Chemistry in the Institute of Terrestrial Ecology

Institute of Terrestrial Ecology

NATURAL ENVIRONMENT RESEARCH COUNCIL

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The Institute of Terrestrial Ecology (ITE) was established in 1973, from the former Nature Conservancy's research stations and staff, joined later by the Institute of Tree Biology and the Culture Centre of Algae and Protozoa. ITE contributes to and draws upon the collective knowledge of the fourteen sister institutes which make up the *Natural Environment Research Council,* spanning all the environmental sciences.

The Institute studies the factors determining the structure, composition and processes of land and freshwater systems, and of individual plant and animal species. It is developing a sounder scientific basis for predicting and modelling environmental trends arising from natural or man-made change. The results of this research are available to those responsible for the protection, management and wise use of our natural resources.

Nearly half of ITE's work is research commissioned by customers, such as the Nature Conservancy Council who require information for wildlife conservation, the Forestry Commission and the Department of the Environment. The remainder is fundamental research supported by NERC.

ITE's expertise is widely used by international organisations in overseas projects and programmes of research.

This leaflet is one of a series describing research which is currently being done by the Institute of Terrestrial Ecology (ITE), a component body of the Natural Environment Research Council. This leaflet not only describes a particular aspect of ITE's research, but also covers the facilities provided and services offered by the Institute in the field of chemistry.

Introduction

The Institute of Terrestrial Ecology, a biological institute within NERC, is concerned with research on terrestrial ecosystems, and many of its projects are at least partly chemical in nature. These call for a contribution from the chemist who may be required to reveal a pathway within an ecosystem, to clarify a physiological process or to examine the impact of man upon the environment. These and other investigations frequently depend upon the measurement of some chemical characteristics and, because the analytical requirements are paramount, most of the chemical staff specialise in this field. There are also a few projects where the chemist is more directly involved in the implementation of the research. The purpose of this booklet is to review the chemical service within ITE, to describe its main research activities and to comment on its organisation and facilities.

All the analytical staff are based at two stations, Merlewood in Cumbria and Monks Wood in Cambridgeshire. The Chemical Sections at these centres are organised to accept service work from all the stations within ITE and operate in a complementary way.

Much of the early research at Merlewood, after the station was established in 1953, was concerned with studies on biological productivity, nutrient cycling and soil processes in woodland and moorland ecosystems. These studies required information on chemical nutrients which had to be produced by the separate research teams themselves. It was soon apparent that the efficiency of this work could be much improved if it was all carried out within one laboratory using common facilities. From this beginning, the Merlewood Chemical Section developed and was providing an extensive analytical service even before the inception of the Institute of Terrestrial Ecology. The Monks Wood Section had its origins in the Toxic Chemicals and Wildlife Section which was created at Monks Wood in the early sixties, shortly after that station was opened. Its function was to investigate the effects of pesticides on wildlife. From the start, chemists were appointed to the group and a capacity to analyse for pesticides was developed.

Laboratories and Equipment

The origin and growth of the Chemical Sections have coincided with considerable technological progress in the field of electronics and instrumentation. The staff have taken full advantage of these developments wherever relevant to the analytical and data processing needs of the laboratories, both of which are well equipped today. In the case of Merlewood, a new block was completed in 1973 in which the entire upper floor of about 300 m^2 is devoted to chemical laboratories. These replaced the somewhat primitive earlier accommodation which was situated in what were the kitchen and servants' quarters of Merlewood when it was a large Victorian mansion. Although this accommodation had been adapted into acceptable laboratories through some ingenious conversions, they ultimately became too cramped as the work load and staff numbers increased. Perhaps it should be mentioned that the servants' quarters did have their advantages — the large meat cutting slabs made perfect balance benches and there was never any danger of sunlight affecting solutions in the graduated glassware!

The senior chemical staff were very fortunate in being allowed to play a major part in the design of the new laboratories. The experience of the 'kitchen' period proved invaluable. Particular attention was paid to the selection and installation of an efficient fume extraction system to handle the toxic vapours and acid digesting operations. Provisions to reduce sample contamination, handle large batches of samples and automate the analytical processes were also incorporated into the design. Subsequent experience has demonstrated the value of allowing the staff to design their own working conditions, a practice which might be more widely followed when building new laboratories.

Monks Wood Experimental Station, in contrast to Merlewood, is a purpose built establishment that was completed in 1963. From the start a small laboratory was set aside for chemical investigations but, as at Merlewood, it proved inadequate as analytical demands escalated. Extra accommodation was added in a piecemeal fashion, but it was not until the reorganisation following the inception of ITE that it was possible to improve and extend the laboratory accommodation in a rational way to meet the needs of the section. The use of large quantities of flammable solvents for the extraction of pesticides, together with poor ventilation, and potential contamination of samples all presented problems that had to be solved.

Both sections are reasonably well-equipped with major instruments and ancillary apparatus. The emphasis in the Merlewood Chemical Section on ecological analyses has resulted in a large investment in

Plate 1. Atomic absorption equipment in use at Merlewood.

Plate 2. Main laboratory, Merlewood, Chemical Section.

flame and atomic absorption equipment, spectrophotometers and automated colorimetric systems. Other instruments available for the more specialised requirements include an X-ray fluorescence spectrometer, elemental analysers, IR and UV spectrophotometers, electro-analytical equipment and thermal instruments. Although both chemistry sections have some capacity for chromatographic analytical techniques, the major development in the use of gas chromatographs has taken place at Monks Wood and all this type of work is now carried out there. Six gas chromatographs are in regular use; one is coupled to a mass spectrograph which is used for identification purposes. Monks Wood is also equipped for atomic absorption analyses. Both sections have their own dedicated data processing systems and, for more demanding calculating requirements, have access to their station computers.

The Contribution of the Chemist

Since the Institute was formed four years ago its research interests have extended and there is a greater emphasis on wider environmental issues than hitherto, especially in relation to contract research. Information is now sought on the responses of plants and animals to environmental pressures and much use is made of surveys and monitoring techniques together with classification studies based on land use capability. More time is also spent on practical problems such as industrial spoil reclamation, amenity planting, recreation impact, plant breeding and specific pollutant effects, to name but a few.

The total number of projects resulting from this wide range of research interests extends into the hundreds. Some of these deal with problems for which chemical factors are hardly relevant but there are many others which depend on the data produced by the chemist to clarify, or even resolve, the issue.

To illustrate the way in which the chemist provides essential support for the ecologist, we might consider a short selection of ITE's research interests.

Ecosystem studies: a number of sites including woodland, heathland, moorland and grassland have been examined in an attempt to quantify the basic ecological processes such as biomass production, decomposition and nutrient cycling. Projects of this type call for the analyses of large numbers of plant and soil samples for essential nutrients.

Plant selection and breeding:	the presence or concentration of some organic compounds has been found to be a valuable way of distinguishing between different genotypes within certain plant species. The use of isoenzymes, polyphenols and monoterpenes in particular have been effective for this purpose. As might be expected, the properties of the compounds within each of these groups are very closely related, so analytical techniques of high resolution are needed.
Reclamation of industrial spoil:	this type of work requires the examination of the spoil material and associated vegetation to check growth conditions and to test for symptoms of deficiencies and toxicity.
Pesticide studies:	experiments and surveys are being carried out using animals and birds to examine the critical dose levels, retention characteristics and effects on reproduction and behaviour resulting from the application of various pesticides. These investigations require regular assays of the pesticides and their residues.
Land use:	surveys and classification studies sometimes require the measurement of chemical and/or physical factors to categorise different land, soil or vegetation classes.
Atmospheric pollution:	the monitoring of sites and the detailed examination of materials likely to be affected by the pollutant are generally needed in these investigations. It may also be necessary to examine pathways into and through the ecosystem for individual pollutants.
Management:	a number of projects deal with the long-term effects of different forms of land management, for example, afforestation. The factors examined include effects of age, density, cropping, and species composition. Indicator tests are needed to monitor any changes.

Routine Services

Sample materials

Such a variety of research problems clearly shows that the chemists must be prepared to examine a wide range of material types and analytical characteristics. For convenience of processing most of the materials can be split into five categories. This classification is determined in most cases by the nature of the subsequent analytical treatment, rather than by ecological considerations as shown below.

Classification of Materials for Analysis

Type	Examples	Initial treatment
Mineral matter	(a) Rock, soils (total analyses), lake sediments	1 Dried at 105°C 2 Finely ground 3 Stored in dry conditions
	(b) Soils (extraction analyses)	1 Dried at 40°C 2 Sieved through 2 mm 3 Stored in dry conditions
Stable organic materials	Vegetation, litter, peat, some animal products	1 Air-dried 2 Finely ground 3 Stored in dry conditions
Labile materials	Animal tissues, some plant materials (dependent on analyses)	1 Low temperature, storage 2 Freeze dried 3 Homogenised
Waters	Sea water, rainwater, surface and ground waters, some biological fluids	Low temperature storage or chemical preservative

As one would expect, no classification system is all-embracing. Other substances occasionally analysed include fertilizers and atmospheric materials and even more exotic materials such as ladies' hair-nets, hand cream, beer, car tyres and ball point pens are not unknown!

Nutrient elements

Almost all the routine estimations of the mineral elements and structural organic components in ecological materials are carried out by the Merlewood Chemical Section, which is organised to handle the large sample numbers generated by statistically controlled field experiments and surveys. Approximately 100,000 chemical determinations are carried out every year by the analytical team. Most of the routine work concentrates on the chemical nutrient elements which are essential ingredients for all plant and animal life. They feature in many of the ITE research projects as indicated in the previous section.

These elements include the well-known traditional macro-nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium together with sulphur. Elements of marginal necessity to a plant such as aluminium and sodium may also be required because of their value in ecosystem studies. Certain minor nutrients including iron, manganese, copper and zinc are frequently estimated whilst cobalt, molybdenum, and boron are only occasionally requested. Further fractionation to determine the nature of the compound or radical present may be necessary, for example whether nitrogen is present as the nitrate, nitrite or ammonium ions or in an organic form. Waters are a special case in which tests for conductivity, oxygen, anions and silica become equally important.

Most of the nutrient analyses are carried out on samples of vegetation and on soil extracts. The latter are produced by shaking the soil samples with a chemical reagent chosen to remove the exchangeable elements which are associated with the finest particles. Although these extractions are arbitrary in their action, their purpose is to simulate the action of the plant roots and give a rough measure of the available 'pool' of nutrients. Vegetation samples have to be ignited or oxidised with powerful oxidising acids to bring the nutrients into solution. This is a potentially hazardous stage carried out under stringent conditions in well-ventilated fume chambers.

Once the samples are in solution they are passed through various dilution and reagent-dispensing stages using semi-automatic devices. These stages are necessary because some of the initial solutions are very concentrated and additional chemical reagents may have to be introduced to aid the reaction and suppress interfering substances. The treated solutions are next transferred to sample loading modules which can then be coupled to the appropriate test instruments. The three instrumental systems used for routine determinations are the Auto Analyzer which is a continuous colorimetric process, the flame photometer and the atomic absorption spectrophotometer.

Three separate Auto Analyzer lines are in use and once loaded their operation is continuous. Different reagents are fed into the system through tubes by a proportioning pump and are mixed with the sample solution using a system of glass coils until optimum reaction conditions are achieved. The coloured solution thus produced is passed through a colorimeter where its colour intensity is measured and transmitted to a chart recorder. Standard and reference solutions are similarly treated.

The flame photometer used for sodium and potassium also runs continuously but it is generally convenient to operate the basically similar atomic absorption spectrophotometer manually. In both these systems the sample solutions are aspirated into a flame and the emitted radiation is measured directly, or after absorption in a hollow cathode discharge beam.

Pesticides

Just as the Merlewood chemists are organised to process large numbers of elemental analyses so the Monks Wood Chemical Section has developed a routine capacity for the assay of organic pesticides. This was inherited from the days when the Toxic Chemicals and Wildlife Section was responsible for research on the effects of organochlorine insecticides on wildlife.

There is a continuing need to monitor pesticide residues in the environment and to watch for the effects of the similar industrial pollutants, such as the polychlorinated biphenyls (PCBs) which find their way into the environment. Vigilance in this field is always necessary because of the continual introduction of new insecticides and herbicides which may not be harmful for human health and agriculture, yet may damage natural habitats and wildlife.

Many of these compounds can be examined using gas chromatography which has become a speciality of the Monks Wood Chemical Section. The basic sequence in much of this work is to grind the samples (frequently animal tissue) with sand and anhydrous sodium sulphate to produce a friable mixture. Chlorinated hydrocarbons are then extracted into an acetone-hexane mixture which is reduced in volume and passed through a clean-up stage, before being further extracted into a small volume of hexane. These extracts are introduced into a gas chromatograph column containing special packing material (stationary phase) which selectively retains the compounds being determined. An eluting gas (mobile phase) is passed through the column and the compounds are separated by partition and eventually eluted from the column. They are measured by a detector head and an integrating recorder gives a final print-out of the results. Standard mixtures are used for calibrating the gas chromatograph.

One of the chromatographs is coupled to the mass spectrograph. In this instrument the elements are separated by their atomic mass-charge ratio which enables their empirical formula to be determined. This is of particular value when dealing with unknown compounds such as toxic residues.

A great deal of attention has been paid to heavy metal pollution in recent years, particularly in relation to human health. However, these pollutants affect the environment in many other ways and the Institute of Terrestrial Ecology has sponsored a number of research projects in

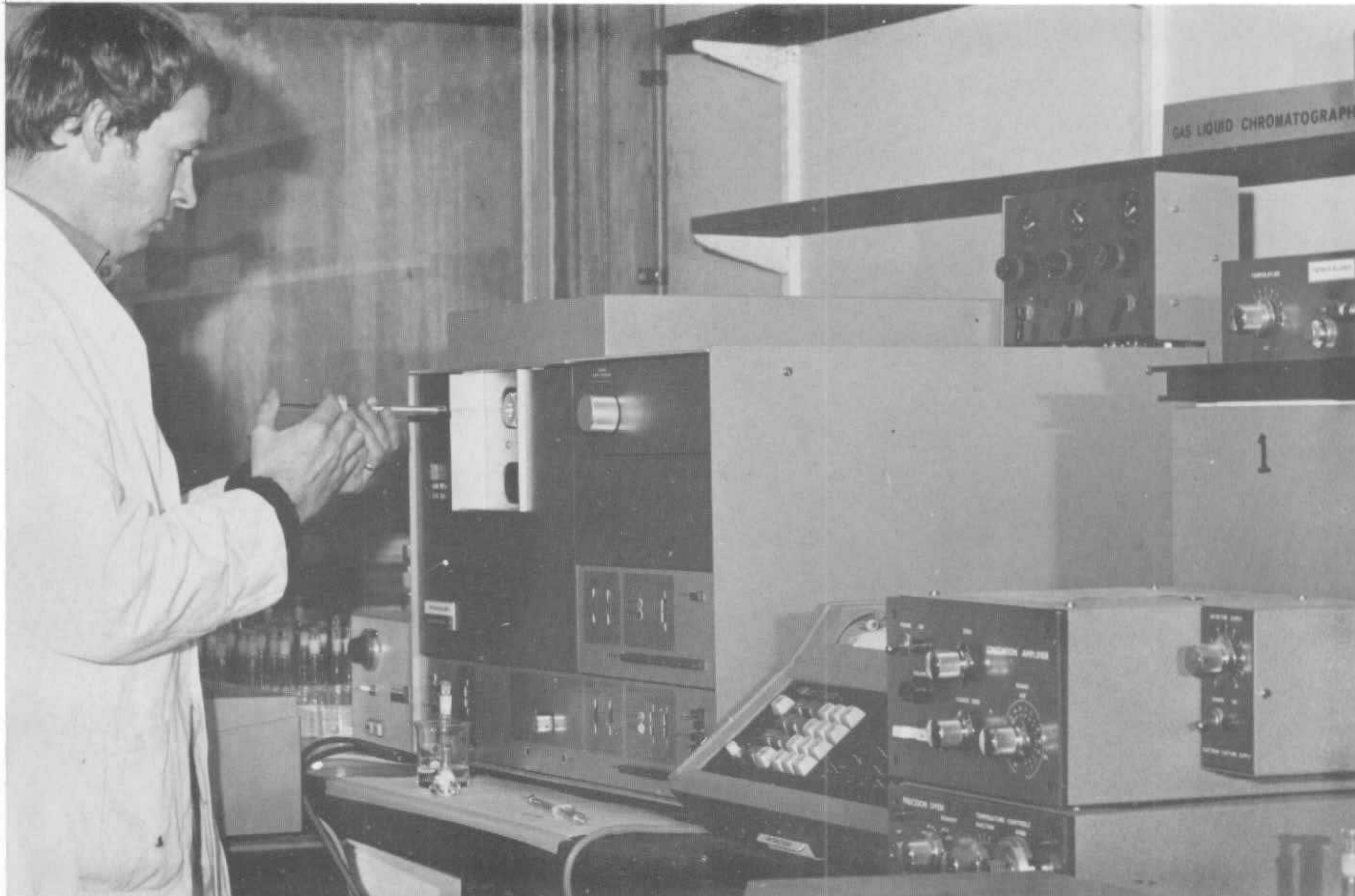


Plate 3. Gas chromatograph in use at Monks Wood.

this field. Apart from providing analytical support for these projects, the Chemical Sections have also carried out various analyses arising from pollution incidents. Toxic elements and materials which have been examined include antimony (birds), arsenic (mammals), cadmium (birds and small mammals), copper (vegetation), chromium (vegetation), lead (birds, mammals, water organisms and vegetation), mercury (birds, water organisms, and vegetation), nickel (vegetation), selenium (mammals) and thallium (small mammals).

Almost all the heavy metal analyses are done by atomic absorption spectrophotometry, often replacing the usual flame with a graphite tube furnace for greater sensitivity. Methyl mercury is a special case which is analysed by gas chromatography, whilst colorimetric techniques are also used for some of the other toxic elements. Both the Merlewood and Monks Wood laboratories are equipped for most heavy metal analyses, which allows flexibility in times of pressure, but usually vegetation samples are processed at Merlewood and animal materials at Monks Wood.

Apart from the pesticides and heavy metals, other pollutants have sometimes to be dealt with on a routine scale. Recently, this has occurred with both sulphur and fluorine. For the total analyses of these elements X-ray fluorescence is used for sulphur and the selective ion electrode for fluorine. If the projects require information about the respective compounds present, then different analytical procedures are used.

Other routine analyses

Apart from plant nutrients and heavy metals, the concentrations of other mineral elements are also determined as required. This is a miscellaneous group but most of these requests originate from research studies concerned with the structure and origin of soils and sediments and from other geologically related investigations. The X-ray fluorescence spectrograph at Merlewood is particularly appropriate for this type of work.

Different groups of analyses are carried out in connection with investigations into biological processes, for example plant decomposition or animal digestibility. Information may then be needed about the gross structural composition of vegetation or the concentration of compounds which are involved in the metabolic changes within an organism. The classes of compounds which have been examined in such studies include carbohydrates, amino acids, fatty acids and polyphenols. Examination of individual compounds within a group invariably involves detailed chromatographic fractionation and is usually confined to a specific research requirement, in contrast to the analysis of proximate organic constituents which is a continuing requirement. The 'proximates' include fats, crude protein, water-soluble carbohydrates, cellulose fractions and lignin which, together with mineral matter, give a primary breakdown of plant material. Traditional chemical extraction and gravimetric procedures are still used for these analyses.

Some of the organic groups have been examined because of their potential value for chemical taxonomy but only monoterpenes have been handled as a routine. In this respect the skills developed in gas chromatography for pesticide assays at Monks Wood have been of particular value.

Another measurement which is often needed for the studies of biological processes is energy content which is usually measured as calorific value. Strictly this is a physical test but its value is determined by the relative concentrations of the organic constituents. Further groups of physical tests which are frequently required are those which measure specific soil properties. Probably the most important test in this group is the sedimentation procedure which is used to fractionate soil particles into size classes (sand, silt and clay).

Analytical Development

A substantial proportion of the time of the senior staff of the two Chemical Sections has to be spent on development work to meet the considerable variety of service requests which are received. Most of this time is spent on testing new procedures and setting up fresh instrumental systems. This work may involve relatively simple modifications to existing procedures but often there is a need for more advanced development work to give entirely new procedures. A book *Chemical Analysis of Ecological Materials* written by senior ITE chemists was published by Blackwell Scientific Publications in 1974 and contains a comprehensive explanation of methods in use in the Institute. It includes details of sample collection procedures and a review of instrumental techniques, together with statistical methods and background information about ecological processes and data. Information on subsequent developments can be obtained by contacting the Senior Analysts.

Some of the current and recent development work is described opposite.

Sample preservation

Since the two laboratories cater for projects in all parts of the country suitable arrangements have to be made for sample despatch. Sometimes inter-station transport is used, but this is not always possible and samples then have to be sent by post or commercial carrier. There is then a risk of deterioration caused by biological changes during transit. Amongst the sample materials likely to be affected are waters, plant material and animal tissues, notably the latter which decompose rapidly after sampling. It is therefore necessary for the chemists to ensure that samples to be sent in for analysis will not change significantly in composition during transit and to devise ways of preventing such changes.

Matters which have received particular attention include:

1. The effectiveness of various preservation additives
2. The effect of low temperature storage on elemental composition of waters
3. Changes with time in the composition of nitrogen and phosphorus fractions in waters
4. Effects of drying on the composition of plant materials
5. The effect of freeze storage on the extractable constituents of soils
6. Preservation of animal tissues, particularly with reference to the presence of pesticides

Routine processing procedures

A considerable amount of effort goes into the refinement of analytical techniques to enable greater numbers of samples to be processed without lowering analytical standards. This applies particularly to the Merlewood Chemical Section since this group is handling very large quantities of plant and soil samples for the basic nutrient elements.

Some improvements become practicable because of advances in instrumentation although it is necessary to decide whether the potential increase in productivity can be used to justify the high capital cost of new equipment. However, other improvements are still possible as a result of laboratory experimentation. As an example, the development of a single digestion technique might be cited. In the determination of the principal nutrient elements in plant materials, it has long been accepted that two separate acid digestion stages are needed to destroy the organic matter and bring everything into solution. One procedure, which employs a mixture of oxidising acids, is used for the mineral elements and phosphorus, whilst a modification of the classical

Kjeldahl digestion method is generally used for nitrogen. The former method cannot be used for nitrogen because of the presence of nitric acid, and the Kjeldahl system is unsuitable for the mineral elements because of the presence of potassium or sodium salts and also because of subsequent analytical interferences from certain catalysts. The single digestion technique developed in the Merlewood laboratories is based on the use of hydrogen peroxide and sulphuric acid as the oxidising media, together with lithium sulphate to raise the temperature, and selenium as the catalyst.

X-ray fluorescence spectrometry

This technique is in regular use in the Merlewood chemical laboratories for the determination of mineral elements including aluminium, barium, calcium, iron, magnesium, manganese, phosphorus, silicon and titanium in soils and plant materials. The soil samples are milled to a fine powder and then fused with sodium tetraborate and sodium carbonate to give a glass disc. Vegetation samples are finely ground and pressed into a disc using a ring mill. The samples are placed in the spectrometer and bombarded with high energy X-rays so that the secondary (fluorescent) intensities of the K lines can be measured. Standard samples of known composition are treated in the same way for calibration.

A particular problem with this analytical method is the correction for matrix interference effects. These effects are usually overcome by analysing similar materials of known composition to serve as standards when drawing the calibration curves. Recent development work has now made it possible to use the computer to compensate for matrix effects. The separate elemental concentrations are applied successively and an iterative procedure is then used to obtain a final correction. The method is now in routine use for the analysis of soils by X-ray fluorescence spectrometry. Recent work has also resulted in the X-ray spectrograph being used for the estimation of particulate sulphur in the atmosphere, the particles being trapped on filter discs.

Reserve nutrients

The fertility of soils is generally assessed by using a chemical extraction or leaching technique in an attempt to measure the levels of chemical nutrients available to vegetation. Several methods are in widespread use for this purpose but are primarily intended to meet agricultural requirements. To some extent, these extraction methods are of value to the ecologist but there is also a need for some kind of index which measures the long-term nutrient potential of a soil. In ecological research the interest is in natural plant communities, and the capacity of the soil to support a particular plant assemblage over many years. Therefore work has been directed towards obtaining a chemical extraction system which will give a suitable 'ecological index'. Extraction systems that take into account factors such as acidity, oxidation, chelation and ionic exchange are now being tried. The response of elements found in contrasting soil types and mineral structures is being examined. Experiments are in hand to correlate possible extraction methods with soil exhaustion tests and continual cropping.

Chemical taxonomy

Monoterpenes are present in relatively high concentrations in the resins of conifers and have been shown to have valuable characteristics for taxonomic purposes, particularly within the genus *Pinus.* Some published material is available describing analytical techniques for separating compounds of this group, but it was found that considerable development work was needed before any of these methods could be used for the routine analysis of pine needle resins. To a certain extent, both Chemical Sections participated in the initial development studies although all the analyses are now carried out in the Monks Wood laboratories.

In the routine analytical procedure the resins are extracted into n-hexane before being introduced into a gas chromatograph. Most of the terpenes are separated on a column with 5% Apeizon on Chromasorb W-AW-DCMS, but, in some cases, a separate packing is needed to achieve adequate separations. For example, Carbowax 20M is used in the case of limonene and B-phelandrene. The running temperature varies between 60°C and 80°C and flame ionisation is used for detection. Difficulties were encountered in the identification of some of the less common monoterpenes although the mass spectrograph proved to be a valuable asset for this purpose. Further problems arose because of the difficulty in obtaining suitable standards for certain monoterpenes and the possibility of preparing some of these compounds in a pure form is now being explored.

There have been a number of attempts to use isoenzymes for identifying variants within a given species. This method is being used at Furzebrook Research Station in the work on ant communication and organisation. Similar work is being carried out on plants at Colney Research Station. So far, the method has been successfully used as a means of 'fingerprinting' the genetic composition of the plants *Puccinellia maritima* and other species are now being examined. The technique used in this work makes use of electrophoresis equipment to separate the isoenzymes as coloured bands on gel slabs. After they have been separated they are removed for photographic recording and in some cases for quantitative measurement with a densitometer. Much of the Colney development work was carried out under guidance from staff at the nearby John Innes Institute, where techniques of this type are extensively used.

Apart from these compounds other groups, notably the flavonoids, have been examined in relation to separate taxonomic requirements. The investigations on flavonoids, carried out at Merlewood, were based on the use of thin layer chromatography to separate the compounds.

New pesticides and herbicides

As new compounds are developed, the Institute may be commissioned to look into their effects on species and habitats of conservation interest. These investigations often involve the chemists in the analyses of animal and plant materials for the presence of the various pesticides and herbicides. Where possible, close liaison is maintained with the manufacturer who is generally able to offer assistance with the analytical methods. Even so, further development work is frequently required to modify a method to meet particular laboratory or instrumental conditions.

Two recent examples of this type of work arose from studies on Asulam, produced by May and Baker, and those on Cyanatryn, produced by Shell Chemicals. Asulam is a herbicide which is now being used to destroy bracken. The analytical problem resolved into finding the appropriate sequences of solvent mixtures to allow maximum recovery of Asulam over a wide concentration range. The final concentration was determined by a spectrophotometric procedure. Cyanatryn, a herbicide being tried out on aquatic habitats, was estimated on the gas chromatograph following appropriate partitioning and concentration stages.

Bomb calorimetry

To measure the energy content of biomaterials in connection with decomposition and digestibility studies a standard adiabatic bomb calorimeter is used. There are instances when modifications to the standard technique have had to be evolved, particularly those involving minute, unstable and relatively incombustible sample materials. Procedural modifications which have been introduced include the use of freeze drying and emulsification to stabilise the samples, the employment of additives to control combustion conditions and the design of a fresh crucible and firing system to eliminate misfiring and to improve precision.

The principal development has been in the construction of a miniature bomb calorimeter. In this instrument a micro bomb is surrounded by an adiabatic enclosure of reduced volume which enables the total water equivalent to be markedly reduced. The use of a transistorised temperature recording system is employed to measure the temperature differential within the relatively small volume of water. The overall precision of this miniature bomb has been found to be an improvement on other micro systems which have been described.

Data processing

All the discussion so far has been related to the development of experimental methods, but there have been concurrent advances in the calculation procedures. To some extent the need to process large numbers of samples, in the Merlewood laboratories in particular, provides the incentive to make use of new electronic processing systems as they become available.

The heart of the calculating system lies in the conversion of the instrument response into meaningful scientific values. This procedure can be very tedious when carried out manually since it is necessary to produce graphs based on known standard concentrations for each sample batch. Nowadays, most laboratories which are handling large numbers of routine samples use one of the following procedures to eliminate the manual stages.

1 Each instrument records samples and standards as peaks on a chart. These are then read on an electronic chart reader, generally linked to a programmable calculator or computer

2 Each instrument has its own built-in microprocessor, or outputs to an integrating module which enables a digital read-out or print-out of the solution concentration to be obtained

3 Each instrument is linked 'on-line' to a master calculator or computer which, with varying degrees of sophistication, processes the data to give a final print-out, or even controls the operation of some instruments

The Merlewood laboratory has used electronic chart readers for many years since this was the natural development for processing the charts from the Auto-Analyzers and flame instruments. The procedure was retained in preference to direct instrumental read-out systems because it was possible to detect and identify analytical faults by visual inspection. By coupling the chart reader to a programmable calculator it has been possible to develop procedures to correct for base-line drift, blank subtractions, curvilinear regressions and to obtain final sample concentrations.

At Monks Wood, with its emphasis on gas chromatography techniques, the need was primarily for an instrumental procedure which could measure peak areas rather than peak heights. For this reason the policy at the laboratory has been to concentrate on the use of integrating modules which can be connected directly to the gas chromatographs.

However, partly in response to the pressure of service work, and partly because of the rapid development in the field of microprocessors, the possibility of following the third option is now being explored. It has already been demonstrated that it is feasible to link all the principal instruments directly to a suitable processing unit and at the same time retain the advantages of the chart inspection arrangement. Ultimately the intention is to develop compatible processing systems in the two laboratories so that programs and data can be interchanged.

Research Projects

So far we have only considered the analytical service functions of the two Chemical Sections. There are, however, a number of research topics which cannot be dealt with as a service. Some of these are assigned as projects to staff within the Subdivision of Chemistry and Instrumentation whereas others are primarily the responsibility of other subdivisions. However, close liaison is maintained throughout the Institute in all chemical topics where there are common interests.

1 SUBDIVISION OF CHEMISTRY AND INSTRUMENTATION

Chemical Data Bank

One of the projects which has concerned the staff of the Merlewood Chemical Section has been the creation and maintenance of a data bank to contain chemical and physical information on indigenous plant species and their associated soils. Chemical analyses have been carried out in the laboratories at Merlewood Research Station for nearly twenty years and during that period over a million separate characteristics have been measured, many of which have been included in numerous publications. Some of these data are relevant only to the work of the original 'customers', but a substantial amount has a wider interest and forms a valuable reservoir of chemical information about our vegetation and soil. As knowledge about the existence of these records has spread so has the number of requests for information. However, because of the mass of data, it is often difficult to locate the precise information required so that the need to develop a computerised data bank becomes apparent.

It is ITE policy to install small computers at its stations and to encourage direct user communication. One of these is available at Merlewood but a way had to be found to enable a relatively large data bank to be handled on a time-shared machine with only a small core storage capacity (4K) available to any one user. This was carried out by taking advantage of a ready access to fixed-head disks (256K each) and magnetic DEC tapes (191K each). An advantage of these tapes is that they provide an almost unlimited capacity. With the emphasis on retrieval, in contrast to data sorting, it was possible to devise a fixed format structure with master and subrecord arrangements which was suitable for the purpose. Although most data are now stored on the DEC tapes it is possible, using a number of retrieval programs, to extract the data quickly and easily in various combinations.

Nutrient Survey of Vegetation

Chemical and environmental variables of some common native plant species are being examined in an attempt to relate plant performance to environmental parameters. Plants and associated soils have been collected over four seasons from sites covering the whole of Britain and then they have been analysed for various nutrients. Multivariate statistical techniques are being used to extract information about the variability of nutrient levels between the sites so that significant correlations between nutrients may be revealed. The validity of the findings with regard to the status of fifteen of the most common species is being considered in detail.

The project has also revealed some of the pitfalls to be avoided in this type of work if the data obtained are to be suitable for the objectives of the survey. Both biological and statistical factors have to be considered when planning the survey.

Chemistry of Aquatic Pollutants

One of the largest of the chemical research commitments is concerned with the effects of selected pollutants on aquatic organisms. This is a project being carried out jointly with the Subdivision of Animal Function. A certain amount of information can be obtained by examining specimens from polluted streams but detailed studies can only be carried out using continuous flow tanks where the treatments can be controlled and varied.

Because of the lack of suitable water from natural sources near to Monks Wood Experimental Station where the investigations are based, it was necessary to make use of water from the mains supply to the station which could be treated to create a suitable 'natural' water.

In order to carry out experiments on the scale required it was estimated that 250 gallons of water would have to be treated each day. Inevitably, the continuous processing of such a large volume of water called for a substantial treatment plant.

Before the plant could be constructed a considerable amount of development work had to be carried out to determine the most appropriate treatment. Initial tests were done in the laboratory and then the operations were scaled up to determine the optimum conditions for operating a treatment plant.

Much of the plant construction was accomplished by the Institute's own engineering staff. In the first stage of the treatment process the water is purified using reverse osmosis, followed by deionisation. The experimental water is then reconstituted by a sequence of chemical dosing, mixing and gasification stages, all controlled through a programmed console. Certain chemical characteristics are monitored to check the water quality. An important advantage of the system is that the treatment can be changed, enabling the composition of the experimental water to be varied at any time.

The next step was to establish the chosen experimental species or communities in the flow tanks. It is intended that many different pollutants will eventually be examined but in the initial studies most attention is being paid to the effects of lead, zinc and cadmium salts, although one project is concerned with aquatic herbicides. In parallel with these station-based experiments additional investigations are being carried out in the River Ecclesbourne in Derbyshire. The water in this river passes over bed rock and eroded sediments rich in lead minerals. These conditions produce aquatic habitats with high lead concentrations which, in many ways, serve as ideal controls for the station experiments.

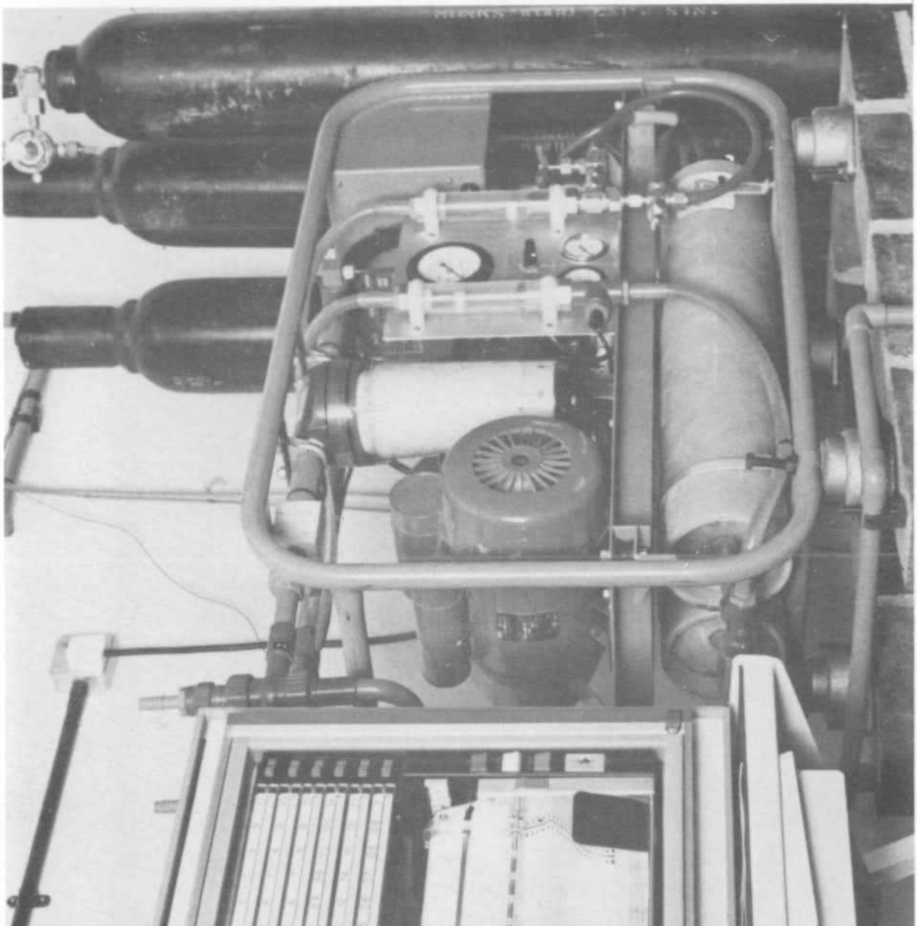


Plate 4. Dosing plant installed for the Continuous Flow Studies, Monks Wood.

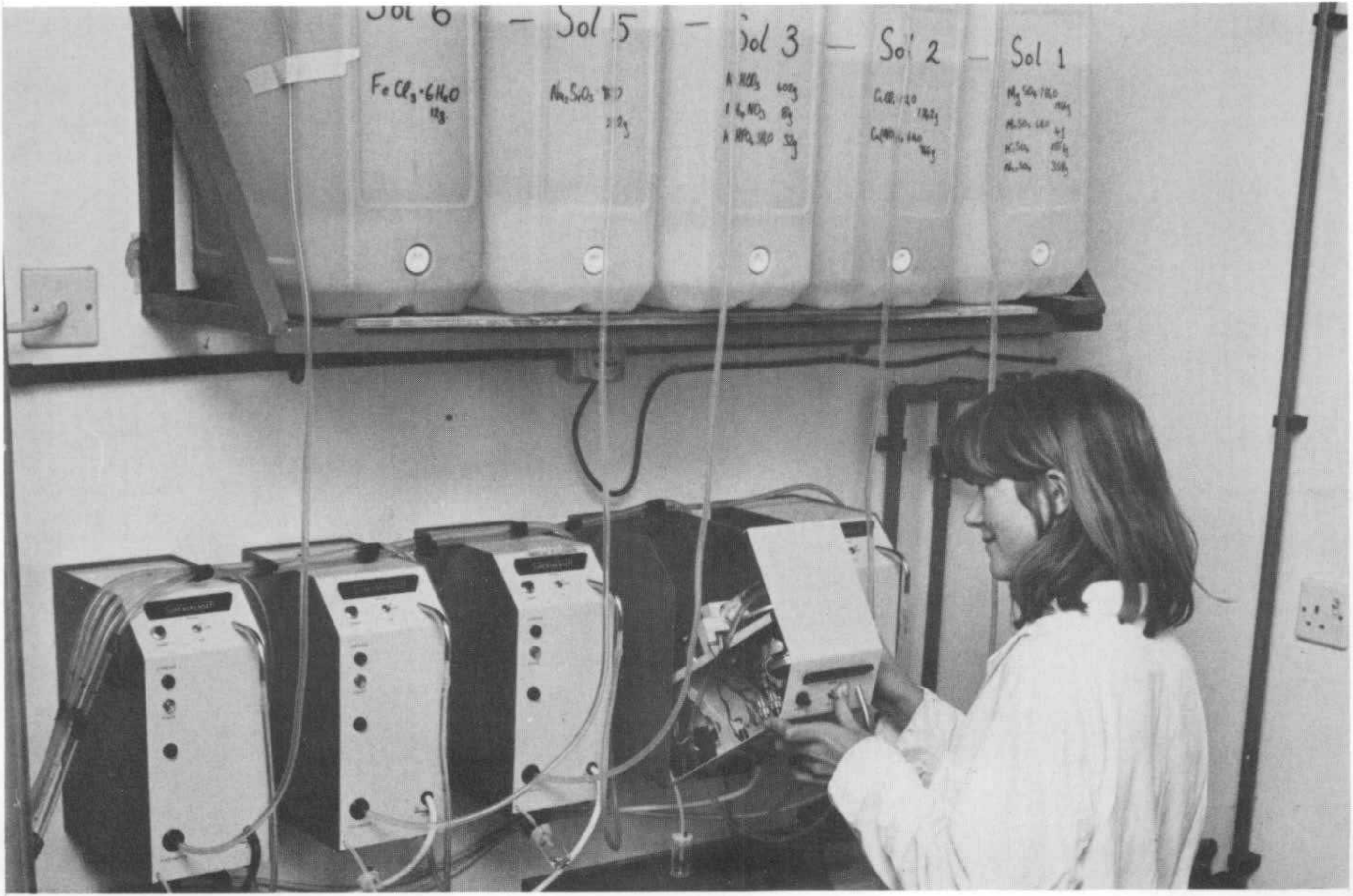


Plate 5. Dosing Plant installed for the Continuous Flow Studies, Monks Wood.

2 OTHER SUBDIVISIONS

Sulphur Pollution (Subdivision of Plant Community Ecology)

There has been a great deal of interest in recent years on the effect and extent of sulphur pollution and the Institute of Terrestrial Ecology is one of the organisations involved in this problem. The emphasis in the Institute's contribution is on the rural atmosphere and on the effect on trees. Following recent surveys in Manchester and Glasgow several tree species were sampled and analysed for various sulphur fractions. Samples were obtained from localities at increasing distances from the urban centres, and examined for 'surface' sulphur, inorganic sulphur and organic sulphur. These experiments established a clear relationship between the levels of surface sulphur, and to a certain extent inorganic sulphur, and the levels of sulphur dioxide in the atmosphere. Further work is being carried out on the distribution and effects of sulphur gases and acid rain in the rural environment, concentrating on Scottish localities.

The principal experimental site for detailed monitoring studies is at Devilla Forest on the north bank of the Forth opposite Falkirk. At this site continuous recording equipment is being used to monitor the concentration of sulphur dioxide (and also of nitrogen oxides and ozone) above the tree canopy. In this way it will be possible to examine diurnal and seasonal variations as well as the reported build up of high concentrations of the gaseous pollutants over short time periods and to relate these to any synergistic effects.

Fluorine pollution (Subdivision of Plant Biology and Vertebrate Ecology)

Another atmospheric pollutant causing concern in some locations is fluorine. This is present in relatively high concentrations in the areas around many industrial establishments, notably aluminium smelters, brickworks, fertiliser plants and steel works. The Institute of Terrestrial Ecology is looking at its accumulation and effects on the vegetation in some exposed locations. Lichens, which are valuable indicators of urban pollution, are also seriously affected by high fluorine concentrations. The concentration of this element as it passes through the faunal food chain is also a matter of some concern.

Effects of pollutants on birds (Subdivision of Animal Function)

Much of the research within the Subdivision of Animal Function is devoted to the effects of exposure to insecticides and heavy metals on avian species. In one project the reported sublethal effects of organochlorine insecticides on the endocrine systems of various birds are being examined further. The purpose is to show which endocrine lesions are being primarily affected, and to establish relationships between the dose and times of exposure to different pesticides both individually and in combination. A separate investigation is focussing on the effects of heavy metals on birds. In particular the storage distribution of heavy metals within the tissue is being examined together with the role of metallo-thionen in limiting the toxicity of heavy metals. The accumulative effect of sublethal concentrations is also being studied.

Phosphorus studies (Subdivision of Soil Science)

Of all the chemical nutrients, the role of nitrogen has attracted the most attention, notably in agriculture. However, in many natural situations, the availability of phosphorus can be the principal limiting factor to plant growth. Unlike most other elements there is often only a limited soil reserve. The importance of understanding more about the mechanisms involving phosphorus has been recognised in the Institute and there are a number of research projects on this topic.

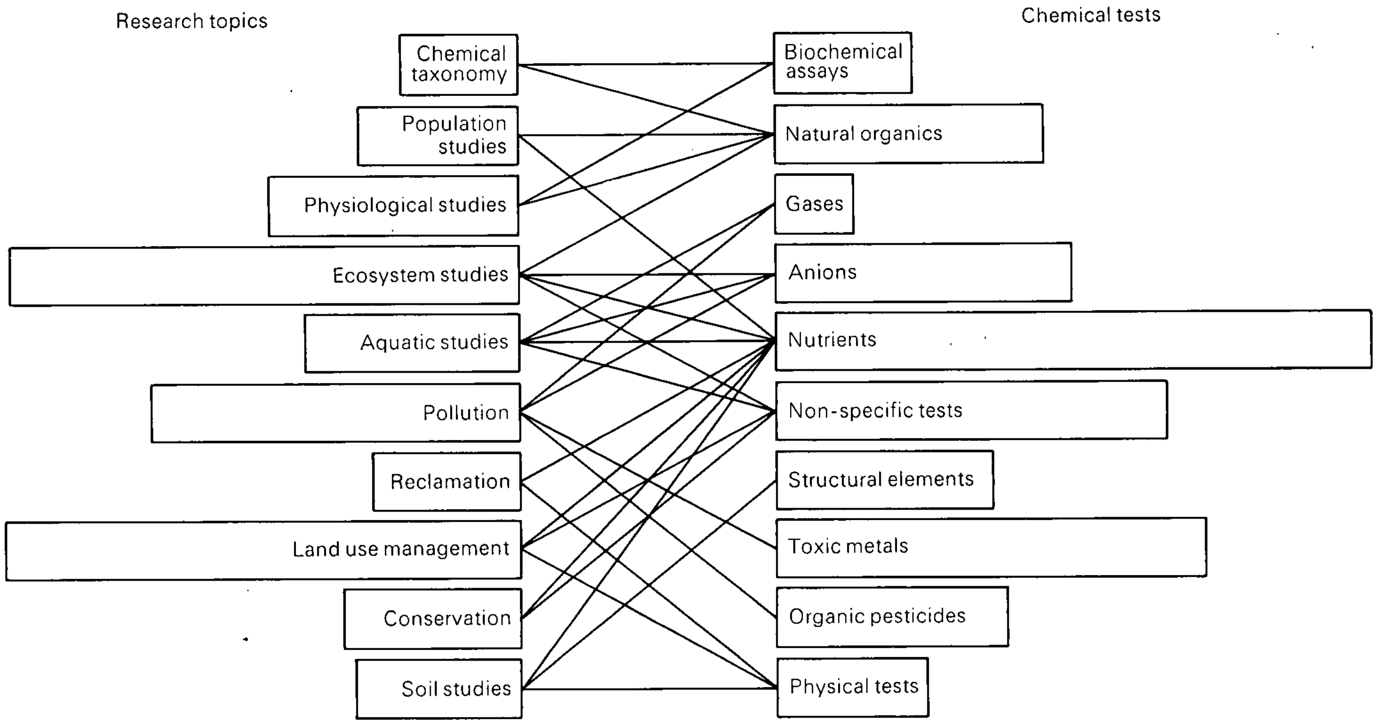
Essentially these studies involve the detailed examination of the processes involved in the circulation of phosphorus in soils with an examination of the different forms in which the element is held, its availability to plants and the amounts carried to different parts of the ecosystem. The availability of phosphorus has been assessed by measuring the movement of the P^{32} radioisotope. This and other soil variables have been examined in a range of soil types and correlations obtained.

The Future of Chemistry in ITE

Clearly, there will always be a need for basic ecological studies which help us to understand our environment. However, with resources being so limited, it will be essential for us to identify the key problems and to concentrate on projects which will have practical benefits. In most subjects, including those involving chemistry, it is easy to produce data which have little real or lasting value.

One fundamental topic which has received much attention in agricultural research, but less in ecology, is the soil-root interface. This includes the capacity to acquire essential nutrients under conditions which may be quite different from those which apply in agriculture. The long term rather than the annual availability of the nutrient elements is an important consideration in this respect and some studies on this

Relationships between ITE research topics and their analytical requirements with relative time spent on each in 1977



subject have already been mentioned. It is also clear that the nutrient levels of plants are governed not just by supply in the soil but by a complexity of environmental factors which need to be examined further.

Plant physiology investigations, and, more recently, pollution research programmes, have included studies on the relationship between the plant and the atmosphere, for example in the work on the effects of sulphur dioxide on rainwater acidity. However, there are still aspects of this interface, with probable ecological implications, that have not yet been researched.

Chemistry taxonomy

It has long been known that certain organic compounds show differences in concentration between genotypes of the same species. In a few cases a particular compound may be apparently confined to one genotype. One example occurs in the cultivars of decorative plants which may be distinguished by their anthocyanin content. Within ecology most taxonomic studies concerned with intra-species variations have concentrated on morphological and other growth characteristics. With the development of new and more sensitive analytical techniques it is now possible to detect fine concentration differences or pick up smaller concentrations. Chemical methods hold out hope of providing more precise and objective ways of identifying genotypes. As already mentioned, some of this type of work is based on the measurements of monoterpenes and isoenzymes and is already being carried out.

Pollution

Greater awareness of the hazards of pollution and the ensuing legislation will lead to the limitation or removal of many of the pollutants which have caused concern in the past, although there will be a continuing need for environmental surveillance because of the persistent nature of many of these substances. There will also be a need to examine new products and assess their likely effects. Following any fresh incident of industrial or agricultural pollution it will be necessary to look at the extent and magnitude of environmental damage but it seems likely that in future more attention will have to be given to studying the mechanisms of damage by pollutants, and to the processes of pollutant accumulation within a species or ecosystem. It will also be desirable to look into ways of neutralising the effects of harmful materials, for example by selection of shelter species to soak up pollutants or through the design of buffer zones to protect sensitive habitats. In any studies of this type, as in all pollution work, the chemist will have an essential role to play.

Land use

The pressure for more home food production, resulting in the increased use of fertiliser and heavier stocking on the land, is another issue which is likely to throw up problems for the Institute's scientists. Already the high levels of phosphate and nitrate used in agriculture are leading to the accelerated development of eutrophication in many lakes and water courses and are also affecting ground waters and potable water supplies. As these nutrient-rich solutions diffuse through the environment, the nature of many of our natural habitats may be changed, perhaps irreversibly. The proposed use of treated sewage on a large scale for increasing the productivity of the land and for bringing more marginal and upland areas into agricultural use could have similar effects. The Institute's chemists have the capability to monitor changes in chemical balance and there is a need for further research into ways of minimising the effects on the ecosystem of intensive food production.

In addition to the need for more food, there is also a demand for more home-produced fibre and fuel and this could influence future ITE research and the contribution of the chemist. Work might be needed on topics such as management practices for maximum fibre yields, the selection of species for high energy yields and the problems of soil depletion through changes in land use. The chemist can make a valuable contribution to land use studies by identifying the parameters which are most appropriate for classifying the different land and water categories. So far, most attention has been paid to land capability surveys although there have also been studies of lakes and estuaries. In the future these areas seem likely to come under greater pressure for food production, water storage or recreation and this will lead to a demand for more detailed studies than hitherto.

Already a great deal of work has been carried out to reclaim old industrial and urban waste areas and this work is likely to grow as the pressures on land increase. Apart from the need to win back the past spoil areas there will be new problems associated with industrial construction and mineral working operations which will require attention. Ways of landscaping or reclaiming disturbed land to minimise any damage will also need to be examined. These studies will in many cases call for support by the chemist when problems of chemical toxicity and deficiency or even of nutrient availability are involved.

New techniques

It is of interest to note that it is often the ease of analysis, or the introduction of a new instrumental technique which determines the attention paid to certain chemical constituents, rather than the actual need. For example, almost all nutrient studies include sodium, which, although usually of limited nutrient significance, is generally determined together with potassium, by flame photometry. Similarly the proliferation of heavy metal research over the last ten years has been possible because of the introduction of atomic absorption techniques which have made the analyses of these elements so much easier. On the other hand some elements, both nutrient and toxic, receive much less attention because they are difficult to determine. The situation with soil extractions and pesticides is much the same. Because the research is built around the easier techniques it may be that some nutrients and pollutants of considerable importance are being overlooked. One of the more important roles of the Institute's chemists over the next few years must be to make an attempt to redress some of these imbalances in ecological research.

Contract Work

Subject to the need to service ITE research the Subdivision of Chemistry and Instrumentation is sometimes prepared to accept contracts for analytical work or other chemical investigations in relation to ecological and environmental problems. Normally contract work is restricted to other government departments, local authorities and universities. Priority is given to requests which are related to current ITE research interests.

If assistance is required it is advisable to write at an early stage of the work to the

Head of the Subdivision of Chemistry and Instrumentation
Merlewood Research Station,
Grange-over-Sands, Cumbria, LA11 6JU.

This requirement applies whether the work is to be carried out in the laboratories at Merlewood or Monks Wood. Early consultation may save time and effort later.

Requests for assistance should be accompanied by brief details of the investigation which has prompted the enquiry, so that the work can be assessed and if appropriate a quotation supplied. Information should also be supplied concerning the type of sample material, sample numbers, consituents to be determined and the urgency of the work.

If the Institute can carry out the work, instructions will be sent covering such matters as sampling procedure, preservation, drying, packing and despatch. A form will also be sent for entering full background information about the samples.

Results are sent out on standardised output sheets. Details of, or reference to, the analytical methods used for the analyses can also be supplied. In addition, it is the practice to send some comments, where applicable, about the interpretation of the data by drawing on the experience and records of the Chemical Sections. It may be possible to obtain related information by referring to the Chemical Data Bank. All results produced by the Chemical Sections are retained in the data bank and may be referred to in conjunction with other data for ecological reference purposes. However, there is no question of supplying a 'customer's' results to another individual without the authority of that 'customer'.

The Subdivision may be approached for advice on any chemical topic in connection with ecological or environmental problems whether or not use is made of the analytical services. Advice can also be provided on analytical techniques. Another service which can be offered by the chemistry department is in analytical training. Although the numbers accepted for training at any one time are strictly limited, it is generally possible to make some suitable arrangements for short or long term training. Further details about these and other services can be obtained by writing to the Head of the Subdivision of Chemistry and Instrumentation.

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