Influence of pesticides on trace element transfer in the soil–solution–plant system

A focus on glyphosate and tebuconazole

Nathan Bemelmans

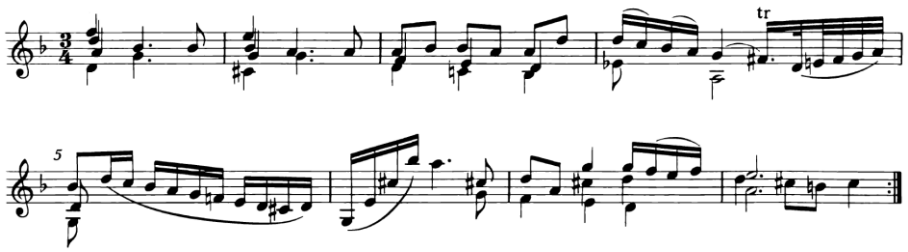
November 2024

Thèse présentée en vue de l'obtention du grade de docteur
en sciences agronomiques et ingénierie biologique

Composition of the jury:
Yannick Agnan (UCLouvain, supervisor)
Lionel Alletto (INRAE)
Maryse Castrec-Rouelle (Sorbonne Université)
Stanley Lutts (UCLouvain, secretary)
Benoît Pereira (EREA)
Marnik Vanclooster (UCLouvain, jury president)

For nitrates are not the land, nor phosphates; and the length of fibre in the cotton is not the land. Carbon is not the man, nor salt nor water nor calcium. He is all these, but he is much more; and the land is much more than its analysis.

John Steinbeck,
The Grapes of Wrath



Johann Sebastian Bach, *Violin Partita No. 2 in D Minor (BWV 1004) : Sarabande (1-8)*

Acknowledgments

It is a major injustice that only one name is featured on the cover of this thesis, an injustice that is quite insufficiently corrected by the title page. This work, such as all theses, is indeed the result of the happy alignment of circumstances and, even more, of the goodwill and the flawless implication of a series of persons.

I therefore wish to thank the F*onds pour la formation à la Recherche dans l'Industrie et l'Agriculture* (FRIA) from the *Fond de la Recherche Scientifique* (FNRS) for financing this thesis. My thanks also go to the members of my jury for the time they have taken to carefully read this work, and review it. I want to underline the kindness and benevolence they have shown during the private defence. Marnik Vanclooster and Benoît Pereira are especially acknowledged for their presence in my support committee thereby providing useful advices. I also want to thank all the students that worked with me on the experiments of this thesis, in chronological order, Loth Synclair Tamo, Rosalie Dejardin and Alexandre Vanden Hautte but also Julie Falys and Adil Thami although I was less implied in their work. Beyond providing useful help in performing experiments, they helped by giving relevant insights on the results and more than anything made the time pass quicker by the quality of their conversation during, sometimes long and laborious, manipulations. The analytical component of this work would not have been carried out properly without the support of Anne Iserentant, Elodie Devos and Laurence Monin for trace element analyses, Claudine Givron for dissolved organic carbon and nitrogen analysis, and Etienne Bodart and Hélène Dailly for the development of the analysis method of glyphosate and AMPA such as the operating of the HPLC. I would also like to thank all other members of the MOCA platform for their

useful advices and the good time shared in the labs and with them the whole administrative team.

A person that was most central all along these four years was my supervisor Yannick Agnan. I am especially thankful for the trust he placed in me while I was still a master student, when he accepted to start this project with me. But mostly for his daily support in all the aspects of this work (experiments design, redaction etc.). I will remember many good moments and exciting conversations shared around chocolate bars and dried apples.

Of course, many challenges in a thesis are related to the scientific aspect but the human factor is key and, on this subject, I had the chance to be able to count on many people that were always there to offer a listening ear during difficult times. Therefore, I want to thank my numerous colleagues of the SOLS lab (and also ELIE), especially Bryan Arbalestrie, Romain Duquenne, Maxime Delvoie and Arnaud Denis those that started their theses at the same moment as me and were already good friends. Arnaud Denis deserves special acknowledgements as I had the privilege of sharing his office for four years. I must admit that this association was full of *clairvoyance* as, beyond the fruitful results in the ELIE sport challenge with a double victory in the office ranking and despite Arnaud's very personal interpretation of the concept of tidiness, it led to the reinforcement of our friendship.

Finally, I desire to acknowledge all those that supported me from outside of the institute. First, my friends that are too many to be cited, I cannot express how grateful I am and it feels like I cannot believe I have so many people of such quality around me. Though I have left the familial nest during the early months of my thesis I could always count on them to have a moral support. And to conclude, the key person without which I could never have brought this project to its accomplishment

is my dear Maud who was always there, even in the most difficult moments to try cheering me up, and bring me her unconditional support.

Table of contents

Abstract

Soils are complex ecosystems affected by various contaminants, including trace elements and pesticides. Despite their low environmental concentration, trace elements pose important risks to human and ecosystem health due to their toxicity and their mobility that is influenced by complex formation with organic compounds. Pesticides commonly found in agricultural soils, such as glyphosate and tebuconazole, can bind trace elements, but the extent to which they affect their transfer to hydrosystem and plant remains poorly understood. Additionally, the role of organic matter in mitigating these effects via competitive binding is unclear.

This thesis aimed to better understand how pesticides may influence trace element transfer from the soil to soil solutions and plants. Therefore, extractability experiments and greenhouse experiments (with *Triticum spelta*) were conducted on four soils with contrasting contamination backgrounds. These experiments assessed how these ligands (formulated and unformulated pesticides as well as a metabolite and dissolved organic matter), alone and in combination, influence trace element extractability and transfer.

Results showed that all organic ligands affected trace element extractability, varying by soil and element. Combined effects were mostly additive or sub-additive, with occasional synergy. In greenhouse experiments, glyphosate showed similar trends to extractability experiments but with less variation between elements. In general, increased anthropogenic contamination reduced organic compounds effects on trace element mobility. Solid organic matter (compost) helped reduce mobility in highly contaminated soils but increased risk in those with low mobile trace element content. Overall, we showed that there is a potential risk

of increased trace element exposure through pesticide use, especially for toxic elements such as Pb, Cd, and As.

1. Introduction

Soils are incredibly complex ecosystems that are highly impacted by human activities, affecting their quality and limiting their ability to fulfil their functions. One of the pressures weighing on soil is contamination. This not only limits the availability of useable land but also directly affects human and ecosystem health, as contaminants spread throughout the environment, are taken up by plants, and end up in food products. Some lands are naturally-enriched with contaminants, but can still pose risks.

There is a wide variety of substances among contaminants, both organic (such as hydrocarbons or microplastics) and mineral compounds. Trace elements (As, Pb, Cd, Cr, Cu, etc.) can be toxic and occur in a wide range of concentrations with significant variations across different elements and soils. These elements are present naturally, resulting from the bedrock alteration, but can also originate from anthropogenic activities, including atmospheric deposition of pollutants or importation as stowaways in fertilizers or organic matters. Yet, contaminants do not behave independently and may interact with each other, potentially amplifying or mitigating their harmful effects. For example, organic compounds may influence soil pH but also form complexes with trace elements. Among organic contaminants, pesticides especially occur in agricultural soils where they are applied. The herbicide glyphosate is one of the most well-known and commonly found pesticides and it has been shown to interact with trace elements through binding processes, forming complexes. However, other pesticides also presented similar trends, such as the fungicide tebuconazole. These interactions could influence the behaviour and the toxicity of trace elements in agroecosystems, altering their chemical properties and thereby affecting their ability to sorb onto soil particles, remain stable in solution, and even cross cell

membranes up to plant accumulation. To date, the influence of contaminants on each other's behaviour remains a blind spot in our understanding of their impacts. In a broader sense, the influence of organic compound addition remains neglected regarding its role on trace elements mobility.

This thesis aimed to deepen our understanding of these processes by studying the influence of pesticides (glyphosate and tebuconazole) on the transfer of a wide variety of trace elements (As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, and Zn) from soil to solution and to plant. Additionally, it explores the potential use of exogenous organic matter as a means to mitigate possible pesticide-driven influence in trace element mobility.

2. State of the art

2.1. Trace elements

2.1.1. Who are they?

Our environment conceals a myriad of chemical elements. Though very variable in their abundance and unequally distributed, every handful of earth, water, or leaves contains them in all their diversity, from the most common to the most exotic. While the Earth's crust is primarily composed of silicon and oxygen, its core is predominantly made of iron. Living organisms are largely composed of carbon, hydrogen, oxygen, and nitrogen. On the other side of the spectrum, there is a series of elements sometimes necessary for the health and metabolism of living organisms, but they all become toxic when reaching higher concentrations, which makes them a challenge for environmental and human health. Some of the most toxic caused sanitary disasters, like the mercury pollution of Minamata (Harada 1995), or have marked the public through notorious contamination such as the lead poisoning that could have caused L. van Beethoven’s deafness (Stevens et al. 2013). More generally, there has been a widespread use of products like ceruse (a lead carbonate) or *Scheele’s Green* (a copper arsenite) throughout the history for such mundane applications as dyes or cosmetics (Drasch 1982; Whorton 2010). Some have even been inscribed in popular culture with, for example, the arsenic suicide of Emma Bovary in Gustave Flaubert’s novel. It is a common misconception among the broader public that trace elements have mainly been a threat to humans since industrialization, but human poisoning by trace elements is an old history. Evidence of arsenic poisoning dates to the Bronze Age (Charles 1967) and a great variety of lead poisonings are reported in the Late Antiquity (Drasch 1982).

As elements are unequally distributed among compartments, the elements that are considered as “trace” vary across the fields of research, as each have produced their definition regarding their needs. In medicine, trace elements are considered referring to concentrations in tissues (Mertz 1981), while in geochemistry, trace elements are commonly defined as elements that are individually representing less than 1‰ of the mass of the Earth's crust (Kabata-Pendias 2011). This definition also includes elements well known as “heavy metals” although this term is considered obsolete because it includes elements with a density lower than the usual threshold of 5 g cm^{-3} (e.g., Ti) as well as metalloids and non-metalloids (As, Sb, and Se; Duffus 2001; Chapman and Holzmann 2007). Trace elements are still a major subject as they constitute valuable ores and are vital for the world’s economy. For example, copper is widely used in conductors and elements like the lanthanides (also known as rare earth elements) are crucial for modern technologies. However, handling of trace elements comes with societal costs due to increased spending in healthcare (Landrigan et al. 2002) but also for loss of surfaces that become out of use or need costly rehabilitation (Khan et al. 2021).

Cycle of trace elements

Trace elements are present in minerals either as major constituents of the structure, like for lead in galena, copper in malachite, mercury in cinnabar, vanadium (and lead) in vanadinite or arsenic in orpiment, or as trace (as impurities) diluted in other elements. In some cases, like chromium, the structure of minerals can be constituted in totality by one element, one then refers as “native element mineral”. More commonly, trace elements are present in rocks to various degrees depending on their type and formation processes, for example, Lovering (1976) notes average lead concentrations of 32 mg kg^{-1} for carbonaceous shales against 11 mg kg^{-1} for limestone and dolomite. Thus, in igneous rocks

(rocks that are derived from a magma), the trace elements content depends on both the initial concentration in the magma and the fractionation processes. When a magma is cooling or heating, minerals are precipitating or melting following the Bowen's reaction series (Bowen 1922); in these processes certain trace elements are preferencially incorporated into minerals while others are excluded (Hart and Allègre 1980). By weathering of these igneous rocks, some trace elements are directly released into water while others, following the pedogenesis process, integrate into soil particles.

Transport through the air is also key (Figure 2.1). Indeed, trace elements are emitted by magmas through volcanic gases at rates exceeding 100 kg, and potentially more than 1000 kg per day of elements such as Pb, Zn and Cu for volcanoes like the Etna or the Kilauea during eruption events (Crowe et al. 1987; Edmonds et al. 2022). They are also massively transported as (or carried by) dust from aeolian erosion that can represent up to 50% of the total input to the atmosphere for elements like Cr, Mn, and V (Patterson and Settle 1987; Rauch and Pacyna 2009; Camarero et al. 2009). Other processes and events, such as forest fires (especially for Cu, Pb, and Zn) but also sea salt sprays (Cu and Pb; Nriagu 1989; Pacyna and Pacyna 2016), represent a less important contribution to the atmosphere (<15% of the total budget; Nriagu 1989). In addition, often overlooked biogenic sources can contribute up to 60% of airborne trace elements in forest regions (like As and Hg; Nriagu 1989), they are mainly transported by spores or pollen. Air transport being so important, the dissemination of trace elements is intimately linked to climate through wind and rain (Jickells et al. 2005).

Beyond these natural sources, human activities also release trace elements in the atmosphere. Important fluxes come from the combustion of fossil fuels, accounting for more than 85% of total anthropogenic emissions of elements like Ni, Sn, or V, but less than 15% for As and Cu (Nriagu and Pacyna 1988), but also from the waste combustion.

Transport is an important source of Pb through the combustion of fuel but also the wear from tires and brakes (Adachi and Tainosho 2004; Pacyna and Pacyna 2016). Finally, the industry and especially the smelting industry, represents 40 to 65% of anthropogenic Cr, Cu, and Zn inputs into the atmosphere but other domains such as cement production are also important contributors (Nriagu and Pacyna 1988). Note, however, that some old data regarding global trace element budget to the atmosphere present important uncertainties, because of estimates based on correlations with other trace elements and the implementation of many regulations that are resulting in decreased anthropogenic trace element emissions (Poulakis et al. 2015; Dimitriou and Kassomenos 2017; Harlavan et al. 2021).

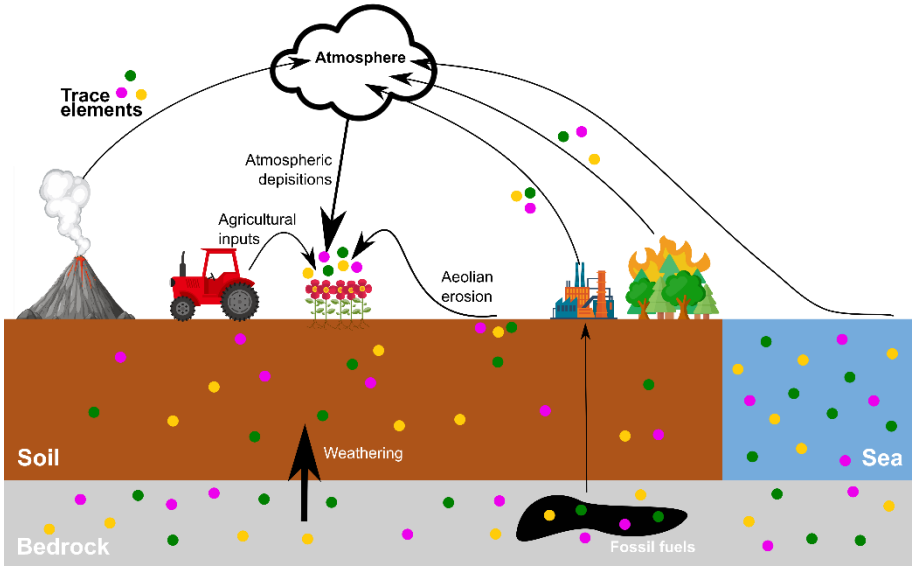


Figure 2.1 Major trace elements fluxes in agricultural contexts

The deposition of all the suspended particles (dusting process) implies that a soil has a composite origin and does not only originate from a single material. Note that the diagenesis of the created secondary materials produces sedimentary rocks, they are secondary materials whose

trace element content is the result of a wide diversity of processes involved in the formation of the primary materials. All these soils can reach back to the mantle in subduction zones. Through hydric erosion these soil particles can be transported to the hydrosystem. In the soil, such as in aquatic ecosystems, trace elements can be incorporated in a variety of organisms including plants and thus run through the food chain and possibly be ingested by humans (Dudka and Miller 1999).

Toxicology

Trace elements can have harmful effects on both human and environmental health. There are two main types of toxicity trace elements are associated with (Goldhaber 2003): acute and chronic toxicity. The first one occurs after a single exposure to a high dose, leading to diseases or causing death in extreme cases. For example, lead can cause delirium (Wani et al. 2015), severe arsenic poisonings cause gastro-intestinal lesions and nausea (Ratnaike 2003), and acute cadmium poisoning can be the cause of renal failures and cardiopulmonary depression (Bernard and Lauwerys 1984). The second one concerns effects consecutive to repeated expositions to low doses. These chronic exposures have been shown to cause a wide variety of diseases including cardio-vascular diseases, anaemia, liver disturbances, or cancer (Calabrese et al. 1985). In addition to the risk posed by pollutants alone, their risk can combine and even have synergetic effects (Altenburger et al. 2000; Cedergreen 2014), the same goes for trace elements (Wu et al. 2016).

In addition to their deleterious effects on human health, trace elements affect the functioning of ecosystems by diminishing microbial biomass (He et al. 2005), they accumulate in fishes and affect their physiology (Shahjahan et al. 2022), but they could also be linked to the decline of forests (Gawel et al. 1996).

2.1.2. Trace elements in agroecosystems

Agroecosystems are crucial for human societies but face numerous pressures, trace elements being one of them. These elements join those that are already present in the soil through various pathways. One primary route is the weathering of the parent materials that all contain trace elements to varying degrees, whether high or low and are then inherited by the soils formed from these parent materials (Adriano 2001; Pacyna and Pacyna 2016).

In addition to these natural sources and processes, human activities have a major influence on trace elements import and cycling. Indeed, airborne trace elements are also massively imported leading to widespread diffuse contaminations, especially around industrial poles and along roads (Bermudez et al. 2012; Biasioli et al. 2012; Modrzewska and Wyszkowski 2014). Besides, there are direct inputs of trace elements into the soil, either through accidental pollution with highly trace element-concentrated materials or, more commonly, through enrichment because of trace elements being unintentionally introduced as stowaways in substances added for other purposes. In agriculture, organic matters that are commonly enriched in trace elements such as sewage sludge, plant compost (Hg, Pb), manure (Mo, Ni, Zn), and other inputs of animal origin are a major source (Belon et al. 2012, Figure 2.2). Another is mineral fertilizers that are produced by mining, such as those containing phosphate (especially for Cd, Cr, and Se). Pesticides can also be a source, though the use of metallic pesticides has decreased, this mainly regards Cu that is the main component of Bordeaux mixture (Adriano 2001; Nicholson et al. 2003; Ballabio et al. 2018). Finally, through time, various products have been discovered to have somehow beneficial properties without being originally destined to an agricultural use. For example, steel industry residues have sometimes been im-

ported in plots to increase cation exchange capacity (CEC), that is defined as the number of negative charges per mass of soil, and bring nutrients. Note that trace elements are persistent contaminants in soil as, in opposition to most organic contaminants, they cannot be removed by degradation.

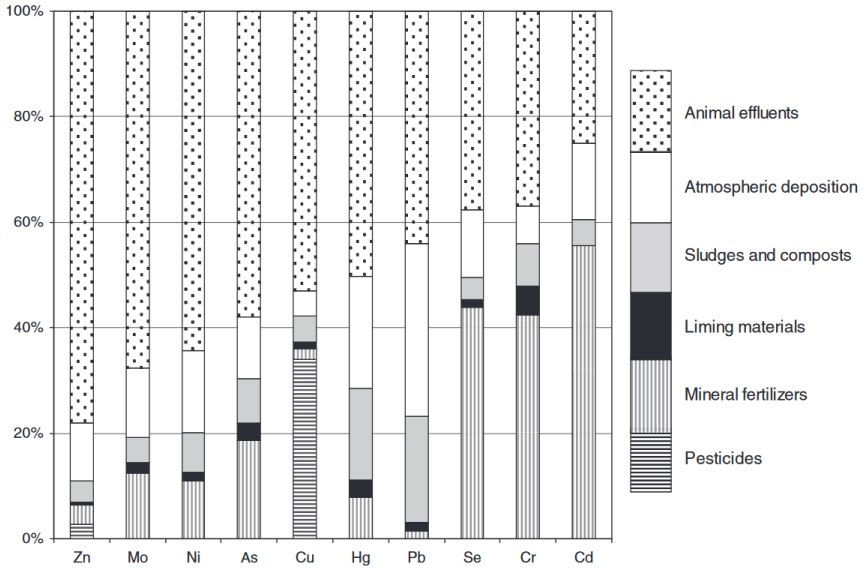


Figure 2.2 Annual contribution of six main contamination sources to the average total amount of 10 trace elements reaching soils from France (Belon et al. 2012).

Legislative aspects

Due to their toxicity, regulations exist to avoid the practice of activities supposed to increase the public exposure to trace elements. In Wallonia, this is part of a corpus of laws called “Décret Sols” (Soils decree). The link between pollutant content (also others than trace elements) and associated authorized category of use is detailed in its annexe I. The only trace elements considered are As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn for five categories of soil use with increasing tolerance for trace elements

(Table 2-1; Gouvernement wallon 2018): natural zones (category I); agricultural uses (category II); residential uses (category III); recreational and commercial zones (category IV); and industrial uses (category V).

If trace element content in a soil is superior to the norm (for recent pollutions, after April 2007), the polluter needs to remediate the site. In the case of older pollution, a risk assessment must be carried out. After remediation, trace element content in soil must be inferior to 80% of the threshold value (Gouvernement wallon 2018).

For trace elements in food products, a European legislation sets limits for As, Cd, Hg, Pb, and Sn in a wide diversity of food products that varies across the elements (EC 2023). For example, Hg is only regulated in fishes, molluscs, cephalopods, food supplements and salt while for Pb is added a wide diversity of agricultural products, beverages, etc.

Table 2-1 Threshold values in soils (in mg/kg of dry matter) associated with trace elements and categories of use (Gouvernement wallon 2018).

	Threshold value by soil use category				
Trace elements	Category I	Category II	Category III	Category IV	Category V
Cd	1.8	1.3	3	10	20
Cr	57	57	78	140	288
Cu	53	53	156	490	600
As	30	30	40	40	65
Ni	87	87	146	350	350
Pb	120	200	200	390	1840
Zn	196	196	415	3000	3000
Hg	1.1	1.1	1.75	5	5

2.1.3. Speciation of trace elements in the soil

Liquid fraction

The soil is a complex system with typically a solid, a liquid (i.e., soil solution), and a gaseous fraction. In addition, it houses a diversity of living organisms able to modify their environment. Coherently, the behaviour of individual atoms varies heavily in function of the soil conditions and the location of the element in the system. In the liquid fraction, both pH and redox conditions play a major role as they determine the level of oxidation of the element and can influence their speciation (distribution of an element amongst defined chemical species in a system, IUPAC 2019) that controls their solubility, precipitation, etc. (Adriano 2001; Kabata-Pendias 2011). Therefore, it is common to represent the speciation of trace elements in function of pH and Eh (i.e., Pourbaix diagram) thermodynamically controlled (Figure 2.3). Then, some elements can be under the form of oxides, hydroxides, etc. (Hooda 2010). They might also be in a free ion form (for example Zn^{2+}) or a hydrated one ($Zn(H_2O)_6^{2+}$). However, in circumneutral soil pH and in soils that are not flooded, most trace elements are in the Me^{2+} form (Me stands for metal), except for As, Mo, and Sb that are usually in the form of oxyanions (like AsO_4^{3-}, $HAsO_4^{2-}$, MoO_4^{2-}, and $Sb(OH)_6^-$, respectively; Adriano 2001; Okkenhaug et al. 2011). Besides, trace elements can also be part of organic molecules (like $HgCH_3$). Note that other substances in solution, such as CO_2 or sulphate, influence the trace element speciation altering the global charge (Pedeferri 2018).

Other components present in the solution can also interact with trace elements. In solution, organic compounds can form complexes with trace elements that can either be stable in solution or precipitate. A complex is defined as *a molecular entity formed by loose association involving two or more component molecular entities—ionic or uncharged—or the corresponding chemical species. The bonding between*

the components is normally weaker than in a covalent bond (IUPAC 2019). In surface chemistry, complexes are termed "inner sphere" when no water molecule is involved in the bond between a surface and a compound. Conversely, when water is involved, the complex is referred to as "outer sphere". It is common in the literature to use the term "chelation" or "chelator". It is a specific case of complexation defines as *the formation or presence of bonds (or other attractive interactions) between two or more separate binding sites within the same ligand and a single central atom* (IUPAC 2019). In addition, colloids that are organic or mineral particles with a diameter between the orders of the nanometre and the micrometres, considered as part of the solution, can also sorb trace elements (de Jonge et al. 2004; Shaheen et al. 2013). This means that they can bind them on their surface, thus serving as an element transporter.

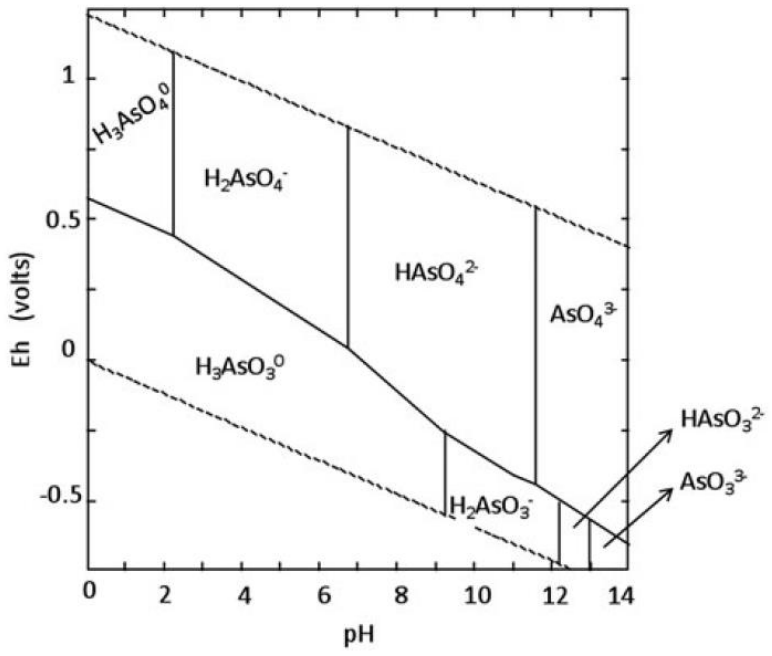


Figure 2.3 Pourbaix diagrams for As in soils (Lombi and Holm 2010).

Solid fraction

In the solid fraction, trace elements can be either occluded in minerals or sorbed onto particle surfaces. When they are occluded, there is a high chance that they were already part of the parent material or have been imported before being occluded because of subsequent processes (Tessier et al. 1979; Adriano 2001). Furthermore, all elements occluded in minerals are facing different circumstances, as some may be more easily released than others depending on the stability of minerals. As an example, oxides might release the trace elements they contain if put in reducible conditions, while more stable (less reactive and difficult to dissolve) minerals like quartz may not.

To determine in which kind of phase the elements are, and thus how easy it is to mobilise them, various procedures called sequential extractions have been elaborated to fit different needs. One was described by Tessier et al. (1979) and distinguishes five fractions that are, from the most to the less mobile: the exchangeable fraction, the fraction bound to carbonates, the fraction bound to iron and manganese oxides, the fraction bound to organic matter, and the residual one. Another, proposed by the Commission of the European Communities Bureau of Reference (BCR) separates an exchangeable, a reducible, an oxidizable and a residual fraction (Žemberyová et al. 2006). Note that, as protocols are not identical, fractions with the same names across protocols (exchangeable, residual) may not be comparable. Moreover, sequential extractions face criticism due to the fact that they were designed for sediments and may not be suited for other contexts of use, such as polluted soils in distinct geochemical contexts. In additions, it has been pointed out that: (1) reagents usually lack selectivity; (2) re-adsorptions can occur throughout the process; and (3) sample pre-treatment could also be a source of errors (Martin et al. 1987; Gleyzes et al. 2002)

Trace elements brought by anthropogenic contaminations tend to be found on the surface of soil particles (Rauret 1998; Sungur et al. 2014).

When they are sorbed via surface charges, they are then called exchangeable, meaning that they are easily transferred to solutions. Those surface charges are especially present on clay minerals (White 2009). In addition to the mineral phase, organic phase can play a major role, mainly due to its important CEC. Beyond its influence on the soil solution, pH influences the number of charges available for the binding of trace elements as H^+ tends to compete with other cations for binding sites. In addition to that, alkaline pH promotes adsorption via inner sphere complexes of elements on a hydroxide form ($MeOH^+$), while lower pH promotes non-specific adsorption (Kim et al. 2015). Beyond the mineral matrix, trace elements are included inside underground living organisms being plants, fungi, bacteria, or animals (Brantley et al. 2001; Kabata-Pendias 2011; Arbalestrie et al. 2022).

2.1.4. Transfer of trace elements in agroecosystems

Soil-plant transfer

Plants play an important role in the cycling of trace elements, through root uptake, accumulation and litter recycling. They can also absorb trace elements from the atmosphere through their aerial parts but also from the soil through their roots. However, a series of barriers regulate trace elements transfer. Plants possess in their roots a Casparian band (or strip), which is a thickening of the endodermis mainly composed of suberin and lignin (Figure 2.4, Taiz and Zeiger; 2006). This boundary prevents the apoplasmic transport (i.e., transport through cell walls without crossing membranes) and forces elements to cross membranes through specific transporters. This process favours the transfer of elements that are either selected by plants or that are much alike others and can use their transmembrane transporter. A well-known example is Cd that uses Ca transporters (Li et al. 2017b). However, some trace elements may cross the barrier through imperfections in the Casparian band, especially at the root tips and zones of lateral roots emergence.

Thus, trace elements may reach the vascular system only by the apoplasmic pathway (Perry and Greenway 1973; Häussling et al. 1988). In addition to the capacity of plants to take trace elements up from the soil, their ability to translocate them to other organs of the aerial part is key for the total mass of element that is taken up. A non-linearity between the concentration of available trace elements and their content in plants was reported and was attributed to the fact that trace elements may be toxic for plants implying saturation processes. All plant species do not have the same ability to physiologically cope with trace elements. Consequently, some that are especially prone to take them up without major physiological failure are called accumulators or in extreme cases hyperaccumulators (Li et al. 2007). They are used to remove trace elements from soils (phytoremediation). Soil-plant transfer is considered critical for the exposure of humans to trace elements through the consumption of contaminated food.

The bioavailability of trace elements, i.e., ability to be taken up by plants, depends on both the chemistry of the elements and their speciation. Indeed, exchangeable trace elements are more bioavailable than occluded ones (Adriano 2001). Beyond the question of the speciation of the element, environmental variables play an important role on trace elements bioavailability.

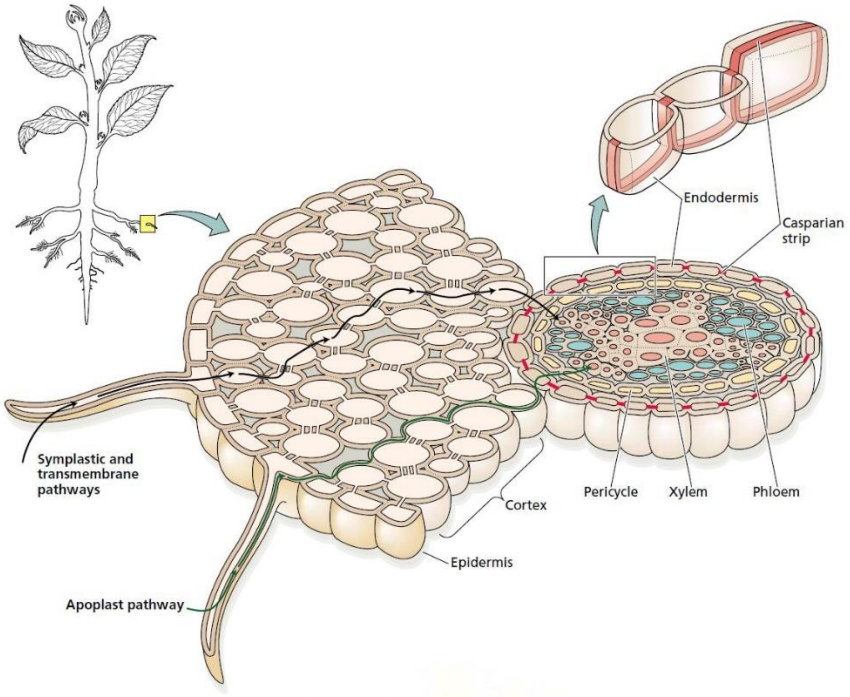


Figure 2.4 Cross section of a plant root and pathways for water and solutes uptake (Taiz and Zeiger 2006).

Transfer to the hydrosystem

Hydrosystem is the other main output for trace elements in agricultural environments. One of the processes that transfer trace elements to the hydrosystem is erosion, it is not insignificant but mainly regards surface horizons. Indeed, eroded soil particles carry trace elements with them whether they are sorbed or occluded and transport them to surface waters (Quinton and Catt 2007, Figure 2.5). The other process is the transfer of trace elements that occurs through their leaching. There are some similarities with the factors that control bioavailability as an increased concentration of the trace element in the solution can also be associated with an increased transferability to water tables. Nonetheless, an important difference resides in the role of colloids that can constitute a vehicle for sorbed trace elements transport (Pédrot et al. 2008). Trace

elements on colloids can represent a significant part of total trace elements, up to more than 85% of the total in solution in presence of leachate contamination (Hooda 2010; Antoniadis et al. 2017). However, many contextual factors play an important role on trace elements transfer to the hydrosystem. One of the parameters is the overall water flux that is a consequence of the climate, but also hydrodynamic properties influence the “motor force” of trace element solubilisation and translocation. For example, in arid climates without irrigation trace element transfer is low. Conversely, a higher level of precipitation will increase the total lixiviated mass of trace elements, while preferential flows decrease the overall volume of the soil that is explored by flowing water, though increasing the rapidity of the transfer of solutes (such as trace elements) from the topsoil to deeper horizons (Kim et al. 2005).

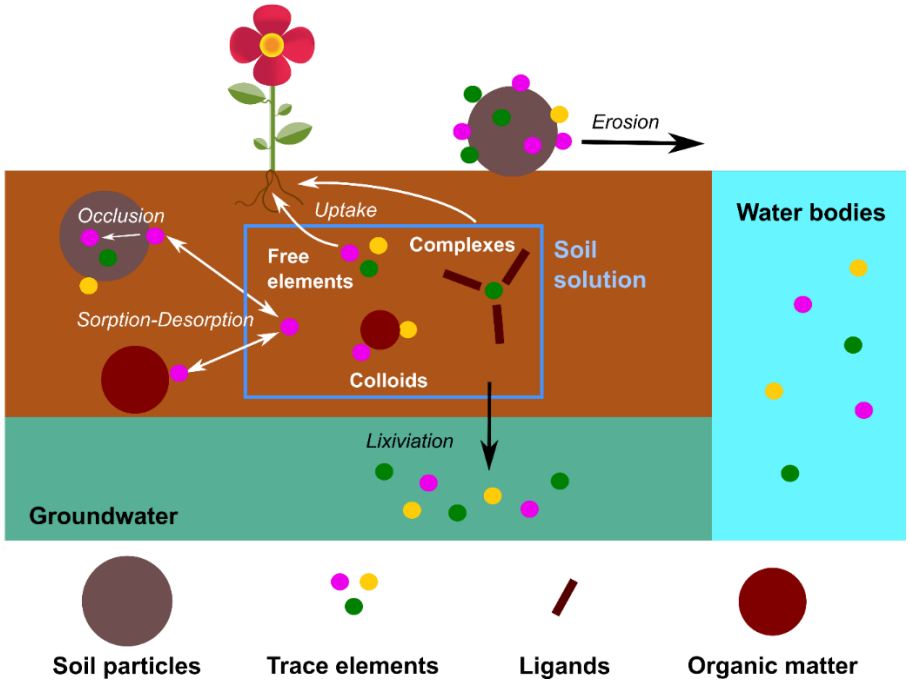


Figure 2.5 Schematic representation of trace element transfer from soil to plants and hydrosystems.

Finally, the physico-chemical properties of the subsoil are of major importance for the transfer of trace elements to the hydrosystem (Helling 1971). The distance between the topsoil and the water tables (also known as vadose zone) varies greatly, as it can be neglectable in very humid ecosystems, such as marshes, but this unsaturated zone can also be tens of meters thick. Then, the capacity of the subsoil to sorb trace elements and the speed of water transfers through the medium can have an important impact on trace element transferability (Tanji and Valoppi 1989; Köhne et al. 2009).

2.1.5. Influence of environmental variables on trace element transfer

The environmental variables that influence the uptake of trace elements are similar as those that influence their speciation, and pH is the first that comes to mind as in soils it derives widely from the solid fraction and the buffer systems that are present. Lower pH are associated with increased trace element mobility, while higher pH are associated with increased trace element retentions, better for nutritional function (Öborn et al. 1995; Kim et al. 2015). This relies on the fact that most of these elements are taken up as cations, opposite behaviours may be observed for anions as surfaces become less negative (or more positive). Consequently, pH is usually controlled by farmers (usually by liming) to avoid too acidic states and ensure correct nutrition (optimal pH depending on the crop considered). However, on the root surrounding, the plant can also have a major influence on the acidity at the microscale by proton extrusion (Adriano 2001). Though this acidification can be associated with mineral element uptake, it is also linked to nitrogen nutrition (Taylor and Bloom 1998). The other major chemical parameter defining the chemical environment, Eh, mainly becomes relevant in soils that are flooded as it creates more reducing conditions. Indeed, in flooded soils (like paddy soils), it has been shown that decreased Eh (more reducing conditions) implied a higher mobility of trace elements

(Kashem and Singh 2001). However, soils may undergo variations in water table depth, implying intermittent flooding, those soils thus endure strong redox condition changes influencing element behaviour, even conditioning their interactions with the organic fraction (Monhonval et al. 2023). Moreover, reducing conditions may lead to the dissolution of mineral phases, such as oxides, releasing trace elements. Nonetheless, even in non-flooded soils, there are humidity variations at the microscale with pores that are less prone to dry. Besides, plants influence the redox potential of their environment through root exudates and associations with microbial communities (Hartmann et al. 2009). A higher temperature also tends to increase trace element uptake and influence their distribution in the plants (Adriano 2001). Moreover, plant related parameters also play a role in their ability to manage trace element uptake. As an example, increased evapotranspiration promoted by a low relative humidity, strong wind or important solar radiation increase trace element uptake by plants through increasing solution uptake (Skidmore et al. 1969; Robinson et al. 2009; Renkema et al. 2012).

In an agricultural context, organic compounds are present because of (1) root and shoot residues of cultivated crop and (2) addition of exogenous organic matter, including manure or plant compost. These inputs enhance the soil stability by structuring it, as decaying roots and incorporated stems provide structural strength and their degradation produces secondary products that favour soil aggregation that also increases water retention capacity (Emran et al. 2012), influencing both organic matter and trace element behaviour. Moreover, organic matter can also directly bring readily available nutrients and store carbon (Minasny et al. 2017). Organic compounds commonly possess charges, mainly negative but also positive, they therefore increase the cation (and anion) exchange capacity and can thus increase soil fertility.

The addition of organic carbon has many ways of influencing trace elements dynamics. First, there is a direct input of trace elements initially

present in exogenous organic matter. For example, compost is composed of plant materials commonly enriched in Cd, manure in Pb or Ni, and sewage sludges in Cu, Cr, and Pb (Sager 2007).

The mobility of trace elements is especially linked to their ability to be either adsorbed on soil particles or solubilised to the soil solution. Organic compounds play a crucial role in both sorption and desorption processes, with distinct influences depending on their nature—whether it is solid, colloidal, or soluble organic matter.

In fact, there are even various competing behaviours in the same category. For soluble organic matters, low molecular weight organic compounds can increase the solubility of trace elements due to soluble complex formation, while heavier organic compounds are more prone to form insoluble complexes that would decrease trace elements availability (Schmitt et al. 2002; Carrillo-González et al. 2006). This property is studied in the context of phytoremediation, where the application of low molecular weight organic compounds is increasing trace elements uptake by plants. Commonly studied compounds are citric acid, malic acid, oxalic acid, or ethylenediaminetetraacetic acid (EDTA; Vassil et al. 1998; Schmidt 2003; Ebrahimian and Bybordi 2014). Note that when complexes become bigger, for example due to heavier ligands, it can decrease the bioavailability due to a lower diffusion through root tissues (Zhao et al. 2016). In plants, complexes are formed to better transport trace elements (Takahashi et al. 2003; Mishra et al. 2020). Moreover, diverse proteins, including transporters, are specifically involved along the soil-plant continuum to control the uptake and translocation of the complexes (Haydon and Cobbett 2007). These soluble organic compounds are produced either indirectly through the decomposition process of the organic matter, or directly through root and microbial exudate production (Awad and Römheld 2000; Reichman and Parker 2005). However, when the organic compounds are colloidal, they cannot be readily taken up by plants, as particles do not cross roots barriers

(Hooda 2010; Antoniadis et al. 2017). Finally, solid organic matter can have antagonistic effects. On one hand, it increases the global CEC and thus the pool of exchangeable trace elements. On the other hand, this increased presence of charges (mostly negative although depending on the pH) favours the trace element sorption in the solution; they are thus less easily taken up. In this case, organic carbon plays the role of a chemical filter. As mentioned, soluble organic compounds also partly derive from solid organic matter, thus potentially producing substances that increase trace element solubility (Borggaard et al. 2019).

2.2. Pesticides in the agroecosystems

2.2.1. Pesticides: then and now

Pest management is not at all a modern concern and, along with magical and religious methods, technical means were already used millennia ago not only for stored food but also for crops. Ancient Roman and Greek texts report that treatment existed against fungi, weeds, insects, or other animals, both preventive and curative. Organic, minerals, used as coatings, sprayed or dispersed by fumigation, there is a wide variety of practices. Among the materials used are hemlock, sulphur and arsenic that would still speak to the contemporary reader, few data however exist on their efficacy and impact on workers and environment health (Smith and Secoy 1975; Levinson and Levinson 1998). During the Renaissance, the use of pesticides was mainly centred on the use of mercury and arsenic (Abubakar et al. 2020).

The major revolution happened at the beginning of the 20th century, with a breakthrough of industrial chemistry in the world of agriculture. The Haber-Bosch process had increased yields by providing new nitrogen fertilizers, and in the forties it is the time for synthetic pesticides to

get into the game after the discovery of dichlorodiphenyl-trichloroethane (DDT) in 1939, thus decreasing the use of former pesticides like arsenates that are however slow to disappear (Nriagu et al. 2007; Matthews 2018; Abubakar et al. 2020). Moreover, progresses in genetics led to the development of high-yielding crop varieties (Evenson and Gollin 2003). Together, these new fertilisers, plant varieties and pesticides combined with a technologizing of irrigation allowed the so-called *green revolution* to take place and cause a drastic increase in the global agricultural production. Considered at the time as a relevant answer to poverty, food shortages and elevated prices in developing countries, this *green revolution* was considerably funded by both private and public foundations and organisations (Cleaver 1972; John and Babu 2021).

Strong criticism has emerged because this system, while strengthening food safety, failed to reduce poverty because of multiple factors. For instance, pesticides caused a strong dependency of farmers to technological/industrial products and was a Trojan horse for imperialism (Cleaver 1972; Yapa 1993). But, beyond the economic point of view, it is the environmental and health cost of the generalization of pesticide use that raised the highest concern. A tipping point was the publication in 1962 by Rachel Carson of the book *The Silent Spring*. This book pointed out the collateral influence of DDT on birds and warned against the risk of the advent of a spring that would be deprived of their song (Carson 1962). It raised public awareness, led to a strong debate on pesticide use, and to modifications in pesticide regulations (Epstein 2014). Nevertheless, since this date, synthetic pesticides have spread widely, they are used in other fields than agriculture, like the maintenance of public space and gardening. Pesticide use is also iconic of the Vietnam war where defoliants have been used for strategic purposes (Young et al. 2004).

Current state of the challenges associated with pesticides

Nowadays, pesticides are still a hot subject of debate, although some that were considered particularly harmful are now banned, their effect still impacts populations through appearance of long-term diseases but also due to the persistence of residues that might have a low degradability, this is for example the case for chlordecone (Dereumeaux et al. 2020). In addition to this inherited patrimony, heated debates rage in the public, political, economic and scientific spheres regarding many active substances and additives. More than any others, glyphosate has become an emblem due to the associated health risks and especially its very widespread use and presence in agricultural soils (Van Bruggen et al. 2018; Silva et al. 2019). In addition, triazoles face criticism for health risks as well as development of resistant fungal species. Indeed, triazoles used in human medicine thus have seen their efficiency decrease (Taxvig et al. 2007; Price et al. 2015; Garcia-Rubio et al. 2017).

Regarding ecological issues, neonicotinoids among others are a subject of great concern for pollinators, not only through their active substances but also their metabolites that may be quite persistent (Main et al. 2020; Singla et al. 2021). Beyond the substances, it is also sometimes the additives that are pilloried and banned as they are sometimes considered more problematic than some active substances. Polyethoxylated alkylamines (POEA), used along with glyphosate are probably the most famous of those controversial additives (Kalyabina et al. 2021). Although the use of mineral pesticides has significantly declined with the rise of synthetic organic alternatives, Cu sulphate remains an exception. When combined with lime to create the Bordeaux mixture, it is still widely used as a fungicide. A reason for the popularity of Bordeaux mixture is its homologation in organic farming, it is thus extensively used, especially in vineyards, leading to widespread Cu contaminations (El Azzi et al. 2013; Ballabio et al. 2018). Nowadays, there is a wide focus on the question of ecosystem services, it has been shown that the presence

of pesticides in the environment decrease the ability of the ecosystem to provide these services (Leenhardt et al. 2023). There is an increasing number of voices claiming that pesticide use must come to an end. However, the modern agriculture (even the organic farming) is highly dependent on pesticides that still bring great benefits (synthetic but also plant-based, Rodríguez-Cruz and Sánchez-Martín 2022). Besides, not all substances should be put in the same basket regarding their impacts. But issues regarding the environmental impact of agricultural practices go far beyond pesticide use. In this context, pesticides are also tools that help address some of those issues. For example, glyphosate can be used in a no-till context in order to prevent a previous cover to grow through the following one. Thus, the understanding of the impact of pesticides on humans and the environment will remain an important challenge for the coming decades.

2.2.2. Pesticide terminology and classification

Pesticides are chemicals that are used to control biological agents, either in an agricultural context or in an urban one. When pesticides are used for plant protection, they are called phytosanitary products, but pesticides are also widely used for material protection (such as roofing), health care, or weeding in a non-agricultural context (e.g., the railway company is the biggest pesticide user in Belgium). They are then, in Europe, classically called biocides in legal texts until 2022 (Wittmer et al. 2010; EC 2022). It highlights the moving character of definitions on the subject. Another classification is focused on the target: herbicides are used against plants, insecticides are used against insects, fungicides are used against fungi, molluscicides are used against molluscs and bactericides are used against bacteria. In these categories, there can be further subdivisions based on the action mode like the IRAC classification of insecticides that gathers the inhibitors of the acetylcholinesterase or the nicotinic the acetylcholine receptor competitive modulators. A last

classification is based on the chemistry of the molecule that is responsible for the pesticide effect, called active substance (EC 2022). For example, the carbamate family includes molecules that are esters based on carbamic acids, the triazoles (including tebuconazole) possess at least a cycle of five atoms of which three are nitrogen and two double bonds, and glyphosate has a structure based on the amino-acid glycine. Both composition and structure of the molecule are responsible for the pesticide effect, as well as for potential side effects, such as the environmental behaviour.

2.2.3. Chemistry of pesticides

Like trace elements, pesticides can be pH sensitive. This happens when the active substance possesses at least one function that can be either protonated or deprotonated. The total charge and the number and location of positive and negative charges change the ability of the pesticide to interact with other charged components of the environment, like surfaces for example, this is known to be an important feature of glyphosate behaviour (Sheals et al. 2002; Figure 2.6). Some pesticides are very soluble in water and can therefore be easily leached, while others have a high solubility in organic solvents/lipids (which usually result in a high ability to be accumulated in adipose tissues; Paasivirta 1998). Another parameter is the volatility (Henry constant) that, when high, exposes the population to higher doses through inhalation and increases aerial dispersion to surrounding environment. Although expressed by different metrics in function of the chosen model, the affinity for sorption on soil and organic matter is key in the retention or transfer of the pesticides by leaching, while DT_{50}, the half-life time, indicates the persistance of the molecule relatively to its sensibility to oxidation by soil microorganisms. There is a strong variability between soils, as given DT_{50} values relate to standard conditions that allow to compare molecules; but environmental factors, such as water content, temperature,

pH, and microbial communities that are inherent to every soil and influenced by agricultural practices, play a role on the kinetics of molecule degradations but also on their transfer (Alletto et al. 2010). Besides, as mentioned for trace elements, the biological component has an influence on the other soil physico-chemical properties at the local scale.

When a pesticide is degraded, it can be fully oxidised to CO_2. Some intermediate molecules can exist along the degradation pathways (Figure 2.7); these molecules are called metabolites or degradation products. Note that some molecules like glyphosate are known for their high degradability (DT_{50} = 17.3 days; Lewis et al. 2016), but their related degradation products can be persistent. The main degradation product of glyphosate, aminomethylphosphonic acid (AMPA), has indeed a high persistence regarding the biodegradability in the soil with a DT_{50} of 114 days (Lewis et al. 2016). Like glyphosate, AMPA is an acid molecule with three pKa. At circumneutral pH it possesses two negative and one positive charge. Under a pH of 5.4 one negative charge is lost (Chen et al. 2009). Some degradation products can be common to many molecules of the same family such as 1,2,4-triazole (1H-1,2,4-triazole) for many azole fungicides, tebuconazole included (Del Puerto et al. 2022). Moreover, the toxicity of degradation products may vary in comparison to the parent molecule. While sometimes degradation decreases the toxicity, the opposite can also happen.

Additional substances are usually used to increase the stability of the commercial products (for example to improve the solubility), their ease of use, or the pesticide effect. For example they can increase the affinity of the active substance for the plant cuticle, thus avoiding loss by direct washing out, these substances usually include surfactants (Kalyabina et al. 2021). These additional molecules are either called co-formulants or

additives and are generally unknown to the users, as the pesticide composition besides the active substance is considered a confidential business information (Mesnage et al. 2019).

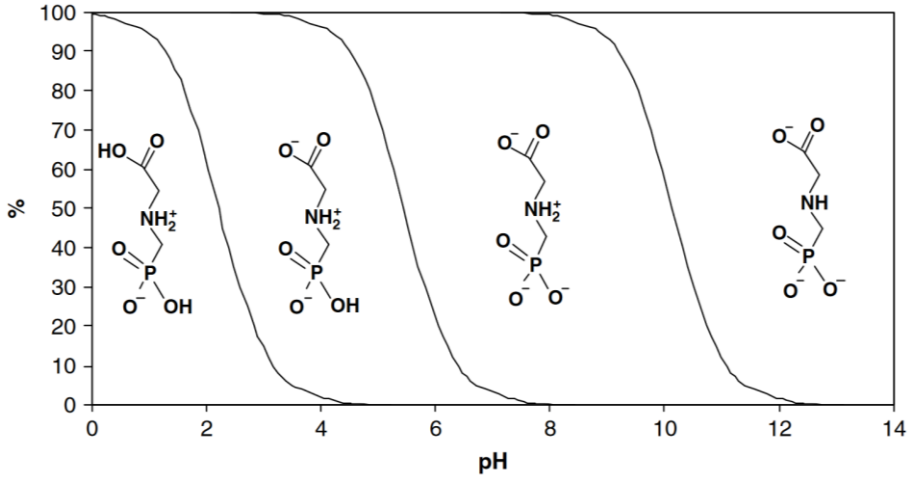


Figure 2.6 Distribution of glyphosate species as a function of pH (Bjerrum diagram). Acid dissociation constants: pKa1 = 2.22, pKa2 = 5.44, and pKa3 = 10.13 (Borggaard and Gimsing 2008).

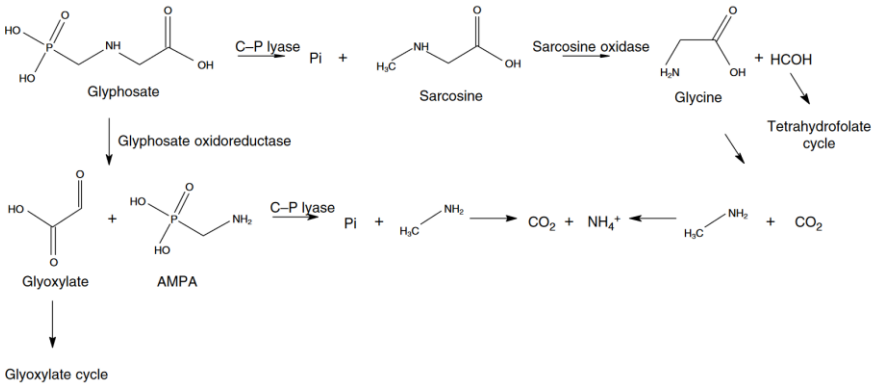


Figure 2.7 Main microbial degradation pathways of glyphosate (Borggaard and Gimsing 2008).

2.2.4. Occurrence and transfer of pesticides in agroecosystems

In 2020, 2.7 Mt of active substances were used worldwide (7.2 Mt in terms of formulated products) which represented 1.8 kg ha^{-1} of cropland (FAO 2022). In Belgium (2020) 5518 t of pesticides were purchased mainly for professional use (97.4%), (SPW 2023). As they do not degrade instantly or form other products, they spread in various compartments of the environment and thus many organisms become exposed, raising concern for both environmental and human health. Starting with the soil, Silva et al. (2019) showed that in agricultural soils, the contamination was very widespread, as 80% of the tested soils representing 10 European countries were contaminated with at least one residue (active substance or degradation product), and 58% had more than one residue (Figure 2.8). Note however that the studied soils were selected among the crops associated with the highest pesticide use and that of the 76 residues that have been searched for, 43 have been found. In many soils, two to five different residues were simultaneously present, though 60% of the residues were considered non-persistent. Fourteen substances were long lasting substances that were already banned, stressing the long-term impact of pesticide application despite a sometimes-contrary feeling. Especially striking is the fact that the glyphosate-AMPA couple was the most present in studied samples just before fungicides, such as tebuconazole and boscalid (Figure 2.8). In France, Froger et al. (2023) confirmed by analysing 47 soil samples that pesticides were massively present in soils as only one contained none. Though agricultural soils were the most contaminated, sampled grasslands and forest were not pesticide free. Again, glyphosate and AMPA (and more generally herbicides) were especially present, but the diversity of pesticides present in single samples was especially striking.

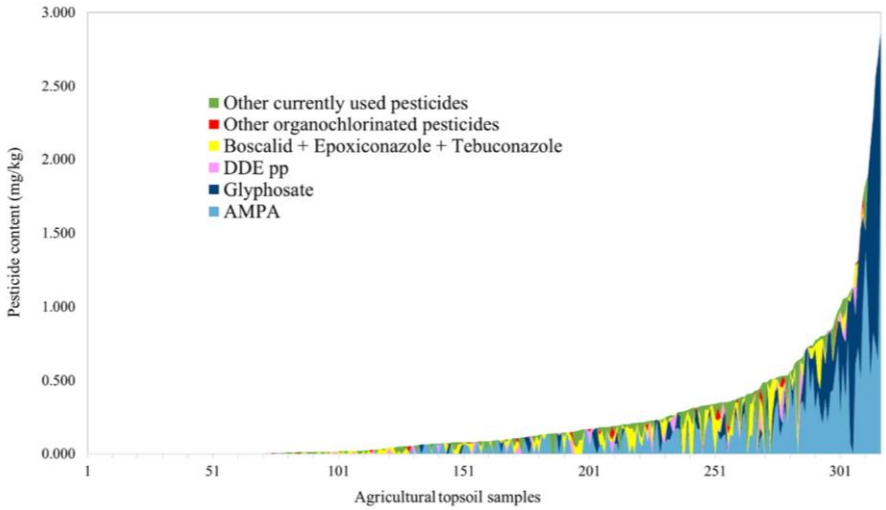


Figure 2.8 Pesticide distribution across 317 EU agricultural topsoil samples. Topsoil samples (numbered from 1 to 317) were organized by increasing total pesticide content (Silva et al. 2019).

Though pesticides are sprayed on a delimited and chosen area, they are scattered in the surroundings by a process that is called pesticide drift (Van Den Berg et al. 1999). This process occurs in two stages: first during pulverization, when wind, particularly turbulence, carries droplets to altitudes higher than the pulveriser, secondly, sprayed molecules are scattered by the wind after their volatilisation (Zivan et al. 2017, Figure 2.9). This process can have a rather large footprint as, for glyphosate, several square kilometres can be impacted (Lucadamo 2018).

From soils, pesticides may be transferred by leaching to the hydrosystem. In Wallonia, pesticides were found at measurable concentration in 70% of the underground water control sites (SPW 2022a) and 28.2% of sites were considered to have a moderate to low quality (18 pesticides were considered). The most problematic sites were mainly located in the north, the region where agriculture is the most intensive (Hesbaye, Brabant Loamy Region and Hainaut Loamy Region). Pesticides also

reach surface water bodies, as worldwide pesticides are commonly found in rivers or lakes, they can by this mean be transported over long distances to reach unpolluted zones (de Souza et al. 2020). Though downward movements of pesticides in the soil are the majority, upward water movements can also carry them up in the soil profile in periods of strong evaporation (Rodríguez-Cruz and Sánchez-Martín 2022). A more direct way for pesticides to reach those surface waters is through erosion of soil particles that have sorbed pesticides (Clement et al. 2023). Loess derived (silt loam) soils with low organic carbon content, such as those in the Brabant Loamy Region, are particularly vulnerable to runoff and soil erosion. Herbicides, sprayed during overland-flow sensitive periods of low vegetation cover, are particularly prone to these transfers.

Instead of being leached, pesticides may also be taken up by plants, through roots but also aerial parts. For pesticides taken up through roots, they follow either a passive pathway and travel along the potential gradient or an active one and are able to use carriers to pass cell membranes (Su and Zhu 2007). As for trace elements, pesticides may travel along the apoplasmic or the symplasmic pathway (Rodríguez-Cruz and Sánchez-Martín 2022). Once taken up, the plant relocates them with modalities that depend on both pesticide chemical properties and plant physiological properties (Huang et al. 2010; Ju et al. 2020). This is the main source of consumer exposure, as it is estimated that Swiss individuals ingest 0.41 g of pesticide for every 1 kg applied to cultures (Juraske et al. 2009; Fantke et al. 2011). Finally, pesticides are taken up by the soil fauna such as earthworms (Krauss et al. 2000). When released in the soil or the underground or surface waters, or when evaporated in the atmosphere, pesticides spread out and are found in the most diverse and common locations, they thus occur in products such as eggs, especially if they are persistent and soluble in fats, or even in dusts (Windal et al. 2009; Harnly et al. 2009).

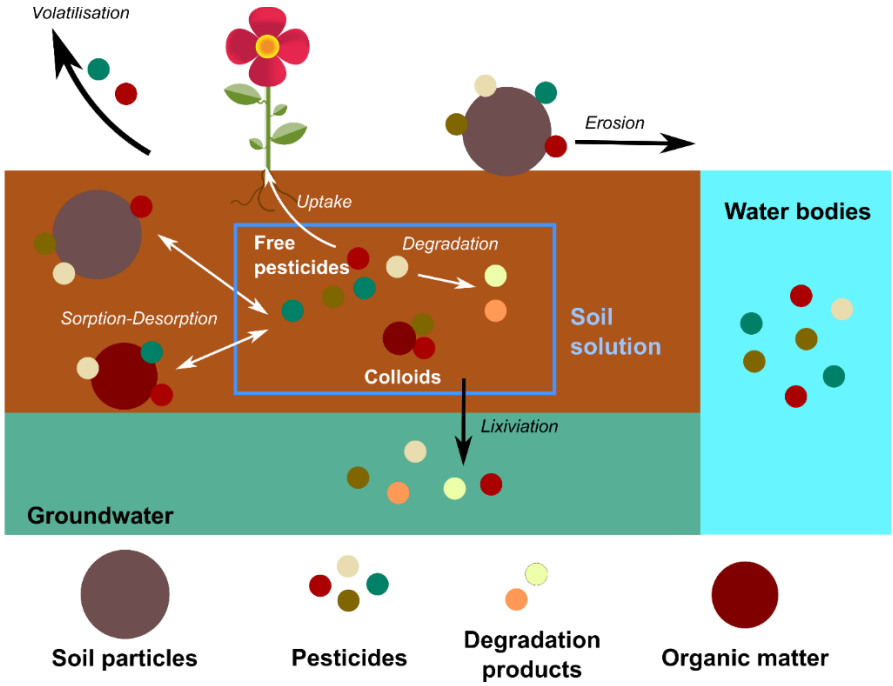


Figure 2.9 Schematic representation of the fate of pesticides in soils.

2.2.5. Influence of pesticides on the transfer of trace elements

Pesticides in the environment and their degradation products are not inert, they interact with their surroundings and other molecules. The presence of specific chemical groups able to form complexes (e.g. amine, carboxylic acid) is a key for these interactions. The complexing ability of some pesticides is long known, such as glyphosate that is co-ordinated to metal ions individually or with multiple ligands (bi- or tri-dentate, Glass 1984; Subramaniam and Hoggard 1988). It was first patented in a series of chelators in industrial uses (Dock Fon Toy et al. 1964; Mesnage and Antoniou 2018), while it was only patented as a pesticide in 1971 (Franz 1974). When sprayed solutions have a high ion concentration (especially Ca and Mg), complex formation commonly diminishes pesticide activity. Glyphosate was also observed to decrease

plant nutrient uptake and, in general, chelation ability of pesticides is supposed to impoverish soils (Cakmak et al. 2009; Tesfamariam et al. 2009; Zobiole et al. 2010; Kaur et al. 2017). Note that complex formation is also sometimes reported to increase pesticide activity, indicating potential synergetic effect between substances (Li et al. 2017a). However, at the turn of the 21st century, the influence of complex formation on the environment and the soil plant system began to be considered. As glyphosate is the most known chelating pesticide, and an important environmental challenge, it gathered most research.

In the environment, mobility of both glyphosate and trace elements are important concerns. Besides, it is known that elements that possess two positive charges can form cationic bond between negative charges on (1) soil particles, minerals such as goethite, montmorillonite, organic compounds, and (2) glyphosate. This has been shown for cations, such as Cd, Cu, Pb, or Fe (Piccolo et al. 1992; Morillo et al. 2000; Maqueda et al. 2002; Zhou et al. 2004; Si et al. 2013). Some of these studies however also observed that when some concentrations of metals are reached, the increase of the pesticide sorption stops, and a decrease is observed. Soil pH plays a role on glyphosate metal interaction and the implication of soil CEC is unclear.

Regarding the influence of glyphosate on the mobility of trace elements, various studies focussed on individual elements with a predominance of Cu that is the most well-known when it comes to interactions with glyphosate and is especially present in agricultural soil due to its import along with pesticides. Thus, studies with Cu-spiked solutions and glyphosate observed and a strong diminution of Cu sorption on montmorillonite goethite or soil (Morillo et al. 1994, 1997, 2002; Maqueda et al. 2002). When increasing glyphosate concentrations were applied, a point could be reached when a drastic decrease of Cu sorption was reached. Though sorption processes are mobilised to explain the influ-

ence of glyphosate on trace elements mobility, many competition processes for specific binding sites also supposedly occur. There is, in these studies, a low mobilisation of native Cu due to the spiking, thus the level and kind of contamination do not play a role, and desorption is not the matter.

Having a basic knowledge of Cu-pesticide interactions, few other elements have been studied following similar protocols highlighting the diminution of the sorption of Cd in presence of glyphosate, an element that causes issues due to its high soil to plant transferability and its contamination in soils (Zhou et al. 2004), but also of Zn that is an essential element and is also broadly imported with metallic contaminations (Wang et al. 2008). Though pH always played an important role on trace element glyphosate dynamics (wide ranges are studied), it is especially striking for Pb as totally inverted results are observed depending on the pH range (Si et al. 2013). As glyphosate is never pulverized alone, but only as a formulated product, there was a need to study the effect of the formulation on the glyphosate-induced trace element desorption (or lower sorption). Thus, Divisekara et al. (2018) showed that Roundup® (a formulated glyphosate-based product) also decreases the sorption of both Pb and Cd on soil and increases their desorption.

In a more environmental perspective, it has been observed that the spraying of formulated glyphosate (Roundup®) on soil columns increased the leaching of Al, As, Cu, Ni and Zn (Barrett and McBride 2006). The presence of an effect on As suggests that anions could also be influenced by glyphosate. This was especially observed in the contaminated soils, highlighting the importance of the contamination status of the soil. In turn, Cu modifies the leachability of glyphosate in soil columns in function of the nature of the soil (calcareous vs granitic); (Dousset et al. 2007). The chelation effect of glyphosate may also influence animals and other environments such as aquatic ones, as

Roundup® has been shown to influence (mainly decrease) trace element uptake of *Ceriodaphnia dubia* (Tsui et al. 2005). The variety of harmful effects associated with glyphosate's chelating ability raises the need to consider this property in a more comprehensive reflection when conducting ecological risk assessments (Mertens et al. 2018). Nowadays, influence of glyphosate on many other potentially toxic trace elements and their transfer to plants is widely unknown.

Glyphosate is usually sprayed before a culture to have a clean surface. But, sometimes, it is sprayed on the culture when transgenic glyphosate resistant species are used. This practice is not allowed in Europe. However, selective herbicides and all other kind of pesticides (fungicides, insecticides, molluscicides, etc.) may be sprayed on the culture and then intervene during plant growth, but their chelating ability is rarely investigated.

Among these pesticides that are sprayed on the cultures, there is the fungicide tebuconazole that is sprayed on crops that are in place and is known to form complexes with Cd, Co, Cu, Ni, and Zn, although these interactions did not happen through charged functions (Evans et al. 2007; Dytrtová et al. 2011; Norková et al. 2012; Li et al. 2015, 2017a; Figure 2.10). In all these complexes, the metal was bound to at least two molecules of tebuconazole. It has been shown that Cu, and thus potentially other trace elements, can increase tebuconazole sorption on soils and organic substances (Čadková et al. 2013). To this day, no study regarding the ability of tebuconazole to influence the solubility of soil trace elements has been carried out despite its widespread presence as residues in European soils (Silva et al. 2019), and there is also a similar lack of research for a wide range of other molecules.

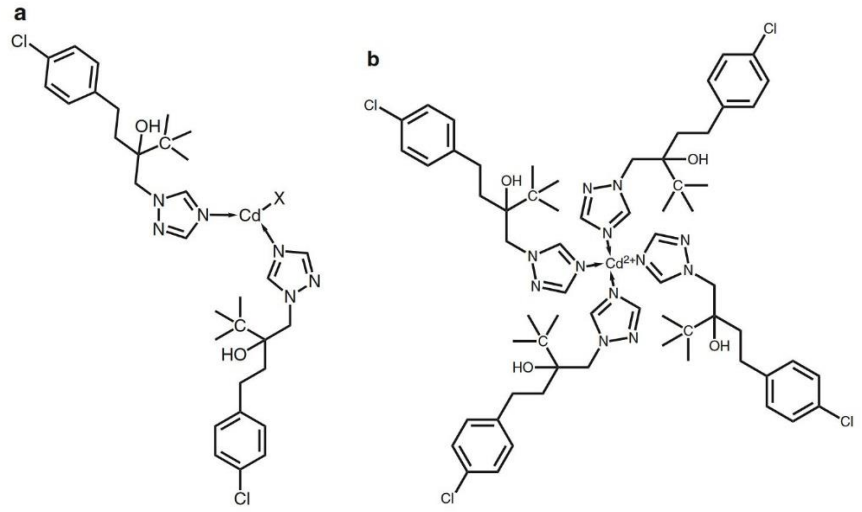


Figure 2.10 Plausible structures of (a) $[CdI(Teb)_2]^+$ and (b) $[Cd(Teb)_4]^{2+}$ complexes (Norková et al. 2012).

3. Objectives and approach of the thesis

The mobility of trace elements in agricultural soils is a major concern for human and environmental health issues. Since pesticides may govern their transfer by complex formation, and as one could suspect it may lead to an increased trace element concentration in waters and plants, **the aim of this thesis is to evaluate their potential to influence the transfer of a series of 15 trace elements (As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, and Zn) from soil to solution and to plant**. These elements were selected for their health concern (nutrients and/or toxic elements) or tracer ability (Ti as conservative element). We also considered the influence of both the contamination level and nature of the soil. Therefore, one herbicide (glyphosate) and one fungicide (tebuconazole) have been selected because of their known ability to form complexes with trace elements and to represent two different types of pesticides with distinct targets and application practices (glyphosate sprayed before cultures while tebuconazole sprayed on crops). The possibility to use organic matter in both solid and dissolved form, to mitigate the pesticide effect on trace element mobility was also considered.

Several sub-questions were tackled in this work:

Are glyphosate and tebuconazole able to influence trace element extractability from the soil? (Chapters I and III)

Are there competitive or synergetic effect when pesticides are present together in solution? (Chapter III)

Does the form of the pesticide (active substance alone, formulated, degradation product) play a role? (Chapters I and II)

Does the level and kind of contamination of the soil play a role on the trace element pesticide interaction? (Chapters I, II, and III)

Is dissolved organic carbon able to counteract the effect of glyphosate on the mobility of trace elements? Or is there a risk of increased trace elements extractability? (Chapter IV)

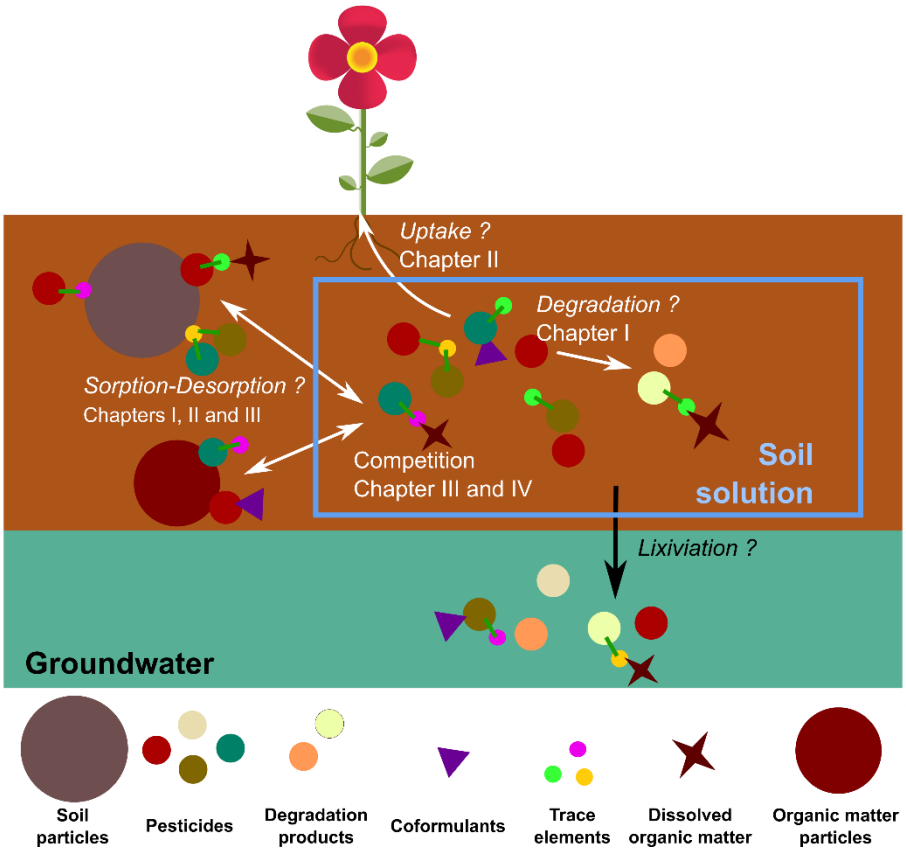


Figure 3.1 Schematic representation of the objectives of the thesis and the chapters where they are discussed

To answer our research questions, we selected four agricultural soils: one uncontaminated (Vieusart), two representing a gradient of anthropogenic trace element contamination (Sainte-Walburge and Bressoux), and one naturally-enriched in trace elements (Aubange). Two different experiments were implemented, representing two levels of complexity. The first, called extractability experiment, was performed in batch conditions in laboratory to evaluate the influence of pesticides alone or combined on the extractability of trace elements. The second, a greenhouse experiment included transfer to soil solution and to the plant (*Triticum spelta*) in presence of glyphosate-based pesticides. To evaluate the role that can be played by organic compounds, dissolved organic matter was included in the extraction experiment and compost addition was incorporated to the greenhouse experiment.

4. Materials et methods

4.1. Study sites

4.1.1. Sites description

For the whole thesis, four study sites have been selected characterised as agricultural soils. They are all located in Wallonia, Belgium, and have been selected to study a trace element contamination gradient, including both natural and anthropogenic contaminations.

Belgium, and especially Wallonia, possesses some climatic, geological, and thus pedological diversity but, in addition to that, there are wide varieties in the anthropic context, as wide differences exist in both population densities and industrial structure (Annexe 1). Indeed, the industrial development of Wallonia throughout the 19th century was strongly linked to fluvial infrastructure and important poles developed along the Sambre and the Meuse, where steel industry and coal extraction flourished leading to widespread trace element contaminations (Figure 4.1). The strong resulting industrial poles are the regions of Charleroi in Western Wallonia and Liege in North-Eastern Wallonia. In the Walloon Brabant, agriculture was dominant despite the presence of small industrial pools for example in Court-Saint-Etienne and Mont-Saint-Guibert. A strong increase of the population happened throughout the 20th century partly due to the proximity of Brussels and the development of Louvain-la-Neuve but it was not associated with a development of heavier industry, no widespread contaminations are thus to be found there. The region of the south and the east of the country remained sparsely populated regarding Belgian standards, they are mostly covered with meadows and forests and did not undergo widespread anthropogenic contaminations.

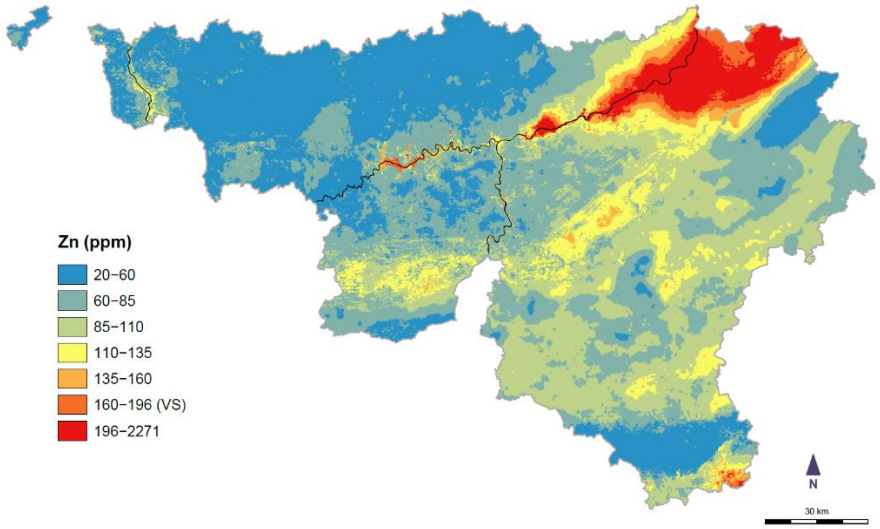


Figure 4.1 Expected Zn content in agricultural soils of Wallonia (Pereira et al. 2018).

Vieusart

The Vieusart soil (50.6799°N; 4.6357°E) has been collected in agricultural fields of the *Centre Alphonse de Marbaix*, an experimental farm belonging to UCLouvain and located in the municipality of Chaumont-Gistoux (Figure 4.3). It is located in the loamy region of Belgium in Walloon Brabant. Throughout history, the loamy region has been known to be very fertile which led originally to an enrichment and to the construction of large farms (i.e., square and castle farms) that became an emblem of the Walloon Brabant. The Vieusart soil has a low content in trace elements (Table 4-1). The soil is silty (Figure 4.2) and developed on a calcariferous loess deposit from the Quaternary. It is classified, in the Belgian classification (SPW 2022b), as an *Aba1* standing for a silty soil (A), with favourable drainage (b), and with a textural B horizon (a) including a deep profile development (1). According to

the World Reference Base for soil resources (WRB), this soil is a Luvisol, under the loess lies a widespread sand formation dating from the Lutecien (known in Belgium as Bruxellien). The soil presented characteristics frequently found in agricultural soils: slightly acidic (pH_{H2O} = 6.5), with a low carbon content (1.25%), and a moderate CEC (11.1 $cmol_c\ kg^{-1}$). Over the period ranging from 1991 to 2020, the mean yearly temperature was of 10.5 °C and the mean annual precipitations were of 790 mm (IRM 2024).

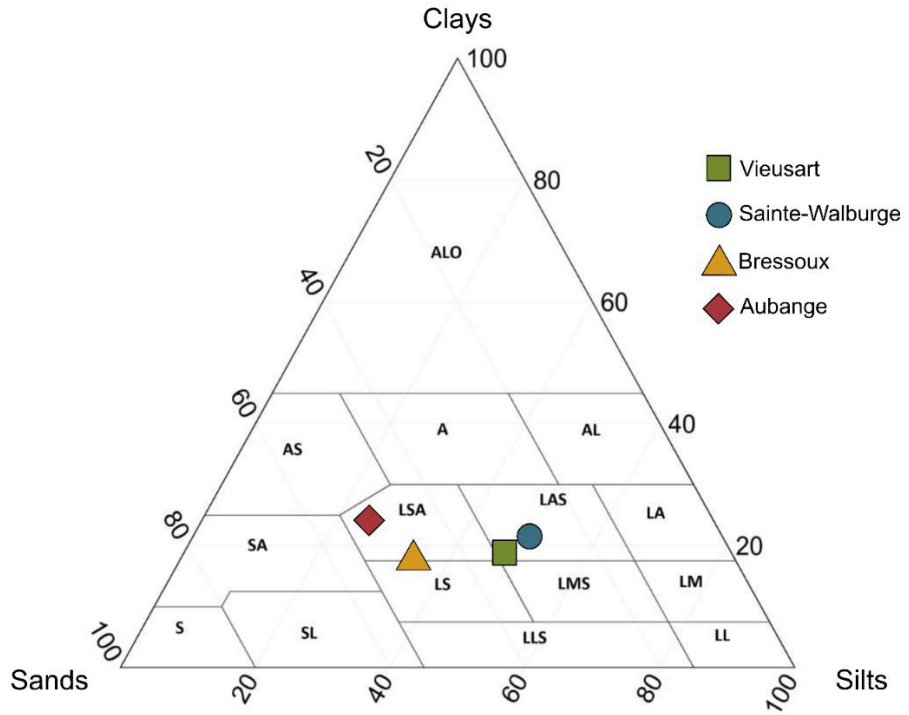


Figure 4.2 Soil texture triangle for the top 20 cm of the sites of Vieusart, Sainte-Walburge, Bressoux, and Aubange, based on the classification established by Jamagne (Thami 2022). LSA stand for clayey sand silt (*limon sablo-argileux*), LAS for sandy clayey silt (*limon argilo-sableux*).

Sainte-Walburge

The Sainte-Walburge study site is in the northern periphery of Liege (50.6644°N; 5.5887°E) and is enclosed in residential districts (Figure 4.3). The field where the soil was collected is directly bordering a slag heap but has also been contaminated by atmospheric deposition. Indeed, the whole region of Liege, being located along the river Meuse, has been an important industrial basin where coal extraction and metal industry were especially developed. This led to a widespread trace element contamination of the region, due to atmospheric depositions (Vandeuren et al. 2023). This soil will be referred to moderately anthropogenically contaminated soil. The soil is silty (Figure 4.2) and developed on flint and chalk from the Maastrichtian (Gulpen formation) and contained a non-neglectable load of coal extraction debris resulting in a higher total carbon content (11.1%, Table 4-1) and soil pH (pH_{H2O} = 7.8). Despite the presence of coal, the CEC was only slightly higher than at Vieusart (15.2 $cmol_c\ kg^{-1}$). On the digital map of Walloon soils (SPW 2022b), it is located between a *Aba1* and a *Abp1* soil (p standing for an absence of profile development), corresponding to a Luvisol or Cambisol according to the WRB classification. Over the period ranging from 1991 to 2020 the mean yearly temperature was of 11.2°C and the mean precipitations were of 854 mm (IRM 2024).

Bressoux

The Bressoux (50.6374°N; 5.6109°E) site located in the south of Liege is the most contaminated by trace elements of the four study sites and will often be referred to as highly anthropogenically contaminated (Figure 4.3). The contamination context is the same as in Sainte-Walburge, with a long-term history of atmospheric depositions (it has been reported that in the past the site was sometimes covered in a thick malodorous mist). In addition, the soil is contaminated with industrial debris. Some of these industrial debris have been brought by farmers to increase the fertility as they contain some nutrients and minerals with high

CEC but also unwanted trace elements. It was however not the main source of the trace elements (Vandeuren et al. 2023). Moreover, the metallic pollutant in those debris showed a very low mobility. The soil is silty with a slight stony load (Figure 4.2) formed of Miocene alluvium but unmapped on the digital map of Walloon soils, although finding itself on the edge of a zone marked as reworked terrain (SPW 2022b), corresponding to a Luvisol according to WRB classification. Due to an important coal load, carbon content was of 15.8% (Table 4-1). The soil pH_{H2O} was of 7.0, and the CEC of 26.9 $cmol_c\ kg^{-1}$. Below the site, a collective vegetable garden has been famous in the media because of the high content of trace elements in the vegetables that were produced (RTBF, 2017). The climatic data are the same as those of Sainte-Walburge.

Aubange

The Aubange site (49.5765°N; 5.7684°E) is located at the extreme south of Belgium. Though having a history of steel industry, it is mainly a rural district (Figure 4.3). The soil is silty with a moderate stony load (Figure 4.2). This soil is developed on the Aubange formation that is a complex of siltstone, sandstone and argillite dating from the Pliensbachian. It is described on the digital map of Walloon soils as a Gbp which stands for a slightly stony silt with a *favorable drainage* and no profile development (SPW 2022b), corresponding to a Cambisol according to the WRB classification. This soil is naturally enriched in Ni, Cr and especially As but another marking characteristic of the soil is the higher iron oxide content caused by a post sedimentary iron accumulation due to a past water table that gives the soil a reddish colour. This partly explains the high As content, As having a high affinity for iron oxides (Suda and Makino 2016). Indeed, the highest As content has been found in so-called ironstones (Smedley and Kinniburgh 2002). The specificity of this soil is that in opposition to both Liege study sites,

the contamination is here natural, trace elements are thus enclosed inside minerals which diminishes their mobility. The soil was characterized by a pH_{H2O} of 7.3, a total carbon content of 2.43%, and a CEC of 20.8 $cmol_c\ kg^{-1}$. (Table 4-1). Over the period ranging from 1991 to 2020 the mean yearly temperature was of 9.4°C and the mean precipitations were of 1088 mm.

Table 4-1 Summary table of the soil properties and total trace element content of the four agricultural study soils in Wallonia, Belgium. The data are the result of the soil characterisations described in the following sections. Exceedances of the regulation for agricultural soils are noted in bold.

	Vieusart	**Sainte-Walburge**	**Bressoux**	**Aubange**
Soil properties				
pH_{H2O}	6.4	7.8	7	7.3
Total carbon content (%)	1.25	11.1	15.8	2.43
CEC ($cmol_c\ kg^{-1}$)	11.1	15.2	26.9	20.8
Trace element content ($mg\ kg^{-1}$)				
As	7.59	14.9	**35.6**	**147**
Ba	261	346	422	197
Cd	0.269	1.87	**6.92**	0.433
Co	7.75	23.4	42.7	63.9
Cr	48.4	**66.0**	**79.9**	**129**
Cu	11.9	**72.7**	**263**	15.2
Mn	283	462	1080	9080
Mo	0.710	2.12	4.40	6.34
Ni	14.8	41.9	73.1	**124**
Pb	20.1	142	**623**	67.7
Sb	0.622	3.35	12.5	1.01
Sn	1.14	12.8	59.9	1.68
Ti	1680	2540	996	259
V	52.2	84.5	115	403
Zn	44.1	**353**	**1350**	**237**

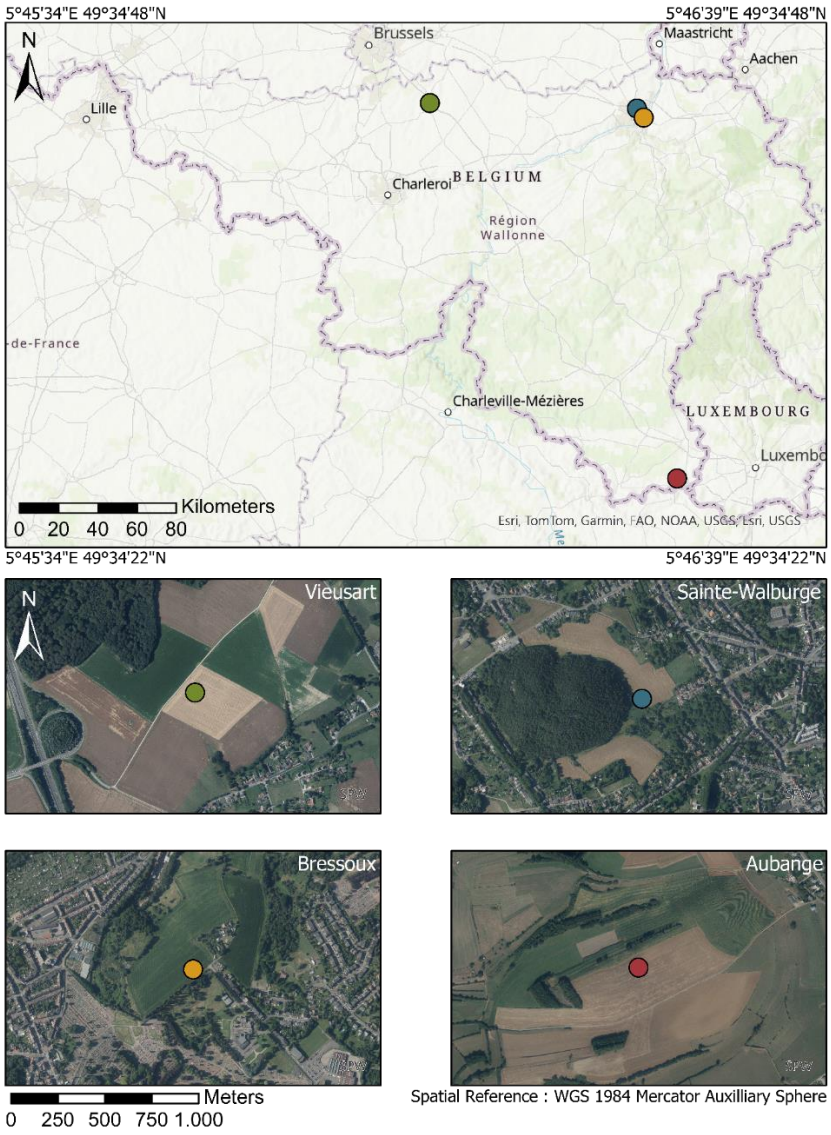


Figure 4.3 Location of the study sites.

4.1.2. Soil sampling and characterisation

Soils have been collected in the 30-upper cm of the four study sites with a shovel and stored in 30-L plastic boxes. Previously the grass had been

removed to collect soil exclusively. Soils used for extraction and greenhouse experiments have been collected at the same location but at different periods.

Soil characterisation required a specific sample preparation, including an air drying at 25 °C for 15 days. For the extraction experiments, a part of the soil has been unpacked (while taking care of not crushing gravels) and sieved through a 2-mm mesh for CEC, soil pH, water and sequential extractions (Annexe 9). For total element analyses (trace elements, C, and N), soil samples have been crushed with a soil grinder (Vibratory Disc Mill RS 200, Retsch, Haan, Germany). For the greenhouse experiment, unpacked soil has been first sieved through a 12 mm mesh for the filling of pots.

4.2. Extractability experiments in laboratory

4.2.1. Experimental setup

To evaluate both the extractability of trace elements and the sorption of glyphosate, 5 g of the four soils, prepared following the above-mentioned protocol have been weighed in a 50 mL polypropylene tube. In this tube, 25 mL of an extracting solution are added (Figure 4.4). This ratio has been established to keep both a certain realism by avoiding an extremely high soil solution/soil ratio and to keep comparability with the literature. Therefore, the same ratio has been used as by Divisekara et al. (2018). Concentrations were chosen in mass and not in molarity as in an agricultural context mass concentration are majorly used in legislative texts, by users and by the scientific community. Studied glyphosate and AMPA concentrations were selected to represent the lowest range tested in the literature for the interactions with trace elements and represent concentrations between sprayed ones and soil solution ones. Tebuconazole concentrations were selected following the ratio of the

maximal dose of tebuconazole that can be sprayed in comparison to the maximal dose of glyphosate that can be sprayed (following Belgian regulations).

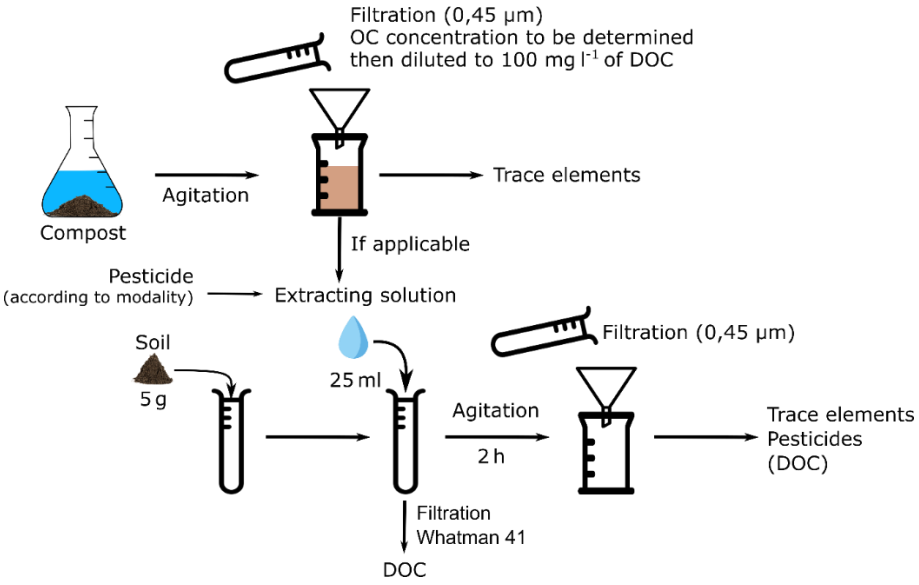


Figure 4.4 General diagram of the extraction experiments including preparation of the DOC stock solution.

These experiments take place in three times. In the beginning, five extracting solutions have been prepared. First, a control solution containing exclusively Milli-Q water. Secondly, a 100 mg L^{-1} (i.e., 0.59 mmol L^{-1}) standard glyphosate solution (*Sigma Aldrich*) that represent a reference glyphosate solution. Thirdly, a 20 mg L^{-1} (i.e., 0.12 mmol L^{-1}) standard glyphosate solution (*Sigma Aldrich*) that will allow to evaluate the effect of a decreased concentration. Fourth, a 100 mg L^{-1} (i.e., 0.59 mmol L^{-1}) formulated glyphosate solution made with *Glyfall plus*® (*Bayer cropscience*) that allows to evaluate the effect of the formulation, as co-formulants may also alter trace elements mobility or glyphosate dynamics. At last, a 100 mg L^{-1} aminomethylphosphonic

acid (AMPA) solution (*Sigma Aldrich*) was prepared. AMPA being the main degradation product of glyphosate, it allows to evaluate the influence of glyphosate degradation on the mobility of trace elements (Table 4-2).

In a second time, experiments were performed with tebuconazole solutions and mixed glyphosate-tebuconazole solutions. One contained analytical standard tebuconazole (*Sigma Aldrich*) at a concentration of 10 mg L^{-1}, three contained commercial formulated tebuconazole (*Tebusip, Sipcam Oxon*) at a concentration of 2, 10, and 100 mg L^{-1} (6.50 10^{-3}, 0.0325 and 0.325 mmol L^{-1} respectively) and one mixed glyphosate-tebuconazole solution with concentrations of 100 and 10 mg L^{-1} respectively.

In a third time, mixed dissolved organic matter (DOM)-glyphosate solutions were produced. They were composed of a 100 mg L^{-1} dissolved organic carbon (DOC) solution with an addition of 100 mg L^{-1} of either unformulated glyphosate ($Gly_{100}DOM_{100}$), formulated glyphosate ($GlyF_{100}DOM_{100}$) or AMPA ($AMPA_{100}DOM_{100}$), corresponding to 0.59, 0.59 and 0.9 mmol L^{-1} respectively. The DOM solution has been produced by extraction of the DOM from compost (composition in Annexe 5), therefore 30 g of compost has been put in 500 mL Erlenmeyers with 200 ml of Milli-Q water. They were placed on an agitation table for 2 h at 125 bpm. They have then been centrifuged at 4000 rpm before filtrating at 0.45 µm before DOC (*Shimadzu Série TOC-L*). All solutions have been prepared using Milli-Q water (Table 4-2).

Once the soil and the solution have been mixed, tubes were put in the dark on agitation tables at 150 bpm for 2 h. After agitation, the tubes have been agitated for 25 min a 4000 rpm, pre-filtered with a *Whatman 41*® filter and then filtrated using a 0.45-µm pore size *Acrodisc*®. One aliquot of the solution has been acidified with 2% HNO_3 for elemental

analysis while another was not (for glyphosate an AMPA measurement). A detachable summary of all modalities with the soils is available in Annexe 2.

Table 4-2 Summary table of the modalities for extraction experiments.

Modality code	**Composition**
Control	Milli-Q water
Single modalities	
Glyphosate20 (or Gly_{20})	Standard glyphosate 20 mg L^{-1} (S*igma Aldrich*)
Glyphosate100 (or Gly_{100})	Standard glyphosate 100 mg L^{-1} (S*igma Aldrich*)
Formulated Glypho-sate100 (or $GlyF_{100}$)	Formulated glyphosate 100 mg L^{-1} *(Glyfall plus®)*
$AMPA_{100}$	Standard aminomethylphosphonic acid (AMPA) 100 mg L^{-1} (S*igma Aldrich*)
Tb_{10}	Standard tebuconazole 10 mg L^{-1} (S*igma Aldrich*)
TbF_2	Formulated tebuconazole 2 mg L^{-1} (*Tebusip®*)
TbF_{10}	Formulated tebuconazole 10 mg L^{-1} (*Tebusip®*)
TbF_{100}	Formulated tebuconazole 100 mg L^{-1} (*Tebusip®*)
DOC_{100}	Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}
Mixed modalities	
$Tb_{10}Gly_{100}$	Standard tebuconazole 10 mg L^{-1} (S*igma Aldrich*) and Standard glyphosate 100 mg L^{-1} (S*igma Aldrich*)
$Gly_{100}DOC_{100}$	Standard glyphosate 100 mg L^{-1} (S*igma Aldrich*) in Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}
$GlyF_{100}DOC_{100}$	Formulated glyphosate 100 mg L^{-1} *(Glyfall plus®) in* Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}
$AMPA_{100}DOC_{100}$	Standard AMPA 100 mg L^{-1} (S*igma Aldrich*) in Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}

4.3. Greenhouse experiments

4.3.1. Experimental setup

The 12-mm sieved soil from the four different study sites was placed in 10-L pots after re-humidification and homogenisation. The use of this fraction implies the possibility of slightly different substrate characteristics in comparison to the characterised fraction (< 2 mm), especially for the soil of Bressoux that has a higher stony load. As there were four modalities and each was performed in triplicates, the total number of pots was of 48. In each pot, 20 g of NPK 9−7−14 fertiliser (i.e., 240 kg_N ha^{-1}) was added to ensure correct plant nutrition. The quantity has been selected to represent an excess in all soils. In 12 pots, soil was mixed with 245 g of green waste compost (i.e., 35 t ha^{-1}) to evaluate if compost had the ability to counteract the potential effect of glyphosate on the mobility of trace elements, had no influence or would increase this effect. This question arises in a context of important promotion of organic matter addition to soils to increase carbon storage as well as soil stability and improve chemical properties. All soils have been mixed after the addition of fertilizers and compost.

The soil pots have been sprayed (on July 28, 2022) with a pesticide solution, three doses were tested to simulate both field doses, and accentuate the visibility of potential effects. The solution was, for all modalities, constituted of 10 g L^{-1} of formulated glyphosate as *Glyfall plus*®. Thus, between modalities concentration was identical while only the total dose varied. The four modalities were: (1) a control without any pulverization; (2) the maximal authorized glyphosate dose in Belgium (3.6 kg ha^{-1}) representing 2.5 mL of sprayed solution (Max); (3) represented three times the pesticide dose of the previous modality (3Max); and (4) the same pesticide dose as the previous one (3Max), but the soil that had been used was mixed with compost (3MAX & OM,

cf. previous section). Fourteen days later (11/08/2022), nine spikelets of spelt (*Triticum spelta*) have been sown per pot, every spikelet containing a random number of seeds (1 to 3). Along the experiment, plants have been watered once a week with 0.8 L of tap water per pot (two times a week at the end of the experiment due to high temperatures and increased evapotranspiration). The day before the soil solution collection, pots were watered with 0.4 L per pot to ensure soil water sampling.

4.3.2. Sample collection

Water solutions were collected every two weeks by using rhizon samplers (10-cm long hydrophilic polyether sulfone membrane with a porosity of 0.15 µm; 19.21.21 F, Rhizosphere, Wageningen, The Netherlands) connected to 50 ml syringes, a stick retaining the syringe's piston ensured the suction. With time, the suction decreased along with the filling of the syringe. Rhizons are stems made out of a 0.15 µm porosity membrane. The hydrophilic polyether sulfone membrane was used to avoid interactions with both organic and mineral compounds. On November 11, 2022, aerial parts were collected, while roots have been collected two weeks later (November 23, 2022) to simulate the harvest at the end of the crop. The plant samples were oven-dried at 50 °C, weighed, and crushed in a plant shredder (Cyclotec 1093 Sample Mill, Foss A/S, Hillerød, Denmark).

4.4. Chemical analyses

4.4.1. Trace elements

Trace elements quantification in solid matrixes (soils, plants) were digested with acids. Therefore, 100 mg of crushed sample was put in Teflon savillexes. In each series a control and reference materials were inserted, a Hawaiian basalt (BHVO-2) for mineral samples and a lichen

(IAEA-336) for plant samples. They allowed to quantify the accuracy of the measures.

For the digestion, 0.5 mL of 65% *suprapure* HNO_3 and 0.5mL of 40% *suprapure* HF were added to the savillex and degassed at ambient temperature, then heated on a hot plate at 90 °C for two days then evaporated completely at 40 °C. Once dry, 1 mL of H_2O_2 was added to the savillex to oxidise organic compounds, they were evaporated completely at 40°C. Afterwards, 1 mL of aqua regia (0.8 ml of 37% HCl and 0.2 ml of 65% HNO_3) was added. Next, 1 mL of 65% HNO_3 was added, then heated at 90 °C for one day. Finally, samples were diluted 12 000 times for mineral matrices and 1200 times for less loaded organic matrices with, first, MilliQ water then with 5% *suprapure* HNO_3 and finally with 2% HNO_3 (for soil matrices alone).

Trace elements in solutions (e.g., soil solutions) were directly analysed by inductively coupled plasma – mass spectrometry (ICP–MS) using an iCAP Q ICP–MS (Thermo Fischer Scientific, Waltham, MA, USA) after acidification to reach 2% HNO_3 (using *suprapure* HNO_3). For solution matrices, we used SLRS-6 (river sample), HM-10 (ICP Forests reference material), or the *certipur* multi-element standard (Sigma Aldrich) reference materials.

In ICP–MS, the analysed solution is injected in the form of an aerosol in an Ar plasma produced by induction, by a solenoid, of a strong time variating magnetic field. At the same time, internal standard (Ru, In, Re) is added to evaluate analytical drifts. In this plasma, elements are ionized and thus become charged. The elements being charged, they can then be accelerated by an electric field, after that they enter a speed selector. The speed selector plunges the elements in both a magnetic and an electric field that cause opposed forces. As the only force that is function of the speed in the Lorentz force (caused by the magnetic field), a single speed of particle is thus giving a perfect compensation

between both forces implying a straight trajectory. The particles that do not have the right speed go out of the system. As trace elements may interfere and produce heavier particles, a helium flux can be used to reduce these interferences (i.e., kinetic energy discrimination or KED). When KED is not used, the mode is considered standard (STD). Finally, elements are separated on their mass to charge ratio by entering a zone with a quadrupole (containing two positive and two negative electrodes) as only the selected mass to charge ratio can pass through the system. The elements that passed through the quadrupole then hit the detector and their number is counted in hits per second. To transform these hits per second into concentration, results are compared with reference solutions where a known concentration of the elements is diluted in a matrix that is similar to the samples. Selected isotopes and methods (KED or STD) are detailed in Annexe 3.

To evaluate the quality of the acid digestions (when applicable) and the analysis, recoveries are measured on reference materials (BHVO-2, IAEA-336, and SLRS-6) using the following formula:

$$Recovery\ (\%) = \frac{C_{Measured}}{C_{Reference}} \times 100$$

Detection limits were measured using concentrations in analytical blanks using the following formula:

$$Detection\ limit = \overline{c_{\mathrm{blank}}} + 3 \times \sigma blank$$

The quantification limit was equal to 3 times the detection limit. Note that the vast majority of samples showed concentrations at least 10-times higher than detection limits.

4.4.2. Pesticides

Glyphosate and AMPA were analysed jointly because, as their structures are very similar, one can use the same procedure. They were quantified by high performance liquid chromatography (HPLC), (1200 series, Agilent Technologies Santa Clara, CA, USA). The general principle of HPLC is based on the separation of substances based on the affinity of the molecules of interests for both the chromatography column and two elutants (A and B). Therefore, an elutant is constantly flowing (rate of 1 ml min^{-1}) through the whole system, including the column (Merck Lichrocart C18 150 × 4.6 mm–5 µm column kept at 35 °C). From t=0 to t=1 min the sample is injected while a mix (25% of B) of the A (pH 7 10 mM phosphate buffer) and the B solvents (45:45:10 acetonitrile:methanol:MilliQ water solution) is flowing, the A solvent is chosen in order to solubilize the compounds of interest, but these compounds still have a higher affinity for the column, they become thus fixed on it. With the time the elutant mix gradually includes a higher amount of a B solvent, the compounds of interest have a higher affinity for this solvent than for the column (100 % of B solvent from t=10 to t=15 min). When the compounds (glyphosate and AMPA) have a higher affinity for the elutants mix, they detach and are transported by the flow to the detectors. The detector used here was a fluorescence detector that emits light in a certain wavelength (260 nm) and measures the emitted light in another wavelength (310 nm) that corresponds to the signature of the compounds that are analysed. These light intensities are converted into signals that are displayed on the chromatogram, showing the intensity of the signal in function of the time. Concentrations are calculated by measuring the area of the obtained spikes on the chromatogram and comparing them to the response of standard solutions. Thus, compounds are separated in time as they all have their own affinity for both elutants and columns and they are extracted from all the other components by their optic characteristics. Finally, the solvent mix goes back

to the original ratio (25% of B from 17 to 22 min) and a new cycle can begin for another sample.

Glyphosate and AMPA being not fluorescent, the molecules were modified by a process called derivatization. By this process, a fluorescent molecule was attached to glyphosate and AMPA. This molecule is FMOC-Cl, its fluorescence comes from the fact that it possesses two aromatic cycles, which are able to absorb and re-emit photons in certain wavelength by resonance. Thus, 1 mL of the samples, previously diluted to match the preferred analytical range (1 to 20 µg L^{-1}) of the method, was inserted in an amber glass vial to avoid photodegradation along with 100 µL of pH 9-borate buffer (3.1 g of H3BO3, 3.75 g of KCl and 0.8 g of NaOH in 100 mL of water). Finally, 200 µL of a FMOC-Cl solution (200 mg L^{-1} in acetonitrile) is added. When the sample had a high Ca and Mg concentration (hard water), EDTA was added to avoid prevented reaction by excessive complex formations. Samples were then injected. The detailed protocol is available in Annexe 4.

The detection limit could go down to 0.2 µg L^{-1} but was variable in function of the series and the load of the solution in organic compounds or ions. It was calculated using the calibration curve of both glyphosate and AMPA as the first point of the calibration curve that showed a signal coherent with the rest of the curve has been considered as the limit of quantification. The limit of detection was a third of this value.

4.4.3. Dissolved organic carbon and total nitrogen

To measure dissolved organic carbon and total nitrogen, considered as present in the water-soluble fraction that is thinner than 0.45 µm and is also associated as the non-purgeable organic carbon, solutions were filtered on a Büchner at 0.45 µm and analysed using a total organic carbon analyser (*TOC-L*, *Shimadzu*, Kyoto, Japan). The process was based on

a oxidation at high temperature followed by the measurement of the produced CO_2 and nitrogen oxidation products (Shimadzu 2011).

4.5. Data processing

For all extraction experiments, concentrations were expressed as extractability or extractability potential.

$$Extractability = \frac{C_{element\ in\ solution} \times V_{solution}}{m_{soil}}$$

$$Extractability\ potential = \frac{C_{element\ in\ solution} \times V_{solution}}{m_{element\ in\ the\ soil}}$$

For the greenhouse experiments, the contribution of compost to the total water extractible pool of elements was evaluated for each element using the following formula.

$$Portion\ of water\ extractible\ element\ brought\ by\ the\ compost = \frac{C_{water\ extractable\ element\ in\ compost} \times m_{compost}}{C_{water\ extractable\ element\ in\ soil} \times m_{soil} + C_{water\ extractable\ element\ in\ compost} \times m_{compost}}$$

The total mass of soil (m_{soil}) was estimated at 13 kg while the mass of compost ($m_{compost}$) was of 245 g.

Data have been analysed using *RStudio* (*2023.06.1*. and *2023.09.1*) and *R.4.0.2*. Statistical comparisons were performed between modalities (two by two) using a Kruskal–Wallis test and a post-hoc Dunn tests with the Bonferroni adjustment (*rstatix* library). In addition, when pools of elements were globally considered, a ratio of 1.2 between mean concentrations in solution or plants of two modalities was considered as minimal to consider a difference (in spite of the question of the statisti-

cal significance). For principal component analysis (PCA) the *FactoMineR* library was used and data have been prepared using the *clr* function (*Compositions* library). Data were represented using barplots on which error bars represent standard deviations. Moreover, ratios between modalities will regularly be represented using matrices of circles. The size of the circles is a function of the ratio that is represented.

5. Chapter I. Influence of glyphosate and aminomethylphosphonic acid on the mobility of trace elements in uncontaminated and contaminated agricultural soils

5.1. Abstract

Glyphosate is one of the most widely used herbicides in the world. In addition to its herbicidal effect, glyphosate is a chelating agent that can form complexes with trace elements. Yet, agricultural soils can be contaminated with both organic and mineral substances, questioning the possible influence of glyphosate application on the trace element mobility. In this context, we specifically studied the extractability of trace elements in uncontaminated and metal-contaminated agricultural soils by adding glyphosate, formulated glyphosate, and aminomethylphosphonic acid (AMPA, a degradation product of glyphosate) in batch experiments from 0 to 100 mg L^{-1}. Results showed that, on average, glyphosate enhanced the extractability of the elements considered (e.g., As, Cd, Cu, Pb, and Zn) at 20 and 100 mg L^{-1}. Surprisingly, the uncontaminated soil highlighted the highest influence of glyphosate compared to the contaminated ones, likely resulting from a higher natural element extractability in the contaminated soils. Although formulated glyphosate presented an overall higher impact than unformulated glyphosate, it was evidenced that AMPA showed lower influence meaning that glyphosate degradation is beneficial to limit deleterious effects.

5.2. Introduction

Glyphosate (N-(phosphonomethyl)glycine), a non-selective post-emergence organophosphorus herbicide that inhibits the biosynthesis of the aromatic amino acids (Pline et al. 2002), is widely used in agriculture. The commercial glyphosate–based products include additional molecules (also called co-formulants) to enhance the herbicide role of the active substance (e.g., by facilitating penetration through the cuticle; (Leaper and Holloway 2000). Unfortunately, the composition of these glyphosate co-formulants are usually unknown due to commercial confidentiality (Mesnage et al. 2019). The common glyphosate concentration in the commercial formulated products is 360 g L^{-1}, then diluted by the farmer for field application. The maximum authorized application of glyphosate depends on both culture and country: for example, in Belgium, it reaches up to 6 L ha^{-1} of 360 g L^{-1} glyphosate solution (i.e., 2160 g ha^{-1}; Phytoweb, 2015).

With an average theoretical half-life of 16 days despite variable data depending on the protocol used (Lewis et al. 2016), glyphosate is considered to be non-persistent in soil. The degradation rate, however, varies according to environmental conditions, such as climate (moisture and temperature), soil microbial activity, soil organic matter (Alletto et al. 2010; Bento et al. 2016), or even formulation (Wilms et al. 2023). Its main degradation product is aminomethylphosphonic acid (AMPA) with a similar chemical structure. AMPA, however, is more persistent in soil with a typical half-life that is ranging widely from 23 to 958 days (Bergström et al. 2011; Lewis et al. 2016; Bento et al. 2016). Due to the widespread use of glyphosate, both glyphosate and AMPA are largely present as pesticide residues in agricultural soils (Silva et al. 2019). In soil, these molecules can interact with soil constituents. Indeed, with its three acid functions (phosphonate, carboxylic, and amino; pKa of 2.6, 5.6, and 10.6, respectively; Sprankle et al. 1975), glyphosate has three

negative and one positive charges in most agricultural soils. This allows binding with ions, such as metals, and form complexes.

Trace elements are mineral elements present in low concentrations in the Earth's crust (< 1‰), including various metals (e.g., Cd, Cu, Zn) and metalloids (e.g., As, Sb) with specific behaviours in the environment (i.e., volatility, solubility, interaction with organic compounds). The occurrence and chemical forms of trace elements in soil depend on the parent material, pedogenesis, and anthropogenic inputs (Adriano 2001). Indeed, trace elements may be brought to soils by fertilizers, exogenous organic matter (e.g., manure, compost, sludge), metal-based pesticides (e.g., Bordeaux mixture), and atmospheric deposition (He et al. 2005; Belon et al. 2012). Despite the metabolic roles played by some elements, all trace elements are toxic for human and environmental health at concentrations exceeding the threshold value. Leaching to the hydrosystem and uptake by plants constitute the two major pathways for human exposure through water and food consumption, respectively (Adriano 2001; Barati et al. 2010; El-Kady and Abdel-Wahhab 2018). Understanding the trace element fate and parameters that control their transfer is thus required for health assessment.

As a chelating agent, glyphosate applied in agricultural soils can modify the trace element mobility, as already observed for major elements, such as Mg, impacting the soil fertility and the crop nutrition (Cakmak et al. 2009; Kaur et al. 2017). Consequently, there is a concern about the potential environmental impact of glyphosate application, particularly in metal-contaminated agricultural soils. Increasing mobility from soil (or mineral surfaces) to solution have already been evidenced for As, Cd, Cu, Ni, Pb, and Zn after adding glyphosate (Morillo et al. 2002; Barrett and McBride 2006; Si et al. 2013; Divisekara et al. 2018) and could promote either the transfer to the plant by increasing the trace element availability (and thus a potential plant contamination), or the

transfer to the hydrosystem (and thus a potential groundwater contamination). However different methodologies were used, especially with unrealistic high glyphosate concentration, making comparison difficult. Moreover, no information is available for some exclusively toxic elements (e.g., Sb and Cr). While glyphosate can enhance the mobility of trace elements, those elements also influence the glyphosate adsorption on soil by the formation of cationic bridges (Morillo et al. 2000; Dousset et al. 2007).

In this study, we aimed to assess the influence of glyphosate concentration (0, 20, and 100 mg L^{-1} of glyphosate) and substance (unformulated glyphosate, formulated glyphosate, and AMPA at 100 mg L^{-1}) on the mobility of trace elements from contrasted soils to soil solution in batch experiment. For that purpose, we measured a set of 15 trace element concentrations after an extraction experiment and considering one uncontaminated and three distinct metal-contaminated agricultural soils collected in Wallonia, Belgium.

5.3. Materials and methods

5.3.1. Study area

Four agricultural soils were collected in Wallonia, Belgium: an uncontaminated soil from Vieusart, Walloon Brabant, two anthropogenically metal-contaminated soils from Sainte-Walburge and Bressoux (moderately and highly contaminated, respectively) in the province of Liege, and a naturally enriched soil from Aubange in Belgian Luxembourg. The Vieusart soil (50.6799°N; 4.6357°E) is a silty soil developed on Quaternary loess characterized by slightly acidic conditions (pH_{H2O} = 6.5), low total carbon content (1.25%), and a CEC of 11.1 $cmol_c\ kg^{-1}$ (Table 4-1). The Sainte-Walburge soil (50.6644°N; 5.5887°E) is a silty

soil developed on flint and chalk from the Maastrichtian (Gulpen formation). The soil was collected at the slag heap foothill and contained a non-neglectable load of coal extraction debris resulting in a higher total carbon content (11.1%) and soil pH (pH_{H2O} = 7.8; Table 4-1). The CEC, however, was slightly higher than at Vieusart (15.2 cmolc kg^{-1}). The Bressoux soil (50.6374°N; 5.6109°E) is a silty with a slight stony load formed of Miocene alluvium. The soil was characterized by inputs of faience debris and industrial residues and presented a total carbon content of 15.8%, a soil pH_{H2O} of 7.0, and a CEC of 26.9 cmolc kg^{-1} (Table 4-1). Due to the industrial history of this region of the city of Liege (mostly steel industry and coal extraction), both Sainte-Walburge and Bressoux were influenced by atmospheric deposition of metal contaminants impacting their total trace element content from Sainte-Walburge (moderately contaminated soil) to Bressoux (highly contaminated soil; Table 4-1). Finally, the Aubange soil (49.5765°N; 5.7684°E) is a silty soil with moderate stony load developed on the Aubange formation, a complex of sandstone, siltstone, and argillite dating from the Pliensbachian with high naturally enriched trace element content, such as As, Ni, and Cr (Table 4-1; Vandeuren et al. 2023). The soil was characterized by a pH_{H2O} of 7.3, a total carbon content of 2.43%, and a CEC of 20.8 $cmol_c$ kg^{-1} (Table 4-1).

5.3.2. Soil sampling and characterization

Soil sampling and sample preparation

On each study site, we collected the top 30 cm soil using a shovel to perform a homogeneous composite sample. The soil samples were then air-dried at 25 °C for 15 days and sieved through a 2-mm mesh. An aliquot was crushed for trace element analyses using a soil grinder (Vibratory Disc Mill RS 200, Retsch, Haan, Germany). Basic soil characterization included soil pH_{H2O} (using a 1:5 ratio), total carbon content

(Elementar vario EL cube, Langenselbold, Germany), and CEC (Metson method with ammonium acetate at pH 7). Due to the absence of carbonates, total carbon content can be assumed as organic carbon content.

Trace element concentration quantification

Before quantification of trace element concentrations, 100 mg of ground soil samples were digested in an ISO 6 cleanroom (Earth and Life Institute, UCLouvain) in a Teflon bottle (Savillex). We applied a four-step procedure (HNO_3/ HF, H_2O_2, HNO_3/HCl, and HNO_3) using suprapure chemicals and high purity Milli-Q water (Arbalestrie et al. 2022). Heating steps were fixed at 90 °C and evaporation steps at 40 °C. The samples were preserved in 2% HNO_3 solutions before analysis.

For all samples, a set of 15 trace elements (As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, and Zn) was quantified in the MOCA platform (Mineral and Organic Chemical Analysis, Earth and Life Institute, UCLouvain) using an ICP–MS (iCAP Q ICP–MS, Thermo Fischer Scientific, Waltham, MA, USA). An internal standard (Ru, In, and Re) was used in each sample for drift instrumental correction. The performance of the procedure was evaluated using two certified soil material (GSS1 and GSS4; Xuejing et al. 1985) in each series. The average recovery ($C_{measured}/C_{certified} \times 100$) was estimated to 100±15% for all analytes, except for Cd (117%), Sb (121%), Mn (78%), Ti (75%), and Ba (70%). Limits of detection were estimated to <0.01 μg kg^{-1}, except for Ti, Ni, and Zn (<0.1 μg kg^{-1}) and for Mo (<1 μg kg^{-1}).

5.3.3. Experimental procedure

Trace element extraction procedure

To test the influence of both glyphosate concentrations and substances on the soil trace element mobility, four extraction solutions were considered: Milli-Q water (control), glyphosate (Sigma Aldrich) at 20 and

100 mg L^{-1} (corresponding to 0.12 and 0.59 mmol L^{-1}, respectively), formulated glyphosate (*Glyfall plus*) 100 mg L^{-1} (corresponding to 0.59 mmol L^{-1}), and AMPA (*Sigma Aldrich*) at 100 mg L^{-1} (corresponding to 0.90 mmol L^{-1}). These concentrations were in the range of concentrations tested in the literature (Wang et al. 2008; Si et al. 2013). Trace element concentrations in the extraction solutions were negligible. Extractions were performed in triplicate in a 50-mL centrifuge polypropylene tubes with 5 g of sieved soil and 25 mL of extraction solution, i.e., 1:5 ratio, adapted from Divisekara et al. (2018). The mixture was then agitated in the dark for 2 h at 150 bpm. After agitation, the tubes were centrifuged for 25 min at 4000 rpm and filtrated using a 0.45-µm pore size Nylon *Acrodisc*®. All extraction solutions have been prepared using Milli-Q water. An aliquot of 1 mL of the filtered solution was diluted 10 times and acidified (2% HNO_3) for trace element analysis, while another aliquot of 10 mL was used for pH measurement and glyphosate and AMPA analysis.

Chemical analyses

Trace element concentrations were measured by ICP-MS as described for soil sample analysis. In each series, the quality was checked using the river water SLRS 6 reference material that presented an average recovery of 100 ± 15%, except for Zn (83%), Cd (60%), and Pb (133%). The concentration of all considered elements were always above the quantification limit.

For glyphosate and AMPA analysis, the solution samples were diluted to reach the range of the analytical method (1 to 20 µg L^{-1}) adapted from ISO 21458 and ISO 116308. Glyphosate and AMPA were derivatized at pH 9 (borate buffer) by an excess of FMOC-Cl. Glyphosate and AMPA were measured by HPLC (1200 series, Agilent Technologies Santa Clara, CA, USA) with a Merck Lichrocart C18 150 ×

4.6 mm–5 µm column kept at 35 °C. Elutants were pH 7 10 mM phosphate buffer (A) and 45:45:10 acetonitrile:methanol:Milli-Q water solution (B). Elution was performed at a flow rate of 1 mL min^{-1} using the following gradient (expressed as solvent B): from 0 to 1 min, 25% B (initial composition); from 10 to 15 min, 100% B; from 17 to 22 min, 25% B. The detection was performed by a fluorescent detector with an excitation at 260 nm and an emission at 310 nm.

5.3.4. Data processing and statistical analyses

Measured trace element concentrations in the extraction solution were expressed as soil extractability (i.e., masse of extracted element by mass of soil) and soil extractability potential (i.e., mass of extracted element by mass of element in soil). Statistical analyses were performed using *R 4.0.2* and *RStudio 2023.06.1*. Statistical comparisons between glyphosate concentrations (0, 20, and 100 mg L^{-1}) or tested substances (unformulated glyphosate, formulated glyphosate, and AMPA at 100 mg L^{-1}) were performed using a Kruskal-Wallis test and a post-hoc Dunn tests including the Bonferroni adjustment (*rstatix* library).

5.4. Results

5.4.1. Trace element extractability in uncontaminated agricultural soil

Trace element content in the uncontaminated soil (i.e., Vieusart) was in the range of the European agricultural soils (Table 4-1; Baize 2000). We first investigated the extractability of four trace elements as contrasted examples: Cu, As, Mo, and Pb (Figure 5.1B–E). They were selected because of their environmental and sanitary relevance (Cu, As, Pb) and contrasted geochemical behaviour (cations vs oxyanions). The element water-extractability (i.e., control modality) was ranged in the

following order: Mo (0.007 μg g_{soil}^{-1}) < As ≤ Pb < Cu (0.75 μg $gsoil^{-1}$). Results showed an overall increase of element extractability with both concentrations of glyphosate (20 and 100 mg L^{-1}) compared to the control, on average 3.5 and 2.0 times higher, respectively, and up to 6.9 times for Pb with 20 mg L^{-1} of glyphosate. We highlighted an increase from 0.0014 to 0.22 μmol L^{-1} for Mo and Cu, respectively, with an addition of 0.59 mmol L^{-1} of glyphosate (i.e., 100 mg L^{-1}), representing an elements/glyphosate molar ratio of 0.05% (considering the four trace elements: Cu, As, Mo, and Pb). Note that only Pb with 20 mg L^{-1} of glyphosate showed a statistically significantly higher concentration than the control ($p < 0.05$). Interestingly, the intermediate glyphosate concentration (i.e., 20 mg L^{-1}) sometimes reached higher tested modality/control extractability ratio than for 100 mg L^{-1} (e.g., for As, Mo, and Pb). Only Mo showed low or a lack of effect, with 1.1 to 1.3 times higher extractability than the control according to the glyphosate concentration. For formulated glyphosate, the results were similar to unformulated glyphosate with only positive effect compared to the control in the following order: Pb (× 1.2) < Mo < Cu < As (× 3.3). The tested modality/control extractability ratios were either higher (for Cu and Mo) or lower (for Pb) compared to glyphosate (Figure 5.1). Finally, the influence of AMPA on the extractability of trace elements was more heterogeneous depending on the element considered, including positive effect for As (× 2.3), negative effect for Pb (× 0.7), and no effect for Cu and Mo (× 1.1).

For > 70% of the considered element-modality combination, the extractability was also enhanced (i.e., modality/control extractability ratio > 1.2, and up to 8.7 for Sn; Figure 5.2). Titanium, V, Cr, Mn, Co, and Ni also presented a similar behaviour as Pb with higher extractability with 20 mg L^{-1} of glyphosate than with 100 mg L^{-1}. Comparing the tested substances with the unformulated glyphosate, results were similar as the four previously mentioned elements: formulated glyphosate

with slightly lower extractability and AMPA with almost no extractability effect, despite no statistically significant differences. Copper and Zn, however, showed higher extractability with: (1) 100 mg L^{-1} of glyphosate compared to 20 mg L^{-1}, and (2) formulated glyphosate compared to unformulated one at 100 mg L^{-1}. Finally, with 100 mg L^{-1} of AMPA, Zn, Sn, and Ba presented the maximum extractability decrease comparted to the control (at least 3 times).

The molecule concentrations were measured in the post-extraction solution (Figure 5.1A). Results indicated a minor part of glyphosate used for the experiment that remained in solution, from 10 (with 20 mg L^{-1} of glyphosate) to 28% (with 100 mg L^{-1} of glyphosate). This part, however, increased to 42% when 100 mg L^{-1} of formulated glyphosate was added. For the same mass concentration of AMPA (i.e., 100 mg L^{-1}), the molecule remaining in solution reached 35%, in-between glyphosate and formulated glyphosate. Note that AMPA in the post-extraction solutions was below detection limit for all unformulated and formulated glyphosate modalities. The average pH values in the post-extraction solutions were ranged from 6.4 to 7.0, representing decrease from the pH of the control ranging between 0 (for 20 mg L^{-1} of glyphosate) and 0.57 (for 100 mg L^{-1} of formulated glyphosate) pH unit.

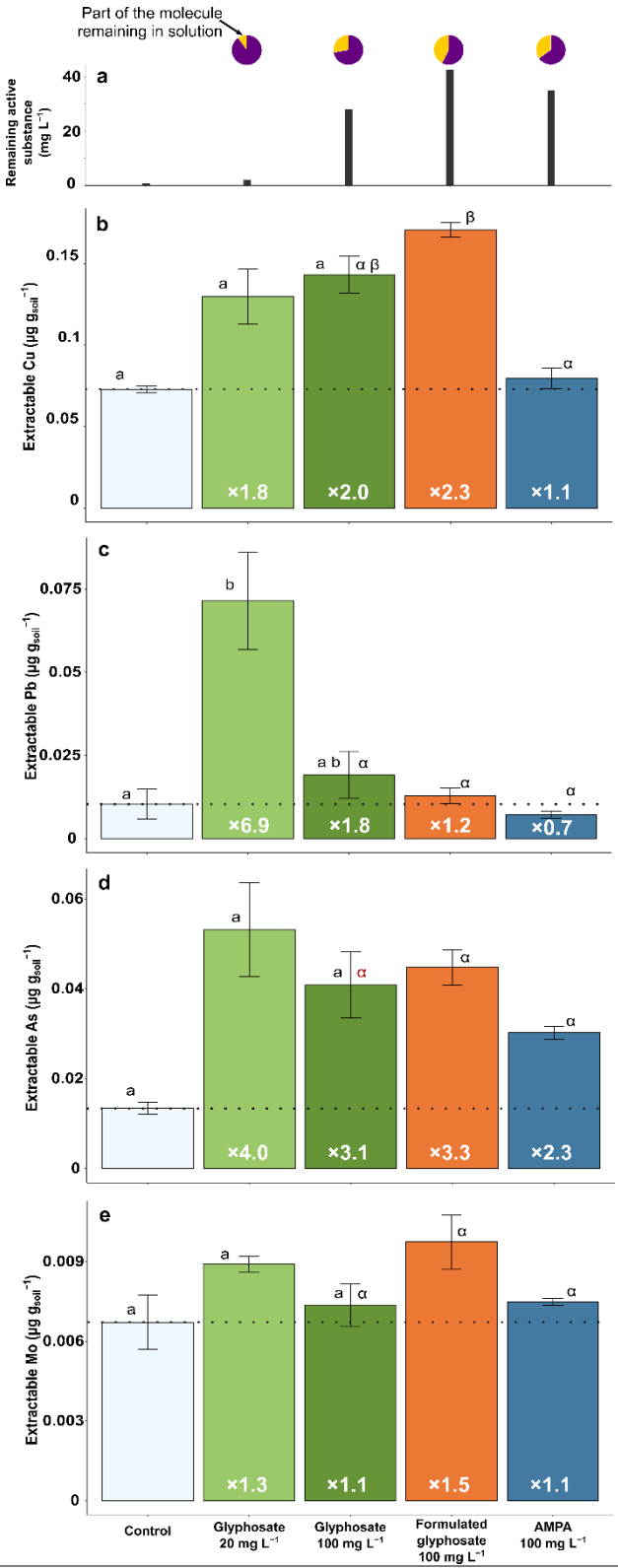

Part of the molecule remaining in solution
a
Remaining active substance (mg L⁻¹)
40
20
0
b
Extractable Cu (µg g$_{soil}$⁻¹)
0.15
0.1
0.05
0
×1.8
×2.0
×2.3
×1.1
c
Extractable Pb (µg g$_{soil}$⁻¹)
0.075
0.05
0.025
0
×6.9
×1.8
×1.2
×0.7
d
Extractable As (µg g$_{soil}$⁻¹)
0.06
0.04
0.02
0
×4.0
×3.1
×3.3
×2.3
e
Extractable Mo (µg g$_{soil}$⁻¹)
0.009
0.006
0.003
0
×1.3
×1.1
×1.5
×1.1
Control
Glyphosate 20 mg L⁻¹
Glyphosate 100 mg L⁻¹
Formulated glyphosate 100 mg L⁻¹
AMPA 100 mg L⁻¹

Figure 5.1 Concentrations (lines) and proportion (pie charts) of molecule remaining in solution after extraction (n = 3; a) and extractability (n = 3) of Cu (b), As (c), Mo (d), and Pb (e) in the uncontaminated soil (Vieusart) following four extraction modalities: control (Milli-Q water), glyphosate (20 and 100 mg L^{-1}), formulated glyphosate (100 mg L^{-1}), and AMPA (100 mg L^{-1}). Numbers indicate the tested modality/ control extractability ratios (the dashed line represents the control value). The Latin and Greek letters on the top of the bars represent the statistically significant differences (Kruskal–Wallis test and Dunn test with Bonferroni adjustment) for concentrations and substances, respectively. Scales were adapted for each element.

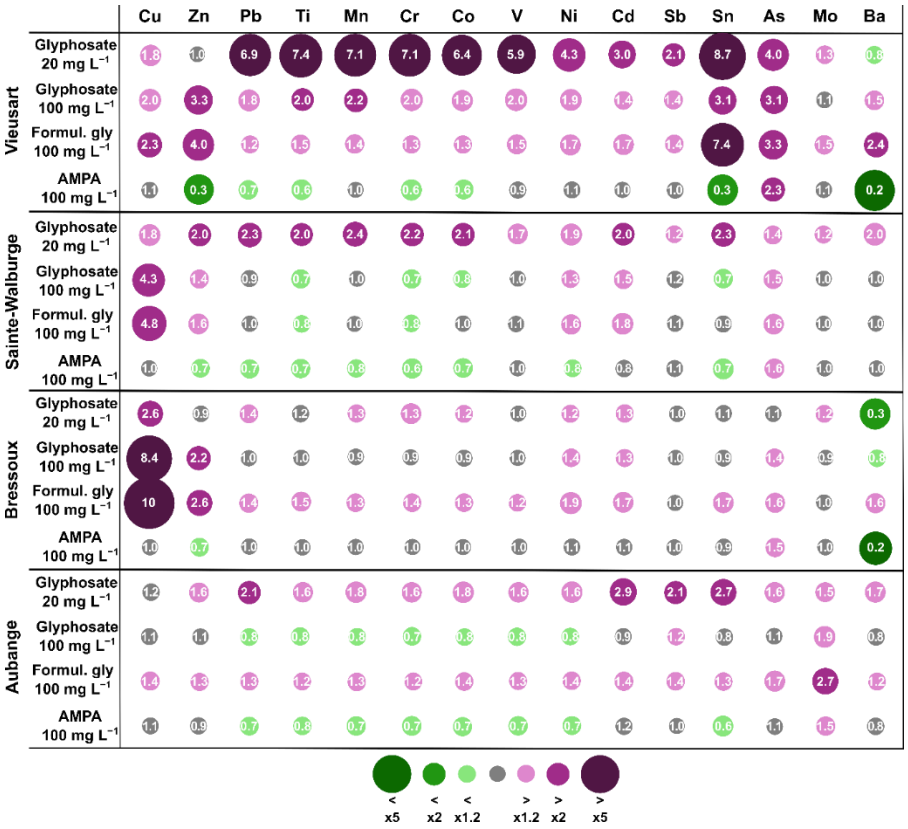


Figure 5.2 Summary of tested modality/control extractability ratios (n = 3) for each studied element in the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils. Colours represent the range of positive (purple) or negative (green) influence compared to the control. Elements were ranked in order to better see shared behaviours between elements.

5.4.2. Trace element extractability in contaminated agricultural soils

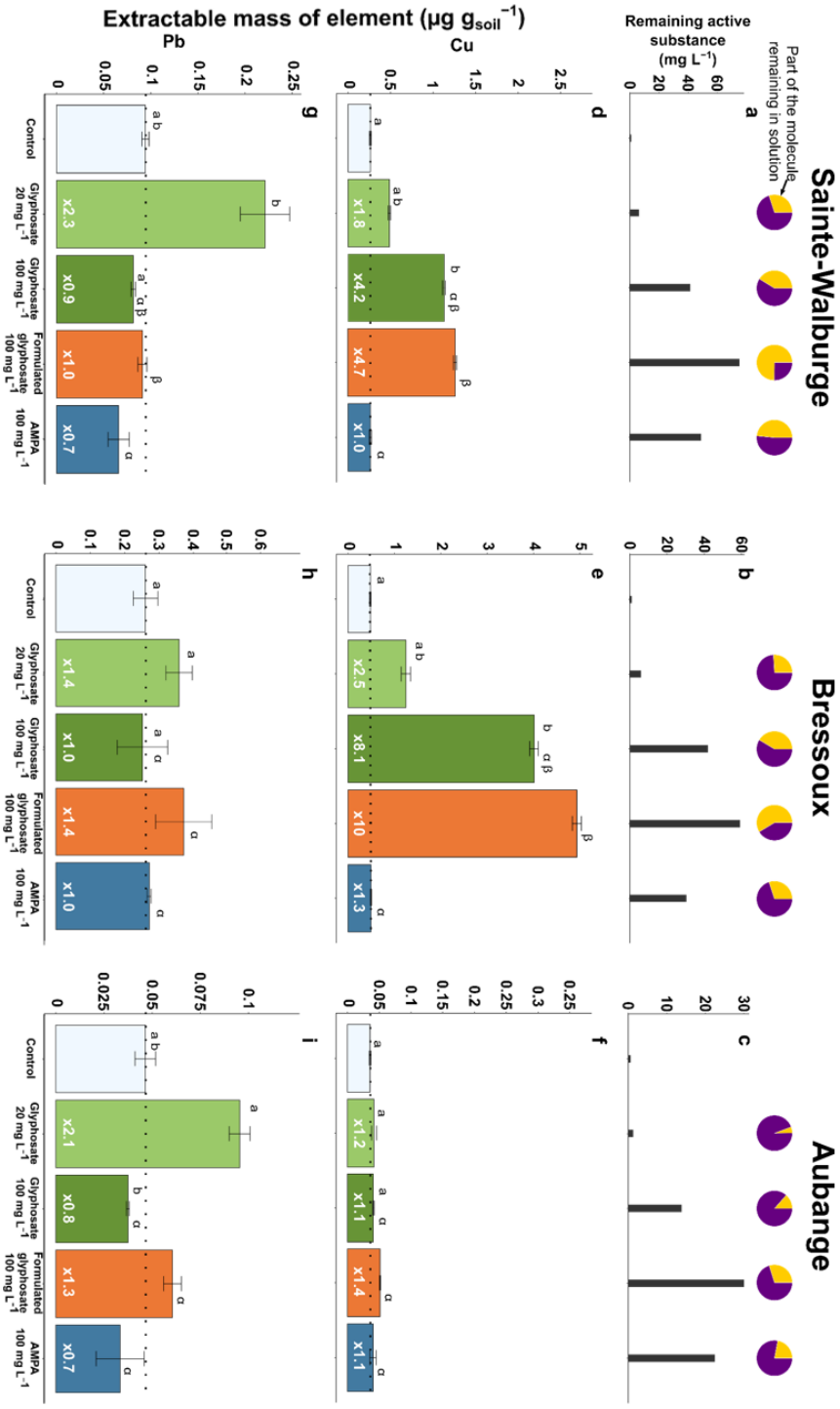
A similar experiment was performed for the two anthropogenically contaminated (Sainte-Walburge and Bressoux) and one naturally enriched (Aubange) soils (Figure 5.3). These three soils indicated elevated trace element content compared to European agricultural soils (Table 4-1;

Baize 2000). The element water-extractability was ranged from Cd in Aubange (0.22 ng g_{soil}^{-1}) to Mn in Aubange (3.8 µg g_{soil}^{-1}), i.e., on average 5.5 times higher than for the uncontaminated soil. Overall, the tested modality/control extractability ratios for the four selected trace elements were between 0.7 (for Pb with 100 mg L^{-1} of AMPA at Sainte-Walburge and Aubange) and 10 (for Cu with 100 mg L^{-1} of formulated glyphosate at Bressoux). This highlighted a higher heterogeneity compared to the uncontaminated soil. We also compared these four trace elements with the glyphosate: according to the soil considered, molar concentration increased from 0.0019–0.0016 to 0.015–11.1 µmol L^{-1} for Mo and Cu, respectively, with an addition of 0.59 mmol L^{-1} of glyphosate (i.e., 100 mg L^{-1}), representing an elements/ glyphosate molar ratio of 0.5–1.9 for the anthropogenically contaminated and 0.009% for the naturally enriched soils (considering Cu, As, Mo, and Pb). Except for Pb with 20 mg L^{-1} of glyphosate, both anthropogenically contaminated soils presented similar patterns between modalities and elements (including comparable extractability ratios for Mo and As), and dissimilar to Aubange. For glyphosate, the extractability was either enhanced or similar compared to the control depending on the tested concentrations. The highest influence was observed for Cu with 100 mg L^{-1} of glyphosate at Bressoux and, to a lesser extent, Sainte-Walburge ($p < 0.05$), while the naturally enriched soil generally showed lower effects ($p > 0.05$). As for the uncontaminated soil, the effect can be evidenced from 100 mg L^{-1} of glyphosate (e.g., Cu at Bressoux, $p < 0.05$) or from 20 mg L^{-1} of glyphosate (e.g., As at Aubange, $p < 0.05$). Surprisingly, the extractability was increased for some trace elements with 20 mg L^{-1} of glyphosate without effect observed with 100 mg L^{-1} of glyphosate: case for Zn in Sainte-Walburge or As in Aubange ($p < 0.05$).

The four selected trace elements presented frequently a positive effect with 100 mg L^{-1} of formulated glyphosate. The tested modality/control

extractability ratios were similar or higher than for unformulated glyphosate (statistically significant differences for As in Sainte-Walburge and Cd in Aubange compared to the control, $p < 0.05$). The influence of AMPA on trace element extractability was generally lower (on average, from × 0.7 to× 1.6 depending on the selected elements and the considered site), with no effect for half of the presented results ($p > 0.05$). As for the uncontaminated soil, Pb also highlighted lower extractability than the control (with the exception of Bressoux).

Similar groups of elements defined in the uncontaminated soil were also observed in the contaminated ones (Figure 5.2): e.g., Ti, V, Cr, Mn, and Co behaved like Pb, with higher extractability with 20 mg L^{-1} of glyphosate than 100 mg L^{-1}. The overall effect of glyphosate, however, was lower in contaminated soils and formulated glyphosate showed higher influence compared to glyphosate (except for Sainte-Walburge). Also, Cu and Zn behave similarly in regard to glyphosate with some exceptions in Sainte-Walburge (Zn more extracted with 20 mg L^{-1} of glyphosate compared to 100 mg L^{-1}, as observed for Cd). AMPA generally presented no effect (for 24 cases) or negative influence (for 17 cases) on element extractability compared to the control. However, differences appeared among contaminated soils: the negative influence was observed in Sainte-Walburge and Aubange, but not in Bressoux (except for Ba and to a lesser extent Zn).


Sainte-Walburge
Bressoux
Aubange
Part of the molecule remaining in solution
Remaining active substance (mg L-1)
Extractable mass of element (µg gsoil-1)
Cu
Pb
Control
Glyphosate 20 mg L-1
Glyphosate 100 mg L-1
Formulated glyphosate 100 mg L-1
AMPA 100 mg L-1
x1.8
x4.2
x4.7
x1.0
x2.5
x8.1
x10
x1.3
x1.2
x1.1
x1.4
x1.1
x1.4
x1.0
x1.4
x1.0
x2.1
x0.8
x1.3
x0.7
x2.3
x0.9
x1.0
x0.7

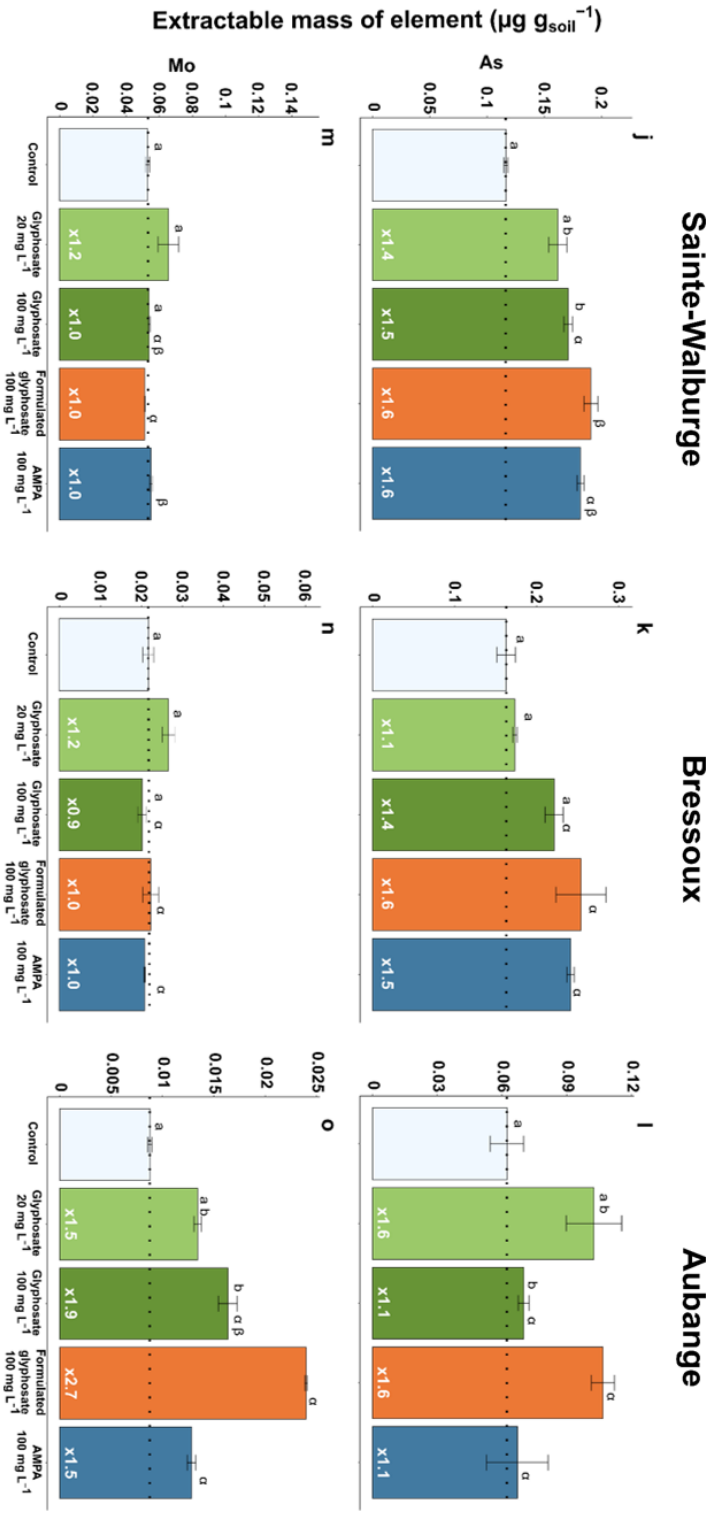

Extractable mass of element (µg g$_{soil}^{-1}$)
Sainte-Walburge
Bressoux
Aubange
As
Mo
Control
Glyphosate 20 mg L^{-1}
Glyphosate 100 mg L^{-1}
Formulated glyphosate 100 mg L^{-1}
AMPA 100 mg L^{-1}
x1.4
x1.5
x1.6
x1.6
x1.2
x1.0
x1.0
x1.0
x1.1
x1.4
x1.6
x1.5
x1.2
x0.9
x1.0
x1.0
x1.6
x1.1
x1.6
x1.1
x1.5
x1.9
x2.7
x1.5

Figure 5.3 Concentrations (lines) and proportion (pie charts) of molecule remaining in solution after extraction (n = 3; a) and extractability (n = 3) of Cu (d, e, f), Pb (g, h, i), As (j, k, l), and Mo (m, n, o) in the three contaminated soils (Sainte-Walburge, Bressoux, and Aubange) following four extraction modalities: control (MilliQ water), glyphosate (20 and 100 mg L^{-1}), formulated glyphosate (100 mg L^{-1}), and AMPA (100 mg L^{-1}). Numbers indicate the tested modality/control extractability ratios (the dashed line represents the control value). The Latin and Greek letters on the top of the bars represent the statistically significant differences (Kruskal-Wallis test and Dunn test with Bonferroni adjustment) for concentrations and substances, respectively. Scales were adapted for each element.

Results of the molecule remaining in the post-extraction solution indicated the same trend in the three contaminated soils: increasing proportion of active substance with increasing glyphosate concentration (on average, ×1.7 from 20 to 100 mg L^{-1}), higher proportion with formulated glyphosate than unformulated glyphosate, and intermediate proportion with AMPA (Figure 5.3). The naturally enriched soil, however, showed on average 3.2 times less glyphosate remaining in solution with 100 mg L^{-1} compared to both Sainte-Walburge and Bressoux. As observed for the uncontaminated soil, no AMPA was measured above the detection limit in the post-extraction solution in the unformulated and formulated glyphosate modalities.

5.4.3. Extractability potential

We finally considered the extractability potential of trace elements (i.e., extractability normalized to soil element concentration) to compare the different soils by eliminating any concentration influence. We thus

compared the extractability potential of each tested modality (20 mg L^{-1} of glyphosate, 100 mg L^{-1} of glyphosate, 100 mg L^{-1} of formulated glyphosate, and 100 mg L^{-1} of AMPA) to the extractability potential in the control (Figure 5.4). Considering the glyphosate influence on the extractability potential of elements (Figure 5.4 a–b), Vieusart depicted an overall higher positive effect compared to the contaminated soils, particularly with 20 mg L^{-1} of glyphosate. For contaminated soils, Sainte-Walburge and Aubange generally presented higher extractability potential than Bressoux with 20 mg L^{-1} of glyphosate, while negative effect was common with 100 mg L^{-1} of glyphosate. Overall, Cu, Zn, Mo, and Sb were the most potentially extractable element in all uncontaminated and contaminated soils considered in this study, whereas Co, Ti, and Sn highlighted the lower extractability potential. Arsenic was the only element with a wide range of extractability potential according to the considered soil. Formulated glyphosate showed similar pattern as unformulated glyphosate with the same concentration (Figure 5.4c), while AMPA decreased the influence on the extractability potential compared to glyphosate, reducing differences observed between the soils studied (Figure 5.4d).

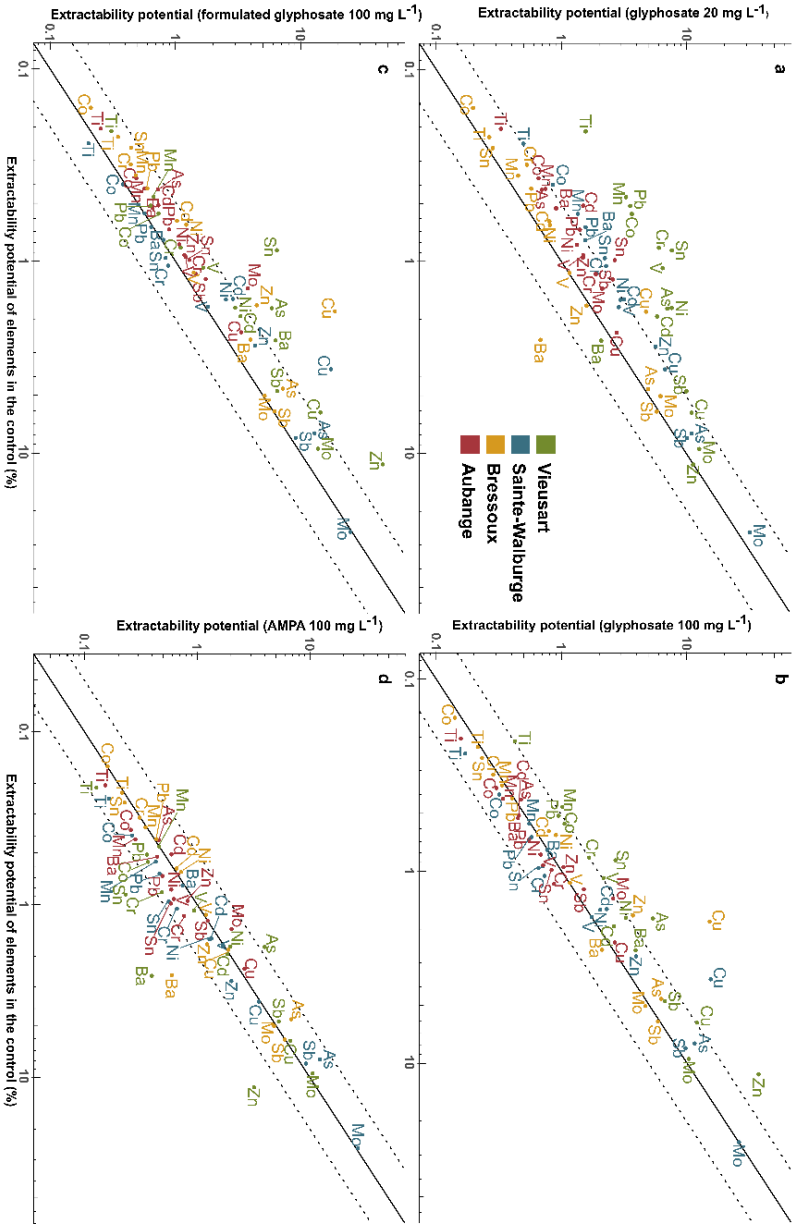


Figure 5.4 Extractability potential of trace elements of each tested modalities compared to extractability potential in the control (n = 3): glyphosate 20 mg L^{-1} (a), glyphosate 100 mg L^{-1} (b), formulated glyphosate 100 mg L^{-1} (c), and AMPA 100 mg L^{-1} (d). The solid line represents the 1:1 ratio, and the dashed lines represent the 2:1 ratio (upper line) and 1:2 ratio (lower line).

5.5. Discussion

5.5.1. Influence of glyphosate on trace element mobility in uncontaminated soil

The application of glyphosate in uncontaminated soil samples frequently highlighted a positive effect on trace element mobility. Indeed, 67% of the tested trace elements showed a mean mobility > 1.2 times higher than the control after adding 20 mg L^{-1} of glyphosate, despite not always statistically significant differences due to the low number of replicates and the statistical tests used. Some of these observations have already been documented (e.g., As, Cd, Cu, Ni, and Pb; Morillo et al. 2000; Barrett and McBride 2006; Divisekara et al. 2018), while, to our knowledge, no information is available in the literature regarding other trace elements. Some trends (Cu > Zn) were in accordance with metal-glyphosate complexation coefficients found in the literature (Madsen, et al. 1978). For some elements (e.g., Ba and Zn), an increasing mobility was only observed when the concentration of glyphosate reached 100 mg L^{-1}. Although these two tested concentrations are not commonly found in soil solutions (glyphosate concentrations are generally below 1 mg L^{-1} in soil solution samples and decrease dramatically with soil depth; Veiga et al. 2001; Vereecken 2005; Borggaard and Gimsing 2008), they are 100- to 500-times lower than the concentrations of the solution directly applied to the field. This suggests that trace elements should mostly be mobilized in the upper soil layers that are exposed to higher glyphosate concentrations after spraying than the commonly reported soil solution ones before dilution by consecutive rain event. We assume that along the vertical transport and the environmental changes with soil depth (such as glyphosate concentration), both trace elements and glyphosate could find new sorption sites. Despite the high heterogeneity of uncontaminated soils, Vieusart soil can be representative of a large range of temperate agricultural soils because of its physical and

chemical characteristics (i.e., silty soil with low organic carbon content).

Among the trace elements considered, we highlighted different groups with comparable masses following the same behaviours regarding their interaction with glyphosate (Figure 5.2): e.g., Ti, Mn, Cr, Co, and V (light elements) or Cd, Sb, and Mo (heavy elements), despite that some elements did not fully fit with this grouping (e.g., Pb). For instance, both Cu and Zn (in between those light and heavy elements) showed frequent complexation to organic matter (Martínez and McBride 1999; Wong et al. 2007; Borggaard et al. 2019) that highlights their affinity for organic compounds, such as organic pollutants (including glyphosate), resulting in element mobility enhancement (Figure 5.2; Barrett and McBride 2006). Note that although organic molecules can have an effect on the trace element extractability, the nature of these molecules may largely influence this mobility. However, water-extractable organic compounds being present in every modality (both in the control and in the pesticide treatments) from the same soil, it becomes possible to determine the role of glyphosate alone. Several other trace element chemical features, however, are pointed out as important factors controlling their sorption capacity, such as electronegativity, charge/radius ratios and related Goldschmidt categories, or hydrolysis constant (Goldschmidt 1937; McBride 1994; Covelo et al. 2004). The element origin (e.g., lithogenic elements such as Co, Mn, Ni, and Ti; Rubio et al. 2000) or their complexation capacity may also contribute to this grouping.

Surprisingly, the effect of glyphosate on the element mobility was higher at 20 mg L^{-1} compared to 100 mg L^{-1} for 11 of the 15 elements considered (especially for Co, Cr, Mn, Pb, Sn, Ti, and V; Figure 5.2). Note that the glyphosate concentration in the post-extraction solution was lower for the 20 mg L^{-1} modality and, despite a higher relative sorption of glyphosate, the total mass of glyphosate that was sorbed on

the soil was still lower than for the 100 mg L^{-1} modality (Figure 5.1). With three negative and one positive charges under common soil pH conditions (from 5.6 to 10.6; Sprankle et al. 1975), sorbed glyphosate could contribute to the soil CEC, and thus promote the cation binding (Figure 5.5 a-b). The antagonist processes between trace element mobilization by free glyphosate and trace element binding by sorbed glyphosate could explain the non-linear response between applied glyphosate concentration and trace element mobility. Nevertheless, as major elements are not considered, it cannot be excluded that increased desorption of elements like Ca and Mg under higher glyphosate concentration releases sites on the CEC contributing to a re-adsorption of trace elements. The absence of such trends in literature data (e.g., for Pb and Cd; Divisekara et al. 2018) may results from more contrasted glyphosate concentrations, from extremely low (up to 2 times lower concentrated compared to our lowest tested values) to high (up to 44 times higher) concentrations, preventing intermediate behaviours. Moreover, only formulated glyphosate was considered in Divisekara et al. (2018) which makes the comparison potentially difficult. Further investigations are thus required to confirm or refute this hypothesis, with particular focus on the structure of the soil-trace element-glyphosate complexes.

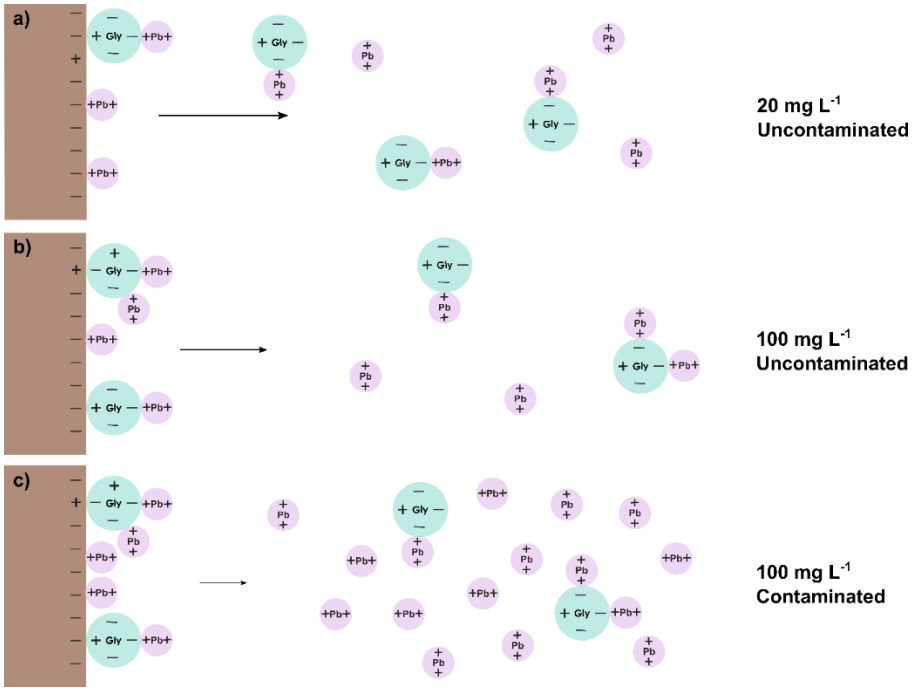


Figure 5.5 Diagram of the influence of glyphosate concentration and soil contamination level on trace element extractability.

5.5.2. Influence of glyphosate on trace element mobility in contaminated soils

Influence of glyphosate on trace element mobility in anthropogenically contaminated soils (i.e., Sainte-Walburge and Bressoux) highlighted similar groups as observed in the uncontaminated soil (Figure 5.2), involving similar geochemical behaviours. The effect of glyphosate, however, was lower compared to Vieusart soil, particularly at 100 mg L^{-1} of glyphosate where no influence was evidenced for 50% of the elements considered. This likely results from the higher extractability of anthropogenically derived trace elements even without glyphosate (up to 25 times more extracted in the control depending on the element considered; Figure 5.1 and Figure 5.3) frequently observed in human-induced contaminated soils (Rauret 1998; Sungur et al. 2014), making the

influence of glyphosate negligible for many elements (Figure 5.5 b-c). Indeed, the overall influence of 20 mg L^{-1} of glyphosate decreased with the contamination level, i.e., Vieusart > Sainte-Walburge > Bressoux. In an absolute point of view, however, the same molar concentration of glyphosate mobilizes a higher proportion of trace elements in anthropogenically-contaminated soils (i.e., trace elements/glyphosate molar ration of 0.5–1.9% vs 0.05% in Vieusart, considering Cu, As, Mo, and Pb). Conversely, Cu presented a higher mobility with glyphosate in anthropogenically contaminated soils compared to Vieusart soil. Again, this may result from the high affinity of Cu with organic compounds (Sposito et al. 1979; Gao et al. 1997; Manceau and Matynia 2010), including glyphosate. Note that Cu belongs to the most mobile elements (along with As, Mo, Sb, and Zn according to the control data; Figure 5.4), we suspect potential environmental and health risks due to available toxic elements in glyphosate-treated soils, especially when Cu is applied as fungicide (Ballabio et al. 2018).

In contaminated soils, the sources of trace elements may largely control the element behaviour. For instance, similar trends between Cd and Zn in the modality/control extractability ratios (Figure 5.2) likely results from a similar industrial source: both are frequently emitted from metallurgical and extractive industry along with Pb and Cu among others (Helios Rybicka 1996; Cox et al. 2002). The nature of soil contamination determines the trace element speciation, and thus the trace element-glyphosate interactions. The interaction between Cu and glyphosate has been extensively studied in regard to both the role of ionic bridge that Cu can form to enhance the glyphosate adsorption (Morillo et al. 2000; Maqueda et al. 2002; Dousset et al. 2007) and the chelating effect of glyphosate on the Cu extractability and sorbability (Glass 1984; Morillo et al. 2002; Barrett and McBride 2006). Yet, we have demonstrated that

Cu, one of the most investigated elements in published glyphosate experiments, exhibits a unique biogeochemical behaviour that cannot be extrapolated to other elements.

In the naturally enriched soil (i.e., Aubange), the influence of glyphosate on the trace element mobility may be related to either the uncontaminated soil (e.g., Cu without drastic enhancement with glyphosate) or anthropogenically contaminated soils (e.g., low influence for the other elements; Figure 5.2). Since the overall mobility of trace elements in Aubange was as low as in the uncontaminated soil (i.e., basically low extractability in the control), glyphosate did not contribute much to the trace element transfer from soil to water.

5.5.3. Influence of formulated glyphosate and AMPA on trace element mobility

When glyphosate is formulated, its influence on trace element mobility depended on the elements considered: lower influence compared to the unformulated glyphosate for lower mass elements (<Ni) and Pb vs higher influence for the other elements (except for Sb with no significant difference; Figure 5.2). We proposed several hypotheses for this contrasted result: (1) a complex formation between glyphosate and the co-formulant that would limit the availability of sorption sites on the glyphosate and, if applicable, the co-formulant (i.e., negative effect); (2) a more important pool of free glyphosate able to form complexes with trace elements due to glyphosate desorption (positive effect); (3) a complex formation between trace elements and the co-formulant, as observed for detergents with charged hydrophilic groups (Chittleborough 1980) that are frequently used as co-formulant, which promotes element mobility (positive effect); or (4) changed surface properties of soil particles due to the co-formulant (positive or negative effect). Indeed,

glyphosate concentration in the solution after the experiment systematically increased in presence of the co-formulant (Figure 5.1 and Figure 5.3). Since there was no evidence for glyphosate degradation (i.e., no significant AMPA production in the solution after the experiment), we suggest a decrease of glyphosate sorption. This is consistent with the most common role of the co-formulant that is to increase lipophilicity, usually by detergent effect; it would thus decrease the glyphosate affinity for charged soil surfaces. The influence of the formulation, however, changed along the contamination level: from an average lower effect compared to unformulated glyphosate in the uncontaminated soil (i.e., Vieusart) to an average higher effect in the highest contaminated soil (i.e., Bressoux) and naturally enriched soil (i.e., Aubange). As glyphosate is always applied as a formulated product in the field, it is expected that investigating unformulated glyphosate in controlled environment leads to overall underestimations in contaminated soils. In addition, some highly toxic elements (e.g., As, Cd, or Pb) are generally more mobile with formulated glyphosate, encouraging the element bioavailability and their adverse impacts on human and environmental health.

In comparison to unformulated and formulated glyphosate, AMPA showed a lower influence on trace element mobility. Here, distinct groups of elements were evidenced, suggesting other processes involved in the trace element AMPA interactions (Figure 5.2). We hypothesize that (1) complexes between trace elements and AMPA might be easily sorbed to the soil (the lack of literature data about AMPA sorption on soil particles prevent any validation) or (2) AMPA plays a role of chelating agent for more mobile elements resulting in empty sites on the exchange complex for less mobile ones (Bloem and Vogler 2018). The concentrations of AMPA remaining in the solution being higher than those of glyphosate in the unformulated experiment (with the exception of Bressoux where there was no negative influence on the trace element mobility), this mainly leads to the second hypothesis.

Note that we compared glyphosate and AMPA with the same mass concentration: considering the molar mass (i.e., 1.5 times lower than the glyphosate) and the fact that one mole of glyphosate is degraded to less than one mole of AMPA because of the multiple degradation pathways (Liu et al. 1991), we overestimate the concentration effect of AMPA compared to glyphosate. Unlike the other elements, AMPA particularly enhanced the mobility of As, one of the rare elements present as oxyanions. As AMPA has two negative charges and one positive charge at pH > 5, it is surprising that AMPA does not promote the extraction of positive ions (i.e., less mobile in presence of AMPA; Figure 5.2), likely resulting from possible sorption processes of the trace element-AMPA complexes to the soil (Barja and dos Santos Afonso 2005). However, Mo also present as oxyanion did not follow the same trend, probably due to distinct electronegativity, an important feature for complex formation. With the exception of As, the degradation of glyphosate to AMPA could thus limit the availability and leaching of trace elements and their harmful effects.

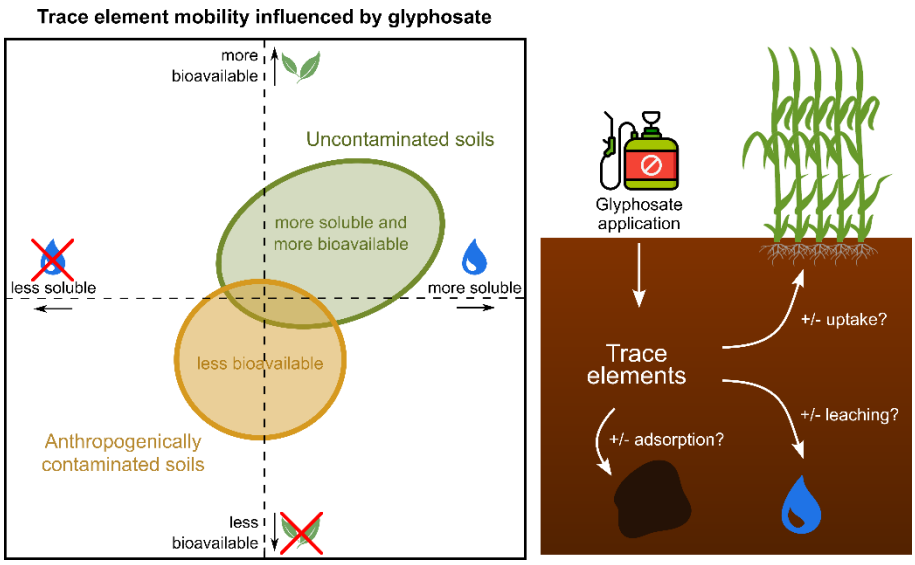
5.6. Conclusion

In a context of multi-contaminated agricultural soils (i.e., both trace elements and pesticides), it is required to understand the role of pesticides, such as glyphosate, on trace element dynamics. In this study, we observed increasing element (particularly for As, Cd, Cu, and Ni) mobility from soil to water with glyphosate solution concentration of 20 and 100 mg L^{-1} in a batch experiment, i.e., glyphosate concentration comprised between solutions applied to the field and soil solutions collected in the field. We thus expect a potential increase of trace element availability after glyphosate treatment. The effect was higher for the uncontaminated soil compared to both the anthropogenically contaminated and naturally enriched soils considered. This likely results from

the higher element extractability without glyphosate in highly contaminated soils. The use of formulated glyphosate, however, exacerbated the trace element mobility thanks to a decrease of glyphosate sorption. As AMPA showed a lower effect, except for As, it appears that degradation of glyphosate is preferable for an environmental point of view. These results need to be validated in more realistic conditions.

6. Chapter II. Glyphosate application may influence the transfer of trace elements from soils to both soil solutions and plants

Graphical abstract



6.1. Abstract

Trace elements dynamics in the soil-plant system depends on multiple parameters, including chelating organic compounds from natural or synthetic organic matters. In this study, we evaluated the influence of one of the most common pesticides—glyphosate—on the mobility of trace elements considering contrasted soils (uncontaminated, anthropo-

genically contaminated, and naturally-enriched) in a greenhouse experiment. Four modalities have been tested: one control without any application, two with different glyphosate doses (1 and 3 times the authorised field dose), and one with compost addition to evaluate its potential ability to mitigate the impact of glyphosate on trace element mobility. Both, trace element and glyphosate concentrations were measured in the soil solutions and trace element contents were determined in plants at the end of the experiment. The results showed that, although glyphosate concentrations rapidly decreased in soil solutions, glyphosate application still influenced the transfer of trace elements to both soil solution (up to 12-times higher) and plant (up to 5.2-times higher). This influence was highly dependent on both the specific elements and the type of soils considered. For instance, in uncontaminated soils, glyphosate especially increased the mobilization of Mn, Co, Zn, Mo, and Pb to soil solution. This effect diminished of 2.5 times on average with increasing soil contamination. A similar trend was observed for the transfer of trace elements from soil to plant (i.e., on average 2.2-times lower in the most contaminated compared to the uncontaminated soil). However, in the naturally-enriched soil, opposing trends were noted between soil solutions and plants. The impact of compost addition on the transfer of trace elements to plants remains unclear: compost enhanced the transfer of trace elements to soil solution in uncontaminated and naturally-enriched soils likely due to trace element input through the compost, but decreased the transfer in anthropogenically-contaminated soils likely due to adsorption processes. Therefore, glyphosate could potentially increase the exposure of trace elements through food consumption and their transfer to the ecosystem, particularly in uncontaminated and weakly contaminated soils. In highly contaminated soils, compost could mitigate the glyphosate-induced enhancement of trace element mobility to soil solutions.

6.2. Introduction

Trace elements, whose content is below 1‰ in the Earth's crust, are naturally present in soils. Additional sources, such as direct application of exogenous organic matter or indirect inputs from industrial atmospheric deposition, may contaminate the agricultural soils (Belon et al., 2012). Although some trace elements are essential for plants and animals, all become toxic above a certain threshold, with particular health concerns for Pb, Cd, Hg, or As (Underwood 1977; Arnich et al. 2012). Since human exposure is closely linked to agricultural products and drinking water, both soil–plant transfer and leaching to groundwater are key processes for health risk assessment (Barati et al. 2010; Kabata-Pendias 2011).

As they encompass various chemical families—metals (e.g., Cd, Cu, Zn) and metalloids (e.g., As, Sb)—trace elements behave differently in the environment depending on their speciation and chemical properties (i.e., volatility, solubility, interaction with organic compounds, etc.; Adriano, 2001; Kabata-Pendias, 2011). Moreover, environmental characteristics, such as soil pH, redox, or cation exchange capacity (CEC), are crucial in controlling the transfer of trace elements from one compartment to another. For instance, the presence of organic compounds in soil—whether added (which may have undergone some modification) or natural—can significantly reduce their transfer due to adsorption processes (Hooda 2010; Pathak et al. 2024). However, adding exogenous organic matter may also introduce extra trace elements to soils (e.g., compost, manure, sewage sludge; Baldantoni et al., 2010; Benke et al., 2008; Berti and Jacobs, 1998). This is especially relevant in the context of climate change, as it is increasingly recommended to add organic matter to soils for carbon storage purposes (Minasny et al. 2017). Additionally, dissolved organic matter from decomposition processes may have a chelating effect, generating a complex that is either

more or less soluble than the ion itself, thus either increasing or decreasing trace element mobility (Schmitt et al. 2002; Carrillo-González et al. 2006). Low molecular weight organic compounds can promote trace element solubility, while higher molecular weight compounds are more likely to form insoluble complexes.

Most pesticides are organic compounds, often present as residues in agricultural soils due to their widespread use (Silva et al. 2019; Bhandari et al. 2020). Some—including glyphosate, a widely used herbicide—are known to have chelating abilities (McBride and Kung 1989; Morillo et al. 2002; Barrett and McBride 2006; Divisekara et al. 2018). Glyphosate, along with its main degradation product, aminomethylphosphonic acid (AMPA), has shown a significant effect on the mobility of several trace elements (Barrett and McBride 2006; Si et al. 2013; Bemelmans et al. 2023). This is primarily due to its multiple charged functional groups (Sprankle et al. 1975): phosphonate (pKa 2.6), carboxylic (pKa 5.6), and amino (pKa 10.6). Additionally, pesticides are frequently applied as formulated products, i.e. with co-formulants that improve their efficacy (Leaper and Holloway 2000; Krogh et al. 2003). These co-formulants are known to influence the mobility of trace elements from soil to soil solution, either directly or indirectly (Bemelmans et al. 2023). Unfortunately, data on their chemical composition are unavailable due to commercial confidentiality, hindering the identification of the processes involved (Mesnage et al. 2019).

The presence of such organic compounds from pesticide application may influence the mobility of trace elements from soil to soil solution and plant. For example, Cakmak et al. (2009) suggest that the translocation of certain elements may be reduced when associated with glyphosate. Indeed, organic acids may be unable to cross cell membranes and thus must follow the apoplastic route until they reach the xylem through imperfections in the Casparian band (Perry and Greenway 1973; Häussling et al. 1988; Schaider et al. 2006). These effects on

trace element transfer are particularly concerning in contaminated soils, although glyphosate has a relatively limited additional effect compared to the initial pool of mobile elements (Bemelmans et al. 2023). Furthermore, organic compounds can affect phytohormones, which also play a role in trace element uptake (Dai et al. 2023; Kumar et al. 2024). As this research topic lies at the interface between organic and mineral soil contamination, it has been underexplored, resulting in a knowledge gap. We hypothesise that the application of glyphosate-based pesticides to soil may increase the mobility of trace elements in both soil solution and plant, but this effect should be weaker than those observed in the laboratory with concentrations higher than the doses allowed in the field.

In this study, we aimed to: (1) assess the influence of pre-sowing glyphosate application on the transfer of trace elements from soil to soil solution and plant; (2) compare the behaviour in uncontaminated and contaminated soils; and (3) evaluate the potential of exogenous organic matter to mitigate the glyphosate effect on trace element transfer. We conducted a greenhouse experiment in which a glyphosate-based pesticide was sprayed on four contrasting soils (varying in both contamination levels and types) at the maximum and 3 times the maximum dose authorised in Belgium.

6.3. Materials and methods

6.3.1. Study area

Four agricultural soils from Wallonia, Belgium, were considered in this study to include a trace element contamination gradient as described by Bemelmans et al. (2023): (1) an uncontaminated soil from Vieusart (50.6799°N, 4.6357°E; loamy soil, pH_{H2O} = 6.5, CEC = 11.1 $cmol_c\ kg^{-1}$,

organic carbon content C_{org} = 1.25%); (2) a moderately anthropogenically contaminated soil from Sainte-Walburge (50.6644°N, 5.5887°E; loamy soil, pH_{H2O} = 7.8, CEC = 15.2 $cmol_c\ kg^{-1}$, C_{org} = 11.1%); (3) a highly anthropogenically contaminated soil from Bressoux (50.6374°N, 5.6109°E; loamy soil, pH_{H2O} = 7.0, CEC = 26.9 $cmol_c\ kg^{-1}$, C_{org} = 15.8%); and (4) a naturally-enriched soil from Aubange (49.5765°N, 5.7684°E; sandy clay loamy soil, pH_{H2O} = 7.3, CEC = 20.8 $cmol_c\ kg^{-1}$, C_{org} = 2.43%). Briefly, the soils of Sainte-Walburge and Bressoux (region of Liege) were significantly impacted by industrial activities, leading to atmospheric deposition and coal-extraction debris inputs that caused trace element contamination. The Aubange soil was developed on sedimentary rocks with high natural content of trace elements (e.g., As, Ni, and Cr).

6.3.2. Experimental procedure

On each study site, soil from the top 30 cm was collected with a shovel and stored in 30-L plastic boxes. Soil samples were air dried at 25 °C for 15 days and sieved through a 12-mm mesh. After homogenising, the sieved soil material was slightly rehydrated before being placed in 10-L pots for the experiment. Two weeks after glyphosate application (*Glyfall Plus*® at 360 g L^{-1}, a commercial glyphosate-based pesticide from *Bayer Cropscience*), nine spikelets of spelt (*Triticum spelta*) per pot have been sown to mimic a field seeding density. In each pot, we added 20 g of NPK 9−7−14 fertiliser (i.e., 240 kgN ha^{-1}) to ensure correct plant nutrition. The experiment was conducted in a greenhouse over a period of 107 days (from July 28, 2022 to November 23, 2022), including 93 days from sowing to harvest. Environmental conditions during the experiment were 17.6 to 36.1 °C for air temperature and 25 to 99% for air humidity.

Four modalities were considered in the experiment: (1) a control with no glyphosate application; (2) a single-dose application of glyphosate

(3.6 kg ha^{-1}, i.e., the maximum annual dose authorised in Belgium; Phytoweb 2015; "Max") representing a realistic situation; (3) a triple-dose application of glyphosate ("3Max") to study the effect of a contrasted dose in case of low glyphosate effect; and (4) a triple-dose application of glyphosate with an addition of 245 g of green waste compost (vegetation debris <15 mm, such as grass, dead leaves and branches, from the Basse-Wavre composting platform, Walloon Brabant) mixed in the soil (i.e., 35 t ha^{-1}, "3Max&OM") to evaluate potential influence of exogenous organic matter on trace element mobility. By comparing glyphosate application modalities to the control, we considered any potential glyphosate residues present in the soil material despite no glyphosate was detected in the control modality. Note that glyphosate application is not toxic for plants when it is already present in the soil due to its foliar action. For glyphosate applications, we manually sprayed 2.5 mL (Max) or 7.5 mL (3Max and 3Max&OM) of a 10 g L^{-1} glyphosate solution using a hand sprayer.

Trace elements water extractability in soils and compost have been evaluated by agitating 1 g of 2 mm-sieved soil with 40 mL of milli-Q water for 16 h. The solutions were analysed before filtering at 0.2 µm.

6.3.3. Sampling procedure

During the experiment, 42 soil solution samples (one per pot, except some missing samples) have been collected 26 days after glyphosate spraying each in 50-mL syringes with rhizon samplers (10-cm long hydrophilic polyether sulfone membrane with a porosity of 0.15 µm; 19.21.21 F, Rhizosphere, Wageningen, The Netherlands). Of these samples, 5 had a volume too low for trace element analysis. For trace element concentrations, an aliquot of every solution was acidified (2% HNO3) and stored in polypropylene tubes prior to ICP-MS analysis.

For glyphosate concentrations, a second aliquot was stored at 4 °C (maximum 7 days) before HPLC analysis.

At the end of the experiment, plant samples were collected by isolating both aerial parts (i.e., by cutting at the collar) and root parts (i.e., by removing soil particles and cleaning thoroughly using a soft brush and demineralized water). Plant samples were oven-dried at 50 °C and then weighted and ground (Cyclotec 1093 Sample Mill, Foss A/S, Hillerød, Danemark). Plant masses are available in Annexe 7.

6.3.4. Chemical analyses

Concentrations of 15 trace elements were measured by ICP-MS (iCAP Q ICP–MS, Thermo Fischer Scientific, Waltham, MA, USA) at the Earth and Life Institute analytical platform (MOCA, UCLouvain, Belgium). The SLRS-6 reference material (river water) was used for quality check; recoveries reached 100 ± 20% for all elements, except for Sb (124%), Zn (131%), and Cd and Sn that were >150% (only relative concentrations between modalities were considered). Limits of detection for soil solutions were between 0.001 and 0.01 µg kg^{-1}, except for Ti, Ni and Zn (between 0.01 and 0.1 µg kg^{-1}). Trace element concentrations were determined in both soil and plant (i.e., root and shoot) samples by ICP-MS after a $HF/HNO_3/HCl/H_2O_2$ digestion procedure in a clean room (Arbalestrie et al., 2022). Reference materials (BHVO-2 and IAEA-336) have been used for quality check of mineral and organic matrices, respectively. Recoveries reached 100 ± 20% for all certified elements, except for Sn (149%) in the BHVO-2, and Ba, Cr, Zn (80–70%), Cd, Cu, Pb (70–60%), and Mo (47%) in the IAEA-336. Limits of detection for solid samples were evaluated to <1 ng kg^{-1}, except for Cr, Cu, Mn, Pb, and V (<0.01 µg kg^{-1}), for Ba and Zn (<0.1 µg kg^{-1}), and for Ti (<1 µg kg^{-1}).

Glyphosate and AMPA were analysed in soil solution samples following the Bemelmans et al. (2023) procedure (adapted from ISO 21458 and ISO 116308). Briefly, both molecules were derivatized at pH 9 (borate buffer) by FMOC-Cl in excess before analysis by HPLC (1200 series, Agilent Technologies Santa Clara, CA, USA) using phosphate buffer/acetonitrile:methanol:Milli-Q water as elutant solutions.

6.3.5. Data treatment

Data were analysed using *R 4.0.2*. Results included triplicates, they were compared to the control (i.e., without glyphosate application) and expressed as tested modality/control concentration ratios for soil solution samples and tested modality/control content ratios for plant samples. Welsh t-tests were performed to evaluate the difference between results for the glyphosate application modalities and controls.

6.4. Results and discussions

6.4.1. Influence of glyphosate on trace element transfer to soil solution

In this section, we will consider indifferently the influence of the glyphosate molecule and the glyphosate-based pesticide. As the experiment was based on formulated glyphosate, one should point out that results can over- or under-estimate the actual influence of glyphosate alone, since anther formulation may provide different results (Bemelmans et al., 2023). We first focused on four contrasted elements: Cu as the best documented element to interact with glyphosate; Pb as a well-known toxic element frequently found in Belgian contaminated soils; and As and Mo as oxyanions in natural Eh-pH soil conditions (Figure 6.1, Annexe 7). In the uncontaminated soil (i.e., Vieusart), the soil solutions collected 26 days after glyphosate spraying presented

higher concentrations for the four selected trace elements in the Max modality than in the control: from 1.3 to 12 times, $p < 0.1$ for two of the eight cases (elements/glyphosate modalities, compost excluded). This has already been reported for few elements (e.g., Cu and Pb) under controlled conditions where glyphosate highlighted metal desorption, resulting in an increased leaching (Barrett and McBride, 2006; Divisekara et al., 2018). When considering the 15 elements studied, we observed that 80% of these elements showed a mean Max/control concentration ratio >1.2 (Figure 6.2). This trend confirms the findings made in extractability experiment using the same soils (Bemelmans et al., 2023), supporting that the enhancement effect of glyphosate still occurs in more complex conditions using field-like concentrations and natural percolation throughout the soil. This suggests that in real conditions, glyphosate may enhance exposure to trace elements through leaching into natural waters.

When the pesticide was applied at the highest dose (i.e., 3Max), no difference was observed for As and Cu concentrations in soil solutions compared to the control, unlike Mo and, to a lesser extent, Pb with higher concentrations (up to 7 times, even if no statistically significant differences were observed; Figure 6.1). This contrasting result for chemically similar elements (Pb and Cu as cations and Mo and As as oxyanions; Hooda, 2010) indicates that the charge is not the only factor that drives the element behaviour. Overall, higher glyphosate concentrations (i.e., 3Max compared to Max) have less influence on trace element mobility (73% of the studied elements; Figure 6.1 and Figure 6.2), resulting in an absence of significant differences compared to the control for the 3Max modality (against 5 with $p < 0.1$ for the Max modality), as already observed in Bemelmans et al. (2023). The hypothesis is that, although chelation and complex formation can enhance trace element solubility, glyphosate may be adsorbed onto soil particles due to its multiple charges (i.e., one positive and three negative; Sprankle et

al., 1975), promoting the fixation of cationic elements on the soil. The formation of ternary complexes involving glyphosate, trace elements, and soil particles has already been documented, but primarily in the context of enhancing glyphosate sorption through cationic bridge formation (Morillo et al. 2000; Wang et al. 2006). Glyphosate degradation over time leads to the AMPA formation, resulting to a reduced influence on trace element mobility (Bemelmans et al. 2023).

Similarly to Vieusart, the anthropogenically contaminated soils exhibited an increased influence of glyphosate on the trace element mobility, though the effect was weaker with only three of the 16 cases (elements/glyphosate modalities, compost excluded) with $p < 0.1$ (Cu, Pb, As, and Mo; Figure 6.1 and Figure 6.2). Indeed, in the moderately contaminated soil (i.e., Sainte-Walburge), 73% of the elements studied were more concentrated in soil solutions in the Max modality than in the control with ratios >1.2 (and up to 3.0). Nonetheless, the average of concentration ratios in soil solutions was much lower, reaching 1.6 in Sainte-Walburge vs 3.2 in Vieusart. The highly contaminated soil (i.e., Bressoux), however, presented a lower influence, including negative effect: >1.2-times lower for 6 elements and >1.2-times higher for only 1 element (Figure 6.2). The effect decreased with the increasing anthropogenic contamination level, resulting from the higher pool of mobile elements already present on deposited anthropogenic particles in contaminated soils (Bemelmans et al., 2023). Thus, the relative contribution of glyphosate remained minor, even in conditions more realistic than extraction experiments. Note that the opposite trend was also observed for Cu in the literature (Barrett and McBride 2006), highlighting the influence of the nature of soil considered. In contrast to Vieusart, higher pesticide dose (3Max) had no influence (Sainte-Walburge) or increased the glyphosate effect (Bressoux; on average, 1.7-times higher) on the potential leaching of trace elements in anthropogenically contaminated soils.

A stronger negative effect was observed in the naturally contaminated soil (i.e., Aubange) where statistically significant differences ($p < 0.05$) were observed for three of the eight cases (element/glyphosate modality, compost excluded; Figure 6.1). Furthermore, eight elements showed the Max modality >1.2-times lower compared to the control (up to 4.2 times) vs only three elements >1.2-times higher (up to 1.9 times; Figure 6.2). It is noteworthy that Aubange presented specific properties: (1) trace element less mobile than for the anthropogenically contaminated soils because they are included in the mineral matrix (Vandeuren et al. 2023); and (2) high iron oxide content promoting the sorption of trace elements alone (Suda and Makino 2016) or glyphosate-trace element complexes onto iron oxides, limiting the trace element mobility (Maqueda et al. 2002). This negative effect was even stronger as the applied dose of glyphosate increased (average concentration ratio of 0.48 for 3Max vs 0.88 for Max), highlighting the importance of the dose considered.

In this study, only sprayed volume of similar glyphosate concentration changed between modalities Max and 3Max. Glyphosate concentrations measured in soil solutions decreased rapidly with time, resulting in concentrations mostly below the limit of detection (<0.67 µg L^{-1}). Indeed, AMPA was detected in 56% of samples when glyphosate was applied (up to 5.8 µg L^{-1}), indicating that glyphosate was degraded throughout the experiment. Since AMPA concentrations were higher in the 3Max modality (up to 2.9) than in the Max modality (up to 0.67), we assume that the influence of glyphosate should be higher in the 3Max modality. Note that AMPA may be partly responsible for the observed effects since it has already been shown to increase the extractability of trace element, although with a weaker effect (Bemelmans et al. 2023). We thus demonstrate that glyphosate may influence trace element dynamics from soil to soil solution even with field-like concen-

trations largely lower than concentrations used in previous experimental conditions (Bemelmans et al., 2023; Divisekara et al., 2018). Note that high air temperature and humidity in the greenhouse may accelerate the pesticide degradation compared to field conditions.

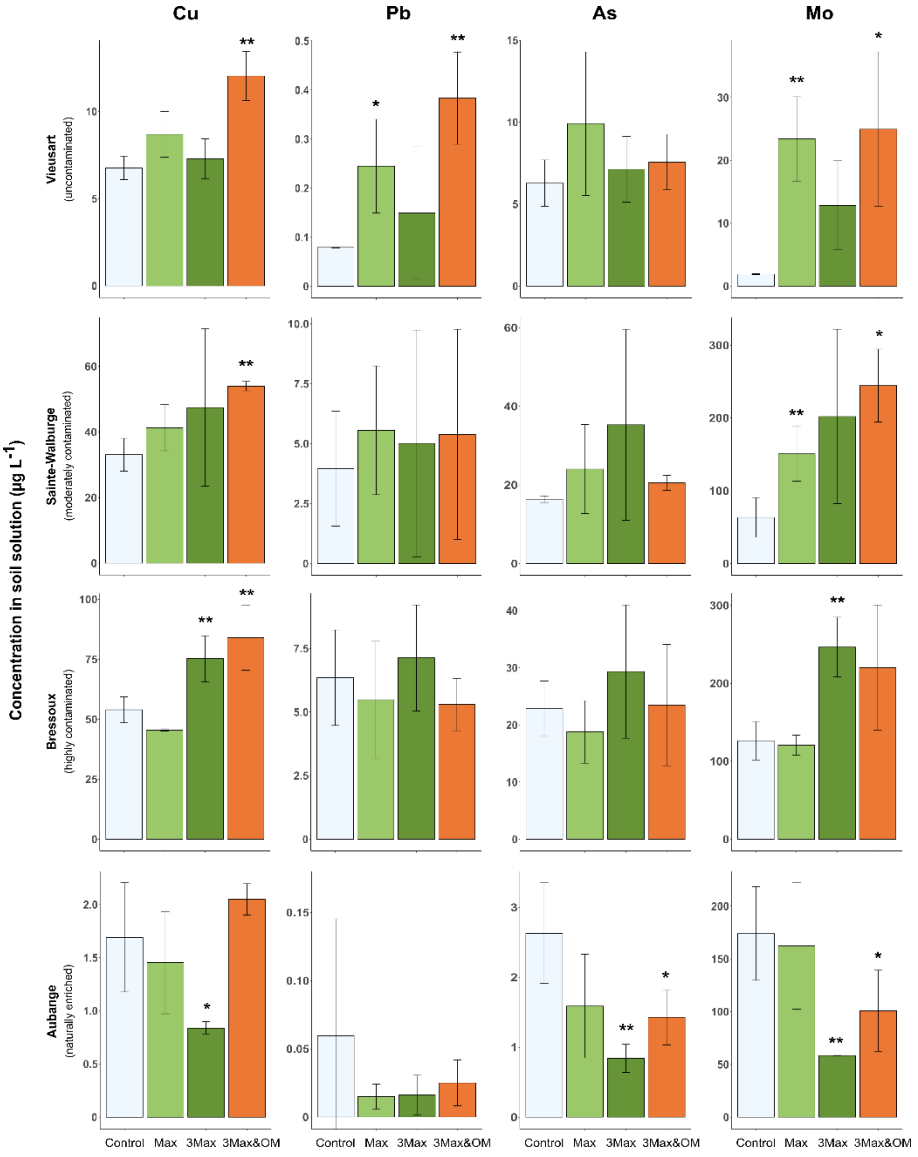


Figure 6.1 Trace element concentrations (n = 3) in soil solutions (26 days after spraying) of the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils considering various modalities: without glyphosate (Control), single dose application of glyphosate (Max), triple dose application of glyphosate (3Max), and triple dose application of glyphosate with an addition of compost (3Max&OM). Scales were adapted for each element. For t-tests with Control, p-value<0.1 = *, p-value<0.05 = ** and p-value<0.01 = ***.

Figure 6.2 Summary of tested modality/control concentration ratios (n = 3) of trace elements in soil solutions of the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils considering various modalities: single dose application of glyphosate (Max), triple dose application of glyphosate (3Max), and triple dose application of glyphosate with an addition of compost (3Max&OM). For t-tests with Control, p-value<0.1 = *, p-value<0.05 = ** and p-value<0.01 = ***. Elements are ranked in the same order as in chapter I.

6.4.2. Influence of glyphosate on trace element transfer to plant

We then studied the influence of glyphosate on trace element transfer to plant. In this section, we only considered plant shoot, as it constitutes the main agronomical interest for Poaceae. The four elements studied for soil solutions have also been selected (Figure 6.3): Cu, Pb, As, and Mo. Results of the uncontaminated soil (i.e., Vieusart) showed that the

glyphosate application (Max modality) slightly increased the transfer of trace elements from soil to plant (from 1.4- to 2.1-times higher than in the control), despite a high heterogeneity between replicates making no significant difference ($p > 0.1$). This increasing availability for plants could result from: (1) a higher mobilisation of trace elements in soil pore water (Bruemmer et al. 1986), as illustrated through soil solutions (Figure 6.1); and/or (2) higher pool of trace elements in the exchangeable fraction (i.e., sorption onto soil exchange capacity without increasing concentration in soil solution). Overall, 67% of the elements studied presented a positive effect (with content ratios >1.2), including both cationic and anionic chemical forms (Figure 6.4). As observed for soil solutions, trace element contents in plant materials were lower in the 3Max modality than in the Max modality ($p < 0.05$ only for Cu), supporting a weaker effect on both soil solution and plant available fraction, and thus the possibility of a non-monotonic effect of pesticides on trace element mobility as a function of concentration.

When increasing the soil contamination level (Vieusart < Sainte-Walburge < Bressoux), we observed a lesser or even negative effect (average Max/control content ratios from 1.7 to 0.8; Figure 6.3 and Figure 6.4). When applying the highest dose (i.e., 3Max), results showed a decrease in content ratios, limiting positive effects in uncontaminated soil and amplifying negative effects in highly contaminated soils. The presence of coal in contaminated soils may promote the sorption of glyphosate-trace element complexes, thanks to the binding ability of both glyphosate and trace elements on coal (Swaine 2013; Ighalo et al. 2021). However, some contrasting trends that occur between soil solution and plant reflect specific biological processes, such as barrier effect of the Casparian band for chemical complexes. This is particularly relevant in contaminated soils, where protective mechanisms are activated to prevent metal saturation in plant (Antoniadis et al. 2017; Mishra et

al. 2020). In addition, this discrepancy may result from the non-representativeness of the soil solution as a bioavailable fraction to plant, but rather an indicator of possible subsequent groundwater contamination (Arbalestrie et al. 2022). Finally, most elements present in the naturally contaminated soil (i.e., Aubange) evidenced a positive effect of glyphosate on plant contents whatever the dose applied (on average, Max/control content ratio of 1.3 and 3Max/control of 1.5), including 47 and 67% of trace elements considered, respectively. Note that the heterogeneity between replicates were still important. Only Cd, Mn, Sb, and Zn were not influenced by glyphosate whatever the dose applied, likely due to their high mobility (e.g., Cd and Zn) that already promotes their leaching.

Contrary to exchanges at the soil-soil solution interface, plant uptake involves slower chemical processes that lead to lower heterogeneity between modalities in plant than in soil solution: plant content therefore constitutes an integrative compartment unlike soil solution. Indeed, element dynamics in the rhizosphere are largely dependent on root and microbial activity (including exudation and siderophores) occurring on a small scale (Neumann and Römheld 1999), such as root acidification by proton extrusion. As low-molecular-weight organic compounds, glyphosate can form stable complexes in solution that could increase plant uptake (such as EDTA, citric acid, oxalic acid, or acetic acid; Ebrahimian and Bybordi, 2014; Qiao et al., 2020; Schmidt, 2003; Vassil et al., 1998). Yet, under a pH of 5.6, glyphosate loses one of its negative charges potentially decreasing its ability to interact with cations (Sprankle et al. 1975), which could explain the trend observed. These local acidic conditions were probably less pronounced in the naturally-enriched soil, which had a higher pH. These complexes can thus enter the plant through imperfections in the Casparian band (Schaider et al. 2006). Note that the plant uptake of glyphosate can also lead to metabolism disruption or flux saturation, resulting in variable trace element

contents in plant (Schmidt 2003; Prasad et al. 2006; Degryse et al. 2009). The complexity of those soil–plant transfer can explain some contradictory trends between plant and soil solution, as in Aubange.

In Europe, only two of the trace elements have a legally controlled content in cereals, Pb and Cd, with a maximum authorised content of 0.2 mg kg^{-1} of fresh material (EU 2024). Considering ca. 80% of water content by mass for Poaceae (Glenn 1987), shoot contents (no data for grains) exceeded in Bressoux (Cd and Pb, control modality) and Sainte-Walburge (Cd, Max modality). Note that these results were based on a single application although several pesticides are applied in several doses and a variety of pesticides is applied through the cultural period (Yang et al. 2024).

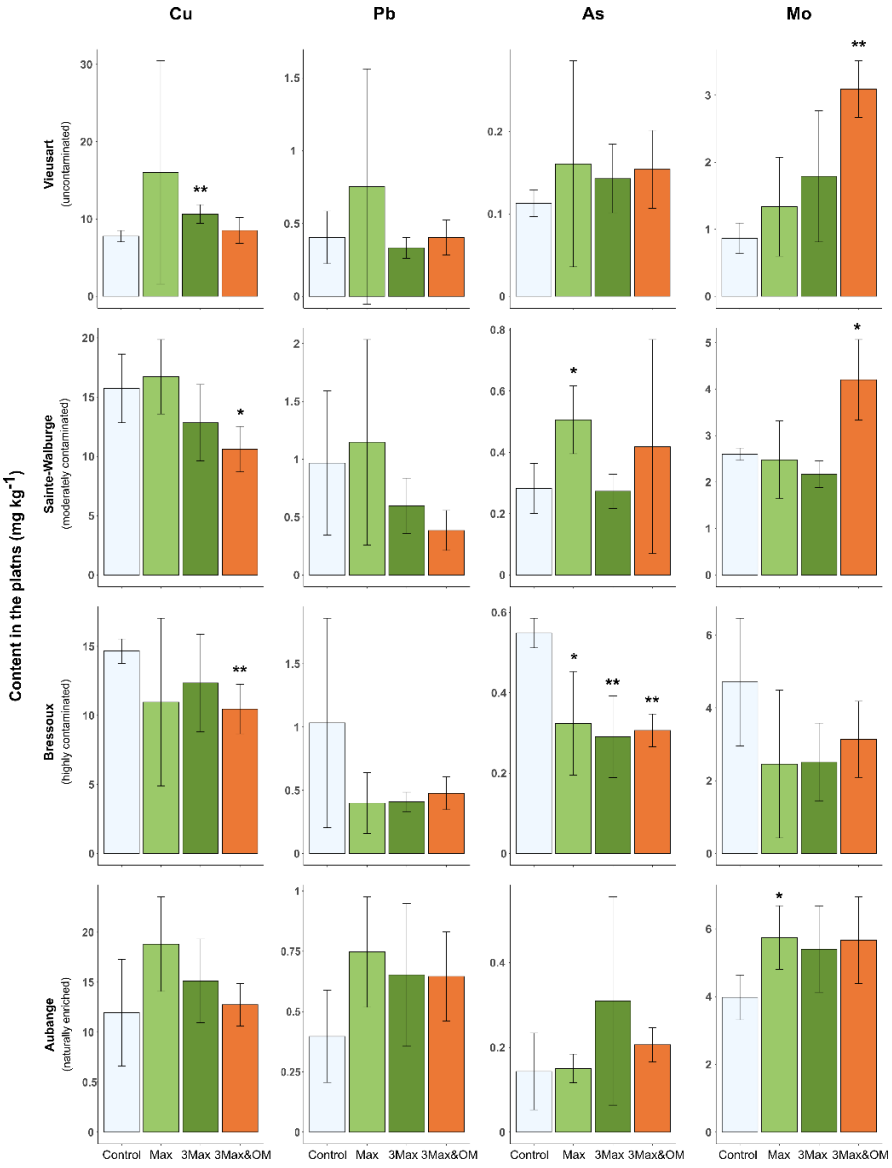


Figure 6.3 Trace element contents (n = 3) in plants (shoot of spelt) that grown in the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils considering various modalities: without glyphosate (Control), single dose application of glyphosate (Max), triple dose application of glyphosate (3Max), and triple dose application of glyphosate with an addition of compost (3Max&OM). Scales were adapted for each element. For t-tests with Control, p-value<0.1 = *, p-value<0.05 = ** and p-value<0.01 = ***.

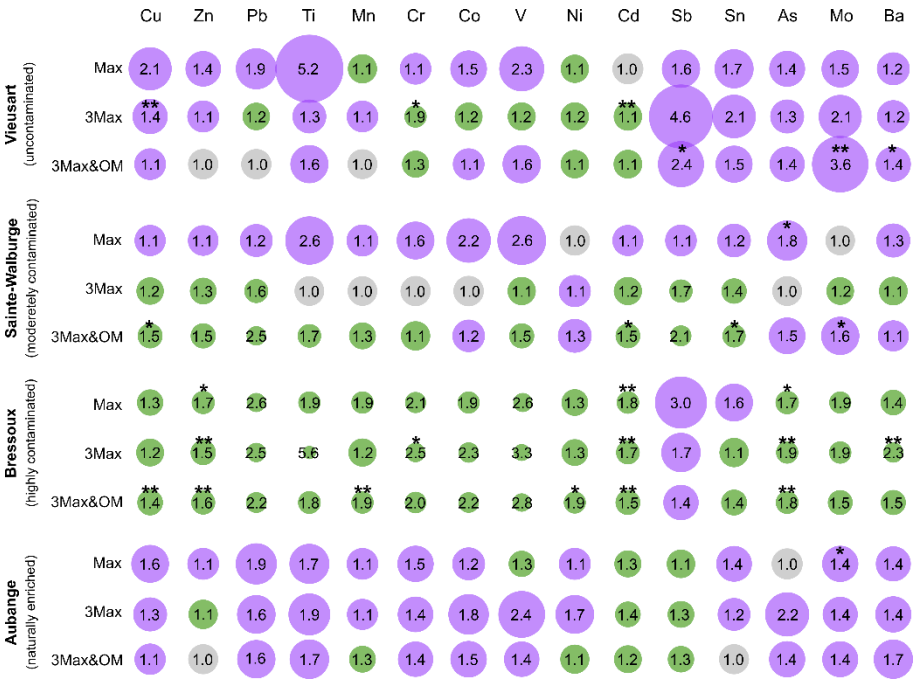


Figure 6.4 Summary of tested modality/control content ratios (n = 3) of trace elements in plants (shoot of spelt) of the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils considering various modalities: single dose application of glyphosate (Max), triple dose application of glyphosate (3Max), and triple dose application of glyphosate with an addition of compost (3Max&OM). Elements are ranked in the same order as in chapter I. For t-tests with Control, p-value<0.1 = *, p-value<0.05 = ** and p-value<0.01 = ***.

6.4.3. Role of organic matter in controlling the glyphosate influence

We finally tested the influence of exogenous organic matter (i.e., compost) input on the glyphosate effect previously evidenced on soil–water and soil–plant transfers. Considering both soils with low trace element mobility (i.e., the uncontaminated and the naturally-enriched soils), we observed overall higher concentrations in the soil solutions with compost addition (Figure 6.1 and Figure 6.2): on average,

3Max&OM/3Max content ratios of 1.8 and 1.7 for Vieusart and Aubange, respectively, including 73 and 93% of trace elements considered for which the ratios were >1.2, respectively. This was mainly observed for Mn (up to 6.5 in Vieusart), Pb, Sn, Cu, Cd, and Ni, including elements particularly toxic. Zinc, however, showed an opposite pattern with a content 2.7-times lower with compost addition, likely resulting from sorption by organic compounds. These observations may result from either (1) an additional pool of trace elements released by the compost itself or (2) soil elements mobilised by low molecular weight organic compounds from the compost via chelating properties (Vaughan et al. 1993; Ahmad and Prasad 2012). Considering the water-extractability data, we estimated the compost contribution of the exchangeable pool between 0.2% (for Sn) and 42% (Mo) in Vieusart (<15% for 73% of the elements). In Aubange, only two elements presented a relative contribution >15% (Zn and Mo with 24 and 27%, respectively), while in the anthropogenically contaminated soils, the contribution was always <12%. We thus hypothesize that the exogenous organic matter can promote the trace element mobilisation, as for most elements their increased concentration is higher than its input. However, the transfer from soil to plant showed more divergent results (Figure 6.3 and Figure 6.4). Indeed, six elements presented 3Max&OM/3Max content ratios >1.2 (V, Mo, Cr, Co, Ti, and Pb) in Vieusart, while Sb, Sn, and Cu were less concentrated with compost addition (>1.2-times lower). Note that only negative effect was observed for five trace elements in the naturally-enriched soil (Ni, V, As, Mn, and Co). We thus consider that the compost can both adsorb or mobilise trace elements. Besides, a possible interaction may exist between organic matter and pesticides: an increased sorption of glyphosate in presence of organic matter could also favour sorption of trace elements-glyphosate complexes (Albers et al. 2009; Ololade et al. 2014; Erban et al. 2018). Since some results were inconsistent between plant and soil solution, we assume that complexes

formed by mobile organic compounds are not always bioavailable for plant.

In the anthropogenically contaminated soils (i.e., Sainte-Walburge and Bressoux), elements were mainly less concentrated in the soil solution with compost addition (between 40 and 47% of elements with a negative effect, respectively, vs only 13% with a positive effect; Figure 6.1 and Figure 6.2). Cobalt, Zn, Ni, and As showed negative effect for both soils, up to 3.9-times lower for Co in Bressoux. Results showed contrasted trends for transfer to plant, including 47% of negative effect in Sainte-Walburge (vs 13% of positive effect), while Bressoux presented 27% for both positive and negative effects (Figure 6.3 and Figure 6.4). Compared to the uncontaminated soil, the addition of organic matter may partially decrease the trace element mobility in the anthropogenically contaminated soil by promoting the sorption effect over their release, which can to a certain extent counteract the effect of glyphosate in the soil solution.

6.4.4. Environmental fate of trace elements after a glyphosate application

Using a greenhouse experiment, glyphosate application has shown to influence the transfer of trace elements from soil to soil solution and plant. Here, we compare the two transfer pathways to assess their possible environmental fate and consequences, both in terms of hydrosystem and food contamination. We thus jointly considered the element concentration ratios between the tested modalities and the control in both soil solution (solubility) and plant (bioavailability; Figure 6.5). For the uncontaminated soil (i.e., Vieusart; Figure 6.5a), 53% of data points (glyphosate application, whatever the application dose) were in the top

right corner, indicating a positive effect on the transfer to both soil solution and plant. The higher solubility may result in a greater element pool in the exchangeable fraction, and thus a higher bioavailability. The main direct risk in uncontaminated soils therefore lies in increased transfer to the plant, although the initial trace element contents are lower. This is especially concerning as plants are especially critical for human ingestion of trace elements (Antoniadis et al. 2017; Natasha et al. 2022). Indeed, contamination of hydrosystem requires crossing the barrier of deeper soil horizons before expecting to affect human health (sampling only performed on the top 10 cm of the soil).

With the increasing anthropogenic contamination level (i.e., Vieusart < Sainte-Walburge < Bressoux), however, we first observed a general decrease of the bioavailability influence, leading to antagonistic effects depending on the contamination status considered (negative effect in contaminated soil versus positive effect in uncontaminated soil; Figure 6.5b-c). Since the evolution on the bioavailability axis was more sensitive than that on the solubility axis, we assume that the risk will mainly be observed in natural waters. At high level of contamination, however, the effects on the soil solution are weaker, ultimately reducing the transfer of trace elements into the environment at the expense of their retention in the soil. Conversely, the behaviour observed in the naturally-enriched soil (i.e., Aubange) indicated a different trend for most elements, with decreasing the solubility effect (up to negative effect) while bioavailability remained unchanged compared to the uncontaminated soil (Figure 6.5d). This likely results from the affinity of the complexes formed with glyphosate and the mineral constituents of the soil, including iron oxides for this specific soil (Maqueda et al. 2002), limiting their solubility without impacting the more efficient root uptake (specific environmental pH and redox condition in the rhizosphere). This results in the same environmental risk as described in the uncontaminated soil without hydrosystem contamination due to higher soil retention. For

both plant and soil solution, the effect of glyphosate on the element mobility, is important and mainly depends on the soil considered (including the contamination type), while the geochemistry of each individual element remains a relatively minor influencing factor.

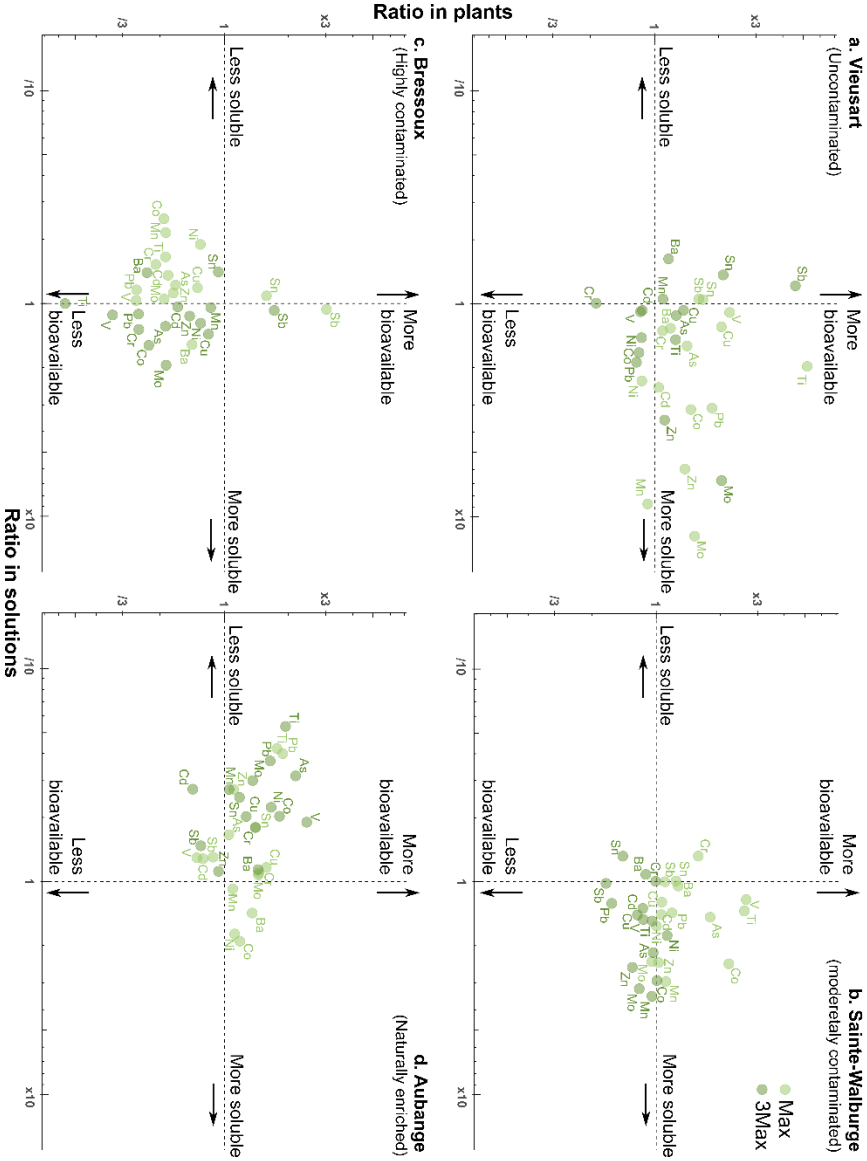


Figure 6.5 Influence of glyphosate application on the environmental transfer of 15 trace elements considered (i.e., tested modality/control concentration ratios in soil solutions and tested modality/control content ratios in plants) in the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils considering various modalities: single dose application of glyphosate (Max, light green) and triple dose application of glyphosate (3Max, dark green).

6.5. Conclusion

Trace elements are a significant concern in agricultural soils worldwide, and given the widespread use of pesticides, their role could be crucial in the dynamics of trace elements in the soil–plant system and human exposure. Firstly, this study demonstrated that glyphosate-based pesticides could increase trace elements transfer in uncontaminated soil and decrease it in highly anthropogenically contaminated soils, likely due to the initially higher pool of mobile elements (Figure 6.6). Pesticide use could thus influence human exposure to harmful trace elements, but specifically in uncontaminated environments. Secondly, distinct trends were generally observed between soil solution and associated plant, particularly in contaminated environments. This requires all possible risks to be taken into account, both in foodstuffs and drinking water depending on the contamination levels. Thirdly, a non-monotonic relationship between added dose and trace element content was observed, highlighting the competition between solubilization and adsorption processes. Finally, compost addition primarily influenced soil solution concentrations and was not effective method for counteracting glyphosate-induced increases in trace element transfer in uncontaminated soils, as it tended to reinforce this effect. However, it may provide some mitigation in contaminated soils. Given the diversity of pesticides used in agriculture, there remains a knowledge gap regarding their combined

effects on trace element dynamics. Also, multiple pesticides application could cumulatively affect trace element mobility and transfer to plant throughout the growing season. Due to the experimental timing, we did not analyse the edible plant parts that constitutes the main organs to consider for a risk assessment. We thus recommend continuing research in this topic, but under more realistic conditions to better assess the associated risks.

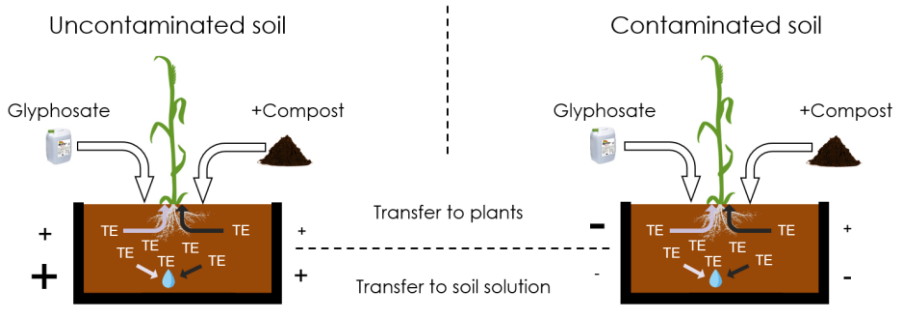


Figure 6.6 Summary of the influence of glyphosate and compost addition on trace element (TE) transfer to soil solution and plants.

7. Chapter III. Combined effects of glyphosate and tebuconazole on trace element extractability from soil to solution

7.1. Abstract

Trace elements, though present at low concentrations in the environment, may reach higher levels in agricultural soils. Then, they can pose, due to their toxicity, significant risks to human and environmental health if their mobility and bioavailability are altered. It is known that trace elements can interact with various organic compounds in soils such as organic amendments but also pesticides of which the use is widespread. Understanding how these substances influence, not only alone but also together, trace element dynamics is crucial for effective soil management and contamination control. The objectives of this research were to investigate, first, how tebuconazole influences the mobility of trace elements in both uncontaminated and contaminated soils, and second, to explore the interactive effects of this substance with glyphosate. Extractability experiments were conducted to assess the potential mobilization of trace elements under varying conditions. The results revealed that tebuconazole significantly influences trace element mobility (Mn increased up to 13 times and Pb up to 11 times), with tebuconazole generally affecting extractability even more than glyphosate. It mainly increased the mobility of the most mobile elements but decreased it for the less mobile ones, while for the intermediate elements the soil was of crucial importance. Moreover, increasing anthropogenic contamination level has been shown to decrease tebuconazole's ability to influence trace element dynamics. This study also

shows that the effects of combining these substances were predominantly additive or less-than-additive, though some synergistic interactions were noted, especially under limiting factors such as important concentration decreases.

7.2. Introduction

Trace elements, defined by their low content in natural environment (<1‰), are potentially harmful to human and environmental health (Adriano 2001). Indeed, despite some essential elements for living organisms (such as Cu or Zn), all are toxic at high doses and can cause a wide variety of diseases (Underwood 1977; Arnich et al. 2012). Agricultural soils may present high contents of these elements, with the associated risk depending on their mobility, i.e., their ability to move from soil to soil solution and to plant (Kabata-Pendias 2004). The mobility of trace elements is a function of their origin (Sungur et al. 2014): natural from parent material alteration vs anthropogenic from both atmospheric deposition (e.g., originated from surrounding industry) and direct input (e.g., manure, sludge, compost, or eventually industrial debris; Bañuelos and Ajwa, 1999). Indeed, trace elements included in minerals are generally less mobile, while manmade materials present potentially more mobile trace elements because of their sorption to surfaces (Sungur et al. 2014). This mobility is also influenced by the environmental conditions, such as soil pH, redox, cation exchange capacity, drainage, as well as the speciation of individual trace elements (mostly divalent cations in common soil pH and redox, except, mainly, As and Mo as oxyanions).

Trace element mobility is also influenced by the formation of organometallic complexes (Violante et al. 2005; Clemente et al. 2010; Ebrahimian and Bybordi 2014). Indeed, complexes can either increase or decrease trace element transfer from soil to soil solution and to plants

depending on their solubility or their capacity to be sorbed on soil particles. The presence of solid or dissolved organic compounds can also modify this mobility. For instance, phytoremediation applications use specific organic compounds, such as citric acid or EDTA, to increase trace element uptake by plants (Meers et al. 2005; Schaider et al. 2006; Ebrahimian and Bybordi 2014). However, other organic substances widely present in agricultural soils, such as organic pesticides, are also able to form complexes with trace elements (Borggaard et al. 2019).

Indeed, both active substances (e.g., glyphosate) and metabolites (e.g., aminomethylphosphonic acid, AMPA, the most common glyphosate metabolite) may modify trace element dynamics, particularly between soil and soil solution, as well as between soil and plant (Cakmak et al. 2009; Bemelmans et al. 2024). Moreover, these molecules are frequently found in agricultural soils (Silva et al. 2019), questioning on the importance on the pesticide-induced transfer in these environments. In addition, there is a discrepancy between the wide range of pesticides used and found in soils (e.g., nearly 450 active substances, safeners, and synergist approved in the European Union; EC, 2024) and the research carried out on interactions with trace elements (i.e., mainly focused on glyphosate, only limited information for other molecules). For instance, tebuconazole is one of these other molecules that complex trace elements, as observed for Cu, but little is known about its ability to influence trace elements mobility (Evans et al. 2007; Čadková et al. 2013; Li et al. 2017a). Another challenge is that commercial products also include substances—co-formulants—to improve their pesticide action. Formulated pesticides show a different influence on trace elements than active substances alone (Bemelmans et al. 2023). If knowledge on the effect of pesticides on trace elements is scarce, knowledge about interactions between multiple active substances and trace elements that may lead to competitive or synergistic effects is, to our knowledge, absent

from the scientific literature. This is especially true when including co-formulants.

In this study, we aimed to investigate the joined influence of glyphosate and tebuconazole on trace elements mobility in soil to evaluate whether this effect is additive or synergistic. Therefore, we compared extractability capacity of pesticides alone (glyphosate and tebuconazole) and a mixture of pesticides in an extraction experiment using four soils with increasing contamination level.

7.3. Materials and methods

7.3.1. Study area

Four agricultural soils were collected in Belgium following a contamination gradient (Bemelmans et al. 2023). The uncontaminated soil was located in Vieusart (50.6799°N; 4.6357°E), slightly acidic (pH_{H2O} = 6.5), with low organic carbon content (1.25%) and a CEC of 11.1 $cmol_c\ kg^{-1}$. The second one was moderately contaminated by human activities, from Sainte-Walburge (50.6644°N; 5.5887°E), characterised by a higher soil pH (pH_{H2O} = 7.8), organic carbon content (11.1%), and CEC (15.2 $cmol_c\ kg^{-1}$). The third one was highly contaminated by human activities from Bressoux (50.6374°N; 5.6109°E), characterised by a soil pH_{H2O} of 7.0, a higher organic carbon content (15.8%), and a CEC of 26.9 $cmol_c\ kg^{-1}$. Finally, a naturally-enriched soil has been collected in Aubange (49.5765°N; 5.7684°E) and was characterised by a pH_{H2O} of 7.3, an organic carbon content of 2.43%, and a CEC of 20.8 $cmol_c\ kg^{-1}$.

7.3.2. Soil sampling and preparation

Thirty litres of the top 30-cm soil layer were collected on an approximative surface of 4 square-meters using a shovel on every study site. All samples were air-dried at 25 °C for 15 days before sieving through a 2-mm mesh. A subsample was crushed with a soil grinder (Vibratory Disc Mill RS 200, Retsch, Haan, Germany) for trace element and organic carbon content analyses. CEC and soil pH_{H2O} were measured on the 2-mm sieved subsample.

7.3.3. Experimental procedure

The implemented experiment followed the protocol of Bemelmans et al. (2023) of which results were extracted to also serve as reference here. Briefly, 5 g of soil sample were added in a 50-mL propylene centrifugation tubes and mixed with 25 mL of extracting solution (1:5 ratio). Tubes were agitated in the dark for 2 h at 150 bpm. Tubes were then centrifuged for 25 min at 4000 rpm and filtered with a 0.45-µm pore size nylon *Acrodisc*®. One sub-sample was acidified at 2% HNO_3 before trace element quantification, while another sub-sample was used for pH measurement and pesticide quantification.

Several extracting solutions were tested using Milli-Q water for their preparation: (1) unformulated tebuconazole (*Sigma Aldrich*) at a concentration of 10 mg L^{-1} ($Tb_{10,}$ equivalent to 32.5 µmol L^{-1}); (2) formulated tebuconazole (*Tebusip*, *Sipcam Oxon*) at three distinct concentrations (2 mg L^{-1} for TbF_2, 10 mg L^{-1} for TbF_{10}, and 100 mg L^{-1} for TbF_{100}, equivalent to 6.5, 32.5, and 325 µmol L^{-1}, respectively); and (3) a mix of unformulated tebuconazole and glyphosate (concentrations of 10 and 100 mg L^{-1}, respectively), $Tb_{10}Gly_{100}$. Our pesticide concentrations were selected according to their concentrations in the application solutions and soil solutions. Higher unformulated tebuconazole concentrations were not possible because of its low water solubility. Also, the

concentration ratio between tebuconazole and glyphosate respected the ratio of pesticide application, as the Belgian regulation fixed maximal yearly dose of tebuconazole of the order of ten times lower compared to glyphosate. All experiments have been performed in triplicates.

7.3.4. Chemical analyses

Trace elements in soil samples were quantified after a four-step digestion (HNO_3/HF, H_2O_2, HNO_3/HCl, and HNO_3) in a ISO 6 cleanroom (Arbalestrie et al. 2022). Trace element (As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, and Zn) quantification in soil and solution samples were performed using a ICP-MS (*iCAP Q ICP–MS*, Thermo Fischer Scientific, Waltham, MA, USA) using internal standards (Ru, In, Re). Quality was checked using the *Certipur* multi element standard (Sigma Aldrich) and the HM-10 (ICP-Forest reference material) for liquid samples. Deviations from certified values were inferior to 10% for all elements except for Zn (19% for *Certipur* and 13% for HM-10). Each element was measured at a concentration above the quantification limit.

Glyphosate and AMPA have been measured by HPLC (*1200 series*, Agilent Technologies Santa Clara, CA, USA) using a Merck Lichrocart C18 150 × 4.6 mm–5 μm column kept at 35 °C, after dilution to reach the range of the analytical method (1 to 20 μg L^{-1}). Samples were derivatized by an excess of FMOC-Cl at pH 9 (borate buffer). Elutants were pH 7 10 mM phosphate buffer (A) and 45:45:10 acetonitrile:methanol:Milli-Q water solution (B). Elution was performed at a flow rate of 1 mL min−1 using the following gradient (expressed as solvent B): from 0 to 1 min, 25% B (initial composition); from 10 to 15 min, 100% B; from 17 to 22 min, 25% B. The detection was performed by a fluorescent detector with an excitation at 260 nm and an emission at 310 nm

7.3.5. Data processing

Trace element concentrations in the extraction solution were expressed as soil extractability (i.e., masse of extracted element by mass of soil). Statistical analyses were performed using *R 4.0.2* and *RStudio 2023.09.1*.

To evaluate both glyphosate-tebuconazole interactions, theoretical additive values have been calculated for trace elements. The theoretical concentration of the trace element was defined by the following equation:

$$Me_{th} = Me_{control} + \left(Me_{Gly100} - Me_{control}\right) + \left(Me_{Tb10} - Me_{control}\right)$$

Me: extractability of the element (usually a metal)

7.4. Results and discussion

7.4.1. Influence of tebuconazole on trace elements mobility in soil

Uncontaminated soil

We first investigated the influence of tebuconazole on trace element mobility in the uncontaminated soil (i.e., Vieusart; Figure 7.1). Ten out of the 15 elements considered (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Sn, and V) were more extracted in presence of tebuconazole than in the control, whatever the concentration and formulation considered (Tb_{10}, TbF_2, TbF_{10}, TbF_{100}). Two elements (Ti and Cr) showed influence only for the TbF_{10} modality, despite an important inter-replicate variability. The elements that were the most affected by tebuconazole were Mn (up to 13 times more concentrated) and Pb (up to 11 times). Interestingly, these elements were not those that are well-documented to form complexes with tebuconazole (e.g., Cd, Co, Cu, Ni, Zn; Evans et al., 2007; Li et al., 2017; Norková et al., 2012). Indeed, the extractability of Ni

was among the least influenced elements by tebuconazole (ratio up to 1.6), showing that complex formation observed in the literature does not predict its influence on the element fate. This confirms the risk of increased trace element release following a tebuconazole application, and particularly for highly concerning elements like Cd and Pb. Another possible cause of trace element release is the competition between these elements and tebuconazole on sorption sites (Redman et al. 2002).

Conversely, three elements (Ba, Mo, and Zn) had their concentration decreased compared to the control (ratio down to 0.22 for Mo). There is a possibility that they take places left vacant on the soil particle surfaces by the desorption of the other elements. Interestingly, some of these were also reported to form complexes with tebuconazole, such as Zn (up to 2.2 times less concentrated). These results are quite different from those following glyphosate application as, except for Ba, no element was less extracted in presence of glyphosate in the same soil. Additionally, the elements that were especially more extracted differ.

Since pesticides are applied with co-formulants, we investigated formulated modalities. Results showed that formulation played little role for all elements (except for Ti), including <30% of relative difference in concentration between formulated and unformulated modalities. For nine elements (Ba, Cd, Co, Cu, Mn, Pb, Sb, V, Zn), the concentration in solution was lower when formulated tebuconazole was used. It should be noted that the concentration of tebuconazole that remained in solution after the extraction was not measured, and thus it is not possible to know the influence of co-formulants on the tebuconazole sorption, and its possible complex formation, as mentioned for glyphosate by Bemelmans et al. (2023). One cannot, however, suppose that all tebuconazole-based pesticides would interact similarly with trace elements. For instance, 30 commercial pesticides containing tebuconazole are currently authorized in Belgium (Phytoweb 2024).

Soil pesticide concentrations changing with time due to degradation and transfer processes, we compared the influence of different tebuconazole concentrations. General trends regarding elements, however, do not allow to conclude for an influence of the dose of tebuconazole across the studied range (2 to 100 mg L^{-1}). This is because, except for Cu and Sb, the concentration of trace elements in solution were not proportional to tebuconazole concentration (modalities TbF_2, TbF_{10}, TbF_{100}). In addition, for Cu and Sb, differences were extremely small (ratios ranging from 1.1 to 1.2 for Sb). This could be attributed to a saturation of the effect of tebuconazole upon the studied tebuconazole concentration range.

Anthropogenically contaminated soils

In the moderately contaminated soil (i.e., Sainte-Walburge), seven elements (Ba, Cd, Co, Cu, Mn, Pb, and Zn) were always more extracted in presence of tebuconazole than in the control, while five elements (Cr, Mo, Sb, Sn, and Ti) were always less extracted in presence of tebuconazole (Figure 7.1). In the highly contaminated soil (i.e., Bressoux), almost the same elements were more extracted (including As and excluding Ba) and less extracted (including Ba and excluding Mo) with tebuconazole. It is worth noting that the number of elements showing either an increase or decrease in concentration in the post-extraction solution was similar for both contaminated soils. However, the average concentration increase (relative to the control) for each affected element was higher in the moderately contaminated soil (2.4 times more concentrated) than in the highly contaminated soil (1.8 times more concentrated).For the elements that were less mobile in presence of tebuconazole, the same is observed as there was more concentration decrease in the moderately contaminated soil (1.8 times less concentrated) compared to the highly contaminated one (1.6 times les concentrated), the difference is however less obvious. Note that the mean concentration

increase in the uncontaminated soil was of 3.5 times and the mean decrease was of 1.9 times; this suggests a decreased effect of pesticide on trace element extractability with increasing anthropogenic contamination levels. This can be attributed to the higher mobility of trace elements in anthropogenically contaminated soils (Sungur et al. 2014). Consequently, the relative contribution of pesticide to the global trace element release becomes less important. Similar results were observed for glyphosate in Bemelmans et al., (2023).

In contaminated soils, the formulation generally decreased the influence of tebuconazole, for ten elements in Sainte-Walburge (difference >0.2 between Tb_{10} and TbF_{10}) and for five elements in Bressoux. This is consistent with the overall lower effect of tebuconazole in these soils. Thus, even if formulation seems to decrease the chelation ability of tebuconazole in contaminated soil, this also decreased with the increasing contamination level (Figure 7.1).

Finally, the effect of concentration was unclear. In Sainte-Walburge, Ba, Cd, Co, Mn, Pb, and Zn were the only elements with differences >0.2 between TbF_2 and TbF_{10} regarding their ratios to the control: all indicated a reduced extraction efficiency when the tebuconazole concentration increased. In Bressoux, the same elements exceeded a difference of 0.2 (except for Ba), showing lower extraction with increasing tebuconazole concentration. As observed for the formulation, lower differences in the highly contaminated soil can be explained by overall lower effects of tebuconazole. This likely results from a saturation of the effect across the concentration range considered, highlighting the need to study lower concentrations.

Naturally-enriched soil

In the naturally-enriched soil (i.e., Aubange), six elements were always more extracted with tebuconazole (Ba, Cd, Co, Cu, Mn, and Pb), six

less extracted (As, Cr, Mo, Ni, Ti, and V; Figure 7.1). Average concentration/control ratios were the lowest in Aubange compared to the other soils. This is indicating that the soil did not only influence the magnitude of the tebuconazole effect, but can also invert it depending on the element, as already observed for glyphosate (Bemelmans et al. 2023) where ratios were also the lowest in Aubange. It could be associated with the high iron oxides content in this soil, as they can sorb both ions and organic compounds (Korshin et al. 1997; Suda and Makino 2016). If this means that tebuconazole spraying could be less hazardous in this soil, it could still increase exposure to harmful elements.

Finally, the formulation played a minor role in the extractability of trace elements in Aubange as only five elements (Cd, Cu, Pb, Sn, and Zn) showed a difference >0.2 between Tb_{10} and TbF_{10} modalities (up to 0.34 for Cd). For all these 5 elements, the use of the formulated pesticide led to a decreased element concentration in the post extraction solution (i.e., less positive effect). The role of concentration among the studied range was also low in Aubange, as only four elements show a difference >0.2 between the TbF_{10} and TbF_2 modalities. For Ba, Mn, and Pb, concentrations in solutions were more important with the lowest tebuconazole concentration (i.e., higher increase).

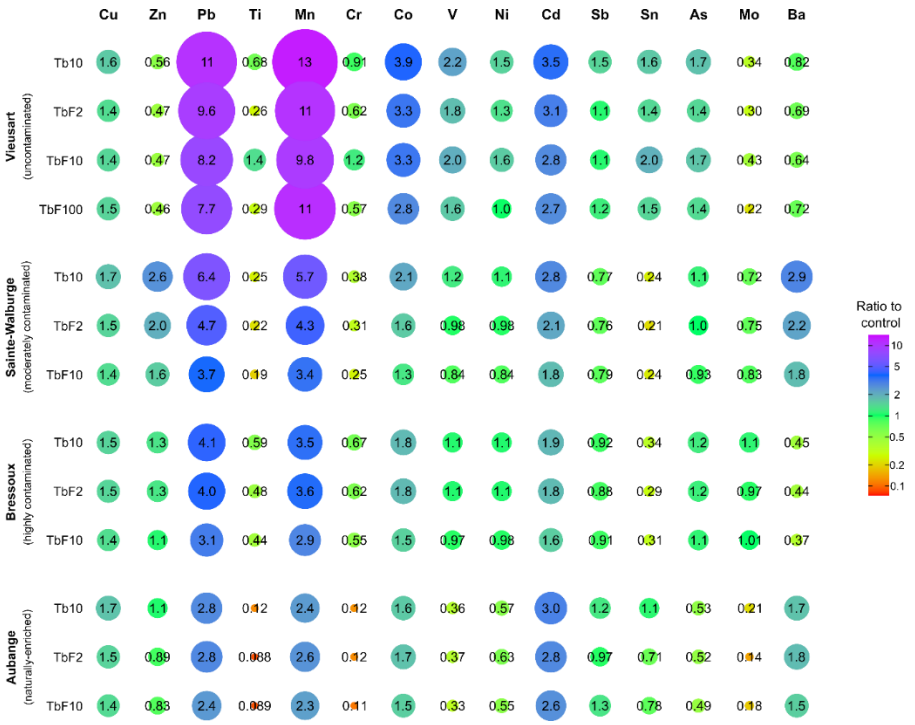


Figure 7.1 Summary of tested modality/control extractability ratios (n = 3) for each studied element in the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils. Colours and size of the circles represent the range of positive or negative influence of modalities in comparison to the control. Elements are ranked similarly as the first data chapter.

Influence of tebuconazole addition across elements

Now that the whole set of elements-soil-modality triads has been presented, we grouped elements with similar behaviours. A first group (Cd, Co, Mn, and Pb) includes elements that were always more extracted in the presence of tebuconazole than in the control. BCR sequential extraction showed that they were among the most mobile elements (i.e., higher proportion of exchangeable or reducible fraction; Annexe 9). Though among the most mobile elements, there are important differences among their speciation in the solid phase. For instance, Cd

reached up to 26% for the exchangeable fraction (and up to 64% for the reducible fraction) while <2% of Pb was in the exchangeable fraction (and 74% in the reducible fraction). This suggests that not only the exchangeable fraction is mobilized during the extraction experiment, the reducible fraction could also play a role by being partially mobilized. Indeed, it is already known that chelation is able to promote the dissolution of oxides (especially for low- crystallinity oxides; Borggaard, 1991; Chang and Matijević, 1983; Nowack and Sigg, 1997). The pH is also known to be a factor influencing the release of exchangeable trace elements and the dissolution of mineral phases. The pH ranged from 5.9 to 6.5 for tebuconazole modalities, which is lower than those observed for glyphosate extractions. However, decrease in pH was not correlated with the effect intensity and thus cannot explain the reduction in element mobility.

A second group of elements (Cr and Ti) showed a clear decreasing trend in element concentration with tebuconazole. These elements, however, were among the less mobile according to the BCR extraction. A third group of elements (As, Ni, and to a lesser extent V) was moderately influenced by tebuconazole in all soils (except in Aubange with a low mobility). Finally, a last group gathered Cu and Sb, with a lower effect of tebuconazole. Other elements (Ba, Mo, Sn, or Zn) have more individual behaviours: they are neither among the most, nor the less mobiles (cf. BCR extraction; Annexe 9). To a lesser extent, the same trend was also observed for glyphosate that showed higher effects for the most mobile elements, which seems contradictory with the decreased effect with increasing anthropogenic contamination level (Bemelmans et al. 2023). It shows that mobility due to the mineralization and mobility due to the geochemistry of elements have not the same influence. The presence of particularly toxic elements, like Cd and Pb, which were the most

mobilized by tebuconazole is especially concerning for human and environmental health, especially since tebuconazole is sprayed on crops during plant growth.

The PCA performed on trace element concentrations in solutions also reflects results observed before (Figure 7.2). The first component (explaining between 45.6% and 76.3% of the data variance depending on the soil considered) highlighted the influence of tebuconazole (all tebuconazole modalities) separated from the control. Only few differences between the different tebuconazole modalities were observed. On the variables projection, elements that were less extracted in presence of tebuconazole for most samples (Ba, Cr, Mo, Sn, Ti) mainly pointed in the direction corresponding to the control modality. Barium was however pointing toward the tebuconazole side, in both Sainte-Walburge and Aubange, in coherence with the ratios observed in Figure 7.1 (increased concentration in presence of tebuconazole). The elements that were clearly detaching themselves as strongly more extractable in presence of tebuconazole, being mainly Mn and Pb, but also Cd and Co systematically pointed toward the tebuconazole modalities.

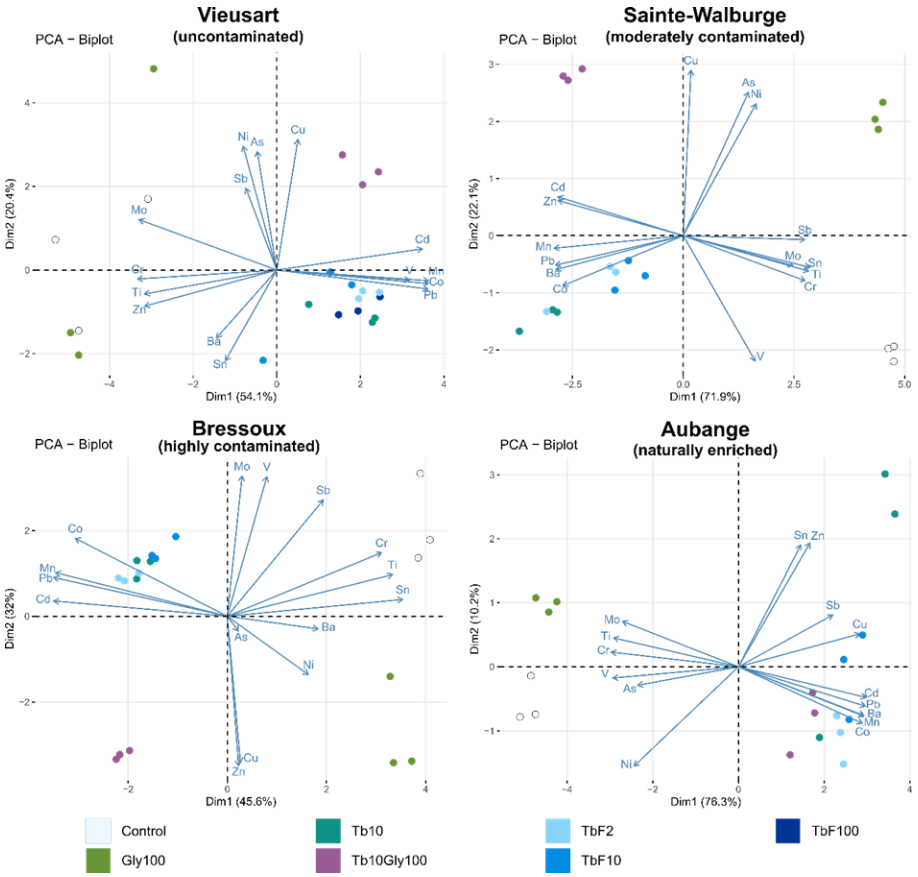


Figure 7.2 Principal component analysis, for the studied soils, of the concentrations of trace elements in the solutions of each modality (n=3).

A focus on four contrasted elements

Four elements were selected based on their distinct behaviours according to the previous results and because of their contrasted geochemistry and sanitary impacts: (1) Pb with major health concern; (2) Cu as the most well-documented element regarding trace element-pesticide interactions; (3) As and (4) Mo as they form oxyanions.

In the post-extraction solution, Pb was more concentrated with tebuconazole with at least half the modalities showing statistically significant differences from the control (Figure 7.3). The influence of tebuconazole was even more important than that of glyphosate, even though glyphosate is better known for its chelating abilities indicating that further research on Pb-tebuconazole interactions should be carried out (Barrett and McBride 2006; Si et al. 2013; Divisekara et al. 2018). In the contaminated soils, the Pb extractability was still strongly increased compared to the control (up to 6.4 times in the moderately contaminated soil), but the effect decreased in the highly contaminated soil (ratio up to 4.1) in line with the general trend from soil to soil. In the naturally-enriched soil, the effect of tebuconazole was even more decreased in comparison to other soils, while staying positive (ratio up to 2.8), probably because of the presence of iron oxides as the exchangeable fraction was lower than in other soils (Annexe 9). Formulation decreased the increase of Pb concentration in the soils where tebuconazole caused the strongest concentration increase, thus limiting its projected harmful effect in an agricultural context.

Molybdenum was up to 4.5-times less present in the soil solution in presence of tebuconazole in Vieusart (Figure 7.3). This suggests that tebuconazole-Mo complexes are less stable in solution than Mo alone. A possibility is that tebuconazole complexes are more prone to be sorbed, but it has also been shown that trace element-tebuconazole complexes can crystallize (Evans et al. 2007). Molybdenum was also less extracted in Sainte-Walburge in presence of tebuconazole than in the control (up to 1.4 times), an effect that is not present anymore in Bressoux, where tebuconazole had nearly no influence on the extractability of Mo. Again, it is consistent with the trends already observed for increasing anthropogenic contamination level. In Aubange, the extractability of Mo was strongly decreased by the presence of tebuconazole (up to 7.1 times). Indeed, trace elements were not mobile in this

soil (the situation is more similar to Vieusart) and, furthermore, the addition of organic substances in Aubange tends to decrease trace element mobility. There was no effect of both formulation and concentration across soils. The fact that Mo behaved differently than Pb could be associated with its charge. Indeed, Mo forms negative groups in solution in opposition to most trace elements that are present as divalent cations. But, if the charge were the determining factor, As, which also forms negative groups, could be expected to exhibit similar behaviour. Arsenic, however, was only up to 1.7 times more extracted in Vieusart and was nearly not influenced by tebuconazole in the anthropogenically contaminated soils (increase of only up to 1.2 times; Figure 7.3). However, for 60% of the modalities, differences were statistically different to various extent. In Aubange, As concentration is decreased by half. Thus, given the divergence with Mo, the theory of the charge does not hold. No effect of formulation or concentration was observed.

Of these four elements, Cu is the only one that is known to form complexes with tebuconazole and it is even known to increase the tebuconazole sorption (Čadková et al. 2013). However, though tebuconazole always increases its extractability with 75% of modalities that show a certain level of statistical significance, they remain low as they are plateauing at 1.7 times the control (Figure 7.3). Again, there was a decreased effect of formulation (up to 20%), and the effect of concentration was negligible. A special feature of Cu was the stability of trace elements concentration increase in presence of tebuconazole. Indeed, this increase did not diminish with higher levels of anthropogenic contamination. A similar pattern was observed with glyphosate, where the concentration even increased further (Bemelmans et al. 2023).

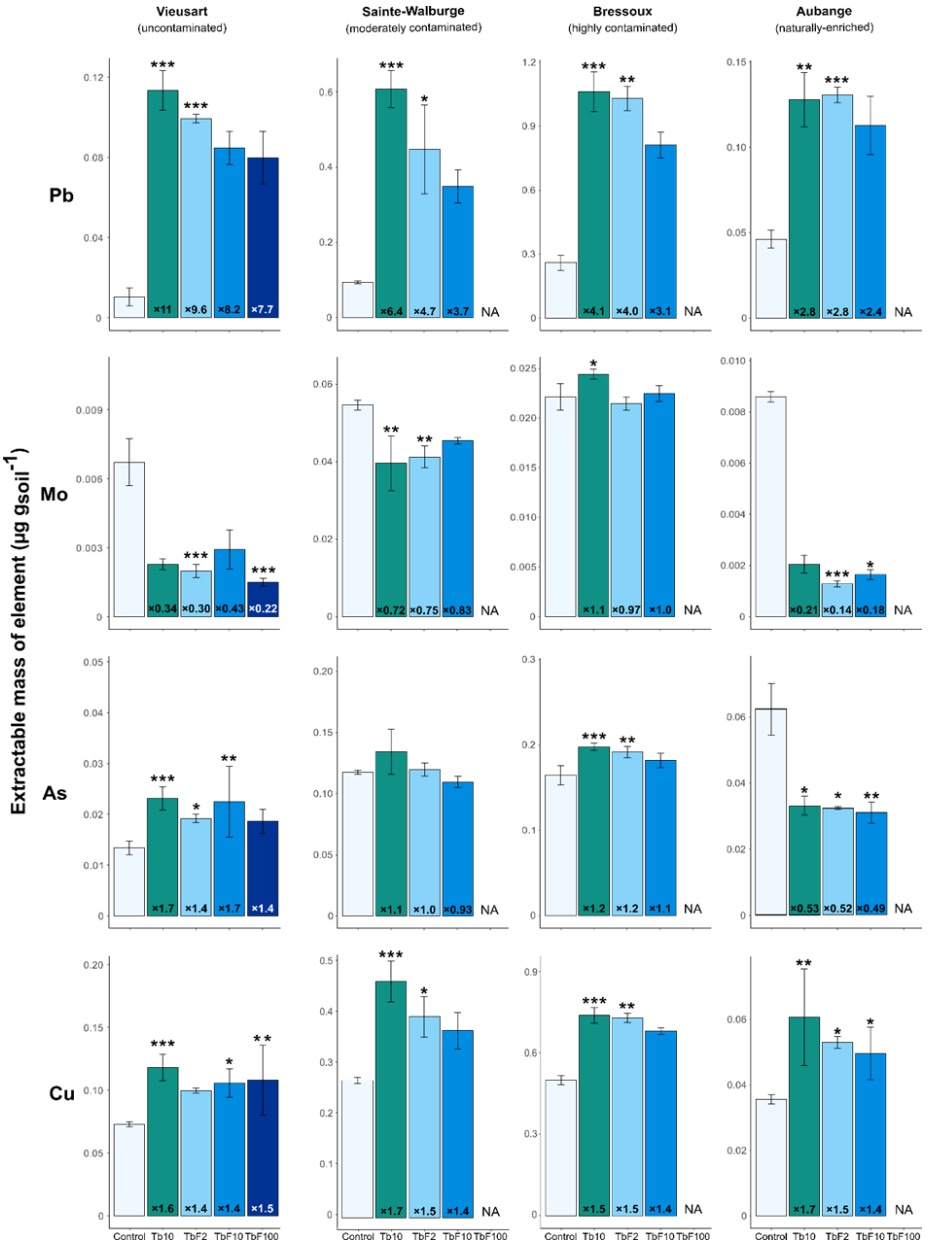

Vieusart
(uncontaminated)
Sainte-Walburge
(moderately contaminated)
Bressoux
(highly contaminated)
Aubange
(naturally-enriched)
Pb
Mo
As
Cu
Extractable mass of element (µg gsoil-1)
Control Tb10 TbF2 TbF10 TbF100

Figure 7.3 Extractability (n = 3) of Pb, Mo, As, and Cu in the soils of Vieusart, Sainte-Walburge, Bressoux, and Aubange following five extraction modalities: control (Milli-Q water), tebuconazole (10 mg L^{-1}), formulated tebuconazole (2, 10, 100 mg L^{-1}). The TbF_{100} modality was only tested in Vieusart. NA stands for not analysed. Scales were adapted for each element. Results of Dunn test for differences between modalities and controls are symbolised by stars (*). One star indicates a p-value inferior to 0.1 two stars, indicate a p-value inferior to 0.05 and three stars inferior to 0.01.

7.4.2. Joined influence of glyphosate and tebuconazole on the extractability of trace elements

Since multiple pesticides were present together in the environment, we then investigated the joined influence of glyphosate and tebuconazole on trace element mobility (i.e., competition, addition, etc.). PCA results (Figure 7.2) showed that, considering all elements, joined effects of tebuconazole and glyphosate led to a combination of both single effects. This was especially visible for the anthropogenically contaminated soils as the map of individuals showed that the $Tb_{10}Gly_{100}$ was located at a position, in comparison to the control, corresponding to the same movement on the first dimension (representing the tebuconazole effect) as Tb_{10} and the same as Gly_{100} on the second dimension (representing the glyphosate effect). As a result, the sign of its score changed on both dimensions in comparison to the control. The fact that in Aubange this effect is less visible can be mainly explained by the lower effect of glyphosate on trace element mobility in comparison to tebuconazole (which is coherent with the lower explanatory power of the second dimension in this soil (10%). But to study more in depth the joined effect of glyphosate and tebuconazole, six elements (As, Cd, Cu, Pb, Sn and Zn) have been chosen to represent the diversity of the behaviours.

Joined effects of glyphosate and tebuconazole were either additive (38% of the elements, with less than 5% of difference compared to the theoretical value that represent the sum of the theoretical concentration increases for both modalities alone, in comparison to the control) or less than additive (50% of the elements), while only two elements (Cd but mainly Cu) showed effects that were sometimes synergistic (meaning more than additive, Figure 7.4). The less than additive effects were found especially in uncontaminated or naturally-enriched soils (Pb, Sn, As). This could be linked with the lower pool of extractable trace elements in these soils, that led to a higher competition of pesticides for the binding of elements. Regarding the individual effects of pesticides on trace element mobility, the opposite trend was observed as pesticides increased more trace element mobility in uncontaminated soils, since in contaminated soils the relative effect of pesticides tended to be more marginal (Bemelmans et al. 2023, 2024). However, this signifies that the more contaminated the soils, the higher the risk to have additive effects. Conversely, less than additive effects were observed in uncontaminated soils with less mobile elements. It is noteworthy that the final concentration with the mixed modality was higher than any other modalities in 46% of the cases. As multi-pesticide contaminated soils are common (Silva et al. 2019), there is thus a risk for an even more increased trace element mobility when pesticides are not present alone. Finally, the influence of tebuconazole on trace elements mobility was stronger than the influence of glyphosate, despite its lower mass concentration and even lower molar concentration resulting from tebuconazole nearly twice higher molecular mass (Lewis et al. 2016).

The Pb concentration in the solution of the mixed tebuconazole-glyphosate modality was lower than the theoretical value in the uncontaminated and the moderately contaminated soils (Vieusart and Sainte-Walburge; Figure 7.4). The effects were thus less than additive, suggesting that both glyphosate and tebuconazole are competing for the complex

formation with extractable elements. However, for both soils, the concentration was even lower than for the tebuconazole alone. This could be due to inter-sample variability, as differences were low in Vieusart, but it also suggests that, when present together, the pesticide's ability to solubilise trace elements might be decreased beyond the competition effect. This might be the consequence of an inactivation of the pesticides by formation of either less soluble complexes between the pesticides together and the metals or the formation of glyphosate-tebuconazole complexes that are thus less able to bind metals, as observed for metal pesticide-organic acid interactions (Helal et al. 2006). In both the highly anthropogenically and naturally-enriched soils (i.e., Bressoux and Aubange, respectively), the Pb concentration was similar between observed and theoretical values (difference <5%), thus indicating a purely additive effect. Similar increased effects in presence of mixed modalities have already been reported in the literature for other substances (Wang et al. 2019).

Tin shows a similar trend as Pb in the uncontaminated and moderately contaminated soils with effects that were mainly less than additive (Figure 7.4). However, in Vieusart, concentrations became even lower than for each individual modality (Tb_{10} and Gly_{100}), while staying superior to the control. Nevertheless, the trend was reversed in Sainte-Walburge and Bressoux, where both tebuconazole and glyphosate decreased Sn extractability to such an extent that the theoretical concentration in Sainte-Walburge became negative (and thus unattainable). If Sn was less present in the mixed modality than in the control, it is not possible to compare this case (diminution of trace elements concentrations) with the cases marked by an increase of trace element extractability. Indeed, the pool of elements was more limited, reaching the boundary of the additive effect hypothesis. In this extreme case, competition for the binding of trace elements increases drastically. The case of Aubange is different, as tebuconazole and glyphosate had opposite influences on

Sn mobility. The theoretical value predicted a small concentration decrease, and it is a small decrease equal to the Gly_{100} modality that is observed. The low influence of the pesticides on Sn mobility in this soil suggest a result caused by experimental variability, though the formation of insoluble metal-tebuconazole-glyphosate complexes cannot be excluded.

For the concentrations considered, Cd mobilized by the mixed modality was nearly equal to the prediction in all soils, which indicates an additive effect, though in Bressoux the effect was slightly less than additive (Figure 7.4). Note that, in Aubange, the Gly_{100} modality had a low effect, it is thus difficult to consider the mixed effect as additive, as tebuconazole only seems to play a role on extractability.

The behaviour of As was similar to Pb and Cd, gathering three elements with high health impact (Needleman and Bellinger 1991; Ratnaike 2003; Järup and Åkesson 2009). There was an equal or slightly less than additive (by 11%) effects of the pesticides in the mixed modality (Figure 7.4). In Aubange, where glyphosate and tebuconazole had opposite effects, the experimental concentration with the mixed modality was intermediate between the individual concentrations of Gly_{100} and Tb_{10}. However, the result was not as low as predicted indicating that in this case a purely mathematical interpretation of the combination of effects was not relevant.

Zinc showed a specific behaviour in Vieusart, as a wide difference was observed between the prediction and the observation (80%), (Figure 7.4). In addition, though an increased concentration was expected in comparison to the control, the result showed an important decrease (2 times) that was identical to the Tb_{10} modality alone. In the anthropogenically contaminated soils, the concentration of the mixed modality was similar to the theory (<7%), like for As and Cd. In the naturally contaminated soil, similarly to Sn, a slight decrease of extractability is

observed in comparison to the control, while both Gly_{100} and Tb_{10} had caused an even slighter increased concentration.

Finally, Cu in the extraction solution was higher than the theoretical value in Vieusart and Bressoux, indicating a synergistic effect of glyphosate and tebuconazole (Figure 7.4). In the moderately contaminated soil, observed concentrations matched the prediction. Of the six chosen elements, Cu is the only one that was more extracted than the theoretical value in three soils. It should be noted that glyphosate had much more influence on Cu extractability than tebuconazole. Synergistic effects can occur when agents are not limited by the same processes, for example due to different abilities to accesses to compartments (Vlachodimitropoulou Koumoutsea et al. 2015). Thus, the addition of a pesticide that itself has a low influence on trace element mobility could have a higher influence than expected due to synergistic effects. In Aubange, the effects of glyphosate and tebuconazole were less than additive, and even inferior to the effect of tebuconazole, like Pb in Vieusart and Sainte-Walburge.

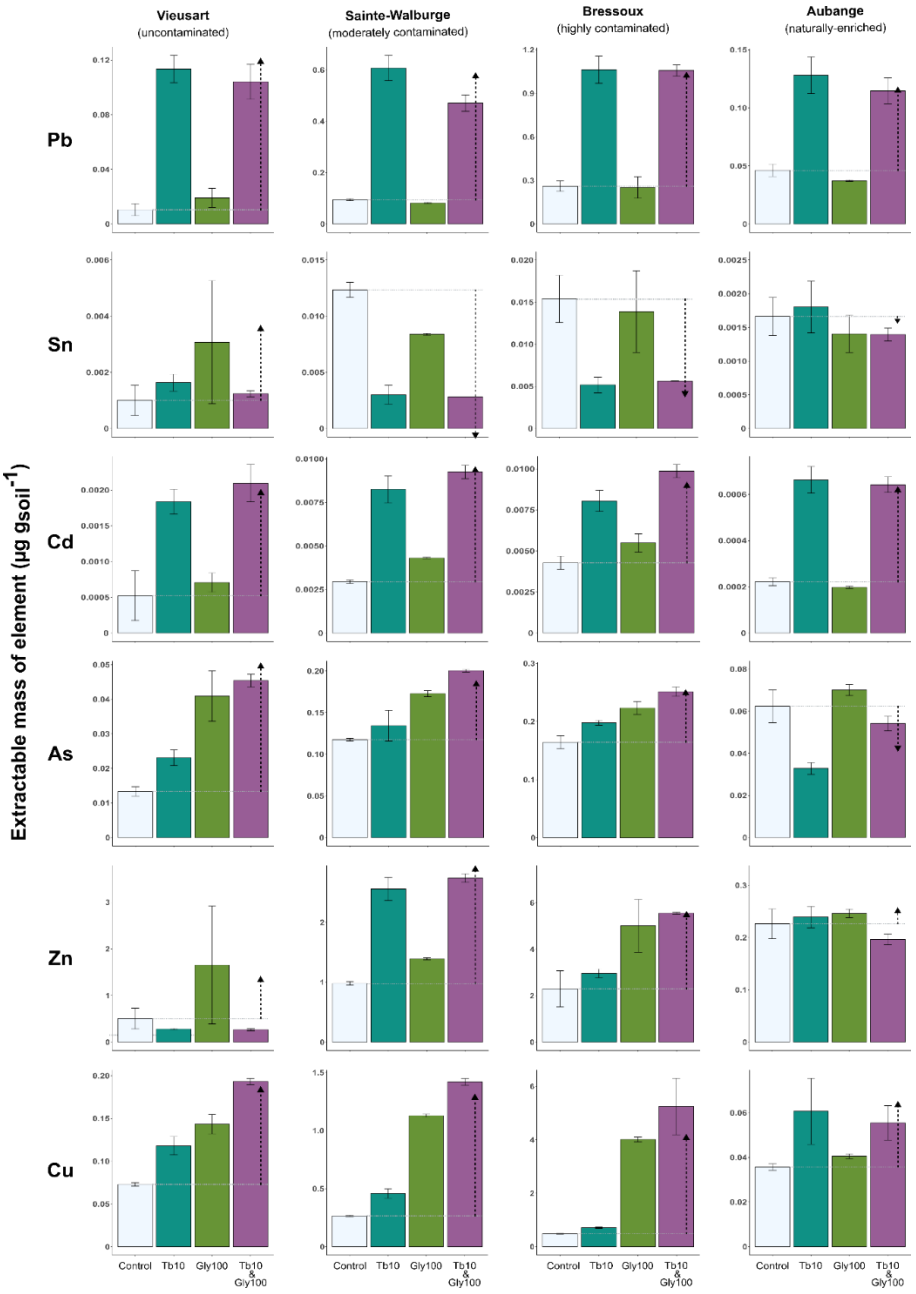

Vieusart
(uncontaminated)
Sainte-Walburge
(moderately contaminated)
Bressoux
(highly contaminated)
Aubange
(naturally-enriched)
Pb
Sn
Cd
As
Zn
Cu
Extractable mass of element (µg gsoil-1)
Control
Tb10
Gly100
Tb10 & Gly100

Figure 7.4 Extractability (n = 3) of Pb, Sn, Cd, As, Zn, Cu in the soils of Vieusart, Sainte-Walburge, Bressoux and Aubange following four extraction modalities: control (Milli-Q water, Bemelmans et al., 2023), tebuconazole (10 mg L^{-1}), tebuconazole (10 mg L^{-1}) + glyphosate (100 mg L^{-1}) and glyphosate (100 mg L^{-1}, Bemelmans et al., 2023). The arrow represents the theoretical value based on additive effects. Scales were adapted for each element.

7.5. Conclusion

As previously observed for glyphosate, tebuconazole could have a major influence on the mobility of trace elements in soils, both increasing the mobility of the most mobile elements, but decreasing the mobility of the less mobile ones. In comparison to glyphosate effect, the behaviour is even more dependent on the element with strongly increased and decreased element concentration for the same modality in the same soil (Pb and Mo). The soil was of major importance for the elements that were more intermediate in terms of mobility. The increase in anthropogenic contamination level decreased the influence of tebuconazole on trace elements mobility, both regarding increases and decreases, as it was observed following glyphosate addition. It indicates that mobility of element one to another and among soils had different influences, while both being crucial. It also implies that projections could be more easily made than for glyphosate for which the mechanisms behind the behaviour of individual elements was more difficult to unravel. If following similar mechanisms, glyphosate can increase (but also decrease) plant uptake of trace elements as well as their transfer to solutions, tebuconazole spraying could possibly increase the transfer of harmful trace elements such as Pb. Thus, experiments including plants need to be carried out in this sense.

As in soils a variety of pesticides and other organic compounds coexist, the other goal of this study was to evaluate the joined influence of pesticides (glyphosate and tebuconazole) on trace elements mobility. Results showed that, though in some cases synergistic effects can happen, purely additive and less than additive effects were the predominant effects observed. It has also been shown that additive effects, considered as the sum of the increased concentrations regarding the control, was relevant to represent the mechanism of small trace element concentration modifications. It was not, however, suitable for representing the mechanisms of important modifications, especially important concentration decreases that led to a strong competition. Real conditions experiments, including undisturbed soil profiles and plants should be carried out to evaluate if extractability trends translate to real soils and plants. At present, one should consider that trace element mobility increases can accumulate, though these accumulations would probably be less than additive they could pose a risk under multiple complexing pesticide spraying, especially for elements like Cd or As.

8. Chapter IV. Combined effects of pesticides and dissolved organic matter on soil trace element extractability

8.1. Abstract

Though trace elements are present at low concentrations in the environment, soils, including the agricultural ones, may be enriched. Due to their toxicity, they can cause important risks to human, but also environmental, health. Especially if their mobility and bioavailability are increased. Since trace elements interact with a broad range of organic compounds in soils, including organic amendments and the widely present pesticides, it is essential to understand how these substances, both individually and together, affect the dynamics of trace elements for effective soil management and contamination control. In this research, we investigated first how dissolved organic matter (DOM) affects the mobility of trace elements in both uncontaminated and contaminated soils, and second, its potential to modulate the effect of glyphosate. Batch extractability experiments were conducted to assess the potential mobilization of trace elements under varying conditions. The results showed that DOM significantly influences trace element extractability. Dissolved organic matter mainly increased the mobility of the most mobile elements but decreased it if for the less mobile ones, while for the intermediate elements the soil was of crucial importance. However, for some elements, the influence of DOM was not clear due to the import of elements through the DOM solution that influenced the equilibriums. Regarding the influence of the soil, increasing anthropogenic contamination levels has been shown to decrease DOM's ability to influence

trace element dynamics. When combining DOM and pesticides, effects were predominantly additive or less-than-additive though some combinations of opposite trends were more intricate to decipher. Finally, DOM did not consistently counteract the increased mobility caused by pesticides, except in specific cases like arsenic contamination and can hardly be considered as a suitable lever against trace element mobility increases.

8.2. Introduction

Present at low concentrations in the environment (<1‰), trace elements may be harmful to human and environmental health (Adriano 2001). Although all of these elements are toxic at high doses and can cause a wide variety of diseases, some are essential for living organisms (e.g., Cu or Zn; Arnich et al., 2012; Underwood, 1977). In agricultural soils, high contents of these elements occur, but their mobility—i.e., their ability to be transferred from soil to soil solution and to plant—is also key regarding the associated risks (Kabata-Pendias 2004). This mobility depends on their origin (Sungur et al. 2014). Trace elements can be naturally present, mainly coming from the alteration of the parent material, but they can also be anthropogenic and thus the consequence of atmospheric deposition (e.g., coming from surrounding industries) and direct input (e.g., manure, sludge, compost, or eventually industrial debris; Bañuelos and Ajwa, 1999). Indeed, trace elements that are included in minerals generally have a lower mobility as they cannot be simply desorbed but are mobilised following partial dissolution, while materials of human origin present trace elements that are potentially more mobile because they are mainly sorbed to the surfaces or included in more degradable (e.g., organic) matrices (Sungur et al. 2014). Environmental conditions, such as the physical and chemical context (soil

pH, redox, cation exchange capacity, drainage, etc.), but also the chemistry of individual trace elements (i.e., speciation of the element, divalent cations or oxyanions) also play a role in trace element mobility.

It has been shown that complexation of trace elements with other organic compounds also largely influences their mobility. Complexes can modify the transfer of trace elements from soil to soil solution, but also to plants (Violante et al. 2005; Clemente et al. 2010; Ebrahimian and Bybordi 2014). It depends on the capacity of these complexes to be either soluble or sorbed on soil particles. There is a wide variety of organic compounds in soils that are able to form complexes with trace elements. Among them are pesticides, which are predominantly organic compounds. Whether they are active substances (e.g., glyphosate, tebuconazole) or metabolites (e.g., aminomethylphosphonic acid, AMPA, the most common glyphosate metabolite), they may influence trace element extraction from soils and even modify the behaviour of trace elements in the soil-solution-plant system (Cakmak et al. 2009; Bemelmans et al. 2024). Besides, these molecules are frequently found in agricultural soils (Silva et al. 2019), along with many others as nearly 450 active substances, safeners, and synergists are approved in the EU (EC, 2024).

But the main pool of organic compounds does not come from pesticides. Natural dissolved organic matter (DOM), that is widespread and also known to influence the solubility of trace elements, is produced by the degradation of organic debris (such as decaying roots) or any imported organic materials (e.g., manure, compost, etc.; Beesley and Dickinson, 2010; Carrillo-González et al., 2006; Borggaard et al., 2019). One could expect an increase of the input of such organic matters as a consequence of the carbon storage capacity of soils and the benefits for them in terms of fertility and stability (Minasny et al. 2017; Page et al. 2020). Indeed, organic carbon management has become key in innovative agriculture practices (Faust et al. 2016; Chenu et al. 2019). One

should add that other organic compounds, in their soluble form, are used in phytoremediation applications (such as EDTA and citric acid). They are added in situ for their ability to increase trace element uptake by plants (Meers et al. 2005; Schaider et al. 2006; Ebrahimian and Bybordi 2014). Knowledge about interactions between pesticides, organic compounds, and trace elements that may lead to increased trace element mobility (and potentially human exposure) through additive or even synergistic effects, or to decreased mobility through competition or the formation of less soluble complexes is, however, lacking.

In this study, we aimed to investigate the influence of the joined effects between DOM and glyphosate (and AMPA) on trace element mobility in soils to evaluate whether it could be competitive, additive or even synergistic. Thus, we would like to evaluate whether DOM could counteract the influence of pesticides on trace elements mobility. In this perspective we also needed to characterise the influence of DOM alone on trace element mobility. Therefore, extractions experiments were performed on soils with increasing contamination levels using either substances alone or combining substances.

8.3. Materials and methods

8.3.1. Study area

Four agricultural soils were collected in Wallonia (Belgium), they form a trace element contamination gradient and are from the same batch as Bemelmans et al. (2023). One was uncontaminated and located in Vieusart (50.6799°N; 4.6357°E), it has acidic conditions (pH_{H2O} = 6.5), has a low organic carbon content (1.25%), and a CEC of 11.1 $cmol_c\ kg^{-1}$. One was moderately contaminated by human activities and was collected in Sainte-Walburge (50.6644°N; 5.5887°E), it had a higher total carbon content (11.1%) and soil pH (pH_{H2O} = 7.8). Its CEC

was slightly higher than in Vieusart (15.2 $cmol_c\ kg^{-1}$). One was highly contaminated by human activities and was collected in Bressoux (50.6374°N; 5.6109°E), it had a total carbon content of 15.8%, a soil pH_{H2O} of 7.0, and a CEC of 26.9 $cmol_c\ kg^{-1}$. Finally, a naturally-enriched soil was collected in Aubange (49.5765°N; 5.7684°E), it was characterized by a pH_{H2O} of 7.3, a total carbon content of 2.43%, and a CEC of 20.8 $cmol_c\ kg^{-1}$.

8.3.2. Soil sampling and preparation

Thirty litres of the top 30-cm soil layer were collected from an approximate surface of 4 square-meters using a shovel on every study site. All samples were air-dried at 25 °C for 15 days before sieving through a 2-mm mesh. A subsample was crushed using a soil grinder (Vibratory Disc Mill RS 200, Retsch, Haan, Germany) for trace element and organic carbon content analysis. Cation exchange capacity and soil pH were measured on the 2-mm sieved subsample

8.3.3. Experimental procedure

The experiments that were implemented followed the protocol of Bemelmans et al. (2023). Some results were extracted from this article to serve as reference here. In short, 5 grams of soil sample were added in a 50-mL propylene centrifugation tubes and mixed with 25 mL of extracting solution (1:5 ratio). Tubes were agitated in the dark for 2 h at 150 bpm. Afterward, tubes were centrifuged for 25 min at 4000 rpm and filtered with a 0.45-µm pore size nylon *Acrodisc*®. One sub-sample was acidified at 2% HNO_3 before trace element quantification and another was used for pH measurement and pesticide quantification.

A first series of experiments considered the effect of DOM alone on trace element extractability. Therefore, a DOM solution was produced

by extracting DOM from 30 g of green waste compost in a 500 mL erlenmeyer using 200 mL of Milli-Q water. After 2-h agitation (125 bpm), DOM samples were centrifuged at 4000 rpm before filtration at 0.45 µm. The sample was then diluted to reach a dissolved organic carbon (DOC) concentration of ca. 100 mg L^{-1}. To evaluate the joined effect of pesticides and DOM, several other extracting solutions were tested based on the same DOM solution but with pesticide addition: (1) DOM and unformulated glyphosate (*Sigma Aldrich*) at a concentration of 100 mg L^{-1} ($Gly_{100}DOC_{100}$, equivalent to 0.59 $mmol_{Gly}$ L^{-1}); (2) DOM and formulated glyphosate (*Glyfall plus*) at a concentration of 100 mg L^{-1} ($GlyF_{100}DOC_{100}$, equivalent to 0.59 $mmol_{Gly}$ L^{-1}); and (3) DOM and AMPA (*Sigma Aldrich*) at a concentration of 100 mg L^{-1} ($AMPA_{100}DOC_{100}$, equivalent to 0.9 $mmol_{AMPA}$ L^{-1}). All experiments were performed in triplicates.

8.3.4. Chemical analyses

The quantification of trace elements in soil samples followed a four-step digestion (HNO_3/ HF, H_2O_2, HNO_3/HCl, and HNO_3) in a ISO 6 cleanroom (Arbalestrie et al. 2022). Trace element (As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, and Zn) quantification in soil and solution samples were performed with an ICP-MS (*iCAP Q ICP–MS*, Thermo Fischer Scientific, Waltham, MA, USA) using internal standards (Ru, In, Re). The quality was checked using the *Certipur* multi element standard (Sigma Aldrich) and the HM-10 (ICP-Forest reference material) for liquid samples. The deviations from certified values were inferior to 10% for all elements, except for Zn (19% for *Certipur* and 13% for HM-10). Each element was measured at a concentration above the quantification limit.

Glyphosate and AMPA were measured by HPLC (*1200 series*, Agilent Technologies Santa Clara, CA, USA) using a Merck Lichrocart

C18 150 × 4.6 mm–5 µm column kept at 35 °C, after dilution to reach the range of the analytical method (1 to 20 µg L^{-1}). Samples were derivatized by an excess of FMOC-Cl at pH 9 (borate buffer). Elutants were pH 7 10 mM phosphate buffer (A) and 45:45:10 acetonitrile:methanol:Milli-Q water solution (B). Elution was performed at a flow rate of 1 mL min^{-1} using the following gradient (expressed as solvent B): from 0 to 1 min, 25% B (initial composition); from 10 to 15 min, 100% B; from 17 to 22 min, 25% B. The detection was performed by a fluorescent detector with an excitation at 260 nm and an emission at 310 nm.

DOC was measured as non-purgeable organic carbon in solution (<0.45 µm) using a total organic carbon analyser (*TOC-L, Shimadzu,* Kyoto, Japan).

8.3.5. Data processing

Trace element concentrations in the extraction solution were expressed as soil extractability (i.e., masse of extracted element by mass of soil). Statistical analyses were performed using *R 4.0.2* and *RStudio 2023.09.1*.

To evaluate glyphosate-DOM interactions, theoretical additive values were calculated for trace elements. The theoretical concentration of the trace element was defined by the following equation:

$$Me_{th} = Me_{control} + \left(Me_{Gly100} - Me_{control}\right) + \left(Me_{DOC} - Me_{control}\right)$$

Me: extractability of the element (usually a metal)

The same formula was used to evaluate theoretical extractability for DOC-glyphosate, DOC-tebuconazole and DOC-AMPA

8.4. Results and discussion

8.4.1. Influence of dissolved organic matter on the extractability of trace elements

General behaviour across soils

To evaluate the joined effect of organic matter and glyphosate on trace elements extractability, we specifically studied DOM and firstly assessed the effect of DOM addition as potential source of trace elements and potential interaction with soil constituents. Indeed, 7 elements were more concentrated in the post extraction solution following the DOC_{100} modality than in the control solution in Vieusart (Table 8-1). For 5 elements, the DOC_{100} stock solution released less than 40% of the amount released by the control (i.e., soil with water), but for Mo, it could release up to 7.5-times more than the control. To ensure that a concentration increase is not the consequence of the trace element addition through the extracting solution, one could consider the *DOC_{100}-corrected value* (post-extraction solution concentration – DOC stock solution concentration).

Among the set of elements, some displayed a clear behaviour regarding DOM addition across soils. Three were always less extracted (Cr, Ti, and V; Figure 8.1), all three among the less mobile elements (BCR extractions; Annexe 9). As no element was, for sure, more extracted in Aubange in comparison to the control, no element can be considered as always more extracted in presence of DOM. However, in the other soils, Cd, Cu, Mn, and Ni were more extracted in presence of DOM. Note that, Cd and Mn are known for the importance of vegetation in their geochemical cycles (green waste compost served for the production of the DOC_{100} solution) and Cu geochemistry is known for its strong control by organic matter that has a strong ability to bind it (Manceau and Matynia 2010). Moreover, Borggaard et al. (2019) demonstrated that

Cu and Cd were especially extracted by dissolved organic compounds, but As was also mentioned because of the dissolution of sorbents like aluminium and iron oxides, it was not observed in this study. In a context of increased organic carbon input in soils, sometimes with the goal to increase the sorption of trace elements, the possibility of increased mobility of some elements needs to be considered. Such high DOC concentrations are seldom reached with compost addition in agricultural soils. But other applied products, such as manure, release more DOM. In addition, greenhouse experiments performed on the same four soils showed that compost could either increase or decrease trace element mobility, with a strong dependence on the soil rather than the specific element considered (Bemelmans et al. 2024).

Table 8-1 Mass of elements in the DOC_{100} stock solution relative to the soil mass and extractability of trace elements under the DOC_{100} modality in the four soils.

	DOC_{100} stock solution ($\mu g\ g^{-1}_{soil}$)	Vieusart ($\mu g\ g^{-1}_{soil}$)	Sainte-Walburge ($\mu g\ g^{-1}_{soil}$)	Bressoux ($\mu g\ g^{-1}_{soil}$)	Aubange ($\mu g\ g^{-1}_{soil}$)
Ti	0.0197	0.119	0.378	0.211	0.0826
V	0.0243	0.0275	0.0975	0.100	0.0464
Cr	0.00547	0.0127	0.0298	0.0216	0.0169
Mn	0.122	0.976	0.733	0.727	1.42
Co	0.00536	0.0114	0.0144	0.0119	0.0101
Ni	0.0234	0.0816	0.151	0.129	0.0801
Cu	0.380	0.477	0.855	1.48	0.393
Zn	0.342	0.236	0.901	3.81	0.202
As	0.0277	0.0179	0.104	0.126	0.0251
Mo	0.0503	0.0277	0.108	0.0653	0.0101
Cd	0.000864	0.00208	0.00675	0.0124	0.00053
Sn	0.00198	0.00219	0.00673	0.0135	0.00264
Sb	0.00258	0.00499	0.0227	0.0568	0.00228
Ba	0.0695	0.270	0.408	0.414	0.0398
Pb	0.0231	0.0235	0.0903	0.370	0.0287

Uncontaminated soil

For Ba, Cr, Ti, V, and Zn in the uncontaminated soil, the addition of DOM led to solution concentrations that were inferior to the control (with a reduction of at least 60%; Figure 8.1), indicating that DOM increased their sorption capacity on the soil (Figure 8.2 b). For As, Mo, and Zn, the final concentration was lower than the concentration in the DOC_{100} stock solution, indicating a net sorption of the elements of the DOC_{100} stock solution by the soil (Figure 8.2 e). In some cases (Pb, Sn, and Sb), the concentration in the post extraction solutions was higher than in the control. However, the DOC_{100} corrected value was lower than the concentration of the control (Figure 8.2 c). In this case, it is not possible to conclude that DOM played a role as the net lower desorption of trace elements from the soil may be the result of the sorption of the important pool of trace elements already present in the solution (brought by the DOC containing solution). The last case is when the concentration is higher than the control even when the contribution of the DOC_{100} stock solution is removed (Cd, Co, Cu, Mn, and Ni; Figure 8.2 d). It indicates an increased solubility due to the chelation by the organic compounds or due to competition for the sorption on the soil. Though the trace elements initially present prevent the quantification of the DOM effect alone, high increases in element mobility were observed (DOC corrected values up to 6.5 for Mn and more than 2 times for Ni and Cd). In this soil, a similar concentration increase was observed for Mn because of compost addition in a greenhouse experiment (Bemelmans et al. 2024).

Anthropogenically contaminated soils

In the anthropogenically contaminated soils, as the general trace element concentration in the control was higher, the contribution of the compost was lower. All elements in the DOC stock solution were less concentrated than in the control, except for Cu in Sainte-Walburge (1.4 times more) and Mo in Bressoux (2.3 times more). For elements that

were less extracted under the DOC_{100} modality (Figure 8.2 b), the decrease of extractability was less pronounced than in the uncontaminated soil (with no element having a ratio <0.4). However, the number of such elements was considerably higher, with 8 elements for Sainte-Walburge (As, Cr, Pb, Sb, Sn, Ti, V, and Zn) and 7 for Bressoux (As, Ba, Cr, Sb, Sn, Ti, and V; Figure 8.1). Only one element in Sainte-Walburge (Co) and two in Bressoux (Co and Mo) were more concentrated in the post-extraction solution than in the control (Figure 8.2 d), but their DOC_{100}-corrected concentrations were lower than those of the control, so they can be reported as neither more nor less extractable. This is mainly the consequence of the lower concentration in the control inducing lower differences between uncorrected and corrected values. A similar number of elements were more extracted with the DOC_{100} modality compared to the control in the anthropogenically contaminated soils (six elements in Sainte-Walburge: Ba, Cd, Cu, Mn, Mo, and Ni; and 6 in Bressoux: Cd, Cu, Mn, Ni, Pb, and Zn) than in the uncontaminated soil, even when corrected values are considered. Nevertheless, though most concentration ratios were in the same range, extreme values like the 6× augmentation that was observed in Vieusart for Mn was not reached. The diminution of the occurrence of extreme values, both low and high, in the contaminated soils is to be associated with the increased pool of mobile elements that consequently decreases the influence of the DOM (Bemelmans et al. 2023, 2024), similar results were observed for tebuconazole. However, in greenhouse experiments, the addition of compost in solid form to the Bressoux soil decreased the trace element concentration, with less differences between elements, highlighting a strong difference between the systems (in pot vs in tubes) and the organic matter form (dissolved vs solid) considered.

Naturally-enriched soil

In the naturally-enriched soil (Aubange), the mobility of trace elements was low as observed in Vieusart, resulting in a higher proportion of trace elements in the DOC_{100} stock solution (up to 10.6 times for Cu). Still, for 8 elements, the concentration in the compost represented less than 40% of the concentration of the control.

The number of elements that were up to 10-times less concentrated than in the control was the most important in Aubange (10 elements; As, Ba, Co, Cr, Mn, Ni, Pb, Ti, V, and Zn; Figure 8.1, Figure 8.2 e). This can be linked to the results of tebuconazole and glyphosate in the same soil as, in Aubange, the addition of organic compounds tends to increase the sorption of elements more than in other soils. This is potentially due to the strong presence of iron oxides that sorb both trace elements (Suda and Makino 2016) and DOM (Gu et al. 1994) and might thus also sorb trace element-DOM complexes. As observed in Vieusart, DOC_{100}-corrected values were sometimes negative, indicating a net transfer of trace elements from the DOC_{100} stock solution to the soil rather than desorption of the elements from the soil. For five elements (Cd, Cu, Mo, Pb, and Sn), the influence of DOM could not be determined because the measured concentrations were superior to the control without correction but lower with the correction. Finally, no element had a DOC_{100}-corrected concentration superior to the control (they would be considered more extractable with DOM), probably for the same reason as the high number of elements that are less mobile in presence of DOM.

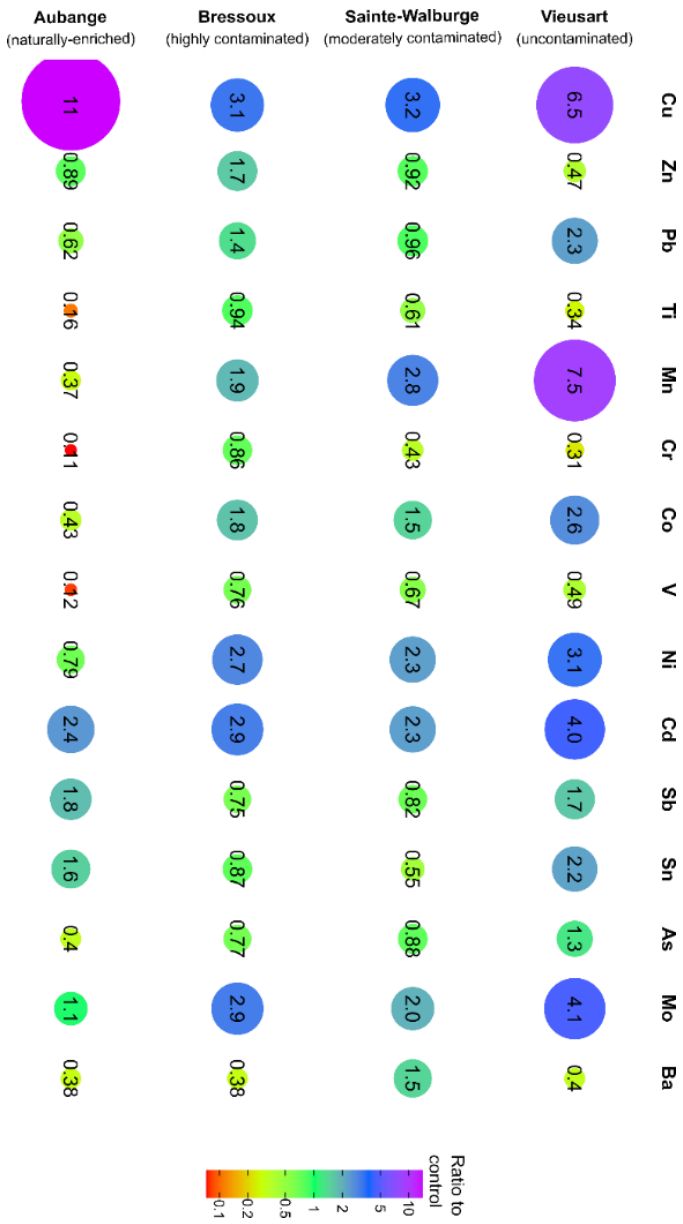


Figure 8.1 Summary of DOC_{100}/control extractability ratios (n = 3) for each studied element in the uncontaminated (Vieusart) and the three contaminated (Sainte-Walburge, Bressoux, and Aubange) soils. Elements are ranked in the same order as in chapter I.

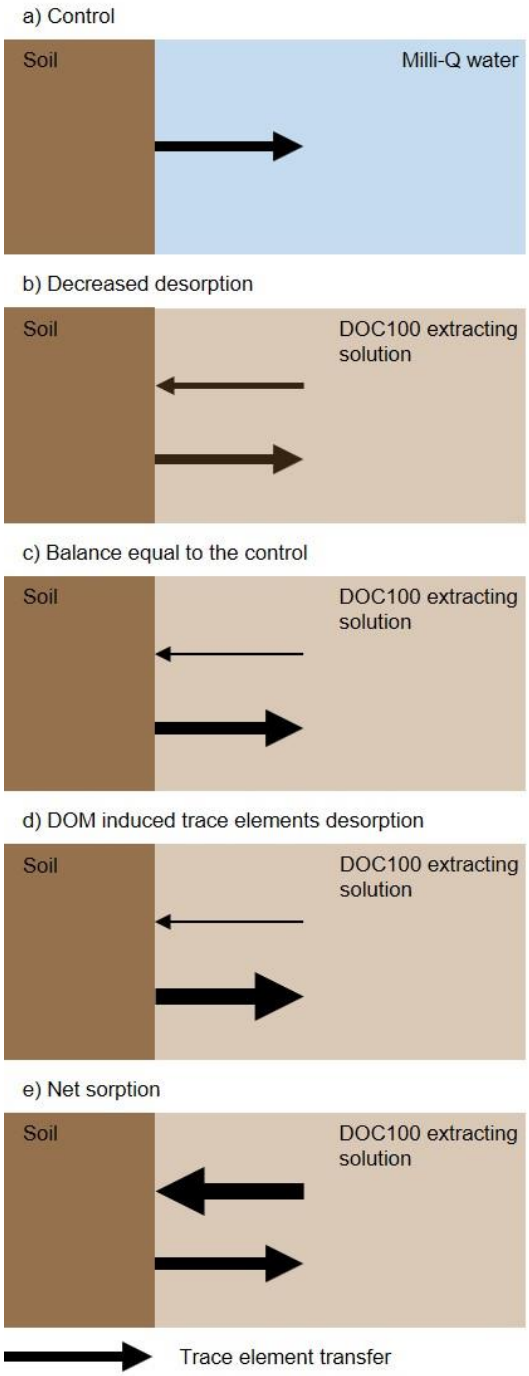


Figure 8.2 Schematic representation of the sorption desorption mechanics of trace elements between soils and solutions.

8.4.2. Influence of dissolved organic matter on the glyphosate-induced trace element extractability modification

To evaluate the behaviour of trace elements under the joined influence of glyphosate and DOM, we will focus on eight elements (As, Cd, Cr, Cu, Mn, Pb, Sn, and Ti) with contrasting behaviours and environmental interest. For mixed DOM-glyphosate or DOM-AMPA modalities, concentrations are compared with a theoretical concentration to determine the concentration corresponding to purely additive effects. Note that, overall, DOC had a small influence on the pH of the solutions as DOC solutions had a neutral pH, the acidity of the solution was thus driven by the soil and the pesticide such as in Bemelmans et al. (2023). As a consequence, the pH of most post extraction solutions mainly ranged between 6.4 and 6.8.

Uncontaminated soil

In the soil of Vieusart, all elements had a predicted concentration for $Gly_{100}DOC_{100}$ modality superior to the control (Figure 8.3). However, for Ti and Cr, two elements characterised by a low mobility in soils (Cornu et al. 1999; Kabata-Pendias 2011), the extractability was decreased (ratios of 0.3), the DOM effect was thus overshadowing glyphosate. One could imagine the formation of DOM-glyphosate-trace element complexes (Daouk et al. 2015) and that they could be less soluble due to their increased mass, even though trace elements-glyphosate complexes (without DOM) were more soluble. Most studies report that, in absence of metals, DOM influences pesticide sorption by many ways, but mainly decreasing its sorption (Lee et al. 1990; Li et al. 2005; Müller et al. 2007). The extractability of all other elements was increased (up to 10.2 times), but with wide variability regarding the difference between observed and theoretical values. A similar additive effect was observed for Cu, and it was less than additive for As, Pb and Sn. For Sn and As, DOM and glyphosate even seemed to neutralize their effect as

extractabilities were below those observed for the Gly_{100} and the DOC_{100} modalities. Synergistic effects were observed for Mn and Cd, increasing the risk of exposure. Such cases could be favoured by the presence of an additional pool of trace elements, coming from the DOC_{100} stock solution (Table 8-1), which were already complexed in solution before the addition of soil and can possibly reduce their sorption. In addition, this pool can be easily desorbed as it is not bound strongly to the soil.

When glyphosate was formulated, extractabilities for Ti and Cr were also governed by DOM that prevented glyphosate from increasing element extractability as much as expected (Figure 8.3). All other elements were from 2.5 (Pb, Sn) to 9 (Mn) times more extracted than in the control. Copper reached again the theoretical concentrations along with Pb, while As and Sn were less extracted than the theoretical value (1.3 times for As and 3.5 times less for Sn). As for unformulated glyphosate, Mn and Cd had concentration higher than the theory (by 10 %). The co-formulants played thus little additional role for DOM-trace elements interactions.

For AMPA, like for the glyphosate modalities, Ti and Cr were less extracted in the mixed $AMPA_{100}DOC_{100}$ modality than in the control (Figure 8.3), but $AMPA_{100}$ and DOC_{100} alone already decreased Ti and Cr mobility by more than half. Therefore, the theoretical concentrations were negative and competition for the binding of free ions by AMPA and DOM was important. Other elements were from 2.1 (Sn) to 8.1 times (Mn) more extracted with the mixed modality than in the control. Manganese, Cd, Sn and Pb were more concentrated than the theoretical value indicating possible synergy. For Cu and As, observed concentrations matched with the theory, while less than additive effects are not observed for elements that are more concentrated in presence of the mixed modality. Though AMPA and DOM were sometimes showing

effects that were not additive, it was the DOM addition that was controlling trace element behaviour.

Anthropogenically contaminated soils

In Sainte-Walburge, the mixed $Gly_{100}DOC_{100}$ modality yielded concentrations below the control for Ti, Cr, Sn, and Pb (Figure 8.3). For the first and the last, concentrations were even lower than predicted (more than 7% difference). For Cr and Sn, however, the reached extractabilities were not as low as the theoretical value, probably due to the competition as well as the consequence of the high sorption capacity that organic compounds would need to diminish as much the solution concentration. Similar results were observed in Bressoux, but with lower mobility influence. For both Cu and As, concentration matched the theory with increases of respectively 1.3 and 6.4 times regarding the control. Finally, Mn and Cd were in both soils, like in Vieusart, more extracted than predicted showing a strong synergistic effect between DOM and glyphosate (up to 35% increase).

If glyphosate was formulated, differences were more important between the anthropogenically contaminated soils, as in Sainte-Walburge trends were similar as for the $Gly_{100}DOC_{100}$ modality but in Bressoux no element was less extracted than in the control under the $GlyF_{100}DOC_{100}$ modality (Figure 8.3). The theoretical value predicted this outcome, as co-formulants were known to enhance trace element extractability. This effect was particularly pronounced for Cu, which exhibited the highest increase in extractability amongst all elements and soils (11 times), except Aubange. However, the observed increases were lower than expected. Copper, which typically demonstrates additive effects with DOM and pesticides in most soils, did not reach the theoretical levels, likely due to enhanced competition for the diminishing pool of sorbed Cu. This scenario mirrors the important and unattainable decreases in extractability observed for elements like Cr in

Vieusart under the $AMPA_{100}DOC_{100}$ modality. For Sn, the expected extractability increase was not reached. It could result from the high uncertainty that existed on formulated glyphosate effect, but it has also been noticed in other cases that DOM can dominate the effect of the associated organic substance, maybe by inhibiting it.

The mixed $AMPA_{100}DOC_{100}$ modality led to lower trace element extractabilities than the other mixed modalities (Figure 8.3) due to the initial influence of AMPA on their extractability (Bemelmans et al. 2023). In most cases, effects of DOM and AMPA were additive or nearly additive, like for As that shows less than additive effects and Mn for which AMPA and DOM showed synergistic effects (but lower than with glyphosate). In Sainte-Walburge exclusively, strongly less than additive effects between AMPA and DOM appeared for Cr and Sn due to the strong predicted extractability decrease and the resulting competition. This difference disappears between theoretical and observed extractability in Bressoux as, following the increased mobility of the trace elements, both DOM and AMPA have lower effects on trace element extractability. Except for Cd, the other elements also showed less extractability deviation in comparison to the control under the $AMPA_{100}DOC_{100}$ modality with the increasing anthropogenic contamination level.

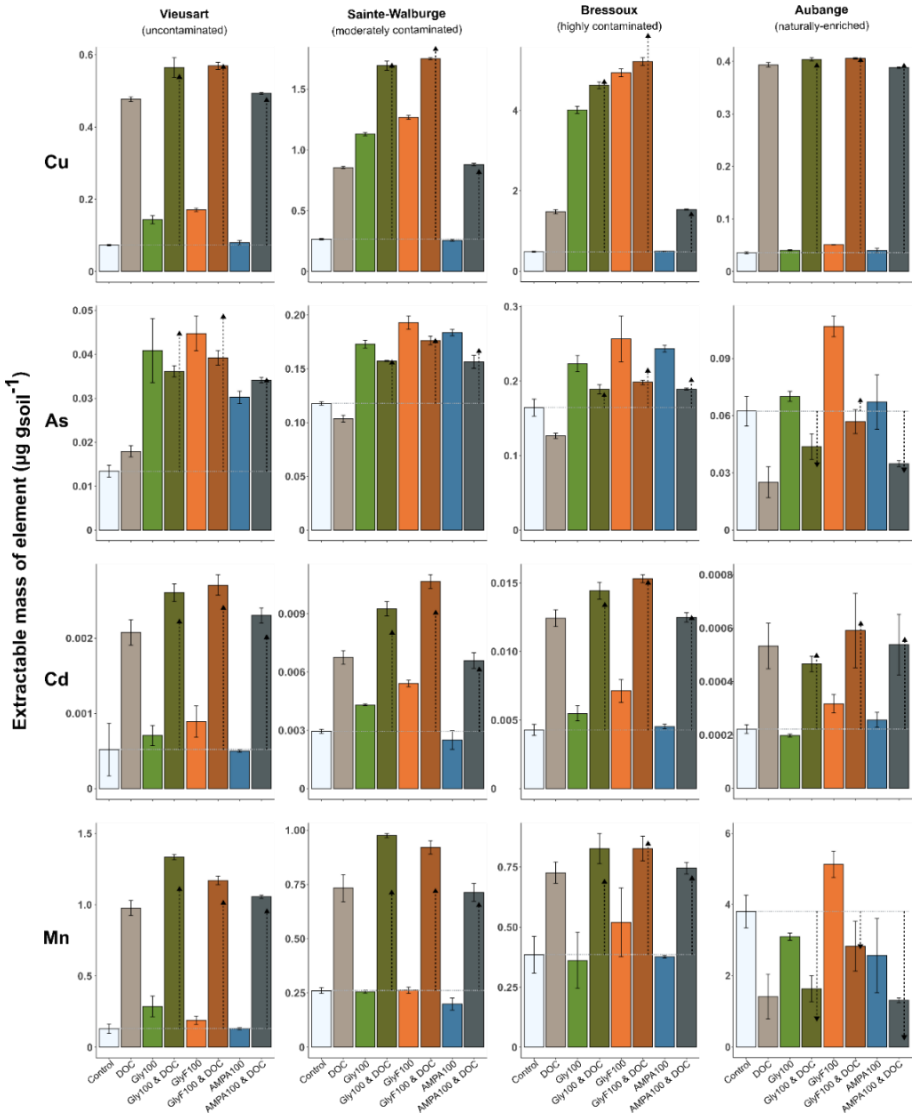
Naturally-enriched soil

In the naturally-enriched soil (Aubange), the mixed $Gly_{100}DOC_{100}$ modality caused a decrease in the extractability of five elements in comparison to glyphosate alone (Figure 8.3). For As, it even shifted from a positive effect to a negative one in comparison to the control. In this soil, both glyphosate and, more importantly, DOC reduced the extractability of a wide range of elements. Thus, some of the predicted decreases in extractability compared to the control led to a strong competition and even unreachable negative values (Ti, Cr). Thus, less than

additive effects were encountered in all five cases (As, Cr, Mn, Pb, Ti). Unlike in Bressoux, Cu extractability increases are attributed to the high relative Cu concentration brought with the DOM in the extraction solution (Table 8-1), this effect of glyphosate and DOM is additive. In this case, glyphosate alone having no influence on Cu extractability, it is safe to consider that only the DOC_{100} operated.

When the DOC_{100} solution was added to the formulated glyphosate, extractability increased and reached the maximum across all elements (with an increase of 11.4 times for Cu; Figure 8.3). While strong competition typically leads to less than additive effects, in this case, the effects were perfectly additive. This was because the concentration of elements introduced by the extracting solution (with DOC_{100}) represented 93% of the final concentration in solution (Table 8-1). DOM had the ability to decrease As extractability more than expected. This is interesting as this soil is highly contaminated in As (Vandeuren et al. 2023). Tin, like in Vieusart and Bressoux, was less extracted than the theoretical value suggesting that the co-formulants interacted with DOM and disactivated themselves. DOM counteracted effectively the effect of formulated glyphosate for As, Cr, Mn, Pb and Ti.

The trends when DOM was added to AMPA were similar to those with glyphosate and DOM for all elements. As a whole, Aubange was marked by the increased number of elements that were less concentrated than in the control under the various modalities.


Vieusart
(uncontaminated)
Sainte-Walburge
(moderately contaminated)
Bressoux
(highly contaminated)
Aubange
(naturally-enriched)
Cu
As
Cd
Mn
Extractable mass of element (µg gsoil-1)
Control
DOC
Gly100
Gly100 & DOC
GlyF100
GlyF100 & DOC
AMPA100
AMPA100 & DOC

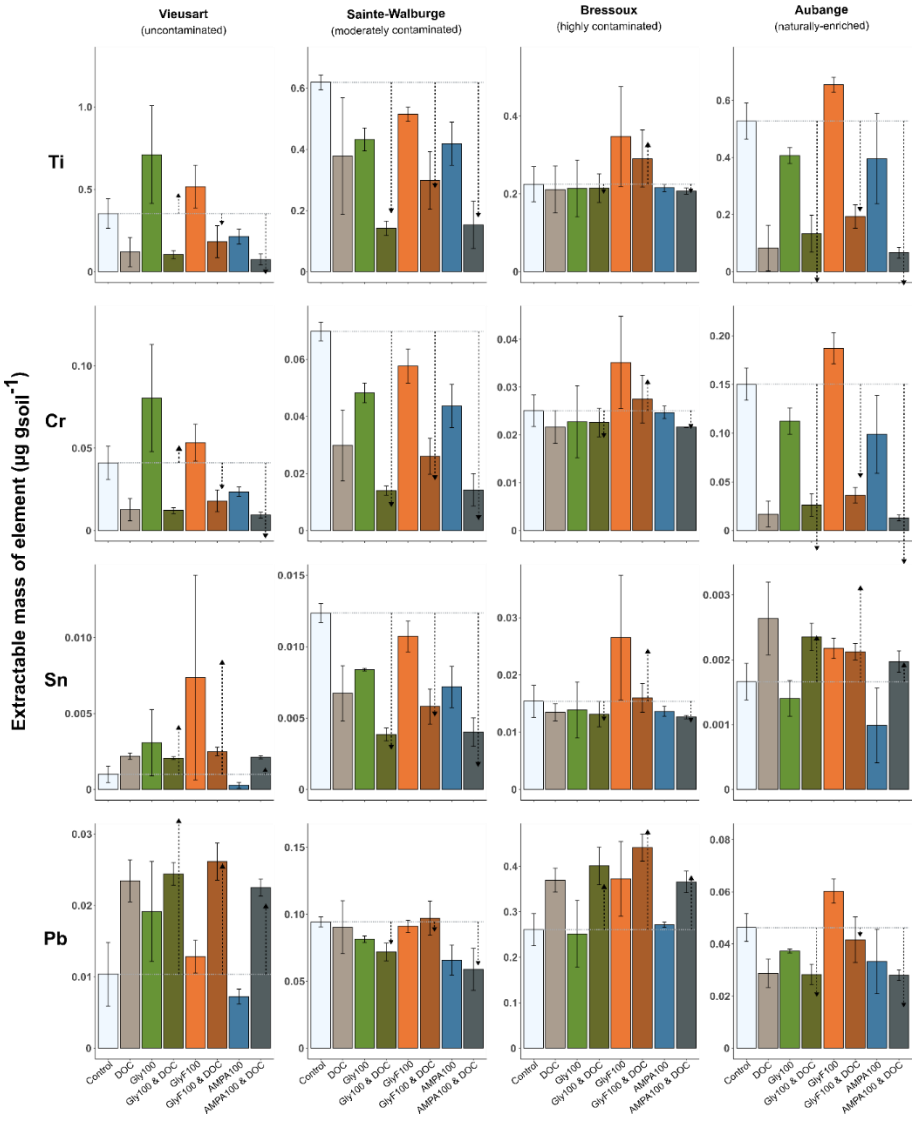


Figure 8.3 Extractability (n = 3) of Cu, As, Cd, Mn, Ti, Cr, Sn, Pb in the soils of Vieusart, Sainte-Walburge, Bressoux and Aubange following eight extraction modalities: control (Milli-Q water), DOC, glyphosate (100 mg L^{-1}), glyphosate (100 mg L^{-1}) + DOC, formulated glyphosate (100 mg L^{-1}), formulated glyphosate (100 mg L^{-1}) + DOC, (100 mg L^{-1}), AMPA (100 mg L^{-1}), AMPA (100 mg L^{-1}) + DOC. The arrows represent the theoretical values based on additive effects. Scales were adapted for each element.

Summary of the influence of the co-occurrences of DOM and pesticides

In all soils except for Bressoux, and for all three mixed DOM-glyphosate modalities, Ti and Cr were less extracted than in the control. Furthermore, in Vieusart and Aubange, the addition of DOC was able to decrease substantially their solubility in comparison to the Gly_{100} and $GlyF_{100}$ modalities. Thus, DOM showed a real potential of mobility diminution and especially of counteraction of glyphosate driven mobility increase. They also showed that strong competition between organic compounds can occur and in extreme cases, where there is a strongly limiting pool of trace elements, the additive effect theory was not relevant.

On the other side, Cu and Cd were always more concentrated under the mixed modalities than the modalities alone, with however diverse circumstances among soils and modalities. Cadmium showed that DOM and other modalities could have synergistic effects, thus increasing the exposure beyond the additive effect, for an element potentially toxic and especially transferable to plants and to the food chain (Satarug et al. 2003; Mendoza-Cózatl et al. 2011). For Cu, additive or near additive effects were observed. Furthermore, the increases of Cu were the most important among elements in coherence with the fact that this element has special affinity for both organic matter and glyphosate (Manceau and Matynia 2010; Bemelmans et al. 2023). Depending on the soil, it was one or the other that was the cause of the important mobility increase. As with the increasing mobility of the Cu across soils, the contribution of the DOC_{100} solution decreased, being taken over by glyphosate that increases Cu mobility more with rising human contamination level (Barrett and McBride 2006; Bemelmans et al. 2023). It indicates that in highly contaminated soils, glyphosate is problematic but DOM, though not compensating, becomes relatively minimal in terms of risk.

Manganese showed predominant synergistic effects of organic compounds when there was an increase of mobility in comparison to the control but, as manganese toxicity is rare and associated with occupational exposure, it is not especially concerning in this context (Adriano 2001). Arsenic and Sn were representative of less than additive or competitive effects as this occurred for most cases. DOM could be used in the specific case of As contamination to reduce its mobility, as it represents an important hazard and can be naturally present (Hughes 2002; Vandeuren et al. 2023). The behaviour of Pb was more erratic, with a combination of additive, synergistic and less than additive effects.

From one soil to another, the same trends were observed for glyphosate in extraction and greenhouse experiments, but also for tebuconazole and DOM addition. This means that, with the increasing level of anthropogenic contamination, the difference between the observed concentrations and the control decreased. With the decreasing difference between the concentrations of a mixed modality and the control, the competition for either the sorption or the solubilization of the trace elements decreased. Consequently, co-influences of pesticides and DOM tended to get closer to the additive effect. The formulation, however, did not play a striking role beyond the one it played in the context of Bemelmans et al., (2023), as formulation does not obviously and consistently influence the way modalities effects are adding themselves. Therefore, it cannot be concluded that the formulant influences DOM-glyphosate-trace element interactions to a major extent.

Regarding the use of DOC to counteract the influence of the pesticides on the trace element mobility, 25% of the concentrations that were increased by glyphosate were decreased by the addition of DOC. However, in 45% of the cases, the mixed pesticide-DOC_{100} modality increased the concentration of the trace element in comparison to the pesticide alone. Therefore, the performed experiment did not allow to conclude to a positive effect of DOM addition for the counteracting of the

pesticide mediated trace element increases. Nevertheless, results show that in most cases, effects of pesticides and DOM are less than additive. Though DOC concentrations of 100 mg L^{-1} represent the upper fringe in agricultural soils (Zhang et al. 2023), they can be reached after the addition of organic inputs (Long et al. 2015). Moreover, Bemelmans et al. (2024) showed that punctual high concentrations of an organic compound can influence trace element mobility, which extends over time even when the concentrations have decreased.

8.5. Conclusion

Dissolved organic matter showed the potential for a major influence on the mobility of trace elements in soils, both increasing the mobility of the more mobile elements and decreasing the mobility of the less mobile ones. This in addition to being the cause of an important direct trace element adjunction. For some elements, the soil was of major importance. When anthropogenic contamination level increased, the influence of DOM on trace element tended to decrease, and this regarding both increases and decreases of mobility. It indicates that the mobility of elements, both relative to each other and across soils, did not have the same influence.

As pesticides, such as glyphosate, and soluble organic compounds from organic matter degradation coexist in soils, the other goal of this study was to evaluate the joined influence of organic compounds on trace elements mobility. Results showed that, purely additive and less than additive effects were predominantly observed. Another finding is that the concept of additive effects was well represented by the sum of the increased concentrations regarding the control, and was relevant to figure the mechanism for small trace element concentration modifications. However, it could not represent the mechanisms of important modifications, especially important concentration decreases that led to a

strong competition due to an insufficient stock of elements. The consequence of the application of such compounds on structured (undisturbed) soil to evaluate its influence on soil solution trace element concentrations should be investigated such as the influence on the transfer to plant. Finally, one can conclude in the risk of an accumulation of the trace element mobility increases until some sort of saturation process occurs. As a consequence, DOM did not appear to be a suitable way to hinder pesticide driven trace element concentration increases, except for specific contamination and soils, such as As in the contaminated soils.

9. General discussion

9.1. Influence of pesticide addition on trace elements mobility depends on the nature of the soil

Both glyphosate and tebuconazole, whatever their chemical form (i.e., formulated or not), including some metabolites, had the ability to increase or decrease trace element mobility in agricultural soils. However, this ability varied greatly according to the soil considered. In this study, soil selection was based on a gradient of anthropogenic and naturally-enriched contaminations. Experiments conducted in controlled conditions (i.e., both laboratory extractions and greenhouse experiments) showed that, whatever the chelating organic compound studied, the increase in soil anthropogenic contamination levels led to a reduced influence of the pesticide. This was observed for trace elements in both solutions and plants. The reduced pesticide influence is attributed to the fact that soils with a higher degree of anthropogenic contamination had a more important pool of exchangeable elements with a high solubility that are readily transferred to soil solutions. The contribution of the chelating agent seems thus negligible. This is similar to adding a lane to a road that only has one vs a road that already possesses four. Another hypothesis suggests that variations between the soils may result from differences in soil characteristics, such as organic carbon content. Indeed, this content followed the contamination gradient, but increased glyphosate sorption on soil organic matter that might have been expected was not observed in this study, while organic matter in the form of compost seemed to influence trace element behaviour. Thus, we could suppose that: (1) the nature of the organic matter in the anthropogenically contaminated soils did not allow organic matter to play a role on pesticide sorption; or (2) organic matter had an influence on trace

elements behaviour, and this influence could not specifically be isolated from other soil characteristics.

The natural trace element enrichment in Aubange (especially in As) results from mineral formation and pedogenetic processes. Consequently, the pool of trace elements readily available represents a small proportion of the total one. Intermediate behaviours have been observed between the uncontaminated and the anthropogenically contaminated soils, with uneven results depending on the modality and the element considered. For instance, in the greenhouse experiment, higher trace element concentration in the soil solution were close to observations made on the highly contaminated soil, whereas plant content behaved like the uncontaminated soil. Aubange was characterized by an important proportion of decreasing effect on trace element mobility resulting from the chelation process. However, this cannot be attributed to the contamination level alone, but also to the high content in iron oxides, known for their sorption capacity. In the future, we suggest considering contrasted naturally-enriched soils to overcome the limitation caused by the fact that it is not possible to have naturally contaminated and uncontaminated soils with the same lithology, while the soils of the contamination gradient were formed on roughly the same parent material. Moreover, for comparability purposes, all soils had a circumneutral pH but, as acidity changes the charge of both glyphosate (that loses negative charges with decreasing pH) and organic compounds, but also the chemistry of the surfaces and the elements, results are difficult to generalise and acid as well as alkaline soils should be studied. Besides, it is known that soil structure (that was omitted here because no experiment on undisturbed soils has been carried out) influences both pesticide and trace element behaviour regarding their transport but also their degradation (by influencing micro-fauna; Nunan et al. 2006; Köhne et

al. 2009). While the main trends are soil-dependent, there are also differences between elements, with some having opposed trends, such as Cu.

9.2. Contrasted pesticide influences depending on the element considered

Though soils played an important role on trace element-pesticide interactions, there were wide variations among elements regarding their interactions with pesticides. For glyphosate, we observed contrasting behaviours between the laboratory extractions and the greenhouse experiments. And though groups of elements might share the same behaviour, others do not relate with each other. Indeed, Cu was the only element that was released in solution in presence of glyphosate with increasing anthropogenic contamination levels. Yet, this could be especially problematic since, despite its low toxicity to mammals, it is known to be harmful for microorganisms. Moreover, agricultural soils can be highly contaminated in Cu due to the use of Bordeaux mixture (Ballabio et al. 2018). Conversely, for many elements, such as Pb, Ti, Mn, Cr, Co, or V, the glyphosate influence was reduced in contaminated contexts. However, results indicate a potential additional risk in uncontaminated sites as highly toxic elements (e.g., Pb, Cd, As) were more transferred to solutions. Nickel, Cd, and to a lesser extent Sb, also shared similar results with regular consistent mobility increases. This is concerning, as Cd is an issue for human health and easily penetrates plants. Arsenic is among the elements that had a singular behaviour and was also among those that were more mobile in the presence of pesticides for most glyphosate modalities. A slight trend appeared that showed that the elements that are most increased tended to be the most mobile. But, this trend failed to be sufficient to explain individual behaviours. Geochemical properties like the charge of ions were not of

greater help. General effects on trace element mobility (whether increase or decrease) were even more important when tebuconazole was added.

These groups, however, did not necessarily translate to the greenhouse experiment, even in the soil solutions. For example, Cu did not present its characteristic trend across soils, Pb was not as much extracted, while Zn and Mo were more. Other elements that exhibited a change in behaviour include Sb and Sn, which showed an increased concentration in the non-anthropogenically contaminated soils during the extraction experiment and no effect in the others, but shifted to either no effect or a decreased concentration across all soils during the greenhouse experiment. Thus, the elements that had their solubility the most increased in concentration in the uncontaminated soil were less harmful in the greenhouse experiment compared to the extraction experiments. The contrast between the uncontaminated soil and the moderately contaminated one was less marked as trace element concentration increases were more important in the moderately contaminated soil during the greenhouse experiment than the extraction experiment. In the plants, new groups of elements emerged, with As, Mo, and Ba showing similar behaviour by increasing in content within the non-anthropogenically contaminated soils, and to a lesser extent, in the moderately contaminated soil. Notably, Cd was the only element that did not show a concentration increase; in fact, its content mostly decreased. This is reassuring, given that Cd poses a risk primarily through plant uptake.

The elements that had their mobility most decreased in solution in presence of tebuconazole were Ti and Cr, the latter being among those that are subject to regulations in Belgian soils, its concentration decrease could be favourable in health terms. Some elements consistently showed an increase in concentration in solution, among them were Cu and Mn. Although Cu (which was moderately increased) is an essential element, it has a regulated concentration in Belgian soils and is known

to be toxic (Uriu-Adams and Keen 2005), though the increase was moderate. Mn is also an essential element that can become toxic at high concentrations (Peterson and Skinner 1931; Miah et al. 2020). But the most concerning elements that were consistently more concentrated in solution in presence of tebuconazole were Co and, above all, Cd and Pb. Indeed, their increases were reaching up to 3.9 times (Co), 3.5 times (Cd) and 10 times (Pb), and Cd and Pb are causing major health concerns (Needleman and Bellinger 1991; Järup and Åkesson 2009). Contrary to glyphosate, where the link between its effect on trace elements and their geochemistry was intricate, tebuconazole-trace element interactions were linked with the mobility of the element, although it could not provide explanation of every element's behaviour. Indeed, the elements that were always less extracted when tebuconazole was applied were among the less mobile (with a high proportion in the soils that was not available), while the most increased were among the most mobile in the soil. For the elements that were intermediate in terms of mobility, the soil was of great importance and the specific drivers of an increased, or a decreased concentration in presence of tebuconazole were difficult to unravel. The behaviour of elements was marked by their individuality making predictions on the risks difficult.

Pesticides are applied as formulated products and not as active substances alone, and for glyphosate the presence of these additives had an influence on trace element mobility. In the uncontaminated soil, the additives decreased the influence of glyphosate on trace element mobility for half of the elements but increased it for the other half. In the other soils, it either had no influence or increased trace element extractability. As the formulation of the pesticide decreased the sorption of glyphosate, it has been concluded that, though the co-formulants could interact directly with the trace elements, there is a possibility that it increases the stability of the complex in solution. For tebuconazole, the formula-

tion seemed to decrease the concentration of the trace elements in comparison to unformulated tebuconazole. As tebuconazole has not been measured in the solution, the effect of the formulation was more difficult to disentangle. Beyond the co-formulants, pesticide degradation produces other substances that may persist in soils. Glyphosate's main degradation product, AMPA, had a low influence on trace element extractability and even decreased it. Thus, favouring the degradation of glyphosate seems favourable. However, this cannot necessarily be extended to other pesticides and their degradation products. Unlike pesticides, degradation products are not directly applied but are gradually produced within the soil profile, resulting in lower concentrations than the active substances.

Regarding the influence of pesticide concentrations, one might expect that increasing the concentration of the active substance would consistently amplify its effects on trace element mobility, whether that means increasing or decreasing trace element concentrations. While this was observed for some elements, such as Cu, it was not always the case. For glyphosate, both in extractability experiments and greenhouse experiments, in both soil solutions and plants, trace element extractability could be higher with a lower concentration. This was explained by the fact that glyphosate possesses charges, both positive and negative, and can thus form bridges and potentially increase soil sorption capacity. The equilibrium between glyphosate mediated solubilization and sorption capacity could thus lean more or less on one way or the other. For tebuconazole, though such trends were observed for elements like Pb and Cd, the influence of the concentration is unclear across the studied range, maybe because of a saturation of the effect. Testing lower concentrations could provide more insights. It also points out that tebuconazole might have an even stronger impact on trace element mobility than glyphosate. In the future, testing pesticide concentrations that are too similar is to be discouraged and either stronger differences should

be considered or a reduction in the diversity of the concentrations could serve to study other parameters. Finally, though the pH variations between control and pesticide modalities was not necessarily correlated with the influences on trace element mobility. An influence of the acidification cannot be fully excluded such as a role of the difference of ionic force of the extracting solutions, it would be interesting to perform experiments that break free from these constraints.

9.3. Influence of pesticide application on the transfer to plants

Since plant consumption is among the main sources of exposure to trace elements, studying the dynamics of trace elements in soil solutions alone is not enough in an environmental or health perspective. Indeed, the diversity of mechanisms that occur between the soil and the edible parts of plants can either promote or inhibit the transfer of certain elements (Arbalestrie et al. 2022). Our greenhouse experiment showed contrasted results depending on the soil considered. Indeed, in three uncontaminated soils, glyphosate mostly increased the plant uptake of trace elements, while this effect diminished with the contamination gradient, up to a mostly negative influence on plant uptake of trace elements in the highly contaminated soil. Although it is difficult to generalize trends for all trace elements, this shows that the greatest risk occurs in uncontaminated soils, meaning low additional impact for human health and the absence of risk associated with edible plants in contaminated contexts. Interestingly, different trends occurred in the naturally-enriched soil between soil solution (negative effect) and plants (positive effect). It confirms that soil solution cannot be considered as the bioavailable fraction.

Other organic substances, such as EDTA or citric acid, have been shown to increase plant uptake of trace elements in contaminated soils,

suggesting that other pesticides than glyphosate might have a similar effect. Since glyphosate was sprayed before the culture and still influences trace element transfer after spraying, other pesticides that are directly applied to the crop as fungicides (e.g., tebuconazole) might have an even greater impact. Therefore, there is a need to test the influence of such molecules on soil-plant transfer of trace elements. Additionally, field experiments are required to validate our observations, especially to measure trace element content in the edible parts (i.e., grains for Poaceae or root vegetables for carrots, turnips, potatoes, or beets).

9.4. Implications for the agroecosystems

In agricultural soils, pesticides may coexist over the long term as residues, and our findings indicate that both glyphosate and tebuconazole can influence trace element mobility. Both these pesticides were shown to be commonly present in European agricultural soils (Silva et al. 2019), raising the risk of potential addition of available trace element to plants or natural waters. Having considered only two molecules in this thesis, we have a partial view of the effects produced on trace elements released to the environment. Yet, we can suspect wider environmental consequences if we consider all the pesticide residues present in agricultural soils. Our results also show that such risks may exist through additive effects and, in some cases, even synergistic effects, particularly in the most anthropogenically contaminated soils where trace elements were highly mobile. However, in most cases, the combined effects of both pesticides were less than additive, suggesting competition processes for the elements or inter neutralization. Although the addition of tebuconazole has sometimes more than neutralized the glyphosate effect on trace element mobility, it cannot be considered that co-occurrence of pesticides would not increase or even decrease the

risks. Consequently, one should be cautious before using a wide diversity of pesticides.

The addition of organic matter, either as solid compost (exogenous organic matter) or as liquid compost extract (resulting from the decomposition process), was considered to evaluate its potential role to counteract the effects of pesticides on trace element mobility, while also serving as a method for carbon storage in soil. Indeed, solid organic matter is known for its sorption capacity, while dissolved organic matter might compete with pesticides for trace elements binding as well as serve as a potential transfer vector after sorption. Contrasting behaviours were evidenced, from positive to negative effect on trace element transfer with increasing contamination gradient and trace element mobility. This could be attributed to contradictory effects: compost may release trace elements of its own composition, but also release chelating organic compounds that can favour trace element solubilization. Thus, addition of compost could be useful in contaminated soils to mitigate the effect of pesticides on trace element transfer, although this effect is less pronounced in these soils. Nevertheless, the potential introduction of additional trace elements through compost must be considered. In plants, the effect of compost was more element-dependent with lower intensity compared to observations performed in solutions. It is thus difficult to promote the use of compost as a general mean to decrease trace element mobility. In parallel, compost extract consistently diminished the transfer of less mobile elements (e.g., Ti or Cr), while favouring the mobilisation of Cd, Mn, Ni or Cu. Quantifying these effects, DOM cannot be considered to effectively counteract the increase in trace element mobility induced by pesticides. The main methodological difficulty of this investigation, however, lies in the pool of trace elements released from the DOM, limiting to accurately assess the role of DOM. Thus, the addition of organic matter that produces large amounts of DOM could increase the risk of trace element exposure, even more

so if DOM is added directly. In the end, it seems that there is an important case dependence regarding the influence of added organic compost that prevents this work of giving categorical advices.

Although pesticides had the ability to increase the presence of harmful trace elements in soil solution, the risk for the hydrosystem remains unclear. If the distance between the soil and the water table is important, there is a high probability that trace elements will be re-adsorbed. However, in lowland soils, particularly those prone to flooding, the risk for the hydrosystem may approach the risk for plants, especially in the context of growing pressure on water bodies. One should add that, beyond the human toxicity perspective that was mainly adopted, the influence of pesticides on the trace element uptake by soil fauna (including microorganisms) remains unclear and could lead to an increased toxicity for this environment.

10. Conclusions et perspectives

10.1. Conclusion

In the context of widespread contamination by pesticides and trace elements, along with increasing pressure on soils, this study aimed to enhance understanding of how pesticides affect trace element mobility in agroecosystems, particularly focusing on their transfer from soil to soil solution and to plant, which in turn could increase human exposure.

The findings indicate that both glyphosate and tebuconazole can either increase or decrease trace element mobility in soil solutions, depending on the contamination context. It was also demonstrated that the relation between pesticide concentration and effect may not be monotonic, as low concentrations of pesticides could lead to increased solubilisation of trace elements.

Furthermore, the use of glyphosate was shown to increase or decrease plant uptake of trace elements, even when applied before sowing. This suggests that application of pesticides on cultures in place, like tebuconazole or glyphosate (on resistant crops), thus resulting in peaking concentrations during the growing season, might influence trace element mobility to an even greater extent, thereby increasing risks.

Additionally, the combined effect of multiple pesticides was observed, with a higher level of anthropogenic contamination generally reducing the impact of pesticides on most trace elements. Elements such as Pb, Cd, As, and Cu were identified as especially concerning in the presence of pesticides if their toxicity is taken in consideration.

The study also evaluated the ability of organic matter to mitigate increased trace element exposure under pesticide influence. While

organic matter can reduce trace element transfer in contaminated soils, it may inadvertently increase human exposure in soils with lower levels of mobile trace elements, particularly when more dissolved organic matter is released. Therefore, the impact of pesticides on trace element mobility should be more thoroughly studied in order to evaluate if it should also be considered in environmental assessments.

10.2. Perspectives

In the present study, only two pesticides were examined, with only one being investigated for soil-plant transfer. There is a clear need to expand this research to encompass a broader range of pesticides. Therefore, the produced dataset (extraction experiments) could be amended with other pesticides to establish links between pesticide characteristics and effects. This could be associated with a study based on digital simulations of pesticide-trace element interactions based on the structure of the pesticides. In parallel, a validation of the mechanisms of pesticide-trace element interactions (and also the competition between pesticides for ligands) should be carried out using extended X-ray absorption fine structure. One should also study their impact on trace element uptake by plants.

Additionally, it is essential to take the structure of the soil in consideration by performing experiments on undisturbed soil columns. After, there is a need to conduct field experiments to better understand how these pesticides are transferred to hydrosystems and how real environmental conditions (rain, temperature, etc.) influence trace element transfers. Extending these studies through the full crop cycle will also provide insights into how pesticide use affects the distribution of trace elements within plants, considering that edible plant parts often represent only a portion of the aerial biomass and that pesticides are not uniformly distributed within the plant. Moreover, diverse array of plant

species should be studied, including root vegetables such as turnips, carrots or potatoes, as the barriers to trace element transfer from soil to roots are fewer than those from roots to aerial parts implying different mechanisms.

If, in this study, transfer to plants and hydrosystem are mainly tackled with a background of human contamination, the influence of pesticide-trace element interactions on soil health and transfer to other critical compartments, such as microorganisms, should be studied. In this environmental perspective, an approach less based on the transfer but more on the joined ecotoxycology of pesticides and trace elements would be an interesting way.

Lastly, given the wide variety of trace element contamination contexts, ranging in magnitude and sources of contamination, but also including natural enrichments that are strongly influenced by the parent material, it is crucial to recognize the unique characteristics of each soil type. This should be linked with a thorough study of the speciation of the elements in the soils through, for example, sequential extractions.

References

Abubakar Y, Tijjani H, Egbuna C, et al (2020) Pesticides, History, and Classification. In: Natural Remedies for Pest, Disease and Weed Control. Elsevier, pp 29–42

Adachi K, Tainosho Y (2004) Characterization of heavy metal particles embedded in tire dust. Environ Int 30:1009–1017. https://doi.org/10.1016/j.envint.2004.04.004

Adriano DC (2001) Trace Elements in Terrestrial Environments. Springer New York, New York, NY

Ahmad P, Prasad MNV (eds) (2012) Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Springer New York, New York, NY

Albers CN, Banta GT, Hansen PE, Jacobsen OS (2009) The influence of organic matter on sorption and fate of glyphosate in soil – Comparing different soils and humic substances. Environ Pollut 157:2865–2870. https://doi.org/10.1016/j.envpol.2009.04.004

Alletto L, Coquet Y, Benoit P, et al (2010) Tillage management effects on pesticide fate in soils. A review. Agron Sustain Dev 30:367–400. https://doi.org/10.1051/agro/2009018

Altenburger R, Backhaus T, Boedeker W, et al (2000) Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri* : Mixtures composed of similarly acting chemicals. Environ Toxicol Chem 19:2341–2347. https://doi.org/10.1002/etc.5620190926

Antoniadis V, Levizou E, Shaheen SM, et al (2017) Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. Earth-Sci Rev 171:621–645. https://doi.org/10.1016/j.earscirev.2017.06.005

Arbalestrie B, Falys J, Bemelmans N, et al (2022) Rare earth elements in an intercropping cover crop to evaluate the trace element transfer from soil to plant. Biogeochemistry. https://doi.org/10.1007/s10533-022-00989-7

Arnich N, Sirot V, Rivière G, et al (2012) Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study. Food Chem Toxicol 50:2432–2449. https://doi.org/10.1016/j.fct.2012.04.016

Awad F, Römheld V (2000) Mobilization of heavy metals from contaminated calcareous soils by plant born, microbial and synthetic chelators and their uptake by wheat plants. J Plant Nutr 23:1847–1855. https://doi.org/10.1080/01904160009382147

Baize D (2000) Guide des analyses en pédologie. Editions Quae, Paris

Baldantoni D, Leone A, Iovieno P, et al (2010) Total and available soil trace element concentrations in two Mediterranean agricultural systems treated with municipal waste compost or conventional mineral fertilizers. Chemosphere 80:1006–1013. https://doi.org/10.1016/j.chemosphere.2010.05.033

Ballabio C, Panagos P, Lugato E, et al (2018) Copper distribution in European topsoils: An assessment based on LUCAS soil survey. Sci Total Environ 636:282–298. https://doi.org/10.1016/j.scitotenv.2018.04.268

Bañuelos GS, Ajwa HA (1999) Trace elements in soils and plants: An overview. J Environ Sci Health Part A 34:951–974. https://doi.org/10.1080/10934529909376875

Barati AH, Maleki A, Alasvand M (2010) Multi-trace elements level in drinking water and the prevalence of multi-chronic arsenical poisoning in residents in the west area of Iran. Sci Total Environ 408:1523–1529. https://doi.org/10.1016/j.scitotenv.2009.12.035

Barja BC, dos Santos Afonso M (2005) Aminomethylphosphonic Acid and Glyphosate Adsorption onto Goethite: A Comparative Study. Environ Sci Technol 39:585–592. https://doi.org/10.1021/es035055q

Barrett KA, McBride MB (2006) Trace Element Mobilization in Soils by Glyphosate. Soil Sci Soc Am J 70:1882–1888. https://doi.org/10.2136/sssaj2005.0415

Beesley L, Dickinson N (2010) Carbon and trace element mobility in an urban soil amended with green waste compost. J Soils Sediments 10:215–222. https://doi.org/10.1007/s11368-009-0112-y

Belon E, Boisson M, Deportes IZ, et al (2012) An inventory of trace elements inputs to French agricultural soils. Sci Total Environ 439:87–95. https://doi.org/10.1016/j.scitotenv.2012.09.011

Bemelmans N, Arbalestrie B, Dailly H, et al (2023) Influence of glyphosate and aminomethylphosphonic acid on the mobility of trace elements in uncontaminated and contaminated agricultural soils. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-023-29660-w

Bemelmans N, Dejardin R, Arbalestrie B, Agnan Y (2024) Glyphosate application may influence the transfer of trace elements from soils to both soil solutions and plants. Chemosphere 367:. https://doi.org/10.1016/j.chemosphere.2024.143603

Benke MB, Indraratne SP, Hao X, et al (2008) Trace Element Changes in Soil after Long-Term Cattle Manure Applications. J Environ Qual 37:798–807. https://doi.org/10.2134/jeq2007.0214

Bento CPM, Yang X, Gort G, et al (2016) Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. Sci Total Environ 572:301–311. https://doi.org/10.1016/j.scitotenv.2016.07.215

Bergström L, Börjesson E, Stenström J (2011) Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil. J Environ Qual 40:98–108. https://doi.org/10.2134/jeq2010.0179

Bermudez GMA, Jasan R, Plá R, Pignata ML (2012) Heavy metals and trace elements in atmospheric fall-out: Their relationship with topsoil and wheat element composition. J Hazard Mater 213–214:447–456. https://doi.org/10.1016/j.jhazmat.2012.02.023

Bernard A, Lauwerys R (1984) Cadmium in human population. Experienca 40:143–152

Berti WR, Jacobs LW (1998) Distribution of Trace Elements in Soil from Repeated Sewage Sludge Applications. J Environ Qual 27:1280–1286. https://doi.org/10.2134/jeq1998.00472425002700060002x

Bhandari G, Atreya K, Scheepers PTJ, Geissen V (2020) Concentration and distribution of pesticide residues in soil: Non-dietary human health risk assessment. Chemosphere 253:126594. https://doi.org/10.1016/j.chemosphere.2020.126594

Biasioli M, Fabietti G, Barberis R, Ajmone-Marsan F (2012) An appraisal of soil diffuse contamination in an industrial district in northern Italy. Chemosphere 88:1241–1249. https://doi.org/10.1016/j.chemosphere.2012.03.081

Bloem E, Vogler I (2018) Influence of graded EDTA applications on mobilization and translocation of trace elements—a soil column experiment. J Plant Nutr Soil Sci 181:220–230. https://doi.org/10.1002/jpln.201700353

Borggaard OK (1991) Effects of Phosphate on Iron Oxide Dissolution in Ethylenediamine-N,N,Nt',Nt'-Tetraacetic Acid and Oxalate. Clays Clay Miner 39:324–328. https://doi.org/10.1346/CCMN.1991.0390313

Borggaard OK, Gimsing AL (2008) Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag Sci 64:441–456. https://doi.org/10.1002/ps.1512

Borggaard OK, Holm PE, Strobel BW (2019) Potential of dissolved organic matter (DOM) to extract As, Cd, Co, Cr, Cu, Ni, Pb and Zn from polluted soils: A review. Geoderma 343:235–246. https://doi.org/10.1016/j.geoderma.2019.02.041

Bowen NL (1922) The Reaction Principle in Petrogenesis. J Geol 30:177–198. https://doi.org/10.1086/622871

Brantley SL, Liermann L, Bau M (2001) Uptake of Trace Metals and Rare Earth Elements from Hornblende by a Soil Bacterium. Geomicrobiol J 18:37–61. https://doi.org/10.1080/01490450151079770

Bruemmer GW, Gerth J, Herms U (1986) Heavy metal species, mobility and availability in soils. Z Für Pflanzenernähr Bodenkd 149:382–398. https://doi.org/10.1002/jpln.19861490404

Čadková E, Komárek M, Kaliszová R, et al (2013) The influence of copper on tebuconazole sorption onto soils, humic substances, and ferrihydrite. Environ Sci Pollut Res 20:4205–4215. https://doi.org/10.1007/s11356-012-1198-0

Cakmak I, Yazici A, Tutus Y, Ozturk L (2009) Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. Eur J Agron 31:114–119. https://doi.org/10.1016/j.eja.2009.07.001

Calabrese EJ, Canada AT, Sacco C (1985) Trace Elements and Public Health. Annu Rev Public Health 6:131–146. https://doi.org/10.1146/annurev.pu.06.050185.001023

Camarero L, Botev I, Muri G, et al (2009) Trace elements in alpine and arctic lake sediments as a record of diffuse atmospheric contamination across Europe. Freshw Biol 54:2518–2532. https://doi.org/10.1111/j.1365-2427.2009.02303.x

Carrillo-González R, Šimůnek J, Sauvé S, Adriano D (2006) Mechanisms and Pathways of Trace Element Mobility in Soils. In: Advances in Agronomy. Elsevier, pp 111–178

Carson R (1962) Silent Spring. Houghton Mifflin Company, Boston

Cedergreen N (2014) Quantifying Synergy: A Systematic Review of Mixture Toxicity Studies within Environmental Toxicology. PLoS ONE 9:e96580. https://doi.org/10.1371/journal.pone.0096580

Chang H-C, Matijević E (1983) Interactions of metal hydrous oxides with chelating agents: IV. Dissolution of hematite. J Colloid Interface Sci 92:479–488. https://doi.org/10.1016/0021-9797(83)90169-8

Chapman PM, Holzmann M (2007) Heavy metal—music, not science | Cell-phone hazards. Environ Sci Technol 41:6–6. https://doi.org/10.1021/es072552o

Charles JA (1967) Early Arsenical Bronzes—A Metallurgical View. Am J Archaeol 71:21–26. https://doi.org/10.2307/501586

Chen Z, He W, Beer M, et al (2009) Speciation of glyphosate, phosphate and aminomethylphosphonic acid in soil extracts by ion chromatography with inductively coupled plasma mass spectrometry with an octopole reaction system. Talanta 78:852–856. https://doi.org/10.1016/j.talanta.2008.12.052

Chenu C, Angers DA, Barré P, et al (2019) Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations. Soil Tillage Res 188:41–52. https://doi.org/10.1016/j.still.2018.04.011

Chittleborough G (1980) A chemist's view of the analysis of human hair for trace elements. Sci Total Environ 14:53–75. https://doi.org/10.1016/0048-9697(80)90126-6

Cleaver HM (1972) The Contradictions of the Green Revolution. Am Econ Rev 62:177–186

Clement T, Bielders CL, Degré A, et al (2023) Soil pitting mitigates runoff, erosion and pesticide surface losses in maize crops in the Belgian loess belt. Soil Tillage Res 234:105853. https://doi.org/10.1016/j.still.2023.105853

Clemente R, Hartley W, Riby P, et al (2010) Trace element mobility in a contaminated soil two years after field-amendment with a greenwaste compost mulch. Environ Pollut 158:1644–1651. https://doi.org/10.1016/j.envpol.2009.12.006

Cornu S, Lucas Y, Lebon E, et al (1999) Evidence of titanium mobility in soil profiles, Manaus, central Amazonia. Geoderma 91:281–295. https://doi.org/10.1016/S0016-7061(99)00007-5

Covelo EF, Couce MLA, Vega FA (2004) Competitive Adsorption and Desorption of Cadmium, Chromium, Copper, Nickel, Lead, and Zinc by Humic Umbrisols. Commun Soil Sci Plant Anal 35:2709–2729. https://doi.org/10.1081/CSS-200036421

Cox SE, Bell PB, Lowther JS, VanMetre, PC (2002) Vertical Distribution of Trace-Element Concentrations and Occurrence of Metallurgical Slag Particles in Accumulated Bed Sediments of Lake Roosevelt. U. S. Geological Survey, Washington

Crowe BM, Finnegan DL, Zoller WH, Boynton WV (1987) Trace element geochemistry of volcanic gases and particles from 1983–1984 eruptive episodes of Kilauea Volcano. J Geophys Res Solid Earth 92:13708–13714. https://doi.org/10.1029/JB092iB13p13708

Dai Z-H, Guan D-X, Bundschuh J, Ma LQ (2023) Roles of phytohormones in mitigating abiotic stress in plants induced by metal(loid)s As, Cd, Cr, Hg, and Pb. Crit Rev Environ Sci Technol 53:1310–1330. https://doi.org/10.1080/10643389.2022.2134694

Daouk S, Frege C, Blanc N, et al (2015) Fluorescence spectroscopy to study dissolved organic matter interactions with agrochemicals applied in Swiss vineyards. Environ Sci Pollut Res 22:9284–9292. https://doi.org/10.1007/s11356-015-4086-6

de Jonge LW, Kjaergaard C, Moldrup P (2004) Colloids and Colloid-Facilitated Transport of Contaminants in Soils: An Introduction. Vadose Zone J 3:321–325. https://doi.org/10.2136/vzj2004.0321

de Souza RM, Seibert D, Quesada HB, et al (2020) Occurrence, impacts and general aspects of pesticides in surface water: A review. Process Saf Environ Prot 135:22–37. https://doi.org/10.1016/j.psep.2019.12.035

Degryse F, Smolders E, Zhang H, Davison W (2009) Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. Environ Chem 6:198. https://doi.org/10.1071/EN09010

Del Puerto O, Gonçalves NPF, Medana C, et al (2022) Attenuation of toxicity and occurrence of degradation products of the fungicide tebuconazole after combined vacuum UV and UVC treatment of drinking water. Environ Sci Pollut Res 29:58312–58325. https://doi.org/10.1007/s11356-022-19691-0

Dereumeaux C, Saoudi A, Guldner L, et al (2020) Chlordecone and organochlorine compound levels in the French West Indies population in 2013–2014. Environ Sci Pollut Res 27:41033–41045. https://doi.org/10.1007/s11356-019-07181-9

Dimitriou K, Kassomenos P (2017) Airborne heavy metals in two cities of North Rhine Westphalia – Performing inhalation cancer risk assessment in terms of atmospheric circulation. Chemosphere 186:78–87. https://doi.org/10.1016/j.chemosphere.2017.07.138

Divisekara T, Navaratne AN, Abeysekara ASK (2018) Impact of a commercial glyphosate formulation on adsorption of Cd(II) and Pb(II) ions on paddy soil. Chemosphere 198:334–341. https://doi.org/10.1016/j.chemosphere.2018.01.155

Dock Fon Toy A, Uhing EH, Forest P (1964) Aminomethylenephosphinic acids, salts thereof, and process for their production

Dousset S, Jacobson AR, Dessogne J-B, et al (2007) Facilitated Transport of Diuron and Glyphosate in High Copper Vineyard Soils. Environ Sci Technol 41:8056–8061. https://doi.org/10.1021/es071664c

Drasch GA (1982) Lead burden in prehistorical, historical and modern human bones. Sci Total Environ 24:199–231. https://doi.org/10.1016/0048-9697(82)90001-8

Dudka S, Miller WP (1999) Accumulation of potentially toxic elements in plants and their transfer to human food chain. J Environ Sci Health Part B 34:681–708. https://doi.org/10.1080/03601239909373221

Duffus DJH (2001) “Heavy Metals”—A Meaningless Term. Chem Int 23:

Dytrtová JJ, Jakl M, Schröder D, et al (2011) Complexation between the fungicide tebuconazole and copper(II) probed by electrospray ionization mass spectrometry: Formation of complexes between tebuconazole and Cu(II) salts. Rapid Commun Mass Spectrom 25:1037–1042. https://doi.org/10.1002/rcm.4957

Ebrahimian E, Bybordi A (2014) Effect of Organic Acids on Heavy-Metal Uptake and Growth of Canola Grown in Contaminated Soil. Commun Soil Sci Plant Anal 45:1715–1725. https://doi.org/10.1080/00103624.2013.875206

EC (2023) Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance)

EC (2022) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, consolidated in 2022

EC (2024) EU Pesticides Database - Active substances. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances. Accessed 11 Aug 2024

Edmonds M, Mason E, Hogg O (2022) Volcanic Outgassing of Volatile Trace Metals. Annu Rev Earth Planet Sci 50:79–98. https://doi.org/10.1146/annurev-earth-070921-062047

El Azzi D, Viers J, Guiresse M, et al (2013) Origin and fate of copper in a small Mediterranean vineyard catchment: New insights from combined chemical extraction and δ65Cu isotopic composition. Sci Total Environ 463–464:91–101. https://doi.org/10.1016/j.scitotenv.2013.05.058

El-Kady AA, Abdel-Wahhab MA (2018) Occurrence of trace metals in foodstuffs and their health impact. Trends Food Sci Technol 75:36–45. https://doi.org/10.1016/j.tifs.2018.03.001

Emran M, Gispert M, Pardini G (2012) Patterns of soil organic carbon, glomalin and structural stability in abandoned Mediterranean terraced lands. Eur J Soil Sci 63:637–649. https://doi.org/10.1111/j.1365-2389.2012.01493.x

Epstein L (2014) Fifty Years Since *Silent Spring*. Annu Rev Phytopathol 52:377–402. https://doi.org/10.1146/annurev-phyto-102313-045900

Erban T, Stehlik M, Sopko B, et al (2018) The different behaviors of glyphosate and AMPA in compost-amended soil. Chemosphere 207:78–83. https://doi.org/10.1016/j.chemosphere.2018.05.004

EU (2024) Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006

Evans PD, Schmalzl KJ, Forsyth CM, et al (2007) Formation and Structure of Metal Complexes with the Fungicides Tebuconazole and Propiconazole. J Wood Chem Technol 27:243–256. https://doi.org/10.1080/02773810701702220

Evenson RE, Gollin D (2003) Assessing the Impact of the Green Revolution, 1960 to 2000. Science 300:758–762. https://doi.org/10.1126/science.1078710

Fantke P, Charles R, Alencastro LF de, et al (2011) Plant uptake of pesticides and human health: Dynamic modeling of residues in wheat and ingestion intake. Chemosphere 85:1639–1647. https://doi.org/10.1016/j.chemosphere.2011.08.030

FAO (2022) Pesticides use, pesticides trade and pesticides indicators – Global, regional and country trends, 1990–2020, FAOSTAT Analytical Briefs. FAO, Rome

Faust DR, Kröger R, Miranda LE, Rush SA (2016) Nitrate Removal from Agricultural Drainage Ditch Sediments with Amendments of Organic Carbon: Potential for an Innovative Best Management Practice. Water Air Soil Pollut 227:378. https://doi.org/10.1007/s11270-016-3075-9

Franz JE (1974) N-phosphonomethyl-glycine phytotoxicant compositions

Froger C, Jolivet C, Budzinski H, et al (2023) Pesticide Residues in French Soils: Occurrence, Risks, and Persistence. Environ Sci Technol 57:7818–7827. https://doi.org/10.1021/acs.est.2c09591

Gao S, Walker WJ, Dahlgren RA, Bold J (1997) Simultaneous sorption of Cd, Cu, Ni, Zn, Pb, and Cr on soils treated with sewage

sludge supernatant. Water Air Soil Pollut 93:331–345. https://doi.org/10.1007/BF02404765

Garcia-Rubio R, Cuenca-Estrella M, Mellado E (2017) Triazole Resistance in Aspergillus Species: An Emerging Problem. Drugs 77:599–613. https://doi.org/10.1007/s40265-017-0714-4

Gawel JE, Ahner BA, Friedland AJ, Morel FMM (1996) Role for heavy metals in forest decline indicated by phytochelatin measurements. Nature 381:64–65. https://doi.org/10.1038/381064a0

Glass RL (1984) Metal complex formation by glyphosate. J Agric Food Chem 32:1249–1253. https://doi.org/10.1021/jf00126a010

Glenn EP (1987) Relationship between cation accumulation and water content of salt-tolerant grasses and a sedge. Plant Cell Environ 10:205–212. https://doi.org/10.1111/1365-3040.ep11602236

Gleyzes C, Tellier S, Astruc M (2002) Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. TrAC Trends Anal Chem 21:451–467. https://doi.org/10.1016/S0165-9936(02)00603-9

Goldhaber SB (2003) Trace element risk assessment: essentiality vs. toxicity. Regul Toxicol Pharmacol 38:232–242. https://doi.org/10.1016/S0273-2300(02)00020-X

Goldschmidt VM (1937) The principles of distribution of chemical elements in minerals and rocks. The seventh Hugo Müller Lecture, delivered before the Chemical Society on March 17th, 1937. J Chem Soc 0:655–673. https://doi.org/10.1039/JR9370000655

Gouvernement wallon (2018) Législation/décret gestion et assainissement sols. http://environnement.wallonie.be/legis/solsous-sol/sol006.htm. Accessed 16 Aug 2024

Gu Baohua, Schmitt Juergen, Chen Zhihong, et al (1994) Adsorption and desorption of natural organic matter on iron oxide: mechanisms and models. Environ Sci Technol 28:38–46. https://doi.org/10.1021/es00050a007

Harada M (1995) Minamata Disease: Methylmercury Poisoning in Japan Caused by Environmental Pollution. Crit Rev Toxicol 25:1–24. https://doi.org/10.3109/10408449509089885

Harlavan Y, Shirav M, Ilani S, et al (2021) The fate of anthropogenic Pb in soils; years after Pb terminated as a fuel additive; Northern Israel. Environ Pollut 271:116319. https://doi.org/10.1016/j.envpol.2020.116319

Harnly ME, Bradman A, Nishioka M, et al (2009) Pesticides in Dust from Homes in an Agricultural Area. Environ Sci Technol 43:8767–8774. https://doi.org/10.1021/es9020958

Hart SR, Allègre CJ (1980) Chapter 4. Trace-Element Constraints on Magma Genesis. In: Physics of Magmatic Processes. Princeton University Press, pp 121–160

Hartmann A, Schmid M, Tuinen D van, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257. https://doi.org/10.1007/s11104-008-9814-y

Häussling M, Jorns CA, Lehmbecker G, et al (1988) Ion and Water Uptake in Relation to Root Development in Norway Spruce (Picea abies (L.) Karst.). J Plant Physiol 133:486–491. https://doi.org/10.1016/S0176-1617(88)80042-7

Haydon MJ, Cobbett CS (2007) Transporters of ligands for essential metal ions in plants. New Phytol 174:499–506. https://doi.org/10.1111/j.1469-8137.2007.02051.x

He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 19:125–140. https://doi.org/10.1016/j.jtemb.2005.02.010

Helal AA, Imam DM, Khalifa SM, Aly HF (2006) Interaction of pesticides with humic compounds and their metal complexes. Radiochemistry 48:419–425. https://doi.org/10.1134/S1066362206040199

Helios Rybicka E (1996) Impact of mining and metallurgical industries on the environment in Poland. Appl Geochem 11:3–9. https://doi.org/10.1016/0883-2927(95)00083-6

Helling CS (1971) Pesticide Mobility in Soils III. Influence of Soil Properties. Soil Sci Soc Am J 35:743–748. https://doi.org/10.2136/sssaj1971.03615995003500050032x

Hooda PS (ed) (2010) Trace elements in soils. Wiley, Chichester, West Sussex

Huang H, Zhang S, Christie P, et al (2010) Behavior of Decabromodiphenyl Ether (BDE-209) in the Soil−Plant System: Uptake, Translocation, and Metabolism in Plants and Dissipation in Soil. Environ Sci Technol 44:663–667. https://doi.org/10.1021/es901860r

Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. Toxicol Lett 133:1–16. https://doi.org/10.1016/S0378-4274(02)00084-X

Ighalo JO, Ajala OJ, Adeniyi AG, et al (2021) Ecotoxicology of glyphosate and recent advances in its mitigation by adsorption. Environ Sci Pollut Res 28:2655–2668. https://doi.org/10.1007/s11356-020-11521-5

IRM (2024) IRM - Climat dans votre commune. In: KMI. https://www.meteo.be/fr/climat/climat-de-la-belgique/climat-dans-votre-commune. Accessed 14 Aug 2024

IUPAC (ed) (2019) The IUPAC Compendium of Chemical Terminology: The Gold Book, 4th edn. International Union of Pure and Applied Chemistry (IUPAC), Research Triangle Park, NC

Järup L, Åkesson A (2009) Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 238:201–208. https://doi.org/10.1016/j.taap.2009.04.020

Jickells TD, An ZS, Andersen KK, et al (2005) Global Iron Connections Between Desert Dust, Ocean Biogeochemistry, and Climate. Science 308:67–71. https://doi.org/10.1126/science.1105959

John DA, Babu GR (2021) Lessons From the Aftermaths of Green Revolution on Food System and Health. Front Sustain Food Syst 5:644559. https://doi.org/10.3389/fsufs.2021.644559

Ju C, Li X, He S, et al (2020) Root Uptake of Imidacloprid and Propiconazole Is Affected by Root Composition and Soil Characteristics. J Agric Food Chem 68:15381–15389. https://doi.org/10.1021/acs.jafc.0c02170

Juraske R, Mutel CL, Stoessel F, Hellweg S (2009) Life cycle human toxicity assessment of pesticides: Comparing fruit and vegetable diets in Switzerland and the United States. Chemosphere 77:939–945. https://doi.org/10.1016/j.chemosphere.2009.08.006

Kabata-Pendias A (2011) Trace elements in soils and plants, 4th ed. CRC Press, Boca Raton

Kabata-Pendias A (2004) Soil–plant transfer of trace elements—an environmental issue. Geoderma 122:143–149. https://doi.org/10.1016/j.geoderma.2004.01.004

Kalyabina VP, Esimbekova EN, Kopylova KV, Kratasyuk VA (2021) Pesticides: formulants, distribution pathways and effects on human health – a review. Toxicol Rep 8:1179–1192. https://doi.org/10.1016/j.toxrep.2021.06.004

Kashem MA, Singh BR (2001) Metal availability in contaminated soils: I. Effects of floodingand organic matter on changes in Eh, pH and solubility of Cd, Ni andZn. Nutr Cycl Agroecosystems 61:247–255

Kaur S, Kumar V, Chawla M, et al (2017) Pesticides Curbing Soil Fertility: Effect of Complexation of Free Metal Ions. Front Chem 5:43. https://doi.org/10.3389/fchem.2017.00043

Khan S, Naushad Mu, Lima EC, et al (2021) Global soil pollution by toxic elements: Current status and future perspectives on the risk assessment and remediation strategies – A review. J Hazard Mater 417:126039. https://doi.org/10.1016/j.jhazmat.2021.126039

Kim R-Y, Yoon J-K, Kim T-S, et al (2015) Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review. Environ Geochem Health 37:1041–1061. https://doi.org/10.1007/s10653-015-9695-y

Kim Y-J, Darnault CJG, Bailey NO (2005) Equation for Describing Solute Transport in Field Soils with Preferential Flow Paths. SOIL SCI SOC AM J 69:

Köhne JM, Köhne S, Šimůnek J (2009) A review of model applications for structured soils: b) Pesticide transport. J Contam Hydrol 104:36–60. https://doi.org/10.1016/j.jconhyd.2008.10.003

Korshin GV, Benjamin MM, Sletten RS (1997) Adsorption of natural organic matter (NOM) on iron oxide: Effects on NOM composition and formation of organo-halide compounds during chlorination. Water Res 31:1643–1650. https://doi.org/10.1016/S0043-1354(97)00007-9

Krauss M, Wilcke W, Zech W (2000) Availability of Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) to Earthworms in Urban Soils. Environ Sci Technol 34:4335–4340. https://doi.org/10.1021/es001137s

Krogh KA, Halling-Sørensen B, Mogensen BB, Vejrup KV (2003) Environmental properties and effects of nonionic surfactant adjuvants in pesticides: a review. Chemosphere 50:871–901. https://doi.org/10.1016/S0045-6535(02)00648-3

Kumar D, Singh R, Upadhyay SK, et al (2024) Review on interactions between nanomaterials and phytohormones: Novel perspectives and opportunities for mitigating environmental challenges. Plant Sci 340:111964. https://doi.org/10.1016/j.plantsci.2023.111964

Landrigan PJ, Schechter CB, Lipton JM, et al (2002) Environmental pollutants and disease in American children: estimates of morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities. Environ Health Perspect 110:721–728. https://doi.org/10.1289/ehp.02110721

Leaper C, Holloway PJ (2000) Adjuvants and glyphosate activity. Pest Manag Sci 56:313–319. https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<313::AID-PS147>3.0.CO;2-3

Lee D-Y, Farmer WJ, Aochi Y (1990) Sorption of Napropamide on Clay and Soil in the Presence of Dissolved Organic Matter. J

Environ Qual 19:567–573. https://doi.org/10.2134/jeq1990.00472425001900030035x

Leenhardt S, Mamy L, Pesce S, Sanchez W (2023) Impacts des produits phytopharmaceutiques sur la biodiversité et les services écosystémiques. éditions Quae

Levinson H, Levinson A (1998) Control of stored food pests in the ancient Orient and classical antiquity. J Appl Entomol 122:137–144. https://doi.org/10.1111/j.1439-0418.1998.tb01475.x

Lewis KA, Tzilivakis J, Warner D, Gree. A (2016) An international database for pesticide risk assessments and management. Hum Ecol Risk Assess Int J 22:1050–1064. https://doi.org/DOI: 10.1080/10807039.2015.1133242

Li J, Xi T, Yan B, et al (2015) Synergistic actions between tebuconazole ligand and Cu(II) cation: reasons for the enhanced antifungal activity of four Cu(II) complexes based on the fungicide tebuconazole. New J Chem 39:9550–9556. https://doi.org/10.1039/C5NJ01845E

Li J, Zhang Y, Yang M, Ma H (2017a) Two novel Co(II) and Ni(II) complexes of tebuconazole with enhanced antifungal activities. RSC Adv 7:33364–33372. https://doi.org/10.1039/C7RA03629A

Li K, Xing B, Torello WA (2005) Effect of organic fertilizers derived dissolved organic matter on pesticide sorption and leaching. Environ Pollut 134:187–194. https://doi.org/10.1016/j.envpol.2004.08.011

Li L-Z, Tu C, Peijnenburg WJGM, Luo Y-M (2017b) Characteristics of cadmium uptake and membrane transport in roots of intact wheat (Triticum aestivum L.) seedlings. Environ Pollut 221:351–358. https://doi.org/10.1016/j.envpol.2016.11.085

Li MS, Luo YP, Su ZY (2007) Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environ Pollut 147:168–175. https://doi.org/10.1016/j.envpol.2006.08.006

Liu C-M, McLean PA, Sookdeo CC, Cannon FC (1991) Degradation of the Herbicide Glyphosate by Members of the Family *Rhizobiaceae*. Appl Environ Microbiol 57:1799–1804. https://doi.org/10.1128/aem.57.6.1799-1804.1991

Lombi E, Holm PE (2010) Metalloids, Soil Chemistry and the Environment. In: Jahn TP, Bienert GP (eds) MIPs and Their Role in the Exchange of Metalloids. Springer New York, New York, NY, pp 33–44

Long G-Q, Jiang Y-J, Sun B (2015) Seasonal and inter-annual variation of leaching of dissolved organic carbon and nitrogen under long-term manure application in an acidic clay soil in subtropical China. Soil Tillage Res 146:270–278. https://doi.org/10.1016/j.still.2014.09.020

Lovering TG (1976) Lead in the Environment. U.S. Department of the Interior, Geological Survey

Lucadamo L (2018) Evaluation of glyphosate drift and anthropogenic atmospheric trace elements contamination by means of lichen transplants in a southern Italian agricultural district. Air Qual Atmos Health 15

Lunk H-J (2015) Discovery, properties and applications of chromium and its compounds. ChemTexts 1:6. https://doi.org/10.1007/s40828-015-0007-z

Madsen, H, Christensen, H, Gottlieb-Petersen, C (1978) Stability constants of copper (II), zinc, manganese (II), calcium, and magnesium complexes of n-(phosphonomethyl) glycine (glyphosate). Acta Chem Scand A 32:79–83

Main AR, Hladik ML, Webb EB, et al (2020) Beyond neonicotinoids – Wild pollinators are exposed to a range of pesticides while foraging in agroecosystems. Sci Total Environ 742:140436. https://doi.org/10.1016/j.scitotenv.2020.140436

Manceau A, Matynia A (2010) The nature of Cu bonding to natural organic matter. Geochim Cosmochim Acta 74:2556–2580. https://doi.org/10.1016/j.gca.2010.01.027

Maqueda C, Morillo E, Undabeytia T (2002) Cosorption of glyphosate and copper (II) on goethite: Soil Sci 167:659–665. https://doi.org/10.1097/00010694-200210000-00004

Martin JM, Nirel P, Thomas AJ (1987) Sequential extraction techniques: Promises and problems. Mar Chem 22:313–341. https://doi.org/10.1016/0304-4203(87)90017-X

Martínez CE, McBride MB (1999) Dissolved and Labile Concentrations of Cd, Cu, Pb, and Zn in Aged Ferrihydrite−Organic Matter Systems. Environ Sci Technol 33:745–750. https://doi.org/10.1021/es980576c

Matthews GA (2018) A History of Pesticides. CABI

McBride M, Kung K (1989) Complexation of Glyphosate and Related Ligands with Iron (III). Soil Sci Soc Am J 53:1668–1673. https://doi.org/10.2136/sssaj1989.03615995005300060009x

McBride MB (1994) Enviromental chemistry of soils. Oxford University Press, New York

Meers E, Lesage E, Lamsal S, et al (2005) Enhanced Phytoextraction: I. Effect of EDTA and Citric Acid on Heavy Metal Mobility in a Calcareous Soil. Int J Phytoremediation 7:129–142. https://doi.org/10.1080/16226510590950423

Mendoza-Cózatl DG, Jobe TO, Hauser F, Schroeder JI (2011) Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. Curr Opin Plant Biol 14:554–562. https://doi.org/10.1016/j.pbi.2011.07.004

Mertens M, Höss S, Neumann G, et al (2018) Glyphosate, a chelating agent—relevant for ecological risk assessment? Environ Sci Pollut Res 25:5298–5317. https://doi.org/10.1007/s11356-017-1080-1

Mertz W (1981) The Essential Trace Elements. Science 213:1332–1338. https://doi.org/10.1126/science.7022654

Mesnage R, Antoniou MN (2018) Roundup Ready! Glyphosate and the Current Controversy Over the World's Leading Herbicide. In: Encyclopedia of the Anthropocene. Elsevier, pp 149–153

Mesnage R, Benbrook C, Antoniou MN (2019) Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food Chem Toxicol 128:137–145. https://doi.org/10.1016/j.fct.2019.03.053

Miah MR, Ijomone OM, Okoh COA, et al (2020) The effects of manganese overexposure on brain health. Neurochem Int 135:104688. https://doi.org/10.1016/j.neuint.2020.104688

Minasny B, Malone BP, McBratney AB, et al (2017) Soil carbon 4 per mille. Geoderma 292:59–86. https://doi.org/10.1016/j.geoderma.2017.01.002

Mishra K, Tandon PK, Srivastava S (eds) (2020) Sustainable Solutions for Elemental Deficiency and Excess in Crop Plants. Springer Singapore, Singapore

Modrzewska B, Wyszkowski M (2014) Trace metals content in soils along the state road 51 (northeastern Poland). Environ Monit Assess 186:2589–2597. https://doi.org/10.1007/s10661-013-3562-z

Monhonval A, Mauclet E, Hirst C, et al (2023) Mineral organic carbon interactions in dry versus wet tundra soils. Geoderma 436:116552. https://doi.org/10.1016/j.geoderma.2023.116552

Morillo E, Maqueda C, Bejarano M, et al (1994) Cu(II)-Glyphosate system: A study by anodic stripping voltammetry and the influence on Cu adsorption by montmorillonite. Chemosphere 28:2185–2196. https://doi.org/10.1016/0045-6535(94)90186-4

Morillo E, Undabeytia T, Maqueda C (1997) Adsorption of Glyphosate on the Clay Mineral Montmorillonite: Effect of Cu(II) in Solution and Adsorbed on the Mineral. Environ Sci Technol 31:3588–3592. https://doi.org/10.1021/es970341l

Morillo E, Undabeytia T, Maqueda C, Ramos A (2000) Glyphosate adsorption on soils of different characteristics. Influence of copper addition. Chemophere 5

Morillo E, Undabeytia T, Maqueda C, Ramos A (2002) The effect of dissolved glyphosate upon the sorption of copper by three selected soils. Chemosphere 47:747–752. https://doi.org/10.1016/S0045-6535(01)00338-1

Müller K, Magesan GN, Bolan NS (2007) A critical review of the influence of effluent irrigation on the fate of pesticides in soil. Agric Ecosyst Environ 120:93–116. https://doi.org/10.1016/j.agee.2006.08.016

Natasha N, Shahid M, Khalid S, et al (2022) Influence of biochar on trace element uptake, toxicity and detoxification in plants and associated health risks: A critical review. Crit Rev Environ Sci Technol 52:2803–2843. https://doi.org/10.1080/10643389.2021.1894064

Needleman HL, Bellinger D (1991) The Health Effects of Low Level Exposure to Lead. Annu Rev Public Health 12:111–140

Neumann G, Römheld V (1999) Root excretion of carboxylic acids and protons in phosphorus-deficient plants

Nicholson FA, Smith SR, Alloway BJ, et al (2003) An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci Total Environ 311:205–219. https://doi.org/10.1016/S0048-9697(03)00139-6

Norková R, Dytrtová JJ, Jakl M, Schröder D (2012) Formation of Tebuconazole Complexes with Cadmium(II) Investigated by Electrospray Ionization Mass Spectrometry. Water Air Soil Pollut 223:2633–2640. https://doi.org/10.1007/s11270-011-1055-7

Nowack B, Sigg L (1997) Dissolution of Fe(III) (hydr) oxides by metal-EDTA complexes. Geochim Cosmochim Acta 61:951–963. https://doi.org/10.1016/S0016-7037(96)00391-2

Nriagu J, Bhattacharya P, Mukherjee A, et al (2007) Arsenic in soil and groundwater: an overview. In: Trace Metals in the Environment. Elsevier, pp 3–60

Nriagu JO (1989) A global assessment of natural sources of atmospheric trace metals. Nature 338:47–49. https://doi.org/10.1038/338047a0

Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333:134–139. https://doi.org/10.1038/333134a0

Nunan N, Ritz K, Rivers M, et al (2006) Investigating microbial micro-habitat structure using X-ray computed tomography. Geoderma 133:398–407. https://doi.org/10.1016/j.geoderma.2005.08.004

Öborn I, Jansson G, Johnsson L (1995) A field study on the influence of soil ph on trace element levels in spring wheat (Triticum aestivum), potatoes (Solanum tuberosum) and carrots (Daucus carota). Water Air Soil Pollut 85:835–840. https://doi.org/10.1007/BF00476933

Okkenhaug G, Zhu Y-G, Luo L, et al (2011) Distribution, speciation and availability of antimony (Sb) in soils and terrestrial plants from an active Sb mining area. Environ Pollut 159:2427–2434. https://doi.org/10.1016/j.envpol.2011.06.028

Ololade IA, Oladoja NA, Oloye FF, et al (2014) Sorption of Glyphosate on Soil Components: The Roles of Metal Oxides and Organic Materials. Soil Sediment Contam Int J 23:571–585. https://doi.org/10.1080/15320383.2014.846900

Paasivirta J (1998) New types of environmental POPs. Toxicol Environ Chem 66:59–70. https://doi.org/10.1080/02772249809358584

Pacyna JM, Pacyna EG (eds) (2016) Environmental Determinants of Human Health. Springer International Publishing, Cham

Page KL, Dang YP, Dalal RC (2020) The Ability of Conservation Agriculture to Conserve Soil Organic Carbon and the Subsequent Impact on Soil Physical, Chemical, and Biological Properties

and Yield. Front Sustain Food Syst 4:31. https://doi.org/10.3389/fsufs.2020.00031

Pathak HK, Seth CS, Chauhan PK, et al (2024) Recent advancement of nano-biochar for the remediation of heavy metals and emerging contaminants: Mechanism, adsorption kinetic model, plant growth and development. Environ Res 255:119136. https://doi.org/10.1016/j.envres.2024.119136

Patterson CC, Settle DM (1987) Review of data on eolian fluxes of industrial and natural lead to the lands and seas in remote regions on a global scale. Mar Chem 22:137–162. https://doi.org/10.1016/0304-4203(87)90005-3

Pedeferri P (2018) Corrosion Science and Engineering. Springer International Publishing, Cham

Pédrot M, Dia A, Davranche M, et al (2008) Insights into colloid-mediated trace element release at the soil/water interface. J Colloid Interface Sci

Pereira B, Vandeuren A, Delmelle P (2018) Rapport final de la convention de recherche intitulée : "Gestion des concentrations de fond élevées à l'échelle régionale : mesure et diagnostic du risque adapté aux spécificités du contexte wallon" (acronyme : CAPASOL 6)

Perry MW, Greenway H (1973) Permeation of Uncharged Organic Molecules and Water Through Tomato Roots. Ann Bot 37:225–232. https://doi.org/10.1093/oxfordjournals.aob.a084678

Peterson WH, Skinner JT (1931) Distribution of Manganese in Foods. J Nutr 4:419–426. https://doi.org/10.1093/jn/4.3.419

Phytoweb (2015) Consulter autorisations de produits phytopharmaceutiques. https://fytoweb.be/fr/produits-phytopharmaceutiques/consulter-autorisations-de-produits-phytopharmaceutiques. Accessed 10 Aug 2023

Phytoweb (2024) Consulter autorisations de produits phytopharmaceutiques. In: Phytoweb. https://fytoweb.be/fr/produits-phytopharmaceutiques/consulter-autorisations-de-produits-phytopharmaceutiques

Piccolo A, Celano G, Pietramellara G (1992) Adsorption of the herbicide glyphosate on a metal-humic acid complex. Sci Total Environ 123–124:77–82. https://doi.org/10.1016/0048-9697(92)90134-E

Pline WA, Wilcut JW, Duke SO, et al (2002) Tolerance and Accumulation of Shikimic Acid in Response to Glyphosate Applications in Glyphosate-Resistant and Nonglyphosate-Resistant Cotton (*Gossypium hirsutum* L.). J Agric Food Chem 50:506–512. https://doi.org/10.1021/jf0110699

Poulakis E, Theodosi C, Bressi M, et al (2015) Airborne mineral components and trace metals in Paris region: spatial and temporal variability. Environ Sci Pollut Res 22:14663–14672. https://doi.org/10.1007/s11356-015-4679-0

Prasad MNV, Sajwan KS, Naidu R (eds) (2006) Trace elements in the environment: biogeochemistry, biotechnology, and bioremediation. CRC/Taylor and Francis, Boca Raton

Price CL, Parker JE, Warrilow AG, et al (2015) Azole fungicides - understanding resistance mechanisms in agricultural fungal pathogens: Mode of action and resistance mechanisms to azole fungicides. Pest Manag Sci 71:1054–1058. https://doi.org/10.1002/ps.4029

Qiao D, Lu H, Zhang X (2020) Change in phytoextraction of Cd by rapeseed (Brassica napus L.) with application rate of organic acids and the impact of Cd migration from bulk soil to the rhizosphere. Environ Pollut 267:115452. https://doi.org/10.1016/j.envpol.2020.115452

Quinton JN, Catt JA (2007) Enrichment of Heavy Metals in Sediment Resulting from Soil Erosion on Agricultural Fields. Environ Sci Technol 41:3495–3500. https://doi.org/10.1021/es062147h

Ratnaike RN (2003) Acute and chronic arsenic toxicity. Postgrad Med J 79:391–396. https://doi.org/10.1136/pmj.79.933.391

Rauch JN, Pacyna JM (2009) Earth's global Ag, Al, Cr, Cu, Fe, Ni, Pb, and Zn cycles: GLOBAL METAL CYCLES. Glob Biogeochem Cycles 23:n/a-n/a. https://doi.org/10.1029/2008GB003376

Rauret G (1998) Extraction procedures for the determination of heavy metals in contaminated soil and sediment. Talanta 46:449–455. https://doi.org/10.1016/S0039-9140(97)00406-2

Redman AD, Macalady DL, Ahmann D (2002) Natural Organic Matter Affects Arsenic Speciation and Sorption onto Hematite. Environ Sci Technol 36:2889–2896. https://doi.org/10.1021/es0112801

Reichman SM, Parker DR (2005) Metal complexation by phytosiderophores in the rhizosphere. In: Biogeochemistry of trace elements in the rhizosphere. Elsevier, Amsterdam, pp 129–156

Renkema H, Koopmans A, Kersbergen L, et al (2012) The effect of transpiration on selenium uptake and mobility in durum wheat and spring canola. Plant Soil 354:239–250. https://doi.org/10.1007/s11104-011-1069-3

Robinson BH, Bañuelos G, Conesa HM, et al (2009) The Phytomanagement of Trace Elements in Soil. Crit Rev Plant Sci 28:240–266. https://doi.org/10.1080/07352680903035424

Rodríguez-Cruz MS, Sánchez-Martín MJ (eds) (2022) Pesticides in Soils: Occurrence, Fate, Control and Remediation. Springer International Publishing, Cham

RTBF (2017) Le plus grand potager collectif de Wallonie, à Bressoux, pollué aux métaux lourds. In: RTBF. https://www.rtbf.be/article/le-plus-grand-potager-collectif-de-wallonie-a-bressoux-pollue-aux-metaux-lourds-9663157. Accessed 14 Aug 2024

Rubio B, Nombela MA, Vilas F (2000) Geochemistry of Major and Trace Elements in Sediments of the Ria de Vigo (NW Spain): an Assessment of Metal Pollutionq. Mar Pollut Bull 40:

Sager M (2007) Trace and nutrient elements in manure, dung and compost samples in Austria. Soil Biol Biochem 39:1383–1390. https://doi.org/10.1016/j.soilbio.2006.12.015

Satarug S, Baker JR, Urbenjapol S, et al (2003) A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 137:65–83. https://doi.org/10.1016/S0378-4274(02)00381-8

Schaider LA, Parker DR, Sedlak DL (2006) Uptake of EDTA-complexed Pb, Cd and Fe by solution- and sand-cultured Brassica juncea. Plant Soil 286:377–391. https://doi.org/10.1007/s11104-006-9049-8

Schmidt U (2003) Enhancing Phytoextraction: The Effect of Chemical Soil Manipulation on Mobility, Plant Accumulation, and Leaching of Heavy Metals. J Environ Qual 32:1939–1954. https://doi.org/10.2134/jeq2003.1939

Schmitt D, Taylor HE, Aiken GR, et al (2002) Influence of Natural Organic Matter on the Adsorption of Metal Ions onto Clay Minerals. Environ Sci Technol 36:2932–2938. https://doi.org/10.1021/es010271p

Shaheen SM, Tsadilas CD, Rinklebe J (2013) A review of the distribution coefficients of trace elements in soils: Influence of sorption system, element characteristics, and soil colloidal properties. Adv Colloid Interface Sci 201–202:43–56. https://doi.org/10.1016/j.cis.2013.10.005

Shahjahan M, Taslima K, Rahman MS, et al (2022) Effects of heavy metals on fish physiology – A review. Chemosphere 300:134519. https://doi.org/10.1016/j.chemosphere.2022.134519

Sheals J, Sjöberg S, Persson P (2002) Adsorption of Glyphosate on Goethite: Molecular Characterization of Surface Complexes. Environ Sci Technol 36:3090–3095. https://doi.org/10.1021/es010295w

Shimadzu (2011) TOC-L Series : SHIMADZU (Shimadzu Corporation). https://www.shimadzu.com/an/products/total-organic-

carbon-analysis/toc-analysis/toc-l-series/index.html. Accessed 15 Aug 2024

Si Y-B, Xiang Y, Tian C, et al (2013) Complex Interaction and Adsorption of Glyphosate and Lead in Soil. Soil Sediment Contam Int J 22:72–84. https://doi.org/10.1080/15320383.2012.714421

Silva V, Mol HGJ, Zomer P, et al (2019) Pesticide residues in European agricultural soils – A hidden reality unfolded. Sci Total Environ 653:1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441

Singla A, Barmota H, Kumar Sahoo S, Kaur Kang B (2021) Influence of neonicotinoids on pollinators: A review. J Apic Res 60:19–32. https://doi.org/10.1080/00218839.2020.1825044

Skidmore EL, Jacobs HS, Powers WL (1969) Potential Evapotranspiration as Influenced by Wind [1]. Agron J 61:543–546. https://doi.org/10.2134/agronj1969.00021962006100040018x

Smedley PL, Kinniburgh DG (2002) A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 17:517–568. https://doi.org/10.1016/S0883-2927(02)00018-5

Smith AE, Secoy DM (1975) Forerunners of pesticides in classical Greece and Rome. J Agric Food Chem 23:1050–1055. https://doi.org/10.1021/jf60202a004

Song NH, Chen L, Yang H (2008) Effect of dissolved organic matter on mobility and activation of chlorotoluron in soil and wheat. Geoderma 146:344–352. https://doi.org/10.1016/j.geoderma.2008.05.031

Sposito G, Holtzclaw KM, LeVesque-Madore CS (1979) Cupric Ion Complexation by Fulvic Acid Extracted from Sewage Sludge-Soil Mixtures. Soil Sci Soc Am J 43:1148–1155. https://doi.org/10.2136/sssaj1979.03615995004300060018x

Sprankle P, Meggitt WF, Penner D (1975) Adsorption, Mobility, and Microbial Degradation of Glyphosate in the Soil. Weed Sci 23:229–234. https://doi.org/10.1017/S0043174500052929

SPW (2023) Utilisation de produits phytopharmaceutiques

SPW (2022a) Pesticides dans les eaux souterraines

SPW (2022b) Carte Numérique des Sols de Wallonie. http://geoportail.wallonie.be/catalogue/38c2a87e-d38a-4359-9899-9d4a6b9f0c2a.html. Accessed 14 Aug 2024

Stevens MH, Jacobsen T, Crofts AK (2013) Lead and the deafness of Ludwig van Beethoven. The Laryngoscope 123:2854–2858. https://doi.org/10.1002/lary.24120

Su Y-H, Zhu Y-G (2007) Transport mechanisms for the uptake of organic compounds by rice (Oryza sativa) roots. Environ Pollut 148:94–100. https://doi.org/10.1016/j.envpol.2006.11.004

Subramaniam V, Hoggard PE (1988) Metal complexes of glyphosate. J Agric Food Chem 36:1326–1329. https://doi.org/10.1021/jf00084a050

Suda A, Makino T (2016) Functional effects of manganese and iron oxides on the dynamics of trace elements in soils with a special focus on arsenic and cadmium: A review. Geoderma 270:68–75. https://doi.org/10.1016/j.geoderma.2015.12.017

Sungur A, Soylak M, Ozcan H (2014) Investigation of heavy metal mobility and availability by the BCR sequential extraction procedure: relationship between soil properties and heavy metals availability. Chem Speciat Bioavailab 26:219–230. https://doi.org/10.3184/095422914X14147781158674

Swaine DJ (2013) Trace Elements in Coal. Butterworth-Heinemann

Taiz L, Zeiger E (2006) Plant physiology, Fourth ed. Sinauer associates, Sunderland (Mass.)

Takahashi M, Terada Y, Nakai I, et al (2003) Role of Nicotianamine in the Intracellular Delivery of Metals and Plant Reproductive Development. Plant Cell 15:1263–1280. https://doi.org/10.1105/tpc.010256

Tanji K, Valoppi L (1989) Groundwater contamination by trace elements. Agric Ecosyst Environ 26:229–274. https://doi.org/10.1016/0167-8809(89)90015-7

Taxvig C, Hass U, Axelstad M, et al (2007) Endocrine-Disrupting Activities In Vivo of the Fungicides Tebuconazole and Epoxiconazole. Toxicol Sci 100:464–473. https://doi.org/10.1093/toxsci/kfm227

Taylor AR, Bloom AJ (1998) Ammonium, nitrate, and proton fluxes along the maize root. Plant Cell Environ 21:1255–1263. https://doi.org/10.1046/j.1365-3040.1998.00357.x

Tesfamariam T, Bott S, Cakmak I, et al (2009) Glyphosate in the rhizosphere—Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. Eur J Agron 31:126–132. https://doi.org/10.1016/j.eja.2009.03.007

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51:844–851. https://doi.org/10.1021/ac50043a017

Thami A (2022) Transfert des éléments traces dans le système sol-plante : Influence d'apports de matières organiques sur des sols agricoles non contaminés et contaminés. UCLouvain

Tsui MTK, Wang W-X, Chu LM (2005) Influence of glyphosate and its formulation (Roundup®) on the toxicity and bioavailability of metals to Ceriodaphnia dubia. Environ Pollut 138:59–68. https://doi.org/10.1016/j.envpol.2005.02.018

Underwood EJ (1977) Trace Elements in Human and Animal Nutrition, 4th edn. Academic Press, New York

Uriu-Adams JY, Keen CL (2005) Copper, oxidative stress, and human health. Mol Aspects Med 26:268–298. https://doi.org/10.1016/j.mam.2005.07.015

Van Bruggen AHC, He MM, Shin K, et al (2018) Environmental and health effects of the herbicide glyphosate. Sci Total Environ 616–617:255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Van Den Berg F, Kubiak R, Benjey WG, et al (1999) Emission of Pesticides into the Air. In: Van Dijk HFG, Van Pul WAJ, De Voogt P (eds) Fate of Pesticides in the Atmosphere: Implications for Environmental Risk Assessment. Springer Netherlands, Dordrecht, pp 195–218

Vandeuren A, Pereira B, Kaba AJ, et al (2023) Environmental bioavailability of arsenic, nickel and chromium in soils impacted by high geogenic and anthropogenic background contents. Sci Total Environ 902:166073. https://doi.org/10.1016/j.scitotenv.2023.166073

Vassil AD, Kapulnik Y, Raskin I, Salt DE (1998) The Role of EDTA in Lead Transport and Accumulation by Indian Mustard. Plant Physiol 117:447–453. https://doi.org/10.1104/pp.117.2.447

Vaughan D, Lumsdon DG, Linehan DJ (1993) Influence of Dissolved Organic Matter On the Bio-Availability and Toxicity of Metals in Soils and Aquatic Systems. Chem Ecol 8:185–201. https://doi.org/10.1080/02757549308035308

Veiga F, Zapata JM, Fernandez Marcos ML, Alvarez E (2001) Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain. Sci Total Environ 271:135–144. https://doi.org/10.1016/S0048-9697(00)00839-1

Vereecken H (2005) Mobility and leaching of glyphosate: a review. Pest Manag Sci 61:1139–1151. https://doi.org/10.1002/ps.1122

Violante A, Ricciardella M, Pigna M, Capasso R (2005) Effects of organic ligands on the adsorption of trace elements onto metal oxides and organo-mineral complexes. In: Biogeochemistry of Trace Elements in the Rhizosphere. Elsevier, pp 157–182

Vlachodimitropoulou Koumoutsea E, Garbowski M, Porter J (2015) Synergistic intracellular iron chelation combinations: mechanisms and conditions for optimizing iron mobilization. Br J Haematol 170:874–883. https://doi.org/10.1111/bjh.13512

Wang K, Liu Y, Song Z, et al (2019) Effects of biodegradable chelator combination on potentially toxic metals leaching efficiency in

agricultural soils. Ecotoxicol Environ Saf 182:109399. https://doi.org/10.1016/j.ecoenv.2019.109399

Wang Y-J, Zhou D-M, Sun R-J, et al (2008) Zinc adsorption on goethite as affected by glyphosate. J Hazard Mater 151:179–184. https://doi.org/10.1016/j.jhazmat.2007.05.060

Wang Y-J, Zhou D-M, Sun R-J, et al (2006) Cosorption of zinc and glyphosate on two soils with different characteristics. J Hazard Mater 137:76–82. https://doi.org/10.1016/j.jhazmat.2006.02.032

Wani AL, Ara A, Usmani JA (2015) Lead toxicity: a review. Interdiscip Toxicol 8:55–64. https://doi.org/10.1515/intox-2015-0009

White RE (2009) Principles and Practice of Soil Science: The Soil as a Natural Resource. Wiley

Whorton JC (2010) The Arsenic Century: How Victorian Britain was Poisoned at Home, Work, and Play. OUP Oxford

Wilms W, Parus A, Homa J, et al (2023) Glyphosate versus glyphosate based ionic liquids: Effect of cation on glyphosate biodegradation, soxA and phnJ genes abundance and microbial populations changes during soil bioaugmentation. Chemosphere 316:137717. https://doi.org/10.1016/j.chemosphere.2022.137717

Windal I, Hanot V, Marchi J, et al (2009) PCB and organochlorine pesticides in home-produced eggs in Belgium. Sci Total Environ 407:4430–4437. https://doi.org/10.1016/j.scitotenv.2008.11.063

Wittmer IK, Bader H-P, Scheidegger R, et al (2010) Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. Water Res 44:2850–2862. https://doi.org/10.1016/j.watres.2010.01.030

Wong JWC, Li KL, Zhou LX, Selvam A (2007) The sorption of Cd and Zn by different soils in the presence of dissolved organic matter from sludge. Geoderma 137:310–317. https://doi.org/10.1016/j.geoderma.2006.08.026

Wu X, Cobbina SJ, Mao G, et al (2016) A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ Sci Pollut Res 23:8244–8259. https://doi.org/10.1007/s11356-016-6333-x

Xuejing X, Mingcai Y, Lianzhong L, Huijun S (1985) Usable Values for Chinese Standard Reference Samples of Stream Sediments, Soils, and Rocks: GSD 9-12, GSS 1-8 and GSR 1-6. Geostand Geoanalytical Res 9:277–280. https://doi.org/10.1111/j.1751-908X.1985.tb00458.x

Yang M, Wang Y, Yang G, et al (2024) A review of cumulative risk assessment of multiple pesticide residues in food: Current status, approaches and future perspectives. Trends Food Sci Technol 144:104340. https://doi.org/10.1016/j.tifs.2024.104340

Yapa L (1993) What are Improved Seeds? An Epistemology of the Green Revolution. Econ Geogr 69:254. https://doi.org/10.2307/143450

Young AL, Giesy JP, Jones PD, Newton M (2004) Environmental fate and bioavailability of agent orange and its associated dioxin during the vietnam war. Environ Sci Pollut Res 11:359–370. https://doi.org/10.1007/BF02979652

Žemberyová M, Barteková J, Hagarová I (2006) The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. Talanta 70:973–978. https://doi.org/10.1016/j.talanta.2006.05.057

Zhang X, Li X, Li Y, et al (2023) The Characteristics of Soil Dissolved Organic Carbon and Their Influences on Metal Solid-Solution Partitioning in Subtropics Agricultural Soils. Water Air Soil Pollut 234:441. https://doi.org/10.1007/s11270-023-06420-7

Zhao C-M, Campbell PGC, Wilkinson KJ (2016) When are metal complexes bioavailable? Environ Chem 13:425. https://doi.org/10.1071/EN15205

Zhou D-M, Wang Y-J, Cang L, et al (2004) Adsorption and cosorption of cadmium and glyphosate on two soils with different characteristics. Chemosphere 57:1237–1244. https://doi.org/10.1016/j.chemosphere.2004.08.043

Zivan O, Bohbot-Raviv Y, Dubowski Y (2017) Primary and secondary pesticide drift profiles from a peach orchard. Chemosphere 177:303–310. https://doi.org/10.1016/j.chemosphere.2017.03.014

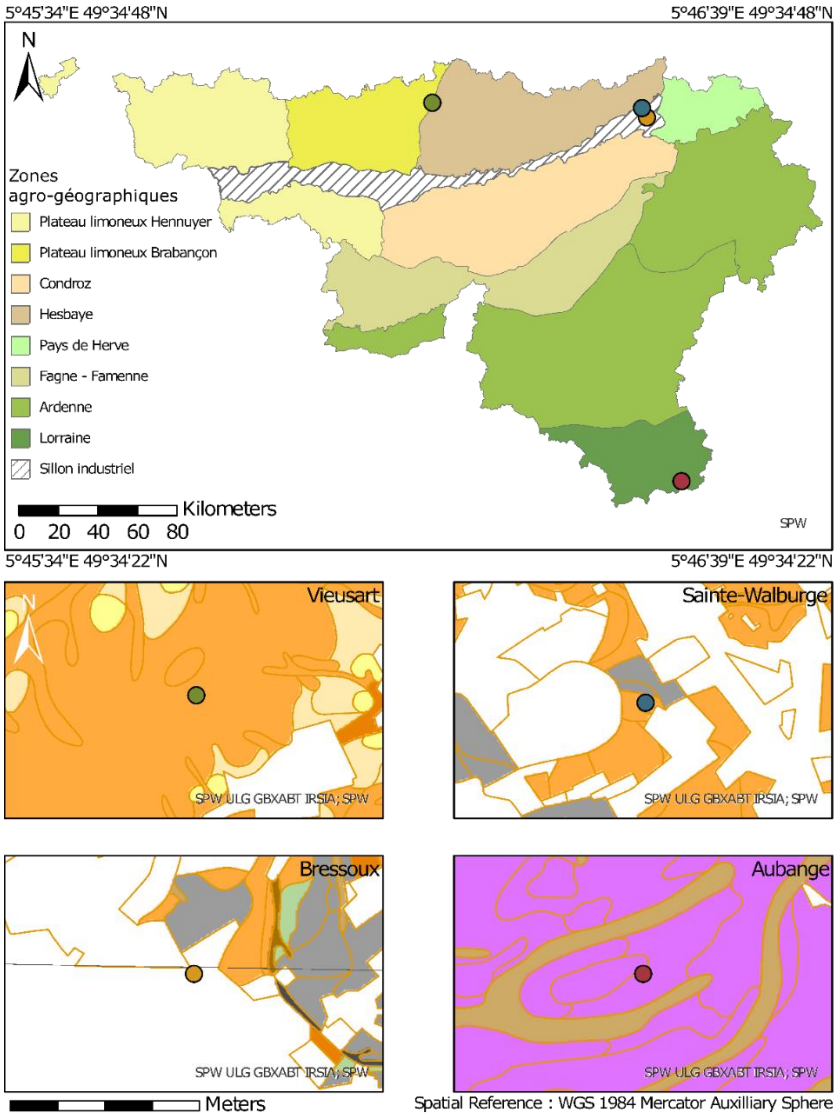
Zobiole LHS, de Oliveira RS, Morgan Huber D, et al (2010) Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. Plant Soil 328:57–69. https://doi.org/10.1007/s11104-009-0081-3

List of figures

List of tables

Annexes

Annexe 1. Map of the soils of the study sites, soil types.



Annexe 2. Summary of the modalities and soils

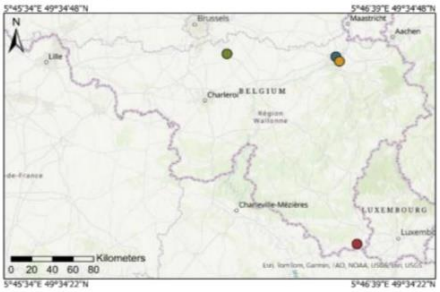
Extractability experiments

Modality code	Colour code	Composition
Control		Milli-Q water
Single modalities		
Gly_{20}		Standard glyphosate 20 mg L^{-1}
Gly_{100}		Standard glyphosate 100 mg L^{-1}
$GlyF_{100}$		Formulated glyphosate 100 mg L^{-1}
$AMPA_{100}$		Standard aminomethylphosphonic acid (AMPA) 100 mg L^{-1}
Tb_{10}		Standard tebuconazole 10 mg L^{-1}
TbF_2		Formulated tebuconazole 2 mg L^{-1}
TbF_{10}		Formulated tebuconazole 10 mg L^{-1}
TbF_{100}		Formulated tebuconazole 100 mg L^{-1}
DOC_{100}		Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}
Mixed modalities		
$Tb_{10}Gly_{100}$		Standard tebuconazole 10 mg L^{-1} and Standard glyphosate 100 mg L^{-1}
$Gly_{100}DOC_{100}$		Standard glyphosate 100 mg L^{-1} in Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}
$GlyF_{100}DOC_{100}$		Formulated glyphosate 100 mg L^{-1} in Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}
$AMPA_{100}DOC_{100}$		Standard AMPA 100 mg L^{-1} in Green waste compost extract (<0.45 µm) 100 mg_{DOC} L^{-1}

Greenhouse experiments

Modality code	Colour code	Modalities
Control		No glyphosate application
Max		Glyphosate application at about 3.6 kg ha^{-1}
3Max		Glyphosate application at about 10.8 kg ha^{-1}
3Max & OM		Glyphosate application at about 10.8 kg ha^{-1} and green waste compost at about 35 t ha^{-1}

Soils



Soil	Colour code	Contamination level
Vieusart		Uncontaminated
Sainte-Walburge		Moderately contaminated
Bressoux		Highly contaminated
Aubange		Naturally-enriched

Annexe 3. ICP-MS – Isotopes measured.

Element	Isotope	Mode of analysis
Ti	47	KED
V	51	KED
Cr	52	KED
Mn	55	KED
Co	59	KED
Ni	60	KED
Cu	65	KED
Zn	66	KED
As	75	KED
Mo	95	STD
Cd	114	STD
Sn	120	STD
Sb	121	STD
Ba	135	STD
Pb	208	STD

STD = Standard

KED = Kinetic Energy Discrimination

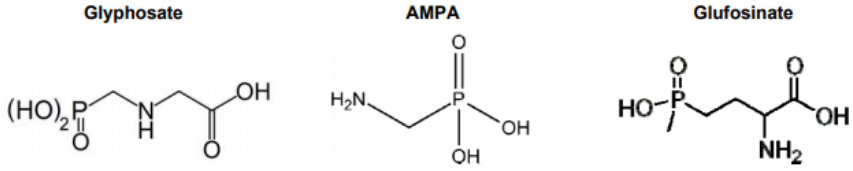
Annexe 4. Analytical method for glyphosate and AMPA.

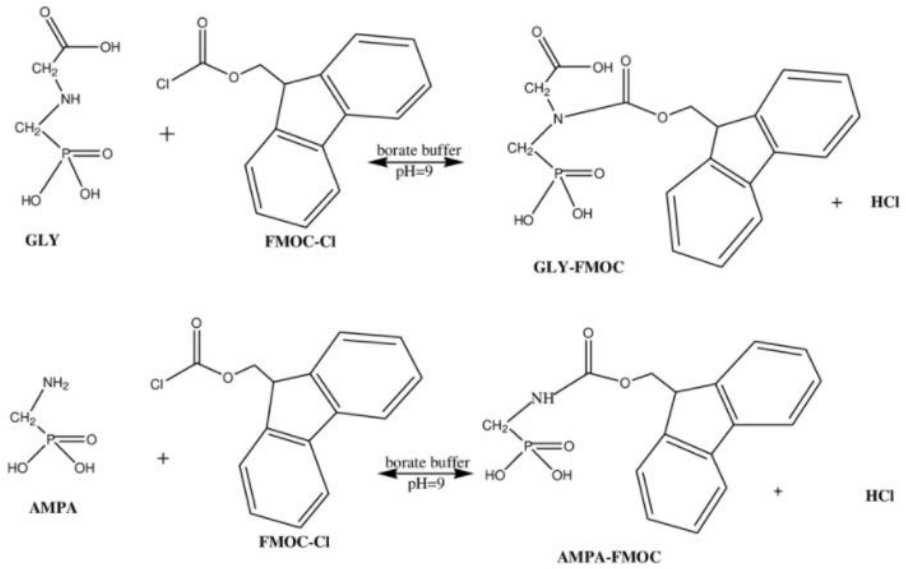
1. Chemical analysis

This procedure describes the analytical method used for the extraction, derivatization, purification and quantification of glyphosate and its metabolite (AMPA) by HPLC-FLD in waters.

After filtration, glyphosate and its metabolite (AMPA) are derivatized using Fmoc to reduce their polarity and achieve retention on reversed-phase columns. After derivatization, excess Fmoc is removed by liquid-liquid extraction with DCM, ether or ethyl acetate. The sample can be purified and concentrated if necessary by SPE (solid phase extraction).

The presence of divalent cations such as Ca, Cu, Fe or Zn (concentration greater than 3M) can lead to an underestimation of glyphosate. To avoid this phenomenon, the addition of EDTA is recommended.





2. Conservation

Derivatized samples can remain 48 hours on the HPLC chain without loss of signal.

3. Materials

- HPLC-FLD
- Amber vials

4. Reactifs

- **EDTA-Na_2**
- **Acetonitrile (ACN)** HPLC grade
- **Methanole (MeOH)** HPLC grade
- **Dichloromethane / Ether / ethyl acetate**
- **Disodium hydrogen phosphate (Na_2HPO_4)**
- **Ortho-phosphoric acid**
- **Na triazide (NaN_3)**
- **Standard glyphosate**

- **Standard AMPA**
- **Fmoc chloride** derivatization grade
- **Boric acid**
- **KCl**
- **NaOH**

- **Borate buffer pH 9** : Weigh 3.1 g of boric acid (H_3BO_3), 3.75 g of KCl and 0.8 g of NaOH, dissolve in 80 ml of water, adjust the pH to 9 with NaOH 1M adjust the volume to 100 ml.
- **EDTA solution 10g/l, pH 9**: Dissoudre 100 mg de EDTA Na_2 dans 9ml d'eau. Ajuster le pH à 9 à l'aide de KOH ou de NOH
- **Borate buffer pH 9 + EDTA** : To 12,5 ml of borate buffer, add 1 ml of EDTA solution
- **Fmoc (200ppm) :** Weigh 10 mg of Fmoc and dissolve in 50 ml of ACN
- **Stock standard solution (100ppm) :** Weigh 10 mg of Glyphosate and AMPA in a 100 ml gauged balloon with a mixture of H_2O/MeOH 75 :25.
- **Stock standard solution (1000ppb) :** Dilute 100x the 100 ppm solution in a mixture of H2O/MeOH 75 :25
- **Elutant A –Phosphate buffer 10 mM pH 7** : Dissolve 1,152 g of H_3PO_4 (85 %) in 900 ml of Milli-Q water. Adjust to pH 7 with NaOH 50%. Add 50 mg of NaN_3 to avoid bacteria developments, complete to reach 1 L and filter on a 0.22 µm membrane.
- **Elutant B** : Acetonitrile 450 ml, Methanol 450 ml, H2O 100 ml. Filter on a 0.22 µm membrane

5. Sampling and procedure

Derivatization by Fmoc (teneur attendue entre 2 – 10 ppb)

- In a 2 ml eppendorf collect 1 ml of sample
- Add 100 µl of borate buffer/EDTA/ pH9
- Ajouter 200 µl de solution Fmoc

- Homogenise and let react at room temperature but protected from light for at least 45 min.
- Filter and transfer in an amber HPLC vial

6. HPLC

Calibration curve ou point 5ppb :

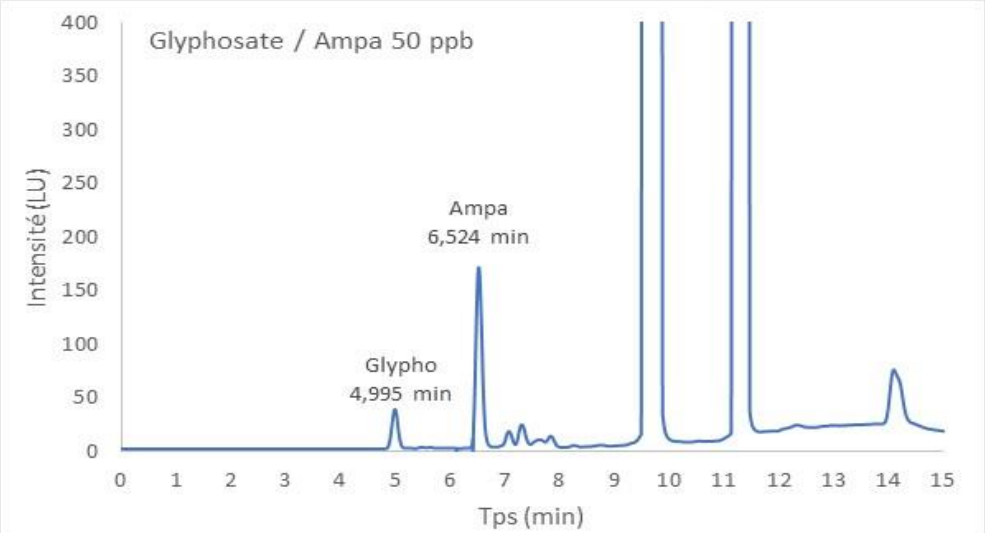
- Prepare the following dilutions in HPLC vials, dilute 100x the stock solution 1000ppb, 100 µl of 1000 ppb stock in 10 ml of Milli-Q water
-

Conc	**Standard 10 ppb**	**H2O mQ**	**Tampon borate/EDTA/ pH 9**	**Fmoc 100ppm**
Bl-Fmoc	-	1000 µl	100 µl	200 µl
0.1 ppb	10 µl	990 µl	100 µl	200 µl
0.5 ppb	50 µl	950 µl	100 µl	200 µl
1 ppb	100 µl	900 µl	100 µl	200 µl
2 ppb	200 µl	800 µl	100 µl	200 µl
5 ppb	500 µl	500 µl	100 µl	200 µl
10 ppb	1000 µl	-	100 µl	200 µl

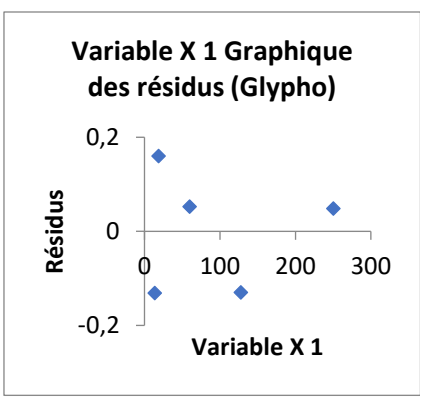
- Let derivatise for 45 min protected from light before injecting

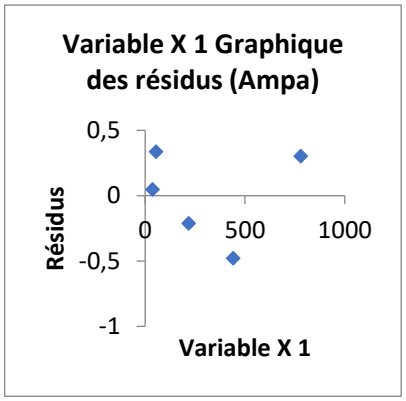
HPLC analysis

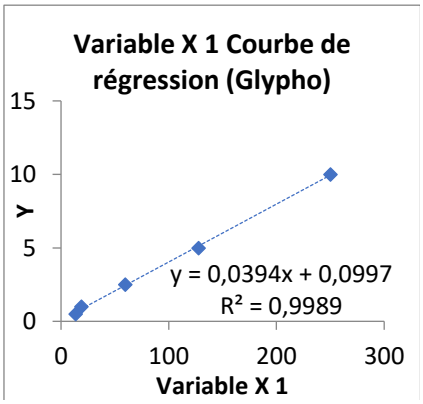
- Column: Merck Lichrocart C18 150 X 4.6mm - 5µm
- Oven 35°C
- Flow : 1 ml/min
- Elution (gradient): start with 30% of B for 1 min, then increase to 100% de B à 10 min. Stay at 100% of B for 3 min, before re-descending to initial conditions in 2 min and equilibrating the column for 5 min.
- FLD detector: excitation at 260 nm and emission at 310 nm – Gain of 12
- Injected volume : 50 µl

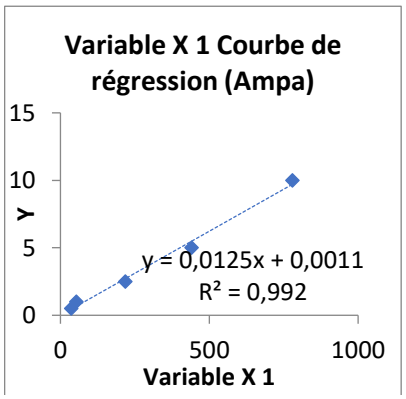


Chromatogram : Glyphosate and AMPA in FLD for a standard solution at 50 ppb (without Fmoc elimination)









7. Calculation and expression of results

- Concentration values are calculated on the calibration curve. The results are expressed in µg L^{-1}.

Annexe 5. Trace element content in the compost.

Element	Trace element content (mg kg^{-1})
Ti	1030
V	23.4
Cr	25.1
Mn	376
Co	11.5
Ni	10.4
Cu	28.1
Zn	168
As	4.14
Mo	1.89
Cd	0.628
Sn	2.08
Sb	0.773
Ba	166
Pb	36.0

Annexe 6. Raw data – chapter I.

Table 1 Mean trace element extractabilities (n=3) across soils for the control, Gly_{20}, Gly_{100}, $GlyF_{100}$ and $AMPA_{100}$ modalities.

		Mean extractability of trace element (µg/g soil)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	Control	3,53E0-1	5,66E-02	4,11E-02	1,31E-01	4,39E-03	2,59E-02	7,29E-02	5,05E-01	1,34E-02	6,71E-03	5,21E-04	1,00E-03	2,95E-03	6,75E-01	1,04E-02
	Gly20	2,61E+00	3,36E-01	2,91E-01	9,26E-01	2,83E-02	1,13E-01	1,30E-01	4,97E-01	5,32E-02	8,90E-03	1,56E-03	8,69E-03	6,18E-03	5,42E-01	7,14E-02
	Gly100	7,11E-01	1,13E-01	8,04E-02	2,87E-01	8,20E-03	4,84E-02	1,43E-01	1,65E+00	4,09E-02	7,37E-03	7,06E-04	3,07E-03	4,16E-03	1,02E+00	1,92E-02
	GlyF100	5,16E-01	8,71E-02	5,34E-02	1,89E-01	5,69E-03	4,45E-02	1,71E-01	2,00E+00	4,48E-02	9,74E-03	8,91E-04	7,38E-03	4,04E-03	1,64E+00	1,28E-02
	AMPA100	2,14E-01	4,91E-02	2,35E-02	1,30E-01	2,84E-03	2,93E-02	7,96E-02	1,41E-01	3,02E-02	7,49E-03	5,00E-04	2,65E-04	3,30E-03	1,03E-01	7,22E-03
Sainte-Walburge	Control	6,19E-01	1,46E-01	6,97E-02	2,61E-01	9,33E-03	6,65E-02	2,66E-01	9,81E-01	1,18E-01	5,46E-02	2,95E-03	1,24E-02	2,79E-02	2,68E-01	9,43E-02
	Gly20	1,26E+00	2,42E-01	1,50E-01	6,25E-01	2,00E-02	1,24E-01	4,87E-01	2,00E+00	1,64E-01	6,75E-02	5,91E-03	2,85E-02	3,36E-02	5,34E-01	2,21E-01
	Gly100	4,32E-01	1,47E-01	4,83E-02	2,56E-01	7,45E-03	8,53E-02	1,13E+00	1,39E+00	1,73E-01	5,55E-02	4,31E-03	8,39E-03	3,27E-02	2,65E-01	8,16E-02
	GlyF100	5,15E-01	1,54E-01	5,76E-02	2,62E-01	8,90E-03	1,06E-01	1,27E+00	1,53E+00	1,93E-01	5,28E-02	5,41E-03	1,07E-02	3,06E-02	2,77E-01	9,10E-02
	AMPA100	4,18E-01	1,44E-01	4,36E-02	1,99E-01	6,20E-03	5,39E-02	2,56E-01	7,12E-01	1,84E-01	5,67E-02	2,50E-03	7,18E-03	3,10E-02	2,57E-01	6,59E-02
Bressoux	Control	2,25E-01	1,32E-01	2,51E-02	3,86E-01	6,79E-03	4,74E-02	4,81E-01	2,29E+00	1,64E-01	2,21E-02	4,28E-03	1,54E-02	7,57E-02	1,08E+00	2,61E-01
	Gly20	2,64E-01	1,33E-01	4,23E-02	4,86E-01	8,42E-03	5,92E-02	1,25E+00	2,14E+00	1,75E-01	2,72E-02	5,55E-03	1,69E-02	7,22E-02	2,87E-01	3,58E-01
	Gly100	2,14E-01	1,35E-01	2,28E-02	3,62E-01	6,00E-03	6,60E-02	4,01E+00	5,01E+00	2,23E-01	2,06E-02	5,49E-03	1,39E-02	7,37E-02	8,90E-01	2,52E-01
	GlyF100	3,47E-01	1,61E-01	3,52E-02	5,20E-01	9,04E-03	8,93E-02	4,94E+00	6,00E+00	2,56E-01	2,27E-02	7,13E-03	2,65E-02	7,74E-02	1,68E+00	3,72E-01
	AMPA100	2,15E-01	1,38E-01	2,47E-02	3,76E-01	6,84E-03	5,26E-02	4,97E-01	1,65E+00	2,44E-01	2,12E-02	4,53E-03	1,36E-02	7,49E-02	2,52E-01	2,71E-01
Aubange	Control	5,28E-01	3,74E-01	1,50E-01	3,81E+00	2,36E-02	1,02E-01	3,57E-02	2,26E-01	6,23E-02	8,79E-03	2,22E-04	1,66E-03	1,26E-03	1,05E-01	4,63E-02
	Gly20	8,48E-01	6,02E-01	2,43E-01	6,76E+00	4,20E-02	1,66E-01	4,19E-02	3,53E-01	1,03E-01	1,34E-02	6,45E-04	4,49E-03	2,59E-03	1,79E-01	9,49E-02
	Gly100	4,06E-01	2,88E-01	1,13E-01	3,10E+00	1,93E-02	8,46E-02	4,05E-02	2,46E-01	7,01E-02	1,64E-02	1,98E-04	1,40E-03	1,53E-03	8,87E-02	3,73E-02
	GlyF100	6,55E-01	4,75E-01	1,87E-01	5,13E+00	3,12E-02	1,34E-01	5,08E-02	2,88E-01	1,07E-01	2,40E-02	3,17E-04	2,18E-03	1,77E-03	1,30E-01	6,03E-02
	AMPA100	3,96E-01	2,51E-01	9,88E-02	2,57E+00	1,65E-02	7,33E-02	4,00E-02	1,96E-01	6,71E-02	1,28E-02	2,56E-04	9,85E-04	1,26E-03	8,73E-02	3,32E-02

Table 2 Standard deviations of trace element extractabilities (n=3) across soils for the control, Gly_{20}, Gly_{100}, $GlyF_{100}$ and $AMPA_{100}$ modalities.

		Standard deviation of the extractability of trace element (µg/g soil)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Control**	8,97E-02	9,41E-03	1,00E-02	3,31E-02	9,79E-04	6,28E-03	2,04E-03	2,22E-01	1,34E-03	1,02E-03	3,48E-04	5,36E-04	3,05E-04	4,74E-01	4,46E-03
	Gly20	6,48E-01	7,62E-02	6,97E-02	1,99E-01	6,64E-03	2,16E-02	1,70E-02	2,58E-02	1,04E-02	2,97E-04	2,73E-04	1,98E-03	5,58E-04	1,20E-01	1,47E-02
	Gly100	2,97E-01	3,72E-02	3,25E-02	7,30E-02	2,79E-03	9,29E-03	1,14E-02	1,26E+00	7,30E-03	8,07E-04	1,34E-04	2,19E-03	7,23E-05	8,24E-01	7,00E-03
	GlyF100	1,31E-01	1,34E-02	1,12E-02	2,95E-02	1,06E-03	5,21E-03	4,63E-03	1,61E+00	3,93E-03	1,02E-03	2,07E-04	6,74E-03	2,50E-04	1,30E+00	2,29E-03
	AMPA100	4,68E-02	3,96E-03	2,96E-03	7,49E-03	1,45E-04	5,27E-03	6,37E-03	1,82E-03	1,44E-03	1,30E-04	1,80E-05	2,06E-04	1,07E-04	7,34E-03	1,06E-03
Sainte-Walburge	**Control**	2,43E-02	6,09E-03	3,30E-03	1,29E-02	3,00E-04	2,12E-03	5,85E-03	2,77E-02	1,63E-03	1,25E-03	9,65E-05	6,57E-04	4,24E-04	7,42E-03	3,84E-03
	Gly20	1,75E-01	2,25E-02	1,72E-02	5,98E-02	2,44E-03	1,15E-02	1,25E-02	1,92E-01	8,01E-03	6,38E-03	3,85E-04	4,04E-03	1,93E-03	4,86E-02	2,62E-02
	Gly100	3,72E-02	1,80E-03	3,44E-03	6,28E-03	4,91E-04	3,86E-04	1,20E-02	1,81E-02	3,76E-03	6,69E-04	4,38E-05	9,08E-05	5,50E-05	2,49E-03	2,46E-03
	GlyF100	2,35E-02	3,05E-03	5,99E-03	1,43E-02	5,46E-04	3,82E-03	1,65E-02	5,91E-02	6,12E-03	2,23E-04	1,73E-04	1,07E-03	9,18E-04	1,30E-02	4,71E-03
	AMPA100	7,01E-02	1,05E-02	7,60E-03	2,75E-02	9,71E-04	6,73E-03	9,17E-03	1,34E-01	3,02E-03	8,16E-04	4,79E-04	1,45E-03	8,22E-04	3,87E-02	1,13E-02
Bressoux	**Control**	4,51E-02	1,02E-02	3,32E-03	7,63E-02	1,13E-03	2,91E-03	1,56E-02	7,77E-01	1,13E-02	1,33E-03	4,07E-04	2,80E-03	6,71E-03	7,76E-01	3,57E-02
	Gly20	7,35E-02	1,50E-02	2,04E-02	7,52E-02	1,20E-03	6,82E-03	1,01E-01	2,32E-01	2,53E-03	1,56E-03	2,11E-04	3,76E-03	7,69E-03	2,38E-02	3,85E-02
	Gly100	7,27E-02	1,03E-02	7,53E-03	1,16E-01	1,71E-03	5,53E-03	9,25E-02	1,14E+00	1,09E-02	1,07E-03	5,59E-04	4,84E-03	2,61E-03	5,61E-01	7,33E-02
	GlyF100	1,28E-01	2,28E-02	9,64E-03	1,43E-01	2,41E-03	7,11E-03	9,83E-02	1,25E+00	3,07E-02	1,97E-03	8,37E-04	1,09E-02	5,14E-03	1,25E+00	8,23E-02
	AMPA100	9,30E-03	3,08E-03	1,29E-03	5,44E-03	3,79E-04	2,46E-03	9,00E-03	2,85E-02	4,35E-03	1,63E-04	1,58E-04	8,40E-04	1,28E-03	3,86E-03	5,70E-03
Aubange	**Control**	6,32E-02	3,96E-02	1,65E-02	4,63E-01	2,85E-03	1,08E-02	1,43E-03	2,83E-02	7,71E-03	1,98E-04	1,67E-05	2,82E-04	2,13E-05	9,46E-03	5,29E-03
	Gly20	5,89E-02	5,45E-02	1,59E-02	7,06E-01	3,96E-03	1,30E-02	4,06E-03	1,34E-02	1,28E-02	3,45E-04	9,42E-05	3,61E-04	1,41E-04	2,02E-02	5,36E-03
	Gly100	2,79E-02	3,01E-02	1,35E-02	1,01E-01	7,81E-04	8,52E-03	1,08E-03	8,23E-03	2,60E-03	9,14E-04	5,09E-06	2,75E-04	4,53E-05	2,36E-03	7,62E-04
	GlyF100	2,64E-02	3,72E-02	1,59E-02	3,71E-01	1,98E-03	1,02E-02	2,47E-04	3,28E-02	5,36E-03	1,15E-04	3,48E-05	1,59E-04	6,07E-05	7,85E-03	4,62E-03
	AMPA100	1,58E-01	9,70E-02	4,00E-02	1,04E+00	6,40E-03	2,57E-02	4,32E-03	2,90E-02	1,43E-02	4,21E-04	2,79E-05	5,76E-04	8,29E-05	2,14E-02	1,23E-02

Annexe 7. Raw data – chapter II.

Table 1 Mean trace elements concentrations in soil solutions (23/08/2022, n=3) across soils for the Control, Max, 3Max and 3Max & MO modalities.

		Mean trace element concentration in soil solution (µg/L)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Control**	5,29E-01	2,85E+01	8,82E-01	3,73E+01	5,41E+00	1,65E+01	6,77E+00	3,22E+02	6,28E+00	1,90E+00	5,05E-01	1,08E-01	5,50E-01	8,15E+02	7,93E-02
	Max	1,04E+00	3,14E+01	1,18E+00	3,25E+02	1,70E+01	3,80E+01	8,69E+00	1,92E+03	9,92E+00	2,34E+01	1,25E+00	1,03E-01	5,23E-01	1,06E+03	2,45E-01
	3Max	7,81E-01	3,11E+01	8,77E-01	3,55E+01	9,18E+00	2,38E+01	7,27E+00	1,13E+03	7,13E+00	1,28E+01	5,44E-01	7,92E-02	4,53E-01	5,02E+02	1,49E-01
	3Max & MO	1,21E+00	3,15E+01	1,08E+00	2,30E+02	1,00E+01	3,14E+01	1,20E+01	4,21E+02	7,57E+00	2,50E+01	8,05E-01	1,97E-01	5,47E-01	9,35E+02	3,83E-01
Sainte-Walburge	**Control**	1,45E+00	1,00E+01	1,77E+00	6,98E+02	1,63E+01	6,14E+01	3,31E+01	1,39E+03	1,64E+01	6,34E+01	9,47E+00	2,03E-01	4,08E+00	2,52E+03	3,96E+00
	Max	1,99E+00	1,22E+01	1,34E+00	2,06E+03	3,97E+01	9,95E+01	4,13E+01	3,34E+03	2,40E+01	1,51E+02	1,35E+01	2,02E-01	4,08E+00	2,64E+03	5,56E+00
	3Max	2,22E+00	1,51E+01	1,76E+00	2,41E+03	4,76E+01	1,10E+02	4,74E+01	3,52E+03	3,53E+01	2,02E+02	1,27E+01	1,54E-01	4,16E+00	2,33E+03	5,01E+00
	3Max & MO	1,69E+00	1,35E+01	1,69E+00	2,21E+03	2,61E+01	7,40E+01	5,40E+01	1,44E+03	2,05E+01	2,45E+02	1,21E+01	1,14E-01	4,52E+00	3,21E+03	5,39E+00
Bressoux	**Control**	1,36E+00	1,39E+01	1,15E+00	2,19E+02	1,02E+01	7,87E+01	5,39E+01	5,12E+03	2,29E+01	1,26E+02	1,81E+01	1,08E-01	1,10E+01	9,79E+02	6,35E+00
	Max	8,19E-01	1,35E+01	7,57E-01	1,02E+02	4,10E+00	4,16E+01	4,55E+01	4,58E+03	1,88E+01	1,21E+02	1,33E+01	9,91E-02	1,17E+01	1,53E+03	5,48E+00
	3Max	1,36E+00	1,58E+01	1,53E+00	2,29E+02	1,61E+01	9,75E+01	7,52E+01	5,87E+03	2,93E+01	2,46E+02	1,88E+01	7,65E-02	1,18E+01	7,02E+02	7,13E+00
	3Max & MO	1,50E+00	1,44E+01	1,48E+00	1,43E+02	4,17E+00	4,74E+01	8,41E+01	3,32E+03	2,35E+01	2,20E+02	1,24E+01	9,52E-02	1,09E+01	1,31E+03	5,30E+00
Aubange	**Control**	1,61E+00	2,29E+00	7,09E-01	1,99E+01	8,07E+00	2,53E+01	1,69E+00	1,45E+03	2,63E+00	1,74E+02	9,12E-02	7,20E-02	1,10E-01	7,82E+01	5,95E-02
	Max	3,81E-01	1,78E+00	6,47E-01	2,15E+01	1,54E+01	4,47E+01	1,45E+00	5,35E+02	1,59E+00	1,62E+02	7,14E-02	4,01E-02	8,44E-02	1,10E+02	1,49E-02
	3Max	3,00E-01	1,21E+00	3,96E-01	7,35E+00	3,99E+00	1,14E+01	8,38E-01	1,30E+03	8,40E-01	5,84E+01	3,36E-02	2,90E-02	7,46E-02	6,88E+01	1,62E-02
	3Max & MO	3,68E-01	1,67E+00	6,06E-01	1,65E+01	6,92E+00	2,41E+01	2,05E+00	1,17E+03	1,43E+00	1,01E+02	7,31E-02	5,56E-02	9,72E-02	9,92E+01	2,50E-02

Table 2 Standard deviation of trace elements concentrations in soil solutions (23/08/2022, n=3) across soils for the Control, Max, 3Max and 3Max & MO modalities.

		Standard deviation of the trace element concentration in soil solution (µg/L)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Control**	9,38E-02	1,05E+00	9,11E-02	2,12E+01	1,69E+00	1,58E+00	6,78E-01	3,02E+02	1,42E+00	6,97E-02	1,83E-01	1,78E-03	3,84E-02	4,16E+02	1,45E-03
	Max	3,28E-01	3,73E+00	2,62E-01	2,32E+02	4,76E+00	9,74E+00	1,32E+00	2,41E+03	4,38E+00	6,71E+00	4,45E-01	6,21E-02	5,24E-02	2,54E+02	9,55E-02
	3Max	3,12E-02	3,42E+00	1,35E-01	3,61E+01	7,23E+00	1,64E+01	1,15E+00	1,12E+03	2,00E+00	7,09E+00	3,24E-01	1,87E-02	7,33E-03	1,54E+02	1,35E-01
	3Max & MO	5,50E-01	1,82E+00	1,35E-01	2,62E+02	1,97E+00	6,76E-01	1,40E+00	2,80E+02	1,70E+00	1,23E+01	2,30E-01	1,10E-01	1,00E-02	4,34E+02	9,41E-02
Sainte-Walburge	**Control**	4,29E-01	1,31E+00	1,41E+00	8,64E+02	7,73E+00	2,86E+01	4,99E+00	8,48E+02	8,41E-01	2,74E+01	4,96E+00	9,10E-02	1,06E-01	9,57E+02	2,40E+00
	Max	6,31E-01	1,58E+00	4,06E-01	1,18E+03	2,69E+01	5,27E+01	7,02E+00	5,35E+02	1,13E+01	3,76E+01	4,74E+00	8,87E-02	1,25E-01	4,26E+02	2,69E+00
	3Max	1,54E+00	6,66E+00	1,25E+00	2,94E+03	5,32E+01	9,43E+01	2,40E+01	2,89E+03	2,43E+01	1,20E+02	7,02E+00	3,28E-02	5,00E-01	3,70E+02	4,73E+00
	3Max & MO	1,81E-01	1,57E+00	3,19E-02	1,71E+02	1,17E+01	1,91E+01	1,43E+00	2,99E+02	1,90E+00	5,01E+01	7,32E-02	3,21E-03	8,24E-02	6,71E+02	4,39E+00
Bressoux	**Control**	2,17E-01	1,07E+00	1,86E-01	1,06E+02	1,41E+00	1,13E+01	5,48E+00	1,60E+03	4,79E+00	2,46E+01	4,74E+00	1,68E-02	1,53E-01	3,88E+02	1,88E+00
	Max	4,57E-02	1,50E+00	8,82E-02	7,91E+01	2,03E+00	1,64E+01	4,57E-01	1,24E+03	5,47E+00	1,30E+01	5,56E+00	2,39E-02	7,09E-01	1,03E+03	2,29E+00
	3Max	1,22E-01	8,59E-01	2,16E-01	8,10E+01	5,64E+00	1,73E+01	9,57E+00	1,30E+03	1,17E+01	3,83E+01	3,36E+00	8,48E-03	6,43E-01	2,44E+02	2,08E+00
	3Max & MO	3,68E-01	6,92E-01	6,03E-01	1,06E+02	3,18E+00	2,56E+01	1,36E+01	1,21E+03	1,06E+01	8,05E+01	4,21E+00	1,95E-02	6,01E-01	5,45E+02	1,03E+00
Aubange	**Control**	2,19E+00	6,18E-01	1,81E-01	1,17E+01	2,02E+00	6,12E+00	5,14E-01	1,81E+03	7,20E-01	4,43E+01	5,11E-02	2,75E-02	1,79E-02	2,65E+01	8,59E-02
	Max	4,66E-02	6,73E-01	2,50E-01	2,48E+00	1,14E+01	3,09E+01	4,82E-01	3,88E+02	7,40E-01	5,98E+01	2,88E-02	6,72E-03	7,92E-03	3,83E+01	9,29E-03
	3Max	3,22E-02	2,10E-01	1,11E-02	3,55E+00	1,51E+00	3,86E+00	5,98E-02	1,00E+03	2,01E-01	1,31E-01	9,79E-03	4,21E-03	3,89E-04	2,95E+01	1,45E-02
	3Max & MO	4,26E-02	1,61E-01	9,72E-02	9,39E+00	8,51E-01	3,98E+00	1,47E-01	8,92E+02	3,95E-01	3,85E+01	2,41E-02	5,98E-03	5,13E-03	2,85E+01	1,69E-02

Table 3 Mean trace elements contents in aerial parts of *Triticum spelta* (n=3) across soils for the Control, Max, 3Max and 3Max & MO modalities.

		Mean trace element content in plants (mg/kg plant)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Control**	6,95E-06	1,02E-07	7,08E-07	5,89E-05	5,17E-08	4,14E-05	7,78E-06	2,07E-05	1,13E-07	8,66E-07	2,10E-07	6,01E-08	2,23E-08	1,31E-05	4,05E-07
	Max	3,62E-05	2,30E-07	7,73E-07	5,47E-05	7,68E-08	3,61E-05	1,60E-05	2,87E-05	1,61E-07	1,34E-06	2,20E-07	1,02E-07	3,60E-08	1,56E-05	7,54E-07
	3Max	8,73E-06	8,79E-08	3,77E-07	6,45E-05	4,37E-08	3,60E-05	1,06E-05	2,30E-05	1,43E-07	1,79E-06	1,84E-07	1,26E-07	1,02E-07	1,53E-05	3,33E-07
	3Max & MO	1,12E-05	1,66E-07	5,61E-07	5,70E-05	5,67E-08	3,64E-05	8,53E-06	2,07E-05	1,54E-07	3,09E-06	1,88E-07	9,10E-08	5,26E-08	1,78E-05	4,05E-07
Sainte-Walburge	**Control**	1,04E-05	1,58E-07	4,26E-07	5,93E-05	7,83E-08	2,14E-05	1,57E-05	7,31E-05	2,82E-07	2,60E-06	5,89E-07	7,52E-08	3,87E-08	2,04E-05	9,67E-07
	Max	2,71E-05	4,19E-07	6,72E-07	6,58E-05	1,72E-07	2,15E-05	1,67E-05	7,49E-05	5,06E-07	2,48E-06	6,22E-07	9,31E-08	4,25E-08	2,59E-05	1,15E-06
	3Max	9,98E-06	1,38E-07	4,23E-07	5,66E-05	7,89E-08	2,41E-05	1,28E-05	5,64E-05	2,73E-07	2,16E-06	5,09E-07	5,24E-08	2,25E-08	1,83E-05	5,98E-07
	3Max & MO	6,25E-06	1,07E-07	4,00E-07	4,63E-05	9,06E-08	2,69E-05	1,06E-05	4,98E-05	4,19E-07	4,20E-06	3,90E-07	4,39E-08	1,80E-08	2,15E-05	3,85E-07
Bressoux	**Control**	1,04E-05	1,82E-07	6,28E-07	4,44E-05	5,27E-08	3,38E-05	1,47E-05	2,18E-04	5,49E-07	4,71E-06	1,30E-06	9,06E-08	3,52E-08	2,36E-05	1,03E-06
	Max	5,52E-06	7,03E-08	2,99E-07	2,36E-05	2,75E-08	2,61E-05	1,10E-05	1,26E-04	3,24E-07	2,46E-06	7,09E-07	1,43E-07	1,06E-07	1,66E-05	3,99E-07
	3Max	1,86E-06	5,45E-08	2,49E-07	3,83E-05	2,33E-08	2,62E-05	1,24E-05	1,50E-04	2,91E-07	2,51E-06	7,87E-07	8,50E-08	6,03E-08	1,02E-05	4,08E-07
	3Max & MO	5,80E-06	6,52E-08	3,11E-07	2,37E-05	2,38E-08	1,77E-05	1,05E-05	1,36E-04	3,06E-07	3,14E-06	8,69E-07	6,53E-08	4,83E-08	1,60E-05	4,77E-07
Aubange	**Control**	6,37E-06	2,93E-07	5,65E-07	1,05E-04	5,02E-08	2,70E-05	1,19E-05	2,77E-05	1,43E-07	3,98E-06	1,10E-07	5,61E-08	2,74E-08	2,64E-06	3,97E-07
	Max	1,13E-05	2,17E-07	8,21E-07	1,15E-04	5,93E-08	3,02E-05	1,88E-05	3,06E-05	1,50E-07	5,75E-06	8,76E-08	7,88E-08	2,42E-08	3,57E-06	7,47E-07
	3Max	1,23E-05	7,13E-07	7,90E-07	1,11E-04	9,11E-08	4,48E-05	1,51E-05	2,60E-05	3,10E-07	5,40E-06	7,84E-08	6,61E-08	2,12E-08	3,80E-06	6,53E-07
	3Max & MO	1,10E-05	4,03E-07	7,77E-07	8,15E-05	7,36E-08	2,50E-05	1,28E-05	2,71E-05	2,07E-07	5,67E-06	9,28E-08	5,52E-08	2,07E-08	4,50E-06	6,46E-07

Table 4 Standard deviations of trace elements contents in aerial parts of *Triticum spelta* (n=3) across soils for the Control, Max, 3Max and 3Max & MO modalities.

		Standard deviation of the trace element content in plants (mg/kg plant)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Control**	2,51E-06	4,25E-08	1,90E-07	1,14E-05	8,95E-09	8,95E-06	7,41E-07	4,40E-06	1,63E-08	2,28E-07	7,38E-09	3,86E-09	4,54E-09	2,06E-06	1,80E-07
	Max	4,53E-05	2,33E-07	4,73E-07	2,64E-05	5,09E-08	1,84E-05	1,44E-05	2,10E-05	1,25E-07	7,38E-07	1,53E-07	8,76E-08	3,06E-08	1,09E-05	8,08E-07
	3Max	5,52E-06	3,97E-08	1,36E-07	9,90E-06	1,54E-08	2,79E-06	1,22E-06	1,99E-06	4,16E-08	9,79E-07	8,47E-09	5,55E-08	5,27E-08	3,31E-06	7,18E-08
	3Max & MO	2,43E-06	5,90E-08	1,20E-07	7,97E-06	1,39E-08	6,94E-06	1,66E-06	3,06E-06	4,72E-08	4,22E-07	5,10E-08	2,59E-08	1,40E-08	2,98E-06	1,20E-07
Sainte-Walburge	**Control**	3,71E-06	5,78E-08	1,71E-07	1,80E-05	2,43E-08	1,76E-06	2,90E-06	2,04E-05	8,15E-08	1,33E-07	1,26E-07	1,74E-08	1,58E-08	9,50E-06	6,23E-07
	Max	2,70E-05	4,22E-07	4,65E-07	5,19E-06	9,47E-08	3,77E-06	3,14E-06	3,06E-06	1,11E-07	8,36E-07	2,63E-08	4,73E-08	2,16E-08	4,08E-06	8,88E-07
	3Max	5,13E-06	6,15E-08	2,15E-07	1,88E-05	1,78E-08	3,21E-06	3,22E-06	6,74E-06	5,57E-08	2,87E-07	8,10E-08	1,01E-08	3,43E-09	3,08E-06	2,40E-07
	3Max & MO	4,52E-06	8,79E-08	1,04E-07	1,99E-05	1,01E-07	4,33E-06	1,92E-06	1,23E-05	3,49E-07	8,65E-07	8,39E-08	1,34E-08	7,61E-09	4,83E-06	1,73E-07
Bressoux	**Control**	5,48E-06	1,22E-07	2,22E-07	7,13E-06	2,98E-08	8,51E-06	8,83E-07	2,88E-05	3,70E-08	1,75E-06	1,82E-07	4,90E-08	1,51E-08	6,15E-06	8,28E-07
	Max	5,00E-06	4,89E-08	1,26E-07	1,88E-05	1,71E-08	1,16E-05	6,08E-06	4,92E-05	1,29E-07	2,03E-06	2,67E-07	8,02E-08	7,73E-08	8,26E-06	2,40E-07
	3Max	3,91E-07	8,21E-09	9,01E-08	1,40E-05	4,85E-10	2,80E-06	3,52E-06	1,52E-05	1,01E-07	1,07E-06	1,08E-07	1,87E-08	1,46E-08	2,87E-06	7,66E-08
	3Max & MO	4,34E-06	7,64E-09	2,06E-08	3,61E-06	2,53E-09	6,94E-06	1,80E-06	8,35E-06	4,09E-08	1,05E-06	1,87E-07	4,24E-09	1,15E-08	2,80E-06	1,29E-07
Aubange	**Control**	3,73E-06	2,48E-07	2,20E-07	2,90E-05	2,69E-08	3,40E-06	5,34E-06	9,08E-06	9,06E-08	6,61E-07	6,89E-08	1,92E-08	1,11E-08	9,33E-07	1,91E-07
	Max	1,09E-05	1,02E-07	1,21E-07	1,51E-05	2,68E-08	8,68E-06	4,73E-06	2,29E-06	3,39E-08	9,38E-07	2,09E-08	2,72E-08	9,55E-09	1,88E-06	2,29E-07
	3Max	3,39E-06	7,07E-07	2,35E-07	2,82E-05	7,14E-08	1,42E-05	4,20E-06	3,19E-06	2,46E-07	1,28E-06	1,27E-08	1,93E-08	4,79E-09	8,74E-07	2,96E-07
	3Max & MO	6,39E-06	1,63E-07	9,56E-08	1,44E-05	1,91E-08	5,99E-06	2,13E-06	5,15E-06	4,02E-08	1,28E-06	2,02E-08	9,42E-09	4,88E-09	1,17E-06	1,85E-07

Table 5 Biomass of aerial parts of plants

		Aerial parts dry mass (g)	
Soil	Modality	Mean	Standard deviation
Vieusart	Temoin	27,1	2,39
	Max	25,5	0,0603
	3Max	26	3,33
	3Max & OM	24,7	4,77
Sainte-Walburge	Temoin	28,7	2,15
	Max	20,5	2,14
	3Max	25,1	4,98
	3Max & OM	25,9	8,75
Bressoux	Temoin	22,6	2,92
	Max	24,1	2,86
	3Max	27	1,63
	3Max & OM	22,2	3,66
Aubange	Temoin	26,4	2,05
	Max	25	1,82
	3Max	25,3	1,44
	3Max & OM	25,1	1,01

Annexe 8. Raw data – chapter III and IV.

Table 1 Mean trace element extractabilities (n=3) across soils for the Tb_{10}, TbF_2, TbF_{10}, TbF_{100} and $Tb_{10}Gly_{100}$ modalities.

		Mean extractability of trace element (µg/g soil)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Tb10**	2,39E-01	1,27E-01	3,73E-02	1,68E+00	1,73E-02	3,88E-02	1,18E-01	2,81E-01	2,31E-02	2,27E-03	1,84E-03	1,63E-03	4,32E-03	5,53E-01	1,13E-01
	TbF2	9,26E-02	1,01E-01	2,53E-02	1,41E+00	1,44E-02	3,43E-02	9,97E-02	2,36E-01	1,92E-02	1,98E-03	1,60E-03	1,41E-03	3,17E-03	4,68E-01	9,93E-02
	TbF10	4,77E-01	1,15E-01	4,94E-02	1,29E+00	1,44E-02	4,11E-02	1,06E-01	2,39E-01	2,25E-02	2,92E-03	1,44E-03	1,98E-03	3,17E-03	4,29E-01	8,46E-02
	TbF100	1,02E-01	9,21E-02	2,33E-02	1,48E+00	1,22E-02	2,62E-02	1,08E-01	2,31E-01	1,86E-02	1,49E-03	1,41E-03	1,46E-03	3,48E-03	4,88E-01	7,98E-02
	Tb10 & Gly100	9,11E-02	1,24E-01	3,00E-02	1,84E+00	1,68E-02	5,04E-02	1,93E-01	2,67E-01	4,54E-02	3,27E-03	2,09E-03	1,24E-03	4,03E-03	5,40E-01	1,04E-01
Sainte-Walburge	**Tb10**	1,54E-01	1,78E-01	2,63E-02	1,49E+00	1,99E-02	7,36E-02	4,59E-01	2,56E+00	1,34E-01	3,96E-02	8,25E-03	3,01E-03	2,14E-02	7,77E-01	6,07E-01
	TbF2	1,36E-01	1,43E-01	2,18E-02	1,13E+00	1,49E-02	6,49E-02	3,91E-01	1,98E+00	1,20E-01	4,12E-02	6,32E-03	2,62E-03	2,13E-02	5,96E-01	4,47E-01
	TbF10	1,19E-01	1,22E-01	1,77E-02	8,83E-01	1,17E-02	5,57E-02	3,64E-01	1,54E+00	1,10E-01	4,54E-02	5,31E-03	2,94E-03	2,21E-02	4,80E-01	3,48E-01
	Tb10 & Gly100	1,31E-01	1,65E-01	2,20E-02	1,44E+00	1,90E-02	1,06E-01	1,42E+00	2,74E+00	2,00E-01	4,40E-02	9,25E-03	2,81E-03	2,42E-02	6,80E-01	4,71E-01
Bressoux	**Tb10**	1,33E-01	1,41E-01	1,69E-02	1,34E+00	1,25E-02	5,03E-02	7,11E-01	2,96E+00	1,98E-01	2,44E-02	8,04E-03	5,18E-03	6,95E-02	4,86E-01	1,06E+00
	TbF2	1,07E-01	1,41E-01	1,56E-02	1,39E+00	1,24E-02	5,03E-02	7,01E-01	2,89E+00	1,92E-01	2,14E-02	7,81E-03	4,44E-03	6,70E-02	4,73E-01	1,03E+00
	TbF10	9,94E-02	1,28E-01	1,37E-02	1,11E+00	1,03E-02	4,62E-02	6,54E-01	2,46E+00	1,82E-01	2,25E-02	6,70E-03	4,76E-03	6,88E-02	3,96E-01	8,12E-01
	Tb10 & Gly100	1,13E-01	1,39E-01	1,61E-02	1,33E+00	1,23E-02	7,23E-02	5,25E+00	5,55E+00	2,51E-01	2,38E-02	9,86E-03	5,64E-03	7,15E-02	5,33E-01	1,06E+00
Aubange	**Tb10**	6,31E-02	1,36E-01	1,88E-02	9,11E+00	3,82E-02	5,80E-02	6,07E-02	2,39E-01	3,28E-02	1,87E-03	6,63E-04	1,80E-03	1,55E-03	1,79E-01	1,28E-01
	TbF2	4,65E-02	1,38E-01	1,81E-02	9,75E+00	4,05E-02	6,39E-02	5,30E-02	2,01E-01	3,21E-02	1,25E-03	6,22E-04	1,18E-03	1,22E-03	1,89E-01	1,31E-01
	TbF10	4,70E-02	1,23E-01	1,62E-02	8,72E+00	3,65E-02	5,65E-02	4,97E-02	1,87E-01	3,08E-02	1,61E-03	5,87E-04	1,30E-03	1,60E-03	1,61E-01	1,13E-01
	Tb10 & Gly100	3,89E-02	1,32E-01	1,57E-02	9,33E+00	3,85E-02	6,73E-02	5,55E-02	1,96E-01	5,42E-02	4,04E-03	6,42E-04	1,40E-03	1,60E-03	1,73E-01	1,15E-01

Table 2 Standard deviations of trace element extractabilities (n=3) across soils for the Tb_{10}, TbF_2, TbF_{10}, TbF_{100} and $Tb_{10}Gly_{100}$ modalities.

		Standard deviation of the extractability of trace element (µg/g soil)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**Tb10**	1,17E-01	2,05E-03	7,59E-03	1,42E-01	6,79E-04	2,68E-03	1,07E-02	1,41E-02	2,28E-03	2,42E-04	1,73E-04	3,05E-04	1,96E-03	3,87E-02	9,88E-03
	TbF2	1,64E-02	1,51E-03	1,27E-03	1,21E-02	6,05E-05	7,95E-04	2,00E-03	1,17E-02	8,33E-04	2,90E-04	4,46E-05	6,41E-05	3,80E-04	1,12E-02	2,18E-03
	TbF10	6,38E-01	4,66E-02	4,30E-02	1,08E-01	4,15E-03	1,53E-02	1,12E-02	4,41E-02	7,01E-03	8,48E-04	5,82E-05	1,26E-03	4,61E-04	6,32E-02	8,22E-03
	TbF100	1,08E-02	1,22E-02	2,81E-03	1,67E-01	1,80E-03	2,03E-03	2,78E-02	3,23E-02	2,36E-03	1,67E-04	1,51E-04	4,40E-04	1,90E-04	5,27E-02	1,31E-02
	Tb10 & Gly100	8,39E-03	1,11E-02	2,15E-03	1,89E-01	1,59E-03	2,39E-03	3,64E-03	2,25E-02	1,84E-03	4,00E-04	2,61E-04	1,10E-04	2,33E-04	6,43E-02	1,29E-02
Sainte-Walburge	**Tb10**	2,62E-02	1,69E-02	2,97E-03	1,17E-01	1,86E-03	7,98E-03	4,03E-02	1,93E-01	1,85E-02	7,07E-03	7,77E-04	8,39E-04	2,50E-03	6,82E-02	4,90E-02
	TbF2	3,06E-02	2,13E-02	4,40E-03	2,53E-01	3,38E-03	7,64E-03	4,02E-02	4,54E-01	5,28E-03	2,84E-03	1,38E-03	1,45E-04	5,27E-05	1,37E-01	1,18E-01
	TbF10	4,16E-03	4,99E-03	1,04E-03	6,84E-02	8,45E-04	2,85E-03	3,15E-02	1,09E-01	4,67E-03	7,90E-04	4,89E-04	4,28E-04	4,85E-04	3,53E-02	4,41E-02
	Tb10 & Gly100	9,95E-03	3,29E-03	4,57E-04	5,96E-02	5,76E-04	1,81E-03	2,96E-02	7,29E-02	1,71E-03	1,86E-03	3,80E-04	2,21E-05	6,00E-04	3,01E-02	3,17E-02
Bressoux	**Tb10**	2,02E-02	5,47E-03	1,36E-03	1,25E-01	1,33E-03	2,88E-03	2,80E-02	1,88E-01	4,25E-03	5,03E-04	6,44E-04	9,25E-04	2,29E-04	3,34E-02	9,37E-02
	TbF2	8,04E-03	6,44E-03	9,91E-04	8,75E-02	7,40E-04	1,36E-03	1,75E-02	1,24E-01	6,44E-03	6,58E-04	2,17E-04	1,82E-04	1,03E-03	2,07E-02	5,73E-02
	TbF10	3,41E-03	4,88E-03	8,70E-04	8,28E-02	8,57E-04	1,72E-03	1,27E-02	1,52E-01	8,28E-03	8,06E-04	5,22E-04	2,22E-04	1,06E-03	2,62E-02	6,11E-02
	Tb10 & Gly100	4,13E-03	3,27E-03	5,60E-05	3,31E-02	2,38E-04	4,20E-04	1,06E+00	5,03E-02	8,08E-03	5,01E-04	3,97E-04	3,33E-05	1,38E-03	1,81E-02	3,86E-02
Aubange	**Tb10**	1,27E-02	2,35E-02	4,08E-03	1,67E+00	6,36E-03	8,06E-03	1,48E-02	2,04E-02	2,77E-03	3,58E-04	5,74E-05	3,84E-04	2,08E-04	2,22E-02	1,58E-02
	TbF2	3,53E-03	5,79E-03	5,17E-04	4,62E-01	1,68E-03	3,66E-03	1,78E-03	5,51E-03	2,00E-04	1,22E-04	1,06E-04	4,53E-05	2,57E-04	9,07E-03	4,60E-03
	TbF10	6,62E-03	1,49E-02	1,56E-03	9,22E-01	3,85E-03	5,58E-03	8,05E-03	1,77E-02	3,28E-03	1,99E-04	9,71E-05	1,35E-04	1,19E-04	2,17E-02	1,71E-02
	Tb10 & Gly100	9,46E-03	1,19E-02	1,75E-03	7,46E-01	3,27E-03	4,28E-03	7,72E-03	1,03E-02	3,48E-03	4,32E-04	3,39E-05	9,45E-05	1,53E-04	1,06E-02	1,10E-02

Table 3 Mean trace element extractabilities (n=3) across soils for the DOC_{100}, $Gly_{100}DOC_{100}$, $GlyF_{100}DOC_{100}$ and $AMPA_{100}DOC_{100}$ modalities.

		Mean extractability of trace element (µg/g soil)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**DOC100**	1,19E-01	2,75E-02	1,27E-02	9,76E-01	1,14E-02	8,16E-02	4,77E-01	2,36E-01	1,79E-02	2,77E-02	2,08E-03	2,19E-03	4,99E-03	2,70E-01	2,34E-02
	Gly100DOC100	1,04E-01	3,88E-02	1,22E-02	1,33E+00	1,48E-02	1,00E-01	5,65E-01	2,38E-01	3,61E-02	2,83E-02	2,61E-03	2,06E-03	6,22E-03	2,56E-01	2,44E-02
	GlyF100DOC100	1,82E-01	4,69E-02	1,80E-02	1,17E+00	1,46E-02	1,05E-01	5,69E-01	3,05E-01	3,92E-02	3,75E-02	2,71E-03	2,50E-03	6,13E-03	2,50E-01	2,62E-02
	AMPA100DOC100	7,40E-02	3,35E-02	9,38E-03	1,06E+00	1,17E-02	8,90E-02	4,93E-01	2,37E-01	3,41E-02	3,39E-02	2,30E-03	2,12E-03	5,58E-03	2,51E-01	2,25E-02
Sainte-Walburge	**DOC100**	3,78E-01	9,75E-02	2,98E-02	7,33E-01	1,43E-02	1,51E-01	8,55E-01	9,01E-01	1,04E-01	1,08E-01	6,75E-03	6,73E-03	2,27E-02	4,08E-01	9,03E-02
	Gly100DOC100	1,42E-01	9,56E-02	1,40E-02	9,75E-01	1,77E-02	1,69E-01	1,69E+00	1,45E+00	1,57E-01	1,00E-01	9,25E-03	3,85E-03	2,66E-02	4,83E-01	7,19E-02
	GlyF100DOC100	2,98E-01	1,12E-01	2,60E-02	9,20E-01	1,83E-02	1,94E-01	1,75E+00	1,69E+00	1,76E-01	1,08E-01	1,07E-02	5,80E-03	2,63E-02	4,31E-01	9,72E-02
	AMPA100DOC100	1,53E-01	9,53E-02	1,43E-02	7,13E-01	1,24E-02	1,35E-01	8,78E-01	6,86E-01	1,56E-01	1,10E-01	6,58E-03	4,01E-03	2,48E-02	3,95E-01	5,89E-02
Bressoux	**DOC100**	2,11E-01	1,00E-01	2,16E-02	7,27E-01	1,19E-02	1,29E-01	1,48E+00	3,81E+00	1,26E-01	6,53E-02	1,24E-02	1,34E-02	5,68E-02	4,14E-01	3,70E-01
	Gly100DOC100	2,15E-01	1,17E-01	2,26E-02	8,27E-01	1,26E-02	1,80E-01	4,63E+00	6,55E+00	1,89E-01	6,35E-02	1,44E-02	1,32E-02	6,14E-02	4,62E-01	4,01E-01
	GlyF100DOC100	2,91E-01	1,24E-01	2,75E-02	8,27E-01	1,37E-02	1,84E-01	5,22E+00	7,06E+00	1,98E-01	6,62E-02	1,53E-02	1,60E-02	6,04E-02	4,52E-01	4,41E-01
	AMPA100DOC100	2,07E-01	1,11E-01	2,16E-02	7,46E-01	1,16E-02	1,32E-01	1,54E+00	3,95E+00	1,89E-01	6,56E-02	1,25E-02	1,26E-02	5,88E-02	4,32E-01	3,66E-01
Aubange	**DOC100**	8,26E-02	4,64E-02	1,69E-02	1,42E+00	1,01E-02	8,01E-02	3,93E-01	2,02E-01	2,51E-02	1,01E-02	5,33E-04	2,64E-03	2,28E-03	3,98E-02	2,87E-02
	Gly100DOC100	1,33E-01	7,34E-02	2,62E-02	1,64E+00	1,25E-02	9,70E-02	4,04E-01	1,92E-01	4,37E-02	1,86E-02	4,66E-04	2,35E-03	2,42E-03	3,91E-02	2,83E-02
	GlyF100DOC100	1,93E-01	1,07E-01	3,63E-02	2,83E+00	1,78E-02	1,22E-01	4,06E-01	2,24E-01	5,68E-02	2,49E-02	5,91E-04	2,12E-03	2,54E-03	6,67E-02	4,16E-02
	AMPA100DOC100	6,66E-02	4,35E-02	1,30E-02	1,31E+00	9,49E-03	8,58E-02	3,88E-01	1,94E-01	3,48E-02	1,71E-02	5,39E-04	1,97E-03	2,23E-03	6,12E-02	2,80E-02

Table 4 Standard deviations of trace element extractabilities (n=3) across soils for the DOC_{100}, $Gly_{100}DOC_{100}$, $GlyF_{100}DOC_{100}$ and $AMPA_{100}DOC_{100}$ modalities.

		Standard deviation of the extractability of trace element (µg/g soil)														
Soil	**Modality**	**Ti**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sn**	**Sb**	**Ba**	**Pb**
Vieusart	**DOC100**	8,89E-02	8,56E-03	6,69E-03	5,22E-02	1,14E-03	5,14E-03	6,52E-03	3,59E-02	1,31E-03	1,37E-03	1,69E-04	2,08E-04	2,03E-04	2,40E-02	2,94E-03
	Gly100DOC100	2,50E-02	2,09E-03	1,78E-03	1,99E-02	2,63E-05	1,70E-03	2,77E-02	3,55E-02	1,24E-03	9,51E-04	1,16E-04	1,04E-04	6,71E-05	5,62E-03	1,57E-03
	GlyF100DOC100	9,63E-02	8,49E-03	6,64E-03	3,12E-02	3,92E-04	1,80E-03	9,78E-03	6,02E-02	1,70E-03	1,77E-03	1,37E-04	2,76E-04	2,19E-04	1,55E-02	2,58E-03
	AMPA100DOC100	3,29E-02	2,36E-03	1,75E-03	1,04E-02	3,72E-04	9,52E-04	2,73E-03	4,58E-03	5,93E-04	1,02E-03	1,01E-04	9,97E-05	1,32E-04	1,18E-02	1,19E-03
Sainte-Walburge	**DOC100**	1,91E-01	1,74E-02	1,24E-02	6,18E-02	1,73E-03	1,45E-03	7,88E-03	1,24E-01	3,15E-03	1,98E-03	3,44E-04	1,94E-03	7,57E-04	2,97E-02	1,98E-02
	Gly100DOC100	2,33E-02	2,86E-03	1,62E-03	1,02E-02	6,69E-06	2,54E-03	3,90E-02	4,42E-02	4,26E-04	2,69E-03	3,76E-04	4,54E-04	6,91E-04	7,37E-02	6,63E-03
	GlyF100DOC100	9,41E-02	8,97E-03	6,25E-03	3,12E-02	7,96E-04	1,47E-03	9,79E-03	8,09E-02	3,84E-03	1,55E-03	3,49E-04	1,23E-03	1,90E-03	1,19E-02	1,27E-02
	AMPA100DOC100	7,71E-02	9,25E-03	5,68E-03	4,11E-02	1,16E-03	3,72E-03	1,10E-02	1,11E-01	5,96E-03	2,93E-03	4,05E-04	9,98E-04	8,04E-04	2,00E-02	1,57E-02
Bressoux	**DOC100**	6,00E-02	5,65E-03	3,42E-03	4,45E-02	1,20E-03	5,84E-03	5,51E-02	1,91E-01	3,66E-03	4,46E-03	6,18E-04	1,47E-03	3,30E-03	2,14E-02	2,61E-02
	Gly100DOC100	3,68E-02	4,33E-03	2,96E-03	6,35E-02	5,90E-04	3,51E-02	8,16E-02	1,90E-01	6,19E-03	8,34E-04	6,16E-04	2,20E-03	1,74E-03	2,11E-02	4,12E-02
	GlyF100DOC100	7,34E-02	7,12E-03	4,97E-03	5,09E-02	1,00E-03	2,77E-02	9,82E-02	1,49E-01	3,07E-03	8,78E-04	2,96E-04	2,51E-03	1,01E-03	4,64E-02	2,99E-02
	AMPA100DOC100	8,22E-03	1,87E-03	6,39E-05	2,45E-02	7,92E-05	2,23E-03	1,54E-02	3,72E-02	1,38E-03	4,27E-04	3,46E-04	3,01E-04	7,30E-04	1,11E-02	2,36E-02
Aubange	**DOC100**	7,97E-02	3,41E-02	1,32E-02	6,28E-01	3,24E-03	9,02E-03	4,31E-03	2,19E-02	8,13E-03	6,85E-04	8,55E-05	5,61E-04	4,10E-05	7,01E-03	5,42E-03
	Gly100DOC100	6,45E-02	2,97E-02	1,16E-02	3,66E-01	2,08E-03	8,81E-03	2,84E-03	1,84E-02	6,76E-03	5,97E-04	2,85E-05	2,09E-04	8,62E-05	8,17E-03	3,81E-03
	GlyF100DOC100	4,18E-02	2,40E-02	7,84E-03	7,05E-01	2,84E-03	2,13E-02	1,51E-03	4,46E-02	6,22E-03	4,15E-04	1,39E-04	1,28E-04	1,66E-04	2,44E-02	8,72E-03
	AMPA100DOC100	1,90E-02	6,24E-03	2,85E-03	6,02E-02	2,80E-04	5,00E-03	1,63E-03	3,50E-02	1,56E-03	2,25E-04	1,13E-04	1,64E-04	1,51E-04	4,39E-02	2,05E-03

Annexe 9. BCR extractions.

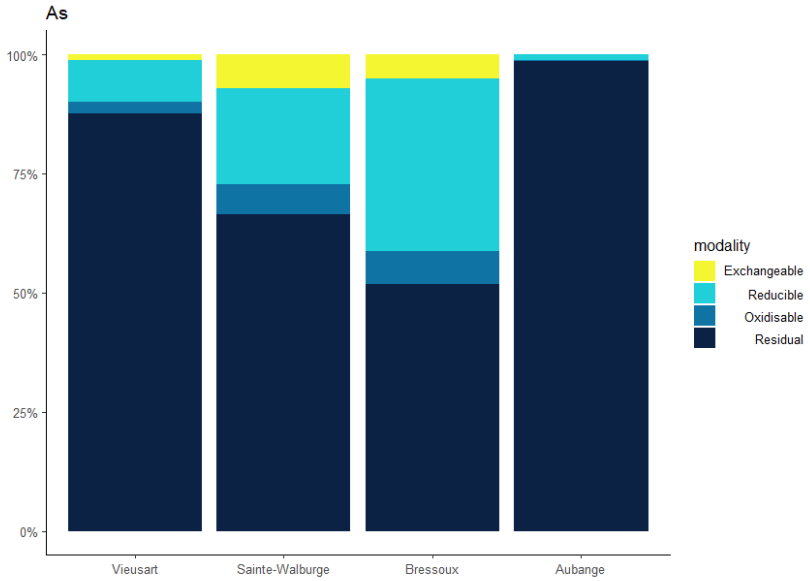
1. Protocol

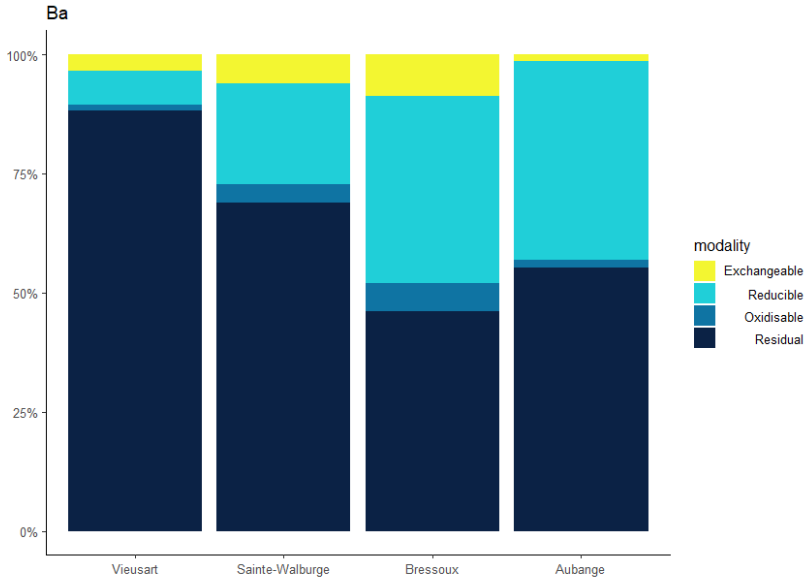
To assess the element distribution in solid phase of each studied soil a modified BCR three-step sequential extraction (Žemberyová et al., 2006) was performed on 1 g of <2 mm soil samples: (1) 40 mL of 0.11 mol L−1 CH3COOH (exchangeable fraction); (2) 40 mL of 0.5 mol L−1 $NH_2OH{\cdot}HCl$ (reducible-iron/manganese oxides fraction); and (3) 2×10 mL of 8.8 mol L−1 H_2O2 before 50 mL of 1 mol L−1 CH_3COONH_4 (oxidizable-organic matter and sulfides fraction). For each extraction step, soil samples were mixed with the extractant at 300 rpm for 16 h using a mechanical shaker (Universal Shaker SM 30 B, Edmund Bühler GmbH, Bodelshausen, Germany). The extracted solutions were then separated from the solid phase by: (1) centrifugation 160 at 4000 rpm for 20 min (Centrifuge Jouan GR4.12, France); (2) filtration with 20-µm Whatman filters; and (3) filtration with 0.2-µm GHP Acrodisc (PSF and nylon, Pall). The precipitates were washed with 20 mL of Milli-Q water before the next step. The residual fraction was obtained for each element by the difference between the total soil content and the sum of the other three fractions.

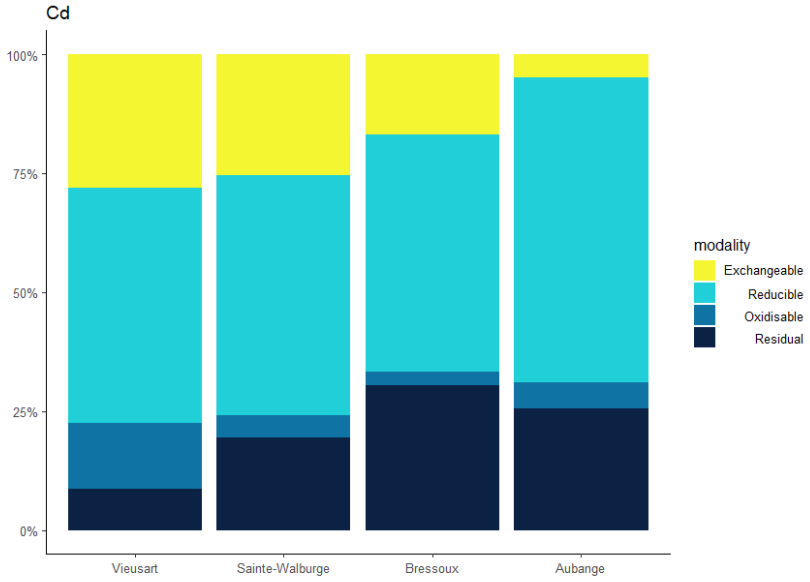
To determine the distribution of elements in the solid phase of each studied soil, a modified BCR three-step sequential extraction (Žemberyová et al. 2006) was conducted on 1 g of soil (sieved at 2 mm). The extraction steps included: (1) 40 mL of 0.11 mol L−1 CH_3COOH for the exchangeable fraction; (2) 40 mL of 0.5 mol L−1 $NH_2OH{\cdot}HCl$ for the reducible fraction associated with iron and manganese oxides; and (3) 2×10 mL of 8.8 mol L−1 H_2O_2 followed by 50 mL of 1 mol L−1 CH_3COONH_4 for the oxidizable fraction, which includes organic matter and sulfides.

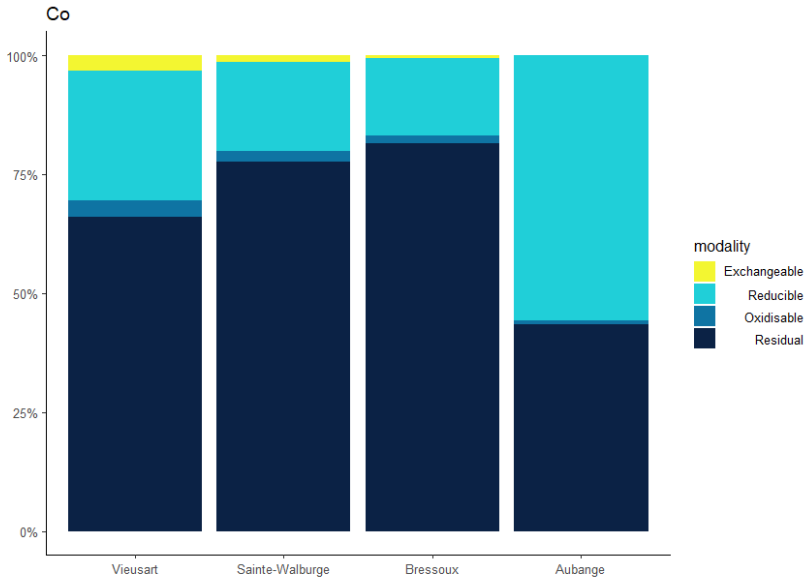
During each extraction step, the soil samples were agitated at 300 rpm for 16 hours using a mechanical shaker (Universal Shaker SM 30 B, Edmund Bühler GmbH, Bodelshausen, Germany). The resulting solutions were then separated from the solid phase by: (1) centrifugation at 4000 rpm for 20 minutes (Centrifuge Jouan GR4.12, France); (2) filtration through 20-µm Whatman filters; and (3) filtration through 0.2-µm GHP Acrodisc filters (PSF and nylon, Pall). The precipitates were rinsed with 20 mL of Milli-Q water before proceeding to the next step. The residual fraction for each element was determined by subtracting the sum of the three extracted fractions from the total soil content.

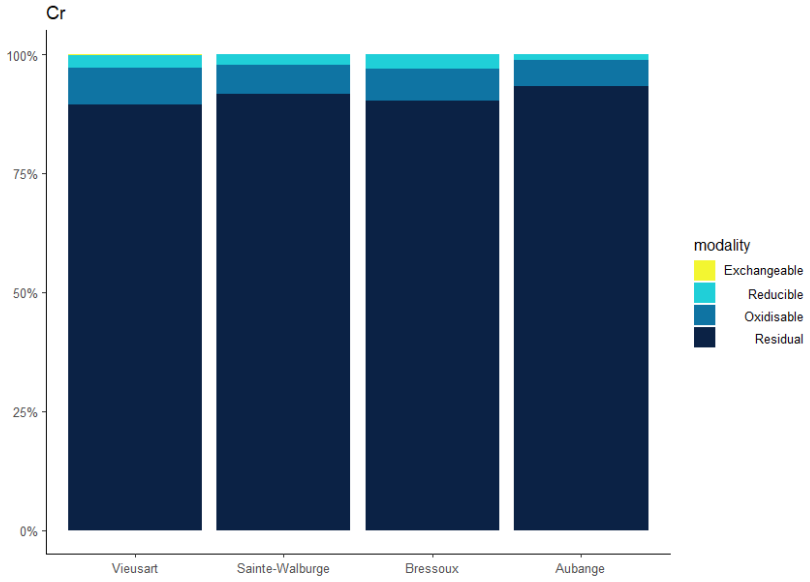
2. Results

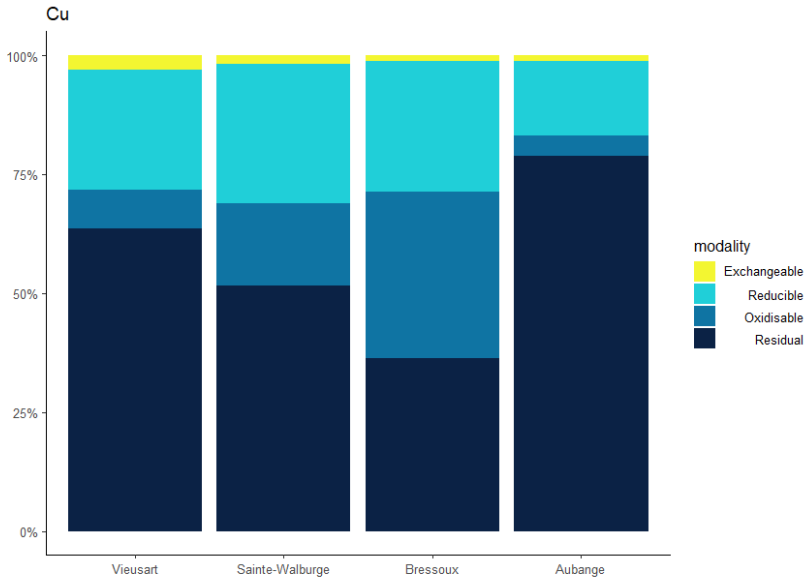

As
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

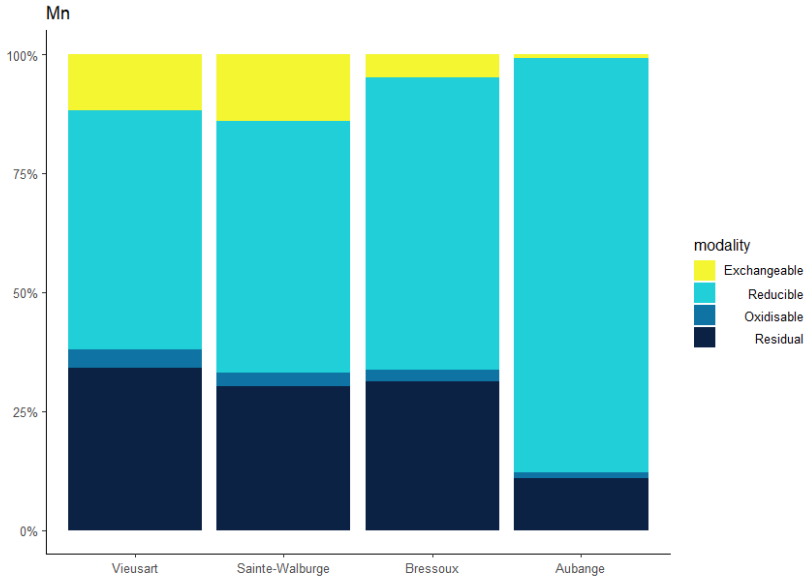

Ba
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

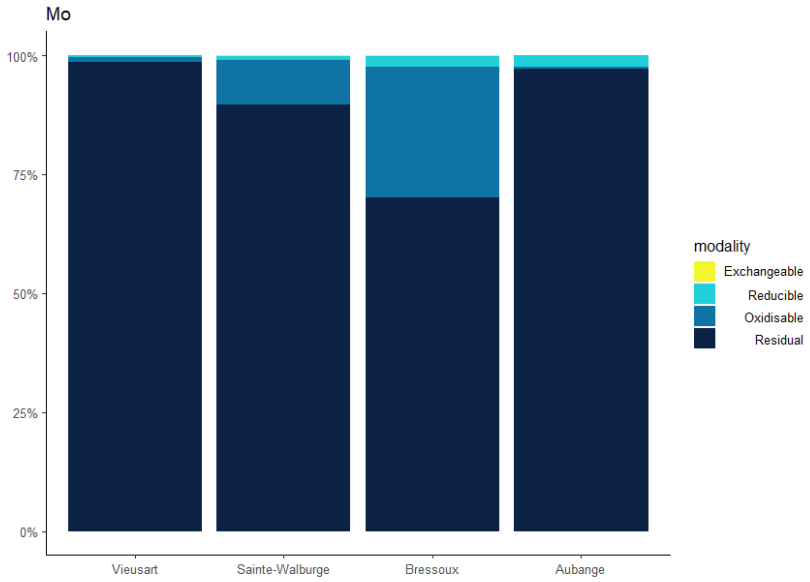

Cd
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

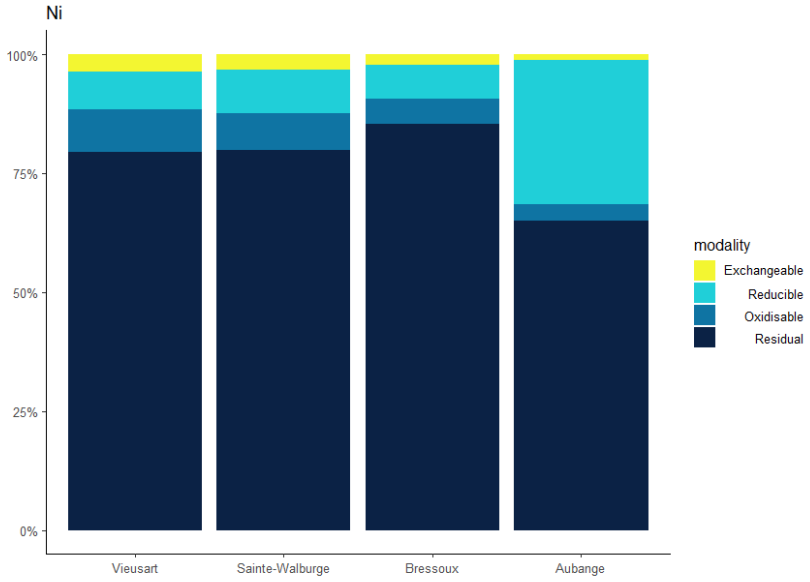

Co
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

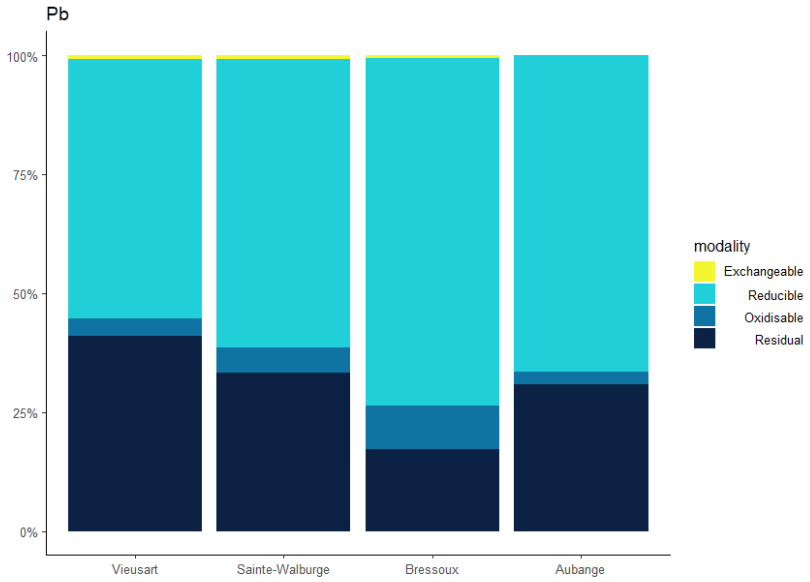

Cr
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

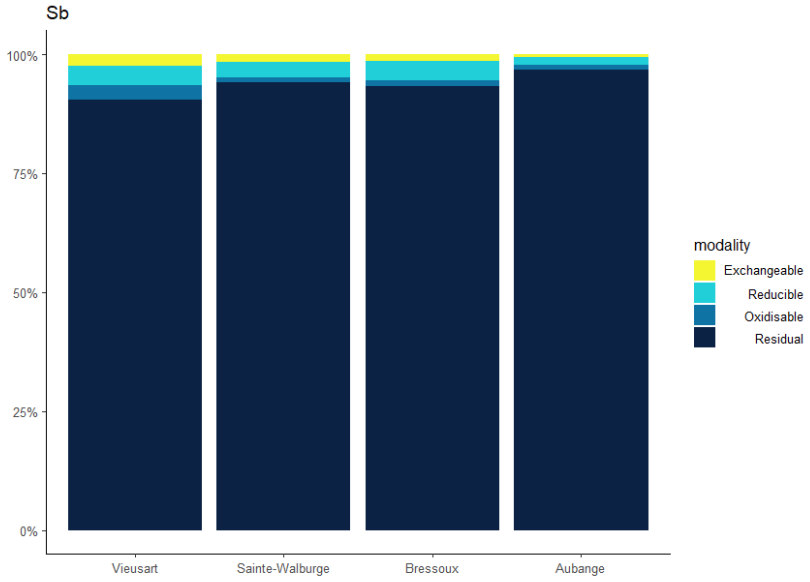

Cu
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

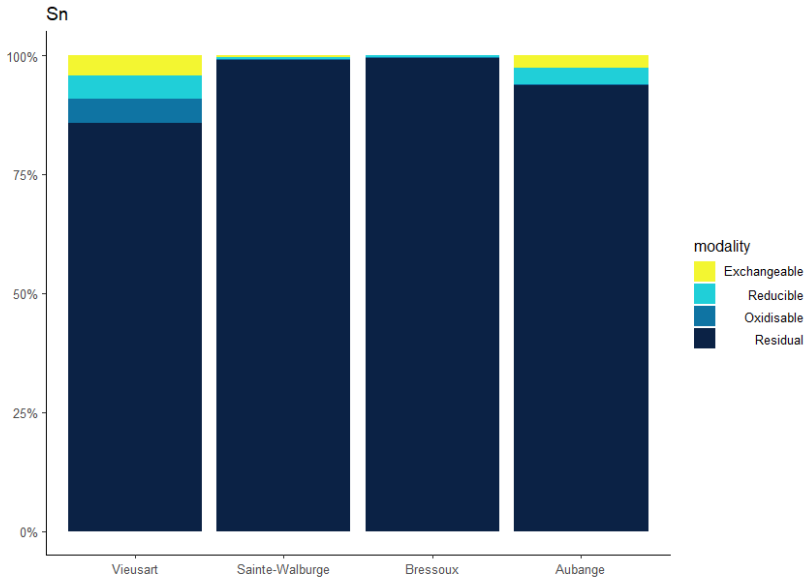

Mn
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

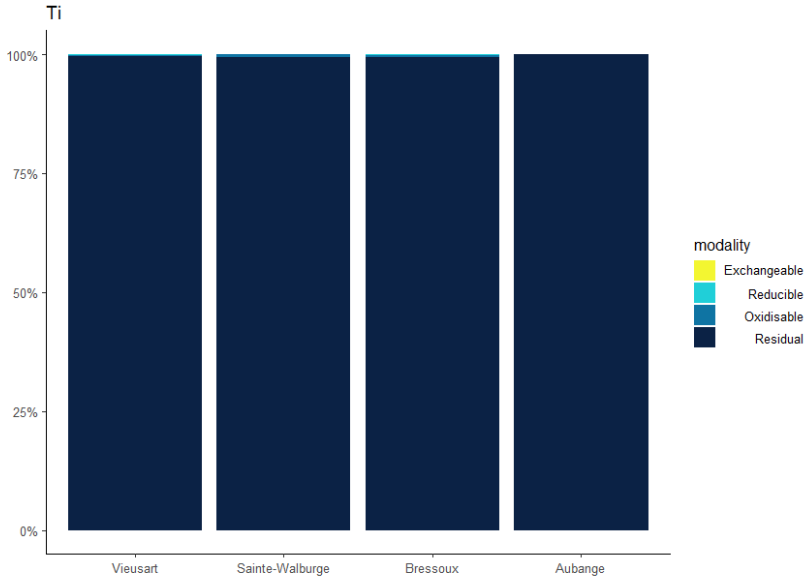

Mo
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

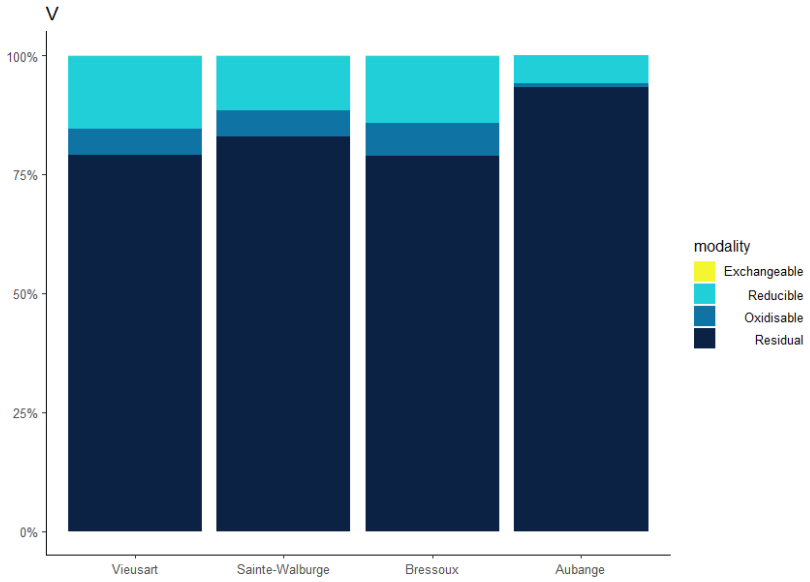

Ni
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

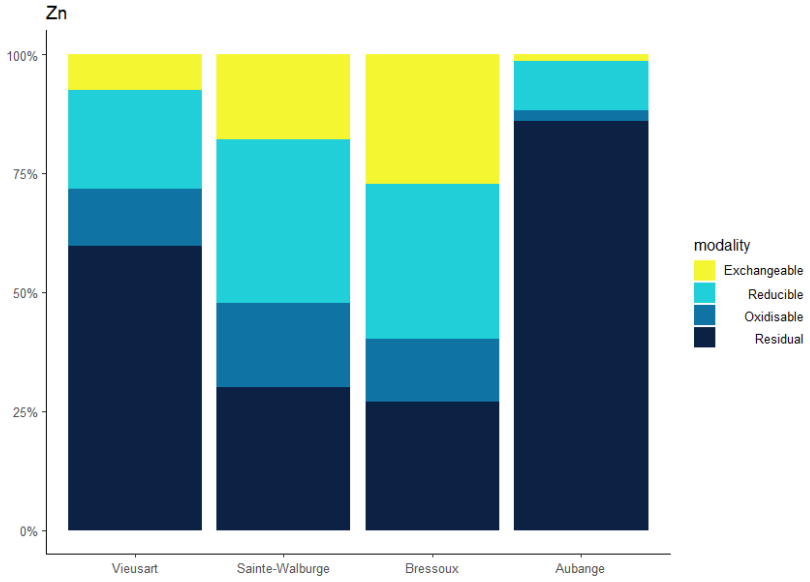

Pb
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual


Sb
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual


Sn
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual


Ti
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual


V
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual


Zn
100%
75%
50%
25%
0%
Vieusart
Sainte-Walburge
Bressoux
Aubange
modality
Exchangeable
Reducible
Oxidisable
Residual

Annexe 10. List of publications.

Articles published in peer-reviewed journals

Arbalestrie Bryan, Thami Adil, Dedoyard Marie, **Bemelmans Nathan**, Agnan Yannick. Challenges in assessing trace element transfer from soil to plant: contribution of environmental proxies for uncontaminated and contaminated agricultural soils. (in prep)

Bemelmans Nathan, Dejardin Rosalie, Arbalestrie Bryan, Agnan Yannick (2024) Glyphosate application may influence the transfer of trace elements from soils to both soil solutions and plants. *Chemosphere* 367:. https://doi.org/10.1016/j.chemosphere.2024.143603

Bemelmans, Nathan, Arbalestrie, Bryan, Dailly, Hélène, Bodart, Etienne & Agnan, Yannick (2023). Influence of glyphosate and aminomethylphosphonic acid on the mobility of trace elements in uncontaminated and contaminated agricultural soils. *Environmental Science and Pollution Research*, 30, 103983-103995. doi :10.1007/s11356-023-29660-w

Monhonval, Arthur, Mauclet Elisabeth, Hirst, Catherine, **Bemelmans, Nathan**, Eekman, Elodie, Schuur Edward & Opfergelt, Sophie (2023). Mineral organic carbon interactions in dry versus wet tundra soils. *Geoderma*, *436*, 116552. doi :10.1016/j.geoderma.2023.116552

Arbalestrie, Bryan, Falys, Julie, **Bemelmans, Nathan**, Thami, Adil, Monin, Laurence, Devos, Elodie & Agnan, Yannick (2022). Rare earth elements in an intercropping cover crop to evaluate the trace element transfer from soil to plant. *Biogeochemistry*, *161*(1), 373–387. doi :10.1007/s10533-022-00989-7

Monhonval, Arthur, Strauss, Jens, Mauclet, Elisabeth, Hirst, Catherine, **Bemelmans, Nathan**, Grosse, Guido, Schirrmeister, Lutz, Fuchs, Matthias & Opfergelt, Sophie (2021). Iron Redistribution Upon Thermokarst Processes in the Yedoma Domain. *Frontiers in Earth Science*, *9*, doi :10.3389/feart.2021.703339

Presentations during conferences with scientific selection committee.

Bemelmans, Nathan, Dejardin, Rosalie, Arbalestrie, Bryan & Agnan, Yannick (2024). Glyphosate application modifies the transfer of trace elements from soils to soil solutions and plants. Communication presented at the Centennial IUSS, Florence.

Thomas, Maxime, Fouché, Julien, Morelle, Charlotte, **Bemelmans, Nathan**, Rochereau, Thomas, Lafrenière, Melissa, Heslop, Joanne K. & Opfergelt, Sophie (2024). Impact of hillslope thermokarst development on mineral-OC interactions: case study in Cape Bounty, Canadian High Arctic. Communication presented at Geologica Belgica Luxemburga International Meeting 2024, Liège, Belgium.

Arbalestrie, Bryan, Van de Casteele, Adrien, Quénéa, Katell, Baudin, François, Anquetil, Christelle, **Bemelmans, Nathan** & Agnan, Yannick (2024). Qualitative characterization and influence of organic matter on the transfer of potentially harmful trace elements in the soil-plant system. Communication presented at Centennial IUSS, Florence.

Bemelmans, Nathan, Dejardin, Rosalie, Arbalestrie, Bryan & Agnan, Yannick (2023). Glyphosate application increases or decreases the mobility of trace elements in agricultural soils depending on the contamination level. Communication presented at ICOBTE/ICHMET, Wuppertal.

Bemelmans, Nathan, Arbalestrie, Bryan & Agnan, Yannick (2023). Impact of glyphosate and aminomethylphosphonic acid on the mobility of trace elements in uncontaminated and contaminated agricultural soils. Communication presented at Goldschmidt, Lyon.

Agnan, Yannick, Arbalestrie, Bryan, Falys, Julie, Dedoyard, Marie & **Bemelmans, Nathan** (2023). Rare earth elements in agroecosystems: interest in the understanding of trace element transfer from soil to plant. Communication presented at ICOBTE/ICHMET, Wuppertal.

Arbalestrie, Bryan, Thami, Adil, **Bemelmans, Nathan** & Agnan, Yannick (2023). Transfer evaluation of potentially harmful elements from uncontaminated and contaminated soils to plant. Communication presented at Goldschmidt, Lyon.

Bemelmans, Nathan, Arbalestrie, Bryan & Agnan, Yannick (2022). Influence du glyphosate et de l'AMPA sur l'extractibilité des éléments traces dans les sols contaminés et non contaminés. Communication presented at Groupe français de recherches sur les pesticides, Namur.

Bemelmans, Nathan, Arbalestrie, Bryan & Agnan, Yannick (2022). Influence of glyphosate and AMPA on the extractability of trace elements in uncontaminated and contaminated soils. Communication presented at Young Soil Scientists Day 2022, Brussels.

Arbalestrie, Bryan, Falys, Julie, **Bemelmans, Nathan**, Thami, Adil & Agnan, Yannick (2022). Use of rare earth elements as tracers for soil–plant transfer of trace elements. Communication presented at Young Soil Scientists Day 2022, Brussels.

Monhonval, Arthur, Opfergelt, Sophie, Mauclet, Elisabeth, Hirst, Catherine, Pereira, Benoît, Vandeuren, Aubry, **Bemelmans, Nathan**, Grosse Guido, Schirrmeister Lutz & Fuchs Matthias (2020). Iron Dynamics during Thermokarst Processes in the Yedoma Domain and Implications for Interactions between Iron and Organic Carbon. Communication presented at AGU FALL MEETING, ONLINE everywhere.

Thomas, Maxime, Opfergelt, Sophie, Monhonval, Arthur, **Bemelmans, Nathan**, Lafreniere Melissa, Heslop Joanne, Fouché Julien & Rochereau Thomas (2019). Impact of modern thermokarst on mineral element release: case study in Cape Bounty, Canada. Communication presented at Artic Week, Paris, France.