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MINISTRY OF AGRICULTURE FISHERIES AND FOOD

DIRECTORATE OF FISHERIES RESEARCH

AQUATIC ENVIRONMENT MONITORING REPORT

Number 20

First Report of the Marine Pollution Monitoring Management Group's Co-ordinating Group on Monitoring of Sewage-Sludge Disposal Sites

Lowestoft 1989

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First Report of the Marine Pollution Monitoring Management Group's Co-ordinating Group on Monitoring of Sewage-Sludge Disposal Sites.

LOWESTOFT 1989

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Foreword

This, the first report of the Co-ordinating Group on the Monitoring of Sewage-Sludge Disposal Sites (CGMSD), describes the progress made by the Group up to the end of 1988.

The Group was established in 1987 when, following a review of existing monitoring, it had become apparent to the Marine Pollution Monitoring Management Group (MPMMG) that UK effort and resources, applied to scientific investigations and monitoring of the impact of sea disposal of sewage sludge, were unco-ordinated and contained many inconsistencies.

The report addresses these problems directly and contains a set of environmental quality objectives (EQOs) with the appropriate environmental quality standards (EQSs) which have common applicability and represent good technical and environmental practice.

The need to harmonise monitoring methodologies and procedures has been recognised and the report contains details of procedures and protocols which have been finalised to date covering the following measures:

(a) fish disease sampling;
(b) method-determined sampling and analytical procedures;
(c) use of microbiology;
(d) benthic community monitoring; and
(e) monitoring of metals in sediments.

Details are given of the monitoring of sludge disposal sites, carried out during 1988 by the various licensees operating throughout the UK and by the licensing authorities (DAFS/DOE(NI)/MAFF). Future reports will assess this monitoring in relation to the established goals but, because these newly-defined aims were not in existence in 1987, no such review is included in this first report.

D. J. Garrod
Director of Fisheries Research
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and Food

1. Introduction

In 1985, the Marine Pollution Monitoring Management Group (MPMMG) established a sub-group to review the practice of monitoring of sewage-sludge disposal sites. The review concluded that much of the work was unco-ordinated and that some, at least, of the monitoring did not yield results that were useful, particularly in relation to the scale of impact of the various disposal operations. The review highlighted substantial differences in approach to monitoring the different sites and questioned whether these should occur. It was also noted that in many cases the goals of monitoring were not clearly specified and that standards by which to assess whether detectable impacts could be regarded as acceptable were, at best, only vaguely stated. In order to rectify these deficiencies, it was suggested that the MPMMG should establish a Co-ordinating Group on Monitoring of Sewage-Sludge Disposal Sites (CGMSD) and that this Group should prepare, with a view to publication, regular reports on the monitoring undertaken in sewage-sludge disposal areas.

The MPMMG endorsed the findings of the sub-group and, in April 1987, agreed to establish the CGMSD. The Group's terms of reference, as defined by the MPMMG, are as follows:

(i) to continue to evaluate sewage-sludge monitoring programmes, and advise on their development and co-ordination, to make best use of available resources, expertise and techniques - where appropriate, recommendations should be made for the termination of ineffective programmes;

(ii) to identify and report upon those areas where research is necessary in support of monitoring;

(iii) to verify methods, develop standard protocols and intercalibrate analyses used in monitoring programmes;

(iv) to formulate environmental quality standards (EQSs) against which monitoring programmes can be designed and results assessed;

(v) to advise on responses to technical issues arising at the Conventions;

(vi) to encourage the production of regular reports on the progress and results of monitoring by those responsible for the conduct of the programmes;

(vii) to produce an annual review of monitoring carried out at all sites, which will be made widely available.

Discussions with the Water Authorities Association and others as to the membership of the CGMSD were not completed until June and the new group first met on 3rd September 1987. Details of the membership and substituted attendances are provided in Annex 1. Between the establishment of the Group and the end of 1988, a total of six meetings took place. This report outlines the progress made up to the end of 1988, reviews some of the monitoring conducted during 1987, gives a brief summary of the intended monitoring during 1988 and compares practice with the aims and objectives defined by the CGMSD.

2. Tasks undertaken by the CGMSD

From the start, the CGMSD was intended to be a group which advises on policy and where the advice is adopted, demonstrates, through its reports, how the advice is being implemented. As such, it was expected that some of the detailed work of defining procedures for monitoring would have to be undertaken by specialist task teams. This indeed proved to be the case and the role of the CGMSD, in relation to the task teams, has been to define their tasks, supervise and guide their progress and promote the implementation of advice and adoption of harmonised procedures.

Arising from its terms of reference, the aims set for itself by the CGMSD at its first meeting were as follows:

(i) to define environmental quality objectives (EQOs) to be met at sewage-sludge disposal grounds and the development of standards by which the meeting of those objectives can be verified;

(ii) to develop detailed guidelines for monitoring using microbiological determinands, biological effects techniques, sediments, biota and water;

(iii) to produce a report on monitoring conducted in 1987 and 1988.

The guiding principle throughout has been that, wherever possible, common goals should be met at all sites and that procedures used for monitoring for a particular purpose should be harmonised. Since the group did not actually start work until the latter half of 1987, it was recognised that the influence which it could exert on monitoring conducted in 1987, and probably also in 1988, would be minimal. The main objective, therefore, was to achieve as many of the aims as was possible in time for them to be implemented during 1989.

In relation to the harmonisation of objectives and methods, it was realised from the outset that different sludge disposal sites have different characteristics and that these would have to be allowed for in the adopted procedures. Thus, for example, it would be pointless to advocate detailed grab surveys and analysis of sediment particle size structure and meiofauna for an area of hard ground. Nevertheless, it was agreed that for most purposes common objectives could be set, even if the standards by which compliance is judged differ according to local circumstances. It was also agreed that, although harmonisation of procedures was the ultimate aim, standardisation of methods was only likely to be essential where the method used actually determines what is measured. In other cases, the aim of overall harmonisation should be subservient to the need for consistency of data with time, provided that the data were justifiable in relation to the defined objectives.

In order to proceed quickly with the detailed work of defining methods, the CGMSD, at its second meeting, agreed to establish specialist task teams to undertake the following tasks:

(i) defining the methods to be used for measuring determinands where the method influences what is measured;

(ii) describing the role of, and methods to be used for, measuring microbiological determinands;

(iii) describing the methods to be used for assessing biological effects, with emphasis initially on effects on macrobenthos;

(iv) describing the methods to be used for measuring chemical contaminants in the marine environment, with special emphasis initially on metals in sediments.

3. Environmental quality objectives and standards

3.1 General comments

In considering objectives and standards, the CGMSD noted that many sludge disposal grounds are sited in areas that are otherwise relatively uncontaminated. In such areas, an unperturbed fauna might be expected and levels of contamination from anthropogenic sources would be so small that they would be virtually indistinguishable from the general background levels in biota or water or geochemically similar sediments elsewhere in the North-Eastern Atlantic. Other disposal grounds are in areas that are influenced by contamination from a variety of nearby land-based sources and some degree of perturbation may already exist in such areas. However, in either type of situation, the overall aim should not be just avoidance of significant effects upon species of importance to economic or conservation interests, but the preservation of conditions as close to natural conditions as is practicable given other activities in the areas concerned.

The use of objectives geared to the preservation of natural conditions is also likely to prove to be more acceptable than those which place immediate human needs above long-term environmental stability.

Another important concept to be considered is the question of irreversible change. It is generally agreed that any change in the environment which is reversible through natural processes is more acceptable than change which is irreversible. In the context of sludge disposal, this could be taken to mean that local organic enrichment or even some enhancement of trace metal levels is acceptable, whereas measurable accumulation of truly persistent and harmful organic chemicals is unlikely to be viewed favourably.

Taken together, the concepts described above offer a workable strategy for sludge disposal to sea combined with genuine environmental protection. It is generally envisaged that objectives should be achieved within the designated disposal area but minor perturbations associated with specific disposal sites may be permitted. In this context, it is clear that definitions are required of what is meant by measurable and harmful. The CGMSD has addressed these questions and the subsequent sub-sections provide details of the conclusions.

3.2 Environmental quality objectives: definitions

It is upon these premises that the following EQOs were proposed by the CGMSD to the MPMMG, which subsequently endorsed them. In order to maintain comparability with objectives in use for fresh waters and estuaries, the objectives for sludge disposal areas are described in terms of 'use' of the areas. These 'uses' include preservation of natural conditions.

	Use	Objective	Notes
3.2.1	Basic amenity use	Maintenance of environmental quality so as to prevent public nuisance arising from aesthetic problems and interference with other legitimate uses of the sea.	This refers to the presence of persistent surface slicks; aesthetic contamination of the sea bed with plastics and other persistent materials; fouling of fishing equipment.
3.2.2	Commercial harvesting of fish and shellfish for public consumption	Maintenance of environmental quality, such that commercial marine fish and shellfish quality shall be acceptable for human consumption, as determined by the appropriate competent authorities (e.g. MAFF).	This objective relates only to the suitability for human consumption; the general health of the fish and shellfish is protected under use (Sub-section 3.2.3).
3.2.3	Protection of commercial species	Preservation of the general well-being of commercially-exploited species.	Probably little different in practice from use (Sub-section 3.2.4).
3.2.4	General ecosystem conservation	Maintenance of environmental quality so as to protect aquatic life and dependent non-aquatic organisms, such that the ecosystem is typical of coastal water with those physical characteristics and latitude.	Depending on the conditions in the area, it may be necessary to allow for a mixing zone within which the EQO would not apply, but for both the water column and benthic environment this should be kept as small as practicable.
3.2.5	Preservation of the natural environment	Outwith the immediate disposal zone, the quality of the receiving environment will be indistinguishable from that of the adjacent estuarine or marine environment.	This limitation on contamination is in line with the decisions taken at the second North Sea Conference.*

3.3 Environmental quality standards: definitions

The means of demonstrating whether the above uses are maintained in any area is by comparison with standards. In most cases, there are no internationally agreed standards by which compliance can be assessed. Indeed, there are few nationally set standards, except for certain of the heavy metals for which water and food standards have been set.

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* Second International Conference on the Protection of the North Sea, London, 1987. Department of the Environment, London.

Accordingly, the CGMSD has listed the criteria by which maintenance of the defined use or EQO can be assessed, together with an indication of how the basis of the standards could be judged, as follows:

	EQO	Criteria	Basis of standards
3.3.1	Aesthetic - no nuisance (Use - Sub-section 3.2.1)	Turbidity	Increase in suspended solids.
		Floatables	Occurrence in standardised surface trawls.
		Persistent sewage debris	Occurrence in benthic trawls. Visual inspection.
3.3.2	Fish and shellfish	Bacterial contamination Chemical contamination	Measured levels to be below those prescribed by public health authorities/MAFF.
3.3.3	Protection of commercial species (Use - Sub-section 3.2.3)	Water column and benthic environment	No significant effect on habitat. Measured levels of potentially toxic materials to be below levels of effect and within any relevant EQS.
		Fish disease	No significant increase in occurrence compared with normal limits in control populations.
3.3.4	Ecosystem - maintenance (Use - Sub-section 3.2.4)	Benthic diversity	Deviation from the control site(s) to be within normal limits.
		Water quality	Dissolved oxygen to exceed, and toxic substances in the water column to be below, levels of effect, and within any EQS set by relevant legislation.
		Sediment quality	Grain size, carbon/nitrogen and toxic substances to be below levels of effect, and within any EQS set by relevant legislation.
3.3.5	Preservation of the environment (Use - Sub-section 3.2.5)	Sediment quality	Minimal percentage change over background levels of metals and other contaminants. No

		continuing upward trends after 'steady state' is achieved.
Outwith zone of immediate effect	Water quality	Must be within any EQS set by relevant legislation.
	Benthic fauna	No deviation from control sites.

It should be noted that the column headed 'basis of standards' is literally that; for example, the extent to which an increase in turbidity is detectable, involves practical and judgemental matters. The details of the way in which it is suggested that these are to be taken into account are described in the subsequent sub-sections.

Progress has been made in defining what the standards should be, or at least how compliance with them can be demonstrated in qualitative terms, for a number of the EQOs specified above. Details are as follows:

(a) Turbidity It is recommended that there be no more than a 5 mg l^{-1} increase in suspended solids at 1 m depth in the receiving water 1 h after disposal. It should be noted that this limit refers to the water into which the sludge was disposed and which may have moved away from the disposal site due to tidal- or wind-driven currents.

(b) Floatables and sewage debris These are to be understood to mean large detrital material of sewage origin, including condoms and tampons. The CGMSD considers that the only acceptable standard is that these should not be found to occur in the area of disposal, either in surface trawls or in bottom trawl, dredge or grab samples. If significant evidence of such solids is found in the area of disposal, screening of the inflow effluent or the outflow sludge will have to be undertaken. Since it is conceivable that not all of the sewage-derived solids are of dumped sludge origin, subsequent compliance with the standard should then be checked, primarily by monitoring sludge quality at the point of loading, to show no retention of solids on a 5 mm sieve.

(c) Fish and shellfish for public consumption National standards already exist for bacterial quality of shellfish, though these may have to be adjusted in the future if the expected EC Directive on shellfish quality is adopted. National standards or guidelines also exist for mercury, lead, copper and zinc. For PCBs, dieldrin, DDT, etc, acceptable daily intakes (ADIs) or provisional tolerable weekly intakes (PTWIs) have been recommended by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) and would be used as guideline values in a UK food consumption context.

(d) Protection of commercial species EQSs for a range of substances have been set in terms of concentrations in the water column. The Biological Effects task team has been charged with assessing whether additional standards can be defined.

In relation to fish diseases, it is recommended that detailed assessments of the position be carried out by the licensing authorities using the procedures laid down by the International Council for the Exploration of the Sea (ICES).

Where sufficient concern exists, over the possible association between one occurrence of fish disease which is higher than normal and an area used for sewage-sludge disposal, detailed studies will be required. In other circumstances a simplified version of the ICES guidelines will suffice. Details of the simplified procedures can be found in Annex 2 to this report.

(e) Ecosystem maintenance and preservation of the environment At present, no standards exist other than the nationally established EQSs for water column concentrations of certain metals and Black-list substances. The Biological Effects and Metals task teams were therefore charged with advising on how to judge compliance with qualitative standards based on no detectable change in the area in question. Details of progress thus far are outlined in Sections 4, 5, 6 and 7.

4. Method-determined parameters

For many determinands, the actual method of analysis which is used, in principle, should have little influence on the result obtained, provided that the method and the operator are capable of achieving adequate accuracy and precision. However, the method of sampling, and in a few instances the method of analysis, may be important. Particular examples likely to be encountered in monitoring of sewage-sludge disposal sites are particle size analysis, total organic carbon and total nitrogen analysis and measurement of redox potential.

Specialists from the MAFF and DAFS laboratories were charged with producing agreed guidelines for the procedures to be used for these determinands. They were asked, also, to produce guidelines for sampling that would be compatible with the procedures to be used for assessing benthos and contaminants in sediments and to consider whether standards for degree of change could be defined for any of the three method-determined parameters in question. Full details of their advice are reproduced in Annex 3.

No standards are considered as appropriate for Eh, nor are they considered to be necessary for particle size. Since all but one of the areas used for sludge disposal are considered as dispersive, the limited accumulation is very unlikely to affect particle size distribution. For organic nitrogen and carbon, a standard to protect marine biota is not considered to be appropriate but, in principle, it is considered that neither the organic carbon nor nitrogen content of sediments should increase significantly. Definition of what is to be regarded as significant will, however, require further work by the licensing authorities.

5. Microbiological determinands

Microbiological determinands can be used as tracers of sewage-sludge deposition. A variety of grounds are monitored in this way, but no common approach has been used, either in relation to methods or interpretation of the data. A specialist task team was appointed to advise on both of these aspects of the use of microbiology. The task team completed its work in mid-1988 and produced a detailed and extensive report. Copies of the full report can be obtained from the licensing authorities in England and

Wales, Scotland or Northern Ireland* but a summary of that report is included at Annex 4. This summary provides all of the necessary basic information on the procedures to be used for sampling, determination and interpretation of the results. Standards are not considered to be appropriate, since the basic use of the methods is to indicate where sewage-sludge deposition occurs, with a view to delineation of the likely area of impact on the defined uses of the area.

6. Biological effects

In order to produce advice on the procedures to be used in monitoring biological effects, a specialist task team was appointed. Recognising that the biological effect for which monitoring is most commonly undertaken is change in the macrobenthic fauna, the task team was initially requested to consider this aspect. The need was considered to be particularly urgent, because the original MPMMG sub-group had highlighted benthic studies as an issue receiving considerable attention but seriously lacking in common approaches and adequate interpretation of the data.

The task team completed its assessment of how to harmonise approaches to monitoring for changes in macrobenthos late in 1988 and its full report is being made available, as a separate publication, by the DAFS Marine Laboratory, Aberdeen. A summary of the main questions which were addressed, including practical descriptions of how to assess the results in relation to the extent of change induced by sewage-sludge disposal, can be found at Annex 5 to this report.

The task team has already paid some attention to other biological effects that might usefully be used in a monitoring context. The group has been given a revised membership in order to allow it to address this subject more fully; it is expected to produce a report during 1989.

7. Chemical determinands

Most sewage-sludge grounds are monitored to check the extent of chemical contamination in the area which is likely to be affected. There has, until now, been no concerted attempt either to check the quality of data obtained by different groups or to harmonise the approaches used in data collection and interpretation.

In order to remedy these deficiencies in relation to metals in sediments, the CGMSD established a task team to review present procedures, to arrange for a check on the intercomparability of produced data, and to advise accordingly. The task team concerned has made good progress in the organisation of an intercomparison exercise for metals in sediments and has produced advice on the methods of pre-treatment and analysis of sediment samples for contaminant metals. That advice has been endorsed by the CGMSD and is reproduced as Annex 6 to this report. The CGMSD has accepted the opinion of the task team that, at the present time, it is not possible to define standards for the protection of marine organisms in terms of concentrations of metals in sediments. The task team has, therefore, been asked to advise on qualitative standards expressed in terms of detectable increase in concentration in the sediments in question. Such standards

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* Details can be obtained from the compiler of this report.

would have to take account of sample and analytical variability, both of which involve complex issues that are unlikely to be resolved until the intercomparison exercise has been completed.

The need for similar work on organic contaminants in sediments has been recognised and, late in 1988, steps were taken to establish a specialist task team to review the issues which are involved and to advise accordingly. It is hoped that it will be possible to include a report on progress in the second report of the CGMSD. The Biological and Chemical task teams will be asked to consider both the need for, and feasibility of, monitoring chemical contaminants in water and biota once they have completed their tasks in relation to sediments.

8. Review of monitoring of sewage-sludge disposal sites as conducted in 1987

Sewage sludge is dumped at sea in twelve areas around the UK. All sites are monitored regularly. The CGMSD was established before plans were finalised for privatisation of the water industry in England and Wales. The intention, at that stage, was to review monitoring conducted in 1987 with a view to assessing the extent to which it would have allowed compliance with EQOs to be demonstrated.

Data were sought from all of the organisations involved in monitoring sewage-sludge disposal sites. These were assessed, in a preliminary manner, with a view to publication of a summary of the conclusions in this report. The basic conclusions of that review confirm the findings of the earlier MPMMG sub-group, in that it is clear that in 1987 considerable disparity existed, both in the programmes conducted and the methods used, as well as in the extent to which the results were interpreted, or could have been interpreted, against common goals.

That being said, so far as conclusions could be drawn from the preliminary review, it was apparent that there was no real evidence that the disposal of sewage sludge has an impact on the marine environment at any site except that in the Clyde estuary. This site is the least dispersive of the sites used for sewage-sludge disposal and, even here, the scale of impact is limited.

The preliminary review of the 1987 programmes, data and findings were considered at the seventh meeting of the CGMSD early in March 1989. At that meeting, it was established that, due to privatisation plans, all references to Water Authorities' data would have to be cleared by a Water Authorities Association verification committee.

It was clear that this would substantially delay publication of this first report of the CGMSD. Since the main aim of publication of this report is to make readily available details of the agreed EQOs and associated EQSs and the details of proposed methods of monitoring, it was decided to abandon the production of a review of 1987 monitoring data and results.

Since the original purpose had been a review of results and interpretation, full details of the programmes conducted have not been provided by all contributors, it has not, therefore, proved possible to provide, in an equable manner, even this limited level of information on 1987 monitoring programmes without delaying the publication of this report.

9. Sewage-sludge disposal ground monitoring activities in 1988

9.1 Introduction

During 1988, monitoring surveys were carried out at the following disposal grounds: Garroch Head, Bell Rock, St Abbs, Tyne, Humber, Thames Estuary (Barrow and South Falls), Nab, Plymouth, Bristol Channel and Liverpool Bay.

A short summary of the various surveys is given in the following sub-sections. The surveys were planned, and in most cases executed, before the various task team protocols were available. Thus, in most cases, established methodologies were used, rather than those now recommended by the CGMSD. In future surveys, there will be a general move towards harmonisation based on the recommendations of the task teams.

9.2 Northumbrian Water survey of the Tyne sewage-sludge disposal ground, summer 1988

(a) Monitoring was conducted for sediment bacteria and metals at sites shown in Figure 1.

(b) Benthos sampling was carried out at 40 stations as shown in Figure 1.

(c) Beam trawl hauls were made at 6 stations as shown in Figure 1. Six surveys are conducted throughout the year at these sites.

(d) Underwater videos were made at sites shown in Figure 1.

9.3 MAFF survey of the Tyne sewage-sludge disposal ground, May 1988

(a) Monitoring was conducted for faecal bacteria, sediment metals, carbon and nitrogen at sites shown in Figure 2.

E. coli and faecal streptococci were assessed in the surface layer of sediment (*ca* 2 mm).

Concentrations of Hg, Cd, Cu, Cr, Ni, Pb, Zn, C and N were determined in the $< 63\ \mu m$ fraction of the surface (0-1 cm) sediments which were collected with a Day grab.

(b) Beam-trawl samples of epibenthos and sludge artefacts were taken at sites shown in Figure 2.

(c) Underwater videos were made in the vicinity of the disposal ground.

9.4 MAFF survey of the Humber sewage-sludge disposal ground, May 1988

(a) Monitoring was conducted for sediment metals, carbon and nitrogen at sites shown in Figure 3.

Concentrations of Hg, Cd, Cu, Cr, Ni, Pb, Zn, C and N were determined in the $< 63\ \mu m$ fraction of the surface (0-1 cm) sediments. Samples were collected with a Shipek grab.

(b) *Modiolus* was collected for metal analysis, using an anchor dredge at sites shown in Figure 4 (see Rees and Nicholson, 1989*).

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* REES, H. L. and NICHOLSON, M. D., 1989. Trends in lead levels in the horse-mussel from the western North Sea. Mar. Pollut. Bull., 20: 86-89.

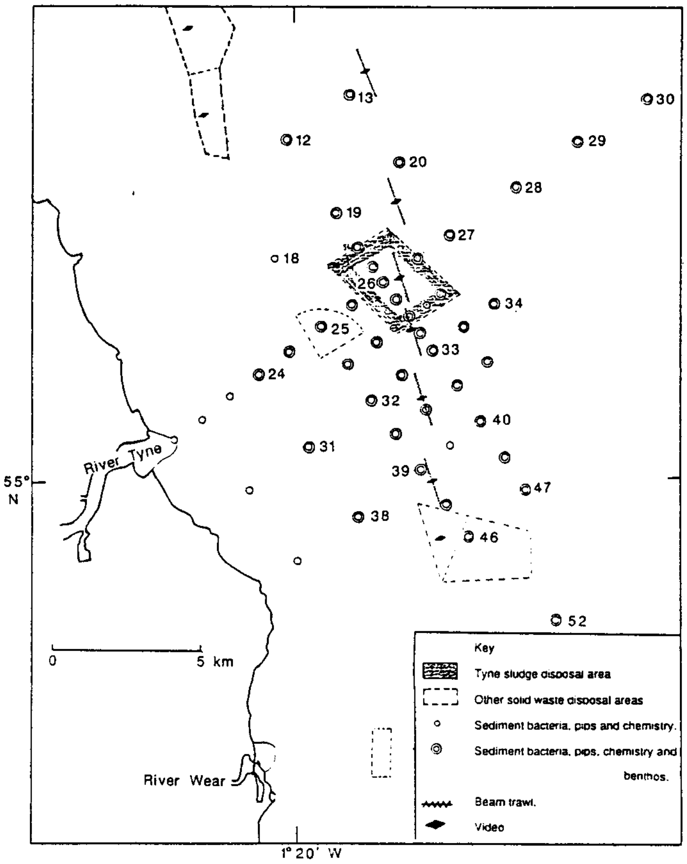

Figure 1 Northumbrian Water survey of the Tyne sewage-sludge disposal ground, summer 1988. (Numbered sites are those previously sampled for benthos (pre-1980) and sediment chemistry.

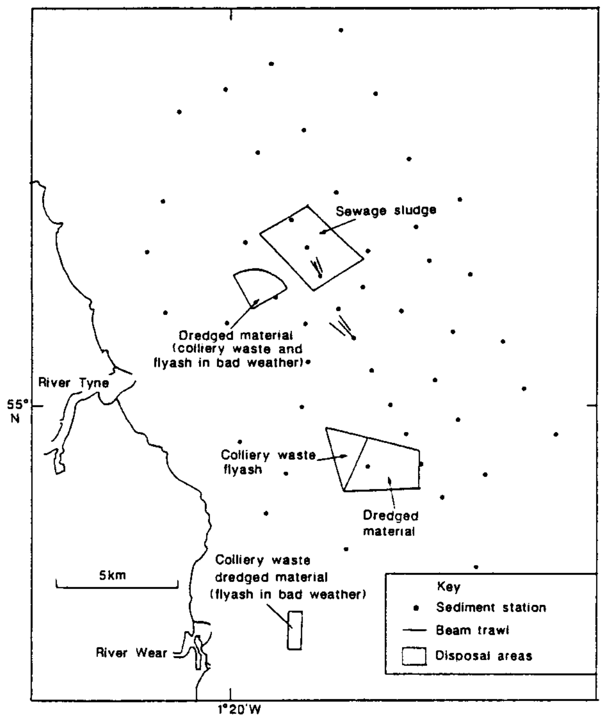

Figure 2 MAFF survey of the Tyne sewage-sludge disposal ground, May 1988.

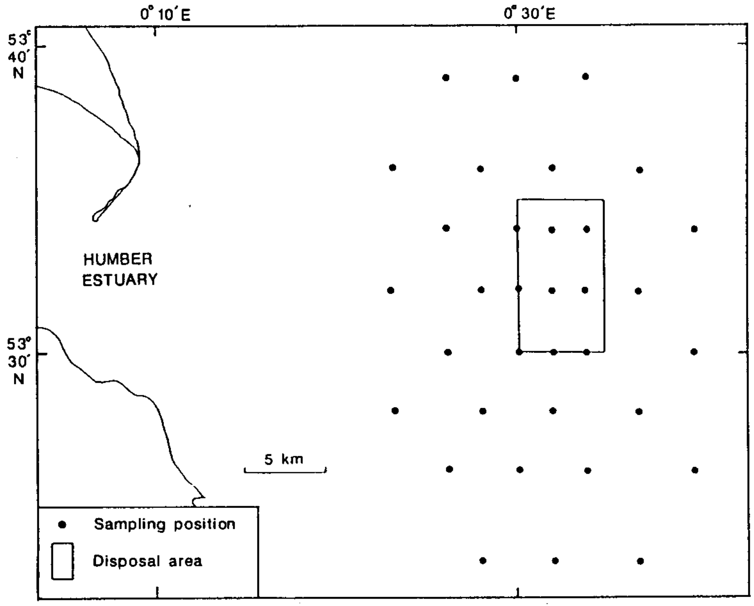

Figure 3 MAFF survey of the Humber sewage-sludge disposal ground, May 1988.

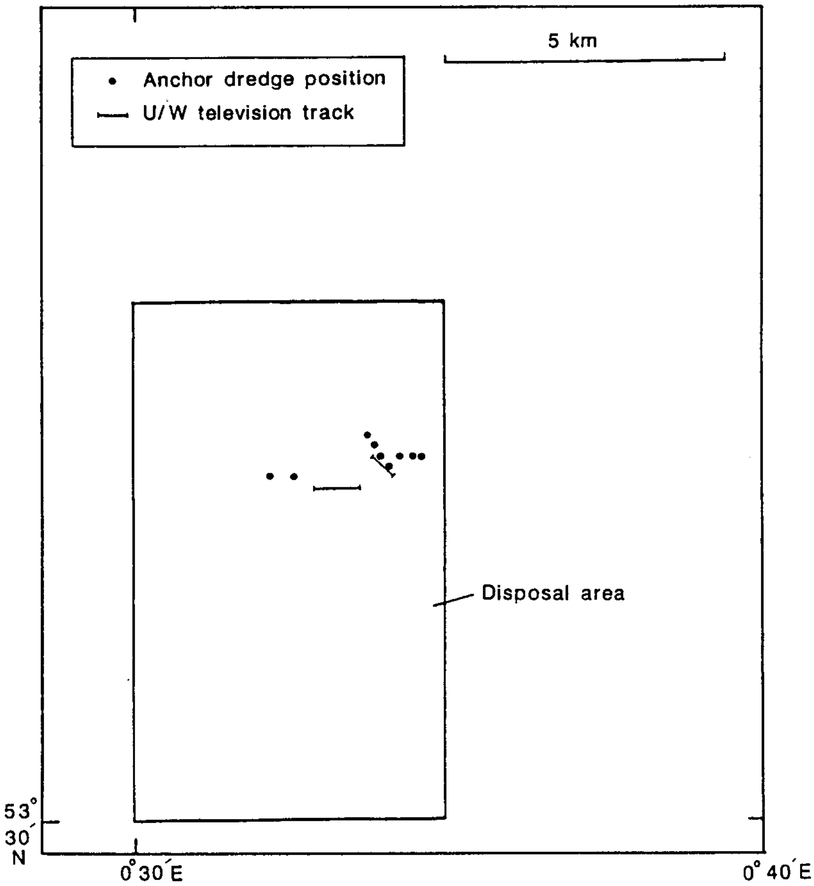

Figure 4 MAFF anchor dredge and video surveys of the Humber sewage-sludge disposal ground, May 1988.

(c) An underwater video camera was deployed in the area of the disposal ground (Figure 4).

9.5 Thames Water/Water Research Centre survey of the Thames (Barrow) sewage-sludge disposal ground, 1988

(a) Seven sites around the East Barrow Sand were sampled for macrobenthos, particle size and organic carbon.

(b) Thirty-six sediment sites around the sludge disposal ground were sampled for organic carbon, metals, coprostanol, faecal bacteria and particle size distribution.

(c) Mussel larvae bioassays were carried out with samples being collected in the wake of discharging vessels.

(d) Fish disease survey work was carried out.

9.6 MAFF survey of the South Falls sewage-sludge disposal ground, December 1988

(a) Monitoring was conducted for sediment metals, carbon and nitrogen at sites shown in Figure 5.

Concentrations of Hg, Cd, Cu, Cr, Ni, Pb, Zn, C and N were determined in the < 63 µm fraction of the surface (0-1 cm) sediments. Samples were collected using a Day grab.

(b) Macrobenthos was sampled at sites shown in Figure 5.

Infauna were retained on a 1 mm seive for identification and enumeration.

9.7 MAFF survey of the Plymouth sewage-sludge disposal ground, December 1988

(a) Monitoring was conducted for faecal bacteria, sediment metals, carbon and nitrogen at sites shown in Figure 6.

E. coli and faecal streptococci were assessed in the surface layer of sediment (*ca* 2 mm).

Concentrations of Hg, Cd, Cu, Cr, Ni, Pb, Zn, C and N were determined in the < 63 µm fraction of the surface (0-1 cm) sediments which were collected using a Day grab.

(b) Macrobenthos was sampled at sites shown in Figure 6.

(c) Beam-trawl samples of epibenthos were taken at sites shown in Figure 6.

9.8 Welsh Water/Wessex Water survey of the Bristol Channel sewage-sludge disposal ground, 1988

(a) Chemical and bacterial quality of sediments collected at sites shown in Figure 7 were assessed.

Clostridia, faecal streptococci and viruses were evaluated in the surface sediments.

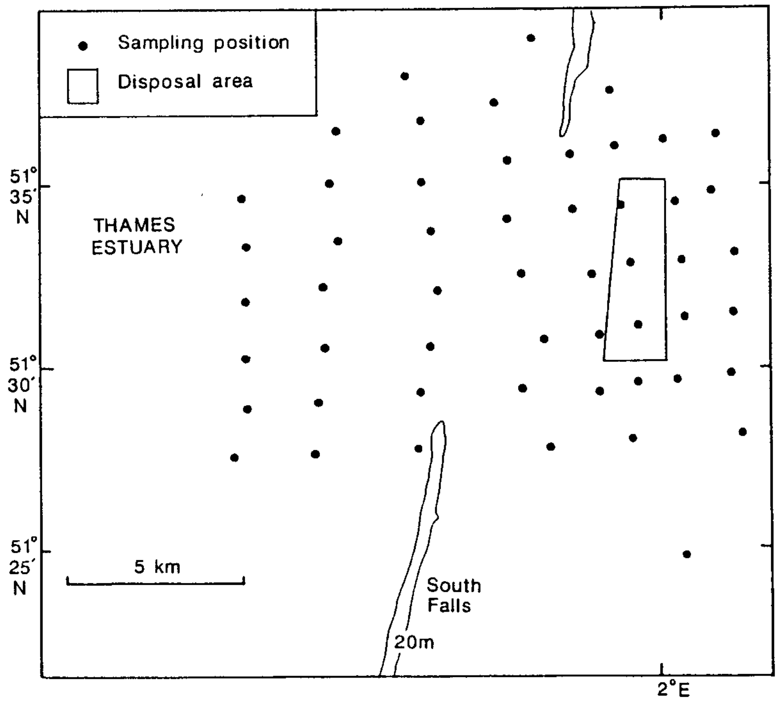

Figure 5 MAFF survey of the South Falls sewage-sludge disposal ground, December 1988.

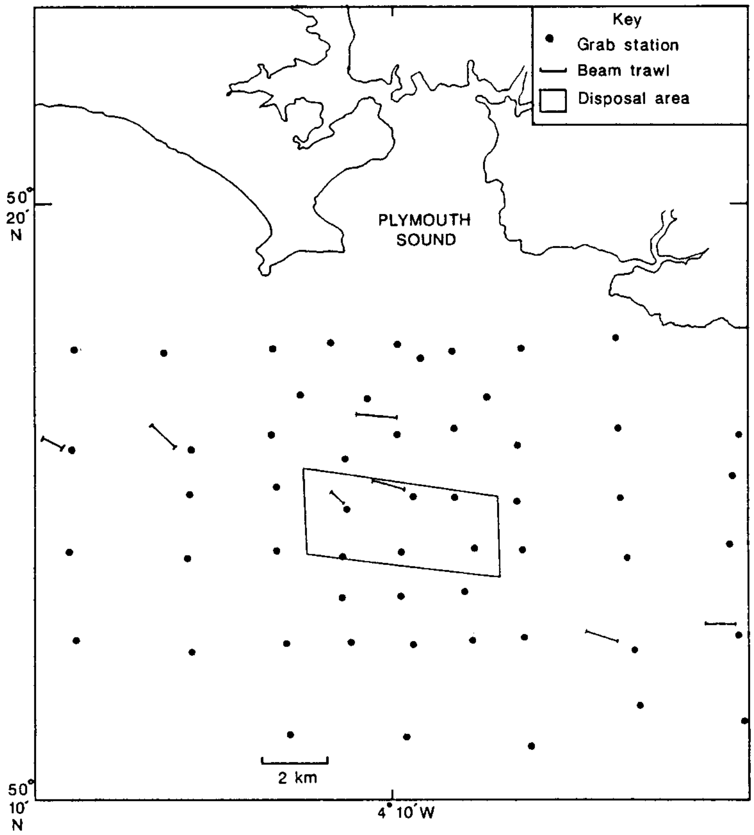

Figure 6 MAFF survey of the Plymouth sewage-sludge disposal ground, December 1988.

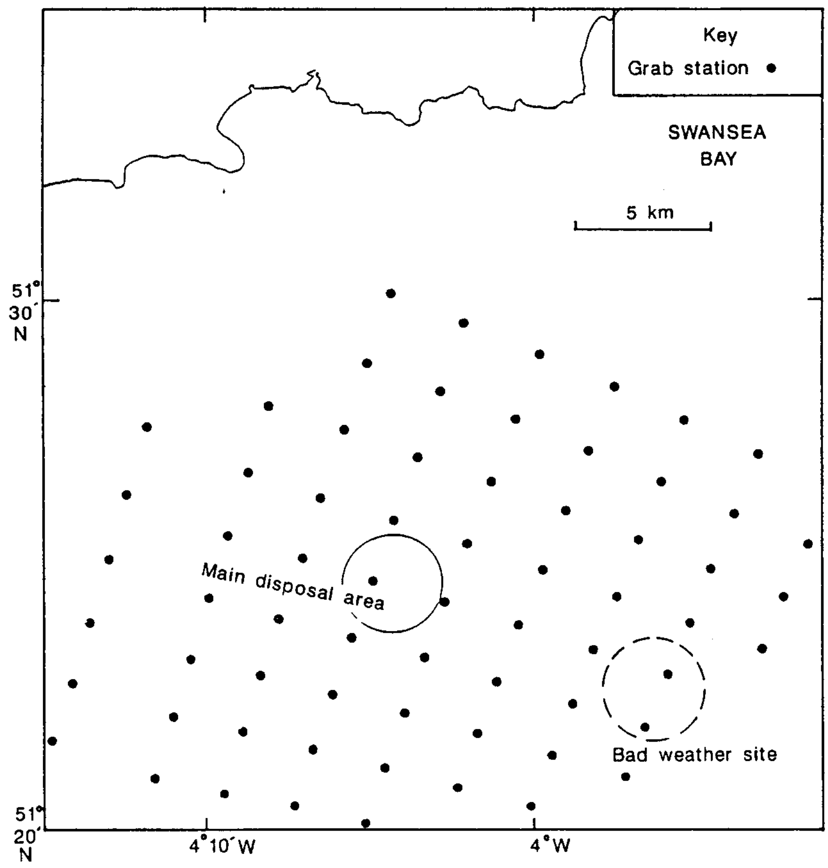

Figure 7 Welsh Water/Wessex Water survey of the Bristol Channel sewage-sludge disposal ground, 1988.

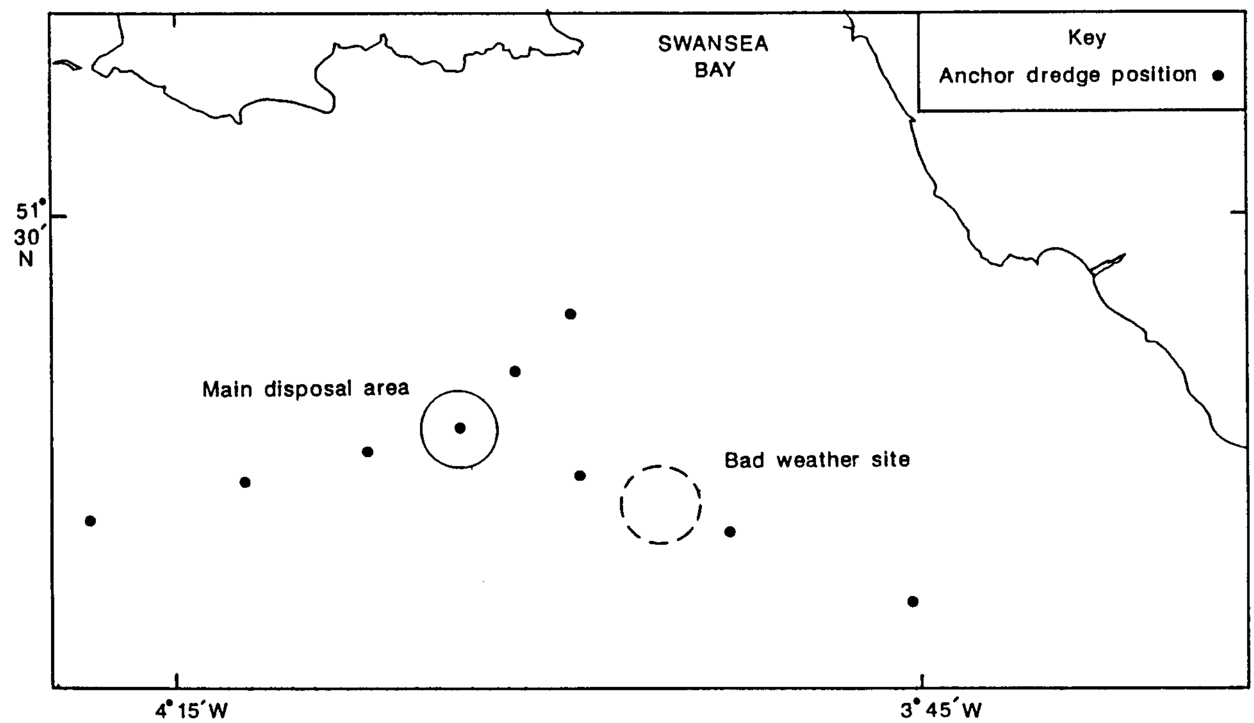

Figure 8 Welsh Water/Wessex Water anchor dredge survey of the Bristol Channel sewage-sludge disposal ground, 1988.

Concentrations of Hg, Zn, Pb, Cd, Ni, Cr, C and N were determined in the < 63 μm fraction of the surface (0-1 cm) sediments.

(b) Benthos was sampled at sites shown in Figure 8.

Infauna and epifauna were identified to species level in anchor dredge samples. Also, CHN analysis and particle size determinations were made on these samples.

(c) An underwater video survey was made of the disposal ground.

(d) Fish populations were surveyed.

9.9 MAFF survey of the Liverpool Bay sewage-sludge disposal ground, September 1988

(a) Monitoring was conducted for metals, carbon and nitrogen at sites shown in Figure 9.

Concentrations of Hg, Cd, Cu, Cr, Ni, Pb, Zn, C and N were determined in the < 63 μm and <90 μm fractions of the surface (0-1 cm) sediments.

(b) An underwater TV camera was deployed at the disposal ground.

9.10 North West Water survey of the Liverpool Bay sewage-sludge disposal ground, September 1988

(a) Monitoring was conducted for sediment metals at sites shown in Figure 10.

Concentrations of Hg, Cd, Cu, Zn, Pb, Cr and Ni were determined in the < 90 μm and 90-500 μm fractions of the surface (0-1 cm) sediments.

(b) Macrobenthos was sampled at sites shown in Figure 10.

Duplicate samples were retained on a 1 mm sieve for macrobenthos identification and enumeration.

(c) Water quality samples were taken at sites shown in Figure 11.

January-April: monthly samples were taken at sites 3, 4, 6, 8, 10, 12 and 14.

May-December: monthly samples were taken at stations 3, 4, 6, 8 and 9.

Surface water samples were analysed for salinity, temperature, dissolved oxygen, suspended solids, nitrate, nitrite, ammonia, phosphate, silicate, chlorophyll and phaeopigments.

An additional bottom sample, from station 10, was collected and analysed for the above determinands during June-October.

(d) Fish were monitored at sites shown in Figure 12.

Hg was determined in muscle from samples of about 30 plaice and whiting.

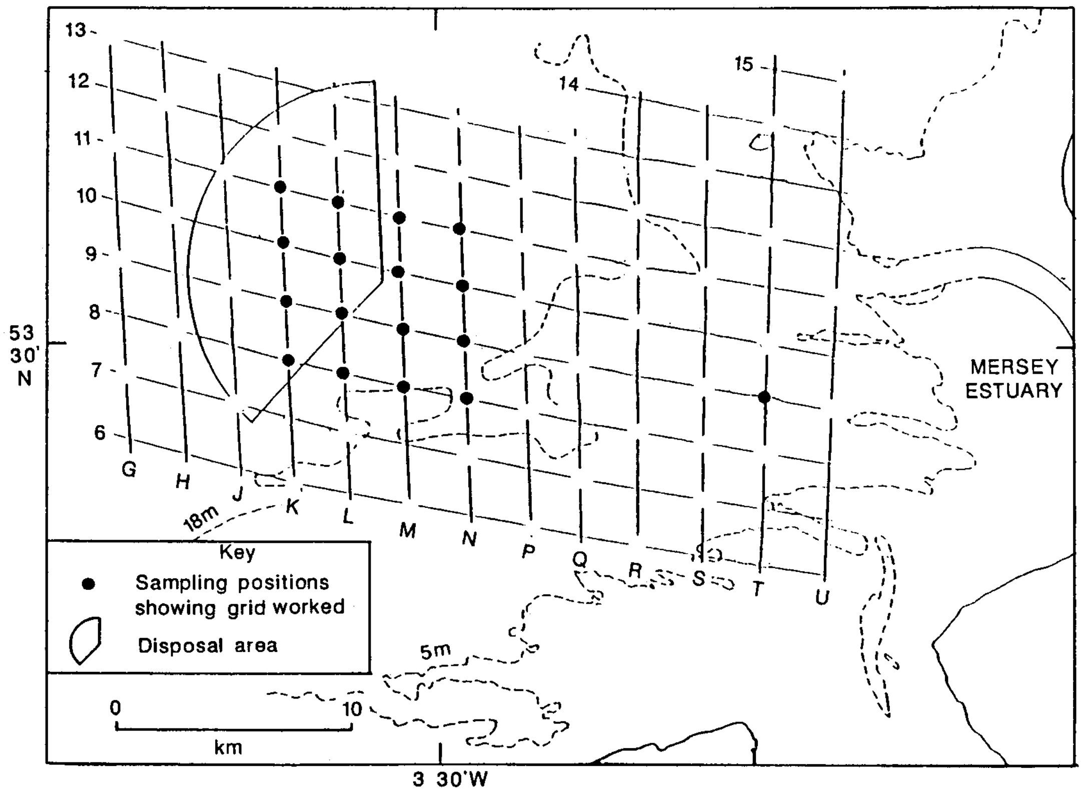

Figure 9 MAFF survey of the Liverpool Bay sewage-sludge disposal ground, September 1988.

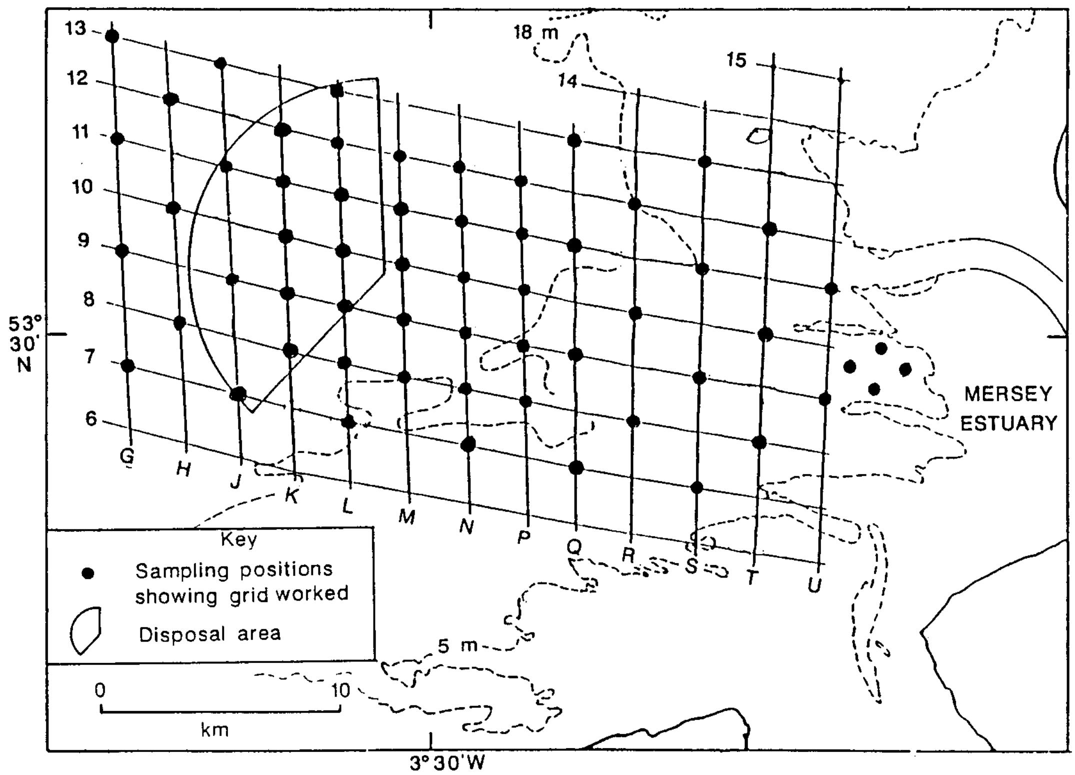

Figure 10 North West Water survey of the Liverpool Bay sewage-sludge disposal ground, September 1988.

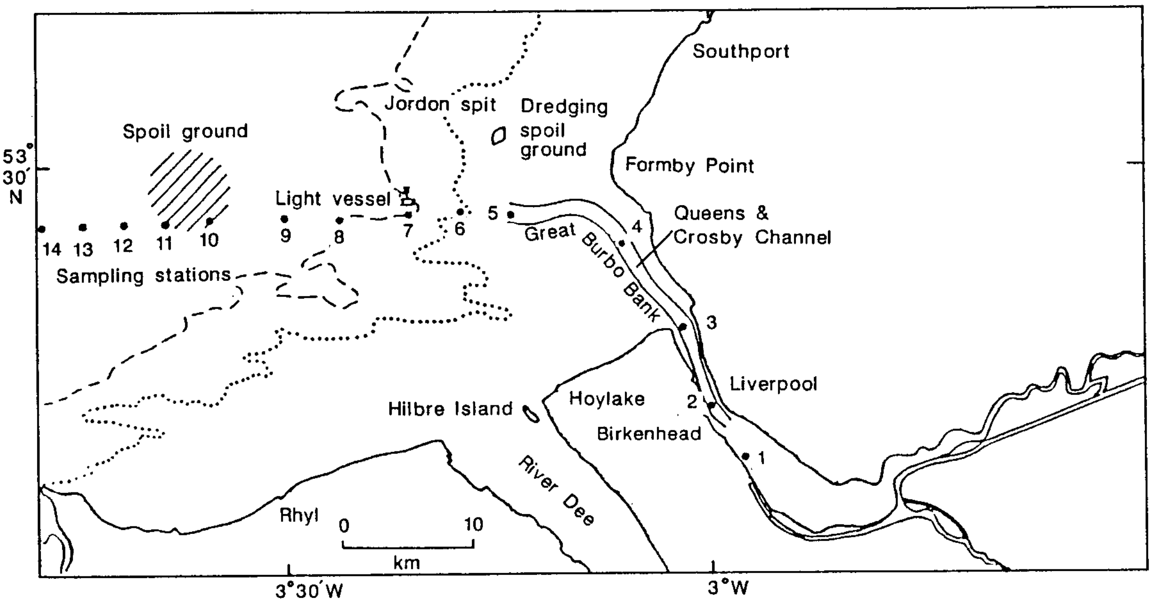

Figure 11 North West Water survey of water quality in Liverpool Bay, September 1988.

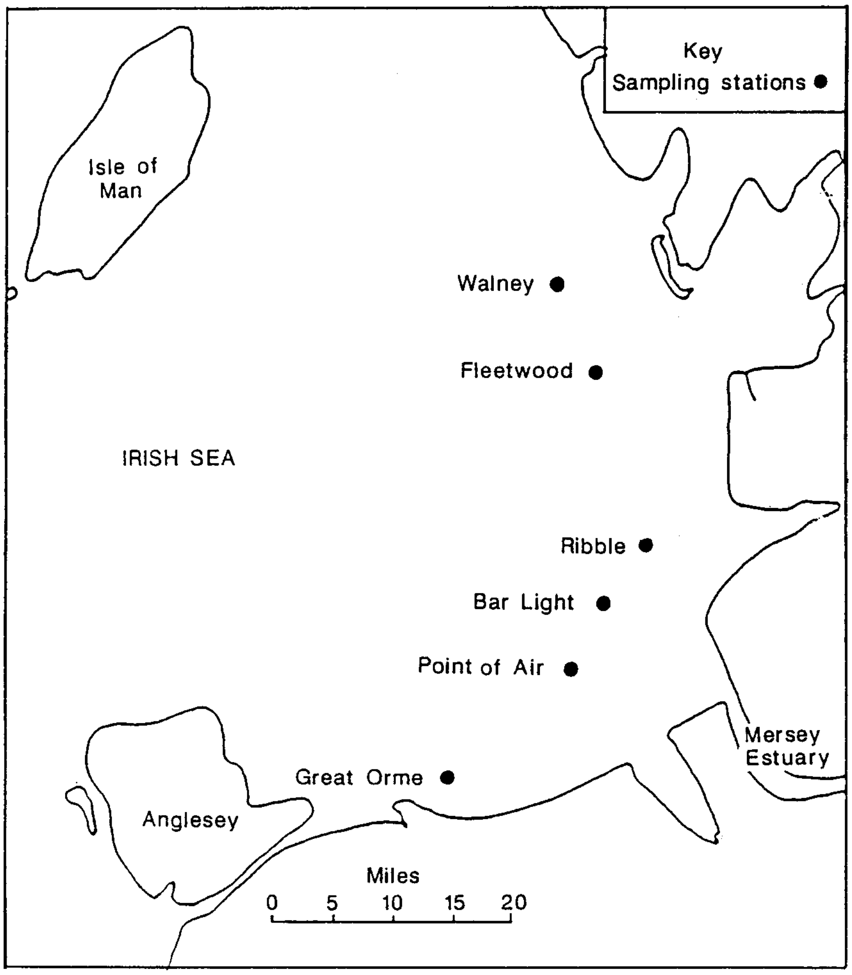

Figure 12 North West Water survey of fish quality in Liverpool Bay, September 1988.

9.11 Forth River Purification Board/Lothian Regional Council survey of the Bell Rock sewage-sludge disposal ground, 1988

(a) One grab sample was taken from each of stations 1, 3, 9, 11, 13, 15, 17, 23 and 25, (Figure 13) and examined for physical appearance, particle size, carbon, nitrogen, heavy metals and organochlorines.

One grab sample was taken from each of stations C (control), 4, 5, 6, 7, 8, 12, 14, 18, 19, 20, 21, 22, 26, 27, 28 and 29 and examined for physical appearance, carbon, nitrogen and heavy metals and, in the case of the sample from station C, for organochlorines.

(b) Two Agassiz trawls and 1 otter trawl sample were taken at each of stations C and 13 (Figure 13). Adult fish in the catch were examined for lesions, histology and microbiology. A full species list was prepared.

Two grab samples were taken at each of stations 1, 3, 9, 11, 13, 15, 17, 23 and 25 (Figure 13) and examined for macrobenthos and fruit pips.

9.12 Forth River Purification Board/Lothian Regional Council survey of the St Abbs sewage-sludge disposal ground, 1988

(a) One grab sample was taken from each of the following stations C, 1, 3, 9, 11, 13, 15, 17, 23, 25, 27 and 29 (Figure 14) and analysed for physical appearance, particle size, carbon, nitrogen and heavy metals (including the < 63 μm element of the sample) and organochlorines.

One grab sample was taken from each of stations 4, 5, 6, 7, 8, 14, 18, 19, 20, 21, 22, 26 and 28 (Figure 14) and analysed as above, but excluding organochlorines.

(b) Two Agassiz trawls and 1 otter trawl samples were taken at station 13 and C (Figure 14). Adult fish in the catch were examined for lesions, histopathology and microbiology. A full species list was prepared.

Two grab samples were taken at each of stations 1, 3, 9, 11, 13, 15, 17, 23, 25, 27 and 29 (Figure 14) and examined for macrobenthos and fruit pips.

9.13 Scottish Marine Biological Association/Strathclyde Regional Council survey of Garroch Head sewage-sludge disposal ground, 1988

(a) Concentrations of Hg, Cd, Cu, Cr, Ni, Pb, Zn, As, Co, Mn, carbon, nitrogen, Eh, pH and organochlorines were determined in sediments from 23 sites (Figure 15).

(b) Oxygen content, salinity and temperature of the water immediately above the sediment surface were analysed at 19 sites (Figure 15).

(c) Macrobenthos was sampled at selected sites (Figure 15).

(d) Epifaunal populations were surveyed at the dumpsite (including demersal fish species) (Figure 15).

(e) Histopathological and microbiological investigations of fish species were carried out (Figure 15).

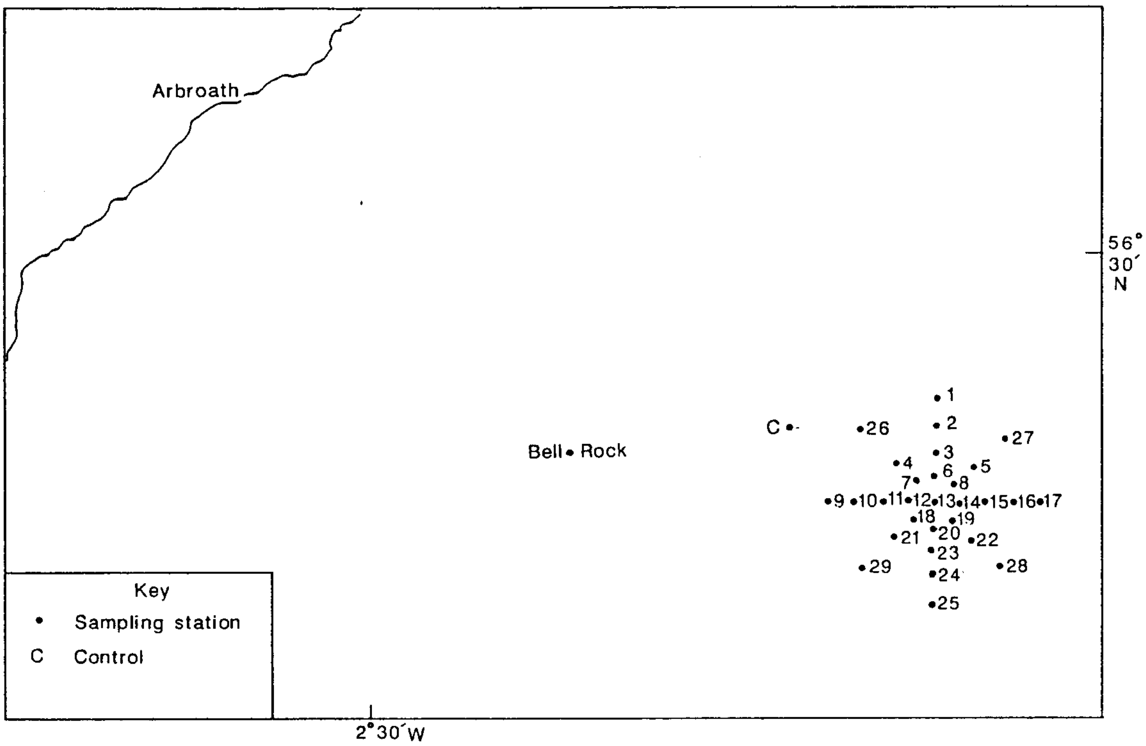

Figure 13 Forth River Purification Board/Lothian Regional Council survey of the Bell Rock sewage-sludge disposal ground, 1988.

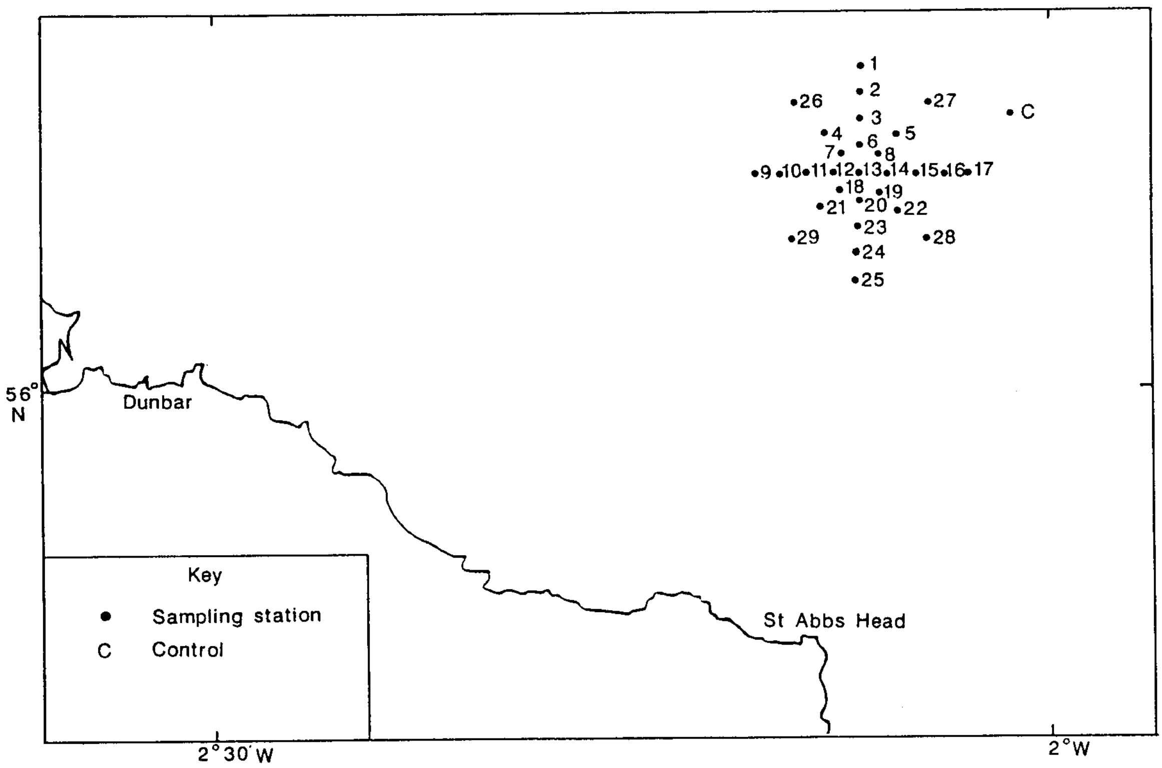

Figure 14 Forth River Purification Board/Lothian Regional Council survey of the St Abbs sewage-sludge disposal ground, 1988.

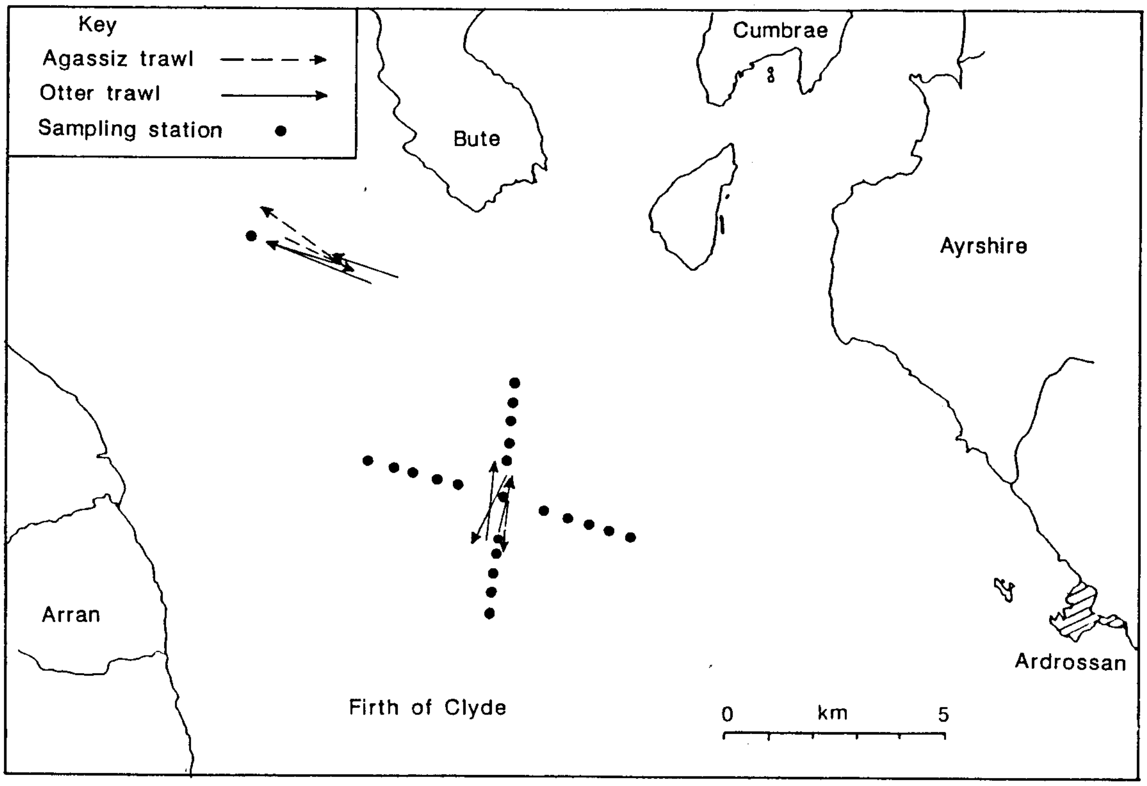

Figure 15 Scottish Marine Biological Association/Strathclyde Regional Council survey of Garroch Head sewage-sludge disposal ground, 1988.

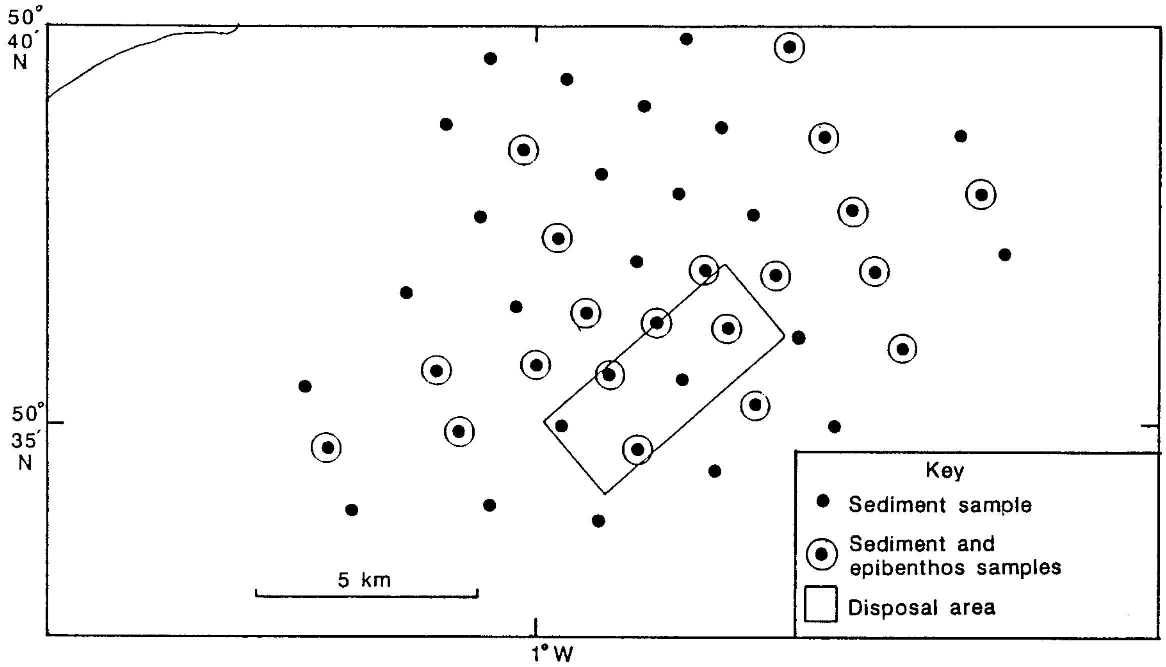

Figure 16 Southern Water survey of the Nab sewage-sludge disposal ground, 1988.

9.14 Southern Water survey of the Nab sewage-sludge disposal ground, 1988

(a) Monitoring was conducted for faecal bacteria and metals, carbon and nitrogen in surface sediments at 44 stations, around the Nab disposal site (see Figure 16).

(b) Epibenthos were sampled at 12 sites (see Figure 16).

9.15 Department of Agriculture and Fisheries for Scotland survey of the Garroch Head sewage-sludge disposal site, 1988

(a) Water samples were collected for investigation of the partitioning of heavy metals, particularly mercury, in soluble and particulate phases of the water column following sewage-sludge disposal activity.

(b) Settlement traps were deployed in the immediate vicinity of the sewage-sludge disposal ground. Following completion of daily sewage-sludge disposal activity, the traps were recovered and samples of the particulate material collected were analysed for heavy metals, total organic carbon and total nitrogen.

(c) Transmissometer studies were undertaken, in conjunction with acoustic profiling studies, to investigate the patterns of dispersion and settlement of sewage-sludge solids.

(d) Sediment samples were collected from a total of 29 sampling locations within the sewage-sludge disposal ground, the original, disused, sewage-sludge disposal ground and a control sampling area, to determine the levels of heavy metals, pesticide residues, total organic carbon, total nitrogen and chlorophyll, and to investigate the levels of faecal coliforms in the sediments.

(e) Sediment samples were collected from 14 of the 29 sampling locations within the sewage-sludge disposal ground, the original, disused, sewage-sludge disposal ground and a control sampling area, to investigate the macrobenthic infauna, particle size distribution and redox potential.

(f) Underwater television studies were undertaken at 14 locations within the sewage-sludge disposal ground, the original, disused, sewage-sludge disposal ground and a control sampling area.

(g) Commercially-exploited fish species were collected from the sewage-sludge disposal ground, the original, disused, sewage-sludge disposal ground and a control sampling area, to determine the levels of heavy metals in the flesh and livers.

(h) The levels of prevalence of fish diseases were investigated at the sewage-sludge disposal ground, the original, disused, sewage-sludge disposal ground and a control sampling area.

(i) The levels of faecal coliforms in the guts of commercially-exploited fish species were investigated at the sewage-sludge disposal ground, the original, disused, sewage-sludge disposal ground and a control sampling area.

9.16 Department of Agriculture and Fisheries for Scotland surveys of the Bell Rock and St Abbs Head sewage-sludge disposal sites, 1988

(a) Sediment samples were collected from 26 sampling locations within both the Bell Rock and St Abbs Head sewage-sludge disposal sites, and from three locations within both the Bell Rock and St Abbs Head control sampling areas, to determine the levels of heavy metals, pesticide residues, total organic carbon and total nitrogen.

(b) Transmissometer studies were undertaken, in conjunction with acoustic profiling studies and water sampling, to investigate the patterns of dispersion and settlement of sewage-sludge solids at the St Abbs Head sewage-sludge disposal ground.

(c) Underwater television studies were undertaken at five locations within both the Bell Rock and St Abbs Head sewage-sludge disposal sites. The locations investigated were those not visited during the 1987 survey.

(d) Commercially-exploited fish species were collected from the Bell Rock and St Abbs Head sewage-sludge disposal sites and the Bell Rock and St Abbs Head control sampling areas, to determine the levels of heavy metals in the flesh and livers.

(e) The levels of prevalence of fish diseases were investigated at the Bell Rock and St Abbs Head sewage-sludge disposal sites and the Bell Rock and St Abbs Head control sampling areas.

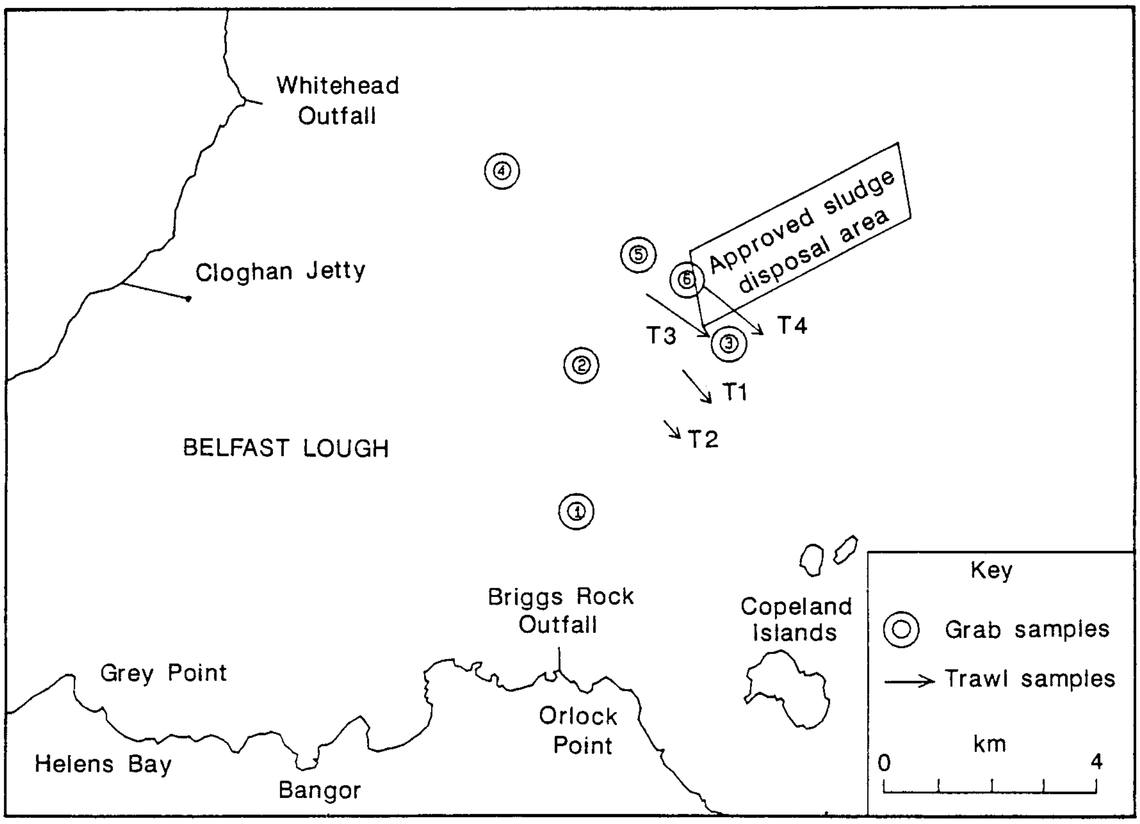

Figure 17 Department of the Environment (Northern Ireland) survey of the North Channel sewage-sludge disposal site, autumn 1988.

(f) Immuno-competence indicators were investigated in commercially-exploited shellfish at the Bell Rock and St Abbs Head sewage-sludge disposal sites and the Bell Rock and St Abbs Head control sampling areas.

9.17 Department of the Environment (Northern Ireland) survey of the North Channel sewage-sludge disposal site, autumn 1988

(a) Benthos, sediment chemistry and particle size analysis were investigated at 6 stations for temporal trend analysis-stratified sampling (Figure 17).

(b) Otter trawl hauls were made at 4 stations for epibenthos. Fish samples were retained for heavy metal analysis (Figure 17).

(c) Scallop were dredged over the general area; scallop muscle tissue was retained for heavy-metal analysis (Figure 17).

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Dr C Pattinson
Attended meetings 1-4 in lieu of Dr Owens

Mr C Chubb
Attended meeting 5 in lieu of Dr Owens

Mr E Bent
Attended meeting 6 in lieu of Dr Owens

Dr K W Wilson
Attended meetings 3-6 in lieu of Dr Harper

Mr J C Davies
Attended meetings 5 and 6 in lieu of Mr Nicholson

Annex 2 Fish disease sampling protocol

A2.1 Introduction

It is recognised that there is a need to undertake monitoring in the vicinity of sewage-sludge disposal sites, to satisfy an EQO that the disposal operations are not having a significantly adverse effect on fish species, particularly those that are commercially exploited. An increase in the incidence of disease symptoms in fish species in the vicinity of the disposal site would be one example of an adverse effect on such species, and a monitoring programme should, therefore, be designed to demonstrate an environmental standard that the level of incidence of routinely monitored fish disease symptoms is not significantly greater in the immediate vicinity of the disposal site than the level of incidence in an adjacently similar area.

Whilst the EQO and associated standard are fairly straightforward, the drafting of a suitable sampling programme is not a simple matter, because the natural prevalence of disease conditions in fish is poorly documented and is subject to spatial, seasonal and host distribution variations. It is considered to be unrealistic to expect the sewage-sludge disposal authorities to investigate these factors. A routine monitoring programme must, therefore, be devised, that attempts to rule out the possible effects of natural differences in disease prevalence, and yet is within the capabilities of the disposal authorities or their contracted monitoring agencies. It is suggested that such a programme should not be designed to identify small variations in disease prevalences, but should be designed as an early-warning monitoring programme to indicate whether a more intensive study is warranted. It is further suggested that it should be the function of the better-equipped licensing authorities to undertake any such intensive study, to complement the routine monitoring undertaken by the disposal authorities.

A2.2 Routine monitoring to be undertaken by the disposal authority

It is recommended that the disposal authority should undertake an annual sampling programme to investigate the levels of incidence of specific pathological conditions in fish species caught in the vicinity of sewage-sludge disposal sites.

A2.2.1 Sampling frequency

The sampling should be undertaken during the same clearly-defined period every year, outwith periods of known migratory or spawning activity. A specified sampling 'window' of 1-3 weeks during the May-June period would be appropriate for most locations.

A2.2.2 Sampling areas

It is recommended that two clearly-defined sampling areas should be investigated, and suggested that the areas should be of approximately 2 nautical miles radius. A sampling area of this size is usually perfectly adequate from the point of view of undertaking trawling operations, and it would be expected to contain sufficient numbers of fish to permit collection of a suitable sample.

One of the sampling areas should be centred on the licensed disposal area, or the area of maximum observed benthic effect, the second sampling area should be adjacent to the disposal area, preferably at 90° to the main tidal flows, but outwith the zone of immediate effect of the disposal operations. This 'control' sampling area ideally would be centred within a maximum of 5 nautical miles of the centre of the 'disposal' sampling area, should be separated from the latter by a minimum of 0.5 nautical miles, and should have similar benthic sediment and fauna to that found in the 'disposal' sampling area.

A2.2.3 Sampling methods

Otter trawl tows (or similar fishing trawls), of maximum one-hour bottom time, should be undertaken in both the 'disposal' and 'control' sampling areas, to collect fish for pathological examination. If there are large amounts of abrasive material caught in the trawls, for example shell material or even dogfish, the trawling time should be reduced. Repeated trawl tows should be undertaken as necessary, at either location, to satisfy the sample requirements detailed below. The trawling speed for all tows should be approximately 2.5 knots, although this obviously will depend upon the exact gear used and the towing vessels. An accurate description of the trawl used, including dimensions, should be included in the sampling report, together with trawling times, positions and speeds.

A2.2.4 Sample requirements

The common dab (*Limanda limanda*) has been widely used for fish disease studies, because the species is fairly widespread in UK waters and there is evidence to suggest that the species is not highly mobile and does not move constantly from one area to another. It is, therefore, recommended that the common dab should be used for sewage-sludge disposal disease studies. Samples of 50 common dabs should be collected from both the 'disposal' and 'control' sampling areas for intensive examination. If possible, all other adult fish caught in the trawls should also be retained for external pathological examination, but the main sampling requirement is to obtain the required flatfish sample. If the required sample of common dabs cannot be readily obtained in any particular area, numbers may be supplemented with, in order of preference, flounder (*Platichthys flesus*), long rough dab (*Hippoglossoides platessoides*) or plaice (*Pleuronectes platessa*). If a mixed flatfish sample is examined, the numbers of each species should be noted. If more than adequate samples are obtained, the fish chosen for examination should be biased to include the larger specimens. Sample fish should always be thoroughly washed before examination, being careful to avoid physically damaging the fish, and examinations should be undertaken within one hour of capture. Individual trawl catches should be examined and reported separately, although summary tables are proposed for 'disposal' and 'control' sampling areas.

A2.2.5 Sample examination

The sample of 50 flatfish should be submitted for external pathological examination. The presence of obvious external tumours, lymphocystis (nodules greater than 1 mm in size), skin hyperplasia/papilloma (greater than 2 mm in size), skin ulcers (open ulcers and ulcers which are not healed with totally regenerated epidermis), and skeletal deformities of the vertebral column, head or fin rays (excluding fin erosion) should be recorded. Any other visible disease conditions or abnormalities observed with regular frequency should also be investigated and reported. Separate lists should be prepared for flatfish caught in individual trawls, undertaken at both the 'disposal' and 'control' sampling areas, detailing the number of fish of every species examined, the size range of fish of every species examined, and the number of fish of every species exhibiting the specific external pathological conditions.

Following external examination, the sample of 50 flatfish should also be examined internally. Any large internal tumours and clearly-defined liver nodules of greater than 2 mm in diameter (excluding cestode and nematode cysts) should be recorded. Any other visible disease conditions or abnormalities observed with regular frequency should also be investigated and reported. Separate lists should be prepared for flatfish caught in individual trawl hauls undertaken at both the 'disposal' and 'control' sampling areas, detailing the number of fish of every species examined, the size range of fish of every species examined, and the number of fish of every species exhibiting the specific internal pathological conditions.

A summary table should be prepared for the 'disposal' and 'control' sampling areas, detailing the incidence of both external and internal pathological conditions. The summary table should indicate the length distribution of fish of all species examined and the mean length of all species examined, and should include single entries for all diseased fish indicating if there is any relationship between the incidence of external and internal pathological conditions.

If it proves possible to externally examine all adult fish caught in the trawls, a further summary table should also be prepared for the 'disposal' and 'control' sampling areas, detailing the number of fish of every species examined, and the number of fish of every species exhibiting the specific pathological conditions. If the pathological conditions appear to be prevalent in a discrete size range of fish of a particular species, this should also be recorded. However, it is not necessary to record the size and size range of all fish examined.

A2.3 Intensive monitoring to be undertaken by licensing authorities

It is assumed that the licensing authority will assist the disposal authority in the planning of routine monitoring surveys, and that the 'check monitoring' will concentrate on the same sampling areas, the same species and the same specified pathological conditions. It is also assumed that, if possible, the 'check monitoring' will be undertaken at the same time of year as that of the routine surveys. There may, however, be circumstances when the licensing authority chooses to modify certain elements of the sampling programme, with a view to perhaps changing the routine monitoring requirements.

Where the 'check monitoring' is likely to differ from the routine monitoring, in that the sampling frequency is unlikely to be annual (given that the licensing authorities are responsible for a number of disposal sites and the number of fish to be examined will be far greater), as far as frequency is concerned, it would seem logical initially to base the sampling frequency on the quantity of sewage sludge which is dumped, with a view to increasing the frequency if routine monitoring indicates that there are any problems. As far as sampling intensity is concerned, it is recommended that all fish should be examined for external pathological symptoms and the results reported, and that the flatfish sample should comprise 100 fish from each of two 5 cm length groups (the smallest to include 2-year+ fish) and 50 larger fish. In addition, it is also recommended that the disease sampling should be closely integrated with investigations of other biotic and abiotic parameters in the 'disposal' and 'control' sampling areas.

Annex 3 Method-determined sampling and analytical procedures

A3.1 Introduction

When collecting samples for the measurement of environmental parameters, and during the measurement of some of those parameters, the method used may have an effect on the results obtained. Whilst many sampling and analytical methods have some influence on the results obtained, this annex addresses those methods where this effect can be marked. Recommendations are made with respect to the use of harmonised or directly comparable methodologies, and sampling and analysis protocols are recommended for routine monitoring surveys of sewage-sludge disposal sites.

A3.2 Sediment sampling

A3.2.1 General comments

Sediment samples are routinely collected from the vicinity of sewage-sludge disposal grounds, to investigate physical, chemical, microbiological and macrobiological characteristics. At the time of sample collection, it is essential to ensure that the choice of sampling device is appropriate for the parameter under study. Commonly used sampling devices and recommendations relating to their use are summarised below.

A3.2.2 Grab samplers

Many grab sampling devices are available, but not all are suitable for routine monitoring surveys of sewage-sludge disposal sites. Recommended grab sampling devices are the Day, Smith-McIntyre, van Veen, and, in exceptional circumstances, the Shipek grab.

The Day, Smith-McIntyre and van Veen grabs have the following recommended characteristics:

(a) the tops of the grabs have lifting flaps, to allow access to the surface of the sediment sample for collection of relatively undisturbed sub-samples for analysis;

(b) the grabs sample an area of 0.1 m^2;

(c) the jaws of the grabs shut tightly, to prevent loss of fine material when the grabs are recovered from the sea bed;

(d) there is a facility to add weights to the grabs, as necessary, to improve the depth of penetration.

All three grab types are suitable for the collection of samples of clay, silt and sand sediments. When sampling very fine sediments, it may be necessary to use the lighter van Veen model to prevent the grab from burying itself in the sediment. When sampling less fine sediments, both the Day and Smith-McIntyre grabs can be used. However, the Day grab is considered to be superior for operational reasons, because sampling in heavy sea conditions with the Smith-McIntyre spring-loaded grab can be difficult and dangerous, particularly if the sampling team have no experience in using this type of grab. The Day model is, therefore, the preferred sampling grab for most clay, silt and sand sediments.

When using the Day, Smith-McIntyre and van Veen grabs, it is recommended that a minimum penetration depth of 5 cm is obtained for fine sediment samples and that the sample volume for all sediment types should be greater than 2.5 litres. It is also recommended that samples obtained should have a relatively undisturbed surface. Smaller volume samples, or samples with a disturbed surface, should be rejected.

When sampling very coarse sediments, such as gravel, or when sampling sediments containing significant quantities of very large materials, such as cobbles, it may prove impossible to collect suitable samples using the Day, Smith-McIntyre and van Veen grabs. In such exceptional circumstances, a modified Shipek grab may be used, although it is possible that fine materials in the sample will be washed out of the grab. Advice on a suitable design of Shipek grab can be obtained from the MAFF Fisheries Laboratory, Burnham-on-Crouch.

In general, grab samplers are suitable for the collection of surface sediment samples for determination of physical, chemical and microbiological parameters. They are also suitable for the collection of quantitative samples for identification of benthic infaunal species. If samples are required for chemical analysis, it is essential that all surfaces that come into contact with the sediment sample are made of stainless steel, and not galvanised or plated steels. Non-stainless steel grabs are, however, suitable for the collection of samples for determination of physical and microbiological parameters, and for the identification of benthic infaunal species. Following collection of any grab sample, it is important to ensure that the grab sampler is thoroughly cleaned (washing with sea water is usually sufficient) to avoid cross-contamination of subsequent samples.

Grab samplers are not suitable for collection of samples for identification of physical, chemical or biological changes with depth in the sediment.

It is recommended that at the time of collection of any grab sample, a sampling log is completed. This should record the date, time and position of the sampling operation and detail the nature of the sample. The latter should include the depth of penetration of the grab and/or the sample volume; a description of the sediment type (including a visual estimate of the proportions of silt/clay, sand, gravel, etc, observations on the structure of the sediment, and comments on artefacts present in the sample); and details of the type of sample(s) collected.

A3.2.3 Core samplers

Grab samplers cannot be used for the study of physical, chemical or biological changes with depth. If such samples are required, a core sampler or box-core sampler (see Sub-section A3.2.4) is essential. Recommended core sampling devices are the Craib core sampler and the SMBA multiple core sampler. For ease of operation, the Craib corer is preferred.

In general, grab samplers are simpler to operate than core samplers, and grabs are, therefore, used for routine collection of surface sediment samples. There are, however, no objections to the routine use of core sampling devices for the collection of samples for physical and chemical analysis, or for the collection of quantitative samples for identification of meiofaunal species. If the samples are being collected for chemical analysis, it is essential that all surfaces that come into contact with the sediment are either stainless steel or inert Perspex/acrylic/polycarbonate. The transparency of the latter also allows the operator to make visual observations on the *in situ* nature of the sample, which can be of particular interest when redox potential is to be measured. Following collection of any core sample, it is important to ensure that the core sampler and core tube are thoroughly cleaned (washing with sea water is usually sufficient) to avoid cross-contamination of subsequent samples.

In common with grab sample collections, it is recommended that a sampling log is completed at the time of core sample collection, recording the date, time and position of the sampling operation, relevant observations on the sediment sample, and details of the type of sample(s) collected.

A3.2.4 Box-core samplers

There is a body of opinion that totally undisturbed near-surface samples can only be obtained using a box corer. However, the requirement for a large vessel to deploy box-coring devices, such as the Reineck or USNEL samplers, and the need for relatively calm weather to operate the samplers, are considered to rule out the use of box-coring devices for routine monitoring surveys of sewage-sludge disposal sites. Sampling requirements associated with such routine surveys can be satisfied using the smaller grab and core sampling devices described above. It should, however, be noted that the above observations do not preclude the use of box-coring devices for such surveys. If such samplers are used, a sampling log should be completed and, in the case of sample collections for chemical analysis, the corer box must be made of stainless steel. Following collection of any box-core sample, it is important to ensure that the box-core sampler is thoroughly cleaned (washing with sea water is usually sufficient) to avoid cross-contamination of subsequent samples.

A3.3 Particle size analysis

A3.3.1 General comments

Determination of sediment type by particle size analysis is essential for the accurate interpretation of benthic infaunal data. In addition, particle size analysis data can be of use when considering the dispersive nature of the study area. However, it is unlikely that a survey designed for routine monitoring purposes will be sufficiently detailed to permit assessment of the dispersive nature of an area, and particle size analysis is not a useful parameter per se for assessing the effects of sewage-sludge disposal. It is, therefore, recommended that particle size analysis need only be undertaken if the benthic infauna are to be investigated.

A3.3.2 Sample collection

Samples for particle size analysis can be collected using one of the recommended grab, core or box-core samplers referred to above. For ease of operation, the grab is preferred. If the nature of the sediment necessitates the use of a Shipek grab, advice on sampling methodology should be sought from the MAFF Fisheries Laboratory, Burnham-on-Crouch.

Immediately following sample device recovery, sub-samples for particle size analysis should be removed from the central area of the surface of the sample using an inert Perspex/acrylic/polycarbonate core tube. The internal diameter of the core tube should be between 2.5 cm and 5 cm. When sampling well-sorted sediments, the depth of the core sub-samples should be the same as the penetration depth achieved when collecting complementary grab samples for benthic infauna determination. When sampling fine, well-sorted, sediments, the minimum sub-sample depth should be 5 cm.

When sampling sediments that are not well-sorted, if a distinct sub-surface biologically inert layer is evident, this layer should be discarded from the core sub-samples collected for particle size analysis. The depth of the core sub-samples retained for analysis should always be recorded.

In survey areas where the sea-bed sediment is heterogeneous in nature, to the extent that the sediment types found in replicate samples collected at the same sampling station can be significantly different, it may be appropriate to collect core sub-samples for particle size analysis from all grab samples collected for benthic infaunal determination. In such circumstances, the maximum internal diameter of the core tube used to collect the sub-samples for particle size analysis should be 2.5 cm.

The core sub-samples for particle size analysis should be transferred in its entirety to a plastic or glass jar, and either preserved with 70% industrial methylated spirit or deep frozen. It should be noted that some sediments contain particles that have a significant water content within the particle structure, and that deep freezing this type of material can result in particle fracture. Seventy percent industrial methylated spirit, is therefore, the preferred method of sample preservation, at least until the sediment nature in the area under study has been thoroughly investigated and deep freezing is found to be acceptable.

If the nature of the sediment precludes sub-sampling using a core tube, sub-samples of the required depth and volume should be removed from the central area of the surface of the sample using a stainless steel or plastic scoop. The depth of the sub-samples should be recorded, and the sub-samples preserved in the manner described.

A3.3.3 Sample preparation and analysis

The method of sample preparation will depend upon the chosen method of sample analysis. A specific method is not, therefore, recommended. Details of appropriate sample preparation methods are contained in the reference literature (Section A3.6).

Following appropriate sample preparation, it is recommended that analysis of that proportion of the sediment with a particle size diameter of greater than 63 µm should be undertaken using either the dry-sieving method described by Buchanan (1984), or a laser diffraction granulometry technique. If a laser diffraction granulometry technique is used, extremely large particles must first be removed by sieving. Dry-sieving is, therefore, the preferred method.

A number of techniques are available for analysis of the silt/clay component (particle size diameter of less than 63 µm). Pipetting, Coulter Counter, Sedigraph and laser diffraction granulometry techniques are commonly used and, with qualifications, all are acceptable.

The pipette technique is described by Buchanan (1984). This technique is time consuming and labour intensive. The Coulter Counter technique can require application of a correction factor to compensate for materials finer than the 'window' of analysis, and care must be taken to ensure that the small sample size required is representative of the whole sample. The latter technique will normally entail analysis of a number of replicates. The Sedigraph technique is claimed by some workers to be less precise than the pipette and Coulter Counter techniques, but Stein (1985) and Singer *et al*. (1988) have compared this method with other techniques and found it to be acceptable. The laser diffraction granulometry technique is claimed by McCave et al. (1986) to be neither accurate nor precise when assessing the clay component of sediments containing significant quantities of clay-sized particles.

Despite the qualifications attendant to the techniques, it is not considered to be desirable to recommend one technique. The purpose of including this parameter in routine monitoring surveys of sewage-sludge disposal sites is to provide the information necessary to interpret benthic infaunal data, and all of the above methods are sufficiently accurate and precise for that stated purpose. It, therefore, must be concluded that all of the above methods can be recommended. To designate a preferred method is also considered to be undesirable, as Coulter Counter, Sedigraph or laser diffraction granulometry equipment may already have been purchased for particle size analysis.

A3.3.4 Data presentation/interpretation

It is recommended that mean and median particle size and the percentage silt/clay component should be reported for all samples analysed. Cumulative particle size frequency curves should also be prepared and reported for all samples, relating the particle size frequency to the Wentworth scale of classification of sediments. This method of data presentation is described by Buchanan (1984). In addition, the inclusive graphic standard deviation (sorting coefficient) should be reported for all samples. This method of data presentation is described by Folk (1974).

A3.3.5 Environmental quality standards

At one UK sewage-sludge disposal site, Garroch Head in the Firth of Clyde, the surface sea-bed sediments have been extensively modified by sewage-sludge disposal and accumulation. Given the dispersive characteristics of other UK disposal sites, the physical nature of the sediments in terms of particle size distribution is unlikely to be significantly altered, if at all. As significant effects on the particle size distribution of the sediments are only likely in the least dispersive locations, it is considered to be inappropriate to assess environmental quality standards for particle size distribution.

A3.4. Total organic carbon and total nitrogen

A3.4.1 General comments

Determination of total organic carbon and total nitrogen in sediment samples can provide useful supportive data when investigating other routinely monitored environmental parameters. Because carbon and nitrogen are major components of sewage sludge, the levels of total organic carbon and total nitrogen in the sediments can also be useful per se when assessing the impact of sewage-sludge disposal operations. It is, therefore, recommended that these parameters should always be included in routine monitoring surveys of sewage-sludge disposal sites.

Investigation of the chemical properties of sediments has shown that the silt/clay component (particle size diameter of less than 63 µm) is particularly important. It is, therefore, recommended that total organic carbon and total nitrogen are determined in this component of the sediment. However, if the silt/clay component is insignificant, total organic carbon and total nitrogen should be determined in the 'whole' sediment.

Where a data series exists for total organic carbon and total nitrogen in 'whole' sediment, both the silt/clay component and the 'whole' sediment should be analysed for these parameters.

A3.4.2 Sample collection

Samples for total organic carbon and total nitrogen analysis can be collected using one of the the recommended grab, core or box-core samplers referred to above. For ease of operation, the grab is preferred. If the nature of the sediment necessitates the use of a Shipek grab, advice on sampling methodology should be sought from the MAFF Fisheries Laboratory, Burnham-on-Crouch.

Immediately following sample-device recovery, sub-samples for total organic carbon and total nitrogen analysis should be removed from the central area of the surface of the sample using an inert Perspex/acrylic/polycarbonate core tube. The internal diameter of the core tube should be between 2 cm and 2.5 cm, and the minimum depth of the sub-samples should be approximately 3 cm. The surface sediment (0-1 cm depth section) of the core sub-samples should be removed from the core tube and transferred to glass jars. Obvious benthic infauna should be removed using forceps, and the sub-samples deep frozen. If the quantity of surface sediment collected in this manner is insufficient for the analyses to be undertaken (see Sub-section A3.4.3), a series of core sub-samples should be collected from the central area of the sample. The surface sediment sections should be transferred to the same glass sample jar, obvious benthic infauna removed, and the composite sub-sample deep frozen. Collection of a sub-sample series from a single sample will preclude the use of a core sampler for collection of sediment for total organic carbon and total nitrogen analysis.

If the nature of the sediment precludes sub-sampling using a core tube, sub-samples of approximately 1 cm depth should be removed from the central area of the surface of the sample, using a stainless steel or plastic scoop. The samples should be treated and preserved in the manner described below.

A3.4.3 Sample preparation and analysis

A3.4.3.1 Total organic carbon

Prior to analysis for total organic carbon, the sample must be separated into appropriately sized fractions. If the 'whole' sediment is to be analysed, samples should be sieved via a 2 mm (2000 µm) pore-size nylon or metal sieve to remove coarse particles. Material retained on the sieve should be discarded. Material passing the 2 mm pore-size sieve is considered to be the 'whole' sediment referred to in Sub-section A3.4.1. If the silt/clay component is to be analysed, samples should be sieved via a 63 µm pore size nylon or metal sieve. Material retained on the sieve should be discarded. Material passing the 63 µm pore-size sieve is considered to be the silt/clay component referred to in Sub-section A3.4.1.

There are two commonly-used separation techniques - dry- and wet-sieving. Dry-sieving is considered to be the more appropriate for fractionation of samples prior to analysis for total organic carbon, for two reasons. Firstly, because it is the simpler technique and can be undertaken by comparatively inexperienced staff; and secondly, because wet-sieving using non-saline water can remove soluble, weakly-bound, organic materials. The recommended method of sample preparation for total organic carbon analysis is, therefore, freeze-drying, homogenisation and dry-sieving fractionation. Air-drying and oven-drying are not considered to be acceptable, because the dried sediment obtained using these techniques cannot be effectively homogenised into discrete particles.

It is recommended that laboratories currently employing the wet-sieving fractionation technique should adopt the freeze-drying/dry-sieving fractionation technique as soon as possible, and by 1 January 1990 at the latest. It is further recommended that those laboratories currently employing the wet-sieving fractionation technique should determine total organic carbon (and total nitrogen, see Sub-section A3.4.3.2) in both wet-sieved and freeze-dried/dry-sieved samples, until the laboratories concerned are satisfied that termination of the wet-sieving technique will not affect any temporal trend analysis of existing data series.

Following sample preparation, it is recommended that the samples are analysed for total organic carbon using a CHN elemental analyser technique. Suitable techniques are described by Byers et al., (1978). Prior to analysis, carbonates must be removed. A commonly-used method is the sulphurous acid technique of Shaw (1959). This involves soaking weighed sediment samples in the acid, followed by hot-plate drying in a fume cupboard at between 80°C and 110°C prior to analysis. Alternatively, weighed sediment samples can be placed in a modified dessicator and exposed overnight to the fumes of concentrated hydrochloric acid, followed by air-drying (at less than 40°C) prior to analysis (DAFS, pers. comm., 1988). Having removed carbonates, the total organic carbon is determined directly by burning the sample in a CHN elemental analyser, at the temperature recommended by the equipment manufacturer. In the case of the commonly-used Perkin Elmer 240 elemental analyser, the combustion temperature is 920°C. Another technique, that is commonly used to compensate for carbonate, involves sub-sampling the prepared sample, combusting one sub-sample in a muffle furnace at 475°C for four hours to remove organic materials, and determining inorganic and total carbon separately, in pre-treated and untreated sub-samples respectively, using a CHN elemental analyser. The total organic carbon level is then derived by subtraction. The method is, however, unreliable if the organic content of the sediment is high, or if the sediment contains a significant component of coal. Providing the latter technique is not used for the inappropriate sediments described, it must be assumed that all three methods of compensating for carbonates are acceptable. Relative assessment of the three techniques is not possible, because a detailed intercomparison of the techniques has not been undertaken. It is, therefore, recommended that, until such an intercomparison is undertaken, the technique employed to compensate for carbonates should be that which is most familiar to the laboratory undertaking the analyses.

It should be noted that, if a data series exists for analyses undertaken using a modification of the Schollenberger chromic acid wet oxidation technique, a change of method may rule out temporal trend analysis of the data. In such circumstances, it is recommended that duplicate sediment samples should be collected, and total organic carbon estimated using both CHN and wet oxidation techniques. If the results obtained from the CHN and wet oxidation techniques are found to be directly comparable, the wet oxidation analyses can be discontinued. If the sediments under investigation are predominantly coarse sands (particularly if there are large quantities of shell waste in the sample), the results obtained using the two techniques are unlikely to be comparable.

A3.4.3.2 Total nitrogen

It is recommended that samples are analysed for total nitrogen using a CHN elemental analyser technique. Using a CHN elemental analyser, total nitrogen can be co-determined with total organic carbon (see Sub-section A3.4.3.1). It will, therefore, be unnecessary to routinely prepare separate sediment samples for total organic carbon and total nitrogen analysis. If separate samples are required for additional analyses, the samples should be prepared in the manner described for total organic carbon (Sub-section A3.4.3.1).

Again, it should be noted that, if a data series exists for analyses undertaken using a modification of the Kjeldahl technique, a change of method may rule out temporal trend analysis of the data. In such circumstances, it is recommended that duplicate sediment samples should be collected, and total nitrogen estimated using both CHN and Kjeldahl techniques. If the results obtained from the CHN and Kjeldahl techniques are found to be directly comparable, the Kjeldahl analyses can be discontinued.

A3.4.4 Data presentation interpretation

A3.4.4.1 Total organic carbon and total nitrogen

It is recommended that total organic carbon and total nitrogen levels in the sediments are reported as a percentage of the prepared sample weight prior to removal of carbonates.

A3.4.4.2 Total organic carbon (TOC): total nitrogen (N) ratio

The determination of total organic carbon in sediments collected from the vicinity of recognised sea disposal sites is often complicated by the presence of coal. This can originate from exposed coal seams, disposal of colliery waste or discharges from coal-burning vessels. Neither the CHN elemental analyser nor the wet oxidation technique makes an allowance for this material, and it can have a significant effect on the results obtained. One method of overcoming this problem, which can provide an indication of the labile/refractory nature of the organic material, is to report the TOC:N ratio for the sample. Because nitrogen is virtually absent from all but the softest coals, an unusually high TOC:N ratio may indicate that coal is present, and the result should, therefore, be interpreted with caution. It is, therefore, recommended that the TOC:N ratios are always reported, in addition to the results of total organic carbon and total nitrogen analyses.

A3.4.4.3 Total protein

Another method of overcoming the coal problem, is to estimate total protein in addition to total organic carbon and total nitrogen. The method and rationale of this technique are described by Buchanan and Longbottom (1970). It is suggested that, in certain circumstances, it would be appropriate to employ this additional method to compensate for the presence of coal in a sediment sample and to report both the TOC:N ratio and total protein levels, in addition to the results of total organic carbon and total nitrogen analyses. The necessity to include this parameter in a routine monitoring survey of a sewage-sludge disposal site should be discussed with the licensing authority.

A3.4.5 Environmental quality standards

Organic carbon, derived from sources such as sewage sludge, exhibits non-conservative behaviour. Levels of total organic carbon in the sediments at disposal locations would, therefore, be expected to vary in a complex fashion related to disposal timing and frequency, and seasonal variations in the rate of recycling of organic material in the sediments. Such variations would have to be taken into account if standards were proposed. As already indicated determination of total organic carbon levels in sediments can also be complicated by the presence of coal. If this refractory carbon component cannot be adequately quantified, it would be unrealistic to propose standards for total organic carbon. One suggested method of quantifying the refractory carbon component is to report the TOC:N ratios for sediment samples, and it is possible that standards could be based on a combination of total organic carbon, total nitrogen and TOC:N ratio data. Before attempting to do so, it would, however, be necessary to compare the available data for UK sewage-sludge disposal sites, initially to decide whether standards were feasible, and then to decide whether a universal standard or site-specific standards would be appropriate. Given the large differences between individual UK sewage-sludge disposal sites, a universal standard might seem unrealistic, but it may be possible to derive a common standard related to values determined in adjacent 'control' areas. Without a desk study of available data, no conclusions can be drawn at the present time.

It is recommended that the licensing authorities should undertake a desk study of available total organic carbon, total nitrogen and TOC:N ratio data for UK sewage-sludge disposal sites, with a view to deciding whether EQSs can be derived. It is also recommended that they should initiate a research study to investigate variations in total organic carbon levels, total nitrogen levels and TOC:N ratios at sewage-sludge disposal sites, both in relation to disposal activity and seasonal changes in the recycling of organic materials. It is further recommended that the research study should investigate changes in total organic carbon levels, total nitrogen levels and TOC:N ratios with depth in sediment samples.

A3.5 Redox potential

A3.5.1 General comments

The redox potential of a sediment sample is the potential measured between a reference and inert metal electrode inserted into the sample, corrected to the potential of a standard hydrogen reference electrode. This potential is normally measured using a double platinum or a platinum and calomel electrode pair, and the results are standardised using a solution of known redox potential. Corrected potentials derived in this manner, are usually referred to as Eh values.

Whitfield (1969) discusses the difficulties involved in the quantitative determination of redox potential in natural aqueous systems, and concludes that, whilst not of precise physico-chemical significance, it is a parameter of operational significance. Natural media containing large quantities of oxidising agents have high Eh values, and those containing large quantities of reducing agents have low Eh values. The determination of Eh in sediments is, therefore, considered to be a useful semi-quantitative indicator of reducing conditions in the sediment sample.

Despite reservations relating to the precision of its measurement and interpretation of the data obtained, Fenchel and Riedl (1970) have used redox potential measurements to categorise the physico-chemical nature of marine sediments, and Eh determinations are routinely included in monitoring surveys to supplement investigations of other environmental parameters. Often, the derived data is found to be extremely valuable when assessing benthic infaunal data, particularly when the benthic environments under study are impacted by significant inputs of organic materials (for example sewage-sludge disposal sites). It is, therefore, recommended that determination of Eh is included, at least at an assessment level, in routine monitoring surveys of sewage-sludge disposal sites (see Subsections A3.5.2 and A3.5.4).

A3.5.2 Sample collection

Samples of marine sediment collected for the determination of Eh must, as far as is practicable, be totally undisturbed, to the extent that the sediment matrix and interstitial water within the sediment should be undisturbed to a depth of at least 10 cm. Obviously, a totally undisturbed sample cannot be guaranteed when samples are collected using remote sampling devices, but it is generally assumed that samples recovered beneath a layer of clear supernatant water satisfy the criterion. The requirement to obtain undisturbed samples with clear overlying supernatant water precludes the use of a grab sampler for determination of Eh, except when sampling the most cohesive sediments. In other sediment types, the jaws of the grab will not provide an effective seal to prevent loss of interstitial and supernatant water. It is also unusual to collect samples of the required 10 cm depth using a grab sampler. The grab is, therefore, not recommended for collection of samples for Eh determination, and it is necessary to use one of the core or box-core samplers referred to previously.

If a Craib corer or SMBA multiple corer is used, redox potential measurements can be made directly in the inert Perspex/acrylic/polycarbonate sampler tube, and results compared with the observed nature of the sediment in the core. This can be very useful, as distinct bands of decaying organic material (with a low Eh) are often clearly visible in otherwise clean sediment samples. If a box corer is used, it is desirable to remove a sub-sample from the box corer, using an inert Perspex/acrylic/polycarbonate core tube to permit visual observation of the nature of the sediment. The minimum internal diameter of the core tube should be 5 cm. (When sampling with a box corer, it is also inconvenient to undertake direct measurement of redox potential *in situ*.) Such sub-sampling increases the possibility of sample disturbance, particularly with regard to loss of interstitial water. Recent German investigations have also suggested that the deeper sediments and interstitial water in a box-core sample can be significantly disturbed, irrespective of sub-sampling. Great care is, therefore, required when determining Eh in sediment samples collected using a box corer. For these reasons, and because box corers are not totally suited to routine monitoring programmes, the recommended sampling device for the collection of sediment samples for Eh determination is the core sampler. For ease of operation, the Craib corer is preferred.

It is accepted that certain sediment types cannot be sampled using a core sampler, and that the determination of Eh in such sediments is unlikely to be warranted. If it is found (or is already known) that the sediments at the disposal site under investigation are not amenable to sample collection using a core sampler, this parameter can be deleted from the sampling protocol.

A3.5.3 Sample preparation and analysis

Following collection of the core sample, the sampler tube should be removed from the sampler frame and redox potential measurements undertaken in situ as soon as is practicable. During any interim period, core samples should be maintained at a constant temperature, which is as close as possible to the ambient temperature of the overlying supernatant water.

Redox potential measurements are made using a suitable electrode system connected to a pH/mV meter. Many manufacturers supply suitably robust electrodes for in situ measurement of redox potential in marine sediments, for example Russell Electrodes Ltd and V A Howe. Advice on electrodes can be obtained directly from the manufacturers, or from the MAFF Fisheries Laboratory, Burnham-on-Crouch and the DAFS Marine Laboratory, Aberdeen. Prior to making redox potential measurements in a core or series of core samples, the electrode system must be standardised against a solution of known redox potential. The recommended choice is ZoBell's solution. This is made up by dissolving 1.087 g of potassium ferricyanide, 1.399 g of potassium ferrocyanide and 7.456 g of potassium chloride in distilled water to a total volume of one litre. The calculated redox potential of this solution is 442 mV.

The electrode system is first thoroughly washed with distilled water and dried and immersed in ZoBell's solution. If the pH/mV meter can be calibrated to a specific value, it should be set to 442 mV. If the meter cannot be calibrated in this manner, the apparent potential should be recorded and the necessary correction factor calculated by addition. This correction factor should be applied to all subsequent sediment potential readings.

Following standardisation of the electrode system, the electrode is lowered into the sample supernatant water, to within 1 cm of the sediment surface. After a one-minute equilibration period, the potential is recorded. The electrode is then lowered into the sediment core using an adjustable screw stand system and, following one-minute equilibrations, readings are taken at the water/sediment interface and at 0.5 cm intervals down the sediment core to a depth of 4 cm. Thereafter, the series of readings is continued at 1 cm intervals to the bottom of the core sample. When a series of readings is completed, the electrode is withdrawn from the sample, thoroughly washed with distilled water, dried and returned to ZoBell's solution prior to undertaking measurements in a further core sample. If necessary, the system is recalibrated prior to the next series of measurements.

A3.5.4 Data presentation/interpretation

Following correction (if necessary) of apparent potentials to the standard potential of ZoBell's solution, it is recommended that Eh data is presented as a graphical profile of redox potential with depth in the core sample. Visual observations on the nature of the core should also be reported. The procedures outlined in this and the previous sub-section, are based on those suggested by Pearson and Stanley (1979).

When undertaking inter-station comparisons of the redox profiles, it is sometimes useful to compare Eh data from specific depth strata. A depth of 4 cm in the core is commonly used, but the precise depth of relevance may be site specific. It is, therefore, recommended that graphical profiles are always reported in addition to any single depth strata measurements.

As indicated in Sub-section A3.5.2, certain sediment types are not amenable to Eh sample collection and determination, and inclusion of this parameter in the sampling protocol is not warranted. In addition, it should be noted that the ability to collect a sample does not automatically imply that investigation of the parameter would be useful. If core samples can be collected but it is found (or has already been found) that there is no evidence of a change in reducing conditions with depth in a sediment sample, it is unnecessary to collect regular samples for Eh determination. In such circumstances, it is suggested that periodic checks on redox potential would be appropriate.

A3.5.5 Environmental quality standards

As already indicated, Eh has no precise physico-chemical meaning. It is, therefore, perhaps, optimistic to consider EQSs. However, a comparison of graphical profiles of redox potential with depth in cores from a number of UK sewage-sludge disposal sites and their corresponding 'control' locations may indicate that there are patterns of change in the redox potential profiles that can be defined in terms of a quantitative standard. Given the vast differences between individual UK sewage-sludge disposal sites, it is likely that a redox potential standard will be inappropriate for many UK disposal sites, and it also seems likely that any numerical standards that can be derived would be site specific. Again, without a detailed desk study of available data, no conclusions can be drawn at the present time.

It is recommended that the licensing authorities should undertake a desk study of available redox potential profile data for UK sewage-sludge disposal sites, initially to identify those disposal sites where adequate data are available, and then to decide whether qualitative or quantitative EQSs can be derived.

A3.6 References

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Annex 4 Procedures for the use of microbiology in monitoring of sewage-sludge disposal sites

A4.1 Introduction

The primary objective of any monitoring programme is to differentiate between detectable variations arising from the disposal of sewage sludge and those which occur naturally in the marine environment.

In this context, it is necessary to adopt the most cost-effective technology available to minimise the wastage of expensive and time-consuming environmental monitoring in the collection of scientifically valid data.

In this respect, microbiological techniques offer a relatively cheap, simple and rapid method of targeting areas of sea bed where the more sophisticated technology should be applied.

A4.2 Use of faecal micro-organisms in the monitoring of marine sewage-sludge disposal sites

A4.2.1 Rationale

Sewage sludge contains a wide range of enteric micro-organisms. The marine environment is alien to their survival although the rate at which they succumb varies considerably between species. For example, *Escherichia coli* will die relatively quickly whereas sulphite-reducing *Clostridium* spores persist for considerably longer periods.

Analytical techniques for the isolation and enumeration of faecal bacteria, thermotolerant coliforms (TTC) such as *E. coli*, Group D streptococci (*Enterococcus*) and sulphite-reducing *Clostridium* spores (S-RC) are well developed. Whilst other faecal indicator organisms such as pathogenic yeasts, amoebae and enteroviruses have been suggested as alternatives, the methodologies for their isolation and identification are either considerably less well-defined, or technically complicated (see Section A4.8).

Hence, it is recommended that routine microbiological monitoring should be based on the isolation and enumeration of the commonly-used faecal indicator organisms.

A4.2.2 Limitations of application

It is proposed that microbiological studies have the following applications for the monitoring of sewage-sludge disposal activities in the marine environment based purely on the analysis of sea-bed sediments:

(i) pre-disposal baseline studies to establish patterns of faecal micro-organism distribution on the sea bed;

(ii) confirmatory assessment of the area and direction of sewage-sludge settlement on the sea bed once disposal has commenced;

(iii) establishment of benchmark patterns of faecal micro-organism distribution on the sea bed for sewage-sludge disposal sites already in use;

(iv) establishment of zones of sewage-sludge settlement on the sea bed by use of microbiological techniques;

(v) limited evidence for the rate of movement of sewage sludge on the sea bed, obtained by using sulphite-reducing Clostridium spores;

(vi) recovery of sites once sewage-sludge disposal activity has ceased, assessed in part by microbiological monitoring.

Microbiological studies to determine the impact of sludge disposal on the water column are NOT recommended unless there is a specific and specialised requirement unique to a particular disposal site.

A4.3 Sampling objectives and frequency of monitoring

For any major descriptive or benchmark surveys, the continuing practice of establishing sampling grids, which take into account the main physical features of the disposal site, is recommended. These grids should extend inshore of the disposal site to assess the potential impact of shoreline and estuarine inputs.

The number of sites used for microbiological sampling should be no less than the number used for sampling for other environmental parameters. Where the objective of the study is to locate areas of sewage settlement sampling will necessarily be much more extensive.

Samples for microbiological analysis should be taken at all stations sampled for other parameters (e.g. sediment chemistry and benthos).

Microbiological studies should only be omitted from surveys of disposal sites when there is no doubt or disagreement that environmental changes are well-characterised and unequivocally linked to sewage-sludge disposal.

Microbiological surveys should be used primarily to establish the spatial distribution of sewage-sludge settlement at a given point in time.

The frequency of microbiological sampling at sewage-sludge disposal sites is entirely site dependent and should be assessed by all parties involved in monitoring the disposal site.

A4.3.1 Sample collection

Whenever possible, there should be a suitable period (e.g. 24 h) of cessation of disposal activity before sampling commences. Sampling should be performed from a vessel other than the sludge disposal vessel.

Grab or core sampling devices should be designed and used in a manner to minimise disturbance of sewage-sludge floccules and other matter at the sediment-sea water interface during sample collection. These devices should collect a standard 0.1 m^2 surface area of sea bed with a minimum penetration of 7 cm. In general, it should not be necessary to disinfect grabbing devices between each sampling site.

When sampling water, the chosen sampling device must use sterile collection vessels and must enable adequate control of the opening and closure of the sampling vessel in order to ensure collection of a representative sample at the appropriate depth.

A4.3.2 Sampling techniques

All procedures must be undertaken in a manner which is as aseptic as practicable in order to avoid contamination of samples.

Sterile equipment should be used for removal and storage of samples before processing.

After collection, samples should be processed as soon as possible. When processing is unavoidably delayed, the samples may be stored for up to 8 h **in the dark** at 4-8°C in a refrigerator. The period of storage time for samples should always be permanently recorded. For enumeration of S-RC spores, samples may be stored for much longer periods in this manner. Freezing of samples should not be undertaken.

A4.4 Microbiological methods

A4.4.1 General comments

Ultrasonication, homogenisation or any other procedure for disaggregation should be fully evaluated before routine use specifically to establish the optimum period of treatment for maximum recovery of faecal micro-organisms.

Methods for isolation and enumeration of TTCs should use a resuscitation stage in order to maximise the recovery of debilitated cells.

Faecal bacteria should be enumerated by membrane filtration (MF) or most-probable-number (MPN) techniques. MF procedures should use gridded cellulose nitrate filters with pore sizes of 0.45 µm. MPN procedures are less desirable than MF techniques due to the inherent statistical variability.

It is recommended that 0.1% peptone water (pH 7.2) should be used as a diluent for all samples to ensure a recovery of debilitated cells which is as high as possible.

A4.4.2 Methodologies for specific micro-organisms: recommendations

It is recommended that the application of TTCs, enterococci, and sulphite-reducing Clostridium (S-RC) spores for routine microbiological monitoring of sewage-sludge disposal sites in the UK, is continued.

It is recommended, as an essential requirement of any sludge disposal monitoring, that established methods are adopted and used unaltered each time.

All media should be tested before use to determine the efficiency for the recovery of each specific micro-organism.

A4.4.3 Thermotolerant coliforms (TTCs)

Presumptive counts of TTCs should be obtained at a selective incubation temperature of 44°C ± 0.5°C for 24 h on an appropriate culture medium. For MF methods, the use of mFC agar (without rosalic acid) or

0.1% lauryl sulphate broth is recommended. Recommended resuscitation steps are pre-incubation at 30°C for 4 h or, pre-incubation of membranes on a non-selective medium. For MPN procedures, minerals modified glutamate broth should be used at 37°C, with sub-cultures taken at 24 and 48 h for presumptive identification of TTCs using Brilliant Green lactose broth incubated at 44°C ± 0.5°C.

A4.4.4 Group D faecal streptococci (enterococci)

Presumptive counts of enterococci should be obtained at a selective incubation temperature of 37°C ± 0.5°C for 48 h on an appropriate culture medium. Incubation at 44°C is not recommended since this may fail to recover all types of enterococci. The use of Slanetz and Bartley medium or KF-streptococcus agar for MF procedures, and use of azide-based commerical media for MPN procedures, is recommended. Sub-cultures should be taken from broths used in MPN procedures to confirm that the visual changes are due to presumptive enterococci. No resuscitation steps are currently recommended.

A4.4.5 Sulphite-reducing *Clostridium* (S-RC) spores

Presumptive counts of S-RC spores should be obtained at a selective incubation temperature of 37°C ± 0.5°C for 24 h on an appropriate culture medium. It is recommended that TSC agar with cycloserine supplement for MF procedures, and meat broth with supplements of sodium sulphite and ferric citrate for MPN procedures, are used. Sub-cultures should be taken from broths used in MPN procedures to confirm that the visual changes are due to presumptive S-RC spores. Spores of S-RC species should be detected after prior treatment of environmental samples at 70-80°C for 10 min to destroy vegetative cells.

Alternative methods may be used if they have been shown to give results comparable to the methods recommended above.

A4.5 Interpretation of microbiological data

In general terms, TTCs and enterococci can be used to indicate the presence of sewage sludge in marine sediments for a relatively short time after disposal. This is due to the relatively short life of these organisms in the marine environment. This concept may change once the impact of the 'viable but non-culturable' phenomenon is researched further (Sub-section A4.8).

Enterococci counts are generally used to complement the interpretation of TTC data. Enterococci in general survive slightly longer than TTCs and so provide a better indication of sewage-sludge settlement especially at more dispersive disposal sites were numbers of TTCs can die-away to levels below detection before settlement of the sewage sludge on the sea bed occurs. It is recommended that microbiological monitoring surveys of sewage-sludge settlement on the sea bed routinely use both counts of TTCs and enterococci for all disposal sites.

Spores of S-RCs can survive in the sea bed sediment for many months after sewage-sludge disposal or transport to the marine environment via riverine input. The prolonged longevity of these organisms poses a problem for monitoring disposal sites located near riverine discharges at the shoreline. Often, it is not possible to distinguish between the zone of sewage-sludge settlement and riverine input on the basis of the counts of these organisms alone. Accordingly, it is recommended that use of S-RC spores is made, additional to TTCs and enterococci, at disposal sites remote from other discharges of similar magnitude. Spores of S-RCs survive for a considerable time in the marine environment and thus are suited for detecting sewage-sludge settlement and potential movement over a period of time.

A4.6 Spatial and temporal trends in microbiological data

A host of environmental factors will ultimately influence the persistence and recovery of each microbial indicator at the sea bed of sewage-sludge disposal sites. These factors include seawater temperature, sediment type, and a plethora of ill-defined, antagonistic properties of the marine environment. In addition, the proportions of TTCs, enterococci and S-RC spores can vary depending on the age and treatment given to the sewage sludge. Accordingly, it is recommended that:

(i) Microbiology is used as a monitoring tool almost exclusively to evaluate the spatial distribution of sewage-sludge deposition on the sea bed; and

(ii) Microbiological data should NOT be used for the derivation and setting of EQSs in attempts to relate such standards to changes in environmental quality of the disposal site.

It is stressed that microbiological data should not be used in isolation to demonstrate whether unacceptable changes or effects are occurring at disposal sites. Such data should be used to augment other discrete and quantitative monitoring data, such as sediment chemistry and benthic analysis, to link environmental perturbation with sewage-sludge settlement on the sea bed.

A4.7 Use of microbiological monitoring of sewage-sludge disposal activity for assessment of risk to human health

Bacteriological monitoring of seafoods, such as fish and some shellfish, is not routinely required unless there is epidemiological evidence to support such a requirement at a specific site. Where such analyses are deemed to be expedient, shellfish should be caught and handled by traditional methods ensuring minimal external or internal damage to the organism. Reference documents, as produced by organisations such as the International Council for the Exploration of the Sea (ICES) Copenhagen, should be consulted for statistical aspects of sampling fish.

Authorities which licence disposal of sewage sludge should maintain surveillance for the development of scallop beds in the vicinity of disposal sites.

There is no routine requirement to examine sewage sludge for occupational health reasons unless specific epidemiological evidence exists of a threat to public health. If required, it is recommended that the sludge is examined using methods described by the appropriate expert working group of the Standing Committee of Analysts within the DOE or by a similar competent organisation.

A4.8 Future research

It is recommended that an urgent assessment is made by a competent research organisation of the phenomenon of 'viable but non-culturable' faecal micro-organisms in marine environments and the influence which this may have on the delineation of zones of sewage-sludge deposition at the sea bed.

The potential use of yeasts and amoebae in sewage-sludge monitoring has not been exploited in the UK. However, it is recommended that the utility of these groups of micro-organisms, in particular the Acanthamoeba species, should be further evaluated for use as monitoring tools at UK sewage-sludge disposal sites. The presence of these longer-lived sewage-sludge-associated micro-organisms may better delineate zones of sludge settlement than E. coli or the enterococci in selected disposal sites. Comparison is required between Acanthamoeba species and S-RC spores for long-term impact assessment of sewage-sludge disposal.

In specific instances, sampling of marine biota for micro-organisms derived from sewage sludge could be necessary at specific sites. It may be desirable to demonstrate the extent of sludge contamination of fish guts when investigating fish abnormalities, or examination of the viscera of shellfish used in caged experiments which determine 'scope for growth'. Such studies are, as yet, of indeterminate value in the overall strategy for use of microbiology as a monitoring tool. A limited programme of research and evaluation is required to attempt to correlate abnormal changes with faecal micro-organism counts in marine biota.

A limited research programme is recommended to assess the extent to which fish and crustaceans harvested for human consumption accumulate faecal micro-organisms at sewage-sludge disposal sites in the UK.

Human enteric viruses may occur in very high numbers in sewage sludge and are known to survive for much longer than faecal bacteria in marine environments. In these respects, the culturable enteric viruses (e.g. polio, coxackie, ECHO) have the potential to provide a better long-term integrated picture of the impact and settlement of sewage sludge at the sea bed. However, lack of technical progress in the development of cheap and rapid procedures for environmental virology continues to hamper the routine use of viruses as a microbiological monitoring tool. New advances in techniques for recovery of viruses from the marine environment are being reported. It is recommended that the value of viral monitoring is kept under review, in the light of these developments.

The potential usage, as a monitoring tool, of microbial tracers (such as Bacillus globigii, and bacteriophage) which are specifically added to sewage sludge, should be fully evaluated.

Annex 5 Procedures for monitoring benthic communities at sewage-sludge disposal sites

A5.1 Introduction

Sewage-sludge disposal sites are monitored to detect changes which may occur as the result of the disposal practice. These sites are usually in dispersing areas and generally include soft substrata which facilitates quantitative benthic monitoring. The objectives for this monitoring, and the biological techniques used currently and advocated for future use, are detailed in the full report prepared by the Biology task team*. This summary is extracted from that report and gives the recommended procedures for the sampling, laboratory analysis and data recording and interpretation of the biological communities in disposal areas. Furthermore, the adoption of these procedures is necessary to ensure conformity of monitoring, data treatment and reporting at all disposal grounds. The use of these standardised procedures is also necessary for the eventual derivation and adoption of EQSs relevant to the benthos.

The recommendations made here, which apply to most grounds but exclude features which may be site specific such as the number of stations sampled, are the minimum which are necessary and do not preclude the use of other methods. The main report gives the rationale behind the recommendations, includes many explanatory remarks and must be consulted for further details.

A5.2 Monitoring programme

The benthic macro-infauna is generally the most significant element in the monitoring of biological effects of sewage-sludge disposal. The sedentary, long-lived nature of these organisms allows them to integrate over time the effects of changes in the sediments which act as a sink for most sludge-associated contaminants. As this component is well studied and amenable to quantitative sampling, spatial and temporal trends at the community level, including any anthropogenic effects, can be determined statistically. Because of these features, the recommendations made here primarily concern the macro-infauna, although mobile epifauna and fish should also be assessed.

In the monitoring programme, four stages are required:

(i) a desk study;

(ii) an exploratory survey;

(iii) a baseline survey; and

(iv) an ongoing survey.

However, in some cases, prior knowledge or limited objectives may remove the need for one or more of these stages. Appropriate environmental parameters must be determined concurrently in all surveys to aid the interpretation of biological data.

In an exploratory survey, only a single sample per station need be taken but with further surveys, in which spatial and temporal statistical

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* To be published in the future, in the Scottish Fisheries Research Report series.

comparisons are to be made, then 3-5 replicates are needed. In the exploratory survey, qualitative or semi-quantitative techniques will determine the extent and intensity of sampling. In general, unless recruitment patterns are being assessed, sampling must occur annually at the same time of year to minimise seasonal variability.

The selection of stations should include both the area of potential impact and a comparable reference area. In any heterogeneous area, a stratified random-sampling strategy will need to be adopted. However, where a grid or transect survey is used, sufficient stations should be included to give at least two stations per habitat type.

A5.3 Sampling procedures

A5.3.1 Demersal fish and benthic epifauna

At sites selected for sampling, a minimum of 3 tows should be taken using either a 2 m beam or Agassiz trawl of a standard design. The distance should be recorded and for most marine conditions should be > 500 m. Still photography and television surveys may provide valuable complementing information. Otter and/or beam-trawls should be used to assess sea-bed sewage debris and to provide fish and epifauna for chemical and histo-/patho-/micro-biological analyses as required. The catch composition (species, abundances of all organisms taken), the length range of fish, details of sewage debris and other unusual features should be recorded. Specimens should be retained for further study as necessary.

A5.3.2 Benthic fauna of hard substrata

Hard substrates are those which are not amenable to quantitative sampling by grab. Such substrates require greater ingenuity in sampling, though the trawls mentioned above will often be suitable for sampling the epifauna. On stony or rocky ground, there is a need for more robust sampling gear. Anchor and naturalist's dredges will provide qualitative information and underwater photography may be used to good effect on these grounds. The selection of large organisms, such as sedentary bivalves, for ecological and bioaccumulation studies can be useful.

A5.3.3 Benthic infauna of soft substrata - quantitative assessment

A 0.1 m^2 Day or van Veen grab, which has lifting flaps for sediment-surface access, should be used and the grab sample volume should be recorded; samples of less than ca. 2.5 litres (5 cm bite-depth) should be discarded. The sediment surface features, smell, texture and colour should be noted for each sample. The grab contents should be placed in a hopper and agitated with sea water and then gently sieved through a 1 mm mesh. However, for the assessment of recruitment or possibly because of local conditions and/or results from previous surveys, a 0.5 mm mesh will be necessary. Delicate or enmeshed specimens should be removed using forceps and the sieve residue should then be fixed in stained and buffered 10% formalin in sea water.

A5.4 Laboratory procedures for benthic infauna

A5.4.1 Sorting

A sieve, at least as fine as that used in the field, should be used when washing the sieve residue to remove the preservative/fixative under forced ventilation. The sample should then be agitated repeatedly with fresh water and the supernatant liquid poured through a mesh to extract all light material. The resulting light and heavy fractions should be placed under water and sorted against a contrasting background and with good illumination. A binocular microscope or illuminated magnifier must be used to sort samples taken with < 1 mm mesh. The extracted fauna should be preserved in either a buffered 5% formalin in sea-water solution or an alcohol/glycerol mixture. In addition, a representative sample of fruit seeds and other sewage-derived artefacts should be retained and counted during sorting. All containers must be well labelled and a record of sorting details should be maintained.

A5.4.2 Identification and recording

In this rapidly-changing field, workers must ensure that taxonomic literature is up to date and that a reference collection is maintained. In order to ensure comparable nomenclature, a standard classification should be used and workers must participate in the formal circulation of literature and in workshops and intercalibration exercises as available. The abundances of complete specimens should be recorded although, in the case of fragments, only anterior ends of specimens should be counted. The colonial, sessile epibenthic taxa (e.g. hydroids, bryozoans) and 'headless' fragments should be recorded on a presence/absence basis. Taxonomic and other information should be recorded and the final data must be compatible to computer entry. In order to use the benthos to assess subtle changes in the environment, specimens should be identified to species level where possible. However, a lower level taxonomic separation can be used in exploratory surveys or to assess gross changes.

A5.4.3 Biomass determination

As biomass changes during preservation, specimens should be left for 3 months to stabilise and then the wet-weight (paper-blotted dry) biomass of the individual taxa and whole samples should be determined. Fragments of specimens should be included in the biomass determination at the highest recognisable taxonomic division. Following this process, wet-weight to dry-weight to ash-free-dry-weight (AFDW) conversions should be derived on representative sub-samples and then applied to the data.

A5.5 Data analysis/archiving/reporting

The data analysis aimed at identifying biological change due to the sludge disposal should answer specific questions, i.e. 'What is the normal situation' and 'can the natural variability be quantified and accounted for'? 'Has there been a deviation from the normal situation' and 'can the deviation be quantified'? 'Is that deviation significant' and 'what is the cause of the deviation'? A final question - 'what is the acceptable change from the normal situation'? is to be addressed in a future report.

In order to answer these questions, the data set provided by study of the benthic infauna should be ordered and analysed using a range of techniques, each of which will provide part of the necessary information. Each of these techniques are freely and widely available so the mechanics of data analysis should be a minor part of the effort involved in any survey. The analysis of benthic data is a rapidly-developing field and given here is the basic suite of the most suitable currently-available techniques.

A5.5.1 Tabulation of raw data

At the initial stage, the data must be collated, preferably using a coding system based on hierarchical taxonomy, and be in a form compatible with returns to regulatory authorities. A matrix should be constructed of taxon abundance per replicate sample and should include quantitatively - and qualitatively - recorded taxa. Where required, a species/station matrix should be constructed after merging replicates. Similarly, a taxon wet-weight biomass per sample and per station matrix should be constructed and used to tabulate the AFDW biomass using independently-derived conversion factors.

A5.5.2 Detection of dominant species

The species recorded quantitatively should be ranked according to abundance and the top-ten taxa, and taxa contributing to the top 50% of the community, should be listed for merged replicates. This allows an assessment of the major elements of the community at each station.

A5.5.3 Determination of basic community variables

Firstly, the primary variables should be summarised to give the total number of identified and quantified taxa (S), total abundance (A) and biomass (B) per station. Secondly, the derived variables from the quantitative data should be calculated; these are the ratios A:S and B:A, which will also identify dominance by certain-sized individuals, and the indices for diversity (Shannon-Weiner, H') and evenness (Pielou, J); H' and J should be computed according to $\log_2$.

A5.5.4 Graphical representation of the community structure

In the case of gradients across disposal grounds, SAB curves should be constructed showing the trends in the major variables. In addition, as a complement to single-figure diversity indices, graphical representations of the community structure should be used to compare stations in time or space. These methods take the form of species/area or ranked species/abundance (or biomass) curves. Rarefaction curves (based on the Sanders method or using the Hurlbert modification) can be constructed for abundances of all species or for species within certain major taxa (e.g. bivalves and polychaetes) in the total community. Another method, the abundance-biomass-comparison (ABC), which uses a k-dominance curve constructed for the abundance and biomass per logarithmically-ranked taxon per station, is currently being evaluated and may produce further information.

A5.5.5 Assessment of inter-station and inter-species relationships

There are many multivariate methods which can be used to group stations according to their faunal attributes and to give species associations according to their distributions. It is essential that several multivariate techniques are used in order to assess the robustness of any associations produced. Also, with all of these techniques, transformation (using log (x + 1) or root-root) and truncation (removal of rare species) of the data should be used as necessary. The methods are divided into classification and ordination techniques. The first recommended classification technique computes the Bray-Curtis similarity measure based on the quantitative data; then a dendrogram should be constructed by the group-average sorting technique. Secondly, the Jaccard similarity measure should be used on the presence/absence records of the total data set and again the dendrogram constructed by the group-average sorting technique. Thirdly, for the simultaneous classification of both samples and taxa, two-way indicator species analysis should be used. This latter classification technique has the further advantage of identifying the species responsible for the separation of station groupings.

The classification techniques should be complemented by the use of an ordination technique, of which two are recommended. These techniques are detrended correspondence analysis and non-metric multi-dimensional scaling and each analysis includes the construction of an ordination plot.

A5.5.6 Assessment of the relationship between faunal and environmental parameters

In order to correlate the faunal and environmental attributes of each station to explain the faunal patterns within the data, non-parametric correlation analysis should be used for environmental variables' data and ordination scores at each station. Also, the environmental data per station should be overlaid onto the ordination plots, identifying station similarities according to their faunal attributes. Finally, dendrograms produced according to the faunal data can be compared with those produced by the environmental data, to give an assessment of the relationship between the two data sets.

A5.5.7 Determination of the significance of spatial and temporal changes

It is necessary to assess the significance of temporal and inter- and intra-site differences identified by the above techniques in the primary and derived parameters. Statistical significance should be identified by non-parametric or, after transforming the data, parametric analyses of variance and covariance. In order to identify the particular stations or years responsible for those differences, multiple-range testing should be used. Also, the significance of changes in patterns generated by multivariate techniques should be assessed.

A5.5.8 Report content

In addition to the data interpretation, each survey report should contain all raw data, taxonomic and faunal notes and all method details, such that the report is free-standing. The report should be freely available.

A5.6 Final comments

The main report, from which the above procedures are taken, reviews the techniques currently in use and makes extensive recommendations concerning a standard approach for monitoring disposal grounds. These procedures are considered to be the best practice for detecting change in the sea-bed communities of the grounds. Many aspects of the monitoring of the biological effects of sludge disposal warrant further study and are discussed more fully in the main report. The study of the benthos will identify change at the community and population levels but that change may also be detected at the individual and sub-individual levels. The latter topics will be addressed by a future task-team.

Annex 6 Procedures for monitoring metals in sediments at sewage-sludge disposal sites

A6.1 Introduction

The main purposes of monitoring metals in sediments at sewage-sludge disposal sites are investigations of the following parameters:

(a) identification of the spatial extent of the impacted area;

(b) quantification of the intensity of the impact; and

(c) subsequent recognition of temporal trends in the extent or intensity of impact.

The main elements of a chemical monitoring programme are as follows:

(i) design of the sampling programme. This would be subject to agreement between the licensing authority and the licensee, would be site specific, and not appropriate for harmonisation at this stage;

(ii) sample collection;

(iii) sample preservation;

(iv) sample preparation;

(v) extraction/separation of the contaminants of interest;

(vi) estimation of the contaminants.

Steps (ii), (iii), (iv) and (v) above should be carried out by standardised procedures whilst techniques at (vi) should be comparable with each other.

A6.2 Sample collection

The main concern in routine programmes is the surface sediments. There is a body of opinion that wholly-undisturbed surface sediments can only be obtained by the box corer. The use of a box corer (Reineck or similar) is unrealistic (in present circumstances) for routine surveys; suitable samples can be obtained from grabs (Day, van Veen or Smith-McIntyre). The grab must be of a top-opening design, of around 0.1 m^2 area, and all surfaces which come into contact with the sample should be made of stainless steel; galvanised or plated steels should be avoided. In certain sediments, the presence of cobbles or larger particles may prevent the jaws of these grabs from closing correctly. In such circumstances, a Shipek grab may be used, although it is likely that losses of fine material will occur with this method. Advice on a suitable design of Shipek grab can be obtained from the MAFF, Fisheries Laboratory, Burnham-on-Crouch. Whenever possible, the grab sample should be sub-sampled by inserting a transparent plastic core tube vertically into the central area of the surface of the sample. This core sub-sample should be extruded from the top of the core tube, and the top 1 cm removed using stainless steel or plastic implements. In appropriate sediments, a Craib corer or box corer may also be used. In sediments where it is not possible to insert a core tube, a surface sample should be taken using a stainless steel or plastic scoop. Details of appropriate sub-sampling techniques for Shipek grab samples are obtainable from the MAFF Fisheries Laboratory, Burnham-on-Crouch. For routine surveys, information on depth profiles in sediments would not normally be required. Grabs would be unsuitable sampling tools for such studies.

It is particularly important to consider and avoid possible sources of contamination of samples. Galvanised or plated steels have already been mentioned, but other sources include rust, which can include metals other than iron, and oils and greases. Equipment should not be excessively lubricated, and it should be noted that some greases in use at sea can contain significant amounts of metals.

A6.3 Sample preservation

For most purposes, samples should be stored deep-frozen in plastic or glass containers. There have been reports of the migration of mercury through plastic bottles during storage of water samples. Thus, although there do not appear to have been reports of similar losses of mercury from sediments, it is thought to be advisable to store samples for mercury determination in glass containers, away from likely sources of mercury contamination. Chemical treatment at sea is not considered to be feasible.

A6.4 Sample preparation

Where possible, chemical analysis should be carried out on the < 63 µm size fraction. In coarse sediments where very little or no material of < 63 µm is present, the < 2000 µm fraction should be taken. In either case, a nylon sieve should be used to make the separation. In circumstances where normalisation of data on a chemical basis is intended (see Section A6.5), whole sediment analysis would be appropriate. When size fractionation was carried out, there was not seen to be any benefit in analysing the < 63 or < 2000 µm fractions.

There are two approaches in use for size fractionation by sieving, and subsequent sub-sampling for analysis:

(a) wet-sieving. The sediment is sieved wet, and the resulting slurry of fine particles is vigorously mixed, and sub-samples of the homogeneous suspension are taken for subsequent analysis; and

(b) dry-sieving. The sediment is freeze-dried, disaggregated, and sieved dry. The fine material is collected, and sub-samples for analysis are taken by weight.

The dry-sieving procedure is recommended as preferable because of the possible effects on weakly-bound trace elements of washing with non-saline water during wet-sieving, and concern over the precision of the wet sub-sampling procedure in inexperienced hands. Not all monitoring agencies may currently have freeze-drying facilities available and it should be noted that air or low-temperature drying of fine-grained sediments would not provide material which would readily disaggregate for subsequent sieving.

Laboratories experienced in wet-sieving may wish to retain this procedure, but should ensure that their results are directly comparable with those obtained by freeze-drying and dry-sieving.

A6.5 Extraction/separation of the contaminants of interest

The primary elements of concern are as follows:

(a) lead, cadmium, zinc, copper, nickel, chromium;

(b) mercury;

(c) arsenic.

Determination of the total metal content of the sediment requires either complete digestion (employing hydrofluoric acid), or determination by a technique such as X-ray fluorescence. Total metal concentrations will exceed those introduced by sludge disposal, and will include metals held in lattice positions in minerals (and highly unlikely to be available to biota). On the other hand, total concentrations are necessary if normalisation of data against 'conservative' elements (such as Al, Ti, Sc, Li) is to be carried out. Total digestion is not recommended in routine monitoring, but it does have a role in research projects.

A6.5.1 Extraction of group (a) - elements - (Pb, Cd, Zn, Cu, Ni, Cr)

Group (a) elements should be extracted from sediments by strong mineral acid digestion. After reviewing the wide range of available procedures, and recognising that differences between procedures are likely to be small, two procedures seem preferable:

(a) *aqua regia* (HNO_3/HCl) digestion; or

(b) nitric acid/hydrogen peroxide digestion

Aqua regia methods for sediment analysis have been described by the Standing Committee of Analysts (DOE, SCA, 1987) and by Harper *et al.* (1989). HNO_3/H_2O_2 would more effectively destroy organic materials, but was a rather more time consuming method. MAFF undertook a series of digestions of duplicate sediment samples from Liverpool Bay to compare *aqua regia* and HNO_3/H_2O_2 procedures, and found no significant differences in the amounts of copper, zinc and lead extracted. However, in keeping with advice from the ICES Advisory Committee on Marine Pollution, 1989 (in press), it is recommended that *aqua regia* digestion should be used. It should be noted that, due to the common occurrence in sediments of chromium in the mineral chromite, extraction of this element from sediments will normally be incomplete, but should be reproducible. Pending results of the planned intercomparison exercises, the methods described by Harper *et al.* (1989) or in the booklet 'Methods for the determination of metals in soils, sediments, and sewage sludge and plants by hydrochloric-nitric acid digestions', (DOE, SCA, 1987), should be adopted.

A6.5.2 Extraction of group (b) element - mercury

Methods discussed for mercury extraction included digestion with:

(a) acidic $KMnO_4$;

(b) bromine monochloride (potassium bromate in hydrochloric acid);

(c) HNO_3/H_2O_2;

(d) *aqua regia*.

All of the above methods have been used successfully in some laboratories, but it was agreed that there was considerable attraction in a procedure applicable to other elements of interest, apart from mercury. The Standing Committee of Analysts (see above) has recommended the use of *aqua regia* for the extraction of mercury from soils, sediments, and sewage sludge. MAFF has demonstrated the equivalence of *aqua regia* and HNO_3/H_2O_2 digestion for Hg extraction. It was, therefore, agreed that *aqua regia* digestion should be recommended, although the method should be subjected to careful testing in the case of contaminated sediments - particularly to assess the ease with which it could be adopted by inexperienced laboratories (see Section A6.6).

A6.5.3 *Extraction of group (c) element - arsenic*

Digestion with HNO_3/H_2SO_4 (Uthe *et al*., 1974) with added vanadium pentoxide is considered to be an acceptable method.

A6.6 Intercomparison of methods

The methods recommended will ultimately need to be applicable to both experienced laboratories and those entering the field for the first time. Nevertheless, it has been decided that initial intercomparison work should be confined to the relatively small group of laboratories represented on the task group, to check the recommendations. This intercomparison is being conducted in the following three parts, reflecting the division of the whole analytical task:

(a) sieving by individual laboratories, digestion and measurement to be carried out centrally;

(b) digestion to be carried out by individual laboratories, and measurement centrally;

(c) sieving, digestion and measurement to be carried out individually.

Sediments, for the inter-comparison of procedures, were collected as follows:

(i) Garroch Head, DAFS, June 1988;

(ii) Forth estuary, LRC/FRPB, May 1988.

Several kilograms were collected from each site and stored deep-frozen. They were then to be used as follows:

A6.6.1 *Assessment of comparability of sieving procedures between laboratories*

Ten kilograms of sediment from Garroch Head would be freeze-dried (DAFS) to provide approximately 3 kg of dry material. This would be split into six equal portions for the participating laboratories (DAFS). Each laboratory would disaggregate its sample, and split it into six equal portions. Each portion would then be sieved separately, and the fine

(< 63 μm) material sent to the central laboratory for analysis (using the agreed procedures) for all of the elements listed in Section A6.6. Analysis was to be carried out by the WRC.

The timetable established for this part of the intercomparison exercise was as follows:

(a) dried sediment to be distributed the week beginning 19 September 1988;

(b) sieved samples to be submitted to the central laboratory by 7 October 1988.

(c) analysis to be completed by the central laboratory by the end of October 1988.

A6.6.2 Assessment of comparability of digestion procedures between laboratories

Sufficient sediment from Garroch Head would be freeze-dried and sieved (DAFS) to provide 250-300 g of material < 63 μm. This would be divided between the participating laboratories. Each laboratory would prepare six replicate digests for the elements of concern, and submit the digests to the central laboratory for estimation of the metal content.

This part of the intercomparison exercise could not proceed until satisfactory results had been obtained from the first part of the exercise which, as of December 1988, was still in progress.

A6.7 References

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