



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

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8

9 *Keywords:* Remediation, unsaturated zone, contaminant transport, perchlorate,  
10 monitoring

11

12 **Abstract**

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



26 vadose zone (<17 m), perchlorate concentration increased, suggesting its mobilization  
27 down through the cross section. Breakthrough of DOC and bromide at different  
28 depths across the unsaturated zone showed limited migration capacity of biologically  
29 consumable carbon and energy sources due to their enhanced biodegradation in the  
30 upper soil layers. Nevertheless, the increased DOC concentration with concurrent  
31 reduction in perchlorate and increase in the chloride-to-perchlorate ratio in the top 13  
32 m indicate partial degradation of perchlorate in this zone. There was no evidence of  
33 improved degradation conditions in the deeper parts where the initial concentrations  
34 of perchlorate were significantly higher.

35

## 36 **1 Introduction**

37

38 In situ bioremediation of a contaminated unsaturated zone (also termed vadose zone)  
39 depends mainly on the ability to control the hydrological, physical and chemical  
40 conditions in the subsurface (Bombach et al., 2010; EPA, 2015; Höhener and Ponsin,  
41 2014). Chemical and hydrological manipulations are primarily aimed at enhancing the  
42 activity of specific indigenous degrading bacteria. The optimal conditions for specific  
43 contaminants' degradation are usually determined in microcosm experiments, where  
44 the preferred electron donor and acceptor for degradation can be controlled and  
45 examined (Gal et al., 2008; Megharaj et al., 2011; Sagi-Ben Moshe et al., 2012). The  
46 optimal degradation conditions, evaluated through laboratory experiments, usually  
47 form the basis for selecting a strategy for in situ remediation in field-scale operations.  
48 Nevertheless, implementation of desired biodegradation conditions in the deep vadose  
49 zone through full-scale field setups requires control of the vadose zone  
50 hydrogeochemical conditions. This is often achieved through either infiltration of



51 water enriched with electron donors or nutrients (EPA, 2004; Frankel and Owsianiak,  
52 2005; Battey et al., 2007), or injection of a gaseous mixture capable of promoting  
53 optimal biogeochemical conditions for microbial pollutant degradation (Evans and  
54 Trute, 2006; Evans et al., 2011). Due to the complex nature of flow and transport  
55 processes in the unsaturated zone, application of water with specific chemical  
56 conditions near land surface does not necessarily result in promoting the desired  
57 geochemical and hydraulic conditions in deeper parts of the vadose zone (Flury and  
58 Wai, 2003; Jarvis, 2007; Allaire et al., 2009; Rimon et al., 2011a). Therefore, in the  
59 vadose zone, and particularly in its deeper parts, a proper understanding of the  
60 transport process is key to the success of in situ remediation operations (Dahan et al.,  
61 2009; Rimon et al., 2011a; Baram et al., 2012a; Kurtzman et al., 2016).

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



75 chemical composition across the unsaturated profile (Rimon et al., 2011a; Dahan et  
76 al., 2014; Turkeltaub et al., 2014, 2016).

77 Perchlorate is an environmental pollutant that is often associated with the  
78 explosives manufacturing industry (Roote, 2001; Urbansky, 2002; Trumpolt et al.,  
79 2005). It is mostly produced, and consequently released to the environment as  
80 ammonium perchlorate. Its high solubility ( $220 \text{ g L}^{-1}$ ) and stability in aerobic  
81 environments makes it very mobile and persistent in the subsurface (Motzer, 2001;  
82 Urbansky and Brown, 2003). Microbial reduction of perchlorate to harmless chloride  
83 and oxygen in the unsaturated zone requires elevated water content, negative redox  
84 potential, available electron donors and the presence of suitable indigenous bacteria  
85 (Coates and Achenbach, 2004). In the vadose zone, natural attenuation and  
86 biodegradation of perchlorate are considered very limited (Gal et al., 2009).  
87 Nevertheless, studies have shown that perchlorate can be metabolized in unsaturated  
88 soil whenever reducing conditions ( $<110 \text{ mV}$ ) (Attaway and Smith, 1993; Shroud and  
89 Parkin, 2006) are achieved and an available electron donor is introduced (Tipton et al.,  
90 2003; Frankel and Owsianiak, 2005; Nozawa-Inoue et al., 2005; Evans and Trute,  
91 2006; Cai et al., 2010).

92 Here, the efficiency of a remediation operation of a perchlorate-contaminated  
93 vadose zone was assessed using a VMS, which provided continuous information on  
94 the chemical composition of the vadose-zone pore water. Promotion of perchlorate-  
95 degrading conditions in the vadose zone was based on infiltration of water enriched  
96 with ethanol (as a source of electron donor) from land surface. Real-time information  
97 on the depth of the enriched water's propagation, along with variations in the  
98 concentrations of perchlorate, chloride and bromide (applied as a tracer), was used to  
99 assess transport and degradation of perchlorate across the unsaturated profile. Water-



100 and ethanol-application strategies were adjusted in each flow phase to obtain real-time  
101 feedback on the chemical and hydrological state of the vadose zone.

102

## 103 **2 Study site**

104

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

118 The stratigraphy of the area is characterized by Neogene and Pleistocene  
119 sediments, mainly of sands and sandstones with interbedding of clay lenses  
120 (Gvirtzman, 2002). The vadose zone lithological profile at the site was assessed again  
121 through a borehole that was drilled at the pilot site in 2012 (Table 1, Fig. 1). Most of  
122 the profile is composed of yellow and red sand layers with low clay content ( $<5 \%$ ),  
123 with interbeds of brown sand containing variable clay content of up to  $11 \%$ . A single  
124  $\sim 1\text{m}$  thick clay layer ( $27.5 \%$  clay content) was observed at a depth of  $13.3 \text{ m}$ . To



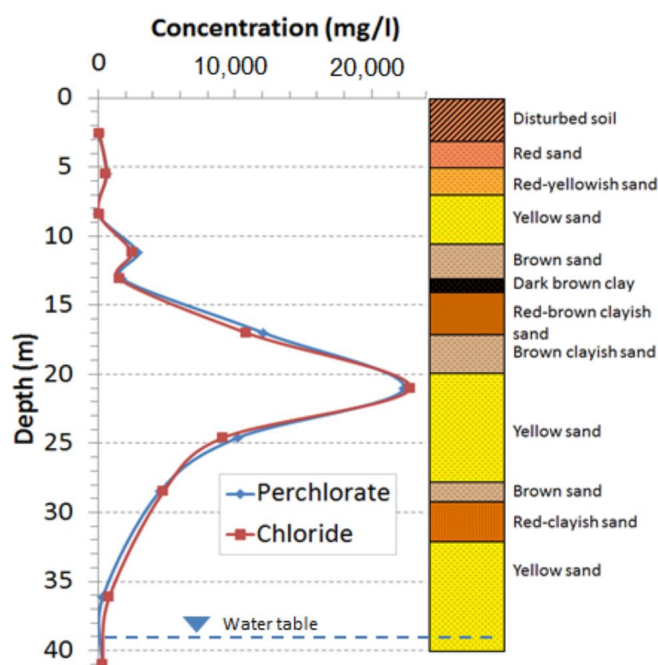
125 improve infiltration capacity in deep sections of the vadose zone during the  
 126 remediation experiment, a shallow clay layer with low permeability, known as  
 127 “nazaz” (Singer, 2007), was removed from a depth of 2.5–3 m by excavation. The  
 128 excavated area, 10 × 30 m, which was primarily assigned for the pilot infiltration  
 129 experiment, was backfilled with the sandy loam from the excavated site after removal  
 130 of the 0.5m thick nazaz layer. This layer is therefore presented in the profile as  
 131 disturbed soil.

132 **Table 1.** *Sedimentological Composition of the Vadose Zone at the Pilot Site*  
 133

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

144 The climate in the area is characterized as subtropical Mediterranean with a hot and  
 145 dry summer from May to October and a colder wet winter from November to April.  
 146 The average air temperature on summer and winter days is 30 °C and 17 °C,  
 147 respectively. The average annual precipitation is 530 mm year<sup>-1</sup>, mostly as rain  
 148 occurring mainly in four to seven rainy episodes during the winter season (IMS,  
 149 2011).

150



151

152 **Figure 1.** Initial concentration profiles of chloride and perchlorate in the vadose zone  
153 pore water under the former waste lagoon, along with the lithological profile.

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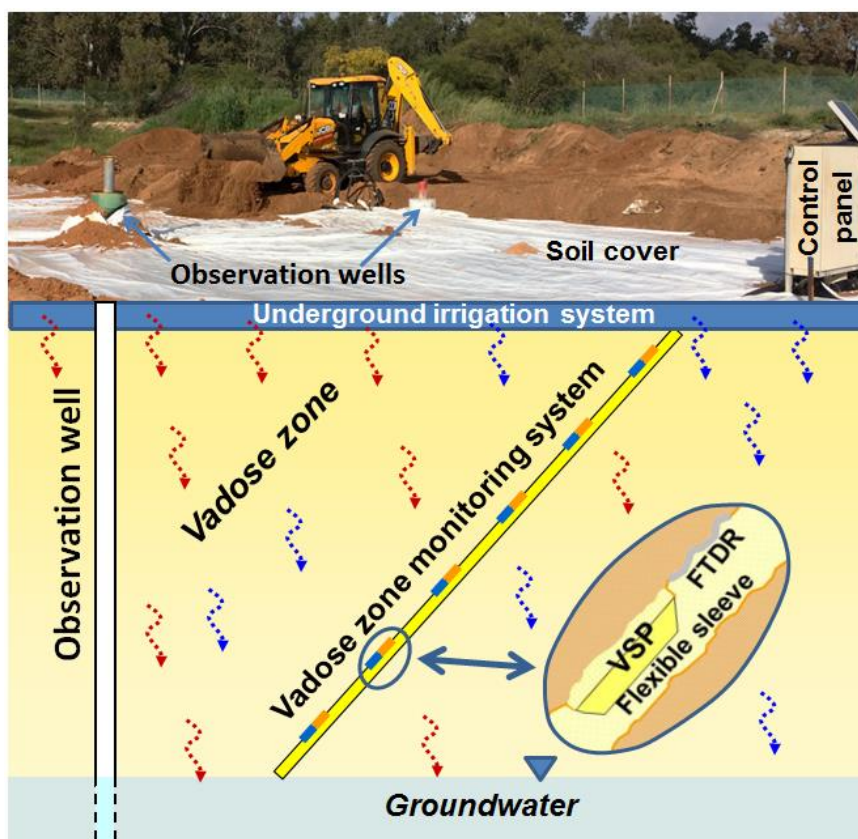
### 155 3 Experimental setup

156

#### 157 3.1 Vadose-zone monitoring setup

158

159 Real-time characterization of flow and transport processes in the vadose zone, as well  
160 as assessment of chemical transformation of the percolating water during the  
161 remediation experiments were carried out with a VMS that was installed across the  
162 entire unsaturated profile, from land surface to a depth of 37 m (Fig. 2). A detailed  
163 description of the VMS, its structure, installation procedure and performance, can be  
164 found in previous publications (Dahan et al., 2009; Rimon et al., 2011a) and in the



165

166 **Figure 2.** Schematic illustration of the vadose-zone monitoring system installed in the  
167 vadose zone under the infiltration pilot site. In the picture above the vadose zone, the  
168 irrigation system at the site is being covered.

169 supplementary material. In particular, the VMS that was used at this site was  
170 composed of a 44m long flexible polyurethane sleeve hosting 11 monitoring units  
171 distributed along its length. Each monitoring unit included: (a) a flexible time-domain  
172 reflectometer (FTDR) sensor for continuous measurement of variations in the  
173 sediment water content (Dahan et al., 2008; Rimon et al., 2007), and (b) vadose-zone  
174 sampling ports (VSPs), which enable frequent sampling of the vadose zone pore water  
175 for chemical analysis (Baram et al., 2012a; Dahan et al., 2009; Rimon et al., 2011b;  
176 Turkeltaub et al., 2016). The VMS flexible sleeve was installed in a 0.16m diameter





177 uncased borehole drilled slanted at a  $55^\circ$  angle (to the horizon) to a vertical depth of  
178 37 m. In addition to the 11 monitoring units that were installed with the VMS, four  
179 additional monitoring units were installed directly in the soil at depths of 0.5 m and  
180 1.5 m. It should be noted that the slanted installation is preferred to ensure that  
181 measurements carried out by each monitoring unit take place in separate undisturbed  
182 sediment columns. In addition, the flexibility of the monitoring sleeve and its filling  
183 with non-shrinking cement grout ensured complete sealing of the borehole void and  
184 prevention of cross-contamination through preferential flow in the borehole.

185

### 186 **3.2 Field setup**

187

188 Water amended with ethanol as the electron donor for perchlorate-reducing bacteria  
189 was infiltrated into the vadose zone through an area of 8 x 30 m at the pilot site using  
190 a drip-irrigation system. Dripping lines with drippers having a nominal discharge rate  
191 of  $2.2 \text{ L h}^{-1}$  were set up in a 0.3 x 0.3 m spatial distribution to create fairly even water  
192 distribution over the area. Accordingly, the total discharge rate of the irrigation  
193 system was set to  $5 \text{ m}^3 \text{ h}^{-1}$ , which is equivalent to an infiltration rate of  $0.02 \text{ m h}^{-1}$ . To  
194 promote anaerobic conditions in the unsaturated zone, a polyethylene liner covered  
195 with soil was placed over the dripper system after its installation. Ethanol was  
196 selected as the electron donor and carbon substrate because it is a natural, soluble  
197 compound that is commonly used by perchlorate-reducing bacteria (Bardiya and Bae,  
198 2011). Moreover, it eliminates the increased soil salinity associated with other  
199 common sources of electron donors such as acetate (Gal et al., 2008).

200

### 201 **3.3 Infiltration experiments**



202

203 Three infiltration experiments with variable amounts of water and ethanol were  
204 implemented at the pilot site over a period of 7 months. To trace the percolating water  
205 across the unsaturated zone, bromide (as KBr) was added to the infiltrating water at  
206 the early stages of the experiment. The infiltration rates, as well as the concentrations  
207 and application sequence were assigned for each experiment with insight gained from  
208 the previous experiment (Table 2). Accordingly, information obtained by the VMS on  
209 depth propagation of the ethanol and tracer and variations in perchlorate and chloride  
210 concentrations across the unsaturated zone during and after each infiltration  
211 experiment were used to adjust the infiltration procedure in the following stage.

212

*Table 2. Infiltration experiment conditions*

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

213

214 The first experiment (8 Aug 2010) consisted of infiltration of 50 m<sup>3</sup> water  
215 (equivalent to 210 mm) (Table 2). The first 6 m<sup>3</sup> were applied as untraced fresh water  
216 with no ethanol to wet the topsoil. This wetting stage is essential to promoting deep  
217 transport and preventing accumulation of tracers and ethanol in the low-flow zone  
218 located on the margins of the dripper's influential zone. Following the initial wetting  
219 phase, 0.4 m<sup>3</sup> of bromide tracer solution (as KBr) at a concentration of 12.5 g L<sup>-1</sup> was  
220 applied, followed by 1 m<sup>3</sup> of water with 5 % ethanol. Immediately after the  
221 application of the carbon and tracer solution, the rest of the water (42.6 m<sup>3</sup>) was  
222 applied to enhance transport of the ethanol and tracers to deeper parts of the vadose  
223 zone.



224           After obtaining the results pertaining to the wetting process, as well as tracer  
225 and ethanol migration in the vadose zone during the first infiltration experiment, a  
226 second experiment was performed (1 Sep 2010). This experiment was conducted with  
227 100 m<sup>3</sup> of water (equivalent to 420 mm). Here the first 7 m<sup>3</sup> of water was injected into  
228 the topsoil as untraced fresh water, followed by 1 m<sup>3</sup> of water with 5 % ethanol, and  
229 then the rest of the water dose (92 m<sup>3</sup>). No tracers were used in this experiment. The  
230 amount of water used after application of the ethanol was doubled to enhance  
231 migration of the ethanol to deep sections of the unsaturated zone.

232           Results from the first two experiments indicated limited migration of tracer  
233 and ethanol to deeper parts of the vadose zone. A third infiltration experiment was  
234 therefore conducted 5 months later with increased discharge of 300 m<sup>3</sup> (equivalent to  
235 1250 mm). This experiment started with 24 m<sup>3</sup> of untraced water followed by 0.4 m<sup>3</sup>  
236 concentrated (50 %) ethanol solution. Then, the rest of the water (275.6 m<sup>3</sup>) was used  
237 to push the ethanol down into the vadose zone. The large quantity of water applied  
238 after the concentrated ethanol solution was designed to enhance quick migration of  
239 the ethanol to deep parts of the vadose zone while minimizing its biodegradation in  
240 the upper soil layers.

241

### 242 **3.4 Analytical procedure**

243

244 Perchlorate was analyzed with a perchlorate ion-selective electrode (ISE; Laboratory  
245 Perchlorate Ion Electrode, Cole-Parmer, USA). All samples measured with the ISE  
246 were adjusted by dilution to a concentration range of 10–100 mg L<sup>-1</sup>. Duplicates were  
247 frequently analyzed by injecting 25 µL sample into a Thermo Scientific™ Dionex™  
248 ion chromatography system (ICS 5000) equipped with Ion Pac AS19 column



249 (detection limit of  $\pm 0.01 \text{ mg L}^{-1}$ ). Because results from the two methods were not  
250 significantly different, most of the data reported here are from the perchlorate  
251 electrode with a detection limit of 1 ppm. Bromide and chloride were analyzed by ion  
252 chromatography with a detection limit of 30 ppb (Gal et al. 2008). Total organic  
253 carbon (TOC) was analyzed to examine the success of delivering carbon to the vadose  
254 zone. Because porewater samples from the vadose zone are obtained through the VSP,  
255 which uses a porous ceramic interface (pore size  $< 2 \mu\text{m}$ ), TOC values reflect DOC.  
256 TOC was analyzed through a combustion TOC analyzer (Teledyne Tekmar, Apollo  
257 9000) with a detection limit of 2 ppm. Ethanol concentration in the vadose zone pore  
258 water was analyzed in a gas chromatograph (Varian, CP3800). Water samples (1.5  
259  $\mu\text{L}$ ) were injected by autosampler. The FID and injector temperatures were set to 270  
260 and 250 °C, respectively. The GC oven temperature was first held at 50 °C for 1 min,  
261 increased to 220 °C at a rate of  $25 \text{ °C min}^{-1}$ , and then held for 4 min. The separation  
262 was performed by Stabilwax® capillary column (60 m, 0.32 mm, 0.25  $\mu\text{m}$ , Restek  
263 Corporation, USA); helium was used as the carrier gas ( $1 \text{ mL min}^{-1}$ ). For  
264 quantification, five external standards were used.

265

#### 266 **4 Results and discussion**

267

268 All of the data obtained by the VMS are presented here as variations in measured  
269 parameters with depth, as commonly done to describe depth profiles. However, to  
270 ensure measurements under undisturbed vertical profiles, the VMS was installed in a  
271 slanted orientation (Fig. 2 and supplementary material). Thus, each monitoring unit  
272 faces an undisturbed profile that is shifted horizontally and vertically from the other  
273 units. Accordingly, although the data are presented as depth profiles, they should be



274 regarded as individual points distributed across the 3D space of the vadose zone  
275 (Dahan et al., 2007; Rimon et al., 2011a).

276

#### 277 **4.1 Water percolation**

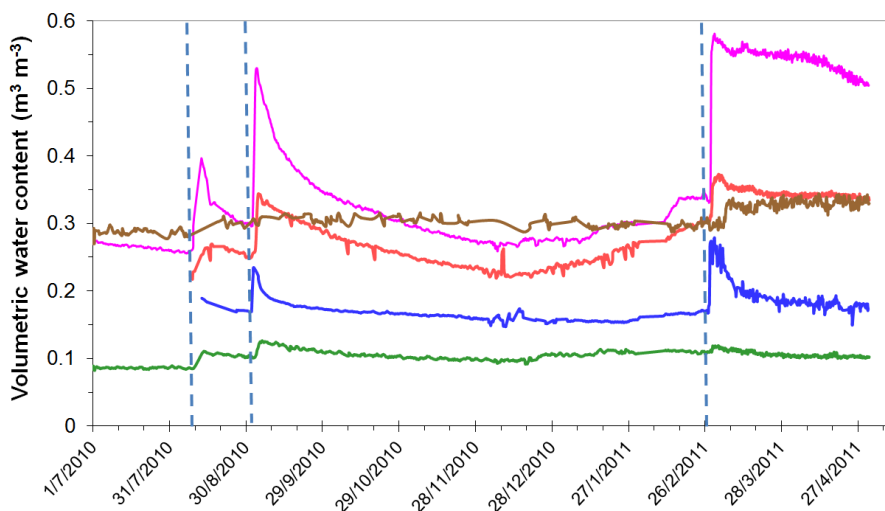
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279 Temporal variations in the vadose zone water content provide a direct indication of  
280 percolation processes in the vadose zone (Rimon et al., 2007; Dahan et al., 2008;  
281 Turkeltaub et al., 2015). Each infiltration experiment launched a wetting wave that  
282 propagated sequentially through the unsaturated zone (Fig. 3). Down-migration of the  
283 wetting wave was expressed as a quick rise in water content followed by a recession  
284 caused by water redistribution and drainage. Referring the wetting sequence in the  
285 vadose zone to the infiltration events on land surface enabled a direct calculation of  
286 the flow velocity across the unsaturated zone (Rimon et al., 2007; Dahan et al., 2008).  
287 All three infiltration experiments produced wetting fronts that moved down the  
288 vadose zone at a velocity of  $\sim 0.18 \text{ m h}^{-1}$ , even though the water volumes that were  
289 used in each experiment were significantly different (50, 100 and  $300 \text{ m}^3$ ).  
290 Observations of regulated flow velocities at constant rates across the vadose zone  
291 under variable surface hydraulic conditions have also been reported in other studies  
292 (Dahan et al., 2008; Amiaz et al., 2011; Rimon et al., 2011a).

293         The high salinity of the deeper parts of the vadose zone ( $>13 \text{ m}$ ) (Fig. 1) limits  
294 the reliability of the TDR technology for measuring water content at those depths  
295 (Nadler et al., 1999). Therefore, variation in water content, as an indication of deep  
296 percolation, is presented here only down to a depth of 11.2 m, where the salinity was  
297 low enough to achieve reliable moisture measurements with the FTDR sensors.  
298 Nevertheless, indications of deep percolation in the deeper layers ( $>13 \text{ m}$ ) are further



299 discussed through the variation in chemical composition of the percolating water  
300 across the entire thickness of the unsaturated zone (40 m).

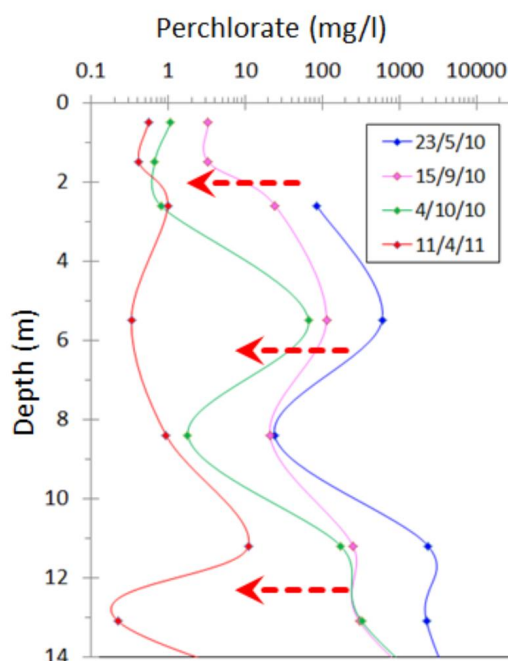


301  
302 **Figure 3.** Temporal variations in sediment water content in the top 13 m of the vadose  
303 zone during the infiltration experiments. Dates are given as day/month/year.

#### 304 305 **4.2 Perchlorate transformation and mobilization**

306  
307 Initial analysis of porewater samples from the vadose zone, prior to initiation of the  
308 infiltration experiments, revealed very high concentrations of perchlorate and  
309 chloride, both reaching maximum values of  $\sim 22,500 \text{ mg L}^{-1}$  (Fig. 1), and total  
310 dissolved solids (TDS) of  $43,000 \text{ mg L}^{-1}$ , at a depth of 21 m. Note that at this stage,  
311 the concentrations of perchlorate and chloride are nearly identical throughout the  
312 entire profile. These high concentrations, sampled by the VMS, are in accordance  
313 with concentration profiles obtained previously in extracts of sediment samples (Gal  
314 et al., 2009).

315



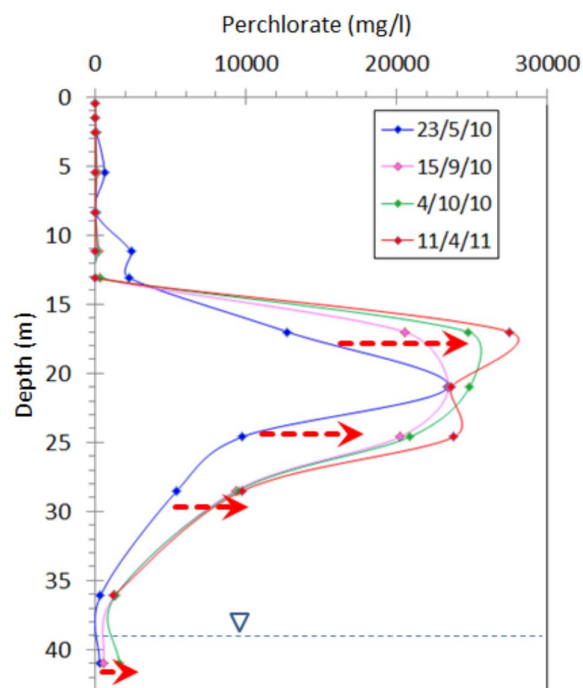
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317 **Figure 4.** Perchlorate concentration profile across the top 13 m of the vadose zone  
318 under the pilot site during the infiltration experiments. The profiles emphasize the  
319 gradual decrease in perchlorate concentration with time. Dates are given as  
320 day/month/year.

321 Frequent sampling of the vadose zone pore water showed dynamic variations  
322 in perchlorate concentration during the percolation experiments. In the upper section  
323 of the vadose zone (0–13 m), perchlorate concentrations decreased dramatically, from  
324 as high as 9000 mg L<sup>-1</sup> to below detection levels (Fig. 4). Such a reduction in  
325 concentration in a relatively thick portion of the vadose zone (13 m) over the short  
326 period of 10 months is clearly desirable and may even be considered a great success.  
327 Nevertheless, closer inspection of the variations in perchlorate concentration in deep  
328 parts of the vadose zone (17–40 m) showed an increase at most of the measurement  
329 points (Fig. 5). Perchlorate concentration rose from 12,700 mg L<sup>-1</sup> to 27,400 mg L<sup>-1</sup> at



330 a depth of 17 m during the same period. A similar increase in concentration was also  
331 found in deeper parts of the cross section at depths of 25, 28, and 36 m. Note that  
332 during this period, an increase in perchlorate concentration was even observed in the  
333 groundwater (represented at a depth of 41 m in Fig. 5). Obviously, the mixed trend in  
334 variations of perchlorate concentration implies that transformation and mobilization  
335 processes take place simultaneously. As such, the conditions for both biodegradation  
336 and mobilization should be examined along with the variation in perchlorate  
337 concentration.



338  
339 **Figure 5.** Perchlorate concentration profile across the entire vadose zone and top  
340 groundwater under the pilot site during the infiltration experiments. The profiles  
341 emphasize the gradual increase in perchlorate concentration with time. Dates are  
342 given as day/month/year.  
343





344 **4.3 Electron donor availability**

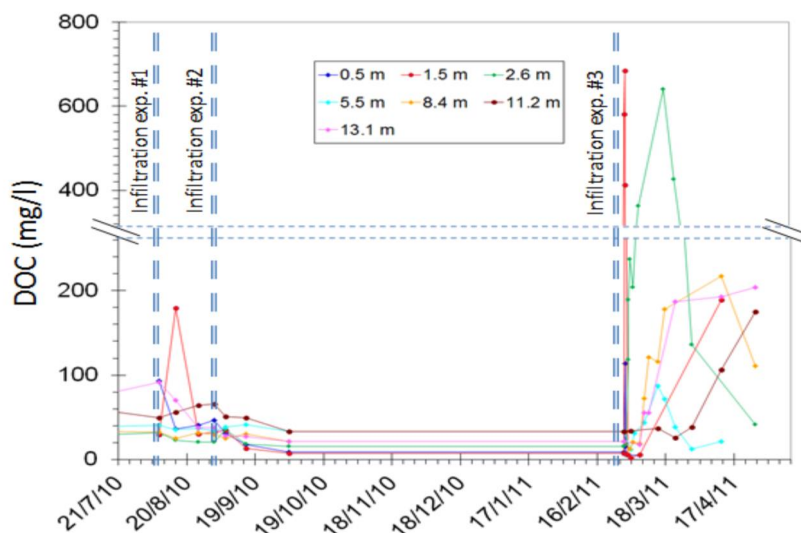
345

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

353 During the first infiltration experiment, an increase in DOC above background  
354 levels was observed only at shallow depths, down to 1.5 m (Fig. 6). No signs of  
355 increasing DOC were observed in the deeper parts of the cross section at this stage.  
356 Twenty-three days later, before initiation of the second infiltration experiment, DOC  
357 values had dropped back down to background levels. This implies that the ethanol  
358 was microbiologically consumed in the soil before it could be leached further down.

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

368



369

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

373 To overcome the limitation of electron donor delivery through the shallow  
374 soils, a third infiltration experiment was designed. In this experiment, the ethanol was  
375 injected in a 0.4m<sup>3</sup> high-concentration (50 %) pulse followed by a large volume of  
376 water. Application of ethanol at a very high concentration was aimed at suppressing  
377 its biological degradation in the shallow soil. The ethanol pulse was introduced after  
378 application of 24 m<sup>3</sup>, the latter to provide high initial wetting conditions under the  
379 ethanol front. Then the ethanol slug was pushed down with 276 m<sup>3</sup> of water. At this  
380 stage of the study, which was conducted 6 months after the previous one, a substantial  
381 increase in DOC was observed in the entire top 13 m of the cross section (Fig. 6).  
382 Obviously, an increase in DOC serving as electron donor is an essential prerequisite  
383 for perchlorate degradation. Apparently, application of ethanol at a high  
384 concentration, which inhibited its degradation in the upper layer, succeeded to drive



385 the ethanol all the way down to 13 m, just above the clay layer. Nevertheless, no signs  
386 of DOC increase were observed below 13 m.

387

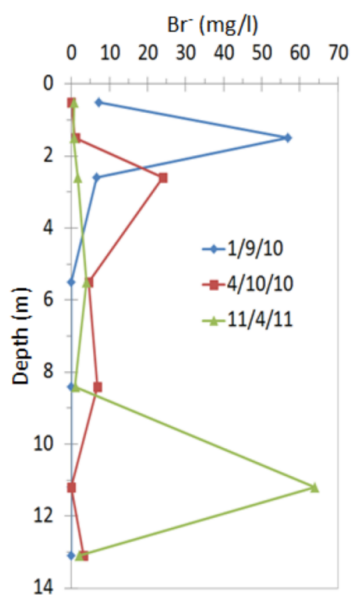
#### 388 **4.4 Transport and degradation**

389

390 The mechanism controlling down-propagation of a non-conservative substance such  
391 as ethanol may be elucidated by looking at the migration pattern of a conservative  
392 tracer such as bromide. Bromide was injected with the percolating water in the early  
393 stages of the first infiltration experiment. Results on bromide migration are presented  
394 here only for the top 13 m, where the background concentrations prior to the initiation  
395 of the infiltration experiment were below detection limits. Concentration profiles  
396 during the infiltration experiments clearly demonstrated sequential progress of the  
397 percolating water across the top 13 m of the unsaturated zone (Fig. 7). Mass balance  
398 calculation of bromide on the basis of the concentration profiles (Fig. 7) and sediment  
399 water content (Fig. 3) on various dates after the infiltration experiment resulted in  
400 high recovery rates of 85–127 %. A comparison of the transport patterns of bromide  
401 and DOC confirmed that biodegradable material such as ethanol is rapidly consumed  
402 in the vadose zone.

403 An increase in chloride concentration in the vadose zone is usually attributed  
404 to evaporation processes near land surface, a mechanism that is unlikely to occur in  
405 this particular setup where the surface is isolated from the atmosphere. Accordingly,  
406 variations in chloride concentration across the vadose zone may be attributed to  
407 chloride mobilization with the percolating water and perchlorate reduction. Therefore,  
408 degradation of perchlorate is expected to result in an increase in chloride mass.

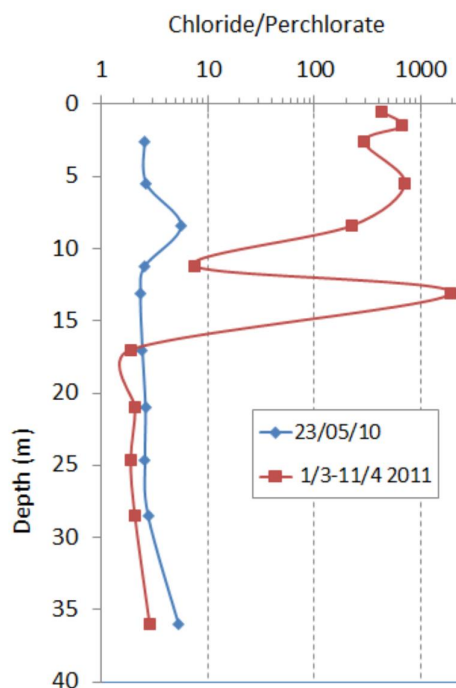
409



410

411 **Figure 7.** Variations in bromide concentration profile across the top 13 m of the  
412 vadose zone during the infiltration experiments. Dates are given as day/month/year.

413 Prior to the infiltration experiments, chloride-to-perchlorate ratios in the  
414 vadose zone were very similar, exhibiting nearly identical profiles (Fig. 1) with  
415 equivalent concentration proportions of 2.4–5.5 (Fig. 8). Following the infiltration  
416 experiment, a significant increase in ionic ratios was observed in the top 13 m, while  
417 in the rest of the profile—from a depth of 17 m to the water table, the concentration  
418 ratio of chloride to perchlorate remained relatively unchanged. Obviously, since both  
419 perchlorate and chloride are very soluble and mobile, infiltration water with a low  
420 concentration of chloride ( $\sim 100 \text{ mg L}^{-1}$ ) and zero perchlorate is also expected to result  
421 in an increased chloride-to-perchlorate ratio, even if no perchlorate degradation takes  
422 place. Since both chloride and perchlorate are very mobile and easily displaced with  
423 the percolating water, quantification of the perchlorate-degradation rate with respect  
424 to its down-leaching is not straightforward.



425

426 **Figure 8.** Chloride-to-perchlorate equivalent concentration ratio profiles before and  
427 after the infiltration experiments. Dates are given as day/month/year.

428

## 429 5 Conclusions

430

431 The infiltration experiments were primarily aimed at improving the environmental  
432 conditions for perchlorate-reducing bacteria across the vadose zone. This included an  
433 increase in water content along the soil profile and amendment of the electron donor.  
434 The results, which were based on continuous monitoring of the entire vadose zone,  
435 exhibited notable variation in the concentrations of perchlorate, DOC and other  
436 solutes in the unsaturated zone. Increased concentrations of DOC with a concurrent  
437 reduction in perchlorate concentration (from thousands to a few milligrams per liter)  
438 and increased chloride-to-perchlorate ratio (from ~2.5 to ~300) in the upper 13 m



439 indicated that perchlorate is partially reduced in this part of the vadose zone. On the  
440 other hand, no evidence of improved reducing conditions was observed in the deeper  
441 parts, where the initial concentrations of perchlorate were significantly higher.  
442 Nevertheless, since assessment of redox conditions in deep vadose zone is not yet  
443 feasible, we can only rely on variations in the chemical composition to assess the  
444 existence of degradative conditions.

445 The limited ability to deliver a soluble electron donor across a  
446 microbiologically reactive medium, such as topsoil, is a major limiting factor for  
447 remediation of the deep vadose zone through gravitational percolation of enriched  
448 solution. Note that temporal variations in the concentrations of perchlorate, as well as  
449 other solutes, in the deep parts of the vadose zone, i.e., under the clay layer at 14 m,  
450 indicate that the clay layer does not play any role in limiting infiltration capacity.  
451 Similar observations on the role of clay layers in infiltration in the unsaturated zone  
452 have been reported in previous publications (Rimon et al., 2007; Baram et al., 2012a,  
453 2012b).

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



463           The study demonstrates that application of vadose-zone monitoring  
464 technology during a remediation operation provides real-time information on the  
465 chemical and hydrological state of the subsurface. Linking the temporal variation in  
466 the chemical composition of the vadose zone pore water, sediment saturation degree  
467 and flow velocities are vital for efficient management of remediation operations.

468

469 **Author contribution:** Ofer Dahan (PI, Vadose zone hydrology) design of the  
470 experimental and monitoring setup. Idan Katz (MSc student) conducted the field  
471 experiment and laboratory analysis. Zeev Ronen (PI, Microbiology) design the bio  
472 treatment setup. Data analysis and manuscript preparation - all coauthors.

473

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

475

476 **Acknowledgments.** The authors wish to express their appreciation Israeli Water  
477 Authority for project funding.

478

479

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