XXXIV. ON THE SELF-PURIFICATION OF RIVERS AND STREAMS.

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It is well known that the purifying action of river-water polluted with sewage is very considerable, as a few miles below the outfall or point of pollution a river may show little or no sign of pollution at all. Purification is effected by sedimentation of the suspended solids, and oxidation of the soluble material. The processes of oxidation give rise to deoxygenation of the river-water, and the extent of deoxygenation depends on the strength of the sewage, the degree of dilution afforded by admixture with the river-water, and the velocity of the river.

If the concentration of oxidisable material be excessive, the river-water will suffer considerable or complete deoxygenation, and a nuisance will result owing to the septic condition caused by the anaerobic decomposition of the organic matter. On the other hand, if there be sufficient dilution, the organic matter can be oxidised and thus destroyed without depriving the river-water of oxygen to any appreciable degree. The suspended matter will also be sedimented in the form of a thin film distributed over a considerable area of river-bed, and no nuisance will thus result through the formation of foul mud-banks.

Recovery from pollution, or self-purification, as it is termed, thus depends on the conditions obtaining with the particular river. Ordinarily towns situated on the same river are sufficiently separated to give time for the river to recover from the effects of the upper pollution before it is subjected to the next. On the other hand, if towns be close together, a nuisance may result, and the river may become unfit to receive a further volume of sewage lower down, until a considerable length of time and dilution from tributaries enable purification to be effected.

As early as 1891 Pettenkofer [see *Roy. Comm.* 1913, p. 65] appreciated the fact that rivers are capable of self-purification and stated that the condition of a river below the point of pollution could be roughly foretold provided the population of the town and the volume of the river were known. He also realised that dissolved oxygen in the river-water played an important part in the process of self-purification.

A certain amount of work has been carried out by the Royal Commission on Sewage Disposal to ascertain the rate of self-purification of polluted river-

waters as measured by the change in the amounts of oxidisable carbonaceous matter and ammonia present, and the production of nitrites and nitrates.

In laboratory experiments it was conclusively shown by Adeney [*Roy. Comm.* 1908] that in mixtures of sewage and river-water the oxidation of the carbonaceous matter precedes that of ammonia. In the case of rivers and streams under natural conditions however the disappearance of the ammonia takes place practically *pari-passu* with the oxidation of the carbonaceous matter [*Roy. Comm.* 1913, p. 63]. This disappearance of ammonia is due to a large extent to absorption by algae and aquatic plants [*Roy. Comm.* 1913, p. 85].

It would also appear that nitrification actually takes place in the polluted river-waters, and this is an additional factor bringing about the disappearance of the ammonia [*Roy. Comm.* 1913, p. 89].

The effect of temperature on the rate of oxidation of organic matter, *i.e.* absorption of dissolved oxygen, was also considered by the Commission [1913, p. 94], especially in relation to the question of standardisation of sewage effluents. Observations were made on the temperatures of different river-waters during the year, and the mean maximum temperature was found to be about 65° F. or 18.3° C. The dissolved oxygen absorption by sewage effluents in five days was found to be much greater at temperatures of 22–24° C. than at 18.3° C., and it was accordingly recommended that in the standardisation of effluents by the dissolved oxygen absorption test a definite temperature should be adopted and that this should be 18.3° C.

Seasonal variation is therefore another factor in the self-purification of rivers. It is clear that owing to the increased rate of oxidation of organic matter due to greater bacterial activity, and the removal of ammonia due to plant development, the process of purification will operate more rapidly during the warmer months. On the other hand, if the river-water be overcharged with sewage, the nuisance may be greater in the summer months than in the winter, as the increased rate of oxidation may lead to deoxygenation of the water.

Although the subject of self-purification of rivers has thus been considerably studied, no work so far seems to have been carried out to ascertain whether the rate and course of purification are influenced by the geological source of the river. Some evidence has already been obtained by the authors [1918] that there is a considerable difference in the rates of oxidation of organic matter in tap-waters and river-waters, the difference being so great that it is necessary to employ the river-water into which the effluent is discharged as the diluting medium in the dissolved oxygen absorption test, in order to obtain results of any value in the standardisation of effluents. It is quite possible that the mineralogical constituents of river-waters may exercise specific influences—either by acceleration or retardation of the oxidation processes.

It may also be expected that the particular types of organic matter derived

from the plant and animal life of rivers and from drainage of land, and the numbers and kind of bacteria and other micro-organisms will have important influences. These however ultimately depend to a large extent on the geological source of the river.

The complex chemical and biological processes going on in river-beds or in the supernatant river-water no doubt have some relation to those going on in soil. There are however differences in the conditions controlling these processes in rivers and on land.

First of all, there is frequently much less humus in a river-bed than in the soil, so that in rivers the chemical and biological processes may be said to be actually going on in a geological bed and uncomplicated by large amounts of decomposed vegetable matter.

Furthermore, in a particular soil there is as a general rule a constancy in the amounts and proportions of the mineralogical constituents. In the case of rivers, on the other hand, except at the spring-head there must be at various times considerable variation, as a river may receive contributions of water from many springs and from drainage off large areas of land of different geological formations, and the relative volume of water from these various sources must depend on climatic conditions and seasonal variations. The conditions obtaining are thus extremely complicated.

Notwithstanding these variations, a river-water may be sufficiently characteristic in chemical composition to distinguish it from other river-waters.

For example, in considering the characters of river-waters in this country, we find that they necessarily depend on the nature of the soils and rocks over which they flow. The rivers of Scotland thus are in general soft, the mountains being composed of granite, gneiss, mica schists, and there is only a small proportion of calcium carbonate. The water from the Cumberland and the Welsh mountains (Silurian rocks) is for the same reason generally soft. In Wales however when the Old Red Sandstone district is reached, marls and limestones are found, and the river-waters are harder. Rivers draining areas in which the Carboniferous Limestone, the Permian Rocks, and New Red Sandstone occur, also yield, as a general rule, somewhat hard water, while river-waters derived from the Lias, Oolitic and Cretaceous Rocks are of necessity hard, because Limestone or Chalk as the case may be are so abundant in these formations.

In draining areas of the various geological strata, rivers carry down an enormous amount of mineral and vegetable matter, which is finally discharged into the sea.

For this reason, river-beds, except possibly near the spring-head, are in general "alluvial" in nature, *i.e.* representative of many geological formations. The mineralogical constitution of a river-bed depends to a large extent on the adjacent geological strata and on those drained by the tributaries of the river, and at different times may be thus liable to considerable variation. Even if the actual mineral constituents in certain cases do not vary much, the physical

and mechanical factors, *i.e.* size and form of particles or texture, would vary and these also would be expected to affect the rate of the chemical and biological processes going on in the river-bed, owing to variations in the degree of aeration.

In the Chalk country, for example, the river-beds will tend to consist of fine-grained calcareous loams or marls, in the Lower Greensand formations these will be replaced by sandy loams, often of coarse texture, sometimes also by peaty loams and fine gravel; again, in the case of a stream from the Lower Greensand formations running over the Atherfield Clay, we should expect to find the clay converted into a sandy clay owing to admixture with the sandy detritus from the Hythe beds. Calcareous, glauconitic and cherty matter might also be found, derived from the Bargate beds, Glauconitic Sandstones, and Chert beds present in the Lower Greensand formation.

There are thus reasons for anticipating that the processes of oxidation of organic and possibly inorganic matter may depend on the quality of the river-water into which it is discharged.

The question is not only of interest to geologists, but is also of importance from the point of view of practical sewage disposal, as, if the oxidation of sewage constituents varies perceptibly in different river-waters, it will be necessary in drawing up new plans for sewage purification to consider the nature of the river into which the effluent is to drain before a standard of purification can be selected.

During the last two years therefore many comparisons of the rates of oxidation in different river-waters of the constituents of sewage or sewage-effluents have been made, and the results of this investigation are given in the present paper. The comparisons have been made by determining the dissolved oxygen absorbed by sewage or sewage effluents when diluted with the various river-waters.

Further experiments supplementary to those described in the previous paper [Cooper, 1918] and comparing the rates of oxidation in the presence of distilled water, various tap-waters and river-waters have also been made.

In addition, some questions of technique bearing on the work and also of importance in the standardisation of effluents are discussed.

The general geological aspect of this work has been studied by the late A. E. C. who unfortunately died in the course of the investigation. In addition, he had proposed to study the mineralogical constitution of the river-beds and to correlate the results with those obtained relating to the processes of oxidation, but this part of the work had only been commenced just before his untimely death and is necessarily not far advanced. His help and enthusiasm in scientific work have been greatly missed. The copious notes on the subject left by him have however been carefully read through, and suitable selections have been incorporated in this paper.

The chemical work has been carried out by E. A. C. and J. A. H. at the Hygienic Laboratory, School of Army Sanitation, Aldershot Command.

1. THE ESTIMATION OF DISSOLVED OXYGEN IN RIVER-WATERS AND DILUTED SEWAGE EFFLUENTS.

The method used for the determination of the dissolved oxygen content was that of Winkler, with the Rideal-Stewart modification [*Roy. Comm.* 1913, p. 93].

By this method it was found that aerated distilled water and tap-waters contained 0.93 parts of oxygen per 100,000 at 18.3° C. or 65° F. This is the correct figure according to Roscoe and Lunt's tables [1889].

It was found however that in the case of aerated clean river-waters the oxygen concentration was often somewhat lower, *e.g.* 0.80 to 0.90, while aerated polluted river-waters, and sewage effluents diluted five times with river-water often only contained about 0.80 parts, sometimes, in fact, only 0.70 parts of oxygen per 100,000. As a general rule bad effluents yielded lower results for oxygen concentrations after aeration than good ones, but this was not always the case.

With bad effluents it would not matter whether these low figures were due to experimental error or were realities, as the results would always condemn the effluents. In the case of good or fairly good effluents, the question is of great importance, as is shown by the following examples:

Sewage Effluents.

Initial oxygen concentration after full aeration	Dissolved oxygen absorption in five days at 18.3° C. Parts per 100,000	
	Assuming the oxygen concentrations are correct	Assuming true oxygen concentration is 0.93
1. Effluents 1.5 dilution:		
1. 0.78	0.50	1.15
2. 0.83	0.85	1.25
Canal-water receiving effluent:		
0.91	0.28	0.30
2. Effluents 1.5 dilution:		
1. 0.74	0.20	0.85
2. 0.77	0.60	1.10
Stream-water receiving effluent:		
0.86	0.11	0.18

In the case of good effluents therefore it makes a very considerable difference whether the initial concentration is in reality 0.93 or is the lower figure actually found by experiment.

Both in the routine standardisation of effluents and the carrying out of the investigation on self-purification of rivers it was therefore essential first of all to go into this question of technique.

The low results could be due to at least three factors:

1. The presence of a substance or substances in sewages diminishing the solubility of oxygen in water (in which case the low results would be realities).

2. The presence of oxidisable substances, using up dissolved oxygen or manganese peroxide in the course of the process of estimation.

3. The presence of substances reacting with iodine in the course of the final titration with thiosulphate.

In the latter two cases the actual results obtained for the oxygen concentration are erroneous.

It was thought possible that the sodium chloride present in sewage effluents roughly to the extent of 12 parts chlorine per 100,000 was the cause of the low results. In a sewage effluent diluted 1.5 with stream-water this would yield a chloride concentration of about four parts chlorine per 100,000. Experiments showed however that this concentration of sodium chloride had no effect on the solubility of oxygen in water.

The possible influence of organic matter was then considered. Peptone was dissolved in boiled distilled water just before full aeration and the dissolved oxygen figure of the solution compared with the normal figure at 18.3° C., obtained under the same conditions for distilled water itself. The amount of peptone to be added was judged from the organic nitrogen figure of a fairly bad effluent and amounted to 0.30 g. per litre.

The results obtained were as follows:

Distilled water alone	...	...	0.94	0.87	Dissolved oxygen, parts per 100,000
Distilled water + 0.3 g. peptone per litre			0.70	0.70	

It is seen that much lower results were obtained with the peptone solution.

Peptone, and also tank-liquor were next added at various stages of the dissolved oxygen test in order to attempt to ascertain the nature of the interference. The results were as follows:

*Dissolved oxygen concentration of boiled distilled water after full aeration
at 18.3° C.*

1. Distilled water alone (control)	...	0.97	1. Distilled water alone (control)	...	0.92
2. 0.3 g. peptone per litre added before aeration	...	0.88	2. One-fifth vol. tank-liquor added before aeration	...	0.85
3. 0.3 g. peptone per litre added immediately after aeration	...	0.82	3. One-fifth vol. tank-liquor added immediately after aeration	...	0.83
4. 0.3 g. peptone per litre added before precipitation of the manganese peroxide	...	0.90	4. One-fifth vol. tank-liquor added before precipitation of manganese peroxide	...	0.75
5. 0.3 g. peptone per litre added just before the peroxide was dissolved by addition of the HCl	...	0.93	5. One-fifth vol. tank-liquor added just before solution of peroxide in HCl	...	0.91
6. 0.3 g. peptone per litre added after addition of the acid	...	0.95	6. One-fifth vol. tank-liquor added after addition of the acid	...	0.84
			7. Tank-liquor alone unaerated contained no dissolved oxygen		

It is seen that the organic matter or other constituents of sewage can affect the results at all stages of the process. The low results do not appear to be due to diminished solubility of oxygen, as the organic matter, etc., exerts its influence after aeration and bottling of the water, and even when added

immediately before the precipitation of the oxygen as peroxide of manganese (probably through decomposition of the latter). It can also affect the titration of the iodine with thiosulphate, probably by reacting quickly with some of the iodine.

The latter point is further illustrated by the following additional results:

	Oxygen concentration of distilled water at 18.3° C.
Distilled water alone	0.90
With addition of tank-liquor just before titration of liberated iodine with thiosulphate	0.84

Further experiment also showed that, if a diluted sewage effluent were aerated and allowed to stand for a short time before estimation of the dissolved oxygen, there was also a marked fall in oxygen concentration:

	Immediately after aeration	Standing 40 mins. after aeration
Oxygen concentration	0.86	0.66
" "	0.88	0.79

Such rapid loss of oxygen must probably be due to chemical oxidation of certain substances, *e.g.* ferrous sulphide and organic matter, and points to this factor as one cause of the low results obtained in estimating the dissolved oxygen content of sewage effluents.

The presence of suspended matter in sewage effluents on the other hand seemed to have only a slight effect on the dissolved oxygen figure:

	Parts per 100,000
Unfiltered	0.84
Filtered	0.87

From these results it would appear that the low results obtained in estimating the dissolved oxygen content of aerated sewage effluents are due to:

1. Rapid oxidation of certain constituents during the estimation.
2. Decomposition of a portion of the manganese peroxide by organic matter.
3. Loss of iodine in the final thiosulphate titration due to reaction with constituents of sewage.

Attempts were next made to destroy the constituents of effluents causing the interference by (1) doubling the amount, and (2) doubling the time of action of the permanganate used in the Rideal-Stewart modification of Winkler's dissolved oxygen test. The permanganate is primarily used to destroy nitrites, which also interfere with the test, but it was thought that it might also be serviceable in destroying the other interfering substances if sufficient concentration or time were allowed.

Satisfactory results were sometimes but not always obtained when the amount of permanganate was doubled:

Oxygen concentration. Parts per 100,000.

Usual amount of permanganate	Double amount
0.87	0.98
0.88	0.94
0.81	0.81
0.71	0.77
0.70	0.81
0.82	0.82
0.88	0.90
0.83	0.91
0.86	0.83
0.88	0.93
0.88	0.935
0.85	0.90

Doubling the time, *i.e.* allowing 40 mins. instead of 20 mins., also did not give good results:

Usual time	Double time
0.83	0.86
0.85	0.84
0.80	0.91
0.74	0.78

There thus seemed to be no simple method of preventing the low results obtained in estimating the oxygen content of sewage effluents.

Experiments were therefore carried out to ascertain if the error still existed after incubation of the diluted effluent at 18.3° C. as carried out in the Rideal-Stewart or dissolved oxygen absorption test. If the interfering substances were largely oxidised or otherwise destroyed during the incubation, there would obviously be a large error in the initial oxygen estimation, but little or none in the final estimation after five days. In this case, the results for the dissolved oxygen figures of the effluents would be low and a considerable error thus introduced in routine work. On the other hand, if the interfering substances were not appreciably destroyed, the error in estimation would be practically the same at the end of five days as at the commencement of the test, and would thus be neutralised, and sufficiently accurate results would be obtained for ordinary purposes.

Experiments were therefore carried out to throw some light on this matter. Aerated diluted effluents were incubated in closed bottles at 18.3° C. and after five days or other suitable period the contents of the bottles were again *reaerated* strictly at the same temperature and the oxygen concentrations estimated and compared with the initial result.

It is seen that as a general rule there was very little difference in the figures, and, thus, although low results are often obtained in the estimation of oxygen in sewage effluents, the error is neutralised and does not interfere with the utility of the dissolved oxygen test.

Initial oxygen concentration at 18.3° C. Oxygen concentration after incubation and aeration at 18.3° C.
 Parts per 100,000

0.91	0.90 (5 days)
0.90	0.90 (5 ")
0.87	0.88 (5 ")
0.86	0.85 (5 ")
0.83	0.82 (24 hours)
0.80	0.80 (3 days)
0.80	0.73 (13 ")
0.88	0.82 (24 hours)
0.88	0.86 (7 days)
0.88	0.90 (5 weeks)
0.87	0.86 (5 days)
0.73	0.73 (4 weeks)
0.85	0.94 (4 ")
0.84	0.87 (5 days)
0.80	0.83 (2 ")

2. THE EFFECT OF TEMPERATURE ON THE DISSOLVED OXYGEN ABSORPTION FIGURES OF EFFLUENTS.

Reference has already been made to the experimental work of the Sewage Commission, showing that the oxidation processes were considerably accelerated by rise in temperature from 18.3° to 22° or 24° C., on account of which it was considered essential to carry out the dissolved oxygen absorption test rigidly at 18.3°, the average maximum temperature of rivers in this country.

Some further experiments have been made on the question of the influence of temperature, and are summarised below:

Dissolved oxygen absorption figures for effluents.
 Parts per 100,000, five days.

10° C.	18.3° C.	22° C.	37° C.
1.10	1.75	—	—
1.20	2.45	—	—
0.30	0.60	—	—
—	0.65	2.10	—
—	9.80	9.70	9.6
—	11.30	11.10	11.00

(In all these experiments the initial aerations were carried out strictly at the particular temperatures selected.)

It is seen that in one case out of three a very much higher result was obtained at 22° than at 18.3°, while in the two other cases at temperatures of 18.3°, 22°, 37°, no differences were observed. At 10° however the oxidation processes went on very much more slowly than at 18.3°, and the results emphasise the necessity of carrying out the test under strictly standardised conditions. For routine work the standard temperature of 18.3° C. recommended by the Commission has been used.

The figures tabulated above are also of interest from the point of view of the general question of river purification, as they tend to show that sewage will have a considerably less polluting effect in the winter months than during the summer, while it will not necessarily cause a greater pollution in rivers in tropical countries than in temperate regions.

3. THE EFFECT OF TIME ON THE DISSOLVED OXYGEN ABSORPTION TEST.

The period of five days was recommended by the Commission for this test [*Roy. Comm.* 1913, p. 78], as it was suspected that unreliable results were obtained if shorter periods were employed, owing to the possibility of a bacterial lag, and a greater influence due to the presence of inhibitory substances in the sewage. The value of the test, however, would be considerably enhanced, if a shorter period than five days would be sufficient. It was therefore considered advisable to carry out more experiments on this point, and the results are tabulated below:

Dissolved oxygen absorbed at 18.3° C.

	48 hours	5 days	Ratio
1. Effluent	0.14	0.30	1:2
Stream above effluent	0.007	0.014	1:2
Stream below effluent	0.02	0.11	1:5
2. Effluent	0.70	1.00	1:1.4
Stream above ...	0.04	0.10	1:2.5
3. Primary effluent ...	1.70	3.00	1:1.7
4. Effluent	0.12	0.95	1:8
Stream above ...	0.12	0.13	1:1
5. Effluent	2.35	All O absorbed	1:?
6. Effluents 1	0.10	0.80	1:8
„ 2	0.00	1.45	1:∞
Stream above ...	0.16	0.29	1:1.8
7. Effluents 1	0.00	0.70	1:∞
„ 2	0.18	>3.45	1:>19
„ 3	0.16	0.75	1:4.7
„ 4	0.46	>3.40	1:>7.4
River above ...	0.11	0.23	1:2
8. Effluents	0.80	0.65	1:0.8
„	1.20	1.55	1:1.3
Stream	0.08	0.26	1:3

The Commission found that the five days' test gave a very useful idea of the polluting effect of an effluent [*Roy. Comm.* 1913, p. 63], and it was thought that if a fairly definite ratio between the two and five days' tests could be obtained, it would be possible to use the two days' test as a standard. The above results however show that this is not the case, as even very bad effluents may give little or no indication of oxygen absorption in two days.

Further experiments using a stronger concentration of effluent, *i.e.* 1 : 1 dilution with river-water, were next carried out. The results are as follows:

			48 hours	5 days	Ratio
1. Effluent	...	...	0.15	1.40	1:9
2. „	...	...	0.56	0.90	1:1.6
Stream above	...	...	0.09	0.15	1:1.7
3. Effluent 1	...	...	0.52	0.65	1:1.2
„ 2	...	...	0.96	1.55	1:1.6
Stream	...	...	0.08	0.26	1:3.2

Even with the increased concentration it is seen there is considerable variation in the ratio, and at the present time there is no alternative but to employ the longer period of five days.

It was thought that it might be possible to accelerate the processes by means of a catalyst, and in this connection the effect of manganese salts has been studied. Small amounts of manganese chloride were added to diluted sewage effluents, the dissolved oxygen contents estimated at the end of two days, and the results compared:

	Dissolved oxygen absorbed in 2 days. Parts per 100,000
Effluent without $MnCl_2$	0.65
„ plus 0.1 % $MnCl_2$	0.02
„ plus 0.025 % $MnCl_2$	0.03

The addition of the manganese salt thus considerably inhibited the absorption of the dissolved oxygen. Further experiments with other catalytic substances are desirable.

4. THE INFLUENCE OF DILUTION.

The Commission [*Roy. Comm.* 1913, p. 80] carried out some experiments bearing on the question of the effect of dilution on the rate of dissolved oxygen absorption by sewage effluents, and found that dilution up to 1 in 3 with tap-water had very little effect. It was thought desirable to carry out some more experiments, and the results are illustrated by the following figures:

Dilution	Dissolved oxygen absorbed by effluent. Parts per 100,000	
1 in 2	0.52	0.96
1 in 5	0.80	1.20

It is seen that the oxygen absorption is slightly increased by increasing the dilution $2\frac{1}{2}$ times. Increasing the dilution from 1 in 5 to 1 in 20 had practically no influence at all, and dilution would thus seem to be not such an important factor as would be expected.

5. THE POSSIBLE INFLUENCE OF INHIBITION FACTORS.

The possible influence of certain constituents, *e.g.* lime, in sewage in inhibiting the absorption of dissolved oxygen was realised by the Commission [*Roy. Comm.* 1913, p. 84]. It was considered quite likely that the dissolved

oxygen test might for this reason under certain conditions give false and low results, especially if the two days' test was used instead of the five days' test. The possibility of ammonia acting as inhibitory agent does not however appear to be discussed. It has been noticed frequently in routine work that an effluent containing as much as 10 parts per 100,000 of ammoniacal nitrogen might yield a dissolved oxygen absorption figure of only about 1.0 and would thus be classified as a satisfactory effluent. Experiments have been therefore carried out to ascertain whether ammonia in such concentrations has any inhibitory action. Ammonium carbonate was used for this purpose:

Diluting medium 1 : 5										Dissolved oxygen absorbed by an effluent in 5 days
Distilled water	...	...	...	...	...	...	...	...	...	13.0
"	containing amm. carb. equivalent to	2.5	parts amm. N per 100,000							13.4
"	"	"	"	"	5.0	"	"	"	"	13.4
"	"	"	"	"	10.0	"	"	"	"	12.6
"	"	"	"	"	20.0	"	"	"	"	12.2
"	"	"	"	"	45.0	"	"	"	"	11.2

It is seen that ammonia in concentrations such as occur in sewage effluents had no perceptible influence on the rate of oxygen absorption. Much larger concentrations in fact had only a small effect.

6. A COMPARISON OF THE CAPACITIES FOR SELF-PURIFICATION OF DIFFERENT RIVER-WATERS.

This comparison has been made by measuring the rate of oxidation of the constituents of sewage effluents or unpolluted river-muds in the presence of various river-waters. The sewage effluents were diluted with the river-waters, and the mixtures incubated at 18.3° C. usually for five days as in the dissolved oxygen absorption test. The amount of oxygen absorbed by the diluted sewage and also by the unpolluted river-water itself being thus determined by Winkler's process, the dissolved oxygen absorption due to the sewage or effluent could be ascertained by difference¹. The experiments with the muds were similarly carried out.

The geological source and nature of the river-bed has been investigated in the case of each river, and the conditions have been correlated with the results of the oxidation experiments. The results are set forth in the following table. Unless otherwise stated, the temperature of the incubation was 18.3°, dilution of effluent 1 : 5, and period of incubation five days.

¹ In the case of tank-effluents, the amounts used were so small and the dilution thus was so great that the actual absorption of dissolved oxygen by the river-water itself was not required, because the small amount of sewage did not affect the estimation of oxygen, and the absorption of oxygen by the tank-effluent could be determined by taking the difference between the oxygen concentrations of the river-water, and river-water + effluent at the end of the period of incubation. This was also the case in the experiments with muds, as before titration the supernatant water was syphoned off from the layer of mud into another stoppered bottle.

Comparison of rate of oxidation in river-waters.

Material used	River (1)			River (2)			
	Dissolved oxygen absorbed, parts per 100,000, by		Character of river-bed at point of sampling	Dissolved oxygen absorbed, parts per 100,000, by		Character of river-bed at point of sampling	
	Effluent when di- luted with this water	River- water alone		Effluent when di- luted with this water	River- water alone		
(a) 1. From Gravel and Upper Bagshot Sands.							
2. From same beds but after river has subsequently run over, first Bracklesham beds, Lower Bagshot and finally London Clay for a considerable distance.							
Land-effluent		1.35	0.19	Gravel and sand	1.70	0.02	Clay
"		0.55	0.19	"	1.85	0.02	"
"		0.00	0.19	"	0.95	0.02	"
"		2.61	0.00	"	2.19	0.11	"
"		2.68	0.00	"	2.05	0.11	"
"		0.94	0.00	"	0.67	0.11	"
"		1.04	0.00	"	0.68	0.11	"
"		1.05	0.09	"	0.95	0.19	"
"		0.60	0.09	"	0.55	0.19	"
"		1.05	0.18	"	1.60	0.23	"
"		1.20	0.08	"	1.10	0.53	"
"		0.65	0.00	"	1.15	0.22	"
"		0.80	0.04	"	0.65	0.13	"
"		1.60	0.04	"	0.45	0.13	"
Tank-effluent							
(dilution 1 in 50)	2 days	13.75	—	"	{ 36.25 30.25 }	—	"
"	1 "	6.50	—	"	11.10	—	"
"	2 "	13.75	—	"	14.75	—	"
"	1 "	17.00	—	"	16.00	—	"
"	1 "	4.00	—	"	6.00	—	"
"	3 "	9.00	—	"	13.40	—	"
"	5 "	10.50	—	"	15.20	—	"
"	2 "	23.50	—	"	21.70	—	"
"	3 "	28.50	—	"	14.50	—	"
"	4 "	34.50	—	"	24.50	—	"
River-mud							
(dilution 1 in 100)		63.3	—	"	70.00	—	"
"		65.0	—	"	58.30	—	"
(b) 1. From Gravel and Upper Bagshot Sands.							
2. From same beds but mixed with river-water from Bracklesham beds.							
Land-effluent		0.35	0.13	Gravel and sand	1.05	0.24	Sand and clay
"		0.65	0.15	"	0.00	0.27	"
Tank-effluent							
(dilution 1 in 50)	2 days	22.25	—	"	10.75	—	Green (Glauc- conitic) clay
"	2 "	26.00	—	"	17.15	—	"
"	2 "	10.75	—	"	11.62	—	"
"	4 "	14.25	—	"	17.00	—	"
Glaucconitic mud from river in Bracklesham beds (dilution 1 in 100)		63.30	—	"	55.80	—	"
"		65.00	—	"	53.70	—	"
Land-effluent		0.65	0.09	"	0.00	0.11	Clay and sand
"		0.50	0.06	"	0.60	0.17	"
(c) 1. From Gravel and Upper Bagshot Sands.							
2. From Lower Bagshot, subsequently running over London Clay.							
Effluent		1.95	0.11	Gravel and sand	3.35	0.17	Clay
"		0.18	0.03	"	1.35	0.22	"

Comparison of rate of oxidation in river-waters.

(g) From Gravel, Sand, and Clay, and from the Chalk.

Material used	Dissolved oxygen absorbed, parts per 100,000, by			Dissolved oxygen absorbed, parts per 100,000, by		
	Effluent diluted with the river-water	River- water alone	Character of river-bed at point of sampling	Effluent diluted with the river-water	River- water alone	Character of river-bed at point of sampling
	River-water from Lower Bagshot Clay, subsequently running over London Clay.			River-water from Chalk subse- quently running over Wool- wich and Reading beds.		
Land-effluent	1.15	0.23	Clay	1.70	0.06	Calcareous gravel
"	0.90	0.23	"	0.80	0.06	"
	River-water from Lower Chalk.			River-water from Folkestone beds subsequently running over Sandgate beds.		
"	2.60	0.09	Marly loam and calcareous gravel	1.45	0.19	Sand
	River-water from Gravel and Up- per Bagshot Sands.			River-water from Chalk subse- quently running over Wool- wich and Reading beds.		
Tank-effluent	1 day	24.9	—	Gravel and sand	32.0	—
(dilution 1 in 50)	2 "	34.4	—	"	41.3	—
"	3 "	40.0	—	"	45.7	—
	River-water from Gravel and Up- per Bagshot Sands, subsequent- ly running over Bracklesham beds, Lower Bagshots and Lon- don Clay.			River-water from Chalk subse- quently running through Wool- wich and Reading beds.		
Tank-effluent	1 day	11.40	—	Clay	6.00	—
(dilution 1 in 50)	2 "	9.50	—	"	8.80	—
"	3 "	10.60	—	"	3.20	—
	River-water from Lower Bagshot subsequently running over Lon- don Clay.			River-water from Chalk subse- quently running over Woolwich and Reading beds (3 different rivers).		
Filter-effluent	1.35	0.22	Clay	1.15	0.05	Calcareous gravel
"	River-water from Gravel and Up- per Bagshot Sands.			0.75	0.02	"
"	0.18	0.03	Gravel and sand	1.08	0.11	"
	River-water from Gravel and Up- per Bagshot Sands.			River-water from Lower Chalk, Upper Greensand and Gault subsequently running over Folkestone beds.		
Tank-effluent	1 day	9.50	—	Gravel and sand	10.25	—
(dilution 1 in 50)	2 "	11.25	—	"	19.87	—
"	3 "	16.75	—	"	22.70	—
	River-water from Folkestone beds subsequently running over Sandgate beds.			River-water from Lower Chalk, Upper Greensand and Gault subsequently running over Folkestone beds.		
Filter-effluent	11.40	0.16	Sand	9.85	0.11	Clay
	River-water from Sandgate beds, Lower Greensand.			River-water from Lower Chalk and Upper Greensand.		
Effluent	0.80	0.04	Sand	0.45	0.00	—

A survey of the tabulated results shows that very considerable differences exist in the rate of oxidation of the constituents of sewage effluents in different river-waters of different geological source. There is however a marked inconsistency in the results, so that it is not yet possible to draw any definite conclusions as to the influence of geological source on the oxidation processes in river-waters. This inconsistency may be expected, as the conditions, *i.e.* the chemical composition and bacterial population of sewage are subject to very great variation, and thus in each experiment new factors may be introduced.

The results so far obtained may be summarised thus:

Although in the case of nearly all the river-waters used for comparisons, close agreement in the rates of oxidation was sometimes observed, yet marked differences were frequently found.

Oxidation proceeded more slowly or more quickly in:

1. River-water from the Gravel and Upper Bagshot Sands than in river-water derived from the same source but subsequently running over successively the Bracklesham beds, Lower Bagshot Sands, and London Clay.
2. River-water from the Gravel and Upper Bagshot Sands than in river-water from the same beds, but mixed with river-water from the Bracklesham beds.
3. River-water from the Folkestone beds than in river-water from the Hythe beds.
4. River-water from the Folkestone beds than in river-water derived from the same beds but subsequently running over the Sandgate beds.
5. River-water from the Gravel and Upper Bagshot Sands than in river-water from the Lower Greensand strata.
6. River-water from Gravel, Sand, and Clay than in river-water from the Chalk.
7. Oxidation proceeded more slowly in river-water from the Gravel and Upper Bagshot Sands than in river-water from the Lower Bagshot Sands and London Clay.

A considerable amount of work has yet to be done in order to interpret these variable results. They indicate however the importance of always strictly using the particular river-water into which an effluent is discharged for dilution of the effluent in Winkler's dissolved oxygen absorption test. This rule should always be adhered to, unless it can be conclusively shown by a long series of experiments that any other river-water, which happens to be more convenient for use, gives results in close agreement.

No work seems to have been carried out previously on the deoxygenating action of the muds of unpolluted rivers. The experiments described in this paper show that such river muds can absorb from 12 to 70 parts per 100,000 of oxygen from river-waters in five days at 18.3° C.

Further observations are given below:

Character of mud	Supernatant water used	Dissolved oxygen absorbed. Parts per 100,000
From clean bed of stream formed by spring-water from Hythe beds thrown out by Atherfield Clay	Stream water	6.00
From stream in Folkestone beds	Distilled water	16.00
From river in Sandgate beds	"	40.00
From river in London Clay	River-water	44.00

It is seen that unpolluted river-muds absorb very considerable amounts of dissolved oxygen, and their potential deoxygenating effect is in every case much greater than that of a sewage effluent of fair quality (2 parts per 100,000). As a general rule however muds of unpolluted rivers will have actually less deoxygenating effect than sewage effluents, because the volume of the superficial deoxygenating layer of mud relative to that of the river is small.

7. COMPARISON OF THE DISSOLVED OXYGEN ABSORPTION OF SEWAGE EFFLUENTS WHEN DILUTED WITH VARIOUS RIVER- AND TAP-WATERS.

In a previous communication [Cooper, 1918] it was shown that the dissolved oxygen absorption figures of effluents were considerably lower when dilution was made with a hard tap-water from the Chalk than with distilled water or river-water taken from the river receiving the effluent. It was thought desirable to obtain more data bearing on this question and comparisons of the rates of oxidation in the presence of: 1. River-water above the outfalls. 2. Tap-water at laboratory. 3. Local tap-water, *i.e.* water-supply running into sewerage.

Some results on these lines have already been given in Section 6 of this paper, as the river-water from the Gravel and Upper Bagshot Sands happened to be derived from the same springs as yielded the laboratory tap-water and the results thus serve as comparisons of dissolved oxygen absorption in the presence of tap-water (Gravel and Upper Bagshots) and the river-waters.

It is seen that the oxygen absorption often varied considerably and quite different results were obtained according as tap- or river-water was used for dilution. Further comparisons are made in the table on p. 362.

It is seen that lower results were frequently obtained with the various hard calcareous waters, thus confirming the previous findings. This however was not invariably the case.

The fact that hard waters do not always inhibit the oxidation processes is further brought out by the following experiments with river-muds and effluents:

Supernatant water					Dissolved oxygen absorption mud or effluent. Parts per 100,000	
1.	Distilled water	...	...	...	16	
	Hard water from Chalk	...	...	...	12	
	"	"	...	...	20	
2.	Distilled water	...	...	...	40	
	" plus solid calcium carbonate	...	...	...	39	
	Hard water from Chalk	...	...	...	30	
					a.	b.
3.	Effluent diluted with distilled water	...	...	...	1.20	0.90
	" " " " plus Oolite				1.05	0.95
	" " " " plus Barytes				0.90	0.75

Dissolved oxygen absorption. Parts per 100,000 of effluent.

	Diluted with river-water receiving effluent	Diluted with laboratory tap-water	Diluted with local tap- water, i.e. water-supply running into sewerage
1.	36.25	28.0	—
	30.25	23.0	—
	(London Clay)	(Hard water from Chalk be- neath Tertiary formations)	
2.	0.55	—	1.90
	(London Clay)		(Hard water from Chalk be- neath Tertiary formations)
	0.00	—	7.75
	(Gravel and Upper Bagshot Sands)		(Hard water from Chalk be- neath Tertiary formations)
	23.0	23.0	—
	(Gravel and Upper Bagshot Sands)	(Hard water from Chalk be- neath Tertiary formations)	
	0.00	0.65	—
	(Bracklesham beds)	(Gravel and Upper Bagshots)	
	0.90	—	1.15
	(Sandgate beds)		(Hythe beds)
	0.30	—	0.35
	(Gravel and Upper Bagshot and Bracklesham beds)		(Chalk beneath Tertiaries)
	20.7	19.5	—
	23.75	24.35	—
	(Gravel and Upper Bagshots)	(Gravel and Upper Bagshots)	
	3.35	1.95	2.80
	(London Clay)	(Gravel and Upper Bagshots)	(Chalk beneath Tertiary for- mations)
	"	"	1.30
			(Tap-water from another local supply, water from Chalk at surface)
	0.65	—	0.45
	(Bracklesham beds)		(Chalk beneath Tertiaries)
	1.90	—	1.05
	(London Clay)		(Chalk beneath Tertiaries and river gravel)
	1.35	0.18	0.90
	(London Clay)	(Gravel and Upper Bagshots)	(Chalk beneath Tertiaries and river gravel)
	"	"	1.05
			(Upper Chalk, another local supply)

8. COMPARISON OF DISSOLVED OXYGEN ABSORPTION OF SEWAGE EFFLUENTS DILUTED WITH RIVER-WATER AND DISTILLED WATER.

In the previous paper [Cooper, 1918] some evidence was offered showing that the dissolved oxygen absorption figures of sewage effluents were higher when distilled water was used for dilution than when river-water was em-

ployed. Since then many other experiments have been made and the results are recorded below:

Dissolved oxygen absorption. Parts per 100,000 of effluent.

1. Diluted with river-water receiving effluent		2. Diluted with dis- tilled water
0.0	(Gravel and Upper Bagshot Sands)	7.65
23.00	" " "	15.50
1.80	" " "	1.50
0.65	" " "	1.25
1.10	" " "	0.95
2.25	" " "	2.40
0.18	" " "	1.30
0.50	" " "	0.85
1.15	(London Clay)	1.25
1.35	"	1.30
2.05	"	2.00
2.35	"	2.75
0.80	(Sandgate beds—Lower Greensand)	0.45
1.25	" " "	1.25
1.95	(Hythe beds—Lower Greensand) ...	1.50
0.70	" " " ...	0.85
1.40	(Folkestone beds—Lower Greensand)	1.40
2.40	" " "	2.40
0.45	(Lower Chalk and Upper Greensand)	0.45
1.15	(Chalk and Tertiaries)	1.30
0.75	" "	1.30
1.08	" "	1.30

The results show that there is sometimes close agreement in the two sets of figures, and in this case for practical purposes it makes no difference whether distilled or river-water is used. In many cases however there is a considerable discrepancy and oxidation may go on either more slowly or more quickly in river-water than in distilled water. In the case of water from the Gravel and Upper Bagshot Sands the inhibitory effect was often very marked, and was greater in fact than shown in the earlier results [Cooper, 1918]. Oxidation also proceeded more slowly in river-waters from the Chalk than in distilled water.

River-water from the London Clay on the other hand as a rule had no perceptible inhibitory or accelerating effect on the oxidation processes. In one experiment however there was an appreciable inhibition. In the case of river-waters from the Lower Greensand (Sandgate, Folkestone, and Hythe beds) the results were variable. The earlier experiments [Cooper, 1918] pointed to a considerable inhibitory action, but in some of the further experiments described above oxidation proceeded either more rapidly in the river-waters or at the same rate approximately as in distilled water.

9. COMPARISON OF DISSOLVED OXYGEN ABSORPTION OF SEWAGE EFFLUENTS
WHEN DILUTED WITH VARIOUS TAP-WATERS.

In the previous communication it was shown that the constituents of sewage effluents oxidised about 5 to 20 times more rapidly in distilled water than in a hard tap-water, and about 25 per cent. faster in distilled than in tap-water from the Gravel and Upper Bagshot Sands.

In the case of a highly ferruginous tap-water from the Folkestone beds there was an initial inhibition which however was no longer noticed when the iron was removed by oxidation and filtration. In the usual five days' dissolved oxygen test, on the other hand, oxidation proceeded at the same rate in the tap-water (filtered or unfiltered) as in distilled water.

Some further observations on the relative rate of oxidation in distilled water, hard tap-waters, and tap-water from the Gravel and Upper Bagshot Sands are given below:

Dissolved oxygen absorbed in five days at 18.3° C.

Material used	Dilution with		
	Distilled water	Hard water	Soft water from Gravel and Upper Bagshot
Primary filter-effluent	1.30	0.90	0.18
" "	1.30	1.05	0.18
" "	—	2.80	1.95
" "	—	1.30	1.95
Tank-liquor	—	7.75	0.00
"	—	23.00	23.00
Land-effluent	—	0.35	0.30

Additional figures affording comparisons of oxidations in distilled water and tap-water from Gravel and Upper Bagshot Sands are given in Section 8, the river-water from these geological formations being derived from the same springs as supplied the tap-water.

A survey of all the results shows that there is very great variation in the relative rate of oxidation of sewage constituents in distilled water and in the tap-waters (soft and hard). It is seen that both tap-waters frequently exercise a very considerable inhibitory effect on the rate of oxidation, and that the hard water may have either a greater or smaller inhibitory action than the soft water. In some cases however there is a fairly close agreement. The results point to the conclusion that the oxidation processes in the artificial methods of sewage purification must be considerably affected by the nature of the water-supply which mixes with the sewage, and also by that of surface-water gaining access to the drainage system. Owing to the great variations it is not possible to draw any helpful conclusions from the results of laboratory work in reference to this question, but it may be pointed out that the possibility of inhibitory action should always be borne in mind in framing new schemes for sewage purification, and arrangements should always be made to render it easily possible to extend the area of filtration, should difficulties be encountered.

10. THE OXYGEN ABSORBED FROM PERMANGANATE OR "TIDY TEST."

The test is used extensively in the chemical examination of water supplies. It is also employed to a certain extent in the standardisation of sewage effluents, although the Sewage Commission [*Roy. Comm.* 1913, p. 74] has reported that it gives a less correct measure of the polluting effect of an effluent than the dissolved oxygen test. Some observations made in the course of water analysis at first suggested that geological factors influenced the permanganate test just as they had been found to affect the Winkler's test. Thus, the loss in strength of the permanganate solution on incubation was occasionally found to be greater in the presence of distilled water than in presence of pure tap-waters (calcareous or soft water supplies).

10 cc. *N/80 potassium permanganate*. 10 cc. 25 % *sulphuric acid*.
100 cc. *water*. Four hours at 37° C.¹

Immediate titration	Titration with thiosulphate after 4 hrs incubation with distilled water	Titration after 4 hrs incubation with pure tap-water
12.4 cc.	11.2 cc.	11.8 cc. (hard water)
15.0	13.2	13.8 "
15.4	15.0	15.3 (soft water)

It would seem that certain constituents of the tap-water inhibit either the natural decomposition of the permanganate or the oxidation of some impurity present in it [Cooper and Heward, 1919]. Experiments were therefore next carried out to ascertain if the rate of oxidation of the constituents of sewage by permanganate were affected by dilution with different tap-waters. For this purpose the Tidy Test was carried out in the usual way.

10 cc. *Tank Liquor (Sedimented Sewage)*. 10 cc. *N/80 permanganate*. 10 cc. 25 % *sulphuric acid*. 90 cc. *water*. Four hours at 37° C.

Oxygen absorbed by Tank Liquor from Permanganate. Parts per 100,000.

Dilution with distilled water	Dilution with hard calcareous water	Dilution with soft peaty water
3.10	3.00	3.50
2.00	2.10	—
7.25	7.40	—
8.00	—	8.20
3.70	—	2.33

The results as a whole show that the permanganate test unlike the dissolved oxygen test is not appreciably affected by the presence of a hard or soft water. It follows from this that the permanganate test gives a more correct measure of the actual amount of oxidisable matter present, and is thus of special value in water analysis. The dissolved oxygen test on the other hand determines the amount of oxidation that can go on under certain defined conditions, and thus measures, not the proportion of oxidisable matter present, but its actual deoxygenating or polluting effect on the diluting water. This test will thus be of special value in the standardisation of sewage effluents.

¹ 37° C. was used as standard temperature as it was more convenient and as there was practically no difference in the results obtained at 27° and 37°.

SUMMARY.

1. Fully aerated sewage effluents and polluted river-waters may contain considerably less dissolved oxygen than distilled water under the same experimental conditions. The low results appear to be due to various factors, and cannot be prevented by any method so far employed. As, however, the oxygen content is still the same after incubation for five days and subsequent re-aeration, there seems to be no appreciable error involved in Winkler's dissolved oxygen absorption test (Rideal-Stewart modification).

2. The oxidation of the constituents of sewage proceeds much more slowly at 10° C. than at the standard temperature of 18.3° C. usually employed.

At temperatures of 22° C. and 37° C. oxidation may proceed either at the same rate as at 18.3° C. or sometimes faster. The results show the necessity of adhering strictly to a definite temperature in the dissolved oxygen absorption test. The temperature of 18.3° C. was recommended by the Sewage Commission, this being the maximum temperature of river-waters in this country.

3. Attempts to shorten the five days' dissolved oxygen absorption test to two days have not been successful, as owing to an initial lag most variable ratios between the two and five days' tests are obtained. A bad effluent in fact may give very little indication of oxygen absorption in two days.

4. Dilution appears to have comparatively little influence on the rate of oxidation of the constituents of sewage.

5. Ammonia up to a concentration of 45 parts per 100,000 has also only a slight inhibitory effect on the rate of oxidation.

6. Oxidation of sewage constituents may proceed at very considerably different rates in river-waters of different geological source. The results are however very variable, and it is not yet possible to draw any definite conclusions as to the influence of geological factors upon the oxidation processes.

While in the case of nearly all river-waters employed, close agreement in the results is sometimes observed, oxidation may be slower or quicker:

(i) In river-water from the Gravel and Upper Bagshot Sands than in river-water from the London Clay, Bracklesham beds, and Lower Greensand formation.

(ii) In river-water from the Folkestone beds than in river-water from the Sandgate and Hythe beds.

(iii) In river-water from Gravel, Sand, and Clay formations than in river-water from the Chalk.

7. Mud from unpolluted rivers contains a considerable amount of oxidisable matter and may have a deoxygenating effect upon the water from 3 to 35 times as great as that of a good standard sewage effluent. The actual volume of the superficial deoxygenating mud relative to that of the supernatant water being small, however, muds from clean rivers will in general have less polluting effect than an effluent.

8. Considerably lower or higher results in the dissolved oxygen absorption test may be obtained when tap-water from the Gravel and Upper Bagshot

Sands is used for dilution than when the river-water into which the effluent actually drains is employed. Sometimes however there is close agreement between the two sets of figures.

9. Lower results are often obtained when hard calcareous tap-waters are used for dilution instead of the river-waters. Occasionally however the results are higher, while sometimes there is no appreciable difference noticed.

10. There may sometimes be close agreement in the dissolved oxygen absorption figures of an effluent diluted respectively with river-water and distilled water.

In many cases however, *e.g.* river-water from the Gravel and Upper Bagshot Sands, Lower Greensand, and Chalk, there is a considerable difference, and oxidation may go on more slowly or more rapidly in the river-water than in distilled water; the river-water thus having an inhibitory or accelerating action as the case may be.

In the case of river-water from the Gravel and Upper Bagshot Sands, and the Chalk, the *inhibitory* action upon the processes of oxidation may be especially marked.

11. The main conclusion to be drawn from the results summarised in 6-10 is that in the standardisation of sewage effluents the river-water into which the effluent is actually discharged must be used for dilution in Winkler's dissolved oxygen absorption test, unless it can be conclusively shown by several experiments that reliable results can be obtained by employing a more convenient diluting medium, *e.g.* another river-water, tap-water, or distilled water.

12. It is very likely that the oxidation processes in artificial methods of sewage purification may be affected by the nature of the water-supply, and by surface water entering the drainage system. Both hard and soft tap-waters may exert a very considerable inhibitory action in laboratory experiments. It is recommended therefore that allowance for possible inhibitory factors be always made in preparing new schemes of sewage purification, so that additional filtering area can be subsequently added, if necessary, with the minimum of trouble and expense.

13. The oxidation by permanganate or Tidy Test, unlike the dissolved oxygen absorption test, does not appear to be affected by the presence of waters of different geological source.

A consideration of the facts leads to the conclusion that the permanganate test is more suitable for the chemical examination of water supplies, while the dissolved oxygen test is more suitable for the standardisation of sewage effluents.

BIBLIOGRAPHY.

Cooper, E. A. and A. E. (1918). *Biochem. J.* 12, 275.

Cooper and Heward (1919). *Biochem. J.* 13, 25.

Roscoe and Lunt (1889). *J. Chem. Soc.* 532.

Royal Commission on Sewage Disposal (1908). *5th Report*, Appendix 6, 9.

— (1913). *8th Report*, 2, Appendix.