4^{a}
		After incubation	8.2 ± 0.2^{a}	6.6 ± 0.5^{a}	2.2 ± 0.04^{a}	1.2 ± 0.1^{a}	3.5 ± 0.1^{a}	2.4 ± 0.2^{a}	5.0 ± 0.04^{a}	4.1 ± 0.5^{a}

Contradictory results of changes in the metal enrichment of POM fractions of various soils in field conditions as well as in case of in vitro studies have been reported (Balabane et al., 1999; Balabane and van Oort, 2002; Boucher et al., 2005a; Labanowski et al., 2007). In the literature the type and magnitude of changes in metal enrichment was found different according to the type of soil and the conditions of study. But generally these studies agreed on the fact that the metal contents of POM fractions changed during their decomposition with time. Selective decomposition of POM fractions or of portions with low metal contents as well as the resistance to degradation of the remaining portions with high metal concentrations may be responsible for increased trace metal enrichment of POM with time (Balabane et al., 1999). With the aging of organic matter in polluted soils, the retention of trace metals by decomposing plant parts from soil solution can take place due to the high reactivity of organic materials with metals (Harter and Naidu, 1995; Ledin et al., 1996). In our conditions during incubation the increased enrichment of the POM fractions in Cu and no increase in the enrichment in other metals might be due to the more selectivity of Cu to be bound to organic matter than other metals, whatever the range of Cu pollution in the soils, as reported in the literature (Stalikas et al., 1999; Carrillo-González et al., 2006). The decrease in Zn and Cd enrichment of POM fractions after incubation for miscanthus soil samples only and not in the annual crop soils can be linked to the fact that in our conditions the soil organic carbon mineralized more in the miscanthus soils than in the annual crop soils whatever the site (Figure 5.2). So it is possible that this favored the release of Cd and Zn poorly linked to POM fractions thus favoring their re-adsorption to other fractions decreasing the enrichment of POM fractions. The fact that enrichment factors of Pb did not change after incubation would be linked to the fact that POM fractions are not preferential soil constituent for Pb retention in the soils. Some specific experiments should have to be done to confirm these hypotheses.

5.2.2. Comparison of trace metal availability of the annual crop soils with POM fractions and without POM fractions

5.2.2.a. Contribution of particulate organic matter associated metals to total soil metal contents of the annual crop soils of both sites

In order to prepare the samples for studying the trace metal availabilities of the soils with and without POM fractions, coarse POM (200-2000µm) and fine POM (50-200µm) fractions were isolated from the annual crop soils of both sites which were the only soils studied here. Quantities of isolated POM were similar as given above in figure 5.1 for soils before incubation. Contribution of the POM fractions to total trace metal contents of the soils was calculated and the results are presented in figure 5.2. It was observed that for the soils of both sites Cu and Cd contents of the POM fractions were more than their Pb and Zn contents in accordance with their enrichment factors. The percentage contribution of POM

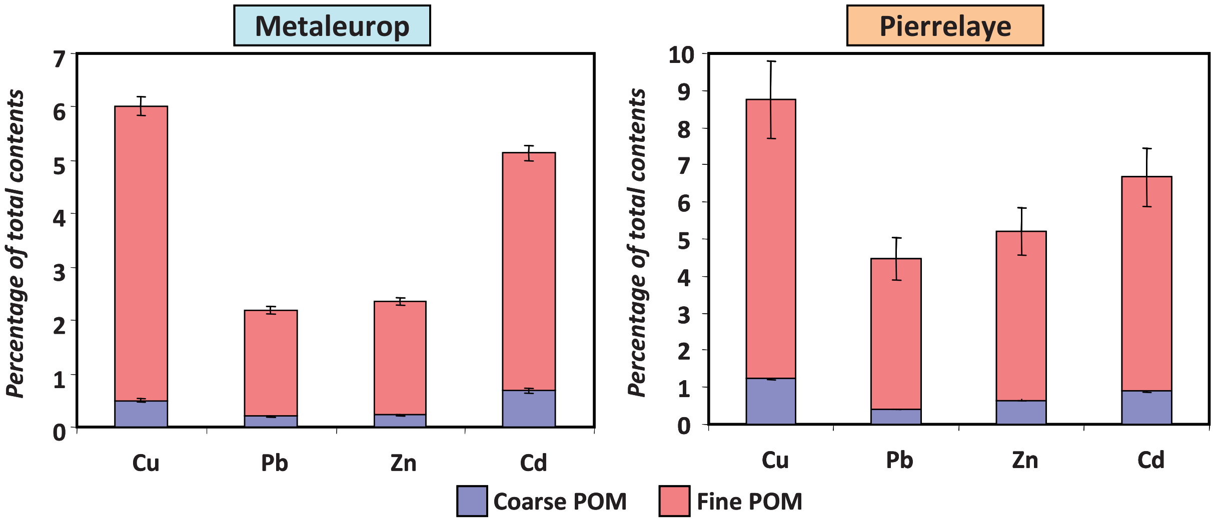


Figure 5.2: *Trace metal contents of the particulate organic matter fractions of the annual crop soils as percentage of total soil metal contents.*

associated metals to the total soil metal contents was more in Pierrelaye soil than in the Metaleurop soils because of their greater quantities of POM. The contribution of the fine POM fractions was more than that of the coarse POM fractions in both soil samples which is not only because of the greater quantities of fine POM fractions than coarse POM fractions (Figure 5.1) but also because fine POM fractions were slightly more enriched in metals than the coarse POM fractions (Table 5.1).

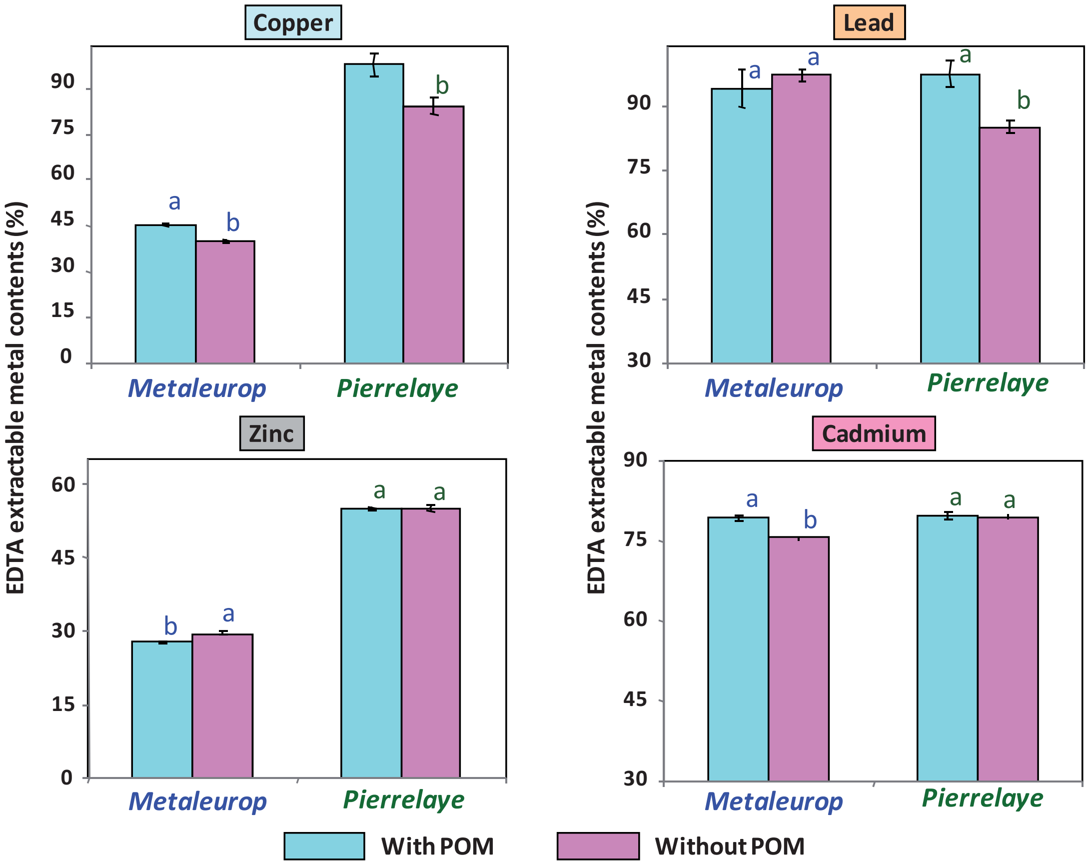


Figure 5.3: *EDTA extractable metal contents of the annual crop soil of both sites at equilibrium. Bar graphs represent mean values of 3 replicates while the error bars represent standard deviation among these replicates. Different letters indicate statistically significant difference (Tukey's test, p < 0.01) in the extractable metal contents of the soils with and without particulate organic matter (POM).*

5.2.2.b. EDTA extractable contents at equilibrium

Figure 5.3 presents the results of the EDTA metal extraction at equilibrium of the annual crop soil samples of both sites with and without POM fractions. It was found that for all the metals studied except Cu the effect of POM isolation was different for the two sites studied. Percentage of Cu availability was less in the soils without POM fractions for both Metaleurop as well as Pierrelaye annual crop soil samples but the decrease in the soil of

Pierrelaye was more than in the soil of Metaleurop. Percentage of Pb availability in the soil samples with and without POM fractions was similar in case of the Metaleurop soil but decreased for Pierrelaye soil. Zn and Cd percentages were similar in the Pierrelaye soil with and without POM. For the Metaleurop soil it was observed that the percentage of Zn availability was more in the soil without POM than in the soil with POM while the percentage of Cd availability was less in the soil without POM.

5.2.2.c. Labile and slowly labile metal pools and their extraction rates constants

Figure 5.4 presents the percentages of the labile and slowly metal pools of the soils under annual crops for the two sites with and without POM fractions. The extraction rate constants of these labile and slowly labile pools are presented in the table 5.2.

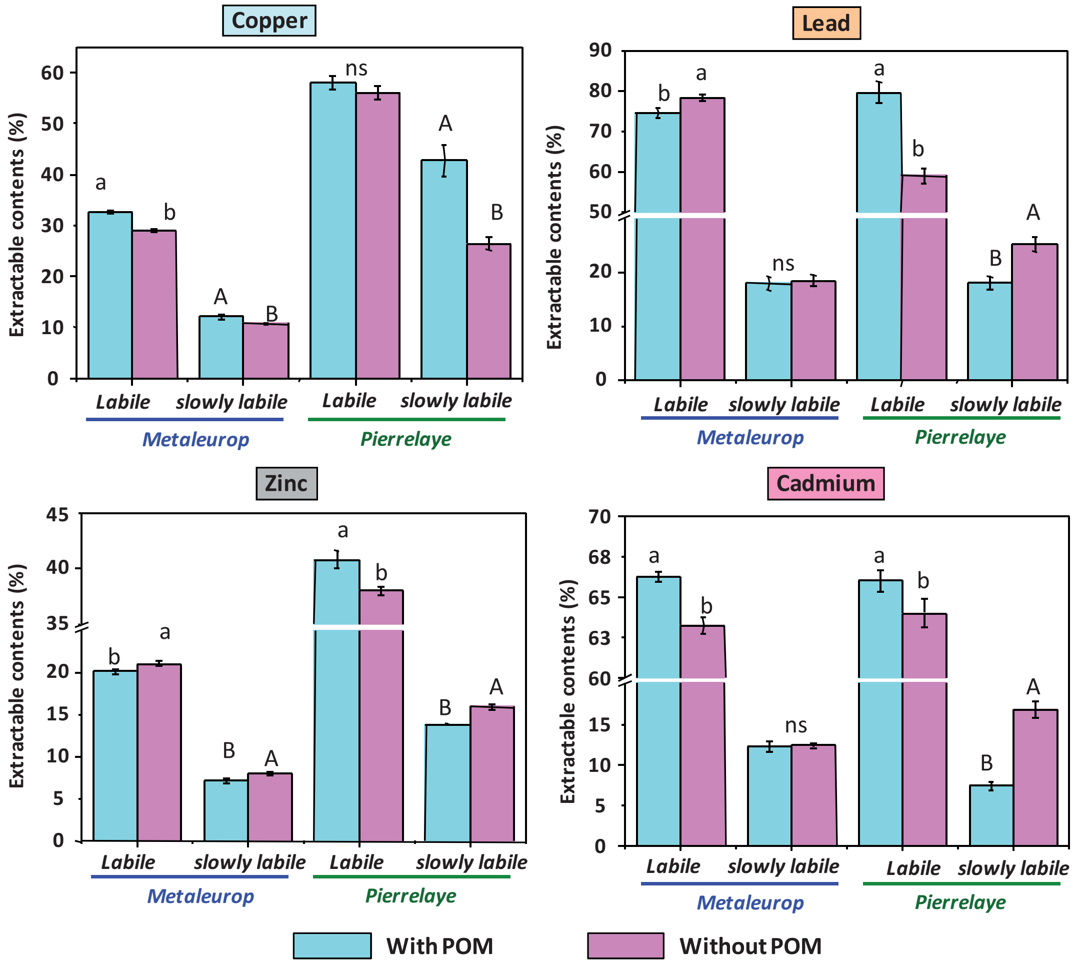


Figure 5.4: *Percentages of labile metal fraction (Q_1), slowly labile metal fraction (Q_2) of the annual crop soils for the two sites with and without particulate soil organic matter (POM). Bar graphs represent mean values of 5 replicates while the error bars represent standard deviation among these replicates. Statistically significant difference (Tukey's test, $p < 0.01$) for each metal fraction of the soils with and without POM is indicated by different letters while "ns" represent non significant differences.*

It was found that for the soil of Metaleurop, POM isolation significantly impacted both labile and slowly labile pools of Cu and Zn while for Pb and Cd only labile pool changed. The labile and slowly labile pool decreased for Cu and increased for Zn after soil POM isolation. For Pb the labile pool increased and for Cd it decreased in the soil without POM. For the soil of Pierrelaye it was found that both labile and slowly labile pools of Pb, Zn and Cd were impacted after POM isolation while for Cu only slowly labile pool changed significantly. For Pb, Zn and Cd the percentages of labile pools were lower in the soil without POM while the slowly labile pools of the soil without POM were higher. For Cu the percentage of slowly labile pool was lower in the soil without POM than the soil with POM but the labile pool was similar indicating that the non-extractable pool increased.

Table 5.2: *Extraction rate constants of labile metal fraction (K_1) and of slowly labile metal fraction (K_2) of the soil samples with and without POM. Mean values ± Standard Deviation (n=5). Different letters in the columns indicate pair-wise statistically significant difference (Tukey's test, $p < 0.01$) for rate constant of labile and slowly labile fractions of the metals for each soil with and without POM.*

Metal			***Silty soil***	***Sandy soil***
Cu	K_1 **(min^{-1})**	***With POM***	0.41 ± 0.003 ^{a}	0.34 ± 0.07 ^{a}
		Without POM	0.37 ± 0.023 ^{b}	0.41 ± 0.03 ^{a}
	K_2 **(min^{-1}, x10^{2})**	***With POM***	0.53 ± 0.03 ^{a}	0.31 ± 0.03 ^{a}
		Without POM	0.57 ± 0.03 ^{a}	0.38 ± 0.04 ^{a}
Pb	K_1 **(min^{-1})**	***With POM***	0.47 ± 0.03 ^{a}	0.24 ± 0.02 ^{b}
		Without POM	0.42 ± 0.01 ^{a}	0.33 ± 0.03 ^{a}
	K_2 **(min^{-1}, x10^{2})**	***With POM***	0.57 ± 0.07 ^{a}	0.61 ± 0.24 ^{a}
		Without POM	0.50 ± 0.04 ^{a}	0.66 ± 0.10 ^{a}
Zn	K_1 **(min^{-1})**	***With POM***	0.23 ± 0.02 ^{a}	0.43 ± 0.04 ^{b}
		Without POM	0.26 ± 0.02 ^{a}	0.52 ± 0.03 ^{a}
	K_2 **(min^{-1}, x10^{2})**	***With POM***	0.26 ± 0.02 ^{a}	0.48 ± 0.03 ^{b}
		Without POM	0.31 ± 0.08 ^{a}	0.66 ± 0.05 ^{a}
Cd	K_1 **(min^{-1})**	***With POM***	0.52 ± 0.03 ^{a}	0.51 ± 0.14 ^{a}
		Without POM	0.52 ± 0.04 ^{a}	0.55 ± 0.07 ^{a}
	K_2 **(min^{-1}, x10^{2})**	***With POM***	0.41 ± 0.05 ^{a}	1.27 ± 0.09 ^{a}
		Without POM	0.47 ± 0.05 ^{a}	1.15 ± 0.14 ^{a}

In the Metaleurop soil, no effect of POM isolation was observed on the extraction rate constants whatever the metals except for the extraction rate of labile Cu which was less in the soil without POM. In Pierrelaye soil, no effect of POM isolation was found for the extraction rates of both labile and slowly labile pools of Cu and Cd. The extraction rates of both pools of Zn and of the labile pool of Pb were more in the soil without POM than that of the soil with POM.

We hypothesized that the POM fractions served as source of metals in the soils when the availabilities of the metals were more in the soils with POM than the soils without POM. Inversely if in the soils without POM the trace metal availability was more than the soils with POM we considered that the POM fractions played a role of sink of metals. The results of the trace metal availabilities of the soils with and without POM fractions studied at equilibrium show that in our conditions the POM fractions served as source of Cu in the annual crop soils of the two sites and of Pb in Pierrelaye soil and Cd in Metaleurop soil because the availabilities of these metals were more in the soils with POM than the soils without POM. For Zn the POM fractions of Metaleurop soil play a role of sink because the Zn availability was less in the soil with POM than the soil without POM. Whereas this experiment at equilibrium suggests that POM fractions had no role in the availability of Zn and Cd in Pierrelaye soil and of Pb in Metaleurop soil. But the results of kinetic extractions highlighted that the POM fractions of Pierrelaye soil contributed to both labile and slowly labile pools of all the metals, so the percentages of both the pools were changed when the POM fractions were isolated from the soil. In case of Metaleurop soil the POM fractions contributed to both labile and slowly labile pools of Cu and Zn, therefore the percentages of both pools were changed when the POM were isolated from this soil. But for Pb and Cd the POM fractions were found to contribute only for labile pool and not for slowly labile pool, because only the percentage of labile pool of these metals changed after POM extraction.

To our knowledge there is no any study reported in the literature which have compared the trace metal availabilities of the soils with POM fractions and without POM fractions. The role of soil organic matter in trace metal availability and their dynamic in the soils is generally studied by the addition of variety of different organic amendments to the soils like composts, sewage sludge and different plant parts etc (Planquart et al., 1999; Clemente et al., 2005; Kidd et al., 2007; Jamali et al., 2009). But assessing the role of organic matter through these methods have certain discrepancies because more metals are brought to the soil through these amendments and the newly added organic matter needs time to be stabilized (Chang et al., 1997; McBride, 2003; Singh and Agrawal, 2008). Specific role of particulate organic matter fractions in the trace metals dynamic have also been reported for the soils amended with different plant debris (Besnard et al., 2001; Sebastia, 2007). Besnard et al. (2001) investigated vineyard soils with Cu contamination and found that among the two most Cu-enriched soil fractions, the POM accumulated up to 2,000 mg/kg of Cu, and the other corresponding clay fraction contained up to 500 mg/kg of Cu. They found that POM was predominantly responsible for the differences in Cu contents between the non-amended and the three amended parcels. In a study aiming the quantification of the reactivity of POM towards Cu in soils with different organic amendment managements, it was found that copper affinities of POM fractions were independent of the size of the fractions of organic inputs. The values of Cu affinities obtained were in the range of those reported for reactive soil organic fractions such as humic substances, highlighting the metal sink function of POM in soils (Sebastia et al., 2008).

Comparison of results of this study with the results of our study on trace metal availabilities of the soils before and after incubation (Figure 4.3) endorses the results found in both cases and better highlights the role of POM fractions as source or sink of metals. In the present study we isolated POM fractions >50µm while in the case of incubations the whole soil organic matter is evolved. Keeping in view the turnover of organic matter in soil we can hypothesize that in this six month time of incubation the particulate organic matter fractions of all sizes were evolved more than the other organic matter fractions. For example, we observed that Cu availability in the annual crop soils of both sites was less in the soils without POM fractions than the soil with POM fractions whereas Cu availability was more in the soils after incubation which shows that during incubation the evolution of soil organic matter served as source of Cu and gave rise to more available Cu in soil. Similar is the case with Pb availability in case of annual crop soil of Pierrelaye. In case of annual crop soil of Metaleurop, Zn availability was more in the soil without POM fractions than the soil with POM fractions whereas Zn availability decreased as a result of soil incubation, highlighting that POM served as sink of Zn and it was more firmly held in the soil in presence of POM so its availability was low.

To further elaborate the role of particulate organic matter fractions in the trace metal availability of the soils, more work is required involving the chemical as well as biological assays of trace metal availabilities.

5.2.3. Bioavailability of copper to the filamentous fungi Trametes versicolor in a liquid medium: Comparison of Cu added in the form of solution and copper linked to soil particulate organic matter

Keeping in view the potential of the functional as well as morphological traits of *Trametes versicolor* based upon the previous work conducted in the laboratory, we used *Trametes versicolor* as a biological test for the assessment of bioavailability of copper linked to particulate organic matter fractions extracted from soil. Bioavailability of copper linked to a fine POM fraction was compared to the bioavailability of copper added as salt solution ($CuSO_4$) in a liquid medium designed for the growing of *Trametes versicolor*.

5.2.3.a. Evolution of the fungal biomass development

Figure 5.5 presents the results of the evolution in the fungal biomass with the time of incubation in the liquid mediums in the presence and absence of added copper. We observed that the development of fungal biomass without added Cu followed a classical curve of fungal biomass development i.e. includes a growth phase (until day 7), a stationary phase near day 7 (although it is not much clear because of the large gap of sampling between day 7 and day 10) and then a declined phase until the end of incubation (day 14). The curves of fungal biomass development in the presence of added copper attained the highest growth at earlier stage (near day 5) compared to the curve of fungal development without Cu addition. These curves were similar whatever the form of added Cu i.e. in solution or linked to the POM. The fungal biomass was lower in both cases of added Cu compared to the case of medium without Cu at decline phase. These results show a difference of fungal growth in the absence or presence of added Cu conditions with an earlier start of decline at day 5 and less biomass at declined stage in added Cu conditions. Fungal growth in the liquid medium in the absence of added Cu depends upon the availability of the energy source and nutrition from the components of the medium and in precedent studies it has been shown that the fungal growth have various characteristics depending on the liquid mediums (Charchar, 2008; Lebrun et al., 2011). The shape of the time-growth curve obtained and the quantity of fungal biomass obtained at different sampling stages in our study are similar to the other studies conducted in our laboratory by using the same culture medium (Charchar, 2008; Lebrun et al., 2011). *Trametes versicolor* as

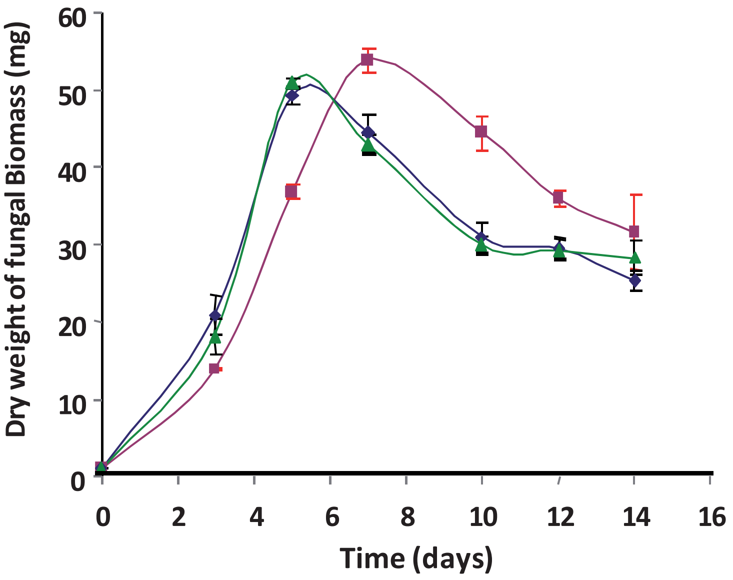


Figure 5.5 : *Evolution in the biomass of Trametes versicolor exposed to different source of copper during laboratory incubations in liquid medium.*

■ *Unexposed to Cu (Cu added as an oligo-element in yeast extracts*).

■ *Exposed to Cu as CuSO$_4$*

■ *Exposed to Cu linked to particulate organic matter (POM)*

other fungi are known to adopt the contaminated conditions and tolerate the elevated levels of pollution (Favero et al., 1991; Gabriel et al., 1996; Cuny et al., 2001; Baldrian, 2003). Biomass development of *Trametes versicolor* in liquid medium remained un-impacted at 20mg/L Cu and was slightly impacted with a 10% decrease in biomass when Cu concentration was 200mg/L (Lebrun et al., 2010). In our study with much less added Cu (100µg/L) the increased speed of biomass growth in the presence of added Cu can be due to the rapid utilization of energy resources and nutrients by the fungi as shown in precedent studies by a more rapid consumption of sugar resources in the liquid medium (Charchar, 2008; Lebrun et al., 2010).

5.2.3.b. Evolution of pH in the liquid mediums

The results of the evolution of pH of liquid mediums with time of incubation are presented in the figure 5.6. It was observed that the pH of liquid medium in the absence of added Cu decreased until the day 10 of incubation with a more rapid decrease until day 5 (decrease of 2 units of pH) and then the decrease was slower. An increase in the pH at the last stage (day 10 onwards) was observed. The pH of medium with added Cu (soluble or Cu-

POM) decreased until day 5 and then started to increase. The pH versus incubation times curves for both situations of Cu addition were similar until day 7 but afterwards the pH of

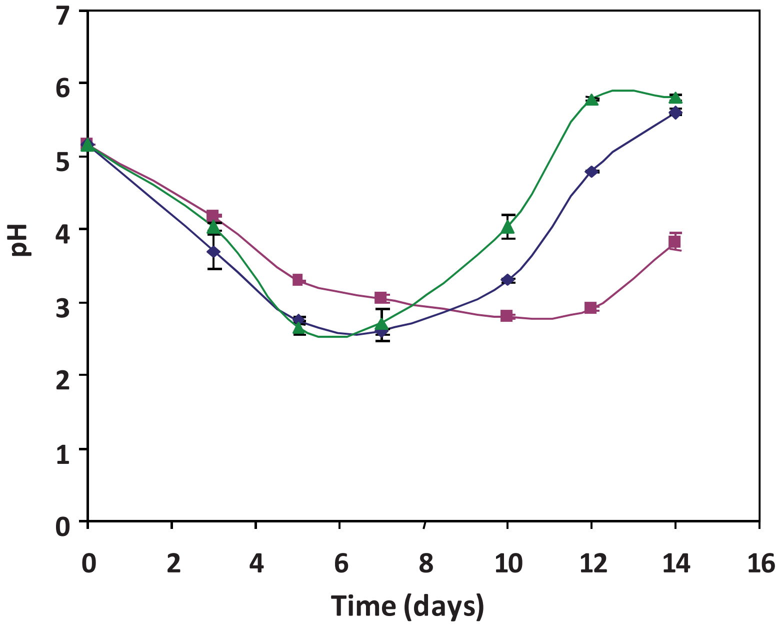


Figure 5.6: *Evolution in the pH of liquid medium because of the activity of Trametes versicolor exposed to different source of copper during laboratory incubations in the liquid medium.*

■ *Unexposed to Cu (Cu added as an oligo-element in yeast extracts*)

■ *Exposed to Cu as* $CuSO_4$ ■ *Exposed to Cu linked to POM*

medium with Cu added in the form linked to POM remained slightly higher than the pH of medium with Cu added in the form of salt in solution. Until day 7, the pH of liquid mediums in the presence of added Cu was less than the medium in the absence of added Cu but afterwards it started increasing and at the end of experiment a difference between mediums of 2 units of pH was found. Different fungi especially white-rot fungi including *T. versicolor* are known to secrete organic acids and more particularly oxalic acids as by product of their metabolism leading to the decrease in medium pH (Dutton and Evans, 1996; Galkin et al., 1998; Mäkelä et al., 2002; Fomina et al., 2005). The larger decrease in the presence of added Cu at earlier stage can be a result of more fungal activity which is consistent with the increased biomass also. The increase in the pH of the liquid mediums at decline stage could be the result of excretion of by-products linked to the cell lyses at this stage because of less availability of energy resources. Moreover it is reported that under nutrition exhaustion like carbon limitation, fungi activate their alternate metabolic pathways associated to new enzymatic secretion profiles (Orth et al., 1991; Lebrun et al., 2010).

5.2.3.c. Evolution of the copper contents of liquid culture mediums

Evolution of the copper contents of the liquid mediums during fungal growth was measured in order to determine the copper availability for the fungi. The copper contents of the liquid mediums at all sampling points during the fungi incubation are presented in table 5.3. On average during the 14 days of fungal growth (6 points of sampling) copper content of the reference liquid mediums were 3.18 ± 1.04 µg/L in the absence of fungi and 3.09 ± 1.53µg/L in the presence of fungi. The source of this copper was from the yeast extract. Cu being an essential element for biological life (Tyler, 1981), precedent assays in the laboratory have shown that this minimum amount contained in the yeast extract was necessary for the fungal growth in the liquid medium. But the similar Cu contents found in the uncontaminated mediums in the presence and absence of fungi show the uptake the Cu if any is not detectable at such low concentrations. This finding is supported by the absence of detectable laccase activity in this medium also (see later Figure 5.8).

Table 5.3: *Evolution of the copper concentrations in the liquid mediums during the exposure of Trametes versicolor to different source of copper contamination in laboratory incubations.*

Time (Day)	Cu in liquid medium (µg L^{-1})					
	Blank (Cu in yeast extract)		Cu added as $CuSO_4$		Cu added as linked to POM	
	control	*with fungi*	*control*	*with fungi*	*control*	*with fungi*
3	2.6	4.8 ± 0.8	96.6	66.9 ± 2.2	82.1 ± 4.5	70.5 ± 5.1
5	3.1	0.7 ± 0.2	93.5	25.1 ± 3.2	88.2 ± 6.5	58.2 ± 3.7
7	2.2	4.1 ± 0.7	97.9	27.9 ± 3.3	98.8 ± 2.3	50.9 ± 1.3
10	3.2	2.4 ± 0.6	122.0	29.9 ± 1.4	97.5 ± 2.0	57.8 ± 4.8
12	2.8	2.4 ± 0.9	88.4	25.2 ± 6.0	89.0 ± 3.7	51.9 ± 3.3
14	5.2	4.2 ± 0.1	88.9	36.1 ± 5.0	105.1 ± 8.8	60.7 ± 3.1
Average:	3.18 ± 1.04 Day 3rd to 14th	3.09 ± 1.53	97.9 Day 3rd to 14th	28.8 ± 3.8 Day 5th to 14th	93.4 ± 3.7 Day 3rd to 14th	55.9 ± 3.2 Day 5th to 14th

In the control liquid mediums with Cu added in the form of POM (but without fungi) an average of Cu content of 93.4 ± 3.7µg/L was found indicating that some Cu has been released from the solid phase of POM into the solution. This is why in the control liquid medium we added Cu in the form of salt solution an average Cu content of 97.9 µg/L in order to be in the same conditions but without POM during the whole period of 14 days of incubation (6 points of sampling). In the Cu contaminated mediums in the presence of fungi, it was found that at the 5th day of fungal growth a maximum of Cu uptake from the liquid medium was attained by the fungi whatever the source of Cu addition (in the form of salt or POM). It was observed that in the presence of fungi during the incubation period from 5th day of growth onwards, average Cu contents of 28.8 ± 3.8µg/L were found in the medium when Cu was added in the form of salt solution. Whereas in the liquid medium with added Cu in the form of POM fractions and in the presence of fungi the average Cu contents in solution in the period from 5th day of growth onwards were 55.9 ± 3.2µg/L (Table 5.3).

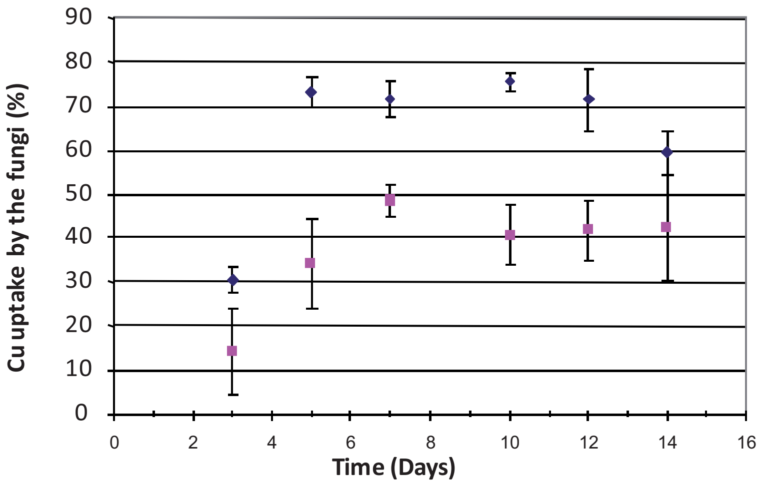


Figure 5.7: *Percentage of Cu accumulation in fungi during laboratory incubation in liquid mediums with Cu contamination added as CuS0$_4$ (100µg/L) and as Cu linked to POM fractions (50-200µm).* 🟦 *Exposed to Cu as CuSO$_4$* 🟪 *Exposed to Cu linked to POM*

The uptake of heavy metals by biomass is usually classified into three categories (Gadd, 1988); (1) cell surface binding, (2) intracellular accumulation and (3) extracellular accumulation. In this experiment we did not measured these compartments separately and thus we will use the general term “uptake”. We have been able to harvest very little amounts of the fungal biomass (<50mg) and it was not feasible to measure the Cu contents of fungal biomass to determine Cu uptake by fungi. So we calculated the Cu uptake by fungi in each conditions of Cu addition by deducting the Cu contents of the medium in the absence of fungi and of the medium in the presence of fungi. These results (Figure 5.7) show that during the period between 5th and 14th day of fungal growth in case of Cu added in the form of salt solution on average 71% of Cu present in solution was taken up by the fungi, whereas in the case of Cu added as POM only 40% of the total Cu present in solution (due to release from POM) was taken up by the fungi. Although the quantities of Cu found in the control mediums in both cases of Cu added were almost similar (97.9 µg/L in case of salt solution and 93.4 ± 3.7µg/L in case of POM), we observed that fungi took up almost double Cu in case of Cu added in the form of solution compared to that when Cu was added initially in the form of POM.

Metal bioaccumulation by fungi is widely reported in the literature and different factors have been discussed (Ehrlich, 1997; Baldrian, 2003; Bayramoglu et al., 2003; Al Abboud and Alawlaqi, 2011; Viraraghavan and Srinivasan, 2011). Fungal cell walls include components with carboxyl, hydroxyl, sulphydryl, amino and phosphate groups which play a major role in sorption of heavy metals and these surface sorptions are supposed to be the most significant mechanism of uptake (Gadd, 1993; Galli et al., 1994; Krantz-Rülcker et al., 1995). In a precedent study in our laboratory the bioavailability of Cu to *Trametes versicolor* was assessed by adding Cu as 1mM $CuSO_4$ in the same culture medium as used in this study. It was shown that the response of the fungi for Cu uptake was dependent on the stage of addition of copper (Charchar, 2008). In case of Cu addition at the growth phase (3rd day of growth), it was observed that Cu uptake increased with the Cu exposition with a highest Cu uptake of 7.5mg Cu/g of dry fungal biomass at the 11th day of Cu exposure. On average 5.65mg Cu/g of dry fungal biomass was taken up during the period between 4th and 11th day. In our case Cu was added at the start of experiment at day 0 and the added content was much lower (250 times less i.e. 100µg/L compared to 249mg/L in the precedent study). We calculated an average of 1.89mg Cu/g of dry fungal biomass in case of Cu added as salt solution. It shows that even if the source of Cu addition is the same, the relation between added Cu and Cu accumulated by the fungi is dependent upon the experimental conditions.

The metal uptake in fungi and other microorganisms have been reported to depend upon many factors i.e. fungal strain, pH of medium, metal speciation, amount of fungal biomass, stage of metal exposure and on temperature to an extent (Singh, 1977; Gadd, 1990; Gadd, 1993; Chang et al., 1997; Kunito et al., 1999; Baldrian, 2003). In our study the conditions being similar for the both sources of Cu, the difference of Cu uptake (Figure 5.7) can be linked to the metal speciation and the pH of medium. In a study of removal of heavy metals using the fungus *Aspergillus niger* Kapoor et al. (1999) revealed that with an increase in pH beyond 4, the negative charge density on the cell surface increases due to deprotonation of the metal binding sites and thus increases biosorption. In our study, the pH of the medium in the case of Cu added as POM was slightly higher than that of Cu added as salt solution during the period for which metal uptake was studied (between 5th and 14th day) but the Cu uptake was more in the case of Cu added as salt solution. So in our case the effect of pH is not the same as that reported in the study about fungus *Aspergillus niger.*

Keeping in view the concept of the environmental bioavailability of metals as described by the norm ISO 17402 (ISO, 2008) we considered this uptake of Cu by fungi as proof of environmental bioavailability of Cu to the fungi as target. The POM fractions used for this experiment have a total metal content of about 500mg Cu kg^{-1} POM. 5mg of this POM fraction had been added to the liquid medium for the experiment. We observed that about 37% of this Cu was released into the liquid medium (93.4 ± 3.7µg Cu/L found in control liquid medium instead of theoretical 250 µg Cu/L in hypothetical case of 100% release of Cu from POM), which corresponds to the fraction potentially available in the liquid medium. About 40% of this potentially available Cu was taken up by the fungi on average at the period of 5th days of exposure onwards (Figure 5.7) corresponding to the part that was not found again in solution and thus can be supposed to be bioaccumulated. Finally, in our conditions we calculated that about 15% of the total Cu contained in POM fraction was found bioavailable i.e. taken up by the fungi, with or without any effect on fungi. Whereas in case of Cu added in the form of salt solution, the bioavailability of the Cu was found to be 71%. So in our conditions, the environmental bioavailability of the Cu added in the form of POM fractions was found to be about five times less than Cu added in the form of salt solution.

5.2.3.d. Evolution of the laccase activities in the liquid mediums

In order to assess that Cu had been already taken up by the fungi we determined the laccase activity in the culture medium. Figure 5.8 presents the laccase activities (Units per mg of dry fungal biomass) in function to the time of incubation. No enzyme activity was observed in the liquid medium with fungal growth in the absence of added Cu. In the presence of Cu a laccase activity could be detected in the liquid medium which attained 0.45U/mg fungal biomass at the 12th day of fungal growth when Cu was added in the form of salt solution. In the same conditions but in the presence of Cu added in the form of POM, we observed a laccase activity which, at 12th day of incubation and onwards, was four times less than the medium with Cu added in the form of salt solution. This result is consistent with the preceding ones and confirmed the bioavailability of Cu when Cu is in the form linked to POM.

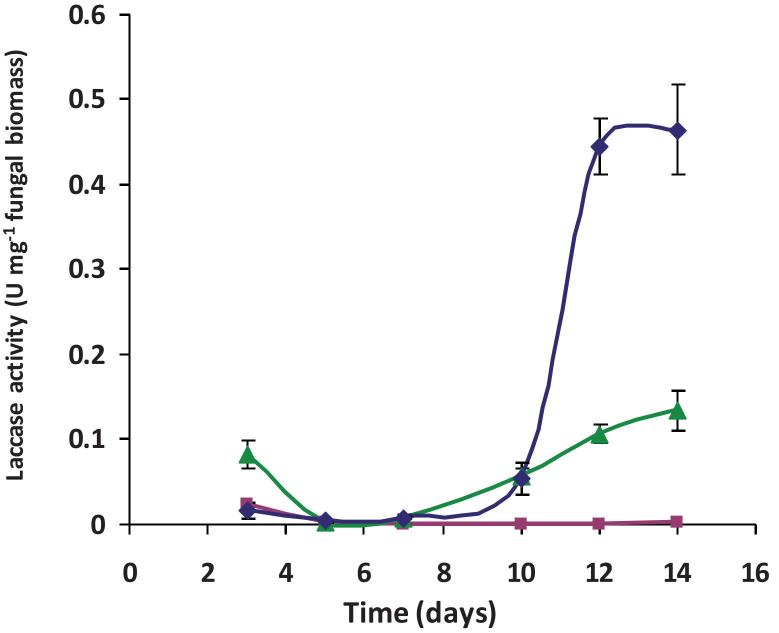


Figure 5.8: *Laccase activities of Trametes versicolor in liquid medium exposed to different source of copper during laboratory incubations in the liquid medium.*

—■— *Unexposed to Cu (Cu added as an oligo-element in yeast extracts)*
—■— *Exposed to Cu as* $CuSO_4$ —■— *Exposed to Cu linked to POM*

Indeed, the presence of Cu induces the production of ligninolytic oxidoreductases like Mn-peroxidase and laccase by different filamentous fungi (Collins and Dobson, 1995; Crowe and Olsson, 2001; Baldrian et al., 2005; Lebrun et al., 2010; Levin et al., 2010). This may be explained by the fact that Cu is a constitutive element of the laccase catalytic site (Mougin et al., 2003). The presence of the laccase activities in the medium with metal addition and not in the control mediums have already been reported in case of *Trametes versicolor* (Charchar, 2008; Lebrun et al., 2010; Lebrun et al., 2011). The stimulation of oxidase production in fungal cultures is the results of metal action at the transcriptional level. Indeed, metal-responsive elements (MREs) have been characterized on the laccase gene in *P. ostreatus* (Giardina et al., 1999) and on a gene cluster coding for Mn- and lignin peroxidases in *T. versicolor* (Johansson and Nyman, 1996). Thus, it has been observed that RNA coding for a laccase is increased in the presence of Cu in *T. versicolor* (Collins and Dobson, 1995).

The comparison of the results of laccase production in different studies is difficult because of the variation in experimental conditions. In the literature, the average values of laccase production determined by oxidation of ABTS in classical culture media and in the presence of well-known organic inducers are ranged between 1.5–15.8 U/mL in Trametes species (Mougin et al., 2002; Wang et al., 2006). In the precedent studies conducted in our laboratory by using the same fungal strain and a specific low complexing culture medium but at relatively high Cu contamination level (1mM), the laccase activities were also found to be in a range between 8-14U/mg dry fungal biomass between 7-10 days of Cu exposure (Charchar, 2008; Lebrun et al., 2011). In our study the Cu exposure was at a stage different from the above mentioned studies i.e. from the start of incubation and the Cu contamination level was about 250times less than the above mentioned studies. We obtained a maximum of 0.45U/mg or 1.4U/mL of laccase activities in the lower range of above mentioned laccase production studies.

The production of laccase activities in the liquid medium in the presence of Cu contamination was reported to be strongly linked to Cu speciation rather than its total contents in the medium (Lebrun et al., 2011). For the inductive potential, these authors established the following hierarchy of form of Cu species: free > inorganic complexed > organic complexed > total. In our study the laccase activities in the liquid mediums with different sources were different but Cu level in the solution was similar (provided that Cu linked to POM did not recharge the solution after displacement of equilibrium). This difference of laccase activities could therefore be linked to the hypothesis that the speciation of Cu in the liquid medium was different in both cases i.e. when released from POM fractions and when added as $CuSO_4$ salt.

The data of laccase activity of the fungi in the Cu added mediums of our study correspond to the toxicological bioavailability of Cu as described by the norm ISO 17402 (ISO, 2008), i.e. the Cu availability inducing a measureable effect on fungi. We found that the laccase activity was four times less in case of Cu addition in the form of POM than in the case of Cu addition in form of salt solution. But quantity of copper taken up by the fungi in case of Cu added as POM was only 2 times less than the Cu uptake by fungi in case of Cu added as salt solution. In other words, the toxicological bioavailability of Cu (bioavailability expressed by an effect) was 2 times less when Cu was added as POM than when Cu was added as salt solution. This difference of magnitude between the Cu environmental and toxicological bioavailabilities in the case of Cu added as POM may be due to the fact that Cu remained adsorbed on fungal mycelium without causing an effect and was not absorbed inside the fungi but also that Cu speciation was not the same after the Cu addition in different forms.

5.3. ***Conclusion***

On the basis of the results obtained in this part of the study we showed that the coarse POM fractions of the polluted soils had rapid turnover and their quantities were reduced during the six months of soil incubation. The enrichment of the fine POM fractions in Cu was increased during soil incubation. In our conditions the POM fractions served as source of Cu in both studied soils and of Pb in Pierrelaye soil and Cd in Metaleurop soil while for Zn the POM fractions of Metaleurop soil play a role of sink for their availability in the soils. The results of the bioavailability study of trace metals linked to POM fractions show that in our study the bioavailability to a living organism i.e. *Trametes versicolor* was lower in case of copper linked to POM fractions than in case of copper added as a salt solution so that it can be suggested that trace metals linked to POM fractions, even bioavailable, may be less bioavailable than metals in solution.

GENERAL CONCLUSION

The main objective of the work presented in this thesis was to assess the effect of land uses on soil trace metals availability in contaminated agricultural soils, using a specific case of perennial bioenergy crops replacing annual crop system. We made the hypothesis that soil organic matter is one of the key labile soil constituent besides the soil texture that controls trace metal availability, and thus which is involved in the effect of land uses. We studied the Cu, Pb, Zn and Cd availability using i) in situ soil samples from the same contaminated parcel but under two different land uses, annual or perennial crop, and ii) a combination of laboratory experiments including chemical extractions at equilibrium and through kinetic extractions and soil incubations as well as separation by physical fractionation of the soil samples in the different size fractions. Metal contents were determined in all the extracts or separates, and comparison of the results through equilibrium and kinetic extractions and linking results to the metal distribution in physical size fractions has allowed us to discuss the concept of trace metal availability.

The study of trace metal availabilities in the soils belonging to the two different sites differing in their texture and origin of pollution have allowed us to highlight that globally the trace metal availabilities of the two soils were different depending on the metal studied. The magnitudes in % of Cu and Zn availabilities were higher in the site of Pierrelaye with the sandy-textured soils rich in organic matter while Pb and Cd availabilities were higher in the other site i.e. Metaleurop with silty-clayed-textured soils. These differences in trace metal availabilities between both sites showed similar trends whatever the approach of assessment used i.e. equilibrium or kinetic extractions. With the physical fractionation method, we also showed that globally the trace metal dynamics in the soils of both sites were different. In the case of the silty-clayed soil the distributions of trace metals and organic carbon in the soil fractions showed the same trends, with a decrease in metal and organic carbon contents with increasing size of soil fractions, a result consistent with the known reactivity of fine fractions. The case of the organic matter rich sandy soils is more complex: metal contents were also found more located in fine size fractions but the organic carbon was more located in coarser fractions. The soil texture as well as the organic content but also the nature of this soil organic matter was involved to interpret the results. Indeed in the case of the Pierrelaye site, the carbon localization in the coarser fractions was, as shown in preceding studies, due to organic coatings around the grain sand-sized fractions related to the nature of the pollution (waste water irrigation). In these soils, results of soil incubation showed that a larger part of the organic matter compared to the site of Metaleurop (with no exogenous organic matter) was not mineralized. It can be thus suspected that the nature of this coarse organic matter is not in favor of reactivity towards metals when looking at results of metal localization in soil fractions.

The comparison of trace metal availabilities of the soils under annual crop and under miscanthus belonging to the two different sites was done to highlight how a change in land use could impact the trace metal availabilities and which are the factors involved. Through the work done, we showed that changing the land use from annual crops to miscanthus influenced the trace metal availabilities and their dynamics but depending on the site, the impacted metals and the trend of change varied. In case of silty clayed soils the trace metal availabilities of Cu and Pb were decreased for the soil under miscanthus and it is interesting to note that these metals are known to be preferentially bound to soil organic matter. At the other site with sandy soils Zn availability decreased and Cd availability increased. In complement it was found that in silty soils both labile and slowly labile pools of Cu and Pb were impacted whereas for sandy soils only the labile pool of Zn and Cd was changed. Finally, it was shown that in the silty clayed soils the decrease in trace metal availabilities of Cu and Pb was accompanied by an increase in localization of these metals in the finer size fractions while for the sandy soils changes in trace metal localization occurred for the metals for which change in metal availabilities was not observed. This apparent large variability of results between the two sites can, however, be consistent with the potential effects of the key soil factors involved in these changes (change in the status of organic matter and absence or presence of tillage) taking into account that the resulting effect will depend on the soil texture and the soil organic matter contents of the corresponding site. Indeed, when the soil is already rich in organic matter, new organic matter due to new management practices will not perturb the soil metal equilibriums as far as for a soil which is not as richer in organic matter. On the other hand, the impact of the absence of tillage for a soil with an initial sandy-texture will not be as consequent as for a soil with a silty-clay sandy texture.

Through the studies of the samples from laboratory incubations we were able to highlight the role of soil organic matter in the trace metal availabilities of these contaminated soils. Through this work we found that the changes in trace metal availabilities and their dynamics as a result of enhanced organic mineralization though laboratory incubations were different for both textured soils. In the same conditions of incubation, the mineralization of the organic carbon was more in the case of soils under miscanthus for both sites. The results from the trace metal availabilities studies at equilibrium showed that the metals for which the availabilities were changed after soil incubation can be divided into two groups, the metals for which the changes in availability were not site and then of texture dependent i.e. Cu and Zn because their availability changed in all soils, and the metals for which the changes were specific of a site and then of texture i.e. Pb and Cd. The only metal for which the change in availability was different according to the land use of soil was Pb. It was also observed that the enhancement in soil organic matter mineralization influenced the trace metal localization in the different size fractions as well as the relations among trace metal availability, trace metal distributions and carbon distribution in the different size fractions.

The use of combined approaches of chemical extractions in bulk samples and metal localization in physical fractions of the soils allowed us to assess the influence of soil organic matter on the trace metal dynamics in soils. This influence was found comparing annual crop and miscanthus soils where for the Metaleurop site with silty clayed soils the changes in trace metals availabilities and in localization towards fine fractions occurred for the same metals while this was not the case for the Pierrelaye site with sandy soils. Similarly, applying this approach on soil samples after laboratory incubation revealed that in case of the Metaleurop soil with silty soils the relationships already observed between trace metal availabilities, trace metal localization and organic carbon localization were lost while in case of the Pierrelaye site with sandy soils, the trace metal availabilities became correlated with the organic carbon localization.

In our work, the effects of changes in the nature of soil organic matter on trace metal availabilities were observed in two ways 1) by comparing in situ soils under annual versus perennial crops, but in this case the effect of change in tillage is also present and 2) by enhancing the soil organic matter mineralization during laboratory soil incubations and in this case only the organic matter was concerned. Our results showed that enhancing mineralization in the laboratory induced an increase in Cu availability whatever the soils or the site, the other metals being differently impacted but the trend was that Pb availability also increased, and Zn and Cd availability decreased. As in situ the results were globally different and opposite (Cu and Pb availabilities decreased in miscanthus soils in silty-clay soils and Zn and Cd availabilities increased in sandy soils) we concluded that the change in land use in our study was not in favor of an enhanced carbon mineralization but rather carbon stock and/or redistribution.

Through our work on particulate organic matter fractions, we have been able to discuss the role of this labile soil organic matter fraction with a rapid turnover as a source or sink of metals by studying the trace metal availabilities of the soils with and without their POM fractions. In our conditions we found that the POM fractions have a role of source of Cu, Pb and Cd depending upon the soil studied and of sink for Zn. Further we developed an experiment aiming to study the bioavailability of copper linked to particulate organic matter (POM) using a fungi as target whose response in the presence of copper corresponded to the activation of an enzyme. The first approach we made in liquid medium revealed that some copper initially linked to POM fractions became bioavailable mostly because of small release in solution, but this copper released was found less bioavailable than the same Cu content in the form of salt solution.

Perspectives

The results of the present study raised several scientific questions, which need attention in order to improve the understanding of the role of soil organic matter in the trace metal availabilities in contaminated soils.

- Linking the soil trace metal availabilities data studied through chemical extractions to the data of trace metal dynamics studies through soil physical fractionation was found an interesting approach. In this work we were not able to get enough data to apply statistical approaches like multiple linear regressions to quantify the relationships among data. But we think that it will be a useful approach to get more insights into the relations between trace metal availabilities of the soils with their localization in physical size fractions.

- In our conditions we observed an influence of land use on trace metal availabilities in the two different studied sites, but the age of miscanthus was three years at the time of sampling, and it should be interesting to follow the study of the influence of land use with longer history of changes in land use, as the trends could change. Indeed, it can be hypothesized that in soils where the trace metal availability decreased, living organisms could come back even in such polluted sites, but the feedback effect of these organisms on the soil trace metal availability should have to be determined.
- In this study only the trace metal availabilities and dynamics of contaminated soils were involved, but including the assessments involving non contaminated soils under the same pedogenetic conditions could be a complementary approach.
- In this study we focused more to the characterization of trace metals availabilities and trace metal dynamics while the changes in organic matter were indirect and thus indicative. Coupling of this study with a study involving quantification of changes in soil organic matter will enhance our understandings of the role of soil organic matter.
- Our physical fractionation method did not involve the separation of clay and fine silt fractions; similarly the separation of POM fractions was done only for the work presented in last part of the thesis. Involvement of these fractions and their inclusive in data interpretation of this study could be complementary.
- Studies involving POM fractions from more soils and studying their role as source or sink of metals is necessary.
- Relations between soil metal availabilities and metal bioavailabilities with soil organisms as target would be interesting to develop.

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List of Presentations/Posters /Publications concerning results of thesis work

Communications.

1. *Relation between metal bioavailability, nature of organic matter and impact on soil organisms in contaminated soils*. **Muhammad Iqbal**, M. Hedde and I. Lamy. SETAC Europe Annual Meeting 2010, May 23-27, 2010, Seville, Spain. (Poster presentation)
2. *Impact du changement d'occupation des sols sur la biodisponibilité des éléments traces métalliques: Cas des sols pollués*. **Muhammad Iqbal.** Annual meeting of ANR Project RESACOR, January 29, 2010. AgroparisTech, Paris. (Oral presentation)
3. *L'offre du sol et la biodisponibilité des métaux dans les sols contaminés.* **Muhammad Iqbal,** A. Bermond and I. Lamy. Seminar on Ecotoxicologie, F.I.R.E. June 11, 2010. University Paris 6, Paris. (Oral presentation)
4. *La biodisponibilité des éléments traces métalliques des sols contaminés: Rôle du statut de la matière organique du sol.* **Muhammad Iqbal.** Annual meeting of Axe 1 of Research, Unité PESSAC-Physico-chemistry and ecotoxicology of contaminated agricultural soil systems. October 15, 2010. INRA de Versailles, Versailles. (Oral presentation)
5. Assessment of changes in trace metal availability of contaminated soils after changes in land use from annual to perennial cropping systems. **Muhammad Iqbal** and I. Lamy. European Geosciences Union General Assembly 2011, April 03-08, 2011, Vienna, Austria. (Poster presentation)
6. Changes in land use of contaminated sandy soils modify their trace metal availability. **Muhammad Iqbal** and I. Lamy. Soil interfaces in a changing world, ISMOM 2011, Montpellier, France. (Poster presentation)
7. Impact du changement d'occupation des sols sur la biodisponibilité des métaux dans les sols contaminés : cas des cultures annuelles vs cultures pérennes à vocation énergétique. **Muhammad Iqbal,** L. Beaumelle, C. Chenu, and I. Lamy. 4ème Séminaire d'Écotoxicologie de l'INRA, 7-9 Novembre 2011, Château de Ravatys, France. (Oral presentation)

Research Article.

1. Impact of energy crops on trace metal availability in contaminated agricultural soils: complementary insights from kinetic extraction and physical fractionation. Muhammad Iqbal, A. Bermond and I. Lamy. (manuscript submitted to Chemosphere)

Résumé

Le changement d'occupation des sols contaminés est susceptible de modifier la nature et la quantité des matières organiques du sol (MOS). Les matières organiques sont connues pour avoir un rôle de source trophique pour les organismes des sols et un rôle de ligand vis-à-vis des éléments traces métalliques (ETM) dans les sols contaminés. Des changements de matières organiques dus aux changements d'occupation des sols contaminés peuvent modifier la spéciation des ETM et leur disponibilité. Mais dans ce cas, le rôle de la matière organique dans le déterminisme de la disponibilité des ETM est peu documenté. L'objectif du travail présenté dans cette thèse était de mettre en évidence le rôle des matières organiques dans la disponibilité des ETM, dans le cas de sols agricoles contaminés après un changement de cultures annuelles vers des cultures pérennes à vocation non-alimentaire. Le travail a concerné l'étude de la disponibilité du Cu, Pb, Zn et Cd dans les sols de deux sites différents en texture et en origine de pollution i.e. le site de Metaleurop (Nord de la France) avec des sols limoneux contaminés par des retombées atmosphériques et le site de Pierrelaye (Région Parisienne) avec des sols sableux contaminés par les apports d'eaux usées brutes de la ville de Paris. Le rôle de la matière organique a été étudié en travaillant 1) sur des échantillons de sols sous deux cultures différentes i.e. sous culture annuelle et culture pérenne à vocation énergétique miscanthus depuis trois ans, 2) sur les échantillons de sol avant et après incubation in vitro afin d'accélérer la minéralisation de la matière organique, et 3) sur des échantillons de sol avec et sans matières organiques particulaires (MOP) i.e. les fractions libres des MOS afin de mettre en évidence leur rôle spécifique. Une approche combinée d'évaluation de la disponibilité par des extractions chimiques des ETM et d'étude de la localisation des ETM dans des fractions granulométriques a été utilisée. Les résultats obtenus montrent que l'impact de l'occupation des sols (sous culture annuelle ou pérenne miscanthus) sur la disponibilité des ETM était différent pour les deux sites étudiés. Pour les sols du site de Metaleurop les résultats ont montré que la disponibilité de Cu et Pb diminuait sous miscanthus tandis qu'une augmentation de leur localisation dans les fractions fines était observée. Par contre aucun changement n'a été observé pour Zn ni Cd dans ce site. Au contraire pour les sols sableux riches en matières organiques de Pierrelaye le changement d'occupation a impacté la disponibilité de Zn et Cd tandis que celle de Pb et Cu n'a pas évolué. Les résultats des sols avant et après incubation montrent que l'impact de la minéralisation des MOS sur la disponibilité des ETM était site dépendant pour Pb et Cd alors que l'effet sur Cu et Zn était similaire pour les sols des deux sites. En travaillant sur les sols avec et sans MOP, nous avons pu observer que les MOP en tant que fraction organique libre participaient à la disponibilité des ETM telle qu'étudiée, et que une partie du cuivre lié aux MOP était disponible pour un organisme dans nos conditions. Ces résultats de disponibilités des ETM dans des sols qui varient avec la nature et la quantité des matières organiques sont discutés.

Mots clés: Cu, Pb, Zn, Cd, sols agricoles contaminés, disponibilité, biodisponibilité, matières organiques, extractions cinétiques, fractions granulométriques, matières organiques particulaires, miscanthus