Transport and degradation of perchlorate in deep vadose zone: implications from direct observations during bioremediation treatment

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Abstract

An in situ bioremediation experiment of a deep vadose zone (~40 m) contaminated with a high concentration of perchlorate (>25,000 mg L^{-1}) was conducted through a full-scale field operation. Favorable environmental conditions for microbiological reduction of perchlorate were sought by infiltrating an electron donor-enriched water solution using drip irrigation underlying an airtight sealing liner. A vadose-zone monitoring system (VMS) was used for real-time tracking of the percolation process, the penetration depth of dissolved organic carbon (DOC), and the variation in perchlorate concentration across the entire soil depth. The experimental conditions for each infiltration event were adjusted according to insight gained from data obtained by the VMS in previous stages. Continuous monitoring of the vadose zone indicated that in the top 13 m of the cross section, perchlorate concentration is dramatically reduced from thousands of milligrams per liter to near-detection limits with a concurrent increase in chloride concentration. Nevertheless, in the deeper parts of the vadose zone (<17 m), perchlorate concentration increased, suggesting its mobilization down through the cross section. Breakthrough of DOC and bromide at different

depths across the unsaturated zone showed limited migration capacity of biologically consumable carbon and energy sources due to their enhanced biodegradation in the upper soil layers. Nevertheless, the increased DOC concentration with concurrent reduction in perchlorate and increase in the chloride-to-perchlorate ratio in the top 13 m indicate partial degradation of perchlorate in this zone. There was no evidence of improved degradation conditions in the deeper parts where the initial concentrations of perchlorate were significantly higher.

1 Introduction

In situ bioremediation of a contaminated unsaturated zone (also termed vadose zone) depends mainly on the ability to control the hydrological, physical and chemical conditions in the subsurface (Bombach et al., 2010; EPA, 2015; Höhener and Ponsin, 2014). Chemical and hydrological manipulations are primarily aimed at enhancing the activity of specific indigenous degrading bacteria. The optimal conditions for specific contaminants' degradation are usually determined in microcosm experiments, where the preferred electron donor and acceptor for degradation can be controlled and examined (Gal et al., 2008; Megharaj et al., 2011; Sagi-Ben Moshe et al., 2012). The optimal degradation conditions, evaluated through laboratory experiments, usually form the basis for selecting a strategy for in situ remediation in field-scale operations. Nevertheless, implementation of desired biodegradation conditions in the deep vadose zone through full-scale field setups requires control of the vadose zone hydrogeochemical conditions. This is often achieved through either infiltration of water enriched with electron donors or nutrients (Battey et al., 2007; EPA, 2004; Frankel and Owsianiak, 2005), or injection of a gaseous mixture capable of promoting optimal biogeochemical conditions for microbial pollutant degradation (Evans et al.,

                                                                 Dahan

2011; Evans and Trute, 2006). Due to the complex nature of flow and transport processes in the unsaturated zone, application of water with specific chemical conditions near land surface does not necessarily result in promoting the desired geochemical and hydraulic conditions in deeper parts of the vadose zone (Allaire et al., 2009; Flury and Wai, 2003; Jarvis, 2007; Rimon et al., 2011a). Therefore, in the vadose zone, and particularly in its deeper parts, a proper understanding of the transport process is key to the success of in situ remediation operations (Baram et al., 2012a; Dahan et al., 2009; Kurtzman et al., 2016; Rimon et al., 2011a).

Assessment of water percolation and solute transport in the vadose zone is considered a major challenge in hydrological sciences. It is often characterized by unstable flow that is highly sensitive to hydraulic, chemical and microbial conditions (Bautersa et al., 2000; Dahan et al., 2009; DiCarlo, 2007; Germann and al Hagrey, 2008; Hallett et al., 2013; Rimon et al., 2011a; Sher et al., 2012; Stumpp et al., 2009). Moreover, the chemical composition of the percolating water [e.g., dissolved organic carbon (DOC), oxygen and nutrients] is subjected to frequent changes due to natural hydroclimatic and biological cycles (Stumpp et al., 2009, 2012). Accordingly, contaminant attenuation in the vadose zone is dependent on the complex hydrological, chemical and biological states of the sediment. Continuous measurements of the hydrological and chemical properties of the unsaturated zone may be achieved with a vadose-zone monitoring system (VMS) (Dahan et al., 2009). The VMS provides high-resolution measurements of variation in sediment water content (Dahan et al., 2008; Rimon et al., 2007) and evolution of the pore water's chemical composition across the unsaturated profile (Rimon et al., 2011a; Dahan et al., 2014; Turkeltaub et al., 2014, 2016).

72 Perchlorate is an environmental pollutant that is often associated with the explosives

73 manufacturing industry (Roote, 2001; Urbansky, 2002; Trumpolt et al., 2005). It is

74 mostly produced, and consequently released to the environment as ammonium

75 perchlorate. Its high solubility (220 g L^{-1}) and stability in aerobic environments makes

76 it very mobile and persistent in the subsurface (Motzer, 2001; Urbansky and Brown,

77 2003). Microbial reduction of perchlorate to harmless chloride and oxygen in the

78 unsaturated zone requires elevated water content, negative redox potential, available

79 electron donors and the presence of suitable indigenous bacteria (Coates and

80 Achenbach, 2004). In the vadose zone, natural attenuation and biodegradation of

81 perchlorate are considered very limited (Gal et al., 2009). Nevertheless, studies have

82 shown that perchlorate can be metabolized in unsaturated soil whenever reducing

83 conditions (<110 mV) (Attaway and Smith, 1993; Shrout and Parkin, 2006) are

84 achieved and an available electron donor is introduced (Tipton et al., 2003; Frankel

85 and Owsianiak, 2005; Nozawa-Inoue et al., 2005; Evans and Trute, 2006; Cai et al.,

86 2010).

87 Here, the efficiency of a remediation operation of a perchlorate-contaminated vadose

88 zone was assessed using a VMS, which provided continuous information on the

89 chemical composition of the vadose-zone pore water. Promotion of perchlorate-

90 degrading conditions in the vadose zone was based on infiltration of water enriched

91 with ethanol (as a source of electron donor) from land surface. Real-time information

92 on the depth of the enriched water's propagation, along with variations in the

93 concentrations of perchlorate, chloride and bromide (applied as a tracer), was used to

94 assess transport and degradation of perchlorate across the unsaturated profile. Water-

95 and ethanol-application strategies were adjusted in each flow phase to obtain real-time

96 feedback on the chemical and hydrological state of the vadose zone.

2 Study site

The study area is located in the central part of the Israeli coastal plain, east of the city of Ramat Hasharon. The site is a former unlined earthen pond that was used to store industrial wastewater for several decades. A hydrogeological survey conducted in the study area revealed substantial perchlorate contamination in the vadose zone and groundwater under the pond area (Gal et al., 2008, 2009). It was concluded that percolation of untreated wastewater from the ponds had crossed the 40m thick vadose zone and created a large perchlorate pollution plume in the underlying phreatic aquifer with concentrations exceeding 1,000 mg L^{-1}. In the vadose zone, however, the investigation revealed extreme perchlorate pollution, reaching concentrations exceeding 2,000 mg kg^{-1} dry soil (equivalent to ~25,000 mg L^{-1} in the sediment pore water), along with high total salinity and chloride concentration exceeding 25,000 mg L^{-1}. Because this area is under consideration for future urban development, remediation of both the vadose zone and groundwater there is of major concern.

The stratigraphy of the area is characterized by Neogene and Pleistocene sediments, mainly of sands and sandstones with interbedding of clay lenses (Gvirtzmen, 2002). The vadose zone lithological profile at the site was assessed again through a borehole that was drilled at the pilot site in 2012 (Table 1, Fig. 1). Most of the profile is composed of yellow and red sand layers with low clay content (<5 %), with interbeds of brown sand containing variable clay content of up to 11 %. A single ~1m thick clay layer (27.5 % clay content) was observed at a depth of 13.3 m. To improve infiltration capacity in deep sections of the vadose zone during the remediation experiment, a shallow clay layer with low permeability, known as "nazaz" (Singer, 2007), was removed from a depth of 2.5–3 m by excavation. The excavated area, 10 × 30 m,

which was primarily assigned for the pilot infiltration experiment, was backfilled with the sandy loam from the excavated site after removal of the 0.5m thick nazaz layer. This layer is therefore presented in the profile as disturbed soil.

The climate in the area is characterized as subtropical Mediterranean with a hot and dry summer from May to October and a colder wet winter from November to April. The average air temperature on summer and winter days is 30 °C and 17 °C, respectively. The average annual precipitation is 530 mm year^{-1}, mostly as rain occurring mainly in four to seven rainy episodes during the winter season (IMS, 2011).

3 Experimental setup

3.1 Vadose-zone monitoring setup

Real-time characterization of flow and transport processes in the vadose zone, as well as assessment of chemical transformation of the percolating water during the remediation experiments were carried out with a VMS that was installed across the entire unsaturated profile, from land surface to a depth of 37 m (Fig. 2). A detailed description of the VMS, its structure, installation procedure and performance, can be found in previous publications (Dahan et al., 2009; Rimon et al., 2011a) and in the supplementary material. In particular, the VMS that was used at this site was composed of a 44m long flexible polyurethane sleeve hosting 11 monitoring units distributed along its length. Each monitoring unit included: (a) a flexible time-domain reflectometer (FTDR) sensor for continuous measurement of variations in the sediment water content (Dahan et al., 2008; Rimon et al., 2007), and (b) vadose-zone sampling ports (VSPs), which enable frequent sampling of the vadose zone pore water for chemical analysis (Baram et al., 2012a; Dahan et al., 2009; Rimon et al., 2011b;

145 Turkeltaub et al., 2016). The VMS flexible sleeve was installed in a 0.16m diameter

146 uncased borehole drilled slanted at a 55° angle (to the horizon) to a vertical depth of

147 37 m. In addition to the 11 monitoring units that were installed with the VMS, four

148 additional monitoring units were installed directly in the soil at depths of 0.5 m and

149 1.5 m. It should be noted that the slanted installation is preferred to ensure that

150 measurements carried out by each monitoring unit take place in separate undisturbed

151 sediment columns. In addition, the flexibility of the monitoring sleeve and its filling

152 with non-shrinking cement grout ensured complete sealing of the borehole void and

153 prevention of cross-contamination through preferential flow in the borehole.

154 **3.2 Field setup**

155 Water amended with ethanol as the electron donor for perchlorate-reducing bacteria

156 was infiltrated into the vadose zone through an area of 8 x 30 m at the pilot site using

157 a drip-irrigation system. Dripping lines with drippers having a nominal discharge rate

158 of 2.2 L h^{-1} were set up in a 0.3 x 0.3 m spatial distribution to create fairly even water

159 distribution over the area. Accordingly, the total discharge rate of the irrigation

160 system was set to 5 m^3 h^{-1}, which is equivalent to an infiltration rate of 0.02 m h^{-1}. To

161 promote anaerobic conditions in the unsaturated zone, a polyethylene liner covered

162 with soil was placed over the dripper system after its installation. Ethanol was

163 selected as the electron donor and carbon substrate because it is a natural, soluble

164 compound that is commonly used by perchlorate-reducing bacteria (Bardiya and Bae,

165 2011). Moreover, it eliminates the increased soil salinity associated with other

166 common sources of electron donors such as acetate (Gal et al., 2008).

167 **3.3 Infiltration experiments**

168   Three infiltration experiments with variable amounts of water and ethanol were

169   implemented at the pilot site over a period of 7 months. To trace the percolating water

170   across the unsaturated zone, bromide (as KBr) was added to the infiltrating water at

171   the early stages of the experiment. The infiltration rates, as well as the concentrations

172   and application sequence were assigned for each experiment with insight gained from

173   the previous experiment (Table 2). Accordingly, information obtained by the VMS on

174   depth propagation of the ethanol and tracer and variations in perchlorate and chloride

175   concentrations across the unsaturated zone during and after each infiltration

176   experiment were used to adjust the infiltration procedure in the following stage.

177   The first experiment (8 Aug 2010) consisted of infiltration of 50 m^3 water (equivalent

178   to 210 mm) (Table 2). The first 6 m^3 were applied as untraced fresh water with no

179   ethanol to wet the topsoil. This wetting stage is essential to promoting deep transport

180   and preventing accumulation of tracers and ethanol in the low-flow zone located on

181   the margins of the dripper's influential zone. Following the initial wetting phase, 0.4

182   m^3 of bromide tracer solution (as KBr) at a concentration of 12.5 g L^{-1} was applied,

183   followed by 1 m^3 of water with 5 % ethanol. Immediately after the application of the

184   carbon and tracer solution, the rest of the water (42.6 m^3) was applied to enhance

185   transport of the ethanol and tracers to deeper parts of the vadose zone.

186   After obtaining the results pertaining to the wetting process, as well as tracer and

187   ethanol migration in the vadose zone during the first infiltration experiment, a second

188   experiment was performed (1 Sep 2010). This experiment was conducted with 100 m^3

189   of water (equivalent to 420 mm). Here the first 7 m^3 of water was injected into the

190   topsoil as untraced fresh water, followed by 1 m^3 of water with 5 % ethanol, and then

191   the rest of the water dose (92 m^3). No tracers were used in this experiment. The

192 amount of water used after application of the ethanol was doubled to enhance

193 migration of the ethanol to deep sections of the unsaturated zone.

194 Results from the first two experiments indicated limited migration of tracer and

195 ethanol to deeper parts of the vadose zone. A third infiltration experiment was

196 therefore conducted 5 months later with increased discharge of 300 m^3 (equivalent to

197 1250 mm). This experiment started with 24 m^3 of untraced water followed by 0.4 m^3

198 concentrated (50 %) ethanol solution. Then, the rest of the water (275.6 m^3) was used

199 to push the ethanol down into the vadose zone. The large quantity of water applied

200 after the concentrated ethanol solution was designed to enhance quick migration of

201 the ethanol to deep parts of the vadose zone while minimizing its biodegradation in

202 the upper soil layers.

203 **3.4 Analytical procedure**

204 Perchlorate was analyzed with a perchlorate ion-selective electrode (ISE; Laboratory

205 Perchlorate Ion Electrode, Cole-Parmer, USA). All samples measured with the ISE

206 were adjusted by dilution to a concentration range of 10–100 mg L^{-1}. Duplicates were

207 frequently analyzed by injecting 25 µL sample into a Thermo Scientific™ Dionex™

208 ion chromatography system (ICS 5000) equipped with Ion Pac AS19 column

209 (detection limit of ±0.01 mg L^{-1}). Because results from the two methods were not

210 significantly different, most of the data reported here are from the perchlorate

211 electrode with a detection limit of 1 ppm. Bromide and chloride were analyzed by ion

212 chromatography with a detection limit of 30 ppb (Gal et al. 2008). Total organic

213 carbon (TOC) was analyzed to examine the success of delivering carbon to the vadose

214 zone. Because porewater samples from the vadose zone are obtained through the VSP,

215 which uses a porous ceramic interface (pore size < 2 µm), TOC values reflect DOC.

TOC was analyzed through a combustion TOC analyzer (Teledyne Tekmar, Apollo 9000) with a detection limit of 2 ppm. Ethanol concentration in the vadose zone pore water was analyzed in a gas chromatograph (Varian, CP3800). Water samples (1.5 μL) were injected by autosampler. The FID and injector temperatures were set to 270 and 250 °C, respectively. The GC oven temperature was first held at 50 °C for 1 min, increased to 220 °C at a rate of 25 °C min^{-1}, and then held for 4 min. The separation was performed by Stabilwax® capillary column (60 m, 0.32 mm, 0.25 μm, Restek Corporation, USA); helium was used as the carrier gas (1 mL min^{-1}). For quantification, five external standards were used.

4 Results and discussion

All of the data obtained by the VMS are presented here as variations in measured parameters with depth, as commonly done to describe depth profiles. However, to ensure measurements under undisturbed vertical profiles, the VMS was installed in a slanted orientation (Fig. 2 and supplementary material). Thus, each monitoring unit faces an undisturbed profile that is shifted horizontally and vertically from the other units. Accordingly, although the data are presented as depth profiles, they should be regarded as individual points distributed across the 3D space of the vadose zone (Dahan et al., 2007; Rimon et al., 2011a).

Prior to detailed discussion on the results a general outline of the rationale behind the experimental setup will be presented here. Three infiltration experiments were conducted with variable amounts of water, ethanol as electron donor, and bromide as a tracer (all of which is presented above in chapter 3.3 *Infiltration experiment*). Nevertheless, the experimental conditions in each experiment were set following the results obtained from the previous stage. The first infiltration experiment was

conducted as a first trial to infiltrate ethanol-enriched water-solution into the unsaturated zone. This experiment was also conducted with bromide as a tracer in order to mark the water front propagation across the unsaturated zone. As will be discussed further on, results the first infiltration experiment indicated that the migration capacity of both ethanol and bromide across the unsaturated profile was very limited. Accordingly a second infiltration experiment was conducted with a double amount of water and the same amount and concentration of ethanol in order to enhanced deep migration of the ethanol down the unsaturated zone. Following the results from the first two experiments a third infiltration experiment was conducted with larger water volumes and higher ethanol concentration in order to avoid quick ethanol degradation in the shallow soil. all of which will be presented and discussed below.

4.1 Water percolation

Temporal variations in the vadose zone water content provide a direct indication of percolation processes in the vadose zone (Rimon et al., 2007; Dahan et al., 2008; Turkeltaub et al., 2015). Each infiltration experiment launched a wetting wave that propagated sequentially through the unsaturated zone (Fig. 3). Down-migration of the wetting wave was expressed as a quick rise in water content followed by a recession caused by water redistribution and drainage. Referring the wetting sequence in the vadose zone to the infiltration events on land surface enabled a direct calculation of the flow velocity across the unsaturated zone (Rimon et al., 2007; Dahan et al., 2008). All three infiltration experiments produced wetting fronts that moved down the vadose zone at a velocity of ~0.18 m h^{-1}, even though the water volumes that were used in each experiment were significantly different (50, 100 and 300 m^3).

264   Observations of regulated flow velocities at constant rates across the vadose zone

265   under variable surface hydraulic conditions have also been reported in other studies

266   (Dahan et al., 2008; Amiaz et al., 2011; Rimon et al., 2011a).

267   The high salinity of the deeper parts of the vadose zone (>13 m) (Fig. 1) limits the

268   reliability of the TDR technology for measuring water content at those depths (Nadler

269   et al., 1999). Therefore, variation in water content, as an indication of deep

270   percolation, is presented here only down to a depth of 11.2 m, where the salinity was

271   low enough to achieve reliable moisture measurements with the FTDR sensors.

272   Nevertheless, indications of deep percolation in the deeper layers (>13 m) are further

273   discussed through the variation in chemical composition of the percolating water

274   across the entire thickness of the unsaturated zone (40 m).

275   **4.2 Perchlorate transformation and mobilization**

276   Initial analysis of porewater samples from the vadose zone, prior to initiation of the

277   infiltration experiments, revealed very high concentrations of perchlorate and

278   chloride, both reaching maximum values of ~22,500 mg L^{-1} (Fig. 1), and total

279   dissolved solids (TDS) of 43,000 mg L^{-1}, at a depth of 21 m. Note that at this stage,

280   the concentrations of perchlorate and chloride are nearly identical throughout the

281   entire profile. These high concentrations, sampled by the VMS, are in accordance

282   with concentration profiles obtained previously in extracts of sediment samples (Gal

283   et al., 2009).

284   Frequent sampling of the vadose zone pore water showed dynamic variations in

285   perchlorate concentration during the percolation experiments. In the upper section of

286   the vadose zone (0–13 m), perchlorate concentrations decreased dramatically, from as

287   high as 9000 mg L^{-1} to below detection levels (Fig. 4). Such a reduction in

concentration in a relatively thick portion of the vadose zone (13 m) over the short period of 10 months is clearly desirable and may even be considered a great success. Nevertheless, closer inspection of the variations in perchlorate concentration in deep parts of the vadose zone (17–40 m) showed an increase at most of the measurement points (Fig. 5). Perchlorate concentration rose from 12,700 mg L^{-1} to 27,400 mg L^{-1} at a depth of 17 m during the same period. A similar increase in concentration was also found in deeper parts of the cross section at depths of 25, 28, and 36 m. Note that during this period, an increase in perchlorate concentration was even observed in the groundwater (represented at a depth of 41 m in Fig. 5). Obviously, the mixed trend in variations of perchlorate concentration implies that transformation and mobilization processes take place simultaneously. As such, the conditions for both biodegradation and mobilization should be examined along with the variation in perchlorate concentration.

4.3 Electron donor availability

Available organic carbon as an electron donor is crucial for perchlorate reduction. To increase the concentration of DOC in the vadose zone, ethanol was mixed with the percolating water during the early stage of each infiltration experiment. Analysis of ethanol and DOC in the water samples from the vadose zone throughout the experiment revealed a similar concentration pattern (1 g ethanol = 2 g DOC). Therefore, we assume that the variation in DOC was due to transport of ethanol or ethanol-degradation products with the percolating water.

During the first infiltration experiment, an increase in DOC above background levels was observed only at shallow depths, down to 1.5 m (Fig. 6). No signs of increasing DOC were observed in the deeper parts of the cross section at this stage. Twenty-three

312   days later, before initiation of the second infiltration experiment, DOC values had

313   dropped back down to background levels. This implies that the ethanol was

314   microbiologically consumed in the soil before it could be leached further down.

315   As a result of the limited transport of ethanol in the first infiltration experiment, a

316   second experiment was conducted with the same mass and concentration of ethanol.

317   However, it was flushed with double the amount of water to promote its quicker

318   migration to deeper layers (Fig. 4). In this experiment, no signs of increasing DOC

319   were observed at any depth. On the contrary, DOC level decreased to values below

320   background levels (Fig. 6). Obviously, the rate of ethanol metabolism in the soil

321   increased following the first experiment, where both water content of the sediment

322   and substrate required for efficient microbial activity increased. As a result, ethanol-

323   degradation efficiency in the topsoil (<0.5 m) was significantly enhanced.

324   To overcome the limitation of electron donor delivery through the shallow soils, a

325   third infiltration experiment was designed. In this experiment, the ethanol was

326   injected in a $0.4 m^3$ high-concentration (50 %) pulse followed by a large volume of

327   water. Application of ethanol at a very high concentration was aimed at suppressing

328   its biological degradation in the shallow soil. The ethanol pulse was introduced after

329   application of 24 m^3, the latter to provide high initial wetting conditions under the

330   ethanol front. Then the ethanol slug was pushed down with 276 m^3 of water. At this

331   stage of the study, which was conducted 6 months after the previous one, a substantial

332   increase in DOC was observed in the entire top 13 m of the cross section (Fig. 6).

333   Obviously, an increase in DOC serving as electron donor is an essential prerequisite

334   for perchlorate degradation. Apparently, application of ethanol at a high

335   concentration, which inhibited its degradation in the upper layer, succeeded to drive

the ethanol all the way down to 13 m, just above the clay layer. Nevertheless, no signs

of DOC increase were observed below 13 m.

4.4 Transport and degradation

The mechanism controlling down-propagation of a non-conservative substance such

as ethanol may be elucidated by looking at the migration pattern of a conservative

tracer such as bromide. Bromide was injected with the percolating water in the early

stages of the first infiltration experiment. Results on bromide migration are presented

here only for the top 13 m, where the background concentrations prior to the initiation

of the infiltration experiment were below detection limits. Concentration profiles

during the infiltration experiments clearly demonstrated sequential progress of the

percolating water across the top 13 m of the unsaturated zone (Fig. 7). Mass balance

calculation of bromide on the basis of the concentration profiles (Fig. 7) and sediment

water content (Fig. 3) on various dates after the infiltration experiment resulted in

high recovery rates of 85–127 %. A comparison of the transport patterns of bromide

and DOC confirmed that biodegradable material such as ethanol is rapidly consumed

in the vadose zone.

An increase in chloride concentration in the vadose zone is usually attributed to

evaporation processes near land surface, a mechanism that is unlikely to occur in this

particular setup where the surface is isolated from the atmosphere. Accordingly,

variations in chloride concentration across the vadose zone may be attributed to

chloride mobilization with the percolating water and perchlorate reduction. Therefore,

degradation of perchlorate is expected to result in an increase in chloride mass.

Prior to the infiltration experiments, chloride-to-perchlorate ratios in the vadose zone

were very similar, exhibiting nearly identical profiles (Fig. 1) with equivalent

concentration proportions of 2.4–5.5 (Fig. 8). Following the infiltration experiment, a significant increase in ionic ratios was observed in the top 13 m, while in the rest of the profile—from a depth of 17 m to the water table, the concentration ratio of chloride to perchlorate remained relatively unchanged. Obviously, since both perchlorate and chloride are very soluble and mobile, infiltration water with a low concentration of chloride (~100 mg L^{-1}) and zero perchlorate is also expected to result in an increased chloride-to-perchlorate ratio, even if no perchlorate degradation takes place. Since both chloride and perchlorate are very mobile and easily displaced with the percolating water, quantification of the perchlorate-degradation rate with respect to its down-leaching is not straightforward.

5 Conclusions

The infiltration experiments were primarily aimed at improving the environmental conditions for perchlorate-reducing bacteria across the vadose zone. This included an increase in water content along the soil profile and amendment of the electron donor. The results, which were based on continuous monitoring of the entire vadose zone, exhibited notable variation in the concentrations of perchlorate, DOC and other solutes in the unsaturated zone. Increased concentrations of DOC with a concurrent reduction in perchlorate concentration (from thousands to a few milligrams per liter) and increased chloride-to-perchlorate ratio (from ~2.5 to ~300) in the upper 13 m indicated that perchlorate is partially reduced in this part of the vadose zone. On the other hand, no evidence of improved reducing conditions was observed in the deeper parts, where the initial concentrations of perchlorate were significantly higher. Nevertheless, since assessment of redox conditions in deep vadose zone is not yet

feasible, we can only rely on variations in the chemical composition to assess the existence of degradative conditions.

The limited ability to deliver a soluble electron donor across a microbiologically reactive medium, such as topsoil, is a major limiting factor for remediation of the deep vadose zone through gravitational percolation of enriched solution. Note that temporal variations in the concentrations of perchlorate, as well as other solutes, in the deep parts of the vadose zone, i.e., under the clay layer at 14 m, indicate that the clay layer does not play any role in limiting infiltration capacity in terms of flow velocity and fluxes. Similar observations on the role of clay layers in infiltration in the unsaturated zone have been reported in previous publications (Baram et al., 2012b, 2012c; Dahan et al., 2009; Rimon et al., 2007; Turkeltaub et al., 2015b).

The attempts to leach the ethanol down into the vadose zone with large quantities of water inevitably drove down-leaching and displacement of the dissolved solutes, including perchlorate. Although there were indications of partial degradation of perchlorate in the upper part of the vadose zone, its downward displacement toward the water table was evident from the sequential increase in perchlorate concentration with depth (Fig. 5). It seems that the entire column of perchlorate mass was pushed down by the percolating water toward the water table, which also resulted in an increased concentration of perchlorate in the observation well, which was located under the infiltration zone.

The study demonstrates that application of vadose-zone monitoring technology during a remediation operation provides real-time information on the chemical and hydrological state of the subsurface. Linking the temporal variation in the chemical

406 composition of the vadose zone pore water, sediment saturation degree and flow

407 velocities are vital for efficient management of remediation operations.

408 ***Author contribution:*** *Ofer Dahan (PI, Vadose zone hydrology) design of the*

409 *experimental and monitoring setup. Idan Katz (MSc student) conducted the field*

410 *experiment and laboratory analysis. Zeev Ronen (PI, Microbiology) design the bio*

411 *treatment setup. Data analysis and manuscript preparation - all coauthors.*

412 ***Competing interest:*** *The authors declare that they have no conflict of interest.*

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567 *Table 1. Sedimentological Composition of the Vadose Zone at the Pilot Site*
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Depth (m)	Description	Clay Content (%)
0–3	Red sand (disturbed)	7.5
3–5	Red sand (Hamra)	5
5–7	Red-yellowish sand	5
7–10	Yellow sand	5
10–13	Brown sand	5
13–14	Dark brown clay	27.5
14–17	Red-brown clayish sand	12.5
17–20	Brown clayish sand	3.75
20–27	Yellow sand	1
28–29	Brown sand	11.75
29–33	Red-clayish sand (Hamra)	3
33–41	Yellow sand	0

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578 *Table 2. Infiltration experiment conditions*

Date	Water Volume (m^3)	Equivalent Water Depth (mm)	Ethanol (l)	Bromide (Kg)
8 Aug 2010	50	210	50	5
1 Sep 2010	100	420	50	-
27 Feb 2011	300	1250	200	-

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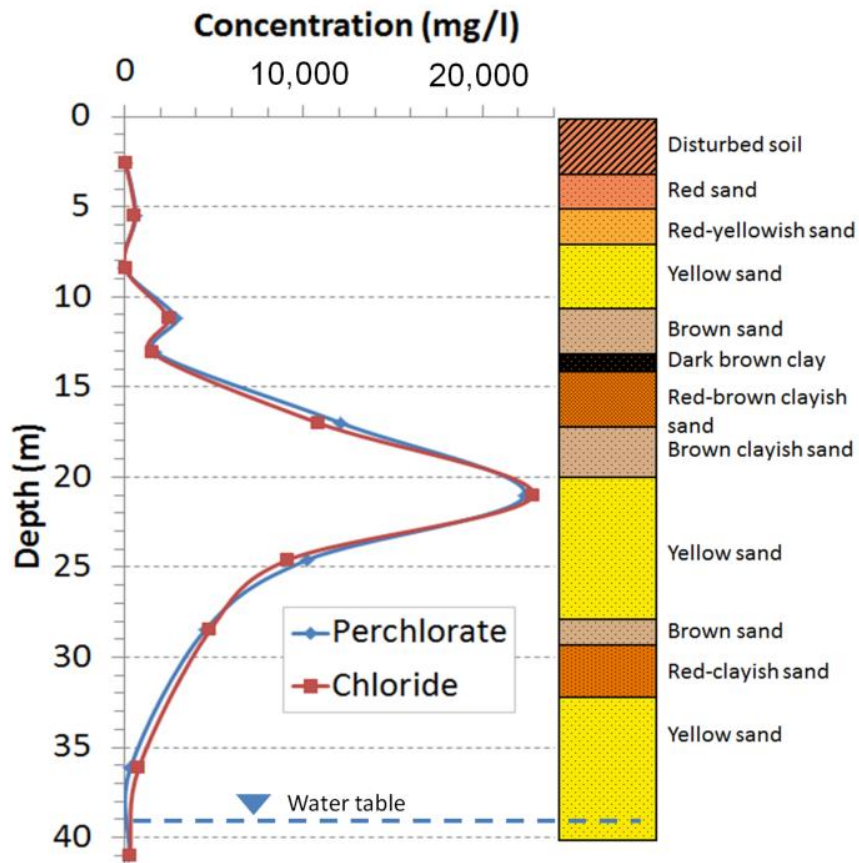
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582   Figure 1. Initial concentration profiles of chloride and perchlorate in the vadose zone

583   pore water under the former waste lagoon, along with the lithological profile.

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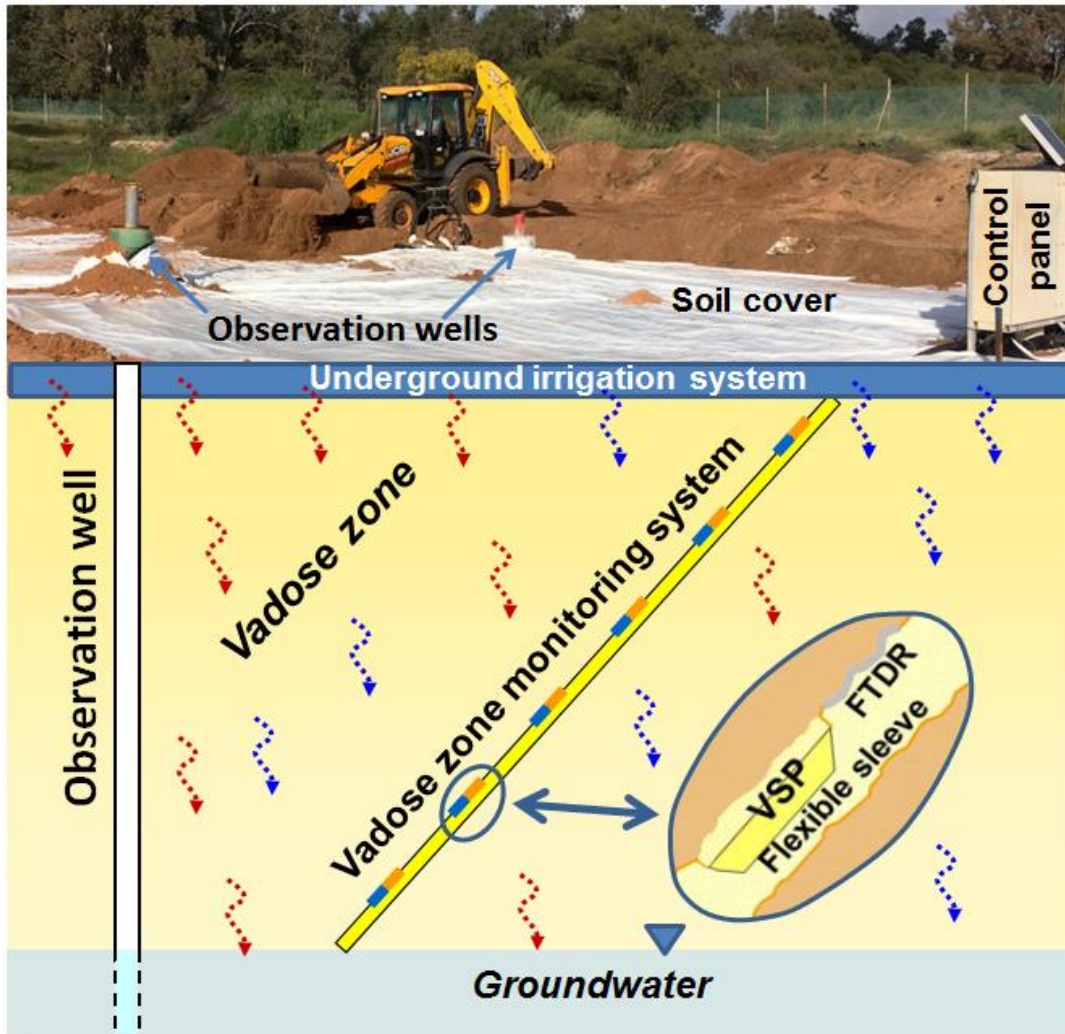
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586 Figure 2. Schematic illustration of the vadose-zone monitoring system installed in the

587 vadose zone under the infiltration pilot site. In the picture above the vadose zone, the

588 irrigation system at the site is being covered.

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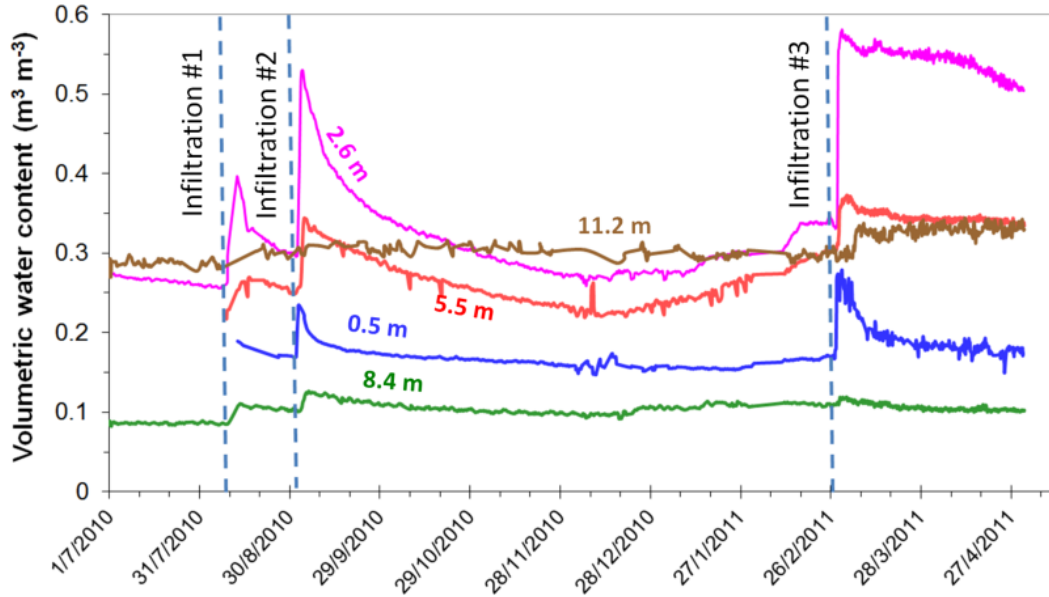
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591 Figure 3. Temporal variations in sediment water content in the top 13 m of the vadose

592 zone during the infiltration experiments. Dates are given as day/month/year.

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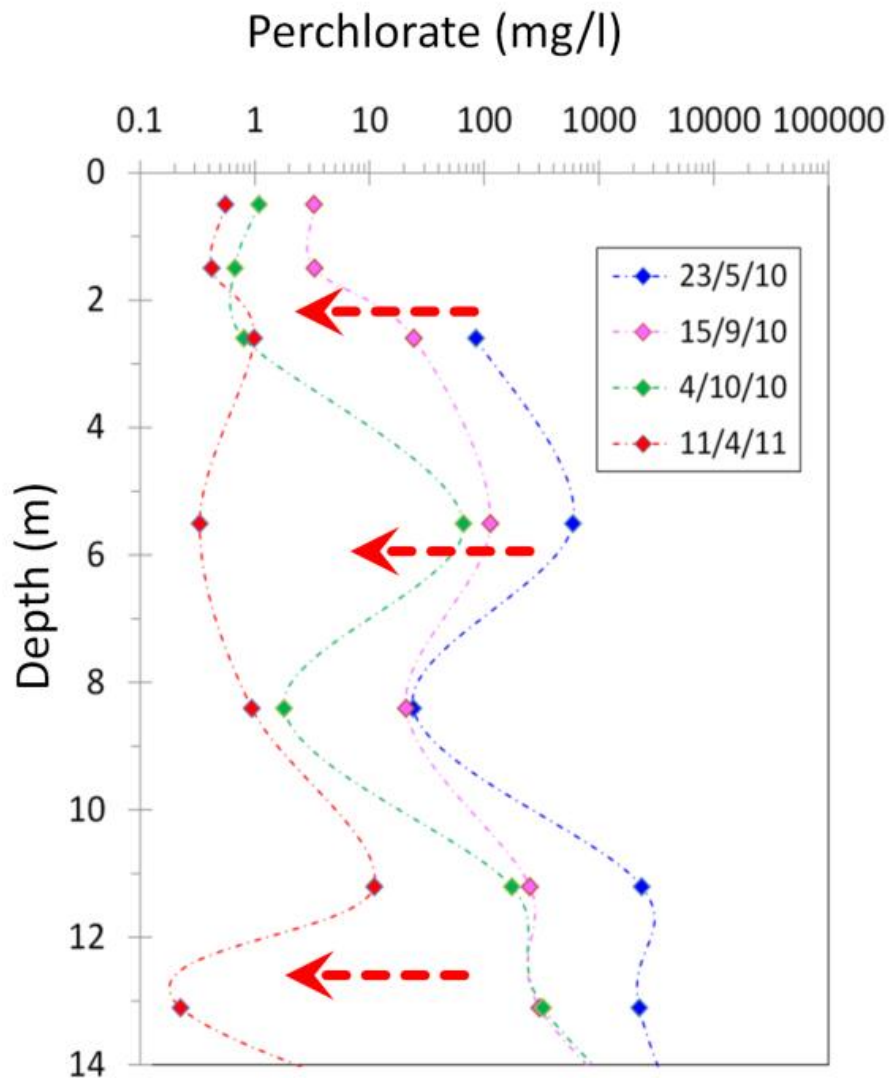
26   Dahan

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595   Figure 4. Perchlorate concentration profile across the top 13 m of the vadose zone

596   under the pilot site during the infiltration experiments. The profiles emphasize the

597   gradual decrease in perchlorate concentration with time (marked in red arrows). Dates

598   are given as day/month/year. Note that data points are aligned in a slanted orientation

599   and interpolated as time intervals.

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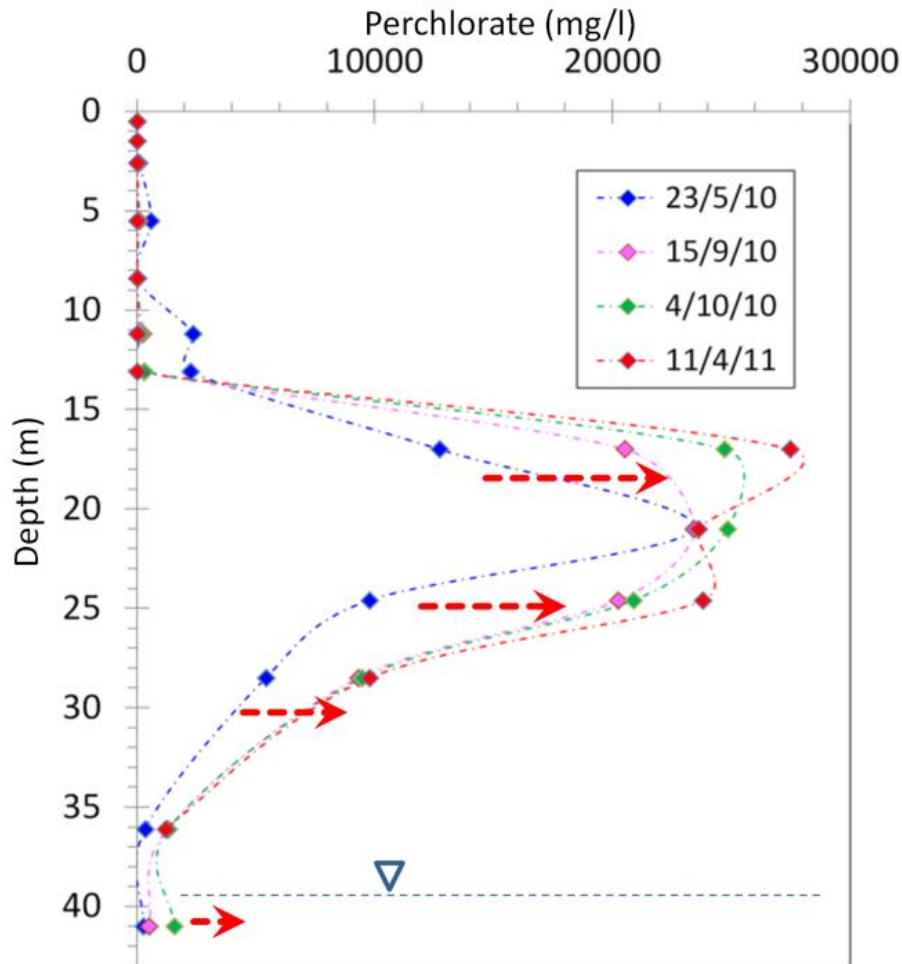
Figure 5. Perchlorate concentration profile across the entire vadose zone and top groundwater under the pilot site during the infiltration experiments. The profiles emphasize the gradual increase in perchlorate concentration with time (marked in red arrows). Dates are given as day/month/year. Note that data points are aligned in a slanted orientation and interpolated as time intervals.

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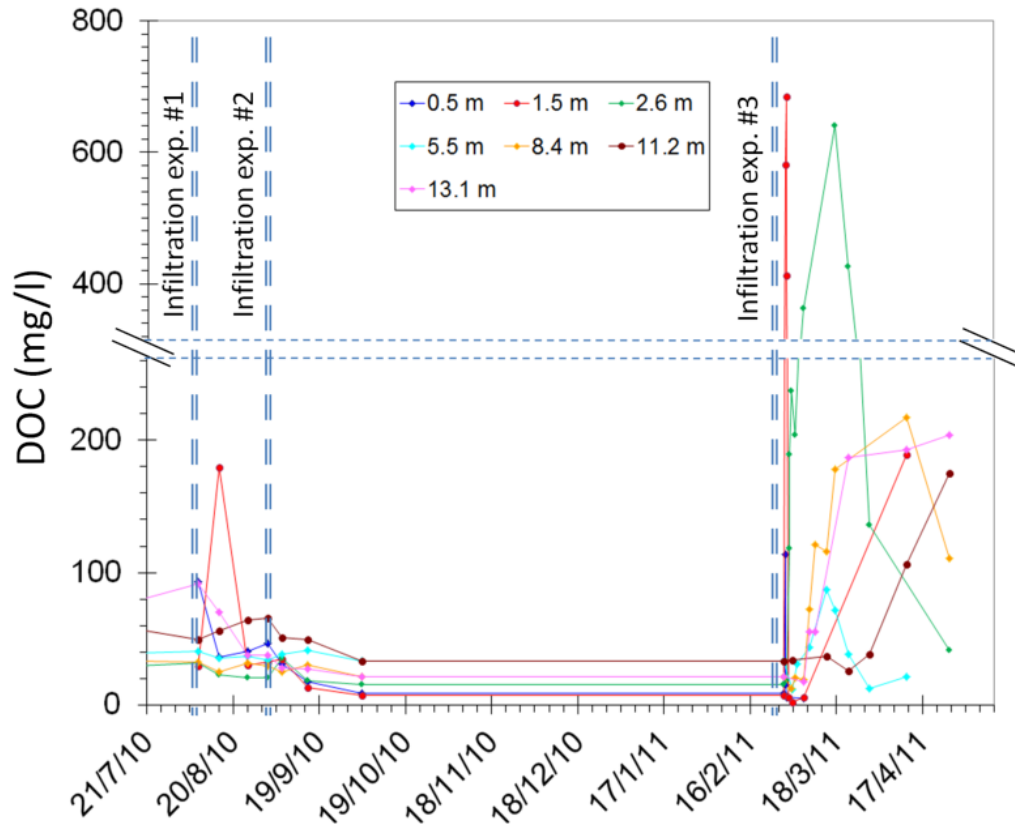
Figure 6. Variations in dissolved organic carbon (DOC) across the top 13 m of the
vadose zone following infiltration of water enriched with ethanol. Dates are given as
day/month/year.

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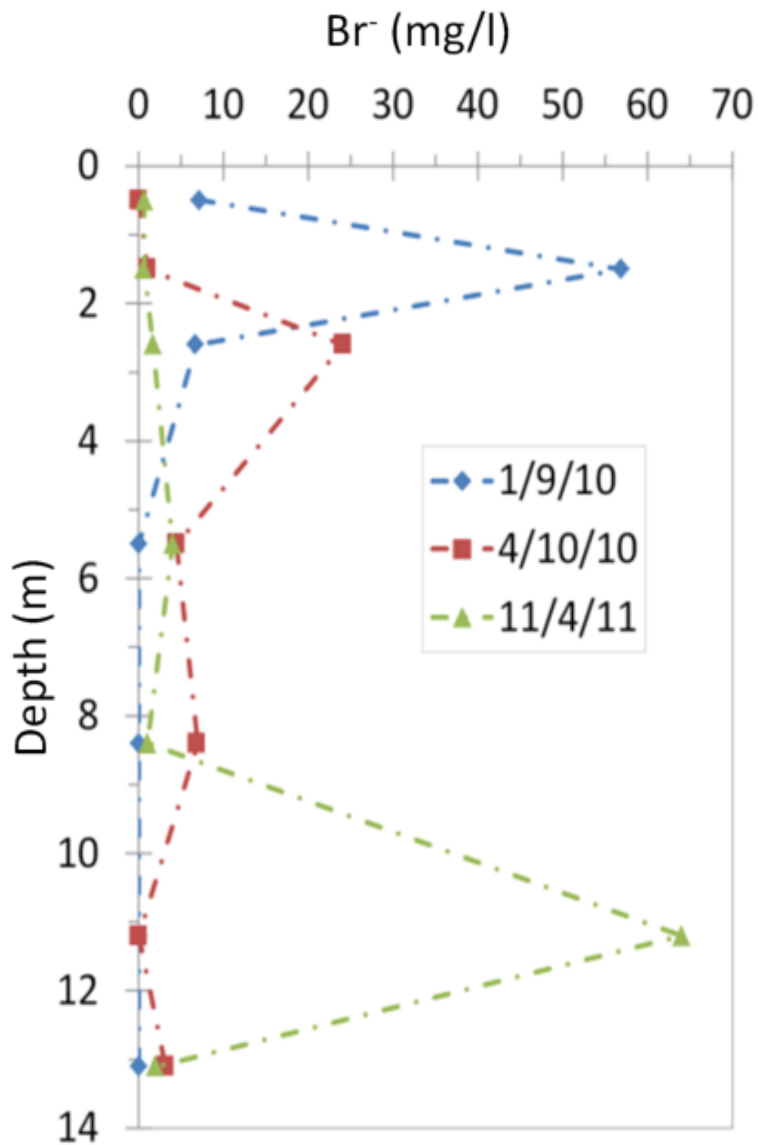
Figure 7. Variations in bromide concentration profile across the top 13 m of the vadose zone during the infiltration experiments. Dates are given as day/month/year. Note that data points are aligned in a slanted orientation and interpolated as time intervals.

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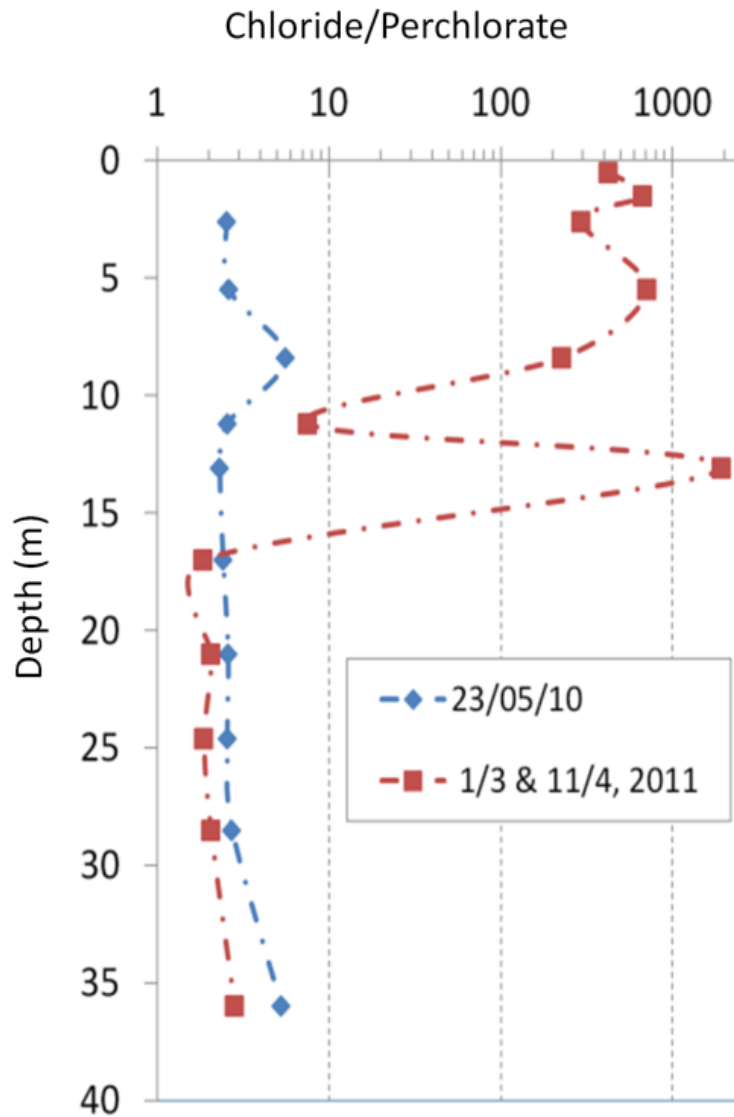
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620 Figure 8. Chloride-to-perchlorate equivalent concentration ratio profiles before and

621 after the infiltration experiments. Dates are given as day/month/year. Note that data

622 points are aligned in a slanted orientation and interpolated as time intervals.

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