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ORIGIN AND ENVIRONMENTAL SIGNIFICANCE OF ORGANIC DEPOSITS IN AQUATIC SEDIMENTS

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SUMMARY: The nutrient status and the lipids from a series of sediment samples taken from various aquaculture productive systems, have been characterized. Noticeable differences were found in the concentration of the lipid extracts although their composition were very similar as analyzed by gas chromatography-mass spectrometry. The most abundant components identified include series of linear and branched alkanes, fatty acids and alcohols, as well as unsaturated fatty acids, phytol, and in minor amounts some ω-hydroxyacids and sterols. Terrigenous plant material, phytoplankton and bacteria were found to be the probable sources of the isolated lipids.

Key words: aquaculture, lipids, sediments, alkanes, fatty acids, sterols.

INTRODUCTION

During the last decade the growing concern in the pollution of aquatic environment has estimulated the adoption of severe controls concerning the origin and fate of organic pollutants entering the water bodies. One way to achieve that control is the analysis of the sedimentary organic deposits, which could reflect the nature and extent of the inputs, both of anthropogenic and/or natural origin, to the system, as well as the variable performance of the biogeochemical cycle in the aquatic environment (1,2).

On the other hand, the study of the sedimentary organic enrichments have additional environmental interest in the case of productive systems. They may have a direct bearing on declining yield of the fisheries through different processes, such are the development of harmful phytoplankton blooms, the biological immobilization of nutrients by aquatic microorganisms, or the selective retention of pollutants in the ponds, which could in turn reach the water streams through the drainage waters (3,4,5).

Considerable literature about the composition and dynamic of the sedimentary organic deposits in unaltered subaquatic ecosystems have been published (6,7 and references therein). Most of these works are based in the so-called biomarker approach, i.e. the analysis of both the quantity and composition of the organic solvents soluble biochemicals or lipidic fraction. However, similar studies on the organic deposits from fish farms have been usually limited to the analysis of the total organic carbon and nitrogen contents. So far, only few works have described in some detail the composition of the organic enrichment in sediments from marine farms (8,9).

This paper is part of a wider study on the seasonal evolution of physico-chemical and biological characteristics of fish aquaculture ponds subjected to different production regimes. The data presented here on the nutritional status and the lipid composition as analyzed by gas chromatography-mass spectrometry aim to describe i) the present conditions of the sediments regarding their environmental quality, and ii) the specific sources of the the lipid compounds isolated from the sediments.

MATERIAL AND METHODS

The sediment samples were taken from the San Pedro river (sample A), which brings water to the marine farm "El Toruño" (Puerto de Sta. Maria, Cadiz, SW Spain) belonging to the Ministry of Agriculture and Fishery of Andalucia; from the Sancti Petri channel, an intertidal sea arm used for shellfish cultures (sample B), and from two different aquaculture ponds located inside the farm (samples C and D). Replicate samples were taken at the sediment surface (0-5 cm layer), immediately frozen to prevent microbial growth, freeze-dried and homogenized to 2 mm.

Total organic matter (TOM), as well as nutrient and heavy metal contents were determined by standard procedures (10).

Finely ground and lyophylized sediment samples (100 g) were Soxhlet extracted with dichloromethane:methanol (2:1) for 72 h. The total lipids extracts were then saponified with 25 ml methanol (10% KOH) and 25 ml toluene and fractionated into neutral and acidic fractions. The acidic fractions were subsequently derivatized with ethereal diazomethane and N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA). The neutral fractions were only silylated with BSTFA. Both fractions were analyzed by direct injection (splitless mode) into a gas

chromatography-mass spectrometry system (GC-MS) (Hewlett Packard 5988A). For the separation of compounds a 25 m (0.32 mm i.d.) SE-52 fused silica capillary column was used. The injector temperature was set at 280 °C and the oven temperature was programmed from 50 °C (1 min initial hold) to 100 °C at a rate of 30 °C/min, and then from 100 °C to 280 °C at 6 °C/min, with 15 min final hold. Helium at a flow rate of 1.5 ml/min was used as carrier gas. Typical mass spectrometer operating conditions were: ion source temperature, 200 °C; electron voltage, 70 eV; mass range, 50-500 amu in 1.2 second/cycles. Identification of individual components was achieved using gas capillary chromatography (on the basis of retention times and coinjection with standards), mass fragmentography (by key single ion monitoring (SIM) for some homologous series: ions at m/e 57 for *n*-alkanes, and m/e 74 for *n*-fatty acid methyl esters), low resolution mass spectrometry and matching with computer stored library spectra (Wiley, NBS).

RESULTS AND DISCUSSION

Table 1 shows the composition of biogenic elements in the samples. Only small variations were found as regard to the nutritional status of the sediments and the heavy metal concentrations, which in all cases were well below any level critical for the development of phytoplankton blooms and the well being of the fish to be cultivated.

Table 1.- Average values for macronutrients, oligoelements and heavy metals contents in the sediments samples ($g\ Kg^{-1}$ dry ash-free sediment)

	A	B	C	D
N	0.005	0.015	0.008	0.009
P	0.004	0.010	0.004	0.009
K	0.130	1.230	4.220	1.313
Mg	0.008	0.706	0.018	0.728
Ca	0.790	0.143	0.818	0.119
Na	0.126	0.182	0.119	0.195
Cu	24	43	36	46
Fe	17000	37000	29000	49000
Mn	277	520	495	1298
Zn	60	78	83	88
Cr	34	88	63	116
Ni	23	49	42	68

The significative variations observed in some values (K, Mg, Fe) reflect most probably the fertilization history of the systems where samples B, C and D were taken. These histories should be carefully considered in fish culture experiments to avoid a well recognized source of error (11).

Table 2 shows the total organic matter (TOM) contents and the concentration of the lipid extracts in the sediments studied. The high organic matter content in the intertidal sediment (B) is due to an accidental recent introduction of sewage (pers. comm.). The low TOM values in the other samples can be explained by the high oxic conditions existing (low water depth, high temperatures) which lead to a poor preservation of the organic matter (12). Although the TOM content in the two aquaculture ponds (C and D) are rather similar, they show striking differences in their lipid concentrations. The pond C also shows a fairly unusual distribution of the neutral and acidic subfractions, for which a convincing explanation is still lacking. The quantitative differences among the lipid extracts are not reflected in their molecular composition since in all cases the distribution of compounds in the neutral and acidic subfractions were rather similar to each other.

Table 2.- Total organic matter (TOM) and lipid contents of the sediment samples.

Sample	TOM(*)	Lipid extracts(**)	Partition of lipids(%)	
			neutrals	acids
A	0.79	6.37	82	18
B	2.78	11.70	52	46
C	1.34	2.40	20	80
D	1.60	11.96	51	48

(*) Total dried sediment=100% w/w
(**) TOM=100% w/w

The chemical composition of the various extracts is summarized in table 3. The neutral fractions are dominated by a series of n-alkanes in the range C_{19}-C_{31}. Maxima around C_{27} typical for waxes from higher plants were found in all samples. Unexpectedly the homolog C_{17} typical from aquatic algal sources was detected only in trace amounts.

Table 3.- Composition of the sediment lipid extract.

	A	B	C	D
n-Alkanes	C_{19}-C_{31}	C_{22}-C_{31}	C_{15}-C_{31}	C_{18}-C_{31}
n-Alcohols	C_{16}-C_{22}	C_{22}-C_{28}	C_{16}-C_{20}	C_{16}-C_{30}
n-Fatty acids	C_{14}-C_{26}	C_{14}-C_{28}	C_{14}-C_{28}	C_{14}-C_{30}
Unsaturated fatty acids	C_{16},C_{17},C_{18}	C_{16},C_{17},C_{18}	C_{16},C_{18}	C_{16},C_{18}
Branched fatty acids	C_{15},C_{17}	C_{15},C_{17}	C_{15}	C_{16}
ω-Hydroxyacids	C_{17}	C_{19}-C_{23}	C_{21}-C_{24}	C_{16}-C_{26}
Sterols (*)	C,Cn,B,Cp, S,ßS,ßSn	C,Cn,B,ßS	C	C,Cn,B,S, ßS,ßSn

(*) C: Cholest-5-en-3ß-ol (*Cholesterol*)
Cn: 5α(H)-Cholestan-3ß-ol (*Cholestanol*)
B: 24-Methylcholesta-5,22-dien-3ß-ol (*Brassicasterol*)
Cp: 24-Methylcholest-5-en-3ß-ol (*Campesterol*)
S: 24-Ethylcholesta-5,22-dien-3ß-ol (*Stigmasterol*)
ßS: 24-Ethylcholest-5-en-3ß-ol (*ß-Sitosterol*)
ßSn: 24-Ethyl-5α(H)-cholestan-3ß-ol (*ß-Sitostanol*)

The presence of phytol, mainly derived from algal chlorophyll *a*, in varying amounts in all sediments can be correlated with the total amounts of algae in the ponds. The absence of phytanoic acid or the isoprenoid C_{18} ketone, derived diagenetically from phytol, shows that these sediments have not been exposed to highly anoxic or reducing conditions.

The fatty acid distribution in all samples ranged from C_{14} to C_{30} and were dominated by the homologue C_{16}. Although the C_{16} fatty acid cannot be used as a specific biomarker since is found in the cell membranes of almost all organisms, the distribution can be adscribed mainly to an algal input, with lower inputs from terrigenous plants.

Bacterial fatty acids ($C_{16:1}$, $C_{18:1}$, anteiso C_{15}) were present in all the samples, but the polyunsaturated fatty acids typical for algal origin were not found.

ω-Hydroxyacids were detected in the samples in the range C_{17}-C_{26}. The maxima at homologs C_{24} and C_{26} clearly suggest cutin and suberin contributions, from higher plants (13). ß-Hydroxyacids characteristic of a bacterial source were detected in some extracts but only in trace amounts.

The sterol distribution varied very little among the samples. This uniformity reflects the dominance of phytoplankton as the primary source of the sedimentary organic matter. Bacteria generally do not biosynthesize much sterol (14). The detection of cholesterol and C_{29} sterols (24-ethylcholest-5-en-3ß-ol and 24-ethyl-5α(H)-cholestan-3ß-ol) might indicate both phytoplankton and terrestrial plant input, respectively (15).

We failure in detecting series of well-known specific ubiquitous organic pollutants of anthropogenic origin, such as polynuclear aromatic hydrocarbons and polychlorinated biphenyls although specific protocols were follow to detect them in the sediment extracts.

In conclusion the distribution of biogenic/nutritive elements, as well as the total amount and composition of lipids in the studied aquatic sediments reveals their high environmental quality and therefore their suitability for the fish production. It is suggested that variable inputs from terrigenous plant material, phytoplankton and microbial biomass are the main sources of the individual biochemicals identified in the different sediments.

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