

## TRANSMISSION ELECTRON MICROSCOPICAL INVESTIGATION OF XYLEM REMAINS TRANSPORTING RADIOACTIVE ELEMENTS IN THE MUD OF LAKE VADKERT

M. KEDVES AND T. SZEDERKÉNYI

*Department of Botany and Department of Mineralogy, Geochemistry and Petrology  
Attila József University H—6701 Szeged, P.O.B. 654, 651, Hungary*

(Received: March 20, 1987)

### Abstract

Using transmission electron microscope method we have established the following: 1. The sub-microscopic structure of the xylem remnants transporting radioactive elements reworked into the mud of Lake Vadkert may be studied in ultra-thin sections. 2. The ultrastructures of the xylem remnants is not the same in the different samples studied. This is the consequence of the different degree of coalification or the taphonomical process. The tendency of the change is towards the homogeneous coal without sub-microscopic structure. 3. The lamellar ultrastructure, described from the secondary xylem of the recent taxa was in some places discernible. 4. There are granules with high electron density in the organic debris enclosing the xylem fragments.

*Key words:* Palynology, Xylotomy, ultrastructure.

### Introduction

In the course of our complex investigations of the mud of Lake Vadkert (KEDVES and KÖRMÖCZI, 1985) we have mentioned that in the Holocene mud of the lake there are dark coloured xylem remnants, too. On the basis of our further investigations it was established that the mud of Lake Vadkert is radioactive (KEDVES and SZEDERKÉNYI, 1985), and the intensity of this radioactivity is twofold of the "usual". Among others we have pointed out the curative effect of the mud. Further, complementary and verificatory investigations were carried out of the mud of Szik-sóstó (Dorozsma, Szeged) and some localities of the backwater of Tisza. Based on these results the transport of the radioactive elements is connected with the rebedded secondary xylem remnants. Referring to this problem here are two basics: SZALAY (1954), p. 310: "Laboratory experiments of the author revealed that decomposing plant debris, peat, lignite, and brown coal have a very high adsorption power and capacity for uranium, which is in fact sufficiently high to explain geochemical enrichment." BREGER, DEUL and RUBINSTEIN (1955), p. 226: "Uranium is not held in the coal by ion exchange but seems to be present in the form of organo-uranium compounds that are soluble at pH less than 2.18". From several point of view it was necessary to carry out detailed investigation of the secondary xylem remnants. The aim of the first investigation with transmission electron microscope was as follows:

1. Are the dark coloured and coalified secondary xylem remnants suitable for transmission EM investigation, e.g.: is it possible to prepare ultra-thin sections for this purpose?

2. When the TEM method is suitable for these xylem remnants, what kind of information can be obtained for the diagenetic process of the secondary wood on the basis of its ultrastructure?

### Material and Methods

Altogether 10 xylem fragments were used for transmission electron microscopical investigations. Among them macroscopically six were stronger, four were in a less degree coalified. The method of the mud samples for microscopical plant remnants was published in a previous paper (KEDVES and KÖRMÖCZI, 1985). In this way, we mention the most important steps: HCl to eliminate carbonates and sulphides; ZnCl<sub>2</sub> solution density about 2 to separate organic matter from the inorganic HF to eliminate residous inorganic materials. After washing, postfixation with OsO<sub>4</sub> aq. dil., embedding in Araldite. The ultra-thin sections were made with glass knives in the EM Laboratory of the Biological Center of the Hungarian Academy of Science in Szeged, with an ultra-microtome of Porter Blum. The pictures were taken in the EM Laboratory of the J. A. University, on a Tesla B-500 electron-microscope, resolution 6 Å. We express our sincere thanks to Dr. I. ROJIK for his kind technical assistance. Among the xylem remnants investigated seven are from sample A/1, in which the colonies of the *Botryococcus* algae occur in predominant quantity. Among the pollen grains, *Phragmites* occur in the highest per cent, the more important of these: Cyperaceae, *Urtica*, *Allium* and Chenopodiaceae. Three secondary xylem remnants from mud sample No. II/4 were selected for TEM investigations. In consequence of the strong biological activity the plant microfossil remnants of this sample decomposed; pollen grains of the Cyperaceae occurred in the largest quantity (cf. KEDVES and KÖRMÖCZI, 1985, Fig. 2, p. 270). The geochemistry of the lignin derivatives (cf. HATCHER et al., 1988) was not studied during our investigations.

### Results

In the investigations with LM method, the dark coloured secondary xylem remnants (Plate I, fig. 1,3) can be well distinguished from the other Holocene plant tissue remnants (Plate I, fig. 2, 4—6), and from the pollen grains, respectively (Plate I, fig. 7—9). Among the Holocene tissue remnants there are parenchym (not lig-

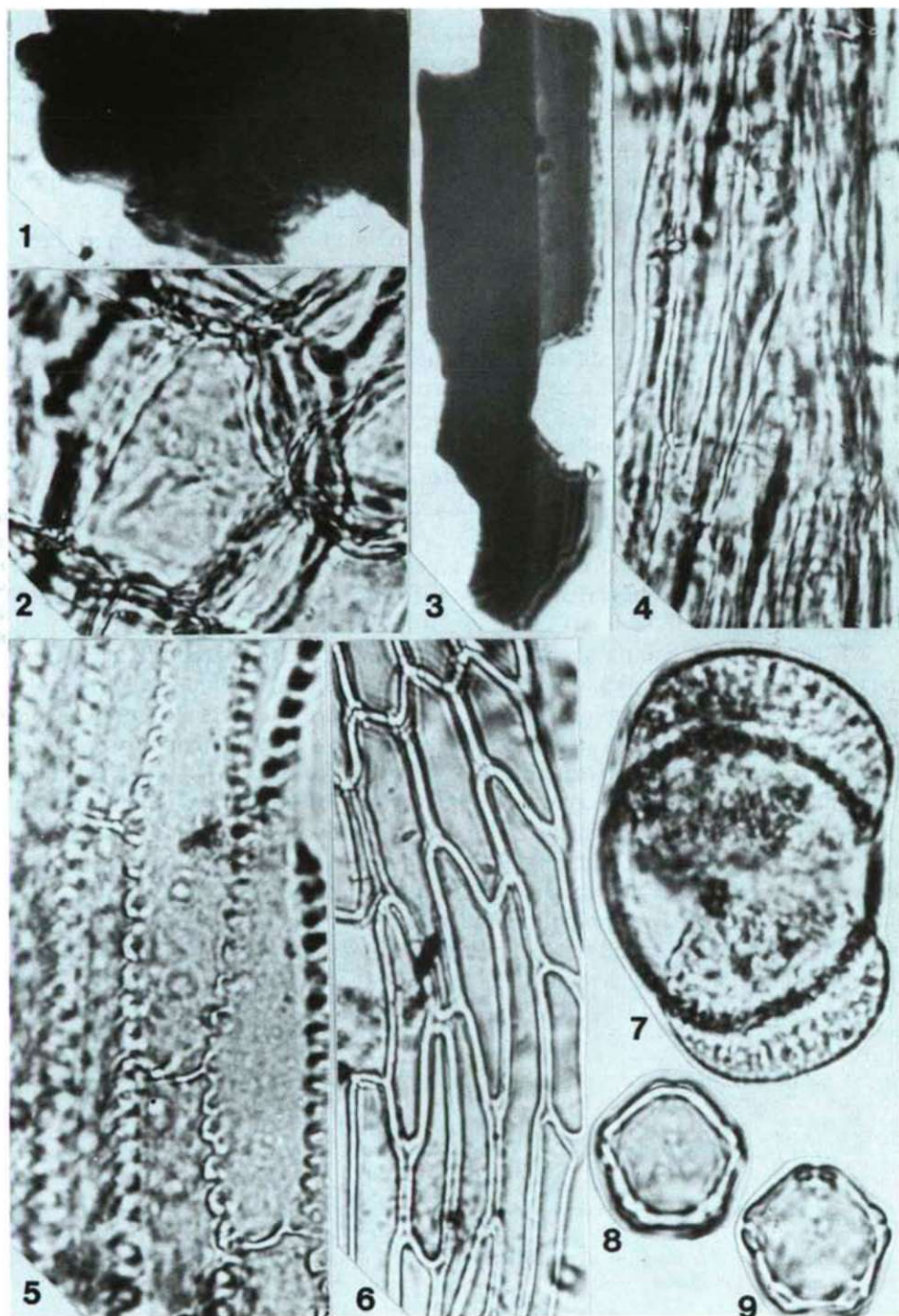
#### Plate I

1—9. LM pictures from the microscopic plant remnants of the mud of Lake Vadkert.

1. Reworked secondary xylem fragment; prep. A/1-1; 11.4/141.7.
2. Holocene parenchym tissue remnant; prep. A/1-2; 11.5/142.4.
3. Reworked less coalified secondary xylem remnant; prep. A/1-1; 19.8/138.4.
4. Holocene tissue remnant with fibrous cells; prep. A/1-2; 16.3/142.8.
5. Holocene epidermis remnant of Gramineae type; prep. A/1-1; 17.5/140.8.
6. Holocene epidermis remnant of dicotyledonous type; prep. A/1-2; 18.5/144.8.
7. *Pinus* pollen; prep. A/1-2; 11.8/142.4.
- 8,9. *Alnus* pollen; prep. A/1-1; 11.9/139.8.

X1000





nified), (Plate I, fig. 2), prosenchyma (Plate I, fig. 4) and epidermis remnants (Plate I, fig. 5, 6), too. Among the dark coloured secondary xylem remnants, which transport the radioactive elements there are some where the vessel structure can be recognized, but several, in all probability because of the high degree of coalification are completely homogeneous. It is regrettable, but rebedded sporomorphs were not observed in the spore-pollen assemblages, what was very important in the determination of the geological age of the rebedded secondary xylem remnants.

With the transmission electron-microscope method the following may be established about the not strongly coalified secondary xylem remnants (Plate II, fig. 1—4 plate III, fig. 1, 2): The lamellar structure of the wall of the secondary xylem can be recognized of course with secondary alterations. Some of the lamellae are darker coloured; there are differences in the osmium affinity. The submicroscopic structure can be established in consequence of the taphonomic process, the preparation procedure, made possible in different degree to study the submicroscopic structure of the wall of the secondary xylem. These structures are in one respect in the dimension of the fibrils which are discernible with transmission electron-microscope (Plate II, fig. 1—4, plate III, fig. 1), on the other hand in the dimension of large biopolymer structures (Plate III, fig. 2).

On the basis of ultrastructure two types can be distinguished at the strongly coalified secondary xylem fragments (plate III, fig. 3, 4, plate IV, fig 1—3, plate V, fig. 1—3):

1. More or less compact remnants, their submicroscopic structure identical on the outer and the inner parts of the debris (Plate III, fig. 3, 4, plate IV, fig. 1, 2). The lamellar structure of the wall of the secondary xylem may be perceived only in some places. There are holes oriented towards the elements of the secondary xylem. In these fragments the fibrillar structure, which is common at the not strongly coalified remnants occur only in some parts, and in a strongly dezorganized condition (Plate IV, fig. 2). Fibrills in the dimension of biopolymers were not detectable. On the xylem remnants there are organic debris, with well defined morphological features, some are electron dense material (Plate III, fig. 4, plate IV, fig. 1).

2. In the case of the second types the ultrastructure of the secondary xylem remnant differs on the outer and the inner part. The inner part is roughly identical with the above-mentioned one, strongly coalified, and its consistence is compact. For the

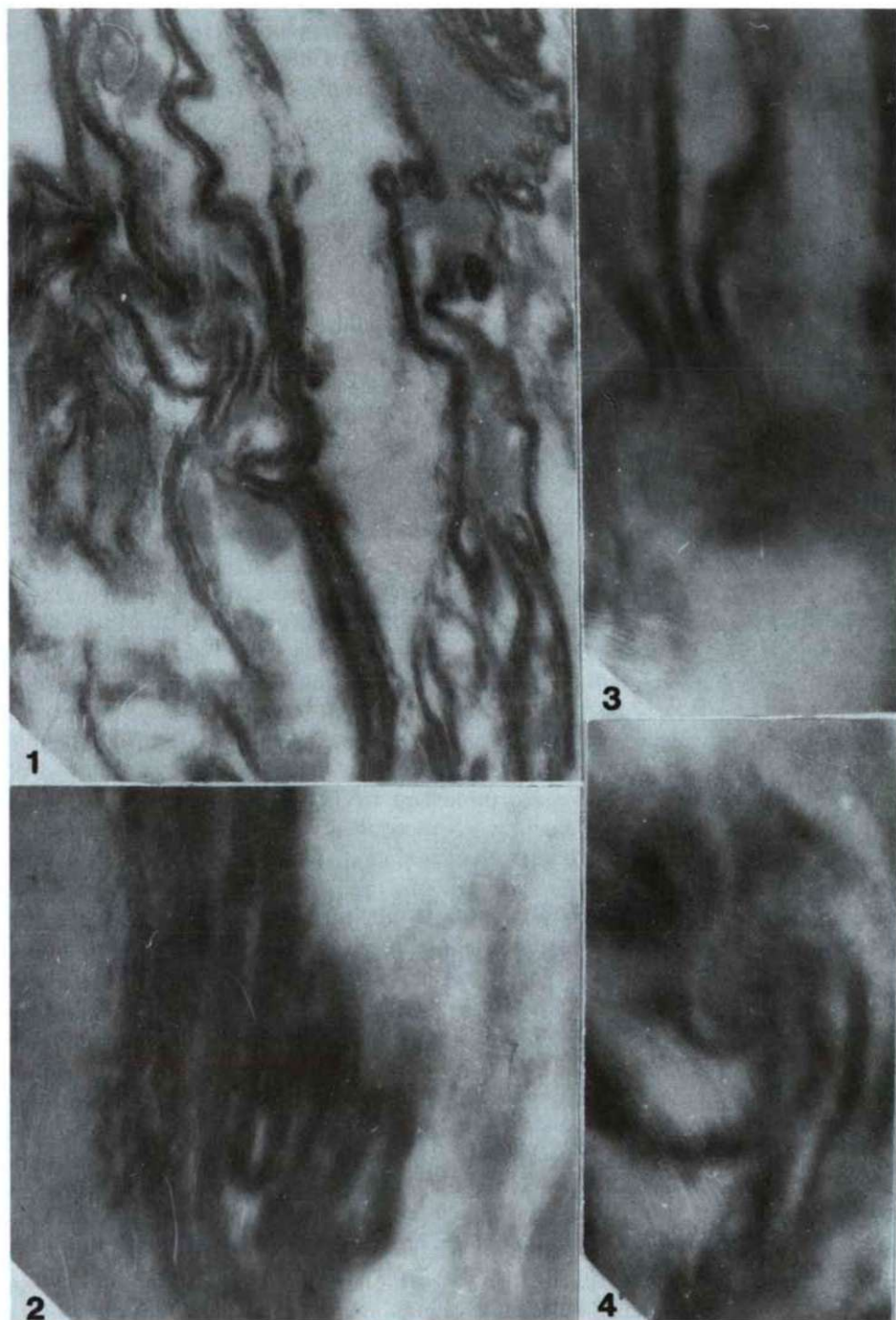
#### Plate II

1-4. Transmission electron microscope pictures from less coalified reworked secondary xylem remnants.

1. General picture from the ultrastructure of the wall of the secondary xylem. Sample No: II-4; block-number: 86/19; x50000.

- 2-4. Ultrastructure of the fibrills. Sample No: II-4; block-number: 86/19; x150000.





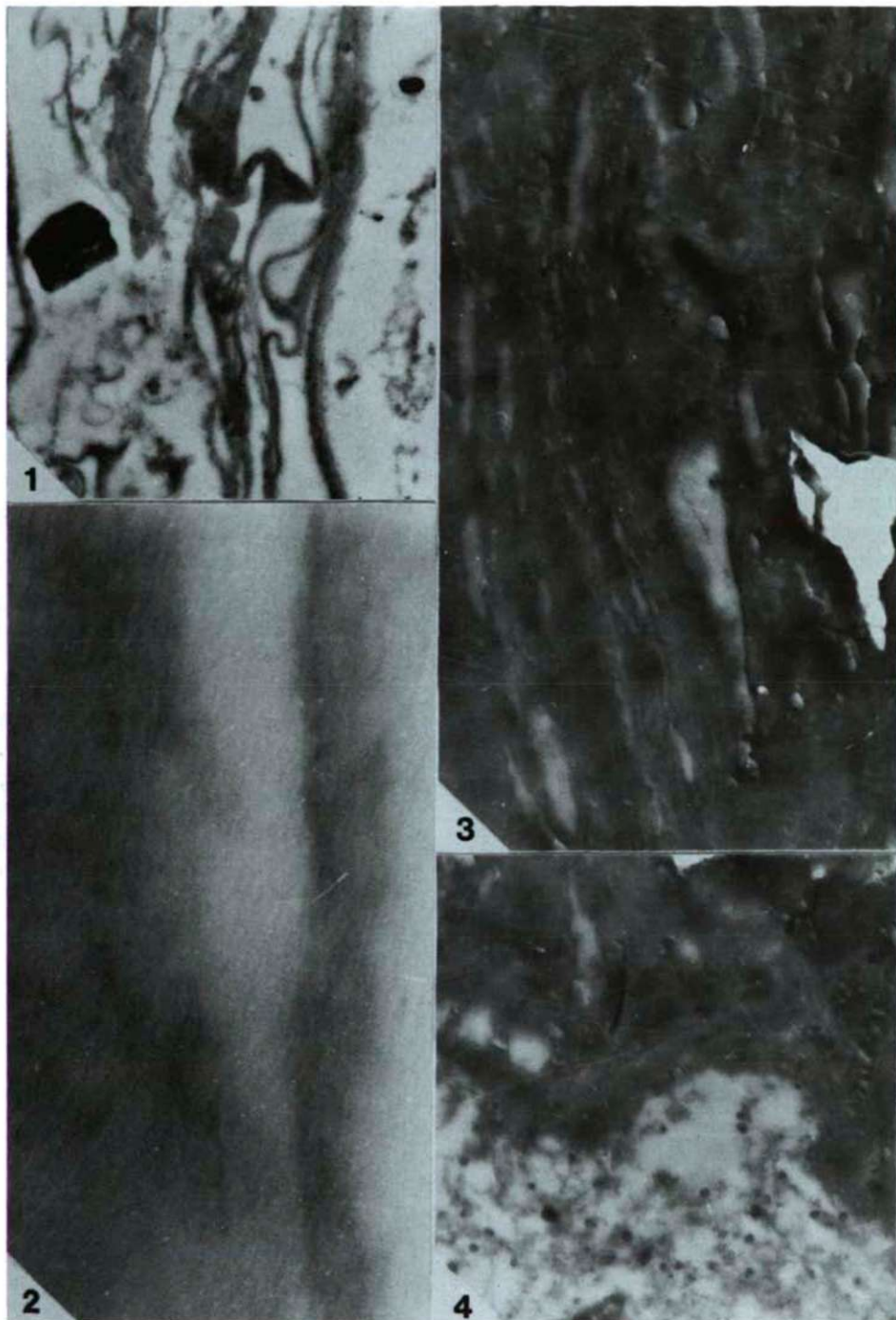
outer part of the secondary xylem fragment a loose structure is characteristic, and in a degraded condition a fibrillar structure can be discernible (Plate IV, fig. 3, plate V, fig. 1, 2). In general, the fibrills are strongly degraded (Plate V, fig. 3).

### Discussion

As regards the fine structure of the secondary xylem several results were published, with several conceptions. FREY—WISLING (1957) emphasized that the ultrastructure of the secondary xylem is useful to solve taxonomic problems. KORÁN and CÔTÉ (1964) established that the ultrastructure of the tyloses is in connection with the mechanism of the growth. FREI et al. (1957) described the ultrastructure of the tracheids of the conifers. CHAFE (1974) in the thickened and primary wall of *Chamaecyparis nootkatensis* (D. DON.) SPACH. observed lamellar structure. PARAMESWARAN and LIESE (1978) in connection with the investigation of the bamboo established the following; p. 7: "Cross section of the wall thickening (w) of protoxylem element evidencing parallel arrangement of microfibrils." Concerning our own results the paper by HARCHE and CATESSON (1985) is particularly important, but it is important concerning all investigations of the xylem; p. 61: "Lignified secondary walls appear compact on ultra-thin sections and their texture is difficult to visualize without a prior extraction of matrix material." In this way the fact for itself that the secondary xylem fragments transporting reworked, coalified radioactive elements are suitable for transmission electron microscopical investigations can be taken as a result. We think it is important to point from the above cited authors the following; p. 61: "Methylamine alone was reported as a good, mild extractant of wood all walls (CZANINSKI et MONTIES, 1982 ROLAND et MOSINIAK, 1983)." The polylamellate structure published by HARCHE and CATESON (1985) was suitable for transmission electron microscopical investigations after the elimination of the polysaccharids. Essentially it is similar to the method of 2-aminoethanol and other solvents and oxidizing agents in the investigation of the biopolymer system of the sporoderm; cf. ROWLEY et al. 1981, SOUTHWORTH, 1974, 1985). The polylamellate structure observed by us (Plate III, fig 2) regarding its dimension and degree is essentially lower than those published in the data in several papers. In this

### Plate III

1. General picture from the ultrastructure of a less coalified secondary xylem. Sample No: II-4, block number: 86/22; x10000.
2. Detail from the fibrillar structure of the wall of the secondary xylem. Sample No: II-4; block number: 86/22; x250000.
3. General picture from the ultrastructure of a coalified secondary xylem. Sample No: A/1, block number: 86/36; x10000.
4. Ultrastructure of the xylem remnant, and of the organic remnants of the embedding sediments. Sample No: A/1; block number: 86/36; x10000.





way during the sedimentation and the preparation method the investigated structure by TEM method was essentially the same as that of the secondary xylem of the recent taxa. In both cases some polysaccharids were degraded.

Using the transmission electron microscope method on fossil leaf remnants BROWN et al. (1978) observed the microfibrillar structure of the cellulose. SMOOT and TAYLOR (1984) established essentially the same; p. 621: "The cell walls of sieve elements in the primary phloem of the Carboniferous fern *Tubicaulis* contain structural features that morphologically resemble cellulose microfibrils in extant plants." P. 622: "The presence of structurally identifiable but chemically altered fibrils in a fossil plant 290 million years old underscores the fidelity of morphological preservation in coal balls and suggests that it may be possible to use fossil material to investigate the evolution of such basic biological phenomena as the organization of cell wall constituents."

The phylogenetical significance of the biopolymer structures of the sporopollenin was pointed by KEDVES (1986) in connection with the degradation of the sporoderm. This molecular — biopolymer evolution may be extended to all remnants of living taxa containing organic materials.

Finally, in connection with the characteristic holes of the coalified secondary xylem remnants it can be noted that during the coalification and fossilization respectively gases may be formed also and this stretched the fibrills of the secondary xylem. In this way the transmission electron microscope method can serve data in the solution of such problems.

## References

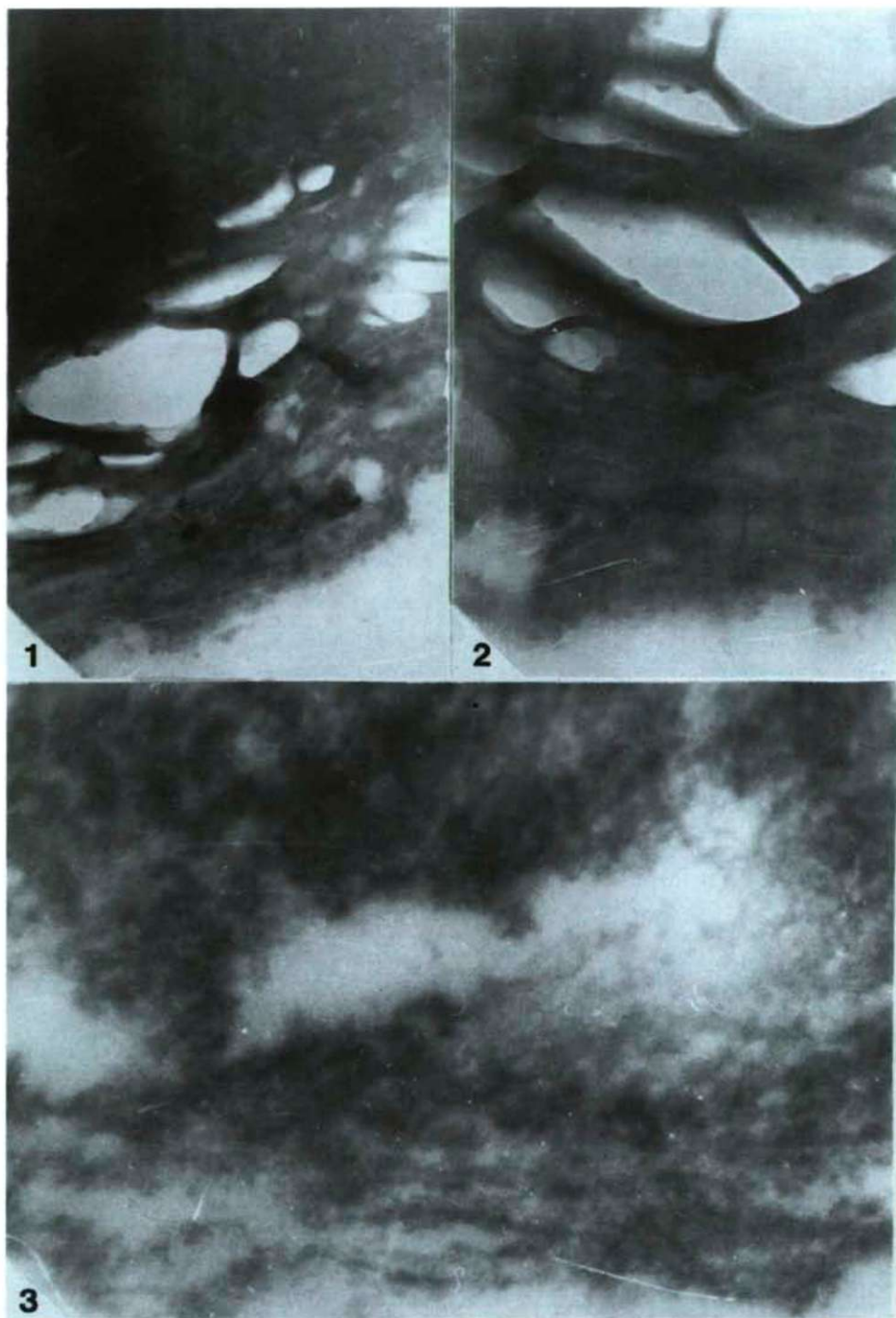
- BREGER, I.A., DEUL, M. and RUBINSTEIN, S. (1955): Geochemistry and mineralogy of an uraniferous lignite. — *Economic Geology* 50, 206—226.
- CHAFE, S.C. (1974): Cell wall thickenings in the ray parenchyma of yellow cypress. — *IAWA Bull.* 2, 3—10.
- CZANISKI, Y. et MONTIES, B. (1982): Étude cytologique ultrastructurale des parois du bois de Peuplier après extraction ménagée. — *C.R. Acad. Sc.* 295, 551—556.
- FREI, E., PRESTON, R.D. and RIPLEY, G.W. (1957): The fine structure of the walls of conifer tracheids. VI. Electron microscope investigation of sections. — *J. Exp. Bot.* 8, 139—146.

## Plate IV

1. Ultrastructure of the secondary xylem remnant and of the organic remnants of the embedding sediment. Sample No: A/1; block number: 86/36; x25000.
2. Detail from the ultrastructure of the degraded fibrills. Sample No: A/1; block number: 86/36; x50000.
3. Ultrastructure of a coalified xylem remnant. The inner part is compact, the outer one is more loose. Sample No: A/1; block number 86/38; x25000.









- FREY—WYSSLING, A. (1957): Use of the electron microscope in wood taxonomy. — IAWA News Bull. 1, 1—4.
- HARCHE, M. and CATESSON, A. M. (1985): Cell wall architecture in alfa (*Stipa tenacissima* L.) fibres. — IAWA Bull. n.s. 6, 61—69.
- HATCHER, P.G., LERCH III, H.E., VERHEYEN, T.V. and WILSON, M.A. (1988): Organic geochemical studies of the early coalification of peat and xylem tissue. — U.S. Geol. Surv. Circul. 1025, 18—19.
- KEDVES, M. (1986): Dégénération expérimentale de la paroi pollinique. — VI Simp. de Palinol. Resumenes, 20.
- KEDVES, M. et 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 of the lake mud. — Acta Biol. Szeged 31, 215—216.
- KEDVES, M. and SZEDERKÉNYI, T. (1986): Investigations on the microscopic plant remnants and the radioactive element content of some samples of the Hungarian Plain. — Acta Biol. Szeged 32, 209—211.
- KÓRÁN, Z. and COTÉ, W.A. (1964): Ultrastructure of tyloses and a theory of their growth mechanism. — IAWA News Bull. 2, 3—15.
- NIKLÁS, K.J., BROWN, R.M., SANTOS, R. and VIAN, B. (1978): Ultrastructure and cytochemistry of Miocene angiosperm leaf tissues. — Proc. nat. Acad. Sci. 75, 3263—3267.
- PARAMESWARAN, N. and LIESE, W. (1978). A note on the fine structure of protoxylem elements in bamboo. — IAWA Bull. 2—3, 28—32.
- ROLAND, J.C. and MOSINIAK, M. (1983): On the twisting pattern, texture and layering of the secondary cell walls of lime wood. Proposal of an unifying model. — IAWA Bull. n.s. 4, 15—24.
- ROWLEY, J.R., DAHL, A.O., SENGUPTA, S. and ROWLEY, J.S. (1981): A model of exine substructure based on dissection of pollen and spore exines. — Palynology 5, 105—152.
- SMOOT, E.L. and TAYLOR, T.N. (1984): The fine structure of fossil plant cell walls. — Science 225, 621—623.
- SOUTHWORTH, D. (1974): Solubility of pollen exines. — Amer. J. Bot. 61, 36—44.
- SOUTHWORTH, D. (1985): Pollen exine substructure. I. *Lilium longiflorum*. — Amer. J. Bot. 72, 1274—1283.
- SZALAY, S. (1954): The enrichment of uranium in some brown coals in Hungary. — Acta Geol. Acad. Sci. Hung. 2, 299—311.

## Plate V

1. Detail from the ultrastructure of the outer loose part of the carbonified secondary xylem remnant. Sample No: A/1; block number: 86/38; x50000.
2. The same detail with larger magnification. Sample No: A/1; block number: 86/38; x100000.
3. Degraded fibrills. Sample No: A/1; block number: 86/38; x100000.