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Eliane Fustec, Eric Chauvet, Gilbert Gas. Lignin Degradation and Humus Formation in Alluvial Soils and Sediments. *Applied and Environmental Microbiology*, 1989, 55 (4), pp.922-926. hal-01264295

HAL Id: hal-01264295

<https://hal.science/hal-01264295>

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To cite this version : Fustec, Eliane and Chauvet, Eric and Gas, Gilbert
Lignin Degradation and Humus Formation in Alluvial Soils and Sediments. (1989) *Applied and Environmental Microbiology*, vol.55 (n°4). pp.922-926. ISSN 0099-2240

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Lignin Degradation and Humus Formation in Alluvial Soils and Sediments

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Received 22 July 1988/Accepted 6 December 1988

The contribution of lignin to the formation of humic compounds was examined in different environments of the terrestrial-aquatic interface in the Garonne River valley in southwestern France. Alluvial soils and submerged or nonsubmerged river and pond sediments containing alder, poplar, or willow [¹⁴C-lignin]lignocelluloses were incubated. After a 49-day incubation period, 10 to 15% of labeled lignins in alluvial soils was recovered as evolved ¹⁴CO₂. In nonsubmerged sediments, 10% of the applied activity was released as ¹⁴CO₂, and in submerged sediments, only 5% was released after 60 days of incubation. In the different alluvial soils and sediments, the bulk of residual activity (70 to 85%) remained in the two coarsest-grain fractions (2,000 to 100 and 100 to 50 μm). Only 2 to 6% of the residual activity of these two coarse fractions was recovered as humic and fulvic acids, except in the case of alder [¹⁴C-lignin]lignocellulose, which had decomposed in a soil collected beneath alders. In this one 55% of the residual activity was extracted as humic substances from the 2,000- to 100-μm fraction. Humic and fulvic acids represented from 6 to 50% of the residual activity in the finest-grain fractions (50 to 20 and 20 to 0 μm). The highest percentages were obtained in soil collected beneath alders and in submerged pond sediment. The contribution of different groups of microorganisms, as well as nutrients and clay content, may influence humic-substance formation in such environments. Physical stability also may be an important factor for complex microbial activity involved in this process.

Humic substances are the major constituent of organic matter in waters and sediments of rivers and lakes. They constitute from 50 to 80% of the dissolved organic carbon in stream and lake waters (25, 30) and from 10 to 60% of total organic carbon in lacustrine sediments (24). Advances in understanding the chemical structure of aquatic humic compounds and their role in relation to water quality and metal complexation were recently reviewed (10). However, the origins of humic compounds in the different aquatic ecosystems are not fully understood. Soil is one source of humic materials which reach the aquatic environments through erosion or underground water. Furthermore, in aquatic ecosystems, allochthonous plant materials, autochthonous organisms, and sewage may be involved in the production of humic substances. The importance of each source of organic matter and the complex reactions which induce the formation of humic substances are dependent on various environmental factors.

Humic-substance formation and its relation to lignin degradation has been studied primarily in soils because of the abundance of this phenolic polymer in terrestrial ecosystems (23, 26, 29). Principally through microbial activity, lignin molecules may be partially degraded, decomposed to smaller units, or degraded to simple phenolic or aliphatic compounds. Phenolic units may also originate through microbial synthesis from nonaromatic sources. Then humic-substance formation in soils occurs through complex mechanisms, including synthesis of reactive phenols, linkage to proteins or other substances, and enzymatic and autoxidative polymerization (20, 21, 26).

Lignin signature of dissolved humic substances in rivers and lakes has been reported, but it has not been possible to

distinguish humic compounds produced in soils from those formed in aquatic systems (16). Real advances in understanding lignin degradation occurred through experiments in which natural or synthetic ¹⁴C-labeled lignins were used in studies of streams and lakes (2, 14) and of freshwater or marine sediments (3, 19, 31). Most studies have focused on the physical and nutritional factors affecting the rate of aerobic lignin mineralization and the microorganisms involved in this process (1, 5, 6, 17). Previous studies on lignin degradation in anaerobic environments led to the conclusion that this material was not decomposed under such conditions (19, 28, 32). However, a slow degradation of labeled lignin to ¹⁴CO₂ and ¹⁴CH₄ was recently observed in the absence of oxygen (4, 11). The contribution of lignin to humic-substance formation in such environments has not yet been considered.

The riparian zone along a large river such as the Garonne River, in the south of France, is characterized by intense exchanges between terrestrial and aquatic ecosystems. This interface system is occupied by successive woody communities dominated by willow along the channel, then by alder, and finally by ash and elm. Poplar plantations often take the place of natural woods. One part of the litter falls on alluvial soils; another part falls directly into the river or into ponds and swamps. In the spring, plant residues at various stages of decomposition in soils may be flushed by floods into the river channel.

The purpose of our previous experiments was to compare the mineralization of willow, alder, or poplar [¹⁴C-lignin]lignocelluloses in waters and sediments of the Garonne River system (8, 18). This paper summarizes results of studies to determine the contribution of degradation products of lignin to humic acid (HA) formation. By using a grain-size fractionation of the incubated soils and sediments, we attempted

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TABLE 1. Characteristics of alluvial soils and sediments

Sample type and source	% in dry soil or sediment								pH	Redox potential	Ratio. C/N	% TOC		Ratio FA/HA
	Coarse sand (2-0.2 mm)	Fine sand (0.2-0.05 mm)	Coarse silt (0.05-0.02 mm)	Fine silt (0.02-0.002 mm)	Clay (<0.002 mm)	CaCO ₃	TOC ^a	N				FA	HA	
Alluvial soils under:														
Willow	0	3.1	25.1	46.8	25.0	8.6	4.45	0.35	7.9		12.7	10.2	8.5	1.2
Alder	0.5	10.1	20.5	40.4	28.5	7.7	5.33	0.42	7.9		12.7	11.6	9.8	1.2
Poplar	5.1	32.9	22.5	22.7	16.8	6.3	3.08	0.22	8.2		13.9	11.0	7.6	1.4
Sediments														
River	0.5	41.0	23.4	18.6	16.5	6.6	2.90	0.20	7.2	+180 mV	14.5	9.5	6.6	1.4
Pond	3.1	14.9	12.9	30.3	38.8	7.1	5.90	0.49	7.6	-64 mV	12.0	11.2	9.0	1.2

^a TOC, Total organic content.

to investigate in greater detail the transformation of raw plant material into complex humic polymers.

MATERIALS AND METHODS

Alluvial soils and sediments. Alluvial soil samples were collected in willow and alder woods and in a poplar plantation in the floodplain of the Garonne River. Sediment samples originated from the river and from a pond (an oxbow lake) 200 m away from the river channel. The main characteristics of the soils and sediments are presented in Table 1.

Preparation of [¹⁴C-lignin]lignocelluloses. The specifically labeled lignocelluloses were prepared by the method of Crawford et al. (13), with [¹⁴C]phenylalanine as the lignin precursor. In May, young branches of *Salix alba*, *Alnus glutinosa*, and *Populus euramericana* (Dode) 1240 were cut with care to prevent air entrance into the plants. A 1-ml portion of solution containing 5 μ Ci of L-[U-¹⁴C]phenylalanine was incorporated into each branch by absorption. The plants were incubated for 1 week in distilled water in a lighted, aerated, thermoregulated enclosure and then barked, freeze-dried, and ground to pass a 500- μ m sieve. The material was extracted four times with distilled water, then with a NaCl-Triton X-100 (2%) solution, and finally with ethanol-benzene (1:2, vol/vol). The residues were rinsed with 100% ethanol and dried under vacuum. Specific activities of the radiolabeled lignocelluloses were determined by burning samples in a Tricarb Sample Oxidizer (Packard Instrument Co.). The percentage of incorporated L-[U-¹⁴C]phenylalanine in proteins associated with the lignocelluloses was determined after digestion with a pronase solution (Sigma Chemical Co.) (12, 27). The esterified labeled phenolics linked with the periphery of lignin macromolecule were extracted with 2 N NaOH solution (22). A modified Klason procedure was used to determine the per-

centage of incorporated label associated with lignin macromolecules (15) (Table 2).

Degradation tests for soils and sediments. By using moisture determinations, enough soil which had been passed through a 2-mm sieve was used to provide 30 g of dry soil. These portions were placed into 250-ml Erlenmeyer flasks. The soil samples were enriched with 30 mg of the corresponding plant material (e.g., labeled lignocelluloses from willow were added to soil collected beneath willows). Three replicates were prepared for each type of lignocellulose. The flasks were connected with an aerated closed system in a dark room at 28°C for 7 weeks. The ¹⁴CO₂ evolved from each flask was absorbed in a 20% ethanolamine solution, and its radioactivity was measured after the addition of Readysolv MP (Beckman Instruments, Inc.).

The sediments which had been passed through a 2-mm sieve were used at two levels of moisture. They were first centrifuged at 25,000 \times g for 20 min to achieve a water content near the field capacity (33 and 58% in the river and pond sediments, respectively). Six portions of each sediment, corresponding to 30 g of dry sediment, were enriched with 30 mg of willow [¹⁴C-lignin]lignocelluloses. Three flasks were placed directly in the incubation room (wet sediment), and three were covered with 200 ml of the corresponding water recovered after centrifugation (submerged sediment). The degradation tests were carried out during 9 weeks as described above for soil samples.

Size fractionation of organic matter. At the end of the experiments, the incubated soils and sediments were submitted to size fractionation through successive wet sievings (7). Four fractions were separated: 2,000 to 100, 100 to 50, 50 to 20, and 20 to 0 μ m.

The water used for each fractionation was concentrated in a rotating evaporator, and the activity of the aliquots was measured. The different fractions were dried for subsequent determination of residual activity and for control of the state of organic matter to be determined by scanning electron microscopy. The 2,000- to 100- μ m fraction contained easily recognizable and little-transformed plant material. In the 100- to 50- μ m and the 50- to 20- μ m fractions, small fragments of plant membranes were observed, but the major part of the organic matter was in the form of fecal pellets. In the finest fraction, black particles not identifiable as plant material were occasionally found. The fractions were then submitted to successive extractions with 0.1 N HCl and 0.1 M sodium pyrophosphate. HA and fulvic acids (FA) were separated through acidification at pH 1.5, and the activity of the aliquots was measured.

TABLE 2. Distribution of ¹⁴C in [¹⁴C-lignin]lignocelluloses

Source of lignocellulose	dpm/mg of lignocellulose	% ¹⁴ C extracted with:		
		Modified Klason procedure	Pronase solution	2 N NaOH
<i>Populus euramericana</i>	6,390	70	2.1	10.0
<i>Alnus glutinosa</i>	3,420	67	4.3	9.0
<i>Salix alba</i>	28,610	96	4.8	14.4

TABLE 3. Distribution of radioactivity from [¹⁴C-lignin]lignocelluloses recovered from alluvial soil after 49 days of incubation^a

Sample type ^a	% Applied activity						
	Grain size fractions				Evolved ¹⁴ CO ₂	Water	Total
	2,000–100 μm	100–50 μm	50–20 μm	20–0 μm			
Willow	30.2 ± 3.4	35.7 ± 2.1	13.2 ± 2.1	6.2 ± 1.7	15.2 ± 1.0	1.2 ± 0.4	101.8 ± 3.9
Alder	26.7 ± 10.7	43.4 ± 7.6	10.3 ± 3.1	4.2 ± 1.0	12.2 ± 0.6	5.7 ± 1.0	102.6 ± 7.6
Poplar	28.3 ± 7.5	44.9 ± 7.7	7.4 ± 0.2	3.0 ± 0.9	9.9 ± 3.0	2.9 ± 0.3	96.4 ± 5.2

^a Alluvial soil collected under willow, alder, and poplar.

RESULTS

In alluvial soils, the total degradation to ¹⁴CO₂ reached about 15% for willow [¹⁴C-lignin]lignocellulose after 49 days of incubation and 12 and 10% for alder and poplar labeled plant material, respectively (Table 3). The total mineralization of willow [¹⁴C-lignin]lignocellulose in sediments appeared to be related not to the habitat but to the moisture conditions (Table 4). After a 60-day incubation period, the decomposition was greater in wet river and pond sediments (10%) than in submerged sediments (4 to 5%).

In alluvial soils, as in sediments, the bulk of the residual activity was recovered in the coarsest fractions (2,000 to 100 and 100 to 50 μm), amounting to from 62 to 73% of applied activity (Tables 3 and 4); 10 to 25% was recovered in the two finest fractions. The activity of water used for sievings varied between 0.4 and 3%, except in the case of water from soil collected beneath alders, in which the amount reached 6%.

Treatment with dilute HCl, which extracted low-weight molecules weakly bound to mineral or organic constituents of soils and sediments, did not permit the recovery of an important percentage of the applied activity. The amounts of HCl-soluble products, expressed as percentage of the residual activity of each fraction, did not appear to be related to the size of particles in alluvial soils (Table 5) as was the case for sediments (Table 6).

The percentage of applied activity recovered from the different grain-size fractions as FA and HA was low, in the range of 0.25% in the 20- to 0-μm fraction of soil collected beneath willows to 15% in the 2,000- to 100-μm fraction of soil collected from beneath alders. But FA and HA represented an increasing percentage of the residual activity from the coarsest to the finest fractions, except in the case of soil collected beneath alders. In this soil the amount of labeled FA and HA was equivalent in the 2,000- to 100-μm fraction and in the 20- to 0-μm fraction (55%). In sediments, the highest percentage of residual activity recovered as FA and HA was in the submerged pond sediment. In the majority of samples, labeled HAs were more abundant than FAs.

The total amount of labeled products recovered in water and in HCl and alkaline extracts represented 5% of applied activity in soil collected beneath willows, 10% in soil collected beneath poplars, 34% in soil collected beneath alders, and between 7.5 and 9.5% in sediments.

DISCUSSION

Alluvial soils occupied by willow and alder communities or poplar plantations originate from the kind of material that is deposited as recent sediments in the river and ponds of ancient meanders, but these soils and sediments have very different ages and water regimes. Under willow trees along water channels, soils may be submerged by floods and by a water table which is never more than 1.5 m below the surface. Alder communities and poplar plantations are not submerged by normal floods, but in the former, soils remain waterlogged, while in the latter, there is better drainage of upper layers. The river sediment in running waters is more aerated than the sediment in standing pond waters. From August to November, most sediments in the river are not submerged.

These studies show that lignin mineralization to ¹⁴CO₂ was relatively high in alluvial soils. About 10 to 15% of ¹⁴C was lost after 7 weeks, the amount dependent on the different labeled materials and incubated soils. In nonsubmerged sediments of the Garonne River and the pond, about 10% of willow [¹⁴C-lignin]lignocelluloses were recovered as ¹⁴CO₂ after 9 weeks of incubation. The degradation was significantly lower in submerged sediments. However, it was close to those previously observed in different freshwater or marine sediments (6, 17, 19).

The advantage of grain-size fractionation in studying the degradation products of labeled lignin in soil and sediments is the separation of less-transformed plant material in the coarsest fraction (2,000 to 100 μm) from more-transformed material in the finest fraction (20 to 0 μm), where it may be bound to mineral particles. In the intermediate fractions (100 to 50 and 50 to 20 μm), the organic matter appears as very finely ground, recognizable plant material or as fecal pellets.

TABLE 4. Distribution of radioactivity from willow [¹⁴C-lignin]lignocelluloses recovered in sediment after 60 days of incubation

Sample source	% Applied activity						
	Grain size fractions				Evolved ¹⁴ CO ₂	Water	Total
	2,000–100 μm	100–50 μm	50–20 μm	20–0 μm			
Wet sediments							
River	50.0 ± 9.6	15.7 ± 0.8	11.8 ± 0.3	8.9 ± 0.2	10.2 ± 1.3	1.8 ± 0.0	98.4 ± 4.3
Pond	45.2 ± 3.7	17.1 ± 1.1	11.8 ± 0.2	12.7 ± 0.6	10.4 ± 0.9	1.0 ± 0.2	99.1 ± 3.6
Submerged sediments							
River	59.1 ± 3.7	10.3 ± 0.8	6.5 ± 1.2	5.5 ± 0.8	4.6 ± 0.9	3.3 ± 0.4	89.3 ± 3.4
Pond	58.1 ± 8.0	13.9 ± 1.5	9.3 ± 1.2	11.1 ± 2.1	4.7 ± 0.6	2.7 ± 0.3	99.8 ± 4.8

TABLE 5. Distribution of radioactivity from willow, alder, and poplar [¹⁴C-lignin]lignocelluloses recovered in acid and alkaline extracts of fractions of alluvial soils

Sample source and grain size (μm) ^a	% Applied activity (% residual activity)			
	HCl extract	FA	HA	Extracted soil
Willow				
2,000–100	0.22 (0.7)	0.66 (2.2)	0.48 (1.6)	28.8 (95.5)
100–50	0.20 (0.6)	0.36 (1.0)	0.38 (1.1)	34.7 (97.3)
50–20	0.21 (1.6)	0.39 (3.0)	0.51 (3.8)	12.1 (91.6)
20–0	0.05 (0.8)	0.24 (3.9)	0.10 (1.6)	5.8 (93.8)
Total	0.68 (0.8)	1.66 (1.9)	1.47 (1.7)	81.5 (95.5)
Alder				
2,000–100	2.72 (10.2)	6.02 (22.5)	8.76 (32.8)	9.2 (34.4)
100–50	0.66 (1.5)	1.88 (4.3)	1.24 (2.8)	39.7 (91.3)
50–20	0.65 (6.3)	2.37 (23.0)	1.49 (14.4)	5.8 (56.2)
20–0	0.62 (14.7)	1.12 (26.6)	1.17 (27.8)	1.3 (30.9)
Total	4.65 (5.5)	11.40 (13.4)	12.65 (14.9)	56.0 (66.1)
Poplar				
2,000–100	0.49 (1.7)	0.79 (2.8)	0.69 (2.4)	26.3 (92.9)
100–50	0.64 (1.4)	0.91 (2.0)	0.96 (2.1)	42.4 (94.4)
50–20	0.48 (6.5)	0.46 (6.2)	0.50 (6.7)	6.0 (80.5)
20–0	0.14 (4.5)	0.25 (8.2)	0.77 (25.5)	1.8 (61.5)
Total	1.75 (2.1)	2.41 (2.9)	2.92 (3.5)	76.5 (91.5)

^a Soil obtained under willow, alder, and poplar.

In alluvial soils, as in sediments, the greatest part of residual activity (62 to 73%) was recovered in the coarsest fractions. For sediments, the activity was essentially in the first fraction (2,000 to 100 μm), while for soils, the highest activity was principally in the second fraction (100 to 50 μm). The percentage of applied activity recovered in the 20- to 0-μm fraction of sediments was, on the average, higher than in soils, especially in soil collected from beneath poplars.

Compared with the applied activity, the percentage of residual activity recovered in HA and FA was very low (0.5 to 2%) in all fractions of soils and sediments except in the coarsest fraction of soil collected beneath alders (15%). It was 2 to 20 times less than in developed soils (21), but the percentage of residual activity recovered in HA and FA increased from the coarsest to the finest fraction in soils and sediments. In the coarsest fraction, which had the highest level of residual activity, only 2 to 5% activity was recovered in alkaline extracts. One exception was noted for soil collected beneath alders, in which 55% of the residual activity was extracted in HA and FA. This result suggested that alder [¹⁴C-lignin]lignocellulose incubated in soil collected beneath alders and still present as recognizable plant material was more transformed into extractable products than were willow and poplar labeled materials.

In the finest fraction, HA and FA represented 9 to 19% of the residual activity in sediments, with the highest percentage in the submerged pond sediment. In soils, the highest activities of HA and FA were in the fine fraction of soil collected beneath alders (54%). The residual activity was two times more in soil collected beneath willows than in soil collected beneath poplars, but the percentage recovered in HA and FA was six times less (5.5 and 34%). In most fractions, the percentage of residual activity in HAs was equal to or higher than that in FAs.

In conclusion, the fate of ligneous plant material produced in a riparian zone could vary, depending on the environment in which the litter was incorporated. Although the soil beneath willows was waterlogged and flooded each year, its

lignin mineralization potentialities seemed to be higher than that of other alluvial soils, but inversely, the humification process was much slower. In soil beneath poplars, a drier region but one with a lower nitrogen and clay content, the transformation of lignin as ¹⁴CO₂ or humic substances was very low. In soil beneath alders, the mineralization of lignin was not very different, but HA and FA formation appeared to be more rapid. In sediments, the moisture conditions seemed to inversely influence the mineralization and humification processes. Mineralization was reduced under submerged conditions, while the highest recovery of humic compounds was observed in submerged pond sediment. In soils, fungi are considered the predominant organisms in lignin degradation and in the humification process. Recent studies indicate that in marine and freshwater ecosystems, bacteria are the predominant degraders of lignocellulose (5, 6). The lesser importance of fungal activity in such environments could explain the fact that the formation of humic compounds from lignin was slower there than in soils.

Besides nutrients and clay content, physical stability could also influence humic-compound formation in such environments. River sediments, as well as soil beneath willows, are greatly disturbed by running waters. Poplar plantations are also disturbed by soil plowing, drainage, and understory cutting. In contrast, soil beneath alders and sediments in ponds provide stable conditions.

The formation of humic substances from lignin is demonstrated, but it occurs to only a limited extent in most environments in a riparian zone. It is especially obvious in those environments directly in contact with running waters, such as river sediments and soil beneath willows. But litter production is higher in willow communities than in other

TABLE 6. Distribution of radioactivity from willow [¹⁴C-lignin]lignocelluloses recovered in acid and alkaline extracts of fractions of sediments

Kind of sediment and grain size (μm)	% Applied activity (% residual activity)			
	HCl extract	FA	HA	Extracted sediment
Wet river				
2,000–100	0.64 (1.3)	0.71 (1.4)	0.77 (1.5)	47.9 (95.8)
100–50	0.55 (3.5)	0.40 (2.5)	0.72 (4.6)	14.0 (89.4)
50–20	0.75 (6.4)	0.33 (2.8)	0.37 (3.1)	10.3 (87.7)
20–0	0.19 (2.1)	0.45 (5.0)	0.51 (5.7)	7.7 (87.2)
Total	2.13 (2.5)	1.89 (2.2)	2.37 (2.7)	80.0 (92.6)
Wet pond				
2,000–100	0.65 (1.4)	0.44 (1.0)	0.64 (1.4)	43.5 (96.2)
100–50	0.50 (2.9)	0.43 (2.5)	0.54 (3.1)	15.6 (91.5)
50–20	0.41 (3.5)	0.28 (2.4)	0.45 (3.8)	10.7 (90.3)
20–0	0.14 (1.1)	0.43 (3.4)	0.66 (5.2)	11.5 (90.3)
Total	1.70 (1.9)	1.58 (1.8)	2.29 (2.6)	81.3 (93.7)
Submerged river				
2,000–100	1.29 (2.2)	0.79 (1.3)	0.81 (1.4)	56.2 (95.1)
100–50	0.47 (4.6)	0.25 (2.4)	0.36 (3.5)	9.2 (89.5)
50–20	0.27 (4.1)	0.20 (3.0)	0.35 (5.3)	5.7 (87.6)
20–0	0.27 (4.9)	0.25 (4.5)	0.34 (6.2)	4.6 (84.4)
Total	2.30 (2.8)	1.49 (1.8)	1.86 (2.3)	75.7 (93.1)
Submerged pond				
2,000–100	0.81 (1.4)	0.49 (0.8)	0.74 (1.3)	56.1 (96.5)
100–50	0.39 (2.8)	0.22 (1.6)	0.44 (3.2)	12.8 (92.4)
50–20	0.17 (1.8)	0.15 (1.6)	0.21 (2.3)	8.8 (94.3)
20–0	1.14 (10.2)	0.68 (6.1)	1.43 (12.8)	7.8 (70.9)
Total	2.51 (2.7)	1.54 (1.7)	2.82 (3.0)	85.5 (92.6)

deciduous forests of the temperate zone (5.4 to 6 t of willow litter ha⁻¹ year⁻¹ plus 2.5 t of herbaceous litter ha⁻¹ year⁻¹) (9). As the upper layers of these soils and sediments are periodically moved and washed by running waters, the terrestrial litter decomposed at the direct interface zone may really contribute to the pool of aquatic humic materials. Moreover, woody communities farther from the river channel, such as alder woods, and sediments of standing water in oxbow lakes may also participate in the enrichment of rivers in humic substances through subsurface water systems.

ACKNOWLEDGMENTS

We thank M. Escautier and D. Lacaze for technical assistance.

This work was supported by Interdisciplinary Research Programme on Environment (PIREN/CNRS) and Regional Research Programme grants.

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