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Influence of agricultural amendments on arsenic biogeochemistry and phytotoxicity in a soil polluted by the destruction of arsenic-containing shells

Fabienne Battaglia-Brunet [a,*], Marina Le Guédard [b,c], Olivier Faure [d], Mickael Charron [a], Daniel Hube [a], Nicolas Devau [a], Catherine Joulian [a], Hugues Thouin [a], Jennifer Hellal [a]

[a] French Geological Survey (BRGM), 3 Avenue Claude Guillemin, 45060 Orléans Cedex 02, France
[b] LEB Aquitaine Transfert – ADERA, 71 Avenue Edouard Bourlaux, CS20032, 33140 Villenave d'Ornon, France
[c] University of Bordeaux, CNRS, Laboratoire de Biogenèse Membranaire (LBM), UMR 5200, 33140 Villenave d'Ornon, France
[d] Mines Saint-Etienne, Univ Lyon, Univ Jean Moulin, Univ Lumière, Univ Jean Monnet, ENTPE, INSA Lyon, ENS Lyon, CNRS, UMR 5600 EVS, Centre SPIN, Departement PEG, F-42023 Saint-Etienne, France

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ABSTRACT

Agricultural soils can contain high arsenic (As) concentrations due to specific geological contexts or pollution. Fertilizer amendments could influence As speciation and mobility thus increasing its transfer to crops and its toxicity. In the present study, field-relevant amounts of fertilizers were applied to soils from a cultivated field that was a former ammunition-burning site. Potassium phosphate (KP), ammonium sulfate and organic matter (OM) were applied to these soils in laboratory experiments to assess their impact on As leaching, bioavailability to *Lactuca sativa* and microbial parameters. None of the fertilizers markedly influenced As speciation and mobility, although trends showed an increase of mobility with KP and a decrease of mobility with ammonium sulfate. Moreover, KP induced a small increase of As in *Lactuca sativa*, and the polluted soil amended with ammonium sulfate was significantly less phytotoxic than the un-amended soil. Most probable numbers of AsIII-oxidizing microbes and AsIII-oxidizing activity were strongly linked to As levels in water and soils. Ammonium sulfate negatively affected AsIII-oxidizing activity in the un-polluted soil. Whereas no significant effect on As speciation in water could be detected, amendments may have an impact in the long term.

1. Introduction

High concentrations of the toxic element arsenic (As) in soils generally originate from mining and industrial activities, long-term applications of As-containing pesticides or the geochemical background. Among industrial activities, storage or destruction of As-bearing molecules used in chemical weapons during the wars has locally resulted in high As concentrations in soils (Bausinger and Preuβ, 2005; Bausinger et al., 2007; Thouin et al., 2016; Hube, 2017).

When soils affected by As pollution are submitted to agricultural practices, arsenic speciation, bio-availability for plants and mobility towards the water phase may be changed. Major phenomena influencing As mobility (Smith et al., 1998) include: (1) pH which influences AsIII and AsV oxy-anions charge, (2) redox conditions, which influence As speciation and the stability of iron oxides that are essential As-bearing phases, and (3) competing substances, that may favour As desorption from solid phases. In particular, phosphate, a structural analogue of AsV can compete with As for sorption on iron oxides (Smith and Naidu, 2009).

Thus, in agricultural soils, fertilizing practices involving phosphate amendments may affect As speciation and mobility. Brackhage et al. (2014) observed an increase of As mobility and uptake by wheat associated to P-fertilization in soil flooding conditions. Conversely, N-fertilization seemed to attenuate As mobility and plant uptake (Brackhage et al., 2014, Van Oort et al., 2017). In addition, agricultural soils are often fertilized with organic matter (OM). Many studies have described geochemical interactions between As and organic matter: modification of As speciation (Redman et al., 2002), formation of soluble complexes (Saada et al., 2003; Redman et al., 2002), competition for sorption sites (Bauer and Blodau, 2006), and influence of OM on

* Corresponding author.
E-mail address: f.battaglia@brgm.fr (F. Battaglia-Brunet).

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microbial AsIII-oxidizing activity (Lescure et al., 2016).

Finally, all types of amendments may impact the structure of soil microbial communities which exert a major influence on As speciation (Yamamura et al., 2009). Bacteria isolated from soils have been shown to oxidize AsIII and/or reduce AsV (Macur et al., 2004; Inskeep et al., 2007; Bachate et al., 2012), or to methylate this toxic metalloid (Huang et al., 2012). Filamentous fungi isolated from contaminated soils are able to reduce AsV and methylate As (Su et al., 2011). Microbial transformations of As in soil have important implications because mobility, toxicity and bioavailability of this metalloid are closely related to its speciation (Smedley and Kinniburgh, 2002). The global AsIII-oxidizing activity of the microflora should tend to reduce the risk of As transfer from soil to surface water or groundwater. This global activity is the result of AsIII-oxidation and simultaneous AsV-reduction, that can occur in aerobic conditions through the activity of As resistance genes. All the modifications due to the amendments can lead to changes in the bioavailability and the toxicity of As for crops.

This study aimed to measure the impact of fertilizing practices on As mobility, speciation and transfer to crops in soils from a former chemical-ammunition-destruction facility dating from the interwar period (1918–1939) and subsequently converted into agricultural land near Verdun, France (Hube, 2017). It is one of the most important historical areas of chemical ammunition destruction of WW I, containing arsenical chemical warfare agents, located in a sensitive zone for agriculture and groundwater. Fertilizers frequently applied for common crops such as barley, corn and wheat (potassium phosphate (KP) fertilizer, ammonium sulfate and OM) were applied to soils in a laboratory-scale experiment to assess their impact on the speciation, mobility and phytotoxicity of As as well as on As transforming microbial communities.

2. Material and methods

2.1. Origin and characterization of soil samples

Soils were sampled from a field near Verdun, France, where there was previously a chemical ammunition destruction facility (Hube, 2017). The field was used as pasture in 2012 and was cultivated from 2012 to 2015 with wheat, barley and corn. Since 2015, it is fallow ground as farming was forbidden when the pollution was detected.

Surface soils (0–20 cm) were sampled with a spade in a highly polluted zone, and in a reference zone 25 m away. Each sample was taken as a composite of 5 points from 3 m × 3 m squares according to the GEMAS protocol (EGS, 2008) but adapted to the small surfaces available. The soils were characterized (Table 1) according to the following methods: pH in water (NF ISO 10390), organic carbon (NF ISO 10694), total nitrogen (NF ISO 13878), Phosphorus (NF ISO 11263), total elements, major and trace elements (extraction with HF+ $HClO_4$, NF ISO 14689-1).

As species in soils were analysed by HPLC-ICP-MS after extraction with 10 mL H_3PO_4 1 M added to 0.4 g of freeze-dried sample, ground and homogenized by sieving (2 mm) and microwave heating (Vergara Gallardo et al., 2001) in a closed system at 120 °C during 20 min (analyses performed by UT2A laboratory, Pau, France). The remaining solution was diluted to 50 mL with ultrapure water and then As species were analysed with HPLC-ICP-MS using quantification by standard additions to avoid matrix effects. As species separation was performed using an anion exchange column (Hamilton PRPX-100) and a mobile phase made of ammonium hydrogen phosphate 15 mM at pH 8.5. These analytical conditions enable the determination of AsIII, AsV and methylated As species (MMA and DMA). Details of the analytical methods are provided in SM1.

Diphenylarsinic acid (DPAA) was analysed by HPLC-DAD and clark I, clark II, Clark oxide, triphenylarsine (TPA), and 9-phenylarsafluorene were analysed by GC–MS (Envilytix, Wiesbaden, Germany, details in SM1). They were detected only in the polluted soil that contained 0.2% DPAA and 0.1% TPA. These two last species were not quantified in the next steps of the study that focused on total As and bio-transformations of AsIII and AsV.

2.2. Microcosm experiments

2.2.1. Preparation

Microcosms were setup in 200 mL polystyrene pots, 50 mm diameter, whose bottoms were perforated with a 0.9 mm needle, to make 13 holes in each pot. In order to retain soil particles in the pot, a fine layer of glass wool was placed at the bottom of each pot and covered with 10 cm^3 of clean Fontainebleau sand. Both glass wool and sand were previously cleaned in 10% HNO_3, rinsed with demineralized water and dried before use.

Each microcosm was filled with 150 g dry soil, with or without amendments. Each condition was tested in triplicate. The quantities of amendments added to the soils in the microcosms were calculated based on the real quantities applied on site (SM2) for KP fertilizer and for ammonium sulfate. A third amendment, organic manure, was chosen as the site was used as a pasture for many years (Table 2). The amount of manure added to the soils in the microcosms was based on real quantities usually applied in cultivated fields, i.e. 10 tons·ha^{-1}.

KP fertilizer (00 18 18) was provided by Soufflet Agri Service (Neuville-aux-Bois, France). It contained 0.32% organic C, 22.4% K_2O, 18.9% P_2O_5, and less than 0.1 g. kg^{-1} of total N. Fertilizer granules were crushed to a powder before application (<100 μm). Biomarine organic amendment (NF U 44-051 provided by Truffaut, France) was used as an organic amendment. It is composed a mixture of vegetal and animal wastes (horse manure, sheep manure, poultry manure, algae, grape marc), containing 33.9% organic C, 31.8 g·kg^{-1} total N, 4% K and 0.6% P. The two solid amendments (KP and manure) were added as powders to the dry soil and mixed for 24 h by rotation to homogenize.

Table 2
Amendments for microcosms. Hypothesis: Depth of amended soil 0.3 m, soil density 1.3.

Amendments	Real average quantity applied per hectare (kg)	kg·ton^{-1} of soil	Mass of amendment in the microcosm, for 150 g of soil (mg)
KP	273	0.07	10.5
Ammonium sulfate	95	0.024	3.6
Organic amendment	10,000	2.56	384

Table 1
Main characteristics of the two studied soils.

Parameter	pH	P_2O_5 (mg·kg^{-1})	K_2O (mg·kg^{-1})	Cd[a] (mg·kg^{-1})	Cr[a] (mg·kg^{-1})	Cu[a] (mg·kg^{-1})	Hg[a] (mg·kg^{-1})
Verdun reference soil	8.3	19.7 (±86.7)	306 (±9.7)	0.4 (±0.01)	30.9 (±16)	25.7 (±1.5)	0.07 (±0.006)
Verdun polluted soil	8.2	182 (±14)	678 (±23)	0.4 (±0.02)	41.4 (±2.9)	74.6 (±3)	1.1 (±0.2)
Parameter	Ni[a] (mg·kg^{-1})	Pb[a] (mg·kg^{-1})	Zn[a] (mg·kg^{-1})	As[a] (mg·kg^{-1})	Mn[a] (mg·kg^{-1})	C %	N %
Verdun reference soil	25.2 (±0.32)	21.9 (±0.05)	111.1 (±2.6)	21.8 (±0.9)	884.5 (±47.4)	1.9 (±0.7)	0.21 (±0.006)
Verdun polluted soil	24.4 (±0.99)	45.8 (±10)	180.3 (±9.9)	983 (±130)	791.9 (±18.7)	2.8 (±0.17)	0.32 (±0.03)

[a] Total element.

Ammonium sulfate was added as a 2.4 $g{\cdot}L^{-1}$ concentrated solution, 1.5 mL in each microcosm during the first watering.

2.2.2. Watering and incubation

Watering was always performed with Mont Roucous mineral water (pH 5.85; 3.1 $mg{\cdot}L^{-1}$ Na^{2+}; 2.4 $mg{\cdot}L^{-1}$ Ca^{2+}; 0.5 $mg{\cdot}L^{-1}$ Mg^{2+}; 2.0 $mg{\cdot}L^{-1}$ SO_4^{2-}; 6.3 $mg{\cdot}L^{-1}$ HCO_3^-; 3 $mg{\cdot}L^{-1}$ NO_3^-), used to simulate rain water.

For the first watering, 53 mL of water were carefully poured on the soil surface. Then, 24 h after the first watering, the microcosms were watered again, sufficiently to recover 20 mL of percolated water in the underlying pot. The quantity of inlet and outlet water was recorded by weighing the recipients. Global aerobic non-saturated conditions were maintained. Microcosms were incubated at 25 °C in the dark, with 80% atmospheric humidity. Soils never dried out during the incubation. Percolated water was filtered at 0.45 µm. Determination of As speciation was performed using an ion exchange method (Kim, 2001), the separated As species and the total As in water were determined by oven-AAS (details in SM1). Soils were watered at the beginning of experiment then after 1 week, 1 month and 3 months.

2.2.3. Final determination of biological parameters

At the end of the experiment (3 months), soils were sampled to determine biological parameters. All measurements were performed in triplicate.

2.2.3.1. Most probable number (MPN) determinations. Active As-transforming microorganisms present in microcosms were enumerated by the following MPN methods. To enumerate the active AsIII-oxidizing microorganisms (Thouin et al., 2016), wet soil (equivalent to 2.5 g dry soil) was placed in a sterile glass erlenmeyer flask with 10 mL of sterile physiological water (9 $g{\cdot}L^{-1}$ NaCl in demineralized water), agitated for 30 min at 25 °C, then sonicated 2 × 20 s at 45 kHz. Triplicate suspensions were prepared for each soil. Soil suspensions were serially diluted in sterile physiological water up to dilution 10^{-7}. CAsO1 mineral medium (Battaglia-Brunet et al., 2002) containing 100 $mg{\cdot}L^{-1}$ AsIII was distributed in Microtest TM Tissue culture plates (96 wells), 250 µL by well. Each well was inoculated with 25 µL of each soil suspension dilution. Five wells were inoculated for each dilution. Culture plates were incubated at 25 °C for 10 days. Presence of AsIII in the wells was revealed by the formation of insoluble white complex AsIII-Pyrrolidinedithiocarbamate (PDC): in each well 150 µL 0.1 M acetate buffer (pH 5) and 100 µL PDC solution (5 $g{\cdot}L^{-1}$) were added. A white precipitate appeared when AsIII was present, i.e. when AsIII-oxidizing bacteria were absent (negative well). Un-inoculated wells served as negative blanks, and wells containing CAsO1 medium with 100 $mg{\cdot}L^{-1}$ AsV served as a positive reference. The number of positive wells for each dilution was determined, and the most probable number of bacteria in dilutions was given by the Mc Grady table for 5 tubes.

To enumerate Active AsV-reducing microorganisms (Thouin et al., 2018), soil suspensions were prepared as described for AsIII-oxidizing microorganisms then diluted in sterile physiological saline solution to a dilution of 10^{-6}. CAsO1 basal mineral medium (Battaglia-Brunet et al., 2002) was complemented with 20 mM lactic acid and AsV (100 $mg{\cdot}L^{-1}$). The medium was distributed in Microtest TM Tissue culture plates (96 wells), 250 µL per well. Each well was inoculated with 25 µL of diluted soil suspension. Five wells were inoculated for each dilution. Culture plates were incubated at 25 °C for 10 days in anaerobic jars with Anaerocult packs (Merck). Presence of AsIII formed in the wells during incubation was revealed as described above. Positive well numbers were determined for each dilution, and the most probable number of AsV-reducing microorganisms was given by the Mc Grady table for five tubes.

2.2.3.2. Activity tests. AsIII-oxidizing tests were performed in 250 mL Erlenmeyer flasks filled with 100 mL of CAsO1 medium (Battaglia-Brunet et al., 2002) supplemented with 100 $mg{\cdot}L^{-1}$/AsIII and inoculated with soil (equivalent to 0.2 g dry weight (DW)). Flasks were incubated at 25 °C in oxidizing conditions under agitation (100 rpm). AsV-reducing tests were performed in 250 mL serum flasks filled with 100 mL of CAsO1 medium supplemented with 20 mM lactic acid, 0.2 $g{\cdot}L^{-1}$ yeast extract and AsV (100 $mg{\cdot}L^{-1}$). Flasks were inoculated with soil (equivalent to 0.2 g DW). Flasks were hermetically closed, flushed with N_2, and incubated at 25 °C in static conditions. Flasks were sampled every day in order to monitor the evolution of AsV concentration: 5 mL of culture were filtered at 45 µm with cellulose acetate filters and frozen at −20 °C until AsIII/AsV separation with the PDC/MIBK method (Battaglia-Brunet et al., 2002, details in SM1), As in the AsV-containing aqueous phase was quantified by flame AAS (Varian, Palo Alto, CA, USA). AsIII-oxidation and AsV-reduction rates were calculated between each point of analyse (evolution of AsV concentration divided by the time between sampling events).

2.3. Toxicity and transfer to plants

Impact of fertilizers on As bioavailability and phytotoxicity was evaluated with the ecotoxicological test AFNOR XP X31-233 (AFNOR, 2012), using the Omega-3 Index based on the analysis of leaf fatty acid composition of *Lactuca sativa* grown under controlled conditions and by measuring the As leaf content in the same plants. Fifteen *Lactuca sativa* seeds were sown in plastic pots filled with 430 g of dry soil, with or without amendment, and control pots containing a standard soil (loam). Each condition was tested in triplicate using the same quantities of amendments as for the microcosm experiment. Amendments were added to the soil at the beginning of the experiment and seedlings were grown for 17 days in a growth chamber under a 16 h/8 h photoperiod (8000 lx or 10000 lx white light intensity) and a 22 °C/16 °C day/night temperature. Pot location inside the growth chamber was randomly changed every 2–3 days to homogenize light exposure and watering. One week after germination, germination rates were determined and the number of young seedlings per pot was reduced. 14 days after germination, the aerial parts of seedlings were weighed, and the first leaf of some plants (or a section of it: 20–200 mg of fresh tissue) used to determine the leaf fatty acid composition, was placed in a glass tube containing 1 mL of a methanol/H_2SO_4 solution. Determination of leaf fatty acid composition was performed as described in Le Guédard et al. (2008). Determination of As concentrations was carried out by harvesting all the plants in each pot used for measuring the leaf fatty acid content. Plants were thoroughly washed in tap water and rinsed three times with deionised water. Plant biomass was then dried at 40 °C to constant weight and ground in a plastic bag to approximately 2 mm, to facilitate the digestion step. Samples (1 g DW) were digested in clean, dry PTFE screw cap vessels in hot concentrated HNO_3, according to Zarcinas et al. (1987) and As concentrations in the extracts were measured by ICP-MS (details in SM1).

2.4. Statistical analysis

Statistical analysis was performed with XLSTAT 2019.3.2.61397. Significance of differences between results of As concentrations, most probable number of bacteria and Omega-3 index were evaluated using the non-parametrical Kruskal and Wallis test, multiple pairwise comparison, at a significance level of 5% followed by a Dunn post hoc test. Correlations between parameters were calculated with XLSTAT 2019.3.2.61397, Pearson (n) correlation matrix, ($p < 0.05$). Data of maximum AsIII oxidation and AsV reduction rates were tested for homogeneity of variance and normal distribution. One-way analysis of variance (ANOVA) and Tukey HSD (Honestly Significantly Different) tests were carried out to test for any significant differences between the means. Differences between means at the 5% level ($p < 0.05$) were

considered significant.

3. Results

Initial soil analyses showed that phosphate concentration was ten times higher in the polluted soil than in the reference soil, and the potassium concentration was two times higher in the polluted soil than in the reference soil (Table 1). Average concentrations of total As were close to 1000 ppm in the polluted soil and to 20 ppm in the reference soil. Concentrations of total As in the percolated water were significantly lower for the reference soil microcosms (1–6 $\mu g \cdot L^{-1}$, Fig. 1 A) compared to polluted soil microcosms (2000–5000 $\mu g \cdot L^{-1}$, Fig. 1B). Although the ratio polluted/reference for total As was close to 50X in the solids, it was in the range of 1000X in the percolated water. This indicated that As was much more mobile in the polluted soil than in the reference soil. An increase of leached As was linked to the addition of KP fertilizer and a decrease of this leached As was linked to the addition of ammonium sulfate amendment in the polluted soil, at the first watering event (day 0, Fig. 1B).

Cumulated amounts of total As leached from the soils (Fig. 2) indicated that total As leached from the polluted soil was 1000X higher than the cumulated As leached from the reference soil. None of the amendments significantly increased or reduced As leaching compared with the blank experiment. A slight increase could be linked to the addition of KP fertilizer in the polluted soil and the lowest values of leached As were obtained with the ammonium sulfate amendment (Fig. 2A and B).

Speciation (quantification of AsIII and AsV) was monitored in water from microcosms containing the polluted soils only, as As concentrations were too low in the reference soil percolation water. AsIII concentrations varied between 5 and 15 $\mu g \cdot L^{-1}$ in these leachates, representing 0.1–0.5% of total As. Total AsIII leached during the experiment (Fig. 2C) ranged from 700 to 1000 ng. These amounts of AsIII represent less than 1% of the leached As, however they are higher than the total amounts of As leached from the reference soil. The amount of total leached AsIII was slightly higher with the KP amendment. However, the difference with other conditions is not statistically significant. Globally, the amendments had no significant influence on As speciation in the leached water during this experiment.

Final pH values (SM3) were close to the initial values, the general tendency being a small decrease during the experiment. None of the amendments induced an increase in pH sufficient to mobilize As.

Numbers of AsIII-oxidizing microorganisms were in the range of 10^4 cells$\cdot g^{-1}$ for the reference soil, and 10^6 cells$\cdot g^{-1}$ for the polluted soil (Fig. 3A), thus 100 times higher in the polluted soil. Numbers of AsV-reducing microorganisms were in the range of 10^6 cells$\cdot g^{-1}$ whatever the soil type (Fig. 3B). Globally cell numbers were slightly lower in the reference soil compared to the polluted soil. However, this difference was less marked than it was for AsIII-oxidizing microorganisms. Non-parametric statistical tests (XLSTAT 2018.2.50583 – Kruskal–Wallis test Two-sample *t*-test and z-test), performed as the comparison of all values with reference soil and all values with polluted soil indicates that the two groups (a and b on Fig. 3) are significantly different for both AsIII-oxidizing and AsV-reducing bacteria. However, no significant influence of the different amendments on the MPN of As-transforming microorganisms could be detected.

Results of the AsIII-oxidizing activity tests (Fig. 4A) clearly showed two trends: one observed with the group of polluted soil conditions, whose microbial communities oxidized AsIII very rapidly, and a second trend observed with the reference soils where kinetics were slower and clearly affected by the amendments. The maximum AsIII-oxidation rate was significantly higher with the polluted than with the reference soil. Considering exclusively the reference soil, the ammonium-amended soil's maximum AsIII-oxidation rate was significantly lower than the non-amended soil, whereas the KP and organic amendments tended to decrease the AsIII-oxidizing rate but not significantly.

Results of the AsV-reducing activity tests (Fig. 4B) indicated a rapid complete reduction of AsV after two days, for all soils. Thus, the AsV-reduction rate was similar for all conditions.

As contents in the lettuce leaves are shown in Fig. 5A and B. As contents in leaves are significantly higher in lettuces grown on polluted soils (7000–9000 $\mu g \cdot kg^{-1}$ DW) than in those grown on reference soils (60–120 $\mu g \cdot kg^{-1}$ DW). As transfer factor from soil to shoot ($TF_{shoot/soil}$) for polluted soil is 2.7-fold higher than for the $TF_{shoot/soil}$ from the reference soil. Thus, as observed with microcosm experiments, As from the polluted soil appeared more mobile and more phytoavailable than As from the reference soil.

While the phosphate concentration in the soil is 10X higher in the polluted soil compared with the reference soil, the phosphorus content in the lettuce leaves was similar for both soils (SM4). Concerning the amendment intake, results showed a significant (except for As in the leaves of plants grown on the reference soil) increase of As and P contents in lettuce leaves in both soils amended with KP (Fig. 5 and SM4). The other amendments did not show any differences with the unamended soil, regardless of the soil type (reference or polluted).

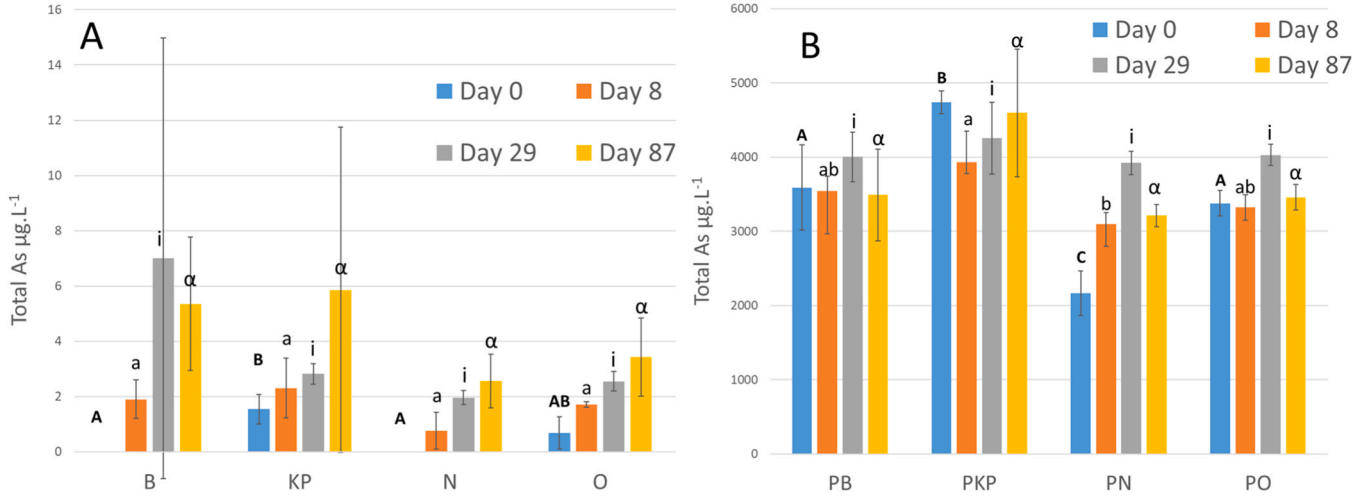

Fig. 1. Evolution of total As concentrations in the leachates. A: reference soil; B: polluted soil. B: blank experiment without amendment; KP: KP fertilizer; N: ammonium sulfate; O: organic amendment. P for polluted. Significance of differences between amendment conditions was evaluated separately for each incubation time using the non-parametric statistical method (details given in material and methods section), T0 (A, B, C); T7 days (a, b, c); T 29 days (i); T 87 days (α). Error bars represent the standard deviation of the means (3 replicates).

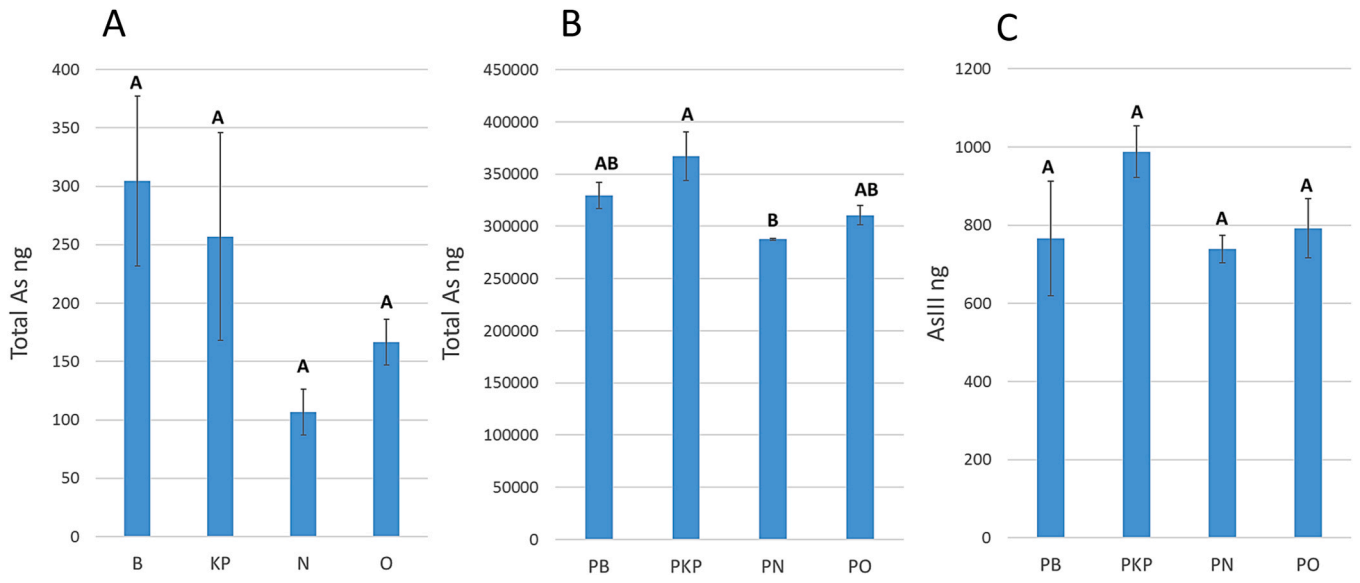

Fig. 2. Cumulated leached total As and AsIII. A: reference soil total As; B: polluted soil total As; C: polluted soil AsIII. KP: KP fertilizer; N: ammonium sulfate; O: organic amendment. P for polluted. Significance of differences between amendment conditions was evaluated separately for each incubation time using a non-parametric statistical method (details in material and methods section). Error bars represent the standard deviation of the means (3 replicates).

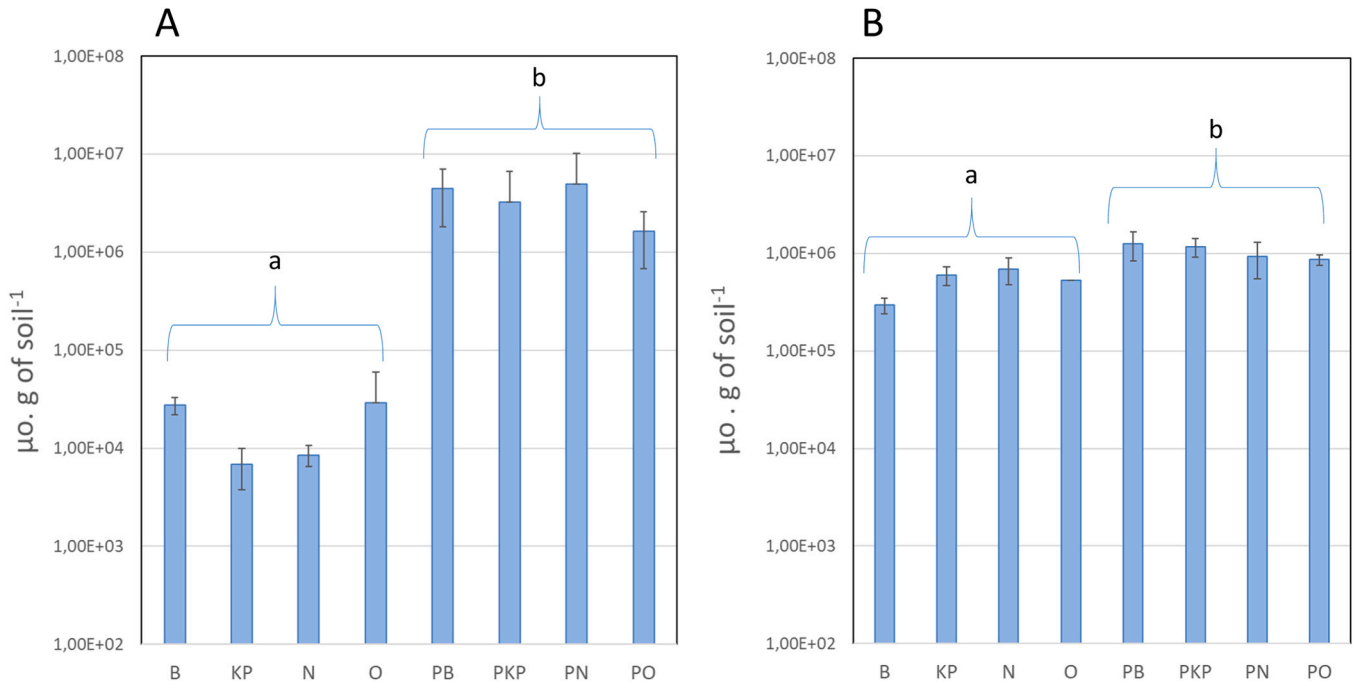

Fig. 3. Most probable numbers of As-transforming microorganisms. A: AsIII-oxidizing microorganisms; B: AsV-reducing microorganisms. KP: KP fertilizer; N: ammonium sulfate; O: organic amendment. P for polluted. Error bars represent the standard deviation of the means (3 replicates). Groups A and B were statistically different according to the Kruskal–Wallis test.

The Omega-3 Index is a standardized biomarker to evaluate the possible toxic effects of contaminants on plants (AFNOR, 2012). This biomarker measures the degradation of polyunsaturated fatty acids and decreases when lipid peroxidation, caused by an excess of reactive oxygen species (ROS) in the presence of contaminants, increases. Results showed that the Omega-3 Index was significantly lower for lettuces grown on the polluted soil compared to the reference soil (Fig. 6). This indicated that lipid peroxidation was higher in lettuces grown on the polluted soil, which was therefore more phytotoxic than the reference soil. This phytotoxicity of As in the polluted soil caused a decrease of the lettuce seedling growth, as shown in SM5 (69.02 mg of fresh weight (FW)·plant^{-1} ± 13.73 and 30.34 mg of FW·plant^{-1} ± 4.97 for reference soil and the polluted soil, respectively). Moreover, while there was no difference between the un-amended and amended reference soils, the Omega-3 Index increased significantly for lettuces grown on polluted soil amended with ammonium sulfate. Thus, addition of ammonium sulfate in the polluted soil seemed to reduce As induced lipid peroxidation.

4. Discussion

Among the parameters controlling the behaviour of As in soils, redox

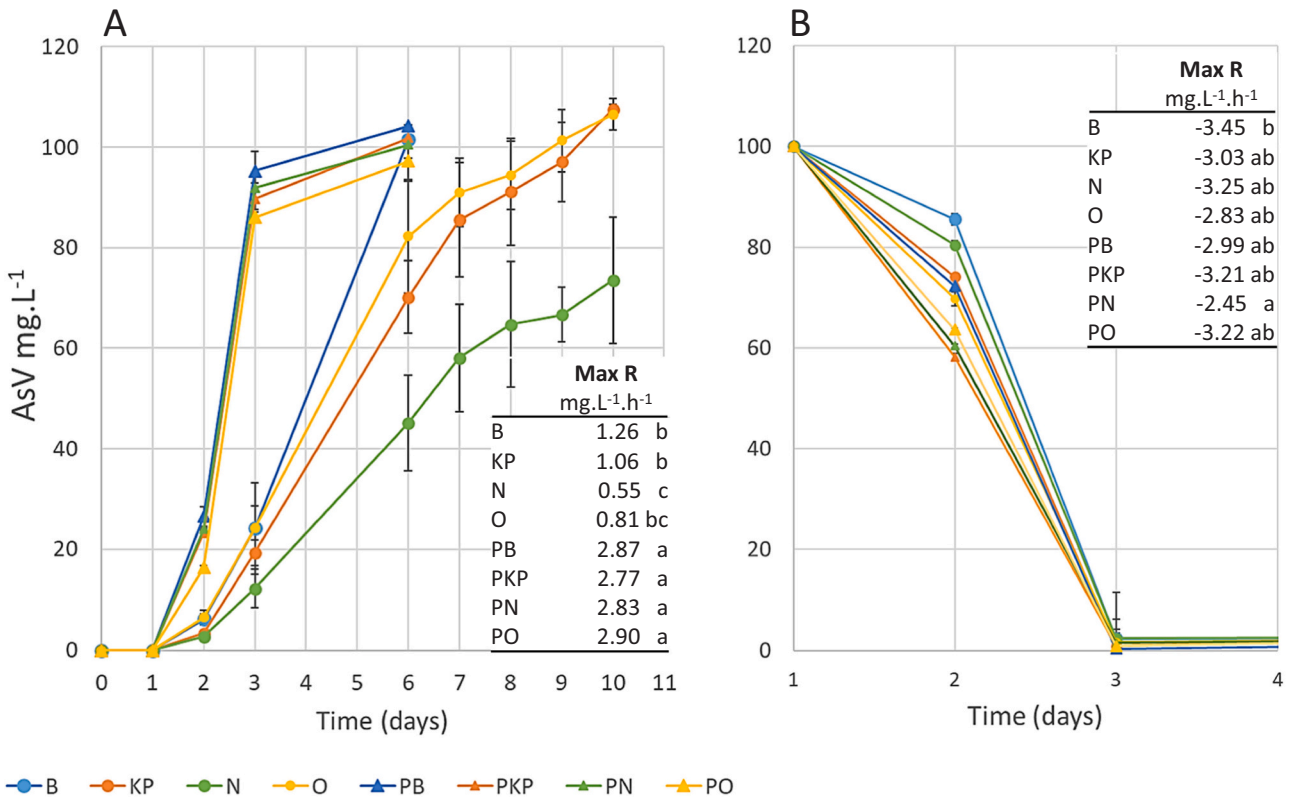

Fig. 4. Activity tests. A: AsIII-oxidizing activities; B: AsV-reducing activities. Error bars represent the standard deviation of the means (3 replicates). KP: KP fertilizer; N: ammonium sulfate; O: organic amendment. P for polluted. AsIII-oxidation and AsV-reduction rates were calculated between each point of analyse (evolution of AsV concentration divided by the time between sampling events). Max R: Maximum rates. Values are the means (n = 3). Values with different letters are significantly different (P< 0.05, ANOVA, Tukey-HSD).

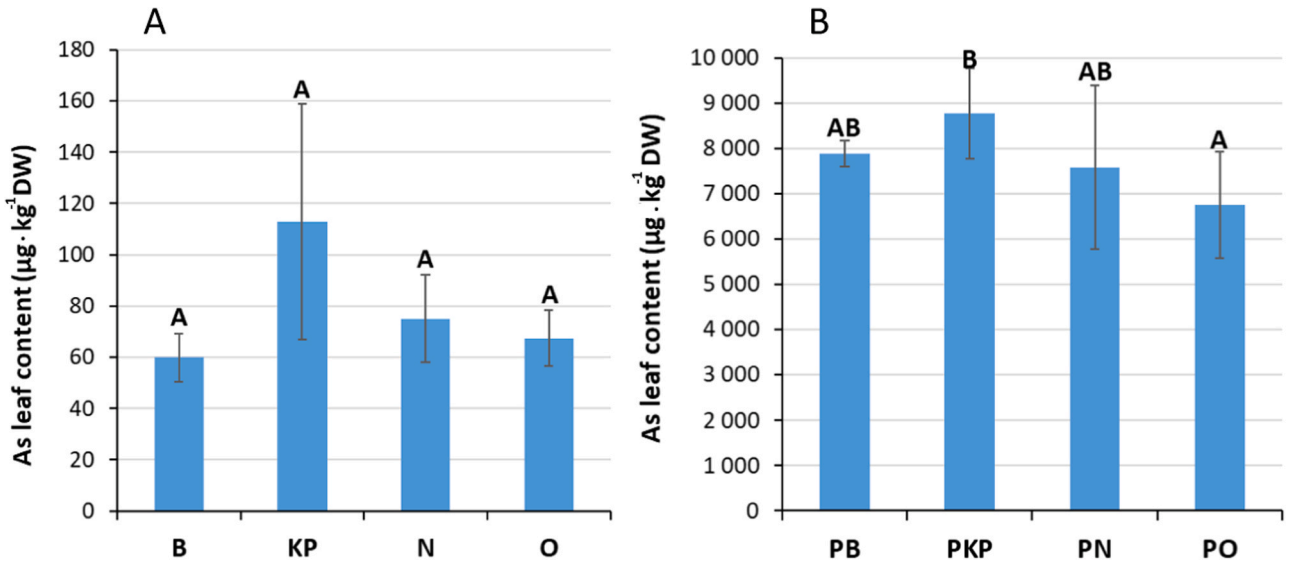

Fig. 5. As content in the lettuce leaves grown on A: the reference soil; B: the polluted soil (P) un-amended or amended with different amendments. KP: KP fertilizer; N: ammonium sulfate; O: organic amendment. P for polluted. Different letters (A or B) indicate significant differences between the different amended soils. Each value is the mean of 3 samples.

conditions play a major role. Experiments performed with microcosm systems allowing control of redox conditions showed that low Eh values induced mobilization of As from a flood plain soil (Frohne et al., 2011), a freshwater marsh delta soil (Shaheen et al., 2016) and an historically contaminated coastal soil (LeMonte et al., 2017). Here, non-saturated conditions were maintained in microcosms, so the mobility of As should mainly be controlled by other factors. Although AsV adsorption on iron oxides is known to decrease with pH (Dixit and Hering, 2003), As mobility in the polluted soil compared to the reference soil was probably not exclusively linked to soil pH (8.2 and 8.3 in the reference and the polluted soil, respectively, Table 1). As mobility could also be related to the higher concentration of phosphate in the polluted soil. Indeed,

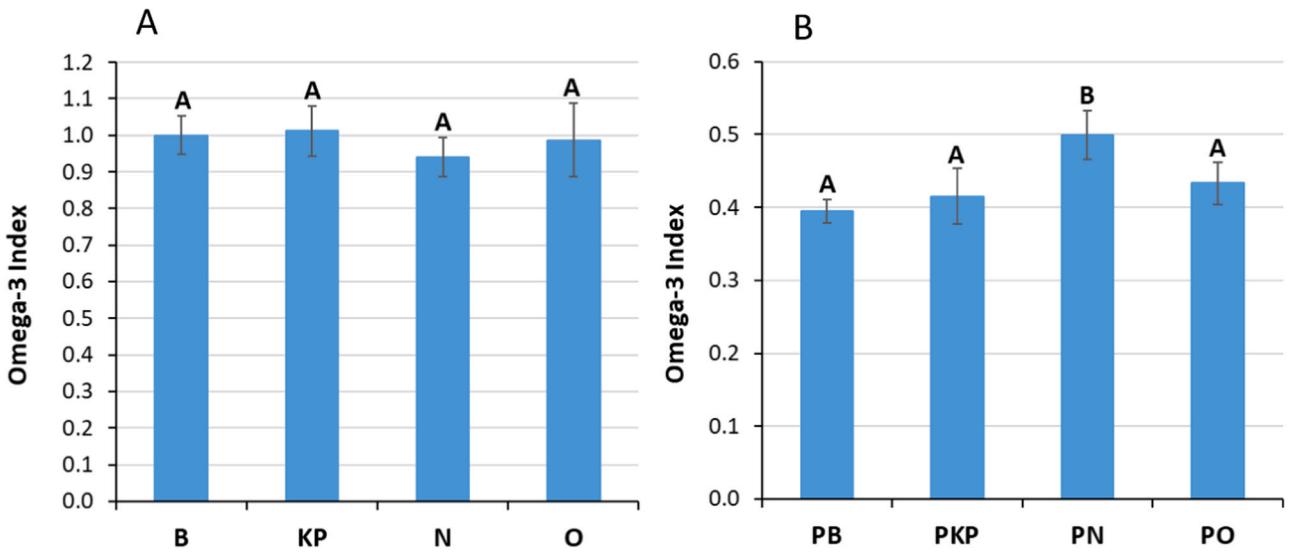

Fig. 6. Omega-3 Index measured in lettuces grown on A: the reference soil; B: the polluted soil (P), un-amended or amended with different amendments. KP: KP fertilizer; N: ammonium sulfate; O: organic amendment. P for polluted. Different letters (A, B or C) indicate significant differences between the different soils un-amended or amended. Each value is the mean of 3 samples.

phosphate concentration was ten times higher (and the potassium concentration two times higher) in the polluted soil than in the reference soil. One hypothesis to explain this is that the farmer might have provided higher quantities of KP fertilizer in this precise area in an attempt to improve crop yields. Indeed plant growth was strongly affected by the undetected pollution in this area of the field. Phosphate is an analogue of arsenate AsV (Smith and Naidu, 2009) which competes with As for adsorption sites on iron oxides. In the percolation water, the highest total As concentrations were always observed with the KP fertilizer and could be linked to the addition of phosphate. Consequently increased As mobility may have increased the toxic impact on plant growth. In addition, as the polluted soil contained both more phosphate and more As than the reference soil, the ratio between the density of adsorption sites and concentrations of both elements was lower in the polluted than in the reference soil. This has previously been observed in soils polluted with As, but with higher doses of phosphate fertilizers (Davenport and Peryea, 1991; Peryea and Kammereck, 1997). In terms of concentration, As in the microcosm percolation water was in the range 2–5 $mg{\cdot}L^{-1}$, in the same range as concentrations already reported in water phases in contact with soils polluted by different sources of As. Thus, Cao et al. (2003) found 5–6 $mg{\cdot}L^{-1}$ of As in water leached from soil polluted with a chromium-copper-arsenic (CCA) pesticide, presenting a total As content of 135 $mg{\cdot}kg^{-1}$, and Qi and Danahoe (2008) found 1 $mg{\cdot}L^{-1}$ of As when they used acid rainwater to leach a soil polluted by historical herbicide application and containing 300 $mg{\cdot}kg^{-1}$ As.

MPN of As-transforming microbes suggested that the pollution induced an increase of the abundance of active microorganisms able to modify As speciation in the soil (Fig. 3). The difference observed between the reference soil and the polluted soil was more marked for AsIII-oxidizing than for AsV-reducing microbes, and that was true both for MPN and activity tests: AsIII-oxidizing microbes were roughly 100 times more abundant in the polluted soil (Fig. 3A), and this soil presented a higher AsIII-oxidizing activity than the reference soil. Thouin et al. (2016) showed that the AsIII-oxidizing rate increased with the As concentration in samples of polluted technosoils, and suggested that the level of As concentration exerted a selective pressure on the microbial community, increasing its global efficiency to oxidize AsIII. Here, whereas amendments did not influence the AsIII-oxidizing activity of the polluted soil (Fig. 4A), the fertilizers exerted a clear influence on this activity in the reference soil. AsIII-oxidizing activity was diminished by the ammonium sulfate amendment (Fig. 4A). Yet, this fertilizer tended to decrease As mobility from soils towards percolation water, in particular at the beginning of the experiment with the polluted soil (Fig. 1B). Our results are in agreement with those of a long-term (1929–2018) experiment studying the effects of agricultural amendments on the behaviour of trace elements. In that experiment, authors showed that ammonium-based fertilization induces an increase of immobilized As in the soil, compared with other types of amendments, due to reduced As leaching (van Oort et al., 2017). This phenomenon might be linked to H^+ production during ammonium oxidation (nitrification). Even if no macroscopic pH decrease was observed here, protonation of the surface hydroxyl groups of iron oxides could increase AsV adsorption on these minerals in the soil (Dixit and Hering, 2003). Reduced As mobility from solids to the water phase might decrease As bioavailability, consequently reducing the selective pressure on the global microbial community and decreasing its efficiency to oxidize AsIII.

The organic fertilizer did not significantly influence As behaviour nor the activity and abundance of As-transforming microbes (Figs. 1–4). In previous studies, OM was reported to induce higher As mobility in soils (Beesley et al., 2014). However, contradictory results were reported about the impact of OM on As mobility (Kumpiene et al., 2008). In terms of effects on microbial transformation of As, Lescure et al. (2016) showed that OM exerted a positive effect on AsIII-oxidation rates from 0 to 0.08 $g{\cdot}L^{-1}$ of organic carbon, then tended to decrease the AsIII-oxidation activity at higher concentrations. Here, the dose of organic fertilizer (0.26%) was probably not sufficient to modify microbial activities.

The solid and liquid phases of the soils contained AsV as the main As form, whereas the MPN and activity of AsV-reducing microbes was important according to Figs. 3 and 4. Even when As is mainly in the form of AsV, it is not a static distribution of AsV between solids and water phases, but rather a dynamic equilibrium, involving both microorganisms oxidizing AsIII and microorganisms reducing AsV. In this system, bioreduction of AsV might play a role in As mobilisation. Turpeinen et al. (1999) have already observed that microbes increased the As mobility, mainly as AsV, from soils incubated in aerated conditions. We found AsV as the main As form leached from the polluted soil, with no significant effect of the amendments on this speciation, in soils that were not saturated with water, i.e. with no limitation of oxygen availability. Our results must be confirmed with further experiments performed with diverse polluted sites, and comparing biotic to abiotic conditions, and

non-saturated to saturated conditions. However, they suggest that microbial parameters would be very sensitive bio-indicators of the dynamics of As concentrations and speciation in the water phase.

Considering the pot experiments with plants, As transfer was significantly more important in lettuces grown on the polluted soil. These results showed that As was highly mobile and as a consequence highly phytoavailable in the polluted soil. This correlated with results from the microcosms where As was shown to be more mobile from soil to water within the polluted soil compared to the reference soil. In lettuces, amendment with KP induced an increase of both As and P contents in lettuce leaves, as already observed by Cao and Ma (2004). Phosphate-induced plant As uptake may have been related to the slight increase of As concentration in the leachate. The lettuces' Omega-3 Index showed that the high As content in the polluted soil increased lipid peroxidation in lettuce leaves compared to the reference soil (Fig. 6). Bustingorri et al. (2017) observed an increase of lipid peroxidation in soybean plants linked to the presence of As. Here, the increase of lipid peroxidation induced by the polluted soil, corresponding to nearly 50% decrease of the Omega-3 index (Fig. 6) was coupled with a decrease of the lettuce seedling growth. Thus, in agreement with findings obtained from previous studies, the excessive production of ROS induced by As exposure promotes lipid peroxidation and causes damage in thylakoid membranes that may lower photosynthetic efficiency (Abbas et al., 2018). In agreement with previous results of As transfer from soil to lettuces (Fig. 5), these results confirm that As is highly phytoavailable and as a consequence highly phytotoxic in the polluted soil.

The amounts of agricultural amendments applied to fields can influence the mobility, bioavailability and toxicity of As. Addition of ammonium sulfate to polluted soil was associated with an increase of the Omega-3 Index (Fig. 6) and as a consequence a reduction of lipid peroxidation in lettuce leaves while no decrease was observed concerning the As uptake. This was not observed in the reference un-polluted soil, suggesting that the decrease of lipid peroxidation is possibly influenced by the contribution of ammonium sulfate to As detoxification. These results are in agreement with several studies showing that, under heavy metal stress, an application of sulfur stimulates plants (Mishra et al., 2008; Duan et al., 2013). In fact, sulfur (S) in higher plants is a vital component for the synthesis of some amino acids (Cysteine and Methionine) and metabolites such as gluthatione (GSH) and phytochelatins (PCs) involved in the detoxification of heavy metals. Some studies relate that As induces ROS production in plants leading to lipid peroxidation (Shukla et al., 2018) and the synthesis of PCs enzymatically synthesized from GSH. Then, PCs form complexes with As before its sequestration into the vacuoles through ABCC transporters (Song et al., 2010). This induction of the biochemical pathways of plant S metabolism increases the S requirement under As stress (Leao et al., 2014; Khare et al., 2017). Therefore, the decrease of lipid peroxidation in lettuces grown on the polluted soil amended with ammonium sulfate may be explained by an improvement of the intracellular As detoxification processes due to an increase of the synthesis of GSH and PCs linked to an increase of S assimilation by plants. Similarly, nitrogen (N) could also play an important role in the detoxification of heavy metals in plants. Indeed, many studies related that, under abiotic stress, nitrate supply stimulates root nitric oxide production (Sun et al., 2010; Simontacchi et al., 2015) which is a bioactive signalling molecule involved in plants' response to heavy metal stress by detoxifying ROS (Hassan et al., 2005; Kaur et al., 2015). Thus, according to these results, it seems that a better N and S nutrition status may protect the plants from As and as a consequence lead to a decrease of oxidative stress. However, more research is needed to fully understand the mechanisms of interactions between ammonium sulfate and As in plants and the importance of ammonium sulfate in detoxification.

5. Conclusions

The addition of fertilizing amendments, at the real average dose applied on the sites, did not strongly influence the speciation and the quantity of mobile As. Observed trends were an increase of As mobility with KP fertilizer, a decrease of mobility with ammonium sulfate amendment, and no effect of organic amendments. The quantity of As mobilised in the percolation water was bioavailable for plants and soil microorganisms. Results of an original combination of active As-transforming bacteria enumeration and As-related microbial activity tests showed that microbial parameters were strongly linked to As levels in water and in soils. In particular, microbial AsIII-oxidizing activity proved to be very sensitive to low doses of ammonium sulfate. Although the impacts of KP and ammonium sulfate on As speciation were insignificant, the small effects observed on plant As-uptake and bacterial activities may have an impact in the long term. Consistent results were observed with microbial and plant parameters, in particular concerning the effect of ammonium sulfate fertilization. These tests could be used as sensitive indicators of As bioavailability and toxicity in soils. Whereas microbial parameters can be quicker to obtain than plant indicators, As bioavailability for plants will always be very important to determine, particularly when these plants are cultivated and consumed.

CRediT authorship contribution statement

Fabienne Battaglia-Brunet: Conceptualization, Writing - original draft, Supervision. **Marina Le Guédard:** Methodology, Investigation, Visualization, Writing - original draft, Editing. **Olivier Faure:** Investigation, Visualization. **Mickael Charron:** Investigation. **Daniel Hube:** Resources. **Nicolas Devau:** Validation, Writing (Editing). **Catherine Joulian:** Investigation, Writing (Editing). **Hugues Thouin:** Methodology, Writing (Editing). **Jennifer Hellal:** Conceptualization, Methodology, Investigation, Writing (Editing).

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124580.

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