

Bound Residue Formation and Chemical Binding in Soil: a Literature Survey

BY

J. Karl C. Nieman

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The following table is the result of a literature search for information about the formation of bound residues from pesticides or hazardous wastes in soils. It is meant to be a chronological summary of representative work done in this area over the last 30 years but certainly does not represent all of the work that has been done. Complete references are listed at the end of the table. Please contact the author at k.nieman@usu.edu with questions, comments, or corrections.

About the author:

Karl Nieman holds a bachelor's degree in Agronomy and a Master's degree in Civil and Environmental Engineering. He is currently pursuing his Ph.D. at Utah State University. His research focuses on the fate of organic chemicals during the bioremediation process.

Reference	Compounds/ methods	Title, Summary, or Findings
Bartha, R. (1971)	propanil 4-chloroaniline Analytical methods: GC, TLC, liquid scintillation	“Fate of herbicide-derived chloroanilines in soil.” Over a 20 day incubation, 73 and 54% of added propanil was bound in treatments of 5ppm and 500ppm respectively. Similar percentages were observed for 4-chloroaniline. Caustic digestions indicated that the majority of the bound residue was the propanil metabolite 3,4-dichloroaniline. The 4-chloroaniline incubations confirmed this possibility.
Stevenson, F.J. (1972)	herbicides Analytical methods: Various-review	“Organic matter reactions involving herbicides in soil.” (A review) focuses on the evidence that organic matter plays a major role in the adsorption of herbicides and that organic matter content is usually the soil factor most directly related to herbicidal behavior. Discusses organic matter composition and formation, and potential reactions with various types of herbicides-conjugation reactions, free radical attack, partial metabolism (activation) and then binding etc. Discusses roles of individual fractions HA, FA etc. Adsorption mechanisms include” ion exchange, protonation, H-bonding, van der Waals forces, and coordination through an attached metal ion. Changes in FTIR spectra are observed when HA is methylated.
Khan, S.U. (1973)	Herbicides (diquat, paraquat) Analytical methods: Sephadex gel, IR, UV	“Interaction of humic substances with bipyridylum herbicides” Humic acid complexation was higher than fulvic acid for both herbicides. IR spectroscopy indicated charge-transfer complexes. HA and FA retained herbicides in amounts that were “considerably less” than their exchange capacities (possible steric hinderance). FTIR data showed a shift in the C-H out of plane bending bands upon complexation.
Khan, S.U. (1974)	diquat, paraquat Analytical methods: UV, IR	“Adsorption of bipyridylum herbicides by humic acid” Adsorption to HA complexed with various cations was studied. The extent of herbicide adsorption on cation-saturated HA’s was inversely proportional to the relative strength of cations binding to HA. IR Spectroscopy showed the band at 1720 (carbonyl of carboxylic acid) diminished while that at 1610 (carboxylate) increased upon herbicide addition. This indicated conversion of COOH to COO- which reacted with the bipyridylum cations to form carboxylate bonds.
Hsu, T.-S., Bartha, R. (1974)	chloroanilines Analytical Methods: liquid scintillation, TLC	“Biodegradation of chloroaniline-humus complexes in soil and in culture solutions.” Incubations of soil-bound chloroanilines released 1% of the ¹⁴ C as ¹⁴ CO ₂ per week. And this rate was not influenced by pH or soil moisture. A glucose addition of 5000mg/kg did inhibit ¹⁴ CO ₂ release. The overall soil respiration rate was not affected by the presence of the bound residue. Residence time of the bound residues was estimated at 2-4 years. Inoculation with <i>P. frequentans</i> or <i>A. versicolor</i> resulted in less than 1.3% mineralization, but in the case of <i>A. Versicolor</i> 25.9% of the residue became associated with the fungal mycelium (0.76% for <i>P. frequentans</i>).

<p>Hsu, T.-S., Bartha, R. (1974)</p>	<p>chloroanilines</p> <p>Analytical Methods: liquid scintillation, GC, IR, TLC</p>	<p>“Interaction of pesticide-derived chloroaniline residues with soil organic matter,” Radiolabeled chloroanilines were incubated with sterilized and non-sterile soils and with extracted humic and fulvic acids. Treatments were solvent extracted and then subjected to alkaline or acid hydrolysis. Binding was rapid and did not appear to be microbially mediated. Pure humic acid was more reactive than soil. Soils were methylated and sponified in an effort to block binding sites with inconclusive results. A free radical inhibitor indicated that free radicals played a minor role in binding. Test with HA monomers showed binding with aldehydes and quinones (potentially hydrolyzable bonds), but not with phenols and carboxylic acids (hydrolysis resistant bonds). Hydrolysis released significant amounts of radioactivity (including parent compound, 35.8 and 44.9% for bound DCA for alkaline and acid hydrolysis respectively) from soil.</p>
<p>Kaufman, D.D., Still, G.G., Paulson, G.D., Bandal, S.K. Eds. (1976)</p>	<p>Pesticides</p> <p>Analytical methods: many</p>	<p>“Bound and Conjugated Pesticide Residues.” This widely referenced book is product of an ACS symposium held in 1975. In the introductory chapter, Kaufman restates a previously proposed definition of bound residue as “that unextractable and chemically unidentifiable pesticide residue remaining in fulvic acid, humic acid, and humin fractions after exhaustive sequential extraction with nonpolar organic and polar solvents” and states three critical questions, 1-What is their nature and/or identity?, 2-What is their significance?, and 3-What is their source? half of the papers deal with pesticide conjugates in plant and animals while the other half deal with pesticide-soil interactions.</p> <p>Conclusions included that detailed studies on bound residues were still in their infancy, there was “considerable confusion” as to what was being measured when radioactive materials were used, and there was still “considerable questions” as to the environmental significance of the bound residues.</p>
<p>Meikle, R.W. et al. (1976)</p>	<p>ditalimfos</p> <p>Analytical methods: liquid scintillation gel chromatography</p>	<p>“Classification of bound residues soil organic matter: Polymeric nature of residues in humic substances.” Humic material was efficiently extracted with DOWEX A-1 chelating agent. Extractions with DOWEX showed radioactivity in the humic acid to be 1.8 times that in the fulvic acid. Extraction with hot NaOH resulted in a 0.6:1 ratio indicating that the extraction was destructive in some way. Humic materials were fractionated with Sephadex gel showing two general sizes, >10,000mw and 2100-3400mw, with associated radioactivity. The majority (70-80%) of the humic material was retained by the Sephadex gel. Specific activities (dpm/mgC) were similar for fulvic and humic acid and were reduced in humin although the authors indicated that activities decreased with increasing molecular weight.</p>

Riley, D., Wilkinson, W., Tucker, B.V. (1976)	Paraquat Analytical methods: various bioassays liquid scintillation other unspecified methods	“Biological unavailability of bound paraquat residues in soil.” This paper focuses on results of many bioassays to show that paraquat is effectively deactivated by soil. Paraquat is a divalent cation and binds strongly to clay minerals, yet can be recovered by boiling with sulfuric acid. The authors concluded that paraquat residues are not available to plants, earthworms, microarthropods, or microorganisms and that there is no risk of bound paraquat becoming freed. Plants are very sensitive to free paraquat responding to concentrations as low as 0.01µg/ml in the soil solution and are therefore good indicators of paraquat toxicity.
Hill, I.R. (1976)	pirimicarb Analytical methods: liquid scintillation	“Degradation of the insecticide pirimicarb in soil-characterization of ‘bound’ residues.” Pirimicarb binding was studied under aerobic and anaerobic conditions. In one soil, incubated for 2 years, bound ¹⁴ C continued to increase until it reached 70%. Another soils showed increasing binding over 5-10 weeks, but then showed a decline in bound residue. Almost all of the radioactivity was extractable with NaOH and was present in the fulvic acid fraction. Drying cycles and grinding resulted in releases of bound material. Extraction with acetate buffer released about half of the residue in one soil that had been incubated anaerobically. The authors concluded that the ¹⁴ CO ₂ had been fixed as ¹⁴ C-carbonate under flooded conditions
Khan, S.U.(1978)	various pesticides Analytical methods: Various-review	“The interaction of organic matter with pesticides” Khan reviews organic matter-pesticide interactions. Two aspects are addressed, 1)adsorption of pesticides by OM, and 2)nonbiological degradation of pesticides by OM. Mechanisms of adsorption and isotherms are reviewed. Classes (ionic, nonionic etc.) of pesticides are discussed. Bound residues are discussed and the need for more research “to determine whether or not the bound residue consists of intact pesticides or their degradation products” is emphasized.
Fuhremann, T.W., Lichtenstein, E.P. (1978)	Methyl Parathion Analytical methods: liquid scintillation	“Release of soil-bound methyl [14C]parathion residues and their uptake by earthworms and oat plants.” Soils were incubated with methyl parathion and then exhaustively extracted (10 times) leaving 32.5% of the applied radioactivity as bound residue. This soil was used to expose earthworms and to grow oats. Earthworms accumulated the residue at a rate that resulted in uptake of 2.7% of the bound residue in 6 weeks and three crops of oats accumulated 5.1% over 6 weeks.
Helling, C.S., Krivonak, A.E. (1978)	Dinitroanilines (butralin, chlornidine, dinitramine, fluchloralin, profluralin, trifluralin) Analytical Methods: liquid scintillation, TLC	“Biological characteristics of bound dinitroaniline herbicides in soils.” Butralin residues created under anaerobic conditions were relatively stable during aerobic incubation. Residues bound to HA were the most degradable with a half-life of 7 years. Bound residues from several herbicides seemed to cause abnormal growth. Residue uptake from dinitroanilines was observed in soy beans, but at levels unlikely to cause phytotoxicity. The benzene-methanol extraction used to produce the residue containing soil appeared to cause an increase in available Mn which in turn caused Mn toxicity to the plants. Prolonged dry storage or autoclaving produced similar results. The authors suggested that the Mn oxidizing community had been inhibited.

<p>Martin, J.P., Haider, K., Linhares, L.F. (1979)</p>	<p>Catechol</p> <p>Analytical methods: Liquid scintillation, TLC</p>	<p>“Decomposition and stabilization of ring-¹⁴C-labeled catechol in soil.” Added catechol was highly reactive in alkaline soils causing 94% to be non-extractable after 1 week in sterilized soil. Neutral and acid soils bound only 20 and 10% respectively. Microbial activity increased binding/polymerization significantly. Phenolase activity was the suspected catalyst. Peroxidase was found to be more efficient for polymerization with model humic compounds.</p>
<p>Bartha, R. (1980)</p>	<p>various pesticides</p> <p>Analytical methods: Various-review</p>	<p>“Pesticide Residues in Humus” Discuss binding of aniline and phenolic compounds to humic materials. DCA residues were found to degrade at a slower rate than organic matter. This could lead to a build up of residues. Evidence was found that bound DCA could be liberated by humus degrading soil fungi and actinomycetes. Humus-degrading soil fungi were capable of releasing intact methoxychlor that had been bound previously. Methyl-parathion residues were also found to be available to oat seedlings and to earthworms.</p>
<p>Fuhr, F., Mittelstaedt, W. (1980)</p>	<p>methabenzthiazuron</p> <p>Analytical Methods: TLC, HPLC Liquid scintillation</p>	<p>“Plant experiments on the bioavailability of unextracted [carbonyl-¹⁴C]methabenzthiazuron residues from soil” After 111 days of incubation, 41% of the herbicide was unextractable (bound). Maize plants planted in the extracted soil contained 0.7% of the original radioactivity after 29 days of growth compared to 2.2% in unextracted soil and 4% in soil with freshly applied herbicide.</p>
<p>Khan, S.U. (1980)</p>	<p>Prometryn</p> <p>Analytical Methods: Liquid scintillation, GC-MS</p>	<p>“Plant uptake of unextracted (bound) residues from an organic soil treated with prometryn.” The same bound residues were used as in Khan and Ivarson, (1981). Oats were planted in the soil (that contained 57.4% of the original radioactivity) and grown for 21 days. The plants removed 0.53% of the bound residues which was distributed into roots shoots and lignin. The paper focuses on the distribution within the plants, but 0.53% of 57.4% may not be highly significant. It depends on the mass initially applied which is not reported.</p>
<p>Parris, G.E. (1980)</p>	<p>Anilines and humic material</p> <p>Analytical methods: GC, IR</p>	<p>“Covalent binding of aromatic amines to humates 1. Reactions with carbonyls and quinones.” The primary imines bind to humate in two phases, an initial, fast, reversible reaction that may be formation of imine linkages with the humate carbonyls and a second slow reaction is proposed to be the formation of amino substituted quinones that is not readily reversible. IR spectroscopy is used to monitor chemical changes in humics as binding proceeds.</p>

<p>Martin, J.P., and K. Haider (1980)</p>	<p>Humic acid precursors; ferulic acid, catechol, amino acids</p> <p>Analytical methods: liquid scintillation</p>	<p>“Comparison of the use of phenolase and peroxidase for the synthesis of model humic acid-type polymers” Incorporation efficiencies of the peroxidase-H₂O₂ system were generally higher than for the mushroom phenolase catalyzed reactions. “it should be noted that the enzymes do not synthesize the polymers but oxidize the phenolic compounds by removal of phenolic-group H or hydrogens to form radicals or quinones. The radicals stabilize by linking together and other aromatic compounds, amino acid, amino sugars, an other substrates with free-NH₂ or -SH groups link into the reactive quinones through nucleophilic addition.”</p>
<p>Khan, S.U., Ivarson, K.C. (1981)</p>	<p>prometryn</p> <p>Analytical Methods: scintillation counting, GC, GC-MS</p>	<p>“Microbiological release of unextracted (bound) residues from an organic soil treated with prometryn.” After one year of incubation, 57.4% of the applied radioactivity had been bound. The extracted soil containing bound residue was inoculated and incubated for 22 days. No residue mineralization was observed, but after the incubation, 27% of the residues were extractable compared to <1% in the uninoculated controls. Released compounds included parent compound and metabolites.</p>
<p>Cheng, H.H., Haider, K., Harper, S.S. (1983)</p>	<p>catechol chlorocatechols</p> <p>Analytical Methods: liquid scintillation</p>	<p>“Catechol and chlorocatechols in soil: degradation and extractability.” Chlorocatechols were degraded to a greater extent than catechol. This was contrary to other findings, but supported by the finding that the catechol was more reactive and formed stable bound residues (25-60% bound) when added to fresh soil and incubated under various conditions. Bound chlorophenol residues ranged from 2-55%. Extractability decreased with increased incubation time in sterilized and unsterilized soils. More than half of the bound residues could be removed with Na-pyrophosphate extraction and were evenly distributed between humic and fulvic acid.</p>
<p>Bollag, J.-M., Minard, R.D., Liu, S.-M. (1983)</p>	<p>4-chloroaniline 3,4-dichloroaniline 2,6-diethylaniline</p> <p>Analytical methods: liquid scintillation TLC, HPLC, MS, proton NMR, UV</p>	<p>“Cross linkage between anilines and phenolic humus constituents.” Anilines were found to cross link with the humic constituents syringic acid, vanillic acid, ferulic acid, and protochatechuic acid in the presence of fungal phenol oxidase. Anilines alone were not transformed by the enzyme. Mechanisms of Parris (1980) are referred to. Products of oxidative coupling are isolated and analyzed by HPLC, MS, and proton NMR.</p>
<p>Freitag, D., Scheunert, I., Klein, W., Korte, F. (1984)</p>	<p>4-chloroaniline</p> <p>Analytical Methods: Scintillation counting, TLC, GC, GLC-MS</p>	<p>“Long-term fate of 4-chloroaniline-¹⁴C in soil and plants under outdoor conditions. A contribution to terrestrial ecotoxicology of chemicals” The majority of the radioactivity was not recovered (67%, assumed to be mineralized), and the bulk of the recovered activity was bound (after chloroform extraction for 60 hrs) to the soil (32.36%) and remained bound for three years. 10% of the unextractable residue was released as parent compound by alkaline hydrolysis.</p>

<p>McCarthy, J.F., and Jimenez, B.D. (1985)</p>	<p>¹⁴C naphthalene, anthracene, benzoanthracene, 3-methylcolanthrene, and benzo(a)pyrene</p> <p>Analytical methods: liquid scintillation, fluorescence quenching</p>	<p>“Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: binding and dissociation” Equilibrium dialysis and fluorescence techniques were used to study sorption of PAH to dissolved Aldrich HA. Results indicated that sorption of B(a)P was fully reversible and that affinity for the DHA was directly related to Kow.</p>
<p>Berry, D.F., and Boyd, S.A. (1985)</p>	<p>3,3'-dichlorobenzidine</p> <p>Analytical methods: liquid scintillation</p>	<p>“Decontamination of soil through enhanced formation of bound residues” Ferulic acid and H₂O₂ caused a significant decrease (65%) in the level of free DCB after extraction with ethylacetate-methanol (3:2). Peroxidase addition had no effect. Vanillic acid and glucose in place of ferulic acid had no effect. “Addition of ferulic acid and H₂O₂ are believed to accelerate the natural process of humus formation which is mediated by indigenous peroxidase enzymes.”</p>
<p>Mac Rae, I.C. (1986)</p>	<p>fenitrothion</p> <p>Analytical Methods: scintillation counting, TLC, GC</p>	<p>“Formation and degradation of soil-bound [¹⁴C]fenitrothion residues in two agricultural soils.” After 65 days of incubation, non-extractable radioactivity represented 73.7 and 59.35% of the added radiolabel in two different soils. The radioactive residue increased over the 65 day incubation. The soils were then mixed with unextracted soil and amended with various carbon sources. All amendments caused an increased release of ¹⁴CO₂ over the control (from 0.5 to 9.02% of the residues were mineralized) and the author suggested that this could be due to stimulated humus degradation.</p>
<p>Dec, J., and Bollag, J.-M. (1988)</p>	<p>catechol, 4-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, PCP</p> <p>Analytical methods: liquid scintillation, HPLC, gel chromatography</p>	<p>“Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid.” Synthetic humic acid was created (from 13 individual components) that incorporated radiolabeled catechol or chlorophenols. The resultant polymer was then incubated with mixed microbial cultures. About 80% of the radioactivity remained bound for all compounds. 2.5-4.1% was found in the cells and was not extractable with acetone. Supernatants containing nonprecipitated fractions of the synthetic polymer contained 6.1 to 14-6%. The majority of the released material was parent compound. The authors propose that there is release of “surface” bound contaminant and retention of residues bound to the “core” HA, but that the small percentage released should not have adverse environmental effects.</p>

McBride, M.B. et al. (1988)	catechol Analytical methods: UV, gel chromatography, ESR, Proton NMR, ¹³ C NMR, IR, O ₂ -monitoring	“Complexation and catalyzed oxidative polymerization of catechol by aluminum in acidic solution” Aluminum cations were shown to catalyze catechol oxidation forming colored polymeric products. UV-visible, NMR, IR, and electron spin resonance spectroscopies were used to characterize the reaction.
Calderbank, A. (1989)	various pesticides Analytical methods: Various-review	“The occurrence and significance of bound pesticide residues in soil.” The nature of soil colloids and potential binding processes (adsorption, chemical interaction) are reviewed. The extent of bound pesticide residues is usually between 20 and 70%. Chemical extraction of residues yields parent compound in many cases (pirimicarb, prometryn, atrazine, simazine, picloram etc.). Aging may involve chemical incorporation into soil humin (no refs. given). If bound residues decay (1 st order) then repeated addition will allow for a plateau concentration if new parent compound is repeatedly applied (when amount degraded is equal to the amount added). It is entirely predictable that the slow release of bound residues...will be of little toxicological or ecological concern in practice.”
Simmons, K.E. et al. (Bollag) (1989)	guaiacol 4-chloroaniline Analytical methods: TLC, MS, proton NMR, HPLC	“Oxidative co-oligomerization of guaiacol and 4-chloroaniline.” Guaiacol (2-methoxyphenol), a model humus constituent was incubated with 4-chloroaniline in the presence of manganese dioxide, tyrosinase, peroxidase, and laccase. All of these resulted in the same five co-oligomeric products. The oxidation of guaiacol was essential to the reactions. Mechanisms could include 1) nucleophilic addition of the aniline to humus quinoid groups or 2) through free-radical coupling reactions.
Dec, J. and Bollag, J. –M. (1990)	chlorophenols Analytical methods: Liquid scintillation, HPLC, gel chromatography, TLC, MS	“Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions.” Laccases and tyrosinase would be preferred over peroxidase in an engineered system since hydrogen peroxide is not required. Polymerization of chlorophenols by all of these enzymes was explored over a range of pH and temperatures. The polymerization ability of peroxidase was much quicker and was not temperature dependent, but the other enzymes also succeeded in polymerization and dechlorination.
Qui, X., McFarland, M.J. (1991)	PAH Analytical methods: Liquid scintillation, HPLC	“Bound residue formation in PAH contaminated soil composting using Phanerochaete Chrysosporium.” Fungal inoculation enhanced B(a)P removal significantly, but the majority of the enhancement was through bound residue formation, not mineralization. Approximately 25% was bound without fungi and 40% was bound with fungi (not statistically different). Residue formation was higher (but not statistically) in the Kidman soil that contained more clay. Looking at the figures, it does not appear that the fungi treated soil was much different than the unamended control except for a drop in extractable B(a)P in the inoculated Kidman soil that is not totally explained by residue formation.
Singh, D.K., and Agarwal,	DDT	“Chemical release and nature of soil-bound DDT residues.” A maximum of 8% of the ¹⁴ C-DDT applied to the soil was

H.C. (1992)	Analytical methods: liquid scintillation HPLC, TLC	considered to be bound (not methanol extractable). Sulfuric acid treatment released 91.5% of the bound residues that consisted primarily of parent DDT.
Bollag, J.-M. and Myers, C. (1992)	Phenols Analines Analytical methods: various-review	“Detoxification of aquatic and terrestrial sites through binding of pollutants to humic substances.” Oxidative coupling may be mediated by enzymes, inorganic chemicals, clay and soil extracts, or occur as a result of auto-oxidation. Phenolic compounds are oxidized to aryloxy radicals by removal of an electron and hydrogen ion from the hydroxyl group. Two free radical intermediates can then couple at the <i>ortho</i> or <i>para</i> positions, but the <i>meta</i> position is non-reactive. Two classes of oxidoreductases that catalyze aromatic coupling: peroxidases (have iron porphyrin and need H ₂ O ₂) and monophenol mono-oxidases (laccases and tyrosinases which contain copper and require bimolecular oxygen). Laccases oxidize phenolic substrates to form anionic free radicals. Tyrosinases carry out a two step reaction by forming the o-diphenol and then oxidizing it to an o-quinone. Bound residue should be released/degraded very slowly and is “unlikely to present an environmental hazard.” In some cases toxic compounds can be formed by enzymatic polymerization (e.g. chlorophenols incubated w/peroxidase formed dioxins and furans)
Bollag, J.-M. (1992)	Phenols Analines Analytical methods: various-review	“Decontaminating soil with enzymes.” A review article. Humification can be enhanced by addition of extracellular enzymes, abiotic catalysts (Al, Fe, Mn), or by altering the physiochemical conditions. Coupling reactions may occur spontaneously in the presence of oxygen at alkaline pH. Oxidoreductases are either peroxidases or polyphenol oxidases (laccases and tyrosinases). Binding depends on many environmental factors that vary with season, climate, soil type, and agricultural practices. “Because of this variability, it is necessary to evaluate the incorporation of each xenobiotic into humus under specific sets of environmental conditions.” Calcareous or acid soil may reduce the activity of phenoloxidases that have pH optimum of 4-7. “All available data indicate that after xenobiotics are incorporated into soil, they are released only minimally and gradually”

Hatcher, P.G., Bortiatynski, J.M., Minard, R.D., Dec, J., Bollag, J-M. (1993)	2-4 Dichlorophenol Analytical methods: ¹³ C NMR, HPLC liquid scintillation	“Use of high-resolution ¹³C NMR to examine the enzymatic covalent binding of ¹³C-labeled 2,4-dichlorophenol to humic substances.” ¹³ C-dichlorophenol was bound to a Minnesota peat humic acid in vitro with horseradish peroxidase. ¹⁴ C-dichlorophenol was used to establish the mass balance for bound and free DCP. 34% of the added ¹⁴ C was bound to the humic acid after acidified water and solvent extractions. Only acidified water was used to extract samples with ¹³ C. ¹³ C NMR results indicated a dispersion of chemical shifts induced by covalent bonding (C-C, ether, or ester bonds), C-C and ester linkages being the most likely (since they yielded the strongest peaks). “A similar methodology can be employed with other pollutants and humic materials to provide much information on the binding of pollutants to soil organic matter.”
Richnow, H.H., Seifert, R., Hefter, J., Kastner, M., Mahro, B., Michaelis, W. (1993)	PAH PCB Analytical methods: GC, GC-MS	“Metabolites of xenobiotica and mineral oil constituents linked to macromolecular organic matter in polluted environments.” Selective chemical degradation techniques (OH-, BCl ₃ , Rh/H ₂) and hydrolysis with Na ¹⁸ OH were used to examine released metabolites from previously extracted soil (ethylacetate and dichloromethane). GC-MS was used to analyze extracts. Phthalic acid, 2-hydroxy-3-naphthoic acid, naphthoic diacids, phenanthrene-4-carboxylic acid, and 9-fluorenone-1-carboxylic acid were identified. Authors concluded that some metabolites form stable ester bonds with humics.
Sims, R.C., Abbot, C.K. (1993)	pyrene benzo(a)pyrene Analytical methods: liquid scintillation	“Evaluation of Mechanisms of Alteration and Humification of PAHs for Water Quality Management” Tracking of radiolabeled carbon indicated little mineralization (<1%) occurred in non-poisoned treatments, although humin associated ¹⁴ C appeared to increase in previously uncontaminated soil in poisoned and non-poisoned treatments. Humin contained 60-70% of the radiolabel (added as pyrene) after 285 days. The combined humic and fulvic acid extract generally contained about 10-20% of the label for both compounds, but was as high as 30% in some cases for B(a)P in poisoned soil. The authors concluded that 70 to 80% of added ¹⁴ C was humified in previously uncontaminated soil and that humification was also the most significant fate in previously contaminated soil.
Dec, J. and Bollag, J-M. (1994)	Chlorinated phenols Analytical methods: liquid scintillation HPLC, coulometric titration	“Dehalogenation of chlorinated phenols during oxidative coupling.” Release of chloride was observed during two oxidative coupling reactions, polymerization and binding to humic acid. Reactions were catalyzed by laccase, horseradish peroxidase, and Birnessite. Dechlorination occurs when the unpaired electron of a free radical is located at the chlorine substituted aromatic carbon. Rates of transformation were higher for Birnessite than for the oxidoreductive enzymes.
Bollag, J-M., Mertz, T., Otjen, L. (1994)	Organic pollutants Analytical methods: Various-review	“Role of microorganisms in soil bioremediation.” A general overview of the bioremediation field. “It appears feasible to effectively detoxify pollutants on a large scale by enhancing natural humification processes or by increasing free radical reactions through the use of oxidoreductive enzymes.”
Eschenbach, A., Kastner,	PAH	“Evaluation of a new effective method to extract polycyclic aromatic hydrocarbons from soil samples.” The extraction

M., Bierl, R., Schaefer, G., Mahro, B. (1994)	Analytical methods: HPLC	involves 3 sonications with acetone and then a saponification extraction with KOH and methanol to release bound residues. The saponification released 20-30% more PAH than the solvent extraction alone. The amount of PAH recovered by saponification increased over 56 days of incubation. The authors propose that saponification cleaves ester bonds and breaks down the macromolecular humic structure thus releasing trapped PAH. ¹⁴ C studies showed that there is another fraction that is not released by saponification.
Dec, J. and Bollag, J.M. (1995)	Chlorinated phenols and anilines Analytical methods: liquid scintillation HPLC, coulometric titration	“Effect of various factors on dehalogenation of chlorinated phenols and anilines during oxidative coupling.” “Short reaction time, limited amount of catalyst, and unfavorable pH conditions may result in an insufficient generation of free radicals and a reduction in dehalogenation of chlorinated phenols.” “...dehalogenation is strictly connected to oxidative coupling and cannot be enhanced independently by adjustment of the reaction conditions.”
Bhandari, A., Novak, J.T., Berry, D.F. (1996)	4-monochlorophenol Analytical methods: liquid scintillation	“Binding of 4-monochlorophenol to soil.” Oxygen enhanced 4-MCP binding in standard batch sorption experiments. 15% of the ¹⁴ C remained unextracted by water and solvent extractions. This was associated with humic and fulvic acids and humin components. Autoclaving the soil reduced binding while addition of H ₂ O ₂ caused a 4 fold increase in binding.
Burgos, W.D., Novak, J.T., Berry, D.F. (1996)	Naphthalene α-naphthol Analytical methods: liquid scintillation TLC	“Reversible sorption and irreversible binding of naphthalene and α-naphthol to soil: elucidation of processes.” “Biologically mediated oxidative coupling accounted for 49.4 + 15% of the nonextractable α-naphthol. Oxidative coupling catalyzed by mineral surfaces accounted for 40.5 ± 15.8% of nonextractable α-naphthol. Over contact times up to 200d, nonextracted naphthalene increased from 1.6% to 11.7% of the initially sorbed material, coincident with the biological production of a more polar metabolite.” “During the degradation of naphthalene and PAHs in general, extracellular metabolite accumulation has been observed...once the partial oxidation of a PAH occurs, the irreversible binding of metabolites can occur.” Irreversible binding may become competitive with biodegradation.
Karimi-Lotfabad, S., Pickard, M.A., Gray, M.R. (1996)	anthracene phenanthrene pyrene Analytical methods: GC, TLC, liquid scintillation, MS	“Reactions of polynuclear aromatic hydrocarbons on soil.” PAHs were added to oven dry soils or montmorillonite clay (140 °C) in methylene chloride. Results indicated that the dry soils and clay were highly reactive, causing oligomerization of the added PAH. Oligomerized anthracene was not bioavailable to an anthracene degrading culture. Soils at moistures above 1% were not reactive indicating that water effectively competes for the reactive sites in the soil.

U.S.EPA (1996)	phenanthrene PCP Analytical Methods: liquid scintillation	“Champion International Superfund Site, Libby, Montana; Bioremediation Field Performance Evaluation of the Prepared Bed Land Treatment System, Volumes I and II” Soil bound pentachlorophenol (PCP) averaged 32% (± 13) after 45 days of incubation followed by repeated (3x) sonication extractions with methylene chloride:acetone (1:1). Phenanthrene bound residues averaged 60% (± 20) after the same treatment. The percentage mineralized to $^{14}\text{CO}_2$ was less than 7% in all cases and binding did not appear to correlate with mineralization.
Bhandari, A., Novak, J.T., Burgos, W.D., Berry, D.F. (1997)	4-chlorophenol 2,4,6-trichloro- phenol pentachlorophenol phenol Analytical methods: liquid scintillation	“Irreversible binding of chlorophenols to soil and its impact on bioavailability.” Phenols were incubated in soil for 2 days. Treatments were oxic-nonautoclaved, oxic-autoclaved, anoxic-autoclaved, and anoxic-autoclaved. Nonextractable residues in oxic-nonautoclaved treatments with phenol were 54% and 43% of the original spike for initial concentrations of 0.53mM and 5.3mM phenol respectively. Binding of chlorophenols ranged from 12% to 25% of the original spike. Binding in the oxic-nonautoclaved soils was “at least twice as high” as in other treatments that were either autoclaved or deprived of oxygen. 50% of the bound 4-CP (or ^{14}C activity originally associated with 4-CP) was bioavailable to a CP degrading culture. The authors propose that the bacteria were either “better extracting agents” or were capable of “decoupling” the bound residue.
Luthy, R.G., et.al. (1997)	Hydrophobic organics (HOC) Analytical methods: Various-review	“Sequestration of hydrophobic organic contaminants by geosorbants.” Sorption through various mechanisms is described. These include absorption into amorphous (“soft”) organic matter or NAPL, absorption into condensed (“hard”) organic matter or combustion residue (soot), adsorption onto water-wet organic surfaces, adsorption to exposed water-wet mineral surfaces, and adsorption into microvoids or microporous minerals with porous surfaces. Oxidative coupling and bound residues were not addressed.
Xie, H., Guetzloff, T.F., Rice, J.A. (1997)	atrazine 2,4-D DDT Analytical methods: liquid scintillation ^{13}C NMR for humic characterization	“Fractionation of pesticide residues bound to humin.” The percentage of ^{14}C bound to soil after methanol extraction depended on the pesticide and where the pesticide was labeled, and ranged from 6.47% for [ring- ^{14}C] p,p' DDT to 44.95% for [ring- ^{14}C] 2,4-D. The MIBK (methylisobutyl ketone) extraction was used to separate humin into bound-humic acid, lipid, and mineral components. The bound-HA fraction and lipid fraction of the humin were the dominant sinks for bound ^{14}C within the humin fraction, although in most cases the entire humin fraction contained less than 30% of the total bound residue (DDT was the exception with 63% of the bound residue being associated with the humin).

Chung, N., Alexander, M. (1998)	phenanthrene atrazine Analytical methods: liquid scintillation	“Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils.” Contaminants were aged in 16 different sterile soils for 0, 20, 60, 120, and 200 days. Bioavailability (to a capable microbial strain) and extractability (with ethanol and water) were assessed. Correlations between extractability and bioavailability were poor. Soxhlet extraction with methanol was also employed, showing that some bound residues were produced after 120 days of sterile incubation (3% bound phenanthrene, and 20% bound atrazine in a high organic matter soil).
White, J.C., Quinones-Rivera, A., Alexander, M. (1998)	phenanthrene DEHP Analytical methods: liquid scintillation HPLC	“Effect of wetting and drying on the bioavailability of organic compounds sequestered in soil.” Wet and dry cycles during aging in sterilized soils decreased bioavailability and extractability of phenanthrene while wetting and drying after the aging process increased availability. Bound phenanthrene residues were not observed in sterilized soils incubated for 174 and 620 days.
Richnow, H.H., Eschenbach, A., Mahro, B., Seifert, R., et al. (1998)	anthracene Analytical methods: GC-MS, IRM- GC-MS	“The use of ¹³C-labelled polycyclic aromatic hydrocarbons for the analysis of their transformation in soil.” ¹³ C-anthracene was repeatedly spiked onto clean soil over 588 days. An initial lag phase (0-49d) was observed after which a bound residue producing phase (49d-138d) was observed, followed by complete mineralization of added anthracene (138d-588d). ¹³ CO ₂ production was monitored along with the isotopic ratio ($\delta^{13}\text{C}[\text{‰}]$) of extracted soil to show bound residue incorporation. Extractable metabolites of anthracene were monitored by GC-MS and IRM(isotopic ratio monitoring)-GC-MS. A lack of ¹³ C enrichment for <i>n</i> -hexadecanoic acid indicated that anthracene was not utilized for anabolic production of bacterial lipids. Alkaline hydrolysis released labeled phthalic acid and hydroxynaphthoic acid.
Drzyzga, O. et al. (1998)	TNT Analytical methods: liquid scintillation HPLC	“Incorporation of ¹⁴C-labeled 2,4,6-trinitrotoluene metabolites into different soil fractions after aerobic and anaerobic-aerobic treatment of soil/molasses mixtures.” A 5 week anaerobic period designed to produce reduced metabolites followed by a 4 week aerobic treatment to promote oxidative coupling was used. 84% of the applied label was humified at the end of both treatments compared to 57% at the end of only the anaerobic phase. The humin fraction contained the majority of the bound residue.
Kohl, S.D., Rice, J.A. (1998)	naphthalene phenanthrene benzo(a)pyrene dichlorobiphenyl tetrachloro- biphenyl Analytical methods: liquid scintillation	“The binding of contaminants to humin: a mass balance.” Compounds were added to soil in methylene chloride and then incubated at various moisture contents for up to 90 days. It is unclear how dry the soils were before they were spiked, but increased binding of naphthalene (approx. 90%) over benzo(a)pyrene (approx. 40%) may indicate that dry soil could have catalyzed binding and polymerization as observed by Karimi-Lotfabad et al. (1996). Bound residues were mainly associated with the soil humin and the bound lipid fraction of the soil humin had the highest affinity for bound residues when normalized for organic carbon content.

Guthrie, E.A., Pfaender, F. K. (1998)	pyrene Analytical methods: liquid scintillation HPLC	“Reduced pyrene bioavailability in microbially active soils.” ¹⁴ C-pyrene bound residues increased in non-poisoned treatments regardless of the ability to mineralize pyrene. Large percentages (40-80%) of the radiolabel were bound even at day 0 (79.6%, the largest value reported). Trends over time were not apparent. Extracted radioactivity from DCM extraction, base extraction (humic and fulvic acids), and the residual non-extractable residue were incubated with a soil capable of pyrene mineralization. Less than 10% of the activity considered bound (the humic and fulvic acids and the soil residual) was mineralized in 28 days.
Eschenabch, A., Weinberg, R., Mahro, B. (1998)	naphthalene anthracene pyrene benzo(a)pyrene Analytical methods: liquid scintillation	“Fate and stability of nonextractable residues of [¹⁴C]PAH in contaminated soils under environmental stress conditions.” Between 10 and 20% (5% for B(a)P) of fungally produced residues were mineralized over a 190 day incubation period. Addition of humus decomposing microorganisms (the fungi <i>Pleurotus ostreatus</i> and <i>Trametes versicolor</i> or the bacterium <i>Rhodococcus erythropolis</i>) had a stimulatory effect on residue mineralization. About 63% of the initially bound material remained unextractable (by solvent and base) regardless of freeze/thaw treatment. Approximately 20% of radioactivity in controls and treatments was removed by solvent, so by some definitions it was not “bound residue” to start with. Increased release (from 1 to 9%) of residue associated ¹⁴ C after treatment with EDTA was concluded to be the result of ¹⁴ C activity associated with colloidal or dissolved organic matter.
Knicker, H., Bruns-nagel, D., Drzyzga, O. et al. (1999)	TNT Analytical methods: ¹⁵ N NMR	“Characterization of ¹⁵N-TNT residues after an anaerobic/aerobic treatment of soil/molasses mixtures by solid-state ¹⁵N NMR spectroscopy. 1. Determination and optimization of relevant NMR spectroscopic parameters.” This is a companion study to Drzyzga et al. (1998) in which solid state NMR was used to show that aniline derivatives and condensation products were present in the TNT-enriched humic acid
Richnow, H.H. et al. (1999)	Anthracene Analytical methods: liquid scintillation MS	“Formation of nonextractable soil residues: a stable isotope approach.” Added information regarding their previous study (Richnow et al., 1998) and focusing on their methodology. Bound residues formed from ¹⁴ C-anthracene (20%) were two times higher than those formed from ¹³ C-anthracene (11%). Reactors with compost amendments formed less bound residue than unamended reactors (10% of added label vs 60% after 138 days). The authors propose that residue formation is a function of microbial metabolism with more acclimated cultures producing less residue.
Nieman, J.K.C., Sims, R.C., Sims, J.L., Sorensen, D.L., Mclean, J.E., Rice, J.A. (1999)	Pyrene Analytical methods: liquid scintillation HPLC	“¹⁴C]Pyrene bound residue evaluation using MIBK fractionation method for creosote-contaminated soil.” Bound residues increased over time (from 3.0% to 10.7%) in biologically active soils over 294 days of incubation. The major increase was observed in the humic acid fraction (increasing from 0.6% to 4.2%). Other humic fractions showed increases while the bound-lipid fraction of the humin did not change over time.

<p>Park, J.-W., Dec, J., Kim, J.-E., Bollag, J.-M. (1999)</p>	<p>chlorophenols</p> <p>Analytical Methods: HPLC</p>	<p>“Effect of humic constituents on the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes and birnessite.” Sixteen different humic constituents were screened with three different enzymes and the mineral birnessite for their effectiveness in chlorophenol coupling. Some enzyme-humic combinations enhanced coupling while others suppressed it. The authors concluded that both the chlorophenol and humic constituent must be in the same oxidized form to react (either free radicals or o-quinones and phenoxide anions). Some combinations promoted similar oxidized forms and thus promoted coupling to humics, and some combinations allowed for different oxidized forms that were not compatible.</p>
<p>Guthrie, E.A., et.al. (1999)</p>	<p>Pyrene</p> <p>Analytical Methods: Solid-state ¹³CNMR Pyrolysis-GC-MS HPLC</p>	<p>“Determination of [¹³C]Pyrene sequestration in sediment microcosms using flash pyrolysis-GC-MS and ¹³C NMR.” Aerated sediments were amended with ¹³C pyrene and incubated for 60 days. Sediments were extracted with methylene chloride and bound residues within humic acid and humin were monitored. Amounts of bound residue increased over time in both fractions, but the majority accumulated in the humin fraction (up to 53% of the added pyrene). Analysis showed that the pyrene was not covalently bound, but had moved into hydrophobic parts of the humic material as biologic activity degraded less polar fractions within the sediments .</p>
<p>Kastner, M., Streibich, S., Beyrer, M., Richnow, H.H., Fritsche, W. (1999)</p>	<p>Anthracene</p> <p>Analytical Methods: Scintillation counting HPLC NMR</p>	<p>“Formation of bound residues during microbial degradation of [¹⁴C]anthracene in soil.” Results showed that anthracene labeled in different positions was metabolized at different rates and formed different levels of bound residues (28.5% for anthracene labeled at 1,2,3,4,4a,5a, and 20.7% for label at C-9). Incubations with ¹⁴CO₂ indicated that it may be a significant source for bound residue formation. In vitro formation of humic acid-like polymers were shown to be formed from PAH metabolites in the presence of catalytic bivalent cations (Fe, Mn, Zn). Bound residues were found in HA, FA, and humin fractions. Compost increased mineralization and decreased residue formation. Bacteria were found to play the major role in anthracene degradation, not fungi. “Most of the nonextractable bound residues which are formed during microbial degradation of PAH result from incorporation of PAH metabolites”. Potential residues: parent compound, metabolites, CO₂, microbial biomass.</p>
<p>Bogan, B.W., Lamar, R.T., Burgos, W.D., Tien, M. (1999)</p>	<p>anthracene fluoranthene benzo(a)pyrene</p> <p>Analytical methods: liquid scintillation</p>	<p>“Extent of humification of anthracene, fluoranthene, and benzo(a)pyrene by Pleurotus ostreatus during growth in PAH-contaminated soils.” Incubation of soils spiked with ¹⁴C-PAH with the fungus resulted in slight increases in the bound residues associated with humic acid and more significant increases (1.5 to 2 times) in the residues associated with the humin fraction, which retained from 10 to 30% of the applied label and was the major sink for residues .</p>

Ressler, B.P., Kneifel, H., Winter, J. (1999)	PAH Analytical Methods: HPLC Gel permeation chromatography Microtox	“Bioavailability of polycyclic aromatic hydrocarbons and formation of humic acid-like residues during bacterial PAH degradation.” When incubated with PAHs, the mixed culture produced a dark brown or black polymerization product that precipitated under acidic conditions like humic acid and was of similar molecular size. Extracellular metabolites were isolated. Association of PAH with the polymerized residues reduced their bioavailability.
Park, J.-A., Dec, J., Kim, J.-E., Bollag, J.M. (2000)	chlorophenol chloroaniline Analytical Methods: HPLC Scintillation counting	“Transformation of chlorinated phenols and anilines in the presence of humic acid.” This is a follow up study to Park et al. (1999). Instead of individual humic acid constituents, whole humic acids were tested with oxidoreductive enzymes and birnessite with either chlorophenols or chloroaniline. Humic acid increased the binding of 4-chlorophenol in most cases, but only reacted with 4-chloroaniline when an excess of humic acid was present. This difference was attributed to the different mechanisms of activation and binding for the two compounds, free radical formation of 4-chlorophenol, and nucleophilic addition of 4-chloroaniline. The authors suggested that the 4-chloroaniline reaction was limited by the availability of carbonyl groups or quinone moieties available for reaction in the humic acid. Reactions with other chloroaniline/chlorophenol enzyme combinations in the presence of humic acids were mixed.
Karimi- Lotfabad, S., Gray, M.R. (2000)	Creosote Analytical Methods: Fluorescence confocal laser scanning microscopy and cryogenic SEM	“Characterization of contaminated soils using confocal laser scanning microscopy and cryogenic-scanning electron microscopy.” Fluorescence of aged creosote contaminated soil was not removed by 6 hours of Soxhlet extraction with methylene chloride. Combustion for 1h at 815 °C resulted in loss of fluorescence. This may have implications regarding bound residues in aged NAPL contaminated soils.
Wunderwald, U. et al. (2000)	Synthetic HA Analytical Methods: NMR, FTIR Gel permeation Chromatography HPLC	“Formation and degradation of a synthetic humic acid derived from 3-fluorocatechol.” The authors discuss the potential alteration of humic acids by introduction of bound residues. Active white-rot fungus and its manganese peroxidase were used to degrade an artificial humic acid that had been synthesized from 3-fluorocatechol. This treatment is proposed to eliminate bound residue containing humic substances that may have detrimental environmental effects including effects on “microorganisms and plants as well as their enzymes interacting with humus.”

References

- Bartha, R. (1971). Fate of herbicide-derived chloroanilines in soil. *J. Agr. Food Chem.*, 19,385-387.
- Bartha, R. (1980). Pesticide residues in humus. *ASM News*, 46,356-360.
- Berry, D.F., and Boyd, S.A. (1985). Decontamination of soil through enhanced formation of bound residues. *Environ. Sci. Technol.*, 19,1132-1133.
- Bhandari, A., Novak, J., T., and Berry, D.F. (1996). Binding of 4-Monochlophenol to soil. *Environmental Science and Technology*, 30,2305-2311.
- Bhandari, A., Novak, J.T., Burgos, W.D., and Berry, D.F. (1997). Irreversible binding of chlorophenols to soil and its impact on bioavailability. *Journal of Environmental Engineering*, 123,506-513.
- Bogan, B.W., Lamar, R.T., Burgos, W.D., and Tien, M. (1999). Extent of humification of anthracene, fluoranthene, and benzo(a)pyrene by *Pleurotus ostreatus* during growth in PAH-contaminated soils. *Letters in Applied Microbiology*, 28,250-254.
- Bollag, J.-M. (1992). Decontaminating Soil with Enzymes. *Environmental Science and Technology*, 26,1876-1881.
- Bollag, J.-M., Mertz, T., and Otjen, L., 1994, Role of Microorganisms in soil bioremediation, *Bioremediation Through Rhizosphere Technology*, American Chemical Society, p. 2-10.
- Bollag, J.-M., Minard, R.D., and Liu, S.-Y. (1983). Cross-linkage between anilines and phenolic humus constituents. *Environ. Sci. Technol.*, 17,72-80.
- Bollag, J.-M., and Myers, C. (1992). Detoxification of aquatic and terrestrial sites through binding of pollutants to humic substances. *The Science of the Total Environment*, 117/118,357-366.
- Burgos, W.D., Novak, J.T., and Berry, D.F. (1996). Reversible sorption and irreversible binding of naphthalene and alpha-naphthol to soil: elucidation of processes. *Environ. Sci. Technol.*, 30,1205-1211.
- Calderbank, A. (1989). The occurrence and significance of bound pesticide residues in soil. *Reviews of environmental contamination and toxicology*, 108,71-103.
- Cheng, H.H., Haider, K., and Harper, S.S. (1983). Catechol and chlorocatechol in soil: degradation and extractability. *Soil Biol. Biochem.*, 15,311-317.
- Chung, N., and Alexander, M. (1998). Difference in sequestration and bioavailability of organic compounds aged in dissimilar soil. *Environ. Sci. and Technol.*, 32,855-860.
- Dec, J., and Bollag, J.-M. (1988). Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid. *Soil Sci. Soc. Am. J.*, 52,1366-1371.
- Dec, J., and Bollag, J.-M. (1990). Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions. *Arch. Environ. Contam. Toxicol.*, 19,543-550.
- Dec, J., and Bollag, J.-M. (1994). Dehalogenation of chlorinated phenols during oxidative coupling. *Environ. Sci. Technol.*, 28,484-490.
- Dec, J., and Bollag, J.-M. (1995). Effect of various factors on dehalogenation of chlorinated phenols and anilines during oxidative coupling. *Environ. Sci. Technol.*, 29,657-663.

- Drzyzga, O., Bruns-Nagel, D., Gorontzy, T., Blotevogel, K.-H., Gemsa, D., and Von Low, E. (1998). Incorporation of ¹⁴C-labeled 2,4,6-trinitrotoluene metabolites into different soil fractions after aerobic and anaerobic-aerobic treatment of soil/molasses mixtures. *Environ. Sci. Technol.*, 32,3529-3535.
- Eschenbach, A., Kastner, M., Bierl, R., Schaefer, G., and Mahro, B. (1994). Evaluation of a new, effective method to extract polycyclic aromatic hydrocarbons from soil samples. *Chemosphere*, 28,683-692.
- Eschenbach, A., Weinberg, R., and Mahro, B. (1998). Fate and stability of nonextractable residues of [¹⁴C] PAH in contaminated soils under environmental stress conditions. *Environ. Sci. Technol.*, 32,2585-2590.
- Freitag, D., Scheunert, I., Klein, W., and Korte, F. (1984). Long-term fate of 4-chloroaniline-¹⁴C in soil and plants under outdoor conditions. A contribution to terrestrial ecotoxicology of chemicals. *J. Agric. Food Chem.*, 32,203-207.
- Fuhr, F., and Mittelstaedt, W. (1980). Plant experiments on the bioavailability of unextracted [carbonyl-¹⁴C]methabenzthiazuron residues from soil. *J. Agric. Food Chem.*, 28,122-125.
- Fuhreman, T.W., and Lichtenstein, E.P. (1978). Release of soil-bound methyl [¹⁴C]parathion residue and their uptake by earthworms and oat plants. *J. Agric. Food Chem.*, 26,605-610.
- Guthrie, E.A., Bortiatynski, J.M., Van Heemst, J.D.H., Richman, J.E., Hardy, K.S., Kovach, E.M., and Hatcher, P.G. (1999). Determination of [¹³C]pyrene sequestration in sediment microcosms using flash pyrolysis-GC-MS and ¹³C NMR. *Environ. Sci. Technol.*, 33,119-125.
- Guthrie, E.A., and Pfaender, F.K. (1998). Reduced pyrene bioavailability in microbially active soils. *Environ. Sci. Technol.*, 32,501-508.
- Hatcher, P.G., Bortiatynski, J.M., Minard, R.D., and Bollag, J.-M. (1993). Use of high-resolution ¹³C NMR to examine the enzymatic covalent binding of ¹³C-labeled 2,4-dichlorophenol to humic substances. *Environ. Sci. Technol.*, 27,2098-2103.
- Helling, C.S., and Krivonak, A.E. (1978). Biological characteristics of bound dinitroaniline herbicides in soils. *J. Agric. Food Chem.*, 26,1164-1172.
- Hill, I.R., 1976, Degredation of the insecticide pirimicarb in soil-characterization of 'bound' residues, in Kaufman, D.D., Still, G.G., Paulson, G.D., and Bandal, S.K., eds., Bound and Conjugated Pesticide Residues, Volume 29: ACS Symposium Series: Washington D.C., American Chemical Society, p. 358-361.
- Hsu, T.-S., and Bartha, R. (1974a). Biodegradation of chloroaniline-humus complexes in soil and in culture solution. *Soil Science*, 118,213-220.
- Hsu, T.-S., and Bartha, R. (1974b). Interaction of pesticide-derived chloroaniline residues with soil organic matter. *Soil Science*, 116,444-452.
- Karimi-Lotfabad, S., and Gray, M.R. (2000). Characterization of contaminated soils using confocal laser scanning microscopy and cryogenic-scanning electron microscopy. *Environ. Sci. Technol.*, 34,3408-3414.
- Karimi-Lotfabad, S., Pickard, M.A., and Gray, M.R. (1996). Reactions of Polynuclear Aromatic Hydrocarbons on Soil. *Environmental Science and Technology*, 30,1145-1151.
- Kastner, M., Streibich, M., Beyrer, M., Richnow, H.H., and Fritsche, W. (1999). Formation of bound residues during microbial degradation of [¹⁴C]anthracene. *Applied and Environmental Microbiology*, 65,1834-1842.

- Kaufman, D.D., Still, G.G., Paulson, G.D., and Bandal, S.K., 1976, Bound and Conjugated Pesticide Residues, in Gould, R.F., ed., ACS Symposium Series, Volume 29: ACS Symposium Series: Washington D.C., American Chemical Society, p. 1-10.
- Khan, S.U. (1973). Interaction of humic substances with bipyridylum herbicides. *Can. J. Soil Sci.*, 53,199-204.
- Khan, S.U. (1974). Adsorption of bipyridylum herbicides by humic acid. *J. Environ. Quality*, 3,202-206.
- Khan, S.U., 1978, The interaction of organic matter with pesticides, in Khan, M.S.a.S.U., ed., Soil Organic matter: new York, Elsevier Scientific pub. Co., p. 136-171.
- Khan, S.U. (1980). Plant uptake of unextracted (bound) residues from an organic soil treated with prometryn. *J. Agric. Food Chem.*, 28,1096-1098.
- Khan, S.U., and Ivarson, K.C. (1981). Microbiological release of unextracted (bound) residues from an organic soil treated with prometryn. *J. Agric. Food Chem.*, 29,1301-1303.
- Knicker, H., Bruns-nagel, D., Drzyzga, O., Von Low, E., and Steinbach, K. (1999). Characterization of 15N-TNT residues after an anaerobic/aerobic treatment of soil/molasses mixtures by solid-state 15N NMR spectroscopy. 1. Determination and optimization of relevant NMR spectroscopic parameters. *Environ. Sci. Technol.*, 33,343-349.
- Kohl, S.D., and Rice, J.A. (1998). The binding of contaminants to humin: a mass balance. *Chemosphere*, 36,251-261.
- Luthy, R.G., Aiken, G.R., Brusseau, M.L., D., C.S., Gschwend, P.M., Pignatello, J.J., Reinhard, M., Traina, S.J., Weber, W.J.J., and Westall, J.C. (1997). Sequestration of hydrophobic organic contaminants by geosorbents. *Environ. Sci. Technol.*, 31,3341-3347.
- Mac Rae, I.C. (1986). Formation and degradation of soil-bound [14C]fenitrothion residues in two agricultural soils. *Soil Biol. Biochem.*, 18,221-225.
- Martin, J.P., and Haider, K. (1980). A comparison of the use of phenolase and peroxidase for the synthesis of model humic acid-type polymers. *Soil Sci. Soc. Am. J.*, 44,983-988.
- Martin, J.P., Haider, K., and Linhares, L.F. (1979). Decomposition and stabilization of ring-14C-labeled catechol in soil. *Soil Sci. Soc. Am. J.*, 43,100-104.
- McBride, M.B., Sikora, F.J., and Wesselink, L.G. (1988). Complexation and catalyzed oxidative polymerization of catechol by aluminum in acidic solution. *Soil Sci. Soc. Am. J.*, 52,985-993.
- McCarthy, J.F., and Jimenez, B.D. (1985). Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: binding and dissociation. *Environ. Sci. Technol.*, 19,1072-1076.
- Meikle, R.W., Regoli, A.J., Kurihara, N.H., and Laskowski, D.A., 1976, Classification of bound residues soil organic matter: polymeric nature of residues in humic substances, in Kaufman, D.D., Still, G.G., Paulson, G.D., and Bandal, S.K., eds., Bound and Conjugated Pesticide Residues, Volume 29: ACS Symposium Series: Washington D.C., American Chemical Society, p. 272-284.
- Nieman, J.K.C., Sims, R.C., Sims, J.L., Seorensen, D.L., Mclean, J.E., and Rice, J.E. (1999). [14C]Pyrene bound residue evaluation using MIBK fractionation method for creosote-contaminated soil. *Environ. Sci. Technol.*, 33,776-781.

- Park, J.-P., Dec, J., Kim, J.-E., and Bollag, J.-M. (2000). Transformation of chlorinated phenols and anilines in the presence of humic acid. *J. Environ. Qual.*, 29,214-220.
- Park, J.-W., Dec, J., Kim, J.-E., and Bollag, J.-M. (1999). Effect of humic constituents ofn the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes or birnessite. *Environ. Sci. Technol.*, 33,2028-2034.
- Parris, G.E. (1980). Covalent binding of aromatic amines to humates. 1. Reactions with carbonyls and quinones. *Environ. Sci. Technol.*, 14,1099-1106.
- Qui, X., and McFarland, M.J. (1991). Bound residue formation in PAH contaminated soil composting using Phanerochaete Chrysosporium. *Hazardous Waste and Hazardous Materials*, 8,115-126.
- Ressler, B.P., Kneifel, H., and Winter, J. (1999). Bioavailability of polycyclic aromatic hydrocarbons and formation of humic acid-like residues during bacterial PAH degradation. *Appl. Microbiol. Biotechnol.*, 53,85-91.
- Richnow, H.H., Eschenbach, A., Mahro, B., Kastner, M., Annweiler, E., Seifert, R., and Michaelis, W. (1999). The formation of nonextractable soil residues: a stable isotope approach. *Environ. Sci. Technol*, 33, 3761-3767.
- Richnow, H.H., Eschenbach, A., Mahro, B., Seifert, R., Wehrung, P., Albrecht, P., and Michaelis, W. (1998). The use of ¹³C-labelled polycyclic aromatic hydrocarbons for the analysis of their transformation in soil. *Chemosphere*, 36,2211-2224.
- Richnow, H.H., Seifert, R., Hefter, J., Kastner, M., Mahro, B., and Michaelis, W. (1993). Metabolites of xenobiotica and mineral oil constituents linked to maccromolecular organic matter in polluted environments. *Advances in Organic Geochemistry*, 22,671-681.
- Riley, D., Wilkinson, W., and Tucker, B.V., 1976, Biological unavailability of bound paraquat residues in soil, in Kaufman, D.D., Still, G.G., Paulson, G.D., and Bandal, S.K., eds., Bound and Conjugated Pesticide Residues, Volume 29: ACS Symposium Series: Washington, D.C., American Chemical Society, p. 307-353.
- Simmons, K.E., Minard, R.D., and Bollag, J.-M. (1989). Oxidative co-oligomerization of guaiacol and 4-chloroaniline. *Environ. Sci. Technol.*, 23,115-121.
- Sims, R. C., and C. K. Abbott. (1993). Evaluation of Mechanisms of Alteration and Humification of PAHs for Water Quality Management. United States Geological Survey report G-1723, USGS, Reston, VA.
- Singh, D.K., and Agarwal, H.C. (1992). Chemical release and nature of soil-bound DDT residues. *J. Agric. Food Chem.*, 40,1713-1716.
- Stevenson, F.J. (1972). Organic matter reactions involving herbicides in soil. *J. Environ. Quality*, 1,333-342.
- U.S. EPA. (1996). Champion International Superfund Site, Libby, Montana; Bioremediation Field Performance Evaluation of the Prepared Bed Land Treatment System, Volumes I and II. EPA/600/R-95/156. U.S.EPA. Washington, DC, 1996.
- White, J.C., Quinones-Rivera, A., and Alexander, M. (1998). Effect of wetting and drying on the bioavailability of organic compounds sequestered in soil. *Environmental Toxicology and Chemistry*, 17,2378-2382.

Wunderwald, U., Kreisel, G., Braun, M., Schulz, M., Jäger, C., and Hofrichter, M. (2000). Formation and degradation of a synthetic humic acid derived from 3-fluorocatechol. *Appl. Microbiol. Biotechnol.*, 53,441-446.

Xie, H., Guetzloff, T.F., and Rice, J.A. (1997). Fractionation of Pesticide Residues Bound to Humin. *Soil Science*, 162,421-429.