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M. J. I. Mattina et al., *Environ. Chem.* **2004**, *1*, 86–89. doi:10.1071/EN04048

Rhizotron Study of Cucurbitaceae: Transport of Soil-Bound Chlordane and Heavy Metal Contaminants Differs with Genera

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Environmental Context. *Many pesticides are difficult to remove from the soil, and remain as persistent pollutants. Some plant species can extract these pollutants from the soil and thereby degrade them, leading to a potential plant-based soil remediation technology. This study examines how plants extract an enduring organic pollutant (chlordane) and heavy metals (zinc, cadmium) from the soil, where they are processed in the plant, and what end-products are generated.*

Abstract. Two Cucurbitaceae, *Cucurbita pepo* L. and *Cucumis sativus* L., were grown in rhizotrons containing soil contaminated with heavy metals and highly weathered chlordane residues. This experimental scheme allowed in situ access to several portions of the soil/plant system. In the root exudates, concentrations of low molecular weight organic acids (LMWOAs) collected from both genera were detected consistently in the order malic > citric > succinic. Xylem sap was collected from severed plant stems over recorded time segments. Chlordane components and elemental analytes in the sap showed distinct, consistent differences between *C. pepo* L. and *C. sativus* L. with regard to chlordane flux, enantiomeric fractions of chlordane components, and heavy metal content. This is the first detailed report of patterns of heavy metals and persistent organic pollutants (POPs) in the contiguous compartments of soil, whole root tissue, xylem sap, and aerial tissue.

Keywords. chlorinated organics — metals — phytoremediation

Manuscript received: 18 May 2004.
Final version: 4 August 2004.

Persistent organic pollutants (POPs) have been found in environmental compartments in every region around the globe. Since many POPs were intended as agrochemicals, the soil compartment remains a large reservoir of the contaminants. Terrestrial plants, therefore, are particularly susceptible to POP contamination. Studies of plant uptake of soil-bound POPs by our group and others[1–3] have established the unique status of *Cucurbita pepo* L. (zucchini) in its ability to accumulate the pollutants in its roots and to transport them to aerial tissues. POPs as structurally diverse as chlordane, DDE, and dioxins and furans, have been detected in *C. pepo* roots, as well as stems, leaves, and fruit. These POPs are organochlorine compounds which are highly hydrophobic ($\log K_{OW} > 6$) and become sequestered as they age in the soil matrix.[4] We have contrasted *C. pepo* L. with another Cucurbitaceae, *Cucumis sativus* L. (cucumber), in which uptake and transport within the plant are considerably lower.[2] Other researchers have suggested different pathways of contaminant uptake for *C. pepo* L. versus *C. sativus* L.[1] In these prior studies conclusions regarding soil/plant interactions, as well as air/plant interactions, have been drawn based on field cultivation conditions and analyses of whole plant tissues—roots, stems, leaves, fruit—and the source contaminant compartment, e.g. soil or air.[5]

Most POPs are recent, anthropogenic, and toxic environmental contaminants; plants have not evolved mechanisms for their uptake and accumulation. Similarly, the plant's accumulation of toxic, soil-borne heavy metals does not derive from a demand of plant physiology. If such contaminants are found in plants, it is reasonable to assume that the analytes are opportunistically crossing the root membrane, probably on the 'coattails' of mechanisms evolved for acquisition of materials essential to the plant's physiology.[6]

Studies of the mechanisms of POPs contaminant release from the soil matrix and uptake by plants are scarce.[7,8] In order to investigate in detail the fate and transport of POPs within the soil/plant system, variations inherent in field studies must be minimized and experiments conducted

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1448-2517/04/020086

preferentially in situ. Such studies are important to assess exposure risks for humans and wildlife accurately and to establish fundamental mechanisms underlying transport of hydrophobic, anthropogenic organic pollutants from the exterior to the interior regions of the plant.

Accordingly, eight rhizotrons[9] were constructed from 48 cm × 12 cm PVC pipe cut in half lengthwise and filled with 3.5 kg of a well characterized, contaminated soil.[10] Very briefly, the soil, derived from a location on the New Haven campus of the Connecticut Agricultural Experiment Station, was treated in 1960 with technical-grade chlordane as part of a crabgrass control experiment. Date and rate of treatment as well as the subsequent history of the site are documented explicitly. The rhizotrons were planted with *C. pepo* L. cv. 'Black Beauty' or *C. sativus* L. cv. 'Marketmore'. Both 'dense' and 'non-dense' planting schemes were used for each cultivar, as summarized in Table 1. The rhizotrons were watered automatically twice daily throughout a six to eight week growing period in the greenhouse.

At the second week of plant growth, root systems had developed sufficiently to allow collection of root exudates for determination of LMWOAs. Quartz fibre filters (no. 1851-032, Whatman) were placed in contact with individual roots in situ.[11] The filters were set on a small piece of polyethylene to isolate them from the surrounding soil and kept moist throughout the four-hour collection period. After collection, [D_6]succinic acid was added as internal standard, and the filters were frozen, freeze dried, and derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluroracetamide (Regis Technologies) to form the volatile esters. Analysis of the esters was by gas chromatography–mass spectrometry (GC-MS). The number of quartz fibre filters analyzed from each rhizotron is given in Table 1.

At six to eight weeks stems of individual plants were severed approximately 1 cm above the level of the soil and stem exudates were collected by means of capillary action.[12,13] Sap collection time and volume were recorded. After collection of the sap, the plants were destructively harvested, and root and aerial plant tissue were collected, washed, and weighed. The plant tissues as well as the pre-planted soil were extracted and analyzed for chlordane components by previously published methods.[5] Chlordane components in the sap fractions were determined by solid phase microextraction fibres (SPME) using a method recently described by us.[14] All chlordane components were quantified using a chiral Gamma-Dex 120 GC column (Supelco) interfaced to a Saturn 2000 ion trap MS (Varian) by ^{13}C internal standard calibration, with the five chlordane-component sum ('TN', *trans*-nonachlor; '±TC', enantiomers of *trans*-chlordane; '±CC', enantiomers of *cis*-chlordane) as the total.[10,15] The chiral chlordane component, MC-5, has been described in previous publications by Falconer and others.[16,17] Chiral heptachlorepoxide (HEPX) is a metabolite of heptachlor, another component of technical chlordane. However, heptachlor was applied as a pesticide independently from technical chlordane. For this reason we have never included it in our quantitation schemes, although it has been consistently included in our calibration standards. Elemental analyses of the soil, plant tissues, and sap were accomplished using inductively coupled plasma atomic emission spectroscopy (ICP-AES) or graphite furnace atomic absorption spectrophotometry (GFAA).[18]

Data from the analysis of root exudates will be discussed first. It was mentioned above that plant acquisition of POPs and toxic heavy metals may be expected to 'piggyback' on existing biochemical pathways. For example, mechanisms evolved by plants for nutrient acquisition (e.g. P, Fe), including the exudation of LMWOA chelators which deconstruct the soil matrix, may concurrently mobilize soil-sequestered POPs and heavy metals.[19] We have hypothesized[6] that the exceptional status of *C. pepo* derives from exudation of large quantities of LMWOAs, in contrast to *C. sativus* for which neither citric nor malic acid was detected in root exudates.[20]

In the present study a large range in the absolute amounts of acids collected on the filters, both within a given rhizotron and between rhizotrons, was noted. For example, the sum of LMWOAs per filter ranged from a low of 9 ng to a high of 8970 ng across the 109 separate filters analyzed in all eight rhizotrons. There was a large standard deviation for the average quantities collected on the filters within a given rhizotron. Because of this wide range, the data are best viewed as providing a qualitative profile of LMWOA exudation and are presented on a relative basis in Fig. 1. Several comments are in order. First, for almost every filter analyzed, malic acid was found in highest quantity, usually an order of magnitude higher than either of the other two acids which were detected

Table 1. Rhizotron study

Rhizotron identification[A]	Description	No. quartz fibre filters
#1, ZND	single *C. pepo* L. plant	17
#2, ZND	single *C. pepo* L. plant	8
#3, ZD	ten *C. pepo* L. plants	19
#4, ZD	ten *C. pepo* L. plants	12
#5, CND	single *C. sativus* L. plant	10
#6, CND	single *C. sativus* L. plant	10
#7, CD	thirteen *C. sativus* L. plants	10
#8, CD	ten *C. sativus* L. plants	13

[A] Z = *C. pepo*; C = *C. sativus*; ND = not dense; D = dense.

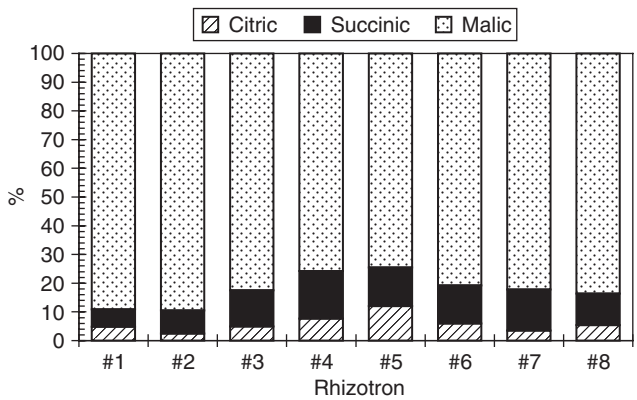

Fig. 1. Relative amounts of LMWOAs in root exudates. See Table 1 for identification of rhizotrons.

in measurable amounts. This contradicts the previous study that reported neither malic nor citric acids in root exudates from *C. sativus*.[20] Second, because of the wide range in the standard deviations, no meaningful statistical comparison of exudation from *C. pepo* versus *C. sativus* was possible. Thus, at this point we cannot test our hypothesis that LMWOA exudation plays a causal role in distinguishing uptake in *C. pepo* and *C. sativus*. Nevertheless, these data provide the first qualitative assessment of LMWOA exudation from Cucurbitaceae in situ.

Next, we consider the distribution of the contaminants among the soil source compartment and various plant compartments as summarized in Table 2. There are several comments to be made regarding these data. First, we note that the 'sum 5' chlordane concentration in the whole root tissue from rhizotron-grown *C. pepo* plants is similar to that from *C. sativus* plants. This contrasts with previous field studies in which the bioconcentration factor ($BCF = C_{roots}/C_{soil}$, where C is concentration) for *C. pepo* was typically eight times larger than that for *C. sativus*.[5]

Next, we note that in spite of its hydrophobicity, chlordane residues were detected in the aqueous plant sap at the low ng mL^{-1} level. Third, we note some interesting patterns of enantiomer fractions ($EF = C_{+en}/[C_{-en} + C_{+en}]$, where the subscripts refer to the (±)-enantiomer) across the values listed in Table 2. The commercial technical-grade chlordane formulation used as an agrochemical contained over 140 separate components, several of which are optically active compounds and exist as two, non-superimposable isomers called enantiomers. If $EF = 0.5$, the two enantiomers are present in equal amounts and the mixture is racemic; if $EF \neq 0.5$, the mixture is non-racemic. EF values for the chlordane residues are the same in the soil and whole root tissue for both *C. pepo* and *C. sativus*. However, there are distinct differences in the EFs for *exo*-HEPX between (a) *C. pepo* sap and the soil, (b) *C. sativus* sap and soil, and (c) the *C. pepo* sap and *C. sativus* sap. Similar comments may be made for the EF values for CC. Particularly noteworthy are EF values for the MC-5 component. This compound is essentially racemic (EF 0.5) in the soil and whole root tissue, but is non-racemic in the sap from *C. pepo* and well below the limits of detection in the sap of *C. sativus*. Obviously, the transport of the chlordane compounds from the external to internal plant regions of this soil/plant system involves enantioselective processes which are distinctly different for the two genera.[14]

Since the sap was collected over carefully recorded time segments, we gain additional insight regarding flows within the plant, as the data in Fig. 2 show. The data in Fig. 2a show the sap flow rate in mL h^{-1} as a function of the root biomass per plant. Apparently, under the conditions of the rhizotron experiments, the osmotically driven xylem exudate flow, J_v, in units of mL h^{-1} g^{-1} of root weight,[12] is equivalent for both *C. pepo* and *C. sativus* (R^2 0.78 for the combined dataset; R^2 0.78 for *C. pepo*; R^2 0.78 for *C. sativus*). If we now consider chlordane, we note that the chlordane flow, in ng h^{-1}, for the two genera correlate very differently with root biomass per plant, as shown in Fig. 2b (R^2 0.56 for the combined dataset). From the slopes of the two lines in Fig. 2b we conclude that the chlordane flux in *C. pepo* (R^2 0.94) is more than three times greater than that in *C. sativus* (R^2 0.95).

Finally, we comment on the differences noted between *C. pepo* and *C. sativus* in regard to transport within the plants for zinc and cadmium. From the data in Table 2 we note that transport of Zn for both genera is similar. The situation is quite different for Cd, with *C. pepo* exhibiting transport (although Cd concentrations are two orders of magnitude less than for Zn), and no transport apparent for *C. sativus*. While it was assumed in earlier work that Zn and Cd transport similarly, the present data are consistent with the recent observation that the transport behaviour of these two Group IIB elements may diverge in closely related plants.[21]

The data from these rhizotron experiments permit us to draw novel and important conclusions regarding the mechanisms of uptake and transport of soil-sequestered

Table 2. Pollutants in various soil/plant compartments

Average values across two rhizotrons for both planting regimes for each genus and each plant compartment

Parameter	Soil	Whole root				Sap				Whole aerial[A]			
		pepo		*sativus*		*pepo*		*sativus*		*pepo*		*sativus*	
		ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
Sum 5[B]	3348	44831	41262	48067	51432	47.9	16.8	27.1	18.9	3059.1	2770.2	1303.1	1127.3
EF HEPX	0.59	0.59	0.60	0.58	0.58	0.64	0.63	0.45	0.43	0.67	0.67	0.44	—[D]
EF TC	0.45	0.48	0.48	0.46	0.46	0.51	0.45	0.5	0.42	0.50	0.48	0.40	0.39
EF CC	0.55	0.55	0.55	0.54	0.55	0.56	0.60	0.48	0.46	0.53	0.55	0.45	0.45
EF MC-5A[E]	0.48	0.48	0.49	0.48	0.48	0.30	0.32	—[C]	—[C]	0.24	0.22	0.47	0.48
Cd[F]	2	11	12.5	6.5	6.5	0.01	0.008	—[C]	—[C]	2	2	—[D]	—[D]
Zn[F]	56	319	117.5	167.5	256.5	0.8	0.7	0.4	0.2	187	226	—[D]	—[D]

[A] Composite of all above soil plant tissue.
[B] Sum of five chlordane components (TN, +TC, –TC, −CC, +CC); ng g^{-1} dry weight for soil and plant tissue, ng mL^{-1} for sap.
[C] Below limits of detection.
[D] No sample available for analysis.
[E] No absolute assignment of MC-5 enantiomers available; enantiomers designated as A and B, see refs [16,17].
[F] Units are μg g^{-1} in soil and plant tissue; μg mL^{-1} for sap.

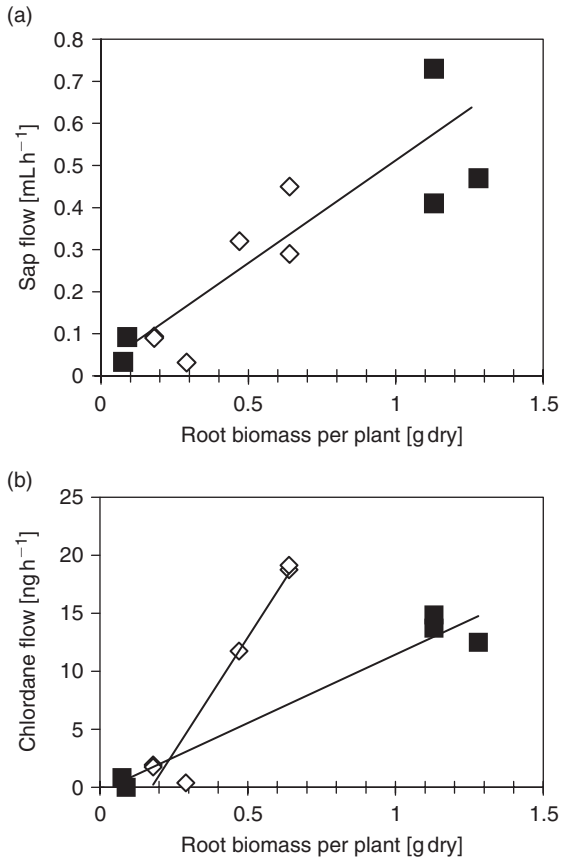

Fig. 2. Flow rate of (a) collected sap and (b) chlordane, as function of root biomass for *C. pepo* (◇) and *C. sativus* (■).

contaminants. First, it is probable that the concentration of contaminant associated with whole root tissue consists of a portion of the contaminant adsorbed to the root surface together with a portion contained within the plant interior. Except for tuberous crops which can be peeled, such as beets, carrots, and potatoes, whole root analysis will be a composite of both portions. Therefore, whole root tissue data should be used cautiously in drawing conclusions regarding contaminant partitioning in soil/plant systems. Second, under the conditions used in these rhizotron experiments, osmotically driven water flow in the xylem of *C. pepo* and *C. sativus* is similar. Chlordane flux in the xylem sap, however, is dissimilar in the two genera, being at least three times greater in *C. pepo*. Furthermore, chlordane transport within the plant for the two Cucurbitaceae investigated exhibits different enantioselective passage for some of the chiral components. Whether the enantioselectivity results from passive diffusion of the chlordane components through root lipid bilayers, through membrane-spanning protein channels, or by means of active transport cannot be concluded from the present data alone. Experiments are in progress to further elucidate the mechanisms of uptake and transport. Finally, we note the distinct difference between *C. pepo* and *C. sativus* in regard to the uptake and transport of Zn and Cd. Whether this difference in elemental transport relates to mechanisms of chlordane transport cannot be determined solely from the present data.

These rhizotron studies provide detailed knowledge of the soil/plant system not available previously, with particular emphasis placed on the difference between water and chlordane flow within the plants for the two genera of Cucurbitaceae. These studies together with data from additional rhizotron studies in progress will provide fundamental knowledge regarding interactions at the rhizosphere, which will impact applied phytoremediation technologies in the future.

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