Molybdenum, especially in Sewage Sludges and Soils by Spectrophotometry 1982

Methods for the Examination of Waters and Associated Materials

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Molybdenum in Sewage Sludges and Soils by Spectrophotometry 1982 Version

Methods for the Examination of Waters and Associated Materials

Two methods for the determination of molybdenum based on the spectrophotometric determination of its thiocyanate complex are described in this booklet. The first, Method A, is suitable for application to sewage sludges and the second, Method B, is suitable for application to soils and related materials.

Contents

Warning to users 2
About this series 3
General Introduction 4

A Molybdenum in Sewage Sludges by Spectrophotometry 6
A1 Performance Characteristics of the Method 6
A2 Principle 6
A3 Interferences 7
A4 Hazards 7
A5 Reagents 8
A6 Apparatus 9
A7 Sample Collection, Preservation and Preparation 9
A8 Analytical Procedure 9
A9 Notes on Interference 10
A10 Checking the Linearity of the Calibration Curve 11
A11 Change in Concentration Range of the Method 11
A12 Sources of Error 11
A13 Checking the Accuracy of Analytical Results 11
A14 References 12

B Molybdenum in Soils and Related Materials by Spectrophotometry 13
B1 Performance Characteristics of the Method 13
B2 Principle 13
B3 Interferences 14
B4 Hazards 14
B5 Reagents 14
B6 Apparatus 15
B7 Sample Collection, Preservation and Preparation 15
B8 Analytical Procedure 15
B9 Checking the Linearity of the Calibration Curve 16
B10 Change in Concentration Range of the Method 16
B11 Sources of Error 17
B12 Checking the Accuracy of Analytical Results 17
B13 References 17

Address for correspondence 17
Membership responsible for this method inside back cover

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Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No. 5, The Biochemical Society, London, which includes biological hazards, and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Service Monograph 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasized that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in '*Analysis of Raw, Potable and Waste Waters*'. These volumes took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological methods
9.0 Radiochemical methods.

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and addenda for booklets in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

T A DICK
Chairman

L R PITTWELL
Secretary

3 February 1983

General Introduction

1. The total molybdenum (Mo) content of most soils varies from 0.1 to 5.0 mg/kg with a mean of around 2 mg/kg although values as high as 50 mg/kg have been recorded. The trace element content of soils, including that of Mo, is largely determined by the combined effects of geochemical and pedological soil-forming processes on their parent rocks. Mo in rocks is preferentially accumulated in minerals containing Ti^{4+} and Fe^{3+} such as sphene, ilmenite, titanomagnetite and biotite which, with the exception of biotite, are resistant to weathering. In granitic rocks with normal molybdenum contents, however, much of the molybdenum is contained in moderately stable feldspar minerals, but concentrations greater than about 2 mg/kg may be largely attributable to molybdenite (MoS_2), which may be soluble in hot aqua regia. In mafic rocks excess molybdenum is chiefly associated with the resistant and stable iron-containing minerals mentioned above. After oxidation and release from primary minerals by weathering the molybdenum as molybdate, is soluble and fairly mobile.

2. Molybdenum is essential to the growth of plants in very small amounts but can be toxic to ruminant animals. Excessive amounts of molybdenum in the diet of grazing cattle interfere with normal copper absorption and utilization to produce an induced or conditioned copper-deficiency. The levels of copper and sulphur in the diet are also important factors. Molybdenosis thus tends to arise when the copper to molybdenum ratio in the herbage is low and dietary sulphur is greater than about 2 g/kg DM (dry matter). Because mixed pasture herbages in Britain often contain less than 10 mgCu/kg DM it is desirable that herbage molybdenum contents do not exceed 3 mg Mo/kg DM.

3. The uptake of molybdenum by plants is affected by a number of factors, particularly soil pH, drainage conditions and organic matter content. The effect of liming on molybdenum uptake by herbage cannot be overemphasized. The increase in soil pH produced by liming can raise the molybdenum content in herbage, especially clovers, several fold. Some 2 to 20 per cent of the total molybdenum content of soil is mobile and available to plants depending upon soil conditions. In problem areas neutral molar ammonium acetate generally extracts around 2 per cent of the total soil molybdenum content and because a level of 0.05 to 0.10 mg/kg extractable molybdenum is the borderline between normal and excessive amounts, then soils having total molybdenum contents of 4 mg/kg or greater could be potentially toxic. Workers in Oregon, USA have found molybdenosis in grazing animals on soils containing 0.4 to 6.0 mg/kg total molybdenum, while in Scotland soils in areas where molybdenum is a problem can contain as little as 4 mg/kg with 0.10 mg/kg or greater extractable by ammonium acetate. The disposal of sewage sludges with unusually high molybdenum contents on agricultural land could therefore raise the content in soils to levels which are potentially hazardous to grazing animals.

4. As the amounts of molybdenum in topsoils are normally around 2 mg/kg and soils containing more than about 4 mg/kg may give rise to problems, there is thus a need for a method which will accurately determine total molybdenum in soils containing less than 1 mg/kg. The molybdenum content in soils can be contained in a variety of forms: in resistant, moderately resistant or easily-weathered primary minerals, in secondary minerals or associated with organic matter. A vigorous digestion procedure using for example hydrofluoric acid (HF), which is able to disintegrate primary silicates is therefore necessary to determine the true total molybdenum content at typical levels. In sewage sludges which normally contain 2 to 40 mg Mo/Kg, (mean 7 mg Mo/kg), most of the molybdenum is likely to be present as molybdate associated with organic matter and a less vigorous digestion of the sludge ash with 4M hydrochloric acid (HCl) is therefore satisfactory.

5. In this booklet two methods are described for the determination of molybdenum; Method A is for the determination of molybdenum in sewage sludges and Method B is for

the determination of molybdenum in soils and related materials. For the reasons given above these methods have different acid pretreatment stages. The analytical finish for both methods is similar and is based on the spectrophotometric measurement of the molybdenum thiocyanate complex.

6. Molybdenum can occur in natural waters but at very low concentrations. If necessary, see Ref 7 in Method A.

A Molybdenum in Sewage Sludges by Spectrophotometry Tentative Method (1982 Version)

Note: Throughout this method molybdenum is expressed as the element Mo.

A1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see General Principles of Sampling and Accuracy of Results 1980, also published in this series)

A1.1	Substance determined	All forms of molybdenum likely to occur in sewage sludges.
A1.2	Type of sample	Dried sewage sludges.
A1.3	Basis of the method	The dried sample is ashed, extracted with acid and the molybdenum reacted with thiocyanate ions to form an amber coloured complex which is extracted into 4-methylpentan-2-one and measured by spectrophotometry.
A1.4	Range of application (a)	Up to 50 mg/kg.
A1.5	Calibration curve (a)	Linear to 50 mg/kg.
A1.6	Total standard deviation (a)	See table below.
A1.7	Limit of detection (a)	Approximately 1 mg/kg based on the within batch standard deviation of sludge containing a low concentration of molybdenum (with 15 degrees of freedom).
A1.8	Sensitivity (a)	40 mg/kg gives an absorbance of approximately 0.6.
A1.9	Bias (a)	No important sources of bias have been detected in normal sludges (b).
A1.10	Interferences (a)	None of the substances tested (see section A3) caused appreciable errors at levels likely to be present in normal sludges.
A1.11	Time required for analysis (a)	Excluding the time for ashing (conveniently carried out overnight) the analytical time for 1-100 samples is typically 2-10 hours.

A1.6 Total standard deviation (a)

Sludge sample	Molybdenum Concentration (mg/kg)	Total Standard Deviation (mg/kg)	Degrees of Freedom
1	4.4	0.3	10
2	9.2	0.5	13
3	28.2	0.9	18

(a) These data were obtained at the Water Research Centre, Stevenage Laboratory (1).
(b) This statement is based upon the satisfactory recovery (97-99% with 95% confidence limits between ± 4 and ± 8%) of spiked molybdenum (5.9 to 58.8 mg/kg) from a typical sewage sludge containing 5.5 mg/kg molybdenum.

A2 Principle

Dried sewage sludge samples are ashed to destroy organic matter and the molybdenum present is extracted into hydrochloric acid solution. An aliquot of the acid solution is treated with a complexing solution and then with potassium thiocyanate to form the amber coloured molybdenum thiocyanate complex which is extracted into 4-methylpentan -2-one (MIBK). The extract is measured spectrophotometrically after

washing with stannous chloride to reduce traces of the iron (III) complex. The method is based on modifications of techniques described by Quin(2) and Lillie(3).

A3 Interferences

A3.1 It is not practical to investigate the effect of potential interfering substances by spiking typical sludges because the spiked substance may not be present in the same form as it would have been had it occurred 'naturally' in the sludge and also a vast range of substances and concentrations are likely to be present in sludges.

A3.2 The effect of certain other substances at equivalent concentrations well above those normally encountered in sludges on the complex formation, extraction and spectrophotometric stages of the method has been investigated by the Water Research Centre(1) and the results are presented in Table A1. Most substances tested did not interfere. Vanadium and tungsten interfered and further investigation showed that extractable green/yellow complexes were being formed which absorbed at a slightly lower wavelength and that the tail of these absorbance peaks was just included with the amber/orange molybdenum complex peak at 470nm. However, at the vanadium and tungsten concentrations normally present in sewage sludges there should be no interference. The positive effect with ferric chloride was found to be due to molybdenum in the ferric chloride.

A3.3 Some other substances which might be expected to interfere(4) eg rhenium were not tested because they are unlikely to be present in significant concentrations in sludges. Although the interference results must be treated with some caution, it is concluded that there is unlikely to be any large or significant interference when applied to normal sludges.

A4 Hazards

The fumes from the muffle furnace may be toxic and should be ducted away. One of the reagents, 4-methylpentan -2-one (MIBK) is flammable and has a harmful vapour (see Section A5.7). It is irritating to the eyes and mucous membranes and is narcotic in high concentrations. It must not be pipetted by mouth.

Table A1

Other substances	Equivalent concentration of other substance (g/kg) (c)	Effect in (mg/kg) molybdenum of other substance at molybdenum concentration of (d)	
		0.0 mg/kg	30.0 mg/kg
Cadmium as Cd^{2+}	1	0.0	0.7
Cobalt as Co^{2+}	1	0.4	-0.1
Manganese as Mn^{2+}	1	0.1	0.4
Vanadium as V^{4+}	1	1.5	1.7
Tungsten as W^{6+}	1	6.0	5.0
Aluminium as Al^{3+}	10	-0.2	0.8
Chromium as Cr^{3+}	10	0.1	0.7
Copper as Cu^{2+}	10	-0.6	0.5
Magnesium as Mg^{2+}	10	-0.1	0.7
Nickel as Ni^{2+}	10	-0.1	0.4
Lead as Pb^{2+}	10	0.0	0.7
Titanium as Ti^{4+}	10	0.0	0.8
Zinc as Zn^{2+}	10	0.1	0.7
Zirconium as Zr^{4+}	10	-0.2	-0.1
Fluoride as F^-	10	-0.2	0.8
Silicon as Si^{4+}	10	-0.3	-0.8
Calcium chloride	1000	0.1	0.2
Ferric chloride (e)	1000	1.3	2.0
Ferric nitrate	1000	0.6	0.7
Potassium chloride	1000	-0.1	0.2
Potassium nitrate	1000	0.2	1.6
Potassium hydrogen phosphate	1000	0.0	0.7
Sodium sulphate	1000	-0.1	0.2

(c) This equivalent concentration was calculated assuming that 0.250 g of dried sludge is ashed, extracted with 5 ml of acid and a 2 ml aliquot of the acid taken for analysis.

(d) If the other substances did not interfere the effect would be expected to lie (95% confidence) within the ranges 0.0 ± 0.8 and 0.0 ± 1.0 mg/kg molybdenum at 0.0 and 30.0 mg/kg molybdenum respectively.
(e) This effect was found to be due to molybdenum in the ferric chloride.

A5 Reagents

All reagents and standard solutions may be kept in glass or polyethylene bottles. Analytical reagent grade chemicals are suitable unless otherwise stated.

A5.1 Water

The water used for preparing reagent and standard solutions should have a molybdenum content that is negligible compared with the smallest concentrations to be determined in samples. Deionized or distilled water is suitable.

A5.2 4M Hydrochloric acid (approximately)

Dilute 360 ± 10 ml of hydrochloric acid (d_{20} 1.18) with water to 1 litre in a measuring cylinder and mix well.

A5.3 15% m/V Ascorbic acid solution

Dissolve 15.0 ± 0.2 g of L-ascorbic acid and 2.0 ± 0.2 g of citric acid monohydrate in water and dilute with water to 100 ml in a measuring cylinder. This solution should be stored in a cool dark place in which case it is stable for 3 days.

A5.4 0.25% m/V Copper (I) chloride solution

Dissolve 0.25 ± 0.01 g of copper (I) chloride in 90 ±1 ml of hydrochloric acid (d_{20} 1.18), add 10 ±1 ml of water and mix well.

A5.5 Reducing/Complexing solution

Dilute 10.0 ± 0.5 ml of 0.25% m/V copper (I) chloride solution with 40 ±1 ml of water, add 50 ±1 ml of 15% m/V ascorbic acid and mix well. This solution should be freshly prepared when required.

A5.6 25% m/V Potassium thiocyanate solution

Dissolve 25 ±1 g of potassium thiocyanate in water and dilute with water to 100 ml in a measuring cylinder and mix well.

A5.7 4-Methylpentan -2-one (MIBK)

This reagent is hazardous — see Section A4. A special grade of this solvent for atomic absorption spectrophotometry was found to be preferable. Analytical and other grades may be suitable but may need to be purified by distillation. MIBK should be stored in an amber glass bottle.

A5.8 10% m/V Stannous chloride dihydrate solution

Dissolve with warming 10 ±1 g of stannous chloride dihydrate in 18 ±1 ml of hydrochloric acid (d_{20} 1.18), cool and dilute with water to 100 ml in a measuring cylinder and mix well.

A5.9 Standard molybdenum solutions

A5.9.1 Solution A 1 ml contains 1 mg molybdenum

Dissolve 1.840 ± 0.005 g of ammonium molybdate tetrahydrate [$(NH_4)_6 Mo_7 0_{24}.4H_2 0$] in water and dilute with water to 1 litre in a calibrated flask. Alternatively use a commercially available molybdenum standard for atomic absorption spectrophotometry.

A5.9.2 Solution B1 ml contains 50 μg molybdenum

Dilute 5.00 ± 0.02 ml of solution A with 4M hydrochloric acid to 100 ml in a calibrated flask.

A5.4.3 Solution C 1 ml contains 2.5 μg molybdenum

Dilute 5.00 ± 0.02 ml of solution B with 4M hydrochloric acid to 100 ml in a calibrated flask. This solution is stable for at least one week.

A6 Apparatus

A6.1 Culture vials borosilicate glass with plastic screw caps fitted with PTFE liners. 100 x 16 mm tubes have been used but other sizes may be suitable.

A6.2 to A6.4 must be capable of accommodating these vials.

A6.2 Muffle furnace for operation at 500°

A6.3 Centrifuge

A6.4 Heating source for operation at 80-90°C, such as a water bath or a block thermostat.

A6.5 Spectrophotometer

For use at 470 nm with 40 mm micro cells and preferably with an automatic sampling cell.

A7 Sample Collection, Preservation and Preparation

Sewage sludge samples should be collected, preserved and prepared according to the methods given in another publication in this series(5). Wet sludges should be dried (oven drying at 105 ± 3°C or freeze drying are suitable), ground to a fine powder and stored in closed polyethylene containers. Residues from total solids or ash determinations may often provide suitable starting materials for the determination of molybdenum.

A8 Analytical Procedure

READ SECTION A4 ON HAZARDS BEFORE STARTING THIS PROCEDURE

Step	Procedure	Notes
	Preparation of the sample	
A8.1	Weigh a culture vial, add about 0.25 ± 0.05 g of dried, ground sample, reweigh and calculate to ± 0.001 g the weight of sample, W, used (note a).	(a) If the residue from an ash determination is used take a quantity of ash equivalent to about 0.25 g of dried sample and start at step A8.3.
A8.2	Place the vial in a cold muffle furnace, increase the temperature to 500 ± 20°C and maintain this temperature for at least 2 hours (note b). Remove from the furnace and allow to cool to less than 80°C.	(b) For sludges 2 hours is usually adequate but is often more convenient to ash overnight. For some sludges, very occasionally, it may be necessary to use ashing aids, such as potassium hydrogen sulphate.
A8.3	Add 5.00 ± 0.03 ml of 4M hydrochloric acid, cap the vial and mix well. Digest at 85 ± 5°C for 15 ± 2 minutes, shaking from time to time to ensure complete extraction. Remove from the heating source and allow to cool to less than 30°C. Loosen the cap and centrifuge until a clear supernatant is obtained. Transfer 2.00 ± 0.02 ml of the supernate to another vial. Proceed to step A8.6.	
	Blank determination	
A8.4	A duplicate blank determination should be run with each batch of determinations using the same batch of reagents as for samples. Add 2.00 ± 0.02 ml of 4M hydrochloric acid to each of two culture vials. Proceed to step A8.6.	
	Calibration standards	
A8.5	Duplicate calibration standards should be run with each batch of determinations using the same batch of reagents as for samples. Add 2.00 ± 0.02 ml of standard molybdenum solution C to each of two culture vials. Proceed to step A8.6.	

Step	Procedure	Notes
	Spectrophotometric stage	
A8.6	To each blank, calibration standard and sample (the 2.00 ml of supernatant liquid from step A8.3) add 2.00 ± 0.02 ml of reducing/complexing solution (note c), mix, add 1.00 ± 0.02 ml of 25% m/V potassium thiocyanate solution, mix, add 5.00 ± 0.03 ml of MIBK, cap and shake for at least 20 seconds.	(c) Other reducing/complexing agents have been suggested to reduce interferences, but have not been tested in this method.
A8.7	Allow the layers to separate and transfer 4.0 ± 0.1 ml of the MIBK layers to another series of culture vials. Add 1.00 ± 0.02 ml of the 10% m/V stannous chloride dihydrate solution, cap and shake for at least 20 seconds (note d). Loosen the cap and centrifuge to produce a clear supernate (note e).	(d) This procedure is to remove any pink interference colour due to iron (III). See Section A9. (e) Care should be taken to ensure that changes producing cloudiness do not occur when the supernate is transferred to the cell. A useful technique is to cool the extracts for 2-3 minutes in a refrigerator and reshake before centrifuging.
A8.8	Set up the spectrophotometer according to the manufacturers instructions using a 40 mm flow through micro cell at a wavelength of 470 nm. Wash the cell with water, acetone and finally MIBK. Adjust the absorbance to zero with MIBK.	
A8.9	Measure and record the absorbances of the blanks, A_{B1} and A_{B2}, the calibration standards, A_{C1}, and A_{C2} and the sample extracts, A_S.	
	Calculation of the result (note f)	
A8.10	The molybdenum concentration, C, (in mg/kg) in the original dried sample is given by:	(f) This calculation assumes a linear calibration curve. Linearity must be checked (see Section A10).

$$C = \frac{A_S - \overline{A}_B}{\overline{A}_C - \overline{A}_B} \times \frac{12.5}{W}$$

where $\overline{A}_B = \frac{A_{B1} + A_{B2}}{2}$

$$\overline{A}_C = \frac{A_{C1} + A_{C2}}{2}$$

W = weight of the dried sample

A9 Notes on Interferences

A9.1 The shaking time necessary to remove the pink iron interference colour when washing with the stannous chloride solution (step A8.7) depends on several factors including temperature, iron concentration, molybdenum concentration and the quality of the MIBK and stannous chloride. The pink colour due to iron is easily distinguished from the amber colour due to molybdenum and the shaking time must be long enough to remove the pink colour. If the pink colour is not removed within 1 minute the technique or reagents are suspect and the analyst will have to take appropriate action. The following suggestions may be useful:

i. add a further 1 ml of stannous chloride solution or double the strength of the solution;

ii. prepare fresh stannous chloride;

iii. redistil the MIBK;

iv. use a dilution (with 4M hydrochloric acid) of the acid extract of the sample.

A9.2 If colours other than amber/orange are present then possible interference should be suspected and the analyst will have to take appropriate action. The following suggestions may be helpful:

i. if pink probably due to iron (see Section A9.1);
ii. if faintly blue probably due to exceptionally high concentrations of cobalt, chromium, nickel or copper which are unlikely to interfere anyway;
iii. if yellow/green probably due to exceptionally high concentrations of vanadium or tungsten which may cause slight interference;
iv. if yellow, green or brown it could be caused by organic matter and the use of an ashing aid should be considered.

A9.3 If an interfering substance is present and its effect can not be overcome by actions suggested above consideration should be given to:

i. scanning the spectrum of the extract and making the measurement at a different wavelength;
ii. use of different complexing agents to mask interference;
iii. use of atomic absorption spectrophotometry as the final determination.

If any of these techniques are used the performance characteristics quoted in Section A1 no longer apply.

A10 Checking the Linearity of the Calibration Curve

A10.1 The procedure given in this section must be carried out on at least two independent occasions before application of the method to any samples. Any significant departure from linearity indicates that the technique is suspect at some stage and the reagents and method should be thoroughly checked.

A10.2 To a series of culture vials add 0.00, 0.40, 0.80, 1.20, 1.60 and 2.00 ml (all $\pm$ 0.01 ml) of standard molybdenum solution C and 2.00, 1.60, 1.20, 0.80, 0.40 and 0.00 ml (all $\pm$ 0.01 ml) respectively of 4M hydrochloric acid. These vials contain respectively 0, 1, 2, 3, 4 and 5 μg molybdenum respectively. Carry out steps A8.6 to A8.9 on these solutions, subtract the blank A_B from each absorbance and plot the corrected absorbances against μg molybdenum. The calibration curve is linear to at least 5μ g molybdenum.

A11 Change in Concentration Range of the Method

If the molybdenum concentration of the dried sample is likely to exceed 50 mg/kg it is recommended that the acid extract from step A8.3 is diluted with 4M hydrochloric acid to an appropriate volume V_1 ml and 2.00 ml of this diluted extract used in step A8.6. The result in step A8.10 must then be multiplied by $\frac{V_1}{5}$.

A12 Sources of Error

The attention which is necessary to pay to sources of error depends on the accuracy required of analytical results. The following sub-sections summarize the main sources of error:

A12.1 Molybdenum content of water and reagents

Distilled or deionized water should not normally contain sufficient molybdenum to introduce a significant error. If blank determinations (step A8.4) give absorbances higher than 0.03 unsuitable water or reagents are probably being used.

A12.2 Interferences

The presence of pink colour due to iron (III) or turbidity due to water precipitation in the final extract (step A8.7) will cause significant positive errors but these can be observed visually and steps taken to minimize their effect. The effect of other substances on the spectrophotometric stage of the procedure may be determined by analysing standards spiked with various concentrations of the potential interfering substance.

A13 Checking the Accuracy of Analytical Results

(For further information see General Principles of Sampling and Accuracy of Results 1980, also published in this series).

Once the method has been put into normal routine operation many factors may subsequently affect the accuracy of analytical results. It is recommended that

experimental tests to check certain sources of inaccuracy should be made regularly. Many types of test are possible and they should be used as appropriate(6). As a minimum, however, it is suggested that a standard solution of molybdenum of suitable concentration be analysed at the same time and in the same way as samples from steps A8.6 onwards. The results should then be plotted on a quality control chart which will facilitate the detection of inadequate accuracy. It is also recommended that a sample of a particular stored homogenized dried sludge be analysed with each batch and the results plotted on a quality control chart.

A14 References

(1) Musselwhite CC, Water Research Centre, *Laboratory Report,* LR 1201, 1980.

(2) Quin BF and Brooks RR, *Anal Chim. Acta* 1975, *74,* 75-78.

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(6) Wilson AL and Cheeseman RV, Water Research Centre, *Technical Report* TR 66, 1978.

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* See also *Additions, Corrections and Index, 1983* (also in this series).

B Total Molybdenum in Soils and Related Materials by Spectrophotometry Tentative Method (1982 Version)

Note: Throughout this method molybdenum is expressed as the element Mo.

B1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see General Principles of Sampling and Accuracy of Results, 1980, also published in this series).

B1.1	Substance determined	All forms of molybdenum.
B1.2	Type of sample	Soils and related materials.
B1.3	Basis of the method	Destruction of the organic matter by dry combustion, extraction of the mineral matter with hydrochloric/hydrofluoric acid mixture followed by spectrophotometric determination of molybdenum as its thiocyanate complex.
B1.4	Range of application	Up to 10 mg/kg.
B1.5	Calibration curve	Linear to 10 mg/kg.
B1.6	Standard deviation (within batch) (a) (b)	

Soil sample	Molybdenum concentration (mg/kg)	Standard deviation (mg/kg)	Degrees of freedom
1	7.57	0.15–0.89	1 - 3
2	0.95	0.04–0.11	1 - 3
3	1.40	0.01–0.12	1 - 3
4	1.15	0.03–0.14	1 - 3
5	1.52	0.01–0.16	1 - 3
6	1.34	0.00–0.21	1 - 3

B1.7	Limit of detection	0.2 to 0.4 mg/kg.
B1.8	Sensitivity	10 mg/kg gives an absorbance of approximately 0.75.
B1.9	Bias	Not known.
B1.10	Interferences	See Section A3.
B1.11	Time required for analysis	The total time for a batch of 12 samples including 2 hours ashing is approximately 7 hours.

(a) The range of standard deviations quoted were obtained by 5 Agricultural Development and Advisory Service (ADAS) laboratories (1).
(b) The pooled standard deviations for soils 1 to 6 are 0.43, 0.08, 0.10, 0.07, 0.10 and 0.09 respectively each with 11 degrees of freedom.

B2 Principle

The organic matter in a finely ground sample is destroyed by dry combustion. The mineral matter is digested with a hydrochloric/hydrofluoric acid mixture and the residue dissolved in hydrochloric acid. The molybdenum in solution is treated with iron (III) and thiocyanate in the presence of a reducing agent to form an amber/orange coloured complex, which is extracted into di-iso-propyl ether and measured spectrophotometrically at 470 nm. (2). The final acidity in the solution to be extracted should not exceed 1.5 M as hydrochloric acid.

B3 Interferences

Substances which are normally present in soils do not interfere with this determination (2). Potential interference from titanium concentrations exceeding 30 mg/kg is minimized by the addition of fluoride (3).

B4 Hazards

B4.1 Hydrofluoric acid (see B5.4) is a dangerous reagent and must not be allowed to contact the skin or eyes. Rubber gloves and goggles or spectacles with polycarbonate lenses must be worn. All operations involving hydrofluoric acid must be carried out in a fume cupboard. 2% calcium gluconate gel must be available. (see Hazards in the Chemical Laboratory, Muir GD, 2nd Edition)

B4.2 Di-iso propyl-ether (see B5.2) is a hazardous chemical because of its tendency to form explosive peroxides. It must be stored in a dark bottle containing a few crystals of stannous chloride.

B5 Reagents

Analytical grade reagents are suitable unless otherwise stated. All reagents and standard solutions should be stored in polyethylene bottles unless otherwise stated.

B5.1 Water

The water used to prepare the reagents and standard solutions should have a molybdenum content which is negligible compared to the lowest concentration expected in sample solutions. Water distilled from an all glass apparatus is normally suitable.

B5.2 Di-iso-propyl ether (d_{20} 0.73)

This reagent is hazardous — see section B4.2.

B5.3 Hydrochloric acid

Ultra high purity hydrochloric acid (d_{20} 1.18) is required.

B5.3.1 6M Hydrochloric acid approximately

Dilute 540 ± 10 ml of hydrochloric acid (d_{20} 1.18) with water to 1 litre. Alternatively distil the same dilution of analytical grade hydrochloric acid, rejecting the initial and final portions of distillate and retaining the middle portions to obtain ultra pure approximately 6M hydrochloric acid. The distillation must be carried out in a fume cupboard and anti-bumping granules must be present in the distillation flask.

B5.3.2 2M Hydrochloric acid approximately

Dilute 330 ± 10 ml of 6M hydrochloric acid with water to 1 litre.

B5.4 Hydrofluoric acid (d_{20} 1.13)

This reagent is hazardous — see section B4.1.

B5.5 0.5% m/V Ferric chloride hexahydrate

Dissolve 0.50 ± 0.01 g of ferric chloride hexahydrate in 100 ± 2 ml of 2M hydrochloric acid.

B5.6 40% m/V Potassium thiocyanate solution

Dissolve 40 ± 1 g of potassium thiocyanate in 100 ± 2 ml of water.

B5.7 40% m/V Stannous chloride dihydrate solution

Dissolve with warming 40 ± 1 g of stannous chloride in 20 ± 1 ml of hydrochloric acid (d_{20} 1.18). Dilute with water to 100 ml in a measuring cylinder. If turbid filter through an 11.0 cm filter paper capable of retaining particles of 2.5μm size. Store in a refrigerator.

B5.8 5% m/V sodium fluoride solution

Dissolve 12.5 ± 0.5 g of sodium fluoride in 250 ± 5 ml of water.

B5.9 Standard Molybdenum Solutions

B5.9.1 Solution A 1 ml contains 1 mg of molybdenum

Dissolve 1.840 ± 0.005 g of ammonium molybdate tetrahydrate [$(NH_4)_6 Mo_7 0_{24}.4H_20$] in water and dilute with water to 1 litre in a calibrated flask. Alternatively use a commercially available molybdenum standard for atomic absorption spectrophotometry.

B5.9.2 Solution B 1 ml contains 50 μg of molybdenum

Dilute 5.00 ± 0.02 ml of solution A with water to 100 ml in a calibrated flask.

A5.9.3 Solution C 1 ml contains 1 μg of molybdenum

Dilute 5.00 ± 0.02 ml of solution B with water to 250 ml in a calibrated flask. Prepare this solution freshly when required.

B6 Apparatus

B6.1 100 ml polypropylene beakers

B6.2 20 ml translucent silica, shallow form evaporating basins with a spout and round bottom.

B6.3 Spectrophotometer for use at 470 nm with 40 mm cells.

B7 Sample Collection, Preservation and Preparation

Soil samples should be collected, preserved and prepared according to the methods given in another publication in this series(4) and be air dried, ground and sieved to less than 2 mm size.

B8 Analytical Procedure

READ SECTION B4 ON HAZARDS BEFORE STARTING THIS PROCEDURE

Step	Procedure	Notes
	Preparation of the samples	
B8.1	Cone and quarter a representative portion of the air dried, sieved (to less than 2 mm) sample. Grind a representative sub-sample (at least 10 g) to less than 150 μm size in a suitable mill, preferably of agate, and weigh 1.00 ± 0.01 g into a 20 ml silica basin.	
B8.2	Place the basin in a cool muffle furnace, increase the temperature to 500 ± 25°C and maintain this temperature for at least 2 hours (note a). Cool the basin to room temperature and quantitatively transfer the residue into a 100 ml polypropylene beaker.	(a) For soils 2 hours is usually adequate but it is more often convenient to ash overnight.
B8.3	Carry out this operation in a well ventilated fume cupboard. Add to the beaker 10 ± 1 ml of hydrochloric acid (d_{20} 1.18) and 10 ± 1 ml of hydrofluoric acid (d_{20} 1.13) (note b). Evaporate to dryness by immersion of the lower half of the beaker in a boiling water bath. Add 25 ± 1 ml of 2M hydrochloric acid and heat gently until the residue is dissolved (approximately 5 minutes). Allow to cool to room temperature.	(b) This reagent is hazardous, see section B4.1.
B8.4	Quantitatively transfer the solution to a 100 ml conical separating funnel. Add 5.0 ± 0.1 ml of 5% m/V sodium fluoride solution and dilute with water to 50 ± 5 ml. Proceed to step B8.7.	
	Blank determination	
B8.5	A blank must be run with each batch of determinations using the same reagents as for samples. Carry out steps B8.3 and B8.4 and proceed to step B8.7.	

Step	Procedure	Notes
	Calibration standards	
B8.6	A duplicate calibration standard must be run with each batch of determinations using the same reagents as for samples. Pipette 10.0 ± 0.1 ml of standard molybdenum solution C into each of two 100 ml conical separating funnels. Add 25 ± 1 ml of 2M hydrochloric acid, 5.0 ± 0.1 ml of 5% m/V sodium fluoride and dilute with water to 50 ± 5 ml. Proceed to step B8.7.	
	Extraction and Spectrophotometric stages	
B8.7	To each funnel add, mixing well between each addition, 1.0 ± 0.1 ml of 5% m/V ferric chloride hexahydrate solution, 1.0 ± 0.1 ml of 40% m/V potassium thiocyanate and 1.0 ± 0.1 ml of 40% m/V stannous chloride dihydrate solution. Add 12.0 ± 0.1 ml of di-iso-propyl ether, shake vigorously by hand for 2.0 ± 0.2 min and allow the layers to separate. Discard the lower aqueous layer and filter the upper ether layer through a 7.0 cm filter paper capable of retaining particles of 8.0 μm size (note b) directly into a 40 mm optical cell.	(b) Alternatively use a suitable phase separation paper.
B8.8	Set up the spectrophotometer according to the manufacturers' instructions. Measure the absorbance of each sample, blank and calibration standard extract at 470 nm using di-iso-propyl ether in the reference cell.	
	Calculation of the Result (note c)	
B8.9	The molybdenum concentration, C, (in mg/kg) in the original dried, sieved sample is given by $C = \frac{A_s - A_b}{A_c - A_b} A \times 10$ Where A_s = the absorbance of the sample A_b = the absorbance of the blank A_c = the mean absorbance of the calibration standard	(c) This calculation assumes a linear calibration curve. Linearity must be checked. See Section 9.

B9 Checking the Linearity of the Calibration Curve

B9.1 The procedure in this Section must be carried out on at least two occasions before application of the method to any samples. Any significant departure from linearity indicates that the technique is suspect at some stage and the reagents and the method should be thoroughly checked.

B9.2 To a series of 100 ml conical separating funnels add 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 ml (all ± 0.02 ml of standard molybdenum solution C. Add to each funnel 125 ± 1 ml of 2M hydrochloric acid and 5.0 ± 0.1 ml of 5% m/V sodium fluoride solution and dilute with water to 50 ± 5 ml. These solutions contain respectively 0, 2, 4, 6, 8 and 10 μg Mo. Carry out steps B8.7 and B8.8, subtract the blank, A_b, and plot the corrected absorbances against μg Mo. The calibration curve is linear to at least 10 μg Mo.

B10 Change in the Concentration Range of the Method

If the molybdenum concentration in the dried soil is likely to exceed 10 mg/kg it is recommended that the acid solution from step B8.3 is diluted with 2M hydrochloric and to an appropriate volume, V_1 ml, and 25 ± 1 ml of this diluted solution used in step B8.4. The result in step B8.9 must be multiplied by $\frac{V_1}{25}$.

B11 Sources of Error

The attention which it is necessary to pay to sources of error depends on the accuracy required of analytical results. The following sub-sections summarize the main sources of error:

B11.1 Molybdenum content of water and reagents

Distilled water should not normally contain sufficient molybdenum to introduce significant error. If the blank determination (step B8.5) gives an absorbance higher than 0.03 unsuitable water or reagents are probably being used.

B11.2 Interferences

The effect of other substances on the spectrophotometric stage of the procedure may be determined by analysing standards spiked with various concentrations of the potential interfering substance.

B12 Checking the Accuracy of Analytical Results

(For further information see General Principles of Sampling and Accuracy of Results 1980, also published in this series).

Once the method has been put into normal routine operation many factors may subsequently affect the accuracy of analytical results. It is recommended that experimental tests to check certain sources of inaccuracy should be made regularly. Many types of test are possible and they should be used as appropriate (5). As a minimum, however, it is suggested that a standard solution of molybdenum of suitable concentration be analysed at the same time and in the same way as samples. The results should then be plotted on a quality control chart which will facilitate the detection of inadequate accuracy. It is also recommended that a sample of a particular stored homogenized dried soil be analysed with each batch and the results plotted on a quality control chart.

B13 References

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(5) Wilson AL and Cheeseman RV, Water Research Centre, *Technical Report* TR66, 1978.

Address for Correspondence

However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this booklet are requested to write to:

The Secretary
The Standing Committee of Analysts
The Department of the Environment
43 Marsham Street
LONDON SW1P 3PY
England

* See also *Additions, Corrections and Index 1983* (also in this series).

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