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## Non-exhaustive extraction techniques (NEETs) for bioavailability assessment of organic hydrophobic compounds in soils

Edoardo PUGLISI<sup>a\*</sup>, Colin J. PATTERSON<sup>b</sup>, Graeme I. PATON<sup>b</sup>

<sup>a</sup> Istituto Chimica Agraria ed Ambientale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy

<sup>b</sup> School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen, Scotland, UK

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Hydrophobic organic compounds (HOCs) have characteristics that are of particular concern due to associated toxicity and environmental persistence. The recalcitrance of these compounds is associated with their high affinity for organic matter and the corresponding tendency to bioaccumulate in fatty tissues.

Organic pollutants have traditionally been studied with exhaustive extraction techniques and these results are often coupled to risk assessment models to add a biological interpretation. Nevertheless, this approach does not take into account two important processes, namely bioavailability and aging. Bioavailability as a term is difficult to define, especially as it has been used in different disciplines. In soil science, it has been defined as “*the accessibility of a chemical for assimilation and possible toxicity*” [1]. Bioavailability as a term is receptor-defined. Environmental receptors include target organisms or soil matrices where mineralisation has occurred.

Bioavailability is a dynamic rather than a static process. Time-dependent losses in bioavailability have been demonstrated for several organic and inorganic chemicals. As HOCs persist within a soil or sediment matrix, compounds become increasingly resistant to desorption and thus less bioavailable. This process, technically called aging, is the result of slow entrapment of chemicals into sites within the soil matrix that are not accessible to the microbial biomass [1]. The nature and extent of aging processes depends on soil and compound properties as a result of physical, chemical and biological interactions. Significant soil and compound properties include pore size, water content, the amount and nature of organic matter, especially in dissolved forms, molecular structure, polarity, water solubility, and the octanol:water partition coefficient.

Bioavailability assessments usually rely upon the quantity of compound readily desorbed by aqueous phase extractions. However, with hydrophobic organic compounds this method has major limitations as very low amounts of these compounds will be in the aqueous soil phase, while a large labile and potentially bioavailable pool remains

in the solid phase [2]. Appropriate extraction techniques should therefore enhance the solubility of the compounds, in order to extract their entire labile fraction. Cyclodextrin and resin extractions look to be suitable approaches for addressing the problem.

Cyclodextrins (Fig. 1a) are cyclic oligomers of -D-glucose formed by the action of enzymes on starch. The three main cyclodextrins are  $\alpha$ -cyclodextrin, which comprises six glucose units,  $\beta$ -cyclodextrin (seven glucose units), and  $\gamma$ -cyclodextrin, with eight glucose units.

Cyclodextrins have a hydrophilic surface and an internal hydrophobic cavity, thus although fairly soluble in water, they are also able to entrap hydrophobic molecules inside their cavity. Correct selection of the particular cyclodextrin molecule is required if the target molecules, namely the specific HOCs, are to substitute the water molecules that occupy the hydrophobic cavity.

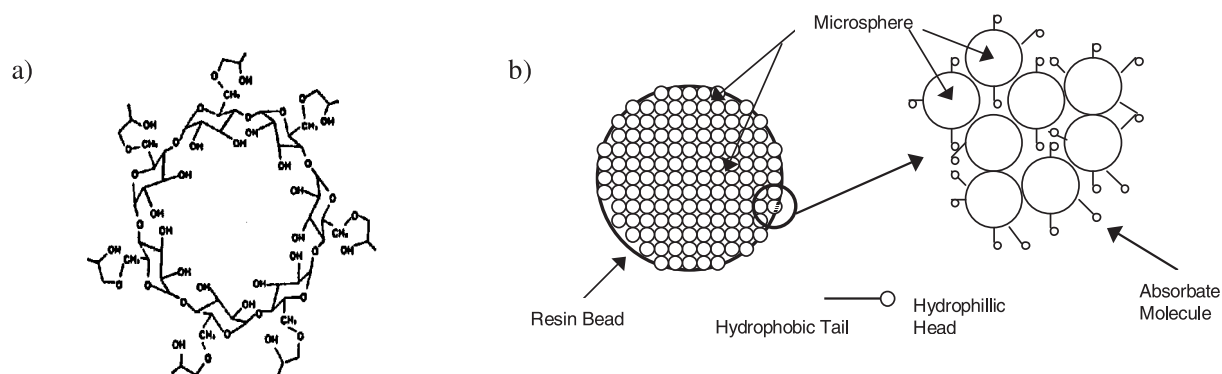
The formation of inclusion complexes and their stability (expressed by a complex stability constant,  $K_a$ ) depend on the type of cyclodextrin and on the physico-chemical properties of the guest molecule. The diameter of the cavity increases from  $\alpha$  to  $\beta$  and  $\gamma$ -cyclodextrin. Hydrophobic organic compounds with a relatively small molecular size will form the strongest complexes with  $\alpha$ -cyclodextrin and the weakest with  $\gamma$ -cyclodextrin. Organic molecules that are too large to form 1:1 complexes can, however, form 1:2 complexes. The hydroxyl groups may be functionalised with hydrophobic (e.g. methyl, propyl) or hydrophilic groups (sulfate, phosphate), to enhance the complex forming ability and selectivity.

The entrapment property of cyclodextrins has inspired considerable basic research, applied science and industrial utilisations in various fields (drugs, foods, agro-chemicals, biotechnological products, etc.). In the environmental field, researchers studied cyclodextrins with two main aims, both dealing with soil contamination; (i) the mitigation of pesticide application and (ii) the assessment of the bioavailability of organic hydrophobic compounds.

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\* Corresponding author: edoardo.puglisi@unicatt.it



**Figure 1.** Non-exhaustive extraction techniques for bioavailability assessments. Hydrophobic compounds may be a) entrapped in the internal cavity of hydroxypropyl beta cyclodextrin (HPCD) or b) adsorbed on the surface of XAD resin. In both cases an enhanced solubility is obtained.

An important contribution to the validation of the use of cyclodextrins in the assessment of the bioavailability of organic compounds such as polycyclic aromatic hydrocarbons (PAHs) was given by Reid et al. [2]. Phenanthrene, pyrene and benzo[a]pyrene were spiked in different soils at increasing concentrations and extracted with a hydroxypropyl- $\beta$ -cyclodextrin (HPCD) solution. A series of concentrations of HPCD and variable contact times were tested; the optimum extraction of PAHs was obtained with a concentration of 50 mM and 20 h of contact. The buffering and pH conditions were tested as well, and the use of unbuffered HPCD solutions was indicated as the more appropriate. In a long-term experiment to assess the aging process, the quantity of PAHs recovered in soil by HPCD extraction was compared with an exhaustive extraction using dichloromethane and butanol. All data were correlated to the mineralisable (bioavailable) fraction determined by means of bacterial strains able to mineralise the compounds. HPCD extraction was shown to be the more reliable tool for predicting the bioavailability of the soil-associated contaminants.

Resins are another method that have been investigated for extracting highly hydrophobic soil-bound compounds. There are various different resins commercially available, including Tenax and Amberlite. Amberlite XAD is a polymeric adsorbent of hydrophobic styrene-divinylbenzene copolymer resin (Fig. 1b). It is supplied as insoluble beads with a 20–60 mesh size and can be obtained in a variety of forms. Commercially, Amberlite XAD has been used for a wide range of processes, which include removal of antibiotics, organic nitrogen, and various aromatic compounds from aqueous streams. Amberlite XAD-2 has also been used to detect narcotics in blood and urine. Other forms of Amberlite XAD such as Supel-pak<sup>TM</sup>-2 can be used for sensitive analytical procedures i.e. detecting, identifying and measuring pesticides, PAHs and other hydrophobic organic molecules in the environment.

A single bead of Amberlite XAD consists of an agglomeration of many very small microspheres, giving a continuous gel phase and a continuous pore phase. The open-cell porous structure allows water to penetrate the pores easily. The macroporous nature of the microspherical resin beads, together with their large surface area and hydrophobicity relative to their mass promotes fast adsorption and high extraction efficiencies of hydrophobic organic compounds from aqueous media [3]. During the adsorption process, the hydrophobic portion of the adsorbate molecule is preferentially adsorbed on the hydrophobic polystyrene surface of the resin, while the hydrophilic

section of the adsorbate remains orientated in the aqueous phase. The compounds being adsorbed by the resin do not penetrate substantially into the microsphere phase, but remain adsorbed at the surface. With appropriate elution techniques, the sorbed compound can be rapidly eluted because of the high rate of diffusion of the elution mobile phase through the porous structure of the bead. As the compound of interest is bound to the outer or inner surfaces of the beads, penetration of the microspheres by the eluting solution is neither required nor necessary. The selectivity and extent of adsorption of soluble organic compounds by Amberlite resins increase as the hydrophobicity of the adsorbate molecule increases.

The large variation in properties of PAHs results in their different affinities for XAD resins. In an experiment carried out by Northcott and Jones [3], phenanthrene, pyrene and benzo[a]pyrene were used. They manipulated the XAD resin system in order to quantify rapid and slowly desorbing compound pools and their corresponding rate constants for compound release. This in turn provided an effective and simple tool for evaluating the desorption behaviour of hydrophobic organic chemicals from soil and sediment.

Non-exhaustive extraction techniques (NEETs) such as cyclodextrins and XAD may represent the way forward in studies of the bioavailability of hydrophobic compounds. Further studies involving ecotoxicological assays (i.e. biosensors), phytotoxicity and mineralisation experiments are mandatory in order to correlate the fractions extracted with NEETs with the biological response of living organisms.

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