SEWAGE POLLUTION OF WATER SUPPLIES

Rapid Methods for its Detection by means of Creatinine and Detergents

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Introduction

THE usual methods for the detection of sewage pollution in water supplies involve either bacteriological and chemical examinations or the addition of fluorescein dye to the drainage system. For the former, bacteriologists, chemists and well-equipped laboratories are required but several days may elapse before the report is available, while the presence of viruses in the water may not be detected. With fluorescein, no specially skilled personnel are required, but there may be considerable delay before the dye appears in the water supply and a search for the drains involved may cause further delay.

The two tests described below can be carried out by a technician in a Service Laboratory using very simple apparatus, take less than two hours from receipt of a sample and can detect pollution likely to contain viruses.

These simple and rapid tests were devised by Abbott for the detection and determination of creatinine (1962) and detergent (1963), the former being present in urine. The basis of the creatinine test is that, as it decomposes under aerobic conditions, with its concentration negligible after about two days its presence in water is therefore definite proof of recent pollution. This does not prove human contamination because all animals secrete creatinine in their urine, but it provides a warning of pollution and underlines the need for treatment by filtration and chlorination.

In localities where detergents are not used there is, of course, no object in testing for them. Also, in countries where so-called "soft" detergents are used, their destruction by organisms will result in negative test results (Hammerton, 1956). Therefore, positive tests for creatinine and detergents indicate past pollution but negative tests for both creatinine and detergents do not guarantee that the water is free from pollution.

Taking Samples

The samples should not be taken in bottles cleaned with detergent, unless they have been cleaned with chromic-sulphuric acid afterwards. The kationic detergents (e.g. Centrimide) are absorbed onto glass; they may contaminate the sample and cause a false positive test result. In hot weather, it is found that creatinine is more rapidly decomposed than in the cold, also bacterial action on the detergents is more rapid at the higher temperatures; therefore, if the sample cannot be tested immediately it is taken, it should be stored in a refrigerator or an ice box.

Test for Creatinine

Reagents:—

1. *Picric acid solution* 1% — Standardize with 0.1 N sodium hydroxide; using phenol red A.R. indicator.
2. *Sodium hydroxide* 0.5 N

3. *Sodium hexametaphosphate chloride solution* — Dissolve 10 gm. each of sodium hexametaphosphate and sodium chloride in distilled water and make up to 100 ml.
4. *Standard creatinine solution* — Prepare a stock solution of 0.1 per cent in 0.1 N. hydrochloric acid; this solution may be kept for 1 month at 0°C. For the working solution, dilute 1 ml. of the stock solution to 100 ml. with distilled water. 1 ml. contains 10 μ gm. of creatinine.

Procedure:—

In eight 50 ml. stoppered volumetric flasks place:—

(1)—0 ml. (2)—2 ml. (3)—4 ml. (4)—6 ml. (5)—8 ml. (6)—10 ml. of standard (working) solution and dilute to 20 ml. Into flasks (7) and (8) place 20 ml. of the sample.

Add 1 ml. of hexametaphosphate-chloride to each and mix.

Add 10 ml. of picric acid solution to flasks number (1)-(7), but omit (8).

Add 5 ml. of 0.5 N sodium hydroxide to each and mix.

Set aside for 1 hour at 20°C, then dilute to 50 ml. and mix.

Measure the optical density in 4 cm. cells against flask number (1) with a 495 m μ . (number 3 or 603) screen using a photelectric absorptiometer. Use the readings of the standard to make a graph which should be a straight line, and read off the concentrations in the two samples (flasks 7 and 8). Deduct that in flask (8) to allow for any colour in the water, and thus find x μ g. of creatinine in 20 ml., therefore 50 x μ g. in 1,000 ml., 50 x $\div$ 1,000 mg./litre or x $\div$ 20 mg./litre. The hexametaphosphate prevents haze with calcium and magnesium salts (hardness) in the water when soda is added. The chloride enhances the colour.

Validation and Investigation

1. *Limits of the test and period of decay of creatinine in samples stored at 21°C.*

Tests on samples taken of Thames water at Vauxhall Bridge

<i>Time of Storage</i>	<i>Sample</i>	<i>Dilution 1 in 100</i>	<i>Dilution 1 in 100</i>
0 hours	0.5 mg./litre	0.1 mg./litre.	Less than 0.1 mg./litre.
24 hours	0.3 mg./litre.	less than 0.1 mg./litre	Less than 0.1 mg./litre.
48 hours	Less than 0.1 mg. per litre in all.		

The limit of the test was found to be 0.1 mg. of creatinine per litre. As urine contains approximately 1,000 to 2,000 mg. of creatinine per litre, the test will show a dilution of about one part of urine in ten thousand parts of water if there is no delay in testing and shows the disappearance of the creatinine in between 24 and 48 hours in a polluted river water.

2. *Period of decay when sample is preserved at 4°C. and 37°C.* Sample of River Thames water taken at Walton-on-Thames and preserved at ambient temperature before testing showed 2.4 mg. of creatinine per litre when tested eighteen hours later. After a further

24 hours incubation, the sample at 4°C. contained 1.4 mg./litre and at 37°C. less than 0.1 mg./litre. After a further 24 hours, the sample at 4°C. contained 0.5 mg./litre and at 37°C. less than 0.1 mg. per litre.

3. Effect of the temperature of storage.

a. Tests on samples taken from Mogden Sewage Works Isleworth

		Creatinine mg. per litre
Original	Sample at 4°C. activated sludge 1st grid	3.65
After 24 hrs	" " " " " "	3.3
After 48 hrs	" " " " " "	2.9
Original	Sample at 21°C.	3.0
After 24 hrs	" " " " " "	1.15
After 48 hrs	" " " " " "	0.4
Original	Sample at 37°C.	2.9
After 24 hrs	" " " " " "	0.8
After 48 hrs	" " " " " "	0.5
Original	Sample at 4°C. final effluent	0.45
After 24 hrs	" " " " " "	0.4
After 48 hrs	" " " " " "	0.2
Original	Sample at 21°C.	0.5
After 24 hrs	" " " " " "	0.4
After 48 hrs	" " " " " "	0.3
Original	Sample at 37°C.	0.2
After 24 hrs	" " " " " "	0.2
After 48 hrs	" " " " " "	0.15

b. Tests on the final effluent from Pirbright Sewage Works

		Creatinine mg. per litre
Original	Sample at 4°C. final effluent	0.6
After 24 hrs	" " " " " "	0.5
After 48 hrs	" " " " " "	0.35
Original	Sample at 21°C.	0.6
After 24 hrs	" " " " " "	0.4
After 48 hrs	" " " " " "	Less than 0.1
Original	Sample at 37°C.	0.4
After 24 hrs	" " " " " "	0.1
After 48 hrs	" " " " " "	Less than 0.1

c. Tests on crude sewage and final effluent from North Surrey Joint Sewage Board Works at Berrylands

		Creatinine mg. per litre
After 18 hrs	Storage. Crude sewage at 4°C.	2.2
After 42 hrs	" " " " " "	1.9
After 66 hrs	" " " " " "	1.9
After 18 hrs	Storage. Crude sewage at 21°C.	1.5
After 42 hrs	" " " " " "	1.2
After 66 hrs	" " " " " "	0.8
After 18 hrs	Storage. Crude sewage at 37°C.	0.3
After 42 hrs	" " " " " "	0.1
After 66 hrs	" " " " " "	Less than 0.1
After 18 hrs	Storage. Final effluent at 4°C.	0.8
After 42 hrs	" " " " " "	0.2
After 66 hrs	" " " " " "	Less than 0.1
After 18 hrs	Storage. Final effluent at 21°C.	0.3
After 42 hrs	" " " " " "	Less than 0.1

These tests show that it is advisable to preserve the sample in a refrigerator if the test cannot be carried out at once.

Test for Detergents

Reagents:—

1. Hydrochloric acid, approximately 2N.
2. Chloroform distilled, free from colour.
3. Methyl green (C.I. No. 42585). Dissolve 0.01 g. in water, and dilute to 500 ml. with distilled water. Extract sufficient of this solution (5 ml. for each test) with 10 ml. portions of chloroform, until a colourless extract is obtained, usually four times. Filter to remove droplets of chloroform.

Procedure :—

To 200 ml. of the sample contained in a 250 ml. glass-stoppered separating funnel (or 250 ml. measuring cylinder) add 5 ml. of the hydrochloric acid, and 5 ml. of the extracted methyl green solution. Add 5 ml. of chloroform, shake vigorously for 30 seconds and allow the layers to separate. Compare the colour of the lower chloroform layer with that given by similar treatment of 200 ml. of distilled water. A green colour indicates the presence of an anion-active material in the sample.

0.001 p.p.m. gives a trace green colour.

0.003 p.p.m. gives a definite green colour.

Validation and Investigation

1. *Limits of the test.* Several detergents were tested, viz:— *Anionic detergents:— QUIX—20 per cent total solids: TEEPOL—24.5 per cent total solids: COMPROX—24.5 per cent total solids: Domestic Detergent Powders:— OMO, DAZ, TIDE. Non-ionic detergents:— LISSAPOL N.D.B. Cationic detergents:— CETRIMIDE.*

All of the above gave a perceptible green colour in the chloroform in the test using 0.001 p.p.m. concentration.

The optical densities of 0.001 p.p.m. solutions, using a 603 (490 mμ.) filter and 4 cm. cells, were as follows:— *QUIX* 0.04, *TEEPOL* 0.04, *COMPROX* 0.085, *OMO* 0.075, *DAZ* 0.070, *TIDE* 0.05 (in London tap water), *LISSAPOL N.D.B.* 0.05, *CETRIMIDE* 0.04. *MANOXOL O. T.* gave an O. D. of 0.015.

The limit of the test is, therefore 0.001 p.p.m. detergent.

Detergent in a sample of final effluent from North Surrey Joint Sewage Works, Berrylands

The sample had an O.D. of 0.27 which, on a *MANOXOL O. T.* basis, would indicate a concentration of 0.018 p.p.m. of detergent. This is a low concentration probably as the detergents used were "soft" detergents. A further sample showed a concentration of 0.022 p.p.m. of detergents. Information from North Surrey Joint Salvage Works, Berrylands said that "soft" detergents were mostly used in the area. According to Eden and Truesdale (1960), 94 per cent of "soft" detergents are removed by the various methods of sewage treatment.

Discussion

These simple test will show definitely whether sewage is polluting water. A negative test result will not, of course, guarantee that the water is safe to drink; any creatinine previously present may have been decomposed by a high temperature and several days storage, while any detergent may have been decomposed by organisms. It should be noted that viruses are likely to filter through soil which will retain organisms such as *E. coli*; work on this aspect is still being continued by Burman (1962). However the two tests described above, if positive, give definite proof of pollution.

Acknowledgement

I am most grateful to Dr. D. C. Abbott for permission to publish his methods of analysis.

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Senior Appointments

Major-General T. M. R. Ahern is to become Director of Medical Services, British Army of the Rhine. He is to be succeeded as Deputy Director of Medical Services Eastern Command by his brother Brigadier D. M. Ahern.

Donal Maurice Ahern was born at Cork on 30th March, 1911, son of the late Lieutenant-Colonel M. D. Ahern, O.B.E., R.A.M.C. Educated at Ampleforth College and at Trinity College, Dublin, he graduated B.A. 1932, M.B., B.Ch. 1933, was commissioned July 1933 as Lieutenant R.A.M.C. Service in India, including active service in Waziristan, followed. In the Second World War, he saw service in Iraq, Burma and North West Europe where he was awarded the D.S.O. in 1944. He was also mentioned in despatches for service in Burma 1942. A Staff College graduate in 1943 he was an Assistant Director General War Office in 1954 before being appointed to H.Q. Allied Forces Northern Europe and later to British Army of the Rhine. He was appointed C.B.E. in 1962. He is married with three children.

Brigadier J. A. D. Johnston, O.B.E., M.C., has been appointed Director of Medical Services, Far East Land Forces.

James Alexander Deans Johnston was born at Glasgow on 28th February 1911. Educated at Woodside and Glasgow University, he graduated M.B., Ch.B. in 1933. Commissioned Lieutenant R.A.M.C. in October 1934 he served in India 1935-40 including operations in Waziristan, being awarded the Military Cross in December 1937. During the war 1939-45, duty with Field Ambulance and Casualty Clearing Station included that of S.M.O. Belsen. He was appointed O.B.E. and mentioned in despatches (Europe) 1945. An ADMS in Malaya and Middle East, he served as an Assistant Director General War Office before becoming Commandant, Depot and Training Establishment, R.A.M.C. He is married with three children.

Alexander Medal

The Alexander Medal and prize of £100 has been awarded to Lieutenant-Colonel J. E. Noble, M.C.Path., M.R.C.S., L.R.C.P., D.T.M.&H., R.A.M.C., of the David Bruce Laboratories, for his series of articles on "The diagnosis and prophylaxis of cholera".