INTEGRATION-OPTIMIZATION OF BIOAVAILABILITY MEASUREMENTS AND OECD 307 TEST TO EVALUATE PERSISTENCE OF ORGANIC POLLUTANTS DURING INCOMPLETE BIODEGRADATION PROCESSES IN SOIL

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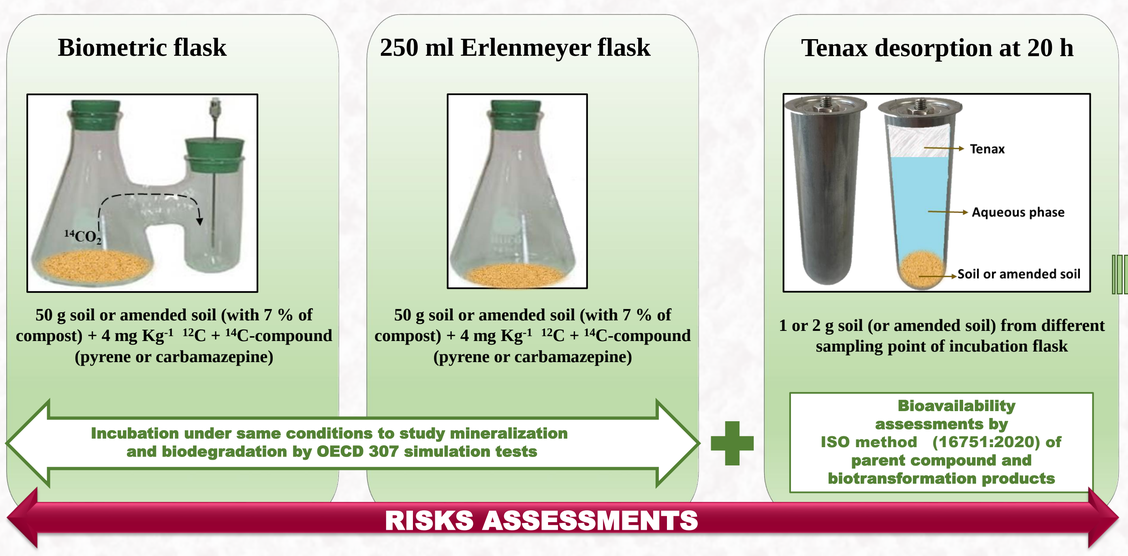
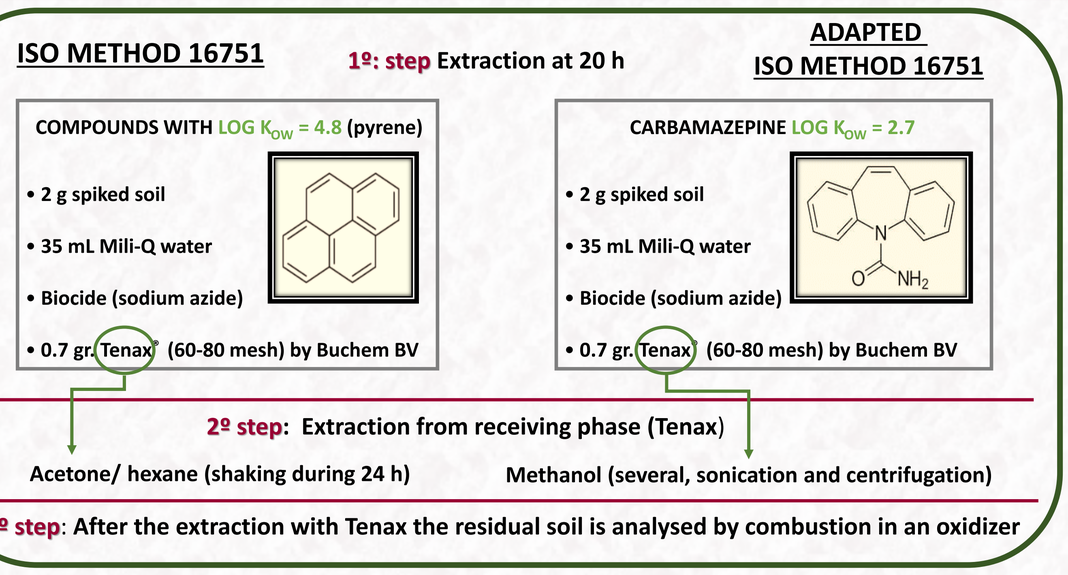
OBJETIVES

The incomplete biodegradation of organic pollutants in soil can rise the environmental risks if the transformation products are more toxic and mobile than the parent compounds. Alternately, the biodegradation products may remain as non-extractable residues, or as slowly-desorbing but still extractable chemicals, which may be less risky than the parent, fast desorbing pollutants. The biodegradation process is itself influenced by the bioavailability of contaminants to the soil microbial populations, so bioavailability and risk assessment should be integrated in persistence studies. The significance of evaluating bioavailability during a biodegradation process has been examined in our previous studies on soil bioremediation, explaining that integrating bioavailability assessment gives a more realistic risk information than using the total contaminant concentrations only. Here, we studied the biodegradation by organic compounds in a prospective risk assessment scenario, with the OECD 307 simulation test incorporating bioavailability assessments by the standardized ISO method (16751:2020).

MATERIALS AND METHODS





RESULTS

PYRENE

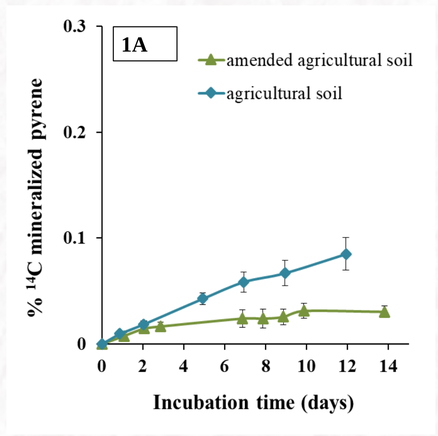


Figure 1A. Mineralization by *P. putida* G7 of ^{14}C-pyrene added to a sterilized soil. Mineralization was less than 0.1 % for both unamended and compost-amended agricultural soil, indicating the cometabolic transformation by this strain. These low extents excluded any losses of ^{14}C from the soil as $^{14}CO_2$. Combustion of the soil led to a complete recovery of ^{14}C after incubation.

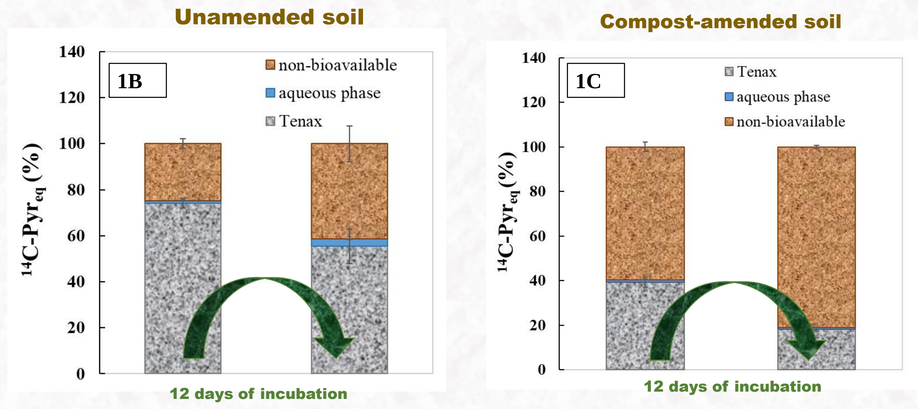


Figure 1B and 1C: Determination of the phase distribution of ^{14}C-Pyr$_{eq}$ (^{14}C-labelled parent compound and metabolites) among soil, water and Tenax in bioavailability assessments for unamended (B) and amended (C) agricultural soil. The bioavailable concentration of ^{14}C-Pyr$_{eq}$ in unamended soil doubled that of the compost-amended soil, what was attributable to the significantly higher TOC content of the amended soil. The increase of ^{14}C-Pyr$_{eq}$ concentration in the aqueous phase after incubation was possibly caused by the metabolites formed, more water soluble than the parent compound. The bioavailability profile changed after incubation: in the amended soil the metabolites only partitioned partially to the Tenax, remaining in the soil as a non-bioavailable fraction.

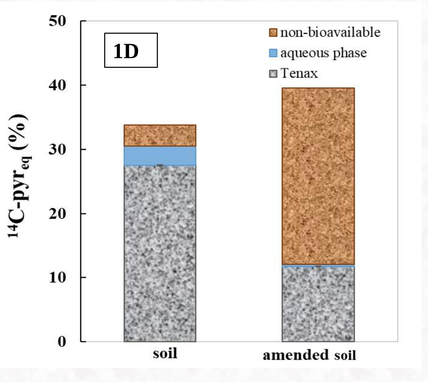


Figure 1D: Distribution (as % of total ^{14}C in soil) of transformation products formed during biodegradation (^{14}C-Pyr$_{eq}$ as metabolites-determined after HPLC fractionation) in the different phases of Tenax extractions. In the case of compost-amended soil, most of ^{14}C-Pyr$_{eq}$ remained in soil as non-bioavailable products.

CARBAMAZEPINE

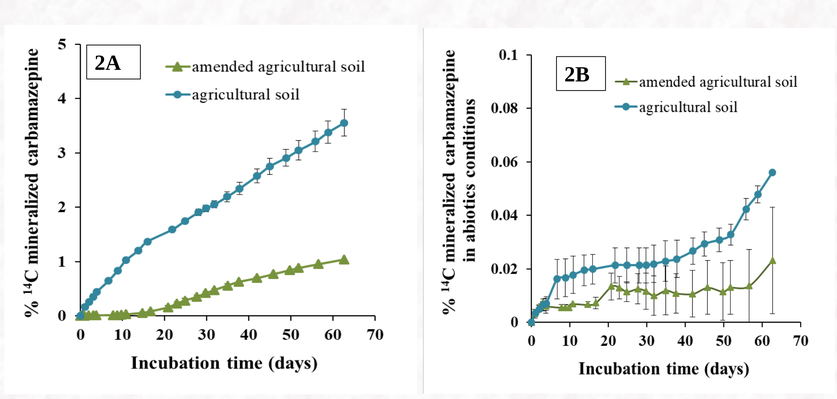


Figure 2A and 2B: Mineralization of ^{14}C-carbamazepine added to a non-sterilized soil (A) and sterilized soil (B). In this case, no inoculation was performed. Radiorespirometry determinations showed very low extents of mineralization in non-sterilized soils (3.55 ± 0.5 % and 1.04 ± 0.01 % for soil and compost-amended soil respectively, 2A). After sterilization, the mineralization extent of carbamazepine decreased further, to 0.05 ± 8*10^{-5} % and 0.02 ± 0.01 for soil and compost-amended soil respectively (2B), indicating the microbial nature of this transformation. The recovery of ^{14}C through combustion was also complete after incubation.

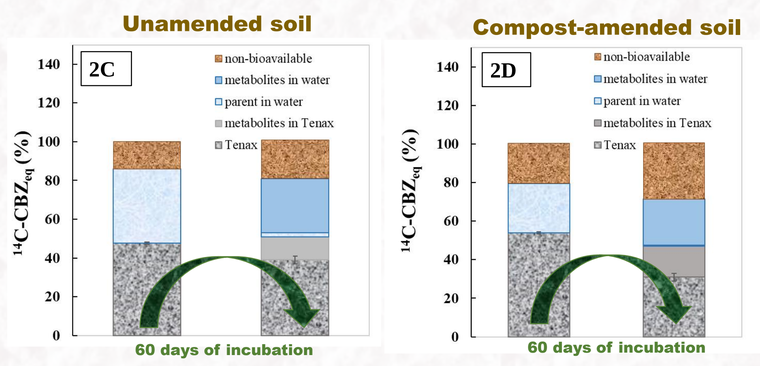


Figure 2C and 2D: Phase distribution of ^{14}C-CBZ$_{eq}$ (^{14}C-labelled parent compound and metabolites) among soil, water and Tenax. for unamended (C) and amended (D) agricultural soil. The non-bioavailable fraction was higher in the compost-amended soil. In both cases, the non-bioavailable fraction increased slightly after incubation, and consequently the fraction of ^{14}C-CBZ$_{eq.}$ decreased in the water (in the case of unamended soil) and Tenax (for the case of amended soil) phases. After the incubation period, the water and Tenax fractions were analyzed by the combined use of liquid scintillation and HPLC fractionation, and the ^{14}C-CBZ$_{eq}$ products (as metabolites) were determined. The aqueous phase contained mostly metabolites for both unamended and amended soil, whereas the parent compound partitioned preferentially into the Tenax fraction.

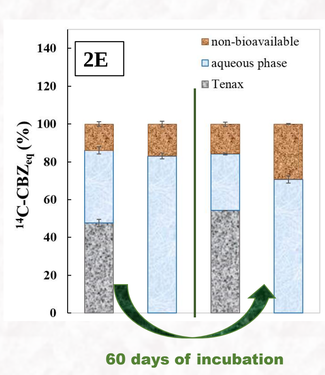


Figure 2E: Change in Tenax extractability of total ^{14}C-CBZeq from unamended agricultural soil after 60 days of incubation, as compared to extraction with only water. Before incubation (left), Tenax did not extract more equivalents than the water only. However, after incubation, significantly more compound was extracted through Tenax. Together with the results after incubation from figures 2C and 2C, these results indicate that the desorption rate of the parent compound had decreased significantly after incubation. Therefore, the Tenax phase acted efficiently as an infinite sink for the parent compound.

CONCLUSIONS

- For the case of pyrene, we show that cometabolism decreased the risks from pyrene in soil because the metabolites remained in soil as slowly desorbing compounds. This risk reduction was favoured by soil amended with compost.
- With carbamazepine, the ISO methodology for bioavailability assessments was useful after an incubation period, and specially with amended soils, where aging and biodegradation led to a significant decrease in the desorption rate of the parent compound. The metabolites formed by cometabolism were present both in the water and Tenax phases, what should be taken into account when incorporating this methodology in risks assessment.

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