ENVIRONMENT AGENCY

The Microbiology of Drinking Water (2004) - Part 11 - Taste, odour and related aesthetic problems

Methods for the Examination of Waters and Associated Materials

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Methods for the Examination of Waters and Associated Materials

This booklet contains advice and guidance on the likely microbiological causes of taste, odour and related aesthetic problems in drinking waters.

Within this series there are separate booklets dealing with different topics concerning the microbiology of drinking water. Other booklets include

The Microbiology of Drinking Water (2002)
Part 1 - Water quality and public health
Part 2 - Practices and procedures for sampling
Part 3 - Practices and procedures for laboratories
Part 4 - Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7)
Part 5 - A method for the isolation and enumeration of enterococci by membrane filtration
Part 7 - Methods for the enumeration of heterotrophic bacteria by pour and spread plate techniques
Part 8 - Methods for the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa* by membrane filtration
Part 10 - Methods for the isolation and enumeration of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment

The Microbiology of Drinking Water (2004)
Part 6 - Methods for the isolation and enumeration of sulphite-reducing clostridia and *Clostridium perfringens* by membrane filtration
Part 9 - Methods for the isolation and enumeration of *Salmonella* and *Shigella* by selective enrichment, membrane filtration and multiple tube most probable number techniques
Part 12 - Methods for the isolation and enumeration of micro-organisms associated with taste, odour and related aesthetic problems

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soil (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical and physical methods
4 Metals and metalloids
5 General non-metallic substances
6 Organic impurities
7 Biological methods
8 Biodegradability and inhibition methods
9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with this booklet are listed at the back of the booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods and details on how to obtain copies are available from the Agency's web-page (www.environment-agency.gov.uk/nls) or from the Secretary.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood
Secretary

February 2004

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

Taste, odour and related aesthetic problems

1 Introduction

Organoleptic problems in drinking waters can be caused by a number of factors. These include: natural products in waters used for abstraction; compounds formed during treatment, storage or distribution; and ingress of materials (to distribution systems) that either react with compounds in the water or are responsible for tastes and/or odours. Many problems related to unacceptable tastes and/or odours (experienced by water undertakers and their customers) are caused by chemical substances. The majority of these substances are used or produced as a consequence of disinfection processes. Taste and/or odours and related aesthetic problems caused by micro-organisms can be significant, particularly for certain types of water sources. Treated water contains a variety of substances, including metabolites from bacteria, micro-fungi and yeasts indigenous to its source and the surrounding soil and vegetation. Often, these substances do not necessarily give rise to water quality problems. As well as causing objectionable tastes and/or odours, the activities of micro-organisms may result in a deterioration of water quality leading to discolouration or other changes. Micro-organisms may also play a role in corrosion processes that occur within water distribution systems. This booklet provides advice and guidance on assessing the most likely microbial causes of taste and/or odour problems. Methods for the detection and identification of micro-organisms are described elsewhere(1). Those for chemical substances and algae are covered elsewhere within this series.

2 Tastes and/or odours in drinking waters

The occurrence of substances which impart undesirable tastes and/or odours in drinking waters is one of the principal causes of complaint by consumers. These substances may be present as a result of artificial or natural processes, and often result from microbial growth and metabolism. The major causes of taste and/or odour problems associated with drinking water supplies are biological activity in source waters, especially by algae; disinfectants used in water treatment, notably chlorine and derived compounds; and biological activity within distribution systems(2, 3). Tastes and/or odours may also be associated with substances present in construction materials and linings, or by the leaching of industrial chemicals into the supply.

One of the most commonly reported complaints made by consumers related to microbial activity is the occurrence of earthy or musty tastes and/or odours. These tastes and/or odours are primarily associated with the production of two compounds, namely geosmin and 2-methylisoborneol. These compounds are metabolites produced by a range of micro-organisms, most notably the actinomycetes (for example *Streptomyces*, *Nocardia* and *Microbispora*), the cyanobacteria (blue-green algae, for example *Oscillatoria, Anabaena* and *Aphanizomenon*) and a number of species of algae (particularly members of the Chlorophyceae and the Bacillariophyceae). These compounds may be released by organisms which are actively growing or through cell lysis and decomposition, and have very low odour threshold concentrations (typically, 0.015 $\mu g l^{-1}$ for geosmin and 0.02 $\mu g l^{-1}$ for 2-methylisoborneol)(4).

A number of tastes and/or odours can also be associated with the metabolism and decomposition of actinomycetes and algae. These tastes and/or odours range from fishy, grassy and woody tastes and/or odours caused by metabolites of sulphur compounds released during decomposition. Sulphur tastes may also be related to microbial reduction of sulphates under anaerobic conditions. Another problem, of an aesthetic nature, is discolouration caused by release of iron compounds resulting from microbial activity in cast-iron mains or iron-rich waters.

Sources of microbially-mediated taste and/or odour and aesthetic problems can be broadly categorised into the following four groups.

2.1 Metabolites produced by microbial activity

As described previously, geosmin and 2-methylisoborneol are produced by members of the actinomycetes, cyanobacteria and green algae, and these organisms are the main sources of earthy and musty tastes and/or odours in drinking waters. These metabolites may be produced in source waters and pass through treatment processes into supply, or may be formed as a result of growth of actinomycetes within a matrix of micro-organisms (a biofilm) growing on the walls of pipes, or on sediments and other deposits within distribution systems. In addition, the presence of moulds has been associated with taste and/or odour problems[5]. There are limited data on the production of geosmin, 2-methylisoborneol, or other taste and/or odour compounds by fungi isolated from water supplies. Geosmin production has been demonstrated for *Chaetomium globosum* and *Basidiobolus ranarumi*[2]. Other metabolites from actinomycetes which impart tastes and/or odours are cadin-4-ene-1-ol (woody/earthy odour) and 2-isopropyl-3-methoxypyrazine (musty/mouldy potato odour)[2].

Various odours have been associated with algae, particularly members of the cyanobacteria. Fishy odours have been related to the production of aldehydes (for example, n-hexanal, n-heptanal and isomers of decadienal) and sulphur-containing compounds. Other substances produced by algae imparting tastes and/or odours are terpenes, aromatic compounds and esters. Fishy odours have also been associated with large populations of some zooplankton (for example, the rotifer *Keratella* and the crustaceans *Cyclops* and *Daphnia*). It is unclear, however, whether the sources of these odours are derived from metabolites produced by these organisms or compounds generated during decomposition of the organisms. Nematodes have been reported[6] to secrete odorous compounds, one of which, when isolated from culture, gives an earthy/musty odour. It has also been reported[6] that some amoebae can cause tastes and/or odours. The significance of these latter sources of taste and/or odour compounds to drinking water supplies remains unassessed.

2.2 Products from microbial decomposition

Bacterial decomposition of organic matter can lead to the production of a range of products, including sulphur-containing compounds, many of which impart tastes and/or odours to waters. Decomposition of algae, particularly following a cyanobacterial bloom in source waters, can result in significant concentrations of mercaptans, dimethyl sulphide and polysulphides, and other volatile sulphur compounds which impart fishy, swampy or septic odours. Some of these compounds are also produced by the putrefaction of proteinaceous material within distribution systems. Species of *Pseudomonas*, *Flavobacterium* and *Aeromonas* have been reported[7] in the production of dimethyl polysulphides resulting in swampy odours. These bacteria have been shown to be capable of producing a number of volatile sulphur compounds, particularly dimethyl disulphide[2]. The mould, *Penicillium caeseicolum*, is also capable of producing dimethyl disulphide[2]. Other odorous compounds are indole and skatole (from tryptophan), several amines (for example, putrescine, cadaverine and β-phenylethylamine) and fatty acids (for example butyric, propionic and stearic acids).

2.3 Hydrogen sulphide from reduction of sulphate

The presence of a microbial population capable of reducing sulphate to hydrogen sulphide can result in a distinctive rotten-egg odour being associated with the water. Under anaerobic conditions, sulphate-reducing bacteria (for example, *Desulfovibrio desulfuricans* and *Desulfotomaculum orientis*) are able to reduce sulphate to sulphite and then to hydrogen sulphide. In distribution systems, other bacteria (for example, species of *Clostridium* and *Bacillus*) may also be involved in the reduction of sulphite to hydrogen sulphide[8]. Further information on the microbial reduction of sulphates can be found elsewhere[9, 10]. Clostridia are also capable of degrading proteins containing sulphur, thereby releasing hydrogen sulphide. Rotten-egg odour problems are often associated with deep groundwater supplies and corrosion of pipes in systems with very low flow rates.

2.4 Discolouration due to iron salts

Microbially-induced corrosion of iron pipes occurs during the reduction of sulphate in the presence of iron resulting in the production of iron(II) sulphide. The subsequent oxidation of this compound to iron(III) oxide (for example, during the growth of iron-precipitating bacteria such as *Gallionella ferruginea*) can result in elevated levels of iron in the water. This may cause discolouration, particularly when clothes are washed in the water or, more dramatically, the production of 'red water'.

The structures of some of the compounds causing tastes and/or odours are shown in Figure 1.

3 Investigation of microbially-mediated taste and/or odour and related aesthetic problems

When attempting to ascribe a microbiological cause to a taste and/or odour problem, adequate evidence should be gathered to establish the possible relationship. Although a microbial cause may be suspected, it is often very difficult to isolate the causative organisms, particularly when the taste and/or odour problem is identified in a location some distance from the original site of production.

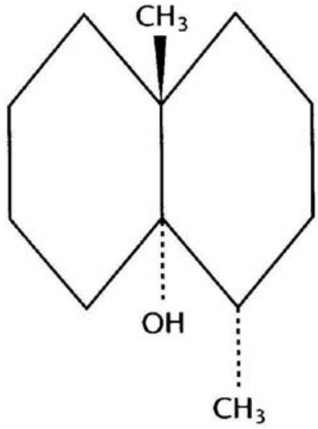
The principal steps to be taken to establish the source of microbially-mediated taste and/or odour problems include the following.

(i) The location and definition of those parts of the distribution system affected by the problem. This may include a qualitative survey to confirm the characteristics of the tastes and/or odours reported by consumers. An assessment of the location and timing of consumer complaints (taking into account likely patterns of water flow) may also be useful in helping to determine whether the cause is microbially-mediated.

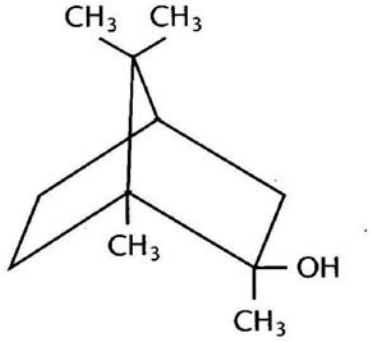
(ii) If, on the basis of any preliminary assessment, it is considered likely that the problem is microbiological in origin, then the types of organisms that may be involved can be selected for investigation. In addition, individual tastes and/or odours can facilitate the identification of possible causes. The isolation and, where feasible, enumeration of the suspected organisms can then proceed.

(iii) Confirmation of the identity of the organisms and, where feasible, confirmation that the organisms are capable of producing the compounds associated with the reported tastes and/or odours. Metabolites from the growth of the organisms may also be recovered and analysed by gas chromatography-mass spectrometry.

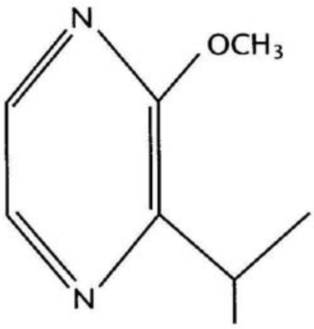
Figure 1 Structures of various compounds associated with taste and/or odour problems



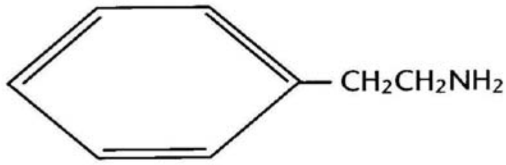
Geosmin



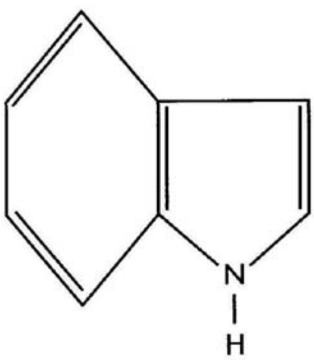
2-Methylisoborneol



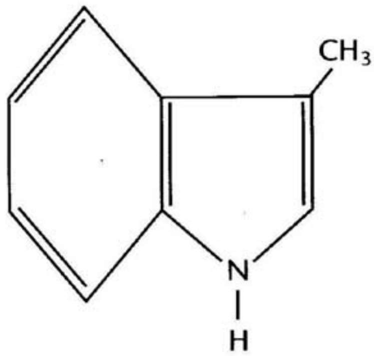
2-Isopropyl-3-methoxypyrazine



ß-Phenylethylamine



Indole



Skatole

$NH_2(CH_2)_4NH_2$

Putrescine

$NH_2(CH_2)_5NH_2$

Cadaverine

When investigating microbially-mediated taste and/or odour and related aesthetic problems, the major groups of organisms to consider include

(i) Heterotrophic colony count bacteria
(ii) Actinomycetes (particularly *Streptomyces* and *Nocardia*)
(iii) Micro-fungi and yeasts
(iv) Sulphate-reducing bacteria (*Desulfovibrio* and *Desulfotomaculum*)
(v) Sulphite-reducing bacteria (*Clostridium*)
(vi) Iron-precipitating bacteria (*Gallionella* and *Leptothrix*)

The choice of organisms to investigate will depend on the assessment of the type of tastes and/or odours present and the nature of the problems encountered. Methods for the detection or enumeration of these micro-organisms are given elsewhere in this series[1, 11, 12].

4 Assessment of tastes and/or odours in drinking water

The receipt of consumer complaints regarding the quality of tap water is often the first indication of taste and/or odour problems in drinking water. These complaints vary in their nature, frequency and timing, and many may result from intentional changes brought about in the source, treatment or distribution of water supplies. These changes include:

(i) the type of source waters (for example surface water and groundwater) and the mixing of these waters in distribution systems;

(ii) the water treatment process, particularly the disinfection process and amounts of residual disinfectant;

(iii) seasonal and weather-related effects, for example stratification within reservoirs, run-off into reservoirs and rivers following heavy rainfall (particularly after a prolonged, dry period);

(iv) the extent and complexity of the distribution system, residence times, secondary disinfection, materials of construction, etc; and

(v) the nature of plumbing within individual buildings, contamination from storage tanks or cross-connections.

There are various causes of objectionable tastes and/or odours in drinking waters. Only some of these are microbiological in origin. An initial assessment of the problem should be carried out to determine the likelihood of the problem being microbially-related. A key factor may be the description of the taste and/or odour being experienced by consumers. Several of the more common taste and/or odour problems arising from micro-organisms are very specific and require little further information before proceeding to microbiological or chemical analysis, for example, the presence of the earthy or musty odour of geosmin or 2-methylisoborneol from the growth of actinomycetes.

There may be localised contributing factors affecting the perceived taste and/or odour of the water and the following should be considered where appropriate:

(i) Anti-splash nozzles (particularly those made from rubber or plastic) that are in a visible state of deterioration. Bacterial and fungal action can give rise to breakdown products from such attachments, which, by themselves or in combination with chlorine in the water, can cause offensive tastes and/or odours.

(ii) Rubber and plastic hoses of the type used to fill drinking water tanks on coaches, caravans, trains, etc may give rise to taste and/or odour problems, as can hoses used to fill drinks-vending machines.

When considering whether a taste and/or odour problem is microbially-related, reference should be made to the descriptions given in Table 1 and elsewhere(3, 13). If the descriptions provided by consumers are non-specific or contradictory, the analysis of samples for qualitative taste and odour may help to define whether the cause is microbiological. However, health and safety considerations should be taken into account. Consideration should be given to the collection of further samples, including those for physical and chemical analysis, and samples from neighbouring properties, if appropriate.

5 Aesthetic problems in drinking water

In addition to problems of tastes and/or odours in drinking water, micro-organisms may also be involved in other processes which can give rise to changes in water quality. These include:

(i) Discoloured water, or staining of appliances, caused by the presence of iron-precipitating bacteria.

(ii) Microbially-induced corrosion of materials and fittings leading to their discolouration and possible failure, the most common form being due to the activity of sulphate-reducing bacteria.

5.1 Iron-precipitating bacteria

Iron-precipitating bacteria (iron bacteria) are a diverse group of micro-organisms widely distributed in fresh and marine waters and in soil. They are capable of transforming soluble iron (and occasionally manganese) into an insoluble form that can cause fouling in boreholes, water treatment plants and distribution systems. Iron bacteria convert soluble iron(II) to insoluble iron(III) which is then deposited within or on the exterior of the bacterial cell.

Bacteria that have been associated with fouling and discoloured water include the following.

(i) The sheathed bacteria that produce sheaths of oxidised iron (and occasionally manganese) surrounding the cells. Oxidised iron, or manganese, is deposited outside the sheaths. The most common examples are *Leptothrix* and *Clonothrix*. Another species, *Sphaerotilus*, does not deposit insoluble iron or manganese on the outside of the sheath but may form large flocs.

(ii) Stalked bacteria, such as *Hyphomicrobium*, *Caulobacter* and *Gallionella*, which may have appendages. *Gallionella* form long, spirally-twisted stalks arising from the centre of the cell. *Hyphomicrobium* and *Caulobacter* may oxidise manganese.

(iii) Bacteria of the Genus *Thiobacillus* are capable of oxidising both sulphur and iron(II).

Bacterially-mediated iron precipitation may give rise to taste and/or odour problems, but more usually result in discoloured water and occasionally, frothing. In distribution systems, the presence of iron bacteria may increase disinfectant demand. Biofilms may develop around iron bacteria and accelerate corrosion of susceptible materials.

Table 1 Sources of the principal tastes and odours encountered in drinking water

Taste and/or odour description	Source	Compound(s)
earthy	actinomycetes, cyanobacteria, micro-fungi	geosmin
musty	actinomycetes, cyanobacteria, micro-fungi	2-methylisoborneol
mouldy, musty	actinomycetes	2-isopropyl-3-methoxypyrazine
woody, earthy	actinomycetes	cadin-4-ene-1-ol
mouldy, musty, "TCP"	methylation of chlorophenols by actinomycetes and micro-fungi	chloroanisoles, especially 2,4,6-trichloroanisole
grassy	green algae	cis-3-hexen1-ol
geranium-like	diatoms	diphenyl ether, trichloramine
cucumber	green algae	trans-2 and cis-6-nonadienal
fruity, fragrant	ozonation	aldehydes (C_7 and above)
petroleum-like or solvent-like	Permeation of petrol, diesel and similar products through plastic pipes	hydrocarbons; 1,3-pentadiene
fishy	green algae, diatoms	n-hexanal; n-heptanal
cod liver oil	green algae	decadienal
fishy	*Dinobryon* (algae)	hepta- and deca-dienals
malodourous sulphur	decomposing cyanobacteria	mercaptans
swampy, fishy	*Pseudomonas* species	dimethyl polysulphides, especially dimethyl trisulphide
rotten eggs	sulphate-reducing bacteria, clostridia	hydrogen sulphide
swampy, swimming pool	chlorination of amino acids	aldehydes (low molecular weight)
medicinal, "TCP"	chlorination of phenol	chlorophenols
medicinal	chloramination	iodinated trihalomethanes
plastic, burnt plastic	polyethylene pipes	phenolic anti-oxidants
chlorinous	disinfection of water	chlorine (free, monochloramine)
ozonous	disinfection of water	ozone (in solution)
swimming pool	disinfection of water	dichloramine

5.2 Microbially-induced corrosion

Sulphur bacteria in water systems are nuisance organisms that cause severe taste and/or odour problems as well as contributing significantly to corrosion, with subsequent discolouration and failure of materials. Under anaerobic conditions, certain sulphate-reducing bacteria produce sulphide and hydrogen sulphide gas. This may trigger electrolytic corrosion on metal surfaces. Under aerobic conditions corrosion may be caused by sulphuric acid produced by sulphur-oxidising bacteria. Biofilm organisms present in the same location may enhance the corrosion by producing organic acids which may also be corrosive.

The main groups of sulphur bacteria include:

(i) Sulphate-reducing bacteria, for example *Desulfovibrio*, *Desulfotomaculum*;

(ii) Sulphur-reducing bacteria, for example *Desulfuromonas*;

(iii) Sulphur-oxidising bacteria, for example *Thiobacillus* (which may also oxidise iron);

(iv) Colourless sulphur bacteria, for example *Beggiatoa*, *Thiothrix*; and

(v) Sulphur-oxidising photosynthetic purple and green sulphur bacteria, for example *Chlorobium* and *Chromatium*.

6 Sampling

The investigation of taste and/or odour complaints may require the adoption of modified procedures to those used for routine microbiological examination of drinking water supplies(14) and should be tailored to suit the nature of the complaint. The number and type of samples, and their locations, need to be carefully considered. For routine microbiological sampling, it is essential that the samples taken are representative of the quality of the water supplied to the property. This may often not be the case when investigating taste and/or odour complaints, since there may be particular conditions, such as, for example, the physical state of the tap, which contribute to the cause of the complaint.

Before taking a representative sample of the incoming water, it may be appropriate, for example to take samples without flushing, disinfecting the tap or removing anti-splash nozzles. A number of field or on-site tests may also need to be carried out in order to help identify the possible cause of a taste and/or odour complaint. These may include temperature(15) (before and after flushing), qualitative taste and odour(4), and chlorine residuals(16). It may be appropriate to take samples for quantitative taste and odour analysis(4) for ascertainment of the extent and severity of the problem.

If a taste and/or odour problem has been established which is not related to local conditions but is considered representative of the distribution supply, then sampling may need to be extended. This should then include the raw source water, process samples within the treatment plant, the treated water entering supply, and representative samples from within the distribution system. Procedures for sampling from hydrants and drinks-vending machines, and the transport and storage of samples are described elsewhere in this series(14). If the problem is associated with algal growth within a raw water reservoir, it may be necessary to undertake depth sampling (for example, in order to advise on an appropriate draw-off points).

6.1 Distribution systems and field tests

When investigating a complaint of taste and/or odour at a consumer's property, the sampling and field testing regime should be tailored to the specific circumstances of the complaint. Indeed, a sample may not even be required. Guidance[3] and the descriptions listed in Table 1 can be used to ascertain the possible origin of the complaint and the category into which it may fall. These include:

(i) chlorinous tastes and/or odours;
(ii) metallic, chemical, solvent; and
(iii) microbiological.

Microbiological analyses are not, generally, appropriate for situations involving chlorinous tastes and/or odours, and metallic, chemical and solvent tastes and/or odours are not considered here. When taking samples, the procedures used should ensure that samples for microbiological examination are representative and are free from extraneous contamination at the time of collection, and that changes during transportation are minimised or eliminated. For certain parameters, for example free chlorine, the concentration may change as a result of the sampling process. For other parameters, such as temperature, values will change as a result of different conditions of storage. To ensure that meaningful results are obtained, it is necessary to carry out such determinations at the time of sampling.

For complaints thought to have a microbiological cause, the sequence of sampling should be as follows.

6.1.1 Water

Without flushing or disinfecting the tap, or removing any attached fitments or point-of-use devices, a first-draw microbiological sample should be taken. The analyses carried out on this sample should reflect the type of taste and/or odour reported. Typically, an estimate of the number of heterotrophic bacteria should be determined. If the reported taste and/or odour is described as earthy or musty, analyses for Actinomycetes and micro-fungi should be carried out. Coliform organisms are not considered to be a cause of these types of complaints and their examination need not necessarily be included at this stage.

A volume of sample (50 - 100 ml) should also be collected into a second clean bottle. The bottle should not impart a taste and/or odour to the sample, nor eliminate the offending taste and/or odour from the sample. This sample can be used for determining the on-site temperature[15] and qualitative taste and odour[4], although due health and safety considerations should be given to tasting the sample before obtaining a satisfactory microbiological examination report.

Any tap attachments should now be removed and the tap cleaned, disinfected and flushed[14]; a second microbiological sample should then be taken. This sample should undergo the same microbiological analysis identified above. A comparison of the results should then give some indication as to whether the problem is localised. Consideration should also be given to the analysis of other determinands, including coliform organisms, and a biological examination, including algae.

After adequate flushing, the residual chlorine content of the water should be determined[16].

Samples of water and deposits intended for the analysis of sulphate-reducing bacteria should be taken in sterile containers and filled to the top to exclude air. Sodium thiosulphate should not be added as this may react with sulphides present in the sample. The determination of pH, redox potential, oxygen concentration, sulphate and sulphide can yield valuable information when investigating the presence of sulphate-reducing bacteria which are able to grow at a redox potential below –100 mV in the absence of oxygen. The presence of sulphide suggests conditions suitable for growth of sulphate-reducing bacteria and their possible presence.

6.1.2 Deposits and sediments

In certain cases, in addition to water samples, it may be useful to examine sediments and deposits since these may be sources of micro-organisms causing taste and/or odour problems. Various types of deposits may be present in the water supply including slime layers (biofilms), tuberculations and sediments. Special procedures are required for sampling such deposits and the techniques used will depend upon their nature. All equipment (for example swabs, spatulas, scoops, etc) used to collect the deposits, and the containers for transporting the material should be clean and sterile. All material collected should be examined as soon as possible.

Direct access to deposits, for example in storage tanks or service reservoirs, may be possible if appropriate parts of the system are drained during the investigation. Deposits on surfaces can be obtained by scraping or swabbing. Firmly adhering deposits can be obtained by scraping with a spatula and transferring the material to a container. Loose deposits can be collected with a scoop and transferred to a suitable container. Swab samples can be taken using cotton- wool swabs (preferably single-pack in a tube). After use, return the swab to its case or place in a suitable container.

Where direct access is not possible, samples of deposits may be obtained by flushing the system from suitable locations such as hydrants. Procedures for disinfecting hydrants before the collection of samples for microbiological examination are describe elsewhere in this series(14). To collect a deposit, a nylon bag of suitable mesh size can be attached to the stand-pipe and the flow increased to dislodge any deposits. The deposits retained in the bag should then be transferred to a suitable container. The water which passes through the nylon bag should be allowed to flow into a tank to enable any fine sediment to settle. This material can be collected after the water has been discarded(17).

6.2 Raw water reservoirs

Tastes and/or odours can be associated with algal growths within impounding or storage reservoirs, or in rivers used as sources of drinking water. Samples should be taken at depths commensurate with the draw-off level. If it is suspected that the origin of any tastes and/or odours may arise from the activity of actinomycetes, then it may be appropriate to take sediment samples for analysis.

6.2.1 Algae

If it is intended to count the number of algal cells and/or identify the species, then water samples should be placed in appropriate vessels containing a suitable preservative, for example Lugol's iodine(18). Alternatively, an indirect assessment of the algal load can be made by an analysis of the chlorophyll content(19); these samples, however, should not be preserved, but should be analysed as soon as practicable to avoid changes in the chlorophyll content.

6.2.2 Other micro-organisms

Samples intended for the detection or enumeration of micro-organisms other than algae should be placed in a suitable container without further treatment, or use of preservative or neutralising agent.

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However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below.

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