EFFLUENT COMPLEXITY, ECOTOXICOLOGICAL RESPONSE AND REGULATORY IMPLICATIONS

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ABSTRACT

Current pollution control strategy in the European Community and elsewhere is increasingly through the use of priority pollutant lists. The application and derivation of these is governed by the concept of environmental capacity. The major legislative instrument with respect to industrial discharges is the permit or consent. The consent system is subject to limitations imposed by the complexity of controlled effluents. It does not take account of the full complexity of effluents commonly discharged to aquatic systems. In addition organisms exhibit a variety of responses, both intra- and interspecific. It is unlikely, therefore, that current strategies will enable comprehensive environmental protection.

KEYWORDS: priority pollutants; effluent complexity; PCBs; environmental protection;

1. INTRODUCTION

The 1990 Ministerial Declaration on the North Sea contained a list of 36 chemicals which is intended to form the framework for pollution reduction in the North Sea [1]. This and priority pollutant lists such as the US EPA list, the EEC "black" and "grey" lists and the UK "redlist" contain those substances for which an unqualified environmental capacity approach is perceived to be inappropriate, in order to facilitate their regulation [2]. The environmental capacity concept with its' basic underlying assumption that natural ecosystems can assimilate a certain loading of otherwise harmful material without loss of structural or functional integrity, has been widely adopted in the formulation of international environmental policy regulating chemical discharges [3].

While priority pollutant lists are attractive to decision makers, however, there are some important limitations. The compilation of a list is heavily reliant on results from single species toxicity tests and uses estimates of environmental half-life and bioaccumulative potential rather than empirical values [2]. Cairns (1989), [4] notes that basic ecotoxicological methods have changed little in 45 years. Single species toxicity test results remain a primary tool of decision makers despite the serious problems inherent in the extrapolation of results to the prediction of

effects in natural systems. Hence, the demonstration of direct causal effect in the aquatic environment is inevitably retrospective. Extensive research and monitoring effort would be required to even partially evaluate the fate and effects of the estimated 100,000 chemicals manufactured in the EEC with the potential to cause environmental problems [5]. Indeed, the UK Department of the Environment justifies the "redlist" in terms of improved utility, insofar as it considers only 26 substances rather than the 129 materials of the EEC "blacklist" to which it is compared [2].

This paper illustrates practical difficulties in controlling discharges to aquatic environments using listing procedures by reference to the complexity of some effluents currently discharged to tidal waters in the north west of England and to the Tees estuary. These simple evaluations demonstrate that an extremely large variety of compounds is discharged to the aquatic environment. Many of these may be detected some distance off shore [6]. No listing system is likely to be able to fully account for such complexity, of both organic and inorganic compounds, without entailing prohibitive monitoring and research costs. Significantly, Agg & Zabel (1990), [5] point out that, in the UK, current monitoring effort will be unable to detect even local reductions in the "redlist" chemical inputs with any degree of statistical reliability.

The complexity of potential response of marine organisms is illustrated by the differential effect of two individual polychlorinated biphenyl isomers (PCBs) on the brown shrimp, Crangon crangon. Around 100 of the 209 theoretically possible PCB isomers are found in typical technical formulations of PCBs. These isomers are known to exhibit differential toxicity in mammalian systems [7]. Although the major chemical companies in the USA, UK, FRG and Japan have ceased production the PCBs are not simply an historical problem. Global equilibrium of these compounds has not yet been reached, and input into the environment continues. In the case of the PCBs, the chemically similar co-planar and non-ortho substituted congeners have been identified as of particular concern in marine mammals [8]. It is thought that some 60% of the PCBs originally produced (1-2 million tonnes) are localised in dumps and landfills or still in use while 35% have been lost to the wider environment. The use of toxicity data derived from mammalian responses, however, is of uncertain utility in predicting effects upon invertebrate animals which differ physiologically in many important respects. These species may be of commercial as well as ecological importance and may be responsible for the transfer of bioaccumulative compounds through the food chain to higher animals. The results presented indicate that haematological effects in the brown shrimp differ with respect to the two PCBs tested. The wider implications of the result are discussed.

2.MATERIALS AND METHODS

2.1 Broad Spectrum Effluent Analysis

1 litre samples of effluent were taken directly from discharge pipelines into glass bottles, previously acid washed and rinsed with pesticide residue analysis grade hexane. Samples were back extracted with two 5ml aliquots of hexane. The sample was acidified to 0.5% with Aristar nitric acid between extractions to facilitate removal of polar species. Emulsions were removed by centrifugation of the extract. Chromatography was carried out on a Hewlett Packard 5890 gas chromatograph with a 5970 mass selective detector and 25m Ultra-1 column programmed from 35 to 260^{o}C

using a 1 microlitre sample. Solvents used were evaluated before use and blank and control tests routinely carried out. Compound identification was by computer matching mass spectra of analytes against the US National Bureau of Standards mass spectral library. Integration threshold and other data processing parameters were kept constant in order to allow the results to be broadly compared. Matches at >90% probability and <90% but >50% probability were recorded.

2.2 Heavy metal analyses

11 samples of effluent were taken directly from the discharge pipeline of a phosphate rock processing plant. The metal content was determined directly in filtered subsamples using inductively coupled plasma arc analysis. The analyses were conducted at Severn Trent Laboratories in the UK.

2.3 Haematological Assay in Crangon crangon

Specimens of brown shrimp Crangon crangon, acclimated to 12+/- 2^{o}C, were exposed in randomised groups of twenty to test solutions containing PCB 15 (4,4'-dichlorobiphenyl) and PCB 77 (3,3',4,4'-tetrachlorobiphenyl) dissolved in acetone carrier solvent at the concentrations indicated in FIGURE 2. Test solutions, including controls, were renewed daily. Mortality was determined every 12 hours. After five days exposure, the animals were subjected to haematological examination.

The test animals were fully exsanguinated into 1ml sterile syringes containing 100 ul of cold cacodylate buffer **[9]** and haemocyte counts were made using improved Neubauer haemocytometers.

3.RESULTS AND DISCUSSION

3.1 Broad Spectrum Effluent analysis

Almost 0.5 million cubic metres of effluents are discharged daily to the Tees Estuary with three major industrial sites contributing around three quarters of the total. There is no regulation either specifically or by group parameter of any organic components in the discharge consents granted by the UK National Rivers Authority with the exception of oils, grease, cyanide and phenols. On average, less than four determinands are routinely monitored for each discharge.

FIGURE 1 shows three traces obtained from the broad spectrum analysis of effluents discharged to the Tees. TABLE 1 records the number of peaks resolved on each trace using the same analysis and integration parameters in each case, together with the results of probability based matching of mass spectra. Few compounds are identified to a high level of probability. For each trace, numbers of individual peaks broadly correspond to the numbers of chemicals present although as the trace becomes more complex the chances that each peak may represent more than one compound due to co-elution increases. This further complicates the identification procedure. The pitfalls of the automated GCMS analysis of environmental samples are reviewed by Millington & Norwood (1986) **[10]**. Apart from possible chromatographic inadequacies, the standard of spectra held in the computer library and the algorithms used for matching can result in a high

potential for false negative and false positive identifications. Only by lowering the probability threshold considerably is it possible to report high match probabilities from an analysis of this kind.

Table 2: Peaks resolved under identical conditions of sample preparation and machine settings from effluent samples taken from the Tees estuary. Peaks matched against the US NBS spectral library at the 90% and 50% level are recorded with the percentage remaining unmatched at each probability. Analytical traces for samples A, B & C are shown in FIGURE 2. Samples D,E & F were taken at different times from the effluent stream of an integrated chemical complex. Total ion chromatograms are not shown for these samples.

SAMPLE	INDUSTRIAL SECTOR	PEAKS RESOLVED	MATCHED > 90% (% NOT MATCHED)	MATCHED > 50% (% NOT MATCHED)
A	Steel	9	4 (55.6)	4 (11.2)
B	Mixed sewage/industrial	22	3 (86.4)	13 (27.8)
C	Chemical/Agrochemical	108	8 (92.6)	46 (50.0)
D	Combined chemical	30	6 (80.0)	16 (26.6)
E	Combined chemical	131	20 (84.7)	46 (49.7)
F	Combined chemical	158	36 (77.3)	79 (13.5)

Effluent components vary considerably according to source. Sample A contained several easily identifiable polynuclear aromatic hydrocarbons. Sample B contains many unidentifiable nitrogenated compounds from methyl-methacrylate manufacture. In the case of sample C, the sewage component contains a large number of biogenic and oil derived compounds which have a confounding effect upon the analysis. The traces all show a high degree of individuality. The results for samples D, E & F, for which traces are not shown suggest that there may be considerable variability with time from a single integrated industrial complex. Full, precise characterisation then, presents a difficult prospect, would require a considerable amount of analytical effort and result in costs well in excess of the $900-2000 per sample already estimated as normal for quantitative routine multi-component analysis **[11]**

There is evidence that some of these chemicals may be detected at appreciable concentration in coastal waters. A variety of industrial chemicals have been reported to be present in the coastal waters adjacent to three industrialised estuaries at the ppt level **[6]**. The chemical "fingerprint" was found to be different in the waters impacted by each of the estuaries, and this is attributed to differences in the industrial base developed on each. Interestingly, these authors note the paucity of ecotoxicological data upon which to base an assessment of the environmental effects of these chemicals.

3.2 Heavy metal analysis

Table 2 shows the controlled consent parameters for a 51 Ml day^{-1} discharge into coastal waters from a phosphate rock processing plant together with the additional elements identified using an ICP Scan. Phosphate rock processing is one of the few industrial sectors to be explicitly excluded from the provisions of the EEC Cadmium Directive. The plant has been identified as a regionally important source of cadmium to the Irish Sea as well as of phosphate **[12]**. The results show that in addition to the consented metal discharges, a wide variety of other metals

are also present in the effluent. Under UK legislation it is not possible to take action with respect to unconsented determinands even though they may be of toxicological significance. The results indicate that the effluent is highly variable in content, with substantial excursions from the consented values. The original application for a "deemed" consent made by the industry to the regulatory authority. included some (but not all) of the parameters determined by the ICP scan reported here. Presumably, omission of these from regulation by consent was deliberate despite their known toxicological significance.

Consented Parameters (Limit values, ppm)		Sample Values (ppm) 1	2	3	Unconsented Parameters	[*] Sample Values 1	2	3
Cu	4.2	8.9	0.35	1.1	Suspended Solids	67,500.0	83.0	60,400.0
Cr	16.0	68.0	4.6	12.5	Al	24.9	N/D	N/D
Zn	32.0	58.0	10.4	26.0	Be	0.5	<0.1	0.2
Ni	3.1	9.3	0.88	2.3	B	1.6	0.2	25.0
Pb	0.3	<0.1	0.1	<0.1	Ag	2.6	N/D	N/D
Hg	0.08	N/A	N/A	N/A	Sr	0.1	0.9	5.4
Cd	2.3	1.5	0.61	2.4	Sn	76.9	0.8	N/A
Anionic Detergents	170.0	25.1	35.0	30.5	Ti	40.1	0.8	8.9
pH	2-8	1.2	1.8	2.3	V	42.9	3.7	N/A

Authorised Flow Rate 51 x 10^6 Litres Day $^{-1}$

Table 2: Analytical results from effluent samples from a phosphate rock processing plant in the UK shown with the consented discharge parameters and those for which no consent conditions are set. [*] Unconsented metal loadings of the effluent were determined using ICP scan and are subject to variation by up to 25% of the given value.

3.3 Haematological parameters in Crangon crangon

The results in Figure 2 indicate that exposure of Crangon crangon to PCB 15 causes a reduction in the number of circulating haemocytes. At the highest concentrations tested, numbers of blood cells were reduced by over half. By contrast, in animals exposed to PCB 77 no significant difference was found between the control tanks and the various treatments. In all cases, the concentration of PCB was below the 96 hour LC50.

Invertebrates lack the specialised T-lymphocytes and specific immunoglobulins characteristic of the immune systems of vertebrates. Immunocompetence depends upon the non-specific, inflammatory responses of the circulating blood cells and body fluids for host defence. In crustaceans, the prophenoloxidase activating (proPO) system, a complement-like enzyme cascade located within the blood cells, has been shown to play a central role in mediating non-self recognition and defence reactivity **[13,14]**. Haemocyte count, therefore, provides a measure of defence capability in crustacean hosts. Exposure to PCB 15 caused a significant reduction in the numbers of circulating haemocytes. This in turn has potentially serious consequences on the ability of exposed animals to maintain homeostatic integrity against disease.

These findings serve to illustrate that important differences may exist in the response of target organisms to closely related chemicals. The PCBs are generally regulated by group parameter quantified as a technical mixture. Recently, there has been a move towards congener specific analysis. PCB congeners 28, 52, 101, 138, 153 and 180 are most often used

as indices since they are considered to represent the major compounds of the main PCB formulations [15]. The toxicological properties of the PCBs in higher animals have been widely studied [7]. Toxic effects may be caused by the intact compounds or by their biotransformation products. The biotransformation products include epoxides as intetrmediates. The rate of epoxidation is highly dependent on the substitution pattern of the biphenyl molecule. Congeners with chlorine atoms in the 3,4- positions are less readily converted to an arene oxide intermediate. In mammals, the toxicity of individual PCB isomers appears to be directly related to the degree to which these substances induce the enzyme aryl hydrocarbon hydroxylase (AHH). The known immunotoxic effects in mammals [16] appear to be mediated by the *Ah* receptor. PCB isomers with a planar molecular configuration, such as PCB 77, have a strong binding affinity for this receptor and are regarded as of particular concern in marine mammals [8].

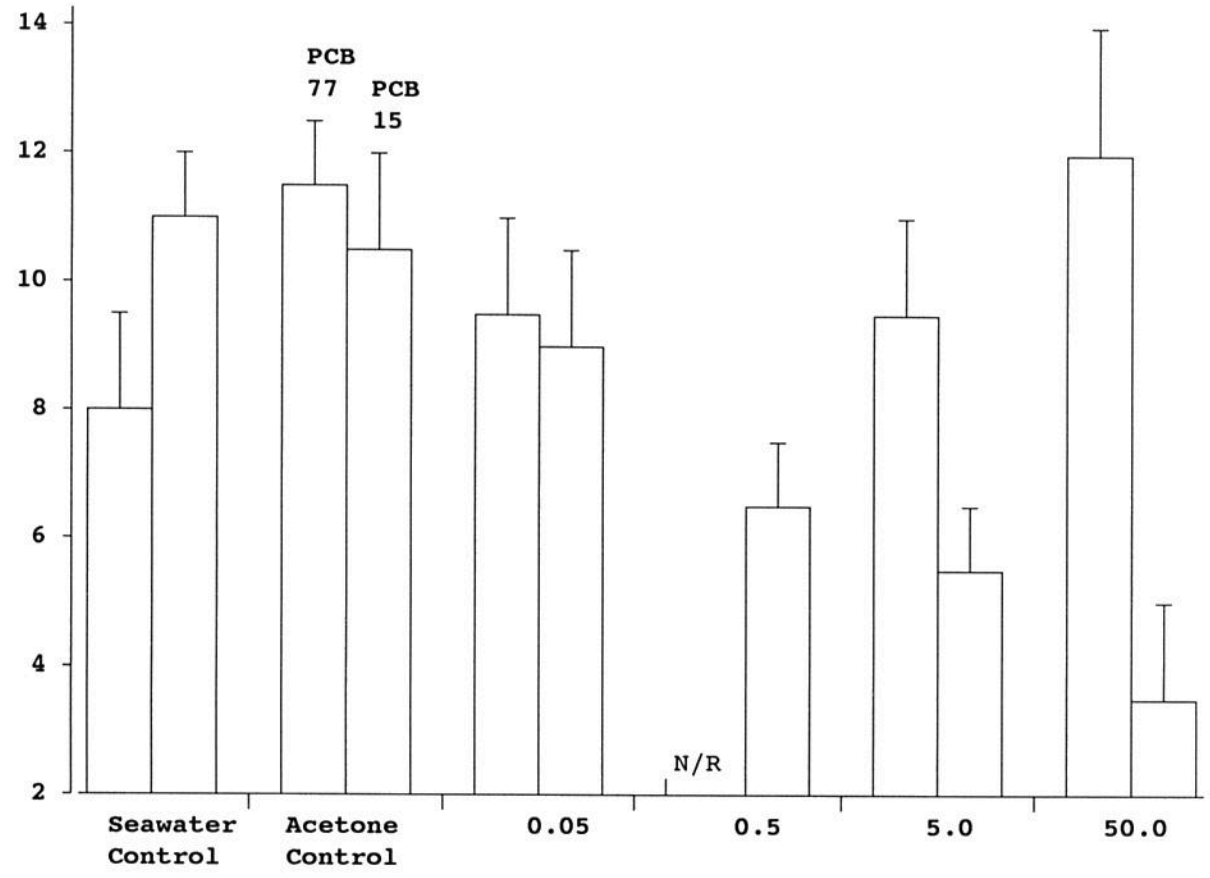

Figure 2: Haemocyte counts from Crangon crangon expressed as cells ml^{-1} x 10^5 in the haemolymph after five days exposure to either PCB 77 or PCB 15 at the indicated concentrations in microgrammes l^{-1}. Vertical bars denote one Standard Error about the mean. (N/R: Not recorded). Left hand bars at each concentration represent PCB 77, right hand bars represent PCB 15.

In invertebrate populations, there is growing evidence that planar PCBs exhibit low toxicity. In the amphipod crustacean Hyalella azteca, concentrations of PCB 77 of up to 2.7ppm resulted in no observed toxic effects although accumulation of 140ppm in tissues was recorded[17]. The findings reported here indicate no sub-acute effects of PCB 77 in Crangon crangon. Taken together, this suggests that knowledge of the relative toxicities of different isomers in mammalian systems cannot be extrapolated to predict effects upon invertebrate species. This has widespread implications for control and monitoring of PCB emissions to the

wider environment. Congener specific analyses need to be extended to embrace those congeners known to be of toxicological as well as quantitative significance. In effect this means that ultimately all congeners will need to be individually quantified in environmental matrices. This will clearly entail considerable effort and expense.

4.CONCLUSION

The failure of consents and permits due to poor characterisation of the controlled effluent is probably common. Added to this are the problems caused by the poor statistical reliability of detection of changes, trends and excursion from the consent values **[18]**. Equally, there are extensive problems in the detection of changes and trends in the wider environment **[5]**. Due to analytical difficulties and the omission of toxicologically active components from consent limitations, there is the potential for widespread failure of the underlying environmental capacity strategy of environmental protection. If it is not possible to identify components of the mixture reliably, then it is simply not possible to estimate the environmental significance of the discharges and thereby assign a notional value to environmental capacity. It is probable that the validity of the concept of environmental capacity itself will probably never be established and that lack of validation appears to be acting as a barrier to the development of adequate methodologies in the field of ecotoxicology **[4]**. Evaluation of eco-system effect is impossible other than in the simplest terms due to the complexity of the effluents and to possible periodic changes in their composition. The variable response of individual species to even closely related chemicals is a further complication of an environmental protection strategy based upon environmental capacity. At the sub-acute level, toxicants may exert profound, though subtle, physiological effects. The haematological response of the brown shrimp Crangon crangon to PCB 15 and PCB 77 illustrates this. These are only two of 209 possible isomers whose inter-specific and intra-specific toxic effects are likely to differ substantially. Simply, it is neither economically possible nor technically feasible to predict the whole ecosystem effect of discharged effluents, or of contaminants introduced by other routes.

High costs of monitoring will be entailed in attempting to protect the environment using an environmenal capacity approach. Monitoring itself does not, however, contribute to solving the problems and, moreover, may not adequately identify important processes taking place in the wider environment. It is highly uncertain, therefore, that present strategies will assure adequate environmental protection. A precautionary approach to environmental protection with a view to eventual zero discharge of chemicals becomes conceptually highly attractive under these circumstances. Such an approach has been widely adopted by various international fora and is discussed in greater detail in a following paper. There is no doubt that translation of a precautionary approach into environmental protection will necessitate far reaching changes in methods of industrial production.

ACKNOWLEDGEMENTS

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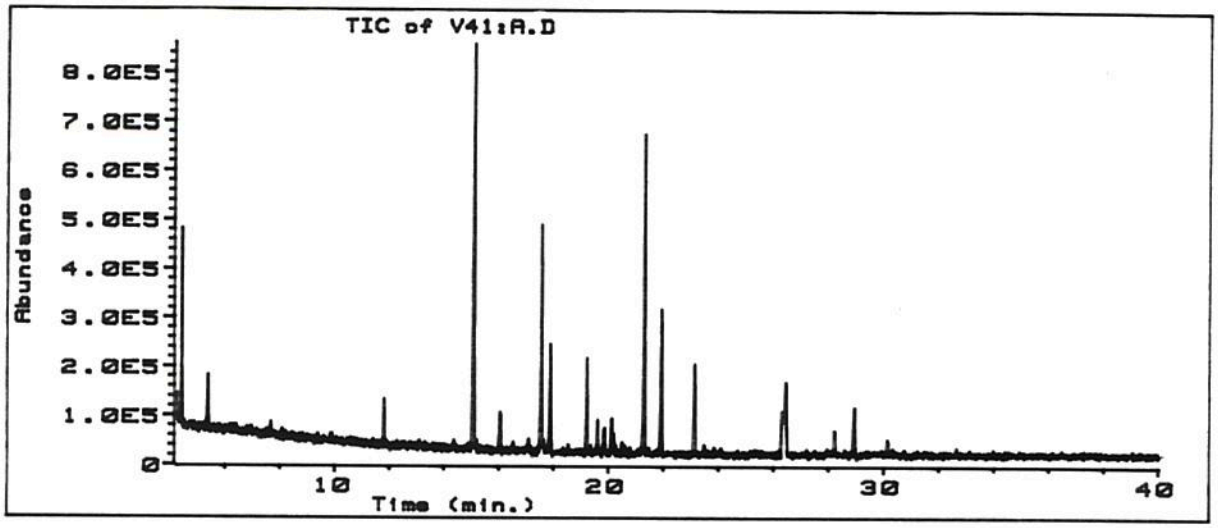

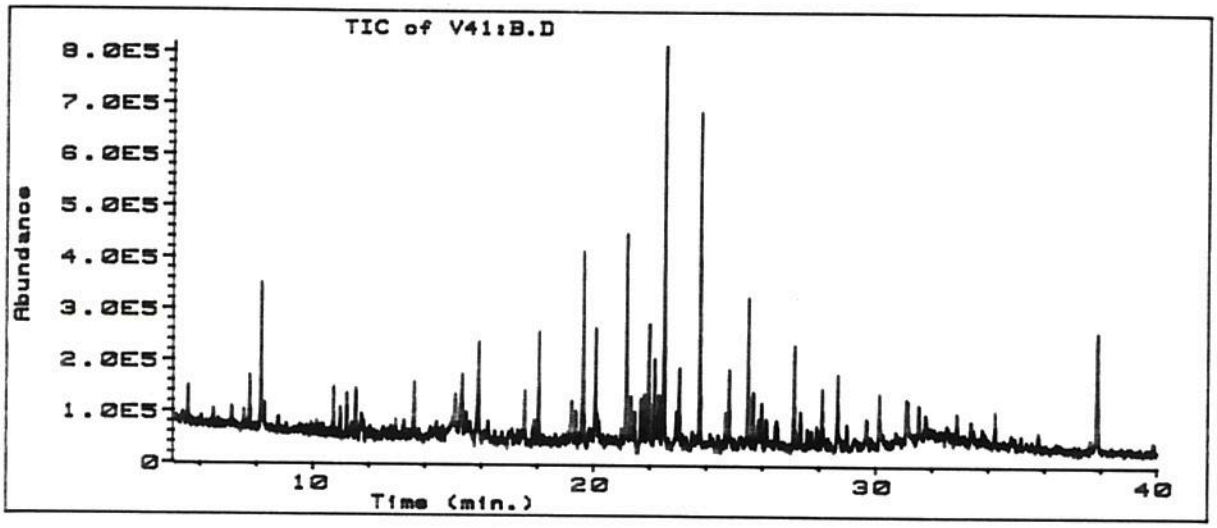

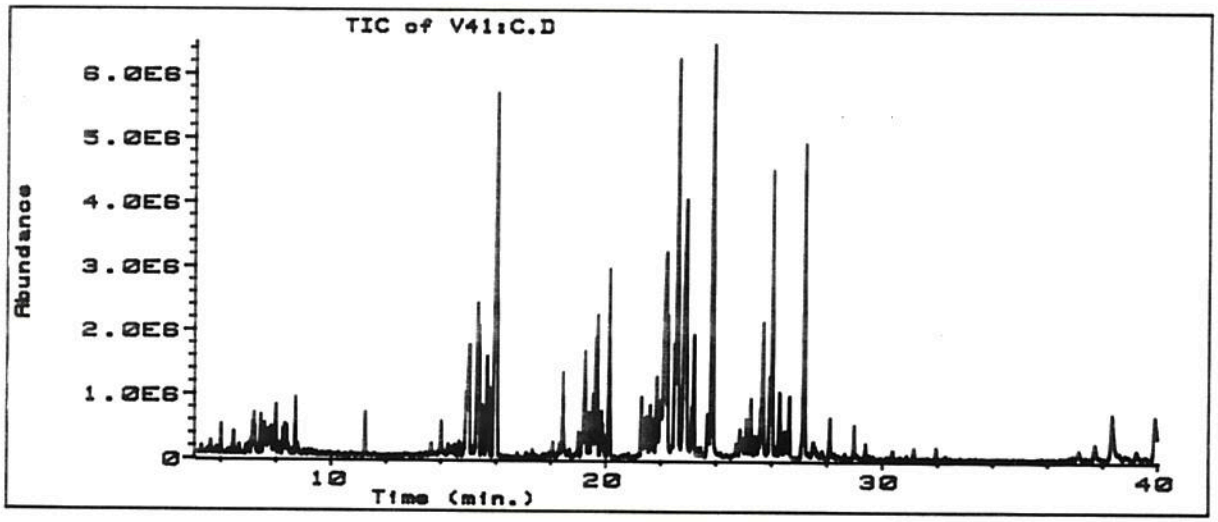

FIGURE 1: A, B & C. Traces derived from the analysis of industrial effluents discharged into the tidal River Tees. These correspond to the details given in TABLE 2. Trace A: Steel manufacture. Trace B: Combined Sewage/Industrial. Trace C: Chemical/agrochemical. Data acquired using the analytical protocol described in the text. The traces indicate considerable variability in the composition of effluents. The figures in Table 1 show that many of the components cannot be identified with any certainty.