Plant uptake of sewage sludge-associated dioxin-like compounds - estimation of levels using a sensitive avian bioassay

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Introduction

Application of sewage sludge to agricultural land is widely recognized as an economic way of recycling nutrients. However, sewage sludges are contaminated with a complex mixture of organic compounds, and the potential bioavailability to plants of toxic organics from sludge may limit sludge utilization. One important group of toxic organics present in sewage sludge is the one with compounds having an Ah-receptor-mediated mechanism of action. These dioxin-like compounds include the PCDDs/Fs, the PCBs and many others. If estimating the risk for plant uptake of the complex mixture of dioxin-like compounds present in sewage sludge by using a chemical analysis approach, important toxic compounds may be overlooked. In addition, levels of dioxin-like compounds provide only part of the information necessary to evaluate their potential biological effects, since the individual compounds differ in toxicity, and the ways they interact are quite unknown. Bioassays can therefore be useful tools for screening the toxic potencies of mixtures of such compounds. By this approach, the integrated effect of all dioxin-like compounds present in the samples is registered, including unknown dioxin-like compounds. An *in vitro* bioassay for such purposes has been developed in our laboratory [1-3]. This assay is based on the fact that chicken embryo livers respond to very low concentrations of dioxins by showing enhanced 7-ethoxyresorufin *O*-deethylase (EROD) activities. By using this bioassay, we estimated the uptake of sludge-borne dioxin-like compounds into carrots, which may represent the worst case scenario, and oil seed rape, which due to its oil content also may accumulate organics. The experiments were done using sludge samples from Swedish sewage treatment plants (STPs) with differing degrees of contamination of dioxin-like compounds. We did one experiment in pots, and one open field experiment.

Materials and methods

Anaerobically digested sewage sludge was collected from STPs in the Swedish cities Stockholm (Henriksdal STP) and Göteborg (Ryaverken STP), from the STPs in the towns Gävle, Uppsala, Huskvarna, Jönköping and Borås and from the STP in the Uppsala suburb Storvreta.

Sewage sludge samples from the Borås STP and from the STP in Stockholm, both having relatively high levels of dioxin-like compounds, as determined in the chicken embryo liver EROD induction bioassay [4], were used in the pot experiment. The sludge-fertilized soil (sludge application rate 9 t (d.w.) per ha) and the control soil (no sludge) were put into plastic pots in which carrot or rape seeds were sown. After 84 and 101 days of outdoor culturing, respectively, the carrots and rape seeds were harvested and extracted and analyzed as described below.

On a normal agricultural plot, having a light clay soil, 50 by 50 cm squares were amended with sewage sludge from the STPs in Gävle, Storvreta, Uppsala, Stockholm, Huskvarna, Jönköping and Göteborg (application rates between 36-55 tons (d.w.)/ha). After 82 and 125 days of culturing, respectively, the carrots were harvested and extracted and analyzed as described below.

Carrots from each treatment group were cut in slices (2-3 mm) in a food mixer and 15 g of rape seeds were taken from each treatment group and pooled. The samples were extracted 2x2 hours with n-hexane in an ultrasonic bath. The extracts were filtered and then eluted through a deactivated (10% H_2O) silica column (10x200 mm) with n-hexane (120 ml) as mobile phase.

The extracts were re-dissolved in DMSO before addition to the organ cultures of livers from chicken embryos. The bioassay was carried out as described in Brunström et al. [5]. Essentially, livers of 8-day-old embryos were exposed to concentration series of the extracts in organ cultures for 48 h (5 livers/concentration). As a positive reference TCDD (10^{-10} M) was used. EROD activities in the livers were determined as described in Brunström et al. [6].

The maximal induction rates of the concentration-response curves for the various samples were generally lower than that of the positive reference (10^{-10} M TCDD). Therefore, the effective concentration values were calculated on the basis of the induction caused by 10^{-10} M TCDD rather than the maximum induction of each sample. The concentration of the sample which caused 25% of the TCDD-induced maximum EROD activity was defined as $EC_{25\,TCDD}$ for the sample. The $EC_{25\,TCDD}$-values were used for the comparisons between samples and for the calculations of the concentrations of bioassay TCDD-equivalents (bio-TEQs) in the samples. The bio-TEQ-concentrations were calculated as:

$$\text{bio-TEQs (pg/g)} = \frac{\text{TCDD EC}_{25}\ \text{(pg/ml)}}{\text{extract EC}_{25\,\text{TCDD}}\ \text{(g/ml)}}$$

The TCDD EC_{25}-value is a mean value calculated from a large number of concentration-response curves for TCDD.

Results and discussion

The pot experiment

The carrot extracts contained very low levels of EROD-inducing compounds, and the rape seeds had levels below the detection limit of the bioassay. The levels in the carrots, expressed as concentrations of bioassay-derived dioxin-like compounds, varied between 0.2 and 0.6 pg bio-

TEQs/g d.w, which may be compared to the level in the control soil (2.1 pg bio-TEQs/g d.w.). Levels in the sewage sludge samples have previously been determined and range from 7 to 109 pg bio-TEQs/g d.w. [4]. The Borås sludge sample and the Stockholm sludge sample which were used in the experiments had 109 and 38 pg bio-TEQs/g d.w., respectively. Levels in the carrots grown in soil applied with sludge from Borås were similar to those in carrots grown in the two control soils (with or without NH_4NO_3) (Fig. 1). The levels of bio-TEQs in the carrots grown in soil amended with sludge from the Stockholm STP were slightly higher than the others (Fig. 1). The reason that the less contaminated sludge from Stockholm yielded more contaminated carrots may be that more easily bioaccumulated dioxin-like compounds were present in this sludge sample, or that other components in the sludge influenced the plant uptake.

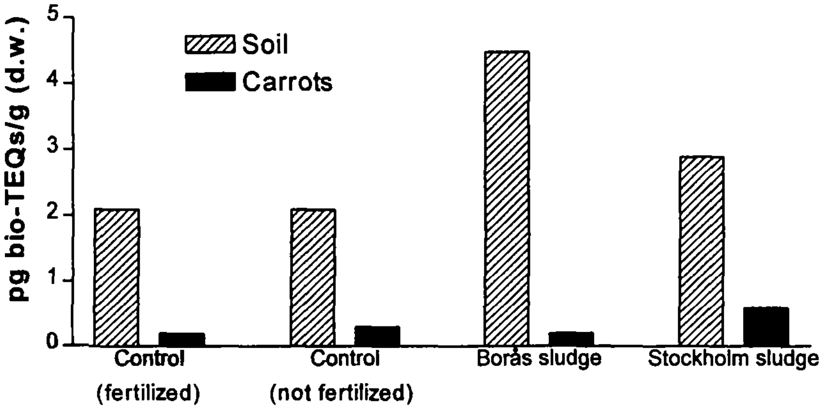

Figure 1. Concentrations of dioxin-like compounds in soil fertilized with sewage sludge (application rates 9 tons/hectare) from Borås and Stockholm and in fertilized/unfertilized control soil and resulting levels of bio-TEQs in carrots grown in these soils.

The field experiment
In the field experiment the levels in the carrots were between 2 and 15 pg bio-TEQs/g d.w. (Fig. 2). This is considerably higher than in the carrots from the pot experiment, and is probably a result of the higher sludge application rates (36-55 t (d.w.)/ha). However, the soil type in this experiment was a light clay soil and in the pot experiment it was a sandy loam.

No correlation was found between applied amounts of sludge-borne dioxin-like compounds and levels of bio-TEQs in the carrots (Fig. 2). Another study has also reported no relationships between HOC concentrations in soil amended with sewage sludge contaminated with PCB and other organics and levels in corn and carrots [7].

Following sludge application, the dioxin-like compounds may have remained sorbed to sludge particles, become dissolved in soil water or transferred to soil solids. This partition process is dependent on the physicochemical properties of the individual compounds and the soil and sludge properties. It is possible that the sludge samples themselves from the STPs had differing properties in this aspect, resulting in differing partitioning between phases, which may have resulted in different carrot bioavailabilities of the dioxin-like compounds. For instance, differing concentrations of organic matter in the sludge may influence this. The organic matter content of sludge has been reported to range from 16 to 65%, and to comprise of a heterogeneous mixture of different constitutents [8]. Thus, the large variability in sludge quality may have impact on plant availability of the dioxin-like compounds. In addition, the composition of dioxin-like compounds in the sludge samples probably differed, and thus their physicochemical properties may have differed, resulting in variable uptake rates into the carrots.

In conclusion, sewage sludge amendment (in moderate application rates) when growing carrots did not yield notably high carrot concentrations of dioxin-like compounds. However, the risk analysis is complicated by the fact that no correlation could be found between application of sludge-borne dioxin-like compounds and resulting levels in the carrots. In addition, the effect of long-term application of sludge on carrot uptake of dioxin-like compounds remains to be investigated.

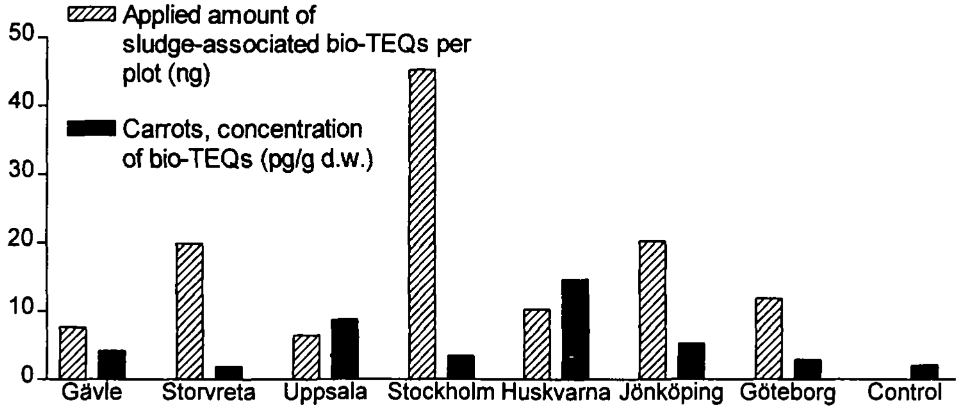

Figure 2. Applied amounts of sludge-associated dioxin-like compounds to the 50 x 50 cm plots on which carrots were grown and resulting levels of bio-TEQs in these carrots. The sludge application rates ranged between 36 and 55 tons/hectare.

Acknowledgements

This study was financially supported by the Swedish Council for Forestry and Agricultural Research. The staff at the Horticultural Research Station, Swedish University of Agricultural Sciences are thanked for their technical assistance.

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