PCDD/PCDF-complexing Compounds in Zucchini

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Introduction

Recent studies show that polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF) accumulate in plants largely through atmospheric deposition, whereas soil-related pathways are neglegible[1-4]. This behavoiur can be ascribed to the high affinity of PCDD/PCDF for soil organic matter and the resulting immobility in soils, as well as to the lack of translocation of the extremely hydrophobic PCDD/PCDF within plants[5-7].
An exception to the above discribed occurence are zucchini for which we could demonstrate an uptake of PCDD/PCDF *via* the roots and subsequent translocation to the shoots[8]. In a follow up experiment we showed that exudates released by zucchini roots were capable of extracting PCDD/PCDF from a contaminated soil. We further found that certain compounds in zucchini root exudates probably attach to PCDD/PCDF molecules forming polar complexes, thus facilitating root uptake as well as transport within the plant of the *per se* extremely hydrophobic PCDD/PCDF[9].

The main objective of the present study was to chemically characterize the substances in zucchini responsible for the mobilization of PCDD/PCDF. For comparative purposes tomato - a plant species with proven minimal soil-plant transfer of PCDD/PCDF[8] - was included in the experiments. In addition to the mobilization of PCDD/PCDF in the root zone, the aspect of translocation within the plant was also investigated.

Experimental

Collection of root exudates. Zucchini and tomato plants were cultivated in glass tubes (2.5 l) with quartz sand (particle size 2.5-3.0 mm) and supplied constantly with nutrient solution. Root exudates were collected six and eight weeks after sowing for zucchini and tomato respectively. The tubes were water logged with 1l of doubly destilled water for 10 min. and subsequently percolated. The percolate was filtered (Whatman GF/D) and concentrated by rotavaporization (9l → 10 ml).
Collection of xylem sap. Zucchini and tomato plants were harvested eight/ten weeks after sowing respectively. At this time the collection of xylem sap was performed. For this purpose, plant shoots were decapitated and silicon hoses fitted to the stumps in order to collect the extruding xylem sap.
Laboratory experiments. To chemically characterize the substance in zucchini responsible for the uptake and translocation of PCDD/PCDF, the collected plant saps had to be factionated. In a preceeding experiment we found that PCDD/PCDF in combination with

zucchini root exudates are less hydrophobic than their K_{ow} values predict. Therefore, the root exudates as well as xylem saps were gradually extracted with solvents of increasing polarity namely, petroleum ether, ethyl acetate, methanol, and water. Prior to the extraction the plant saps were spiked with ^{14}C-TCDD so that, by means of liquid scintillation, the fraction containing the complexing substance could be determined.

Results

In Figure 1 the partitioning of TCDD from zucchini and tomato root exudates and from water into solvents of increasing polarity is depicted. There is no difference in the partitioning of ^{14}C-TCDD spiked to water or to tomato root exudates. After the extraction with petroleum ether in a separation funnel, more than 98% of the applied activity could be detected in the petroleum ether phase for both variants. A completely different behaviour can be observed for TCDD in combination with zucchini root exudates, where only 10% partitioned into the petroleum ether phase. The majority of the applied TCDD was methanol extractable.
While TCDD in tomato root exudates and in water shows a partitioning behaviour which is in accordance with the K_{ow}-value, the affinity of TCDD to methanol in zucchini root exudates suggests that a complex consisting of the hydrophobic TCDD and a relatively non-polar substance (soluble in methanol) had been formed, thus verifying the results of our preceeding experiment[9].

As for the mobilization of PCDD/PCDF in the soil, the transport within the plant is only feasible if the extreme hydrophobicity of PCDD/PCDF is compensated. As already pointed out, this can possibly be achieved by the formation of a complex between PCDD/PCDF and a polar compound. Therefore, xylem sap was included in the investigations.The fractionation of the xylem sap was performed in a similar way to the method applied for root exudates.

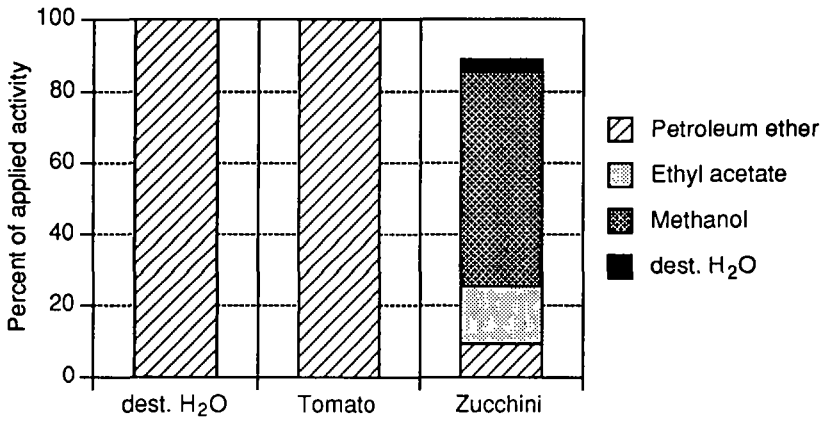

Figure 1: Partitioning of TCDD from H_2O and root exudates into solvents of different polarity; measured as ^{14}C-activity

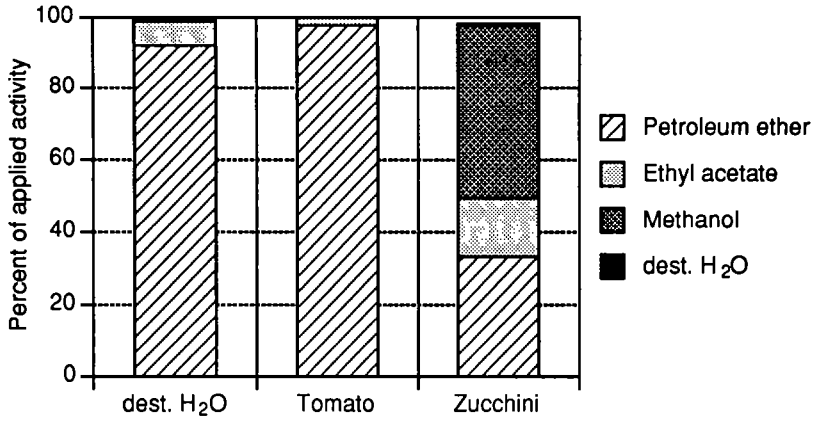

Figure 2: Partitioning of TCDD from H_2O and xylem sap into solvents of different polarity; measured as ^{14}C-activity

The results of the gradual xylem sap extraction with different solvents are shown in Figure 2. While tomato xylem sap did not alter the polarity of TCDD in zucchini xylem sap approximately 50% of the spiked TCDD partitioned into the methanol phase. Similar results were obtained with leaf extracts of zucchini which are summarized in Figure 3. Again, the majority of the spiked TCDD was methanol extractable. The latter property of the zucchini leaf extract suggests that the compound responsible for the uptake and translocation of PCDD/PCDF is ubiquitous in the whole zucchini plant.

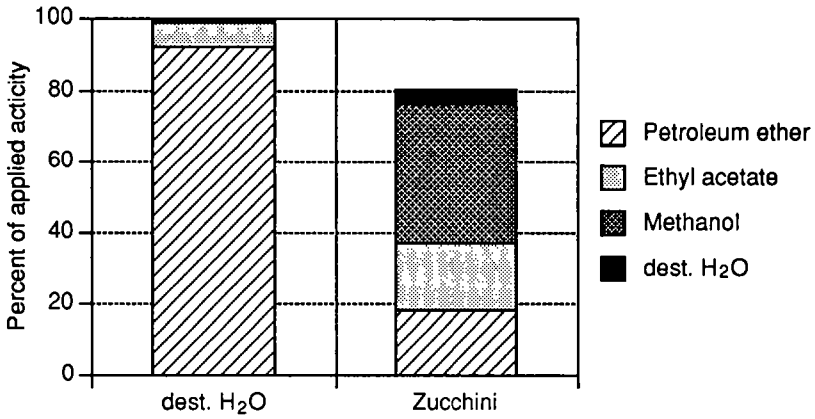

Figure 3: Partitioning of TCDD from H_2O and zucchini leaf extracts into solvents of different polarity; measured as ^{14}C-activity

Conclusions

The objective of the study was to chemically characterize the PCDD/PCDF mobilizing substance in zucchini plants. The gradual extraction of zucchini and tomato root exudates with solvents of increasing polarity confirmed that certain compounds in zucchini root exudates compensate the hydrophobicity of TCDD. Since TCDD is not likely to form chemical bonds, this behaviour is probably due to the formation of complexes between the compound in question and the TCDD molecule. Of the applied solvents the TCDD complex showed the highest solubility in methanol. This suggests that the relevant substance might be a glycosidic phenolic compound.
As could be demonstrated by the extraction of leaf extracts, the PCDD/PCDF complexing substance seems to be distributed in the whole zucchini plant.

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