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Technical Note

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

Y. Foucault a,b,c, T. Lévêque a,b,d, T. Xiong a,b, E. Schreck e, A. Austruy a,b, M. Shahid f, C. Dumat a,b,*

a Université de Toulouse, INP-ENSAT, Avenue de l’Agrobiopôle, 31326 Castanet-Tolosan, France
b UMR 5245 CNRS-INP-UPS, EcoLab (Laboratoire d’écologie fonctionnelle), Avenue de l’Agrobiopôle, BP 32607, 31326 Castanet-Tolosan, France
c STCM, Société de Traitements Chimiques des Métaux, 30 Avenue de Fondevye, 31200 Toulouse, France
d Agence de l’Environnement et de la Maîtrise de l’Energie, 27 rue Louis Vica, 75737 PARIS Cedex 15, France
e UMR 5563 CNRS/UPS/IRD/CNES GET, 14 avenue Avenue Edouard Belin, 31400 Toulouse, France
f Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari, Pakistan

HIGHLIGHTS

- Green manures plants were tested for quality restoration of soils polluted by metal(loid)s.
- Bioavailability and ecotoxicity of metal(loid)s were measured.
- Borage and mustard improve polluted soil quality.
- Phytoremediation decreases ecotoxicity and quantity of bioaccessible metal(loid)s.

ABSTRACT

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

Keywords:
Green manure plants
Metal(loid)s
Polluted soil
Ecotoxicity
Bioaccessibility
Phytoremediation

1. Introduction

In many countries, the regulation was recently reinforced to improve the management of (eco)toxicity due to chemicals uses (Schreck et al., 2013). Total quantity of lead emitted into the environment strongly decreased last years (Cecchi et al., 2008). But in the world numerous brownfields and kitchen gardens are polluted (Bacigalupo and Hale, 2012). However, the recovery of urban brownfields is required and the possibility of healthy soil gardening becomes an important issue (Foucault et al., 2012).

Phytoremediation techniques modify total and/or bioavailable soil metal(loid) concentrations in relation with compartmentalization and/or speciation (Butcher, 2009). Soil (micro)biology is improved (Kidd et al., 2009), large machinery and excavation equipment are not needed and soil erosion is reduced. Green manure undemanding plants, usually used in agriculture to improve soil fertility thanks to a high rhizosphere activity (Zotarelli et al., 2012) appear as good candidates for phytoremediation, as certain mustard species (Kim et al., 2010). Otherwise, in addition to the measure of total metal(loid) soil concentrations, ecotoxicity and availability measures (Denys et al., 2007) are needed. As these parameters depend on physicochemical soil properties (Foucault et al., 2013), due to their rhizosphere activity (Vamerali et al., 2010; Shahid et al., 2011, 2013), green manure plants could change metal(loid) impact. For the first time to our knowledge, green manure plants were...
therefore tested for phytoremediation on various polluted soils, with both metal(loid) human bioavailability and ecotoxicity assessment in relation with soil characteristics.

2. Materials and methods

2.1. Soils sampling, preparation and characterization

Various polluted soils were prepared from a highly contaminated sandy top soil (call T) collected from a secondary lead smelter located in Toulouse with pH = 7, CEC (cation exchange capacity) = 8.9 cmol(+)/kg(-1), CaCO3 = 8 g kg(-1), metal(loid) concentrations (mg kg(-1)) Pb 39,800 ± 796, As 288 ± 6, Cu 286 ± 6, Cd 18 ± 1, Zn 294 ± 6and Nb 2095 ± 42. Soils were cleaned of roots and visible plant materials, dried and sieved under 2 mm. According to Leveque et al. (2011), soil T was mixed with two unpolluted loamy calcic top cambisol profiles, with metal(loid) concentrations close to the natural geochemical background and noticed C1, in order to prepare the metal(loid) concentrations ranges: 400 mg Pb kg(-1) (C2) and 825 mg kg(-1) (C1). Soil1 has a high OM (Organic Matter) content (44.7 g kg(-1)); pH = 6.5, CEC = 12.3 cmol(+)/kg(-1) and a low CaCO3 content (16.1 g kg(-1)); while soil2 is basic (pH = 8.3), carbonated (98 g kg(-1)) with low OM content (12.5 g kg(-1)). Before and after treatment, CEC, OM and CaCO3 contents and pHe were measured respectively according to NF X 31-130, ISO 10694, ISO 10693 and ISO 10390, as the total metal(loid) concentrations were determined by ICP–OES IRIS Intrepid II XDDL, after mineralization with agua regia (HNO3, HCl, ratio 1:3 v/v) according to ISO 11466. The detection limits of Pb, Cd, Sb, and Zn were 0.3, 0.2, 0.2, 1.3 and 2.2 μg L(-1). The accuracy of measurements was checked using a certified reference material 141R (BCR, Brussels).

2.2. Ecotoxicity tests

2.2.1. Germination tests

For each soil condition, plants of borage (Borago officinalis), phacelia (Phacelia staal) and white mustard (Sinapis alba L.) were cultivated in pots in five replicates. Germination tests and growth assays were performed to investigate soil phytotoxicity (Ma et al., 2010). Seeds were immersed in a 10% sodium hypochloride solution for 10 min to ensure surface sterility (Lin and Xing, 2007) and rinsed with deionised water. Then, 200 g dry weight of soils were placed in plastic pots: 8 cm (top) in diameter and 7 cm in height with some drain holes on the bottom. Germination was determined by visual seedling emergence and recorded after 8 d of treatment in each replicate, with 10 mL of test solution. Organisms were fed 2 h before the experiment. The multiwall plate placed in the incubator at 20 °C in darkness. The mobility of D. magna was recorded after 24 and 48 h, and inhibition rate was calculated. Microorganisms’ validity was verified by reference toxin (K2Cr2O7) according to the norm specifications.

2.3. Soil respiration measurement

Soil respiration (CO2 efflux) was measured in situ before and after treatment with a LICOR 6400 portable photosynthesis system (infrared gas analyzer, IRGA) fitted with a LICOR 6000-9 soil respiration chamber (LICOR, Lincoln NE). To minimize soil surface disturbances, a 10 cm diameter soil PVC collar (about 81 cm² area) was installed (1–2 cm deep) 1 d before the measurements, in each pot in a cleared area (Han et al., 2007). CO2 flux is computed based on a running average of change in CO2 concentration with time as CO2 refills the chamber to a described concentration above ambient concentration (Yim et al., 2002). The process is repeated through three cycles and the intermediate flux data are fit with a regression, which is then used to calculate soil respiration (μmol CO2 m(-2) s(-1)) at ambient CO2 (Ramsey et al., 2005). The average measurement taken at each pot was used to report soil respiration.

2.4. Plant experiments

For each experimental condition, 5 kg of soil were placed in pots in a greenhouse. 10 seeds of each species were sown per pot after 10 min immersion in H2O2 (10%) to ensure surface sterilization (Lin and Xing, 2007). After 10 d of germination, only 3 seedlings of the most uniform plants were kept in each pot to perform crops assays for 10 wk (Gong et al., 2001). Roots and shoots were then separated. Samples were washed with deionised water to remove potentially surface contamination (Evangelou et al., 2007) and oven-dried 48 h at 40 °C. The dry weight was determined and the plant parts were grinded to homogenize particle size. Then, they were mineralised 4 h in a 1:1 mixture of HNO3 and H2O2 at 80 °C (Schreck et al., 2011). Metal(loid) concentrations in plant samples were finally measured by ICP–OES (IRIS Intrepid II XDDL) The accuracy of acidic digestion and analytical procedures was checked using Virginia tobacco leaves (CTA-VTL-2, ICHTJ) as reference. All analyses were realised in triplicate.

2.5. Evaluation of metal(loid) phytoavailability with CaCl2 extraction

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

2.6. Lead bioaccessibility

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

2.7. Statistical analysis

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

3. Results

3.1. Ecotoxicity of soil samples

The three plants grow on the polluted soils without observed phytotoxicity symptoms, but the GI and root length of borage decreased when soil Pb concentration increased. However, root length was different according to the type of soil: roots were between 2 and 3 cm longer for soil1 than in soil2. Concerning the other species, root length decreased from 12 to 10 cm in soil1 and from 12.7 to 8.7 cm in soil2 for mustard; and for phacelia, from 11 to 7 cm and from 13.5 to 8.6 cm respectively in soil1 and soil2. RRE also decreased with lead concentrations and ratios varied between 55% and 100% for the two species. Daphnia test for borage showed a higher ecotoxicity after 48 h than after 24 h of contact with the polluted soils. Mobility inhibition was comprised between 55% and 100% for the two species. Daphnia test for borage showed a higher ecotoxicity after 48 h than after 24 h of contact with the polluted soils. Mobility inhibition was comprised between 55% and 100% for the two species.

The same trend was observed for mustard and no influence of phacelia culture was observed on daphnia mobility.

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

Fig. 1a and b concerning borage experiments respectively for soil1 and soil2, shows total soil metal(loid) concentrations reduction during plant-soil contact function of the nature and initial metal(loid) concentration. In the soil2, the decrease was similar for the conditions C1 and C3 with concentrations of lead and antimony reduced by 55–60% respectively, from 33% to 49% for Cd and more modestly for Zn (from 23% to 27%) and Cu (from 9% to 19%). Decrease of metal(loid) concentrations was less pronounced in soil1, except for the condition C3. The variation of Sb was 17%, 21% for Cd and was almost zero for lead in soil1-C2. These values increased up to 92% for Sb, 53% for Cd and 42% for Pb in soil1-C3. Except for antimony, recorded variations for soil1 were lower than those in the soil2. Concerning mustard, variations of metal(loid) concentrations were close to those of borage, except for lead where the registered decrease was on average twice lower (maximum was 51% in soil1 and 44% in soil2). Finally, no changes were observed with phacelia. Borage and mustard induced changes of CEC, pH, and CaCO3 in soil1. At the beginning of the experiment, differences in soil parameters were explained by the dilution step. CaCO3 content increased from 16 to 71, from 12.5 to 31.1 and from 46 to 148.4 g g\(^{-1}\) respectively for C1, C2 and C3. CEC varied from 7.8 to 10.4 cmol kg\(^{-1}\) and pH increased from 5.7 to 8.1. Under mustard crop, same trends were registered and values were also within the same range.

3.3. Metal(loid) concentrations in roots and shoots

Table 1 presented metal(loid) concentrations in borage shoots and roots. Cu and Zn were mostly present in shoots, between 26 and 188 mg kg\(^{-1}\) and between 70 and 196 mg kg\(^{-1}\) respectively, and were not detected in roots except for soil2-C2 with concentrations close to 2 mg kg\(^{-1}\). The same trend was recorded for Cd with concentrations up to 16 mg kg\(^{-1}\). Conversely, lead and antimony were up to 20 times more concentrated in the roots. Cu and Zn were principally found in mustard shoots (up to 69 mg Cu kg\(^{-1}\) and 260 mg Zn kg\(^{-1}\)). A same trend was found for cadmium, with maximum concentrations in shoots of 11.1 mg kg\(^{-1}\) in soil1. Conversely to borage, lead concentrations were higher in mustard shoots: up to 1131 mg kg\(^{-1}\) in soil2. Only Sb followed the same trend for borage and mustard whose concentrations in roots reached 229 and 218 mg kg\(^{-1}\) for soil1 and soil2 respectively. Amounts of metal(loid)s recorded for phacelia both in shoots and roots were very low accordingly to low variation of metal(loid)s in soils.

3.4. Soil respiration

Depending of soil characteristics and initial metal(loid) concentrations, soil respiration only increased with borage (see Fig. 2) and mustard. Concerning borage, CO2 flux initially ranged from 0.84 to 1.57 μmol CO2 m\(^{-2}\) s\(^{-1}\) between soil1-C1 and C4, but only from 0.64 to 0.85 μmol CO2 m\(^{-2}\) s\(^{-1}\) between soil2-C1 and C3. Similarly, after 10 wk of culture, the amplitude was higher for soil1. Moreover, soil respiration increased with metal(loid) concentrations. For soil1 (μmol CO2 m\(^{-2}\) s\(^{-1}\)): 1.7 (C3), 0.5 (C2) and 0.26 (C1), and for soil2 (μmol CO2 m\(^{-2}\) s\(^{-1}\)): 1.6 (C3), 1.5 (C2) and 1 (C1).

3.5. Metal(loid) bioavailability

Experiments focused on lead: phytovailability varied from 0 to 8 or 10 mg kg\(^{-1}\) respectively for soil2 and soil1. Concerning soil1, even if the condition C2 initially presented the highest extracted fraction (66 mg kg\(^{-1}\)), after treatment, lead concentration was 0.3, 6.7 and 10.4 mg kg\(^{-1}\), respectively for C1, C2 and C3. In soil2, Pb-contents were initially below the limit detection for C1 and C2, and reached 8.6 mg kg\(^{-1}\) for C3. Then, concentrations for C1 and C2 are around 4.8 mg kg\(^{-1}\), while it was 1.8 mg kg\(^{-1}\) in C3. Pb quantities extracted under mustard were in the same range, but not detected for phacelia.
Amounts potentially accessible for human were higher than those available for plants. In the soil₁ lead bioaccessibility was initially 35%, 63% and 35% respectively for C₁, C₂ and C₃. These ratios increased after treatment with borage to reach 46%, 92% and 75% respectively for C₁, C₂ and C₃. A linear trend was observed for the soil₂ lead bioaccessibility increased with soil concentration: from 8% to 15% (C₁) and from 291 to 362 mg kg⁻¹ (C₂) and from 250.4 to 5.9 mg kg⁻¹ (C₃). However, when results are expressed as quantities, taking into account the modification of lead concentrations in soils, two behaviours were distinguished in function of soil nature. For soil₁, the fraction of extracted lead grown with the treatment: from 251 to 359 mg kg⁻¹ (C₂) and from 291 to 362 mg kg⁻¹ (C₂); conversely, for soil₂ bioaccessible fraction decreased from 110 to 89 mg kg⁻¹ (C₂) and from 444 to 323 mg kg⁻¹ (C₃).

4. Discussion

4.1. Improvement of soil quality by ecological engineering

Borage, mustard and phacelia were able to grow on various polluted soils. A trend for germination reduction was however observed as roots are directly in contact with pollutants (Schreck et al., 2011). Root length was influenced by lead concentration (R² = 0.56 and 0.59 for soil₁ and soil₂ respectively), as RRE was too (R²-soil₁ = 0.74; R²-soil₂ = 0.62). Soil characteristics also affected root length as shown by correlations: (i) in the soil₁, R² = 0.73 for pH and OM content and 0.63 for CEC; in soil₂, R² = 0.83, 0.66 and 0.69 with pH, OM and CaCO₃ content respectively. Root toxicity was lower in soil₂ because, as noted by Birkefeld et al. (2007), lead oxide particles incubated in calcareous soil can be covered by a crust of lead carbonate. Soil respiration significantly increased after phytoremediation, certainly in relation with both changes of abiotic (Han et al., 2007) and biotic factors as microbial activity.

4.2. Evolution of environmental and sanitary risks

In soil₁, the concentration of phytoavailable lead is related to the soil concentration (R² = 0.95 and 0.94 at 0 and 10 wk respectively). Moreover, as for soil respiration and root toxicity, CaCO₃ amount is influent: R²-soil₁ = 0.89 and R²-soil₂ = 0.95 at the end of experiment. Soil properties can strongly modify metal bioavailability (Kidd et al., 2009). Soil₁ showed an CaCO₃ content increase and a more basic pH which can lead to a reduction of metal(loid) availability. Lead bioaccessibility (in%) increased during the culture-period and was statistically correlated with total lead-concentrations (R²-soil₁ = 0.61 and 0.95 at 0 and 10 wk respectively; R²-soil₂ = 0.75 initially and 0.98 after treatment), and accordingly to Caboche (2009), with the soil characteristics: pH and CEC for soil₁; carbonate and OM content for soil₂. However, a lower lead quantity was bioaccessible after 10 wk in soil₂ conversely to soil₁. Moreover, soil ecotoxicity measured by sensitive daphnia test was reduced by the treatment, certainly due to rhizosphere activity.

4.3. Suitability of green manure crops for ecological restoration of polluted soils

Metal(loid) compartmentalisation in the plants depend both on pollutant and plant type: copper and zinc were mainly concentrated in shoots; lead and antimony were mainly concentrated in roots of borage but in shoots of mustards. According to, bio-concentration and translocation factors are respectively defined as BCF (Bio-Concentration Factor) = (Q₁ + Q₂)/Q₁ and TF (Translocation Factor) = Q₂/Q₁: where Q₁, Q₂, Q₃ are average metal(loid) quantities (in mg kg⁻¹) respectively in soil, roots and shoots. BCF > 1 indicates that the plants accumulate the pollutants and BCF < 1 indicates excluder plants (Arshad et al., 2008). Accordingly to Evangelou et al. (2007), using PbNO₃ spiked soils, calculated BCF for borage was below 1 for Pb and Sb. BCF was far above 1 for Cu and Zn (and Cd in a lesser extent) (Table 2), which are essential elements for plants (Zheng et al., 2011). TF calculated for borage were below 1 for Pb, Sb and Cd (except for soil₁-C₂) and above 1 for Cu and Zn. Borage stabilises lead and antimony into its roots (McGrath and Zhao, 2003). Zou et al. (2011) showed that metal(loid)s are unevenly distributed in roots, where different root tissues act as

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<th>Pb</th>
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<td>Soil₁ (a)</td>
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**Table 1**

Metal(loid)s concentrations in shoots and roots (mg kg⁻¹ DW): (a) borage; (b) mustard. Values are given as mean of five replicates with three seedlings each. *DL: Detection Limit (= 0.1 mg kg⁻¹).
barriers to apoplastic and symplastic transport, thereby restricting transport to the shoots. Borage extracts and translocates Cu and Zn and could therefore be used for remediation of polluted vineyards (Banas et al., 2010). Calculated TF confirmed that mustard was relevant for phytoextraction (Bareen and Tahira, 2010). Finally, for all the performed experiments, the soil-plant metal/(loid) transfer was influenced by the soil type, as metal/(loid) availability may change according to interactions with the soil matrix (Niemeyer et al., 2012; Shahid et al., 2012).

5. Conclusions and perspectives

Unlike to phacelia, borage and mustard can improve soil respiration, reduce total and bioavailable metal/(loid) quantities and their ecotoxicity. For lead and antimony, contrasted mechanisms were developed: phytoextraction with storage in shoots and phytostabilization with storage in roots respectively for mustard and borage. Thanks to these treatments, metal/(loid) transfer was influenced by the soil type, as metal/(loid) availability may change according to interactions with the soil matrix (Niemeyer et al., 2012; Shahid et al., 2012).

Acknowledgements

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References


Table 2

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