BIOGEOCHEMICAL STUDIES IN SOUTH EAST ASIA BY USE OF HERBARIUM MATERIAL.

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in Chemistry
at Massey University.

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ABSTRACT.

A biogeochemical survey of Indonesia was undertaken analyzing herbarium samples by atomic absorption. (Indonesia was chosen because it has been well surveyed botanically, but poorly surveyed geologically.)

Background concentrations of copper, nickel and zinc were determined using various species, predominantly members of the families Flacourtiaceae and Violaceae.

Rinorea bengalensis (Wall.) O.K. (Violac.) was discovered as a hyperaccumulator of nickel (Brooks et al., 1977) and this led to an in depth study of R. bengalensis and other species of the genus. From this study two areas of doubtful geology were predicted as containing ultrabasic rocks, R. javanica (Bl.) O.K. was shown to be a hyperaccumulator of nickel and R. albersii Engl. was found to yield a very high cobalt/nickel concentration ratio.

Three hyperaccumulators of nickel were discovered after analysis of a selection of herbarium samples collected on Obi Island by Dr. E. de Vogel. (They were: Myristica laurifolia Spruce ex DC var. bifurcata (Myrist.); Planchonella oxyedra Dubard (Sapotac.); Trichospermum kjellbergii Burret (Tiliac.).)
Ambon Island was predicted as consisting of ultrabasic substrates.

Plants collected from Salajar Island were shown to contain anomalously high concentrations of copper possibly due to anomalous copper concentrations in the soil.

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GENERAL INTRODUCTION.

With approximately 200,000,000 plant specimens stored in the world's herbaria, it at first seems curious that few attempts have been made to analyse this material chemically. However, when the size of sample required for some classical analytical techniques is considered, the reluctance of herbaria to donate samples becomes very easy to understand. In fact, because of the size of the samples required for analysis of dried material, much of the early use of herbaria by geochemists relied on the use of information stored in the co-operating herbarium rather than the use of plant material itself. (e.g. Persson, (1956) used herbarium data sheets to find the collection localities of "copper mosses" and thereby was able to pin-point copper anomalies in the substrate). Chenery (1948) however, did use dried herbarium plant samples for analysing aluminium concentrations in plant tissues.

Now that analysis can be carried out with very small samples, particularly using the techniques of flame (Walsh, 1955) and flameless (Kirkbright, 1971) atomic absorption spectrophotometry, chemical analysis of herbarium material now becomes feasible. Potential uses of this material now fall into three major categories:-

Taxonomic - species differentiation on the basis of trace element concentration as in the work of Brooks, McCleave and Schofield (1977) with the genus *Nyssa*.

Phytochemical - studying accumulation of elements by certain plant species e.g. the study of nickel accumulators and hyperaccumulators (more than 1000 μg/g nickel) by Brooks *et al*. (1977).

Geological - the use of plant samples for biogeochemical work and mapping of geological anomalies. Although this field was touched on by Brooks *et al*. (1977), much remains still to be done. Work of this nature can fall into two categories : geobotanical, where the visual appearance of the species present or the general presence or absence of certain species can be related to the availability of trace element in the soil; and biogeochemical where plant material is analysed for its elemental content. (The work of Persson (1956) could be described as geobotanical and that of Chenery (1948) as biogeochemical.)

The aim of this study was to carry out a biogeochemical study covering a limited area of the world's surface. The desirable characteristics for the study area were that it would have to be poorly mapped geologically (or else there would be little point in attempting to carry out mapping) but relatively well surveyed botanically, so that a large volume and variety of specimens could be called upon. The area itself must contain a rich flora, (an area of desert would be most unsuitable for any biogeochemical studies). Finally, it would be desirable if those parts of the area that were geologically mapped were known to contain ultrabasic rocks (associated with high nickel, cobalt, chromium, vanadium and manganese concentrations), since the biogeochemical method must first be tested in areas of known geology. Two areas come to mind as being suitable for a study of this nature: South East Asia (particularly in the region of Indonesia) and South America. As this laboratory already contained a large number of samples of the genera Homalium and Hybanthus collected from Malesia (Indonesia, Malaysia and the Philippines), South East Asia was picked as a general area for the study with particular attention to be paid to Indonesia. (See Figure 0-1.)

The aims of the project were as follows: Firstly, it was hoped to predict the geology of certain areas and fill in gaps in the geological mapping of the area. Unfortunately any mapping done, could only be guesswork as the only way to know the precise mineral composition of the soil would be to analyse the soil. The second aim was to discover previously unknown sites of mineralization. Again these predictions would have to be checked with soil and rock analyses in the area. Finally, it was hoped that new mineral accumulating species could be discovered, particularly further hyperaccumulators of nickel (Brooks et al., 1977). Such species are of great interest to phytochemists, and even to chemical engineers because of the obvious potential of being able to imitate technologically, the low energy methods of extraction by plants of heavy metals from their ores.

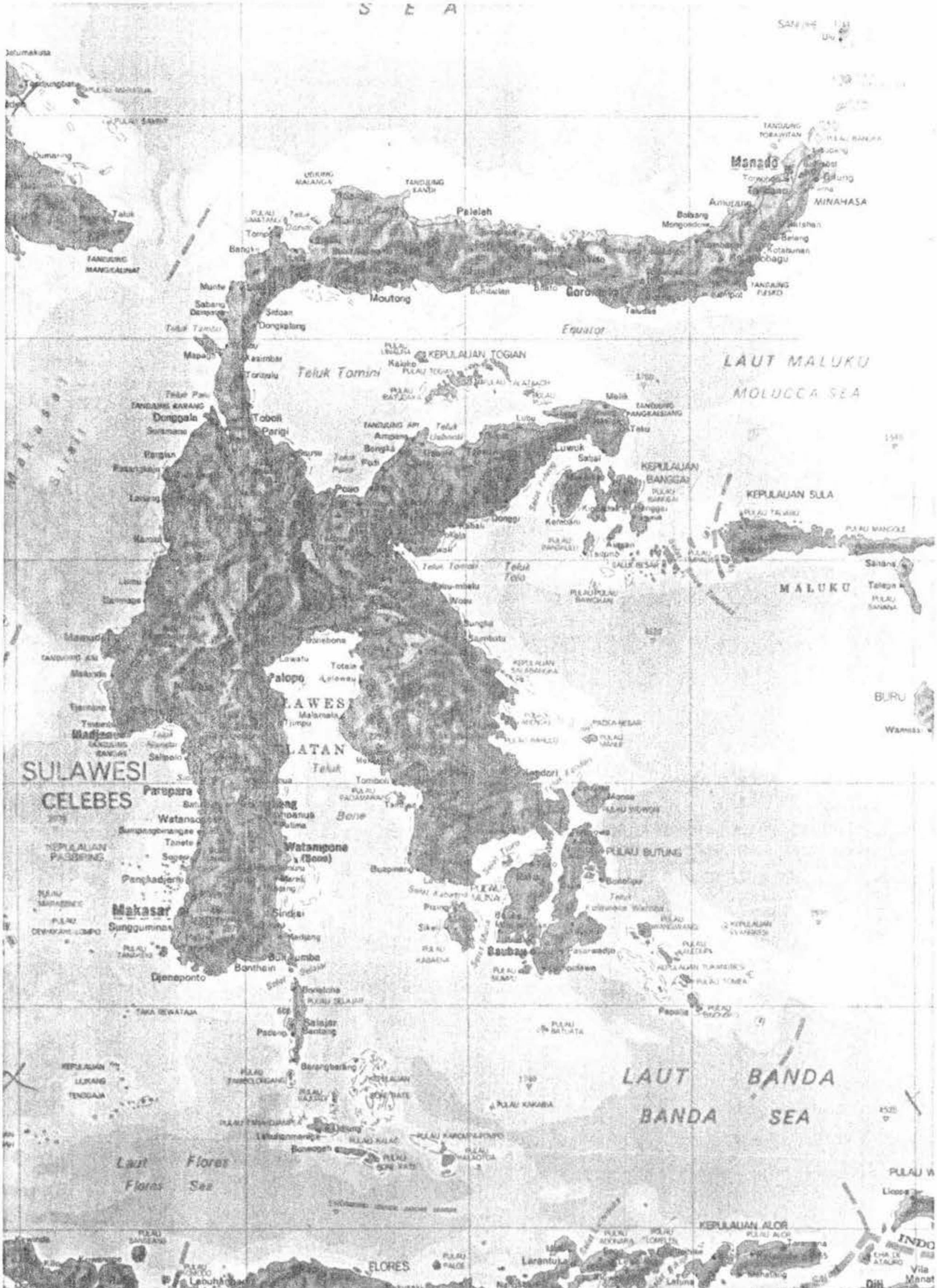
Although this project had the disadvantage that soil and rock types could only be predicted and checks would have to be made on the site, it did have the advantage of cheapness compared with the enormous cost of field work. It was expected that pinpointing favourable sites for subsequent in situ investigation would be the major benefit of this work.

The results of this survey are presented in this thesis.

FIGURE 0-1.

MAP OF THE CELEBES, INDONESIA

(Nickel mining carried out in this Region.)


S E A
Manado
MINAHASA
Paleleh
Moutong
Equator
KEPULAUAN TOGIAN
Teluk Tomini
LAUT MALUKU
MOLUCCA SEA
Donggala
Toboli
Parigi
Poso
Luwuk
KEPULAUAN BANGGAI
KEPULAUAN SULA
MALUKU
Palopo
BURU
SULAWESI
CELEBES
Parepare
Watampone (Bone)
Bone
PULAU BUTUNG
Makasar
Sungguminasa
Djeneponto
Bonthain
Baubau
LAUT BANDA
BANDA SEA
Laut Flores
Flores Sea
KEPULAUAN ALOR
FLORES
Larantuka
KEPULAUAN SOLOR
Dili
Atapupu
PORT. TIMOR

SECTION I.

GEOGRAPHY AND BOTANY OF THE MALAY ARCHIPELAGO.

Although this section deals in general with the chain of islands known as the Malay Archipelago, (Malesia) specific reference will also be made to those islands that make up the Republic of Indonesia, as the majority of the samples studied were collected originally in that area.

A. GEOGRAPHY

The Malay Archipelago comprises many islands of greatly varying shapes and areas, situated near the equator, and forms a broken chain from South East Asia in its northwest extremity to just north of eastern Australia in the south western extremity (See Figure I-1.)

1. Indonesia.

Indonesia is made up of a large portion of the Malay Archipelago and has been described by Woodman (1955) as follows:

"--- 3000 islands strung along the Equator for 3000 miles --- all sizes and shapes; small atolls built up by millions of coral polyps; island volcanoes; islands of undisturbed green jungle; islands where no foreigner has wandered and unknown to most Indonesians; islands that hug the coast of thickly populated Java; a string of islands appearing like a row of distant steamers along the coast of Sumatra; hundreds of islands in the Moluccas famous for the spices and cloves and pepper which first attracted visitors from the western world; islands that are rich in tin; islands thickly covered with rubber trees; islands rich in oil. Throughout these islands and islets the sun always shines; there is no winter, no spring, no autumn, only a wet season and a dry."

The land surface area of Indonesia is 736,469 square miles (1,885,361 square kilometres) (making it the fifteenth largest territorial unit in the world). This land supported a population of 118,460,000 in 1971 (Anon,1975).

One of the problems arising from the dispersed nature of the country is that it is hard to unify the people. When the republic was formed in 1949 it contained many differing groups of people and this was one of the problems the new government set out to remedy. (Woodman, 1955).

2. Effect of the Sea Floor.

A study of ocean depths in the vicinity of the Malay Archipelago reveals three zones. At either end of the chain, shallow waters are found and deeper waters occur in the central region.

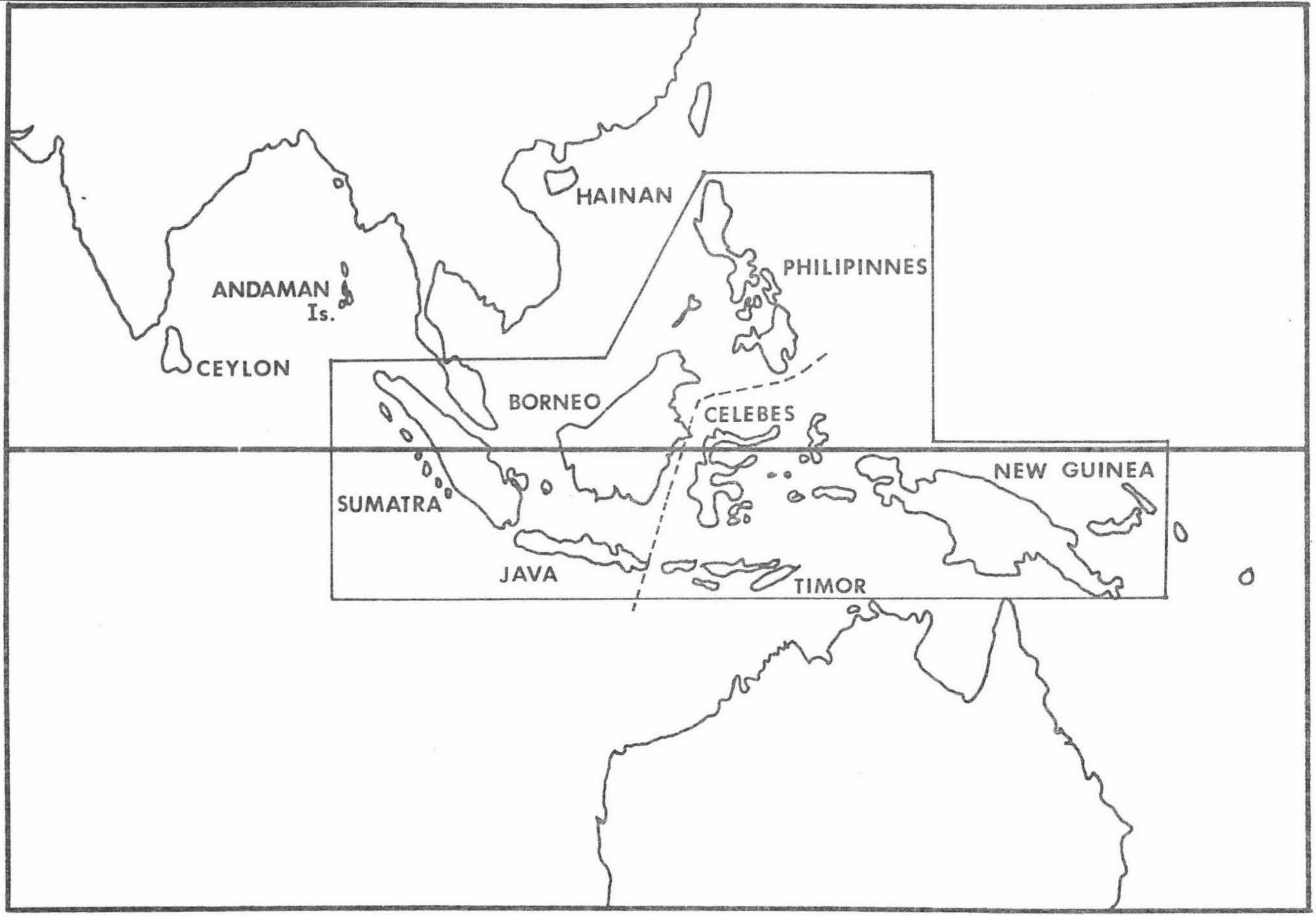
Java, Bali, Sumatra and Borneo arise out of a continental shelf called the Sunda shelf which is believed to have once been part of the Asian mainland.

Similarly the Sahul shelf runs from the Australian coast. New Guinea and Aru are part of this.

When Alfred Russel Wallace visited the area over a century ago (Wallace 1869) he noticed that the western islands of the Archipelago contained species of plants and animals similar to those found in Asia, whereas those found in the eastern islands were more like the Australian species. In the central regions (the Celebes and the Moluccas) a mixture of Asian and Australian species was found. On the basis of these species differences he drew "Wallace's line" (shown on Figure I-1), which marked the eastern boundary of the zone which contained only Asian species. When the Sunda shelf was later mapped it was found that the outer edge approximated "Wallace's line" (Good,1974).

FIGURE I-1.

Area of studies (enclosed by solid line).
"Wallace's line" shown as dotted line.


HAINAN
PHILIPINNES
ANDAMAN Is.
CEYLON
BORNEO
CELEBES
NEW GUINEA
SUMATRA
JAVA
TIMOR

3. Indonesian Natural Resources.

Indonesia has long been known for its natural resources as shown by the following statistics (Anon, 1975).

(i) Mining - Minerals mined in the Republic of Indonesia in 1973 were:-

Bauxite	1,229,375	tonnes
Gold	352.1	kg.
Silver	8,832	kg.
Nickel	867,046	tonnes*
Tin	22,204	tonnes

*Nickel production has now greatly increased as a result of exploration of vast lateritic reserves in the Malili - Soroako area of South-central Celebes.

(ii) Exports in the year 1973 comprised mainly fish, coffee, tea, pepper, tobacco, lumber, palm oil, tin, nickel ore, crude oil and petroleum products.

B. CLIMATE AND VEGETATION.

1. Climate.

Generally the climate could be described as maritime equatorial (high temperatures, except at high altitudes, and heavy rainfall all year round). However, parts of western Indonesia experience periods of exceptionally heavy rain coinciding with the north east or south west monsoons.

The eastern half of Java, Bali, southern Celebes and Nusa Tenggara have clearly marked dry seasons caused by the southeast monsoon (which changes direction to become the southwest monsoon over western Indonesia) in the months June, July, September and October. An example of these effects is cited in "The Far East and Australasia" (Anon 1975).

"Thus whereas in Pontianak, situated almost exactly on the equator on the west coast of Kalimantan (originally Borneo) the monthly mean temperature varies from only 25.6°C in December to 26.7°C in July, and no month contributes less than 160 mm (July) or more than 399 mm (December) to the total annual rainfall of 3200 mm. Surabaya in eastern Java , while showing even less variation in mean monthly temperature, which fluctuates between 26.1°C throughout the year, has four months (December - March) each year with over 241 mm of rain, and four others (July - October) with less than 51 mm each out of an annual total of 2735 mm."

2. Vegetation.

In Sumatra and Java the northern slopes of the high ranges are comparatively dry. Java is also moister in the west than the east and these differences have an effect on the vegetation types. The moister side consists of evergreen, temperate rainforests usually overgrown with hanging mosses. The drier side has a middle zone of grasslands and woods with small bushes with leathery foliage further down the mountains and only poor grasslands near the summits.

Borneo has wide coastal plains, with those on the Asiatic side covered with fields and fallow lands. The central highlands are densely wooded, thinning out to scrub at high altitudes. The narrow plateaus of the backbone of the Celebes display typical savannahs.

For a discussion of the effects of climate upon vegetation see Hardy (1920) and Anon (1975).

C. PLANT SPECIES OF THE MALAY ARCHIPELAGO.

The flora of the Malay Archipelago is one of the richest in the world; comprising well over 10,000 species of which a significant percentage are endemic. With such an enormous flora, a "blanket programme" was scarcely feasible and initial attention was paid to three genera known to favour or tolerate nickeliferous substrates. Brooks *et al*. (1977) have shown that many hyperaccumulators ($>$1000 ug/g dry weight) of nickel are found in the families Flacourtiaceae and Violaceae, particularly in the genera *Homalium* and *Hybanthus*.

The families Flacourtiaceae and Violaceae were therefore given special attention in this survey and are described below.

Following the discovery of hyperaccumulation of nickel by *Sebertia acuminata* (Jaffré *et al*. 1976) it was also decided to include the Sapotaceae.

1. Flacourtiaceae.

This family has been described by Sleumer (1954) and his notes were a valuable guide in this work.

The family contains nineteen genera that are found in the area under study. (See Table I-1.)

(i) *Ahernia*.

One species is found in the Philippines and the Moluccas but it was not used in this study.

(ii) *Erythrospermum*.

One species grows throughout the area but was not studied.

(iii) *Scolopia*.

Sleumer (1954) recognizes seven species found throughout the region and two of these were studied.

S. luzonensis, shrub or a small tree, grows in Borneo, the Philippines and the Celebes in dry thickets and secondary forests up to an altitude of 680 m. It will grow in clayey soils, on sandy beaches or limestone rocks.

S. spinosa, tree, grows in primary and secondary subforest, up to an altitude of 1100 m, in Sumatra, Bangka, the Malay Peninsula, Java, Borneo, Palawan and Talaud Island. The wood is used for fencing and home building and the fruit is edible.

(iv) *Itoa*.

One species grows in the area and it was studied. *I. stapfii*, tree (25-40 m), grows in the Celebes, the Moluccas and on Japen Island (West Irian) in old primary and secondary subforest. The heart-wood is used for house construction.

TABLES I-1, I-2, I-3.

MALESIAN GENERA OF THE FAMILIES FLACOURTIACEAE (I-1) VIOLACEAE (I-2) AND SAPOTACEAE (I-3).

Genera, numbers of Malesian species in each genus and the distribution of the genus are listed.

TABLE I-1.

Genus	No. of Species.	Distribution.
Ahernia	1	Philippines, Halmahera.
Erythrospermum	1	Throughout Malesia.
Scolopia	7	Throughout Malesia.
Itoa	1	Celebes, Moluccas, West Irian.
Paropsia	1	Sumatra, Malay Peninsula.
Hydnocarpus	31	Throughout Western Indonesia.
Scaphocalyx	2	Malay Peninsula.
Pangium	1	Throughout Malesia.
Trichadenia	1	East Malesia.
Ryparosa	16	New Guinea.
Eleutherandra	1	Sumatra, Borneo.
Homalium	23	Throughout Malesia.
Bennettiodendron	1	Sumatra, Java.
Hemiscolopia	1	Sumatra, Bangka, Java.
Xylosma	4	Throughout Malesia.
Osmelia	3	Throughout Malesia.
Flacourtia	7	Throughout Malesia.
Pseudosmelia	1	Moluccas.
Casearia	60	Throughout Malesia.

TABLE I-2.

Genus	No. of Species.	Distribution.
Rinorea	12 - 13	Throughout Malesia.
Agatea	1	New Guinea.
Hybanthus	1	Throughout Malesia.
Viola	18	Throughout Malesia.

TABLE I-3.

Genus.	No. of Species.	Distribution.
Planchonella	34	New Guinea, Java, Sumatra Moluccas, Borneo.
Chrysophyllum	4	New Guinea.
Manilkara		New Guinea, Sumatra, Java, Celebes.
Mimusops		Throughout Malesia.
Madhuca	1	Throughout Malesia.

From Van Royen (1957) and Vink (1958)

(v) Paropsia.

One species is known to grow in Sumatra and the Malay Peninsula but it was not studied.

(vi) Hydnocarpus.

Thirty one species grow throughout Western Indonesia. Two species were studied.

H. heterophylla philippinensis, tree, grows in the low altitude forest of Borneo, the Philippines and the Celebes.

H. sumatrana, tree, grows in the rain forests of Sumatra, Java, Borneo, the Celebes and the Philippines on ground that is never inundated, usually at altitudes of 30 to 200 m.

(vii) Scaphocalyx.

Two species of this genera are found on the Malay Peninsula but neither were studied.

(viii) Pangium

One species grows in the area and it was studied.

P. edule, tree, grows in isolated clumps throughout the Malay Archipelago on all types of ground, generally at an altitude of less than 300 m. The fruit is edible and the wood used for construction purposes.

(ix) Trichadenia.

One species grows in the east of the region and it was used in this project.

T. philippinesis, tree, grows in the Philippines, the Celebes, the Moluccas and New Guinea. It is found in primary or old secondary forest usually at an altitude of 5 to 100 m.

(x) Ryparosa.

There are sixteen species of this genera found in New Guinea but none were studied.

(xi) Eleutherandra.

One species grows in Sumatra and Borneo, but wasn't studied.

(xii) Homalium.

Twenty three of the species of this genera are listed as commonly found in various parts of the Malay Archipelago and of these twenty three, eleven were studied and they are listed below.

H. tomentosum, tree, grows in mixed and teak forests in Sumatra, Java, Madura, the Kangean Archipelago and the Lesser Sunda Islands. It is often found growing at low altitudes on periodically dry ground. The wood is used for match manufacture.

H. barandae, tree, grows at low and medium altitudes in the forests of the Philippines.

H. foetidum, tree, is a very common species that grows over a wide area including Sumatra, the Malay Peninsula, Borneo, the Philippines, the Celebes, the Moluccas and New Guinea. It often grows along river banks, in thickets or rain forests, at an altitude of 20 to 200 m. The tree produces a hard, dense timber that is used for constructing houses and bridges, and making combs.

H. caryophyllaceum, small tree, grows in humid forests or brushwood vegetation in the Malay Peninsula, Sumatra, Bangka, Java, Borneo and the Celebes. In Java it is sometimes seen as a hedge plant.

H. villarianum, tree up to 8 m in height, grows in the forest of the Philippines up to an altitude of 400 m.

H. undulatum, tree, 10 - 20 m, grows on the Malay Peninsula in evergreen forests. It is often found on rocky limestone hills up to an altitude of 300 m.

H. bracteatum, tree, grows in the low altitude primary forests of the Philippines.

H. celebicum, tree, grows in the Celebes and Buton Island in primary mixed forests at an altitude of 30 to 550 m. The wood is excellent for home building.

H. grandiflorum, tree 10 - 30 m, is found in the lowland forests of Sumatra, the Malay Peninsula and Borneo. The wood is hard and difficult to work.

H. minahassae, tree up to 35 m, grows on lowlying, level ground in the primary forest of the Celebes and the Moluccas. The timber is used for house construction.

(xiii) *Bennettiodendron*.

One species grows in Sumatra and Java but it was not analysed.

(xiv) *Hemiscolopia*.

One species is found in Sumatra, Bangka and Java and it was not studied.

(xv) Xylosma.

Four species are known throughout the Malay Archipelago and two were analysed.

X. papuanum, tree up to 6 m, found in the Moluccas and New Guinea growing at an altitude of 400 - 1600 m. in the forests. The wood is very tough.

X. luzonense, large shrub or tree (10m), a rare species but it is found in individual specimens or small clumps scattered over a wide area comprising the Philippines, the Celebes, the Moluccas and New Guinea.

(xvi) Flacourtia.

This genus contains seven species that grow throughout the area and four were analysed.

F. inermis, unarmed tree, is cultivated as a fruit tree in Sumatra, the Malay Peninsula, Java, Borneo, the Celebes, the Moluccas and New Guinea. It grows up to an altitude of 1300 m.

F. zippelii, slender tree, grows in moist ground and clayey soil in the primary and secondary rain forests of the Philippines, the Celebes, the Moluccas and New Guinea.

F. rukam, small tree, is widely distributed but scattered. It can be found in evergreen primary or secondary forest, often along the bank of a river. The fruit is edible and the roots and the juice of the young fruit are used for medicinal purposes.

F. indica, small tree up to 15 m., grows in most areas but is not known in Sumatra. Usually it grows in open places that have a definite dry season. It is cultivated for its fruit and the medicines extracted from it.

(xvii) Osmelia.

Three species grow throughout the Malay Archipelago but only one was studied.

O. philippina, shrub or tree 5-8 m., grows in the primary forests of Sumatra, the Malay Peninsula, Borneo, the Philippines, the Celebes, the Moluccas and New Guinea. The wood is hard, odourless and tasteless and used in the North Celebes for house construction.

(xviii) Pseudosmelia.

One species is known to grow in the Moluccas and it was analysed.

P. moluccana, shrub or treelet $2\frac{1}{2}$ - 3 m., is common in the forests of the Moluccas at an altitude of 200 to 800 m.

(xix) Casearia.

Approximately sixty species of this genus are known to grow throughout the Malay Archipelago and four of them were studied.

C. papuana, shrub 1 - 1½ m., grows in New Guinea in rain forests or fringing forests on rocky slopes at an altitude of 100 to 500 m.

C. hosei, tree or shrub 3 - 5 m., grows in primary forests in Borneo and the Celebes up to an altitude of 1500 m.

C. grewiaefolia var deglabrata, shrub or tree, grows in most areas or open spaces in primary and secondary forests, preferably in a nonshaded locality. It will grow in all regions.

C. halmaherensis, treelet or tree, scattered through the young forests of the Moluccas.

2. VIOLACEAE.

This family contains the genus Hybanthus which has provided three hyperaccumulators of nickel. The family is described by Jacobs and Moore (1971).

Four genera are commonly found in the Malay Archipelago and each will be dealt with in turn. (See Table I-2.)

(i) Rinorea.

A genus with twelve or thirteen species in the vicinity of the Malay Archipelago. Many species of Rinorea were analysed, including some from Africa (where they are more common) and this section of the project is dealt with in Section IV where the characteristics of the genus will be discussed.

(ii) Agatea.

One species is known to grow in New Guinea but it was not analysed.

(iii) Hybanthus.

One species grows throughout the region and it was studied.

H. enneaspermus, herb 15 - 60 cm. tall, does not grow in the Celebes but is found in most other regions. It will grow in monsoon areas, on roadsides, in grasslands and pastures and will tolerate open or shaded sites.

(iv) Viola.

Eighteen species of this genus grow throughout the area and four were studied.

V. betonicifolia, herb, grows in the grasslands or open montane woodlands of Sumatra, Java, the lesser Sunda Islands, the Celebes, the Philippines and New Guinea.

V. mearnsii, herb, grows along streams and in montane forests in the Philippines and the Celebes.

V. pilosa herb, found in Sumatra, Java, the Lesser Sunda Islands, the Celebes and the Moluccas. It grows at an altitude of 1100 to 3300 m., in grasslands and alpine woods.

V. kjellbergii, herb, found at high altitudes (2000 to 3540 m) in the alpine heath and grasslands of the Celebes and New Guinea.

3. SAPOTACEAE.

There are five genera of Sapotaceae that will be dealt with here (See Table I-3).

(i) *Planchonella*.

There are thirty four species of this genus that grow in New Guinea, Sumatra, the Philippines, Borneo, Java and the Moluccas. Of these thirty four species, twenty-nine grow in New Guinea. (The species is also well represented in New Caledonia.)

(ii) *Chrysophyllum*.

This genus has four species that grow in New Guinea but *Chrysophyllum* species are rare in other areas of the Malay Archipelago.

(iii) *Manilkara*.

Species of this genus grow in Central and Southern America, Central Africa, India, Indochina, Sumatra, Java, the Celebes, New Guinea and New Caledonia.

(iv) *Madhuca*.

This genus is confined in area to India, Indochina, most of the Malay Archipelago and New Caledonia.

(v) *Mimusops*.

This genus has four species in Central and Southern America, thirteen in Central Africa, two in India, one in the Malay Archipelago and four on the Islands of the Pacific Ocean.

SECTION II.

ANALYTICAL TECHNIQUES.

A. INTRODUCTION.

For the purposes of this study, analytical techniques were required that could detect trace metal concentrations in minute samples. (The samples required by the techniques used, must, of necessity, be small due to the rare and valuable nature of some of the botanical specimens investigated).

However, for analysis of the samples, a delicate balance had to be struck between a volume sufficiently large for multielement analysis, and a small enough dilution factor to allow easy detection.

It was decided to ash samples with a dry weight between 0.02g and 0.05g and dissolve the ash in 1 ml. of acid. (This gives a dilution factor of between fifty and twenty.)

It was hoped to investigate concentrations of the four elements cobalt, copper, nickel and zinc. Brooks (1972) lists the following typical background values of these and other elements in plant ash. (dry weight values are obtained by dividing values for ash by fifteen):

Cobalt :- 9 µg/g ash weight (~ 0.5 µg/g dry weight)
Copper :- 180 µg/g ash weight (~ 10 µg/g dry weight)
Nickel :- 65 µg/g ash weight (~ 4 µg/g dry weight)
Zinc :- 1400 µg/g ash weight (~ 90 µg/g dry weight)

Assuming a dilution factor of twenty to fifty, the following elemental concentrations could be expected from typical samples:

Co, 0.01 - 0.03 µg/ml.
Cu, 0.24 - 0.60 µg/ml.
Ni, 0.07 - 0.23 µg/ml.
Zn, 1.87 - 4.67 µg/ml.

The desired analytical technique must therefore be capable of detecting concentrations in the range 0.01 to 5 µg/ml.

B. SAMPLE PREPARATION.

Dried, preserved leaf samples were received from herbaria throughout the world (see acknowledgements) and from these, small samples (approximately 0.02 to 0.05g) were accurately weighed out on a Mettler H6 balance (capable of weighing to 0.0001 g) and placed in 12 mm x 75 mm borosilicate test-tubes (care must be taken at this stage so that any implements such as scissors or tweezers that come into contact with the samples do not contain any of the element to be analyzed so as to minimize possible sources of contamination.)

Samples were ashed in a muffle furnace at 500°C and the ash was dissolved in 1 ml. of 2M redistilled hydrochloric acid.

C. <u>FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY</u>.

This technique was first used by Walsh (1955) and involves shining an intense spectrum of the element under investigation into an atom cloud produced by aspirating a solution into a flame. Flames have certain desirable properties as an atomization source. (Kirkbright, 1971).

(i) They are relatively free from memory effects, noisefree and simple to operate.

(ii) Burner systems are predominantly small, durable and inexpensive.

(iii) There is a variety of available flame gas mixtures : C_2H_2/air (the most common flame, used for Fe, Mn, Cu, Zn, Co, Ni, Pb and many other elements); propane/air (used for Na and K); C_2H_2/N_2O (for V and Al); Ar/H_2 (for Se and As).

(iv) Flame chemistry and atomization processes are fairly well understood.

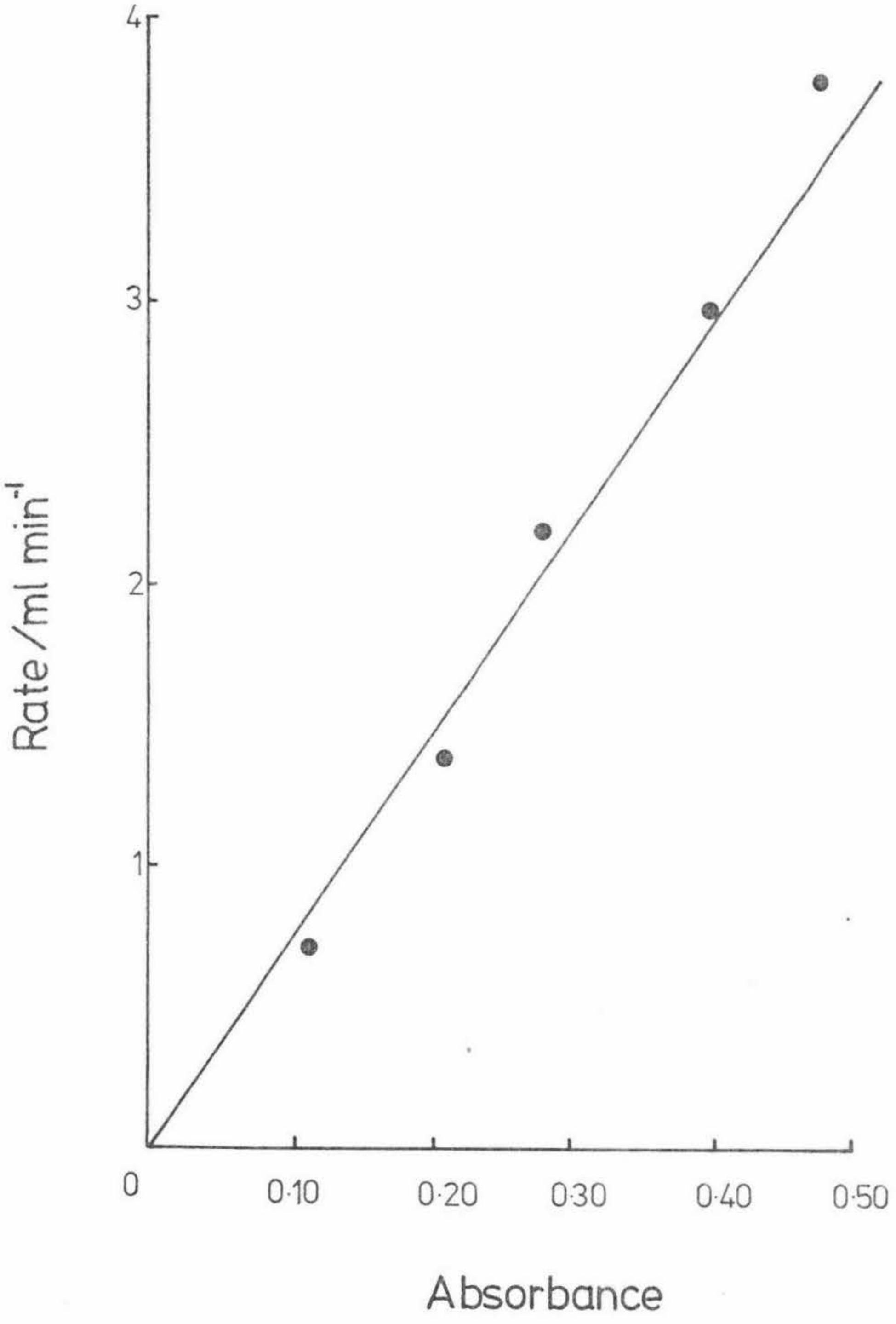
However flames are inefficient in that approximately 95% of the solution that is nebulized is wasted and never gets to the flame. Consequently 1 ml of solution would only just be sufficient for five analyses.

It was hoped that the volume required for each analysis could be reduced by slowing down the rate of nebulization. However, when a standard solution containing 1 µg/ml zinc was nebulized into an air/acetylene flame and the absorbance was measured at 213.9 nm at varying flow rates, the absorbance was found to decrease with decreasing nebulization rate (Figure II-1). Therefore if the flow rate was decreased, lack of sensitivity would become a problem.

It was then decided to nebulize solutions into the flame at a rate of 3.8 ml/min. At this rate, minimum detection limits of about 0.1 µg/ml could be reasonably expected for all four elements (0.05 µg/ml under good conditions) which meant that copper and zinc could be analyzed by flame atomic absorption but only some nickel values would be detectable and only anomalous cobalt values. Therefore samples were analyzed for copper, nickel and zinc by this technique and a more

FIGURE II-1.

GRAPH SHOWING EFFECT ON SENSITIVITY OF REDUCING ASPIRATION RATE.



4
3
2
1
0
0·10
0·20
0·30
0·40
0·50
Rate /ml min⁻¹
Absorbance

sensitive technique was required for low nickel concentrations and the detection of cobalt.

D. FLAMELESS ATOMIC ABSORPTION SPECTROPHOTOMETRY.

As mentioned in the previous section, the flame as an atomization source is wasteful of sample and not sensitive enough for low nickel and cobalt values. A technique that used less sample and was more sensitive, would, therefore be advantageous. Such a system is flameless atomic absorption spectrophotometry which uses electric power of low voltage (0 to 10 volts) and high current (200 to 300 amps) to heat a minute sample. These electrothermal atomizers fall into two categories : (i) rods and filaments using techniques based on the work of West and Williams (1969), (ii) tubular furnaces based largely on the pioneering work carried out by L'vov in the period 1959-1970. (See L'vov, 1961 and 1969).

1. Carbon rod atomiser.

Early investigations were carried out by placing 5 µl samples in a drilled cavity in a carbon rod prepared, in the laboratory, from FX9I grade Poco-Spectroscopic electrode graphite. Unfortunately these were found to have a short lifespan and tended to break in half after thirty to fifty firings, making analysis a tedious and expensive process. For this reason the carbon furnace was used.

2. Carbon furnace.

Tubular furnaces treated with a coating of pyrolytic carbon were supported in the working head of the carbon rod atomizer by two shaped graphite support rods. This system was found to be more durable than the carbon rod.

E. INSTRUMENTAL PARAMETERS.

1. Flame Atomic Absorption.

Solutions were analyzed by flame atomic absorption using a Varian Techtron AA5 Atomic Absorption Spectrophotometer fitted with a model BC6 Background Corrector using a Hydrogen continuum lamp.

2. Flameless Atomic Absorption.

Study of absorption using various parameters gave identical optimum conditions for nickel and cobalt as follows:

Dry 3.6 volts 22 seconds
Ash 5.2 volts 10 seconds

Atomize 6.2 volts 2.6 seconds.

3. Analysis lines.

Samples were analyzed at the following absorption lines:

Cobalt 240.7 nm.
Copper 324.7 nm.
Nickel 232.0 nm.
Zinc 213.9 nm.

All concentrations were expressed on a dry weight basis using micrograms of the element per gram of leaf as the unit (µg/g).

F. PRECISION OF THE EXPERIMENTAL TECHNIQUE.

An investigation was made into the precision or reproducibility of a technique that relied on the analysis of a small section of the leaf of a plant.

An electron probe analysis was made of a leaf of *Rinorea bengalensis* which had been found to contain a high nickel concentration. The object of this analysis was to determine if the nickel concentration was uniform across the leaf. Plate II-1 is a micrograph of 0.2 mm^2 of the leaf, the lighter coloured area is a secondary vein. The dark line across the photo delineates the path of the electron probe and the jagged white line is a graph of the relative nickel concentration across the analysis line. It can be seen that the nickel concentration varies across the leaf with the greatest concentrations in this case just beside the secondary vein. From this photo it is obvious that if a small section of the leaf is taken the absolute amount of nickel in that section and therefore, the concentration value derived, will depend upon the part of the leaf that the segment came from.

Small circles were punched out of plant leaves with a paper punch and analyzed for nickel concentration. The mean concentration and standard deviation were calculated for each leaf. Four separate determinations were made with leaves 1-3 coming from the same specimen.

Leaf 1. - Twenty sections were placed in test tubes, 1 section per tube. Average sample weight 0.006g. Nickel concentration 552 ± 12 (2.2%) µg/g dry weight.

Leaf 2. - Thirty sections were placed in test tubes, 2 sections per tube. Average sample weight 0.012g. Nickel concentration

PLATE II-1.

ELECTRON PROBE ANALYSIS OF NICKEL
IN <u>RINOREA BENGALENSIS</u>.

536 ± 20(3.8%) µg/g dry weight.

Leaf 3. - Thirty sections were placed in test tubes, 3 sections per tube. Average sample weight 0.019g. Nickel concentration 518 ± 27 (5.2%) µg/g dry weight.

Leaf 4. - Twenty eight sections were placed in test tubes, 4 sections per tube. Average sample weight 0.025g. Nickel concentration 125 ± 5(4%) µg/g dry weight.

From these analyses it would appear that there is a 2-5% relative standard deviation in the concentration of a trace element depending on the part of the leaf analysed.

The total error is of course a function partly of the analytical method and partly of the natural variation of the nickel content within the leaf itself. Since the reproducibility of the analytical technique was of the order of ± 2-3%, it would appear that for samples weighing at least 6 mg (20 mm^2), the within-leaf error is negligible. This at first sight seems to contradict the finding of the electron probe. However that survey was carried out on an area of 0.2 mm^2 compared with an area of 20 mm^2 for the smallest leaf samples tested chemically. Provided that major veins are ignored in taking leaf samples, reproducibility should be satisfactory for replicates taken from the same leaf. Leaf-to-leaf variability is another factor for consideration, but was not evaluated because of the lack of sufficient sample material.

SECTION III.

A BIOGEOCHEMICAL SURVEY OF THE MALAY ARCHIPELAGO.

A. INTRODUCTION.

Leaf samples were analysed for three elements(Copper, nickel and zinc) in order to assess background and anomalous concentrations of these three elements in common species. The majority of the plant species studied were from the families Flacourtiaceae and Violaceae which were studied because they were known to include hyperaccumulators of nickel. The genus Albizia, was also studied due to the discovery of Albizia sp nov. growing over the porphyry copper deposit at Bougainville. (Cole, 1971).

When a concentration was found that was considered to be high, one of two steps was taken:-

(i) If the high value seemed to be characteristic of the species, the species was further investigated.

(ii) If the high concentration appeared to be a site anomaly, the geology of the location was investigated by the use of geological maps (where available) and by analysing further samples collected from the area, if these could be obtained.

B. THEORY OF DISTRIBUTIONS OF TRACE ELEMENT DATA.

Liebscher and Smith (1968) found that distributions of trace elements in animal tissues could be divided into two categories: normal (symmetric about the arithmetic mean) and lognormal (symmetric about the geometric mean). Normal distributions were associated with those elements that were known to be essential to the growth or nutrition of the organism and lognormal distributions were associated with non-essential and mildly toxic elements. On the basis of these results Liebscher and Smith proposed that the type of distribution could be used as a guide to whether an element was essential or non-essential.

Timperley et al. (1970) investigated elemental concentration distributions in plant species in order to discover which elements were normally distributed and which were lognormally distributed. Their method of determining the distribution type was to calculate the median, the arithmetic mean, and the geometric mean and to compare the median with the two means. When the median was a better approximation to the arithmetic mean than the geometric mean, the distribution was considered to be normal. When the reverse was true, the distribution was considered to be lognormal.

TABLE III-1.

TRACE ELEMENT CONCENTRATIONS (μg/g dry weight) IN EIGHT MALESIAN SPECIES.

TABLE III-1.

SPECIES.	No. of SPECIMENS	Ni Range.	Ni Med	Ni a.m.	Ni g.m.	Cu Range	Cu Med.	Cu a.m.	Cu g.m.	Zn Range.	Zn Med.	Zn a.m.	Zn g.m.
Albizia saponaria Blume	12	<1-15	<1	2.23	0.94	1-18	5.5	7.07	4.91	17-310	51.5	74.90	46.97
Homalium caryophyllaceum (Z et. M) Benth	57	<1-45	2	3.28	1.63	1-68	5	9.98	6.54	11-2479	47	121.05	52.04
Homalium celebicum (Roxb.) Benth	14	<1-45	1	5.46	2.09	4-60	30	32.00	25.71	74-1546	493.5	565.86	402.00
Homalium foetidum Benth	112	<1-88	3	6.45	3.05	2-208	18	28.21	17.83	5-3812	127	295.45	114.50
Homalium grandifolium Benth	23	<1-8	<1	2.02	1.10	4-49	8	9.30	8.39	12-369	89	100.70	81.36
Homalium minahassae kds	11	<1-18	3	6.23	3.24	13-177	69	58.55	53.02	110-7586	313	968.9	341.6
Homalium tomentosum Benth	51	<1-26	<1	1.86	0.91	4-29	8	9.59	8.63	8-1522	72	146.33	95.21
Hybanthus enneaspermus (L) F.v.M.	29	<1-138	<1	8.07	1.53	3-154	8	19.90	11.13	17-7857	98	768.83	152.20

Unlike Liebscher and Smith (1968), Timperley et al. (1970) could not find a relationship between the type of distribution and the essentiality of the element concerned.

Timperley et al. (1970) investigated elemental concentrations in seven New Zealand species. Copper, molybdenum and zinc are considered to be essential for plant nutrition (Bowen, 1966) and lead, nickel and uranium are considered non-essential. Therefore the seven copper, one molybdenum and six zinc populations investigated by these authors would be expected to be normally distributed and have an arithmetic mean close to the median. However this occurred for only two distributions, copper in Nothofagus menziesii and zinc in Quintinia acutifolia and in both these cases the difference between the arithmetic and geometric means was small.

C. RESULTS AND DISCUSSION.

Seventy-five species were analyzed and of these, eight were sufficiently well represented (ten or more specimens) to be statistically analysed and they will be discussed as "ubiquitous species" and the remaining sixty-seven species are treated as "non-ubiquitous species."

1. Ubiquitous Species.

As described in Timperley et al. (1970), the criterion for deciding whether a distribution was normal or lognormal was the relationship between the median and one or other of the means. If there is a link between essentiality and normality of distribution, copper and zinc would be expected to be normally distributed in the eight ubiquitous species as they are essential elements in plants and, as a non-essential element, nickel would be expected to be lognormally distributed.

The eight species analysed for the three elements copper, nickel and zinc were:- Albizzia saponaria Blume (Legumin), Homalium caryophyllaceum (Z et M.) Benth (Flacourtiac), Homalium celebicum Kds, Homalium foetidum (Roxb.) Benth, Homalium grandiflorium Benth, Homalium minahassae Kds, Homalium tomentosum Benth and Hybanthus enneaspermus (L) F.v.M (Violac). Table III-1 lists each species with the number of specimens analysed and the range, median and arithmetic and geometric means of the concentrations of each element.

Upon statistically analysing the twenty four data populations it was discovered that only three were normally distributed and they were copper and zinc in Homalium celebicum and copper in Homalium minahassae. It should be noted that in the case of the two normally distributed populations in Homalium celebicum, the arithmetic mean and the geometric mean are relatively similar and the median lies between the two, but is closer to the arithmetic mean. In the case of copper in Homalium minahassae the median is definitely closer to the arithmetic mean.

Liebscher and Smith postulated that a normal distribution arose in the concentration values of an essential trace constituent because the uptake of the element was controlled by the organism so that the concentration fell in a narrow optimum range. For non-essential elements, an organism has no control over uptake and the toxic threshold is the upper limit of accumulation. Consequently essential elements tend to be distributed with small ranges and standard deviations and non-essential elemental distributions tend to have large ranges and definite positive skews that tend to disappear when logarithmic transformations are made.

Ahrens (1954) gave a method of measuring the spread of a range of values when he stated, "The abundance of an element in an igneous rock is always greater than its most prevalent concentration; the difference may be immeasurably small or very large and is determined solely by the magnitude of the dispersion of its concentration." When Ahrens mentions "the abundance" he is referring to the arithmetic mean and the geometric mean when he speaks of the "most prevalent concentration." He demonstrated that standard deviations greater than 0.4 are proportional to a.m./g.m. Using this information the prediction can be made that essential elements will have a smaller ratio of the arithmetic mean to the geometric mean then non-essential elements. (The ratios of arithmetic means to geometric means are shown in Table III-2). Table III-2 shows that the lowest values of the ratio exist for copper. Values for nickel are all higher than the copper values and the zinc values cover a wide range. The low values (less than 1.50) contain the three normal populations (copper and zinc in Homalium celebicum -1.21 and 1.40 respectively and copper in Homalium minahassae (1.28) but they also contain some lognormal populations, copper (1.12) and zinc (1.24) in Homalium grandifolium and copper in Homalium tomentosum (1.12). In the case of Homalium grandifolium the value of the ratio of nickel values was also low (1.78 the lowest of the nickel values) so either the plants that supplied the specimens were growing

TABLE III-2.

RELATIONSHIP BETWEEN ARITHMETIC AND GEOMETRIC MEANS IN ELEMENTAL CONCENTRATION DATA.

TABLE: III-2

SPECIES.	NICKEL			COPPER			ZINC		
	a.m. (µg/g)	g.m. (µg/g)	a.m./g.m.	a.m. (µg/g)	g.m. (µg/g)	a.m./g.m.	a.m. (µg/g)	a.m. (µg/g)	g.m./g.m.
Albizia saponaria	2.23	0.94	2.38	7.07	4.91	1.48	74.90	46.97	1.59
Homalium caryophyllaceum	3.28	1.63	2.02	9.98	6.54	1.55	121.05	52.04	2.31
Homalium celebicum	5.46	2.09	2.62	32.00	25.71	1.21	565.86	402.00	1.40
Homalium foetidum	6.45	3.05	2.12	28.21	17.83	1.58	295.45	114.50	2.58
Homalium grandifolium	2.02	1.10	1.87	9.30	8.39	1.12	100.70	81.36	1.24
Homalium minahassae	6.23	3.24	1.94	68.55	53.02	1.28	968.9	341.6	2.84
Homalium tomentosum	1.86	0.91	2.05	9.59	8.63	1.12	146.33	95.21	1.54
Hybanthus enneaspermus	8.07	1.53	5.29	19.90	11.13	1.78	768.83	152.20	5.04

on very similar substrates, or this species has greater control over trace element uptake than some other species. The very low value for the ratio of the arithmetic mean to the geometric mean for copper in Homalium tomentosum could be a sign that the distribution tends toward normality.

Anomalous elemental concentrations were determined by plotting the data on probability paper (Tennant and White, 1959). The values for each of the twenty four distributions were ranked and the concentrations (µg/g) were plotted (on a linear scale for normal populations or a logarithmic scale for lognormal populations) against the cumulative percentage of values less than or equal to the particular concentration. On these graphs each population is represented by a straight line. Where two straight lines occur, two populations are indicated and their point of intersection is the threshold value that forms the boundary between the two populations. (See Figures III-1, III-2, III-3, III-4 for representative graphs). Where two populations were present, the specimens that fell in the upper population were considered anomalous and these anomalous values could be indicative of mineralization, or of rock types with elevated levels of the elements concerned.

2. Non-ubiquitous species.

As well as the eight species previously mentioned, sixty seven were analysed which had fewer than ten specimens. Table III-3 lists these species family by family and notes the number of specimens and the mean and range for each of the three elements copper, nickel and zinc. Due to the small sizes of the populations it was not possible to distinguish low and high populations within a species and so identification of anomalies must be on an arbitrary basis. Anomalous values were predicted using the criteria of copper and nickel concentrations ≥ 50 µg/g and zinc levels ≥ 1000 µg/g. These levels were set deliberately high in order that errors of prediction should be minimized.

3. Sites of particular interest.

(i) Nickel.

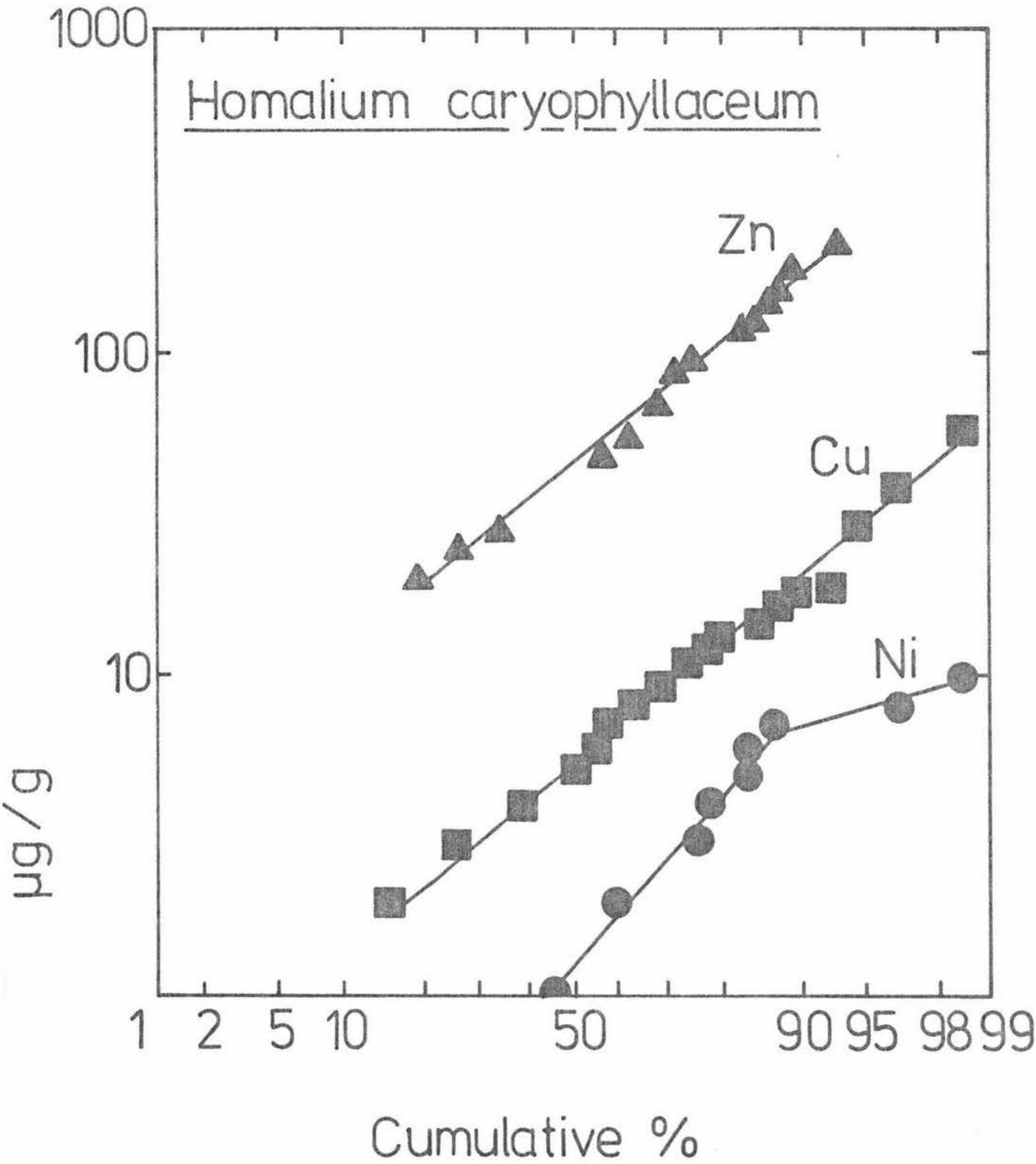
Listed in Table III-4 are sites for all the specimens that were considered to contain an anomalous concentration of nickel either because a cumulative frequency graph showed them to fall in an upper population or because they exceeded an arbitrary value of nickel

FIGURES III-1, III-2, III-3, III-4.

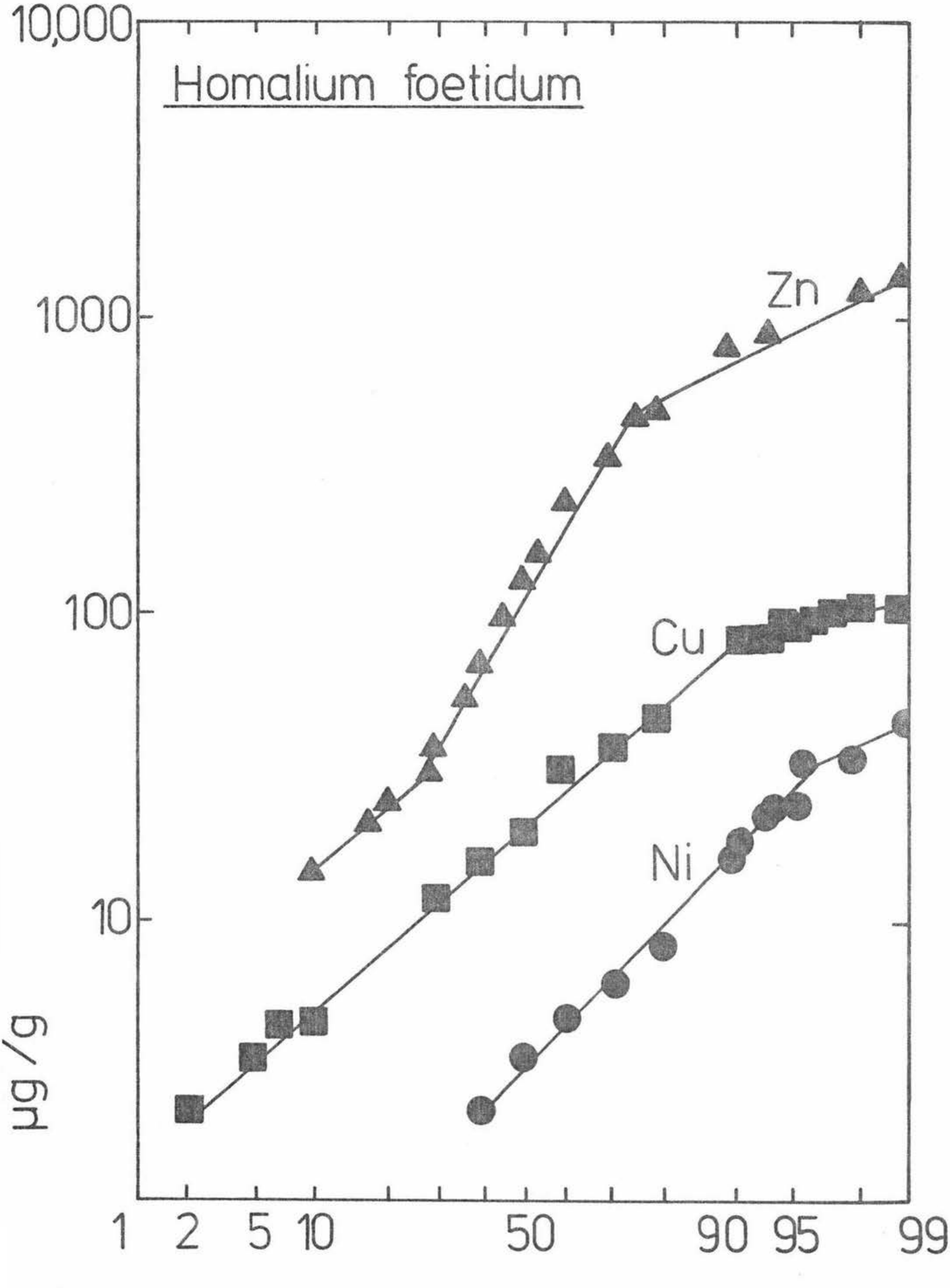
CUMULATIVE FREQUENCY GRAPHS SHOWING TRACE ELEMENT CONCENTRATIONS.

Species are:

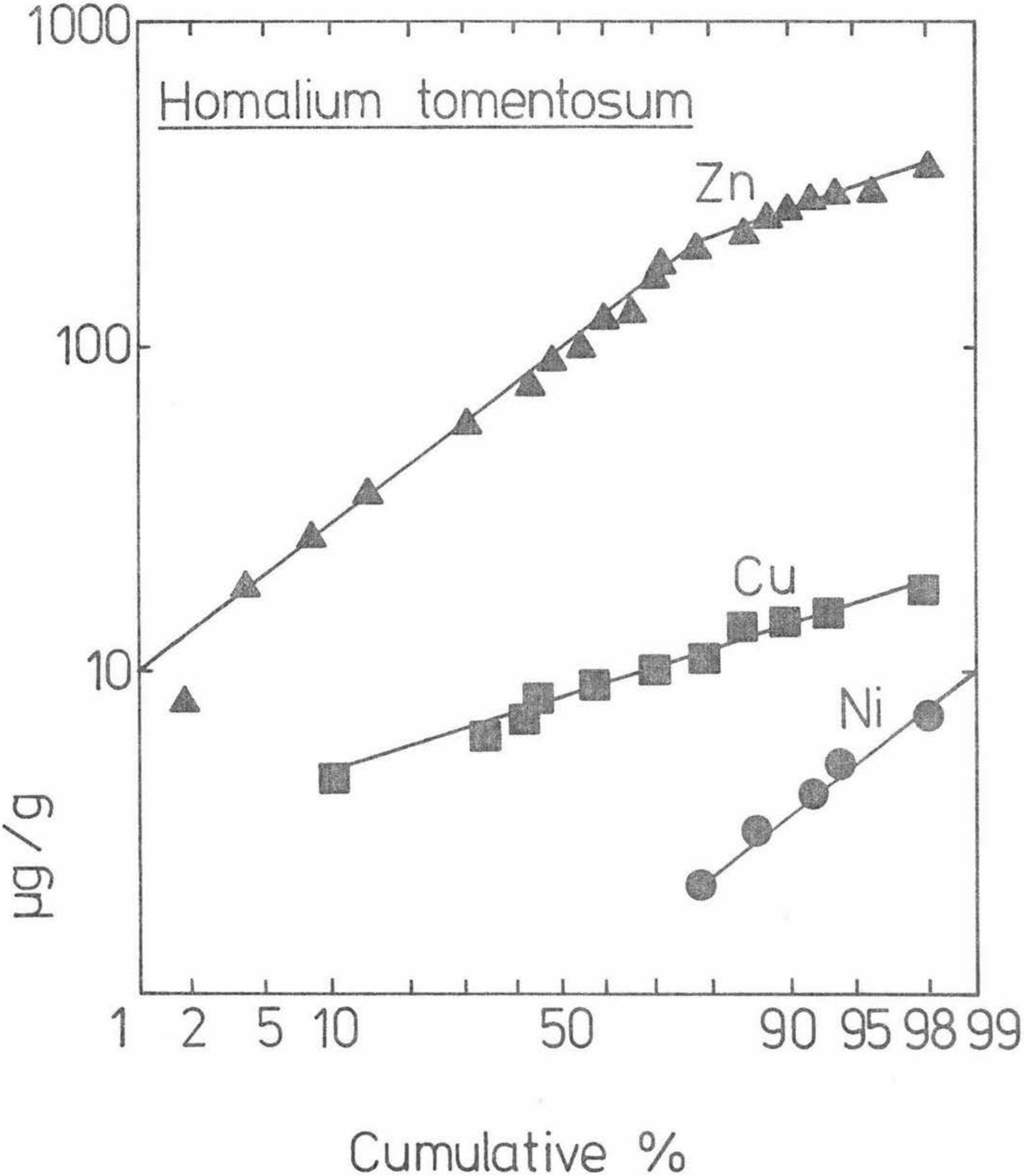
Homalium caryophyllaceum	III-1
Homalium foetidum	III-2
Homalium tomentosum	III-3
Hybanthus enneaspermus	III-4

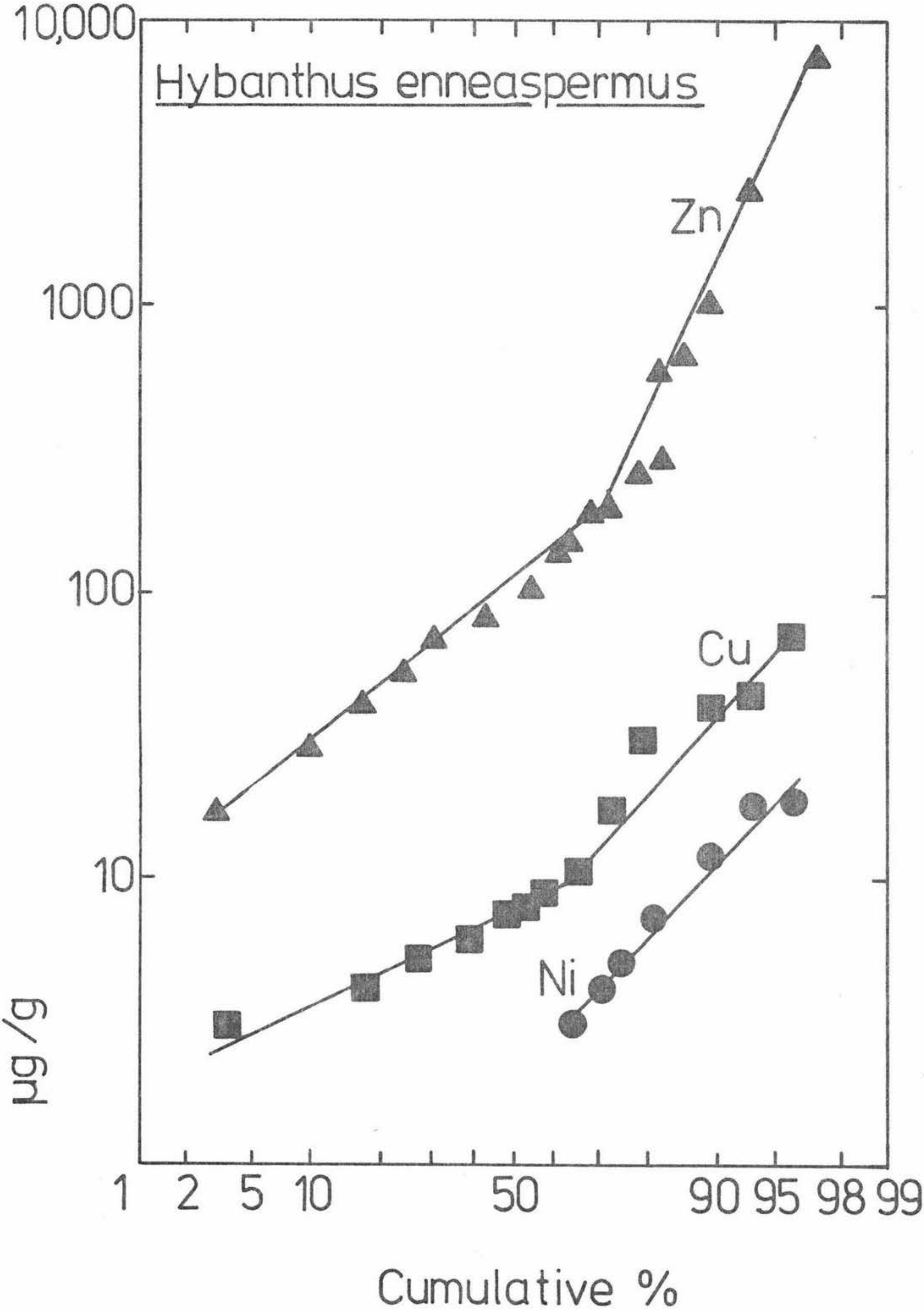


Homalium caryophyllaceum
Zn
Cu
Ni
1000
100
10
µg / g
1 2 5 10 50 90 95 98 99
Cumulative %



Homalium foetidum
Zn
Cu
Ni
10,000
1000
100
10
µg /g
1
2
5
10
50
90
95
99
Cumulative %


Homalium tomentosum
Zn
Cu
Ni
1000
100
10
µg / g
1
2
5
10
50
90
95
98
99
Cumulative %



Hybanthus enneaspermus
Zn
Cu
Ni
10,000
1000
100
10
µg /g
1
2
5
10
50
90
95
98
99
Cumulative %

TABLE III-3.

TRACE ELEMENT CONCENTRATION ($\mu g/g$ dry weight)

IN NON-UBIQUITOUS MALESIAN SPECIES.

TABLE III-3

N.B. In calculating means <1 is equated with 0.5.

SPECIES	No.	Nickel		Copper		Zinc.	
		Range	Mean	Range	Mean	Range	Mean
Family: - Caryophyllaceae							
Cerastium indicum Wright & Arn.	2	<1-<1	0.5	33-33	33	67-241	154
Family: - Cornaceae							
Alangium griffithi Harms	2	<1-<1	0.5	11-14	12.5	19-145	82
Alangium javanicum Wangerin	3	<1-7	2.3	6-22	12.7	19-276	111.7
Family: - Flacourtiaceae							
Casearia glabra Roxb.	2	<1-<1	0.5	6-12	9	40-112	76
Casearia grewiaefolia var *deglabrata* Koord & Val.	7	<1-50	15.4	4-21	9.7	19-1000	311.9
Casearia halmaherensis v Sloot	2	<1-3	1.8	5-8	6.5	44-46	45
Casearia hosei Merr.	1		2.6		15		172
Casearia papuana Sleum.	1		0.5		8		60
Flacourtia indica Merr.	2	<1-3	1–8	10-19	14.5	102-276	189
Flacourtia inermis Miq.	5	<1-47	11.2	3-12	7.0	98-357	224.4
Flacourtia rukam Zoll & Mor.	5	<1-35	8.6	5-14	8.0	66-165	110.0
Flacourtia zippelii v Sloot.	4	<1-9	2.6	4-16	10.8	23-130	75.5
Homalium barandae Vid.	2	<1-<1	0.5	10-11	10.5	60-102	81
Homalium bracteatum Benth.	1		1		5		167
Homalium cochinensis (Lour.) Douce	4	<1-13	3.6	10-23	17.0	100-162	119.8

continued...

Table III-3 continued...

Species	No	Nickel		Copper		Zinc	
		Range	Mean	Range	Mean	Range	Mean
Homalium dasyanthum Warb.	3	<1-<1	0.5	4-8	5.7	20-46	35.3
Homalium dictyoneurum Hance	1		27		7		20
Homalium fagifolium Benth	1		0.5		11		115
Homalium fallax v Sloot.	6	<1-4	2.4	4-16	9.3	23-5107	1054.7
Homalium frutescens King.	1		10		15		71
Homalium glabrifolium Geddes.	1		0.5		2		11
Homalium griffithianum Kuz.	3	<1-<1	0.5	6-10	8.3	56-69	63.3
Homalium hainanense Gagnep.	1		3		3		29
Homalium hoheri Merr.	1		0.5		6		95
Homalium myriandeum Merr.	1		0.5		6		12
Homalium propinquium Clarke.	1		0.5		14		34
Homalium undulatum King	2	<1-<1	0.5	3-4	3.5	12-56	34.0
Homalium villarianum Vid.	3	2-2	2.0	3-31	14.3	24.53	36.0
Hydnocarpus heterophylla Var. philippinensis Sleumer	1		127		8		128
Hydnocarpus sumatrana (Miq) Koord.	3	<1-168	59.2	6-10	7.7	17-1370	565.05
Itoa stapfii (Koord) Sleum.	1		0.5		30		27
Osmelia philippina (Turcz) Benth.	3	<1-9	3.3	11-19	15	66-371	185.3
Osmelia sp.	2	1-3	2.0	8-13	10.5	202-391	296.5
Pangium edule Reinw.	5	<1-108	24.0	13-70	32.6	26-267	148.2
Pseudosmelia moluccana Sleum.	2	<1-<1	0.5	8-13	10.5	85-104	94.5
Scolopia crenata Clos.	1		21		7		60

Table III-3 continued...

Species	No.	Nickel Range	Nickel Mean	Copper Range	Copper Mean	Zinc Range	Zinc Mean
Scolopia luzonensis Warb.	1		36		11		28
Scolopia spinosa Warb.	1		4		9		251
Trichadenia philippinensis Merr.	8	<1-12	3.6	3-20	8.8	28-608	198.4
Trichadenia sp.	2	1-3	2.0	3-18	10.5	122-210	166.0
Xylosma papuanum Gilg.	1		0.5		7		54
Xylosma luzonense Clos.	2	<1-5	2.8	5-12	9.5	24-279	150.5
Family: - Labiateae							
Ajuga bracteosa Wall.							
Plectranthus Scutelleroides Blume	1		66		27		519
Plectranthus teysmanii Miq.	2	<1-3	1.8	10-11	10.5	58-70	64
Plectranthus sp.	1		52		26		690
Family: - Leguminosae							
Albizzia chinensis Merr.	1		0.5		3		27
Albizzia falcata Backer	1		0.5		5		43
Albizzia lebbeck Benth.	3	<1-3	1.3	4-6	4.7	13-121	74.0
Albizzia libbechoides Benth.	1		3		4		616
Albizzia minahassae Koord.	2	4-8	6.0	8-10	9.0	206-275	240.5
Albizzia odoratissima Benth.	1		0.5		1		17
Albizzia procera Benth.	5	<1-5	2.7	4-11	7.6	13-769	256.4
Albizzia retusa Benth.	3	1-6	2.7	3-14	7.3	100-308	172.7
Albizzia rotundata Blume	1		13		10		518
Albizzia tomentella Miq.	3	<1-1	0.7	2-8	4.7	21-374	220.7

continued...

Table III-3 continued...

Species	No.	Nickel		Copper		Zinc	
		Range	Mean	Range	Mean	Range	Mean
Family:- Sapotaceae							
Palaquium amboinense Burck	5	<1-1	0.6	6-11	7.8	14-133	66.8
Palaquium bataanense Merr.	4	<1-387	109.6	3-7	5.0	27-242	110.5
Palaquium luzoniense Vidal	5	1-51	14.6	6-8	7.4	8-436	193.8
Planchonella moluccana Burck (H.J. Lam)	4	1-14	5.3	5-6	5.7	36-254	135.7
Planchonella obovoida Lam	2	1-1	1	2-5	3.5	134-150	142
Family: - Violaceae							
Rinorea bengalensis (Wall) O.K.	2	5350-5750	5550	6-17	11.5	197-992	594.5
Viola betonicifolia J.E. Smith	1		0.5		10		88
Viola kjellbergii Melchior	1		0.5		7		130
Viola mearnsii Merr.	1		11		23		460
Viola pilosa Blume	1		0.5		4		93

concentration. Also included in the table is one specimen of *Hybanthus enneaspermus* which had more than ten times the concentration of nickel of any other specimen of that species. When the sites of the specimens listed in Table III-4 are plotted on a map (Figure III-5) certain areas appear that could contain high levels of nickel in the soil. It is satisfying to note that five of these specimens were collected at Oesu, Malili a centre of nickel mining. The other sites marked on the map may or may not represent nickeliferous substrates and only a detailed geological survey could discover the exact nickel levels. However these anomalous results may be a guide to suitable sites for geological exploration.

(ii) Copper and zinc.

Biogeochemical surveys have been shown to be less reliable for essential elements than non-essential ones (Timperley *et al*. 1970) and as copper and zinc are both essential elements in plants it was decided that more reliable predictions about substrate could be made if copper and zinc were considered jointly. Table III-5, therefore lists specimens that appear to have anomalous concentrations of both copper and zinc. The sites of these anomalies are shown on Figure III-6. From this map it appears possible that there could be copper zinc mineralization in some parts of Indonesia and the islands in the vicinity of Halmahera could prove particularly interesting.

D. CONCLUSIONS.

Although Liebscher and Smith found that normality and lognormality could be used to determine whether an element was essential or not in human tissues, there appears to be no definite method of determining essentiality in plant tissues. However, normality of distribution and/or a low value of the ratio of the arithmetic mean to the geometric mean could indicate an essential element.

This study has pinpointed various regions in South East Asia that may contain higher than normal levels of various metals. However this study can do no more than predict areas likely to contain mineralization to some extent. Predictions can also be made about geology but in both cases, final proof can only be made by *in situ* inspections.

TABLE III-4

SAMPLES CONTAINING ANOMALOUSLY HIGH CONCENTRATIONS OF NICKEL.

TABLE: III-4.

Species	Site	µg/g Ni
Homalium caryophyllaceum	Sandakan, North Borneo,	8
	Sandakan, North Borneo,	7
	Kinabatangan River, North Borneo,	10
	Butong, Borneo,	8
	Boseli Sei Besor, S.E. Borneo,	45
Homalium celebicum	Tuntans, Banggai, Celebes	7
	Tomohon, Celebes,	8
	Kayu wutu, Minahassa, Celebes,	45
Homalium foetidum	Oesu, Malili, Celebes,	88
	Oesu, Malili, Celebes,	31
	Ambon, Moluccas,	32
	South East Borneo,	30
	South East Borneo,	44
Homalium minahassae	Tual, Kai Island, South Moluccas	11
	Tanimbar Island, South Moluccas	18
	Otimmer, Tanimbar Island, South Moluccas	11
	P. Jamdera, Tanimbar Island, South Moluccas	12
Hybanthus enneaspermus var *deglabrata*	Golo, Philippines	138
	Kabaena Island, Celebes	50
Hydnocarpus heteroplylla philippinensis	Boeta, Banggai, Menado, Celebes,	127
Hydnocarpus sumatrana	Latooe, Kolaka, Celebes	168
Panguim edule	Bone Moena Island, Celebes	108

continued....

Table III-4 continued...

Plectranthus scutelluroides	Kolonedale, Celebes	66
Palaquium bataanense	Oesu, Malili, Celebes	387
Palaquium luzoniense	Oesu, Malili, Celebes	51
Rinorea bengalensis	Kolaka, Lataoe, Celebes	5350
	Kawata, Malili, Celebes	5750

TABLE III-5.

SAMPLES CONTAINING ANOMALOUSLY HIGH

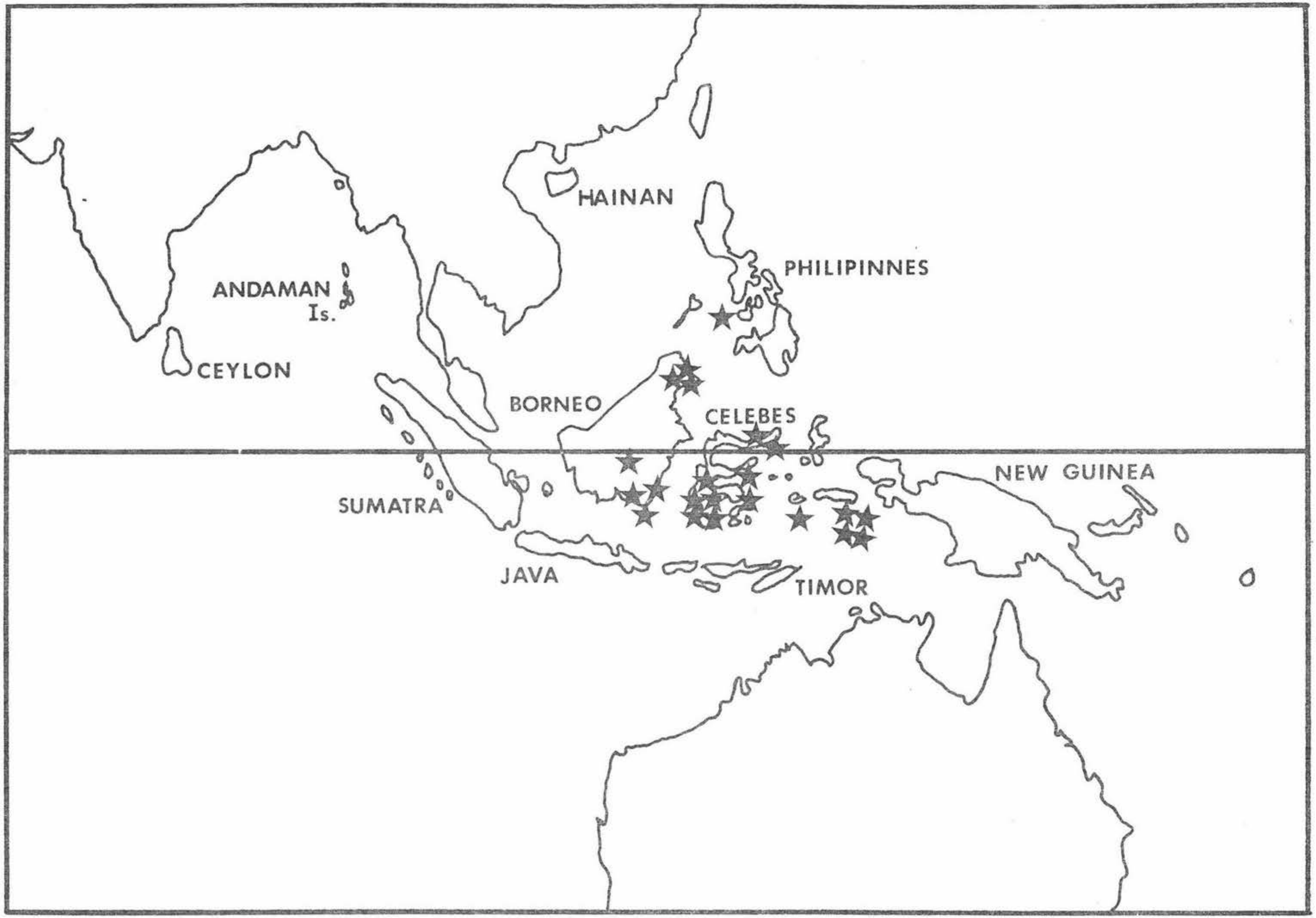
CONCENTRATIONS OF COPPER <u>AND</u> ZINC

Table III-5.

Species	Site	µg/g Cu	µg/g Zn
Homalium foetidum	Lagandi, Butung Island, Celebes	92	3817
	Pandu, Minahassa, Celebes	112	1370
	Tobelo, Halmahera	88	976
	Ambon, S. Moluccas	102	457
Hybanthus enneaspermus	Ternate, Halmahera	71	7500
	Ternate, Halmahera	29	2464
	Kai Island	154	989
	Kai Island	37	679
	Lombok Island	44	581

FIGURE III-5.

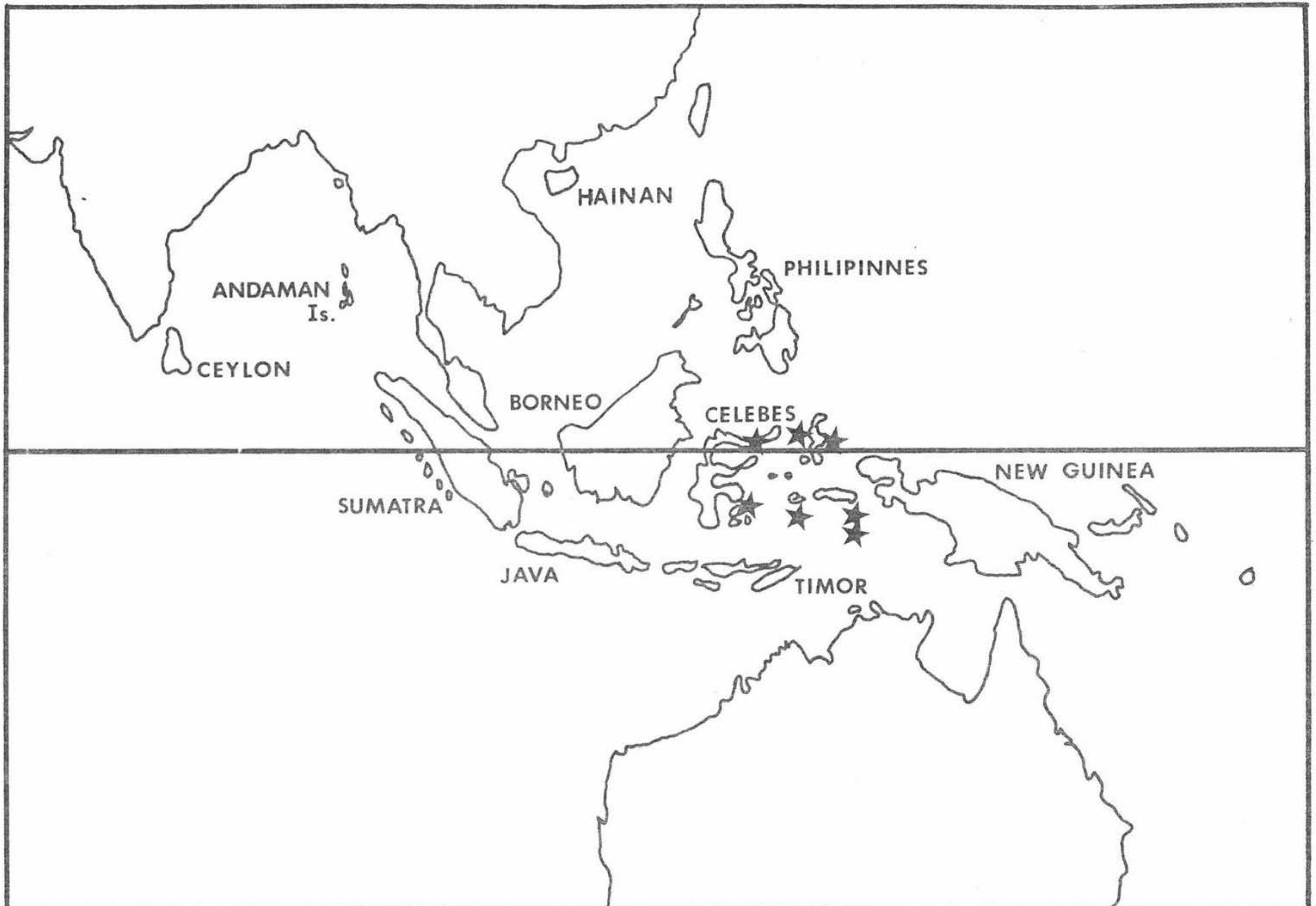
COLLECTION SITES OF PLANT SPECIMENS CONTAINING ANOMALOUSLY HIGH NICKEL CONCENTRATIONS.



HAINAN
PHILIPINNES
ANDAMAN Is.
CEYLON
BORNEO
CELEBES
NEW GUINEA
SUMATRA
JAVA
TIMOR

FIGURE III-6

COLLECTION SITES OF PLANT SPECIMENS CONTAINING ANOMALOUSLY HIGH COPPER <u>AND</u> ZINC CONCENTRATIONS.


HAINAN
PHILIPINNES
ANDAMAN
Is.
CEYLON
BORNEO
CELEBES
NEW GUINEA
SUMATRA
JAVA
TIMOR

SECTION IV.

ACCUMULATION OF COBALT AND NICKEL BY SPECIES OF THE GENUS *RINOREA*.

A. INTRODUCTION

As mentioned in Table III-3 two specimens of Rinorea bengalensis (Wall.) O.K., both from the Celebes, were found to contain over 5000 µg/g Nickel on a dry weight basis:- (5350 µg/g for a sample from Lataoe, Kolaka and 5750 µg/g from a sample from Kawata, Malili). This clearly qualifies R. bengalensis for classification as hyper-accumulator of nickel (Brooks et al., 1977) and, therefore a study of all available specimens of the genus Rinorea was undertaken with particular emphasis upon the species R. bengalensis.

For the purposes of this study all samples were analysed for cobalt and nickel by flame atomic absorption spectrophotometry.

B. TAXONOMY OF THE GENUS RINOREA

Jacobs and Moore (1971) describe the genus Rinorea as comprising approximately two hundred species, mostly small shrubs or small trees. These species grow mainly in tropical regions with over half the species being endemic to Africa. The plants are found predominantly in the understorey of primary rainforests, at very low altitude, occasionally up to 1000 m., on various soils including limestone. Unfortunately a satisfactory subdivision of the entire genus has not yet been made. (Jacobs and Moore, 1971).

C. RINOREA BENGALENSIS - A HYPERACCUMULATOR OF NICKEL.

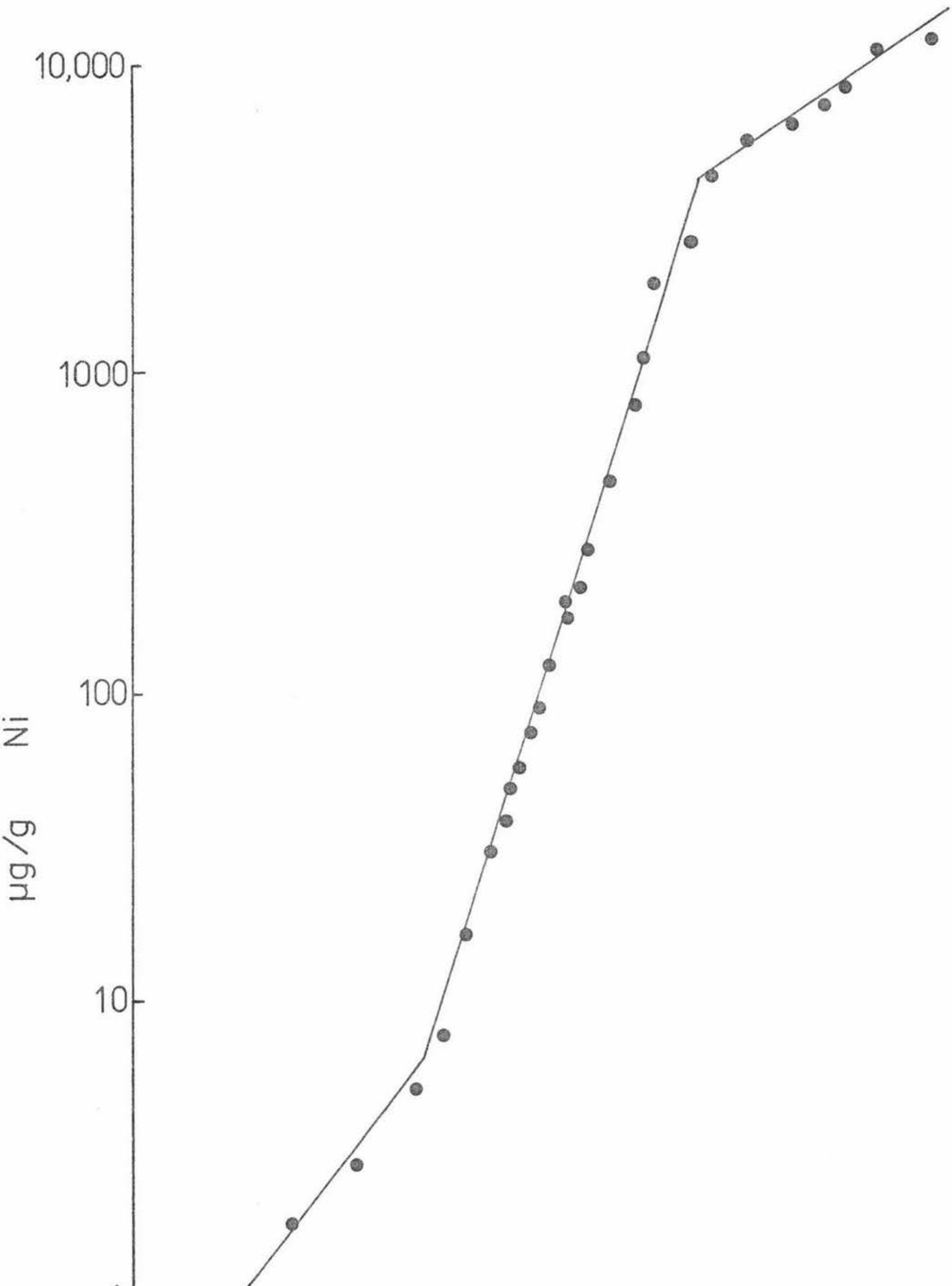
The discovery of two specimens of R. bengalensis from the Celebes containing in excess of 5000 µg/g nickel guarantees this species a place in the growing list of hyperaccumulators of nickel. (Brooks et al., 1977). A further eighty nine samples of this species were analysed for calcium, cobalt and nickel.

The plant is found in the understorey of mixed rainforest, as a shrub or tree (up to 20 m high) in Ceylon, Southern India, Burma, the Indochinese Peninsula, Hainan, the Andaman Islands, Malesia except Southern Sumatra and Java, Northern Queensland, Melanesia and the Caroline Islands.

FIGURE IV-1

CUMULATIVE FREQUENCY GRAPH FOR NICKEL

IN *RINOREA BENGALENSIS*.



10,000
1000
100
10
1
µg/g Ni
1 2 5 10 20 30 40 50 60 70 80 90 95 98 99
Cumulative Percentage

TABLE IV-1

HIGH NICKEL VALUES (>4000 $\mu g/g$) IN

RINOREA BENGALENSIS.

TABLE IV-1.

Collection Locality:	Known Ultra-basics	µg/g Ni
Dalman, Nabire, West Irian	No	17750
Paracale, Camarines Province, Luzon Philippines	yes	13750
Paracale, Camarines Province, Luzon, Philippines	yes	10500
Mt. Kinabalu, British North Borneo	yes	5650
Mt. Kinabalu, British North Borneo	yes	4750
Cycloop Mts. Tanah Merah Bay, Hollandia, New Guinea	yes	9000
Mt. Kinabalu, British North Borneo	yes	7150
Mt. Kinabalu, British North Borneo	yes	8775
Tapul, Paragua I., Palawan, Philippines	yes	5900
Mt. Giting-Giting Philippines	yes	8000
Paluan, Mindoro, Philippines	yes	8000
Road to Klamono, Sorong Dist., New Guinea	No	12000
Lataoe, Kolaka, The Celebes	yes*	5350
Kawata, Malili, The Celebes	yes*	5750

* Near nickel mining areas.

1. Population Studies.

Ninety-one samples of this species were available for analysis and the nickel concentrations for these ninety-one samples were ranked and plotted on cumulative frequency paper as described in Section III. The resultant graph (figure IV-1) appears to indicate the presence of three populations. The lower population comprises approximately 26% of the total samples and contains values below 6.8 µg/g. The upper population (which is the population of most interest) contains only 17% of the specimens and refers to individuals containing over 4400 µg/g of nickel (dry weight). (See table IV-1 and figure IV-2 for collection localities of these specimens.)

2. Determination of ultrabasic areas.

As ultrabasic areas are, potentially, very valuable it would be desirable if we could decide upon certain criteria that distinguish specimens of R. bengalensis growing on ultrabasic substrates from other specimens of the species.

Of the fourteen specimens listed in Table IV-1, twelve were collected from known ultrabasic areas and the remaining two samples came from areas of uncertain geology. The first criterion decided upon was that samples containing more than 4000 µg/g nickel can be considered to have been collected in ultrabasic areas. From the study of collection localities of known geology, it was clear that nearly all specimens containing over 2000 µg/g nickel were also found on ultrabasic substrates. This cut off point of 4000 µg/g is therefore conservative.

Cobalt/nickel ratios were calculated for all samples of R. bengalensis and these can be found (grouped on a substrate basis) in Table IV-2. For ultrabasic substratesas a very low cobalt/nickel ratio was found and this led to the second criterion for determining samples of R. bengalensis growing on ultrabasic substrates I.e a cobalt/nickel ratio of 0.01 or less. Therefore for the purposes of this study any specimen from an area of unknown or doubtful geology containing more than 4000 µg/g nickel (dry weight) and with a cobalt/nickel ratio of 0.01 or less was considered to have been collected from an ultrabasic area. Two samples fall into this category, one from Dalman, Nabire, West Irian and one from the road to Klamono in the Sorong district, New Guinea.

Reference to the Indonesian Geological Survey (G.S.I.) Bandung was made in both these cases. G.S.I. had no information on ultrabasics

FIGURE IV-2

COLLECTION LOCALITIES OF SAMPLES

OF *RINOREA BENGALENSIS*.

(Concentration ranges shown.)

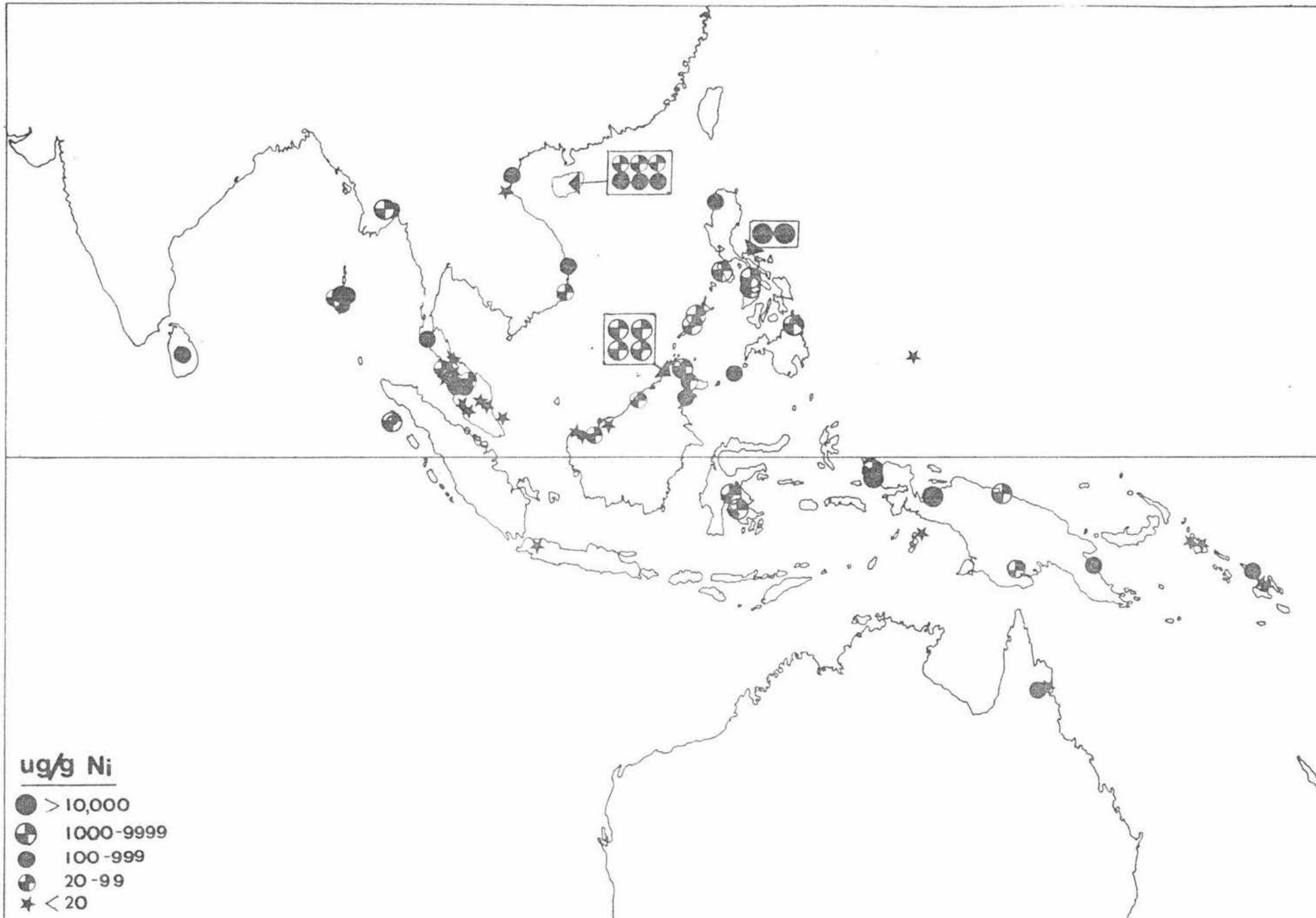

ug/g Ni
>10,000
1000-9999
100-999
20-99
<20

TABLE IV-2.

NICKEL AND COBALT CONCENTRATION (μg/g dry weight)

IN RINOREA BENGALENSIS.

TABLE IV-2.

	No. of Samples	Cobalt		Nickel		Co/Ni
		Mean	Range	Mean	Range	
Ultrabasic rocks	21	87	6-545	6857	836-17,500	0.01
Limestones	12	12	1-33	113	2-560	0.11
Other Sedimentary Rocks	14	75	3-290	674	3-3000	0.11
Basic Rocks	11	27	1-217	103	1-550	0.26
Acid Rocks	5	16	5-29	20	2-56	0.80
Unknown	28	38	0.5-300	177	1-2000	0.21
Overall	91	51	0.5-545	1810	1-17,500	0.03

in the Nabire area. The only regional map available at this institute was incomplete. However, gross extrapolation of a belt of ultrabasic intrusives in northwest West Irian indicates the likelihood of ultrabasic rocks under the alluvial basin (near Nabire) adjacent to Japen Island.

There is also some doubt as to whether ultrabasic rocks are known at the precise locality in the Sorong area of northwest West Irian.

It would appear therefore, that the *R. bengalensis* survey has resulted in the discovery of at least one, and possibly two, previously unrecorded areas of ultrabasic rocks.

The final proof as to whether or not these collection localities are ultrabasic, will of course depend on *in situ* examination of the sites, and even if they do contain ultrabasic rocks, there will not necessarily be nickel mineralization within them. It is, however, noteworthy that the sample of *R. bengalensis* from Nabire contained the highest nickel content (1.75% dry weight) so far recorded for this species.

3. Relationship between Nickel and Calcium.

Unlike nickel, calcium is not a trace element and is found in large quantities in plants. The question arose as to whether extraordinarily high nickel concentration would cause a lowering of the concentrations of major elements like calcium. Figure IV-3 is a plot of μg/g nickel against % calcium. There is a very highly significant relationship ($p < 0.001$) between the two variables ($r = -0.53$ for 89 degrees of freedom). It would appear then that increasing concentrations of nickel have a depressant effect on the concentration of the major element calcium.

From figure IV-3 it can be seen that when different symbols are used to denote different substrates for the various specimens, those growing on ultrabasics are grouped together (as are others such as on limestone) and so high nickel and low calcium concentrations in a specimen of *R. bengalensis* could provide additional evidence that suggests that the specimen was growing on an ultrabasic substrate.

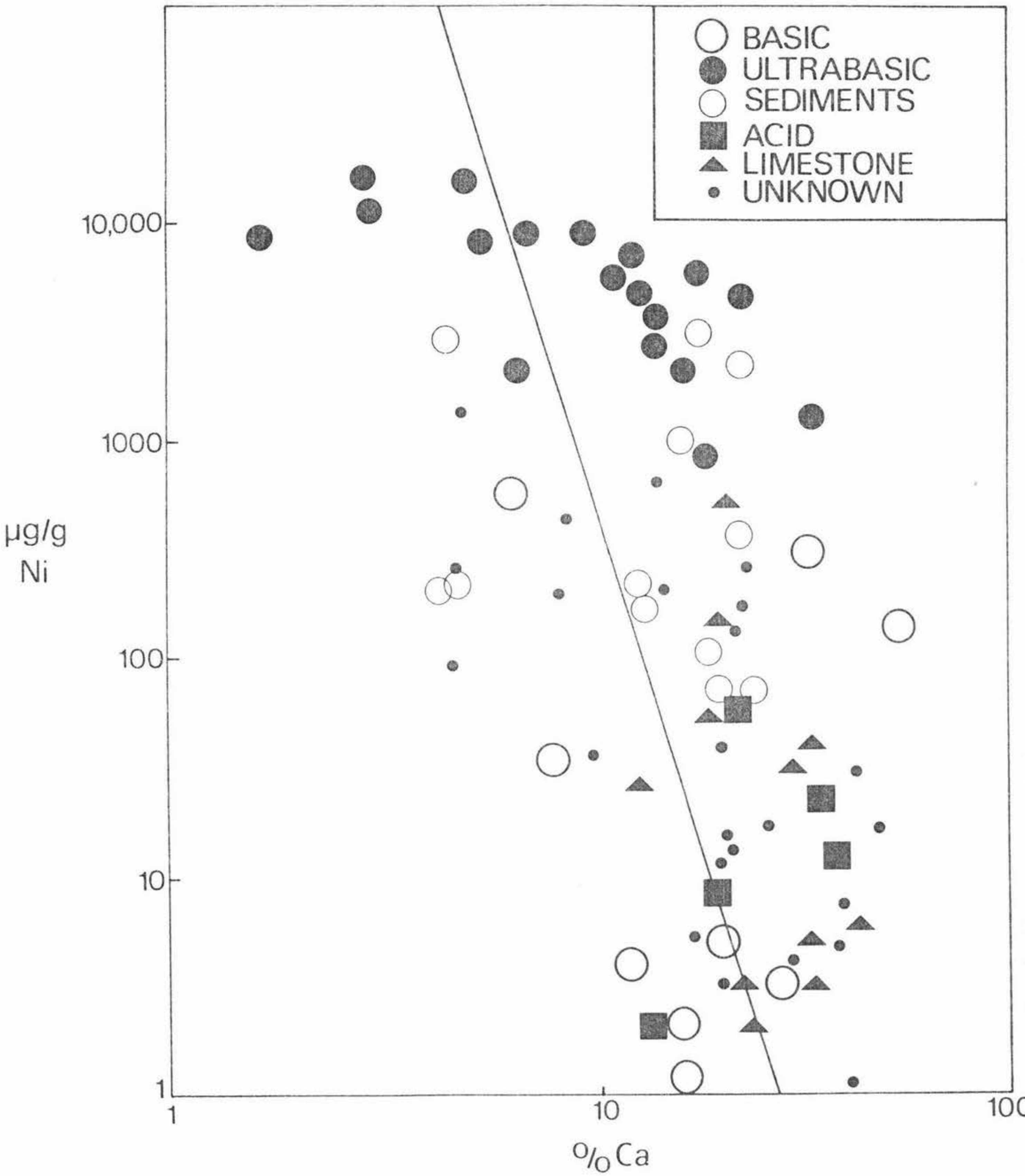
D. OTHER RINOREA SPECIES.

After studying the species *R. bengalensis* a study of some of the remaining species of the genus *Rinorea* was made. Specimens were analysed for cobalt and nickel only, and flame atomic absorption was

FIGURE IV-3.

NICKEL CONCENTRATIONS IN SPECIMENS OF
<u>RINOREA BENGALENSIS</u> EXPRESSED AS A FUNCTION
OF THE CALCIUM CONTENT.

The inverse relationship is very highly significant ($p < 0.001$, $\underline{r} = -0.53$).


BASIC
ULTRABASIC
SEDIMENTS
ACID
LIMESTONE
UNKNOWN
10,000
1000
100
10
1
µg/g
Ni
1
10
100
% Ca

used for the analyses. Table IV-3 list all species analysed.

1. Rinorea javanica - a hyperaccumulator of nickel.

Of the four samples of R. javanica analysed, the one from Kinabalu National Park in Borneo had extremely high concentrations of both nickel and cobalt.

A nickel concentration of 2170 µg/g places this species in the hyperaccumulator class using the arbitrary standard set by Brooks et al. (1977) of a nickel concentration of greater than 1000 µg/g. This means that R. javanica joins R. bengalensis as the second hyperaccumulator of nickel in the genus.

The same specimen contained 670 µg/g of cobalt which is an extremely high concentration. (0.6 µg/g would be a typical value - Brooks, 1972). Only two species have previously been found to contain a higher concentration of cobalt than this and they are:-

Haumaniastrum robertii 10,222 µg/g Brooks (1977)

Nyssa sylvatica var. biflora 845 µg/g Kubota et al. (1960)

2. High cobalt levels from Tanzania.

Samples of the two closely related species R. albersii and R. albersii var puberula that had been collected in Tanzania were analysed and found to contain more cobalt than nickel. Normally the reverse is the case and it can be seen from Table IV-3 that the cobalt/nickel ratio is usually very low but in the case of R. albersii var puberula the ratio hits a very high value of 200.

Although cobalt deposits are not recorded for Tanzania, cobalt ores are known in nearby Katanga (Duvigneaud, 1959) and in Southern Uganda, just to the north of Tanzania (Jones, 1972). It is therefore possible that these Tanzanian Rinorea species were growing in soils rich in cobalt.

E. Conclusion.

The discovery of two specimens of R. bengalensis from the Celebes with over 5000 µg/g nickel, has lead to; the discovery of two hyperaccumulators of nickel (R. bengalensis and R. javanica), criteria for identifying ultrabasic substrates, two possible new ultrabasic areas (Dalman, Nabire and Sorong) and a possible source of cobalt and a new cobalt indicator in Tanzania.

TABLE IV-3.

COBALT AND NICKEL CONCENTRATIONS

($\mu g/g$ dry weight) IN RINOREA SPECIES.

TABLE IV-3.

Species	No.	Locations	Cobalt Mean	Cobalt Range	Nickel Mean	Nickel Range	Co/Ni
accommanthera Gagnep	1	Vietnam	3		0.5		6.00
afzelii Engl.	1	Cameroun	1		4		0.25
albersii Engl.	18	Tanzania	24	0.5-96	6	0.5-19	4.00
albersii Eng. var. puberula Engl.	1	Tanzania	100		0.5		200.00
amaniensis Engl. ex Brandt	1	Tanzania	77		56		1.38
anguifera (Lour.) O.K.	9	Malaya, Cambodia, Sumatra, Sabah	9	0.5-72	3	1.4	3.00
ardisiiflora (Welw.) O.K.	1	Tanzania	53		74		0.71
aruwinensis Engl.	2	Zaire	0.5	0.5-0.5	6	0.5-12	0.08
australica C.T.W.	2	Australia	1	0.5-20	22	0.5-43	0.05
bengalensis (Wall.) O.K.	91	S.E. Asia	51	0.5-545	1810	1-17,500	0.03
bipindensis Engl.	1	Cameroun	0.5		13		0.04
brachypetala (Turcx.)O.K.	2	Nigeria, Liberia	0.5	0.5-0.5	0.7	0.5-1	0.71
brevipes (Benth.) Blake	1	Guyana	4		7		0.60
castaneifolia Bondar	1	Brazil	15		2		7.50
castilloi Merr.	1	Philippines	0.5		0.5		1.00
comosa (King) Merr.	1	Laos	0.5		0.5		1.00
convallariiflora Brandt	1	Kenya	0.5		0.5		1.00
crenata Blake	1	Costa Rica	4		4		1.00
dasyadena Robyns	1	Panama	2		4		0.50
densiflora Bartlett	1	Brit.Honduras	2		8		0.25
dentata	5	Angola, Cameroun, Liberia	6	0.5-25	7	0.5-28	0.86
elliotii De Wild	1	Sierra Leone	0.5		0.5		1.00
elliptica (Oliv.)O.K.	9	Kenya, Tanzania	1	0.5-2	1	0.5-2	1.00
falcata (Mart.) Ktze	1	Peru	1		6		0.17
fasciculata (Turcz.) Merr.	1	Solomons	5		6		0.86
ferruginea Engl.	1	Tanzania	0.5		2		0.25
flavescens (Aubl.) Ktze	2	Brazil	1	0.5-2	6	4-8	0.17

gazensis (Bak.J.)Brandt	8	Kenya, Tanzania	0.5	0.5-0.5	1	0.5-3	0.50
glandulosa (Elm.) Merr.	1	Hainan	0.5		21		0.02
gracilipes Engl.	2	Cameroun	7	4-11	8	8-8	0.87
guatemalensis (Watts.) Bartl.	2	Mexico	1	0.5-1	10	2-18	0.10
guianensis Aubl.	4	Brazil	1	0.5-2	3	1-6	0.66
horneri (Korth.) O.K.	48	S.E. Asia	3	0.5-41	6	0.5-60	0.50
hummelli Sprague	1	Mexico	2		20		0.10
ilicifolia (Welw. ex Oliv.) O.K.	1	Kenya	0.5		0.5		1.00
ilispaiei Jacobs	1	Sabah	0.5		2		0.25
javanica (Bl.) O.K.	4	Malaya, Sumatra, Java, Borneo	172	3-670	545	2-2170	0.32
kamerunensis Engl.	1	Cameroun	0.5		7		0.07
kibbiensis Chipp.	1	Ghana	0.5		2		0.25
lanceolata (Wallich) O.K.	3	Malaya, Java, Sumatra	0.7	0.5-1	6	1-15	0.11
liberica Engl.	2	Liberia	20	0.5-40	7	2-11	2.86
lindeniana (Tul.) O.K.	3	Colombia, Guyana, Surinam	2	0.5-2	8	0.5-18	0.25
longicuspis Engl.	2	Cameroun	4	0.5-6	2	1-2	2.00
longiracemosa (Kurz.) Craib	3	Java, Sabah, Hainan	0.5	0.5-0.5	4	2-5	0.13
longisepala Engl.	1	Cameroun	37		12		3.08
macrocarpa (Mart.) Ktze	1	Brazil	7		2		3.50
macrophylla (Decne) O.K.	1	Sumatra	1		2		0.50
marginata (Tr. & Pl.) Rusby	1	Columbia	1		0.5		2.00
melanodonta Blake	1	Columbia	1		0.6		1.67
microdon Brandt	1	Liberia	22		12		1.83
multinervis Brandt	1	Zaïre	1		15		0.07
oblanceolata Chipp	1	Sierra Leone	11		4		2.75
oblongifolia (Wright) Marq. ex Chipp	2	Sierra Leone, Ghana	18	17-19	8	7-10	2.25
paniculata	1	Brazil	1		1		1.00
passoura Ktze	1	Peru	3		0.8		3.75
physiphora (Mart.) Baillon	1	Brazil	2		0.9		2.22
prasina Stapf.	1	Liberia	2		1		2.00

pubiflora (Benth.) Sprague & Sandw.	4	Guyana Brazil,	1	0.5-3	2	0.5-4	0.50
pubipes Blake	1	Costa Rica	5		14		0.36
quangtriensis Gagnep	1	Vietnam	18		3		6.00
racemosa (Mart.) Ktze	1	Brazil	4		27		0.15
riana (DC) Ktze	4	Surinam, Guyana	1	0.5-1	7	0.5-21	0.05
sclerocarpa (Burgesd.)	1	Sumatra	1		2		0.50
sessilis (Lour.) Ktze	1	Hainan	2		1		2.00
solomonensis (Rech.) Melch.	1	Solomons	8		2		4.00
sprucei (Eichl.) Ktze	1	Guyana	2		4		0.50
squamata Blake	1	Panama	3		14		0.21
subintegrifolia (C.B.)O.K.	1	Cameroun	2		12		0.17
subumbellata (Brandt	1	Tanzania	0.5		3		0.17
sylvatica (Seem.) Ktze	2	Panama, Colombia	8	6-12	4	2-7	3.00
virgata (Thw.) Ktze	1	Hainan	5		0.8		6.25
viridifolia Rusby	1	Peru9	6		8		0.75
welwitschii (Oliv.) O.K.	3	Cameroun, Zaire	0.5	0.5-0.5	5	0.5-10	0.10
zenkeri Engl.	1	Cameroun	2		22		0.09

SECTION V.

THE FLORA OF OBI ISLAND.

A. INTRODUCTION.

A series of dried leaf samples that were from a collection made by Dr. E. de Vogel in a known ultrabasic region in the vicinity of Jikodolong on Obi Island in the North Moluccas was supplied for this project. (The collection site is located at approximately 1.25° S. and 128.15° E.)

As the collection area contains mainly ultrabasic rocks, any species collected there that had the ability to accumulate nickel would be expected to have amassed considerable quantities of nickel. Because of this, any potential hyperaccumulators would show up in the analysis of these samples.

The intention was to analyse all the supplied material and then analyse further samples from elsewhere in South East Asia, of any genus that contained a new hyperaccumulator of nickel in order to confirm that the species was, in fact, a hyperaccumulator, to identify previously unrecorded ultrabasic areas, and to check whether any other species in the genus qualified as hyperaccumulators of nickel.

B. THE DE VOGEL COLLECTION.

Sixty seven samples representing sixty different species were ashed and the ash was dissolved in 2 ml. of 2M redistilled hydrochloric acid. The solutions were then analysed for cobalt, chromium, nickel and manganese by flame atomic absorption. As chromium uptake is very slight in plants because of its toxicity (Brooks, 1972), the chromium concentration was useful as a guide to possible contamination of the samples by soil. Any sample that contained more than 10 $\mu g/g$ chromium was considered to be contaminated and values for the other elements for those samples were considered to be unreliable. There were three samples with more than 10 $\mu g/g$ chromium:-

Number 4289 Machaerina glomerata
Number 4265 Hydnophytum formicarum
Number 4230 Schizomeria serrata

Table V-1 lists, on a family-by-family basis, the sixty seven specimens along with their concentrations of cobalt, nickel and manganese in $\mu g/g$. (Chromium is omitted because most specimens had undetectably low chromium concentrations.) Inspection of Table V-1 shows three specimens with more than 1000 $\mu g/g$ nickel this placing them in the hyperaccumulator of nickel category.

1. Myristica laurifolia var bifurcata.

This specimen contained 1110 µg/g nickel. As this only just exceeds 1000 µg/g nickel, it was decided that on any substrate except an extremely nickel-rich one, this species would not be likely to fall into the hyperaccumulator of nickel category. For this reason it was considered that an in depth study of the genus Myristica was not warranted.

2. Planchonella oxyedra.

A specimen of this sample contained 19,600 µg/g (1.96%) nickel and as this was even higher than the levels found in Rinorea bengalensis (see section IV) a study of the genus Planchonella was carried out in the hope of discovering further nickel accumulators.

3. Trichospermum kjellbergii

There were two specimens of this species analysed, one contained 657 µg/g nickel which is high but not in the hyperaccumulator class and one containing 3770 µg/g nickel. The genus Trichospermum was also investigated more fully.

C. COBALT AND MANGANESE LEVELS IN SPECIMENS FROM OBI ISLAND.

1. Cobalt.

There were two specimens with more than 100 µg/g cobalt. (Such levels are extremely rare in vegetation - see discussion on R. albersii in Section IV). The two specimens in question are:-

Number 4230 Schizomeria serrata containing 262 µg/g cobalt, but this sample could be contaminated by soil as it contains 59 µg/g chromium.

Number 4233 Trichospermum kjellbergii containing 350 µg/g cobalt. This is the same sample that contained the very high levels of nickel. The high nickel and high cobalt could be indicative of a lateritic substrate.

2. Manganese.

A typical manganese concentration in plant samples is about 300 µg/g on a dry weight basis (Brooks, 1972), but our values showed an extremely scattered range (3 µg/g - 2620 µg/g). Two samples contained

TABLE V-1.

ELEMENTAL CONCENTRATIONS (μg/g) IN PLANTS

(de Vogel collection) FROM OBI ISLAND.

TABLE V-1.

Species	Ref.	Concentrations Co	Ni	Mn
Anacardiaceae				
Buchanania amboinensis Miq.	4295	<1	15	25
Annonaceae				
Cyathocalyx biovulatus Boerl.	4310	<1	<1	29
Mezzetia leptopoda Hook.f. et Thoms.	4250	<1	25	295
	4300	<1	2	71
Apocynaceae				
Alyxia stellata Roem.et Schult.	4239	3	29	910
Cerbera manghas L.	4251	1	6	39
Araliaceae				
Gastonia papuana Miq.	4229	31	40	1225
Araucariaceae				
Agathis alba Foxworthy s.sp. *corneensis*	4226	<1	5	33
Cyperaceae				
Gahnia aspera Spreng.	4320	<1	9	121
Machaerina glomerata (Gaudich.) Koyama	4271	5	95	95
	4289	26	276	345
Dilleniaceae				
Dillenia ovalifolia Hoogl. var. *sericea*	4236	2	423	690
Dipterocarpaceae				
Vatica papuana Dyer	4307	<1	5	224
Epacridaceae				
Styphelia abnormis J.J.Smith	4276	6	63	108
Gnetaceae				
Gnetum gnemon L. var.*domesticum*	4235	2	113	415
Goodeniaceae				
Scaevola oppositifolia Roxb.	4274	95	121	1790
Guttiferae				
Garcinia microphylla Merr.	4337	<1	8	133
Leguminaceae				
Desmodium umbellatum DC	4223	<1	5	60
Intsia palembanica Miq.	4254	<1	6	8
Liliaceae				
Dianella nemorosa Lam.	4321	1	2	488
Smilax australis A.Cunn. ex DC	4262	2	57	53

TABLE V-1 continued...

Species	Ref.	Co	Ni	Mn
Melastomataceae				
Melastoma polyanthum Blume	4278	<1	23	820
Monimiaceae				
Kibara macrophylla Benth.	4243	<1	18	286
Myristicaceae				
Horsefieldia glabra Warb.	4240	<1	20	136
	4253	<1	31	3
Horsefieldia roxburghii Warb.	4298	<1	3	79
	4303	3	3	68
Knema tomentella Warb.	4306	<1	1	111
Myristica laurifolia Spruce ex DC var. *bifurcata*	4325	56	1110	889
Myrsinaceae				
Rapanea densiflora Mez.	4279	<1	10	122
Myrtaceae				
Decaspermum fruticosum Forst.	4241	2	95	34
	4275	1	197	33
Eugenia acutangula L.	4294	6	28	111
Leptospermum flavescens Sm.	4283	1	11	1310
Rhodamnia cinerea Jack.	4238	3	66	47
Nepenthaceae				
Nepenthes maxima Reinw.	4286	7	65	191
Nepenthes mirabilis Druce.	4335	1	17	390
Nepenthes cf. *reinwardtiana* Miq.	4285	9	66	380
Olacaceae				
Gomphandra mappiodes Valeton	4301	<1	6	43
Orchidaceae				
Pseuderia foliosa Schlecht.	4272	4	109	15
Piperaceae				
Piper caninum Blume	4318	2	4	40
Pittosporaceae				
Pittosporum ferrugineum Dryand. in Ait.	4266a	1	100	133
Rhamnaceae				
Alphitonia incana Roxb.Teijsm. et Binn.	4280	2	25	712
Colubrina asiatica Brongn.	4350	<1	3	35
Ventilago oblongifolia Blume	4258	<1	2	4

TABLE V-1 continued...

Species	Ref.	Co	Ni	Mn
Rhizophoraceae				
Bruguiera gymnorrhiza Lam.	4299	< 1	3	168
Rosaceae				
Parastemon versteeghii Merr. et Perry	4314	< 1	2	13
Rubiaceae				
Guettarda speciosa van Eeden ex Steud.	4261	3	19	32
Hydnophytum formicarum Kurz.	4265	19	412	118
Psychotria longicauda Valetron	4339	1	12	2620
Rutaceae				
Tetractomia obovata Merr.	4346	2	59	520
Santalaceae				
Casearia glabra Roxb.	4225	< 1	36	81
Dendrotrophe varians Miq.	4344	5	34	407
Sapindaceae				
Guioa patentinervis Radlk.	4228	3	63	960
Sapotaceae				
Palaquium ridleyi King et Gamble	4333	1	2	24
	4342	1	3	29
Planchonella firma Dubard	4332	< 1	3	2200
Planchonella oxyedra Dubard	4268	34	19,600	110
Saxifragaceae				
Schizomeria serrata Hochr.	4230	262	590	1900
Sterculiaceae				
Commersonia bartramia Merr.	4277	< 1	26	621
Heritiera littoralis Dryand. in Ait.	4260	1	7	12
Tiliaceae				
Elaeocarpus cf *gjellerupi* Pulle in Lorentz	4227	3	16	295
Trichospermum kjellbergii Burret	4233	350	3770	1600
	4259	21	657	257
Urticaceae				
Celtis paniculata Planch.	4263	1	4	8
Gironniera subaequalis Planch.	4328	< 1	50	46
Verbenaceae				
Gmelina lepidota Scheff.	4338	< 1	6	720

in excess of 2000 µg/g manganese and these were:-

Psychotria longicauda 2620 µg/g manganese.

Planchonella firma 2200 µg/g manganese.

Such concentrations of manganese are rare as Jaffré (1977) pointed out. He discovered that in soils of pH <6.5 only 6.41% of plant specimens contained manganese concentrations in excess of 2000 µg/g manganese.

D. THE GENUS PLANCHONELLA.

The genus Planchonella is unfortunately less ubiquitous than the genus Rinorea and this study could not cover such a wide area as that of the genus Rinorea, previously described. We were however supplied with a further eighteen specimens of P. oxyedra and fourteen samples representing five other species of the genus (see Table V-2). The study confirmed P. oxyedra as a hyperaccumulator of nickel with two more samples falling into this category (a sample from Sukarnapura, West Irian containing 4800 µg/g and another from Hoetoemoeri Ambon containing 6700 µg/g). As can be seen from Figure V-1, samples of this species either contained less than 100 µg/g nickel or more than 1000 µg/g. If this trend could be found to exist throughout the species no matter how many more samples are eventually analysed, it would be an extremely useful characteristic as it would facilitate instant identification of highly nickeliferous substrates.

The other five species of the genus Planchonella that were analysed (P. aneityensis, P. firma, P. pittosporifolia, P. pyrulifera, and P. vitiensis) showed no tendency whatever to accumulate nickel. Although this may indicate that only one species in the genus (P. oxyedra) has the ability to accumulate nickel, the number of species analysed is too small to eliminate the possibility that other hyperaccumulation might be found in this genus.

E. THE GENUS TRICHOSPERMUM.

Like the genus Planchonella, the genus Trichospermum proved to be extremely rare and only seventeen samples representing five species were available for this study. (See Table V-2). The specimen of Trichospermum kjellbergii from Obi Island was the only species from the genus Trichospermum which was a hyperaccumulator.

TABLE V-2

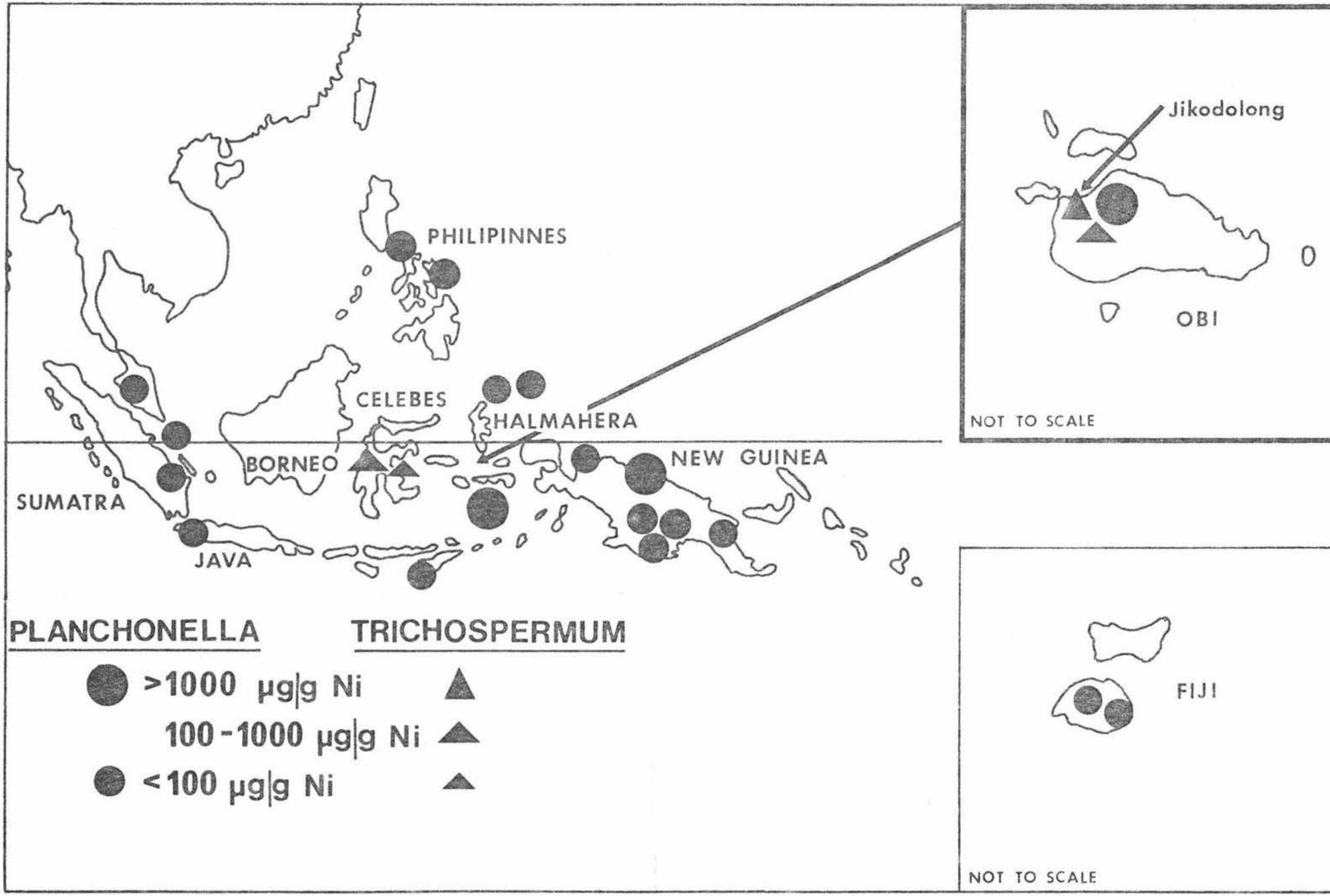
ELEMENTAL CONCENTRATIONS (µg/g) IN SPECIES OF <u>PLANCHONELLA</u> AND <u>TRICHOSPERMUM</u> FROM SOUTHEAST ASIA AND OCEANIA.

TABLE V-2.

Species	No.	Location	Co concn		Ni concn	
			Mean	Range	Mean	Range
Planchonella aneityensis(Guill.)Lam	1	New Hebrides	0.4		0.7	
Planchonella firma Dubard	1	Obi	1		3	
Planchonella oxyedra Dubard	19	S.E.Asia	15	0.2-240	1640	0.5-19,600
Planchonella pittosporifolia Elmer	6	Philippines	1.1	0.3-1.8	5	0.4-18
Planchonella pyrulifera (Gray) Lam	2	Fiji	1	0.3-1.5	3	1-5
Planchonella vitiensis Gillespie	4	Fiji	0.5	0.1-1.6	4	2-6
Trichospermum javanicum Blume	9	Borneo	0.8	0.2-2.9	2	0.8-8
Trichospermum kjellbergii Burret	4	Indonesia	94	0.5-350	1257	16-3770
Trichospermum kursii	1	Malaya	0.3		0.4	
Trichospermum psilocladum Merr.& Perry	1	Sarawak	1.6		2	
Trichospermum quadrivalve Merr.	2	New Guinea	2.1	1.5-2.7	8.5	4-13

FIGURE V-1.

COLLECTION LOCALITIES FOR *PLANCHONELLA OXYEDRA* AND *TRICHOSPERMUM KJELLBERGII*.



PHILIPINNES
CELEBES
HALMAHERA
BORNEO
NEW GUINEA
SUMATRA
JAVA
Jikodolong
OBI
NOT TO SCALE
FIJI
NOT TO SCALE
PLANCHONELLA
TRICHOSPERMUM
>1000 µg|g Ni
100 - 1000 µg|g Ni
<100 µg|g Ni

F. ACCUMULATION OF NICKEL - A PRIMITIVE FEATURE?

Using the advancement index of Sporne (1969), Chenery and Sporne (1976) postulated that the plant accumulators of aluminium were predominantly from the more primitive families. An investigation was undertaken to see if the same was true for nickel accumulators.

Table V-3 lists the nine non-contaminated species, that contained more than 100 µg/g nickel along with the advancement index of their families. Sporne(1970) states that the average advancement index for families in the Malay Peninsula and Java is 56.7. From Table V-3 it can be seen that five of the specimens are from families more primitive than the average. Two of the families are relatively advanced and two are not listed in Sporne's (1969) paper. It would appear, then, nickel accumulation is not solely a characteristic of primitive species but it may be more prevalent in the more primitive species.

G. THE FAMILY SAPOTACEAE.

Planchonella oxyedra, one of the two nickel hyperaccumulators discovered at Jikodolong, Obi Island, is a member of the family Sapotaceae as is Sebertia acuminata, a hyperaccumulator discovered by Jaffré and his co-workers (1976). Because of the occurrence of two hyperaccumulators in this single family, a biogeochemical study was undertaken on the family Sapotaceae. As they are most common in the region of New Caledonia (van Royen 1957 and Vink 1958) most of our samples are from the island of New Caledonia.

The family Sapotaceae is known for the milky sap contained in many genera and species. In the case of Sebertia acuminata the sap contains extraordinarily high concentrations of nickel (11.2% wet weight - Jaffré et al., 1976). The sap of P. oxyedra was not analysed but it is possible that, that too contains extraordinary concentrations of nickel.

A further seventy specimens were analysed representing forty-five species from fourteen different genera. The result of this study are listed in Table V-4. None of these seventy specimens had a nickel content exceeding the arbitrary 1000 µg/g limit set by Brooks et al., (1977). There were however, ten samples, representing eight species, containing more than 100 µg/g nickel. These eight species, though not hyperaccumulators, definitely have the ability to accumulate nickel and are as follows:

TABLE V-3

THE ADVANCEMENT INDEX OF FAMILIES

WITH NICKEL CONCENTRATIONS

IN EXCESS OF 100 μg/g.

TABLE V-3.

SPECIES	FAMILY	μg/g NICKEL	ADVANCEMENT INDEX*
Gnetum gnemon var. domesticum	Gnetaceae	113	not listed
Scaevola oppositifolia	Goodeniaceae	121	76
Myristica laurifolia var bifurcata	Myristicaceae	1110	30
Decaspermum fruticosum	Myrtaceae	197	27
Pseuderia foliosa	Orchidaceae	109	not listed
Pittosporum ferrugineum	Pittosporaceae	100	73
Planchonella oxyedra	Sapotaceae	19,600	42
Trichospermum kjellbergii	Tiliaceae	3,770	40
Trichospermum kjellbergii	Tiliaceae	657	40

*From Sporne (1969)

TABLE V-4.

NICKEL CONCENTRATIONS (μg/g dry weight)
IN SPECIES OF THE FAMILY SAPOTACEAE.

TABLE V-4.

SPECIES.	Number.	COLLECTION SITE	µg/g Ni. Range.	Mean.
Bureavella endlicheri	1	Rivière Bleue, New Caledonia		23
Bureavella wakere	1	Tonaaman, New Caledonia		3
Chrysophyllum lanceolatum	3	Sumbawa, New Guinea	2-9	5
Chrysophyllum roxburghii	3	Palawan, The Celebes	4-7	5.3
Corbassona deplanchei	3	New Caledonia	23-36	27.7
Gymnanthera paniculata	1	Luzon, Philippines		4
Iteiluma leptostylidifolia	1	Voh, New Caledonia		3
Iteiluma pimfolia	1	Paagaumevi, New Caledonia		4
Iteiluma rheophytopsis	1	Goro, New Caledonia		60
Leptostylis filipes	1	Boulinda, New Caledonia		30
Leptostylis goroensis	1	Goro, New Caledonia		16
Leptostylis grandiflora	1	Petchicara, New Caledonia		13
Leptostylis micrantha	2	Kopeto, New Caledonia	33-37	35
Leptostylis petiolata	3	New Caledonia	24-259	112.7
Niemeyra calausoe	1	Valle' de la Thy, New Caledonia		3
Ochrothallus blanchonii	1	Kaala, New Caledonia		3
Ochrothallus francii	1	Curone, New Caledonia		3
Ochrothallus litsuflorus	1	Mt Kaala, New Caledonia		13
Ochrothallus sarlinii	1	Petchicara, New Caledonia		12
Ochrothallus schmidii	1	Mt. Maoya, New Caledonia		164
Ochrothallus wagopensis	1	Massif Ton-Nom, New Caledonia		3
Planchonella cinerea	1	Nefaui, New Caledonia		7
Planchonella contermina	2	Voh, New Caledonia	8-11	9.5

continued..

TABLE V-4 continued...

SPECIES.	Number.	COLLECTION SITE	µg/g Ni. Range	Mean
Planchonella crassinervia	2	New Caledonia	7-436	221.5
Planchonella danikeri	2	New Caledonia	148-247	197.5
Planchonella dictyoneura	2	New Caledonia	3-6	4.5
Plancholtnella kuebiniensis	5	New Caledonia	4-242	78.6
Planchonella microphylla	1	Rivière Bleue, New Caledonia		3
Planchonella proyensis	1	Yaté, New Caledonia		71
Planchonella reticulata	2	New Caledonia	3-19	11
Planchonella vieillardii	2	Katepahie, New Caledonia	5-6	5.5
Pycnandra carinicostata	3	New Caledonia	3-21	9.7
Pycnandru chartaceci	1	Plaine des Lacs, New Caledonia		3
Pycnandra cf. linti vena	1	Rivière Bleue, New Caledonia		8
Pycnandra comptonii	1	Montagne des Sources, New Caledonia		177
Pycnandra decandra	1	Taam, New Caledonia		23
Pycnandra griseosepala	1	New Caledonia		47
Pycnandra kaalensis	1	Voh, New Caledonia		9
Rhamnoluma lecomtei	1	Tontouta, New Caledonia		102
Sarcosperma paniculatum	4	Celebes, New Guinea, Borneo	2-8	4.3
Sarcosperma sp.	1	New Guinea		4
Sarcosperma uittienii	1	Sumatra		5
Sebertia gatopensis	2	New Caledonia	137-754	445.5
Trouettea heteromera	1	Rivière Bleue, New Caledonia		4
Trouettea lissophylla	1	Kaala, New Caledonia		4

Leptostylis petiolata collected at Voh, New Caledonia (259 µg/g Ni)

Ochrothallus schmidii collected at Mt. Maoya, New Caledonia (164 ug/g Ni)

Planchonella crassmeria collected at Massif du Humboldt, New Caledonia (436 µg/g Ni)

Planchonella danikeri collected at Mt. Koala, New Caledonia (148 µg/g Ni)

Planchonella danikeri collected at Mt. de Poum, New Caledonia (247 µg/g Ni)

Planchonella kuebiniensis collected at Mt. Doré, New Caledonia (242 µg/g Ni)

Pycnandra comptonii collected at Mt. des Sources, New Caledonia (177 µg/g Ni)

Rhamnoluma lecomtei collected at Tontouta, New Caledonia (102 µg/g Ni)

Sebertia gatopensis collected at Me Ori, New Caledonia (754 µg/g Ni)

Sebertia gatopensis collected on the Route de Yaté, New Caledonia (137 µg/g Ni)

The above eight species, could, therefore be considered as accumulators of nickel even though they are not hyperaccumulators since "normal" plants even if growing over ultrabasic substrates do not usually have a nickel content exceeding 50 µg/g.

This study did not find further hyperaccumulators of nickel in the family Sapotaceae, but it would appear that the ability to tolerate nickeliferous substrates and to accumulate nickel is found in various species of the family and there may be more hyperaccumulators of nickel still to be discovered.

H. CONCLUSION.

On receipt of Dr. de Vogel's collection of specimens from Obi Island, an attempt was made to discover new hyperaccumulators. After the original analysis, further analysis of the genera Planchonella and Trichospermum and a study of the family Sapotaceae, three hyperaccumulator species have been identified (Myriatica laurifolia var. bifurcata, *Planchonella oxyedra* and *Trichospermum kjellbergii*). Also noted are thirteen species that have the ability to accumulate in excess of 100 µg/g nickel on a dry weight basis:- *Gnetum gnemon* var. *domesticum*; *Scaevola oppositifolia*; *Decaspermum fruticosum*; *Pseuderia foliosa*; *Pittosporum ferrugineum*; *Leptostylis petiolata*; *Ochrothallus schmidii*; *Planchonella crassinervia*; *Planchonella danikeri*; *Planchonella kuebinensis*; *Pycnandra comptonii*; *Rhamnoluma lecomtii* and *Sebertia gatopensis*.

Sporne's (1969) advancement index was used to study the relationship between primitiveness and the ability to accumulate nickel. Most of the species that tended to accumulate nickel were from the more primitive families.

SECTION VI.

THE FLORA OF SALAJAR ISLAND.

A. INTRODUCTION.

It has been established, mainly from work in Central Africa, that species of the Labiatae are tolerant to copper mineralization. Such examples are the "copper flower," of Zaïre - Haumaniastrum robertii (Durvigneaud and Denaeyer-de-Smet, 1963) and the "copper flower" of Zaïre, Zambia and the Rhodesia - Becium homblei (Howard-Williams, 1970). The latter has been used to detect copper mineralization in the "Copper Belt" (Anon, 1959) and is a good example of the use of a specific species for geobotanical prospecting.

Because of the usefulness of the Labiatae in prospecting for copper in Central Africa, the heavy metal content of species from this family growing in Indonesia was established, in order to see whether this might indicate the presence of copper (or other metals) in the substrate.

When W.M. Docteurs van Leeuwen visited the Island of Salajar in May 1913 he discovered species from this family growing there. These were deposited at the Utrecht herbarium. Specimens of each of Coleus atropurpureus and Cymaria acuminata obtained from this herbarium contained anomalous concentrations of copper and zinc. Samples of these same species from other localities outside of Salajar did not contain the high levels of copper and zinc found in that Island and so a study of samples of other species collected on Salajar was undertaken. However, the island of Salajar has not been botanically surveyed to any great extent. The largest collection of specimens is the one collected by Docteurs van Leeuwen which is stored at the Utrecht herbarium and this is the collection that provided the samples for analysis. The other major collection is that of Teijsmann which is smaller in number and to be found at Bogor and at Leiden.

TABLE VI-1

NICKEL, COPPER AND ZINC CONCENTRATIONS

IN PLANTS COLLECTED BY W.M. DOCTEURS VAN LEEUWEN.

TABLE VI-1

SPECIES	SPECIMEN NUMBER	µg/g Ni	µg/g Cu.	µg/g Zn.
Family: - Compositae				
Vernonia actaea Kost.	DVL 1320	1	14	238
Vernonia actaea Kost.	DVL 1423	7	7	15
Vernonia actaea Kost.	DVL 1466	4	4	17
Vernonia actaea Kost.	DVL 1937	1	300	6380
Family:- Labiatae				
Coleus atropurpureus Benth.	DVL 1444	38	19	95
Coleus atropurpureus Benth.	DVL 1651	16	195	226
Coleus atropurpureus Benth.	DVL 1888	1	500	1700
Cymaria acuminata Decne.	DVL 1883	250	275	19500
Hyptis capitata Jacq.	DVL 1554	1	14	27
Hyptis capitata Jacq.	DVL 1644	42	146	267
Hyptis capitata Jacq.	DVL 1762	100	80	1525
Hyptis suaveoleus Poit.	DVL 1647	34	45	227
Family:- Leguminosae				
Albizzia saponaria Blume.	DVL 1357	1	7	15
Albizzia saponaria Blume.	DVL 1447	12	12	60

Biographical information upon the life of Docteurs van Leeuwen can be found in Flora Malesiana (Series 1 Volume 1) and is as follows:

Willem Marius Docteurs van Leeuwen was born at Batavia in Java in 1880. He was educated at Amsterdam University where he took his Ph Dr's degree in 1907. During 1908 and 1909 he was an entomologist at the General Experiment Station at Salatiga in Central Java. He then taught Natural History in Semarang (1909-1915) and Bandung (1915-1918). He was Director of the Botanic Gardens at Buitenzorg from 1918 until 1932 and also Extraordinary Professor at the Medical College, Batavia from 1926 to 1932. Upon returning to Holland in 1932 he became a Lecturer at Amsterdam and a Professor in 1942.

During his term of office in Java, his studies especially covered the field of general biology, symbiosis of ants and plants, flower biology, seed dispersal, investigations on the mountain flora and the succession in the new vegetation of the isle of Krakatau.

B. THE ISLAND OF SALAJAR.

The island of Salajar measures 71 km long and 11 km wide (at the widest point) (Anon,1944). It is located approximately between longitudes 120°26'E and 120°34'E and between latitudes 5°47'S and 6°30'S. A terraced mountain chain traverses the island and descends steeply to a rock coral-fringed coast on the east side but descends more gradually to the west coast.

Docteurs van Leeuwen (1937) described the geology of Salajar as consisting of a kernel of sandstones and marls covered by very young coral. Once the soil has been cultivated it often becomes denuded of its humus.

C. THE FIRST SAMPLES FROM THE DOCTEURS VAN LEEUWEN COLLECTION.

The first fourteen samples to arrive from the Docteurs van Leeuwen collection arrived in answer to a general request for samples from families and genera that had previously been discovered to contain accumulators of various metals. Those samples that arrived were studied for three different reasons:-

1. Four samples of *Vernonia actaea* were studied beacuse they belong to the family Compositae which includes the nickel accumulating species *Dicoma niccolifera* (Wild 1970 and 1971).

TABLE VI-2.

NICKEL, COPPER AND ZINC CONCENTRATIONS IN

SPECIES OF THE GENUS COLEUS.

TABLE VI-2.

SPECIES	COLLECTION LOCALITY	µg/g Ni	µg/g Cu	µg/g Zn
C. atropurpureus	Indochina	1	15	175
C. atropurpureus	New Guinea	1	13	25
C. atropurpureus	Philippines	3	9	25
C. atropurpureus	Malaysia	15	7	103
C. atropurpureus	Singapore	9	9	130
C. atropurpureus	Singapore	2	7	77
C. atropurpureus	Malaysia	4	13	75
C. scutellarioides	Java	67	67	84
C. scutellarioides	Sumatra	15	15	59
C. scutellarioides	Java	71	71	98
C. scutellarioides	Sumatra	17	3	41
C. scutellarioides	Ceram	3	10	154
C. scutellarioides	Philippines	1	25	50
C. scutellarioides	Sumatra	1	9	53

TABLE VI-3.

NICKEL, COPPER AND ZINC CONCENTRATION IN SPECIES OF THE GENUS *CYMARIA*.

TABLE VI-3

SPECIES	COLLECTION LOCALITY	µg/g Ni	µg/g Cu	µg/g Zn
C. acuminata	Philippines	<1	7	24
C. acuminata	Philippines	<1	11	484
C. acuminata	Philippines	<1	3	14
C. acuminata	Philippines	<1	10	32
C. acuminata	Papua	<1	13	15
C. acuminata	Papua	<1	12	40
C. acuminata	India	<1	14	12
C. elongata	Nicobar Island, Bay of Bengal	<1	7	13
C. acuminata	Bali	<1	5	53
C. sp.	Hainan	<1	4	17
C. dichotoma	Hainan	<1	6	15
C. dichotoma	Hainan	<1	3	6
C. acuminata	Hainan	<1	4	12
C. acuminata	Philippines	5	7	36
C. acuminata	Bali	20	51	40
C. acuminata	Moluccas	<1	6	116
C. acuminata	Philippines	<1	14	7
C. acuminata	Philippines	6	6	31
C. acuminata	Java	<1	5	20
C. acuminata	Philippines	14	14	41
C. acuminata	Java	<1	28	167

2. Eight samples from the Labiatae were analysed because this family contains a number of species tolerant to copper (see above).

3. The genus *Albizzia* was requested because of the discovery of *Albizzia* sp *nov* growing over the Bougainville porphyry copper deposit (Cole, 1971). This local species was rarely found in areas where soil copper values were not anomalous.

Concentrations of copper, nickel and zinc are listed in Table VI-1 for the fourteen specimens analysed in an initial collection. Of particular interest because of their anomalous copper and zinc levels are: DVL 1937, *Vernonia actaea*; DVL 1888, *Coleus atropurpureus*; DVL 1883, *Cymaria acuminata*. For the purposes of this study *Coleus atropurpureus* and *Cymaria acuminata* were considered to be of the greatest interest as it was though copper accumulation may be a characteristic of several genera and species of the family Labiatae. Therefore a request was made for further samples of specimens from these two genera which had been collected from other areas outside of the Salajar group.

D. THE GENERA COLEUS AND CYMARIA.

Fourteen samples of the genus *Coleus* and twentyone of the genus *Cymaria* were supplied for this investigation. Copper, nickel and zinc concentrations are listed in tables VI-2 (*Coleus*) and VI-3 (*Cymaria*). The extremely high copper levels found in the samples from Salajar Island were not repeated. Only three specimens of the thirty-five could be considered to contain anomalous levels of copper: two samples of *Coleus scutellarioides* from Java (67 and 71 µg/g copper) and one sample of *Cymaria acuminata* from Bali (51 µg/g copper). Although these three specimens do contain anomalous copper values, they are only slightly anomalous and the plants would not be considered to be accumulating excessive amounts of copper, in contrast to the samples from Salajar.

None of the thirty-five samples could be considered to contain zinc levels that were in any way anomalous. In fact, the zinc levels were generally low.

Because of the uniqueness of high copper and/or zinc concentrations in these two species from Salajar, it was decided to investigate copper and zinc concentrations in other species that Docteurs van Leeuwen had collected on Salajar Island.

E. COPPER AND ZINC CONCENTRATIONS IN SAMPLES FROM SALAJAR ISLAND.

A further seventy-six samples of plants collected by Docteurs van Leeuwen were received and analyzed for copper and zinc by flame atomic absorption in a manner similar to that previously described. Attention was paid more to concentration levels of copper than zinc as zinc concentrations in plants tend to cover a wide range of values. The concentration of copper and zinc in each sample (in µg/g on a dry weight basis) is listed in Table VI-4.

The seventy-six samples just mentioned took the total number of specimens from the Docteurs van Leeuwen collection analysed to ninety of which eighty were collected on the Island of Salajar. (Of the original fourteen samples only four; DvL 1888 Coleus atropurpureus DvL 1883 Cymaria acuminata, DvL 1762 Hyptis capitata and DvL 1937 Vernonia actaea, were from that Island). When the copper concentrations of the eighty samples from the Island of Salajar are plotted on a histogram (Figure VI-1) an arbitrary division into three subpopulations can be made.

1. Subpopulation 1 - "Normal" concentrations.

On this island it would appear that concentrations of copper of less than 200 µg/g can be considered as normal for the local flora. Previously in these studies, copper concentrations in excess of 50 µg/g have not been encountered. Therefore by the standard of other areas in the region of Indonesia even many of these "normal" concentration values are in themselves above average. This would seem to indicate that copper is either more prevalent in the soil or more easily available to the flora on the Island of Salajar than in many sites in Indonesia.

2. Subpopulation 2 - "Accumulators" of copper.

Samples with 200-500 µg/g copper could be considered to accumulate anomalously high concentrations of copper in their foliage. Therefore the suggestion is made that species that can be found to contain copper concentrations in this range should be called accumulators of copper. (See Table VI-5).

TABLE VI-4.

COPPER AND ZINC CONCENTRATIONS IN PLANTS COLLECTED ON SALAJAR ISLAND.

TABLE VI-4

SPECIES	NUMBER (DvL)	µg/g Cu.	µg/g Zn.
Family:- Amaranthaceae.			
Aerva scandens Wall.	1873	395	5526
Allmania nodiflora R.Br.	1862	178	947
Cyathula prostrata Blume.	1842	553	17475
Family:- Annonaceae.			
Artabotrys odoratissimus R.Br.	1904	31	563
Uvaria littoralis Blume.	1690	46	92
Family:- Aristolochiaceae.			
Aristolochia tagala Cham.	1817	25	2030
Family:- Begoniaceae			
Begonia sp.	1746	68	68000
Family:- Euphorbiaceae.			
Acalypha caturus Blume.	1765	66	221
Alchornea rugosa Muell - Arg.	1654	28	640
Alchornea rugosa Muell - Arg.	1850	47	314
Breynia sp.	1877	160	427
Bridelia lanceifolia Merr.	1835	28	75
Claoxylon sp.	1694	31	60
Codiaeum variegatum Blume.	1764	23	205
Euphorbia plumerioides Teysm.	1894	185	5770
Excoecaria agallocha L.	1856	34	400
Excoecaria agallocha L.	1931	23	205
Glochidion molle Blume.	1816	44	117
			continued...

Table VI-4 continued...

SPECIES	NUMBER. (DvL)	µg/g Cu.	µg/g Zn.
Glochidion rubrum Blume.	1827	18	245
Glochidion zeylanicum Juss.	1727	22	265
Homalanthus populneus O.K.	1716	35	70
Macaranga hispida Muell-Arg.	1754	36	11860
Macaranga hispida Muell-Arg.	1755	150	675
Strophioblachia fimbricalyx Boerl.	1879	14	512
Family:- Guttiferae.			
Calophyllum inophyllum L.	1848	8	328
Family:- Loranthaceae			
Amylotheca stenopetala Dans.	1822	61	1220
Ginalloa arnottiana Korth.	1712	6	74
Scurrula fusca G. Don.	1709	16	78
Family:- Menispermaceae			
Stephania forsteri A. Gray.	1792	79	316
Family:- Mimosaceae			
Pithecolobium junghuhnianum Benth	1805	19	260
Pithecolobium umbellatum Benth	1933	134	4285
Family:- Moraceae			
Fatoua japonica Blume.	1828	180	12000
Ficus benjamina L.	1886	7	1640
Ficus hispida L.F.	1889	<5	200
Ficus retusa L.	1757	3	41
Ficus superba Miq.	1725	12	470
Streblus asper Lour.	1872	6	86

continued...

Table VI-4 continued...

SPECIES	NUMBER (DvL)	µg/g Cu.	µg/g Zn.
Family:- Moringaceae			
Moringa oleifera Lamk.	1868	183	7120
Family:- Nyctaginaceae			
Pisonia aculeata L.	1869	258	430
Family:- Papilonaceae - Caesalpinioideae			
Bauhinia binata Blanco.	1932	48	2250
Cassia alata L.	1852	55	97
Cassia sophora L.	1662	131	153
Cassia sophora L.	1826	333	1530
Cassia tora L.	1708	41	83
Family:- Papilionaceae-Papilionatae			
Abrus precatcrius L.	1686	167	9170
Crotolaria ferruginea Grah.	1800	21	1150
Derris scandens Benth.	1790	49	12770
Derris scandens Benth.	1892	96	22800
Desmodium laxiflorum DC.	1655a	167	153
Desmodium umbellatum DC.	1940	22	227
Desmodium zonatum Miq.	1794	20	564
Dioclea umbrina Benth.	1775	22	76
Dolichos falcatus Klein.	1802	276	490
Flemingia strobilifera R.Br.	1824	30	6800
Mucuna pruriens DC.	1776	46	11540
Phylaceum bracteosum Benn.	1756	102	307
Pongamia pinnata Merr.	1941	132	790

continued...

Table VI-4 continued...

SPECIES	Number (DvL)	µg/g Cu.	µg/g Zn
Psophocarpus tetragonolobus DC.	1788	103	12410
Rhynchosia acuminatissima Miq.	1795	82	2800
Sesbania grandiflora Pers.	1926	158	2370
Tephrosia zollingeri Pers.	1664	28	2770
Teramnus labialus Spreng.	1691	70	256
Uraria lagopotiodes Desv.	1818	58	423
Uraria Sp.	1915	129	1650
Family:- Piperaceae			
Peperomia pellucida H.B.K.	1841	300	25720
Peperomia Sp.	1797	5	188
Piper betle L.	1741	65	458
Piper caninum Blume.	1724b	7	2750
Piper caninum Blume.	1745	22	161
Piper caninum Blume.	1807	<10	326
Family:-Pittosporaceae			
Pittosporum moluccunum Blume.	1914	10	360
Family:- Rosaceae			
Rubus alcaefolius Poir.	1732	27	870
Rubus rosaefolius Sm.	1735	170	5680
Family:- Santalaceae			
Exocarpus latifolius R.Br.	1928	12	93
Family:- Urticaceae			
Laportea ruderalis Gaud.	1897	600	4650
Pilea sp.	1697	21	300

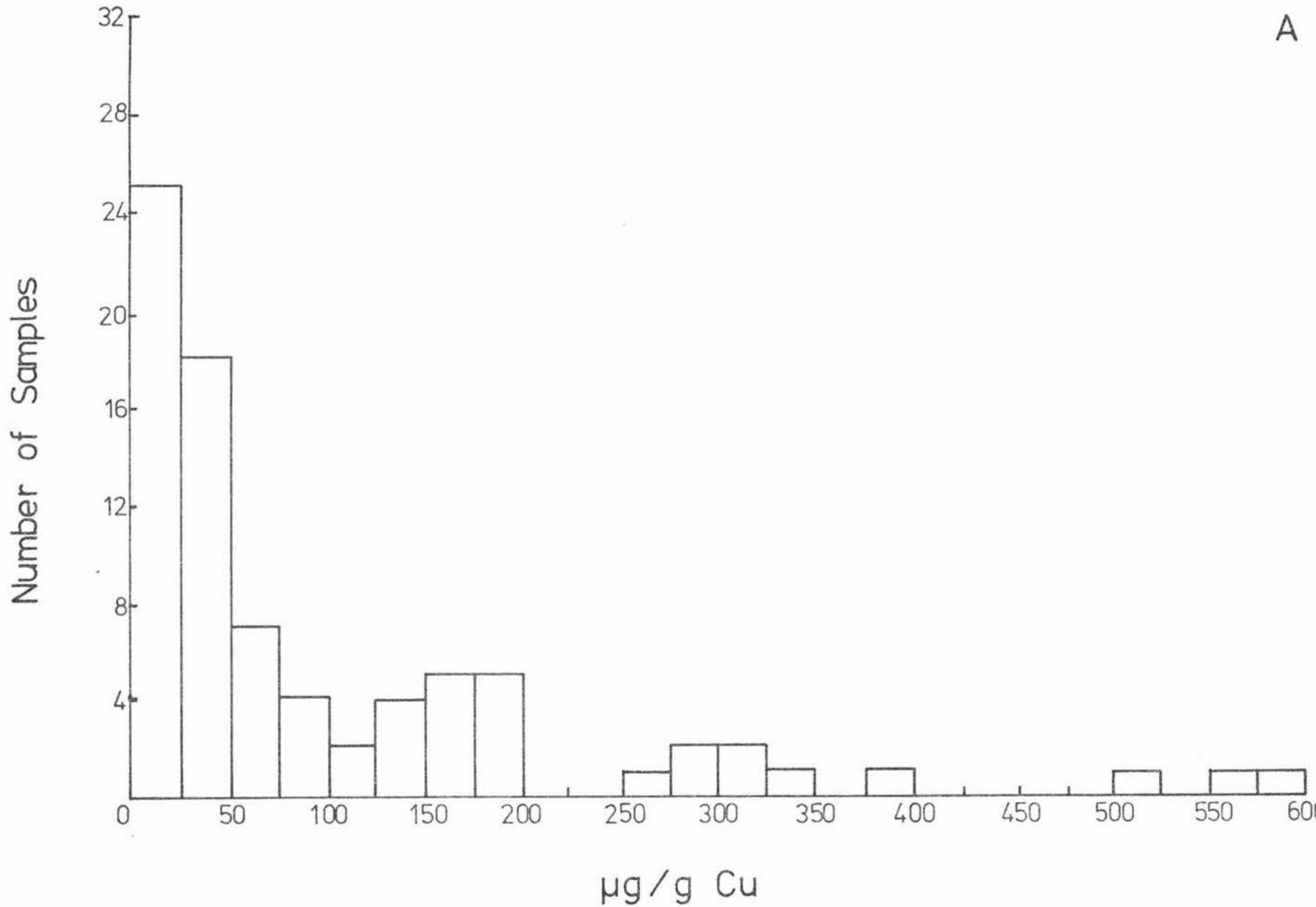
FIGURE VI-1.

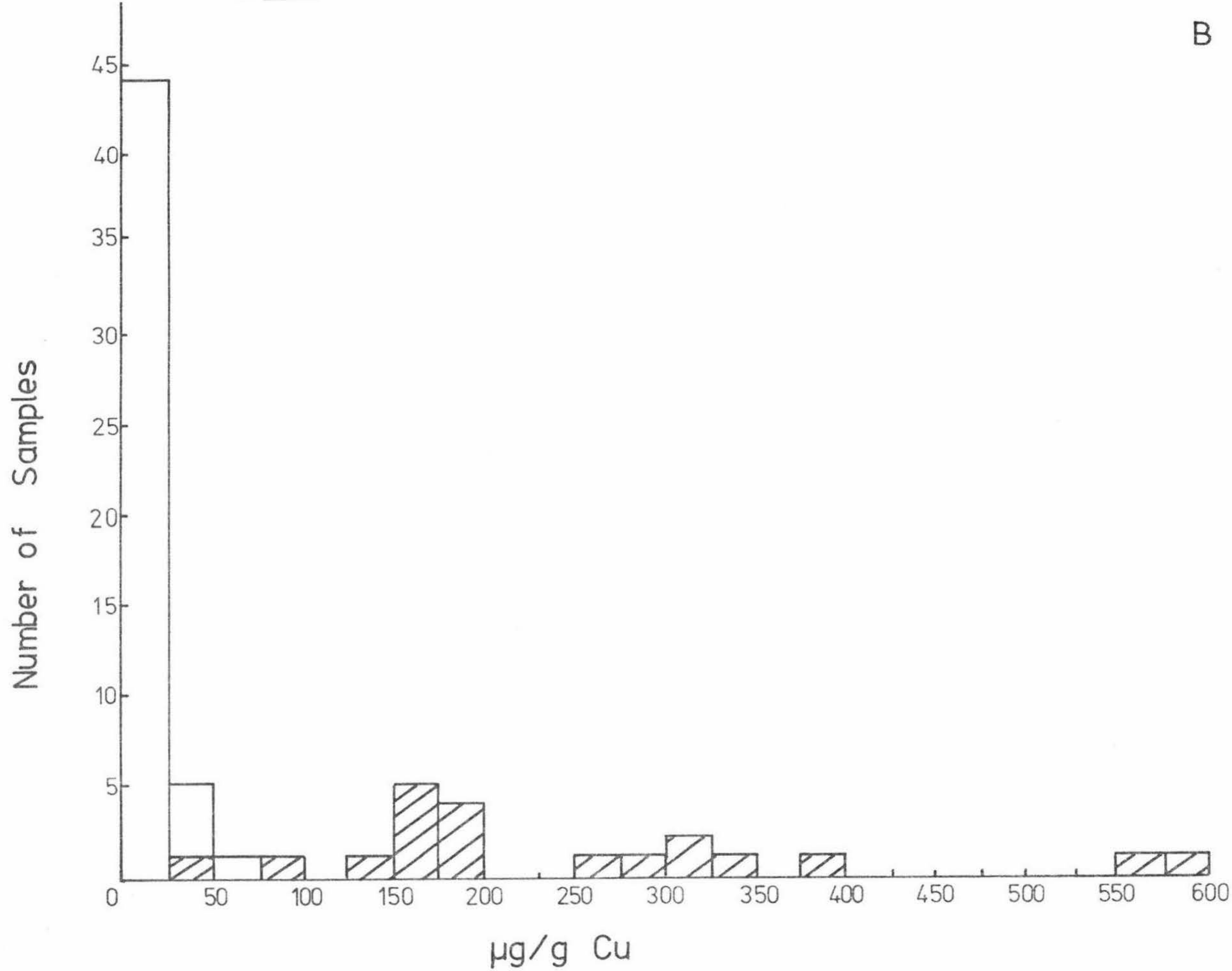
HISTOGRAM SHOWING COPPER VALUES FROM PLANT SPECIMENS COLLECTED ON SALAJAR ISLAND.

A. All plants from Salajar Island are shown.

B. Plants from Salajar Island are shown (▨) with plants of the same species from other species also shown for comparison.

A


Number of Samples
32
28
24
20
16
12
8
4
0
50
100
150
200
250
300
350
400
450
500
550
600
µg/g Cu


B
Number of Samples
45
40
35
30
25
20
15
10
5
0
50
100
150
200
250
300
350
400
450
500
550
600
µg/g Cu

3. Subpopulation 3 - "Hyperaccumulators" of copper.

Following the example of Brooks et al., (1977) in calling a plant species with dried leaf samples containing in excess of 1000 μg/g nickel a hyperaccumulator of nickel, it is suggested that any species found to contain in excess of 500 μg/g copper in its foliage should be termed a "hyperaccumulator of copper." (See table VI-6).

4. Comparison with the African Copper Belt.

Applying the suggested divisions to results reported by Duvigneaud and Denaeyer-de-Smet (1963) brings to light the rarity of accumulators and hyperaccumulators of copper. These two workers undertook a biogeochemical survey in Katanga (in the African Copper Belt). Only five of their species (growing in extremely copper rich areas) would fall into one of the two upper subpopulations:- Becium aureoviride ssp. lupotense 210 μg/g Cu (accumulator) Pandiaka metallorum 740 μg/g Cu (hyperaccumulator) Ascolepis metallorum 1200 μg/g Cu (hyperaccumulator) Silene cobalticola 1660 μg/g Cu (hyperaccumulator) Haumaniastrum robertii 1960 μg/g Cu (hyperaccumulator).

Again the evidence strongly suggests that the concentrations of copper in the flora of Salajar represents a definite anomaly.

F. SAMPLES OF SPECIES THAT CONTAINED COPPER ANOMALIES COLLECTED OUTSIDE OF SALAJAR ISLAND.

As was done after the discovery of high copper concentrations in Coleus atropurpureus and Cymaria acuminata, further samples of the species with high copper concentrations were requested from regions other than Salajar Island. Again the anomalous copper concentrations that were found on Salajar Island were not repeated in samples from other collection sites. (See table VI-7).

It is therefore obvious that copper uptake by plants growing on Salajar Island was higher than copper uptake by the same species growing elsewhere. There are three possible explanations for this higher uptake of copper:

(i) Pollution of the soil of Salajar Island by copper at the time of collection, or pollution of the specimens since collection. These two suggestions can be discounted as there was no industry or any other likely pollution source on Salajar Island back in 1913 and the handling and storage of samples by the herbarium at Utrecht in no way involves copper. When the herbarium sheets for some of these samples with high

TABLES VI-5 and VI-6.

COPPER ACCUMULATING (VI-5) AND HYPERACCUMULATING SPECIES AND THEIR COLLECTION LOCALITIES.

TABLE VI-5

SPECIES	NUMBER	µg/g Cu	COLLECTION DATE	COLLECTION LOCALITY
Vernonia actaea	1937	300	29/5/1913	South Salajar
Cymaria acuminata	1883	250	28/5/1913	South Salajar
Aerva scandens	1873	395	28/5/1913	South Salajar
Pisonia aculeata	1869	258	28/5/1913	South Salajar
Cassia sophora	1826	333	25/5/1913	Near Benteng
Dolichos falcatus	1802	276	24/5/1913	Near Benteng
Peperomia pellucida	1841	300	25/5/1913	Near Benteng

TABLE VI-6

SPECIES	NUMBER	µg/g Cu	COLLECTION DATE	COLLECTION LOCALITY
Coleus atropurpureus	1888	500	28/5/1913	South Salajar
Cyathula prostrata	1842	553	25/5/1913	Near Benteng
Laportea ruderalis	1897	600	28/5/1913	South Salajar

copper concentrations were analysed, they contained 4-18 $\mu g/g$ Cu. If the samples had been contaminated, contamination of the sheets should have ocurred too.

(ii) Copper is more available to plants growing on Salajar Island than elsewhere. This is unlikely because Salajar Island contains predominantly limestone substrates (Docteurs van Leeuwen, 1937) which are pH-basic and copper has an extremely low mobility in basic substrates (Andrews-Jones, 1968) which depress rather enhance its availability to the flora growing on the substrate.

(iii) The concentration of copper in the soil is anomalously high and the anomalies in plant copper levels are a reflection of this. This appears to be the most plausible explanation and so the collection localities of the specimens with anomalously high copper concentrations should be investigated *in situ* for soil copper concentrations.

G. COLLECTION LOCALITIES.

With the aid of the precise collection dates of these samples and the report of Docteurs van Leeuwen's collection trip (Docteurs van Leeuwen, 1937) it was possible to determine collection localities for the specimens with copper concentrations in excess of 200 $\mu g/g$. (These collection localities are listed in Tables VI-5 and VI-6). The information on Docteurs van Leeuwen's trip is contained in the following report (Docteurs van Leeuwen, 1937):

"May 17th. Overnight the ship sails back to Salajar, where we arrive at 5 o'clock in the afternoon.

"The next few days are spent with arranging the collections and making preparations for an excursion to the mountains of Salajar.

"May 20th. With many coolies we leave early in the morning; the way first leads through coco plantations and mangrove forests, and along shaded roads into the hills. The vegetation gradually becomes richer but yet it is a poor remnant of what formerly it must have been. By 12 o'clock we arrive at the pasanggrahan (Government resthouse for visitors), where we stay a few days in order to collect plants in the neighbourhood. The pasanggrahan is situated at an altitude of about 300 m, just above a campong Bitombang, so that the surroundings are cultivated for the greater part. At the back of the house we have a view of the highest mountain of the Island, the Bontanoharoe.

"May 22nd. We continue our way upwards, after a few hours climbing along steep paths we reach the top of the Bontanoharoe about 600 m

TABLE VI-7.

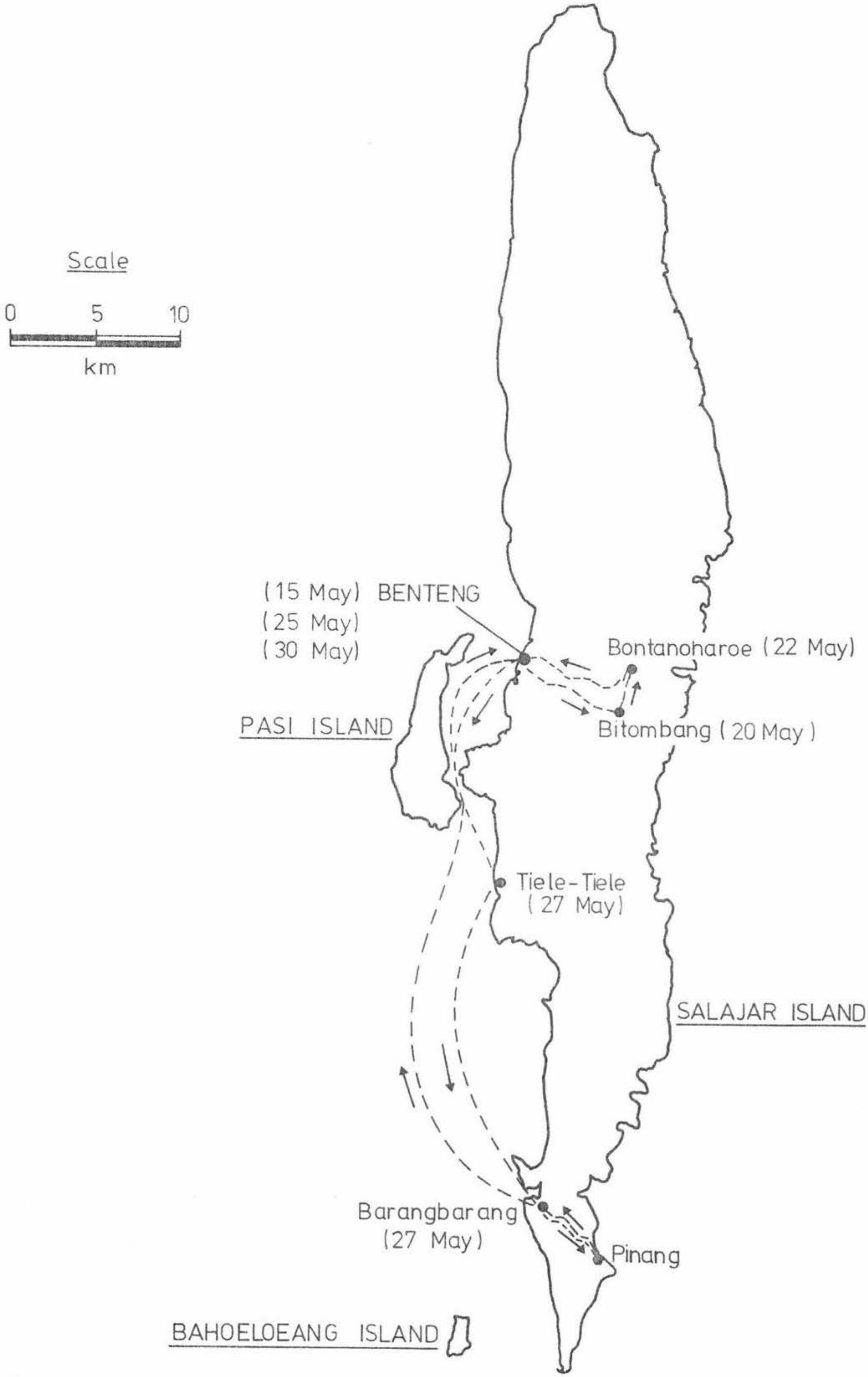
COMPARISON OF COPPER CONCENTRATIONS FROM SALAJAR ISLAND WITH COPPER CONCENTRATIONS IN THE SAME SPECIES COLLECTED ELSEWHERE.

TABLE VI-7.

	Salajar µg/g Cu	Other Regions Range (µg/g Cu)	Mean (µg/g Cu)
Abrus precatorius	167	8-17	12.0
Aerva scandens	395	7-33	17.0
Allmania nodiflora	175	15-32	23.5
Cassia sophora	131; 333	6-18	11.7
Cyathula prostrata	553	8-18	11.7
Desmodium laxiflorum	167	6-39	17.0
Dolichos falcatus	276	6-10	7.3
Euphorbia plumerioides	185	4-8	6.0
Fatoua japonica	180	4-23	13.3
Hyptis capitata	80	20-33	26.5
Laportea ruderalis	600	9-43	20.7
Macaranga hispida	36; 150	4-5	4.3
Moringa oleifira	183	12-19	15.3
Peperomia pellucida	300	16-22	19.0
Pisonia aculeata	258	9-51	26.3
Rubus rosaefolius	170	7-13	10.0
Sesbania grandiflora	158	4-14	8.0
Vernonia actaea	300	10-11	10.5

FIGURE VI-2.

MAP OF SALAJAR ISLAND, SHOWING THE ROUTE OF W.M. DOCTEURS VAN LEEUWEN.


Scale
0 5 10
km
(15 May) BENTENG
(25 May)
(30 May)
Bontanoharoe (22 May)
PASI ISLAND
Bitombang (20 May)
Tiele-Tiele
(27 May)
SALAJAR ISLAND
Barangbarang
(27 May)
Pinang
BAHOELOEANG ISLAND

above sea-level. On a ridge, covered with grass, between remnants of the virgin forest, is a small hut, from where we have a beautiful view of the west side of the island. The top itself is flat, and covered with wood, with alternate grass and shrub wilderness. Psidium guajava has run wild everywhere and is fructifying amply. In the evening the wild boars feast upon the fallen fruits. Towards the east coast the country goes down steeply, and the slopes bear but little vegetation. This part of the Island is richer in plants than any other visited so far. For several days we make excursions and collect as many plants as possible.

"May 25th. We go back to Benteng along a ridge running in a northwestern direction; at first the way leads down gradually, but nearer to the plain the slope becomes steeper. The ridge is very narrow, with on both sides perpendicular walls; everything is quite white owing to the limestone; the growth of plants is extremely poor.

"May 26th. The day is spent with preparing an excursion to the southern point of Salajar; the chief of a campong there, of Barang-barang, goes home tomorrow by proah, and I may join him.

"May 27th. In a small heavily laden flying proah we leave Benteng early in the morning, at first rowing, later on by sail. We sail close under the west coast, which consists of steep limestone rocks bearing may Pandanus and Cycas. The sea undermines the coast, everywhere are caves, and large blocks of rock lie spread in the sea. Halfway, at Campong Tiele - Tiele, we take a heavier proah and before long we are sailing again southwards over a rough sea. At half past six we reach the Campong Tonkè-Tonkè from where we reach Barang-Barang after a quarter of an hours walk. We put up our campbeds in the house of the chief of this Campong, and soon retire behind the mosquito curtain, for this part is known for its many mosquitos and malaria.

"The next few days we make excursions in the neighbourhood, and amongst the others right across the Island towards the east side where a few small campongs are situated, Bonesela and Pinang. The greater part of the south point is covered with thin wood and along the coast here and there with mangrove forests.

"May 30th. We leave at 8 o'clock in the morning sailing before a stiff breeze, and thus we reach Benteng at half past two."

Those plant samples containing more than 200 µg/g copper can be divided into two groups on a locality basis. The larger group contains the species Vernonia actaea, Cymaria acuminata, Aerva scandens, Pisonia aculeata, Coleus atropurpureus and Laportea ruderalis which were all collected in the south of the island in the region of Barangbarang and Pinang (See Figure VI-2). The other group contains: Cassia sophora, Dolichos falcatus and Cyathula prostrata and these three species were all collected on the trip from Bontanoharoe to Benteng. (See figure VI-2). It would appear, therefore, that suitable areas for carrying out an investigation of soil copper levels in the hope of finding copper mineralization would be an area east of Benteng heading slightly south into the mountains or on a west to east transect of the south of the island in the vicinity of Barangbarang.

H. CONCLUSION.

Extremely high levels of copper have been found in various plant species growing on Salajar Island but the values were not repeated in the same species collected elsewhere. The anomalies appear to be probably due to mineralization as no known copper pollution exists and the predominant substrates are limestones in which copper mobility is low. Two areas seem to be worthy of an *in situ* investigation for copper; near Benteng in central Salajar and near Barangbarang in South Salajar.

GENERAL CONCLUSION.

When the results of this thesis are studied in the light of the original aims it can be seen that these have largely been fulfilled. Many of the results that it was hoped to attain have been achieved in some form or another. Moreover, the work on the Docteurs van Leeuwen collection from Salajar Island has supplied the unexpected result of the possible discovery of copper anomalies on the island.

The first aim of the project was to predict geology in various regions of Southeast Asia. Predictions were indeed made (although they will need confirmation by _in situ_ inspection) and were as follows:

(i) Areas of possibly anomalous nickel, copper and zinc concentrations in the substrate were delineated as a result of the data obtained during the routine biogeochemical survey involving seventy-five Malesian species (chiefly from the families Flacourtiaceae and Violaceae).

(ii) It was discovered that elemental analysis of _Rinorea bengalensis_ could be used to differentiate the substrates that the specimens had been collected from and it was even possible to predict the presence of ultrabasics in areas where such rocks were not known previously (Nabire and Sorong in West Irian).

(iii) With the aid of _Planchonella oxyedra_ it was possible to predict the presence of previously unrecorded ultrabasic rocks on Ambon Island (South Moluccas).

(iv) Analysis of the Docteus van Leeuwen collection from Salajar led to the prediction that the island might contain copper anomalies in rocks of specific localities.

The discovery of ultrabasics was the second aim of the project and as just mentioned, three specimens that were collected from areas of doubtful geology yielded results suggesting that they were collected off ultrabasics.

It was hoped this study would lead to the discovery of new metal accumulating plant species, particularly hyperaccumulators of nickel (Brooks _et al_., 1977). Not only did this project bring to light five previously unknown hyperaccumulators of nickel (Brooks _et al_., 1977) but it also led to the discovery of three hyperaccumulators of copper. The hyperaccumulators of nickel are _Rinorea bengalensis_ Wall. (O.K.), _R. javanica_ (Blume) O.K., _Myristica laurifolia_ Spruce ex DC var. _bifurcata_, _Planchonella oxyedra_ Dubard and _Trichospermum kjellbergii_

Burret. The hyperaccumulators of copper are Coleus atropurpureus Benth, Cyathula prostrata Blume and Laportea ruderalis Gaud.

Biogeochemical prospecting using herbarium specimens has therefore proved successful in a region that is botanically well surveyed but poorly surveyed geologically. Hopefully in the years to come the technique will fulfill its potential as a useful biogeochemical method and will prove equally successful in some other region - (e.g. South America.)

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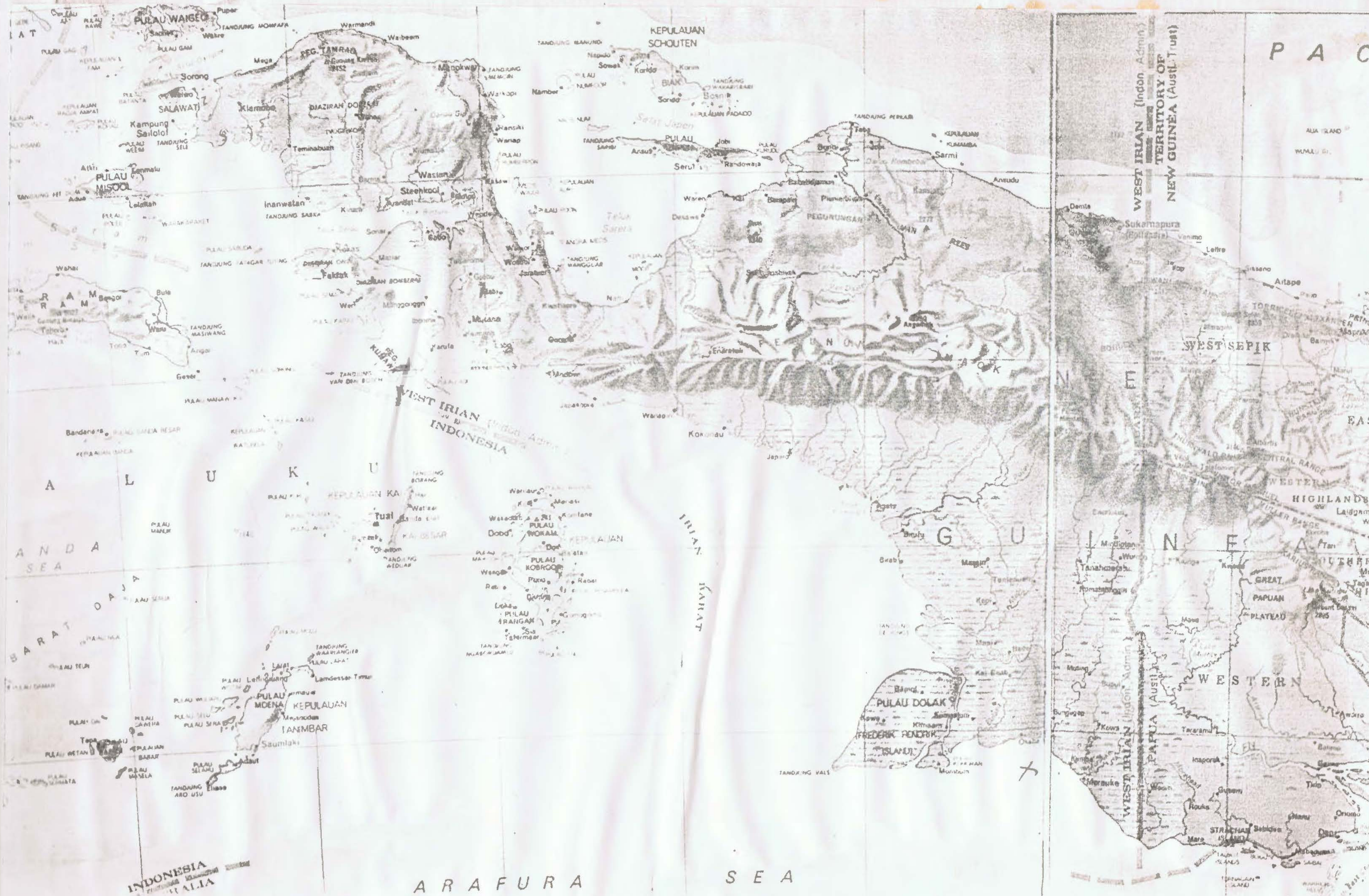

PULAU WAIGEO
Sorong
SALAWATI
Kampung Sailolof
PULAU MISOOL
KEPULAUAN SCHOUTEN
BIAK
Manokwari
Steenkool
Wasior
Inanwatan
Fakfak
Kaimana
Sarmi
Sukarnapura
WEST IRIAN (Indon. Admin.)
TERRITORY OF NEW GUINEA (Austl. Trust)
WEST SEPIK
Aitape
HIGHLANDS
GREAT PAPUAN PLATEAU
WESTERN
PAPUA (Austl.)
PULAU DOLAK
FREDERIK HENDRIK ISLAND
Merauke
KEPULAUAN KAI
Tual
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Saumlaki
KEPULAUAN ARU
WEST IRIAN
INDONESIA
IRIAN BARAT
ARAFURA SEA
BANDA SEA
NEW GUINEA
MALUKU
PAC

WEST IRIAN