Production of reference soils for ecotoxicological field studies using supercritical CO_2-extraction.

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This work reports on the extraction of cadmium from contaminated soils using supercritical carbon dioxide (SC-CO_2). To this purpose, two soils with different characteristics - mainly in clay and organic matter content - were freshly spiked with a Cd-salt and subjected to SC-CO_2 extraction (SFE) at different conditions. As SC-CO_2 was unable to extract heavy metals, a complexing agent had to be added which both binds cadmium and is soluble in SC-CO_2. Three different complexing were investigated, i.e. ethylenediamine, hexafluoroacetylaceton and Bis(2,4,4-trimethylpentyl)monophosphinic acid (Cyanex 302). It is found that Cyanex 302 results in the highest extraction efficiency. The extraction efficiency is soil type dependent. The soil, containing more clay minerals and organic matter, retains more Cd.
Both soils were extracted at the optimal conditions and were subjected to different ecotoxicological tests, including a bacteriological test, a plant test and a test with compost worms. The bacteriological test and the plant test with Garden cress confirm that less Cd is available in the soil after SFE. However, also some changes in soil nutrients are found affecting the growth of the Garden cress. Compost worms that obtain their food both from the soil pore water and by ingestion of soil particles, are less affected by the slight changes in soil characteristics induced by SC-CO_2 in the control cultures and, as a result, benefit more from the lower Cd-content of the soil.

INTRODUCTION

Ecotoxicological field studies of contaminated soils are often hampered by the lack of non-contaminated matched controls. These are soils with identical characteristics as the contaminated ones, that can be used to generate the reference values for ecotoxicity testing. Being able to refer to such reference soils is crucial, because soils are very heterogeneous and only minute differences in soil composition may affect the bioavailability and hence the toxicity of pollutants. As a result, an extraction technique is needed that removes the contaminants from the soil without introducing major changes in soil characteristics.
It is well-known that compounds can be extracted from a solid matrix at relatively mild conditions using supercritical carbon dioxide (SC-CO_2). This extraction treatment (SFE) does not leave a residue, because the remaining CO_2 becomes gaseous and evaporates from the matrix. Although SFE has been applied extensively for the extraction of organic compounds, only limited information is available on the extraction of metals.
In this paper, it is investigated whether SFE can be applied to extract cadmium from contaminated soils. The research is performed on two soils and the optimal extraction conditions are determined. The extracted soils are subjected to different ecotoxicological tests, including a bacteriological test, a plant test and a test with compost worms.

I - MATERIALS AND METHODS

Materials

Two soils were selected, based on their textural properties and on their lack of heavy metal contamination. The soil Borris2 originates from Denmark and consists of 39.5 wt% of fine sand particles smaller than 200 μm. Its clay content is 6.9 wt%. The soil Kettering originates from Great Brittan and consists of only 13.2 wt% of fine sand particles smaller than 200 μm. Its clay content amounts to 24.5 wt%.
The metal-complexing ligand ethylenediamine was purchased at Merck; hexafluoroacetyl-acetone (HFA) was purchased at Sigma-Aldrich. The ligand Bis(2,4,4-trimethylpentyl)-monophosphinic acid (Cyanex 302) was kindly provided by Cytec Industries.

Methods and analysis

The two soils were intentionally doped with cadmium using a $CdCl_2$-solution. The applied concentration range was from 0 mg/kg up to 500 mg/kg. The volume of the spiking solution was taken large enough so as to assure uniform doping (volume ratio of 0.2). The contaminated soils were stored at 25 ^oC during 2 weeks in a open recipient and further dried in an oven. After drying the soil was homogenized.

The extractions were performed on a laboratory scale apparatus (ISCO SFX^{TM}220), designed for a maximum pressure of 510 bar and a maximum temperature of 150^oC. In the apparatus, the CO_2-fluid was pressurized and heated before entering the extraction chamber. After leaving the extraction cell, the CO_2-fluid was depressurized over a heated capillary restrictor and the extract was collected in a vial filled with methanol.

Following extraction procedure was followed:

- The extraction cell of 10 ml was filled with (oven dried) soil, to which 1 ml of the metal-complexing ligand was added. At both sides of the extraction cell, glass beads were added to prevent clogging and to ensure a uniform distribution of the SC-CO_2.
- The cell was mounted in the apparatus and heated to the required temperature.
- The cell was pressurized to 200 bar with CO_2 for 5 minutes (static mode), after which it was dynamically extracted for 1 hour at a rate of 2 ml/minute (measured as liquid CO_2 at 2 ^oC and 200 bar).
- The extraction cell was depressurised and an additional ml of the metal-complexing ligand was added to the soil.
- The extraction was continued for another hour.

The plant growth inhibition test was performed with garden cress (*Lepidium Sativum*). The tests were performed in a conditioned room (25^oC), which had a 12/12 hours light regimen. For each soil and contamination level, 4 replica's of each 2 g were distributed over the test vessels and in each replica, 5 seeds were sown. The soils were irrigated on a daily basis. Five days after more than 50% of the seeds had germinated, the number of plants and the biomass of the emergent plant tissues were determined.

The lethality tests of the compost worms (*Eisenia Foetida*) was performed on 3 replica's of the soils (20g). In each replica, two adult compost worms of similar weight were introduced.

The soils were kept at 80% of the water holding capacity of the soils. After 7 days, the weight loss and the mortality rate was determined. A weight loss of more than 25% is considered irreversible and as a consequence will result in mortality of the test organism.

The Biomet® assay was developed in order to quantify the bioavailable metal fraction in environmental matrices. In the Biomet assay the lux genes of the marine bacteria *Vibrio fisheri* have been put under control of the promoter region of those genes that give rise to heavy metal-resistance in the soil bacterium *Alcaligenes eutropus*. Different strains have been constructed with unique sensitivities to one or two heavy metals. In the current test set-up a suspension of the contaminated soil was inoculated with the Cd sensitive strain AE1433. Bioluminescence was measured using a luminometer (Luminoscan, Berthold) and quantified using a calibration curve.

The cadmium content of the samples was analysed using inductive coupled – atom emission spectrometry (ICP-AES). The samples were destructed with concentrated HNO_3. The residue was dissolved in 5 ml of 0.1N HNO_3. The error related to the destruction and analysis procedure was 9%.

II – EXPERIMENTAL RESULTS & DISCUSSION

Extraction efficiency

It is known that the selection of ligands is a key factor in the efficiency of the extraction process. Many ligands have been developed. However, most of them are used for ambient extraction with aqueous or organic solvents. Most ligands are classified in four classes, based on the type of reaction that occurs between the metal and the ligand. The classes are acid ligands, acid chelating ligands, ion exchangers and solvating ligands.
A suitable ligand for SFE has to fulfil several requirements. Ideally, the ligand strongly complexes with the metal under investigation, and the formed complex is highly soluble in the supercritical fluid to facilitate the extraction. In literature, only a limited number of ligands have been tested for SFE. These are mainly ß-diketones, dithiocarbamates and organo-phosphate systems.[1]
Initially, the ligand ethylenediamine was used as a substitute for ethylenediaminetetraacetic acid (EDTA). Ethylenediamine is known to form complexes with heavy metals in an aqueous solution. The molecule is smaller than EDTA, and is expected to have a higher solubility in SC-CO_2. However, a white precipitate was found when ethylenediamine was brought in contact with SC-CO2, probably due to a reaction. Therefore, the ligand was abandoned.
In this work two promising ligands were investigated, i.e. hexafluoroacetylaceton (HFA) en Bis(2,4,4-trimethylpentyl)-monothiophosphinic acid (Cyanex 302). HFA belongs to the ß-diketones, a class of ligands that has been studied extensively in SC-CO_2. HFA is fluorinated. This ensures that the compound is mainly in the enol-form which promotes complex-formation, and that the metal-complex has a higher solubility in the supercritical CO_2-fase.[2] The compound is found to extract cobalt efficiently from doped stainless steel beads.[3] Cyanex 302 is known to form Cu-complexes which are soluble in SC-CO2. Moreover the ligand is known to extract cadmium from doped fly ash and dry sand with SFE.[4]

Table 1: Cd-content of the soil after SFE at 200 bar and 35°C, using HFA or Cyanex 302 as a metal-complexing ligands.

Metal-complexing ligand	Cd-content (mg/kg)	Removal efficiency (%)
Control – no extraction	213 +/- 30	
Supercritical CO_2 + HFA	147	31
Supercritical CO_2 + Cyanex 302	117	45

The Borris soil (contaminated with 250 mg Cd/kg) was extracted according to the procedure outlined above. The extraction was performed at 200 bar and 35 °C (supercritical CO_2). The extracted soil was analysed for Cd and the results are shown in Table 1. Five control samples were measured independently and the variation in Cd-content was found to be 14%. With a destruction and measurement error of 9%, this suggests that the doping was not completely homogeneous or that leaching occurred in time.

The table shows that Cd was extracted for both ligands (HFA and Cyanex 302). The removal efficiency for Cyanex 302 was roughly 45%, and larger than in the case of HFA. The error on the determination of the removal efficiency was 20%.

Table 2: Influence of different extraction conditions on the Cd-removal. The extraction was performed without ligand and with the ligand Cyanex 302. The extraction with supercritical CO_2 (200 bar and 35°C) was compared with subcritical CO_2 (200 bar and 27°C).

Extraction condition	Cd-content (mg/kg)	Removal efficiency (%)
Control – no extraction	498+/-45	
Supercritical CO_2 (no ligand)	472	5
Supercritical CO_2 + Cyanex 302	378	24
Subcritical CO_2 + Cyanex 302	432	13

In addition, the influence of different extraction conditions was investigated (see Table 2). Each result represents the mean of 15 independent extractions. On this occasion the soil was spiked to a final concentration of 500mg/kg. Table 2 shows that only 5% of cadmium is extracted when no ligand is added. The result is in agreement with the data of Kersch on the removal of Cd from dry sand [5]. If Cyanex 302 was added, the extraction proceeded more efficiently at 35°C (supercritical CO_2) than at 27°C (subcritical CO_2).
It should be noticed, that for supercritical CO_2 - Cyanex 302, the extraction efficiency depends on the concentration of doping. The value obtained for a soil doped with 500 mg Cd/kg (see Table 2), is approximately half the value obtained for a soil doped with only 250 mg Cd /kg (see Table 1). This suggests that the extraction is incomplete, especially for the soil with the highest concentration of Cd. Presumably, Cd may be removed to a lager extent, by increasing the extraction time or amount of ligand. This requires further investigation.

The Kettering soil, doped with 500 mg Cd/kg, was extracted with supercritical CO_2 at 35°C and 200 bar using the ligand Cyanex 302 (see Table 3). After extraction, the soil was found to be wet and entangled, forming a dense cake. Weight measurements of the extraction cell revealed that 35% of the ligand was still present. The inspection of the extraction cell after the extraction, indicated that the supercritical CO_2-fluid did not penetrate the soil completely,

but mainly flowed along the edges of the soil. It seemed not possible to extract the clay soil in a dynamic extraction.

Table 3: Comparison of the extraction of Cd from the soils Borris2 and Kettering. The mass difference due to the extraction step is shown.

Soil type	Mass difference	Cd-content	Removal efficiency
Boris		498	
Boris	0.00	378	24
Kettering	0.66		
½ Kettering		495	
½ Kettering	0.06	437	12

When in a subsequent experiment the structure of the clay soil was loosened by mixing it with 50 wt% of white sand, the extraction proceeded more efficiently and the residual Cyanex 302 was found to be less than 3%.
The Cd-analysis of the extracted soils is shown in Table 3. It is clear that cadmium was extracted less efficiently in the clay soil. The removal efficiency only amounted 12% compared to 24% for Borris2 at the same conditions.

Ecotoxicological results

The remaining bioactive amount of Cd in the extracted soils was determined with the bio-sensor Biomet (strain AE1433). The test indicated that 90% of the doped Cd was bio-available in the unextracted soil. This value is unexpectedly high and suggests that the partition of Cd between the solid and liquid phase had not reached equilibrium at the time of analysis.

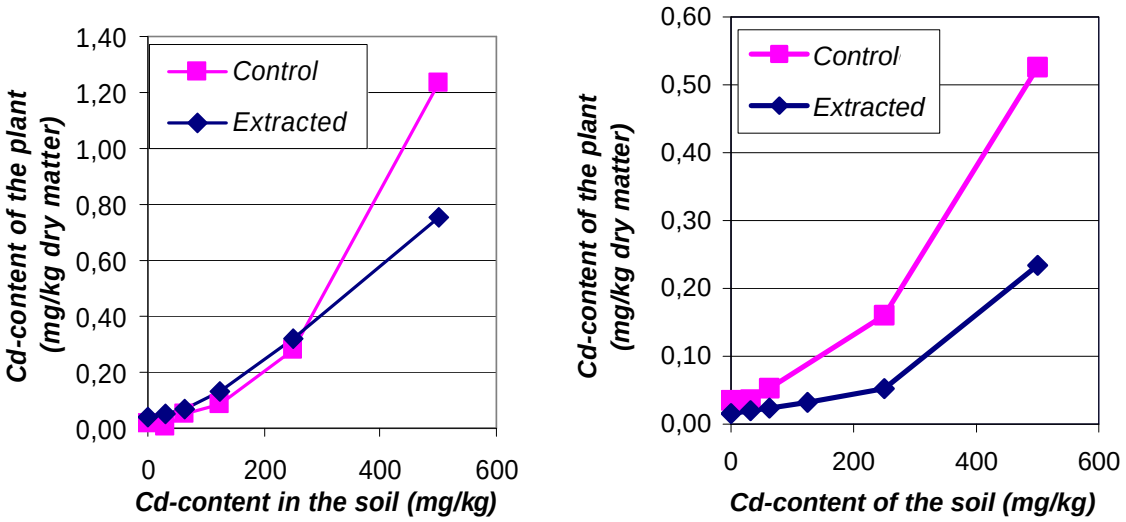
Table 4: Bioactive amount of Cd as a function of the extraction condition for a sandy soil (Borris2), as determined with the biosensor Biomet.

Extraction condition	Cd-content (mg/kg)	Removal efficiency (%)
Control – no extraction	444	
Supercritical CO_2 (no ligand)	432	3
Supercritical CO_2 + Cyanex 302	358	19
Subcritical CO_2 + Cyanex 302	395	11

Table 5: Bioactive amount of Cd as a function of the extraction condition for a clay soil (Kettering mixed with quartz sand (1/1)) as determined with the biosensor Biomet.

Extraction condition	Cd-content (mg/kg)	Removal efficiency (%)
Control - no extraction	460	
Supercritical CO_2 + Cyanex 302	358	22

Figure 1: Cd uptake in Garden cress grown on a sandy soil (Borris2; left-hand panel) and a clay soil (Kettering mixed with quartz sand (1/1); right-hand panel). Both figures show the results on the non-extracted soil and on the soil extracted with SC-CO_2 using Cyanex 302.



The efficiency of SFE to extract Cd from soils was also checked by comparing seedling emergence and plant biomass in control and SFE extracted soils. In the sandy soil no significant reduction in seedling emergence nor biomass was observed with increasing concentrations Cd in the soil samples. However, the average biomass of plants grown on control soils was slightly higher (be it not statistically significant) from those grown on extracted soil. This may reflect a decrease of soil nutrients (Ca, K, Fe and Mg) in the extracted soils. The uptake of Cd in Garden cress grown on the sandy soil is shown in Figure 1 (left-hand panel). A reduced availability of Cd is only seen in the extracted soils both at the highest applied concentration. Because SFE had been shown to be very difficult to perform on the Kettering soil, and because admixture of quartz sand had been shown to provide a solution, the plant tests were also performed on a Kettering/quartz sand (1/1) mixture. No significant differences in seedling emergence and plant biomass could be calculated for increasing Cd concentrations in the soil samples. Moreover, both endpoints did not differ between the control and the extracted soils. This is in agreement with the analytical results on the soil composition which had shown no significant reduction of soil nutrients in the clay soil after SFE. The uptake of Cd in the plants grown on Kettering soil (1/1 mixture with quartz sand) is shown in Figure 1 (right-hand panel). Cd uptake was shown to be reduced in SFE extracted soil over the entire concentration range studied. However, as for the sandy soil only in the 500 mg/kg samples this difference was statistically significant.

Contaminant exposure pathways are entirely different in plant and animal species. Therefore also a soil invertebrate test was included to test the efficiency of ligand mediated SFE to remove the bioavailable fraction of Cd in soils. Both mortality and loss in biomass were considered. The results for Borris2 soil are shown in Table 6. As can be seen from the table earthworms remained healthy and did not loose a significant amount of weight in non-contaminated Borris2 Soils. Spiking with Cd to a final concentration of 500 mg/kg induced a significant mortality of 33%. Moreover the remaining animals showed a significant weight

loss of 37%. From previous experience a weight loss of more than 25% is considered irreversible an will eventually lead to death. This effect is significantly reduced after the soil sample is extracted with SFE-Cyanex 302. No more lethal effects are seen and loss of weight remains below the level of irreversible effects. The weight of loss is significant and may be due to a residual amount of Cyanex 302. Finally, EDTA washing was shown to be completely inadequate because 100% mortality of the earthworms occurred when the animals were exposed to EDTA treated soil.

Table 6: Mortality and loss of biomass in earthworms exposed to Borris2 soil without spiking. Spiked with Cd at a final concentration of 500 mg/kg , spiked with Cd at a final concentration of 500 mg/kg but extracted with SFE-cyanex 302 or EDTA (aqueous).

Type	Mass reduction	Dead
Control – undoped	8 %	0%
Control – doped	37%	33 %
Extracted – SC-CO2 - Cyanex 302	18 %	0 %
Extracted – EDTA	100 %	100 %

CONCLUSIONS

This work reports on the extraction of cadmium from a contaminated soils using supercritical carbon dioxide (SC-CO_2). To this purpose, a clay and sandy soil were freshly spiked with a Cd-salt and subjected to SC-CO_2 extraction (SFE) at different conditions. As SC-CO_2 alone, was unable to extract heavy metals, several complexing agents were added. The ligand Bis(2,4,4-trimethylpentyl)-monophosphinic acid (Cyanex 302) resulted in the highest extraction efficiency. Furthermore, the highest Cd removal efficiency was obtained by extracting the sandy soil with SC-CO_2 at 200 bar and 35^{o}C.
Both soils were extracted at the optimal conditions and were subjected to different ecotoxicological tests, including a bacteriological test, a plant test and a test with compost worms. The bacteriological test and the plant test with Garden cress confirmed that less Cd was available in the soil after SFE. However, also some changes in soil nutrients were found affecting the growth of the Garden cress. Compost worms that obtain their food both from the soil pore water and by ingestion of soil particles, were less affected by the slight changes in soil characteristics induced by SC-CO_2 in the control cultures and, as a result, benefited more from the lower Cd-content of the soil.

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