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David Houben, Philippe Sonnet. Metal immobilization and nitrate reduction in a contaminated soil amended with zero-valent iron (Fe⁰). *Ecotoxicology and Environmental Safety*, 2020, 201, pp.110868. <10.1016/j.ecoenv.2020.110868>. <hal-02900977>

HAL Id: hal-02900977

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Submitted on 16 Jul 2020

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Metal immobilization and nitrate reduction in a contaminated soil amended with zero-valent iron (Fe⁰)

David Houben^{a*}, Philippe Sonnet^b.

^aUniLaSalle, AGHYLE, 19 Rue Pierre Waguet, 60026, Beauvais, France

^bEarth and Life Institute, Université Catholique de Louvain, Croix Du Sud 2/L7.05.10, 1348, Louvain-la-Neuve, Belgium

*Corresponding author: david.houben@unilasalle.fr

Abstract

Technologies based on zero-valent iron (Fe⁰) are increasingly being used to immobilize metals in soils and remove metals and nitrate from waters. However, the impact of nitrate reduction on metal immobilization in metal contaminated soils has been poorly investigated so far. Here, different concentrations of Fe⁰ filings (1%, 2% and 5%; wt%) were applied to a metal contaminated soil. The resulting nitrate reduction and metal (Cd and Zn) immobilization was investigated using a column leaching experiment for 12 weeks. Corrosion of Fe⁰ filings and precipitation of Fe oxyhydroxydes (FeOOH) on the surfaces of the filings were observed using SEM-EDS and EMPA-WDS at the end of the experiment. Compared to the untreated soil, total nitrate amounts released were lowered by 47%, 59% and 87% in the presence of 1%, 2% and 5% of Fe⁰, respectively. Concomitantly with nitrate reduction, Cd and Zn concentrations in leachates were strongly alleviated in the presence of Fe⁰, which was partly attributed to the rise of soil pH subsequent to nitrate reduction. More importantly, biotests with *Lupinus albus* L. revealed that the mechanisms involved in metal immobilization are stable to root-induced acidification. However, Fe⁰ was not efficient to reduce Cd concentration in *Lolium multiflorum* Lam., indicating that root processes other than acidification may re-mobilize metals.

Keywords

Heavy metals; Immobilization; Nitrogen; Pollution; Remediation; Zero-valent iron

1. Introduction

Over the last decades, the *in situ* immobilization of metals has attracted considerable interest for remediating contaminated soils (Kumpiene et al., 2019). *In situ* immobilization is a technique that aims at preventing metals from spreading to proximal waters and reducing

bioavailability without excavation and offsite disposal of contaminated soil (Palansooriya et al., 2020). One method to achieve this aim is to modify the physicochemical properties of the metals by using soil amendments (Houben et al., 2012). As reviewed by Komárek et al. (2013), numerous studies have focused on the potential of various iron-bearing additives to immobilize metals in soil. Among these additives, zero-valent iron (Fe⁰) appears to be one of the most cost-effective because, unlike other Fe containing compounds, it is cheaply available in large quantities as an industrial by-product (Jiang et al., 2018; Xue et al., 2018a). Over the years, Fe⁰ particles have proved to successfully remove metals from water due to their strong sorption or reducing abilities (Arshadi et al., 2014; Boparai et al., 2013; Xu et al., 2012). However, the use of Fe⁰ for the *in situ* immobilization of metals such as Cd or Zn in soil or sediment has been rare (Gil-Díaz et al., 2014; Houben et al., 2012; Huang et al., 2018). Application of Fe⁰ in soils may immobilize metals through its oxidation which increases the number of scavenging micropores (Lombi et al., 2003), promotes metal sorption, cementation, reduction, complexation and (co)precipitation (Shokes and Moller, 1999; Smith, 1996) and removes dissolved organic carbon (DOC) from the soil solution (Houben et al., 2012; McBride and Martinez, 2000).

On the other hand, Fe⁰-based technologies have generated significant interest for the removal of various substances from contaminated groundwaters (Fu et al., 2014). Long known to occur (Young et al., 1964), the chemical nitrate reduction in Fe⁰-containing medium is becoming increasingly attractive due to its easy operation and high performance (Liu and Wang, 2019). The reduction of nitrate to ammonium in the presence of Fe⁰ (Equation (1)) takes place spontaneously in conditions ranging from slightly alkaline to acidic (Cheng et al., 1997).



In constructed wetlands, Fe⁰-based amendment was recently found to enhance simultaneously nitrate reduction and metal removal (Jia et al., 2020). However, the authors concluded that further investigation was needed to reveal the complex relationships between these two processes. In groundwater, Su et al. (2014) found that the presence of nitrate might affect Pb removal by Fe⁰ due to change in water pH following nitrate reduction. Since nitrate is also ubiquitous in soils, one can wonder to what extent the application of Fe⁰ in contaminated soils also leads to nitrate reduction and, in turn, affects metal behavior. The objective of this study was therefore to gain insight into the relationship between nitrate reduction and metal mobility and bioavailability in soils amended with Fe⁰.

2. Materials and methods

2.1. Study soil

The study soil is located at Prayon (Liège province, eastern part of Belgium). From the 1930s to the 1970s, the site was intensively subjected to Cd- and Zn-bearing atmospheric fallouts. A total mass of 50 kg of surface soil (0–7 cm) was obtained by composite sampling of a 20 × 20 m² area that was colonized by metal-tolerant plant species (*Agrostis capillaris*, *Viola calaminaria*, *Noccaea caerulescens*, *Armeria maritima* subsp. *halleri*). As exhaustively described in Couder et al. (2015), the soil properties are pH = 4.8, cation exchange capacity = 38 cmol_c kg⁻¹, organic C concentration is 216.5 g kg⁻¹ and total Zn and Cd concentration are 7400 mg kg⁻¹ and 150 mg kg⁻¹, respectively. After sampling, the soil was dried at room temperature and sieved through a 2-mm nylon sieve. The sieved soil was then stored at 4°C prior to use.

2.2. Treatments

Fe⁰ was introduced in the form of alloy-free Fe⁰ filings from a metalworking lathe in the Nuclear Physics Institute (UCL, Belgium) machine shop. Acid dissolution and X-ray diffraction analyses revealed that Fe⁰ filings contained 99.5% α -Fe⁰ iron with minor Mn and Ca contents (Houben et al., 2012). Sub-samples of soil were treated with Fe⁰ filings as follows (wt%): untreated polluted soil (UNT); Fe⁰ filings 1% (Fe⁰-1%); Fe⁰ filings 2% (Fe⁰-2%); Fe⁰ filings 5% (Fe⁰-5%). Homogeneous mixing of soil and Fe⁰ filings was obtained by agitation in a plastic flask during 2 h just prior to use.

2.3. Leaching column design

The column design has been previously described in Houben et al. (2013). Briefly, PVC columns (7.5-mm diameter; 120-mm height) were fitted with a multi-holes drilled bottom end cap and connected to a funnel channeling the leachates into a 250-ml high-density polyethylene (HDPE) collector bottle. A Whatman No. 41 paper filter was fitted within each cap to prevent any loss of material. In the column, a 10-mm layer of quartz wool was inserted under the substrate to ensure free drainage. A similar 10-mm layer of quartz wool and a fritted glass disk were placed at the top of the soil column to distribute the supplied water evenly over the entire surface and to avoid disturbance of the soil surface by the water droplets. All apparatus were acid washed (pH 3, HCl) and rinsed with deionized water before use. Each column was filled with 50 g (dry weight) of the soil/Fe⁰ filings mixture. All the experiments were carried out in

triplicate. The columns were arranged in a randomized design in a controlled room and kept in the dark at constant temperature (18°C) and humidity (80%). Prior to leaching, the soil columns were allowed to equilibrate at 75% of the water holding capacity (WHC) for two weeks.

After the equilibration period, 17 ml of deionized water was supplied manually by pipette to each column four times a week. The leachates were collected beneath the columns every two weeks for twelve weeks.

2.4. Leachates analysis

At each date of leachate collection, the volume of solution was determined by weighing the HDPE collector bottles. A small aliquot was then set aside for pH determination and the remaining solution was filtered at 0.45 µm (Pall, Supor 450 membrane). Concentrations of Cd and Zn were measured by inductively coupled plasma–atomic emission spectroscopy (ICP-AES; Jarrell Ash). The concentrations of inorganic NO₃⁻ and NH₄⁺ were determined by ionic chromatography (HPLC-Dionex).

2.5. Fe⁰ filings characterization

At the end of the column experiment, the mixtures were removed from the columns. Fe⁰ filings were then isolated from Fe⁰-5% subsamples by magnetic separation and selected individual grains were impregnated with epoxy and polished. These polished sections were observed and analyzed semi-quantitatively using a field emission gun scanning electron microscope (FEG-SEM; Zeiss Ultra55) and an in-lens secondary electron detector. The FEG-SEM was equipped with an energy-dispersive X-ray spectrometer (EDS) for element detection and element mapping (silicon drift detector, Quantax, Bruker). Voltage was set at 25 kV. Image and element raster maps were imaged using Esprit software (Bruker). Quantitative analysis of element concentration was performed by punctual analysis on the polished sections using a Cameca SX 50 electron microprobe equipped with four wavelength-dispersion spectrometers (EMPA-WDS). Operating conditions were an accelerating voltage of 15 kV and a beam current of 20 nA. Counting time was 30 s.

2.6. Metal fractionation

Subsamples of soil and Fe⁰-5% were recovered at the end of the leaching experiment for metal fractionation characterization using the BCR three-step sequential extraction scheme ([Rauret et al., 1999](#)). This method makes it possible to fractionate the metals into the operationally defined exchangeable, acid-soluble and specifically adsorbed fraction (F1), iron and manganese

oxyhydroxide-bound fraction (F2), and organic- and sulfide-bound fractions (F3). In addition, a fourth step consists in extracting the residual fraction bound to the mineral matrix (F4). As described in Houben et al. (2013), the supernatant was filtered (0.45 μm) after each separation by centrifugation and the Cd, Zn, and Pb concentrations in the solution were measured by ICP-AES.

2.7. Biotest experimental device

The design of the biotest used in this study was described in details by Houben and Sonnet (2015). The principle of this device is similar to that used by other authors (Bravin et al., 2012; Couder et al., 2015; Gómez-Suárez et al., 2020) and consists in separating plant roots from soil with a 20- μm polyamide mesh to facilitate the collection of plants and rhizosphere. Briefly, 6 sterilized (10 min in 2 mol L⁻¹ H₂O₂) seeds of *Lupinus albus* L. or 1.5 g sterilized seeds of *Lolium multiflorum* Lam., per pot were germinated in demineralized water for three days in darkness. Then, the demineralized water was replaced with nutrient solution and the seedlings were allowed to grow for 7 days under a photoperiod of 16 h (photon flux density of 120–180 $\mu\text{mol m}^{-2} \text{s}^{-1}$), constant temperature (20°C) and relative humidity (95%). The chemical composition of the nutrient solution expressed in mmol L⁻¹ was: 1 Ca(NO₃)₂·4H₂O, 0.5 KCl, 0.25 K₂SO₄, 0.05 MgCl₂, 0.05 MgSO₄, 0.05 NaH₂PO₄, and 0.08 H₃BO₃. This pre-growth period allowed the formation of a dense root mat at the surface of the 20 μm -polyamide mesh.

At the completion of this pre-growth stage, plant compartments were transferred onto a thin layer of 14-day pre-incubated UNT or Fe⁰-5% treatment (about 2-mm thick, 7.5 g dry soil), which was connected to a demineralized water reservoir by a polyamide cloth in order to maintain constant soil moisture by capillarity. Unplanted control treatments, in which the soil had been incubated in similar devices without plants (thereafter called bulk soil), were also part of the design. In total, 24 such devices were implemented: 2 treatments (UNT and Fe⁰-5%) x 3 crop conditions (*L. multiflorum*, *L. albus*, and bulk soil) x 4 replicates. After 14 d of contact, plants were harvested, dried at 60 °C, weighed and crushed. The concentration of Cd and Zn in shoots was then determined by ICP-AES after mineralization by HNO₃ and *aqua regia* digestion. Cropped soil (rhizosphere) and uncropped soil (bulk soil) were collected, dried at ambient temperature and then characterized for soil pH (soil to water ratio of 1:5).

2.8. Statistical analyses

Average results for the different treatments were compared using one-way analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) for multiple comparisons. Prior to ANOVA, homogeneity of variances was tested using Levene's test. Pearson's correlation coefficients (r) were calculated to determine the relationships between Cd and Zn concentrations and nitrate concentration or pH in leachates. All statistical analyses were performed using R software version 3.5.0 (R Core Team, 2017) and the package Rcmdr (Fox, 2005).

3. Results and discussion

3.1. Nitrate and ammonium leaching

The total nitrogen quantity released by each column was calculated by summing up the amounts of nitrate and of ammonium leached from the column during the span of the experiment. Nitrite was not included in the sum because it always occurred in negligible concentrations ($<0.1 \text{ mg L}^{-1}$). At the end of the experiment, each treatment yielded approximately the same total quantity of nitrogen (Fig. 1). However, the proportion of nitrate progressively decreased as the concentration of amendment increased (Fig. 1). The total leached nitrate amount was reduced by 47%, 59% and 87% when 1%, 2% and 5% of Fe^0 were applied to the soil, respectively. The decrease of nitrate release occurred at the very beginning of the experiment (Fig. 2a). This suggests that nitrate reduction due to the corrosion of Fe^0 is a rapidly-occurring phenomenon in soil and much similar, in that respect, to what has been found for nitrate-rich waters (Choe et al., 2000). It is also likely that the nitrate reduction was favored by the acidic nature of the present study soil as it was demonstrated that NO_3^- reduction efficiency is higher at low pH (Suzuki et al., 2012). From the sixth week to the end of the experiment, nitrate concentration in leachates from the untreated soil was very low (Fig. 2b) and similar to that collected beneath treated soils which can be explained by the rapid leaching of nitrate due to its high mobility in soils (Cameron et al., 2013).

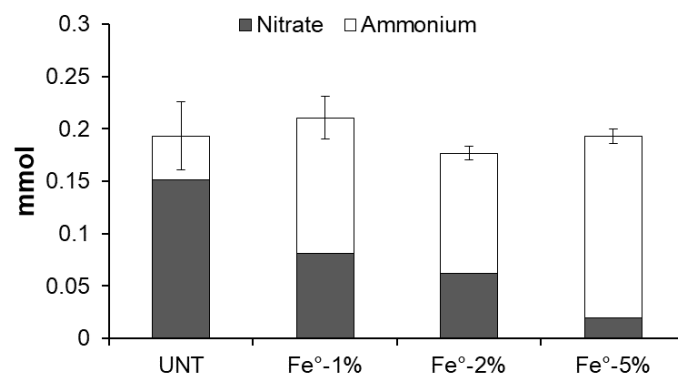


Fig. 1. Total amount of nitrogen and distribution into nitrate and ammonium forms released by the untreated soil (UNT) and the soils amended with 1% (Fe⁰-1%), 2% (Fe⁰-2%) or 5% (Fe⁰-5%) of Fe⁰. Standard error (n = 3) is presented for the total nitrogen amount.

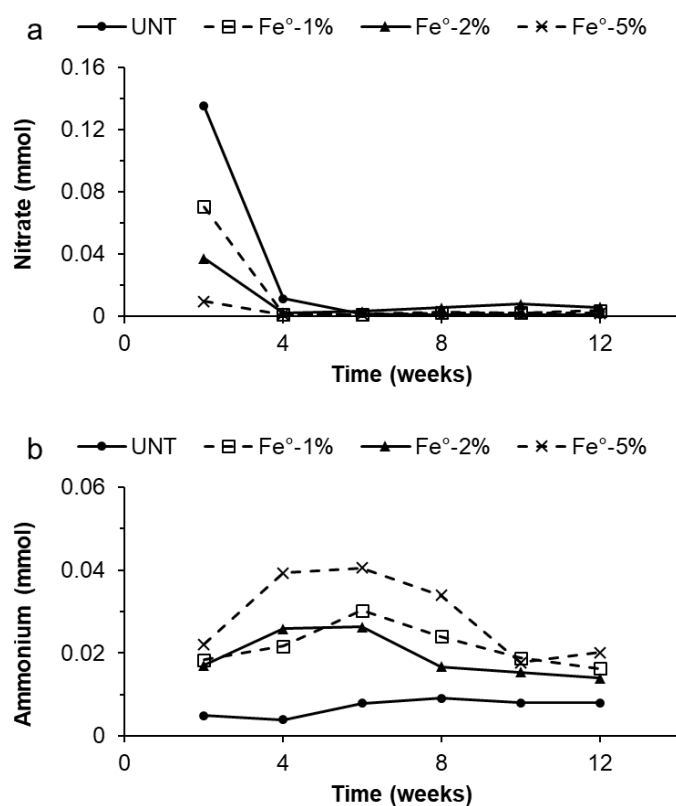


Fig. 2 Evolution of nitrate (a) and ammonium (b) amounts in leachates from the untreated soil (UNT) and the soils amended with 1% (Fe⁰-1%), 2% (Fe⁰-2%) or 5% (Fe⁰-5%) of Fe⁰. Each point represents the average of three replicates.

The lack of any significant difference ($p > 0.05$) in the total nitrogen recovery for each treatment (Fig. 1) suggests that the nitrate was stoichiometrically reduced to ammonium. This is in agreement with previous studies reporting that ammonium ions in nearly stoichiometric amounts are the final end-products of nitrate reduction in the presence of Fe⁰ (Huang et al., 1998; Su and Puls, 2004). However, in contrast with studies on contaminated water, the

ammonium and nitrate concentrations were not inversely related (Fig. 2a and b). Ammonium being a cation, it can be expected that its leaching was slowed down as it interacted with negative exchange sites at the surface of both soil constituents (Cameron et al., 2013) and newly formed iron oxides (Westerhoff and James, 2003). As a result, several successive percolation events were necessary to leach out the total amount of produced ammonium.

3.2. Fe oxide formation

SEM-EDS analysis provided evidence of Fe⁰ corrosion as Fe⁰ particles were coated by a soft layer of a banded mixture of Fe and oxygen (O) revealing the presence of iron oxides and hydroxides (Fig. 3). Silicon (Si), which indicates the presence of soil particles, was only detected beyond this layer. It was thus concluded that the banded layer was formed by direct replacement of Fe⁰ by corrosion products. Quantitative analysis by EMPA-WDS (Fig. S1) revealed that the banded layer was on the average composed of 60 wt% of Fe. This weight proportion is close to that of Fe in Fe oxyhydroxides (FeOOH; 63 wt% of Fe) such as goethite (α -FeOOH) and lepidocrocite (γ -FeOOH). Since our experimental set-up was open and the soil was not saturated by water, aerobic conditions were maintained all throughout the experiment. Our results are thus congruent with those found by Huang and Zhang (2005) that showed that lepidocrocite is the preferential Fe⁰ corrosion product during nitrate reduction as long as dissolved oxygen is present.

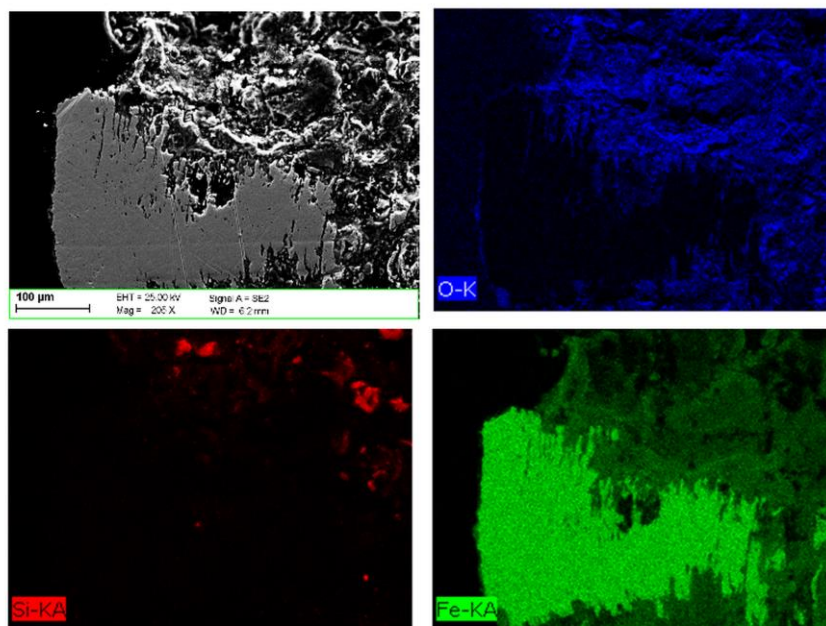


Fig. 3. Representative scanning electron microscope (SEM) images of the polished cross-section of Fe⁰ filings and color coded SEM-EDS element dot-maps (blue for oxygen, red for silicon and green for iron) illustrating the corrosion of a Fe⁰ grain. Si dot-map was used to detect the presence of any particle from the native soil material.

Previous studies suggested that layers of iron oxides could passivate Fe⁰ surface and, thereby, limit mass transport of nitrate to the reactive surface where the exchange of electrons are facilitated (Bae et al., 2018; Luo et al., 2010; Westerhoff and James, 2003). Nevertheless, Huang et al. (2003) showed that iron oxide coating did not impede nitrate reduction by Fe⁰, provided that sufficient aqueous Fe²⁺ was present in the system. Since the release of Fe²⁺ by Fe⁰ particles depends on their size and their proportion in the system (Furukawa et al., 2002), these two parameters should be carefully considered for any further investigations on nitrate reduction by Fe⁰ in soils.

3.3. Metal immobilization

Numerous studies have investigated the ability of Fe⁰ to immobilize metals in soils (Guan et al., 2015; Xue et al., 2018a). Hanauer et al. (2011) reported that Fe⁰ has initially no binding capacity for ions. However, as shown in the present study (Fig. 3, Fig. S1), once Fe⁰ is applied to soil, it rapidly oxidizes leading to the formation of iron oxides onto which metals may quickly sorb (Smith, 1996) or coprecipitate (Lambrechts et al., 2011; Martinez and McBride, 1998). Fig. 4 shows that Zn and Cd concentrations in the first leachate were strongly reduced in the presence of Fe⁰, confirming its rapid immobilizing effect. At the end of the experiment, the total amount of leached Zn was reduced by up to 73%, 87% and 98% in the leachate in the presence of 1%, 2% and 5% of Fe⁰ respectively, while the total of leached Cd was reduced by up to 48%, 66% and 83% in the leachate from soil amended with 1%, 2% and 5% of Fe⁰, respectively. Zinc and Cd leaching attenuation by zero-valent iron amendment was thus very effective and slightly more efficient for Zn. Several studies have reported that the adsorption affinity for metal ions to oxides was higher for Zn than for Cd (Boparai et al., 2013; Trivedi and Axe, 2000), which was attributed to the lower ionic radius of Zn (0.074 nm for Zn, 0.097 nm for Cd) and its lower first hydrolysis product (9.0 for Zn, 10.1 for Cd) (Bruemmer et al., 1988). In addition, Buekers et al. (2008), suggest that the higher ionic radius of Cd also limits its penetration into oxyhydroxide. The higher Zn immobilization compared to Cd was also confirmed by sequential extractions (Fig. 5). The application of Fe⁰ induced a shift of both Cd and Zn from the lowest stable F1 fraction to the most stable F4 fraction, which can be explained by metal sorption to the Fe⁰ surface, during which the further oxidation of Fe⁰ promotes the growth of the oxide shell and shrinkage of the core, resulting in the embedment of the adsorbed metal into the Fe oxide/Fe⁰particle (Khum-in et al., 2020; Phenrat et al., 2019). However, unlike Zn, Cd was also enriched into the less stable F2 fraction. This suggests that a significant part of the Cd adsorbed to newly-formed Fe oxides was not further embedded into the oxide shell and could therefore

be re-mobilized under change in soil conditions (Houben and Sonnet, 2015; Phenrat et al., 2019).

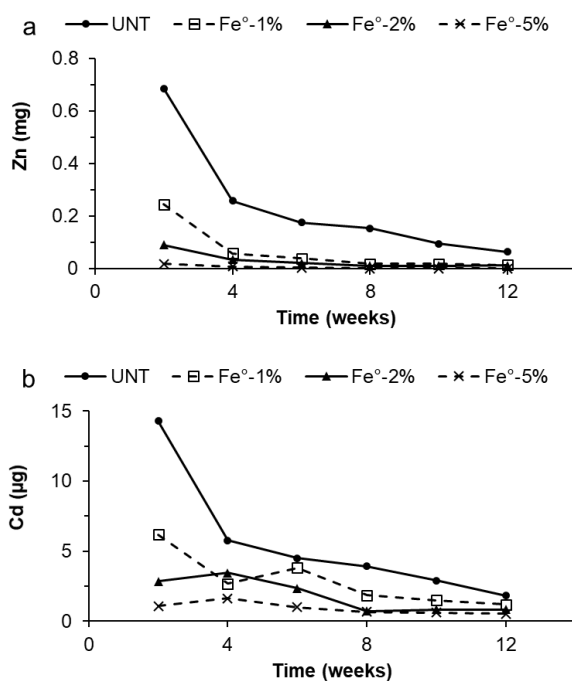


Fig. 4. Evolution of Zn (a) and Cd (b) amounts in leachates from the untreated soil (UNT) and the soils amended with 1% (Fe⁰-1%), 2% (Fe⁰-2%) or 5% (Fe⁰-5%) of Fe⁰. Each point represents the average of three replicates.

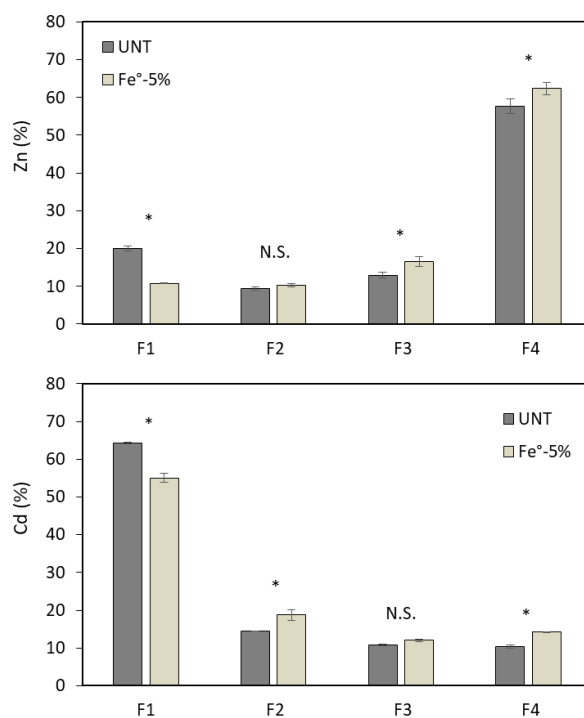
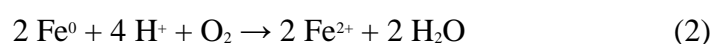


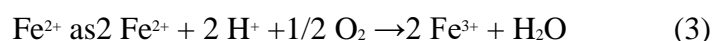
Fig. 5. Solid phase fractionation of Zn and Cd into the acid-soluble (F1), reducible (F2), oxidizable (F3) and residual (F4) fractions. Values are average ($n = 3$) \pm standard error. * significant at $p < 0.05$. N.S. not significant.

3.4. Coupling nitrogen reduction and metal immobilization

Soil contamination with metals is well known to reduce or even inhibit nitrification (i.e. the microbial conversion of ammonium to nitrite and then to nitrate) (Baath, 1989; Yeates et al., 1994). As a result, by reducing metal availability to nitrifiers, the use of immobilizing amendments usually leads to increasing nitrification (Kostov and Van Cleemput, 2001; Zhang et al., 2017). In the present study, the oxidation of Fe⁰ hindered the positive effect of metal immobilization on nitrification as the amount of NH₄⁺ and NO₃⁻ gradually increased and decreased, respectively, with increasing levels of Fe⁰ into the soil. Several studies have reported that nitrification might increase metal mobility by releasing H⁺ (Basta and McGowen, 2004; Chang and Broadbent, 1980). However, the effect of nitrate reduction to ammonium on metal mobility has been poorly investigated so far (Su et al., 2014). Here, the positive correlation between nitrate and Zn and Cd concentrations ($r = 0.89, p < 0.001$ and $r = 0.87, p < 0.001$, respectively) suggests that metal immobilization might be related to nitrate reduction. In acid media, an increase of soil pH after the addition of Fe⁰ in presence of oxygen is usually observed and attributed to the following reaction



and the subsequent oxidative transformation of



In addition, nitrate reduction, as found in the present study, may also contribute to increase the medium pH (Equation (1)) due to both alkaline release from iron corrosion and acidity consumption from nitrate reduction by Fe⁰ (Tang et al., 2012). For instance, using Fe⁰ packed column, Westerhoff and James (2003) found that pH increased with growing mass nitrate removals. Moreover, Su et al. (2014) showed experimentally that the addition of nitrate increased the efficiency of Fe⁰ to remove Pb from groundwater because the pH increase brought about by nitrate reduction promoted Pb precipitation. In the present study, the strong correlation between pH and Zn and Cd concentrations ($r = 0.89, p < 0.001$ and $r = 0.85, p < 0.001$, respectively; Fig. S2) in leachates suggests that similar mechanisms occur not only in waters but also in soils. The rise in soil pH can increase Zn and Cd immobilization by favoring their precipitation, decreasing their solubility and promoting their adsorption due to the increase of

the net negative charge of variably charged soil constituents (Bradl, 2004; Lindsay, 1979). It is also likely that the higher pH brought about by nitrate reduction improves metal sorption on Fe⁰-derived iron oxides themselves since their deprotonation with increasing pH promotes Zn and Cd chemisorption (Boparai et al., 2013; Stahl and James, 1991). This agrees Xue et al. (2018b) who found that, in alkaline sediments, the rise of pH following Fe⁰ application increased Cd (and Pb) immobilization due to stronger electrostatic affinity between iron oxides and the positively charged metal ions.

3.5. Metal uptake by plants

In the bulk soil, the application of Fe⁰ showed a significant liming effect (Fig. 6), which can be partially attributed to nitrate reduction, as previously discussed. However, this liming effect was reduced in the presence of *L. multiflorum* and even disappeared with *L. albus* as its rhizospheric pH in the presence and in the absence of Fe⁰ was similar and significantly lower than the bulk soil pH (Fig. 6). The main process responsible for change of rhizosphere pH is the release of H⁺ or OH⁻ by roots to compensate charge imbalance due to unequal uptakes of cation and anions (Hinsinger et al., 2003). In particular, N uptake by roots greatly impacts rhizosphere pH as a higher uptake of NH₄⁺ relative to NO₃⁻ increases the release of H⁺ from roots (Gómez-Suárez et al., 2020). As a result, by promoting the reduction of NO₃⁻ into NH₄⁺, the application of Fe⁰ increased the uptake of NH₄⁺ relative to NO₃⁻, which explains the pH decrease in Fe⁰-amended rhizosphere compared to the bulk soil.

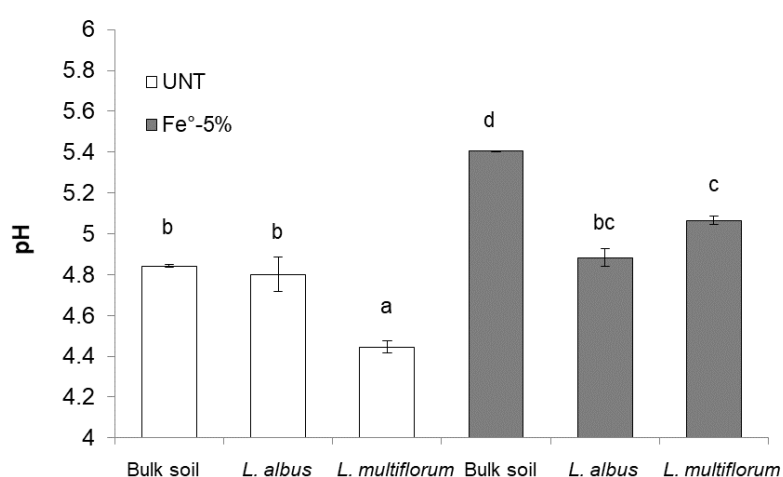


Fig. 6. pH in the bulk soil and in the rhizosphere of *Lupinus albus* and *Lolium multiflorum* in the control and Fe⁰-5% treatment. Values are average (n = 4) ± standard deviations. Columns with the same letter do not differ significantly at the 5% level according to the Tukey's multiple comparison test.

Rhizosphere acidification due to uptake of nitrogen in the form of ammonium instead of nitrate has been previously found to increase solubility and uptake of Zn and Cd by plants (Cheng et al., 2016; Loosemore et al., 2004). However, in the present study, although root activity induced a re-acidification of the rhizosphere, Zn and Cd concentrations in *L. albus* were lower in the presence of Fe⁰ (Table 1). This suggests that the mechanisms involved in metal retention by Fe⁰ are stable to acidification. According to Lombi et al. (2003) who found similar results with red mud, this might be due to solid-phase diffusion of metals into the lattice of Fe oxides or migration into micropores.

Table 1. Concentration of Cd, Zn and Fe in shoot of *L. albus* and *L. multiflorum* in the absence (UNT) and in the presence of 5% Fe⁰ (Fe⁰-5%). For each plant species, metal concentration. Values are average (n = 4) ± standard error.

	<i>L. albus</i>			<i>L. multiflorum</i>		
	UNT	Fe ⁰ -5%	<i>P</i> value	UNT	Fe ⁰ -5%	<i>P</i> value
Cd (mg kg ⁻¹)	0.29 ± 0.05	0.12 ± 0.01	<0.001	6.9 ± 0.4	9.0 ± 0.5	<0.001
Zn (mg kg ⁻¹)	146 ± 16	60 ± 2	<0.001	381 ± 26	288 ± 20	<0.001
Fe (mg kg ⁻¹)	39.9 ± 3.7	71.9 ± 12.6	<0.001	33.3 ± 4.1	807 ± 142	<0.001

Compared to *L. albus*, *L. multiflorum* showed much higher metal concentrations in shoots (Table 1). These result agree with Römer et al. (2000) who found that Cd concentration in 13 days old plants grown on Cd-contaminated soil was 55 times lower in *L. albus* (0.1 mg kg⁻¹) than in *L. multiflorum*(5.5 mg kg⁻¹). These authors demonstrated that the lower uptake of Cd by *L. albus* was due to the secretion of high amounts of citrate which complex Cd in the rhizosphere, restricting thereby its uptake by roots. In addition, unlike Zn, Cd concentration in *L. multiflorum* was unexpectedly not alleviated by the presence of Fe⁰, suggesting that *L. multiflorum* remobilized Cd. Unlike *L. albus*, *L. multiflorum*, as a graminaceous species, releases phytosiderophores to acquire Fe (Takagi et al., 1984). Phytosiderophores are secreted through efflux transporter (Nozoye et al., 2010) and then solubilize Fe in the soil, forming Fe-phytosiderophore complexes that are absorbed into root cells through a Fe-phytotransporter (Curie et al., 2001). Several studies have shown that such Fe-complexing compounds can efficiently dissolve Fe from poorly oxides such as ferrihydrite, lepidocrocite and goethite (Kraemer, 2004; Reichard et al., 2007) and remobilize metals or prevent them from sorption onto these constituents (Neubauer et al., 2002). Since sequential extractions revealed that, unlike Zn, Fe⁰ application induced a Cd shift from the acid-soluble (F1) to the Fe/Mn-bound (F2) fraction, it is likely that the high uptake of Fe by *L. multiflorum* (Table 1) through

phytosiderophore secretion dissolved newly-formed Fe oxides which in turn released previously-immobilized Cd. Therefore, although Cd immobilization mechanisms in the presence of Fe⁰ are stable under acidification, our data suggest that other processes, including root secretion, might suppress them. The perspective is thus to better constrain the role of root exudates on the effectiveness of Fe⁰ to immobilize metals in the long run. Interestingly, by contrast to *L. albus* which shows similar Zn/Cd shoot ratios between the control and the Fe⁰-5% treatment (503 and 500, respectively), *L. multiflorum* grown in the untreated soil had a Zn/Cd ratio 1.7 times higher than that when it grew in the presence of Fe⁰-5% (55 and 32, respectively), suggesting possible interactions between Zn and Cd (Peng et al., 2018). It has been shown that Zn acts as a competitive ion for Cd absorption and high Zn concentration in solution often inhibits Cd uptake by roots (Hart et al., 2005; Sarwar et al., 2015). As a result, the strong immobilization of Zn by Fe⁰ might have reduced its inhibiting effect on Cd uptake by *L. multiflorum*, leading also to higher Cd concentration in plants.

4. Conclusion

This study reveals that nitrate reduction and metal immobilization are coupled in soil amended with Fe⁰. Indeed, by increasing the soil pH, nitrate reduction and iron corrosion favors metal immobilization. More importantly, Zn concentrations in *L. albus* and *L. multiflorum* were strongly decreased in the presence of Fe⁰ despite a re-acidification of their rhizosphere. This indicates therefore that immobilizing mechanisms are stable under acidification. Similar results were found for Cd in *L. albus* but not in *L. multiflorum*, suggesting that Cd can be re-mobilized by other rhizospheric processes, possibly the secretion of phytosiderophores. These findings may have significant implications in the field of soil remediation but further studies are required to elucidate the processes responsible for potential re-mobilization of metals.

Credit Author Statement

David Houben: Conceptualization, Methodology, Investigation, Writing - original draft, Philippe Sonnet: Conceptualization, Resources, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank P. Populaire for technical assistance, A. Iserentant and C. Givron for analytical assistance, J. Wautier for EMPA assistance and L. Ryelandt for SEM-EDS assistance.

Appendix A

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoenv.2020.110868>.

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Appendix A. Supplementary data

Metal immobilization and nitrate reduction in a contaminated soil amended with zero-valent iron (Fe^0)

David Houben and Philippe Sonnet

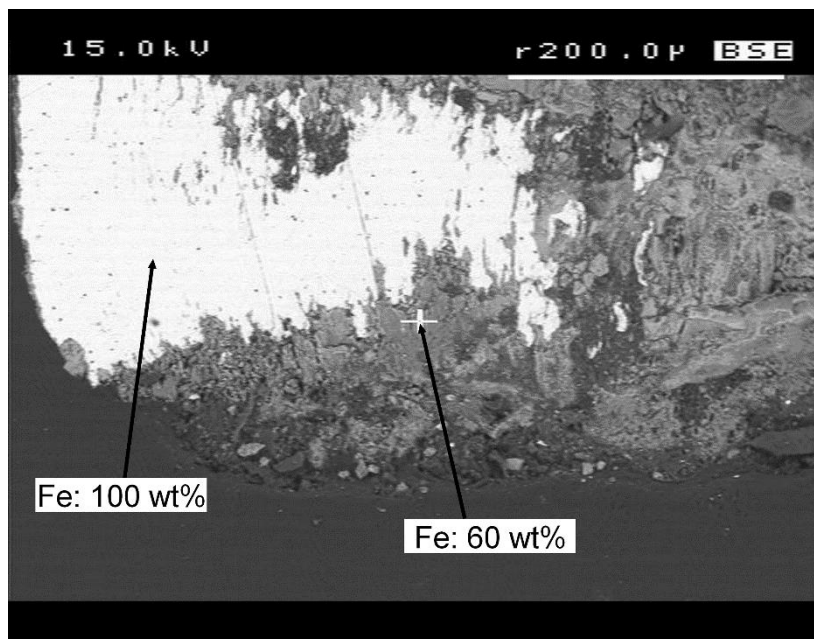


Fig. S1. Representative back-scattered electron image of the polished cross-section of Fe^0 filings obtained by EMPA-WDS. The light area is uncorroded Fe^0 while the darker coating consists of Fe oxyhydroxides (FeOOH). Percentage values represent the average iron content in points indicated by arrows.

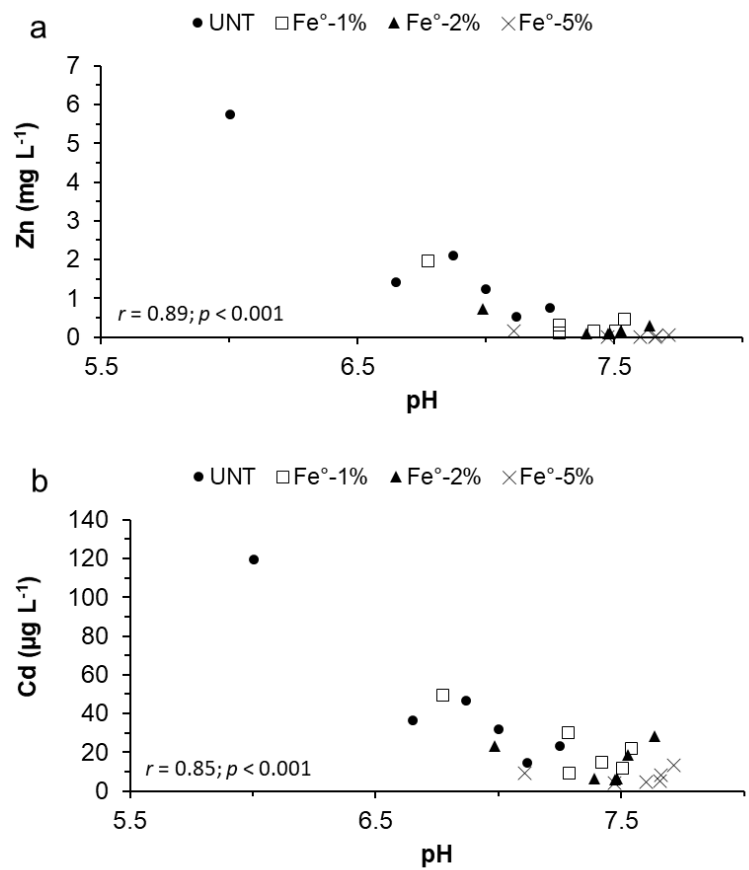


Fig. S2. Relationship between pH and concentrations of Zn (a) and Cd (b) in leachates. Marked correlation coefficient (r) is the Pearson's correlation coefficient calculated for the whole dataset of measurements.