1   **Potential Impact of Flowback Water from Hydraulic Fracturing on**

2   **Agricultural Soil Quality: Metal/Metalloid Bioaccessibility, Microtox**

3   **Bioassay, and Enzyme Activities**

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Abstract

Hydraulic fracturing has advanced the development of shale gas extraction, while inadvertent spills of flowback water may pose a risk to the surrounding environment due to its high salt content, metals/metalloids (As, Se, Fe and Sr), and organic additives. This study investigated the potential impact of flowback water on four representative soils from shale gas regions in Northeast China using synthetic flowback solutions. The compositions of the solutions were representative of flowback water arising at different stages after fracturing well establishment. The effects of solution composition of flowback water on soil ecosystem were assessed in terms of metal mobility and bioaccessibility, as well as biological endpoints using Microtox bioassay (*Vibrio fischeri)* and enzyme activity tests. After one-month artificial aging of the soils with various flowback solutions, the mobility and bioaccessibility of As(V) and Se(VI) decreased as the ionic strength of the flowback solutions increased. The results inferred a stronger binding affinity of As(V) and Se(VI) with the soils. Nevertheless, the soil toxicity to *Vibrio fischeri* only presented a moderate increase after aging, while dehydrogenase and phosphomonoesterase activities were significantly suppressed with increasing ionic strength of flowback solutions. On the contrary, polyacrylamide in the flowback solutions led to higher dehydrogenase activity. These results indicated that soil enzyme activities were sensitive to the composition of flowback solutions. A preliminary human health risk assessment related to As(V) suggested a low level of cancer risk through exposure via ingestion, while holistic assessment of environmental implications is required.

Keywords: fracturing fluids; metal mobility; microbial toxicity; enzyme activity; soil interaction.

1. Introduction

The technology breakthrough of hydraulic fracturing (fracking) has expanded the natural gas extraction from shale reserves, of which the global volume is estimated to be over 200 trillion m^3 (Kargbo et al., 2010; Kuuskraa et al., 2013). China plans to produce 300 billion m^3 of shale gas by 2020 (China's State Council, 2015). In spite of the huge economic benefit it may bring, fracking has raised substantial concerns on human health and environmental impact, such as air pollution (Bunch et al., 2014; Moore et al., 2014) and water contamination (Warner et al., 2013; Vengosh et al., 2014). Depending on the geological conditions of the shale formation, the fracking process requires varying amount of fracking fluid from 8000-19,000 m^3 per well, which is composed of about 90% water, 6-9% proppant and 0.5-2% chemical additives such as gelling agents, friction reducers, and surfactants (Gregory et al., 2011; Vidic et al., 2013; Vandecasteele et al., 2015).

About 10-70% of the injected fluid returning to the surface prior to gas recovery is known as flowback water, while the produced water is generated during gas production (Gregory et al., 2011; Vidic et al., 2013; Vengosh et al., 2014). These wastewaters contain elevated concentration of dissolved organic carbon (up to 590 mg L^{-1}) from the chemical additives in fracking fluids. Naturally present substances such as radioactive elements, metals/metalloids, and brine salts from the shale formations also result in highly concentrated total dissolved solids (up to 350,000 mg L^{-1}) (Barbot et al., 2013; Kondash et al., 2013; Lester et al., 2015). The release of fracking chemicals into the environment could happen at any stage during shale gas development from drilling to waste disposal due to accidental spills or operational failures (Ziemkiewicz et al., 2014; Vandecasteele et al., 2015). In particular, more than three quarters of the spills may

62 involve flowback and produced water as revealed by the latest study (McLaughlin et al., 2016),

63 and the associated risks to water resources have aroused extensive interests (Ferrar et al., 2013;

64 Warner et al., 2013; Vengosh et al., 2014). Recent studies have reported the positive

65 relationships between the composition in fracking wastewaters and the occurrence of disinfection

66 by-products (Hladik et al., 2014). Elevated concentrations of arsenic, selenium, and strontium

67 (Fontenot et al., 2013; Abualfaraj et al., 2014) were also found in surrounding streams of shale

68 gas formations. However, contamination in soil environment exposed to the spills of flowback

69 and produced water is poorly understood in the existing literature (Annevelink et al., 2016).

70

71 In view of the elevated salt contents and organic additives in the fracking wastewater, the

72 physico-chemical properties of soil such as viscosity and pH may be altered (Stringfellow et al.,

73 2014). Our recent work has demonstrated that the leaching of flowback solutions may alter the

74 transport channels in soil (Chen et al., 2016). Besides, the chemical interactions between

75 fracking wastewater and soil environment may lead to eco-toxicological risks (Stringfellow et al.,

76 2014; Annevelink et al., 2016). It has been shown that the biocide glutaraldehyde is largely

77 sorbed in soil and it forms cross-linking with polyacrylamide (PAM), which is a friction reducer

78 used in fracking fluids that can remain stable in soil over six months (Caulfield et al., 2002;

79 McLaughlin et al., 2016). The presence of salts in the flowback water also prevents the

80 biodegradation of polyethylene glycol, which is a surfactant employed in fracking fluid

81 (McLaughlin et al., 2016). The complexity of the flowback water composition as well as the

82 increasing reports of accidental spills have aggravated the potential risks to our soil environment

83 and human health (Ziemkiewicz et al., 2014). Accidental spills of fracking fluids affect colloid

84 mobilization in the unsaturated zone (Sang et al., 2014), while fracking activity often occurs near

85　　the agricultural area and poses a higher risk to agricultural soil (McLaughlin et al., 2016).

86　　However, the implications of chemical toxicity of flowback water on soil organisms and

87　　functionalities of soil microbial communities such as enzyme activities of dehydrogenase and

88　　phosphomonoesterase still remain uncertain.

89

90　　Therefore, this study investigates the metal mobility/bioaccessibility and soil health by

91　　simulating spills of flowback water on representative surface soils collected in the vicinity of

92　　shale wells. Under acidic and oxidizing conditions in the flowback solution, As(V) and Se(VI)

93　　would be the predominant form and susceptible to mobilization (Phan et al., 2015; Parnell et al.,

94　　2016). Soil quality was assessed in terms of biological endpoints of the Microtox bioassay and

95　　soil enzyme activities, which are rapid and reliable approaches to assessing the chemical toxicity

96　　to organisms and the microbial activity in the soil ecosystem (Pandey and Singh, 2006; Maisto et

97　　al., 2011; Lyubun et al., 2013). Potential human health risk of accidental spills to neighbouring

98　　farmers is also estimated in this study.

99

100　　**2. Materials and Methods**

101　　*2.1 Soil sampling and synthetic flowback solutions*

102　　The variations of soil properties on resultant metal mobilization and soil quality were

103　　investigated with four types of surface soils sampled from shale gas regions, namely Anda,

104　　Bayan, Binxian and Daqing, in Songliao Basin, China. The shale formation also overlapped with

105　　an elevated-arsenic-risk region (Rodriguez-Lado et al., 2013), which may increase the risk of the

106　　mobility and bioaccessibility of arsenic when exposed to the spills of flowback solutions. The soil

107　　samples were ground and passed through 2 mm sieve. The four soils were sampled from

108   agricultural land, where the pH ranged from 8.1 to 9.3, reflecting the prevalence of alkaline soils in

109   this area. The content of total organic carbon (TOC) and amorphous Fe were measured, as they

110   are known to provide major adsorption sites for arsenate (As(V)) and selenate (Se(VI)), and may

111   influence the metal mobilization (Goldberg and Johnston, 2001; Peak and Sparks, 2002; Feng et

112   al., 2013). Anda (AD) and Bayan (BY) soil contained similar level of TOC (25.3 mg g^{-1} and 24.8

113   mg g^{-1}, respectively), while the amorphous Fe in BY soil (45.0 mg g^{-1}) was much higher than

114   that in AD soil (21.0 mg g^{-1}). Binxian (BX) and Daqing (DQ) soil contained a relatively low

115   amount of TOC (10.2 mg g^{-1} and 12.4 mg g^{-1}, respectively), and the amorphous Fe content was

116   comparatively high in BX soil (38.7 mg g^{-1}) but much less in DQ soil (9.36 mg g^{-1}) (Table 1).

117

118   The synthetic solutions were prepared according to the characteristics of Day 1 and Day 14

119   (flowback water) as well as Day 90 (produced water) effluent waters from the comprehensive

120   report (Hayes, 2009). In this study, they were referred to synthetic flowback solutions. As

121   summarized in Table 2, they represented significant temporal variation in ionic strength, iron

122   content, and scaling (precipitation) in the field. Polyacrylamide (0.088% v/v) and ethylene glycol

123   (0.043% v/v) were added as commonly used fracturing additives (information obtained from

124   FracFocus database; www.FracFocus.org). The presence of As(V) and Se(VI) has been widely

125   reported in fracking fluids and aroused environmental concerns (Balaba and Smart, 2012;

126   Fontenot et al., 2013; Abualfaraj et al., 2014), therefore, 100 μg L^{-1} concentrations of assurance

127   grade As(V) and Se(VI) (SPEX CertiPrep, USA) were spiked into flowback solutions (Sun et al.,

128   2017). The presence of Sr(II) and Fe(III) in flowback solutions was also studied for their

129   potential environmental impact. The soil quality and the associated risks of metals/metalloids of

130   concern (i.e., As(V), Se(VI), Sr(II), and Fe(III)) were accordingly investigated in the case of spill

131   and leakage of flowback water near the ground surface.

132

133   *2.2 Metals/metalloids Leaching and Human Health Risks Assessment*

134   As the soil samples were obtained from the agricultural land of Northeast China Plain, where rice

135   is one of the dominating crops (USDA, 2014) and flooded environment is prevalent in rice paddy

136   fields, the fate of chemicals under saturated condition was investigated in this study. According

137   to a sudden spill scenario where spill volume is 1000 gallon (3785.4 L) over an impacted area of

138   0.1 acre (404.7 m^2) (Gradient, 2012), the solid-to-liquid ratio was calculated to range from 7 to

139   28 g L^{-1} assuming the bulk density of soil of 1.3 x 10^3 kg m^{-3} and the topsoil thickness of 5 to 20

140   cm. Therefore, the median solid-to-liquid ratio (17.5 g L^{-1}) was rounded up to 20 g L^{-1} and

141   adopted in this study. Each type of soil was mixed with Day 1, Day 14, and Day 90 flowback

142   solutions (400 mL), respectively, in 500 ml polypropylene centrifuge bottles at a soil-to-solution

143   ratio of 20 g L^{-1}. The samples were shaken by an end-over-end shaker at 20 rpm for 24 h, and

144   then incubated for 30 d at room temperature with daily shaking by hand for 1 min. At the end of

145   30 days, the artificially aged samples were separated by centrifugation at 4000 rpm. The

146   supernatant solutions were acidified with concentrated hydrochloric acid to pH less than 2 and

147   stored at 4 °C. The concentrations of As(V) and Se(VI) were determined by Atomic Absorption

148   Spectrometry with vapour generation accessory (VGA-AAS, Agilent VGA77, limit of detection

149   1 µg L^{-1}), while Sr(II) and Fe(III) were measured by AAS (limit of detection 1 mg L^{-1}). The

150   separated soils were air-dried (for subsequent biological analyses) in an incubator at room

151   temperature with a relative humidity of 20%. The control soils were aged at the same conditions

152   with deionized water instead of flowback solutions. Portions of the dried soils was assessed by

153   Synthetic Precipitation Leaching Procedure (SPLP, US EPA 1312) and European Council Waste

154 Acceptance Criteria (ECWAC, prCEN/TS 12457-3) tests, simulating the mobility under leaching

155 of acidic rainwater and unbuffered deionized water, respectively (Tsang et al., 2013a; 2013b).

156

157 In regard of human health risks in the vicinity of sensitive regions, the bioaccessibility of As(V),

158 Se(VI), Sr(II), and Fe(III) from the control and flowback-water-contaminated soils for human

159 receptors was assessed by an *in vitro* simplified physiologically based extraction test (SBET) for

160 estimating the bioaccessible metals in a simulated gastric environment (Drexler and Brattin,

161 2007; Beiyuan et al., 2016). The non-carcinogenic and carcinogenic risks of As(V), Se(VI), and

162 Sr(II) were characterized using the SBET results and US EPA standard methods. The hazard

163 quotients (HQ) of a specific metal element from the exposure routes via accidental soil ingestion

164 were added to determine a hazard index (HI) (US EPA, 1997; US EPA, 2001). The human risks

165 were adjusted by the bioaccessible fraction (BAF) rather than the total metal concentration, and

166 the corresponding hazard index (HI$_B$) was derived (Luo et al., 2012; Beiyuan et al., 2016). Three

167 major exposure pathways of heavy metals in soils were considered in this study: (a) ingestion; (b)

168 inhalation; and (c) dermal absorption (De Miguel et al., 2007), while only (a) and (c) were of

169 concern for As(V), Se(VI), and Sr(II) in flowback water contamination. Detailed calculations are

170 shown in the Supplementary Information.

171

172 *2.3 Ecological impact on soil quality*

173 The Microtox® bioassay and selected soil enzyme activity tests were carried out on the soils after

174 one-month exposure to flowback solutions. The soil toxicity was examined by the Microtox®

175 Basic Solid-Phase Test (AZUR, Model M500 Analyzer), which detects changes to specific

176 luminescent bacteria (*Vibrio fischeri*). The EC$_{20}$ was adopted to represent the soil concentration

177   at 20% effects on *Vibrio fischeri* bioluminescence upon 30-min exposure, which provides more

178   sensitive endpoints (Fulladosa et al., 2005). Dehydrogenases and phosphomonoesterase activities

179   were examined as a relatively rapid and easy procedure to assess soil health, which reflect the

180   total metabolic activity of soil microorganisms and regulated phosphorus metabolism in soils,

181   respectively (Lyubun et al., 2013; Beiyuan et al., 2017). Dehydrogenases activity was

182   determined by standard assay that reduced 2,3,5-triphenyltetrazolium chloride to

183   triphenylformazan, of which the intensity at 485 nm was detected by UV/Vis spectrophotometer

184   (Biochrom, Libra S35). Since all the soils used in this study were alkaline soils, the predominant

185   alkaline phosphomonoesterase activities were determined by measuring the *p*-nitrophenol

186   released from *p*-nitrophenyl phosphate using UV/Visible spectrophotometer at 410 nm.

187

188   **3. Results and Discussion**

189   *3.1 Mobility of metals/metalloids in soils after exposure to flowback solutions*

190   The mobility of As(V) and Se(VI) in the soils incubated with Day 1 flowback solution was more

191   than 50% and 5 times higher than those in the control soils when subject to DI water leaching

192   and acidic rainfall, respectively (Figure 1a-d). The mobility of both metalloids drastically

193   decreased with increasing ionic strength of flowback solutions (Table 2). The concentrations of

194   extracted As(V) from the soils incubated with Day 14 and Day 90 flowback solutions were even

195   lower than those from the control (Figure 1a&c). This suggested an unexpected suppression of

196   metal mobility by the chemical composition of the flowback solutions. The results may be

197   explained by possible change of binding phases in soil or formation of precipitates with elevated

198   cation concentrations in the flowback solutions, particularly for Ba(II), Ca(II), and Fe(III) as

199   shown in previous studies on arsenate (Zhang and Selim, 2005; Zhu et al., 2005). Besides, the

200 concentrations of As(V) in the ECWAC and SPLP tests were relatively comparable despite the

201 difference in soil-to-solution ratios (0.1 and 0.05 kg L^{-1}), indicating that As(V) was probably

202 desorbed from the soils via dissolution of acid-soluble minerals. Selenate showed similar

203 extraction patterns to As(V), but nearly 10-times higher leachate levels than the latter, suggesting

204 its weaker binding with the soils, which was also in line with our previous results of column

205 studies on metal transport (Chen et al., 2016).

206

207 The significance of soil characteristics on metal mobility seems to be less important than solution

208 chemistry of flowback water in this study. For instance, the metal concentrations in the leachates

209 from the BY and BX soils, which had abundant amounts of amorphous iron (oxy)hydroxides and

210 clay minerals, did not differ much from those of the sandy DQ soil (Figure 1a-d). Although these

211 contents proved to have a high affinity for the sorption and immobilization of As(V) and Se(VI)

212 (Peak and Sparks, 2002; Dixit and Hearing, 2003), this may be comparatively less important than

213 the effects of anionic PAM and elevated ionic strength in the flowback solutions.

214

215 As shown in Figure 2a&c, the mobility of Fe(III) was similar to that of As(V) and Se(VI). It was

216 the highest after aging with Day 1 flowback solution, even although the concentration of Fe(III)

217 significantly increased from Day 1 to Day 90 flowback solutions. It has been reported that Fe(III)

218 precipitates as $Fe(OH)_3$ in the flowback water (Lester et al., 2015), which probably induces the

219 co-precipitation and surface-precipitation with As(V) and Se(VI), thereby reducing their mobility

220 as observed in this study. In contrast, the leachate concentration of Sr(II) was significant and

221 increased from the Day 1 to Day 90 flowback solutions (Figure 2b&d), indicating a large

222 sorption capacity of Sr(II) in the four soils yet sorbed Sr(II) was relatively easy to leach out by

223 neutral or slightly acidic solutions. This result supports the previous findings that the sorption of

224 Sr(II) in soil mainly depends on ion exchange and the sorbed Sr(II) exists in labile fraction

225 (Twining et al., 2004; Gil-García et al., 2008), even in the matrix of fracking solutions.

226

227 *3.2 Bioaccessibility of metals/metalloids in soils after exposure to flowback solutions*

228 Figure 3a&b showed that the BAF values of As(V) and Se(VI) in all types of soils decreased

229 gradually with flowback solution exposure from Day 1 to Day 90. Interestingly, the

230 bioaccessible As(V) in all soil samples aged with the same flowback solution (except AD soil)

231 showed comparable BAF values regardless of the amount of Fe (oxy)hydroxides. The amounts

232 of As(V) sorption in each soil did not significantly vary among various flowback solutions as

233 indicated by mass balance calculation (Table S1). It is known that As(V) bounded by iron

234 (oxy)hydroxides could be released in the gastric solution (i.e., 0.4 M glycine in this study) in the

235 gastrointestinal tract (Smith et al., 2008). While As(V) would also co-precipitate with $Fe(OH)_3$

236 on soils during aging (Lester et al., 2015), the results of this study suggested that this fraction

237 was possibly bioaccessible as well. Consequently, the BAF values of As(V) showed little

238 variation among soils by glycine extraction. The BAF value over 100% (i.e., bioaccessible

239 concentration was higher than spiked total concentration) for the AD soil confirmed the presence

240 of indigenous As(V) in an elevated-arsenic-risk region. In addition, the decreasing BAF values

241 from Day 1 to Day 90 fracking solutions may suggest that the aquatic chemistry had an effect on

242 metal bioaccessibility. Previous bioaccessibility studies revealed a decrease of soluble and

243 exchangeable fractions with time during soil aging (Tang et al., 2007; Quazi et al., 2010), while

244 this study indicated that the solutions with higher ionic strength may favour the metal binding

245 with less-extractable fractions.

246

In contrast to the higher mobility of Se(VI) than As(V) (Figure 1a&b), the bioaccessibility of

Se(VI) was generally lower than As(V) (Figure 3a&b). This could be attributed to the capability

of glycine in the gastric solution for solubilizing more iron (oxy)hydroxides and consequently

more associated As(V) was extracted (Meunier et al., 2010). There was an absence of a clear

relationship between sorption and bioaccessibility of As(V) and Se(VI) (Figure S1), which

corroborated the lack of correlation between the mobility (i.e., ECWAC and SPLP) and

bioaccessibility (i.e., SBET) due to the different conditions in the measurements (Yang et al.,

2002). The important implication of these findings is that although the risk of groundwater

contamination by metal leaching is much lower in Day 14- and Day 90-contaminated soil, the

relatively high BAF values in all soil samples may result in human health risk (as exemplified in

subsequent section).

258

Moreover, the bioaccessible Se(VI) was much lower in the BX and DQ soils (approximately

10%) than in the BY and AD soils (about 40%) (Figure 3b). Both BY and AD soils had the

highest amount of TOC (Table 1), which, however, has been reported to inhibit Se

bioaccessibility to plants (Johnsson, 1991). The relatively high BAF values for Se(VI) in these

soils indicated that the controlling factors may differ for bioaccessibility to plants and humans.

The results of bioaccessible Fe(III) and Sr(II) related to the impacts of flowback solutions were

inconclusive as a considerable amount of Fe(III) may come from indigenous soil (Table S1),

while substantial concentrations of Sr(II) in the flowback solutions (up to 3320 mg L^{-1}) may

override the effects of different soil properties (Figure 3c&d).

268

269   *3.3 Effects of flowback solutions on soil toxicity and functions*

270   Bioavailability and soil properties are known to affect toxicity of metals in soils (Maisto et al.,

271   2011). A mild increase of toxicity (i.e., a lower EC_{20} value) was observed in the soils aged with

272   flowback solutions compared to the control (Table 3). Arsenic(V) is known to hinder the

273   generation of ATP and consequently inhibits *Vibrio fischeri* bioluminescence emission (Rubinos

274   et al., 2014). On the other hand, the EC_{20} values were approximately the same among flowback

275   solutions in each soil. This could be related to the soil-metal interactions in Day 14 and Day 90

276   flowback solutions, because they could limit the metal bioavailability to *Vibrio fischeri* (Tsiridis

277   et al., 2006; Rubinos et al., 2014). This result was in accordance with lower mobility and

278   bioaccessibility in soils incubated with Day 14 and Day 90 flowback solutions than with Day 1

279   flowback solution.

280

281   In addition, the compositional difference in the Day 1, 14, and 90 flowback water may alter the

282   toxicity significantly, as the additive, synergistic, or antagonistic effects of heavy metals on

283   toxicity was dependent on the concentration of each metal in the mixture (Tsiridis et al., 2006).

284   Stimulation of light emissions was detected in BY soil, which was probably due to the organic

285   compounds in the soil matrix that favoured the bioluminescence of *Vibrio fischeri* (Tang et al.,

286   2012), hence overcoming the effects of flowback solutions. Although AD soil had a similar TOC

287   content as BY soil (Table 1), indigenous arsenic exerted toxicity to AD soil, which was validated

288   by the mobility result (Figure 1) and Microtox bioassay (Table 3) in the control group.

289

290   As shown in Figure 4a, dehydrogenase activity was suppressed in soils with flowback solutions

291   of higher ionic strength. Metal contamination could disrupt soil functions by uncoupling the

292 formation of enzyme-substrate complexes, or masking the active groups on enzymes (Gianfreda

293 et al., 2005). Reduced dehydrogenase activity in soils was consistent with the increasing metal

294 contents in flowback solutions. Since As(V) hampers ATP generation, it affects the total

295 oxidative activity catalyzed by dehydrogenase (Rubinos et al., 2014). However, there was an

296 increase of dehydrogenase activity in the incubation with Day 1 flowback solution in BX and

297 DQ soil. A recent study on the impacts of fracking additives on agricultural topsoil showed an

298 increased ATP concentration in the presence of PAM (McLaughlin et al., 2016), which may

299 suggest an increasing microbial activity due to the utilization of the nitrogen source from PAM.

300 It has been shown that PAM is stable in soil and water without degradation over six-month

301 incubation (Caulfield et al., 2002; McLaughlin et al., 2016). Therefore, the presence of PAM

302 may promote dehydrogenase activity, while its beneficial effect was offset in Day 14 and Day 90

303 flowback solutions.

304

305 Similarly, phosphomonoesterase activity was significantly suppressed after incubation with Day

306 90 flowback solution (Figure 4b). Phosphate transporters could also take up As(V) because of

307 similar chemical and structural properties between phosphate (electron configuration of

308 phosphorus: [Ne] $3s^23p^3$) and arsenate (electron configuration of arsenic: [Ar] $3d^{10}4s^24p^3$), and

309 competition between arsenate and phosphate in phosphatase activity was reported in soils (Rosen

310 and Liu, 2009; Rubinos et al., 2014). A slight increase of phosphatase activity was found in some

311 soils after incubation with Day 1 flowback solution. Lyubun et al. (2013) also reported an

312 increase of soil phosphatase activities in As-contaminated soil, which could be also attributed to

313 analogous functions between phosphate and As(V) or the increase of phosphate-solubilizing

314 bacteria in soil. Varied microbial communities established in soils during aging with flowback

315   solutions may also contribute to a slight-to-moderate increase of dehydrogenase and

316   phosphomonoesterase activity in some soils with Day 1 solution (Mohan et al., 2013; Cluff et al.,

317   2014). In contrast to the lower mobility and bioaccessibility with Day 14 and Day 90 flowback

318   solutions, the more adverse influence on soil enzyme activities with Day 14 and Day 90 solution

319   indicated the contents of flowback solution should also be accessed by soil microflora. These

320   results revealed that flowback solutions were able to alter the soil microbial functions and the

321   influence varied with soil type, while only a gentle increase of toxicity was determined by

322   Microtox bioassay. A summary of the chemical and biological impact of various flowback

323   solutions on the soil environment was given in Table 4.

324

325   The carcinogenic risk assessment was based on the As(V) concentration in soils after one-month

326   aging with flowback solutions. Among the four concerned metals in this study, As(V) is

327   classified as a known human carcinogen by the Integrated Risk Information System of US EPA.

328   The results revealed that the adjusted carcinogenic risk posed by As(V) ranged from 4.56×10^{-7} to

329   2.09×10^{-6}, of which more than 85% was attributed to exposure via ingestion (Table S2).

330   According to the US EPA (2011), the cancer risks derived in this study would be considered

331   acceptable (10^{-4} – 10^{-6}), so that remediation might not be needed immediately. As for non-

332   carcinogenic risks related to the metals present in this study (As(V), Se(VI) and Sr(II)), ingestion

333   was also the dominant pathway followed by dermal contact and inhalation. The non-cancer

334   hazard index representing the aggregated non-carcinogenic effects of metals was smaller than 1

335   in this study, which meant that concern about an increase of health hazard was less likely (US

336   EPA, 2011). Although this study has provided an approximate assessment of human health risks,

337   the evaluation needs to be validated by site-specific exposure pathways. Potential synergistic or

338 antagonistic effects associated with the complex matrices and other components in the flowback

339 water should be taken into account in order to provide a more comprehensive evaluation of the

340 environmental risks.

341

342 **4. Conclusions**

343 During hydraulic fracturing, inadvertent spills of flowback water have been reported. Here, we

344 investigated the impact of contaminated flowback waters on environmental quality, soil

345 ecological health, and human health risk exposed to the surrounding soils of representative shale

346 gas areas in China. Both the mobility and bioaccessibility of As(V) and Se(VI) decreased as the

347 ionic strength in flowback solution increased, indicating a change of metal-binding phases with

348 varying solution pH and composition. The results revealed a mild increase of soil toxicity

349 (Microtox bioassay) compared to the control after one-month aging with flowback solutions,

350 while the dehydrogenase activity and phosphomonoesterase activity were significantly decreased

351 with increasing ionic strength in flowback solutions. A preliminary human health risk assessment

352 implied no immediate need for remediation due to As(V) and Se(VI). This study demonstrates

353 that the temporal change of flowback water compositions leads to different environmental

354 implications, which deserve more comprehensive evaluation for a holistic understanding of the

355 environmental impact of fracking process.

356

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