XXXI. AN INVESTIGATION INTO THE CAUSES OF DEATH OF SALMON AND SEA TROUT SMOLTS IN THE ESTUARY OF THE RIVER TEES.

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INTRODUCTION.

THE Tees was formerly a noted salmon river, large numbers of both salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) being caught by rods in the upper reaches and in nets in the estuary. For some years past however there has been considerable mortality amongst adult fish whilst attempting to pass through the estuarine reaches on their way to the spawning grounds, and again when descending to the sea as kelts after spawning. In addition, a large proportion of the young salmon and sea trout in the smolt stage are killed during their seaward migration in the months of April, May and June. This mortality is undoubtedly brought about by the heavy pollution to which the estuary is now subjected. The polluting material includes large volumes of sewage and the waste liquors from numerous industrial plants on the estuary banks. The work to be recorded was undertaken to determine the relative importance of these different types of polluting materials in contributing to the toxicity of the estuarine water into which they are discharged.

An account of the physical features and tidal conditions of the estuary has already been published by the Water Pollution Research Board [1931]. The limit of tidal action is some 25 miles from the mouth, salt water penetrating about 15 miles from the sea at high water during spring tides. For a distance of approximately 12 miles from the mouth the estuary channel is navigable and is maintained, by dredging, at a depth of not less than 12 feet at low water. The banks here consist either of artificial training walls, or of wharves and similar structures. It is into this section of the estuary that the bulk of the pollution, both of domestic and industrial origin, is discharged. Above the navigable reaches the channel is undredged and runs between natural or mainly natural banks.

Polluting effluents.

The industries of Tees-side are concerned predominantly with the production of iron and steel and the majority of toxic discharges arise, directly or indirectly, from these processes. Other miscellaneous effluents are, in

comparison with these, of but minor importance and do not contribute appreciably to the toxicity of the estuary water.

The main types of polluting material discharged are:

(1) *Crude sewage*. Crude sewage is discharged from all the towns on the banks of the estuary, the largest being Middlesbrough (pop. 136,000) and Stockton (pop. 67,200); the total contributing population is approximately 275,000. Water carriage of sewage is now employed throughout practically the whole of the area.

(2) *Coke-oven effluents*. Coke-oven effluents are produced in considerable volumes during the manufacture of the large quantities of coke used locally in iron smelting. Two methods are employed for the recovery of ammonia from the coke-oven gas; the direct and semi-direct processes giving rise to two types of effluent:

(A) *Coke-oven effluent, direct ammonia recovery*. In this type of installation the heavy tar is removed from the crude coke-oven gas at a temperature of 65 to 70° before the liquor condenses. The hot gas then passes through a saturator where the ammonia is converted into sulphate. The waste liquor consists of a mixture of the condensate from the water-cooled condensers and the washing and cooling water from the jet condensers. The liquor then flows through settling tanks to remove naphthalene and a small quantity of light oil and is afterwards discharged into the estuary.

Table I.

	(A) parts per 100	(B) parts per 100
Chloride as HCl	1.65	0.153
Alkalinity as CaO	0.014	0.20
Ammonia (free)	0.003	0.024
Ammonia (fixed)	0.002	0.003
Thiocyanate (as CNS)	nil	0.010
Thiosulphate (as S)	0.007	0.027
Cyanide (as HCN)	0.004	nil
Sulphur (as H ₂ S)	trace	trace
Monohydric phenols (as C ₆ H ₅ OH)	0.0105	0.184
Total tar-acids	0.0125	0.250
Oxygen consumed from N/80 } in 4 hours	0.057	0.494
permanganate at 80° F. } in 3 minutes	0.020	0.315

The sulphur content of (A) may be as high in some samples as 0.01% H₂S.

(B) *Coke-oven effluent, semi-direct ammonia recovery*. In this type of plant the ammonia liquor which is deposited in the water-cooled condensers is separated from tar and is mixed with the condensate from the water-cooled condensers. The mixed liquor is distilled with steam in an ammonia still, with the addition of lime. The gases from the ammonia still are mixed with the crude coke-oven gas at a point in the gas main between the tar extractor and the saturator. The hot spent liquor from the ammonia still is discharged into the estuary.

Analyses, carried out by the Government Chemist, of samples of effluents

(A) and (B) are given in Table I (A) and (B). The total volume of effluents discharged per 24 hours is, very approximately, (A) 10,200 cubic metres and (B) 280 cubic metres.

(3) *Spent pickle liquor*. Pickle liquors are produced as the result of cleaning steel sheets or wire in concentrated hydrochloric acid, before galvanising. The following is an analysis of a spent pickle liquor (Table II).

Table II.

	Parts per 100
Total iron as Fe	16.05
Ferric iron	0.40
Ferrous iron	15.65
Free hydrochloric acid as HCl	3.13

In some works the free acid is neutralised with lime before discharge.

The volume of effluent produced varies widely according to trade conditions and, in addition, part of it is disposed of locally for iron recovery so that an average figure for the rate of discharge cannot be given.

The effect of polluting discharges.

After being discharged, polluting material is carried up and down the estuary under tidal action and it has been calculated that under normal conditions of freshwater flow from the upper river it may remain in the estuary for several days before finally reaching the open sea. During this time both sewage and industrial effluents undergo biological, or in the case of pickle liquors, chemical oxidation at the expense of the dissolved oxygen of the estuary waters. A belt of water, in the central portion of the estuary, moving to and fro under tidal action is thus permanently partially deoxygenated. The degree of deoxygenation has been found to depend in some measure on the freshwater flow and on certain other factors but mainly on the water temperature.

The possibility of migrating smolts being killed by dissolved oxygen deficiency was therefore one which had to be considered.

The lower limit of dissolved oxygen under which smolts can live for periods of several days does not seem to have been determined; such a determination would require the use of a large-scale deoxygenating apparatus and has not been attempted during the present investigation. It was found possible, however, to maintain a supply of running water having a dissolved oxygen content of 58 to 64 % of the saturation value (temperature 15.5°) and rainbow trout (*Salmo irideus* Gibb.), whose viability is of the same order as that of smolts, lived comfortably in this water for a period of 4 days. During the early part of the smolt migration of 1930, when the water temperature was low, smolts were found to be dying in water having a dissolved oxygen content of not less than 70 % of saturation. It appeared then that the mortality was due, at least in part, to some factor other than oxygen deficiency.

Investigations in progress at the Tees Survey Laboratory at Barnard

Castle and not yet published, have shown that fresh sewage or sewage undergoing aerobic decomposition when diluted with river water has no toxic action on fish for short periods.

Of the main types of trade wastes discharged it is improbable that pickle liquors contribute to the directly toxic substances in the estuary water except perhaps in restricted areas. The ferrous chloride they contain appears to be readily precipitated as insoluble compounds on admixture with the alkaline water of the estuary, since soluble iron salts have not been detected in the general body of the estuary waters. The p_H of the estuary water lies between that of the fresh water (about 7.0) and that of the sea (about 8.3) and is not appreciably affected by the free acids discharged.

A more detailed examination of the coke-oven effluents was therefore undertaken, with the object of determining the relative toxicity of the constituents of the two types.

The source of the toxicity of coke-oven effluents.

Rainbow trout, about 2" to 4" in length and 1 to 2 years old were used in all cases as the test animals. When placed in a solution containing a lethal concentration of a toxic substance they first display symptoms which are to some extent characteristic for the poison to which they are exposed. In all cases they eventually lose their equilibrium, after which they sometimes recover but finally remain in an overturned position. The length of time from their first exposure to the solution to this final overturning point is taken as a measure of the toxicity of the solution. All experiments were carried out in wide-mouthed vessels, a volume of either 12 or 20 litres of solution being used. In all cases the dissolved oxygen concentration was found, by analysis, to be near the saturation value and the hydrogen ion concentration was, if necessary, adjusted by the addition of acid or alkali, until the solution was approximately neutral.

The following experiments were carried out with a sample of direct process effluent having approximately the same composition as that given in Table I (A).

1. Toxicity of effluent: a 1 % solution caused trout to overturn in approximately 6 minutes.
2. The effluent was distilled in neutral solution: a 1 % solution of the distillate was approximately as toxic as the undistilled effluent.
3. The effluent was distilled in alkaline solution: a 1 % solution of the distillate was non-toxic for periods up to 60 minutes.
4. The residue from (3) was distilled in acid solution: a 1 % solution of the distillate was approximately as toxic as the undistilled effluent.
5. The effluent was distilled in acid solution: a 1 % solution of the distillate was approximately as toxic as the undistilled effluent.
6. The residue from (5) was distilled in alkaline solution: a 1 % solution of distillate was non-toxic for periods up to 60 minutes.

It appeared from these experiments that the toxic constituents of the effluent were volatile, and were capable of distillation from acid or neutral, but not from alkaline solution. Volatile neutral bodies and basic substances cannot have contributed appreciably to the toxicity of the effluent. An aqueous solution (A) was then prepared of the following composition:

Table III.

	g. per 100 cc.
Sodium chloride	2.73
Sodium thiosulphate	0.0345
Ammonium chloride	0.009
Phenol	0.0105
Mixed xylenols	0.002
Potassium cyanide	0.01

This mixture contains the main constituents of the waste liquor in the concentrations given in Table I (A). The toxicity of the artificial and natural effluents to trout was found to be approximately the same.

A series of solutions was then made up of the same composition as solution A (Table III), except that each constituent was in turn omitted, and the toxicities of these mixtures were determined. It was found that the toxicity was in each case practically the same as the complete solution A except when potassium cyanide was omitted, when a 1 % solution of the mixture was non-toxic under the conditions of the experiment. Moreover, a solution of pure potassium cyanide, in the concentration in which it occurs in the effluent, was found to have about the same toxicity as the complete effluent.

After the removal of cyanide by precipitation and filtration (as AgCN by the addition of AgNO_3) the liquor was non-toxic in a 1 % solution, the toxicity being restored to its original value by the addition of a quantity of potassium cyanide equivalent to the amount of cyanide removed. It appeared, then, that the cyanide fraction was the main toxic constituent of a typical direct process coke-oven waste liquor.

A similar investigation of the toxicity of the main groups of constituents of a typical semi-direct process coke-oven effluent was then carried out. The composition of the effluent was approximately the same as that given in Table I (B) and a 1 % solution of it caused trout to overturn in about 6 minutes. The effluent was distilled under the same conditions as those described for the direct process liquor and the toxicity to trout of the various distillates was determined. It was found that the poisonous constituents were volatile in acid or neutral, but not in alkaline solution. Double distillations showed that volatile neutral bodies did not appreciably contribute to the toxicity and it was concluded that the group of tar-acids was mainly responsible. It will be seen from Table I (B) that cyanide did not occur in the semi-direct process effluent examined, but that tar-acids were present in a very much larger concentration than in the direct process effluent.

It had now been shown that cyanides were the chief toxic constituents of

direct process, and tar-acids of semi-direct process, coke-oven effluents, and it was decided to determine the concentrations of these substances, together with the dissolved oxygen concentration, in the estuary waters where the mortality of smolts occurred during the 1931 migration.

In preparation for this work, some questions which appeared to have a bearing on the problem of smolt mortality were investigated and methods which might yield further information were examined; the main lines of investigation are summarised below.

The toxicity of potassium cyanide and p-cresol.

The toxicities to rainbow trout of various concentrations of potassium cyanide at temperatures of 5° and 10·5 to 12·5° and similar data for solutions of *p*-cresol (taken as a representative of the complex group of tar-acids) at 5° are shown in Fig. 1. In all these experiments 12 litres of solution were used and

Fig. 1.

the time taken by two fish out of four to overturn in it was observed. It will be noticed that, at the same temperature, *p*-cresol is very much less toxic than potassium cyanide; thus, trout overturned in less than 1 hour in a solution of potassium cyanide equivalent to 0.014 g. CN per 100,000 cc., but did not overturn in 1 hour in *p*-cresol at a strength of 0.5 g. per 100,000 cc.

It was found that given concentrations of potassium cyanide were more toxic at the higher temperature than at the lower. This relationship was more fully investigated over a wider range of temperature and it was found that the toxicity (represented by the reciprocal of the average time of overturning) was approximately proportional to the water temperature, over the temperature range 5 to 20°.

The toxicity of solutions of cyanide in water unsaturated with dissolved oxygen was also investigated and it was found that a solution of potassium cyanide became more toxic as the oxygen content was reduced.

The removal of cyanides from dilute solutions.

A method was worked out whereby cyanide in small concentration could be removed from the complex mixture of substances present in the waters of the estuary, and the effect of its removal on the toxicity of the water determined. Formaldehyde in sub-lethal concentration was added to combine with any cyanide present to form compounds which were non-toxic in the concentrations examined.

The effect of adding formaldehyde to the following substances was determined.

Potassium cyanide. Concentrations up to 1 g. CN per 100,000 cc. rendered non-toxic.

Phenol. Concentrations up to 1 g. phenol per 100,000 cc. No change in toxicity.

p-cresol. Concentrations up to 1.5 g. *p*-cresol per 100,000 cc. No change in toxicity.

Coke-oven effluents. 1 % solutions. No change in toxicity of semi-direct process effluent where the toxicity is due to tar-acids. Toxicity of direct process effluents, due to cyanide, destroyed.

Tees estuary water. Taken from the neighbourhood of industrial outfalls. Toxicity destroyed or materially reduced in cases where cyanides were found by analysis to be present.

It was decided, therefore, to examine samples of estuary water by this method during the 1931 smolt migration, in order to assess the importance of cyanides in contributing to their toxicity.

The examination of the colour of the gills of trout.

During the smolt migration of 1930, about 600 salmon and sea trout smolts, which had been picked up dead in the estuary or had been poisoned by known toxic substances in the laboratory, were examined and an attempt was made to determine the cause of their death from their *post mortem* appearance, but with no success, owing largely to the uncertainty as to how long the fish had been dead before examination. It was observed however that the gills of trout, when being poisoned in the laboratory by solutions of cyanides, or by effluents containing cyanides, appeared to be of a brighter colour than those of normal fish. Experiments were therefore carried out in which the gill colours of trout and sea trout smolts exposed to various known poisons were compared with a graded series of seven colours ranging from scarlet (Colour No. 2) to dark crimson (Colour No. 16). A definition of these colours on two colour scales is given in Note 1 at the end of this paper (p. 283). It was found that the gill colour became darker than that of normal fish when both rainbow trout and sea trout smolts were subjected to poisoning by tar-acids, naphthalene or dissolved oxygen deficiency but became markedly lighter when exposed to toxic con-

centrations of cyanides. It was decided therefore to match the gill colours of smolts found dying in the estuary during the migration of 1931 and to compare these with the gill colours of healthy smolts.

The 1931 smolt migration.

Smolt netting, carried out near the upper limit of salt water in the estuary, showed that smolts were migrating past that point from April 23rd to June 5th, 1931. In 1930 it was observed that the death of smolts occurred mainly, if not entirely, at high water in the reaches of the estuary between about 33,000 ft. and 73,000 ft. from the sea (*cf.* Fig. 2). In 1931 sampling was carried out daily

Fig. 2. Position of toxic zone at high tide and distribution of dead smolts at low tide in the Tees Estuary 1931.

in these reaches from April 25th to June 5th at about the time of high water, the following determinations being made.

1. Salinity: by titration with silver nitrate against standard sea water.
2. p_H value: colorimetrically.
3. Temperature.
4. Dissolved oxygen concentrations: by Winkler's method [1929] without preliminary oxidation.
5. Tar-acids (in most samples): by the method of Fox and Gauge [1920].
6. Cyanide: by phenolphthalin or silver nitrate methods (see note, p. 283).
7. Toxicity and toxicity due to cyanides: for this determination duplicate samples, each of 20 litres, were taken at each station. One sample was untreated: to the other 1.5 cc. of 40 % formaldehyde solution were added to remove cyanides, and the time taken by one rainbow trout to overturn in each solution was observed. The dissolved oxygen concentration was found to be approximately the same in the treated and untreated samples both at the beginning and the end of the toxicity determinations.

The results of the toxicity determinations are given in Table IV.

Table IV.

No. of samples Non-toxic: untreated Toxic: with formalde- hyde	No. of samples Non-toxic: untreated Non-toxic: with form- aldehyde	No. of samples Toxic: untreated Non-Toxic: with formaldehyde	No. of samples Toxic: untreated Toxic: with form- aldehyde
5	140	130	8

Thus of 138 samples which were originally poisonous the toxicity of 130 was removed by formaldehyde. It will be noticed that 5 samples were innocuous untreated but were rendered toxic by the addition of formaldehyde. This is explained by the fact that the quantity of formaldehyde added (3 parts per 100,000) was only just below the minimum concentration toxic to trout, and incomplete mixing might result in a layer containing sufficient formaldehyde to be toxic. It is probable that some of the cases where toxicity was not removed by formaldehyde were also due to this factor. Other explanations can be advanced to account for the death of three fish in this group.

One case: the test fish used was a large sea trout smolt. It was removed in a helpless condition and subsequently died after having reduced the oxygen concentration to 13 % of the saturation value.

Two cases: both on the same day. The fish storage tanks had become foul and several fish died in them. The presumption is that the trout used for the experiment were in an unhealthy condition.

The experimental evidence therefore points to the conclusion that in practically all the samples examined, the toxicity, where present, was due to cyanides, this toxicity being destroyed by the addition of formaldehyde.

In Table V are shown the percentages of toxic samples found in various ranges of cyanide concentration.

Table V.

Concentration of cyanide; g. CN per 100,000 cc.	No. of samples	No. of toxic samples %	No. of non-toxic samples %
0	84	13	87
0-002	37	19	81
0-003-0-004	45	62	38
0-005-0-006	32	84	16
0-007-0-008	15	93	7
0-009-0-01	12	100	0
>0-01	23	100	0

The accuracy of the methods of determination of cyanides is discussed in a note at the end of this paper where it is shown that the concentration of cyanide found by analysis is, in general, lower than the amount actually present, especially at low concentrations of CN. The figures given in Table V are therefore only comparative.

The values of tar-acids found by analysis in the samples were generally of the order of 0-01 to 0-03 part per 100,000 the maximum observed value being

0.05. These concentrations are much lower than the minimum concentration of tar-acids toxic to trout (Fig. 1).

The dissolved oxygen concentration of the estuary waters during the period of migration was generally high for the time of year, owing to the abnormally low water temperature. The concentrations found in water in which smolts were actually observed to be dying are given in Table VI.

Table VI.

Dissolved oxygen concentration % saturation	Temperature range (° C.)	No. of days on which these con- centrations were observed
> 70	10-14	2
60-70	10.5-13	2
50-60	11-12	7
40-50	11-13	3

It has been mentioned above (p. 275) that rainbow trout have been kept under laboratory conditions for 4 days in water having a dissolved oxygen concentration of 58 to 64 % of the saturation value. Other experiments have shown that trout are not killed in short periods in water containing only 20 % of the oxygen saturation value; similar results were obtained by Gardner [1926]. During the present investigations in 4 cases where smolts were used as test fish they were unharmed after 14 hours' exposure to formaldehyde-treated solutions in which they had reduced the oxygen content to 19-34 % of saturation. It is improbable therefore that the oxygen deficiency in the estuary was sufficient to have caused, by itself, the death of smolts.

Occasional samples of estuary water were aerated continuously during the period in which trout were exposed to them. In some cases the oxygen concentration rose nearly to the saturation value, but of these all samples which were found to contain cyanides remained toxic to trout.

During the migration, numbers of salmon and sea trout smolts were observed in an overturned condition or swimming feebly near the surface of the water: these were netted and their gill colours were compared with the colours of the standard chart (p. 283). Some of these smolts were transferred to fresh-water tanks in the laboratory and the gill colours of the now healthy fish were recorded. The results are given in Table VII.

Table VII.

	Salmon smolts		Sea trout smolts	
	Dying	Normal	Dying	Normal
No. of smolts from which records were taken	66	40	131	176
Average gill colour	8.5	12.5	7.1	11.7

The brightening of the gill colour observed in the dying smolts was similar to that found in the laboratory in cases of cyanide poisoning.

From the whole of the data presented, it is concluded that cyanide is the main factor responsible for the smolt mortality in the estuary. Lehmann [1926], in his investigation on the Leine, has pointed out the hitherto underestimated importance of cyanides in the study of the pollution of rivers by industrial effluents.

The position of the toxic belt of the estuary during 1931 at about high water each day from April 29th to June 5th is shown in Fig. 2 together with the distribution of dead smolts on the banks at low water. The position of the toxic belt at high water appears to be determined mainly by the tidal range, partly by the volume of fresh water entering the estuary from the upper river and possibly by some other factors. The majority of smolts were killed at about the time of high water, and in dying they usually congregated near the banks, where they were left stranded as the tide ebbed. In general therefore they were found at low water in reaches which had been occupied by toxic water (the toxicity of which has been shown to be due to the presence of cyanide) at high tide.

The exceptions are probably explained by the fact that the position of the toxic zone was determined at only one high tide, occurring in daylight, of the two daily tides, and dead smolts were collected only during the daylight low water period. It is to be expected that roughly half the dead smolts found were killed during the unobserved high water period. Since the tidal range of the two daily tides is in general different, the position of the toxic belt and thus the distribution of dead smolts would be correspondingly altered.

Note 1. The gill colour scale.

The colours of the chart used for matching the gills of trout and smolts, referred to on p. 279, have been standardised by means of a Lovibond tintometer, modified for the examination of opaque objects (Table VIII). The nearest corresponding colours from Ridgeway's Colour Chart [1886] are also given for comparison.

Table VIII.

Gill colour scale colour No.	Lovibond tintometer units			Ridgeway's colours
	Red	Yellow	Blue	
2	15.8	1.5	0	Geranium red
4	15.3	1.5	0	
6	14.2	1.9	0	
8	12.8	1.9	0.3	Burnt carmine
10	11.0	2.5	1.3	
12	9.5	2.5	2.6	
14	8.9	2.8	3.4	Indian purple
16	6.0	2.8	4.0	

The comparison by the Lovibond tintometer was carried out by Mr Strafford of British Dyestuffs Corporation.

Note 2. The determination of cyanides.

The sample is distilled in acid solution and the concentration of CN in the distillate is determined by a modification of the phenolphthalin method due to Weehuizen [1905], an account of which will shortly be published. The reaction is not known to be given by any substance, other

than cyanides, likely to be present in the Tees Estuary, but it is inhibited by sulphides, which, if present, must be removed from the sample before distillation. If more than 0.01 g. CN per 100,000 cc. is found to be present, the estimation is checked by the silver nitrate method [Liebig, 1851]. The percentage difference between duplicate determinations by the two methods varies from 5 to 25 %, the larger discrepancies occurring in samples having a low CN concentration. A relatively large loss of CN occurs during the distillation of the sample which is common to both methods. Thus, a series of determinations of estuary water, originally cyanide-free, to which a known concentration of potassium cyanide, equivalent to 0.01 g. CN per 100,000 cc. had been added, gave results varying from 0.0035 to 0.006 g. CN per 100,000 cc. in different samples of estuary water.

The Prussian blue method is not sufficiently delicate for the estimation of the low concentrations of cyanides found in the estuary. Estimations of CN by the Prussian blue method have, however, been carried out on coke-oven effluents, and the values obtained compared with the concentrations determined by the phenolphthalin method in dilutions of these effluents in estuary water (Table IX).

Table IX. *g. CN per 100,000 cc.*

1	2	3	4	5
Concentration of CN in effluents by Prussian blue	Concentration of CN in diluting estuary water by phenolphthalin	Concentration of CN in 0.25 % solution of effluent in estuary water by phenolphthalin	Observed concentration of CN in solution, due to added effluent (3)-(2)	Calculated concentration of CN in solution due to added effluent. From (1)
8.8	0.006	0.024	0.018	0.022
8.4	0.007	0.028	0.021	0.021
8.4	0.007	0.03	0.023	0.021
8.7	0.006	0.025	0.019	0.022

SUMMARY.

1. The causes of the mortality amongst salmon and sea trout smolts migrating through the Tees estuary have been investigated.
2. The main directly toxic substances discharged into the estuary are tar-acids and cyanides from coke-oven effluents.
3. The smolt mortality has been shown to be due chiefly to cyanides.

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