4. EXPERIMENTAL INVESTIGATIONS ON HUNGARIAN TERTIARY LIGNITES I.

M. KEDVES

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, H-6701, P. O. Box 993, Szeged, Hungary

Abstract

An experimental, combined research program is planned on Hungarian Tertiary lignites. LM and EM methods will be used to investigate the organic matter of the secondary wood remnants. Partial dissolution method will also be used. This paper presents the methological considerations and some preliminary LM results on two samples.

Key words: Xylotomy, partial dissolution, Tertiary, Hungary.

Introduction

There are several papers concerning the anatomy with LM method of Hungarian lignites; HOLLENDONNER (1931), HARASZTY (1933, 1953, 1957, 1958), SÁRKÁNY (1943), MAÁCZ (1955), STIEBER (1955), SIMONCSICS (1956), GREGUSS (1959), KEDVES (1959). In 1967 GREGUSS published a monograph of the anatomy of the fossil *gymnosperm* woods in Hungary, including all kinds of fossilized secondary xylem.

Later, the importance of the dark coloured wood fragments was recognized in the transport of the radioactive elements in the Hungarian Holocene sediments (KEDVES and KÖRMÖCZI, 1985, KEDVES and SZEDERKÉNYI, 1985, 1988). KEDVES and SZEDERKÉNYI (1988) described the ultrastructure of the xylem remnants transporting radioactive elements reworked into the mud of Lake Vadkert in Hungary. In this paper the following facts were established:

1. The ultrastructure of the xylem remnants is not identical in the different samples investigated. This is the consequence of the different degree of coalification, or the taphonomical processes.
2. The lamellar ultrastructure was in some places discernible.
3. Granules with high electron density are in the organic debris enclosing the xylem fragments.

Taking into consideration the importance of the ultrastructure data of the xylem remnants in the reconstruction of the sedimentation processes, a new research program was projected to the LM and EM investigations on Hungarian, mostly Tertiary lignites. The aim of this paper is to present the basic methods of the projected investigations together with the first results of the LM morphology of two lignite samples.

Materials and Methods

About 25–30 samples of lignite from different localities and ages (Miocene to Pliocene) are planned for combined investigations. The samples were collected and investigated previously with two kinds of research concepts and methods:

1. Paleoecological, Geological and Paleontological (*Mollusca, Ostracoda, Nannoplankton*, etc.), supported by Grant OTKA 1/5, T 007482, responsible for the program Dr. M. SZÓNOKY (Department of Geology and Paleontology of the J. A. University, Szeged, Hungary).
2. Organic Geochemistry concept supported by Grant OTKA 1/5, T 007429 responsible Dr. M. HETÉNYI (Department of Mineralogy, Petrography and Geochemistry of the J. A. University, Szeged, Hungary).

The methods used for the wood anatomy are the following:

1. LM studies

1.1. The so-called classical thin sections were made for the determination of the botanical affinities of the lignite remnants and to establish the preservation and the Paleoecology of the samples investigated.

1.2. These data were completed with those of the macerations material with SCHULTZE or DUBERT mixture. The remains of the macerations were coloured with Toluidine Blue.

2. Experimental LM and TEM investigations

2.1. The first step was to eliminate the inorganic components of the xylem fragments, with HCL aq. dil. and HF aq. dil.

2.2. After carefully washing the organic material in distilled water, the samples were broken into small fragments. This material was shaken in distilled water in a measuring testtube of 100 ml. After 5 second of deposition the small lignite pieces containing liquid part overflew. This matter was shaken again and 5-5 ml of woody fragments containing water were measured for experimental studies.

2.3. Three kinds of material were investigated.

2.3.1. The so-called zero material without experiment.

2.3.2. 0.2 ml diethylamine was added to the 5 ml xylem fragments containing water.

2.3.3. 0.2 ml merkaptoethanol was added to the 5 ml xylem fragments containing water.

2.4. The material, prepared by the previous methods is ready for partial dissolution. This was made in a thermostat at 30 °C during 30 days.

2.5. After this partial dissolution the woody fragments were carefully washed with distilled water.

2.6. For LM investigations the fragments were again coloured with Toluidine Blue, the slides were mounted in glycerine-jelly, hydrated at 39.6%.

2.7. For TEM studies fixation was done in Millonig buffered 1% osmium tetroxide for 1 hr. After fixation the material was washed in a 0.2 M Millonig (osmium-tetroxide-free) phosphate buffer overnight. Dehydratation was performed in an ascending series of ethanol in 15 min. steps, including uranyl acetate staining in 70% ethanol. The samples were embedded in Durcupan (Fluka) araldite epoxy resin in gelatin capsules and polymerized at 56 °C thermostat for 3 days. The ultrathin sections will be made in the Electron Microscopical Laboratory of the Institute of Biophysics of the Biological Center of the Hungarian Academy of

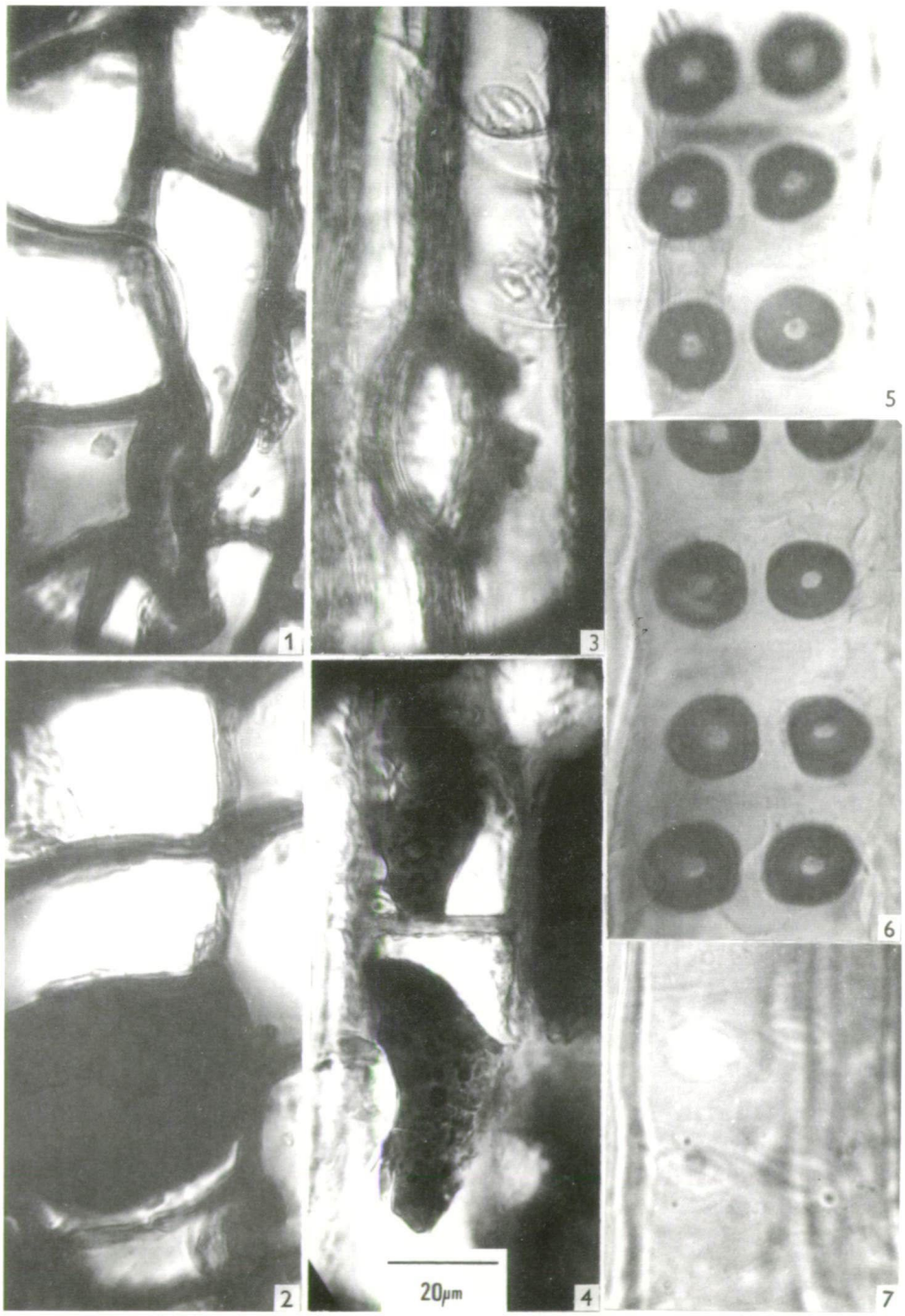

Plate 4.1.

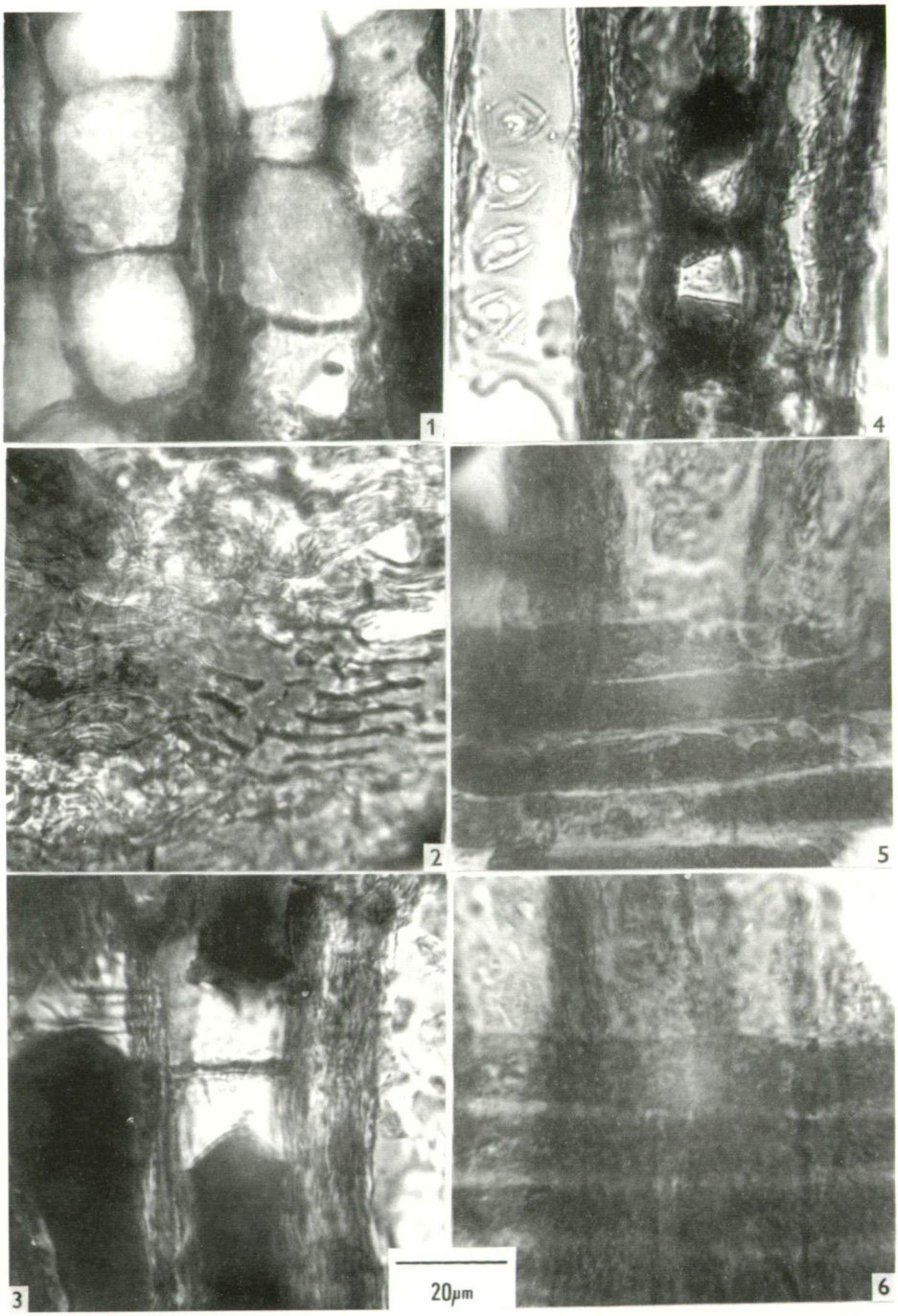

Plate 4.2.

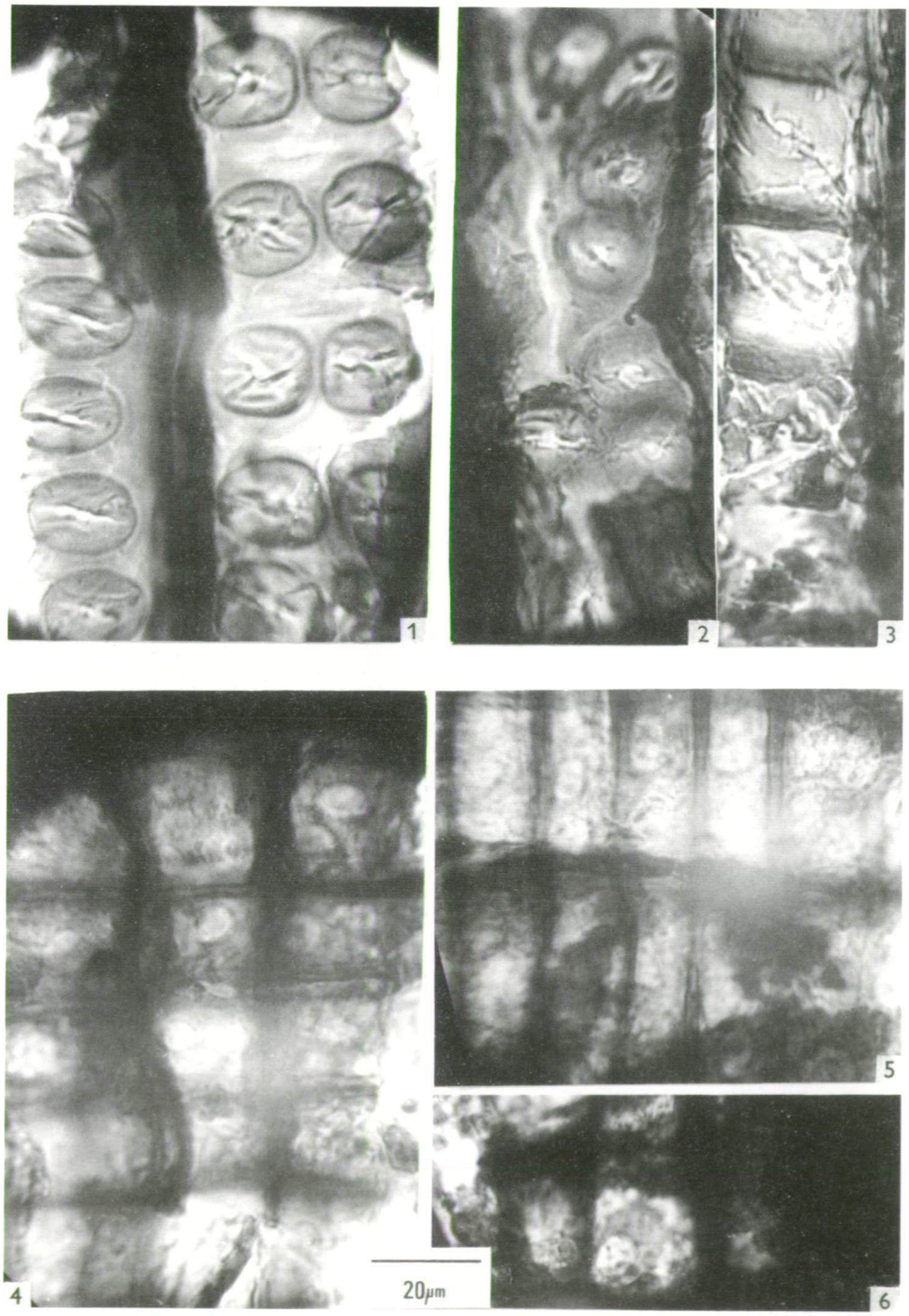

Plate 4.3.

Sciences on Porter Blum ultramicrotome. The TEM pictures will also be taken in this Laboratory.

In this paper the results of the LM investigations of the following two samples are presented:

Sample, No 1.

Locality: Salgótarján, Miocene. The woody samples are deposited in the collection of the Department of Geology and Paleontology of the J. A. University, Szeged, number: UC_2 1047. The samples were handed over for my investigations by Dr. M. SZÓNOKY.

Sample, No 2.

Locality: Sopron, Lower Pannonian, brick factory, collected by Dr. M. SZÓNOKY on 15.04.1988, the samples are deposited in the Department of Geology and Paleontology of the J. A. University, Szeged.

Results

Sample, No 1 (Plate 4.1., figs. 1–7, plate 4.3., figs. 1–3)
Sequoioxylon gypsaceum (GÖPPERT) GREGUSS 1967

Wood anatomical characteristic features important from taxonomic points of view are well preserved. The non-experimental material was investigated on thin sections (Plate 4.1., figs. 1–4) and macerated with the DUBERT's mixture (Plate 4.1., figs. 3–7). The longitudinal parenchyma cells contain resin drops (Plate 4.1., figs. 2,4). The horizontal wall of the longitudinal parenchyma cell is smooth without thickenings (Plate 4.1., fig. 4). In some places there are bordered pits on the tangential side of the tracheids (Plate 4.1., fig. 3). There are bi-seriate bordered pits on the radial wall of the tracheids. Bars of Sanio or crassulae are present (Plate 4.1., figs. 5,6). Cross-field pits are mostly two or more of taxodioid type (Plate 4.1., fig. 7). After dissolution with diethylamine (Plate 4.3.,

Plate 4.1.

1–7. *Sequoioxylon gypsaceum* (GÖPPERT) GREGUSS 1967
1,2. Cross sections of the tracheids.
3,4. Tangential sections.
5,6. Bordered pits and bars of Sanio.
7. Cross-field pits.

Plate 4.2.

1–6. *Sequoioxylon medullare* GREGUSS 1967
1,2. Cross sections of the tracheids.
3,4. Tangential sections.
5,6. Radial sections.

Plate 4.3.

1–3. *Sequoioxylon gypsaceum* (GÖPPERT) GREGUSS 1967, partially dissolved tracheids.
1. Dissolution with diethylamine.
2,3. Dissolution with merkaptoethanol.
4–6. *Sequoioxylon medullare* GREGUSS 1967, partially dissolved xylem.
4,5. Dissolution with diethylamine.
6. Dissolution with merkaptoethanol.

fig. 1) and merkaptoethanol (Plate 4.3., figs. 2,3) important alterations may be observed by the LM method, too. Particularly at the pits of the radial wall of the tracheids, and the cross-field pits lost their original LM morphology.

Sample, No 2 (Plate 4.2., figs. 1–6, plate 4.3., figs. 4–6)
Sequoioxylon medullare GREGUSS 1967

The preservation of the tissue of the secondary wood is not identical on a small piece of the lignite sample (Plate 4.2., figs. 1,2). A relatively well preserved part is illustrated in fig. 1, in cross-section. In the 2nd picture in contrast to this the original structure is hardly damaged and compressed at this part of the lignite. The most important LM morphological characteristic features of the lignite sample are the following: The longitudinal parenchyma cells contain resins, its longitudinal wall is smooth (Plate 4.2., fig. 3). There are bordered pits on the tangential wall of the tracheids (Plate 4.2., fig. 4). The bordered pits of the radial wall of the tracheids are damaged, uni- or bi-seriate (Plate 4.2., figs. 5,6). The ray cells are in general filled with dark content, therefore they cannot be so easy established (Plate 4.2., figs. 5,6). There are 3–5 small cross-field pits (Plate 4.2., figs. 5,6). The diethylamine (Plate 4.3., figs. 4,5) and the merkaptoethanol (Plate 4.3., fig. 6) altered the morphological characteristic features of the lignite tissue. The cell contents were dissolved and the size and the character of the cross-field pits have been altered (Plate 4.2., figs. 4–6).

Discussion and Conclusions

As it was emphasized previously, this paper is a preliminary report of a new research program, with the attempt to elaborate the basic methods for the series of investigations. But based on our previous experiences of the partially dissolved spores and pollen grains, the ultrastructure may change without alterations in the LM morphology. It is worth of mentioning, that there are important alterations in the LM morphology of the two lignite samples investigated in consequence of both organic solvents. In this way it is presumable to get some information to the fine structure of the wall and sometimes the resin and other contents of the Hungarian lignites.

Acknowledgements

This work was supported by Grant OTKA 1/7 T 014692.

References

GREGUSS, P. (1959): Xylotomische Untersuchungen an Braunkohlenfunden von Várpalota. – Acta Biol. Szeged. *5*, 1–46.

GREGUSS, P. (1967): Fossil Gymnosperm woods in Hungary. – Akadémiai Kiadó, Budapest.

HARASZTY, Á. (1933): A gyöngyösi és a rózsaszentmártoni lignitek mikroszkópos vizsgálata. – Bot. Közl., 185–189 (Hungarian with German Summary)

HARASZTY, Á. (1953): A Petőfibánya barnaszeneinek mikroszkópos vizsgálata. – M. T. A. Biol. Oszt. Közl. *2*, 245–257 (Hungarian with German Summary)

HARASZTY, Á. (1957): Die mikroszkopischen Untersuchungen der Xylite von Hidas. – Ann. Univ. Sci. Budapestiensis Sect. Biol. *1*, 71–87.

HARASZTY, Á. (1958): Recherches anatomiques sur les xylites d'âge tortonique de Herend-Szentgál (Hongrie occidentale). – Acta Bot. Acad. Sci. Hung. *4*, 233–256.

HOLLENDONNER, F. (1931): A Kőszeg-pogányvölgyi lignit mikroszkópos vizsgálata. – M. T. A. Mat. és Term. Tud. Ért. *48*, 731–738.

KEDVES, M. (1959): Xylitbestimmungen aus den Bohrungen bei Ganna und Herend. – Acta Biol. Szeged. *5*, 17–23.

KEDVES, M.–KÖRMÖCZI, L. (1985): Sur les problèmes de conservation des sporomorphes dans des conditions différentes. – An. Asoc. Palinol. Leng. Esp. *2*, 263–271.

KEDVES, M. and SZEDERKÉNYI, T. (1985): The importance of the spore-pollen investigations in the recognition of the radioactive element content of the lake mud. – Acta Biol. Szeged. *31*, 215–216.

KEDVES, M. and SZEDERKÉNYI, T. (1988): Transmission electron microscopical investigation of xylem remains transporting radioactive elements in the mud of Lake Vadkert. – Acta Biol. Szeged. *34*, 71–81.

MAÁCZ, J. (1955): Xylituntersuchungen aus dem Kohlenrevier von Borsod. – Acta Biol. Szeged. *1*, 41–44.

SÁRKÁNY, S. (1943): Várpalotai lignit növényszövettani vizsgálata. – Földt. Közl. *73*, 449–458. (Hungarian with German Summary)

SIMONCSICS, P. (1956): Braunkohlenpflanzen aus dem Kohlenrevier von Nógrád. I. Über eine Fusit von Kányás. – Acta Biol. Szeged. *2*, 59–62.

STIEBER, J. (1955): Királdi alsó miocén famaradványok xylotomiai vizsgálata. In: ANDREÁNSZKY and KOVÁCS: Gliederung und Ökologie der jüngeren tertiärfloren Ungarns. – Budapest.