STUDIES OF ARSENIC, COPPER AND LEAD IN THE SOILS OF THE TAMAR VALLEY

Stephen John Haswell, B.Sc.

A thesis submitted to the Council for National Academic Awards in part fulfilment of the requirements for admittance to the Degree of:

DOCTOR OF PHILOSOPHY

Plymouth Polytechnic,
Department of Environmental Sciences,
Drake Circus,
Plymouth,
Devon. PL4 8AA

In collaboration with:

Ministry of Agriculture, Fisheries & Food,
Agricultural Development & Advisory Service,
Staplake Mount,
Starcross,
Exeter. EX6 8PE

Applied Geochemistry Research Group,
Imperial College,
London SW7.

September 1983

ACKNOWLEDGEMENTS

I am grateful and indebted to my supervisors, Professor K.C.C. Bancroft and Dr. P. O'Neill, for their guidance and encouragement throughout my work and in the presentation of this thesis.

I thank the many colleagues and associates that have helped me, in particular Dr. L. Brown for his help and guidance, Mr. I. Doidge for his invaluable geographical knowledge of the sampling area, Joanna Eddy for her microbiological assays, and Mr. A. Hopkins for his electronic wizardry.

I gratefully acknowledge the receipt of funding from the Devon Education Authority.

Finally, I thank my wife, Olenka, for her patience and support through the period of this work, and for the typed presentation of this thesis.

RADIX SANITATIS TELLUS

(The root of man's well-being lies in the soil)

STUDIES OF ARSENIC, COPPER AND LEAD IN THE SOILS OF THE TAMAR VALLEY

Stephen John Haswell

ABSTRACT

A more detailed soil survey than had previously been carried out in the Tamar Valley area of South West England confirmed higher than normal background levels of arsenic, copper and lead. These elevated background levels are attributed to the weathering of mineralized bed rock. Anomalous values were found directly above mineralized veins and in areas of past mining activity. Effects of aerial pollution are indicated by the combination of sodium dithionite and nitric acid extraction techniques, from which elemental association with secondary iron and sulphide minerals may be determined. Enhanced levels resulting from aerial pollution were found in a zone a few hundred metres around mine sites. This work has investigated elemental associations in soils. Using water, acetic acid, EDTA and sodium dithionite extraction methods, soluble copper and lead were found to be associated with exchangeable weak inorganic complexes, whilst arsenic is associated with secondary iron. When selecting a single extraction technique for the quantification of potentially soluble copper and lead EDTA is recommended, whereas for arsenic sodium dithionite should be used. The three elements follow the order arsenic > copper > lead for complexation with inorganics and secondary iron, whilst lead > copper > arsenic for organic associations. An HPLC/UV/GFAAS interface was constructed and used to separate and detect organo-copper and -lead species in soil pore waters. Up to 90% of the soluble copper and lead was found associated with low molecular weight polar dissolved organic compounds, especially citric and malic acids. Arsenic species were separated and detected on a HPLC/FAAS hydride generation interface. In aerobic soils arsenate was the predominant soluble species in the pore water. Where mineralization and mining activities have elevated arsenic levels arsenite and monomethylarsonic acid were found. In anaerobic soils the arsenite species predominates.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

1.1 The Tamar Valley

The River Tamar flows over and through various geological and geographical features from its source some 60 kilometres north of Plymouth (Figs. 1.1, 1.2, 1.3). This project is based on a study area of approximately 30 square kilometres, where the river flows through a steeply sloping valley (Fig. 1.2). Today there is extensive market gardening and afforestation in the area, but historically it was a site of intensive mining for metalliferous minerals (1).

The Upper Devonian sediments and Lower Carboniferous rocks underlying most of the area have been thermally metamorphosed by the granitic batholithic intrusions of Bodmin Moor and Dartmoor (Fig. 1.3). Metalliferous deposits or lodes have fracture-filled the country rock in parallel belts of generally east-west orientation (Fig. 1.4). These initial deposits were followed later by north-south intrusions (Fig. 1.4) as fold relaxation occurred (2). The sequence of mineral deposition is controlled by (a) the magma temperature and pressure, and (b) the crystallization temperature of individual minerals. The resulting lode deposition sequence produced zones of mineralization with arsenic deposited in the hotter inner regions, followed by copper and finally lead mineralization in the cooler outer zones (1). Of the 205 reported arsenic minerals (3) Arsenopyrite, FeAsS, or mispickel was the only economic form of arsenic to be extracted in South West England (1). Mispickel contains up to 46% arsenic and is generally found in lodes with east-west trends. Arsenopyrite was usually processed on site, the ore being crushed, roasted, and the vapours condensed as arsenic oxides, As_2O_3, As_2O_5, on the walls of flues; this process yielding a 99% pure compound. The ruined remains of these processes are evident at Gawton and Devon Great Consols mines (Fig. 1.4) where flues, chimneys and spoil tips are still present. Copper forms some 225 minerals (4), but only chalcopyrite, $CuFeS_2$, was extracted in the south west (1). The ores were smelted in South Wales after concentration on site by various gravity separation methods. Lead, which forms 235 minerals (5), was extracted principally as galena, PbS, which contained small quantities of silver. The lead/silver lodes were deposited in a north-south trend

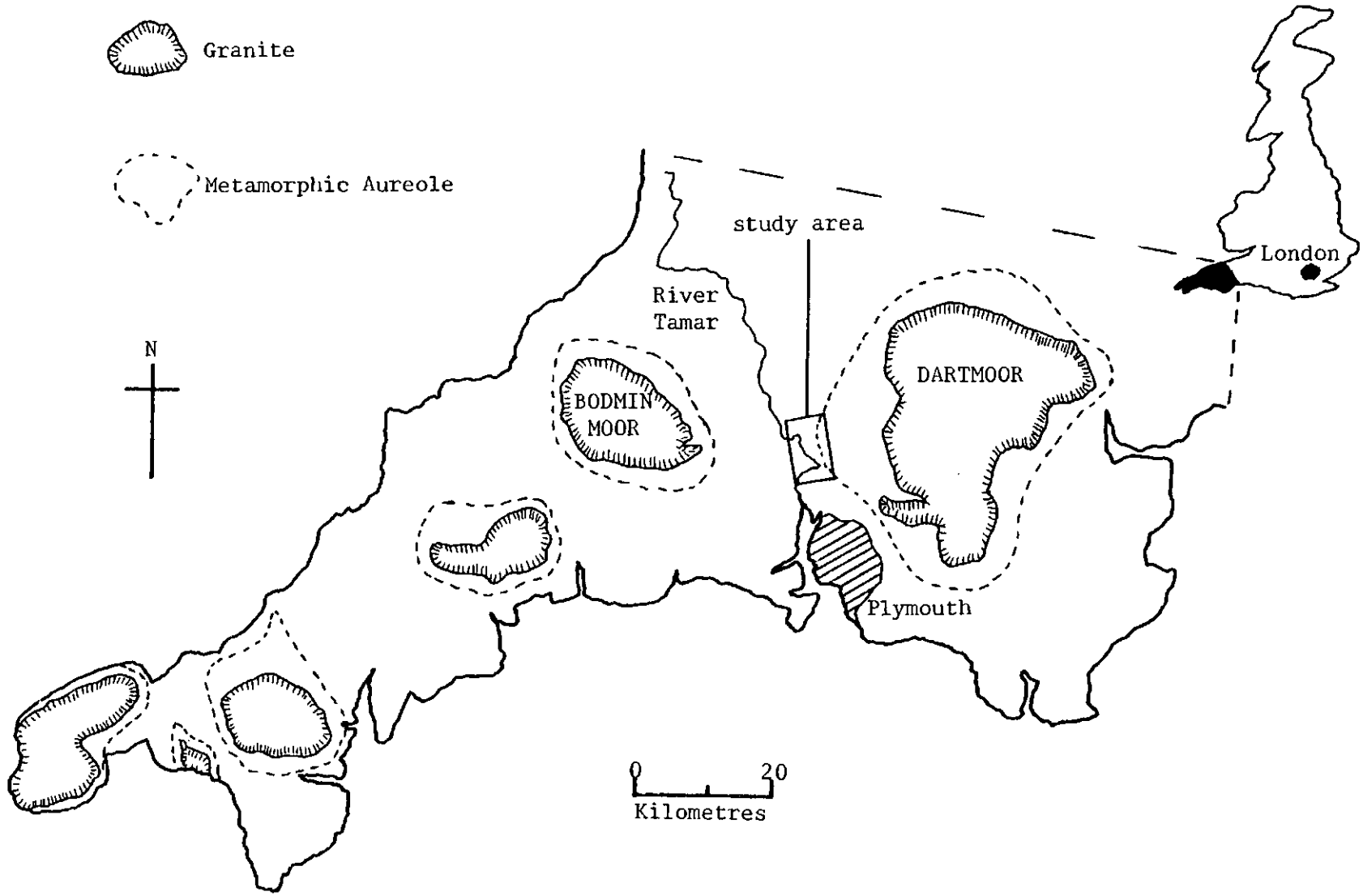


Fig. 1.1

The main granitic intrusions of South West England

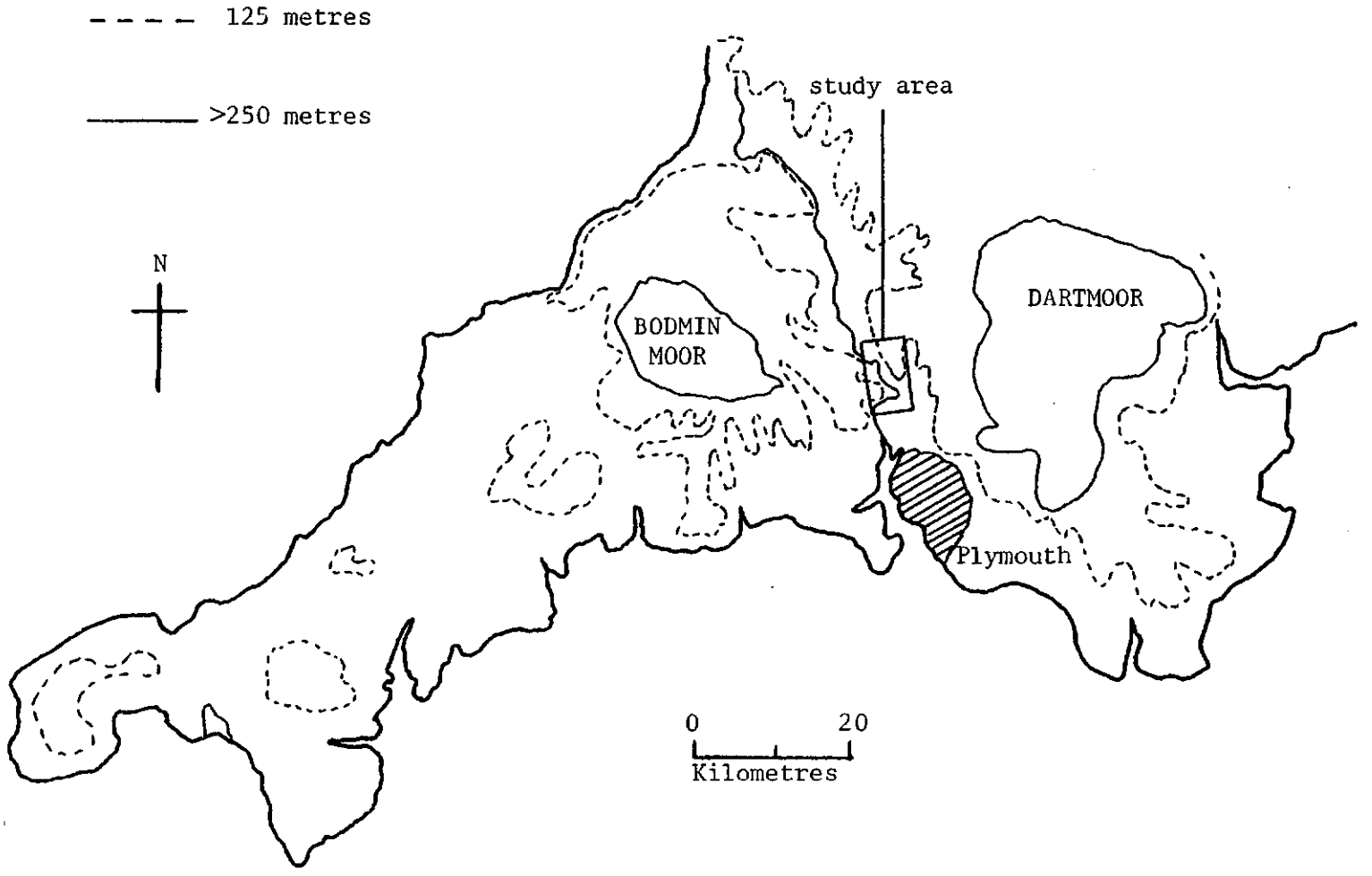


Fig. 1.2

The main topographic features of South West England

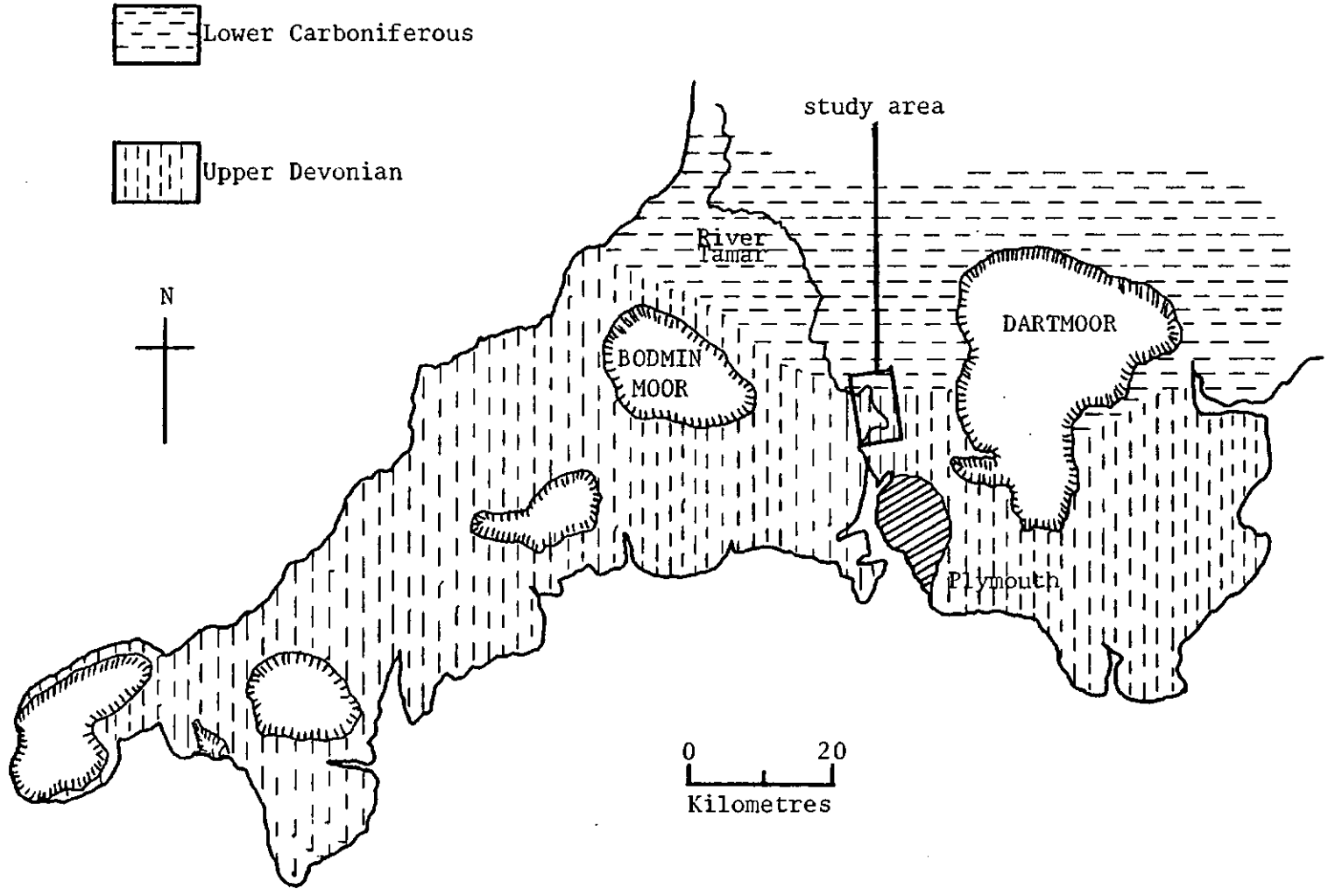


Fig. 1.3

The geology of South West England

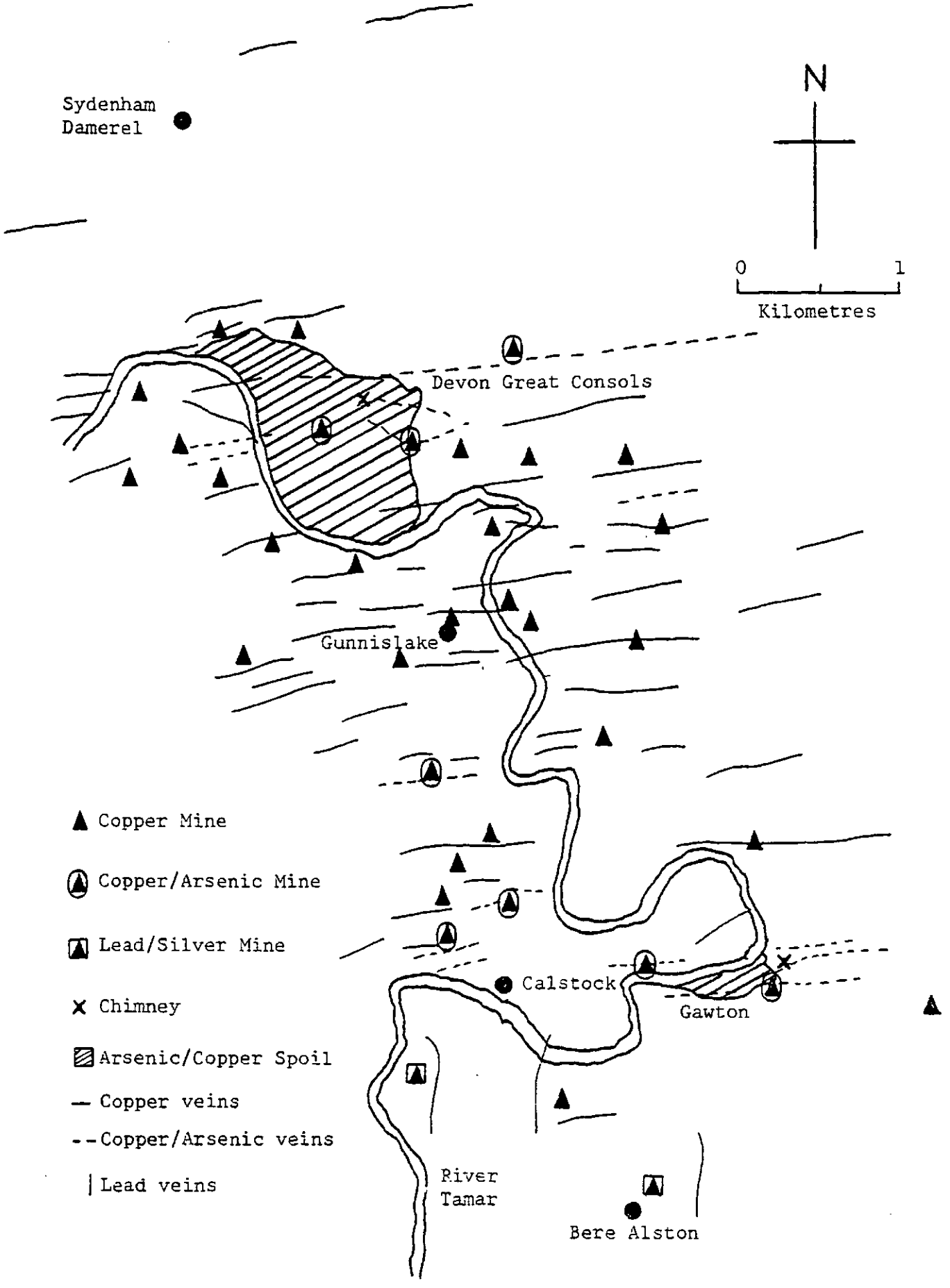


Fig. 1.4

The metalliferous deposits of the Tamar Valley study area

at a later time than the arsenic and copper. The lead, extracted since Roman times, was often smelted on site (6).

The soils reflect the geological, topographical and climatic conditions of the area, being mainly typical brown earths of the Highweek and Tavistock soil series (7) renamed the Denbigh and Trusham series (Fig. 1.5). Generally, the soils are shallow, having a rock dominant horizon at approximately 50cm, and are interspersed with small patches of gleyed brown earths of the Ivybridge series. Typical podzolic soils of the Dartington series, renamed the Manod series, dominate the steep valley slopes where gleying is also evident. The valley floor consists of pockets of Conway, Yeolland Park and Exe (renamed the Wharfe) series soils (Fig. 1.6). The mineralization of the area has led to naturally elevated levels of some non-ferrous metals in the soils with further local enhancement of these levels due to the various mining and extractive activities which have occurred in historical times (8-18).

1.2 Elemental mobility in soils

The ease or relative mobility with which elements move in soils can be divided into various chemical and physical processes. The major environmental factors controlling mobility in the aqueous phase are acidity (pH) and redox (Eh) conditions. Levinson (19) and Garrels and Christ (20) give detailed accounts on the construction and applications of Eh-pH diagrams and their importance to elemental mobility. Associated with Eh and pH is the adsorption of metals by the hydrous oxides of iron and manganese, this mechanism is again fully discussed by Levinson (19). In addition to the variables mentioned above, mobility is also related to (i) the presence and activity of micro-organisms, (ii) the solubility of compounds and complexes which metals form with anions and organic matter, (iii) the presence of dissolved gases such as CO_2 and O_2, and (iv) the mechanical factors such as permeability, porosity, grain size, soil water and ground water movements.

The relative mobility and speciation of soluble elements in soils, together with the solid/liquid equilibria, are important factors in the mechanisms of elemental plant uptake (21, 22).

Fig. 1.5

The soils of the Tamar Valley study area

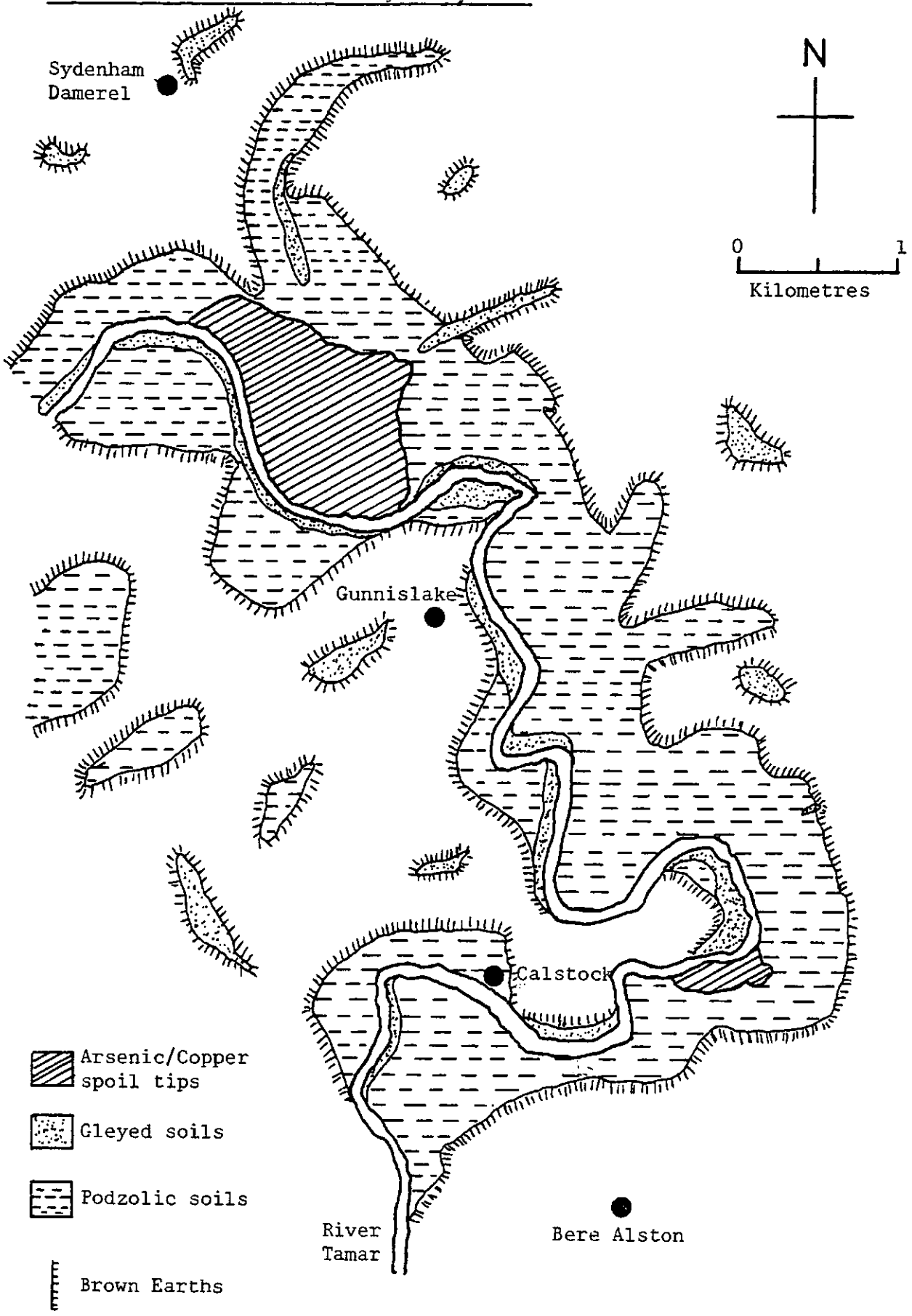
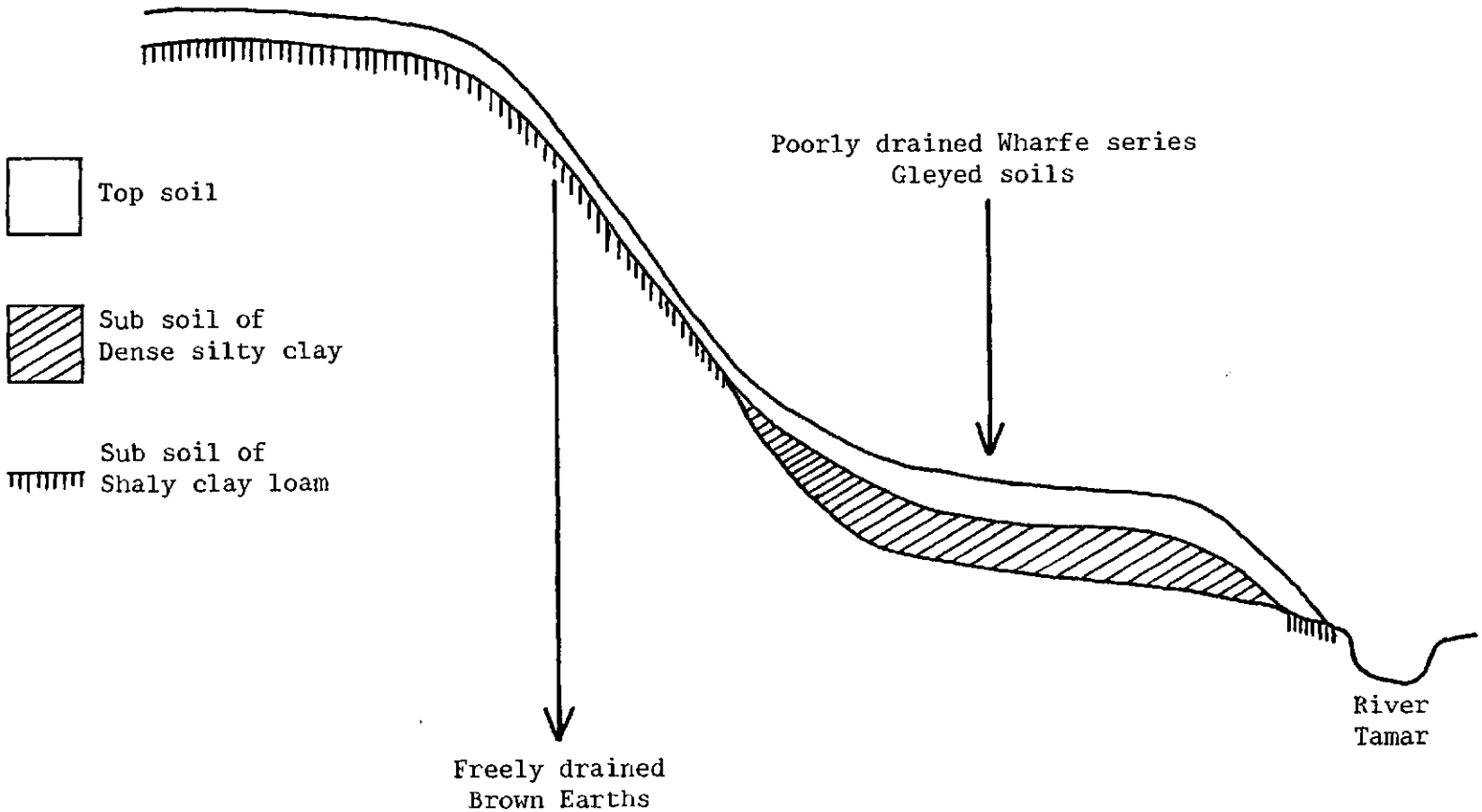


Fig. 1.6

Topography and soil types found in the study area



1.3 Determination of elements associated with soil phases

The numerous phases present in soils for elemental retention such as weakly and strongly sorbed ions, metal carbonates, sulphides, sulphates, oxides, hydroxides and organometallic compounds, makes determination of elements in individual phases difficult. The problem is further complicated by the fact that many phases are not crystalline, but are amorphous or adsorbed surface layers (23-27). Rather than attempting to characterize each soil phase, the relative extractability of various reagents has been defined as the type of chemical leaching necessary to liberate a fraction of a particular element. Using this approach soils and their associated trace elements can be characterized by a comparative degree of susceptibility to chemical conditions rather than by the relative amounts of specific elemental forms within each phase. Many chemical reagents have been used for the extraction of elements, especially heavy metals, from specific soil fractions such as organic matter, clays, silicates and sesquioxides (28-34). The purpose of these techniques has been to identify the distribution of elements with reference to solubility, mobility, availability, exchangeable, residual and total levels. Commonly these terms are ambiguous and the inter comparison of methods is not feasable, however, general trends are seen to exist.

1.4 Determination of soil water soluble/dissolved fractions

Few authors (35-40) determine the soluble or dissolved elemental concentrations in soils as the relative concentration of the dissolved fraction compared to exchangeable or total levels. Commonly most techniques are based on dry soils. Soil solution concentrations and elemental speciation are of interest in the study of mobilization and plant availability mechanisms, and are thus relevant to such problems as plant uptake selectivity, functional metabolic role and toxicity patterns of elements (35). Two methods at present used to extract pore waters directly are (a) low pressure squeezing (35), and (b) centrifugation (36-40). Pressure squeeze techniques require presoaking of soils for 24 hours prior to extraction, this will however alter redox potentials (19), introduce contamination into the samples (36), and allow microbial reduction to occur (19, 41), all undesirable in

speciation studies. The sample yield by centrifugation will be dependent on water content and soil structure. Fluorocarbon solvent extraction methods have been demonstrated to achieve substantially increased yield on sand type soils (36).

Centrifugation was considered to offer a rapid and simple method of obtaining soil pore waters on site. These samples may be preserved for soluble speciation analysis by immediate filtration (0.45 μm filter).

1.5 Determination of available and exchangeable elemental levels in soils

Table 1.1 summarizes many of the reagents which have been used for the determination of elements associated in available or exchangeable forms. These terms are often ill-defined and as can be seen from Table 1.1, 1M ammonium acetate is used for both available (30, 42-44) and exchangeable extractions (39, 45, 46). The two most common reagents used for available determinations are EDTA (31, 32, 42, 43, 45, 47, 48) and acetic acid (42, 47, 48) with the range of elements covered by these extractants being extensive. Comparisons carried out on extraction methods for plant available forms indicate 0.05M EDTA pH7 (42, 43) is the most representative, with the pH and EDTA concentration being critical (32, 45). Individual reagents for specific elements have been reported (47, 48), but these do not appear to be significantly different from those for the EDTA methods. Determination of exchangeable forms is achieved most successfully with 1M ammonium acetate (39, 40, 45, 49, 50). Where concentrations of various elements are low, solvent extraction techniques may be incorporated as a concentration step (30, 47, 51, 52).

1.6 Determination of total elemental levels in soils

Table 1.2 summarizes the various reagents used to determine total elemental concentrations in soils. Single acids and various mixtures are reported to extract 95% $\pm$ 10% of most elements. Comparative studies by independant workers (10, 55-57) gave close agreement of extraction efficiencies (36, 55-59), indicating the data obtained for soil

Table 1.1

Available and exchangeable extraction methods for elements from soil matrices

Available

Reagents	Elements	Ref.
0.05M EDTA (pH7)	Cu, Zn, Mn, Pb, Fe, Mo	30-32, 41, 47, 69
(pH4)	Cu	48
0.02M EDTA	Cu	43
0.02M EDTA + 5% ammonium chloride	Cu	43
1M ammonium chloride	As, Fe	37, 53
2.5% acetic acid (pH7)	Cu, Zn, Mn, Co, Pb	42, 47, 54
5% acetic acid	Pb, Zn, Cu, Cd	6
0.5M acetic acid	Zn	48
0.005 DTPA + 0.1M calcium chloride (pH 7.3)	Zn, Fe, Mn, Cu, Pb, Zn, Fe, Mn	42, 47
1M ammonium chloride	As	37
1M ammonium nitrate	Mg	48
1M ammonium acetate	Pb, Cu, Cd, Co, Mg, Ni, Pb, Zn, Mo	30, 42-44
0.5M HCl	Al, Fe, Mn, Cu, Co, Ni	69
0.1M HCl	Fe, As, Cd, Cu, Pb, Zn	57

Exchangeable

1M ammonium acetate	Zn, Cu, Cr, Hg, Fe, Ni, Pb	39, 45, 46, 50
1M ammonium acetate + 0.5M magnesium acetate	Zn, Pb, Cu, Cd, Mn, Fe	49
0.05M calcium chloride	Cu	29

Table 1.2

Total extraction of elements from soil matrices

Reagents	Elements	Ref.
Perchloric	Cd, Cu, Pb, Zn, As, Ni	10, 43, 44, 74
Hydrofluoric	Cu	29
Nitric	Cu, Ni, Cd, Zn, Pb, As	18, 42, 56-60
Nitric + ammonium bromide	Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Zn	60, 74, 75
Hydrochloric sealed tube	As, Sb, Bi	61, 62
Hydrochloric	As	59, 63
Perchloric/Hydrofluoric	-	47, 69
Perchloric/Nitric	Cu, Pb, Zn, Ni, Cd, Cr	56, 58, 61, 69, 76
Perchloric/Sulphuric	As	77
Nitric/Hydrofluoric	Cu, Pb	55, 72
Nitric/Sulphuric	As, Al, Fe, Zn, Cr, Cu, Pb, Ni	56, 58, 78, 79
Nitric/Hydrochloric	Pb	42, 55
Slurries	Cr, Cu, Mn, Zn, Ni, Pb, As	49, 66, 67
Ammonium oxalate	-	43

properties and specific elemental content is of significance when using these techniques.

It should be noted that Karumanos (42) showed that 1M nitric acid could extract 98% lead from one soil type, whereas 6M nitric acid was required for other soils. Other authors have reported that the extraction efficiencies of reagents varies with the elements being investigated; thus Luoma (57) showed that hot concentrate nitric acid extracted 100% lead, copper and zinc from one soil type studied, whilst Bradley (60) used the same procedure to extract 80% lead, 60-80% copper and 100% zinc from different soils.

Total arsenic extraction by hydrochloric acid is favoured by many authors (58, 61-63) prior to hydride generation. Interferences with hydride formation by other acids have been reported (64, 65), but are considered of minor importance. In contrast to the acid digestion methods direct slurry analysis has been reported to be a comparable technique (58, 66, 67), being both simple, cheap and rapid.

1.7 Sequential extraction methods for elemental associations in soils

The principle of sequential soil analysis was described by Jackson (68) as part of his procedures for the dispersion of soil minerals. Various authors (28, 29, 33, 34, 46, 49, 50, 69) have used sequential extraction to quantify elemental levels associated with various defined soil fractions or sites. Exchangeable and total extraction methods have been discussed previously. Organic fractions are reported to be extracted fully by hydrogen peroxide (29, 49, 50, 70), and pyrophosphate (34, 46, 70) methods. The role played by iron and manganese oxides as scavengers of heavy metals has led to various extraction techniques specific for these oxides (28, 33, 71-73). Hydrous oxides of iron and manganese are ubiquitous in soils as coatings on other materials and as colloidal particles, and their strong chemical influence as scavengers is far out of proportion to their concentration (33). Table 1.3 shows typical methods used in fractioning iron and manganese forms. None of these methods are reported to remove pyrite or other sulphide mineral forms (72). Comparative studies indicate oxalate extractions are similar to the 0.25M hydroxylamine hydrochloride method (33), for the

Table 1.3

Extraction reagents used for specific iron and manganese extractions

Mn oxides	0.1M hydroxylamine hydrochloride	39, 45, 46, 50
	0.1M hydroxylamine hydrochloride in 0.01M nitric acid	43
Fe and Mn oxides	0.1M hydroxylamine hydrochloride and acetic acid	34
Amorphous Fe oxides	0.25M hydroxylamine hydrochloride	43, 49, 57
	Ammonium oxalate	28, 43, 57, 68, 75
Crystaline Fe	Sodium dithionite	29, 33, 73
	Sodium dithionite (Citrate Buffer)	43
	Citrate - dithionite - bicarbonate	28
Free Fe	5% sodium hydrosulphite in 0.15M sodium citrate	68, 72
	0.05M calcium chloride	71

determination of amorphous iron oxides. The results of these extraction methods may find use in indicating metal anomalies that are not related to mineralization, but are caused by either the scavenging action of iron and manganese oxides or the anthropogenic inputs of elements in their oxide forms.

1.8 Sites associated with arsenic, copper and lead in soils

Arsenic, copper and lead in the soil occurs either naturally through weathering of rock effected by mineralization or by anthropogenic inputs (6, 80). The demand for arsenical pesticides notably monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) has resulted in significant accumulations of arsenic in some soils (81-88). The predominant mechanism for arsenic retention or fixation in soils (> 90%) is sorption associated with clays (77, 89), and/or sequioxides (28, 41, 90) (Table 1.4). The addition of phosphorous to soils has been shown to (i) have no effect on arsenic sorption (91), (ii) cause enhancement (92), or (iii) produce suppression (93, 94). The mechanisms by which these various effects on solubility of arsenic levels may occur do not appear to have been explained satisfactorily. Copper is adsorbed by manganese oxides (29, 54), iron oxides (95, 96), organic matter (29, 97), and clay minerals (98, 99). McLaren and Crawford (29) have shown sorption onto soil constituents follows the order, manganese oxides > organic matter > iron oxides > clay minerals. Accumulation of copper in upper organic horizons of soil profiles is significant where surface humus has developed (100, 101). The highest concentrations of lead in the soil profiles generally occur in the surface horizons (11, 102-104), probably due to biological cycling and/or atmospheric fallout (105). Dust from mine spoil tips has been shown to contaminate surface soils (9, 11, 106, 107). Adsorption of lead onto manganese and iron oxides (108, 109), and enrichment in the soil clay fraction (71, 109-111) have been reported. The role of organic matter is considered to be the principal mechanism in lead fixation in soils (71, 111) due to the high stability constant of the Pb^{2+} ion with chelating groups (112). Investigation into the association of lead with humic (113) and fulvic acids (114-119) have confirmed the stability of these complexes which are pH and ionic strength dependent (113).

Table 1.4

Correlation of the stability partition coefficients of arsenic onto various sediments and soils (90)

K_S	Arsenite	Arsenate	Monomethylarsonic acid
1×10^5	Ferric Oxide	Activated Alumina	
1×10^4		Ferric Oxide Aluminum Oxide	Ferric Oxide
1×10^3	Activated Alumina Activated Bauxite Illite	Activated Carbon Octadecylamine-Sand Mixture	Octadecylamine-Sand Mixture
1×10^2	Dodecanethiol-Sand Mixture Montmorillonite	Kaolinite (ph5-7) Illite (pH5-9) Montmorillonite (pH5-9)	Activated Carbon Sand-Red Clay Mixture Red River Clay; Mississippi Clay Illite; West Baton Rouge Soil
1×10^1	Kaolonite		Alexandria Sand; Montmorillonite; Kaolinite; Thompson Creek Sand; Fine Sand
1×10^0	Mason Sand	Kaolinite (pH9)	
	No sorption observed	No sorption observed	No sorption observed
	Stearic Acid-Sand Mixture Octadecylamine-Sand Mixture	Mason Sand Stearic Acid-Sand Mixture Dodecanethiol-Sand Mixture	Stearic Acid-Sand Mixture Dodecanethiol-Sand Mixture

K_S = stability partition coefficient

1.9 Organo-complexes of arsenic in soil solutions and aqueous systems

The speciation of trace elements in natural waters is important in the processes of dissolution, accumulation and their retention in the aqueous phase, thus effecting the overall mobility and availability of such elements to flora and fauna (120-122).

The chemical species of arsenic present in aqueous media depend on physical and chemical characteristics of the soil, and soil water, e.g. pH, Eh, dissolved oxygen, organics and phosphorus concentrations, clay and sesquioxide abundance. Fig. 1.7 shows an Eh-pH diagram for arsenic (123). Under aerobic conditions arsenate (As(V)) prevails, whereas under anaerobic conditions the predominant aqueous species is arsenite (As(III)). Bohn (124) suggests that the boundaries given in the diagram are less rigid than illustrated due to kinetic hinderance resulting from matrix effects (i.e. organics, phosphorus, etc.), thus arsenate, once it has formed, occupies a larger area than that shown. Porter and Peterson (18) indicate that arsenate predominates in soil water extracts with arsenite representing usually less than 10%.

The biomethylation of inorganic arsenicals and the reduction of alkyl arsenicals by soil microorganisms to form volatile alkylarsines are reported (41, 125-129) (Fig. 1.8). The release of alkylarsines from soils by volatilization is reported by Hiltbold (130) to be 40-60%, whereas a more conservative estimate of 17-35% is given by Sandberg and Allen (131). The microbial activity in soils have been demonstrated to be of significance in the methylation and thus removal of arsenic from the soil system (131-133). Additional losses from surface soils by leaching of inorganic and organic arsenicals into ground and surface waters is most marked for sandy soils (74, 134). The relative solubilities of the species will effect the leaching process with the sulphides being more resistant than the oxide forms (135), however, Tammer (136) reports the weathering of sulphides to the oxide forms may occur.

The separation of inorganic (arsenate, arsenite) and methylated derivatives (monomethylarsonic acid, dimethylarsinic acid and trimethylarsine oxide) are well reported in the literature. The

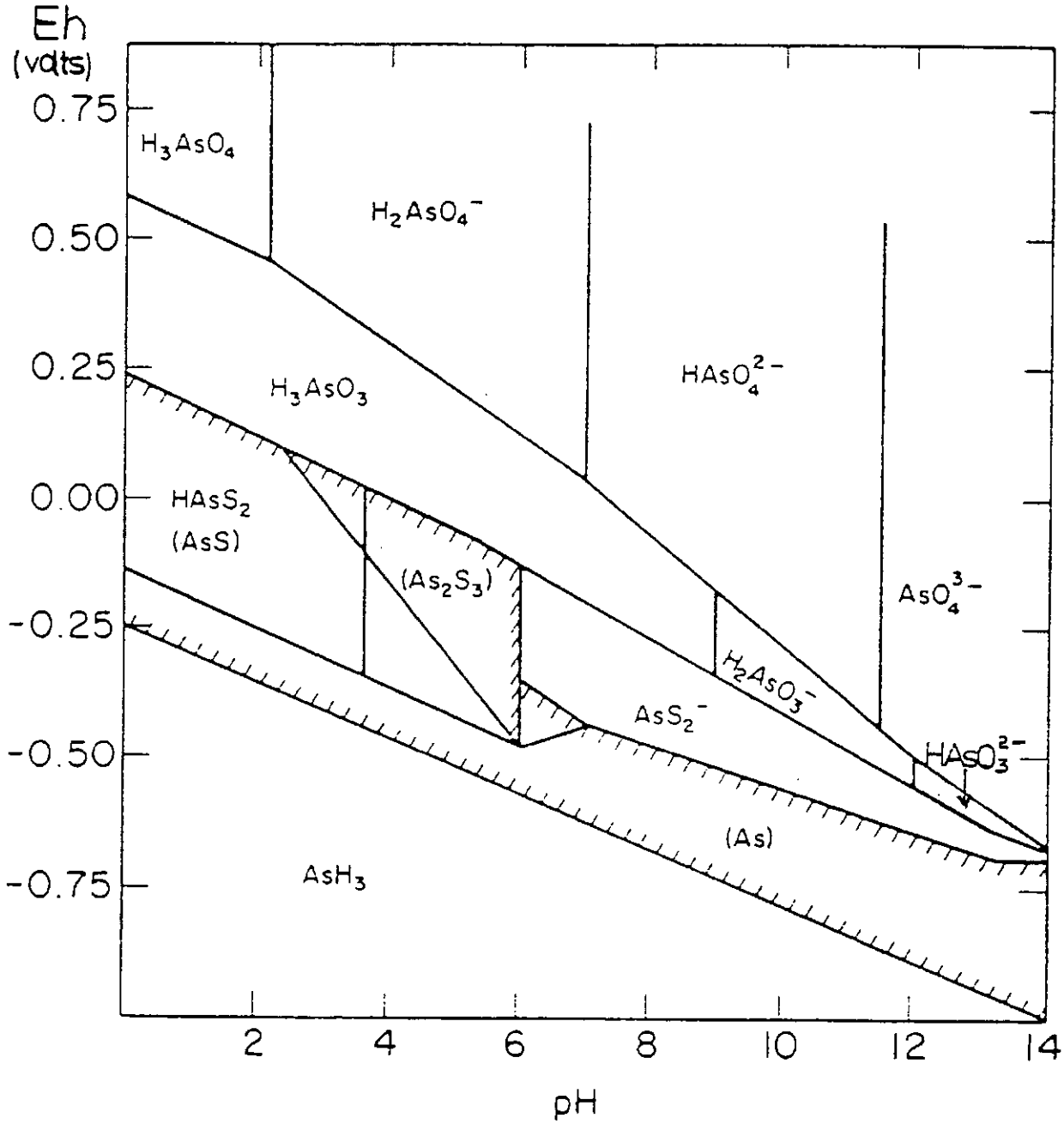
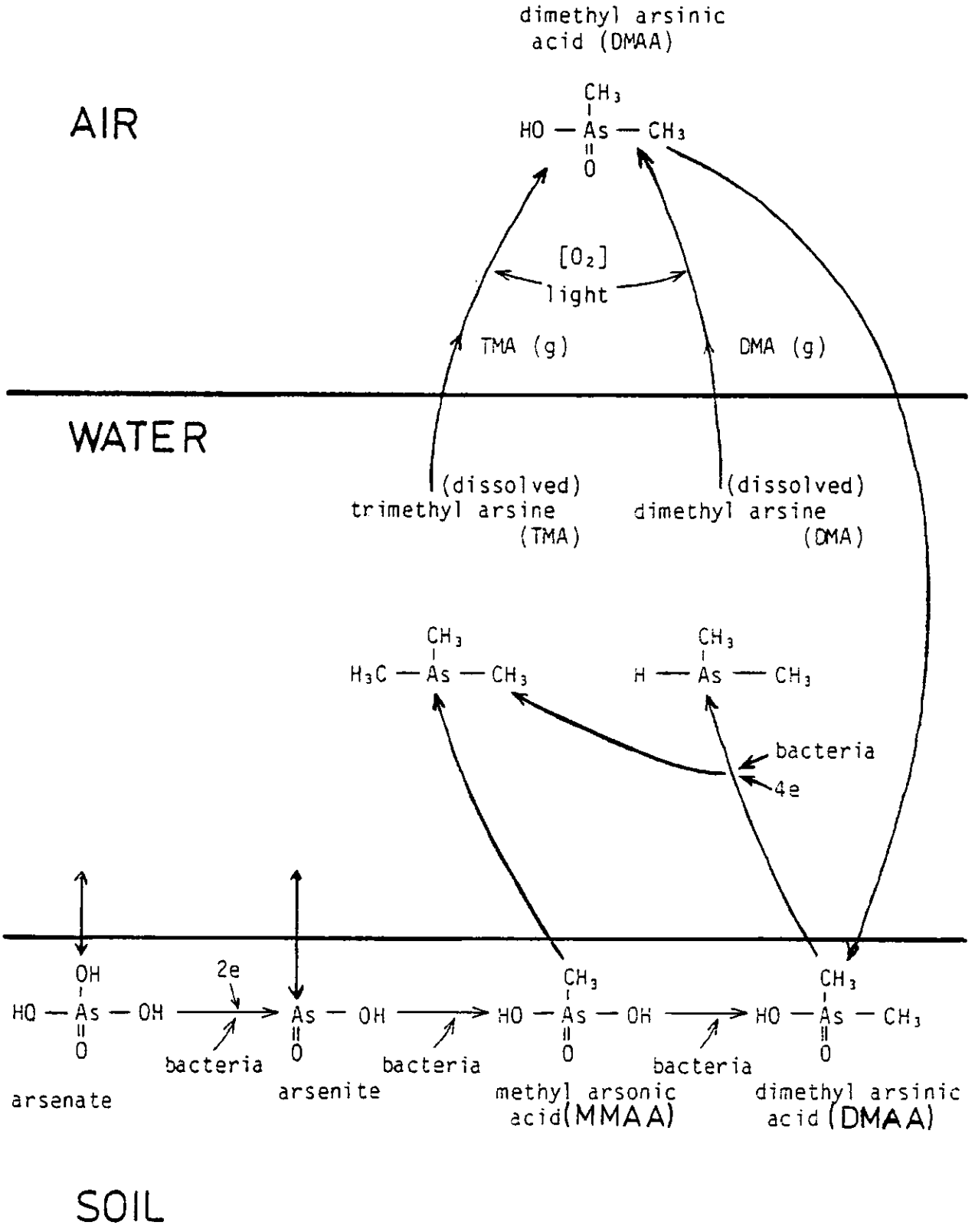


Fig. 1.7

Eh-pH diagram for arsenic at 25°C and 1 atmosphere (123)

Fig. 1.8

Proposed microbiological cycle of arsenic (129)



techniques described include selective reduction (76, 137, 138), cation (37, 38, 85, 139-141), anion (88, 142, 143), and combined ion exchange (144, 145), paper chromatography (146), column chromatography (LC); cation (102), anion (97, 102, 103), and reverse phase (147-149), high-performance liquid chromatography (HPLC); cold trap hydride generation (150-153); gas chromatography (GC) (154-159), and acid/ solvent extractions (144, 160-163).

Methods of detection for arsenic have been extensively reviewed (164-169), these include graphite furnace atomic absorption spectrometry (GFAAS), direct and indirect flame atomic absorption spectrometry (FAAS), colourmetric analysis (162, 170, 171), neutron activation, emission spectrometry and inductively coupled plasma (172, 173), electrometric methods (174-176), x-ray procedures and atomic fluorescence spectrometry. In speciation studies various methods for coupling separation with detection techniques have been investigated. Separation by LC/HPLC has been coupled (i) directly with GFAAS (147, 173, 177, 178), and hydride generation (88) methods using interfaces which allow continuous flow, and (ii) indirectly by fraction collection with subsequent GFAAS or hydride generation analysis (38, 76, 141, 170, 172, 179). Cold trap sequential hydride generation techniques have been described (150-153, 168, 180), these may incorporate the use of silica tubes suspended in the FAAS optical path to increase elemental residence time (38, 88, 174, 181). Typical detection limits for the methods described above are 0.1-1 ng arsenic. In a comparative study by Freeman et al (182), GFAAS and hydride generation methods were found to give similar accuracy and precision, however, interferences with the hydride technique by certain matrices are reported (38, 76, 153, 180-183), but Hinners (153) indicates these may be insignificant with cold trap methods. Analysis of soil samples with LC (38, 141, 184) and HPLC (142, 147, 148, 179) separation coupled GFAAS detection has indicated the presence of arsenate, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA). Takanatsu et al (84) has reported that in addition to arsenate, MMAA and DMAA, two other unidentified organo-arsenic compounds, are present in soil extracts.

1.10 Organo-complexes of copper and lead in soil solutions and aqueous systems

To date studies on organo-copper, -lead complexes in soils and natural waters have been concerned with humic (185-188) and fulvic acids (189-191). Humic and fulvic acids are derived from the leachate of soils and decaying plants. They are usually described as polymeric polyelectrolytes of ill-defined composition (192). Humic acids have been discussed extensively (193-195) and their association with metals including the formation of organo-metallic complexation has been investigated (196-199). Fulvic acids have been isolated by various workers and semi-characterized (200, 201) with their molecular weights estimated to range from 300-2000 with an average of 633 (202). Humic and fulvic acid complexes with copper and lead are reported to have high stability constants (203), these being greater for fulvic complexation (204-206). These highly stable organo complexes are believed to represent over 90% of the available copper in soil systems (207, 208). Detailed information on the chemical nature of the compounds referred to as fulvic acids has not to date been obtained, consequently little is known about the structure of the compounds covered by this definition (209). Various authors (210-216) have shown that the chemistry of dissolved trace metals in aqueous systems is significantly effected by the presence of low molecular weight (< 1000) organic compounds. Bloomfield and Sanders (205) report that the small soluble molecular weight organic compounds have a greater holding capacity for metals whilst Renhold *et al* (217) conclude that amino acids, for example copper-proline complexes, are important in (i) the solubilization of copper in soils, and (ii) promote mobilization in root zone areas, thus effecting plant uptake (218).

To evaluate organo-metallic interactions in aqueous systems it is necessary to know (i) the amount of organic matter present, (ii) the molecular and chemical nature of the dissolved organic compounds, and (iii) some indication of the nature of the organo-metallic interactions. Models based on theoretical and to a less extent experimental data have been used to determine speciation in aquatic systems (219-226). These studies have indicated that speciation calculations based on stability constant data differ significantly from both the concentrations

and species observed under environmental conditions (210, 211-214, 227-230). Such models can, however, indicate the most likely species of copper and lead to be present (210, 229, 231). Little is known about the kinetics of formation of these complexes. They are reported to be labile (211) with equilibration rates between metals and fulvic acids not appreciably slower than for those of conventional aqueous mixing, with the reactions being readily reversible. Pagenkopf (232) states that the life time of a metal complex in a hetrogeneous system is controlled by the rate of complex formation, dissociation and exchange with the solid phase with first order dissociation rate constants for copper chelates estimated to be approximately 2 sec^{-1}.

Often total organic carbon (TOC) values are obtained for aqueous systems, however, the limitations on information with respect to the fraction of the TOC which occurs in particulate form rather than in the dissolved fraction have been illustrated (227). Attempts to obtain information on the dissolved organic matter (DOM), namely that which will pass through a 0.45 μm filter pore size, and particulate organic matter have been made (227, 233, 234). Physiochemical methods have been used to fractionate trace metal species in aqueous systems into their charge, size, stability or structional characteristics, these have included ultrafiltration (235-239) differential spectrophotometry (238), dialysis (239, 240), ion exchange (241, 242), gel chromatography (45, 228, 243), solvent extraction (244-246), ion-selective electrodes (ISE) (247-250), and anodic stripping voltammetry (ASV) (244, 251-253).

ASV has been the most popular technique used in metal speciation studies for elements such as copper, lead, cadmium and zinc, and has been extensively reviewed (244, 251-253). Problems with the technique have been reported from surface active compounds such as humic, fulvic acids and colloids effecting metal adsorption onto the mercury electrode (254-256), and signal enhancement resulting from tensammetric waves (257, 258). In addition it is necessary to (i) control the ionic strength of a sample by either adding a buffer solution or bubbling carbon dioxide through it, and (ii) remove dissolved oxygen from the system by bubbling nitrogen and/or carbon dioxide through the sample. ASV has been useful in obtaining information on the relative amounts of labile metals present in an aqueous sample often referred to as the

bioavailable forms (248, 259). Total metal levels are obtained by such a system after sample treatment with acid (260) or ultraviolet irradiation (245), however, detailed identification of organic compounds associated with the labile metals have not been obtained by this technique.

Ion exchange separation of very labile, moderately labile, slowly labile and inert fractions have been reported (231). These relative ambiguous divisions indicate little of the chemical nature of the complexed metals, however, Gamble et al (241) indicates that copper-fulvic acid complexes are based on carboxylic group interactions.

Ion selective electrodes (ISE) have been widely used in titration studies on the metal organic complexing ability, and for obtaining stability constant data (247-249). The complex matrix effects experienced with environmental samples have resulted in various reports of non-Nernstain responses when an ISE has been used for metal determinations (261-263).

The methods indicated and discussed above currently used in organo-metallic speciation studies suffer from at least one of the following problems (a) poor sensitivity, (b) adsorption of metal or organic matter onto working surfaces, (c) lengthy, repetitive procedures, (d) inclusion of complexed metal in results for uncomplexed metal, (e) disruption of equilibrium conditions, (f) restriction to only a few metal elements, and (g) restriction on working pH often somewhat different from those of the raw sample. The use of reverse phase chromatography (264-266) for the separation of polar dissolved organic compounds (PDOCs) suggests that with suitable specific detectors many of the restrictions of other methods indicated above can be eliminated or minimized. Lee (266) reports the separation of dissolved organic compounds into various size fractions and indicates that polar low molecular weight fulvic type compounds are significant in copper complexation. Mills and Quinn (264, 265) suggest that 50-70% copper in sea water is associated with dissolved organic carbon of molecular weights < 1000, and that the nature of the exchange sites undergo rapid cycling, indicating the complexity of the kinetic reactions between copper and organic binding sites. Copper analysis in the

above sited work (164-166) was achieved by fraction collection of post column aliquots, with subsequent detection by graphite furnace atomic absorption spectroscopy (GFAAS). The results obtained from these studies (264-266) suggest that PDOCs are of greater importance in the complexation of labile metals than humic acids in the soluble phase of soil waters (267). Tetraalkyl forms of lead have been studied using coupled GC/FAAS (178) and HPLC/GFAAS (268) to separate and detect tetra and trialkyl lead species, however, these compounds are rapidly degraded in the environment (167, 269). Brinckman et al (149). used HPLC/GFAAS to separate and detect mixtures of organo-metallic compounds containing hexaphenyldilead. The methylation of lead in soils (270), and leaching in acid soils (271) are reported to be insignificant in lead removal from the system.

1.11 The presence of polar dissolved organic compounds in soils

The film of capillary water within soil aggregates and on the surface of soil particles contains relatively high concentrations of organic acids (272) associated with the proliferation of microorganisms around roots due to the exudation of substrates (273-275). The complexation capacity of these low molecular weight acids increases with the presence of -OH, $-NH_2$, and additional -COOH functional groups in the order shown in Table 1.5, thus citric > malic > lactic > formic acid in complexation ability. The presence of these acids in the rhizosphere and leaf litter of soils (276-281) has been associated with bacteria, fungi, algae and lichens, which are reported to release such compounds (Table 1.6). Species of particular importance are bacteria of the genus Pseudomonas and Bacillus, and fungi of the genus Asperigillus and Penicillium (267, 282, 283). Suggestions are that many of these acids are associated with the Krebs cycle (284) and that often the build-up of various compounds such as malic acid occurs when the cycle is blocked by inhibitors such as arsenic (285). Published concentrations for these acids in soil pore waters are few, Putilina and Varentsor (286) report concentrations for citric, oxalic, formic, lactic and malic acids in the range 0.01-10 mg l^{-1}, whereas more specific values for citric 1.6-2.7 mg l^{-1}, malic 0.2-4.8 mg l^{-1}, lactic 1.4-5.9 mg l^{-1} and formic 0.3 μg l^{-1} are given by Bonneau and Souchier (287) and Robert and Rozzague-Karimi (288).

Table 1.5

Organic acids - classification by functional groups (267)

Type	General formula	Acids investigated in this study
Carboxylic acids	$R-COOH$	Formic, acetic, propionic, butyric, oleic, benzoic
α-amino acids	$R_2-C(R_1)(NH_2)-COOH$	Aspartic
α-hydroxy acids	$R-CH(OH)-COOH$	Glycolic, lactic, gluconic, ascorbic
β-hydroxy acids	$R-CH(OH)-CH_2-COOH$	Salicylic, glucuronic, galacturonic
α-keto acids	$R-C(=O)-COOH$	Pyruvic, 2 ketogluconic
Dicarboxylic acids	$R(COOH)_2$	Oxalic, malonic, succinic, glutaric, fumaric, phthalic
Polycarboxylic hydroxy acids	$R_1-C(OH)(COOH)-C(R_2)(R_3)-COOH$	Citric, malic, tartaric

Table 1.6

Microbes reported to cause biodegradation and metabolic products (267)

Acid-producing microbes	Metabolic product
Bacteria	
Pseudomonas fluorescens	2 ketogluconic acid
Pseudomonas aeruginosa	
Pseudomonas striata	
Other Pseudomonas species	2 ketogluconic acid
Clostridium pasteurianum	
Erwinia freundii	lactic acid
Achromobacter species	
Bacillus mucilaginous, subsp. siliceus	
Bacillus megatherium De Bary	
Bacillus oligonitrophilus	
Bacillus salivarius	
Bacillus cereus	(formic acid
Bacillus polymyxa	(acetic acid
Bacillus licheniformis	(butyric acid
	(lactic acid
Bacillus circulan	
Undefined bacterial species	
Actinomycetes	
Nocardia species	succinic acid
Other species	
Fungi	
	(oxalic acid
	(fumaric acid
	(citric acid
Aspergillus niger	(glycolic acid
	(gluconic acid
	(succinic acid
Aspergillus flavus	
Aspergillus awamori	
Other Aspergillus species	
Penicillium notatum	
Penicillium simplicissimum	citric acid
Penicillium species	citric acid
Schwanniomyces occidentalis	
	(oxalic acid
Spicaria species	(acetic acid
	(formic acid
Botrytis species	citric acid
Cephalosporium species	citric acid
Trichoderma species	citric acid
Fusarium species	
Hormodendrom species	
Muco species	
Margarinomyces species	

2.1 Atomic absorption theory

Atomic absorption spectrometry (AAS) is an analytical method for the determination of elements based upon the absorption of radiation by free atoms. The subject has been the theme of various books (289-291) and the limitations and advantages of this technique have been clearly discussed (292-295). An atom is said to be in the ground state when its electrons are at their lowest energy levels, when energy is transferred to a population of such atoms by means of thermal or electrical excitation, transfer takes place by means of collision processes. The amount of energy transferred may vary considerably from atom to atom, resulting in a number of different excitation states throughout the population. The subsequent emission spectrum produced contains a number of different frequencies and hence a complex spectrum. The reverse process of absorption also occurs if light of any of these frequencies is passed through a vapour containing specific atoms, thus the electromagnetic radiation will be absorbed in performing the process of excitation. As atomic absorption corresponds to transitions from low to higher energy states of ground state atoms, the degree of absorption will depend on the population in the lower state. When thermodynamic equilibrium prevails the population of a given level is determined by Boltzmann's law:

$$Nm = \frac{Nn\ gm}{gn} \exp\left[-\frac{(Em-En)}{kT}\right] \qquad (1)$$

where:

N = the number of atoms in the ground (n) and excited (m) states with energies En and Em

gn and gm = the statistical weights of the ground and mth state atoms respectively where $g = 2m + 1$

k = the Boltzmann constant

T = the temperature in Kelvin

A low proportion of atoms exists in the first excited state even at temperatures of 3000K, indicating that absorbance of radiation other

than that originating from a transition from ground state would be very small. Absorptions involving the ground state are known as the resonance lines. One of the main advantages of atomic absorption is that the absorption spectra is relatively simple, unlike the complex spectra for emission.

Atomic absorption shows an exponential relationship between the intensity I of transmitted light and the absorption path length l, similar to Beer-Lambert's law in molecular absorption spectrscopy:

$$I = I_o \exp(-k\nu l) \quad (2)$$

where I_o is the intensity of the incident light beam and $k\nu$ is the absorption coefficient of frequency ν. In quantitative spectroscopy absorbance A is defined by:

$$A = \text{Log}(I_o/I) \quad (3)$$

Thus from Beer-Lambert's law and the absorbance equation we obtain the linear relationship (2 + 3):

$$A = k\nu l \log_e \nu \quad (4)$$
$$= 0.4343\, k\nu l$$

The validity of making absorption measurements depends on the relationship between absorption and the concentration of the absorbing atoms. This relationship follows the classical dispersion theory:

$$k\nu\, d\nu = \frac{\pi e^2}{mC} N\nu f \quad (5)$$

where:

$k\nu$ = the absorption coefficient at frequency ν

m = electronic mass

e = electronic charge

N = the number of atoms per ml capable of absorbing energy in the range ν to $\nu + d\nu$

f = the oscillation strength, that is the effective number of free electron oscillators per atom of specific element responsible for the absorption effect produced by the incident radiation

In practical terms $k\nu$ in equation 5 is the proportional number of atoms per ml, therefore absorbance A in equation 4 is proportional to analyte concentration.

As atomic absorption and atomic emission lines have the same wavelength, the narrowness ($\approx$ 0.002nm) of atomic lines is a positive advantage, as the chances of an accidental overlap of an atomic absorption line of one element with an atomic emission line of another is almost negligible. The uniqueness of overlaps often known as the 'lock and key' effects (296) is responsible for the very high selectivity enjoyed by atomic absorption spectroscopy. The amount of radiation isolated by the conventional monochromator, and thus viewed by the detector, is not significantly reduced by the very narrow atomic absorption signal, therefore the amount of atomic absorption seen using a continuum source is negligible. The major disadvanatages of AAS when using a line source such as a hollow cathode lamp are absorption from molecular species and scattering of radiation from particulates. The latter known as non-specific absorption is a particular problem at shorter wavelengths (< 250nm) and can lead to positive errors. A second beam of continuum radiation to correct for non-atomic absorption is widely used. When using a continuum source (e.g. a deuterium arc or a hydrogen hollow cathode lamp) the amount of atomic absorption observed is negligible, but the same amount of non-specific absorption is seen. Thus if the signal observed with the continuum source is subtracted from that observed with the line source the error is corrected for. Background correction is now generally a simultaneous, automated feature on commercial instruments.

2.2 Electrothermal atomization

The theory and practice of electrothermal atomization for AAS has been discussed in detail by numerous authors (289-291, 297). The electrothermal atomizer is generally a small cyclindrical carbon furnace or cell which can be raised to a high temperature by resistive heating. In order to atomize sample material such furnaces must be capable of being raised to temperatures in excess of 2000°C for most elements, and up to 3000°C for the so-called refractory elements.

The great sensitivity of electrothermal atomizers in AAS arises from their ability to retain a substantial proportion of the atomized analyte element in the observation zone for a longer period of time than for a flame. Sensitivity is therefore increased when the formation of atoms in the optical path occurs at a greater rate than their removal. This effect follows a typical maximum minimum profile, with the high rate of atom formation showing a peak, after which time the formation of atoms drops below that for their removal. At the absorbance maximum:

$$\left(\frac{dN}{dt}\right)_{\text{formation}} = \left(\frac{dN}{dt}\right)_{\text{removal}} \qquad (6)$$

if N is the number of atoms at time t, dN/dt is the rate of change of the number of atoms.

The mechanism by which free atoms are produced in such a cell depends on a number of factors. These include the compounds being present in the cell at time of atomization, the material from which the furnace tube is made, the atmosphere present in the cell, the rate of increase and final temperature of the cell.

The faster the rate of heating the higher the density of the atoms which will be formed in the transient atomic cloud. This lead to improved analytical sensitivity, in addition, electrothermal atomizers do not suffer from the poor nebulization efficiencies, rapid dilution in the expanding flame gases and short residence times of a conventional flame atomization system. Improvements in detection limits with electrothermal atomizers over flame atomizers are in the range 100-1000 fold, with detection limits typically 0.1-1 ng ml^{-1}. The power supply controlling the furnace can be programmed so as to dry the sample after injection, ash at an intermediate temperature ($\approx$ 600°C) and atomize it. The temperature and duration of each of these steps can be controlled over a wide range. Optimizing the operating conditions of the furnace is essential, drying must be achieved without problems from 'spitting', ashing of any organic matter must be complete with minimal loss of volatile analyte, and atomization temperatures which produce a rapid peak should be selected. The furnace should be operated with background

correction as background absorption caused by broad band absorption of radiation by molecules and the presence of alkali metals is often at the 90% level in furnaces.

Commercial electrothermal atomizers are constructed to accept small 10-100 µl discrete liquid samples and the heating of such devices is typically not continuous. Thus when the analytical features of a furnace are required with a continuous flow system, such as the eluate from a chromatographic system, an interface capable of eluate storage is required.

2.3 Hydride generation

A number of elements such as arsenic are difficult to analyse for by flame AAS due to the interferences at the low wavelengths of their primary resonance lines (164-169). The generation of the volatile covalent hydride by reaction with borohydride solution replaces the inefficient nebulization of the conventional flame atomization system and separates the atoms from their liquid matrix, thus improving the detection limit for most elements. Arsine generation will vary according to valence state, the +5 state giving poorer responses than the +3 state. Practically this problem is minimized by ensuring arsenic in the sample is all in the +3 state, this is usually achieved by making the sample up to 6M HCl. The use of a narrow-bore silica tube mounted coaxially with the resonance beam over an air-acetylene flame into which the hydride is flushed and decomposed, increases even more the sensitivity of this method by increasing the residence time of atoms in the optical path. A transverse flow of nitrogen at the ends of the tube to ensure that liberated hydrogen does not burn in the light path is also a design feature of this method (298). Inter-elemental interference effects have been reported on the actual hydride formation step. Elements easily reduced by sodium borohydride (e.g. silver, gold, copper, nickel) give rise to the greatest suppressions. Interferences in the actual atom cell have not been documented. Although most instruments now operate with simultaneous background correction, this is not necessary using the narrow-bore silica tube method.

2.4 High performance liquid chromatography theory

High performance liquid chromatography (HPLC) like other forms of chromatography (299-301) involves the separation of the components of a mixture by virtue of differences in the equilibrium distribution (K) of the components between two phases:

$$K = \frac{\text{concentration of component in stationary phase}}{\text{concentration of component in mobile phase}} \qquad (7)$$

The basic HPLC system consists of four components, a pump to move the solvent through the system, an injector for depositing the sample at the head of the column, a column filled with a suitable packing material, and a detector or detectors to visualize the eluted components.

In normal and reverse phase chromatography the separation is carried out by a liquid mobile phase with either a solid stationary phase or a liquid phase bonded to a solid support which reversibly sorbs the solute molecules. In normal chromatography the stationary phase is polar (e.g. silica gel, porous glass beads or alumina), the mobile phase being relatively non-polar (e.g. hexane or chloroform). The solvent molecules in the mobile phase compete with the solute molecules for sites on the stationary phase or adsorbent. In order that a solute molecule can be adsorbed onto the stationary phase a solvent molecule must first be displaced from the surface. If the adsorbent possesses a polar surface non-polar groups will have little affinity for the surface and will not displace the solvent molecules, therefore they will not be retained. Polar functional groups or groups capable of hydrogen bonding will have a strong affinity for the surface and will be strongly retained. In reverse phase chromatography the stationary phase is non-polar (e.g. octadecylsilane C18 coated polymer beads) and a polar mobile phase (e.g. water, ethanol, dilute acid) would be used. The separation is based on a liquid-liquid partition and depends on the comparative solubility of the sample molecules in the solvent and in the liquid coating on the solid support. Thus non-polar compounds are retained more strongly than those with polar characteristics.

Ion exchange involves the substitution of one ionic species for another. The stationary phase consists of a rigid matrix, the surface of which carries either a negative charge (cation exchange) or a positive charge (anion exchange). For example in anion exchange the positive ion exchange sites will attract and hold negative counter ions (Y^-) and sample anions (X^-) may exchange with these counter-ions (Y^-). The complementary process occurs for cation exchange chromatography. Ion exchangers can be further divided into weak or strong, anion or cation exchangers according to the nature of the functional groups in the resin. Carboxylic acid (-COOH) functional groups and tertiary amine ($-CH_2N^+HMe_2\ \bar{O}H$) groups are used for weak acid and weak base anion exchangers respectively, whilst strong cation exchanges would contain sulphonic acid ($-SO_3H$) groups for an acidic type exchanger, or tetra-alkylammonium ($-CH_2\overset{+}{N}Me_2\ \bar{C}l$) groups for a strong basic type exchanger.

Migration of component molecules may be assumed to occur only when the molecules are in the mobile phase. The rate of migration of a component is inversely proportional to its distribution coefficient. For example components with a high distribution coefficient in the stationary phase will move more slowly through, or be retained by the column, and hence be separated from the components with a lower distribution coefficient in the stationary phase. Without this difference in distribution and by inference a differential rate of migration, no separation can be achieved, thus bands of separated components move through the column at rates less than the mobile phase velocity. The ratio of the two velocities is known as the retardation factor (R):

$$R = \frac{\text{rate of movement of the sample band}}{\text{rate of movement of the mobile phase}} \qquad (8)$$

and is related to the equilibrium distribution coefficient. The time of elution of the peak maximum is called the retention time, this being a function of the mobile phase velocity. The volume of mobile phase required to elute a component from the column, the retention volume (V_R), is given by:

$$V_R = F \times t_R \quad (9)$$

where F is the volume flow rate of the mobile phase and t_R is the retention time.

Column efficiency or number of theoretical plates (N) of a chromatographic system may be defined from a single chromatographic band or peak:

$$N = 16(t_R/w)^2 \quad (10)$$

where t_R is the retention time and w is the peak width at the base line measured in units of time. Often comparative column efficiencies are expressed as the height equivalent to a theoretical plate or plate value H:

$$H = L/N \quad (11)$$

where L is the length of the column and H measures the efficiency of the column per unit length, small H values indicates more efficient columns. Various components contribute to the overall theoretical plate determinations, these are:

(a) Longitudinal diffusion - this is an effect of column packing, where diffusion of a compound is restricted physically by the column packing material.

(b) Stationary phase mass transfer - this is analogous to retention on an adsorptive surface, in that a certain average time is required to adsorb and desorb the molecule.

(c) Mobile phase - (i) a moving flow of fluid through a packed bed undergoes eddy diffusion and lateral mass transport by diffusion/convection, and (ii) where the mobile phase is trapped in porous spherical particals and becomes stationary.

The solvent efficiency is quantified in the terms of relative retention (α) for a two component system:

$$\alpha = \frac{t_{R2}}{t_{R1}} = \frac{K_2}{K_1} \quad (12)$$

where t_{R1} and t_{R2} are the retention times of two compounds 1 and 2 for which their equilibrium constant is given as K_1 and K_2 respectively.

The relationship to the equilibrium constants K_1 and K_2 indicates the thermodynamic nature of the system which can be more fundamentally shown as:

$$\Delta \ (\Delta \ G^{\theta}) \ = \ -RT \ \ln\alpha \qquad (13)$$

where $\Delta \ (\Delta \ G^{\theta})$ is the difference in free energies of distribution of the two components.

The ability for particular stationary and mobile phases to produce a separation or resolution is ultimately a function of the thermodynamics and kinetics of the system. The degree of resolution required will be determined to some extent by the nature of the chromatographic analysis performed.

2.5 HPLC/GFAAS interface

Figs. 2.1 and 2.2 show the schematic representations of the interface as used for copper, lead (Fig. 2.1) and arsenic (Fig. 2.2) determinations. The HPLC system consists of a Waters 6000A solvent delivery system, either a Waters U6K or a Rheodyne 7125 injection valve fitted with 1000 µl sample loops, attached to appropriate columns. When required a Pye-Unicam LC ultra-violet spectrophotometer was used. The column eluate was then injected into an Instrumentation Laboratories IL151 Atomic Absorption Spectrophotometer. The IL555 furnace was modified so that an injector (Fig. 2.3) could be fixed to the face plate and aligned with the cuvette sample injection opening. In addition the vertical access port was replaced by a borosilicate glass tube, which allowed nitrogen to be blown into the chamber via a stainless steel lance to speed up cooling. The increased gas flow reduced the cooling time to about 20 seconds.

The interface consists of two Altex (4 way) slide injection valves with pneumatic actuators. The sample (76.6 µl) and co-analyte (5 µl) loops were of 0.8mm I.D. Teflon Tubing cut to appropriate lengths. All other inter-connecting tubing was of 0.33mm I.D. Teflon. In the

Fig. 2.1

Schematic diagram of HPLC/GFAAS interface system for analysis of copper and lead

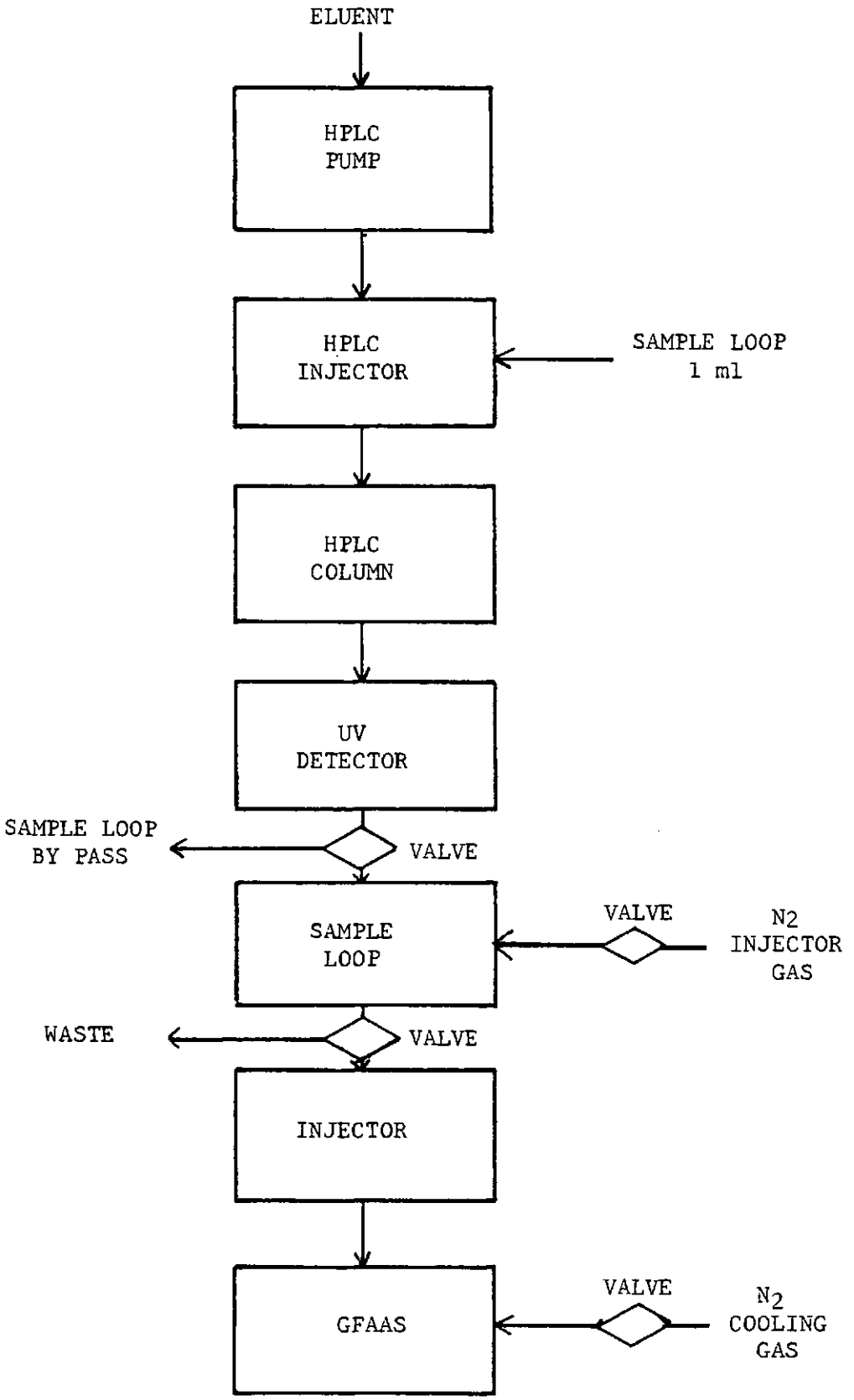


Fig. 2.2

Schematic diagram of HPLC/GFAAS interface system for analysis of arsenic

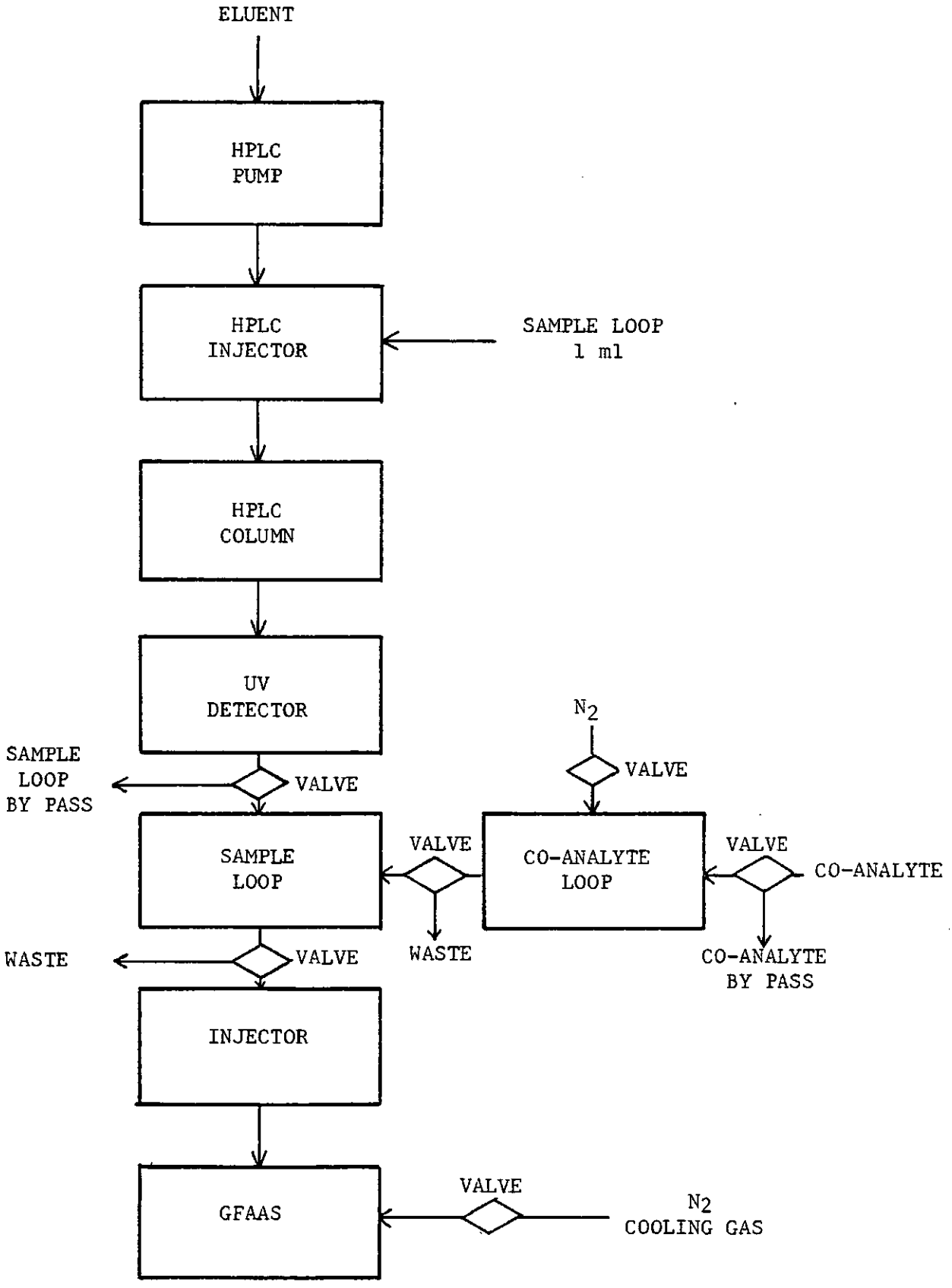
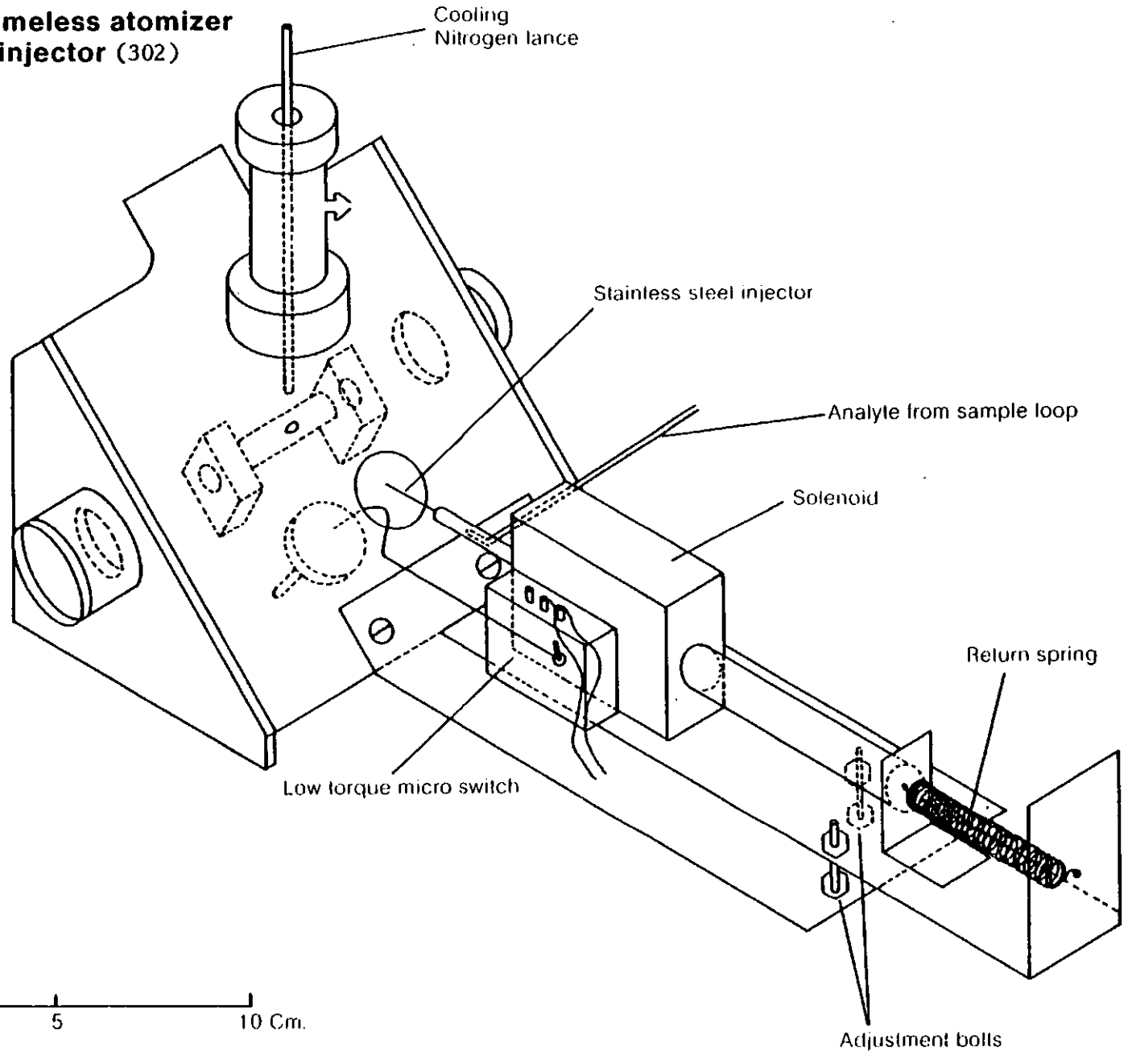


Fig. 2.3

IL 555 Flameless atomizer with auto-injector (302)



case of arsenic, delivery of the co-analyte and the sample was by nitrogen pressure through a 1/16th inch OD 316 stainless steel tube activated by a solenoid, the co-analyte, by following the sample through the system into the cuvette, reduced the possibility of inter-sample contamination. When determinations were carried out for copper and lead the co-analyte loop remained empty.

In the first instance the interface was controlled by an Ohio Scientific Super Board II computer. This computer controlled all aspects of the interface and data acquisition. The computer was modified by the addition of two input and two output ports.

All external functions of the interface were controlled by the output ports via 24 volt relays. The external functions controlled by the computer were:

1) Injection of sample
2) Control of sampling valves
3) Activation of the atomic absorption spectrometer
4) Control of cassette recorder for data storage
5) Resetting of the spectrometer and activating the N_2 cooling valve
6) Operation of on-line printer (Trend 800)

Internal functions of the computer controlled by the output ports were controlled directly through semiconductor buffers. The internal functions were:

1) Starting analog to digital convertor (A/D)
2) Selection of the analog input

The two input ports were used to accept data from the A/D convertor and status lines. The A/D convertor allowed the determination of peak area from atomic absorption signal and the determination of furnace temperature. The status lines were used to inform the computer of the following:

1) Whether the furnace door was open and ready for next injection cycle

2) When the atomization of the sample was about to occur so data acquisition might begin.

The outputs from the UV detector and from the GFAAS were also recorded on chart recorders attached to the respective systems. It was thought that because the computer was manufactured to be compatable with the USA 60 H_Z supply, this must be the reason why it was prone to cut out when operated on the 50 H_Z U.K. power supply. Thus the Ohio computer was replaced with an electronic control system capable of performing the analysis sequence outlined previously, without however the data acquisition facility. The results obtained were recorded on standard chart recorders and measurements of peak height were taken.

Blowing the sample into the hot cuvette has a number of advantages, namely:

(a) a larger volume of sample can be accommodated by the cuvette as vapourisation of solvent occurs almost immediately, thus increasing the effective sensitivity;

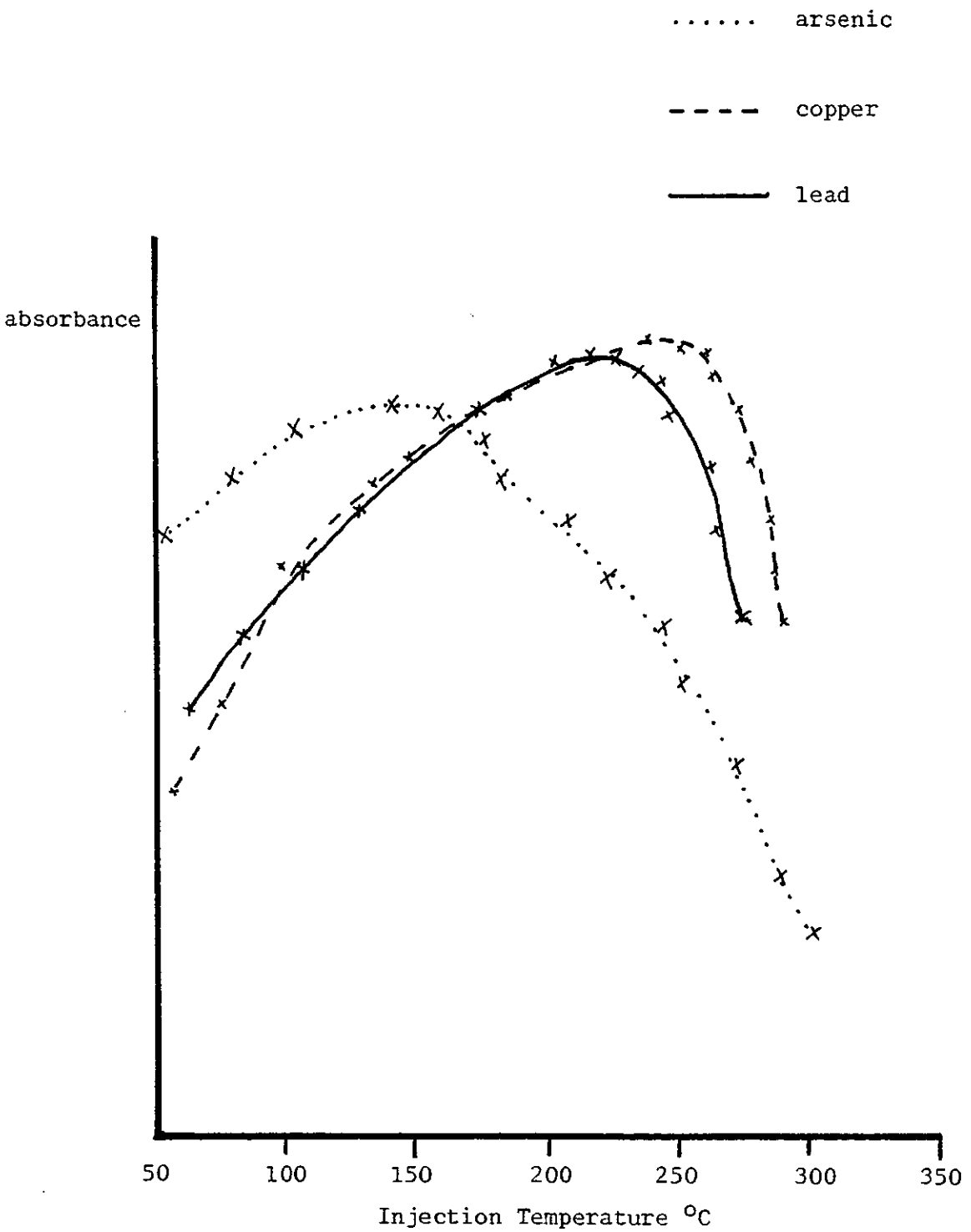
(b) the analysis sequence time for any one determination is reduced both by shortening the drying time and decreasing the cooling range. These effects plus the increased rate of cooling achieved by introduction of extra nitrogen coolant gas reduces the total cycle time from over three minutes to approximately 50 seconds;

(c) sensitivity, as shown in Fig. 2.4, passes through a maximum with injection temperatures between 100 and 150°C for arsenic (III) chloride, 175-225°C for lead (II) nitrate, and 200-250°C for copper (II) nitrate, all other instrument variables being held constant.

The sample was introduced into the cuvette at 125°C $\pm$ 10°C for arsenic, and 180°C $\pm$ 10°C for copper and lead. A loss of approximately 5% of the signal when using these temperatures was considered acceptable as the shape of the optimization graphs at the maximum point suggest a rapid loss in sensitivity at temperatures greater than the maximum point.

Fig. 2.4

Temperature injection optimization graphs for arsenic copper and lead



Calibration curves for arsenic, copper and lead are shown in Figs. 2.5, 2.6 and 2.7. Typical linear working ranges were 10 ng, 60 ng and 20 ng with detection limits of 0.5 $\mu g\ l^{-1}$, 0.4 $\mu g\ l^{-1}$ and 0.6 $\mu g\ l^{-1}$ for arsenic, copper and lead respectively, the detection limits being based on two standard deviations for ten replicate determinations. The blanks and standard arsenic, copper and lead solutions were fed to the interface sample loop by a peristaltic pump, rather than through a HPLC system. The programmes used, including manual conditions used in early development work, are given in Table 2.1.

2.6 HPLC/FAAS continuous hydride interface

Fig. 2.8 illustrates the interface used for arsenic determinations by continuous hydride generation. The HPLC system consists of a Waters 6000A solvent delivery system, and Waters U6K injection valve fitted with a 1000 µl sample loop, attached to two anion exchange columns in series. A peristaltic pump flow rate 1.6 ml min^{-1} and two borosilicate glass auto analyser Y pieces are used to entrain the hydrochloric acid (6M) and borohydride solution (4% in 0.1M sodium hydroxide solution) into the column eluate flow 4 ml min^{-1}. Reduction took place in the short mixing coil and the arsine was liberated in a glass gas-liquid separator, and flushed by nitrogen into a heated quartz tube (Fig. 2.8). Detection of arsenic was achieved with a Pye-Unicam SP9 Spectrometer fitted with a hollow cathode lamp detection wavelength 193.6nm, band pass 1nm, with background correction mode on. Peak height and area values were collected using a chart recorder and a Hewlett Packard reporting integrator.

Optimizations using the univariate technique (303) were carried out for (i) the hydrochloric acid and borohydride concentrations (Fig. 2.9, 2.10), (ii) the flows of auxiliary and feed nitrogen gas (Fig. 2.11). Concentrations of 6M hydrochloric acid and 4% borohydride were selected as they give similar peak heights for acceptable losses in sensitivity for all four arsenic species, and in addition assured complete reduction for a wide range of arsenic concentrations. A nitrogen feed flow of 0.3 l min^{-1} and an auxiliary flow of 15 l min^{-1} were adopted as at these rates a reasonable signal was obtained with protection against sudden surges of hydrogen combusting within the

Fig. 2.5

Calibration curve of peak area against weight of arsenic standard with 0.5% nickel nitrate co-analyte

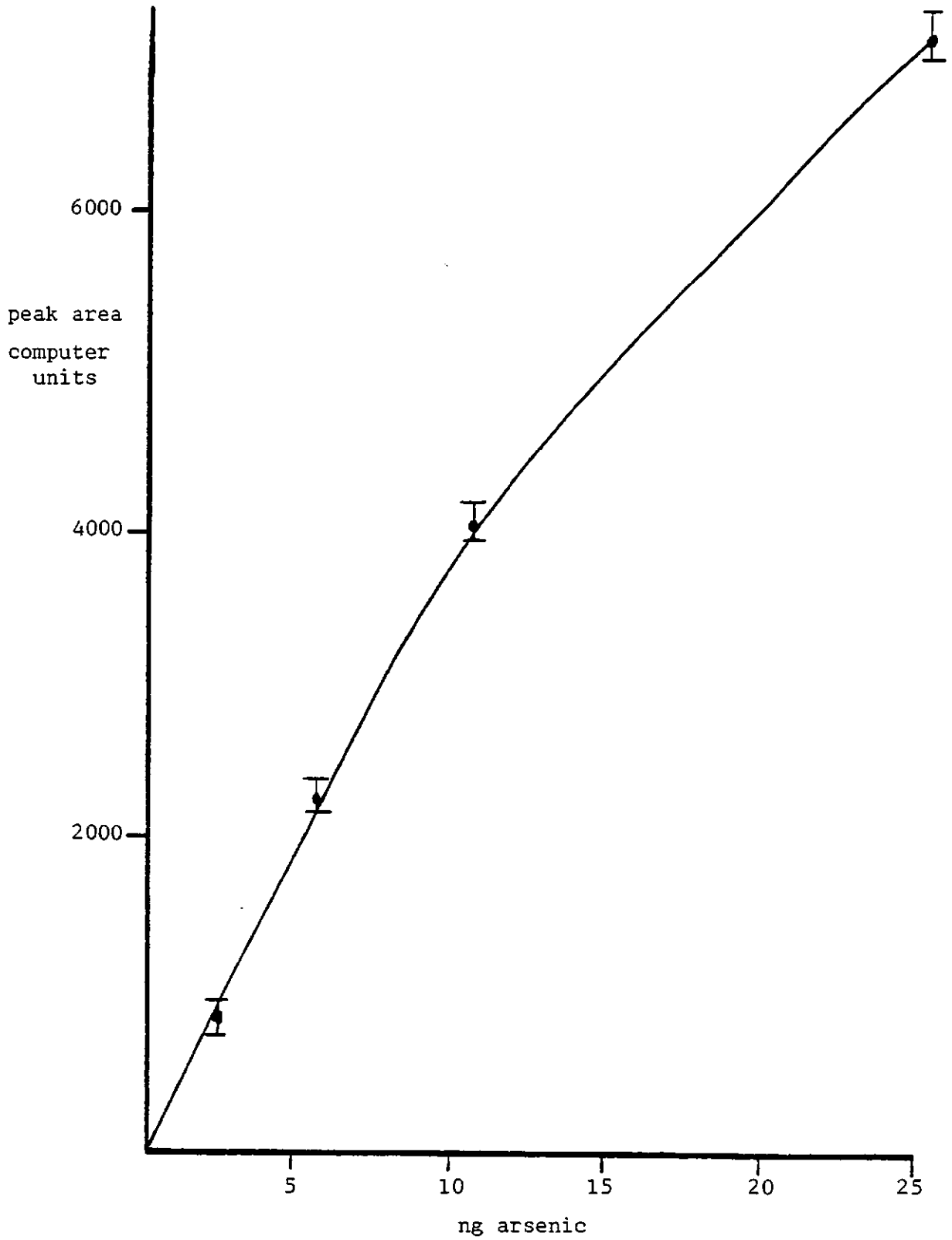


Fig. 2.6

Calibration curve of peak height against weight of copper nitrate standard

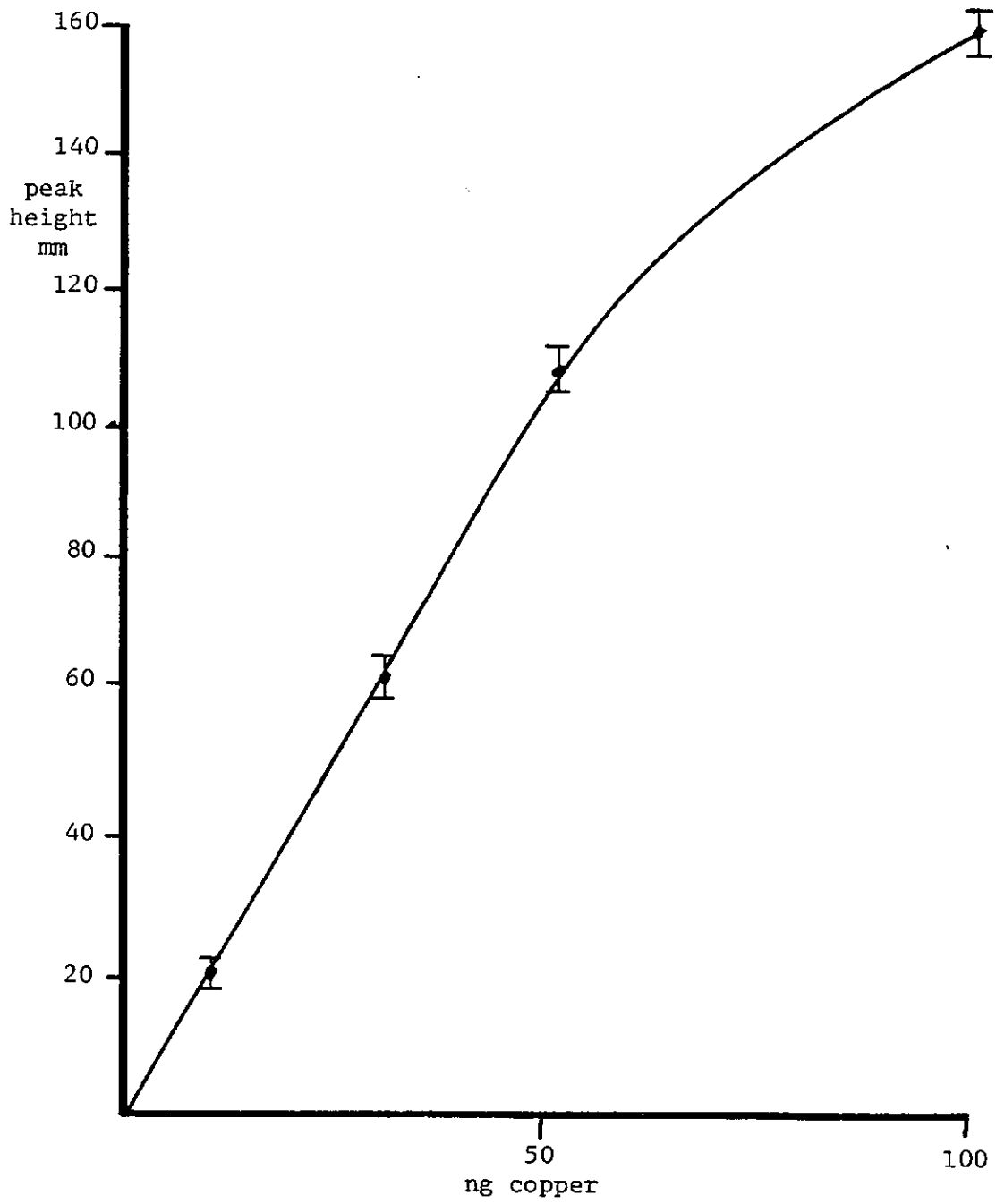


Fig. 2.7

Calibration curve of peak height against weight of lead nitrate standard

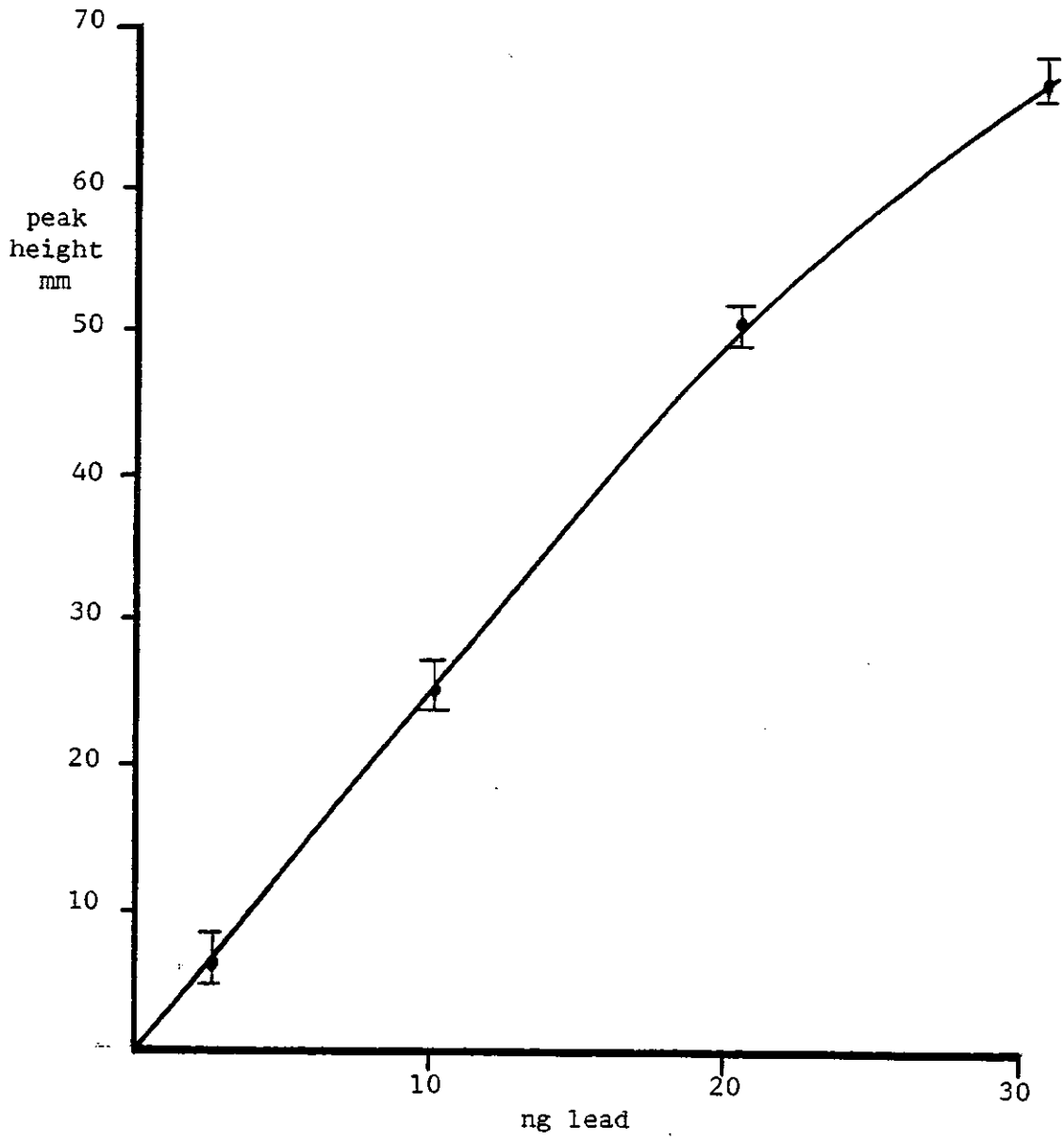


Table 2.1

Graphite furnace atomic absorption spectrometer conditions for the determination of arsenic copper and lead

Element	Lamp	Wave length nm	Band pass nm
Arsenic	Hollow cathode	193.6	1
Copper	Hollow cathode	324.7	1
Lead	Hollow cathode	283.3	0.5

(a) Manual injection (arsenic copper and lead)

Temperature ^{o}C	75	100	350	700	1800
Time seconds	20	25	25	25	5

(b) Interface injection

Arsenic

injection: volume 76.6 µl + 5 µl 0.5% nickel nitrate, pressure 16 psi, temperature 125 $\pm$ 10^{o}C

Temperature ^{o}C	175	270	1900
Time seconds	10	5	5

Copper

injection: volume 76.6 µl, pressure 16 psi, temperature 180 $\pm$ 10^{o}C

Temperature ^{o}C	175	270	1900
Time seconds	10	5	5

Lead

injection: volume 76.6 µl, pressure 16 psi, temperature 180 $\pm$ 10^{o}C

Temperature ^{o}C	175	350	700	2000
Time seconds	10	5	5	5

Fig. 2.8

Schematic diagram of HPLC/FAAS continuous flow interface for the analysis of arsenic

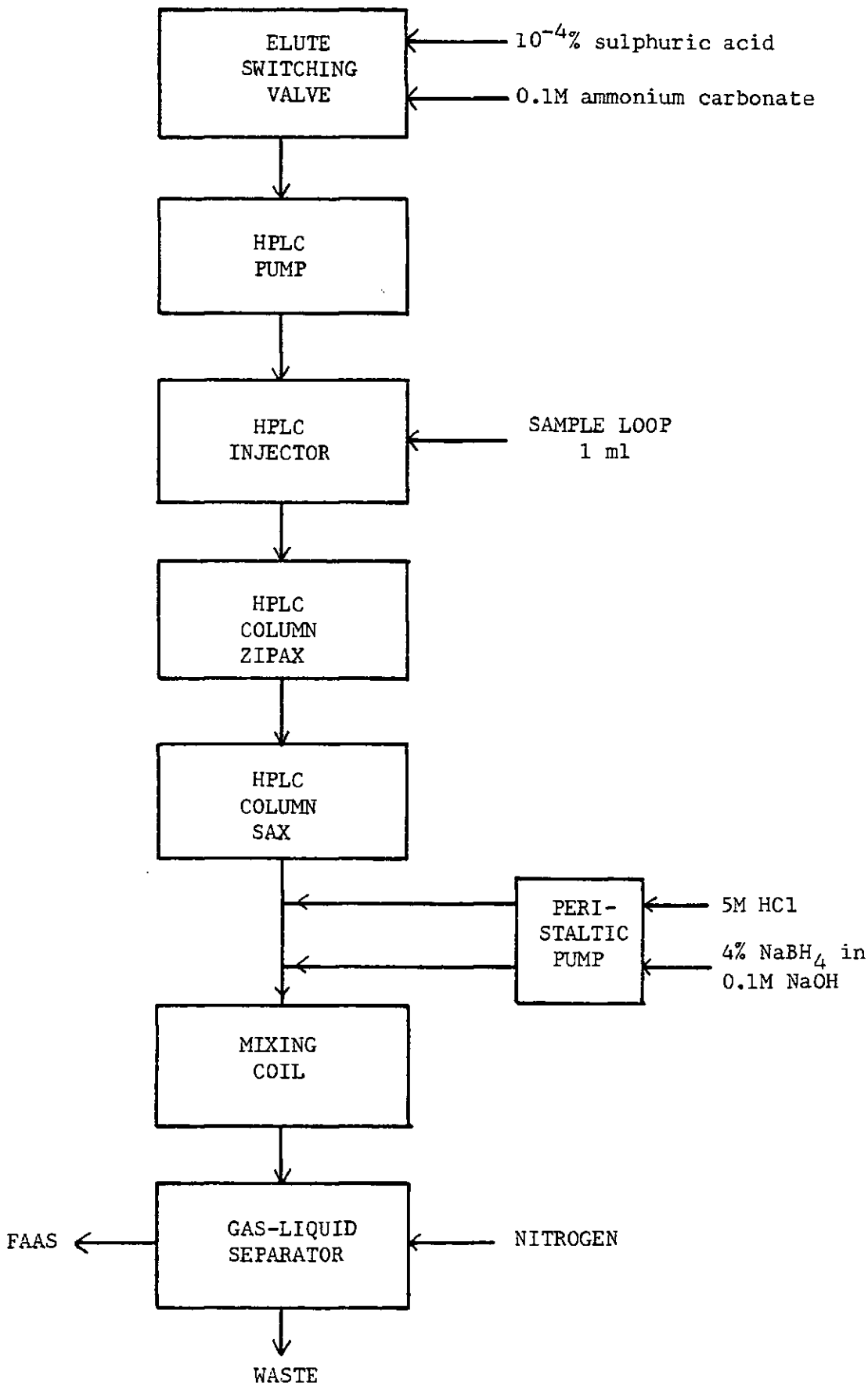


Fig. 2.9 Optimization of hydrochloric acid concentration

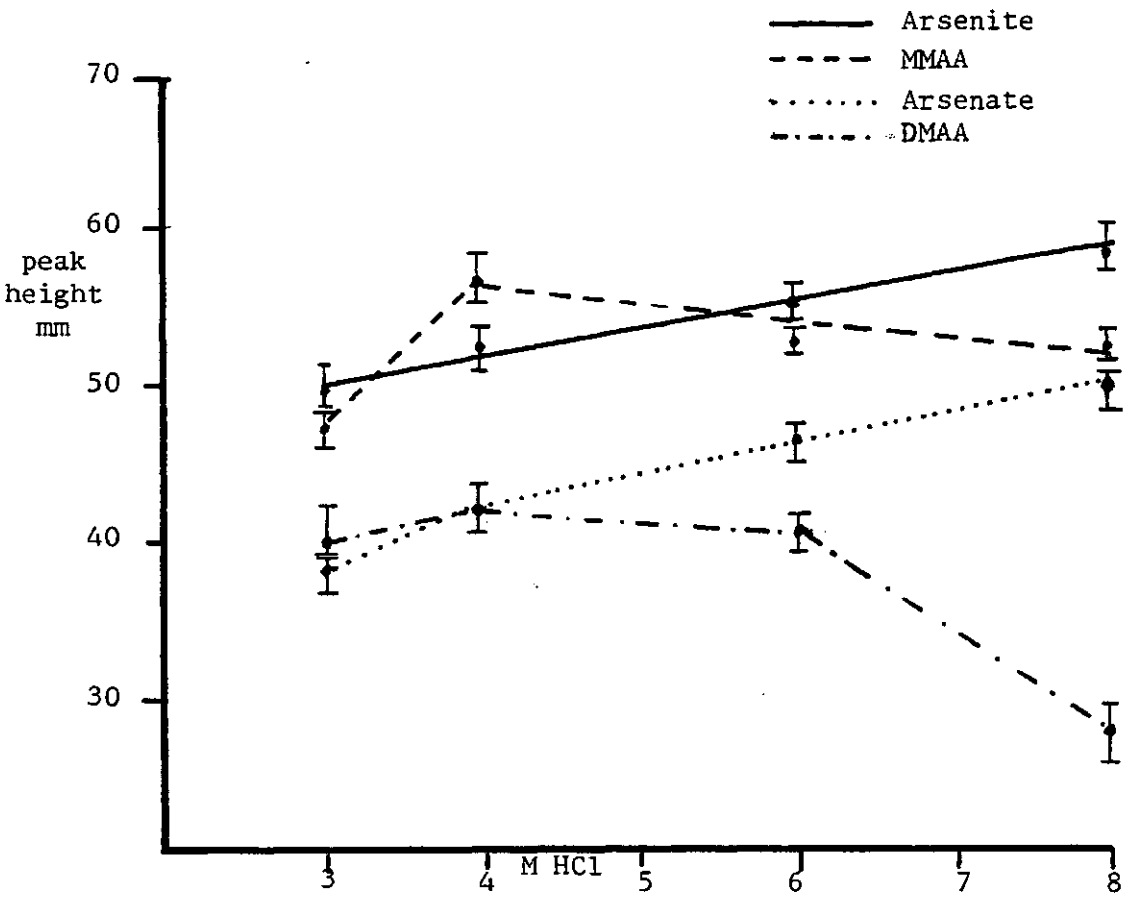


Fig. 2.10 Optimization of sodium borohydride concentration

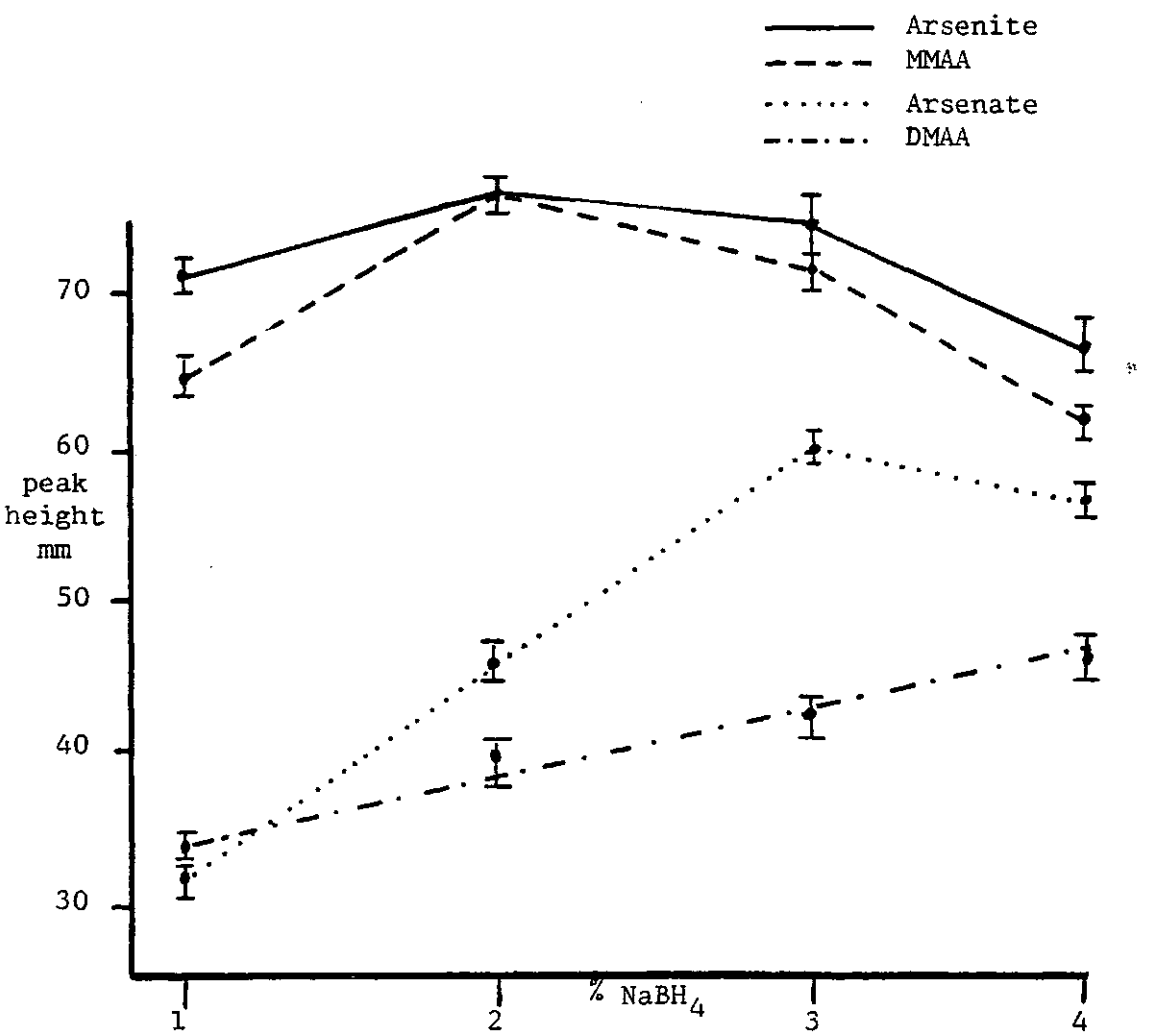
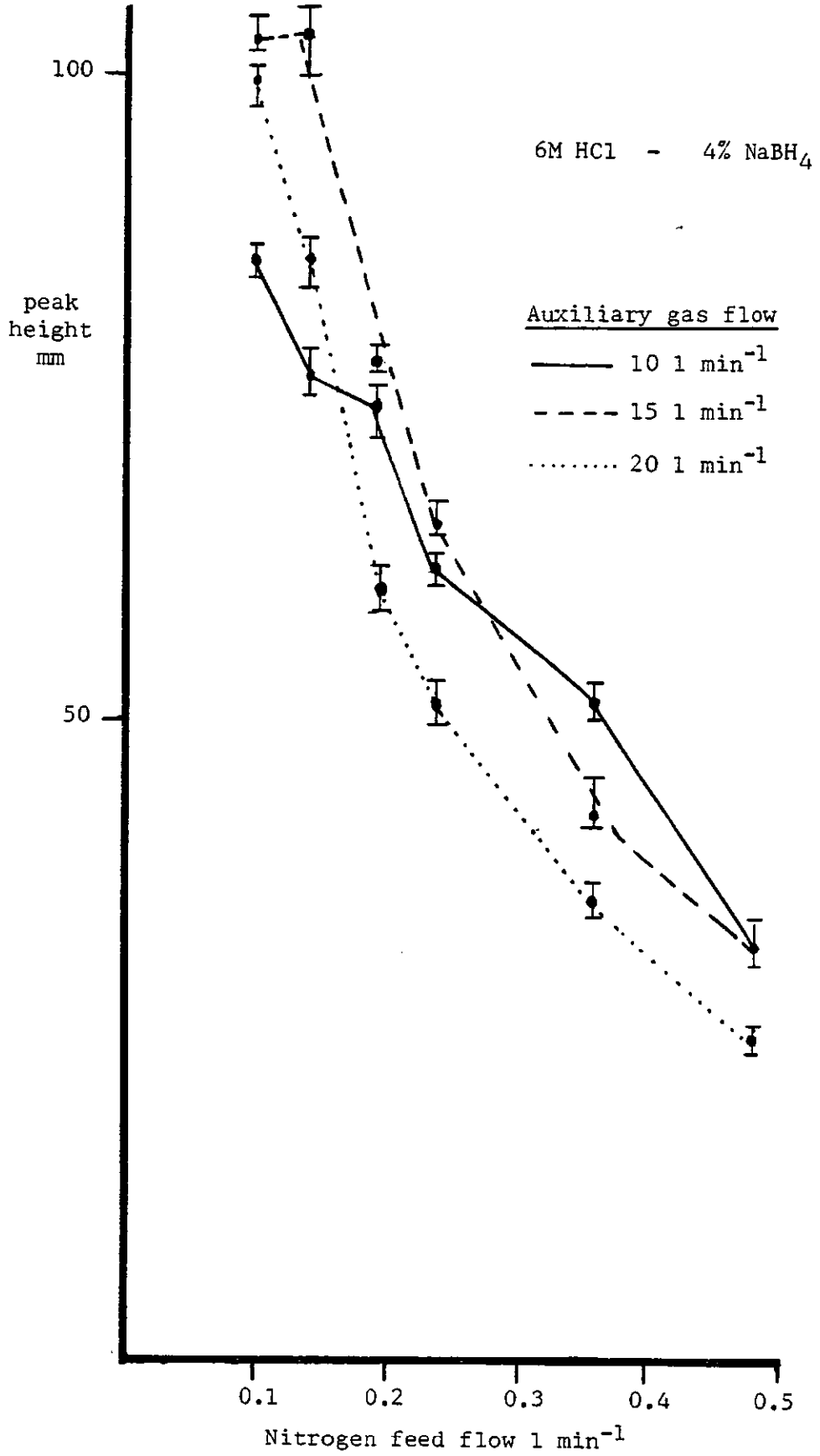


Fig. 2.11

Optimization of auxiliary and feed nitrogen gas



quartz tube, caused by decreases in arsine production as a result of variations in sample concentrations.

Calibration curves for arsenite, arsenate, dimethylarsinic acid and monomethylarsonic acid at the optimized conditions are shown in Fig. 2.12. For all species the curves were found to be linear up to 80 ng of arsenic, with detection limits of 1.2 $\mu g\ l^{-1}$ (0.12 ng), 2.8 $\mu g\ l^{-1}$ (0.28 ng), 1.5 $\mu g\ l^{-1}$ (0.15 ng) and 2.4 $\mu g\ l^{-1}$ (0.24 ng) arsenic for arsenite, dimethylarsinic acid, monomethylarsonic acid and arsenate respectively. Table 2.2 summarizes the optimum conditions selected in this section of the work.

The continuous hydride generation method was used in preference to FAAS because of its improved sensitivity and lack of interference problems. The rapid continuous flow analysis obtained by the hydride technique described, enabled HPLC flow rates in the order of 2-5 ml min^{-1} to be used, this resulted in a more rapid and compatable method for interfacing of the HPLC to AAS than could be obtained by the GFAAS technique.

A full summary of the instrumentation, elution systems, standards and reagents are included in Table 2.3. Analar quality chemicals and deionised distilled water was used throughout this work.

2.7 Nitric acid digestion

One gram of air dried sieved soil (< 180 μm) was weighed into a borosilicate glass tube and placed in a Tectator Digestion System 40 with a 1006 heating unit and a 1008 control unit. This system consists of an electrically heated aluminium block capable of accepting 40 samples. To each tube 15 ml of concentrated nitric acid is added and the temperature programmed to rise to 145°C in two hours, it then being held constant for a further two hours. After digestion the contents of the tubes were filtered through a Whatman (Qualitative 1) filter paper into a 100 ml volumetric flask and made up to volume. The hot nitric acid digestion described above was found to remove 100% $\pm$ 2% arsenic, 96% $\pm$ 3% copper, 60% $\pm$ 7% lead and 70% $\pm$ 4% iron from certified sediment samples (Table 2.4). Elements associated with the

Fig. 2.12

Calibration curve of peak height against weight of arsenic standards on the continuous HPLC/FAAS interface

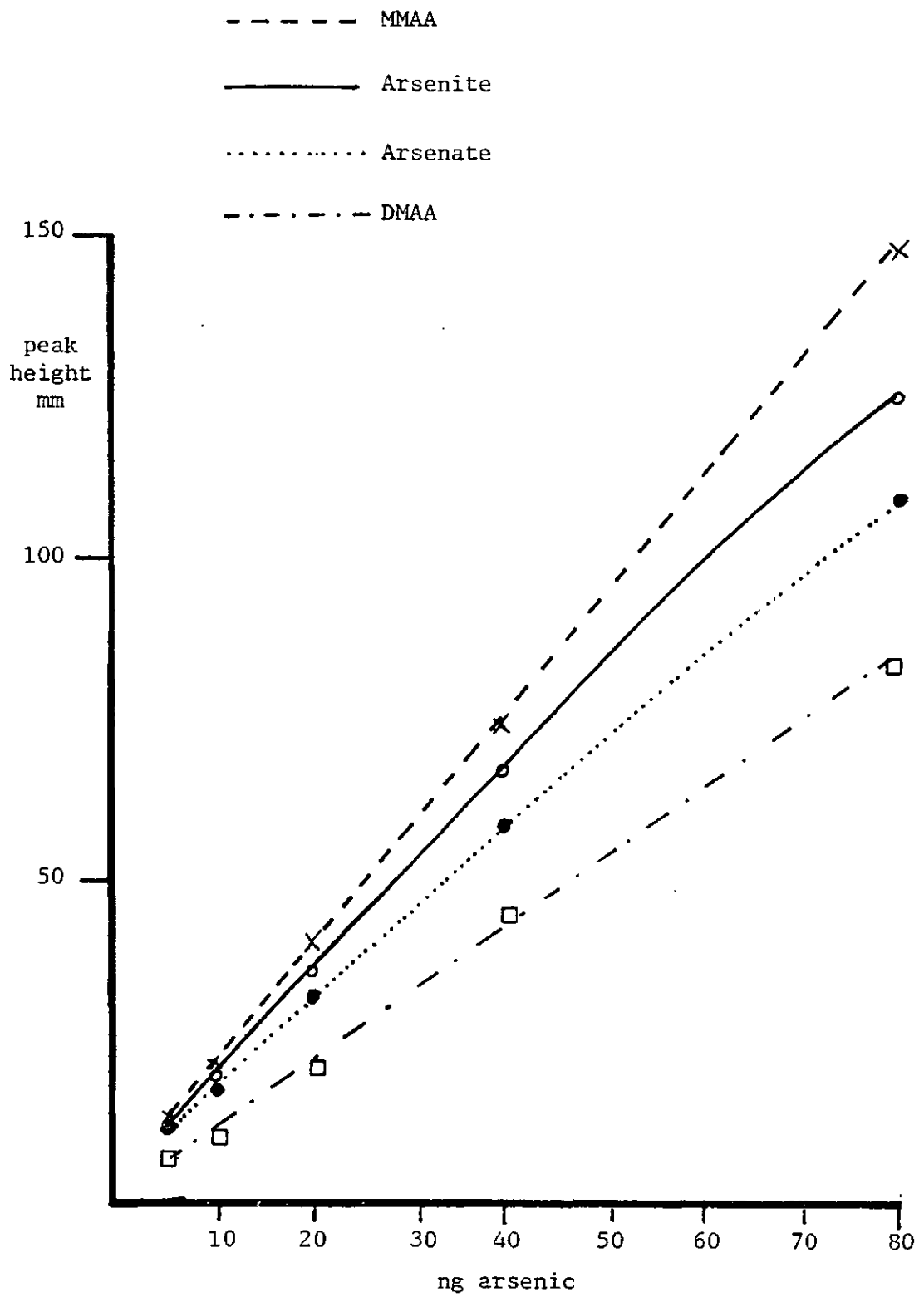


Table 2.2

Operating conditions for the arsenic calibration curves using the FAAS interface

Peristaltic Pump Rates

Sample feed 2.4ml min^{-1}

Sodium Borohydride (4%) 1.6ml min^{-1}

Hydrochloric acid (6M) 1.6ml min^{-1}

Atomic Absorption Spectrometer Conditions

Lamp:	Hollow cathode (7 mV)
Band pass:	1nm
Wave length:	193.7nm
Background correction:	ON

Table 2.3

Summary of the instrumentation and equipment used in this work

HPLC pumps and injectors

Pumps

Perkin-Elmer Series 2

Waters Series 6000A solvent delivery system

Injectors

Waters U6K injector valve

Rheodyne 7125 injector valve

Samples were loaded using a SGE 100 µl syringe

Columns

Hypersil octadecylsilane 5-7 µm:
slurry packed into a stainless steel Shandon 250mm x 5mm id column

Hypersil octadecylsilane 3-5 µm:
slurry packed into a stainless steel Shandon 250mm x 5mm id column

Vydac 3021C46 anion exchange 10 µm
Perkin-Elmer 200mm x 5mm id

Strong anion exchange BAX10 5 µm:
slurry packed into a stainless steel Shandon 250mm x 5mm id column
(supplier Benson Co. Ltd. Nevada USA)

Zipax ion-exchange 40 µm:
slurry packed into a stainless steel Shandon 100mm x 5mm id column
(supplier Dupont)

Hamilton PRP I octadecylsilane (styrene based) 10 µm:
slurry packed into a stainless steel Shandon 100mm x 5mm id column

Eluents

0.02% orthophosphoric acid in water

0.002% orthophosphoric acid in water

0.0002% orthophosphoric acid in water

0.0004% orthophosphoric acid in water

0.1% formic acid in water

0.01% formic acid in water

0.02% formic acid in water

continued ...

Eluents (continued)

10^{-4}% sulphuric acid in water

0.1M ammonium carbonate

0.01M ammonium formate

0.01M ammonium phosphate

0.2% propionic acid in water

0.01M sodium dihydrogen phosphate - 0.1M disodium hydrogen phosphate

0.05M sodium dihydrogen phosphate

0.01M sodium dihydrogen phosphate

0.1M sodium sulphate

UV detectors

Perkin-Elmer LC75 with scanning facility

Pye LC UV

Recorders

Perkin-Elmer 023

Linseis LS 24

Integrator

3390A reporting integrator Hewlett Packard

Atomic absorption spectrometers

Pye-Unicam SP9

Instrument Laboratory 151 fitted with an instrument laboratory 555 electrothermal atomizer

Table 2.4

Extraction efficiencies of hot nitric acid digestion on standard sediments

	Arsenic $\mu g\ g^{-1}$	Copper $\mu g\ g^{-1}$	Lead $\mu g\ g^{-1}$	Iron %
MESS-1	10.3	24.0	21.0	2.3
	10.6	24.0	20.0	2.3
	10.4	24.0	20.0	2.3
	10.4	25.0	19.0	2.3
	10.3	24.0	20.0	2.3
	10.5	24.0	20.0	2.3
$\bar{x}$	10.4	24.1	20.0	2.3
SD	0.11	0.41	0.63	8.1×10^{-3}
Certified*	10.6 ± 1.2	25.1 ± 3.8	34.0 ± 6.1	3%
Recovery	(98%)	(96%)	(59%)	(76%)
BESS-1	11.2	18.0	10.5	2.5
	11.6	18.0	10.0	2.5
	11.4	18.0	10.0	2.5
	11.1	17.0	11.0	2.5
	11.0	18.0	11.0	2.5
	11.2	18.0	10.0	2.5
$\bar{x}$	11.2	17.8	10.4	2.5
SD	0.21	0.41	0.5	8.1×10^{-3}
Certified*	11.1 ± 1.4	18.5 ± 2.7	22.7 ± 3.4	3.8%
Recovery	(101%)	(96%)	(46%)	(66%)

* National Research Council Canada Marine Sediment reference materials MESS-1 and BESS-1
Supplied by the Marine Analytical Chemistry Standards Program, Division of Chemistry, National Research Council, Ottawa, Canada

inert silicate matrix of the soil may not be removed by this method (69), thus hot nitric acid extraction is considered to represent the total levels of environmental importance.

2.8 Sodium dithionite extraction

Half a gram of air dried sieved soil (< 180 μm) was weighed into a 50 ml plastic centrifuge tube, together with 0.5g sodium dithionite and 25 ml of citrate buffer (72). The contents were then shaken for 30 minutes at 50°C. After centrifugation (3000 rpm 1000 x g, 2 minutes) the supernatant was transferred to a 50 ml volumetric flask, the residue being washed with 10 ml of 0.1M HCl by shaking for 10 minutes. The sample was once again centrifuged with the supernatant being added to the volumetric flask, which was then made up to volume. The sodium dithionite extraction will remove elements from soils held with varying degrees of complexation and those specifically associated with the hydrous oxide coatings (73), to give a measure of the soluble, inorganic, originally complexed and co-precipitated elements. Thus the proportion of elemental levels specifically associated with secondary crystalline precipitate coatings may be compared to those with the residual iron extracted by nitric acid.

2.9 Acetic acid extraction

Five grams of air dried sieved soil (< 180 μm) was weighed into a plastic bottle to which 40 ml of 5% acetic acid was added and the contents shaken overnight. The samples were then filtered through a Whatman (Qualitative 1) filter paper into a 50 ml volumetric flask and made up to the mark with water. This method will displace (a) water soluble ions, (b) ions held on cation exchange sites, e.g. clay surfaces, and (c) weakly bound inorganic complexes (6).

2.10 Ethylenediaminetetraacetic acid (EDTA) extraction

Five grams of air dried sieved soil (< 180 μm) was weighed into a plastic 50 ml bottle with 20 ml of 0.05M (pH7) EDTA and shaken for one hour. The samples were then filtered through a Whatman (Qualitative 1) filter paper into a 25 ml volumetric flask and made

up to the mark with water. This method will displace (a) water soluble ions, (b) ions held on cation exchange sites, e.g. clay surfaces, and (c) strongly bound inorganic and organic complexes (30-32).

2.11 Water extraction

Five grams of air dried sieved soil (< 180 μm) was weighed into a 50 ml plastic centrifuge tube, to which 25 ml of distilled water was added. The contents were then shaken for one hour and then centrifuged (3000 rpm 1000 x g, 2 minutes), the supernatant being collected and used for elemental determinations. This extraction removes the water soluble component of soils in accordance with their relative solubilities.

2.12 Repeated water extractions

One gram of air dried sieved soil (< 180 μm) was weighed into a plastic 50 ml centrifuge tube, to which 10 ml of water was added and shaken for one hour, after which time it was centrifuged (3000 rpm 1000 x g, 2 minutes) and the supernatant removed and analysed for elemental concentrations. A further 10 ml of distilled water was added to the residue and the procedure repeated. The repeated extraction technique is designed to give an indication of the potentially available water soluble forms of elements present in soils. The repeated removal of the soil water solution and its replacement by fresh distilled water could be considered to approximate to the removal of metal ions by organisms and plant root systems. Thus the subsequent dissolution of the metal ions bound to various soil components represents the mobilization of the elements into the soluble form.

2.13 Soil pore water extraction

Surface soil samples (0-10cm) were placed in a 100 ml plastic centrifuge tube and centrifuged on site (3000 rpm 1000 x g, 1 minute) to extract a suitable volume of pore water (≈ 0.5 ml). This was then drawn up in a plastic syringe and immediately filtered (Whatman WCN pore size 0.45 μm) using a Millipore Swinex apparatus into an acid

washed vial and the pH taken. Samples were stored as soon as possible at 4°C in the dark, with the subsequent elemental determinations made within 24 hours.

SOIL EXTRACTION METHODS AND SPATIAL DISTRIBUTION PATTERNS OF COPPER LEAD AND ARSENIC IN SOILS OF THE TAMAR VALLEY

3.1 Comparison of different extraction methods for copper, lead and arsenic from soils

Soil pore waters were extracted from thirteen soil samples, which were subsequently air dried and sub divided to allow five extraction procedures to be carried out on each sample (see methods section). The air dried, sieved (< 180 μm) samples were rewetted with distilled water and shaken for one hour to enable soluble forms of copper, lead and arsenic to equilibriate, the water was then extracted as the supernatant after centrifugation and the elemental concentrations determined. Four selected samples (R, S, I, Q) representing a range of elemental soil concentrations were repeatedly wetted and centrifuged to remove the water over a 24 hour period. At each stage the distribution between the solid and soluble elemental forms was allowed to readjust. The summation of the data for these extractions represents the potentially solubilizable available forms of copper, lead and arsenic, these values as a percentage of the total levels extracted can be compared to those obtained for single extraction procedures and may indicate the relative significance of reagents, such as acetic acid (5%) and EDTA (0.05M pH7), when used as a one time extractant for the determination of available forms. As indicated in the methods section acetic acid extracts weakly bound inorganic complexed ions, EDTA extracts strongly bound inorganic/organic complexed ions, and sodium dithionite extracts strongly bound inorganic/organic complexed and secondary crystalline precipitated iron. Hot nitric acid extraction represents the total extractable elemental levels of environmental importance.

3.2 Copper and lead

Table 3.1 summerizes the data for copper determinations obtained from the various extractions previously outlined. The water and acetic acid extractions removed ten to one hundred times the copper levels found in pore waters. EDTA extractions removed one hundred to one thousand times the amount of copper found in the pore waters. The repeated water extractions (Table 3.2, Fig. 3.1) indicate the removal of readily

Table 3.1

Copper concentrations $\mu g\ g^{-1}$ obtained by various extraction techniques

Sample No.	Pore Water	Extractions				
		Water	Acetic Acid	EDTA	Sodium Dithionite	Nitric Acid
G (w)	0.04	0.3	1.2	13	20	52
H (p)	0.05	0.2	0.6	9	19	40
I (p)	0.07	1.5	1.9	13	35	110
J (w)	0.05	0.7	2.9	28	42	230
K (p)	-	1.4	2.0	12	15	49
L (w)	-	0.6	4.0	20	36	87
M (w)	-	1.5	3.5	21	29	88
N (p)	0.09	1.4	3.4	19	26	82
O (w)	0.05	0.9	0.8	9	32	140
P (p)	0.05	0.8	0.9	8	30	110
Q (p)	0.1	1.4	2.2	16	28	140
V (p)	0.06	0.4	1.3	52	43	130
W (w)	0.08	0.08	120	400	726	1400

p = permanent pasture

w = woodland

Table 3.2

Copper concentrations $\mu g\ g^{-1}$ for soil water extractions

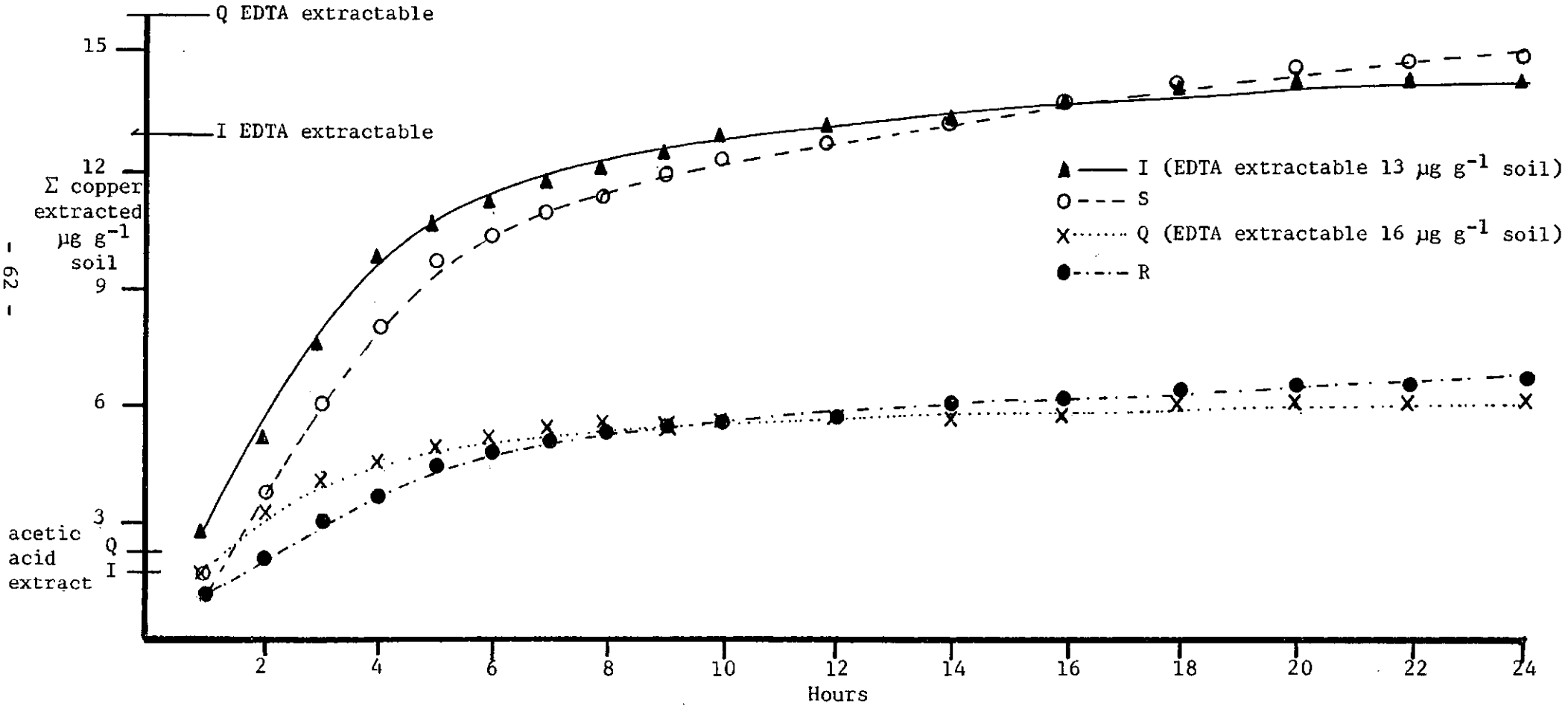
Hours	R (w)	Q (p)	S (p)	I (p)
1	1.2	1.7	1.7	2.8
2	0.9	1.9	2.3	2.3
3	0.9	0.5	1.9	2.3
4	0.7	0.5	2.0	2.5
5	0.8	0.3	2.0	0.8
6	0.3	0.2	0.5	0.5
7	0.3	0.2	0.5	0.4
8	0.2	0.1	0.4	0.3
9	0.2	0.1	0.4	0.3
10	0.2	0.1	0.4	0.3
12	0.2	0.1	0.4	0.4
14	0.2	0.1	0.4	0.4
16	0.2	0.1	0.5	0.3
18	0.2	0.1	0.5	0.2
20	0.1	0.2	0.4	0.2
22	0.1	0.1	0.4	0.2
24	0.1	0.1	0.3	0.2
Σ	6.8	6.5	15.0	14.4

p = permanent pasture

w = woodland

Fig. 3.1

A plot of copper extracted by repeated water extractions with time



soluble forms of copper. When the weights of copper extracted by acetic acid and EDTA are superimposed on the repeated water extraction curves (Fig. 3.1) the acetic acid values relate to the steeper section of the curves, whilst the EDTA values correspond to the tailing off region of the graph where there is a decline in the availability of soluble forms of copper. This suggests that in the region of acetic acid extraction there is a good supply of readily soluble forms of copper, which is not apparent in the EDTA extraction region. The indication from these results is that the readily soluble forms of copper relate more closely to those removed by the acetic acid, namely weakly bound inorganically complexed, rather than the strongly bound inorganic and organic complexed forms removed by EDTA.

The extraction of copper present with secondary crystalline iron indicated by the sodium dithionite extraction was observed to be up to twice the EDTA values and approximately half the nitric acid concentrations, suggesting the presence of considerable amounts of insoluble copper with secondary iron precipitates.

Table 3.3 summarizes the data for lead determinations obtained from the various extraction methods. The water and acetic acid extractable levels give similar values, both being an order of magnitude greater than those for the pore waters. The repeated water extractions (Table 3.4, Fig. 3.2) indicate a trend similar to copper, with most of the soluble lead being related to the weak inorganic type complexes. The sodium dithionite extraction suggests that little lead is associated with secondary iron as the EDTA extraction removed similar concentrations.

The concentrations of copper and lead extracted by the EDTA method are greater than those for the repeated water extractions. In the case of copper a close approximation between the two methods is found, this confirms that EDTA extractions are a good indicator of readily available forms of copper. In the case of lead the correspondence between the repeated water and EDTA extractions are not so good.

Table 3.3

Lead concentrations $\mu g\ g^{-1}$ obtained by various extraction techniques

Sample No.	Pore Water	Extractions				
		Water	Acetic Acid	EDTA	Sodium Dithionite	Nitric Acid
G (w)	0.04	0.2	0.1	17	22	30
H (p)	0.04	0.2	0.1	14	21	30
I (p)	0.1	0.3	0.2	50	47	120
J (w)	0.04	0.2	0.1	42	34	100
K (p)	0.02	0.1	0.1	13	23	50
L (w)	0.02	0.3	0.5	19	24	31
M (w)	0.03	0.2	0.2	11	35	40
N (p)	0.06	0.2	0.3	31	25	58
O (w)	0.05	0.1	0.1	29	31	90
P (p)	0.05	0.2	0.2	20	32	110
Q (p)	0.05	0.2	0.2	12	29	91
V (p)	0.02	0.2	0.2	13	57	120
W (w)	0.02	4.5	5.1	83	132	240

p = permanent pasture

w = woodland

Table 3.4

Lead concentrations $\mu g\ g^{-1}$ for soil water extractions

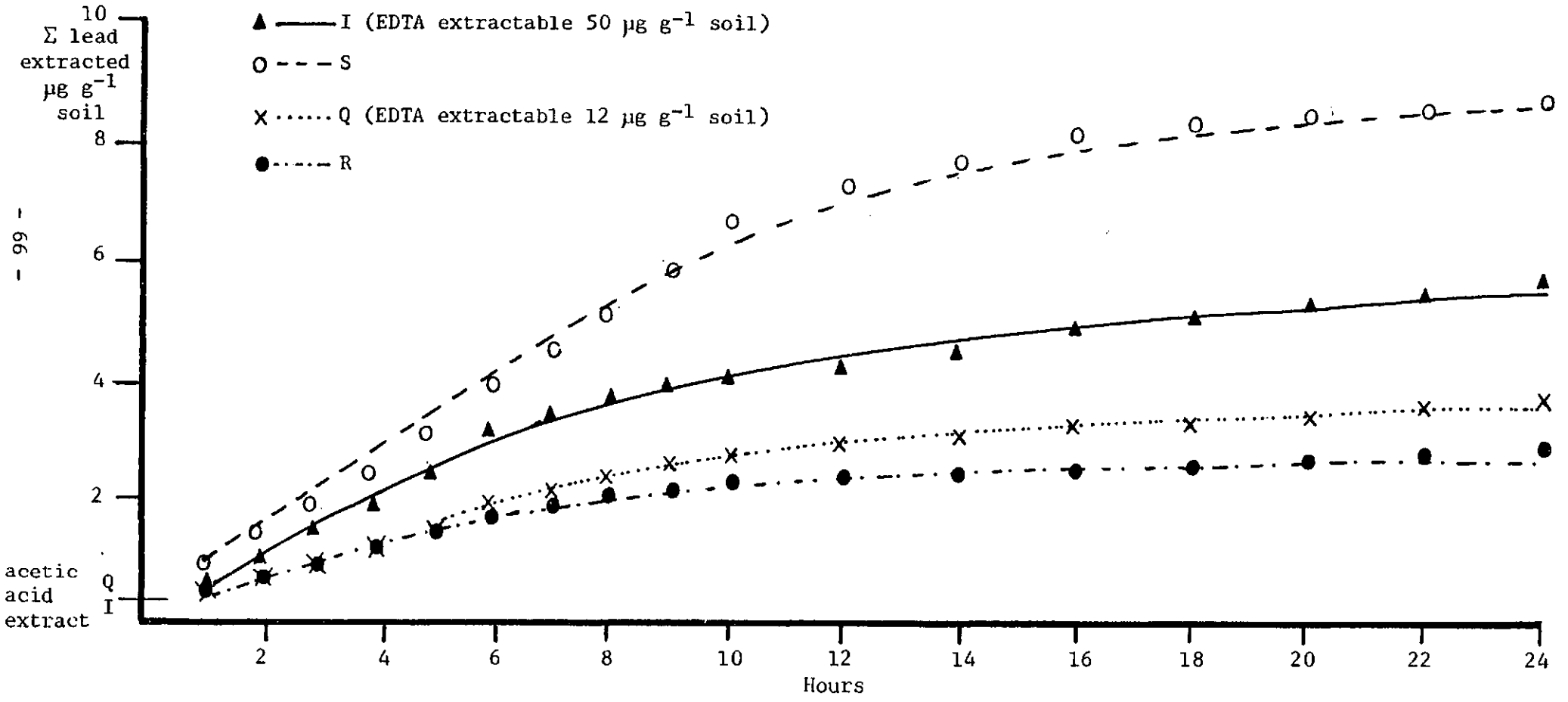
Hours	R (w)	Q (p)	S (p)	I (p)
1	0.4	0.4	0.7	0.5
2	0.3	0.3	0.7	0.6
3	0.3	0.3	0.6	0.5
4	0.3	0.3	0.6	0.5
5	0.2	0.3	0.7	0.4
6	0.2	0.3	0.7	0.3
7	0.2	0.2	0.6	0.3
8	0.2	0.3	0.7	0.4
9	0.1	0.3	0.7	0.3
10	0.1	0.2	0.8	0.3
12	0.1	0.2	0.5	0.3
14	0.1	0.1	0.4	0.3
16	0.1	0.1	0.4	0.3
18	0.1	0.1	0.3	0.2
20	0.1	0.1	0.1	0.3
22	0.1	0.1	0.1	0.2
24	0.1	0.1	0.1	0.2
Σ	3.0	3.7	8.7	5.9

p = permanent pasture

w = woodland

Fig. 3.2

A plot of lead extracted by repeated water extractions with time



3.3 Arsenic

Table 3.5 summarizes the data for arsenic determined from the various extraction methods. The values obtained for the pore waters correlate well with the values for the water extraction method. Extractions by acetic acid and EDTA gave values comparable to each other, being an order of magnitude greater than those for the pore water. The repeated wetting of samples (Table 3.6, Fig. 3.3) indicated that soluble extractable arsenic is greater than values obtained by the acetic acid or the EDTA extraction methods. Water extractions carried out on samples after a sodium dithionite extraction did not contain any arsenic. This suggests that arsenic associated with secondary precipitated iron is relatively soluble. Thus a single extraction with sodium dithionite is a good indicator of potentially soluble levels of arsenic in soils.

3.4 Spatial distribution patterns of copper, lead and arsenic in soils of the Tamar Valley

Soil samples were collected from 128 sites in the Tamar Valley (Fig. 3.4). The spatial distribution of soil 'A' horizon data (0-5cm) obtained by hot nitric acid and sodium dithionite for copper, lead and arsenic are shown in Figs. 3.5-3.10 (data appendix A). The data followed a characteristic positively skewed lognormal distribution common for geochemical data containing two populations, for example, the data for lead is shown in Figs. 3.11 and 3.12. The formula used to choose the number of intervals into which the data was sub divided is:

$$[K] = 10 \times \text{Log}_{10} N \qquad (14)$$

where $[K]$ = the interval, and N is the number of observations in the data. To obtain the interval width the largest numerical value is divided by $[K]$.

The criteria for the numerical contour construction (Figs. 3.5-3.10) was based upon background, threshold and anomalous values. One of the major objectives of regional geochemical surveys is to establish the

Table 3.5

Arsenic concentrations $\mu g\ g^{-1}$ obtained by various extraction techniques

Sample No.	Pore Water	Extractions				
		Water	Acetic Acid	EDTA	Sodium Dithionite	Nitric Acid
G (w)	0.04	0.02	0.7	0.2	5	15
H (p)	0.03	0.02	0.5	0.2	9	14
I (p)	0.11	0.32	2.6	2.5	18	130
J (w)	0.12	0.1	3.4	2.3	19	160
K (p)	0.05	0.04	1.0	0.6	9	16
L (w)	0.05	0.03	0.7	0.5	6	16
M (w)	0.02	0.03	1.1	1.1	5	19
N (p)	0.04	0.03	0.5	0.5	11	62
O (w)	0.04	0.08	0.7	0.9	10	90
P (p)	0.04	0.04	0.4	0.7	11	76
Q (p)	0.03	0.02	0.5	0.8	10	57
V (p)	0.08	0.1	2.2	2.2	10	49
W (w)	0.32	0.58	4.8	2.3	66	880

p = permanent pasture

w = woodland

Table 3.6

Arsenic concentrations $\mu g\ g^{-1}$ for soil water extractions

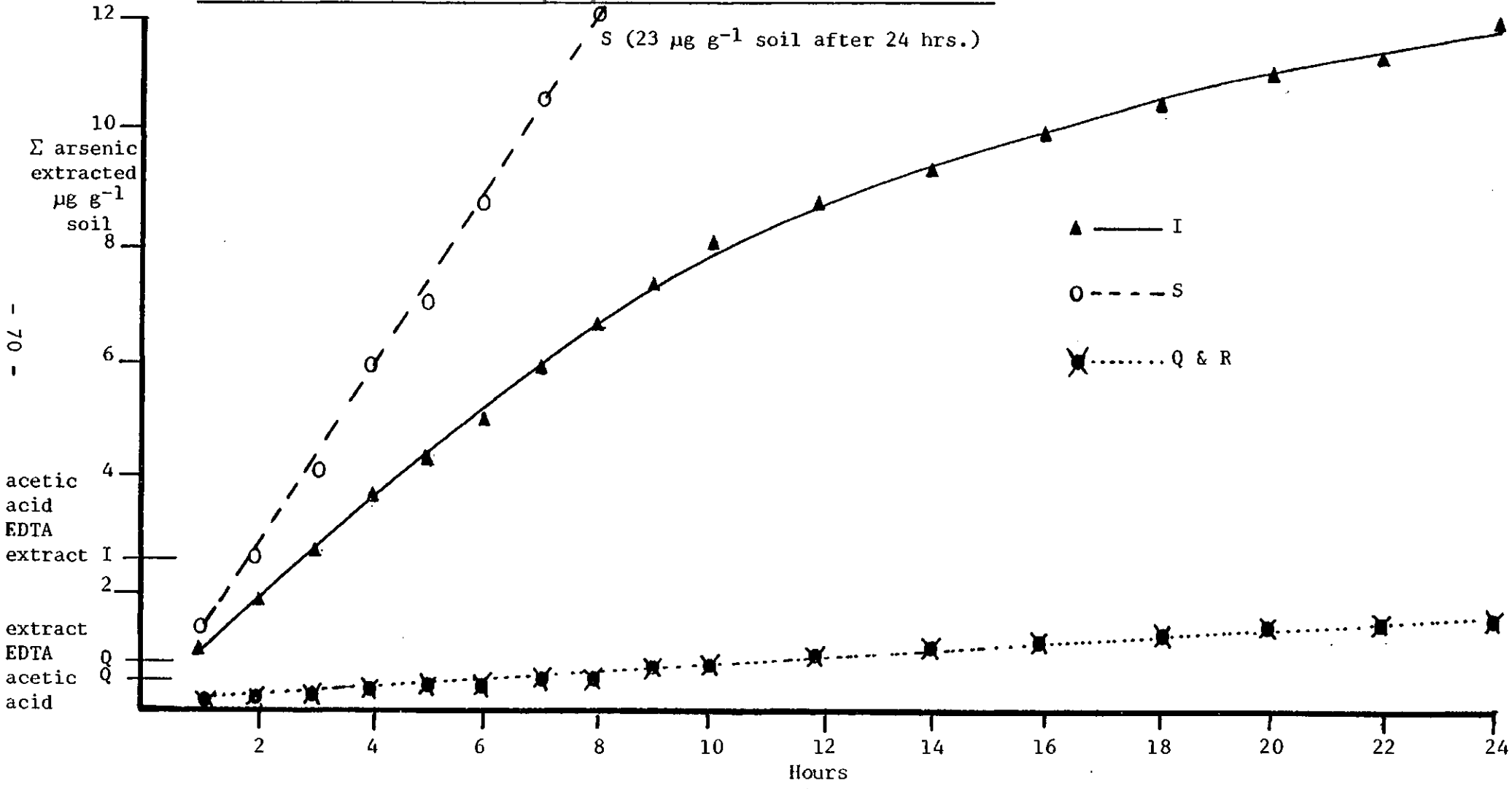
Hours	R (w)	Q (p)	S (p)	I (p)
1	0.1	0.1	1.5	1.1
2	0.1	0.1	1.1	1.0
3	0.1	0.1	1.6	0.7
4	0.1	0.1	1.9	1.1
5	0.1	0.1	1.2	0.7
6	0.1	0.1	1.8	0.7
7	0.1	0.1	1.6	0.7
8	0.1	0.1	1.5	0.7
9	0.1	0.1	1.4	0.8
10	0.1	0.1	1.5	0.7
12	0.1	0.1	1.1	0.6
14	0.1	0.1	1.0	0.7
16	0.1	0.1	1.1	0.5
18	0.1	0.1	1.1	0.6
20	0.1	0.1	1.2	0.5
22	0.1	0.1	1.3	0.5
24	0.1	0.1	1.1	0.6
Σ	1.7	1.7	23.0	12.2

p = permanent pasture

w = woodland

Fig. 3.3

A plot of arsenic extracted by repeated water extractions with time



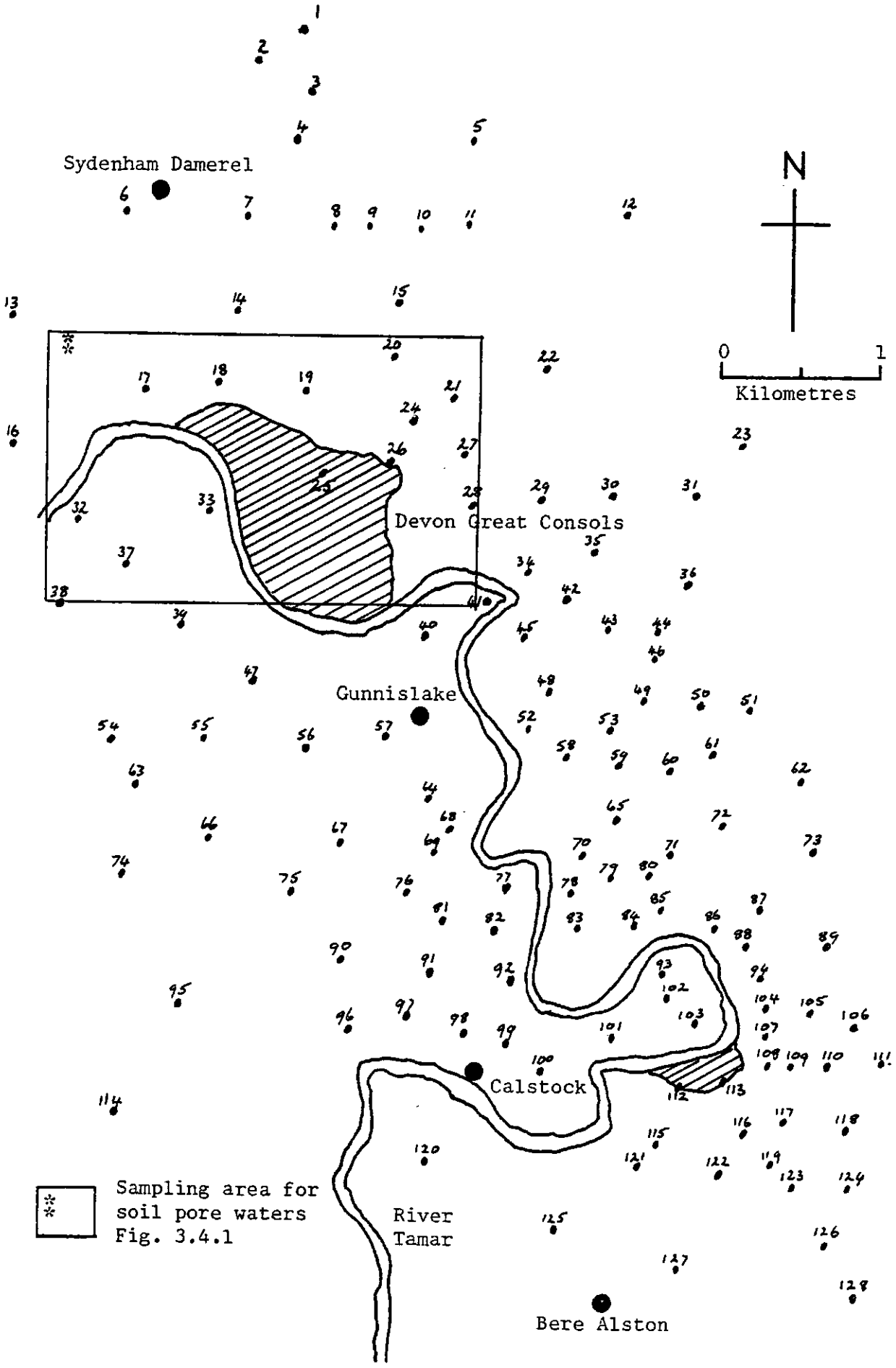


Fig. 3.4

Soil sampling sites in the Tamar Valley study area

Fig. 3.4.1

Soil pore water sampling sites

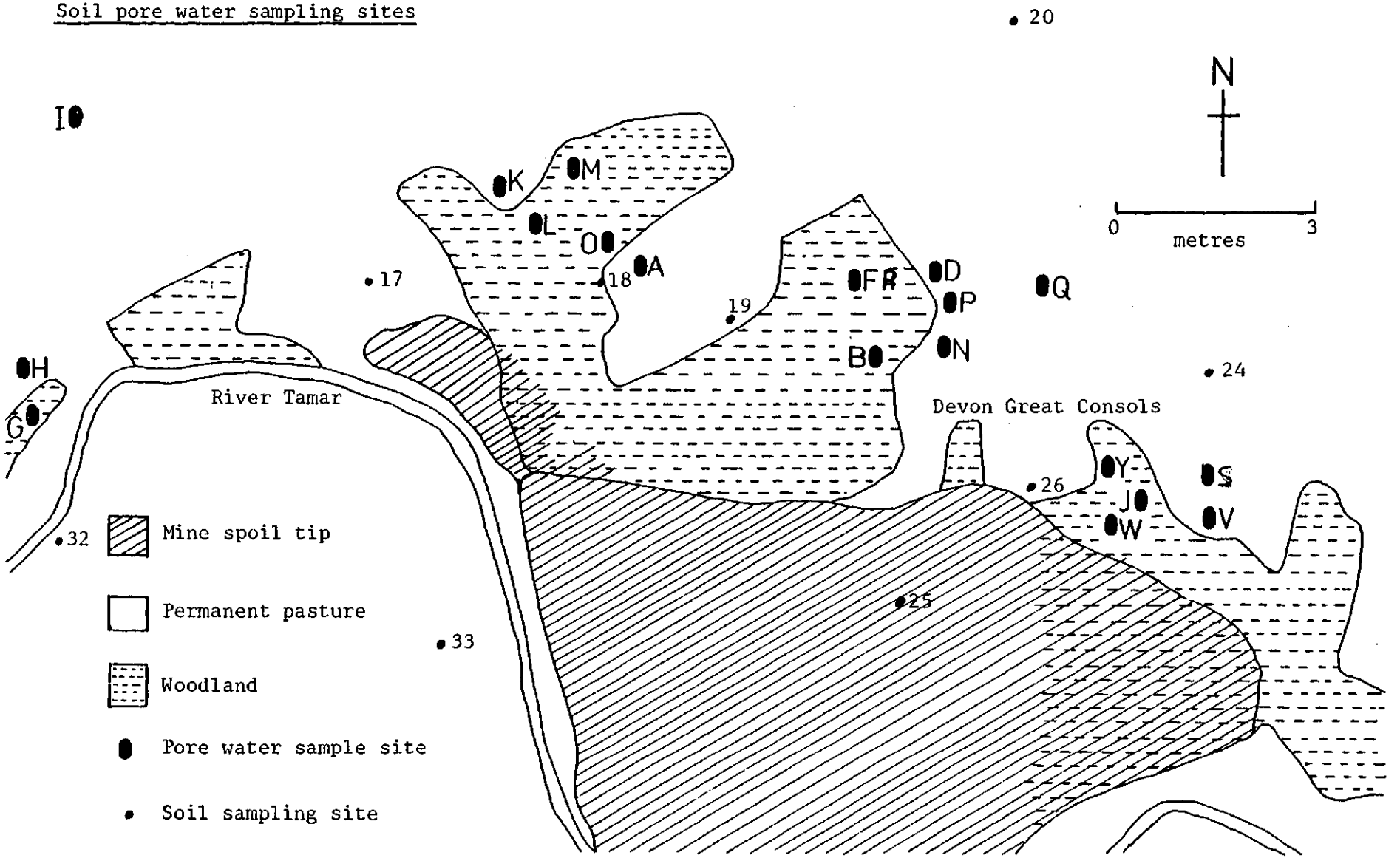


Fig. 3.5

Contour distribution patterns for soil 'A' horizon copper levels extracted by nitric acid

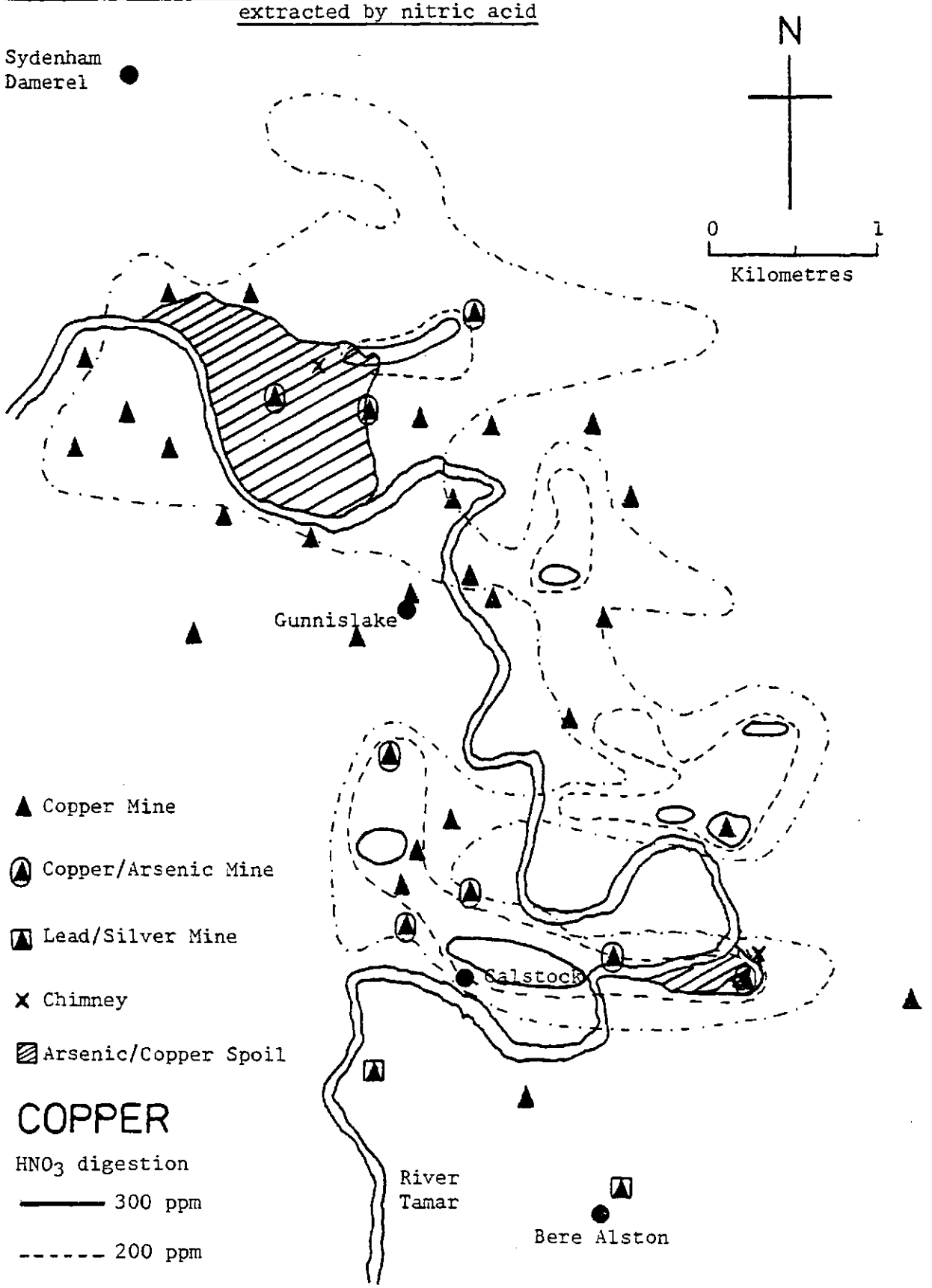
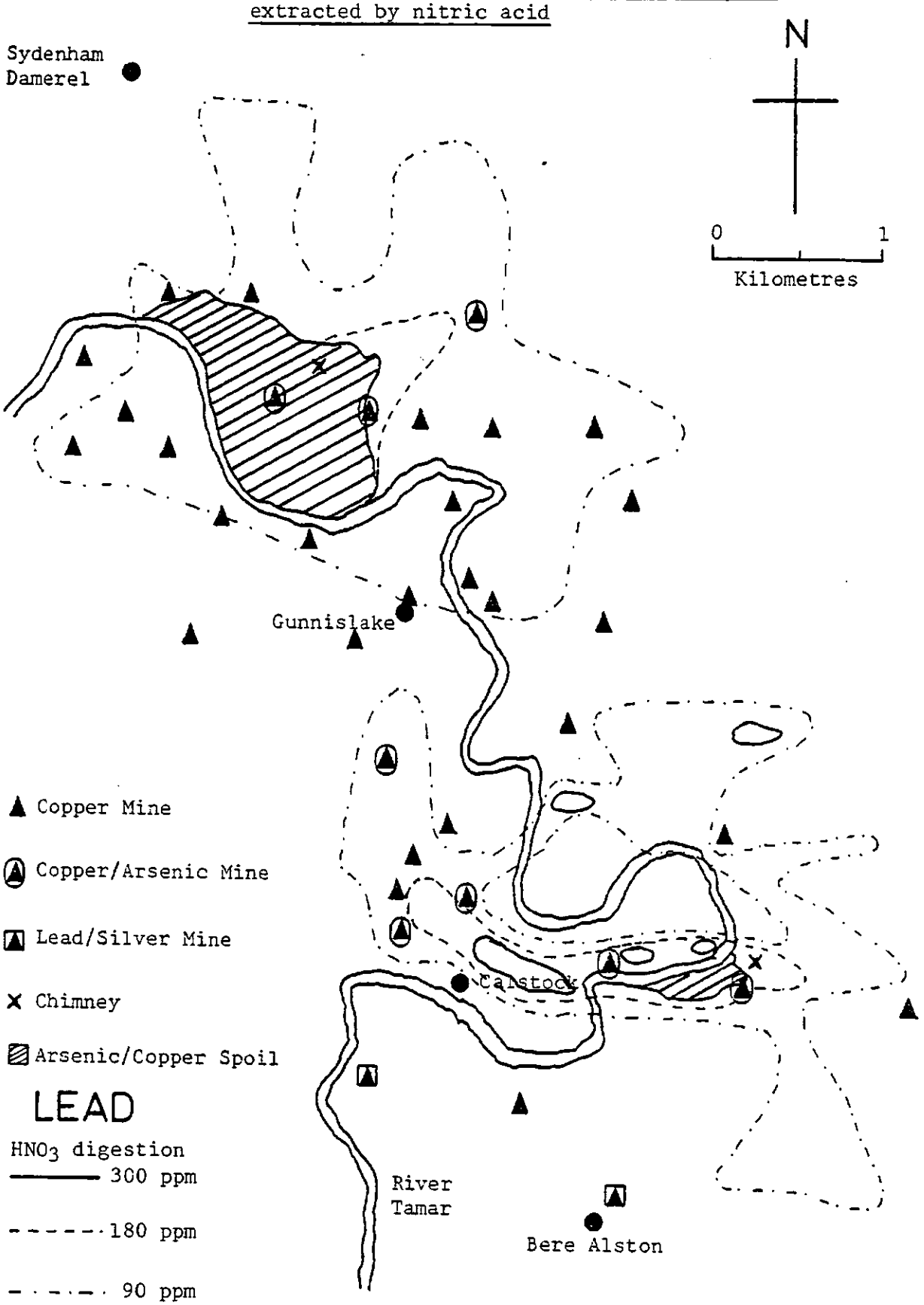


Fig. 3.6

Contour distribution patterns for soil 'A' horizon lead levels extracted by nitric acid



Mean background 87 ppm

Fig. 3.7

Contour distribution patterns for soil 'A' horizon arsenic levels extracted by nitric acid

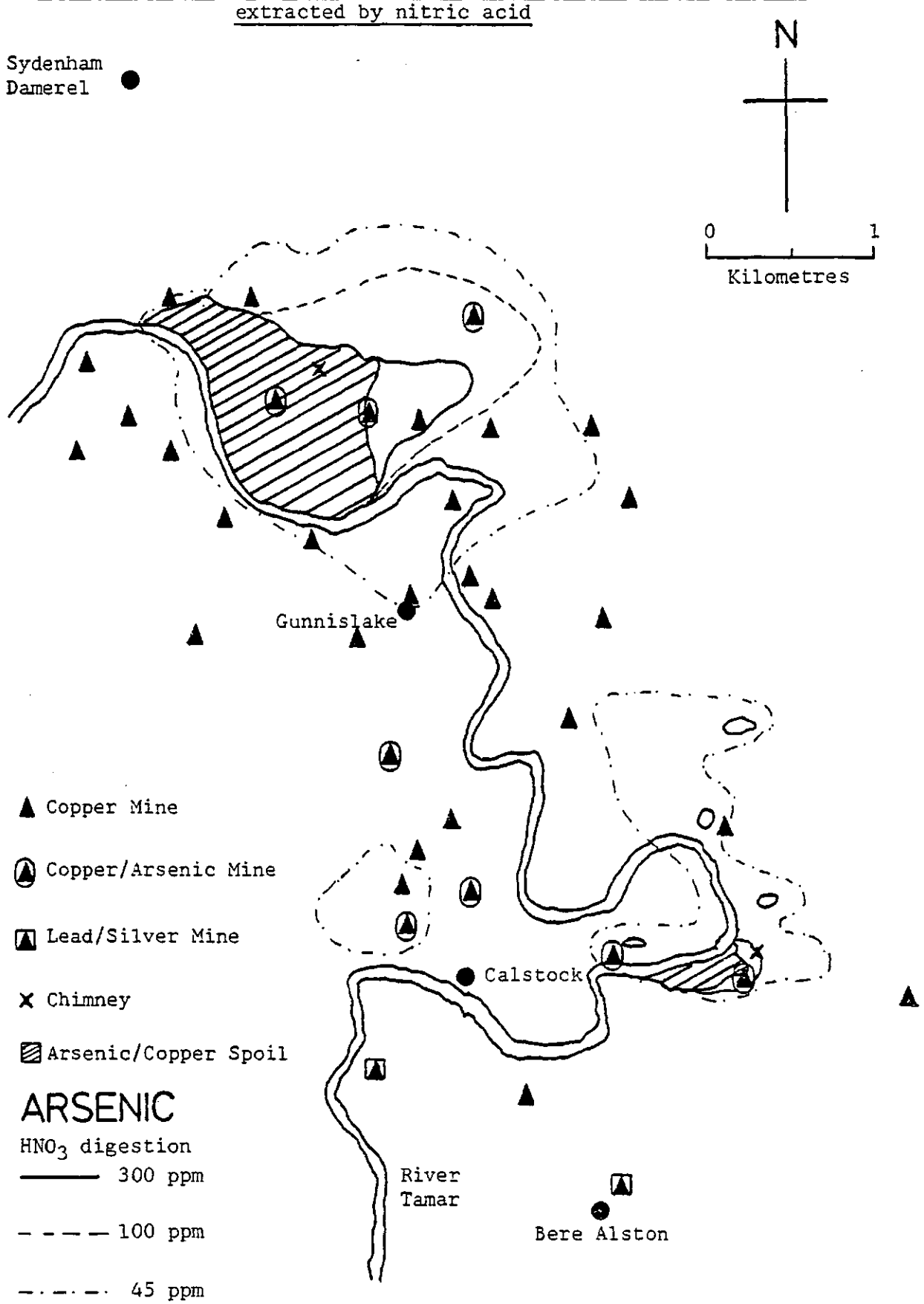


Fig. 3.8

Contour distribution patterns for soil 'A' horizon copper levels extracted by sodium dithionite

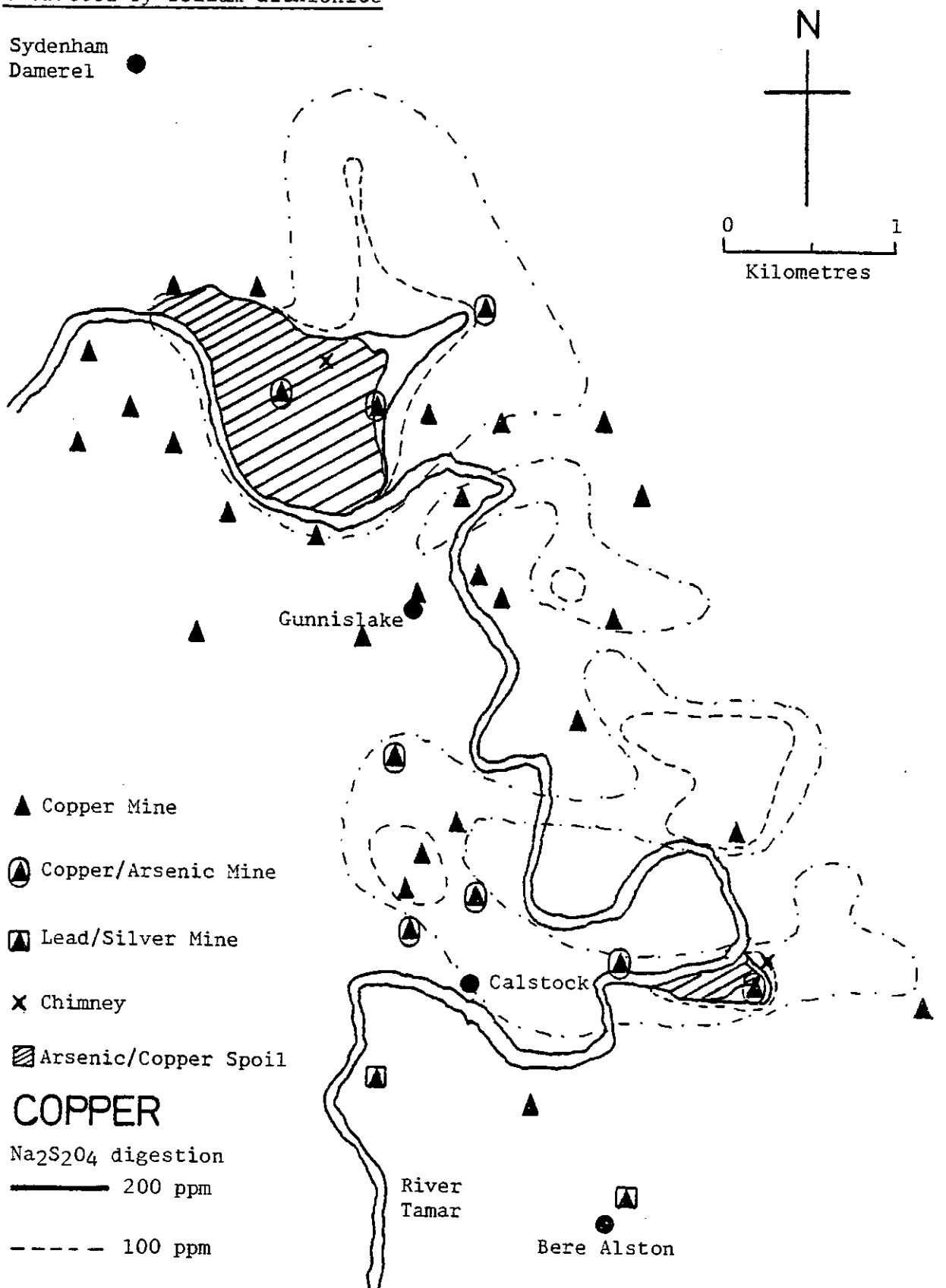


Fig. 3.9

Contour distribution patterns for soil 'A' horizon lead levels extracted by sodium dithionite

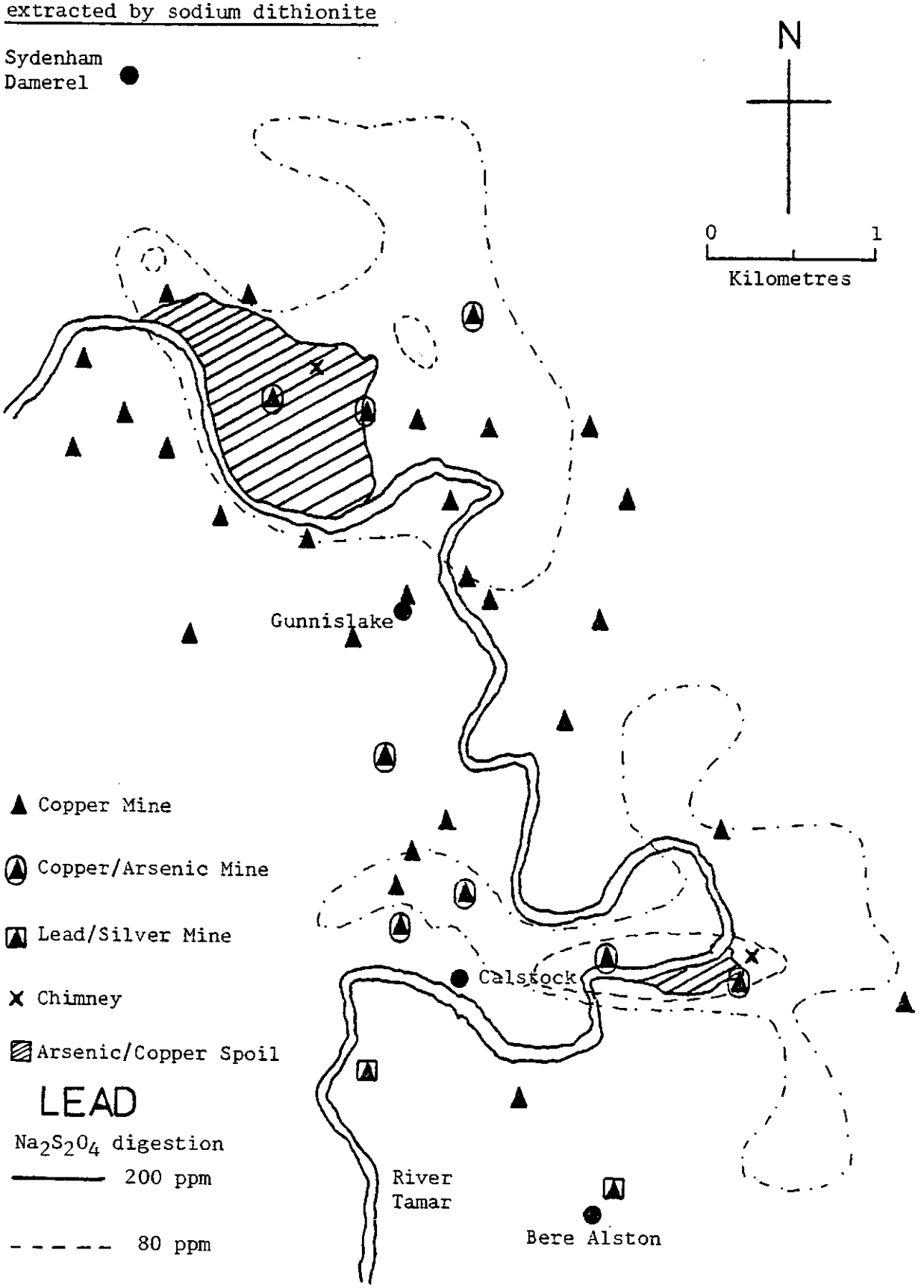


Fig. 3.10

Contour distribution patterns for soil 'A' horizon arsenic levels extracted by sodium dithionite

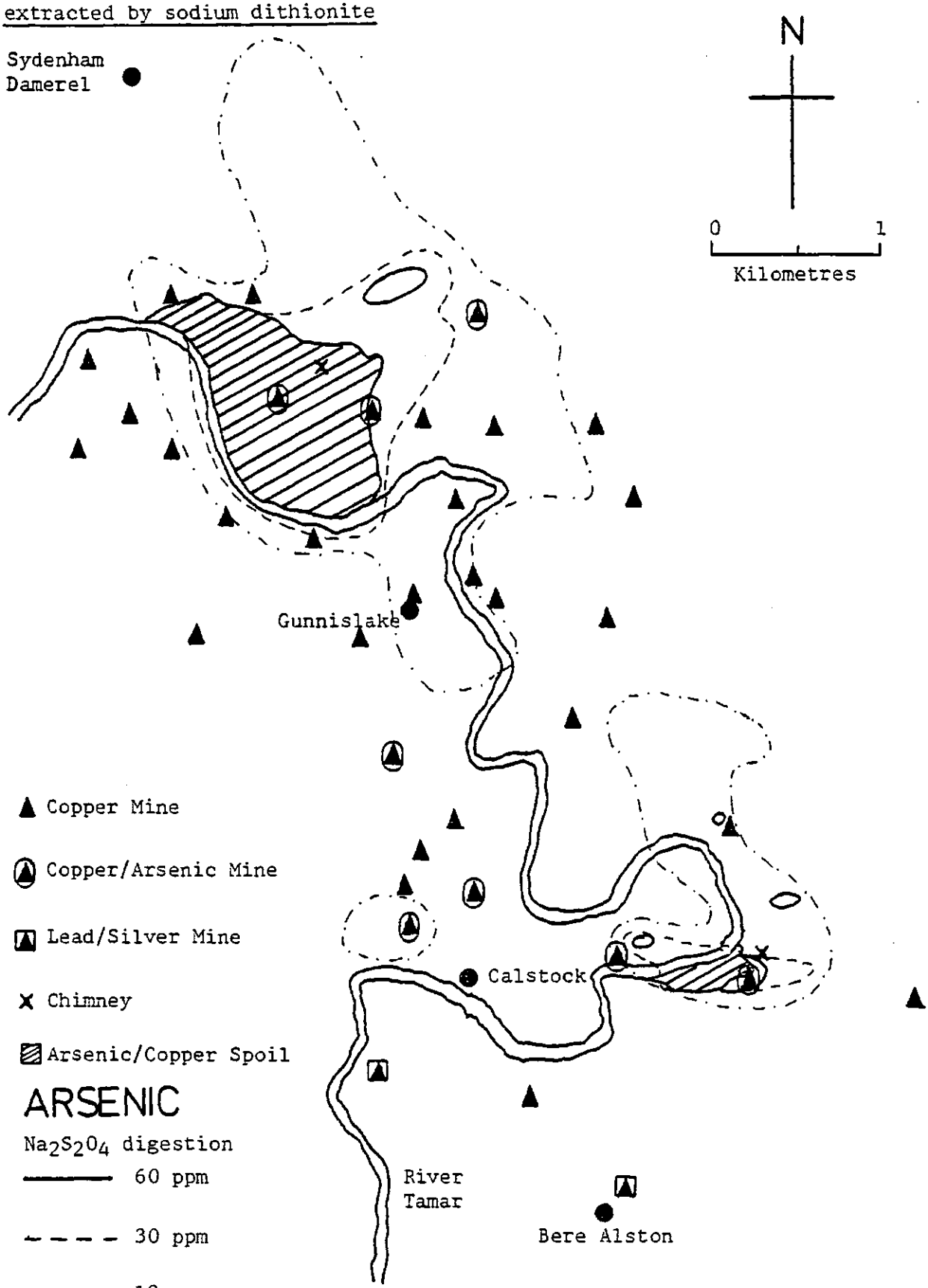
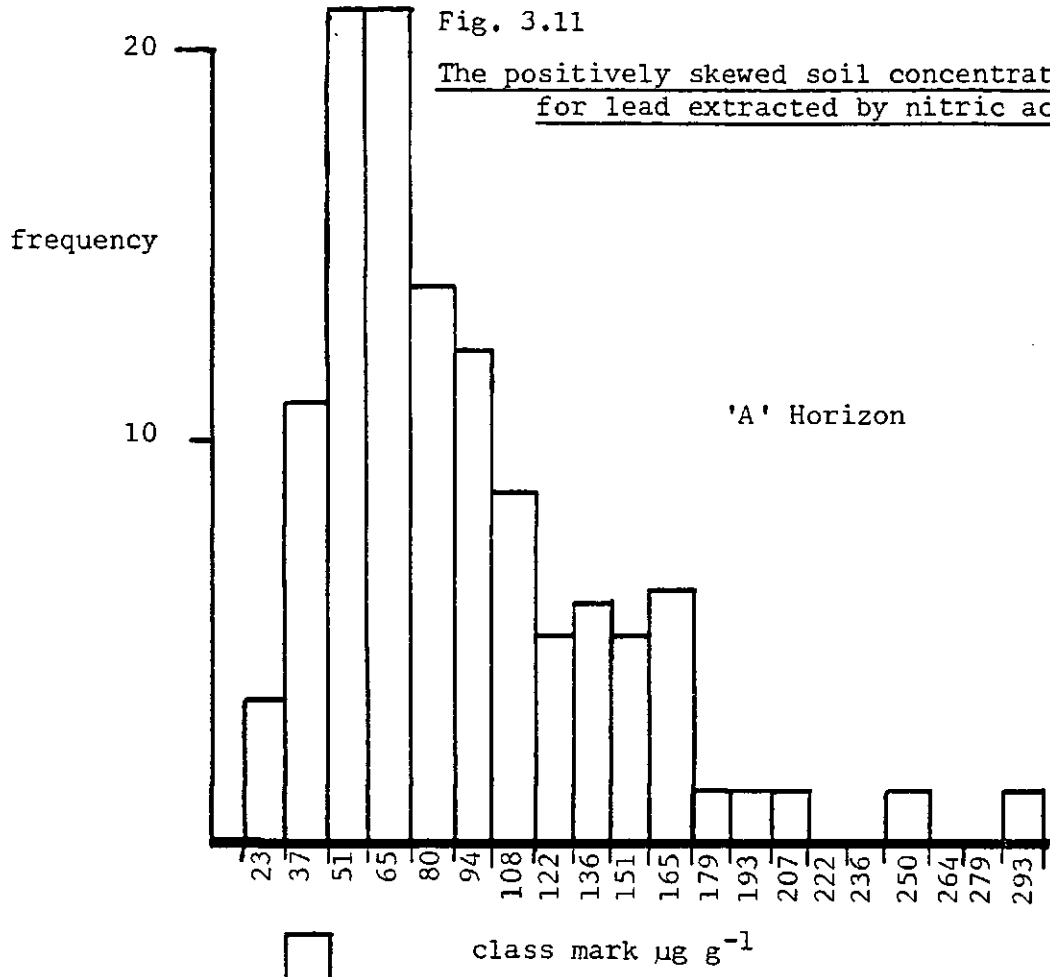


Fig. 3.11

The positively skewed soil concentration data for lead extracted by nitric acid



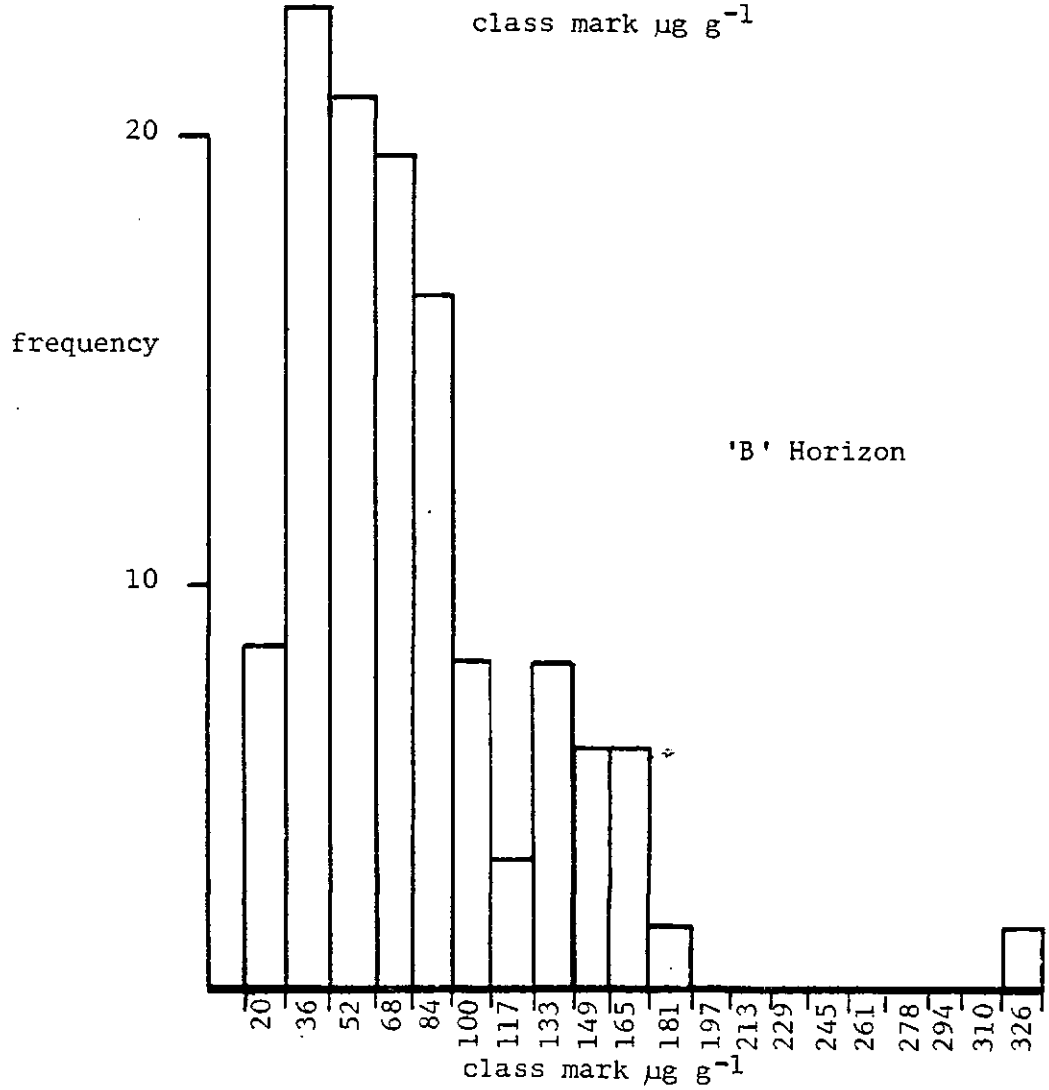
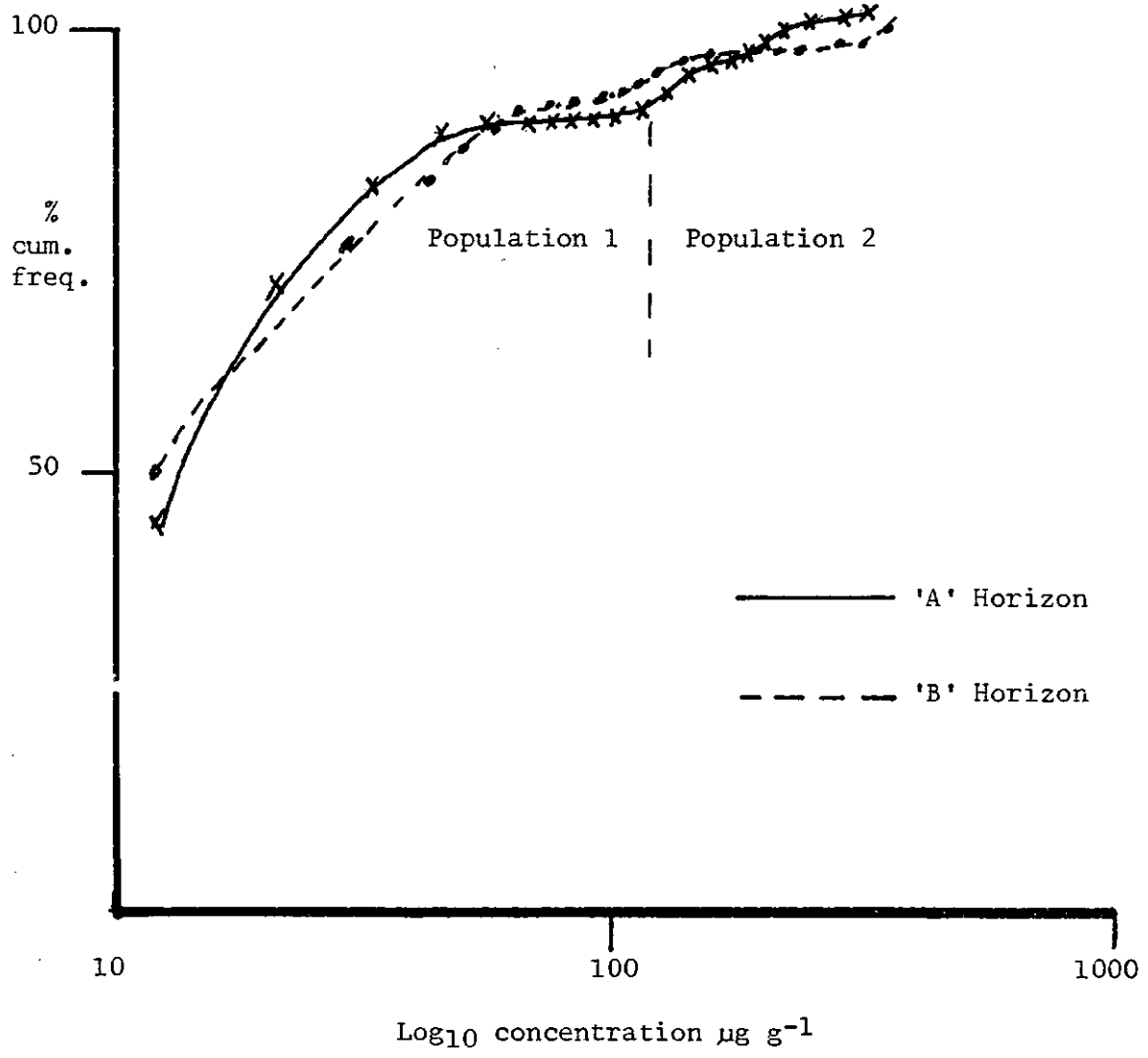


Fig. 3.12

Percentage cumulative frequency against soil lead concentrations extracted by nitric acid



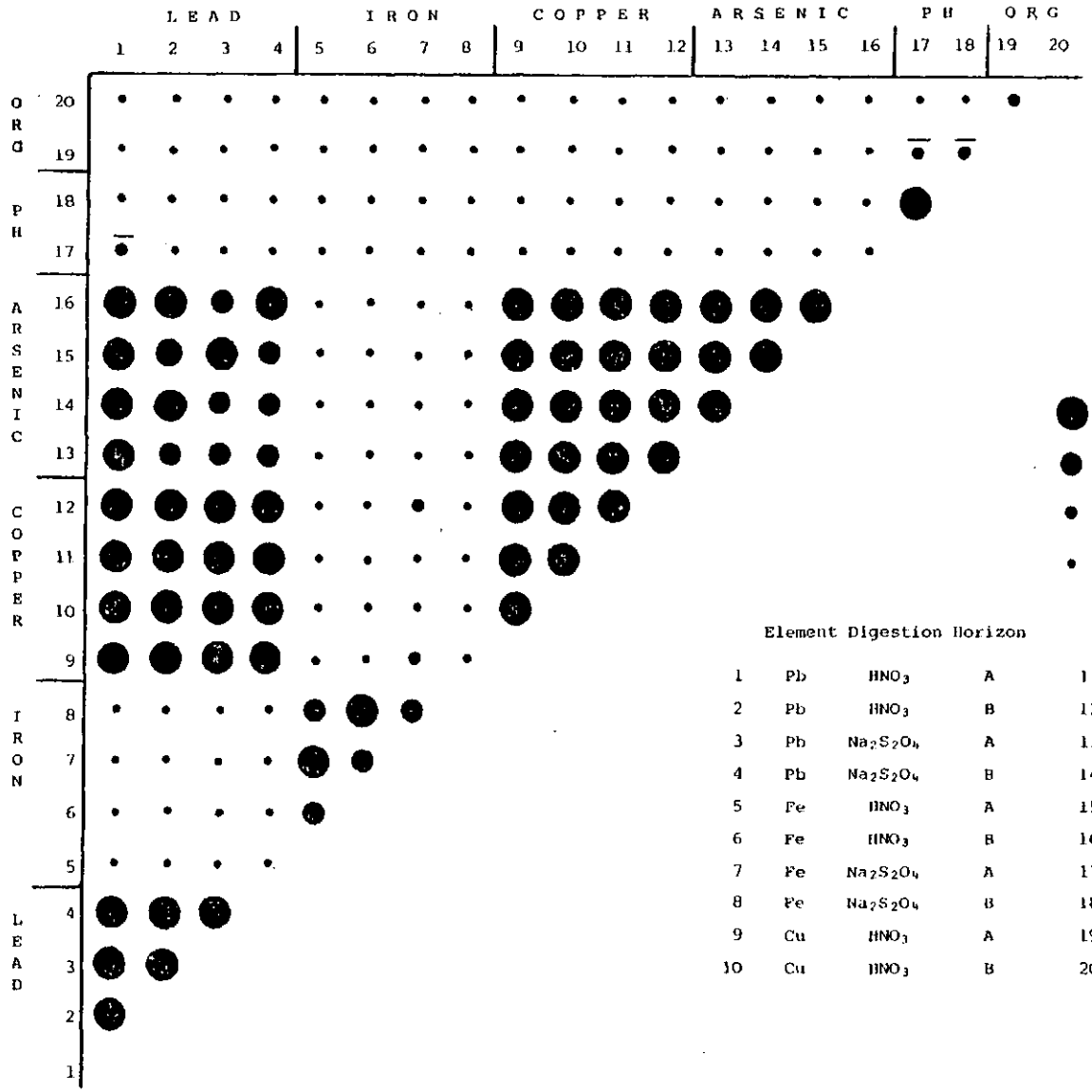
background variation of an element. The background may be considered to be the normal range of concentrations for an element, or elements, in an area. Once a regional background has been obtained the threshold and anomalous values may be determined. The threshold is the upper limit of normal background values and is obtained statistically from the data, it follows that anomalous values are those above a predetermined threshold. The effects of mineralization on the background values is better illustrated when the higher mean value is used rather than the mode. A high correlation (Fig. 3.13) between soil 'A' and 'B' horizons is obtained for the data, and only 'A' horizon data has been used for the distribution diagrams. As indicated the 'A' horizon samples were collected from a 0-5cm depth, however, as the 'B' horizon is often poorly defined in the soils studied, where no obvious horizon was present samples from the base of the profile were collected.

3.5 Hot nitric acid digestion

The hot nitric acid digestion is reported to extract sulphide associated elements and 80-100% of the total levels present (69). Six replicate extractions with hot nitric acid on certified sediment reference material (Table 2.4) resulted in removal of 100 $\pm$ 2% arsenic, 96 $\pm$ 3% copper, 60 $\pm$ 7% lead and 70 $\pm$ 4% iron. Soil copper (Fig. 3.5) is generally seen to reflect the underlying mineralization which follows an east-west orientation (Fig. 3.14). Two variations from this pattern are observed, (i) the area to the east of Sydenham Dameral has elevated soil copper levels extending north of the historically known copper veins (Fig. 3.14), indicating possible unrecorded mineralization; (ii) the distribution of copper mineralization is extensive on both sides of the River Tamar (Fig. 3.14), certain areas however are not defined by the distribution contours (Fig. 3.5), this is thought to be a function of low sampling density in these areas. Lead displays a similar spatial pattern (Fig. 3.6) and strong correlation with copper (Figs. 3.5, 3.13). Reports (1) on copper mining activities in the survey area indicate that chalcopyrite ($CuFeS_2$) and pyrite (FeS_2) were commercially extracted, with no reference being made to lead minerals. The lead associated with copper shown by this survey is thought to be either related to uneconomic deposits and/or

Fig. 3.13

DATA CORRELATION MATRIX



	Element	Digestion	Horizon		Element	Digestion	Horizon
1	Pb	HNO_3	A	11	Cu	$Na_2S_2O_4$	A
2	Pb	HNO_3	B	12	Cu	$Na_2S_2O_4$	B
3	Pb	$Na_2S_2O_4$	A	13	As	HNO_3	A
4	Pb	$Na_2S_2O_4$	B	14	As	HNO_3	B
5	Fe	HNO_3	A	15	As	$Na_2S_2O_4$	A
6	Fe	HNO_3	B	16	As	$Na_2S_2O_4$	B
7	Fe	$Na_2S_2O_4$	A	17		pH	A
8	Fe	$Na_2S_2O_4$	B	18		pH	B
9	Cu	HNO_3	A	19	Organic		A
10	Cu	HNO_3	B	20	Organic		B

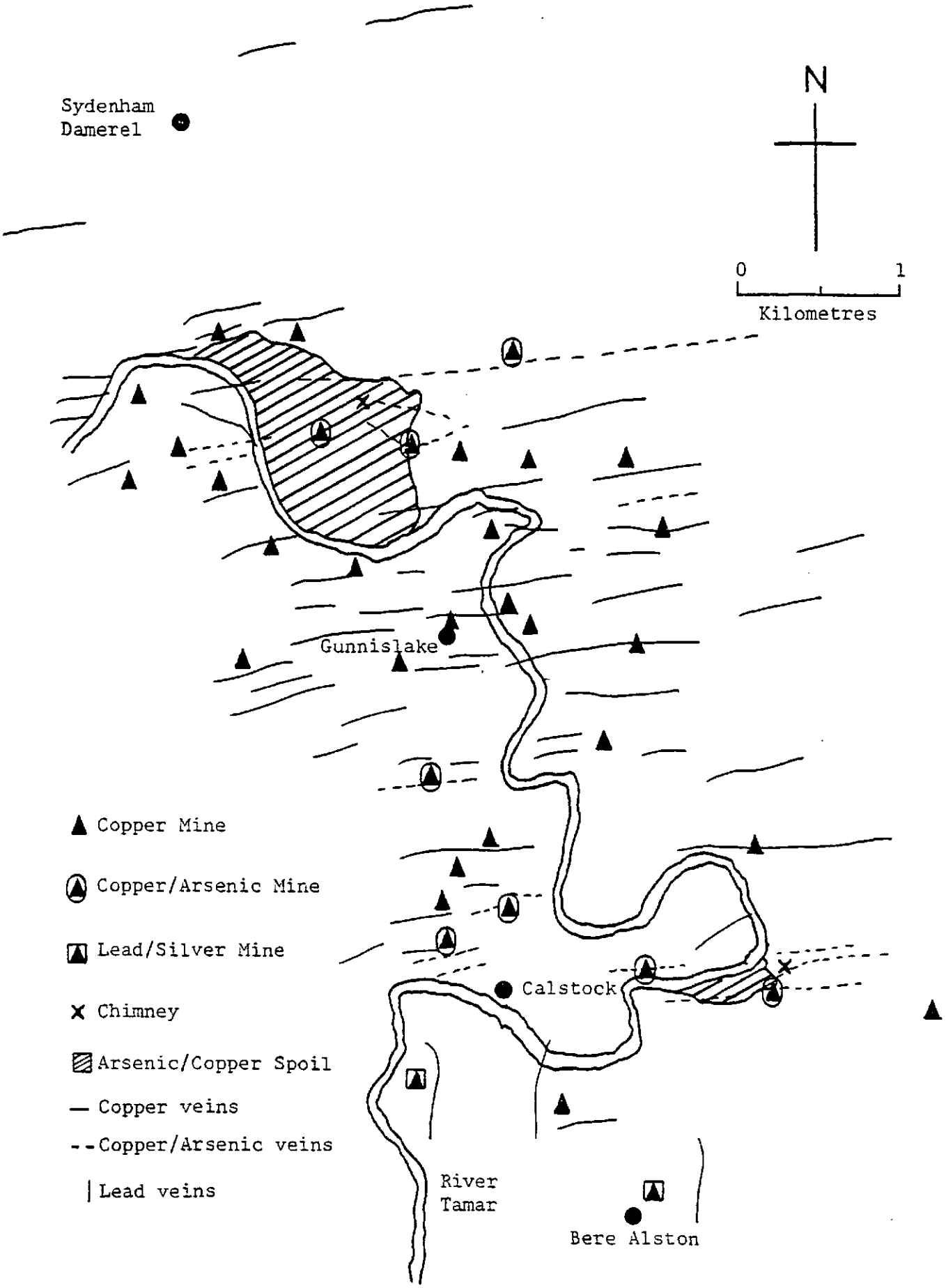


Fig. 3.14

The metalliferous deposits of the Tamar Valley study area

lead associated with pyrite mineralization (304). Lead levels in the region to the north-east of Bere Alston correspond to lead, lead/silver veins (Fig. 3.14). It is noted that there is no copper distribution (Fig. 3.5) corresponding with this region, unlike lead which is found associated with copper mineralization (Fig. 3.6). This may be a reflection of the later deposition of lead and silver or a geochemical deposition (1) effect corresponding to zones emanating from the granite mass in the order arsenic, copper and lead. Thus lead may be present with arsenic and copper mineralization, but the latter two will have been removed from solution by precipitation and will therefore, not be found in mineralized forms with lead. Arsenic displays a smaller geographical (Fig. 3.7) and mineralized distribution (Fig. 3.14) than that of copper and lead. A more circular distribution pattern most noted around the large spoil tips in the north section of the area may indicate that the soils have been modified by aerial and physical dispersion of arsenic subsequent to the mining activity. The largely unvegetated tips are unconsolidated and are therefore exposed to wind erosion and transportation. Evidence of aerial pollution of arsenic, resulting directly from the smelting, giving a characteristic contaminant plume pattern emanating from the source chimney, was not evident, however, it is reasonable to suppose regional contamination during processing will have elevated the surrounding soil arsenic levels. The use of spoil tip material by farmers as hardcore, and for field levelling, introduced a physical dispersion problem, but often these areas are characterized by their poor vegetation growth and must be considered atypical of the natural soil levels. The mean background values used for copper, lead and arsenic 106, 89 and 45 $\mu g\ g^{-1}$ respectively in the soils of the survey area are similar to those reported by previous workers (6, 10), being significantly higher than those for non-mineralized areas, typically 25.8 (Cu), 29.2 (Pb) and 11.3 (As) $\mu g\ g^{-1}$ (305).

3.6 Sodium dithionite digestions

Data from the sodium dithionite digestions is reported to reflect elemental association with secondary crystalline iron oxides (33, 73). The naturally occurring sulphide minerals of arsenic will slowly be weathered into the soil matrix and small amounts will eventually be

converted by microbial and chemical processes to the more soluble oxides of arsenic (3). They will then be adsorbed onto the surfaces of precipitates of secondary crystalline iron oxides (306), leading to a more elevated background value than that obtained for a non-mineralized area (305). Soil samples which give above the background levels for the sodium dithionite extraction indicate either an additional input of arsenic oxide, or a more rapid weathering process is taking or has taken place. It has been suggested that atmospheric dispersion may contribute a substantial input in areas surrounding the site of past mining activity. It is unlikely due to the situation of these sites (i.e. based towards the valley bottom) that hydromorphic transportation of on site weathered materials will have any major effect on the secondary iron soil levels above the spoil tips. The distribution patterns of copper (Fig. 3.8), lead (Fig. 3.9) and arsenic (Fig. 3.10), are similar to those obtained with the hot nitric acid digestion (Figs. 3.5-3.7). Arsenic and lead show slight broadening northwards, a possible indication of wind dispersion from spoil tips and smelting activities, this data however gives no conclusive evidence of these effects. The relative percentages of copper, lead and arsenic extracted by sodium dithionite to that for hot nitric acid are 54%, 39% and 35% respectively. Copper and lead are reported to be associated with clay and organic matter (70, 73, 111, 307), whilst copper, lead and arsenic are all associated with iron and aluminium oxides (33, 73). The copper, lead, arsenic profile distributions (Table 3.7) and data correlation (Fig. 3.13) are of little practical use in illustrating their association with iron, as (a) the relative variation in iron levels is too small to be significant in the matrix correlation calculation, and (b) the absolute levels of iron are greatly in excess of those for the trace elements (100-1000 times) examined, thus from experimental evidence the high percentages of copper, lead and arsenic corresponding to secondary crystalline iron oxides 35-54%, indicate iron concentrations are adequate to offer an abundance of absorption sites. There was no indication of any correlation for copper, lead and arsenic with organic matter or pH (Fig. 3.13). Organic matter in the 'A' horizon gave a negative correlation with pH (Fig. 3.13), indicating as might be expected that as organic content increased the pH decreased.

Table 3.7

Soil profile data
(see Fig. 3.15 page 90)

Pit No.	Depth cm	pH	% Organic	Hot nitric acid digestion				Sodium dithionite digestion			
				µg g^{-1} Cu	µg g^{-1} Pb	µg g^{-1} As	% Fe	µg g^{-1} Cu	µg g^{-1} Pb	µg g^{-1} As	% Fe
1	0	4.6	14.5	88	86	16	2.8	36	29	5.0	2.5
	10	4.5	10.8	98	85	17	3.6	41	27	4.8	2.0
	20	5.1	9.1	90	71	16	3.9	34	29	6.0	2.4
	30	5.5	6.9	65	60	18	4.1	22	22	3.0	2.4
	40	5.8	6.6	51	57	9	3.7	18	20	3.1	2.6

continued ...

Pit No.	Depth cm	pH	% Organic	Hot nitric acid digestion				Sodium dithionite digestion			
				µg g^{-1}			%	µg g^{-1}			%
				Cu	Pb	As	Fe	Cu	Pb	As	Fe
2	0	3.8	17.0	92	110	124	5.1	62	71	83	3.7
	10	3.9	12.6	82	100	95	5.5	57	69	62	4.6
	20	4.0	11.4	103	110	129	5.9	70	72	81	4.1
	30	4.2	7.9	80	80	108	5.9	37	39	56	4.3
	40	4.2	6.6	75	37	56	5.9	36	13	21	4.6
	50	4.2	6.3	65	37	20	5.8	27	15	7	4.5
	58	4.2	6.7	60	31	24	6.0	25	12	8	4.3
3	0	5.5	12.6	314	720	13	3.9	83	180	5.0	2.9
	10	5.7	9.6	124	740	14	3.8	23	160	4.3	2.8
	20	6.0	9.6	126	720	14	3.6	17	140	5.3	2.3
	30	6.1	7.1	46	280	14	4.1	12	59	5.1	2.3
	40	6.4	5.3	44	110	16	4.5	11	31	4.2	2.4
	50	6.5	5.1	41	100	18	4.4	12	27	3.9	2.3

continued ...

Pit No.	Depth cm	pH	% Organic	Hot nitric acid digestion				Sodium dithionite digestion			
				μg g^{-1} Cu	μg g^{-1} Pb	μg g^{-1} As	% Fe	μg g^{-1} Cu	μg g^{-1} Pb	μg g^{-1} As	% Fe
4	0	6.5	15.5	57	65	14	4.5	24	24	3.0	3.3
	10	6.5	13.1	53	57	16	4.7	22	19	5.0	3.4
	20	6.5	10.9	51	61	18	4.9	25	21	4.5	3.2
	34	6.4	8.2	53	58	15	5.0	21	17	3.7	3.7
5	0	5.3	18.7	152	210	64	4.7	98	140	45	3.2
	10	5.3	12.3	180	230	60	5.0	130	160	43	3.3
	20	5.7	9.9	175	190	45	5.0	120	110	26	3.0
	30	5.9	9.1	62	90	16	5.1	27	42	8	2.8
	40	5.9	8.5	60	83	14	5.5	21	37	5	3.2

continued ...

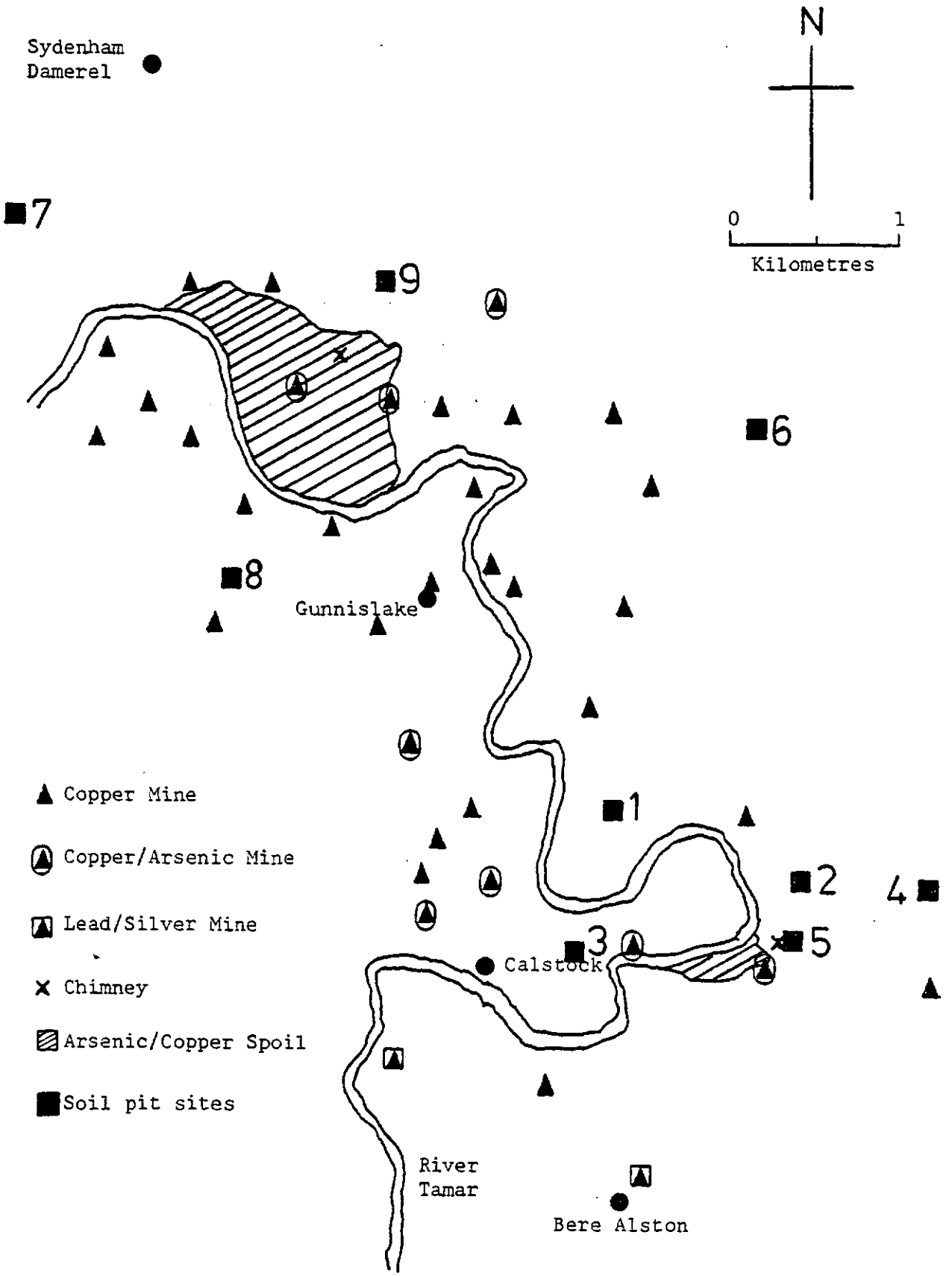
Pit No.	Depth cm	pH	% Organic	Hot nitric acid digestion				Sodium dithionite digestion			
				µg g^{-1}			%	µg g^{-1}			%
				Cu	Pb	As	Fe	Cu	Pb	As	Fe
6	0	6.0	18.0	65	82	23	4.0	27	31	8.0	2.3
	10	6.1	12.1	64	77	17	4.0	25	29	6.6	2.4
	20	6.0	8.3	62	77	11	3.9	21	28	4.3	3.1
	30	6.0	6.5	69	74	14	4.6	23	27	5.6	3.4
7	0	6.0	14.3	40	45	19	5.9	18	18	6.7	3.4
	10	6.2	23.0	39	46	15	4.7	16	19	6.0	3.2
	20	6.4	9.6	40	48	15	4.9	16	18	5.7	3.0
	30	6.3	4.3	32	36	13	4.8	12	15	4.6	3.2
	40	6.3	5.6	32	38	10	5.0	11	16	3.1	3.3
	50	6.4	3.8	31	45	9	4.7	10	18	3.0	3.3
	60	6.6	3.1	34	32	9	4.6	12	15	3.2	3.1

continued ...

Pit No.	Depth cm	pH	% Organic	Hot nitric acid digestion				Sodium dithionite digestion			
				µg g^{-1}			%	µg g^{-1}			%
				Cu	Pb	As	Fe	Cu	Pb	As	Fe
8	0	6.3	13.1	61	48	28	2.1	61	19	9.7	1.7
	10	6.4	11.0	65	51	17	2.3	65	21	6.2	1.6
	20	5.7	9.7	66	48	24	2.2	66	18	8.0	1.7
	30	5.8	5.1	47	30	17	1.9	47	14	6.0	1.2
	40	5.9	5.1	36	28	20	1.7	36	10	6.3	1.2
	50	5.9	5.4	33	27	13	1.5	33	9	5.7	1.3
9	0	6.0	17.0	170	98	120	5.2	120	73	86	2.9
	10	6.0	10.3	170	93	110	5.7	120	61	84	3.3
	20	5.5	7.5	160	86	110	5.3	91	49	79	4.2
	30	5.1	5.2	150	84	97	3.3	83	35	61	2.9
	40	4.9	3.1	130	67	61	3.7	58	27	27	2.8
	50	5.0	3.8	94	64	63	4.0	41	24	21	2.3

Fig. 3.15

Soil pit locations



3.7 Soil profiles and the distribution of copper, lead and arsenic

At selected points in the survey area (Fig. 3.15) soil profiles were collected to represent non-mineralized and non-aerial polluted sites (Table 3.7, profiles 4, 6 and 7). These sites indicate copper, lead, arsenic and iron homogeneaity for soils varying in depth from 34 to 60cm, these results are in agreement with those published and reviewed by Bowen (305). Surface enrichment (0-20cm) occurred for copper, lead and arsenic in regions effected by direct mineralization (Table 3.7, profiles 3, 8) and/or probable surface contamination (Table 3.7, profiles 2, 5, 9), except for profile 3 (Table 3.7) where arsenic showed lower values at surface increasing with depth. High levels in the upper layers in soil overlying mineralization do not follow the expected or reported trend of McKenzie (108), who indicates elevation in the lower region of the profile, Karin (100), however, suggests that in shallow silty soil surface enrichment may occur, but the mechanism is still unclear. In these particular samples it is probable that surface contamination may be responsible for the observed profile dispersion and that mineralization is either too deep or the shallow soils are well mixed at depth. Due to the relative concentrations of arsenic and iron no significant association was observed in the soil profiles, however, 35% of the total arsenic present is known to be related with the iron.

3.8 General discussion

The results obtained for the different extraction methods indicate that soluble copper and lead are associated mainly with exchangeable weak inorganic complexes. Arsenic, however, does not show this trend, but is associated with secondary crystalline precipitated iron. When selecting a single extraction technique for the quantification of potentially soluble copper and lead EDTA is recommended, whereas for arsenic sodium dithionite should be used. The three elements follow the order arsenic > copper > lead for complexation with inorganic and secondary iron, and the order lead > copper > arsenic for organic associations.

Little detailed work has been published on the distribution patterns of trace elements in soils developed on the Upper Devonian and Lower Carboniferous sediments effected by the igneous intrusions associated with the granite masses of Dartmoor and Bodmin Moor. Davies (6) collected soil samples from pastures and gardens from within and outside the mineralized area, and reported no regional variations for cobalt, iron or manganese, but within the mineralized area cadmium, copper, lead, silver and zinc levels were abnormally high. Typical background values for copper and lead were 58 and 110 $\mu g\ g^{-1}$ with anomalous values of 684 and 522 $\mu g\ g^{-1}$. A more recent survey by Colbourn (10) gives background values for arsenic, copper and lead in the Tamar Valley as 27 (As), 62 (Cu) and 121 (Pb) $\mu g\ g^{-1}$ and levels for upland mining areas as 385 (As), 314 (Cu) and 215 (Pb) $\mu g\ g^{-1}$. The background values obtained in this work for arsenic, copper and lead were 45 (As), 106 (Cu), 89 (Pb) $\mu g\ g^{-1}$, with anomalous values of 380 (As), 430 (Cu), 300 (Pb). Although the anomalous values are in close agreement with Colbourn's results (10), both sets of data are approximately half of those values given by Davies (6), in addition the background values given by both Colbourn (10) and Davies (6) are less than those obtained in this study. In all cases the background values are higher than those reported as normal background values by Bowen (305) for non-mineralized areas typically 11 (As), 26 (Cu) and 29 (Pb) $\mu g\ g^{-1}$. The percentage elemental recoveries obtained in this study for the hot nitric acid digestion were 100 $\pm$ 2% arsenic, 96 $\pm$ 3% copper and 60 $\pm$ 7% lead; with results based on elemental extractions from certified reference material (Table 2.4). Colbourn (10) and Davies (6) both used strong hot acid extractions to obtain total elemental levels, but no details are given on the percentage recovery of the techniques or the statistical manipulation carried out on the results.

Direct comparison of the results of these various surveys are therefore difficult, however, it is clear that the soils within the defined study area contain naturally elevated levels of arsenic, copper and lead, and that intra study comparisons of elemental levels may be used to obtain dispersion pattern characteristics. The results for this survey and those reported by Davies (6) indicate significant elevations in the soil elemental levels due to mineralization and

past mining activity. Davies (6) suggests that it would be useful to differentiate between the effects of mine pollution and those of the regional mineralization. In this work the specific extraction techniques incorporating sodium dithionite and nitric acid have been used to identify these differences. Sodium dithionite will extract elements associated with precipitated secondary crystalline iron oxides (74), which are indicative of transported minerals, whereas hot nitric acid will extract both insitu weathered minerals and elementals associated with secondary iron (58).

3.9 Conclusions

The granitic intrusions of Dartmoor and Bodmin Moor have resulted in metalliferous deposits within the fractured country rocks of the Tamar Valley. The soils resulting from the weathering of these rocks have above normal regional background values for arsenic, copper and lead (305). These elevated levels can generally be attributed directly to the weathering of the mineralized bed rock. Anomalous values in the soils are found directly above mineralized veins and in areas of past mining activity. Using the specific extraction techniques of sodium dithionite and nitric acid the elemental association with secondary iron and sulphide minerals may be determined. The combination of the two extraction techniques enables differentiation to be made between enhanced soil levels resulting from aerial pollution (sodium dithionite) and mineralized bed rock weathering (nitric acid - sodium dithionite). Aerial pollution results from either (a) wind blown material from the surface of barren tips, or (b) atmospheric fall-out from past smelting activities. Where aerial pollution has been observed in the data only a limited zone of contamination of a few hundred metres from the mine centre has been found.

The mines in the study area are generally located towards the bottom of the sloping valley edges and along the valley floor. Most of the sample collection sites were topographically above the mines, thus hydromorphic dispersion is considered to play a minor role in these results. It is acknowledged, however, that significant transportation of arsenic, copper and lead by surface and sub surface waters does occur along the valley floor. Studies on these waters and the

sediments/water column of the River Tamar indicate significant elemental transportation from these mining sites (57, 308, 309, 310).

As approximately 90% of the soil in the study area is under permanent pasture it may be of interest to future workers to obtain data on the elemental concentrations of herbage and animal products from within the high and low zones defined by this work, in order to obtain information on the significance of the observed distribution levels.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION ULTRA-VIOLET AND ATOMIC ABSORPTION DETECTION OF ORGANO-COPPER, -LEAD SPECIATION IN SOIL PORE WATERS

4.1 Separation of polar dissolved organic compounds

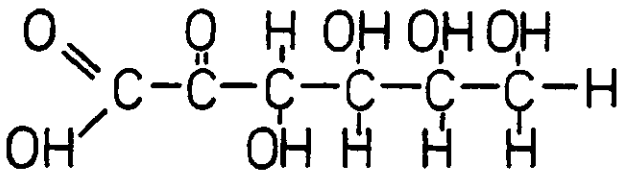
Reverse-phase HPLC offers the potential for separation and, with suitable detectors, the analysis of a wide variety of polar organic and organo-metallic compounds. The retention on an octadecylsilane ODS C18 stationary phase is best achieved when ionization is suppressed, thus allowing separation according to the non-polar properties of the compounds. As the polar organic ligands of interest in pore waters are acidic (287), i.e. citric acid, Fig. 4.1, the eluent system chosen to suppress ionization would also need to be acidic. Various elution systems were examined, these included ammonium formate (0.01M pH6.1), ammonium phosphate (0.01M pH6.1), propionic acid (0.2% v/v pH3.6), combined sodium dihydrogen phosphate (0.01M) - disodium hydrogen phosphate (0.01M pH6.1), and orthophosphoric acid (0.02% v/v pH2.6). Isocratic elution systems of orthophosphoric acid (OPA) and ammonium formate enabled examination of polar dissolved organic compounds (PDOCs) over a wide range of polarities, whilst giving minimal background signals with the GFAAS. The OPA system resolved the more polar compounds, whilst the ammonium formate separated those of less polar characteristics. Compounds with carboxylic and hydroxyl groups have non specific ultra-violet (UV) absorbance in the λ 200-230 nm range. A wavelength of 215 nm was selected in this study as at lower wavelengths interference from non specific absorption became significant and at higher wavelengths loss of sensitivity impaired the detection of certain compounds.

Using a single Hypersil ODS (3-5 µm 250 x 5mm) column, the orthophosphoric elution system (OPA 0.02% v/v pH2.6) flow rate (2 ml min^{-1}) and a UV detector (λ215 nm) the retention volumes of 29 amino and carboxylic acids were determined, Table 4.1. In addition the detection limits for these compounds with retention volumes less than 8 ml are also shown. Compounds with retention times > 8 ml on the OPA elution system, but which have shorter retention times on the ammonium formate elution

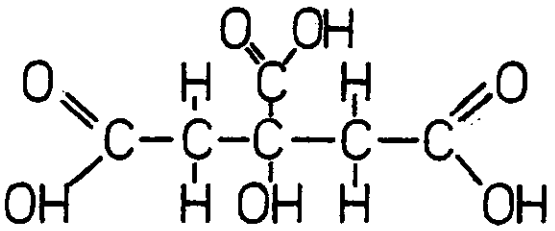
Fig. 4.1

Examples of the structure of polar organic ligands of relevance to this study

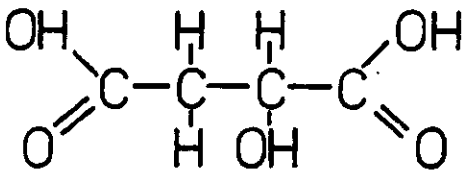
2 Ketoglutamic acid



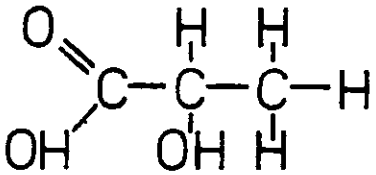
Citric acid



Malic acid



Lactic acid



Formic acid

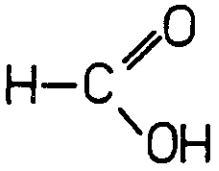


Table 4.1

The retention volumes and detection limits of organic compounds on the single column system

	Retention Volumes	Detection Limits	
	ml	ng	mgl^{-1}
Oxalic acid	2.00	11.3	0.1
Gluceronic acid	2.20	35.4	0.35
Cystine	2.30	35.0	0.35
2-Ketoglutamic acid	2.30	21.0	0.21
Pyruvic acid	2.50	12.0	0.12
Tartaric acid	2.55	10.4	0.10
Glycine	2.60	35.4	0.35
Urea	2.60	25.0	0.25
Hydroxoproline	2.65	6.7	0.06
Allantoin	2.70	14.0	0.14
Glutamine	2.75	13.0	0.13
Glycollate	2.75	14.4	0.14
Cysteine	2.80	31.0	0.31
Formic acid	3.00	86.0	0.86
Succinate	3.10	86.0	0.86
Citrulline	3.20	23.0	0.23
∝ Amino butyric acid	3.50	39.6	0.39
Malic acid	3.60	90.0	0.9
Malonic acid	3.70	95.0	0.95
Oxoglutaric acid	3.70	18.3	0.18
Lactic acid	4.00	180	1.8

continued ...

	Retention Volumes	Detection Limits	
	ml	ng	mgl^{-1}
Citric acid	4.40	150	1.5
Creatine	4.65	13.9	0.14
Proline	4.70	13.0	0.13
Ascorbic acid	4.80	17.5	0.17
Creatinine	5.30	24.7	0.24
Acetic acid	5.35	218	2.8
ɣ Amino Butyric acid	5.40	22.0	0.22
Methionine	7.60	2.2	0.02
Butyric acid	>8.00		
Resorcinol	>8.00		
Phenylalanine	>8.00		
Fumaric acid	>8.00		
Propionic acid	>8.00		
Phenyl-acetic acid	>8.00		
Valeric acid	>8.00		
Crotonic acid	>8.00		
Phloroglucinol	>8.00		
Succinic acid	>8.00		
Hippuric acid	>8.00		
Benzoic acid	>8.00		
Phenol	>8.00		
Salicylic acid	>8.00		
Quinol	>8.00		
Uric acid	>8.00		

system were observed to have little association with copper and were therefore not considered further (Fig. 4.2). The best separation of the PDOCs (Fig. 4.3a) was obtained with the three column system using two Hypersil ODS columns in series, followed by a Hamilton PRP I column held at 28.5°C. The total elution volume for this separation at a flow rate of 1.5 ml min^{-1} was 27 ml. A similar analysis carried out on a single Hypersil ODS column (Fig. 4.3b) gave poor resolution. When interfacing the continuous eluate flow from a HPLC to a discrete operating mode of the GFAAS, the limiting factor in total sample consumption is the GFAAS cycle time. A typical GFAAS cycle time of 3 minutes was reduced to less than 50 seconds by the modifications described in section 2.5. With this reduced cycle time, a HPLC eluent flow rate of 0.1 ml min^{-1}, and a 76.6 µl sample loop on the interface > 92% of the eluate was analysed. This almost total eluate consumption allowed for more accurate quantification and better resolution of samples compared to the previous methods of Brinckman et al (147), Fry et al (173) and Vickery et al (178), which typically analyse 50-60% of eluate. The three column system described above is not suitable for interfacing with the GFAAS due to the long retention volumes involved, which would result in a single analytical run lasting approximately four hours. The single Hypersil ODS column, with an eluent flow rate of 0.1 ml min^{-1}, gave acceptable resolution (Fig. 4.3c) and an analytical run time of approximately one hour. Therefore, the single column system was used for determining the association with metals with the organic compounds in the soil pore waters. Using the three column system the UV trace at λ 215 nm of standards can be seen in Fig. 4.4 with the retention times and detection limits shown in Table 4.2. Aliquots of the pore water used in Fig. 4.3a were spiked with (a) citric and succinic (Fig. 4.5a), (b) malic and fumaric (Fig. 4.5b), (c) formic and lactic (Fig. 4.5c), (d) allantoin and acetic (Fig. 4.5d) acids, to confirm the identification and quantification of the peaks observed in Fig. 4.3a.

The UV analysis of a pore water on the single column system (Fig. 4.6a) was found to contain five recognisable PDOCs. Confirmation of these compounds was achieved on the basis of their retention volumes, Table 4.3, and coincidence peaks with coinjection of standards (Fig. 4.6 b, c and d). Repeat runs carried out on the three column

Fig. 4.2

The U.V. absorbance and copper distributions for a soil pore water using (a) orthophosphoric acid and (b) ammonium formate elution systems

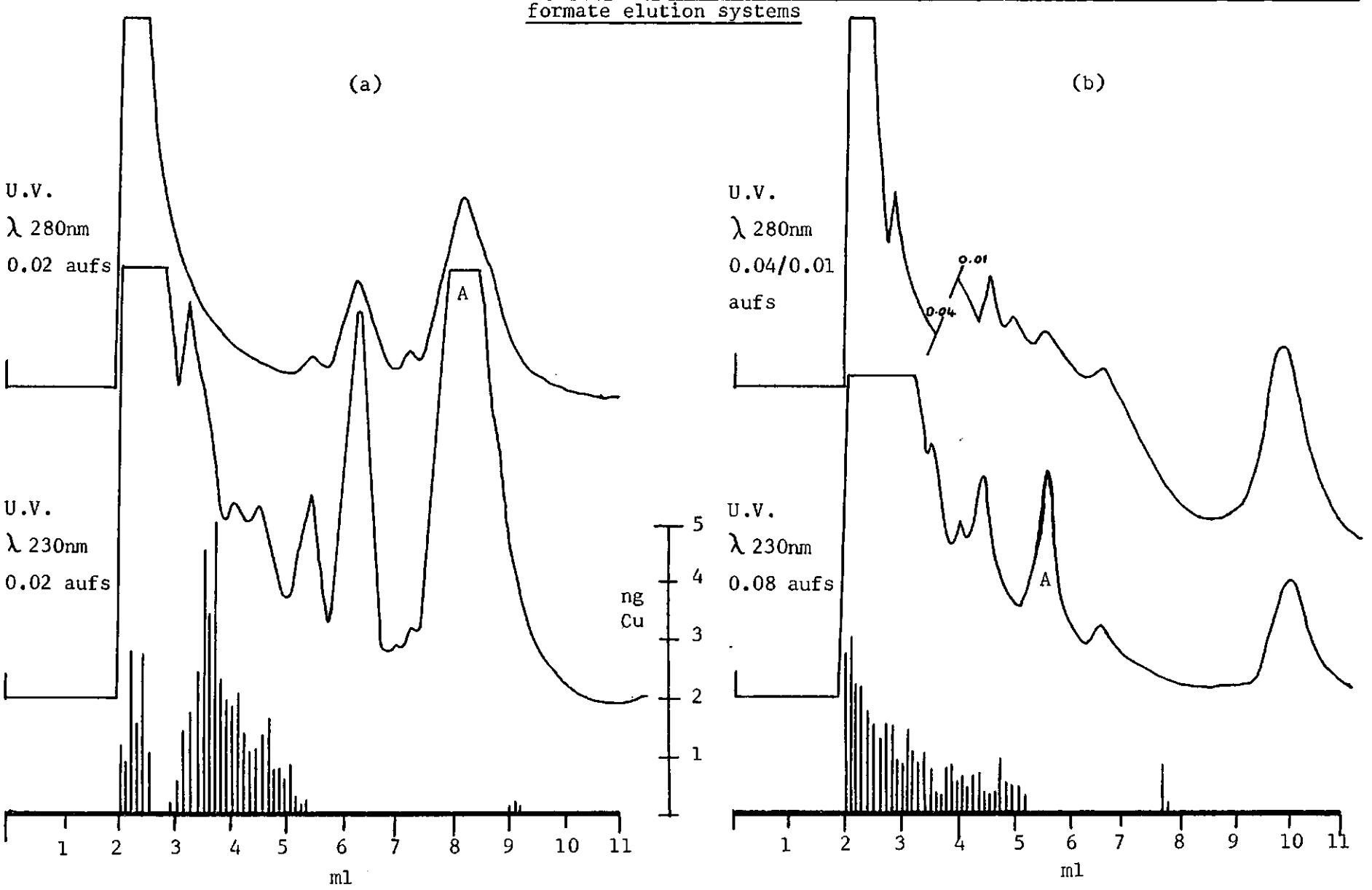


Fig. 4.3

U.V. absorbance of soil pore waters on the three and one column systems

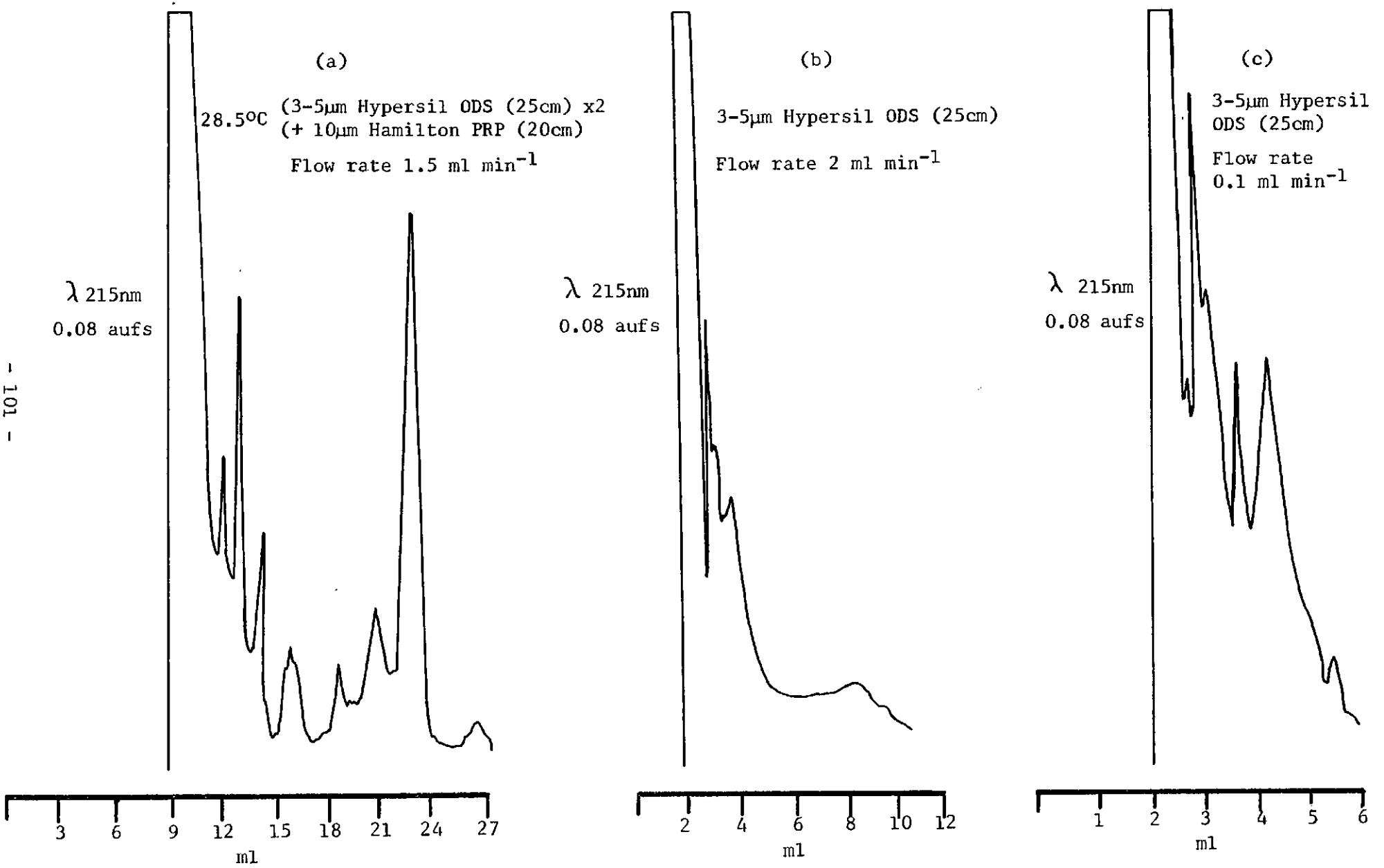
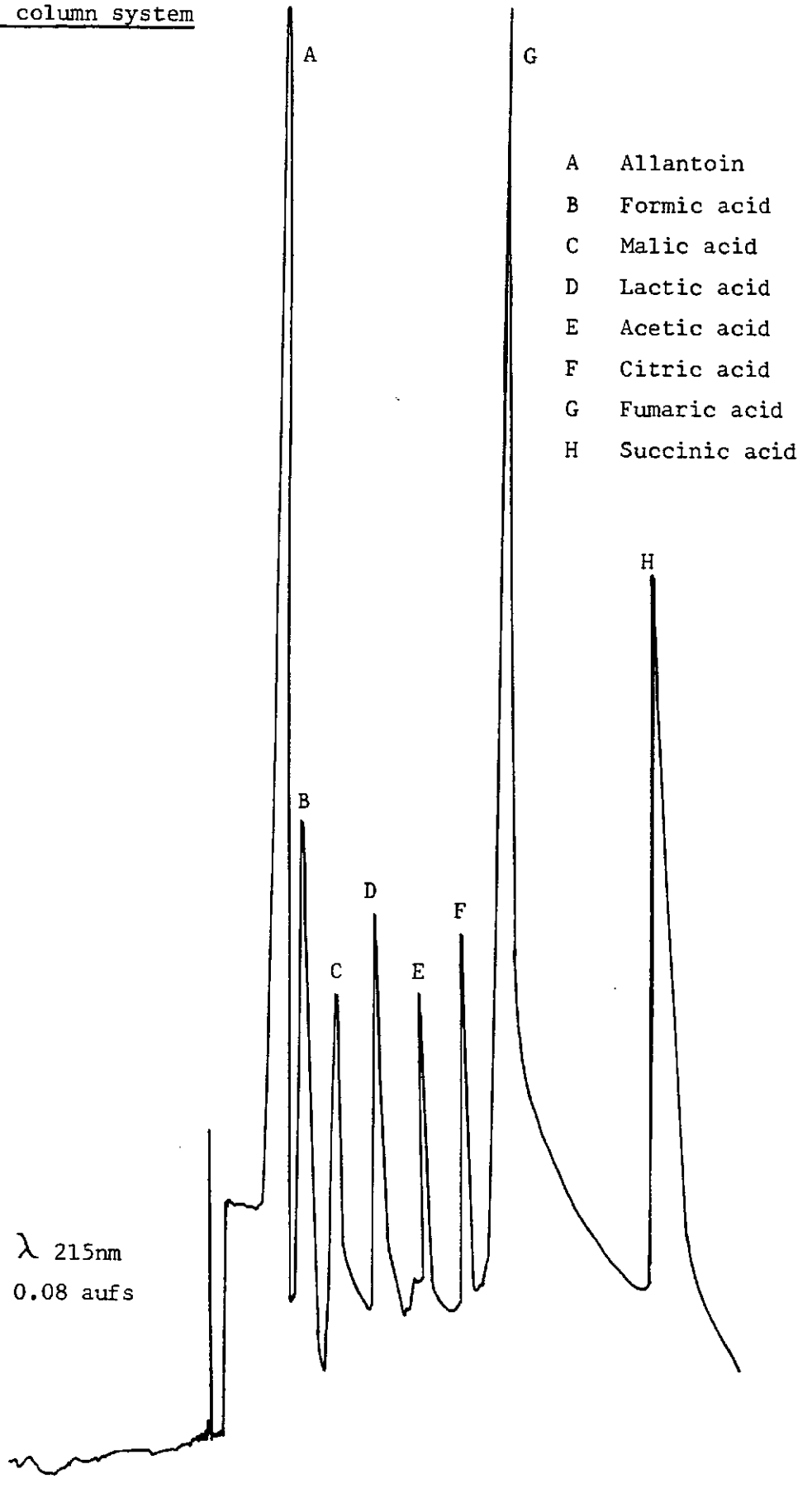


Fig. 4.4

U.V. absorbance of a mixture of organic acid standards separated on the three column system



		mgl^{-1}
A	Allantoin	10
B	Formic acid	10
C	Malic acid	10
D	Lactic acid	20
E	Acetic acid	20
F	Citric acid	10
G	Fumaric acid	2
H	Succinic acid	20

Table 4.2

Retention volumes and detection limits of organic compounds on the three column system

	Retention Volumes	Detection Limits	
	ml	ng	mgl^{-1}
Allantoin	12.0	1	0.01
Formic acid	13.5	5	0.05
Malic acid	15.0	5	0.05
Lactic acid	16.8	100	1
Acetic acid	18.9	100	1
Citric acid	21.0	50	0.5
Fumaric acid	23.1	1.3	0.01
Succinic acid	29.4	13	0.13

Fig. 4.5

U.V. absorbance of a soil pore water separated on the three column system spiked with organic acid standards

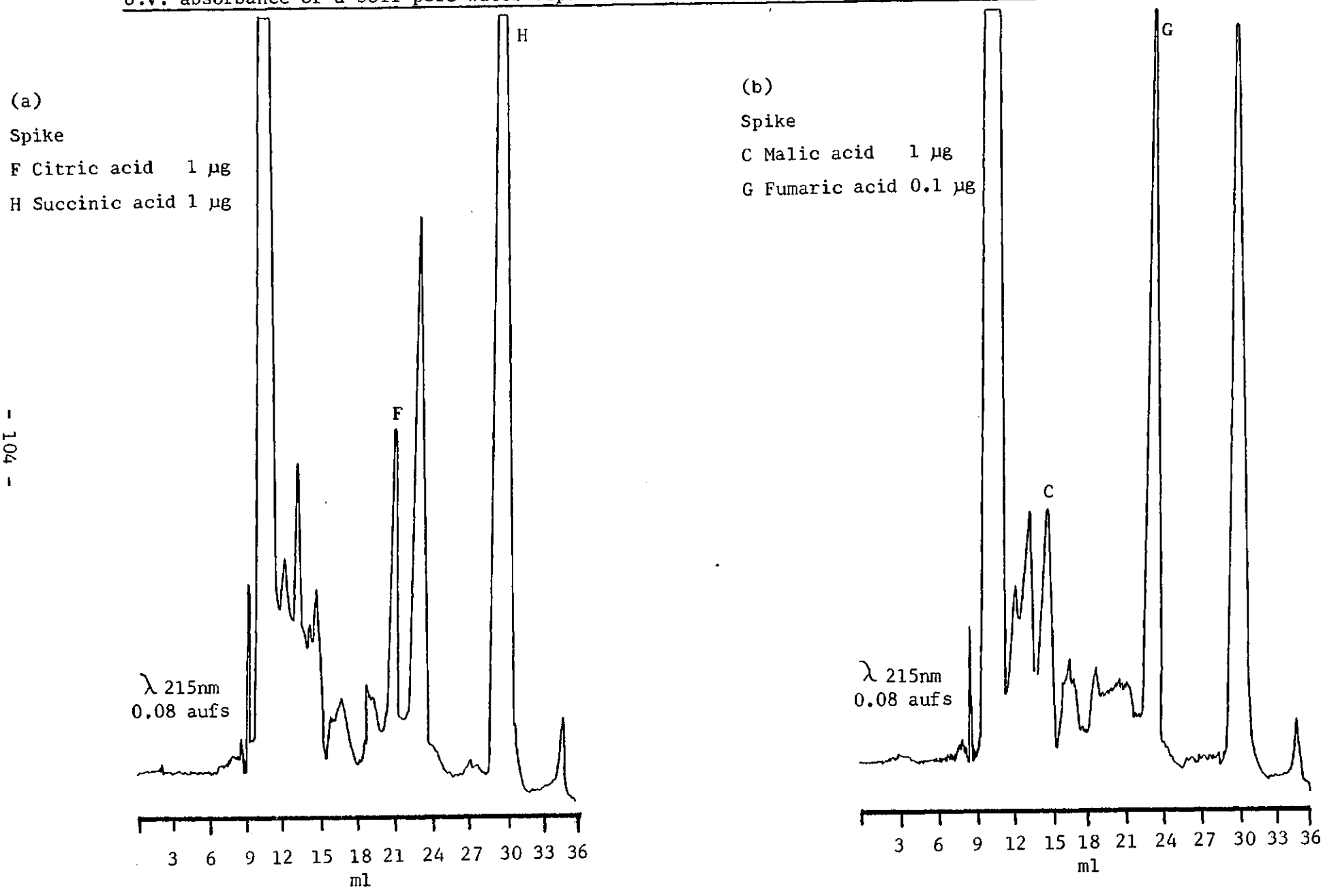
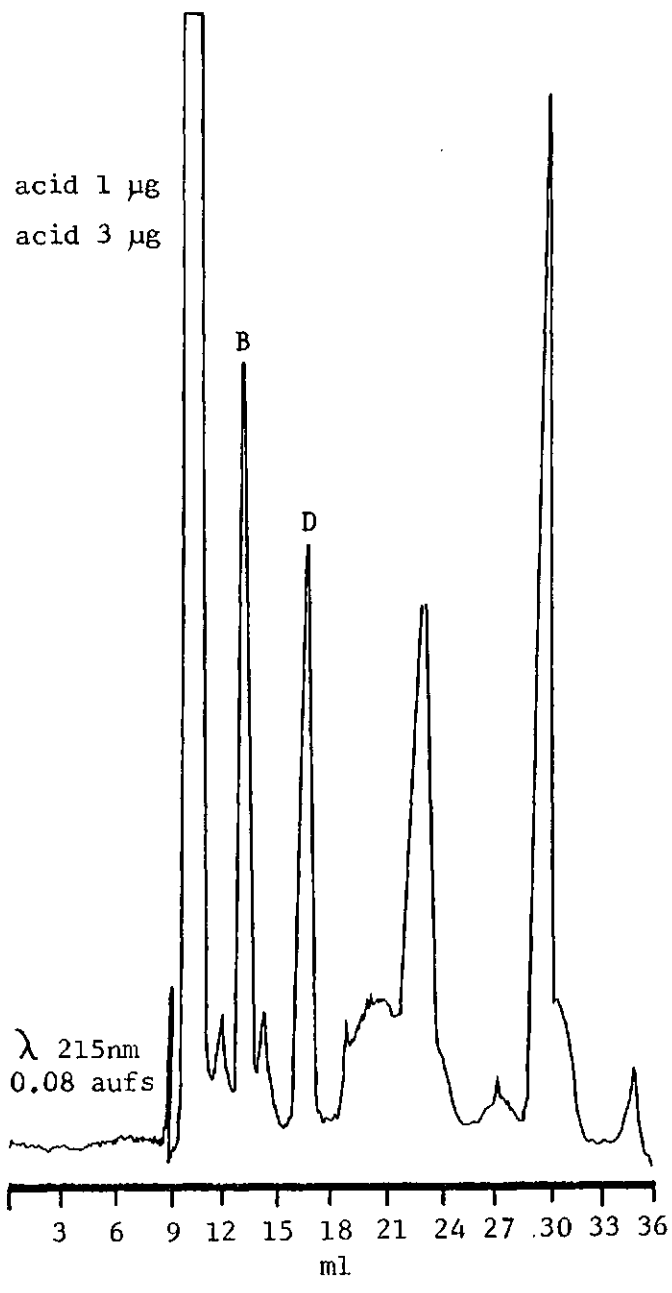


Fig. 4.5 (continued)



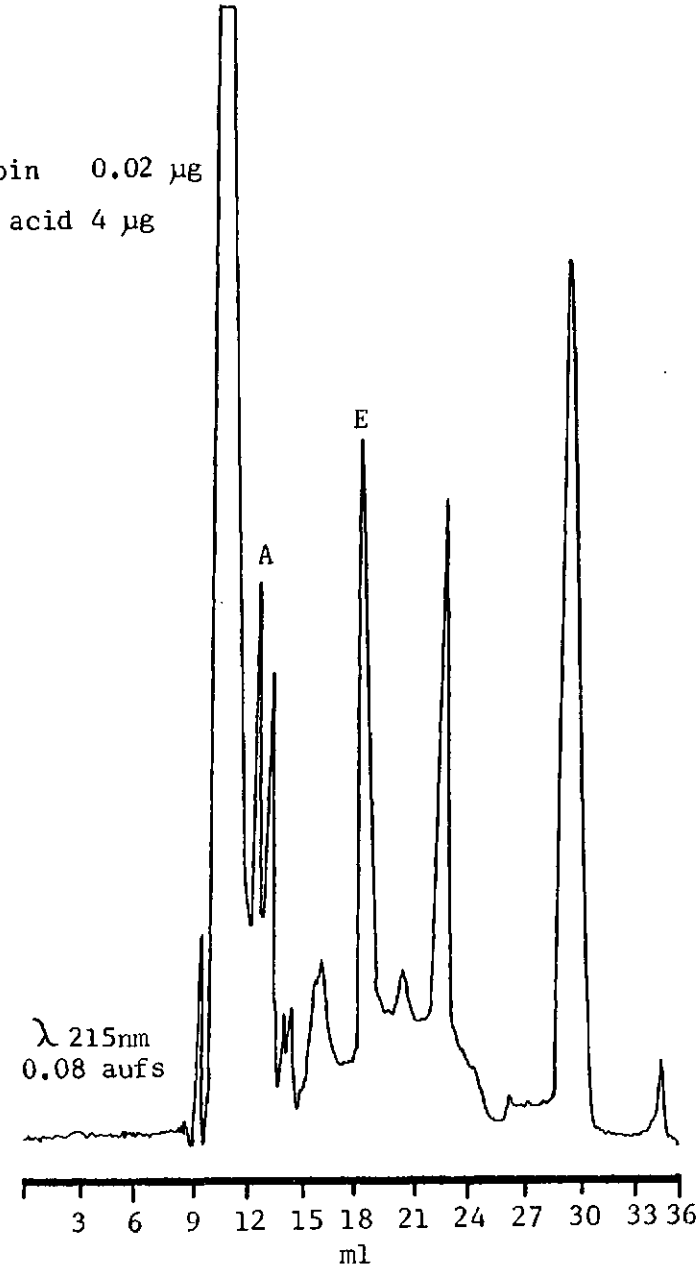


Fig. 4.6
U.V. absorbance of a soil pore water separated on the one column system spiked with organic acid standards

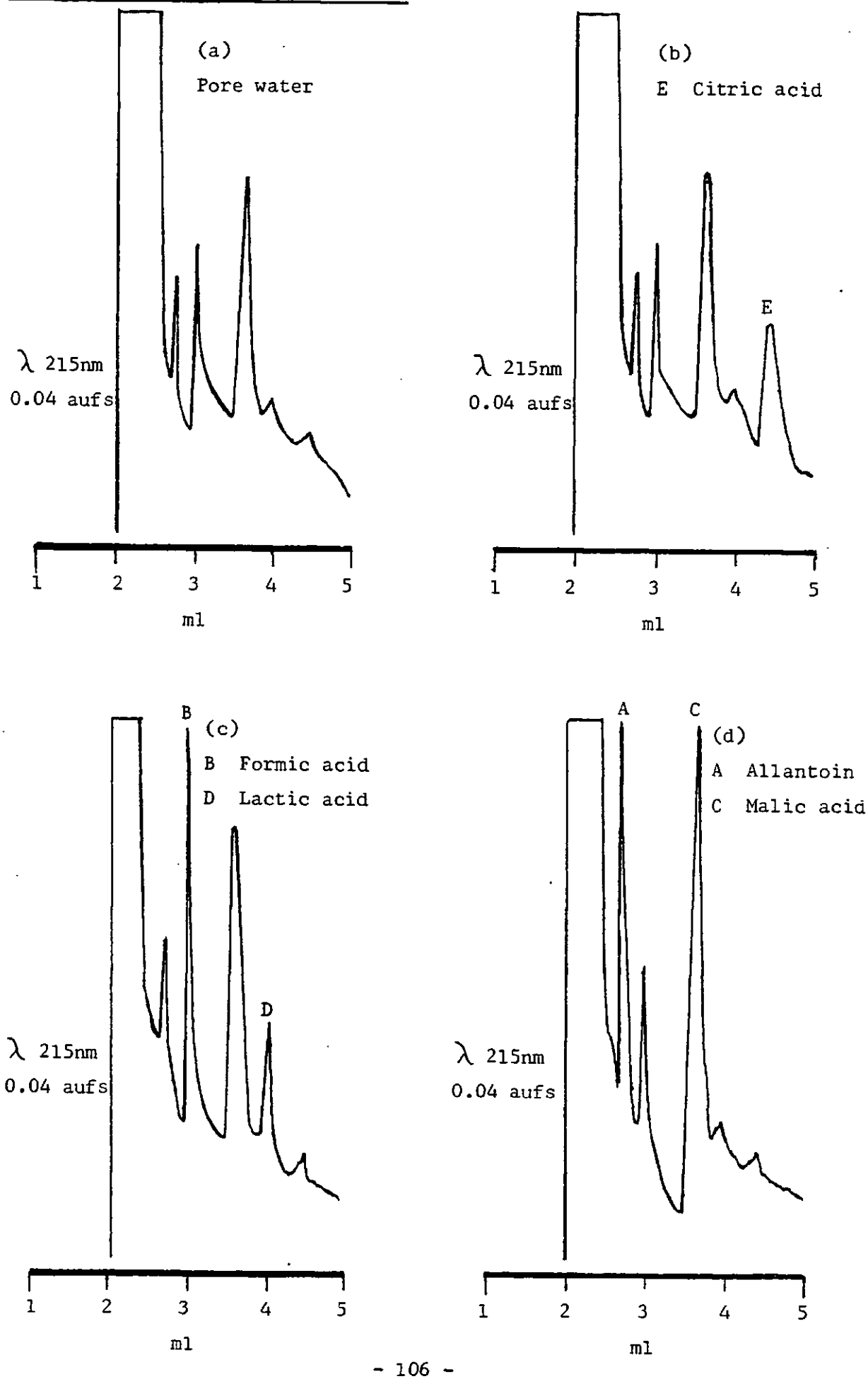


Table 4.3

Comparison of retention volumes and detection limits obtained by the three and one column systems

	Retention Volumes ml		Perkin-Elmer U.V. 3 col.		PYE U.V. 1 col.	
	3 col.	1 col.	ng	mgl^{-1}	ng	mgl^{-1}
Allantoin	12	2.70	1	0.01	14	0.14
Formic acid	13.5	3.00	5	0.05	86	0.86
Malic acid	15.0	3.60	5	0.05	90	0.9
Lactic acid	16.8	4.00	100	1	180	1.8
Acetic acid	18.9	5.35	100	1	281	2.8
Citric acid	21.0	4.40	50	0.5	150	1.5

system gave similar results with quantification usually agreeing to within ± 20%. The quantification of a soil pore water by both the three column and single column systems can be seen in Table 4.4. Variations of ≈15% were achieved, except for the case of allantoin where a substantial deviation of 50% was observed. In all cases the values for the single column system were higher. These variations are thought to be due to lack of resolution and reduced sensitivity when using the single column system (Table 4.3).

4.2 Development of an HPLC with direct UV/indirect GFAAS detectors for the separation and detection of a copper-citrate complex

The initial development of GFAAS as an element specific detector involved fraction collection of the HPLC column eluate. This development was concerned primarily with the use of standards of copper (II) nitrate, citric acid and the formation of copper (II)-citrate complexes under laboratory conditions. Fig. 4.7 shows the UV and copper GFAAS results using Hypersil ODS (5-7 µm 250 x 4mm) and OPA (0.02% pH2.65) elution system for standards. The GFAAS operating conditions were as given in the manufacturer's manual for waste water determinations (Table 2.1a).

Initially fractions were collected, which encompassed the entire nitrate and citrate peaks (Fig. 4.7a). It was found that the dilution factor associated with gross fraction (0.5 ml) collection of the citrate peak resulted in the copper being below the detection limit. Subsequent work involved the collection of smaller fractions (60 µl) (of which an aliquot of 20 µl was injected) for the entire run. This gave increased resolution of copper peaks without reducing the copper concentrations of individual fractions by too great an amount (e.g. Fig. 4.7f).

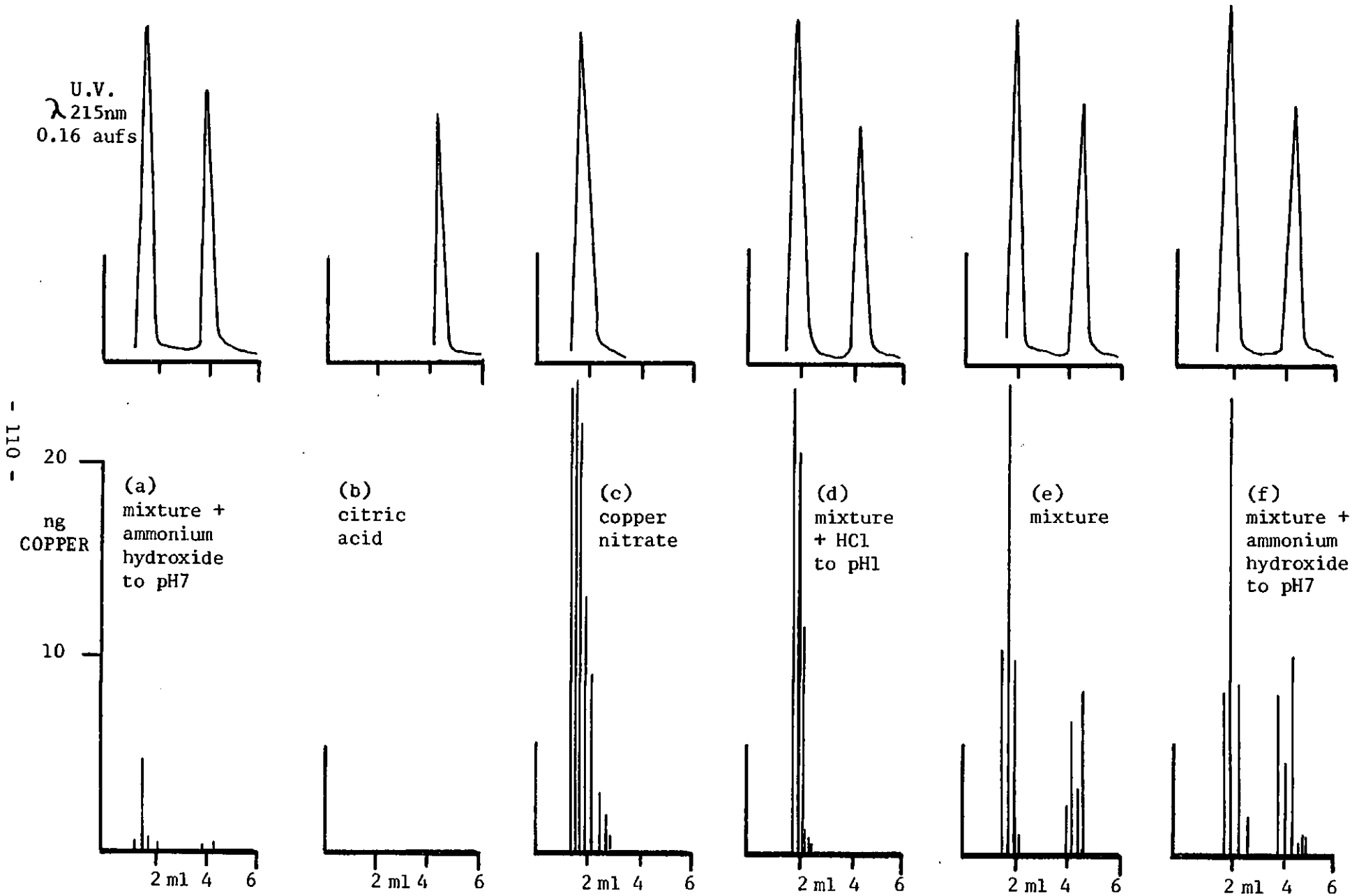
The chromatograms of citric acid and copper nitrate standards by UV were characteristic (Fig. 4.7b, c). The GFAAS analysis indicated that the citrate standard is copper free (Fig. 4.7b). The copper nitrate standard showed a copper distribution corresponding to the UV signal (Fig. 4.7c). In all cases where mixtures of copper nitrate and citric acid were used the nitrate UV peak reduced

Table 4.4

Comparison of the quantification obtained for soil pore water analysed by the three and one column systems

	3 column		1 column	
	ng	mgl^{-1}	ng	mgl^{-1}
Allantoin	10	0.1	20	0.2
Formic acid	600	5.9	660	6.6
Malic acid	330	3.3	400	4
Lactic acid	160	1.6	200	2
Citric acid	160	1.6	160	1.6

Fig. 4.7 U.V. absorbance and copper distributions for manually collected HPLC fractions



Mixture = citric acid 10 mg l^{-1} + $Cu(NO_3)_2$ 1 mg l^{-1} - eluate aliquots (a) 0.5 ml (b-f) 60 µl

in size and the citrate UV peak retention decreased. Broadening of the UV citrate peak was noted (Fig. 4.7d, e, f) in comparison to the citric standard (Fig. 4.7b). Formation of copper-citrate complexes was indicated by the copper becoming associated to varying degrees with the citrate UV peak.

Calculations based on the concentration, pH and copper stability constants for the eluent suggested that certain polar dissolved organic compounds as ligands (e.g. citric acid, methionine) might form copper complexes which would remain substantially undissociated during column chromatography. In these calculations (311) it was assumed instant equilibria occurred, and no allowance was made for possible stationary phase adsorption. Approximately 95% of the total copper in a standard could be detected by this method.

4.3 Conditions for HPLC with direct UV/GFAAS detection

Fig. 4.8 shows the UV and copper GFAAS results for the manually collected (a) and interfaced (b) techniques, using a Hypersil ODS column and orthophosphoric acid elution system previously described. Direct total column efluent analysis was carried out by use of the interface (section 2.5). When a mixture of copper nitrate and citric acid standards were passed through the column, the copper originally associated with the nitrate peaks was found to be associated with both nitrate and citrate peaks. 82% of the copper originally associated with the nitrate peak was found to coincide with the citrate peak, using the manual technique the corresponding figure was 83% with the interface. This redistribution of the copper indicates the possible formation of a copper citrate complex. Comparison of the total copper added to the column with the total copper determined in the eluate, when correcting for volume losses in the interface, gave 97 $\pm$ 5% recovery.

4.4 Analysis of soil pore waters for copper using HPLC with direct UV/direct and indirect GFAAS detection

Fig. 4.9 shows the UV and copper GFAAS results for environmental soil pore water samples. The soil pore water was collected from the south-

Fig. 4.8 Comparison of U.V. absorbance and copper distributions by manual and automatic HPLC/GFAAS techniques

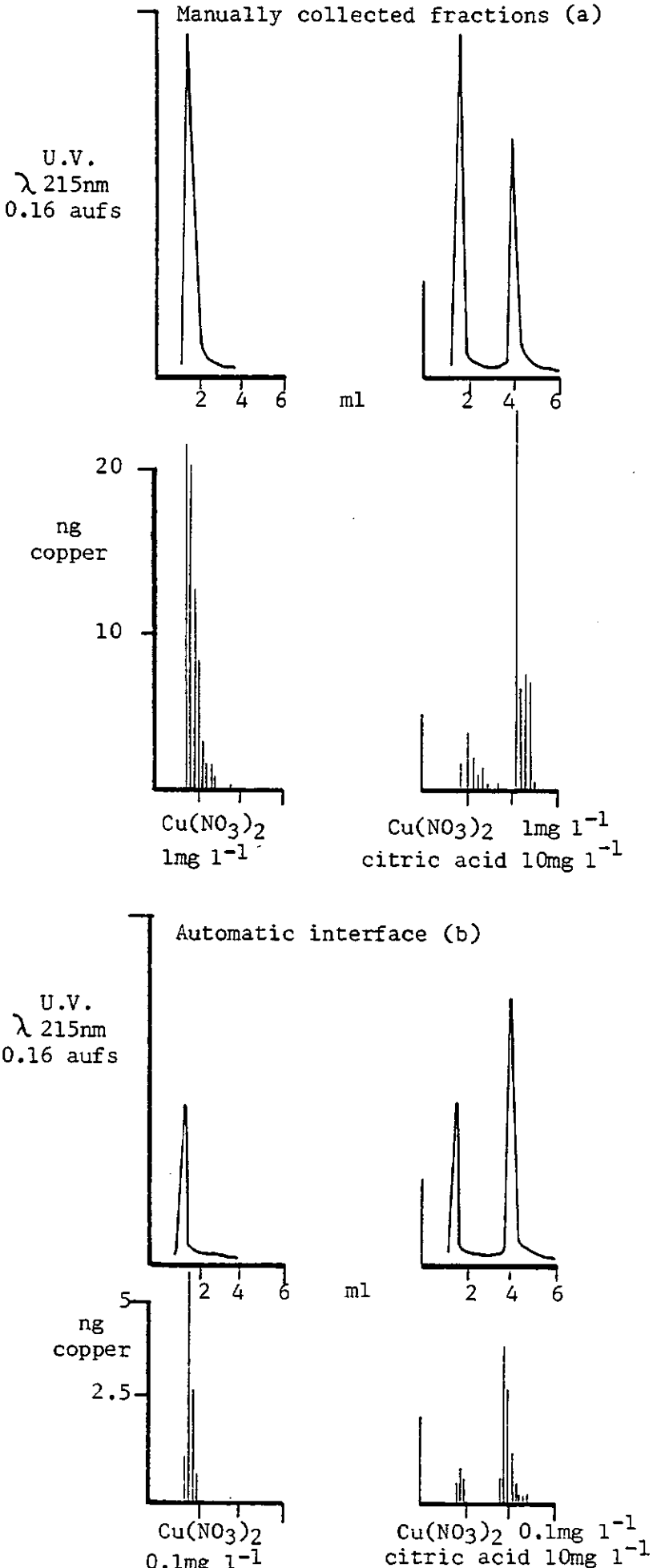
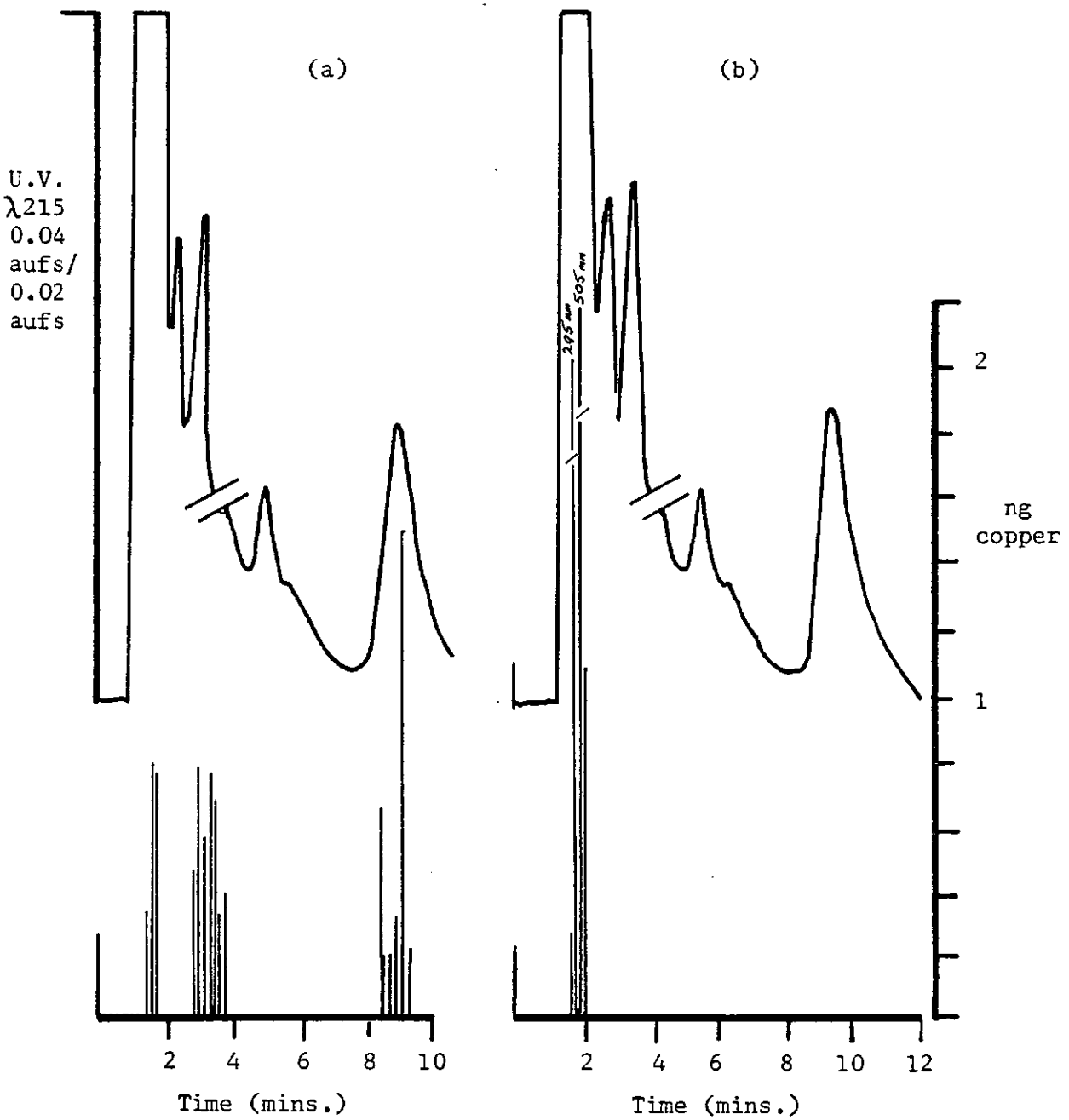


Fig. 4.9

U.V. absorbance and copper distribution for a soil pore water using (a) minimal sample preservation and (b) sample acidification



west region of the survey area (Fig. 1.1). 70% Of the total dissolved copper could be accounted for with ≈ 10% being associated with the unretained inorganic/highly polar dissolved organic molecules (Fig. 4.9a). The addition of hydrochloric acid (10M 8 ml l^{-1}) to filtered samples resulted in > 90% of the total dissolved copper being associated with the unretained polar inorganic/organic species (Fig. 4.9b), indicating the destruction of the complexes between copper and the polar dissolved organic compounds in the sample.

Fig. 4.2 shows the separation of a soil pore water using two elution systems, 0.02% orthophosphoric acid (Fig. 4.2a) and 0.01M ammonium formate (Fig. 4.2b), which examine two 'windows' of polarity. The total mass of copper in 100 µl of sample was 81 ± 3 ng, of which 60 ± 10 ng and 70 ± 12 ng were determined using orthophosphoric acid and ammonium formate respectively. When eluting with orthophosphoric acid (Fig. 4.2a) the copper peak association with the solvent front may include inorganic and very polar dissolved organic copper species. The next 4 ml of eluate contained copper which was assumed to be complexed with less polar ligands. Ammonium formate (Fig. 4.2b) will not elute inorganic copper at the solvent front, this is found to be the case when copper nitrate is injected onto the HPLC column, i.e. the retention volume was found to be > 12 ml.

The further analysis of larger numbers of soil pore waters using the single column HPLC system interfaced with GFAAS showed that the copper was not always associated with the same PDOC in the same proportions (Figs. 4.10, 4.11). In the majority of pore waters association of the copper with citric acid and neighbouring eluting compounds was found (Fig. 4.10a). The complexing effect of citric acid was emphasized by adding a mixture of copper (II) nitrate and citric acid to the pore water (Fig. 4.10b). The proportion of copper associated with the solvent front, inorganic and highly polar species, was 16% before the standard addition, with the remaining 84% of the copper being associated with the citric acid peak. In the original pore water the total copper content was 43 µg l^{-1}, as determined by direct injection into the GFAAS and 42 µg l^{-1}, as determined by summation of the copper peaks after HPLC separation (Fig. 4.10a). In the latter case, a correction to take account of the volume of eluate not injected into the GFAAS

Fig. 4.10 The U.V. absorbance and copper distribution of (a) soil pore water Y and (b) pore water Y spiked with copper and citric acid

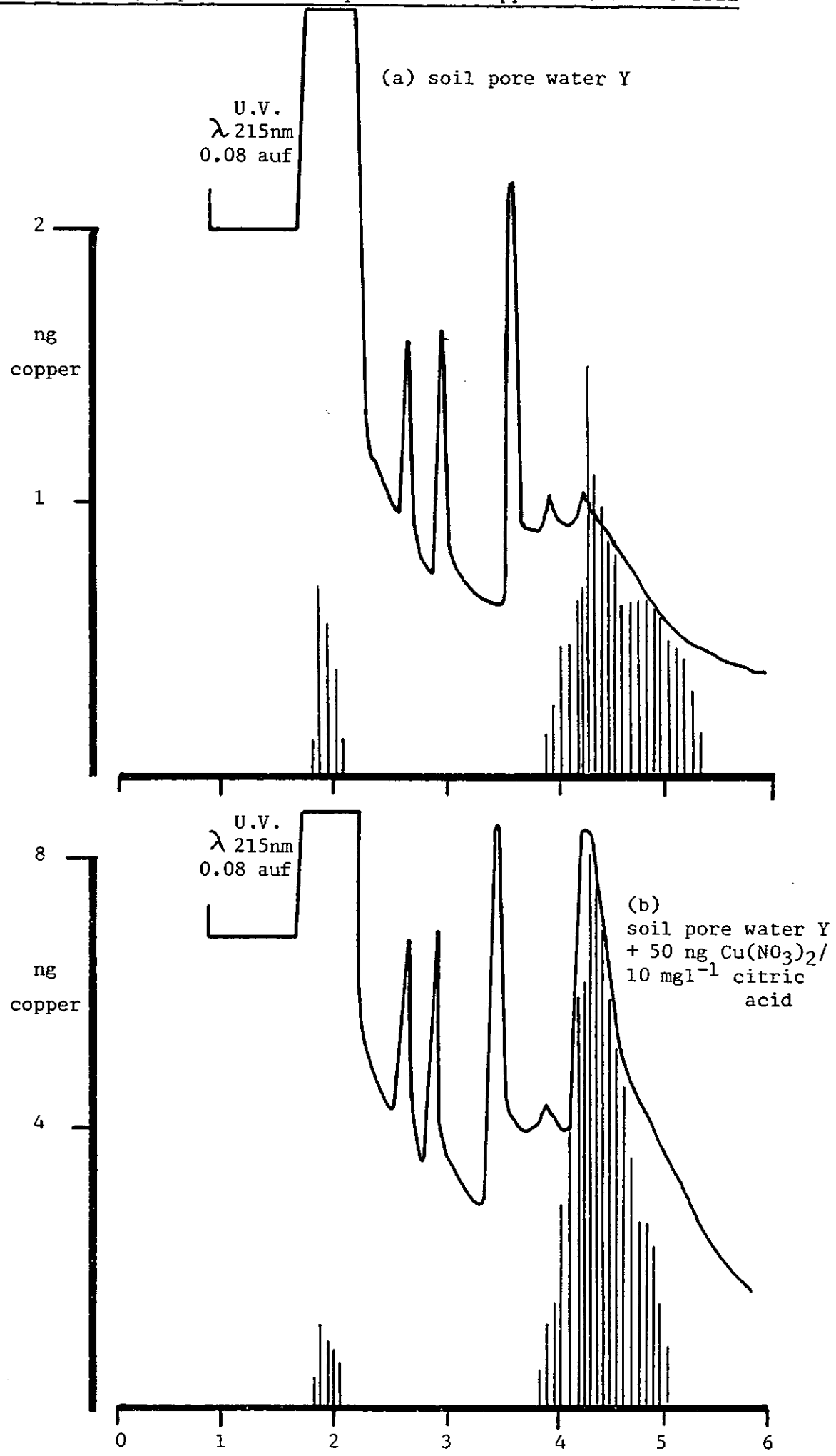
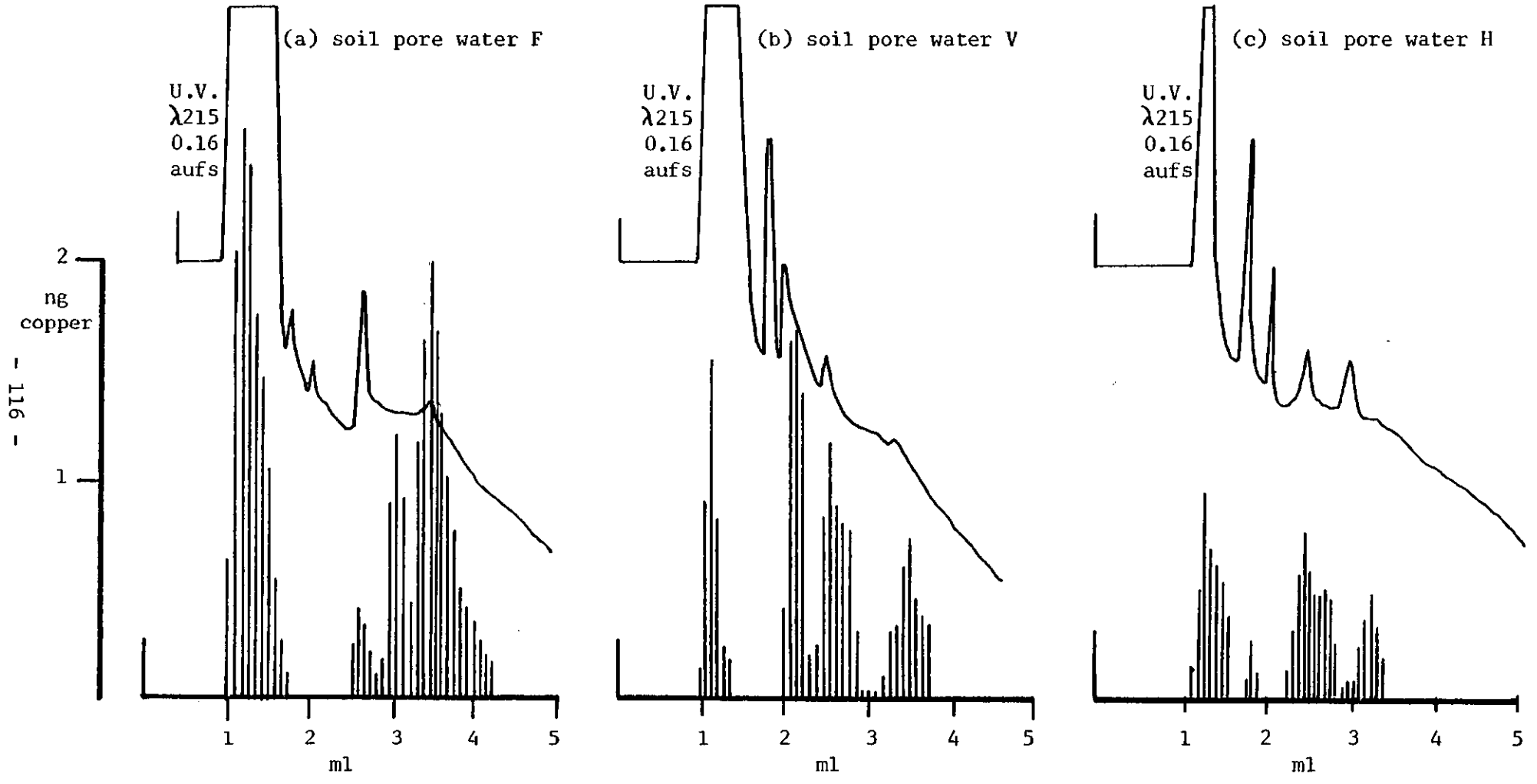


Fig. 4.11

U.V. absorbance and copper distributions for various soil pore waters



was applied, and this raised the measured value of 39 μg l^{-1} to 42 μg l^{-1}. When 50 ng of copper (II) ion and 3 μg citric acid were added to the soil pore water (Fig. 4.10b) the total copper content was determined to be 65 μg (theoretical value 64 μg). This was equivalent to a concentration of 217 μg l^{-1} compared to a calculated value due to addition of 209 μg l^{-1}, with similar percentage distributions between the solvent front and citric acid peaks, as were obtained before the standard addition.

A number of the more extreme distributions found in the soil pore waters examined are illustrated (Fig. 4.11) and the copper levels are quantified in Table 4.5. Citric acid/copper association was found in all the samples, but the association of copper with allantoin, formic and lactic acid occurred rarely. Malic acid was second only to citric acid in the number of samples which indicated association between it and copper.

4.5 Analysis of soil pore waters for lead using HPLC with direct UV and direct GFAAS detection

In the previous section details on the development of the separation and detection techniques have been described. Typical results for organo-lead determinations on soil pore waters can be seen in Fig. 4.12. As with copper, lead does not always associate to the same degree with the sample PDOCs in individual samples. Between 17-30% of the lead is found to occur with the inorganic and very polar organic compounds, Table 4.6 summerizes the data for pore waters analysized. Confirmation of the presence of observed peaks was achieved by coinjections of organo-lead complexes made up in aqueous solutions. The retention volumes of these complexes formed from 5 ng of lead (II) nitrate mixed with 1 μg of organic acid are given in Fig. 4.13. These retention volumes also correlate with those observed for these compounds in the copper work.

4.6 Comparison of the observed experimental data with the calculated theoretical predictions of organo-copper, -lead complexes

The observed distribution patterns for the copper and lead in various

Table 4.5

Amount of copper in soil pore waters as determined by GFAAS after HPLC separation on the single column system

HPLC peak	Amount of copper in soil pore water					
	Sample F $\mu g\ l^{-1}$	% of total	Sample V $\mu g\ l^{-1}$	% of total	Sample H $\mu g\ l^{-1}$	% of total
Solvent front	64	53	13	21	13	30
2 ketoglutamic acid/cystine					5	12
Allantoin					2	5
Formic acid			19	30		
Malic acid	5	4	20	32	16	37
Lactic acid	13	11				
Citric acid	39	32	11	17	7	16

Fig. 4.12

U.V. absorbance and lead distributions for various soil pore waters

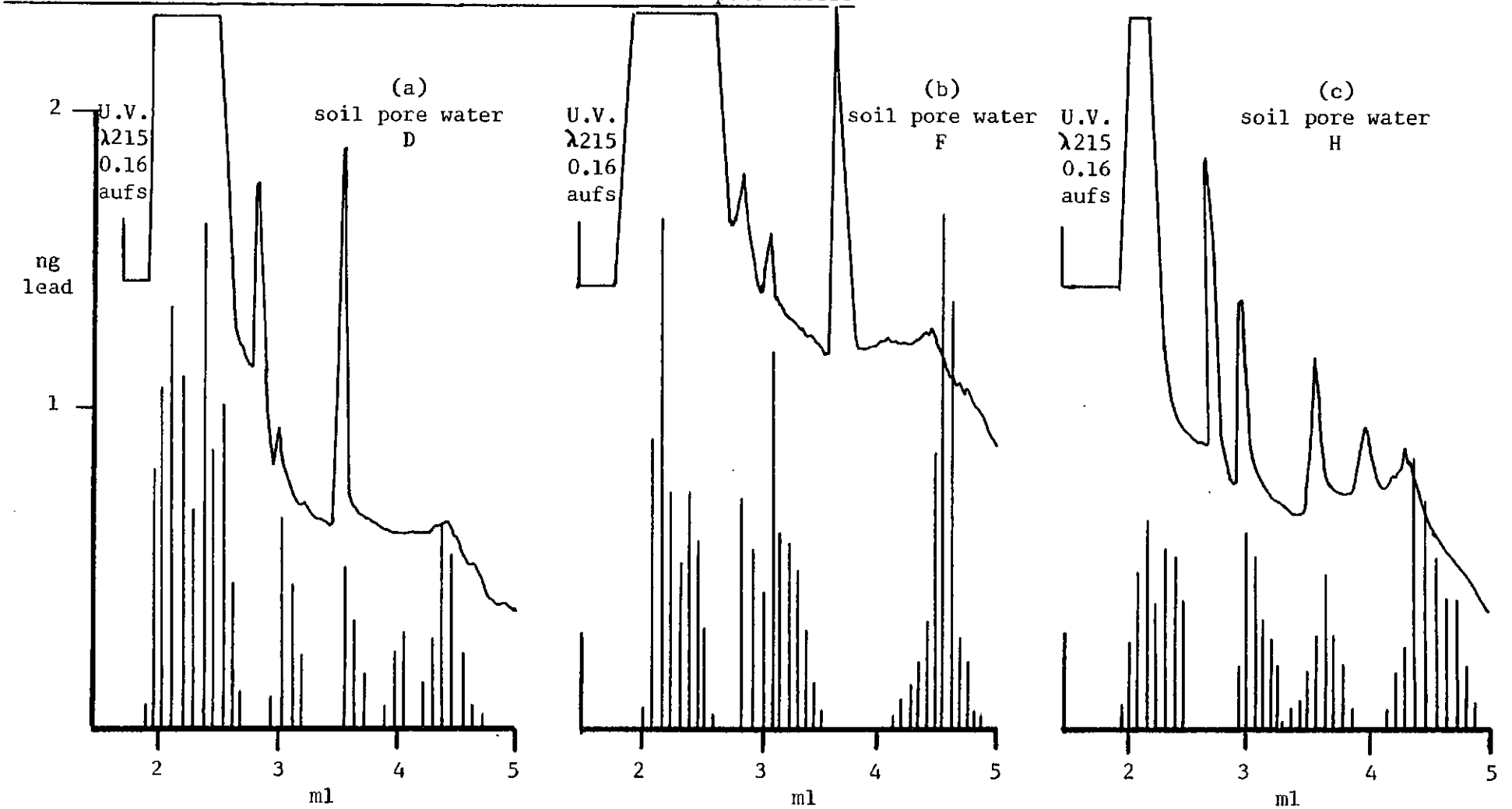


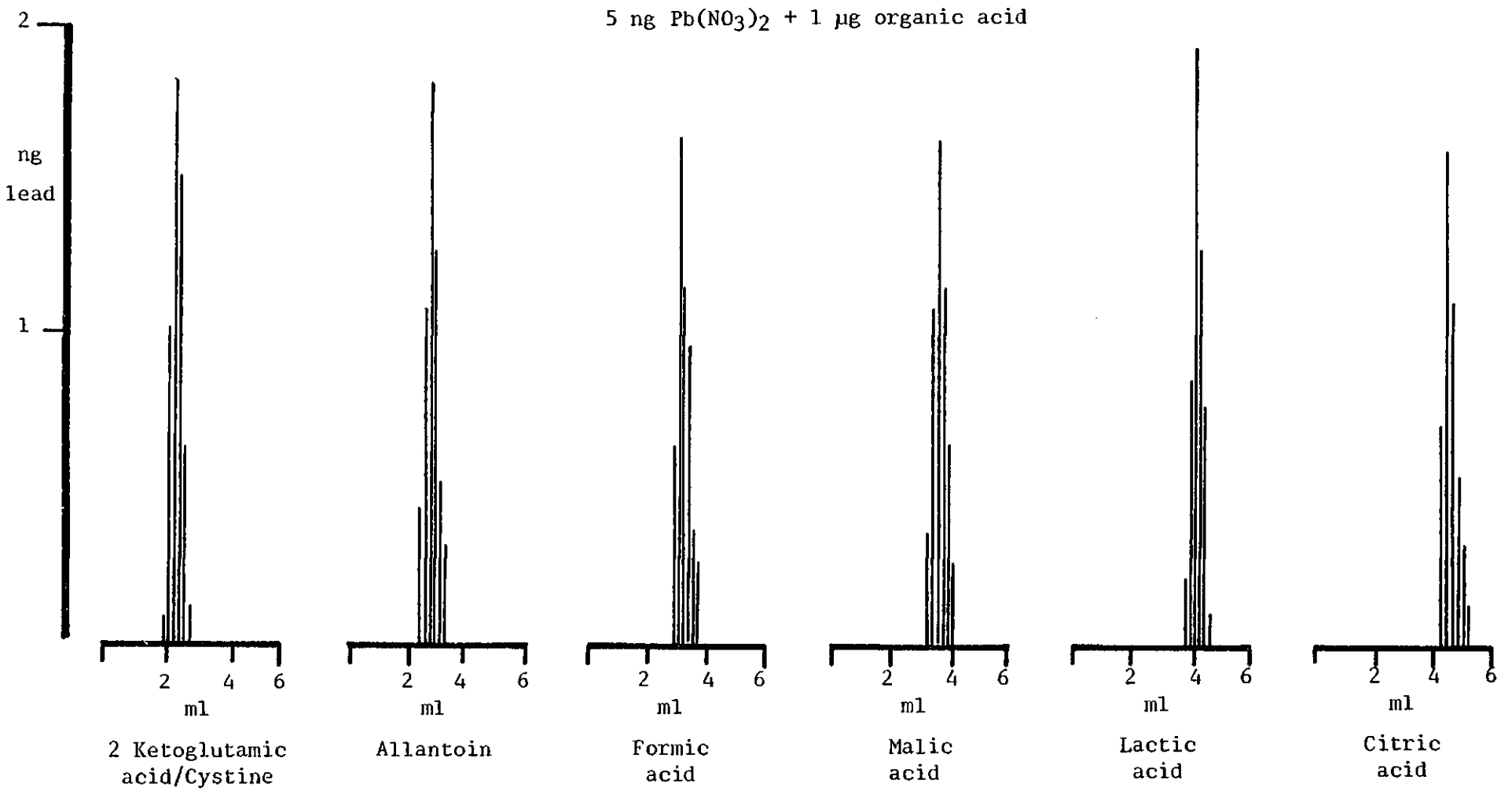
Table 4.6

Amount of lead in soil pore waters as determined by GFAAS after HPLC separation on the single column system

HPLC peak	Amount of lead in soil pore water					
	Sample D $\mu g\ l^{-1}$	% of total	Sample F $\mu g\ l^{-1}$	% of total	Sample H $\mu g\ l^{-1}$	% of total
Solvent front	18	30	12	24	6	16
2 ketoglutamic acid/cystine	16	28	5	10	6	16
Allantoin			5	10		
Formic acid	6	10	9	17	7	17
Malic acid	5	9			5	14
Lactic acid	3	5				
Citric acid	10	17	20	39	13	35

Fig. 4.13

Retention volumes of mixtures of inorganic lead and organic acids standards



pore waters suggest citric and malic acid are the major ligands in the system. The variation, however, between samples indicates the kinetics of these systems are complex and that the system is not in equilibrium. This effect is further confirmed when experimental observed data is compared to theoretically calculated data. For a mixture containing organic and inorganic ligands (L) calculations based on thermodynamic data for a metal ligand (ML) can be performed using standard data. In a system containing numerous ligands individual complexation with a metal can be expressed as the side reaction coefficient σ_{ML}, and can be calculated from the formula:

$$\sigma_{ML} = \log\beta\,[L]^n \qquad (15)$$

where $\log\beta$ is the conditional or cumulative stability constant of the complex and n is the complex coordination number. The sum of the side reaction coefficient $\bar{\alpha}$ can be used to calculate the percentage metal ligand complexed from the equation:

$$\bar{\alpha} = \sigma_{ML}\,\frac{[M] = \text{concentration of metal}}{[ML] = \text{concentration of complex}} \qquad (16)$$

This gives some information on the free ligand remaining after complexation. The assumptions made in the calculation above are that:

1) the ligand species are in excess;
2) the ligand species determined are the most important in the system;
3) the physochemical conditions, i.e. pH, Eh, ionic strength and temperature are ideal for complex formation;
4) only soluble species react.

Table 4.7 lists the conditional stability constants used in the calculation shown in this work (312). The calculated theoretical percentage ligand formation for individual pore waters of formic, malic, lactic and citric acid (313) are shown in Table 4.8, these indicate the predominant ligands expected in the samples analysed. From the experimental data (Table 4.9) the calculated ligand concentrations (314) for soil pore waters are given in Table 4.10. The calculated side reaction coefficients σ for copper and lead are

Table 4.7

Conditional stability constants Log β values (312)

		Copper	Lead	Iron
Formic acid		2.00	1.65	3.1
Malic acid	ML	3.42	3.70	7.1
	MHL	2.00	2.45	
Lactic acid	ML	2.55	1.99	
Citric acid	ML	5.9	4.08	11.5
	MHL	3.42	2.97	
	MH_2L	2.26	1.51	
OH	n = 1	6.00	6.29	11.81
	n = 2	10.70	10.88	22.33
CO_3		6.75	7.00	9.72

Table 4.8

Theoretical percentage ligand formation (313)

Sample No.	pH	Formic acid		Malic acid			Lactic acid		Citric acid			
		% HL	% L	% H_2L	% HL	% L	% HL	% L	% H_3L	% H_2L	% HL	% L
A	6.5	-	100	-	2	98	-	100	-	-	13	87
B	6.8	-	100	-	1	99	-	100	-	-	7	93
D	6.5	-	100	-	2	98	-	100	-	-	13	87
F	5.0	3	97	1	34	66	4	96	-	16	70	14
H	6.4	-	100	-	2	98	-	100	-	-	16	84
V	6.4	-	100	-	2	98	-	100	-	-	16	84
Y	6.2	-	100	-	3	97	-	100	-	-	24	76

Table 4.9

Summary of the data obtained for soil pore waters

	Sample A (p) µM			Sample B (w) µM		
	Organic	Copper	Lead	Organic	Copper	Lead
Inorganic/highly polar	-	5.0×10^{-1}	1.1×10^{-1}	-	6.6×10^{-1}	1.3×10^{-1}
2 Ketoglutamic acid	ND	ND	1.1×10^{-1}	ND	ND	1.3×10^{-1}
Allantoin	3.3	ND	ND	9.5	ND	ND
Formic acid	180	ND	8.6×10^{-2}	91	ND	1.9×10^{-1}
Malic acid	50	ND	1.9×10^{-1}	56	ND	1.0×10^{-1}
Lactic acid	ND	ND	ND	ND	ND	ND
Citric acid	ND	1.0	1.7×10^{-1}	ND	4.4×10^{-1}	1.2×10^{-1}
Total in filtered sample	-	2.4	6.3×10^{-1}	-	1.7	6.9×10^{-1}
Total detected by separation	-	1.5	6.7×10^{-1}	-	1.1	6.6×10^{-1}

continued ...

	Sample D (p) µM			Sample F (w) µM		
	Organic	Copper	Lead	Organic	Copper	Lead
Inorganic/highly polar	-	8.6×10^{-1}	8.5×10^{-2}	-	1	6.1×10^{-2}
2 Ketoglutamic acid	ND	ND	8.0×10^{-2}	ND	ND	2.7×10^{-2}
Allantoin	3.5	ND	ND	1.4	ND	2.7×10^{-2}
Formic acid	57	6.9×10^{-2}	2.9×10^{-2}	68	ND	4.3×10^{-2}
Malic acid	110	1.3×10^{-1}	2.6×10^{-2}	61	7.1×10^{-2}	ND
Lactic acid	ND	1.4×10^{-1}	1.5×10^{-2}	7.4	2.0×10^{-1}	ND
Citric acid	ND	4.2×10^{-1}	4.6×10^{-2}	7.6	6.1×10^{-1}	9.8×10^{-2}
Total in filtered sample	-	2.8	2.8×10^{-1}	-	2.6	2.4×10^{-1}
Total detected by separation	-	1.6	2.8×10^{-1}	-	1.9	2.6×10^{-1}

continued ...

	Sample H (p) μM			Sample V (p) μM		
	Organic	Copper	Lead	Organic	Copper	Lead
Inorganic/highly polar	-	2.0×10^{-1}	3.0×10^{-2}	-	2.1×10^{-1}	-
2 Ketoglutamic acid	ND	7.2×10^{-2}	2.9×10^{-2}	ND	ND	-
Allantoin	12	2.4×10^{-2}	ND	5.9	ND	-
Formic acid	300	ND	3.1×10^{-2}	230	3.0×10^{-1}	-
Malic acid	27	2.5×10^{-1}	2.5×10^{-2}	18	3.1×10^{-1}	-
Lactic acid	170	ND	ND	15	ND	-
Citric acid	15	1.1×10^{-1}	6.2×10^{-2}	15	1.7×10^{-1}	-
Total in filtered sample	-	7.8×10^{-1}	1.9×10^{-1}	-	1.0	-
Total detected by separation	-	6.6×10^{-1}	1.8×10^{-1}	-	9.7×10^{-1}	-

continued ...

	Sample Y (w)		
		μM	
	Organic	Copper	Lead
Inorganic/highly polar	-	1.1×10^{-1}	1.6×10^{-2}
2 Ketoglutamic acid	ND	ND	1.1×10^{-2}
Allantoin	3.5	ND	ND
Formic acid	500	ND	2.6×10^{-2}
Malic acid	100	ND	1.3×10^{-2}
Lactic acid	29	ND	ND
Citric acid	30	5.9×10^{-1}	3.1×10^{-2}
Total in filtered sample	-	7.2×10^{-1}	1.1×10^{-1}
Total detected by separation	-	7.1×10^{-1}	9.8×10^{-2}

ND = not detectable

p = permanent pasture

w = woodland

Table 4.10

Ligand concentrations (mol l^{-1}) (314)

Sample No.		A	B	D	F	H	V	Y
Formic acid		1.8×10^{-4}	9.1×10^{-5}	5.7×10^{-5}	6.5×10^{-5}	3.0×10^{-4}	2.3×10^{-4}	5.7×10^{-4}
Malic acid	HL	1.3×10^{-5}	5.6×10^{-6}	2.2×10^{-6}	2.1×10^{-5}	5.4×10^{-7}	3.6×10^{-7}	3.0×10^{-6}
	L	6.6×10^{-4}	5.5×10^{-4}	1.1×10^{-4}	4.0×10^{-5}	2.6×10^{-5}	1.8×10^{-5}	9.7×10^{-5}
Lactic acid	HL	-	-	-	3.0×10^{-7}	-	-	-
	L	-	-	-	7.1×10^{-6}	1.7×10^{-4}	1.5×10^{-5}	2.9×10^{-5}
Citric acid	H_2L	-	-	-	1.2×10^{-6}	-	-	-
	HL	-	-	-	5.3×10^{-6}	2.4×10^{-6}	2.4×10^{-6}	7.2×10^{-6}
	L	-	-	-	1.1×10^{-6}	1.3×10^{-5}	1.3×10^{-5}	2.3×10^{-5}
$[CO_3]^{2-}$		1.1×10^{-7}	2.9×10^{-7}	1.1×10^{-7}	2.0×10^{-10}	8.2×10^{-8}	8.2×10^{-8}	4.0×10^{-8}
$[OH]^-$		3.2×10^{-8}	6.3×10^{-8}	3.2×10^{-8}	1.0×10^{-9}	2.5×10^{-8}	2.5×10^{-8}	1.6×10^{-8}

expressed numerically and as a percentage distribution in Tables 4.11-4.14. These are compared to the percentage distribution observed for the environmental data for copper and lead given in Tables 4.15 and 4.16.

The effect of iron complexation at the concentration range determined (1.8-7.0 mg l^{-1}) in various pore water samples indicates total complexation occurs with the free hydroxal groups and that no apparent competitive complexation for the other organic ligands takes place. This suggests that the organic ligands are present in the samples in excess. This is confirmed by reducing the observed ligand concentrations by a factor of one thousand, when the recalculated distribution was negligible.

Soil pore water Y gave a good comparison of percentage distribution for the calculated and observed data, with 82% of copper calculated to be with citric acid and 97% for the observed. The expected calculated inorganic percentage of 16% was not observed in the experimental data (2%), but minor amounts of copper are seen associated with formic, malic and lactic acids.

Samples F, H and V all showed significant variations between the calculated and the observed data. From stability constant data 88-98% of the copper is predicted to be associated with citric and malic acids. The observed values are for citric acid 18-32%, and for malic acid 4-38%. The inter sample variations and comparison of percentage distribution for observed and theoretical data suggest strongly the system is not in thermodynamic equilibrium. In systems such as soil pore waters meta-stable conditions may occur with true thermodynamic equilibrium only being reached when competing activation energy barriers can be crossed. Lindsay (315) suggests that the major factors effecting equilibrium are (i) the removal of ions from the soil solution causing partial desorption of similar ions from the exchange complex, (ii) either supersaturation of solution with respect to any mineral which may precipitate or undersaturation which may dissolve minerals, and (iii) microorganisms absorbing and releasing soluble substrates within the soil solution. In addition rainfall, drainage and fertilizer usage will all have some effect on the thermodynamic equilibrium of

Table 4.11

Side reaction coefficients for copper

Sample No.		A	B	D	F	H	V	Y
Formic acid		1.8×10^{-2}	9.1×10^{-3}	5.7×10^{-3}	6.5×10^{-3}	3.0×10^{-2}	2.3×10^{-2}	5.7×10^{-2}
Malic acid	HL	1.3×10^{-3}	5.6×10^{-4}	2.2×10^{-4}	2.1×10^{-3}	5.4×10^{-5}	3.6×10^{-5}	3.0×10^{-6}
	L	1.7	1.5	2.0×10^{-1}	1.1×10^{-1}	6.2×10^{-2}	4.6×10^{-2}	2.5×10^{-1}
Lactic acid		-	-	-	2.5×10^{-3}	6.0×10^{-2}	5.3×10^{-3}	1.0×10^{-2}
Citric acid	H_2L	-	-	-	-	2.2×10^{-4}	-	-
	HL	-	-	-	1.4×10^{-2}	6.3×10^{-3}	6.3×10^{-3}	1.9×10^{-2}
	L	-	-	-	8.4×10^{-1}	10	10	18.1
$[CO_3]^{2-}$		6.4×10^{-1}	1.62	6.4×10^{-1}	1.5×10^{-3}	4.7×10^{-1}	4.6×10^{-1}	2.3×10^{-1}
$[OH]^-$		3.2×10^{-2}	6.3×10^{-2}	3.2×10^{-2}	1.0×10^{-3}	2.5×10^{-2}	2.5×10^{-2}	1.6×10^{-2}
$\bar{\alpha}$		3.4	4.1	1.9	2.0	11.7	11.6	18.7

Table 4.12

Side reaction coefficients for lead

Sample No.		A	B	D	F	H	V	Y
Formic acid		8.0×10^{-3}	4.1×10^{-3}	2.5×10^{-3}	2.9×10^{-3}	1.3×10^{-2}	1.0×10^{-2}	2.5×10^{-2}
Malic acid	L	3.3	2.8	5.2×10^{-1}	2.0×10^{-1}	1.3×10^{-1}	8.8×10^{-2}	4.9×10^{-1}
Lactic acid		-	-	-	6.9×10^{-4}	1.7×10^{-2}	1.5×10^{-3}	2.8×10^{-3}
Citric acid	H_2L	-	-	-	3.9×10^{-5}	-	-	-
	HL	-	-	-	5.0×10^{-3}	2.2×10^{-3}	2.2×10^{-3}	6.7×10^{-3}
	L	-	-	-	1.3×10^{-2}	1.5×10^{-1}	1.5×10^{-1}	2.7×10^{-1}
$[CO_3]^{2-}$		2.2×10^{-1}	5.6×10^{-1}	2.2×10^{-1}	5.1×10^{-4}	1.6×10^{-1}	1.6×10^{-1}	7.9×10^{-2}
$[OH]^-$		6.2×10^{-2}	1.2×10^{-1}	6.2×10^{-2}	1.9×10^{-3}	6.9×10^{-2}	4.9×10^{-2}	3.1×10^{-2}
$\bar{\alpha}$		4.6	4.5	8.1	1.2	1.5	1.5	1.9

Table 4.13

Percentage distribution of side reaction coefficients for copper

Sample No.		A	B	D	F	H	V	Y
1 Formic acid	% of 1-4	1.0	0.6	2.7	0.7	0.3	0.2	0.3
	% of 1-6	0.7	0.3	0.6	0.7	0.3	0.2	0.3
2 Malic acid	% of 1-4	98.9	99.4	97.3	11.1	0.6	0.5	1.4
	% of 1-6	71.5	46.3	23.1	11.1	0.6	0.4	1.4
3 Lactic acid	% of 1-4	-	-	-	0.3	0.6	0.1	0.1
	% of 1-6	-	-	-	0.2	0.6	0.1	0.1
4 Citric acid	% of 1-4	-	-	-	88.0	98.5	99.2	98.2
	% of 1-6	-	-	-	87.7	93.9	94.7	97.0
5 $[CO_3]^{2-}$		26.4	51.4	72.6	0.1	4.4	4.4	1.2
6 $[OH]^-$		1.3	2.0	3.6	0.1	0.2	0.2	0.1

Table 4.14

Percentage distribution of side reaction coefficients for lead

Sample No.		A	B	D	F	H	V	Y
1 Formic acid	% of 1-4	0.2	0.1	0.5	1.3	4.2	4.1	3.2
	% of 1-6	0.2	0.1	0.3	1.3	2.5	2.2	2.8
2 Malic acid	% of 1-4	99.8	99.9	99.5	90.4	42.0	34.8	61.1
	% of 1-6	91.8	80.1	64.5	89.5	25.3	19.1	53.7
3 Lactic acid	% of 1-4	-	-	-	0.3	5.2	0.6	0.3
	% of 1-6	-	-	-	0.3	3.2	0.3	0.3
4 Citric acid	% of 1-4	-	-	-	7.9	48.5	60.5	35.3
	% of 1-6	-	-	-	7.8	29.2	33.2	31.0
5 $[CO_3]^{2-}$		6.2	16.2	27.5	0.2	30.5	34.6	8.7
6 $[OH]^-$		1.7	3.5	7.6	0.9	9.3	10.6	3.4

Table 4.15

Percentage distribution for the copper determinations made on soil pore waters

Sample No.		A	B	D	F	H	V	Y
1 Formic acid	% of 1-4	-	-	9.1	-	-	38.5	-
	% of 1-7	-	-	4.3	-	-	30.3	-
2 Malic acid	% of 1-4	-	-	17.1	8.0	69.4	39.7	-
	% of 1-7	-	-	8.0	3.8	38.1	31.3	-
3 Lactic acid	% of 1-4	-	-	18.4	22.7	-	-	-
	% of 1-7	-	-	8.6	10.6	-	-	-
4 Citric acid	% of 1-4	100.0	100.0	55.3	69.2	30.5	21.8	100.0
	% of 1-7	66.6	40.0	25.9	32.4	16.8	17.2	82.5
5 Inorganic/highly polar		33.3	60.0	53.1	53.1	30.5	21.2	15.7
6 2 Ketoglutamic acid		-	-	-	-	11.0	-	-
7 Allantoin		-	-	-	-	3.6	-	-

Table 4.16

Percentage distribution for the lead determinations made on soil pore waters

	Sample No.		A	B	D	F	H	V	Y
1	Formic acid	% of 1-4	19.3	46.3	25.0	30.5	26.3	-	37.1
		% of 1-7	17.8	28.3	10.3	16.8	17.5	-	26.8
2	Malic acid	% of 1-4	42.6	24.4	22.4	-	21.2	-	18.6
		% of 1-7	28.5	14.9	9.2	-	14.1	-	13.4
3	Lactic acid	% of 1-4	-	-	12.9	-	-	-	-
		% of 1-7	-	-	5.3	-	-	-	-
4	Citric acid	% of 1-4	38.1	29.2	39.6	69.5	52.5	-	44.3
		% of 1-7	25.5	17.9	16.4	38.3	35.1	-	31.9
5	Inorganic/highly polar		16.5	19.4	30.2	23.8	16.9	-	16.5
6	2 Ketoglutamic acid		16.5	19.4	28.5	10.5	16.4	-	11.3
7	Allantoin		-	-	-	10.5	-	-	-

the soil pore water. The indications are that competing metals, pH (315), chlorides and other anions (316) may also effect the equilibria of these systems (317), and that the presence of aluminium, silicon, iron and manganese dissolved in pore waters will have insignificant effects (315).

The pore waters in this study were extracted from soils under both woodland (pore waters B, F, Y) and permanent pasture (pore waters A, D, H, V). The soil type under both these forms of cultivation were brown earths. Data obtained indicated no obvious differences in elemental levels or associations with specific polar dissolved organic compounds between sites with differing vegetation cover. The high pore water elemental levels followed the trends found in the soils, which contain elevated levels of copper and lead as a result of weathering of bed rock effected by mineralization. No apparent trends between either pH or organic matter content and the presence of polar dissolved organic matter were observed for both woodland and permanent pasture soils for the limited number of samples examined.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION WITH ULTRA-VIOLET AND ATOMIC ABSORPTION DETECTION OF ORGANO-ARSENIC COMPOUNDS IN SOIL PORE WATERS

5.1 Detection of arsenic compounds by UV

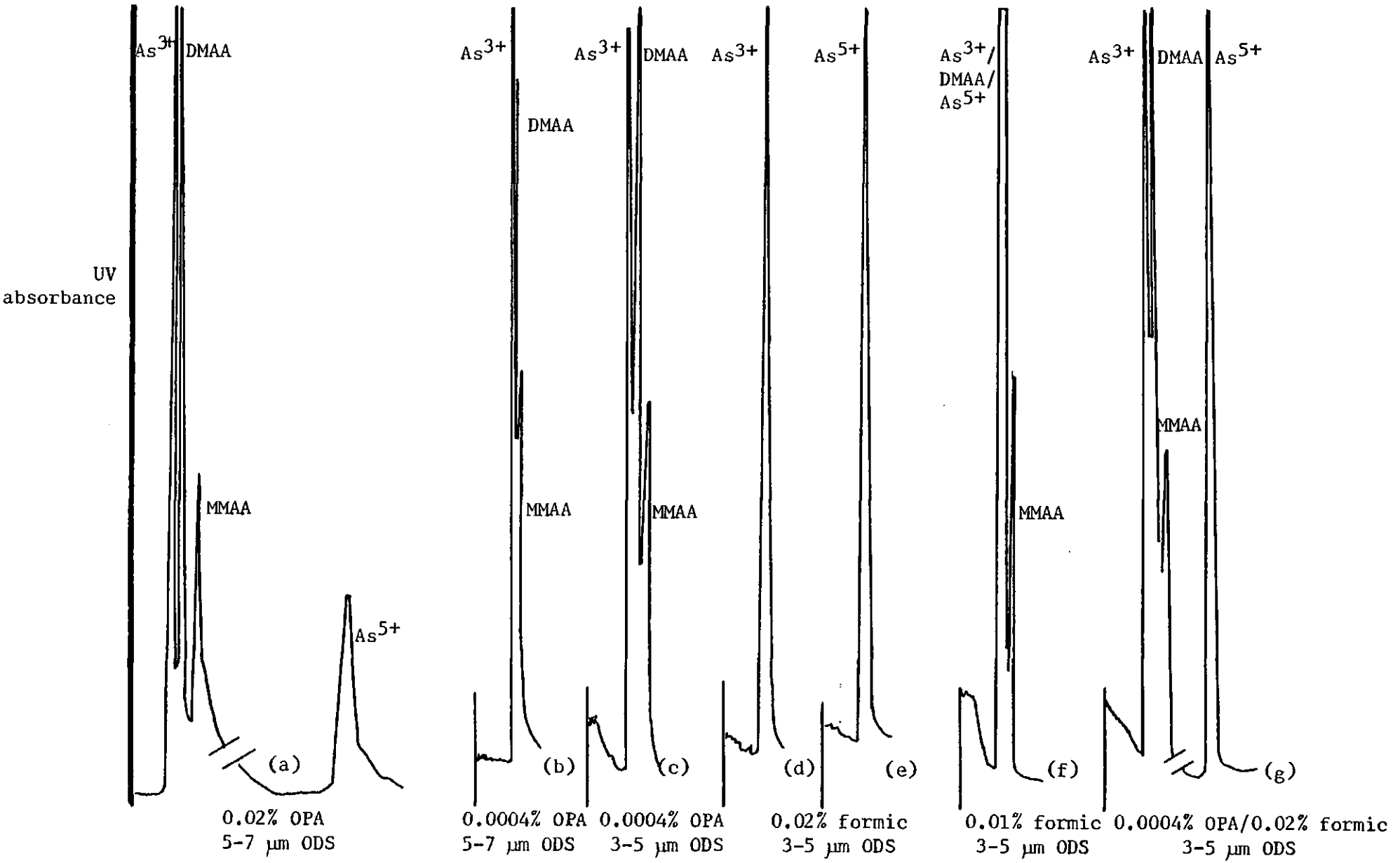
All arsenic compounds analysed were found to absorb in the UV at the following wavelengths, disodium arsenate λ max 195 nm, sodium arsenite λ max 192 nm, monomethylarsonic acid (MMAA) λ max 193 nm and dimethylarsinic acid (DMAA) λ max 192 nm. UV detection was used initially in order to achieve rapid analysis time for the development of chromatographic systems.

5.2 Development of a chromatographic system for the separation of organo-arsenic compounds

Fig. 5.1a shows the separation achieved on a 5-7 µm ODS column using 0.02% orthophosphoric acid elution system. This elution system was found to be incompatible with the GFAAS, as phosphate interfered with the arsenic signal. Reduction of the orthophosphoric concentration to 0.0004% to minimize GFAAS interference resulted in loss of separation of arsenite and dimethylarsinic acid on the 5-7 µm ODS column (Fig. 5.1b). Separation was achieved with the increased efficiency of the 3-5 µm ODS column (Fig. 5.1c). The long retention time of arsenate on the 3-5 µm ODS column with 0.0004% orthophosphoric was shortened by changing the elution system to formic acid (0.02%), which elutes arsenate immediately without causing interference to the GFAAS signal (Fig. 5.1e). The use of formic acid (0.01%) to separate arsenite, dimethylarsinic acid, monomethylarsonic acid and arsenate, resulted in arsenite, dimethylarsinic acid and arsenate coincidental peaks (Fig. 5.1f). A stepped orthophosphoric acid (0.0004%) formic acid (0.02%) elution system with the 3-5 µm ODS column was able to separate the four compounds without significant interference in the GFAAS signal for concentrations of 100 ppm As (Fig. 5.1g). Further work carried out at lower concentrations (100 ppb As) using the proposed elution system with the GFAAS for detection indicated problems of column degradation, modification and sorption by phosphorus and arsenic, probably on uncapped silanated sites. The use of paired ions

Fig. 5.1

UV absorbances of arsenic species separated on a single ODS column with various elution systems

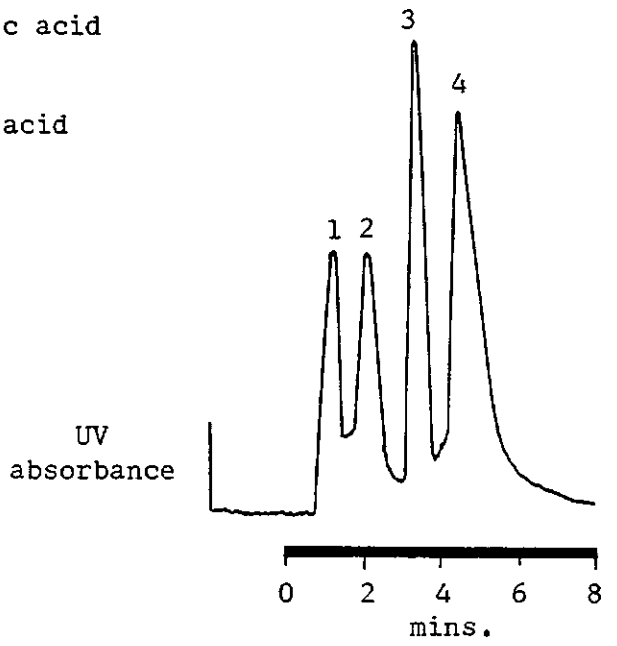


with this system, such as dodecylsulphonic acid, give a background signal too large for the IL151 correction facility.

Using again the Hypersil ODS (3-5 μm 250 x 4mm) column a second isocratic elution system, sulphuric acid (1.8 x 10^{-5}M) was investigated. This was found to give reproducable separation for standards of arsenate, monomethylarsonic acid, arsenite and dimethylarsinic acid (Fig. 5.2).

Fig. 5.2
The separation of arsenic species on a single ODS column with sulphuric acid as the eluent

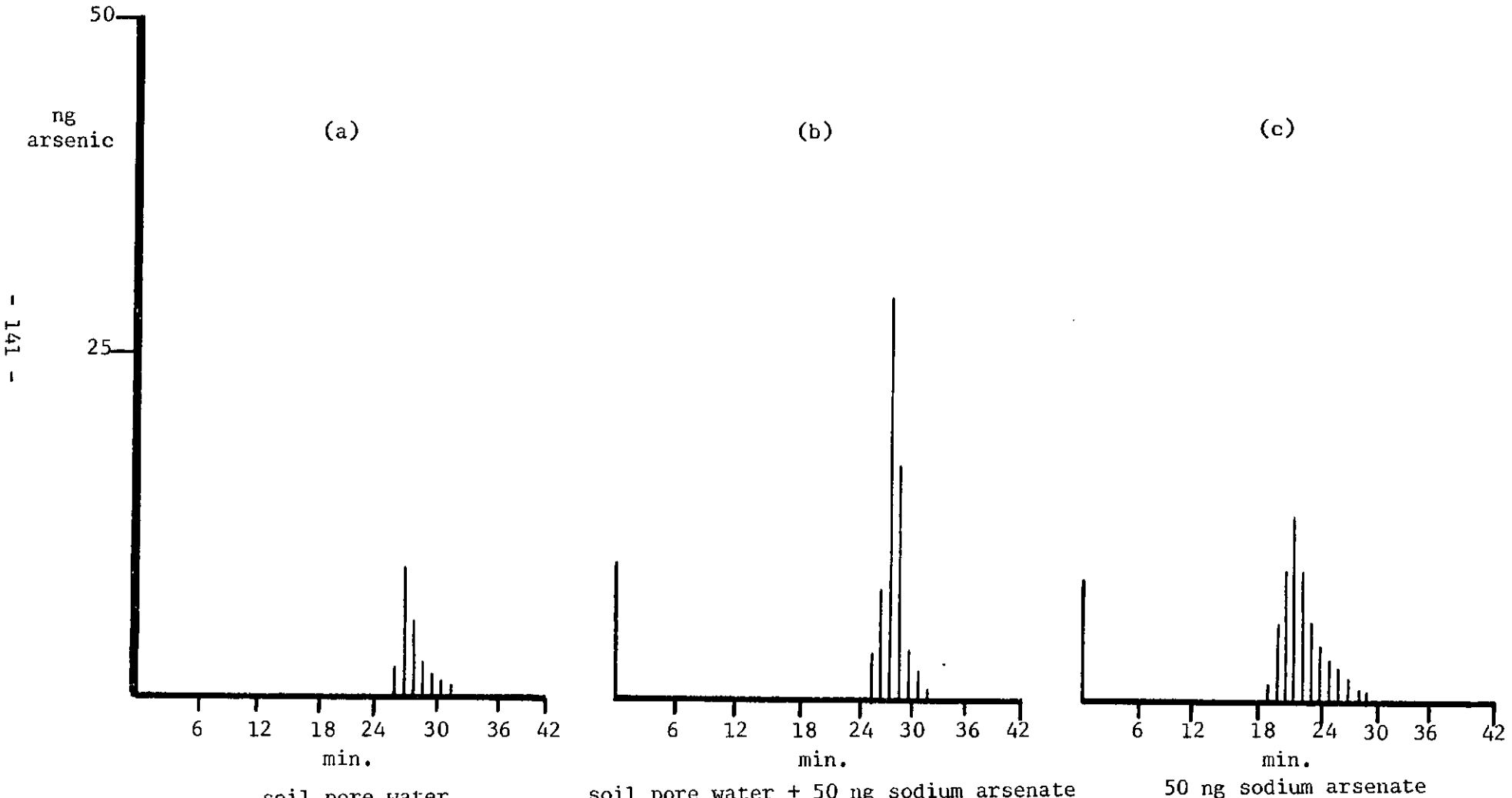
1 sodium arsenate
2 monomethylarsonic acid
3 sodium arsenite
4 dimethylarsinic acid



Using the Hypersil ODS column and sulphuric acid elution system described above, a soil pore water from the Gawton area (Fig. 1.2) was analysed. The chromatogram for this sample can be seen in Fig. 5.3a. A coinjection with sodium arsenate gave a quantitative identification of the peak (Fig. 5.3b), however, an injection of a single standard of sodium arsenate resulted in a change in retention times (Fig. 5.3c). The shift in retention times of the arsenate in the soil pore water by comparison to standards was thought due to interference effects resulting from the sample matrix. This technique was therefore rejected as a method for assessing arsenic speciation in soil pore waters.

Fig. 5.3

Analysis of a soil pore water by HPLC/GFAAS interface using an ODS column with the sulphuric acid elution system



Separations on anion exchange columns were examined as possible ways to overcome the uncertainty in reproducability observed with the Hypersil ODS columns. A Vydac 3021C46 silica based anion exchange column (10 μm) gave poor separations when examined with various elution systems, however, a resin based strong anion exchange (SAX) BAX10 (5 μm) column with an ammonium carbonate (0.1M) elution system gave acceptable separations. The additional use of a precolumn packed with Zipax, a silica based anion exchange material (40 μm) and a step elution system of sulphuric acid (10^{-4}%)/ammonium carbonate (0.1M), resulted in a preconcentration step on the Zipax, which in addition acted as a guard column, resulting in a reproducable separation on the SAX column (Fig. 5.4). Spiking experiments have shown that the four peaks are due to arsenite (1), dimethylarsinic acid (2), monomethylarsonic acid (3) and arsenate (4) respectively. Initial experiments showed that arsenite is not retained on either of the columns, even in the sulphuric acid elution system. Because of the arbitrary nature of switching over eluents, retention times for the last three peaks are best measured with respect to the last peak. Fig. 5.5 shows the use of the preconcentration facility of this system for the analysis of a soil pore water in which there are low concentrations of arsenic species present. It was noted that no apparent loss in resolution occurs when preconcentration was used.

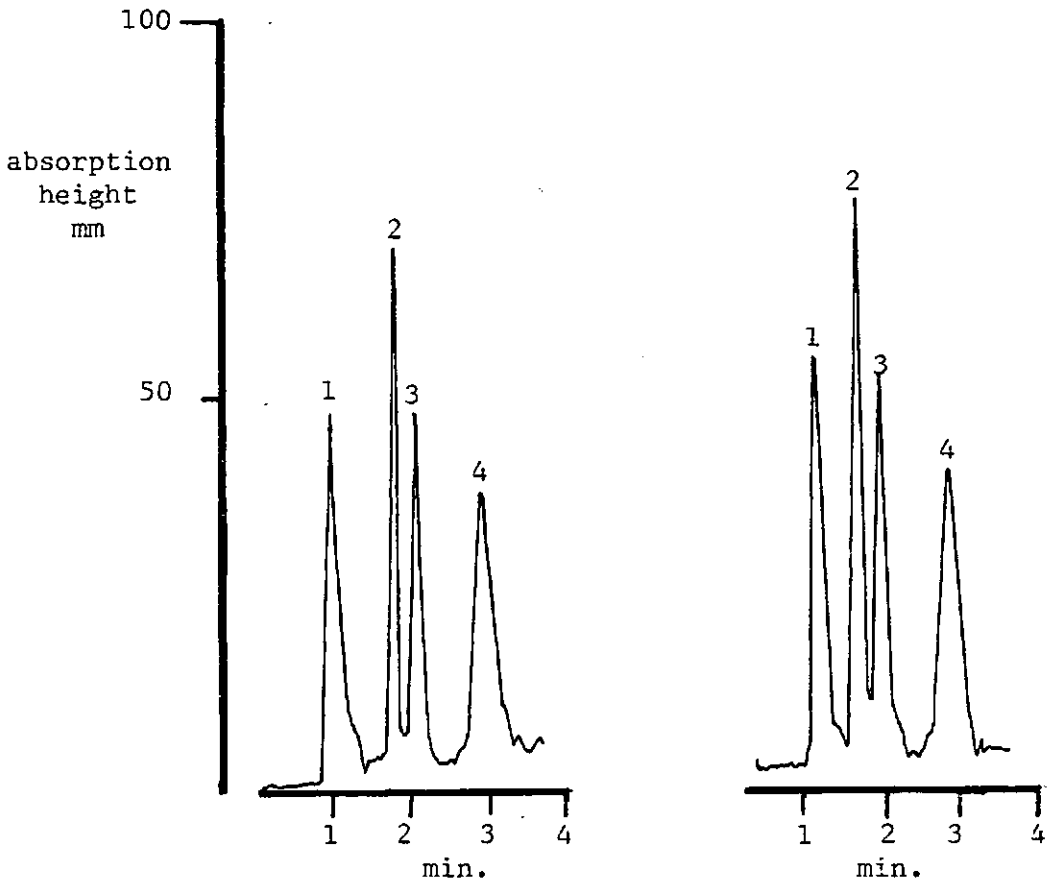
5.3 Sample storage

Soil pore waters were collected from a bog area not directly effected by arsenic mineralization. These samples were treated in two ways: (i) filtered (< 0.45 μm) on site, and (ii) sealed unfiltered in an air-tight container and filtered (< 0.45 μm) just prior to analysis, samples from both treatments were stored in the light at room temperature. The samples filtered on site (Fig. 5.6, Table 5.1) indicated the samples may be stored for up to 5 hours with < 10% loss of arsenite compared to 20% for samples stored unfiltered (Fig. 5.7, Table 5.2). For both samples within 12 hours 90% of arsenite was oxidized to arsenate, with both dimethylarsinic acid and monomethylarsonic acid showing losses from the system. All subsequent pore water samples analysed were filtered on site at time of collections.

Fig. 5.4

Separation of arsenic species on anion exchange columns with continuous arsine generation and atomic absorption detection

It should be noted that although the retention times of the latter three components are dependent on the eluent change over time, their relative separations are consistent with each other.



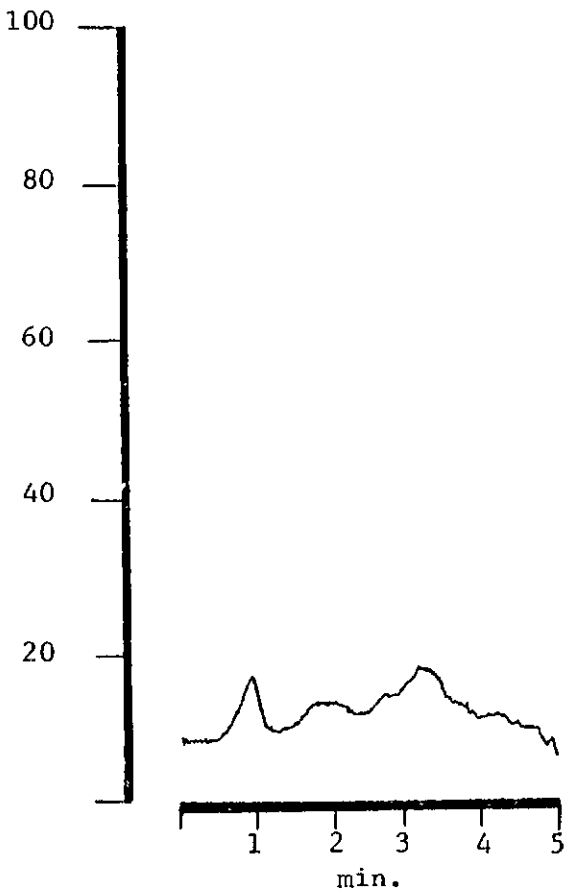
Peak

1	arsenite
2	dimethylarsinic acid
3	monomethylarsonic acid
4	arsenate

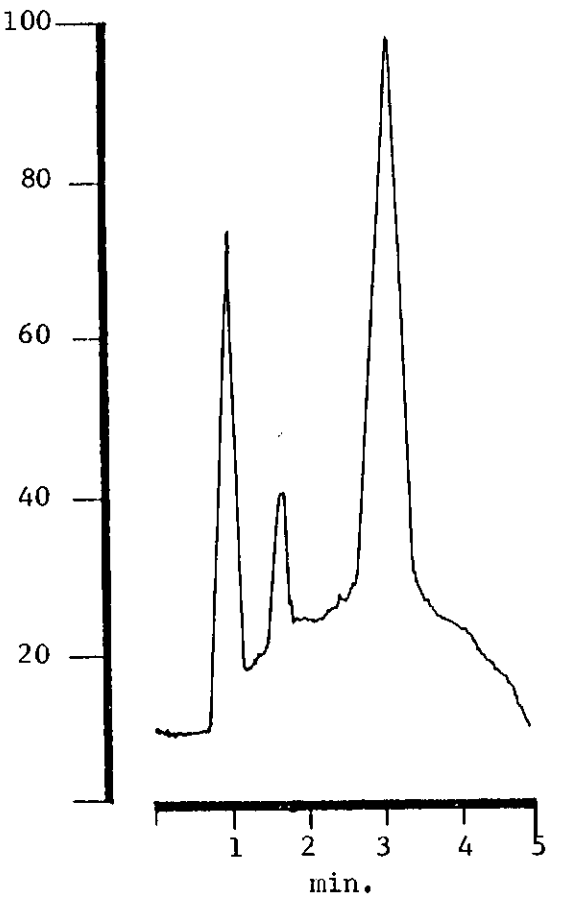
10 ng of each species injected

Fig. 5.5

Preconcentration of arsenic species in a soil pore water on the Zipax anion exchange column



100 µl injection of soil pore water



1 ml injection of soil pore water

Fig. 5.6

Changes in arsenic species for samples filtered on site and stored at room temperature in the light

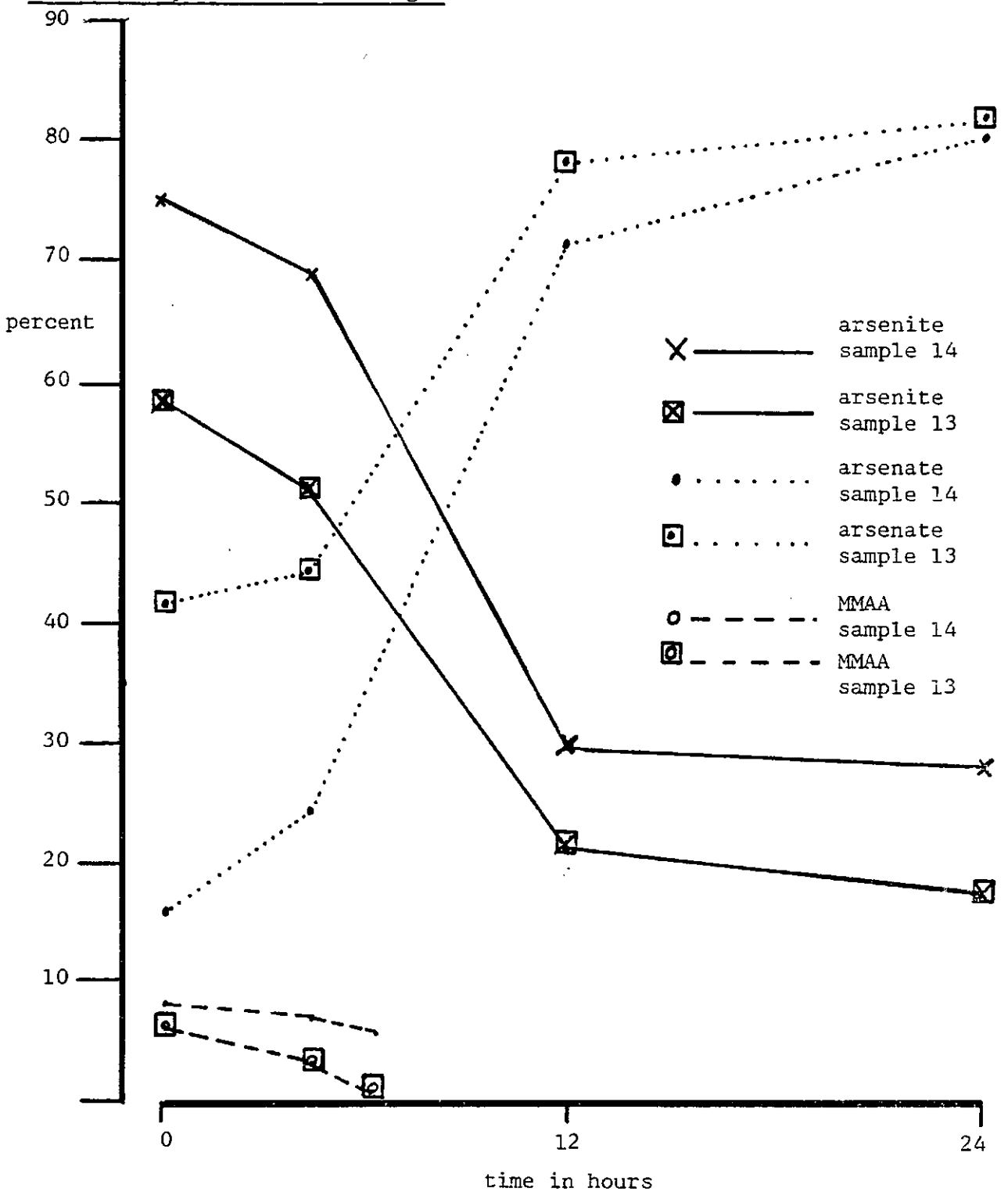


Table 5.1

Concentrations of arsenic species in soil pore water samples filtered at time of collection and stored in the light at room temperature

Hours	Sample No.	Arsenite $\mu g\ l^{-1}$	DMAA $\mu g\ l^{-1}$	MMAA $\mu g\ l^{-1}$	Arsenate $\mu g\ l^{-1}$	Σ of species $\mu g\ l^{-1}$
1	13	10	-	1	7	17
	14	15	-	2	3	20
	15	2	-	-	13	14
5	13	10	-	1	9	19
	14	15	-	1	5	21
8	15	2	-	-	14	16
12	13	4	-	-	16	20
	14	6	-	-	15	21
24	13	3	-	-	14	17
	14	6	-	-	17	21

Fig. 5.7

Changes in arsenic species for samples unfiltered stored at room temperature in the light and filtered prior to analysis

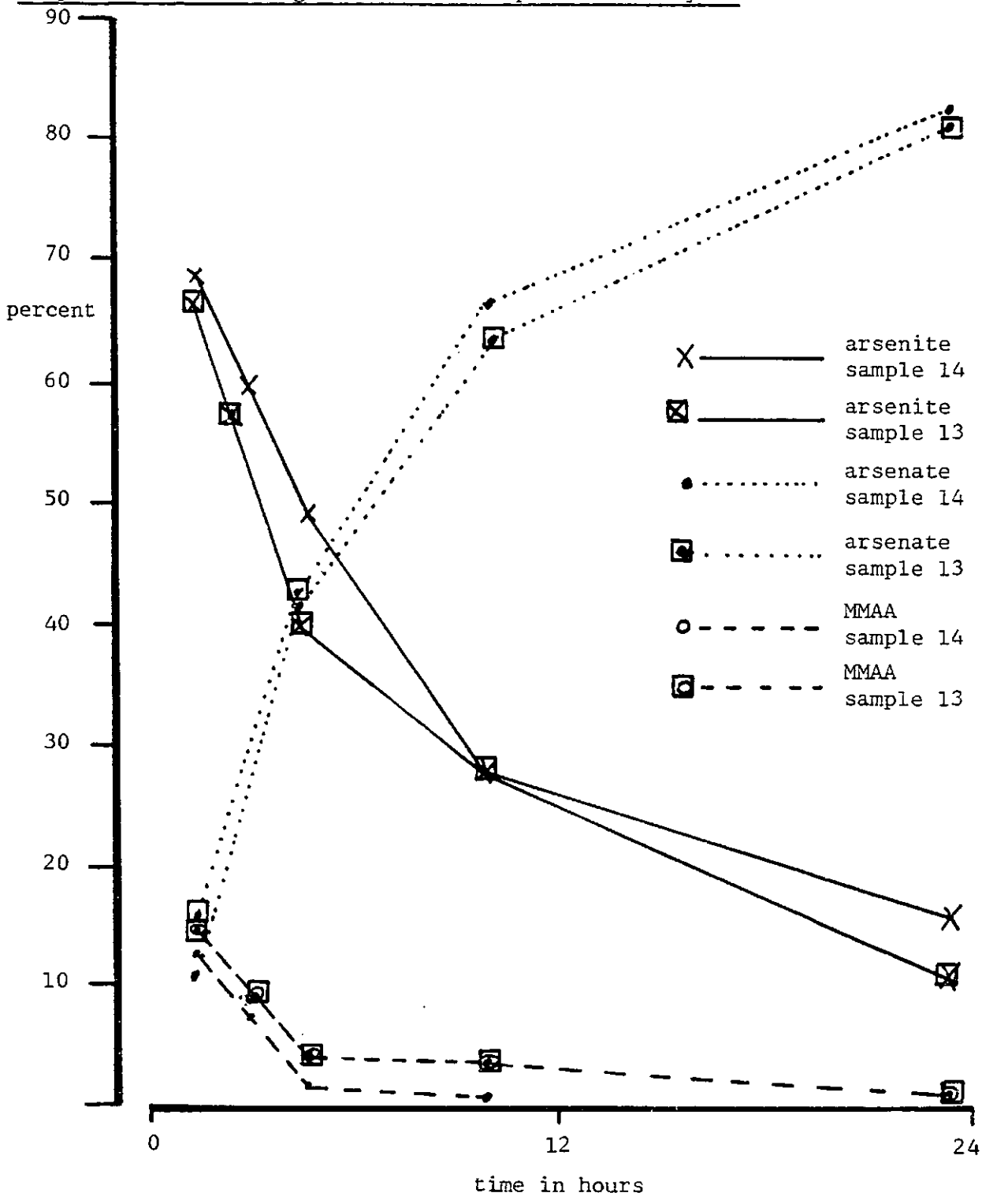


Table 5.2

Concentrations of arsenic species in soil pore water samples stored unfiltered in the light at room temperature and filtered prior to analysis

Hours	Sample No.	Arsenite $\mu g\ l^{-1}$	DMAA $\mu g\ l^{-1}$	MMAA $\mu g\ l^{-1}$	Arsenate $\mu g\ l^{-1}$	Σ of species $\mu g\ l^{-1}$
3	13	13	-	3	3	20
	14	16	-	4	3	23
4.5	13	13	-	2	6	21
	14	14	-	2	6	23
6	13	9	-	1	10	22
	14	10	-	1	9	20
11	13	6	-	1	12	19
	14	6	-	1	14	21
24	13	2	-	1	15	18
	14	4	-	-	18	22

5.4 Arsenic speciation in soil pore waters from mineralized and unmineralized areas

By using the survey data for arsenic soil levels, presented in section 3.4, soil pore waters were collected from mineralized, unmineralized and unmineralized but water logged sites (Fig. 5.8); the levels and species found are shown in Table 5.3.

In all aerobic soil the predominant species($\approx$ 90%)present in the soil pore waters was found to be arsenate, this agrees with other published work (16). Where mineralization has enhanced the soil levels (samples 1, 2, 3, 4) the presence of monomethylarsonic acid is observed. These results are not in complete agreement with Woolson (87), who reported the presence of dimethylarsinic acid as the major organo-arsenic compound to be present, the results he reported, however, were for soils treated with organo-arsenic pesticides. The range of arsenite in samples 1, 2, 3 and 4 is not noticeably different from that obtained for unmineralized areas (samples 5, 6, 7, 8). This suggests a possible threshold concentration for arsenite may exist after which point monomethylarsonic acid is microbially produced (Fig. 5.9). The pH of these soil pore waters are typically 4.1-5.3. Samples 9, 10, 11 and 12, also from a mineralized area (Fig. 5.8), have a similar range of arsenate levels to samples 1, 2, 3 and 4, however, comparison of the samples containing the highest total levels of arsenic (samples 1, 2, 9 10) indicate that samples 9 and 10 have less monomethylarsonic acid than those levels determined in samples 1 and 2. This reduction of monomethylarsonic acid in samples 9 and 10 corresponds to elevated levels of arsenite, this relationship is also apparent in samples with intermediate total arsenic levels, namely samples 4, 11 and 12. Thus in samples where arsenic levels are elevated the arsenite concentrations may either (a) stay similar to those in non elevated samples, in which case the presence of monomethylarsonic acid is observed, or (b) increase 2-5 times the levels normally observed, in which case no or very little monomethylarsonic acid is observed. As the mechanism of methylation of arsenite to monomethylarsonic acid is controlled solely by micro-organisms (Fig. 5.9), it is postulated that the build up of toxic arsenite in the soils by either chemical (Fig. 5.10) or microbial

Fig. 5.8

Sample sites for soil pore waters used in arsenic species determinations

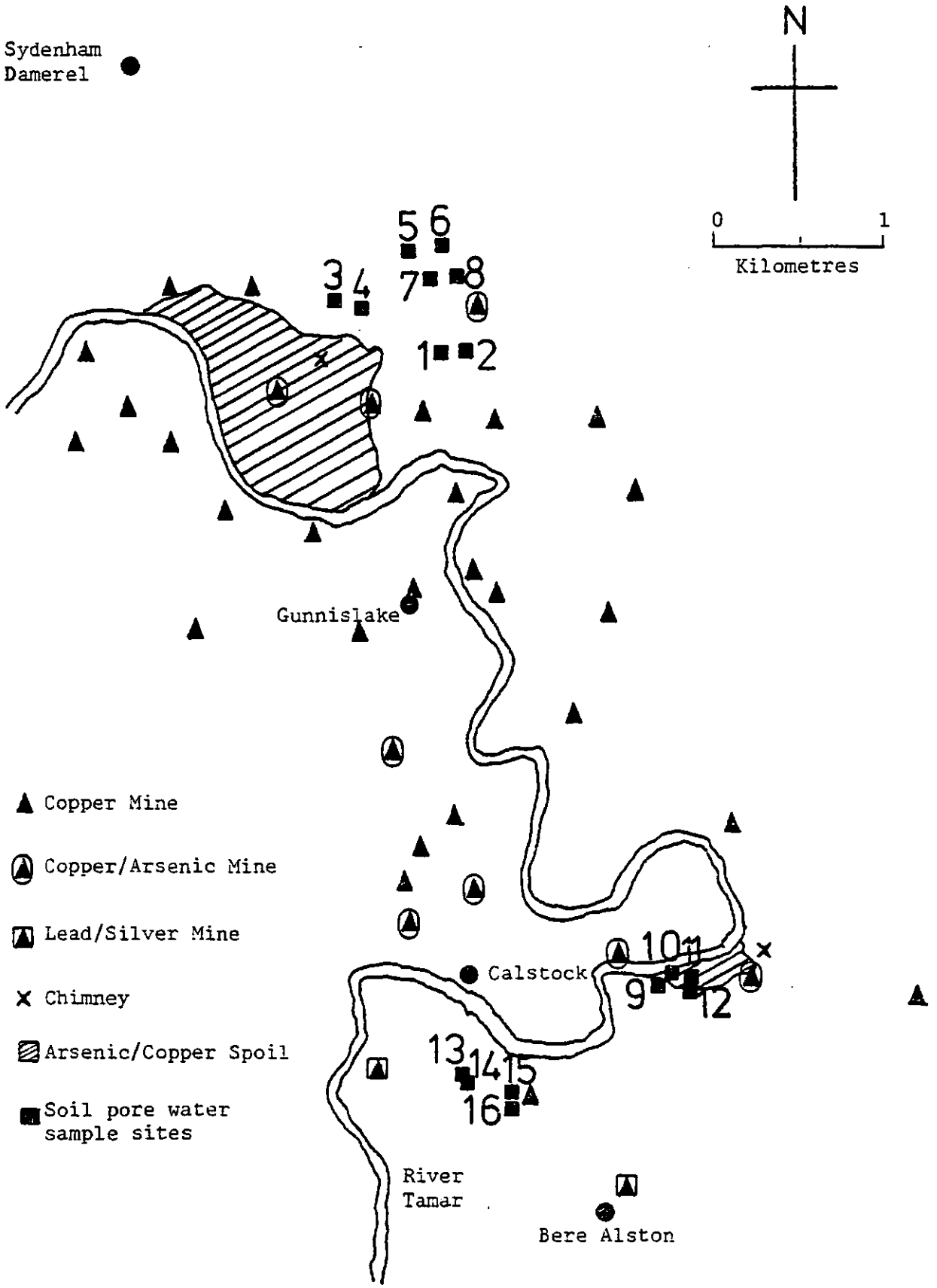


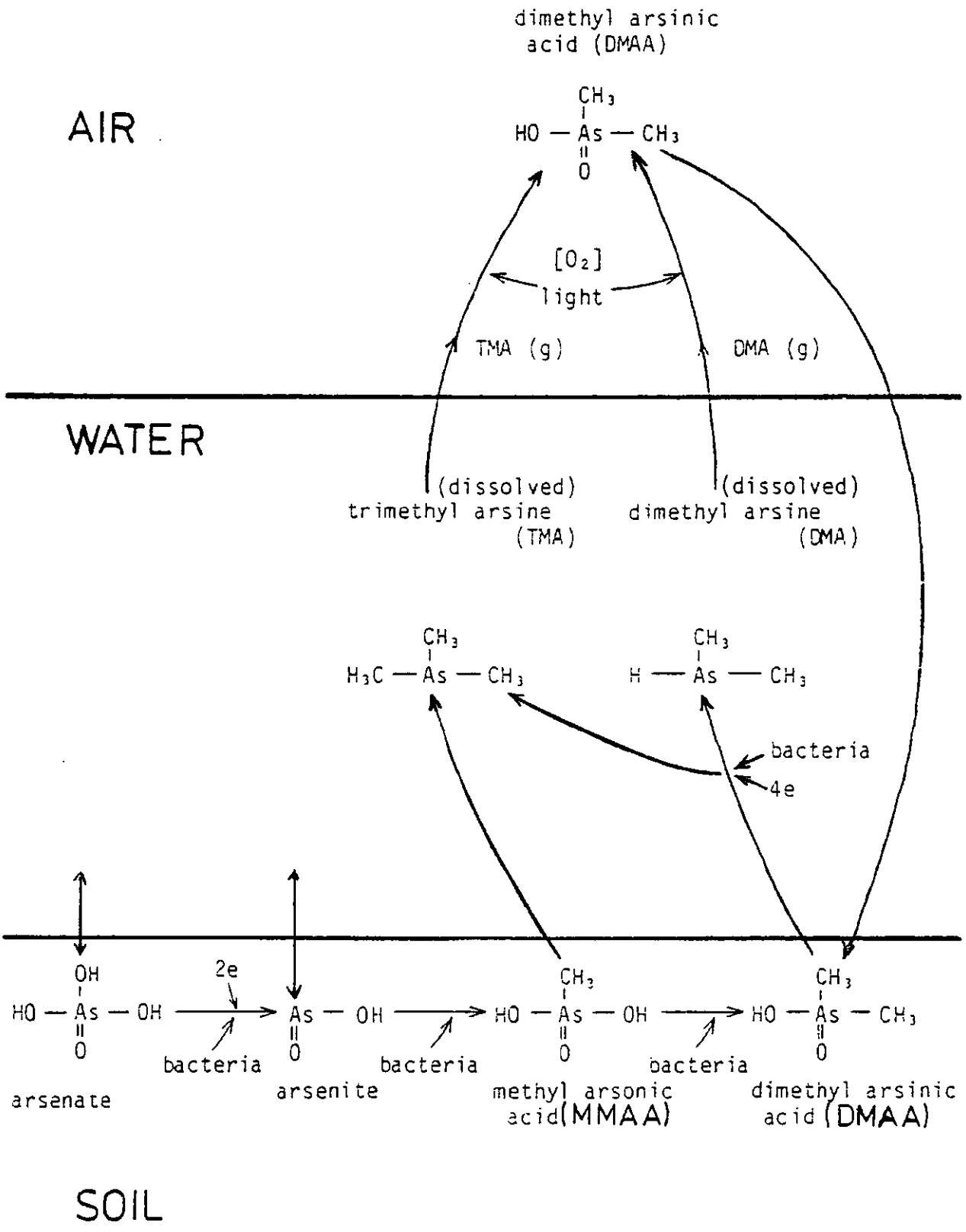
Table 5.3

Concentrations of arsenic species in soil pore water samples

Sample No.	pH	Arsenite µg l^{-1}	DMAA µg l^{-1}	MMAA µg l^{-1}	Arsenate µg l^{-1}	Σ of species µg l^{-1}	Total by hydride µg l^{-1}	GFAAS µg l^{-1}
1	3.9	3	-	22	210	235	240	210
2	4.2	2	-	19	210	231	-	210
3	5.0	2	-	11	84	96	-	100
4	5.3	8	-	4	79	91	-	110
5	5.7	2	-	-	35	37	40	86
6	5.4	1	-	1	34	36	-	79
7	5.6	7	-	-	49	57	59	52
8	5.5	2	-	-	44	46	45	54
9	4.1	6	-	7	200	212	220	210
10	4.0	7	-	8	210	230	220	230
11	4.9	11	-	-	80	91	93	150
12	5.1	8	-	-	150	160	170	180
13	4.3	10	-	1	7	17	17	21
14	4.4	15	-	2	3	20	22	18
15	5.5	2	-	-	13	14	13	11
16	5.2	1	-	-	15	16	15	10

Fig. 5.9

Proposed microbiological cycle of arsenic (129)



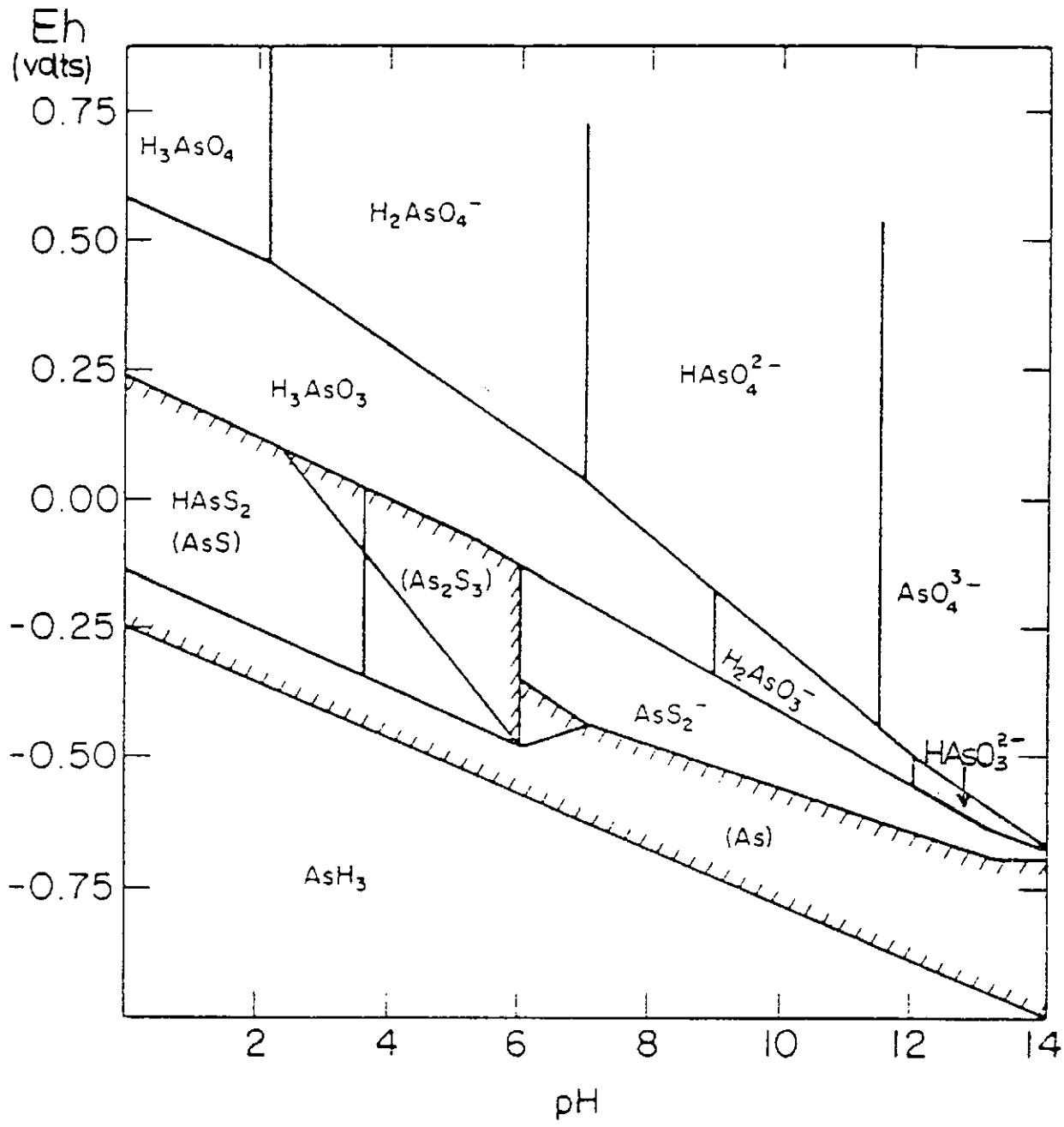


Fig. 5.10

Eh-pH diagram for arsenic at 25°C and 1 atmosphere (123)

(Fig. 5.9) reduction may inhibit the activity of microorganisms involved in the methylation process.

The typical total levels of arsenic found in unmineralized aerobic soils (samples 5, 6, 7, 8, 15, 16) are 16-56 μg l^{-1} compared to 80-215 μg l^{-1} for the previously discussed mineralized soils. The presence of monomethylarsonic acid in the samples from unmineralized areas is not observed, except in one sample (sample 6), which is unusual in that the arsenite level is low, this may be some unique microbial or soil chemistry effect.

Samples 13 and 14 were collected from anaerobic soils in an unmineralized area (Fig. 5.8). The arsenite which represents 59% and 77% of the total arsenic present by contrast to ≈ 10% for aerobic soils is in agreement with previously published results, which suggest arsenite in anaerobic soils may be up to 80% (318). The chemical conditions in the aerobic and anaerobic environment are shown in Fig. 5.10, these suggest the presence of arsenite will be elevated due to chemical reducing conditions. Although microbial reduction of arsenate to arsenite is reported (127) at the redox potential experienced in anaerobic soils (i.e. < 0.00 volts), the chemical reduction overrides the microbial conversion of arsenate to arsenite (124). Thus any microorganism feedback mechanism controlling the toxic arsenite concentrations will be impaired in water logged soils. The low levels of monomethylarsonic acid in these samples may be due to volatilization or reduced microbial activity (127, 130).

Comparison between total determined arsenic by species summation and by single analysis on the hydride agree to within ± 5% and between total hydride and GFAAS ± 10%, with the exception of one sample in which the hydride total was only 45% of the GFAAS values.

SUMMARY AND CONCLUSIONS OF ARSENIC, COPPER AND LEAD SPECIES IN SOIL PORE WATER

6.1 Summary

The soil pore water is the medium through which all the dynamic functions of a soil system operate, as can be illustrated by Fig. 6.1. It is the aqueous phase through which plants absorb their nutrients (flux 1). Small quantities of plant constituents and root exudates are also released back into the soil pore water (flux 2). Ions in the soil pore water are buffered by those adsorbed onto soil surfaces or held by exchange sites (fluxes 3 and 4). Removal of ions from the soil pore water causes partial desorption of similar ions from the exchange sites. The extracting reagents commonly used to quantify the elemental association in this soil reservoir are acetic acid and EDTA.

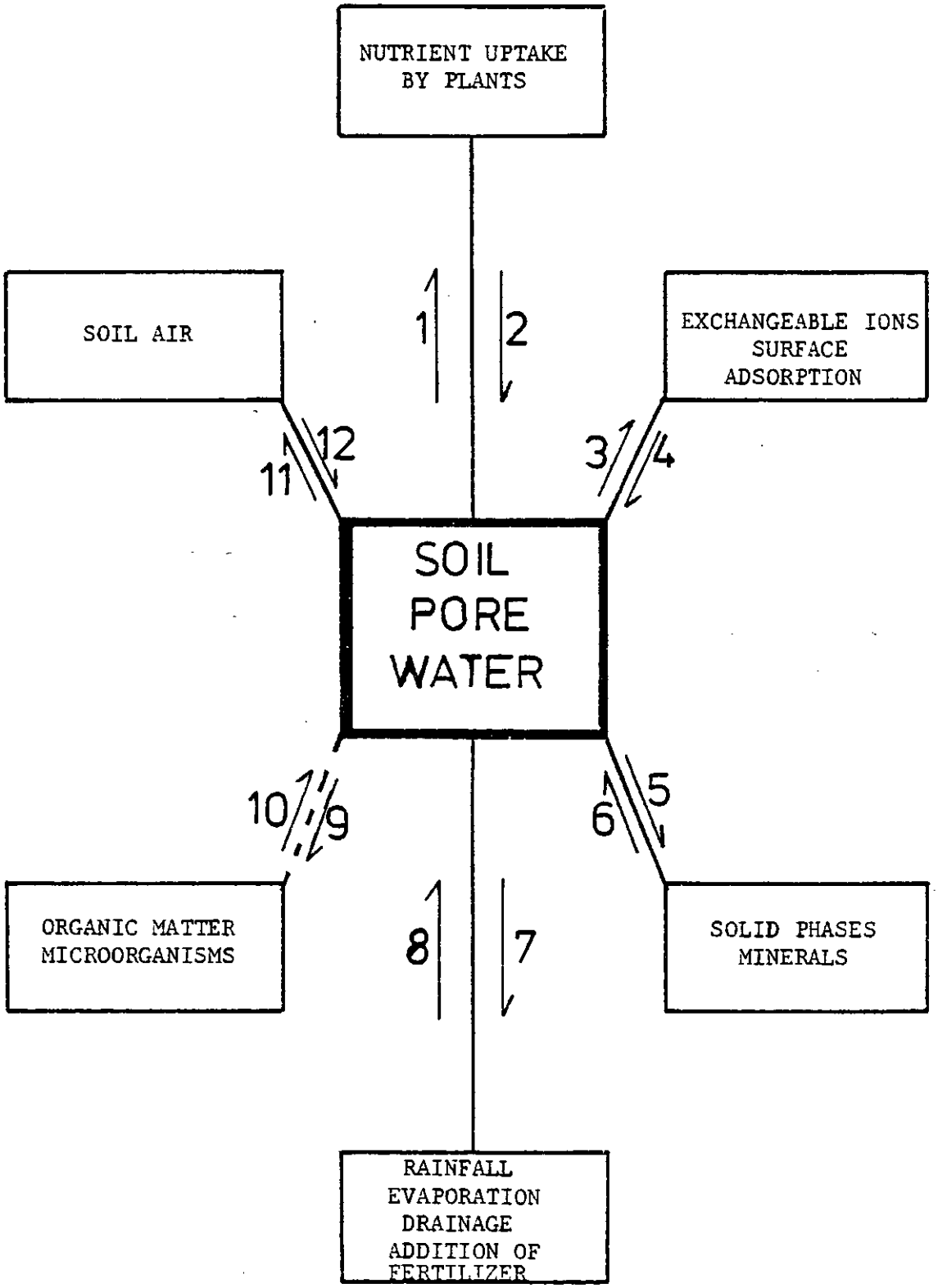
Soils contain numerous minerals, some of which are crystalline, others are amorphous. These minerals impose limits on the chemical composition of the soil pore water. If the soil pore water becomes supersaturated with respect to any mineral that mineral can precipitate (flux 5) until equilibrium is attained. Similarly if the soil pore water becomes undersaturated with respect to any mineral present in the soil, that mineral can dissolve until equilibrium is attained (flux 6). Sodium dithionite and nitric acid are generally used to determine the extent of elemental association with the amorphous and crystalline minerals.

Fluxes 7 and 8 depict several dynamic processes that may occur in soils. For example rainfall adds water that dilutes the soil pore water (flux 8). Excess water may drain from the soil profile and carry with it salts and other dissolved constituents (flux 7), in order to simulate these fluxes experimentally repeated water extractions on soils were carried out.

Organic matter and microorganisms also affect the equilibrium relationships in soils. Living organisms remove constituents from soil pore water and incorporate them into their body tissues

Fig. 6.1

A model of the dynamic equilibria that occur in soils (315)



(flux 9). Similarly extra cellular excretions and nutrients released during the decomposition of organic matter or upon the death of the organism (flux 10) contribute to the soil pore water. These fluxes are connected with a broken line to indicate that true equilibrium relationships are generally not achieved, but are modified by the metabolic energy relationships of microorganisms that mediate many of these reactions. Assessment of the elemental association with organic matter is typically carried out by use of extracting reagents, such as EDTA;with sodium hydroxide commonly used for humic and fulvic acids.

Gases may either be released to the soil air (flux 11) or dissolved in the soil pore water (flux 12). In soils, plants and microorganisms generally utilize oxygen as an electron acceptor and give off carbon dioxide from metabolic processes. In water logged soils the exchange of oxygen and carbon dioxide is greatly restricted because diffusion rates in water are $\approx 10^{-4}$ those in air. As the oxygen in the soil is depleted the soil becomes reduced. The solubility product of the carbon dioxide will effect the relative pH of the soil solution, the greater the level of carbon dioxide saturation the lower the pH will tend to be, carbon dioxide will therefore buffer the acids in the soil pore water. It is generally considered that it is the weathering of the mineral phase of the soil that determines solely the composition of the soil pore water. It should be clear, however, from Fig. 6.1 that the solubility of the mineral phase is effected by subsequent addition and removal of elements by other mechanisms. Often the rates of dissolution and precipitation of soil minerals are so slow that true equilibrium is not attained, consequently both kinetic and thermodynamic factors must be considered.

For a number of soil samples the soil solution was removed and replaced by fresh distilled water, this repeated water extraction experiment simulated fluxes 7 and 8 in Fig. 6.1. The indications from these results are that the major short term supply of readily soluble copper and lead is via flux 4. Subsequent extractions exhaust the supply of soluble ions, suggesting that the reservoir of surface adsorbed and exchangeable ions is only able to hold a limited supply of readily soluble copper and lead. The results for arsenic in the

soils studied indicate that the readily soluble arsenic associated with the amorphous mineral reservoir is a factor of 10 times larger compared to the levels for copper and lead.

The use of distilled water in this experiment simplifies somewhat the complex matrix of a natural soil pore water. It is not considered, however, that this technique will incur any significant errors in the assessment of readily soluble arsenic, copper or lead.

Analysis of the organo-copper, -lead species in pore waters indicates the importance of polar dissolved organic compounds such as citric acid and malic acid, which may complex up to 90% of total soluble copper/lead in a soil pore water. The supply of these organic compounds to the pore water is by fluxes 2 and 10, and it is believed that their production is via root and microorganism secretions. A full understanding of the true role of these organic compounds as complexing agents in soil pore water is not yet clear. The concentration of soluble organo-arsenic, -copper and -lead species in local soil pore waters is dependent on the influence of mineralization, the redox potential of the soil pore water and past mining activities. Arsenic species in particular are controlled by the redox conditions of the soils, i.e. when the soil is aerobic arsenate predominates, whereas in anaerobic soils arsenite levels become significant. When arsenic soil levels are high due to mineralization and past mining activity monomethylarsonic acid is observed. Its presence may be a response of microorganisms to the toxic levels of arsenite in the soils, which when reaching a threshold cause microbial methylation to occur. It should be stressed that the mechanisms of arsenic speciation in soils are not well defined, but it is suggested that fluxes 9 and 10 together with the soil solution redox potential are important in these processes.

6.2 Conclusions

The current literature and results from this work illustrate the lack of detailed information at present available on the composition of soil pore waters. The kinetic nature of the system has been illustrated by comparison of theoretical calculations with experimental data obtained.

Mills and Quinn (264, 265) report that 50-70% dissolved copper in sea water is associated with unspecified dissolved organic compounds with molecular weights < 1000, whilst Lee (266) indicates that fulvic type compounds are significant in copper complexation. The results obtained in this work are in agreement with these previous reports (264-266) and show that up to 90% of the soluble copper and lead in soil pore waters are associated with these low molecular weight polar dissolved organic compounds. The development of an interface between a high performance liquid chromatograph and a graphite furnace atomic absorption spectrometer, described in this thesis, gives the advanced analytical capability for the specific identification of these previously ill-defined low molecular weight polar dissolved organo-metallic complexes. Citric and malic acids appear to be the predominant complexing agents in these soil pore waters.

Further work is needed to identify the role of polar dissolved organic compounds in the processes of mobilization and availability of nutrients in soil systems. The few samples analysed to date give limited information on these soil processes. In order to evaluate more fully the specific role of polar dissolved organic compounds the following proposals are made:

1) To identify any correlations between elemental plant levels and both elemental and polar dissolved organic compound concentrations in soil pore waters. It will be necessary to look at natural and land managed systems, as well as carrying out controlled studies. These studies may be achieved by hydroponic and pot experiments. The hydroponic system reduces the multi-variable system to a simple solution/plant root system, removing the solid and humic acid phases from the experiment. Under experimental conditions combinations of elemental, organic acid and nutrient, in particular phosphate, concentrations, together with plant and microbial species can be more strictly controlled. Assessment of the importance of mineral and organic matter may be achieved with similar pot or lysimeter experiments to those outlined for the hydroponic experiments. In addition the soil texture and structure may be investigated, together with the pH, temperature and redox potential of the soils. Comparison of results obtained in these

experiments to environmentally obtained data will ultimately be necessary to evaluate the role of polar dissolved organic compounds in plant uptake mechanisms. Variations in soil type, land usage, climatic differences and crop varieties will require careful investigation.

2) To identify any role of organo-metallic complexes in the mechanism of root transfer and transportation of elements within the plant. This may be achieved by sampling from just outside and within the root system and from other areas of the growing plant under hydroponic, pot and natural conditions.

3) Identify the role of bacteria, in particular of the species bacillus and pseudomonas, and fungi of the species penicillium, in the generation of organic acids related to the mobilization of elements through complexation. This may be achieved by using selective culture mediums.

The development, described in this thesis, of an interfaced high performance liquid chromatograph, continuous flow hydride generation atomic absorption spectrometer system, permits the rapid detection of reducible arsenic species in soil pore waters at low concentrations. In aerobic soils the pore water arsenic concentrations are dominated by the arsenate species. Where mineralization and past mining activities have elevated the total soluble arsenic levels arsenite and monomethylarsonic acid species are observed. Arsenic speciation in anaerobic water logged soils is markedly different from those of aerobic soils, with the arsenite species representing the greater percentage of the total soluble arsenic present. The role of micro-organisms and redox potentials in the reduction and methylation processes may further be examined under controlled pot and lysimeter experiments.

REFERENCES

1. Dines, H.G., 'The metalliferous mining region of South-West England', vol. 1 and 2, 2nd edition, H.M.S.O., 1969.

2. Edmonds, E.A., McKeown, M.C., and Williams, M., 'South-West England', 3rd edition, British Regional Geology, H.M.S.O., 1969, 130.

3. Baur, W.H., and Onishi, H., 'Handbook of Geochemistry', ed. Wedepohl, K.H., Springe-Verlag Berlin (Heidelberg), 1978, 33-D-2.

4. Zemann, J., and Wedepohl, K.H., 'Handbook of Geochemistry', ed. Wedepohl, K.H., Springe-Verlag Berlin (Heidelberg), 1978, 29-D-11.

5. Sahl, K., Doe, B.R., and Wedepohl, K.H., 'Handbook of Geochemistry', ed. Wedepohl, K.H., Springe-Verlag Berlin (Heidelberg), 1978, 82-D-9.

6. Davies, B.E., Oikos, 1971, 22, 366.

7. Hogan, D.V., Soil Survey Record No. 44, 'Soils in Devon III', sheet SX47 (Tavistock), Adlard & Son Ltd. (Dorking), 1977.

8. Aguilar-Ravello, A.M., 'The regional distribution of arsenic in South-West England', unpub. Ph.D thesis, Univ. of London, 1974.

9. Alloway, B.J., and Davies, B.E., Geoderma, 1971, 5, 197.

10. Colbourn, P., Alloway, B.J., and Thornton, I., Sci. Total Environ., 1975, 4, 359.

11. Davis, B.E., Geoderma, 1976, 16, 183.

12. Thomas, R., 'Arsenic pollution arising from mining activities in South-West England', unpublished, ADAS (Starcross), 1977.

13. Millman, A.P., Geochem. Cosmochim. Acta, 1957, 12, 85.

14. Peterson, P.J., Benson, L.M., and Porter, E.K., 'Heavy metals in the Environ. Int. Conf.', London, Sept. 1979, 198.

15. Porter, E.K., and Peterson, P.J., Sci. Total Environ., 1975, 4, 365.

16. Thompson, A.J., and Thoresby, P., Analyst, 1977, 102, 9.

17. Benson, L.M., Porter, E.K., and Peterson, P.J., J. Plant Nutr., 1981, 3, 655.

18. Porter, E.K., and Peterson, P.J., Environ. Pollut., 1977, 14, 255.

19. Levinson, A.A., 'Introduction to Exploration Geochemistry', 2nd edition, Applied Publishing Ltd. (USA), 1980, ch. 3.

20. Garrels, R.M., and Christ, C.L., 'Minerals, Solutions and Equilibria', Harper and Row, 1965.

21. Goodman, B.A., and Linehan, D.J., 'The soil-root interface', ed. Harley, J.L., and Scott Russell, R., Academic Press (London), 1979, 68.

22. Mitchell, R.L., Geol. Soc. Am. Bull., 1972, 83, 1069.

23. Krom, M.D., and Sholkovitz, E.R., Geochim. Cosmochim. Acta, 1978, 42, 607.

24. Forbes, E.A., Posner, A.M., and Quirk, J.P., J. Soil Sci., 1976, 27, 154.

25. Berner, R.A., Am. J. Sci., 1967, 265, 773.

26. Donnell, T.W., and Merrill, L., Chem. Geol., 1977, 19, 167.

27. Murray, J.W., Geochim. Cosmochim. Acta, 1975, 39, 505.

28. Jacobs, L.W., Syers, J.K., and Keeney, D.R., Soil Sci. Soc. Amer. Proc., 1970, 34, 750.

29. McLaren, R.G., and Crawford, D.V., J. Soil Sci., 1973, 24, 172.

30. Petrov, I.I., At. Spectrosc., 1980, 1, 47.

31. Davies, B.E., Sci. Tot. Environ., 1978, 9, 243.

32. Lindsay, W.L., and Norvell, W.A., Soil Sci. Soc. Amer. Proc., 1969, 33, 62.

33. Chao, T.T., and Theobald, Jr., P.K., Economic Geology, 1976, 71, 1560.

34. Chester, R., 'Pollution analysis of the sea and waterways', Paper given at Royal Society of Chemistry Meeting 5/6th April (Portsmouth) 1982.

35. Phipps, D.A., Effect of heavy metal pollution on plants, vol. 1 - Effects of trace metals on plant function, ed. Lepp, N.W., Applied Sci., 1981, ch. 1.

36. Batley, G.E., and Giles, M.S., 'Contaminants and Sediments', vol. 2, ed. Baker, R.A., Ann Arbor Science (Michigan), 1980, 101.

37. Iverson, D.G., Anderson, M.A., Holm, T.R., and Stanforth, R.R., Environ. Sci. Technol., 1979, 13, 1491.

38. Iverson, D.G., Anderson, M.A., Holm, T.R., and Stanforth, R.R., 'Contaminants and Sediments', vol. 2, ed. Baker, R.A., Ann Arbor Science (Michigan), 1980, 29.

39. Adams, D.D., Darby, D.A., and Young, R.J., 'Contaminants and Sediments', vol. 2, ed. Baker, R.A., Ann Arbor Science (Michigan), 1980, 3.

40. Boust, D., and Saas, A., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 709.

41. Woolson, E.A., and Kearney, P.C., Environ. Sci. Technol., 1973, 7, 47.

42. Karamanos, R.E., Bettany, J.R., and Rennie, D.A., Can. J. Soil Sci., 1976, 56, 37.

43. Mishra, P.C., Mishra, M.K., and Misra, S.G., Indian J. Agric. Sci., 1973, 43, 609.

44. Viro, P.J., Soil Sci., 1955, 79, 459.

45. Butterworth, F.E., and Alloway, B.J., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 713.

46. Gatehouse, S., Russell, D.W., and Van Moort, J.C., J. Geochem. Explor., 1977, 8, 483.

47. David, D.J., Prog. Analyt. Atom. Spectrosc., 1978, 1, 225.

48. Jordan, W.J., Alloway, B.J., and Thornton, I., J. Sci. Fd. Agric., 1975, 26, 1413.

49. Badri, M.A., and Aston, S.R., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 705.

50. Forstner, U., Calmano, W., Conradt, K., Jaksch, H., Schimkus, C., and Schoer, J., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 698.

51. Pedersen, B., Willems, M., and Jørgensen, S.S., Analyst, 1980, 105, 119.

52. Magnusson, B., and Westerland, S., Anal. Chem. Acta, 1981, 131, 63.

53. Fassbender, H.W., Ambio, 1975, 4, 134.

54. McKenzie, R.M., Aust. J. Soil Res., 1967, 8, 235.

55. Harrison, R.M., and Laxen, D.P.H., Water Air Soil Pollut., 1977, 8, 387.

56. Delcarte, E., and Piret, T., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 709.

57. Luoma, S.N., and Bryan G.W., Sci. Total Environ., 1981, 17, 165.

58. Reboredo, F., Carrondo, M.J.T., Ganho, R.M.B., and Oliveira, J.F.S., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 587.

59. Wauchope, R.D., At. Absorption Newslett., 1976, 15, 64.

60. Bradley, R.I., Geoderma, 1980, 24, 17.

61. Hale, M., J. Geochem. Explor., 1981, 15, 307.

62. Pahlavanpour, B., Thompson, M., and Thorn, L., Analyst, 1980, 105, 756.

63. Sandhu, S.S., Analyst, 1981, 106, 311.

64. Brown, Jr., R.M., Fry, R.C., Moyers, J.L., Northway, S.J., Denton, M.B., and Wilson, G.S., Anal. Chem., 1981, 53, 1560.

65. Kang, H.K., and Valentine, J.L., Anal. Chem., 1977, 49, 1829.

66. Fuller, C.W., Hutton, R.C., and Preston, B., Analyst, 1981, 106, 1266.

67. Stupor, J., and Ajlec, R., Analyst, 1982, 107, 144.

68. Jackson, M.L., 'Soil chemical analysis - advanced course', 2nd edition published by author (Madison), 1969, 27.

69. Valin, R.V., and Morse, J.W., Mar. Chem., 1982, 11, 535.

70. Petruzzelli, G., Guidi, G., and Lubrano, L., Water Air Soil Pollut., 1978, 9, 263.

71. Riffaldi, R., Levi-Minzi, R., and Soldatini, G.F., Water Air Soil Pollut., 1976, 6, 119.

72. Coffin, D.E., Can. J. Soil Sci., 1963, 43, 7.

73. Learned, R.E., Chao, T.T., and Sanzolone, R.F., J. Geochem. Explor., 1981, 15, 563.

74. Chisholm, D., Can. J. Plant Sci., 1972, 52, 583.

75. Schachter, M.M., and Boyer, K.W., Anal. Chem., 1980, 52, 360.

76. Godden, R.G., and Thomerson, D.R., Analyst, 1980, 105, 1257.

77. Stewart, J.W.B., Bettany, J.R., Rennie, D.A., Huang, P.M., and McKercher, R.B., Int. Atomic Energy Agency, 1975, 23.

78. Arafat, N.M., and Glooschenko, W.A., Analyst, 1981, 106, 1174.

79. Thompson, A.J., and Thoresby, P.A., Analyst, 1977, 102, 9.

80. National Research Council Canada, 'Effects of arsenic in the Canadian environment', NRCC No. 15391, Canada (Ottawa), 1978, ch. 5.

81. Braman, R.S., 'Arsenical Pesticides', ed. Woolson, E.A., American Chemical Society (Washington D.C.), 1975, 108.

82. Peoples, S.A., 'Arsenical Pesticides', ed. Woolson, E.A., American Chemical Society (Washington D.C.), 1975, 1.

83. Richardson, C.W. Price, J.D., and Burnett, E., J. Environ. Qual., 1978, 7, 189.

84. Takamatsu, T., Aoki, H., and Yoshida, T., Soil Sci., 1982, 133, 239.

85. Wauchope, R.D., and Yamamoto, M., J. Environ. Qual., 1980, 9, 597.

86. Woolson, E.A., Environ. Health Perspect., 1977, 19, 73.

87. Woolson, E.A., Weed Sci., 1977, 25, 412.

88. Woolson, E.A., and Aharonson, N., J. Assoc. Off. Anal. Chem., 1980, 63, 523.

89. Johnson, L.R., and Hiltbold, A.E., Soil Sci. Soc. Amer. Proc., 1969, 33, 279.

90. Abdelghani, A.A., Reimers, R.S., Anderson, A.C., Englande, Jr., A.J., Lo, C.P., and Sharcatpanahi, M., 'Inter. Conf. heavy metals in the environment', Amsterdam, September 1981, 665.

91. Goodroad, L.L., and Caldwell, A.C., J. Environ. Qual., 1979, 8, 493.

92. Woolson, E.A., Axley, J.H., and Kearney, P.C., Soil Sci. Soc. Amer. Proc., 1973, 37, 254.

93. Liversey, N.T., and Huang, P.M., Soil Sci., 1981, 131, 88.

94. Wagner, H., Muench. Beitr. Abwasser. Fisch. Flussbial., 1977, 27, 125.

95. Forbes, E.A., Posner, A.M., and Quirk, J.P., J. Soil Sci., 1976, 27, 154.

96. Le Hiche, H.H., and Weir, A.H., J. Soil Sci., 1963, 14, 225.

97. McLaren, R.G., and Crawford, D.V., J. Soil Sci., 1974, 25, 111.

98. Farrah, H., and Pickering, W.F., Water Air Soil Pollut., 1978, 9, 491.

99. Heydemann, A., Geochim. Cosmochim. Acta, 1959, 15, 305.

100. Karim, H., Sedberry, J.E., and Miller, B.J., Commun. Soil Sci. Plant Anal., 1976, 7, 437.

101. Panin, M.S., Agrokhimiya, 1972, 2, 126.

102. Sugden, T.M., Philos. Trans. R. Soc., London, Ser. A, 1979, 290, 469.

103. Reaves, G.A., and Berrow, M.L., J. Sci. Food Agric., 1979, 30, 1.

104. Goldschmidt, V.M., J. Chem. Soc., 1937, 1, 655.

105. Koeppe, D.E., Effect of heavy metal pollution on plants, vol. 1 - effects of trace metals on plant function, ed. Lepp, N.W., Applied Sci., 1981, ch. 2.

106. Colbourn, P., and Thornton, I., J. Soil Sci., 1979, 52, 127.

107. Hemphell, D.D., Marienfeld, C.J., Reddy, R.S., and Pierce, J.O., Arch. Environ. Health, 1974, 28, 190.

108. McKenzie, R.M., Aust. J. Soil Res., 1980, 18, 61.

109. National Research Council Canada, 'Effects of lead in the environment', NRCC No. 16736, Canada (Ottawa), 1979.

110. Short, N.M., J. Geol., 1961, 69, 534.

111. Soldatini, G.F., Riffaldi, R., and Levi-Minzi, R., Water Air Soil Pollut., 1976, 6, 111.

112. Harrison, R.M., and Laxen, D.P., Chem. in Britain, 1980, 16, 316.

113. Jackson, K.S., and Skippen, G.B., J. Geochem. Explor., 1978, 10, 117.

114. Heinricks, H., and Mayer, R., J. Environ. Qual., 1977, 6, 402.

115. Zimdahl, R.L., and Skogerboe, R.K., Environ. Sci. Technol., 1977, 11, 1202.

116. Alloway, B.J., Gregson, M., Gregson, S.K., Tanner, R., and Tills, A., 'Heavy metals in the environment Int. Conf.', London, September 1979, 545.

117. Verloo, M., and Cottenic, A., Pedologic, 1972, 22, 174.

118. Saar, R.A., Diss. Abstr. Int., 1980, 41, 2199.

119. Saar, R.A., and Weber, J.H., Environ. Sci. Technol., 1980, 14, 877.

120. Batley, G.E., Trace element speciation in surface waters and its ecological implications, ed. Leppard, G.G., Plenum Press, New York, 1983.

121. Cantillo, A.Y., and Segar, D.A., 'Heavy metals in the environment Int. Conf.', Toronto, 1975, 183.

122. Norvell, W.A., Soil Sci. Soc. Am., 1972, 115.

123. Ferguson, J.F., and Gavis, J. Water Res., 1972, 6, 1259.

124. Bohn, H.L., Soil Sci., 1976, 121, 125.

125. Braman, R.S., Environ. Health Perspect., 1977, 19, 1.

126. Cox, D.P., and Alexander, M., Bull. Environ. Contam. Toxicol., 1973, 9, 84.

127. Cox, D.P., 'Arsenical Pesticides', ed. Woolson, E.A., American Chemical Society (Washington D.C.), 1975, 81.

128. McBride, B.C., and Wolfe, R.S., Biochemistry, 1971, 10, 4213.

129. Wood, J.M., Science, 1974, 183, 1049.

130. Hiltbold, A.E., 'Arsenical Pesticides', ed. Woolson, E.A., American Chemical Society (Washington D.C.), 1975, 53.

131. Sandberg, G.R., and Allen, I.K., 'Arsenical Pesticides', ed. Woolson, E.A., American Chemical Society (Washington D.C.), 1975, 124.

132. Quastel, J.H., and Scholefield, P.G., Soil Sci., 1953, 75, 279.

133. Von Endt, D.W., Kearney, P.C., and Kaufman, D.D., J. Agric. Food Chem., 1968, 16, 17.

134. Akins, M.B., and Lewis, R.J., Soil Sci. Soc. Amer. J., 1976, 40, 655.

135. Wedepohl, K.H., 'Handbook of Geochemistry', Springe-Verlag Berlin (Heidelberg), 1978, 3/33.

136. Tammer, P.M., and de Lint, M.M., Neth. J. Agric. Sci., 1969, 17, 128.

137. Deuel, L.E., and Swaboda, A.R., Soil Sci. Soc. Amer. Proc., 1972, 36, 276.

138. Nakahara, T., Anal. Chim. Acta, 1981, 131, 73.

139. Aggett, J., and Aspell, A.C., Analyst, 1976, 101, 341.

140. Terashima, S., Anal. Chim. Acta, 1976, 86, 43.

141. Yamamoto, M., Soil Sci. Soc. Amer. Proc., 1975, 39, 859.

142. Ricci, G.R., Shepard, L.S., Colovos, G., and Hester, N.E., Anal. Chem., 1981, 53, 610.

143. Ficklin, W.H., Talanta, 1983, 30, 371.

144. Maher, W.A., Anal. Chim. Acta, 1981, 126, 157.

145. Pacey, G.E., and Ford, J.A., Talanta, 1981, 28, 935.

146. Sachs, R.M., Micheal, J.L., Anastasia, F.B., and Wells, W.A., Weed Sci., 1971, 19, 412.

147. Brinckman, F.E. Jewett, K.L., Iverson, W.P., Irgolic, K.J., Ehrhardt, K.C., and Stockton, R.A., J. Chromatogr., 1980, 191, 31.

148. Fish, R.H., Brinckman, F.E., and Jewett, K.L., Environ. Sci. Technol., 1982, 16, 174.

149. Brinckman, F.E., Blair, W.R., Jewett, K.L., and Iverson, W.P., J. Chromatogr. Sci., 1977, 15, 493.

150. Andreae, M.O., Anal. Chem., 1977, 49, 820.

151. Braman, R.S., Johnson, D.L., Foreback, C.C., Ammons, J.M., and Bricker, J.L., Anal. Chem., 1977, 49, 621.

152. Crecellius, E.A., Anal. Chem., 1978, 50, 826.

153. Hinners, T.A., Analyst, 1980, 105, 751.

154. Beckermann, B., Anal. Chim. Acta, 1982, 135, 77.

155. Taimi, Y., Bostick, D.T., Anal. Chem., 1975, 47, 2145.

156. Butts, W.C., and Rainey, Jr., W.T., ibid., 1971, 43, 538.

157. Daughtrey, Jr., F.H., Fitchett, A.W., and Mushak, P., Anal. Chim. Acta, 1975, 79, 199.

158. Fukui, S., Hirayama, T., Nohara, M., and Sahagami, Y, Talanta, 1983, 30, 89.

159. Odanaka, Y., Matano, O., and Goto, S., J. Agric. Food Chem., 1978, 26, 505.

160. Buchet, J.P., Lauwerys, R., and Roels, H., Int. Arch. Occup. Environ. Health, 1980, 46, 11.

161. Pahlavanpour, B., and Thompson, M., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 661.

162. Yashui, A., Tsutsumi, C., and Toda, S., Agric. Biol. Chem., 1978, 42, 2139.

163. Subramanian, K.S., and Meranger, J.C., Anal. Chim. Acta, 1981, 124, 131.

164. Brooks, R.R., Ryan, D.E., and Zhang, H., Anal. Chim. Acta., 1981, 131, 1.

165. Horlick, G., Anal. Chem., 1982, 54, 276R.

166. Majors, R.E., Barth, H.G., Lochmüller, C.H., Anal. Chem., 1982, 54, 323R.

167. Mushak, P., J. Anal. Toxicol., 1977, 1, 286.

168. Van Loon, J.C., Anal. Chem., 1979, 51, 1139A.

169. Robbins, W.B., and Caruso, J.A., Anal. Chem., 1979, 51, 889.

170. Johnson, D.L., and Pilson, M.E.Q., Anal. Chim. Acta, 1972, 58, 289.

171. Norris, L.A., Canutt, P.R., and Neuman, J.F., Bull. Environ. Contam. Toxicol., 1983, 30, 309.

172. Morita, M., Uehiro, T., and Fuwa, K., Anal. Chem., 1981, 53, 1806.

173. Fry, R.C., Denton, M.B., Winson, D.L., and Northway, S.J., Anal. Chem., 1977, 49, 1413.

174. Overby, L.R., Bocchieri, S.F., and Fredrickson, R.L., J. Assoc. Off. Agric. Chem., 1965, 48, 17.

175. Subramanian, K.S., Leung, P.C., and Meranger, J.C., Intern. J. Environ. Anal. Chem., 1982, 11, 121.

176. Henry, F.T., and Thorpe, T.M., Anal. Chem., 1980, 52, 80.

177. Stockton, R.A., and Irgolic, K.J., Intern. J. Environ. Anal. Chem., 1979, 6, 313.

178. Vickrey, T.M., Buren, M.S., and Howell, H.E., Anal. Lett., 1978, 12, 1075.

179. Iadevaia, R., Aharonson, N., and Woolson, E.A., J. Assoc. Off. Anal. Chem., 1980, 63, 742.

180. Howard, A.G., and Arbab-Zavar, M.H., Analyst, 1981, 106, 213.

181. Crock, J.G., and Lichte, F.E., Anal. Chim. Acta, 1982, 144, 223.

182. Freeman, H., Uthe, J.F., and Flemming, B., At. Absorption Newslett., 1976, 15, 49.

183. Welz, B., and Melcher, M., Anal. Chim. Acta, 1981, 131, 17.

184. Miller, J.D., and Koizumi, H., Am. Lab., 1979, 11, 35.

185. Abdul-Halim, A.L., Evans, J.C., Rowlands, C.C., and Thomas, J.H., Geochim. Cosmochim. Acta, 1981, 45, 481.

186. Boyd, S.A., Sommers, L.E., Nelson, D.W., and West, D.X., Soil Sci. Soc. Amer. J., 1981, 45, 745.

187. Brogan, J.C., Orbital, 1966, 1, 26.

188. Goodman, B.A., and Cheshire, M.V., J. Soil Sci., 1976, 27, 337.

189. Cheshire, M.V., Berrow, M.L., Goodman, B.A., and Mundie, C.M., Geochim. Cosmochim. Acta, 1977, 41, 1131.

190. Piccolo, A., and Stevenson, F.J., Geoderma, 1982, 27, 195.

191. Rainville, D.P., and Weber, J.H., Can. J. Chem., 1982, 60, 1.

192. Steelink, C., J. Chem. Ed., 1977, 54, 599.

193. Kononova, M.M., Soil organic matter - its nature, its role in soil formation and in soil fertility, 2nd edition, Pergamon Press, Oxford, 1966.

194. Hayes, M.H.B., and Swift, R.S., The chemistry of soil constituents, ed. Greenland, D.J., and Hayes, M.H.B., Whiley, London, 1978, ch. 3.

195. Wershan, R.L., and Pinckney, D.J., 'Contaminants and Sediments', vol. 2, ed. Baker, R.A., Ann Arbor Sci., USA, 1978, ch. 12.

196. Chen, Y., Senesi, N., Schnitzer, M., Geoderma, 1978, 20, 87.

197. Petruzzelli, G., Guidi, G., Lubrano, L., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 686.

198. Stevenson, F.J., Soil Sci., 1977, 123, 10.

199. Siegel, A., Organic compounds in aquatic environment, New York, 1971, 265.

200. Weber, J.H., and Wilson, S.A., Water Res., 1975, 9, 1079.

201. Dawson, H.J., Hrutfiord, B.F., Zasoski, R.J., and Ugolini, F.C., Soil Sci., 1981, 132, 191.

202. Wilson, S.A., and Weber, J.H., Chem. Geol., 1977, 19, 285.

203. Irving, H., and Williams, R.P.J., J. Chem. Soc., 1953, 3192.

204. Stevenson, F.J., Soil Sci. Soc. Amer. Proc., 1976, 40, 665.

205. Bloomfield, C., and Sanders, J.R., J. Soil Sci., 1971, 28, 435.

206. Schnitzer, M., and Skinner, S.I.M., Soil Sci., 1967, 103, 247.

207. Geering, H.R., and Hodgson, J.F., Soil Sci. Soc. Am. Proc., 1969, 33, 54.

208. Hodgson, J.F., Lindsay, W.L., and Trierweiler, J.F., Soil Sci. Soc. Am. Proc., 1966, 30, 723.

209. MacCarthy, P., and O'Cinneide, S., J. Soil Sci., 1974, 25, 329.

210. Florence, T.M., Talanta, 1982, 29, 345.

211. Lee, J., and Jonasson, I.R., J. Geochem. Explor., 1983, 18, 25.

212. Hart, B.T., and Davies, S.H.R., Estuarine, coastal and shelf science, 1981, 12, 353.

213. Kremling, K., Wenck, A., and Osterroht, C., Mar. Chem., 1981, 10, 209.

214. Wallace, Jr., G.T., Mar. Chem., 1982, 11, 379.

215. Zsolnay, A., Mar. Chem., 1977, 5, 465.

216. Saar, R.A., and Weber, J.H., Environ. Sci. Technol., 1982, 16, 510A.

217. Reinhold, K., Aldag, R., and Scheffer, F., Trans. Int. Congr. Soil Sci., Bucharest, 1964, 4, 1117.

218. Lepp, N.W., Effect of heavy metal pollution on plants, vol. 1, Effects of trace metals on plant function, ed. Lepp, N.W., Applied Sci., 1981, ch. 4.

219. Garrels, R.M., and Thompson, M.E., Am. J. Sci., 1962, 260, 57.

220. Kester, D.R., and Pytkowicz, R.M., Limnol. Oceanogr., 1969, 14, 686.

221. Morel, F., and Morgan, J.J., Environ. Sci. Technol., 1972, 6, 58.

222. Zirino, A., and Yamamoto, S., Limnol. Oceanogr., 1972, 17, 661.

223. Florence, T.M., and Batley, G.E., Talanta, 1976, 23, 179.

224. Long, D.T., and Angino, E.E., Geochim. Cosmochim. Acta, 1977, 41, 1183.

225. Mantoura, R.F.C., Dickson, A., and Riley, J.P., Estuarine Coastal Mar. Sci., 1978, 6, 387.

226. Lu, J.C.S., and Chen, K.Y., Environ. Sci. Technol., 1977, 11, 174.

227. Reuter, J.H., and Perdue, E.M., Geochim. Cosmochim. Acta., 1977, 41, 325.

228. Mantoura, R.F.C., and Riley, J.P., Anal. Chim. Acta., 1975, 78, 193.

229. Mouvet, C., and Bourg, A.C.M., Water Res., 1983, 6, 641.

230. Florence, T.M., and Batley, G.E., Talanta, 1977, 24, 151.

231. Figura, P., and McDuffle, B., Analyt. Chem., 1979, 51, 120.

232. Pagenkopf, G.K., Am. Chem. Soc. Symp. Ser., 1978, 82, 372.

233. Larsen, R.A., Freshwater Biol., 1978, 8, 91.

234. Wetzel, R.G., Limnology, Saunders, 1975, 538.

235. Buffle, J., Deladoey, P., and Haerdi, W., ibid., 1978, 101, 339.

236. Laxen, D.P., and Harrison, R.M., Sci. Total, Environ., 1981, 19, 59.

237. Florence, T.M., Biochem. J., 1980, 189, 507.

238. Tuschall, Jr., J.R., and Brezonik, P.L., Anal. Chim. Acta., 1983, 149, 47.

239. Beneš, P., Water Res., 1980, 14, 511.

240. Hart, B.T., and Davies, S.H., Aust. J. Mar. Freshwater Res., 1977, 28, 105.

241. Gamble, D.S., Schnitzer, M., and Hoffman, I., Can. J. Chem., 1970, 48, 3197.

242. Ardakani, M.S., and Stevenson, F.J., Soil Sci. Soc. Amer. Proc., 1972, 36, 884.

243. Steinberg, C., Water Res., 1980, 14, 1239.

244. Florence, T.M., and Batley, G.E., CRC Crit. Rev. Anal. Chem., 1980, 9, 219.

245. Batley, G.E., and Farrar, Y.J., Anal. Chim. Acta., 1978, 99, 283.

246. Slowey, J.F., Jeffery, L.M., and Hood, D.W., Nature, 1967, 214, 377.

247. Buffle, J., Greter, F.L., and Haerdi, W., Anal. Chem., 1977, 49, 216.

248. Bresnahan, W.T., Graut, C.L., and Weber, J.H., Anal. Chem., 1978, 50, 1675.

249. McCardy, J.K., and Chapman, S.A., Water Res., 1979, 13, 143.

250. Cheam, V., and Gamble, D.S., Can. J. Soil Sci., 1974, 54, 413.

251. Nürnberg, H.W., Valenta, P., Mart, L., Raspor, B., and Sipos, L., ibid., 1976, 282, 357.

252. Nürnberg, H.W., Sci. Total Environ., 1979, 12, 35.

253. Whitfield, M., Chemical Oceanography, eds. Riley, J.P., and Skirrow, G., 2nd edition, Academic Press, London, 1975.

254. Florence, T.M., Anal. Chim. Acta., 1980, 119, 217.

255. Van Leeuwen, H.P., Anal. Chem., 1979, 51, 1322.

256. Oehme, M., Talanta, 1979, 26, 913.

257. Jacobsen, E., and Lindseth, H., Anal. Chim. Acta, 1976, 86, 123.

258. Batley, G.E., and Florence, T.M., J. Electroanal. Chem., 1976, 72, 121.

259. Florence, T.M., Water Res., 1977, 11, 681.

260. Batley, G.E., and Florence, T.M., Mar. Chem., 1976, 4, 347.

261. Midgley, D., Anal. Chim. Acta, 1976, 87, 7.

262. Pungor, E., Toth, K., Popy, M.K., Polos, L., Malilssa, H., Graserbauer, M., Hoke, E., Ebel, M.F., and Persy, K., Anal. Chim. Acta, 1979, 109, 279.

263. Gamble, D.S., Underdown, A.W., and Longford, C.H., Anal. Chem., 1980, 52, 1901.

264. Mills, G.L., Hanson, Jr., A.K., and Quinn, J.G., Mar. Chem., 1982, 11, 355.

265. Mills, G.L., and Quinn, J.G., Mar. Chem., 1981, 10, 93.

266. Lee, J., Water Res., 1981, 15, 507.

267. Davey, P.W., Wakerley, D.S., and Le Roux, N.W., Report No. LR 380(ME), Warren Spring Laboratory, Dept. of Industry, June 1981.

268. Vickrey, T.M., Howell, H.E., Harrison, G.V., and Ramelow, G.J., Anal. Chem., 1980, 52, 1743.

269. Röderer, G., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 250.

270. Mattigod, S.V., 'Int. Conf. heavy metals in the environment', Amsterdam, September 1981, 721.

271. Tyler, G., Water Air Soil Pollut., 1978, 9, 137.

272. Zvyaginser, D.C., Bull. Ecol. Res. Com. (Stockholm), 1973, 17, 61.

273. Dommergues, Y.R., and Krupa, S.V., 'Interactions between non-pathogenic soil microorganisms and plants', Elsevier Sci., Netherlands, 1978.

274. Newman, E.I., Biol. Rev., 1978, 53, 511.

275. Cataldo, D.A., and Wildung, R.E., Sci. Total Environ., 1983, 28, 159.

276. Muir, J.W., Morrison, R.I., Brown, C.J., and Logon, J., J. Soil Sci., 1964, 15, 220.

277. Berthelin, J., and Dommergues, Y., Rev. Ecol. Biol. Sol., 1972, 9, 397.

278. Bruckert, S., and Jacquin, F., Soil Biol. Biochem., 1969, 1, 275.

279. Johnston, H.W., N.Z. J. Sci. Technol., 1954, 36, 281.

280. Mortensen, J.L., Soil Sci. Soc. Am. Proc., 1963, 27, 179.

281. Pareek, R.P., and Gaur, A.C., Plant Soil, 1973, 39, 441.

282. Rowatt, J.W., and Katznelson, H., J. Applied Bacteriol., 1961, 24, 164.

283. Webley, D.M., and Duff, R.B., Plant and Soil, 1965, 22, 307.

284. Srivastava, K.S., and Kamal, Indian J. Mycol. Plant Pathol., 1980, 10, 23.

285. Beatty, J.T., Gest, H., J. Bacteriol., 1981, 148, 2.

286. Putilina, V.S., and Varentsor, I.M., Chem. Erde., 1980, 39, 298.

287. Bonneau, M., and Souchier, B., Constituents and Properties of Soils, Academic Press, London, 1982, 201.

288. Robert, M., Razzague-Karimi, Bull. Group Franc. Argiles., 1974, 26, 307.

289. Kirkbright, G.F., and Sargent, M., Atomic absorption and fluorescence spectroscopy, Academic Press, London, 1974.

290. Price, W.J., Spectrochemical analysis by atomic absorption, Heyden, London, 1979.

291. Ebdon, L., An introduction to atomic absorption spectroscopy, Heyden, London, 1982.

292. Ottaway, J.M., Proc. Anal. Div. Chem. Soc., 1979, 16, 64.

293. Van Loon, J.C., 'Int. Conf. heavy metals in the environment', Toronto, 1975, 349.

294. Mertz, W., Ultratrace metal analysis in biological sciences and the environment, ed. Risby, T.H., American Chemical Society, Washington D.C., 1979, 1.

295. Hamilton, E.I., Applied Soil Trace Elements, ed. Davis, B.E., Wiley, 1980, ch. 2 and 3.

296. Walsh, A., Spectrochim. Acta, 1955, 7, 108.

297. L'Vov, B.V., Atomic absorption spectrochemical analysis, Adam Hilger, London, 1970.

298. Thompson, K.C., and Thomerson, D.R., Analyst, 1974, 99, 595.

299. Hamilton, R.J., and Sewell, P.A., Introduction to high performance liquid chromatography, Chapman and Hall, London, 1977.

300. Billingham, N.C., Practical high performance liquid chromatography, ed. Simpson, C.F., Heyden, London, 1976.

301. Stock, R., and Rice, C.B.F., Chromatographic Methods, Chapman and Hall, London, 1974.

302. Haswell, S.J., Stockton, R.A., Bancroft, K.C.C., Irgolic, K.J., O'Neill, P., and Rahman, A., in preparation.

303. Michel, R.G., Coleman, J., and Winefordner, J.D., Spectrochim. Acta, 1978, 33B, 195.

304. Davis, B.E., 'Applied soil trace elements', Wiley, 1980, ch. 9.

305. Bowen, H.J.M., Environmental Chemistry, The Royal Society of Chemistry, vol. 2, 1980, ch. 3.

306. Loganathan, P., and Burau, R.G., Geochim. et Cosmochim. Acta, 1973, 37, 1277.

307. McLaren, R.G., and Crawford, D.V., J. Soil Sci., 1973, 24, 1560.

308. Klumpp, D.W., Peterson, P.J., Environ. Pollut., 1979, 19, 11.

309. Langston, W.J., J. Mar. Biol. Ass. U.K., 1980, 60, 869.

310. Bland, S., Ackroyd, D.R., Marsh, J.G., and Millward, G.E., Sci. Total Environ., 1982, 22, 235.

311. Mantoura, R.F.C., Institute for Marine Environmental Research, Plymouth, personal communication.

312. Smith, R.M., and Martell, A.E., Critical Stability Constants, vol. 4, inorganic complexes, Plenum Press, 1976.

313. Butler, J.N., Ionic equilibrium, a mathematical approach, Addison Wesley, USA, 1964.

314. Turner, D.R., Whitfield, M., Dickson, A.G., Geochim. Cosmochim. Acta, 1981, 45, 855.

315. Lindsay, W.L., Chemical Equilibria in Soils, Wiley and Sons, USA, 1979.

316. Vuceta, J., and Morgan, J.J., Environ. Sci. Technol., 1978, 12, 1302.

317. Jackson, K.S., and Skippen, G.B., J. Geochem. Explor., 1978, 10, 117.

318. Duble, R.L., Holt, E.C., McBee, G.G., Weed Sci., 1968, 16, 421.

APPENDIX A

Individual values obtained for soil samples collected from the Tamar Valley study area and used in chapter 3

Table I

Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$
1	50	33	150	65	200	97	180
2	48	34	93	66	100	98	510
3	46	35	90	67	210	99	270
4	52	36	65	68	330	100	310
5	61	37	110	69	280	101	180
6	54	38	63	70	47	102	55
7	46	39	62	71	230	103	110
8	160	40	210	72	5200	104	92
9	170	41	95	73	50	105	53
10	110	42	240	74	49	106	57
11	75	43	75	75	92	107	34
12	51	44	100	76	370	108	150
13	40	45	79	77	100	109	110
14	33	46	56	78	230	110	72
15	66	47	61	79	62	111	57
16	51	48	320	80	110	112	480
17	140	49	140	81	220	113	100
18	57	50	180	82	94	114	48
19	150	51	60	83	88	115	56
20	130	52	62	84	120	116	51
21	99	53	130	85	430	117	52
22	48	54	66	86	1300	118	42
23	43	55	72	87	63	119	98
24	180	56	45	88	70	120	43
25	1600	57	61	89	71	121	64
26	54	58	95	90	140	122	64
27	2400	59	140	91	530	123	99
28	240	60	57	92	350	124	44
29	200	61	63	93	71	125	52
30	110	62	75	94	92	126	75
31	120	63	61	95	79	127	59
32	52	64	79	96	27	128	48

Table II

Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$
1	52	33	180	65	170	97	63
2	31	34	66	66	77	98	300
3	56	35	56	67	210	99	430
4	54	36	69	68	370	100	41
5	41	37	110	69	500	101	120
6	51	38	75	70	37	102	43
7	33	39	71	71	330	103	74
8	170	40	170	72	4400	104	60
9	270	41	450	73	52	105	48
10	65	42	1400	74	43	106	53
11	56	43	83	75	77	107	66
12	41	44	100	76	570	108	60
13	34	45	110	77	96	109	99
14	28	46	70	78	290	110	82
15	67	47	45	79	60	111	45
16	47	48	350	80	78	112	150
17	55	49	110	81	210	113	52
18	54	50	170	82	56	114	39
19	170	51	59	83	51	115	46
20	120	52	76	84	220	116	59
21	130	53	110	85	330	117	45
22	49	54	51	86	990	118	37
23	32	55	63	87	61	119	130
24	94	56	62	88	75	120	41
25	3500	57	33	89	45	121	60
26	45	58	93	90	130	122	60
27	620	59	180	91	290	123	94
28	240	60	45	92	150	124	39
29	240	61	48	93	66	125	49
30	78	62	50	94	78	126	84
31	77	63	47	95	130	127	61
32	52	64	85	96	24	128	54

Table III

Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$
1	26	33	42	65	110	97	34
2	26	34	26	66	37	98	85
3	24	35	53	67	100	99	81
4	33	36	27	68	140	100	83
5	37	37	24	69	57	101	80
6	23	38	26	70	27	102	21
7	18	39	20	71	140	103	57
8	84	40	82	72	450	104	62
9	130	41	65	73	19	105	42
10	57	42	63	74	18	106	24
11	40	43	23	75	23	107	29
12	42	44	37	76	98	108	98
13	18	45	40	77	56	109	73
14	12	46	37	78	82	110	54
15	43	47	25	79	27	111	31
16	19	48	110	80	58	112	280
17	44	49	92	81	180	113	44
18	23	50	48	82	41	114	26
19	40	51	24	83	36	115	25
20	86	52	27	84	57	116	23
21	75	53	44	85	170	117	35
22	32	54	25	86	310	118	19
23	23	55	34	87	28	119	46
24	120	56	13	88	51	120	21
25	480	57	26	89	35	121	27
26	24	58	48	90	94	122	34
27	980	59	48	91	94	123	45
28	62	60	19	92	130	124	22
29	86	61	29	93	32	125	18
30	47	62	23	94	73	126	39
31	37	63	48	95	49	127	48
32	12	64	29	96	19	128	26

Table IV

Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$	Sample No.	Copper $\mu g\ g^{-1}$
1	27	33	49	65	93	97	28
2	29	34	20	66	42	98	68
3	32	35	29	67	290	99	75
4	39	36	23	68	190	100	12
5	36	37	22	69	72	101	110
6	28	38	23	70	21	102	20
7	10	39	25	71	150	103	33
8	110	40	73	72	350	104	25
9	120	41	180	73	23	105	32
10	31	42	430	74	23	106	21
11	26	43	27	75	23	107	34
12	25	44	40	76	120	108	21
13	12	45	54	77	47	109	63
14	10	46	24	78	69	110	63
15	39	47	17	79	24	111	23
16	17	48	150	80	33	112	91
17	30	49	70	81	130	113	26
18	22	50	37	82	28	114	21
19	42	51	20	83	18	115	21
20	69	52	29	84	97	116	27
21	81	53	44	85	120	117	32
22	29	54	10	86	220	118	17
23	19	55	24	87	29	119	60
24	41	56	33	88	55	120	24
25	1400	57	11	89	21	121	21
26	17	58	30	90	33	122	30
27	320	59	55	91	98	123	48
28	79	60	16	92	58	124	20
29	110	61	23	93	29	125	27
30	39	62	19	94	49	126	39
31	19	63	35	95	51	127	46
32	10	64	21	96	16	128	27

Table V

Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$
1	70	33	110	65	100	97	190
2	63	34	160	66	85	98	250
3	73	35	140	67	100	99	300
4	72	36	82	68	50	100	720
5	30	37	110	69	170	101	240
6	45	38	35	70	34	102	45
7	110	39	44	71	160	103	330
8	91	40	110	72	2000	104	110
9	89	41	50	73	43	105	74
10	120	42	160	74	46	106	65
11	150	43	76	75	63	107	81
12	30	44	81	76	160	108	210
13	45	45	100	77	60	109	230
14	47	46	70	78	380	110	130
15	64	47	44	79	54	111	59
16	58	48	160	80	150	112	530
17	140	49	76	81	110	113	120
18	64	50	73	82	120	114	63
19	110	51	67	83	86	115	67
20	51	52	82	84	92	116	58
21	150	53	44	85	150	117	52
22	63	54	44	86	150	118	62
23	39	55	50	87	48	119	130
24	98	56	43	88	98	120	47
25	310	57	48	89	130	121	98
26	46	58	71	90	99	122	84
27	270	59	48	91	210	123	170
28	120	60	70	92	180	124	46
29	92	61	84	93	37	125	43
30	72	62	98	94	58	126	130
31	68	63	55	95	85	127	130
32	43	64	61	96	110	128	93

Table VI

Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$
1	63	33	110	65	70	97	130
2	55	34	76	66	40	98	97
3	76	35	140	67	35	99	330
4	63	36	74	68	60	100	100
5	21	37	92	69	250	101	320
6	20	38	30	70	46	102	51
7	88	39	36	71	150	103	160
8	97	40	57	72	1000	104	31
9	91	41	160	73	59	105	83
10	60	42	160	74	43	106	58
11	110	43	73	75	34	107	59
12	25	44	77	76	130	108	83
13	32	45	73	77	34	109	78
14	20	46	64	78	430	110	130
15	67	47	36	79	36	111	32
16	34	48	130	80	110	112	100
17	130	49	69	81	70	113	83
18	55	50	81	82	81	114	51
19	170	51	56	83	57	115	71
20	41	52	76	84	88	116	59
21	160	53	35	85	80	117	48
22	58	54	42	86	93	118	57
23	41	55	35	87	44	119	150
24	64	56	32	88	100	120	46
25	440	57	27	89	36	121	110
26	36	58	78	90	81	122	91
27	160	59	45	91	150	123	180
28	90	60	66	92	45	124	41
29	100	61	60	93	63	125	59
30	69	62	61	94	32	126	130
31	75	63	25	95	130	127	150
32	44	64	84	96	73	128	84

Table VII

Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$
1	22	33	25	65	34	97	38
2	30	34	36	66	42	98	87
3	32	35	54	67	62	99	120
4	32	36	31	68	49	100	180
5	31	37	34	69	40	101	83
6	34	38	14	70	9	102	13
7	59	39	15	71	73	103	87
8	61	40	41	72	58	104	71
9	34	41	15	73	18	105	43
10	74	42	39	74	23	106	24
11	71	43	28	75	21	107	53
12	30	44	29	76	49	108	140
13	18	45	51	77	22	109	160
14	17	46	40	78	60	110	48
15	24	47	16	79	18	111	19
16	27	48	91	80	41	112	320
17	81	49	30	81	48	113	44
18	20	50	60	82	29	114	19
19	23	51	23	83	29	115	19
20	26	52	23	84	48	116	21
21	61	53	30	85	65	117	27
22	25	54	22	86	21	118	20
23	32	55	20	87	15	119	48
24	73	56	21	88	48	120	17
25	51	57	19	89	90	121	37
26	20	58	25	90	37	122	27
27	58	59	27	91	38	123	64
28	21	60	25	92	53	124	15
29	36	61	32	93	24	125	22
30	23	62	34	94	40	126	47
31	31	63	14	95	36	127	46
32	17	64	21	96	31	128	39

Table VIII

Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$	Sample No.	Lead $\mu g\ g^{-1}$
1	19	33	31	65	27	97	31
2	25	34	19	66	15	98	31
3	30	35	52	67	36	99	71
4	26	36	27	68	17	100	27
5	10	37	27	69	58	101	130
6	23	38	16	70	14	102	17
7	48	39	14	71	61	103	49
8	57	40	26	72	47	104	12
9	20	41	37	73	15	105	53
10	38	42	52	74	17	106	17
11	43	43	25	75	19	107	37
12	18	44	26	76	32	108	37
13	15	45	31	77	14	109	51
14	9	46	32	78	64	110	39
15	25	47	13	79	11	111	10
16	16	48	82	80	34	112	48
17	74	49	32	81	48	113	34
18	29	50	52	82	19	114	12
19	36	51	20	83	20	115	23
20	19	52	21	84	49	116	20
21	68	53	21	85	32	117	26
22	26	54	16	86	18	118	17
23	28	55	14	87	17	119	63
24	24	56	17	88	56	120	16
25	78	57	9	89	15	121	32
26	17	58	27	90	39	122	30
27	32	59	22	91	49	123	54
28	22	60	20	92	36	124	14
29	44	61	28	93	26	125	18
30	20	62	31	94	20	126	43
31	40	63	11	95	45	127	46
32	15	64	18	96	22	128	28

Table IX

Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$
1	21	33	22	65	40	97	49
2	24	34	58	66	20	98	31
3	29	35	21	67	26	99	31
4	29	36	23	68	52	100	13
5	23	37	22	69	48	101	380
6	23	38	15	70	18	102	12
7	17	39	20	71	34	103	58
8	26	40	49	72	400	104	120
9	33	41	53	73	17	105	17
10	24	42	96	74	17	106	14
11	19	43	28	75	17	107	30
12	22	44	26	76	31	108	64
13	19	45	30	77	30	109	52
14	15	46	16	78	20	110	15
15	35	47	17	79	14	111	12
16	28	48	35	80	43	112	520
17	26	49	33	81	18	113	36
18	22	50	20	82	120	114	20
19	45	51	19	83	16	115	15
20	22	52	26	84	36	116	15
21	110	53	17	85	40	117	18
22	18	54	15	86	120	118	17
23	19	55	22	87	16	119	30
24	120	56	10	88	19	120	15
25	330	57	28	89	27	121	16
26	15	58	20	90	65	122	19
27	100	59	24	91	28	123	32
28	350	60	21	92	27	124	14
29	130	61	21	93	19	125	14
30	15	62	16	94	32	126	14
31	17	63	25	95	26	127	21
32	10	64	34	96	41	128	29

Table X

Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$
1	38	33	26	65	26	97	37
2	30	34	44	66	37	98	27
3	31	35	17	67	18	99	27
4	31	36	14	68	11	100	18
5	23	37	21	69	59	101	380
6	23	38	19	70	17	102	10
7	16	39	24	71	32	103	62
8	38	40	51	72	480	104	24
9	78	41	160	73	17	105	14
10	19	42	180	74	14	106	15
11	14	43	18	75	12	107	36
12	24	44	30	76	31	108	14
13	9	45	26	77	20	109	20
14	11	46	20	78	22	110	17
15	56	47	15	79	14	111	14
16	17	48	30	80	64	112	60
17	30	49	36	81	10	113	36
18	15	50	26	82	23	114	16
19	53	51	19	83	9	115	19
20	29	52	14	84	50	116	12
21	90	53	24	85	180	117	15
22	17	54	12	86	50	118	17
23	21	55	22	87	15	119	32
24	63	56	19	88	22	120	17
25	420	57	13	89	23	121	20
26	9	58	18	90	64	122	18
27	200	59	25	91	18	123	33
28	130	60	15	92	19	124	15
29	130	61	11	93	18	125	16
30	16	62	19	94	15	126	16
31	16	63	25	95	10	127	17
32	9	64	31	96	37	128	18

Table XI

Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$
1	6	33	11	65	18	97	15
2	4	34	16	66	3	98	8
3	8	35	10	67	4	99	10
4	15	36	8	68	6	100	5
5	6	37	7	69	14	101	70
6	4	38	5	70	3	102	4
7	13	39	7	71	13	103	21
8	11	40	14	72	28	104	80
9	19	41	14	73	5	105	11
10	11	42	24	74	7	106	3
11	10	43	7	75	5	107	13
12	7	44	10	76	8	108	45
13	7	45	9	77	7	109	37
14	4	46	6	78	6	110	9
15	14	47	7	79	4	111	4
16	9	48	10	80	20	112	360
17	13	49	9	81	3	113	12
18	8	50	8	82	7	114	5
19	14	51	6	83	5	115	6
20	14	52	10	84	13	116	3
21	60	53	10	85	12	117	4
22	10	54	4	86	70	118	5
23	6	55	9	87	4	119	8
24	90	56	2	88	11	120	5
25	35	57	10	89	11	121	5
26	7	58	12	90	27	122	5
27	43	59	10	91	8	123	8
28	32	60	6	92	6	124	5
29	26	61	8	93	7	125	6
30	6	62	4	94	19	126	6
31	6	63	9	95	3	127	5
32	4	64	17	96	12	128	6

Table XII

Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$	Sample No.	Arsenic $\mu g\ g^{-1}$
1	9	33	13	65	9	97	16
2	5	34	9	66	2	98	9
3	7	35	9	67	8	99	13
4	16	36	6	68	7	100	4
5	4	37	7	69	18	101	71
6	4	38	6	70	3	102	4
7	5	39	7	71	13	103	26
8	8	40	12	72	14	104	8
9	33	41	28	73	6	105	10
10	9	42	44	74	6	106	4
11	8	43	4	75	4	107	16
12	7	44	9	76	8	108	5
13	3	45	7	77	7	109	8
14	3	46	5	78	5	110	10
15	18	47	5	79	3	111	5
16	5	48	10	80	19	112	37
17	10	49	9	81	5	113	8
18	6	50	7	82	6	114	4
19	19	51	7	83	3	115	6
20	16	52	11	84	11	116	3
21	48	53	11	85	3	117	4
22	8	54	5	86	29	118	5
23	4	55	8	87	3	119	7
24	21	56	4	88	8	120	5
25	38	57	6	89	8	121	4
26	4	58	11	90	30	122	5
27	50	59	10	91	6	123	8
28	18	60	8	92	3	124	5
29	28	61	5	93	6	125	7
30	5	62	5	94	4	126	6
31	7	63	7	95	2	127	5
32	5	64	19	96	16	128	8

Table XIII

Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %
1	3.7	33	3.7	65	4.3	97	3.2
2	2.4	34	3.5	66	2.0	98	3.3
3	3.7	35	4.0	67	3.1	99	3.1
4	3.1	36	4.0	68	2.9	100	3.9
5	2.6	37	3.2	69	4.3	101	5.0
6	2.4	38	1.4	70	4.3	102	4.2
7	4.0	39	4.9	71	4.6	103	4.9
8	4.4	40	3.9	72	8.0	104	5.0
9	3.7	41	3.8	73	2.9	105	4.3
10	3.4	42	1.1	74	1.9	106	4.5
11	4.2	43	3.7	75	2.9	107	1.6
12	2.2	44	3.1	76	3.0	108	4.7
13	6.0	45	1.0	77	4.5	109	4.0
14	1.7	46	2.9	78	4.5	110	4.5
15	2.7	47	4.5	79	4.3	111	4.7
16	4.2	48	5.0	80	3.3	112	7.0
17	3.4	49	3.5	81	2.5	113	4.3
18	3.3	50	4.0	82	4.7	114	2.1
19	4.5	51	3.7	83	2.9	115	4.0
20	2.6	52	3.1	84	3.5	116	4.0
21	4.0	53	2.7	85	6.0	117	4.4
22	4.2	54	2.1	86	8.0	118	4.5
23	2.4	55	1.6	87	4.2	119	5.0
24	5.0	56	1.2	88	3.8	120	3.7
25	4.6	57	2.1	89	2.8	121	3.7
26	3.8	58	3.8	90	3.9	122	4.4
27	4.1	59	3.2	91	3.5	123	4.2
28	5.0	60	3.3	92	3.2	124	4.5
29	4.4	61	3.7	93	4.3	125	3.2
30	4.1	62	3.7	94	4.4	126	4.7
31	3.2	63	1.9	95	2.4	127	4.5
32	3.0	64	2.7	96	2.9	128	4.3

Table XIV

Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %
1	3.8	33	3.4	65	4.4	97	3.6
2	2.5	34	4.4	66	2.3	98	3.4
3	4.0	35	4.4	67	2.1	99	3.6
4	3.7	36	4.7	68	2.7	100	4.4
5	2.5	37	3.8	69	5.0	101	5.0
6	2.4	38	1.5	70	4.8	102	4.6
7	4.8	39	3.1	71	4.7	103	4.4
8	4.2	40	4.8	72	13.0	104	6.0
9	3.7	41	6.0	73	3.2	105	4.4
10	4.1	42	6.0	74	1.7	106	5.0
11	4.6	43	4.2	75	3.0	107	3.8
12	1.9	44	4.7	76	3.4	108	5.0
13	4.6	45	3.4	77	4.9	109	4.4
14	5.0	46	3.2	78	4.8	110	4.6
15	4.2	47	3.2	79	4.8	111	5.0
16	4.8	48	5.0	80	4.9	112	5.1
17	4.9	49	3.9	81	3.0	113	4.7
18	5.0	50	4.1	82	4.9	114	2.0
19	5.0	51	3.9	83	3.7	115	4.5
20	3.9	52	3.8	84	4.1	116	4.9
21	4.4	53	3.1	85	7.0	117	4.8
22	4.2	54	2.3	86	5.0	118	4.6
23	2.1	55	1.8	87	3.9	119	4.5
24	4.0	56	2.9	88	3.9	120	3.1
25	5.0	57	1.5	89	4.7	121	4.3
26	4.6	58	4.0	90	4.0	122	4.6
27	4.7	59	3.6	91	3.5	123	4.3
28	4.6	60	3.9	92	3.0	124	5.0
29	4.6	61	4.0	93	4.8	125	3.6
30	4.3	62	4.0	94	5.0	126	4.8
31	4.2	63	2.1	95	2.6	127	4.2
32	3.4	64	3.1	96	3.8	128	4.5

Table XV

Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %
1	2.8	33	3.1	65	3.2,	97	2.3
2	1.4	34	2.0	66	2.7	98	2.3
3	3.1	35	2.5	67	2.7	99	2.2
4	2.3	36	2.4	68	2.2	100	2.9
5	1.4	37	2.5	69	3.2	101	2.1
6	1.7	38	0.8	70	3.0	102	3.2
7	3.1	39	3.3	71	3.7	103	3.8
8	3.3	40	2.4	72	6.0	104	3.8
9	2.7	41	2.9	73	2.0	105	3.1
10	2.6	42	0.7	74	2.4	106	3.4
11	3.2	43	2.7	75	2.1	107	0.9
12	0.9	44	2.2	76	2.4	108	3.3
13	3.5	45	0.6	77	3.5	109	2.6
14	1.1	46	2.0	78	3.2	110	3.6
15	2.0	47	3.6	79	3.6	111	3.6
16	3.9	48	3.9	80	2.3	112	4.2
17	2.6	49	2.8	81	1.2	113	3.4
18	2.0	50	3.1	82	3.4	114	1.7
19	3.3	51	2.7	83	2.6	115	2.8
20	2.0	52	2.1	84	2.5	116	3.1
21	3.2	53	2.6	85	4.0	117	1.2
22	3.2	54	1.8	86	5.0	118	3.4
23	2.7	55	0.9	87	3.2	119	4.2
24	3.0	56	0.8	88	2.2	120	2.0
25	3.7	57	1.8	89	2.0	121	2.3
26	2.9	58	3.1	90	2.4	122	2.0
27	3.1	59	2.3	91	2.2	123	3.5
28	3.4	60	2.0	92	3.6	124	3.2
29	3.0	61	2.6	93	3.2	125	2.4
30	3.2	62	2.3	94	2.8	126	3.8
31	2.3	63	1.0	95	1.7	127	2.8
32	2.3	64	2.0	96	2.1	128	3.1

Table XVI

Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %	Sample No.	Iron %
1	2.6	33	2.6	65	2.9	97	2.7
2	2.3	34	2.4	66	2.7	98	2.6
3	2.5	35	2.6	67	2.0	99	2.8
4	1.7	36	3.4	68	2.7	100	2.4
5	1.7	37	2.8	69	3.0	101	2.8
6	1.0	38	0.9	70	3.2	102	3.7
7	2.3	39	2.2	71	2.8	103	3.0
8	2.9	40	3.6	72	7.0	104	4.3
9	2.3	41	3.8	73	2.3	105	2.2
10	2.6	42	3.8	74	2.0	106	3.7
11	3.1	43	3.1	75	2.3	107	2.1
12	0.3	44	2.7	76	2.5	108	3.2
13	3.1	45	2.0	77	3.4	109	3.2
14	3.1	46	2.1	78	2.3	110	2.7
15	2.7	47	2.3	79	2.5	111	3.8
16	3.3	48	3.5	80	3.0	112	3.8
17	2.8	49	2.7	81	2.2	113	2.7
18	3.3	50	2.8	82	3.4	114	1.7
19	4.0	51	2.9	83	2.6	115	3.2
20	2.5	52	2.7	84	2.2	116	3.5
21	2.9	53	2.3	85	2.4	117	0.7
22	2.6	54	1.9	86	3.1	118	3.4
23	2.1	55	1.0	87	2.8	119	3.1
24	2.4	56	2.0	88	1.3	120	2.3
25	2.9	57	1.4	89	3.8	121	1.6
26	3.4	58	3.1	90	2.4	122	2.9
27	3.3	59	2.6	91	2.3	123	2.7
28	3.0	60	2.5	92	2.4	124	3.7
29	2.7	61	2.4	93	3.6	125	2.0
30	3.5	62	2.3	94	4.0	126	3.2
31	1.9	63	2.7	95	2.6	127	2.3
32	2.6	64	2.3	96	2.2	128	2.3

Table XVII

Sample No.	pH	Sample No.	pH	Sample No.	pH	Sample No.	pH
1	6.6	33	5.9	65	5.9	97	5.3
2	7.1	34	3.4	66	6.3	98	5.4
3	5.6	35	5.4	67	5.7	99	5.2
4	5.2	36	6.0	68	5.1	100	5.5
5	5.3	37	5.2	69	5.8	101	5.8
6	6.8	38	6.1	70	6.2	102	5.7
7	5.8	39	5.4	71	5.6	103	6.3
8	6.2	40	5.4	72	5.9	104	3.8
9	6.0	41	5.9	73	5.3	105	6.0
10	5.2	42	3.8	74	5.2	106	6.5
11	5.5	43	5.6	75	4.7	107	3.6
12	5.4	44	6.0	76	5.5	108	5.3
13	6.0	45	3.8	77	4.8	109	4.8
14	3.5	46	6.1	78	5.9	110	5.6
15	6.0	47	5.6	79	4.0	111	3.5
16	5.6	48	5.2	80	3.9	112	3.4
17	7.2	49	6.0	81	6.2	113	6.5
18	3.8	50	6.0	82	6.2	114	5.5
19	5.9	51	6.7	83	4.6	115	6.1
20	5.5	52	5.8	84	4.7	116	5.1
21	5.5	53	3.8	85	4.7	117	5.9
22	6.2	54	6.4	86	6.2	118	5.2
23	5.7	55	5.2	87	6.1	119	5.0
24	6.0	56	5.6	88	6.5	120	5.4
25	3.6	57	6.3	89	3.4	121	5.7
26	7.4	58	4.8	90	6.1	122	6.4
27	4.4	59	6.0	91	5.6	123	5.6
28	5.0	60	5.8	92	4.9	124	4.1
29	5.8	61	6.4	93	5.7	125	6.1
30	6.9	62	7.2	94	3.4	126	5.6
31	6.1	63	5.4	95	6.1	127	5.6
32	6.2	64	6.2	96	6.0	128	4.8

Table XVIII

Sample No.	pH	Sample No.	pH	Sample No.	pH	Sample No.	pH
1	6.3	33	5.4	65	6.0	97	5.3
2	6.5	34	4.1	66	5.9	98	5.0
3	5.5	35	5.6	67	5.4	99	5.1
4	5.4	36	6.0	68	4.9	100	6.5
5	5.1	37	5.4	69	5.7	101	6.1
6	6.3	38	5.7	70	5.8	102	6.1
7	6.3	39	5.6	71	4.8	103	6.1
8	6.3	40	6.0	72	5.9	104	4.2
9	5.4	41	5.4	73	5.5	105	6.1
10	5.8	42	4.0	74	5.0	106	6.4
11	5.8	43	5.9	75	5.0	107	4.1
12	5.0	44	6.0	76	5.6	108	5.9
13	6.6	45	4.0	77	5.1	109	5.6
14	4.1	46	5.8	78	5.9	110	5.0
15	6.3	47	5.0	79	4.1	111	3.8
16	5.9	48	5.2	80	4.2	112	3.8
17	7.2	49	6.0	81	5.9	113	6.9
18	4.2	50	6.2	82	6.1	114	5.4
19	6.2	51	6.1	83	5.8	115	6.0
20	5.6	52	5.3	84	6.8	116	5.1
21	5.8	53	5.8	85	5.5	117	6.1
22	6.3	54	6.0	86	6.5	118	5.5
23	5.5	55	5.1	87	6.2	119	5.6
24	5.0	56	5.1	88	7.2	120	5.0
25	4.4	57	5.9	89	3.8	121	5.9
26	5.1	58	4.1	90	5.8	122	6.6
27	4.9	59	4.1	91	5.2	123	6.0
28	5.0	60	5.9	92	5.0	124	4.8
29	4.8	61	5.8	93	5.5	125	6.1
30	5.9	62	6.5	94	3.6	126	5.8
31	6.4	63	5.1	95	5.7	127	5.8
32	5.8	64	6.0	96	5.7	128	4.8

Table XIX

Sample No.	Organic %	Sample No.	Organic %	Sample No.	Organic %	Sample No.	Organic %
1	11.7	33	14.3	65	10.9	97	15.3
2	23.0	34	25.0	66	13.3	98	12.4
3	11.5	35	12.2	67	18.3	99	10.4
4	13.5	36	17.9	68	14.3	100	12.6
5	10.6	37	12.8	69	18.4	101	14.3
6	23.0	38	17.4	70	13.9	102	10.3
7	10.4	39	9.3	71	13.3	103	15.5
8	17.3	40	16.5	72	5.3	104	17.0
9	16.9	41	7.8	73	12.2	105	30.0
10	18.3	42	75.0	74	25.0	106	15.5
11	16.4	43	11.2	75	27.0	107	73.0
12	13.1	44	10.7	76	8.7	108	18.7
13	14.3	45	69.0	77	22.0	109	18.0
14	53.0	46	28.0	78	11.4	110	22.0
15	10.4	47	13.7	79	18.4	111	20.7
16	10.6	48	21.0	80	31.0	112	27.0
17	11.3	49	15.6	81	17.3	113	12.4
18	25.0	50	13.5	82	11.3	114	17.0
19	15.0	51	10.4	83	14.5	115	17.1
20	13.2	52	24.0	84	12.7	116	17.4
21	15.4	53	18.4	85	22.0	117	10.4
22	15.7	54	9.3	86	33.0	118	13.3
23	18.0	55	15.5	87	23.0	119	16.5
24	17.0	56	10.8	88	17.3	120	11.7
25	6.6	57	13.1	89	42.0	121	18.4
26	9.3	58	17.0	90	21.0	122	12.2
27	33.0	59	13.7	91	10.6	123	12.8
28	15.3	60	10.8	92	10.4	124	27.0
29	9.2	61	14.7	93	8.7	125	15.3
30	16.8	62	18.4	94	26.0	126	9.2
31	14.7	63	16.9	95	15.6	127	11.7
32	17.1	64	21.0	96	15.1	128	15.0

Table XX

Sample No.	Organic %	Sample No.	Organic %	Sample No.	Organic %	Sample No.	Organic %
1	7.5	33	4.0	65	7.0	97	6.7
2	15.1	34	2.4	66	8.1	98	7.3
3	7.2	35	7.4	67	7.1	99	8.6
4	8.2	36	6.5	68	5.6	100	5.1
5	10.6	37	9.7	69	10.6	101	10.7
6	17.3	38	7.1	70	9.7	102	6.4
7	9.1	39	5.1	71	9.2	103	5.0
8	11.6	40	13.9	72	4.0	104	6.7
9	5.0	41	5.7	73	6.5	105	10.3
10	12.9	42	27.0	74	11.6	106	8.2
11	10.3	43	10.6	75	9.3	107	14.3
12	9.6	44	9.3	76	5.3	108	8.5
13	3.1	45	13.7	77	8.7	109	10.9
14	5.6	46	11.5	78	10.3	110	7.4
15	8.7	47	5.7	79	10.1	111	12.8
16	8.9	48	7.3	80	11.9	112	9.8
17	8.7	49	8.6	81	5.7	113	7.8
18	7.3	50	7.0	82	6.1	114	5.1
19	11.4	51	6.7	83	6.6	115	9.2
20	7.1	52	11.4	84	10.3	116	12.9
21	11.8	53	9.2	85	3.7	117	6.8
22	12.8	54	9.1	86	16.5	118	9.6
23	8.2	55	7.1	87	11.8	119	10.7
24	3.8	56	9.3	88	11.4	120	5.1
25	8.6	57	5.4	89	16.8	121	7.1
26	8.6	58	8.6	90	5.0	122	8.6
27	5.0	59	6.4	91	7.1	123	11.5
28	8.6	60	8.8	92	3.1	124	12.2
29	8.7	61	6.1	93	7.0	125	9.6
30	9.9	62	9.7	94	12.5	126	9.0
31	9.3	63	8.6	95	6.5	127	7.5
32	8.2	64	16.5	96	5.7	128	12.3

APPENDIX B

Publications and presented papers

Haswell, S.J., Studies on elevated heavy metal levels in soils of South West England, presented to and published in the proceedings of the South West England Soils Discussion Group, 1983. 2b

Haswell, S.J., O'Neill, P., Bancroft, K.C.C., Association of lead with polar dissolved organic compounds in soil pore waters, Int. Conf. heavy metals in the environment, Heidelberg, 1983, 2, 1215. 6b

Brown, L., Haswell, S.J., Rhead, M.M., O'Neill, P., Bancroft, K.C.C., Initial studies on the application of high performance liquid chromatography to determine organo-copper speciation in soil pore waters, in press, Analyst, 1983. 10b

Haswell, S.J., O'Neill, P., Bancroft, K.C.C., The development of HPLC/GFAA spectrometer interface for the analysis of organo-copper complexes in soil pore waters, presented at the SAC Conference Edinburgh, 1983.

Tye, C.T., Haswell, S.J., O'Neill, P., Bancroft, K.C.C., The determination of arsenic species by coupled high pressure liquid chromatography/hydride generation atomic absorption spectrophotometry including the use of a pre-concentration column - in preparation.

Haswell, S.J., Stockton, R.A., Bancroft, K.C.C., Irgolic, K.J., O'Neill, P., and Rahman, A., Interfacing of HPLC and GFAAS apparatus for element specific detection - in preparation.

STUDIES ON ELEVATED HEAVY METAL LEVELS IN SOILS OF SOUTH WEST ENGLAND

by S.J. HASWELL

Introduction

Instances of elevated soil levels of heavy metals are of general concern with reference to mineralization[1] and sewage sludge disposal[2]. Locally levels of heavy metals related to mineralization and past mining activity have been well studied[3,4], however, although numerous studies on land disposal of sewage sludge and toxic metal levels have been carried out[5,6] little or no data is available for soils used in this locality. This paper reports the results of recent research into (i) elemental enrichment, and (ii) distribution patterns for local soils arising from land disposal of sewage sludge and mineralization effects.

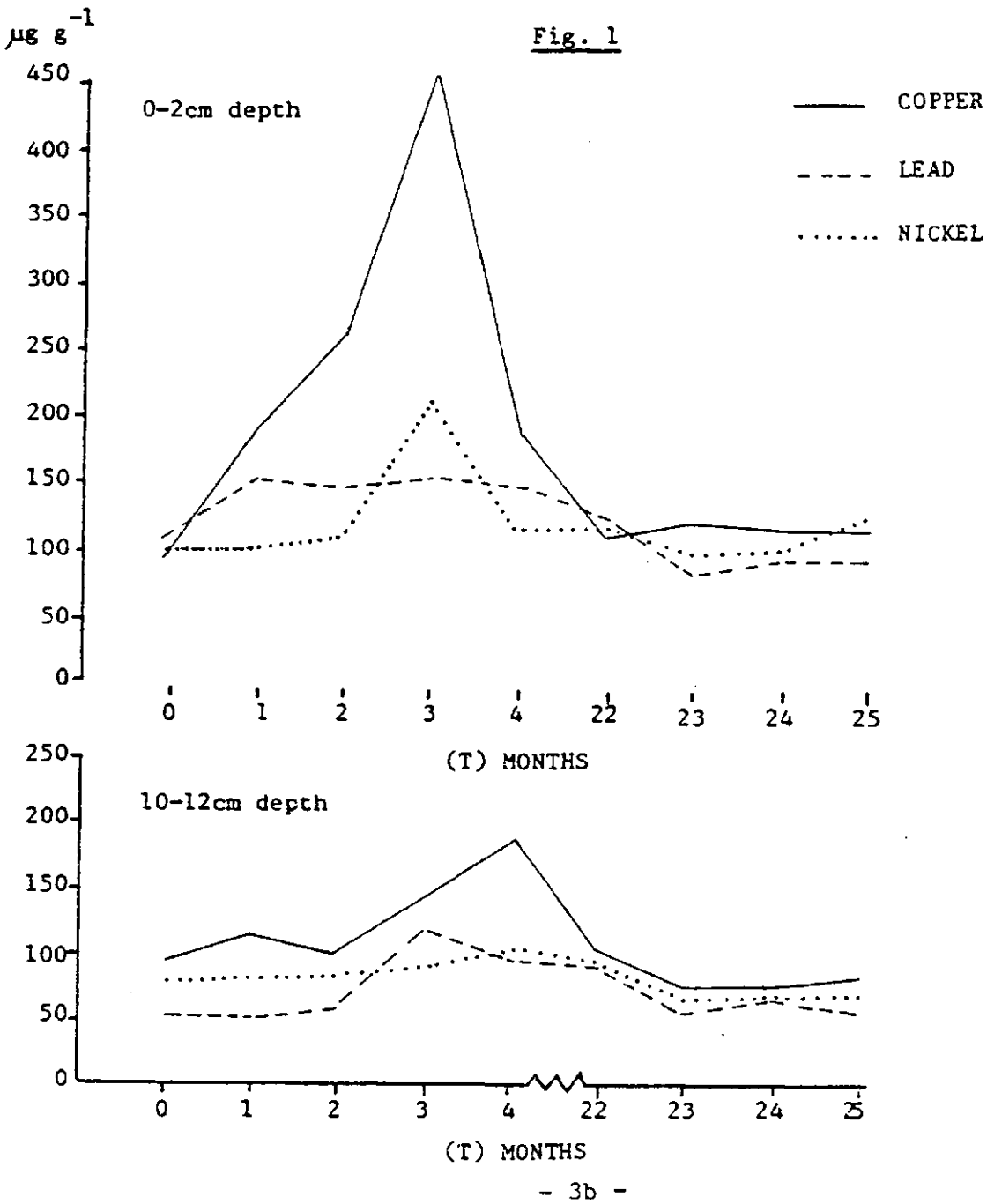
Land disposal of sewage sludge

The distribution of a number of heavy metal elements (copper, nickel and lead) through a soil profile has been investigated with respect to time after an initial application of surface sewage sludge was made onto permanent pasture land. The experimental site at Hatt was dressed with 108 dm^3 per 4 m^2 of sewage sludge giving in a zinc equivalent of 705 Kg ha^{-1}. Samples of soil (Denby series pH 6.9) from two depths 0-2cm and 10-12cm were periodically collected and air dried. A sample of the $< 180\ \mu m$ sieved fraction was digested in hot nitric acid (145°C for 4 hours) and analysed for elemental concentration by flame atomic absorption spectroscopy. Fig. 1 shows the variations in concentrations of copper, lead and nickel after treatment with time for the 0-2 and 10-12cm depths. Surface levels of copper and nickel both show a maximum after 2-3 months, dropping back to pretreatment levels by 22 months. A maximum for lead at the 0-2cm depth is not apparent, however, one is observed at 3 months for the 10-12cm

depth, indicative of lead mobility down the profile. A subsequent maxima at the 10-12cm depth occurs for copper after 4 months, however, nickel does not reflect this trend, elemental levels at the 10-12cm depth return to pretreatment levels after 24 months.

In this study heavy metals present in a surface dressing of sewage sludge appear to be removed from the soil profile within a period of 24 months, with removal patterns reflecting their relative mobilities. It is concluded that rapid break down of organic matter, relief and climate were the dominant factors in the removal of copper, lead and nickel from the soil profile.

Fig. 1



Effects of mineralization in the Tamar Valley

Enhancement of elemental levels of arsenic, copper and lead in the soils of the Tamar Valley may be considered to be a result of either natural weathering of mineralized bed rock or surface pollution resulting from extraction procedures. Over an area of some 30 square kilometres of the Tamar Valley soil samples at a density of approximately 5 per square kilometre were collected from the A and B soil horizons. Two chemical extraction procedures on the air dried $< 180\ \mu m$ sieved fraction were carried out a) hot nitric acid digestion ($145^{\circ}C$ 4 hours) to obtain total levels, and b) sodium dithionite extraction to remove secondary iron and associated elements; all determinations were made by atomic absorption spectroscopic techniques. Typical levels found in the soil are given in Table 1, and can be seen to reflect relatively high background values compared to the national values. The soil extract concentrations were plotted to give spatial distribution patterns for each element soil horizon and extraction procedure. In addition the data was processed statistically to obtain inter variable correlation and determination of background, threshold and anomalous values.

Table 1

Elemental Soil Levels $\mu g\ g^{-1}$

		As	Cu	Pb
NATIONAL	a	11.3	25.8	29.2
TAMAR VALLEY	b	27	90	121
(NON MINERALIZED)	c	34	100	90
TAMAR VALLEY	b	385	2450	215
(MINERALIZED)	c	340	2500	326

a BOWEN, H.T.M., ENVIRONMENTAL CHEMISTRY, VOL. 2, 1980, ch. 3

b THORNTON, I., and WEBB, J.S., APPLIED SOIL TRACE ELEMENTS, Ed. DAVIES, B.E., 1980

c HASWELL, S.J., 1982

The results indicate that bed rock mineralization and subsequent natural weathering cause elevated elemental soil levels in overlying soil, however, the radical dispersion area of this effect was minimal (less than 100 metres). In specific areas where active arsenic smelting had taken place larger areas of surface contamination from mine spoil are observed, however, results from the sodium dithionite extraction indicated that aerial dispersion from smelter stacks was of no significance. At one particular farm in the area, where mine spoil has been used for repairing hedges, numerous cattle deaths have been attributed to direct ingestion of this material.

In conclusion the results of this extensive elemental soil survey in the Tamar Valley indicated (1) higher than national background soil levels with localized elevation in areas effected directly by bed rock mineralization, (2) that extraction processes for arsenic although extensive in the late 19th century caused localized contamination from overburden disposal, but no significant aerial contamination.

References

1 Davis, B.E., Applied Soil Trace Elements, Wiley, 1980

2 Department of the Environment, Report of the Working Party on the disposal of sewage sludge to land, H.M.S.O., London, 1977

3 Davis, B.E., Geoderma, 1976, 16, 183

4 Porter, E.K., and Peterson, P.J., Sci. Total Environment, 1975, 4, 365

5 Beckett, P.H.T., and Wollen, E., Environ. Pollut., 1979, 20, 215

6 Silvicru, D.J., and Sommers, L.E., J. Environ. Qual., 1977, 1, 47

ASSOCIATION OF LEAD WITH POLAR DISSOLVED ORGANIC COMPOUNDS IN SOIL PORE WATERS

S.J. Haswell, P. O'Neill, K.C.C. Bancroft

ABSTRACT

The development of reverse phase high performance liquid chromatography (HPLC) techniques suitable for the analysis of polar dissolved organic compounds (PDOC's) with the use of ultra violet absorption spectroscopic detectors has enabled us to separate and quantify a number of carboxylic and amino acids. The interfacing of the HPLC/u.v. system to a graphite furnace atomic absorption spectrophotometer (GFAAS) allows aliquots of the HPLC eluate to be monitored for lead. The interface is designed so that over 90% of the HPLC eluate is used in the lead analysis. Soil pore waters were collected by on-site centrifugation and immediately filtered through a 0.45 μm millipore filter. Aliquots of pore water were injected onto an ODS column with an orthosphosphoric acid elution system. The eluate passed through a u.v. detector set at 215 nm and via the automatic injector into the GFAAS.

The pore waters were collected from soils in the Tamar Valley, a mineralised area west of Dartmoor in S.W. England. Total lead levels in the waters varied between 30 μg dm^{-3} and 142 μg dm^{-3} (mean 45 μg dm^{-3}). The majority (50-80%) of the soluble lead was associated with citric, malic and formic acids, though the proportions associated with each varied. The remaining 20-40% of the soluble lead eluted with the solvent front as do inorganic and very highly polar organic species.

INTRODUCTION

A satisfactory detection method for metal speciation in environmental waters is a prerequisite for the understanding of both metal mobility and its availability to biological systems (ref. 1,2,3). The development of reverse-phase high-performance liquid chromatography (HPLC) techniques suitable for the analysis of polar dissolved organic compounds (PDOC's) suggested that, with suitable specific detectors, such techniques might afford an insight into organo-lead speciation in natural waters. We report the results detailing the application of molecular and atomic spectroscopic techniques to the determination of PDOC-Pb complexes, in soil pore waters. As well as the high molecular weight compounds such as polysaccharides, peptides, lipids and humic substances, there is a wide range of low molecular weight metabolites produced in soils by micro-organisms and plant roots (ref. 4). These low molecular weight compounds which are polar and soluble in aqueous solutions are of particular interest, as they are more likely to be directly available to organisms. There have been recent reports that significant quantities of various heavy metals are associated with organic species other than those normally described as humic and fulvic acids (ref. 5,6,7).

Department of Environmental Sciences, Faculty of Science, Plymouth Polytechnic, Drake Circus, Plymouth, Devon. PL4 8AA, England.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Series 2 Liquid Chromatograph equipped with a Pye LC ultra-violet (u.v.) detector set at 215 nm and a Perkin-Elmer 023 recorder with a Waters U6K injector valve employed to deliver samples onto a Hypersil 3-5 um octadecylsilane (ODS) column (250mm x 5mm i.d.) was used. For lead detection the column was interfaced to a graphite furnace atomic absorbance spectrophotometer (GFAAS) (Instrument Laboratory 151 spectrophotometer with an IL 555 electrothermal atomizer) using a microprocessor controlled interface based on a previous design (ref. 8).

Sample preparation

Microbial activity can cause rapid changes in the composition of PDOC's after collection and during storage. To obtain pore waters, soil samples were centrifuged on site (typically 2000g for 60 sec.), this process being completed as rapidly as possible after soil sample collection (i.e. minutes). All samples were immediately filtered (Millipore Swinnex fitted with Whatman WCN millipore filters, 0.45 µm) to reduce microbial activity. Storage for periods of 24 hours in a refrigerator (4°C) showed no obvious compositional changes for the samples studied, but storage for longer than 48 hours is not recommended. Unfiltered samples were found to deteriorate within 1 hour of collection. Acidification of collected samples caused dissociation of many PDOC-Pb complexes and was therefore avoided.

Chromatographic conditions

With regard to the chromatographic resolution of PDOC-Pb complexes it was considered that the technique utilised would need to be able to maintain the integrity of the sample and allow simultaneous assessment of both a variety of groups of compounds (carboxylic acids, amino acids, etc.) and the presence of any metal complexes with such compounds. Gas chromatography (GC) techniques and HPLC techniques requiring ion exclusion, ion exchange or pre-column derivatisations do, by their nature, alter the composition of the sample and thus could not be applied to metal speciation studies. Reverse-phase HPLC using an ODS stationary phase offered the potential for analysis of a wide variety of polar organic and organo-metallic compounds and was therefore chosen. Reverse-phase columns will retain polar compounds most effectively when their ionisation is suppressed. As many of the polar organic ligands present in pore waters are acidic, i.e. citric acid, the eluent system chosen to suppress ionisation was also acidic (0.02% v/v orthophosphoric acid pH 2.6). The relative slowness of the GFAAS detection system reduced the rate of eluate analysis to approximately 6 ml hr^{-1}.

GFAAS operating conditions

The operating conditions used are given in Table 1. The injection of eluate into the atomizer at elevated temperatures allowed great volumes (up to 100 µl, if required) to be injected with consequent increase in sensitivity, and together with additional nitrogen cooling of the graphite cuvette, a cycle time of 50 seconds was achieved. An injection procedure commencing at 175°C was used during this study. The introduction of the sample into a warm cuvette by pressurized nitrogen

appears to give instantaneous drying with no evidence of splattering at the nitrogen pressure used. With lead nitrate fed into the sample loop of the interface by means of a peristaltic pump the limit of detection for lead was determined to be 0.6 μg dm^{-3} with the calibration curve showing linearity up to 20 μg dm^{-3}.

RESULTS AND DISCUSSION

The pore waters were collected from soils in the Tamar Valley, a mineralised area west of Dartmoor in S.W. England. Total lead levels in the waters varied between 30 μg dm^{-3} and 142 μg dm^{-3} (mean 45 μg dm^{-3}). Fig. 1 illustrates the results obtained for one of the pore waters. The distribution of lead and the concentrations of the associated organic compounds are given in Table 2. This is a typical example of the pore waters examined in which the majority (60-80%) of the soluble lead was associated with citric, malic and formic acids, though the proportions associated with each varied between samples. The remaining 20-40% of the soluble lead eluted with the solvent front as do inorganic and very highly polar organic compounds. Microorganisms known to release the acids associated with lead, genus Bacillus and Pseudomonas (ref. 9), were identified in the unfiltered soil pore water. These results indicate the apparent importance of dissolved low molecular weight organic acids on the mobility and availability of lead in soils.

REFERENCES

1. J.C. Van Loon, Analyt. Chem., 51, 1139A, 1979.
2. T.M. Florence, Talanta, 29, 345, 1982.
3. J. Lee and I.R. Jonasson, J. Geochem. Explor., 18, 25, 1983.
4. D.C. Zvyaginsev, Bull. Ecol. Res. Com., 17, 61, 1973.
5. B T. Hart and S.H.R. Davies, Estuarine Coastal and Shelf Science, 12, 353, 1981.
6. G.L. Mills, A.K. Hanson, J.E. Quinn, W.R. Lammela and N.D. Chasteen, Mar. Chem., 11, 355, 1982.
7. J. Lee, Water Res., 15, 307, 1981.
8. R.A. Stockton and K.J. Ingolic, Intern. J. Environ. Anal. Chem., 6, 313, 1979.
9. C.H. Collins, Microbial Methods, (Butterworth, London, 1967).

TABLE I

Atomic absorption conditions for Pb determinations by flameless operation with auto interface control.

Lamp: Hollow Cathode Wavelength : 283.3 nm; Band pass : 0.5 nm;

Injection temp : 175°C ; injection pressure : 16 p.s.i.; injection vol : 76.6 μl

temp°C	175	350	700	2000
time sec.	10	5	5	5

FIG. 1

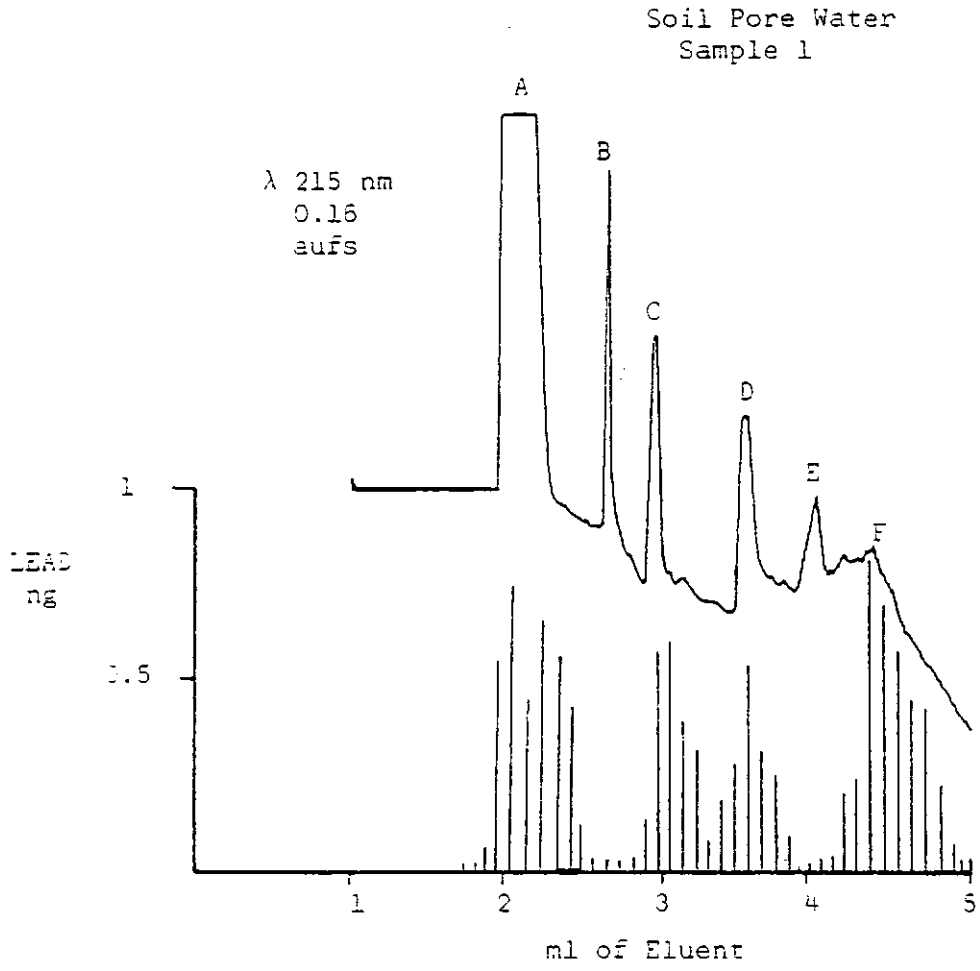


Fig. 1. The U.V. and lead distribution for a soil pore water. U.V. Peaks A = inorganic/highly polar organic compounds, B = allantoin, C = formic acid, D = malic acid, E = lactic acid, F = citric acid.

TABLE II

Quantification and distribution data for soil pore water 1 (Fig. 1)

Total lead in filtered sample : 39 μg dm^{-3}
Total lead determined from GFAAS peaks : 37 μg dm^{-3}

uv peak	A	B	C	D	E	F
% lead	33	-	18	14	-	35
organic acid	-	allantoin	formic	malic	lactic	citric
conc of organic acid mMX10^{-2}	-	1.2	30	2.7	17	1.5

Initial studies on the application of high-performance liquid chromatography to determine organo-copper speciation in soil pore waters

Leslie Brown, Stephen J. Haswell, Michael M. Rhead, Peter O'Neill, Keith C.C. Bancroft.

John Graymore Chemical Laboratory, Department of Environmental Sciences, Plymouth Polytechnic, Drake Circus, Plymouth, England. PL4 8AA.

The use of reverse-phase high-performance liquid chromatographic techniques with molecular and atomic spectroscopic detectors to determine organo-copper complexes in soil pore water is described. Polar dissolved organic compounds and associated copper complexes are separated using either a single Hypersil ODS column or two Hypersil ODS columns and a Hamilton PRP 1 column in series. Quantification was achieved using ultra violet detectors for the organic molecular species and graphite furnace atomic absorption spectrometry for the copper.

Keywords: Polar organic-copper complex determinations; high-performance liquid chromatography; ultra-violet/atomic absorption spectroscopic detection; soil pore waters.

A satisfactory detection method for metal speciation in environmental waters is a prerequisite for the understanding of both metal mobility and its availability to biological systems[1,2,3]. The development of reverse-phase analysis of polar dissolved organic compounds (PDOCs) suggested that, with suitable specific detectors, such techniques might afford an insight into organo-copper speciation in natural waters. We report the preliminary results detailing the application of molecular and atomic spectroscopic techniques to the determination of PDOC-Cu complexes, particularly in soil

pore waters. As well as the high molecular weight compounds such as polysaccharides, peptides, lipids and humic substances, there is a wide range of low molecular weight metabolites produced in soils by micro-organisms and plant roots[4]. The film of capillary water within soil aggregates and on the surface of soil particles contain relatively high concentrations of potentially complexing organic acids associated with the proliferation of micro-organisms in this environment. Bonneau and Souchier[5] have reported the presence of citric, malic, lactic and formic acid in soil pore waters. The low molecular weight compounds which are polar and soluble in aqueous solutions are of particular interest, as they are more likely to be directly available to organisms. There are many areas of the U.K. where copper deficiency in crops, particularly wheat and barley, occurs[6]. It has been found that total copper levels in soils are of little diagnostic value but Na-EDTA extractions, which are thought to extract organically complexed copper, can be used to indicate copper availability. Knowledge of the specific compounds involved in copper uptake is lacking and the correlation between actual uptake and "Na-EDTA extractable" copper is not good[3].

The strong chelating properties of low molecular weight organic acids and their production by micro-organisms play an important part in the bioweathering of rocks and the production of fresh soils[7]. There have been recent reports that significant quantities of copper are associated with organic species other than those normally described as humic and fulvic acids[8,9,10]. The direct interfacing of high-performance liquid chromatography (HPLC) with ultra violet molecular absorption and graphite furnace atomic absorption spectroscopy, as reported here, provides a sensitive method for the separation and quantification of a number of polar organic compounds and the copper associated with them.

Experimental

Reagents

All organic acids and standards were of Analar quality.

Distilled water: deionised, distilled water.

HPLC eluent: 0.02% V/V orthophosphoric acid in distilled water (pH 2.6)

Microbiological assay: substrates used were dextrose agar (P.D.A.) and nutrient agar.

Apparatus

A Perkin-Elmer Series 2 Liquid Chromatograph equipped with an ultra-violet (UV) detector (either a Perkin Elmer LC 75 with scanning facility or a Pye LC UV) set at 215 nm was used. These were connected to a Waters U6K injector valve, Perkin-Elmer 023 recorders and either a single column (Hypersil 5 µm octadecylsilane (ODS)) (250 mm x 5 mm i.d.) or three columns (two Hypersil 5µm ODS and one Hamilton 10 µm PRP 1) (250 mm x 5 mm i.d.) in series in a constant temperature (28.5^{0}C) enclosure. For copper detection either (i) discrete eluate fractions (60 µl) were collected and an aliquot (20 µl) analysed by manual injection into a graphite furnace atomic absorbance spectrophotometer (GFAAS) (Instrument Laboratory 151 spectrophotometer with an IL 555 electrothermal atomiser), or (ii) the column was interfaced to the GFAAS using a microprocessor controlled interface based on a previous design[11] (Fig. 1). The injector consisted of a pneumatic Altex slider injection valve and a solenoid controlled syringe needle. The syringe needle was made of 1/16 OD 316 stainless steel. Nitrogen pressure (16 psi) was used to deliver the sample from the sample loop through the syringe needle into the cuvette. A microprocessor was used to control the injection sequence; including operation of the Altex valve, door opening, and activation of the solenoid to force the needle through

the door port into the cuvette. Further details of the design of the injector will be published elsewhere. Table 1 lists the GFAAS operating conditions.

Procedure

(i) Sample preparation

Microbial activity can cause rapid changes in the composition of PDOC s after collection and during storage. All samples were immediately filtered (Millipore Swinnex fitted with Whatman WCN filters, 0.45 μm) to reduce microbial activity. Storage for periods of 24 hours in a refrigerator (4 °C) showed no obvious compositional changes for the samples studied, but storage for longer than 48 hours is not recommended. Acidification of collected samples will cause dissociation of many PDOC-Cu complexes and was therefore not used. For soil samples, on site centrifugation (typically 2000 g for 60 sec) was used to extract the pore water, this being completed as rapidly as possible after core collection (i.e. within minutes).

(ii) Selection of GFAAS operating conditions

When GFAAS determinations were carried out manually on collected eluate fractions, standard operating conditions were used (Table 1(a)). The direct interfacing of the HPLC-GFAAS systems required both a reduction in the HPLC flow rate (to 0.1 ml min^{-1}) and an increase in eluate volume injected into the GFAAS to minimise the quantity of unanalysed eluate. The operating conditions used are given in Table 1(b). The injection of eluate into the atomiser at elevated temperatures allowed (a) greater volumes (up to 100 μl, if required) to be injected, with consequent increase in sensitivity; (b) reduction in analysis cycle time from 160 to 90 sec. Additional nitrogen cooling of the graphite cuvette was incorporated into the system and this further reduced the cycle time to 50 sec. The temperature of commencement

of the injection cycle affected the size of the absorption signal (Fig. 2). An injection procedure commencing at 250°C was used during this study. The introduction of the sample by pressurised nitrogen appears to give instantaneous drying with no evidence of splattering at the nitrogen injection pressure used. Higher gas pressures do cause this problem. With copper nitrate fed into the sample loop by means of a peristaltic pump the limit of detection for copper was determined to be 0.4 $\mu g\ \ell^{-1}$ and the calibration curve was linear up to 60 $\mu g\ \ell^{-1}$.

Results and Discussion

With regard to the chromatographic resolution of PDOC-Cu complexes it was considered that the technique utilised would need to be able to maintain the integrity of the sample and allow simultaneous assessment of both a variety of groups of compounds (carboxylic acids, amino acids, etc.) and the presence of any metal complexes with such compounds. Gas and gas-liquid (GC and GLC) techniques and HPLC techniques requiring ion exclusion, ion exchange or pre-column derivatisations do, by their nature, alter the composition of the sample and thus could not be applied to metal speciation studies. Reverse-phase HPLC using an ODS stationary phase offered the potential for analysis of a wide variety of polar organic and organo-metallic compounds and was therefore chosen.

Reverse-phase columns will retain polar compounds most effectively when their ionisation is suppressed. As many of the polar organic ligands present in pore waters are acidic, e.g. citric acid, the eluent system chosen to suppress ionisation was also acidic (0.02% v/v orthophosphoric acid pH 2.6). The relative slowness of the GFAAS detection system reduced the rate of eluate analysis to approximately 6 mℓ hr^{-1}. The isocratic elution with orthophosphoric acid was suitable for certain PDOCs up to citric acid Table 2. The slower elution of the PDOCs: with longer retention times than citric acid would have required analysis time of greater than seven hours. A less acidic eluent

was utilised for these less polar compounds. Ammonium formate (0.01 M, pH 6.1) was found to be suitable because it gave a reduced signal to noise ratio for GFAAS analysis as compared to the other chromatographically suitable eluents tested i.e. combined sodium dihydrogen phosphate (0.01 M) - disodium hydrogen phosphate (0.01 M) (pH 6.1) and ammonium phosphate (0.01 M) (pH 6.1). However, as no significant quantities of copper were determined with the later eluting compounds, only the use of the orthophosphoric acid eluting system is described in the present paper.

The use of a single Hypersil ODS column at the higher flow rates (2 ml min^{-1}) gave some separation of the PDOCs (Fig. 3 (a)), but the resolution was poor. Better resolution was obtained (Fig. 3 (b)) when the eluent flow rate was reduced to a value (0.1 ml min^{-1}) suitable for use with the GFAAS detection system. The best separation of the PDOCs (Fig. 3 (c)) was attained with the three column system, using two Hypersil ODS columns in series, followed by a Hamilton PRP 1 column held at 28.5°C. This three column system was not suitable for interfacing with the GFAAS because the longer retention times would extend the time for a single analysis to nearly four hours involving over 250 injections compared to about one hour with about 70 injections using a single column. The retention volumes and detection limits for a number of PDOCs on the single column system are given in Table 2. Those compounds whose retention volumes were greater than 4.4ml were not subsequently considered as the HPLC-GFAAS interface was not operated to investigate their presence. Greater resolution was obtained with the three column system, Table 3, and this was used to confirm the presence and concentration of some compounds. Again, only the compounds of particular interest in this paper are given in Table 3 and fuller details of all compounds which can be resolved will be published separately. The three column system was especially useful for acids that were present in amounts close to or below the detection limit for the single column system.

Having confirmed the separation achieved by running mixtures of standard compounds, soil pore waters were examined. One soil pore water (Fig. 4 (a)) contained five recognisable PDOCs when run on the single column system. These compounds were identified on the basis of their retention volumes, Table 2. Standard solutions of pairs of the proposed compounds were added to aliquots of the pore water. The coincidence of the respective peaks (Fig. 4 (b)), (c), (d)) confirmed the presence of the compounds and allowed them to be quantified. Repeat runs carried out on the three column system gave similar results usually agreeing to within ±20%. However, there were a number of cases where the quantification of some individual PDOCs based on the single column system deviated substantially from that obtained using three columns. The superior qualities of the 3 column system with respect to chromatographic separation made the quantification more precise and its use is essential for the determination of PDOCs.

Initial development of the use of the GFAAS as an element specific detector involved fraction collection of the HPLC column eluate. In order to ensure that adequate resolution of the peaks was obtained, and to reduce the dilution effects from non-copper containing eluate, 60 µl fractions were collected. 20 µl Aliquots of these were manually injected into the GFAAS. This procedure was used for a study of the stability of copper (II) - citric acid complexes when passed through a Hypersil ODS column in the presence of orthophosphoric acid (Fig. 5). A series of mixtures were eluted and comparison of the UV and GFAAS results indicates that the addition of citric acid to a copper(II) solution will cause the formation of a copper(II) - citrate complex (Fig. 5 (b), (c)). Calculations based on the concentration, pH and copper stability constants with the orthosphosphoric acid eluent system indicated that citric acid would form copper complexes which would remain substantially undissociated during column chromatography. It was assumed that instant

uilibria occurred, and no allowance was made for possible stationary
ase adsorption. These assumptions appeared to be justified by the formal
reement between the theoretical stability and the actual stability under
e HPLC separation conditions used.

e acidification of the copper(II) - citric acid mixture with hydrochloric
id, as might be done to preserve an environmental sample after collection,
ve no sign of the presence of the complex (Fig. 5 (d)). This illustrates
e importance of the use of minimum pre-analysis preservation treatment
en attempting speciation studies.

all subsequent work the micro-processor controlled interface between the
LC/UV detector and the GFAAS was used.

e further analysis of larger numbers of soil pore waters using the single
lumn HPLC system interfaced with GFAAS showed that the copper was not
ways associated with the same PDOCs in the same proportions (Figs. 6, 7).
the majority of pore waters association of the copper with citric acid
d neighbouring eluting compounds was found (Fig. 6 (a)). The complexing
fect of citric acid was emphasised by adding a mixture of copper (II)
trate and citric acid to the pore water (Fig. 6 (b)). The proportion of
pper associated with the solvent front, inorganic and highly polar species,
s 16% before the standard addition, with the remaining 84% of the copper
ing associated with the citric acid peak. In the original pore water the
tal copper content was 43 $\mu g\, \ell^{-1}$ as determined by direct injection into
e GFAAS and 42 $\mu g\, \ell^{-1}$ as determined by summation of the copper peaks
ter HPLC separation (Fig. 6 (a)). In the latter case, a correction to
ke account of the volume of eluate not injected into the GFAAS was applied,
d this raised the measured value of 39 $\mu g\, \ell^{-1}$ to 42 $\mu g\, \ell^{-1}$. When 50 ng
copper (II) ion and 3 µg citric acid were added to the soil pore water

(Fig. 6 (b)) the total copper content was determined to be 65 µg (theoretical value 63 µg). This was equivalent to a concentration of 217 µg ℓ^{-1} compared to a calculated value due to addition of 209 µg ℓ^{-1}, with similar percentage distributions between the solvent front and citric acid peaks, as were obtained before the standard addition.

A number of the more extreme distributions found in the soil pore waters examined are illustrated (Fig. 7) and the copper levels are quantified in Table 4. Citric acid/copper association was found in all the samples but the association of copper with allantoin, formic and lactic acids occurred rarely. Malic acid was second only to citric acid in the number of samples which showed association between it and copper. The unfiltered pore waters were examined for the presence of micro-organisms that might produce the organic acids found and micro-organisms with these properties (genus Bacillus and Pseudomonas) were identified[12].

The methods we have described are proving very useful in our studies of the factors which affect PDOC/metal associations in the soils of the Tamar Valley area. Promising results are being obtained with metals other than copper and also in a study of the effects of adding sewage sludge to pasture land. As well as being used to study soil pore waters the Hypersil ODS HPLC-GFAAS system can be applied to other natural and polluted water systems.

Acknowledgements

The authors wish to thank Mr. A. Hopkin for help in the design and construction of the HPLC-GFAAS interface unit. In addition we should like to thank Drs. L. Ebdon and P. Jones, Plymouth Polytechnic, and Drs. J.J. Cleary, R.F.C. Mantoura and A. Nelson, Institute for Marine Environmental Research, Plymouth, for their assistance in the preparation of this paper.

References

1. Stevenson, F.J. and Ardakani, M.S., In:- Micronutrients in agriculture (Ed. Mortvedt J.J., Giordano P.M. and Lindsay W.L.) Soil Science of America, Inc., Madison, USA. 1972, 79.
2. Van Loon, J.C. Analyt. Chem., 1979, 51, 1139A.
3. Goodman, B.A. and Lineham, D.J., In:- The soil-root interface (Ed. Harley, J.L. and Scott Russell, R.) Academic Press, London, 1979, 68.
4. Zvyaginsev, D.C. Bull. Ecol. Res. Com. (Stockholm), 1973, 17, 61.
5. Bonneau, M. and Souchier, B. Constituents and properties of soils, Academic Press, London, 1982.
6. Caldwell, T.H., In:- A.D.A.S. Advisory Paper No. 17, M.A.F.F., 1976, 12.
7. Dacey, P.W., Wakerley, D.S. and Le Roux, N.W. Report No. LR 380 (ME), Warren Spring Laboratory, Dept of Industry, June 1981.
8. Hart, B.T. and Davies, S.H.R. Estuarine, Coastal and Shelf Science, 1981, 12, 353.
9. Mills, G.L., Hanson, A.K., Quinn, J.E., Lammela, W.R. and Chasteen, N.D. Mar. Chem., 1982, 11, 355.
10. Lee, J. Water Res., 1981, 15, 507.
11. Stockton, R.A. and Irgolic, K.J. Intern. J. Environ. Anal. Chem., 1979, 6, 313.
12. Collins, C.H. Microbial Methods, Butterworth, London, 1967.

Table 1 GFAAS operating conditions

(a) Manual injection, Standard operating conditions:-

Wavelength, 324.7 nm; Bandpass, 1 nm; Injection volume, 20 µℓ;

Temperature Programme

T°C	75	100	350	700	1800	1800
Time (sec)	20	25	25	25	0	5

(b) Direct interfacing conditions:-

Wavelength, 324.7 nm; Bandpass, 1 nm; Altex injection value; nitrogen injection pressure, 16 psi; Temperature for initiation of injection procedure, 250°C; Sample loop injection volume, 76 µℓ

Temperature Programme

T°C	175	200	300	1000	1800	1800
Time (sec)	5	0	10	10	0	5

Table 2 Retention volumes of polar dissolved organic compounds on single column Hypersil 5 µm ODS system with 0.02% (V/V) orthophosphoric acid

	Symbol in Figs. 3,4	Retention Volume (ml)		Symbol in Figs. 3,4	Retention Volume (ml)
Oxalic acid		Less than 2.65			
Glucuronic acid		"	Citrulline		3.20
Cystine		"	α A butyric acid		3.50
2 Ketoglutamic acid		"	Malic acid	C	3.60
Pyruvic acid		"	Malonic acid		3.70
Tartaric acid		"	Oxoglutaric acid		3.70
Glycine		"	Lactic acid	D	4.00
Urea		"	Citric acid	E	4.40
Hydroxopoline		"			
Allantoin	A	2.70			
Glutamine		2.75			
Glycollic acid		2.75			
Cysteine		2.80			
Formic acid	B	3.00			

25 other compounds had longer retention volumes

Table 3 Retention volumes and detection limits of some polar dissolved organic compounds using two Hypersil 5 µm ODS columns and a Hamilton 10 µm PRP 1 column in series at 28.5°C with 0.02% (V/V) orthophosphoric acid, flow rate 1 ml min^{-1}.

	Symbol in Figs. 3,4	Retention Volume (ml)	Detection limit (ng) (100 ul injection)
Allantoin	A	12	1
Formic acid	B	13.5	5
Malic acid	C	15.0	5
Lactic acid	D	16.8	100
Citric acid	E	21.0	100

Table 4 Amount of copper in soil pore waters as determined by GFAAS after HPLC separation using a Hypersil 5 µm ODS column. Eluent 0.02% (V/V) orthophosphoric acid, flow rate 0.1 ml min^{-1}. See Fig. 7 for chromatographic separations.

HPLC peak	Amount of copper in soil pore water					
	Sample 7(a) µgCu ℓ^{-1}	% of total	Sample 7(b) µgCu ℓ^{-1}	% of total	Sample 7(c) µgCu ℓ^{-1}	% of total
Solvent front	64	53	13	21	13	30
2 ketoglutamic acid/cystine					5	12
Allantoin					2	5
Formic acid			19	30		
Malic acid	5	4	20	32	16	37
Lactic acid	13	11				
Citric acid	39	32	11	17	7	16

Fig. 1. Schematic representation of the microprocessor controlled HPLC/GFAAS interface

Fig. 2. The absorbance signals for 100 µl (5 ng) copper solution injected via the interface into a cuvette at various initial wall temperatures

Fig. 3. Separation characteristics of various PDOCs using 0.02% (V/V) orthophosphoric acid elution system with (a) a single Hypersil 5 µm ODS column with eluent flow rate 2 ml min^{-1}; (b) a single Hypersil 5 µm ODS column with eluent flow rate 0.1 ml min^{-1}; (c) two Hypersil 5 µm ODS columns in series with one Hamilton PRP 10 µm column at 28.5°C with a flow rate of 1 ml min^{-1}
A - allantoin; B - formic acid; C - malic acid; D - lactic acid; E - citric acid

Fig. 4. UV absorbance signals of various PDOCs separated from a soil pore water on a Hypersil 5 µm ODS column with a 0.02% (V/V) orthophosphoric acid elution system (a); with identification and quantification by co-injections of (b) citric acid (1 µg)-E; (c) formic acid (1 µg)-B and lactic acid (1 µg)-D; (d) allantoin (0.05 µg)-A and malic acid (0.3 µg)-C.

Fig. 5. The UV absorbance and copper distribution patterns of copper-citrate mixtures, with separation on a Hypersil 5 µm ODS column using 0.02% (V/V) orthophosphoric acid, (a) citric acid (10 mg ℓ^{-1}); (b) copper nitrate (1 mg ℓ^{-1}); (c) copper nitrate (1 mg ℓ^{-1}) - citric acid (10 mg ℓ^{-1}) mixture pH 7; (d) copper nitrate (1 mg ℓ^{-1}) - citric acid (10 mg ℓ^{-1}) mixture pH1. Aliquots (60 µl) of column eluate were manually collected with fractions (20 µl) of these aliquots analysed for copper by GFAAS.

Fig. 6. The UV absorbance and copper distribution patterns of a soil pore water (a) using a Hypersil 5 µm ODS column with 0.02% (V/V) orthophosphoric acid elution system flow rate 0.1 ml min^{-1} and the HPLC/GFAAS interface; (b) the same pore water spiked with 50 ng copper and 3 µg citric acid.

Fig. 7. The distribution of copper in various pore waters using UV and the HPLC/GFAAS interface with separation on a Hypersil 5 µm ODS column with 0.02% (V/V) orthophosporic acid elution system, eluent flow rate 0.1 ml min.

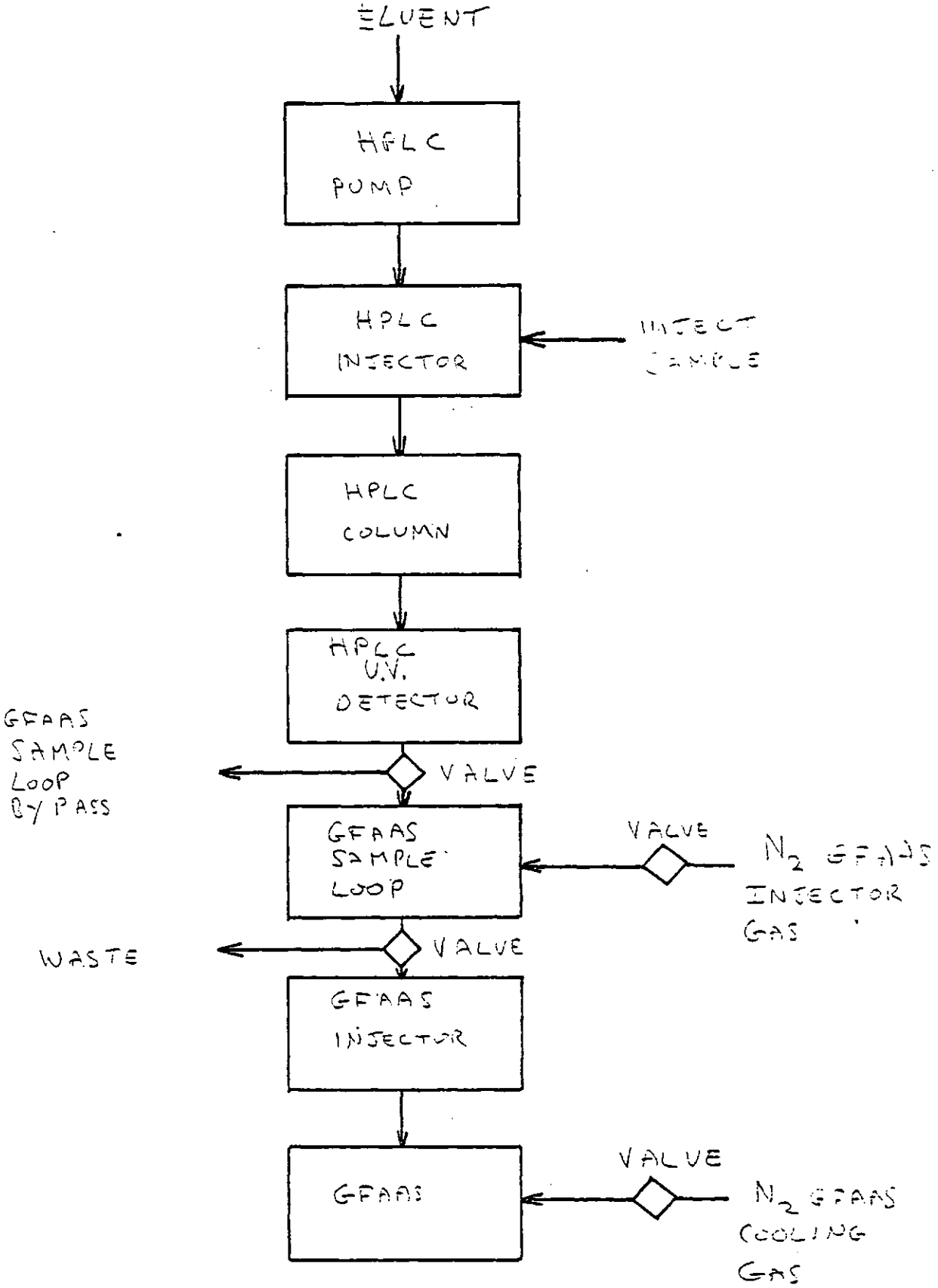
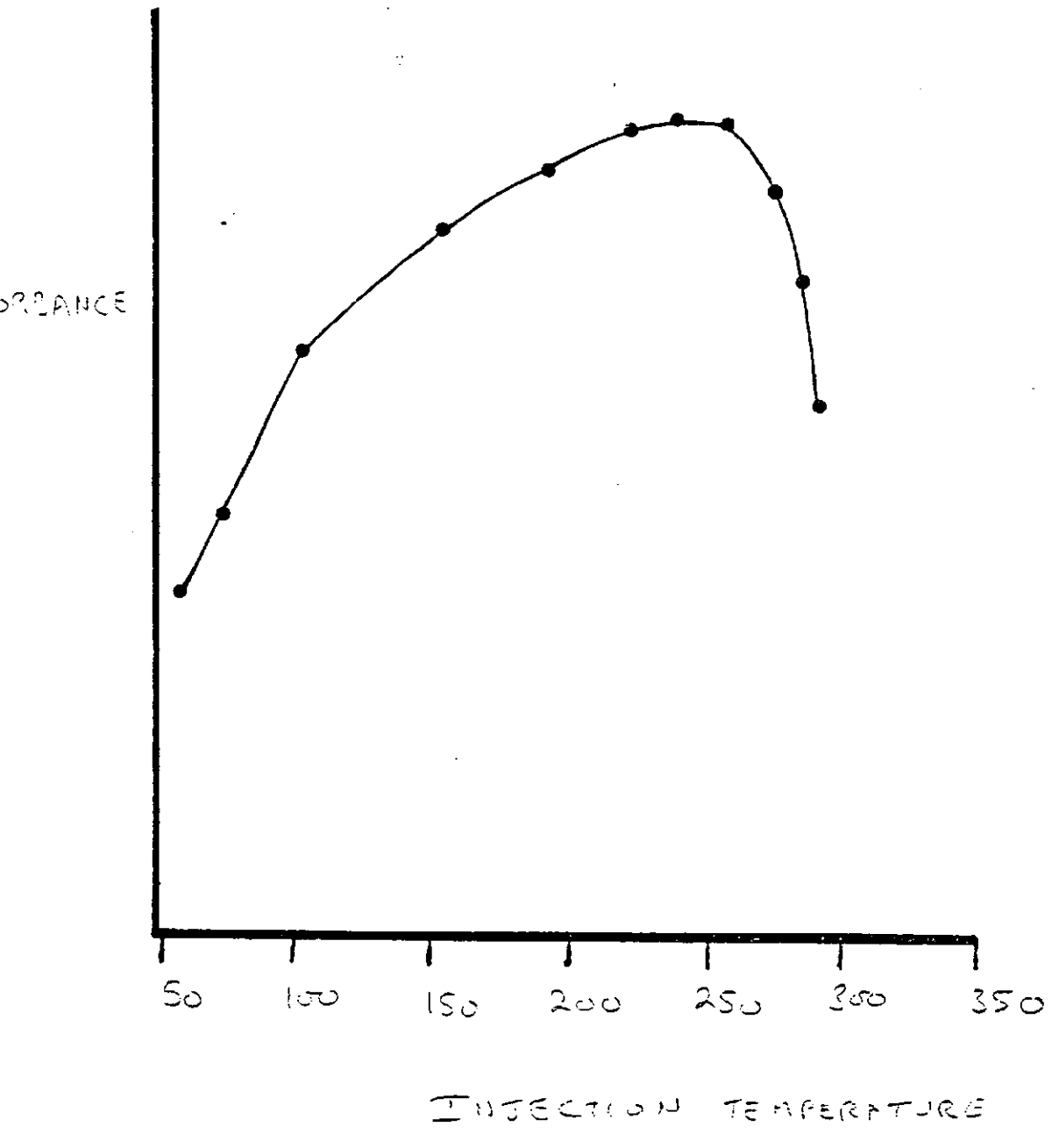

ELUENT
HPLC PUMP
HPLC INJECTOR
INJECT SAMPLE
HPLC COLUMN
HPLC U.V. DETECTOR
GFAAS SAMPLE LOOP BY PASS
VALVE
GFAAS SAMPLE LOOP
VALVE
N_2 GFAAS INJECTOR GAS
WASTE
VALVE
GFAAS INJECTOR
GFAAS
VALVE
N_2 GFAAS COOLING GAS

Figure 2



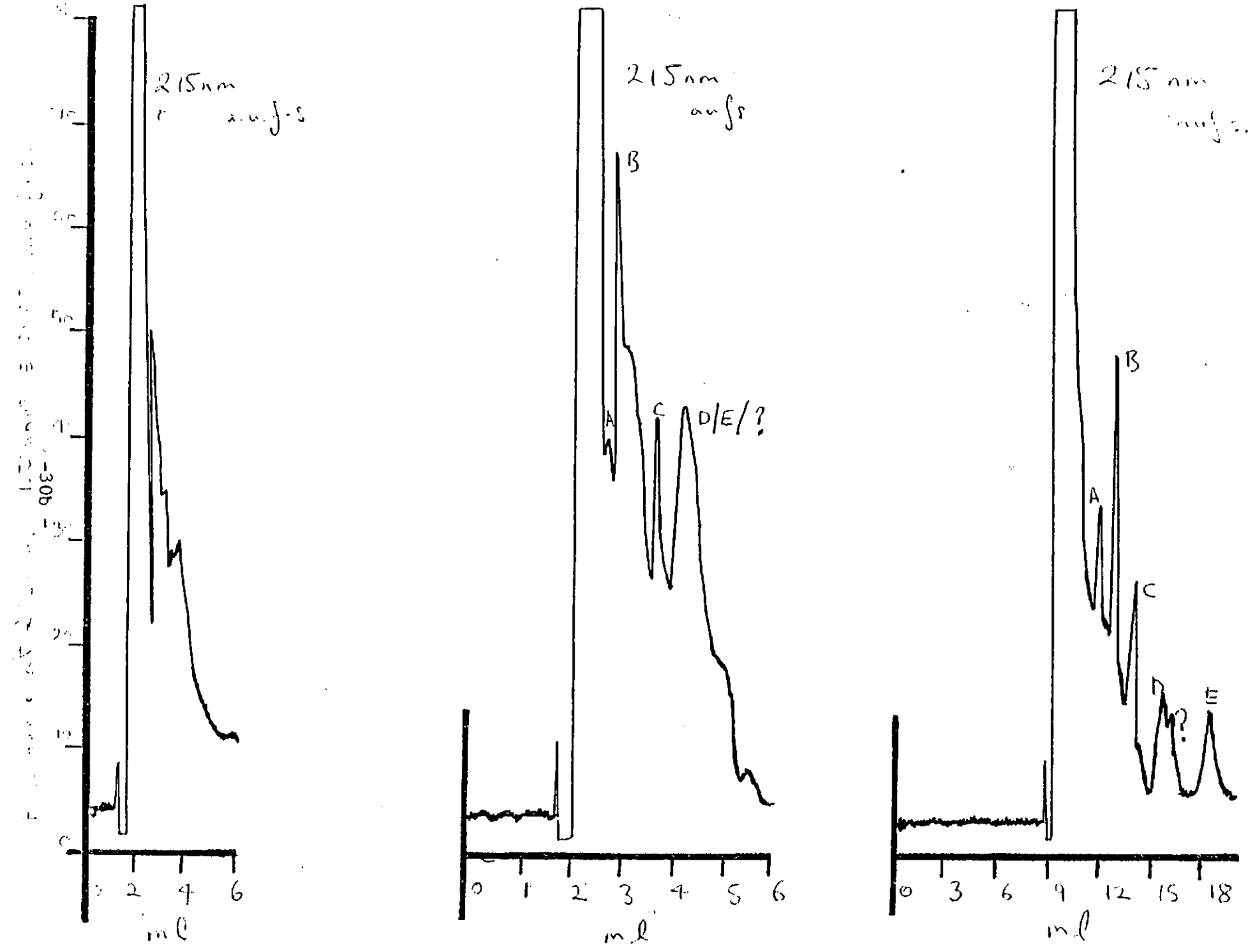
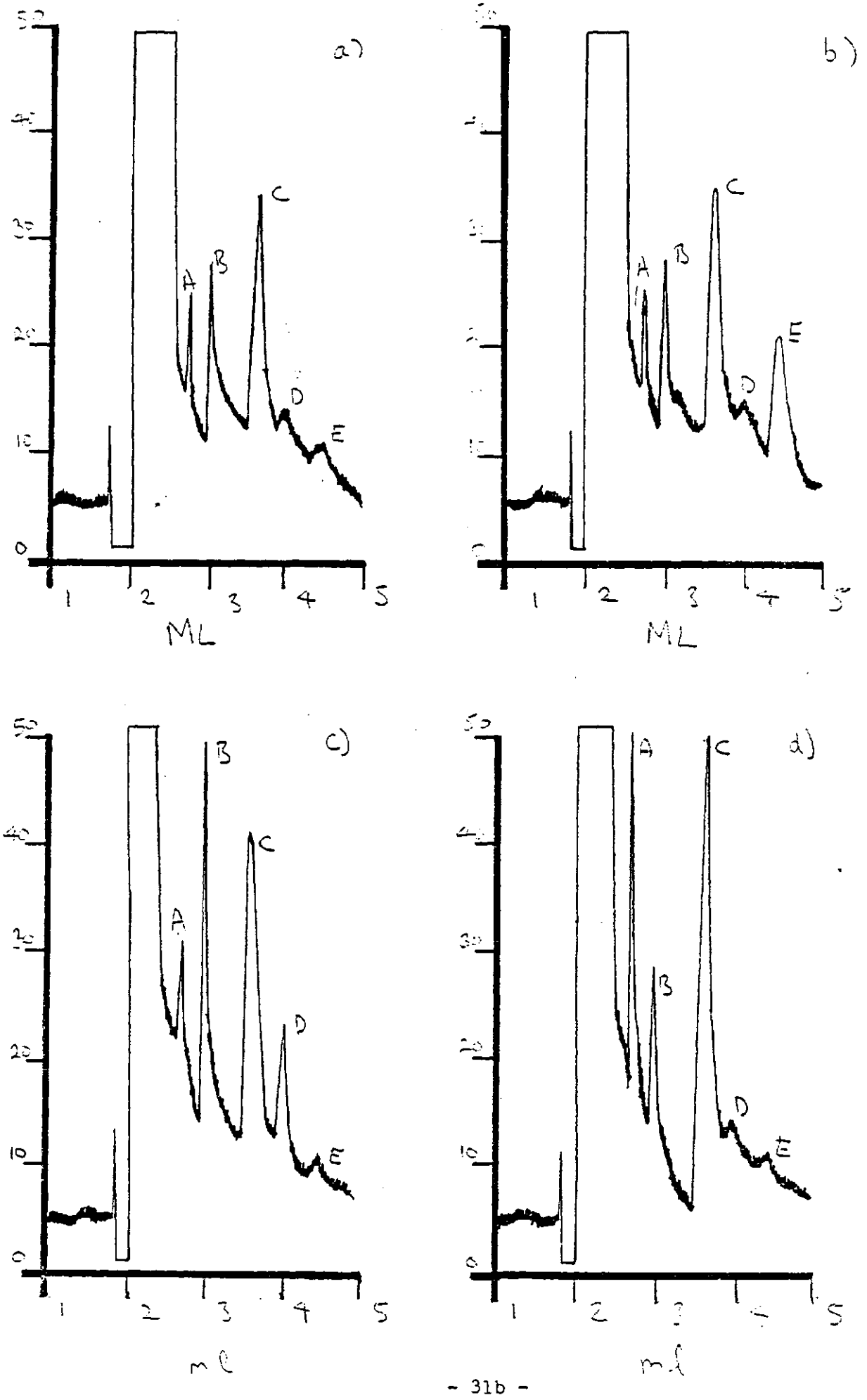

215nm
a.u.f.s
-30b
0
1
2
3
4
5
6
ml
215nm
a.u.f.s
A
B
C
D/E/?
0
1
2
3
4
5
6
ml
215nm
A
B
C
D
?
E
0
3
6
9
12
15
18
ml

Figure 4



- 32b - COPPER

ng

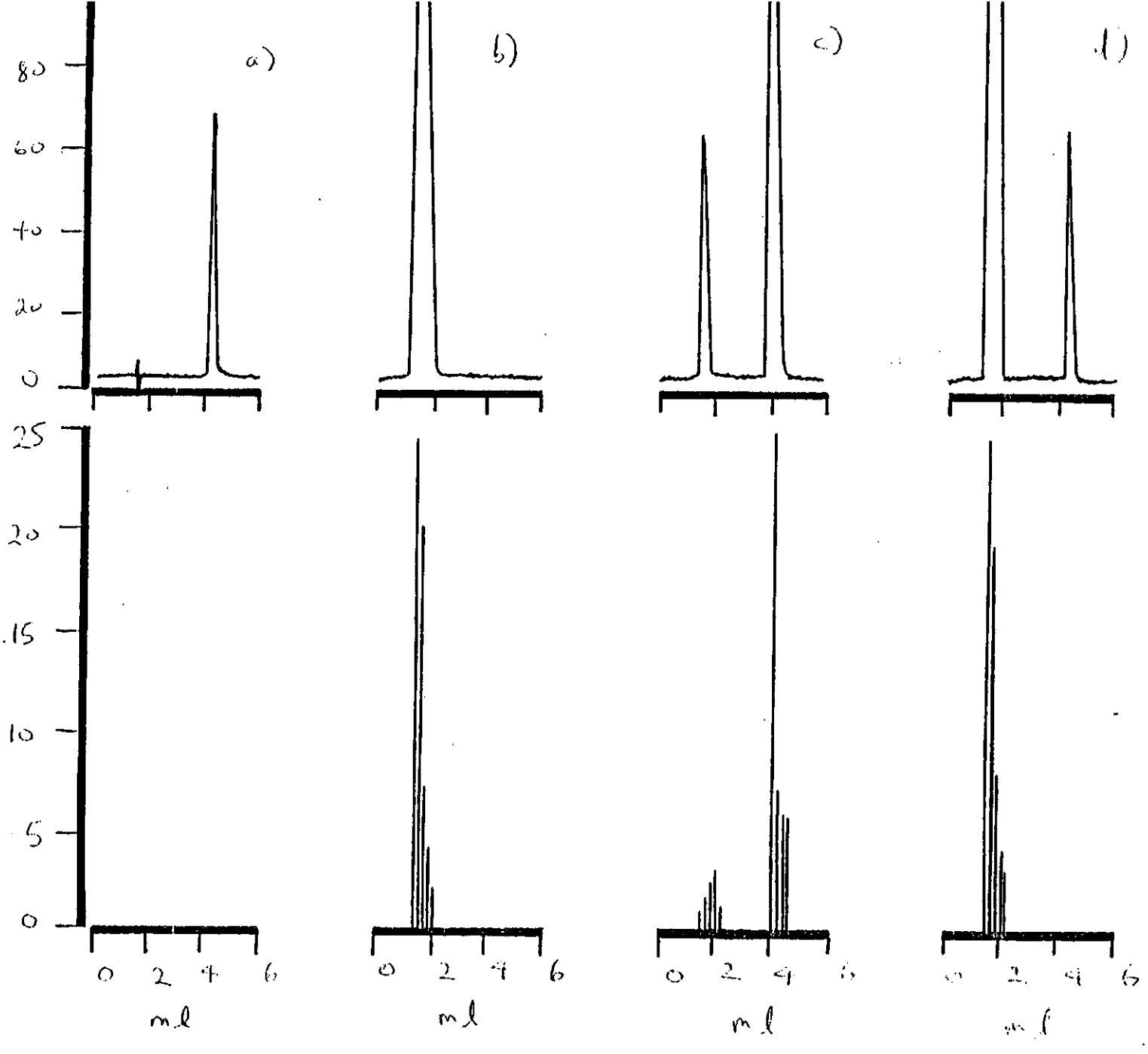
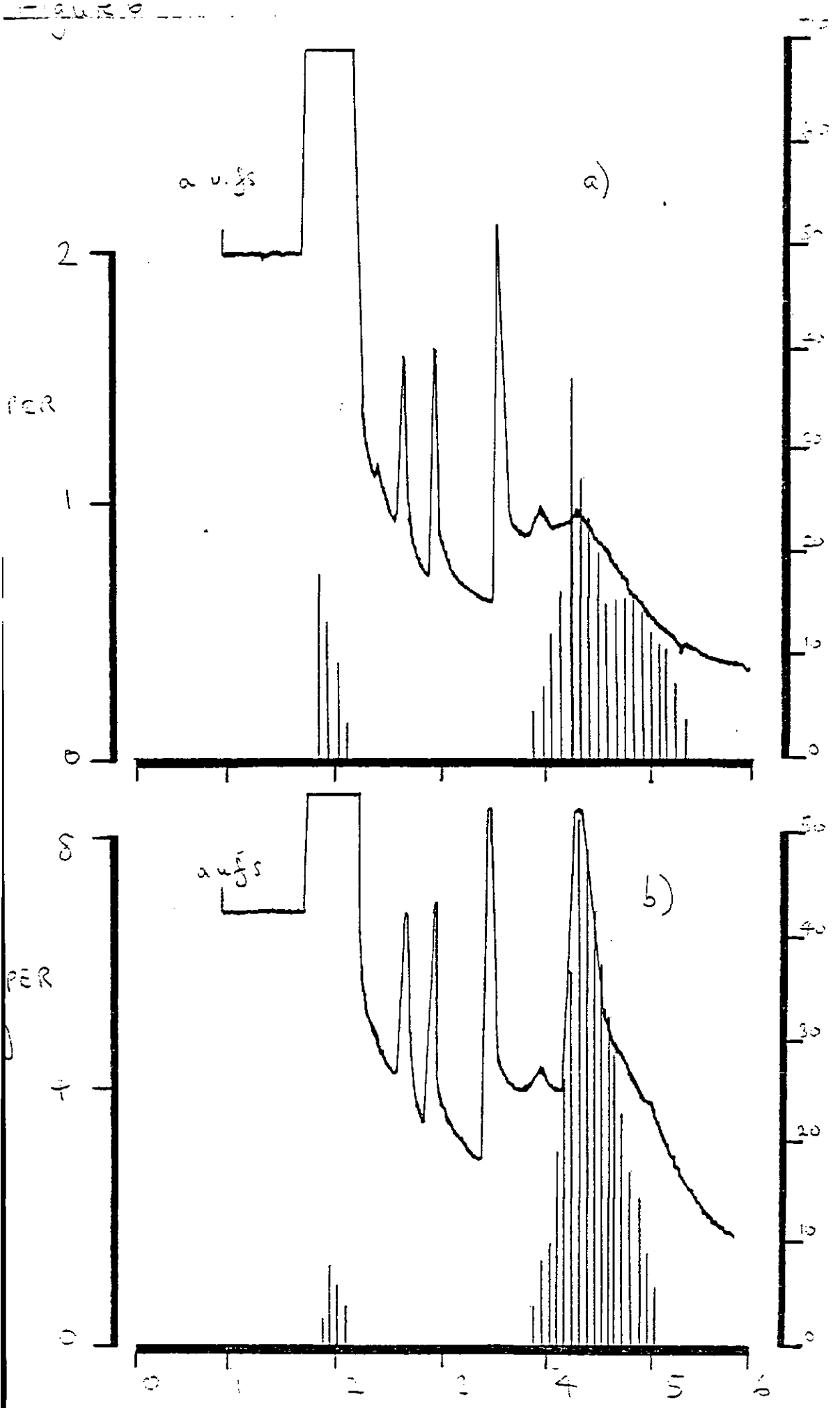
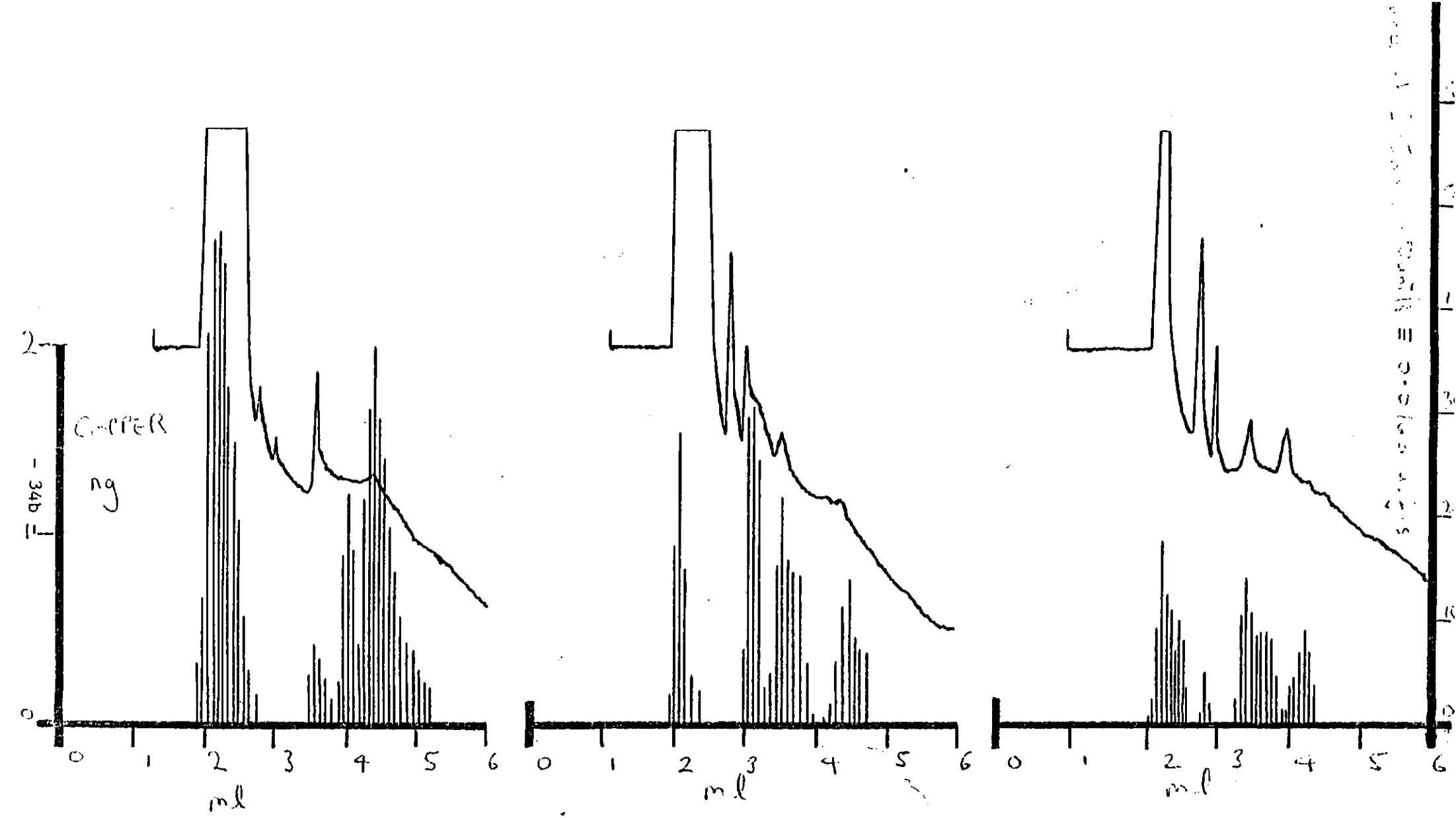


Figure 6




COPPER
ng
2
0
0 1 2 3 4 5 6
ml
0 1 2 3 4 5 6
ml
0 1 2 3 4 5 6
ml