Comparison of NER formation in soil (OECD 307) and water-sediment (OECD 308) degradation study using ^{14}C labelled phenanthrene.

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Introduction

Biodegradation data of chemicals using standard OECD tests are required during chemical safety assessment under different regulations. OECD 307 [1] and OECD 308 [2] are examples of such higher tier standard biodegradation tests in soil and water-sediment matrices. In these tests, formation of Non extractable residues (NER) is common and can be quantified only using isotopic labelled test chemicals. Information on the potential risk posed by NER is required while performing Persistency assessment under the REACH regulation. Type II NER i.e. covalently bound fraction and Type III NER: i.e. the biogenic fractions pose no risk to the environment [3]. Type I NER i.e. the physically entrapped fraction is potentially remobilizable and poses a risk to the environment. Silylation extraction has been proposed for the assessment of Type I NER [3]. In this study, we compare total NER and Type I NER formed in soil and water-sediment biodegradation test using ^{14}C labelled test item phenanthrene.

Materials and Method

OECD 307:

- 2 soils (Table 1),50g dry weight (dw) soil sample
- Test carried using flow through setup
- Test item application using co-solvent
- NaOH and ethylene glycol traps to capture mineralized and volatilized fraction, respectively
- Sterile control (using autoclaving) samples
- Sample incubation in dark at 20 ± 2°C for 103 days

OECD 308:

- 2 sediments (Table 1), 70-80g (dw) sediment, Sed.:water ratio(1:3 V/V)
- Test carried using an improved closed setup [4]
- Test item application using 0.01% (v/V) co-solvent
- NaOH and tenax traps to capture mineralized and volatilized fraction
- Sterile control (using autoclaving) samples
- Sample Incubation in dark at 20 ± 2°C for 120 days

NER Evaluation:

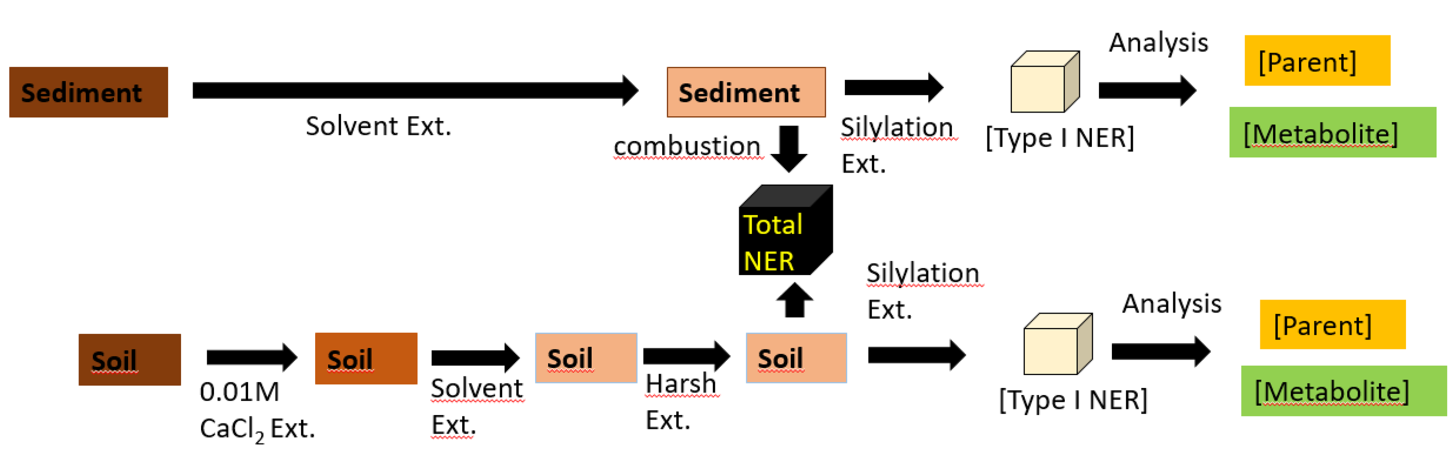

Figure 1: Extraction schemes used for soil and sediment samples before NER quantification and Type I NER analysis. Unlike for soil samples, the terminal harsh extraction step was skipped for sediment samples as the extraction recovery was <5% AR for some exemplary sediment samples.

Table 1: The characteristics of soils and sediments with high and low organic carbon (OC) content used for biodegradation studies in soil (OECD 307) and water-sediment system (OECD 308).

	Sand (%)	Silt (%)	Clay (%)	OC (%)
Low OC Soil	76.70	17.20	6.10	0.80
High OC Soil	17.71	57.49	24.80	3.05
Low OC Sed.	89.68	6.75	3.57	0.76
High OC Sed.	9.22	74.62	16.16	4.13

Results and Discussion

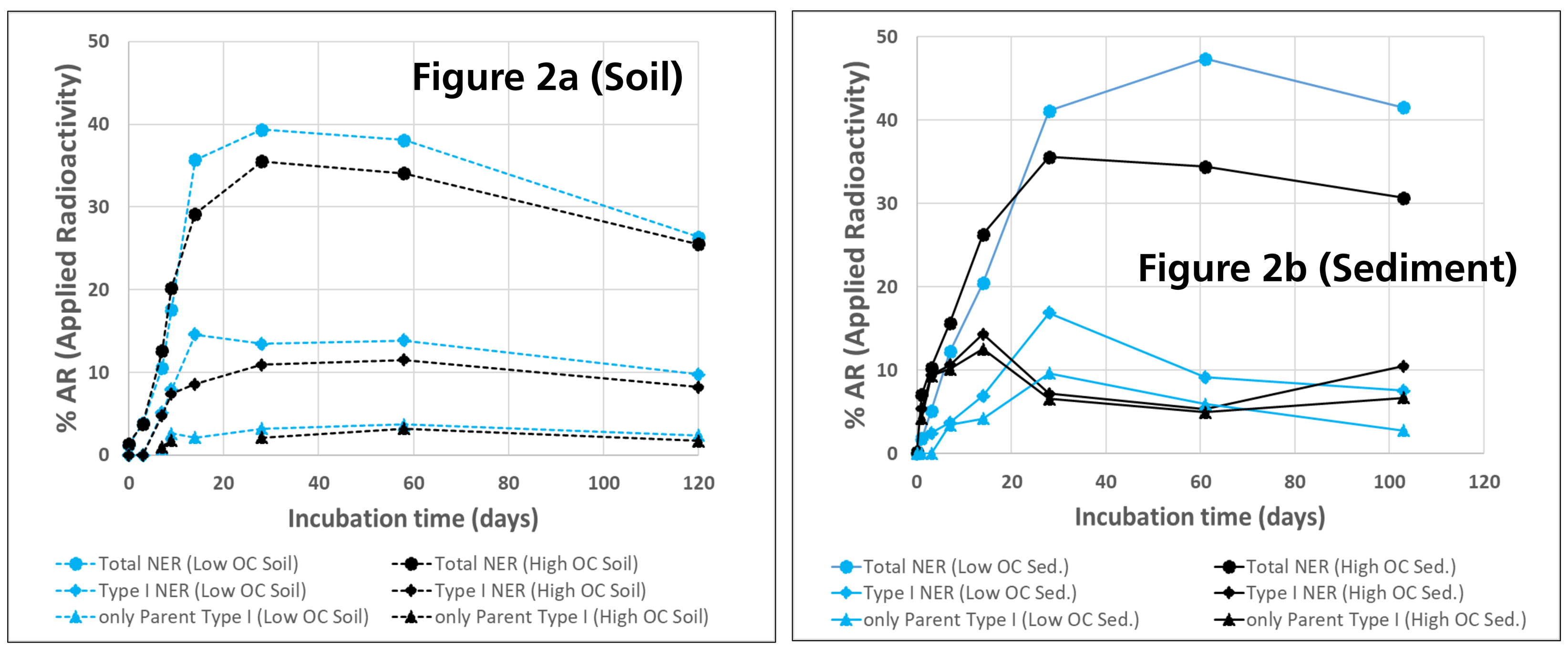

Figure 2a and 2b show the distribution of total NER fraction, Type I NER fraction and parent fraction analysed in Type I NER in high (black) and low OC (blue) soil and sediment degradation tests respectively. Generally, for low OC samples higher total NER fraction was observed in sediment (Figure 2b) in relation to soil (Figure 2a) study. However, for high OC samples the total NER was comparable in both soil and sediment studies. Total NER formation in sterile control samples was 0.2% AR in soil (at 126 d) and 4.4 - 10 % AR in sediment (at 103 d) (data not shown in figure). Overall reduction in total NER formation is observed in both soil and sediment towards the end of the study. Higher total NER fraction is observed in low OC in comparison to high OC soil and sediment. Thus the total NER formed was inversely related to OC content of the soil and sediment and mineralisation observed (Figure 3). The fraction of parent analysed in the Type I NER was relatively higher in sediment in comparison to the soil study.

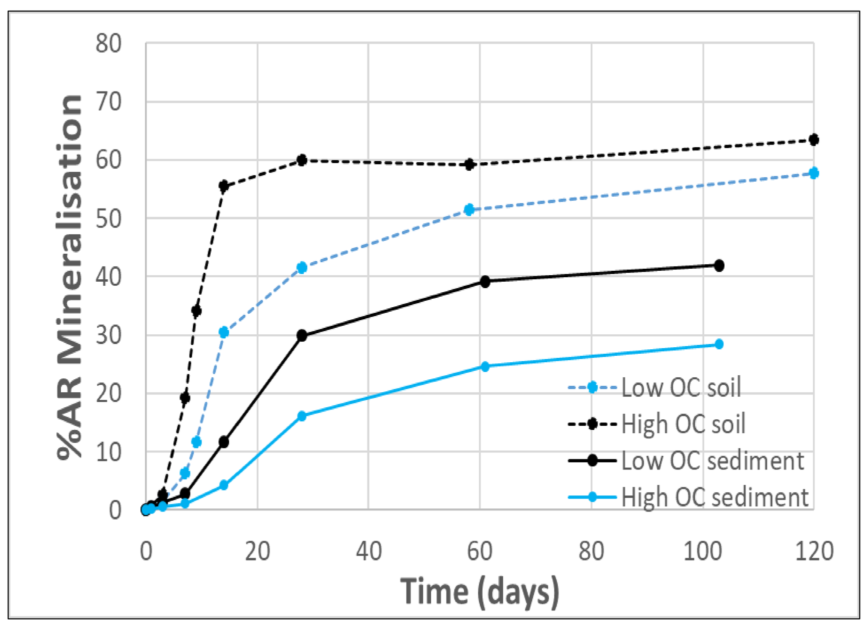

Figure 3: Mineralisation of ^{14}C labelled phenanthrene in soil and water-sediment degradation study. In general higher mineralisation was observed in soil in relation to sediment. Higher mineralisation in high OC in relation to low OC soil and sediment respectively.

Table 2: Calculation of degradation kinetics of phenanthrene in soil and water-sediment (total system) for three different NER treatment scenarios (A , B and C). The calculations were performed with CAKE software. Only the results of best fit model have been presented.*

$DegT_{50\ (days)}$	A*	B*	C*
Low OC soil	9.6	13.2	10.3
High OC soil	7.7	9.3	8.9
Low OC sed.	19.6	21.5	19
High OC sed.	15.6	20	19.4

***A:** considers the total NER fraction as degraded
***B:** considers silylation extracts as non degraded
***C:** considers only analysed parent fraction in silylation extract as non degraded

Higher $DegT_{50}$ is expected when total NER is considered as non degraded e.g. $DegT_{50}$ of 51.6 d and 41.5d for high and low OC sediment, respectively

Conclusion

- For low OC sediment higher total NER formation observed in sediment in comparison to soil degradation study of ^{14}C labelled phenanthrene.
- Higher fraction of parent phenanthrene residue measured in Type I NER for sediment in comparison to soil (potential impact of different extraction regimes)
- Total NER formation was inversely proportional to organic carbon content and mineralisation observed in both soil and sediment.
- The $DegT_{50}$ estimated with different NER considerations results in only slight difference (0.4 - 4.4d). Higher $DegT_{50}$ is expected considering total NER as non-degraded.

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