APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1986, p. 552–558
0099-2240/86/030552-07$02.00/0

Vol. 51, No. 3

Biotransformations of Chloroguaiacols, Chlorocatechols, and Chloroveratroles in Sediments

MIKAEL REMBERGER, ANN-SOFIE ALLARD, AND ALASDAIR H. NEILSON*
Swedish Environmental Research Institute, S-100 31 Stockholm, Sweden

Received 19 July 1985/Accepted 26 November 1985

The occurrence of trichloro- and tetrachloroguaiacols, -catechols, and -veratroles and their transformation was studied in freshwater and brackish water sediments putatively exposed to bleachery discharge. The samples contained both chloroguaiacols and chlorocatechols, of which >90% could not be removed by simple extraction. The bound concentrations varied and ranged from 550 μg kg of organic C^{-1} for 3,4,5-trichloroguaiacol to 8,250 μg kg of organic C^{-1} for tetrachlorocatechol. Chlorinated substrates added to the aqueous phase were rapidly bound to the sediment with K_p values between 1.3 and 2.8 ml kg of organic C^{-1} for the chloroguaiacols and chloroveratroles and 22 to 36 ml kg of organic C^{-1} for the chlorocatechols. Sediment samples incubated aerobically brought about O-methylation of 4,5,6-trichloroguaiacol to 3,4,5-trichloroveratrole in a yield of ca. 25%. Under anaerobic conditions, however, de-O-methylation of both the chloroguaiacols and chloroveratroles took place with synthesis of the corresponding chlorocatechols. In separate experiments, the chlorocatechols were not completely stable under anaerobic conditions, but their ultimate fate has not yet been resolved. Sediment which had been autoclaved twice at 121°C for 20 min was unable to bring about any of these transformations; we therefore conclude that they were mediated by biological processes. These results emphasize that, in determining the fate of chloroguaiacols and related compounds discharged into the aquatic environment, the cardinal roles of sorption to the sediment phase and of the oxygen tension must be taken into account. We propose a hypothetical guaiacol cycle to accommodate our observations.

A range of polychlorinated guaiacols and catechols is produced during the production of fully bleached chemical pulp (13). Even after biological treatment of the effluents, some of these chlorinated compounds may be discharged into the aquatic environment (12, 15) and have been found in samples of water and sediment collected from areas subjected to such discharge in the Gulf of Bothnia (T.-M. Xie, K. Abrahamsson, E. Fogelqvist, and B. Josefsson, Environ. Sci. Technol., in press) and in water samples from Finnish lakes (22).

We have previously shown in laboratory experiments with pure cultures that a number of taxonomically different bacteria were able to bring about the O-methylation of chlorinated guaiacols and phenols with the production of neutral veratroles and anisoles (17). In an attempt to obtain an environmental perspective on the possible significance of these observations, we examined the toxicity of these compounds and their metabolites to fish (18) and evaluated a number of factors which might influence O-methylation under natural conditions—the effect of substrate concentrations, growth conditions, and cell density (1). A realistic assessment of the environmental significance of these observations requires, however, an analysis of the extent to which they occur in natural systems.

Since a number of chlorinated guaiacols and catechols have been identified in sediment samples from the Gulf of Bothnia, it is clearly important to take into account transformations which take place in the sediment phase. We felt that the inherent difficulties of in situ experiments made the use of these unattractive for the kind of studies we envisaged. We decided therefore to conduct laboratory studies using natural sediment samples incubated under various conditions relevant to environmental situations. Sediment samples were spiked with chloroguaiacols and chloroveratroles and incubated under both aerobic and anaerobic conditions. Analyses were carried out on the aqueous and sediment phases to determine the rate of transformation of the substrates and their ultimate fate. All of the compounds studied were rapidly sorbed to the sediments; under aerobic conditions, O-methylation of chloroguaiacols occurred at rates compatible with those found in laboratory studies with pure cultures. Under anaerobic conditions, de-O-methylation took place, and significant differences were observed in the persistence of the chlorinated catechols. An overview of the results is presented in the form of a hypothetical guaiacol cycle (see Fig. 5).

MATERIALS AND METHODS

Sediment samples. Sediment samples were collected from a freshwater lake (sediment A) and from localities in the Baltic Sea (sediment B) and the Gulf of Bothnia (sediment C). All of these localities were in the neighborhood of factories producing fully bleached chemical pulp. The Baltic Sea sample was a fine sand with a low content of organic material. The other two samples consisted of fine clay particles and had a high content of organic carbon; the sample from the Gulf of Bothnia had a high content of cellulose fibers. The dry weight was determined from the loss during heating at 150°C for 3 days to constant weight, and the amount of organic carbon was pragmatically defined as the ignition loss during 5 h at 550°C. These values have been used to express all substrate concentrations in the sediment phase.

Analytical and identification procedures. Although analysis of substrates in the aqueous phase presented no problems and was carried out by methods used previously (1), it became clear early in this study that it is not possible to extract chloroguaiacols, chloroveratroles, or chlorocate-

* Corresponding author.

chols quantitatively from sediment samples by simple extractive procedures. The following precedures were designed to provide satisfactory extraction without destruction of the sensitive chlorocatechols and to remove organic sulfur compounds which seriously interfere with the gas chromatographic (GC) analysis.

Meticulous attention was paid to the purity of all of the solvents used, and the following were used throughout: hexane and acetonitrile, pesticide analysis quality (J. T. Baker Chemical Co.), and *t*-butyl methyl ether distilled in glass (Burdick and Jackson Laboratories Inc.). GC and GC-mass spectroscopic analyses were carried out as described previously (1); the synthesis of the reference compounds has already been described (17), and all of them had a purity of >99% by GC analysis.

Extraction, analysis, and identification of chlorinated compounds in sediment samples. We have arbitrarily defined as free the fraction of the compounds which could be removed by direct extraction with organic solvents. The fraction which could be released only after the more exhaustive extraction procedure we have designated as bound. Since the procedures we have used are pragmatic, it is not possible to establish absolutely the total amounts of the substrates bound to the sediment.

It was important to establish that, in step (ii) below, significant loss of the sensitive catechols did not occur. We accomplished this by showing that the total amounts of free and bound catechols using the combined steps (i) and (ii) differed by less than ±15% from those obtained by direct extraction with acetonitrile in the absence of alkali.

(i) Extraction of free phenolic compounds. Sediment (10 g, wet weight) was mixed with deionized water (5 ml), the pH was lowered to ca. 1, and the slurry was extracted for 10 min with 3 ml of hexane–*t*-butyl methyl ether (2:1) containing 0.15 μg each of 2,3,4,5-tetrachlorophenol and pentachlorobenzene per ml as internal standards and further extracted for 10 min with 3 ml of the solvent mixture lacking internal standards. The total extracts were dried (Na_2SO_4), a trace of solid ascorbic acid was added, and portions were acetylated by the standard procedure (1).

(ii) Extraction of bound phenolic compounds. Sediment (10 g, wet weight) was mixed with methanol (5 ml), 10 M KOH (3 ml), and ascorbic acid (1 ml, 1.14 M). The slurry was stirred at room temperature for 45 min and then centrifuged (750 × *g*, 15 min, 15°C). The methanol-water phase was removed and saved, and the sediment was extracted twice further with 0.2 M KOH (5 ml and 3 ml) for 45 and 30 min, respectively. All three alkaline extracts were combined. Water (5 ml) and a solution of $CuSO_4$ (0.2 ml, 1 M) were added, and the mixture was extracted twice with hexane–*t*-butyl methyl ether as described in (i) above. The organic extracts contained the neutral (nonphenolic) compounds. The aqueous phase was acidified in an ice bath with 1 ml of H_2SO_4 (98%) and extracted twice with hexane–*t*-butyl methyl ether lacking internal standards. Phenolic compounds in the organic phase were analyzed as described above for the free fraction.

Only limited quantities of the chloroguaiacols and chlorocatechols were available for identification. We therefore relied upon a GC comparison of two independently prepared derivatives—the *O*-acetates and the *O*-trimethylsilyl ethers. For the chloroguaiacols, identification was additionally based on a comparison of the mass spectra of the *O*-trimethylsilyl ethers with those of reference compounds. Previous studies (1, 17) had shown the adequacy of identification based on these criteria.

Analysis of sediment samples from the transformation and binding experiments. In view of the large number of samples which had to be analyzed, a simplified version of the above procedure was used. An estimate of the efficiency of the extraction procedure was desirable and was carried out as follows. A compensation standard (2,4,6-tribromophenol; 100 μl of a 2-μg ml^{-1} solution in methanol) was added immediately before extraction and analysis. By using a sample of the pure 2,4,6-tribromophenyl acetate, we could show that the acetylation procedure was quantitative so that we could make an estimate of the effectiveness of the extraction procedure. This was 85 to 95% for the 10-g samples and >95% for the 3-g samples. No serious error due to irreversible binding of the substrates to the sediments was therefore introduced in these relatively short-term experiments.

Sediment samples (10 g, wet weight) were mixed with acetonitrile (4 ml), and a solution of ascorbic acid was added (1 ml, 1.14 M), followed by the compensation standard (2,4,6-tribromophenol; 100 μl, 2 μg ml^{-1}). The mixture was set aside overnight at 4°C, H_2SO_4 (0.25 ml, 98%) was added, and the whole was shaken for 1.5 h. Water (10 ml) was added, and the mixture was extracted first for 15 min with 3 ml of hexane–*t*-butyl methyl ether (2:1) containing 2,3,4,5-tetrachlorophenol and pentachlorobenzene as internal standards and then further for 10 min with the hexane–*t*-butyl methyl ether mixture lacking internal standards. The organic extracts were combined, a trace of solid ascorbic acid was added, and the sample was rapidly dried (Na_2SO_4) and immediately acetylated as described previously (1).

Binding of chlorinated substrates to sediments. Experiments were carried out in screw-cap tubes (20 by 125 mm) with Teflon-lined caps. Sediment (3 g, wet weight) was introduced into a set of tubes, followed by 12 ml of sterile unsupplemented VV 2 medium (containing low concentrations of N and P, but lacking a source of organic carbon) (17). This medium has been successfully used for isolation and growth of bacterial strains from brackish water localities in the Baltic Sea and Gulf of Bothnia (1). The experiment was started by introducing the substrates (prepared as sterile concentrated solutions in VV 2) so that the initial concentrations in the aqueous phase were 100 μg $liter^{-1}$. The contents of the tubes were mixed, and the tubes were incubated at 22°C on a horizontal shaker (100 strokes per min). After various times, tubes were removed, and the phases were separated by centrifugation (1,500 × *g*, 15 min, 15°C) and analyzed. Control tubes without added substrate were incubated simultaneously. Concentrations of the substrates in the aqueous phase were calculated as micrograms per liter, and those in the sediment phase were calculated as micrograms per kilogram of organic C; our K_p values (which are the quotient of these concentrations) are not therefore dimensionless, but have the dimension of $(density)^{-1}$, i.e., liters per kilogram of organic C.

Demonstrations of the biological nature of transformation of chloroguaiacols and chloroveratroles. Experiments were carried out as described above in 25-ml screw-cap tubes. After introduction of the sediment and unsupplemented VV 2 medium, half of the tubes were autoclaved on 2 successive days (121°C, 20 min). Substrate was then added to all of the tubes (100 μg $liter^{-1}$ in the aqueous phase), the contents were mixed, and the tubes were incubated without agitation at 22°C for 14 days. After incubation, the contents of the tubes were separated by centrifugation as above, and the phases were analyzed. Control tubes without added substrate were incubated simultaneously. Since the substrates

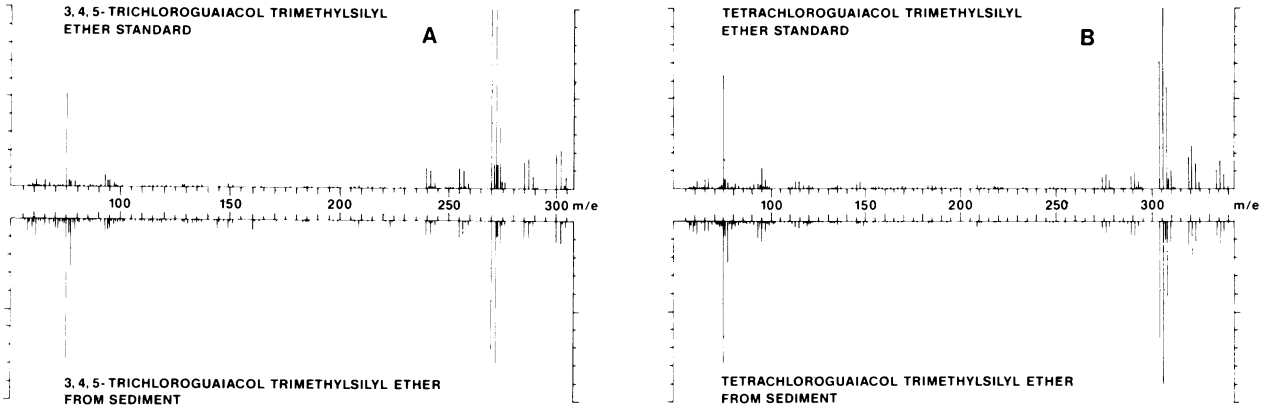

FIG. 1. Comparison of the mass spectra of the *O*-trimethylsilyl ethers of chloroguaiacols isolated from sediment samples with those of authentic compounds.

were added after autoclaving the sediment, the possibility that the substrates were chemically altered during the heat treatment, e.g., by polymerization, can be excluded.

Transformation of chlorinated substrates by sediment samples. Experiments were carried out at 22°C with 10-g samples of sediment and 40 ml of unsupplemented VV 2 medium. The substrates were added to provide initial concentrations in the aqueous phase of 100 μg liter^{-1}. Experiments under aerobic conditions were carried out in conical flasks which were shaken on an orbital shaker (ca. 100 rpm); for anaerobic experiments, 100-ml screw-cap bottles were used. These were flushed with N_2 after the addition of the substrates and capped with metal screw caps fitted with Teflon-coated rubber liners. We assumed that anaerobic conditions occurred for a number of reasons: (i) the geometry of the contents of the bottles (42 by 47 mm), (ii) the density of organisms in the sediments, (iii) the fact that the known products of aerobic metabolism were produced, if at all, in negligible amounts, and (iv) after 2 days, measurement of the potential between a platinum electrode and a standard saturated calomel electrode varied for different samples from −100 to −300 mV at 22°C. Analysis for concentrations of the substrates and metabolites was carried out after separation of the phases by centrifugation as described above. The partition of the substrates and their metabolites between the sediment and aqueous phases was such that only low concentrations could be detected in the aqueous phase. The data have therefore been related to the amount of organic carbon in the sediment phase.

RESULTS

Identification of endogenous substrates in sediment samples. 3,4,5-Trichloro- and tetrachloroguaiacols and -catechols were identified in all of the sediment samples: their structures were confirmed from the identity of the relative GC retention times of their *O*-acetates and *O*-trimethylsilyl ethers with those of authentic compounds (1) and for the chloroguaiacols additionally from the identity of the mass spectra of their *O*-trimethylsilyl ethers with those of reference compounds (Fig. 1). For the trichloroguaiacol derivative, the parent ion peaks occurred at *m/e* values of 297.9, 299.9, and 301.9 as required for $C_{10}H_{13}Cl_3O_2Si$, and for the tetrachloroguaiacol derivative at *m/e* values of 331.8, 333.8, 335.8, and 337.8 as required for $C_{10}H_{12}Cl_4O_2Si$. The concentrations of free and bound compounds found in a freshwater sediment sample (A) and a brackish water sample (B) are given in Table 1. During prolonged storage of sample C under anaerobic conditions at 17°C, the concentrations of the total 3,4,5-trichloro- and tetrachloroguaiacol fell from 3.2 and 1.8 to 1.3 and 0.8 mg kg of organic C^{-1} after 120 days and thereafter remained constant during a further 100 days. Neither 4,5,6-trichloroguaiacol nor 3,4,5-trichloro- or tetrachloroveratrole was found in any of the sediment samples.

Binding of chloroguaiacols, chlorocatechols, and chloroveratroles to sediment. The experiments were carried out with sediment B. All of the compounds were rapidly sorbed from the aqueous phase at an initial concentration of 100 μg liter^{-1}. Sorption of the chlorocatechols was extremely rapid and was essentially complete within 15 min; values for the partition coefficient between the sediment and aqueous phases were therefore calculated from concentrations measured after 1 h. The chloroguaiacols and chloroveratroles were bound less rapidly, so that K_p values were calculated from measurements 4 h after additions of the sorbate were made (Table 2). In all cases, it can be assumed that a pseudoequilibrium had been reached and that, in view of the short duration of the experiments, interference from biological activity was of negligible significance. At the times used for calculating the K_p values, the sum of the concentrations in the aqueous and sediment phases of the chlorocatechols, chloroguaiacols, and chloroveratroles exceeded 90% of the concentrations initially added, whereas the concentrations of the endogenous chlorocatechols and chloroguaiacols extracted from control sediment samples under the conditions employed were <5% of those used in the binding experiments.

Demonstration of biological activity in transformation of

TABLE 1. Concentrations of free and bound chloroguaiacols and chlorocatechols from (A) a freshwater and (B) a brackish water locality

Compound	Concn (μg kg of organic C^{-1})			
	Locality A		Locality B	
	Free	Bound	Free	Bound
3,4,5-Trichloroguaiacol	210	2,400	<10	1,870
Tetrachloroguaiacol	180	1,590	<10	930
3,4,5-Trichlorocatechol	270	3,700	<10	1,390
Tetrachlorocatechol	<10	8,250	<10	2,430

TABLE 2. Partition coefficients between the aqueous and sediment phases (sample B) for chlorocatechols, chloroguaiacols, and chloroveratroles

Compound	K_p (ml kg of organic C $^{-1}$)
3,4,5-Trichlorocatechol	22.2
Tetrachlorocatechol	36.1
4,5,6-Trichloroguaiacol	1.3
Tetrachloroguaiacol	1.4
3,4,5-Trichloroveratrole	1.6
Tetrachloroveratrole	2.8

chloroguaiacols and chloroveratroles. Experiments were carried out with sediment B. With twice-autoclaved sediment samples spiked with 4,5,6-trichlorguaiacol and 3,4,5-trichloroveratrole, the concentrations of the substrates were essentially constant (ca. ± 8%) during 14 days of incubation. In both experiments, the concentration of the endogenous 3,4,5-trichlorocatechol fell slightly. In the corresponding experiments with unautoclaved sediment, both substrates were degraded to 3,4,5-trichlorocatechol; these results were confirmed and extended in further experiments whose details are given below. It was therefore concluded that these transformations are mediated by biological—and probably microbiological—processes.

Transformation of exogenous chlorinated substrates by sediment samples under various conditions. Two different sediment samples were used; one (A) was collected from a freshwater lake and contained ca. 20% organic matter (dry weight) and high concentrations of chloroguaiacols and chlorocatechols, and the other (B) came from the Baltic Sea and had only 0.9% organic matter (dry weight) and much lower concentrations of the chloroguaiacols and chlorocatechols. Essentially identical results were obtained from experiments with both samples, but for ease of illustration we have chosen those experiments with the low-carbon sediment B. The results of an experiment in which the sediment samples were spiked with 4,5,6-trichloroguaiacol (100 μg liter^{-1} in the aqueous phase) and incubated aerobically are given in Fig. 2. The results of a series of experiments carried out with 4,5,6-trichloro- and tetrachloroguaiacol and 3,4,5-trichloro- and tetrachloroveratrole incubated anaerobically are given in Fig. 3, and results from similar experiments with 3,4,5-trichloro- and tetrachlorocatechol are given in Fig. 4. In all cases, the initial substrate concentrations in the aqueous phases were 100 μg liter^{-1}.

DISCUSSION

The data presented here show that substantial concentrations of chloroguaiacols and chlorocatechols, putatively originating from bleachery effluents, may be found in sediment samples collected from areas in the neighborhood of the discharge. 4, 5, 6-Trichloroguaiacol, which is a relatively minor component of bleachery effluents, was not found in any of the sediment samples. On the other hand, a hitherto unidentified compound was widely distributed in samples from brackish water localities; the *O*-acetate of this had a GC retention time very close to that of 4,5,6-trichloroguaiacol, although it could clearly be distinguished from this by decreasing the temperature of the column. These results encompassing samples from a freshwater lake, the Gulf of Bothnia, and the Baltic Sea confirm and extend the observations of Xie et al. (in press).

It is important to emphasize that the greater part (>90%) of the chloroguaiacols could not be removed from sediment samples by simple solvent extraction (6); for the chlorocatechols, however, this fraction was much more variable and was sometimes much lower. The total concentrations of chloroguaiacols and chlorocatechols in the sediment samples cannot of course be determined absolutely. The magnitude of the bound fractions may, however, plausibly be correlated with the demonstrations that all of the substrates were rapidly and tightly bound to the sediments (Table 2). These observations are consistent with extensive data on the role of sorption in determining the extractability of pesticides from soil (33) and recent results on the sorption of phenolic (9, 23, 32, 34) and neutral (10) compounds to sediments. The cardinal role of organic carbon in the sediment phase (9, 23) and of humic acids in the aqueous phase (14) supports the use of organic carbon in studies involving binding to and transformation in the sediment phase.

We should point out that, whereas our K_p values provide a pragmatic measure of the sorption of the compounds, they cannot be directly compared with octanol-water partition coefficients for several reasons: (i) the multiphase system is not in true thermodynamic equilibrium, (ii) transport into the sediment is governed not only by physicochemical adsorption to inorganic material but also by active uptake into the biota, and (iii) sediments vary widely in structural composition and the amount of organic carbon present (our use of total organic carbon to normalize the results is purely empirical). We feel that some published studies have not sufficiently taken into account the significance of these factors. Although the importance of ionic strength has been examined in other studies (23, 32), we feel that our data from experiments with a mineral base medium of low ionic strength are valid for situations prevailing in freshwater lakes and the brackish water of the Baltic Sea and the Gulf of Bothnia.

At an early stage of this investigation, we were confronted with two apparently conflicting observations. (i) We had shown the presence of tri- and tetrachloroveratrole in the liver fat of wild fish captured from areas subjected to the discharge of chloroguaiacols and chlorocatechols (18). (ii) On the other hand, examination of sediment samples did not reveal the presence of chloroveratroles, and such samples

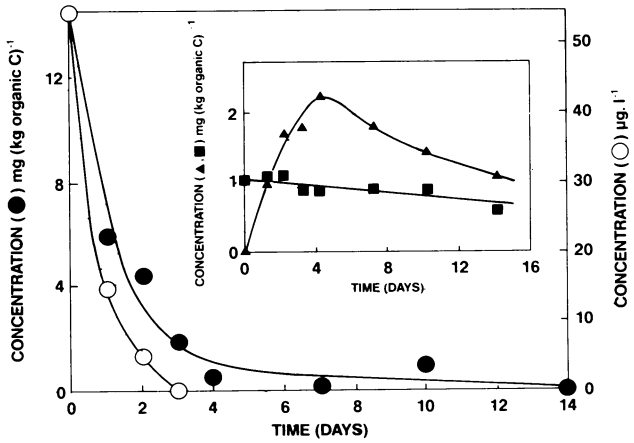

FIG. 2. Transformations in a sediment sample incubated aerobically with 4,5,6-trichloroguaiacol (100 μg liter^{-1} in the aqueous phase). Symbols: (●, ▲, ■) sediment phase; (○) aqueous phase; (●, ○) 4,5,6-trichloroguaiacol; (▲) 3,4,5-trichloroveratrole; (■) 3,4,5-trichlorocatechol.

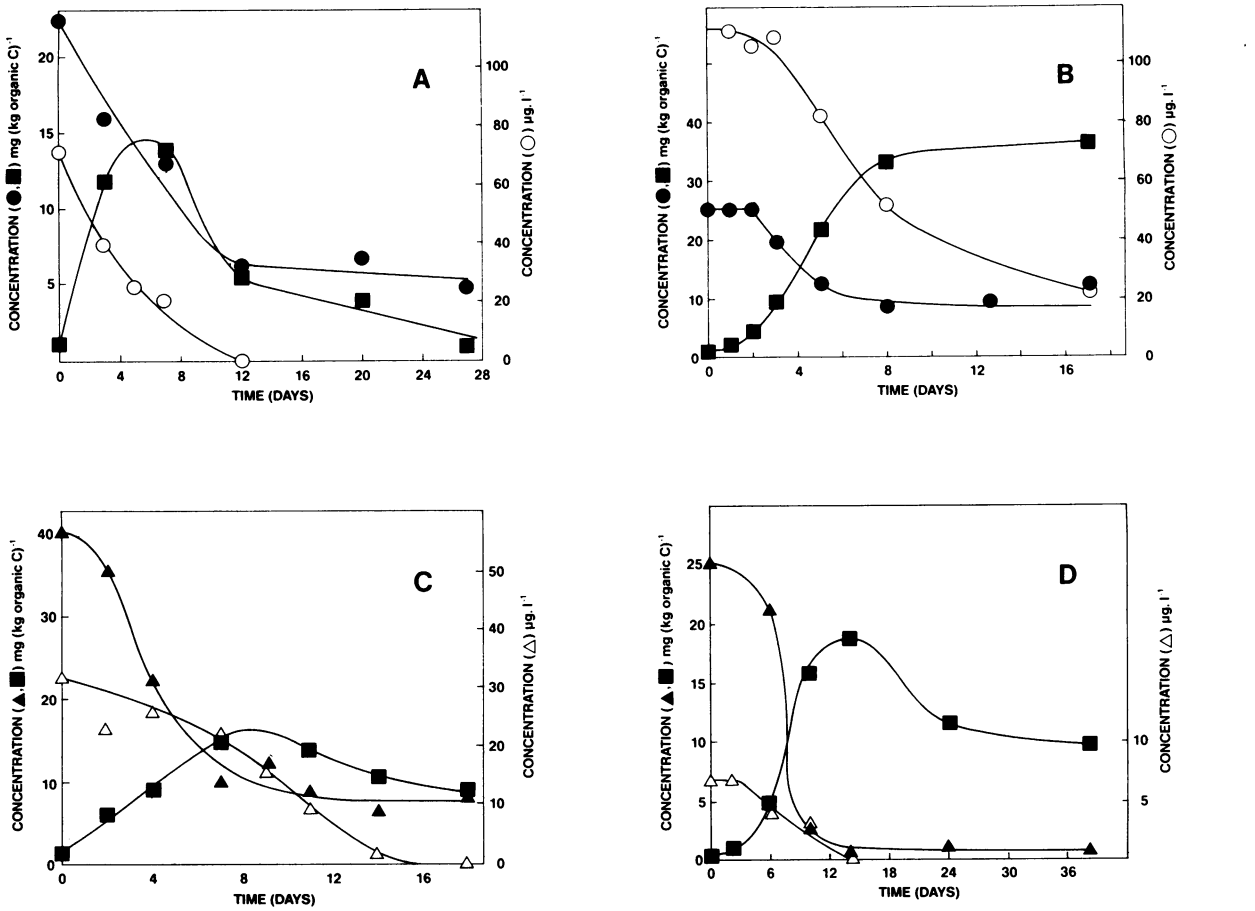

FIG. 3. Transformations in a sediment sample incubated anaerobically with various substrates (100 µg liter^{-1} in the aqueous phase). A, 4,5,6-Trichloroguaiacol; B, tetrachloroguaiacol; C, 3,4,5-trichloroveratrole; D, tetrachloroveratrole. Symbols: (●, ▲, ■) sediment phase; (○, △) aqueous phase; (●, ○) guaiacols; (▲, △) veratroles; (■) catechols.

were dominated by the occurrence of chloroguaiacols and chlorocatechols.

We feel that we can reconcile these apparently conflicting observations by taking into account two significant functions in the sediment phase: (i) the role of higher organisms in transport processes and (ii) anaerobic transformations by bacteria.

Under aerobic conditions, chloroguaiacols and chlorocatechols are transformed by bacterial activity into chloroveratroles (1, 17); we have now shown that these metabolites were sorbed onto the sediments. Mediated, for example, by oligochaetes and polychaetes, these lipophilic metabolites may then be transported up to the sediment-water interface, where they may be accumulated in bottom-feeding fish. Indeed, the importance of oligochaetes in the transport and concentration of chlorobenzenes and chlorobiphenyls within the sediment phase has been demonstrated (11, 21), and organisms such as *Limnodrilus hoffmaesteri*, and in freshwater *Tubifex tubifex* and *Potamothrix hammoniensis*, are widely distributed in our study areas (5).

The fate of bound chloroguaiacols and chloroveratroles in the sediment phase is a complex and dynamic process which is influenced by, among other factors, the oxygen tension. Anaerobic sediments occur in large areas of the Baltic Sea, the Gulf of Bothnia, and polluted areas in freshwater lakes and certainly prevail in our experiments. We therefore postulate that, under anaerobic conditions, de-O-methylation of both chloroguaiacols and chloroveratroles took place with the concomitant synthesis of chlorocatechols. Although in separate experiments with chlorocatechols con-

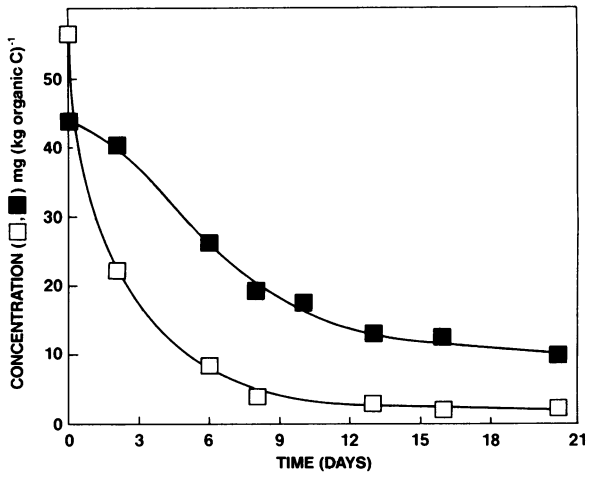

FIG. 4. Transformation of 3,4,5-trichlorocatechol (□) and tetrachlorocatechol (■) in a sediment sample incubated anaerobically. Substrate concentrations in the aqueous phase were 100 µg liter^{-1}.

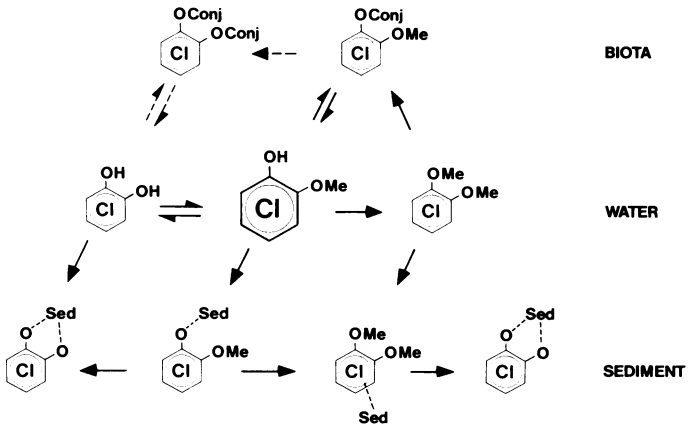

FIG. 5. Hypothetical guaiacol cycle. Abbreviations: OMe, methoxy; OConj, conjugated with glucuronate or sulfate; Sed, sediment.

siderable loss of the substrate occurred without the accumulation of detectable intermediates, variable—although significant—concentrations of both chloroguaiacols and chlorocatechols remained at the end of our experiments. All of these observations are consistent with the existence of these compounds in Baltic Sea, Gulf of Bothnia, and freshwater lake sediments and underline the delicate equilibrium between the chloroguaiacols which are discharged initially and their metabolic products, chloroveratroles and chlorocatechols. Our experiments are, however, unable to determine whether there is a threshold concentration below which biodegradation does not occur (3, 28) or whether during prolonged incubation with the sediment samples there is a major decrease in the bioavailability of the substrates due, for example, to irreversible sorption (6).

Two observations support the possibility that anaerobic bacteria are able to carry out the types of transformation which we have suggested: (i) the gram-positive *Acetobacterium woodii* (2) and a taxonomically distinct gram-negative organism (7) are able to de-O-methylate aromatic methoxy compounds with the formation of catechols and acetate; and (ii) *Pelobacter acidigallici* (24) is able to ferment a range of trihydroxybenzenes, and a consortium of anaerobic bacteria (31) use catechol and 1,4-dihydroxybenzene as growth substrates. A plausible basis for the proposed transformations therefore exists. Attention should, however, also be drawn to the possibility that anaerobic dechlorination comparable to that which has been demonstrated in a number of chlorobenzoates (25, 30) might occur.

It is important to evaluate the relative significance of biotic and abiotic processes in bringing about the transformations observed. We suggest that biotic reactions were of primary importance for the following reasons: (i) for aerobic transformations, the appropriate bacteria capable of carrying out O-methylation of chloroguaiacols had been isolated in pure culture from the sediments examined (1); (ii) under anaerobic conditions all of the substrates were unchanged during incubation with autoclaved sediments for lengths of time extending over the period during which the critical transformations were observed in untreated sediment. We also suggest that our data are better interpreted on the basis of biotic transformations than on a decrease of their chemical accessibility during the incubations. Two principal arguments are (i) that sorption of all of the substrates to the sediments was an extremely rapid reaction which was essentially complete in a few hours in contrast to the slower rates of transformation of the substrates and (ii) that during incubation of the chloroguaiacols and chloroveratroles there was a concomitant accumulation of the corresponding chlorocatechols.

Our data show that even substrates which are tightly bound to sediments are nonetheless accessible to biological transformation. This view apparently conflicts with studies in both the terrestrial (20) and aquatic (8) systems, which showed substantial protection of bound substrates to microbial attack. We do not feel, however, that our data provide unequivocal evidence on the relative role of degradation in the sediment and aqueous phases; an adequate evaluation requires a detailed study of the transport processes between the phases and must also take into account higher organisms.

It should be emphasized that all of the transformations discussed here were carried out by organisms existing naturally in the sediment samples and at the expense of endogenous sources of carbon; the only supplements were provided by the mineral-base medium—principally nitrogen and phosphorus—which may be limiting under certain natural conditions.

We therefore conclude that a realistic evaluation of the fate of chlorinated guaiacols and chlorocatechols discharged into the aquatic environment, and of the chloroveratroles produced by bacterial O-methylation, must take into account the substantial fraction of all of these compounds which may be bound to and undergo transformation in the sediment phase.

Recent studies have demonstrated the significant role of sorption in removing xenobiotics applied to the aqueous phase, both from the sediment phase (8, 27, 29) and from humic acids in the aqueous phase (26). Similar conclusions have been drawn for abiotic processes (16). The significance of comparable processes in terrestrial systems exposed, for example, to pesticides has long been appreciated (4, 6, 20, 34). Collectively, all of these results support the views expressed here.

On the basis of the experiments described here, previous results on the aerobic transformation of chloroguaiacols by bacteria (1, 17), results on the bioconcentration of chloroveratroles by fish (18), and unpublished data on the metabolism of chloroveratroles in fish, we provisionally propose a guaiacol cycle (Fig. 5). This attempts both to summarize current observations and to indicate areas which require further examination. Among these, the isolation of the appropriate anaerobic organisms and the study or their metabolic activities in pure culture will be given the highest priority.

Environmental hazard assessments, particularly of more recalcitrant compounds, should therefore take into account both the bioavailability of the compounds—including their sorption to sediments—and the cardinal role of oxygen tension (19).

ACKNOWLEDGMENTS

This work has received partial support from the Research Committee of the Swedish National Environment Protection Board and from the Swedish Paper and Pulp Association.

LITERATURE CITED

1. **Allard, A.-S., M. Remberger, and A. H. Neilson.** 1985. Bacterial O-methylation of chloroguaiacols: effect of substrate concentration, cell density, and growth conditions. Appl. Environ. Microbiol. **49**:279–288.
2. **Bache, R., and N. Pfennig.** 1981. Selective isolation of

Acetobacterium woodii on methoxylated aromatic acids and determination of growth yields. Arch. Microbiol. **130**:255–261.
3. **Boethling, R. S., and M. Alexander.** 1979. Effect of concentration of organic chemicals on their degradation by natural microbial communities. Appl. Environ. Microbiol. **37**:1211–1216.
4. **Bollag, J.-M., and M. J. Loll.** 1983. Incorporation of xenobiotics into soil humus. Experientia **39**:1221–1230.
5. **Brinkhurst, R. O., and B. G. M. Jamieson.** 1981. Aquatic oligochaeta of the world. University of Toronto Press, Toronto.
6. **Corwin, D. L., and W. J. Farmer.** 1984. Nonsingle-valued adsorption-desorption of bromacil and diquat by freshwater sediments. Environ. Sci. Technol. **18**:507–514.
7. **Frazer, A. C., and L. Y. Young.** 1985. A gram-negative anaerobic bacterium that utilizes *O*-methyl substitients of aromatic acids. Appl. Environ. Microbiol. **49**:1345–1347.
8. **Heitkamp, M. A., J. N. Huckins, J. D. Petty, and J. L. Johnson.** 1984. Fate and metabolism of isopropylphenyl diphenyl phosphate in freshwater sediments. Environ. Sci. Technol. **18**:434–439.
9. **Isaacson, P. J., and C. R. Frink.** 1984. Nonreversible sorption of phenolic compounds by sediment fractions: the role of sediment organic matter. Environ. Sci. Technol. **18**:43–48.
10. **Karickhoff, S. W., D. S. Brown, and T. A. Scott.** 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res. **13**:241–248.
11. **Karickhoff, S. W., and K. R. Morris.** 1985. Impact of tubificid oligochaetes on pollutant transport in bottom sediments. Environ. Sci. Technol. **19**:51–56.
12. **Kovacs, T. G., R. H. Voss, and A. Wong.** 1984. Chlorinated phenolics of bleached kraft mill origin. An olfactory evaluation. Water Res. **18**:911–916.
13. **Kringstad, K., and K. Lindström.** 1984. Spent liquors from pulp bleaching. Environ. Sci. Technol. **18**:236A–248A.
14. **Landrum, P., S. R. Nihart, B. J. Eadie, and W. S. Gardner.** 1984. Reverse-phase partition separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. Environ. Sci. Technol. **18**:187–192.
15. **Leuenberger, C., R. Coney, J. W. Gradyon, E. Molnar-Kubica, and W. Giger.** 1983. Schwer abbauare organische Stoffe in Abwässern der Zellstoffherstellung: Auftreten und Verhalten in einer biologischen Klärlage. Chimia **37**:345–353.
16. **Macalady, D. R., and N. L. Wolfe.** 1985. Effects of sediment sorption and abiotic hydrolysis. I. Organophosphorothioate esters. J. Agric. Food Chem. **33**:167–173.
17. **Neilson, A. H., A.-S. Allard, P.-Å. Hynning, M. Remberger, and L. Landner.** 1983. Bacterial methylation of chlorinated phenols and guaiacols: formation of veratroles from guaiacols and high-molecular-weight chlorinated lignin. Appl. Environ. Microbiol. **45**:774–783.
18. **Neilson, A. H., A.-S. Allard, S. Reiland, M. Remberger, A. Tärnholm, T. Viktor, and L. Landner.** 1984. Tri- and tetrachloroveratrole, metabolites produced by bacterial *O*-methylation of tri- and tetrachloroguaiacol: an assessment of their bioconcentration potential and their effects on fish reproduction. Can. J. Fish. Aquat. Sci. **41**:1502–1512.
19. **Neilson, A. H., A.-S. Allard, and M. Remberger.** 1985. Biodegradation and transformation of recalcitrant compounds, p. 29–86. *In* O. Hutzinger (ed.), The handbook of environmental chemistry, vol. 2, part C. Springer-Verlag, Berlin.
20. **Ogram, A. V., R. E. Jessup, L. T. Ou, and P. S. C. Rao.** 1985. Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. **49**:582–587.
21. **Oliver, B. G.** 1984. Uptake of chlorinated organics from anthropogenically contaminated sediments by oligochaete worms. Can. J. Fish. Aquat. Sci. **41**:878–883.
22. **Paasivirta, J., K. Heinola, T. Humppi, A. Karjalainen, J. Knuutinen, K. Mäntykoski, R. Paukku, T. Piilola, K. Surma-Aho, J. Tarhanen, L. Welling, H. Vihonen, and J. Särkka.** 1985. Polychlorinated phenols, guaiacols and catechols in environment. Chemosphere **14**:469–491.
23. **Schellenberg, K., C. Leuenberger, and R. P. Schwarzenbach.** 1984. Sorption of chlorinated phenols by natural sediments and aquifer materials. Environ. Sci. Technol. **18**:652–657.
24. **Schink, B., and N. Pfennig.** 1982. Fermentation of trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a new strictly anaerobic non-sporeforming bacterium. Arch. Microbiol. **133**:195–201.
25. **Shelton, D. R., and J. M. Tiedje.** 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. **48**:840–848.
26. **Shimp, R., and F. R. Pfaender.** 1985. Influence of naturally occurring humic acids on biodegradation of monosubstituted phenols by aquatic bacteria. Appl. Environ. Microbiol. **49**:402–407.
27. **Spain, J. C., P. H. Pritchard, and A. W. Bourquin.** 1979. Effects of adaptation on biodegradation rates in sediment/water cores from estuarine and freshwater environments. Appl. Environ. Microbiol. **40**:726–734.
28. **Spain, J. C., P. A. Van Veld, C. A. Monti, P. H. Pritchard, and C. R. Cripe.** 1984. Comparison of *p*-nitrophenol biodegradation in field and laboratory test systems. Appl. Environ. Microbiol. **48**:944–950.
29. **Subba-Rao, R. V., and M. Alexander.** 1982. Effects of sorption on mineralization of low concentrations of aromatic compounds in lake water samples. Appl. Environ. Microbiol. **44**:659–668.
30. **Suflita, J. M., J. Stout, and J. M. Tiedje.** 1984. Dechlorination of (2,4,5-trichlorophenoxy)acetic acid by anaerobic microorganisms. J. Agric. Food Chem. **32**:218–221.
31. **Szewzyk, U., R. Szewzyk, and B. Schink.** 1985. Methanogenic degradation of hydroquinone and catechol via reductive dehydroxylation to phenol. FEMS Microbiol. Ecol. **31**:79–87.
32. **Westall, J. C., C. Leuenberger, and R. P. Schwarzenbach.** 1985. Influence of pH and ionic strength on the aqueous-nonaqueous distribution of chlorinated phenols. Environ. Sci. Technol. **19**:193–198.
33. **Westcott, N. D., and B. L. Worobey.** 1985. Novel solvent extraction of lindane from soil. J. Agric. Food Chem. **33**:58–80.
34. **Yost, E. C., and M. A. Anderson.** 1984. Absence of phenol adsorption on goethite. Environ. Sci. Technol. **18**:101–106.