Bioremediation Of Agricultural Soils

Editor

Juan C. Sanchez-Hernandez

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Preface

Quality of agricultural soil is under permanent threat of degradation. Environmental phenomena such as erosion, landslides, flooding, decline of organic matter, loss of biodiversity, and pollution are considered the major threats to the quality of agricultural soils. Among them, chemical contamination is the most alarming and stealthy phenomenon because of its long-term adverse impact on soil biodiversity and functioning, ultimately affecting crop productivity.

Agricultural soil receives a wide variety of environmental contaminants through multiple input pathways. Pollutants such as polycyclic aromatic hydrocarbons, polychlorinated and polybrominated biphenyls, pesticides and fertilizers, metals, and more recently, pharmaceuticals and personal care products, reach the soil through irrigation with reclaimed wastewater, application of sewage sludges and agrochemicals to combat respectively nutrient deficit of soil and pests, or even by atmospheric deposition. Occasionally, land destined to agriculture is historically contaminated by toxic chemicals such as metals; this is a frequent challenge in countries with a high mining activity (e.g., Chile), or in urban farming. In addition, in recent years, compelling evidence in recent years show new families of contaminants in soil. This is the case of engineered nanomaterials and microplastics whose environmental fate and toxicity are nowadays topics of increasing concern in the scientific community. Plastic pollution of soil is particularly relevant if we consider that plastics may contain endogenous toxic chemicals that release during their degradation, or they may accumulate and transport exogenous contaminants bound on their surface.

Among the components of the chemical cocktail that contaminate the agricultural soil, pesticides and fertilizers are the main inducers of its deterioration. Decades of intensive research have led to a more sustainable use of agrochemicals that control pests and increase crop yield with a minimum impact in the environment. To keep this idea of sustainability, many methodologies for monitoring pesticide residues in the environment and a vast number of toxicity testing procedures and currently available to managing agricultural pesticides. However, new emerging issues strike this equilibrium in the coming years. For example, recent studies suggest that growing of biofuel crops and the climate change will be two global threats that will increase the agrochemical consumption with unpredictable side-effects in the agroecosystem.

Nowadays, environmental remediation technology needs innovative clean up strategies with a double scope, i.e., the removal of contaminants (and toxicity) from the soil and keeping soil quality as a preventive measure. In this context, bioremediation (i.e., the use of living organisms to remove or inactivate environmental contaminants) provides an attractive approach for removal of chemical stressors and, in turn, improving biological and chemical parameters of soil quality. Scientific literature is plenty of example of bioremediation methodologies to clean soil using microorganisms and plants. Although, most of the case studies of bioremediation have been performed in metal-contaminated sites, there is a growing concern in using *in situ* bioremediation strategies for cleaning up soil contaminated by pesticides. This is the main scope of this book, i.e., to provide the reader with a set of *in situ* bioremediation methodologies that, besides recovering contaminated soils, increase and maintain its quality. The book is set in three parts that collect 13 chapters written by experts in their field.

The first part (**Chemical Stressors in the Agroecosystem**) will introduce the main chemical stressors of current concern in agriculture, offering a cutting-edge knowledge on their environmental fate and toxicity. Four chapters will deal with the most common pesticides and fertilizers used in conventional agriculture, providing a general vision about sources of contamination and potential environmental risks. Particular attention will be put on plastic debris and microplastics as emerging pollutants in soil, and to urban agriculture as an increasing option of sustainable agriculture although not exempt from contamination and risk to human health. The second part (***In situ* Bioremediation**) will provide an up-to-date knowledge on the major *in situ* bioremediation approaches to clean up polluted soils. Readers will find seven chapters that describe the most common bioremediation strategies (e.g., phytoremediation, biostimulation and bioaugmentation) and their principal achievements and limitations. In this part of the book, we describe emerging in situ bioremediation methodologies compatible with sustainable agriculture and the concept of bioeconomy. Among them, vermicompost, biochar and earthworms appear as promising and complementary remediation strategies. Finally, the third part (**Biological Methodologies for Monitoring Bioremediation**) deals with methodologies to be used in the evaluation of bioremediation effectiveness. The major goal of any remediation action is the decrease of concentrations of environmental contaminants below regulatory limits. However, this end does not mean necessarily that soil deterioration disappears. Indeed, adverse effects on the biological components of soil still may persist because of long-term exposure to low-level contamination. The last section will cover this important issue, occasionally forgotten in the remediation programs, describing the most innovative methodologies for monitoring soil degradation through toxicity testing, biomarkers, and soil enzyme activities.

It is expected that the reader finds in this book inspiration for developing novel ways and strategies for the *in situ* bioremediation of agricultural contaminated soils. The interesting point around the multiple strategies discussed in this book coming from the ecological interactions and synergistic effects that can be developed between the biological actors involved in bioremediation, from microorganisms and plants to soil fauna (e.g., earthworms).

Finally, I am very grateful to acknowledge the efforts of all contributors that made this book comes the light, several colleagues from the academic and business sectors for their criticism and suggestions. I also extend my appreciation to the Editorial Department of CRC Press for continuous assistance.

Toledo, November 2018 **Juan C. Sanchez-Hernandez**

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Part 2
In situ Bioremediation

Chapter 5

Metal Contamination in Urban Soils

Use of Nature-Based Solutions for Developing Safe Urban Cropping

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The Urban Soil Specificities

In the urban areas, the soils are, most of the time, stripped, filled, mixed, compacted and supplemented with artificial materials. Soil profiles are enormously modified, leading to high spatial and vertical heterogeneity (Meuser 2010). At the same time, a strong spatial heterogeneity characterizes the urban crop soil from physical, chemical

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and biological aspects (Morel et al. 2005, Béchet et al. 2009). This heterogeneity can be explained by a wide range of applications aimed for the welfare of citizens (support for buildings, road infrastructure, recreational areas, kitchen gardens and parklands) (Blanchart et al. 2017). In addition, urban soils are known to have peculiar characteristics such as unpredictable layering, poor structure, and high concentrations of persistent contaminants such as trace metals (Kabata-Pendias 2010). Eventually, many studies have measured high bulk densities in urban soils (up to 2 g/cm^3) due to a compaction phenomenon (Baumgartl 1998, Jim 1998, Morel et al. 2005).

As opposed to agricultural soils, urban soils could have either lost their structures (i.e., soil aggregation) and/or accumulated pollutants because of the presence of large natural- and/or anthropogenic-sourced particles (El Khalil et al. 2008, Nehls et al. 2013). Urban soil also differs from the agricultural one by the fact that the former is more strongly influenced by: (i) continuous and intense anthropogenic contaminating activities, (ii) contamination as the result of a higher loads of contaminants (Biasioli et al. 2006) and (iii) the age of soil (Morel et al. 2005). In effect, soils play an important role in maintaining the environmental quality as they can act as both source and sink for pollutants that can easily affect human health (De Kimpe and Morel 2000). From a chemical point of view, urban crop soils are characterized by heterogeneous values of pH and alkalinity due to carbonates (Morel et al. 2005). Yet, plants require certain chemical elements to complete their life cycles (Da Silva and Williams 2001, Knecht and Göransson 2004). Commonly, soil contains nutrients that are directly absorbed by plants as inorganic compounds, or organic nutrients that need to be mineralized to generate inorganic forms easily assimilable by plants. Nevertheless, nutrient uptake by plants is highly affected not only by the chemical form of inorganic compounds but also by the soil properties. For example, K^+, Na^+, NO_3^-, and NH_4^+ ions are absorbed rapidly, whereas PO_4^{-3}, SO_4^{-2}, Ca^{+2}, and Mg^{+2} ions are absorbed more slowly (Tisdale et al. 1985). Similarly, Scharenbroch et al. (2005) found that old urban soils had significantly greater values for weak Bray P (24%), strong Bray P (51%), and K (45%) than newer urban soils. However, Joimel et al. (2016) observed that the extractable phosphorous ratio of the anthropized urban soils was slightly close to the natural soils, in particular forestry and agricultural soils. These elements are naturally present in the soil. Hence, the chemical composition of a soil is inherited from the geological (named parental) material from which the soil has grown, more or less modified by pedogenic evolution without human intervention (background level) (Baize et al. 2007). The presence and heterogeneity of trace metals in urban soils show the relative influence of background and inputs from external factors due to land use (e.g., industrial activity or traffic emissions) (Bechet et al. 2016, Dumat et al. 2017). The most common trace metals in the urban area are Cd, Cu, Ni, Pb and Zn (Dudka et al. 1996). Overall, urban soils are currently at a low or medium level of metal pollution, as is the case, for instance, of Chinese soils contaminated with Cd (Wu et al. 2016). Jacobs et al. (2017) reported that urban soil contamination with trace metals is a major obstacle to the development of urban agriculture because of the high risk of metal accumulation by plants up to toxic levels for humans.

An alternative to reduce metal bioavailability and toxicity in urban soils is phytoremediation, a gentle strategy that maintains the agronomical properties of soils and preserves its function. Phytoremediation can be suggested, therefore, as a low-cost and environmentally friendly strategy to remediate urban soils contaminated with trace metals with two major aims: (i) to maintain the crop potential of soils, and (ii) to reduce the huge amounts of slightly contaminated soils that are commonly excavated and evacuated from the cities where they become wastes. Nature provided several essential services so-called ecosystem services. Plants provide many of these services, and we can optimize the delivery of some of them by growing the appropriate plants in the appropriate media. Phytoremediation is a way to preserve or restore some of these services: (i) a regulation service, (ii) a supply service owing to raw materials it generates for energy and/or metal recycling, and (iii) a cultural service with its contribution to the greening of cities and contribution to urban landscapes.

Crop Activities in the Urban Context

Urbanization and the urban sprawl led to changes in land use, which can be characterized by the growth of built-up areas and a loss of farmland (Cai 2000, Liu et al. 2003, Tan et al. 2005, Li et al. 2016). Thus, urban agriculture becomes a strategy for reducing the ecological footprint of cities, providing some food to the inhabitants, and reconnecting people with nature. Indeed, urban collective gardens (allotment gardens, community gardens, shared gardens) are growing in the last decades (Pourias et al. 2016). For example, in UK, over 78,827 people waited for a plot to develop urban farming (Campbell and Campbell 2010). Moreover, time for authorization does not facilitate the development of urban agriculture. For instance, in the city of Nantes (France), the time to obtain a plot is between 3 to 5 years. In developing countries, urban agriculture is the main option to produce food, whereas in developed countries its scope is quite different; it also provides recreational activities and an educational function (Dubbeling et al. 2010).

The French national scientific research project "JASSUR" (ANR-12-VBDU-0011) studied practices, functions and risks associated with urban gardens. A socio-technical characterization of gardening practices was carried out to evaluate the potential of urban garden to produce foodstuff. The results showed that the food supply service was workable, but the productivity of the system varied (https://www6.inra.fr/jassur). In some cases, the productivity of urban gardening was equivalent to those typically recorded for agricultural lands.

Besides urban collective gardens, urban microfarm is catching the attention of worldwide society. Microfarms are defined like small-sized commercial market gardens, which share some important characteristics: cultivated acreage smaller than official recommendations for market gardening (below 1.5 ha); community-oriented marketing through short-supplying chains; wide diversity of plants cultivated with more than 30 crops per farm to promote biodiversity; and low level of mechanization and investment (Morel and Léger 2016). Their productions are sold through short supplying chains by directly selling to consumers or with only one intermediary (Aubry and Chiffoleau 2009). The production of these microfarms is often low. Indeed, productivity is the second goal because they are multifunctional and propose multiple social activities. Moreover, a great part of the production is donated or auto consumed (Daniel 2017).

What Can We Do against Metal-Contaminated Urban Soils?

Metal contamination of urban soils is incompatible with its use for cropping or for recreational activities (Pascaud et al. 2014a, Mombo et al. 2016). In this situation, management options must be considered to restore its adequacy through reduction of human exposure to pollutants (MEEM 2017). Among these management strategies, removing the source of pollutants (e.g., excavation of the soil), reducing the concentration of pollutants in the soil, or their transport capability (e.g., immobilization, trapping, precipitation, complexation, or reactive barriers) and reducing pollutant availability (e.g., insulating membranes) are currently the most used to limit human exposure to pollutants.

In case of urban allotment gardens, excavating the contaminated soil (*ex situ* management) and its replacement by uncontaminated topsoil is the most used strategy by urban planners. This approach can be assumed as a form of the precautionary principle. For urban planners, removal of the contaminated soil indeed solves the problem immediately and definitively, thereby avoiding any responsibility in case of not properly cleaning-up the soil. But two issues emerge: (i) the availability and the quality of the marketed topsoil used for the soil replacement and (ii) the disposal of low-contaminated soils after removing from the urban allotment garden. This waste soil can be also used for very specific uses such as road base course. In the context of experimental urban gardens in Paris, Badreddine et al. (2017) developed a procedure to manage safe gardening activities on variously polluted urban soils.

In situ management of polluted soils is undoubtedly more sustainable, especially options friendly to the soil quality devoted to crop activities that allow maintaining their physical, chemical and biological characteristics. Among the possible options, reducing the transfer of metals from soil to crops to guarantee the regulatory threshold is technically feasible, but it is not widely accepted by society and legislators (notably in Europe) as long as trace metals remain in the soil. In China, where 20% of agricultural land is currently contaminated (Zhao et al. 2015) and is insufficient to feed the population, several experiments to exploit contaminated lands were undertaken (Tang et al. 2012). Nonetheless, the consumption of contaminated crops is not the only risk for the consumer. It must be kept in mind that the risk of direct soil consumption (Denys et al. 2007, Pascaud et al. 2014b) and inhalation of soil particles, in particular by children, may be higher than the health risk resulting from consuming contaminated vegetables (see Chapter 5 for more details).

This chapter provides an overview on the gentle methods for managing crop soils contaminated by trace metals in the context of urban farming and allotment gardens, with particular emphasis on *in situ* solutions. These *in situ* methods of remediation will be illustrated by some case studies performed by our research group. Lastly, some innovative experiments performed in agricultural areas are also discussed in the context of their potential application in urban agriculture.

Management of Urban Agricultural Soils

In situ Methods for Reducing Transfer of Trace Elements from Soils to Plants

Phytostabilization is a relevant method to reduce the transfer of trace metals from soils to plants, and to avoid their dispersion in the environment (e.g., erosion, transport of airborne soil particles). However, the use of non-food producing plants (Linger et al. 2002, Khan 2003, 2005) leads to change the soil use unless phytostabilization involves the culture of vegetables (in association or as an intermediate culture).

Recently, several studies have attempted to seek potential solutions that enable growing healthy vegetables. For example, the intervention in the physicochemical properties of the soil such as the pH has an immediate effect on tracing metal mobility (Kalkhajeh et al. 2017, Tedoldi et al. 2017, Brimo et al. 2018). On the other hand, the addition of organic amendments not only improves soil quality but also reduces trace metal mobility (Mench and Martin 1991, Mench et al. 1999, 2000, Tang et al. 2012, Austruy et al. 2016). Trace metals are generally adsorbed by carbonates, organic matter, Fe-Mn oxides and primary or secondary minerals (Ross 1994). However, fertilizers occasionally contain by-products or contaminants in their formulation that may be an environmental risk to soil quality and plant health. This is the case of commercial phosphates that contain Cd in their formulation, although some effort is being addressed to improve the quality of this kind of fertilizer (French agency for environmental quality and human health, ANSES). Bioaugmentation of soil with microorganisms is able to immobilize metals by both sorptive and precipitation mechanisms (Volesky and Holan 1995). Many studies have demonstrated that bioaugmentation with (symbiotic) microorganisms may reduce metal uptake by plants (Joner et al. 2000, Karagiannidis and Nikolaou 2000, Lovely and Lloyd 2000, Tonin et al. 2001, Jézéquel et al. 2005, Jézéquel and Lebeau 2008). In this context, bioaugmentation using mycorrhizae is a promising strategy in the bioremediation of metal-contaminated soils (Lebeau et al. 2008, Aghababaei et al. 2014). These microorganisms are abundant in species diversity, with a marked variation in morphology and physiology (Selosse and Tacon 1998). In general, ectomycorrhizal fungi are associated mostly with trees, whereas about 94% of angiosperms establish symbiosis with endomycorrhizal fungi (Brundrett 2009). The arbuscular mycorrhizal fungi have been the most studied group, which is implied in the solubilization of inorganic phosphorus and subsequent transfer to the host plant together with water and other nutrients. In this symbiotic relationship, the fungi obtain photosynthetic carbon compounds from the plant (Smith and Smith 2011, Li et al. 2013). The arbuscular mycorrhizal fungi have been also been studied as biological vectors in the bioremediation of metal-contaminated soils. They play an important role in the soil-plant interface because of their capability to be either a barrier or an enhancer of metal transfer, using a large variety of metabolic pathways (binding in fungal wall, excretion of organic acids and glomalin, among others) (Amir et al. 2014).

These metabolic pathways suggest two different strategies for reducing mobility and toxicity of trace metals in the soils. First, the so-called phytoextraction of trace metals, which is facilitated by the presence of arbuscular mycorrhizal fungi and requires a certain resistance of the target plants to the metals (Amir et al. 2008, Lebeau et al. 2008, Danh et al. 2014). Second, the so-called phytoimmobilization of trace metals that results in the accumulation of these chemicals in the rhizosphere by the joint action of root secretions and the physical barrier by arbuscular mycorrhizal fungi (Lebeau et al. 2008, Rangel et al. 2014). The latter strategy obviously raises a protective mechanism to the plant, a topic that has been intensively tackled in the context of urban agriculture (Pierart 2016).

Phytoextraction

The scope of phytoextraction is the removal of trace metals from the soil by repeatedly harvesting plant biomass from a polluted site. Harvesting is prolonged in time until metal concentrations in the soil are below the regulatory threshold. In their review, McGrath and Zhao (2003) highlighted that metal-accumulating plants are still being sought, and that the phytoextraction capability of high hyperaccumulator plants still needs to be validated in field conditions (Greenland project, e.g., Cundy et al. 2015).

In situ phytoextraction is a preferred strategy for a set of advantages: (i) it is a gentle remediation method that maintains the agronomical properties of the soil (Gerhardt et al. 2009); (ii) it is not expensive compared to other bioremediation procedures such as bioaugmentation with microorganisms (Garbisu and Alkorta 2001); (iii) it is a relatively simple approach (Angle and Linacre 2005); and (iv) it is a socially welcome method (Lambert et al. 2000).

Phytoextraction has, however, several limitations. First, it takes a long time, which is certainly the main restrictive factor explaining why phytoremediation has not gained popularity as a bioremediation methodology. Nevertheless, the duration of phytoextraction can be shortened by increasing the mobility of metals (e.g., pH modification, using complexing agents such as synthetic agents or naturally produced agents from microorganisms inoculated in soil (Lebeau 2011)). Second, phytoextraction is only workable in the topsoil; otherwise, trees can be used to reach deeper layers of contaminated soils (Gerhardt et al. 2009). Third, disposal of plant residues after harvesting arise an environmental problem. Only the recycling of nickel-accumulating plants is at an advanced stage of development for its disposal (Chaney et al. 2018). Finally, phytoextraction must be validated at full scale, which is still in a premature stage of development (Greenland project, Cundy et al. 2015).

Assessing the Performance of Nature-Based Solutions

Plants and trees in urban areas (green spaces) are being increasingly recognized for their capacity not only to support biodiversity conservation (Goddard et al. 2010), but also to generate additional environmental, economic, and social benefits (Haase et al. 2014, Kabisch et al. 2015). Also, they promote the functioning of ecosystems as essential backbones to climate change mitigation and adaptation (European Commission 2015). Currently, the urban vegetation forms a part of the nature-based solutions (NBS) scheme. Most researchers agree that the NBS concept is a rational strategy for promoting the ecological restoration and enhancement of biodiversity, as well as the maintenance of the urban structure (e.g., Kabisch et al. 2016, Maes et al. 2017). In addition, NBS can be characterized by the use of nature in tackling challenges previously cited and conserving biodiversity in a sustainable manner (Balian et al. 2014).

The concept of urban performance indicators comprises those relevant indicators of changes in the soil and water quality related to environmental stressors such as chemical contamination (Whitford et al. 2001, Dyckhoff and Allen 2001). Environmental performance indicators are predominantly integrated to regulating ecosystem services and refer to biodiversity such as vegetation cover (Kabisch et al. 2016). Many authors defined the term of bioindicators that are living organisms such as plants, planktons, animals, and microbes, which are utilized to screen the health of the natural ecosystem in the environment (Gerhardt 2002, Holt and Miller 2010, Parmar et al. 2016). The abiotic indicator comprises temperature, saltiness, stratification, and pollutants, pH, water content, organic matter content, bulk density of the soil, type of soil (e.g., sand peat, clay), and degree of pollution with metals and organic pollutants, but also the type of management (agriculture, application of manure and/or fertilizer, nature, recreation area, etc.) and vegetation (crop rotation). Regarding biotic indicator, it is defined as the abundance and diversity of nematodes, earthworms, enchytraeids, and micro-arthropods, nitrifying activity, microbial functions, genetic diversity, total activity and numbers of bacterial cells (Breure 2004). Heink and Kowarik (2010) indicated that the term ‘‘indicator’’ is frequently used for the interface between science and policy. There is still a great demand for clear definitions of technical terms in science and policy.

There are many examples in the scientific literature on the use of indicators to assess the performance of phytomanagement using vegetation. Pérez de Mora et al. (2011) proposed the use of soil microbial activity and community composition as suitable indicators of phytoremediation actions in metal-contaminated soils. In addition, Parraga-Aguado et al. (2013) established significant relationships between the outcomes of some ecological indices (e.g., heterogeneity of the plant communities, number of different species) and those coming from (i) some physicochemical properties of soil (electrical conductivity, equivalent calcium carbonate, total nitrogen, organic carbon), and (ii) the water extractable ions and dissolved organic carbon. Regarding trace metal phytoextraction, McGrath and Zhao (2003) demonstrated that both high biomass yields and metal hyperaccumulation are required to make the process efficient.

CASE STUDIES FOR THE *IN SITU* TRACE ELEMENT BIOREMEDIATION

Reducing the Trace Metal Transfer from Soil to Crops

Arbuscular Mycorrhizal Fungi-Based Biofertilizers

This case study describes an experiment conducted under greenhouse condition to evaluate if it was possible to use local arbuscular mycorrhizal fungi-based biofertilizers to decrease the transfer of trace metals between contaminated peri-urban soil and edible crops. Two metal-contaminated soils (Cd, Pb and Sb) were compared for their different trace metal origin, which was either anthropic (from Bazoche, France—BZC) or geogenic (from Nantes, France—NTE). A full description of these soils is detailed in Pierart (2016). In brief, the study examined the growing leek in these soils using a pot experimental design: half pots received a biofertilizing solution (called Biofertilization) containing arbuscular mycorrhizal fungi spores isolated from trap crops grown on a mix of these soils. The scope of this fertilization was that urban gardeners could easily prepare such biofertilizer from their own cultivated pots. The second half pots were not biofertilized (control pots). For Cd, biofertilization had a significant opposite result in trace metal concentration in the roots between both soils, with an increase in the NTE soil and a decrease in the BZC soil (Fig. 1A and 1D). On the other hand, biofertilization had no effect on Pb accumulation in the roots, and in the aerial parts of the plant (Fig. 1B and 1E). However, Pb increased significantly in the leaves of leeks grown in the BZC soil (anthropic contamination), probably because of an increase of metal translocation from root to leaves. In the case of Sb (Fig. 1C), biofertilization decreased its concentration in leaves, with significant results only on BZC soil. Sb concentration in root was found under the detection limit, which suggests a full transference from root to leaves in both soils.

For these three trace metals, the human bioaccessible fraction was estimated in edible parts of the plant to assess human health risk by food ingestion (Fig. 1F). A significant fraction of Cd in leek leaves was observed to be gastrically bioaccessible (85%), while Pb was significantly less bioaccessible (~ 60%). Sb bioaccessibility was lower, ranging between 15 and 33%. The biofertilization treatment significantly increased the bioaccessible fraction of Sb in leeks cultivated in the NTE soil (13% compared with controls). In BZC soil, a slight increase was also observed (5.5%); however, these results were not statistically significant.

This case study highlights the lack of understanding of the role of arbuscular mycorrhizal fungi in trace metal transfer from soil to plant. Furthermore, it shows that a case-by-case approach ought to be performed instead of applying a standardized bioaugmentation method, even if arbuscular mycorrhizal fungi were shown to reduce the transfer of trace metal under fully controlled conditions (see the review by Lebeau et al. 2008). Therefore, the balance between advantages and limitations needs to be assessed carefully when using these organisms.

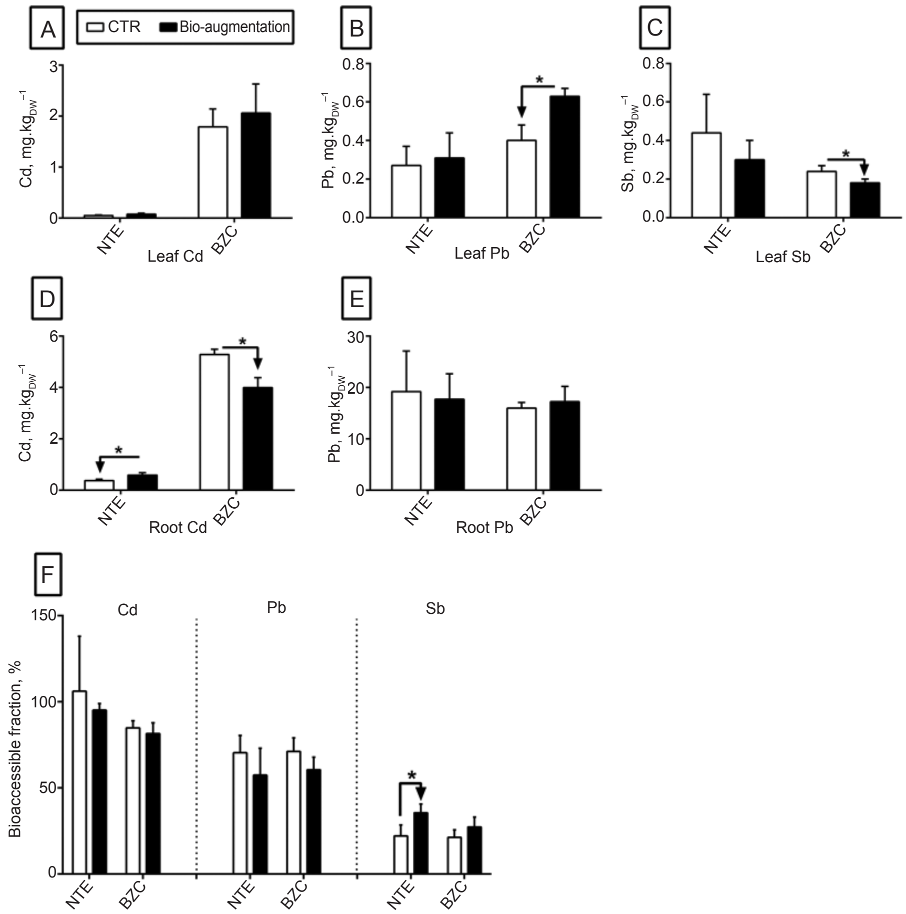

Fig. 1. Trace metal accumulation (mg/kg dry mass) in leek leaf [A-Cd, B-Pb, C-Sb] and root [D-Cd, E-Pb] in natural (control, CTR) and biofertilized soil (arbuscular mycorrhizal fungi, Bio-augmentation) from Bazoche (BZC) or Nantes (NTE). Bioaccessible fraction of each metal [F] is expressed as the ratio between the bioaccessible trace metal concentration and the total trace metal concentration. Sb was under the detection limit in leek root. Significant differences are indicated with * (T-test, α = 5). (Results taken from Pierart et al. 2018.)

Green Manure Plants for Improving Environmental Quality and Fertility

The regeneration of brownfield sites in urban areas is a major challenge for the sustainable development of cities. In effect, these sites are generally localized in the center of the cities and could be transformed in new ecological areas with micro-farms or collective gardens used to produce consumed vegetables. Management and conversion of these large urban sites, imposed by regulations, however, require the development of tools for assessing environmental and health risks, and sustainable remediation techniques. For example, Foucault et al. (2013) developed bioavailability and ecotoxicity tests to improve the classification of contaminated soils, focusing their

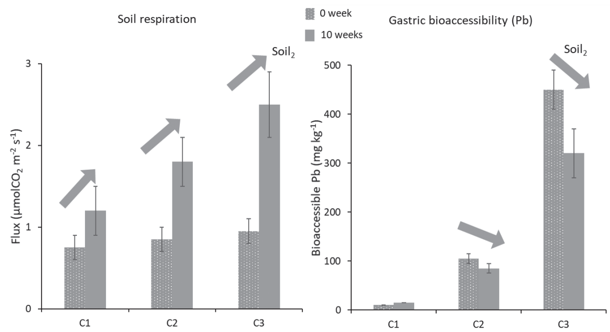

Fig. 2. Soil respiration (Lycor apparatus) and lead bioaccessibility (Barge procedure) variation during 10 weeks of phytoremediation treatment with borage. C1, C2, and C3 are the Pb concentrations in soil, which correspond to 0, 400 and 800 mg/kg dry mass of polluted soil (Data taken from Foucault et al. 2013).

study on a lead battery recycling site in Toulouse (France), which was characterized by historical Pb pollution and other metals (Uzu et al. 2011). Moreover, green manure plants (borage, mustard and phacelia), commonly used in agriculture and gardens, were tested by Foucault et al. (2013) for re-functionalization of polluted soil. It is well-known that these green manures improve biological and physicochemical properties of soils (soil respiration, microorganism biomass) with root system and large production of root exudates. The mechanisms involved in the fate of pollutants in the rhizosphere and associated microorganisms were studied in controlled experiments with the industrial soil, then the tested plants were used directly on the field in order to promote the ecosystem services. Soil respiration and human bioaccessibility of pollutants measured before and after soil remediation in order to assess the soil quality with a global environmental-health approach demonstrated the efficiency of green manure plants for soil remediation. Actually, borage (Fig. 2) improved soil respiration, reduced metal toxicity and the amount of total and gastric bioaccessible lead in soil, respectively, by phytostabilisation (borage) and storage in the roots (Pb and Sb).

Depending on metal speciation (the chemical forms in which a metal is present in the soil) as well as the type of soil and plant species, the environmental fate of metal greatly differs. Metal speciation can also be influenced by the agronomic characteristics of the soil and microbial activity in the rhizosphere. A molecular screening and meta-analysis of microbial genomics have helped to highlight the differences in bacterial communities studied by the level of metal concentration, plant species and characteristics of the soils studied (Foucault 2013).

In the context of polluted soils potentially suitable for urban agriculture, green manure plants appear an interesting tool to develop in the coming years because of their soil fertility capability, decreased metal bioavailability, and human bioaccessibility and metal ecotoxicity. After the first step of experimentation in controlled conditions, long term phytoremediation actions at the field scale are currently being performed using borage and also for comparing the vegetation which grows spontaneously on the site of the lead recycling factory. Moreover, borage plants are not perennial, they should be harvested to avoid the release of metal(loid)s in soil, and treated in a waste treatment unit (Foucault et al. 2013).

Combination of Amendments and Fertilizers in Metal-Polluted Soil

Agricultural soils may contain important levels of trace metals. For instance, the origin of Cd in agricultural soils is mainly due to the high utilization of phosphate fertilizers, which are enriched with this metal (Grant et al. 2013). Lowering metal phytoavailability, it is therefore possible to grow plants consumed by humans on soil slightly or moderately polluted by using the phenomena of adsorption (on clay minerals, wollastonite, biochars or black carbon, etc.), complexation (organic matters) or precipitation (calcareous amendments) of metals, in order to stabilize these pollutants and reduce the soil-plant transfers. Chemical speciation and compartmentalization of elements indeed alter their phytoavailability and (eco)toxicity. In China, for instance, with the aim of reducing human exposure to Cd, several materials (clay minerals, wollastonite, biochars) were tested in order to immobilize cadmium in cultivated soil. The questions asked concerned the specificity and sustainability of these added materials. The study by Wu et al. (2016) is an example on how mineral-based amendments may significantly reduce the phytoavailability of toxic metals. They applied the mineral wollastonite (calcium inosilicate, $Ca_3[Si_3O_9]$), which is a commercial product currently available in China, to Cd-polluted soils. This mineral reduced the Cd mobility in the soil, thus reducing its phytoavailability and toxicity. Therefore, wollastonite applications in cadmium-contaminated soils can reduce cadmium accumulation in plants, although the main limitation is the synchronous immobilization of micronutrients which may affect, in turn, the plant growth.

In an attempt to mitigate this disadvantage of using wollastonite, Wu et al. (2016) applied Zn- and Mn-fertilizers in wollastonite-amended soils to promote the growth and fitness of amaranth (*Amaranthus tricolor* L.). The plants were cultivated under three different treatments: Cd-contaminated soil with a micronutrient fertilizer, Cd-contaminated soil amended with wollastonite, and Cd-contamination soil amended with both wollastonite and micronutrient fertilizer. The following variables were measured: the plant biomass, photosynthesis parameters, and total Cd, Mn and Zn concentrations. This latter variable was measured also in soil samples. The results of that study demonstrated that application of wollastonite decreased the concentrations of Cd, Zn, and Mn in the plant, as well as their availability in the soil. Moreover, this mineral fertilization increased the gas exchange ability of plants. However, wollastonite treatment reduced the chlorophyll concentration in the leaves, and it had no positive influence on plant biomass. In contrast, Mn and Zn fertilization following wollastonite application corrected these two parameters, so plant biomass and photosynthetic ability significantly increased. This combination of fertilizers also reduced Cd phytoavailability more efficiently, probably because of the competition phenomenon. Therefore, this laboratory study is an example on how synergistic improvement could be tacked by combination of a mineral amendment for metal immobilization and a micronutrient fertilizer for reducing nutrient deficit in the plant.

Cultivating Vegetable While Phytoextraction

Cropping using Low Trace Metal-Accumulating Vegetables

Firstly, it should be reminded that phytoremediation, especially phytoextraction, is one of the most common methods for the *in situ* remediation of metal-contaminated soils. Moreover, one of the main weaknesses of phytoremediation is that it takes a long time to significantly reduce the concentration of toxic metals in the soil (Pilon-Smith 2005). To partially solve this limitation, some studies have used phytoextraction together with vegetables with a low capability of metal absorption this latter is linked to human consumption. The association between phytoextraction and crops, and even setting up phytoextraction during inter-cropping seasons, are two practical options to avoid metal toxicity from soil, without taking precaution for reducing metal concentrations. An example of this functional association is the study by Bouquet et al. (Pers. Commun.) performed in an allotment garden in Nantes (France). The soil in this allotment garden is contaminated by Pb (ca. 170 mg kg^{-1} dry mass of soil), so there was a high risk of garden closure for cropping purpose. Moreover, the most workable solution was the excavation of topsoil that displayed an acceptable agronomic quality. To avoid these two measures, an *in situ* 2-years experiment was launched in July 2015. Some vegetable species and Indian mustard (*Brassica juncea*) were cultivated in rotation in this allotment garden. The Indian mustard was used as phytoextraction species. The Pb concentrations in the edible parts of tomatoes, winter cabbages, leeks and potatoes were under the EEC 466/2001 regulatory threshold set at 0.1 mg kg^{-1} of fresh matter (0.3 mg kg^{-1} for cabbage) (Fig. 3). For green beans, Pb concentrations in pods were close to the threshold (0.1 mg kg^{-1}). Biomass production was very low on those plots (7.25 times lower compared to others). Such a cropping system was already tested with success by associating the metal accumulator plant *Sedum alfredii* and a low metal-accumulating cultivar of maize (Xiaomei et al. 2005).

The phytoextraction efficiency of Pb was very low (ca. 2 mg kg^{-1} dry mass of aerial parts). The geogenic Pb availability partly explains this low phytoextraction efficiency (Bouquet et al. 2017). These same authors showed that the addition of EDTA increased significantly the Pb concentration in shoots of *B. juncea*, up to 26 times in comparison with the control sample. Bouquet et al. (2017) estimated that if the phytoextraction capability of *B. juncea* led to a Pb concentration in shoots of 45 mg kg^{-1} dry mass, then 604 years are required to remove the non-residual Pb, but much less if only the bioavailable fraction of Pb is considered. Although EDTA displays sublethal toxicity to plants at concentrations as low as micromolar (Shahid et al. 2012), phytoextraction, in the presence of this metal chelator, could be a complementary alternative (Lebeau et al. 2008).

An unexpected finding was to find a high accumulation rate of Pb in aerial parts, i.e., stems and leaves, of tomato (much more than in *B. juncea*), whereas the metal concentrations in fruits were under the EEC regulatory threshold. With the aim of examining species-specific differences of Pb accumulation in several varieties of *Brassica juncea* and *Solanum lycopersicum* (Tomato), a study by Bouquet et al. (2018) cultivated these vegetables using a hydroponic system and exposed to

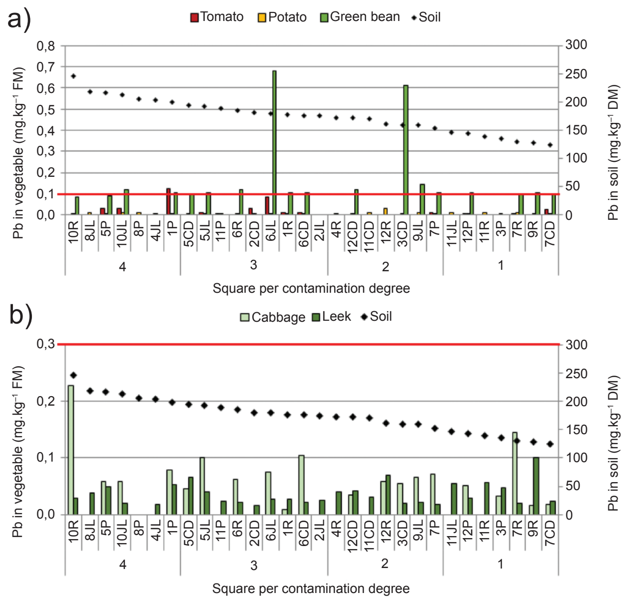

Fig. 3. Lead amount (mg kg^{-1} of fresh matter (FM)) measured in (a) fresh vegetables and (b) leafy vegetables compared to lead content measured in soil before cropping experiment and regulatory thresholds for vegetables (red line). Note: try to correct the number in the Y-axis. Decimals indicated with point instead of comma. Also, try to define all the treatments; otherwise, this figure is not needed.

realistic phytoavailable Pb concentrations frequently found in soil, i.e., 20 mg L^{-1} and 40 mg L^{-1}. In these conditions, tomato plant was a "hyperaccumulator", with Pb concentrations in shoots as high as 500 mg kg^{-1} dw and 2000 mg kg^{-1} dw in the plants exposed to 20 and 40 mg L^{-1}, respectively. Concentrations in *S. lycopersicum* shoots were 50 to 100 times higher than those measured in *B. juncea* shoots, regardless of Indian mustard cultivar involved.

Associating Plants that Compete for Trace Metals in Soil

Another option for maintaining cropping in metal-contaminated soil with minimum risk for human exposure consists in reducing the transfer of trace metals from the soil to plants, by creating competition for the metal uptake in favor of hyperaccumulators. Some results were already published mainly regarding crops, not vegetables co-cultivated with hyperaccumulators. Intercropping with Indian Mustard (*Brassica juncea*) led to a decrease in Cd concentration by 57.1% in alfalfa cultivated in soils while it increased by 14.5% in the aboveground parts of *B. juncea* (Su et al. 2008, Xin-Bo et al. 2009). A co-cropping system with the Cd- and Zn-hyperaccumulator *Sedum alfredii* and a low-accumulating crop (*Z. mays*) was set up in a rice field historically irrigated with Pb- and Zn-enriched water from mining activities. In that study, Wu et al. (2007) showed a removal of heavy metals by *S. alfredii*, whereas safe corn for animal feed was produced allowing farmers to continue their agricultural activities. Cd-accumulating varieties of oilseed rape could also reduce Cd uptake by co-cropped cabbage. Unfortunately, final concentrations remained high (Liu et al.

2007). However, the Cd concentrations in cabbage depended on the metal speciation in the soil (De-Chun et al. 2010).

As compared to low metal-accumulating plant species, the beneficial effect of associating a hyperaccumulator species with a normal crop species was not always achieved as shown by Yu et al. (2014), who used an oilseed rape-rice rotation. Barley (*Hordeum vulgare*) co-cropped with *Noccea caerulescens* decreased Zn concentration in barley, but simultaneously increased Cd concentration (Gove et al. 2002). A similar finding was reported by Jiang et al. (2010) for the co-cropping of *N. caerulescens* and ryegrass. Comparing these results with a co-cropping using *Thlaspi arvense* (non hyperaccumulator), they suggested that the high concentration of Zn mobilized by the hyperaccumulator was used by itself, whereas it did not require Cd, explaining why the co-planted crop accumulated less Zn and more Cd.

Co-cropping of two non-hyperaccumulating plant species may alter the metal speciation in soil leading to a decrease in trace metal accumulated in harvested plants. For example, *Cunninghamia lanceolate*, as inter-crop plant, decreased the concentrations of Cu, Mn and Pb in tea leaves (Xue and Fei 2006). Similarly, Wu et al. (2003) showed that wheat/rice intercropping could reduce Cd concentration in wheat grain while it increased in rice grain. Taken together these studies suggest that this association cropping between a metal hyperaccumulator and plants intended for human consumption reduces the risk of human exposure to toxic metals.

Urban Soil Cleaning using Nature-Based Solutions: Anticipating New Regulations?

Cleaning of contaminated soils using *ex situ* procedures faces stronger regulation constraints than *on site* or *in situ* processes (soil respectively excavated or not and kept on the site itself). Indeed, according to the European legislation (European Waste Framework Directive 2006), contaminated soils are considered waste as soon as they are transported out of their original site. However, cleaning of soil is possible *ex situ* using specific treatment facilities as long as the corresponding industrial legislation and soil/waste traceability is strictly enforced to these procedures. However, phytoremediation needs both space and time, which might not be compatible with management costs.

An option could be to use the public or private space, becoming an opportunity to create green areas of urban concern. Especially, phytoremediation could be introduced when creating new quarters or in the frame of the redevelopment of existing ones, like in polluted harbor area in Amsterdam (Wilschut et al. 2013), and in the case of polluted vacant lands in Canadian municipalities (Todd et al. 2016). Developers and urban planners would thus need to anticipate such *ex situ* treatment in the planning and developing process and the regulation should evolve to allow such option. Of course, there is a need for traceability of soil movement and quality. Also, the quality of the receiving soil and local water (surficial, groundwater) should not be altered, and health risks be limited. With the rate of urbanization, green areas take a particular place in the management of the city. Thus, the use of phytomanagement, including phytoremediation, could be a solution to the need of the city dwellers for nature and vegetation in the urban environment (Boutefeu et al. 2005, Blanc 2009, Cheverry and Gascuel 2009, Bourdeau-Lepage and Vidal

2012). Therefore, the phytomanagement may be an important element to include in the regulation of the urban environment. Indeed, the vegetation should allow the decrease of the temperature during the night (Hardin and Jensen 2007, Cameron et al. 2014, Doick et al. 2014, Foissard 2015). Another function is the possibility to attenuate sound reverberations (Balaÿ 2013). In addition, it is generally accepted that urban vegetation improves air quality. For example, Setälä et al. (2013) studied the ability of urban park/forest vegetation to remove air pollutants (NO_2, anthropogenic volatile organic compounds and particle deposition) in two Finnish cities (Helsinki and Lahti), suggesting that urban vegetation is a suitable biological target to remove air pollutants. Eventually, phytomanagement can contribute to the infiltration of the rain water and thus limit the risk of floods due to the huge impermeable surface in cities (Cheverry and Gascuel 2009, Carré and Deutsch 2015).

Principles of circular economy, as the UK waste strategies WRAP—Waste & Resources Action Programme (Wrap 2010), should help making such options workable, i.e., introduce phytomanagement in the redevelopment of quarters in cities. To ensure that soil quality and use are compatible, a health risk approach can be applied as that used for polluted soil management in countries such as USA (Clay 1991), France (MEEM 2017) and Taiwan (Lai et al. 2010), or for soil reuse within land management (Bodemdecreet 2006, Vlarebo 2008, Blanc et al. 2012, Coussy et al. 2017). Indeed, such options could help to reduce the amount of excavated materials (soil) landfilled. For instance, 45 million of excavated soil (and subsoil) is expected in the Grand Paris Express Project. The objective of this project is to reuse 70% of soil (Richard 2017). Middle size cities are also concerned by the preservation of the soil resource. This is the case of the Nates city (France), where the second part of the Ile de Nantes redevelopment project is expected to produce around 100,000 tons per year of excavated soils over 15 yrs (Jeanniot et al. 2014). The redevelopment project could apply phytoextraction on green areas welcoming low to middle contaminated excavated soils.

Conclusions

The construction and the use of urban soil for cropping depend on specific rules. It is essential to determine in advance if remediation strategies, such as phytoremediation, are needed because many urban soils have high concentrations of trace metals such as Cd, Cu, Mn and Zn, compared with soils from rural areas. Yet, urban agriculture or cropping is increasing worldwide. Nature-based solutions, more especially phytotechnologies, appear to be promising solutions to manage these contaminated urban soils. However, the main limitation of such methods is the long duration that the procedure takes to decrease metal concentrations in the soil up to safety levels for human health. To avoid that, low trace metal-accumulating vegetables (secure cropping system) may be co-cultivated with metal hyperaccumulating plants (*in situ* phytoextraction). This association cropping would allow the soil to be reusable for cultivating vegetables without any regulatory constraint. When excavation of soil is the best option due to time constraints, it is expected that the regulations evolve and validate the opportunity to clean up soils—only when moderately contaminated—by means of phytotechnologies before soils are re-used for vegetable cropping.

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