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Plant uptake of environmental contaminants: applications in phytoscreening

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PLANT UPTAKE OF ENVIRONMENTAL CONTAMINANTS: APPLICATIONS IN PHYTOSCREENING

by

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ABSTRACT

Plants interact directly with their surroundings, extracting nutrients and water from the subsurface to support growth and development of the plant. Through the roots, plants also exude and uptake numerous chemicals. Many of the pathways can also be used by environmental contaminants to be translocated to above ground plant tissues. Such uptake of contaminants has proven useful in remediation and phytoscreening – the use of plants to delineate contaminant plumes. Sampling of trees at contaminated field sites has been used to identify areas of groundwater contaminated with a variety of chlorinated solvents. The use of plants as contaminant biosensors requires understanding of their interactions with the environment. Meteorological variables result in fluctuating water and contaminant fluxes through plants, manifested by seasonal trends in contaminant concentrations in tree trunks. While the application of phytoscreening for chlorinated solvents has been successful, numerous other organic contaminants may be candidates. Chemical properties such as hydrophobicity, molecular weight, and hydrogen bonding were shown to explain uptake of organic compounds by plants. Beyond organic compounds, potential exists for phytoscreening of inorganics. One example is perchlorate, a soluble oxyanion readily available to plant roots. A greenhouse study showed proportional response of tree sap perchlorate concentrations to dosing solution perchlorate. At a field site, perchlorate in tree cores generally reflected areas of groundwater perchlorate contamination. Collectively, phytoscreening is a low-impact, sustainable approach to plume delineation viable for a wide range of environmental contaminants.

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1. INTRODUCTION

1.1. EMERGING AND FUGITIVE CONTAMINANTS

Industrial development and anthropogenic activities have resulted in a fugitive legacy threatening the quality and quantity of the most fundamental needs of living things: water and food. While quality can be broadly defined, this dissertation focuses on sustainably assessing emerging and fugitive contaminants (EFCs). Human exposure to and biomagnification of the escalating number of EFCs are pervasive and public concerns are justified, as evidenced by EFCs in human breast milk and blood.[1, 2] The contamination of food and water are linked via plants, which sit at the base of many food chains. The potential contamination of food chains via EFCs in water is expected to rise over time due to concurrent irrigation with increasingly contaminated surface waters, irrigation with treated wastewater[3, 4] and growth in urban agriculture,[5] particularly on brownfields.[6]

1.2. CONTAMINANT UPTAKE & TRANSLOCATION BY PLANTS

While chemical transport in plants is not a new area of research, surprisingly little about how organic chemicals are transported into roots is known at a mechanistic level. Aboveground transport, such as particle deposition[7-9] and losses due to chemical volatility[10] have been widely studied and modeled. Plant active transport of ionic salts through transport proteins has also been widely studied for nutrients and contaminants. Others have studied contaminant transport and fate in various media, such as groundwater, sediment and air, leading to advances in understanding (e.g., bioavailability and bioaccessibility)[11].

Terrestrial plants have evolved intricate root tissues to provide support and optimize mass transfer of nutrients and water efficiently, even when scarce. To meet the latter requirement, roots must be permeable to solutes of interest, which can also allow a variety of pollutants to enter the root. Thus, the plant has the potential to translocate pollutants (i.e. EFCs).[12-14]

The translocation of compounds by plants is generally assessed using the transpiration stream concentration factor (TSCF)[15-17] – a measurement of a compound's ability to passively cross the root membrane.

$$\mathrm{TSCF} = \frac{C_{\mathrm{Shoot}}}{C_{\mathrm{Media}}}$$

Where:

C_{Shoot} is the aqueous chemical concentration in the plant shoot xylem

C_{Media} is the aqueous chemical concentration in the growing medium

The measurement of the TSCF is complicated by variations in experimental setup (i.e. pressure chamber vs. hydroponic tissue analysis), plant type, exposure duration and the use of radiolabelled compounds (measurements may include degradation products).[17] In addition, a variety of other concentration ratios are also reported in literature, such as the root concentration factor (RCF),[18] grass/soil accumulation factor (GSAF),[19] leaf concentration factor (LCF)[20] and stem concentration factor (SCF).[21] Some of these, such as the RCF, measure sorption processes rather than translocation.

1.3. MODELING CONTAMINANT UPTAKE BY PLANTS

Modeling chemical fate and transport in plants has generally followed two approaches: correlating the TSCF with hydrophobicity (octanol-water partitioning [log K_{OW}]) or compartment-based models. Simpler models relating the TSCF and log K_{OW} have generally demonstrated bell-shaped curves, where moderately hydrophobic compounds (log K_{OW} of 1-3) show the greatest uptake,[16, 17, 22, 23] although a recent paper described a sigmoidal model where significant uptake of hydrophilic compounds was observed.[17] Hydrophilic 1,4-dioxane is another example of a compound that better fits the sigmoidal model.[24] Additional chemical dimensions must be added to the assessment to better understand the transmembrane transport and translocation, as evidenced by poly-parameter linear free energy relationships (pp-LFERs) that better explain other environmental partitioning processes as compared to single-parameter log K_{OW} relationships.[25, 26]

Compartment-based models have greater flexibility, data output and accuracy, although they generally are overly parameter-intensive for screening a multitude of compounds.[27-34] These models rely on estimates of partitioning coefficients for the various compartments, which are generally considered to be dominated by lipid content,

although this has recently been scrutinized.[35, 36] In addition, these partitioning coefficients are generally one-parameter (log K_{OW}) relationships. Further complicating both TSCF measurements and modeling efforts is the transient nature of plant-contaminant system, although some recent modeling progress has been made.[37] In practice, these parameter-intensive models are best suited not for screening but for use as detailed fate and exposure assessment tools in compound- and site-specific applications where potential exposure is a known concern.

1.4. PLANT UPTAKE OF EMERGING CONTAMINANTS

Despite decades and billions of dollars in research, assessment and remediation, groundwater contaminants persist and are growing in diversity. A new, proactive approach is needed to sustainably address risk presented by emerging contaminants (ECs). The EPA has listed a number of compounds and compound classes as ECs,[38, 39] including 1,2,3-trichloropropane (TCP), perchlorate, 1,4-dioxane, 2,4,6-trinitrotoluene (TNT), dinitrotoluene (DNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), N-nitroso-dimethylamine (NDMA), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs).

Many of these compounds, such as PPCPs are prevalent in the environment, particularly in surface water and of concern in water reclamation and reuse.[40-42] Others, such as TCP and energetic compounds are frequently encountered in soil and groundwater.[43] In general, concerns have been raised about the long-term health effects of these compounds. [44] While these compounds have widely varying physicochemical properties, they can be recalcitrant both to typical treatment methods and in the environment.[45]

Uptake of ECs by plants has been recently investigated by several groups, often producing data and reaching conclusions that are difficult to compare due to differences in experimental setup and reported resultseven when the target ECs are similar. Yoo et al.[19] measured perfluoroalkyl acids (PFAAs) and fluorotelomer alcohols (FTOHs) in grasses, finding a negative correlation between the $\log_{10}$ transformed grass/soil accumulation factor (GSAF) and molecule chain length. Stahl et al.[46] reported linear

correlations between $\log_{10}$ concentrations of PFOS and PFOA in plants and in spiked soils. Felizeter et al.[47] found a positive correlation between chemical hydrophobicity and $\log_{10}$ transformed root concentration factor (RCF) for a variety of PFAAs. The small, but measurable, TSCFs were found to generally increase with hydrophobicity, with the exception of the more hydrophilic perfluorobutanoic acid (PFBA), which was found to have the largest TSCF (ca. 0.8).

PPCPs have also been incorporated by plants, as demonstrated by Calderon-Preciado et al.,[48] who found 51-99% removal of six PPCPs by lettuce and spath after a 30-day incubation period. Tanoue et al.[49] measured translocation in 10 of 13 pharmaceuticals dosed hydroponically to pea and cucumber. Moderately hydrophobic compounds, such as carbamazepine exhibited greatest translocation. Carbamazepine, triclosan and triclocarban are among the PPCPs most frequently reported found in aboveground plant tissues.[50-52] Boxall et al.[53] measured translocation of veterinary medicines by plants, but found no relationship with log K_{OW}. Eggen et al.[54] observed translocation of metformin, with seed concentration factors reaching 1.5 for turnip rape, but found minimal translocation of ciprofloxacin or narasin. These experimental studies do provide uptake information, but are quite resource-intensive.

Other emerging compounds have also been found in plant tissues. NDMA was removed by phytovolatilization and rhizodegradation in hydroponically grown willows and poplars.[55] Explosives such as TNT, RDX and HMX have been measured in plant tissues.[20, 56, 57] Li et al.[58] observed translocation of two brominated flame-retardants, tetrabromobisphenol A and hexabromocyclododecane, by cabbage and radish. The relatively limited conclusions are compound-plant-experiment specific and highlight the need for a more fundamental, high-throughput approach that can more efficiently predict and reproducibly measure plant uptake and translocation of future ECs, without the ambiguity of experimental design and reporting methods.

1.5. PLANT UPTAKE OF FUGITIVE CONTAMINANTS

Fugitive organic compounds contaminate countless field sites across the world. Many of these contaminants are suitable for phytoremediation via direct uptake by plant roots. Many constituents of petroleum products, such as benzene, toluene, ethylbenzene and xylenes (BTEX), have been shown to translocate in plants.[16, 59-61] Some pesticides

and herbicides may also translocate, such as atrazine.[62, 63] While the magnitude of contaminant translocation by plants is generally species-independent, some cucurbits can translocate hydrophobic contaminants such as dibenzo-*p*-dioxins.[64, 65] However, the majority of this dissertation will focus on plant uptake of chlorinated solvents, particularly tetrachloroethylene (PCE), trichloroethylene (TCE) and the reductive dechlorination product *cis*-1,2-dichloroethylene (cDCE). These contaminants were widely used as industrial solvents and in dry-cleaning in the 20th century.[66, 67] Denser than water, these compounds sink to the bottom of aquifers and partition into the groundwater at low concentrations due to their modest solubility (see Table 1.1). These compounds have also been widely reported to be translocated by plants.[68-73]

Table 1.1. Physiochemical properties for common chlorinated solvents[74]

Compound	*Molecular Mass (g/mol)*	*Density (g/cm^3)*	*Henry's Constant (dimensionless)*	*Log K_{OW}*
PCE	165.8	1.62	1.2	2.88
TCE	131.4	1.46	0.48	2.42
cDCE	96.9	1.27	0.22	1.86

1.6. PHYTOSCREENING

In the late 1990's, Vroblesky et al. determined that chlorinated solvent concentrations in trees could be used as an indicator of groundwater contaminated with chlorinated solvents.[75] This observation started the field of phytoscreening, which uses tree contaminant concentrations to delineate areas of contaminated groundwater. This method generally has low environmental impact, allows for rapid data collection and is particularly useful at forested sites.[12] Phytoscreening has been employed at numerous sites, generally providing useful delineation of groundwater plumes.[76-82] To measure chlorinated solvents in trees, a tree core is usually taken with an increment borer.[80] A variety of factors such as tree species, season, side of tree, recent rainfall, depth to groundwater and subsurface heterogeneities have been shown to affect the relationship between tree core and subsurface contaminant concentrations.[59, 76, 77, 79, 80, 83-88]

Phytoscreening has also been attempted for inorganics, such as heavy metals, but with poor performance, thought to be due to species-dependent factors.[89]

2. GOALS AND OBJECTIVES

The overall goal of this research was to advance the field of phytoscreening by better understanding the role of plant-environmental factors and through examining EFCs for phytoscreening potential.

- Objective 1: Evaluate the influence of tree-specific factors on chlorinated solvent concentrations in plants
    - Hypothesis: Chlorinated solvent concentrations in trees will be minimally affected by properties such as tree species and tree diameter.
- Objective 2: Assess the influence of weather on chlorinated solvent concentrations in trees
    - Hypothesis: Chlorinated solvent concentrations in trees will vary seasonally, depending on factors such as evapotranspiration.
- Objective 3: Develop a fundamental model to better predict uptake and translocation of organic contaminants
    - Hypothesis: Organic contaminant uptake and translocation can be better predicted with the inclusion of molecular descriptors such as hydrogen bonding and molecular weight in addition to hydrophobicity.
- Objective 4: Test the ability of phytoscreening for perchlorate
    - Hypothesis: Phytoscreening for perchlorate may be possible, but will likely be more influenced by tree-specific factors.

PAPER

I. PHYTOSCREENING WITH SPME: ANALYSIS OF VARIABILITY

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ABSTRACT

Phytoscreening has been demonstrated at a variety of sites over the past 15 years as a low-impact, sustainable tool in aiding delineation of groundwater contaminated with chlorinated solvents. Collection of tree cores is rapid and straightforward, but relatively low concentrations in tree tissues requires sensitive analytics. Solid-phase microextraction (SPME) is amenable to the complex matrix while allowing for solvent-less extraction. Here, we examine the potential for competitive sorption both in laboratory experiments and examination of field data. Through sampling and analysis of approximately 2,000 trees at numerous field sites, we also examine the effect of tree genus and diameter on measured contaminant concentrations. Collectively, while variables these variables were found to significantly affect site-adjusted perchloroethylene (PCE) concentrations, the magnitude of these effects were small. Analysis of replicate data showed no correlation with relative standard deviation (RSD) and wood type or tree diameter, with an overall median RSD of 30%. Collectively, these findings suggest SPME is an appropriate technique for analyzing chlorinated solvents in tree tissues.

INTRODUCTION

Over the past decade, phytoscreening has been used at numerous sites to aid in subsurface contaminant plume delineation, particularly for chlorinated solvents such as trichloroethylene (TCE) and perchloroethylene (PCE).[1-9] Phytoscreening can provide valuable data to aid in more accurate and more sustainable site assessments,[10, 11] although the contaminant concentrations measured in trees are an indirect measure of groundwater contaminant concentrations.

Phytoscreening relies on the assumption that in the field, local groundwater cVOC concentrations are correlated with nearby tree cVOC concentrations. However, some have implicated time of year,[12] tree diameter,[13, 14] tree genus[4, 5, 7] and tree physiology[15] as additional explanatory variables. These factors may affect contaminant uptake rates, loss rates, distribution *in planta*, and/or analytical quantification.

To measure chlorinated solvent concentrations in trees, various *in vitro* and *in planta* methods have been utilized, such as heated headspace,[3, 5, 7, 9] *in planta* passive sampling,[16] *in planta* portable gas-chromatography-mass spectrometry (GC-MS), and headspace solid-phase microextraction (HS-SPME).[2, 6, 16] SPME can yield lower detection limits, but may be subject to competitive sorption from the large number of volatile and semi-volatile compounds in trees. Both heartwood and sapwood contain numerous terpenoid compounds. Wajs et al.[17, 18] used SPME to measure mono- and sesquiterpenes in *Picea abies*, finding more than 100 volatile and semi-volatile compounds. SPME has been used by others to identify endogenous compounds emitted by plants.[19, 20]

In this paper, we characterize the extent of competitive sorption between tree compounds and cVOCs on SPME fibers. To further examine the potential for competitive sorption, tree core data gathered from several field sites are examined for competitive sorption. These field data are also used to examine the effect of tree diameter and genus on cVOC concentrations and replicate precision.

METHODS

All tree core samples were analyzed using HS-SPME coupled with gas chromatography and electron capture detection (GC-μECD). This method was developed

specifically for sampling tree cores for cVOCs using a polydimethylsiloxane (PDMS) fiber.[2] For use with a carboxen composite fiber (CAR/PDMS), the inlet temperature was raised from 230°C to 290°C to improve desorption of the microporous fiber. For both fibers, a tree core sample was placed into a 20-mL vial and the headspace was extracted for 5 minutes with the SPME fiber. Separation occurred on a 10-m long VOCOL column, ramped from 40°C to 160°C over 6 minutes. The SPME fiber was calibrated externally through equilibration with spiked water, allowing a xylem water concentration to be reported. The method was particularly sensitive for TCE and PCE, with detection limits in the low ng/L range.

For analysis of benzene, toluene, ethylbenzene and xylenes (BTEX), a similar method was used.[21] Briefly, a CAR/PDMS fiber was desorbed at 290°C into a 30-m long HP-5 column, followed by detection by flame ionization.

To test the potential for competitive sorption on the PDMS and CAR/PDMS fibers, tree cores were obtained from three tree genera at Vienna, MO. The genera included oak (*Quercus*), elm (*Ulmus*) and cedar (*Juniperus virginiana*). Three cores were taken from three different specimens of each genera using an increment borer[3] and contained no detectable levels of cVOCs. All cores contained both sapwood and heartwood to best resemble a typical core sample. The genera were selected to span a wide range of aromatics found in trees. The elm wood contained comparatively few aromatics, while the cedar contained numerous mono- and sesquiterpenoid compounds.

For each wood core, the SPME fiber first sampled a water standard spiked with 80 ng/L PCE, 1.8 µg/L TCE and 120 µg/L cDCE to determine the baseline response. The fiber was then exposed to a clean wood core for 5 minutes, but was not desorbed. Instead, the fiber then sampled a water standard as before. If no competitive sorption were occurring, the response of the fiber was expected to remain near the baseline. Competitive sorption would be expected to reduce the response of the fiber. All data were analyzed used repeated measures analysis of variance (ANOVA) through proc GLM in SAS 9.2 (SAS Institute, Cary, NC).

Phytoscreening was performed at 39 sites in Europe and North America from 2009-2013, sampling a total of 1,913 trees for cVOCs. When possible, tree diameter and genus were recorded. Diameters are available for 1,539 samples, ranging from 4 to 168

cm, while tree genus is available for 1,477 samples. Only genera containing a minimum of 10 detections were considered in the statistical models. Genera were classified into different wood types: conifer, ring-porous and diffuse-porous.[22] All samples were analyzed using either the PDMS or CAR SPME fiber as described above.

Groundwater data were unavailable at the necessary resolution to explore their correlation with tree core cVOC concentrations. Instead, tree diameter, tree genus and wood type were taken as explanatory variables for analysis by ANOVA in SAS 9.2 using proc GLM (SAS Institute, Cary, NC). Of the cVOCs, only PCE and TCE were considered in the statistical analysis due to the limited number of detections for other compounds. PCE and TCE concentrations were site-normalized by subtracting the site contaminant geometric mean from the $\log_{10}$ of the cVOC concentration. This transformed concentration was used as the dependent variable. Linear regression was performed using proc REG in SAS after taking the square root of tree diameter to stabilize residual variance.

To explore precision of the tree core samples, duplicate samples were generally obtained every 10-20 samples 1-3 cm away from the previous tree core. Relative standard deviations (RSD) were calculated from each duplicate and measured contaminant. Two explanatory variables – tree diameter and wood type – were examined in SAS. Only compounds with greater than 30 observations were considered in the analysis (i.e., chloroform [CF], TCE and PCE). A Box-Cox transformation was used to improve the normality of the RSDs (proc TRANSREG). The optimum lambda values were 0.5, 0.25 and 0.25 for CF, TCE and PCE, respectively. The effect of both explanatory variables was tested using proc GLM with the transformed dependent variable.

RESULTS AND DISCUSSION

No substantial drift in baseline response was observed over time, indicating the desorption time and temperature was sufficient to clean the fiber. The elm tree showed the least amount of influence on the fiber, with percent change values generally close to, or above, zero. Both oak and cedar exhibited mild competitive sorption (see Figure 1). This interaction between tree genus and competitive sorption was significant for TCE and

the PDMS fiber and PCE and the CAR fiber (see Table 1). The PDMS fiber exhibited significant competitive sorption for both cDCE and TCE.

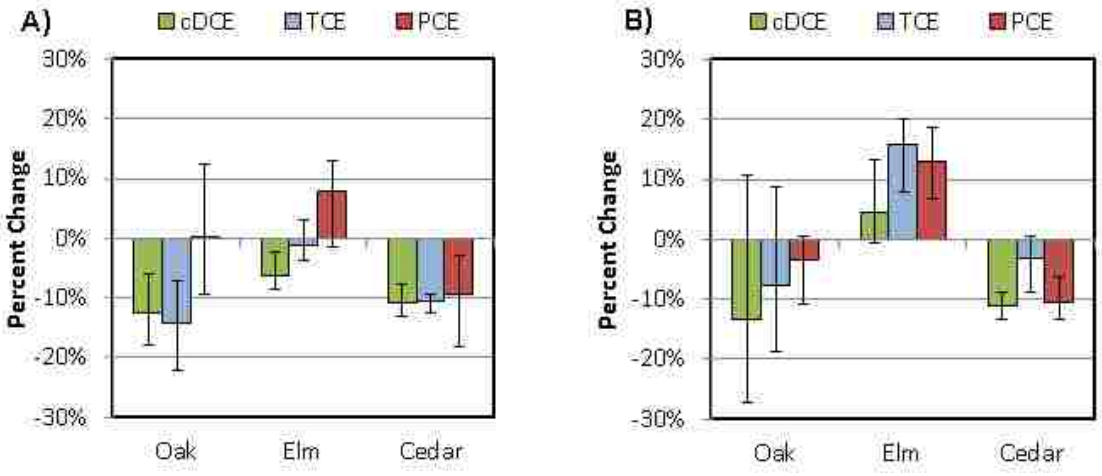

Figure 1. Competitive sorption of cVOCs and tree cores on A) PDMS and B) CAR/PDMS SPME fibers. Percent change characterizes the reduction in GC response after exposure to the corresponding tree core. Error bars denote the range of three samples.

Table 1. p-values from repeated measures ANOVA

Fiber	Compound	Tree*Comp	Comp	Tree
PDMS	cDCE	0.27	0.0005**	0.092
	TCE	0.049*	0.0022**	0.13
	PCE	0.14	0.78	0.35
CAR	cDCE	0.25	0.15	0.42
	TCE	0.068	0.81	0.19
	PCE	0.004**	0.60	0.99

*Significant at α=0.05
**Significant at α=0.01

Using the SPME method, TCE was detected in 509 (27%) of samples while PCE was detected in 1,056 (55%) of samples. Due to limited analytical sensitivity, cDCE was only detected in 75 (4%) of samples. Concentrations spanned several orders of magnitude, as shown by Figure 2. The maximum concentrations recorded were 110 µg/L,

880 µg/L, and 2.6 mg/L for PCE, TCE and cDCE, respectively. These concentrations are far below the aqueous solubility of PCE, TCE and cDCE (140 mg/L, 1.1 g/L and 5.1 g/L, respectively),[23] which is likely the result of several factors. First, the tree is likely not exclusively translocating water saturated with chlorinated solvents. Second, loss mechanisms such as phytovolatilization from the trunk are likely reducing trunk concentrations.[24] In addition, toxicity may reduce the uptake of highly contaminated water. Chlorinated ethene concentrations able to reduce transpiration by 50% are approximately an order of magnitude lower than the solubilities listed above.[25, 26]

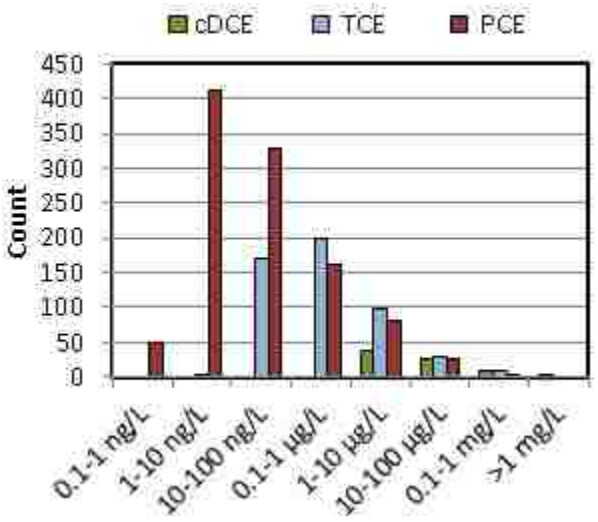

Figure 2. Histogram of tree core concentrations. Note that data are left-censored by the method detection limit.

Forty different genera of trees were sampled at the field sites, although six genera (*Acer*, *Fraxinus*, *Liquidambar*, *Populus*, *Quercus* and *Ulmus*) represented approximately 2/3 of the samples (see Table 2).

Table 2. cVOC detections by genus

Genus	Trees Sampled	cDCE Detections	TCE Detections	PCE Detections	Wood Type
Abies	4	0	3	4	Conifer
Acer	202	0	44	74	Diffuse-porous
Aesculus	6	0	6	6	Diffuse-porous
Ailanthus	2	0	0	2	Ring-Porous
Albizia	2	0	0	0	
Alnus	3	0	0	3	Diffuse-porous
Bauhinia	5	0	0	5	
Betula	17	0	0	15	Diffuse-porous
Carpinus	3	0	3	3	Diffuse-porous
Carya	11	0	2	1	Ring-Porous
Catalpa	7	0	0	3	Ring-Porous
Cedrus	8	1	2	8	Conifer
Celtis	12	0	3	6	Ring-Porous
Cercis	5	0	0	0	
Cornus	1	0	0	0	Diffuse-porous
Corylus	1	0	1	1	Diffuse-porous
Cupressus	4	1	3	4	Conifer
Elaeagnus	0	0	0	0	
Eucalyptus	8	0	0	8	
Fagus	12	0	2	11	Diffuse-porous
Fraxinus	126	0	34	80	Ring-Porous
Gleditsia	2	0	0	0	Ring-Porous
Juglans	43	0	8	22	Ring-Porous
Juniperus	84	0	0	60	Conifer
Liquidambar	69	4	25	27	Diffuse-porous
Melia	1	0	0	0	
Morus	9	0	0	3	Ring-Porous
Ostrya	1	0	1	1	Diffuse-porous
Picea	2	0	2	2	Conifer
Pinus	30	2	6	14	Conifer
Platanus	43	0	18	41	Diffuse-porous
Populus	174	36	107	152	Diffuse-porous
Prunus	33	0	4	8	Ring-Porous
Pyrus	1	0	1	1	
Quercus	272	7	72	131	Ring-Porous
Robinia	1	0	1	1	Ring-Porous
Salix	62	12	40	51	Diffuse-porous
Sequoia	1	0	0	1	Conifer
Taxodium	20	6	12	13	Conifer

Tilia	25	0	5	16	Diffuse-porous
Triadica	1	0	0	1	
Tsuga	33	0	2	32	Conifer
Ulmus	131	0	11	36	Ring-Porous
Total Conifers	186	10	30	138	
Total Ring-Porous	649	7	135	293	
Total Diffuse-Porous	619	52	252	401	
Total	1477	69	418	847	

ANOVA revealed genus significantly affected PCE concentration ($p=0.0012$), but not TCE concentration ($p=0.058$). Mean comparisons performed using Tukey adjustment showed *Platanus* had significantly higher PCE concentrations as compared to *Acer* ($p<0.001$), *Betula* ($p=0.0011$), *Populus* ($p=0.027$), and *Salix* ($p=0.0095$). TCE concentrations were higher for Platanus as well, but the trend was not statistically significant. Both PCE and TCE were detected in *Platanus* with greater frequency than average, with PCE detected in 41 of 43 *Platanus* trees (95%) and TCE detected in 18 of 43 *Platanus* trees (42%). This statistical observation is hypothesized to result from *Platanus* preferentially growing in areas with shallow groundwater. No other significant genera relationships were observed, so *Platanus* was represented by a binary flag in regression analysis.

Both xylem type and tree diameter significantly affected adjusted PCE concentrations ($p=0.021$ and $p=0.016$, respectively). TCE concentrations were not affected by either xylem type ($p=0.19$) or tree diameter ($p=0.55$). Mean comparisons for PCE xylem type using Tukey adjustment revealed diffuse-porous wood was significantly greater than ring-porous wood ($p=0.025$), so a binary flag was used to identify trees containing diffuse-porous wood. The two flags and tree diameter were used to develop a multiple linear regression model. All three variables significantly and positively affected tree PCE concentrations (see Table 3). However, these three variables explained a small proportion of the data variance, with an adjusted R^2 of 0.031.

Table 3. Regression statistics for explanatory PCE model

Variable	*Parameter Estimate*	*p-Value*
Intercept	-0.369	0.003
Platanus Flag	0.611	0.002
Diffuse-Porous	0.198	0.006
√Diameter	0.0477	0.016

The minimal amount of variation explained by tree diameter, genus and wood type implies these variables have a minor effect on tree cVOC concentrations. This statement assumes a sufficiently large sample of trees has been taken such that trees of different genera and diameters appear randomly distributed across the collective contaminated sites. This assumption is more valid for tree genera with numerous samples, such as oak and ash. The analysis also assumes that any single tree genus at one site behaves similarly at another site. For the purposes of competitive sorption and wood composition, this is likely a valid assumption. However, traits such as rooting depth are also function of the soil type,[27, 28] a factor not well captured by this analysis.

In addition to the PCE, TCE and cDCE, other volatile compounds were also observed in trees, although at lower frequency. These compounds include 1,1-dichloroethene, dichloromethane (DCM), CF, carbon tetrachloride, bromodichloromethane (BDCM), 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1,2-trichlorotrifluoroethane (CFC-113), 1,2-dichlorobenzene, 2,2,2-trichloroethanol and BTEX. Other analytic methods, such as the CAR/PDMS SPME fiber, may be more sensitive for many of these analytes.

The relatively wide range of compounds measured in plants allows comparison with physicochemical properties. Figure 3 and Figure 4 show the relationship between median tree contaminant concentration and hydrophobicity and air-water partitioning, respectively. Note that in these figures, the lower error bar is largely a function of the method detection limit (MDL). The MDL also influences the median value as the data distribution is strongly skewed towards lower values. In the figures, both the median and 95th percentile values appear negatively correlated with hydrophobicity over the range studied. The relationship between maximum tree concentration and Henry's constant is

less obvious, but a decreasing upper bound is evident, particularly for low values of Henry's constant. In both plots, larger circle correlate to a larger number of detections. 95th percentile values for larger samples should be considerably closer to the population value and should be considered more reliable estimates. As a point of reference, for several contaminants the 95th percentile values exceed the corresponding maximum contaminant level (MCL) for drinking water.[29]

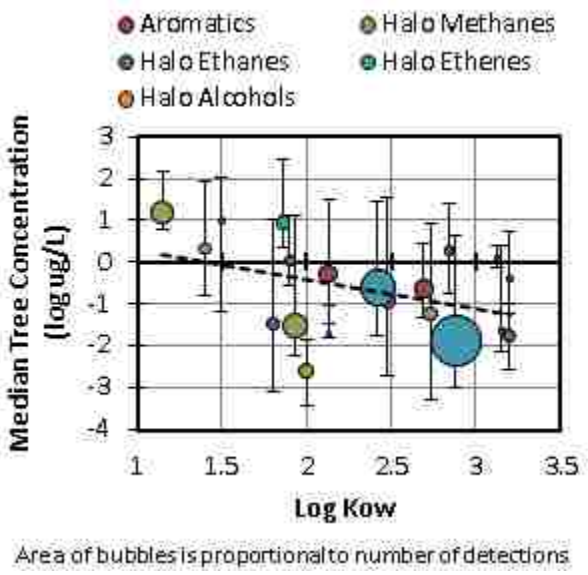

Figure 3. Correlation between hydrophobicity and tree contaminant concentrations. Error bars denote the 95th and 5th percentile values measured.

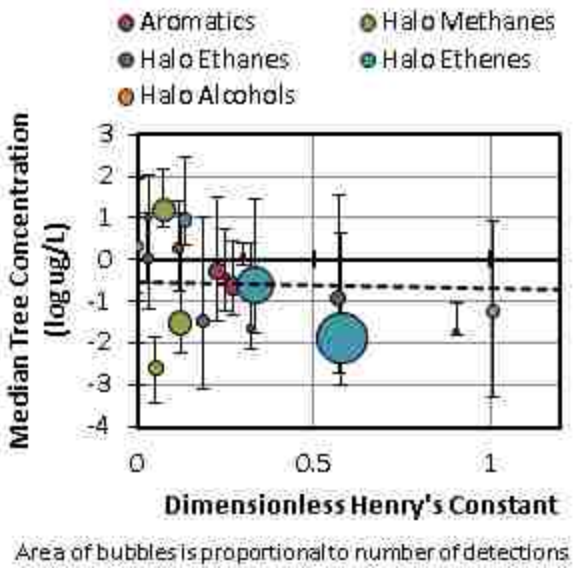

Figure 4. Correlation between Henry's constant and tree contaminant concentrations. Error bars denote the 95th and 5th percentile values measured. CFC-113 included, but not shown for clarity (K_H ca. 12).

From duplicate tree core sampling across the field sites, 230 RSDs were analyzed to examine the effects of tree type and tree diameter. The overall median RSD was 30%, with the 95th percentile RSD of 137% and the 5th percentile RSD of 2.6%. Statistical analysis of CF, TCE and PCE revealed neither tree diameter (p=0.73, 0.14, 0.13, respectively) or wood types (p=0.94, 0.20, 0.08, respectively) were significant explanatory variables. Figure 5 shows the RSD summary by wood type.

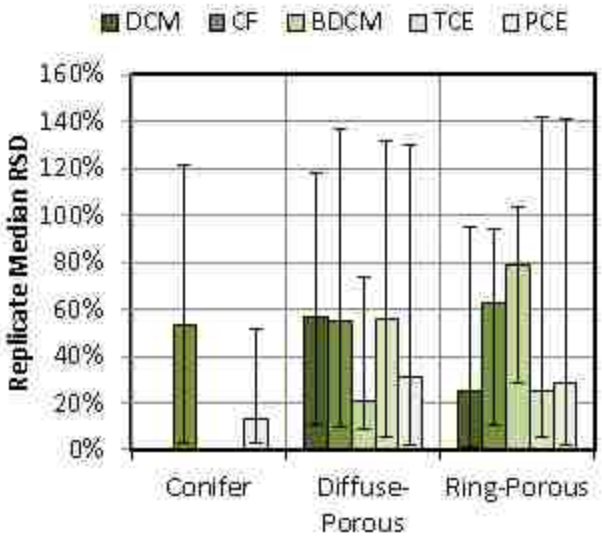

Figure 5. Effect of wood type on replicate RSD. Only wood type-chemical combinations with five or more measurements are shown. Error bars denote 95th and 5th percentiles.

These results reveal that the type of tree sampled does not significantly affect the analytic precision. We initially hypothesized that conifers may have different precision from the deciduous woods due to their differing structure and chemical content. Conifer wood, constructed of smaller tracheids, is more homogenous than angiosperm wood, potentially allowing for increased replicate precision. However, conifer wood also contains a greater abundance and diversity of terpenoid compounds, potentially creating matrix interferences. While the PCE RSD data suggest a trend towards lower RSD for conifers, the trend is not significant and few other compounds possess enough samples to statistically test this hypothesis further. Of the 220 RSD measurements, only 30 belong to conifers.

Collectively, these findings suggest that SPME is a suitable tool for *in vitro* phytoscreening across numerous tree species. While some matrix effects are distinguishable in laboratory-controlled experiments, the magnitude of these effects is much less than the typical range of cVOC concentrations observed at a site. However, these findings cannot address the variability in cVOC concentrations resulting from environmental factors such as seasonality and soil type.

ACKNOWLEDGEMENTS

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II. CHLORINATED SOLVENTS IN TREES: SEASONAL VARIATIONS IN CONCENTRATIONS

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ABSTRACT

Long-term monitoring (LTM) of chlorinated solvent plumes is costly and time-consuming, particularly at phytoremediation sites. The use of trees as sensors of groundwater contamination (i.e., phytoscreening) has been widely described, although the use of trees to provide long-term monitoring of such plumes (phytomonitoring) has not been well described or utilized, largely due to unexplained sources of variability. To help explain this variability we developed an *in planta* sampling method to obtain high-frequency measurements of chlorinated ethenes in trees. The dataset reveals that these contaminant concentrations rise with transpiration in the spring and decrease in the fall. Recent rainfall provided negligible dilution of contaminant concentrations in trees. These data begin to explain the seasonal variations of contaminant concentrations observed in trees, providing a foundation to advance the implementation of phytomonitoring.

INTRODUCTION

Sites contaminated with chlorinated solvents (cVOCs) often require long-term monitoring (LTM), a time-intensive and costly aspect of site cleanup. The Department of Defense (DoD) estimated their LTM costs at more than $100 million annually.[1] Although some LTM optimization has occurred,[2] LTM still often relies on labor-intensive monitoring wells for measuring groundwater contaminant concentrations. To measure groundwater cVOC concentrations passive sampling approaches have emerged as a low-energy alternative to active sampling of monitoring wells. In general, passive sampling has the added benefit of measuring an average concentration over the deployment period in addition to measuring the bio-available concentration of the contaminant.[3]

Phytoforensics combines passive sampling and solar-powered groundwater pumping to understand groundwater contaminants using contaminant concentrations found in plants growing above plumes.[4] The high sorption capacity of wood[5] for many organic contaminants allows for passive sampling of a tree's transpiration stream. Solar-powered groundwater removal by trees makes phytoforensics a relatively sustainable, but indirect, method for groundwater analysis. In one phytoforensic approach, termed phytoscreening, shallow cVOC plumes have been delineated using cVOC concentrations found in trees.[6-8] Additionally, when combined with traditional groundwater data, tree phytoscreening has been shown to reduce uncertainty in the conceptual site model.[9]

Similar to phytoscreening, phytomonitoring is a phytoforensic approach to long-term monitoring. In practice, concentrations in trees could be monitored yearly by taking tree cores or inserting a passive sampling device into the tree.[10, 11] Such a practice would be ideal for sites employing phytoremediation, as LTM requirements may be longer in duration and sampling points are readily available. Phytomonitoring also provides direct evidence of phytoremediation efficacy. Previous studies have suggested phytomonitoring as a low-impact alternative to traditional methods, but have also highlighted potential limitations.[12] Recent rainfall has been reported to decrease contaminant concentrations in the tree, as uncontaminated surface water may be transpired by the tree.[12, 13] Contaminant concentrations in trees have also been shown to fluctuate seasonally,[14] as uptake of cVOCs from the groundwater is dependent on the amount of transpired water and on the

fraction of groundwater transpired, while the loss of contaminants is a diffusive process through the bark and leaves.[15]

The effect of seasonality on tree cVOC concentrations has been demonstrated at field sites, albeit at low temporal resolution. Sorek et al. observed TCE concentrations approximately one order of magnitude greater in the summer than in the winter for rosewood (*Dalbergia sisso*, 46 cm in diameter) and laurel fig (*Ficus microcarpa*, 37 cm in diameter) sampled during five different months.[6] Such seasonal variations have been reported for baldcypress (*Taxodium distichum*),[8] cottonwood (*Populus deltoides*)[16] and Russian olive (E*laeagnus angustifolia*)[16]. However, TCE concentrations in an oak (*Quercus* sp., 40 cm in diameter) and pine (*Pinus* sp., 68 cm in diameter) tree were largely unchanged between August and November.[17, 18] The application of phytoscreening using datasets over time has also been hindered by the large amount of temporal variability. At a phytoscreening site where sampling occurred during a summer event and a fall event, Wahyudi et al. noted that temporal variability accounted for 83% of the total variability in tree core chloroethene concentrations.[19] Despite such variability, active remediation techniques at this site likely precluded statistically significant differences between growing and non-growing season tree core chloroethene concentrations.[20] To better understand the effects of such variables described above, a larger dataset was needed. Here we detail the collection of such a dataset and describe the effects of various explanatory variables. Without detailed understanding of these variables' effects on tree cVOC concentrations, substantial uncertainty will limit the value of phytomonitoring data.

METHODS

Traditional methods of tree sampling for cVOCs remove a small core sample from the tree, which is later analyzed by gas chromatography (GC).e.g., [17] This method is generally unsuitable for this study, as an excessive number of cores would be removed from the tree to obtain high frequency (i.e., weekly – monthly) temporal data, severely damaging the tree and introducing variability in the sampling. To address this concern, *in planta* solid-phase microextraction (SPME) was utilized to repeatedly sample individual trees.[21] SPME is a solvent-less extraction technique that easily interfaces with GC[22] and

is particularly appropriate for headspace sampling of VOCs,[23] as high diffusion coefficients in air lead to rapid equilibrium. For comparison to the *in planta* SPME method, tree cores were taken approximately 1 – 2 per year and analyzed used headspace SPME.

In planta sampling required construction of a resealable sampling port. The sampling port was constructed from a brass pipe fitting, a 3/8-inch hex plug, with a 70-gauge hole (0.71 mm diameter) drilled through. The hole was plugged with 22-gauge stainless steel wire (0.64 mm diameter) when not in use (see Figure 1). This small amount of clearance restricted diffusive flux through the port to the same order of magnitude of diffusive flux escaping from a similar area of the tree.[24] To create sealed headspace in the tree, 19/32-inch (15 mm) holes were drilled to a depth of 1/3 the tree radius. The ports were approximately 1 meter above the ground surface.

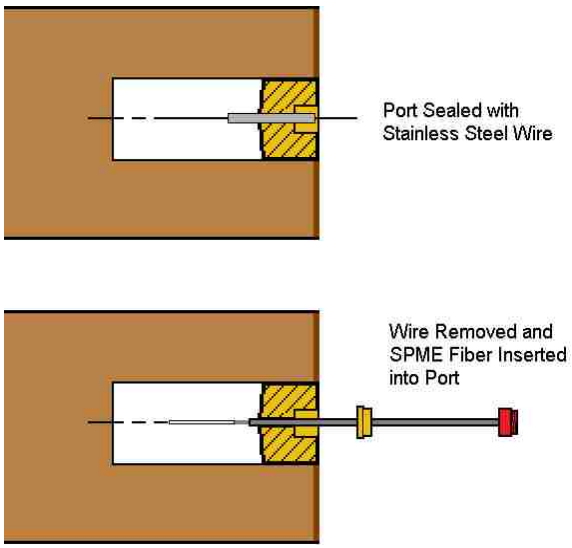

Figure 1. Schematic of SPME sampling port and *in planta* sampling process.

For *in planta* SPME sampling, a 100-µm polydimethylsiloxane (PDMS) fiber was used. PDMS has high affinity for non polar compounds[25] and has been used previously to measure cVOC concentrations in trees.[7, 26] Five-minute extraction periods were sufficient

to reach equilibrium at typical sampling temperatures, although below 5°C the extraction time was extended to six minutes.

The SPME fiber was desorbed into a 7890 Agilent GC with a VOCOL® column (Supelco). Complete GC conditions, calibration procedure and method detection limits have been described previously.[7] Calibrations were performed approximately monthly using 10-mL water standards in 20-mL vials spiked with cVOCs. Such a calibration allowed direct calculation of cVOCs in the transpiration stream assuming a local equilibrium existed.

cVOC PDMS:air partitioning coefficients are highly dependent on temperature, complicating field sampling. Partitioning into the fiber is an exothermic process, leading to increased PDMS:air partitioning coefficients at lower temperatures. However, a majority of the heat of partitioning results from the heat of condensation as compared to the heat of mixing.[27] Therefore, if the enthalpy of mixing is neglected, the effect of temperature on water-fiber partitioning is negligible, which is the approach taken here.

To estimate tree transpiration, daily reference evapotranspiration (ET_0) was calculated using the procedure described by the Food and Agriculture Organization (FAO) of the United Nations.[28] ET_0 estimates the amount of water evapotranspired by a hypothetical grass reference crop and has been shown to be directly proportional to sap flux in isolated trees.[29] ET_0 is driven by the solar radiation, R_n, and other factors such as vapor pressure deficit, wind, humidity, temperature and pressure. Meteorological data were obtained from nearby weather stations,[30, 31] and calculation details are described further in Appendix A and FAO document.[28]

Calculated ET is only realized if sufficient water is available for uptake and functioning leaves can evaporate the water. At this study's field site, groundwater was 1-2 meters below the ground surface, so the trees were assumed to have ample water supply. However, during a drought year of 2013, several trees exhibited water stress. During the period of leaf drop or leaf emergence, ET was linearly scaled from 100% to 0% of ET_0 by visual inspection of the trees.

Schuman Park in Rolla, MO was used as the field site for this research. The park is immediately down-gradient from a former dry cleaning site contaminated with tetrachloroethylene (PCE) and trichloroethylene (TCE). The reductive dechlorination

product, *cis*-1,2-dichloroethylene (cDCE) is also present at the site. The park contains a number of baldcypress (*Taxodium distichum*) and northern red oak (*Quercus rubra*) trees. Additionally, the site is approximately a quarter-mile from the Missouri S&T Environmental Research Center analytical labs, allowing field sampling without use of a portable GC. Groundwater at the site is shallow, <2 meters below ground surface near the trees and is contaminated with low, but steady, levels of PCE and TCE. In the nearest monitoring well (see Figure 2), quarterly groundwater monitoring data revealed concentrations of PCE ranged from 0.17 to 0.96 mg/L and TCE ranged from 29 to 158 µg/L (4/2009 to 8/2011).

Figure 2. Plan view of the field site showing the five sampled trees. The red ellipse denotes the likely source area. Groundwater flow is towards the park to the east.

Sampling at the field site occurred weekly to monthly beginning June 2010 and is reported here through January 2014. Note that some trees contained additional ports for replication purposes. Ports were occasionally retired due to a continual presence of sap filling the port. The likelihood of a port filling with sap appeared to individual specific. Every several months tree tissue was drilled away from the surface of the port to prevent the tree from healing over the port. Ports were not moved within the xylem, allowing the

port to gradually sample deeper into the xylem. Details regarding the sampled trees can be found in Table 1.

Table 1. Ports and trees sampled at field site

Port Number	*Tree Number*	*Tree Type*	*Diameter (cm)*	*Sampling Period*
1	1	Baldcypress	48	6/2010-10/2012
2W	2	Baldcypress	51	6/2010-1/2014
2N				6/2010-1/2014
2E				12/2012-1/2014
3	3	Northern Red Oak	44	6/2010-11/2012
4	4	Northern Red Oak	36	6/2010-1/2014
5S	5	Baldcypress	48	10/2011-1/2014
5N				1/2013-1/2014

Sampling any single port could be accomplished in approximately 20 minutes. After sampling, the SPME fiber was retracted and capped with Teflon during the five-minute transport to the GC. While transporting the capped SPME fiber, losses were reduced by placing the capped SPME fiber into a cooler containing ice.

RESULTS & DISCUSSION

Concentrations of cVOCs in the trees were found to vary in a seasonal manner. Figure 3 shows the seasonal variability in cVOC concentrations in Port 2W. Also shown is the method quantitation limit (MQL) for TCE, defined as 10 times the method detection limit. The MQL for PCE is approximately an order of magnitude lower than the MQL for TCE (not shown). Concentrations of cDCE remained near the MQL throughout the experiment and will not be discussed further. For visual comparison, the figure also includes an 11-day centered moving average of ET_0. The filled circles denote concentrations measured in tree core samples.

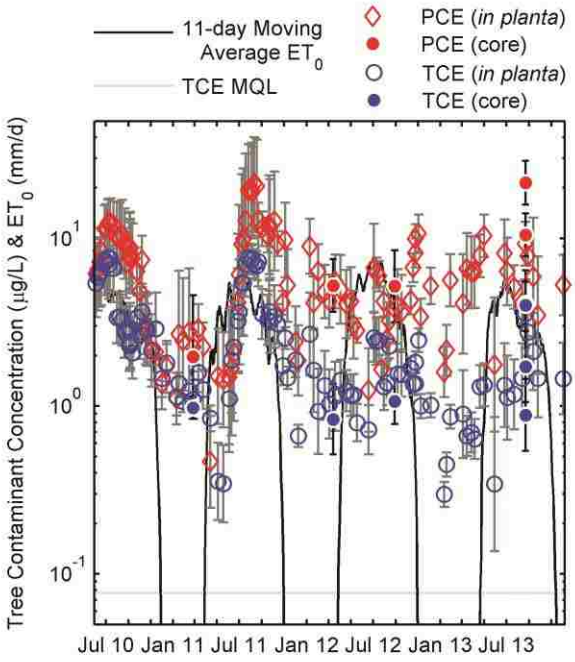

Figure 3. Seasonal variation in Port 2W cVOC concentrations. Error bars denote the 95% prediction interval resulting from error in the calibration curve.

The data shown in Figure 3 show a strong increase in PCE and TCE concentrations following the commencement of transpiration as estimated by ET_0. The increase in contaminant concentration is less pronounced during the summer of 2012, thought to be a result of a severe drought that year. Total precipitation in 2012 was 42 cm less than the annual average of 122 cm. Such an effect may also result from the port's location in the tree. Sampling ports were not moved, but rather sampled the same xylem, which progressed from sapwood-dominated to heartwood-dominated over years.

Sampling Port 2N, 2E, 5S and 5N, exhibited a very similar trend as shown in Figure 3 (see Appendix A). Concentrations of PCE and TCE increased as ET_0 increased during leaf emergence in the spring. Contaminant concentrations increased until ET_0 dropped in late summer. As ET_0 dropped, contaminant concentrations reduced during fall, while remaining nearly constant over winter. Some variability was observed between replicate ports in the same trees, although such variability has been previously documented.[32]

Concentrations of cVOCs in trees 1, 3 and 4 were much lower, making identification of clear trends more difficult. Figure 4 shows the seasonal variation of PCE in Port 4 (TCE not detected). Fluctuations in concentrations were less pronounced than in Tree 2, but a similar trend exists. Concentrations of PCE were highest in the summer and corresponded to periods of peak ET_0. Concentrations in winter appeared relatively constant. Figure 4 also shows a unique spike in PCE concentration on 8/9/2013, which occurred after a period of unusually heavy rain. Between the measurements on 8/3/2013 and 8/9/2013, 209 mm of rain fell, with 117 mm falling on 8/7/2013. The substantial rainfall led to widespread flooding at the field site, with water levels approximately ½ meter above ground surface near the lake. We hypothesize that the heavy rainfall displaced an area of highly contaminated water or non-aqueous phase liquid (NAPL) towards the roots of Tree 4, resulting in the substantial spike in PCE.

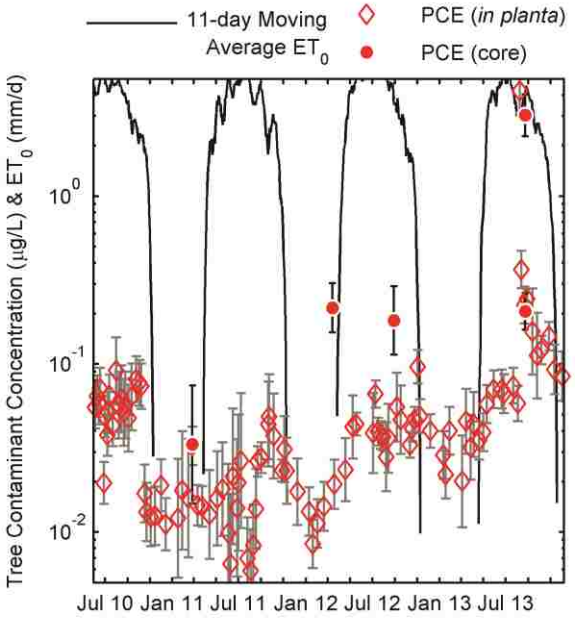

Figure 4. Seasonal variation in Port 4 cVOC concentrations. Error bars denote the 95% prediction interval resulting from error in the calibration curve.

The general correlation between transpiration and concentration was anticipated given previous experimental and field work. Nietch et al used a mesocosm experiment to demonstrate that Baldcypress tree transpiration was correlated to TCE flux from the system.[14] Tree cores collected seasonally from contaminated field sites have exhibited similar seasonal trends to the data presented here, with contaminant concentrations highest during seasons of greatest transpiration.[17] The collective dataset is shown in Figure 5, which includes all ports in Trees 2, 4 and 5. For clarity, a 3-point centered moving average of measured concentration data is shown for both PCE and TCE while ET_0 is an 11-day centered moving average. The baldcypress data (upper lines) show a similar seasonal pattern, with cVOC concentrations climbing during summer. This climb appears to coincide relatively close or slightly after the onset of transpiration. A slight retardation would not be unexpected due to the hydrophobicity of the contaminants. However, a more likely explanation may be errors in approximating actual transpiration by ET_0. The oak PCE response (lowest line) deviates substantially from the baldcypress response. A number of plausible explanations exist, such as an inability of the port to accurately measure cVOC concentrations in ring-porous oak. The ring-porosity of the oak may also affect the seasonal fluctuations in cVOC concentration, as the wood has considerable anisotropy and water is largely conducted in the outermost ring(s).

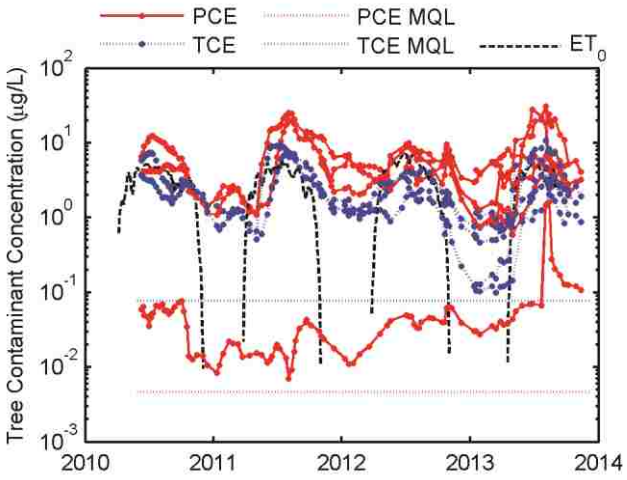

Figure 5. Concentrations of PCE and TCE for six ports installed in 3 trees. Data are a running average spanning 3 measurements.

The loss of cVOCs during fall and winter has been widely attributed to volatilization from the trunk.[8, 15, 33-35] Contaminant degradation *in planta* also represents a potential loss mechanism,[36, 37] although degradation rates have not been measured at field scale. Several authors have estimated diffusional loss rates. For a 40-cm diameter tree contaminated with TCE, half-life due to volatilization was calculated to be 37 days at 20°C using the Fruit Tree model, which assumes contaminants in the trunk are well-mixed.[20, 38] At a temperature of 0°C, the half-life increased to 102 days due to increased TCE partitioning to condensed phases. An alternative approach models diffusional losses during the winter using a cylindrical model, initially at a uniform concentration diffusing out to a clean atmosphere. This model is appropriate when ET_0 is minimal during the winter, resulting in a diffusion-dominated process. This diffusion problem was solved by Crank, resulting in the following equation:[39]

$$\frac{C - C_1}{C_0 - C_1} = 1 - \frac{2}{a}\sum_{n=1}^{\infty} \frac{\exp(-D\alpha_n^2 t) \cdot J_0(r\alpha_n)}{\alpha_n \cdot J_1(a\alpha_n)}$$

Where:

C is the concentration at radius r

C_1 is the initial concentration

C_0 is the ambient concentration

a is the cylinder radius

D is the analyte diffusivity in the cylinder

α_ns are the roots of $J_0(a\alpha_n) = 0$, the Bessel function of the first kind and order zero

Assuming the port measures the contaminant concentration at 90% of the tree radius and using wood diffusion coefficients from literature,[24] diffusional losses are estimated to be minimal during the winter (half-life ca. 1 year for TCE). However, this calculation is quite sensitive to the chosen radius and diffusivity.

In the observed data, cVOC concentrations drop more rapidly in early fall, indicative of higher diffusion out of the tree, possibly from warmer temperatures, higher

trunk permeability or the presence of convection. Recent research has shown that tree trunk permeability increases during the growing season to allow vapor transport of respiratory compounds such as CO_2, O_2 and water vapor.[40] This seasonal fluctuation in trunk permeability may explain the sharp drop of cVOCs in the trunk as ET_0 decreases in the fall as well as the subsequent nearly constant concentration of cVOCs during the winter months, when trunk permeability is comparatively low.

Recent rainfall has been reported to decrease tree cVOC concentrations resulting from the uptake of less-contaminated rainwater. This phenomenon was best demonstrated by Vroblesky et al., through an artificial irrigation experiment.[12] After a simulated 50-mm rain, TCE concentrations in a 75-cm diameter eastern cottonwood (*Populus deltoides*) tree dropped by 20-30% when measured 1-3 days post-irrigation. The effect of recent rainfall at this field site was assessed using two different predictor variables: total rainfall between SPME measurements and total rainfall during the day prior to sampling. The $\log_{10}$ of the total rainfall data was taken to stabilize the variance for regression analysis. Neither approach was significantly associated with changes in cVOC concentration between sampling events (all regression p-values >0.25; see SI for regression plots).

The lack of a significant concentration change due to recent rainfall, while not supportive of the findings of Vroblesky et al.,[41] is explainable by the large amount of chemical mass partitioned to the wood tissue. Assuming a typical xylem unit contains 1 g of dry wood, 1 mL water, and 1 mL air, approximately 97% of the PCE and TCE is partitioned into the woody tissue (see SI for calculation details). Assuming the incoming transpiration stream is void of cVOCs after a rainfall and a local equilibrium exists between the transpiration stream and solid tissues, numerous pore-volumes of transpired water are required to reduce the total contaminant mass in the xylem. For TCE, the 20 – 30% reduction in total contaminant mass observed by Vroblesky et al. would require 9-14 pore volumes of clean water (see Appendix A for calculation details).

As the effects of environmental variables on tree cVOC concentrations are better understood, phytomonitoring is likely to gain usability in practice. From this work, sapwood cVOC concentrations appear well correlated to transpiration, suggesting phytomonitoring of sapwood should occur during periods of similar transpiration rates

each year. Concentrations in the heartwood appear more buffered, suggesting this tissue may be more appropriate for LTM. At this site, recent rainfall appeared to have minimal impact on tree cVOC concentration, particularly when compared to seasonal variation. As additional phytomonitoring data are gathered, seasonal variations may be predictable from widely available meteorological data, increasing the efficacy of phytomonitoring. Phytomonitoring has the potential to reduce the environmental impacts, monetary costs and temporal requirements for long term monitoring of chlorinated solvents plumes, although seasonal fluctuations in cVOC concentration need to be predictable for effective implementation.

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NOTES

The authors declare no competing financial interests.

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III. PLANT TRANSLOCATION OF ORGANIC COMPOUNDS: MOLECULAR & PHYSICOCHEMICAL PREDICTORS

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ABSTRACT

The root – soil boundary represents one of the largest global biotic-abiotic mass-transfer interfaces and is a primary pollutant entry point to the food chain. This interface is also critically important in phytoremediation efforts and herbicide design. Experimental data and single parameter models have resulted in the current understanding that moderately hydrophobic organic compounds are most likely to be translocated by plants, although recent evidence indicates plants can also translocate some hydrophilic compounds. Molecular descriptors initially applied for drug discovery and for trans-membrane migration in mammalian systems, were applied here to determine the physicochemical domains and weighted desirability functions to identify compounds amenable to translocation by plants. Considering molecular descriptor cutoffs defined in this work, chemicals likely to be translocated by plants more closely resemble those able to cross the blood-brain barrier as compared to the intestine. Desirability functions were also used to generate quantitative estimates of plant translocation and these results revealed similarities to the human system as well. Knowledge of the physicochemical domain encompassing plant-translocatable contaminants from this work allows *in silico* screening of emerging contaminants for better estimates of exposure.

INTRODUCTION

Plant roots interact with a wide variety of subsurface chemicals, transporting nutrients and chemical signals while excluding most detrimental compounds. However, roots are not a perfectly selective barrier, allowing agrochemists to develop useful systemic pesticides able to enter the plant through the root.[e.g., 1] In addition, the plant root has also been employed to remove subsurface contaminants (i.e., phytoremediation). The ability of some environmental contaminants to cross the root membrane also presents concern for food safety and contaminant exposure, as terrestrial plants sit at the base of many food chains. Assessing this exposure pathway by solely gathering exhaustive experimental data, while effective, is overly resource intensive and unsustainable, particularly when considering the diversity and number of anthropogenic chemicals being generated in abundance. Conversely, a tiered approach utilizing physiochemical knowledge integrated into *in silico* predictive tools provides a more efficient and robust approach to assessing plant uptake and potential exposure of emerging and fugitive compounds.

Organic chemical uptake and translocation by plants has been studied intensely since the 1950's, generally describing uptake using the transpiration stream concentration factor (TSCF), a ratio of chemical concentration in the xylem pore-water to the chemical concentration in the feed solution.[2] Typically, models relate the TSCF to hydrophobicity (i.e., octanol-water partitioning [log K_{ow}]), generally demonstrating bell-shaped curves, where moderately hydrophobic compounds (log K_{ow} of 1-3) show the greatest uptake.[3-5] However, some hydrophilic compounds readily translocate in plants,[e.g., 6] explainable by a sigmoidal relationship between log K_{ow} and TSCF.[7, 8] This discrepancy at low log K_{ow} reveals the limited ability of log K_{ow} to accurately explain translocation of organic contaminants.

The study of organic compound transport through biological barriers is not limited to plant systems. Lipinski's landmark paper[9] on assessing pharmaceutical uptake (i.e., "drug-likeness") showed orally administered compounds fell into a specific range of physicochemical properties, i.e. physicochemical domains. Lipinski's "Rule of Five" states an orally administered compound is likely to absorbed by the human intestine if the compound has 5 or fewer hydrogen bond donors, 10 or fewer hydrogen bond acceptors, a

molecular mass of less than 500 Daltons and a log K_{ow} of less than 5. The rule of five has been quite successful in predicting the absorption, distribution, metabolism and excretion (ADME) of compounds and has been integrated into the high-throughput screening (HTS) approach to expedite drug discovery.[10] Similar rules have been developed for compounds crossing other biological interfaces, such as the blood-brain barrier (BBB), skin and plant cuticle.[11, 12]

Rule-based cutoffs, while simple to implement, provide no quantifiable measure of "drug-likeness". To provide such a measure, desirability functions[13, 14] have been used in several drug development contexts.[15-17] Desirability functions allow different types of independent variables operating on different scales to be rescaled, weighted, and combined into a single quantitative function that provides the maximum information content using the fewest number of descriptors. Herein, we use Lipinski's framework and desirability functions to describe chemical transport across another important biological organ: plant roots.

METHODS

A comprehensive selection of TSCFs was compiled from literature.[1-7, 18-34] The data include 196 TSCF measurements of 110 unique compounds measured using 21 plant genera and varying experimental methodologies. TSCF measurements were not included if any of the following conditions were noted: metabolism of the parent compound *in planta* was demonstrated, measurements included metabolites (e.g., ^{14}C), no evidence was provided of reaching steady state, the TSCF was calculated improperly (e.g., including analyte concentrations in roots), roots were damaged prior to dosing (intentionally or accidentally), substantial depletion of dosing solution occurred (>50%), or additional modes of exposure were included (e.g., particle deposition). Although an effort was made to remove measurements where *in planta* chemical degradation occurred, the presence of such a loss mechanism would result in an under-reported TSCF value. A table of TSCF values utilized is available in Appendix B. Molecular descriptors – log K_{ow}, H-bond donors (HBD), H-bond acceptors (HBA), molecular weight (MW), rotatable bonds (ROT) and polar surface area (PSA) – were obtained using the ACD/PhysChem Suite as implemented by ChemSpider.[35] Statistics describing range and

intercorrelation of the descriptor variables are available in Appendix B. TSCF values were averaged for each unique compound and weighted histograms were constructed by counting the average TSCF of compounds falling in each specified range (see formula below).

$$n = \sum_{i=1}^{m} \overline{TSCF_i}$$

Where:

n is the weighted count of compounds falling in the specified range

m is the number of compounds falling in the specified range

$\overline{TSCF_i}$ is the average TSCF of compound *i* falling in the specified range

These weighted histograms capture the information carried by the numerical TSCF value while reducing sensitivity to outliers. Average TSCFs have also been calculated, but are more variable, particularly when very few compounds are available in the corresponding range.

Beta regression[36, 37] was performed in SAS 9.1 using a previously developed macro.[38] TSCFs values greater to or equal to one were disregarded for this analysis, as beta regression requires values to belong to (0,1), making Beta regression particularly well suited for analyzing TSCF data. The macro uses the independent variables to fit mean and precision parameters, rather than shape parameters, to allow straightforward interpretation. Models were compared using the Aikake Information Criteria (AIC)[39] and Bayesian Information Criteria (BIC).

Desirability functions were developed following the approach of Bickerton et al.[15] Each histogram was fitted with an asymmetric double sigmoidal function.

$$D(x) = a + \frac{b}{1 + \exp\left(-\frac{x - c + \frac{d}{2}}{e}\right)} \cdot \left[1 - \frac{1}{1 + \exp\left(-\frac{x - c - \frac{d}{2}}{f}\right)}\right]$$

Where:

D(x) is the desirability function for each molecular descriptor, x

a, b, c, d, e, and f are fitting parameters

Desirability functions were combined to calculate the quantitative estimate of plant translocation ($QEPT_w$) given a set of weights. An example calculation of the QEPT for carbamazepine is shown in Appendix B.

$$QEPT_w = \exp\left(\frac{\sum_{i=1}^{n} w_i \ln D_i}{\sum_{i=1}^{n} w_i}\right)$$

Where:

w_i is a weighting factor belonging to [0,1]

D_i is the desirability function for molecular descriptor i

Weights were determined by searching the entire domain in increments of 0.05 to maximize the information content as measured by Shannon entropy (SE).

$$SE_w = -\sum_{i=1}^{n} QEPT_w \log_2 QEPT_w$$

Where:

SE_w is the Shannon entropy for a set of weights

RESULTS AND DISCUSSION

The weighted histograms reveal a range of molecular descriptors notably similar to those of Lipinski (see Figure 1) and provide increased knowledge regarding plant translocation of organic compounds. Box and whisker plots also demonstrate the predictive power of the molecular descriptors (see Appendix B). Moderately hydrophobic compounds are most likely to be translocated by plants, as observed in previous research,[3, 4, 7] with most translocatable compounds exhibiting a log K_{ow} between one and four. The molecular mass histogram demonstrates that translocatable compounds generally have a molecular mass of less than 350 Da, below Lipinski's cutoff of 500 Da. Hydrogen bond donor and acceptor histograms appear to have cutoffs around four and seven, respectively. Again, these values are lower than Lipinski's published cutoffs, five and ten respectively. Collectively, the assessment of published TSCF data indicate plant roots are a more restrictive barrier than the human intestine, particularly for organic compounds exhibiting hydrogen bonding. This suggests that hydrogen bond interactions with the polar cell wall of the plant tissues may be an important process restricting trans-membrane migration.

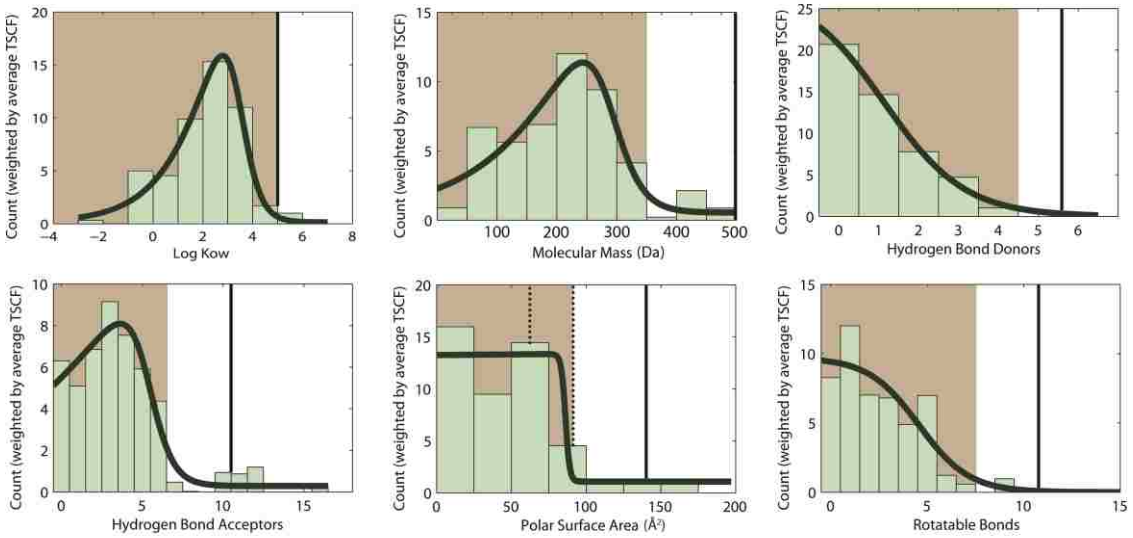

Figure 1. Histograms of TSCF values for hydrophobicity, molecular mass, hydrogen bonding, polar surface area (PSA) and rotatable bonds where the solid line indicates Lipinski's "Rule of Five" cutoffs[9] or approximate cutoffs for orally bioavailable compounds in rats (10 or fewer rotatable bonds, PSA $\leq 140 Å^2$).[40] The shaded area delineates the region of physicochemical properties that yield readily translocatable compounds in plants. The dotted lines indicate two cutoffs proposed for high brain permeation.[41, 42] Solid curves are the fitted desirability functions.

Polar surface area (PSA) and rotatable bonds are two other molecular descriptors often used in drug screening, particularly for compounds able to cross the BBB.[43] The PSA cutoff for plant-translocatable compounds (Figure 1) matches the cutoff proposed by van de Waterbeemd et al. for central nervous system (CNS) drugs.[42] In addition, CNS drugs generally possess fewer hydrogen bond donors (0 – 3) than non-CNS drugs and are generally less flexible (i.e., fewer rotatable bonds).[44] In the plant system, PSA and rotatable bond cutoffs of $90 Å^2$ and 7, respectively, are notably lower than corresponding cutoffs for the human intestinal system. These findings, when combined with the results from hydrophobicity, molecular mass and hydrogen bonding indicate that chemical transport across the root is more similar to that of the BBB rather than the less restrictive intestine.[45]

The importance of hydrogen bonding in addition to log K_{ow} was also demonstrated by beta regression. Two models were fit, one using the TSCF predicted by

the sigmoidal log K_{ow} relationship[7] and another model with a PSA term added (predicted TSCF*PSA/100). The additional PSA term reduced the AIC (-19 to -34) and the BIC (-5.7 to -18), indicating an improved model. The mean parameter estimates are the log-odds of an increase in TSCF per unit increase in parameter, showing that increased PSA is correlated with a decrease in TSCF.

As with Lipinski's rule of five, caution should be exercised when applying these predictive physicochemical domains, as they are not a substitute for detailed exposure data collection and modeling. Rather these domains encompass a majority of compounds with high translocation potential, thereby providing qualitative guidance. In addition, relatively few compounds exist outside the cutoffs with reported TSCFs, likely due to the difficulty in obtaining such measurements and an unfortunate disinclination to publishing "negative" results. Of the 18 compounds outside of the cutoffs, four have TSCFs greater than 0.2, implying a substantial misclassification, although all four compounds arise from a single manuscript. Whether these compounds truly exist in the tails of the physiochemical distribution or if other chemical-plant interactions are present, such as active transport, remains unclear. However with the predictive physiochemical domains of translocation now defined, 'outliers' can be better identified and unique plant uptake or chemical translocation can be more clearly identified. As an example, members of the Cucurbitaceae family have shown a unique ability to translocate hydrophobic compounds such as organochlorines and PAHs.[46] Identifying such an outlier is not possible without first understanding the true physiochemical distribution of translocatable compounds. Note that although the dataset used to generate these histograms is more than an order of magnitude smaller than that of Lipinski, the quantitative TSCF value provides considerably more information than Lipinski's binary dataset, where any potential drug entering Phase 2 efficacy studies was considered to exhibit sufficient solubility and permeability.

Desirability functions (Figure 1) fit to the data were scaled by the maximum value (see Appendix B) and weighted to identify the relative importance of each molecular descriptor. Exploration of the weight-space revealed Shannon entropies were relatively comparable over the best hundred weight combinations, so an average was taken. Table shows the resulting weights for both the maximum Shannon entropy and an average of

the best 100 weights. For comparison, the analogous weights are shown from Bickerton et al.[15] developed for orally bioavailable compounds (i.e., quantitative estimate of drug-likeness [QED]). Note that aromatic rings (AROM) and number of structural ALERTS[47] were not considered in this analysis. Aromatic rings were omitted due to the limited range of data available, while ALERTS represent functional groups known to cause toxicity in humans. While toxicity is a concern for identifying viable drug candidates, human toxicity is not likely to be highly correlated to translocation in plants.

The weightings in Table 1 show good agreement between plant translocation and oral bioavailability, as log K_{ow}, MW and HBD all show large weightings. Conversely, HBA and PSA provide minimal additional information. ROT provide additional information for orally bioavailable drugs, likely due to ROT's correlation with toxicity.[15, 48]

Table 1. Optimized desirability function weightings

	Source	*Kow*	*MW*	*HBD*	*HBA*	*PSA*	*ROT*	*AROM*	*ALERTS*
$QEPT_{Max}$	This Study	0.65	0.9	0.75	0	0	0	*	*
$QEPT_{100}$	This Study	0.56	0.76	0.64	0	0	0	*	*
QED_{Max}	15	0.25	0.5	0.5	0	0	0.5	0.25	1
QED_{1000}	15	0.46	0.66	0.61	0.05	0.06	0.65	0.48	0.95

* descriptors not included in analysis, see text for details
$QEPT_{100}$ is an average of the 100 highest scoring QEPT weights
QED_{1000} is an average of the 1,000 highest scoring QED weights

For validation, a set of 42 unpublished TSCF measurements was obtained from the Utah Water Research Laboratory database.[7, 49] These measurements were obtained using the pressure chamber technique and span a range of molecular descriptors. 36 measurements with no rule-based violations had an average TSCF of 0.67. Six measurements with one or more violations had an average TSCF of 0.22. The calculated QEPT values for the validation set are shown against the measured TSCF in Figure 2 along with traditional predictions of TSCF. Although all models have a similar RMSE (ca. 0.3) and MAE (ca. 0.25), the traditional models perform poorly for hydrophilic

compounds, predicting either very low or very high TSCFs depending on the model. The QEPT shows improved accuracy for such compounds, although very little improvement in prediction range is evident.

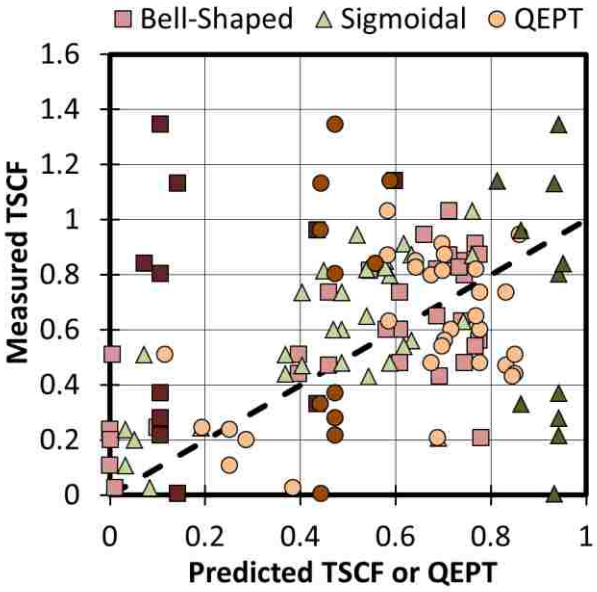

Figure 2. Validation set TSCFs compared against QEPT and bell-shaped[3] and sigmoidal[7] log K_{ow} model predictions of TSCF. Darkly shaded data points represent hydrophilic compounds with a log K_{ow} less than one.

Use of the TSCF, measured or predicted, should involve other fate and transport considerations, both in the plant and in the surrounding environment. Contaminant bioavailability and microbial degradation in the rhizosphere are among numerous abiotic and biological environmental processes that must be considered when evaluating collective transport and exposure pathways for environmental pollutants. A majority of plant uptake data used to build these models was generated using hydroponic studies, generally providing maximum bioavailability. Under more field-like conditions, contaminants may fail to enter the plant due to retardation, biodegradation and redox conditions in the subsurface.[e.g., 50]

Plant-dependent variables, such as rooting depth, are of importance in assessing a contaminant translocation by plants.[e.g., 51] In addition, these root-dependent properties pertaining to chemical transport may vary at the cultivar level.[e.g., 52] A plant's ability to detoxify xenobiotics (i.e., phytotransformation) can also limit persistence or bioaccumulation of environmental contaminants, particularly those susceptible to cytochrome P450 attack.[53] Conversely, weak acids and bases can locally accumulate in plant tissues in their disassociated forms due to differences in pH across tissues.[54, 55] Phloem sap pH is generally basic (ca. 8), while the vacuole pH is near 5.5, creating compartments where less permeable disassociated compounds can accumulate. A similar decrease in compound permeability is possible after hydroxylation or conjugation as evidenced by the plant hormone abscisic acid.[56] In addition, the incredible diversity of the plant kingdom and environmental interactions (e.g., stress, growing conditions) can create variability in rates and activity of the above processes.

The likeness of pharmaceutical physicochemical cutoffs and desirability function weightings with those presented here demonstrates the similarity between plant roots and human membrane systems, particularly the BBB. A level of similitude is to be expected as these systems manage the transport of nourishment, biochemical signals, dissolved gasses and water between highly sensitive organs and a relatively uncontrolled, potentially infectious environment using lipophilic barriers. These barriers, Casparian strips in the plant endodermis and tight junctions in animal epithelia are fundamental barriers in higher organisms.[57]

Ultimately, this improved understanding of mass transfer has several important implications for fields from agricultural chemistry to contaminant remediation to food safety and public health. This environment-food pathway may be the limiting step of exposure, as the physicochemical domain for plant translocation is wholly inclusive of the domain proposed by Lipinski, implying compounds translocated by plants are also orally bioavailable and may cross the BBB. The root membrane may be the leading protective barrier prohibiting subsurface, anthropogenic pollutants from entering the anthroposphere.

In addition, plants may be useful tools for assessing exposure potential. Plants have already been used in sustainable remediation protecting human health (i.e.,

phytoremediation[58]) and sensing of subsurface contamination (i.e., phytoforensics[59]) to delineate polluted environments and reduce the risk fugitive contaminants pose to public health. Plant roots may also prove to be a useful assay for risk assessment and drug discovery. Collectively, better understanding of plant-contaminant interactions and fundamental understanding of plant processes continues to provide vital clues, greater insight, and inspiration as we search for "greener" approaches to protect human health and the environment.

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NOTES

The authors declare no competing financial interest.

SUPPORTING INFORMATION

TSCF data, TSCF statistics, TSCF boxplots, beta regression statistics and desirability function parameters are available. This information is available free of charge via the Internet at http://pubs.acs.org/journal/estlcu.

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IV. PHYTOSCREENING FOR PERCHLORATE: RAPID ANALYSIS OF TREE SAP

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ABSTRACT

Perchlorate presents an environmental health risk due to its widespread use, high solubility in water, and ability to interfere with thyroid function in humans. Delineating such contaminant plumes is difficult and time consuming, particularly in forested areas. Phytoscreening, the analysis of tree contaminants for plume delineation, has been previously applied to shallow chlorinated solvent groundwater plumes. To test the potential of phytoscreening for perchlorate, a sensitive centrifugation method coupled with ultrafast liquid chromatography tandem mass spectrometry (UFLC-MS/MS) detection was developed. An initial hydroponic greenhouse test using willow cuttings demonstrated concentrations of perchlorate in tree sap were proportional to the perchlorate dosing concentration. Eighty tree cores obtained in the field contained measureable amounts of perchlorate and the distribution of perchlorate in trees reflected the distribution of perchlorate in the groundwater. The secondary data (tree cores) were loosely correlated with the primary data (groundwater) as demonstrated by cokriging and regression. Phytoscreening of perchlorate was sufficiently accurate to be used as a screening tool to delineate areas of groundwater contaminated with perchlorate.

INTRODUCTION

Perchlorate (ClO_4^-) is a chemical historically used in the manufacturing of explosives and rocket propellants for the defense and aerospace industries. Perchlorate and its salts have also been used in various industrial applications such as manufacturing of matches, airbag inflators, safety flares and fireworks.[1] Because perchlorate is readily soluble in water, if spilled perchlorate can be transported vast distances in groundwater. Additionally, natural sources of perchlorate have also been reported, particularly in deposits of sodium nitrate in northern Chile historically used as a fertilizer.[2, 3]

Recently, the US EPA conducted occurrence studies (Unregulated Contaminant Monitoring Regulation 1 program) and found perchlorate contamination in both groundwater and surface waters serving as drinking water sources for more than 16 million people in at least 26 states nationwide in USA.[4] Perchlorate has been detected in over 4 % of public water systems nationally at the level of greater than or equal to 4 µg/L.[4] Due to perchlorate's link with decreased thyroid hormone output,[1] the USEPA is establishing a national primary drinking water regulation for perchlorate.[5] The states of California and Massachusetts have set a maximum contaminant level for perchlorate in drinking water of 6 µg/L and 2 µg/L, respectively.[6, 7]

The relatively recent interest in perchlorate environmental occurrence and fate has led to several publications of perchlorate uptake by plants, recently reviewed by Seyfferth and Parker.[8] Numerous laboratory studies have also demonstrated the potential for phytoremediation of perchlorate through direct uptake or rhizodegradation under anoxic conditions.[8] Perchlorate has been found in aboveground plant tissues of many plants growing at contaminated sites. At such field sites, concentrations of perchlorate in stems have been reported as high as 6 mg/kg.[9, 10] Concentrations of perchlorate are often much higher in the leaves, with concentrations reported in the range of 190-5,557 mg/kg for several weed species.[10, 11] For comparison, average leaf perchlorate concentrations have been reported as high as 38.8 mg/kg at a naturally contaminated site.[12]

Phytoscreening is a phytoforensic tool that uses plant sampling to delineate areas of contaminated groundwater.[13] Phytoscreening has been widely employed at sites contaminated with chlorinated solvents,[14-17] but application of phytoscreening for inorganics is lesser studied. Phytoscreening of cadmium, copper, nickel and zinc at one

field site showed significant correlations between soil and wood metal concentrations for 4 of 8 combinations of metal and plant genus.[18] For example, willow zinc concentrations were positively correlated to zinc in the soil (R^2=0.725, n=7), but poplar zinc concentrations were not (R^2=0.007, n=15). A phytoscreening effort for arsenic, cadmium, chromium, copper, nickel and zinc found differences in tree core metal concentrations between tree species were greater than differences between a background site and a contaminated site.[19] Phytoscreening of chlorinated solvents has yet to be shown to be highly dependent on plant species, likely due to the passive nature of uptake of these pollutants.[20, 21] For metal uptake by plants, active transport is likely required, leading to distinct species-metal interactions, often conceptualizing plants as either hyperaccumulators, excluders and bioindicators of a certain metal.[22] For the purposes of phytoscreening, bioindicator species would be most desirable.

Uptake of perchlorate by plants is thought to occur through nitrate transporters. Experiments with lettuce (*Lactuca sativa*) and varying anions showed reduced uptake of perchlorate when nitrate concentrations increased from 4 to 12 mM (~250 mg/L to 750 mg/L), although the degree of competition depended on the variety of lettuce.[23] The presence of sulfate (1-10 mM) and chloride (5-15 mM) did not affect perchlorate uptake. Increasing pH or bicarbonate ion also reduced uptake of perchlorate, indicating ClO_4^-/H^+ cotransport was occurring. Another group of researchers working with lettuce also demonstrated competitive uptake of perchlorate with nitrate, again with differences between varieties.[24] Competition with chloride was also demonstrated.

In this research, a greenhouse studied was designed to test the feasibility of phytoscreening for perchlorate. While perchlorate uptake may differ by species due to differences in transporters, perchlorate is much more bioavailable than heavy metals, which have had limited success in phytoscreening, likely allowing perchlorate to be more amenable to phytoscreening. Previous research investigating leaf perchlorate concentrations has shown substantial seasonal variations,[25] precluding the use of leaves for perchlorate phytoscreening. Hydroponic testing of wetland plants has shown stem perchlorate concentrations to be proportional to dosing concentration, although the tests were performed at high concentrations (20 – 500 mg/L) and some growth inhibition was present at high concentrations.[26] To test phytoscreening in the lab, willow clones were

grown hydroponically and dosed with a range of perchlorate concentrations. To analyze perchlorate in tree sap, a rapid, sensitive analytical method was developed. The method was also applied to phytoscreening at a field site with extensive perchlorate groundwater contamination.

METHODS

To rapidly analyze tree sap perchlorate concentrations for phytoscreening, a centrifugation method was developed. Tree xylem sections (cores or de-barked cuttings) were placed into a 1.5 mL centrifuge tube and then frozen for two hours. Upon thawing, the tubes were centrifuged at 30,000 g to remove sap from the xylem tissue. A 25-µL aliquot of sap was taken and diluted 4x with mobile phase and spiked with isotopically labeled perchlorate ($NaCl^{18}O_4$) as the internal standard. The liquid was then filtered through a 4-mm diameter, 0.2-µm pore nylon filter.

To detect perchlorate in sap and dosing solution, an ultrafast liquid chromatography tandem mass spectrometry (UFLC-MS/MS) method was developed. A 2.1 x 250 mm IonPak (Dionex IonPak®AS-21) column and Shimadzu UFLC system were used for the separation. The sample injection volume was 20 µL. The mobile phase was 200 mM methylamine in water with a flow rate of 350 µL/min. Detection was performed using a 4000Q Trap MS/MS system operated in a multiple-reaction monitoring mode (MRM) with ESI-negative ionization. The quantification ion pair was m/z 98.7/82.9 amu and m/z 100.9/84.8 amu was used as the confirmation ion pair. The ion pair for isotope labeled perchlorate was 106.9/89 amu. The method performed optimally using the following parameters: Ion source temperature 500^{o}C, ion spray voltage -4500 v, auxiliary gas 30 psi, nebulizer gas 40 psi, curtain gas 25 psi, dwell time 150 ms, DP (V) -5, EP (V) -10, CE (V) -38, CXP (V) -15. Using these parameters, the instrument calibration was linear from 0.2 to 200 µg/L with an instrument detection limit of 0.1 µg/L in water.

A greenhouse study was performed to test the viability of phytoscreening under ideal conditions. Laurel-leaf willow (*Salix pentandra*) cuttings were obtained from locally grown clones. All cuttings were ~20 cm in length and between 7.5 and 9 mm in diameter to fit into 1.5-mL centrifuge tubes for later sap centrifugation. The willows were

grown in 10% modified Hoagland's solution for 30 days to allow shoot and root development. To encourage shoot development near the top of the cutting, the upper 5 cm of the cutting was brushed with a 10 mg/L solution of indole-3-butyric acid.

Established cuttings were placed into 1-L jars of 10% modified Hoagland's solution dosed with differing amounts of perchlorate (see Appendix C for photos). The experimental design included two factors: perchlorate concentration and exposure duration. Perchlorate concentration had levels of 10 µg/L, 100 µg/L, 1 mg/L and 10 mg/L. Exposure duration had levels of 7 days, 14 days and 21 days. Each treatment combination was performed in triplicate and controls without perchlorate were also grown. Control perchlorate concentrations remained below the detection limit throughout the experiment. During harvest, the willow trunks were sectioned into three 2-cm long pieces to increase the amount of sap. The three portions of sap were combined, diluted, spike with internal standard and filtered prior to injection on the UFLC-MS/MS.

The field site was the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas.[10] This former military base historically manufactured trinitrotoluene, rocket motor propellants, pyrotechnics and ammunition. The site was placed on the USEPA National Priorities List in 1990 due to groundwater contamination by various compounds such as chlorinated solvents and perchlorate. The areas of interest for this study are Area 16, a former landfill, and Area 18/24, a former burning ground and unlined evaporation pond (Figure 1). In both areas, a Record of Decision (ROD) was signed in 1995 resulting in removal of contaminated soil and/or water, capping and extraction of groundwater.

LHAAP is generally situated above the Wilcox Group, which consists of fine- to medium-grained sands interbedded with considerable clay and lignite.[27] Groundwater at the site is unconfined with levels that fluctuate seasonally. The topography is relatively flat and groundwater flow is generally towards the Harrison Bayou, but seasonal fluctuations occur. In Area 18/24 groundwater is generally at depths of 5 – 20 feet (1.5 – 6 meters), and groundwater in Area 16 is generally 5 – 10 feet (1.5 – 3 meters) below ground surface. The southeastern edge of the former landfill in Area 16 sits in the 100-year floodplain.

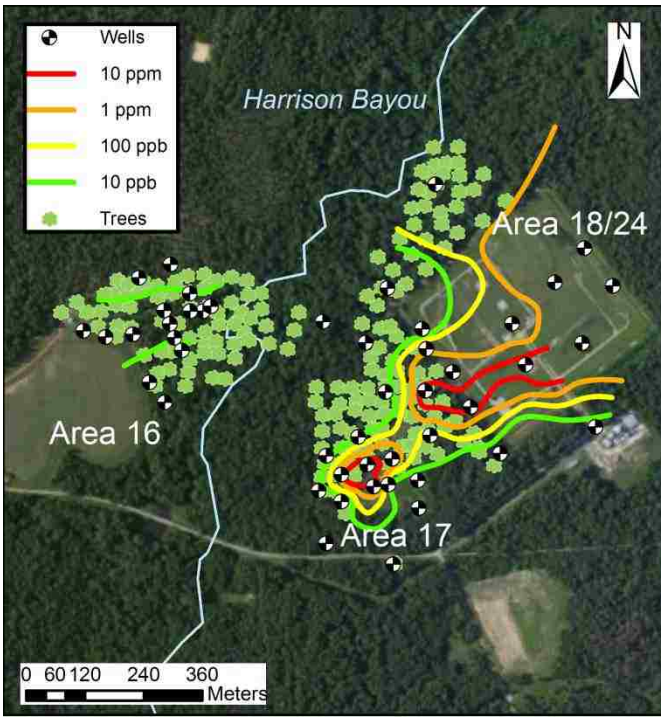

Figure 1. LHAAP site map with sampled trees and groundwater perchlorate isoconcentration contours krigged from the depicted shallow groundwater wells.

Tree cores were taken in the vicinity of Areas 16, 17, 18/24 during June 18-28, 2012 (Figure 1). Trees were selected from predetermined 30 x 30 meter grid nodes. The sampled tree near the node was required to have a diameter at breast height (DBH) greater than 10 cm. Preference was given to hardwoods over softwoods for consistency. The location, diameter and genus were recorded for all 183 trees. Cores were taken using a 5-mm increment borer to a depth of 8 cm following published methods.[28] The increment borer was rinsed with deionized water after each tree coring. Field duplicates were collected every ten samples. The samples were shipped overnight on ice for analysis at Missouri S&T.

Historical groundwater perchlorate concentrations were available from 50 wells screened in the shallow aquifer (Figure 1). Because the wells were sampled at varying frequencies, 226 measured perchlorate concentrations were averaged over the period 2007 – 2012. Wells frequently sampled over this period showed no discernable trend in perchlorate concentrations.

All data were $\log_{10}$ transformed prior to statistical analysis to improve homoscedasticity. ANOVA (proc GLM) and regression (proc REG) analysis of the data were performed in SAS 9.1. Pairwise comparisons were performed using LSMEANS with a Tukey adjustment. Errors-in-variables regression followed the approach of Fuller[29], using the modified estimator 3.1.20. Geospatial mapping and kriging were performed in ArcGIS 10.1. A spherical semivariogram was used for all kriging with a lag size of 10 meters. A nugget was included to explain measurement error of approximately one order of magnitude for perchlorate in groundwater. The model was parameterized through iterative cross-validation.

RESULTS AND DISCUSSION

The centrifugation method performed adequately to remove sap from the wood specimens. On average, 19% (standard deviation of 7%) of the water present in tree samples was removed by centrifugation. Dilution and filtration of the sap resulting in a method detection limit of 1 μg/L. Spike recoveries showed sufficient analytical accuracy (Table 1).

Table 1. Spike recoveries for perchlorate in tree sap

	Spike (μg/L)	n	Average Recovery (Range)
Willow Cuttings			
	5	2	86.4% (84.4 – 88.5%)
	10	2	100% (92.9 – 108%)
Field Site Tree Cores			
	2.5	3	84.0% (79.5 – 92.9%)
	125	4	101% (99.5 – 102%)

Duplicate tree core samples from LHAAP showed modest variability. Perchlorate was below detection limits for in both samples for half of the 18 duplicate tree cores taken. Only six duplicates resulted in detections for both samples, yielding a relative percent difference (RPD) of 51%. To examine potential sources of variability, duplicate injections of sap were performed, resulting in an RPD of 3.2% (n=6). Duplicate

centrifugations of the same tree core were also performed, resulting in an RPD of 25.2% (n=8), suggesting much of the variability results from heterogeneities in the perchlorate concentrations in the tree core. Such heterogeneities have been demonstrated in tree core VOC concentrations for trees growing above heterogeneous contaminant plumes.[30]

All trees showed no signs of toxicity during the three-week experiment. At harvest, trees contained 49% water with a standard deviation of 4%. Over the experimental period, the transpiration rate increased from 30 mL/day to 100 mL/day as the plants grew (see Appendix C).

Concentrations of perchlorate in tree sap were well correlated to the dosing perchlorate concentration (Figure 2). ANOVA revealed both the dosing concentration ($p<0.0001$) and the day of harvest ($p=0.0043$) both significantly affected perchlorate concentrations in tree sap. Trees harvested after 14 days of exposure had significantly reduced concentrations as compared to 7 and 21 days of exposure ($p=0.037$ and $p=0.0042$, respectively). Adding a flag for harvest after 14 days to a regression model provided minimal additional explanatory power, with an adjusted R^2 of 0.993, improved from 0.990 with dosing solution as the sole explanatory variable. This indicates exposure duration is of little practical importance in this experiment.

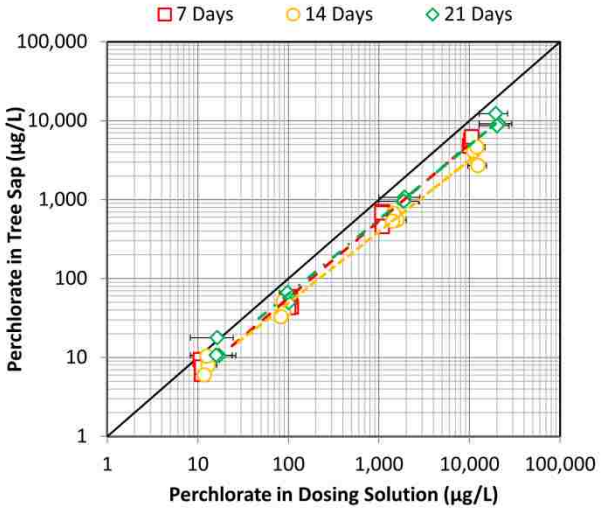

Figure 2. Correlation between tree and dosing solution perchlorate concentrations for each exposure duration. The black line indicates a 1:1 fit and the horizontal error bars denote the range of exposure concentrations during the week prior to harvest (n=2).

The overall ordinary least squares fit for the data shown in Figure 2 demonstrates that trees can potentially be used as biosensors of perchlorate contamination in water. The data do not indicate accumulation of perchlorate in stems over time, as has been shown for leaves. The regression fit yielded a slope near unity, indicating a nearly proportional response. Using an errors-in-variables regression approach, the slope of the regression fit increases slightly, to 0.946. The standard error of the slope estimate increases to 0.0303, resulting in a confidence interval of (0.885, 1.01).

$$\log_{10} C_{sap} = 0.941(0.0160) \log_{10} C_{water} - 0.133(0.0456)$$

Where:

C_{sap} is the sap perchlorate concentration (μg/L)

C_{water} is the water perchlorate concentration (μg/L)

Values in parenthesis represent standard errors

The data are in agreement with another experimental dataset involving hydroponic dosing of wetland plants. He et al.[26] found concentrations of perchlorate in stem tissue were correlated with perchlorate in dosing solution. Their data falls above the extrapolated regression line in this study, suggesting species dependency (See SI).

From the 201 tree cores collected at LHAAP, 86 trees had detectable levels of perchlorate. The maximum perchlorate concentration measured was 1.6 mg/L in a 13-cm diameter oak tree. Qualitatively, concentrations of perchlorate found in trees resided in regions of groundwater contaminated with perchlorate, particularly outside of Area 16 (Figure 3). A krigged tree sap perchlorate plume also resembled the krigged groundwater perchlorate plume (see Appendix C).

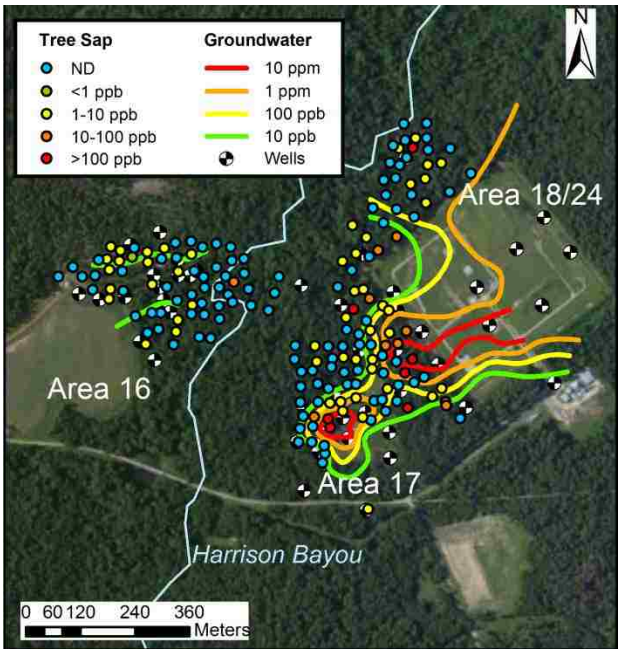

Figure 3. Measurements of tree perchlorate overlaid on groundwater perchlorate isoconcentration contours.

Cokriging is a theoretically useful technique for combining two spatial datasets that share a common spatial distribution.[31] If tree perchlorate concentrations are related to groundwater perchlorate concentrations, then the spatial covariance between the two variables will be noticeable. Figure 4 demonstrates the positive covariance between groundwater and tree sap perchlorate concentrations. Very few data pairs are available at close distances, resulting in a sparse data cloud. When relatively few points are available for cokriging, accurate parameterization of the semivariograms is difficult.[32] Nevertheless, cokriging can be used to generate a more detailed plume map due to the additional sample data (see Appendix C).

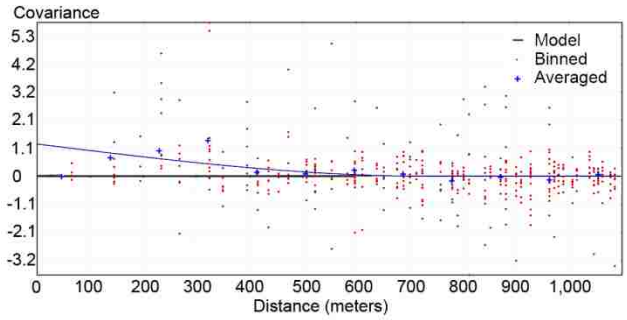

Figure 4. Cross-covariogram between groundwater and tree sap perchlorate concentrations

An alternative is to transform the secondary data (i.e., tree perchlorate concentrations) into primary data (i.e., groundwater perchlorate concentrations) via a regression equation.[32, 33] To create the regression equation, wells were paired with all trees within a 30-m radius. Paired data revealed that tree perchlorate concentrations in Area 16 were not correlated with groundwater perchlorate concentrations and are not further considered (see Appendix C). The lack of significant correlation may be due the limited range of the explanatory variable in Area 16. In Areas 17/18/24, trees perchlorate concentrations were weakly correlated with groundwater perchlorate concentrations ($p = 0.011$, $R^2 = 0.22$, see Appendix C). Using the regression equation developed, tree perchlorate concentrations were transformed into groundwater concentrations and the resulting dataset was krigged (Figure 5). The resulting data show an inherently more detailed picture due to the higher resolution of the dataset. The general features of the plume remain similar to the groundwater perchlorate plume, particularly in the hotspot in Area 17. The plume in Area 17 shows less connection to the plume emanating from Areas 18/24, which is also shifted slightly north in the combined dataset.

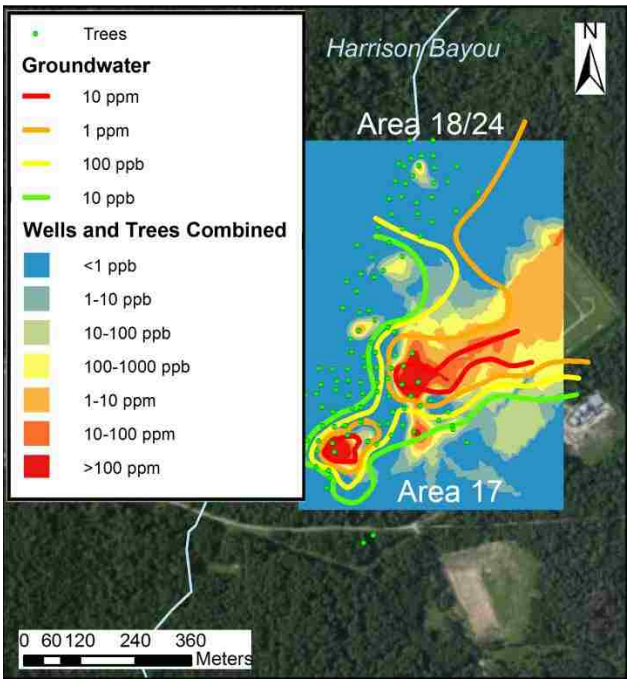

Figure 5. Krigged combined dataset of tree and well perchlorate.

While the correlation between tree sap and groundwater perchlorate is statistically significant at this site, the correlation is rather poor. The greenhouse experiment suggests this variability is not due to concentration-dependent uptake or temporal variability. The potential competition of perchlorate with nitrate is unlikely to be occurring at this site, with the concentration of nitrate in the groundwater at ~0.5 mg/L, lower than demonstrated for competition. Other plant-environment interactions are likely reducing the strength of the correlation, as has been demonstrated for phytoscreening of VOCs. Differential uptake of clean rainwater between trees may be particularly problematic, which has been demonstrated with trichloroethene (TCE).[34] For TCE, much of the contaminant is partitioned to lignin,[35] providing a reservoir of contaminant not likely present for perchlorate. Additionally, trees may metabolize perchlorate at varying rates,[36, 37] although the relative importance of this pathway is debatable given the numerous observations of perchlorate in plants. Other factors such as rooting depth, depth to groundwater, and redox conditions will strongly affect the applicability of phytoscreening at other field sites.

The use of secondary data, such as tree cores, in site investigations can provide additional site detail and reduce the overall uncertainty in contaminant distribution. As the true distribution of contaminants at a site is never completely known, investigators can only attempt to reduce uncertainty in a cost effective manner. The approach of tree coring offers mobility and sampling speed, particularly in heavily forested areas such as LHAAP. At this field site, phytoscreening was capable of resolving the general shape and intensity of the groundwater perchlorate plume in a rapid, non-invasive fashion.

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SECTION
3. CONCLUSIONS

The use of plants as biosentinels of environmental contamination presents a number of potential advantages over traditional sampling, but is a field still in its infancy. Environmental contamination has traditionally been measured directly in the media of interest, rather than indirectly through other sensors such as plants. However, traditional sampling in some areas has begun to be replaced by more complex sampling methods to better assess the true exposure. This is particularly evident in the area of heavy metal contamination in soils and hydrophobic contaminants in sediments and soils. For the former, a variety of extraction techniques have been proposed to better assess labile metals, rather than total metals, in an effort to better quantify metals available to the receptor of interest. Likewise, for ultra-hydrophobic contaminants in sediments passive sampling is increasingly used to better estimate the bioavailable fraction of contaminants. In a similar manner, the contaminants measureable in plants may better reflect the availability of contaminants to certain receptors. Perhaps more certainly, plants have the ability to average contaminant concentrations over temporal and spatial dimensions, providing a measure of contamination inherently more valuable than a point measurement.

Despite the potential benefits of plant sampling for contaminants, a wide variety of technical limitations remain. While some understanding has been advanced for plant uptake of organic compounds, mechanisms for uptake of iongenic compounds are still poorly understood. Similarly, mechanisms of uptake for metals remain relatively elusive, particularly in plants of practical interest. Furthermore, although a number of different types of compounds were detected in plants in this work, a vast number of chemicals will be unlikely to enter a plant due to factors beyond the root membrane. Redox, rooting depth and microbial degradation are critical factors affecting the potential for uptake of numerous chemicals. For chemicals able to endure the journey into the plant transpiration stream additional complicating factors arise. As demonstrated in this research, the pumping rate of trees is variable, affecting the contaminant concentrations and fluxes.

Loss rates such as phytodegradation and phytovolatilization are also dependent on factors such as plant type, plant size and weather.

Even with the current limitations of plant sampling, phytoforensics provides a useful secondary data source for many contaminants at a number of field sites. The sampling methods are minimally invasive and rapid, particularly useful in wooded areas where traditional groundwater assessment techniques are problematic. Sensitive analytics are generally required to measure such trace levels of contamination in complex matrices. When used properly, phytoforensics can provide a useful set of screening data to better assess contaminated sites.

4. RECOMMENDATIONS FOR FUTURE RESEARCH

4.1. PREDICTING PLANT UPTAKE OF ORGANIC COMPOUNDS

A broadly applicable *in silico* model of plant translocation of chemicals is needed to focus on the physiology and morphology of roots, the limiting step in water transport,[90] which is hypothesized to also limit chemical uptake. Before reaching the xylem tissue and translocating throughout plant's vascular system, water and the associated contaminants must negotiate several barriers providing resistances to transport.

Water travels through one of three pathways to reach the xylem: symplastic, transcellular or apoplastic (see Figure 4.1). Collectively, the symplastic and transcellular pathways are referred to as the cell-to-cell path, as both require water to travel predominately through living cells. The apoplastic pathway features transport through intercellular spaces through the cortex tissue of the root. Transport through the intercellular spaces has traditionally thought to be arrested at the endodermis, a ring of cells consisting of lipophilic (suberized[91]) Casparian bands. Recent research has shown that the Casparian bands may be slightly penetrable to water and solutes, a pathway termed the apoplastic bypass.[92] Detailed understanding of water transport through plant roots improves our fundamental understanding of contaminant transport in roots.

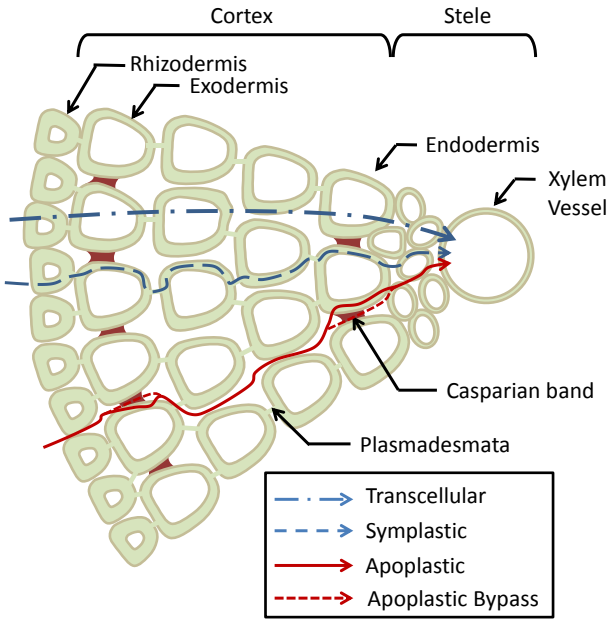


Figure 4.1. Transport paths for water and solutes in root

In this proposed research, a fundamental model considering the available chemical transport pathways will be developed. With such a model, TSCFs can be more accurately predicted from physicochemical properties such as molecular size and hydrogen bonding parameters. TSCFs have been shown to be quite variable for hydrophilic compounds,[17] which likely arises from differences in plant apportionment of flows (e.g., symplastic vs. apoplastic bypass) and chemical interactions such as hydrogen bonding. Consider two pharmaceuticals with similar log K_{OW} (~2.2): carbamazepine and gliclazide. We would hypothesize gliclazide, with its larger number of hydrogen bond acceptors (6 vs. 3) and correspondingly higher polar surface area (87$Å^2$ vs. 46 $Å^2$) will be translocated less effectively than carbamazepine. This hypothesis is supported with laboratory uptake findings of Tanoue et al.[49] (TSCFs: 0.23 vs. 0.69).

Using the following approach, we will develop a quantitative, predictive model of the TSCF using the one-dimensional advection-diffusion equation. First, consider the Péclet number, which can be used to determine the mass transport regime operating in roots. The flux of water on the order of 10^{-7} m/s[93] and chemical diffusivities in water are at most 10^{-5} cm^2/s.[94] Effective diffusivities through the tortuous symplastic and

apoplastic pathways are of the magnitude 10^1-10^2 times smaller than diffusivities in water.[95, 96] The diameters of the fine roots that conduct a majority of water uptake are 0.1 to 1 mm.[97]

$$\mathrm{Pe} = \frac{Lv}{D} \sim \frac{10^{-4} m \cdot 10^{-7} \frac{m}{s}}{10^{-9} \frac{m^2}{s} \cdot 10^{-2}} = 1$$

This Péclet number indicates that the root has evolved in such a way to allow both advection and diffusion to play a role in mass transport, perhaps for reasons of signal transport from roots to leaves (e.g., abscisic acid[98]). Note that during periods of lower transpiration (e.g., night), diffusion will dominate mass transfer. However, this construction of the Péclet number ignores two additional resistances that may be important for some compounds: the unmixed boundary layer (UBL) and the plasma membrane (MEM), as both symplastic and apoplastic transport require crossing a cell membrane (see Figure 3). The apoplastic bypass represents a parallel path that bypasses k_{MEM}. This pathway may be important for compounds with high membrane resistance, although the magnitude of apoplastic bypass flow is thought to be small, unless the roots are mechanically injured.[92, 99] The cell-to-cell pathway reduces to either the apoplastic or the symplastic pathway for membrane impermeable or membrane permeable compounds, respectively.

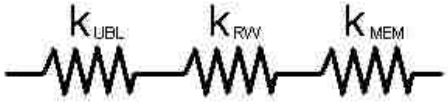


Figure 4.2. Root resistance model, where the resistance of apoplastic or symplastic flow is k_{RW}.

From various membrane studies, the thickness of the UBL is at most 1 mm and possibly as low as 100 µm.[100-102] Such a small thickness is negligible in context of the resistance presented by the tortuous symplastic and apoplastic pathways. The third resistance is the cell membrane, which is traditionally associated with the decrease in TSCF for hydrophilic compounds.[16, 22] While human cell membrane permeability data (or *in vitro* surrogate data) are widely available, plant cell membrane permeability data is sparse. Broadly, these two membrane systems are similar in structure and function, despite some differences such as plants' use of sterols (instead of cholesterol) to regulate the permeability of their more unsaturated fatty acyl chain phospholipids.[103] Plant cell membrane permeability is positively correlated with hydrophobicity,[27, 104-106] although, for hydrophilic compounds, other interactions such as hydrogen bonding and pKa are likely to affect membrane permeability, as demonstrated in human models, but yet to be applied to plant systems.[107, 108] Mammalian membrane permeability has been extensively investigated for drug discovery, leading to various *in vitro* assays. The Caco-2 cell line has been widely used in permeability assays, leading to numerous models, including poly-parameter linear free energy relationships (pp-LFERs).[109-112] Another permeability assay, the parallel artificial membrane permeation assay (PAMPA), uses artificial membranes that mimic human intestine to accomplish HTS of candidate drugs.[113] PAMPA permeability has been correlated to three molecular properties: pKa, log K_{OW} and polar surface area (PSA).[114] Permeability models developed in this objective will explore the applicability of *in vitro* assays to describe plant cell membrane permeability, a notably lacking aspect of plant transport understanding.

Mass transfer of compounds can also be slowed by partitioning to the root tissues. Traditionally, hydrophobic compounds have been thought to exhibit low TSCFs due to this retardation during transport in roots. As the root is continually growing,[115] the zone of greatest water uptake also shifts, likely preventing sorption equilibrium from ever being reached. Further complicating quantitative retardation estimates for EFCs is the lack of robust partitioning data.

4.2. PREDICTING PARTITIONING TO PLANT MATERIALS

Partitioning coefficients are necessary components of environmental models to explain mass flows from compartment to compartment and storage in each. Knowledge

of chemical mass in various environmental compartments is also crucial in exposure modeling, but prediction of partitioning coefficients is critically lacking for plant systems. Much like chemical partitioning to soil, the plant is a mixture of phases and materials, leading to simplifications (such as partitioning to plant lipids) that do not capture the complexity of the system, particularly when dealing with unique solutes that are not well predicted by single parameter relationships (i.e., log K_{OW}). Partitioning also varies by temperature and plant tissue composition, which varies by climate and species, respectively. These complexities in partitioning are better addressed by poly-parameter linear free energy relationships (pp-LFERs).

Historically, root-water partitioning coefficients (K_{RW}) along with many other plant tissue-water partitioning coefficients have been calculated using the root concentration factor (RCF)[27, 34] – the ratio of the compound in the root to that in the feed water. RCFs have been positively correlated to log K_{OW}.[22]

RCFs are less than optimum partitioning coefficients due to the difficulty in removing soil particles from roots and the difficulty of verifying equilibrium in such a dynamic system combining growth with changing flow paths as the root grows and develops In addition, log K_{OW} has been found to inadequately explain partitioning process for hydrophilic compounds, particularly those participating in hydrogen bonding.[25] pp-LFERs better explain partitioning across a broader chemical space[25] and have been used to predict partitioning between a number of materials and chemicals,[116] such as plant cuticles and agrochemicals.[26, 117] LFERs, described by Abraham and others, feature a linear combination of five solute parameters that can be used to explain a free energy based property, such as partitioning.[25, 118]

$$\log SP = c + rR_2 + s\pi_2^H + a\sum \alpha_2^H + b\sum \beta_2^H + vV_x$$

Where:

log *SP* is the solvation property of interest, such has partitioning or the enthalpy of partitioning

R_2 is excess molar refraction of the solute

π_2^H is the dipolarity/polarizability of the solute

$\sum \alpha_2^H$ is the hydrogen bond acidity of the solute
$\sum \beta_2^H$ is the hydrogen bond basicity of the solute
V_x is McGowan's molecular volume (cm^3/mol/100) of the solute

Lower-case letters represent solvent properties and are often estimated by multiple linear regression. McGowan's volume is occasionally replaced by the log of the hexadecane-air partitioning coefficient, particularly when describing interactions between condensed phases.[25, 119] Solute descriptors are available for more than 5,000 compounds and can be predicted using ACD's Absolv program.[112, 120, 121]

To yield a more accurate and flexible root-water partitioning coefficient, a composite plant root model will be developed. The root is considered to be constructed of water, wax, lignin, cellulose, lipid, phenolics, non-structural carbohydrates and ash. In biologists' effort to understand construction costs of various plant materials, compositions of various plant tissues are tabulated in the literature, allowing species-specific K_{RW} to be determined (see Table 4.1).[122, 123]

$$K_{RW} = \rho + f_{wax}K_{wax} + f_{lignin}K_{lignin} + f_{cellulose}K_{cellulose} + f_{lipid}K_{lipid} + \cdots$$

Where:

K_{RW} is the root water partitioning coefficient (L/kg)
ρ is the percent water of the root (L/kg)
f_{wax} is the fraction of the wet root mass that is wax
K_{wax} is the wax water partitioning coefficient (L/kg)
f_{lignin} is the fraction of the wet root mass that is lignin
K_{lignin} is the lignin water partitioning coefficient (L/kg)
$f_{cellulose}$ is the fraction of the wet root mass that is cellulose
$K_{cellulose}$ is the cellulose water partitioning coefficient (L/kg)
f_{lipid} is the fraction of the wet root mass that is lipid
K_{lipid} is the lipid water partitioning coefficient (L/kg)

Table 4.1. Composition of plant roots by life form (mg/g dry weight) from ref 122

	Proteins	Lipids	Phenolics	Cellulose	Lignin	Waxes	Carbo-hydrates	Ash
Trees	53	24	83	415	151	142	27	106
Shrubs	53	27	45	508	129	139	35	62
Grasslands	77	14	8	580	165	40	57	60

Most of the above partitioning coefficients are not currently available as pp-LFERs, but only as functions of log K_{OW}, or not available at all.[124-126] These lacking data and models prevent accurate prediction of partitioning coefficients for emerging or unmeasured compounds. Conversely, partitioning to both storage and membrane lipids as well as proteins has been explained using pp-LFERs.[127-129] In this work, plant root lipids will be considered as membrane lipids.

Figure 4.3 shows preliminary data from the composite partitioning model against traditional estimates of partitioning for a number of EFCs found in plants. The model uses log K_{OW} relationships for cellulose,[125] lignin[124] and waxes[126] and pp-LFER relationships for lipids[127] and proteins.[129] Note that while many compounds follow traditional K_{OW} models (single parameter), several outliers exist due to substantial interactions with membrane lipids. To develop partitioning coefficients that are more broadly applicable, pp-LFERs will be constructed to explain partitioning to basic plant materials, such as waxes, lignin and cellulose. These pp-LFERs will allow more accurate partitioning coefficients to be generated for different plant tissues, for different plant species and at different temperatures.

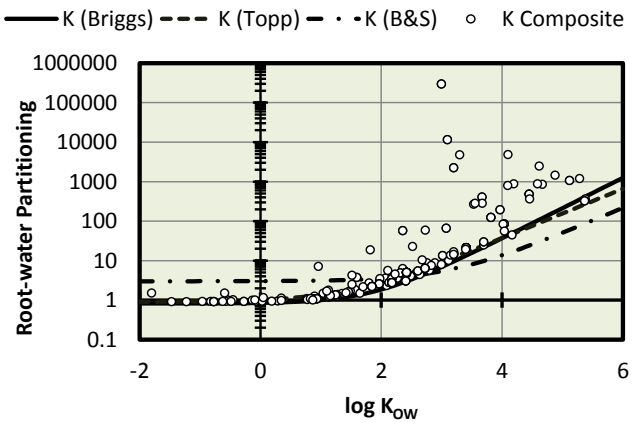


Figure 4.3. Root water partitioning coefficients derived from RCFs of Briggs,[22] Topp[130] and Burken & Schnoor (B&S)[16] compared to the composite model for trees and selected EFCs.

4.3. FATE OF PERCHLORATE IN PLANTS

Perchlorate has been widely measured in the leaves of plants growing above contaminated sites, particularly in the leaves.[131, 132] The fate of this perchlorate upon leaf drop is uncertain. Tan et al.[133] measured perchlorate in leaves prior to and after leaf drop, finding a 2 – 4 fold reduction in perchlorate concentrations after leaf drop. Such a drop in concentration could result from leaching of perchlorate due to rainfall on fallen leaves. However, since perchlorate has been shown to compete with nitrate transporters[134] and nitrate can be remobilized from leaves in fall, some perchlorate may be stored in woody tissues prior to leaf drop. To test such a hypothesis, trees would be dosed with perchlorate and leaf perchlorate concentrations would be periodically measured. Trees could be induced into senescence while monitoring leaf perchlorate concentrations. A decrease in leaf perchlorate concentrations (once corrected for leaf water content) would be indicative of perchlorate remobilization.

APPENDIX A.

Supporting Information:

Chlorinated Solvents in Trees: Seasonal Variations in Concentrations

REFERENCE EVAPOTRANSPIRATION

Reference evapotranspiration (ET_0) was calculated following the FAO Penman-Monteith equation.[1]

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

Where:

ET_0 is the reference evapotranspiration (mm/day)

R_n is the net radiation at the crop surface (MJ/m^2/day)

G is the soil heat flux density (MJ/m^2/day)

T is the air temperature at 2 m height (°C)

u_2 is the wind speed at 2 m height (m/s)

e_s is the saturation vapor pressure (kPa)

e_a is the actual vapor pressure (kPa)

e_s - e_a is the saturation vapor pressure deficit (kPa)

Δ is the slope vapor pressure curve (kPa/°C)

γ is the psychrometric constant (kPa/°C)

ET_0 was calculated on a daily time step. The psychrometric constant was calculated by:

$$\gamma = \frac{c_p P}{\varepsilon \lambda} = 0.665 \cdot 10^{-3} P$$

Where:

c_p is the specific heat at constant pressure, $1.013 \cdot 10^{-3}$ MJ/kg/°C

P is the atmospheric pressure (kPa)

λ is the latent heat of vaporization, 2.45 MJ/kg

ε is the ratio of the molecular mass of water vapor to dry air, 0.622

In the FAO Penman-Monteith equation, the average temperature is defined as the mean of the maximum and minimum daily temperatures. The mean temperature is used in the calculation of the slope of the saturation vapor pressure curve.

$$\Delta = \frac{4098\left[0.6108 \cdot \exp\left(\frac{17.27T}{T+237.3}\right)\right]}{(T+237.3)^2}$$

Where:

T is the average temperature (°C)

Estimation of the net energy reaching the plant surface requires adjustment to the measured solar radiation. Long-wave radiation lost by the soil surface must be subtracted as well as any light reflected by the surface. Extraterrestrial radiation is used to estimate the degree of cloud cover and is calculated from geographic and day-of-year data. The inverse relative distance between the earth and sun is given by:

$$d_r = 1 + 0.033 \cos\left(\frac{2\pi}{365}J\right)$$

Where:

d_r is the inverse relative distance between the earth and sun

J is the day of the year, where January 1 is 1 and December 31 is 365 or 366

The solar declination (δ) is given by:

$$\delta = 0.409 \sin\left(\frac{2\pi}{365}J - 1.39\right)$$

The sunset hour angle (ω_s) is given by:

$$\omega_s = \arccos(-\tan\varphi \tan\delta)$$

Where:

φ is the latitude (radians)

Using the above parameters, the extraterrestrial solar radiation can be calculated.

$$R_a = \frac{24 \cdot 60}{\pi} G_{sc} d_r (\omega_s \sin\varphi \sin\delta + \cos\varphi \cos\delta \sin\omega_s)$$

Where:

R_a is the extraterrestrial solar radiation (MJ/m^2/day)

G_{sc} is the solar constant, 0.0820 MJ/m^2/min

The extraterrestrial solar radiation undergoes some attenuation through the atmosphere, yielding the clear-sky solar radiation.

$$R_{so} = (0.75 + 2 \cdot 10^{-5} z) R_a$$

Where:

R_{so} is the clear-sky solar radiation (MJ/m^2/day)

z is the elevation above sea level (m)

The above theoretical incoming solar radiation is compared to the measured value to estimate cloudiness. Cloudiness, vapor pressure and temperature are used to estimate the net long-wave radiation emitted by the surface.

$$R_{nl} = \sigma \left(\frac{T_{max}^4 + T_{min}^4}{2} \right) \left(0.34 - 0.14\sqrt{e_a}\right) \left(1.35 \frac{R_s}{R_{so}} - 0.35\right)$$

Where:

R_{nl} is the net outgaining long-wave radiation (MJ/m^2/day)

σ is the Stefan-Boltzmann constant, $4.903 \cdot 10^{-9}$ MJ/K^4/m^2/day

e_a is the actual vapor pressure (kPa)

R_S/R_{SO} is the relative shortwave radiation, bounded by [0,1]

The measured net shortwave radiation must be corrected for reflection.

$$R_{ns} = (1 - \alpha) R_s$$

Where:

R_{ns} is the net shortwave radiation (MJ/m^2/day)

α is the albedo of the hypothetical grass reference crop, 0.23

R_S is the measured incoming solar radiation (MJ/m^2/day)

The net radiation (R_n) can then be calculated as:

$$R_n = R_{ns} - R_{nl}$$

The final term in the energy balance, the soil heat flux (G), was neglected for the daily time step. The wind speed measured at the NWS weather station is taken at 10m, higher

than required for the FAO Penman-Monteith equation. The following equation was used to adjust the wind speed.

$$u_2 = u_z \frac{4.87}{\ln(67.8x - 5.42)}$$

Where:

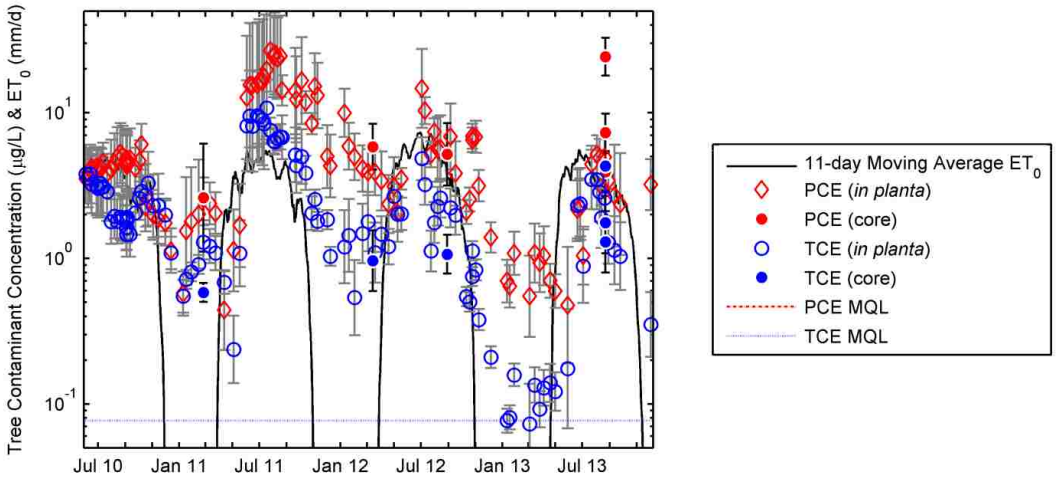
u_2 is the wind speed at 2 meters above ground surface (m/s)

u_Z is the wind speed measured at x meters above ground surface (m/s)

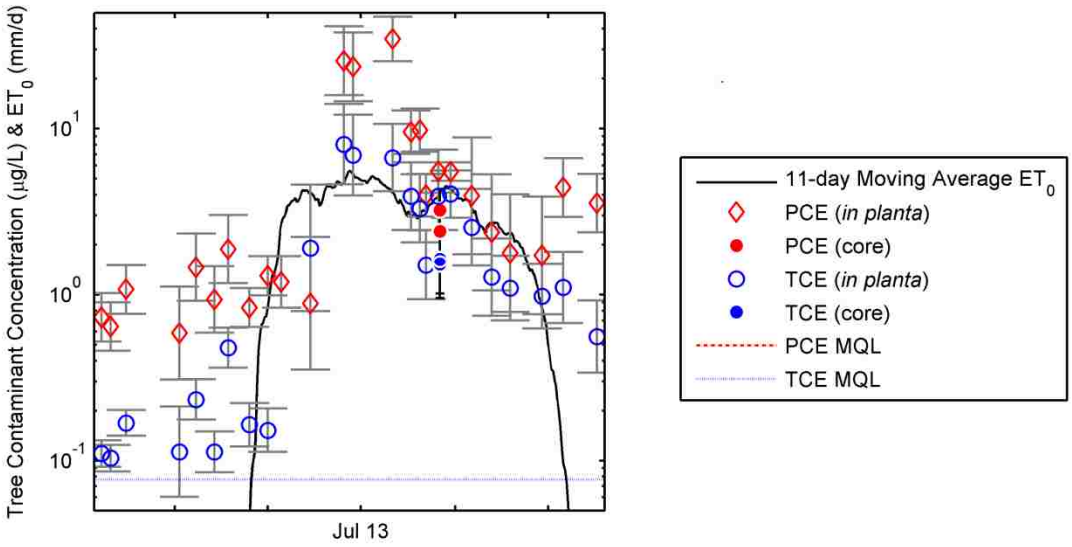
Measured Input Property and Source

Property	*Source*
Pressure (P)	NWS Vichy Weather Station
Maximum Daily Temperature (T_{max})	NWS Vichy Weather Station
Minimum Daily Temperature (T_{min})	NWS Vichy Weather Station
Incoming Solar Radiation (R_S)	Agricultural Weather Station at Cook Station
Actual Vapor Pressure (e_a)	Agricultural Weather Station at Cook Station
Saturation Vapor Pressure (e_s)	Agricultural Weather Station at Cook Station
Average Wind Speed (u_Z)	NWS Vichy Weather Station

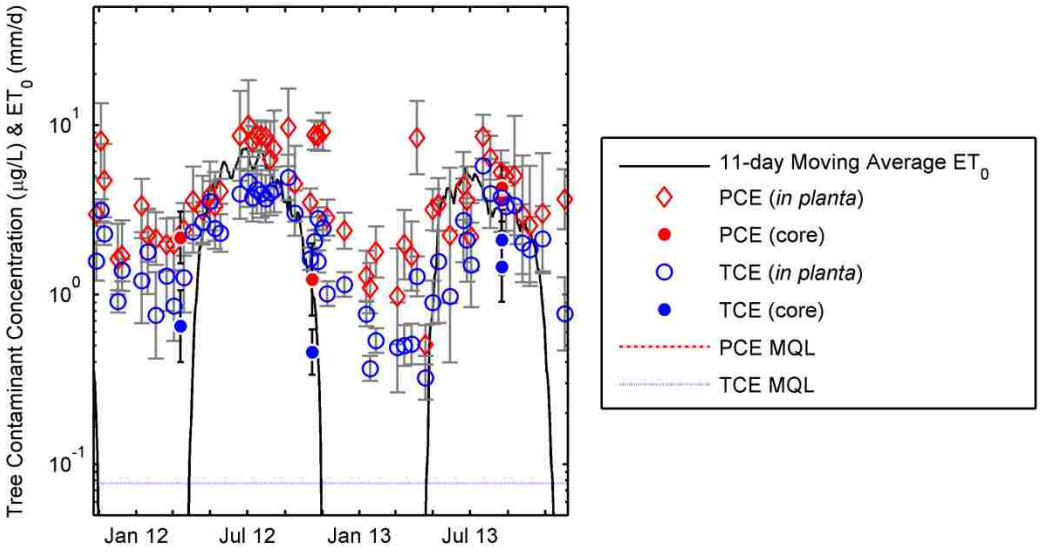
ADDITIONAL PLOTS OF PORT CONCENTRATIONS



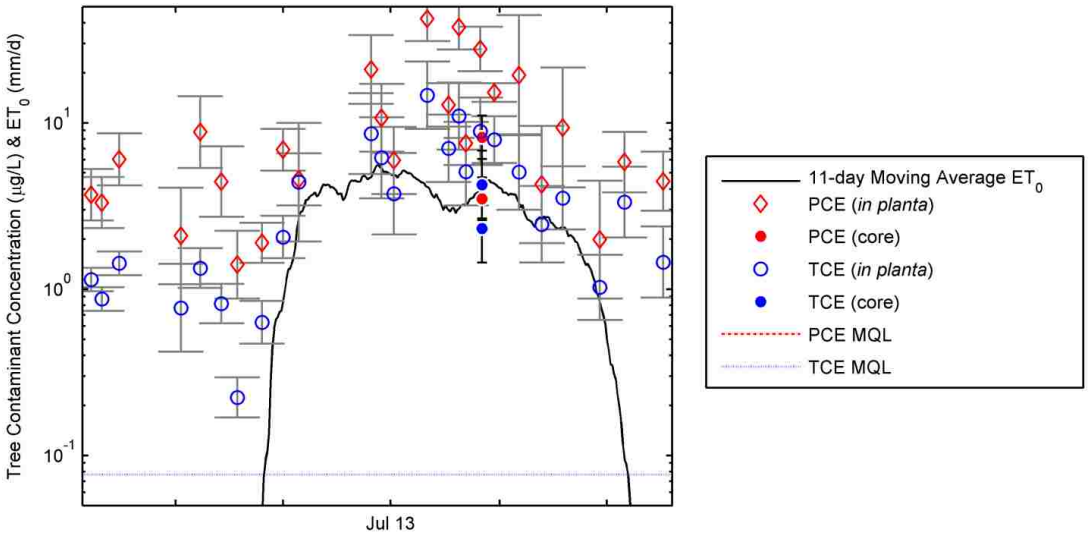
cVOC concentrations in port 2N. Error bars denote the 95% prediction interval.



cVOC concentrations in port 2E. Error bars denote the 95% prediction interval.



cVOC concentrations in port 5S. Error bars denote the 95% prediction interval.



cVOC concentrations in port 5N. Error bars denote the 95% prediction interval.

CVOC CONCENTRATION REDUCTIONS BY RAINFALL

To assess the role of rainfall in tree cVOC concentrations, a tree-core sized xylem section was examined. The tree core was constructed of three compartments: dry wood, water, and air. In tree cores analyzed by our lab, 1 g of dry wood, 1 mL of water and 1 mL of air were representative of most tree cores. A mass balance was constructed to account for all the contaminant mass present.

$$m_t = C_{wood} m_{wood} + C_a V_a + C_w V_w$$

Where:

m_t is the total chemical mass in the defined system (g)
C_{wood} is the chemical concentration in dry wood (g/kg)
m_{wood} is the mass of dry wood (kg)
C_a is the chemical concentration in the air (g/L)
V_a is the volume of air (L)
C_w is the chemical concentration in the water (g/L)
V_w is the volume of water (L)

Literature partitioning coefficients[2, 3] were used to convert chemical concentrations between phases.

$$K_H = \frac{C_a}{C_w}$$

Where:

K_H is Henry's constant (dimensionless)

$$K_{ww} = \frac{C_{wood}}{C_w}$$

Where:

K_{ww} is the wood-water partitioning coefficient (L/g)

To assess the percent of chemical mass in each compartment mass in each compartment was divided by the total contaminant mass.

$$\%m_{water} = \frac{V_w}{V_a K_H + V_w + m_{wood} K_{ww}}$$

$$\%m_{air} = \frac{V_a}{V_a + \frac{V_w}{K_H} + \frac{m_{wood} K_{ww}}{K_H}}$$

$$\%m_{wood} = \frac{m_{wood}}{\frac{V_a K_H}{K_{ww}} + \frac{V_w}{K_{ww}} + m_{wood}}$$

The resulting chemical mass distributions are shown in the below table.

Percent chemical mass in each compartment for varying wood compositions

Dry Wood Mass (g)	Air Volume (mL)	Water Volume (mL)	% Mass in Air			% Mass in Water			% Mass in Wood		
			cDCE	TCE	PCE	cDCE	TCE	PCE	cDCE	TCE	PCE
1	1	1	1.3	0.8	1.1	10	2.5	1.9	89	97	97
1	0.5	1	0.7	0.4	0.6	10	2.5	1.9	90	97	98
1	2	0.5	2.7	1.7	2.2	5.0	1.3	0.9	92	97	97

Assuming the influent transpiration stream contains no contaminant and equilibrates with the local tissue contamination, the total contaminant mass removed by transpiration of rainwater can be estimated, as shown in the below table.

Removal of TCE by transpiration of rainwater per pore-volume of water transpired

Pore Volume	Initial Contaminant Mass	Contaminant Mass Removed	Contaminant Mass Remaining
1	100%	2.5%	97%
2	97%	2.4%	95%
3	95%	2.4%	93%
4	93%	2.3%	90%
5	90%	2.3%	88%
6	88%	2.2%	86%
7	86%	2.2%	84%
8	84%	2.1%	82%

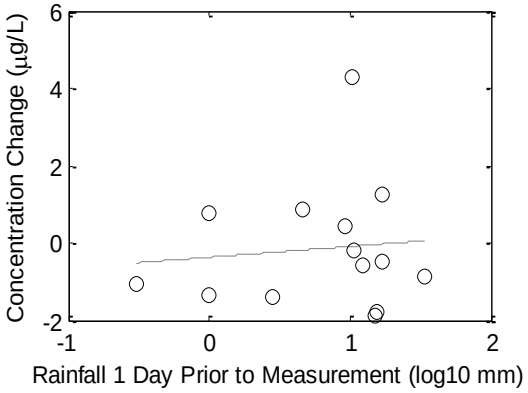
9	82%	2.0%	80%
10	80%	2.0%	78%
11	78%	1.9%	76%
12	76%	1.9%	74%
13	74%	1.9%	72%
14	72%	1.8%	70%
15	70%	1.8%	68%
16	68%	1.7%	67%
17	67%	1.7%	65%
18	65%	1.6%	63%
19	63%	1.6%	62%
20	62%	1.5%	60%
21	60%	1.5%	59%
22	59%	1.5%	57%
23	57%	1.4%	56%
24	56%	1.4%	54%
25	54%	1.4%	53%
26	53%	1.3%	52%
27	52%	1.3%	50%
28	50%	1.3%	49%
29	49%	1.2%	48%
30	48%	1.2%	47%

The above calculations assume that the transpiration water is in equilibrium with the surrounding woody tissues. Using effective diffusivities from literature[3] the diffusion length in one hour can be estimated.

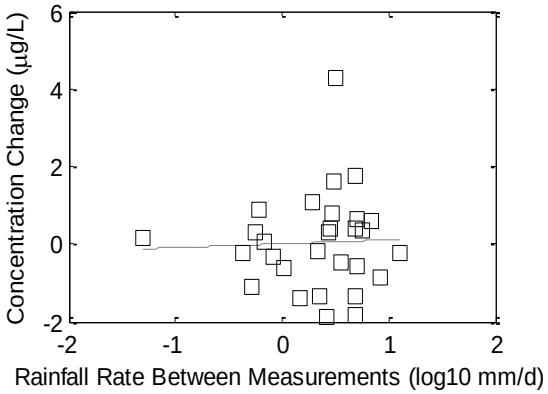
$$L = 2\sqrt{Dt} = 2\sqrt{3.2 \cdot \frac{10^{-8} cm^2}{s} \cdot 1hr} \approx 200\mu m \text{ (for PCE)}$$

This diffusion length is on the order of magnitude of the diameter of large xylem vessels.[4] Such a calculation would suggest that for xylem arrangements where conduits are regularly spaced, thereby minimizing the distance to an active conduit, PCE and TCE are likely at equilibrium locally. It seems possible that some arrangements of xylem, such as diffuse-porous woods, may result in relatively long diffusion distances to an active conduit. In such cases, the transpiration stream may not be at equilibrium with woody tissue, particularly under high transpiration velocities.

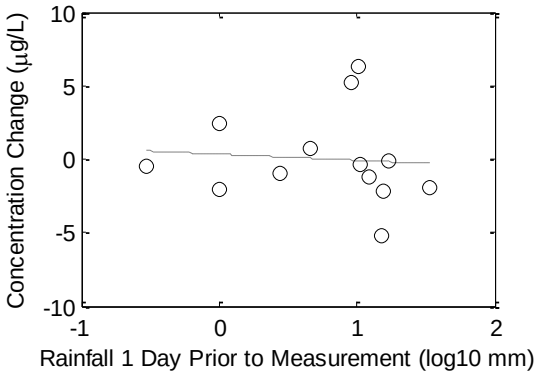
MEASURED CVOC CONCENTRATION REDUCTIONS BY RAINFALL



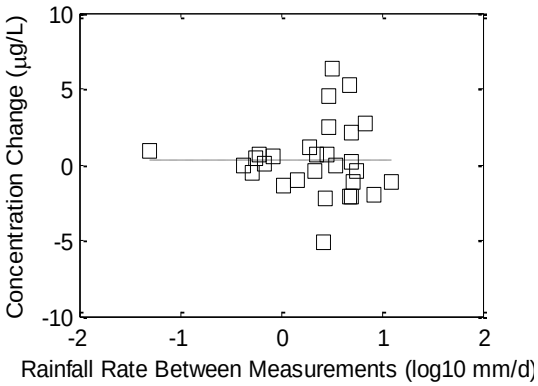
Slope = 0.28; p-value = 0.73



Slope = 0.10; p-value = 0.84

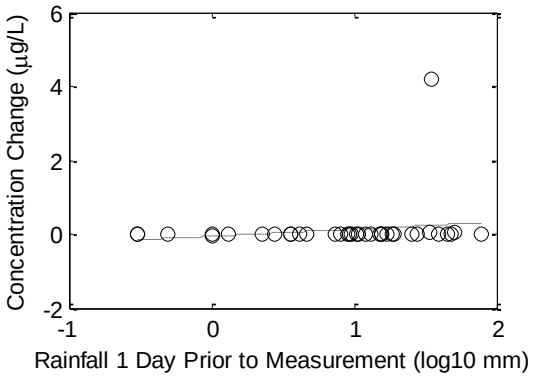


Slope = -0.42; p-value = 0.78

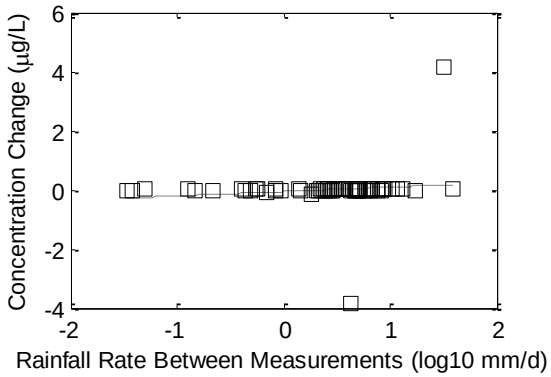


Slope = -0.017; p-value = 0.99

Port 6 TCE (top) and PCE (bottom)

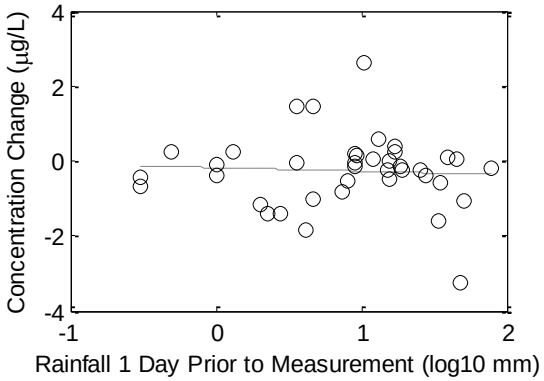


Slope = 0.19; p-value = 0.31

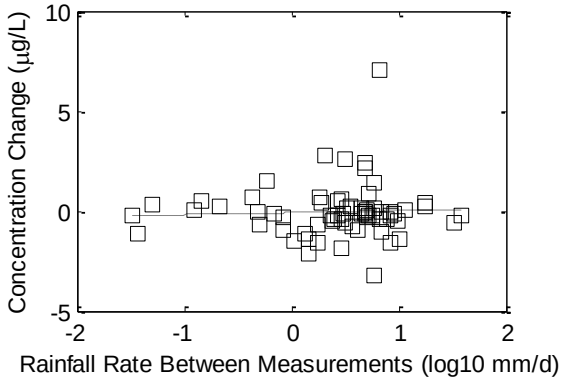


Slope = 0.15; p-value = 0.27

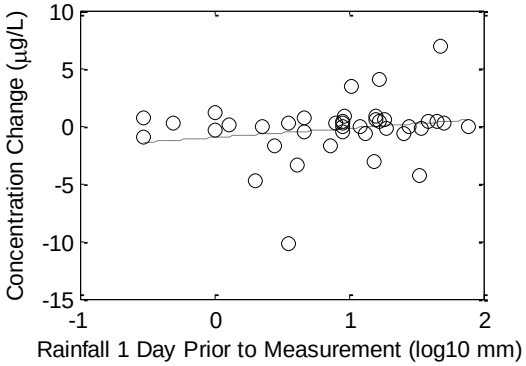
Port 5 PCE



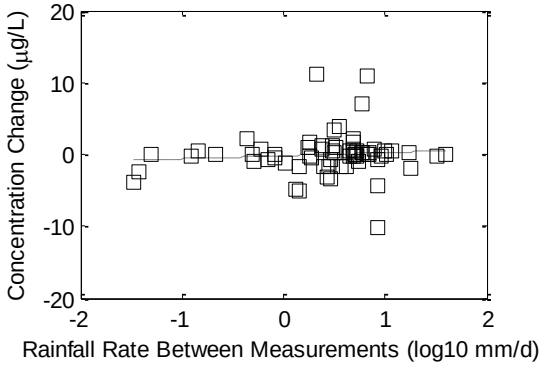
Slope = -0.096; p-value = 0.71



Slope = 0.098; p-value = 0.71

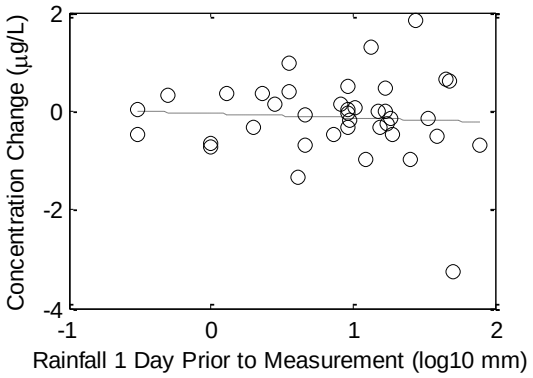


Slope = 0.80; p-value = 0.24

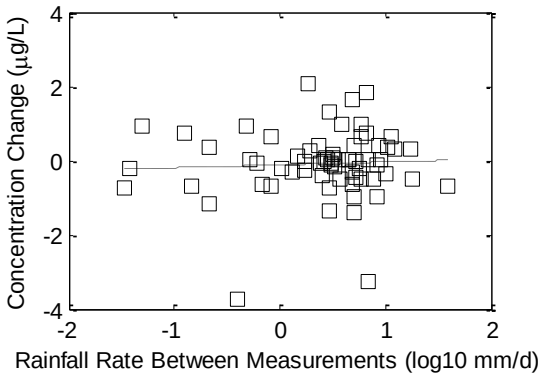


Slope = 0.43; p-value = 0.46

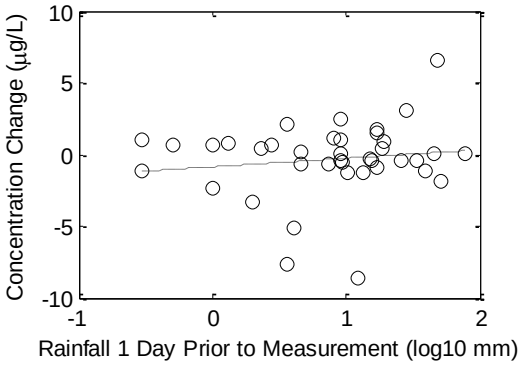
Port 3 TCE (top) and PCE (bottom)



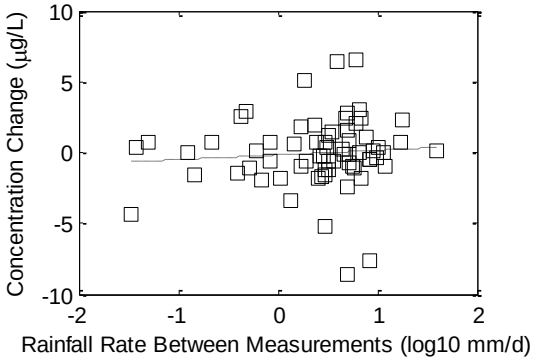
Slope = -0.087; p-value = 0.69



Slope = 0.077; p-value = 0.67



Slope = 0.59; p-value = 0.40



Slope = 0.33; p-value = 0.50

Port 2 TCE (top) and PCE (bottom)

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1. Allen, R. G.; Pereira, L. S.; Raes, D.; Smith, M., *Crop evapotranspiration - Guidelines for computing crop water requirements*. Food and Agriculture Organization of the United Nations: 1998.

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APPENDIX B.

Supporting Information:

Plant Translocation of Organic Compounds: Molecular & Physicochemical Predictors

DESCRIPTOR VARIABLE STATISTICS

For the compounds used to build the model, simple statistics and a correlation matrix are shown in the below tables. Most descriptors show a mild degree of intercorrelation, with the exception of PSA, which is highly correlated with HBA (r=0.93).

Simple statistics for descriptor variables

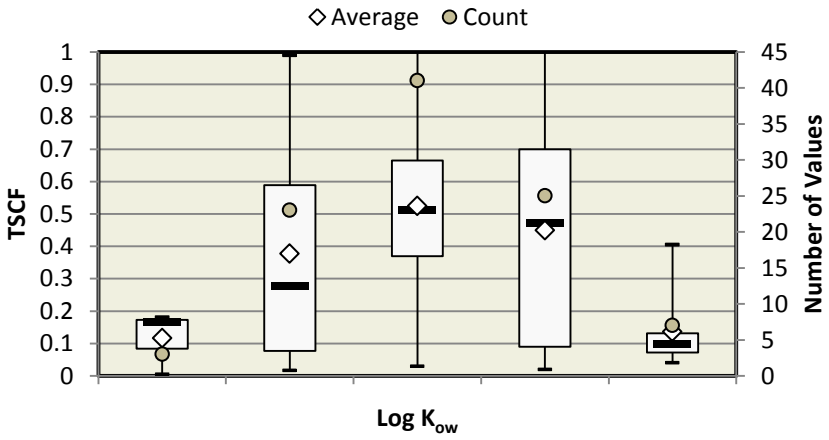
Variable	N	Mean	Std Dev	Minimum	Maximum
Log K_{ow}	110	2.18	1.75	-2.7	6.0
MW	110	229.3	95.1	32	616
HBD	110	1.21	1.29	0	6
HBA	110	3.60	2.87	0	16
Rot	110	3.10	4.19	0	36
PSA	110	53.0	42.4	0	196.2

Pearson correlation matrix for descriptor variables

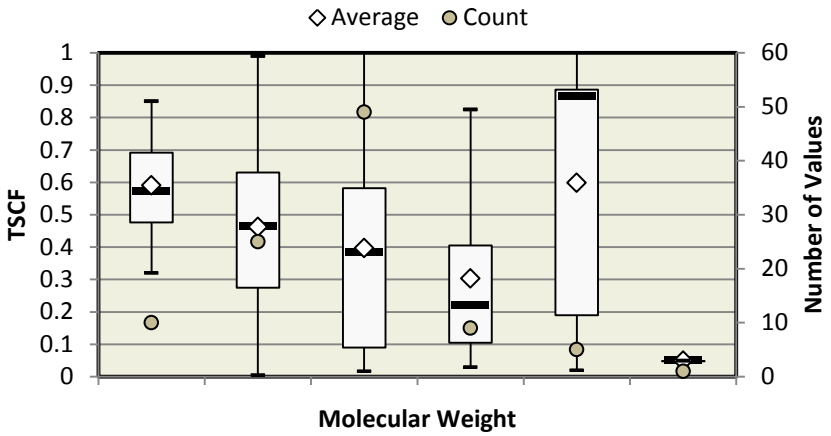
	Log K_{ow}	MW	HBD	HBA	Rot	PSA
Log K_{ow}	1	0.39	-0.35	-0.34	0.23	-0.45
MW	0.39	1	0.20	0.62	0.67	0.51
HBD	-0.35	0.20	1	0.36	0.08	0.58
HBA	-0.34	0.62	0.36	1	0.42	0.93
Rot	0.23	0.67	0.08	0.42	1	0.31
PSA	-0.45	0.51	0.58	0.93	0.31	1

TSCF BOXPLOTS

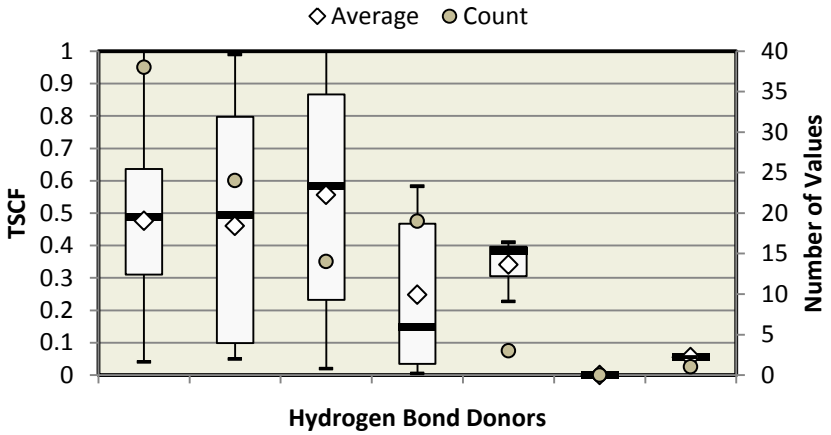
Boxplots were created to illustrate the trends and variability of the average TSCF within each domain. The largest TSCFs are generally observed with compounds exhibiting moderate hydrophobicity, small molecular mass and few hydrogen bonding groups. Note that ranges with few measured values have substantial distributional uncertainty



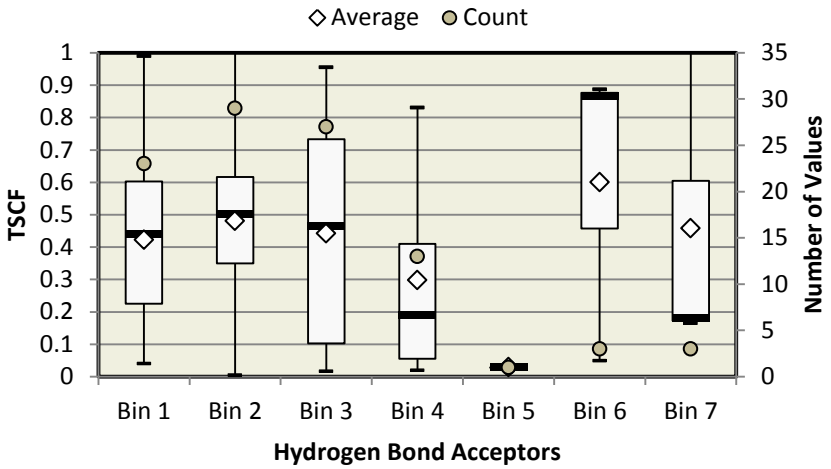
Boxplot for hydrophobicity, where the box represents the 25th to 75th percentile and whiskers are maximum and minimum values of TSCF (left axis). Circles indicate number of compounds in each range (right axis).



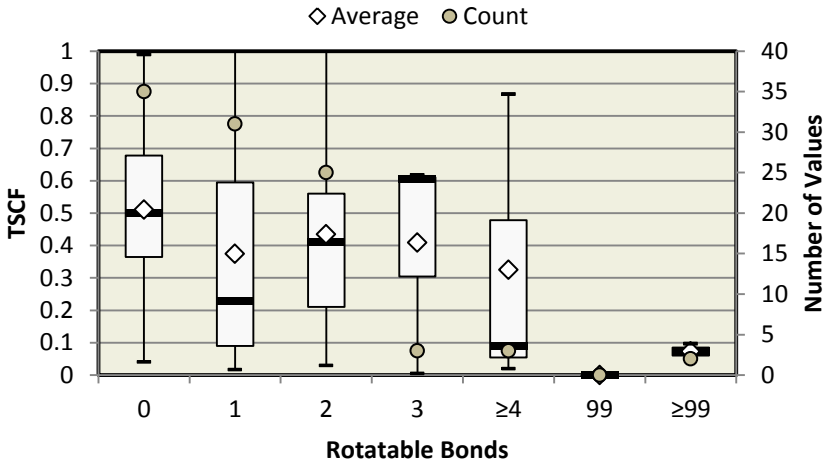
Boxplot for molecular mass, where the box represents the 25th to 75th percentile and whiskers are maximum and minimum values of TSCF (left axis). Circles indicate number of compounds in each range (right axis).



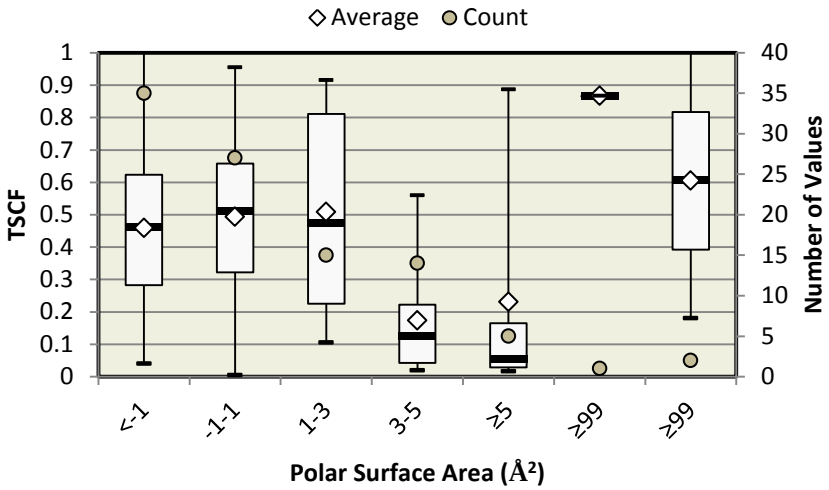
Boxplot for H-bond donors, where the box represents the 25th to 75th percentile and whiskers are maximum and minimum values of TSCF (left axis). Dots indicate number of compounds in each range (right axis).



Boxplot for H-bond acceptors, where the box represents the 25th to 75th percentile and whiskers are maximum and minimum values of TSCF (left axis). Dots indicate number of compounds in each range (right axis).



Boxplot for rotatable bonds, where the box represents the 25th to 75th percentile and whiskers are maximum and minimum values of TSCF (left axis). Dots indicate number of compounds in each range (right axis).



Boxplot for polar surface area, where the box represents the 25th to 75th percentile and whiskers are maximum and minimum values of TSCF (left axis). Dots indicate number of compounds in each range (right axis).

BETA REGRESSION

Only significant terms were left in the models, resulting in parameter estimates shown in the below table. In the log K_{ow} model, increases in predicted TSCF[1] were strongly correlated with increasing measured TSCF. However, increases in predicted TSCF were also correlated with less precision (i.e., more variance) in the measured TSCF. The addition of the PSA term explains additional variation, allowing the parameter estimate for predicted TSCF to increase. Compounds with large predicted TSCFs and large PSA result in lower measured TSCFs and increased variance.

Parameter estimates and significance for beta regression model

Model	Mean Parameter			Precision Parameter		
	Descriptor	Estimate	p-value	Descriptor	Estimate	p-value
Log K_{ow}	Intercept	-0.99	<0.0001	Intercept	1.27	<0.0001
	Sigmoidal	1.5	<0.0001	Sigmoidal	-0.96	<0.0001
Log K_{ow} + PSA	Intercept	-1.1	<0.0001	Intercept	1.1	<0.0001
	Sigmoidal	2.3	<0.0001			
	Sigmoidal *PSA	-1.0	0.003	Sigmoidal *PSA	-0.89	0.0045

DESIRABILITY FUNCTIONS

Desirability function parameter estimates

Parameter	D_{Kow}	D_{HBA}	D_{HBD}	D_{ROT}	D_{MW}	D_{PSA}
a	0.156	0.286	2.48E-09	4.77E-04	0.540	1.08
b	45.3	20.5	54.8	19.4	32.1	24.4
c	3.33	5.33	0.348	0.0258	286	59.6
d	0.152	8.01E-04	1.57	9.07	2.21E-05	53.1
e	1.36	4.95	9797	5870	99.6	5170
f	0.375	0.710	1.01	1.31	24.1	1.42
$D(x)_{i,max}$	15.9	8.09	20.7	9.42	11.4	13.3

QEPT EXAMPLE CALCULATION

For clarity, here we demonstrate the calculation of the QEPT for carbamazepine, a compound often observed to translocate in plants.[2-5] The only descriptor variables required are those which were given a nonzero weight. For carbamazepine, the log K_{OW}, MW and HBD are 2.67, 236 and 2, respectively. First, the desirability functions are calculated for each descriptor using the parameter estimates in Table .

$$D_{Kow}(x) = \frac{\left[1 - \frac{1}{1 + \exp\left(-\frac{2.67 - 3.33 - \frac{0.152}{2}}{0.375}\right)}\right]}{15.9} \cdot \left(0.156 + \frac{45.3}{1 + \exp\left(-\frac{2.67 - 3.33 + \frac{0.152}{2}}{1.36}\right)}\right) = 0.995$$

$$D_{MW}(x) = \frac{\left[1 - \frac{1}{1 + \exp\left(-\frac{236 - 286 - \frac{2.21 \cdot 10^{-5}}{2}}{24.1}\right)}\right]}{11.4} \cdot \left(0.540 + \frac{32.1}{1 + \exp\left(-\frac{236 - 286 + \frac{2.21 \cdot 10^{-5}}{2}}{99.6}\right)}\right) = 0.991$$

$$D_{HBD}(x) = \frac{\left[1 - \frac{1}{1 + \exp\left(-\frac{2 - 0.348 - \frac{1.57}{2}}{1.01}\right)}\right]}{20.7} \cdot \left(2.48 \cdot 10^{-9} + \frac{54.8}{1 + \exp\left(-\frac{2 - 0.348 + \frac{1.57}{2}}{9797}\right)}\right) = 0.394$$

The QEPT is then calculated using the weights combined with the desirability values determined above.

$$QEPT_{100} = \exp\left(\frac{0.56 \ln 0.995 + 0.76 \ln 0.991 + 0.64 \ln 0.394}{0.56 + 0.76 + 0.64}\right) = 0.73$$

The resulting QEPT demonstrates a high likelihood of translocation.

TSCF VALIDATION DATA

Simple statistics for validation data

Variable	Minimum	Maximum
Log K_{ow}	-0.63	6.0
MW	74	616
HBD	0	2
HBA	0	10
Rot	0	36
PSA	0	103.3

TSCF RAW DATA

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Triasulfuron	0.81	1.91	401.1	2	10	5	140.8	Soybean	6
Rimsulfuron	1.35	2.22	431.0	2	12	5	183.3	Soybean	6
Triasulfuron	0.92	1.91	401.1	2	10	5	140.8	Soybean	6
Bentazone	0.69	2.80	240.0	1	5	1	74.9	Soybean	6
Bentazone	0.78	2.80	240.0	1	5	1	74.9	Soybean	6
Primisulfuron-methyl	0.55	3.33	468.3	2	11	9	154.2	Soybean	6
Rimsulfuron	1.10	2.22	431.0	2	12	5	183.3	Soybean	6
Bentazone	0.92	2.80	240.0	1	5	1	74.9	Soybean	6
Triasulfuron	0.93	1.91	401.1	2	10	5	140.8	Soybean	6
Fenpropimorph	0.50	5.13	303.0	0	2	5	12.5	Soybean	6
Imazalil	0.95	3.56	296.0	0	3	6	27.0	Soybean	6
Fenpropimorph	0.31	5.13	303.0	0	2	5	12.5	Soybean	6
Imazalil	0.58	3.56	296.0	0	3	6	27.0	Soybean	6
Imazalil	0.32	3.56	296.0	0	3	6	27.0	Soybean	6
Primisulfuron-methyl	1.50	3.33	468.3	2	11	9	154.2	Soybean	6
Primisulfuron-methyl	0.55	3.33	468.3	2	11	9	154.2	Soybean	6

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Rimsulfuron	0.64	2.22	431.0	2	12	5	183.3	Soybean	6
Fenoxaprop	1.00	3.12	333.0	1	6	5	81.8	Soybean	6
Dimethoate	0.45	1.37	229.0	1	4	5	114.8	Soybean	7
Metalaxyl	0.58	1.76	279.1	0	5	6	55.8	Soybean	7
Carbendazim	0.86	1.52	191.0	2	5	1	67.0	Soybean	7
Simazine	0.82	2.28	201.0	2	5	2	62.7	Soybean	7
Linuron	0.91	3.13	248.0	1	4	2	41.6	Soybean	7
Iprodione	0.78	3.10	329.0	1	6	2	69.7	Soybean	7
Penconazole	0.71	4.64	283.0	0	3	5	30.7	Soybean	7
Aclonifen	0.42	3.28	264.0	2	5	4	81.1	Soybean	7
Carbendazim	0.97	1.52	191.0	2	5	1	67.0	Soybean	7
Aclonifen	0.53	3.28	264.0	2	5	4	81.1	Soybean	7
Dimethoate	0.67	1.37	229.0	1	4	5	114.8	Soybean	7
Fenoxaprop	0.40	3.12	333.0	1	6	5	81.8	Soybean	7
Iprodione	0.87	3.10	329.0	1	6	2	69.7	Soybean	7
Linuron	1.00	3.13	248.0	1	4	2	41.6	Soybean	7
Metalaxyl	0.63	1.76	279.1	0	5	6	55.8	Soybean	7
Penconazole	0.60	4.64	283.0	0	3	5	30.7	Soybean	7

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Simazine	0.76	2.28	201.0	2	5	2	62.7	Soybean	7
RDX	0.17	-2.04	222.0	0	12	3	147.2	Poplar	8
Aniline	0.32	1.14	93.1	2	1	1	26.0	Poplar	8
Phenol	0.48	1.54	94.0	1	1	1	20.2	Poplar	8
Nitrobenzene	0.30	1.92	123.0	0	3	1	45.8	Poplar	8
Benzene	0.74	2.18	78.0	0	0	0	0.0	Poplar	8
Trichloroethene	0.75	2.57	129.9	0	0	0	0.0	Poplar	8
Atrazine	0.57	2.64	215.1	2	5	2	62.7	Poplar	8
Toluene	0.63	2.72	92.1	0	0	0	0.0	Poplar	8
Ethylbenzene	0.79	3.23	106.1	0	0	1	0.0	Poplar	8
m-Xylene	0.78	3.27	106.0	0	0	0	0.0	Poplar	8
1,2,4-trichlorobenzene	0.04	3.82	179.9	0	0	0	0.0	Poplar	8
Pentachlorophenol	0.04	5.12	263.9	1	1	1	20.2	Poplar	8
SD207573	0.22	1.34	319.4	3	5	5	81.8	Soybean	9
SD208380	0.24	1.96	289.2	2	4	4	61.6	Soybean	9
SD208213	0.55	2.19	275.4	3	2	4	44.5	Soybean	9
SD98319	0.58	2.71	289.4	3	2	5	44.5	Soybean	9
SD204691	0.72	3.20	246.2	0	2	3	18.5	Soybean	9

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
SD205857	0.51	2.86	268.2	0	3	4	27.7	Soybean	9
SD204689	0.47	3.34	264.2	0	2	3	18.5	Soybean	9
SD96638	0.35	3.87	278.4	0	2	4	18.5	Soybean	9
SD204690	0.52	3.71	260.2	0	2	3	18.5	Soybean	9
SD204328	0.50	4.37	288.4	0	3	4	35.5	Soybean	9
SD95481	0.08	3.90	274.2	0	2	4	18.5	Soybean	9
SD208586	0.19	3.86	408.5	2	6	5	76.7	Soybean	9
Trichloroethene	0.21	2.57	129.9	0	0	0	0.0	Poplar	10
1,4-dioxane	0.72	-0.26	88.1	0	2	0	18.5	Poplar	11
Nonylphenol	0.07	6.04	220.2	1	1	9	20.2	Wheatgrass	12
Nonylphenol tetraethoxylate	0.05	5.20	396.3	1	5	21	57.2	Wheatgrass	12
Nonylphenol nonylethoxylate	0.05	4.02	616.4	1	10	36	103.3	Wheatgrass	12
HMX	0.18	-2.73	296.1	0	16	4	196.2	Poplar	13
Nonylphenol	0.01	6.04	220.2	1	1	9	20.2	Wheatgrass	14
Nonylphenol tetraethoxylate	0.03	5.20	396.3	1	5	21	57.2	Wheatgrass	14
Nonylphenol nonylethoxylate	0.02	4.02	616.4	1	10	36	103.3	Wheatgrass	14
Aldoxycarb	0.19	-0.37	222.0	1	6	4	93.2	Barley	15
Oxamyl	0.21	-0.47	219.0	1	6	4	96.3	Barley	15

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Acetone O-methylcarbamoyloxime	0.28	-0.13	130.1	1	4	2	50.7	Barley	15
Aldicarb	0.54	0.92	190.1	1	4	4	76.0	Barley	15
Benzaldehyde O-methylcarbamoyloxime	0.67	1.49	178.0	1	4	3	50.7	Barley	15
4-chlorobenzaldehyde O-methylcarbamoyloxime	0.94	2.27	212.0	1	4	3	50.7	Barley	15
3,4-dichlorobenzaldehyde O-methylcarbamoyloxime	0.51	2.89	246.0	1	4	3	50.7	Barley	15
3-phenoxybenzaldehyde O-methylcarbamoyloxime	0.27	3.38	198.1	0	2	3	26.3	Barley	15
3-(3,4-dichlorophenoxy)benzaldehyde O-methylcarbamoyloxime	0.06	4.59	266.0	0	2	3	26.3	Barley	15
3-mesylphenylurea	0.05	-0.12	214.2	3	5	2	97.6	Barley	15
Phenylurea	0.47	0.84	136.1	3	3	1	55.1	Barley	15
4-fluorophenylurea	0.47	1.13	154.1	3	3	1	55.1	Barley	15
3-(methylthio) phenylurea	0.22	1.57	182.2	2	3	2	66.4	Barley	15

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
4-chlorophenylurea	0.50	1.78	170.0	3	3	1	55.1	Barley	15
4-bromophenylurea	0.55	1.99	214.0	3	3	1	55.1	Barley	15
3,4-dichlorophenylurea	0.37	2.67	204.0	3	3	1	55.1	Barley	15
4-phenoxyphenylurea	0.47	2.80	228.0	3	4	3	64.4	Barley	15
4-(4-bromophenoxy)phenylurea	0.11	3.70	306.0	3	4	3	64.4	Barley	15
Simazine	0.90	2.28	201.0	2	5	2	62.7	Barley	16
Simazine	0.87	2.28	201.0	2	5	2	62.7	Barley	16
Simazine	0.93	2.28	201.0	2	5	2	62.7	Barley	16
Simazine	0.87	2.28	201.0	2	5	2	62.7	Barley	16
2,4-D	0.14	2.43	220.0	1	3	3	46.5	Barley	16
Atrazine	0.75	2.64	215.1	2	5	2	62.7	Barley	16
Diuron	0.81	2.68	232.0	1	3	1	32.3	Barley	16
Simazine	0.90	2.28	201.0	2	5	2	62.7	Barley	16
Chloroxuron	0.11	3.20	290.0	1	4	3	41.6	Bindweed	17
Chloroxuron	0.07	3.20	290.0	1	4	3	41.6	Gallant Soldier	17
Sulfolane	0.15	-0.43	120.0	0	2	0	42.5	Cattail	18
Diisopropanolamine	0.00	-1.05	133.0	3	3	6	52.5	Cattail	18

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Methyl tert-butyl ether	0.65	1.30	88.1	0	1	1	9.2	Poplar	19
4,4-sulfonyldianiline	0.38	0.99	248.1	4	4	4	94.6	Bean	20
Sulphathiazole	0.02	0.05	255.0	3	5	2	121.7	Bean	20
Sulphadiazine	0.15	-0.07	250.1	3	6	2	106.4	Bean	20
Sulphanilamide	0.23	-0.67	172.0	4	4	2	94.6	Bean	20
Sulphacetamide	0.10	-0.96	214.0	3	5	2	97.6	Bean	20
Sulphapyridine	0.02	0.47	249.1	3	5	2	93.5	Bean	20
Sulphaguanidine	0.06	-0.76	214.1	6	6	3	132.9	Bean	20
Heptachlor	0.13	5.46	373.3	0	0	0	0.0	Corn	21
Heptachlor	0.17	5.46	373.3	0	0	0	0.0	Wheat	21
Heptachlor	0.14	5.46	373.3	0	0	0	0.0	Tomato	21
Heptachlor	0.08	5.46	373.3	0	0	0	0.0	Bell Pepper	21
Heptachlor	0.07	5.46	373.3	0	0	0	0.0	Pumpkin	21
Heptachlor	0.18	5.46	373.3	0	0	0	0.0	Cucumber	21
Heptachlor	0.08	5.46	373.3	0	0	0	0.0	Cabbage	21
Heptachlor	0.05	5.46	373.3	0	0	0	0.0	Chinese Cabbage	21
Heptachlor	0.11	5.46	373.3	0	0	0	0.0	Lettuce	21

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
N-Nitrosodimethylamine	0.31	-0.50	74.0	0	3	1	32.7	Poplar	22
Trimethoprim	0.41	0.79	290.3	4	7	5	105.5	Cucumber	3
sulfamonomethoxine	0.04	-0.04	280.1	3	7	3	115.6	Cucumber	3
sulfamethoxazole	0.02	0.89	253.0	3	6	2	106.6	Cucumber	3
sulfadimethoxine	0.03	1.48	310.3	3	8	4	124.8	Cucumber	3
crotamiton	1.50	3.10	203.3	0	2	3	20.3	Cucumber	3
gliclazide	0.23	1.57	323.4	2	6	2	86.9	Cucumber	3
carbamazepine	0.69	2.67	236.2	2	3	0	46.3	Cucumber	3
losartan	0.02	3.57	422.9	2	7	9	92.5	Cucumber	3
cyclophosphamide	0.89	0.23	261.1	1	4	5	51.4	Cucumber	3
Ketoprofen	0.10	2.81	254.3	1	3	4	54.4	Cucumber	3
1,1,1-trichloroethane	0.27	2.35	131.9	0	0	0	0.0	Saltceder	23
1,1,1-trichloroethane	0.86	2.35	131.9	0	0	0	0.0	Saltceder	23
1,1,1-trichloroethane	0.64	2.35	131.9	0	0	0	0.0	Poplar	23
1,1,1-trichloroethane	0.83	2.35	131.9	0	0	0	0.0	Poplar	23
1,1,1-trichloroethane	0.77	2.35	131.9	0	0	0	0.0	Poplar	23
1,1,1-trichloroethane	0.84	2.35	131.9	0	0	0	0.0	Poplar	23
1,1,1-trichloroethane	0.25	2.35	131.9	0	0	0	0.0	Saltceder	23

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
1,1,1-trichloroethane	0.29	2.35	131.9	0	0	0	0.0	Poplar	23
Chloroform	0.27	1.94	117.9	0	0	0	0.0	Poplar	23
Chloroform	0.22	1.94	117.9	0	0	0	0.0	Poplar	23
Dichloromethane	0.49	1.41	83.9	0	0	0	0.0	Poplar	23
Ethylether	0.67	1.04	74.1	0	1	2	9.2	Poplar	23
Ethylether	0.90	1.04	74.1	0	1	2	9.2	Poplar	23
Methyl tert-butyl ether	0.63	1.30	88.1	0	1	1	9.2	Saltceder	23
Toluene	0.33	2.72	92.1	0	0	0	0.0	Poplar	23
Toluene	0.25	2.72	92.1	0	0	0	0.0	Poplar	23
Trichloroethene	0.10	2.57	129.9	0	0	0	0.0	Poplar	23
Trichloroethene	0.58	2.57	129.9	0	0	0	0.0	Poplar	23
Trichloroethene	0.29	2.57	129.9	0	0	0	0.0	Poplar	23
Trichloroethene	0.10	2.57	129.9	0	0	0	0.0	Poplar	23
Trichloroethene	0.26	2.57	129.9	0	0	0	0.0	Saltceder	23
1,1,1-trichloroethane	0.44	2.35	131.9	0	0	0	0.0	Soybean/Tomato	1
1,1,2,2-tetrachloroethane	0.36	2.33	165.9	0	0	1	0.0	Soybean/Tomato	1
1,2-dichloroethene	0.51	2.14	96.0	0	0	0	0.0	Soybean/Tomato	1
1,2-dichloropropane	0.63	2.01	112.0	0	0	1	0.0	Soybean/Tomato	1

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
1,4-dioxane	0.98	-0.26	88.1	0	2	0	18.5	Soybean/Tomato	1
Benzene	0.59	2.18	78.0	0	0	0	0.0	Soybean/Tomato	1
Caffeine	0.83	-0.63	194.1	0	6	0	58.4	Soybean/Tomato	1
Carbon tetrachloride	0.44	2.92	151.9	0	0	0	0.0	Soybean/Tomato	1
Chloroform	0.69	1.94	117.9	0	0	0	0.0	Soybean/Tomato	1
Dichloromethane	0.46	1.41	83.9	0	0	0	0.0	Soybean/Tomato	1
Methanol	0.88	-0.69	32.0	1	1	0	20.2	Soybean/Tomato	1
Methyl tert-butyl ether	0.82	1.30	88.1	0	1	1	9.2	Soybean/Tomato	1
Pentachlorophenol	0.07	5.12	263.9	1	1	1	20.2	Soybean/Tomato	1
Phenanthrene	0.15	5.55	178.1	0	0	0	0.0	Soybean/Tomato	1
N-nitrosodimethylamine	0.97	-0.50	74.0	0	3	1	32.7	Soybean/Tomato	1
Nonylphenol	0.18	6.04	220.2	1	1	9	20.2	Soybean/Tomato	1
Nonylphenol nonylethoxylate	0.07	4.02	616.4	1	10	36	103.3	Soybean/Tomato	1
Nonylphenol tetraethoxylate	0.21	5.20	396.3	1	5	21	57.2	Soybean/Tomato	1
Pyrene	0.04	5.00	202.1	0	0	0	0.0	Soybean/Tomato	1
Sulfolane	0.86	-0.43	120.0	0	2	0	42.5	Soybean/Tomato	1
tert-butyl alcohol	0.80	0.58	74.1	1	1	1	20.2	Soybean/Tomato	1
Tetrachloroethene	0.30	3.07	163.9	0	0	0	0.0	Soybean/Tomato	1

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Toluene	0.64	2.72	92.1	0	0	0	0.0	Soybean/Tomato	1
Trichloroethanol	0.99	0.97	147.9	1	1	1	20.2	Soybean/Tomato	1
Trichloroethene	0.43	2.57	129.9	0	0	0	0.0	Soybean/Tomato	1
1,2,3-trichlorobenzene	0.01	3.77	179.9	0	0	0	0.0	Wheat	24
1,2,3-trichlorobenzene	0.02	3.77	179.9	0	0	0	0.0	Tomato	24
2,4-dinitrotoluene	0.78	2.08	182.0	0	6	2	91.6	Wheat	24
2,4-dinitrotoluene	1.16	2.08	182.0	0	6	2	91.6	Tomato	24
Atrazine	1.14	2.64	215.1	2	5	2	62.7	Wheat	24
Atrazine	0.90	2.64	215.1	2	5	2	62.7	Tomato	24
Diclocymet	0.71	3.84	312.0	1	3	4	52.9	Spinach	25
Diclocymet	0.39	3.84	312.0	1	3	4	52.9	Soybean	25
Diethofencarb	0.54	2.97	267.0	1	5	7	56.8	Spinach	25
Diethofencarb	0.35	2.97	267.0	1	5	7	56.8	Soybean	25
Diniconazole-M	0.59	4.34	325.0	1	4	5	50.9	Spinach	25
Diniconazole-M	0.33	4.34	325.0	1	4	5	50.9	Soybean	25
Furametpyr	0.63	3.07	333.1	1	5	2	56.2	Spinach	25
Furametpyr	0.30	3.07	333.1	1	5	2	56.2	Soybean	25
Procymidone	0.73	2.93	283.0	0	3	1	37.4	Spinach	25

Chemical Name	TSCF	Log K_{ow}	Molecular Weight	HB Donors	HB Acceptors	Rotatable Bonds	PSA ($Å^2$)	Plant	Source
Procymidone	0.69	2.93	283.0	0	3	1	37.4	Soybean	25
Pyriproxyfen	0.09	5.50	321.1	0	4	7	40.6	Spinach	25
Pyriproxyfen	0.19	5.50	321.1	0	4	7	40.6	Soybean	25
Nitrobenzene	0.82	1.92	123.0	0	3	1	45.8	Ash	26
Nitrobenzene	0.75	1.92	123.0	0	3	1	45.8	Honeysuckle	26
Nitrobenzene	0.74	1.92	123.0	0	3	1	45.8	Poplar	26
Nitrobenzene	0.60	1.92	123.0	0	3	1	45.8	Russian Olive	26
Nitrobenzene	0.76	1.92	123.0	0	3	1	45.8	Soybean	26
RDX	0.16	-2.04	222.0	0	12	3	147.2	Poplar	27

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APPENDIX C.

Supporting Information:

Phytoscreening for Perchlorate

EXPERIMENTAL PHOTOS

Trees and 1-L reactors prior to week 1 harvest

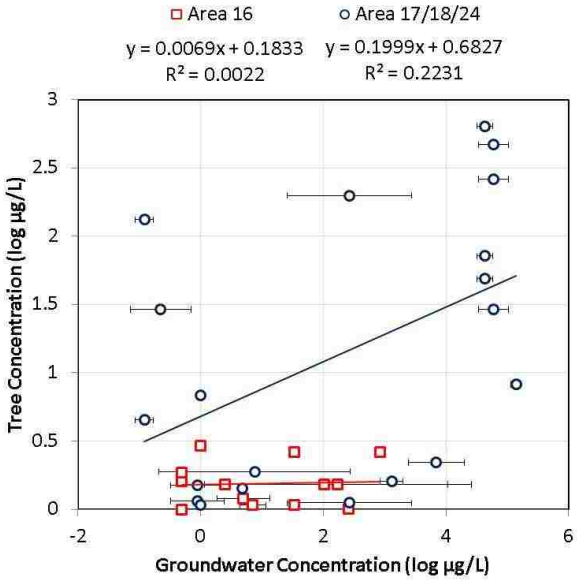
Trees and 1-L reactors after week 2 harvest

Trees and 1-L reactors prior to week 3 harvest

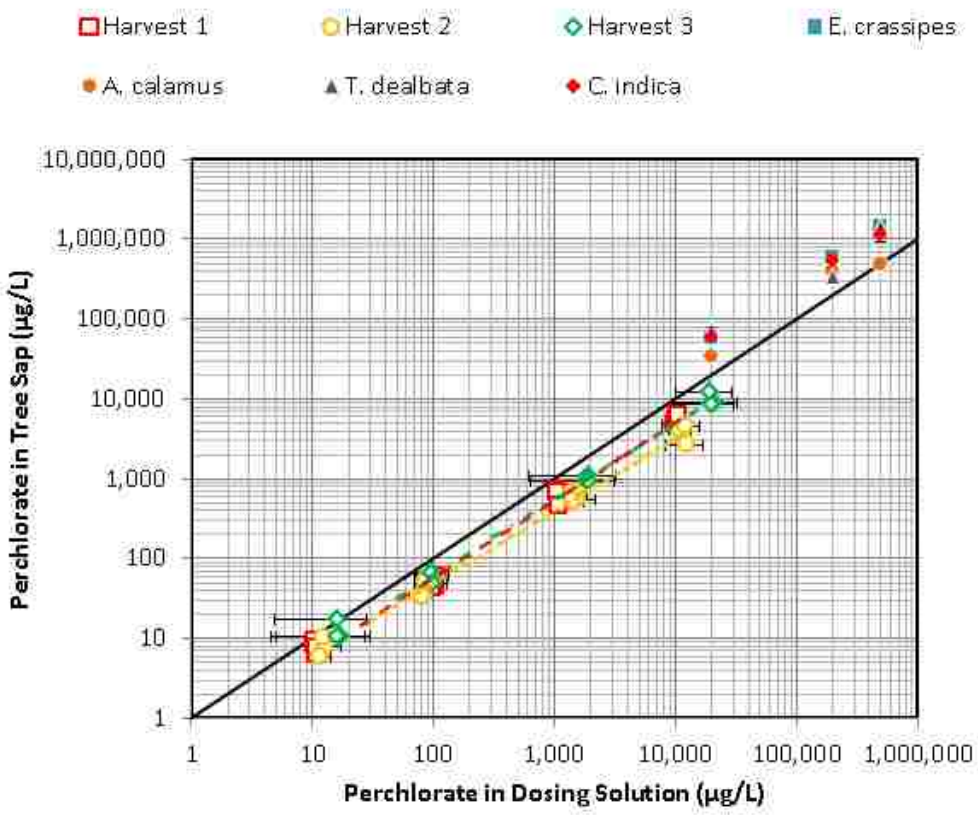
Water usage by greenhouse trees

Exposure Period	**Water Transpired (mL)**	
	Average	Standard Deviation (n=13)
7 Days	223	55.6
14 Days	928	166
21 Days	2065	264

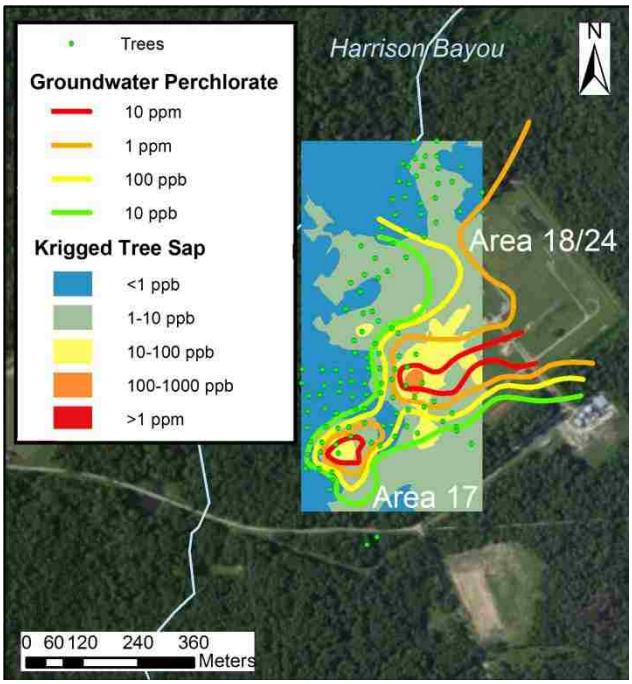
PLANT-WATER DOSING RELATIONSHIPS



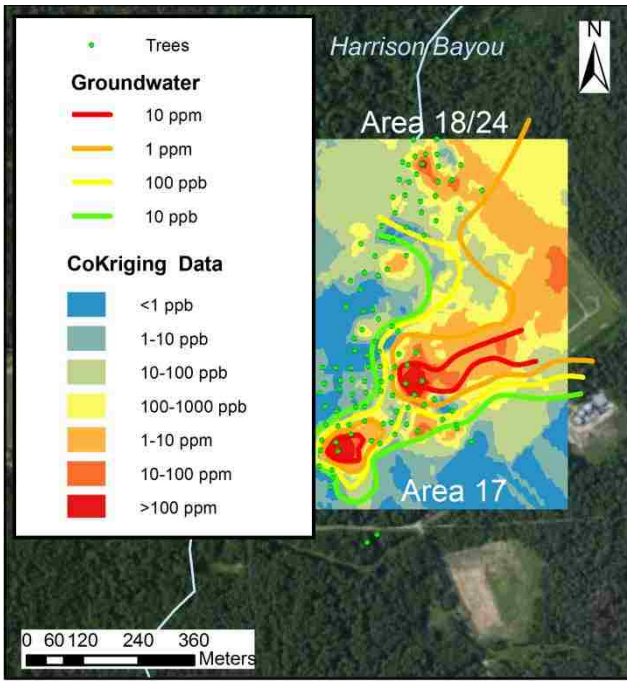
Paired groundwater well and tree perchlorate concentrations



Correlation between tree sap and dosing solution perchlorate concentrations for each exposure duration. The black line indicates a 1:1 fit and the horizontal error bars denote the range of exposure concentrations during the week prior to harvest (n=2). Solid data points represent experimental data from wetland shoots grown hydroponically.[1] For these wetland species, concentrations were converted from mg/kg fresh weight to mg/L sap by assuming a 50% water content and negligible perchlorate mass in all non-aqueous phases.



LHAAP site map with krigged tree sap perchlorate and groundwater perchlorate isoconcentration contours.



LHAAP site map with cokrigged tree sap and groundwater perchlorate overlaid with groundwater perchlorate isoconcentration contours

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