PCDD/PCDF-MOBILIZING COMPOUNDS IN ROOT EXUDATES OF ZUCCHINI

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Introduction

High affinity to the soil organic matter and extreme hydrophobicity (log $K_{O/W}$ > 4) are the main physicochemical characteristics of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) limiting their mobility in soils, and the translocation within plants[1-3]. Recent studies with many plant species show that PCDD/PCDF accumulate mainly by atmospheric deposition[4-7]. As an exception, uptake of PCDD/PCDF via the roots and subsequent translocation to the shoots was demonstrated for zucchini (*Cucurbita pepo* L.)[8]. Root exudates of zucchini plants grown in sand culture are able to mobilize PCDD/PCDF from contaminated soils (Fig. 1A). In contrast, no mobilization of PCDD/PCDF was detectable compared to a control of distilled water, when root exudates of tomato - a plant species with proven minimal soil-plant transfer of PCDD/PCDF - were used for soil extraction. Probably, certain compounds in the root exudates attach to PCDD/PCDF, forming polar adducts, thus facilating root uptake as well as transport within the plant of the *per se* extremely hydrophobic PCDD/PCDF. A chemical characterization of these compounds was the aim of the present study

Materials and Methods

Plant culture and exudate sampling: Zucchini and tomato plants were cultivated in glass tubes (2.5 L) filled with quartz sand (particle size 3.5 - 3.0 mm), and supplied constantly with nutrient solution by use of a peristaltic pump. Periodic collection of root exudates was started 6 weeks after sowing. The tubes were submerged for 60 min with 1 L of distilled water and subsequently percolated. The percolate was filtered (Whatman GF-D), vacuum concentrated (9 L□10 mL) and stored at -80°C. Insoluble precipitates were removed by centrifugation. Xylem sap was collected 10 weeks after sowing. Plant shoots were decapitated, silicon tubes were fitted to the stumps, and the extruding xylem sap was collected in a bottle which was cooled to 2°C, and subsequently stored at -80°C.

Laboratory experiments: ^{14}C-tetrachlorodibenzo-p-dioxin (TCDD) was used as model-substance to test the ability of differentially fractionated samples of root exudates and xylem sap to bind PCDDD/PCDF. 5 µl of ^{14}C-TCDD was added to 10 mL of the exudate sample (final TCDD concentration = 50 pg ml^{-1}) and incubated on a shaker for 2 hours in the dark. Subsequently, ^{14}C-TCDD was extracted by petrolether, and partition of the radioactivity in the organic and aqueous phase was determined by liquid scintillation counting. Methanol fractionation of exudate samples was carried out with cold (-20°C) methanol (final concentration: 80% v/v) to separate hydrophilic macromolecules from low-molecular weight and lipophilic compounds. Precipitated macro-molecules were collected by centrifugation and redissolved in H_2O. Methanol was evaporated from the soluble fraction, and the ability to bind TCDD was tested for both fractions. Selective separation of proteins was achieved by trichloroacetic acid (TCA) precipitation (12% w/v). After

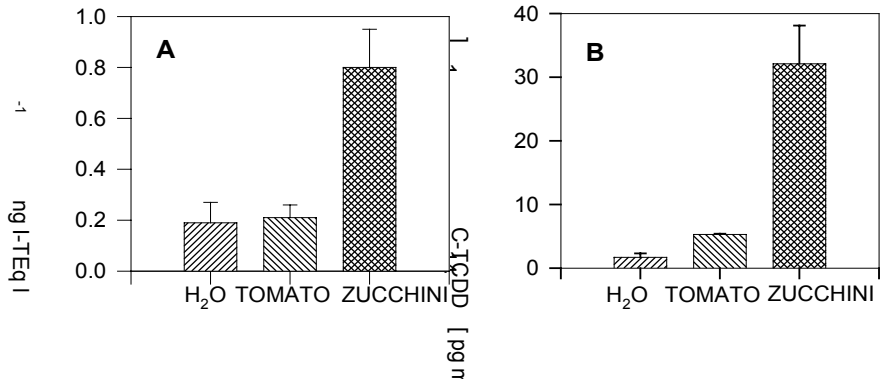
centrifugation, the precipitate was neutralized with NaOH, redissolved in H_2O, and binding of TCDD was tested for both theTCA-insoluble fraction and the TCA soluble supernatant. Gel-permeation chromatography of the macromolecular fractions was performed on a Sephadex G-50 column (Eluent: 20 mM Tris-HCl, 100 mM NaCl pH 8.0; flow rate: 0.9 ml min^{-1}; temp.: 5°C), and SDS-polyacrylamide electrophoresis according to Schägger and v. Jagow[9].

Results and Discussion

^{14}C-tetrachlorodibenzo-p-dioxin (TCDD), employed as a model-substance to test the ability of root exudates to bind PCDD/PCDF, was almost completely re-extracted from water and from root exudates of tomato by use of petrolether as a lipophilic solvent. However, in root exudates of zucchini about 65% of the applied ^{14}C-TCDD remained in the aqueous phase after petrolether extraction (Fig. 1B). The results suggest that certain compounds in the root exudates of zucchini attached to theTCDD molecules forming polar adducts, which increased the water solubility of TCDD. Similar results were obtained with other organohalogen compounds such as ^{14}C-hexachlorobiphenyl , and ^{14}C-hexachlorobenzene (not shown).

Figure1A: PCDD/PCDF concentrations in distilled water and in root exudates of tomato and zucchini used for extraction (2 h) of a contaminated soil [14530 ng I-TEq kg^{-1} dry matter]

Figure1B: Amounts of exogenously applied ^{14}C-TCDD [50 pg ml^{-1}] remaining in distilled water, and in root exudates of zucchini and tomato after petrolether extraction.



^{14}C-TCDD was also retained in xylem sap and in tissue extracts of zucchini, suggesting an ubiquitous distribution of PCDD/PCDF-binding compounds in the whole zucchini plant (Table 1). After methanol-precipitation of root exudates, xylem sap and tissue extracts, only small amounts of ^{14}C-TCDD remained in the methanol-soluble fractions containing low molecular weight and lipohilic compounds. The major proportion of ^{14}C-TCDD was bound in the methanol-insoluble fraction of root exudates and xylem sap where macromolecules such as polysaccharides and proteins are predominant (Table 1)

Table 1: Distribution of ^{14}C-TCDD [pg ml^{-1}] in methanol-[80% v/v]-soluble and -insoluble fractions of root exudates, xylem sap, and tissue extracts of zucchini after petrolether extraction.

Fraction	Root-exudate	Xylem-sap	Extract Root	Extract Leaf-petiole	Extract Fruit
total sample	**31.5** ± 4.0	**18.2**	**57.2**	**50.8**	**44.8**
methanol soluble fraction (low molecular and lipophilic compounds)	**7.6** ± 2.7	**1.8**	**12.1**	**4.6**	**1.5**
methanol insoluble fraction (macromolecular compounds)	**34.7** ± 13.0	**13.7**	n.d.	n.d.	n.d.

n.d. = not determined since complete resolubilisation was not possible

Trichloroacetic acid (TCA) (12% w/v) was used for selective precipitation of proteins. ^{14}C-TCDD was predominatly retained in the TCA-insoluble fraction of the root exudates (Table 2), suggesting that PCDD/PCDF-binding compounds in root exudates of zucchini are polypeptides.

Table 2: Distribution of ^{14}C-TCDD [pg ml^{-1}] in TCA-[12% w/v] -soluble and -insoluble fractions in root exudates of zucchini (2 independent replicates).

Whole root exudate	TCA-soluble fraction	TCA-insoluble fraction	TCA [12% w/v] control
29.7 24.9	11.0 11.4	22.8 25.5	3.1 2.3

After separation of methanol-insoluble compounds in the root exudates of zucchini by gel-permeation chromatography (GPC),^{14}C-TCDD was retained in 4 fractions (F1- F4, Fig. 2). Maxima of UV absorption at 280 nm, which is characteristic for proteins, corresponded with the maxima of ^{14}C-TCDD bound in the different fractions (F1, F4). GPC of xylem sap revealed binding of ^{14}C-TCDD in up to 8 fractions (data not shown).

Proteins in the TCDD-binding fractions of root exudates and xylem sap were subsequently concentrated by TCA precipitation and further analyzed by SDS-PAGE[9] (not shown). Similar polypeptide patterns were found in whole root exudates and in xylem sap as well. The analysis of ^{14}C-TCDD-binding fractions obtained after GPC, revealed the presence of several polypeptides with corresponding bands in a molecular-weight range between 55 and 70 KDa, both in root exudate and xylem sap samples.

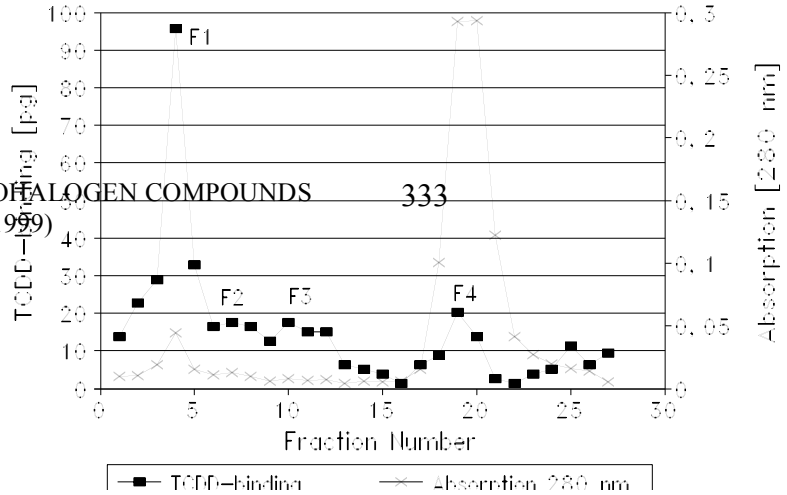


Fig. 2: Gel-permeation chromatography (Sephadex G-50) of methanol-insoluble compounds in root exudates of zucchini**.** Binding of ^{14}C-TCDD (F1-F4) and UV-absorption at 280 nm.

Conclusions

Insolubility in methanol (Table 1) and in dilute TCA (Table 2) as well as UV absorption at 280 nm (Fig. 2) indicate that PCDD/PCDF binding compounds in root exudates of zucchini are polypeptides. Corresponding polypeptide patterns in the TCDD-binding fractions of both root exudates and xylem sap, suggest that roots of zucchini may release PCDD/PCDF-binding polypeptides into the rhizosphere which are subsequently taken up by the roots and translocated to the shoot via xylem transport[8)]. The mechanisms of PCDD/PCDF-mobilization from the soil matrix as well as the mechanisms for root-uptake of PCDD/PCDF-binding polypeptides are still unknown.

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