CETESB

COMPANHIA DE TECNOLOGIA DE SANEAMENTO AMBIENTAL

Studies on plants' resistance ..

PROJETO BRASIL-ALEMANHA

VEGETATION MODULE

1992 SUMMARY REPORT

BY CETESB'S GROUP

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DOCUMENTO

TIPO	DATA	ORIGEM	Nº PÁGINA/V.	Nº MAPAS
Relatório Parcial	Fev/93	DPTE		

TÍTULO DO DOCUMENTO

STUDIES ON PLANTS' RESISTANCE TO POLLUTANT STRESS

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ASSINATURA/CARIMBO/DATA

CLASSIFICAÇÃO DE SEGURANÇA

☐ EXTERNA ☐ INTERNA

☐ RESERVADA

PALAVRAS CHAVES

POLLUTANT STRESS, RESISTENCE MECHANISMS, RESISTANT SPECIES, SENSITIVE SPECIES, ATMOSPHERIC POLLUTION

CÓDIGO E TÍTULO DO PROJETO

PROJETO 31.65.00 - EFEITOS DA POLUIÇÃO ATMOSFÉRICA SOBRE A VEGETAÇÃO NO ESTADO DE SÃO PAULO

DISTRIBUIÇÃO INTERNA

ÁREAS/Nº DE CÓPIAS

USO DA BIBLIOTECA

CLASSIFICAÇÃO DE ASSUNTO	Nº DOCUMENTO	VISTO / CARIMBO / DATA

A4-00071

33-10-040/I

RESUMO

OBSERVAÇÕES

USO DA BIBLIOTECA

LOCAL	EDITORA

IDIOMA

PORTUGUÊS [ ] INGLÊS [X] ESPANHOL [ ] FRANCÊS [ ] ALEMÃO [ ] ITALIANO [ ] ______ [ ]

SÉRIE

A4-00071

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ACKNOWLEDGEMENTS

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ABSTRACT

This research is an activity in the vegetation module of the ENV 3 - "Air Pollution and Vegetation Damage in the Tropics - The Serra do Mar Example", Brazilian/German Programme.

In order to investigate the Atlantic Forest plants' resistance to Cubatão's atmospheric pollutants, 15 resistance and 8 sensitive species were selected to chemical, anatomiacal and physiological comparative studies.

Three field research areas were selected according to Cubatão air quality produced by CETESB and the evolution study of the vegetation by aerial photographs.

This report is a presentation of the methodology adopted and the preliminary results to enable a correlation with the date from the other activities of this project.

Based on the chemical analyses it was possible to identify three F^- and S accumulator species: **Tibouchina pulchra**, **Ficus insipida** and **Cecropia glazioui**. **Miconia cabucu** which do not occur in areas with high F^- atmosphere concentration can be considered as a S accumulator.

The anatomical studies indicate some characteristics that might be correlated with plant's resistance, such as presence of subepidermal aqueous parenquim, presence of crystals and phenolic compunds. This correlation will be verified with the proceding of the research.

The gas exchange data and the biochemical analyses will verify the presence on of non visible injuries (metabolism imbalance) in accumulator species.

The fumigation experiments will enable the characterization of visible injuries by anatomical studies and the evaluation of the tolerance limits.

This research will support the evaluation of the plants responses to pollutant stress and will facilitate the vegetation recuperation projects in Serra do Mar - Cubatão, São Paulo. It will also help the pollution diagnosis and pollution control activities performed by CETESB.

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STUDIES ON PLANTS' RESISTANCE TO POLLUTANT STRESS

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INTRODUCTION

In order to investigate the Atlantic Forest plants' resistance to Cubatão's atmospheric pollutants, we have initially opted for experiments under controlled conditions:

- in loco portable chambers having filtered and unfiltered air (for fluoride experiments);

- fumigation chambers (for SO_2 and O_3 experiments);

- particulate matter experiments in greenhouses.

The data obtained would have been compared with field work data. Nevertheless, the difficulties in the construction and operation of the equipment used as well as the delay in obtaining the field work data, forced us to change the direction of the research of to obtain more conclusive results by the end of this project period.

In order to make comparative studies of resistance mechanisms and to reduce the experimental error, samples were taken and analyses were made with the greatest possible number of species and parameters. Samples were taken in 1991/92 to identify, by chemical analyses, the pollutant accumulator species within the resistant species group.

Biochemical, anatomical and physiological comparative studies are being done aiming to elucidate the resistance mechanisms of these plants. Based on these results the fumigation experiments will be reactivated.

The reintroduction of the sensitive species - virtually extinct in the polluted areas - was done in 3 sites of the Serra do Mar (fig. 1: Vale do Moji-1, Caminho do Mar-2, Vale dos Pilões-3), to make comparative studies of development, pollutant accumulation and visible injuries. This was achieved by planting 3 experimental woods of these species with 1200 specimens of 35 different species from Dec., '92 to Feb., '93. 8 of the sensitive species are being chosen to be tested in the same manner as the resistant plants mentioned above (table 1a).

We believe it will be possible to correlate these results with those obtained by the other activities of this project and discuss the principal resistance mechanisms of the plants studied.

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OBJECTIVES

To evaluate the tolerance limits of selected plant species to atmospheric pollutants.

To evaluate the morphological, physiological and biochemical responses of species sensitive and resistant to pollutants stress.

To understand the resistance mechanisms present in the selected species.

MATERIAL AND METHODS

After establishing which of the species were sensitive (those died due to pollution) and which were resistant (which survived in the polluted study area) 15 representative resistant species were selected from the native vegetation from 1985 to 1987 (table 1a). Among these, we have initially selected 7 species for detailed studies (table 1b).

Three field research areas were selected (figure 1) according to Cubatão - SP air quality data produced by CETESB in the eighties and the evolution study of the vegetation by aerial photographies (vegetation module, activity 7). These sites are under the same conditions of climate.

AREA 1: Vale do Moji (VM) - Affected by fertilizing industries' emissions, mainly F^-, NH_3, NO_x and particulate sulphates, nitrates and phosphates.

AREA 2: Caminho do Mar (CM) - Affected by petroleum refinery emissions specially hidrocarbons, SO_2 and NO_x.

AREA 3: Vale dos Pilões (VP) - Less polluted area, utilized as control.

The areas selected for the resistance studies coincide with some of the passive monitoring sites (activity 3) to make the correlation of all this data viable in the future. There is an air quality continuous monitoring in the areas 1 and 3 under chemical module supervising.

The experiment design was based on the pollutants' chemical nature, including the investigation of F^-, SO_2 and NO_x effects (figure 5), O_3 (Figure 6) and particulate matter (figure 7).

Chemical and Biochemical Analyses

The plant sampling consisted of mature leaves in standardized position for each specie with 3 replicates (3 specimens/specie/site). The samples for chemical analysis were washed in deionized water. Figure 8 illustrates the leaf preparation procedures and analyses.

The analysed chemical parameters were N, S, P, K, Ca, Mg and F^-. The fluoride concentration analyses were performed by CETESB and the others, including the soil analyses, were done by LAGRO

(Agronomical Laboratory in Campinas - SP) under the supervision of Dr. Fernando Jose Hass.

The biochemical analyses will be done by the Biochemistry Department of U.S.P. (São Paulo University) under the supervision of Dra. Ursula Maria Lanfer Marquez. The methodology which will be used for free amino acids determination to be used is adapted from Spackman et al, 1958 and Moore & Stein, 1963. The methodologies for soluble sugars, fenolic compounds and ascorbic acid determination has been tested in the same laboratory.

Anatomical Leaf Studies

The anatomical leaf studies were started using the resistant species from the control area (VP) and from the CETESB greenhouse in order to characterize the morphology of the leaves that had no injury. The data obtained will be correlated with the morphology of injuries on leaves of plants from polluted sites and fumigation experiments. The anatomical characteristics which might be related to the resistance mechanisms will also be investigated .

The leaves were preserved in 70% ethanol. The transversal sections were handmade and the thinner ones were selected with a magnifying glass and prepared for double coloring with basic fuchsin and alcion blue.

Paradermal sections were obtained by different techniques depending on the plant specie: Separating the epiderms with hydrochloric acid; taking an epiderm impression with a thin layer of colorless enamel; scratching one of the epiderms by using a razor blade to obtain only the opposite epiderm.

The paradermal sections made the counting and measuring of the stomatos possible. For this we utilized an optical microscope with a reticulated ocular; the amplification varies from 400 to 630 times. Forty measures of each specie were taken.

Gas Exchange

Trying to obtain more information about stomato aperture and correlate it to avoidance mechanisms, we have accompanied the stomato aperture of seven studied species for 24 hours in the VM and VP sites. The methodology used was xylol infiltration (Molisch, 1912 In Slavik, 1974). This field study took place in the summer under high humidity conditions and good air quality as well.

In order to obtain data such as net photosynthetic rate, stomatal conductance, foliar temperature and active photosynthetic radiation, an Infra-red Gas Analyser was used under the supervision of Prof. Dr. José Antonio P. V. de Moraes, through the CETESB/UFSCar (São Carlos Federal University) agreement.

Compensation curves were done under the green trimming plastic net to control the radiation on the leaf.

The plants analysed by these methodologies (xylol and infra-red) will then be previously placed in vases and acclimatized. They will then be sent to the three study sites to make comparative studies under monitored conditions.

Particulate Matter

Circular pieces of 1.5cm in diameter were extracted from the middle region of fresh leaf collected in Vale do Moji. These leaf disks were dried under vacuum, at 40°C.

At the I.P.T. (Technological Research Institute of the State of São Paulo) Electronic Microprobe Laboratory, one sample of about 1 cm^2 was cut from the adaxial face of each specie. They were then fixed with double-faced adhesive tape using carbon paint to trace boundaries and make eletrical contact with the holder. The carbon metalization was done in a conventional vaccum metalizer.

The Scanning Electronic Microscope (S.E.M.) used was a microprobe JEOL, model JXA-50A, of the Metallurgy Division of the Instituto de Pesquisas Tecnológicas de São Paulo S.A. - I.P.T..

The observations generally were made at 15 keV excitation potential, observing the whole surface and doing point analyses of the particles found at 0.5 nA maximum absorbed current. All the obtained photos are secondary electron images (S.E.I.).

Soil Characterization of Experimental Sites

To characterize the 3 research sites, physical, chemical and microbiological analyses of the soil were carried out (tables 2 and 3; figures 2 to 4).

This soil data received only a superficial interpretation because it was destined only to support the resistance plant studies.

The three study sites are similar in physical aspects. The variability observed on the soil texture and color (table 2) are characteristic of unstable litosol areas as found in the Serra do Mar.

The organic matter (OM) content is similar in VP and VM and lower in CM (figure 3b). Nevertheless, they can be considered normal for the region. The low cation exchange capacity (CEC) of these soils (fig. 3b) may be attributed to the sandy texture and low organic content levels (oscillating within the range of 1.2 to 2.6%).

A pronounced soil acidity (fig. 4a), as well as a strong leaching of Ca, Mg, K and Na (fig. 2b), were observed in the three sites and this is characteristic of Serra do Mar soils.

An evident phosphorus increase was observed in the Vale do Moji (fig. 4b) resulting from particulate phosphate emissions from fertilizing industries. The chemistry module data confirm the high P-input in this area.

Sulphur: The majority of species show concentrations from 0.10% to 0.40% in the 3 sites (figure 10). These data are considered normal (Eaton, F.M. In Chapman, H., 1966). However, **Miconia**, **Phylodendron** and **Tibouchina** show higher concentrations, which have been mentioned by some authors as being values found in specimens from industrial areas (Eaton, F.M.; 1966).

Table 5 shows the foliar S ratio between the polluted and control sites. **Cecropia glazioui**, **Tibouchina pulchra**, **Miconia cabucu** and **Ficus insipida** present significantly higher S concentrations in the polluted sites, the highest concentrations being found in Caminho do Mar. These results are possibly due to atmospheric absorption in addition to soil absorption.

Table 6 shows the N/S foliar ratio in the 3 sites. The normal ratio is approximatly 15/1 (Stewart, 1969) and all these values are very low, specially in Caminho do Mar. These data indicate an S evident accumulation in leaves, that might be prejudicial for the plant's metabolism.

Phosphorus: The greater number of species show P concentrations between 0.10% and 0.25% (figure 11), which is considered normal (Binghan, F.T. In Chapman, H., 1966). The intraspecific differences can't be considered significant due to the high standard deviation.

It is important to mention that the highest P soil concentration was found in the Vale do Moji site, whereas the P soil concentration in Vale do Pilões and Caminho do Mar are almost the same. The P soil accumulation in Vale do Moji is possibly due to fertilizing industry emissions, but the P foliar concentrations do not reflect this.

Potassium: The expected K variation is from 0.5% to 5% (Ulrich, A. and Ohki, K. In Champman, H., 1966). The concentrations appearing in the studied species are within this range (figure 12). The intraspecific differences cannot be considered significant due to the high standard deviation.

It is important to mention that the highest K soil concentration occurs in the Vale do Pilões, whereas the K soil concentration in the Vale do Moji and Caminho do Mar are almost the same.

Calcium: The Ca range that is considered normal is between 0.086% and 2% (Champman, H.D., 1966). The Ca concentrations found are within this range (figure 13). It is not evident that the Ca concentrations in the Vale do Moji species are lower than in the Caminho do Mar species, not reflecting the Ca soil levels.

Magnesium: According to Embleton, T.W. (Champman, H., 1966), normal plants show a dry weight Mg concentration between 0.20% and 0.25%.

The Mg content in **Ficus** in the Vale dos Pilões, in **Phylodendron** in the Caminho do Mar and Vale dos Pilões and in **Tibouchina** in the Caminho do Mar was lower than this range but it cannot be considered as deficient (figure 14). It's important to mention that the Mg soil concentration isn't reflectec on the Mg foliar levels.

Fluoride: The normal fluoride amount in the plant leaves which grow in non saline soil and unpolluted areas is within the range 2 to 20ppm, according to Pushnik and Miller (1990). The majority of the species show concentrations higher than this range, including some species from Vale dos Pilões (control site) and the highest F concentration is found in the species from VM (fig. 15). Comparing the 1992 data (foliar F^- concentration) wiht the results obtained in preceding years in the same sites (CETESB, 1986, 1991) we observe a F^- accumulation in the Vale do Moji species. **Ficus insipida** and **Cecropia glazioui** are those with higher F^- concentration values (table 7). **Piper cernuum** and **Phylodendron imbe** show lower F^- concentration values.

The fluoride concentration in **Tibouchina** from Vale dos Pilões was unexpected in view of the preceding results. To confirm these data, the plant collection and analyses will be repeated.

Based on these chemical analyses it was possible to identify three F and S accumulator species: **Tibouchina pulchra**, **Ficus insipida** and **Cecropia glazioui**. **Miconia cabucu** which do not occur in areas with high F^- atmosphere concentration can be considered as a S accumulator.

2. Biochemical Analyses

The leaves were collected and freeze dried. The methodologies are being tested at U.S.P..

Leaves with and without wax were tested for free amino acids determination and no significant differences were found.

These results and the gas exchange data will verify the presence of non visible injuries (metabolism imbalances) in accumulator species.

3. Anatomical Studies

The results from paradermal and transversal sections are synthesized on tables 8, 9 and 10 and photos 1 to 7. Regarding the ecological characterization of species based on foliar anatomy it is possible to verify that:

a. The studied species are mesophytes, except **Costus**, that presents hygrophyte characteristics because it has stomatos on both leaf surfaces.

b. **Cecropia**, **Tibouchina**, **Ficus** and **Miconia** present a very developed photosynthetic apparatus, typical in heliophytes. Young **Miconia** plants withstand darkness due to their low light compensation point (see gas exchange).

c. **Piper**, **Costus** and **Phylodendron** present sciophyte characteristics.

d. The stomato dimensions are very similar among the species (except **Tibouchina**) and probably this characteristic isn't importat to the ecological aspects as well as to pollution resistance (table 9).

Some species studied have been showing characteristics which are probably linked to atmospheric pollution resistance:

3.1. Presence of subepidermal aqueous parenquim:

This characteristic is correlated to the light excess protection and appears in **Ficus, Piper** and **Costus** (photos 1, 2 and 3). It consists of a supplementary barrier to contain the pollutant flux into the foliar mesophile. The future anatomical studies of plants under fumigation can elucidate the function of this tissue on the mesophile's chlorophylled cells.

3.2. Presence of crystals:

Despite the fact that chemical composition of leaf crystals is normally correlated to calcium carbonates and oxalates, the hypothesis of calcium fluoride crystal formation can not be dismissed. The possibility of fluoride precipitation by apoplastic calcium which would prevent injuries in clorophyll tissues, will be tested by determining the chemical nature of crystals in leaves from polluted areas.

3.3. Presence of phenolic compounds:

Significant differences in total phenol leaf concentrations in polluted and unpolluted sites have not been verified (CETESB, 1986 and 1991). However, in a preliminary analysis, the low tannin levels in **Miconia cabucu** leaves from Caminho do Mar was observed when compared with leaves from Vale dos Pilões.

The anatomical sections of **Miconia cabucu** leaves from the unpolluted site (photo 4) confirm the pronounced presence of phenolic compounds, probably related to tannins.

The hypothesis that tannins give some protection to cells against oxidant pollutants will be verified during 1993 by biochemical analyses and fumigation experiments, and it will be correlated to herbivory parameters.

3.4. Stomato density (table 10):

Higher stomato density is apparently related to higher gas exchange flux by the leaves (see gas exchange) and to higher foliar pollutant concentrations.

Difficulties in obtaining of **Cecropia** and **Miconia** paradermal sections have still impeded more detailed stomato studies of these species. More sensitive and resistant species will be investigated during 1993 and 1994, including visible injuries to the anatomy observed in the field and in laboratory fumigation experiments.

4. Gas Exchange

Because of the initial difficulties in obtaining the equipment to measure gas exchange in field work, we have opted for an indirect method to verify the stomato aperture. This method is based on xylol infiltration through the stomatos. In spite of inaccuracy in measuring the stomatal opening rate, the first results (figure 16) qualitatively reflect the gas penetration capacity in the leaf mesophile.

This field study took place in the summer without hydric constraint.

Assuming that the leaf infiltration period is the same as the period in which the exchange is more intense, we observed that the species showing a larger period of exchanges between leaf and atmosphere (**Cecropia**, **Tibouchina** and **Ficus**) presented higher pollutant accumulation. The species showing lower pollutant accumulation (**Piper**, **Phylodendron** and **Costus**) have more restricted stomato apertures. The excess of **Miconia**'s tricoms has impeded the obtention of the xylol infiltration tests results in this specie.

The observed stomatal density of the plants studied (table 11) seems to be correlated to the S and F gaseous pollutant concentration capacity of the leaves. In other words, the species with higher stomatal density and higher xylol infiltration are the S and F accumulator species (figure 17). These hypotheses will possibly be confirmed when the anatomical and gas exchange studies are finished, especially stomatal conductancy measures.

The field photosynthesis determination started in Nov., 92 with various resistant species. The large variability observed in photosynthetic activity parameters (radiation, leaf temperature, stomatal conductance, etc.) did not permit the detection of significant differences between the polluted areas and the control one. Nevertheless, valuable information about physiological characterization was obtained from the species studied, such as the light response curve of **Cecropia**, **Ficus** and **Tibouchina** from Vale do Moji (figure 18) and some estimates concerning the light compensation point (figure 19 and table 12). Observing these data, we verify that **Costus**, **Piper**, **Phylodendron** and **Miconia** have the lower light compensation-point typical of shadow plants whereas **Ficus** and **Cecropia** are heliophyte, showing high light compensation points.

Tibouchina has the larger range between light compensation and saturation-points, a capacity which makes it suitable to recolonize the degraded areas in Serra do Mar.

Monitoring gas exchange simultaneously in the three studied sites and extending this investigation to sensitive species (during 1993) will possibly characterize pollution effects in photosynthesis, respiration and transpiration and correlate them to possible resistance mechanisms.

5. Particulate Matter

The adaxial foliar surfaces of **Ficus**, **Phylodendron**, **Costus** and **Piper** from Vale do Moji were studied in this stage. **Cecropia**, **Tibouchina** and **Miconia** could not be investigated because of an abundant amount of leaf tricoms. Based on the images obtained with the S.E.M. (photos 8 to 15) and the chemical composition obtained from the X-Ray microprobe, it was possible to make the following observations:

a. **Ficus** and **Costus** cuticles were whole, making it possible to verify a strong epicuticular wax deposit pattern (photos 8, 9 and 10).

b. **Phylodendron** and **Piper** leaf surfaces (photos 11, 12 and 13) showed alterations which can be related to some particle deposits, mainly metallic ferrum.

c. In all studied species, calcium sulfate crystals (in higher or lower concentration) were found on the leaf surface (photos 13 and 14). Nevertheless, contrary to what was observed by Adams et al (1990) in cabbage, these crystals seem not to be related to injuries in the epiderm of the material which we have examined. These authors observed the $CaSO_4$ crystal formation in the epicuticular wax after carrying out simulated acid rain experiments on the cabbage plants.

d. Most of the observed particles in the epiderm, regardless of the species, were enveloped in epicuticular wax. Photo 15 shows the traces left by the X-Ray microprobe on a metallic ferrum particle due to the wax which envelops this particle. The enveloping of particulate matter in wax probably prevents more serious damages to the epiderm. The wax extraction to be realized in the next research stage, will enable us to quantify the particulate matter retained by epicuticular wax and to evaluate the importance of the phenomenon in epiderm protection. The continuation of these studies will also permit the evaluation of the occurence of the epicuticular wax erosion by the rain contaminated with acid pollutants.

FUTURE PERSPECTIVES

1. To extend the chemical analyses to all selected species (table 1a) and to determine which species (sensitive and resistant) are accumulators and which are not.

To extend the biochemical analyses to all selected species (table 1a) in order to verify which species are resistant or sensitive.

2. To complete **Cecropia** and **Miconia** uninjuried leaf anatomical studies and extend the anatomical studies to all selected species (table 1a).

3. To reactivate the fumigation experiments in order to characterize the visible injuries by anatomical studies, and to evaluate the tolerance limits.

4. To investigate the chemical nature of the crystals present in the leaves, and correlate them to possible resistance mechanisms.

5. To verify the hypothesis that tannins give some protection to cells against oxidant pollutants.

6. To study gas exchange with infra-red gas analyser in sensitive and resistant species placed in vases and sent to the 3 sites

(VM, VP and CM). To correlate this study to pollutant accumulation by plants and their biochemical alterations.

7. To extract the epicuticular wax to quantify it and identify it's components. To evaluate the wax importance in the epiderm protection.

8. To continue the particulate matter study in order to evaluate the occurrence of the epicuticular wax alterations by rain contaminated with acid pollutants.

DESIRED IMPACTS

This research will enable us to evaluate the plant responses to pollutant stress and will facilitate the vegetation recuperation projects in Serra do Mar - Cubatão - SP. It will also help the pollution diagnosis and pollution control activities performed by CETESB.

Table 1a: Species from Atlantic Florest selected for studies on plants's resistance to pollutant stress

	SPECIE	FAMILY	HABIT
RESISTANT	*Cecropia glaziou*	Cecropiaceae	tree
	Costus spiralis	Zingiberaceae	herb
	Cupania oblongifolia	Sapindaceae	tree
	Ficus enormis	Moraceae	tree
	Ficus insipida	Moraceae	tree
	Hedychium coronarium	Zingiberaceae	herb
	Miconia cabucu *	Melastomataceae	tree
	Miconia pyrifolia	Melastomataceae	tree
	Miconia theaezans	Melastomataceae	small tree
	Monstera adamsonii	Araceae	creeping herb
	Philodendron bipinatifidum	Araceae	shrub
	Philodendron imbe	Araceae	creeping herb
	Piper cernuum	Piperaceae	shrub
	Syagrus pseudococos	Palmaceae	palm
	Tibouchina pulchra	Melastomataceae	tree
SENSITIVE	*Chrysophilum ramiflorum*	Sapotaceae	tree
	Cryptocharia moschata	Lauraceae	tree
	Euterpe edulis	Palmaceae	palm
	Ocotea odorifera	Lauraceae	tree
	Psidium	Myrtaceae	tree
	Pterocarpus violaceus	Fabaceae	tree
	Virola oleifera	Myristicaceae	tree
	Zigia ramiflorum	Mimosaceae	tree

* this specie was extinguished in VM

Table 1b: Species selected to detailed investigations

CODE	SPECIES
1	Cecropia glazioui Snethl.
2	*Costus spiralis* (Jacq.) Roscoe
3	*Ficus insipida* Willd.
4	*Miconia cabucu* Hoehne
5	*Phylodendron imbe* Schott
6	*Piper cernuum* Vell.
7	*Tibouchina pulchra* Cogn.

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Table 2: Soil Physical Characteristics

PARAMETERS	SITES		
	CM	VM	VP
Color(Munsel Notation)	7,5 YR 7/4	10 YR 6/3	10 YR 7/4
Apparent Density	1,2	1,19	1;18
Absolute Density	2,35	2,5	2,35
Granulometry:			
200 - 20mm	0	1,90%	0
20 - 2mm	0	0,90%	0
2 - 0,2mm	44,60%	35,50%	56,40%
0,2 - 0,02mm	25,90%	33,10%	32,30%
0,02 - 0,002mm	23,70%	18,60%	6,40%
<0,002mm	5,80%	12,80%	4,90%

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Table 3: Soil microbiological characteristics

SITES	N - Cicle							C - Cycle				S - Cycle			Fungi	
	N fixing bacteria		Ammonifying	Nitrifying bacteria		denitrifying	proteolytic	cellulolytic microorganism		hemicellulolytic	amylolytic	sulphate reducing	Thiobacillus	actinomycetes		
	anaerobic	aerobic	bacteria	NH3->NO2	NO2- ->NO3-	bacteria	bacterias	aerobic	anaerobic	microorganism	bacterias	bacteria	thioparus		mold	yeasts
	** M.P.N/g of dry weight													C.F.U./g of dry weight		
Caminho do Mar	1,0*10^4	3,7*10^2	>=3,2*10^8	2,6	<2	>=3,2*10^8	8,9*10^5	30	9,9*10^4	3,0*10^6	9,9*10^4	<2	<2	68	4,0*10^2	1,2*10^4
Vale do Moji	1,3*10^4	4,4*10^2	1,5*10^7	79	<2	>=3,8*10^8	3,6*10^5	3,9*10^3	4,7	1,7*10^5	1,9*10^4	<2	<2	1,6*10^2	1,7*10^3	1,4*10^4
Vale dos Pilões	0,3*10^4	2,2*10^2	2,7*10^7	51	5,1	>=3,0*10^8	2,9*10^6	55	<3	1,4*10^5	2,7*10^2	<2	<2	1,3*10^3	1,4*10^3	1,5*10^4

* C.F.U./g of dry weight = colony forming units by gram of dry weight
** M.P.N./g of dry weight = most probable number by gram of dry weight

NOTE: methods of analysis based on POCHON, J. & TARDIEUX, P. - Techniques d'analyse en Microbiologie di Sol, 1962

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Table 4: N Ratio of polluted and control sites

	CM/VP	VM/VP
Cecropia glaziloui	0,90	0,80
Costus spiralis	0,80	0,90
Ficus insipida	1,20	1,30
Miconia cabucu	0,80	----
Phylodendron imbe	0,90	1,20
Piper cernuum	0,70	0,70
Tibouchina pulchra	1,30	1,40

Table 5: S ratio of polluted and control sites

	CM/VP	VM/VP
Cecropia glaziloui	2,0	1,5
Costus spiralis	1,5	1,1
Ficus insipida	2,6	2,6
Miconia cabucu	2,9	----
Phylodendron imbe	1,8	0,7
Piper cernuum	1,0	1,1
Tibouchina pulchra	3,1	2,2

Table 6: N/S Foliar ratio

SPECIES	CM	VM	VP
Cecropia glaziloui	6	7	13
Costus spiralis	5	8	10
Ficus insipida	6	6	13
Miconia cabucu	1	---	4
Phylodendron imbe	2	6	4
Piper cernuum	4	3	5
Tibouchina pulchra	1	2	3

Table 7: F- Ratio of polluted and control sites

	CM/VP	VM/VP
Cecropia glaziloui	0,74	1,90
Costus spiralis	0,70	1,14
Ficus insipida	0,90	3,30
Miconia cabucu	1,30	----
Phylodendron imbe	0,95	1,54
Piper cernuum	0,93	1,70

Table 8 - Anatomical analyses

SPECIES	CRISTALS				SUBEPIDERMICAL AQUEOUS PARENQUIM	VENTRALDORSUM PARENQUIM (number of layers)	STOMATO	FENOLIC SUBSTANCE	CUTICLE (um)
	drus.	rafid.	prism.	cist.					
Ficus insipida	+	+	+	+	+	palisade 1 - 2 layers lacunose +- 20 layers	hypostomatal	0	sup. 3 inf. 4
Miconia cabucu	+	0	0	0	0	palisade 1 layer lacunose 4 - 5 layers	hypostomatal	+++	sup. 5 inf. 2
Tibouchina pulchra	+	0	0	0	0	palisade 1 layer lacunose 8 - 9 layers	hypostomatal	0	sup. 5 inf. 3
Phylodendron imbe	+	+	0	0	0	palisade 3 - 4 layers lacunose +- 12 layers with large lacuns	hypostomatal	++	sup. 9 inf. 8
Piper cernuum	0	+	0	0	+	palisade 1 layer lacunose 3 - 4 layers	hypostomatal	++	sup. 1 inf. 1
Costus spiralis	0	0	0	0	+	palisade 1 layer lacunose 4 - 5 layers	amphistomatal	+	sup. 5 inf. 3
Cecropia sp	+	0	0	0	0	palisade 1 layer lacunose 3 - 4 layers	hypostomatal	++	sup. 8 inf. 2

+ presence of the characteristic
0 ausence of the characteristic

Table 9: Stomatic Measurements: measurement of mayor and minor diameter of guard cell

SPECIES	MAYOR DIAMETER	MINOR DIAMETER
Ficus insipida	22,8u	3,8u
Tibouchina pulchra	15,2u	3,6u
Phylodendron imbe	30,4u	11,2u
Piper cernuum	38,0u	11,2u
Costus spiralis	30,4u	7,5u

Table 10: Stomato density on epiderm

SPECIES	STOMATOS/mm2
Ficus insipida	260 +- 11,4
Tibouchina pulchra	1117,6 +- 75
Philodendron imbe	94,3 +- 8,6
Piper cernuum	94,3 +- 14,3
Costus spiralis	inferior epiderm 141,4 +- 5,4
	superior epiderm 48,6 +- 8,6

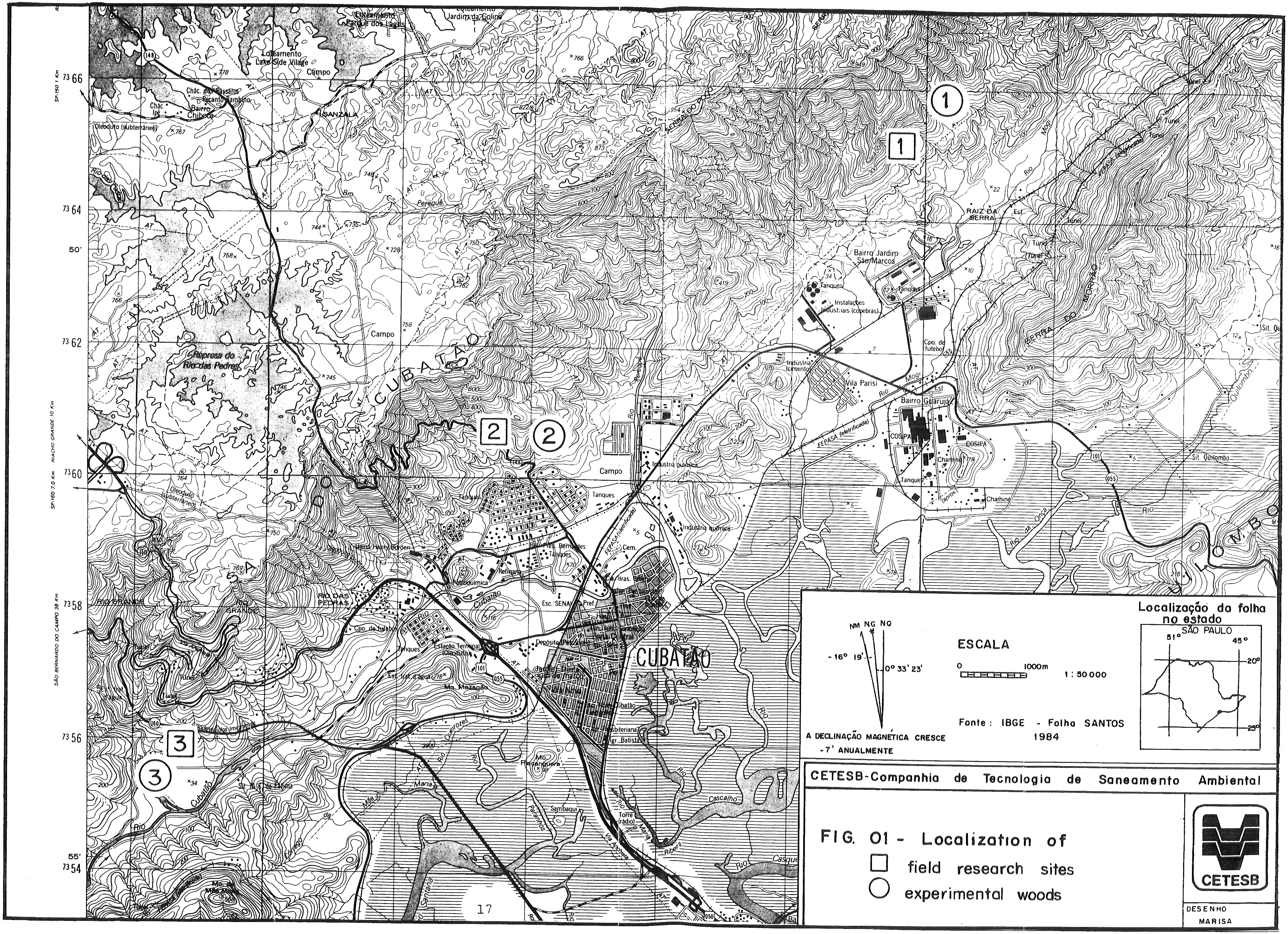


FIG. 01 - Localization of
□ field research sites
○ experimental woods

Fig. 2a - Soil Analysis

Cubatao-SP - 1992

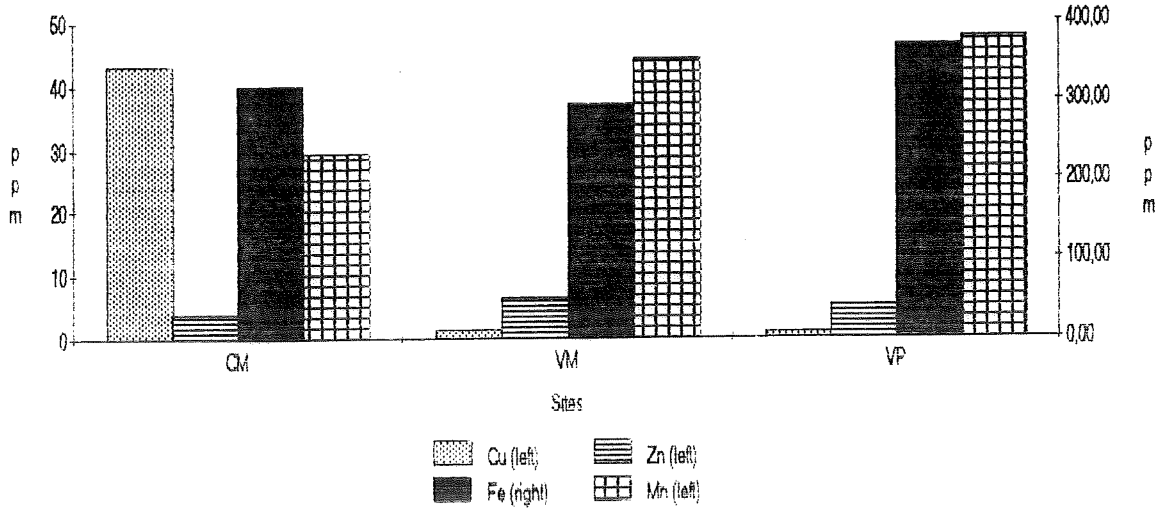


Fig. 2b - Soil Analysis

Cubatao - SP - 1992

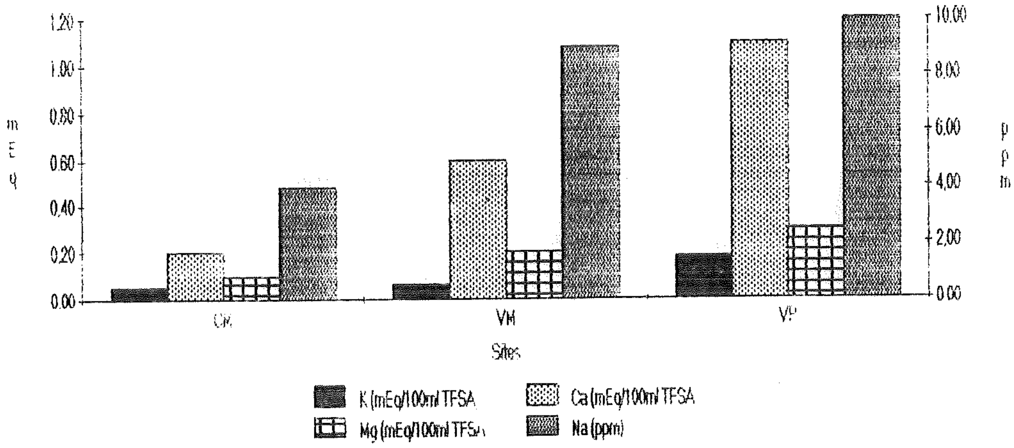


Fig. 3a - Soil Analysis

Cubatao-SP - 1992

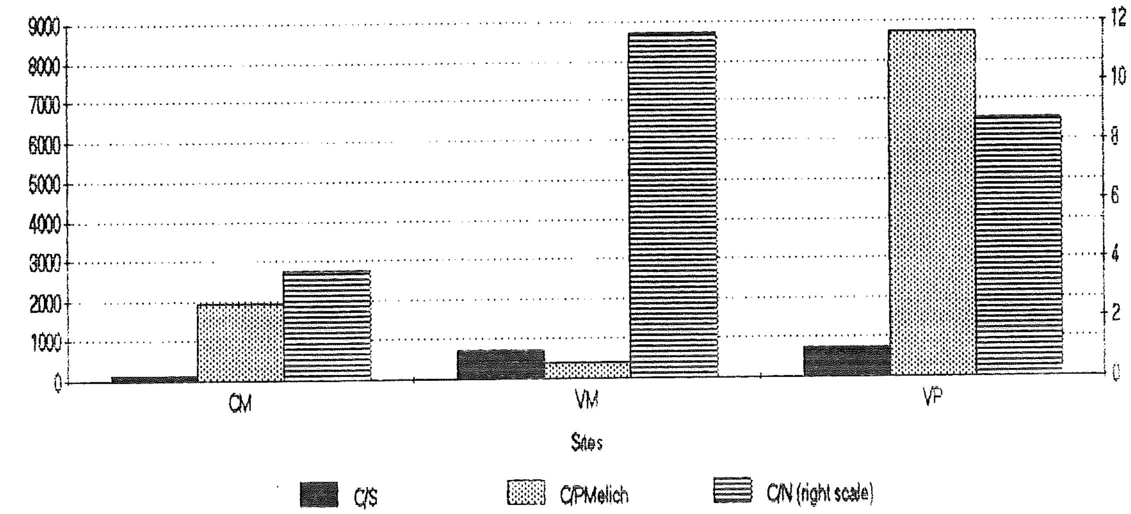


Fig. 3b - Soil Analysis

Cubatao-SP - 1992

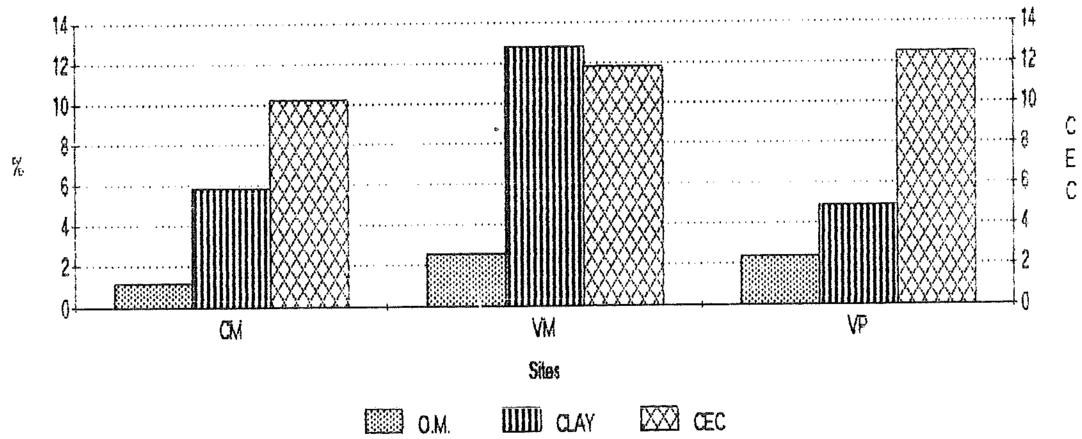


Fig. 4a - Soil Analysis

Cubatao-SP - 1992

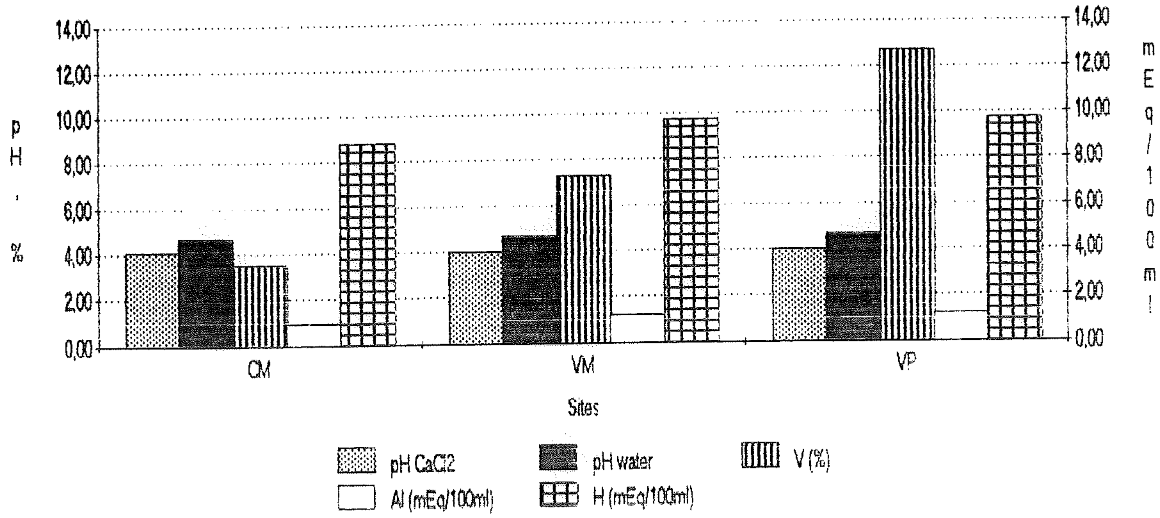
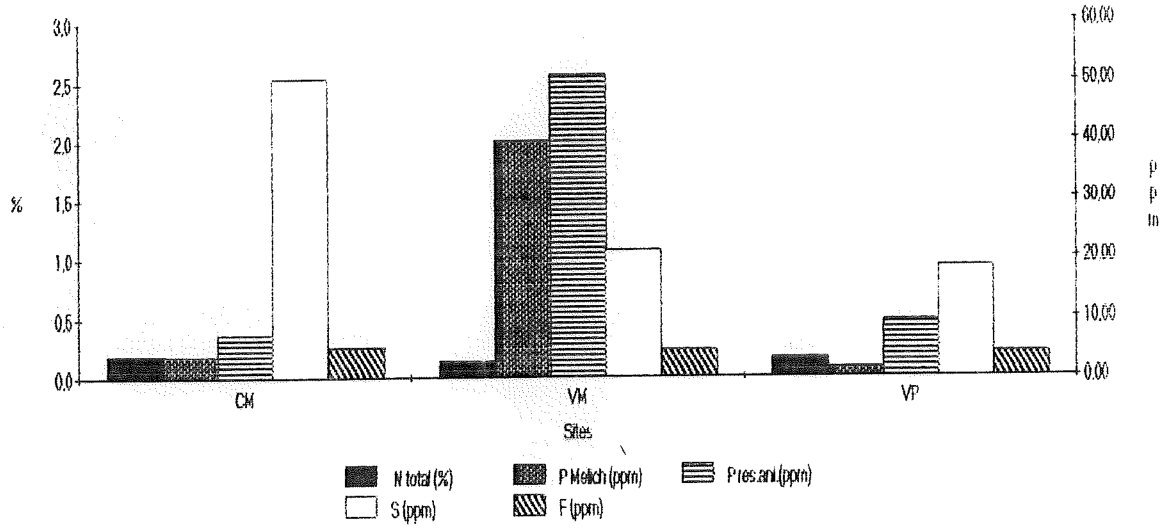


Fig. 4b - Soil Analysis

Cubatao-SP - 1992



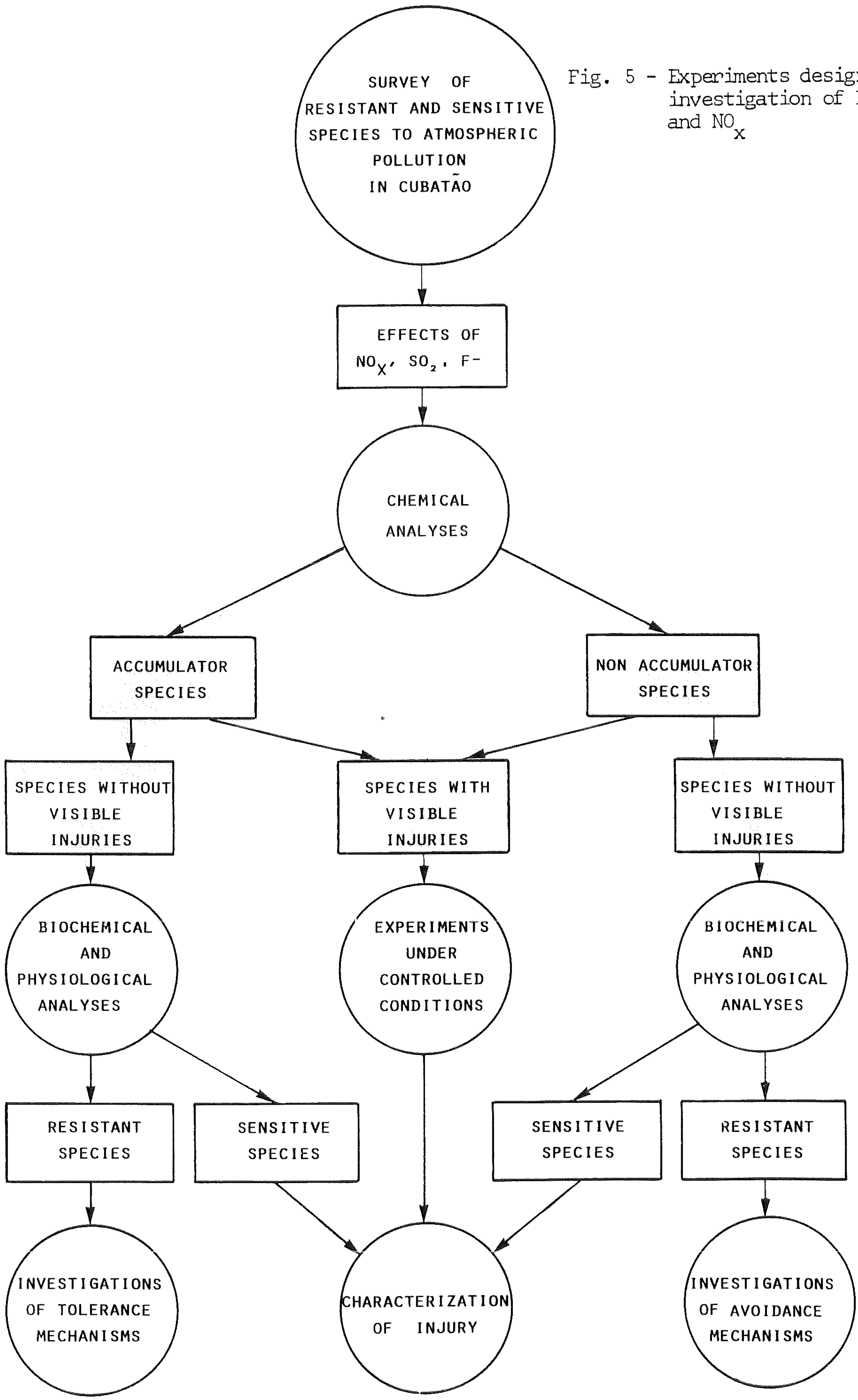
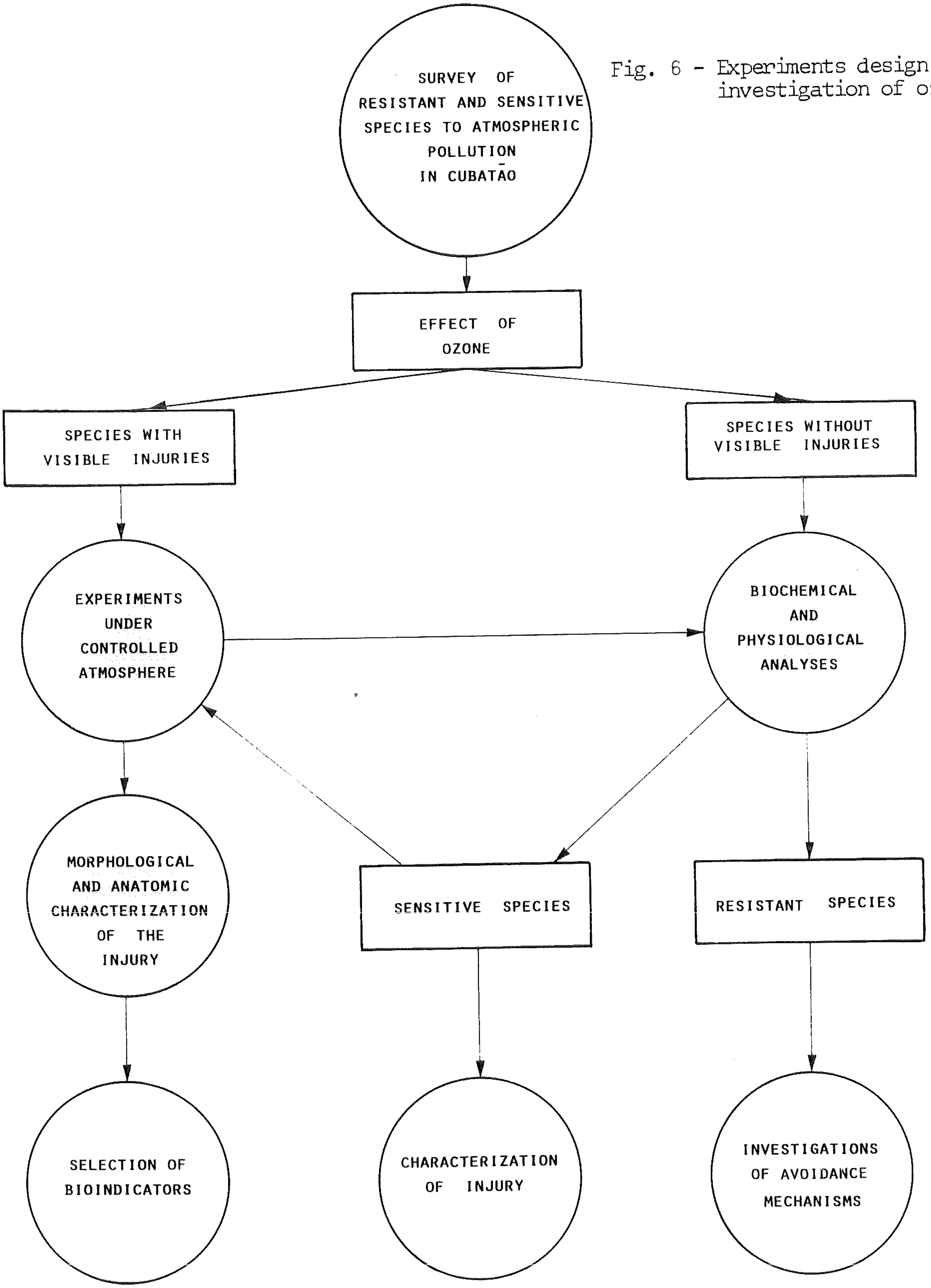


Fig. 5 - Experiments design for investigation of F^-, SO_2 and NO_x

Fig. 6 - Experiments design for investigation of ozone



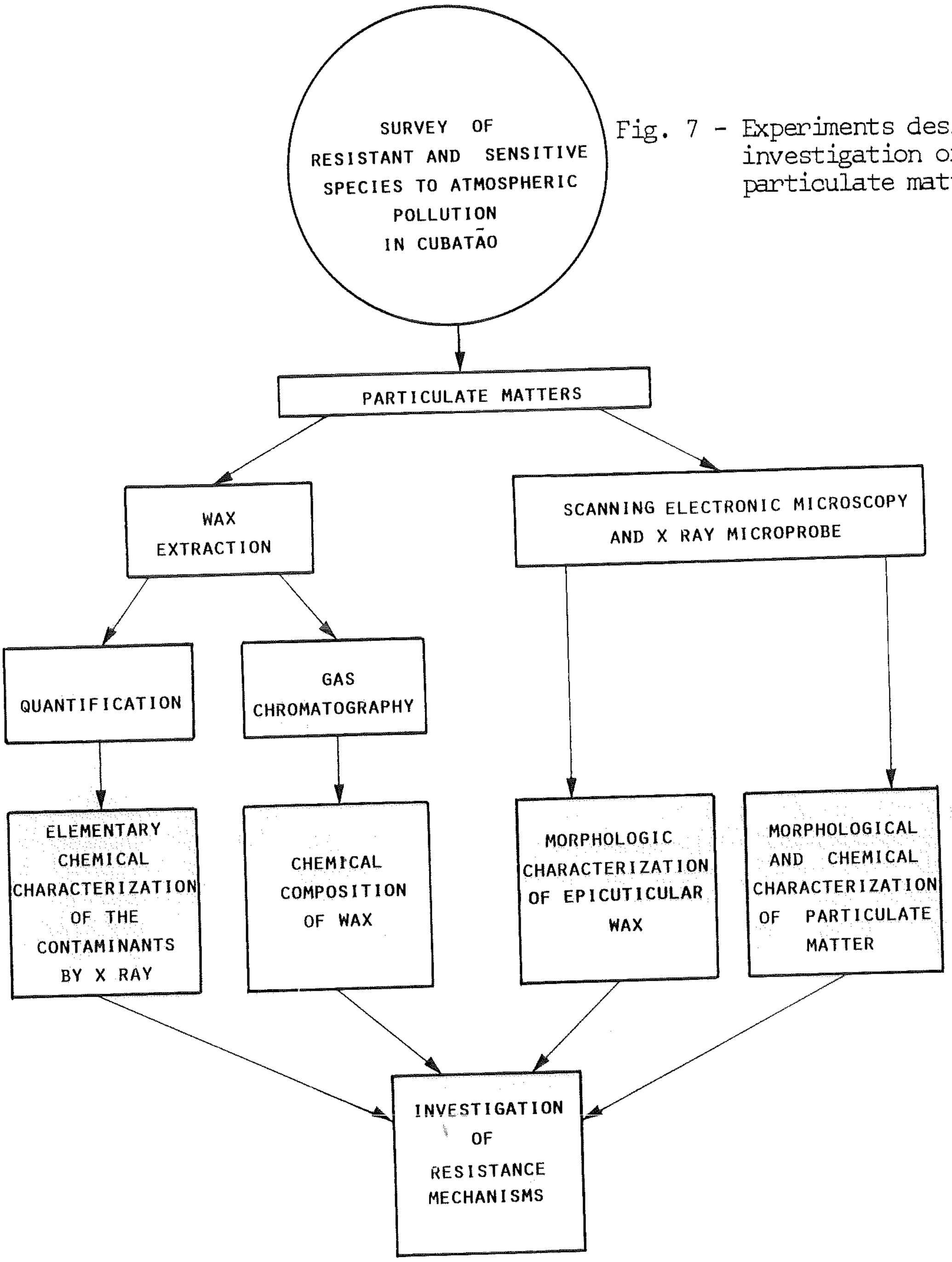


Fig. 7 - Experiments design for investigation of particulate matters

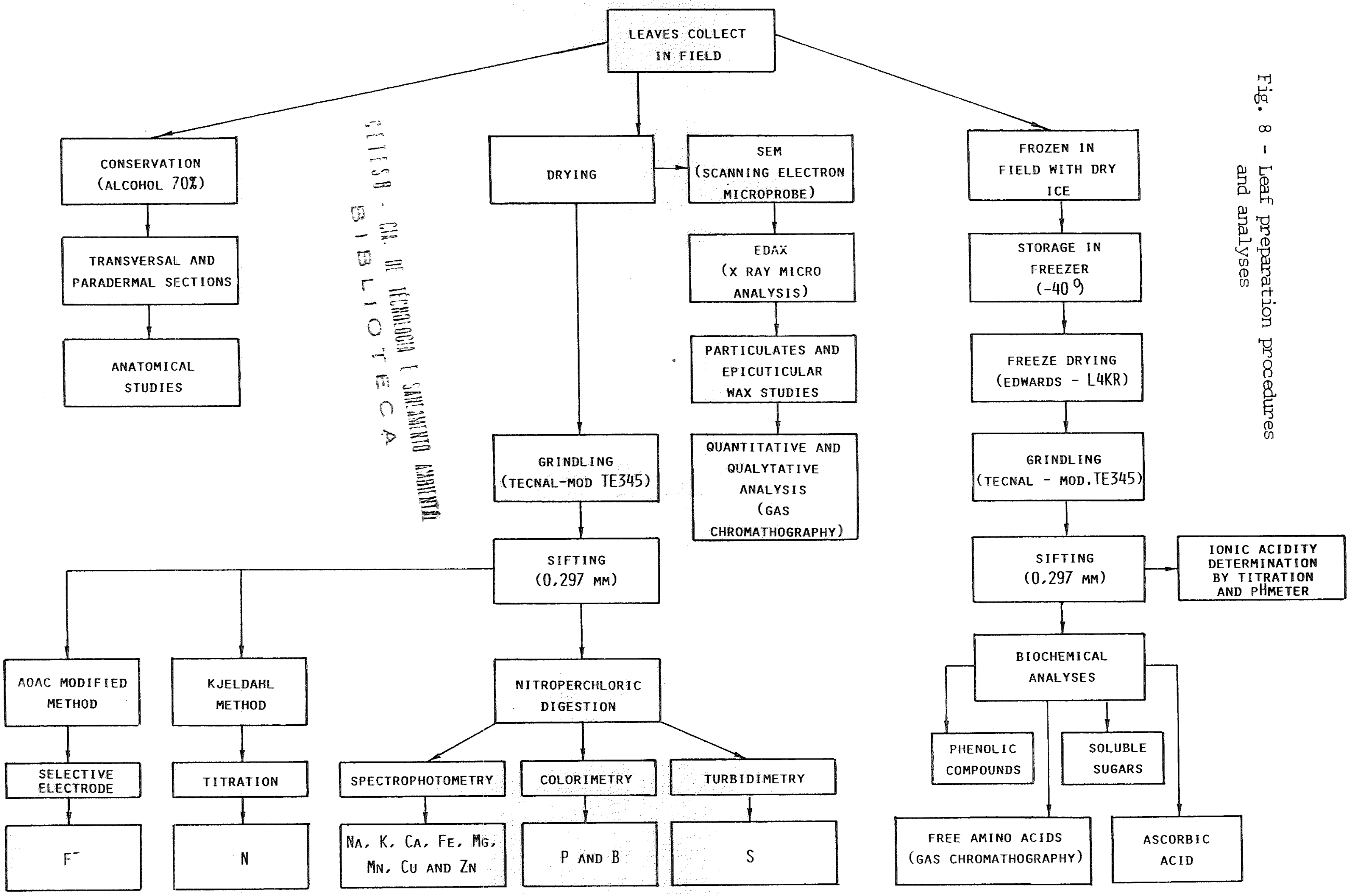


Fig. 8 - Leaf preparation procedures and analyses

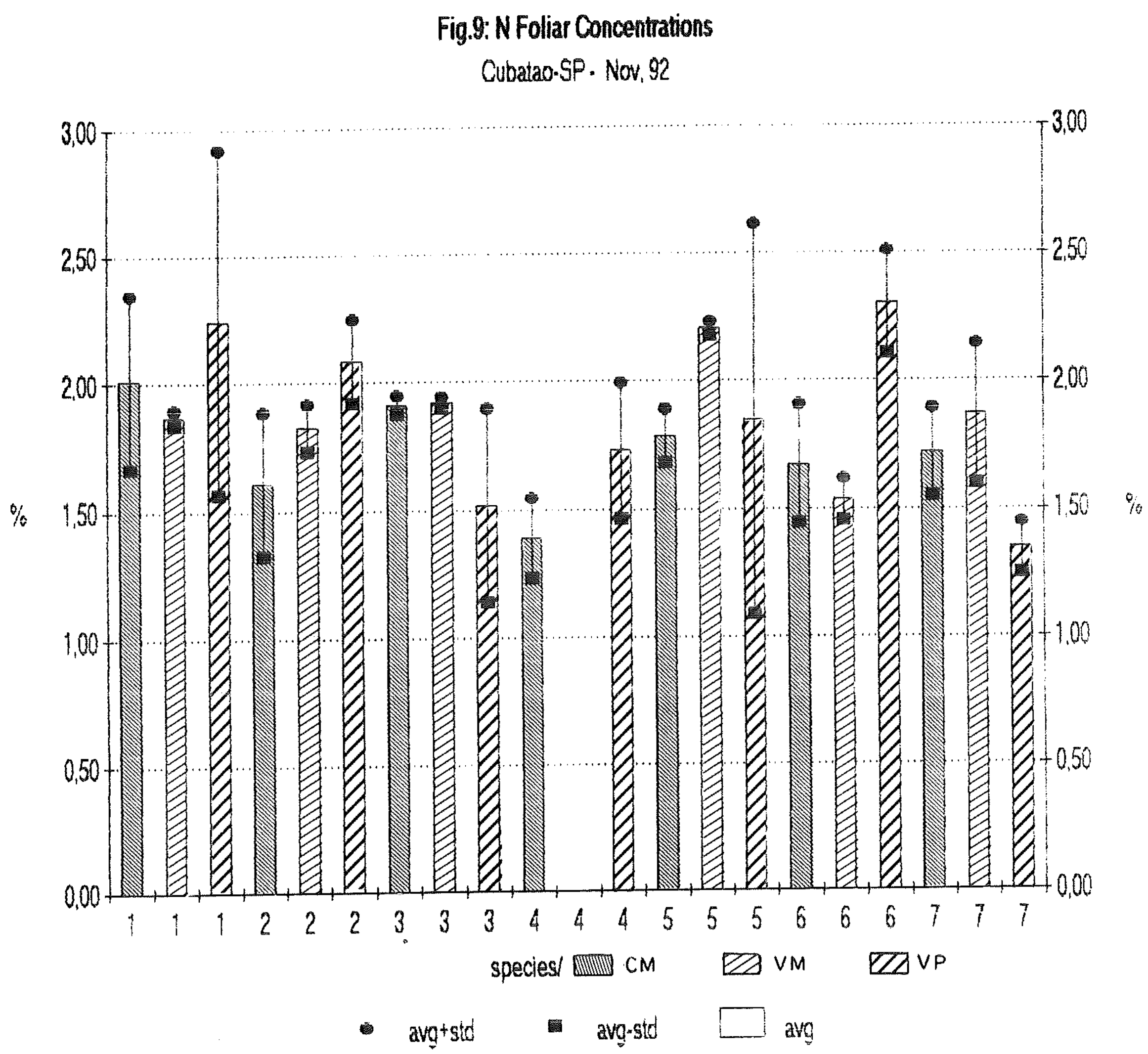


Fig.9: N Foliar Concentrations
Cubatao-SP - Nov, 92
3,00
2,50
2,00
1,50
1,00
0,50
0,00
%
1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7
species/ CM VM VP
avg+std
avg-std
avg

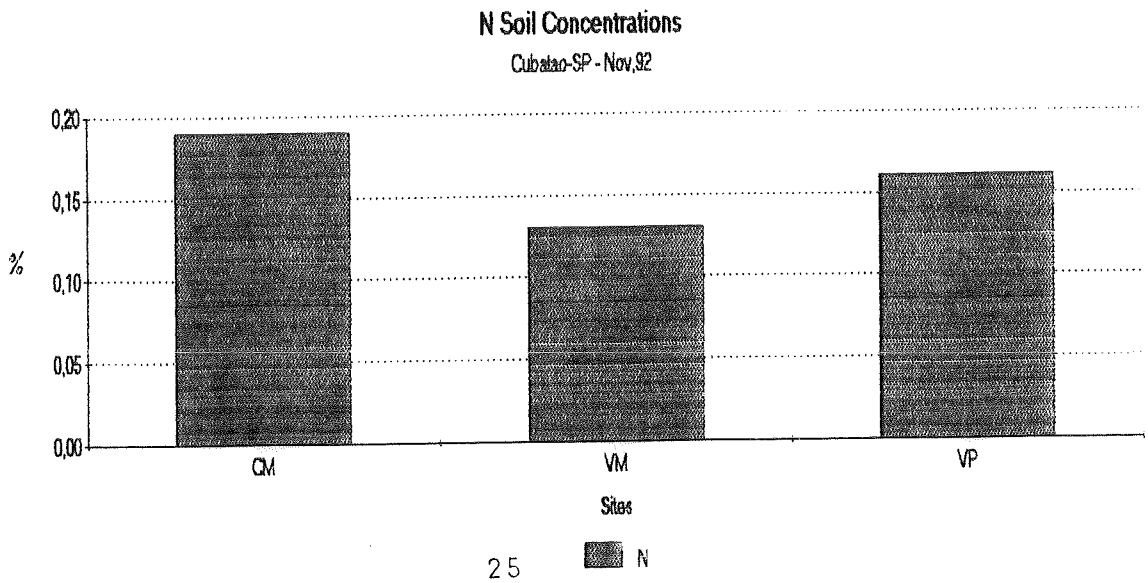
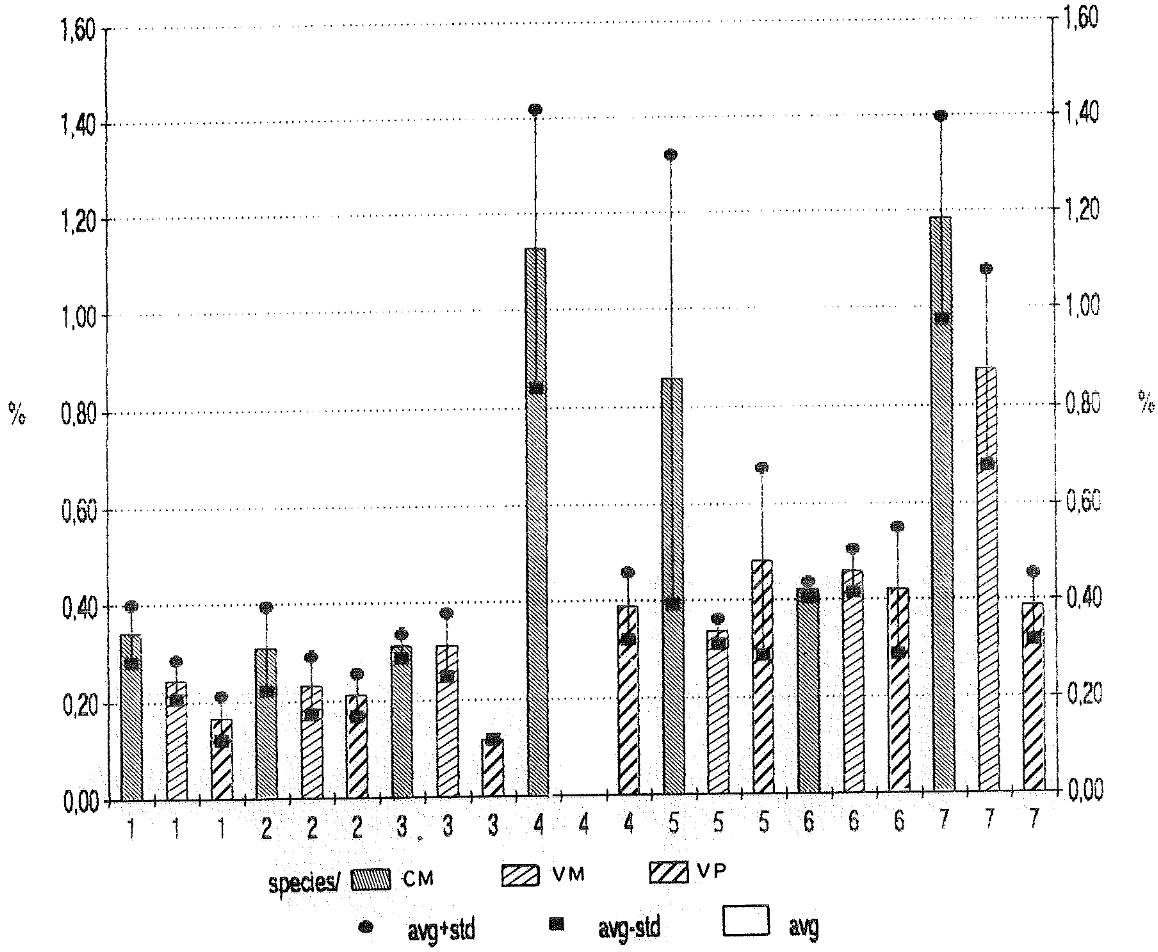

N Soil Concentrations
Cubatao-SP - Nov,92
0,20
0,15
0,10
0,05
0,00
%
CM
VM
VP
Sites
N

Fig. 10: Sulphur Foliar Concentratios

Cubatao-SP - Nov,92



S Soil Concentrations

Cubatao-SP - Nov,92

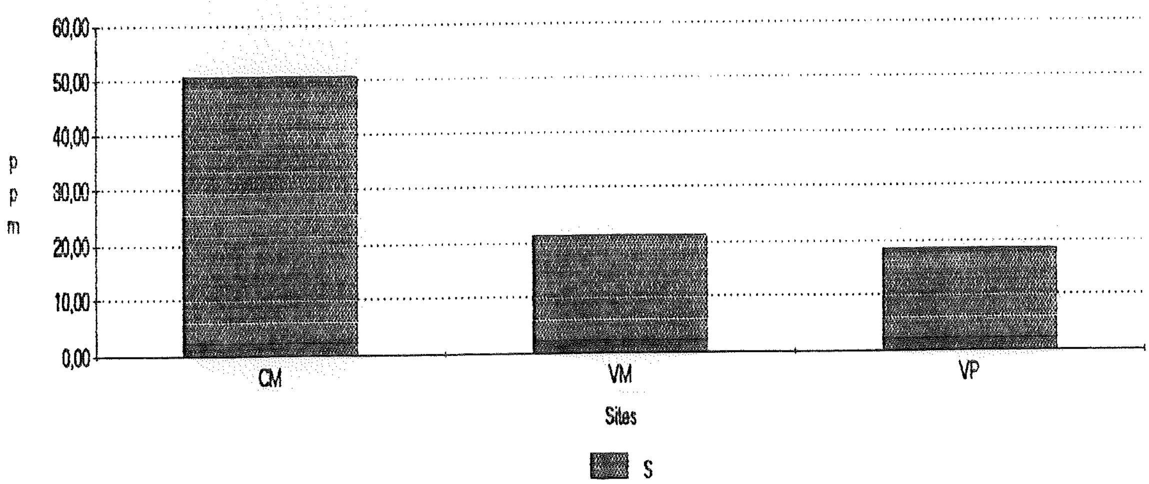
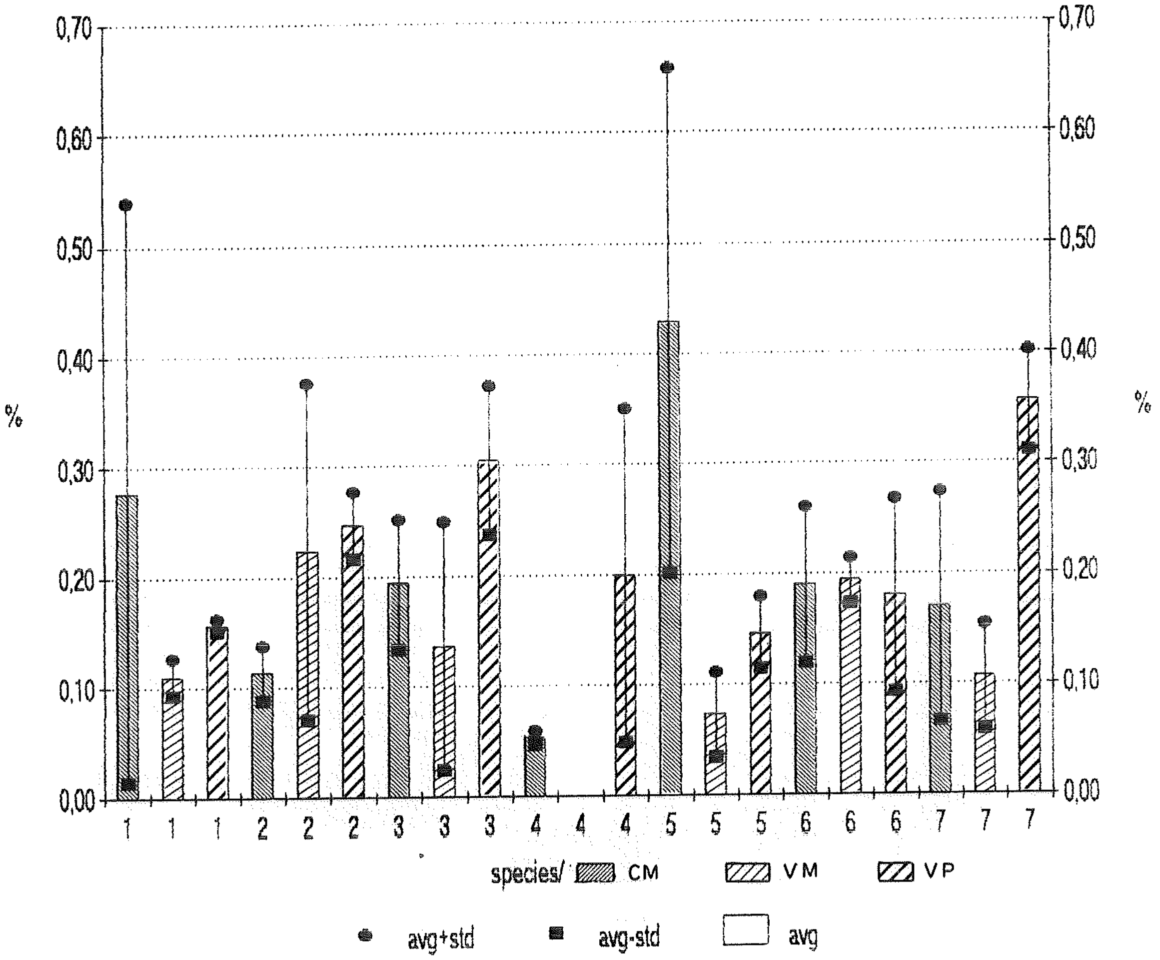


Fig.11: P Foliar Concentrations

Cubatao-SP - Nov, 92



P Soil Concentrations

Cubatao-SP - Nov,92

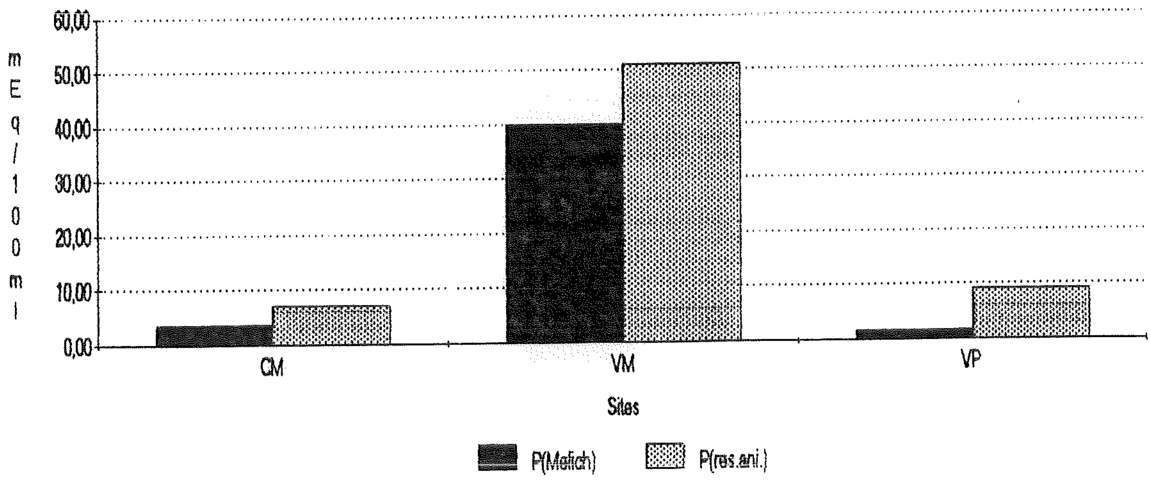
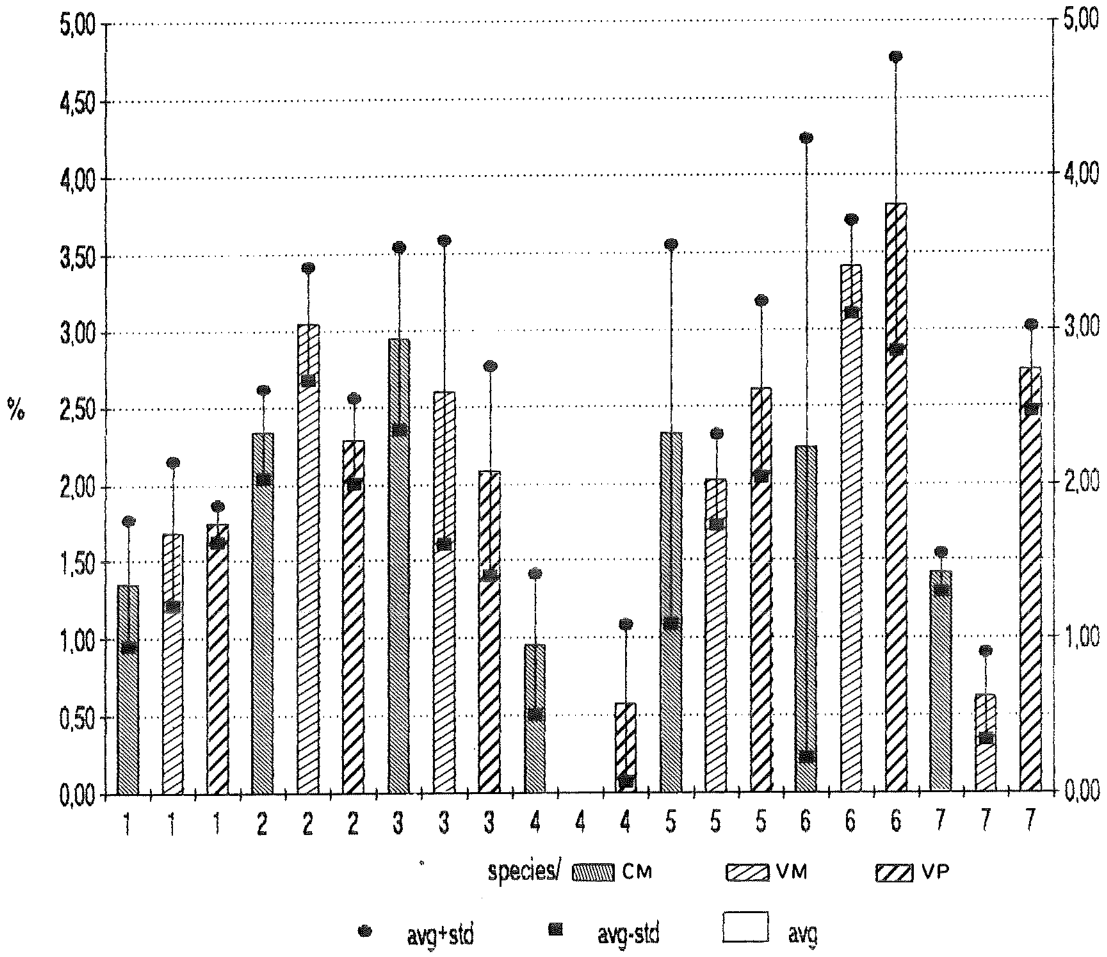


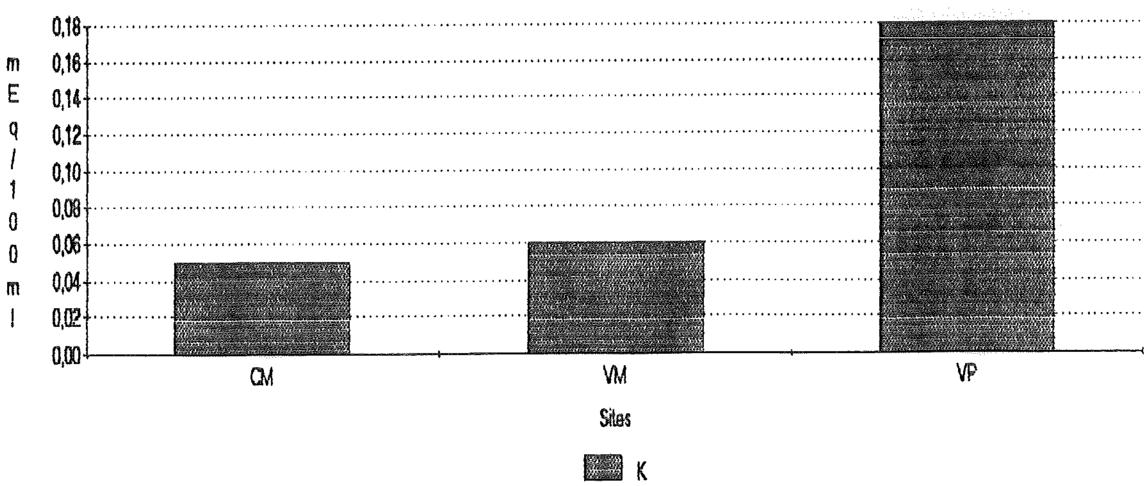
Fig. 12: K Foliar Concentrations

Cubatao-SP - Nov,92



K Soil Concentrations

Cubatao-SP - Nov,92



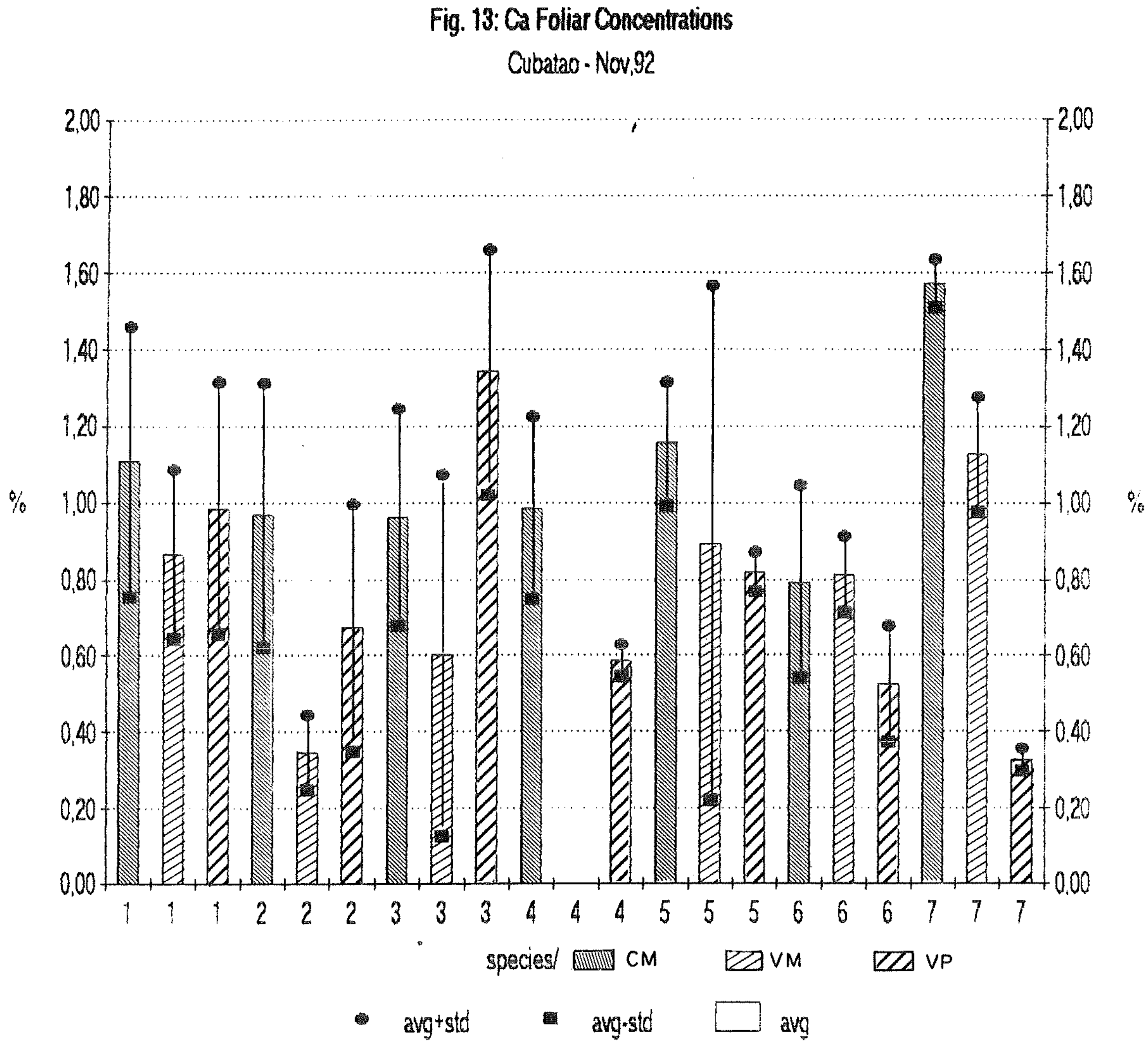
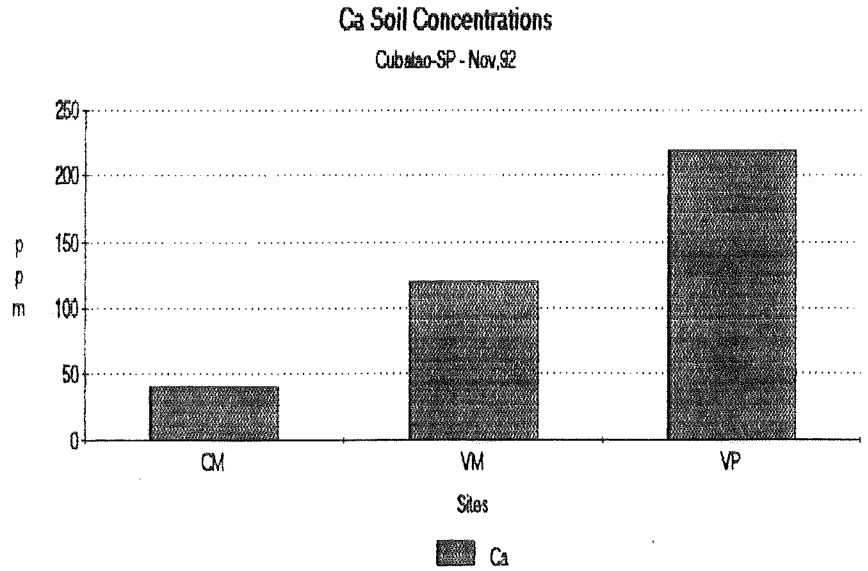


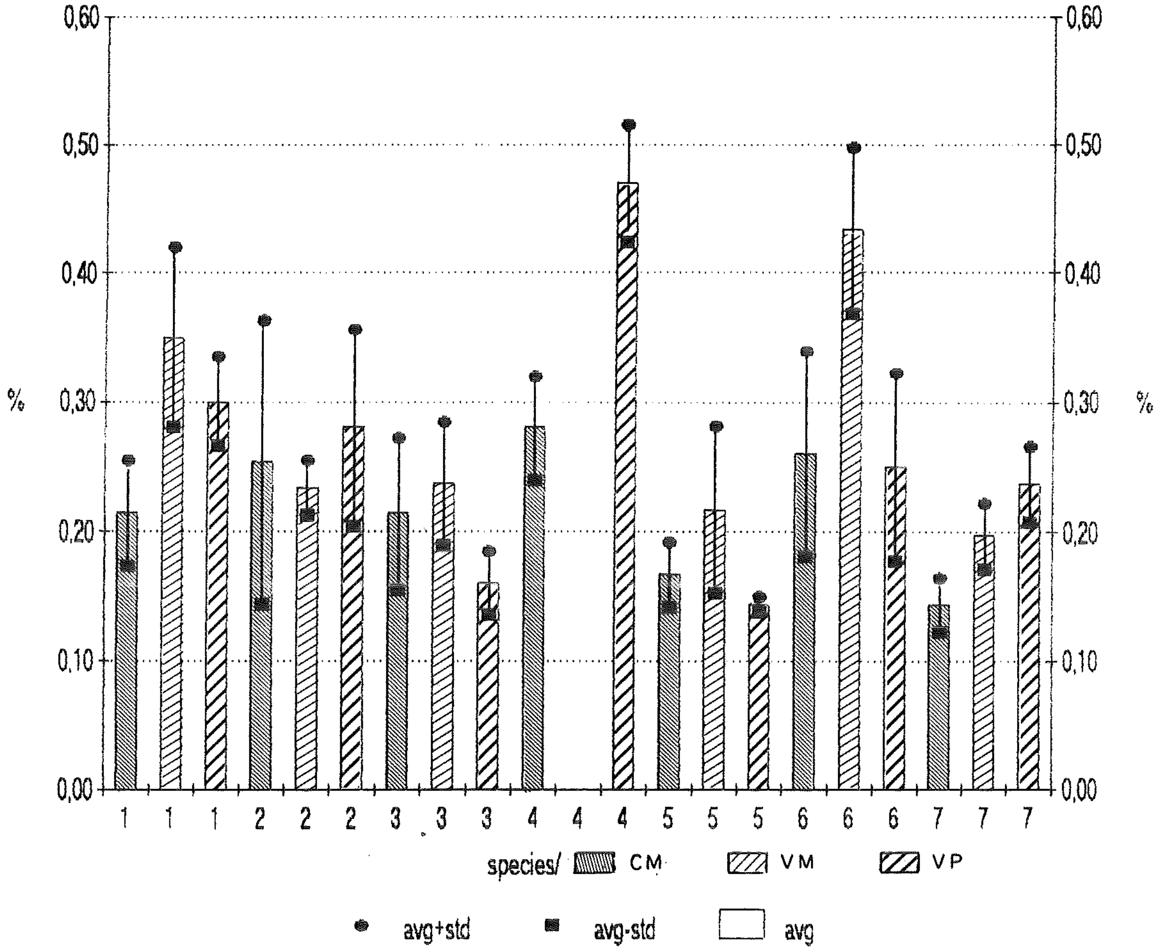
Fig. 13: Ca Foliar Concentrations
Cubatao - Nov,92
2,00
1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
%
1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7
species/ CM VM VP
avg+std
avg-std
avg


Ca Soil Concentrations
Cubatao-SP - Nov,92
250
200
150
100
50
0
p
p
m
CM
VM
VP
Sites
Ca

33-10-040/1

Fig.14: Mg Foliar Concentrations

Cubatao-SP - Nov, 92



Mg Soil Concentrations

Cubatao-SP - Nov,92

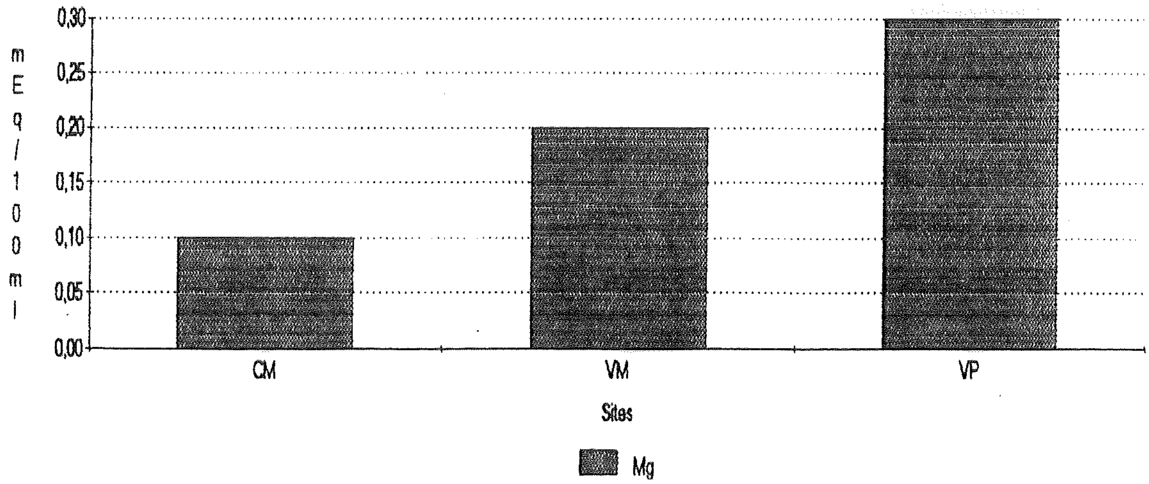
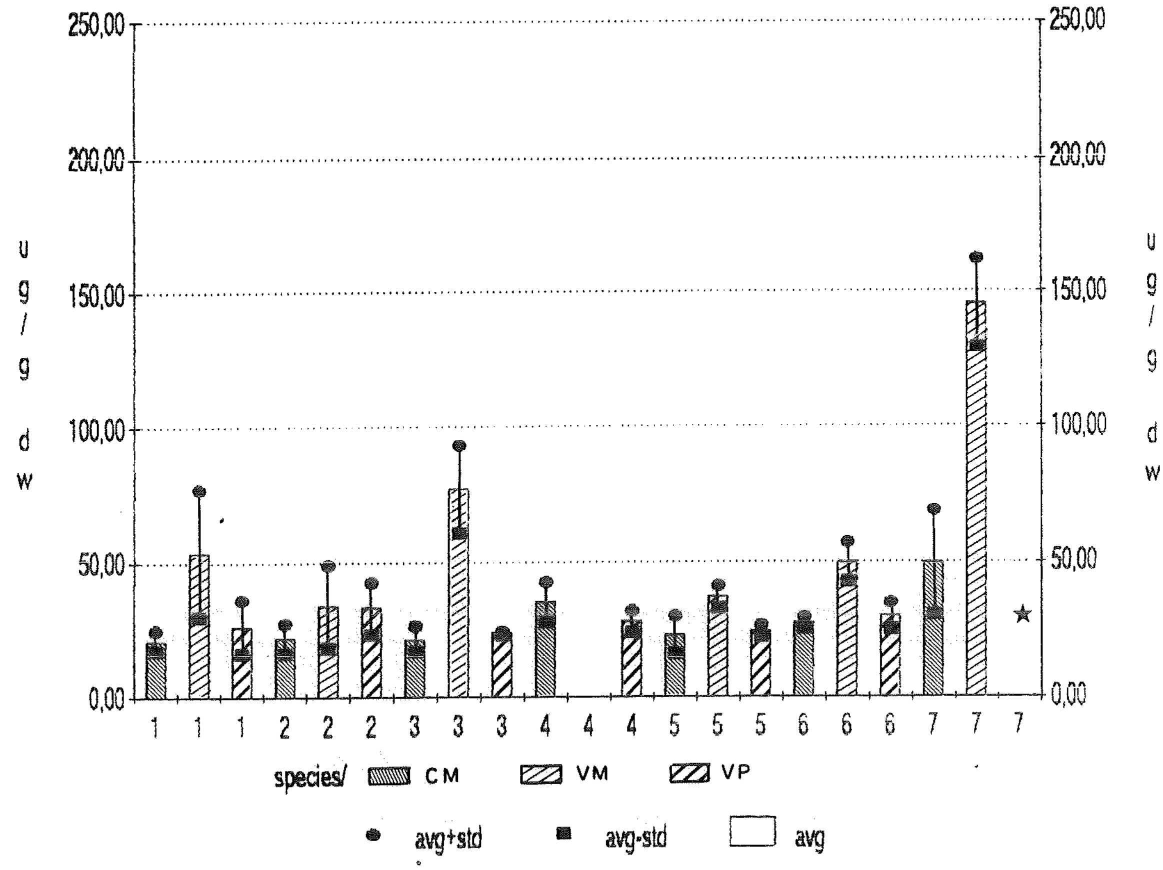


Fig.15 - Fluoride Foliar Concentrations

Cubatao-SP - Nov,92



★ OBS.: The fluoride concentration in **Tibouchina** from Vale do Pilões was unexpected in view of the preceding results. T confirm these data, the collection and analyses will b repeated.

Figure 16: Xylol infiltration

═══ with infiltration, field survey 7 and 8/04/92

▬▬▬ with infiltration, field survey 20 and 21/08/92

---- infered data

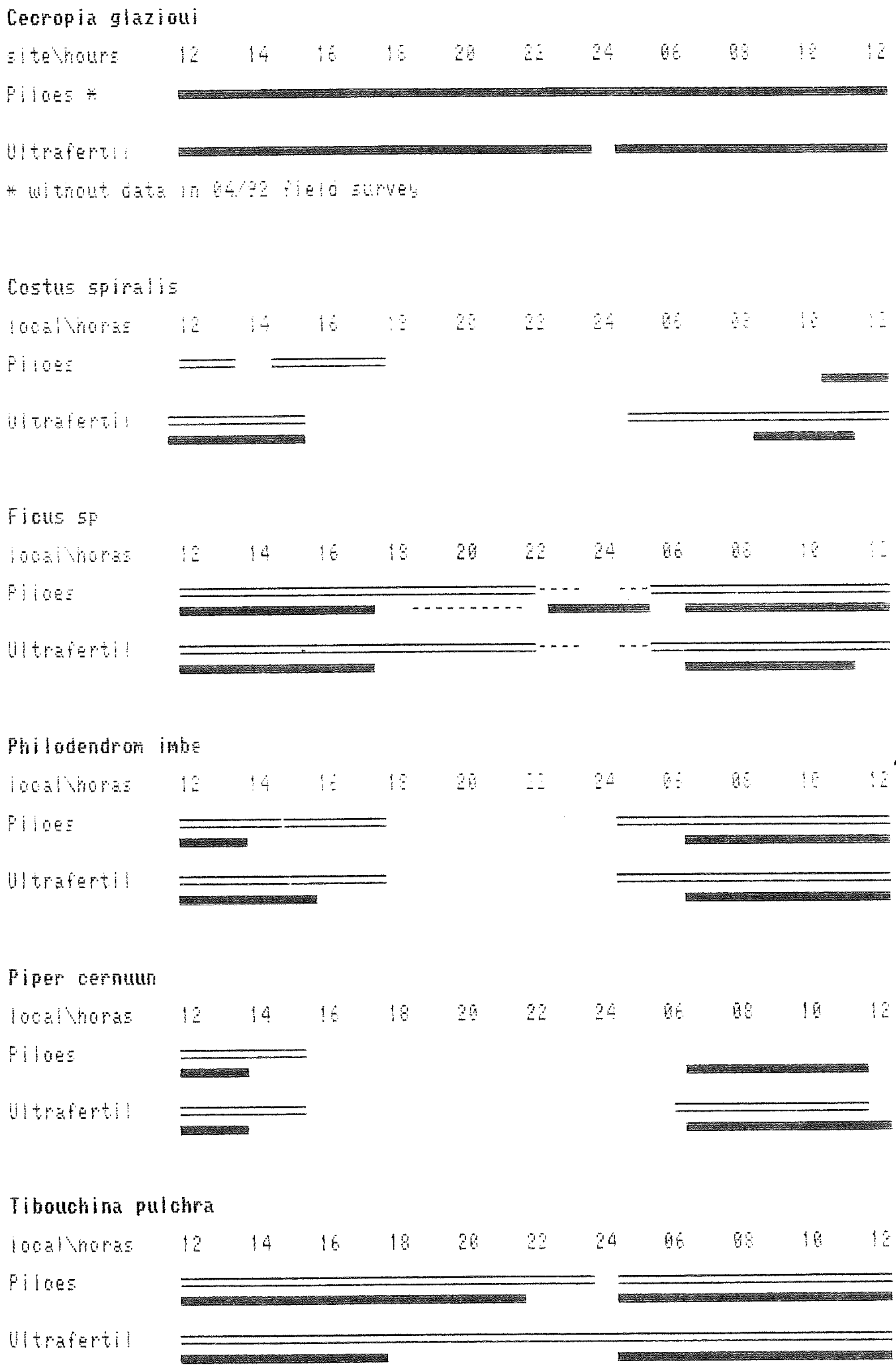
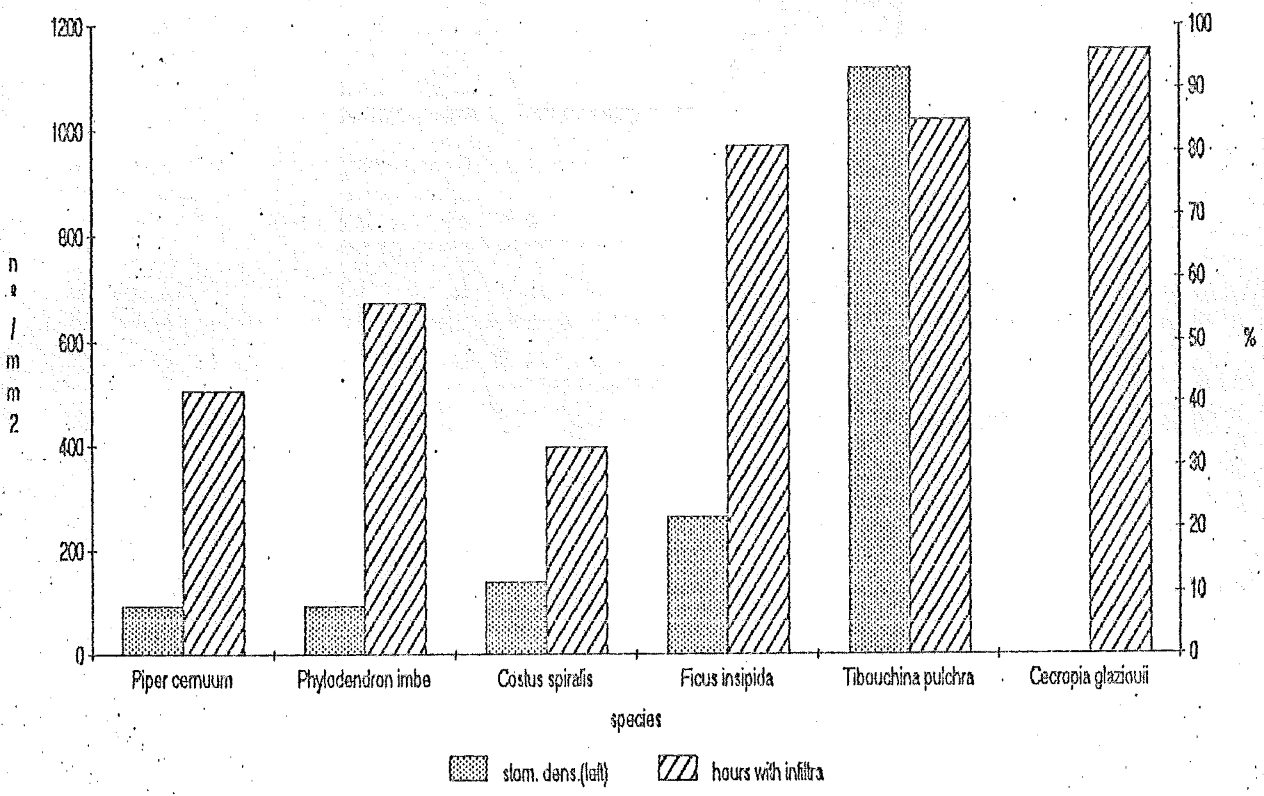


Table 11: Xylol Infiltration and Stomato Density

SPECIES	% OF HOURS WITH XYLOL INFILTRATION	STOMATO DENSITY(nº/mm2)
Piper cernuum	42	94
Phylodendron imbe	56	94
Costus spiralis	33	141
Ficus insipida	81	260
Tibouchina pulchra	85	1117
Cecropia glaziouii	96	

Fig. 17: Xylol Infiltration and Stomato Density



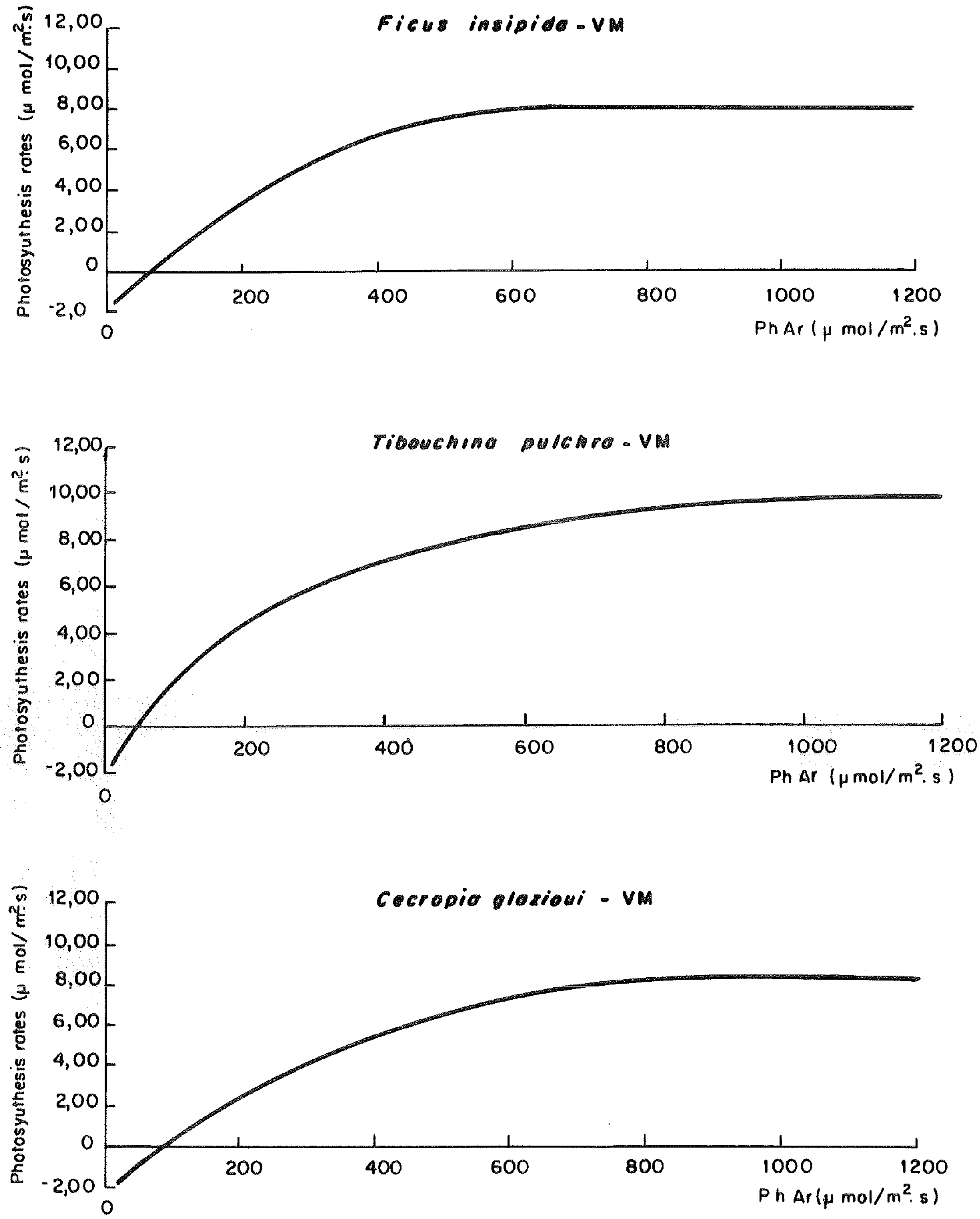
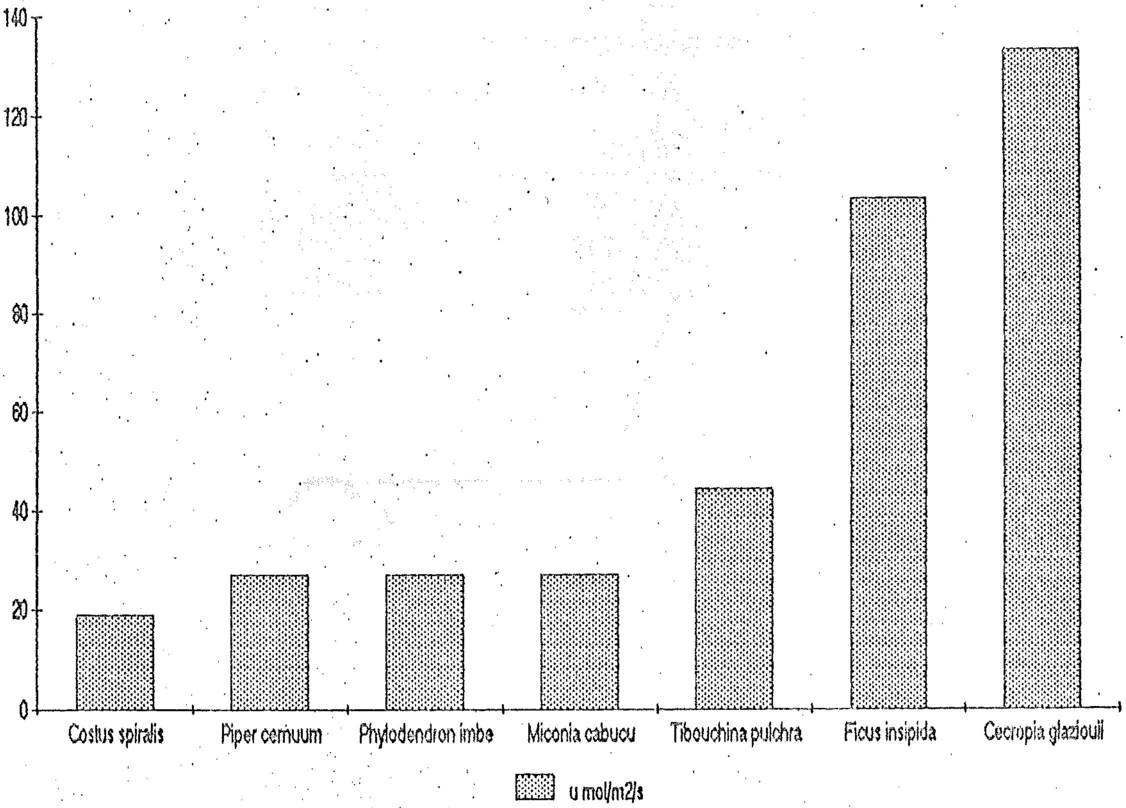


Fig. 18 - Saturation curves

Table 12: Light compensation point

SPECIES	u mol/m2/s
Costus spiralis	19
Piper cernuum	27
Phylodendron imbe	27
Miconia cabucu	27
Tibouchina pulchra	44
Ficus insipida	103
Cecropia glaziouii	133

Fig. 19: Light Compensation Point



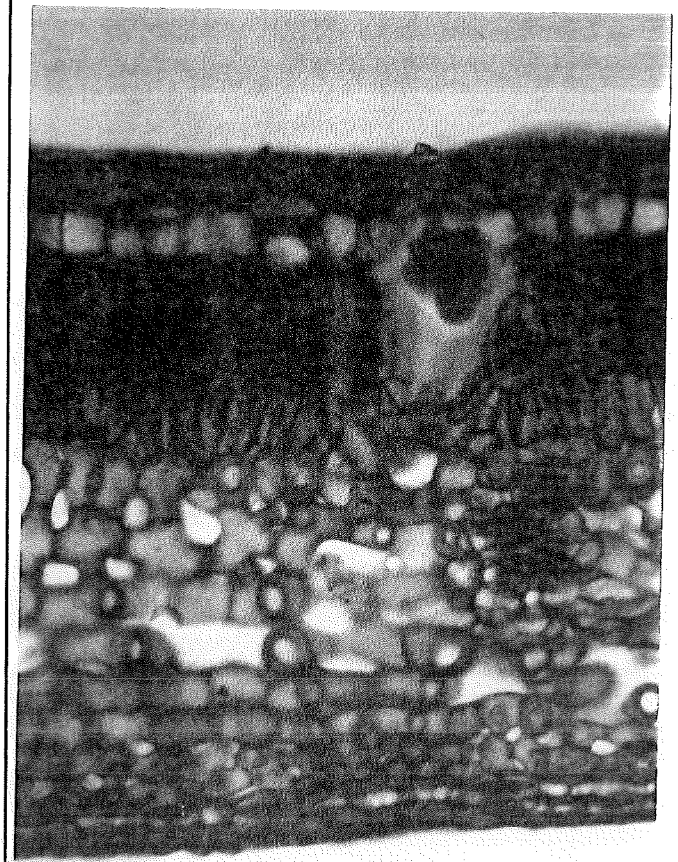
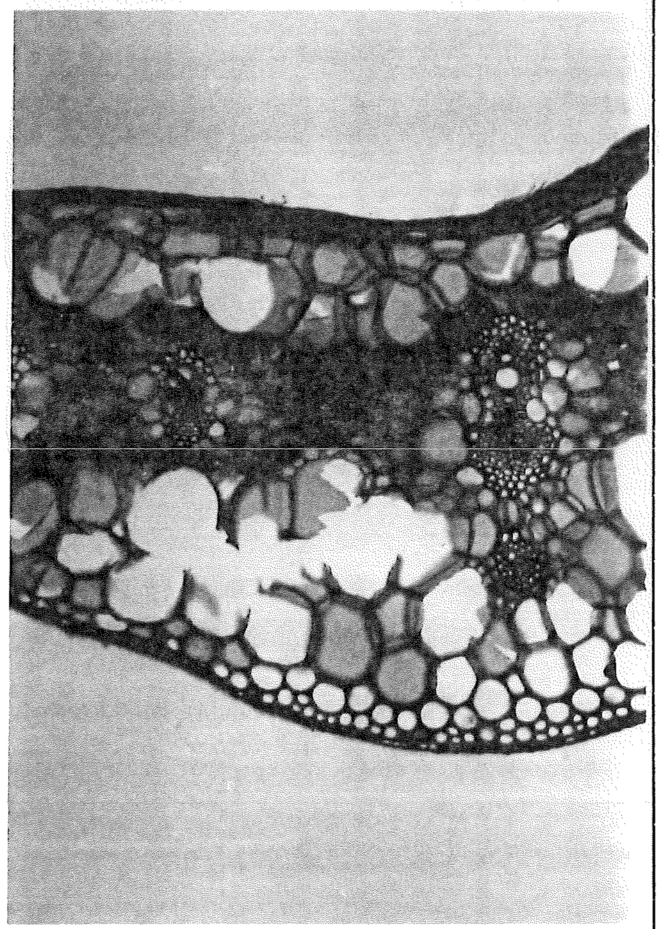


PHOTO 1 - TRANSVERSAL SECTION OF Ficus insipida

PHOTO 2 - TRANSVERSAL SECTION OF Costus spiralis



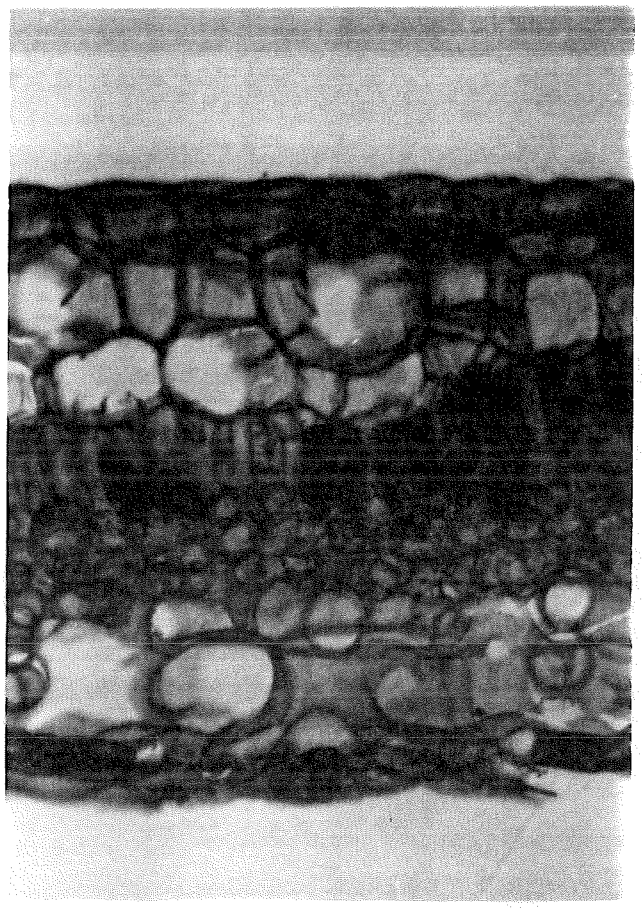


PHOTO 3 - TRANSVERSAL SECTION OF <u>Piper cernuum</u>

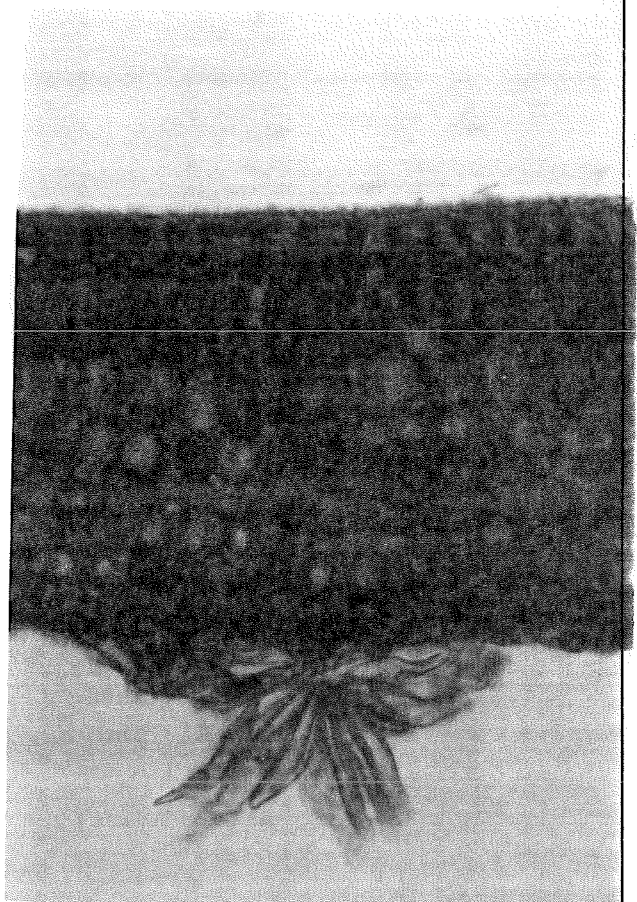


PHOTO 4 - TRANSVERSAL SECTION OF <u>Miconia cabucu</u>

PHOTO 5 - TRANSVERSAL SECTION OF *Cecropia glazioui*

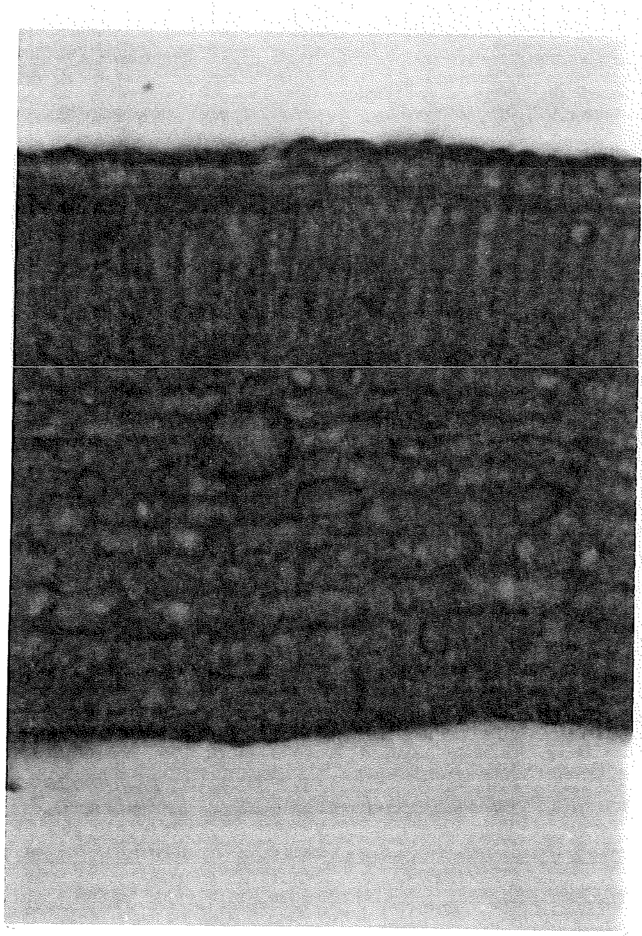


PHOTO 6 - TRANSVERSAL SECTION OF *Tibouchina pulchra*

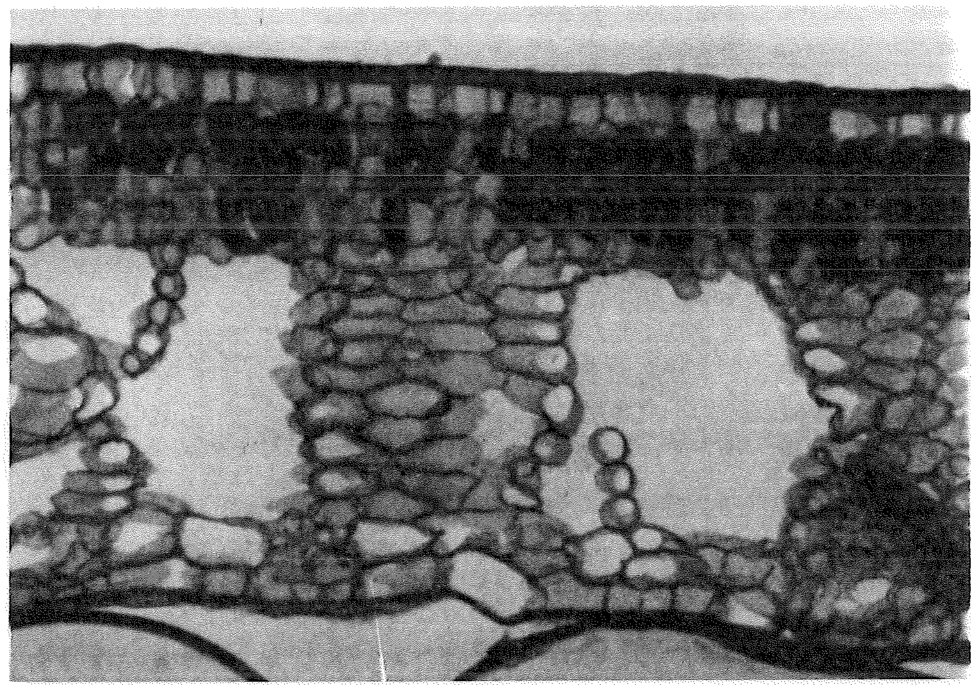


PHOTO 7 - TRANSVERSAL SECTION OF
<u>Phylodendron imbe</u>

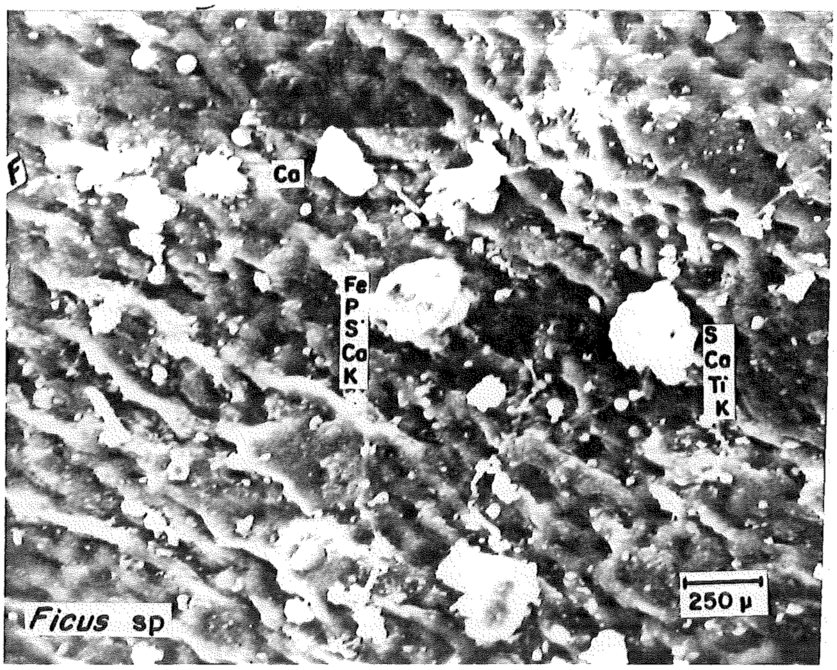


PHOTO 8 - SEI OF ADAXIAL SURFACE
Ficus sp

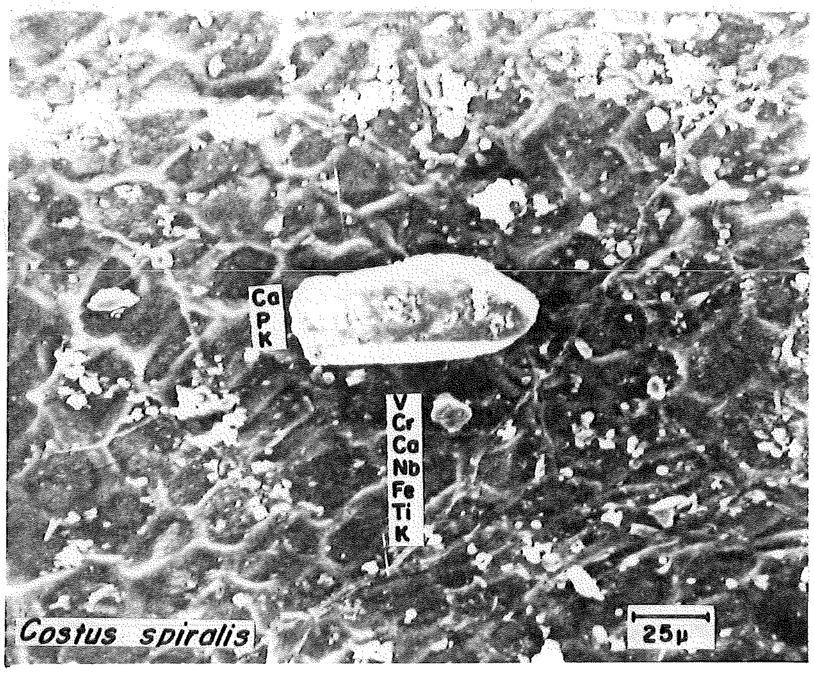


PHOTO 9 - SEI OF ADAXIAL SURFACE
Costus spiralis

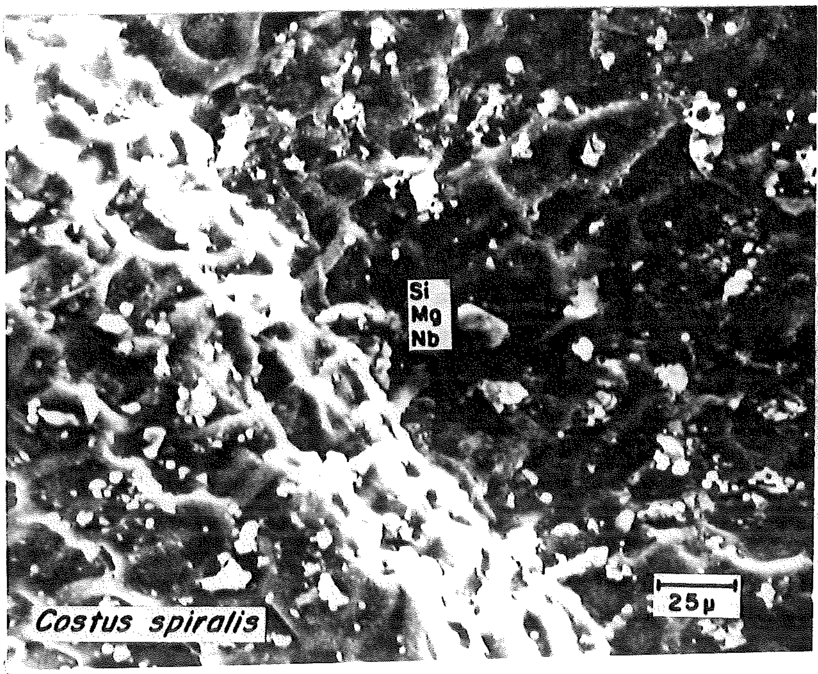


PHOTO 10 - SEI OF ADAXIAL SURFACE
Costus spiralis

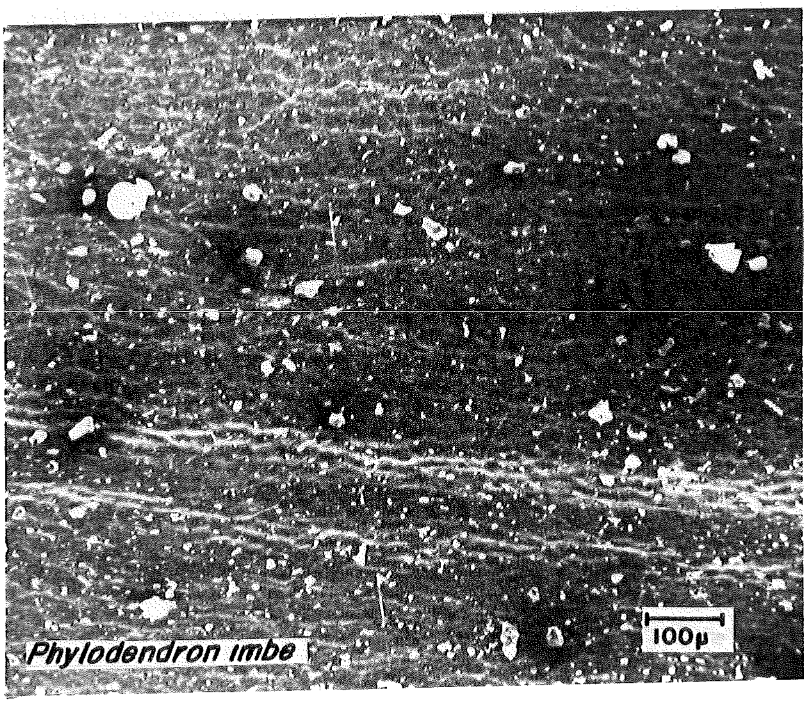


PHOTO 11 - SEI OF ADAXIAL SURFACE
Phylodendron imbe

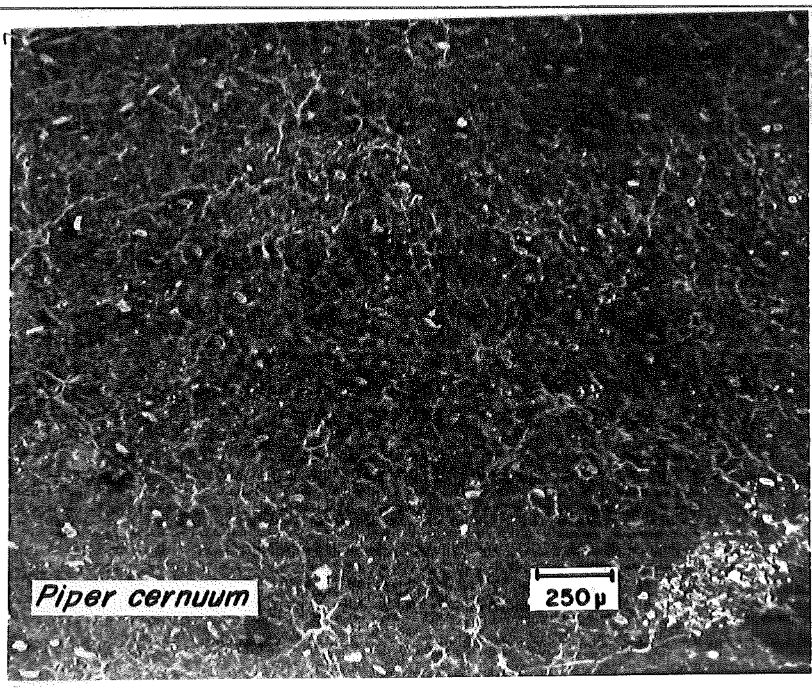


PHOTO 12 - SEI OF ADAXIAL SURFACE
Piper cernuum

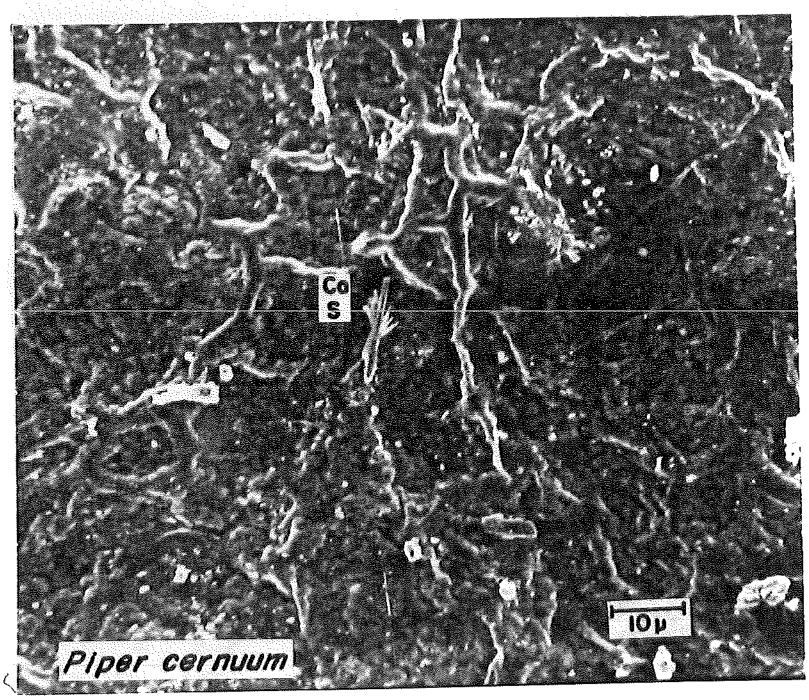


PHOTO 13 - SEI OF ADAXIAL SURFACE
calcium sulfate crystals

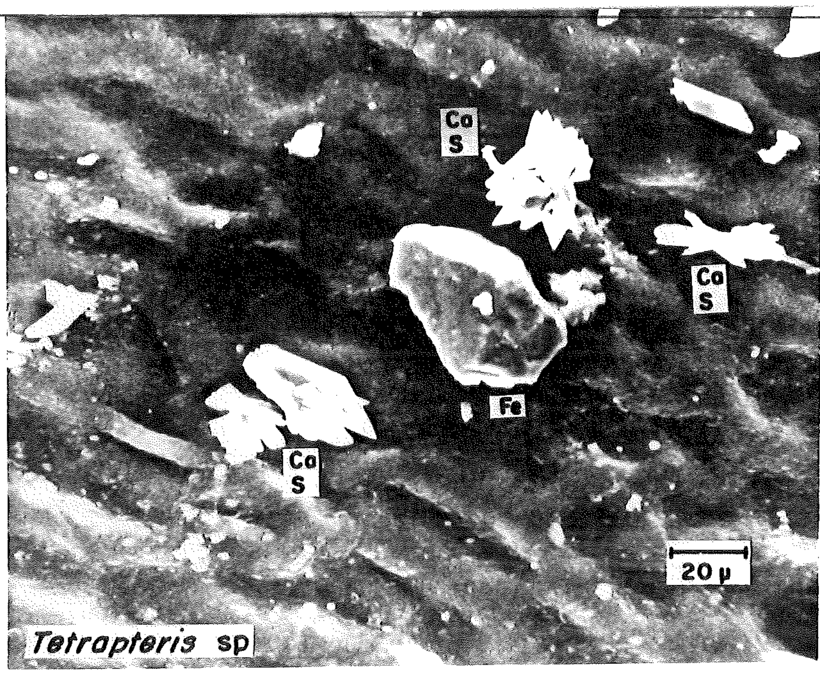


PHOTO 14 - SEI OF ADAXIAL SURFACE
calcium sulfate crystals

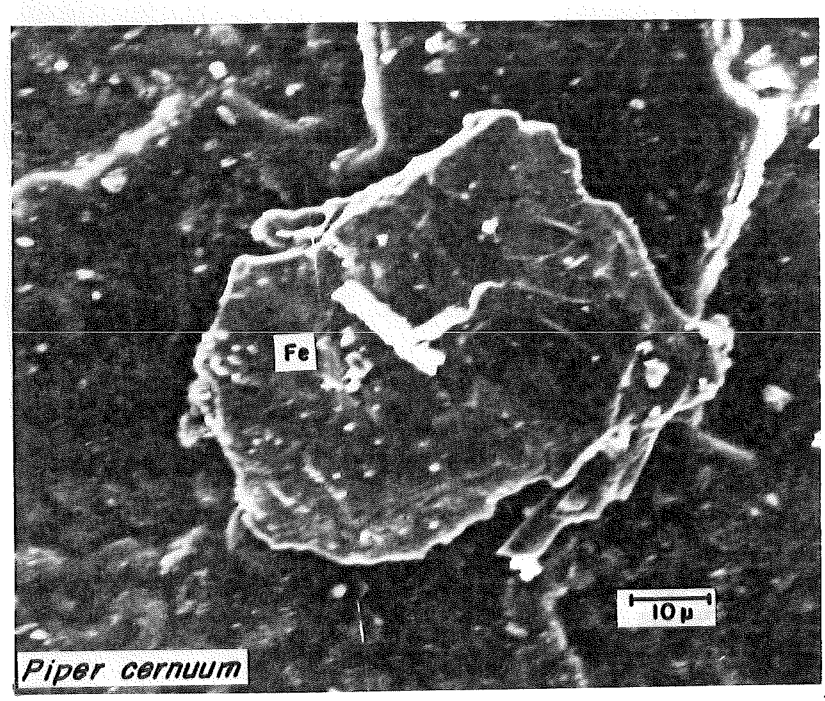


PHOTO 15 - SEI OF ADAXIAL SURFACE
Metallic ferrum particle

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