SOIL-LIPIDS IN CULTURAL LAYERS

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Abstract

This paper deals with some aspects of the effects of human activities on soil-lipid composition. There are several ways to characterize or classify changes in soils due to the impact of human activities. Until recently, the organic part of the soil has been analysed as a total organic content only. The main purpose of this paper is to draw attention to the possibilities of further analysis, using experience from fields such as organic geochemistry and chromatography. Included are decomposition of organic matter in soil, turnover time in natural strata, effects of human activities such as agriculture, dwelling and food handling, and some examples from Vendel par., Uppland, Sweden.

Introduction

In January 1996 a project called *Svealand in the Vendel and Viking Periods* (SIV) was started (Arrhenius & Herschend 1995), with fundings from the Bank of Sweden Tercentenary Foundation. The subtitle of this project is *Settlement, Society and Power*, reflecting that the study of social rank will be a major aim. As food and eating habits are strong inter- and intra-cultural markers (see for example Douglas 1978) they will be among the topics studied (Lidén 1995:26–27; Isaksson & Hansson 1995:32–33). Of course, traditional archaeological analyses of excavated finds and features will be used, but also scientific methods. My contribution will be the chemical analysis of organic remains, and this paper is a brief presentation of the approach adopted and some preliminary results. A more thourough methodological presentation is published elsewhere (Isaksson 1996).

The organic chemical analyses of charred organic remains found adhering to potsherds, other charred organic remains and organic residues incorporated into the ceramic during manufacture or use, are today quite well-established if not yet routine (Skibo 1992; Heron & Evershed 1993; Hansson & Isaksson 1994; Mills & White 1994). The advantage of these materials are that they are readily connected with culinary practices, more or less protected from contamination and to some extent also from decomposition (Evans 1990:8; Heron & Evershed 1993:253). Soils provid a quite different story.

The organic constituent of soils

The organic part of soils derive from living organisms, such as plants, animals and microorganisms. They are responsible for the specific organic compounds found in soils. These compounds may be unchanged, partly decomposed substances or compounds synthesised during decomposition (Morrison 1969:569).

The rate of decomposition of organic materials in a soil is a function of climate and the chemical composition of the compounds. The processes are most rapid in wellaerated, moist and near-neutral soils, and slowest in cold, humid environments with high water-table and acidic condition. In tropical regions, for example, the supply rate is slower than the decomposition rate. In colder regions, with a mean temperature of less than about 25°C, the production is higher than the decomposition (Holmén 1992:247). On a Global scale, however, estimates indicates that only 0.1% of total primary production is ultimately preserved (Hedges & Prahl 1993:238). Also, the organic matter is not only a component left-over from former life, it also plays an active role in the formation and diagenesis of soils and sediments (Welte 1969:264).

Human influences

It is clear that a variety of human activities may influence the composition of soil-organics, either directly or indirectly. Indirect effects are human alterations of the natural input, mainly from vegetation. Examples are clearing of forest for grazing and slash-and-burn agriculture. Direct effects involves things like manuering and waste-handling. On a dwelling-site it should also be possible to trace food-handling and other activities, such as production of natural products like tars and pitches.

Analyses of archaeological soils

Analyses of soils used today in archaeological science, both for site-localization and investigation purposes, include phosphate analysis, single element analysis (for example iron), acidity, electric and magnetic proporties, trace elements and multi-element analyses. Usually the organic part of soils is only treated as a bulk-material, analysed by loss on ignition for total organic content. But as stated above a lot of information may lie hidden in the organic part. To establish a firm correlation between an organic compound found in the soil and its history, knowledge of structure is needed (Eglinton 1969:20; Summons 1993:4; Hegdes & Prahl 1993:238). This is due to the fact that both general and specific features operate simultaniously in the biosynthetic pathways of living organisms, producing organic compounds with information individually encoded into the structure (Summons 1993:4). This calls for an organic chemistry approach, a qualitative search for compounds signified by stability and uniqueness, so called "biomarkers" (Eglinton 1969). Only a few papers has been published utilizing this approach on archaeological soil-samples (for example Bethell et al 1994; Nolin et al 1994). In comparison with measurements of bulk chemical properties, such as elemental composition, biomarkers afford several advantages for source analysis. These advantages include finer resolution of source contribution, additional environmental and diagenetic information and, finally, source distinctions based on molecular-level analyses often afford order-of-magnitude greater sensitivity (Hedges & Prahl 1993:238).

Lipids as "biomarkers"

For a substance to have a biogeochemical significance in the sence of a "biomarker" certain requirements must be fulfilled, apart from stability and uniqueness. The significance of a particular substance is influenced by per capita production, abundance of producers, rate of decomposition, nature of degradation products and mobility of both original and transfered states. How do lipids cope with these requirements?

Lipids are produced by virtually all living organisms, serving as energy storage, in membranes and as protective coating. Several lipid classes show uniqueness derived from different biosynthetic pathways of living organisms. For example, the hydrocarbon squalene is a universal precursor molecule but can be transformed into a great variety of product molecules, depending on the class of organism producing it (Summons 1993:4). The covalent carbon-carbon bonds in lipids are stable and often persist through accumulation and diagenesis of sediments (Eglinton 1969:21). Several degeneration products still carry significant biological signals (Bethell et al 1994), and mobility is low due to the insolvability of lipids in water (Heron et al 1991). Lipids are, it seems, well suited to serve as "biomarkers".

Post-depositional effects on lipids

The decompositional processes should neither be over- nor underestimated. Nothing but well-founded knowledge is needed (Hedges & Prahl 1993:238). Processes observed up till today includes hydrolysis, hydration, autoxidation, decarboxylation, β-oxidation and microbal attacks (Eglinton 1969:21; Heron & Evershed 1993:251–255; Mills & White 1994:34–35). In the end all organic matter in soils looses their biological signals and returns to the global carbon cycle. But this is in many parts of the world a lengthy process, even on a geological time-scale. It has also been shown, both in laboratory simulations and natural settings, that concentrations of individual molecule types almost never drop to zero during diagenesis (Hedges & Prahl 1993:239). The compounds may be changed and broken, but still carry a detectable signal in hard covalent carbon-carbon structures.

Drawing on experiences from organic geochemistry, the lipid compounds listed in table 1 may be of interest. The property of retaining biological signals in structure through diagenesis, may be exemplified by cholesterol and its decomposition products 5α-cholestane and coprostane (5β-cholestane), which have been found in ancient sediments (Eglinton 1969:34–35), and coprostanol (5β-cholestan-3β-ol) and 5α-cholestan-3β-ol, which have been used to interpret the function of archaeological pit features (Bethell et al 1994).

The chemical decomposition, in comparison to microbal, is quite systematical. The early diagenesis of organic matter in soils may, however, be taken to mainly represent decomposition by microbal metabolism (Hedges & Prahl 1993:238). Eventhough typically selective in their initial and intermediate stages (Hedges & Prahl 1993:238–239), these microbiological activities are problematic (Morrison 1969:570), and may be unique to each site. The thourough investigation of the soils microbiological fauna and flora, and especially its contribution to lipid decomposition should be part of any serious soil-lipid investigation.

POTENTIAL BIOMARKER LIPID CLASSES	
Lipid class	Ocurrance
n-alcanes	higher plants
branched & cyclic componds, f. ex. isoprenoids	plants & animals
triterpanes/triterpenoids	plants
steranes/steroids	plants & animals
waxes	mainly plants

Table 1. Potential biomarker lipid classes and their main natural occurance.

Decomposition experiments

Based on the questions at issue in the SIV-project, foodstuffs where selected for decomposition experiments. Soils collected from the site being excavated were used. Thereby the reference material is exposed to a relevant microbiological flora and fauna. These experiments are well under way but not yet concluded.

Sampling strategies

A correct sampling technique is the most important exersice of any scientific enterprise. This comes from the simple but true analytical rule; Garbage in, Garbage out. Errouneous results and disputes between archaeologists and scientists, when applying scientific methods in archaeology, often seem to be the result of deficient communication. At the Archaeological Research Laboratory, Stockholm University, this problem is solved by demanding and providing both scientific and archaeological training for most personnel and all students (Arrhenius 1996).

At the excavation at Vendel, Vendel par., Uppland, Sweden (Isaksson & Arrhenius 1995; 1996), soil-samples are taken at every squaremeter of the cultural layer. This will provide a rough picture of differences in the composition of soil-organic material through-out the site, helping in the archaeological interpretations.

Finds and features are registered and digitalized from day to day. For this purpose we use a totalstation, a laptop computer and the DBase IV and Microstation Field Software packages. This enables quick references to be made to up-to-date chorological relations of finds and features, for example indications of culinary activities. In these areas the soil-sampling is intensified.

The samples need to be taken as soon as possible after being exposed to air and sunlight. To avoid contamination from tools, shoes and hands it is crucial that everyone working at the site is fully aware of the risks. In wait of analysis the samples are stored dark, air-tight and frozen.

Way of analysis

The sampling strategy described above is producing an enormous amount of samples, and a quick and efficient screening-method is needed. A total lipid extract is attained by ultrasonification (2x15 minutes) of the samples in chloroform and methanol (2:1). The produced extracts are screened on high-preformance thin layer chromatography plates using one or two solvent systems in one dimension. This enables classeparation of complex lipid mixtures and multi-sample resolution in a single run. Separated lipids are charred, using an oxidizing agent, appearing as dark deposits. The HPTLC-plates are then scanned and digitalized. This serves to document the results and enables futher analysis, using the IMIX image-processing software. To spot low amount lipid classes pseudocoloration of the computer image is significant. This is due to the limitation of the human eye to separate between gray levels. By transforming the grayscale to a pseudocolor-scale it is possible to bring out week stains undetectable in grayscale. It is also possible to attain a line-profile presentation, for example to present the results as a chromatogram with the relative intensity of separated compounds as a function of the retention factor (Rf). Samples deemed interesting, by comparison to the results of the decomposition experiments, are picked out for analysis by gas chromatography/mass spectrometry (GC/MS). The GC/MS provides structural data of separated compounds, thus enables the positive identification of biomarkers.

Some preliminary work and results.

A bulksample of the cultural layer at Vendel has been analysed for grain size, total organic content, both per grain-size class and totally, and extractable lipids per grain-size class. The total organic content was determined to 7.4±0.3% (n=4) by ignition at 750°C for one hour.

The grain-size investigation was done by water-sieving in the range <4.0 mm to >0.071 mm. Two samples of 100 g were analysed. The results are presented in table 2 and figure 1. The total organic content shows a quite even increase with decreasing

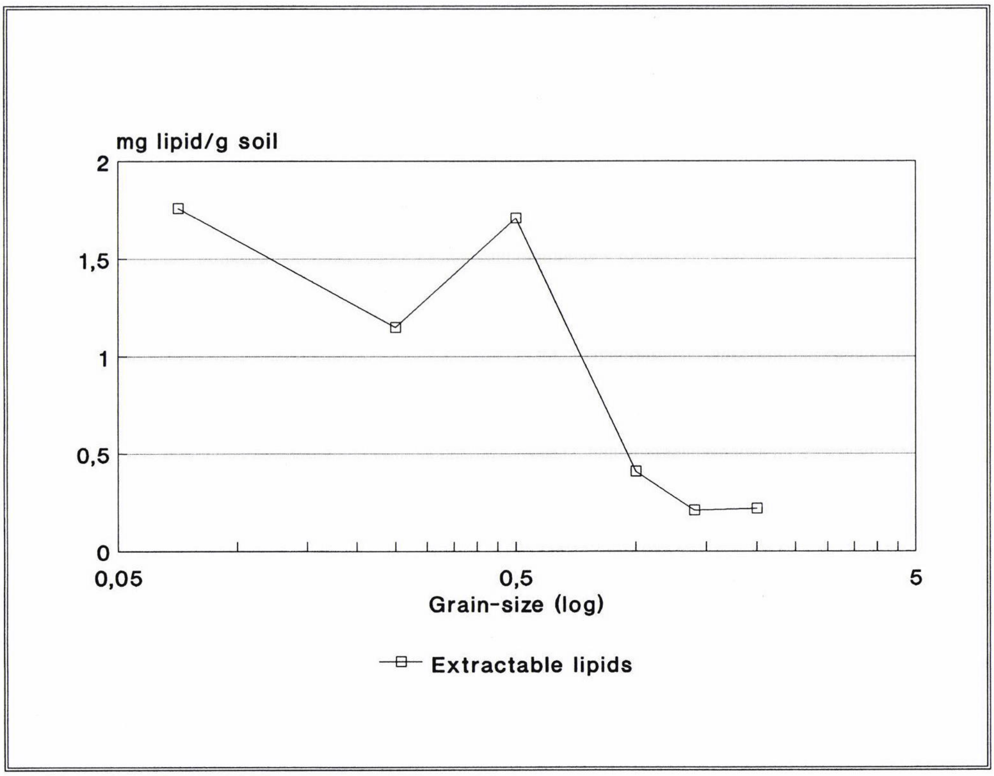

Figure 1. The distribution of grain-sizes and total organic content of the different fractions of the cultural layer of Vendel.

Grain-size fraction (mm)	Weight distribution (%)	Total organic content (%)	Exractable lipids (mg/g)
2.8	3.6	2.3	–
2.0	6.4	4.2	0.22
1.4	7.4	3.3	0.21
1.0	8.4	3.4	0.41
0.50	22.6	3.9	1.71
0.25	23.6	5.3	1.15
0.071	20.0	8.2	1.76
<0.071	8.0	–	–

Table 2. Results from the grain-size analysis. The figure for grain-size fraction <0.071 was received by normalizing the sum of the weight distribution to 100%.

grain-size. About 1.5g of each fraction was used for lipid extraction, as described above. The amount of extractable lipids shows a more uneven distribution (figure 2), with a sudden increase from grain-size class 0.5 mm.

The lipid extracts were anlysed for neutral lipid classes by HPTLC (Silica gel 60, Merck 5631) according to Henderson & Tocher (1992), using a hexane:diethyl ether:acetic acid (80:20:2, by volume) solventsystem. The number of separated lipid classes was more numerous and of distinctively higher relative amounts in the 0.25 and 0.071 mm fractions (figure 3a–c).

These results reflect the larger adsorption area per weight in the finer fractions. Adsorption to mineral surfaces has been suggested as a protective matrix for organic compounds in soils (Hedges & Prahl 1993:239; Heron & Evershed 1993:253). It is

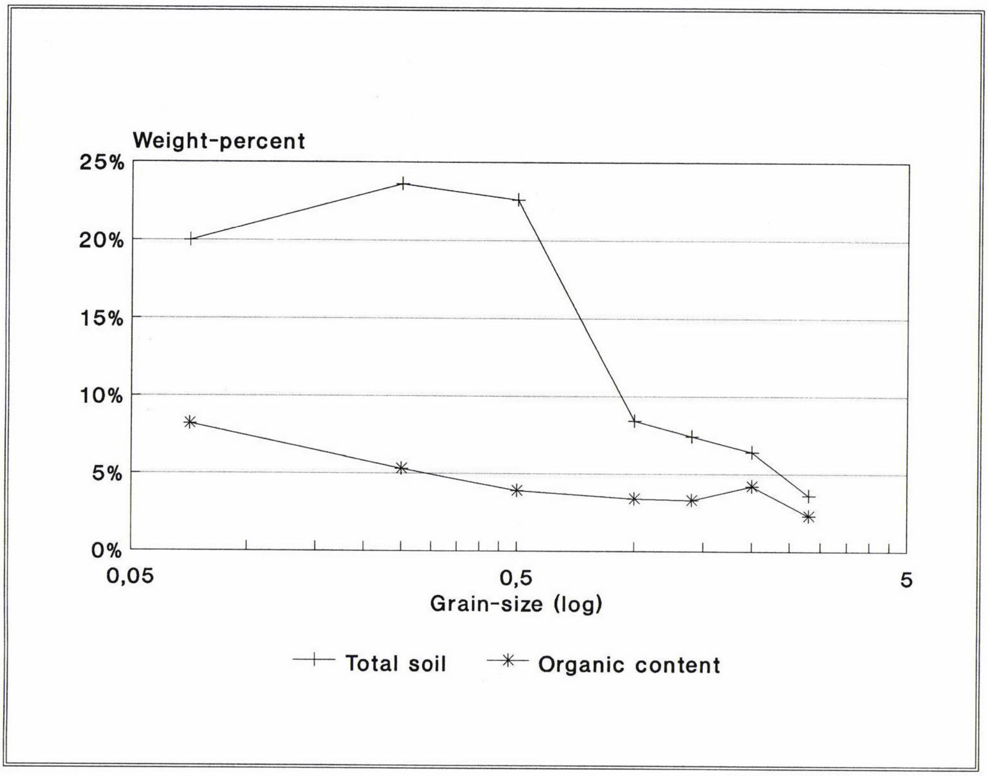

Figure 2. The distribution of extractable lipids of the grain-size fractions.

though unclear if ordinary extraction methods are sufficient enough to release compounds deposited in that way (Hedges & Prahl 1993:243). However, from this it might be suggested that sieving should be included in the soil-lipid analysis protocol, only using soil fractions below 0.5 mm. But more samples have to be investigated and the extracted lipids characterized by GC/MS to thoroughly investigate any compositional differences between the different fractions.

Recent top-soils are being collected to build a reference library of soil-lipid compositions. The samples are collected from areas with differing land-use and differing vegetation. About 5 g soil was accurately weighed and used for analysis. Neutral lipid classeparations show promising differences in composition of extracted lipids, reflecting the variation in organic material input.

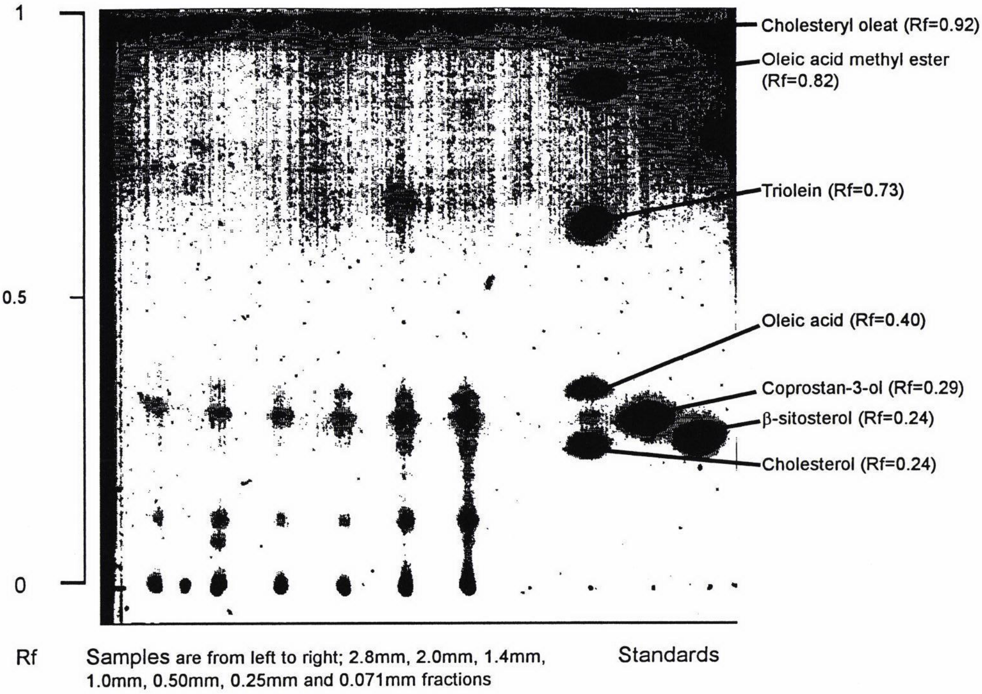

Figure 3a. Filtrated computer-image of the HPTLC-plate. Several of the weaker spots were not visible prior to filtration. To the left are the separated lipids from the different fractions. To the right are the separated standard solutions (Sigma).

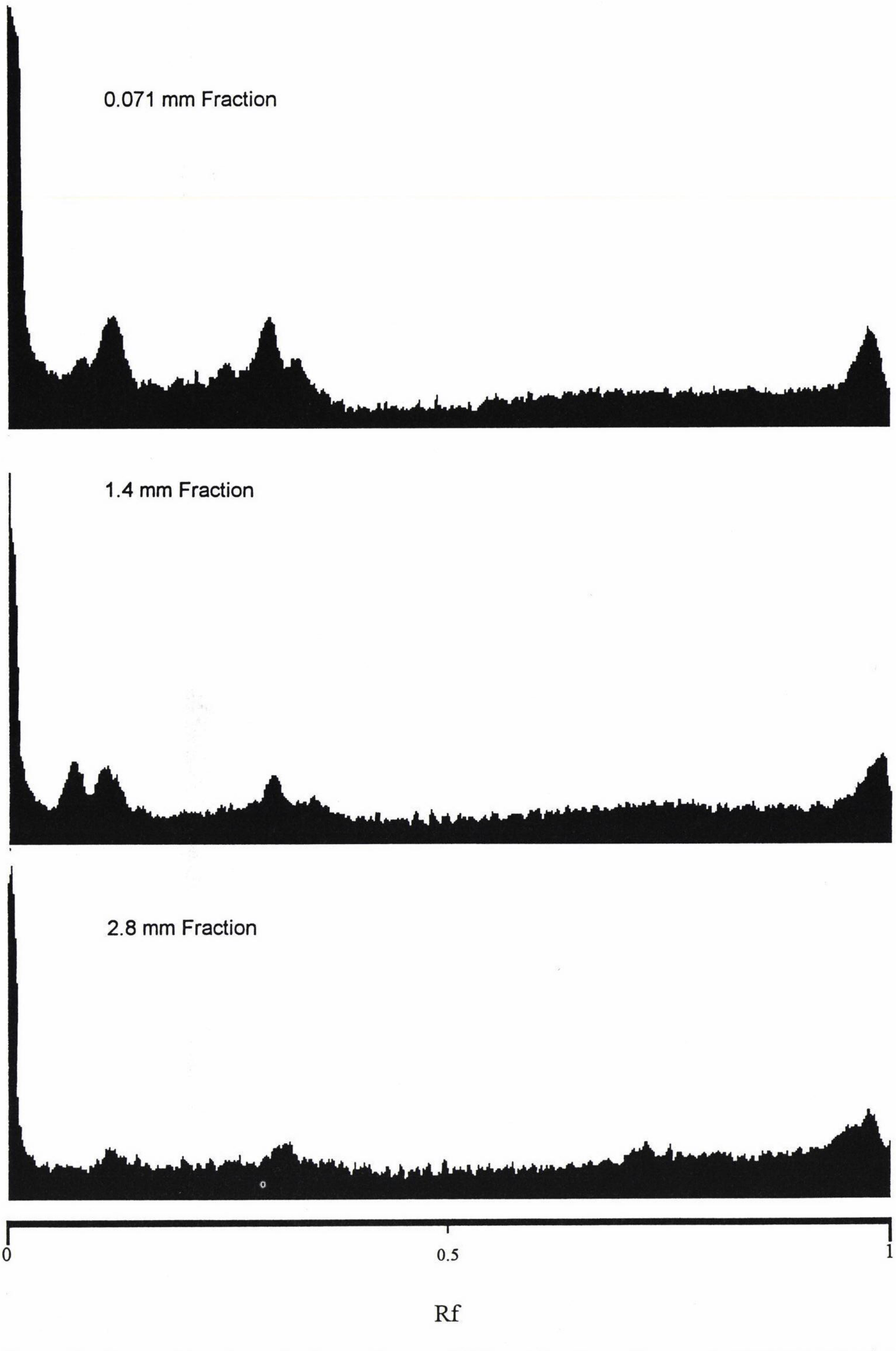

Figure 3b. Line-profiles along eluation pathways of different fractions. The x-axis represents retention factor (Rf) and the y-axis is relative intensity. It is clear that the number of peaks (separated lipid classes) increases with decreasing grain-size.

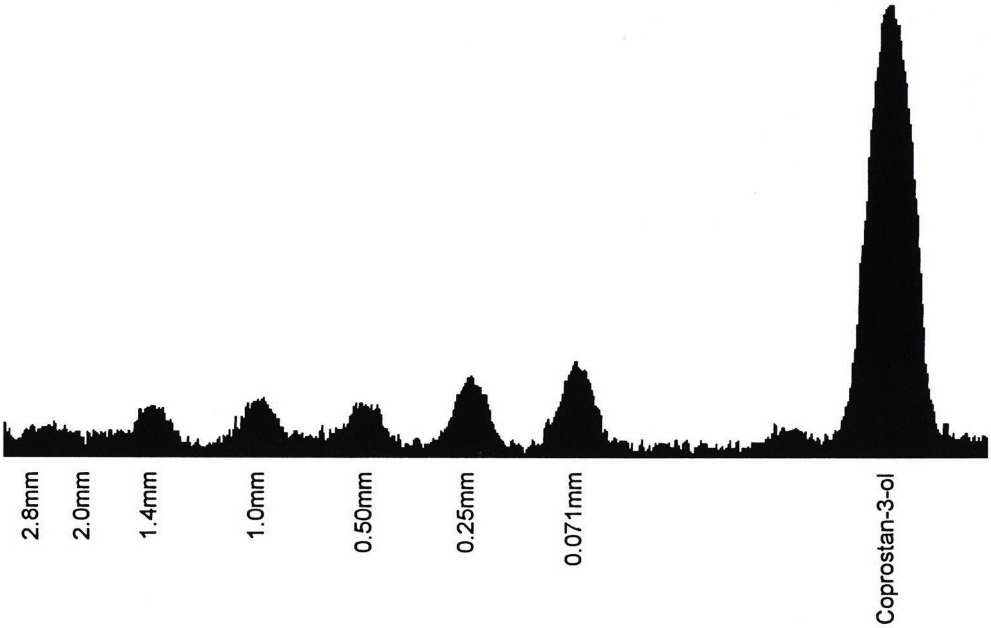

Figure 3c. A line-profile at retention factor 0.29 of the HPTLC-plate presented in figure 3a. The x-axis represents samples and the y-axis the relative intensity of peaks at the same retention factor. Note the higher relative intensity in fraction classes 0.25 and 0.071mm.

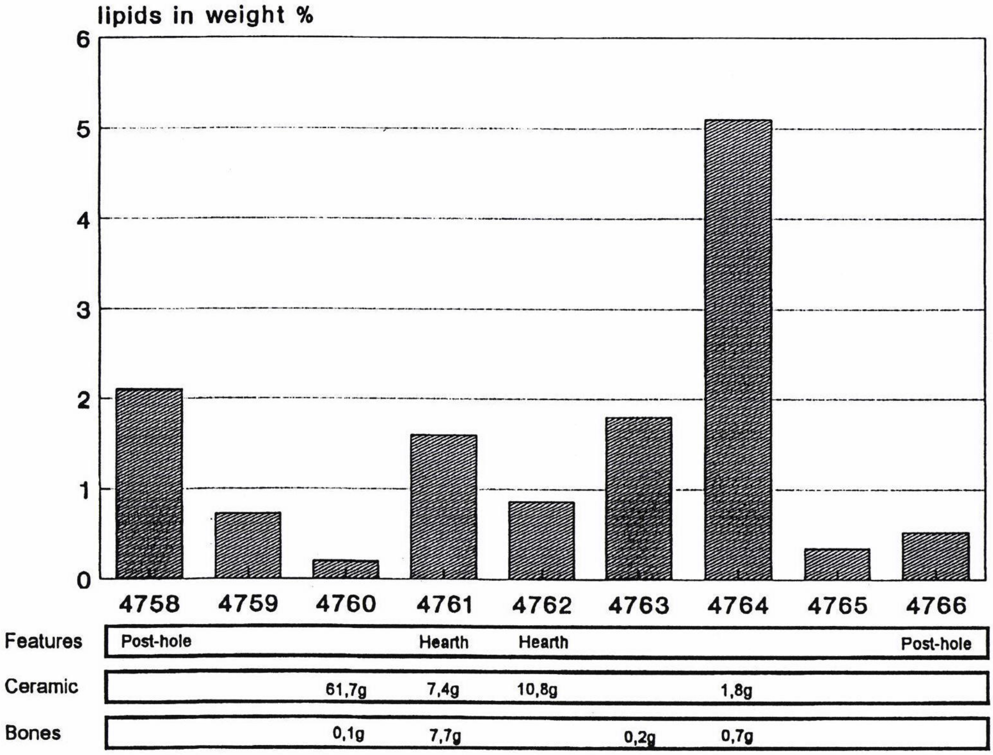

Figure 4. Distribution of extractable lipids along a east-west line at the Vendel-site. The distance between samples is 1m.

Having just started working on the squaremeter-samples of Vendel, a line of samples has been analysed for extractable lipid content and neutral lipid classeparation. About 5 g soil was accuratly weighed and used for analysis. The bar-chart in figure 4 show the variation of lipid content of the different samples. The boxes beneath the bar-chart indicate what kind of features was found in these squares, the amount of ceramic found and the amount of burnt bones. The neutral lipid classeparation shows interesting variations in the lipid composition (figure 5), which need to be futher analysed before any solid statements can be made. The results are, however, very encouraging, an example of which is the variation in lipid classes at a Rf equal to the coprostanol standard (figure 6).

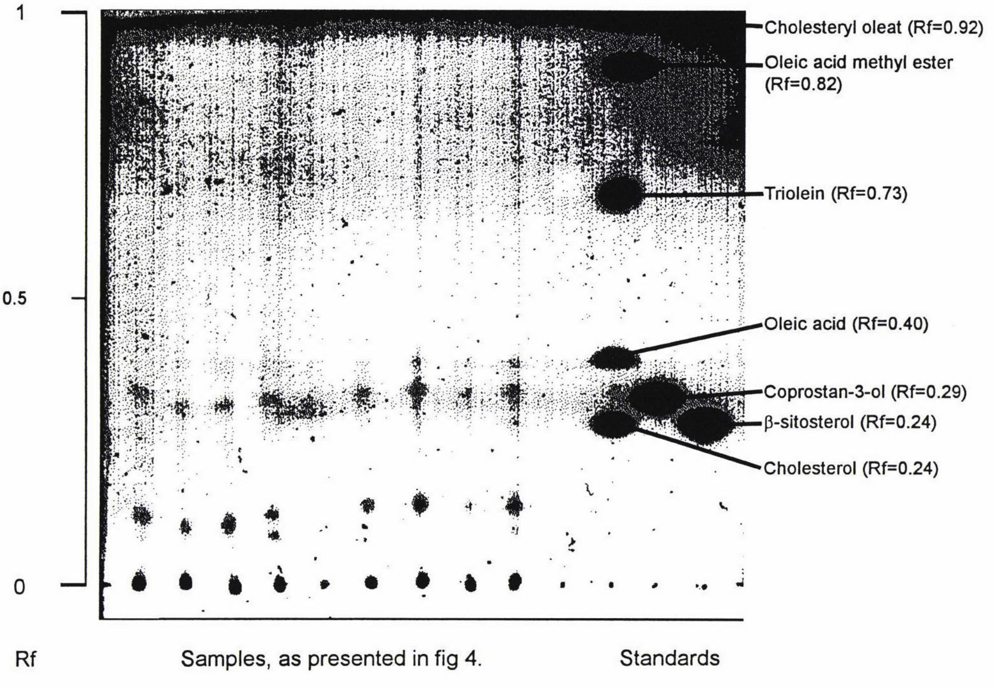

Figure 5. Filtrated computer-image of the HPTLC-plate. To the left are soil-samples, presented in the same order as in fiugure 4 and 6. To the right are standards (Sigma).

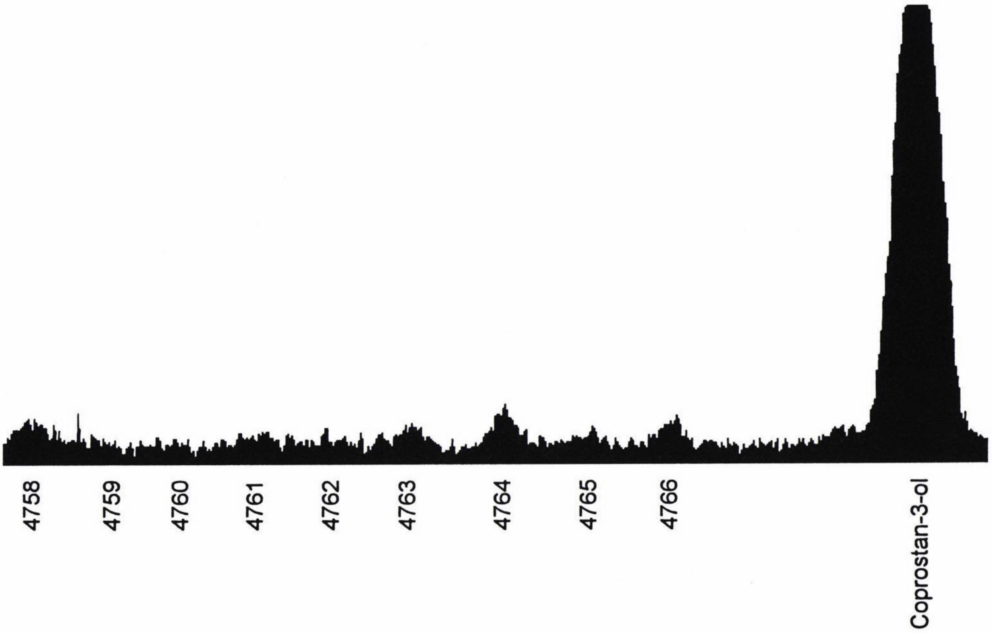

Figure 6. A line-profile at retention factor 0.29 of the HPTLC-plate presented in figure 5.

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