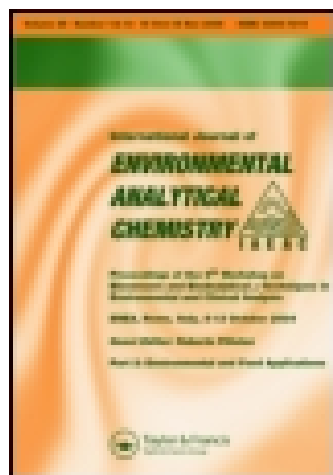


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## International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and  
subscription information:

<http://www.tandfonline.com/loi/geac20>

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Published online: 23 Sep 2006.

To cite this article: I. Scheunert, M. Mansour & F. Andreux (1992) Binding of Organic Pollutants to Soil Organic Matter, International Journal of Environmental Analytical Chemistry, 46:1-3, 189-199, DOI: [10.1080/03067319208027009](https://doi.org/10.1080/03067319208027009)

To link to this article: <http://dx.doi.org/10.1080/03067319208027009>

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# BINDING OF ORGANIC POLLUTANTS TO SOIL ORGANIC MATTER

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*(Received, 13 March 1991)*

Binding of organic pollutants to water-soluble, low-molecular weight humic substances increases their mobility in soil and their leaching, whereas binding to high-molecular weight humic substances results in unextractable residues. Water-soluble pollutants in leachate may be bound to low-molecular weight humic material, or may be free water-soluble conversion products that are slowly released from a soil-bound form, as shown for a conversion product of the insecticide aldrin. Unextractable soil-bound 4-chloroaniline was isolated by supercritical methanol extraction 16 years after application of the phenylurea herbicide buturon to soil. Biodegradation and bioavailability of unextractable soil-bound residues are greatly reduced as compared to the free compounds. For some chlorinated anilines and phenols, biomineralization and plant uptake was shown to be highest for residues bound in fulvic acids and lowest for those in humic acids. Model experiments demonstrate that chlorinated anilines form covalent bonds with humic acid precursors.

**KEY WORDS:** Soil-binding, leaching, unextractable residues, supercritical fluid extraction, bioavailability, covalent bonds, pesticides.

## INTRODUCTION

Binding of organic pollutants to soil organic matter is of paramount importance for the ecotoxicological evaluation of pollutants in soil. Binding of the pollutant to soil organic matter is accompanied by a drastic change in its mobility, leaching and bioavailability, including biodegradation and uptake by plants and soil fauna. Multiple binding mechanisms are effective between organic pollutants and soil organic matter, namely physical, physico-chemical, and chemical, most of them being widely unexplored. Organic pollutants may be bound to low-molecular weight, water-soluble humic substances, as well as to high-molecular insoluble ones. In the first case, mobility in soil and leaching from soil are promoted, resulting in the presence of pollutants in leachate, even if the non-bound pollutant is water-insoluble. In contrast, in the second case mobility in soil and leaching from soil are strongly reduced; the same applies to degradability and bioavailability. Even the extractability of the pollutants' residues by organic solvents is strongly inhibited; therefore, these

residues are called "unextractable" soil-bound residues. Due to the steady molecular changes and rearrangements within humic macromolecules, there is a steady transition between the residues bound to lower- and those bound to higher-molecular weight humic substances.

In the present paper, examples for both soluble and insoluble soil-bound organic chemicals are presented.

## EXPERIMENTAL

### *Lysimeter studies*

Lysimeter studies were performed in boxes  $60 \times 60 \times 60$  cm, constructed from water-resistant plywood and placed in an outdoor pit. The base of the boxes contained holes to permit the drainage of percolate water that was collected in a metal splash tray.  $^{14}\text{C}$ -ringlabelled aldrin and buturon were applied to the top soil layers at rates used in normal agricultural practice, and crops were grown. Leachate and soil samples were assayed for radioactivity. Experiments were continued for 15–16 years. The radioactive substances in leachate were isolated and identified. For detailed description, see reference<sup>1</sup>.

### *Supercritical fluid extraction*

For supercritical fluid extraction of soil-bound residues, soil from a lysimeter treated with buturon- $^{14}\text{C}$  was sampled from a 0–10 cm depth, extracted with methanol in a Soxhlet to remove extractable radioactivity, and subjected to fluid extraction with methanol compressed by a high-pressure liquid chromatography pump through a preheated capillary into the HPLC column that contained the preextracted soil. The conditions were as follows: pressure, 150 atm; temperature,  $250^\circ\text{C}$ ; time: 2 h; extracted soil mass: 10 g. The fluid extract was separated by chromatographic methods, and the main radioactive component was isolated and identified by GC-MS<sup>2</sup>.

### *Biodegradation and bioavailability of unextractable soil-bound pollutants*

4-Chloroaniline- $^{14}\text{C}$ , 2,4,6-trichloroaniline- $^{14}\text{C}$ , and 2,4,6-trichlorophenol- $^{14}\text{C}$  were applied, in aqueous solution or suspension or with the aid of a minimum amount of acetone, to 1.5 kg-portions of air-dried agricultural soil. The initial concentration was about  $2 \mu\text{g g}^{-1}$ , soil humidity was adjusted to 20%. The soils were stored under outdoor conditions, protected from rain, and the water lost by evaporation was restored daily. After 7 to 25 weeks, the soils were extracted exhaustively with methanol in a Soxhlet extractor, reextracted with aqueous acetate buffer solution (pH 4.6), and extracted again with methanol. Separation of the extracted soil into various inorganic and organic fractions was performed according to literature<sup>3</sup>.

In order to study the biomineralization of soil-bound residues, extracted soil or soil fractions were mixed with fresh soil (1:1). The mixtures were suspended in water (soil:water 1:5) and agitated for 28 days at  $25^\circ\text{C}$ . Oxygen was passed through the

apparatus discontinuously on every third day, and  $^{14}\text{CO}_2$  evolved was collected in one or two traps containing a liquid scintillation cocktail with a basic  $\text{CO}_2$ -absorber<sup>4</sup>.

In order to study the plant uptake of soil-bound residues, extracted soil fractions were mixed with fresh soil. Two barley grains were planted into 50 g-portions of the mixtures. After 14 days, the plants were assayed for radioactivity by liquid scintillation counting of  $^{14}\text{CO}_2$  formed after combustion of the plants<sup>5</sup>.

#### *Model reactions for binding of xenobiotics to soil organic matter*

0.27 mMole catechol, 0.36 mMole 4-chloroaniline, and various quantities of catalysts in 100 ml phosphate buffer solution (pH 6.8) were incubated at 25°C for 6 h. The reaction mixture was extracted with two 25 ml-portions of dichloromethane. The organic phase was treated with anhydrous sodium sulfate and, after filtration, subjected to HPLC. The chemical structure of the main reaction product was elucidated by NMR, IR, and HPLC-MS<sup>6</sup>.

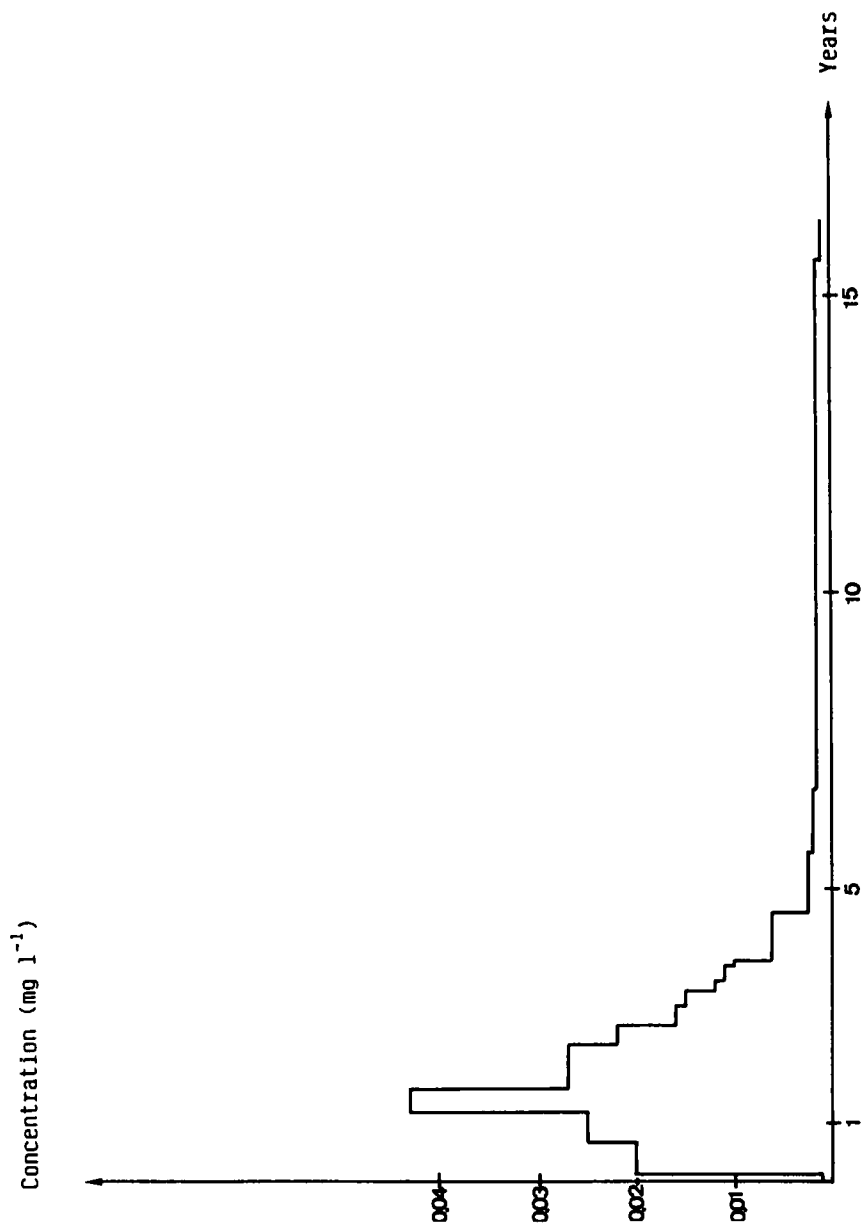
## RESULTS AND DISCUSSION

#### *Organic pollutants in leachate*

Organic pollutants in leachate may be related to the phenomenon of binding to soil organic matter in two ways: first, leachate may contain the pollutants bound to water-soluble humic substances. Second, it may contain free pollutants previously bound to high-molecular weight soil components and slowly released thereof. As to the first case, the chemical structures of the complexes formed between organic pollutants and soluble humic material are widely unknown. In most studies using  $^{14}\text{C}$ -labelled pollutants, they are noted as fully water-soluble, unidentified radioactive products, as in the case of lysimeter studies with atrazine- $^{14}\text{C}$ <sup>7</sup>.

After application of atrazine- $^{14}\text{C}$  to outdoor lysimeters, the percolation water from soil after 4 months contained, besides unchanged atrazine, varying amounts of de-ethyl atrazine, de-isopropyl atrazine, de-ethyl de-isopropyl atrazine, hydroxy-atrazine, de-ethyl hydroxy-atrazine, de-isopropyl hydroxy-atrazine, and a large proportion of unidentified water-soluble substances which are interpreted as atrazine or its metabolites bound to soluble humic material. After 13 months, the ratios between the radioactive components in leachate had changed: the proportion of unidentified  $^{14}\text{C}$ -substances was much smaller. This fact may be attributed to a shifting of atrazine and its metabolites bound to soluble humic material towards insoluble bound residues by further proceeding of the polymerization of building blocks containing the xenobiotics. These higher polymers are insoluble and do not appear in the leachate. The increase in the ratio of soil-bound residues as compared to soluble products with time has been demonstrated in earlier studies<sup>8</sup>.

Figure 1 gives an example of the slow release of a xenobiotic bound to insoluble organic material, resulting in a very slow leaching of the xenobiotic. The insecticide aldrin is not used any more in most of the industrialized countries since many years.



**Figure 1** Concentrations of radioactivity in leachate (depth about 60 cm) after application of aldrin-<sup>14</sup>C to the top soil layer (0–10 cm depth) (mg equivalent to aldrin; dosage: 2.9 kg ha<sup>-1</sup>).

However, it continues to persist in soil, at least in form of conversion products. Figure 1 shows the leaching of  $^{14}\text{C}$  from a lysimeter in a 60 cm depth after the application of aldrin- $^{14}\text{C}$  to the top soil. The radioactivity reached a peak of about  $0.04 \text{ mg l}^{-1}$  (equivalent to aldrin) after about 1 year, then decreased slowly and was detectable even after 15 years in leachate<sup>9</sup>. The radioactivity in leachate was isolated and identified. It consisted of only one substance which was not aldrin but a highly polar degradation product, namely dihydrochlordene dicarboxylic acid<sup>10</sup>, as shown in Figure 2.

Although three major conversion products are formed from aldrin in soil, namely dieldrin, photodieldrin and the product of cleavage of the non-chlorinated ring, dihydrochlordene dicarboxylic acid, only the latter compound, is leachable from soil. On the other hand, in soil about 8% of the radioactivity recovered was unextractable by organic solvents, i.e., bound to soil organic matter. Upon dissolution of humic acids by dilute alkali, a large portion of this bound radioactivity was dissolved and shown to be dihydrochlordene dicarboxylic acid also<sup>1</sup>. It may be concluded that the long-term slow leaching of this substance is due to a long-term slow release from the soil-bound state.

#### *Unextractable soil-bound residues*

The binding of xenobiotics to insoluble, high-molecular weight humic material is by multiple mechanisms that are poorly understood. In many cases, the xenobiotic may be entrapped in cavities of the organic macromolecule and is released when the humic acids are dissolved in diluted alkali, as in the example presented above. Other methods to dissolve unextractable xenobiotic residues from soil are high-temperature distillation<sup>11</sup> and supercritical fluid extraction<sup>12</sup>. The latter method was applied to aged residues of the phenylurea herbicide buturon applied to an outdoor lysimeter in a  $^{14}\text{C}$ -labelled form<sup>13</sup>.

After 16 years, only 12% of the total  $^{14}\text{C}$  recovered could be extracted by normal Soxhlet extraction with methanol; the remaining radioactivity was bound in soil. However, by supercritical methanol half of this radioactivity could be brought into solution. This radioactivity was not due to the parent compound but to its metabolite 4-chloroaniline (15% of  $^{14}\text{C}$  in soil) (Figure 3)<sup>2</sup>.

It is assumed that the bound residues released by the two methods discussed are not fixed by covalent bonds. However, between xenobiotics and soil organic matter covalent bonds may be formed, from which the xenobiotic can be liberated in the laboratory only by chemical methods such as hydrolysis or degradative oxidation, or which are not accessible even to hydrolytic or oxidative attack. 4-Chloroaniline is an example also for bonds of this kind. Part of unextractable  $^{14}\text{C}$ -residues in soil after application of 4-chloroaniline- $^{14}\text{C}$  was released after alkaline hydrolysis followed by steam distillation<sup>14</sup>.

When considering the binding of organic chemicals in soil, it should not be overlooked that inorganic soil constituents also may play a significant role in binding. If the soil containing bound  $^{14}\text{C}$  from  $^{14}\text{C}$ -labelled xenobiotics is separated, by well-known methods, into humic soils, fulvic acids, humin and inorganic fractions,

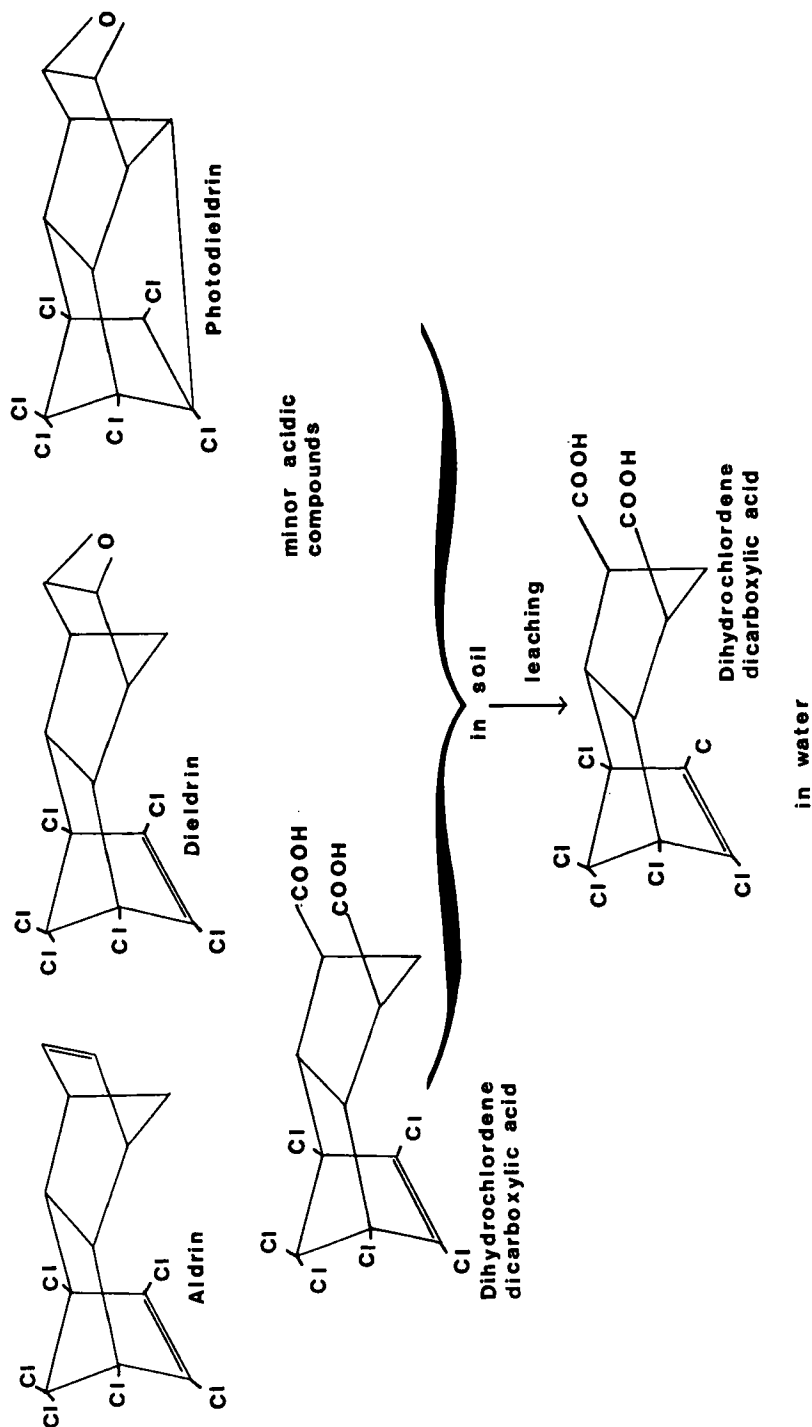
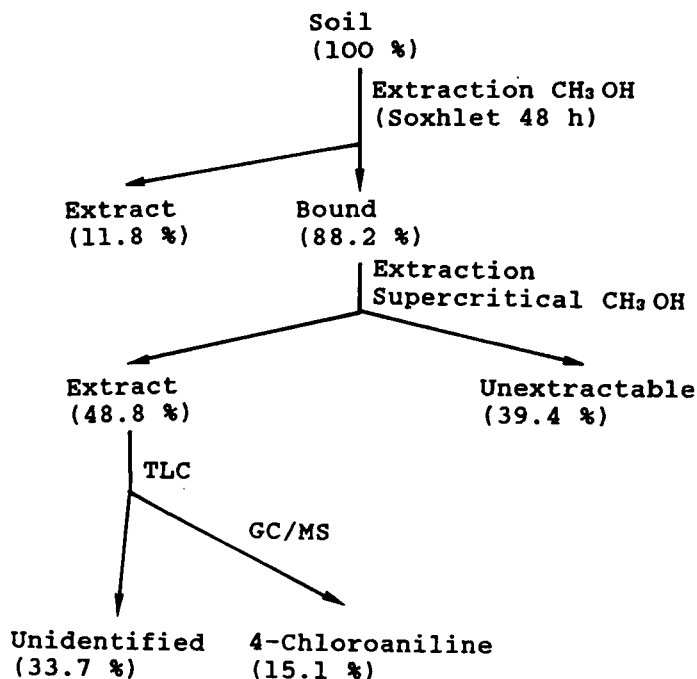


Figure 2 Conversion products of aldrin in soil and leachate.



**Figure 3** Supercritical fluid extraction of soil-bound residues of the phenylurea herbicide buturon- $^{14}\text{C}$ , 16 years after application.

in general  $^{14}\text{C}$  is detected in all of these fractions. The ratios between the portions bound in each of these soil fractions depend on the kind of chemical as well as on the time of exposure<sup>15</sup>.

#### *Biodegradation and bioavailability*

Besides the question of chemical identity of the bound residues of the xenobiotic and of the site of binding, the question of biodegradability and bioavailability is decisive for the evaluation of its toxicological and ecotoxicological significance. In the following, examples are given for some chlorinated anilines and phenols. Both chemical classes show a high tendency to be bound in soil. They were applied to soil in a  $^{14}\text{C}$ -labelled form. After several weeks, the extractable portion of  $^{14}\text{C}$  was removed, and the bound residues were assayed for their biomineralization by fresh soil.

Figure 4 shows the mineralization of soil-bound  $^{14}\text{C}$ -labelled 2,4,6-trichlorophenol, 2,4,6-trichloroaniline, and 4-chloroaniline to  $^{14}\text{CO}_2$ . It can be seen that both anilines have a slow mineralization rate whereas the phenol is mineralized much better<sup>4</sup>. It must be noted that  $\text{CO}_2$  bound as carbonate is not included in this figure. Mechanisms for the binding of unchanged, chlorine-containing phenols have been demonstrated by Bollag *et al*<sup>16,17</sup>.

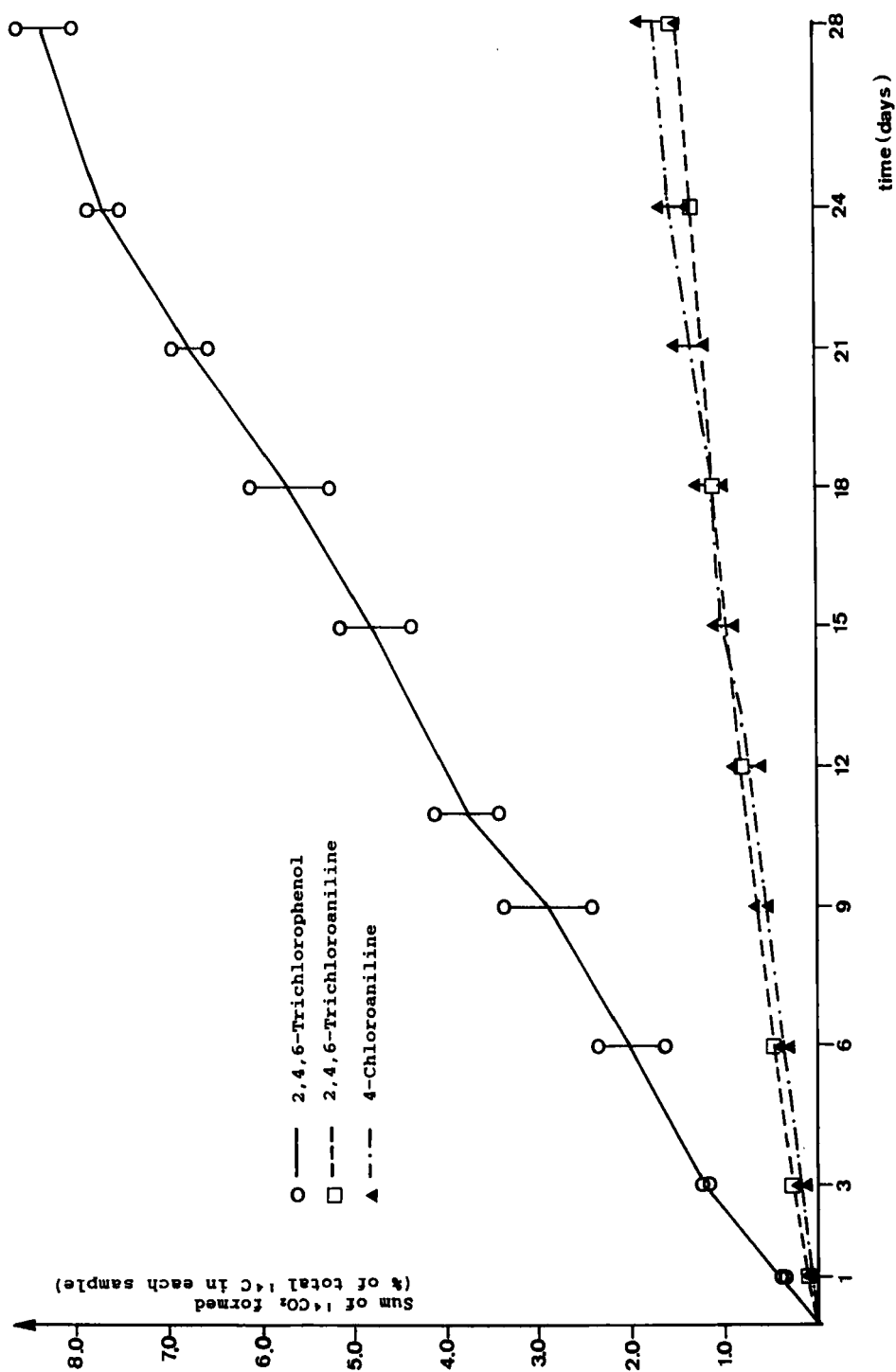
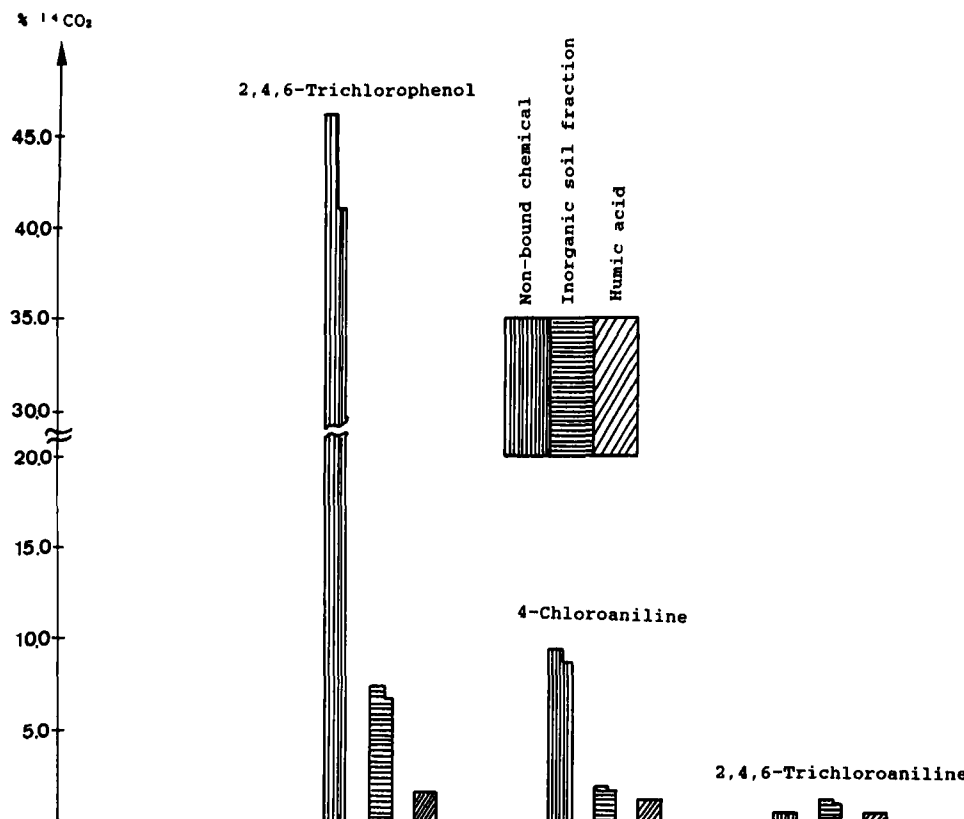


Figure 4 Aerobic biomineralization of unextractable  $^{14}\text{C}$  in soils treated with  $^{14}\text{C}$ -chemicals.

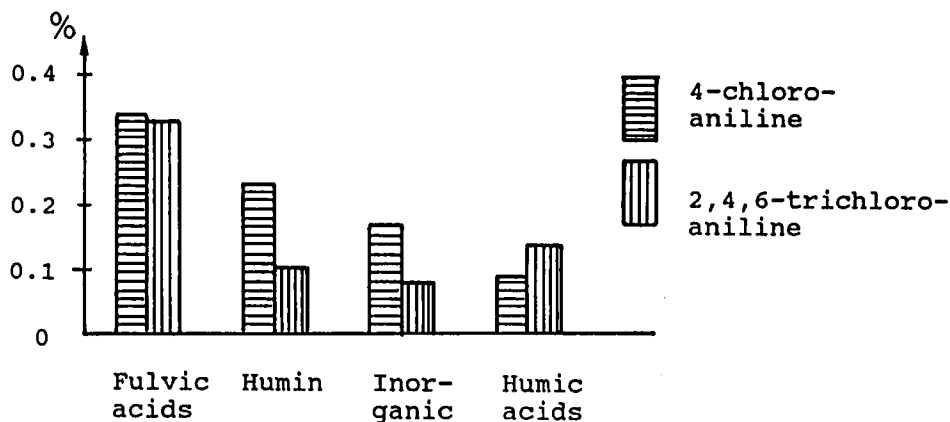


**Figure 5** Aerobic biomineralization of unextractable  $^{14}\text{C}$  in inorganic and organic fractions of soil treated with  $^{14}\text{C}$ -chemicals, in comparison with biomineralization of non-bound  $^{14}\text{C}$ -chemicals.

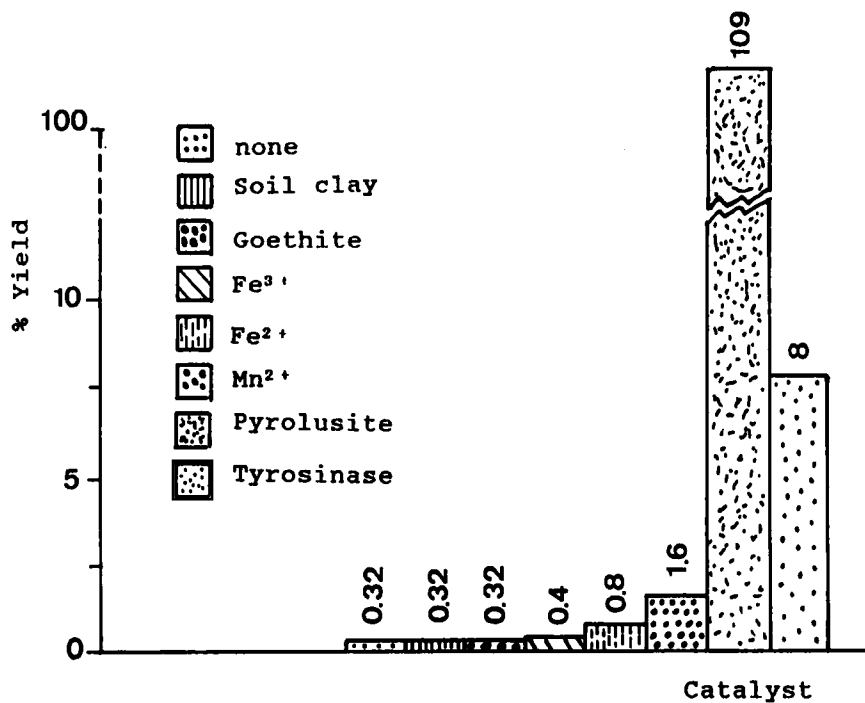
In order to compare the biomineralization of residues bound in different soil fractions, the soil containing bound residues was divided, by well-known methods, into humic acid, fulvic acid, humin, and inorganic fractions.

Figure 5 compares mineralization rates of the pure chemicals with those bound to inorganic soil fractions and to humic acid. Here,  $\text{CO}_2$  bound as carbonate is included. It is evident that biomineralization is by far highest with the pure, non-bound chemical, is lower with the chemically bound to inorganic fractions, and lowest with that to humic acid. Residues bound to fulvic acid—which are not shown in this figure for reasons of clarity of presentation—have a biomineralization rate significantly higher than those to humic acid or to inorganic fractions.

Figure 6 demonstrates the uptake of residues of chlorinated anilines bound to fulvic acids, humin, inorganic fractions, and humic acids by barley seedlings. The figure reveals that the bioavailability of bound residues to plants is generally low. It is highest for the residues bound to fulvic acids and lowest for those bound to humic acids, whereas the uptake from humin and from inorganic fractions is medium<sup>5</sup>. This



**Figure 6** Uptake of unextractable  $^{14}\text{C}$  by barley seedlings from inorganic and organic fractions of soil treated with  $^{14}\text{C}$ -labelled chlorinated anilines (in % of  $^{14}\text{C}$  in each soil fraction).



**Figure 7** Yields of anilinoquinone formed from catechol and 4-chloroaniline in the presence of various catalysts.

agrees well with the findings related to availability to microorganisms, i.e., biomineralization, as discussed above.

### *Model reactions for binding of xenobiotics to soil organic matter*

A number of model reactions to demonstrate covalent binding of xenobiotics to soil organic matter were provided by Bollag and his group<sup>16-19</sup>. In our laboratory, the binding of 4-chloroaniline to the humic acid precursor catechol could be demonstrated. Oligomers are formed, which may be regarded as building blocks for humic acid polymerization.

The main product was shown to be an anilinoquinone (4,5-bis[4-chlorophenyl-amino]3,5-cyclohexadiene-1,2-dione)<sup>6</sup>. The reaction is catalyzed both by enzymes and by inorganic compounds present in soil. The oxidizing enzyme tyrosinase and the mineral pyrolusite exhibit the best catalyzing capacities (Figure 7)<sup>6</sup>.

## CONCLUSION

In spite of the high importance of soil-binding of pollutants for the ecotoxicological evaluation of chemicals, the processes involved are still poorly understood. More research in this field is urgently needed.

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