DANMARKS OG GRØNLANDS GEOLOGISKE UNDERSØGELSE RAPPORT 2009/79

Identification of moler (clayey diatomite) in feed additives

Application of scanning electron microscope analysis for the identification of fossil diatoms in feed additives

Stig A. Schack Pedersen & Jørgen Kystol

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Prepared for DAMOLIN A/S

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Introduction

Diatomite is a geological well known material, which occur naturally as a light, whitish deposit in Cenozoic successions (geological strata younger than 65 mill. years). To be characterized as a diatomite, the sediment must contain more than 50% diatoms, which are microscopic opaline algae that lived as plankton in the near surface water level (Fig. 1). In Denmark the most famous diatomite is the “moler”, which is the Danish term for a clayey marine diatomite deposited in the period between the uppermost Paleocene and earliest Eocene about 55 to 53 million years ago. Moler is sometimes translated to English as mo-clay. The term mo is an old word meaning fine and whitish, and the Danish word for clay is ler, so moler is a reasonable description of a typical lithology of diatomite.

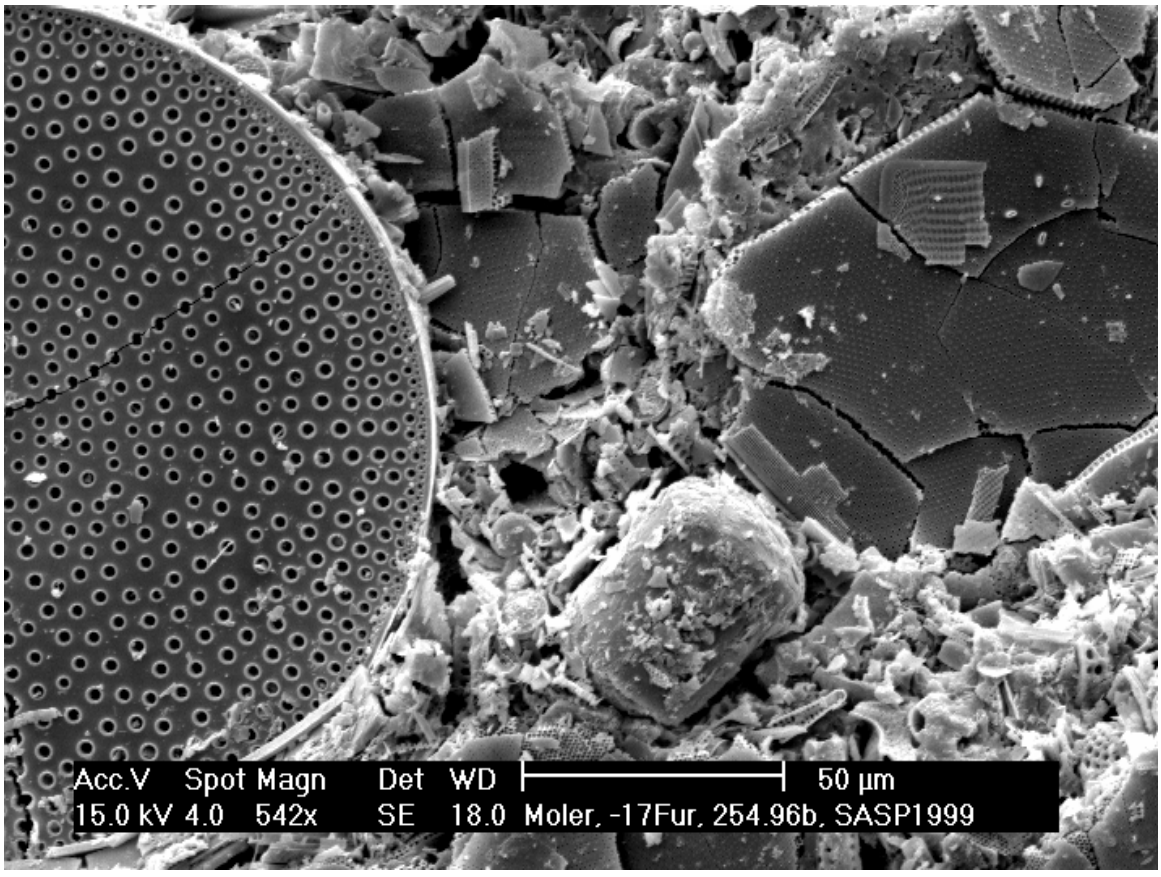


Figure 1. *Scanning electron microscope picture of clayey diatomite. In the left side of the picture the half part of one of the largest diatoms with a diameter of 0.2 mm is seen, and in the right side fragments of a various diatom species are accumulated. Note the radial organisation of pores on the diatom surface. The sample represents Lower Moler-Series (close to ash layer -17) on Fur. This is indicated in the black bar in the lower part of the picture, here as well as in Fig. 2 and 3.*

In the formal stratigraphy moler constitutes the Fur Formation (Pedersen & Surlyk 1983). This formation is c. 60 m thick, of which mainly the middle 30 m are exploited as raw material. The uppermost part of the formation is too densely packed with volcanic ash layers to be exploited, and the lowermost part is buried too deeply to be excavated. The trade name of the raw material is **moler**, and it is a unique occurrence in the western Limfjorden region, with the most famous outcrops on the northern part of Mors and Fur.

The excavation of moler has taken place for more than hundred years. The production is very stabile, which can be demonstrated by the statistics over the last 20 years that shows an average yearly production of 200 000 m^3 moler. The traditional products manufactured have been isolation bricks and absorbing granules. The majority of the absorbing granules production is to day sold for cat litter, although it was originally designed for industrial applications such as absorption of oil spills, inflammable liquids, and grease among others. The new alternative products are micro granules (the Diamol products), which can be used as fillers, binders and absorbers. Moreover the micro granules can be used as carriers and as feed additive. The last mentioned application is the focus of this report.

DAMOLIN A/S has been asked to demonstrate a method that can identify the presence of moler in the feed additive. The suggested method is to identify the diatoms or fragments of the diatoms´ opaline frustules under scanning electron microscope (SEM). This report therefore documents the application of this identification method and demonstrates the use of the SEM instrumentation in connection with an element identification attachment (EDAX).

Diatoms in the moler

More than two thirds of the moler constitutes diatom frustules (Pedersen 1981; Pedersen et al. 2004). In the sediment the frustules only constitutes biogenic opal A. In the X-ray diffractograms the content of opal A in a diatomite samples can be estimated by calculation of the area of the opal bulge (Pedersen et al. 2004), which is only possible for a reasonable amount of opal in the sample. However, the identification of the diatoms by their easily recognizable biogenic structure in the scanning electron microscope is the most secure way of tracing moler in a specimen. In the moler about 100 different species of diatoms have been identified. They can be subdivided into two main groups, namely the small forms (20–40 µm) and the large forms (100–200 µm). The small forms are dominated by the *Stephanopyxis* species (Fig. 2), and the large forms are dominated by the *Coscinodiscus* species (Fig. 3). In the laminated part of the Fur Formation it is often the *Coscinodiscus* sp. that constitutes the distinctive 1–2 mm thick clear white laminae (Fig. 4).

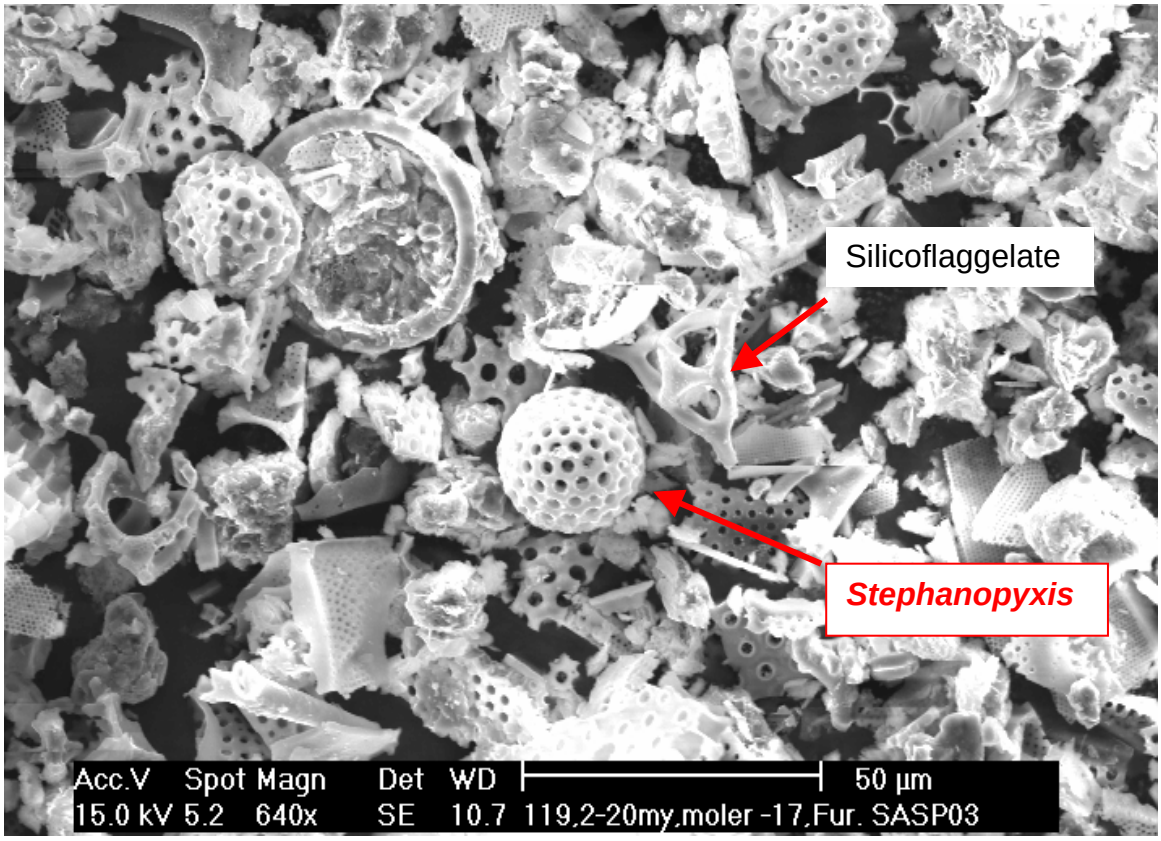


Figure 2. *SEM photomicrograph of diatomite dominated by* ***Stephanopyxis sp.*** *The silicoflaggelates occur abundantly in the diatomite and are useful as bio-stratigraphic markers.*

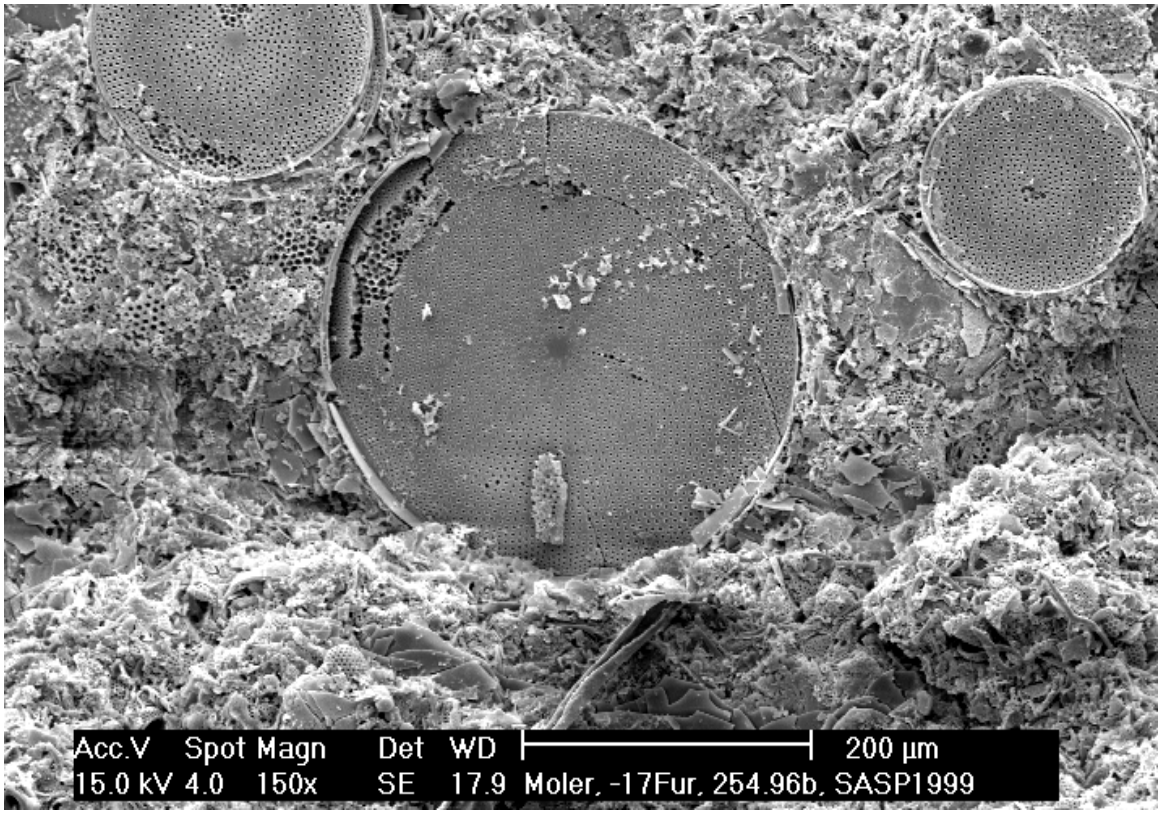


Figure 3. *SEM photomicrograph of well preserved* ***Coscinodiscus*** **sp.** *The radial pattern of the pore holes is very easily recognized. Note the girdle band surrounding the diatoms.*

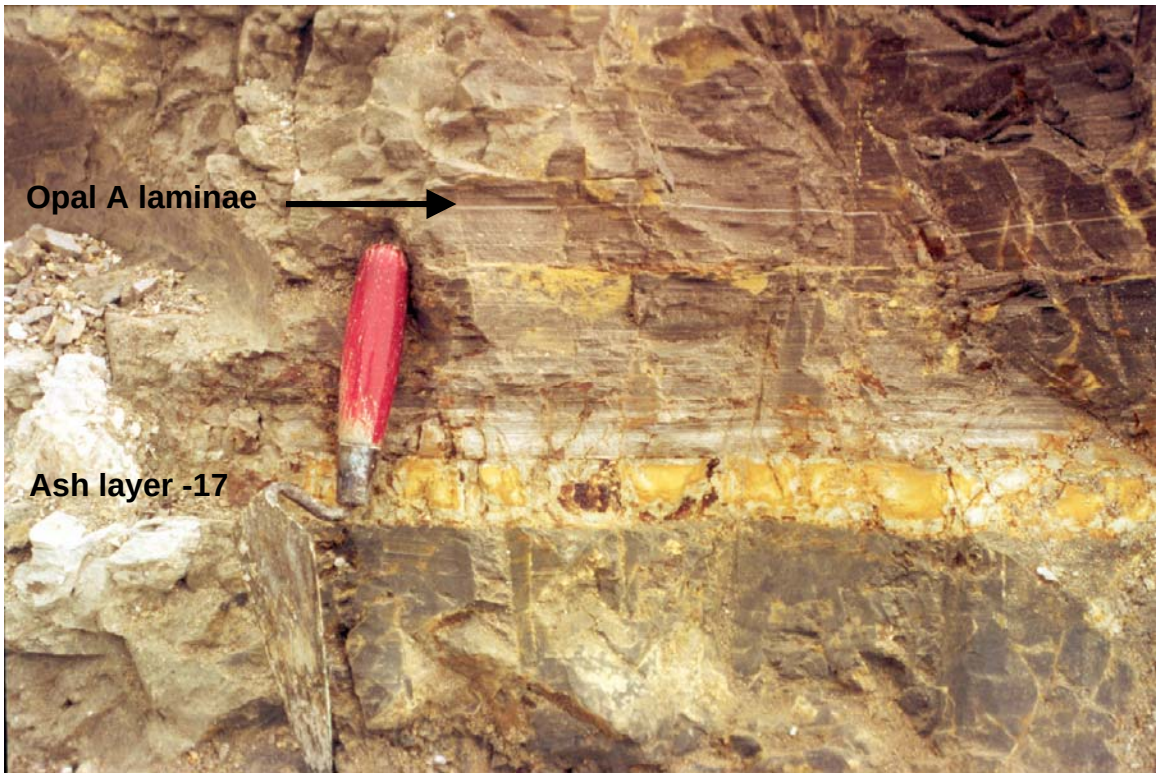


Figure 4. *The moler is characterised by two types of sedimentary structures: 1) the laminated type and 2) the structure-less type. The laminated type is here seen above one of the well known yellow coloured ash layers (-17) in the Fur Formation. Below the ash layer the diatomite is structure-less due to bioturbation.*

Diagnostic features for identification of diatom fragments

The easiest way to identify the presence of diatomite in a sample is of course to recognize the presence of a complete fossil diatom like for example the ball shaped *Stephanophyxis* sp. seen in Fig. 2. However, fragments of the larger diatoms are much more frequent, which are still easily identified due to their organic structure (Fig. 5). The organisation of pores on the fragment surfaces is so distinctive for the diatoms that it can not be mistaken for some thing else. Furthermore the extension of the pores as small tubes is also a diagnostic feature that can be observed in the fragments randomly orientated in a material sample. Other micro-fossils have a completely different organic structure, which of course is the basis for defining various groups of biological species. A large group of micro-fossils will be related to the additive of chalk. Amongst those the coccolites will be very frequently represented. However, they are very easily distinguished by their organic structures and moreover they can be recognised by their chalk skeleton when tested with the EDAX analysis (Fig. 11–13).

For the comparison of the fragments to be expected to appear in the feed sample a test series were carried out on the Diamol micro granules. By this test it is obviously seen that one should only expect to find diatom fragments in the feed additive (Fig. 6–9).

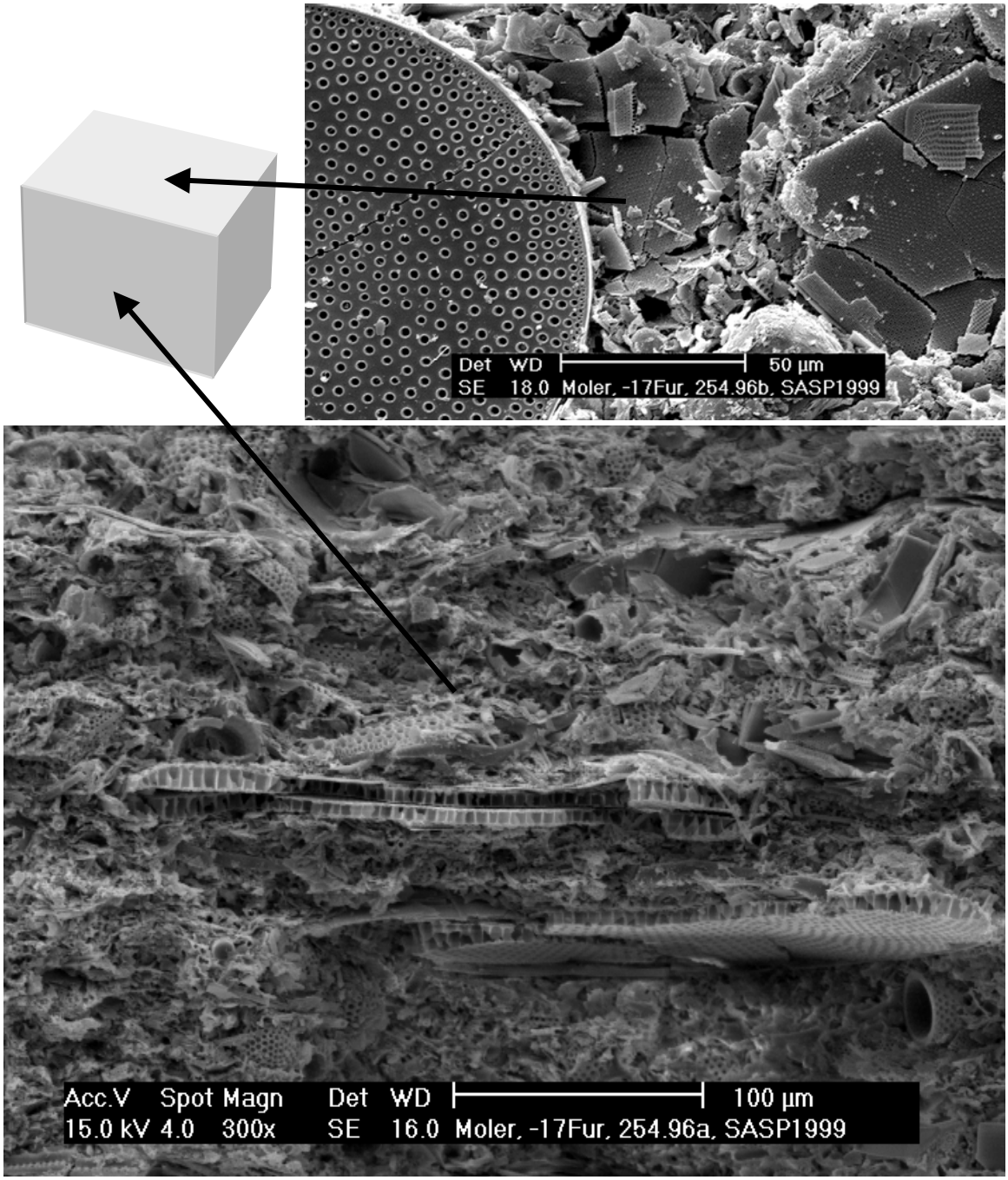


Figure 5. *Planar view and cross-section view of the structures in a laminated diatomite. In the planar view all the circular pores on the surface of the diatom form a structure, which is easily and uniquely identified. The cross-section view shows the double-walled structure of the dish-shaped diatoms and how the pores form small, parallel tubes between the surfaces. The large diatoms are all Coscinodiscus sp.*

The scanning electron microscopy analyses

For the evaluation and verification of the scanning electron microscopy analysis method a number of feed samples were examined in scanning electron microscopy supported with a semi quantitative element analysis (EDAX). A small portion of each sample (1–5 g) was mounted on a “chair” and coated with carbon under vacuum. A few samples were coated with gold for testing the quality of images, but this did not improve the quality of the scanning microscopy. The coated samples are then mounted in the vacuum chamber in the SEM, where they are beamed by the electron source to create the electron images, which are scanned and transmitted to the display on the digital screen. The instrument used is a Philips XL40 electron microscope equipped with two ThermoNoran EDS-detectores. For the semi-quantitative element quantification (EDAX) the instrument is connected to a ThermoFischer-Noran System Six supported by software version 2.1.

The examination of the samples started with the analyses of the raw material product that original is supplied to the feed compound as additive. These products are according to their grain size named Diamol 20G and Diamol 100G. The Diamol 20G is the most coarse grained (Fig. 6), but according to the SEM analysis it is obvious that the small grains about 200 µm in size consist of aggregates of diatom fragments only about 5–20 µm in size (Fig 7 and 8). The much finer product Diamol 100G is only a powder product (Fig. 9 and 10).

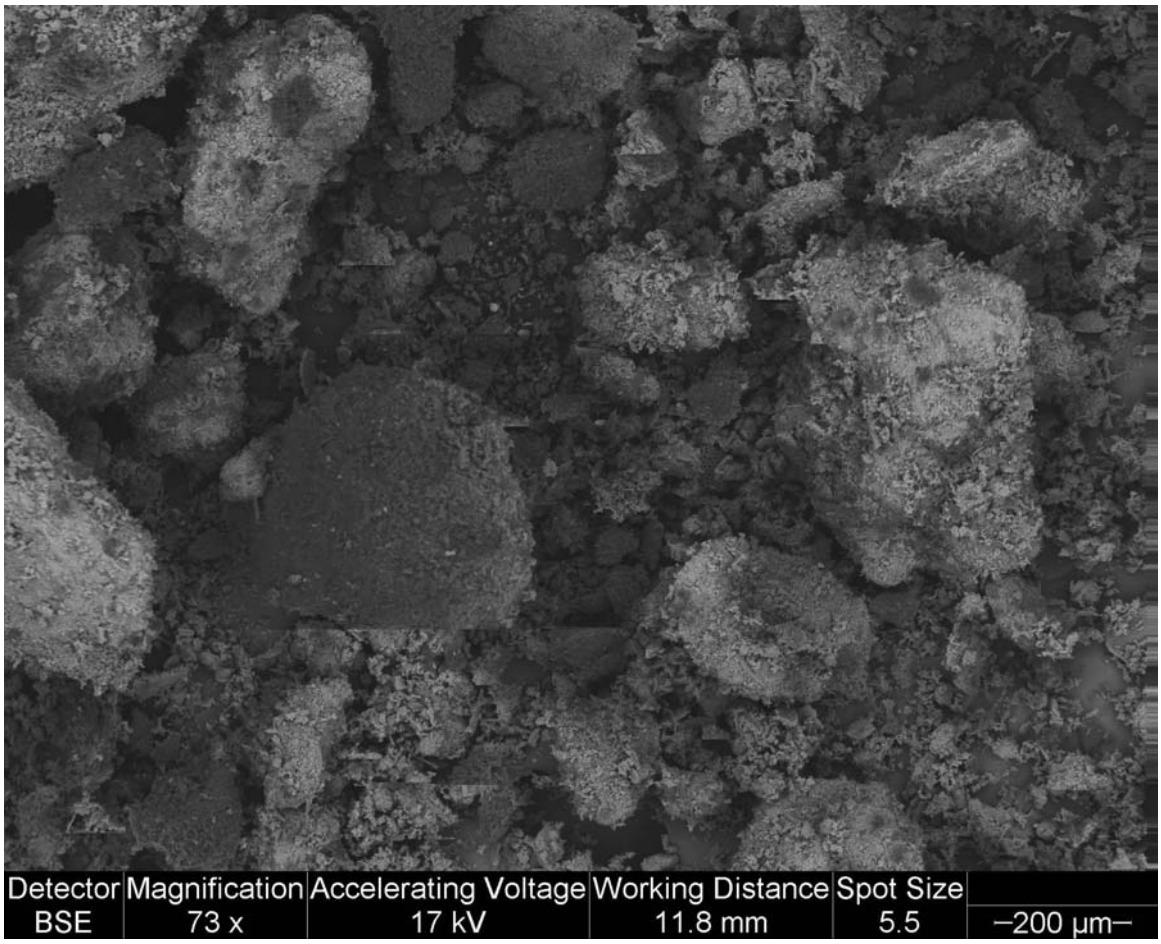


Figure 6. *The typical aggregated structure of the micro granules product Diamol 20G.*

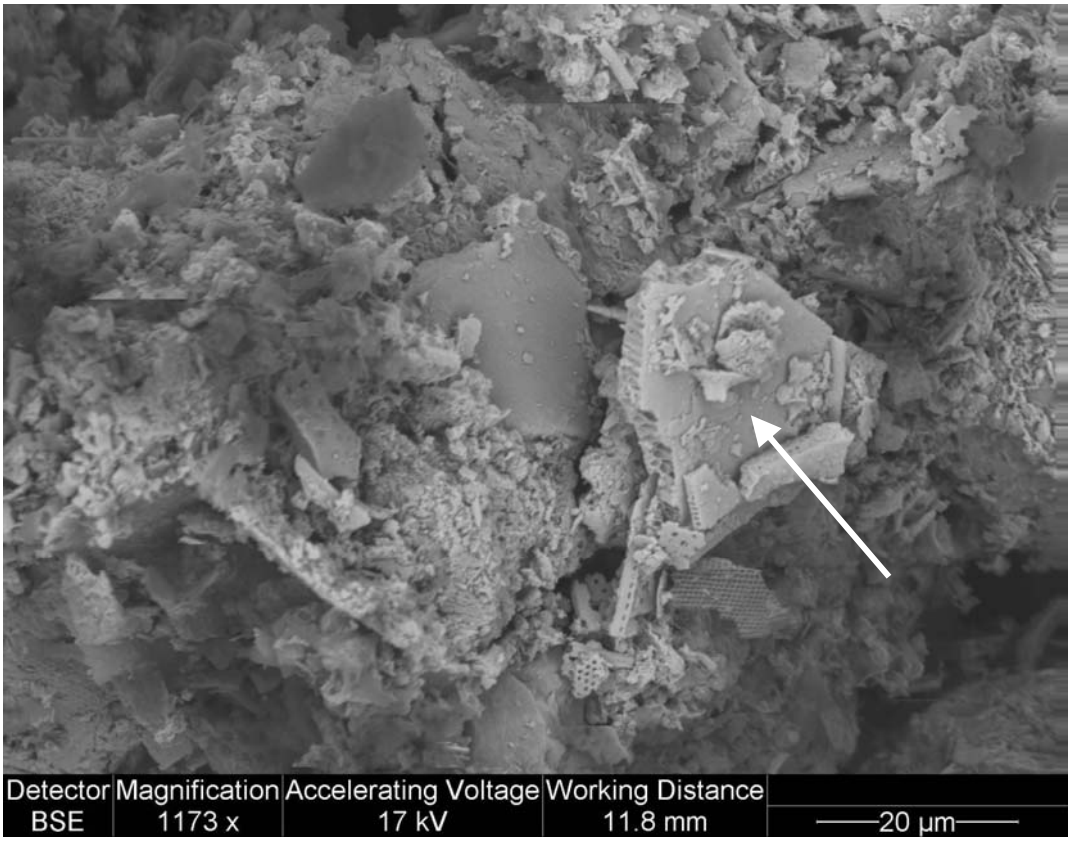


Figure 7. *Fragments of Coscinodiscus sp. (white arrow) in Diamol 20G, which are compacted to form the aggregates shown in Fig. 6. Compare the fragments with the diatom frustules in Fig. 5.*

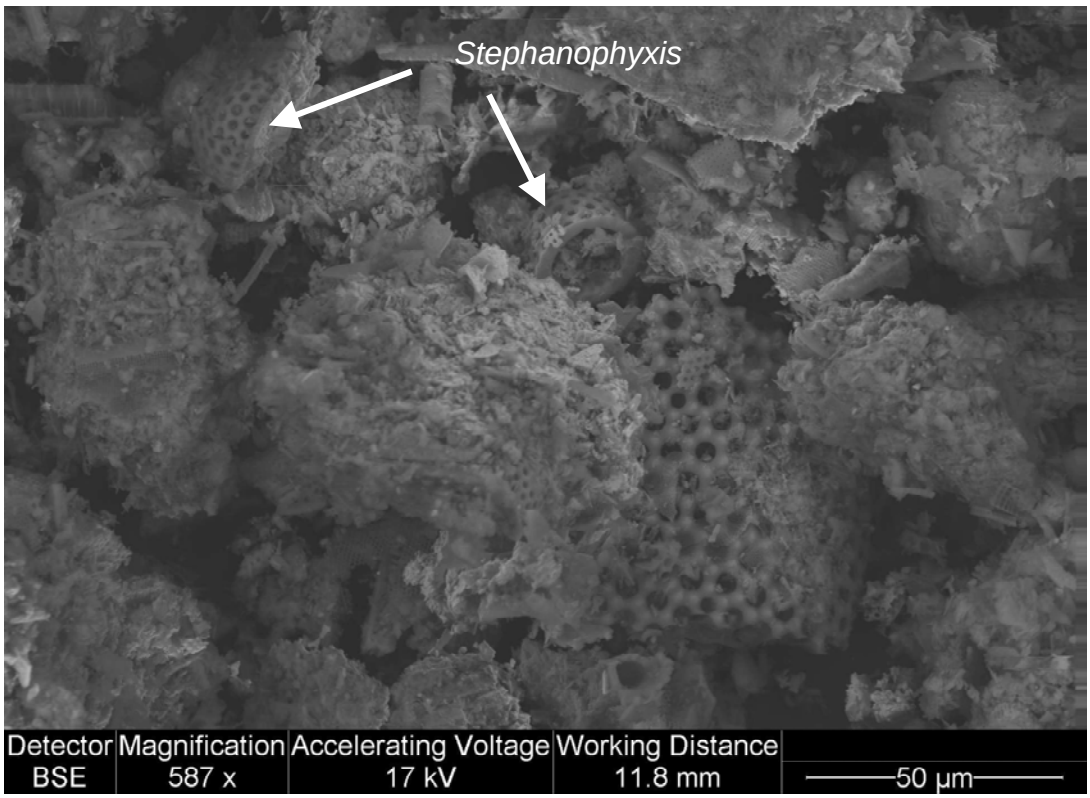


Figure 8. *Aggregates of diatom fragments observed in the Diamol 20G. Two well preserved species of Stephanophyxis are indicated with the white arrows.*

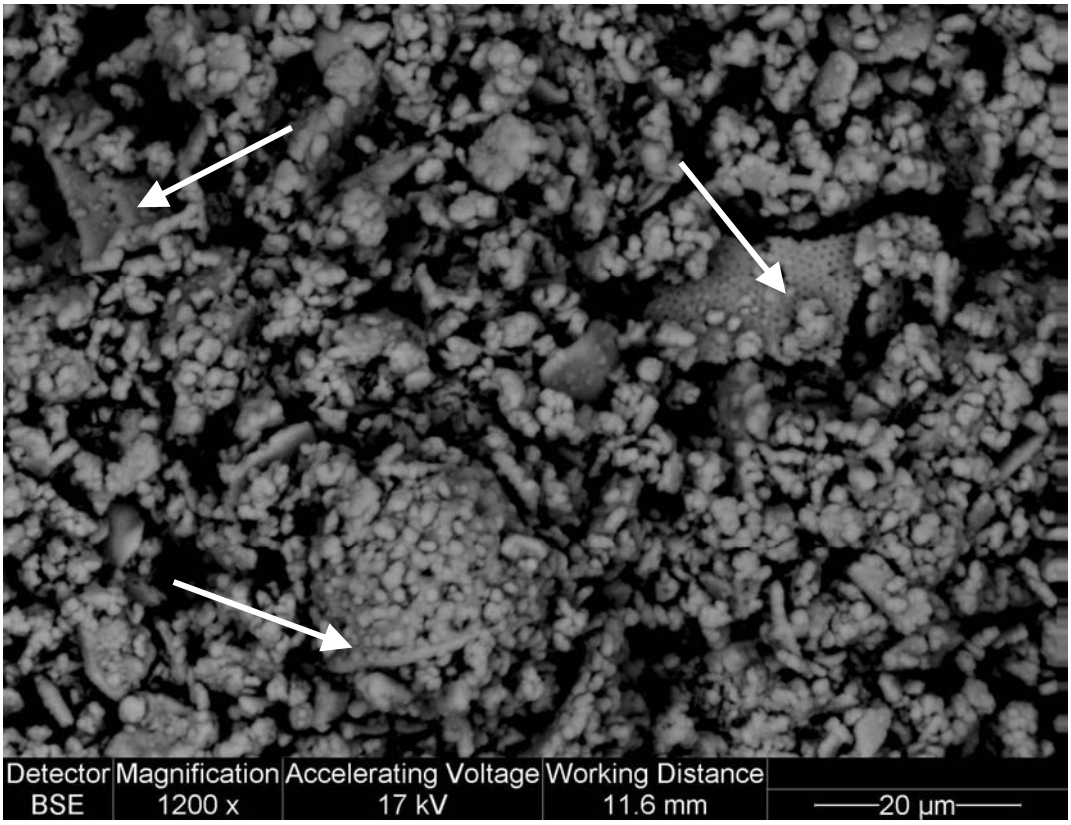


Figure 9. *The diatom fragments identified in this fine grained Diamol 100G are indicated with white arrows. Note the size of fragments is smaller than 10 µm.*

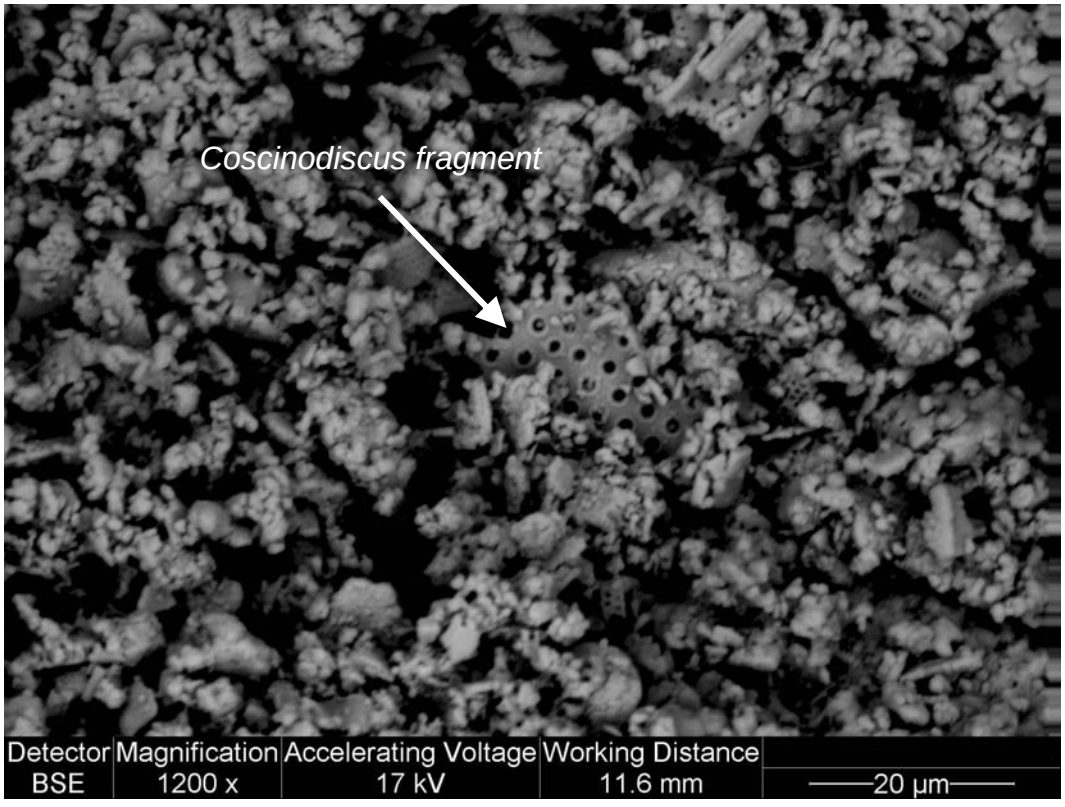


Figure 10. *An easily identified fragment of a Coscinodiscus sp. is here seen in the centre of the SEM image of sample Diamol 100G.*

Identification of diatom fragments in feed samples

The first sample to be tested was a Vilomix feed-mix sample, which contain 3% Diamol additive. Furthermore this sample contains a considerable amount of chalk additive. The diatoms were easily recognised (Fig. 11), and the coccolites indicating the chalk additive appeared frequently. For the demonstration of the element analysis, the EDAX support, both the diatom fragments and the coccolites were tested, which gave very satisfactory results (Fig. 12 and 13). The points analysed on the diatom fragment (pt1 in Fig. 12 and 13) gave 95 % SiO_2 , which is the expected content for opal A. The analysis points pt2, pt4 and pt5 gave 82–95 CaO, which is expected for coccolites and other chalk-skeleton fragments. Pt3 in Fig. 12 and 13 is a framboid pyrite crystal with the composition FeS_2, which is also evident from the semi-quantitative element analysis. Note that the ion-sulphide pyrite is a very good electrical conductor, wherefore the framboid appears with high light indicating a high electrical charge on the surface. The high electrical charge of certain points is a factor that can play a complicated role during the documentation scanning. Sometimes a nice fragment of a diatom is so light that it can easily be brought out of focus due to the “electronic wind” in the electromagnetic field (Fig. 24).

Blind test of feed additive samples

The Vilomix sample was used as a demonstration sample, where the content of moler was known prior to the analysis. Additional ten samples were blind tested for identification of fragments of diatoms. However, it was known that the sample had been added a certain amount of Diamol. Due to theoretical considerations feed sample without Diamol supply was not analysed. In the discussion of the method the theoretical aspects of this will be further commented on.

The blind test samples were only identified by their numbers, and the analyses were concentrated on documentation of the presence of diatom fragments.

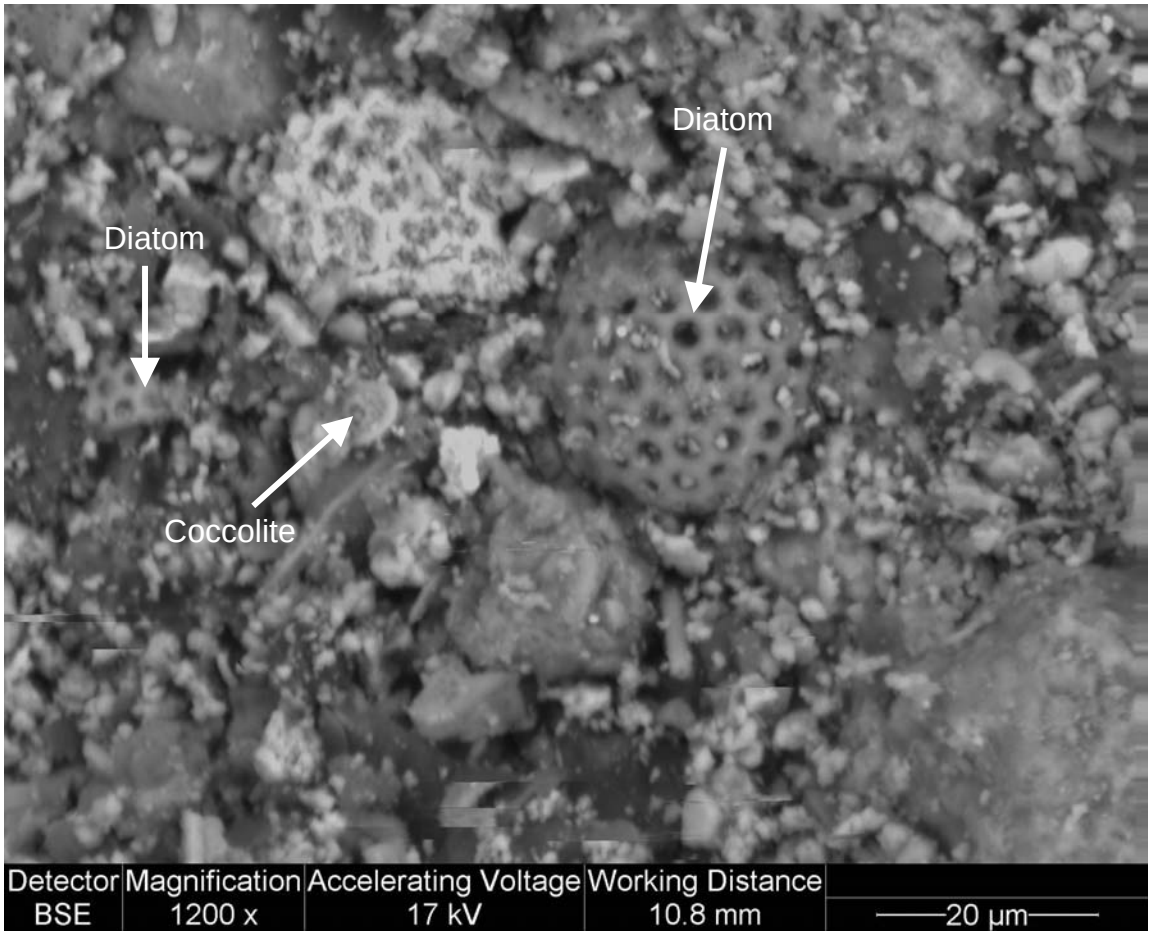


Figure 11. *The scanning electron microscopy image of the Vilomix sample. In Fig. 12 the points tested by the EDAX element analysis are marked, and in Fig. 13 the corresponding element analyses are shown.*

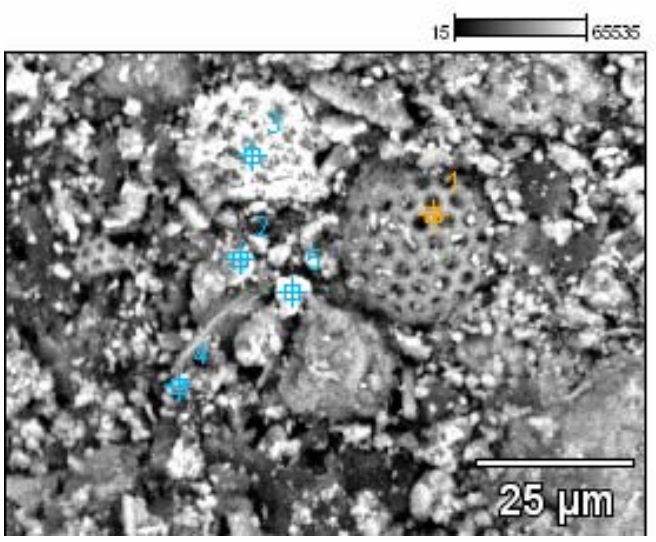
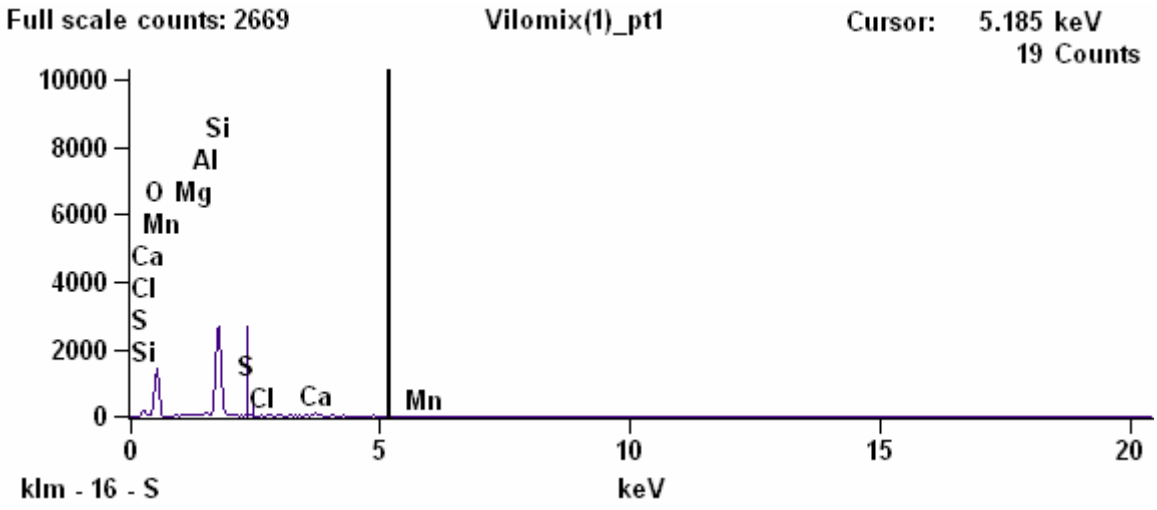


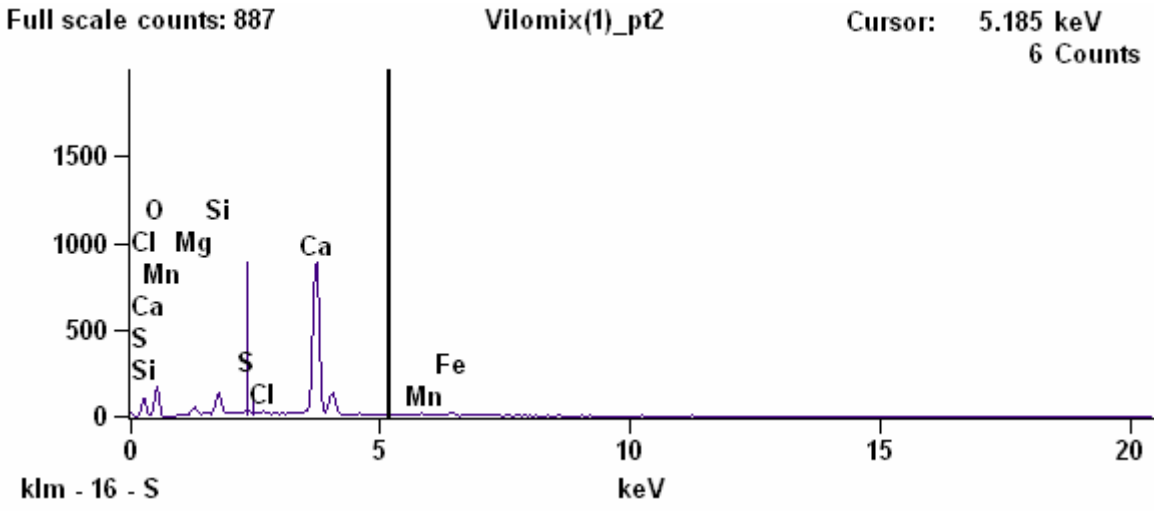
Image Name: Vilomix(1)

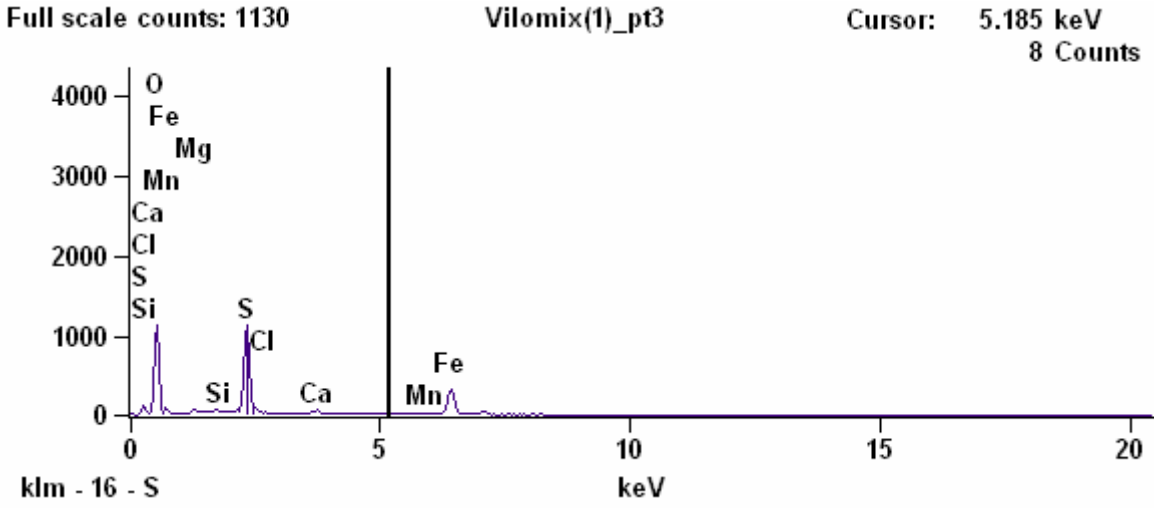
Accelerating Voltage: 17.0 kV

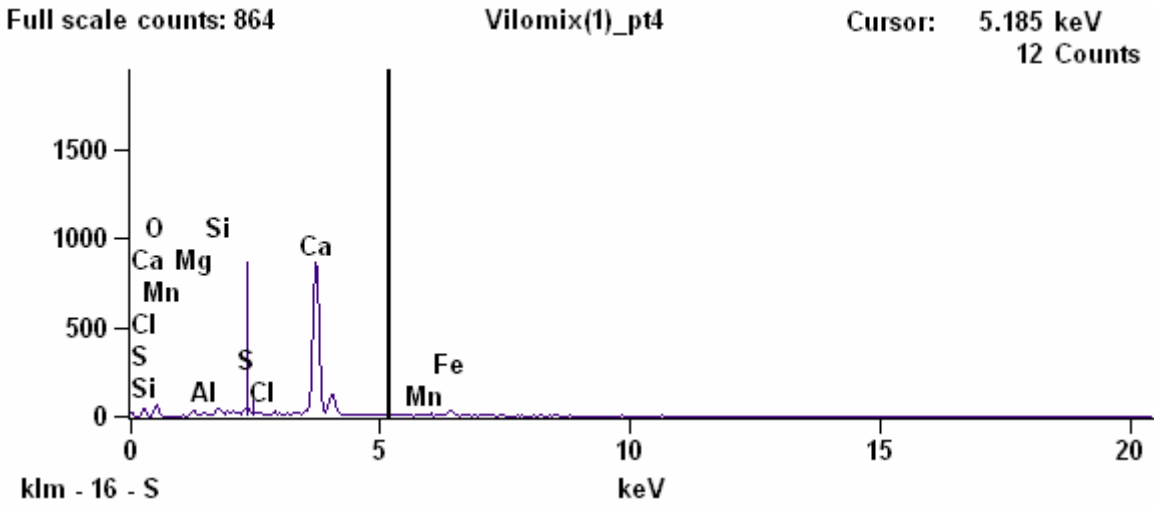
Magnification: 1200

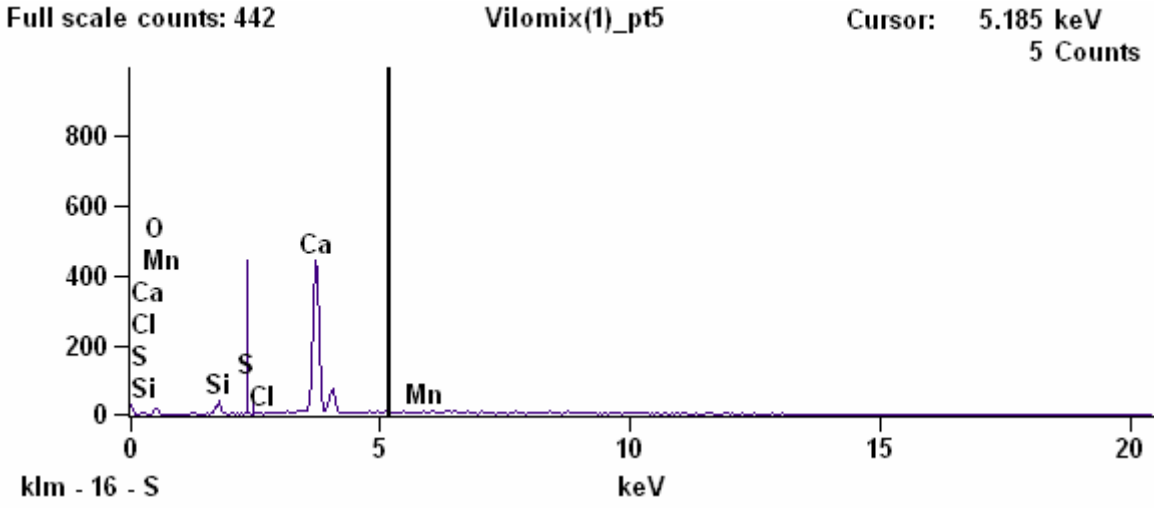
Figure 12. *The EDAX analyses points in the Vilomix(1) sample.*


Full scale counts: 2669
Vilomix(1)_pt1
Cursor: 5.185 keV
19 Counts
10000
8000
6000
4000
2000
0
Si
Al
O Mg
Mn
Ca
Cl
S
Si
S
Cl
Ca
Mn
0
5
10
15
20
klm - 16 - S
keV


Full scale counts: 887
Vilomix(1)_pt2
Cursor: 5.185 keV
6 Counts
1500
1000
500
0
O
Si
Cl
Mg
Ca
Mn
Ca
S
Si
S
Cl
Fe
Mn
0
5
10
15
20
klm - 16 - S
keV


Full scale counts: 1130
Vilomix(1)_pt3
Cursor: 5.185 keV
8 Counts
4000
3000
2000
1000
0
O
Fe
Mg
Mn
Ca
Cl
S
Si
S
Cl
Si
Ca
Fe
Mn
0
5
10
15
20
klm - 16 - S
keV





Compound %

		MgO	Al2O3	SiO2	SO3	Cl	CaO	MnO	Fe2O3
Vilomix(1)_pt1	0.00	0.52	1.21	94.91	0.00	0.00	3.36	0.00	
Vilomix(1)_pt2	0.00	3.09		7.82	1.94	0.54	82.11	0.43	4.07
Vilomix(1)_pt3	0.00	1.49		0.87	57.90	0.19	1.51	0.32	37.73
Vilomix(1)_pt4	0.00	1.75	0.38	2.93	4.35	0.14	83.78	0.00	6.65
Vilomix(1)_pt5	0.00			4.08	0.58	0.00	95.34	0.00	

Figure 13. *The semi-quantitative element distribution diagrams for the five points (pt1–pt5) analysed in the Vilomix(1) sample.*

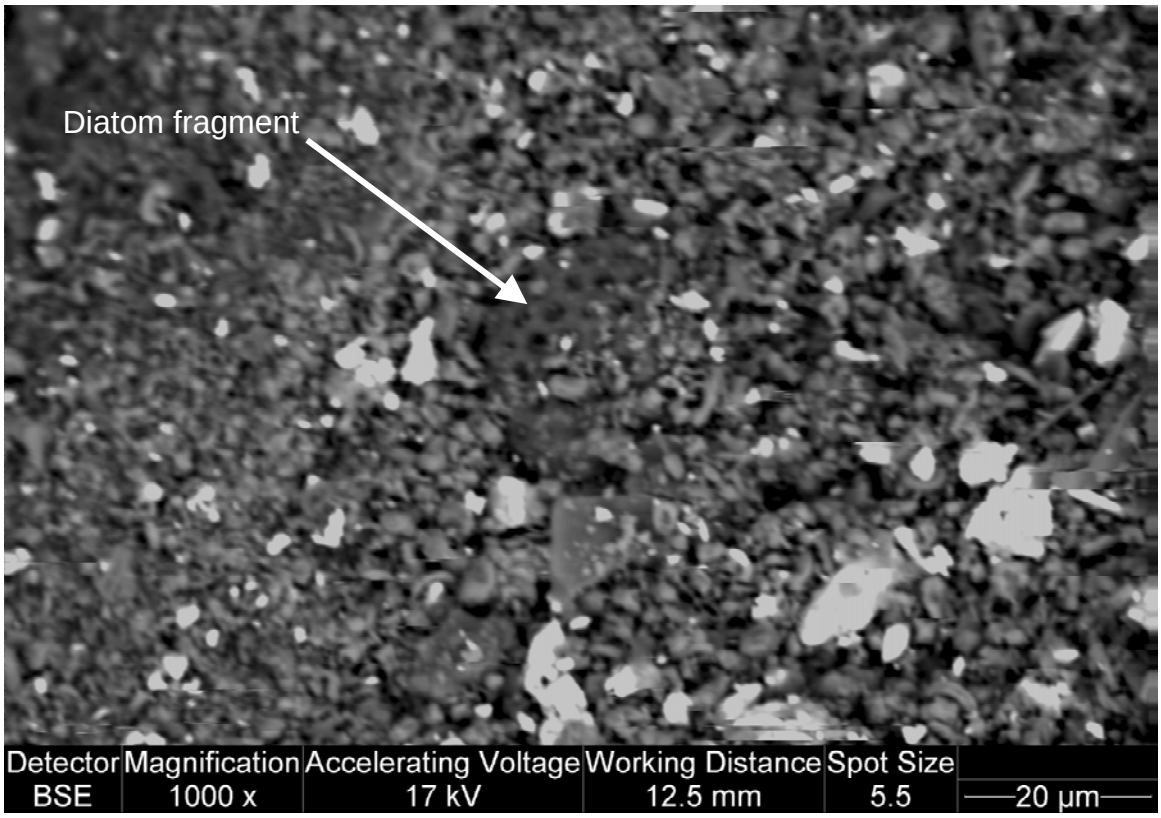


Figure 14. *Scanning electron microscopy of sample 100709. The white arrow points to the fragment element-analysed in Fig. 15 and 16.*

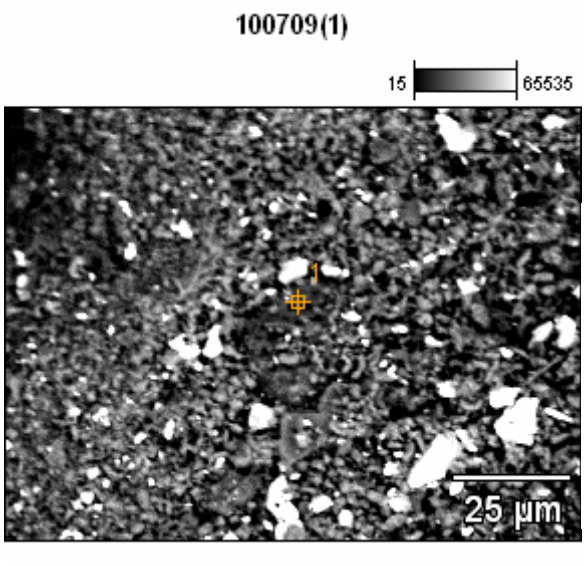
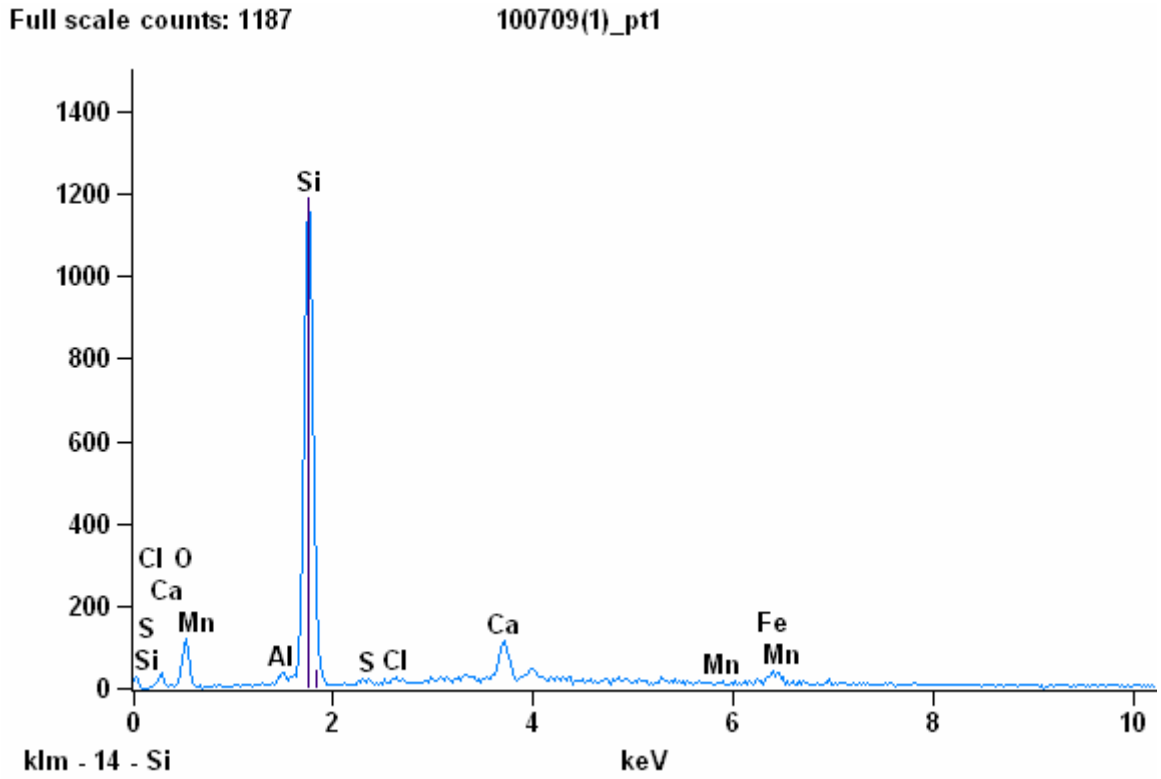


Image Name: 100709(1)

Magnification: 1000

Figure 15. *Position of the element analysis of sample 100709.*



Compound %

		Al2O3	SiO2	SO3	Cl	CaO	MnO	Fe2O3
100709(1)_pt1	0.00	1.17	80.53	1.42	0.64	8.78	0.28	7.18

Figure 16. *EDAX element analysis of point pt1 in Fig. 15. The high SiO_2 content evidently supports the identification of the opal A in the analysed point.*

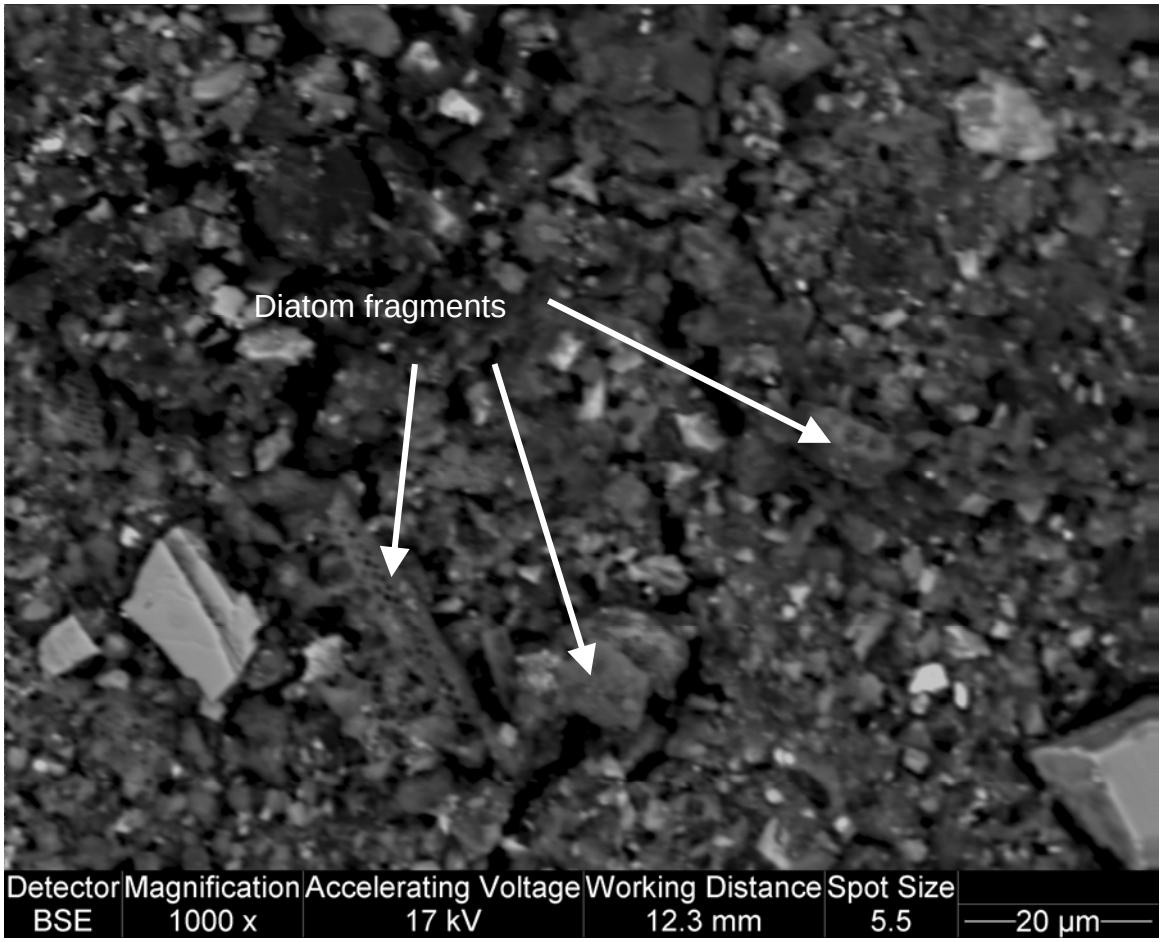


Figure 17. *Scanning electron microscopy of sample 01847. The white arrow points to the fragment element-analysed in Fig. 18 and 19.*

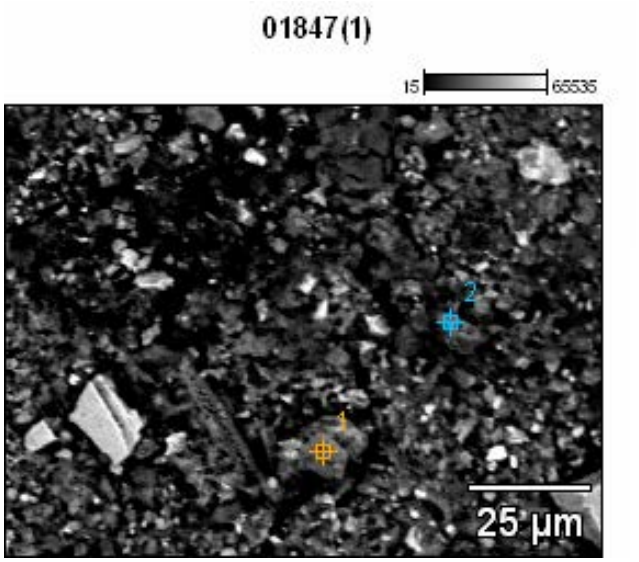
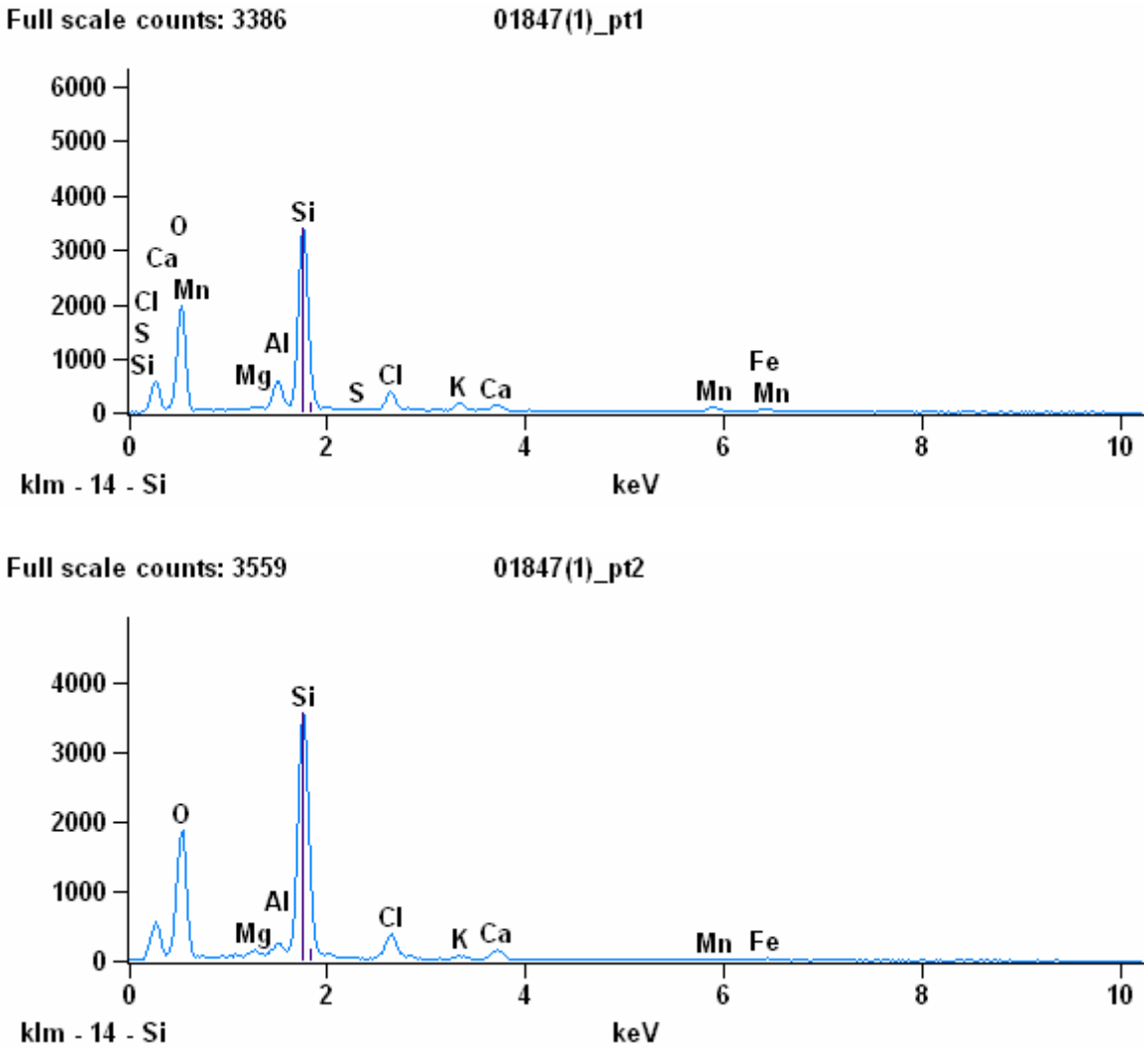


Image Name: 01847(1)

Magnification: 1000

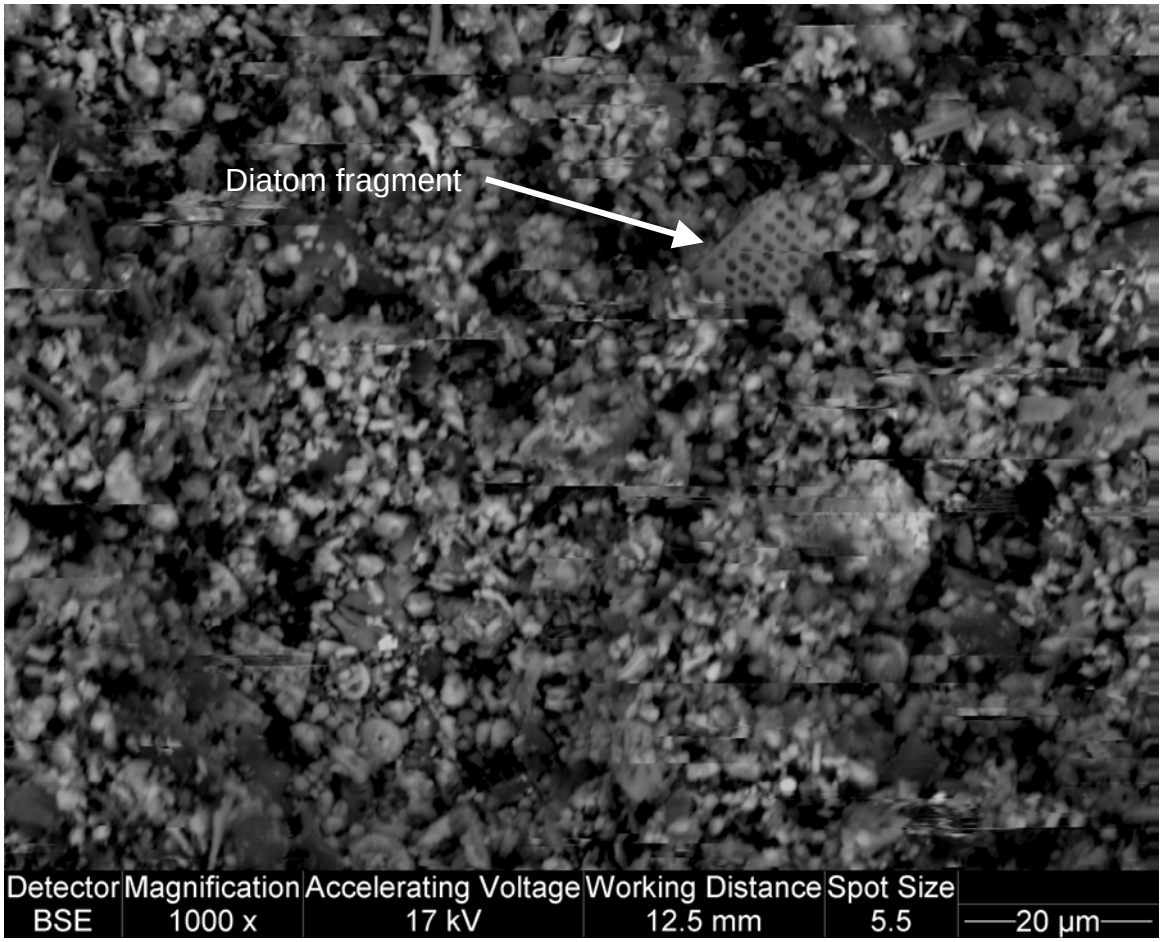
Figure 18. *Position of points analysed in sample 01847.*

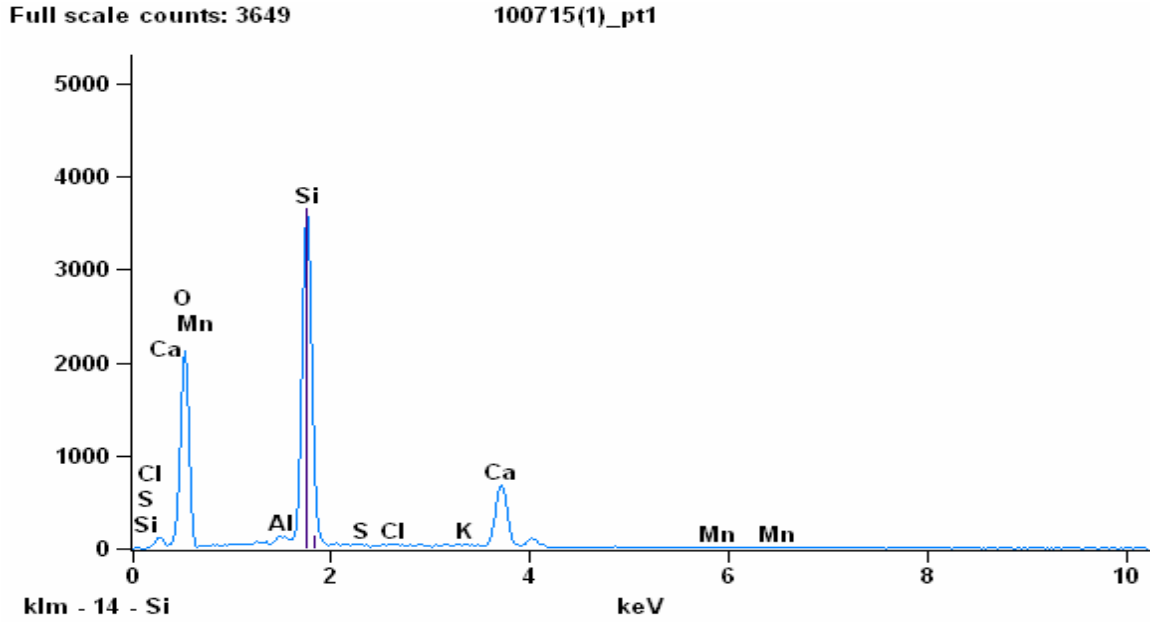


Compound %

		MgO	Al2O3	SiO2	SO3	Cl	K2O	CaO	MnO	Fe2O3
01847(1)_pt1	0.00	0.36	8.94	70.41	0.13	5.01	2.96	3.05	4.92	4.22
01847(1)_pt2	0.00	1.52	2.81	80.13	0.15	6.79	1.24	3.99	0.82	2.54

Figure 19. *EDAX element analysis of the points pt1 and pt2 in Fig. 18. The high SiO_2 content evidently supports the identification of the opal A in the analysed points.*





Compound %

		Al2O3	SiO2	SO3	Cl	K2O	CaO	MnO
100715(1)_pt1	0.00	1.07	76.55	0.02	0.35	0.20	21.64	0.16

Figure 20. *Scanning electron microscopy of sample 100715 and the corresponding element analysis of the point at the white arrow is shown below.*

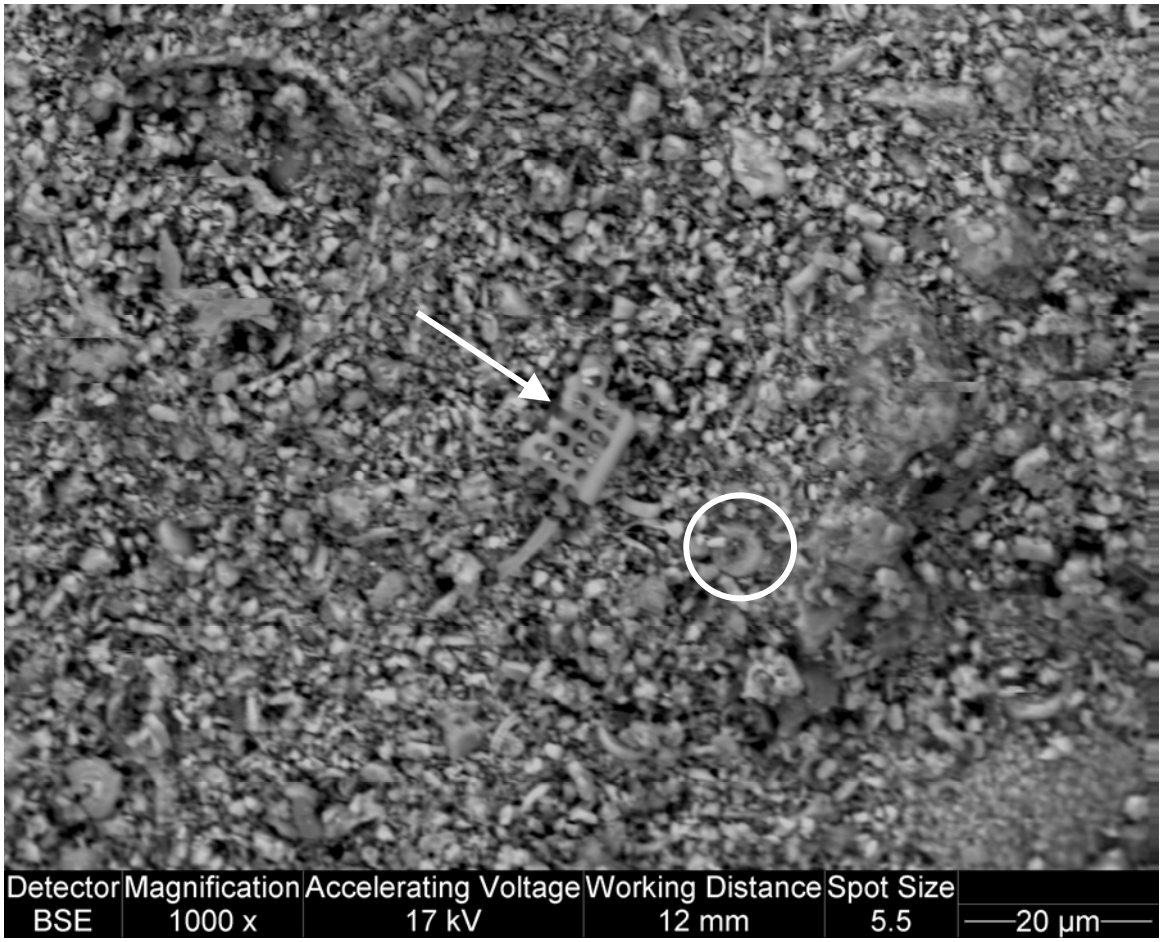


Figure 21. *Scanning electron microscopy of sample 233005. The white arrow points to the diatom fragment element-analysed in Fig. 22 and 23. Note that a large number of chalk granules occur in this sample amongst which the coccolite easily identified is encircled.*

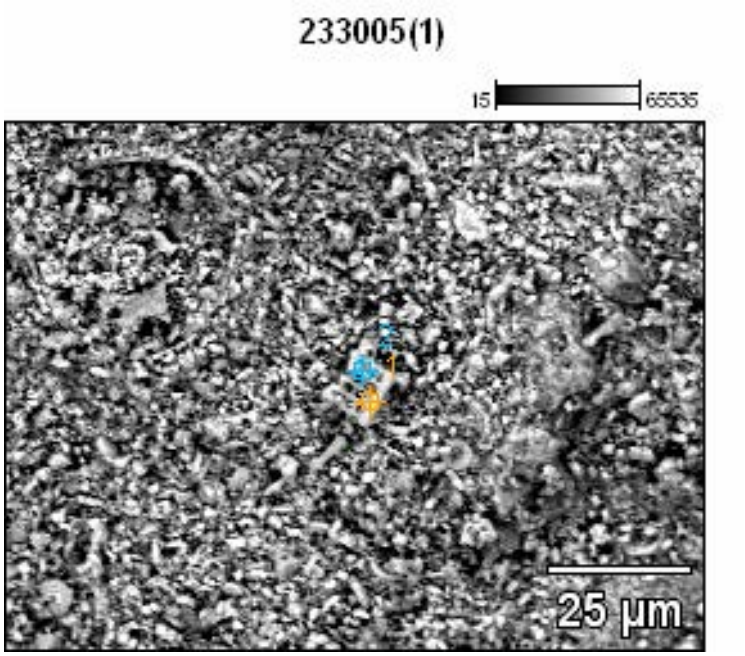
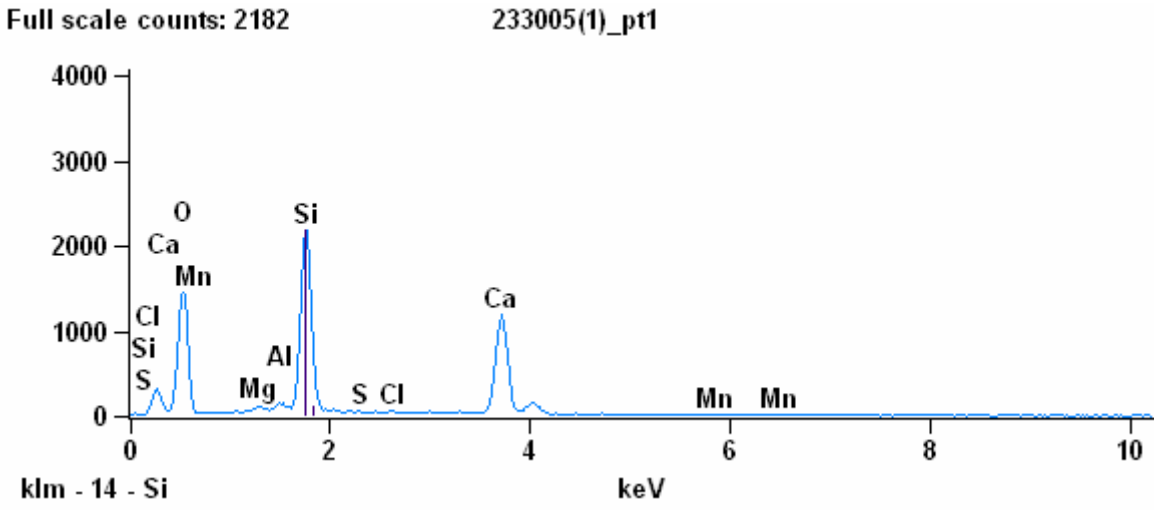
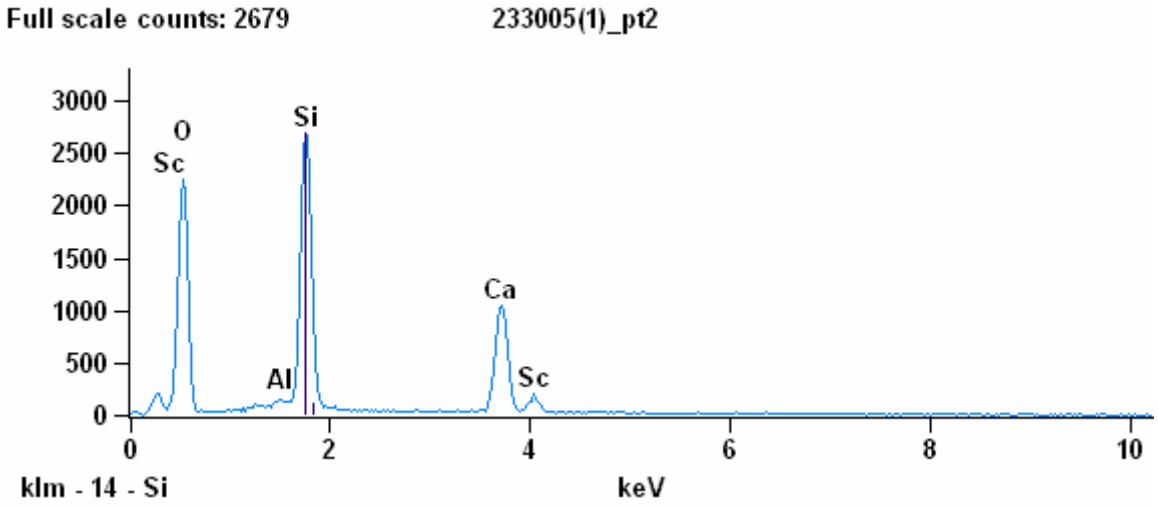


Figure 22. *Position of the two points analysed in sample 233005.*





Compound %

		MgO	Al2O3	SiO2	SO3	Cl	CaO	Sc2O3	MnO
233005(1)_pt1	0.00	1.23	1.56	56.16	0.02	0.22	40.47		0.34
233005(1)_pt2	0.00		1.04	64.28	0.08	0.03	34.12	0.46	0.00

Figure 23. *In sample 233005 the SiO2 content in the diatom fragment appears in the analyses. However, due to the open structure a considerable contamination from the background show up as well in the form of CaO deriving from the numerous fragments of chalk and coccolites.*

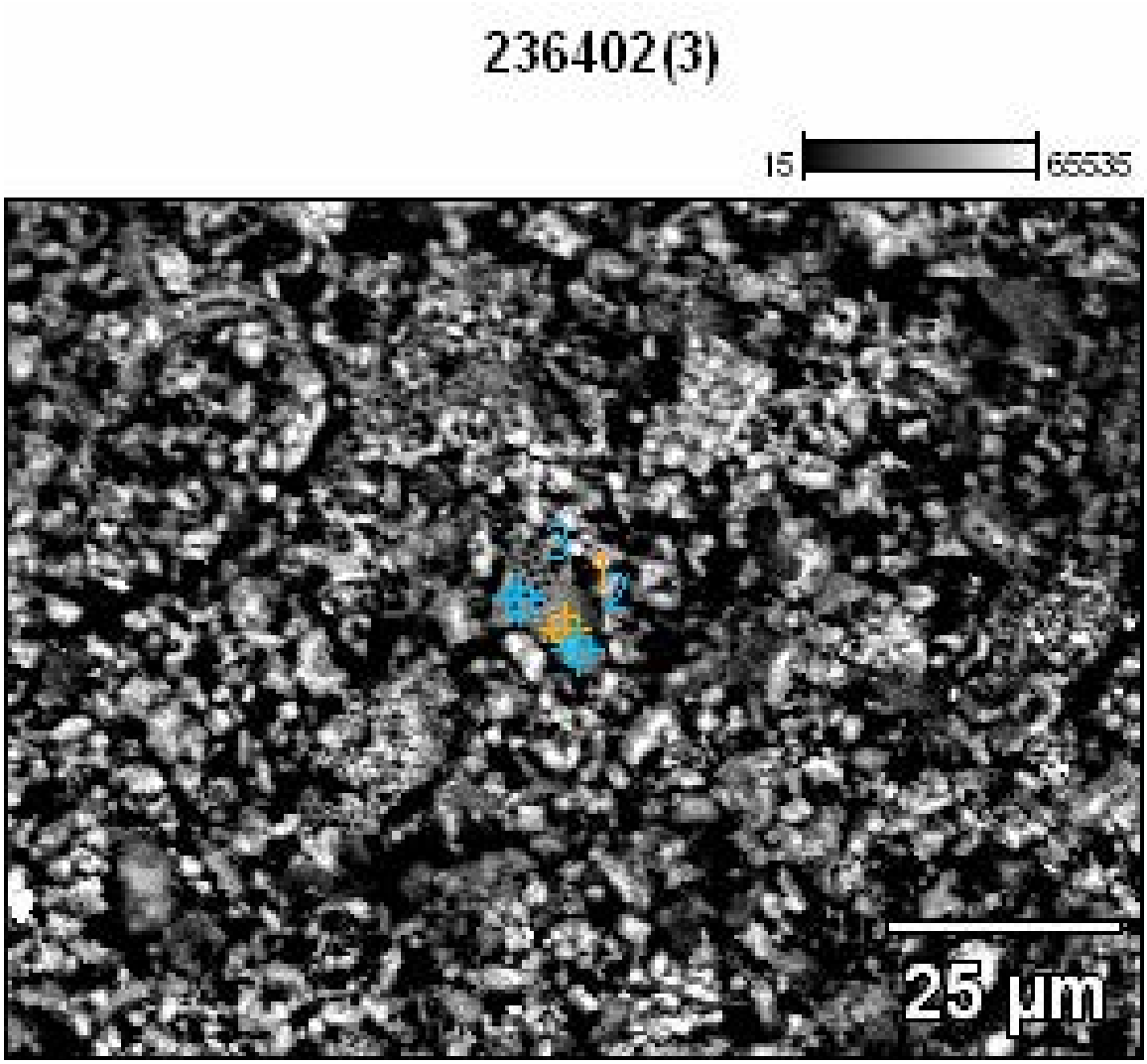
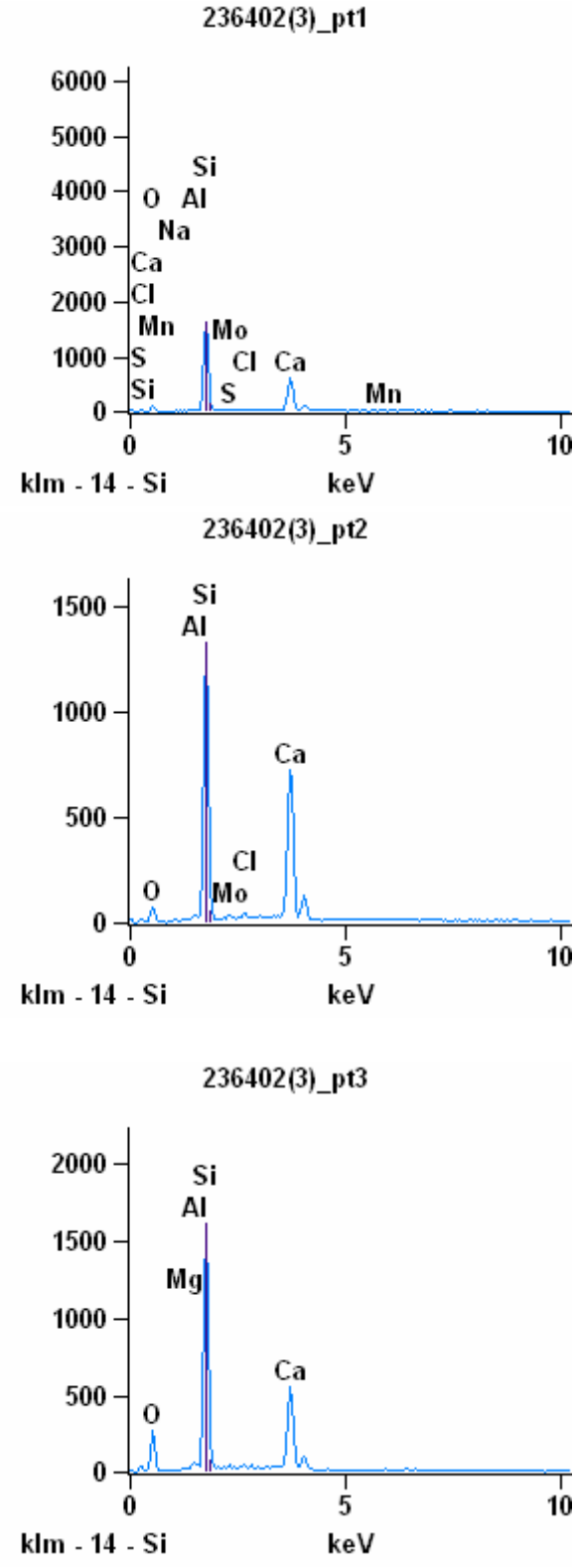


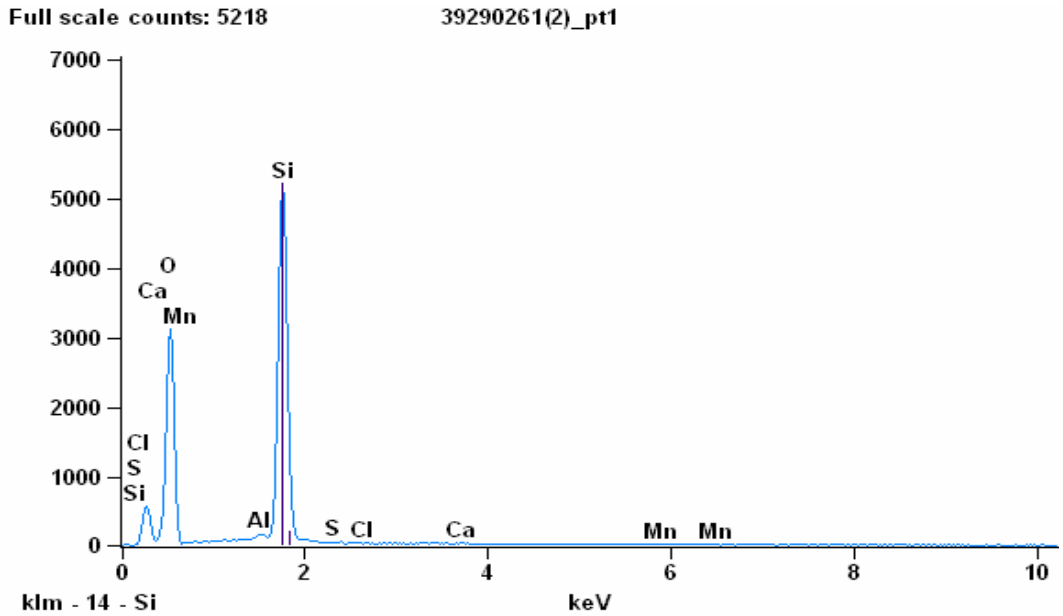
Figure 24. *The analyses of very light (thin) fragments of diatoms in sample 236402. This sample illustrates the problem with very light fragments, which due to the electronic charge of the particles are easily moved out of focus in the scanning microscope. When the image with details in high resolution should be processed, the analysed fragment had disappeared. The element analyses in Fig. 25 did however, show the dominans of SiO_2.*



						Compound %				
		Na2O	MgO	Al2O3	SiO2	SO3	Cl	CaO	MnO	MoO3
236402(3)_pt1	0.00	0.27		0.87	64.26	0.14	0.53	32.21	0.34	1.39
236402(3)_pt2	0.00			0.61	53.76	0.57	0.38	43.70	0.37	0.61
236402(3)_pt3	0.00		0.42	1.16	65.76	0.43	0.20	30.89	1.14	

Figure 25. *The EDAX analyses of the three points in sample 236402.*

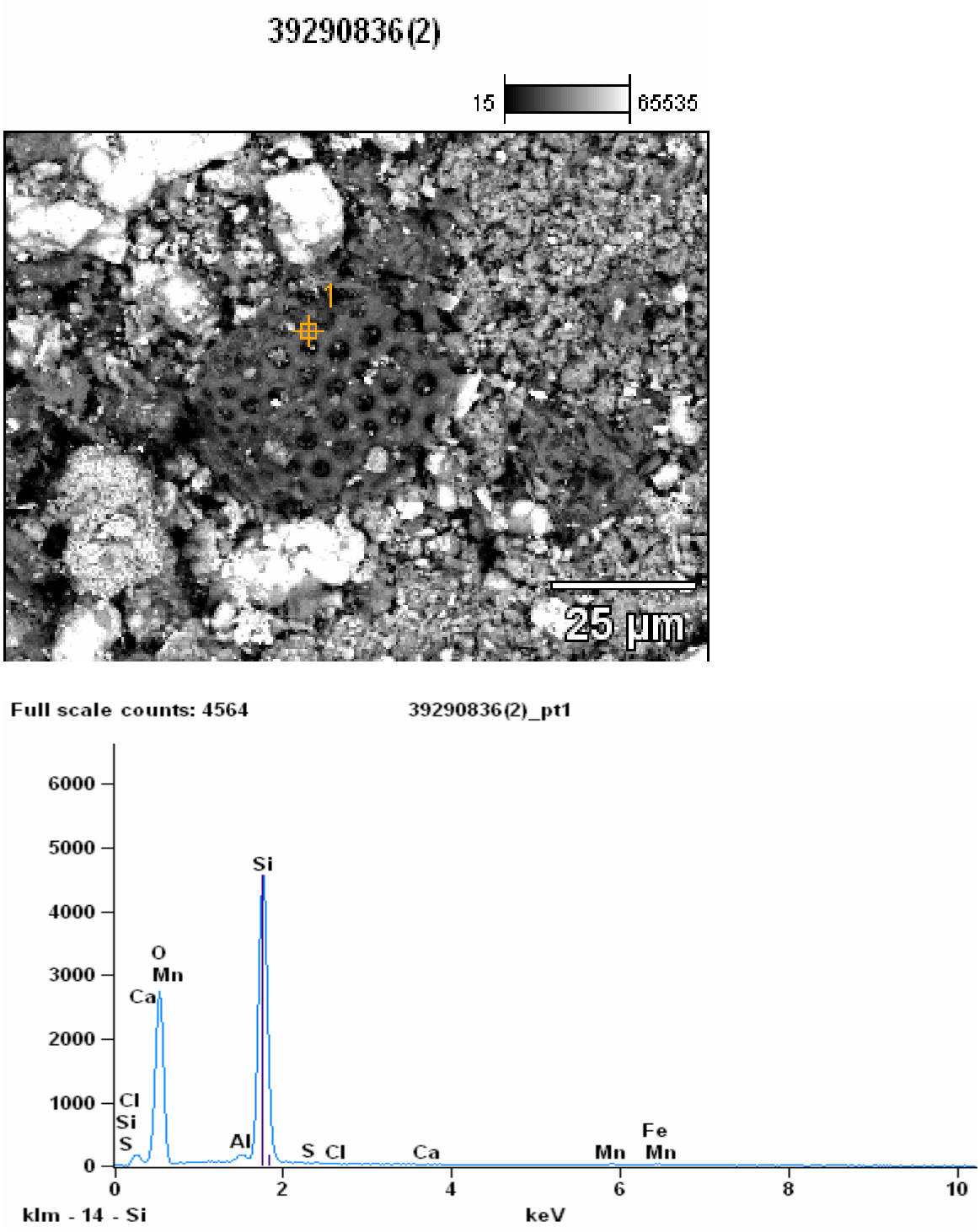
Figure 26. *In the scanning electron microscopy of sample 39290261 the fragments of diatoms are easily recognized. The position of the EDAX analysis of the fragment in the centre of the image is shown with a circle.*



Compound %

		Al2O3	SiO2	SO3	Cl	CaO	MnO
39290261(2)_pt1	0.00	0.67	98.50	0.41	0.03	0.29	0.10

Figure 27. *EDAX analysis of the fragment in Fig. 27, which indicates nearly pure opal A.*



Compound %

		Al2O3	*SiO2*	*SO3*	*Cl*	*CaO*	*MnO*	*Fe2O3*
39290836(2)_pt1	0.00	1.45	95.30	0.00	0.03	0.00	1.58	1.64

Figure 28. *The EDAX analysis of a relative large fragment of a diatom in sample 39290836 shows a satisfactory composition of SiO_2.*

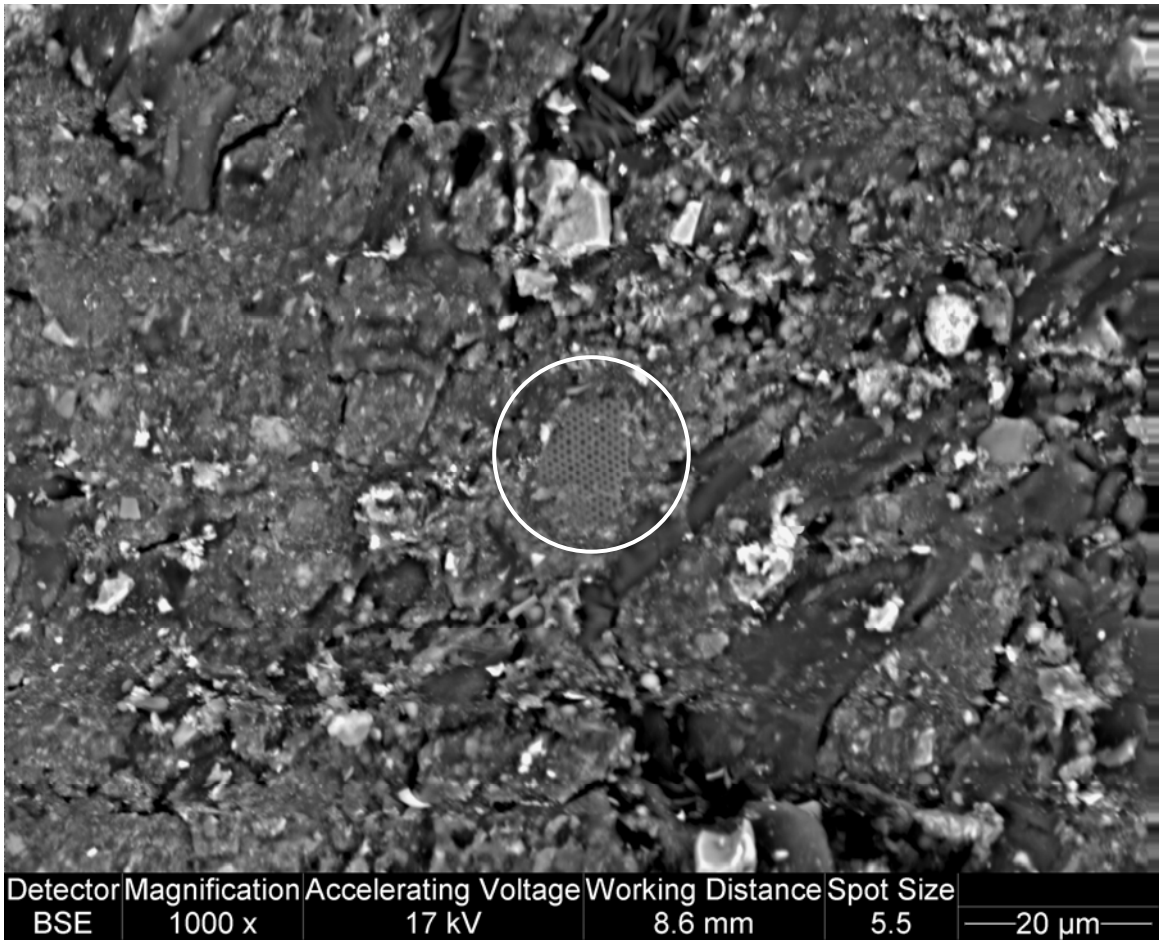
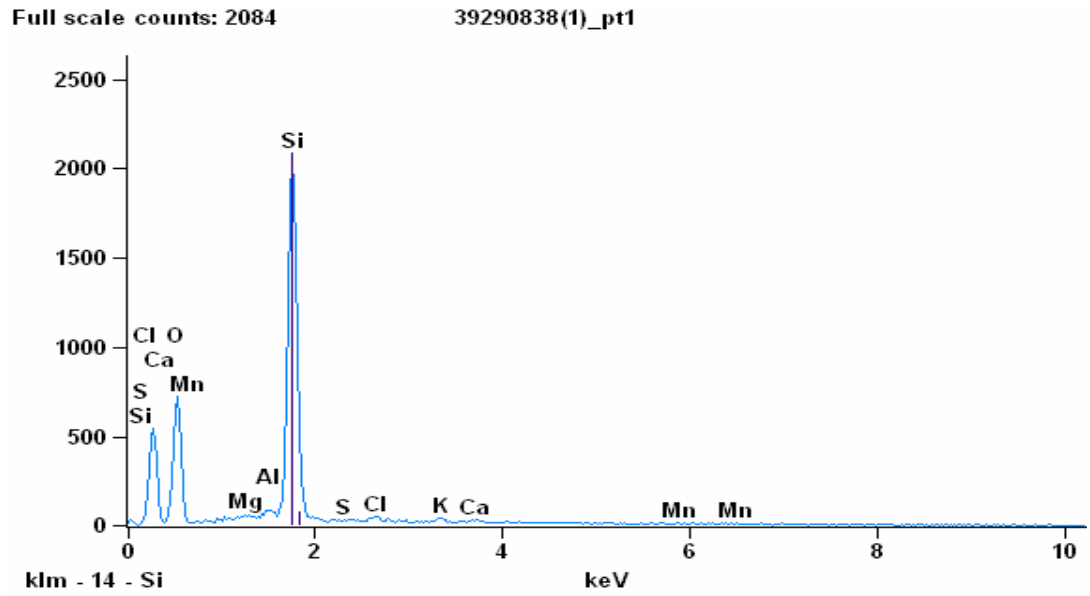


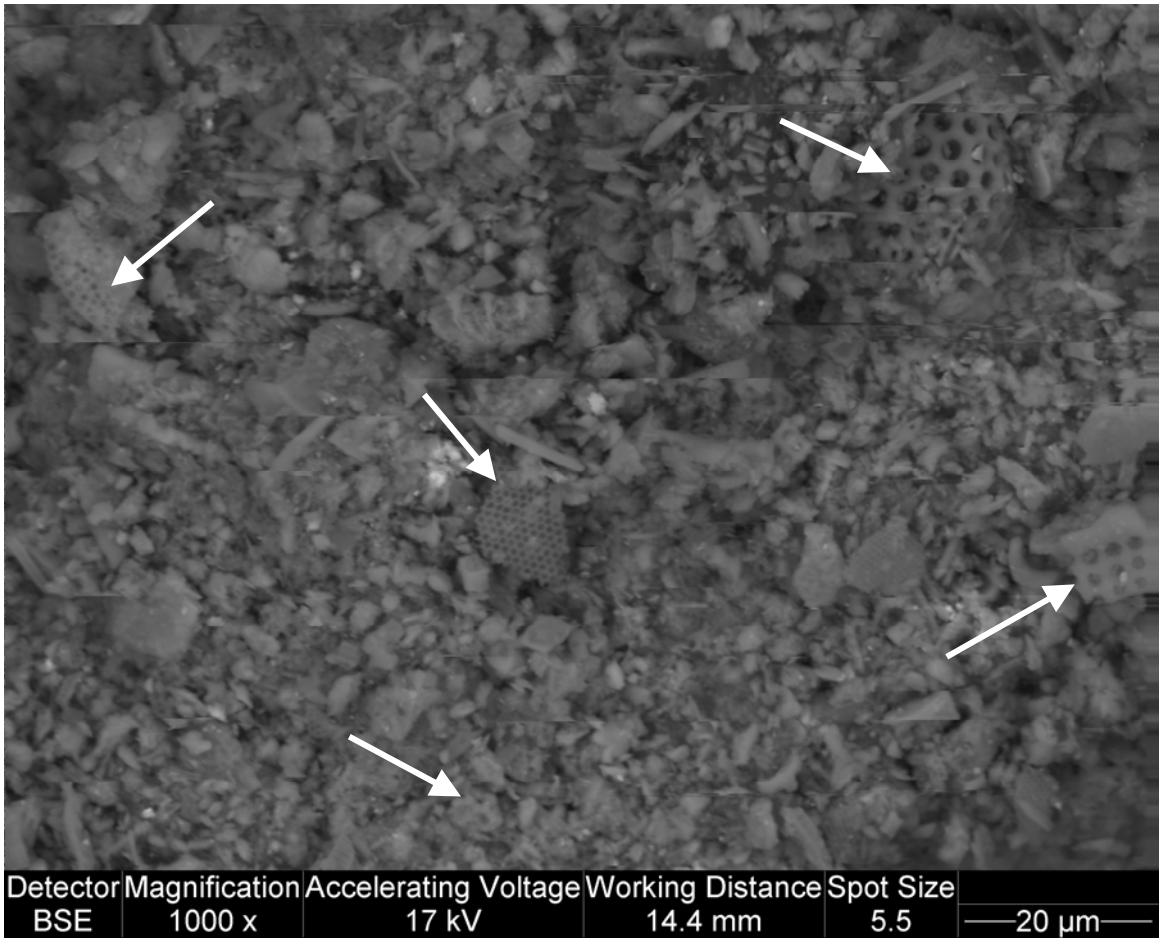
Figure 29. *A very thin platy fragment of a diatom (encircled) was recognised in sample 39290838. The EDAX analysis is shown in Fig. 30.*



Compound %

		MgO	Al2O3	SiO2	SO3	Cl	K2O	CaO	MnO
39290838(1)_pt1	0.00	0.91	1.19	94.74	0.12	0.83	1.12	1.04	0.05

Figure 30. *The EDAX analysis of sample 3929038 shows a reasonable opal A composition.*



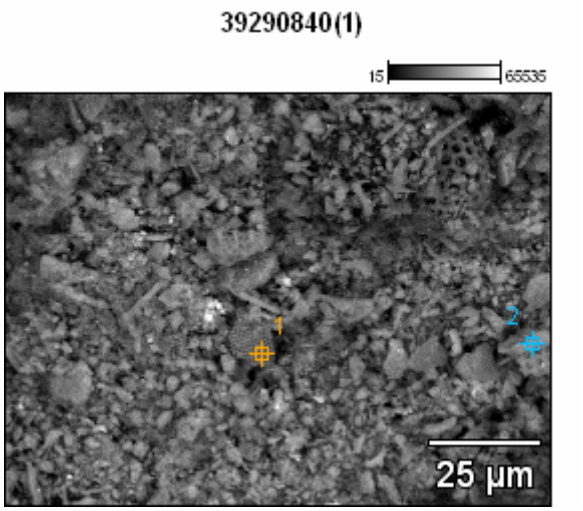
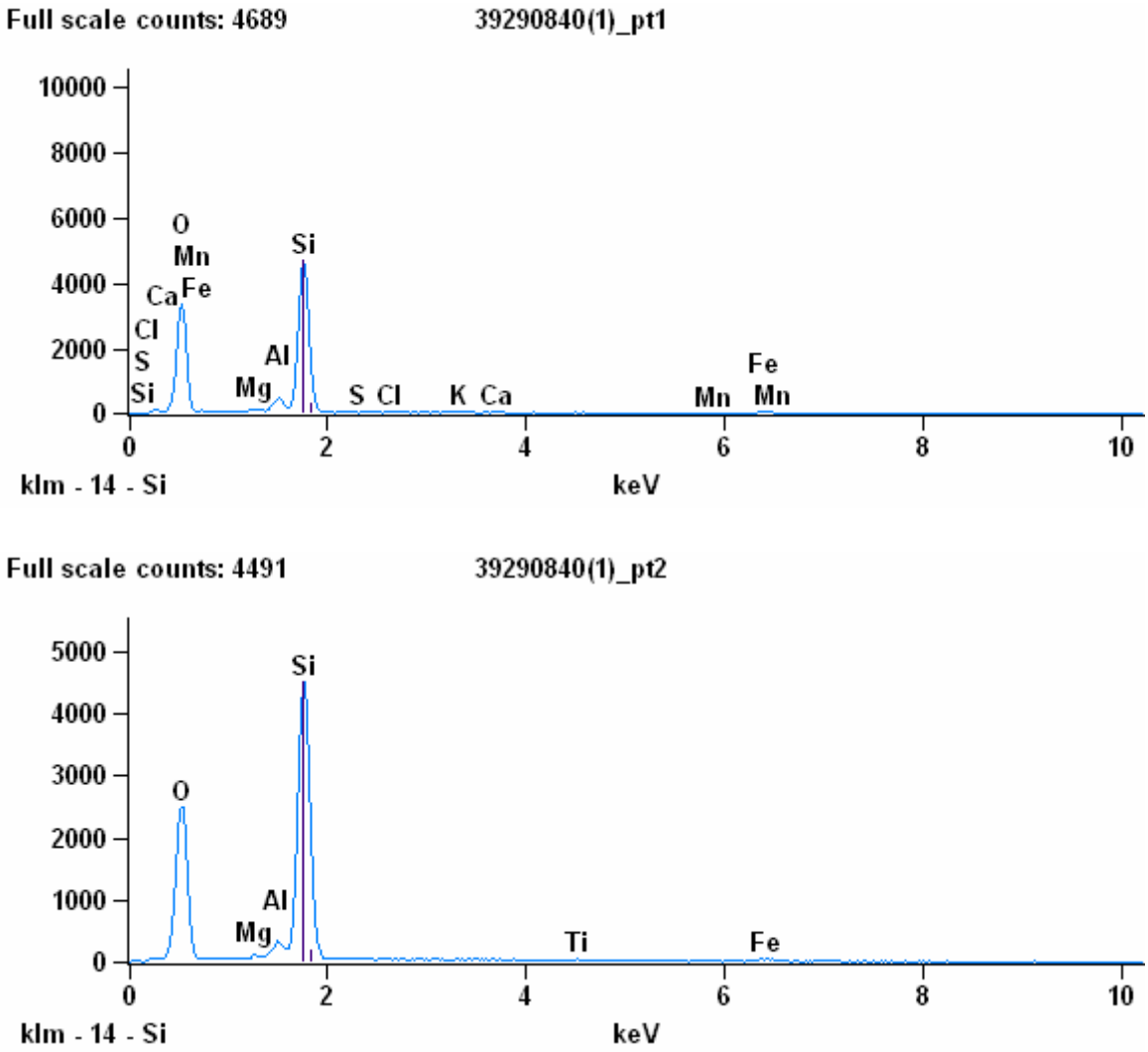


Figure 31. *Scanning electron microscopy of sample 39290840. The white arrows in the upper picture point to a number of various types of diatom fragments. In the small picture below the position of the two points are shown for which an EDAX analysis is provided in Fig. 32.*



Compound %

	MgO	Al2O3	SiO2	SO3	Cl	K2O	CaO	TiO2	MnO	Fe2O3
39290840(1)_pt1	0.92	5.96	87.02	0.00	0.03	0.93	0.46		0.07	4.61
39290840(1)_pt2	0.34	3.16	92.66	0.18	0.04		0.01	0.56	0.00	3.04

Figure 32. *The EDAX analyses of the two points in sample 39290840. Although the fragments were very thin and very small the element analyses show a reasonable SiO_2 composition.*

Discussion

The presented investigation has demonstrated the ability of testing the presence of moler (diatomite) in a feed sample by visual identification of fragments of diatoms in a scanning electron microscopy of the sample. The identification is easily carried out in a common scanning electron microscope with the application of the general procedure image focusing and storage. The method requires a scientist qualified for identification of not only diatoms but also other micro organism and mineral compounds appearing in sediment samples. Most geologists will with a minor training be able to carry out this type of analysis.

The method is furthermore well supported with the semi-quantitative element analysis EDAX, which is a common facility connected to modern scanning electron microscopes (SEM). Therefore it is recommended that the identification method includes the element analysis of the fragments identified in the microscope as demonstrated in this report.

The technical procedure of identifying one sample demands about two hours of work with preparation and coating the sample and the search for one small fragment amongst hundred in the images. The time consuming search for a fragment will of course increase, when the amount of Diamol granules becomes smaller and smaller. Therefore it is unreasonable to suggest the method to by applied for a negative test, i.e. to prove that moler is not in a sample. The simple question is here: how many hours will be necessary for the satisfactory answere: No diatoms were found!

Conclusion

The scanning electron microscopy has been demonstrated to be a very useful method for the identification of moler (diatomaceous material) in a feed sample. The method is based on two important functions:

1) The visual identification of organic structured fragments of diatoms in the sample in a scanning electron microscope (SEM).
2) The identification is geochemically supported by EDAX analysis to prove the correct composition of the diatom frustules fragments.

The method can easily be applied by common geochemical laboratories equipped with a SEM-EDAX instrument. Meanwhile, the method requires a geologist with experience in identifying micro fossils in a SEM.

References

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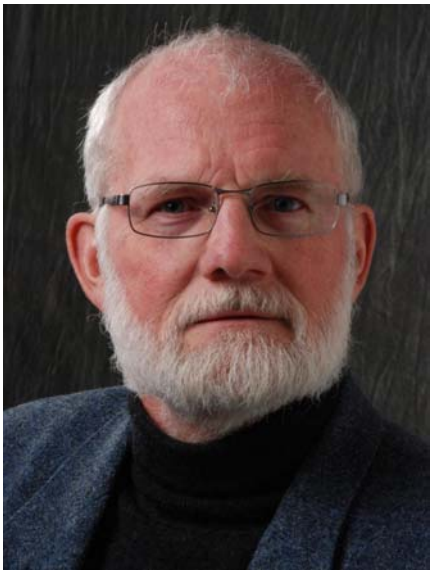
Appendix



Stig Asbjørn Schack Pedersen

This report is the result of a scientific research project conducted by Stig Asbjørn Schack Pedersen, who is senior scientist at the Geological Survey of Denmark and Greenland. S.A.S. Pedersen graduated from University of Copenhagen with a master degree and a Ph.D. degree in geology. He has also defended the honourable degree of Doctor of Sciences (on structural geology of glaciotectonics). His work on glaciotectonics is very relevant for the occurrences and distribution of the moler, the clayey diatomite in northern Denmark. For more than 25 years S.A.S. Pedersen has been working with problems related to the occurrences of moler. He is an expert in industrial minerals and has been investigating diatomite deposits in Denmark, Hungary, Greece and Romania. His list of publications includes more than 100 papers and technical reports about moler, diatomite deposits and related industrial mineral occurrences. The geological survey has appointed Dr. S.A.S. Pedersen as the manager of moler investigations and consultant of the raw material authorities in the ministry and counties.

Jørgen Kystol

The co-author of this report is the senior chemist at GEUS Jørgen Kystol, who is civil engineer and a classic analytical chemist. For more than 20 years Jørgen Kystol has been responsible for the geochemical laboratory providing the X-ray fluorescence analyses and managing the analytic facilities in SEM-EDAX instrumentation.