



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

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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 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 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 m . To



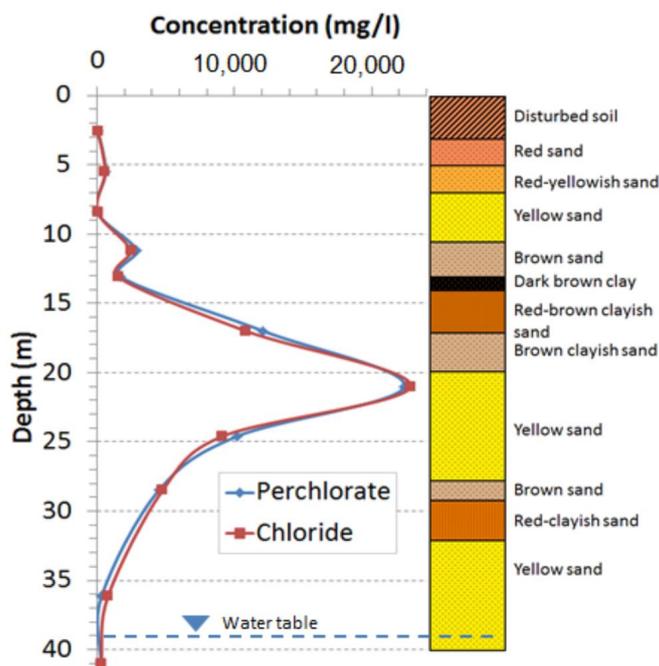
125 improve infiltration capacity in deep sections of the vadose zone during the
126 remediation experiment, a shallow clay layer with low permeability, known as
127 “nazaz” (Singer, 2007), was removed from a depth of 2.5–3 m by excavation. The
128 excavated area, 10 × 30 m, which was primarily assigned 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⁻¹, 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.

154

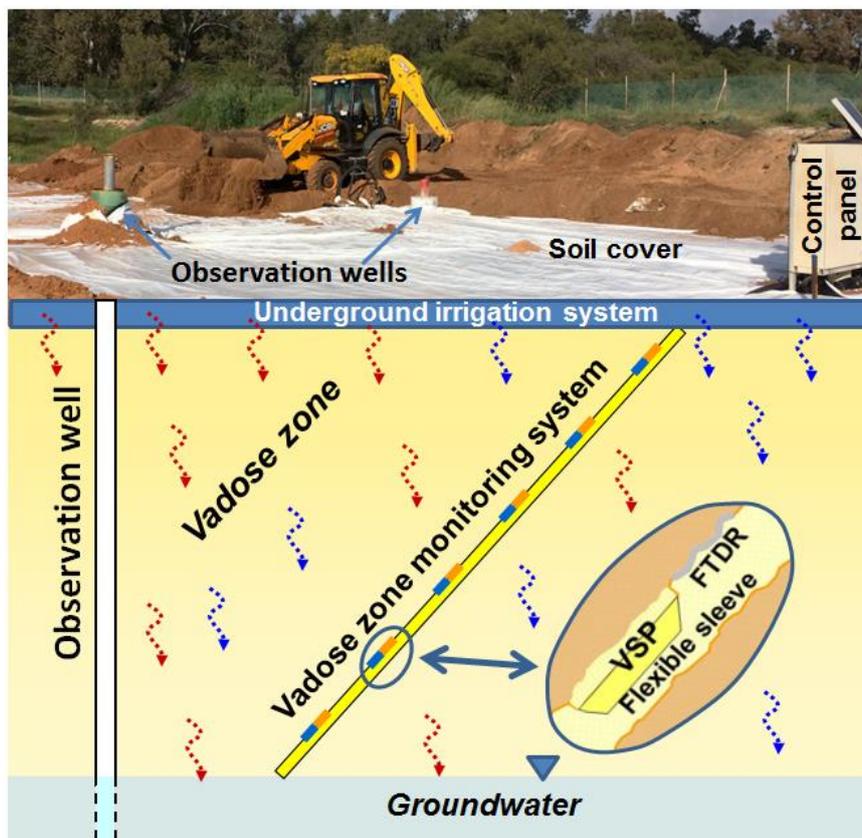
155 3 Experimental setup

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° 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 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 m³ h⁻¹, which is equivalent to an infiltration rate of 0.02 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 ³)	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 m³ water
215 (equivalent to 210 mm) (Table 2). The first 6 m³ 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 m³ of bromide tracer solution (as KBr) at a concentration of 12.5 g L⁻¹ was
220 applied, followed by 1 m³ of water with 5 % ethanol. Immediately after the
221 application of the carbon and tracer solution, the rest of the water (42.6 m³) 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 m³ of water (equivalent to 420 mm). Here the first 7 m³ of water was injected into
228 the topsoil as untraced fresh water, followed by 1 m³ of water with 5 % ethanol, and
229 then the rest of the water dose (92 m³). 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 m³ (equivalent to
235 1250 mm). This experiment started with 24 m³ of untraced water followed by 0.4 m³
236 concentrated (50 %) ethanol solution. Then, the rest of the water (275.6 m³) 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⁻¹. 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 μL) 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 °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 μm , Restek
263 Corporation, USA); helium was used as the carrier gas (1 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**

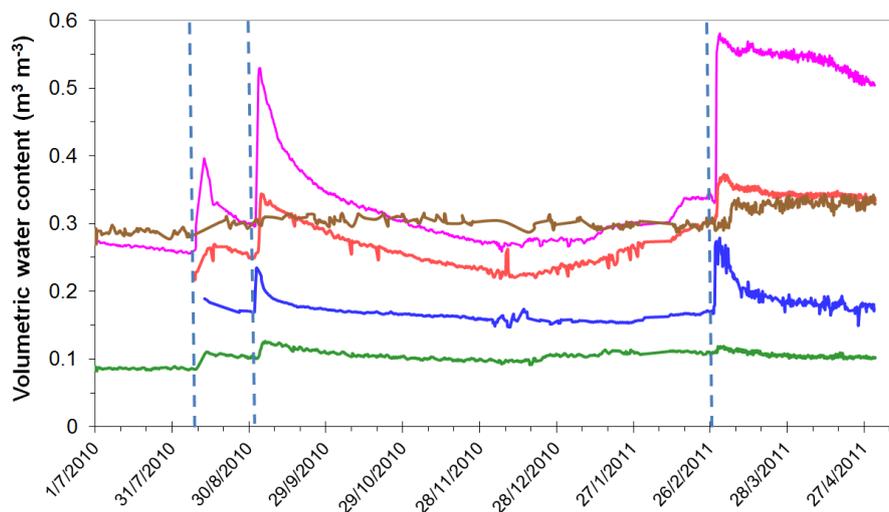
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279 Temporal variations in the vadose zone water content provide a direct indication of
280 percolation processes in the vadose zone (Rimon et al., 2007; Dahan et al., 2008;
281 Turkeltaub et al., 2015). Each infiltration experiment launched a wetting 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 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).

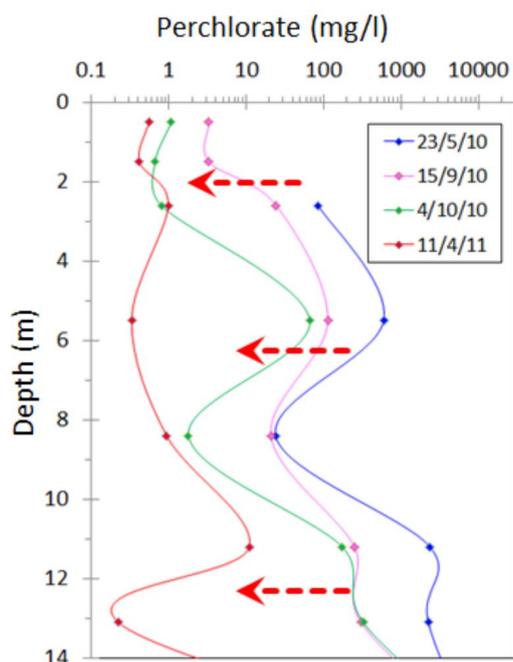


301
302 **Figure 3.** Temporal variations in sediment water content in the top 13 m of the vadose
303 zone during the infiltration experiments. Dates are given as day/month/year.

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



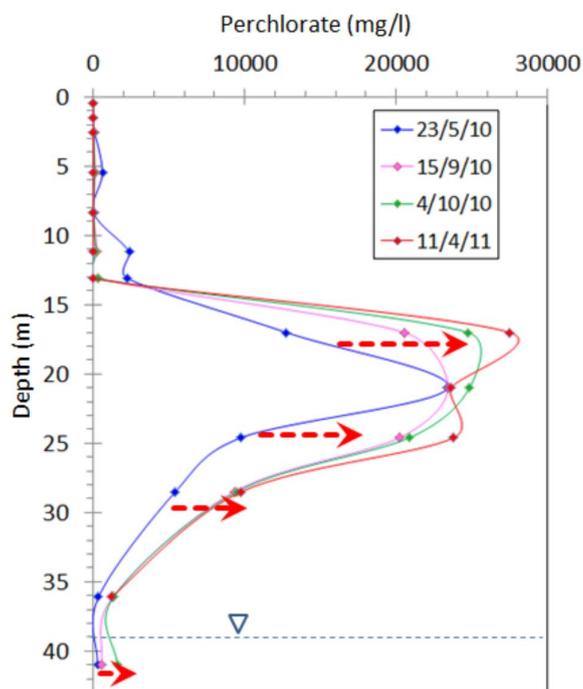
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317 **Figure 4.** Perchlorate concentration profile across the top 13 m of the vadose zone
318 under the pilot site during the infiltration experiments. The profiles emphasize the
319 gradual decrease in perchlorate concentration with time. Dates are 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⁻¹ 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⁻¹ to 27,400 mg L⁻¹ 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**

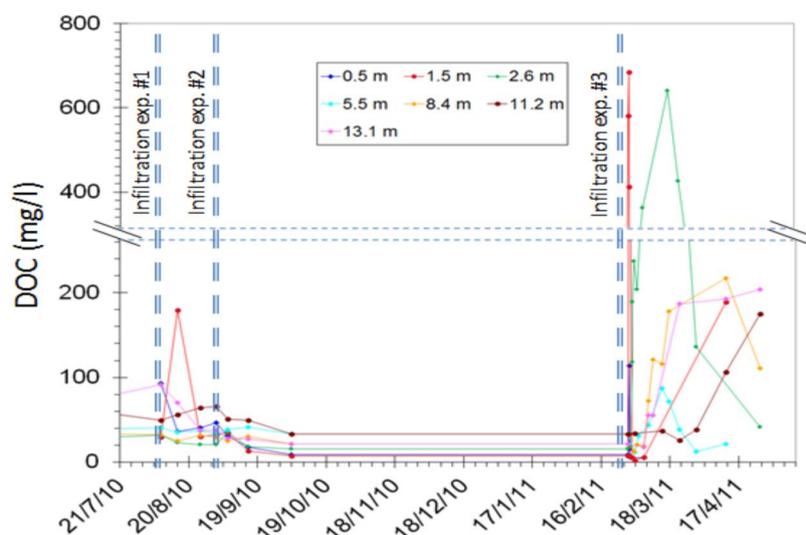
345

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 0.4m³ 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 m³, the latter to provide high initial wetting conditions under the
379 ethanol front. Then the ethanol slug was pushed down with 276 m³ 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

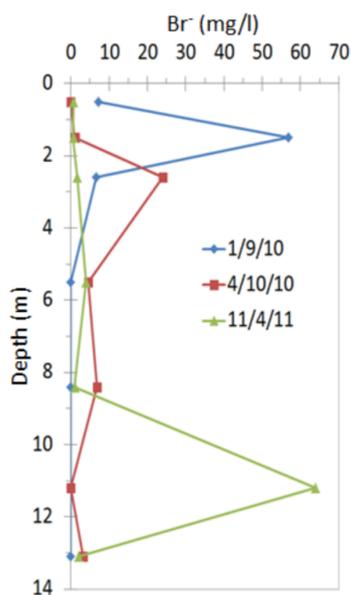
388 **4.4 Transport and degradation**

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.

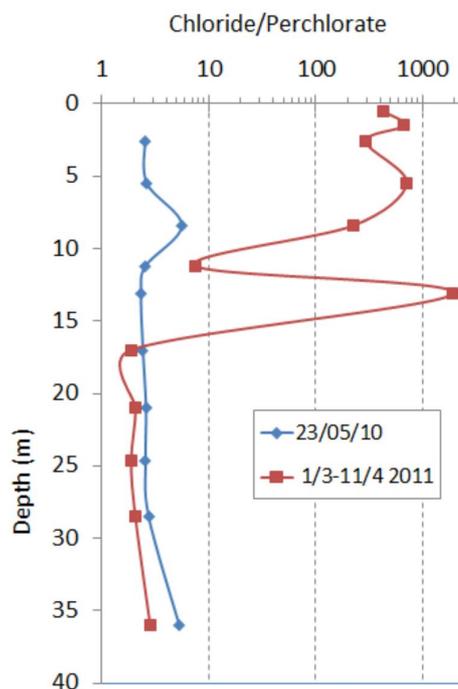
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410

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

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478

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