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Uptake of perchlorate in terrestrial plants

Lu Yu, Jaclyn E. Cañas, George P. Cobb, William A. Jackson, and Todd A. Anderson*

Department of Environmental Toxicology, The Institute of Environmental and Human Health, Texas Tech University and Texas Tech University Health Sciences Center, Box 41163, Lubbock, TX 79409-1163, USA

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Abstract

Cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), and soybean (*Glycine max*) were used to determine uptake of the perchlorate anion (100 ppb) from sand. Plants were watered with different ratios of Hydrosol (a diluted solution of Peters All-Purpose Plant Food) to Milli-Q water (18 MΩ) to determine if the presence of other nutrients (such as nitrate) influenced perchlorate uptake. Perchlorate concentrations in sand and plant tissues were determined weekly. Perchlorate uptake was observed in all three plant species. In most experiments, perchlorate was completely depleted from sand in which plants were growing. Perchlorate concentrations in lettuce were also significantly higher than those in cucumber and soybean ($P<0.0001$). Perchlorate concentrations in sand decreased at a higher rate at lower ratios of Hydrosol to Milli-Q, indicating that plant (cucumber) uptake of perchlorate is influenced by the presence of external nutrients. The results of an 8-week uptake study in cucumber and a 6-week uptake study in lettuce suggest that a threshold perchlorate concentration is reached: for cucumber, 150 ppm and for lettuce, 750 ppm. Although the presence of external nutrients decreases the rate of perchlorate uptake by plants, significant concentrations of perchlorate occur in aboveground plant tissues even after relatively short periods of growth. The potential for trophic transfer of perchlorate from soil to higher organisms through plants exists.

Keywords: Perchlorate; Vegetation; Plant uptake

1. Introduction

Perchlorate has attracted increasing attention since it was detected in drinking water supplies in Nevada, Utah, and California in the late 1990s. Now perchlorate is known to exist in ground and surface water of >20 states (USEPA, 2002), primarily because of its past use in the production of explosives, pyrotechnics, and blasting formulations (Herman and Frankenberger, 1998). The major health concern with perchlorate is interference with normal thyroid function. Perchlorate can competitively inhibit iodide uptake, reducing thyroid hormone production and further affecting normal metabolism, growth, and development of organisms (USEPA, 1998). Perchlorate is a stable ion that may affect humans and other animals through multiple pathways of exposure. Recent debates have highlighted the potential for perchlorate to be translocated from the environment to plants (Urbansky, 2000a).

There are limited data concerning the uptake of perchlorate into agricultural products through irrigation with contaminated water or from application of fertilizers that may contain perchlorate. Recent studies have shown that certain plant species can take up perchlorate. Susarla et al. (1999) evaluated 13 vascular plants and found that all plants, with the exception of waterweed and duckweed, were capable of rapid uptake of perchlorate. Another study reported uptake of perchlorate by salt cedars in the Las Vegas Wash (Urbansky et al., 2000b). Nzengung et al. (1999) showed that woody plants are capable of decontaminating water containing perchlorate and can mediate the transformation of perchlorate into chloride. High concentrations of perchlorate in vegetation were reported in an assessment at the Longhorn Army Ammunition Plant (Smith et al., 2001). In a recent study, perchlorate has been shown to accumulate and to be slowly reduced inside poplar tree tissues (Aken and Schnoor, 2002).

*Corresponding author. Fax: +1-806-885-4577.
E-mail address: todd.anderson@ttu.edu (T.A. Anderson).

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Susarla et al. (1999) suggested that plant species, perchlorate concentration, substrate, anionic nutrients, chloride ion, and stage of plant maturity have considerable influences on movement of perchlorate from soil solution into plants. There are plant uptake data for contaminants that should behave similarly to perchlorate (namely pertechnetate, TcO_4^-) in the environment (Cataldo et al., 1983; Krijger et al., 2000). These data indicate plant uptake, as well as relationships to external nutrients like nitrate.

Because of the potential importance of trophic transfer of perchlorate from plants to animals, including humans, this study was undertaken to evaluate uptake of perchlorate into three types of plants: cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), and soybean (*Glycine max*). The time course of perchlorate uptake and the distribution of perchlorate in sand, leaves, and roots over time were determined. The influence of soil nutrient levels on perchlorate uptake into plants was also determined using Hydrosol, a commercial fertilizer.

2. Materials and methods

2.1. Reagents and standards

A 100 μg/mL certified sodium perchlorate solution (Accustandard, Inc.) was purchased to prepare perchlorate calibration standards. Sodium hydroxide was purchased from Fisher Scientific and diluted to make the eluent required for the ion chromatography (IC). Hydrosol is a diluted solution of Peters All-Purpose Plant Food.

2.2. Uptake experiments

Cucumber, lettuce, and soybean were selected for the uptake experiments. Seeds of plants were placed in polystyrene cups containing 50 or 100 g Ottawa sand (Fisher Scientific) and grown in an incubator at 22°C (photoperiod 15 h light/9 h dark). The cups were covered with petri dishes to avoid excessive loss of water. Our initial studies indicated that plant germination was not affected by as much as 1000 ppb perchlorate (ng perchlorate/g sand); because 100 ppb is a more environmentally relevant concentration, all uptake experiments were conducted at this concentration. The experimental design varied according to different plant species. For cucumber, three 4-week experiments were conducted in the presence of varying ratios of Hydrosol, a diluted solution of Peters All-Purpose Plant Food: (1) 100% Hydrosol, (2) Hydrosol:Milli-Q water (50:50), and (3) Hydrosol:Milli-Q water (25:75). Because we were concerned about the lack of nutrition for proper plant growth, another experiment in which cucumber was watered with 100% Milli-Q water lasted only 2 weeks. An 8-week cucumber uptake study was also conducted in which the sand was respiked with perchlorate after week 4. Lettuce was grown in the presence of 100% Hydrosol for 6 weeks. For soybean, two 4-week experiments were conducted: (1) 100% Hydrosol and (2) Hydrosol:Milli-Q water (50:50). There were four plants in each treatment group at every sampling point. Four control plants of the appropriate species (no perchlorate) were included for each of the respective uptake experiments. An additional control with perchlorate and no plant was also sampled each week to assess possible microbial transformation of perchlorate in the sand.

Plants were removed each week and sectioned into two parts: portion of plant above sand level (primarily leaves) and portion of plant below sand level (primarily roots). Each portion of the plant sample was weighed, rinsed with water, and allowed to dry before extraction (described later). The amount of perchlorate remaining in sand was also determined weekly by adding a known volume of Milli-Q water (18 MΩ) to the cups and extracting the contents by mechanical agitation. Sand extracts were analyzed by IC (described later).

2.3. Tissue extraction and extract cleanup

All plants were extracted in 11-mL cells using Milli-Q water (18 MΩ) with a Dionex (Sunnyvale, CA) Accelerated Solvent Extractor (ASE 200) using the following procedure (Anderson and Wu, 2002). Cells were heated for 5 min at 100°C, filled with Milli-Q water, and pressurized to 1500 psi. Total extraction time was 15 min. At the completion of the extraction procedure, extract volume was recorded. For all plants, 1.0 mL of extract volume was cleaned with alumina solid-phase extraction (SPE) cartridges and then diluted to 5 mL with Milli-Q water. Finally, the diluted extracts were filtered with a 0.45 μm Acrodisc into 5-mL IC vials.

2.4. Analysis

Analysis of perchlorate ion was conducted using a Dionex DX-500 Ion Chromatography System equipped with a GP50 pump, a CD20 conductivity detector, and an AS40 automated sampler. Peaknet was used to control the system. Ion separation was made with a Dionex IonPac AS16 (4 mm) analytical column. The flow rate of eluent, 100 mM sodium hydroxide, was 1.0 mL/min. The injection loop volume was 1000 μL, and the runtime for perchlorate analysis was 12 min. An anion self-regenerating suppressor was used for suppressed conductivity detection. An eight-point standard curve was constructed for calibration standards of 2.5, 5, 10, 20, 50, 100, 200, and 500 ppb (ng/mL). Computer-generated peak areas were used to determine perchlorate

concentrations. Using the method described earlier, the detection limit for perchlorate in water was 1 ng/mL.

2.5. Data analysis

All statistical tests were conducted using SAS software (Version 8). Comparisons of mean perchlorate concentrations between planted sand and unplanted sand were conducted using Student's *t*-test. Two-way ANOVA was conducted to evaluate the effect of species on perchlorate uptake; Duncan's multiple range test was used after the ANOVA.

3. Results and discussion

Analysis of control samples (planted and unplanted cups without perchlorate) at the end of each experiment indicated that perchlorate was not detected in planted sand, roots, or leaves. In addition, loss of perchlorate from sand in the unplanted controls was negligible. For the uptake experiments, perchlorate was removed from sand by plants (Fig. 1) and taken up in aboveground vegetation (Table 1) in all of the tests conducted.

In the 4-week cucumber uptake experiment in the presence of Hydrosol, perchlorate concentration in the planted sand decreased to nondetectability at week 3. There was an 11% decrease in the unplanted sand during the same 4 weeks. Considerable uptake of perchlorate into cucumber leaves (Table 1) was observed. Similar results were obtained for the lettuce and soybean perchlorate uptake experiments. Comparisons of planted sand and unplanted sand in each of these experiments showed significant differences in perchlorate concentrations (P ranged from <0.0001 to 0.0401). There was only a slight decrease of perchlorate in unplanted sand over time, suggesting that microbial transformation of perchlorate in the experimental system was negligible.

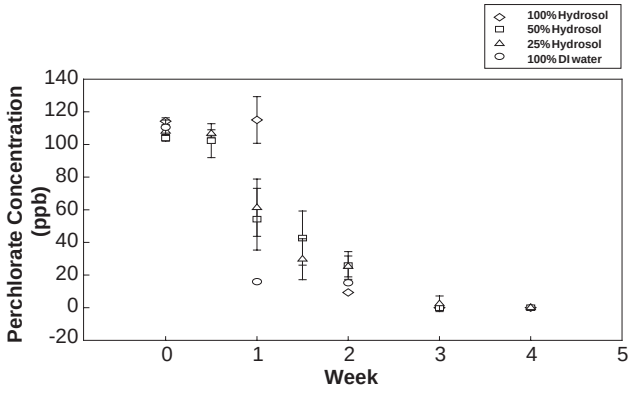

Fig. 1. A comparison among sand concentrations of four cucumber perchlorate uptake experiments in the presence of varying ratios of Hydrosol to water. Error bars represent one standard deviation of the mean.

In this study, the potential effects of external nutrients were assessed by comparing perchlorate concentrations in planted sand, leaves, and roots grown in the presence of various ratios of Hydrosol to Milli-Q water. Evidence indicated that increased nutrient levels decreased the rate of perchlorate uptake into vegetation. For the cucumber experiments, sand concentrations of perchlorate in the presence of plants grown with 100% Hydrosol decreased at the lowest rate, whereas those in 100% Milli-Q water decreased at the fastest rate. Concentrations of perchlorate in the sand of experiments conducted with mixtures of Hydrosol and water decreased at a rate between those of pure Hydrosol and pure water (Fig. 1). Consistent with the sand data, perchlorate uptake in leaves was the greatest when Hydrosol was not used to water the plants (Table 1). Although these perchlorate concentration differences in leaves may be due to decreased leaf mass because of a lack of nutrient(s), there is still an increased potential hazard for higher organisms due to the perchlorate concentration achieved in cucumber leaves. The differences in uptake could be due to certain nutrients in Hydrosol, especially nitrate, that may compete with perchlorate for uptake into plants; the presence of nitrate may essentially block perchlorate uptake. It is possible that perchlorate could only be taken up after most of the nitrate is removed. With fewer of these nutrients in sand, perchlorate was more available to accumulate in the plant. As a result of the sampling protocol (weekly), perchlorate was rarely detected in roots. More frequent sampling of roots would be necessary to detect perchlorate before it translocates to leaves. A similar competition effect was observed in the soybean experiments, although in contrast to the cucumber experiments, the removal of perchlorate from sand occurred almost at the same rate for 100% Hydrosol and Hydrosol:Milli-Q water at a 50:50 ratio.

In the 8-week perchlorate uptake study, samples were respiked with 100 ppb perchlorate after week 4. Concentrations in leaves peaked at week 3 at $\sim$150 ppm and then peaked again at week 6 (at $\sim$150 ppm) after the respike. It seems that the plants reached a maximum threshold of perchlorate, especially because the sand data indicated that there was still perchlorate available for uptake. This suggests that plants do not have the ability to hyperaccumulate perchlorate once a maximum burden in leaves is reached. At that point, the plant may begin to exude, transform, or transpire perchlorate. In the lettuce experiment, perchlorate concentrations in the leaves reached 750 ppm at week 4 and then decreased to 20 ppm at week 5, further supporting the idea of perchlorate exudation, transformation, or transpiration from leaves.

Table 1
Distribution of perchlorate in the test systems

Plants		Sample time	Perchlorate concentration (ppb)				Mass of perchlorate in plant system (ng)
			Unplanted sand	Planted sand	Leaves	Root	
Cucumber	Hydrosol (H)[a]	Week 0	111 ± 5[b]	114 ± 2[b]	NA	NA	11,191 ± 251[b]
		Week 1	NA	115 ± 14[c]	34,963 ± 9052[c]	261,162 ± 38,915[c]	13,065 ± 1644[c]
		Week 2	108 ± 0.68[c]	9[d]	20,665[d]	150,763[d]	2739[d]
		Week 3	NA	ND[b]	22,083 ± 17,135[b]	ND[b]	1204 ± 894[b]
		Week 4	99[d]	ND[b]	41,060 ± 32,011[b]	ND[b]	2258 ± 1656[b]
	H[a]:Milli-Q (50:50)	Week 0	104 ± 2[b]	104 ± 2[b]	NA	NA	10,402 ± 202[b]
		Week 0.5	102 ± 10[b]	102 ± 10	NA	NA	10,218 ± 944[b]
		Week 1	94[d]	54 ± 19[e]	98,049 ± 44,689[e]	313,891 ± 51,375[e]	8331 ± 3548[e]
		Week 1.5	100[d]	43 ± 17[e]	159,903 ± 33,529[e]	ND[e]	8351 ± 1775[e]
		Week 2	106[d]	26 ± 8[e]	267,739 ± 19,193[e]	ND[e]	12,016 ± 1697[e]
		Week 3	65[d]	ND[e]	126,518 ± 56,962[e]	ND[e]	6500 ± 3084[e]
		Week 4	85[d]	ND[e]	139,058 ± 20,553[e]	ND[e]	9249 ± 1126[e]
	H[a]:Milli-Q (25:75)	Week 0	108 ± 2[b]	108 ± 2[b]	NA	NA	10,802 ± 247[b]
		Week 0.5	107 ± 2[b]	107 ± 2[b]	NA	NA	10,671 ± 166[b]
		Week 1	99 ± 7[b]	61 ± 18[c]	127,398 ± 31,067[c]	5495 ± 77,771[c]	9447 ± 3457[b]
		Week 1.5	109[d]	30 ± 13[e]	201,549 ± 22,315[e]	ND[e]	7546 ± 1607
		Week 2	108[d]	25 ± 6[e]	190,623 ± 16,033[e]	9416 ± 18,833[e]	8000 ± 1462
		Week 3	72[d]	2 ± 5[e]	314,375 ± 55,700[e]	ND[e]	11,965 ± 2943
		Week 4	102[d]	ND[e]	219,093 ± 32,953[e]	ND[e]	10,615 ± 1523
	Milli-Q water	Week 0	110 ± 5[c]	110 ± 5[c]	NA	NA	5523 ± 225[c]
		Week 1	85[d]	16 ± 0.90[c]	118,077 ± 11,280[c]	ND[c]	4147 ± 517[c]
		Week 2	98 ± 4[c]	15.21[d]	202,425[d]	ND[d]	1137[d]
	Hydrosol[a]	Week 0	106 ± 4[b]	106 ± 4[b]	NA	NA	10 578 ± 417[b]
		Week 0.5	96 ± 3[e]	96 ± 3[e]	NA	NA	9670 ± 1101[e]
		Week 1	94 ± 5[c]	55 ± 4[b]	81,606 ± 13,548[b]	ND[b]	7843 ± 986[b]
		Week 1.5	128[d]	41 ± 9[e]	114,389 ± 7192[e]	19,515 ± 39,031[e]	8115 ± 1889[e]
		Week 2	85[d]	57 ± 19[e]	99,913 ± 13,830[e]	33,664 ± 67,327[e]	10,136 ± 3774[e]
		Week 3	96[d]	22 ± 16[e]	142,051 ± 10,501[e]	ND[e]	10,873 ± 3163[e]
		Week 4	62 ± 3[c]	6 ± 5[b]	101,659 ± 45,349[b]	ND[b]	7649 ± 2928[b]
		Week 5	184[d]	36 ± 22[c]	119,380 ± 61,927[c]	ND[c]	19,153 ± 6768[c]
		Week 6	164[d]	47 ± 22[b]	146,559 ± 915[b]	ND[b]	20,096 ± 5576[b]
		Week 7	238[d]	45 ± 20[e]	102,079 ± 9322[e]	ND[e]	17,118 ± 4873[e]
		Week 8	149[d]	20 ± 17[e]	79,780 ± 31,284[e]	ND[e]	14,282 ± 3557[e]
Lettuce	Hydrosol[a]	Week 0	69 ± 2[b]	69 ± 2[b]	NA	NA	3444 ± 77[b]
		Week 1	70[d]	72 ± 2[f]	115,656[d]	ND[d]	5174 ± 67[f]
		Week 2	82[d]	68 ± 8[f]	241,268 ± 16,642[e]	ND[d]	5104 ± 888[e]
		Week 3	55[d]	72 ± 19[f]	70,314 ± 23,193[f]	ND[f]	6109 ± 1557[f]
		Week 4	43[d]	8 ± 11[f]	753,784 ± 32,577[f]	ND[f]	5223 ± 1635[f]
		Week 5	79[d]	ND[f]	20,762 ± 6239[f]	19,256[d]	2480 ± 448[f]
		Week 6	71[d]	ND[b]	31,871 ± 14,367[b]	ND[d]	1688 ± 173[b]
Soybean	Hydrosol[a]	Week 0	12 ± 3[b]	128 ± 3[b]	NA	NA	6416 ± 146[b]
		Week 1	114 ± 9[e]	83 ± 8[e]	12,995 ± 6313[e]	9309 ± 18,618[e]	5935 ± 909[e]
		Week 2	77 ± 3[e]	11 ± 10[e]	15,323 ± 3584[e]	28,838 ± 22,590[e]	3643 ± 1774[e]
		Week 3	62 ± 5[e]	8 ± 2[b]	17,774 ± 3949[b]	ND[b]	2724 ± 481[b]
		Week 4	67 ± 4[e]	18 ± 16[e]	14,497 ± 3050[e]	ND[e]	2804 ± 1548[e]

Week 0 indicates immediate extraction after perchlorate application. NA = sample was not collected at this time point. ND = perchlorate was not detected.

[a] Diluted solution of Peters All-Purpose Plant Food. Main components include nitrate, phosphate, magnesium, iron, copper, manganese, zinc, and molybdenum.

[b] Sample size ($n = 3$).

[c] Sample size ($n = 2$).

[d] Sample size ($n = 1$).

[e] Sample size ($n = 4$).

[f] Sample size ($n = 5$).

Although accumulation of perchlorate in leaves was observed in all plant species, there were significant differences in the ability of plants to take up perchlorate. Perchlorate concentrations in leaves were compared among cucumber, lettuce, and soybean experiments all treated identically (100% Hydrosol). Lettuce exhibited the highest accumulation of perchlorate at 750 ppm, followed by cucumber (41 ppm), and soybean (18 ppm). Lettuce showed a greater ability to accumulate perchlorate than the other two species. This difference is not completely explained by a lower plant mass for lettuce (the denominator in the concentration calculation) compared with the other two species. Significant differences ($P<0.0001$) were observed for comparisons between perchlorate concentrations in lettuce vs. cucumber and soybean. There was no significant difference in perchlorate concentrations between cucumber and soybean.

Condensation was observed on petri dishes covering the cups containing plants. This condensation was included in our analysis. Because water was only observed on petri dishes covering the cups containing plants, the condensation is not due to evaporation from the sand, but rather transpiration from plant leaves. In the cucumber experiments, each water sample collected from the petri dishes contained perchlorate, whereas in the soybean experiments, none of the water samples contained perchlorate. Additional studies are needed to explain this process, but it appears that some plants can transpire perchlorate.

A perchlorate mass balance was calculated for each plant uptake experiment. In some experiments, there was an 80% loss of total mass by the end of the experiment. In other experiments, there was little perchlorate lost (<10%) during the study period (Fig. 2). The primary reason for differences in mass balance among the experiments seems to be excessive rinsing of dried samples. After drying, leaves and roots were rinsed with Milli-Q water before extraction to remove perchlorate attached to the external surface of plants. Rinse water was analyzed for perchlorate. If there was perchlorate present in the rinse water, the plant was rinsed again until no perchlorate was found in the rinse water. In some of the first experiments, plants were rinsed several times before extraction. Because of its high water solubility, perchlorate may migrate from plants to water during rinsing, thus removing perchlorate from plants and contributing to loss of total perchlorate mass. Therefore, experimental uncertainty was increased in data from the cucumber experiments in pure Hydrosol and pure water. Other variables that may also contribute to the loss of perchlorate include (1) plant-mediated transformation of perchlorate to chloride, and (2) expiration of perchlorate with transpiration water from plant leaves.

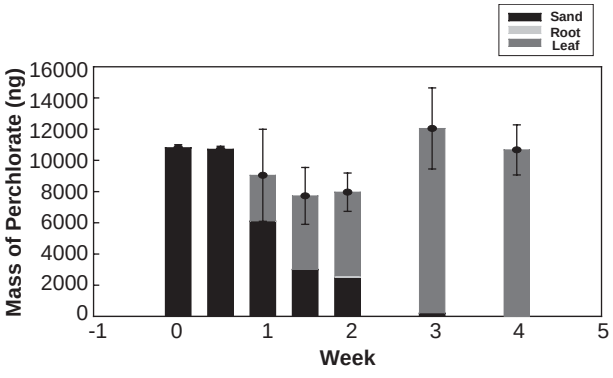

Fig. 2. Perchlorate distributions among soil, root, and leaves from a 4-week cucumber (*C. sativus* L.) perchlorate uptake experiment in the presence of Hydrosol:Milli-Q water (25:75). Error bars represent one standard deviation of total perchlorate.

Cucumber, lettuce, and soybean demonstrated their potential to take up perchlorate from contaminated sand. There was a significant perchlorate concentration burden for cucumber and lettuce. Results also suggest perchlorate depletion from sand, and subsequent uptake into leaves is strongly influenced by the presence of nutrients in the sand. Plant species also affected perchlorate accumulation; the highest perchlorate concentration was achieved in lettuce. Plants in perchlorate-contaminated areas or crops grown with perchlorate-contaminated water represent an important route of perchlorate exposure to higher organisms, including humans. However, external nutrients in soil appear to reduce the levels of perchlorate in plants.

Acknowledgments

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