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AQUATIC PRODUCTIVITY: ISOTOPIC TRACER AIDED STUDIES OF CHEMICAL-BIOLOGICAL INTERACTIONS

REPORT AND RECOMMENDATIONS OF AN ADVISORY GROUP
JOINTLY CONVENED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE
FOOD AND AGRICULTURE ORGANIZATION
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1. REPORT

1.1. Introduction and objectives

Inland waters (ground water, lakes, reservoirs, streams, rivers and their estuaries) are especially subject to the accumulation and effects of trace contaminants and the problems have attracted attention at international level (e.g., see Report of the UN Conference on the Human Environment, Stockholm, 1972; UN July, 1972; FAO/SIDA, Effects of intensive fertilizer use on the human environment, FAO Soils Bulletin No. 16, Rome, 1972; UNESCO, International Coordinating Council of the Programme on Man and the Biosphere, Final Report, Paris, 1971, Project No. 5, Ecological effects of human activities on the value and resources of lakes, marshes, rivers, deltas, estuaries and coastal zones, Paris, 1971; ECOSOC, Proceedings of a Seminar organized by the Committee on Water Problems of the United Nations Economic Commission for Europe, UN, ECOSOC, WATER/SEM. 1/2, 1974).

The complexity and magnitude of the problems of inland water quality are not surprising. Firstly, inland water bodies are, unlike the open oceans, relatively small captive sinks for the by-products from man's activities, thus leading to a possible accumulation of trace contaminants and abnormal levels of nutrients. The biodegradation of some initially oil-soluble pesticide residues can, for example, lead to the formation of more polar and relatively water-soluble metabolites. Secondly, growing populations and industrialization lead to increasing demands for these limited inland water bodies for fisheries, agriculture, industry, water traffic, for domestic purposes, and for leisure. Such demands can clearly be conflicting.

These trends will inevitably exacerbate the problems within the immediate future except for the unlikely introduction of adequate and timely counter-measures on a global scale.

Measurement and study of the nature, levels and trends of biological activity are important as an indicator of the overall impact of man's activities when the spectrum of trace contaminants may be unknown or unsuspected. Moreover, the complexity of the aquatic ecosystems, the different abilities of strains (especially populations of unicellular organisms and mesofauna) to develop resistance or otherwise respond to contaminants make it difficult to predict the consequences, even on the basis of known contamination. (E.g., see Dunstan, W.M., Problems of measuring and predicting influence of effluents on marine phytoplankton, Environmental Science and Technology, 1975, 9, (7), pp. 635-638). This notwithstanding, water quality criteria, current and projected monitoring programmes are largely based on chemical and physical analyses. The importance of trace contaminant-aquatic organism interactions has however attracted attention and several important techniques developed: E.g.,

1.1.1. A cholinesterase-substrate preparation has been used to indicate the inhibitory activity of organophosphorus and carbamate insecticide residues in water (e.g., Gamson, et al., Environmental Science and Technology, 1973, 7, (13), pp. 1137-1140).

1.1.2. Heterotrophic activity of microorganisms of river water samples has been used as a eutrophication indicator and assayed by the addition of carbon-14 labelled glucose and measurement of $^{14}CO_2$ release on incubation (Albright and Wentworth, Environmental Pollution, 1973, 5, (1), pp. 59-72: See also Wright, R.T. and Hobbie, J.E., Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology, 1966, 47, 447-464).

1.1.3. As an essential nutrient of aquatic biota ^{32}P-labelled phosphate has been used for measuring uptake and concentrations in comparative studies of eutrophic and oligotrophic lakes (Takatalo and Miettinen in 5th RIS Symposium, Helsinki, 1969).

1.1.4. The effects of pesticide residues on fresh water algae has been measured by incubation with ^{14}C-labelled sodium carbonate as an indicator of reduced algal carbon assimilation in the presence of residues (Stadnyk, et al., Bulletin Env. Contamination and Toxicology, 1971, 6, (1), pp. 1-8).

1.1.5. The suppression with added copper of nitrogen fixation by the blue-green algae of a eutrophic lake has been followed in parallel with the effects on photosynthesis using ^{14}C-labelled bicarbonate as carbon source (Horne and Goldman, Science, 1974, 183, pp. 409-411).

1.1.6. The use of cyclotron-produced radioactive nitrogen-13 has greatly extended the sensitivity of denitrification studies in water and sediments. Basic knowledge of the denitrification process may lead to better mangement and control of aquatic nitrogen problems (Goldman, C.R., reported to the meeting):

The present Joint FAO/IAEA Advisory Group was convened: Firstly; to appraise the role of isotopic tracer techniques in studying trace contaminant-biota interactions, especially at the levels of "autotrophic and micro-heterotrophic activity which appear to be the key to an understanding of the status of inland water quality (see IBP Handbook No. 12, A Manual on methods for measuring primary production in aquatic environments, Second Edition, Edited by R.A. Vollenweider, Published by the International Biological Programme, 7 Marylebone Road, London, N.W., England, Blackwell Scientific Publications, Oxford and London, 1974) and their potential for change under pressure of man's activities (see 1.2. below). Secondly; to consider the possible usefulness of a Joint FAO/IAEA programme of coordinated research designed to develop and apply such techniques to the problems of aquatic ecosystem quality protection.

1.2. Technical Background

An undisturbed inland aquatic ecosystem effectively tends to assume a "steady-state" biologically, chemically and hydrologically. Its chemistry (the concentrations of natural solutes, nutrients, trace contaminants, particulates, dissolved oxygen, carbon dioxide, pH, nature of the effective surface layers of the sediment or "bottom", etc.) and hydrology are a function of the physical parameters (depth, dimensions, temperature, etc.) of the inputs from watershed drainage, direct precipitation, subterranean sources, and losses through surface drainage, evaporation, etc. Its chemistry almost invariably provides for the support of a biological spectrum of inter-dependent aquatic animal and plant species. A fresh-water body of "high" quality is, ideally, one able to support healthy balanced populations of desirable organisms such as edible fish and crustacea, and to provide an acceptable source of potable water, e.g. by satisfying the water quality critera recommended by WHO (See "International Standards for Drinking Water", WHO, Geneva, 1971).

As a result of man's activities there may be relatively rapid changes in chemistry which disturb the "steady-state" and can lead to undesirable changes in sensitive and dependent biota. Dystrophication can represent an extreme result. Such undesirable changes are not only a function of the chemistry but the net result of the chemical-biological interactions. Chemical examination alone is not sufficient to predict biological changes or vice-versa. Thus, in one case a phytoplankton population can be stimulated by a quantitative increase in nitrate and phosphate, but not in another, because certain secondary, but essential micronutrients, are deficient and limiting, or present in excess and toxic. Similarly, an arthropod population may be reduced or eliminated by an unsuspected pollutant in one case, despite an otherwise adequate supply of food, but not in another pollutant-free case. Particularly important in this context is the response of microorganisms, especially photosynthesizing phytoplankton, to macronutrients such as nitrogen and phosphorus and micronutrients such as trace metals. When added nutrients do favour the growth of certain populations of algae this can be at the expense of desirable populations at higher trophic levels of the food chain.

One important and relatively little studied aspects is that the ability of the various biota themselves to deal with undesirable contaminants, e.g., the ability of the normal fauna and flora of the system to absorb and degrade biochemically trace contaminants such as those from pesticide residues or the ability to survive because the normally sensitive strain has developed resistance. Certain organochlorine pesticide residues can indeed inhibit the grwoth of phytoplankton and undergo bioconcentration. However, the process may be more important as a means of removing the residue from the upper productive aquatic layers to the sediment, than as a threat to the aquatic carbon dioxide-oxygen balance (Portmann, J.E., The bioaccumulation and effects of organochlorine pesticides in marine animals, Proc. Royal Soc. B., London, 1975, 189, 291-304). Some algae are responsible for nitrogen fixation and this can represent a significant and undesirable input to the nitrogen inventory. Conversely, sediment bacteria may significantly eliminate nitrogen by denitrification.

The evidence reviewed by the Advisory Group confirmed that the fundamental biological chemical interactions in the complex chains of events leading to reduction in water quality tend to be at the autotrophic and micro-heterotrophic levels. It is at these levels that relatively simple nutrients can be utilized by phytoplankton and other microorganisms and so support useful food chains or food webs.

The use of radio-labelled substrates can provide a direct measure of the biological utilization of such nutrients, their turnover and release, and for indicating their response or potential response to changes in nutrient status or to the presence of trace contaminants as a result of man's activities. (E.g., see Radiolabelled substrates for studying biological effects of trace contaminants, Technical document published by IAEA, Vienna, 1975, IAEA-170). On the other hand the use of isotopically labelled contaminants provides a powerful and often unique tool for studying the effects of an aquatic species on the fate and, therefore, significance of the contaminant itself to other species or to the ecosystem as a whole. Moreover, recent improvements in the supply of, and methodology for using, stable isotopes as tracers now suggest important potentialities for aquatic studies under field conditions (see FAO/IAEA Isotope Ratios as Pollutant Source and Behaviour Indicators, Symposium Proceedings Series, IAEA, Vienna, 1975, STI/PUB/382).

1.3. Problems and techniques

1.3.1. Aquatic nitrogen and agriculture

Large quantities of nitrogen exist in various "sinks" in geological structures, atmosphere, soils, organic materials, water, etc. However, in soils and water the forms of nitrogen readily usable by plants or aquatic organisms (the nitrate and ammonia nitrogen) are very often in short supply and are limiting to growth. Increasing the available supply of nitrate or ammonia in soil or water will usually result in an increased productivity.

Rice culture ~~Agriculture~~ applies appreciable quantities of nitrogen fertilizers to improve soil productivity and yield of crop.

This practice of fertilization is accepted as being essential to crop production in order to obtain adequate food to meet the demands of an an ever increasing population. Fertilizer use, worldwide, is expected to increase and so is the quantity of nitrogen used per hectare.

Since fertilizer nitrogen is never utilized completely by crops (50% over-all uptake efficiency is an estimated average) this unused nitrogen as well as nitrogen from decomposition of crop residues can be a source of nitrogen moving to a water body. The actual contribution of fertilizer nitrogen to the productivity of a given water body has been difficult to assess with any real degree of certainty.

The contribution of fertilizer nitrogen and the fate or decomposition of fertilizer applied nitrogen is being studied in the field and in the laboratory by use of nitrogen isotopes in two ways (1) uptake of ^{15}N-nitrogen from ^{15}N-enriched fertilizer nitrogen or (2) uptake of ^{15}N from ^{15}N-depleted ^{14}N-fertilizer nitrogen. Both the ^{15}N-enriched fertilizer and the ^{15}N-depleted fertilizer are commercially available and both are being used to study movement, losses, efficiency, and final disposition of the fertilizer applied nitrogen. There is also the possibility of using accurate measurements of the small but significant changes in natural $^{15}N/^{14}N$ ratios to indicate the behaviour or history of nitrogen in soil or water.

With the aid of these studies using N-isotopes, agriculture should be able to manage better its fertilizer and water inputs and reduce its contribution to the over-all water pollution and water quality problem due to nitrogen (See FAO/IAEA Effects of agricultural production on nitrates in food and water with particular reference to isotope studies, Panel Proceedings Series, IAEA, Vienna, 1974, STI/PUB/361).

Nitrogen metabolism is exceedingly important in aquatic as well as agricultural communities. Freshwater and estuarine aquatic ecosystems collect large quantities of nitrogen from terrestrial sources and under some circumstances undergo serious eutrophication as a result to nitrous oxide (N_2O) or molecular nitrogen (N_2). This is largely an anaerobic process which remains poorly understood and almost un-quantified in natural waters and their sediments. The availability of cyclotron-produced radioactive ^{13}N has greatly extended the sensitivity of studies of denitrification in water and sediments and promises to provide a much greater understanding of this important process.

The large quantity of energy used in producing nitrogen fertilizer for intensive agriculture is an increasingly important part of the world energy crisis. Basic knowledge of the denitrification process may also lead to better management of nitrogen in the context of agriculture as well as in the context of aquatic pollution control.

1.3.2. Aquatic ecosystems in arid zones of developing countries

In spite of the great importance of inland water bodies of developing countries for their fishing economies, only some have been the subject of limnological investigations. Studies of Egyptian and Iraqi inland water bodies have identified several problems in need of further investigation.

Increases in human population and industrial growth in most of the developing countries have also exacerbated the problems of pollution of inland water bodies. Many of these bodies have already become polluted to the extent of causing decreased fish production and public health hazards. These water bodies receive pollutants from different sources, e.g. domestic sewage, industrial wastes, pesticides, fertilizers, and oil spills. The problems appear to be particularly important and deserving attention in arid zones, e.g., in countries of the Near Eastern Region where there is rapid industrial development and where evaporation rates, and therefore the concentration effects are relatively high. The Group felt that international scientific cooperation in this respect would be of particular value, especially in the provision of training in the use of isotopic tracer techniques as indicators of aquatic status and biological activity.

1.3.3. Micronutrients in aquatic ecosystems

Micronutrients are well established as important components of aquatic environments. They may limit primary productivity through deficiency or toxicity. Both of these effects may be greatly modified by the chemistry of a water body, especially the presence of organic matter able to chelate trace metals.

In field and laboratory experiments designed to bioassay the deficiency, toxicity, or availability of micronutrients on the biota of aquatic environments, the uptake of labelled substrates is generally the best method of measuring the response of the organism to chemical changes of the aquatic environment.

Trace metal micronutrients may limit autotrophic and auxotrophic primary productivity by deficiency (i.e. as limiting nutrients) or by their toxicity. Toxic effects may effect other organisms in the food chain directly or indirectly (through autotrophic organisms).

Both deficiency and toxicity effects may be greatly modified by the chemistry, especially the presence of organic matter with ability to chelate trace metals. Eutrophic waters may be subject to high inputs of trace metals and organic matter. The organic matter may include man-made chelators such as the polyphosphates used as detergent intermediates. Nitrilotriacetic acid (NTA), a polyphosphate substrate, is a powerful chelator of trace metals and this property must be taken into account when assessing the effects on the environment of replacing polyphosphates with NTA in detergent preparations.

As eutrophication proceeds, the balance between trace metal deficiency and toxicity will change. The net effect on aquatic biota cannot be easily predicted: The increased concentration of trace metals may be potentially toxic, but effectively counteracted by the organic matter also present in increased concentration. Chelation is an important area of limnology which has been relatively neglected, but may be amenable to bioassay techniques.

Vitamins and especially vitamin B12 are micronutrients for about 50% of algae. Comparative studies of the vitamin B12 concentration in lakes and reservoirs indicate that this vitamin could be used as a measure of eutrophication. The concentration of vitamin B12 is relatively high in eutrophic waters. Two factors are involved: (a) increased input into the lake from sewage effluents, (b) a change in the balance between biological consumers and producers of vitamin B12. Bacteria and some algae, mainly blue-green algae are producers of vitamin B12. Other algae, mainly diatoms and dinoflagellates, are the main consumers.

Vitamin B12 therefore reflects the input to heterotrophic activity and algal species composition of a water body. Vitamin B12 may be measured by bioassay and by an isotope dilution method using ^{57}Co-labelled vitamin B12. The method has many practical advantages over more conventional bioassays for this vitamin.

1.3.4. Assays of microbiological activity

Primary (autotrophic) production*) is the most important characteristic of an inland water body. The rate of decomposition of algal detritus and the turn-over rate of limiting nutrient elements (usually P or N) are also important aquatic parameters. Knowledge of these basic parameters is essential to the rational development of eutrophication control.

The radiocarbon-14 assay technique for primary production is well established (see 1.1). Further work on technical details was not considered urgent by the Group. The method can with modification also be used to determine the limiting nutrient or to check the influence of pollutants (heavy metals, pesticides) including the effects of thermal pollution. If a large number of samples occur the ^{14}C-method may be too laberious. On the other hand primary production data can usefully be supplemented by relatively quick fluorescence assay of chlorophyll to indicate biomass (see also ATP technique below).

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*) the term primary production is used here in the sense explained by R.A. Vollenweider in the International Biological Programme Handbook No. 12 (see 1.1), pp. 1 and 41.

Together with productivity estimations the most important chemical and meterological parameters of the lake must also be measured. Heterotrophic production (dark CO_2 uptake) and heterotrophic activity assays (e.g. by using ^{14}C-labelled glucose or acetate as substrate) of the bacteria (see below) as well as macrophytic production have to be considered.

Heterotrophic microbial productivity assays estimate biomass productivity of the heterotrophic bacteria and fungi with a limited contribution from algae. In practice, the incubation of water samples in the dark in the presence of $^{14}CO_2$ is the radiometric method of assay. This heterotrophic productivity is sensitive to the presence of many inorganic and organic nutrients as well as pollutants.

A useful assay of biomass is the ATP technique. ATP does not significantly persist extracellularly and a reasonably constant proportion exists between total cellular biomass and ATP content. It is an excellent means of determining biomass of microorganisms within aquatic systems. ATP can readily be determined by measuring light emission (usually with a photomultiplier-scintillation type counter assembly) upon the additions of a standardized luciferin-luciferase preparation to the water sample.

Heterotrophic microbial activity assay is based upon the utilization and mineralization of dissolved organic matter by heterotrophic microbes (with the aid of radiolabelled organic metabolites) and data interpreted on the basis of Michaelis-Menten enzyme kinetics. Quantitative estimations of turnover times and maximum velocities of mineralization and utilization of dissolved organic matter are determined. This technique yields valuable estimates of the impact of both natural (e.g. alluvial silt) and man-made (e.g. pollutant) effects upon the activity of the heterotrophic microflora.

1.3.5. Enyzmic methods for the determination of water quality

Enyzmic methods lie between chemical and biological methods (bio-assays). Ideally, they combine the advantages of the chemical methods (specificity, reproducibility) with the advantages of the biological methods (direct biological significance). The test organism in the bio-assay is represented by an enzyme system which can, if correctly chosen, give biologically-relevant information in a standardized or quantified way.

Two kinds of enzymic assay are possible: -

(a) determination of specified substances or group of substances with the aid of added enzymes, or

(b) determination of specified enzymes or groups of enzymes with the aid of added substances (substrates).

Examples of determinations as mentioned under (a) which have been described in literature include: determination of ammonia, urea, uric acid, cholinesterase inhibitors (organophosphoric acid esters and carbamates), ATP, H_2O_2 and several carbohydrates. The use of radio-isotopically-labelled substrates has been described for the measurement of cholinestrase activity by means of (^{14}C-carboxy) acetylcholine as the substrate. This enables enzyme activity to be determined with minimal dilution of the tissue or enzyme preparation, very short incubation times (of the order of seconds), and very low initial substrate concentrations (e.g. 10^{-6}M). Such techniques provide extremely sensitive indicators of enzyme inhibition by substances such as carbamate insecticides which behave as reversible enzyme inhibitors. The Group noted that the exploitation of these techniques is one subject of an ongoing Joint FAO/IAEA coordinated research programme: "Biological side-effects of foreign chemical residues in food and agriculture", IAEA Technical Document No. 174, Vienna, 1975.

Examples of determination as mentioned under (b) include oxidative/reductive enzymes (e.g. nitrate-reductase, nitrogenase, glucose-6-phosphate-dehydrogenase, catalase), phosphatases, esterases (e.g. acetylcholinesterase), proteases, glucosidases and transaminases. These determinations can be carried out in water samples or in aquatic organisms (bacteria, algae, fishes) and can in that way give information on the conditions of the water itself (self-regenerating power, eutrophication, suitability for aquatic life) or of the organisms living in it.

2. CONCLUSIONS

Although a large number of biological-chemical interactions play a critical role in the stability of an aquatic ecosystem, "primary production" is of paramount importance.

Until recently water contamination by pesticides had attracted relatively little attention because of their lipophilic character and relative insolubility in water. Analyses of particulate-free surface water invariably suggest concentrations below the thresholds of toxicity. However, these compounds tend to accumulate in the sediment or to be absorbed onto particulate matter in which case they resist breakdown or biotransformation. This condition creates a hazard to zooplankton and other benthic organisms which are the first to be affected. Bio-accumulation, "ecological" magnification, and transfer of toxic material to higher trophic levels can then occur progressively. The result is a disturbance in the balance of the ecosystem as a whole. Available isotopic tracer techniques have provided a useful and powerful tool for studying these mechanisms quantitatively.

Similarly, by studying the behaviour, persistence and degradation of stable or radio-isotopically labelled contaminants under field or simulated field conditions (e.g. see Metcalf, R.L., A laboratory model ecosystem for evaluating the chemical and biological behaviour of radiolabelled micropollutants. FAO/IAEA/WHO, Comparative studies of food and environmental contamination, Symposium Proceedings Series, IAEA, Vienna, 1974, STI/PUB/348, pp. 49-63) the very important effects of the living organism on trace contaminants, and, therefore, on their biological significance can also be studied.

Labelled substrate techniques have already been developed and well-tested for measuring primary productivity as well as certain micro-heterotrophic productivity and activity in one case at least for the determination of toxic pesticide residues (see above).

The distribution, composition and population densities of the organisms present and associated changes can be determined and monitored over a period of time using existing techniques (e.g., See IBP Handbook

No. 12 - Reference given in section 1.1). Similarly, the analytical methodology (especially by GLC techniques) for pesticide residues in various media including water was well established (E.g., see Guidelines on analytical methodology for pesticide residue monitoring, Federal Working Group on Pest Management, Washington, D.C., 20460, 1975, pp. I-1 to XVI-5).

The Group concluded that a combination of these radiometric and complementary techniques would provide for a useful and effective "integrated" approach to the problem of determining status of an aquatic ecosystem and of predicting its capacity to respond to added trace contaminants and nutrients.

However, the safe and effective application of the isotopic-tracer aided techniques appeared to be the less generally exploited and understood despite their importance. There was a need to provide training and coordinated guidance in their exploitation, especially for making aquatic quality studies in developing countries.

3. RECOMMENDATIONS

Against the background above and; (a) recognizing the importance of inland water quality protection in both developing and developed countries; (b) noting the unique value and potential importance of isotopic tracer techniques for determining the autotrophic and micro-heterotrophic status of water bodies and their likely response to man-caused chemical additives, whether nutrients or pollutants; and (c) noting the facilities of the Joint FAO/IAEA, Land and Water Development, and Fisheries Resources and Environment Divisions, addressed the following recommendations to the Directors General of FAO and IAEA: -

3.1. To assist scientists of developing countries, by means of Fellowship Training and Scientific Visits, to exploit these techniques for the identification, study and eventual control of inland water quality problems under the local relevant conditions.

3.2. To initiate a coordinated international programme of research involving institutes of developed and developing countries with the following objectives: -

3.2.1. To develop, standardize and apply isotopically labelled substrate techniques for comparative assays of primary autotrophic and micro-heterotrophic production and decay.

3.2.1.1. To develop, standardize and apply compementary isotopically-labelled contaminant or nutrient techniques to determine their fate and persistence.

3.2.2. To use these techniques to obtain comparable data on the current status of water bodies and changes to be expected as a result of man-caused chemical additives.

3.3. To ensure that an appropriate section in the projected FAO/IAEA training manual under the general title "Use of isotope techniques for studying chemical residue problems of food, agriculture and fisheries" is devoted to isotopic tracer and labelled substrate techniques for studying chemical and biological interactions of aquatic ecosystems.

4. PUBLISHED AND AD-HOC PAPERS STUDIED BY THE GROUP

4.1. Albright, L.J.
Use of the heterotrophic activity technique for assaying pollutant effects upon the heterotrophic microflora of natural waters.

4.2. Albright, L.J.,and Wilson, E.M.
Sub-lethal effects of several metallic salts-organic compounds combinations upon the heterotrophic microflora of a natural water. (Paper published, Water Research, Vol. 8, pp. 101-105, Pergamon Press, 1974, Great Britain).

4.3. Albright, L.J., Wentworth, J.W., and Wilson, E.M.
Technique for measuring metallic salt effects upon the indigenous heterotrophic microflora of a natural water.
(Paper published, Water Research, Vol. 6, pp. 1589-1596, Pergamon Press, 1972, Great Britain).

4.4. Albright, L.J., and Wentworth, J.W.
Use of the heterotrophic activity technique as a measure of eutrophication (Paper published, Environmental Pollution, Vol. 5, 1973, pp. 59-72, Applied Science Publishers, Ltd. England, 1973).

4.5. Ayers, R.S.
Agricultural Pollution

4.6. Bagge, P. and Lehmusluoto, P.O.
Phytoplankton primary production in some Finnish coastal areas in relation to pollution.
(Paper published, Merentutkimuslait. Julk./Havsforskningsinst. Skr. No. 235, 1974, pp. 3-18).

4.7. Baker, M.H., and Daisley, K.W.
Vitamin B_{12} and selected trace metals in three freshwater reservoirs, with brief details of an assay method using ^{57}Co-labelled vitamin B_{12}.

4.8. Bernardo, B.C., and Köteles, G.J.
IAEA environmental activities in the European Region, Co-operative programme - countries in the Danube Catchment area.

4.9. Canton, J.H., and Greve, P.A.
A laboratory model system for the study of the accumulation of chemicals in the aquatic food chain, exemplified on α-hexachlorocyclohexane.
(Paper presented at the International Symposium "Recent Advances in the Assessment of the Health Effects of Environmental Pollution", sponsored by EEC, WHO and EPA, Paris, 24-28 June, 1974).

4.10. Ernst, D.E.W.
Eutrophication and primary production of Northern German shallow lakes.

4.11. Goldman, C.R.
Denitrification in the aquatic system.

4.12. Greve, P.A.
Potentially hazardous substances in surface waters, Part I. Pesticides in the River Rhine, Part II. Cholinesterase inhibitors in Dutch surface water.
(Paper published, The Science of the Total Environment, Elsevier Publishing Company, Amsterdam, The Netherlands, 1972 (1)).

4.13. Greve, P.A.
Enzymatic methods for the description of water quality.

4.14. Lehmusluoto, P.O., and Pesonen, L.
Eutrophication in the Helsinki and Espoo sea areas measured as phytoplankton primary production.
(Paper published, OIKOS Supplementum 15: 202-208, Copenhagen, 1973).

4.15. Lehmusluoto, P.O.
Algal assay procedure in use in Finnland.
(Proceedings from a Nordic Symposium "Algal Assays in Water Pollution Research", Oslo, 25-26 October 1972, Nordforsk Secretariat of Environmental Sciences, Publication 1973:2).

4.16. Lehmusluoto, P.O.
Detection of eutrophication and pollution by the carbon-14 primary production technique.

4.17. Perry, A.S.
Pesticides in aquatic organisms.

4.18. Saad, M.A.H.
Observations on the problems of pollution in Shatt-al-Arab, Iraq.

4.19. Saad, M.A.H.
Effect of pollution on the sediments of Lake Mariut, Egypt.
(Paper published, Rapp. Comm, int. Mer Mèdit., 21, 3, pp. 125-127, 1972).

4.20. Saad, M.A.H.
Distribution of phosphates in Lake Mariut, a heavily polluted lake in Egypt.
(Paper published, Water, Air, and Soil Pollution, 2 (1973), 515-522).

4.21. Saad. M.A.H., and Ezzat, A.
Effect of pollution on the blood characteristics of *Tilapia Zillii G.*
(Paper published, Water, Air and Soil Pollution, 2 (1973) 171-179).

4.22. Saad, M.A. H.
Influence of organic pollution on Lake Mariut, a highly eutrophicated lake South of Alexandria.
(Paper published, Rev. Intern. Oceanogr. Med., Tome XXXIV, 1974).

4.23. Saad, M.A.H.
Catastrophic effects of pollution on Egyptian waters near Alexandria.
(Paper published, Atti 5°, int. oceanogr. med. Messina, 553-572, 1973).

4.24. Winteringham, F.P.W.
Introductory note to FAO/IAEA Advisory Group on Isotopic Tracer-Aided Studies of Inland Water Eutrophication and Pollution.

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