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Microcosm-scale biogeochemical stabilization of Pb, As, Ba and Zn in mine tailings amended with manure and ochre

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Abstract:

Mine tailings are major sources of metals and metalloids in the environment, making the physical and geochemical stabilization of tailings a serious environmental challenge. With a view to facilitate the development of covering vegetation and of decreasing the mobility of Pb in the acid tailings of a former Ag-Pb mine, laboratory microcosm experiments were performed to enable comparison of the effectiveness of several treatments. Tailings were mixed with 5 % by weight of ochre, an iron-rich material produced during the treatment of a coal mine water, and with cow manure (0, 0.15, 1 and 2 % by weight), either solely or in combination. They were then submitted to weekly watering over 84 days. All treatments raised the pH values from 4 to values between 7 and 8 and induced a strong decrease in the total dissolved Pb concentration in the percolating water (from 13–15 $mg.L^{-1}$ to less than 0.5 $mg.L^{-1}$). Several processes seemed to be involved in the immobilization of Pb by the amendments: precipitation as hydroxide, sulfate, carbonate and phosphate, and adsorption on iron hydroxides. A transient increase was observed in both Pb mobility and functional microbial diversity with 1% and 2% manure, with a peak after 28 days of incubation. This peak corresponded to an Average Well Color Development (AWCD) in Biolog™ Ecoplates increase from 0.5 to 0.8 with 1% manure and from 0.6 to 1.5 with 2% manure. However, at the end of experiment, Pb immobilization was strengthened by 2% manure and microbial functional biodiversity fell back, with AWCD values of 0.5 and 0.8 for 1 % and 2% manure, respectively. Other toxic elements present in the tailings, namely As, Zn and Ba, were not strongly mobilized by the treatments, although cow manure slightly increased the leaching of Ba and As, which maximum concentrations in the leaching water reached 65 µg.L^{-1} Ba and 9 µg.L^{-1} As. All amendments improved the growth of ryegrass, which maximum dry biomass ranged from 38 mg/microcosm without amendment to 155 mg/microcosm with 0.15% manure. The results provide key information about the biogeochemical processes driving the mobility of Pb, As, Zn and Ba in acid mine tailings during the first 84 days following their amendment with iron-rich ochre and manure.

Key words: mine tailings, metals, arsenic, amendments, iron oxide-hydroxide, cow manure, microbial processes

Introduction

Mining activities have generated massive amounts of solid wastes throughout the world and every year the sector continues to mobilize some 50 x 10^9 tons of rocks. Huge amounts of solid waste, known as tailings, are produced when the valuable fractions of these materials are processed to extract the desired mining resources (Douglas and Lawson, 2000). For example, the extraction of metals such as Cu, Ni or Au can produce up to 1,000 tons of tailings for one kilogram of pure element. These wastes are usually stored behind tailings dams close to the extraction sites, often with inadequate management, especially on older mining sites. Tailings may contain large fractions of sulfide materials that are subject to oxidation when exposed to air and water. Their leaching by rainwater often induces the mobilization of metals and metalloids towards the surrounding environmental compartments, including groundwater, surface water, soils and sediments (Fuge et al., 1993; Paulson, 1997). Moreover, runoff water can carry downstream metal-rich solid particles that may become soluble if the water characteristics or redox conditions change.

Stabilization of mine tailings is facilitated by amendments that adsorb, complex or (co)-precipitate inorganic pollutants (Kumpiene et al., 2008) and metals and metalloids can be rendered less mobile by amendment of tailings with organic or inorganic materials. In general, amendments not only decrease the leachability of pollutants but also stimulate plant growth, and the resulting covering vegetation contributes to stabilizing tailing particles. Amendments have the additional advantage of being inexpensive and readily available in large quantities, since they derive from agricultural or industrial by-products (Guo et al., 2006). Chemical and inorganic amendments that have been used widely include alkaline and phosphate materials (Hooda and Alloway, 1996; Derome, 2000; Le Forestier et al., 2017). Alkaline materials have been shown to reduce the solubility of divalent metals such as Pb and Cu by increasing pH and phosphate materials to efficiently stabilize Pb by ionic exchange and precipitation of pyromorphite-type minerals [$Pb_5(PO_4)_3X$; X = F, Cl, B or OH] (Mc Gowen et al., 2001; Kumpiene et al., 2008). Organic amendments improve soil agronomic properties and can contribute to reduced mobility of metals by pH buffering (Zeng et al., 2011). A number of organic materials have been tested as amendments for polluted soils: compost, municipal biosolids, peat, chipped wood, composted sewage sludge, manure (Basta and Sloan, 1999; Li et al., 2000; Brown et al., 2003; Hattab et al., 2015), and biochars (Oustrière et al., 2016; Lebrun et al., 2016; Lahori et al., 2017; Norini et al., 2019). However, while alcalinization reduces the mobility of divalent metals, it may also induce the mobilization of As, a toxic metalloid frequently present in mine tailings. As pH increases, the efficiency of the adsorption of arsenate (As(V)) by iron oxides is reduced (Dixit and Hering, 2003). The difference in chemical behavior between metal cations and oxi-anions such as As(V) must therefore be taken into account in the initial choice of amendments to stabilize mine tailings. An alternative is amorphous iron hydroxide (ferrihydrite), which can be an effective sorbent for both cation and anion pollutants (Kumpiene et al., 2008). In fact, Martin and Ruby (2003) have observed efficient stabilization of both Pb and As in a smelter-contaminated soil by 5 % ferrihydrite together with 0.5 % of calcium phosphate. Iron oxides are produced as waste materials, sometimes in huge amounts, during the treatment of mine waters such as those from coal mines. Doi et al. (2005) have shown that iron-oxide rich sludge materials from mining sites, containing 24 % to 62 % of total iron, could be effective in stabilizing As in polluted soils. Treatments not only decreased soil As leachability but also reduced the uptake of As by radishes planted in amended soils. Nielsen et al. (2011) showed a significant, long-term decrease of As concentration in the pore water of a soil polluted by As and Cr and amended with 5 % of an iron-rich water-treatment residue, mainly composed of ferrihydrite. More recently, Olimah et al. (2015) tested four different iron-rich wastes produced in mine water treatment plants for the remediation of As-polluted soils. Their results suggest that this treatment is efficient in decreasing As mobility in water. However, the benefits in terms of uptake of As by plants and plant growth were not significant. The prime focus of these previous studies was As as the main target pollutant of the remediation strategy. In addition, chemical data alone do not take account of the effects of pollutants on the soil's habitat function. The microbiological status of a soil, considered as an indicator of its potential to sustain microbiological activity, can be used to assess the effectiveness of chemical stabilization of metals and metalloids by amendment (Pérez de Mora et al., 2006).

The work reported here investigated the potential for and mechanisms of biogeochemical stabilization of mine tailings of which the principal pollutant was Pb, associated mainly with As, Zn, and Ba. The tailings were amended with iron oxides from a coal mine water treatment plant, combined or not with different doses of cow (*Bos taurus*) manure to improve the agronomic properties of the material. Laboratory microcosm leaching experiments simulating percolation of rainwater were designed to evaluate the efficiency of stabilization of the main pollutants in relation to the biogeochemical evolution of the amended tailings.

Material and methods

Characteristics of the mine tailings

Tailings samples were taken from the site of a former Ag and Pb mine at Pontgibaud (Puy de Dôme, France; 45°47’29”N 2°49’18”E) operating since antiquity but with a peak of activity in the second half of the XIXth century (Cottard, 2010). The mine is located in a medium-altitude mountainous area (altitude 750 m). Mine residues (3 m^3) were recovered over a depth of 0–60 cm from a mine waste dump, using a power shovel. Residues were taken from seven discrete zones within a 25 m^2 area. The material was coarsely mixed with the shovel and transported to the laboratory for the stabilization experiments.

The details on all characterization and analytical methods (detection limits, analytical quality controls) are given in the chemical analysis section below and in the SM1. The grain size distribution of the tailings was determined by wet processing sieving. Only 7 % (by weight) of the material was less than 60 µm, and that 76 % was in the 315 µm to 2 mm range. The > 2 mm fraction represented 2 %, and the remaining fraction (15 %) was in the 60–315 µm range. The pH of the tailings – consisting mainly of quartz, orthoclase and phyllosilicate – was 4.9 (measured in water according to ISO 10390). Pb bearing phases were detected by X-ray diffraction (XRD): anglesite ($PbSO_4$) and beudantite (($PbFe_3(AsO_4)(SO_4)(OH)_6$); a solid solution between plumbojarosite, pure SO_4 pole, and segnitite, pure AsO_4 pole). Anglesite and beudantite had already been detected in tailings from the same mine district (Pascaud et al., 2014). The concentrations of the main constituents were: 26,432 mg.kg^{-1} Pb, 265 mg.kg^{-1} Zn, 1,063 mg.kg^{-1} Ba, 1,134 mg.kg^{-1} As, 820 mg kg^{-1} P_2O_5, 1.5 % Fe_2O_3, 81 % SiO_2 and 1.54 wt% SO_4. CaO and MgO concentrations were less than 1 %. Characteristics of organic matter are given in Table 1. Standard leaching testing in demineralized water (ISO/TS 21268-2) resulted in 19.6 mg.L^{-1} Pb, 2.3.µg.L^{-1} As, and 0.17 mg.L^{-1} Zn (Fe and Ba concentrations, were below the detection limits, respectively 0.1 mg.L^{-1} and 10 µg.L^{-1}).

Amendments

The ochre – iron oxide-hydroxide, mainly composed of goethite – was produced in a coal mine water treatment plant in France, by cascade aeration and sedimentation of the iron precipitates generated in a settling pond. Samples of concentrated sludge taken from the settling pond were dried gently at 30 °C and then ground in a mortar and sieved at 100 µm. The initial water content was 92 %. Size particle distribution before drying was analyzed with a laser granulometer (Malvern); it showed 86.5 % of particles smaller than 10 µm, 98 %

smaller than 89 µm and 100% smaller than 200 µm. The particle fraction most present was the 2–6 µm group. The pH in water (measured according to ISO 10390) of the dried ochre was 8.1. The concentrations of the main constituents were: 1.99 % C, 1.81 % H, 0.69 % S 0.66 % TOC, 1.39 % MinC, 261,232 $mg.kg^{-1}$ Fe, 10.1 $mg.kg^{-1}$ Pb, 505.7 $mg.kg^{-1}$ As, 14,717 $mg.kg^{-1}$ Zn and 69.8 $mg.kg^{-1}$ Ba. N was not detected. Standard leaching testing in demineralized water (ISO/TS 21268-2) resulted in 0.4 $mg.L^{-1}$ Fe, 0.23 mg L^{-1} Pb, 2.5 $\mu g.L^{-1}$ As, 44.1 $mg.L^{-1}$ dissolved organic carbon (DOC) and 4.4 $mg.L^{-1}$ dissolved organic nitrogen (DON) in the leachate. Zn was below the quantification limit, i.e. 0.01 $mg.L^{-1}$.

Cow manure was collected from a farm near Orléans (France); its pH in water (measured according to ISO 10390) was 9.9. The manure was dried gently at 30°C, ground in a mortar and sieved at 2 mm. The concentrations of the main constituents were: 35.40 % C, 4.51 % H, 2.48 % N, 0.18 % S, 28.95 % TOC, 2.17% MinC, 2.82 $mg.kg^{-1}$ Pb, 0.75 $mg.kg^{-1}$ As, 53.8 $mg.kg^{-1}$ Zn and 78.2 $mg.kg^{-1}$ Ba. Standard leaching testing (ISO/TS 21268-2) gave 0.33 $mg.L^{-1}$ Pb, 7 $\mu g.L^{-1}$ As, 0.33 $mg.L^{-1}$ Zn, 0.27 $mg.L^{-1}$ Ba, 1,130 $mg.L^{-1}$ DOC and 131 $mg.L^{-1}$ DON in the leachate.

Microcosm leaching experiments

Microcosms were prepared in 200 mL polystyrene pots (50 mm diameter) of which the bottoms were perforated with a 0.9 mm needle, 13 holes for each pot. To retain soil particles in the pots, a fine layer of glass wool was laid at the bottom and covered with 10 cm^3 of clean Fontainebleau sand. Both glass wool and sand were cleaned prior to use in 10 % HNO_3, rinsed with demineralized water and dried before use.

The pots were watered to simulate rainwater, all watering being performed with Mont Roucous mineral water (pH 5.85, 3.1 $mg.L^{-1}$ Na^+, 2.4 $mg.L^{-1}$ Ca^{2+}, 0.5 $mg.L^{-1}$ Mg^{2+}, 2.0 $mg.L^{-1}$ SO_4^{2-}, 6.3 $mg.L^{-1}$ HCO_3^-, 3.0 $mg.L^{-1}$ NO_3^-).

Each microcosm contained 150 g of air-dried material sieved at 2 mm. The different amendment conditions tested were as follows: tailings without amendment (T); tailings + 5 wt% ochre (TO); tailings +5 wt% ochre + 0.15 wt% manure (TOM 0.15%); tailings +5 wt% ochre + 1 wt% manure (TOM 1%); tailings +5 wt% ochre + 2 wt% manure (TOM 2%). These proportions were chosen based on the results of preliminary tests in slurries, that were performed to determine the minimum amendments concentrations allowing immobilization of both Pb and As (data not shown). The material for each condition was prepared by mixing the dry amendments with the tailings in a rotating agitator for 2 hours. Nine microcosms of each condition were then prepared.

For the first watering, 30 mL of water were sprinkled carefully onto the soil surface. Then, 72 h later, the microcosms were watered again with 25 mL of water (solid/liquid ratio of 6). The quantity of water added and draining out was recorded by weighing. Global aerobic non-saturated conditions were maintained. Microcosms were incubated in a Plant Growth Chamber (MEMMERT HPP750 IPP PLUS) at 25°C, in the dark, with 80 % of air-water saturation. The soils were never dried during incubation.

Watering with 25 mL (solid/liquid ratio of 6) water was then performed once a week. The percolated water was filtered at 0.45 µm. A sample was acidified with a drop of concentrated HNO_3 for total As and Pb analyses. The remaining filtered solution was stored in a refrigerator for complementary analyses.

Three microcosms of each of the amendment test conditions were sacrificed for analysis after incubation periods of 7, 28, and 84 days. When the microcosms were sacrificed, their contents were carefully mixed in sterile bags with a spatula and samples were taken to determine the moisture and for biological and molecular analyses (0.5 g of soil in sterile tubes stored at -20 °C). The remaining soil was stored at 5°C. Each time microcosms were sacrificed, As, Ba, Pb and Zn were analyzed in the percolated water.

Chemical analyses

The details on all analytical methods (detection limits, analytical quality controls) are given in SM1.

Metals and metalloids in the tailings were determined by ICP/AES (Ameteck Spectro apparatus, Arcos model) after dissolution according to NF X31-147. Sulfate concentration in the tailings was determined as per NF ISO 11048. Metals and metalloids in the ochre were determined by ICP-MS (Agilent 8900-Triple Quad apparatus) after complete dissolution by hydrofluoric acid attack (HNO_3 65 %, HCl 37 % and HF 40 %) (US-EPA 3052 method modified). Metals and metalloids in the manure were determined by ICP-MS after complete mineralization (heating at 600 °C for 3 h, followed by mineralization in 65 % HNO_3 and 37 % HCl) (US-EPA 3015A method).

Different chemical analyses were carried out on the solutions from leaching tests and on the filtered percolated water (0.45 µm) from microcosms. As, Pb and Ba were analyzed by oven

AAS (Varian AA220Z), and Zn by flame AAS (Varian AA22FS). pHs of percolated water were measured. Ion chromatography (IC), using a 940 Professional IC Vario instrument (Metrohm) equipped with conductivity detectors, was used to quantify major ions (Li^+, Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, PO_4^{2-}). Anions were separated with a Metrosep A Supp 16 ionic resin column (150 mm × 4 mm) and cations with a Metrosep C6 (150 mm × 4 mm). Only certain ions were detected: Cl^-, Br^-, NO_3^-, SO_4^{2-}, Li^+, Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}. DOC and DON concentrations were determined using a TOC 5050/SSM 5000-A elemental analyzer (Shimadzu).

Measurements of total carbon (C), nitrogen (N), hydrogen (H) and sulfur (S) were obtained using an elemental flash pyrolyser analyzer (Flash 2000, Thermo Fischer Scientific). Total organic carbon (TOC) and mineral carbon (MinC) were determined by Rock-Eval pyrolysis (Rock-Eval 6 Turbo, Vinci Technologies). The moisture content of the samples was evaluated according to NF ISO 11465.

Biological analyses

The functional diversity of soil microbial communities was assessed using Biolog™ Ecoplates community-level substrate utilization assay. This method enables study of the metabolic capability of soil microbial communities to utilize a variety of individual carbon sources, as well as of the communities' physiological properties (Insam and Goberna, 2004). Each Biolog™ Ecoplates contained three replicate wells of 31 different carbon sources, including carbohydrates, carboxylic acids, amino acids, amines, polymers, phenolic acids, and a control. Soil samples were suspended in a (1:4 ratio) sterile saline solution (0.85 % w/v NaCl), agitated (60 rpm) for 30 min at 25 °C, sonicated at 45 kHz twice for 20 s, and agitated (60 rpm) again at 25 °C overnight. The soil suspensions were then centrifuged at 3,000 x g for 10 min.

Triplicates of each treatment were pooled taking 200 µL of each triplicate, and resuspended in 17.4 mL of the sterile saline solution (0.85 % w/v NaCl). Each Biolog™ Ecoplates well was filled with 150 µL of the suspension. The Biolog™ Ecoplates were incubated at 25 °C and color development in each well was recorded as optical density (OD) at 590 nm, according to the protocols described by Garland (1997). The absorbance values of the samples were monitored using an Omega SPECTROstar (BMG Labtech) microplate spectrophotometer at t=0 and 168 h (7 days). The well absorbance values were adjusted by subtracting the average absorbance of the control well (water only) from the absorbance measured at t=0. A well was considered as positive when OD590nm in well − OD590nm in the control well > 0.25. Negative readings (OD < 0) were set to zero for all subsequent analyses. The potential metabolic diversity from the Biolog™ Ecoplates was evaluated by functional richness S', expressed as the number of substrates used on each plate (i.e. number of positive well numbers); microbial activity was expressed according to the Garland and Mills method (1991) as Average Well Color Development (AWCD), as follows: AWCD = ΣODi/31, where ODi is the optical density value for each well. The Shannon-Weaver index (H') was calculated as follows: $H' = -\Sigma Pi \times \ln Pi$, where Pi is the ratio of activity for a particular carbon source (ODi=OD590nm in each well-OD590nm in the control well) to the sum of activities on all substrates (ΣODi).

As-transforming microorganisms were enumerated by the Most Probable Number (MPN) method, detailed in Thouin et al. (2016) for As(III)-oxidizing and in Thouin et al. (2018) for As (V)-reducing bacteria, modified as follows: the weight of soil dispersed in 10 mL of sterile saline solution was 2.5 g instead of 0.25 g.

Total soil DNA was extracted in triplicate from 0.5 g of wet weight of each soil sample, using the FastDNA® SPIN Kit for soil (MP Biomedicals) according to the manufacturer's protocol. Extracted dsDNA was quantified by fluorimetry using a Quantus Fluorometer (Promega) with the Promega Quantifluor®, per the manufacturer's recommendation.

Bacterial communities were estimated by real-time quantitative PCR using universal primer sets 341F (5'-CCTACGGGAGGCAGCAG-3') and 515R (5'-ATTACCGCGGCTGCTGGCA-3'). Real-time quantitative PCR was run in a CFX Connect (BioRad) and was performed in 20 µL reaction volumes containing 10 µL of 2× iQSYBR Green SuperMix (Bio-Rad), supplemented with 0.16 µL of each primer (50 ρmol.μL^{-1}), 0.2 µL of T4 bacteriophage gene 32 Product (500 ng.μL^{-1}) (MP Biomedicals), and 2 µL of template DNA (10 times dilution series of plasmid standard of *Pseudomonas putida* (KT2440) and environmental samples DNA). Positive control *Pseudomonas putida* (KT2440) quantified gene copy or water served as positive and negative controls, respectively. The amplifications were carried out with the following temperature profiles: step one heated to 95°C (3 min), followed by 35 cycles (30 s of denaturation at 95 °C, 30 s at the primers specific annealing temperature (60 °C), 30 s of post-elongation at 72 °C and 30 s at 80 °C for plate read). Finally, at the end of amplification, a melting curve analysis was performed by measurement of the SYBR Green signal intensities during a 0.5 °C temperature increment every 10 s from 65 °C to 95 °C.

SEM observations

The structure and elemental composition material samples were analyzed by scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) using a TM 3000 (Hitachi) unit, operating at 15 kV accelerating voltage, coupled to a Swift ED 3000 X-Stream module (Hitachi). The samples were air dried, deposited on a carbon adhesive and directly analyzed. The acquisition time for EDS point analyses was 300 s and between 15 and 30 minutes for maps with a resolution of 512 x 384 pixels. The AZtecEnergy Analyser Software displays and interprets X-ray data to provide accurate and reliable analysis without standard (Burgess et al., 2007).

Plant growth test

Plant growth tests were performed with rye-grass (*Lolium perenne* L.). The content of the three replicates of microcosms sacrificed after 28 days of experiment was pooled and then re-distributed in three 200 mL polystyrene pots. Twelve *Lolium perenne* L. seeds were placed onto the soil surface of each pot and the soils were watered with 20 mL of Mont Roucous mineral water. The pots were incubated in a phytotron of which the parameters were adjusted as follows: 60 % air-water saturation, 16 h of light (white fluorescent light 500–600 µm m^{-2} s^{-1}) at 25 °C alternating with 8 h of darkness at 18 °C). The pots were watered each time surface drying was observed. After 51 days of incubation, the number of surviving plants was counted for each microcosm and plant biomass was evaluated after plant drying (roots and shoots separately).

Statistics

All statistical tests were conducted with R 3.2.3 (R Development Core Team, 2014). Inter-species averages were used to analyze these data. ANOVA and a post-hoc test for homogeneity of variance were performed. Multiple comparisons were performed by a post-hoc Tukey-HSD test at different levels, to compare the five test microcosm conditions between samples for a given duration (7, 28 or 84 days) with 95 % confidence level. Different letters in tables and in figures indicate the significant differences between samples according to the test.

To facilitate interpretation of the large dataset, a multivariate statistical analysis was performed by principal component analysis (PCA) applied to the data matrix formed by 23 variables, namely total metal and metalloid concentrations and major ions in the percolation water, constituents of organic matter, pH, and measured biological parameters of the five amendment conditions tested, at 7, 28 and 84 days.

Results

Percolated water composition

In the non-amended tailings, the pH value was low and stable after 28 days (4.26 ± 0.08; n=13; SM.2). In the amended conditions, pH remained close to neutral over the 84 days of the experiment. pH values in TO, TOM 0.15%, TOM 1% and TOM 2% were respectively 7.49 ± 0.12, 7.67 ± 0.04, 7.46 ± 0.04 and 7.37 ± 0.06 (n=39; SM.2).

The ion composition of tailings percolation water was dominated by sulfate, potassium, nitrate and sodium (SM.3). Microcosms with ochre (TO, TOM 0.15%, TOM 1%, and TOM 2%) were characterized by high concentrations of sulfate, nitrate, sodium, calcium and magnesium, with respective values above 2 $g.L^{-1}$, 40 $mg.L^{-1}$, 400 $mg.L^{-1}$, 450 $mg.L^{-1}$, and 80 $mg.L^{-1}$. Percolation water from the pots containing manure was also characterized by higher chloride, ammonium and potassium concentrations. After 7 days, [Cl^-], [NH_4^+], and [K^+] in TOM 2% reached, respectively, 581.6 ± 38.2 $mg.L^{-1}$, 12.5 ± 1.1 $mg.L^{-1}$, and 894.8 ± 46.5 $mg.L^{-1}$.

Total dissolved concentrations of As, Zn and Ba were determined respectively at 7, 28 and 84 days and every week for Pb for the five tested conditions. Pb concentrations decreased significantly, from 15 $mg.L^{-1}$ in the tailings to < 0.4 $mg.L^{-1}$ in the amended samples (Fig. 1 and Fig. 2). While, for TO and TOM 0.15%, Pb concentration always remained in the 50–100 $\mu g.L^{-1}$ range, in the presence of 1% and 2% manure, Pb concentration increased to 300–40 $\mu g.L^{-1}$ during the first 28 days of the experiment. It then decreased and even dropped below the detection limit with 2% manure (Fig. 2).

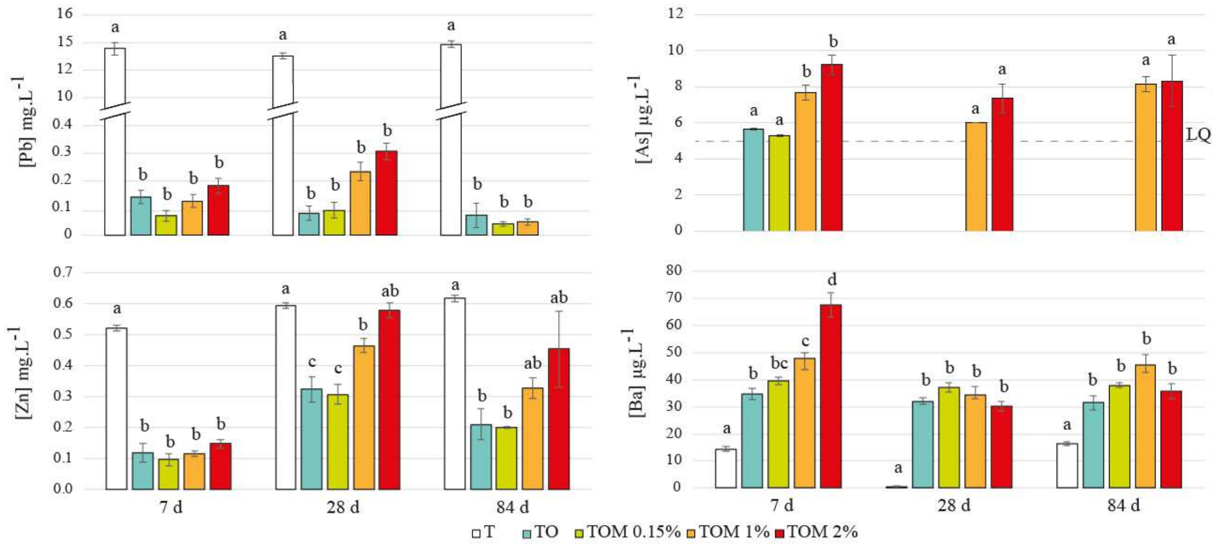


Figure 1: Total dissolved concentrations of Pb, As, Zn and Ba in the percolation water of the five test-condition microcosms after 7, 28 and 84 days of experiment.. Multiple comparisons were performed by a post-hoc Tukey-HSD test at different levels $P < 0.05$ (n=3), to compare the five test microcosm conditions between samples for a given duration (7, 28 or 84 days) with 95% confidence level. Different letters indicate significant differences between samples according to the test.

T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure. Error bars indicate the standard error (n=3).

Zn concentrations decreased significantly in the percolated water from amended tailings after 7 days (Fig. 1). However, later in the experiment, and particularly for the TOM 1% and TOM 2% conditions, Zn concentrations tended to increase. As was not detectable in the percolation water from the non-amended tailings (<0.5 µ.L^{-1}), whereas with 1% and 2% of manure As concentration was in all cases >0.5 µg.L^{-1}. All the amendments induced a significant increase in concentration of Ba in percolation water. Maximum Ba concentration, 67.6 ± 4.39 µg.L^{-1}, was observed in TOM 2% after 7 days, whereas after 28 and 84 days of incubation, no difference was observed between amended conditions.

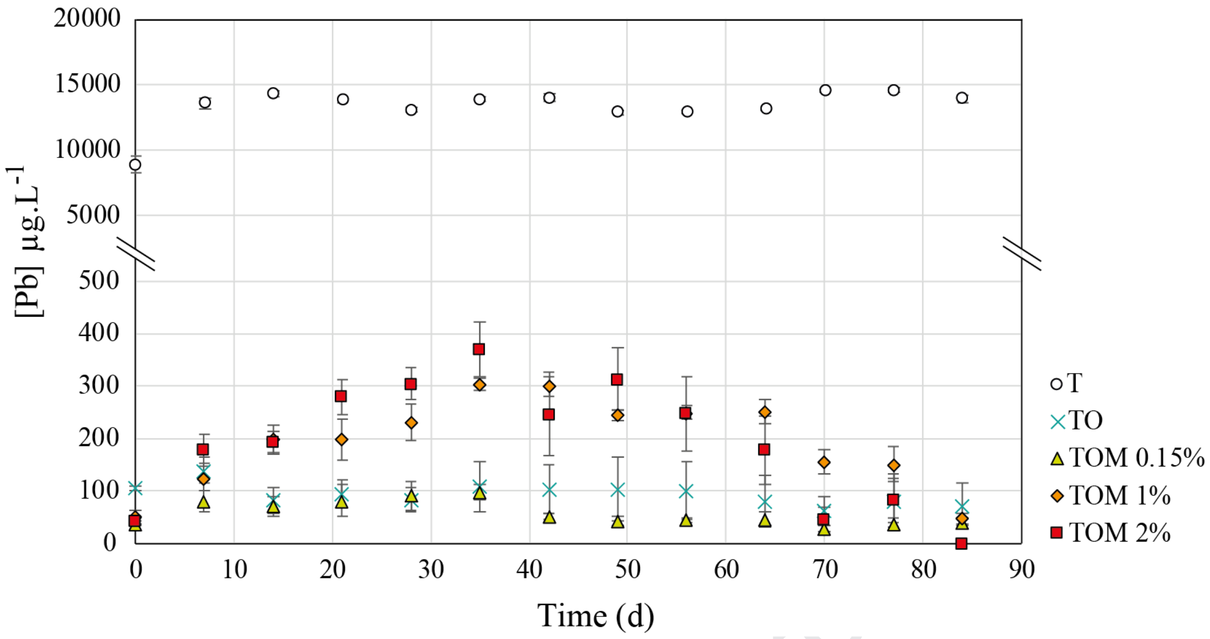


Figure 2: Temporal evolution of Pb total dissolved concentration in the percolation water of the five test-condition microcosms during experiment (in days, d). Error bars indicate the standard error with n=3.

T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

Soil chemical and morpho-chemical characterization

Total C, TOC, MinC, total N, H and S in the soil of the five test microcosm conditions at 7, 28 and 84 days were measured by elemental flash pyrolyser analyzer and Rock-Eval pyrolysis (Table 1). C and N contents were very low in the tailings throughout the experiment. S content did not increase with the addition of ochre or manure. C, H, and N levels were higher in the samples amended with manure, and increased proportionally with the proportion of manure. These parameters did not evolve during the experiment.

Table 1 : Concentrations of the main constituents of organic matter analyzed in the five test-condition microcosms at days 7, 28 and 84 (d) by [a]Flash pyrolyser analyzer and [b] Rock-Eval 6.

TOC: total organic carbon. MinC: mineral carbon. T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

	%	T	TO	TOM 0.15%	TOM 1%	TOM 2%
0 d	**C [a]**	0.05	0.16	0.20	0.53	1.22
	H [a]	0.18	0.17	0.21	0.29	0.34
	N [a]	0.01	0.02	0.01	0.03	0.08
	S [a]	0.56	0.59	0.56	0.76	0.76

	TOC [b]	0.09	0.05	0.07	0.20	0.62
	MinC [b]	0.04	0.13	0.17	0.44	0.44
7d	**C** [a]	0.05	0.20	0.53	0.66	0.69
	H [a]	0.15	0.23	0.29	0.26	0.31
	N [a]	0.62	0.41	0.76	0.48	0.60
	S [a]	0.01	0.01	0.03	0.04	0.04
	TOC [b]	0.07	0.13	0.17	0.37	0.45
	MinC [b]	0.04	0.12	0.13	0.29	0.32
28d	**C** [a]	0.15	0.32	0.46	0.46	0.74
	H [a]	0.22	0.30	0.25	0.25	0.30
	N [a]	0.01	0.02	0.03	0.03	0.05
	S [a]	0.53	0.78	0.56	0.56	0.51
	TOC [b]	0.09	0.16	0.17	0.27	0.44
	MinC [b]	0.04	0.12	0.13	0.20	0.26
84d	**C** [a]	0.03	0.22	0.34	0.60	0.66
	H [a]	0.15	0.22	0.25	0.25	0.25
	N [a]	0.01	0.01	0.02	0.03	0.04
	S [a]	0.54	0.41	0.51	0.43	0.33
	TOC [b]	0.05	0.14	0.18	0.34	0.64
	MinC [b]	0.03	0.12	0.16	0.22	0.21

Ochre and manure were first observed with SEM, to identify their micro-morphological characteristics and chemistry. A selection of samples from the microcosms was studied in detail: tailings +5% iron hydroxide (TO), tailings +5% iron hydroxide +2% manure (TOM 2%) at 7 days (Fig. 3), 28 days and 84 days of incubation. For these samples, the most outstanding result was the high amount of Pb tied to iron hydroxides brought by the ochre, in all TO and TOM conditions.

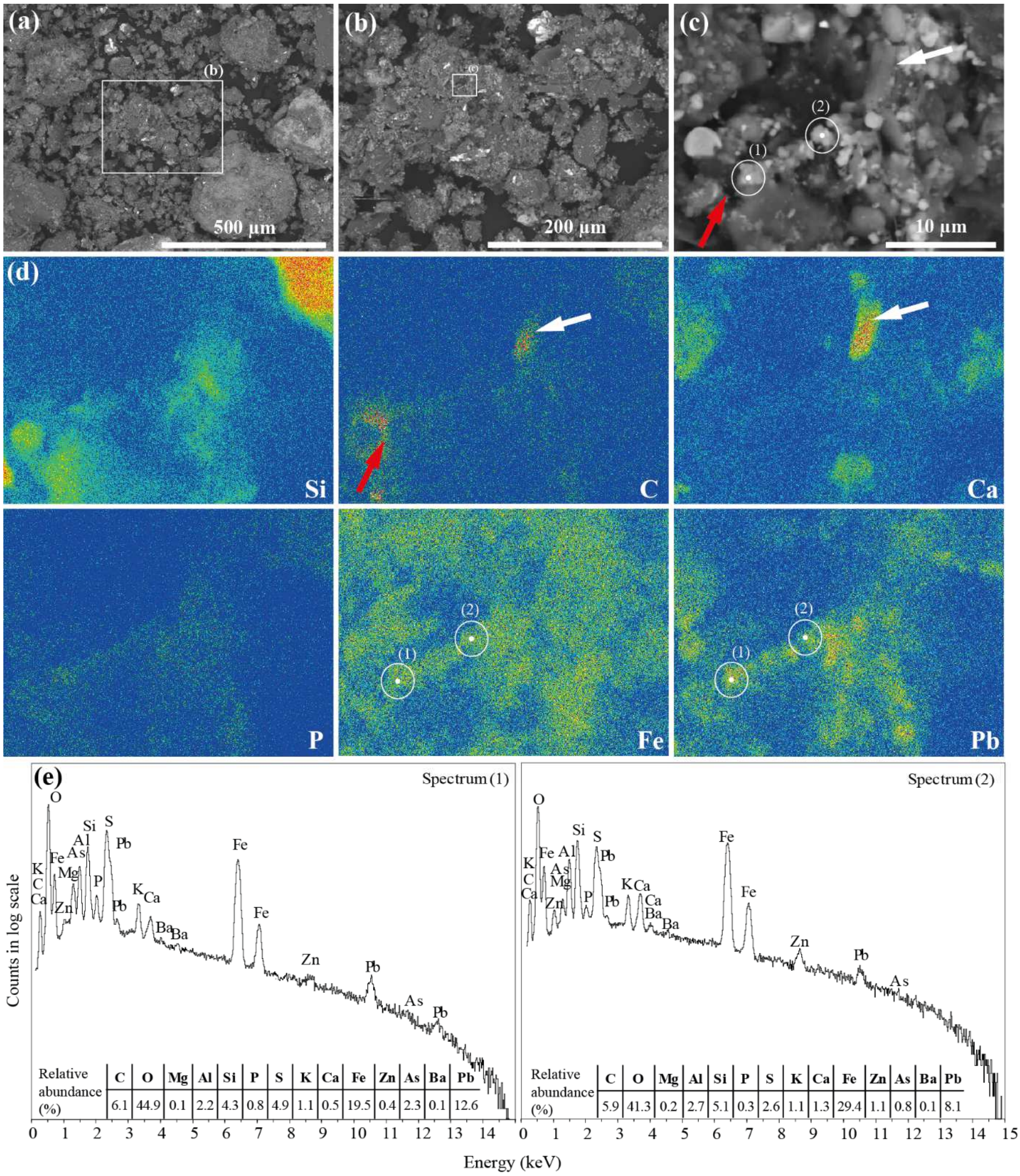


Figure 3: SEM observations from TOM 2% at 7 days (a, b and c). (d) Elemental maps from backscattered electron image (c). Maps show small iron-rich particles, which were Pb carriers (targets (1) and (2)). White arrow show $CaCO_3$ without Pb and red arrow show carbon fragments. (e) EDS point analyses of two Pb-rich particles represented by the targets (1) and (2). (The acquisition time for EDS point analyses was 300 s and 15 for maps).

In the tailings amended with ochre only (TO), the few sludge iron-rich particles larger than 10 µm (less than 15 % of the mud according to the laser granulometer) did not fix Pb efficiently (SM.4), unlike the vast majority of the sludge particles (SM.4), smaller than

10 µm, which contained on average 9 % Pb (Standard Deviation (SD) = 2.96; n = 15) and also 1.5 % of As (SD = 0.75; n = 15).

Locally, concentrations reached 12.6 % for Pb and 2.3 % for As (Fig. 3c and e, spectrum 1). This sludge, rich in Pb and As, also contained S: 4.9 % and 2.6 % in the analyses presented in Fig. 3e, and 3.5 % as an average of 15 point analyses (SD = 1.5; n = 15).

Carbon fragments identifiable with certainty as manure particles were not found in the microcosm amended with manure, even in the microcosm incubated for 7 days only. Particles evoked possible morphologies or textures of manure fragments (arrangements reminiscent of alignments of cells in plant tissues) but contained very low amounts of carbon not allowing direct comparison with intact manure. Nevertheless, these pieces, enriched in phosphorus compared to the other components (soil, ochre), were rich in varied Pb-rich minerals also containing S, P and As. The loosely packed agglutinate shown in Fig. 3 was a commonly found situation, with carbonaceous matter mixed intimately with iron-rich ochre and fine soil particles. Carbon in such agglutinates reached 40 % locally (Fig. 3d, red arrow), but the distribution of Pb clearly matched with Fe.

Scarce Pb-Ca rich particles were present in microcosms amended with manure, even after 7 days of incubation. Ochre contained $CaCO_3$ rod-like particles that remained easily recognizable in the TO and TOM samples but, unlike the Ca-rich particles, they did not contain any trace of Pb, as shown in Fig. 3d (C and Ca maps, white arrows).

Biological characterization

Total microbial biomass, measured by the total double-strand DNA (Table 2), showed that the addition of 2% of manure significantly increased the microbial biomass present in the microcosms after 7 and 28 days of incubation ($p<0.05$). Even if, overall, the data are not significant, the addition of amendment induced an increase in the amount of dsDNA, regardless of incubation time. However, after 84 days of incubation, the TOM 1% and TOM 2% conditions showed a significantly higher microbial biomass ($p<0.05$).

The proportion of bacteria in the microbial community is expressed as 16S rDNA gene copies (Table 2). Bacteria were significantly influenced by addition of 1% of cow manure in samples after 7 days of incubation ($p<0.05$). The variations were not significant in the other conditions, but amendments did tend to increase the amount of bacteria. After 28 days, the amount of bacteria quantified increased with the amount of amendment. After 84 days, all samples contained an equivalent amount of 16S rDNA gene copies, with the T sample having more bacteria than at the beginning of the experiment.

The abundance of As(III)–oxidizing microbes in the solid phases after 84 days (Table 2), tended to decrease with amendment, the decrease being significant for the highest manure concentration (2%). Conversely, the abundance of As(V)-reducing microorganisms tended to increase with the addition of amendments. This increase was not significant for the conditions with manure because of large differences between the samples. However, a significant increase was observed between the T and TO amendment conditions.

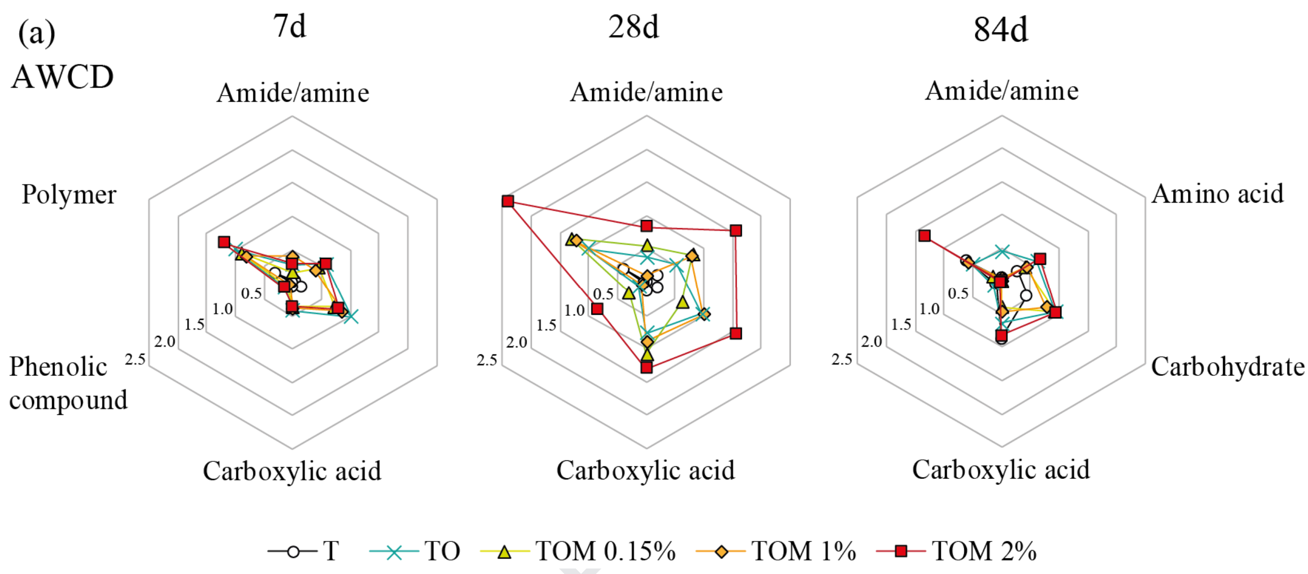
Table 2 : Total soil DNA, bacterial communities quantification, and As(III)-oxidizing and As(V)-reducing bacteria enumerated by the Most Probable Number (MPN) method in the five test-condition microcosms after 7, 28 and 84 days of experiment (d). Analysis of variance was by post-hoc Tukey-HSD test at different levels P <0.05 (n=3). Different letters indicate the differences between samples for a given time.

T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

		T	**TO**	**TOM 0.15%**	**TOM 1%**	**TOM 2%**
7 d	Total dsDNA ng.dw soil^{-1}	298 b	497 b	1282 b	3687 ab	9273 a
	16S rDNA gene copies.g dw soil^{-1}	8.9E+6 b	1.5E+7 ab	1.6E+7 ab	2.3E+7 a	1.0E+7 ab
28 d	Total dsDNA ng.dw soil^{-1}	123 b	558 b	1746 b	4722 b	13,485 a
	16S rDNA gene copies.g dw soil^{-1}	4.2E+6 c	1.0E+7 bc	1.6E+7 b	2.5E+7 a	2.4E+7 a
84 d	Total dsDNA ng.dw soil^{-1}	90 b	304 b	1100 b	5907 a	7396 a
	16S rDNA gene copies.g dw soil^{-1}	1.1E+7 a	2.5E+7 a	7.5E+7 a	1.9E+7 a	2.3E+7 a
	MPN of As(III)-oxidizing microorganisms microorganisms.g^{-1} dw soil	1.7E+5 a	1.3E+5 ab	1.4E+4 ab	1.9E+4 ab	1.5E+4 b
	MPN of As(V)-reducing microorganisms microorganisms.g^{-1} dw soil	1.2E+1 b	5.9E+4 a	1.4E+4 ab	1.1E+4 b	1.1E+4 b

Average well color development (AWCD) and diversity of the microbial communities in the solid phases

The ability of fast growing heterotrophs present in the samples (mainly composed of rapid growing gram-negative bacteria) to utilize different C substrates are presented with the functional richness (S'), the AWCD calculated per substrate and for the whole Biolog™ Ecoplates, and with the Shannon-Weaver diversity index (H') (Fig. 4, SM.5). The AWCD is an expression of the microbial activity in the sample and integrates cell density and diversity in substrate utilization, while H’ expresses the diversity of the microbial communities and S’ the number of degraded compounds. The measurements of S’ provide information about microbial activities without providing any information on the type of substrate used. The community-level physiological profile (CLPP) demonstrates changes in the structure and function of microbial communities with the addition of amendments, and with time.



(b)	7d					28d					84d				
	T	TO	TOM 0.15%	TOM 1%	TOM 2%	T	TO	TOM 0.15%	TOM 1%	TOM 2%	T	TO	TOM 0.15%	TOM 1%	TOM 2%
Average functional richness (S')	4	25	22	23	22	13	25	26	21	29	17	25	14	14	19
Average well color development (AWCD)	0.1	0.6	1.5	0.5	0.6	0.2	0.8	0.8	0.8	1.5	0.5	0.7	0.5	0.5	0.8
Shannon-Weaver diversity index (H')	2.9	3.3	3.3	3.2	3.2	2.7	3.1	3.1	3.1	3.2	2.8	3.2	2.7	2.9	3.0

Figure 4: (a) Radar diagrams of the bacterial average well color development (AWCD) of all classes of carbon substrates, identified by Biolog™ EcoPlates for the five test-condition microcosms after 7, 28 and 84 days of experiment (n=3). (b) Metabolic profiling of samples using Biolog™ EcoPlates based on pattern of utilization based on average functional richness (S'), mean of AWCD, and Shannon-Weaver diversity index (H') of substrates and on functional diversity.

T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

Over time, only a few substrates supported the growth of microbial communities, especially those belonging to the carbohydrates and polymers chemical groups, while amides/amines and phenolic compounds were not (or were only slightly) degraded by the bacteria that catabolize the C-sources of the Biolog™ Ecoplates (Fig. 4, SM.5).

After 7 days of incubation, the relative abundance based on AWCD for each substrate (Fig. 4, SM.5) indicated quantitative differences in the use of 31 carbon sources between the samples without amendment and those amended with iron hydroxide sludge and cow manure. The AWCDs of the tailings amended with iron hydroxide and iron hydroxide-cow manure were greater than those of the tailings without amendments. These samples also showed the highest values for functional richness S' and Shannon diversity index H'. This indicates a greater rate of substrate utilization (catabolic potential) by the microbial community and a greater functional diversity in the amended tailings than in the non-amended tailings. Even though the functional richness value for the TOM 0.15% sample was not the highest, its Shannon-Weiner diversity index and its AWCD value were the highest from amongst the amended samples. After 28 days of incubation, TOM 2% differed from the other samples with greater values for S', AWCD (for each substrate and for the whole Biolog™ Ecoplates) and H' (Fig. 4, SM.5). The S', AWCD, and H' values were greater for the T sample at day 28 than at day 7. After 84 days of incubation, the AWCD (for each substrate and for the whole Biolog™ Ecoplates) had decreased for all amended samples.

The CLPPs indicated by Biolog™ Ecoplates data were affected by amendment and by incubation time. Again, the diversity parameters of T increased (Fig. 4, SM.5). The TO sample appeared to have the greatest microbial community-level substrate utilization diversity with the highest S' and H' values. The TO samples stood out at this time step with communities able to catabolize amides/amines. The AWCD remained highest for the TOM 2% sample.

Phytotoxicity test

Ryegrass survival was improved by amendment but a concentration of 1% and 2% manure tended to reduce plant germination (Fig. 5). The highest survival rate for ryegrass was for the TO and TOM 0.15% amended samples. The addition of ochre and cow manure contributed to the development of roots, since the plants did not develop roots (or did so poorly) when they grew on tailings without amendment. There were significant differences in total plant biomass between plants growing on tailings without amendment and the other samples (statistical data not shown). TO and TOM 0.15% amendments favored higher plant biomasses, while TOM 1% and TOM 2% were least favorable for the development of ryegrass shoots (Fig. 5).

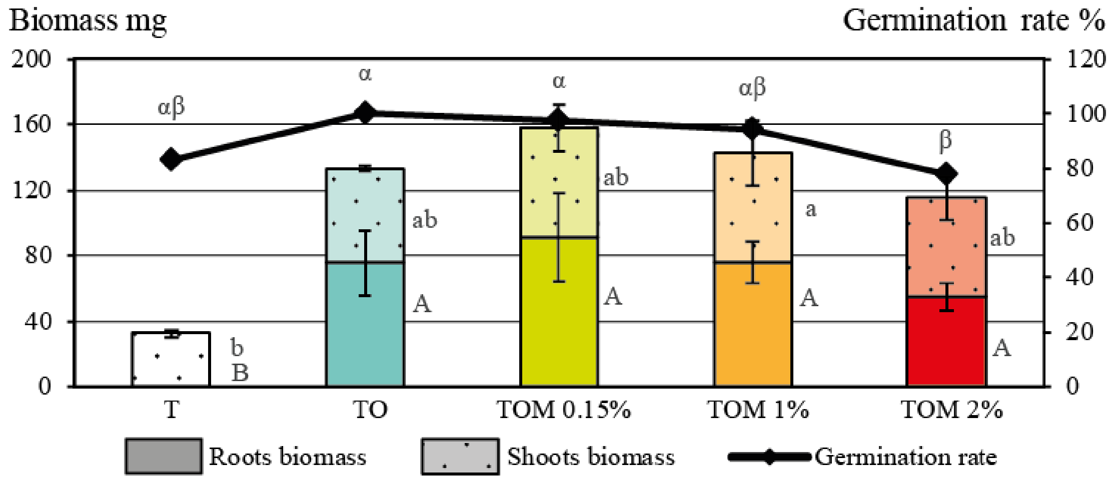


Figure 5: Germination rate (n=12) and average biomass dry weight (DW) of ryegrass (mg) (n=3). Multiple comparisons were performed by a post-hoc Tukey-HSD test at different levels $P < 0.05$ (n=3), to compare the five microcosm conditions with a 95% confidence level. Different letters indicate significant differences between samples for each parameter according to the test (capital letters for roots biomass, minuscule letters for shoots biomass and Greek letters for germination rate).

T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure

Discussion

The tailings sample from the Pontgibaud mine used for the experiment was characterized by relatively high content of metals and metalloids with Pb, As and Ba at respectively 26,432 $mg.kg^{-1}$; 1,134 $mg.kg^{-1}$; and 1,063 $mg.kg^{-1}$. These were higher contents than those found in other tailings from the Pontgibaud mine (Pascaud et al., 2014) but were of the same order of magnitude as those from other former mines in the Massif Central (Bodénan et al., 2004; Courtin-Nomade et al., 2016). Tailings from the Pontgibaud mine, like other mining wastes, were acidic (pH=4) (SM.2) (Pascaud et al., 2014), a condition that contributed to the high mobility of Pb (Fig.6), with concentrations reaching 14,000 $\mu g.L^{-1}$ in percolation water in the microcosms (Fig. 2). Sulfates and Pb were the major chemical species in solution. It seems that these species solubility were controlled by the stability of anglesite and beudantite, the Pb sulfates detected in the initial material.

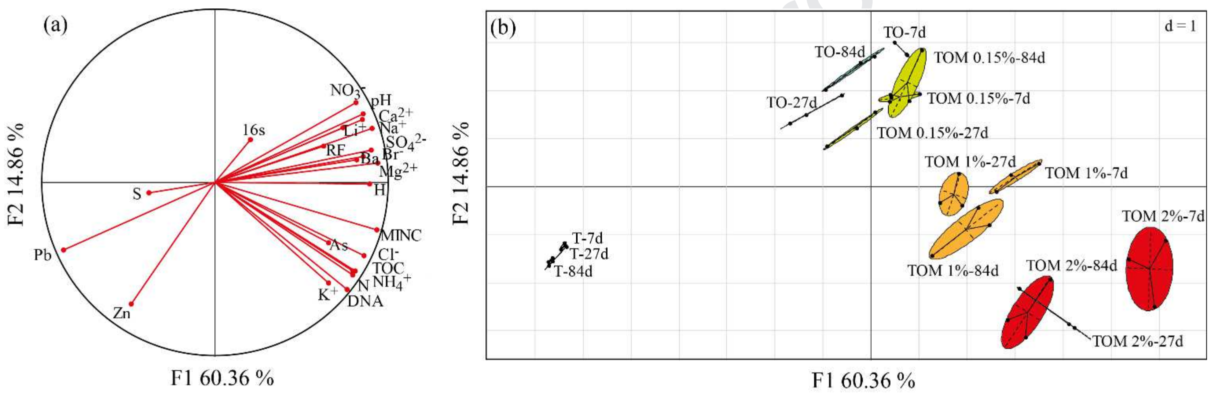


Figure 6: Correlation circle (a) and principal component analysis (PCA) (b) applied to the data matrix formed by 23 variables, namely: total metals and metalloid (Pb, Zn, Ba, and As) concentrations and major ions (Cl^-, Br^-, NO_3^-, SO_4^{2-}, Li^+, Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}) in the percolation water, constituents of organic matter (TOC, MinC), pH, DNA quantification, bacterial abundance (16S), and parameters from Biolog™ EcoPlates (RF,H') of the five test-condition microcosms after 7, 28 and 84 days of experiment.

T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

The tailings had low microbial biomass and both microbial functional richness and diversity were poor. Moreover, the germination of *Lolium perenne L.* was greatly inhibited by this material. The characteristics of the tailings – very low pH, low content of nutrients (organic carbon content between 0.05 and 0.09 %) and severe inorganic contamination – seemed to contribute to their specific biological properties. Londry and Sherriff (2005) have shown that microbial activities are limited by carbon availability in this type of material, and that revegetated areas can provide a source of organic matter. The current geochemical state of the tailings limits the development of both microorganism activity and vegetation cover.

Pb was the most concentrated pollutant in the tailings and in the pore water of this mine site. Immobilization of Pb was the first aim of the amendments. Phosphate amendments are often used to reduce the mobility of Pb by ionic exchange and precipitation of pyromorphite (Brown et al., 2005; Kumpiene et al., 2008). Such amendments were not tested because of the high As content of the tailings, phosphate being able to compete with arsenate for sorption sites (Manning and Goldberg, 1996; Darland and Inskeep, 1997). However, the manure may have introduced some phosphate that contributed to the precipitation of a minor fraction of the mobile As whereas, in our microcosms, Pb immobilization seemed to be mainly linked to the ochre amendment (Fig.6). The addition to soils of iron oxide-hydroxide wastes (ochres) from water treatment facilities is known to induce immobilization of Pb as a result of pH increase (Doi et al., 2005; Nielsen eat al., 2011; Olimah et al. 2015). In the experiment, the ochre induced a rise in pH to neutral values in the amended microcosms. This phenomenon, together with the efficient sorption capacity of goethite (Wu et al., 2003, Rahimi et al., 2015), was beneficial for the stabilization of Pb. SEM observations of Pb associated with iron-rich particles of ochre supported this hypothesis. In order to evaluate the proportion of Pb precipitation in the amended tailings, the geochemical PHREEQCi 3.0.6.7757 code was used with the minteq.v4 thermodynamic database, by integrating the complete chemistry of the percolation water (SM.6). Simulation performed with the data from the amended conditions indicated no precipitation of Pb minerals. However, when Pb concentration was adjusted to that of the non-amended tailings, the simulation predicted the Pb hydroxide and Pb sulfate precipitation. This phenomenon, made possible by the high quantity of sulfate brought with ochre, could also contribute to the immobilization of Pb, this hypothesis being in line with the observation by SEM-EDS of relatively high S concentrations associated with Pb.

One process that may control soluble Ba is the precipitation of barite in the case of high sulfate content (Ippolito and Barbarick, 2006). The PHREEQC simulation sustained the occurrence of this phenomenon with the addition of ochre (SM.6). Also, barite had already been detected in the Pontgibaud mine tailings (Pascaud et al., 2014). A second process was linked to the presence of As, which favored the formation of $BaHAsO_4^-$ and $Ba_3(AsO_4)_2$ (Lu and Zhu, 2011).

Although all amendments induced a strong decrease in Pb concentration in the water phase, the evolution of Pb concentration differed with the composition of amendments. In the presence of 1% and 2% manure, Pb concentration increased during the first 28 days of the experiment. It then decreased and even dropped below the detection limit with 2% manure

(Fig. 2). In parallel, the maximum of metabolic potential for degradation of organic molecules was observed after 28 days of incubation (Fig. 4, SM.5). These results suggest a relationship between the dynamics of mobile Pb and the evolution of the organic amendment. Previous studies have shown that the solubility of Pb is at a minimum at pH 6, and that at higher pH values soil organic matter is more soluble, inducing the release of organo-Pb complexes (Wang and Benoit, 1996; Sauvé et al., 1998). This type of soluble complex may have been produced in our manure-amended microcosms, as they both had pH higher than 6 (between 7 and 8) and had the highest organic matter concentration for this experiment. In addition to these dissolved species, the formation of Pb-bearing colloids is expected in this pH range: Klitzke et al. (2008) observed an increase of both colloidal and dissolved Pb when they raised the pH of a highly polluted forest soil that was rich in organic matter. The effect of pH on the mobility of colloids is linked to the change of soil surface charge, decreasing inter-particle attraction. Colloidal soil minerals are mobilized by changes in charges and stabilized by organic coating. During the microcosm experiment, the more mobile fraction of organic matter from manure may have been released during the first 28 days of experiment, inducing the mobilization of both Pb-organic molecule complexes and organic-coated Pb colloids. Bacterial activity may have contributed to these processes through the biodegradation of insoluble molecules with high molecular weights into more soluble small metabolites. The assumed kinetics of manure biodegradation in our experiment fitted well with those observed in previous laboratory studies: Bernal and Kirchmann (1992) observed a maximum rate of mineralization of pig manure in soil during the first month of incubation, and Calderon et al. (2005) showed that the maximum degradation rate of cow manures occurred during the first 8 weeks of contact with soil.

Immobilization of As by ochre in soils polluted with As has already been observed (Doi et al., 2005; Nielsen eat al., 2011; Olimah et al. 2015). However, the experiment described here was able to show that ochre can simultaneously decrease Pb and As leachability. As was not greatly mobilized during the experiment, its concentration in the percolated water always remaining lower than the standard for drinking water (10 $\mu g.L^{-1}$). However, in every case, As concentration was higher in the manure-amended conditions than in T and TO conditions (Fig. 1). In parallel, the range of concentration of As(III)-oxidizing microorganisms tended to be lower in the manure-amended microcosms. Previous studies have shown that organic matter can negatively influence microbial As(III)-oxidizing activities (Challan-Belval et al., 2009; Bachate et al., 2012; Lescure et al. 2016). The abundance of As(V)-reducing organisms was highest in TO and TOM 0.15% manure, and of the same (lower) range in the other conditions. The increase in pH appeared to stimulate growth of microorganisms able to reduce As(V). Taking into account that As(V) adsorption on iron oxides is optimum at pHs lower than 6, whereas As(III) adsorption is favored at pHs above 7, the evolution of As-transforming communities with amendments was quite positive in terms of As adsorption on solid phases. Globally, the total number of As-transforming microbes was higher in the conditions without manure, i.e. when As was found in lower concentration in the percolating water, suggesting that they were involved in biogeochemical processes favoring As stability.

Quantification of microorganisms by measuring the total DNA and 16S rDNA gene copies number showed that tailings without cow manure and iron hydroxide sludge had lower microbial biomass than tailings with amendment whatever the time step. Similarly, microbial diversity, evaluated by average functional richness (S'), AWCD and Shannon-Weaver diversity index (H'), was lower for tailings without amendment. These results are not surprising since, typically, tailings are known to have low diversity of microbial species and functions and low microbial biomass and activity. Thus, Chen et al. (2005) considered that microbial metabolic activity in tailings is lower than in soils. Tailings can therefore be considered as an extreme and geochemically dynamic environment exerting a strong selection pressure on microbial communities that are suited to stressful conditions and considered as extremophiles, since the microorganisms rely on chemical energy from minerals in tailings due to the lack of organic matter (Schimel et al., 2007). Therefore, it is not surprising that the microbial communities in this experiment strongly metabolized complex and more recalcitrant C sources such as Tween 80, Tween 40, α-cyclodextrin, and glycogen.

Organic matter is a crucial component of the soil sorption complex that immobilizes toxic metals and metalloids and increases the soil's capacity to store and supply nutrients. Increasing organic matter content of tailings by supplying cow manure may contribute to increasing biomass and microbial activity. In this experiment, after 7 days of incubation, the cow manure and the ochre input provided the tailings with more biomass and diverse microbial communities. Microorganisms were thus shown to respond rapidly to environmental changes. The ability of the microbial communities to use substrates seems to have been increased by the ochre supply alone, since there was little or no difference between the TO and TOM samples. Amendments may induce shifts in the soil microbial communities (Pérez de Mora et al., 2006). In a long-term fertilization study of a soil completely eroded and with a very low organic matter content, Zhong et al. (2010) showed that amendment with manure led to shifts in C utilization patterns and increased soil microbial functional diversity. The differences in AWCD for each substrate observed on day 7 between microcosms with or without amendment may be partially due to nutrients (i.e. major ions) brought by the amendments and consumed by microorganisms. However, according to Bolan et al. (2017), microbial diversity increases with increase in pH in the tailings, as neutrophilic microorganisms are more diverse than acidophilic ones. The application of cow manure and ochre increased pH and major ion content while improving the carbon and hydrogen status of the mine tailings, thus increasing the biomass of microorganisms and their activities. It has already been shown that the C/N ratio of soil also has an impact on microbial community dynamics (Wang et al., 2015).

The greater richness and diversity of microbial communities on day 28 for the TOM 2% sample may be due to the higher percentage of amendment of this sample. Thus, 2% amendment led to a greater supply of major ions and possibly of viable microorganisms. The reduction in the activity of microorganisms over time in the other amended microcosms may be due to the decrease of nutrients available for the microflora. This is in agreement with a study on the composting of cow manure, the organic matter contained in the manure being gradually decomposed by microorganisms (Wang et al., 2018). The tailings without amendments remained at the lowest community-level substrate utilization level.

Amendments should contribute to decreasing the availability of metals and metalloids and should enhance microbial biomass and diversity, but they must also allow revegetation of the tailings. This is why a test of *Lolium perenne L.* (perennial ryegrass) growth was conducted to evaluate the effect of cow manure and ochre sludge on the phytotoxicity of the tailings. Ryegrass is one of the highest quality forage grasses and its primary use is for pasturing cattle and sheep. This herbaceous plant is also routinely used to investigate plant growth responses (germination, root and shoot growth) to changing environmental conditions (Gregory et al., 2014). While ryegrass roots had serious difficulties in growing in the tailings without amendments – probably because of acidity, toxicity of metals and metalloids and/or nutrient deficiency – amendments appeared to facilitate plant growth. However, ryegrass seemed to prefer the test condition with 0.15% of cow manure. This result highlights that an excess of nutrients might not be beneficial, and that organic amendment dose should be optimized for the specific plant species selected for the remediation strategy.

Conclusion

The results of the microcosm experiments indicate the efficiency of combining ochre and cow manure amendments to greatly decrease Pb solubility in acidic mine tailings while avoiding any dramatic mobilization of other elements, namely As, Zn and Ba. Stabilization of As-rich mine tailings may be an interesting way to make use of the sludge produced during the treatment of the iron-containing coal mine waters. Short-term monitoring of the amended tailings revealed a transient increase in both Pb leaching and functional microbial diversity, both phenomena linked to the maturation of manure. However, 84 days after the amendment event, Pb immobilization was strengthened by 2% manure, whereas the benefits of this amendment on functional microbial biodiversity were greatly attenuated. This latter result shows that amendments alone are not sufficient to induce an enduring improvement in the soil microbial biodiversity necessary for the development of its functions. However, the amendments were shown to stimulate the growth of ryegrass, and the colonization of tailings by plants should contribute to improving their structure and functions. Further experiments could throw light on the long-term biogeochemical evolution of amendments in the context of plant cover development. In addition, it will be necessary to take account of on-site management practices since, as the amendments would be applied only at the surface of the tailings dumps, there is a strong need to investigate the evolution of the underlying material. With future upscaling of the process in mind, this study provides key information about the biogeochemical processes that drive the dynamics of inorganic pollutants in acidic mine tailings amended with iron-rich ochre and organic matter.

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References

Bachate, S. P., Khapare, R. M., Kodam, K. M., 2012. Oxidation of arsenite by two β-proteobacteria isolated from soils. Appl. Microbiol. Biotechnol. 93, 2135–2145. https://doi.org/10.1007/s00253-011-3606-7

Basta, N. T., Sloan, J. J., 1999. Bioavailablility of Heavy Metals in Strongly Acidic Soils Treated with Exceptional Quality Biosolids. J. Env. Qual. Abstract. 28, 633-638. https://doi.org/10.2134/jeq1999.00472425002800020029x

Bernal, M. P., Kirchmann, H., 1992. Carbon and nitrogen mineralization and ammonia volatilization from fresh, aerobically and anaerobically treated pig manure during incubation with soil. Biol. Fertil. Soils. 13, 135-141. https://doi.org/10.1007/BF00336268

Bodénan, F., Baranger, P., Piantone, P., Lassin, A., Azaroual, M., Gaucher, E., Braibant, G., 2004. Arsenic behaviour in gold-ore mill tailings, Massif Central, France: hydrogeochemical study and investigation of in situ redox signatures. Appl. Geochem. 19, 1785–1800. https://doi.org/10.1016/j.apgeochem.2004.03.012

Bolan, N. S., Kirkham, M. B., Ok, Y. S., 2017. Spoil to soil: mine site rehabilitation and revegetation. CRC Press, Boca Raton, USA. https://doi.org/10.1201/9781351247337

Brown, S., Chaney, R. L., Hallfrisch, J. G., Xue, Q., 2003. Effect of biosolids processing on lead bioavailability in an urban soil. J. Environ. Qual., 32, 100-108. https://doi.org/10.2134/jeq2003.1000

Brown, S., Christensen, B., Lombi, E., McLaughlin, M., McGrath, S., Colpaert, J., Vangronsveld, J., 2005. An inter-laboratory study to test the ability of amendments to reduce the availability of Cd, Pb, and Zn in situ. Environ. Pollut. 138, 34–45. https://doi.org/10.1016/j.envpol.2005.02.020

Burgess, S. R., Statham, P.J., Holland, J., Chou, Y., 2007. Standardless quantitative analysis using a drift detector: what accuracy is possible from live and reconstructed data? Microscopy and Microanalysis.13, 1432-1433. DOI: https://doi.org/10.1017/S1431927607072637

Calderon, F. J., McCarty, G. W., Reeves, J. B. III, 2005. Analysis of manure and soil nitrogen mineralization during incubation. Biol. Fertil. Soil. 41, 328–336. https://doi.org/10.1007/s00374-005-0843-x

Challan-Belval, S., Garnier, F., Michel, C., Chautard, S., Breeze, D., Garrido, F., 2009. Enhancing pozzolana colonization by As(III)-oxidizing bacteria for bioremediation purposes. Appl. Microbiol. Biotechnol. 84, 565–573. https://doi.org/10.1007/s00253-009-2077-6

Chen, C. L., Liao, M., Huang, C. Y., 2005. Effect of combined pollution by heavy metals on soil enzymatic activities in areas polluted by tailings from Pb-Zn-Ag mine. J. Environ. Sci. 17(4), 637–640. https://doi.org/1001-0742(2005)04-0637-04

Cottard, F., 2010. Résultat des caractérisations complémentaires effectuées sur différents milieux dans le district minier de Pontgibaud (63), BRGM/RP-58571-FR, 78 p., 12 fig., 8 tabl., 4 ann.

Courtin-Nomade, A., Waltzing, T., Evrard, C., Soubrand, M., Lenain, J.-F., Ducloux, E., Ghorbel, S., Grosbois, C., Bril, H., 2016. Arsenic and lead mobility: From tailing materials to the aqueous compartment. Appl. Geochem. 64, 10–21. https://doi.org/10.1016/j.apgeochem.2015.11.002

Darland, J. E., Inskeep, W. P., 1997. Effects of pH and phosphate competition on the transport of arsenate. J. Env. Qual. 26, 1133-1139 https://doi:10.2134/jeq1997.00472425002600040027x

Derome, J., 2000. Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilization. Environ. Pollut. 107, 79–88. https://doi.org/10.1016/S0269-7491(98)00183-3

Dixit, S., Hering, J. G., 2003. Comparison of Arsenic(V) and Arsenic(III) sorption onto iron oxide minerals: implications for arsenic mobility, Environ. Sci. Technol. 37, 4182–4189. https://doi.org/10.1021/es030309t

Doi, M., Warren, G., Hodson, M. E., 2005. A preliminary investigation into the use of ochre as a remedial amendment in arsenic-contaminated soils. Appl. Geochem. 20, 2207–2216. https://doi.org/10.1016/j.apgeochem.2005.08.006

Douglas, I., Lawson, N., 2000. In: A Handbook of Industrial Ecology, ed. RU Ayers, LW Ayers, Cheltenham, UK/Northampton, MA: Elgar, 351–364.

Fuge, R., Pearce, F. M., Pearce, N. J. G., Perkins, W. T., 1993. Geochemistry of Cd in the secondary environment near abandoned metalliferous mines, Wales. Appl. Geochem. 8, 29–35. https://doi.org/10.1016/S0883-2927(09)80006-1

Garland, J. L., Mills, A. L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351–2359. https://doi.org/0099-2240/91/082351-09$02.00/0

Garland, J. L., 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol. 24, 289–300. https://doi.org/10.1111/j.1574-6941.1997.tb00446.x

Gregory, S. J., Anderson, C. W. N., Arbestain, M. C., McManus, M. T., 2014. Response of plant and soil microbes to biochar amendment of an arsenic-contaminated soil. Agric. Ecosyst. Environ. 191, 133–141. https://doi.org/10.1016/j.agee.2014.03.035

Guo, G. L., Zhou, Q. X., Ma, L. Q., 2006. Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: a review. Environ. Monit. Assess. 116, 513–528. https://doi.org/10.1007/s10661-006-7668-4

Hattab, N., Motelica-Heino, M., Faure, O., Bouchardon, J. L., 2015. Effect of fresh and mature organic amendments on the phytoremediation of technosols contaminated with high concentrations of trace elements. J. Environ. Manage. 159, 37–47. https://doi.org/10.1016/j.jenvman.2015.05.012

Hooda, P. S., Alloway, B. J., 1996. The effect of liming on heavy metal concentrations in wheat, carrots and spinach grown on previously sludge-applied soils. J. Agric. Sci. 127, 289–294. https://doi.org/10.1017/S0021859600078448

Ippolito, J. A., Barbarick, K. A., 2006. Biosolids affect soil barium in a dryland wheat agroecosystem. J. Environ. Qual. 35, 2333–2341. https://doi.org/10.2134/jeq2006.0076

Insam, H., Goberna, M., 2004. Use of Biolog for the community level physiological profiling (CLPP) of environmental samples. A.D.L. Akkermans, J.D. van Elsas, F.J. DeBrujin, I.M. Head, G.A. Kowalchuck (Eds.), Molecular Microbial Ecology Manual. Detection, Identification and Classification of Microbes Using Other Methods, Kluwer Academic Publishers, pp. 853-860.

ISO 10390 (2005) Soil quality - Determination of pH.

ISO/TS 21268-2 (2007) Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 2: Batch test using a liquid to solid ratio of 10 l/kg dry matter.

Klitzke, S., Lang, F., Kaupenjohann, M., 2008. Increasing pH releases colloidal lead in a highly contaminated forest soil. Eur. J. Soil Sci. 59, 265–273. https://doi.org/10.1111/j.1365-2389.2007.00997.x

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cu, Cr, Pb and Zn in soils using amendments – a review. Waste Manage. 28, 2015–225. https://doi.org/10.1016/j.wasman.2006.12.012

Lahori, A. H., Guo, Z., Zhang, Z., Li, R., Mahar, A., Awasthi, M. K.; Shen, F., Sial, T. A., Kumbhar, F., Wang, P., Jiang, S., 2017. Use of biochar as an amendment for remediation of heavy metal-contaminated soils: prospects and challenges. Pedosphere. 27, 991–1014. https://doi.org/10.1016/S1002-0160(17)60490-9

Le Forestier, L., Motelica-Heino, M., Le Coustumer, P., Mench, M., 2017. Phytostabilisation of a copper contaminated topsoil aided by basic slags: assessment of Cu mobility and phytoavailability. J. Soils Sediments. 17, 1262–1271. https://doi.org/10.1007/s11368-015-1299-8

Lebrun, M., Macri, C., Miard, F., Hattab-Hambli, N., Motelica-Heino, M., Morabito, D., Bourgerie, S., 2016. Effect of biochar amendments on As and Pb mobility and phytoavailability in contaminated mine technosols phytoremediated by *Salix*. J. Geochem. Explor. 182, 149–156. http://dx.doi.org/10.1016/j.gexplo.2016.11.016

Lescure, T., Moreau, J., Charles, C., Ben Ali Saanda, T., Thouin, H., Pillas, N., Bauda, P., Lamy, I., Battaglia-Brunet, F., 2016. Influence of organic matters on AsIII oxidation by the microflora of polluted soils. Environ. Geochem. Health. 38, 911–925. https://doi.org/10.1007/s10653-015-9771-3

Li, Y.-M., Chaney, R. L., Siebielec, G., Kerschner, B. A., 2000. Response of Four Turfgrass Cultivars to Limestone and Biosolids-Compost Amendment of a Zinc and Cadmium Contaminated Soil at Palmerton, Pennsylvania. J. Env. Qual. Abstract. 29, 1440-1447. https://doi.org/10.2134/jeq2000.00472425002900050010x

Londry, K. L., Sherriff, B. L., 2005. Comparison of Microbial Biomass, Biodiversity, and Biogeochemistry in Three Contrasting Gold Mine Tailings Deposits. Geomicrobiol. J. 22, 237–247. https://doi.org/10.2134/jeq2000.00472425002900050010x

Lu, P., Zhu, C., 2011. Arsenic Eh–pH diagrams at 25 °C and 1 bar. Environ. Earth. Sci. 62, 1673–1683. https://doi.org/10.1007/s12665-010-0652-x

Manning, B. A., Goldberg, S., 1996. Modeling Competitive Adsorption of Arsenate with Phosphate and Molybdate on Oxide Minerals. Soil Sci. Soc. Am. J. 60, 121. https://doi.org/10.2136/sssaj1996.03615995006000010020x

Martin, T. A., Ruby, M. V., 2003. In situ remediation of arsenic in contaminated soils. Remediation. Winter 2003, 21–32. https://doi.org/10.1002/rem.10092

Mc Gowen, S. L., Basta, N. T., Brown, G. O., 2001. Use of Diammonium Phosphate to Reduce Heavy Metal Solubility and Transport in Smelter-Contaminated Soil. J. Env. Qual. Abstract. 30, 493-500. https://doi.org/10.2134/jeq2001.302493x

NF X31-147 (1996) Qualité des sols - Sols, sédiments - Mise en solution totale par attaque acide.

Nielsen, S. S., Petersen, L. R., Kjeldsen, P., Jakobsen, R., 2011. Amendment of arsenic and chromium polluted soil from wood preservation by iron residues from water treatment. Chemosphere. 84, 383-389. https://doi.org/10.1016/j.chemosphere.2011.03.069

Norini, M. P., Thouin, H., Miard, F., Battaglia-Brunet, F., Gautret, P., Guégan, R., Le Forestier, L., Morabito, D., Bourgerie, S., Motelica-Heino, M., 2019. Mobility of Pb, Zn, Ba, As and Cd toward soil pore water and plants (willow and ryegrass) from a mine soil amended with biochar. J. Env. Manage. 232, 117-130. https://doi.org/10.1016/j.jenvman.2018.11.021

Olimah, J. A., Shaw, L. J., Hodson, M. E., 2015. Does ochre have the potential to be a remedial treatment for As-contaminated soils? Environ. Pollut. 206, 150–158. https://doi.org/10.1016/j.envpol.2015.06.011

Oustrière, N., Marchand, L., Galland, W., Gabbon, L., Lottier, N., Motelica, M., Mench, M., 2016. Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Sci. Total Environ. 566–567, 816–825. https://doi.org/10.1016/j.scitotenv.2016.05.091

Pascaud, G., Leveque, T., Soubrand, M., Boussen, S., Joussein, E., Dumat, C., 2014. Environmental and health risk assessment of Pb, Zn, As and Sb in soccer field soils and sediments from mine tailings: solid speciation and bioaccessibility. Environ. Sci. Pollut. R. 21, 4254–4264. https://doi.org/10.1007/s11356-013-2297-2

Paulson, J. A., 1997. The transport and fate of Fe, Mn, Cu, Zn, Cd, Pb and SO4 in a groundwater plume and in downstream surface waters in the Coeur d'Alene Mining District, Idaho, U.S.A. Appl. Geochem. 12, 447–464. https://doi.org/10.1016/S0883-2927(97)00013-9

Pérez de Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P., Schloter, M., 2006. Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. Soil Biol. Biochem. 38, 327–341. https://doi.org/10.1016/j.soilbio.2005.05.010

Rahimi, S., Moattari, R.M., Rajabi, L., Derakhshan, A.A., Keyhani, M., 2015. Iron oxide/hydroxide (α,γ-FeOOH) nanoparticles as high potential adsorbents for lead removal from polluted aquatic media. J. Ind. Eng. Chem. 23, 33–43. https://doi.org/10.1016/j.jiec.2014.07.039

Sauvé, S., McBride, M., Hendershot, W., 1998. Soil Solution Speciation of Lead(II): Effects of Organic Matter and pH. Soil Sci. Soc. Am. J. 62, 618–621. https://doi.org/10.2136/sssaj1998.03615995006200030010x

Schimel, J., Balser, T. C., Wallenstein, M., 2007. Microbial stress□response physiology and its implications for ecosystem function. Ecology. 88, 1386–1394. https://doi.org/10.1890/06-0219|

Thouin, H., Le Forestier, L., Gautret, P., Hube, D., Laperche, V., Dupraz, S., Battaglia-Brunet, F., 2016. Characterization and mobility of arsenic and heavy metals in soils polluted by the destruction of arsenic-containing shells from the Great War. Sci. Total Environ. 550, 658–669. https://doi.org/10.1016/j.scitotenv.2016.01.111

Thouin, H., Battaglia-Brunet, F., Norini, M. P., Le Forestier, L., Charron, M., Dupraz, S., Gautret, P., 2018. Influence of environmental changes on the biogeochemistry of arsenic in a soil polluted by the destruction of chemical weapons: a mesocosm study. Sci. Total Environ. 627, 216-226. https://doi.org/10.1016/j.scitotenv.2018.01.158

Wang, E. X., Benoit, G., 1996. Mechanisms Controlling the Mobility of Lead in the Spodosols of a Northern Hardwood Forest Ecosystem. Environ. Sci. Technol. 30, 2211–2219. https://doi.org/10.1021/es950590e

Wang, X., Cui, H., Shi, J., Zhao, X., Zhao, Y., Wei, Z., 2015. Relationship between bacterial diversity and environmental parameters during composting of different raw materials. Bioresour. Technol. 198, 395–402. https://doi.org/10.1016/j.biortech.2015.09.041

Wang, K., Yin, X., Mao, H., Chu, C., Tian, Y., 2018. Changes in structure and function of fungal community in cow manure composting. Bioresour. Technol. 255, 123–130. https://doi.org/10.1016/j.biortech.2018.01.064

Wu, Z., Gu, Z., Wang, X., Evans, L., Guo, H., 2003. Effects of organic acids on adsorption of lead onto montmorillonite, goethite and humic acid. Environ. Pollut. 121, 469–475. https://doi.org/10.1016/S0269-7491(02)00272-5

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., Zhang, G., 2011. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ. Pollut. 159, 84–91. https://doi.org/10.1016/j.envpol.2010.09.019

Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., Shen, W., 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil. 326, 511–522. https://doi.org/10.1007/s11104-009-9988-y

- Ochre from coal mine water treatment increases pH of acid tailings
- Pb is stabilized and As is not mobilized in tailings by ochre and manure amendment
- Manure induces mobilization of Pb for 35 days then its stabilization
- Microbial functional biodiversity is transiently increased by amendment
- Ochre favors short-term growth of ray-grass on tailings