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Revised manuscript with accepted corrections

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

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15

16 **Abstract**

17 Agricultural soils can contain high arsenic (As) concentrations due to specific geological
18 contexts or pollution. Fertilizer amendments could influence As speciation and mobility thus
19 increasing its transfer to crops and its toxicity. In the present study, field-relevant amounts of
20 fertilizers were applied to soils from a cultivated field that was a former ammunition-burning
21 site. Potassium phosphate (KP), ammonium sulfate and organic matter (OM) were applied to
22 these soils in laboratory experiments to assess their impact on As leaching, bioavailability to
23 *Lactuca sativa* and microbial parameters. None of the fertilizers markedly influenced As

24 speciation and mobility, although trends showed an increase of mobility with KP and a
25 decrease of mobility with ammonium sulfate. Moreover, KP induced a small increase of As in
26 *Lactuca sativa*, and the polluted soil amended with ammonium sulfate was significantly less
27 phytotoxic than the un-amended soil. Most probable numbers of AsIII-oxidizing microbes and
28 AsIII-oxidizing activity were strongly linked to As levels in water and soils. Ammonium
29 sulfate negatively affected AsIII-oxidizing activity in the un-polluted soil. Whereas no
30 significant effect on As speciation in water could be detected, amendments may have an
31 impact in the long term.

32

33 **Key words:** arsenic, soil, water, fertilizers, microorganisms, omega 3 index

34

35

36 **1. Introduction**

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

42 When soils affected by As pollution are submitted to agricultural practices, arsenic speciation,
43 bio-availability for plants and mobility towards the water phase may be changed. Major
44 phenomena influencing As mobility (Smith et al., 1998) include; (1) pH which influences
45 AsIII and AsV oxy-anions charge, (2) redox conditions, which influences As speciation and
46 the stability of iron oxides that are essential As-bearing phases, and (3) competing substances,

47 that may favour As desorption from solid phases. In particular, phosphate, a structural
48 analogue of AsV can compete with As for sorption on iron oxides (Smith and Naidu, 2009).

49 Thus, in agricultural soils, fertilizing practices involving phosphate amendments may affect
50 As speciation and mobility. Brackhage et al. (2014) observed an increase of As mobility and
51 uptake by wheat associated to P-fertilization in soil flooding conditions. Conversely, N-
52 fertilization seemed to attenuate As mobility and plant uptake (Brackhage et al., 2014, Van
53 Oort et al., 2017). In addition, agricultural soils are often fertilized with organic matter (OM).
54 Many studies have described geochemical interactions between As and organic matter:
55 modification of As speciation (Redman et al., 2002), formation of soluble complexes (Saada
56 et al., 2003; Redman et al., 2002), competition for sorption sites (Bauer and Blodau, 2006),
57 and influence of OM on microbial AsIII-oxidizing activity (Lescure et al., 2016).

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

70 This study aimed to measure the impact of fertilizing practices on As mobility, speciation and
71 transfer to crops in soils from a former chemical-ammunition-destruction facility dating from

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

80

81 **2. Material and methods**

82 **2.1. Origin and characterization of soil samples**

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

87 Surface soils (0-20 cm) were sampled with a spade in a highly polluted zone, and in a
88 reference zone 25 m away. Each sample was taken as a composite of 5 points from 3 m × 3 m
89 squares according to the GEMAS protocol (EGS 2008) but adapted to the small surfaces
90 available. The soils were characterized (Table 1) according to the following methods: pH in
91 water (NF ISO 10390), organic carbon (NF ISO 10694), total nitrogen (NF ISO 13878),
92 Phosphorus (NF ISO 11263), total elements, major and trace elements (extraction with HF +
93 HClO₄, NF ISO 14689-1).

94 As species in soils were analysed by HPLC-ICP-MS after extraction with 10 mL H₃PO₄ 1 M
95 added to 0.4 g of freeze-dried sample, ground and homogenized by sieving (2 mm) and
96 microwave heating (Vergara Gallardo et al., 2001) in a closed system at 120°C during 20 min

107 (analyse performed by UT2A laboratory, Pau, France). The remaining solution was diluted to
 108 50 mL with ultrapure water and then As species were analysed with HPLC-ICP-MS using
 109 quantification by standard additions to avoid matrix effects. As species separation was
 100 performed using an anion exchange column (Hamilton PRPX-100) and a mobile phase made
 101 of ammonium hydrogen phosphate 15 mM at pH 8.5. These analytical conditions enable the
 102 determination of AsIII, AsV and methylated As species (MMA and DMA). Details of the
 103 analytical methods are provided in SM1.

104

105 **Table 1.** Main characteristics of the two studied soils. (*) total element.

Parameter	pH	P ₂ O ₅ mg.kg ⁻¹	K ₂ O mg.kg ⁻¹	Cd* mg.kg ⁻¹	Cr* mg.kg ⁻¹	Cu* mg.kg ⁻¹	Hg* mg.kg ⁻¹
Verdun Reference soil	8.3	19.7 (+/-6.7)	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* mg.kg ⁻¹	Pb* mg.kg ⁻¹	Zn* mg.kg ⁻¹	As* mg.kg ⁻¹	Mn* 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)

106

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

112

113 2.2. Microcosm experiments

114

115 2.2.1 Preparation

116 Microcosms were setup in 200 mL polystyrene pots, 50 mm diameter, whose bottoms were
 117 perforated with a 0.9 mm needle, to make 13 holes in each pot. In order to retain soil particles

118 in the pot, a fine layer of glass wool was placed at the bottom of each pot and covered with 10
 119 cm³ of clean Fontainebleau sand. Both glass wool and sand were previously cleaned in 10 %
 120 HNO₃, rinsed with demineralized water and dried before use.

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

127 **Table 2.** Amendments for microcosms. Hypothesis: Depth of amended soil 0.3 m, soil density
 128 1.3.

129

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

130

131 KP fertilizer (00 18 18) was provided by Soufflet Agri Service (Neuville-aux-Bois, France). It
 132 contained 0.32 % organic C, 22.4 % K₂O, 18.9 % P₂O₅, and less than 0.1 g.kg⁻¹ of total N.
 133 Fertilizer granules were crushed to a powder before application (< 100 μm). Biomarine
 134 organic amendment (NF U 44-051 provided by Truffaut, France) was used as an organic
 135 amendment. It is composed a mixture of vegetal and animal wastes (horse manure, sheep
 136 manure, poultry manure, algae, grape marc), containing 33.9 % organic C, 31.8 g.kg⁻¹ total N,

137 4 % K and 0.6 % P. The two solid amendments (KP and manure) were added as powders to
138 the dry soil and mixed for 24 h by rotation to homogenize. Ammonium sulfate was added as a
139 2.4 g.L⁻¹ concentrated solution, 1.5 mL in each microcosm during the first watering.

140

141 2.2.2. Watering and incubation

142 Watering was always performed with Mont Roucous mineral water (pH 5.85; 3.1 mg.L⁻¹
143 Na²⁺; 2.4 mg.L⁻¹ Ca²⁺; 0.5 mg.L⁻¹ Mg²⁺; 2.0 mg.L⁻¹ SO₄²⁻; 6.3 mg.L⁻¹ HCO₃⁻; 3 mg.L⁻¹ NO₃⁻),
144 used to simulate rain water.

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

154

155 2.2.3. Final determination of biological parameters

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

158

159 2.2.3.1. Most Probable Number (MPN) determinations

160 Active As-transforming microorganisms present in microcosms were enumerated by the
161 following MPN methods. To enumerate the active As^{III}-oxidizing microorganisms (Thouin et

162 al., 2016), wet soil (equivalent to 2.5 g dry soil) was placed in a sterile glass erlenmeyer flask
163 with 10 ml of sterile physiological water (9 g.l^{-1} NaCl in demineralized water), agitated for 30
164 min at 25°C , then sonicated 2 x 20 s at 45 kHz. Triplicate suspensions were prepared for each
165 soil. Soil suspensions were serially diluted in sterile physiological water up to dilution 10^{-7} .
166 CAsO1 mineral medium (Battaglia-Brunet et al., 2002) containing 100 mg.L^{-1} AsIII was
167 distributed in Microtest TM Tissue culture plates (96 wells), 250 μL by well. Each well was
168 inoculated with 25 μL of each soil suspension dilution. Five wells were inoculated for each
169 dilution. Culture plates were incubated at 25°C for 10 days. Presence of AsIII in the wells was
170 revealed by the formation of insoluble white complex AsIII-PyrrolidineDithioCarbamate
171 (PDC): in each well 150 μL 0.1 M acetate buffer (pH 5) and 100 μL PDC solution (5 g.L^{-1})
172 were added. A white precipitate appeared when AsIII was present, i.e. when AsIII-oxidizing
173 bacteria were absent (negative well). Un-inoculated wells served as negative blanks, and wells
174 containing CAsO1 medium with 100 mg.L^{-1} AsV served as a positive reference. The number
175 of positive wells for each dilution was determined, and the most probable number of bacteria
176 in dilutions was given by the Mc Grady table for 5 tubes.

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

186 most probable number of AsV-reducing microorganisms was given by the Mc Grady table for
187 five tubes.

188 2.2.3.2. Activity tests

189 AsIII-oxidizing tests were performed in 250 mL Erlenmeyer flasks filled with 100 mL of
190 CAsO1 medium (Battaglia-Brunet et al., 2002) supplemented with 100 mg.L⁻¹/ AsIII and
191 inoculated with soil (equivalent to 0.2 g dry weight). Flasks were incubated at 25°C in
192 oxidizing conditions under agitation (100 rpm). AsV-reducing tests were performed in 250
193 mL serum flasks filled with 100 mL of CAsO1 medium supplemented with 20 mM lactic
194 acid, 0.2 g.L⁻¹ yeast extract and AsV (100 mg.L⁻¹). Flasks were inoculated with soil
195 (equivalent to 0.2 g of dry weight). Flasks were hermetically closed, flushed with N₂, and
196 incubated at 25°C in static conditions. Flasks were sampled every day in order to monitor the
197 evolution of AsV concentrations: 5 mL of culture were filtered at 45 µm with cellulose acetate
198 filters and frozen at -20°C until AsIII/AsV separation with the PDC/MIBK method (Battaglia-
199 Brunet et al., 2002, details in SM1), As in the AsV-containing aqueous phase was quantified
200 by flame AAS (Varian, Palo Alto, CA, USA). AsIII-oxidation and AsV-reduction rates were
201 calculated between each point of analyse (evolution of AsV concentration divided by the time
202 between sampling events).

203

204 **2.3. Toxicity and transfer to plants**

205 Impact of fertilizers on As bioavailability and phytotoxicity was evaluated with the
206 ecotoxicological test AFNOR XP X 31-233 (2012), using the Omega-3 Index based on the
207 analysis of leaf fatty acid composition of *Lactuca sativa* grown under controlled conditions
208 and by measuring the As leaf content in the same plants. Fifteen *Lactuca sativa* seeds were
209 sown in plastic pots filled with 430 g of dry soil, with or without amendment, and control pots
210 containing a standard soil (loam). Each condition was tested in triplicate using the same

211 quantities of amendments as for the microcosm experiment. Amendments were added to the
212 soil at the beginning of the experiment and seedlings were grown for 17 days in a growth
213 chamber under a 16 h/8 h photoperiod (8000 lx or 10000 lx white light intensity) and a
214 22°C/16°C day/night temperature. Pot location inside the growth chamber was randomly
215 changed every 2 to 3 days to homogenize light exposure and watering. One week after
216 germination, germination rates were determined and the number of young seedlings per pot
217 was reduced. 14 days after germination, the aerial parts of seedlings were weighed, and the
218 first leaf of some plants (or a section of it: 20 to 200 mg of fresh tissue) used to determine the
219 leaf fatty acid composition, was placed in a glass tube containing 1 mL of a methanol/H₂SO₄
220 solution. Determination of leaf fatty acid composition was performed as described in Le
221 Guédard et al. (2008). Determination of As concentrations was carried out by harvesting all
222 the plants in each pot used for measuring the leaf fatty acid content. Plants were thoroughly
223 washed in tap water and rinsed three times with deionised water. Plant biomass was then dried
224 at 40 °C to constant weight and ground in a plastic bag to approximately 2 mm, to facilitate
225 the digestion step. Samples (1 g Dry Weight) were digested in clean, dry PTFE screw cap
226 vessels in hot concentrated HNO₃, according to Zarcinas et al. (1987) and As concentrations
227 in the extracts were measured by ICP-MS (details in SM1).

228

229 **2.4. Statistical analysis**

230 Statistical analysis were performed with XLSTAT 2019.3.2.61397. Significance of
231 differences between results of As concentrations, most probable number of bacteria and
232 Omega-3 index were evaluated using the non-parametrical Kruskal and Wallis test, with
233 multiple pairwise comparison using Dunn's procedure, two tailed test, at a significance level
234 of 5% followed by a Dunn post hoc test. Correlations between parameters were calculated
235 with XLSTAT 2019.3.2.61397, Pearson (n) correlation matrix, ($p < 0.05$). Data of maximum

236 AsIII oxidation and AsV reduction rates were tested for homogeneity of variance and normal
237 distribution. One-way analysis of variance (ANOVA) and Tukey HSD (Honestly
238 Significantly Different) tests were carried out to test for any significant differences between
239 the means. Differences between means at the 5% level ($P < 0.05$) were considered significant.

240

241 **3. Results**

242 Initial soil analyses showed that phosphate concentration was ten times higher in the polluted
243 soil than in the reference soil, and the potassium concentration was two times higher in the
244 polluted soil than in the reference soil (Table 1). Average concentrations of total As were
245 close to 1000 ppm in the polluted soil and to 20 ppm in the reference soil. Concentrations of
246 total As in the percolated water were significantly lower for the reference soil microcosms (1
247 to 6 $\mu\text{g.L}^{-1}$, Figure 1A) compared to polluted soil microcosms (2000 to 5000 $\mu\text{g.L}^{-1}$, Figure
248 1B). Although the ratio polluted/reference for total As was close to 50X in the solids, it was in
249 the range of 1000X in the percolated water. This indicated that As was much more mobile in
250 the polluted soil than in the reference soil. An increase of leached As was linked to the
251 addition of KP fertilizer and a decrease of this leached As was linked to the addition of
252 ammonium sulfate amendment in the polluted soil, at the first watering event (day 0, Figure
253 1B).

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

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

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

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

284 Results of the As^{III}-oxidizing activity tests (Figure 4A) clearly showed two trends: one
285 observed with the group of polluted soil conditions, whose microbial communities oxidized
286 As^{III} very rapidly, and a second trend observed with the reference soils where kinetics were
287 slower and clearly affected by the amendments. The maximum As^{III}-oxidation rate was
288 significantly higher with the polluted than with the reference soil. Considering exclusively the
289 reference soil, the ammonium-amended soil gave a value of maximum As^{III}-oxidation rate
290 significantly lower than the non-amended soil, whereas the KP and organic amendments
291 tended to decrease the As^{III}-oxidizing rate but not significantly.

292 Results of the As^V-reducing activity tests (Figure 4B) indicated a rapid complete reduction of
293 As^V after two days, for all soils. Thus, the As^V-reduction rate was similar for all conditions.

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

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

306 The Omega-3 Index is a standardized biomarker to evaluate the possible toxic effects of
307 contaminants on plants (AFNOR XP X31-233, 2012). This biomarker measures the
308 degradation of polyunsaturated fatty acids and decreases when lipid peroxidation, caused by

309 an excess of reactive oxygen species (ROS) in the presence of contaminants, increases.
310 Results showed that the Omega-3 Index was significantly lower for lettuces grown on the
311 polluted soil compared to the reference soil (Figure 6). This indicated that lipid peroxidation
312 was higher in lettuces grown on the polluted soil, which was therefore more phytotoxic than
313 the reference soil. This phytotoxicity of As in the polluted soil caused a decrease of the lettuce
314 seedling growth, as shown in SM5 ($69.02 \text{ mg of FW.plant}^{-1} \pm 13.73$ and $30.34 \text{ mg of FW.plant}^{-1}$
315 ± 4.97 for reference soil and the polluted soil, respectively). Moreover, while there was no
316 difference between the un-amended and amended reference soils, the Omega-3 Index
317 increased significantly for lettuces grown on polluted soil amended with ammonium sulfate.
318 Thus, addition of ammonium sulfate in the polluted soil seemed to reduce As induced lipid
319 peroxidation.

320

321 **4. Discussion**

322 Among the parameters controlling the behaviour of As in soils, redox conditions play a major
323 role. Experiments performed with microcosm systems allowing control of redox conditions
324 showed that low Eh values induced mobilization of As from a flood plain soil (Frohne et al.,
325 2011), a freshwater marsh delta soil (Shaheen et al., 2016) and an historically contaminated
326 coastal soil (LeMonte et al., 2017). Here, non-saturated conditions were maintained in
327 microcosms, so the mobility of As should mainly be controlled by other factors. Although
328 AsV adsorption on iron oxides is known to decrease with pH (Dixit and Hering, 2003), As
329 mobility in the polluted soil compared to the reference soil was probably not exclusively
330 linked to soil pH (8.2 and 8.3 in the reference and the polluted soil, respectively, Table 1). As
331 mobility could also be related to the higher concentration of phosphate in the polluted soil.
332 Indeed, phosphate concentration was ten times higher (and the potassium concentration two
333 times higher) in the polluted soil than in the reference soil. One hypothesis to explain this is

334 that the farmer might have provided higher quantities of KP fertilizer in this precise area in an
335 attempt to improve crop yields. Indeed plant growth was strongly affected by the undetected
336 pollution in this area of the field. Phosphate is an analogue of arsenate AsV (Smith and Naidu,
337 2009) which competes with As for adsorption sites on iron oxides. In the percolation water,
338 the highest total As concentrations were always observed with the KP fertilizer and could be
339 linked to the addition of phosphate. Consequently increased As mobility may have increased
340 the toxic impact on plant growth. In addition, as the polluted soil contained both more
341 phosphate and more As than the reference soil, the ratio between the density of adsorption
342 sites and concentrations of both elements was lower in the polluted than in the reference soil.
343 This has previously been observed in soils polluted with As, but with higher doses of
344 phosphate fertilizers (Davenport and Peryea, 1991; Peryea and Kammereck, 1997). In terms
345 of concentration, As in the microcosm' percolation water was in the range 2-5 mg.L⁻¹, in the
346 same range as concentrations already reported in water phases in contact with soils polluted
347 by different sources of As. Thus, Cao et al. (2003) found 5-6 mg.L⁻¹ of As in water leached
348 from soil polluted with a chromium-copper-arsenic (CCA) pesticide, presenting a total As
349 content of 135 mg.kg⁻¹, and Qi and Danahoe (2008) found 1 mg.L⁻¹ of As when they used
350 acid rainwater to leach a soil polluted by historical herbicide application and containing 300
351 mg.kg⁻¹ As.

352 MPN of As-transforming microbes suggested that the pollution induced an increase of the
353 abundance of active microorganisms able to modify As speciation in the soil (Figure 3). The
354 difference observed between the reference soil and the polluted soil was more marked for
355 AsIII-oxidizing than for AsV-reducing microbes, and that was true both for MPN and activity
356 tests: AsIII-oxidizing microbes were roughly 100 times more abundant in the polluted soil
357 (Figure 3A), and this soil presented a higher AsIII-oxidizing activity than the reference soil.
358 Thouin et al. (2016) showed that the AsIII-oxidizing rate increased with the As concentration

359 in samples of polluted technosoils, and suggested that the level of As concentration exerted a
360 selective pressure on the microbial community, increasing its global efficiency to oxidize
361 As^{III}. Here, whereas amendments did not influence the As^{III}-oxidizing activity of the
362 polluted soil (Figure 4A), the fertilizers exerted a clear influence on this activity in the
363 reference soil. As^{III}-oxidizing activity was diminished by the ammonium sulfate amendment
364 (Figure 4A). Yet, this fertilizer tended to decrease As mobility from soils towards percolation
365 water, in particular at the beginning of the experiment with the polluted soil (Figure 1B). Our
366 results are in agreement with those of a long-term (1929 to 2018) experiment studying the
367 effects of agricultural amendments on the behaviour of trace elements. In that experiment,
368 authors showed that ammonium-based fertilization induces an increase of immobilized As in
369 the soil, compared with other types of amendments, due to reduced As leaching (van Oort et
370 al., 2017). This phenomenon might be linked to H⁺ production during ammonium oxidation
371 (nitrification). Even if no macroscopic pH decrease was observed here, protonation of the
372 surface hydroxyl groups of iron oxides could increase As^V adsorption on these minerals in
373 the soil (Dixit and Hering, 2003). Reduced As mobility from solids to the water phase might
374 decrease As bioavailability, consequently reducing the selective pressure on the global
375 microbial community and decreasing its efficiency to oxidize As^{III}.

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

384 The solid and liquid phases of the soils contained AsV as the main As form, whereas the
385 MPN and activity of AsV-reducing microbes was important according to Figures 3 and 4.
386 Even when As is mainly in the form of AsV, it is not a static distribution of AsV between
387 solids and water phases, but rather a dynamic equilibrium, involving both microorganisms
388 oxidizing AsIII and microorganisms reducing AsV. In this system, bioreduction of AsV might
389 play a role in As mobilisation. Turpeinen et al. (1999) have already observed that microbes
390 increased the As mobility, mainly as AsV, from soils incubated in aerated conditions. We
391 found AsV as the main As form leached from the polluted soil, with no significant effect of
392 the amendments on this speciation, in soils that were not saturated with water, i.e. with no
393 limitation of oxygen availability. Our results must be confirmed with further experiments
394 performed with diverse polluted sites, and comparing biotic to abiotic conditions, and non-
395 saturated to saturated conditions. However, they suggest that microbial parameters would be
396 very sensitive bio-indicators of the dynamics of As concentrations and speciation in the water
397 phase.

398 Considering the pot experiments with plants, As transfer was significantly more important in
399 lettuces grown on the polluted soil. These results showed that As was highly mobile and as a
400 consequence highly phytoavailable in the polluted soil. This correlated with results from the
401 microcosms where As was shown to be more mobile from soil to water within the polluted
402 soil compared to the reference soil. In lettuces, amendment with KP induced an increase of
403 both As and P contents in lettuce leaves, as already observed by Cao and Ma (2004).
404 Phosphate-induced plant As uptake may have been related to the slight increase of As
405 concentration in the leachate. The lettuces' Omega-3 Index showed that the high As content in
406 the polluted soil increased lipid peroxidation in lettuce leaves compared to the reference soil
407 (Figure 6). Bustingorri et al. (2017) observed an increase of lipid peroxidation in soybean
408 plants linked to the presence of As. Here, the increase of lipid peroxidation induced by the

409 polluted soil, corresponding to nearly 50 % decrease of the Omega3 index (Figure 6) was
410 coupled with a decrease of the lettuce seedling growth. Thus, in agreement with findings
411 obtained from previous studies, the excessive production of ROS induced by As exposure
412 promotes lipid peroxidation and causes damage in thylakoid membranes that may lower
413 photosynthetic efficiency (Abbas et al., 2018). In agreement with previous results of As
414 transfer from soil to lettuces (Figure 5), these results confirm that As is highly phytoavailable
415 and as a consequence highly phytotoxic in the polluted soil.

416 The amounts of agricultural amendments applied to fields can influence the mobility,
417 bioavailability and toxicity of As. Addition of ammonium sulfate to polluted soil was
418 associated with an increase of the Omega-3 Index (Figure 6) and as a consequence a reduction
419 of lipid peroxidation in lettuce leaves while no decrease was observed concerning the As
420 uptake. This was not observed in the reference un-polluted soil, suggesting that the decrease
421 of lipid peroxidation is possibly influenced by the contribution of ammonium sulfate to As
422 detoxification. These results are in agreement with several studies showing that, under heavy
423 metal stress, an application of sulfur stimulates plants (Mishra et al, 2008; Duan et al, 2013).
424 In fact, sulfur (S) in higher plants is a vital component for the synthesis of some amino acids
425 (Cysteine and Methionine) and metabolites such as glutathione (GSH) and phytochelatin
426 (PCs) involved in the detoxification of heavy metals. Some studies relate that As induces ROS
427 production in plants leading to lipid peroxidation (Shukla et al., 2018) and the synthesis of
428 PCs enzymatically synthesized from GSH. Then, PCs form complexes with As before its
429 sequestration into the vacuoles through ABC transporters (Song et al., 2010). This induction
430 of the biochemical pathways of plant S metabolism increases the S requirement under As
431 stress (Leao et al., 2014; Khare et al., 2017). Therefore, the decrease of lipid peroxidation in
432 lettuces grown on the polluted soil amended with ammonium sulfate may be explained by an
433 improvement of the intracellular As detoxification processes due to an increase of the

434 synthesis of GSH and PCs linked to an increase of S assimilation by plants. Similarly,
435 nitrogen (N) could also play an important role in the detoxification of heavy metals in plants.
436 Indeed, many studies related that, under abiotic stress, nitrate supply stimulates root nitric
437 oxide production (Sun et al, 2010; Simontacchi et al., 2015) which is a bioactive signalling
438 molecule involved in plants' response to heavy metal stress by detoxifying ROS (Hassan et
439 al., 2005; Kaur et al., 2015). Thus, according to these results, it seems that a better N and S
440 nutrition status may protect the plants from As and as a consequence lead to a decrease of
441 oxidative stress. However, more research is needed to fully understand the mechanisms of
442 interactions between ammonium sulfate and As in plants and the importance of ammonium
443 sulfate in detoxification.

444

445

446 **5. Conclusions**

447 The addition of fertilizing amendments, at the real average dose applied on the sites, did not
448 strongly influence the speciation and the quantity of mobile As. Observed trends were an
449 increase of As mobility with KP fertilizer, a decrease of mobility with ammonium sulfate
450 amendment, and no effect of organic amendments. The quantity of As mobilised in the
451 percolation water was bioavailable for plants and soil microorganisms. Results of an original
452 combination of active As-transforming bacteria enumeration and As-related microbial activity
453 tests showed that microbial parameters were strongly linked to As levels in water and in soils.
454 In particular, microbial As^{III}-oxidizing activity proved to be very sensitive to low doses of
455 ammonium sulfate. Although the impacts of KP and ammonium sulfate on As speciation were
456 insignificant, the small effects observed on plant As-uptake and bacterial activities may have
457 an impact in the long term. Consistent results were observed with microbial and plant
458 parameters, in particular concerning the effect of ammonium sulfate fertilization. These tests

459 could be used as sensitive indicators of As bioavailability and toxicity in soils. Whereas
460 microbial parameters can be quicker to obtain than plant indicators, As bioavailability for
461 plants will always be very important to determine, particularly when these plants are
462 cultivated and consumed.

463

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471

472 **Figure captions**

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

480

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

487

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

493

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

500

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

505

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

511

512

513 **References**

514 Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N., Khan, M., Amjad, M., Hussain M.
515 Natasha, 2018. Arsenic Uptake, Toxicity, Detoxification, and Speciation in Plants:
516 Physiological, Biochemical, and Molecular Aspects. Int J Environ Res Public Health. 15, 59.
517 <https://doi.org/10.3390/ijerph15010059>

518 AFNOR, 2012. In: Standard X31 233, Quality of Soil, Contaminated Soil Effects on Fatty
519 Acid Composition of Lactuca Sativa Leaves. AFNOR, Paris.

520 Agnihotri, A., Seth, C.S., 2016. Exogenously applied nitrate improves the
521 photosynthetic performance and nitrogen metabolism in tomato (*Solanumlycopersicum* L. cv
522 *Pusa Rohini*) under arsenic (V) toxicity. Physiol. Mol. Biol. Plants, 22, 341–349.
523 <https://doi.org/10.1007/s12298-016-0370-2>

524

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

528

529 Battaglia-Brunet, F., Dictor, M-C., Garrido, F., Crouzet, C., Morin, D., Dekeyser, K., Clarens,
530 M., Baranger, P., 2002. An arsenic(III)-oxidizing bacterial population: selection,
531 characterization, and performance in reactors. *J. Appl. Microbiol.* 93, 656–667.
532 <https://doi.org/10.1046/j.1365-2672.2002.01726.x>

533

534 Bauer, M., Blodau, C., 2006. Mobilization of arsenic by dissolved organic matter from iron
535 oxides, soils and sediments. *Sci. Total Environ.*, 354, 179-190.
536 <https://doi.org/10.1016/j.scitotenv.2005.01.027>

537

538 Bausinger, T., Preuß, J., 2005. Environmental remnants of the first world war: soil
539 contamination of a burning ground for arsenical ammunition. *Bull. Environ. Contam.*
540 *Toxicol.*, 74, 1045–1053. <http://dx.doi.org/10.1007/s00128-005-0686-z>.

541

542 Bausinger, T., Bonnaire, E., Preuß, J., 2007. Exposure assessment of a burning ground for
543 chemical ammunition on the Great War battlefields of Verdun. *Sci. Total Environ.*, 382, 259–
544 <http://dx.doi.org/271>. <https://doi.org/10.1016/j.scitotenv.2007.04.029>

545

546 Beesley, L., Inneh, O.S., Norton G.J., Moreno-Jimenez E., Pardo T., Clemente R., Dawson J.
547 J.C., 2014. Assessing the influence of compost and biochar amendments on the mobility and
548 toxicity of metals and arsenic in a naturally contaminated mine soil. *Env. Pol.* 186, 195-202.
549 <https://doi.org/10.1016/j.envpol.2013.11.026>

550

551 Brackhage, C., Huang, J.-H., Schaller, J., Elzinga, E.J., Dudel, E.G., 2014. Readily available
552 phosphorous and nitrogen counteract for arsenic uptake and distribution in wheat (*Triticum*
553 *aestivum* L.). Sci. reports, 4, 4944. <https://doi.org/10.1038/srep04944>

554

555 Bustingorri, C., Noriega, G., Lavado, R. S., Balestrasse, K., 2017. Protective effect exerted by
556 soil phosphorus on soybean subjected to arsenic and fluoride, Redox Rep. 22, 353-360.
557 <https://doi.org/10.1080/13510002.2016.1276253>

558

559 Cao, X., Ma, L.Q., Shiralipour A., 2003. Effects of compost and phosphate amendments on
560 arsenic mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris vittata* L.. Env.
561 Pol. 126, 157-167. [https://doi.org/10.1016/S0269-7491\(03\)00208-2](https://doi.org/10.1016/S0269-7491(03)00208-2)

562

563 Cao, X.D., Ma, L.Q., 2004. Effects of compost and phosphate on plant arsenic accumulation
564 from soils near pressure-treated wood. Env. Pol. 132, 435-442.
565 <https://doi.org/10.1016/j.envpol.2004.05.019>

566

567 Davenport, J.R., Peryea, F.J., 1991. Phosphate fertilizers influence leaching of lead and
568 arsenic in a soil contaminated with lead arsenate. Wat. Air. Soil Poll., 57-58, 101-110.
569 <https://doi.org/10.1186/s40064-015-0868-z>

570

571 Dixit, S., Hering, J. G., 2003. Comparison of Arsenic(V) and Arsenic(III) sorption onto iron
572 oxide minerals: implications for arsenic mobility, *Environ. Sci. Technol.* 37, 82-4189.
573 <https://doi.org/10.1021/es030309t>

574

575 Duan, G., Liu, W., Chen, X., Hu, Y., Zhu, Y., 2013. Association of arsenic with nutrient
576 elements in rice plants. *Metallomics Integr. Biomatel. Sci.* 5, 784-792.
577 <https://doi.org/10.1039/c3mt20277a>

578

579 EGS, 2008: EuroGeoSurveys Geochemistry Working Group. EuroGeoSurveys geochemical
580 mapping of agricultural and grazing land in Europe (GEMAS) – field manual. Norges
581 geologiske undersøkelse NGU report 2008.038, 46 pp.
582 https://www.ngu.no/upload/Publikasjoner/Rapporter/2008/2008_038.pdf

583

584 Frohne, T., Rinklebe, J., Diaz-Bone, R.A., Laing, G.D., 2011. Controlled variation of redox
585 conditions in a floodplain soil: Impact on metal mobilization and biomethylation of arsenic
586 and antimony. *Geoderma*, 160, 3–4, 414-424.
587 <https://doi.org/10.1016/j.geoderma.2010.10.012>

588

589 Hassan, M.J., Wang, F., Ali, S., Zhang, G., 2005. Toxic effect of Cd on rice as affected by
590 nitrogen fertilizer form. *Plant Soil*. 277, 359–365. <https://doi.org/10.1007/s11104-005-8160-6>

591

592 Haynes R.J., Swift R.S., 1989. Effect of rewetting air-dried soils on pH and accumulation of
593 mineral nitrogen. *J. Soil Sci.* 40, 341-347. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2389.1989.tb01278.x)
594 [2389.1989.tb01278.x](https://doi.org/10.1111/j.1365-2389.1989.tb01278.x)

595

596 Huang, H., Jia, Y., Sun, G.X., Zhu Y.G., 2012. Arsenic speciation and volatilization from
597 flooded paddy soils amended with different organic matters. *Environ. Sci. Technol.* 46, 2163-
598 2168. <https://doi.org/10.1021/es203635s>

599

600 Hube, D., 2017. Industrial-scale destruction of old chemical ammunition near Verdun: a
601 forgotten chapter of the Great War, *First World War Studies*,
602 <https://doi.org/10.1080/19475020.2017.1393347>

603

604 Kaur, G., Singh, H.P., Batish, D.R., Priyanka, M., Kohli, R., K., Rishi, V., 2015. Exogenous
605 nitric oxide (NO) interferes with lead (Pb)-induced toxicity by detoxifying reactive oxygen
606 species in hydroponically grown wheat (*Triticum aestivum*) roots. *PLoS ONE* 10, e0138713.
607 [d https://doi.org/10.1371/journal.pone.0138713](https://doi.org/10.1371/journal.pone.0138713)

608

609 Khare, D., Choi, H., Huh, S.U., Bassin, B., Kim, J., Martinoia, E., Sohn, K.H., Paek, K.-H.,
610 Lee, Y., 2017. *Arabidopsis* ABCG34 contributes to defense against necrotrophic pathogens by
611 mediating the secretion of camalexin. *PNAS*, 114, 1–9.
612 <https://doi.org/10.1073/pnas.1702259114>

613

614 Kim M.J., 2001. Separation of inorganic arsenic species in groundwater using ion exchange
615 method. *Bull. Environ. Contam. Toxicol*, 67, 46-51. <https://doi.org/10.1007/s001280089>

616

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

620

621 Inskeep, W.P., Macur, R.E., Hamamura, N.H., Warelow, T.P., Ward, S.A., Santini, J.M.,
622 2007. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes.
623 *Environmental Microbiology*, 9, 934-943. <https://doi.org/10.1111/j.1462-2920.2006.01215.x>

624

625 Leão, G.A., Oliveira, J.A., Farnese, F.S., Gusman, G.S., Felipe R.T.A., 2014. Sulfur
626 metabolism: Different tolerances of two aquatic macrophytes exposed to arsenic. *Ecotox.*
627 *Environ. Safety*, 105, 36-42. <https://doi.org/10.1016/j.ecoenv.2014.03.011>

628

629 Le Guédard, M., Schraauwers, B., Larrieu, I., Bessoule, J.J., 2008. Development of a
630 biomarker for metal bioavailability: the lettuce fatty acid composition. *Environ. Toxicol.*
631 *Chem.*, 27, 1147-1151. <https://doi.org/10.1897/07-277.1>

632

633 LeMonte, J.J., Stuckey, J.W., Sanchez, J.Z., Tappero, R., Rinklebe, J., Sparks, D.L., 2017.
634 Sea Level Rise Induced Arsenic Release from Historically Contaminated Coastal Soils. *Env.*
635 *Sci. Technol.*, 51, 5913-5922. DOI: 10.1021/acs.est.6b06152

636

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

641

642 Macur, R.E., Jackson, C.R., Botero, L.M., McDermott, T., Inskeep, W.P., 2004. Bacterial
643 populations associated with the oxidation and reduction of arsenic in an unsaturated soil.
644 *Environ. Sci. Technol.*, 38, 104-111. <https://doi.org/10.1021/es034455a>

645

646 Mishra, S., Srivastava, S., Tripathi, R.D., et al., 2008. Thiol metabolism and antioxidant
647 systems complement each other during arsenate detoxification in *Ceratophyllum demersum L.*
648 *Aquat. Toxicol.*, 86, 205-215. <https://doi.org/10.1016/j.aquatox.2007.11.001>

649

650 Peryea, F., Kammereck, R., 1997. Phosphate-Enhanced Movement of Arsenic Out of Lead
651 Arsenate-Contaminated Topsoil and Through Uncontaminated Subsoil. *Water, Air, & Soil*
652 *Poll.* 93, 243–254. <https://doi.org/10.1023/A:1022196704677>

653

654 Qi, Y., Donahoe, R.J., 2008. The environmental fate of arsenic in surface soil contaminated
655 by historical herbicide application. *Sci. Tot. Environ.*, 405, 246-254.
656 <https://doi.org/10.1016/j.scitotenv.2008.06.043>

657

658 Redman, A.D., Macalady, D.L., Ahmann, D., 2002. Natural organic matter affects arsenic
659 speciation and sorption onto hematite, Environ. Sci. Technol., 36, 2889-2896.
660 <https://doi.org/10.1021/es0112801>

661

662 Saada, A., Breeze, D., Crouzet, C., Cornu, S., Baranger, P., 2003. Adsorption of arsenic(V) on
663 kaolinite and on kaolinite-humic acid complexes. Role of humic acid nitrogen groups.
664 Chemosphere, 51, 757-763. [https://doi.org/10.1016/S0045-6535\(03\)00219-4](https://doi.org/10.1016/S0045-6535(03)00219-4)

665

666 Shaheen, S.M., Rinklebe, J., Frohne, T., White, J.R., DeLaune, R.D., 2016. Redox effects on
667 release kinetics of arsenic, cadmium, cobalt, and vanadium in Wax Lake Deltaic freshwater
668 marsh soils. Chemosphere, 150, 740-748. <https://doi.org/10.1016/j.chemosphere.2015.12.043>.

669

670 Shukla, T., Khare, R., Kumar, S., Lakhwani, D., Sharma, D., Asif, M. H., Trivedi P.K. 2018.
671 Differential transcriptome modulation leads to variation in arsenic stress response in
672 *Arabidopsis thaliana* accessions. J. Hazard. Mater. 351 1–10.
673 <https://doi.org/10.1016/j.jhazmat.2018.02.031>

674

675 Simontacchi, M., Galatro, A., Ramos-Artuso, F., Santa-Maria, G.E., 2015. Plant survival in a
676 changing environment: the role of nitric oxide in plant responses to abiotic stress. Front. Plant
677 Sci., 6, 977. <https://doi.org/10.3389/fpls.2015.00977>

678

679 Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behavior and distribution of
680 arsenic in natural waters. *Appl. Geochem.*, 17, 517-568. [https://doi.org/10.1016/S0883-](https://doi.org/10.1016/S0883-2927(02)00018-5)
681 2927(02)00018-5

682

683 Smith, E., Naidu, R., Alston, M.M., 1998. Arsenic in the soil environment: a review. *Adv.*
684 *Agron.*, 64, 149-195. [https://doi.org/10.1016/S0065-2113\(08\)60504-0](https://doi.org/10.1016/S0065-2113(08)60504-0)

685

686 Smith, E., Naidu, R., 2009. Chemistry of inorganic arsenic in soils: kinetics of arsenic
687 adsorption–desorption. *Environ. Geochem. Health.*, 31, 49–59.
688 <https://doi.org/10.1007/s10653-008-9228-z>

689

690 Song, W.Y, Park, J., Mendoza-Cózatl, D.G., Suter-Grotemeyer, M., Shim, D., Hörtensteiner,
691 S., Geisler, M., Weder, B., Rea, P.A., Rentsch, D., Schroeder, J.I., Lee, Y., Martinoia, E.,
692 2010. Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin
693 transporters. *Proc. Natl Acad Sci U S A.*, 107, 21187-21192.
694 <https://doi.org/10.1073/pnas.1013964107>

695

696 Su, S., Zeng, X., Bai, L., Li, L., Duan, R., 2011. Arsenic biotransformation by arsenic-
697 resistant fungi *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and
698 *Fusarium oxyporum* CZ-8F1. *Sci.Tot. Environ.*, 409, 5057-5062.
699 <https://doi.org/10.1016/j.scitotenv.2011.08.039>

700

701 Sun, L.R., Hao, F.S., Lu, B.S., Ma, L.Y., 2010. Multiple roles of nitric oxide in root
702 development and nitrogen uptake. *Plant Signal. Behav.*, 12, e1274480.
703 <https://doi.org/10.1080/15592324.2016.1274480>

704

705 Thouin, H., Le Forestier, L., Gautret, P., Hube, D., Laperche, V., Dupraz, S., and Battaglia-
706 Brunet, F., 2016. Characterization and mobility of arsenic and heavy metals in soils polluted
707 by the destruction of arsenic-containing shells from the Great War. *Sci. Tot. Environ.*, 550,
708 658–669. <https://doi.org/10.1016/j.scitotenv.2016.01.111>

709

710 Thouin, H., Battaglia-Brunet F., Norini M.P., Le Forestier L., Charron M., Dupraz S., Gautret
711 P., 2018. Influence of environmental changes on the biogeochemistry of arsenic in a soil
712 polluted by the destruction of chemical weapons: a mesocosm study. *Sci. Tot. Environ.*, 627,
713 216-226. <https://doi.org/10.1016/j.scitotenv.2018.01.158>

714

715 Turpeinen, R., Pansar-Kallio, M., Häggblom, M., Kairesalo, T., 1999. Influence of microbes
716 on the mobilization, toxicity and biomethylation of arsenic in soil. *Sci. Tot. Environ.*, 236,
717 173-180. [https://doi.org/10.1016/S0048-9697\(99\)00269-7](https://doi.org/10.1016/S0048-9697(99)00269-7)

718

719 Van oort, F., Paradelo, R., Proix, N., Breuil, S., Delarue, G., Trouvé, A., Baize, D., Monna,
720 F., Richard, A., 2017. Arsenic et vieilles parcelles. *Etude et Gestion des Sols* 24, 99-126.
721 <http://www.fabricemonna.com/wp-content/uploads/2017/11/2017-egs.pdf>

722

723 Vergara Gallardo, M., Bohari, Y., Astruc, A., Potin-Gautier M., Astruc, M., 2001. Speciation
724 analysis of arsenic in environmental solids reference materials by high-performance liquid-
725 chromatography-hydride generation-atomic fluorescence spectrometry following
726 orthophosphoric acid extraction. *Anal. Chim. Acta*, 441, 257-268.
727 [https://doi.org/10.1016/S0003-2670\(01\)01114-X](https://doi.org/10.1016/S0003-2670(01)01114-X)

728

729 Yamamura, S., Watanabe, M., Yamamoto, N., Sei, K., Ike, M., 2009. Potential for microbially
730 mediated redox transformations and mobilization of arsenic in uncontaminated soils.
731 *Chemosphere* 77, 169-174. <https://doi.org/10.1016/j.chemosphere.2009.07.071>

732

733 Zarcinas, B.A., Cartwright, B., Spouncer, L.R., 1987. Nitric acid digestion and multi-element
734 analysis of plant material by inductively coupled plasma spectrometry. *Com. Soil Sci. Plant*
735 *Anal.*, 18, 131-146. <https://doi.org/10.1080/00103628709367806>

Figure 1

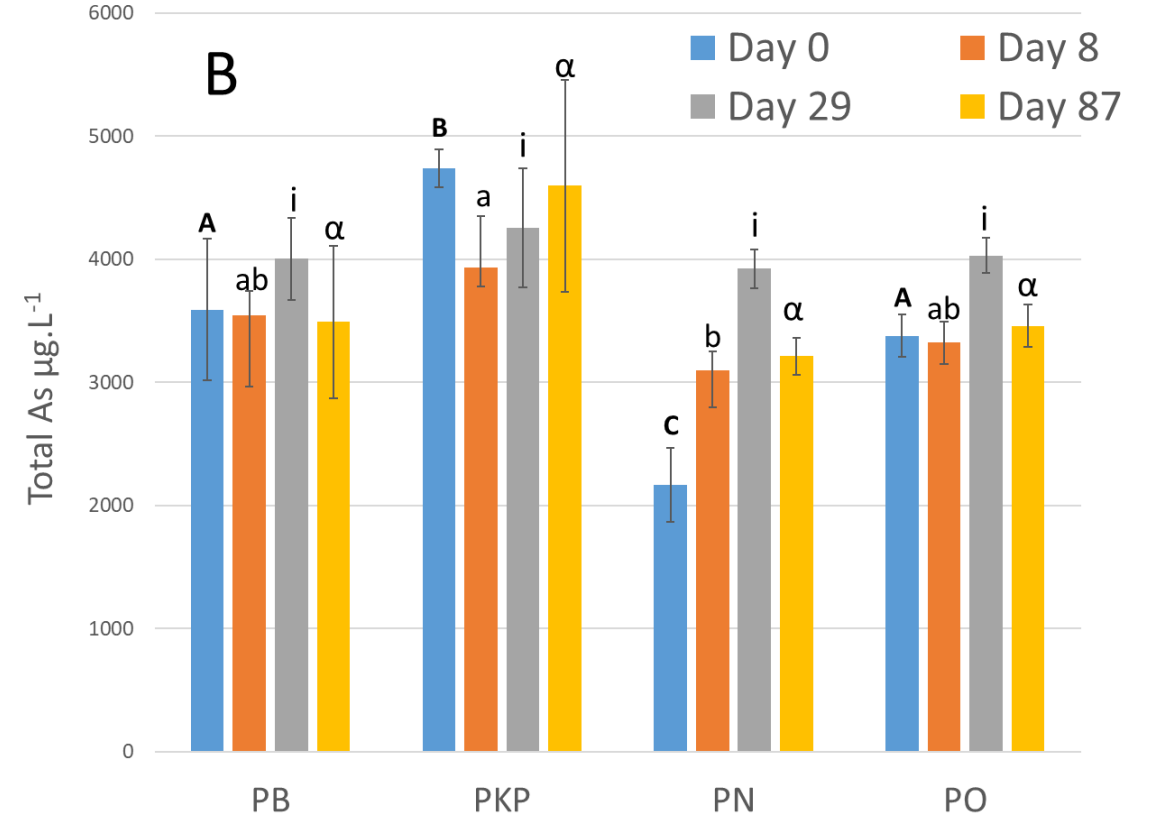
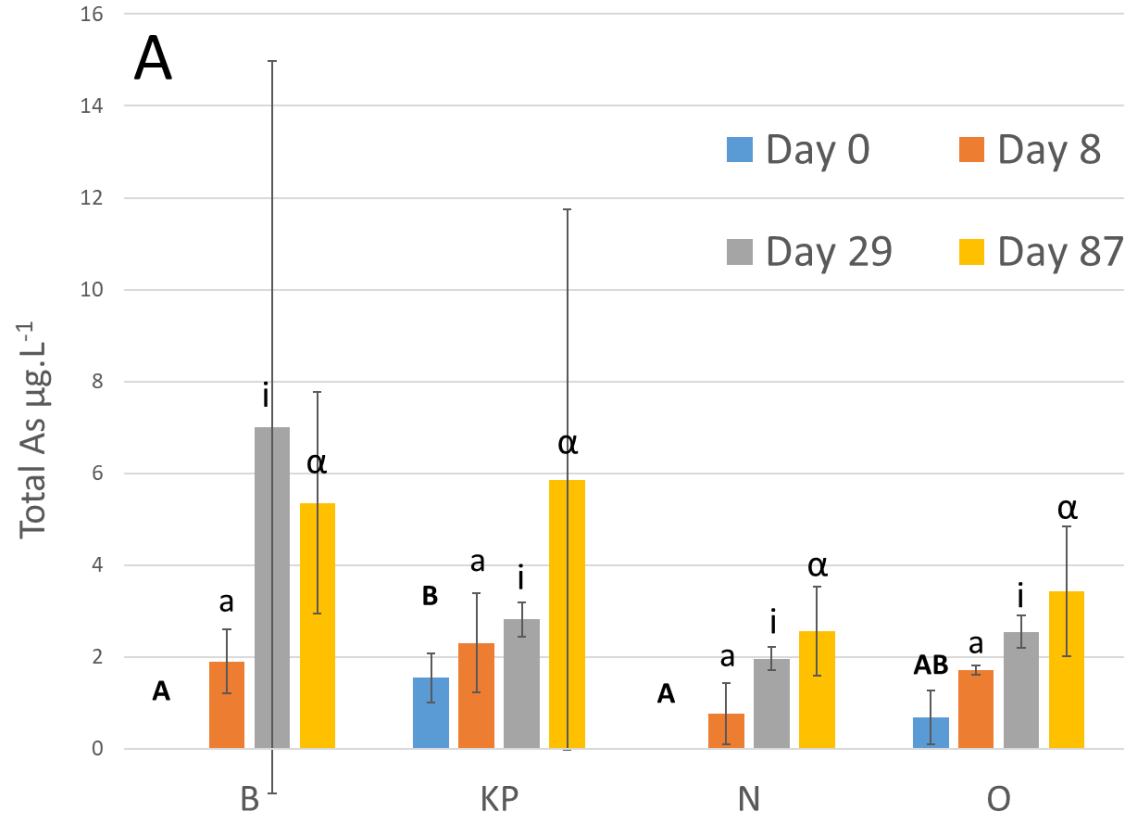


Figure 2

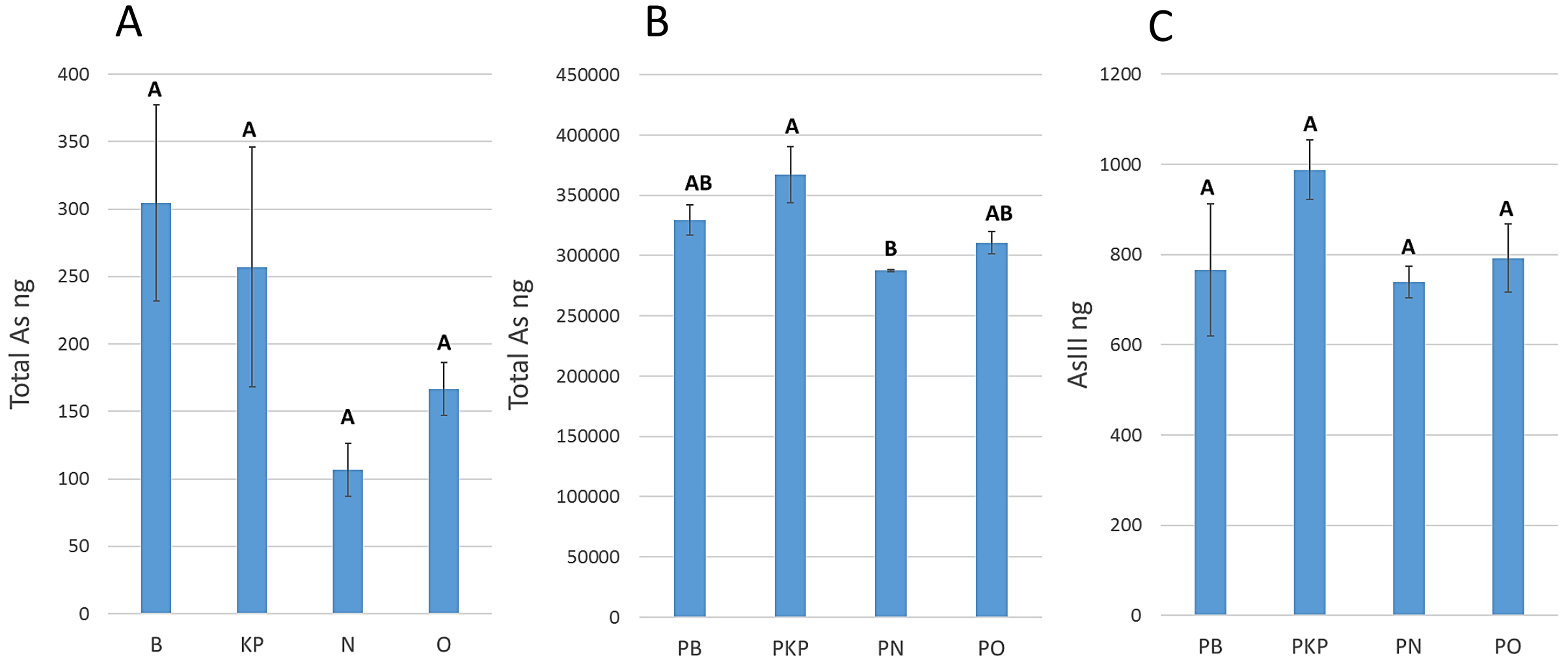


Figure 3

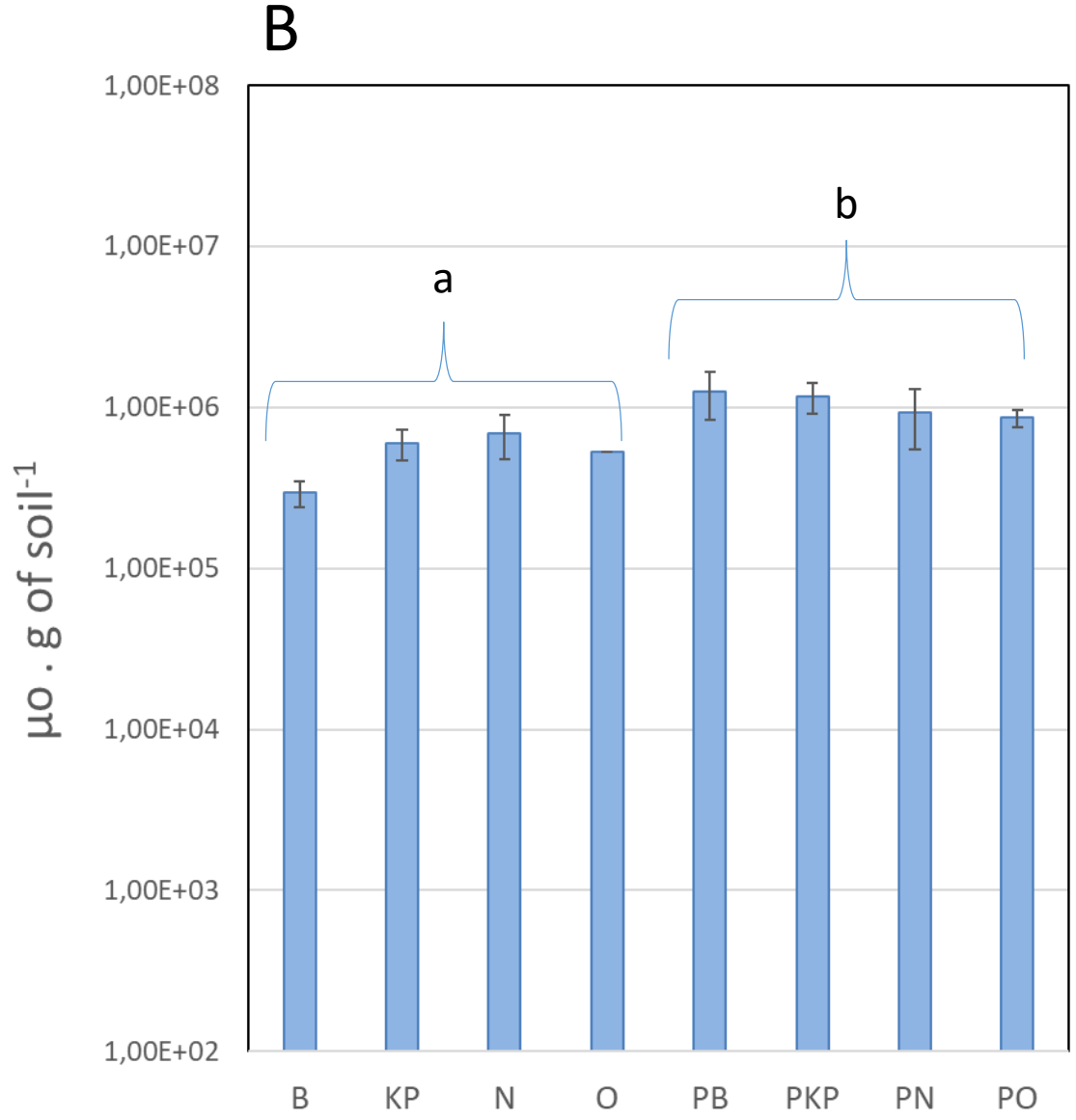
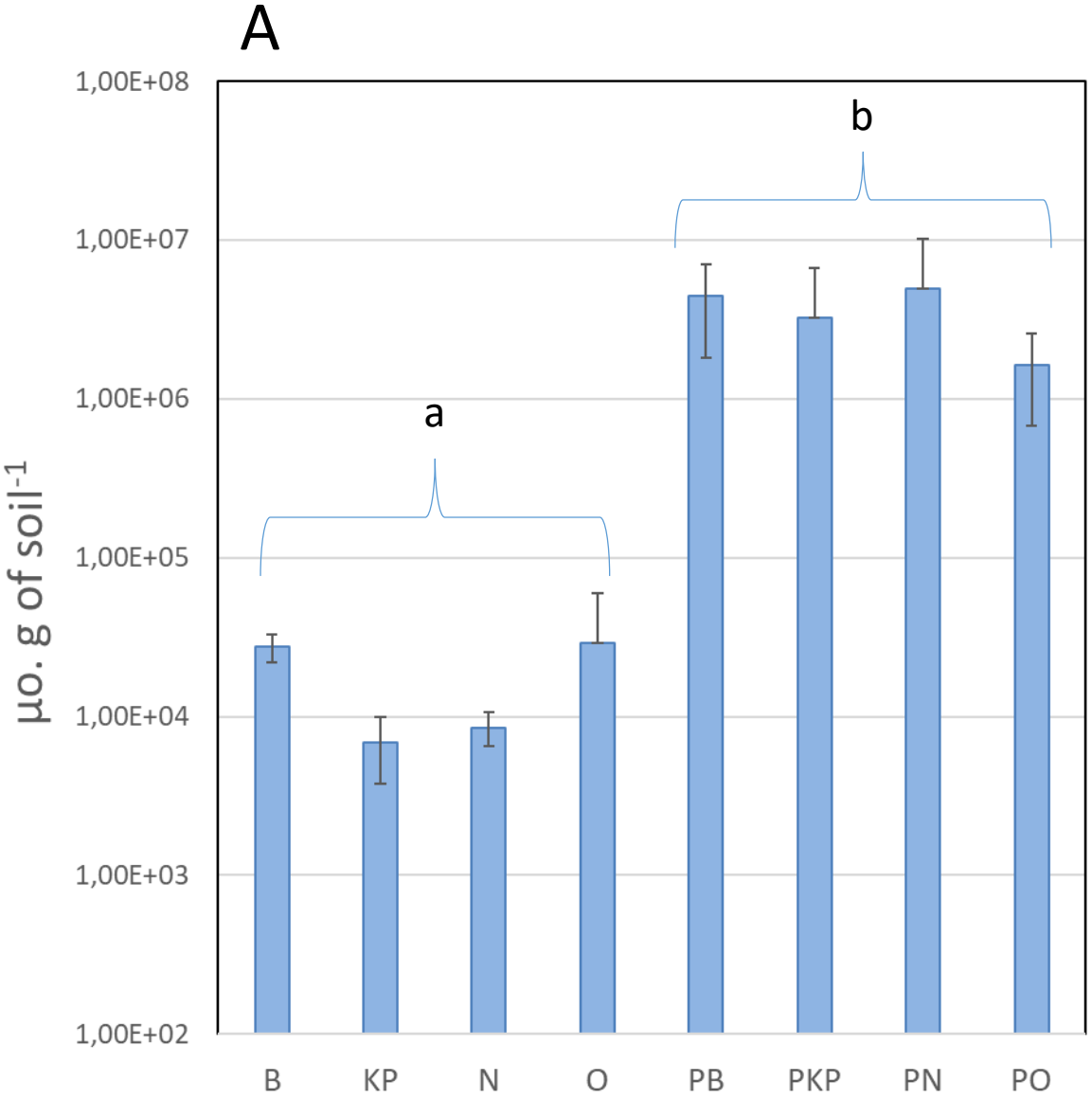
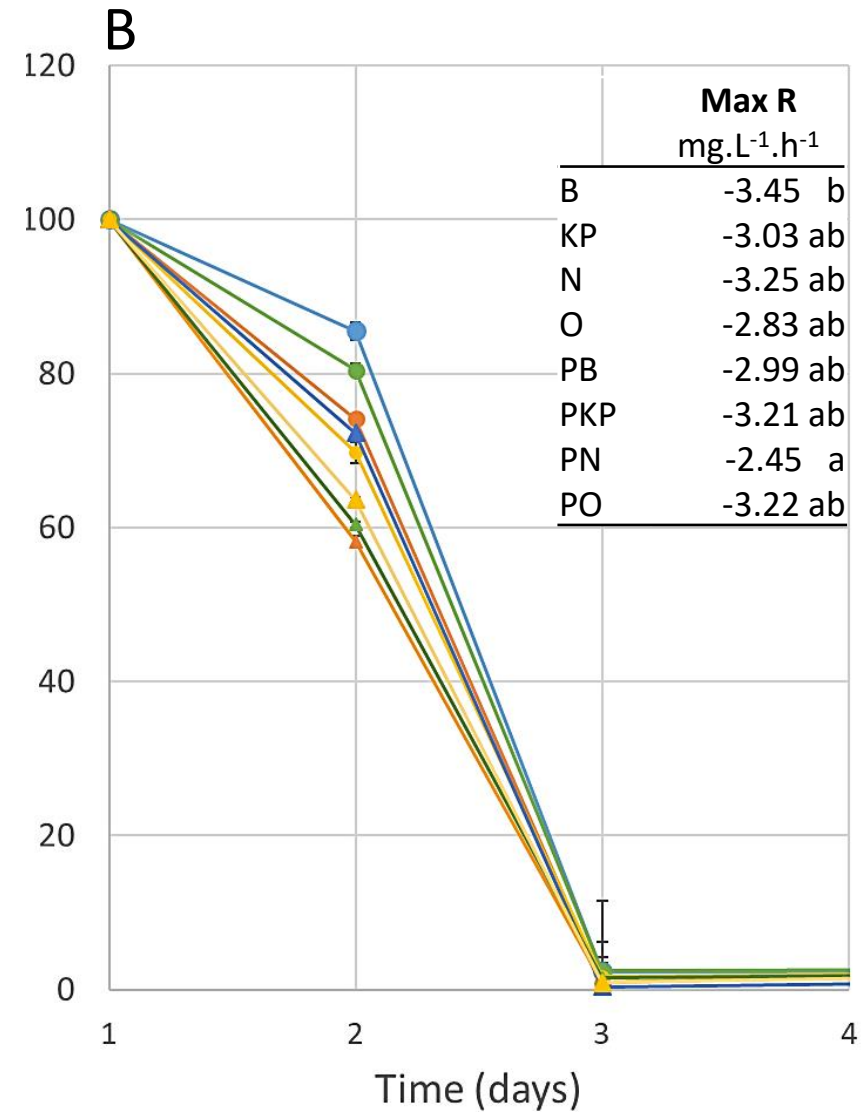
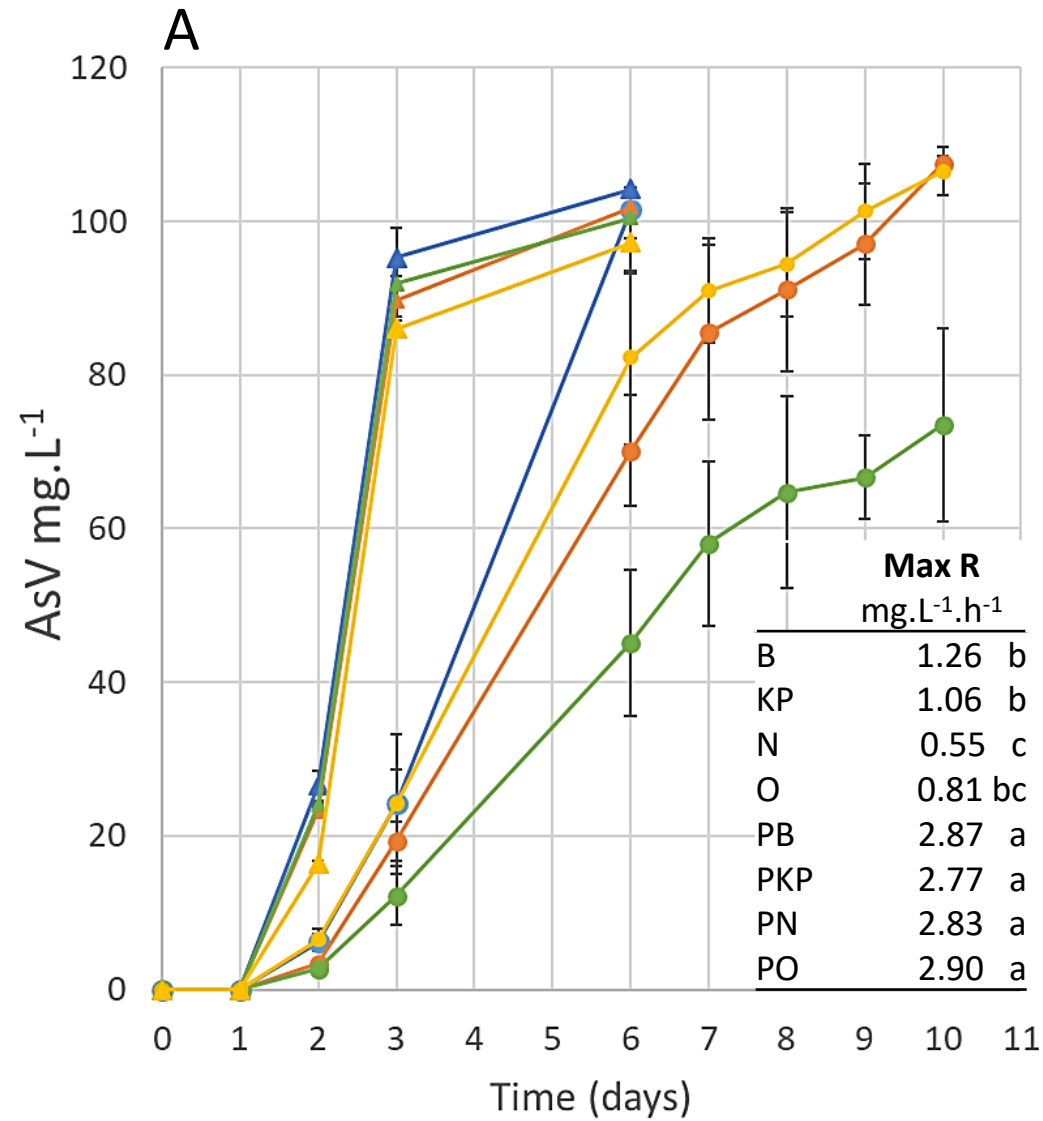


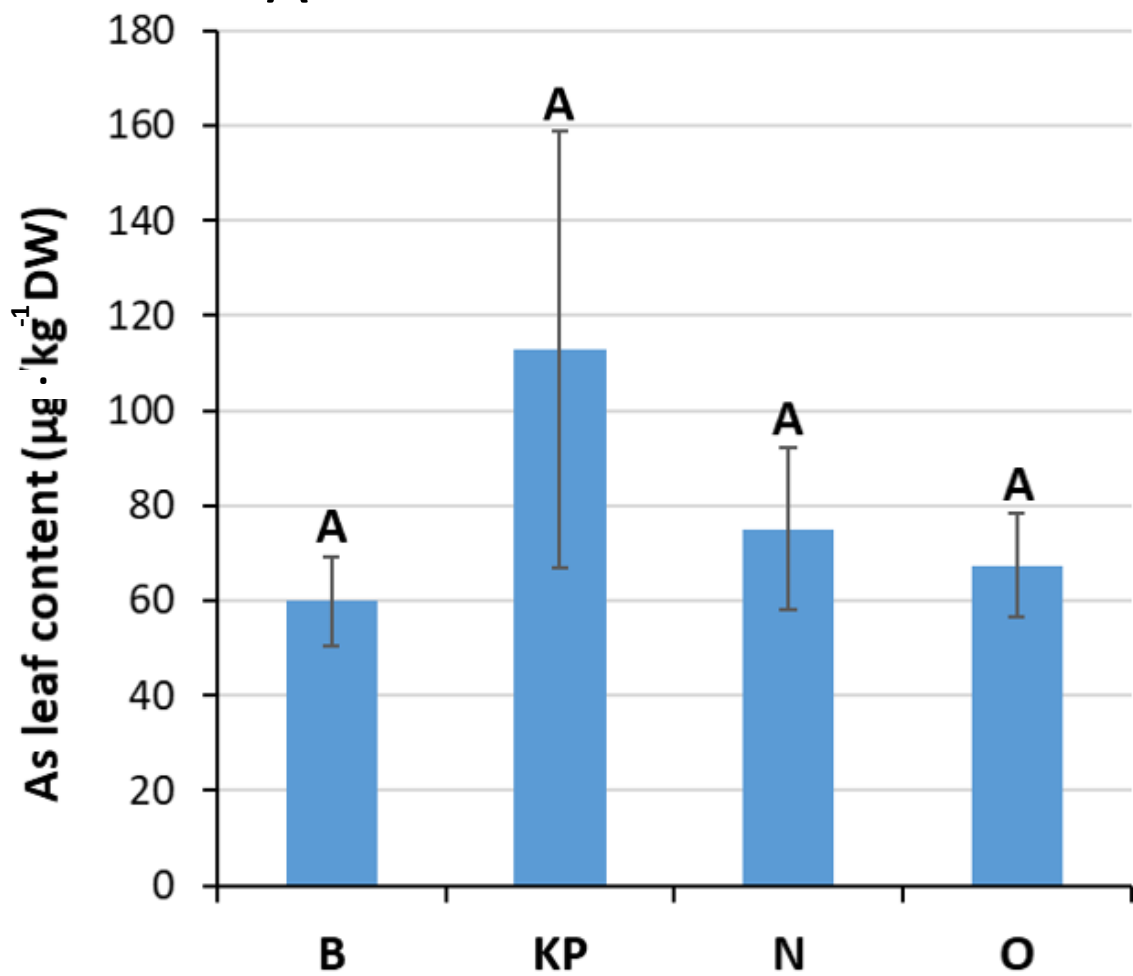
Figure 4



● B ● KP ● N ● O ▲ PB ▲ PKP ▲ PN ▲ PO

Figure 5

A



B

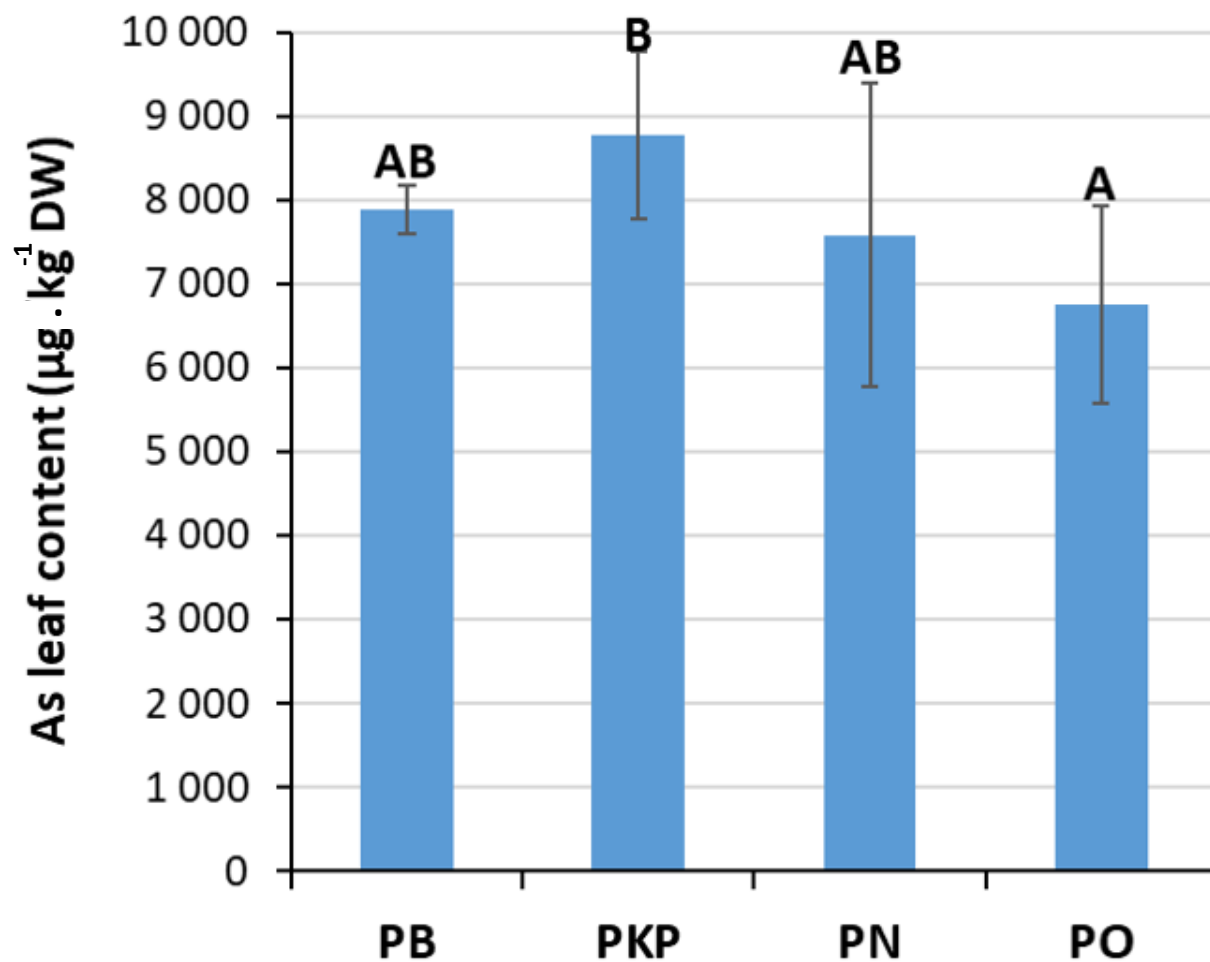
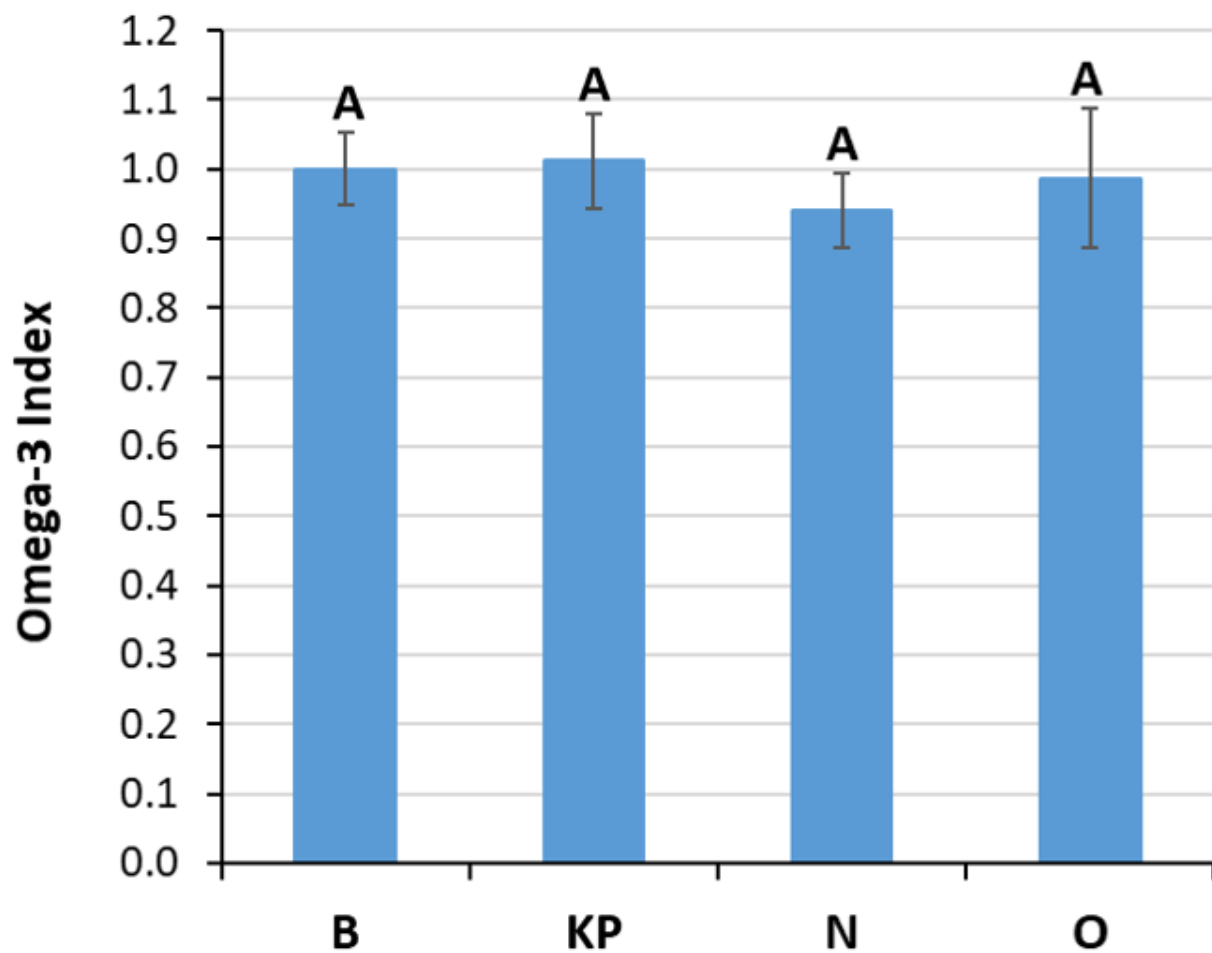
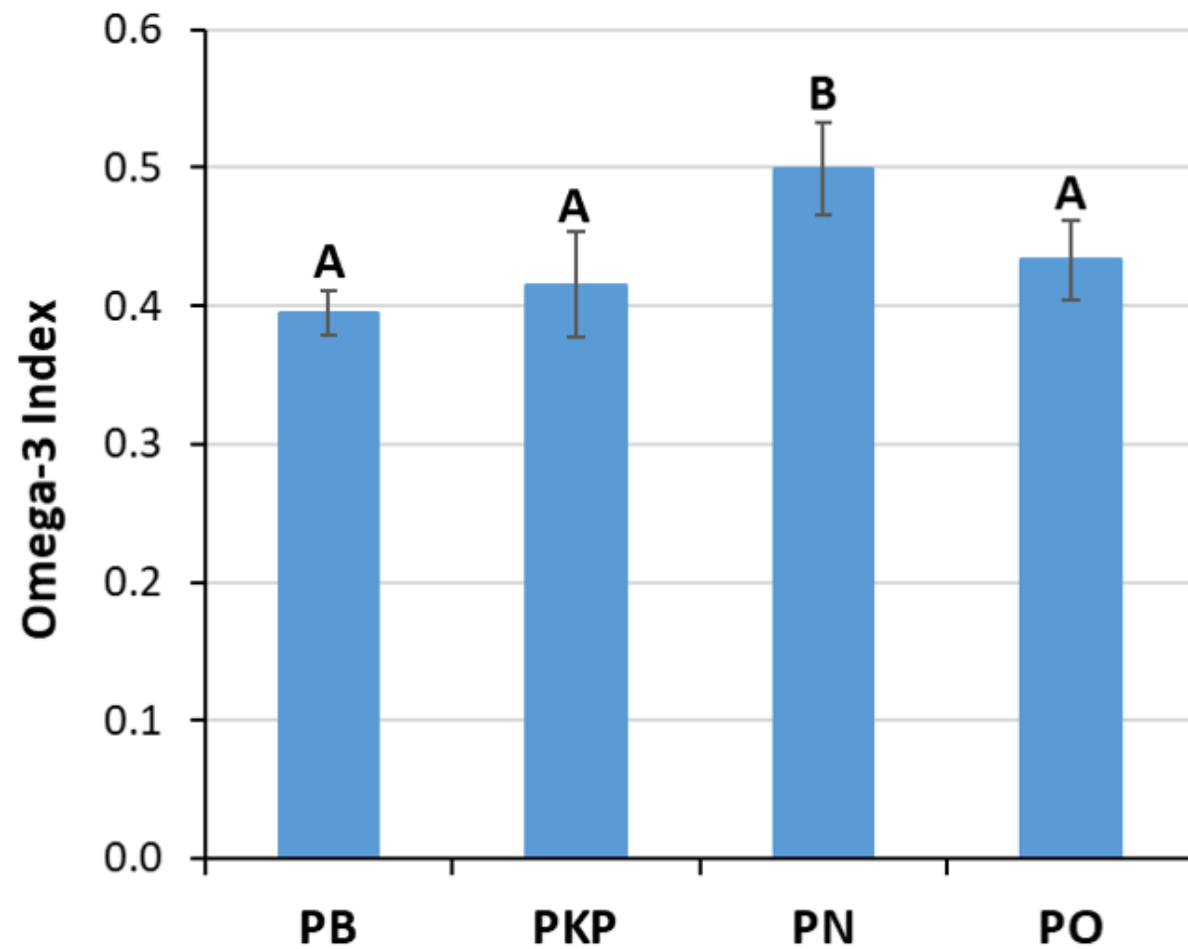


Figure 6

A



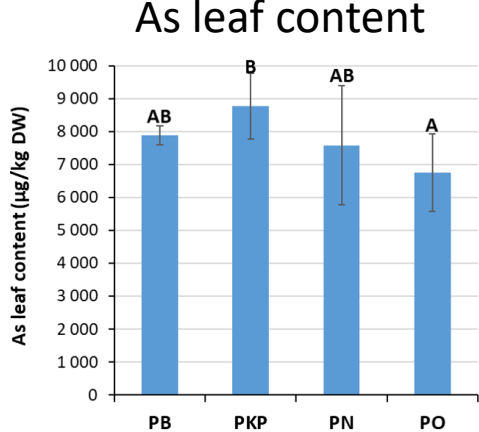
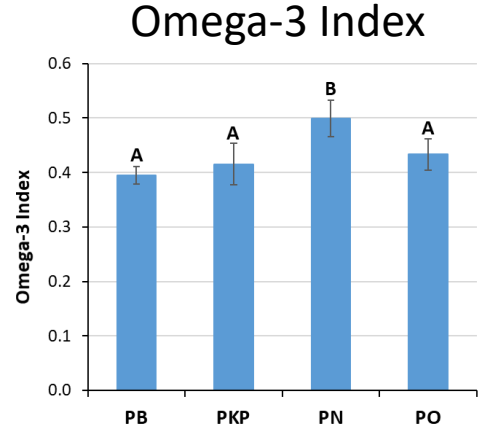
B



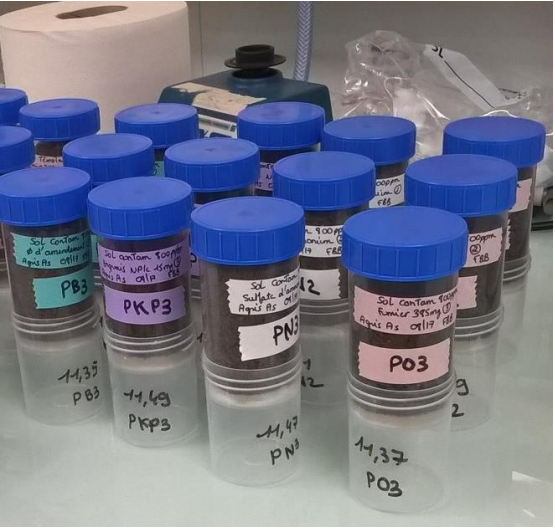
Phytotoxicity tests
Omega3-index with
lettuce



**Agricultural
amendments KP, N, O**



Soil
Polluted with As



Microcosm experiments:
Transfer toward water

