

1 Effectiveness of various sorbents and biological oxidation in the removal of arsenic species from
2 groundwater

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14 Environmental Context

15 Arsenic contamination of aquifers is a worldwide public health concern and several technologies
16 have been developed to reduce arsenic concentrations below the limit imposed by World Health
17 Organization. We investigated the efficiency of new and conventional materials for arsenic removal
18 from groundwater and found that iron-based sorbents have great affinity for arsenic even if
19 groundwater composition can depress their ability to bind arsenic. Moreover, we showed that the
20 use of microorganisms can enhance the efficiency of adsorption in the removal of arsenic from
21 groundwater.

22

23 Abstract

24 Batch experiments were conducted to evaluate As(III) and As(V) adsorption capacity of five
25 sorbents (*i.e.*, biochar, chabazite, ferritin-based material, goethite and nano zero-valent iron) in
26 artificial systems at autoequilibrium pH (MilliQ water without adjusting the pH) and at circa neutral
27 pH (*i.e.*, Tris-HCl, pH 7.2). At autoequilibrium pH, the effects of sorbents on removal of 200 µg L⁻¹
28 As ranged from very high efficiency for iron-based sorbents to ineffectiveness for biochar and

29 chabazite. When tested at circa neutral pH, the sorbents were capable to remove between 17% and
30 100% of As(III) and between 3% and 100% of As(V) in the following order of sorbents: biochar <
31 chabazite < ferritin-based material < goethite < nano zero-valent iron. The study also highlighted
32 that chabazite, a ferritin-based material, and nano zero-valent iron oxidized As(III) to As(V) and
33 that the ferritin-based material was also able to reduce As(V) to As(III). When tested in naturally
34 arsenic-contaminated groundwater, a marked decrease in the removal effectiveness of nano zero-
35 valent iron and goethite occurred (60% and 12%, respectively), due to possible competition with
36 phosphates and manganese.

37 The usefulness of a biological oxidation step was evaluated in one-phase process (As(III) bio-
38 oxidation in conjunction with As(V) adsorption) and in two-phase process (As(III) bio-oxidation
39 followed by As(V) adsorption), both in As(III)-spiked Tris-HCl and in natural groundwater
40 systems. As(III) oxidation was performed by resting cells of *Aliihoeflea* sp. strain 2WW, and
41 arsenic adsorption by goethite. In the one-phase process As removal in Tris-HCl was >95%, while
42 in groundwater it decreased to 85%. More effective was the two-phase process that removed up to
43 95% As in groundwater leaving in solution $6 \mu\text{g L}^{-1}$ arsenic, thus meeting the limit of $10 \mu\text{g L}^{-1}$
44 imposed by World Health Organization.

45 These results can be used in the scaling up of a two-phase treatment, with bacterial oxidation of
46 arsenic used in combination with goethite sorption.

47

48 Introduction

49 Extensive arsenic pollution in groundwater affects highly populated areas in such a serious way that
50 arsenic became one of the major public health problems. Consequently a wide range of technologies
51 has been tried for the removal of arsenic from drinking water; the most common techniques utilize
52 the processes of oxidation, co-precipitation, adsorption onto sorptive media, ion exchange and
53 membrane techniques.^[1,2] Among these, the adsorption of arsenic onto natural and synthetic
54 materials have been broadly studied and it represents one of the most common treatment

55 technologies practiced by the public drinking water systems in large municipal treatment plants and
56 in small devices for small communities.

57 The adsorption of arsenic onto natural and synthetic materials is a low cost, high efficiency process.

58 A wide range of materials for aqueous arsenic removal is available nowadays. Sorbent based on
59 iron oxides/hydroxides and on activated alumina are the most common conventional materials;
60 other metal oxides-based sorbents, such as manganese dioxides, titanium dioxide, zirconium oxides,
61 have been proposed, together with clays, natural zeolites and calcite.^[3]

62 A broad range of materials that are coming from agricultural or industrial processes, and that are
63 easily accessible and low-cost have been evaluated in the screening of new sorbents. Materials
64 derived from biological sources have also been studied as low cost sorbents, such as loaded orange
65 waste gel,^[4] agricultural residue rice polish,^[5] iron-modified bamboo charcoal,^[6] bagasse fly ash.^[7]
66 Some fresh and immobilized plant biomasses gained a significant interest for their ability to
67 passively adsorb arsenite, thereby avoiding the pre-oxidation step.^[8,9] Mineral byproducts such as
68 magnesia-loaded fly ash cenospheres and manganese-loaded fly ash cenospheres,^[10] Zr(IV) iron
69 modified red mud^[11] have also been reported as promising sorbents for the removal of arsenic.

70 Metallic iron as zero-valent iron nanoparticles has been used in the past for the treatment of As-
71 contaminated groundwater and also as a reactive medium in a number of field scale experiments.^[12]

72 Similarly to inorganic iron nanoparticles, iron storage proteins (i.e. ferritin) are nanostructures that
73 can encapsulate in the form of a nano-cage several compounds, such as phosphate.^[13] To the best of
74 our knowledge, no evidence have been reported on arsenic removal capability of ferritin.

75 The main disadvantages of As adsorption onto solid materials are the direct competition for
76 available adsorption sites between arsenic and other oxyanions in the water,^[14] and the general need
77 of a pre-oxidation step to transform arsenite to arsenate.^[12]

78 In fact, the two more common forms of arsenic in water show completely different patterns of
79 dissociation, as a consequence of the predominance of the neutral species H_3AsO_3 for arsenite
80 [As(III)] at pH 2-8 and of the single negatively charged $H_2AsO_4^-$ for arsenate [As(V)] at pH values

81 3-6.^[15] Arsenite is therefore more difficult to be removed by the positively charged surfaces of
82 sorbents. This is a crucial point because the revised drinking water standard for arsenic imposes to
83 reduce arsenic concentrations to less than $10 \mu\text{g L}^{-1}$ ^[16] and consequently there is a dramatic demand
84 for oxidation technologies that effectively convert arsenite into arsenate prior to its removal.^[17]
85 Biological oxidation of As(III) by microorganism has recently received lot of attention as a
86 sustainable alternative to the use of chemical oxidants.^[18,20]

87 In the present study natural minerals (goethite, chabazite), a pyrolysis byproduct (biochar) and iron-
88 based nanomaterials (ferritin-based material and zerovalent iron) were examined at their best
89 effective dose for their ability and selectivity in removing As(III) and As(V) from water. The aim of
90 the first part of the work was to explore the potential of conventional and novel materials as
91 sorbents for As(III) and As(V) and their oxidizing power with respect to As(III). We performed
92 batch experiments in two conditions: (i) without controlling pH in order to evaluate the sorbents in
93 the actual case of small devices with a high ratio sorbent/water; (ii) at circa neutral pH simulating
94 real groundwater conditions. Furthermore, in order to evaluate the interaction between chemical
95 removal by sorbents and As(III) biological oxidation, the effect of an As oxidizer selected bacterial
96 strain on the sorption properties of a high effective As(V) sorbent was evaluated in the artificial
97 system and then assessed on a natural As-rich groundwater.

98

99 Materials and methods

100

101 *Sorbents and chemicals*

102 Sorption experiments were conducted with five materials: biochar, chabazite, ferritin-based material
103 goethite (FeOOH), and nano zero-valent iron (Fe⁰) NZVI. Biochar was from Agrindustria snc, and
104 derived from pyrolysis of pinewood; the sample used in adsorption experiments was milled and
105 successively prehydrated in Tris-HCl buffer (5 mM, pH 7.2) or MilliQ water for 24hrs.

106 The chabazite-rich tuff was obtained by Verdi S.p.A. and contained 60% (w/w), chabazite, 25%
107 (w/w) volcanic glass and traces of phillipsite, K-feldspar and biotite, with particles <200µm.
108 Biochar and chabazite were free from significant amount of soluble arsenic (<0.5 µg L⁻¹ and 4 µg L⁻¹
109 for biochar and chabazite, respectively).

110 Ferritin-based material was provided by BiAqua B.V. (The Netherlands) and the protein is
111 stabilized onto sand, used as carrier (2.74 mg ferritin g⁻¹ dry sand).

112 The goethite used in this study was from Sigma Aldrich, and had a specific surface area of 11.6
113 m²g⁻¹.

114 A commercial zero-valent iron (NANOIFER 25) was supplied by the NANOIRON s.r.o and
115 consisted of aqueous dispersion of Fe⁰ nanoparticles stabilized by an inorganic modifier.

116 Arsenate and arsenite solution were prepared by spiking MilliQ water and Tris-HCl buffer (5 mM,
117 pH 7.2) with 200 µg L⁻¹ As(III) or As(V) from stock solutions of 1000 µg L⁻¹ NaAsO₂ or
118 Na₂HAsO₄. (Sigma).

119

120 *Groundwater sample*

121 The groundwater sample used in the experiments was collected from an As-contaminated well in
122 the Northern part of Italy (Cremona, Lombardy). Physico-chemical characterization revealed that
123 the groundwater sample was anoxic (with an E_h value of -113 mV, and no dissolved oxygen) and
124 had the following physicochemical characteristics: temperature of 15 °C; pH value of 7.6; CaCO₃
125 282 mg L⁻¹; organic C 2.11 µg L⁻¹; dissolved S-SO₄ 267 µg L⁻¹; dissolved P- PO₄ 312 µg L⁻¹;
126 dissolved N-NO₃ 685 µg L⁻¹; dissolved N-NH₄ 2680 µg L⁻¹; dissolved Fe 760 µg L⁻¹; dissolved Mn
127 97 µg L⁻¹. The arsenic concentration in the sample was 171 µg L⁻¹, with As(III) as the main As
128 species.

129

130 *Resting cells preparation*

131 The biological arsenite oxidation step was carried out by addition of resting cells of *Aliihoeflea* sp.
132 strain 2WW. Resting cells of the strain were able to oxidize As(III) in Tris-HCl.^[21]
133 The bacterial strain was grown for 48 h in mineral medium (BBWM) supplemented with sodium
134 lactate (40 mmol L⁻¹) (BBWM-L) at 30°C in shaking condition at 150 rpm. BBWM consisted of:
135 solution A (g L⁻¹): KH₂PO₄ 0.04; K₂HPO₄ 0.04; NaCl 1.0; (NH₄)₂SO₄ 0.4; trace element solution 2
136 mL. The pH of solution A was 6.5. Solution B (g L⁻¹): CaCl₂ 0.2; MgSO₄ 0.2. Solutions A and B
137 were sterilized separately by autoclaving. Equal volumes of solutions A and B were mixed after
138 cooling and then supplemented with 1% (v/v) vitamin solution. Vitamin solution was filter
139 sterilized and contained (mg L⁻¹): *p*-aminobenzoic acid 5; biotin 5; folic acid 2; pyridoxine-HCl 1;
140 riboflavin 5; thiamine 5; nicotinic acid 5; pantothenic acid 5; vitamin B12 0.1. The pH was adjusted
141 to 8.0. After growth, cells were centrifuged at 10,000 rpm, 10 °C for 30 min. Cell pellet was washed
142 three times with Tris-HCl (5 mM, pH 7.2) and resuspended in Tris-HCl (5 mM, pH 7.2). This cell
143 suspension served as inoculum in order to obtain a final cell density of about 10⁷ cell mL⁻¹.

144

145 *Adsorption experiments in artificial and natural systems*

146 Sorbents were tested at their most effective dose, identified in a preliminary screening carried out
147 with different amounts of each sorbent. Based on these results, the following quantities of sorbents
148 (g 50 mL⁻¹) were used: biochar 0.2, chabazite 1.0, goethite 0.2, ferritin-based material 11.4 and
149 zero-valent iron (NZVI) 0.05.

150 Adsorption experiments in artificial systems were performed in polypropylene tubes with the
151 addition of 50 mL Tris-HCl (5mM pH 7.2) or MilliQ water, spiked with 200 µg L⁻¹ As(III) or
152 As(V), chosen on the base of arsenic content of the groundwater used in the present study. **Batch**
153 **experiments were prepared in aerobic condition, with the exception of those with NZVI that were**
154 **filled under anaerobic condition in Nitrogen Dry Box (Plas Labs, Inc.) to prevent Fe⁰ oxidation. The**
155 **tubes were closed with cotton plug in order to allow gaseous exchange.**

156 Adsorption experiments in natural systems were performed with 50 mL natural As-contaminated
157 groundwater in the presence of goethite (0.2, 1.0 g) and of NZVI (0.05 g).

158 A preliminary check of a time course of the groundwater pH had shown a dramatic increase in pH
159 values during 24 h of exposure to air (from 7.8 to 8.5), due to the evolution of dissolved CO₂
160 **Consequently, batch experiments with natural As-contaminated water were prepared under**
161 **anaerobic condition in Nitrogen Dry Box (Plas Labs, Inc.) and tubes were closed with plastic plugs.**

162 The effect of biological arsenite oxidation was evaluated in artificial and natural systems. Resting
163 cells of *Aliihoeflea* sp. strain 2WW were added to 50 mL Tris-HCl or contaminated groundwater
164 either in the absence or presence of goethite. A set of tubes with goethite only and one without
165 addition of inoculum and goethite were used as controls.

166 A one- and two-phase process was compared by adding the bacterial cells either together with 0.2
167 and 1.0 g goethite (one-phase treatment) or by adding the bacterial cells 48 h before the addition of
168 0.2 and 1.0 g goethite followed by 48 h incubation (two-phase treatment).

169 All the experiments were incubated on a rotary shaker in the dark at 15 °C, chosen on the base of
170 groundwater temperature measured on site. The pH was monitored at the beginning and at the end
171 of the experiments using a Radiometer Copenhagen PHM210-pH meter. At the end of the
172 experiments 20 mL of the suspensions were collected from each tube, centrifuged, filtered over
173 nitrocellulose membranes (\varnothing 0.22 μ m) and acidified with HNO₃ to achieve a final concentration of
174 2% (v/v).

175

176 *Analytical methods*

177 **Total arsenic was determined in 5 mL of samples previously acidified with HNO₃. For speciation of**
178 **arsenic forms, As(V) and As(III) species were separated on the basis of their selective retention on a**
179 **WATERS Sep-Pak® Plus Acell Plus QMA cartridge (Waters, MA, USA): As(V) is retained in the**
180 **cartridge, while allowing As(III) to pass through and to be collected. The procedure was performed**
181 **according to Kim et al.:^[22] 5 mL of non-acidified samples were passed through the cartridge and**

182 the flow-through (containing As(III)) was collected. The cartridge retaining As(V) was then washed
183 with 0.16M HNO₃ to elute As(V) from it.

184 Arsenic contents (total As, As(III) and As(V)) were determined by inductively coupled plasma–
185 mass spectrometry (ICP-MS) (Agilent technologies, USA). Standards of As for concentrations
186 ranging from 0 to 1 mg L⁻¹ were prepared from sodium arsenite NaAsO₂ (Sigma Aldrich, USA).
187 For all the measures by ICP–MS an aliquot of a 2 mg/L of an internal standard solution (⁴⁵Sc, ⁸⁹Y,
188 ¹⁵⁹Tb, Agilent technologies, USA) was added both to samples and calibration curve to give a final
189 concentration of 20 mg L⁻¹. The instrument was tuned daily with a multi-element tuning solution for
190 optimised signal-to-noise ratio.

191 Dissolved Mg, Ca, Mn, Fe and P content in the contaminated groundwater sample before and after
192 the one-phase process were determined by ICP-MS; procedure was the same as that used for
193 determining total arsenic.

194

195 *Statistical analysis*

196 Data represent the mean values obtained from at least three replicates of each experiments. The
197 values were subjected to Student t-test (p<0.05) and to one-way ANOVA with Tukey-b test using
198 the SPSS version 20.0.

199

200 **Results**

201

202 *As(III) and As(V) removal by sorbents in artificial systems*

203 Batch test studies with sorbents were conducted at autoequilibrium pH (MilliQ water) and at neutral
204 pH (Tris-HCl solution).

205 Sorbents modified the pH of As-spiked MilliQ water at different values: pH 10.2 (biochar), pH 8.7
206 (chabazite), pH 6.4 (ferritin-based material), pH 5.8 (goethite) and pH 8.7 (NZVI). The effects of
207 sorbents on As(III) and As(V) removal from MilliQ water are reported in Figures 1a and 1b,

208 respectively. Removal of arsenic from biochar and chabazite had no significant effect on As(III)
209 and As(V) mobility; on the contrary ferritin-based material, goethite and NZVI were able to remove
210 both As(III) and As(V). Ferritin-based material and goethite showed a higher affinity for As(V)
211 than As(III); while no detectable As remained in both the As(III) and As(V)-spiked solutions after
212 1h contact with NZVI. Checking a 50-fold concentrated As-spiked MilliQ solutions added with
213 0.05 g of NZVI, a higher affinity of NZVI for As(III) than As(V) was found: the remaining As(III)
214 and As(V) in solution accounted for 4.3 mg L⁻¹ and 6.8 mg L⁻¹, respectively.

215 Results of As(III) and As(V) adsorption experiments conducted in Tris-HCl buffered solutions are
216 reported in Figure 2a and 2b, respectively.

217 Preliminary trials showed that arsenic adsorption onto goethite was not significantly affected by the
218 presence of Tris-HCl (data not shown). At neutral pH all tested sorbents induced a statistically
219 significant decrease of As(III) concentration, the most drastic effect being exerted by ferritin-based
220 material, goethite and NZVI. Similarly to As(III), ferritin-based material, goethite and NZVI
221 efficiently immobilized As(V), while a slight removal of As(V) by biochar and chabazite was
222 observed. Goethite showed a greater affinity for As(V) than for As(III).

223 When As(III) was the initial arsenic species in the buffer solution, As(V) was detected at the end of
224 the adsorption experiments in the presence of ferritin-based material, NZVI and, to a lesser extent,
225 chabazite (Table 1), suggesting an abiotic oxidation of As(III). Conversely, As(V) seemed not to be
226 reduced by sorbents, with the exception of ferritin-based material, that induced a reduction of one
227 third of the total soluble arsenic recovered in the solution.

228

229 *Adsorption of As(III) and As(V) from natural system by goethite and NZVI*

230 Arsenic removal efficiency of NZVI and goethite was evaluated in groundwater sample. The water
231 pH in the control and in the treatments with sorbents were as follows: pH 7.5 (control), pH 7.1
232 (goethite) and pH 7.7 (NZVI).

233 Control without sorbents did not show changes in the amount and speciation of arsenic (Fig. 3);
234 these indicated that no apparent changes of arsenic speciation due to biotic or abiotic reactions
235 occurred in the time of the experiments. The performance of both sorbents was worse in the natural
236 than in the artificial system: As immobilization by NZVI and by goethite were reduced by 60% and
237 12%, respectively, as compared with those in MilliQ water. Speciation of the soluble arsenic at the
238 sampling time indicated that NZVI promoted a complete oxidation of remaining As(III), while no
239 detectable As(V) was recovered in the goethite treatment, thus confirming the absence of chemical
240 or biological activities towards arsenic in the presence of goethite.

241

242 *Effect of bio-oxidation of As(III) on As removal in artificial and natural systems*

243 Due to the inability of goethite to oxidize As(III) in the groundwater, thus leading to arsenic
244 concentration higher than the WHO limit, a biological As(III) oxidation step was considered in one-
245 and two-phase experiments.

246 Preliminary one-phase experiment in As(III)-spiked Tris-HCl solution (Fig. 4a), indicated that the
247 combination of As(III) bio-oxidation and adsorption by goethite resulted in a high efficient removal
248 of As (>95%), decreasing soluble As concentration to $8 \mu\text{g L}^{-1}$. At the end of the experiment, As(V)
249 was the only detectable arsenic form in solution, indicating that the ability of the cells to oxidize
250 As(III) was not affected by the presence of goethite. Resting cells of strain 2WW converted As(III)
251 to As(V) completely, whereas goethite without cells removed approximately 85% of initial As(III).

252 One-phase treatment was tested in natural system (*i.e.*, As(III) contaminated groundwater) (Fig. 4b).

253 Strain 2WW was able to completely oxidize $150 \mu\text{g L}^{-1}$ As(III) present in groundwater; in the
254 absence of 2WW cells, goethite adsorbed As(III) present in the groundwater at a comparable level

255 of artificial system (85% removal). The combined As(III) bio-oxidation and adsorption process was
256 not able to enhance arsenic removal, as observed in artificial system. This effect may be attributable
257 to competition of other ions for goethite sorption sites. Changes in groundwater ion compositions
258 during the time course of the experiment are reported in Table 2. A dramatic decrease of soluble
259 iron concentration occurred in all tubes at the end of the incubation, while manganese and
260 phosphorous concentration decreased in the presence of goethite. Neither calcium nor magnesium
261 was removed in any treatment and dissolved carbon concentrations were negligible (data not
262 shown). When the amount of goethite was increased from 0.2 g 50 mL⁻¹ to 1.0 g 50 mL⁻¹, no dose
263 effect on As removal was evidenced either in the presence and in the absence of 2WW cells.

264 The two-phase system approach applied to natural system was tested in the presence of two
265 different goethite doses: 0.2 and 1.0 g 50 mL⁻¹ (Figure 5). In the presence of goethite 0.2 g 50 mL⁻¹
266 the two-phase system led to 38.5 µg L⁻¹ soluble arsenic, comparable with data obtained in the one-
267 phase system (26 µg L⁻¹ soluble arsenic). When goethite dose was increased to 1.0 g 50 mL⁻¹, As
268 removal was >95%, thus lowering As concentration at 6 µg L⁻¹, evidencing a dose effect on As
269 removal. When in the presence of 2WW cells, As(V) was the only arsenic species in solution in all
270 the systems (Figure 5).

271

272 Discussion

273

274 Groundwater contamination by arsenic may occur under both reducing and oxidizing conditions,
275 and the ratio of As(III) to As(V) can vary significantly, depending on the condition of *in situ*
276 oxidation state of water.^[23] Moreover, groundwater-treatment plants for drinking water can treat
277 groundwater as it is or after an oxygenation step. Therefore, the choice of the best sorbent for As
278 removal from water must take into consideration its affinity for the species of arsenic to be
279 removed. Low cost and high available materials could be good candidate as point-of-use sorbents to
280 mitigate As polluted groundwater.

281 In our study we tested low cost sorbents such as biochar, chabazite and goethite, and compared
282 them with high efficient, but highly operational complex materials (*i.e.*, nano zero-valent iron and
283 ferritin-based material). Batch experiments without controlling pH allow evaluating the sorbents in
284 the actual case of small devices, with a high ratio sorbent/water. Autoequilibrium pH values led
285 systems from mildly acid to highly alkaline and the effects of sorbents on As removal by water
286 ranged from very high efficiency to ineffectiveness. When tested at circa neutral pH simulating real
287 groundwater conditions, almost all the tested materials showed to remove both species of arsenic
288 from As-spiked buffer solutions, from 4% to 100%, depending on the sorbent and on the As
289 species. Biochar was recently proposed as a low-cost adsorbent in water treatment;^[24] nevertheless
290 no studies are reported on As retention by biochar in natural water. At pH 7.2 a significant but small
291 removal of arsenic by biochar was detected, with a more favorable adsorption of As(III) versus
292 As(V), in agreement with the ability of biochar in the adsorption of heavy metals.^[25] Rise in pH
293 seemed to have an adverse effect on biochar efficiency. Arsenate retention to biochar can be
294 attributed to the same mechanism that allows phosphorus adsorption, as postulated by Beesley and
295 Marmiroli,^[26] biochar higher ability to remove arsenite than arsenate could be due to outer surfaces
296 and inner porous micro-structures that explain retention.^[27]

297 The zeolite used in this study was mainly chabazite, which is reported to be more effective than
298 other zeolitic rocks in removing arsenic from waters.^[28] At autoequilibrium pH chabazite induced a
299 10% reduction of As(III) concentration and showed no effect on As(V). At neutral pH the removal
300 percentages of As(III) and As(V) were 30% and 6%, respectively. In our study a small amount of
301 As(V) was retrieved in the solution at the end of the experiment, although Lièvreumont et al.
302 suggested that the high As(III) sorption capacity of chabazite was due to abiotic oxidation of
303 As(III).^[29]

304 As expected, iron-based sorbents showed the highest adsorption capacity in artificial system.
305 Ferritin-based material was recently proposed as a new bionanotechnological system for phosphate
306 removal from waters;^[13] to the best of our knowledge, no studies are reported on As removal by

307 ferritin-based material in natural systems. At autoequilibrium pH and at circa neutral pH, ferritin-
308 based material was capable to adsorb As(III) and As(V) in range from 70% to 78%. Particularly,
309 arsenate retention to ferritin-based material can be due to the same mechanisms involved in
310 phosphates adsorption.

311 Arsenic adsorption rate onto NZVI reaches 100% both for As(III) and As(V) already after 1 h
312 contact of As-spiked solutions with a 20-fold lower amount of sorbent than goethite. Adsorption
313 process by using NZVI can remove both arsenate and arsenite simultaneously, without pre-
314 oxidation step, and such process does not require the use of additional chemical reagents.^[30] A
315 removal ability of NZVI was found by Kanel et al. on a minute time scale, explained by As
316 adsorption onto corrosion products formed by heterogeneous reactions onto NZVI surface.^[31]

317 In our artificial system goethite removed 72% of 200 $\mu\text{g L}^{-1}$ As(III) and 98% of 200 $\mu\text{g L}^{-1}$ As(V),
318 bringing down As(V) level below the threshold limit of 10 $\mu\text{g L}^{-1}$. The higher affinity of goethite
319 for As(V) suggests that oxidation of As(III) to As(V) is required in the treatment of anoxic/suboxic
320 groundwater where As(III) can be the most abundant species. In line with this, arsenic adsorption
321 onto goethite was deeply enhanced (>95% of As removal) when the biological oxidation step was
322 introduced in a one-phase treatment. For the As(III) oxidation in groundwater the findings from this
323 study reveal that a biological process performed with resting cells of strain 2WW can be utilized as
324 an alternative to a chemical oxidants.

325 Our findings were in agreement with Lièvreumont et al. who studied As removal process by using
326 Tris-HCl solution in order to exclude competition between oxyanions (organic or inorganic ligands
327 such as phosphate) and As(V) for sorption sites.^[29] In accordance to this hypothesis, a decrease in
328 the efficiency of As removal from groundwater was observed in the natural system during one-
329 phase process.

330 Among the coexisting ions, present in similar or much higher concentrations than arsenic, Fe(II)
331 oxidation followed by precipitation is known to promote As removal from water via adsorption and
332 co-precipitation. Moreover the formation of ternary goethite-Fe-As complexes can increase

333 adsorption of As(III) by goethite.^[32] In our experiments without goethite addition the decrease of
334 soluble Fe concentration in the groundwater sample at the end of the experiment suggests that Fe(II)
335 was chemically and/or biotically oxidized by O₂ or by O₂-respiring bacteria, with a consequent
336 precipitation as (oxy)hydroxides; at the same time only a slight decrease in As concentration of the
337 groundwater was detected. Among other ions, manganese and phosphate showed a high affinity for
338 goethite, being strongly removed from water. As for arsenic, manganese, which is naturally present
339 in water in reduced form, can be retained on goethite by selective adsorption. The authors
340 evidenced that Mn adsorption on hematite followed by Mn oxides production took up the potential
341 sorption sites for As(V) and resulted in a decrease of As(V) removal. Moreover, phosphate and
342 arsenate compete primarily for a similar set of surface sites on goethite.^[34] Meng et al. demonstrated
343 that at high phosphate concentrations (i.e. > 400 µg L⁻¹), arsenic removal is not efficient, at least for
344 an initial arsenic concentration 50 µg L⁻¹.^[35] In our study, after strain 2WW completely oxidized
345 As(III), a PO₄/AsO₄ molar ratio of 6.0 was detected in the water sample indicating a competitive
346 effect of phosphate on As(V) adsorption. Phosphate naturally present in the studied groundwater
347 can firstly be adsorbed to goethite and saturated the sorption site of goethite, hindering biologically
348 formed As(V) to be adsorbed to sites pre-occupied.

349 In order to limit the effects of competing ions on As removal and thus meeting the threshold limit of
350 10 µg L⁻¹ As for drinking water, biological As(III) oxidation and goethite adsorption were
351 performed separately (two-phase process). Differently from one-phase process, As(III) removal
352 increased up to 96%, suggesting that the oxidation and adsorption steps must be performed
353 separately. Our results are in agreement with previous findings on similar treatments that
354 incorporate a biological transformation of arsenic and subsequent adsorption by different materials
355 such as zero valent iron,^[36] kutnahorite mineral sorbent,^[29] and activated alumina.^[37]

356

357 **Conclusions**

358

359 Experimental results showed that many materials can be used for the treatment of As-polluted
360 water, even if only iron-based sorbents are able to remove arsenic from water to levels below $10 \mu\text{g}$
361 L^{-1} , to assure the water quality as recommended by government health agencies. Among them, nano
362 zero-valent iron and goethite have the highest As removal efficiency. Along with the excitement
363 over the prospects of nanotechnology, there have been increasing concerns regarding risks to public
364 health that exposure to nanomaterials poses especially after disposal.^[38] In this perspective and
365 considering the high efficiency of iron-based materials, the use of non nano-scale sorbents (i.e.,
366 zero-valent iron filings, natural iron oxide goethite) for drinking water treatment plants might be
367 advantageous.

368 This study showed that ferritin-based material could be considered an interesting iron-based
369 sorbent, because it has the advantage to be regenerable, allowing a cost-effective solution for the
370 end-user.

371 Among the other tested sorbents, the use of biochar require further investigations to evaluate the
372 role of raw materials, process conditions and feasible treatments to improve its ability to remove
373 As(III), as biochar is a highly available, of wide geographically distribution and low cost material.

374 The study also highlights that some of the studied sorbents can modify soluble As speciation (i.e.,
375 chabazite, ferritin-based material, nano zero-valent iron). Consequently the choice of the adsorption
376 technique must take into account not only the main As species in the water, but also changes in the
377 As chemical form that could occur during treatments, due to oxygenation of water, contact with
378 highly reactive sorbents, microbial processes.

379 Moreover this study highlights that the effectiveness of sorbents decrease in natural system,
380 particularly that of nano zerovalent iron. Because removal of As(V) by goethite is more efficient
381 than As(III) adsorption, there is a need for a pre-oxidation step that can enhance operational costs.

382 In this sense, biological oxidation methods are considered to be a suitable approach to overcome
383 these problems.

384 These results pointed out that two main factors affected the arsenic removal from groundwater and
385 should be considered in the scaling up of a treatment system: i) the quantity of sorbent in relation to
386 arsenic and competing ion concentration; ii) the separation of As(III) oxidation and As(V)
387 adsorption steps.

388

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392

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481 4–21.
- 482
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- 484 Figures Legend

485

486 **Fig.1** Soluble As before and after contact of 50 mL of 200 $\mu\text{g L}^{-1}$ As(III)- (a) and As(V)- (b) spiked
487 MilliQ water with biochar (0.2 g), chabazite (1.0 g), ferritin-based material (11.4 g), goethite (0.2 g)
488 and NZVI (0.05 g). Experimental time: biochar, chabazite, ferritin-based material, goethite: 48 h;
489 NZVI: 1 h. The asterisk indicates statistical significance at $p=0.05$ (single asterisk) and $p=0.01$
490 (double asterisk) as compared with time 0, determined by the Student's *t* test.

491

492 **Fig.2** Soluble As before and after contact of 50 mL of 200 $\mu\text{g L}^{-1}$ As(III)- (a) and As(V)- (b) spiked
493 Tris-HCl with biochar (0.2 g), chabazite (1.0 g), ferritin-based material (11.4 g), goethite (0.2 g)
494 and NZVI (0.05 g) Experimental time: biochar, chabazite, ferritin-based material, goethite: 48 h;
495 NZVI: 1 h. The asterisk indicates statistical significance at $p=0.05$ (single asterisk) and $p=0.01$
496 (double asterisk) as compared with time 0, determined by the Student's *t* test.

497

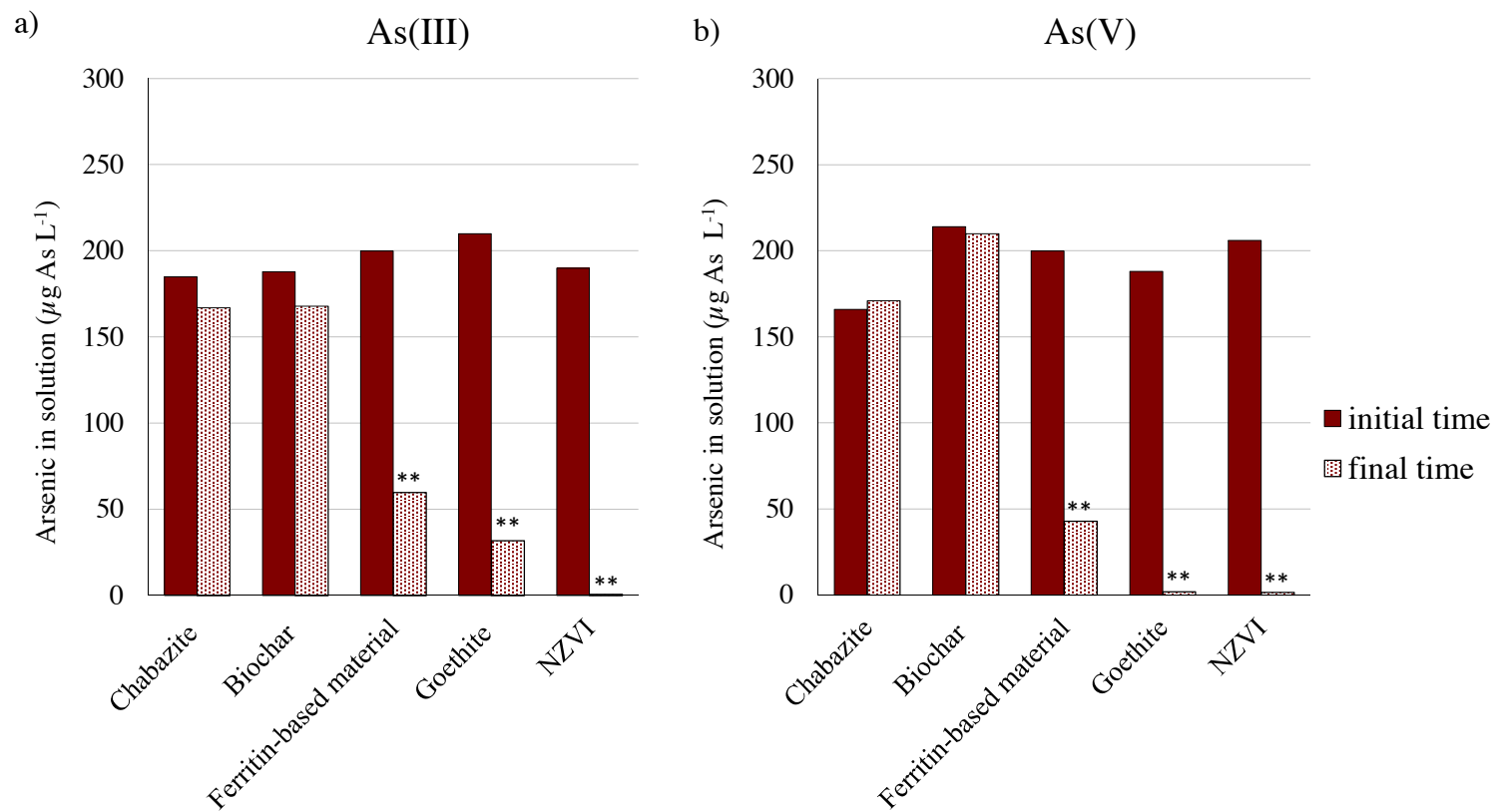
498 **Fig.3** Total As, As(III) and As(V) concentration in the As contaminated groundwater sample after
499 contact with sorbents: NZVI (0.05 g 50 mL^{-1} , experimental time:1 h), goethite (0.2 g 50 mL^{-1} ,
500 experimental time: 48 h). Error bars represent the standard deviations of four replicates.

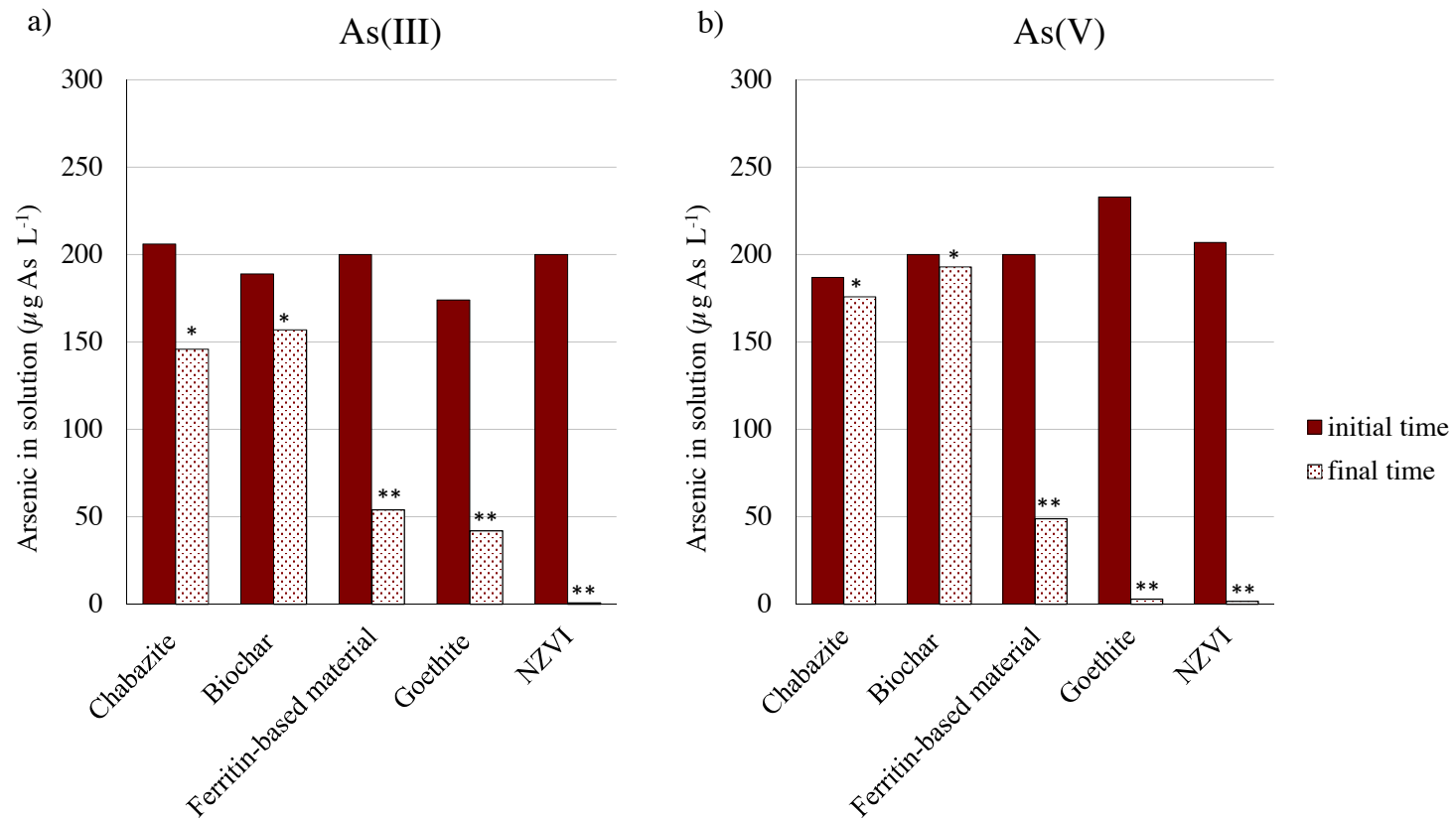
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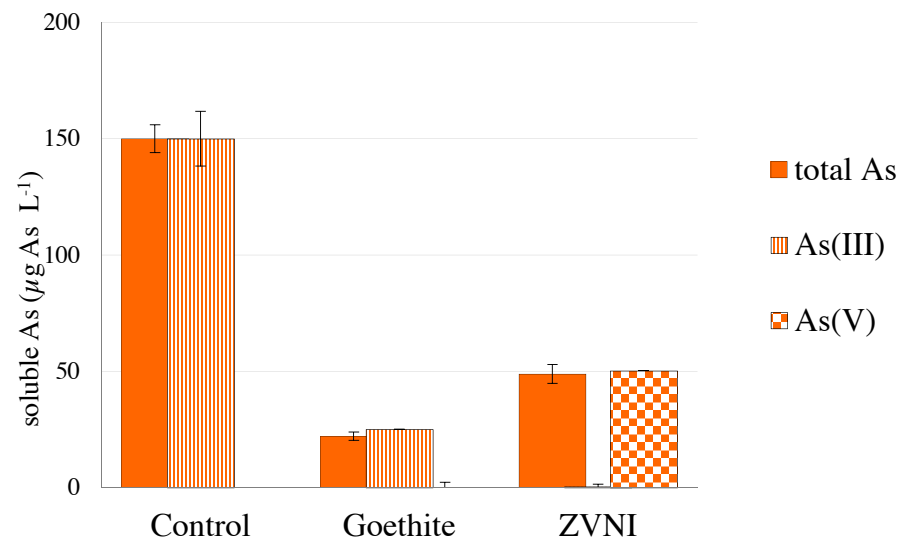
502 **Fig.4** Effect of As oxidizing strain 2WW on total As, As(III) and As(V) in 200 $\mu\text{g L}^{-1}$ As(III)-
503 spiked Tris-HCl (artificial system) and in contaminated groundwater (natural system) after 48 h
504 incubation. Error bars represent the standard deviations of quadruplicate experiments each with 4
505 replicates. GW= As-contaminated groundwater

506

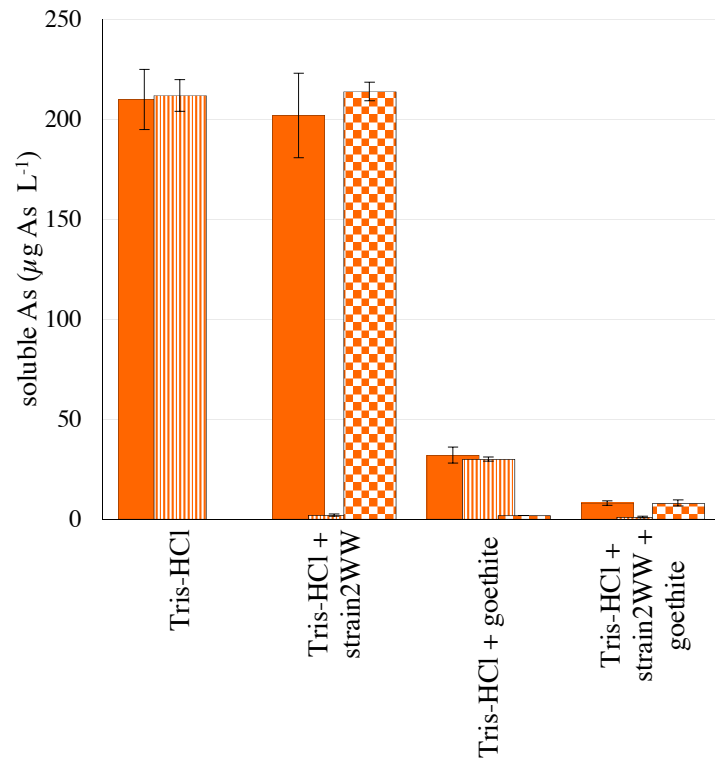
507 **Fig. 5** Effect of biological As(III) oxidation carried out in one- phase and two-phase process on
508 total As concentration in contaminated groundwater after 48 h incubation. In the table is reported
509 As speciation in 1.0 g goethite experiments.



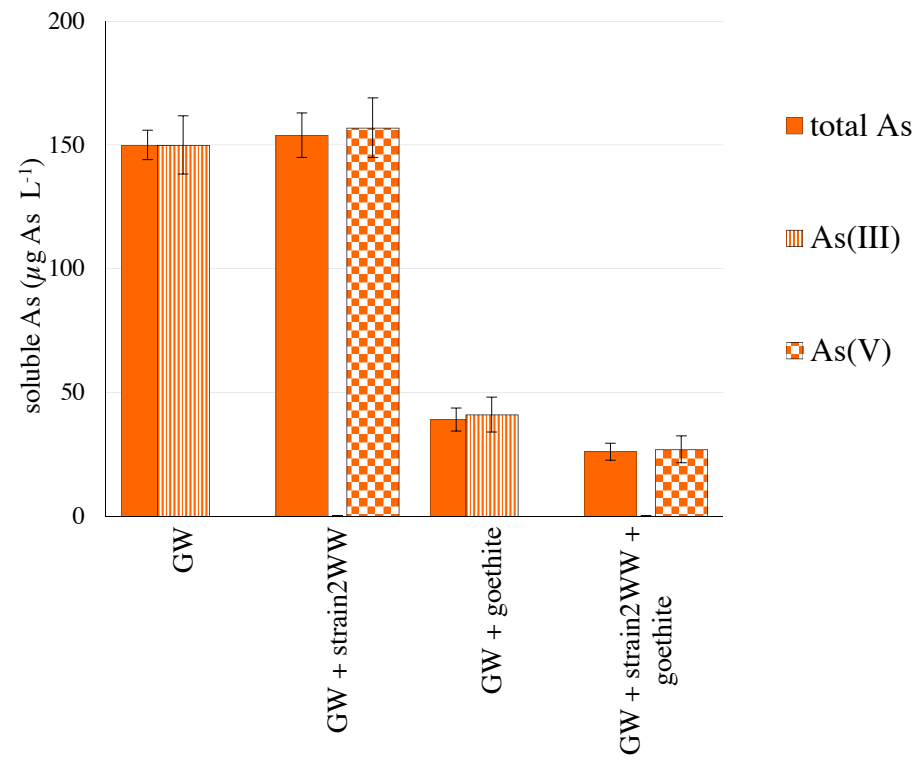




a) artificial system



b) natural system



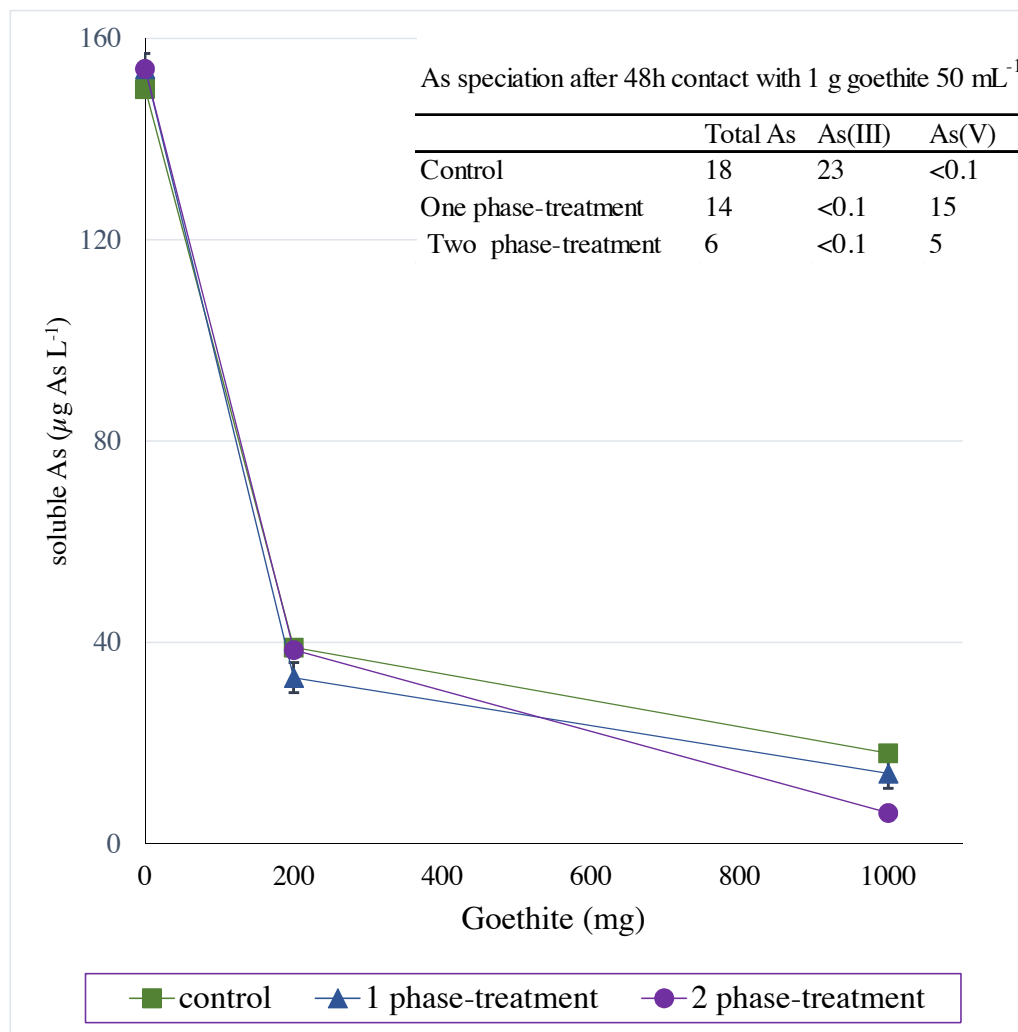


Table 1. Speciation of soluble arsenic ($\mu\text{g L}^{-1}$) retrieved after contact with sorbents (mean \pm standard deviation, n =3).

	As(III) spiked Tris-HCl			As(V) spiked Tris-HCl		
	Total	As(III)	As(V)	Total	As(III)	As(V)
Chabazite	190 \pm 1	180 \pm 9	17 \pm 6	nd ^A	nd	nd
Ferritin-based material	54 \pm 7	42 \pm 3	23 \pm 0.3	37 \pm 10	30 \pm 5	15 \pm 7
Goethite	43 \pm 4	40 \pm 6	2.2 \pm 0.7	5.0 \pm 1.5	<0.1	4.5 \pm 2.8
NZVI	4.4 \pm 0.1	2.8 \pm 0.3	1.1 \pm 0.5	2.7 \pm 0.4	0.4 \pm 0.3	0.7 \pm 0.0

^A not determined**Table 2. Total arsenic and main coexisting ions ($\mu\text{g L}^{-1}$) in natural groundwater sample before and after 48 h incubation**

	As		Mn		Fe		P	
GW ^A at initial time	171	c	97	b	760	b	312	b
GW at final time	150	b	84	b	151	a	373	b
GW + strain 2WW	154	b	97	b	186	a	269	b
GW + goethite	39	a	1.6	a	185	a	< 10	a
GW + strain 2WW + goethite	26	a	1.4	a	179	a	< 10	a

Values followed by the same lower case letters denote those not significantly different in each column ($P < 0.05$); ^AGW= As-contaminated groundwater