EDS ANALYSIS OF POLLEN WALL SURFACES OF *VERNONIA MONOSIS* CL. (ASTERACEAE) AND POLLEN-SOIL CONCENTRATION OF ELEMENTS

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Abstract

Pollen materials of *Vernonia monosis* Cl., a small tree belonging to the Compositae, collected from different distributional sites in the Western Peninsula of India were subjected to x-ray microanalysis of elements with a Kevex energy-dispersive spectrometer.

The present study comprises three parts : the chemistry of wall surfaces, unprocessed pollen and its relation to that of the soils underlying the trees and a third section pertaining to the addition and extraction of elements onto and out of the pollen wall surfaces by different chemical processing treatments.

Introduction

Energy dispersive spectrometry in conjunction with the scanning electron microscope, is a convenient method for elemental analysis of plant tissues ((Dengler & Lin, 1980; Chong & Harder, 1980; Bennett & Sangster, 1982), fungal hyphae (Kunoh & Ishizaki 1980) and algae (Tillberg, Rowley & Barnard, 1980). Various methods of extraction, detection and quantitative assay of minerals in pollen and spores have been reviewed (Stanley & Linskens, 1974). The significant amount of silicon in pollen was detected by EDS analysis (Crang & May, 1974; Vasanthy & Pocock, 1980; Pocock & Vasanthy, 1984). Tryon and Lugardon (1978) provide a review of silicon occurrence in Bryophyte and Pteriodophyte spores. In the present investigation we have analysed the wall surfaces of the pollen of *Vernonia monosis* Cl. from different distribution loci in South India (Map 1 & Table I) and in some cases, soils collected from close to the bases of these Compositae trees. As the pollen mineral content, especially the microelements, appears to be, to some extent, reflective of plant nutrient supply (Stanley & Linskens, 1974), our investigation may help to understand and correlate the soil-plant-pollen relationships. In the ensuing paragraphs, as part of the introduction, we will briefly review current views and knowledge of certain basics of pedology relevant to the plant-soil relationship and the root absorption of nutrients.

The consideration of ecological significance in the light of analysis of mineral constituents of the soil is one of the current fields of pedological investigation. Prevailing bioclimatic and local factors in addition to the ancient weathering history are factors partly governing pedogenesis: the formation and evolution of weathering complex of soil. In tropical countries, the soils, at times, carry the traces of past climatic variations. Local influence of secondary plant associations or degraded ones, resulting from human interference with the climatic vegetation upon soil is considerable. Such secondary associations could affect the biochemical cycles of the nutrient elements and the humification

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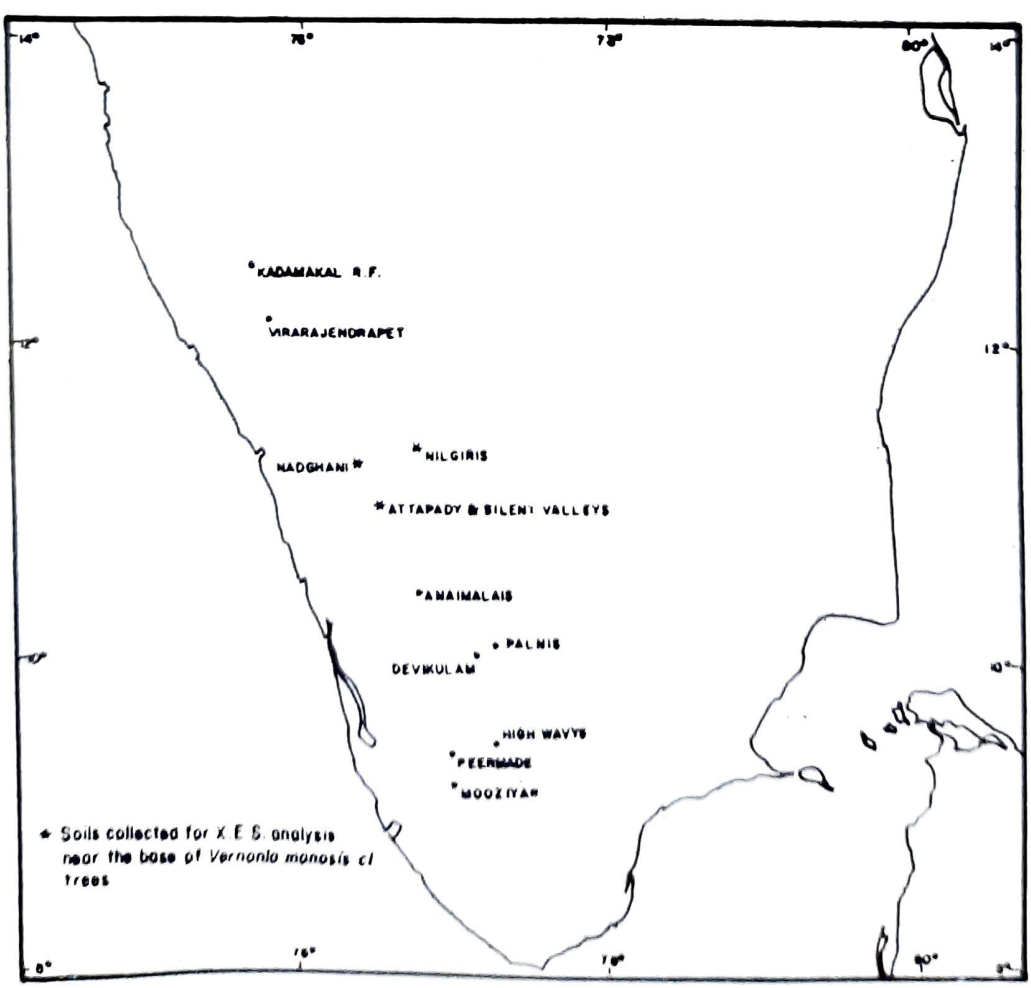
processes, resulting in a type of pedogenesis differing significantly from the predicted climatic type. *Vernonia monosis* Cl. is commonly found growing in disturbed, or secondary, forest fringes.

Soil is a very complex medium which forms a nutritional store for plants. As physical chemical and biological properties partly control the physiology of plant nutrition, the concept of exchangeable ions that cations and anions adsorbed by colloids are capable of being absorbed by roots, either directly or from soil solution, is a significant development in soil chemistry. The phenomenon of ion-exchange in which the soil plays the role of both source and sink for ions in soil solution. "Pedoclimate" as defined by Bonneau (1979) is the combined effect of its atmosphere, temperature and moisutre content over a particular area, the three components of pedoclimate being temperature, water regime and oxygen content. Pedoclimatic factors such as weathering, microbial activity and organic matter development, form and availability of elements to root systems and root activity, influence the evolution and functions of soils and soil-plant relationships.

The mineral nutrition of plants implies the availability of minerals in suitable form at the contact surfaces of roots. Plant nutrition is a complex and dynamic process depending chiefly upon the root-profiles, the root-soil interface and the mode of entry of ions into roots. A number of soil processes such as aeration, the water content of soil and temperature (involving changes in elements and absorption by roots) could alter the availability of elements to plants. Absorption by roots (controlled by the mobility of ions in the liquid phase, their absorption properties and diffusion coefficients), root exploration varying with plant species, the nature and depth of soil, the conditions in the soil-solution medium,



Map 1. Distribution loci of *Vernonia monosis* cl

porosity and texture of soil, soil reserves and their nutritional properties, water parameters (movement condition and its movement through the soil) and pedoclimate have all been discussed by Blanchet (1979).

Distribution and Ecology of *Vernonia monosis*

Vernonia monosis C. B. Cl. is a small tree, 10-30 m in height, widely distributed in the Western Ghats, usually above 1000 m, from Coorg and the Bababudan hills of Mysore to the Nilgiris, Anamalais and the hills of Travancore. In the Biligirirangan hills (link between the Western and Eastern Ghats), it is growing in the Sholas, or montaine evergreen forests, on the eastern slope at about 1600 m (Coll. M. Deshayes & B. R. Ramesh, HIFP). The pollen samples used in this study are from different distributional sites or loci along the western part of the Indian peninsula from Kadamakal reserve forest in Coorg (12 31′ N; 75 38′ E) to Moozhi Ar (9 19′ N; 77 04′ E), their altitudinal range being 500 m through 1900 m. Two other related tree Composites occur in southern India:

—*Vernonia shevarpyensis* C. B. Cl. is restricted to one locus in the Eastern Ghats: Shevaroy hills..

—*Vernonia travancorica* Hook. f. is endemic to the hills of Travancore and Tinnevelly, the southern extremes of the Western Ghats.

Vernonia monosis is an heliophilous tree thriving in openings (forest clearings), in forest fringes or in ecotonal areas (transitional zones intermediate between forest & savanna). It frequently occurs as a secondary vegetation but never grows inside the dense forests, nor as undergrowth of forests; rainfall of its habitats is generally about 2000 mm. In the Nilgiris at 1000-1100 m and in Nadughani at 900 m it is found near streams, by the roadside, where the vegetation has been vulnerable to human interference. Work on the ecological association of other plants with *V. monosis*, based upon the field observations of J. P. Pascal (1974-1982) and Vasanthy (1978, 1984) is continuing.

Method : EDS elemental analysis of pollen and soils

Pollen from the indehisced anthers of mature flower buds were isolated without recourse to any rehydrant or fixative so as to circumvent any leaching or redistribution of soluble ions, such as Ca, Mg, K and Na. These were fixed onto carbon stubs employing double sided tape as adhesive. Similarly pollen isolated in distilled water, pollen treated with ethyl alcohol, and with 49% hydrofluoric acid, acetolysed, acetolysed and chlorinated and boiled in 10% KOH for 30 mins. were prepared for EDS analysis to investigate the effects of these treatments upon the chemistry of pollen wall.

Soil samples from four locations (Table 2) were collected close to the base of trees down to 70 cm using a geological borer. Each sample was dried at room temperature and passed through a 2 mm mesh screen before crushing. The crushed material was passed through a 200 μm sieve prior to mounting on a carbon stub. In mounting, the carbon stub which has been treated with double sided adhesive tape, is pressed firmly into the powder to be analyzed, the excess being removed by blowing with compressed air. This produces an homogeneous, random coating to the stub which is necessary for reliable analysis.

X-ray elemental microanalyses of the pollen wall surfaces of interapertural (lophate) areas were run with a Kevex 7000 energy dispersive spectrometer connected to an ETEC scanning electron microscope. An activating voltage of 30 kv was employed to analyze a pollen wall area of about 1 μm. Since the analyses were based upon constant count, the collecting time varied from sample to sample. The energy dispersive spectrometer detects

the elements with an atomic number of 11 (Na) or higher in the Periodic Table. A count of 200,000 was employed for pollen wall analysis and 50,000 for soil-sediment analyses, where a larger scan area could be utilised (see Text-figs 1, 2). The results were digitised, smoothed and normalised to obtain a numerical printout in the form of elemental relative abundance (cf. Trautman & Pocock, 1983).

Pollen wall surface materials

The scanning electron micrographs (Pl. 1, figs. 4-5) of unprocessed pollen dissected out of the indehisced anthers of mature flower buds, without any rehydrant or fixative, show the pollen surface covered with materials obscuring the surface details and shape of the spinules. Lipid droplets were observed in pollen isolated in distilled water, sticking to the surface, especially in the apertural areas (Pl. 1, figs. 1-3) and becoming dispersed in the glycerine jelly mountant among the pollen grains.

The concentration of an element in plant tissues differs with the species, climatic conditions and the portion of the plant selected for analysis. Since we have chosen to analyse unprocessed pollen and its surface materials for mineral content, it is pertinent to discuss here the sources of materials covering the pollen wall surface during pollen maturation :

—The tapetal influence over the transport of materials to the pollen grain appears to be considerable. Though these materials may not be the direct derivatives of tapetum, they could have passed through the tapetum.

—Pollenkitt is an oily layer, found on the outside of mature pollen grains in many insect pollinated species. It is mainly an hydrophobic lipid containing species-specific carotenoids.

—Lipidophilic globules are extended from plastids into the periplasmodial cytoplasm and this lipid material is eventually deposited on the pollen grain surface. This material is separate and distinguishable from the lipid extruded from within the pollen grain.

—Tryphine (a transitory material) appears to be a complex mixture of hydrophilic substances and derived from the breakdown of the tapetal cells during the final stages of anther maturation.

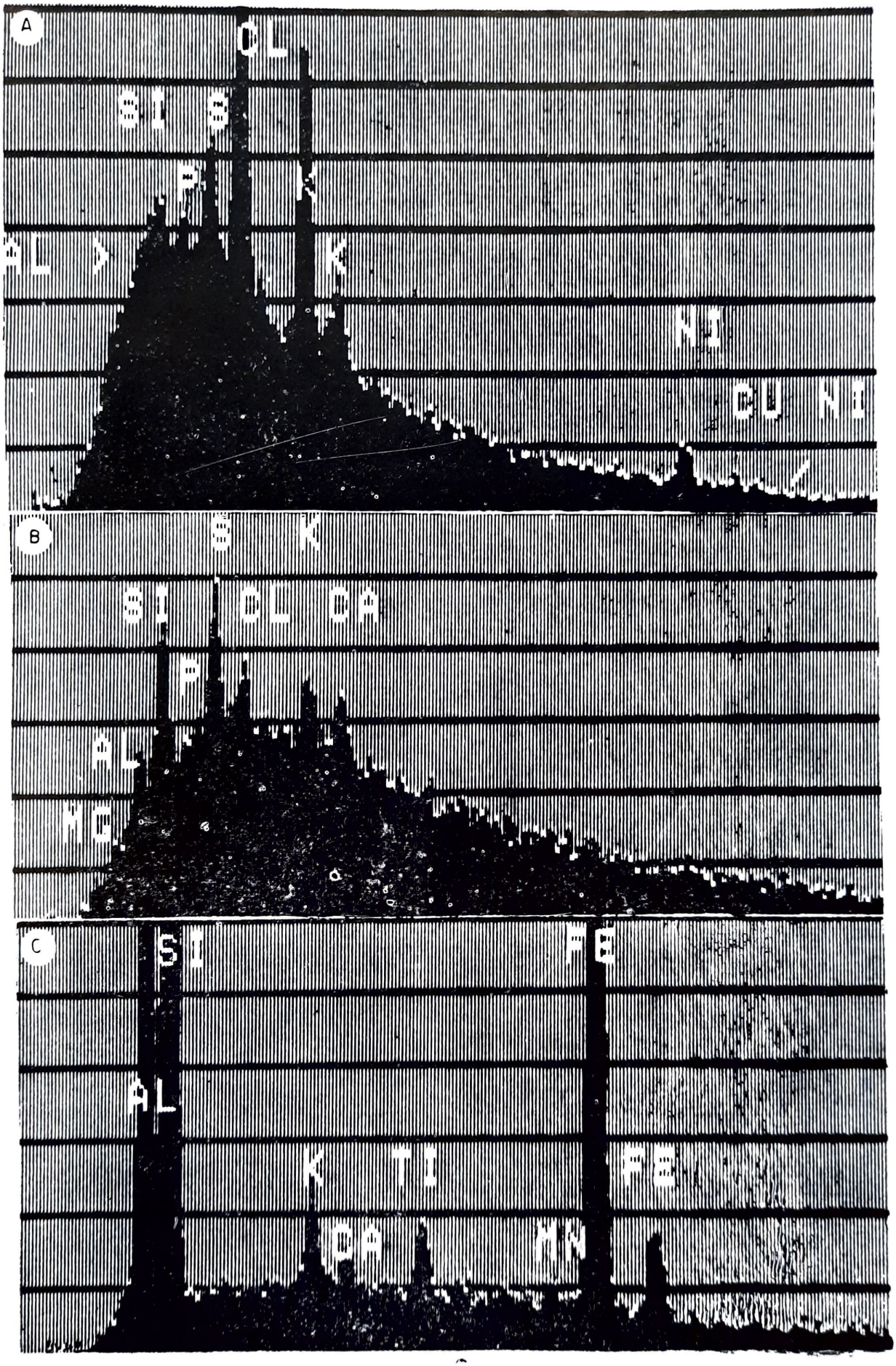
—Although the apparent contribution of the tapetum to the developing microspores is evident, the products and mode of contribution are still unclear.

—Upon initial hydration of dry pollen grains, lipids are dispersed into the hydrating medium in *Tagetes patula* of the Compositae, which compares closely with what we observe with *Vernonia monosis* pollen.

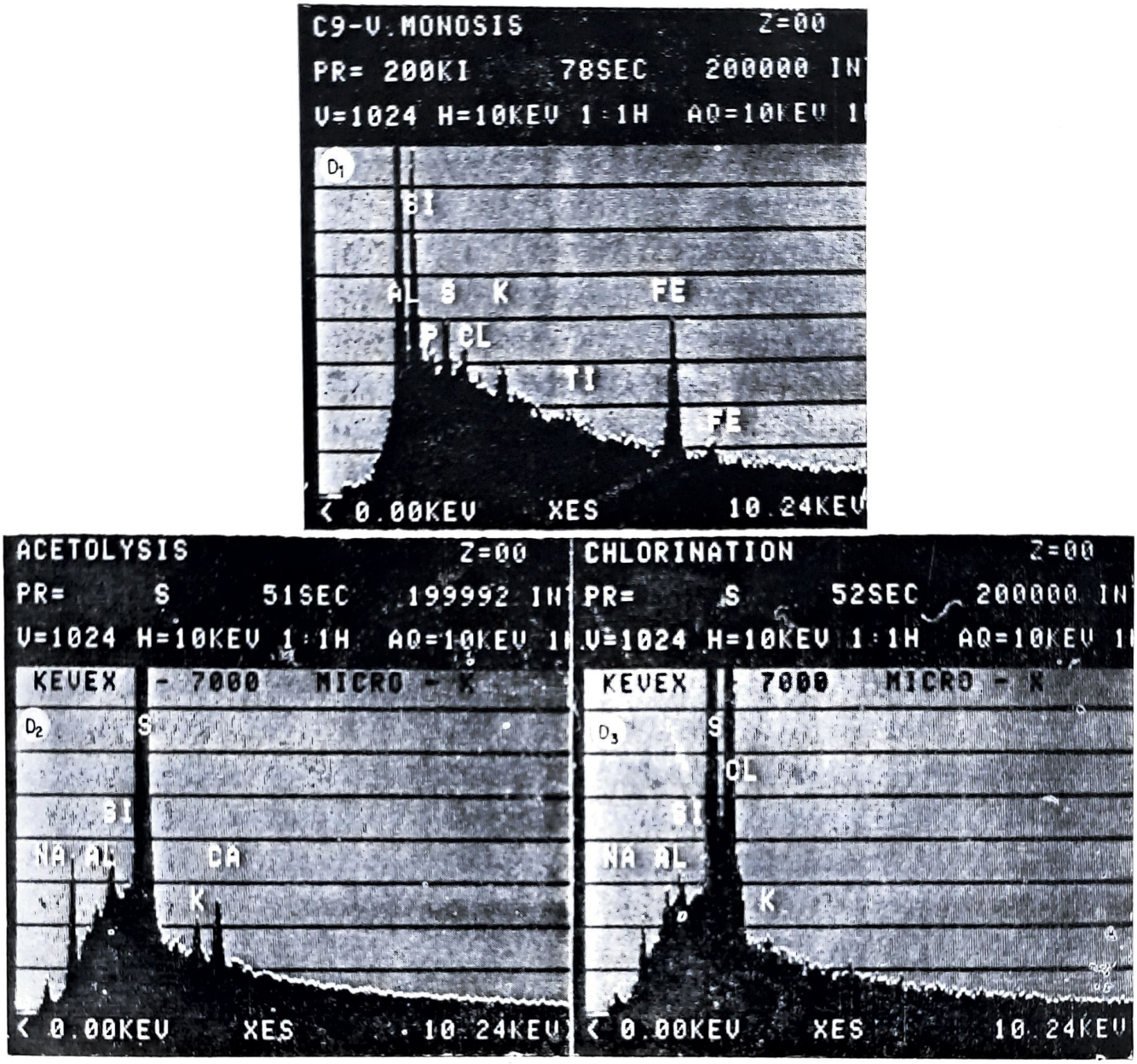
—In many angiosperm families the pollen wall conveys enzymes and other proteins. The protein load of the exine is, as with the surface materials, derived from the anthers. From the above findings and surmises (reviewed in Echlin, 1971; Heslop-Harrison, 1968, 1979), we may infer conjecturally that the surface materials of unprocessed pollen of *Vernonia monosis*, which was analysed for elemental content were derived more from or via the anther tissue than from within the pollen cytoplasm.

Results and discussion

Our present study comprises three parts. The first part concerns the chemistry of unprocessed pollen wall surfaces of *Vernonia monosis*, the second with the influence upon it by the chemistry of soils underlying the trees. The third section concerns the addition and extraction of elements onto and out of the pollen wall by different chemical processing treatments.



Text-fig. 1—X-ray elemental analyses : A. Unprocessed pollen from poisoned herbarium specimen (Palnis : Top Station F. Blasco, HIFP); B. Unprocessed and unpoisoned pollen (Nilgiris : Thoodoor Mattam); C. Soil, 0-15 cm depth from Nilgiris : Thoodoor Mattam.



Text-fig. 2—X-ray elemental analyses of *Vernonia monosis* pollen from Attapady Valley. D1. Unprocessed. D2 : Acetolysed. D3 : Acetolysed and chlorinated.

Soil analyses

Fourteen elements : Na (absent to negligible in these non-marine sediments), Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe and Cu have been detected (Table 2). Al, Si, and Fe are the principal elements encountered in these soils. Quantitative analysis of Nadghani soil (100 gms) by Dr Janel and team indicates the following absolute abundances of these elements:

Al (as Al_2O_3) : 14.78%
Si (as SiO_2) : 22.54%
Fe (as Fe_2O_3) : 10.51%

The amount of phosphorus appears to be very poor to negligible in all of the samples studied. Frequently the rain forest margin appears to be limited by sudden changes in

edaphic, rather than climatic, conditions. These are notably connected with areas of low soil fertility and especially by dearth of soil phosphates.

The "weathering complex" of soil contains, besides clay minerals, (silicates), oxides and hydroxides of iron, aluminium, manganese, silicon and titanium. These elements could have been liberated during the weathering of primary minerals in soluble (ionic) form or complexed with organic materials intervening in the reaction. Upon liberation, the basic cations, calcium, potassium, magnesium and sodium are frequently lost from the soil by leaching; whereas the cations of Fe, Al and Mn and silica undergo reactions in the soil which result in the formation of both amorphous and crystalline compounds which lessen their loss by drainage. Specific roles of aluminium and iron, despite certain almost identical geochemical properties, are quite different. In the ionic form aluminium is more associated with physiochemical behaviour than iron and is also, in part, responsible for soil acidity. Weathering releases silica in a soluble state (monosilicic acid) which transforms into amorphous and crystalline form. Unlike Fe and Al, a significant part of soil silica is recycled by vegetation and returned in the form of bio-opal (opal phytoliths) in humus-rich horizons. The soluble and exchangeable form of Manganese (Mn) is assimilated by plants. Titanium in hydrolysed ferrallitic soils is accumulated in considerable quantities and can be mobilized by organic complexing. Magnesium is leached much more strongly than Calcium (Bonneau & Souchier, 1982).

Pollen wall analysis

In our first experiment herbarium specimens of pollen of *Vernonia monosis* were obtained from ten different distributional sites (Table 1). Relative abundance determinations were made on eleven elements detected on the surfaces of pollen dissected out of anthers in dry condition. These elements were Mg, Al, Si, P, S, Cl, K, Ca, Fe, Ni and Cu. Table 1 summarizes the numerical value of qualitative spectra obtained by EDS analysis. The numerical value of counts may be indicative of, or approximate to, the value of the differential concentration of elements of the pollen surface materials. Since the numerical values are indicative of relative abundance and not absolute quantitative concentrations, we could not positively infer that they are indicators of inhomogeneous elemental distribution on the pollen wall surfaces. Soil conditions may influence the content of normal mineral nutrients, such as Ca, Fe, and Mn (Spitzer *et al.*, 1981).

In the second investigation, pollen and soil samples were collected from the same locations. The materials were dried at room temperature without using any plant poisons, which were present in the herbarium materials employed for the previous experiment. The results are shown on Table 2. Aluminium and silicon are relatively abundant in both pollen and soil samples.

Graph 1 illustrates the relative abundance counts of P, S, K, Ca and Cl. It shows in a striking manner that the concentrations of these elements in pollen are much higher than in the associated soil samples. This is supported by observations that plant ash contains a much higher concentration of these elements than soils supporting the plants themselves.

Soil and its bearing upon plant nutrition

Earlier works on plant nutrition indicate that mineral composition of a plant does not totally correspond to that of the soil in which it grows, since all soil constituents are not equally available to the plant nor absorbable by the roots. In other words, the percentage of an element in plant tissues may be correlated with the availability of that element to

TABLE 1 - Elemental analyses of 10 pollen samples of *Vernonia monosis* Cl. - Relative abundance counts of detectable elements on pollen wall

ALTITUDE m	COLLECTION DATA	K	Mg	Ca	Fe	Al	Si	Ni	Cu	P	S	Cl
1100	COORG: KADAMAHAL RESERVE FOREST FRINGE, NEAR GALLIBEDU, J.P. PASCAL 841,2.3.1977, HIFP.	1.22	0.12	0.72	0.46	2.01	5.26	7.16	0.47	0.46	10.37	72.46
920	COORG: VIRARAJENDRAPET, P.F. FYSON, FEB. 1916, PCM.	17.23	2.08	4.72	0.52	3.21	16.11	7.28	0.60	7.57	15.01	24.56
1700	NILGIRIS: ON THE WAY TO OOTY, G. VASANTHY 62, 17.4.1978, HIFP.	15.45	0.86	0.71	0.38	2.91	10.61	22.66	1.57	0.67	23.19	21.84
900	* NEAR GUDALORE: NADGHANI, S. ARAVAZY, 10.2.1980, HIFP.	13.28	0.41	0.40	1.58	4.03	5.33	22.10	1.69	4.40	8.06	36.67
1000	ANAIMALAIS: SHOLAYAR RANGE, CHALAKUDY, J.P. PASCAL 1378,18.2.1979, HIFP.	19.44	1.44	0.36	0.79	1.50	9.76	7.65	0.09	4.57	23.30	31.18
1900	PALNIS: TOP STATION, F. BLASCO 1150, 24.2.1972, HIFP.	34.30	0.87	3.26	0.35	0.24	2.15	3.40	0.56	2.54	11.25	41.07
1600	KERALA: DEVIKULUM, DR. T.E. OCT. 1938, PCM.	17.96	1.23	0.13	0.63	1.20	10.02	8.86	1.20	5.94	27.02	25.80
1550	HIGH WAVYS: TRAVANCORE, F. BLASCO, 24.4.1972, HIFP.	2.89	0.05	0.57	1.15	0.41	2.84	35.49	2.12	0.16	0.43	48.99
1300	KERALA: PEERMEDE, P.K. BHAT & U.J. PHILIP 9069, 17.2.1967, PCM.	33.71	3.95	3.26	0.43	2.54	5.28	3.51	0.07	13.84	11.27	22.34
500	SABARI GIRI: NEAR MOOZHI AR, J.P. PASCAL 718, 6.2.1976, HIFP.	35.47	1.51	1.43	0.63	0.35	2.60	1.38	0.25	2.99	9.17	45.09

ABBREVIATIONS: HIFP, HERBARIUM, INSTITUT FRANCAIS; PONDICHERRY; PCM, PRESIDENCY COLLEGE, MADRAS.
* EXCEPT POLLEN MATERIAL FROM NADGHANI, REST WERE FROM HERBARIUM SPECIMENS POISONED WITH MERCURIC CHLORIDE DISSOLVED IN ALCOHOL

the roots in the medium of growth. The concentration of each nutrient is, however, believed to differ with species, climatic conditions, the chemical composition of soil and the physical features of prevailing environment. The interesting review by Tiagi and Aery (1981) includes the following points:

—The concentration of a given element varies in different organs of the same plant species.

—The concentration of certain elements in a plant is dependent upon the amount of those elements present in the soil, provided that the soil concentration is not so great as to be toxic to the plant.

—"Luxury consumption" of certain elements (absorbing more than the nutritional requirements) is due to the excessive availability of these elements in the soil.

—The difference in the elemental contents among different plant species is more pronounced than among their underlying soils.

—Plant ash contains much more of certain elements than the soil itself.

The major constituents of living tissue are carbon, oxygen and nitrogen. Twelve elements—B, S, Zn, P, Mn, Ca, Sr, Cu, K, Ba, Se and Ag—are generally considered biophilic elements, since the ratio of the amount of these elements in the plant over the amount in the soil is greater than 0.1. Mo, Mg and Fe, although less concentrated in plant tissues, are also biologically indispensable (Brooks, 1972). Trace elements—Cu, Fe, Mn, B and Mo are essential to the growth of plants although tissues need and contain them in concentrations of a few ppm to a few parts per thousand. The availability of these elements to plants depends greatly upon soil pH.

TABLE 2 - EDS Analyses of soils underlying *Vernonia monosis* Cl. trees and their unprocessed pollen

DEPTH OF SOIL SAMPLES CHOSEN FOR EDX	LOCALITIES AND ALTITUDES	MATERIALS AND DATE OF COLLECTION	ELEMENTS DETECTED AND THEIR RELATIVE ABUNDANCE COUNTS												
			Mg	Al	Si	P	S	K	Ca	Mn	Fe	Cu	Na	Cl	Tl	Cr
20 - 30 cm	NEAR ATTAPADY VALLEY 1050 m	SOIL (3) 12.2.84 pH 5.0-5.1	0.19	38.2	41.6	0	0.24	1.17	0.68	0.08	19.2	0.1	0	0.06	1.41	0.06
30 - 40 cm																
40 - 50 cm		POLLEN 12.2.84	0.19	39.1	22.5	1.58	5.84	3.82	1.26	0.05	21.5	0.54	0	2.66	1.21	0.03
30 - 40 cm	SILENT VALLEY 1250 m	SOIL (3) 12.2.84 pH 4.6-5.3	0.18	33.5	38.7	0	0.33	1.5	0.32	0.06	23.5	0.05	0	0.16	1.92	0.06
40 - 50 cm																
50 - 60 cm		POLLEN 12.2.84	0	29.8	23.1	3.39	9.3	7.96	3.92	0.63	2.88	0.67	0.5	21.7	0.98	0.21
0 - 15 cm	NILGRIS THOODOOR MATTAM 1550 m	SOIL (3) 8.2.84 pH 5.1-5.8	0.07	20.6	54.5	0	0.14	1.68	0.34	0.11	21.3	0.11	0.01	0.04	1.6	0.04
10 - 20 cm																
20 - 30 cm		POLLEN 8.2.84	1.1	10.4	22.2	1.95	26.4	10.2	9.54	0	3.85	0.37	0	13.8	0.21	1.25
20 - 30 cm	NAGHANI 900 m	SOIL (3) 15.11.83 pH 4.5-5.3	0.22	29.5	44.4	0	0.37	0.75	0.56	0.09	23.7	0.03	0	0.03	0.95	0.13
30 - 50 cm																
50 - 70 cm		POLLEN 10.2.84	0.54	2.63	2.27	9.81	9.97	51.3	3.68	0.5	0.39	0.51	0.42	16.2	0.36	0.34

NOTE: THE ARITHMETIC AVERAGE OF ELEMENTAL RELATIVE ABUNDANCE COUNTS OF 3 SOIL SAMPLES FOR EACH OF 4 LOCALITIES IS GIVEN.

Mineral content of pollen

The works on pollen chemistry are summarized and reviewed by Stanley (1971) and Stanley and Linskens (1974), the more important points from these reviews being:

—The presence of K, Na, Ca, Mg, N, P, S, Al, Cu, Fe, Mn, Ni, Zn, Cl, Si, and B in pollen-grains.

—Variations in both essential and non-metabolic elements may be due to specific differences or to environmental differences during maturation and dehiscence.

—Pollen mineral content could (especially in the case of microelements) be, to some extent, reflective of plant nutrient supply.

—High mineral contents in pollen have a direct bearing upon sites of plant distribution.

—Elements such as Si and Al, which are passively absorbed by the roots and other tissues of plants are similarly accumulated passively by the pollen.

—Mineral elements in cell-organelles are involved in physiological functions of pollen. Fe, Co and Mn are the essential requirements for the activity of certain enzymes.

—Levels of inherent elements and organic molecules vary throughout the year and also with the site in which the plant developed.

On complex substrates, factors such as soil texture, pH, Eh, organic carbon content, metal speciation and the presence of other ions affect the uptake and accumulation of metals by plants (reviewed by Taylor & Crowder, 1983 a & b). Their recent investigations on metal uptake by *Typha latifolia* are concerned with the pollen-soil concentration of elements. The chief conclusions of this study are:

—1. Pollen was the only plant tissue which failed to show a correlation with Mn in the soil-sediment.

—2. Fe showed significant correlation between soil-sediment metal concentration and virtually all plant tissues, including pollen.

—3. Pollen and female flowers were the only plant parts showing a significant correlation with total soil-sediment Zn and Ca.

In the following passages the nutritional role of the elements detected on the surfaces of pollen wall of *Vernonia monosis* and their occurrence and abundance in soil are discussed.

Silica—Two contrasting hypothetical mechanisms for silica deposition are physiochemical mechanical factors and metabolic events (Sangster, 1983). The review of the occurrence of silica in soils, plants and animals by Jones and Handrek (1967) deals with a number of aspects of silica in soil-plant association:

—The concentration of monosilicic acid in solution in soils is largely controlled by an adsorption reaction dependent upon pH.

—Iron and aluminium oxides lower the concentration of silica in solution on soil in proportion to their adsorptive capacities in simple systems.

—The concentration of silica in plants varies linearly with the concentration of monosilicic acid in soil solution in the range 7-67 ppm SiO_2.

—Ignoring possible differences due to soil type and composition, recent work supports the concept that plants take up different amounts of silica depending upon their botanical affinities. The Gramineae, for example, take up 10 to 20 times more silica than Leguminosae and other dicotyledons. Most recent studies lay stress on the differences in silica accumulation among different plant species.

—Silica can be absorbed into the transpiration stream by non-relative processes, where it is carried to different tissues and deposited most abundantly where water loss is highest.

—Wherever cell walls are observed to be thickened with cellulose or lignin they are impregnated with silica.

—The association of silica with cell wall constituents tends to make that wall more resistant to enzymic degradation which accompanies penetration of the cell wall by fungal hyphae.

—Despite these functions, silica cannot be regarded as an essential element for plant growth.

Silicon accumulation in the pollen surfaces of *Vernonia monosis* varies in relative abundance counts between 2.15 and 23.40.

Aluminium—The principal ionic form is the hydrated ($6H_2O$) in Al^{3+} which appears in an exchangeable form in soils and soil solutions in acid media (pH of$<$5.0). Aluminium ions are in part responsible for the acidity of soils and often dominate its exchange capacity. This can, at times, exert a toxic effect upon vegetation. The Si/Al ratio of soil samples ranges between 1.1 and 2.6 (EDS), whereas with pollen the ratios show much greater variation. The Si/Al ratios of pollen samples freshly collected from Silent Valley and near Attapadi Valley are 0.78 and 0.58 respectively, showing a greater accumulation of aluminium against silicon. Stanley and Linskens (1974) have discussed the passive accumulation of the elements Al and Si.

Iron—Iron is essential for chlorophyll formation, nutrient uptake, carbohydrate metabolism and enzymic activities. One of the trace elements of plant tissues, iron, needs to be combined in small amounts with plant tissues. As previously mentioned, the availability of trace elements depends greatly upon soil pH. Although the Si/Fe ratio of soils is between 1.65 and 2.55; those of pollen wall surfaces may be as great as 30.98. The very

high amount of iron (21.58) in the pollen sample collected near Attapadi Valley is surprisingly high.

Potassium—A major element in the lithosphere potassium, because of its solubility, is considered exchangeable and therefore available to plants. The downward movement of this element during its fixation by secondary clay minerals ensures a reserve of potassium for plants. Potassium is an important and indispensable element for plant growth and reproduction. Potassium requirements of plants are larger than for any other cation. Except for two (1.22 & 2.89) of the 14 pollen samples, its accumulation is considerable (3.82) (21.68) 51.30), whereas in soil it is low (0.75-1.68) because of its high solubility and mobility.

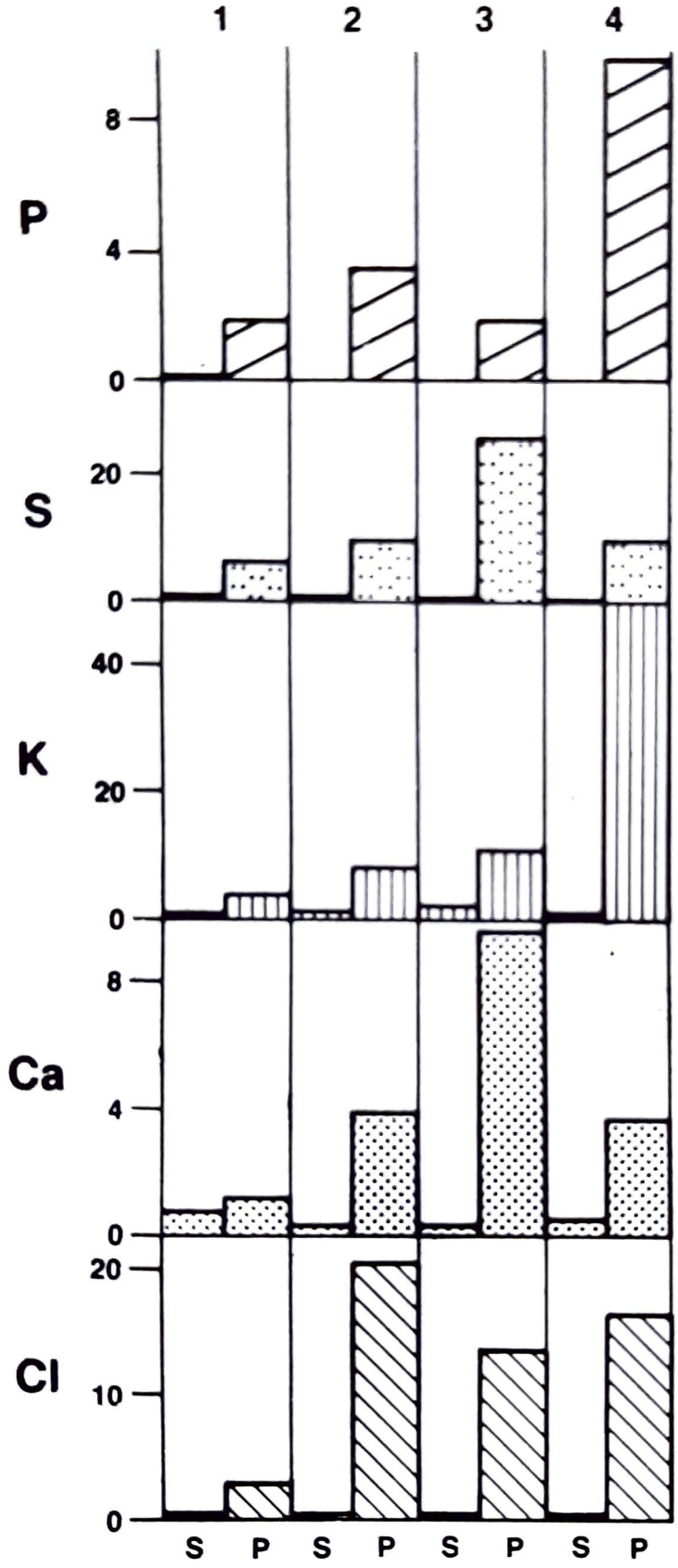
Calcium and Magnesium—Calcium and magnesium are exchangeable ions and are adsorbed onto colloids. Calcium plays a dominant role in the physical behaviour of soils and promotes better growth and normal composition to the tissues of many plants. Calcium is the main component of the cell wall. The occurrence of calcium oxalate is common in the ovary walls of all of the South Indian Vernoniaceae, including *Vernonia monosis*. Magnesium is a component of chlorophyll and its presence in soil is essential for all crops and trees.

Phosphorus—Phosphorus is immobile and its uptake by plants depends not only upon the available reserves but also upon the colonization of soil by root systems. Phosphorus assists in the synthesis and transformation of sugars by phosphorylation and in the composition of nucleo-protein. The state of the soil profile, its humidity (moisture relation), temperature, calcium supply, and biological activity (organic matter) are also factors that help to determine phosphorus nutrition in relation to its reserves. Phosphorus accumulation on pollen surfaces is high (0.46 to 13.64) compared to the low content in associated soils (0.0 to 0.006).

Sulphur—This is an essential major element in the growth of plants and is present in varying quantity in all soils. Much higher than normal quantities are frequently observed in peats and arid soils. Organic sulphur undergoes an annual cycle of variation leading to the formation of sulphates, the main source of sulphur for plants. Sulphur is a building material for the formation of proteins and other organic constituents of plants. Sulphur in pollen surface materials is much greater than in the associated soils (Graph 1).

Manganese—A constituent of ferromagnesian minerals, manganese is liberated from rocks during weathering in the form of soluble and exchangeable $Mn^{?+}$ and, in this form, is available for assimilation by plants. Biodegradation of organic matter frees the manganese in the soluble and exchangeable Mn^{2+} form. As a trace element regulating plant growth and development, manganese is essential for the growth of seedlings. The relationship of iron to manganese is more significant than the absolute concentration of the element in regulating plant growth.

Nitrogen and Carbon—These elements are not detectable with the EDS equipment available to the authors but the total carbon (Anne's method) and total nitrogen (Kjeldahl's method) were determined (C/N ratios 8.0-14.0) for soil samples of Nadghani and Attapady (*V. monosis* sites) by P. Janel and team at the French Institute, Pondicherry. Nitrogen acts to limit the growth possibilities allowed by the system and the plant acts as a "sink" which determines the amount of nitrogen needed. Nitrification in forests is more dependent upon C/N ratio than upon pH. Nitrogen is an essential element in plant growth and its concentration within plant tissues is normally maintained within narrow limits. It primarily influences the development of leaf areas which subsequently control other growth and metabolic activities (Ingestad & Lund, 1979). Nitrogen has the greater effect



GRAPH 1 – Relative abundance of selected elements in soils (S) and pollen (P) from; 1 - Near Attapady Valley 1050 m; 2 - Silent Valley; 3 - Nilgiris:Thoodoor Mattam 1500 m; 4 - Nadghani, 900 m (cf. Table 2).

upon growth, affecting both cell number and size. Phosphorus has similar, but less pronounced effects and potassium least effect upon growth, affecting mainly cell size (see review by Okusanya, 1983).

Aluminium and silicon appear to show soil-pollen surface correlations in three of the four samples analysed (Table 2). The relative abundance counts of P, S, K, Ca and Cl are significantly higher on the pollen surfaces than in associated soil samples (Graph 1.). These observations are supported by the observation that plant ash carries significantly higher concentrations of these elements than associated soils.

Effects of various processing treatments upon the elemental composition of pollen wall

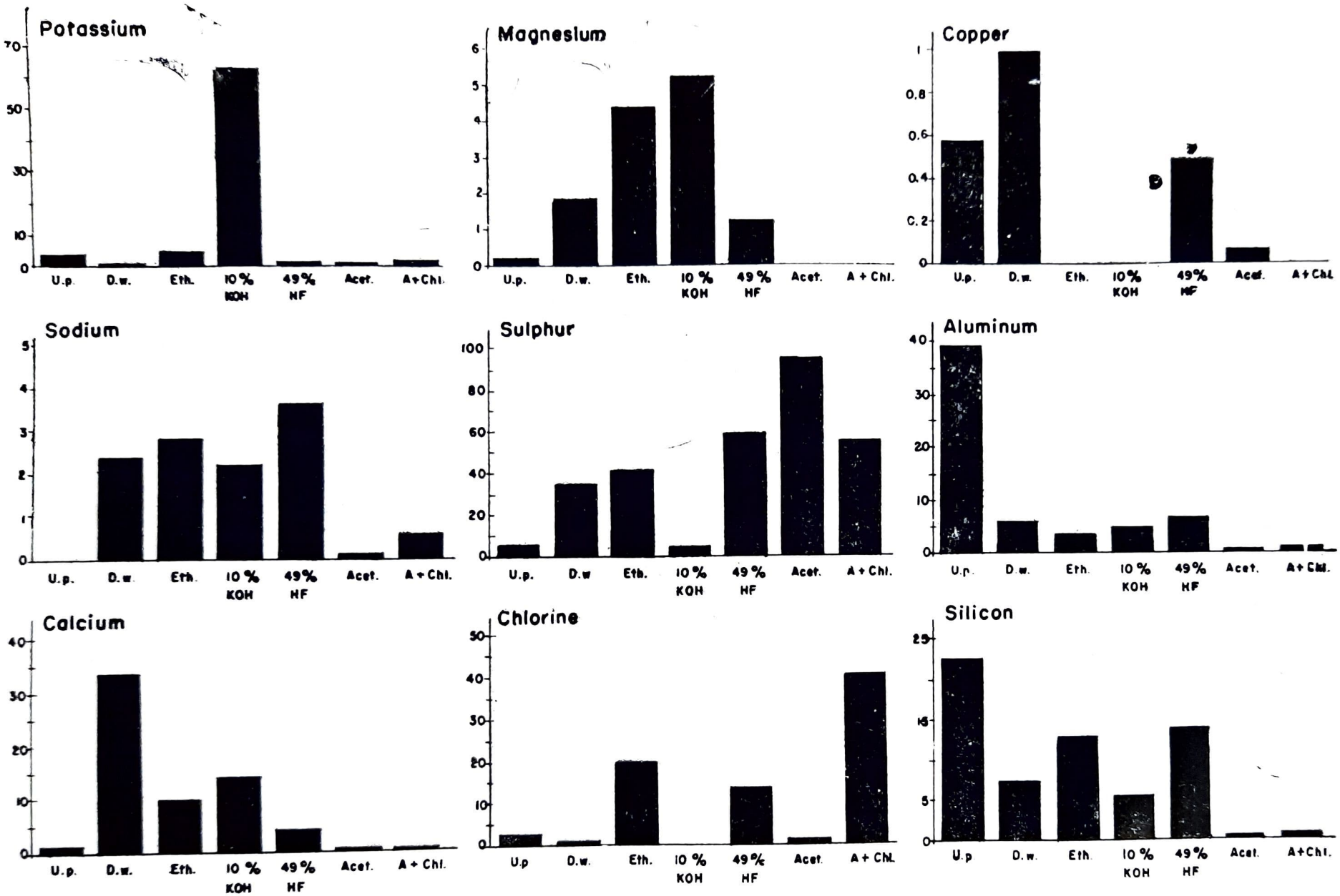
Our third investigation concerns the effect of standard palynological processing techniques upon the elemental composition of pollen wall materials and the significance of the results with regard to pollen chemistry (Table 3 & Graph 2). Before considering the results, it must be noted that the elements Ca, Mg, K, and Mn can be leached or redistributed by solvents such as water and fixatives or rehydrants. Sodium and magnesium are almost undetectable in unprocessed materials but possibly due to the removal of protective coating of surface materials and lipids, become significantly more abundant following treatment with water, ethanol and HF. Acetolysis and acetolysis followed by further treatments remove virtually all of these elements from the pollen wall. Calcium, a cell

TABLE 3 - Effects of processing upon pollen wall chemistry (collected near Attapady Valley, 1050 m)

No	SAMPLE	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Fe	Cu
1	UNPROCESSED	0	0.19	39.18	22.57	1.58	5.84	2.66	3.82	1.26	1.21	21.58	0.58
2	DIST. WATER	2.4	1.9	5.99	7.52	5.94	35.6	0.78	0.85	33.7	1.68	6.35	0.99
3	ETHANOL	2.81	4.41	3.82	13.1	0	41.6	20.2	4.67	10.21	0.24	0	0
4	10% KOH, 30 min 100 °C	2.21	5.3	5.12	5.7	0	4.25	0	63.3	14.26	0.1	1.78	0
5	49% Hf, 12 hr	3.62	1.28	6.75	13.9	0	58.56	13.78	0.9	4.51	1.2	0	0.49
6	ACETOLYSIS	0.09	0.017	0.297	0.636	0	95.6	1.255	0.64	0.412	0.06	0.432	0.06
7	ACETOL. + CHLOR.	0.57	0	0.93	0.77	0.99	55.2	40.6	1.09	0.26	0	0.019	0
8	ACETOL. + 49% Hf. 12 hr	0.17	0.59	0.31	1.54	0.009	88.87	1.13	1.43	2.83	0.02	0.02	0

NOTE: SAMPLES 3 - 8 IN THE TEST TUBE AFTER TREATMENTS WERE WASHED ONLY TWICE WITH DIST ILLED WATER AND AIR-DRIED PRIOR TO EDS.

wall component, shows an increase following initial treatments, probably as a result of the pollen wall being exposed by the removal of pollen-kitt. Acetolysis and further processing, as might be expected, reduce the concentration of this element. Potassium is a highly soluble, mobile element and its incorporation into the pollen wall surface following treatment with 10% KOH is striking (an increase from 3.8 to 63.3%). Potassium on the



ABBREVIATIONS : U.p. = Unprocessed; D.w. = Isolated in distilled water; Eth. = Ethanol treated; 10% KOH = Potassium hydroxide; 49 % HF = Hydrofluoric acid treated; Acet. = Acetolysed; A + Chl. = Acetolysed and Chlorinated.

GRAPH 2.– Effects of processing on pollen-wall chemistry (see Table 3)

pollen wall may be residual in nature, resulting from only two washings in distilled water after KOH treatment. Prolonged washing with slow running deionized water might remove the residual potassium.

Extraneous, or non-essential, elements such as silicon and aluminium are accumulated passively by plant tissues since they are passively absorbed by the roots. Silicon is abundant in unprocessed pollen, less so after successive processing treatments. It is somewhat surprising that it is not completely removed by digestion in 49% HF. This is probably the result of protection by enclosing organic materials. Acetolysis, on the other hand, removes the bulk of this element. Aluminium is quite abundant in unprocessed material, but is gradually removed by successive processing treatments.

Phosphorus is concentrated on pollen surfaces compared with the amount present in soils. It is gradually removed by successive processing procedures.

Sulphur content of pollen surfaces gradually increases with initial treatments, possibly being rendered mobile by the solvents employed and migrating outward to the surface of the grain. Acetolysis dramatically increases the concentration of this element, the result of adsorption from sulphuric acid employed in the process. It is reduced by chlorination, but not by subsequent HF treatment.

Chlorine concentration is, as might be expected, drastically reduced by treatment with KOH and increased by chlorination. Prolonged washing with slow running deionized water might possibly remove the added chlorine.

Iron shows moderately high concentration on unprocessed pollen wall surfaces but is removed by succeeding steps in pollen processing.

Copper is present in traces in unprocessed pollen and in material suspended in water but is removed by chemical treatments, except digestion in HF, to which copper is, to some extent, resistant.

Titanium, an element universally present in small quantities, is present in unprocessed pollen wall surfaces, but is removed by subsequent treatment.

Our conclusions from this last experiment are as follows:

—Removal of pollenkitt and lipids with solvents during processing results in an increase in Na, Ca, and Mg in the surface layers.

—HF (49%) treatment does not remove all of silicon from pollen wall surfaces. Prolonged treatment of unprocessed pollen in concentrated (70%) HF at 100°C is yet to be tried as an effective agent for removing silicon from pollen surfaces.

—Acetolysis, KOH treatment and chlorination, respectively, enhances the concentration of S, K and Cl in pollen wall surfaces: their concentration might change after prolonged washing in slow running deionized water.

—Extraction of certain elements from pollen wall surfaces and incorporation of certain other elements (eg. Na, S, Cl & K) may be indicative of selective adsorption of these elements by the sporopollenin of the pollen wall is conjectural. We wonder if chemical analysis of fossil exines could yield data that would help to elucidate certain peculiarities of sediments from which fossil pollen is isolated.

—Finally, by way of warning to those employing EDS methods of analysis, gold coating of samples prior to analysis not only masks the P and S peaks, but can modify the entire spectral response, making the estimation of relative abundance unreliable. The lesson "coat with carbon or not at all".

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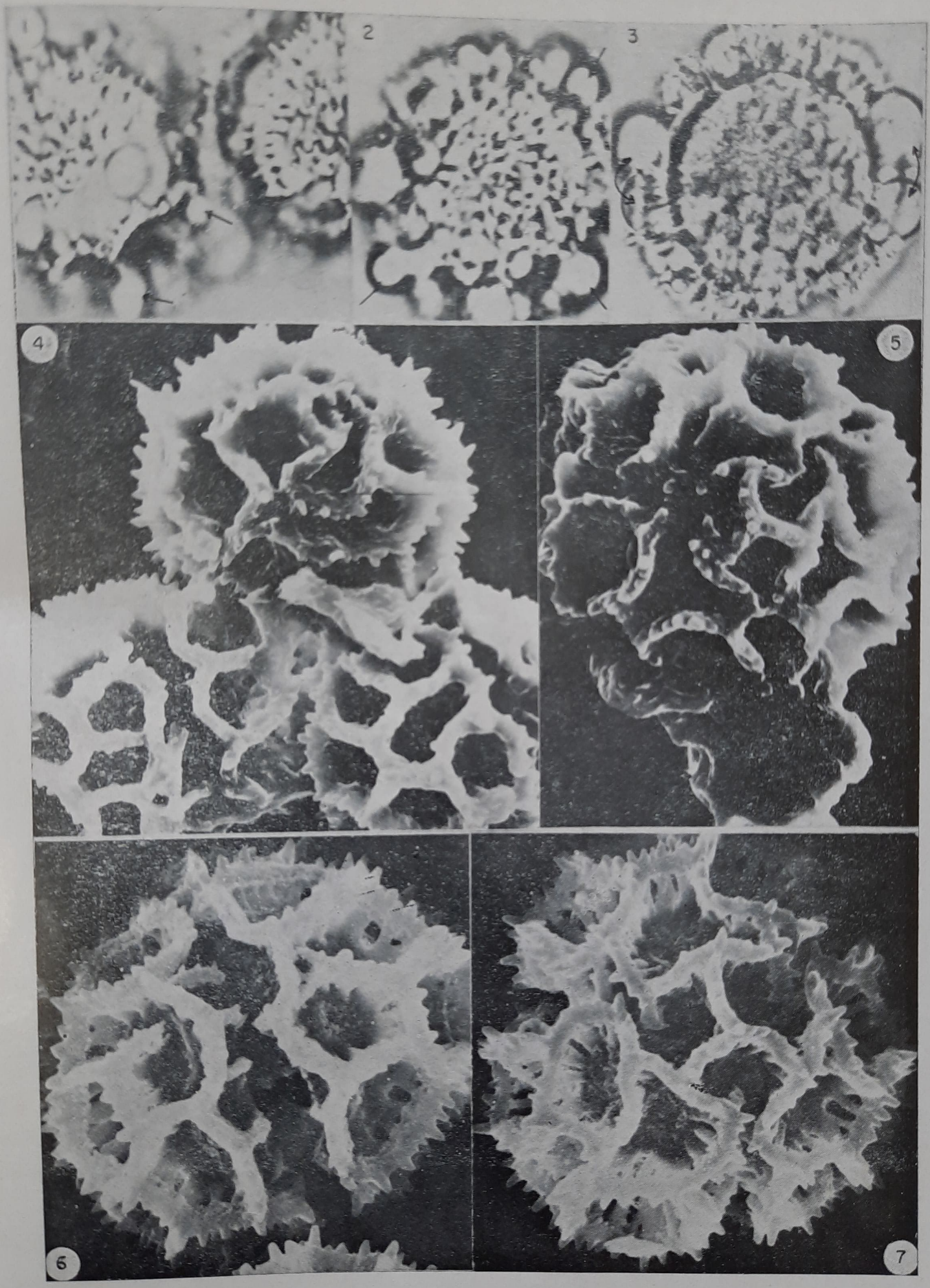
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Explanation of Plate

Colpororate, echinolophate pollen of *Vernonia monosis* Cl.

1-3. LM (×1000) : pollen rehydrated in distilled water, with expanded apertures and dispersing lipids (arrows) ×1300 & ×1000.

4-5. (×1300 & ×1000) SEM : Untreated pollen dissected out of anthers : note the surface materials :

6-7. SEM (X2000) : Acetolysed pollen in equatorial and polar views without surface materials.