1 **Transport and degradation of perchlorate in deep vadose zone: implications**

2 **from direct observations during bioremediation treatment**

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8 **Abstract**

9 An in situ bioremediation experiment of a deep vadose zone (~40 m) contaminated

10 with a high concentration of perchlorate (>25,000 mg L^{-1}) was conducted through a

11 full-scale field operation. Favorable environmental conditions for microbiological

12 reduction of perchlorate were sought by infiltrating an electron donor-enriched water

13 solution using drip irrigation underlying an airtight sealing liner. A vadose-zone

14 monitoring system (VMS) was used for real-time tracking of the percolation process,

15 the penetration depth of dissolved organic carbon (DOC), and the variation in

16 perchlorate concentration across the entire soil depth. The experimental conditions for

17 each infiltration event were adjusted according to insight gained from data obtained

18 by the VMS in previous stages. Continuous monitoring of the vadose zone indicated

19 that in the top 13 m of the cross section, perchlorate concentration is dramatically

20 reduced from thousands of milligrams per liter to near-detection limits with a

21 concurrent increase in chloride concentration. Nevertheless, in the deeper parts of the

22 vadose zone (<17 m), perchlorate concentration increased, suggesting its mobilization

23 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 Owsiniak, 2005), or injection of a gaseous mixture capable of promoting optimal biogeochemical conditions for microbial pollutant degradation (Evans et al.,

48 2011; Evans and Trute, 2006). Due to the complex nature of flow and transport

49 processes in the unsaturated zone, application of water with specific chemical

50 conditions near land surface does not necessarily result in promoting the desired

51 geochemical and hydraulic conditions in deeper parts of the vadose zone (Allaire et

52 al., 2009; Flury and Wai, 2003; Jarvis, 2007; Rimon et al., 2011a). Therefore, in the

53 vadose zone, and particularly in its deeper parts, a proper understanding of the

54 transport process is key to the success of in situ remediation operations (Baram et al.,

55 2012a; Dahan et al., 2009; Kurtzman et al., 2016; Rimon et al., 2011a).

56 Assessment of water percolation and solute transport in the vadose zone is considered

57 a major challenge in hydrological sciences. It is often characterized by unstable flow

58 that is highly sensitive to hydraulic, chemical and microbial conditions (Bautersa et

59 al., 2000; Dahan et al., 2009; DiCarlo, 2007; Germann and al Hagrey, 2008; Hallett et

60 al., 2013; Rimon et al., 2011a; Sher et al., 2012; Stumpp et al., 2009). Moreover, the

61 chemical composition of the percolating water [e.g., dissolved organic carbon (DOC),

62 oxygen and nutrients] is subjected to frequent changes due to natural hydroclimatic

63 and biological cycles (Stumpp et al., 2009, 2012). Accordingly, contaminant

64 attenuation in the vadose zone is dependent on the complex hydrological, chemical

65 and biological states of the sediment. Continuous measurements of the hydrological

66 and chemical properties of the unsaturated zone may be achieved with a vadose-zone

67 monitoring system (VMS) (Dahan et al., 2009). The VMS provides high-resolution

68 measurements of variation in sediment water content (Dahan et al., 2008; Rimon et

69 al., 2007) and evolution of the pore water's chemical composition across the

70 unsaturated profile (Rimon et al., 2011a; Dahan et al., 2014; Turkeltaub et al., 2014,

71 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,

121   which was primarily assigned for the pilot infiltration experiment, was backfilled with

122   the sandy loam from the excavated site after removal of the 0.5m thick nazaz layer.

123   This layer is therefore presented in the profile as disturbed soil.

124   The climate in the area is characterized as subtropical Mediterranean with a hot and

125   dry summer from May to October and a colder wet winter from November to April.

126   The average air temperature on summer and winter days is 30 °C and 17 °C,

127   respectively. The average annual precipitation is 530 mm year^{-1}, mostly as rain

128   occurring mainly in four to seven rainy episodes during the winter season (IMS,

129   2011).

130   **3 Experimental setup**

131   **3.1 Vadose-zone monitoring setup**

132   Real-time characterization of flow and transport processes in the vadose zone, as well

133   as assessment of chemical transformation of the percolating water during the

134   remediation experiments were carried out with a VMS that was installed across the

135   entire unsaturated profile, from land surface to a depth of 37 m (Fig. 2). A detailed

136   description of the VMS, its structure, installation procedure and performance, can be

137   found in previous publications (Dahan et al., 2009; Rimon et al., 2011a) and in the

138   supplementary material. In particular, the VMS that was used at this site was

139   composed of a 44m long flexible polyurethane sleeve hosting 11 monitoring units

140   distributed along its length. Each monitoring unit included: (a) a flexible time-domain

141   reflectometer (FTDR) sensor for continuous measurement of variations in the

142   sediment water content (Dahan et al., 2008; Rimon et al., 2007), and (b) vadose-zone

143   sampling ports (VSPs), which enable frequent sampling of the vadose zone pore water

144   for chemical analysis (Baram et al., 2012a; Dahan et al., 2009; Rimon et al., 2011b;

Turkeltaub et al., 2016). The VMS flexible sleeve was installed in a 0.16m diameter uncased borehole drilled slanted at a 55° angle (to the horizon) to a vertical depth of 37 m. In addition to the 11 monitoring units that were installed with the VMS, four additional monitoring units were installed directly in the soil at depths of 0.5 m and 1.5 m. It should be noted that the slanted installation is preferred to ensure that measurements carried out by each monitoring unit take place in separate undisturbed sediment columns. In addition, the flexibility of the monitoring sleeve and its filling with non-shrinking cement grout ensured complete sealing of the borehole void and prevention of cross-contamination through preferential flow in the borehole.

3.2 Field setup

Water amended with ethanol as the electron donor for perchlorate-reducing bacteria was infiltrated into the vadose zone through an area of 8 x 30 m at the pilot site using a drip-irrigation system. Dripping lines with drippers having a nominal discharge rate of 2.2 L h^{-1} were set up in a 0.3 x 0.3 m spatial distribution to create fairly even water distribution over the area. Accordingly, the total discharge rate of the irrigation system was set to 5 m^3 h^{-1}, which is equivalent to an infiltration rate of 0.02 m h^{-1}. To promote anaerobic conditions in the unsaturated zone, a polyethylene liner covered with soil was placed over the dripper system after its installation. Ethanol was selected as the electron donor and carbon substrate because it is a natural, soluble compound that is commonly used by perchlorate-reducing bacteria (Bardiya and Bae, 2011). Moreover, it reduce potential increase in soil salinity associated with other common sources of electron donors such as acetate (Gal et al., 2008).

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

240   conducted as a first trial to infiltrate ethanol-enriched water-solution into the

241   unsaturated zone. This experiment was also conducted with bromide as a tracer in

242   order to mark the water front propagation across the unsaturated zone. As will be

243   discussed further on, results the first infiltration experiment indicated that the

244   migration capacity of both ethanol and bromide across the unsaturated profile was

245   very limited. Accordingly a second infiltration experiment was conducted with a

246   double amount of water and the same amount and concentration of ethanol in order to

247   enhanced deep migration of the ethanol down the unsaturated zone. Following the

248   results from the first two experiments a third infiltration experiment was conducted

249   with larger water volumes and higher ethanol concentration in order to avoid quick

250   ethanol degradation in the shallow soil. all of which will be presented and discussed

251   below.

252   **4.1 Water percolation**

253   Temporal variations in the vadose zone water content provide a direct indication of

254   percolation processes in the vadose zone (Rimon et al., 2007; Dahan et al., 2008;

255   Turkeltaub et al., 2015). Each infiltration experiment launched a wetting wave that

256   propagated sequentially through the unsaturated zone (Fig. 3). Down-migration of the

257   wetting wave was expressed as a quick rise in water content followed by a recession

258   caused by water redistribution and drainage. Referring the wetting sequence in the

259   vadose zone to the infiltration events on land surface enabled a direct calculation of

260   the flow velocity across the unsaturated zone (Rimon et al., 2007; Dahan et al., 2008).

261   All three infiltration experiments produced wetting fronts that moved down the

262   vadose zone at a velocity of ~0.18 m h^{-1}, even though the water volumes that were

263   used in each experiment were significantly different (50, 100 and 300 m^3). Additional

264 information on calculation procedure of flow velocities may be found in to

265 supplementary material. Observations of regulated flow velocities at constant rates

266 across the vadose zone under variable surface hydraulic conditions have also been

267 reported in other studies (Dahan et al., 2008; Amiaz et al., 2011; Rimon et al., 2011a).

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

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

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

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

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

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

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

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

276 **4.2 Perchlorate transformation and mobilization**

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

278 infiltration experiments, revealed very high concentrations of perchlorate and

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

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

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

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

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

284 et al., 2009).

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

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

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

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 high correlation between the two. Theoretically one gram per liter of ethanol is equal 0.52 gram per liter of soluble carbon. However, in the site the dissolved carbon composes of ethanol its oxidation products (such as acetate) as well as other soluble microbial metabolites that can also serve as electron donors. Thus, DOC provides a better knowledge on the availability of electron donors in the soil pore water. Accordingly, we assume that the variation in DOC during the experiments was due to transport of ethanol or ethanol-degradation products with the percolating

water (for further details correlation between DOC and ethanol concentration see supplementary material).

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 days later, before initiation of the second infiltration experiment, DOC values had dropped back down to background levels. This implies that the ethanol was microbiologically consumed and mineralized to inorganic carbon in the soil before it could be leached further down.

As a result of the limited transport of electron donor, ethanol, in the first infiltration experiment, a second experiment was conducted with the same mass and concentration of ethanol. However, it was flushed with double the amount of water to promote its quicker migration to deeper layers (Fig. 4). In this experiment, no signs of increasing DOC were observed at any depth. On the contrary, DOC level decreased to values below background levels (Fig. 6). Obviously, the rate of ethanol metabolism and mineralization in the soil increased following the first experiment, where both water content of the sediment and substrate required for efficient microbial activity increased. As a result, ethanol-degradation efficiency in the topsoil (<0.5 m) was significantly enhanced.

To overcome the limitation of electron donor delivery through the shallow soils, a third infiltration experiment was designed. In this experiment, the ethanol was injected in a $0.4 m^3$ high-concentration (50 % volume percentages) pulse followed by a large volume of water. Application of ethanol at a very high concentration was aimed at suppressing its biological degradation in the shallow soil. The ethanol pulse was introduced after application of 24 m^3, the latter to provide high initial wetting

339 conditions under the ethanol front. Then the ethanol slug was pushed down with 276

340 m^3 of water. At this stage of the study, which was conducted 6 months after the

341 previous one, a substantial increase in DOC was observed in the entire top 13 m of the

342 cross section (Fig. 6). Obviously, an increase in DOC serving as electron donor is an

343 essential prerequisite for perchlorate degradation. Apparently, application of ethanol

344 at a high concentration, which inhibited its degradation in the upper layer, succeeded

345 to drive the ethanol all the way down to 13 m, just above the clay layer. Nevertheless,

346 no signs of DOC increase were observed below 13 m.

347 **4.4 Transport and degradation**

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

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

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

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

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

353 of the infiltration experiment were below detection limits. Concentration profiles

354 during the infiltration experiments clearly demonstrated sequential progress of the

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

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

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

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

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

360 in the vadose zone.

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

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

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

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

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

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

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

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

369 concentration proportions of 2.4–5.5 (Fig. 8). Following the infiltration experiment, a

370 significant increase in ionic ratios was observed in the top 13 m, while in the rest of

371 the profile—from a depth of 17 m to the water table, the concentration ratio of

372 chloride to perchlorate remained relatively unchanged. Obviously, since both

373 perchlorate and chloride are very soluble and mobile, infiltration water with a low

374 concentration of chloride ($\sim$100 mg L^{-1}) and zero perchlorate is also expected to result

375 in an increased chloride-to-perchlorate ratio, even if no perchlorate degradation takes

376 place. Since both chloride and perchlorate are very mobile and easily displaced with

377 the percolating water, quantification of the perchlorate-degradation rate with respect

378 to its down-leaching is not straightforward.

379 **5 Conclusions**

380 The infiltration experiments were primarily aimed at improving the environmental

381 conditions for perchlorate-reducing bacteria across the vadose zone. This included an

382 increase in water content along the soil profile and amendment of the electron donor.

383 The results, which were based on continuous monitoring of the entire vadose zone,

384 exhibited notable variation in the concentrations of perchlorate, DOC and other

385 solutes in the unsaturated zone. Increased concentrations of DOC with a concurrent

386 reduction in perchlorate concentration (from thousands to a few milligrams per liter)

387 and increased chloride-to-perchlorate ratio (from ~2.5 to ~300) in the upper 13 m

388 indicated that perchlorate is partially reduced in this part of the vadose zone. On the

389 other hand, no evidence of improved reducing conditions was observed in the deeper

390 parts, where the initial concentrations of perchlorate were significantly higher.

391 Nevertheless, since assessment of redox conditions in deep vadose zone is not yet

392 feasible, we can only rely on variations in the chemical composition to assess the

393 existence of degradative conditions.

394 The limited ability to deliver a soluble electron donor across a microbiologically

395 reactive medium, such as topsoil, is a major limiting factor for remediation of the

396 deep vadose zone through gravitational percolation of enriched solution. Note that

397 temporal variations in the concentrations of perchlorate, as well as other solutes, in the

398 deep parts of the vadose zone, i.e., under the clay layer at 14 m, indicate that the clay

399 layer does not play any role in limiting infiltration capacity in terms of flow velocity

400 and fluxes. Similar observations on the role of clay layers in infiltration in the

401 unsaturated zone have been reported in previous publications (Baram et al., 2012b,

402 2012c; Dahan et al., 2009; Rimon et al., 2007; Turkeltaub et al., 2015b).

403 The attempts to leach the ethanol down into the vadose zone with large quantities of

404 water inevitably drove down-leaching and displacement of the dissolved solutes,

405 including perchlorate. Although there were indications of partial degradation of

406 perchlorate in the upper part of the vadose zone, its downward displacement toward

407 the water table was evident from the sequential increase in perchlorate concentration

408 with depth (Fig. 5). It seems that the entire column of perchlorate mass was pushed

409 down by the percolating water toward the water table, which also resulted in an

410 increased concentration of perchlorate in the observation well, which was located

411 under the infiltration zone.

412 Enhancing biodegradation of contaminants in the vadose zone while minimizing their

413 down migration into groundwater is a major challenge in remediation operations that

414 involves water infiltration. Although in this study we have observed dramatic

415 reduction in perchlorate concentration in the top 13 m of the unsaturated zone

416 following the infiltration of ethanol enriched water solution, we can't state that the

417 reduction in perchlorate concentration is only due to bio-degradation and exclude

418 partial down leached to deeper parts of the vadose zone. Accordingly, perchlorate

419 degradation vs. migration process were investigated thourhg the temporal variation in

420 perchlorate concentration with respect to variations in concentrations of ethanol,

421 which was consumed in the subsurface, Br, which is conservative tracer, and

422 variations in the chloride concentration, which is perchlorate final degradation

423 product. All of which provided indicator hints to the question on the degradation vs

424 leaching.

425 The study demonstrates that application of vadose-zone monitoring technology during

426 a remediation operation provides real-time information on the chemical and

427 hydrological state of the subsurface. Linking the temporal variation in the chemical

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

429 velocities are vital for efficient management of remediation operations.

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

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

432 *experiment and laboratory analysis. Lior Avishai (MSc student) conducted data*

433 *analysis and modeling of flow and transport in the unsaturated zone. Zeev Ronen (PI,*

434   *Microbiology) design the bio treatment setup. Data analysis and manuscript*

435   *preparation - all coauthors.*

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441 **References**

442

443 Allaire, S. E., Roulier, S. and Cessna, A. J.: Quantifying preferential flow in soils: A
444 review of different techniques, J. Hydrol., 378(1–2), 179–204,
445 doi:10.1016/j.jhydrol.2009.08.013, 2009.

446 Amiaz, Y., Sorek, S., Enzel, Y. and Dahan, O.: Solute transport in the vadose zone
447 and groundwater during flash floods, , 47(October), 1–10,
448 doi:10.1029/2011WR010747, 2011.

449 Attaway, H. and Smith, M.: Reduction of perchlorate by an anaerobic enrichment
450 culture, J. Ind. Microbiol., 12(6), 408–412, doi:10.1007/BF01569673, 1993.

451 Baram, Arnon, S., Ronen, Z., Kurtzman, D. and Dahan, O.: Infiltration Mechanism
452 Controls Nitrification and Denitrification Processes under Dairy Waste Lagoon, J.
453 Environ. Qual., 41(5), 1623–1632, doi:10.2134/jeq2012.0015, 2012a.

454 Baram, S., Arnon, S., Ronen, Z., Kurtzman, D. and Dahan, O.: Infiltration Mechanism
455 Controls Nitrification and Denitrification Processes under Dairy Waste Lagoon, J.
456 Environ. Qual., 41(5), 1623–1632, doi:10.2134/jeq2012.0015, 2012b.

457 Baram, S., Kurtzman, D. and Dahan, O.: Water percolation through a clayey vadose
458 zone, J. Hydrol., 424–425, 165–171, doi:10.1016/j.jhydrol.2011.12.040, 2012c.

459 Bardiya, N. and Bae, J.-H.: Dissimilatory perchlorate reduction: A review, Microbiol.
460 Res., 166(4), 237–254, doi:10.1016/j.micres.2010.11.005, 2011.

461 Battey, T. F., Shepard, A. J. and Tait, R. J.: Soil Flushing Through a Thick Vadose
462 Zone Perchlorate Removal Documented at Edw, in AGU, American Geophysical
463 Union, Fall Meeting 2007, abstract #H33E-1685., 2007.

464 Bautersa, T. W. J., DiCarlob, D. A., Steenhuisa, T. S. and Parlangea, J.-Y.: Soil water
465 content dependent wetting front characteristics in sands, J. Hydrol. 231–232, 231–
466 232, 244–254, 2000.

467 Bombach, P., Richnow, H. H., Kästner, M. and Fischer, A.: Current approaches for
468 the assessment of in situ biodegradation., Appl. Microbiol. Biotechnol., 86(3), 839–
469 52, doi:10.1007/s00253-010-2461-2, 2010.

470 Cai, H., Eramo, A. G. A. G., Evans, P. J. P. J., Fricke, R. and Brennan, R. A. R. A.: In
471 Situ Bioremediation of Perchlorate in Vadose Zone Soil Using Gaseous Electron
472 Donors: Microcosm Treatability Study, WATER Environ. Res., 82(5), 409–417,
473 doi:10.2175/106143009X12487095237198, 2010.

474 Coates, J. D. and Achenbach, L. A.: Microbial perchlorate reduction: rocket-fueled
475 metabolism., Nat. Rev. Microbiol., 2(7), 569–80, doi:10.1038/nrmicro926, 2004.

476 Dahan, Talby, R., Yechieli, Y., Adar, E., Lazarovitch, N. and Enzel, Y.: In Situ
477 Monitoring of Water Percolation and Solute Transport Using a Vadose Zone

478   Monitoring System, Vadose Zo. J., 8(4), 916–925, 2009.

479   Dahan, O., Shani, Y., Enzel, Y., Yechieli, Y. and Yakirevich, A.: Direct
480   measurements of floodwater infiltration into shallow alluvial aquifers, J. Hydrol.,
481   344(3–4), 157–170, 2007.

482   Dahan, O., Tatarsky, B., Enzel, Y., Kulls, C., Seely, M. and Benito, G.: Dynamics of
483   flood water infiltration and ground water recharge in hyperarid desert, Ground Water,
484   46(3), 450–461, doi:10.1111/j.1745-6584.2007.00414.x, 2008.

485   Dahan, O., Babad, A., Lazarovitch, N., Russak, E. E. and Kurtzman, D.: Nitrate
486   leaching from intensive organic farms to groundwater, Hydrol. Earth Syst. Sci.
487   Discuss., 10(7), 9915–9941, doi:10.5194/hessd-10-9915-2013, 2014.

488   DiCarlo, D. A.: Capillary pressure overshoot as a function of imbibition flux and
489   initial water content, Water Resour. Res., 43(8), 2007.

490   EPA: How to Evaluate Alternative Cleanup Technologies for Underground Storage
491   Tank Sites: A Guide for Corrective Action Plan Reviewers., edited by S. W. and E. R.
492   5401G, www.epa.gov/oust/pubs/tums.htm, 2004.

493   EPA: Remediation Technologies-Tools and resources to assist in contaminated site
494   remediation, http://www.epa.gov/superfund/remedytech/remed.htm, 2015.

495   Evans, P. J. and Trute, M. M.: In Situ Bioremediation of Nitrate and Perchlorate in
496   Vadose Zone Soil for Groundwater Protection Using Gaseous Electron Donor
497   Injection Technology, Water Environ. Res., 78(13), 2436–2446,
498   doi:10.2175/106143006X123076, 2006.

499   Evans, P. J., Fricke, R. A., Hopfensperger, K. and Titus, T.: In Situ Destruction of
500   Perchlorate and Nitrate Using Gaseous Electron Donor Injection Technology, , (4),
501   103–112, doi:10.1111/j1745, 2011.

502   Flury, M. and Wai, N. nu: Dyes as Tracers for Vadose Zone Hydrology, Rev. if
503   Geophys., 41, 1–37, 2003.

504   Frankel, A. and Owsianiak, L.: In-situ anaerobic remediation of perchlorate-impacted
505   soils. [online] Available from: http://www.environmental-expert.com (Accessed 13
506   November 2014), 2005.

507   Gal, H., Ronen, Z., Weisbrod, N., Dahan, O. and Nativ, R.: Perchlorate
508   biodegradation in contaminated soils and the deep unsaturated zone, Soil Biol.
509   Biochem., 40(7), 1751–1757, doi:10.1016/j.soilbio.2008.02.015, 2008.

510   Gal, H., Weisbrod, N., Dahan, O., Ronen, Z. and Nativ, R.: Perchlorate accumulation
511   and migration in the deep vadose zone in a semiarid region, J. Hydrol., 378(1–2),
512   142–149, doi:10.1016/j.jhydrol.2009.09.018, 2009.

513   Germann, P. F. and al Hagrey, S. A.: Gravity-Driven and Viscosity-Dominated
514   Infiltration into a Full-Scale Sand Model, Vadose Zo. J., 7(4), 1160,
515   doi:10.2136/vzj2007.0172, 2008.

516 Gvirtzmen, M.: Israel Water Resources: Chapters in Hydrology and Envirmental
517 Sciences, Yad Ben-Zvi Press, Jerusalem.301 p (in Hebrew)., 2002.

518 Hallett, P. D., Karim, K. H., Glyn Bengough, a. and Otten, W.: Biophysics of the
519 Vadose Zone: From Reality to Model Systems and Back Again, Vadose Zo. J., 12(4),
520 doi:10.2136/vzj2013.05.0090, 2013.

521 Höhener, P. and Ponsin, V.: In situ vadose zone bioremediation., Curr. Opin.
522 Biotechnol., 27, 1–7, doi:10.1016/j.copbio.2013.08.018, 2014.

523 IMS: Israel Meteorological Service- official Climate Data Base, 2011.

524 Jarvis, N. J.: A review of non-equilibrium water flow and solute transport in soil
525 macropores: Principles, controlling factors and consequences for water quality, Eur. J.
526 Soil Sci., 58(3), 523–546, doi:10.1111/j.1365-2389.2007.00915.x, 2007.

527 Kurtzman, D., Baram, S. and Dahan, O.: Soil-aquifer phenomena affecting
528 groundwater under vertisols: A review, Hydrol. Earth Syst. Sci., 20(1), 1–12,
529 doi:10.5194/hess-20-1-2016, 2016.

530 Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N. and Naidu, R.:
531 Bioremediation approaches for organic pollutants: a critical perspective., Environ.
532 Int., 37(8), 1362–75, doi:10.1016/j.envint.2011.06.003, 2011.

533 Motzer, W.: Perchlorate: Problems, Detection, and Solutions, Environ. Forensics,
534 2(4), 301–311, doi:10.1006/enfo.2001.0059, 2001.

535 Nadler, A., Gamliel, A. and Peretz, I.: Practical aspects of salinity effect on TDR-
536 measured water content: A field study, SOIL Sci. Soc. Am. J., 63(5), 1070–1076,
537 1999.

538 Nozawa-Inoue, M., Scow, K. M. and Rolston, D. E.: Reduction of perchlorate and
539 nitrate by microbial communities in vadose soil, Appl. Environ. Microbiol., 71(7),
540 3928–3934, doi:10.1128/AEM.71.7.3928-3934.2005, 2005.

541 Rimon, Y., Dahan, O., Nativ, R. and Geyer, S.: Water percolation through the deep
542 vadose zone and groundwater recharge: Preliminary results based on a new vadose
543 zone monitoring system, Water Resour. Res., 43(5), 1–12,
544 doi:10.1029/2006WR004855, 2007.

545 Rimon, Y., Nativ, R. and Dahan, O.: Physical and Chemical Evidence for Pore-Scale
546 Dual-Domain Flow in the Vadose Zone, Vadose Zo. J., 10(1), 322,
547 doi:10.2136/vzj2009.0113, 2011a.

548 Rimon, Y., Nativ, R. and Dahan, O.: Vadose Zone Water Pressure Variation during
549 Infiltration Events, Vadose Zo. J., 10(3), 1105, doi:10.2136/vzj2010.0061, 2011b.

550 Roote, S. D.: First Edition Ground-Water Remediation Technologies Analysis
551 Technology Status Report : Perchlorate Treatment Technologies , First Edition,
552 Pittsburgh, PA., 2001.

553 Sagi-Ben Moshe, S., Dahan, O., Weisbrod, N., Bernstein, a., Adar, E. and Ronen, Z.:

Biodegradation of explosives mixture in soil under different water-content conditions, J. Hazard. Mater., 203–204, 333–340, doi:10.1016/j.jhazmat.2011.12.029, 2012.

Sher, Y., Baram, S., Dahan, O., Ronen, Z. and Nejidat, A.: Ammonia transformations and abundance of ammonia oxidizers in a clay soil underlying a manure pond, FEMS Microbiol. Ecol., 81(1), 145–155, doi:10.1111/j.1574-6941.2012.01347.x, 2012.

Shrout, J. D. and Parkin, G. F.: Influence of electron donor, oxygen, and redox potential on bacterial perchlorate degradation., Water Res., 40(6), 1191–9, doi:10.1016/j.watres.2006.01.035, 2006.

Singer, A.: The Soils of Israel, Springer Verlag, Berlin, Germany., 2007.

Stumpp, C., Maloszewski, P., Stichler, W. and Fank, J.: Environmental isotope (δ18O) and hydrological data to assess water flow in unsaturated soils planted with different crops: Case study lysimeter station "Wagna" (Austria), J. Hydrol., 369(1–2), 198–208, doi:10.1016/j.jhydrol.2009.02.047, 2009.

Stumpp, C., Stichler, W., Kandolf, M. and Šimůnek, J.: Effects of Land Cover and Fertilization Method on Water Flow and Solute Transport in Five Lysimeters: A Long-Term Study Using Stable Water Isotopes, Vadose Zo. J., 11(1), 0, 2012.

Tipton, D. K., Rolston, D. E. and Scow, K. M.: Transport and biodegradation of perchlorate in soils, J. Environ. Qual., 32(1), 40–46, 2003.

Trumpolt, C. W., Crain, M., Cullison, G. D., Flanagan, S. J. P., Siegel, L. and Lathrop, S.: Perchlorate: Sources, uses, and occurrences in the environment, Remediat. J., 16(1), 65–89, doi:10.1002/rem.20071, 2005.

Turkeltaub, T., Dahan, O. and Kurtzman, D.: Investigation of Groundwater Recharge under Agricultural Fields Using Transient Deep Vadose Zone Data, Vadose Zo. J., 13(4), doi:10.2136/vzj2013.10.0176, 2014.

Turkeltaub, T., Kurtzman, D., Bel, G. and Dahan, O.: Examination of groundwater recharge with a calibrated/validated flow model of the deep vadose zone, J. Hydrol., 522, 618–627, doi:10.1016/j.jhydrol.2015.01.026, 2015a.

Turkeltaub, T., Kurtzman, D., Russak, E. and Dahan, O.: Water Resources Research, Water Resour Res., 4840–4847, doi:10.1002/2015WR017273.Received, 2015b.

Turkeltaub, T., Kurtzman, D. and Dahan, O.: Real-time monitoring of nitrate transport in the deep vadose zone under a crop field – implications for groundwater protection, Hydrol. Earth Syst. Sci., 20(8), 3099–3108, doi:10.5194/hess-20-3099-2016, 2016.

Urbansky, E. T.: Perchlorate as an E n v i r o n m e n t a l C o n t a m i n a n t, , 9(3), 187–192, 2002.

Urbansky, E. T., Brown, S. K., Urbansky and Brownwn, K.: Perchlorate retention and mobility in soils, J. Environ. Monit., 5(3), 455, doi:10.1039/b301125a, 2003.

591   *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

602   *Table 2. Infiltration experiment conditions*

Date	Water Volume (m³)	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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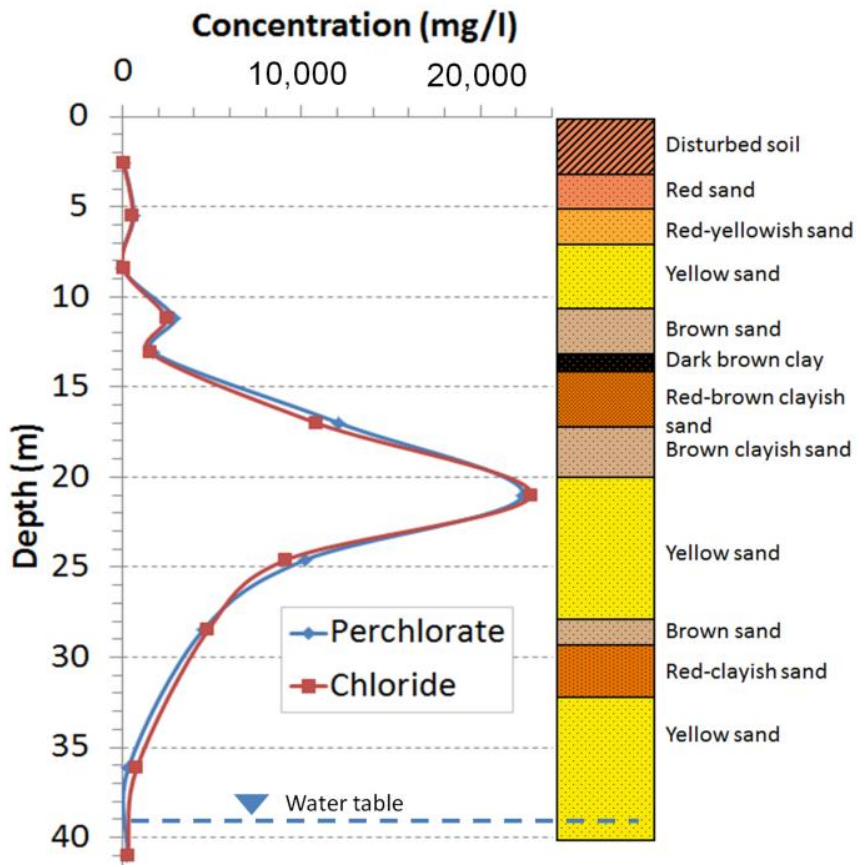
Figure 1. Initial concentration profiles of chloride and perchlorate in the vadose zone

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

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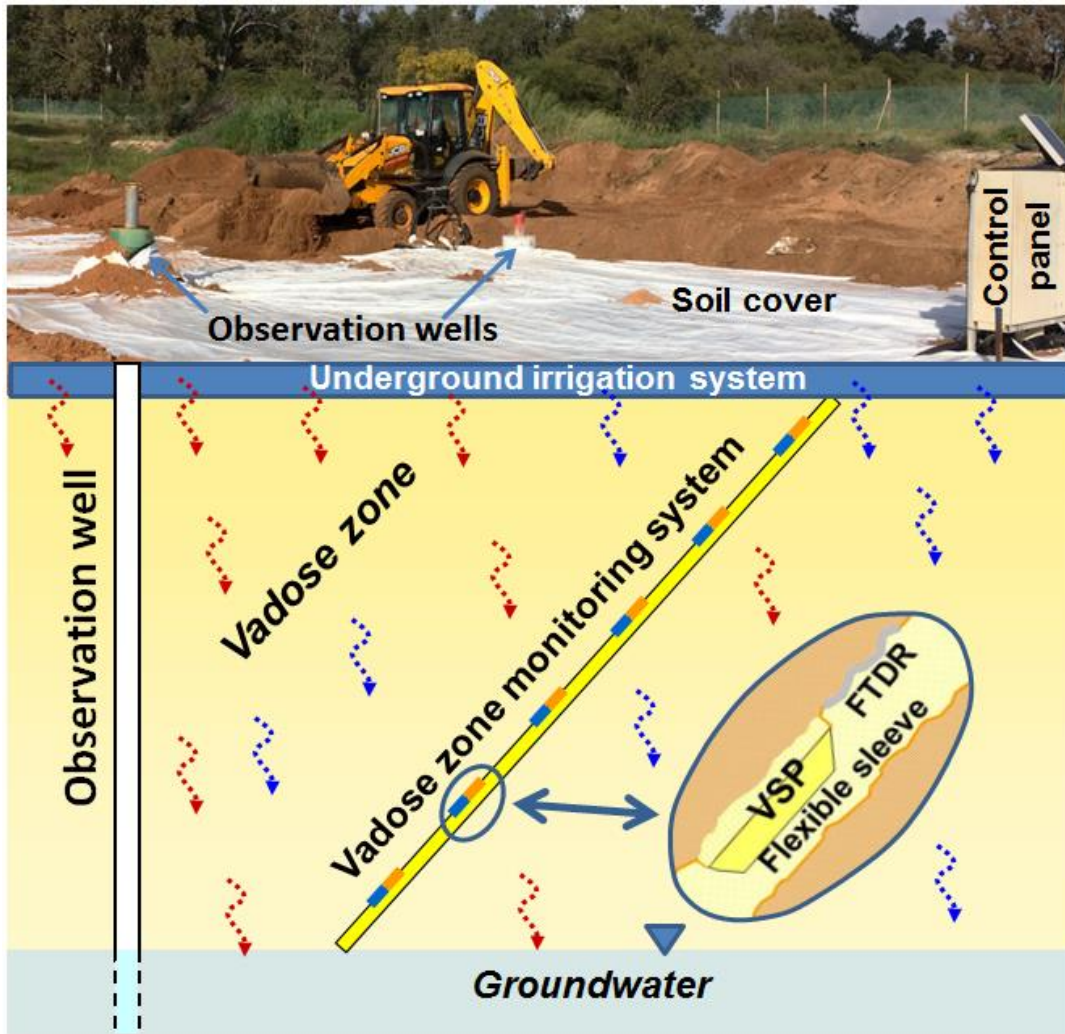
Figure 2. Schematic illustration of the vadose-zone monitoring system installed in the vadose zone under the infiltration pilot site. In the picture above the vadose zone, the irrigation system at the site is being covered.
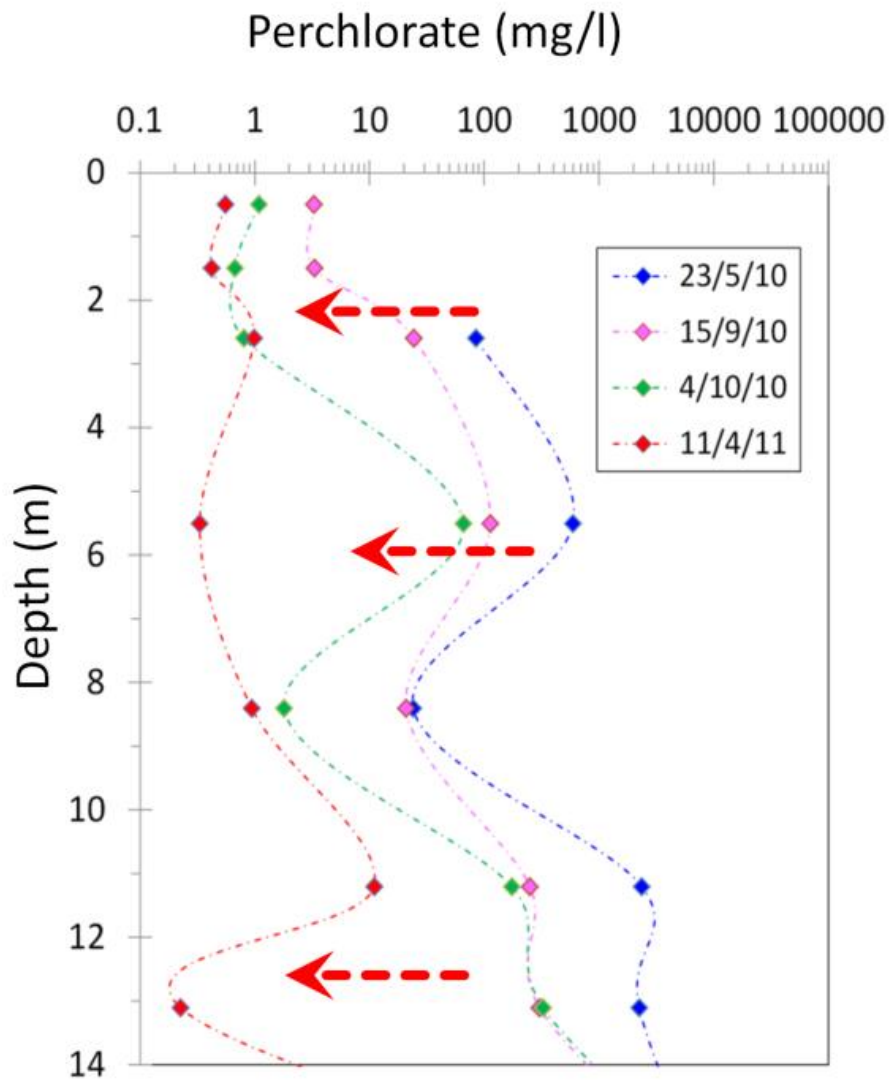
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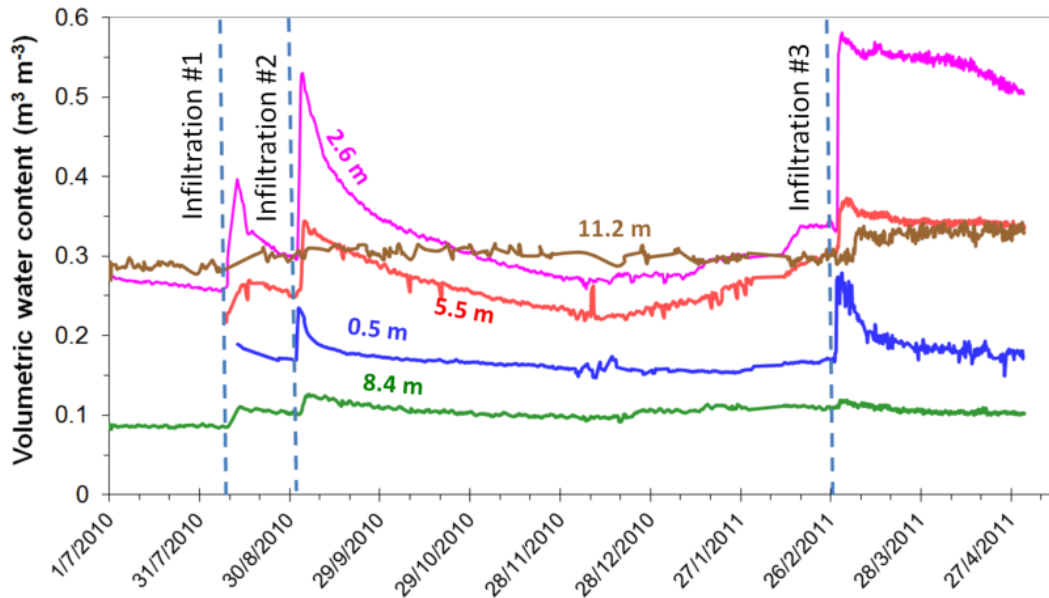
Figure 3. Temporal variations in sediment water content in the top 13 m of the vadose
zone during the infiltration experiments. Dates are given as day/month/year.

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Figure 4. Perchlorate concentration profile across the top 13 m of the vadose zone
under the pilot site during the infiltration experiments. The profiles emphasize the
gradual decrease 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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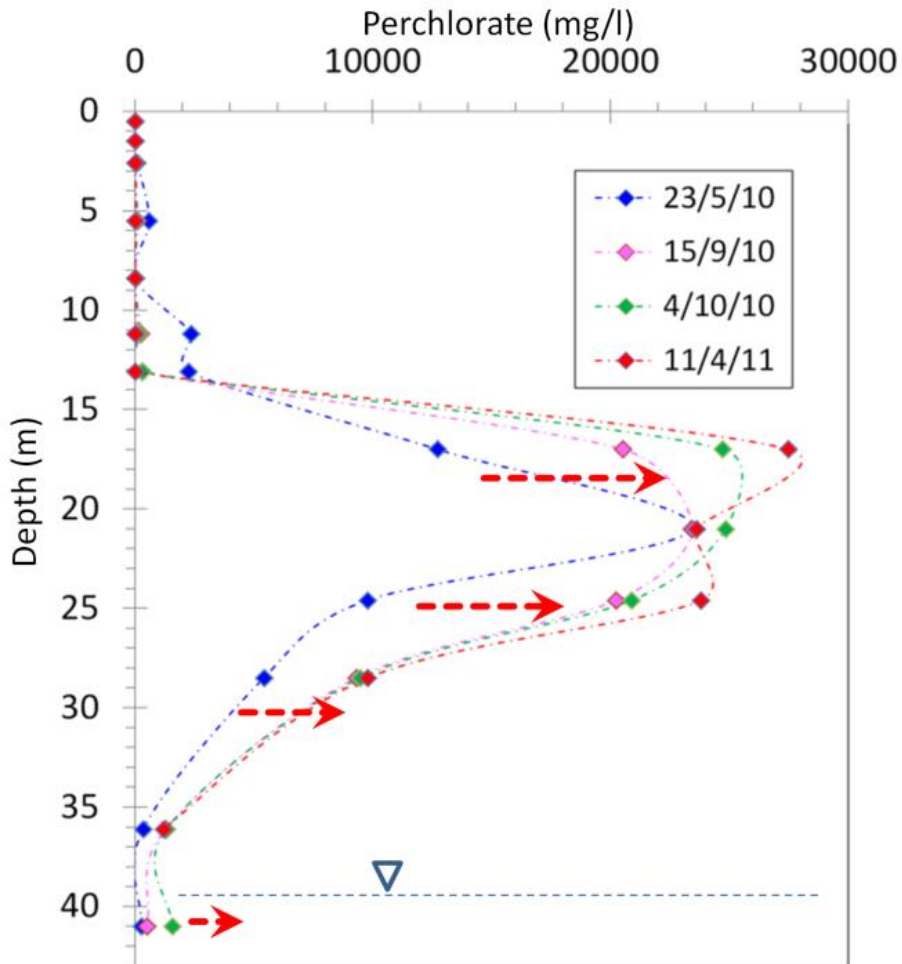
Figure 5. Perchlorate concentration profile across the entire vadose zone and top groundwater under the pilot site during the infiltration experiments. The profiles emphasize the gradual increase in perchlorate concentration with time (marked in red arrows). Dates are given as day/month/year. Note that data points are aligned in a slanted orientation and interpolated as time intervals.
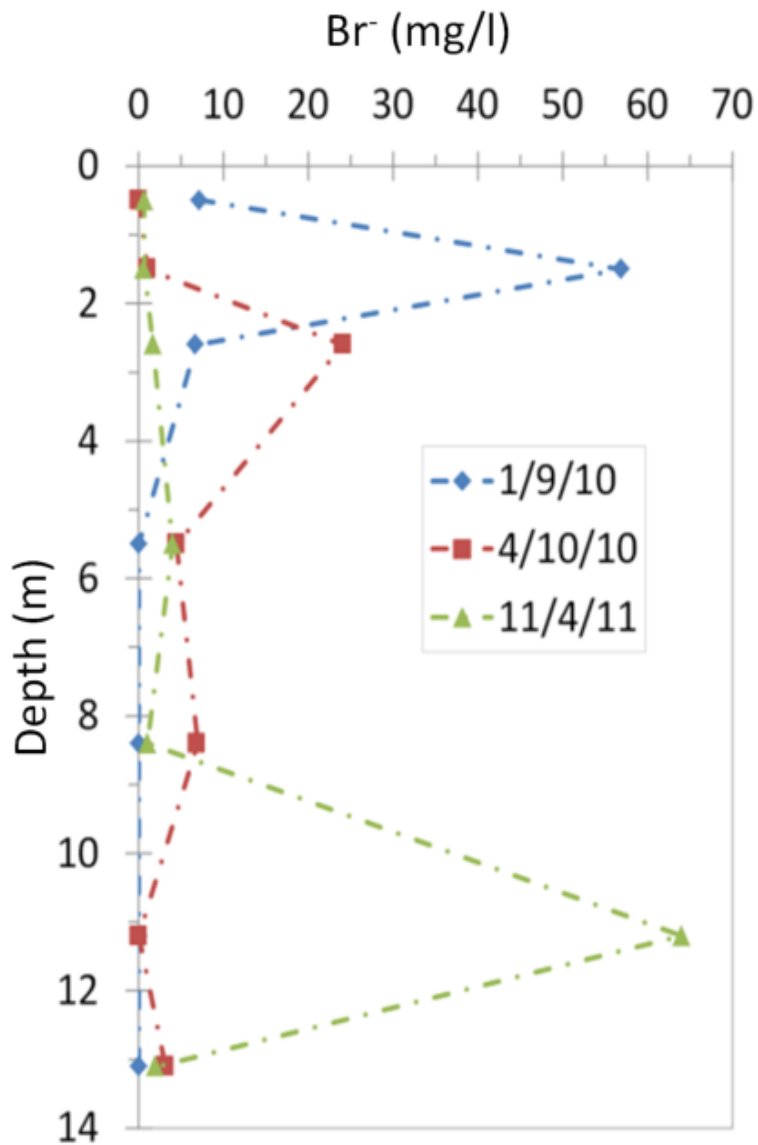
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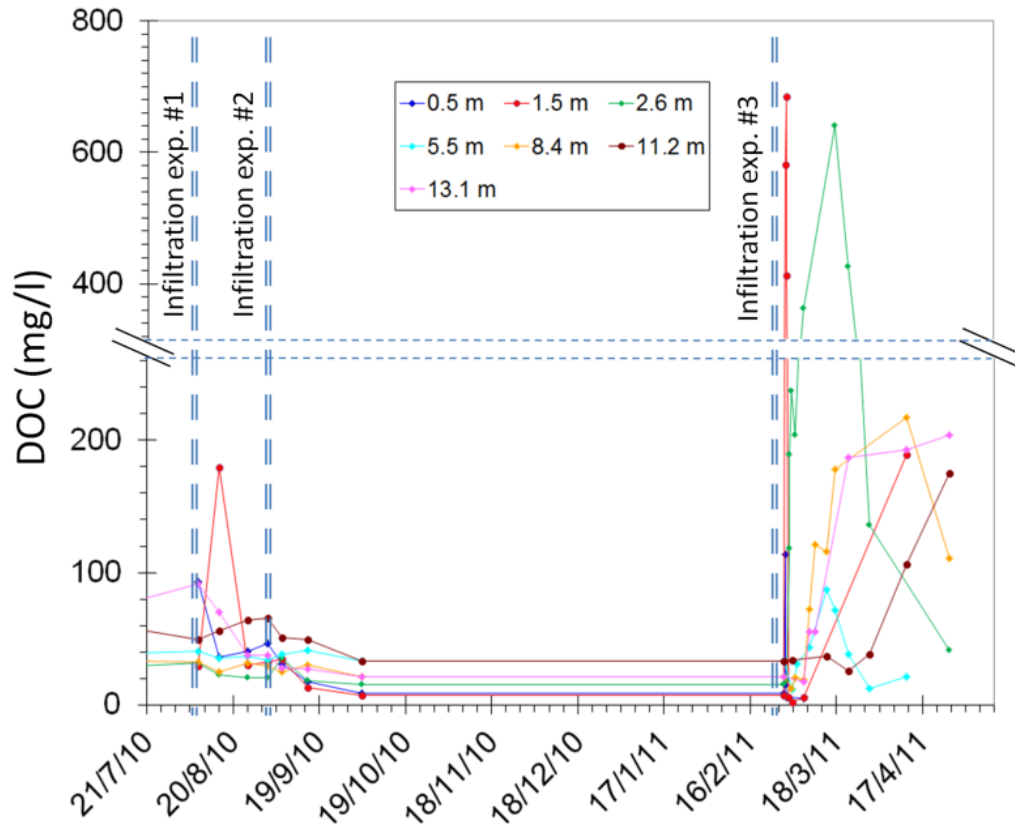
Figure 6. Variations in dissolved organic carbon (DOC) across the top 13 m of the
vadose zone following infiltration of water enriched with ethanol. Dates are given as
day/month/year.

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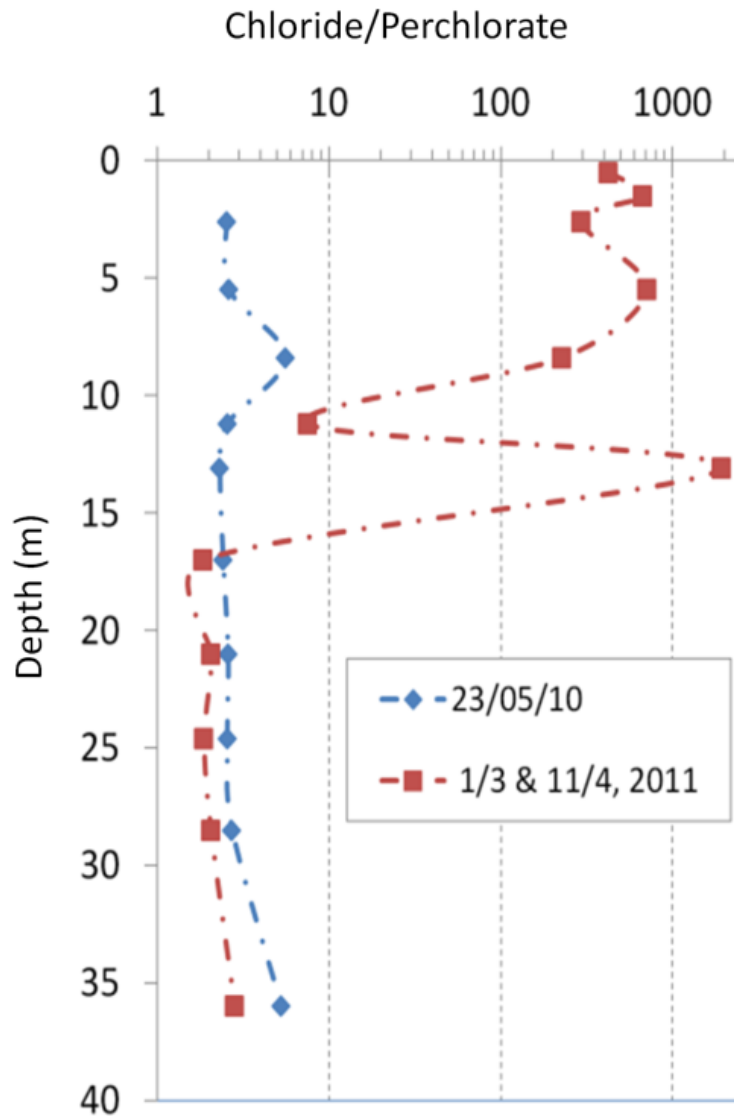Figure 7. Variations in bromide concentration profile across the top 13 m of the vadose zone during the infiltration experiments. Dates are given as day/month/year. Note that data points are aligned in a slanted orientation and interpolated as time intervals.

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