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SUPERINDUCTION IN THE DR-CALUX BIOASSAY BY EXTRACTS FROM SOIL SAMPLES TAKEN DURING A SOIL BIOREMEDIATION PROCESS

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Introduction

The use of mechanism-specific bioassays has a large potential in many areas, including toxicity characterisation of contaminated soil. Also during different types of remediation techniques of soil, bioassays serve a useful purpose, since they may shed some light on changes in bioactivity of soil pollutants before, during and after a specific remediation process. Bioremediation of polluted soil is now an established technique and efficient bioremediation techniques exist for the removal of polycyclic aromatic hydrocarbons (PAHs). However, more needs to be elucidated about formation of more toxic metabolites during such remediations. Many PAHs are also AhR agonists [1, 2], which makes bioassays for dioxin-like compounds useful tools in this context. One of the most common bioassays is the DR-CALUX, which has been adapted in our lab for the study of dioxin-like compounds in soil. In this project the DR-CALUX dioxin bioassay [3] was used to study dioxin-like toxicity before, during and after bioremediation of PAH-contaminated soil in Sweden.

Methods and Materials

PAH-contaminated soil was bioremediated at Sydkraft SAKAB AB in Kumla, Sweden. Proprietary organic amendments with specific particle size and nutrient profile were added (2% of total soil volume) for optimal conditions for the contaminant-degrading microorganisms. The treatment was performed during the spring and summertime 2002 and was done in a large tent at temperatures between 25 and 45 °C. Soil samples were collected after 0, 7 and 66 days of treatment. The samples were extracted (24h soxhlet with toluene) and cleaned up using deactivated silica or sulphuric acid- and KOH impregnated multilayer silica. The deactivated silica removes macromolecules, while the multilayer silica also removes acid degradable compounds such as most PAHs. The DR-CALUX cells were exposed for the extracts (0 to 400 mg soil d.w/ ml) in triplicates for 24 hours. TCDD (0 to 300 pM) was used as a reference. Dioxin-like compounds induce luciferase, which can be detected by measurements of luminescence. The DR-CALUX bioassay detects all compounds having dioxin-like effects in the samples, including novel Ah receptor agonists.

Results and Discussion

The samples contained very high levels of dioxin-like compounds as determined by the DR-CALUX. The dioxin-like activity of the deactivated silica cleaned-up extract decreased with 21% during the treatment time (table 1). Almost all of the activity was due to acid-degradable compounds, since only 1-2% of the dioxin-like effect remained after eluting the extracts through a sulphuric acid- and KOH impregnated multilayer silica. The extract cleaned up with sulphuric acid- and KOH impregnated multilayer silica did not display any reduction in dioxin-like activity during the soil bioremediation, which is not surprising, since the classical dioxin-like compounds (PCDDs/Fs, coplanar PCB etc) are resistant to biodegradation. Chemical analysis showed that

very high PAH concentrations were present, and that they were reduced by 75-80% after 66 days of treatment.

From the concentration-response curves for the soil extracts, CALUX-derived toxicity equivalents (CALUX-TEQs) were estimated. Using chemical analysis data for the PAHs in combination with CALUX-specific relative potency values for PAHs [1], PAH-derived TEQ concentration in the soil samples were calculated. These chemical PAH-TEQs were compared with the CALUX-TEQs (table 1). In the CALUX assay, both extracts cleaned up using deactivated silica or sulphuric acid- and KOH impregnated multilayer silica were tested. The deactivated silica removed macromolecules, while the multilayer silica also removed acid-degradable compounds such as most PAHs. The comparison between these two fractions indicates the proportion of the total bio-TEQ that may be attributed to acid-degradable (i.e. non-persistent) compounds. The difference in CALUX-TEQs between the fraction from the deactivated silica gel clean-up and the corresponding fraction from the multilayer clean-up constitutes the easily degradable fraction of the sample, probably containing most of the PAHs. This difference is shown as CALUX PAH TEQs in table 1. It should be noted that this degradable fraction can contain many other compounds except PAHs, so the naming of this fraction to CALUX PAH TEQs just shows that most of the PAH probably are present in it. The CALUX PAH TEQ concentration may be compared to the chemical PAH-TEQ. After the treatment only 21% of the CALUX PAH TEQs had disappeared in contrast to 67% for the PAHs determined by chemical analysis (chemical PAH TEQs). This suggests that the CALUX bioassay detected presence of non-analysed PAHs or other AhR agonists in the final soil sample. These may be PAH metabolites that act as AhR agonists. Oxy-PAHs such as benzanthrone and benz[a]anthracene-7,12-dione have in vitro AhR-mediated activity [1].

Table 1. CALUX derived TEQs[1,2,3] and chemical[4] in the bioremediated soil.

	7 days of treatment (pg/g)	**66 days of treatment (pg/g)**
CALUX TEQ (deactivated)[1]	60 900	48 300
CALUX TEQ (multilayer)[2]	1000	990
CALUX PAH TEQ[3]	59 900	47 310
Chemical PAH TEQ[4]	79 500	26 200

[1] CALUX TEQs in deactivated silica cleaned extracts.
[2] CALUX TEQs in multilayer (sulphuric acid impregnated) cleaned extracts.
[3] Difference between CALUX TEQs in deactivated silica gel cleaned extracts[1] and CALUX TEQs in multilayer cleaned extracts[2]. This is an estimation of CALUX TEQs due to non persistent compounds, such as PAHs. For convenience, this difference is called CALUX PAH TEQs, even though the authors are fully aware that many other compounds than PAHs may be present in the non-persistent fraction.
[4] PAH concentrations from chemical analysis multiplied with PAH-REPs (relative potency factors) for the US EPA priority 16 PAHs derived from Machala et al [1].

A superinduction (higher maximum induction than the reference TCDD) of luciferase was observed in the deactivated silica gel cleaned extracts from day 7 (fig. 1) and day 66 (data not shown). In multilayer cleaned extracts no superinduction was observed. Thus, the factor causing superinduction was trapped in the multilayer silica columns, thus being degradable or trapped in very acidic or alkaline conditions. The identity of this factor in the soil samples is unknown to us, but several cellular mechanisms leading to superinduction of CYP1A1 have been described in the

literature. Superinduction of CYP1A1 gene expression has been showed to occur after exposure to TCDD and cycloheximide, probably as a result of decreased degradation of agonist-activated AhR [4]. The superinduction by cycloheximide is suggested to be due to the protein synthesis inhibition properties of this compound. This implicates that a cycloheximide sensitive factor, controlling the removal of agonist-activated AhR from the nucleus is affected by cycloheximide. Another mechanism that would result in a superinduction is presence of proteasome inhibitors (e.g. lactacystin), which may stop the ubiquitin-proteasome pathway-mediated degradation of AhR [5]. Superinduction of EROD has been reported by 12-O-tetradecanoylphorbol-13-acetate (TPA) (2.8- and 2.2-fold increase) in MCF-7 cells treated with TPA for 72 or 96 hours compared to MCF-7 cells treated with TCDD alone [6]. The mechanism behind this superinduction was not discussed by the authors. A potentiation interaction was also observed when testing corticosteroids, that alone induced a very weak response in the CALUX, but greatly enhanced the induction by TCDD [7]. It remains to be elucidated if the superinduction observed by us also has significance for in vivo dioxin-like toxicity of the soil extracts described in this study.

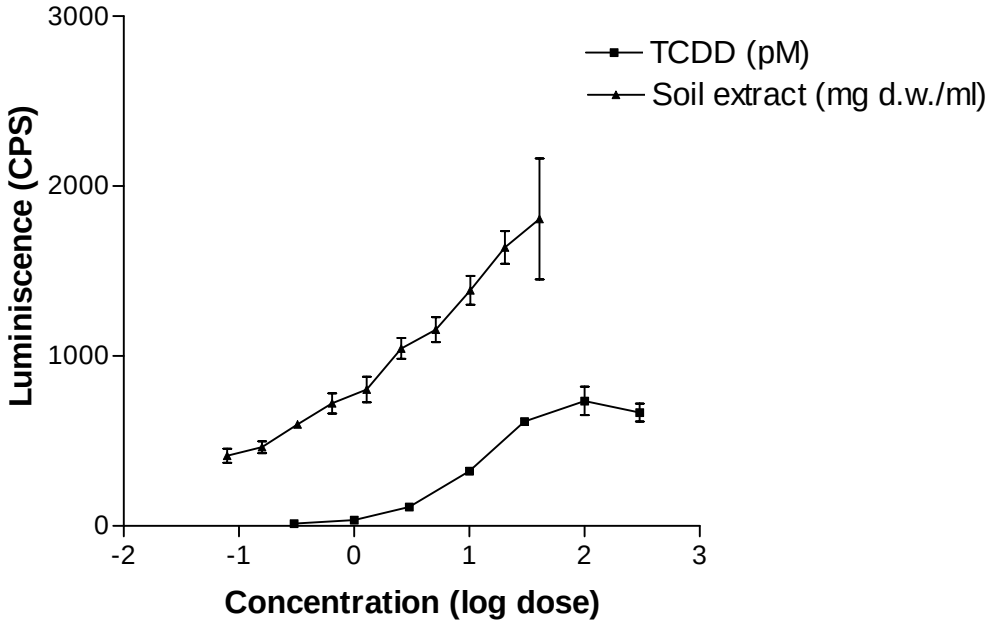

Figure 1. Superinduction in the DR-CALUX by silica gel cleaned up extracts from soil taken after 7 days of bioremediation treatment. CPS=counts per second.

Conclusions

The high DR-CALUX-TEQs levels indicate that very high concentrations of AhR agonists were present in the soil samples. The superinduction observed was due to acid- or alkaline-degradable compounds, unknown at this stage. Of the total CALUX TEQ levels in the soil samples from both sampling occasions, around 98% were due to acid- or alkaline-degradable compounds. The

CALUX bioassay detected presence of non-analysed PAHs or other AhR agonists in the final soil sample, indicating that metabolites with AhR agonist properties may have been formed during the bioremediation. No reduction in persistent dioxin-like compounds was observed during the bioremediation.

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