The Origin of Black 'Sludge' from a Deer Island (New Brunswick) Beach

V. Zitko and S. Robinson

Biological Station
St. Andrews, N. B. E0G 2X0

September 1996

Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 2365

Fisheries and Oceans / Pêches et Océans

Canada

Canadian Manuscript Report of
Fisheries and Aquatic Sciences 2365

September 1996

The Origin of a Black 'Sludge' from a Deer Island (New Brunswick) Beach

by

V. Zitko and S. Robinson
Fisheries and Oceans Canada
Maritimes Region
Biological Station
St. Andrews, New Brunswick E0G 2X0 Canada

Cat. No. Fs 97-4/2265E ISSN 0706-6473

Correct citation for this publication:

Zitko, V., and S. Robinson. 1996. The origin of black 'sludge' from a Deer Island (New Brunswick) beach. Can. Manuscr. Rep. Fish. Aquat. Sci. 2365: iii + 14 p.

ABSTRACT

Zitko, V., and S. Robinson. 1996. The origin of a black 'sludge' from a Deer Island (New Brunswick) beach. Can. Manuscr. Rep. Fish. Aquat. Sci. 2365: iii + 14 p.

Black sludge found on the beach in Richardson Cove, Deer Island, is a resuspended anoxic sediment. It is not directly related to aquaculture and is most likely the consequence of high organic loading from other sources. The sludge and reference samples were characterized by loss on ignition, ultraviolet and visible spectra of dichloromethane and methanol extracts, ultraviolet spectra of reaction products with sulfuric acid, and by thin layer chromatography. Laboratory experiments on biodegradation of polysaccharides, lipids, canthaxanthin and astaxanthin (carotenoids used by aquaculture), were also performed. These carotenoids are relatively resistant to anaerobic degradation, but canthaxanthin (carotenoid currently in use by the local aquaculture industry) was detected only in sediment under an active aquaculture site.

RÉSUMÉ

Zitko, V., and S. Robinson. 1996. The origin of a black 'sludge' from a Deer Island (New Brunswick) beach. Can. Manuscri. Rep. Fish. Aquat. Sci. 2365: iii + 14 p.

La boue noire trouvée sur la plage de l'anse Richardson, Deer Island, et un sédiment anoxique remis en suspension. Sa présence n'est pas directement associée à l'aquiculture et est vraisemblablement due à une charge organique élevée provenant d'une autre source. Des échantillons de boue et des échantillons témoins ont été caractérisés par perte par calcination, analyse spectroscopique dans l'ultraviolet et le visible d'extraits traités au dichlorométhane et au méthanol, analyse spectroscopique dans l'ultraviolet des produits obtenus par réaction avec l'acide sulfurique et chromatographie sur couche mince. Des expériences en laboratoire de biodégradation des polysaccarides, des lipides, de la canthaxanthine et de l'axtaxanthine (caroténoïdes utilisés en aquiculture) ont également été réalisées. Ces caroténoïdes sont relativement résistants à la dégradation anaéroibie, mais la canthaxanthine (caroténoïde utilisée par l'industrie aquicole locale) n'a été détectée que dans des échantillons de sédiments prélevés sous un site aquicole actif.

BEACH SAMPLING ON DEER ISLAND

BACKGROUND

On August 1, 1995, Mr. Larry Foster, President of the Charlotte County Clam Diggers Association, contacted the St. Andrews Biological Station to report that he had a sample of mud with some dead soft-shell clams (*Mya arenaria*) from a beach near Richardson, Deer Island, below Marvin Newman's sea urchin processing plant. He had taken the sample because the clams were apparently dying on the beach according to reports from his harvesters. The mud sample was retrieved from his cold room at the Bocabec clam plant, transported to the St. Andrews Biological Station and placed in the freezer. The sample was very fluid, with a few dead clams in the bottom of the bag. There was a strong smell of hydrogen sulfide despite the sample being double-bagged. Discussions with Mr. Foster and a few diggers at the clam plant about the incidence of this "black mud" around the area indicated it was fairly prevalent. They all felt that it was being produced by the aquaculture sites. Because of the uncontrolled collection and storage of the sample from Mr. Foster, further samples were collected from the beach in question.

GENERAL OBSERVATIONS

On August 9, 1995, S. Robinson visited Richardson (Fig. 1) to observe the general area and have a look at the aquaculture site during high tide. Discussion with a couple of workers at the salmon aquaculture site about the flow characteristics of the area indicated the water comes in the main entrance and generally splits towards either Lords Cove or Richardson. They were skeptical about all the salmon food winding up on the shore. They mentioned that during the warmest times of the year when the fish are feeding rapidly, they are feeding the salmon several tons of food a week. Their lease area was almost fully occupied.

SAMPLING

Two sites were chosen for comparison of core samples - the Richardson site below Newman's sea urchin processing plant, the source of the original complaint, and another site on the other end of the island in Cummings Cove, Western Passage, to act as a reference. There was an established salmon farm at the Cummings Cove site and we chose a sampling area near the northeastern part of the cove where there was an accumulation of mud and algae. The beach had a medium grade and went from a coarse sand in the upper intertidal area to a muddy sand with some rocks in the lower intertidal area. Houses were located on steep embankments at the top of the beach. Groundwater percolated onto the middle of the beach. There was a strong growth of green algae, especially *Enteromorpha intestinalis* (Fig. 2, a, b). (*Enteromorpha* spp. have been shown to be an indicator of increased nutrient loading.) Four standard sediment cores approximately 20 cm in depth were taken at the Cummings Cove site, placed in labelled, plastic bags and immediately placed in a cooler with ice. Photographs were taken of the area in general.

At the Richardson site, the beach in front of the sea urchin plant was quite rocky with a gradation from ledge to soft mud at the lower intertidal area. There was a great deal of *E. intestinalis* on the beach from the upper to the lower intertidal area. There was a strong odor of decomposition on the beach. Mr. Marvin Newman was present during the sampling and indicated where Larry Foster had taken his sample. The lower intertidal area and shallow subtidal area were comprised of a mud layer (light gray in color) over a gravel base. Four cores were taken in this area and treated as described above. In addition, a bulk sample of the surface mud was taken by scooping up approximately 100 mL of the top 2-3 cm and placing it in a plastic bag which was then immediately put on ice. Photographs were also taken at this site (Fig. 2, c, d).

Samples were then transported back to the St. Andrews Biological Station where they were placed in the walk-in freezer for subsequent analyses.

A sample was also collected by sea urchin divers from just outside the weir in Doctors Cove, Deer Island. The sample was scooped from the bottom in a plastic container and transported back to the St. Andrews Biological Station where it was frozen until analysis.

OBSERVATIONS AND IMPRESSIONS

Both of the sites examined showed signs of eutrophication based on the presence of the *Enteromorpha* in the intertidal zone, especially prevalent at the Richardson site. The highest densities of algae were found in the most protected areas (i.e. in the lee of a point or in areas which were primarily depositional). Visual examination of the Richardson area (including Lords Cove) indicated there was a general proliferation of the alga,

Enteromorpha intestinalis. The distribution of this alga was not just located at the water's edge, but tended to concentrate at the mid to high-water mark suggesting there was nutrient loading occurring from the surrounding land areas as well. High algal levels were also noted near the area known as the "boat shop", between Richardson and Lords Cove.

CHEMICAL ANALYSES

CHARACTERISTICS OF SEDIMENT SAMPLES

Chemical analyses of the samples (Table 1) were initiated on August 10, 1995. Sample 4 (Table 1) was collected on August 18, 1995, by V. Zitko, for comparison. Sample 5 was obtained from S. Robinson on October 27, 1995, samples 6-9 were subsampled from Dr. Barry Hargrave's (BIO) box cores on September 21, 1995, and sample 10 was collected by Mr. A. McIntyre below a net pen on December 19, 1995. Some characteristics of the samples are listed in Table 1.

The loss on ignition is a measure of the concentration of total organics. UV absorbance of the DCM extract is also a measure of the presence of organic compounds. It is particularly sensitive to aromatic hydrocarbons, carotenoids, and other organics absorbing UV light. Methanol extracts chlorophyll and related compounds, and a variety of other organics. Spectra of the methanol extracts (Fig. 3, 4) contain absorption maxima at 664 and 416 nm. The absorbance at these wavelengths is a measure of the concentration of chlorophyll-related compounds. Absorbance at the former wavelength was also converted to phaeophytin concentration, for comparison with literature values. Shallow water sediments usually contain phaeophytin at < 10 μg/g, basin-floor sediments at 4-100 μg/g (Orr et al. 1958). The similarities and differences in the spectra of the methanol extracts of the sediment samples can be visualized by projecting the spectra on the planes of the principal components (Fig. 5, 6). It can be seen that the Richardson core (R core) is somewhat similar to that from the active aquaculture site (aqua). Spectra from the former aquaculture and from the reference sites are relatively similar and form a cluster. The remaining four spectra (Table 1, samples 1, 3, 4, 5) are considerably different from each other as well as from the spectra mentioned above. As can be seen from Table 1, the Richardson samples have a much lower loss on ignition than the sediment from the aquaculture site.

Table 1. Samples analyzed and some of their characteristics.

		Absorbance of extracts per (g/mL)					
	Ignit. loss	DCM	MeOH		Phaeo&		Fluoresc 'streak'
Sample	(%)	330 nm	664 nm	416 nm	(μg/g)	Cx#	on TLC
1 Richardson 'sludge'	13.73	19.69	10.15	27.29	290	-	+
2 Richardson core	8.76	14.96	8.65	39.29	247	-	+
3 Cummings Cove core	4.24	3.56	3.76	12.54	107	-	-
4 SABS core	5.74	1.64	0.76	2.94	22	-	-
5 Doctors Cove grab	5.76	36.00	2.56	10.88	73	-	+++
6 Former aquaculture site 1*	10.69	3.28	3.11	14.00	89	-	+
7 Former aquaculture site 2*	9.33	2.45	4.03	20.17	115	-	+
8 Reference site 1	8.93	2.19	2.66	12.26	76	-	+
9 Reference site 2	9.18	2.45	1.85	8.78	53	-	+
10 Active aquaculture site	33.95	16.04	16.89	47.15	483	+	-

& phaeophytin a (from absorbance at 664 nm, k = 35 L/(gcm) (Orr and Grady 1957).
# canthaxanthin.
* vacated 4 yr ago.
DCM = dichloromethane, MeOH = methanol.

MODEL EXPERIMENTS

To obtain additional criteria for the determination of the origin of the Richardson 'sludge', anaerobic biodegradation of aquaculture feed and of its lipid, carotenoids, polysaccharides, and proteins, and of extracts of macroalgae, was investigated.

CAROTENOIDS

In model experiments with the anaerobic degradation of feed lipid at about 20°C in the laboratory, astaxanthin and canthaxanthin were still present after 44 d. On the other hand, astaxanthin was not detectable in any of the sediment samples and canthaxanthin was detected only in the sample from the active aquaculture site (Table 1). Canthaxanthin is the main carotenoid used now by the industry to give red color to salmon tissue. This finding is a surprise. The authors were under the impression that canthaxanthin had been replaced by astaxanthin about 2 yr ago.

LIPIDS

In the model experiments, triglycerides were detectable after 14 d but not after 29 d. In comparison, triglycerides were not detectable in the sediment samples. The lipid biodegradation products left a characteristic 'signature' on thin layer chromatography (TLC), which remained essentially unchanged for another 16 d. A similar 'signature', but depleted in fatty acids, was observed in the extract of the sediment from the active aquaculture site.

POLYSACCHARIDES AND OTHER HIGH MOLECULAR WEIGHT COMPOUNDS

Starch (from feed extract) was not detectable by iodine after 7 d, but some high molecular weight compounds remained and were further modified, after another 12 d, primarily by degradation of proteins. Starch was not detectable in the sediment samples, but all samples contained some high molecular weight compounds.

Sugars and amino acids were detected in hydrolyzates of anaerobic sediments (Plunkett 1957). In the present work, a reaction with concentrated sulfuric acid (Zitko and Rosik 1961), developed originally for pentoses, hexoses, and uronic acids, was extended to humic compounds, lignins, and proteins, and was used to analyze the high molecular weight compounds. The same technique was used previously to characterize high molecular weight compounds in foam collected in the St. Croix estuary. The results, summarized from the 200-350 nm spectra by Principal Component Analysis (Zitko 1994), indicate that the profile of the high molecular weight organic compounds in Richardson 'sludge' resembles more a mixture of polysaccharides and humic substances found in the foam from the St. Croix estuary and decomposed algae, than biodegraded feed (Fig. 7, 8).

NON-POLAR COMPOUNDS

Fluorescing spots and streaks on thin layer chromatography (TLC), suggesting the presence of polynuclear aromatic hydrocarbons (PAHs), were observed in dichloromethane (DCM) extracts of all samples except those from Cummings Cove and SABS. The Doctors Cove sediment contains fluorescent compounds, some of which may not be PAH. The concentration of elemental sulfur appears unusually high in this sample. The Doctors Cove sample also contains sharp pieces of gravel, possibly indicating a recent dumping. This sample was submitted by the Deer Island group because of an unusually high incidence of 'albino' sea urchins in the area. If the incidence continues, sediment samples from the area should be examined in more detail.

CONCLUSIONS

The Richardson sludge is a resuspended anoxic sediment. It contains a high concentration of chlorophyll-related substances such as phaeophytin a. The absence of canthaxanthin indicates that the 'sludge' is not related to aquaculture and may be generated by high organic loading from other sources in the area, or by rotting algae. It is, of course, possible that the 'sludge' is a product of a more extensively degraded aquaculture waste, no longer containing canthaxanthin and proteins. Regular monitoring of Richardson in 1996 may provide a better picture of the sequence of events leading to the appearance of the 'sludge', and may lead to a more definitive identification of the source.

The project provided an interesting experience in the characterization of organic compounds in sediments, and of their behavior during biodegradation. Additional work may be carried out once a mass spectrometer is operational. Analytical techniques for dealing with aquaculture-associated organics should receive continuing attention.

ACKNOWLEDGMENTS

Drs. R.H. Peterson and D.J. Wildish commented on the manuscript. Ms. Marilynn Rudi and Ms. Brenda Best edited and formatted the manuscript.

REFERENCES

Orr, W. L., and J. R. Grady. 1957. Determination of chlorophyll derivatives in marine sediments. Deep-Sea Res. 4: 263-271.

Orr, W. L., K. O. Emery, and J.R. Grady. 1958. Preservation of chlorophyll derivatives in sediments off southern California. Bull. Am. Assoc. Petrol. Geol. 925-958.

Plunkett, M. A. 1957. The qualitative determination of some organic compounds in marine sediments. Deep-Sea Res. 4: 259-262.

Zitko, V. and J. Rosik. 1961. Spektrophotometrische Bestimmung des Pektins und begleitender Polysaccharide. Die Nahrung, 5: 491-505.

Zitko, V. 1994. Principal component analysis in the evaluation of environmental data. Mar. Pollut. Bull. 28: 718-722.

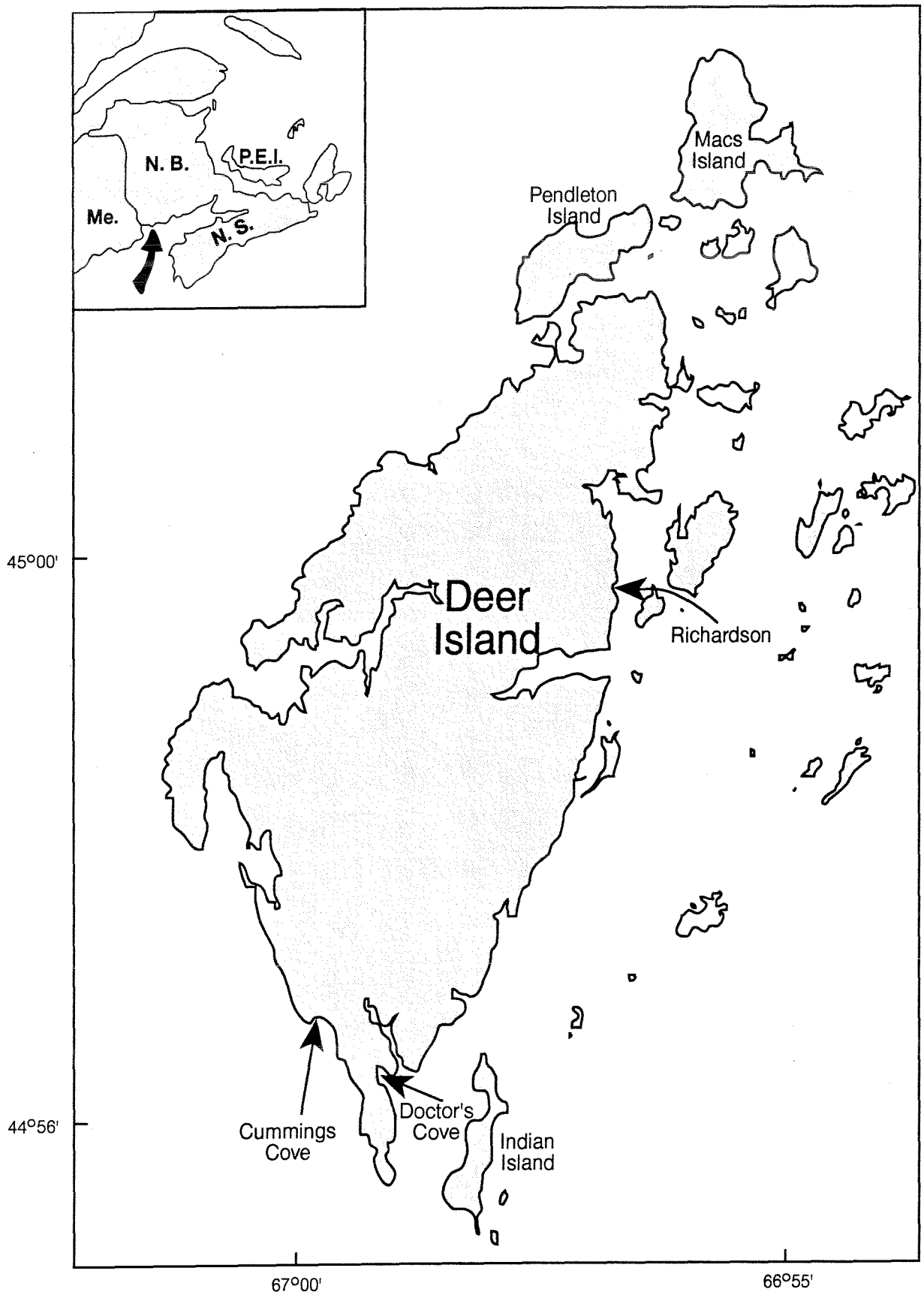

Figure 1. Map of Deer Island indicating the Cummings Cove and Richardson sampling sites.

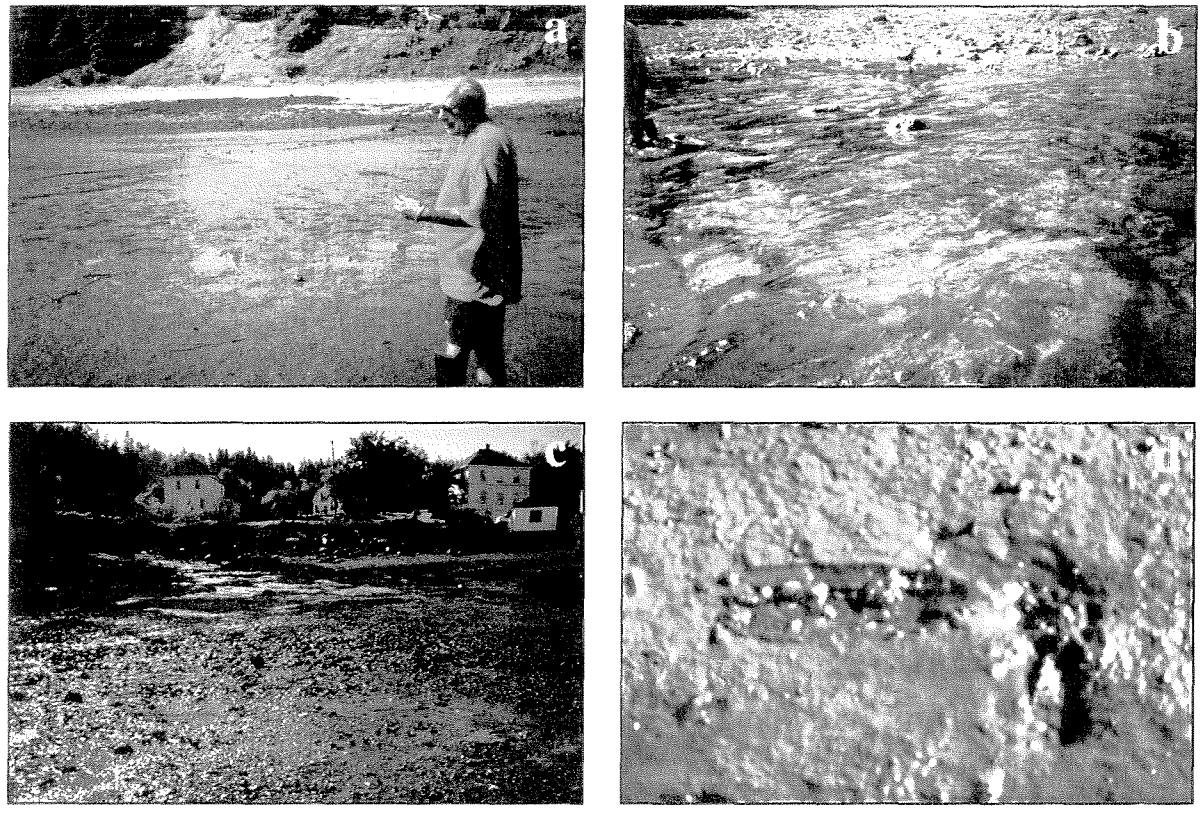

Figure 2. a) Photo of Cummings Cove looking north from low water mark. b) Photo of algae on beach at Cummings Cove in northwest section. c) Photo of beach at Richardson looking up from the low water mark. Note the build up of algae near the shore. d) Photo of the enriched organic mud at the low water mark of the Richardson beach. The area of view is about 30 cm across.

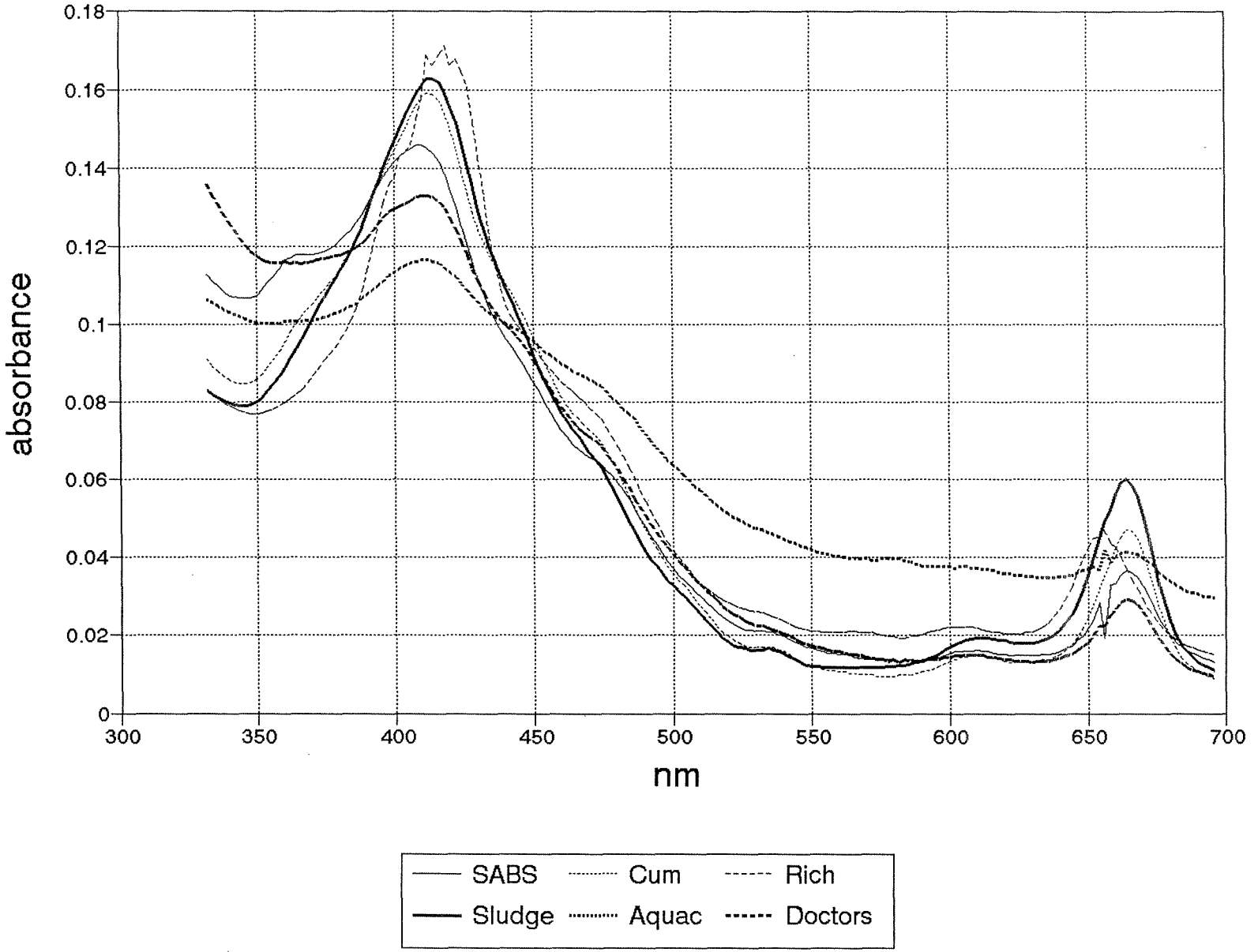

Fig. 3. UV/visible spectra of methanol extracts. For spectra identification see Table 1.

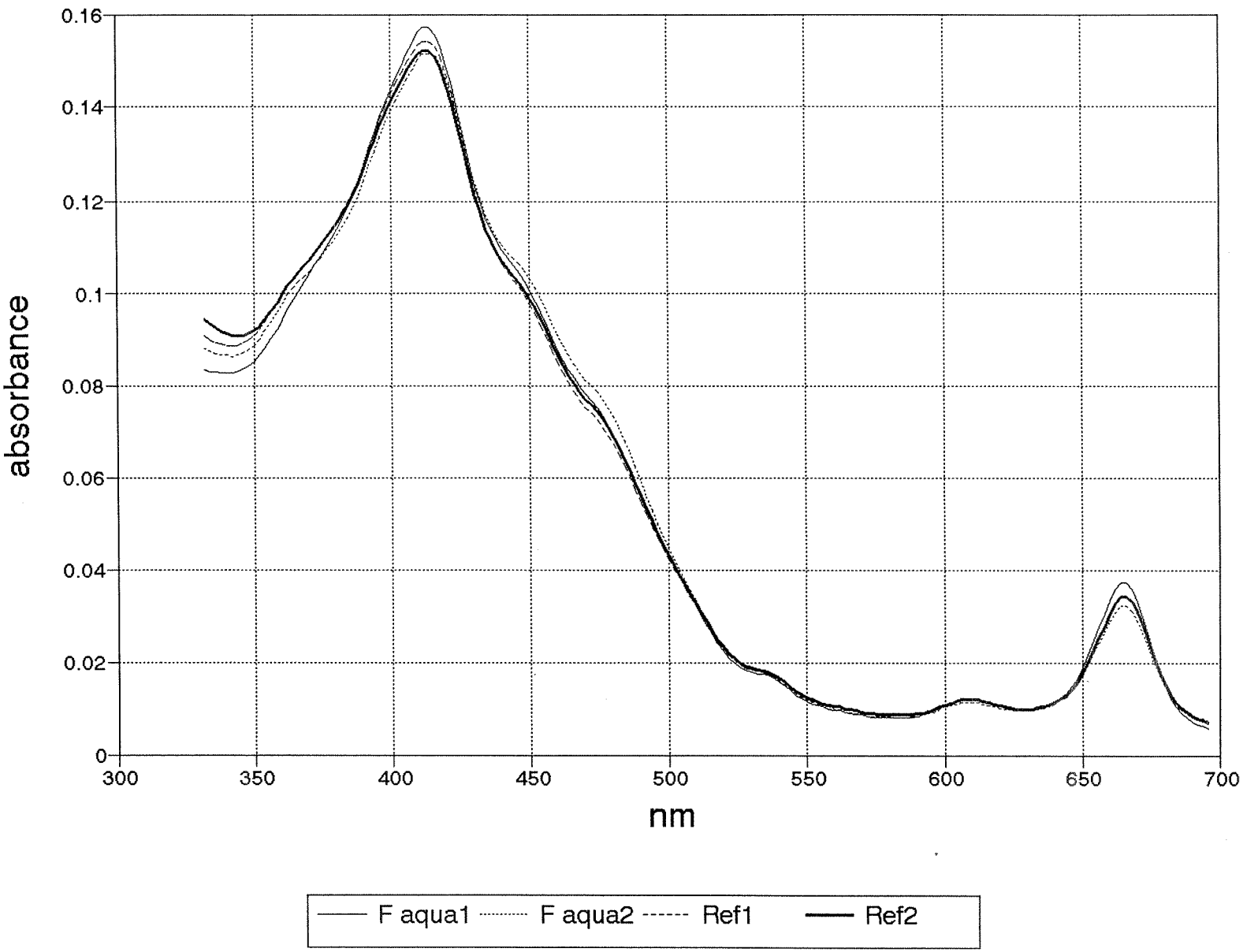

Fig. 4. UV/visible spectra of methanol extracts. For spectra identification see Table 1.

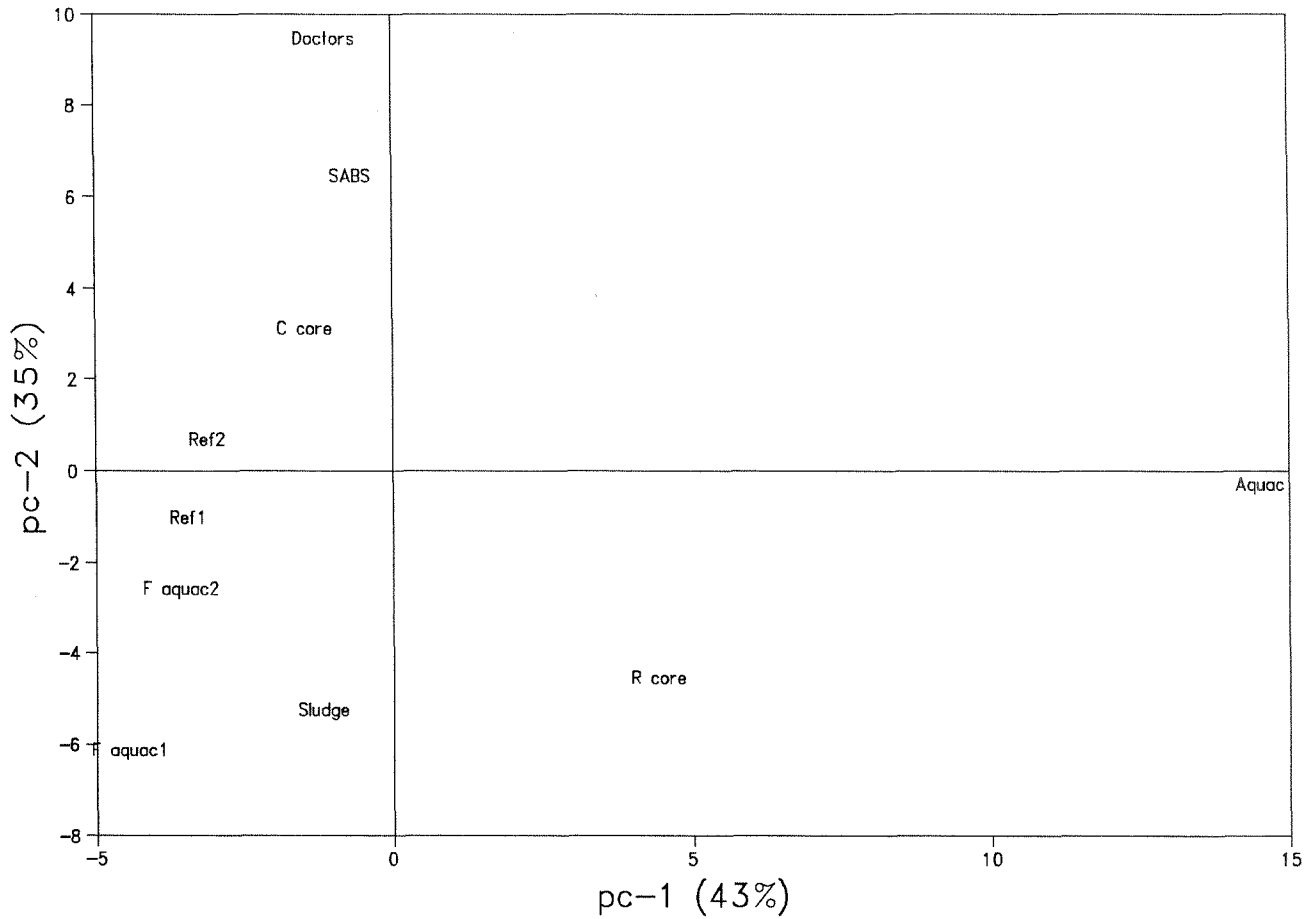

Fig.5. Projection of UV/visible spectra on the plane of the first two principal components. For spectra identification see Table 1. The distances between the projections are inversely proportional to the similarity of the spectra. Fractions of the original variance, accounted for by the individual principal components, are indicated on the axes.

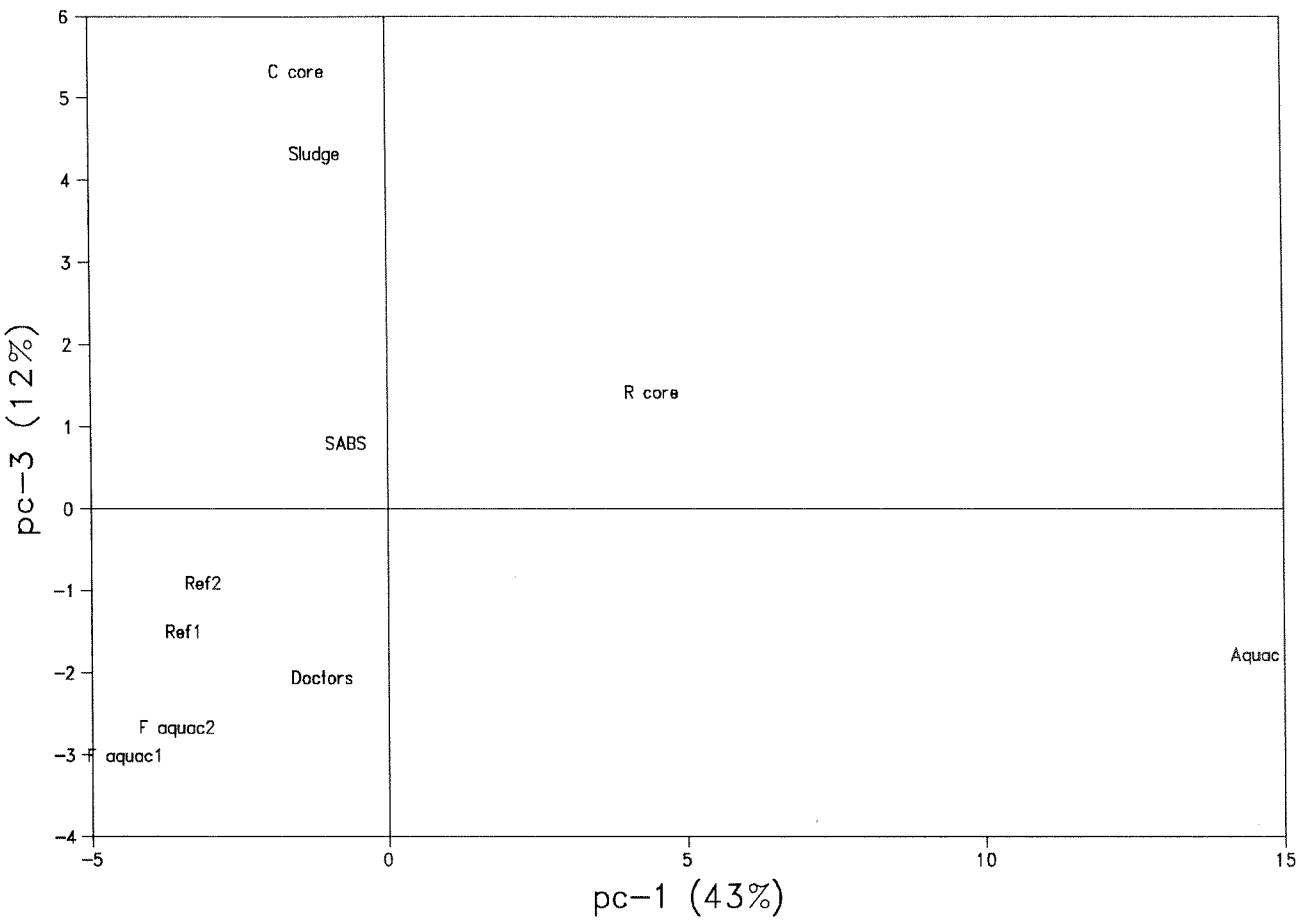

Fig. 6. Projection of UV/visible spectra on the plane of the first and the third principal component. For spectra identification see Table 1. The distances between the projections are inversely proportional to the similarity of the spectra. Fractions of the original variance, accounted for by the individual principal components, are indicated on the axes.

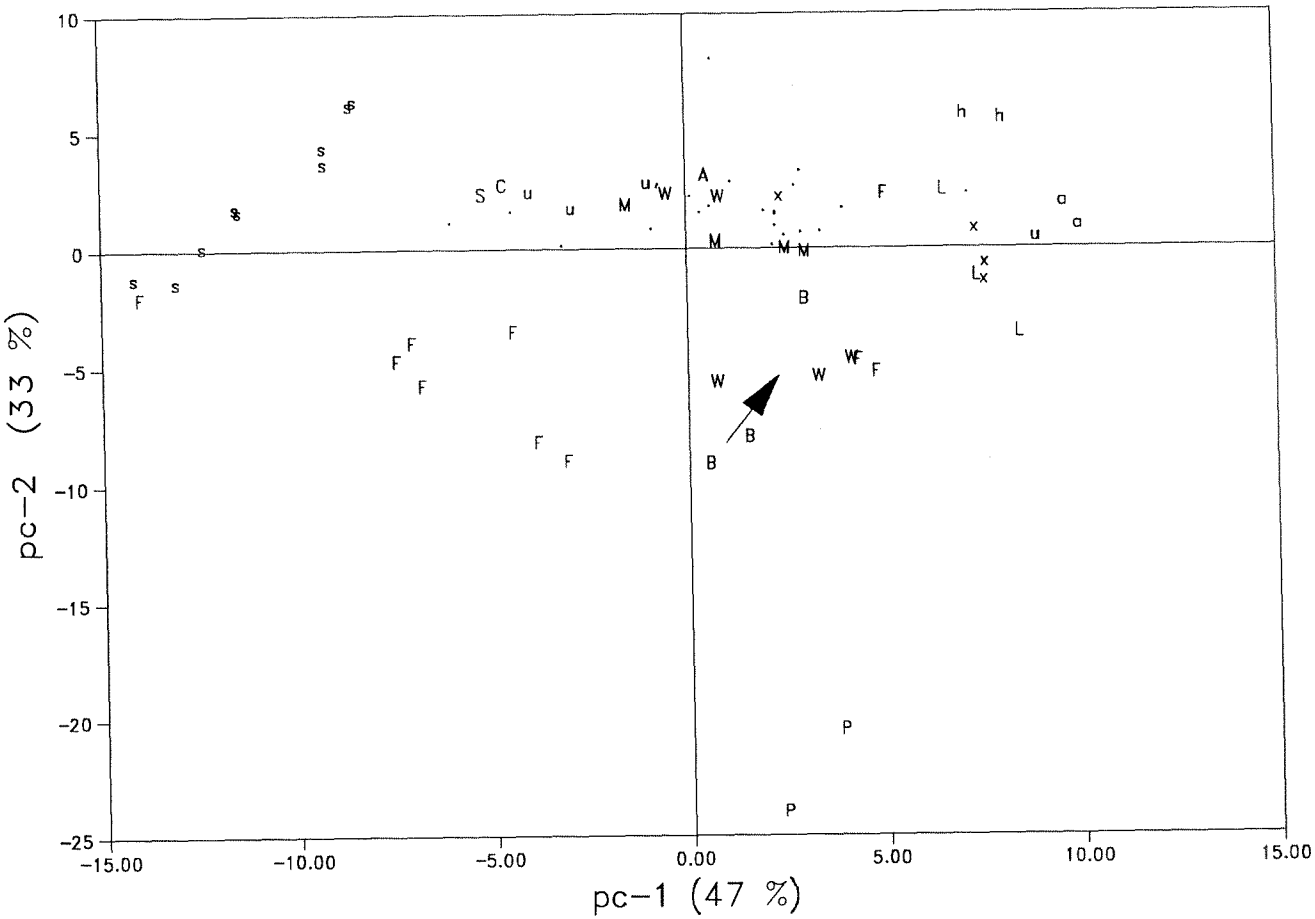

Fig. 7. Projection of the spectra of the reaction products of high molecular weight compounds with concentrated sulfuric acid, on the plane of the first two principal components. The distances between projections are inversely proportional to the similarity of the spectra. Fractions of the original variance, accounted for by the individual principal components are indicated on the axes. The symbols are: P protein, F feed, B biodegraded feed, W aqueous extract of sediment, M Richardson Cove sludge, C water extract of Cummings Cove core. S water extract of SABS core, A extract of algae, a biodegraded extract of algae, s sugar, u uronic acid, h humic acid, L lignin, x high molecular weight compounds from pulpmill effluent, '.' foams from the St. Croix estuary. The arrow indicates the progress of the biodegradation of feed.

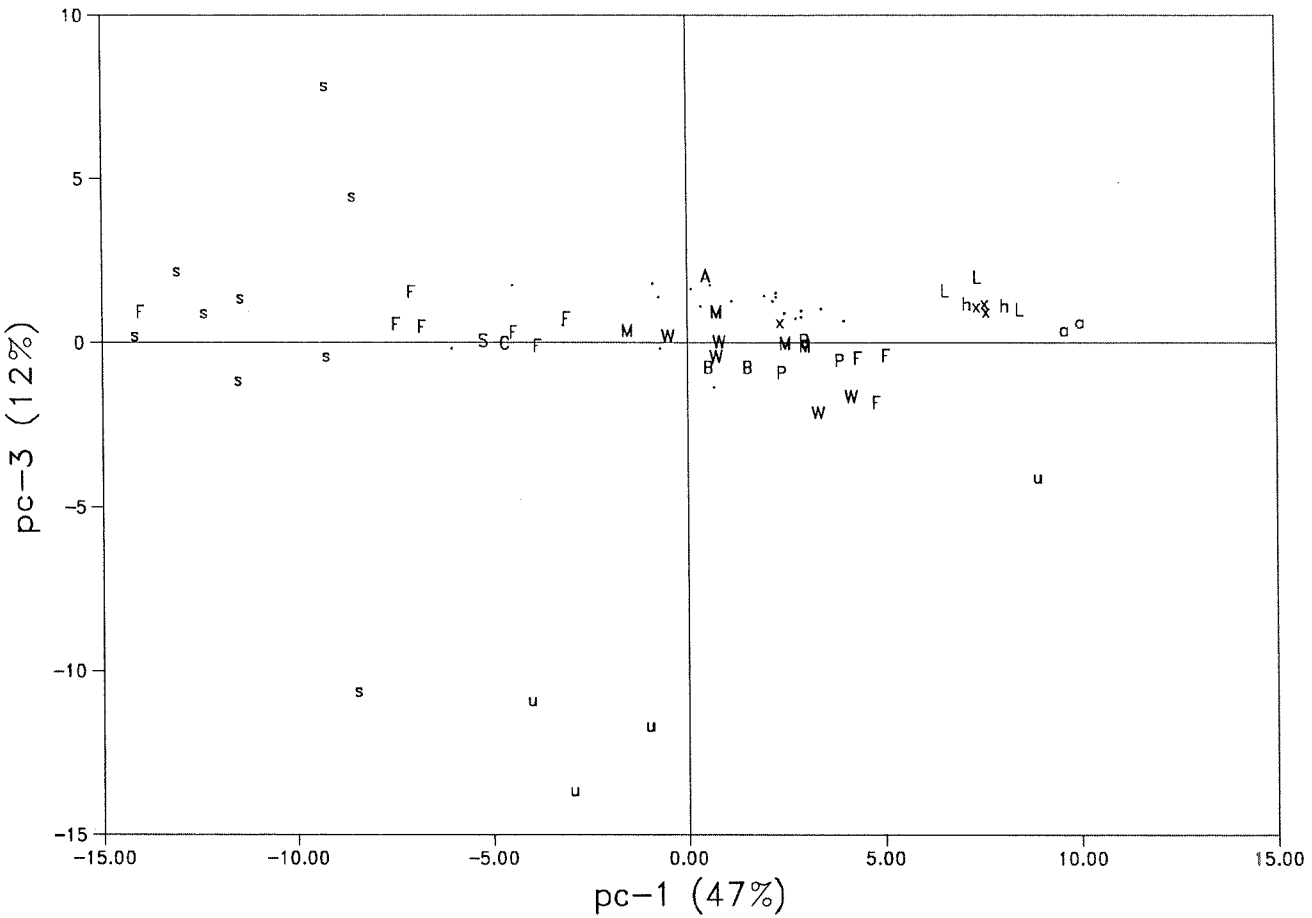

Fig. 8. Projection of the spectra of the reaction products of high molecular weight compounds with concentrated sulfuric acid, on the plane of the first and the third principal component. The distances between projections are inversely proportional to the similarity of the spectra. Fractions of the original variance, accounted for by the individual principal components are indicated on the axes. The symbols are: P protein, F feed, B biodegraded feed, W aqueous extract of sediment, M Richmond Cove sludge, C water extract of Cummings Cove core. S water extract of SABS core, A extract of algae, a biodegraded extract of algae, s sugar, u uronic acid, h humic acid, L lignin, x high molecular weight compounds from pulpmill effluent, '.' foams from the St. Croix estuary.

APPENDIX

SEDIMENT CHARACTERIZATION

Drying and extraction

Sediment samples 1-5, and 10 (Table 1) were centrifuged on a Sorvall GLC-2 centrifuge at 3000 rpm and freeze-dried. The remaining samples were just freeze-dried. Loss on ignition was determined by ashing 0.5-0.7 g of the sediments at 550°C overnight. IR spectra of the freeze-dried sediments were obtained in KBr matrices on a Perkin-Elmer model 467 IR spectrophotometer. Subsamples of the sediments (1 g) were placed in Pasteur pipets and extracted with dichloromethane (10-13 mL), followed by methanol (12-15 mL). UV/visible spectra of the extracts were determined on a HP model 8452A Diode array spectrophotometer.

Portions of samples 1-4, and 10 were washed repeatedly after centrifugation with distilled water, the washings collected, characterized by UV spectra, dialyzed when necessary, and freeze-dried. The dialysis was carried out in Spectra/Por #6 Membrane tubings, flat width 18 mm (Spectrum Medical Industries Inc., 1100 Rankin Road, Houston TX 77073-4716) against distilled water.

Thin layer chromatography (TLC)

The extracts were also examined by TLC on Whatman K5A silica plates. The solvent systems were: (a) petroleum ether-diethyl ether-acetic acid 80:20:1, (b) benzene, and (c) ethyl acetate-hexane 35:75. The compounds were detected visually under normal or UV light, by charring with sulfuric acid or by spraying with (a) anisaldehyde (0.5 mL) in methanol (85 mL), glacial acetic acid (10 mL) and concentrated sulfuric acid (5 mL), and heating for 10 min at 110°C, or (b) silver nitrate (0.1 g) in acetone (190 mL) and phenoxyethanol (10 mL), drying the plate for 15 min at room temperature, and exposing it to UV light at 254 nm.

Polysaccharides and other high molecular weight compounds

These substances were characterized by the reaction in 70 % sulfuric acid (Zitko and Rosik 1961). Distilled water (1 mL) was placed on 5 mL of concentrated sulfuric acid in a 15-mL centrifuge tube. A small amount (< 1 mg) of solid sample was added, the contents were mixed by carefully inverting the centrifuge tube several times, and the tube was placed in a boiling water bath for 5 min, cooled to room temperature, and the UV spectrum (200-360 nm) was recorded. If the absorbance exceeded 1.5 (1 cm cell), the solution was diluted with the sulfuric acid blank.

Chemometrical calculations

Relationships among samples were visualized by projecting the UV spectra of their methanol extracts and, for polysaccharides and related compounds, of the spectra of their reaction products in sulfuric acid, on the planes of the first three principal components (Zitko 1994). AT MATLAB was used for the calculations and Quattro Pro to prepare the graphs. Spectra of the methanol extracts, recorded from 340 to 700 nm, were reduced by a factor of 3 by the MATLAB signal processing method 'decimate', before the principal component projections. The 200 to 350 nm portion of the sulfuric acid spectra was used as such.

MODEL EXPERIMENTS

Biodegradation of polysaccharides

Lipids were removed from about 100 g of dry feed by washing with hexane. The defatted dry feed (10 g) was extracted with warm seawater (3 x 100 mL). The residue was centrifuged off and freeze-dried. A portion of the washings (100 mL) was centrifuged and dialyzed. The remainder was inoculated with a small amount of anoxic sediment from the beach near the Biological Station and kept in a tightly closed Mason jar in the laboratory. The centrifuged residue, as well as whole defatted feed, and torn sections of macroalgae, also collected near the Biological Station, were treated in the same way.

Biodegradation of lipids and carotenoids

The lipid extracted from the feed was recovered by evaporation in a rotary evaporator. A portion of the lipid (5 g) was mixed with sand (20 g). A part of this mixture (9 g) was mixed with freshly collected anoxic sediment (10 g), placed in a jar with seawater, tightly closed, and kept in the laboratory. Atlantic salmon eggs from another project (Miramichi River Environmental Assessment Committee) were similarly extracted, mixed with a portion of freeze-dried sample 2, inoculated with a portion of the feed biodegradation solution, and also kept in a tightly closed jar in the laboratory.

Aliquots (about 50 mL) were withdrawn at intervals, extracted with dichloromethane and the extracts were examined by UV spectrophotometry and by TLC.