UNION INTERNATIONALE DES RADIOECOLOGISTES
INTERNATIONAL UNION OF RADIOECOLOGISTS

COMMISSION DES COMMUNAUTES EUROPEENNES
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ROLE OF MICROORGANISMS ON THE BEHAVIOUR OF RADIONUCLIDES IN AQUATIC AND TERRESTRIAL SYSTEMS AND THEIR TRANSFER TO MAN

Proceedings of a Workshop held in Brussels, 25-27 April 1984,
in cooperation with the
Institut Royal des Sciences Naturelles de Belgique.

Editors : E.BONNYNS-VAN GELDER
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Thanks are due to the following institutions for the financial support provided in organizing this Workshop.

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OPENING ADDRESS

Prof.MISONNE, Director Kon.Belg.Inst.voor Natuurwetenschappen.

Ladies and Gentlemen,

It is my pleasure to welcome you - on behalf of the Direction and all the Staff of our Institute - to this Workshop on radionuclides and micro-organisms. I am indeed very happy to do so, not only because this is the first Symposium to be held in the new part of this building, but also for the following reason : if we are sometimes reproached - in these times of economic difficulties - to do research for its own sake, this remark certainly does not apply to works that will be presented and discussed here during the next three days, and which are closely related to the preservation of our environment.

Of course, during your stay, you will be free to visit our Institution. You may perhaps notice that it has two main parts : the "Museum" and the "Institute". The "Museum" part consists of exhibition rooms, offices of the Educational Service and the Documentation department, a lecture room, and finally, the Auditorium where we are right now.
I would particularly like to recommend you to visit the Iguanodons, the spiders vivarium, the Carpentier collection which is on display on the fourth floor, and also our temporary exhibitions.

The second part, or the "Institute", consists of research laboratories and administrative and technical services. There are four scientific Departments : Paleontology, Recent Vertebrates, Recent Invertebrates and the Department which concerns itself most closely with your work : the Department of Biology.

Ever since man has been adding induced radioactivity to the natural background radioactivity, a number of new problems have arisen, of which the main one stems from the long half-life of many radioisotopes. These treaten to spread into the environment, whatever precautions we may take. Moreover, we even witness the apparition of artificial isotopes such as 99-Technetium, which is not only radioactive but also chemically toxic. Since all these isotopes can concentrate along the food chains, they are higly dangerous for the organisms situated at the end of the trophic chains - and therefore for Man too.

If abundant literature can be found on the phenomenon of radionuclide concentration in macroscopic organisms, very little has been written, on the other hand, on the role of micro-organisms.
It is therefore in this regard mainly - and apart from purely scientific

aspects of the question - that your work is so important for the health of humankind.

May I then wish to all of you much success in your work, and at the same time, a very pleasant stay in Brussels.

OPENING ADDRESS

Prof. C.MYTTENAERE., C.E.C. Bruxelles

Ladies and Gentlemen,

It is a great pleasure on behalf of the Commission of the European Communities and on behalf of the IUR, to welcome the participants to this Workshop on the role of microorganisms on the behaviour of radionuclides in aquatic and terrestrial systems and their transfer to man. In so doing, I would also like to thank the Belgian Royal Institute of Natural Sciences which assisted us very efficiently with the organization of the meeting.

For health reasons, Prof.AUERBACH, President of the UIR, was constraint to stay at home but he wished us a very good meeting.

In a number of energy forecasting surveys carried out at both national and international level, nuclear energy is regarded as a vital source of electricity production, at least for the next few decades, even if other news methods of energy production are successfully developed. Like other industrial processes, the various phases of the nuclear fuel cycle produce waste, a large quantity of which, in this case, is radioactive.

There is therefore a growing interest in the study of the behaviour of radionuclides in the environment, particularly in connection with the increasing number of nuclear power plants and their associated fuel cycle facilities and with the establishment of repositories to meet their radioactive waste disposal needs.

Since a number of years the Commission has taken a close interest in the problems of contamination of the environment. The information has been helpful to radioecologists undertaking the study of the environmental radiocontamination and has contributed to the production of data in the different fields considered and in turn has contributed to the safeguarding of the quality of human life. Since several years the Commission is now helped within its task by the UIR.

Any assessment of the long-term behaviour of the long-lived radionuclides is based on the determination of the factors influencing solubility and on the form of soluble species in substrates. These factors include the concentration and chemical form of the element entering the substrate, the influence of substrate properties on the elemental distribution between the solid and liquid phase and the effect of various processes, on the kinetics of sorption reactions, radionuclides concentration and the form of soluble and insoluble chemical species in living organisms.

Current mathematical models based on thermodynamic data and solubility equilibria are not sufficient for a satisfactory description of the behaviour of long-lived radionuclides. The possible effects of the biological activity on the behaviour of long-lived radionuclides is not well known or ignored and few models have considered the role of these organisms on the radionuclides transfer in the foodchain.

From the results of limited studies of substrate chemistry, microbiology and availability of radionuclides and by reference from studies of the behaviour of trace metals in the environment, it may be concluded that the microflora could play a significant role in transformation governing the form and the long-term solubility and behaviour of radionuclides in soils, waters and living organisms.

Few information is now available on the role of living organisms in sediments, soils, plants and animals. The role of the microflora and microfauna must thus be viewed as one contributory factor among a number of highly important physicochemical and biological phenomena influencing the behaviour of the radionuclides.

The two major objectives of the proposed seminar are :

- to provide an opportunity for scientists involved in radioecological studies to exchange informations on the role of micorfauna and microflora on the long-term availability of the radionuclides,

- to make available the more recent results in this field, and particularly in the modelling of pathways for the assessment of environmental impacts from nuclear facilities.

The papers presented during this meeting will surely serve as a guide not only for those interested in radioecology studies but also in the whole subject of environmental behaviour of nutrient elements.

I am very glad to welcome the speakers and participants to such a symposium which surely will be beneficial to all of you and that will facilitate an exchange of ideas on the actions and developments that are taking place in our respective countries.

The sponsoring organizations wish to express their thanks to the staff of the Belgian Royal Institute of Natural Sciences, whose efforts contributed greatly to the success of the meeting. Special gratitude is due to Dr. VAN DER BEN who acted and played a central role in the conduct of the meeting.

I trust, Mr.Chairman, Ladies and Gentlemen, that you will find the conference facilities in Brussels to your liking, and that after the working sessions you will be able to relax and have a chance to enjoy the delights of our small country.

Ladies and Gentlemen, I wish you a profitable and enjoyable stay.

SESSION I

Marine and estuarine microorganisms

SESSION II

Freshwater microorganisms

SESSION III

Terrestrial soil microorganisms

SESSION IV

Microflora in higher trophic levels

SESSION V

Panel Discussion

SESSION I : Marine and estuarine microorganisms

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THE RADIOECOLOGICAL ROLE OF MARINE BACTERIOPLANKTON

F. AZAM

Institute of Marine Resources, A-018, Scripps Institution of Oceanography
La Jolla, California USA 92093

1. INTRODUCTION

Recent discovery of a large and productive bacterial flora in the sea indicates that bacteria may play a quantitatively significant role in the flows of matter, energy and pollutants in the marine ecosystems.[1-5] In the past, bacteria were thought to have a role only as remineralizers while matter and energy were thought to flow mainly through the grazing food chain (algae→herbivores→carnivores). It now appears that free-living heterotrophic bacteria are important secondary producers. Also, in oligotrophic oceans the photosynthetic cyanobacteria (rather than diatoms and other algae) may be responsible for most of the primary productivity.[11] Furthermore, bacteria in the sea are avidly preyed upon by a variety of animals.[7-10] These observations have led to the development of a new paradigm of the microbial foodweb with a significant bacterial component.[3,5] It appears that 1/3 to 1/2 of the oceanic primary production is utilized by heterotrophic bacterioplankton and the resulting bacterial secondary production is channelled through a microbial foodweb consisting of protozoans and other bacterivores ("microbial loop").[3] This new paradigm has implications for the foodweb transfer and biogeochemical behaviour of radionuclides.

In this paper I will address the question: Could bacteria play a significant role in radionuclide distributional patterns and foodweb biomagnification in the sea? Because this research area is new, there is

little information in the literature on radionuclide dynamics in the microbial loop. I will discuss probable scenarios, and suggest research needs and methodology for quantitative studies with radionuclides.

2. BACTERIOPLANKTON ABUNDANCE, BIOMASS, AND CELL-SURFACE AREA

By using epifluorescence microscopy, realistic estimates of bacterial abundance and biomass for many coastal and oceanic environments have been made.[1] This subject has recently been reviewed.[9,10] In general, the bacterial abundance is highest in the euphotic zone (generally 10-20% of plankton biomass; $0.5 - 2 \times 10^{12}$ bacteria m^{-3}; 50-200 mg wet-weight m^{-3}), declining gradually with depth to 10-30% of these values at 1000m. Since phytoplankton biomass is limited to the top 50-100 m, bacterial biomass at depth dominates the microbial biomass.

In radioecological context, it is important to note that marine bacteria in nature are much smaller than the cultured bacteria. Most bacteria in the seawater are 0.2 μm to 0.6 μm in equivalent spherical diameter[6], or 10-100 times smaller in volume than bacteria grown in enriched media. Therefore they have a very large surface to volume ratio. Calculations show that roughly 80% of bio-surface in the euphotic zone is due to bacteria.[5] If bacterial cell surface reactivity for radionuclides is assumed to be similar to that for other marine plankton then bacteria could, roughly speaking, account for 80% of radionuclide surface adsorption of all biota. From first principles, then, the volume-concentration factors (VCF) of radionuclide uptake for bacteria might be even higher than those for microalgae. Preliminary results show that marine bacteria could have VCF of 10^6. Since bacterial uptake/adsorption is the main mechanism of injection of radionuclides into the microbial loop, it is important to obtain data on VCF of radionuclides using natural bacterial assemblages (methodology is discussed later).

3. BACTERIAL SECONDARY PRODUCTION AND ITS FATE

If bacteria take up radionuclides with high VCF, then it becomes important to inquire how fast bacterial biomass is produced and what is the fate of this biomass, to quantify the role of bacteria in radionuclide dynamics. In the last five years there has been much interest in studying the dynamic state of bacterioplankton biomass.[2,4] Use of new methods has shown that the average doubling time of bacterial biomass is on the order of 0.5-2 days in

the coastal euphotic zone, and one to a few days in the open sea. In the coastal euphotic zone the measured bacterial production rates would require 1/3 to 1/2 of the primary production to support it. Thus, bacterioplankton are a major route (comparable in magnitude to the phytoplankton→herbivore link) in the material and energy flux in the pelagic marine foodweb. It is important, therefore, to find out what implications this route might have for radionuclide fluxes in the ecosystem.

In radioecological context bacterioplankton production channels a significant fraction of the primary production into bacterial cells, which are very small and do not sink unless associated with larger sinking particles (microscopic evidence shows >90% of bacteria to be free-living). Therefore, any radionuclide associated with free-living bacteria will remain in the same layer and not be subject to vertical transport.

4. THE FATE OF BACTERIOPLANKTON: CONSIDERATIONS FOR RADIONUCLIDE DYNAMICS

Recent studies using natural populations of bacterioplankton (rather than larger, cultured bacteria) show that bacterivory is wide-spread among organisms ranging in size from few μm (heterotrophic microflagellates; "μ-flagellates") to many cm (bivalves, salps). μ-Flagellates are the most effective known bacterivores in the water column (reviewed recently)[7,9] but whether they consume most of the daily bacterial production depends on their abundance. Larger animals may supplement their diet by feeding on bacteria, and autolysis and other unstudied fates of bacterioplankton may also be significant.

The efficiency of radionuclide transfer could vary greatly depending on the foodweb transfer pathway. The bacterial biomass consumed by μ-flagellates is likely to follow a high mineralization pathway (due to multiple trophic level transfers): bacteria→μ-flagellates→ciliates→ copepods→fish. This pathway would not be expected to transfer a significant fraction of bacterial biomass to fish. It is possible that bacteria-associated radionuclides are largely mineralized (regenerated, solubilized) in this pathway.

It is known that radionuclides such as Am and Pu do not enter the cells but are adsorbed on cell surface.[12] μ-Flagellates are themselves single-cell organisms which prey on bacteria by phagocytosis, and this may be a unique mechanism for the transfer of surface-bound radionuclide into μ-flagellate cytoplasm.

Trophic transfer of some bacterial biomass may also occur via short pathways. The larvacean *Oikopleura dioica* can obtain its body-weight equivalent of carbon by consuming bacteria.[8] Since *Oikopleura* can be eaten by juvenile fish,[8] the bacteria→*Oikopleura*→fish route would be short and efficient for the transfer of bacteria-associated radionuclides to fish. Likewise, bacteria may be consumed directly by bivalve molluscs.

5. MICROBIAL LOOP AND VERTICAL TRANSPORT OF RADIONUCLIDES

The organisms comprising the microbial loop (bacteria, μ-flagellates, ciliates) are all small. The fecal material produced within the microbial loop is also small, sinks slowly, and is likely to be mineralized within the mixed layer. The operation of the microbial loop should tend to retard the vertical transport of radionuclides from the surface waters. The operation of the grazing chain, however, should tend to accelerate the vertical transport of material including algae-associated radionuclides. This is because the animals comprising the grazing food chain (and their feces) are larger and sink more rapidly.

6. THE ROLE OF BACTERIA IN ORGANIC PARTICLE HYDROLYSIS: IMPLICATIONS FOR RADIONUCLIDE DYNAMICS

Do particle-attached bacteria, in the process of remineralizing the organic matter of the particle, liberate particle-bound radionuclides? Or, might bacteria growing on sinking particles in fact scavenge more radionuclides from the seawater thereby enriching the particle with respect to radionuclides? Both processes probably occur on sinking particles, but little is known about their relative magnitude.

Bacteria do not directly utilize particulate organic matter (POM); it must first be hydrolysed by exoenzymes (secreted by attached bacteria) to DOM. During POM hydrolysis, radionuclides may be released. Since DOM is almost exclusively utilized by bacteria any part of POM which bacteria can solubilize (not necessarily take up immediately) becomes inaccessible to particle grazers. In this respect bacteria compete with herbivores and other particle grazers. It is tempting to hypothesize that attached bacteria act primarily to solubilize POM, by secreting excess hydrolytic enzyme in the particle microenvironment, thus enhancing DOM availability for bacterioplankton. In the context of the release of particle-associated radionuclides, bacterial "hyper-solubilization" of organic

matter may be an important process, leading to rapid release of particle-associated radionuclides. Given the central importance of rapidly sinking particles to radionuclide vertical transport, it is critical to study the mechanisms of bacteria-POM interactions and attendant radionuclide dynamics.

7. METHODOLOGY

It is essential that radionuclide-bacteria interactions are studied in a realistic experimental system and in an ecosystem context, for the results to be of value in radioecological models. In general, this means that one does not rely on cultured bacteria for VCF determinations. Furthermore, the fate of bacterioplankton in a "complete ecosystem" should ideally be studied to elucidate the ecosystem transfer/biomagnification pathways for radionuclides. The use of mesocosms to study "captive ecosystems" is an attractive idea. For VCF measurements, the use of "seawater cultures" (batch or continuous) has potential. Here, natural assemblages of bacterioplankton are grown in particle-free, unenriched seawater. Also, recently developed methods for studying population dynamics of bacterioplankton should prove useful in quantifying radionuclide fluxes through the microbial loop.[1-4] The methodology for studying bacterial processes in the sea is rapidly improving, and it should have applications in microbial radioecology.

8. CONCLUSIONS

The arguments presented here suggest that marine bacterioplankton may play a significant role in ecosystem dynamics of radionuclides introduced in the sea. Bacteria are important in material fluxes in the sea and, unlike phytoplankton, are present at all depths. The role of bacteria in radionuclide dynamics on rapidly sinking particles is a critical issue worthy of research emphasis at a mechanistic level. The need for realistic experimental systems and an ecosystem context is stressed.

9. REFERENCES

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Session : I/A

Paper by : F. Azam

Comment by : E.K. Duursma

Text of comment
or author's answer :

Your remark on the fact that Am is adsorbed to the bacteriaplankton is supported by the fact that the CF otherwise hardly could be 10^6 since bacteria are 90% water and the organic tissue would be unable to go so far. Perhaps in the future a blank containing inert 0.2 µm solid particles would be possible to carry out.

I agree that intracellular accumulation to achieve CF = 10^6 from 26 pM ^{241}Am (the concentration used) would mean a very high value of 26 mM. This may be an improbably high concentration. There is another reason to think that the association of ^{241}Am with bacteria is adsorption : the uptake is essentially eliminated at pH 5 (although I cannot exclude the possibility of physiological damage to cells causing this).
As for the use of 0.2 µm solid particles : might the surface chemistry be sufficiently different to make it less than perfect control.

CONCENTRATION OF RADIONUCLIDES BY MARINE PHYTOPLANKTON

N.S. FISHER

International Laboratory of Marine Radioactivity
I.A.E.A.
Musée Océanographique
MC 98000 Monaco

ABSTRACT

Marine phytoplankton, belonging to different taxonomic groups and maintained in unialgal clonal cultures, have been exposed to 14 different radionuclides (^{60}Co, ^{65}Zn, ^{95m}Tc, ^{109}Cd, ^{110m}Ag, ^{113}Sn, ^{203}Hg, ^{210}Pb, ^{210}Po, ^{235}Np, ^{237}Pu, ^{241}Am, ^{242}Cm and ^{252}Cf) under identical conditions. Up to seven different algal species were compared for any given metal. The kinetics of bioaccumulation were assessed for each metal and each species, with up to 13 different concentrations per metal. Cellular uptake of metal was generally rapid and increased hyperbolically with time. In all cases, an apparent equilibrium was reached within two days with respect to metal partitioning between particulate (ie., cell) and dissolved phases. Concentration factors for all metals on a volume/volume basis (ie., moles μm^{-3} cell divided by moles μm^{-3} ambient seawater at time of equilibrium) were determined, and ranged from <10 for Tc and Np to $>10^5$ for Pu, Am, Cm and Cf. There was relatively small (ie., <1 order of magnitude) variation among algal species for the concentration of any metal. An hypothesis is presented to explain the reactivities of the different metals.

Cellular accumulation of metal proceeded by passive adsorption onto cell surfaces, with dead cells accumulating metal similarly to live cells. Accumulation in the algae was always directly proportional to the metal concentration in solution. Phytoplankton may serve to introduce metals into marine food webs as well as to mediate the vertical transport of the metals in marine systems.

CONTENTS

1 EXPERIMENTAL PROTOCOL

A series of laboratory experiments was conducted to explore the bioconcentration of select radionuclides in marine unicellular algae. Representative species of the major taxonomic algal classes were investigated, including the centric diatom Thalassiosira pseudonana, the chlorophyte Dunaliella tertiolecta, the cyanophytes Synechococcus sp. and Oscillatoria woronichinii, the prasinophyte Tetraselmis chuii, the coccolithophore Emiliania huxleyi, and the dinoflagellate Heterocapsa pygmaea. The radionuclides examined were ^{60}Co, ^{65}Zn, ^{95m}Tc, ^{109}Cd, ^{110m}Ag, ^{113}Sn, ^{203}Hg, ^{210}Pb, ^{210}Po, ^{235}Np, ^{237}Pu, ^{241}Am, ^{242}Cm, and ^{252}Cf.

Experiments were conducted with living and heat-killed cells to determine whether cellular accumulation of the metals was metabolically mediated. Cultures were also exposed to radionuclides in the light and in the dark to see if illumination directly influences metal uptake. Typically, sterile-filtered Mediterranean surface sea water was inoculated with known quantities of cells and immediately exposed to the radionuclides. Care was taken to construct metal "budgets" for all flasks over time to monitor partitioning of the metals between dissolved, particulate, and flask wall phases. Periodically, cells were filtered out of their media using Nuclepore polycarbonate filters and the radioactivity of the filters determined using gamma spectrometry (except for ^{210}Po and ^{210}Pb-- see ref. 1). Cell counts were made throughout the experiments and results expressed as metal accumulated per cell. Blank values, obtained from uninoculated control flasks, were generally negligible, and were subtracted from the culture values. Since the dimensions of the cells were also known[2], it was possible to calculate concentration factors at equilibrium on wet weight, and surface area bases. For example, a volume/volume concentration factor (VCF) is defined as atoms metal per μm^3 cell/atoms metal μm^3 dissolved in water. Details of experimental protocol, counting methods and calculations are given elsewhere[2-4].

2 RESULTS

Fig. 1 presents a typical set of uptake curves, in this case for cellular accumulation of ^{241}Am by seven algal clones belonging to six species.

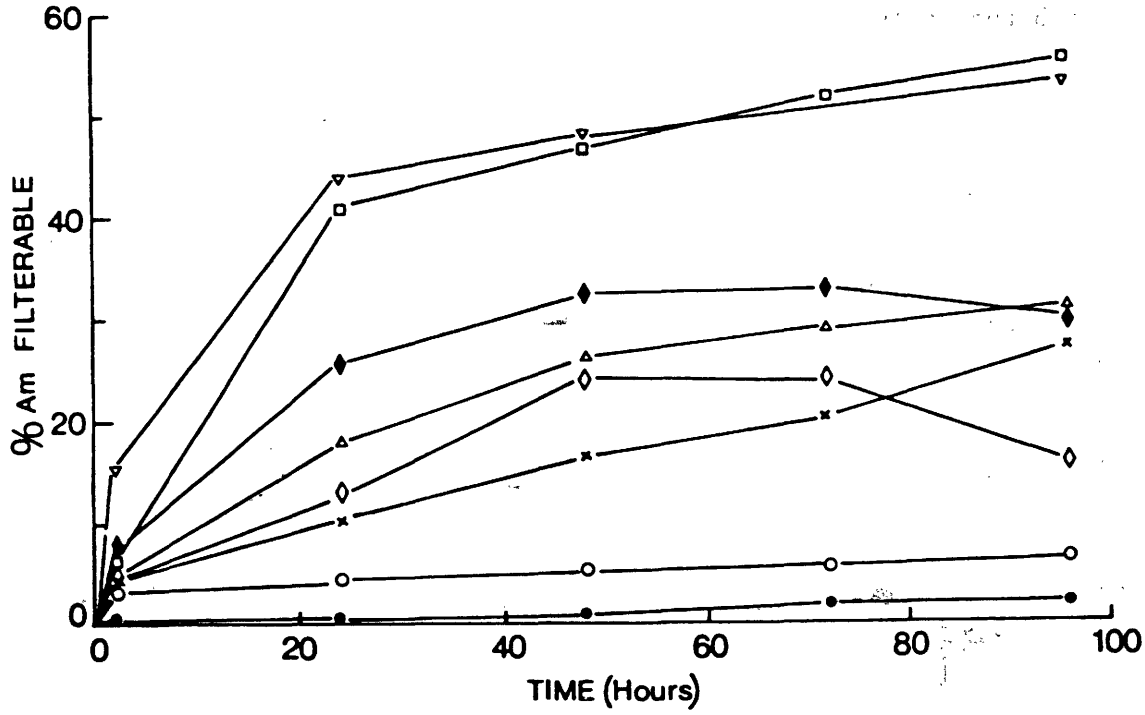

FIGURE 1 Fraction of water column ^{241}Am removable by 1-μm Nuclepore filtration of algal cultures over 4 day period. (•) control--no cells; (O) O. woronichinii; (◇) E. huxleyi (no coccoliths); (◆) E. huxleyi (with coccoliths); (□) T. pseudonana; (△) D. tertiolecta; (▽) H. pygmaea; (X) T. chuii. ^{241}Am (in dilute HNO_3) was added to the culture flasks at 83 pM at time zero.

Radionuclide accumulation was generally complete within 1-2 days, as shown for example for ^{109}Cd uptake by the coccolithophore E. huxleyi (Fig. 2). The experimental results generally showed that light had no direct effect on algal accumulation of the nuclides[2], that live and dead cells concentrated equal amounts of radionuclides, and the metal accumulation per cell was related to the dissolved metal concentration (see, for example, Fig. 3 for results with ^{109}Cd, ^{65}Zn, ^{110m}Ag and ^{203}Hg) in accordance with Freundlich adsorption isotherms:

$$\log C_m = a \log C_t + b \qquad \text{(Eq. 1)}$$

where C_m = moles cell^{-1} at time of equilibrium
and C_t = total metal concentration at t_o. Thus adsorption to cell surfaces appears to be the principal process by which these metals associate with the cells.

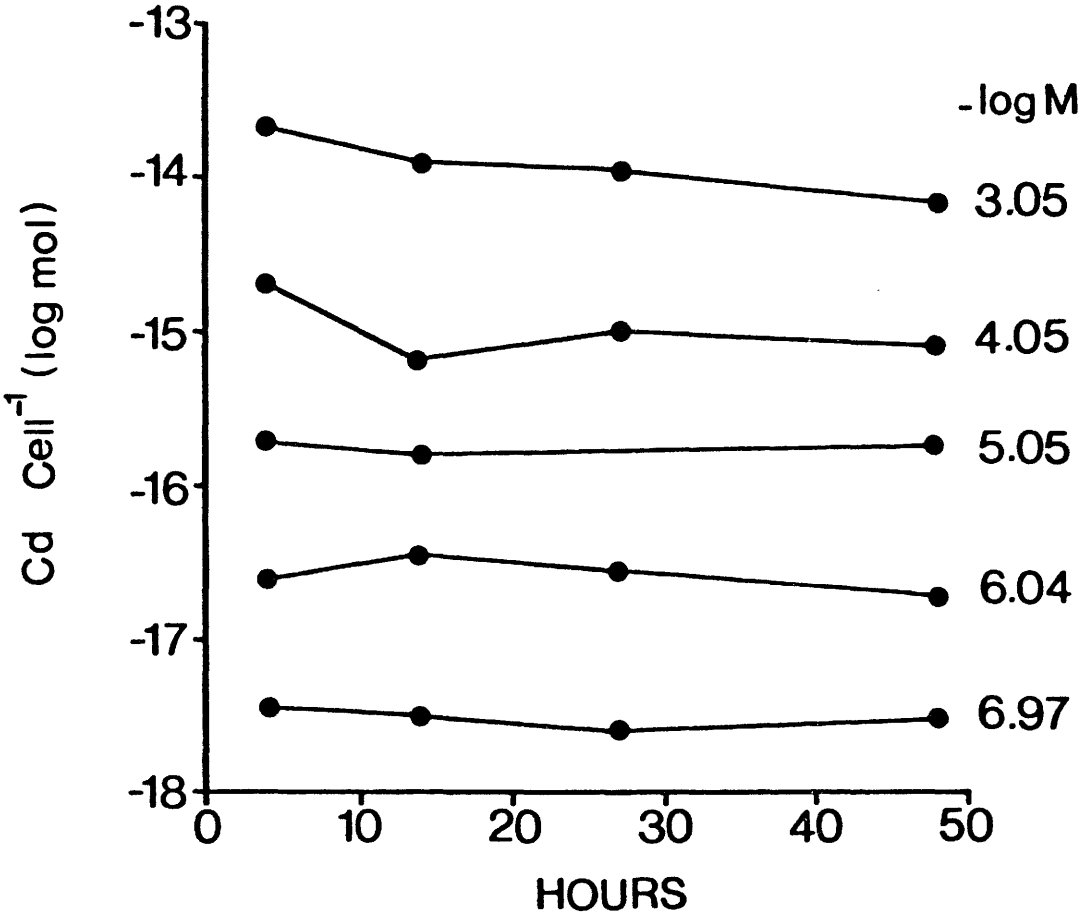

FIGURE 2 Cd content of Emiliania huxleyi cells exposed to different Cd concentrations over a 48 h period. Note that the Cd accumulation by these cells was essentially complete at 4 h.

Using equilibrium data, a compilation of VCFs is presented in Table 1. The range of VCFs is from not significantly different from zero (eg., ^{95m}Tc and ^{235}Np) to greater than 10^5 (for ^{237}Pu, ^{241}Am, ^{242}Cm, and ^{252}Cf). There were modest (generally less than one order of magnitude) differences in

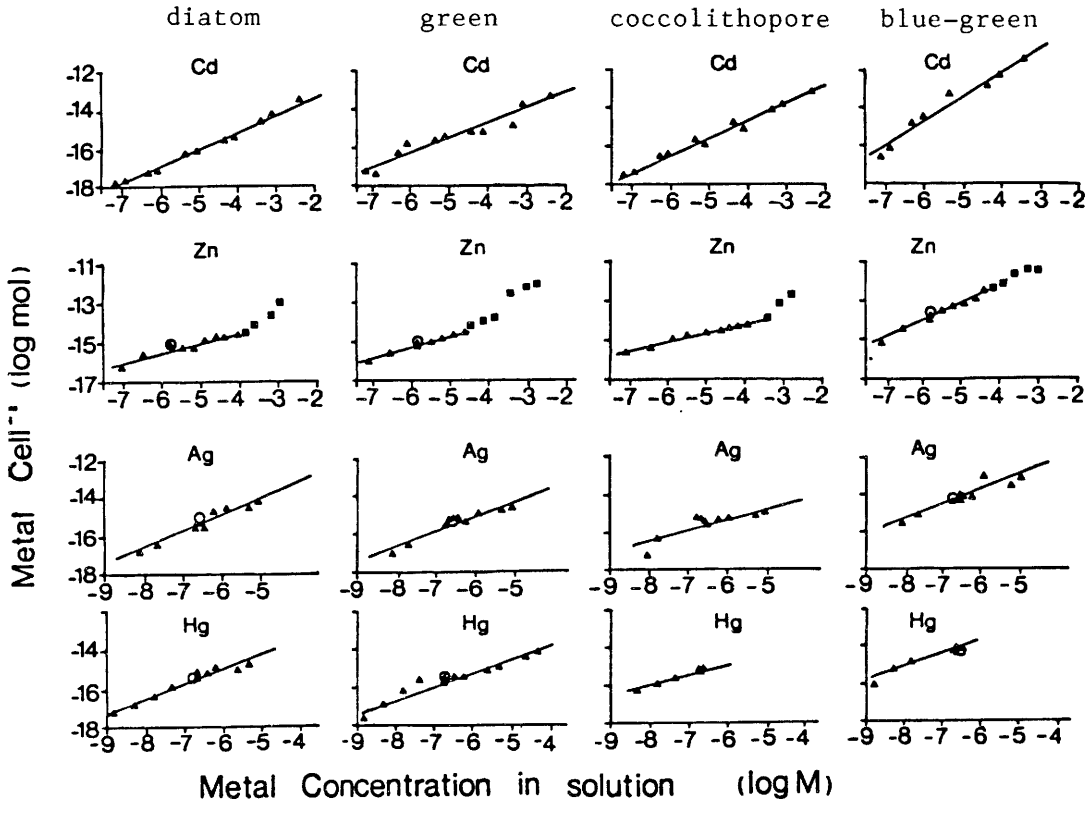

FIGURE 3 Cd, Zn, Ag, and Hg contents of cells of a diatom, a green alga, a coccolithophore, and a blue-green alga as a function of external metal concentrations. Each data point is the mean plateau concentration at time of no change. Triangles denote live cells, circles denote heat-killed cells (for diatom, green and blue-green); squares denote heavy precipitation of Zn.

concentration factors observed among species for any one radionuclide. The enormous differences noted in VCFs among the elements are in large part attributable to the differential speciation of the metals in seawater (eg., Tc exists as the pertechnetate anion: TcO_4^-) although Hg, Ag, and Cd, for example, all exist in seawater principally as the chlorides[5] but have very different reactivities for algal surfaces. Such differences may be related to atomic properties such as polarizing power (ionic charge^2/ionic radius) which influences the metal affinity for various ligands[5].

3 IMPLICATIONS

Considering the broad range of concentration factors observed, dictated largely by the metal and to a much smaller extent by the algal species, (Table I), an attempt was made to find some basis on which to predict the bioaccumulation of the metals by phytoplankton in seawater. The results, taking mean VCFs and pooling with other concentration factor values reported for other algae and other elements[6] suggest that the metal concentration factors in phytoplankton are exponentially related to the solubility

TABLE 1 Volume/volume concentration factors (VCFs) of metals in marine phytoplankton. nd = not determined.

	diatom	green	coccolithophore	prasinophyte	dinoflagellate	Osc blue-green	Syn blue-green
Co	1.0 E3	nd	nd	nd	nd	nd	4.0 E3
Zn	1.2 E4	1.0 E4	4.6 E3	nd	nd	5.2 E3	3.2 E4
Tc	1.0 E1	< 1.0 E0	< 1.0 E0	2 E0	1.7 E1	8.0 E0	nd
Cd	3.0 E2	1.0 E3	3.7 E2	nd	nd	1.0 E3	nd
Ag	3.4 E4	1.3 E4	2.4 E4	nd	nd	6.6 E4	nd
Sn	1.1 E5	nd	nd	nd	nd	nd	7.9 E5
Hg	9.3 E4	3.2 E4	9.5 E4	nd	nd	7.6 E4	1,3 E6
Pb	2.9 E4	7.6 E3	nd	nd	nd	nd	nd
Po	1,2 E5	4.3 E4	nd	nd	nd	nd	nd
Np	< 1.5 E2	< 1.5 E2	<1.5 E2	<1.5 E2	nd	<1.5 E2	<1.5 E2
Pu	6.3 E5	2.2 E5	1.6 E5	4.0 E4	nd	1.7 E5	nd
Am	6.9 E5	1.8 E5	1.1 E5	3.0 E4	3.8 E5	3.0 E4	5.8 E5
Cm	6.4 E5	1.2 E5	2.1 E5	nd	1.2 E5	2.6 E5	nd
Cf	6.2 E5	4.1 E5	3.2 E5	9.0 E4	nd	1.3 E5	nd

products of the metal hydroxides (from ref. 7) (Fig. 4). Moreover, the reactivity of metals for particles (as reflected in the phytoplankton VCF data) correlates exponentially with the toxicity of the metals for phytoplankton (eg., EC_{50}'s for cell division rates), so that highly reactive metals (eg., Hg) are very toxic (Fig. 4; ref. 4). Therefore, metal toxicity is linearly related to solubility products of the metal hydroxides (Fig. 4). Finally, since metals which are highly reactive for particles (like phytoplankton) would behave less conservatively in seawater than

unreactive metals, the mean oceanic residence times of metals should be inversely proportional to their affinity for particles. Fig. 4 shows that the VCFs of metals in phytoplankton correlate significantly ($P < .001$) with their mean oceanic residence times (from refs. 8,9; Am and Pu residence times calculated from data in refs. 10,11). Whitfield and Turner[12] and Yamamoto et al.[13] showed similar correlations. Thus, it would appear possible to predict the degree of bioconcentration of metals in phytoplankton, based on affinity for hydroxyl groups, and therefore predict the toxicity and oceanic residence times of the metals as well.

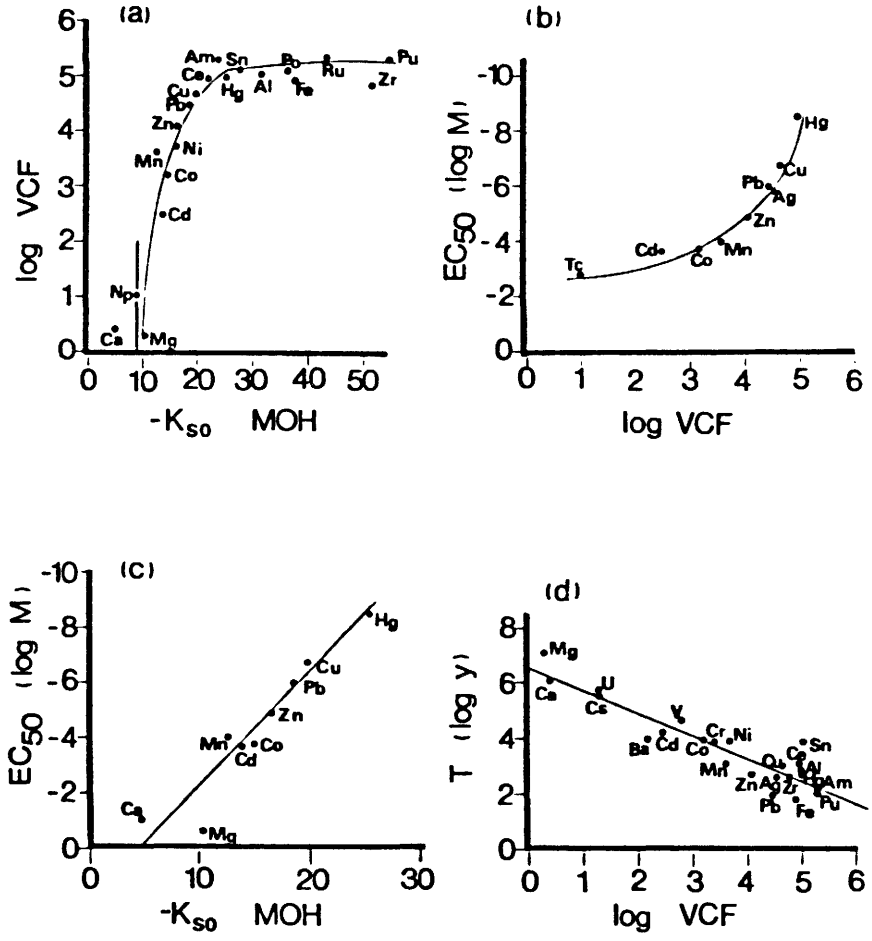

FIGURE 4 (a) Correlation of log VCF of various metals in marine phytoplankton with solubility products of the corresponding metal hydroxides; r = .844. (b) Correlation of sublethal toxicity (EC_{50} metal concentration) of metals with log VCF; r = .929. (c) Correlation of toxicity of metals with solubility products of the metal hydroxides; r = .945. (d) Correlation of mean oceanic residence times (T) of metals with log VCF in marine phytoplankton; r = .915.

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Session : I/A

Paper by : Fisher

Comment by : R. Bittel (France)

Text of comment
or author's answer :

Pensez-vous que les résultats obtenus avec Pu237 (forte activité spécifique) soient valables pour Pu239 (faible activité spécifique)?

The two isotopes have similar chemistries. Moreover, previous experiments at the Monaco laboratory have shown that they behave identically, with repeat to marine organisms.

Session : I/A

Paper by : N.S. Fisher

Comment by : A. Cremers

Text of comment
or author's answer :

A. It appears that, in the cases studied, you are dealing with the formation of some sort of surface complex. I suggest you try to correlate CF values with the first hydrolysis constant of the various elements.

B. My second comment refers to the contents of the abstract stating that there is no effect of humic or fulvic acid. This is somewhat surprising in that one would expect some sort of competitive effects.

A. I have done this, and the results are comparable to those of solubility products.

B. I too was surprised that humic or fulvic acids of marine origin, had no significant effect on the bioaccumulation of Am or Pu in marine phytoplankton, even when present at greater than natural concentrations. Similar uptake of these elements was comparable in UV-irradiated and unirradiated seawater, and algal exudates had no appreciable effect on Am or Pu uptake by algae. EDTA, added to seawater at equimolar concentrations to the humic acids, had an appreciable effect on complexation of these elements.

Session : I/A

Paper by : Fisher N.

Comment by : J. Vangenechten

Text of comment
or author's answer :

Can you comment about the pH-effect on the high correlation between the affinity of the metals for the cells and for the hydroxides ?

I have not specifically examined the effect of pH on metal accumulation by phytoplankton in seawater, since seawater tends to be well-buffered at pH 8. I would imagine that intake would be far less reactive at lower pH's (i.e. < 6). In freshwater, I have shown that ^{241}Am adsorbs the surfaces readily at pH > 7, but at pH 4-5, adsorption is hardly detectable.

Session : I/A

Paper by : Fisher

Comment by : I.McKinley

Text of comment
or author's answer :

Given the varying slopes of the Freundlich isotherms shown, how is the VCF defined ?

The VCF is calculated from the lowest concentration used.

The VCF is thus experiment specific ?

Yes, for the experimental conditions and laboratory temperature, light levels etc. The laboratory measurements do, however, agree with field measurements.

THE TRANSFER OF RADIONUCLIDES FROM SEDIMENTS TO PELAGIC SYSTEMS

E.I. HAMILTON

Institute for Marine Environmental Research,
Prospect Place, The Hoe, Plymouth, Devon PL1 3DH, U.K.

ABSTRACT

For the marine environment a major objective of radiological protection concerns the contribution to the radiation dose man receives from radionuclide contaminants derived from marine ecosystems (principally along foodchains), and any effects upon marine biota. For operational purposes considerable emphasis is placed upon various models (conceptual, applied, predictive) in order to provide information which can be used in decisions and control measures for the protection of man and the environment from undesirable hazards due to contaminant releases. The immediate concern is to provide protection over several decades and hopefully also for the next ∿500y. Therefore, most models deal with average values based upon a consideration of first-order terms. Our understanding of long term processes which take place in the seas is often very poor, and until improved many of the models are of limited value. For local releases and short term events, which may be important to the nuclear industry, we also lack sufficient understanding of the nature of marine processes and how they interact with the release of contaminants.

Considerable advances are required in marine sciences in order that models used for purposes of radiological protection may be based upon more quantitative information. The research requires long term stability, access to sophisticated technology and closer cooperation between marine scientists and those concerned with radiological protection. Webb (1984) comments on a need to direct effort from refining science of investigation toward the explanation of science which is required by politicians, the general public and environmental groups. As a short term measure this is essential, but if taken too far it could generate unwarranted complacancy; an ultimate acceptable solution to many areas of concern can only be provided by substantial improvements in fundamental science.

Here I consider some areas of uncertainty with reference to effluents from the British Nuclear Fuels (BNFL) reprocessing plant at Sellafield, UK, which has labeled vast areas of the sea, but to no apparent detriment to marine ecosystems. An opportunity is provided to consider the value of radionuclide tracers in order to improve our knowledge of long term interactions between radionuclides and marine ecosystems; attention is focussed mainly on the long lived radionuclides Pu and Am. Improvements in the capability to predict concentrations (and therefore calculate dose) of relevant contaminants depends on obtaining further knowledge of critical processes to test the importance of various processes and assumptions (GESAMP 1983).

1. SOURCE TERM

If it can be assumed that the physical and chemical composition of the source term has no effect upon the transfer of radionuclides, then their transfer in the marine environment can be determined from a consideration of stable element analogues. In marine systems radionuclides are considered as dissolved forms and those associated with particulate materials; the use of first-order terms is acceptable as the mass of radionuclide associated with transfer of one form to another is relatively small. However, radionuclides do have properties related to their formation; initially they will be carrier free and it will take some time, after release into the sea, before they are fully integrated into those marine pathways which are characteristic for their stable element analogues. The process of radioactive decay can also exert an influence; for example, the selective loss of 234-U from 238-U, 238-Pu from 239-Pu, as a consequence of alpha particle recoil, also the decay of a parent nuclide to a daughter of a different element can create instability at the site of deposition.

Because of artifacts in preparatory and analytical schemes limitations are imposed upon identifying what is in true solution, and what is present in particulate form. Hamilton (1983, 1984) identified the presence of hot particles derived from BNFL which were present in local estuarine sediments and at the sea surface, also hot particles which were formed by biological processes; the former have mean particle diameters of < 10 μm, and the latter up to several millimeters. Many of the small particles contain $\sim$1 pCi of alpha activity which is of interest to radiological protection (ICRP 31). In sediments, hot particles tend to contribute to $\sim$ 10% of the total alpha activity, but there are problems in identifying < 1 μm hot particles because of entrainment in flocs and absorption of activity in such matrixes. Nuclear weapon testing also produces hot particles (Mamuro and Matsunami 1967, Adams et al 1960), but their stability and transfer in the sea is not unknown. Along the Cumbrian coast the highest levels of radioactivity are associated with fine grained sediment derived from marine and terrestrial sources. An association of alpha and gamma emitters in individual hot particles indicates a stable source derived from the BNFL effluent.

In the absence of any quantitative data for the composition of the BNFL source term it is noted while the Pu/Am ratio has decreased from $\sim$ 9 in 1977 to $\sim$ 2.5 in 1980, in sediments and mussels near the source (Hunt 1979, 1980, 1981, 1982) between 1977-1981 the ratio has been rather constant, namely 1.3 ± 0.2 and 1.5 ± 0.3 respectively. For mussels taken from the Esk estuary ($\sim$ 12 km from the source) the Pu/Am ratio for 1977, 1978 and 1979 was 4.7, 1.2 and 0.3 respectively (Hamilton and Clifton 1982) and values for local sediment for the same intervals were 1.7, 1.4 and 1.2. Evaluation of the data illustrates that the pattern of change in the source term is reflected by sediment and mussels (filter feeders) but the Pu:Am ratio is different to that of the source term. I conclude that at the site of release a change occured when the effluent entered the sea, and the product is stable in the marine environment; possible changes in the ratio with increase in distance from the discharge site are being considered. The characteristic ratios currently found for sediment and biota are not those for pure fuel element debris. While acknowledging the presence of dissolved and particulate forms of Pu and Am the significance of the solid phases in the transport of Pu and Am are not known with any

certainty.

2. GEOCHEMICAL CONSIDERATIONS

Under high temperature and pressure regimes, as an igneous rock magma solidifies the elements become partitioned into various mineral phases; entry is largely controlled by ionic size and charge. Sediments represent low temperature suites of rock-mineral debris, and to a large extent the characteristic parent rock element associations are retained and are of diagnostic value in identifying the presence of the parent rock type. Data for the partitioning of radionuclides in modern diagenetic mineral phases has not been described. By using various pairs of elements the signature of a rock can be identified. Therefore by an examination of element abundances in sediment debris in the gut of a filter feeder such as the mussel Mytilus edulis, the mass of sediment can be determined, but of more interest is the extent to which the composition differs from that of the parent sediment, thus shedding some light upon possible processes common to the mussel which have altered the composition, for example the use of an element by the mussel for a particular biological process. For present purposes it is assumed that the aluminium content of individual tissues of the mussel reflects the presence of sediment; concentrations range from $\sim$ 7000ppmAl in the digestive gland to <80ppm in the muscle. For mussels from the Esk estuary based upon Al levels a total sediment load of 15.3mg is calculated; using iron as an index a load of 14.9mg sediment, and on the basis of ashed water insoluble residues of total soft tissues a value of 15.4mg sediment is obtained. Of all the stable elements of the Periodic Table the rare earth elements (REE) are most likely to follow the same pathways as Pu and Am; the only other group of elements of possible interest are Ca Sr Ba Ra. By using the concentration of Al to subtract the sediment load the following observations are made;

i) Greater than 90% of the total La load is not removed, while all the Ce is lost. Hence the animal contains a pool of labile La which is not related to the La held in mineral structures as determined by the La/Ce ratio; because of the very large numbers of La atoms present, compared to the very few of Am and Pu, this element could provide a suitable carrier for the heavy radionuclides.

ii) Carrying out the same procedure for Ba the subtraction leaves none in mussel tissues, with the exception of the byssal threads and periostracum which probably obtain their Ba from seawater.

iii) When the same calculation is performed using the Am and Pu content of the bulk sediment, >90% of each radionuclide cannot be accounted for on the basis of sediment load. Hence the mussel while taking in Pu and Am labelled sediment also contains another source which is not directly related to bulk sediment. Analysis of intestinal faecal source debris was shown to contain unaltered sediment Pu and Am concentrations. Two possibilities can account for the non-sediment Pu and Am, the first entry of these elements in dissolved phases from seawater and the second preferential uptake by the mussel of a phase enriched in these elements, for example that associated with organic detritus.

iv) 200-molecular weight cut-off dialysis membrane tubes containing deionised water were emplaced into anaerobic surface sediments of the Esk for two days. The dialysate was removed and then oxygenated to precipitate iron (ie transfer of ferrous iron across the membrane in the dissolved state). The precipitate contained 34% iron, 2,4% Mn, 30% organic material and 24ppm La (see Tipping and Ohnstad (1984) who found

similar compositions for amorphous iron particles in lake sediments); no Ce was detected once more indicating the presence of a labile form of La; the dialysate also contained Am and Pu. Therefore the anaerobic surface sediments of the Esk contain a mobile pool of La which is a suitable carrier for Am and Pu either as colloids or in true solution.

There are numerous reports that heavy elements (Pu, Am, Th, Pb, Th, Pb, Po, see Santischi et al 1983) are associated with particulate materials which determines the mobility of these elements, but little is known of the processes whereby they become associated with particulate phases. The flux of particulates to estuaries and near shore waters is dominated by seasonal influences; Sieglo and Helz (1981) have shown that winter colloidal debris is dominated by detrital clay minerals and iron oxides, while in the summer organic detritus derived from biological processes is dominant; Hamilton and Clarke (1984) have also described such features for the Esk. In a recent accidental discharge of effluent by BNFL very high levels of radionuclides were found associated with "organic materials"; in the Esk detrital coal debris is also slightly enriched in alpha emitters.

There is considerable evidence that organic compounds associated with surfaces, together with colloids, play an important role in the transfer of radionuclides in the marine environment. The real problem concerns an identification of the nature of real surfaces, for example structure, ligands present, redox and pH conditions at the surface; current research tends to be directed towards a consideration of the bulk composition of materials.

By co-precipitation Wong et al (1978) have separated Am, Eu, Sb, Bi, Ru, Rh and Co from seawater onto freshly prepared manganese dioxide; at the sediment water interface of the Esk there is a flux of Mn which is mainly present at the turn of the tide because of resuspension of anaerobic organic rich sediments, and could provide a suitable surface for adsorption and co-precipitation of many elements. Eakins and Gomm (1968) obtained a quantitative recovery of all alpha emitters by adsorption onto glass fibre filter paper; the rate of uptake was increased in the presence of NaCl. Apart from the surfaces of silica grains in the estuary, there are many sources of silica (hydrated) which could provide suitable surfaces for uptake of radionuclides. While quartz grains are attractive, because of abrasion as a result of movement it is unlikely that any deposit will accumulate to any appreciable thickness, but in the Esk because of the much greater mass of sand compared with fine sediment the process could provide a significant flux of Am and Pu into overlying waters; in low energy regions where organic rich muds dominate adsorption may be more significant. Robinson (1970) has described the uptake of Pu by proteins which are ubiquitous in the marine environment. The tanned proteins of the byssal threads and periostracum of the mussel are, relative to other tissues, associated with high concentrations of Pu and Am; the distribution is diffuse, therefore uptake from seawater is most likely. There are many types of surfaces present in the sea and it is clear that uptake of dissolved species and particulate forms does take place. It would be useful if the nature of the surfaces could be identified and, if present, any common parameter responsible for uptake was identified. While the diffuse component can perhaps be understood, the particulate forms are more difficult to comprehend, unless of course they consist of very finely macerated organic debris which "sticks" indiscriminantly to surfaces. One further possibility worth investigating is the presence of barium sulphate in marine systems, especially in estuaries at the turbidity maximum. Sill (1969) has illustrated the versatile nature of this

compound in coprecipitation and adsorption of heavy radionuclides, often in a very selective manner. Apart from riverine inputs of dissolved barium interacting with sulphate ions of seawater, in the reduction of sulphate in anaerobic sediments a flux of labile barium is available. Bioturbation at the surface of sediments could release barium to overlying water and precipitate the sulphate which would be a suitable source which has a very large surface area for uptake of elements.

From a consideration of the cosmogenic relationships between heavy radionuclides in natural siliceous melts, under reducing conditions, Pu+3 is more rapidly incorporated into mineral lattices than either U+4 or Th+4; a priori actinides are assumed to substitute for Ca+2 (ionic radius Ca+2-1.01, Am+3-0.99, Am+4-0.89, La+3-1.06, Th+3-1.05, Th+4-0.98, U+3-1.02 and U+4-0.03) because of the existence of Ca-lanthanide phosphates in meteorites (Benjanin et al 1983). In igeneous rocks Ca+2 also plays a key role in the partition of REE, especially in late stage hydrothermal conditions and early high temperature phases, eg apatites. In marine systems the association is not so direct as these elements have to cross biological barriers (membranes) before they are available for incorporation into tissues and exoskeletons; skeletal debris of fish is often enriched in U, Th, REE, but mainly as a result of adsorption onto exposed surfaces. Folsom et al (1975) note the uptake of Pu onto the surfaces of algae in the oceans. Guary and Fraizer (1975) do not observe any progressive enrichment of Pu through marine foodchains. Pentreath and Lovett (1976) note a concentration factor of <1 for Pu and ≅5 for Am in fish over seawater for BNFL derived effluent. Shanbhay and Morse (1982) note that calcareous shells have an affinity for Am; adsorption is high, and independent of the ratio of solid to dissolved phases; synthetic aragonite has a x40 greater uptake than synthetic calcite which is related to the Mg content of the calcite; the observed behaviour of Am with calcium carbonate in shallow waters is predicted from the ratio of aragonite: Mg-calcite in sediments.

Seawater contains ∿52ugBa/1 (Chow and Goldberg 1960) of which ∿5% is in the undissociated form; at a depth of 5000m, because of pressure effects, there is a 2.5-fold greater concentration of Ba. The Ba (and Ra) depth concentration profiles in seawater are similar to those for nutrients indicating an association with the biochemical cycle, especially with sulphate ions which could precipitate $BaSO_4$ and simultaneously scavenge REE and actinides in both dissolved and particulate phases. In oceanic depth profiles the increase in Ba is greater than that for Ra (Chan et al 1976), with a preference for refractory phases rather than transport by labile organic compounds. In the sea REE's are associated with calcareous foraminifera tests, but possibly with Fe-Me surface coatings rather than an involvement with the Ca biological pathways. In laboratory experiments Shen et al (1983) describe the rapid coagulation of Pu in estuaries, the rate of which increases with salinity and the preser . of humic compounds and iron rich precipitates Anderson et al (1983) show that while Th and Pa do not show any preferential fractionation effects in nearshore marine particulates, this does not occur for oceanic particles, hence they are possibly different. Hunter (1983) has described an equilibrium model for scavenging of reactive metals in relation to the presence of organic coatings; the oceanic residence time for Th is ∿22y and -180000y for Cd (Brewer et al 1981); humics may be a major component of surface coatings and the chemical characterization of the surface of marine particulates is urgently required. Balistrieri et al (1981) note the ubiquitous presence of a polymeric organic film on surfaces which are immersed in seawater and are dominated by carboxyl-COOH and phenolic-OH functional groups which are

common to humic substances. Hayase and Tsubota (1983) note that humic and fulvic acids have surface active substances; any particles in the sea-surface microlayer can therefore acquire an organic coat whether it be derived from natural products or oil slicks.

3. BIOLOGICAL CONSIDERATIONS

In tracing the transport pathways of heavy radionuclides from coastal waters to the open oceans with distance, the contribution of land derived organic coated particulates should decrease; for the upper layers of the seas biological debris should predominate, while at depth organic depleted material should occur. Aarkrog et al (1983) have shown that BNFL 137-Cs 134-Cs derived radionuclides can be detected in the Barent and Greenland Sea and in the East Greenland polar current; the transport time from Sellafield is 6-8y. As Cs is conservative in seawater the distribution is a reflection of water movements; there is no complimentary data for Cs associated with particulate phases. In the transport of radionuclides over large distances their pathways must cut across many different biologicel systems and they may or may not interact with them. Off the Hebridian shelf-edge Kremling (1983) has described strong salinity gradients (fronts) which mark the boundary between coastal and oceanic waters; these gradients are associated with a dramatic increase in inorganic nutrients and dissolved trace metals (Cd, Cu, Mn, 226-Ra) which reflects the mobilisation of partly reduced organic rich sediment. The BNFL effluent "plume" intercepts these regions and interactions are possible between radionuclides and particulate phases which may control the passage of species to the open oceans. Holligan et al (1983), from a study of satellite photographs, show that along the continental shelf high reflectance from substances present in surface waters contain large amounts of coccolithophores (Emiliania huxleyi). Apart from providing potentially vast surfaces for adsorption of elements the concentration of calcium can reach 40g Ca_2CO_3 m^{-2} for the upper 60m of water; in areas the animals cover 7200km of the sea and represent 7.2×10^4 tonnes of Ca. As the lysocline for calcium dissolution in the N Atlantic is at c4600m there should be (over shelfs) a net deposition of tests to bottom sediments. As the blooms tend to occur in the same geographical regions for defined times of the year they can be sampled by ships. Therefore for the UK (and maritime Europe), radionuclides derived from land sites have first of all to survive the conditions existing in estuaries and near shore waters, and then "penetrate" regions of high particulate loading on the continental shelf.

In the open ocean there are abundant data which describe the rapid transport of radionuclides in faecal pellets from surface waters to the ocean bottom (Osterberger et al 1967, Higgo et al. 1977). Based upon the pioneering work of A.P. Vinodragov (1953) for the concentration of elements in marine organisms, supplemented by more recent compilations of data by Martin and Knauss (1976) and Eisler(1981), and the distribution of of radionuclides in marine ecosystems by Polikarpov (1966) the involvement of elements in marine organisms can now be considered, albeit in a cursory manner as most analyses are for total animals which will include gut contents and those elements associated with surfaces rather than as a result of an involvement in metabolic processes.

Consideration of the Ca→Sr→Ba→Ra→REE→actinide pathways in biological processes is of interest as both Sr and Ba are found in Radiolaria (Acantharia-$SrSO_4$ skeletons) and deep sea rhizopods such as the Xenophyophora which contain intracellular deposits of $BaSO_4$ (Vinogradov,

1953, Goodhay and Nott 1982). Their involvement in the transport of radionuclides in the oceans could be significant, however an artifact may be present in the interpretation of available data as the tests of Radiolaria and Acantharia dissolve with storage and may be missing in some collections. Phaeodarians Radiolaria (Takahashi et al 1982?, see also Renz 1976), dissolve in the deep oceans and hence are not preserved in bottom sediments; in the dissolution process more refractory forms of elements such as discrete phases or colloids may be formed and be transported to the ocean floor. Bowen (1971) also notes that the strontium skeletons of Acantharia may be more abundant in the Atlantic than has been realised. In some of our own studies in the Atlantic (Williams, Hamilton 1984) it seems possible that Acantharia may be quite abundant in the Atlantic, but based upon observations of the relative abundance of net samples taken at the Weather Ship-India at the time of collection, after 12y none remain in the sample bottles; after 4y only c50% remain but little loss appears to have occured after preservation of ∿3 months. Acantharia are also found in warm coastal waters (Schreiber et al 1963). There is also a possibly significant riverine input of Ba and Sr as many freshwater microorganisms, eg desmids, are enriched in these elements and contribute to estuarine particulate debris. Bowen and Sugihara (1961), after careful examination of long lived radionuclides in the surface water of the oceans note the presence of 90-Sr in seawater in the dissolved state, while lanthanide radionuclides are associated with particles and not organisms. Harley (1956) and Miyake (1955) note high concentrations of lanthanide radionuclides in plankton of the Pacific, possibly because less surface area is available for uptake by terrestrial derived particulate debris.

In order for models to describe, or account for the distribution of radionuclides in the marine environment to be of use they have to be valid (or at least realistic) for the next ∿100y. However such spans of time are beyond the realms of realistic predication for marine processes because the basic information is not available. While some of the processes discussed here may be of second order importance, one major issue which is seldom considered in modelling is the question of climatic change.

Since 1948 there has been a ∿50% decrease in the abundance of zooplankton in the N Atlantic (Robinson 1984); this is possibly accompanied by a significant decrease in the mass of phytoplankton while the depletion in fish stocks for most major fishing areas is well documented. There is no evidence that the decrease in biomass has been compensated for by an increase in other species, hence for the open oceans at least the total load of particulate debris must also have decreased. Whether or not the process of scavenging is such that the impact frequency has also increased or saturation has never been achieved and therefore a reduction in the load of particulate debris in the sea is unimportant. However, if there has been a real decrease in the concentration of particulate debris then this should effect the transport of radionuclides in the oceans. Over the past 1000-2000y the climate in many parts of the world has changed dramatically, eg the ruins of Petra (Jordan) and Carthage (N.Africa) illustrate the drastic changes which have occured. For the UK over the past 100y climatic change has given rise to distinctly warmer conditions, the 1800 freezing over of rivers (and coastal waters) no longer occurs. Such events albeit they are slow to take place, will influence the transport of radionuclides which are released into the sea by altering the biological composition of the seas and the concentration of particulate phases. In any realistic planning for future siting of nuclear effluents

it is worthwhile considering such matters, but until we understand more about the processes taking place in the sea today many of the factors used in models are enlightened guesses, but which, to a first order of approximation may be sufficient for our present and immediate needs. Apart from long term climatic change, acute episodes of unusual conditions, eg droughts or high rainfall, can influence the local fate of radionuclides in estuaries and near shore marine ecosystems. In the Irish Sea as most of the Pu and Am is contained in, or associated with, bottom sediments we need to determine whether or not any realistic natural processes can redistribute the radionuclides such that they enter the food chain, or are deposited in tidal regions and impose an unacceptable risk to man from ionising radiation.

4. MICROORGANISMS

Many microorganisms obtain nutrients through uptake of dissolved species across cell walls; the process discriminates against transfer of many radionuclides, especially those of the heavy elements. Other microorganisms are associated with pinocytotic and exocytotic actions and can engulf particulate debris; a large proportion of macro forms derive food by ingestion but the process of food selection is often very specific. An additional process for element uptake is by adsorption onto external surfaces and the process appears to take place whether the animals are dead or alive. Fisher et al (1983), in laboratory experiments, explain the uptake of Pu and Am onto marine phytoplankton by passive adsorption to cell surfaces with equilibrium between cells and water being reached in 3-4 days; uptake was directly proportional to the number of suspended particles and isotopic concentration in the cells. Clifton (1983) confirmed these findings for three species of phytoplankton common to the Irish Sea and hence relevant to the effluent from BNFL. Some bacteria such as Zoogloea ramigera (Dugan and Pickrum 1972) have a high affinity for uptake of Cs, ZnCu, Co and Fe on extracellular polymers, which directly, or as a result of degradation, may alter the chemical form of the elements.

Microorganisms can initiate process of organic degradation within very specialised microenvironments; although the total biomass may be small the rate of recycling may be significant. In the sea while the total biomass of zooplankton may have been decreasing for decades, the importance of other particulate forms, such as protozoa and other small organisms, together with the inorganic carbon resuspended from bottom sediments in disturbed areas (eg estuaries, shelf seas) is poorly known, mainly because of the conventional methods of collection. Therefore it is difficult to determine whether or not there is a net directional loss of particulate (biotic and abiotic) to the bottom sediments or whether the major process concerns continuous recycling in the water column, except for the deep ocean. In shallow inshore waters the role of the meiofauna in the recycling of bottom deposited detritus and associated bioturbation is largely unknown. In laboratory experiments Miramand et al (1982) note high uptake of Pu and Am by the amphipod Corophium v, compared with that for the polychete annelid Arenicola m and the bivalve mollusc Scrobicularia p which is confirmed for natural conditions by Hamilton and Clarke (1984). Uncertainties in the role of microorganisms as they influence transfers and rate of processes in the marine environment is possibly related to uncertainties in the distribution, abundance and biology of such species.

Overall the great areas of uncertainty concern representative sampling of available biological species in the sea, discrete sampling from ships can be misleading, while aerial photography, using satellite images can at least provide information for distributions of some species in surface waters.

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Session : Marine and Estuarine Microorgan.

Paper by : E. Hamilton

Comment by : W. Kühn, FRG

Text of comment
or author's answer :

Could you give a few comments about your Detector ?
Did you use normal Films or Nuclear Trac-Detectors with which one can see direct the α-particles of the Pu ? They have a very characteristic form of absorption with nuclear Trac-detectors.
What was the type of the film ?

The di-electric detector used in this work is CR-39 (polymer of oxydi-2 1-ethane diyl di-2-propenyl ester of carbonic acid). It is a plastic which records the passage of α particles which are then revealed by etching in 6.5 N NaOH at 85°C for 6 h. The process is non-photographic. The technique is discussed in detail in E.T. Hamilton and R.J. Clifton, Int. J. Applied Isotopes and Radiation, Vol. 32, pp. 313-324 (1981).

Session : I/A

Paper by : E. Hamilton

Comment by : L. Rossi

Text of comment
or author's answer :

Is the distribution of radioactivity on the surfaces of a dead leaf homogeneous? Are the distributions of radioactivity among different leaves significantly different ?

The α activity consists of a diffuse and particle distribution. The former is associated with the surface of the actual leaf and the latter with attached hot-particles of fine grained sediment. For tannin rich oak leaves the diffuse distribution of α-activity is greater than that of fresh leaves where the surface cuticle is intact.

Session : I/A

Paper by : Hamilton E.

Comment by : C. Myttenaere

Text of comment
or author's answer :

If we believe in the results presented, "the importance of the surface phenomena may we model the behaviour of the radionuclides as soon as they are fixed on organisms applying a simple dispersing model ?

In relation to the overall flushing of radionuclides on surfaces in an estuary, they are considered to follow a non-conservative pathway hence should be amenable to description by a first order simple dispersion model, that is they will be transported according to particle size, shape and density. Second order times consider any uptake by biota, or preferential description in fine grained sediments which introduce a time related to biological retention and excretion. For an estuary and any associated salt marsh there is a need to consider their biological processes which takes place at the sediment/water interface through resuspension in relation to redox changes. Our shelf seas there is need to consider restricted processes arising from resuspension of bottom sediment especially in areas of upwelling i.e. shelf edge.
Microorganism processes are considered to be important when redox changes occur, i.e. the anoxic to oxic conditions.

Session : I/A

Paper by : E. Hamilton

Comment by : M.A. Abdallah

Text of comment
or author's answer :

Can it not be misleading to use the word "uptake" when cellular accumulation of metal proceeds by adsorption into the cell surfaces?
Generally this word is used for active transport.

I agree. I prefer to restrict "uptake" in relation to biological processes; to indicates the ability of an element to cross cell membranes. For non-translocated radionuclides the term "association" seems more proper ; sometimes radionuclides are adsorbed, entrained or attached to surfaces in active processes such as the presence of ligands which attract by chemical and physical processes.
Unfortunately, the marine literature is repleted with vague terminology : for example filtration through 0.45 μ (or 0.22 μ) filter removes solids ; the term sediment is used without an attention to its composition which often is of paramount importance in the behaviour of element transport and their characteristics. Most people using such terms offer the excuse "we will know what it means", "it is a technical term"...

Session : I

Paper by : E. Hamilton

Comment by : S. Bonotto

Text of comment
or author's answer :

Is any evidence showing a vertical transport of radionuclides from the sediments to the upper layer of the water column?

In estuaries there is transport from sediments to the water column as a result of tidal revert, bioturbation, storms, etc. The magnitude of the transport has a seasonal character.
In shelf seas, especially at the shelf edge recent work indicates restricted transport mediated by a hitherts unrealized high abundance of microorganisms. In the deep seas some restricted transport probably also occurs but with the tendency of non degradable debris to be transported to bottom sediments. In general the geochemical and biochemical behaviour of each radionuclide (i.e. element) has to be considered separately.

LABORATORY STUDIES ON THE ROLE OF MARINE UNICELLULAR ALGAE IN ACCUMULATING RADIONUCLIDES AND TRANSFERRING THEM TO PRIMARY CONSUMERS

Wang Yongyuan, Xiao Yusheng, Zhou Mingjiang and Teng Wenfa

Institute of Oceanology, Academia Sinica, Qingdao, China

ABSTRACT

Laboratory experiments have been carried out on accumulation of ^{65}Zn, ^{60}Co, ^{124}Sb, ^{137}Cs and ^{203}Hg by marine unicellular algae, Phaeodactylum tricornutum, Chlorella sp. and Platymonas sp., and on the transfer of ^{65}Zn from P. tricornutum to Mytilus edulis and the transfer of ^{203}Hg from Platymonas sp. to Artemia salina. The effects of biomass of algae, different concentrations of radionuclide in sea water, simultaneous presence of other nuclide and environmental factors (photoperiod, temperature and salinity) on uptake of the radionuclides for the algae are studied. The relative importance of the food pathway in the uptake of ^{65}Zn and ^{203}Hg for the animals, the transfer coefficients of the nuclides from the algae to the animals and the possibility of bioamplification of the nuclides in the transfer process are also investigated.

INTRODUCTION

Researchers in radioecology pay much attention to marine unicellular algae [1,2,3] both because of their high ability to take up radionuclides from sea water, thus playing an important role in the fate of the radionuclides in the marine environment, and also because of the potential risk of their transferring the radionuclides to primary consumers, therefore transporting the radionuclides into marine food chains and ultimately humans.

The uptake of radionuclides by marine organisms is affected by many biological, chemical and environmental factors[3]. So it is necessary to investigate the effects of the factors such as biomass of algae, concentration of radionuclides, photoperiod, temperature, salinity etc. in examining the role of marine unicellular algae in accumulating radionuclides from sea water.

To assess the role of marine unicellular algae in transferring radionuclides to primary consumers, three aspects should be considered: the relative importance of food (algae) and sea water in uptake of the nuclides for the consumers, the transfer coefficient from the algae to the animals and the possibility of bioamplification of the radionuclides through the transfer process.

In this paper, several accumulation experiments of radionuclides in marine unicellular algae subjected to different factors, and two transfer experiments concerned with the three aspects mentioned above are reported, and the role of marine unicellular algae in accumulating and transferring radionuclides are discussed.

MATERIALS AND METHOD

I. Materials

1. Unicellular algae: *Phaeodactylum tricornutum*, *Chlorella* sp. and *Platymonas* sp. were provided by our institute.
2. Filter-feeder (primary consumers): *Mytilus edulis*, 5-6 cm long, were collected from Jiaozhou Bay, Qingdao; *Artemia salina*, with an average length of 1.44 mm were incubated from commercial eggs in our laboratory.
3. Radionuclides: $^{65}ZnCl_2$, $^{60}CoCl_2$, $^{124}Sb(NO_3)_2$ and $^{137}CsCl$ were bought from the Institute of Atomic Energy, Academia Sinica; $^{203}HgCl_2$ was purchased from Amersham International, England.

4. Instruments: a SA-41 spectrometer (made in France), an N-530G scaler (made in England) and a FH-408B scaler (made in China) were used to detect radioactivities.

II. Method

1. Experiments on accumulation: Marine unicellular algae were separately cultured in sea water labelled with ^{65}Zn, ^{203}Hg or ^{124}Sb alone and with ^{60}Co + ^{137}Cs or ^{65}Zn + ^{203}Hg combined, and were subjected to different affecting factors (see Table 1).

TABLE 1. Experiments on Accumulation

Algae	Radionuclide	Affecting Factor
Phaeodactylum tricornutum	^{65}Zn	Biomass of algae (8, 5, 2 x 10^6 cells ml^{-1})
P. tricornutum	^{124}Sb	Concentration of radionuclide (0.711, 0.295, 0.071 μci l^{-1})
P. tricornutum	^{65}Zn + ^{203}Hg	Interaction between nuclides
Platymonas sp.	^{203}Hg	--------
Chlorella sp.	^{60}Co + ^{137}Cs	Photoperiod (24, 12, 0 hr illumination)
Chlorella sp.	^{60}Co + ^{137}Cs	Temperature (35, 28, 21°C)
Chlorella sp.	^{60}Co + ^{137}Cs	Salinity (35, 30, 20°/oo)

The algae, were examined periodically to determine the radioactivities accumulated. The ability of algae to take up radionuclides was expressed as C.F. (concentration factor) or radioactivities per gram of the algae (both were calculated in wet weight).

2. Experiments on transfer: Each transfer experiment (^{65}Zn from *P. tricornutum* to *M. edulis* and ^{203}Hg from *Platymonas* sp. to *A. salina*) consisted of two groups, in one of which the animals were exposed to radioactive sea water only, and in another to labelled food (algae) only, so that the relative importance of food and water in uptake of radionuclides by the animals could be examined clearly. Each transfer experiment was conducted over 36 h.

The transfer coefficients of ^{65}Zn and ^{203}Hg from algae to the consumers and the ratios of the nuclide contents in the animals to that in algae were calculated from the group exposed to labelled food only.

RESULTS AND DISCUSSION

I. Experiments on Accumulation of radionuclides

1. Effects of biomass of Phaeodactylum tricornutum on uptake of ^{65}Zn by the algae and on retention of ^{65}Zn in experimental sea water are shown in Fig. 1.

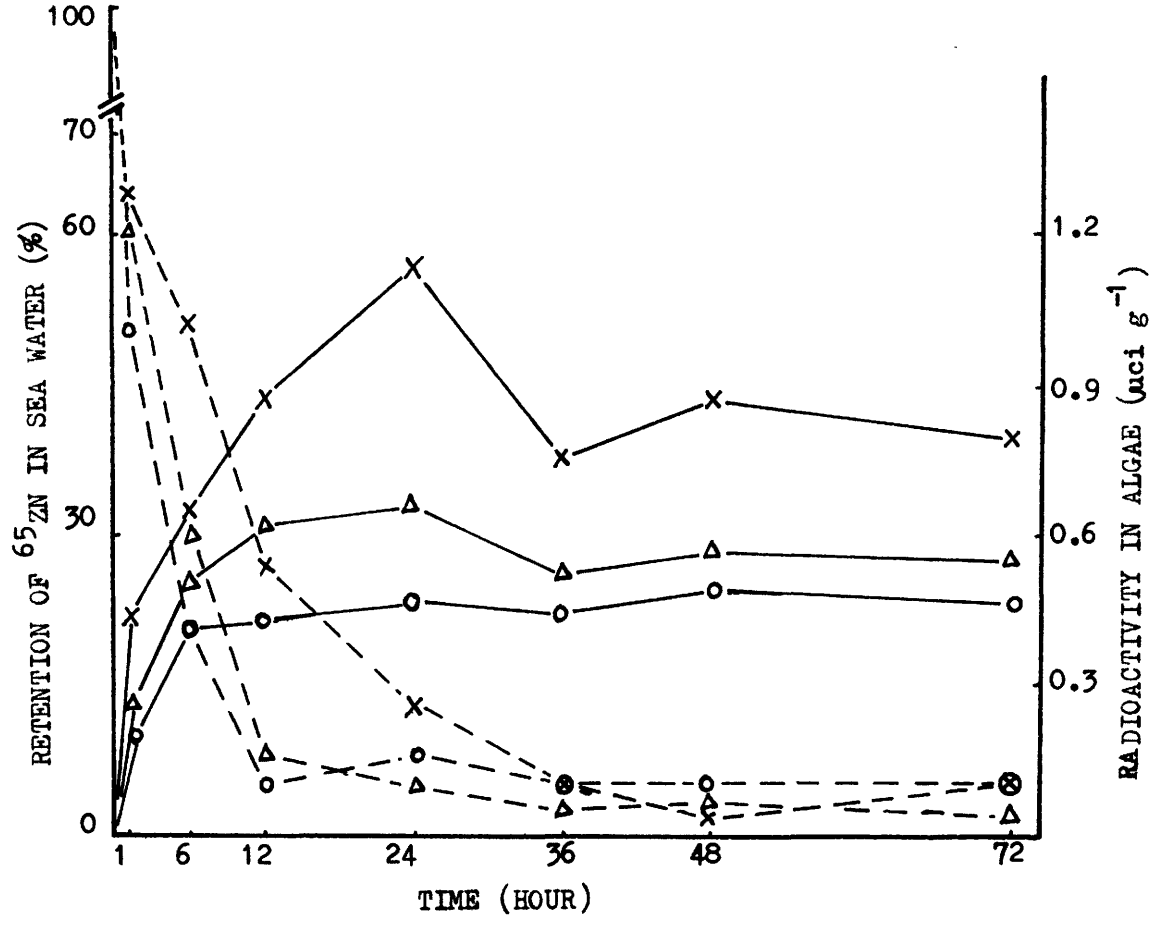

FIGURE 1 Phaeodactylum tricornutum. Effects of biomass on uptake and retention rates of ^{65}Zn. (x _____ x & x ----- x 200, Δ _____ Δ & Δ ----- Δ 500, o _____ o & o ----- o 800, $x10^4$ cells ml^{-1})

The uptake of ^{65}Zn by P. tricornutum and the retention of ^{65}Zn in sea water were both inversely proportional to the cell density of the alga.

P. tricornutum had a high ability to take up ^{65}Zn like Nitzschia closterium[1], the concentration factors are over 1×10^4 (after 24 hours)

for all three groups (200, 500 and 800 x 10^4 cells ml^{-1}). The concentrations of ^{65}Zn in the water dropped so much that it could hardly be detected after 24 hours.

Even though the biomass of marine unicellular algae in the natural environment is much lower than in our experiments, the role of marine algae to remove radionuclides from sea water may still be very important because of their short alternation of generations.

2. Effects of variations in concentration of ^{124}Sb on uptake of it by P. tricornutum are shown in Fig. 2.

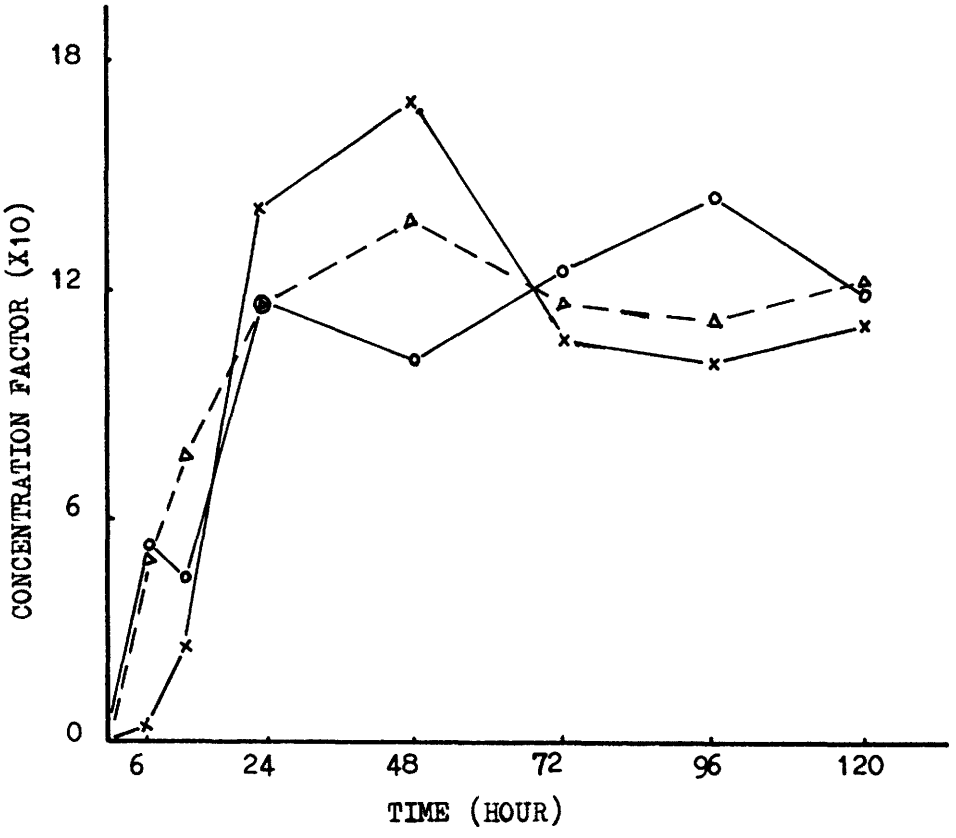

FIGURE 2 Phaeodactylum tricornutum. Effects of different concentration of ^{124}Sb on uptake of the radionuclide. (x _____ x 0.071, o _____ o 0.295, Δ ----- Δ 0.711 μci l^{-1})

The variations in concentration of ^{124}Sb in sea water (0.071, 0.295 and 0.711 μci l^{-1}) had no effect on the uptake of the nuclide for the algae, with final concentration factors of 112.2, 119.3 and 121.3, correspondingly.

3. Effects of interaction between ^{65}Zn and ^{203}Hg on uptake by P. tricornutum are shown in Fig. 3.

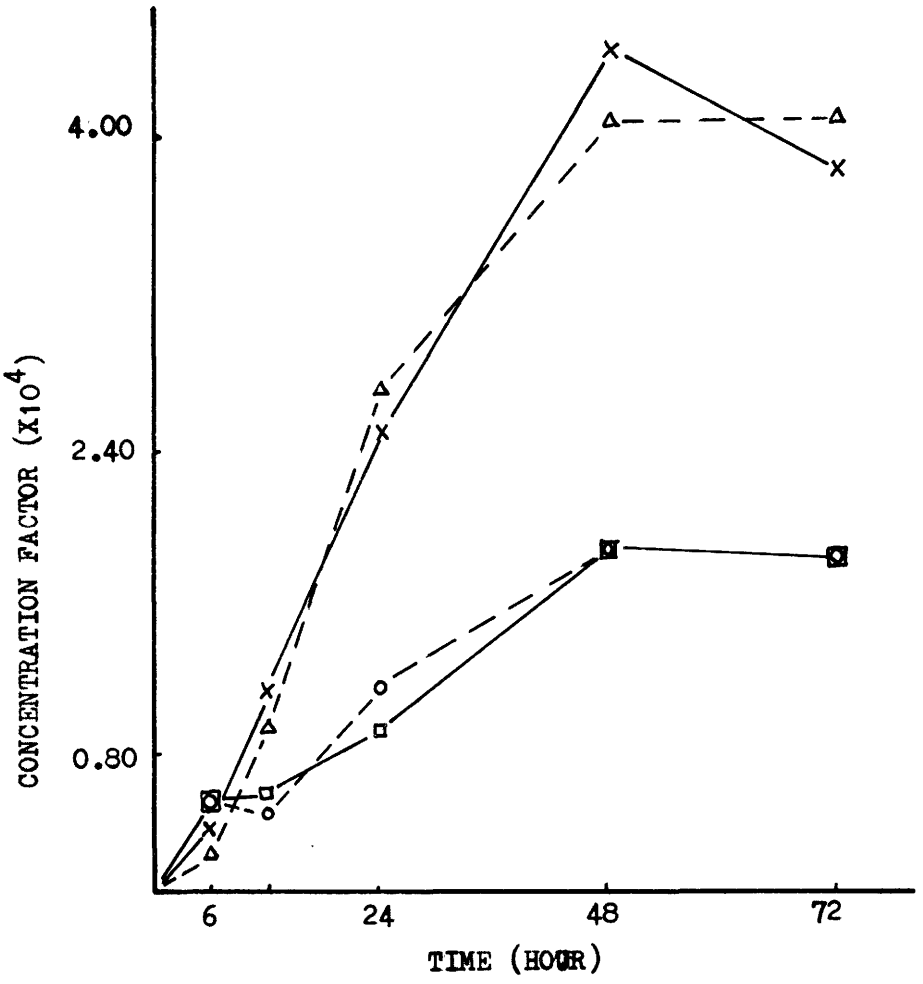

FIGURE 3 Phaeodactylum tricornutum. Effects of interaction between ^{65}Zn and ^{203}Hg on uptake of them from the algae. (Δ ----- Δ Zn in mixture, x _____ x Zn singly, o ----- o Hg in mixture, □ ——— □ Hg singly)

There is neither synergism nor antagonism between ^{65}Zn and ^{203}Hg. The presence of zinc does not affect the uptake of mercury and vice versa.

Zn, Hg, Cu and Cd are major heavy metal pollutants in the marine environment. Previous researches indicated that there was synergism between Zn and Cu[4] and antagonism between Hg and Cd[5]. Our experiments, however, imply that there were no such phenomena between Zn and Hg at least for P. tricornutum.

4. Effects of photoperiod, temperature and salinity on uptake of ^{137}Cs and ^{60}Co by Chlorella sp. are listed in Table 2.

TABLE 2. Concentration factors (C.F.) for ^{137}Cs and ^{60}Co in Chlorella sp. under different environmental conditions*

Environmental Conditions	Concentration Factors	
	^{60}Co	^{137}Cs
Photoperiod (hr illumination)		
24	1138	5.33
12	1021	3.80
0	26.6	0.31
Temperature (^{o}C)		
35	644	3.49
28	540	3.27
21	515	2.68
Salinity ($^{o}/_{oo}$)		
35	622	1.33
30	635	1.60
20	442	2.63

*Values of C.F. listed are on the fifth day of the experiments

Longer photoperiods can enhance the accumulation of both ^{137}Cs and ^{60}Co by Chlorella sp., which confirms the conclusion of Chu[6] in his experiments with ^{32}P. The increased accumulation of ^{60}Co with higher temperatures by the algae is to be expected as higher temperatures speed up the metabolic rate of ^{60}Co, a necessary element for marine algae[7]. The reason that higher temperature enhances uptake of ^{137}Cs is not obvious, because no biological functions of ^{137}Cs have been found so far[8]. The slower uptake of ^{137}Cs at higher salinities may be due to the relatively higher content of potassium, a competing element to caesium[9], in the sea water at a higher salinity.

The effects of salinity on uptake of ^{60}Co are somewhat irregular and the causes need further investigation.

5. The role of marine algae in removing radionuclides from sea water can be expressed by the formula

$$R = \frac{1}{1+CF \cdot W} \times 100\%$$

where, R: retention of radionuclides in sea water

W: biomass of algae in grams per ml

C.F.: concentration factor

Our experiments show that the formula can predict the retention of ^{60}Co in the experimental sea water quite well (see Fig. 4).

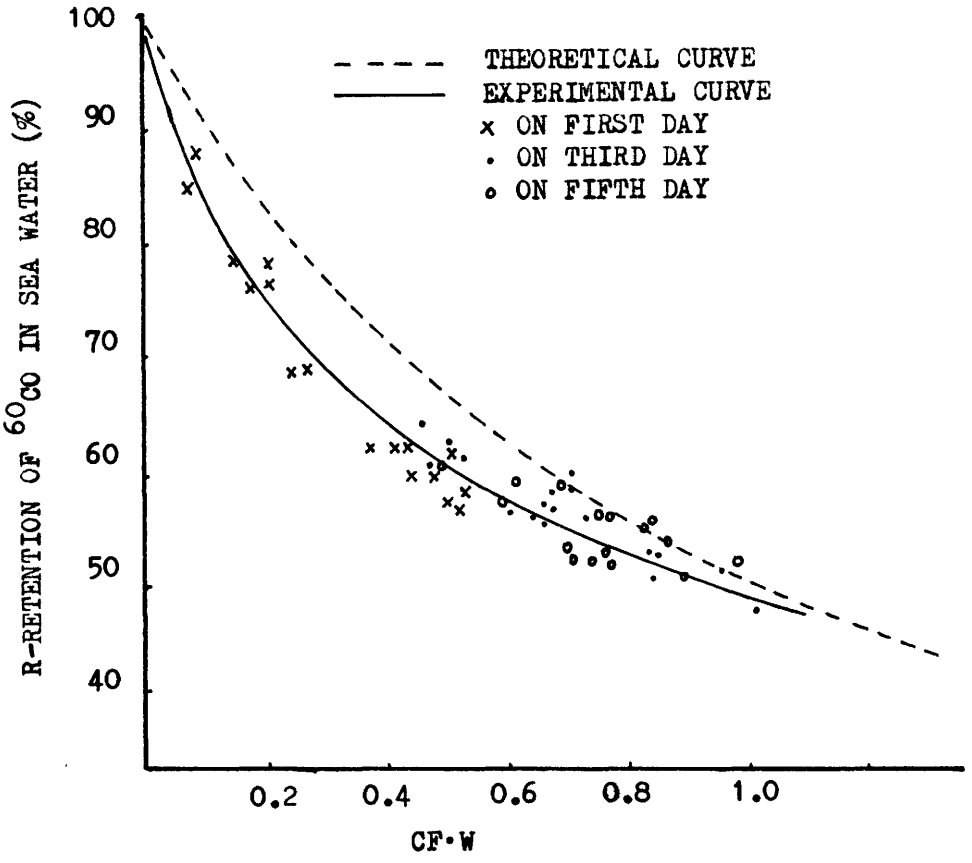

FIGURE 4 Chlorella sp. The relation between retention rate of ^{60}Co in sea water and concentration factors and the biomass

Our formula is rewritten from Polikarpov's formula[1] which is used to calculate how much radionuclide in sea water is accumulated by marine organisms, while our formula is used to indicate how much of the radionuclide remains in the sea water, and has a simpler form.

II. Experiments on transfer of radionuclides

The results of experiments on transfer of ^{65}Zn from P. tricornutum to Mytilus edulis and ^{203}Hg from Platymonas sp. to Artemia salina are shown in Table 3.

TABLE 3 The transfer of ^{65}Zn and ^{203}Hg from algae to primary consumers

Radionuclides	^{65}Zn	^{203}Hg
Algae	P. tricornutum	Platymonas sp.
Primary consumers	M. edulis	A. salina
Radioactivity accumulated from algae by consumers ($x10^{-3}$ µci g^{-1})	18.32	63.0
Radioactivity in medium to which consumers exposed ($x10^{-3}$µci l^{-1})		
algal solid[a]	4.6	32
dissolved[b]	331.2	35
dissolved/algal solid	72.0	1.09
Transfer coefficient, algae→consumer (%)	14.4	9.9
Ratios of radioactivities in consumer to that in algae	0.019	0.011

[a]Concentration of radionuclides associated with algae

[b]Concentration of radionuclide in seawater (calculated from the group exposed to labelled seawater) required to achieve the same activity in consumers as that derived from algae[10]

Young[11] reported that food was a more important source of ^{65}Zn in molluscs than water. However, Renfro[12] indicated that uptake of ^{65}Zn from food did not prevail over uptake from water in crustaceans. Our experiments prove that for the mollusc, Mytilus edulis, uptake of ^{65}Zn from food is more important than from water (dissolved: algal solid=72.0, see Table 3) and for uptake of ^{203}Hg in the crustacean, Artemia salina, accumulation from water or food are both equally as important (dissolved: algal solid=1.09).

Table 3 also illustrates that the transfer coefficient of ^{65}Zn from P. tricornutum to M. edulis (14.4%) does not differ greatly from that of ^{203}Hg from Platymonas sp. to A. salina (9.9%) regardless of the remarkable difference between the relative importance of the food pathways. The values of both transfer coefficients are comparable with those of Cd, 10%[13] and V, 7%[14], but much lower than that of methyl mercury, 70-90%[15].

Hg is commonly considered to be bioamplified through marine food chains[16]. Our results, however, indicate inorganic mercury is unlikely to be amplified through the food chain at least from Platymonas (algae) to Artemia (primary consumer). The phenomenon that the organisms at higher

levels of the food chain have more mercury than those at lower levels is probably due to the direct uptake of mercury from sea water by the animals [15] or due to the amplification of methyl mercury, which is proved to have a higher transfer coefficient[15], through marine food chains.

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Session : I/A

Paper by : M. Zhou

Comment by : N. Fisher

Text of comment
or author's answer :

Two questions :
a. How did you calculate concentration factors, if the dissolved Zn decreased toward zero often a day or two? That is, at equilibrium, what value do you use as a denominator?
b. What molar concentrations of Zn and Hg were used in the Zn/Hg interactions experiments ? If the atom levels were very low, one would not expect any competitive binding interactions, and this may explain your failure to detect such an interaction.

a. We use the concentrations of Zn in sea water at the moment of equilibrium to calculate the concentration factors.

b. May be, it is an explanation to the results in our experiments on the interaction between Zn and Hg. But, for Hg, the atom levels are quite higher (5 ppb) than that in normal marine environment (< 0.5 ppb), so we think our results may be more practical to explain the situation in the nature.

Session : I/A

Paper by : M. Zhou

Comment by : S. Bonotto

Text of comment
or author's answer :

Work on interaction between radionuclides is important because in nature "multielement" pollutions occur. Are you studying also interactions between other radionuclides?

No.
All we have done on the interaction between radionuclides is between Zn and Hg so far. But we will work on others in the future.

Session : I General Discussion

Paper by : E.J.Hamilton

Comment by : C.Myttenaere

Text of comment
or author's answer :

I wonder if the problem has not to be considered in the following way : the column of water. The microorganisms playing a limited role. The surface properties being responsible of the behaviour of the radionuclides. The sediments in which the living organisms could play a role based on their metabolism (biological oxidation, reduction modification of the chemical forms).

I agree. However, we know so little of the role of microorganisms in the seas ; at time of the year they can be present in large numbers and together with waste products and dead cells thus may be important for some elements. In sediments, especially the sediment/water interface release conditions and thus changes can be important, especially for fine grained organic rich sediments. However, with present knowledge, I believe that the surface properties of microorganisms are more important than their biological and biochemical properties.

Session : I/A

Paper by : General discussion

Comment by : A. Cremers

Text of comment
or author's answer :

One of the points which has been emphasized in this morning discussion is the importance of physico-chemical effects (adsorption versus absorption). A comment I would like to make is that CF values as such register some form of steady state of what is obviously a very dynamic system. Whether we are in effect dealing with adsorption equilibria should preferably be controlled by reversibility tests.

Session : I/A

Paper by : General Discussion

Text of comment
or author's answer : E.K.Duurma, Chairman

1) To Fisher : For those radionuclides, which have a stable counterpart, could you comment on the partition coefficient (= concentration factor) for the distribution of the stable counterparts between plankton and sea water, based on literature data ?

Culture experiments generate concentration factor data comparable with those which can be calculated mainly reliable stable element data for plankton (e.g. Martin and Knauen) and for sea water (e.g. Bruland, Patterson, Edmond, etc.). Moreover, the fractionation of ^{241}Am in my laboratory research using natural plankton communities was nearly identical to measurements of Holm and Fukai who examined natural ^{241}Am in Mediterranean particulates. This similarity holds even though the radioisotope culture experiments utilized ^{241}Am concentrations 9 orders of magnitude greater than natural ^{241}Am concentrations.

2) There has been a question on the pH effect on the concentration factors and I would like to comment on this. Any pH change will for sea water affect the solubility product of the radionuclides involved and thus, as Dr Fisher has shown, the concentration factors.

3) Another point has been mentioned concerned the chelation or complexing by dissolved organic ligands. We should realize that for sea water many situations are different than those in fresh water. It is easily possible to calculate that, with the major presence of Ca and Mg in sea water, there is a dominant competition, which rarely allows trace elements to be complexed. Cu may, as is well documented, occur in chelated form, but trace elements with lower stability constants than Cu, rarely do.

MECANISMS OF RADIOECOLOGICAL PROCESSES, THEIR MODELS AND RADIATION EFFECTS IN THE HYDROBIONTS OF THE BLACK SEA.

G.G.Polikarpov, V.N.Egorov, A.Ja.Zesenko, V.G.Tsytsugina, L.G.Kulebakina, N.S.Risik, D.D.Ryndina, G.E.Lazorenko, L.A.Radchenko, I.I.Rudneva, A.V.Parkhomenko, N.V.Demina, S.M.Fedorik

Institute of Biology of South Seas, Sevastopol, 335000, U.S.S.R.

ABSTRACT

At the present stage of the radioecological investigations the Department of Radiation and Chemical Biology (Institute of Biology of South Seas, Acad.Sci.Ukr.SSR) pays the main attention to generalized criteria (ecosystems reliability, zonality of the dose rates field of the ionizing radiations a.s.o.), to determination methods of the marine ecosystems state, to the dynamic models of ecological capacity in relation to radionuclides, to biochemical and physio-chemical mechanisms of the radionuclides fates as well as ionizing radiations effects.

Proposed by us the dose field structure receives the furthur substantiation. The good coincidence of the results of emphirical determination of the zones boundaries with the results of the calculation of these boundaries has been shown by the application of the universal step of the organization and periodicity of the critical states in the living nature development equaled to e^e (Zhirmunsky and Kuzjmin, 1982) to mentioned above dose field structure.

The mathematical model of the self-purification of the ocean photic layer against radioactive and chemical pollutions. Elaboration and application of the radioecological integral method by the disequillibrium of ^{238}U-^{234}Th allowed to determine the natural production of suspension and to estimate the flux of the suspension outflow into the aphotic zone. Without such data calculations of the purification rates of the different regions in the ocean against radioactive and chemical contaminants are impossible.

Cytogenetic and biochemical research in the pure and contaminated regions showed that chromosomes aberrations and protein composition changes are appeared in crustaceans Gammarus marinogammarus olivii as well as processes of the adaption to new conditions are occured.

Long-term dynamics of the decrease of the radioactivity levels in the international zone of the Black Sea near the Danube delta has been proved. A character of the atoms microdistribution of the natural uranium in marine organisms has been revealed.

Distribution of many metals radionuclides in biochemical fractions of the green and brown algae as well as ionizing radiations influence upon this distribution and upon viscosity parameters has been shown in the experimental conditions. The phenols role in element uptake by marine sea-weeds

has been established.

Radiation effects have been described at the cell, organismic and population levels of organization of the Black Sea hydrobionts. The effective doses low level for Artemia salina was established (up to 5 Gray) and the combination of the dose of gammaradiation, temperature and salinity, which stimulate of survival, growth and development of this crustacean species was found out.

Estimation of the water-bodies ecological capacity in relation to radioactive and chemical pollutants is provided as well as the idea of the marine ecosystems reliability under the antropogenic action is elaborated.

REMARQUES SUR LES PROBLEMES POSES PAR L'INCIDENCE DES MICRO-ORGANISMES DU MILIEU (SOLS, EAUX) SUR LES TRANSFERTS DES TRANSURANIENS DANS L'ENVIRONNEMENT. CONSEQUENCE POUR LE CALCUL DE LA DOSE PAR INGESTION CHEZ L'HOMME

R. BITTEL

Institut de Protection et de Sûreté Nucléaire, Département de Protection Sanitaire, Service d'Etudes Appliquées de Protection Sanitaire, BP n° 6 - 92260 Fontenay-aux-Roses (FRANCE)

1. INTRODUCTION

La vie microbienne des milieux physiques, des chaînes alimentaires et du milieu organisé a une incidence certaine sur les transferts des éléments et de certains radionucléides (^{99}Tc par exemple)[1]. Il est donc nécessaire de tenter une quantification en ce qui concerne le cas des plutoniums et du neptunium - 237. Il s'agit en effet de radionucléides dont l'absorption intestinale donne lieu actuellement à de vives discussions, en raison de l'incertitude concernant le paramètre f_1 (coefficient de transfert intestinal) en fonction des états de valence, des formes physico-chimiques et des "effets de masse"[2-3-4].

2. RAPPELS PHYSICO-CHIMIQUES

2.1 Neptunium

Dans les solides[4], en particulier les roches consolidées, Np est à l'état tétravalent sous forme d'oxyde NpO_2, homologue de UO_2, de PuO_2 et d'autres oxydes de terres rares avec lesquels il réalise des solutions solides dont le comportement physico-chimique global est imposé par

l'oxyde dont la proportion est quantativement dominante. En solution[4], la valence la plus stable est la valence V à laquelle correspond l'ion monovalent NpO_2^+ dont le comportement s'écarte des autres transplutoniens et se rapproche de celui des ions Na^+, Ca^{2+}, UO_2^{2+}, Sr^{2+}.

2.2 Plutonium

Les plutoniums envisagés sont : ^{239}Pu, ^{238}Pu et ^{237}Pu. Il s'agit d'émetteurs alpha d'activités spécifiques croissantes. On a longtemps admis que les valences les plus stables correspondaient à Pu (IV) et à Pu (III). Il semble que les valences V et VI puissent exister en solution (PuO_2^+ et PuO_2^{2+}) en équilibre favorable avec les autres formes, si le milieu est oxydant[5] et si l'activité alpha n'est pas trop élevée (cependant des solutions de PuO_2^+ de concentration 10^{-2}M sont stables pendant quelques jours)[6].

3. RESISTANCE DES MICRO-ORGANISMES AUX RAYONNEMENTS ALPHA ET A DIVERSES CONTRAINTES EXTREMES :

L'intervention des micro-organismes dans la géochimie de l'uranium[7-8] et dans la bio-lixiviation des minerais suppose une certaine résistance aux toxicités chimiques de U et de Ra d'une part, et une suffisante résistance aux rayonnements, en particulier α. En milieu marin et estuarien, différentes souches de levures résistantes au plutonium 237 (Pu d'activité massique élevée) ont été mises en évidence. De même, des actinomycètes résistant dans le sol aux dérivés solubles de Pu IV[9] ont été trouvés. Cependant, les micro-organismes hétérotrophes utilisant comme source d'énergie les matériaux d'enrobage de déchets alpha ne paraissent avoir qu'une résistance réduite. Il faut également signaler que la destruction de certaines matrices organiques des déchets libère du desférol, qui avec Pu (IV) donne des complexes solubles[10]. La toxicité vis à vis des micro-organismes serait imputable plus aux effets de l'irradiation qu'à des phénomènes de toxicité chimique, ce qui mériterait d'être précisé expérimentalement. Divers micro-organismes présents dans des eaux minérales naturelles résistent à des températures relativement élevées et à des pH très bas (bactéries thermo-acidophiles)[10]. D'autres sont barophiles, susceptibles de subsister à plusieurs centaines d'atmosphères et à 4°C[11].

Ces exemples suggèrent une survie active de certaines formes microbiennes dans les conditions extrêmes réalisées au voisinage des déchets dans les formations géologiques profondes (continentales et océaniques)[11-12].

4. CONTRAINTES DU MILIEU VIS A VIS DES TRANSURANIENS

Le développement de micro-organismes dans le milieu tendent à modifier les caractéristiques de ce dernier, en particulier le pH, le redox, la complexation, les équilibres hétérogènes entre phases solides et phase liquide. Bien que le milieu soit tamponné vis à vis de ces actions, l'incidence sur le comportement du plutonium et du neptunium ne doit pas être négligé à priori.

4.1 pH

Les modifications du pH du milieu (origine microbienne) résultent d'un grand nombre d'actions dont l'ampleur est souvent limité par le pouvoir tampon.

Vont dans le sens d'une acidification, la libération de gaz carbonique (respiration), la production d'acides organiques, l'oxydation du soufre et des sulfures en sulfates, des composés azotés en nitrates, la "fixation" de l'azote gazeux, la formation d'acides humiques et fulviques (sols et eaux). Vont dans le sens d'une alcalinisation, la sulfatoréduction et la nitratoréduction. Un exemple en est l'acidification de la rhizosphère (- 0,5 unité pH) qui conduit à une mobilisation accrue d'un facteur 10 des ions alcalins-terreux[13]. En ce qui concerne les dépôts de déchets alpha dans des formations terrestres profondes, la résistance de certains micro-organismes aux conditions extrêmes et la faiblesse du pouvoir tampon du milieu accepteur doit inciter à étudier ces problèmes cas par cas.

4.2 Complexations

La vie microbienne du milieu produit différents composés qui sont susceptibles de complexer ou de chélater les transuraniens. C'est ce qui existe en particulier au voisinage immédiat des racines[14]. Dans les tissus des végétaux en place, les transuraniens migrent et se localisent dans

certains organes de la plante, en fonction d'équilibres physico-chimiques propres à chaque espèce végétale et à chaque type cultural. Les ensilages qui servent d'aliments aux animaux de ferme sont le siège de phénomènes bactériens[15] qui, en en modifiant les caractéristiques édaphiques générales, ont une incidence certaine sur la complexation des éléments traces et donc de leurs transferts dans la chaîne alimentaire.

4.3 Redox

Dans le milieu naturel, le potentiel d'oxydo-réduction résulte d'équilibres qui mettent en cause les macro-éléments, ces derniers imposent alors les niveaux des potentiels d'oxydo-réduction. D'autre part, le potentiel d'un système redox se trouve modifié du fait d'une complexation.

Un aspect très important de l'oxydo-réduction est, pour les transuraniens, la dismutation. Il est essentiel de noter que, pour Pu V, Pu VI, Np V, la dismutation implique un remaniement moléculaire avec une cinétique lente. Il faut, en outre, souligner que dans les conditions naturelles, les variations de pH et celles des potentiels d'oxydo-réduction ne sont pas indépendantes. Par exemple, dans les rizières irriguées par submersion, le potentiel d'oxydo-réduction diminue et parallèlement le pH du sol inondé augmente[17].

4.4 Equilibres hétérogènes (phase liquide/phases solides)

Les équilibres hétérogènes revêtent des aspects variés. En se limitant aux aspects dépendant de la microbiologie, il faut citer :

- la complexation sous formes anioniques ou non chargées, non sorbées par les colloides électronégatifs du milieu ;
- l'oxydation jusqu'à des formes cationiques sorbées par les colloides du milieu ;
- l'oxydation microbiologique conduisant à des formes peu solubles ou à des colloides polymères neutres ou électropositifs retenus par les colloides du milieu (filtration ou sorption).

Les micro-organismes du milieu tendent eux-mêmes à se sorber aux

frontières entre les milieux solides et la phase liquide : c'est donc au voisinage des interfaces que ces phénomènes sont les plus intenses. Les ions PuO_2^+, PuO_2^{2+}, NpO_2^+ entrent en concurrence avec les cations monovalents et bivalents dominants par leur masse dans le milieu : la sorption de ces transuraniens se trouvera fortement diminuée du fait de cette concurrence.

5. STABILISATION DES VALENCES PAR LA VIE MICROBIENNE DU MILIEU

Les états de valence différents des transuraniens et leurs équilibres dépendent de divers facteurs. En ce qui concerne les facteurs microbiologiques, on peut distinguer à priori :

- les facteurs thermodynamiques déterminés par les pH, les niveaux d'oxydo-réduction et les complexations ;
- les facteurs cinétiques prenant en compte les vitesses de réaction (équilibres entre les formes ioniques simples et formes complexées ou covalentes, "solubilisation" d'oxydes du fait d'une complexation) et l'incidence de l'effet de masse[18].

Les ions PuO_2^+, PuO_2^{2+} et NpO_2^+ se comportent de manière assez voisine vis à vis des divers facteurs microbiologiques du milieu :

. ils existent dans de vastes zones de pH et d'oxydo-réduction,

. ils sont des ions oxygénés complexes mono et bi-valents ; par suite, ils se complexent peu avec d'autres anions minéraux et organiques. Leur stabilité intrinsèque et la lenteur des réactions covalentes d'oxydo-réduction défavorisent le passage vers d'autres valences ioniques. Il semble donc que, vis à vis de ces trois formes ioniques, les facteurs microbiologiques n'aient qu'une action très lente. Il est possible de penser qu'il ne s'agit pas d'une action stabilisatrice, mais plutôt d'une tendance à la déstabilisation, elle-même très lente. En absence de données expérimentales, le problème reste entier.

Il est clair que si la valence IV du plutonium se trouve stabilisée, c'est à l'état des complexes et en particulier de complexes solubles dans

les conditions naturelles. Il importe de souligner que l'action complexante des composés résultant de la vie microbienne répartit ses effets sur les différents cations présents dans le milieu en fonction et des masses respectives et de la stabilité des différents complexes possibles : sauf cas particulier (concentrations relatives en transuraniens, fortes intensités de vie microbienne aux limites des résistances au rayonnement alpha, concentrations ioniques faibles en macroéléments et oligoéléments), l'incidence de ce phénomène de complexation est peut-être beaucoup moins intense que prévu ; cependant, il est probablement important dans les cas de milieux pauvres en cations mobiles et localement au voisinage des racines. Dans ce cas, il peut rendre compte d'une augmentation significative d'une absorption racinaire.

6. TRANSFERT VERTICAUX DANS LE MILIEU

Il peut s'agir de mouvements descendants en milieux lacustres et marins en association avec la migration des détritus biologiques, de mouvements descendants ou ascendants dans les sols, les sous-sols et les formations géologiques. Des mouvements ascendants susceptibles de se produire en milieu marin doivent également être pris en considération (remontée en association avec des microbiontes adaptés au gradient d'oxydoréduction. Actuellement, les divers problèmes sont seulement posés.

7. CONCLUSION

Les mobilités des diverses formes du plutonium se rangent de la manière suivante : $PuO_2^+ \geqq PuO_2^{++} \geqq$ Pu IV complexé > Pu^{3+} >> Pu (IV) >> oxydes divers. En ce qui concerne Np, l'ordre semble : NpO_2^+ > NpIV et VI compléxés >> autres formes.

Il semble donc, que, par la production de substances complexantes, par des modifications de pH et de redox, la vie microbienne augmente la mobilité des plutoniums et du neptunium - 237. En absence de données expérimentales permettant de mieux préciser quantativement les facteurs de transferts dans ces différentes conditions biophysicochimiques, il est possible de penser que le facteur d'erreur dû à ces phénomènes est du même ordre de grandeur que les facteurs d'erreurs acceptés actuellement dans le calcul des conséquences sanitaires.

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ROLE OF UNICELLULAR ALGAE IN THE BEHAVIOUR OF TRITIUM IN THE AQUATIC SYSTEMS

G. Arapis, G. Nuyts, A. Bossus, S. Bonotto, G. Koch, G.B. Gerber and R. Kirchmann

Département de Radiobiologie, C.E.N.-S.C.K., B-2400 Mol, Belgium

Unicellular algae, which are ubiquitous organisms and thus present also in waters near nuclear facilities, may play a significant role in the behaviour of tritium in the aquatic systems. It is known that these photosynthetic organisms incorporate tritium from tritiated water (by splitting of the water molecules) into their organic matter. Once organically bound, tritium may be eventually transferred to higher trophic levels in the food chain.

Three unicellular green algae (*Acetabularia acetabulum*, *Chlamydomonas reinhardi* and *Dunaliella bioculata*) were grown in culture medium added with tritiated water or with tritiated precursors. The incorporation of tritium into the organic matter was studied by micro-combustion analysis. Its distribution, at the molecular level, was studied by extraction procedures as well as by column chromatography and electrophoresis. It is of interest to note that microalgae, grown in tritiated water, may excrete part of the fixed tritium into the external medium, in organic form. Moreover, organic tritium may be transferred from the algae to animals (fresh water and sea water mussels) which use them as food. The retention of tritium in the mussels was studied by transferring labeled animals into non-radioactive medium.

INTRODUCTION

Aquatic microorganisms (algae, bacteria, protozoa, etc.), which are ubiquitously distributed in the ecosystems, and thus present also near the sources of tritium release, would incorporate this radionuclide in their organic matter. Tritium metabolism was so far studied mainly in photosynthetic bacteria and in unicellular algae growing actively (see recent literature in ref.1). Although tritium is generally not concentrated by freshwater or by marine algae[1,2], some exceptions are known. In *Chlorella pyrenoidosa*[3] some compounds related to the tricarboxylic acid cycle had R values in the range 0.9-1.05 and citrate had a R value of 1.81 in *Anacystis nidulans*[4].

In previous papers[1,5,6] we have studied the incorporation of tritium in several unicellular algae and a simple model to explain its transfer into the organic compartments of the cells has been conceptualized[6]. The distribution of tritium into the various cell components was studied by selective extraction procedures as well as by polyacrylamide gel electrophoresis[1,6]. In this paper, we summarize the most significant recent results on incorporation and biological effects of tritium, obtained with three unicellular green algae (*Acetabularia acetabulum*, *Chlamydomonas reinhardii* and *Dunaliella bioculata*) grown under laboratory conditions. Moreover, some preliminary results on transfer of tritium through an experimental aquatic food chain are briefly reported and discussed.

2. INCORPORATION OF TRITIUM

Incorporation of tritium was studied on three unicellular algae (*Acetabularia acetabulum (= A. mediterranea)*, *Chlamydomonas reinhardii* and *Dunaliella bioculata*) which were used also in previous investigations on tritium metabolism[1,5,6,7,8]. The algae were grown in culture medium added with a high concentration of tritiated water(207.9 μCi ml^{-1}) or with ^{3}H-leucine (3.2 - 5.6 μCi ml^{-1}). After repeated washing with normal medium, the algae were extracted 3 min. at 100°C (boiling water) with 2% SDS (sodium dodecyl sulphate) dissolved in Tris buffer (62 mM Tris-HCl,5% β-mercaptoethanol and 8 mM Na_2-EDTA, pH 6.8). The amounts of tritium incorporated into the extracted proteins are reported in table 1 and table 2. The results show that it is necessary to utilize much more tritium (37-65 times more) in the form of tritiated water than in organic form (^{3}H-leucine) to obtain specific activities of about the same order of magnitude. The extracted proteins were successively analysed by molecular sieving chromatography on Sephacryl S-300 (Pharmacia) gel. This method has revealed the presence of labeled proteins of different molecular weight (ranging from about 180 to 15 kdaltons) and of small peptides (results not shown).

As reported in a previous paper[1], labeled proteins were found in the external medium of *Chlamydomonas reinhardii* CW 15^+ cultures, a finding which suggest that polypeptides may be released by algal cells. Analysis by polyacrylamide gel electrophoresis has shown the presence of two major bands, corresponding to proteins having respectively a molecular weight of 42 and 14.5 kdaltons.

TABLE 1. INCORPORATION OF TRITIUM INTO THE PROTEINS EXTRACTED FROM *ACETABULARIA*, *CHLAMYDOMONAS* AND *DUNALIELLA*, GROWN IN THE PRESENCE OF TRITIATED WATER (207.9 μCi/ml).

Alga	Proteins (mg)	Radioactivity (nCi)	Spec. Activity (nCi mg^{-1})
Acetabularia acetabulum	2.02	639	316
Chlamydomonas reinhardii	10.3	1617	157
Dunaliella bioculata	4.42	744	168

TABLE 2. INCORPORATION OF TRITIUM INTO THE PROTEINS EXTRACTED FROM *ACETABULARIA*, *CHLAMYDOMONAS* AND *DUNALIELLA* GROWN IN THE PRESENCE OF ^{3}H-LEUCINE.

Alga	^{3}H-leucine (μCi ml^{-1})	Proteins (mg)	Radioactivity (nCi)	Spec. Activity (nCi mg^{-1})
Acetabularia acetabulum	5,6	2.5	1940	776
Chlamydomonas reinhardii $CW15^+$	3,2	3.84	321	84
Dunaliella bioculata	3,2	3.7	514	139

3. BIOLOGICAL EFFECTS OF TRITIUM

The biological effects of tritium (supplied as tritiated water, from 0 to 1000 μCi ml^{-1}) were studied on *Acetabularia* as well as on *Chlamydomonas* and *Dunaliella* and the main results were already published[9]. Nevertheless, it is of interest to mention that, in *Acetabularia*, tritium, at doses of 5-50 Gy, inhibits morphogenesis, reduces cap diameter and induces some morphological anomalies. On the contrary, no significant effects on growth were observed for *Chlamydomonas* and for *Dunaliella* (Fig.1.).

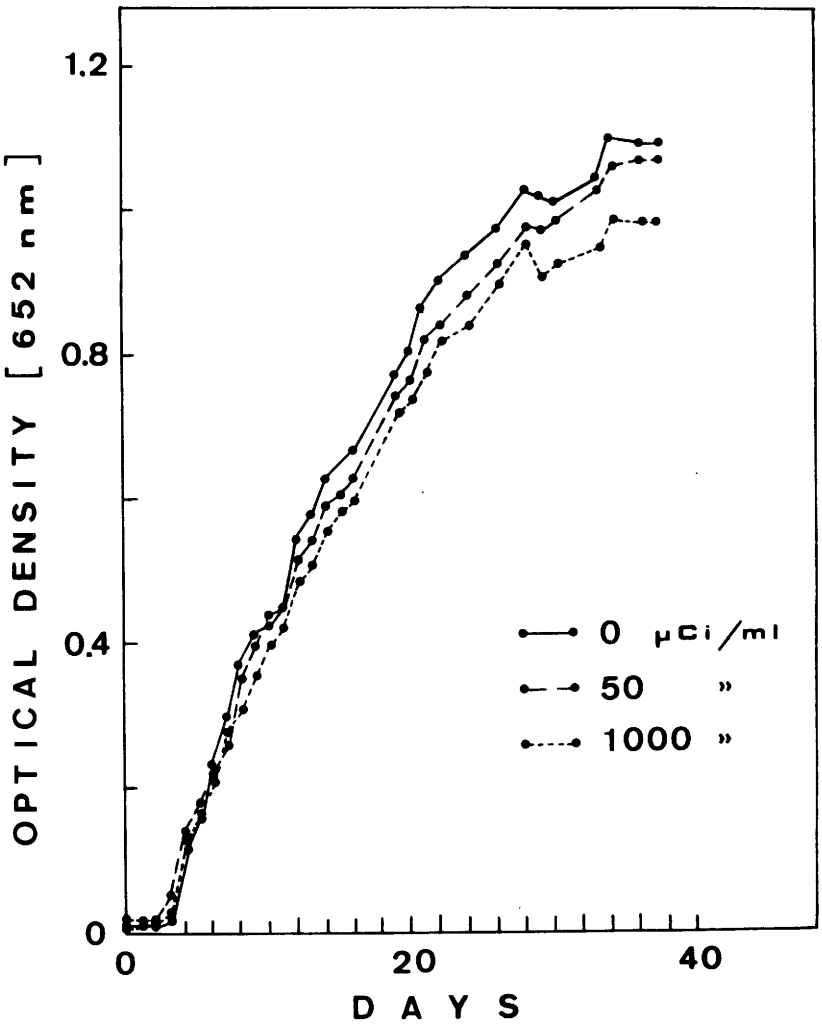

Figure 1. *Dunaliella bioculata*. Growth of the cells in normal culture medium without or with respectively 50 and 1000 µCi ml^{-1} of tritium (HTO). (from ref.9).

4. TRANSFER OF TRITIUM THROUGH AN EXPERIMENTAL AQUATIC FOOD CHAIN

Microalgae (*Chlamydomonas reinhardii*) labeled with tritiated water (1000 µCi ml^{-1}) or with tritiated leucine (3.5 µCi ml^{-1}) were given to seawater mussels[1] (*Mytilus edulis*) or to fresh water mussels (*Anodonta* sp., *Unio* sp. and *Dreissenia polymorpha*)as a single meal.The animals where then reported in normal filtered sea water, which was renewed every 3-4 days. As a function of time, groups of mussels (*Mytilus* and *Anodonta*) were dissected and their organs analysed for tritium content by the micro-combustion procedure[10]. It has been found that, after ingestion of tritiated algae, most of the radioactivity was initially present in the hepatopancreas. Later on, the radioactivity of this organ decreased, part of tritium being lost, part being probably transferred to other organs. Since most tritium (70% or more) is lost during the report of the animals into normal water, no

biomagnification of tritium occurs in this simple aquatic food chain. A complete report on this work shall be published elsewhere.

5. DISCUSSION

Results obtained with three unicellular green algae (*Acetabularia*, *Chlamydomonas* and *Dunaliella*), supplied with tritiated water or with ^{3}H-leucine, showed that tritium becomes incorporated into organic macromolecules of biological importance as well as into small organic molecules. Much less organic tritium is necessary than tritiated water to obtain labeled proteins of comparable specific activity. This result shows that unicellular algae are able to utilize (and eventually accumulate) exogenous organic compounds, being thus facultative heterotrophs. Thus, if organic tritium is released into the aquatic system, it would be probably accumulated by the algal population. This hypothesis is supported by a recent work on the fixation of organic tritium, present in the effluents of a nuclear power plant, by the fresh water microalga *Scenedesmus obliquus*[11] and by investigations on the uptake of tritiated organic precursors by *Acetabularia acetabulum* and *Dunaliella bioculata*[7,8]. Once incorporated into organic compounds, tritium will follow obviously the fate of organic matter in the ecosystems. Though animals grazing on unicellular algae consume the whole plants, the fate of ingested tritium would depend to a large extent on its chemical form. In support of this hypothesis is the finding, by Japanese authors[12], that the retention time of tritium in brine shrimps is longer when the animals are fed with tritiated diatoms (containing ^{3}H-labeled organic compounds) than when they are supplied with tritiated water and fed with unlabeled microalgae. Simple experimental aquatic food chains, such as that utilized in this work, may be useful to compare the retention time of tritium supplied in different chemical forms. Though we cannot exclude accumulation of tritium in some particular molecules (as observed in *Chlorella pyrenoidosa*[3] and in *Anacystis nidulans*[4]), our results suggest that no biomagnification of tritium, through our experimental food chain, occurs, due to the fact that a large part of radioactivity is rapidly lost by the animals.

As reported in a previous work[9], biological effects of tritium can result mainly from beta-rays radiations or from transmutation of tritium into helium. Our investigations show that *Chlamydomonas* and *Dunaliella* cells are little or no affected by tritium even at very high concentrations, which are several orders of magnitude higher than those found in natural aquatic systems, in which tritium toxicity would be extremely difficult (if not impossible) to detect. On the contrary, in *Acetabularia* some biological effects were detected. However, again the concentrations of tritium were very high in comparison with those found in nature.

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UPTAKE, DISTRIBUTION AND BIOLOGICAL EFFECTS OF TECHNETIUM IN AQUATIC MICROORGANISMS

Z. Moureau[a], B. Mania[a], M. Tuaux[a], J. Van Baelen[b], C. Verthé[c], M.N. Maquet[c], J.M. Bouquegneau[c], M. Cogneau[d], D. van der Ben[a], C.M. Vandecasteele[e], C. Myttenaere[e], S. Bonotto[b]

[a] *Institut Royal des Sciences Naturelles de Belgique, B-1040 Bruxelles, Belgium ;*

[b] *Département de Radiobiologie, C.E.N.-S.C.K., B-2400 Mol, Belgium ;*

[c] *Laboratoire d'Océanologie, Université de Liège, B-4000 Liège, Sart Tilman, Belgium ;*

[d] *Laboratoire de Chimie Inorganique et Nucléaire, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium ;*

[e] *Laboratoire de Physiologie Végétale, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium.*

Aquatic microorganisms, living in the water column as well as on various substrates and on sediments, are thought to play an important role in the biogeochemical cycle of man-made substances. Technetium is a long-lived artificial radionuclide which may be found in measurable amounts in the aquatic systems near the reprocessing plants. It may thus interfere with aquatic biota, including both microscopic (bacteria, ciliates, micro-algae etc.) and macroscopic (large algae and aquatic higher plants and various species of animals) organisms. An experimental work was done on the uptake, distribution and biological effects of technetium on a marine bacterium (*Flavobacterium halmephilum*), a ciliate (*Uronema marinum*), a marine alga (*Dunaliella bioculata*) and a fresh water alga (*Chlamydomonas reinhardi*). In most experiments the microorganisms were labeled with ^{95m}Tc-pertechnetate. Distribution of ^{95m}Tc in these microorganisms was studied both at the cellular and at the molecular level, by using selective extraction procedures, column chromatography and electrophoresis. The biological effects on growth and development were studied by using ^{99}Tc-pertechnetate (up to 100 µg x ml^{-1} ^{99}Tc).

1. INTRODUCTION

In a previous paper the role of microorganisms on the behaviour of radionuclides in aquatic and terrestrial systems and their transfer to man was discussed on the basis of recent literature[1]. It is known that the aquatic systems are inhabited by a broad population of microorganisms, mainly constituted by numerous species of bacteria, fungi, microalgae, protozoa and microzooplankters. All these microorganisms interact with the abiotic (organic and inorganic materials and sediments) and the biotic (different species of biota) components of the external medium, where they find the nutrients necessary to sustain their life. Since bacteria are eaten by microflagellates, which in turn are preyed by microzooplankters[2], radionuclides taken up by them might be transferred to higher trophic levels and eventually to man. Radioactive materials incorporated into microalgae and other aquatic plants may behave in a similar way, since plant cells are grazed by a number of animals. It was thus of interest to study the interaction of technetium, a long-lived radionuclide produced by fission[3], with four aquatic microorganisms : a bacterium (*Flavobacterium halmephilum*), a bacteriovorous ciliate (*Uronema marinum*) and two microalgae (*Dunaliella bioculata* and *Chlamydomonas reinhardii*). The most significant results on technetium uptake and distribution and on its biological effects are summarized and briefly discussed in this paper.

2. UPTAKE OF TECHNETIUM UNDER LABORATORY CONDITIONS

Two isotopes of technetium were used in our laboratory investigations : ^{99}Tc (half-life of 2.1×10^5 years) and ^{95m}Tc (half-life of about 60 days). Both were supplied to the microorganisms in the form of pertechnetate. The utilization of ^{95m}Tc, which has a very high specific activity, has permitted to lower considerably the concentration of this element in the external medium (fg to pg ml^{-1}). Table 1 shows the concentration factors of ^{95m}Tc in *Flavobacterium halmephilum*, *Uronema marinum*, *Chlamydomonas reinhardii* and *Dunaliella bioculata*. In contrast with the three other microorganisms investigated, *Dunaliella bioculata* was unable to concentrate ^{95m}Tc (C.F. = 0.3) even after a 40 days incubation. Experiments with *Uronema marinum*, supplied with ^{99}Tc-pertechnetate, have revealed that at higher mass concentrations of technetium in the medium, the concentration factor decreases (results not shown), a result which was previously found also in the unicellular marine alga *Acetabularia acetabulum*[4].

3. DISTRIBUTION OF TECHNETIUM AT THE CELLULAR AND AT THE MOLECULAR LEVEL

The distribution of technetium in the microorganisms was studied by disrupting the cells in a press, by submitting the homogenate to a differential centrifugation and by analysing the supernatant (crude extract) by column chromatography[5,6], which allow separation of various macromolecules from low molecular weight compounds. The utilization of a gel of Sephacryl S-300 (Pharmacia) and of a buffer having a high ionic strength (2 M NaCl, 5 M Urea, 0.02 M Tris-HCl, pH 7.5) has permitted to separate large molecules (mostly proteins) from low molecular weight compounds and from pertechnetate. Control experiments have shown that $^{95m}TcO_4^-$ is eluted as a single peak after about 4 Vo volumes. The comparison of optical density (215 and/or 280 nm) with radioactivity profiles has shown that ^{95m}Tc was present in few (from 3 to 4) main peaks , which correspond respectively to a first group of high molecular weight compounds (probably a mixture of large protein and nucleic acid molecules), to a second group of lower molecular weight mole-

TABLE I. ^{95m}Tc CONCENTRATION FACTORS IN FOUR SPECIES OF AQUATIC MICROORGANISMS.

Microorganisms	^{95m}Tc concentration in the culture medium (pg/ml)	Incubation Time (days)	Concentration (1) Factor
Bacteria			
Flavobacterium halmephilum	1.18	7	88
Flavobacterium halmephilum (2)	1.15	7	42
Ciliates			
Uronema marinum	1.15	7	126
Algae			
Chlamydomonas reinhardii CW 15+	0.96	11	90
Dunaliella bioculata	1.05	40	0.3

(1) The concentration factor is defined as the ratio : $\frac{\text{pCi/g fresh matter}}{\text{pCi/ml medium}}$

(2) Grown together with *Uronema marinum* and separated by differential centrifugation (10 min., 12.000 xg).

cules (mostly constituted by proteins) and to ^{95m}Tc-pertechnetate and/or ^{95m}Tc bound to a class of small organic molecules. Work is in progress to characterize the various substances separated by molecular sieving. The above results suggest that technetium is able to penetrate inside the cells and to become eventually incorporated into organic compounds of biological importance (mostly into proteins). In order to verify this hypothesis, bacterial cells (*Flavobacterium halmephilum*) were labeled with ^{95m}Tc or ^{99}Tc, extensively washed and treated with lysozyme[7]. At the end of the treatment, the samples were cooled (4°C), centrifuged (10 min, 12,000 xg) and the resulting supernatants and sediments were counted in a Packard auto-gamma spectrometer (Model C.5986). The results, reported in table 2, show that lysozyme treatment increases significantly (about two times more than in control samples) the release of technetium, a finding which seems to suggest that this element had entered, at least in part, the bacterial cells. Analysis of the released radioactive materials has revealed that only 2-15% of the radioactivity is bound to dialysable compounds having a molecular weight $\leqslant$ 12.000 daltons (the dialyzer tubing retaining the larger molecules). However, even after enzymatic lysis, most of the radioactivity remained associated with the sediment, which was mainly constituted by large fragments of bacterial cells (results not shown, obtained with the scanning electron microscope).

TABLE 2. EFFECT OF LYSOZYME TREATMENT ON THE RELEASE OF TECHNETIUM BY *FLAVOBACTERIUM HALMEPHILUM*.

Experiment	Treatment	Radioactivity (nCi)			
		^{95}Tc		^{99}Tc	
		Supernatant	Sediment	Supernatant	Sediment
1	Control	2.48	30.92	-	-
	Lysozyme	8.28	31.29	-	-
2	Control	1.96	24.07	-	-
	Lysozyme	3.86	21.41	-	-
3	Control	-	-	35.10	116.12
	Lysozyme	-	-	67.37	93.70

4. BIOLOGICAL EFFECTS OF TECHNETIUM

The biological effects of technetium were studied by adding to the culture medium ^{99}Tc-ammonium pertechnetate, at concentrations ranging from 0 to 10 µg ml^{-1} (*Flavobacterium halmephilum* and *Uronema marinum*) or from 0 to 100 µg ml^{-1} (*Dunaliella bioculata* and *Chlamydomonas reinhardii*). At the concentrations utilized, ^{99}Tc had no clear-cut effects on the growth of the four species of microorganisms investigated. A variability was, however, observed in the response of the microorganisms to technetium treatment, with positive or negative effects. New experiments are necessary to verify if this phenomenon is provoked by the pertechnetate molecule, which contains an ammonium group,rather than by the technetium itself.

5. DISCUSSION

The potential importance of microorganisms in controlling or altering the biological availability of radionuclides in the aquatic and terrestrial ecosystems has been recognized (see recent literature in ref.1). However, information on the interaction of microbes with long-lived radionuclides remains rather scarce. Our results show that four different species of aquatic microorganisms are capable of taking up technetium, which becomes incorporated into cell components of different molecular weight. Three of them (*Flavobacterium halmephilum*, *Uronema marinum* and *Chlamydomonas reinhardii*) concentrate technetium (C.F. ranging from 42 to 126), whereas one (*Dunaliella bioculata*) does not. The concentration factors reported in table 1 for *Flavobacterium halmephilum* and *Uronema marinum* are higher than those previously found[8], a fact which might be due mainly to differences in the culture conditions. Since *Flavobacterium halmephilum* and *Uronema marinum*, as well as microalgae, are eaten by other organisms, part of technetium taken up by these microorganisms might be transferred through the food chain, as shown in a previous experimental work[9]. However, till now, no biomagnification of technetium in food chains has been reported[9].

Though grazers consume the whole cells (for example cell membrane, organites and cytoplasm), the fate of the ingested radioactivity may depend upon its chemical form. It was thus of interest to know if technetium contaminates only the outside of the microorganisms or if it penetrates inside the cells, becoming eventually incorporated into organic compounds of biological importance. The results obtained with extraction procedures, chromatographic analysis and enzymatic treatment (lysozyme) show that part of the technetium supplied to the microorganisms is localized inside the cells. The chemical speciation of technetium in the cells is being analysed.

In spite of the relatively high concentrations of technetium used in our experiments, no clear-cut biological effects were observed. These results confirm and extend those reported in a previous paper[8]. The observed resistance of *Dunaliella bioculata* and *Chlamydomonas reinhardii* to high concentrations of ^{99}Tc-pertechnetate (up to 100 μ ml^{-1}) is in good agreement with the findings of Gearing et al.[10], who reported that the growth of two green microalgae (*Chlorella sorokiniana* and *Dunaliella tertiolecta*) was not inhibited even at 600 μg ^{99}Tc ml^{-1}. However, the same authors found that the nonsulfur purple bacterium *Rhodospirillum rubrum* was strongly inhibited already at 1 μg ^{99}Tc ml^{-1}. It seems thus that the response of aquatic microorganisms to technetium (pertechnetate) may vary considerably from one species to another.

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Session : I/B

Paper by : Z. Moureau

Comment by : W. Kühn

Text of comment
or author's answer :

We have no stable Tc-element and one should clear express, that your bio-positive effect is coming from the chemical behaviour of Tc and its compounds and not from β-rays of the Tc-isotope. You agree with me ?

Yes, I agree.

Session : I/B

Paper by : Z.Moureau

Comment by : M.J.Frissel

Texxt of comment
or author's answer :

You showed some positive effects of the application of Tc on the growth of one of the organisms. This surprises me. Is this effect statistically significant ? Were the NH_4^+ levels in all experiments identical ?

The positive effect is statistically significant inside one particular experiment. However, some variability was found between different experiments, so that more data are necessary to reach a safe conclusion.
The levels of NH_4^+ depended upon the concentration of pertechnetate.
The biological effects of NH_4^+ remain to be investigated.

Session : I/B

Paper by : Z.Moureau

Comment by : M.A. Abdallah

Text of comment
or author's answer :

Have you performed growth experiments using variable amounts of ammonium salts possessing anions of the same size as pertechnetate?

Not yet.

The retention of radioactivity in the chromatographic fraction of the cytosol would suggest that pertechnetate anion remains bound to any macromolecule in an anion binding site and then any anion of its size would play the same role.
However, one cannot rule out the possibility that technetium is transported into the cytoplasm where it can change its state of oxidation (like molybdenum in the case of nitrogenases). In this case pertechnetate cannot be replaced by any anion of its size.

Disruption of ^{95m}Tc labelled bacteria with a press and successfully chromatographic analysis of the extract as well as treatment of labelled microbial cells with lysozyme seem to suggest that ^{95m}Tc is able to cross the cell membrane. If this is the case it could change the state of oxydation, and become bound to some organic compounds.

FUNGAL FLORA OF MARINE MACROALGAE AND ITS ECOLOGICAL SIGNIFICANCE

F. CINELLI[1,3], V. CUOMO[2], G. PARDI[1], U. SALGHETTI[1], F. VANZANELLA[2], S. DE RANIERI[3] and P. BELCARI[3]

1 Centro Interuniversitario di Biologia Marina, Livorno (Italy)
2 Ciba-Geigy S.p.A., Torre Annunziata, Napoli (Italy)
3 Istituto di Biologia Marina, Università di Pisa (Italy)

ABSTRACT

Fungi inhabiting the marine macroalgae *Laurencia obtusa*, *Cystoseira compressa*, *Ulva rigida*, *Pterocladia capillacea*, *Chondria tenuissima*, *Peyssonnelia rubra*, *Peyssonnelia rosa-marina*, *Halimeda tuna*, *Udotea petiolata*, *Acrothamnion preissii*, *Bornetia secundiflora*, *Aeodes marginata*, *Codium vermilara*, *Palmophillum crassum*, *Cladostephus verticillatus* and *Spherococcus coronipifolius* were studied.

The investigation was carried out to study : a) the occurrence of fungal parasitic or saprobic flora of collected macroalgae ; b) the role of the recorded fungal flora on the break-down of the seaweeds and on the behaviour of radionuclides in the marine coastal ecosystem.

The results have shown that the majority of the fungal flora inhabiting the investigated algae belongs to Higher Fungi (Ascomycetes and Deuteromycetes), a small part only being constituted of Lower Fungi (Thraustochitrid Fungi).

These preliminary observations are a first account of a wider study on the occurrence of fungi in the benthic algal and phanerogamic vegetation of the Tuscany coasts and on the role of marine fungi on the break-down of organic matter in the sea, including seaweed waste, and on the behaviour of radioactive pollutants.

2. MATERIALS AND METHODS

2.1. Sampling of seaweeds

The seaweeds were collected along the Leghorn coasts, during the winter 1983/84.

They were separated genus by genus, put in plastic bags and carried to the laboratory inside thermic containers at a temperature not exceding 10°C.

2.2. Isolation of fungi

In the laboratory the fungi were immediately isolated. Alternatively the algal samples were put at 4°C. Before the isolation, the algal samples were also cleaned three times with sterile seawater.

2.2.1. Higher Fungi

Three different techniques were used to grow and evidentiate marine fungi :
a) fragments of seaweeds were incubated for four weeks at room temperature, inside Petri dishes, on sterile filter paper Watman N.1 (diameter 90 mm), humidified with sterile seawater added with chloramphenicol (1 g l^{-1}). As soon as fungi development was observed (generally after 5 days), mycelium and spores were transferred into Petri dishes containing Corn Meal Agar and Glucose Yeast Agar (2) for further growth ;
b) fragments of seaweeds up to 0,4 cm^2 in size (2 or 3 parts of the same seaweed) were put in Petri dishes containing Mycological Agar Difco and Oat Meal Agar and then incubated at 25°C for 2 weeks. As soon as fungi development appeared (generally after 5 days), isolation was carried out as in paragraph a) ;
c) samples of seaweeds, species by species, were put inside plastic box of 9x13x5 cm on paper "Kleenex" humidified with sterile seawater and incubated at 20°C about for 20 weeks. For isolating and growing the fungi we followed the procedures by Jones (3,4,5).

2.2.2. Lower Fungi

The seaweeds cleaned as above mentioned, were washed again with sterile seawater added with penicillin (1.5 g l^{-1}) and streptomycin sulfate (5 g l^{-1}). The isolation procedures of Gaertner (6) and Harrison (7) were used.

3. RESULTS

The majority of the fungal flora (8,9,10,11) isolated from fronds of seaweeds (Fig.1) resulted to be epiphitic and to belong to the Ascomycetes and Deuteromycetes (Higher Fungi) (Table 1).

FIGURE 1. Fronds of the red macroalga *Laurencia obtusa*, one of the many seaweeds investigated in this study.

Epiphitic fungi may grow on different substrates. However, in the case of the genus *Ulva*, a particular relationship seems to exist between the algae and few marine fungi, which are found almost exclusively on their fronds.

Most of the Higher Fungi reported in table 1 have a probable land origin.

Of interest is the presence of Lower Fungi (Thraustochytriaceae) on the majority of the seaweeds (see Table 1).

TABLE I. Higher Fungi (Ascomycetes and Deuteromycetes) and Lower Fungi isolated from seaweeds.

FUNGI	ALGAE
Ascomycetes	
Corollospora maritima Wederman	*Ulva rigida, Aeodes marginata, Cystoseira compressa, Chondria tenuissima*
Lulworthia sp.	*Ulva rigida*
Chaetomium globosum Kunze	*Cystoseira compressa*
Corollospora intermedia Schmidt	*Chondria tenuissima, Halimeda tuna*
Deuteromycetes	
Alternaria maritima Suth	*Cladostephus verticillatus, Ulva rigida, Codium vermilara*
Gliomastix murorum (Corda)Hughes	*Pterocladia capillacea*
Acremonium pinkertoniae	*Udotea petiolata*
Penicillium sp. and *Aspergillus sp.*	*Laurencia obtusa, Cystoseira compressa, Ulva rigida, Pterocladia capillacea, Chondria tenuissima, Peyssonnelia rubra, P. rosa marina, Halimeda tuna, Udotea petiolata, Acrothamnion preissii, Aeodes marginata, Codium vermilara, Palmophillum crassum, Bornetia secundiflora, Cladostephus verticillatus* and *Sphaerococcus coronopifolius*
Stachibotrys atra Corda	*Codium vermilara*
Stemphylium sp.	*Pterocladia capillacea*
Phoma sp.	*Halimeda tuna, Chondria tenuissima*
Dendryphiella salina (Suth)Pugh et Nicot	*Ulva rigida, Laurencia obtusa*
Cladosporium halgarum Cooke et Mossee in Cooke	*Pterocladia capillacea, Codium vermilara, Palmophyllum crassum*
Doratomyces stemonitis Corda	*Codium vermilara, Cladosphus verticillatus*
Gliocladium roseum Bain	*Codium vermilara, Cladostephus verticillatus*
Pytium maritimum Hohnk	*Pterocladia capillacea, Cystoseira compressa*

Phialophora fastigiata (Lager Bert et Melin) Conant	*Acrothamnion preissii, Halimeda tuna, Chondria tenuissima*
Scopulariopsis brevicaulis	*Ulva rigida, Pterocladia capillacea*
Pyrenochaeta rubi Cavara	*Chondria tenuissima, Codium vermilara*
Thraustochytriaceae	
Thraustochytrium roseum Goldstein	*Laurencia obtusa, Peyssonnelia rubra, Ulva rigida*
Thraustochytrium sp.	*Laurencia obtusa, Ulva rigida, Peyssonnelia rubra, Chondria tenuissima, Udotea petiolata, Codium vermilara, Cladostephus verticillatus, Spherococcus coronipifolius*
Thraustochytrium kineii Gaertner	*Ulva rigida, Cladostephus verticillatus*
Schizochytrium aggregatum Goldstein et Belscky	*Halimeda tuna, Palmophyllum crassum, Aeodes marginata*
Thraustochytrium multirudimentale Goldstein	*Codium vermilara, Spherococcus coronipifolius, Cystoseira compressa*
Thraustochytrium aureum Goldstein	*Acrothamnion preissii, Chondria tenuissima*

4. DISCUSSION

4.1. Higher Fungi

Fungi as *Penicillium*, *Aspergillus*, *Fusarium*, *Gliocladium*, etc., having a probable land origin, are not only epiphitic, but few of them are saprobic, as reported also by Chesters et *al.* (12) and Haythorn et *al.* (13). The fungi *Corollospora marittima*, *Corollospora intermedia*, *Dendryphiella salina* are prevalently marine and found to live especially on genus *Ulva*, *Halimeda*, *Chondria*.

Their saprobic activity may be related to the presence of cellulose and laminarine in the Ulvaceae and to their specific enzymes (cellulase and laminarase).

4.2. Lower Fungi

The occurrence of these organisms was ascertained on the majority of the examined seaweeds. In general the Lower Fungi are widespread and live not only on seaweeds and leaves of angiosperms but also on invertebrates (molluscs, coelenterates) and are found in marine waters and sediments.

These organisms seem to play a very important role on the coastal environment.

The fungal flora found on the seaweeds may play a role not only on the decomposition of organic matter, but also on the general behaviour of radionuclides. It is unknown, at the present time, which are the relative amounts of radioactivity taken up respectively by the seaweed fronds and by the inhabiting fungal flora.

In recent papers the behaviour of ^{3}H in the red seaweed *Laurencia obtusa* and the brown seaweed *Cystoseira compressa* was studied (14,15,16). Fronds were incubated for increasing periods of time in tritiated water (5 $\mu Ci\ ml^{-1}$). It was found that in both algae tritiated water was rapidly taken up and released and that a small percentage of tritium (1-5%) was incorporated into the total organic matter of the fronds. Since the seaweeds collected in the sea are inhabited by Higher and Lower Fungi, it is possible that part of the tritium was incorporated into them. Moreover, the fungi developing on the algae might use algal tritiated compounds for their growth. As the fungi are grazed by marine animals, tritium originally incorporated into seaweeds might thus be transferred indirectly to higher trophic levels. It is obvious, however, that for animals grazing the entire fronds of the seaweeds, mainly a direct transfer of tritium from the alga to the animal would occur. Work in progress on this topic in our laboratory should reveal the role of the fungal flora on the behaviour not only of tritium but also of other long-lived radionuclides having an interest for radioprotection.

5. ACKNOWLEDGEMENTS

This work was supported in part by contract CEC nr BIO-519-I(S).
We thank Professor S. Bonotto (Department of Radiobiology, C.E.N./S.C.K., Mol, Belgium) for fruitful discussions.

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EFFECTS OF NITROSYL COMPLEXES OF RUTHENIUM ON ESCHERICHIA COLI K12 AND YEASTS AND THE UPTAKE OF THESE COMPLEXES BY MARINE BACTERIA

JANE F. GIBSON*, ROBERT K. POOLE*, MARTIN N. HUGHES† and JOHN F. REES

Departments of Microbiology* and Chemistry†, Queen Elizabeth College, London W8 7AH, and Environmental Safety Group, Harwell Laboratory, Oxfordshire

ABSTRACT

The uptake of ruthenium nitrosyl complexes by marine bacteria is not significant under normal conditions, as the bacteria cannot compete effectively with sediment for the complexes. In the absence of sediment, and when present in large numbers, the bacteria could bind ruthenium nitrosyl complexes.

The effect of growth of Escherichia coli, Saccharomyces cerevisiae and Schizosaccharomyces pombe of a number of nitrosyl complexes of ruthenium has been studied. The complexes $[RuNO(OH)_3]$, $[RuNOCl_3]$, $[RuNO(NO_3)_3(H_2O)_2].2H_2O$ and $Na_2[RuNO(NO_2)_4OH].2H_2O$ had no measurable effect on E.coli at concentrations of 10^{-3}M and below, although the yeasts were more sensitive to these complexes. It has been shown for E.coli, by spheroplast swelling techniques, that these complexes cannot cross the cytoplasmic membrane, which could account for their lack of toxicity.

1. INTRODUCTION

Ruthenium, a fission product of uranium, is discharged into the sea from nuclear waste reprocessing plants, such as that at Sellafield.[1] The ruthenium in the effluent is present mainly as nitrato and nitro complexes of nitrosylruthenium(II), although on standing these ligands will be aquated to give the species $[Ru(NO)(H_2O)_5]^{3+}$, which contains an NO^+ nitrosyl group.

Ruthenium has been regarded as one of the troublesome radioactive elements in routine discharges from Sellafield, even though the total amounts of ruthenium discharged have been small. In 1981, British Nuclear Fuels Ltd reported 9.2 mg ^{103}Ru and 4.2 g ^{106}Ru discharged to sea from Sellafield,[2] while Hunt[3] found ruthenium levels to be 2×10^{-10} g ^{106}Ru kg^{-1} coastal sediment, and approximately 4×10^{-11} g ^{106}Ru kg^{-1} in Porphyra. Nevertheless, there have been instances previously where ruthenium has been concentrated by marine organisms, notably the laver bread alga[1], and so it was important to consider also whether ruthenium compounds could be concentrated by marine and other bacteria, and then to assess the competition between bacteria and sediment for the binding of ruthenium.

2. EXPERIMENTAL

2.1 Ruthenium Complexes and Laboratory Test Organisms

The nitrosyl complexes were prepared by literature methods.[4,5] Commercial ruthenium trichloride and simulated effluent were obtained from Johnson Matthey. Escherichia coli K12 (NCIB 11825) was grown as described by Scott et al.[6] Saccharomyces cerevisiae N.C.Y.C. 431 and Schizosaccharomyces pombe 972 h^- were grown at 30°C, as described by Barnett and Ingram[7] and Poole et al,[8] respectively. Methods used in growth and physiological experiments with these organisms are described under Results and Discussion.

2.2 Analysis

Protein was assessed by the method of Lowry et al[9] using bovine plasma albumin as standard. Ruthenium was determined[10] by Atomic Absorption spectroscopy on a Perkin-Elmer 280 instrument.

2.3 Preparation of Iron(III) Hydroxide

This was precipitated by adding ammonia to aqueous iron(III) chloride, collected by centrifugation, washed in distilled water and seawater (twice) and resuspended and diluted in seawater. Iron was determined by A.A. analysis of samples to which concentrated nitric acid (0.25 mL per 10 mL sample) had been added.

2.4 Sediment

This was collected by filtration, through 25 μm pore size filters, of up to 600L of seawater at Weymouth, Dorset, or as the top aerobic sediment from tanks continuously supplied with seawater. Uptake of ruthenium was identical in both cases. Sediment suspensions were washed with 0.6M nitric acid and twice with seawater before use. Filtration of about 50L of seawater gave about 390 mg of sediment (dry weight), obtained by drying at 105°C. Marine bacteria were grown from sediment on a medium containing nutrient agar in seawater.

2.5 Uptake Experiments

The first step by which ruthenium reaches the human food chain may involve

uptake by bacteria. Thus studies on ruthenium uptake by marine organisms were initiated. Sediments and/or cells were suspended in 200 to 500 mL of membrane-filtered seawater at 15°C. After one day, a day-old solution of $[RuNO(NO_3)_3(H_2O)_2]$ in seawater or a simulated effluent in 2.7M nitric acid was added to give a ruthenium concentration of 10^{-4}M. Sodium hydrogen carbonate was added to restore the pH to 8. Samples (10 to 30 mL) were centrifuged, the pellets being washed with 3 mL distilled water and 0.5M HCl (5 mL) prior to treatment with a mixture of concentrated acids (HNO_3, $HClO_4$, H_2SO_4; 3:2:1). Supernatants, washings and pellets were analysed for ruthenium; that in the supernatant and water washings were classed as free ruthenium and the remainder as bound ruthenium.

Enrichment solutions added to sediment suspensions contained (per litre, final concentration) yeast extract (0.1 g) or NH_4Cl (0.56 g), and cellobiose (2.4 g). They were prepared in seawater and sterilised. Tripolyphosphate treatment involved the addition of sodium tripolyphosphate (5 mg L^{-1}) and shaking with glass beads. Cytophage L1 enzyme preparation was used as a source of lytic enzymes. The enzyme preparation (12 mg) was added to a suspension of 90 mg sediment in 300 mL seawater.

3. RESULTS AND DISCUSSION

3.1 Uptake of nitrosyl complexes by marine sediments and bacteria

In the marine environment, bacteria are found mainly in association with sediment. The present study attempted to determine the most important sorptive sites for ruthenium and to differentiate between uptake by sediment particles, uptake by iron(III) hydroxide contained within sediments and more particularly, uptake by bacteria present in sediments. An aged solution of nitrosylruthenium, prepared by dissolving $[RuNO(NO_3)_3(H_2O)_2].2H_2O$ in seawater and leaving for a day, was used in the experiments. Nitrosylruthenium complexes undergo hydrolysis and polymerisation reactions in solution[11,12]. These reactions do not result in the loss of the nitrosyl group which is extremely stable. However, it is important to note that because of hydrolysis and polymerisation reactions that take place in seawater, the chemical form of the ruthenium solution will always be dependent on its age and on its original form. Furthermore, the extent of uptake by sediments and marine organisms is very dependent on the chemical form of the ruthenium[11,13,14]. Aged solutions of nitrosyl-ruthenium were therefore used to simulate more closely the environmental situation. Sediment suspensions were found to take up nitrosylruthenium (Figure 1). Uptake was characterised by a relatively rapid initial phase, followed by a slow secondary phase.

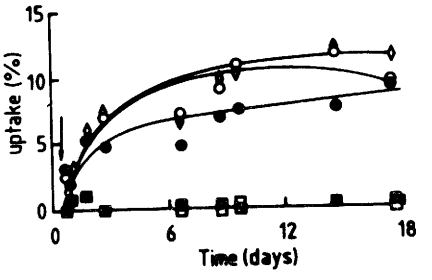

FIGURE 1 Uptake of $[Ru(NO)(NO_3)_2(H_2O)_2]$ (10^{-4}M, added at arrow), aged in seawater, by fine filter (0.45 μm) material (□), enriched fine filter material (■), coarse filter sediment (○), enriched coarse filter sediment (●) and surface sediment (◇). Iron content (ppm), initial

dry weight (mg mL^{-1}) and A_{540} of the suspension were: 0.13, 0.02, 0.03 (□), 0.22, 0.025, 0.13 (■), 4.7, 0.3, 0.41 (○), 3.6, 0.29, 0.44 (●), 2.5, 0.36, 0.48 (◇). Filtered material was at 100-fold its concentration in the sea.

Untreated sediment suspensions took up approximately 3.4 μg ruthenium per mg dry weight of sediment (10% of the total ruthenium) after 12 days. Most of this ruthenium could be removed by washing with acid, suggesting surface binding of the ruthenium by the sediment. When the sediment was enriched with nutrients to increase the bacterial numbers, no increase in uptake of ruthenium was observed, despite observed increases in biomass. Indeed, portions of sediment suspension that had been killed by autoclaving, digested with lytic enzymes, or treated with sodium tripolyphosphate showed the same uptake as portions that had been left untreated or enriched with nutrients. Treatment with tripolyphosphate was presumed to lessen the association of bacteria with sediment.

While increasing bacterial numbers had no effect on uptake by sediment suspension, increasing the concentrations of other components of the sediment enhanced uptake. The sediments used contained 2 to 5 p.p.m. iron, presumably as $Fe(OH)_3$. Addition of further $Fe(OH)_3$ greatly enhanced the uptake (Figure 2). Addition of clay (100 mg mL^{-1}) but not fine sand (0.98 mg mL^{-1}) after 3 days increased the uptake from about 5% to 15% of the total.

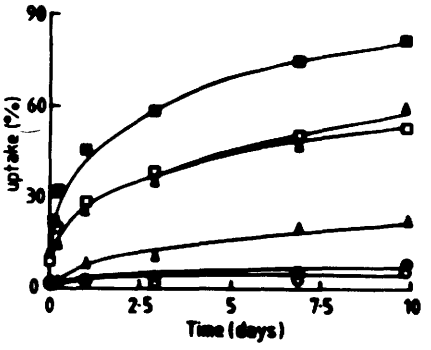

FIGURE 2 Uptake of $[Ru(NO)(NO_3)_3(H_2O)_2]$ (10^{-4}M), aged in seawater, by acid-washed sediment (0, 0.9 ppm $Fe(OH)_3$), and surface sediment supplemented with $Fe(OH)_3$, to final concentrations 4.3 (●), 16.5 (△), 52 (□) and 170 (■) ppm respectively. ▲ shows uptake by 49 ppm $Fe(OH)_3$ alone. Dry weight (mg mL^{-1}) and A_{540} of the acid washed sediment were 0.31 and 0.29; values for the supplemented suspensions were 0.37 and 0.45.

Although bacteria did not appear to be important in uptake of nitrosylruthenium when associated with sediment, they were capable of uptake when present on their own in large numbers. Addition of simulated ruthenium effluent (10^{-4}M in ruthenium) to a concentrated suspension of marine organisms (1.07×10^9 viable cells mL^{-1}) grown from sediment on nutrient agar in seawater resulted in an uptake of 11% of the total ruthenium after six days. Addition of this simulated ruthenium effluent to a sediment suspension (2.6 ppm Fe; 0.25 mg dry wt ml^{-1}) resulted in an uptake of about 7% of the ruthenium. Possible explanations of the apparent inability of bacteria to concentrate ruthenium under normal conditions are their low numbers or the masking by sediment of any uptake. The latter situation could easily be envisaged if the cells were buried in the sediment so that their surfaces were not available for binding. To investigate this

further, uptake of simulated ruthenium effluent by a suspension of cells and Fuller's earth (clay) was compared with that of the same concentration of cells alone, or the same concentration of Fuller's earth alone (Figure 3). Fuller's earth supplemented with cells took up a similar amount of ruthenium as Fuller's earth alone, despite some 13% uptake by the cells alone. This is consistent with the theory that uptake of ruthenium by bacteria is masked by the presence of sediment particles which are mainly responsible for the ruthenium uptake from the aqueous phase.

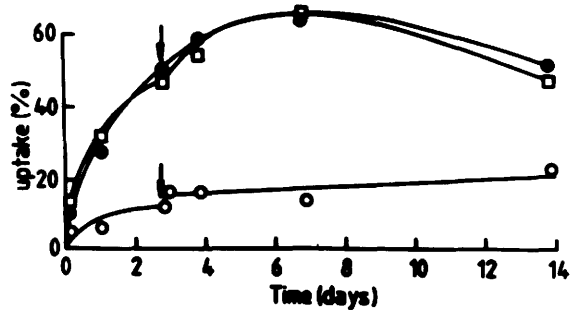

FIGURE 3 Uptake of simulated ruthenium effluent (10^{-4}M) by marine organisms (O; initial A_{540}=, 0.25), Fuller's earth (●, 1.44 mg mL^{-1}) and marine organisms plus Fuller's earth (□). At the time indicated by the arrow, further cell suspension was added to (○) and (□).

3.2 Effect of ruthenium nitrosyl complexes on test organisms

The following complexes were used: $[Ru(NO)(OH)_3]$; $[Ru(NO)Cl_3]$, $[Ru(NO)(NO_3)_3(H_2O)_2].2H_2O$; $Na_2[Ru(NO)(NO_2)_4(OH)].2H_2O$. They had no measurable effect on growth or cell size of E.coli at concentrations of 10^{-3}M and below. The yeasts were more sensitive. Thus the growth rate of S.pombe was inhibited by $[Ru(NO)Cl_3]$ (> 10^{-5}M), which caused a decrease in cell volume at concentrations about 10^{-4}M, and by $[Ru(NO)(NO_3)_3(H_2O)_2].2H_2O$ (> 10^{-3}M). No effect was found for $Na_2[Ru(NO)(NO_2)_4OH].2H_2O$ at 10^{-3}M. The response of the budding yeast Sacch. cerevesiae differed. Complete inhibition of growth, with reduced cell volumes, was observed at 10^{-3}M for $[Ru(NO)Cl_3]$ and $Na_2[Ru(NO)(NO_2)_4OH].2H_2O$, while $[Ru(NO)(NO_3)_3(H_2O)_2].2H_2O$ had no effect at this concentration.

3.3 Transport of nitrosylruthenium complexes across the cytoplasmic membrane of E. coli

The lack of toxicity of the nitrosyl complexes to E. coli could be related to the absence of uptake into the cell. This was studied by monitoring the swelling of spheroplasts prepared by removing the outer membrane from E.coli [15,16] Ion transport and associated water movements cause the spheroplasts to swell, which can be monitored by the decrease in light scattering. Swelling only occurs if the overall transport process is electrically neutral and involves no nett pH change. Spheroplasts were stable in isoosmotic KNO_3, but swelling was observed on addition of valinomycin which acts as an ionophore for K^+. Nitrate can pass across the membrane[15]. Addition of valinomycin to spheroplasts suspended in isoosmotic $K_2[Ru(NO)(NO_2)_4OH]$ did not cause swelling showing that the $[Ru(NO)(NO_2)_4OH]^{2-}$ anion is a non-permeant species. Thus passage of K^+ across the membrane does not occur, because a charge imbalance would arise. Confirmation that the spheroplasts were still osmotically sensitive at this stage was obtained

by demonstrating that swelling occurred on addition of the detergent Triton.

Addition of Et_3PbOAc and the proton carrier CCCP should cause swelling of spheroplasts suspended in isosomotic bromide salts provided the cation can cross the cytoplasmic membrane. This arises from the mediation by the organolead cation of a Br^--OH^- antiport.[16] However, swelling of spheroplasts was not observed on addition of triethyllead acetate and CCCP to spheroplasts suspended in an isoosmotic $[Ru(NH_3)_5NO]Br_3$-NaBr mixture, showing that the complex cation cannot cross the cytoplasmic membrane. It appears therefore that the lack of toxicity of these nitrosylruthenium species to E.coli is primarily due to their inability to cross the cytoplasmic membrane.

4 CONCLUSIONS

Bacteria could concentrate ruthenium compounds in the environment either by uptake into the cell or by 'biosorption' onto the cell surface. The former process could lead to greater selectivity. However the present work shows that the lack of toxicity of both anionic and cationic nitrosyl complexes of ruthenium towards the test organisms may be attributed to the inability of these complexes to cross the cytoplasmic membrane. While surface binding to marine bacteria does occur under laboratory conditions, it is apparent that nitrosylruthenium complexes bind more strongly to sediments, which appear to prevent completely binding to bacteria. It appears unlikely therefore that bioaccumulation of ruthenium nitrosyl compounds can occur in the environment through marine bacteria.

5 ACKNOWLEDGEMENTS

We thank the S.E.R.C. and the United Kingdom Atomic Energy Authority (Harwell) for a C.A.S.E. award to J.F.G.

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Session : I/B

Paper by : J. Gibson

Comment by : S. Bonotto

Text of comment
or author's answer :

You showed that marine bacteria are incapable of taking up Ru-compounds. Is that a general rule ? Because many different species live into the sea, and differences between them could eventually occur. Thank you for your comment on this point.

In fact, we showed that marine bacteria present either as "fine sediment", or as a bacterial suspension do take up ruthenium coumpounds when present in sufficient numbers. Any uptake is readily masked by clay and sediment material however. The osmotic swelling experiments have been performed so far only with E. coli, which was used because of the extensive background information available on soluble uptake systems.

Session : I/B

Paper by : R.K. Poole

Comment by : Bors

Text of comment
or author's answer :

You just showed a complex formation with sodium bromide. Could you imagine such complexation with sodium iodide also ?

The experiments to which you refer involve monitoring the osmotic behaviour of E. coli spheroplasts when suspended in iso-osmotic solutions such as 0.5 M-sucrose or 0.25 M-NH_4Br. Swelling (detected spectrophotometrically) occurs only when both anionic and cationic components of the suspending medium penetrate the membrane leading to electroneutral solute uptake, followed by extensive water movement to retain iso-osmolarity. Thus, in the case of NH_4 Br, although NH_4^+ is a penetrant species (as NH_3), Br^- is not and spheroplasts are stable unless triethyllead acetate is added. This compound facilitates halide (Br^-)- H^+ exchange and causes swelling. The specific experiment to which you refer was designed to test the permeability of the membrane to the cationic ruthenium penta ammine nitrosyl complex which is only sparingly soluble. Thus, spheroplasts were suspended in a mixture of 0.037 M ruthenium compound and 0.0176 NaBr, giving a total osmolarity equivalent to 0.5 M sucrose. The slide was not meant to imply a complex formation with sodium bromide. There was no special radiochemical interest in chosing bromide or any halide. Sodium bromide merely provided a non-penetrant cation and the same anion as was present in the ruthenium compound.

Session : I/B

Paper by : J. Gibson, R. Poole, M. Hughes, J. Rees

Comment by : T. Sibley

Text of comment
or author's answer :

It is somewhat surprising that fine sediments do not accumulate ruthenium. Could you tell me how you isolated that sediment fraction and what the sediment concentration was in these experiments ?

Seawater from Weymouth was collected at a depth of 15 m and filtered through a Millipore 142 mm prefilter (pore size 25 µm) to give "coarse sediment". Subsequent passage through a 0.45 µm pore size filter gave "fine sediment". 50 litres seawater gave 390 mg "coarse sediment" but very little "fine sediment". However, both sediments were resuspended at 100-times their concentration in the sea. In a typical experiment dry weights of coarse and fine sediments used were 30 and 2 mg in 100 ml. These experiments therefore given only an indication of the relative contributions of sediment types as they exist in the marine environment.

Transfer of seston to zooplankton in marine ecosystems

M. Tackx

Delta Institute for Hydrobiological Research

Introduction

When considering zooplankton feeding as one of the pathways involved in the transfer of substances in marine ecosystems, it is important to study the composition and size distribution of the seston: firstly, the elements in which one is interested may be associated with different types and sizes of particles; secondly, particle size and composition are important factors in relation to the feeding process of the zooplankton itself.
High speed cinematographic studies have recently revealed that copepods use two different mechanisms for feeding. Small particles (< 12 µm) are collected by continuous low amplitude movements of the second maxillae and combing of the appendages. Larger particles are detected and collected individually by means of a specific sequence of movements of several mouthparts (Price et al., 1983). This feeding mode enables copepods to collect particles from larger volumes of water than would be possible by strict filter-feeding. It also enables them to select and reject certain types of particles (Paffenhöfer et al., 1982).
In the future these cinematographic techniques will probably be of great use to determine on which type of particles copepods and other zooplankton organisms feed precisely. For quantitative grazing measurements we have, at the moment however, still to rely on more indirect methods.

Methods and results.

This paper presents some data on zooplankton grazing measured in the Eastern Scheldt, an estuary in the south-west of the Netherlands.
Fig. 2 shows the results of a grazing experiment carried out by Daro (Fig. 1) using an in situ C-14 method (Daro, 1978). The seston was devided into 3 size classes: <25, 25-100 and > 100 µm diameter. 6 Measurements were performed in the course of the day. Ingestion rates measured for Acartia tonsa, the dominant copepod in the area are higher on both the <25 and

> 100 µm size class than on the 25-100 µm one. As the former size classes contain the highest chlorophyll concentrations, these results indicate an opportunistic feeding behaviour.
To measure grazing pressure on different particle size classes we have used the counting method (Fuller & Clarke, 1936) as described by Tackx & Francke (1983)
Fig. 3 shows a typical example of the results obtained. Filtering rates (a measure for the grazing pressure) calculated per size class shows that feeding takes place over a wide size-range (4 to 90 µm) and increases with particle size.

Discussion

The presented grazing results show that feeding by A. tonsa occurs on a broad size range and in an opportunistic way. From microscopical analysis, the principal components of the Eastern Scheldt seston are known: phytoplankton species, uniformely shaped detritus particles, aggregates composed of numerous small particles and sand (Bakker et al., 1983). Regular counting and measuring of these components shows that considerable seasonal changes occur, both in total size distribution and in relative abundance of the different components (Bakker et al., in prep.)
During the period in which zooplankton is active in the estuary (march - september), the animals encounter varying combinations of particle types and sizes. This implies that the uptake of elements associated with these different particles may also vary seasonally.

Acknowledgements

My thanks are due to C. Bakker and M.H. Daro for providing information on seston composition and C-14 grazing measurements respectively. J.W. Francke carried out Coulter analysis and drew the figures. E.K. Duursma and C. Bakker critically read the manuscript, and E.S. Nieuwenhuize typed it.

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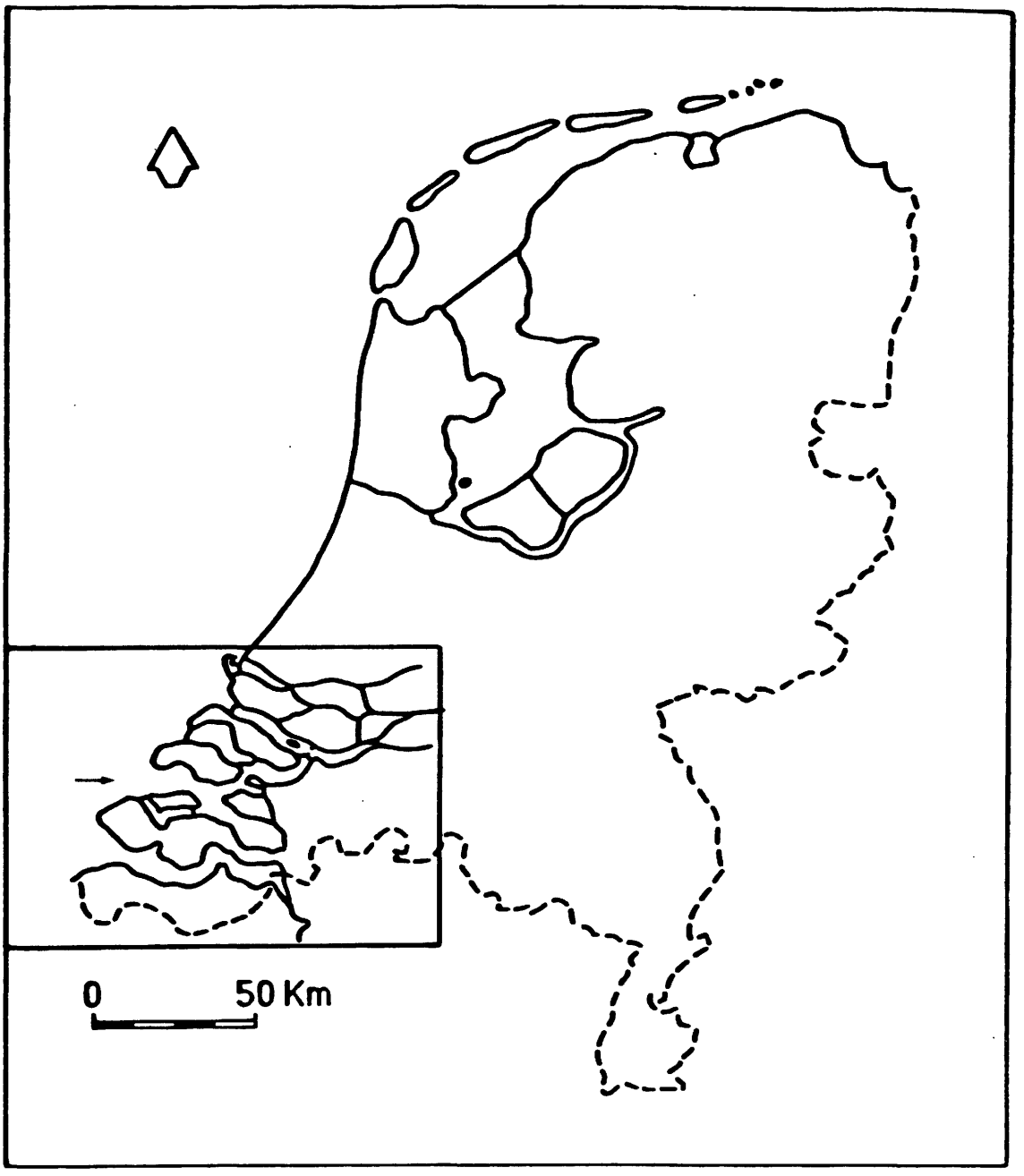


FIG. 1

Localisation of the Eastern Scheldt (⟶) in the Delta area of the s.w. Netherlands.

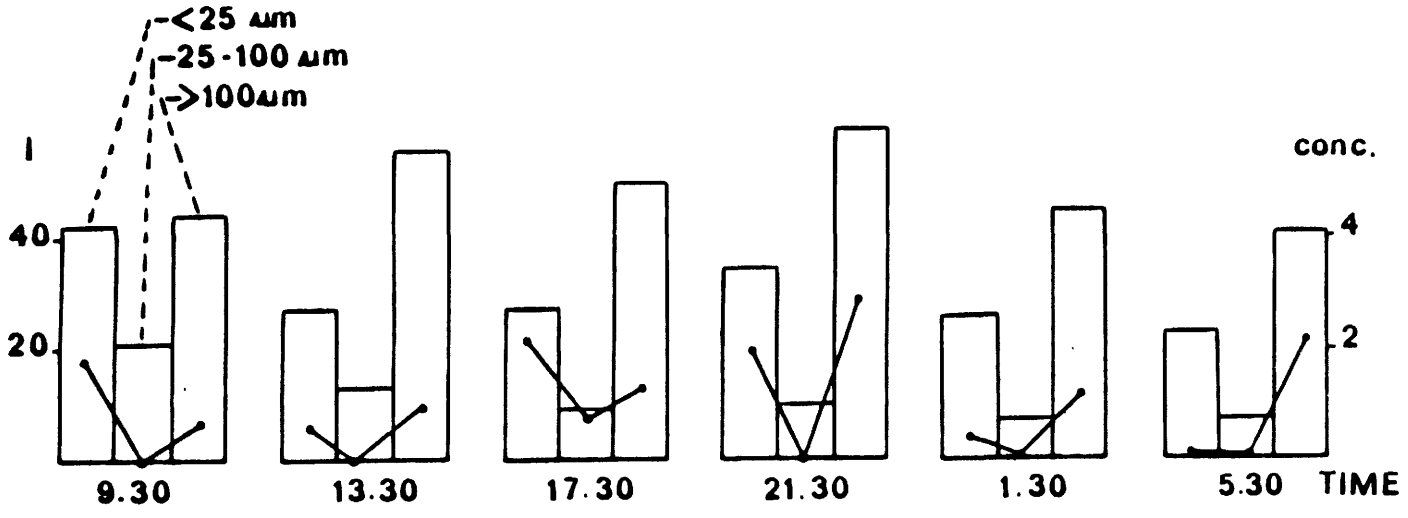


Fig. 2 Ingestion rates (I) of A. tonsa on 3 size fractions of Eastern Scheldt seston, measured with the C-14 method. Chlorophyll concentration in each size class (bars) in mg m^{-3} (right scale); Ingestion rate (left scale) in µg C.ind^{-1} hr^{-1}

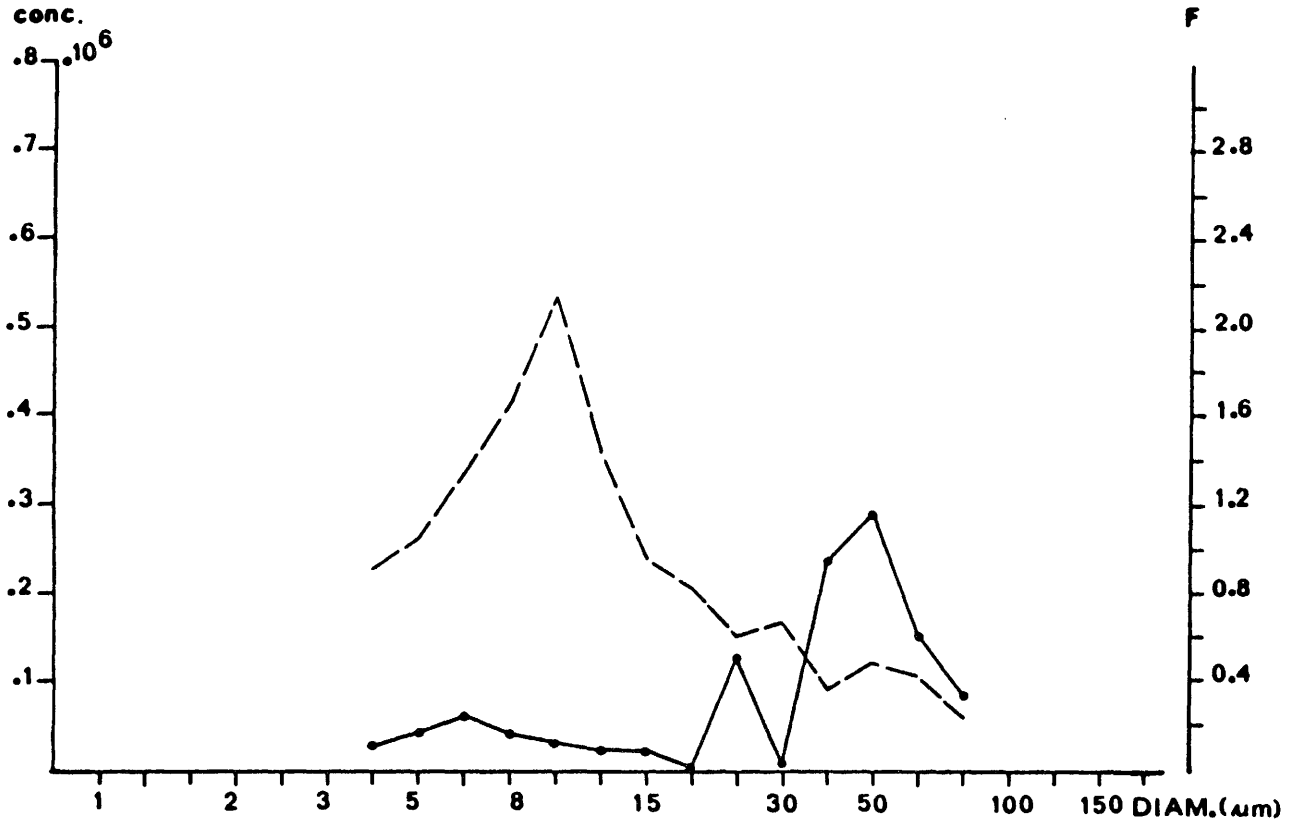


Fig. 3 Feeding pattern of A. tonsa on Eastern Scheldt seston, measured with the counting method.

—— —— particle concentration ($\mu m^3.ml^{-1}$)

•——• Filtering rate (F, ml ind^{-1} h^{-1})

absis: particle diameter

Session : I/B

Paper by : M. Tackx

Comment by : S. Bonotto

Text of comment
or author's answer :

Do you know if Acartia tonsa is digesting all the particles or if it rejects some of them in the external medium ? Would it be possible eventually to quantify this rejection process ?

Faecal pellet studies of several species of copepods have shown that indeed some particles are found to pass intact through the gut.

SESSION II : Fresh-water microorganisms

Chairman	J.PIERI
Co-Chairman	T.H.SIBLEY

SOME DYNAMIC ASPECTS OF THE PLANKTONIC FOOD CHAIN IN LAKES

G. BALVAY

Station d'Hydrobiologie Lacustre I.N.R.A.
75, Avenue de Corzent, B.P. 11 F, F-74203 THONON LES BAINS

ABSTRACT

When studying concentration factors of any pollutant along the different trophic levels in an ecosystem, attention must be paid to the dynamic aspects of the functioning of the food chain. Various factors can act upon the quantity, quality and speed of matter transfers, and can cause changes in the consumption activities of the animals involved.

CONTENTS

1 INTRODUCTION

Living organisms exhibit to a different extent the property to accumulate in their tissues non- or partly biodegradable substances. In a contaminated ecosystem, due to the phenomenon of biological magnification, a pollutant concentrates progressively along the successive links of the food chain (Figure 1). The concentration of poorly-biodegradable pollutant reaches its maximum value in the upper trophic level; the ratio between two consecutive levels is expressed as the concentration factor. This concentration factor is affected by many processes, including the interactions within the trophic web, relationships between the organisms and their environment, and the behavior of the organisms.

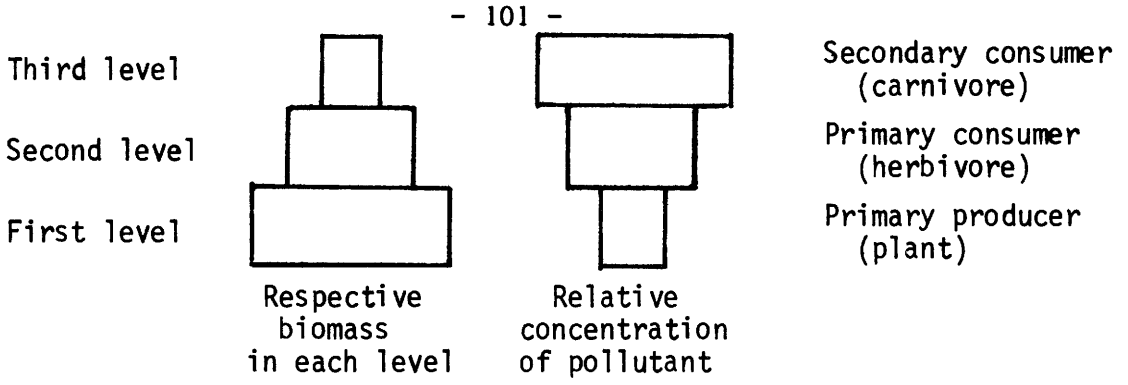


FIGURE 1 Bioaccumulation along a simplified trophic chain.

2 THE TROPHIC CHAIN IN THE PELAGIC ENVIRONMENT

2.1 Different Patterns of Feeding Behavior

Numerous zooplanktonic species filter water with a ciliated apparatus (Ciliata and many Rotatoria) or with setae-supplied appendages (most Cladocera and Calanoida). Under the term filter-feeders are included different zooplanktonic herbivores, feeding upon a mixture of phytoplankton, bacteria and detritus.

Some species of zooplankton are detritivores, ingesting mainly nonliving organic particles with attached bacteria; such as the rotifers *Filinia longiseta, Anuraeopsis fissa, Hexarthra mira* or *Pompholyx sulcata* [1].

Other animals catch their vegetal or animal prey. Although generally algivorous, some rotifers (e.g., *Synchaeta, Polyarthra*) are not filter-feeders but micropredators. All the true carnivorous species are considered as predatory zooplankton, including some Rotatoria (*Asplanchna, Ploesoma*), some Cladocera (*Leptodora, Bythotrephes*), most of the Cyclopoids (mainly the older copepodids and the adult stages) and the phantom midge larva *Chaoborus*.

This classification according to diet preference is obviously inexact because food spectra can vary from strict stenophagy to polyphagy. In addition to herbivores, carnivores and detritivores, there are also omnivores. It is sometimes difficult to define the diet of some species such as the Cladocera which eat phytoplankton as well as bacteria or the detritus-bacteria complex.

2.2 Structure of the Trophic Chain

The production of organic matter in any ecosystem is determined by the functioning of the trophic chain, a complex system of interactions connecting the different biological components of the biocenosis.

In a lacustrine ecosystem, plankton forms only one link of the trophic web, but the role of such pelagic organisms is very important as initiators

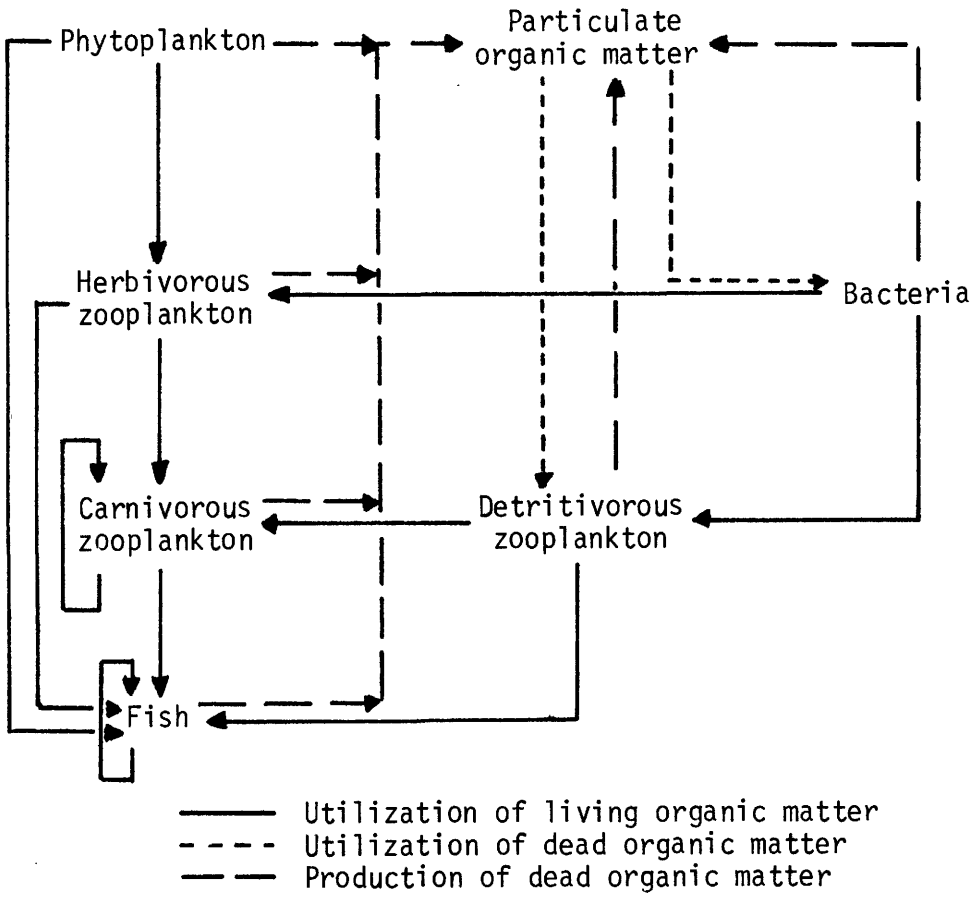


FIGURE 2 Simplified trophic chain in a lake.

of the food chain,thus elaborating organic matter by auto- or heterotrophy, and providing food for the next consumer level.

The elaboration of organic matter starts with phytoplankton primary production. Algae cells are eaten mainly by filter-feeding zooplankton (Rotatoria,Cladocera,Calanoida); herbivorous zooplankton becomes the prey of fish and carnivorous zooplankton, Cyclopoida and some Cladocera which in turn are eaten by fish. This is the direct path of production in the pelagic environment. Matter and energy flows are shortened with phytoplanktonophagous fish, or lengthened by additional levels of predatory zooplankton or fish[2](Figure 2). All these organisms, if they are not preyed as living food by their own consumers, produce dead organic matter which is in turn used as food by detritivorous invertebrates and bacteria. As living food, detritivores are preyed upon by carnivorous animals, whereas bacteria are ingested by filter-feeding organisms. The two last pathways create a recycling system for non-living organic matter,thus departing from the direct flow of organic production.

In general, the direct pathway is the most important one in an oligo-

trophic lake, whereas the recycling pathways tend to predominate as eutrophication increases.

2.3 Losses of Matter and Energy along the Food Chain

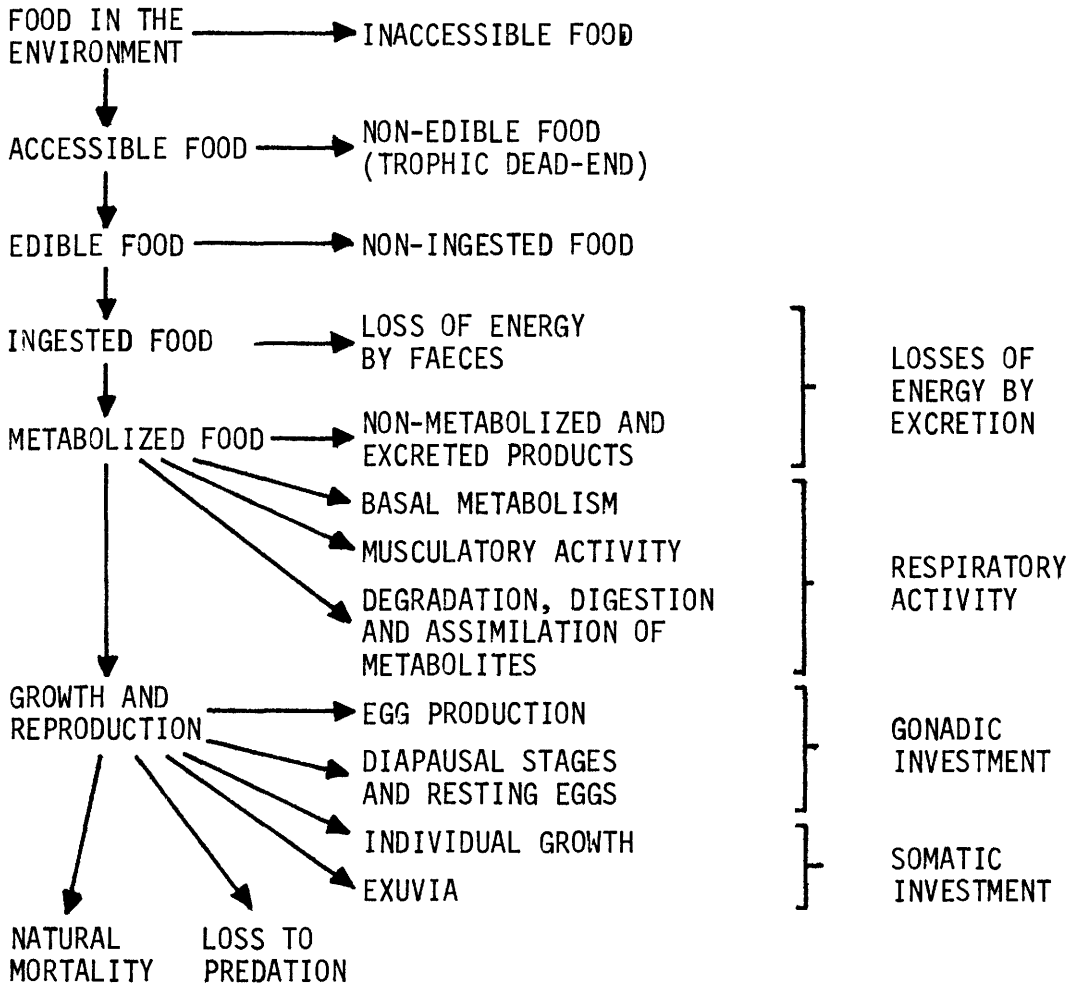


FIGURE 3 Schematized losses of energy for any level of the food chain.

Losses of energy appear in and between all levels of the food chain, reducing the efficiency of the production[2,3] (Figure 3). For example, the energy produced in successive levels of the food chain has been determined for different types of lakes[4,5] (Table I): due to the losses of energy in each link, the global efficiency of the trophic chain is quite low; fish yield amounts to only 0.3 to 0.6 per cent of the total primary production.

3 PREDATOR - PREY RELATIONSHIP

The diet of an animal is generally plurispecific in the presence of a natural assemblage of plankton, since monospecific foods can produce problems for the growth and reproduction of the predator. A monospecific food appears to be generally deficient in meeting all nutritional requirements.

TABLE I Estimation of the net production (in kcal.m^{-2}) during the growing season (about 150 days for lake Driviaty, and 180 days for the other lakes).

	Lake Naroch	Lake Batorin	Lake Myasto	Lake Driviaty
Primary production				
Phytoplankton	486.6	1758.0	1574.0	1200
Macrophytes	594.0	66.0	135.0	100
Periphyton	500.0	40.0	100.0	120
Bacterioplankton	169.0	344.0	401.0	442
Zooplankton				
Filter-feeders	55.4	138.0	116.7	120
Predators	19.5	53.8	44.6	31
Benthos				
Non-predatory	12.3	11.5	3.3	11
Predatory	0.7	1.4	0.7	2.5
Fish				
Plankton-feeders	2.2	0.9	2.4	7.5
Benthos-feeders	1.3	6.0	2.6	
Predators	0.9	1.4	1.4	1.5

The proper utilization of a single food seems to depend on substances supplied by other kinds of food present in the environment. TAUB and DOLLAR[6] showed the nutritional inadequacy of *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* as food for *Daphnia pulex*. This cladoceran could not be maintened indefinitely on such monospecific foods.

3.1 Spatial and Temporal Segregation

As the year progresses, the circulation of matter and the flow of energy vary in intensity (both in quantity and rate) between the different levels of the trophic chain. The phytoplankton - zooplankton relationship depends on the biological cycle, the behavior of the organisms and also on the physical and chemical characteristics of the aquatic environment.

All the food existing in a given ecosystem may not be immediatly or easily accessible to its consumer, owing to possible differences of location according to layers of the water column or to different ecological habitats, or due to the respective seasonal dynamics of both predator and prey. The consumer and its food must occupy the same spatial location simultaneously to allow trophic utilization of the prey.

In nature, three mechanisms are known which obviate competition or predation: different vertical or horizontal distributions, different seasonal dynamics and functional specialization for different foods. According

to the predator's or the prey's perspective, vertical migration acts to promote or avoid contacts. Likewise, a shift in time of abundance peaks of predator and potential prey results in reduced predation. Similarly, the successive appearance of species preying on the same food limits competition between these predators, but also increases the duration of the predation season. As a general rule, the same species may be ingested by a great variety of predators, as demonstrated by WILLIAMSON[7].

3.2 Factors influencing Susceptibility to Predation

A varying proportion of the food accessible can be unavailable for a given consumer for different reasons:

- Size of the prey: small animals (e.g., *Ceriodaphnia, Bosmina*) are caught to a lesser extent than are bigger ones (e.g., *Daphnia*) in the same environment;
- Prey morphology: after encounter and attack, the strike efficiency of the predator (*Cyclops*) is not only related to the size, but also to the shape of the prey; elongated animals such as *Diaptomus* are more easily caught than are rounded ones such as *Bosmina*;
- Body ornementation: spines or other protrusions in algae (e.g., *Staurastrum*) and rotifers (e.g., *Kellicottia, Brachionus*), and elongated (helmeted) forms of *Daphnia* provide reduced susceptibility to predation. *Brachionus calyciflorus* is able to radically change its morphology, producing long spines when coexisting with its predator *Asplanchna*[8];
- Texture and structure of prey: unmanageability can result from rigid cell walls (algae), reinforced integument, or morphological protection with a gelatinous sheath (*Holopedium gibberum*);
- Taste: after initial ingestion, the taste of an unpalatable alga may be sufficient to cause release of the cell unharmed, or to prevent further predation;
- Toxicity: blue-green algae such as *Microcystis aeruginosa* produce toxins which can be fatal to aquatic invertebrates and fish and even to cattle drinking from water with heavy blooms of these blue-green algae;
- Undigestibility: some green and non-toxic blue-green algae such as *Sphaerocystis schoeteri* protected by a gelatinous sheath can pass through the gut of planktonic animals and emerge viable;
- Visibility: to avoid predation, some species like *Diaphanosoma brachyurum* exhibit reduced pigmentation. However, for some transparent Cladocera, the strong visual contrast of the brown ephippia increases predation; ephippial *Daphnia* are quickly removed and are thereafter relatively scarce in the

plankton of Lake Geneva (Lac Léman).

- Behavior: animals possessing visual, mechanical, or chemical receptors exhibit various escape responses such as

reduced swimming speed (small species)

active escape response (*Daphnia pulex, Diaptomus*)

spiralling escape trajectories (*D. pulex, Ceriodaphnia reticulata*)

"dead man" response, with no movement and/or passive sinking (*Bosmina longirostris, Chydorus*).

3.3 Filter-feeding Behavior

3.3.1 Size-Selection of Food particles

Filter-feeders discriminate among particles on the basis of size, texture, shape and taste. The upper and lower size limits of particles that can be collected or managed are determined by the characteristics of the filter apparatus. Since particles greater than or equal to 0.8 µm in diameter can be retained by most filter-feeders, all small algae (nanoplankton) are available for ingestion. The upper size limit of ingested particles is directly related to the size of the consumer.

Using seven species of Cladocera, BURNS[9] established the formula $D = 22L + 4.87$ where D (in µm) is the diameter of the largest plastic bead ingested and L (in mm) the carapace length.

Sometimes bigger particles may be ingested; the largest planktonic *Daphnia* can ingest whole 45 µm spherical particles and even larger non-sperical ones if they are elongated and pliable. PORTER and *al.*[10] reported the unusual ingestion of *Paramecium caudatum* (200 µm in length) by *Daphnia magna*.

Non-ingested algae are often large, filamentous or colonial species, or have rigid cell walls, spines or other ornementation. These unmanageable algae clog the filtering appendages. Cladocera reject such particles by the movements of the post-abdominal claws, and the pseudo-faeces can be reused by detritivores and bacteria.

When the phytoplankton is dominated by algae within the size range of particles (about 40 µm) ingested by large filter-feeders, primary production is well used. If the phytoplankton is dominated by large algae, primary production is poorly used, and benefits primarly detritivores and bacteria. In this case the zooplankton community is dominated by smaller detritivorous filter-feeders.

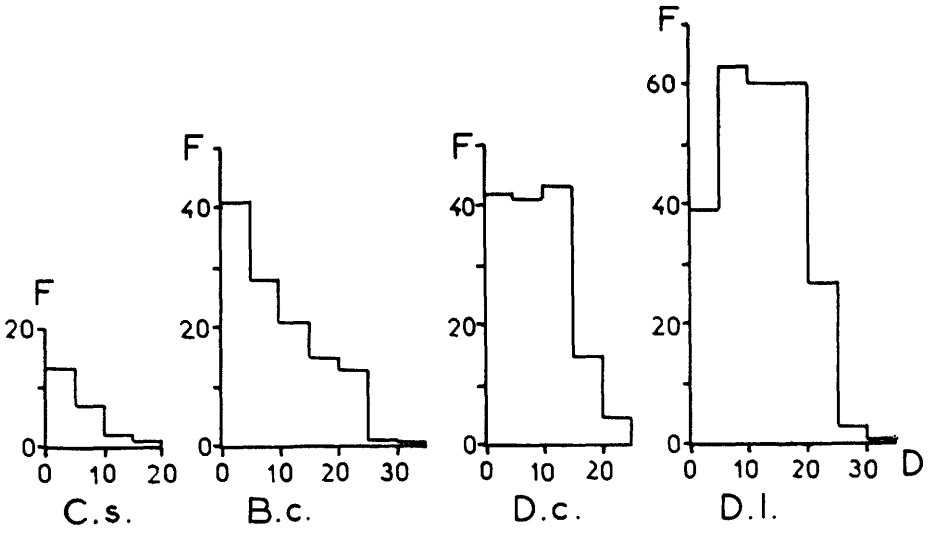


FIGURE 4 Filtering rates F (in $\mu l \cdot ind^{-1} \cdot h^{-1}$) of *Chydorus sphaericus* (C.s.), *Bosmina coregoni* (B.c.), *Daphnia cucullata* (D.c.) and *D. longispina* (D.l.) in respect to particles of various size ranges (Diameter D in μm) in June 1973.

Filter-feeders show preferences within the size spectrum of ingestible prey for food particles of a particular size[11] (Figure 4). As a general rule, the food spectrum is extended towards larger particles as the size of the consumers increases.

3.3.2 Nature of the Food

Exemples of the feeding specificity of some rotifers have been published and reviewed by POURRIOT[1]. When offered various foods of suitable size, *Hexarthra fennica* had a clear preference for Chlorococcales, *Ascomorpha ovalis* for Dinoflagellates, and *Synchaeta lakowitziana* for Chrysomonades; *Anuraeopsis fissa*, *Hexarthra mira* and *Conochilus unicornis* ate exclusively detritus and bacteria.

Some filter-feeders are able to separate living and dead cells[12]; the detritivorous rotifer *Keratella cochlearis* preferentially ingested heat-killed in comparison to living *Chlamydomonas reinhardtii*. In the presence of senescent cells of *Chlorella vulgaris*, *Daphnia magna* decreased both filtering rate (from 2.7 - 3.4 $ml^{-1} \cdot h^{-1}$ to 0.7 $ml^{-1} \cdot h^{-1}$) and maximum feeding rate (from 30 - 60 x 10^4 cells.h^{-1} to 3 x 10^4 cells.h^{-1})[13].

3.3.3 Composition of the Prey Complex

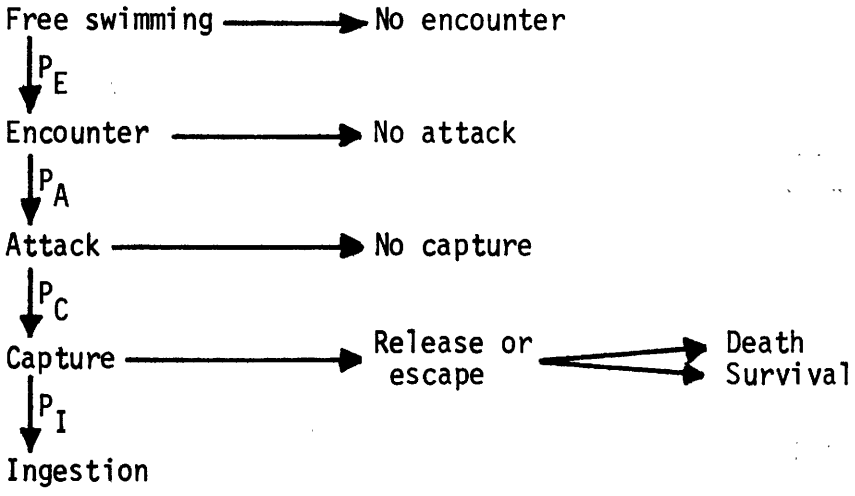
The consumption of a particular food species can be affected by the presence of other species within the food mixture[14]. Ingestion of *Euglena gracilis*

by *Brachionus calyciflorus* was greatly decreased in the presence of even small densities of the yeast *Rhodotorula glutinis*. Ingestion rates of *R. glutinis*, on the other hand, were independant of the quantity of algae present.

In an experimental mixture of particles of various sizes where the total biomass of each particle type was equal, daphnids chose the more abundant prey (i.e., the smaller particles). However, in mixtures with equal numbers of particles of each size type, the bigger particles were selected preferentially[15].

3.4 Predatory Behavior

The amount of available food used by any predator is related to the facility of the catch. The act of predation can be characterized as a series of sequential events leading to ingestion or escape or renunciation of the prey[16]:



The product of the conditionnal probabilities of success associated with each event gives the probability of the successful interaction (P_{SI}): $P_{SI} = P_E \times P_A \times P_C \times P_I$ where P_E = probability of encounter and P_A, P_C and P_I respectively = probability of attack given encounter, capture given attack and ingestion given capture.

The probability of the successful capture improves with the age of the predator; for example, for pike fry, this probability increases from about 30 per cent during the first week of active feeding to 80 per cent after two weeks[17].

The feeding response of the predatory rotifer *Asplanchna girodi*, when starved for about two hours, gives some indication on these different events[18] (Table II).

TABLE II Feeding response of *Asplanchna girodi* to the Dinoflagellate *Peridinium* and to the rotifer *Keratella cochlearis*.

Number of	Encounters	Attacks	Captures	Ingestions
Peridinium	62	13	13	0
K. cochlearis	13	12	7	6

GERRITSEN and STRICKLER[19] estimated the encounter rate (Z_H),

$$Z_H = \frac{\pi R^2 N}{3} \times \frac{U^2 + 3V^2}{V} \quad \text{for } V > U$$

or $$Z_H = \frac{\pi R^2 N}{3} \times \frac{V^2 + 3U^2}{U} \quad \text{for } U > V,$$ where Z_H = prey's encounter rate with predators (in predators encountered per prey per unit time), R = the predator's encounter radius, N = density of predators, U and V respectively mean swimming speeds of prey and predator. While hunting prey, *Chaoborus* adopts a characteristic "ambush strategy" with an encounter radius of about 5 mm[20].

When predator and prey populations share the same habitat, encounter probabilities depend on the relative density of both populations as well as on behavioral and sensorial characteristics of each species. In the pelagic environment, planktonic animals exhibit patchy rather than random distribution in space, and they swim in various directions with given mean speeds. The predator and the prey may also have a distance of perception that varies according to direction.

From the prey's perspective, the best strategy is to avoid encounters entirely, using seasonal and spatial segregation. Minimization of encounter rate can be obtained by the prey decreasing its speed, which thereby decreases the predator's encounter radius. A prey animal can swim slowly, creating as few mechanical disturbances in the water as possible. However, this behavior may impose constraints for the prey's own needs for mating or feeding.

An electivity index (or Ivlev's Index) $I = \frac{S - E}{S + E}$ gives an indication of the prey preference to the predator, using the abundance percentage of the prey in the environment (E) and in the stomach content (S).

The value of this index ranges between -1 (total refusal, avoidance of the prey) and +1 (complete selection or active choice), 0 thus indicating passive ingestion. GUISET[21] determined values for the electivity index of some rotifers (Table III).

TABLE III Electivity index for some Rotatoria.

	Predator			
	Asplanchna priodonta	*Asplanchna girodi*	*Asplanchna brightwelli*	*Eloesoma hudsoni*
Polyarthra remata	0.34	-0.10	0.27	0.54
P. dolicho./vulgaris	-0.45	0.22	0.28	0.10
Anuraeopsis fissa	0.95	-0.08	0.82	
Asplanchna priodonta	-1	-0.93	-0.29	0.08
Tricho. pusilla/similis	0.67	0.45	0.67	0.96
Keratella cochlearis	-0.37	-0.14	0.02	-0.32
Synch. pectinata/grandis	-1	0.62	0.97	0.21

4 THE CONSUMPTION ACTIVITY

Two terms are conventionally used to describe the quantitative feeding activities of grazing zooplankton: filtering rate (clearance rate) and feeding rate (ingestion).

4.1 Filtering Rate

This parameter is ideally an estimate of the volume of water processed by an animal (or per unit animal weight) during an interval of time when the animal is feeding. However filter-feeders are seldom 100 per cent efficient in the removal from water of suspended particles. Rotatoria readily reject particles already collected or exclude particles before processing. Filtering rate is measured as the volume of water containing the number of cells or biomass actually ingested by the animal per unit time.

Filtering rates of Rotatoria vary between less than 0.1 to 50 $\mu l.ind^{-1}.h^{-1}$, depending upon food density and food type. Most published clearance rates range from 1 to 10 $\mu l.ind^{-1}.h^{-1}$ in the 20-25°C temperature range[14]. For Cladocera, the highest values measured during laboratory experiments were respectively 144, 81.6, 74 and 23 $ml.ind^{-1}.day^{-1}$ for *Daphnia middendorffiana, D. magna, D. pulex* and *D. longispina*, as reviewed by CHAMP and POURRIOT[22]. *In situ* rates are generally lower; *D. longispina* filters up to 4.5 $ml.ind^{-1}.day^{-1}$, and *Bosmina coregoni* 1 $ml.ind^{-1}.day^{-1}$ [23], *D. galeata* and *Ceriodaphnia quadrangula* filter 6.4 and 4.6 $ml.ind^{-1}.day^{-1}$ respectively[24].

4.2 Factors affecting Filtration Rates

Filtration rates may be affected by both biotic and abiotic factors, such as: size and age of the consumer, its reproductive state and sex;

temperature, light intensity, dissolved oxygen concentration.

4.2.1 Biotic factors

Animal size: Filtration rate (F) increases with the size of the animals. For different species of *Daphnia* at a given temperature, F ($ml.ind^{-1}.h^{-1}$) is directly related to body length[25]: $F = 0.153\ L^{2.16}$ at 15°C, (L in mm), $F = 0.208\ L^{2.80}$ at 20°C, $F = 0.202\ L^{2.38}$ at 25°C.

Filtering rate also depends on the surface area of the filter appendages; this area varies according to species, even among individuals of the same size: the filtrating rates of *Daphnia rosea* are higher than those of *D. magna*[26], and in general a daphnid filters more than a calanoid of the same weight[27].

Physiological State and Sex: Egg-carrying females of *Eudiaptomus gracilis* have a 20-25 per cent higher filtration rate than females without eggs[28,29]; in addition, *Diaptomus* females exhibit a more active filtration rate than do males[23]. Non-ovigerous females of *Daphnia schoedleri* have a slightly lower filtration rate than do males[30], but the mean filtration rate for matures females increases with the number of eggs and ranges from 0.8 to 1.35 $ml.ind.^{-1}h^{-1}$.

Diel changes in consumption activity: The filtering rate of filter-feeders has been found to vary with day[11] (Figure 5).

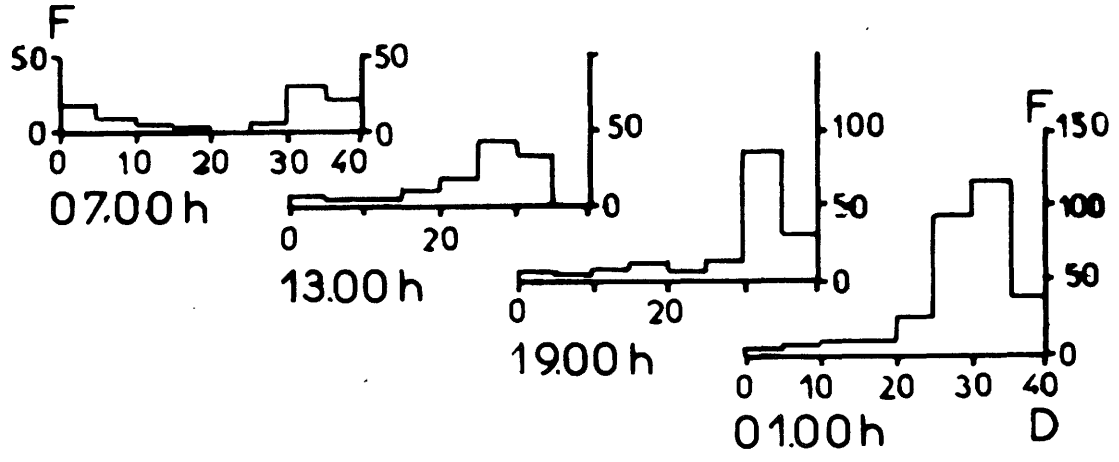


FIGURE 5 Filtering rate F ($\mu l.ind^{-1}.h^{-1}$) of *Eudiaptomus graciloides* in respect to particles of various sizes (diameter D in μm) at various times of day.

Several predatory species exhibit diurnal feeding periodicity similar to that observed for filter-feeders. Both *Tropocyclops prasinus* and *Cyclops bicuspidatus* have higher predation rates at midnight than at noon[31] (Table IV). Furthermore, *T. prasinus* exhibited a higher predation rate at 20 m,

and *C. bicuspidatus* was more efficient at a depth of 5 m.

TABLE IV Predation rates in numbers of prey consumed per predator per hour (A) and per day (B).

	Predator	Depth and time 5 m 12.00	5 m 24.00	20 m 12.00
A	*T. prasinus*	0.025	0.204	0.172
	C. bicuspidatus	0.048	0.142	0.019
B	*T. prasinus*	0.60	4.91	4.13
	C. bicuspidatus	1.17	3.41	0.45

Food concentration: As reviewed by POURRIOT et al.[34], the decrease in filtering rate when food concentration increases above a critical level has been widely reported for all types of zooplankton: cladocerans[22,35], calanoids[36], rotifers[14], and also ciliates[37] (Figure 6).

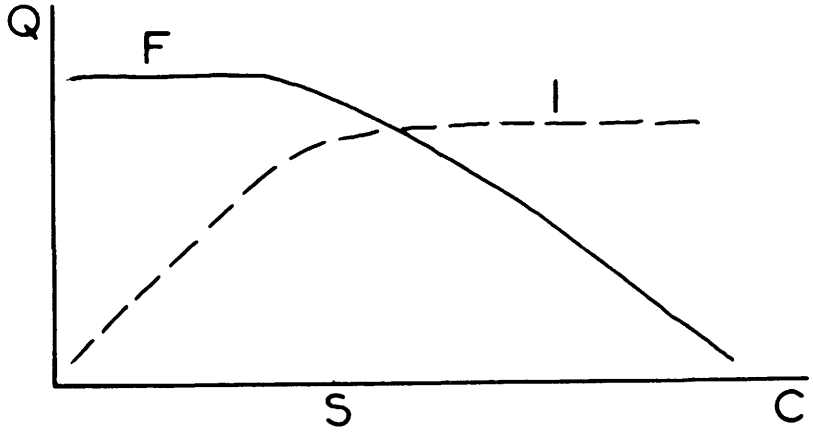


FIGURE 6 Changes in filtration rate (F) and in ingestion (I) for herbivorous zooplankton with increase in food concentration (C) above and below a certain threshold (S). Q = filtration or ingestion rates per animal per hour.

As a general trend, filtration and feeding rates are correlated with food concentration. Even at low food concentration, filtration rate is sufficiently high to permit Cladocera to satisfy their demand for energy. At high food concentrations, filtration rate is minimal, but remains high enough to satisfy oxygen requirements[32].

Food concentration also exerts important effects upon egg production[33] (Figure 7).

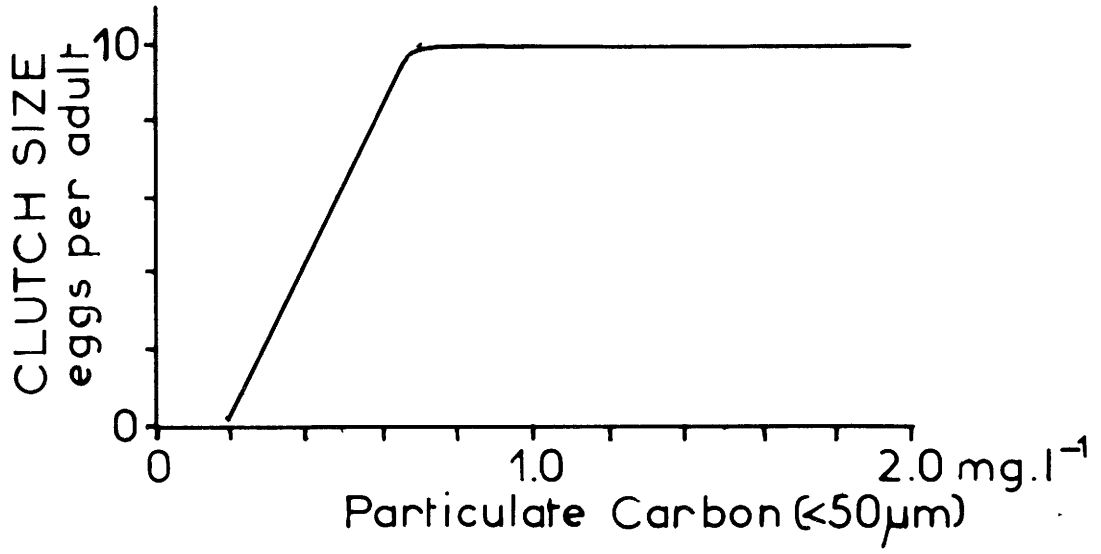


FIGURE 7 Dependence of fecundity of *Daphnia* on availability of ingestible carbon.

In *Daphnia*, there is a threshold food concentration below which no eggs are produced, and a maximum concentration above which there is no further increase in fecundity.

Incipient limiting food concentrations (above which there is no increase in filtering rate) vary with species of filter-feeders and with the kind and size of food[14,22] (Table V).

TABLE V Influence of food type on the feeding dynamics of some Rotatoria[14] and some Cladocera[22].

Species Food Type	Critical concentration (Cells density)
Brachionus plicatilis	
Chlamydomonas	1.5×10^5 cells.ml^{-1}
Chlorella	2.1×10^6 cells.ml^{-1}
Brachionus calyciflorus	
Aerobacter	10^{12} cells.ml^{-1}
Euglena	$1\text{-}2 \times 10^4$ cells.ml^{-1}
Daphnia magna	
Escherichia coli	2×10^6 cells. l^{-1}
Saccharomyces cerevisiae	$0.05\text{-}0.2 \times 10^6$ cells.l^{-1}
Tetrahymena pyriformis	1100 cells.l^{-1}
Daphnia middendorffiana	
Chlamydomonas reinhardtii	5×10^6 cells.l^{-1}
Daphnia rosea	
Rhodotorula glutinis	0.1×10^6 cells.l^{-1}
Daphnia longispina	
Bacteria	0.4×10^6 cells.l^{-1}
Bosmina coregoni	
Bacteria	0.4×10^6 cells.l^{-1}

4.2.2 Abiotic Factors

The most important abiotic factor is water temperature. Optimum temperatures vary according to species[25] (Table VI).

TABLE VI Mean maximum filtering rate (ml. mg dry weight^{-1}.h^{-1}) for different species of *Daphnia*.

Daphnia species	Temperature		
	I5°C	20°C	25°C
D. magna	8.0	I6.6	I9.0
D. schoedleri	I2.6	I5.9	II.3
D. pulex	I3.6	I5.9	I2.8
D. galeata	I0.3	24.3	27.9

For the arctic *Daphnia middendorffiana*[38], acclimated to cold waters, maximum filtering rates occur between 11 and I3°C.

Notholca squamula breaks open the cell frustules of the diatom *Asterionella formosa* and removes the content ; at I0°C, this raptorial rotifer consumes on average 11.5 cells.h^{-1} (= 2.2 x I0^3 µg dry weight. ind.$^{-1}$.h^{-1}), but only 3.2 cells.ind^{-1}.h^{-1} (= 6.1 x I0^4 µg dry weight. ind^{-1}.h^{-1}) at 6°C[39].

Many other abiotic factors such as light intensity, water transparency and the presence of inorganic suspended material can act on feeding and filtering rates.

4.3 Feeding Rates

Feeding rate (quantity of food eaten per consumer per unit time) is influenced by the same factors than act on filtering rates.

For predatory animals as well as for herbivores, feeding rates are affected by the species composition of the food present. In the presence of two prey species, *Chaoborus trivittatus* specializes on its preferred food (*Diaptomus*) when resources are abundant[20] (Figure 8).

Daily food rations for both herbivores and carnivores, are related to the nature and density of the prey, the age of the consumer and the ambient temperature. For herbivorous organisms, the daily ration is related to filtration rate, and thus varies with the factors that influence filtration rate.Above the threshold food concentration, the inverse relation between filtration rate and food density stabilizes the food ration. Below this critical level, filtration rates are maximum, and the quantity of food

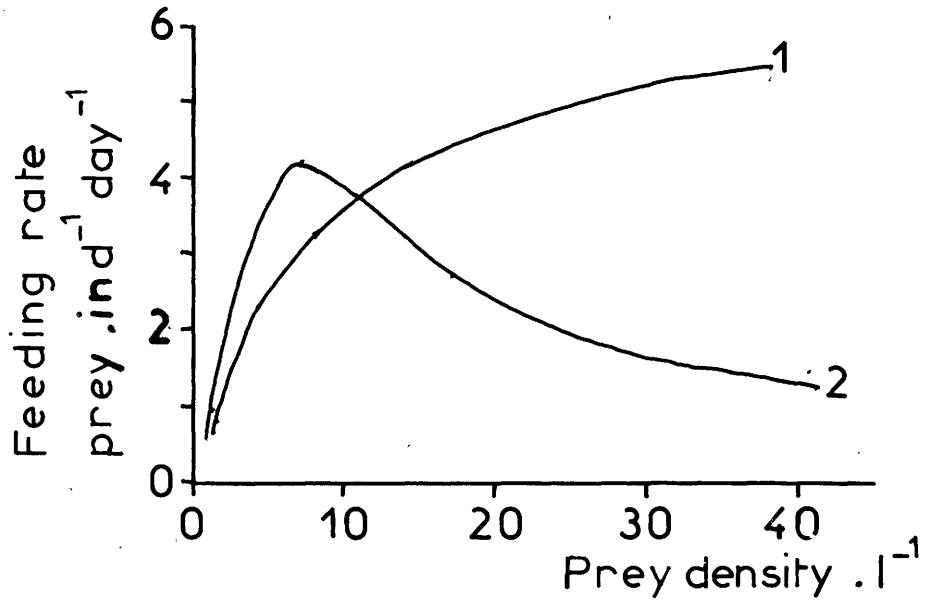


FIGURE 8 Diffential functional responses to *Diaptomus* (1) and *Daphnia* (2) preyed by fourth instar *Chaoborus trivittatus*.

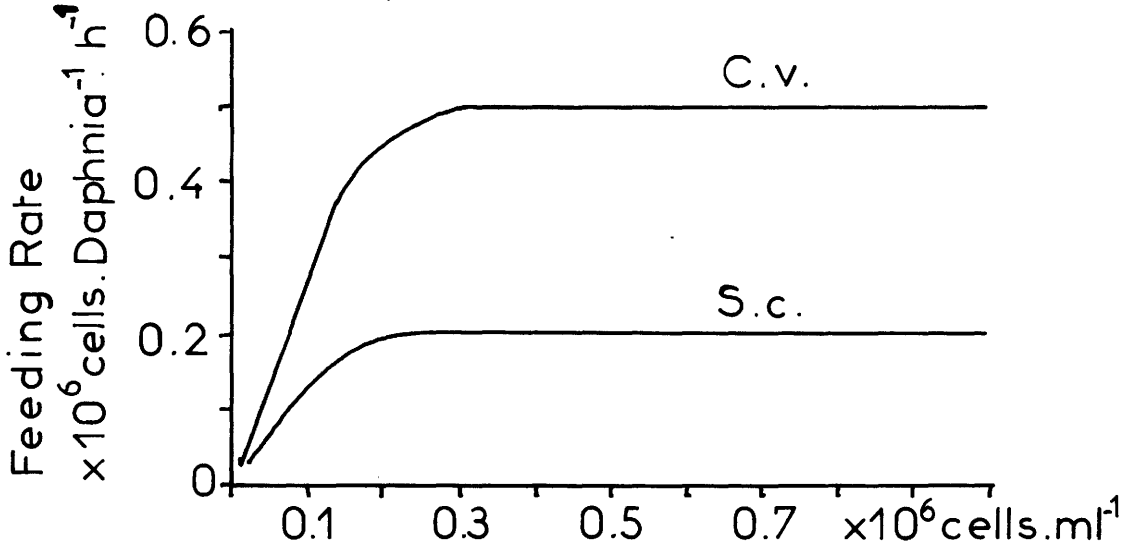


FIGURE 9 Feeding rate of *Daphnia magna* on *Chlorella vulgaris (C.v.)* and *Saccharomyces cerevisiae (S.c.)* at various levels of food concentration (redrawn from McMAHON and RIGLER[13]).

ingested is directly proportional to the food concentration[13](Figure 9).

When measuring ingestion rates, attention must be paid to gut passage time, varying, for example from complete gut renewal in 3 - 5 minutes for *Daphnia cucullata, D. hyalina* and *D. galeata*[29] to about 45 -60 minutes for *D. magna*[40].

Diel changes: Feeding rates like filtering rates, can exhibit diurnal variations. CHISHLAM et al.[38] showed a bimodal diel rhythm in feeding rates for *Daphnia middendorffiana*, with maximum values at 14.00 h and at midnight, when the daily temperature cycle passed through the mean daily temperature (Figure 10).

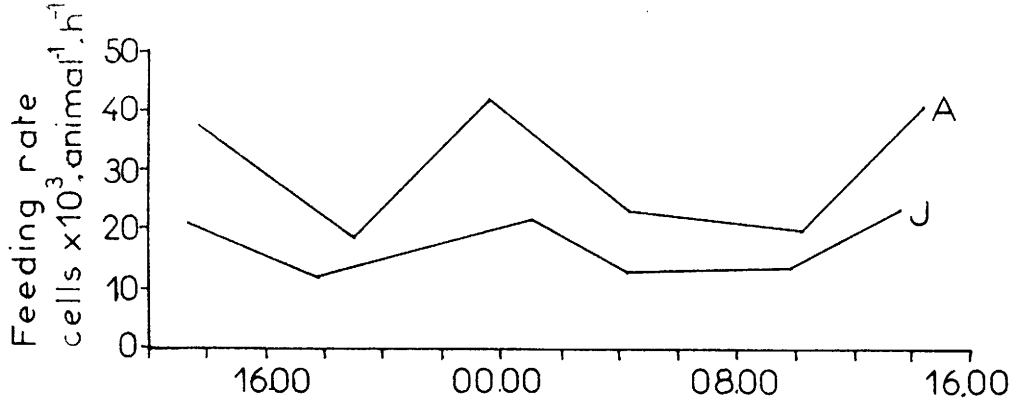


FIGURE 10 Feeding rate of *Daphnia middendorffiana* as a function of time of day, in July (J) and August (A).

The calculated ratio of maximum to minimum daily feeding rates ranges from near 1 for *Ceriodaphnia*[24] and small *Daphnia pulex*[41] to more than 25 for large *D. pulex* [41,42].

Prey density; Prey density has a demonstrable effect on feeding rate. *Daphnia schoedleri* fed at a rate proportionnal to food concentration up to about 10,000 *Ankistrodesmus* cells per ml [30]. Above this incipient limiting level, feeding rate remained constant at about 10,000 cells.ind^{-1}.h^{-1}.

In the same manner, predation rate for *Cyclops vicinus* showed a linear increase up to a prey concentration of 250 ind. 500 ml^{-1}. Above this level, the predation rate seemed to be constant at about 20 ind. Cyclops^{-1}.day^{-1}. In this species, the daily food ration is higher for ovigerous females than for females without eggs or for males[43]. Moreover, females ate mainly nauplii and rotifers, whereas males preferred copepodids.

The daily food ration of the predatory cladoceran *Leptodora kindtii* is about 30 per cent of its body weight (or 0.147 cal.ind^{-1}.day^{-1}); a population of mature *L. kindtii* consumed 15-43 per cent of the production of the non-predatory cladocerans in eutrophic lake Mikolajskie. Working in other lakes, both HALL[45] and WRIGHT[46] have estimated the consumption of the net production of the *Daphnia* population at 25-35 per cent by this same predator.

The dipteran larva *Chaoborus flavicans* is able to reduce the daily

production of crustacean zooplankton by about 50 per cent[47]. It is easy to imagine the devastating effects on zooplankton abundance when *Chaoborus* and *Leptodora* act together in a lake, the former feeding on both predatory and non-predatory zooplankton, the latter preying mainly on herbivorous forms.

It has well known that a high density of predators induces changes in the size distribution of the prey population, leading to the elimination of the larger prey animals, thus favoring the smaller organisms.

5 ASSIMILATION OF INGESTED FOOD

Among the different so-called "efficiencies" defined to estimate food utilization, two of the most often used are K_1 and K_2, where K_1 = Production / Ingestion and K_2 = Production/ Assimilation.

Numerous values for K_1 and K_2 are given by KAJAK and HILLBRICHT-ILKOWSKA[48]. According to ALIMOV[19], the amount of energy accumulated by aquatic invertebrates is generally 2.5 times the energy content of their definitive biomass. Mean values of K_2 during the life span time of an individual are close to 0.4; for populations, K_2 is approximatively 0.26, and for animal communities it is about 0.2. Increasing complexity of the biological system is accompanied by a decline in the efficiency with which the animals utilize assimilated food energy for production.

The following data give an indication of the fate of ingested matter, according to the theoritical diagram where values are expressed in percentage of food ingested[34] (and compared to I = 100 %):

Food ingested I → Assimilation A → Production P K_1 = P/I
(I = 100 %) → Excretion E → Respiration R K_2 = P/A

Brachionus rubens

I → A = 30 % → P = 15 % = K_1
  → E = 70 % → R = 15 % K_2 = 0.50

Brachionus plicatilis[50]

I → A = 19,4 % → P = 11.5 % = K_1
  → E = 80.6 % → R = 7.9 % K_2 = 0.59

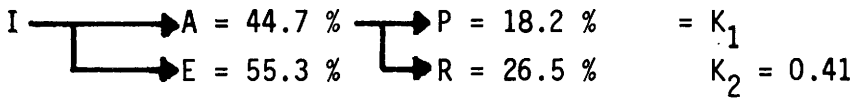
Daphnia ambigua

I → A = 28.2 % → P = 11.1 % = K_1
  → E = 71.8 % → R = 17.1 % K_2 = 0.39

Leptodora kindtii[44]

I → A = 87 % → P = 39 % = K_1
  → E = 13 % → R = 48 % K_2 = 0.45

Planktonic filter-feeding crustacean community[51]



K_1 and K_2 for a particular species are closely related to the physiological state of animals and to experimental conditions, such as food concentration and water temperature[34]. During the growth of *Macrocyclops albidus*, K_1 decreased from 0.50 to 0.20 with successive copepodid stages[52]; a similar decline in K_1 has also been observed with the growth of *Ceriodaphnia reticulata*[53].

6 CONCLUSION

In nature, the great diversity of size in both foods and consumers allows a continuous functioning of the trophic chain and optimum use of the organic matter produced at any production level (Figure 11)[34].

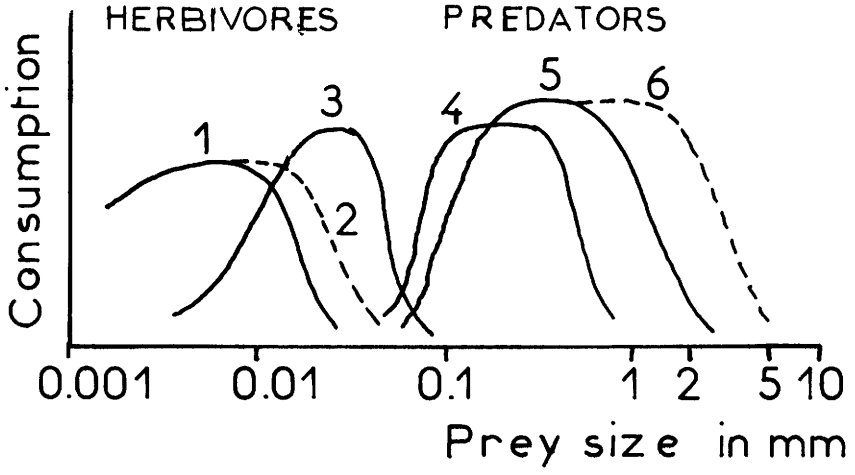


FIGURE 11 Partitioning in the use of food resources according to food size for various planktonic invertebrates (1-Rotatoria; 2-Cladocera; 3-Calanoida; 4-Asplanchna; 5-Predatory crustacea; 6-Chaoborus).

When studying the fate of matter entering or leaving the different levels of the trophic chain, difficulties quickly arise because each organism functions according to its own individual characteristics, which may cause it to react in a different way even as compared to very closely related animals.

Comparison of results obtained from different species in various environments must take into account both biotic and abiotic experimental conditions. Accurate estimation of the concentration factors in a trophic chain must be based on a sound knowledge of the biological interactions that govern matter and energy flows.

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Session : II

Paper by : Balvay

Comment by : M.J.Frissel

Text of comment
or author's answer :

Can you say something more on the units of the vertical axis of the last figure ?

In Fig.11, consumption is expressed in non-comparable arbitrary units.

Session : II

Paper by : Balvay

Comment by : S.Bonotto

Text of comment
or author's answer :

I would like to know of you have found evidence for "biomagnification" in the planktonic food chains ?
Secondly, as experimental food chains are useful, to follow the transfer of radionuclides, could you give some sqggestions for possible food chains to use under laboratory conditions ?

Existence for biomagnification has been prevously proved in marine and fresh-water planktonic food chains, mainly rrelated to heavy metals.
In lake Léman, biomagnification has been proved for some heavy metals (Hg, Cd, Pb, Sn) and pollutants (PCB).
Experimental food chains exhibit biomagnification, but these results are generally higher than those obtained from "in situ" food chains, due to the natural complexity of the trophic web.

Session : II

Paper by : Balvay

Comment by : C.Myttenaere

Text of comment
or author's answer :

Filtering may be considered as a very important mechanism in the decontamination of waters. Do you have any idea about the total quantity of water filtered by living organisms in a lake ecosystem ?

I keep in mind that in some lakes, filtratery 300 plankton can filtrate in one day about the total volume of lake water, but only during a short space of time when the maximum abundance peak of filtrators appears, in Spring or in Summer.

BILAN DE L'UTILISATION DES CULTURES D'ALGUES POUR LA DETECTION DES RADIO-ELEMENTS PRESENTS DANS LES EFFLUENTS DES INSTALLATIONS NUCLEAIRES

M. Bourdon[1], R. Kirchmann[2], J. Binet[3], E. Fagniart[2], A. Colette[1]

[1] *Institut de Botanique, Université de Liège, Sart Tilman, 4000 Liège, Belgique*

[2] *Département de Radiobiologie, C.E.N.-S.C.K., 2400 Mol, Belgique*

[3] *Institut d'Hygiène et d'Epidémiologie, 1050 Bruxelles, Belgique*

Introduction

En raison de la présence de certains radioéléments dans les effluents liquides provenant de centrales nucléaires, l'impact de ces rejets sur la productivité des algues d'une part, et sur la contamination radioactive résultant des radionucléides rejetés, d'autre part, a été recherchée (1-2-3). En effet, après culture sur effluents de centrale nucléaire, une série de radionucléides est fixée par les algues.

Cependant, tous les radionucléides présents dans l'effluent ne se retrouvent pas dans les algues, exemple : ^{124}Sb, ^{125}Sb, ^{144}Ce. Par contre, certains radioéléments sont observés dans les algues après culture alors qu'ils n'ont pas été détectés, en raison de leur faible concentration dans l'effluent mélangé avec le milieu de culture, exemple : ^{57}Co, ^{54}Fe, ^{186}Re, ^{65}Zn.

Il est par ailleurs intéressant d'observer que par l'utilisation de la technique de cultures d'algues, les paires de radioisotopes ^{58}Co et ^{60}Co d'une part et ^{134}Cs et ^{137}Cs d'autre part, sont présents dans les mêmes rapports de concentration dans les algues et dans les effluents (4).

Les tritium et carbone 14 sont des cas particuliers, ces radionucléides pouvant être à la fois présents dans les effluents sous forme ionique et sous forme organique. Les cultures d'algues sur effluents permettent de détecter ces molécules organiques biologiquement disponibles, d'en évaluer la fraction et par là, d'estimer l'impact radiologique. La culture d'algues sur effluents constitue donc un test de disponibilité biologique. Il faut d'autre part remarquer que la signification radiologique des rejets de ^{14}C par les installations nucléaires n'a été pleinement reconnue que depuis quelques années. L'importance du ^{14}C est due à sa longue persistance dans la biosphère et au fait qu'il est incorporé, à partir du $^{14}CO_2$, à la matière végétale par la photosynthèse. Dans le cas du tritium, cette méthode a été appliquée dès 1972 sur diverses catégories

d'effluents et les résultats ont fait l'objet de plusieurs publications (5-6-7).

En ce qui concerne le ^{14}C, la méthode n'est appliquée que depuis 1982 aux effluents provenant de centrales électronucléaires.

Dans la présente communication, nous rappellons le principe de la méthode, quelques résultats marquants, obtenus antérieurement, sont montrés à titre exemplatif ainsi que les résultats récents relatifs à la disponibilité biologique du tritium et du carbone 14.

Matériel et Méthodes

Scenedesmus obliquus a été choisi comme matériel biologique parce qu'il s'agit d'une algue commune d'eau douce, unicellulaire, représentative d'un des premiers maillons de la chaîne trophique dans les écosystèmes aquatiques. De plus, la multiplication de ce "végétal-test" est rapide et les conditions de culture aisément contrôlables.

Expérimentation en conditions contrôlées

1. Culture d'algues en vue de la détermination de la disponibilité biologique du tritium des effluents.

Un schéma du dispositif expérimental pour la culture de l'algue Scenedesmus obliquus en conditions contrôlées est donné dans la figure 1 ci après :

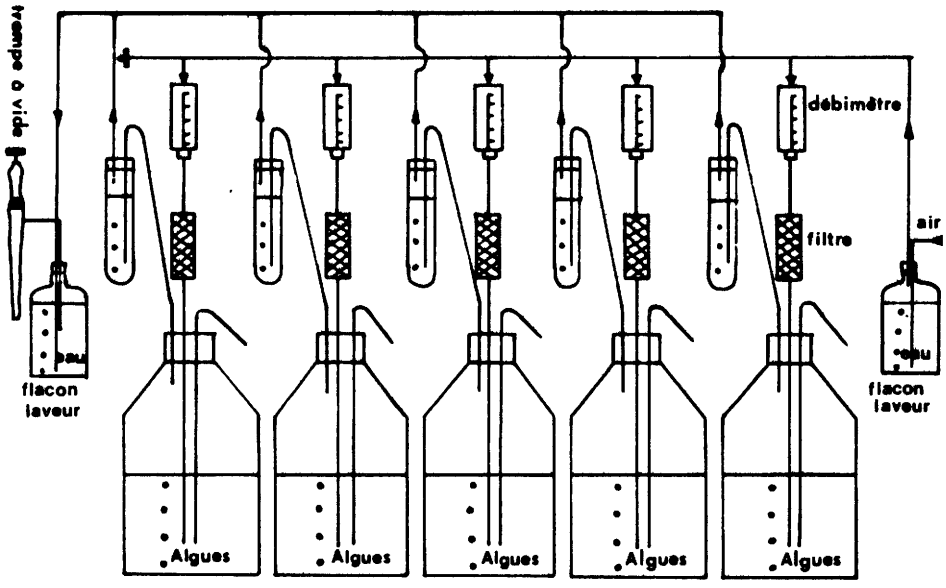

Figure 1 — Dispositif expérimental pour la culture d'algues.

***Figure 1**. — Algae culture apparatus.*

Les flacons de culture contiennent chacun 5l de milieu de culture (selon Hans et al., 1961) (8) et sont soumis à un régime d'éclairement de 12h/jour.

De l'air comprimé, épuré,est insufflé dans les flacons de culture. Le débit est règlé à 1,5 l min^{-1}.

Des flacons laveurs retiennent les vapeurs d'eau tritiée entraînées par la circulation d'air ; un tube immergé dans la culture permet d'obtenir des échantillons du milieu pour le comptage des algues.

Les algues sont cultivées dans les effluents liquides dilués et dans des milieux témoins. Leur croissance est suivie par comptage à la cellule de Thomas durant une période de 18 à 20 jours de culture.

Après culture, les algues sont traitées selon le schéma suivant :

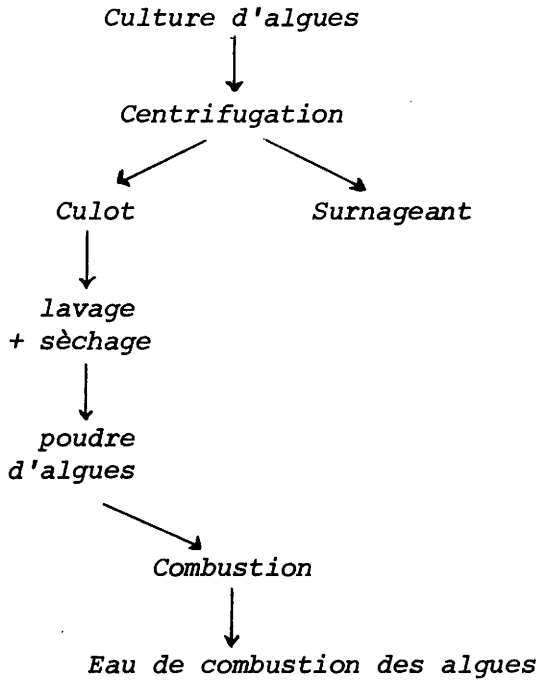

Fig.2. - Fractionnement des algues.

Ce dispositif expérimental a été utilisé pour les cultures de Scenedesmus sur des effluents filtrés (sur filtre millipore) et dilués ou non provenant des centrales nucléaires de Doel, Tihange et Chooz (6-7).

En outre, des effluents d'installations nucléaires complexes (site Mol-Dessel) ont été testés suivant le même schéma expérimental.

2. Culture d'algues en vue de la détermination de la disponibilité biologique du Carbone 14 dans des effluents.

La procédure expérimentale est identique à celle décrite ci-dessus. Néanmoins, il est nécessaire dans le cas présent, de faire subir à l'échantillon un pré-traitement en vue de différencier les formes organiques et inorganiques du carbone. On a procédé jusqu'à présent à une décarbonatation de la façon suivante : dans un premier stade on procède à l'addition dans l'effluent d'une solution (2×10^{-3} M) d'hydroxyde de baryum. Après repos de 2 heures, le précipité est séparé par filtration sur millipore ; l'excès de baryum en solution est ensuite éliminé sous la forme de carbonate de baryum par addition de carbonate de potassium (0,27 $g.l^{-1}$). Le filtrat est alors mélangé (1:2) dans le milieu de culture des algues.

Résultats et Discussion

Une sélection des résultats expérimentaux obtenus antérieurement à partir d'effluents de diverses origines sont présentes dans le tableau 1. L'examen de ce tableau montre que les rapports entre l'activité de l'eau de combustion des algues et du milieu de culture sont proches de 1 dans le cas des effluents provenant de Tihange et de Chooz : on peut donc déduire que le tritium est présent essentiellement sous forme d'eau tritiée. Par contre, les valeurs élevées de ce rapport dans le cas des effluents de Doel et du CEN/SCK ne peuvent s'expliquer que par la présence d'une certaine proportion de molécules organiques tritiées en solution qui sont assimilées et accumulées par les algues de la culture.

Les teneurs comparées en tritium et en carbone-14 des algues cultivées sur des effluents mensuels rejetés en 1982 par la centrale nucléaire de Tihange 1 sont indiqués dans le tableau 2.

On constate que les concentrations en carbone-14 sont en général environ la moitié de celles en tritium.

Cette constatation est surprenante si l'on considère que les niveaux en tritium, étant similaires à ceux de l'eau de combustion des algues, sont aisément mesurables dans l'effluent brut.

En ce qui concerne le carbone-14, cela n'est pas le cas. Nous en concluons que le carbone-14 mesuré dans les algues cultivées dans ces effluents provient essentiellement de molécules organiques de carbone-14 en solution.

Afin de vérifier cette hypothèse, nous avons procédé à un pré-traitement des effluents avant culture ainsi qu'il est indiqué dans la section matériel et méthode.

Les résultats préliminaires de ces expériences sont repris dans le tableau 3.

Ces résultats indiquent qu'une partie du carbone-14 est présente dans l'effluent brut sous forme inorganique. L'autre partie étant présente sous forme organique biologiquement disponible.

La proportion relative de chacune des fractions est en cours d'évaluation.

En conclusion, cette méthode est intéressante non seulement pour la recherche de la détermination des produits d'activation et de fission tels que Co, Cs, Mn mais surtout pour la mise en évidence de formes organiques du tritium et probablement du carbone-14 ainsi que les premiers résultats l'indiquent.

Remerciements

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Tableau 1. Incorporation par les algues du tritium présents dans des effluents filtrés.

Origine	Effluent nr échant.	Activité init. du mil. de culture nCi/ml	Teneur en ^3H de mat. sèche des algues nCi/gMS	Taux d'incorp. (en % de l'act. de l'effl. filtré)	Activité de l'eau de comb. des algues nCi/ml	Rapport $\frac{THO\ combustion}{^3H\ milieu\ de\ culture}$
Doel	DE 11783	2,83	4,9	$2,2.10^{-3}$	8,93	3,16
	DE 11785	1,17	10,06	$2,1.10^{-2}$	21,46	18,28
	DE 11787	1,62	35,67	$4,3.10^{-2}$	76,09	47,08
Tihange	TIE 12289	5,13	2,15	$2,0.10^{-3}$	4,60	0,90
Chooz	GE 7678					
	non dilué (infecté)	31,45	8,53	$4,4.10^{-4}$	18,18	0,58
	dilué 2x	16,57	6,80	$6,9.10^{-3}$	14,49	0,87
	dilué 5x	6,74	6,16	$1,6.10^{-2}$	13,13	1,95
	dilué 10x	3,21	1,13	$4,3.10^{-3}$	2,41	0,75
Waste (CEN/SCK)	W 80 (9-10) dil 10x	0,053	1,29	1,22	2,14	40,37

Tableau 2. Incorporation par les algues du tritium et du carbone-14 présents dans les effluents de Tihange 1 (année 1982).

	^{3}H		^{14}C	
Mois	*pCi/gMS*	*pCi/gH*	*pCi/gMS*	*pCi/gC*
Avril 1982				
Control sample	*619*	*9378*	*1006*	*1977*
Effluent dilué 2x	*2319*	*35131*	*3203*	*6293*
Mai 1982				
Control sample	*500*	*7583*	*842*	*1654*
Effluent dilué 2x	*2129*	*32265*	*1750*	*3438*
Juin 1982				
Control sample	*414*	*6272*	*624*	*1226*
Effluent dilué 2x	*2121*	*32136*	*1018*	*2001*
Juillet 1982				
Control sample	*416*	*6303*	*299*	*588*
Effluent dilué 2x	*1298*	*19666*	*518*	*1017*
Août 1982				
Control sample	*416*	*6311*	*411*	*808*
Effluent dilué 2x	*5283*	*80050*	*2001*	*3931*
Septembre 1982				
Control sample	*292*	*3901*	*313*	*615*
Effluent dilué 2x	*4945*	*74919*	*984*	*1933*
Octobre 1982				
Control sample	*224*	*3401*	*NS*	*NS*
Effluent dilué 2x	*5359*	*81197*	*2577*	*5063*
Novembre 1982				
Control sample	*186*	*2826*	*NS*	*NS*
Effluent dilué 2x	*3522*	*53364*	*2941*	*5778*
Décembre 1982				
Control sample	*245*	*3719*	*NS*	*NS*
Effluent dilué 2x	*4878*	*73909*	*8426*	*16555*

Tableau 3. *^{14}C concentration in algae from PWR liquid effluents after treatment, specific activity of dry matter pCi/gC.*

Echantillons	*avril-mai 1983 Tihange II*	*juin-juillet 1983 Tihange II*	*juin-juillet 1983 Tihange I*	*juin 1983 SENA*
Précipité de ^{14}C sous forme inorganique				
Témoin	*NS*	*NS*	*NS*	*NS*
Effl. nr 1 dil 3x	*616*	*357*	*567*	*597*
Effl. nr 2 dil 3x	-	*269*	*644*	*316*
Après culture dans les algues				
Témoin non traité	*25*	*130*	*NS*	*50*
Effl. dil. 3x non traité	*1830*	*1900*	*400*	*3000*
Effl. nr 1 dil. 3x traité	*5500*	*3500*	*1800*	*1500*
Effl. nr 2 dil. 3x traité	*non analysé*	*3900*	*non analysé*	*non analysé*

DETECTION OF MICROORGANISMS IN PRIMARY COOLING SYSTEMS OF NUCLEAR REACTORS

M. Mergeay(1), M. Bourdon(2), W. Horsten(1), R. Kirchmann(1)

(1) Département de Radiobiologie, C.E.N.-S.C.K., 2400 Mol, Belgique
(2) Laboratoire de Radioécologie, Institut Botanique, Université de Liège, B-4000 Sart Tilman, Belgique

The reactor BR2 of the Belgian Nuclear Center is a material testing reactor with a neutronic flux of 5×10^{14} neutrons/cm^2 sec. A few years ago, the reactor was stopped for many months in order to replace its beryllium matrix. During this period, the water was resting, stagnant in the vessel, the primary cooling circuit and the pool ; this led to a concern about the possibility of microbial corrosion on the metallic surface. Water samples were taken and analysed for sulphate reducing bacteria which were detected in small quantities at various points in the system, especially in the bottom of the pool. We then went on to look for aerobic micro-organisms able to survive in such drastic conditions,paying particular attention to the primary cooling circuit. In this cooling system, when the reactor is running, water is continuously deionized through passage on resins (cationic and anionic), degassed (removal of CO_2 and O_2 : after four days, oxygen concentration is at 50 µg/l) and circulated about 40 times per hour through the lamina of the fuel elements : the total volume of water is about 150 m^3 and the water flow is of 6000 m^3/h.

First observations made during the shut-down of the reactor and also while the reactor was functioning, indicated that microorganisms were present chiefly in the purification by-pass (water flow : 20-30 m^3/h)

and seem to be associated with the resins. It is possible that radioactive products fixed on resins induce some radiolysis of resins and consequently can release organic molecules which may possibly be used as a carbon source. However, these resins are regularly regenerated (every four or six months) by treatment with either NaOH or HNO_3 ; such treatment of course impeded the long term establishment or adaptation of microbial populations in the primary cooling systems and in the pool. The water of the pool is also permanently deionized, regularly removed and renewed and exposed to radiations as shown by the visible Cherenkov effect. During the shut-down of the reactor, the water of the pool is in contact with the primary cooling circuit through the open vessel ; this causes some exchange of microbial populations.
The whole system (pool and primary cooling circuit) seems thus to be a suitable environment to look for radioresistant and/ or oligotrophic microorganisms. (2)

As a first step to approach this ecosystem, we limited ourselves to plate counts of aerobic heterotrophs. During the shut-down of the reactor, viable counts of bacteria from samples taken from the pool and the primary cooling circuit (in the purification by-pass) did not exceed 5.10^2 c.f.u./ml.

Some strains were isolated and tested for sensitivity to ultraviolet radiation. Among 13 strains tested, three bacterial strains and one red yeast exhibited some resistance to UV. The viable count of the red yeast was unchanged under UV exposures up to 500 Joules per square meter. A few weeks after the sample containing the red yeast (strain WH11) was taken from the pool, the reactor was again put into operation. Further samples were taken and one from the purification by-pass of the primary loop, contained almost exclusively this red yeast. (3)

In a further sampling campaign, carried out when the reactor was in operation, samples were taken from different parts of the reactor a)downstream of the reactor itself (at the entrance of the purification by-pass) c.f.u. were lower than 5 per ml.b)in the purification by-pass, c.f.u. were around 10^2 - 2.10^2 per ml but these small populations, estimated by the

means of viable counts on plates, decreased if the sample was taken after extensive blow-off of the pipes used for water sampling. After a couple of days without sampling, the populations were increasing to their previous levels. In fact, the numerous pipes connected to the primary cooling circuit constitute "reservoirs" of small microbial populations which are maintained during the whole time that the reactor is functioning. It was of interest to compare the populations of some of these "reservoirs". From samples taken at different places of the purification by-pass, 50 colonies were replicated on agar plates containing a low salt minimal medium and various carbon sources (glucose, mannitol, lactate, gluconate, histidine, proline, D-alanine, parahydroxybenzoate, pelargonate, azelate ...). The analysis of the replicas shows that the populations are either poorly related or unrelated and that they contain mostly non fermenting heterotrophs. Other remote pipes which had not been blown-off for years were found to contain coliforms which were not detected elsewhere in the primary loop during the functioning of the reactor. (5) (6)

Our observations are , first , very preliminary and therefore it is difficult to draw any ecological conclusions and , secondly, are limited to aerobic heterotrophs, which are probably only a small proportion of the microbial ecosystem. Nevertheless, the work has allowed the detection of some microorganisms with peculiar characteristics such as radioresistance and oligotrophy ; our observations aimed also to attract attention to the possibilities of such a system for various taxonomic, genetic, physiological or ecological purposes. For example : bacteria able to survive in such harsh environments may be suitable hosts for natural or recombinant plasmids containing information about resistance to xenobiotics (heavy metals, pesticides ...) ; they could therefore be used for pollution abatement in polluted waters or in some waste digestors. (4) (1)

Further research should focus on detection of microorganisms which are not represented in our plate counts such as anaerobes, microaerophiles (especially in rarely blown-off pipes), facultative anaerobes, obligate oligotrophs and obligate autotrophs.(2)

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Session : II

Paper by : M. Mergeay

Comment by : Bors

Text of comment
or author's answer :

You isolated a yeast with a very high radioresistance, could it deal with a new mutant strain due to irradiation?

It is possible, however, the permanent treatment of the water of the cooling systems (including regeneration of resins with NaOH and HNO_3, etc.) does not favour a selection process by genetic means of radioresistant microorganisms inside the system although it cannot be excluded.

Session : II

Paper by : M. Mergeay

Comment by : J. De Brabander

Text of comment
or author's answer :

Have there been studies about which "foods" are found in the reactors, where bacteria can live on. I ask this because it is generally known that the water in the reactors has to be very purified and demineralised. If not, do you think such study would be worth doing, and if yes, which were the results?

The system is indeed really oligotrophic and does not sustain very large populations however dust (in the pool), breakdown of resins (in the purification by-pass) and dead microorganisms should feed to some extent.

Session : II

Paper by : M. Mergeay

Comment by : Francis

Test of comment
or author's answer :

I was wondering whether you have used acridine organe direct counts of bacteria in your studies with cooling water. We have found 2 to 3 orders of magnitude over plate counts in resins and in influent and effluent waters from resin.

Session : II

Paper by : M. Mergeay

Comment by : Azam

Text of comment
or author's answer :

Plate counts are probably greatly under-estimating the population size of bacteria you measure. I think it will be most valuable to use microscopy : particularly epifluorescens microscopy acridine orange stained (or DAPI stained) samples. For ex. : see Hobbic, Jasper, Doley - 1977 (App. Environm. Microbiol.). Furthermore, dead or alive bacteria could be determined by microautoradiography or by ETS staining (Packard or Ittariaga work ; I do not remember the ref.). I strongly urge microscopy for your work.

At this stage, our main purpose was to know if microorganisms can be detected in the primary cooling system of BR2 reactor and to use such a biotope to isolate peculiar strains. Plate counts on the other hand are required to discriminate some populations from the taxonmical point of view.

DONNEES EXPERIMENTALES SUR LE ROLE DU SEDIMENT ET DE MICROORGANISMES DANS LE TRANSFERT DE RADIONUCLEIDES EN EAU DOUCE

A. LAMBRECHTS, L. FOULQUIER ET J.P. BAUDIN

CEA, IPSN, DERS, SERE, SRTCM
Laboratoire de Radioécologie des Eaux Continentales
Centre d'Etudes Nucléaires de Cadarache
13115 Saint Paul-Lez-Durance, FRANCE

RESUME

L'incorporation de trois radionucléides dans des chaînes trophiques met en évidence le rôle du sédiment et des microorganismes. 90 % de la teneur en ^{137}Cs de larves de chironomes proviennent du sédiment ; des carpes absorbant ces larves ont une concentration 100 fois supérieure à celle qu'elles auraient par le transfert direct par l'eau.
A l'inverse, des annélides fixent davantage de ^{65}Zn par l'eau que par le sédiment.
Des anodontes mises en contact pendant de longues périodes avec des particules sédimentaires en suspension ont des concentrations très faibles en ^{137}Cs alors qu'elles se contaminent rapidement et fortement à partir de cellules de levure de bière marquées.
Le transfert du ^{137}Cs et du ^{65}Zn de microalgues vers des crustacés planctoniques est responsable de plus de 80 % de la teneur du prédateur alors que le transfert du ^{60}Co reste faible dans cette même chaîne.
Des poissons absorbant des daphnies ou des gammares ont des taux de rétention du ^{137}Cs, du ^{60}Co ou du ^{65}Zn de l'ordre de 10 %. Ce taux pourrait être lié à la qualité et la quantité de nourriture absorbée comme au nombre et à la fréquence des repas.

1 - INTRODUCTION

Le cycle des éléments minéraux, et de leurs isotopes radioactifs, dans les chaînes biologiques englobe les échanges entre l'eau et le sédiment, leur fixation par les producteurs primaires et leur transfert aux prédateurs des différents niveaux trophiques[1]. Dans les études de laboratoire, la contamination radioactive des organismes aquatiques n'est souvent envisagée que sous l'angle du transfert direct des radionucléides à partir de l'eau. Un tel transfert est caractérisé par sa cinétique et le facteur de concentration qui en résulte. Les travaux que nous menons depuis 20 ans[2] montrent que les facteurs de concentration obtenus dans les expériences de laboratoire sont en général inférieurs à ceux mesurés in situ. Cette différence peut provenir des transferts trophiques. On montre ainsi que la fixation du ^{137}Cs, du ^{60}Co et du ^{65}Zn par les hydrobiontes est accrue s'ils sont en présence de sédiment ou de nourriture contaminée [2 à 13]. Par exemple, suivant le mode de contamination de la carpe par du ^{137}Cs on obtient un facteur de concentration de 4 par l'eau seule, de 25 en présence de sédiment et de 200 si le poisson se nourrit de larves de chironomes qui vivent dans ce sédiment[11]. En tenant compte de certains paramètres écologiques, on peut quantifier les différentes voies d'échanges et proposer un modèle de transfert du radiocésium vers les poissons[5, 11].

Nous avons cherché à mettre en évidence le rôle du sédiment et de microorganismes planctoniques dans l'incorporation du ^{137}Cs, du ^{60}Co et du ^{65}Zn dans des chaînes trophiques simplifiées.

Nous nous proposons de rassembler ici quelques données expérimentales qui constituent une première approche à l'étude globale du cycle bio-géochimique des radionucléides.

Ces résultats montrent qu'il est nécessaire d'entreprendre des recherches sur le rôle de la microflore, de la microfaune et du plancton qui constituent un maillon peu connu et pourtant essentiel du transfert des éléments radioactifs.

2 - ROLE DU SEDIMENT ET D'ORGANISMES BENTHIQUES DANS LE TRANSFERT DES RADIONUCLEIDES

2.1. Transfert du césium du sédiment vers la carpe

La possibilité de contamination des poissons par le ^{137}Cs à partir du sédiment est une question complexe et controversée dans la bibliographie[12]. Nous avons montré que les facteurs de concentration sont huit fois plus

importants quand des carpes[4] ou des poissons-chats[13] sont contaminés en présence de sédiment que lorsqu'ils se trouvent en eau seule. Le facteur de concentration des carpes serait plus élevé lorsque le sédiment est frais que lorsqu'il est passé à l'étuve à 60° C pendant 48 heures[4] ; cependant, des conditions différentes de nutrition dans ces deux dernières expériences nous conduisent à rester prudent sur le rôle éventuel des microorganismes benthiques.

A l'inverse, une autre expérience qui étudie le transfert indirect du ^{137}Cs dans les maillons d'une chaîne trophique apporte des enseignements plus intéressants.

2.1.1. Transfert du césium 137 d'un sédiment frais vers des larves de chironomes

L'expérience est réalisée dans 26 béchers en plastique contenant chacun 250 g de sédiment frais du Rhône (ayant une concentration en ^{137}Cs de $4,4 . 10^4$ Bq . g^{-1} frais), 40 ml d'eau du Rhône inactive et 40 à 100 larves de chironomes. Les béchers sont prélevés au bout de temps de contact allant de 1 à 10 jours. Une analyse de variance montre que le temps de contact n'a pas d'influence sur les concentrations en césium des larves dont la moyenne est de 1660 $\pm$ 380 Bq . g^{-1} frais.

GERKING et Coll.[14] trouvent également que les teneurs des larves de chironomes atteignent un plateau dès le premier jour de contamination et pensent que 70 % de l'activité des larves sont dus au sédiment contenu dans leur tractus digestif.

D'après KAJAK et Coll.[15], il faut entre 1 et 5 heures pour qu'une larve, à jeun, emplisse son tube digestif, la vitesse de remplissage dépendant de la taille et de l'état physiologique des larves, comme de la quantité et de la qualité de la nourriture disponible et de l'oxygénation du milieu. Ces auteurs trouvent dans le tube digestif des larves 28,5 % de détritus, 0,4 % de particules minérales, 1,4 % de débris animaux et 69,8 % d'algues. Le pourcentage des algues présentes dans l'intestin des larves est toujours plus élevé que dans le sédiment. Ils estiment qu'une larve peut absorber entre 3000 et 24000 cellules d'algues par jour.

Si le sédiment, comme dans le lac SNIARDWY en Finlande[15], contient 600000 cellules par cm^2 et 10000 larves par m^2, ce sont entre 0,5 et 10 % de la population des algues qui sont ingérées quotidiennement par les larves de chironomes. GERKING et Coll.[14] estiment de leur côté que les chironomes ingèrent en sédiment sec l'équivalent de 9 % du poids de leur corps, ce qui

correspond en sédiment frais à environ 20 % de leur poids. Ainsi des larves pesant entre 8 et 35 mg absorberaient entre 1,5 et 7 mg de sédiment par jour. Ils estiment que 10 % de la teneur en césium 134 des larves seraient dus au transfert direct par l'eau et 90 % par le sédiment. C'est également notre opinion pour le césium 137[11].

Dans notre expérience[11], le facteur de transfert (F.T.) - qui représente le rapport de la concentration en ^{137}Cs des larves à celle du sédiment - est de 4.10^{-2}.

Un tel facteur semble faible, mais étant données les fortes teneurs en radio-césium du sédiment, une quantité importante de ce radionucléide va pouvoir transiter dans les chaînes trophiques comme nous allons à présent le montrer.

2.1.2. Transfert du césium 137 des chironomes vers les carpes[11]

Les chironomes de chacun des béchers de l'expérience précédente sont répartis en 10 lots et distribués à 10 carpes pesant 6 g en moyenne. Chaque carpe reçoit 21 repas (pesant chacun entre 0,1 et 0,3 g) pendant les 38 jours d'expérience. La quantité de nourriture distribuée maitient constant le poids des carpes mais ne permet pas un grossissement normal. La teneur en radio-césium des poissons augmente globalement en fonction du temps avec des variations qui dépendent de l'activité et de la fréquence des repas. On peut définir un facteur de rétention qui est le rapport de la teneur en césium des carpes à la teneur cumulée de la nourriture ingérée. Dès le 3° jour, un équilibre s'instaure entre les phénomènes d'ingestion et d'excrétion. Le facteur de rétention (F.R) est alors de 0,131 $\pm$ 0,025.

On peut émettre l'hypothèse que, dans la nature, un carpillon de 6 g mangerait environ 2 g de larves de chironomes par jour[11]. Si le facteur de rétention était également dans ce cas de 0,131, la teneur des carpes, se nourrissant de larves ayant 1660 $Bq.g^{-1}$ frais de césium 137, serait en 23 jours =

$$1660\ Bq.g^{-1} \cdot 2\ g \cdot 23\ j \cdot 0{,}131 = 1.10^4\ Bq$$

La carpe, ayant grossi, pèserait 8,7 g, sa concentration serait de $\frac{1.10^4}{8{,}7}$ = 1150 $Bq.g^{-1}$ frais et son facteur de concentration par rapport à l'eau (3,8 $Bq.ml^{-1}$) serait de 300, soit 75 fois supérieur au FC de carpes contaminées par l'eau seule ou 12 fois supérieur à celui de carpes contaminées par le sédiment.

Plusieurs voies de transfert du ^{137}Cs vers le poisson existent et

peuvent cumuler leurs effets[11]. La voie trophique semble être prépondérante dans ce transfert, surtout lorsqu'elle intègre un maillon aussi important que les larves de chironomes.

Les remarques de KAJAK et Coll.[15] laissent supposer que les microalgues du sédiment contribuent fortement à la contamination des larves et constituent un vecteur important dans l'incorporation du radio-césium dans les biocoenoses aquatiques.

2.2. Transfert du zinc 65 vers une annélide polychète[16]

Une expérience a été entreprise avec les constituants d'un étang saumâtre méditerranéen. Dans des béchers contenant 250 g de sédiment et 100 ml d'eau de l'étang, on introduit 5 à 6 vers (Nereis diversicolor, Muller). L'eau est contaminée par une solution de $5,2.10^4$ Bq de ^{65}Zn en $ZnCl_2$.

Le radiozinc se fixe rapidement dans le sédiment comme le montre COUGHTREY et Coll[17]. La concentration des vers augmente pendant 40 jours, tant qu'une activité résiduelle subsiste dans l'eau, puis atteint un palier. L'eau constituerait ainsi le vecteur essentiel du transfert du radiozinc vers les Nereis. C'est ce que l'on vérifie en introduisant des annélides dans des récipients contenant 250 g de sédiment préalablement marqué avec $7,8.10^4$ Bq de ^{65}Zn.

Dans ces conditions, après 54 jours, la concentration des vers est de 27 $Bq.g^{-1}$ frais, valeur 12 fois plus faible que celle obtenue au 40° jour de l'expérience précédente, malgré une activité plus élevée du milieu.

Le transfert par le sédiment serait responsable, à 40 jours, d'environ 6 % de la concentration en ^{65}Zn des annélides, le transfert direct par l'eau assurant 94 % de cette concentration.

3 - ROLE DES ORGANISMES DANS LE TRANSFERT DES RADIONUCLEIDES

3.1. Transfert du césium 137 vers un mollusque et un crustacé

3.1.1. Transfert du césium 137 de la levure de bière vers des anodontes[18]

Dans une série d'études sur la radiocontamination de bivalves d'eau douce (Anodonta cygnea, L.) par le césium 137 il avait semblé intéressant de tenir compte de la propriété de ces mollusques de filtrer l'eau et de s'alimenter à partir de particules en suspension. RICE[19] montre que le taux de filtration dépend du nombre, de la taille et de la digestibilité des particules, des diatomées étant, par exemple, davantage retenues que des algues

de petites taille (Nannochloris, Chlorella).

Dans une première expérience, 10 anodontes sont placées dans un aquarium contenant 5 kg de sédiment contaminé de manière homogène par $1,8.10^6$ Bq de ^{137}Cs et 20 litres d'eau inactive. On agite périodiquement l'eau de l'aquarium de manière à remettre en suspension les particules sédimentaires. Les anodontes sont prélevées au bout de 220 jours. Seule la coquille a une concentration relativement élevée, liée aux phénomènes d'adsorption du ^{137}Cs. Les organes internes, y compris ceux impliqués dans la filtration de l'eau, ont des concentrations faibles ($\simeq$ 1 $Bq.g^{-1}$ frais). Trois facteurs peuvent expliquer ces résultats : la turbidité (entraînant la fermeture des valves et l'arrêt de la filtration), la taille et la nature minérale des particules. La filtration ne serait pas un phénomène passif, les anodontes pouvant trier et n'absorber que les particules en suspension ayant une valeur nutritive.

Une deuxième expérience est alors entreprise.
Dans un aquarium contenant 30 litres d'eau et 10 kg de sédiment, 9 anodontes sont nourries pendant 38 jours par 14 repas de levure de bière lyophilisée ayant une concentration en ^{137}Cs de $7,4.10^4$ $Bq.g^{-1}$ sec.

En fin d'expérience il y a 82 % de l'activité introduite dans le sédiment, 12 % dans l'eau et 6 % dans les bivalves. Les concentrations en radio-césium sont 66 $Bq.g^{-1}$ frais pour les organes internes avec un maximum pour la masse musculaire (110 $Bq.g^{-1}$ frais), les organes qui interviennent dans la filtration de l'eau et le tractus digestif. Les moules, qui rejettent les particules minérales, retiennent au contraire la levure de bière. Afin de tester l'influence du cumul des 2 sortes de particules, on essaie de contaminer d'autres anodontes par la remise en suspension du sédiment utilisé dans cette dernière expérience.

Ce sédiment a une activité de $8,5.10^5$ Bq essentiellement constituée par de la levure de bière déposée sur le fond. On ajoute 5 anodontes et 30 litres d'eau inactive. En procédant à une agitation tous les 3 jours pendant 1 minute, on remet périodiquement en suspension les particules.

Après 50 jours d'expérience, les anodontes ont des concentrations moindres, spécialement au niveau des organes internes (4 fois plus faibles). Ce n'est pas dû à l'activité totale disponible (1.10^6 Bq dans un cas et $0,8.10^6$ Bq dans l'autre), ni à la durée des expériences (même ordre de grandeur dans les 2 cas). Seul, le mode de contamination intervient. La turbidité importante provoque pendant des durées variables la fermeture des valves et une contamination plus faible des moules.

On peut en conclure que conformément aux travaux de RICE[19], les bivalves ne se contaminent pas à partir des particules argileuses qu'ils rejettent, alors qu'ils absorbent des particules organiques en suspension dont ils retiennent le ^{137}Cs.

Il faudrait connaître le mode d'alimentation des moules, tant d'un point de vue qualitatif que quantitatif et refaire ces expériences avec des microorganismes vivants plus proches de l'alimentation habituelle des anodontes que ne l'est la levure de bière.

3.1.2. Transfert du césium 137 d'une microalgue vers un crustacé

Plusieurs auteurs ont étudié le transfert du radiocésium à travers les maillons de chaînes alimentaires contenant des microalgues et des crustacés. KING[20] utilise des Chlamydomonas et des daphnies, WILLIAMS et Coll.[21] des Euglena et des daphnies. Nos expériences ont été réalisées avec des Chlorella.

Les microalgues sont introduites dans des fioles stérilisées contenant 400 ml de milieu de culture et contaminées à l'aide de $7,4.10^4$ Bq de ^{137}Cs en CsCl. On laisse les chlorelles se développer un mois avant d'effectuer des prélèvements hebdomadaires. De 26 à 46 jours on constate une grande stabilité des concentrations de l'eau ($1,8.10^2$ Bq.ml^{-1}) et des algues (2.10^4 Bq.g^{-1} frais). Le facteur de concentration est de 90, ce qui est en concordance avec les données bibliographiques[22].

Des chlorelles contaminées dans les mêmes conditions sont données en 3 fois à des daphnies contenues dans 2 cristallisoirs. On introduit ainsi 1.10^4 Bq de ^{137}Cs dans chaque cristallisoir. Après 27 jours, les cladocères ont des teneurs en ^{137}Cs de 90 et 150 Bq respectivement (et des concentrations de 290 et 340 Bq.g^{-1} frais). Le facteur de rétention (rapport des teneurs des daphnies à celles des algues) est respectivement de 0,009 et 0,015, ce qui signifie que les crustacés n'ont retenu au maximum que 1,5 % de l'activité des chlorelles. S'agit-il d'un transfert trophique chlorelles -daphnies ou d'un transfert indirect chlorelles-eau-daphnies ?

En contaminant des daphnies directement à partir de l'eau[3, 11] on obtient un facteur de concentration d'environ 25.

Si les algues se décontaminaient totalement dans l'eau des cristallisoirs où vivent les daphnies (5 litres) la concentration maximum de l'eau serait de $\frac{1.10^4}{5.10^3} = 2$ Bq.ml^{-1}.

Pour un FC de 25, les daphnies pourraient avoir une concentration de 50 Bq.g^{-1} frais au lieu des 290 à 340 Bq.g^{-1} frais mesurés. On peut en

conclure qu'il y a bien transfert trophique et que les microalgues jouent un rôle important dans le transfert du radiocésium vers les daphnies.

3.2. Transfert du césium 137 des daphnies vers des poissons

3.2.1. Transfert du césium 137 des daphnies vers des guppys[23]

Des daphnies ayant une teneur en ^{137}Cs de $1,9.10^4$ Bq sont données à manger à 9 guppys (Lebistes reticulatus, Peters) d'un poids total de 7,13 g. La teneur des poissons, 2 jours après le repas est de $1,2.10^4$ Bq, soit 67 % de celle des daphnies (F.R. = 0,67). Les guppys sont à leur tour donnés à manger à 3 anguilles, en 1 seul repas, qui 7 jours plus tard auront une teneur égale à 39 % de celle des guppys.

Cette expérience montre un transfert important du ^{137}Cs dans les trois maillons de cette chaîne. Les facteurs de rétention sont élevés, les poissons étant maintenus à jeun avant le repas unique, et les rapports de biomasse entre les proies et prédateurs semblant très faibles.

3.2.2. Transfert du césium 137 des daphnies vers des carpes[3, 11]

Deux carpes de 5 g, maintenues en eau inactive dans des aquariums individuels, reçoivent chacune 5 repas constitués par 25 daphnies contaminées en ^{137}Cs.

En 10 jours d'expérimentation, les poissons retiennent 9 % de l'activité des daphnies (FR = 0,09).

Cette expérience, comme la précédente, présente l'inconvénient de ne comporter qu'un petit nombre de repas et de ne pas respecter la pyramide des biomasses entre proies et prédateurs. Après 5 repas le facteur de rétention est 7 fois plus faible. D'autres expériences[3] réalisées sur des durées de 2 mois pendant lesquels 10 carpes sont nourries quotidiennement avec des daphnies séchées montrent que le facteur de rétention se stabilise rapidement à une valeur faible mais constante (FR = 0,034 $\pm$ 0,004) indiquant un état d'équilibre entre l'assimilation et l'excrétion du ^{137}Cs. Le niveau du facteur de rétention pourrait dépendre de la qualité, de la fréquence et du nombre des repas comme de la quantité de nourriture absorbée.

3.3. Transfert du cobalt 60 vers un crustacé[6, 7, 8]

Le transfert du ^{60}Co a été étudié dans une chaîne trophique "chlorelles - daphnies", le protocole expérimental distingue la contamination des organismes par l'eau[6], l'eau + la nourriture[7] et la nourriture seule[8]. La

contamination des algues est effectuée dans des conditions similaires à celles exposées dans le paragraphe 3.1.2. Les chlorelles ont alors un facteur de concentration moyen de 565 ce qui est en accord avec les données bibliographiques[6, 17, 24].

Des daphnies nourries avec ces chlorelles ont des teneurs qui sont en relation avec le niveau d'activité de la nourriture. Il est vraisemblable que la teneur en cobalt des cladocères soit essentiellement liée à la présence de chlorelles non digérées dans le tube digestif. Cette hypothèse est étayée par le fait que cet organe apparaît nettement coloré en vert à travers l'exosquelette transparent des daphnies.

Le rapport de la concentration des prédateurs à celle de la nourriture est d'environ 2.10^{-3} ce qui indique une assimilation très faible. Le rapport des teneurs en cobalt indique une rétention inférieure à 1%.

Des daphnies contaminées à la fois par l'eau et par la nourriture[7] ont un facteur de concentration de 10 après plusieurs mois de contamination. Contrairement à ce que l'on avait constaté pour le ^{137}Cs le vecteur nourriture est peu important dans le transfert du ^{60}Co vers les daphnies.

Des carpes nourries pendant 6 semaines par des daphnies contaminées en cobalt ont leur teneur qui augmente pendant 4 semaines et se stabilise. Le facteur de transfert (rapport des concentrations entre prédateur et proie) est de 0,12, le facteur de rétention est de $0,09 \pm 0,01$ ce qui est identique à la valeur trouvée avec le ^{137}Cs pour des daphnies vivantes. La teneur des carpes est fortement corrélée à la teneur des repas. Deux paramètres viennent compliquer l'interprétation des résultats. En effet, toutes les carpes n'ont pas été contaminées simultanément et la concentration en radiocobalt des daphnies a varié au cours de l'expérience, probablement en liaison avec les modifications des formes physicochimiques du ^{60}Co dont une fraction importante passe sous forme anionique.

La plus grande prudence est nécessaire dans l'interprétation de ces résultats et de nouvelles expériences sont indispensables pour mieux comprendre le transfert de ce radionucléide dans les chaînes trophiques.

3.4. Transfert du zinc 65 vers du plancton

Dans le cadre d'une étude sur le cycle du zinc dans l'étang de l'Olivier[10], le transfert du ^{65}Zn vers le phyto et le zooplancton a été abordé. Dans l'expérience le phytoplancton est composé de cyanophycées et de diatomophycées, le zooplancton étant uniquement constitué de copépodes.

On utilise 8 erlenmeyers dont 4 contiennent 1 litre d'eau et du phytoplancton de l'étang de l'Olivier et 4 contiennent 1 litre d'eau filtrée à 0,45 µm. On ajoute environ 0,2 g frais de zooplancton dans les 8 flacons qui sont maintenus à 15° C. Les 4 récipients de chaque série reçoivent respectivement une concentration croissante de ^{65}Zn en chlorure de zinc (1,5 ; 3 ; 5 et 6 . 10^4 $Bq.l^{-1}$). Les erlenmeyers sont prélevés au bout de 7 jours (tableau I).

TABLEAU I - Caractéristiques des prélèvements effectués à 7 jours dans l'étude du transfert du ^{65}Zn vers le plancton

	NUMERO DU FLACON	1	2	3	4	5	6	7	8
EAU	Concentration t_0 Bq.	12	29	47	64	12	29	47	64
	Concentration t_7 ml^{-1}	2	2	4	5	9	18	35	50
PHYTO-PLANCTON	Teneur (Bq)	8440	14570	28590	31780	-	-	-	-
	% activité initiale	90	50	60	50	-	-	-	-
	Concentration ($Bq.g^{-1}$ frais)	109600	137400	119600	132400	-	-	-	-
	FC t_0	9130	4740	2550	2070	-	-	-	-
	FC t_7 jours	54810	68710	29910	26490	-	-	-	-
ZOOPLANCTON	Teneur (Bq)	510	1460	1560	2280	370	930	2760	3100
	% activité initiale	4	5	3	4	3	3	6	5
	Concentration ($Bq.g^{-1}$ frais)	1700	5180	5540	7200	2170	4970	7600	10500
	FC t_0	140	180	120	110	180	170	160	160
	FC t_7 jours	850	2590	1380	1440	240	280	220	210

L'eau qui avait une activité initiale équivalente dans les flacons 1 et 5, 2 et 6, 3 et 7, 4 et 8 a une concentration à 7 jours beaucoup plus faible dans les 4 premiers récipients où se trouvent les producteurs primaires. Les teneurs du phytoplancton montrent bien qu'il y a fixation du ^{65}Zn par les algues qui retiennent entre 50 et 90 % de l'activité initiale introduite.

Les facteurs de concentration du phytoplancton varient de 26000 à 69000 à 7 jours ce qui est conforme aux données bibliographiques[17, 19]. L'examen des facteurs de concentration suggère qu'ils pourraient évoluer en sens inverse de la concentration initiale en zinc de l'eau, mais nous ne disposons pas d'un nombre suffisant de valeurs pour le vérifier statistiquement.

Le zooplancton retient entre 3 et 7 % de l'activité initiale introduite dans les flacons et sa concentration évoluerait dans le sens de la concentration initiale de l'eau, il en résulte que les facteurs de

concentration par rapport à t_0 sont tous du même ordre de grandeur (153 ± 22).

Si l'on compare les FC calculés à 7 jours, on note une grande diversité entre les 2 séries d'erlenmeyers. Lorsque l'eau est filtrée, donc en l'absence de phytoplancton, les copépodes ont des FC de l'ordre de 240.

En présence de microalgues, ces crustacés ont des FC de l'ordre de 1570, soit presque 7 fois plus. Les flacons de la 2° série (5 à 8) traduisent le transfert direct du ^{65}Zn de l'eau vers le zooplancton, tandis que ceux de la 1° série (1 à 4) représentent à la fois le transfert par l'eau et la nourriture. S'il y a cumul des voies de transfert, il est possible d'évaluer la part qui revient à chacune d'elle, les pourcentages étant proportionnels au rapport des concentrations[11]. Ainsi l'eau est responsable de $\frac{240.100}{1750}$ = 15 % de la concentration en radiozinc des copépodes et la nourriture de 85 %. Cela recoupe les remarques effectuées par RICE[19] concernant la contamination en zinc d'Artemia par l'eau et par des cellules de Carteria.

BAUDIN[9], dans une autre expérience où le copépope Calanipeda aquadulcis est contaminé dans un aquarium où se développent des algues microscopiques, trouve un facteur de concentration de 1650 en 15 jours.

Comme on ne connait pas la quantité et la teneur en zinc des algues absorbées par le zooplancton, il n'est pas possible de calculer le facteur de rétention d'un maillon à l'autre de la chaîne trophique. Néanmoins les microalgues semblent jouer un rôle capital dans l'incorporation du zinc dans les biocoenoses.

Une algue filamenteuse, Cladophora sp. très abondante dans l'étang de l'Olivier fixe intensément le zinc 65[10]. Les amas considérables de cette algue sont peuplés d'une faune importante de crustacés et de gastéropodes. Une expérience[10] étudie le transfert du ^{65}Zn des algues à des sphéromes et à des physes. Ces invertébrés accumulent le radionucléide, très rapidement chez les sphéromes et avec un équilibre à partir de 15 jours chez les mollusques. JENSEN[25] pense que les sphéromes absorbent non seulement les algues mais également la microfaune qui s'y abrite. Nous ne pouvons apporter confirmation dans ce domaine et pensons qu'une grande proportion de la concentration en ^{65}Zn des sphéromes doit provenir des cladophores.

BAUDIN[26] a montré que le transfert du ^{65}Zn (par la voie trophique) de gammares vers des anguilles se traduit, après l'ingestion de 11 repas échelonnés sur 69 jours, par un taux de rétention moyen de 22 %.

Le taux de rétention du ^{65}Zn pourrait diminuer quand augmentent le nombre et la fréquence des repas consommés. Ainsi, des carpes nourries par du tissu mou de limnées[27] (26 repas distribués en 40 jours) ont un taux de rétention du radiozinc de 16 % vers le 4° jour et devenant inférieur à 10 % après le 13° jour.

4 - CONCLUSION

Ces quelques données expérimentales mettent en évidence l'importance des transferts trophiques dans la radiocontamination des mollusques, des crustacés ou des poissons. Dans le cas du ^{137}Cs ou du ^{65}Zn la voie trophique peut être responsable de plus de 80 % de l'activité des prédateurs. Ces données rejoignent les constatations faites par les écotoxicologistes concernant le transfert des polluants chimiques tels que les métaux lourds, les dérivés du mercure ou les organochlorés[28, 29, 30, 31].

Le transfert essentiel des radionucléides de l'eau vers les poissons se fait par l'intermédiaire du plancton et des crustacés de petite taille. De la même façon, le transfert direct du ^{137}Cs du sédiment vers les poissons semble possible mais relativement faible en comparaison du transfert indirect par des chaînes trophiques du type "microalgues - larves de chironomes - poisson".

Les microorganismes de l'eau et du sédiment occupent une position clé dans l'incorporation des radionucléides dans les biocoenoses. Nos premières données expérimentales permettent juste d'en déceler l'importance. Il conviendrait de porter des efforts sur ces premiers maillons des chaînes alimentaires.

La connaissance des échanges à ce niveau est indispensable à la compréhension, la quantification et la modélisation du cheminement des radionucléides dans les écosystèmes d'eau douce.

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Session : II

Paper by : Lambrechts

Comment by : Fisher

Text of comment
or author's answer :

Did you allow the copepode to empty their guts after feeding on Zn-contaminated phytoplankton. The high concentration factor you report may simply represents the presence of a "hot" bolus of food in the copepods gut and does not necessarily give a true retention efficiency. Also, these animals will have guts much fullen, on average immediately after feeding than would the same animals in natural water.

No, we do not let the copepods to empty their digestive tractus. Because it was a first approach of this food-chain and we wanted to know, what really occurs in ponds.
I think you are right, if later we would like to analyse the physiological processes.

Session : II

Paper by : Lambrechts

Comment by : C.Myttenaere

Text of comment
or author's answer :

Are you sure to have the possibility to extrapolate your results to the field conditions ?

It was the object of previous studies.
The transfer of 137-Cs was followed in an experimental ecosystem constituted by water, sediment, chironomid larvae , daphnia and carp.
An experimental study was done at every exchange level, allowing us to calculate a transfer equation.
An experimentation showed that different exchanges could cumulate their own effect. So we used the equations in a predictions model of fish contamination.
The results were coherent with fish concentrations measured in Rhône River. It shows the influence of several factors as lenght of contamination, age and alimentary habits of fishes etc... so one can make predictions for field conditions.
I plane to realise such model for other nuclides- and specially 60-Co, includin g additional compartments as primary productors.

Session : II

Paper by : Lambrechts

Comment by : A.Cremers

Text of comment
or author's answer :

You stated that the sediment had a very high Cs-activity, so I suppose that the Cs-solid-liquid distribution. Therefore, it is of some importance caracterize the sediment in terms of mineralogy.
The effect of drying the sediments on transfer may be due to fixation of Cs in illitic fractions.
In addition, the solid-liquid distribution of Cs may change upon ingestion sinci it is essentially ruled by K levels biological fluids.

I agree whit your comment, it's why I said that one would be carefull about meaning of results of experiment where sediment is oven-dried.

Session : II

Paper by : A.Lambrechts

Comment by : J.P.Descy

Text of comment
or author's answer:

Question 1 :
In the experiment with 65-Zn, where natural phytoplankton has been used :
- what are the culture conditions of the algae ?

Question 2 :
In the calculation of the C.F.water → phytoplankton, did you take into account that one important part of the fresh weight consist of siliceous "shells" of diatoms ?
It would be better to refer to the organic weight in the cases where diatoms are an important part of the algal biomass.

For Q.1. : The algae where not grown in a culture solution. We collected plancton in the pond, separate phytoplancton and zooplancton through nets and put them in aquariums.

For Q.2. : We didn't take into account the weight of siliceous shells of diatoms. It was a first approach of zinc tranfers in plancton compartments as I have already said.

Session : II

Paper by : Lambrechts

Comment by J.C.Guary

Text of comment
or author's answer :

I have some comments and questions concerning the interpretation of the results presented here, and more generally about some problems of methodology posed by such a comparative study of several uptake routes.

1) You have said that the 137-Cs uptake by carps is 8 - 10 higher via sediment route than via water route. But, to be sure that cesium is really absorbed in both cases, it would be more valuable to compare muscle of the fishes. Since you compare total fishes, are you sure that the guts do not contain any contaminated sediment of the time of the radioactivity measurement ? And that you are not measuring, in fact, the 137-Cs fixed on this sediment ?

2) Same question in the case of chironomous larvae which can "absorb" more 137-Cs from sediment than from water ?

3) Are you sure that the difference in the degree of contamination of carps fed with daphnies or chironomous larvae is only due to the nutritional value of the material ingested and not simply to the contaminated sediment contained into the digestive tract of the chironomous larvae ?

4) My last question concerns the 137-Cs accumulation in the soft parts of the freshwater mussel from sediment, yeast + sediment resuspension. When you say that total soft parts of mussels fed with yeast resuspension are more contaminated than with sediment resuspension, and that is the result of an absorption of this organic material, I am not really convinced.
I could be more simply the result of the counting of 137-Cs fixed on the mussels'gills, acting as a trap for the yeast suspension, since you don't give proof of a real assimitation of 137-Cs contaminated yeast by the mussels?

Answering to your first question I would say that experimentation lasted during 42 days, and we measured cesium activity every two days, I could not, then kill the fish and make dissections each time. In fact we know that carps have a special organ in its throat to filter sediment particles, but this filter is not absolute and very little quantities of sediment could transits into digestive track.
We evaluate that 0.02 g of sediment could be absorbed during 42 days. The transit time in the gut is less than 3 days. As the total activity of fish increases, I think it was also a real absorption of cesium.

To answer your second question I can say that 70% of chironomous activity was in the digestive tractus. I made an other experiment where I gave to carps chironimid larvae contaminated by water cesium. The retention factor was 0.13 too. Soo carps are contaminated by the larvae more than by sediment they content.

3) We made dissection of mussels. In fact gills have a high cesium concentration, but the highest activity in the second experiment, where anodonta were contaminated by yeast was found in muscular mass, proving that cesium was really metabolised.
In the third experiment a part of yeast activity could have been fixed by sediment but I really think as I said that the closing of the valve according to turbidity and the capacity of mussels to make choices in relation with size and nutritive value was the main reason for the lower contamination of anodonta.

INTERACTION BETWEEN TECHNETIUM AND NITROGEN FIXING ORGANISMS.

C.M. VANDECASTEELE[1], A. DELMOTTE[1], J. HENROT[1], C. VAN HOVE[1] and M. COGNEAU[2].

[1] Laboratoire de Physiologie Végétale
Université Catholique de Louvain
Place Croix du Sud, 4 B-1348 LOUVAIN-LA-NEUVE.

[2] Laboratoire de Chimie Nucléaire
Université Catholique de Louvain
Chemin du Cyclotron, 2 B-1348 LOUVAIN-LA-NEUVE.

ABSTRACT

The reason why nitrogen fixing microorganisms, grown with atmospheric nitrogen as sole N-source, are more sensitive to technetium than other microorganisms and even than the same organisms grown on nitrate has been investigated in a blue-green alga (*Anabaena cylindrica*) and in a free living bacteria (*Azotobacter chroococcum*).

The results show that Tc inhibits the nitrogenase activity at rather low concentrations in the culture medium (from 1 μM) and that this effect is observed after a short incubation time while total growth is still not affected.

The subcellular localisation of Tc in a crude extract of contaminated bacteria shows that this element is bound in an unspecific way to proteic compounds. Nevertheless the way it inhibits the nitrogenase enzymatic system is still unknown but could be tentatively explained by the substitution of molybdenum by technetium at the active site of this enzyme.

INTRODUCTION

The effects of technetium-99, an artificial element principally issued from the nuclear fuel cycle, have already been investigated on several microorganisms (1,2,3,4). Most of the tested organisms showed low sensitivity to this chemotoxic, rather than radiotoxic, radiopollutant and accumulation factors generally did not exceed 20 on a fresh weight basis.

However this is not the case when nitrogen fixing bacteria and cyanobacteria are grown with atmospheric nitrogen as sole N-source. In such conditions, these organisms show higher sensitivity thanothers and tend to take up higher amounts of the Tc present in the medium. Furthermore, when the nitrogenase enzymatic system is located in differentiated cells like heterocysts in *Anabaena* filaments, the autoradiography reveals higher accumulation in these specialized cells than in vegetative cells (5).

These results suggest a specific interaction between Tc and nitrogenase activity. The inhibition of nitrogenase activity has been tested using the acetylene technique and preliminary experiments have been performed in an attempt to localize Tc in a crude extract of contaminated bacteria.

MATERIALS AND METHODS

Two nitrogen fixing organisms have been studied in culture conditions previously described (5) : *Anabaena cylindrica* LEMM., a filamentous cyanobacteria and *Azotobacter chroococcum* BEIJERINCK, a free living bacteria.

Growth was estimated by optical density measurements at 650 nm (*Anabaena*) or 690 nm (*Azotobacter*) using a Klett-Summerson colorimeter.

Nitrogenase activity was assayed by acetylene reduction : the culture vials were fitted with "Suba-seal" rubber stoppers and acetylene was injected to obtain a 0.1 atm partial pressure in air. Gas samples (100 µl) were analyzed periodically for C_2H_2 and C_2H_4 content using an Intersmat IGC 112F gas chromatograph with FID and "Porapak T" in a 3.5 mm Ø x 0.80 m column.

Technetium-99 was provided to the medium as $^{99}TcO_4^-$ (Amersham or NEN). In localisation experiments, molybdenum and sulfur were traced using $^{99}MoO_4^=$ (IRE) and $^{35}SO_4^=$ (Amersham) respectively.

Radioactivity measurements were performed by γ spectrometry (^{99}Mo) and β spectrometry after humid digestion of the samples (^{99}Tc and ^{35}S) in a Packard Autogamma 5220 and Tri-Carb 2450 respectively.

Localisation of incorporated Tc in subcellular fractions of *A. chroococcum* has been performed on bacteria incubated from 4 to 6 hours in a culture medium contaminated at a subtoxic Tc level (0.5 µM). The bacterial cells were collected by centrifugation, washed with uncontaminated medium and suspended in a phosphate buffer (30 mM ; pH 7.5) in the ratio of 5 ml per g of cell paste. This suspension was frozen (-30°C) and the bacteria were disrupted in a X-press (LKB) under 20,000 psi. After thawing the suspension was centrifuged (20' x 20,000 g) and separated into supernatant and pellet. The pellet was resuspended in phosphate buffer containing 0.2% of Triton X-100 as a cleansing agent and centrifuged in the same conditions, while the supernatant was dialysed against buffer (cut off = 10,000 D). The fraction remaining in the dialysis bag was centrifuged (30' x 27,000 g) and the supernatant was passed through a DEAE cellulose (Whatman DE-52) column (16mm ∅ x 18 cm) and eluted under NaCl gradient from 0.0 to 0.5 M NaCl in buffer. Elution was monitored at 280 nm and collected fractions were analysed for ^{99}Tc, ^{99}Mo and ^{35}S content.

RESULTS AND DISCUSSION

In order to understand the very high sensitivity of diazotrophic microorganisms when grown on N_2 as compared to other microorganisms or to the same organisms grown on NO_3^-, we measured the effect of Tc on N_2 (C_2H_2) reduction.

The first figure shows the inhibition of nitrogenase activity in *A. cylindrica* as a function of time and Tc contamination level. With a concentration of 3.2 µM TcO_4^- in the culture medium, a moderate reduction of ethylene evolution rate is observed, whereas, with concentrations of 10 µM and higher, nitrogenase activity is reduced to less than 1% as compared to the control.

This inhibition is correlated with a drastic increase of the heterocysts to vegetative cells ratio, from 3% (control) up to 20% (100 µM treatment) (6), which is characteristic of N-starved diazotrophic filamentous cyanobacteria (5).

Inhibition of N_2 (C_2H_2) reduction by Tc has also been observed with *A. chroococcum* : nitrogenase activity is reduced by Tc concentrations of 1 µM and higher (unpublished results).

Moreover, a 25 µM Tc contaminated culture (fig. 2) shows a complete inhibition of nitrogenase activity after one hour incubation while growth remains unaffected for at least two and a half hour after contamination.

A possible explanation of the observed effects of Tc on nitrogen fixation should be a substitution of the nitrogenase molybdenum by Tc. Such substitutions have already been reported for other metals (vanadium, tungsten) in nitrogenase and nitrate reductase, with partial or total loss of the enzymatic activity (7, 8, 9). As the chemical properties of Mo and Tc are quite similar, the hypothesis of a replacement of the first one by the second on the active site of nitrogenase should not be excluded.

To investigate this hypothesis, we tried to isolate the organic compounds responsible for the binding of Tc from contaminated bacteria. The distribution of ^{99}Tc in the different fractions obtained is presented in figure 3.

Of the total activity accumulated in the cell paste, 15% are found in the deterged pellet associated with cell walls, membranes and undisrupted cells, while 79% are recovered in the cytosoluble fraction. Another 14% are lost by dialysis of the cytosoluble fraction as free Tc or Tc bound to low molecular weight molecules (< 10,000 D).

The supernatant obtained after centrifugation of the solution remaining in the dialysis bag contains 60% of the total activity accumulated by bacteria. This fraction has been chromatographied on DEAE cellulose using a NaCl gradient from 0.0 to 0.5 M in the phosphate buffer. Most of the Tc is removed from the column by 0.3 M NaCl in the buffer.

The spectra obtained for O.D. (280 nm) and the distribution of ^{99}Tc, ^{99}Mo and ^{35}S are presented in figure 4. A close parallelism is observed between the absorbance spectrum and the distributions of Tc and S (and thus proteins), except for very acidic compounds supposed to be nucleic acids. Moreover, a shoulder in the Tc spectrum seems to correspond to the Mo peak.

Precipitation tests performed on the cytosoluble fraction of Tc contaminated bacteria show that 85% of the activity is removed from the supernatant by acetone treatment and that 90% of the Tc is precipitated by ammonium sulfate. This indicates an association of Tc with the proteic fraction.

CONCLUSIONS

From these results, it appears that Tc incorporated in bacteria is principally bound to proteins in an unspecific way but also that nitrogenase is specifically inhibited. This last observation explains the higher sensitivity of diazotrophic microorganisms grown without combined nitrogen source. The reason for this inhibition can be temptatively explained by a substitu-

tion of Mo by Tc at the enzyme active site but this hypothesis has to be confirmed and is under investigation.

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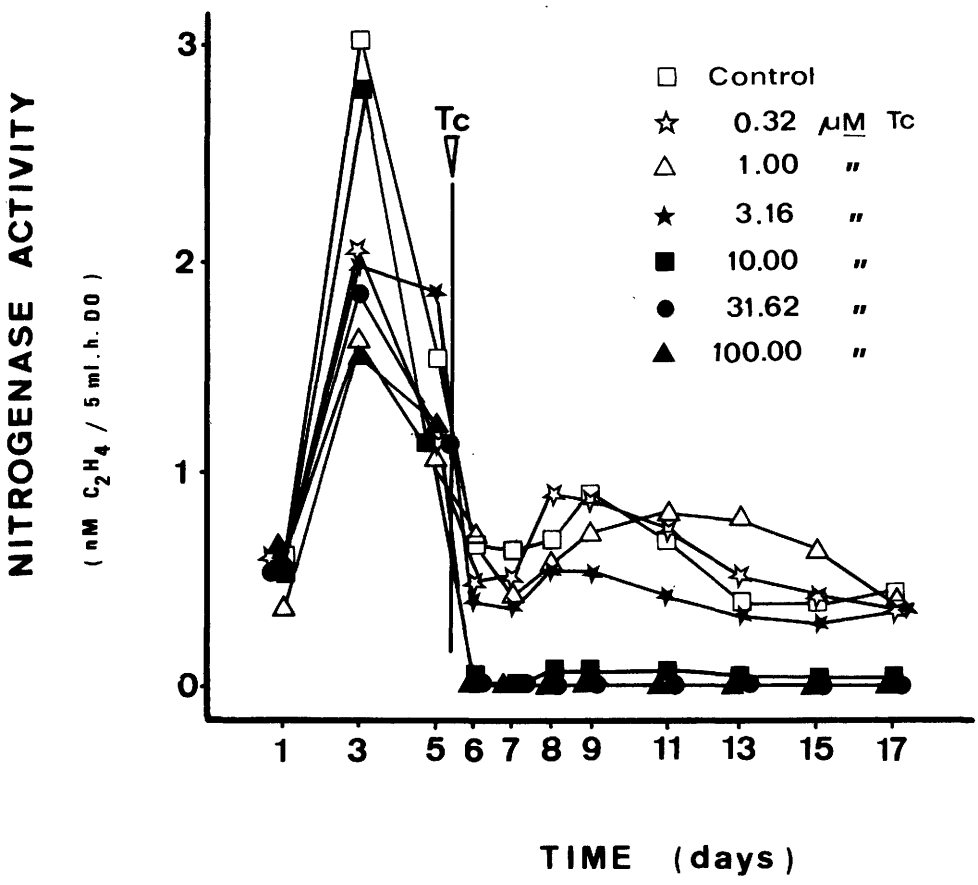

FIGURE 1. Effect of technetium concentrations in the medium on nitrogenase activity (C_2H_2 reduction) of *A. cylindrica*. Tc is added at the time indicated by the arrow.

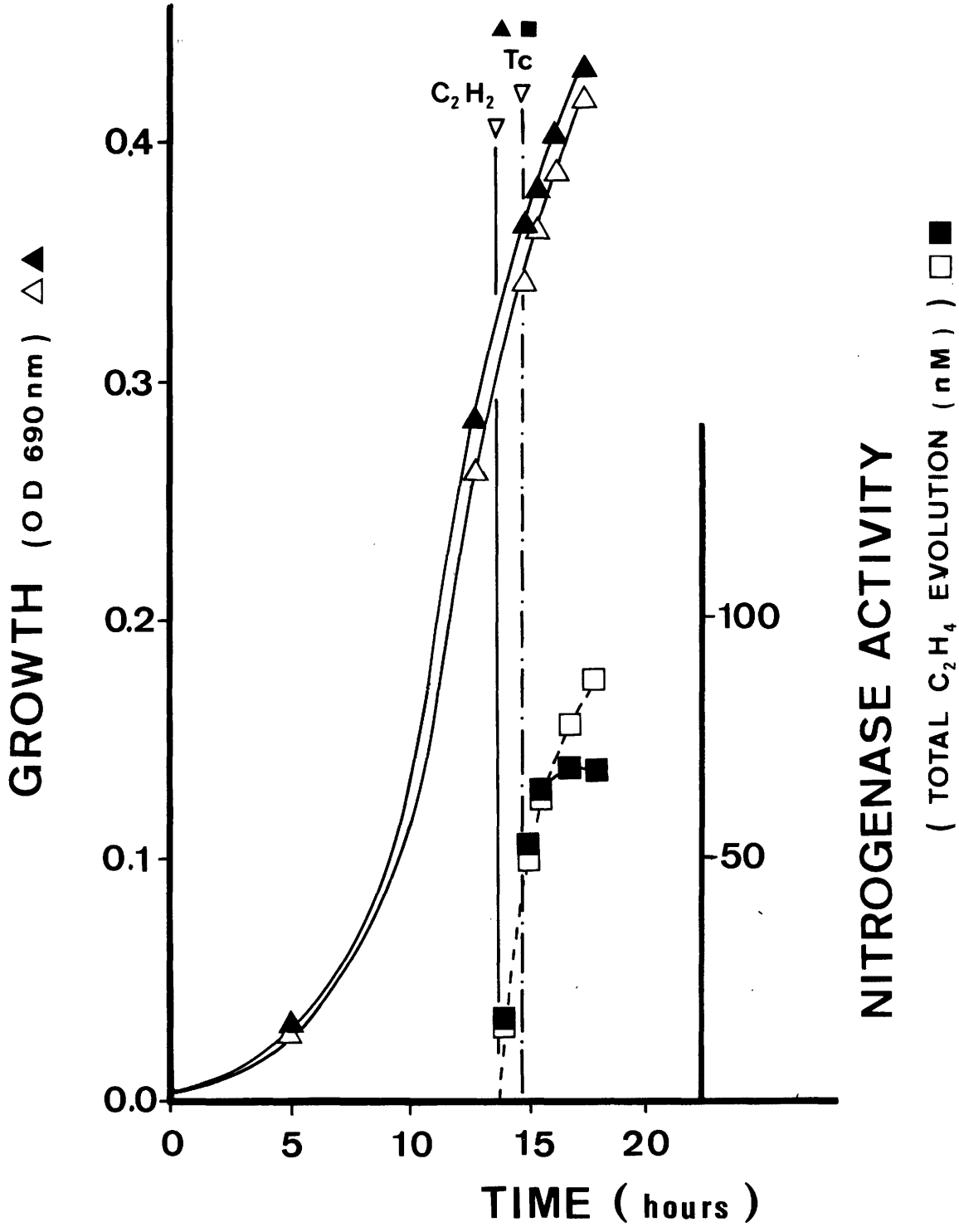

FIGURE 2. Effect of technetium (25 μM) on growth and nitrogen fixation (C_2H_2 reduction) of *A. chroococcum*.
Acetylene and Tc are added at times indicated by the arrows.

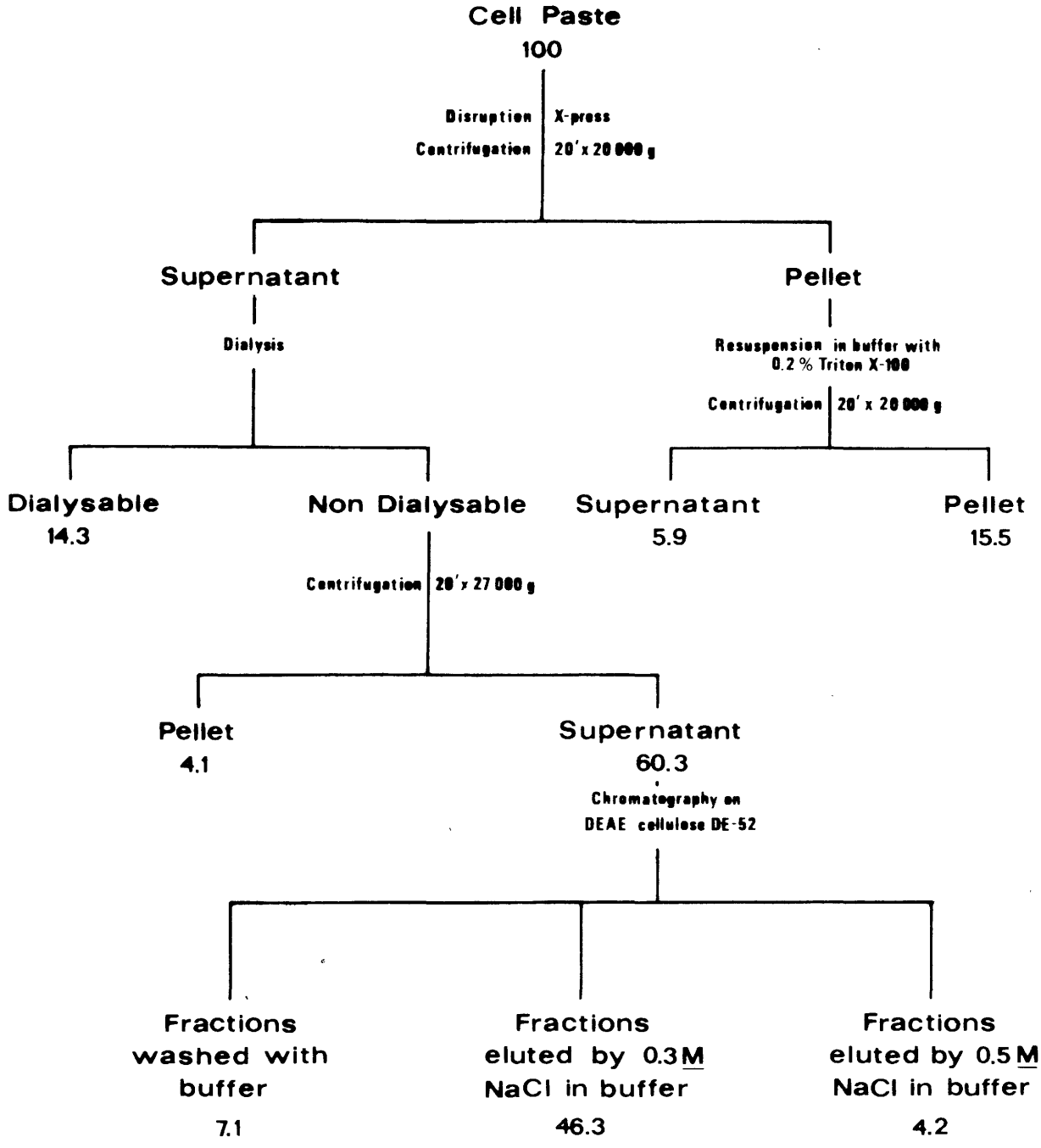

FIGURE 3. Subcellular fractionation of technetium contaminated *A. chroococcum*. The distribution of Tc (in % of the total activity found in the cell paste) is given for the different fractions.

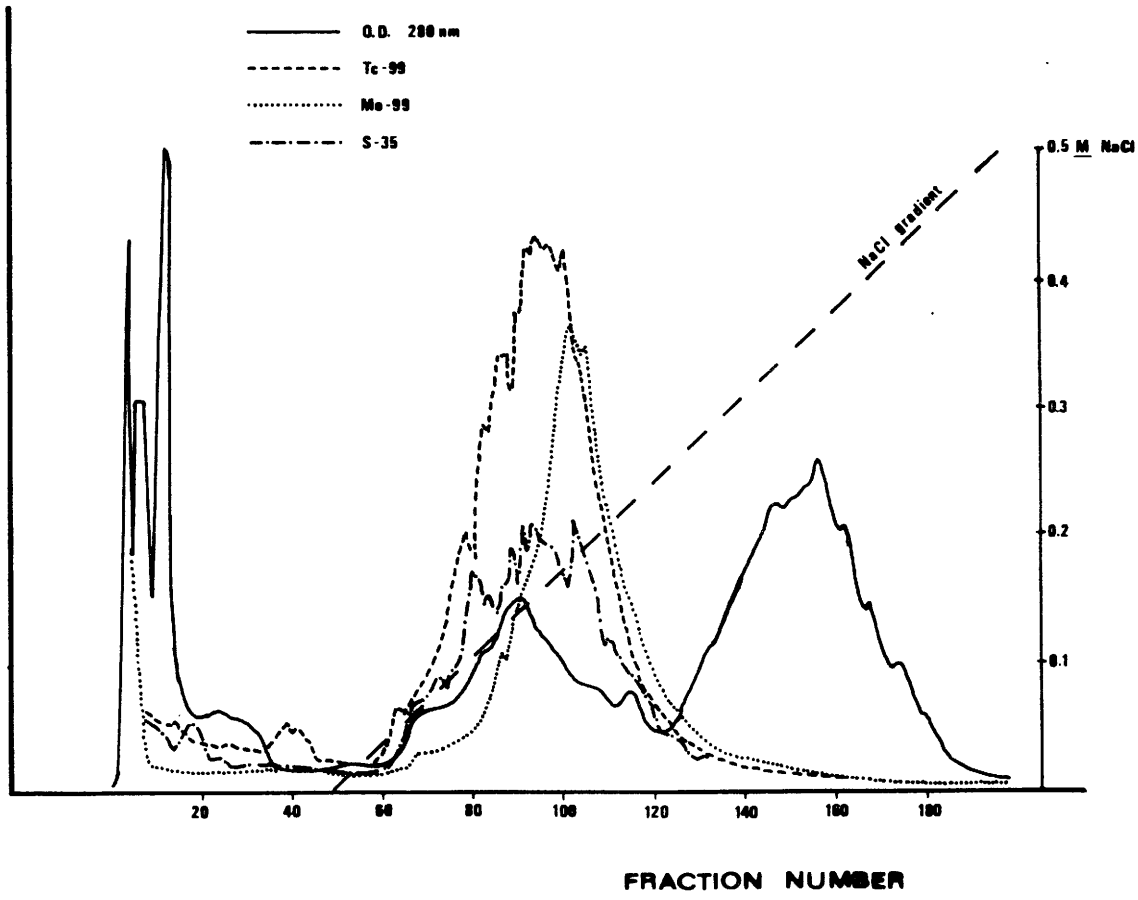

FIGURE 4. Chromatography on DEAE cellulose of a dialysed cytosoluble extract of technetium contaminated *A. chroococcum* under NaCl gradient from 0.0 to 0.5 M in phosphate buffer (30 mM ; pH 7.5): optical density (280 nm) spectrum and Tc, Mo and S distributions in the collected fractions.

Session : II

Paper by : C.M.Vandecasteele

Comment by : A.A.Abdallah

Text of comment
or author's answer :

Is it certain that Tc takes the place of Mo in nitrogenase ?

The lack of activity does not proveit sufficiently ?

The inhibition of the N_2-ase activity can be explained by other hypothesis of course, for example by the binding of Tc on the enzyme in another site than the Mo site with subsequent inactivation of the enzyme, but any way as we consider that other metals are able to take the place of the Mo at the N_2-ase component I active site and since we consider that Tc and Mo have quite similar chemical properties the hypothesis of a substitution of Mo by Tc seems to be very probable and we are currently trying to confirm it, of course if we fail to confirm this, we have already other possible explanations that we will have to test.

Session : II

Paper by : C.M.Vandecasteele

Comment by : Bors

Text of comment
or author's answer :

It would be interesting to known how is the distribution of Tc in blue-green algae, is it located more in the heterocysts, then it is known that nitrogenase is located in the heterocyst also. Is it no contradiction between the inhibition of nitrogenase and an increase of the number of heterocyst you reported ?

Autoradiographic studies have shown a preferential accumulation of Tc into heterocysts when N_2-ase is located too.

There is no contradiction between N_2-ase inhibition and the increase of the heterocysts ratio : when the filamentous cyanobacteria are N-starved it has been demonstrated that lack of internal nitrogen induces the differenciations of vegetative cells into heterocysts. In our case, the N-starvation is not due to a lack of available N but to the inhibition of N_2-ase and to the subsequent unability of the blue green algae to utilize the available dinitrogen and this gives rise to a N-starved like behaviour.

Session : II

Paper by : C.M.Vandecasteele

Comment by : W.Kühn

Text of comment
or author's answer :

If Tc interacts specifically with nitrogenase - Mo-Fe-Complexes one should investigate this fact with Mössbauerspectroscopy; we investigated Azotobacter-Enzymes by Mössbauer-research at low temperatures. We can support you in that experiments.

I completely agree with your comment and we will try to perform such analysis. But we need first to isolate the molybdoferredoxin, what we are currently busy with.

Session : II

Paper by : C.M.Vandecasteele

Comment by : N.Fisher

Text of comment
or author's answer :

In extrapolating your toxicological results to the natural environment, you needed around 10 µM of Tc to elicit as significant response, yet the Tc atom concentrations in even the most contaminated water (e.g. in the Irish Sea off Sellafield) are some 6 orders of magnitude lower.
What revelance do your results have to natural conditions ?

The Tc levels we used are higher than what we find in the environment except for accidental conditions. But we observed a higher uptake in diazotrophs grown in nitrogen fixing conditions. Furthermore the N_2-ase is affected by Tc, at quite high Tc level if we consider environmental conditions, quite low if we consider the sensitivity of other microorganisms.
So the study of the mechanisms responsable of the inhibition of N_2-ase following Tc contamination are interesting at a physiological point of view but will may be allow us to explain the higher uptake we observed for N_2-fixing bacteria in N_2-fixing conditions and this is of radiological interest.

Session : II

Paper by : C.M.Vandecasteele

Comment by : M.Hughes

Text of comment
or author's answer :

Have you studied the effect of added Mo on inhibition by Tc?

The interactions between Tc and Mo are actually under study as absorption is concerned as well as considering the nitrogenase activity.
The preliminary results we have obtained show that increasing the Mo level in the medium does not affect the Tc uptake nor the nitrogenase inhibition by Tc.

Session : II

Paper by : C.M.Vandecasteele

Comment by : S.Bonotto

Text of comment
or author's answer :

You concluded that nucleic acids are not binding technetium. Are such nucleic acids,DNA or RNA ?

Secondly, do you have evidence for binding of Tc to polysaccharides ?

As nucleic acids are concerned, we have not try to distinguish between DNA and RNA.
The results show that the proteic fraction is responsible for the Tc binding in the cytoplasm of contaminated bacteria but we have no informations concerning the polysaccharides.

ROLE OF MACROINVERTEBRATES AND MICROFUNGI IN THE TRANSFER OF 32-P ALONG DETRITUS FOOD CHAINS IN RIVERS

L.ROSSI[1], P.CALOW[2] and L.NOBILE
[1]Dipart. di Genetica e Biol.Molecolare,Sez.Ecologia, Univ.di Roma,00185 Roma,Italy
[2]Dept. Zoology,University of Glasgow, Glasgow G12800 United Kingdom

1 INTRODUCTION

Phosphorus is a major factor limiting the productivity of freshwater ecosystems and relatively high concentrations of its isotop 32-P,may be found in the organisms involved in detritus systems. In fact, the supply and transfer of this element in freshwater depend crucially on the transport of material through the detritus food chains[1] .Microorganisms,microfungi in particular,and macroorganisms are involved as prey/ /predator systems in the detritus food web, but their specific rôles in P-transfer are not fully understood[2].On the other hand,the trophic niches of detritivores are strongly affected by competition as well as predation which control the coexistence equilibria in the ecosystems[3].Hence,the recycling rate from detrital sediments of P-supply could depend on regulation mechanisms of detritivorous populations as well as their food requirements.In present paper,we describe the fate of 32-P utilized by single microfungal species present on natural detritus in a lotic ecosystem,with two main aims:A) to establish the involvement of microfungi, detritivores and invertebrate predators, in 32-P transfer along detritus food chains; B) to determine the rôle of invertebrate predation on coexistence of detritivores and on P-dynamics.

2 MATERIAL AND METODS

The experiments were carried out *in situ* in a Scottish river (R.Kelvin,Glasgow).Five major species of fungi, initially isolated from natural litter in the river, were used: *A.niger*, *C. herbarum*,*P.proliferum*,*P.cyclopium*,*M.mucedo*.The detritivores were the isopod, *Asellus aquaticus* and the pulmonate snail, *Lymnaea peregra*,both of which are known to eat fungi on leaf litter and both of which were the most abundant macroinvertebrate species in the R.Kelvin at the time of experiments(July -October).The predators were *Dendrocoelum lacteum*(Platyhelmin thes,Tricladia) and *Erpobdella octoculata*(Annelida,Hirudinea) again natural predators in the river at the time of study.The experiments were performed in enclosures(20x60x80cm),were the abundance of predators could be manipulated.Five alder leaf

packs(4.5g dry weight),each incubated for 15 days with a single species of fungus,were added to every enclosure,but only one leaf pack was labelled with 32-P per enclosure.These leaf packs were labelled during their incubation period in Enrlenmeyer flasks containing 1.88 μCi/ml of 32-P as H_3PO_4.The enclosure were replicated in pair but one of the pair contained no predators.Hence,10 enclosures were used for each experiment--5 to account for labelling all the fungi x 2 to account for presence and absence of predators.These were left in the shallow water of the river for 7 days,after which all animals that had colonised the leaf packs were manually separated and 32-P activity was monitored in the animals and in the packs themselves using liquid scintillation techniques.Samples of animals and leaf packs were oven dried before radioassay(60°C per 24h.) and weighed to determine the activity density and trophic transfer index[4].

3 RESULTS AND DISCUSSION

The five used fungi show different uptakes of radiophosphorus from the water where they were incubated with leaf packs(TABLE 1).This seems related to different growth rates of fungi on alder leaf litter .In fact,the mean activity densities(AD) of the fungus strains range between 0.176 μCi/g d.w. for M. mucedo and 0.018 μCi/g d.w. for A.niger.These are the strains more and less abundant respectively,on leaf litter in R.Kelvin at the time of the experiments.Three out of the five considered fungi act as important sources for 32-P for trophic chains.Where C.herbarum,P.cyclopium and M.mucedo are sources for 32-P,both AD and % of labelled animals are relatively very high(>>0.1 μCi/g d.w.) (TABLE 1).These results are related to the food niches of detritivores(prey).In fact,marked intra--interspecific variability for the exploitations of various fungi is observed in both Asellus and Lymnaea populations (e.g.%L and AD are distributed with significant heterogeneity among the enclosures,FIGURE 1).Although both detritivorous species are involved in 32-P transfer,the AD and TTI of Asellus are always greater than those of Lymnaea. Moreover,correlation is significant between the AD of Asellus population and correspondent AD of predator population($r=0.92$, $p<0.05$). The rates and importances of the routes of 32-P transfer are significatively modified by presence of predators in the trophic system.In fact,total uptake of radiophosphorus of detritivores is reduced by 3/4 in the enclosures with predators. Exactly,detritivores transfer 70% of isotope were predators are excluded and only 19% where predators are abundant(FIGURE 2).This was due to reductions of the trophic niche and dynamism of the Asellus populations in the enclosures with preda-

TABLE I Labelled fungus and detritivores with their predators in each enclosure. P=predators present: NP=predators excluded. Nc=mean numbers of animals sampled on leaf packs in each enclosure; %L=% labelled animals: AD=mean activity density as μCi/g d.w.; TTI=trophic transfer index. $\approx$ =significative heterogeneity within distribution; * =significative difference between distribution joined by bar ($p < 0.05$); ns=not significant. χ^2-test.

Enclosures			A	B	C	D	E	
Labelled Fungi on leaf-packs			Aspergillus niger	Cladosporium herbarum	Pytium proliferum	Penicillium cyclopium	Mucor mucedo	
		AD	$\approx$ 0.018	0.044	0.068	0.108	0.176	
Asellus aquaticus	P	Nc	ns 66	49	65	51	54	ns
		%L	$\approx$ 18	29	68	58	22	*
		AD	$\approx$ 3.3	4.1	2.4	3.1	6.1	*
		TTI	$\approx$ 183	93	35	29	37	*
	NP	Nc	ns 54	52	62	67	76	
		%L	$\approx$ 62	83	94	96	69	
		AD	$\approx$ 13.2	14.1	21.1	30.1	28.3	
		TTI	$\approx$ 733	320	310	279	161	
Lymnaea peregra	P	Nc	ns 25	18	30	24	27	ns
		%L	$\approx$ 76	62	--	18	74	*
		AD	$\approx$ 2.3	2.0	--	1.9	4.4	*
		TTI	$\approx$ 129	46	--	17	25	*
	NP	Nc	ns 18	23	21	26	15	
		%L	$\approx$ 69	62	--	22	76	
		AD	$\approx$ 1.9	1.5	--	1.9	4.1	
		TTI	$\approx$ 105	33	--	17	23	
Predators	P	Nc	ns 15	14	16	17	10	
		%L	$\approx$ 16	72	23	61	85	
		AD	$\approx$ 0.01	0.18	0.01	0.32	1.00	
		TTI	$\approx$ <.01	0.06	<.01	0.13	0.19	

tors.In fact, in those experimental conditions the Asellus shows significative decrements of the 32-P uptakes and %labeled animals(FIGURE 1).On the other hand,the presence of invertebrate predators facilitate the coexistence of the detriti-

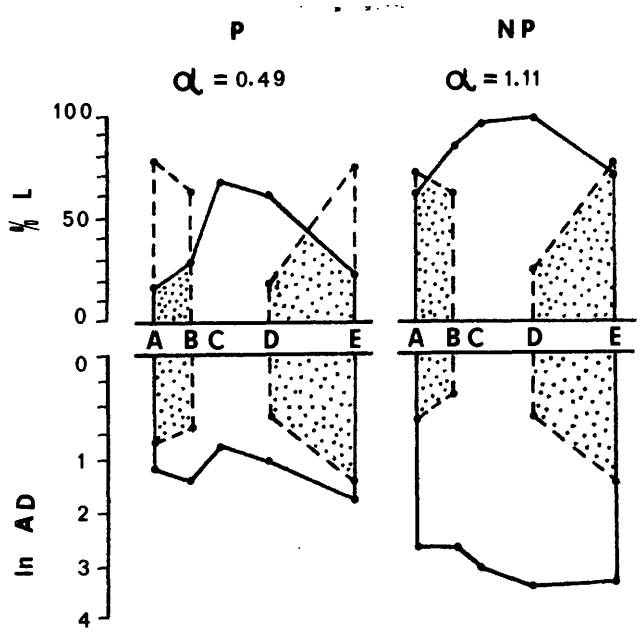

FIGURE 1 Trophic preferences (%L)and uptake of 32-P (lnAD), of native A.aquaticus (——)and L.peregra (---)with respect to 5 labelled fungi.The fungi(capital letters as in TABLE 1) are ranked in according to AD of leaf packs. P=predators present; NP=predators excluded; α=coefficient of interspecific competition; the shaded areas are the food niche overlaps.

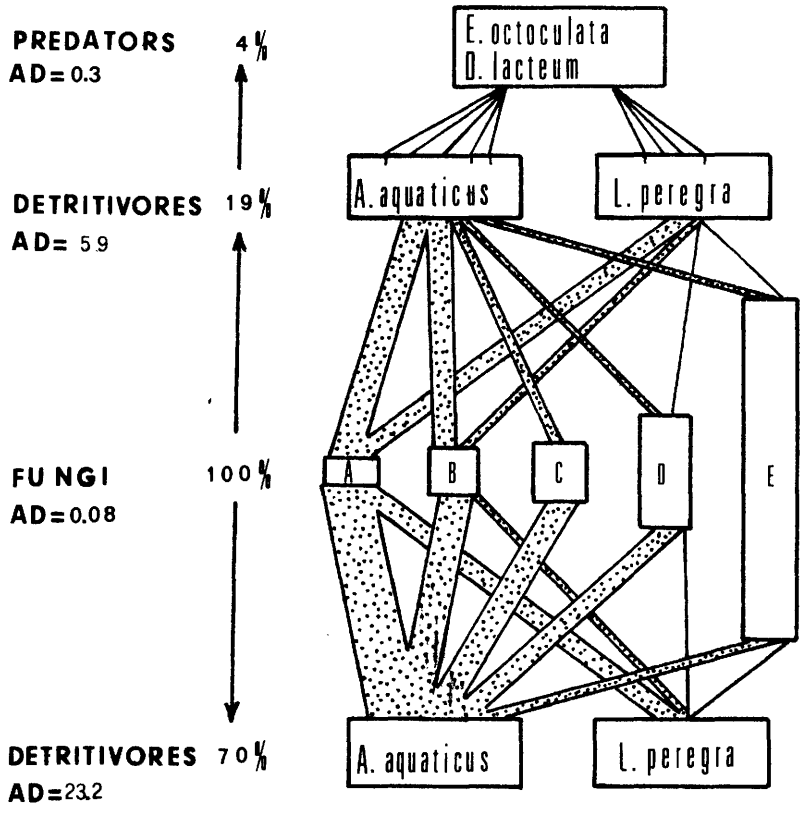

FIGURE 2 Transfer of 32-P along detritus food chains with predators present(upper)or excluded(lower)in R. Kelvin.The lines indicate the extend of trophic transfer from food sources.

vores reducing their trophic overlap(α =0.49).Without predators the large overlap(α =1.11)should not allow stable coexistence and also the potential routes of transfer throughout the ecosystem of phosphorus in general and radioisotope in particular should be reduced.

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ABSTRACT

We describe the use of 32-P in investigating the transfer of phosphorus through trophic chains constituted by microfungi, detritivores and invertebrate predators.Our aim will not only be to use 32-P as a tracer for understanding the overall phosphorus dynamics of the system, but also to consider the involvment of microfungi and invertebrates in the cycling of radiosotopes. The experiments were carried out in situ in a Scottish river (R.Kelvin,Glasgow).They were performed in enclosures (sacks) where the abundance of prey and invertebrate predators could be manipulated.Five leaf packs, each inoculated with a single species of fungus, were added to every sack, but only one fungus was labelled with 32-P per sack. The sacks were replicated in pairs but one of the pair contained no predators.Hence, 10 sacks were used for each experiment - 5 to account for labelling all the fungi x 2 to account for presence and absence of predators.These were left in the river for 7 days, after which 32-P activity was monitored in the animals that had colonised the packs and the packs themselves using liquid scintillation techniques.From these experiments we found that only 3 out of 5 of the fungi acted as sources for 32-P for the food chains in the enclosures and this was related to the food niches of the detritivores. Moreover,the routes and the rates of transfer were modified by predation and this could be ascribed to predator-mediated shifts in the food niches of A.aquaticus.

Session : II General Discussion

Comment by : Azam

Text of comment
or author's answer :

1. Follow up on a comment on the importance of microbial surface in radionuclide uptake : the importance of surface is self-evident if one considers it in relation to both adsorption and in uptake by specific transport system.
   In the absence of precise knowledge of the nature of interaction between the microorganism and radionuclide, it is reasonable to assume that the interaction will be a function of the cell surface area.
   One hopes however that this starting point will be persued further to determine the actual mechanism of interaction at physiological/biochemical level.
2. Regarding radionuclide distribution within the sub-cellular fractions : the problem of redistribution during cell breakage and fractionation can be serious. Perhaps supplementary evidence (e.g. by micro-analysis) would be desirable.

SESSION III : Terrestrial soil microorganisms

III/A	Chairman	W.KÜHN
	Co-Chairman	S.STRACK
III/B	Chairman	A.CREMERS
	Co-Chairman	P.BOVARD

A REVIEW OF MODELS FOR THE GROWTH AND DISAPPEARANCE RATES OF MICROORGANISMS IN SOIL

M.J.Frissel[1)], J.A.van Veen[2)] and A.M.A.van der Linden[2)]
1) National Institute of Public Health (RIVM), Bilthoven, The Netherlands
2) ITAL Research Institute, Wageningen, The Netherlands

ABSTRACT

There are three important mechanisms by which microorganisms play a role to the behaviour of radionuclides in soil. 1) Microorganisms consume material which contains radionuclides. The radionuclides become altered within the microorganism, upon release they remain altered. 2) Microorganisms produce organic compounds which may react with radionuclides. 3) Microbiological activity may change the redox potential of an ecosystem and thus induce changes of the chemical form of radionuclides.

There exists a wealth of models to describe the biogeochemistry, but only part of these models consider microorganisms as a system variable. The growth rate of microorganisms depend on the availability of substrate. Easily accessible substrate allows a fast growth. Several models take care of the differences in availability as well as of the changing composition of substrate with time. Decay of microorganisms may be due to starvation or due to grazing by predators. There exist models for both mechanisms. The models discussed are scientific ones, i.e. they are developed to provide understanding, rather then for management purposes. They are operational, well documented and verified for particular cases. Tests for wide ranges of conditions (validation) are usually lacking.

Part of this research has been carried out under CEC contract BIO 325 NL.

1. INTRODUCTION

There are several mechanisms by which microorganisms affect the behaviour of radionuclides. The three most important ones are:

1) Microorganisms consume material which contains radionuclides. The radionuclides may become altered within the microorganism and therefore, after release from the microbial cell, the radionuclides may have a different chemical form.
2) Microorganisms produce organic compounds which are able to react with the radionuclides. Examples are carbon dioxide which causes the formation of soluble complexes with transuranics and the production of chelates and other organic complexing agents which form easily complexes with radionuclides of the transition metal type or earch alkali type (e.g. siderophores, Akers[1]).
3) Microorganisms induce anaerobic conditions and inherently a decrease of the redox potential. A dramatic change of the properties of many radionuclides will be the result.

The general knowledge of the first two processes has not yet reached a stage that generally applicable models describing the processes can be developed. It can, however, be assumed that both processes are positively related with microbial activity, i.e. the growth and decay of microorganisms. The mechanism of the third process is rather well known, although models which predict anaerobic conditions are still rather empiric. Only the relation between the chemical form of most radionuclides and the redox potential is well known. The latter topic is, however, outside the scope of this paper. The microbiological products represent only a small fraction of all soil organic material. The major part consists of humic material, which is not well defined but can be considered as the end product of a series of chemical condensation reactions of low and high molecular weight organic compounds. The latter ones may include microbiological products, root exudates, and other organic products. Humic material is negatively charged and adsorbs all cations (M) in the sequence $M^{+} < M^{++} < M^{+++}$. The exchange capacity may be as high as 3 $meq.g^{-1}$. Particularly, colloidal compounds are strongly adsorbed. Humic acid and fulvic acid can be obtained from humic material by extracting with a strong NaOH solution, these compounds are not considered here. The main aim of this publication is to inform on the state of the art of modelling the growth and decay of microorganisms in soil.

2. A WEALTH OF MODELS

There exists a wealth of models which describe the geochemical reactions in soil but not all of them include the microorganisms explicitely.
There are e.g. many models on the behaviour of nitrogen in soil which describe processes as mineralization, immobilization of nitrogen, nitrification and denitrification without specifying the microbiological population. The same can be said for many models which describe the course of the organic matter in soil. Yet, the latter models provide a better understanding than the nitrogen models because the decomposition rates, e.g. in kg C per year state in fact explicitely what amounts of carbon have been consumed by microorganisms. Assuming a 40 percent incorporation of the carbon in the produced microorganisms provides an idea of the quantity produced. Interpretation of Bolin's[2] world carbon balance in this way shows us that annually 36.10^{15} g of microorganisms are produced. Naturally they decay with almost the same rate.

There are, however, also many models in which the microorganisms are explicitely included.

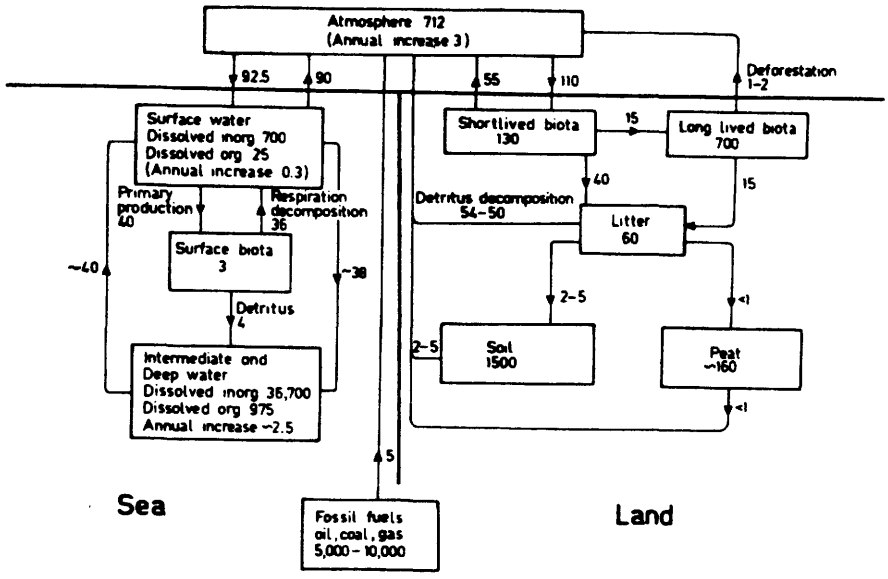

FIGURE 1 Size of carbon reservoirs (in 10^{15} g) and fluxes (in 10^{15} g yr^{-1}) for that part of the cycle that is in a state of comparatively rapid turn-over (Bolin[2]). Reproduced with the permission of Scope.

3. MICROBIOLOGICAL MODELS

Within this context a model is considered as a microbiological one when at least one type of microorganism is explicitely included as a system variable. Almost all microbiological models take into account the availability of substrate as a source for the growth of microorganisms. Figure 2 shows an example of a model in which four different types of chemical compounds serve as substrate for the microorganisms, here called biomass. The plant residues remaining in the soil (roots, straw, green manure) are subdivided into four components: proteins, sugars, cellulose and lignin. Some of the biomass products are transferred to a resistant biomass pool that does not further contribute to the cycle. The biomass utilizes the four components with different specific rate factors. As long as there are sufficient protein and sugar components the biomass grows fast. When these components becomes depleted there is only slow growth. When also lignin and cellulose are depleted the biomass decays. The advantage of the model is that the specific rate factors can easily be determined by laboratory experiments. Also specific mechanisms as e.g. catabolic repression (i.e. a specific preference for one of the components and inhibition of others) can be included. The disadvantage of the model is that it is hardly possible to analyse the soil organic matter fraction and to determine the pools of proteins, sugars, cellulose and lignin.

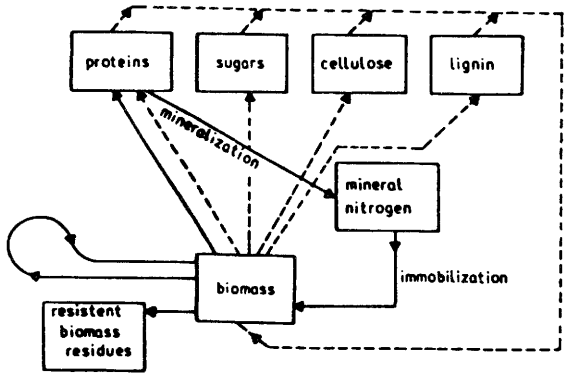

FIGURE 2 The growth of microorganisms (biomass) on four different types of chemical compounds. Full lines: nitrogen flux; dotted lines: carbon flux (Frissel and Van Veen[3]).

Another model of the same authors (Van Veen and Frissel[4]) is shown in figure 3. The organic matter is now divided into 6 pools: Well decomposable C containing fresh material, slowly decomposable C containing fresh material, C + N containing decomposable material, well decomposable C containing active soil organic material, resistent C + N containing active soil organic material and old organic material. The latter subdivision is rather well accepted by many soil scientists, but it remains difficult to subdivide the organic material in the soil to the proper fraction. Two versions of the model exist, a linear one in which the growth rate of the microorganism is independent of

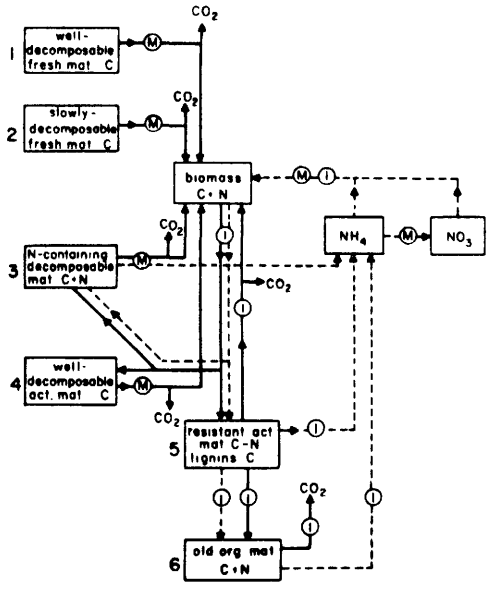

FIGURE 3 The growth of microorganisms (biomass) on five different types of organic material. The old organic matter is supposed not to be used by the microorganisms. Full lines: carbon flux; dotted lines: nitrogen flux (Van Veen and Frissel[4]).

the size of the microbial population and another one in which the growth, within certain limits, is proportional to the quantity of microorganisms already present. This results in Michaelis-Menten or Monod like equations of the form: $\frac{dB}{dt} = V_{max} \frac{S}{K_s + S} \cdot B$ in which B = biomass, S = substrate, K_s = Michaelis-Menten constant, V_{max} = specific maximum growth rate and t = time This has proved to be a satisfactory description of the substrate dependent growth of microorganisms under steady-state conditions. However, in soil with its fluctuating environmental conditions, often the M-M equation appears not to be correct. A model almost similar to the one of Van Veen and Frissel has been developed by Juma and Paul[5]. They distinguish 7 organic fractions. A similar model is that of Bosatta[6] who takes into account only two organic matter pools, one with only carbon and another one with carbon and nitrogen. Bosatta applied his model to forest systems. Another model of this type is Phoenix, developed by McGill et al.[7]. From a chemical point of view the substrate is less well defined as in the previous models. From a practical point of view this model is easier. It contains e.g. the pools 'metabolic litter' and 'structural litter', these are pools which are easily recognized. The microorganisms are subdivided into two groups: the bacteria and actinomycetes and the fungi. The specific rate factors for both groups are different. As a consequence, when easily decomposable material is most abundant the growth of bacteria and actinomycetes dominates, when cellulose is most abundant the growth of fungi dominates.

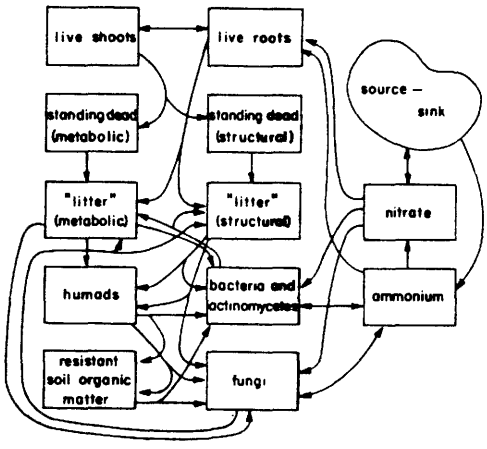

FIGURE 4 The growth of microorganisms according to model PHOENIX (McGill et al.[7]). Two different types of microorganisms are included. Reproduced with the permission of Pudoc (Wageningen).

In particular for those who want to model the interaction between microorganisms and radionuclides these models are important. Conceptually, it is acceptable that this interaction is proportional to the growth rate, irrespective of the fate of the microorganisms. A consequence of the growth models is that when all available substrate have been used the microorganisms will decay. 'Old organic material' is nowhere considered as a suitable substrate, consequently in a deep repository, after some time, there will be

no microorganisms left. Although it may be overdone to assume sterility, no growth of any significance must be expected. But also in a top soil (e.g. shallow land burial) the microbial population might soon reach a very low level when the supply of organic matter decreases. Van Veen et al.[8] suppose a protection mechanism which prevents that the bacteria population decreases below particular limits. This brings us to the question of how the decay of bacteria is simulated. Microbiologists are used to consider a maintenance energy, i.e. to maintain the population as a particular level, substrate is required which consequently is not utilized for growth. Without this minimum supply the population decays. A more general view is that some microroganisms use part of their neighbours as a source of energy. If any, only a small fraction of the carbon products released from lysed cells are incorporated into new microorganisms, the remaining part of the carbon is converted into CO_2 to provide energy. The population decreases therefore without supply of substrate. In top soils it is, however, more realistic to assume that the microorganisms are grazed, i.e. consumed by predating organisms such as nematodes, protozoa and arthropodes. In fact one has to model an important part of the food web in the soil. An example of such a model is shown in figure 5. Grazing acts as a feed back mechanism which sets an upper limit to the microbial population. From a modelling point of view a handsome approach because it prevents instability of the system. An underlimit for the microbial population follows from the consideration that the microorganisms which are present within narrow cracks and holes cannot be grazed because the predating soil animals cannot reach them.

4. ANAEROBIC SYSTEMS

As said a system becomes anaerobic when all oxygen is used by the microorganisms. The oxygen can, however, easily be replenished by diffusion of oxygen from the atmosphere or from oxygen containing water. When the microbiological growth rate is known it is easy to calculate the oxygen consumption rate. The only process which has to be modelled then is the oxygen diffusion. For a homogeneous system e.g. a sediment of a lake, this can be done with a relatively simple linear diffusion model. This provides no special difficulty. For a soil which contains pores of various dimensions, crumps and granules, the situation is, however, very complicated (Van Veen and Frissel[4]). Inside the granules and crumps as well as relatively far from the pores the system may become anaerobic while the other part of the system is aerobic. With increasing water content the diffusion through the pores decreases and near water saturation it is almost negligable. The calculation of the gas diffusion through a pore system which is close to saturation is a very difficult one and a generally accepted model is lacking. Most calculations use instead emperical calculations between the degree of water saturation and anaerobiosis (Leffelaar[10]).

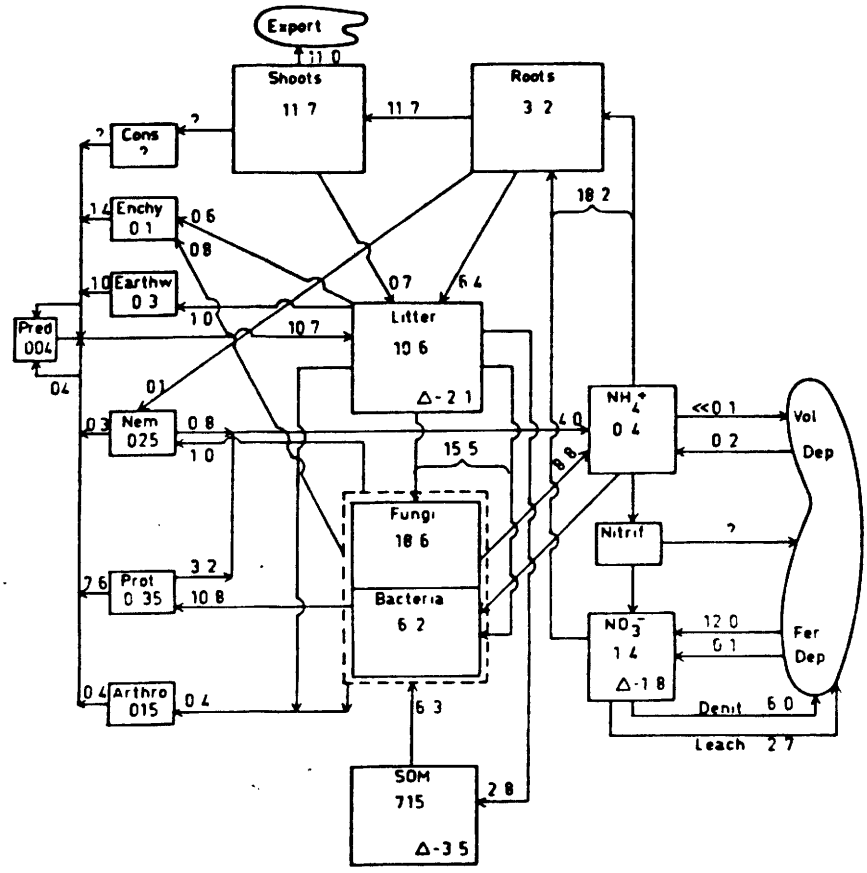

FIGURE 5 A soil food web model for barley receiving 120 kg N $ha^{-1}yr^{-1}$. Compartment abbreviations are as follows: cons (above ground consumers), enchy (enchytraeids), earthw (earthworms), pred (predatory founa), nem (nematodes), prot (protozoa), arthro (soil micro- and macro-arthropods), som (soil organic matter), nitrif (nitrifying bacteria), vol (volatilization), dep (dry and wet deposition), fer (fertilizer), denit (denitrification), leach (leaching). Compartment values (g N m^{-2}) are mean annual estimates except for shoots and roots which are peak standing crop values. Delta symbols indicate net changes, otherwise compartments are assumed to be in steady-state. All flows are expressed as g N m^{-2} yr^{-1} (Rosswall and Paustian[9], reproduced with the permission of Martinus Nijhoff).

5. PROGRAMMING AND MODEL VALIDATION

With the exception of the models of Bolin[2] and of Thomas and Paustian[9], which are budgeting models, the models are dynamic ones. The development of a mathematical model which describes the dynamics involves four to five steps. The first step is the description of each separate dynamic process by a rate equation.

A typical set of equations, for the model shown in figure 3, for the growth of that part of the biomass involved in the decomposition of component x is

$$dB_x/dt = dB_{r,x}/dt.B.C_x/C_T$$

where $dB_{r,x}/dt$ = relative growth rate of biomass on component x; B is total microbial biomass; C_x is carbon present in component x; C_T is carbon present in the four components.
The relative growth of the biomass is calculated by:

$$dB_{r,x}/dt = V_{m,x}/(K_{m,x} + C_x)\cdot C_x \cdot f(temp.,moist.)\cdot f(NO_3^-,NH_4^+)$$

where $V_{m,x}$ and $K_{m,x}$ are the Michaelis-Menten constants; f(temp.,moist.) is a function that reduces the growth rate for suboptimal environmental conditions; $f(NO_3^-,NH_4^+)$ is a function that sets $dB_{r,x}/dt$ to zero if neither NO_3^- nor NH_4^+ is available. If NO_3^-, NH_4^+, or both are available, $f(NO_3^-,NH_4^+)$ is equal to 1.

The second step involves the integration of this type of equations. Usually the equations are so complicated that only numerical integration can be used. This implies computer programming. There are special programming languages, such as CSMP (Continuous System Modelling Program) which facilitate this programming very much, but also languages such as FORTRAN are widely used.

A third step is the verification, i.e. a check to verify that the mathematical model describes the physical-biological model correctly.

The fourth step is the determination of the parameters, initial conditions and other numerical values. For complex models one has to rely for many of its parameters on literature references. Inconsistencies of data, lacking parameters, not well defined conditions belong to the difficulties one will met. When these four steps have been completed, it is possible to use the program but one important final step is still missing: the validation. Sofar, only part of the existing models have been tested, sometimes with surprising success. Failures have been reported too. But if one defines validation as testing under wide ranges of conditions, one must conclude that there is no model, that has sufficiently been validated.

Hoffman et al.[11] explain that there exists an important difference between radioecological scientific models and risk assessment models. Within soil microbiological models such differences also exist; they might be divided into scientific and management models (Frissel and Van Veen[12]). In fact all the mentioned models belong to the scientific ones.

6. CONCLUSION

There exists a wealth of models which describe the growth and decay of soil microorganisms. They provide a fair understanding and, consequently, they will be very helpful to each one who intends to model the role of microorganisms on the behaviour of radionuclides. Many of the models are operational, well documented and tested, but not validated.

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Session : III/A

Paper by : M. Frissel

Comment by : W. Kühn

Text of comment
or author's answer :

You mentioned, that the models work , they have been tested ans verified. Can you make short remarks about the methods you verified the models?

Verification includes a check of the computer codes on the mathematical corrections and testings of the codes on a few case studies of which experimental data are present.
Validation includes a systematic check for wide ranges of conditions such as the pH, organic matter content, type of soil, way of fertilization, etc. In general, unsufficient data sets were available for validation.

Session : III/A

Paper by : Frissel

Comment by : Cremers

Text of comment
or author's answer :

The effects of the microbial population can perhaps be expressed in terms of an additional geochemical phase which competes with the other geochemical sinks or adsorption sites and the rates of mobilization would then be ruled by the distribution of the radionuclides between the different sinks.

Yes, this can be done, although I do not think that this is an optimal procedure. A model should reflect the reality as good as possible and as such growth and decay should be included in the model as such. Considering the microorganisms as a sink (as a function of conditions, as temperature, moistures context, other pools, etc.) does certainly not optimize our understanding. For practical purposes, or in situations where the microbial impact is very small it may be an acceptable solution.

Session : III/A

Paper by : Frissel

Comment by : McKinley

Text of comment
or author's answer :

The models presented are designed for use in a soil environment to what extent can these be applied to a deep geological / high level waste environment ?

This question was discussed by Dr Frissel and several other members of the audience but was not resolved and this topic was postponed till the later general discussion.

Session : III/A

Paper by : Frissel

Comment by : Wirth

Text of comment
or author's answer :

I always feel a little bit uncomfortable when I see very detailed models. There is always the question of getting acurate data for all these transfer coefficients. From fall-out we know that simply models give more reliable data than sophisticated ones. My question is : did you also develop simple models and if yes, can you say something about the accuracy of the results?

2nd question : do you really think that you will get good data for your detailed model ?

It depends entirely on the situation which has to be described whether a simple or complicated model provides better results.
For the description of immobilization of N, nitrification, denitrification, volatilization of ammonia rather complicated models with small time intervals are required.
For the mineralization and the organic matter content of soil as a function of time, simpler models can be used.

Session : III/A

Paper by : M. Fissel

Comment by : A.J. Francis

Text of comment
or author's answer :

What parameters do we need to develop microbial process model in the transport of radionuclides that can be coupled to geochemical and hydrological models ?

The following processes and parameters should be identified :
- the production of complexing agents by microorganisms = quality and quantity, expressed as fucntion of type of substrate, environmental conditions, etc.
- chemical equilibrium of the particular complexing agents and the particular radionuclides.
- the chemical and microbiological stability of the complexing agents (i.e. their lifespan).

Session : III/A

Paper by : Frissel

Comment by : N. Christofi

Text of comment
or author's answer :

Will radiolysis generate oxidizing conditions for the breakdown of organic carbon (high molecular weight) after available (labile) organic carbon has been utilised ?
This question is related to my work on deep disposal of radioactive waste where backfills of clay may be used.

If backfills are being used, which will be sensitive for radiation, yes. But, I do not expect that, backfills will probably contain no or very small amounts of organic matter, I expect e.g. no effects for with a few percent (very old) organic matter.

HT/HTO-CONVERSION OF SOIL ORGANISMS: EXPERIMENTAL RESULTS AND ECOLOGICAL STUDIES

H. Förstel and F. Führ

Institute of Radioagronomy, Nuclear Research Center Jülich, Fed. Rep. Germany

ABSTRACT

HT in the air above a soil volume is taken up rapidly, but only if the biological activity is not cancelled by sterilisation. The activity of dry soil can be activated by wetting, thereafter the HT-turnover rate increases. An addition of glucose does not enhance the HT-turnover of the soil. The main reaction product of HT is water, only 0.5 % of the activity is bound as non-exchangable tritium. The HT-uptake of soil cores from the field of an agricultural area also shows a rapid uptake of HT, even after freezing the samples.

INTRODUCTION

Tritium (^3H) is produced by natural atmospheric processes[1]. The release of tritium by human activity (reactors, reprocessing plants) is continually increasing. At least the handling of tritium by fusion technology enhances the anthropogeneous source above the natural level[2]. If tritium is bound as water it is rapidly diluted in the global water cycle[3]. Emitted as HT gas, its pathway is different. At first glance the radiotoxicity of HT for man is about 10^4 times lower than that of HTO, but HT may enter the local ecosystem at the soil surface and at the leaves. Geochemical considerations[4] as well as studies of the HT-uptake by soil[5,6,7] and plants have focussed attention on the soil as the main HT sink.

This paper describes an experimental circuit for the observation of the HT loss from air and for the study of the pathway of tritium in the soil. For two reasons the system was designed as a gas-tight circuit: to fulfil safety precautions and to obtain a complete balance of the tritium.

EXPERIMENTAL CONDITIONS

Parabraunerde (loess type) from an agricultural area near Jülich (Fed. Rep. Germany) was stored dry and activated by the addition of water (40 % maximum water capacity, i.e. about 18 % water content). Usually 0.5 kg was used (reaction vessel at 65 % relative air humidity, 293 K).

The reaction system (Figure 1) was under slightly lower than atmospheric pressure and made from glass and stainless steel only. The air turnover rate was about 40 l per hour. The water vapour is frozen out behind the reaction vessel at 210 K (separation of HT and HTO, protection of the ionisation chamber).

The ionisation chamber has a short response time, 95 % efficiency and 250 ml volume. The walls are made of stainless steel, the electrode of nickel. Barriers of aluminium improve the flow pattern and consequently the signal. The resistance is read by a teraohmmeter (Knick, 10^6-10^{16} Ω, corresponding

to 0.2-1 Mbq per liter air, 500 V). HT content of the air and signal are linearly dependent. On leaving the ionisation chamber the air is re-wetted by a dew point trap and fed into the reaction vessel by a gas distributor.

Between the dew point trap and reaction vessel a silicon rubber inlet allows the injection of HT by a syringe. The HT source is a large gas reservoir, also closed by a silicon rubber membrane (covered with mercury). 0.5 ml syringe volume contains 2 M Bq.

All tritium activities are measured by liquid scintillation counting in the form of water. HT gas is burned to water over a CuO-catalysator at 920 K in a stream of pure oxygen. HTO is separated from the soil by threefold vacuum distillation (re-wetting the soil with inactive water). The remaining activity, which is liberated by burning as described above, is defined as (non-exchangable) organically bound material (OBT).

RESULTS

Sterilized soil

Soil was sterilized at least twice by heat treatment within three days. The success of the treatment was tested by a routine method for the counting of soil organisms: soil was shaken well with sterile water and the supernatant was grown on three different nutrient agar plates. No organisms could be detected after sterilisation (in comparison to a rich microorganism content before).

Figure 2 compares the HT-concentration of air above a sterilized and an active soil: only a small and strictly limited loss of HT can be observed.

HT-uptake after activation of mixed soil

Dry soil does not show a turnover of HT (water content 0.8 %). Activated by the addition of water the activity of HT turnover increases continuously up to four weeks (Figure 3). It is not influenced by the addition of 1 g glucose per liter wetting solution.

All the tritium injected into the circuit can be detected at the end of the observation time: in twelve experiments of a series 101.2 ± 1.8 % of the original tritium activity was found. The loss of HT in the air is mainly converted to HTO. Nearly all the HTO remains in the soil, only 1 % of the HTO can be detected in the cooling trap in front of the ionisation chamber. As well as the observation of no HT loss in the presence of sterile soil, the constancy of the HT concentration in an empty but wet apparatus demonstrates that the mechanism of HT oxidation in the air is negligible.

Surprisingly, the HT/HTO-turnover in a well-mixed soil leads to a nearly homogeneous distribution of HTO in the soil column (about 8 cm in height).

Figure 3 also demonstrates that the HT loss from air can be described by a simple exponential equation. The time constant k may serve as a parameter to calculate the deposition velocity v_g by

$$v_g = k \cdot \frac{V}{A} \; [\text{cm s}^{-1}], \text{ where}$$

V: volume, A: area.

The deposition rates of well-mixed, water-activated soil are between 0.005 and 0.05 cm s^{-1}.

A further confirmation of the role of microorganisms is the delay of the development of the HT-conversion activity during storage of soil under refrigerator conditions.

HT uptake of undisturbed soil cores

Cores from freshly ploughed soil were taken from the field. The results in Table I show that the mixing of the upper air compartment does not limit the reaction condition. Even after freezing the soil sample converted its activity. Storage for two days under laboratory temperature increased the activity. The deposition rates measured on native soil cores are comparable to data from well-mixed soil. In undisturbed agricultural soil a distinct decrease of the activity within the first ten centimeters can be observed (Figure 4).

TABLE I: Observations of the HT turnover of soil cores from an agricultural area: parabraunerde/March 1984/1.3 MBq per test.

test condition	v_g (cm s^{-1})	soil mass (kg)
gas flow		
- usual conditions	0.026	1.6
- additionally stirred	0.026	1.6
stored		
- 86 h at 255	0.006	1.1
- 48 h at 295	0.035	1.5

CONCLUSIONS

The experiments observing a well-mixed soil have demonstrated that as well sterilized as dry soil do not take up HT. The addition of water alone, not the addition of glucose, increases the HT turnover actively. The first reaction product is HTO mainly. Only less than 1 % is taken up into the OBT. The experiments suggest that HT can penetrate easily into the soil and is converted there. The product HTO is diluted in the soil water pool and is not transported rapidly back to the water vapour of the air above the soil column. This observation may point to a different behaviour of HT and HTO under field conditions.

The loss of activity after sterilisation and after drying supports the hypothesis that the HT/HTO turnover is made by microorganisms. The observations of an annual cycle of the HT uptake of soil in the field[7] is an additional argument. In the field not the temperature but the water content of soil seems to be the most important parameter.

The experiments reported above describe the first step of the HT loss from air. There may be a subsequent slow, but radiotoxicologically important uptake into the OBT.

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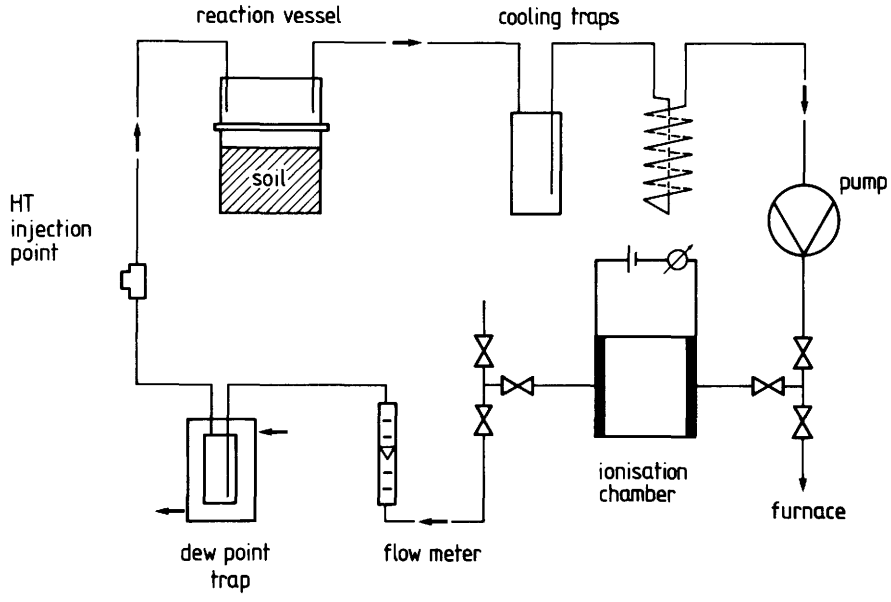

FIGURE 1 Scheme of the gas-tight circuit.

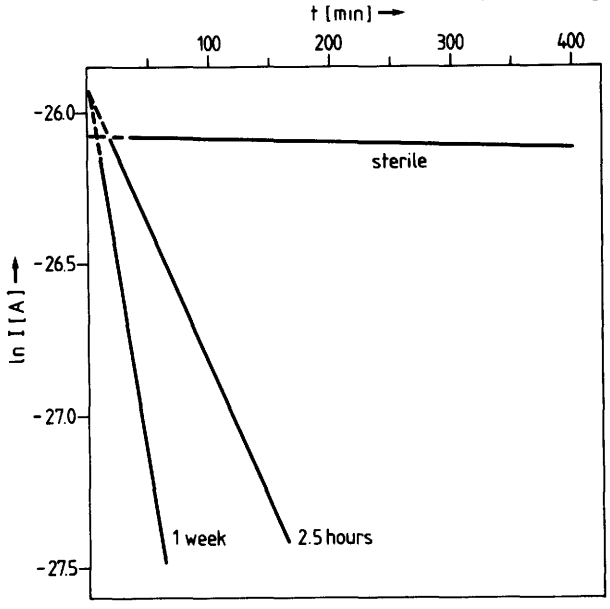

FIGURE 2 HT loss from air (sterile soil, wetted soil stored at 295 K for 2.5 h and 1 week).

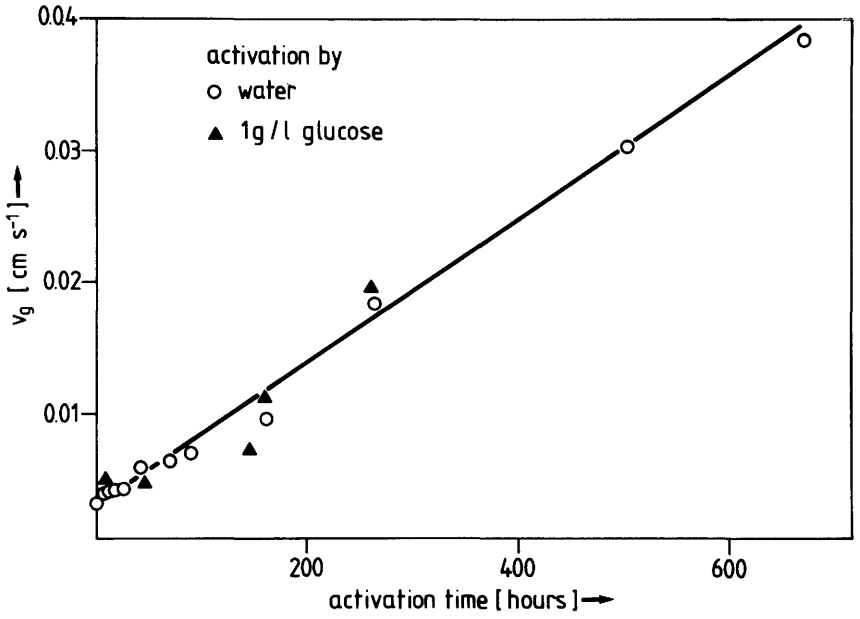

FIGURE 3 Increase of the deposition rate during the storage of soil (parabraunerde) under 295 K (activation by distilled water or glucose solution).

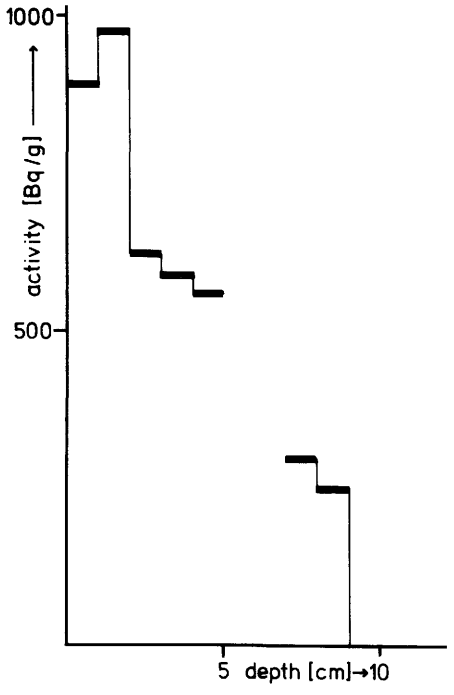

FIGURE 4 HTO profile of a agricultural soil taken from the field (March 1984) after test with HT gas in the reaction circuit (reaction time about one hour).

Session : III/1

Paper by : H. Förstel

Comment by : S. Bonotto

Text of comment
or author's answer :

To have a complete picture, I think that it would be interesting to consider also the production of hydrogen by microorganisms and plants and the possibility of HT formation from HTO.

Seiler et al.(Z.Pflanzen.Bodemkd.,140 (1977) 257 - 272)
have demonstrated that some German soil types mainly take up H_2 and CO. Only under special anaerob conditions, the production of H_2 and CO exceed, the consumption. This happens in non-aerated layers. In general they suppose that the consumption is the dominating process.
The production of plants with many hopes observed by the " alternative energy " research, is a special case of photosynthesis, a leakage of the photosynthetic apparatus under certain physiological conditions.
For radioecological models one should know the range of conditions for the HT-turnover by soil (organisms). The generation of HT from tritiated water seems to be only a small path for the radioecological behaviour of tritium, but should take into account for special situations (e.g. wet land).
Between HT-turnover and HT-production seems to be one difference : HT is a specific substance, bound to specific organisms (enzymes). Its product HTO is thus diluted in the water pool of the soil. The H2 produced then can only have a diluted tritium content.

Session : III/A

Paper by : Förstel

Comment by : R. Kirchmann

Text of comment
or author's answer :

With your experimental system, would it be possible to produce enough OBT for measurement as THO after combustion ?

The first step of the HT-uptake by soil (organisms) is the oxidation to water. Only 0.5% of the tritium converted under our conditions is found as OBT (organically bound tritium). As we use 2 MBq HT this is a small amount, but as a whole a quantity enough. We are able to use more HT and to enhance therefore the OBT-tritium.
The more difficult problem is the distinction between HT-oxidation and HT-binding directly to the organic material. Tritium has a rapid isotopic exchange with bound hydrogen. We eliminate the exchangeable-bound tritium by subsequent dilution with inactive water to obtain firmly a well-defined material. For radioecological considerations, one must also include the exchangeable tritium, which in our experiments (as a very small fraction) is included in the HTO fraction.
It seems worth to notice that there is no remarkable HTO-fraction bound to the soil particles. At least it can be extracted by vacuum distillation and inactive dilution easily. The soil water seems to be a single compartment, different fraction exchanges with each other quickly.

Session : III/A

Paper by : H. Förstel

Comment by : R. Kirchmann

Text of comment
or author's answer :

Have you any idea about the site of localization of the derived from HT, in soil ?
Is this THO behaved as linked water in the cell of microorganism and then is not participating in the soil-water free pool?

From the experimental observations, we did find only 1% of the HTO oxidized from HT in the cooling trap before the ionisation chamber, a slow exchange between the HTO after turnover and air water vapour must be taken into account. The HT seems to diffuse rapidly into the upper centimeters of the soil, to be converted there and will be diluted in the water pool of the soil. A special form as linked water must not be proposed. The relation between water vapour and fluid water in the soil seems to be sufficient to explain the low exchange of HTO back to the air.

Session : III/A

Paper by : Förstel

Comment by : Bors (FRG)

Text of comment
or author's answer :

Concerning release at HT from the soil what would be the situation if you would compare different soils differing in water capacity ?

The results, reported by Garland and Cox 1980 (Water Air Soil Pollution 14, 103-114), demonstrate the importance of the water content of soil. One effect may result from the limitations of the diffusion if the soil pores will be filled with fluid. Another effect may be the change in the microenvironmental grew and living conditions of soil organisms. In addition of fluid may dissolve and distribute nutrients. Additionally, the availability of oxygen (low water solubility) may be a third factor.

At the same precipitation conditions soils of different water capacity should differ in their status, as described above.

Our observation, that dry soil does not show a HT-turnover, must be also taken into account. During dry seasons the upper centimeters of the soil often lose much of their water content.

Session : III/A

Paper by : Förstel

Comment by : Martens

Text of comment
or author's answer :

On the first sight it may be surprising that the addition of glucose did not increase the oxidation potential of the soil population. But the addition of glucose will induce the proliferation of a special glucose-population. These microorganisms are not necessarily the responsible ones for the oxidation of HT. Only special groups of the soil population will mediate this reaction.

I agree with the comment.
We have cultivated soil organisms on different types of nutrient agar. The organisms, taken directly from a suspension of our parabraunerde had lost their HT-turnover activity thereafter, this negative result demonstrates that one must take into account biological details very intensively.
This is also the difficulty of re-inoculation experiments. The classical techniques of the cultivation of microorganisms select certain species or at least systematic groups of organisms. Even a suspension and extraction results in a fractionation of the spectrum of microorganisms, especially of those, which depend on special microenvironmental conditions.

STUDIES OF THE MICROBIOLOGICAL INFLUENCE ON THE BEHAVIOUR OF IODINE-125 IN HUMUS SOIL*

S. STRACK and A. MÜLLER

Kernforschungszentrum Karlsruhe
Hauptabteilung Sicherheit/Radioökologie,
D-7500 Karlsruhe 1
Federal Republic of Germany

ABSTRACT

Laboratory experiments with I-125 have been performed to gain more insight into the microbiological influences on iodine accumulation in humic soil layers. Solutions of $Na^{125}I$ have been added to small packed soil columns, both non-treated and submitted to special treatment. The water-extractable iodine fractions were measured in a liquid-scintillation spectrometer. Non-treated soil with the natural microbiological cover shows an immobilization which can be described sufficiently by a sum of three exponential functions, obviously representing biotic as well as abiotic absorption or binding processes. The results discussed indicate that certain soil microorganisms (preferably bacteria or actinomycetes) cause the rapid immobilization (half-period 0.37 h). A 'medium' and a 'slow' compartment with half-periods of 26 h and 330 h respectively seem to be governed by physico-chemical processes; however, a 'microbiological' acceleration of the slow fixation process can be observed also.

*Work supported by the Commission of the European Communities, Contract BIO-B-489-82-D

1 INTRODUCTION

Airborne long-lived iodine radioisotopes (I-129) released from nuclear installations accumulate in the upper soil layers after deposition[1]. These soil layers with a high content of humic substances and soil microorganisms seem to act as a long-term reservoir of iodine. It has been observed also that the extent of the bio-availability for plants is affected by the biological activity in the soil[2]. For risk assessments it is therefore necessary to clarify the microbiological influences on the immobilization and mobilization processes in the soil. This aim has been pursued by laboratory experiments, which have been performed with I-125 using small columns packed with humic soil collected in the vicinity of the KfK.

2 MATERIAL AND METHODS

The soil material of the H-horizon was collected in a forest at the KfK (fig. 1). A whole-year study of the microflora at the sample site provided an insight into the microbiological situation and its seasonal variations in the soil used in the experiments[3]. The columns were prepared by packing 5 g wet soil material into small cotton stoppered plastic tubes adjusted to a water content of 20 %. Sterilization was performed by autoclaving the soil (121 °C, 20 min). Sterilization tests were performed and the number of organisms was determined using the dilution plate technique. In most experiments about 500 Bq were applied in the form of NaI in solution with an inactive carrier.

During the experiments the soil samples were kept at a constant water content by supplying distilled water to compensate for the daily loss by evaporation. Before leaching, the water content was increased up to the maximum water capacity of the soil. The water-soluble iodine was then eluted with 5 ml of distilled water. After filtration 1 ml of the solution was taken for measurements of the I-125 in a liquid-scintillation spectrometer.

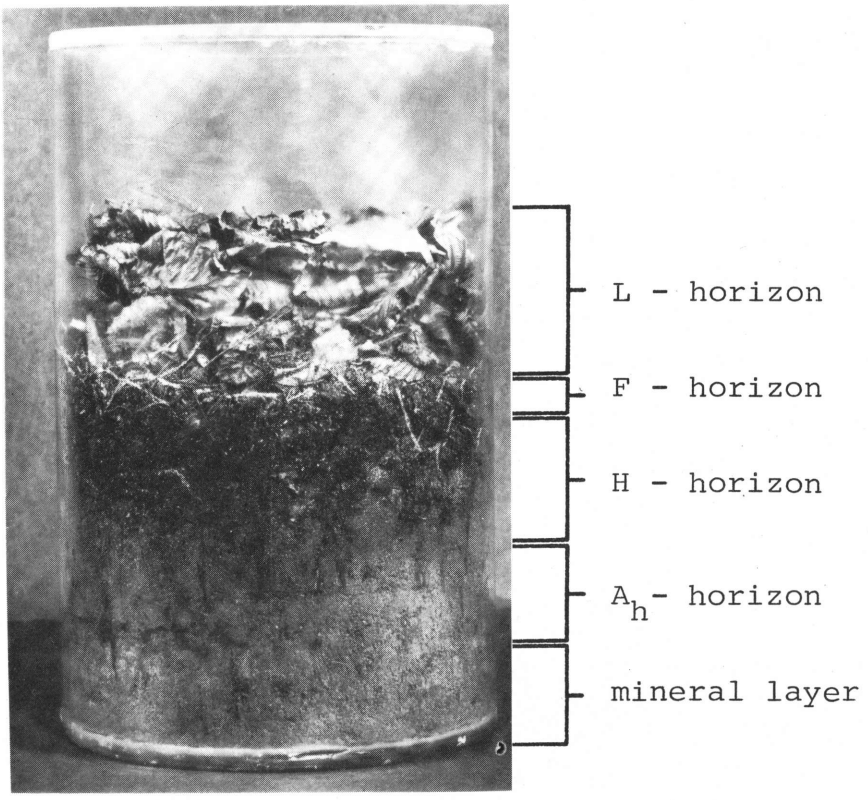

FIGURE 1 Profile of the soil at the sampling point. In the experiments material of the H-horizon was used.

3 RESULTS AND DISCUSSION

Preliminary experiments with non-treated soil and with sterilized soil by autoclaving performed in comparison have shown that the immobilization of iodine is evidently reduced in sterilized soil[4]. The fraction extractable by distilled water is increased by one order of magnitute related to non-treated soil. This finding seems to be an indication that fixation is governed by an active biological process, however, thermal treatment has an effect also on the inorganic soil matrix and the non-living organic substances. Therefore, it has to be taken into account that variations in the physico-chemical properties may be responsible for the observed effect.

3.1 Sterilization and re-inoculation of the soil

In long-term experiments with a higher frequency of sampling, particularly within the first few hours of the experiment, the absorption behaviour was examined, comparing non-treated soil with sterilized soil, and sterilized soil which was re-inoculated by living microorganisms. In figure 2 the water extractable fractions (in percent of the applied iodine) are plotted versus time on a semi-logarithmic scale. The courses of immobilization indicate that they can be described by sums of exponential functions (eq. 1). Graphical analysis of the data in combination with a mathematical regression analysis (least square fit) resulted in the parameters (intersects and slopes) given in table I.

$$Y_t = C_r e^{-\frac{\ln 2}{\tau_r}} + C_m e^{-\frac{\ln 2}{\tau_m}} + C_s e^{-\frac{\ln 2}{\tau_s}} \qquad (1)$$

TABLE I Compartment sizes (intersects) and half-periods of the iodine immobilization in treated and non-treated soils.

	C_x compartment sizes (%)			τ_x half-periods (h)		
	rapid	medium	slow	rapid	medium	slow
non-treated	93.4	4.2	2.4	0.37	26	330
sterilized	-	32	68	-	23	205
sterilized/ re-inocul. (main. fungi)	3	32	65	0.37	22	102
steril./re-inoculated by bact.	8	26	66	0.44	20	181

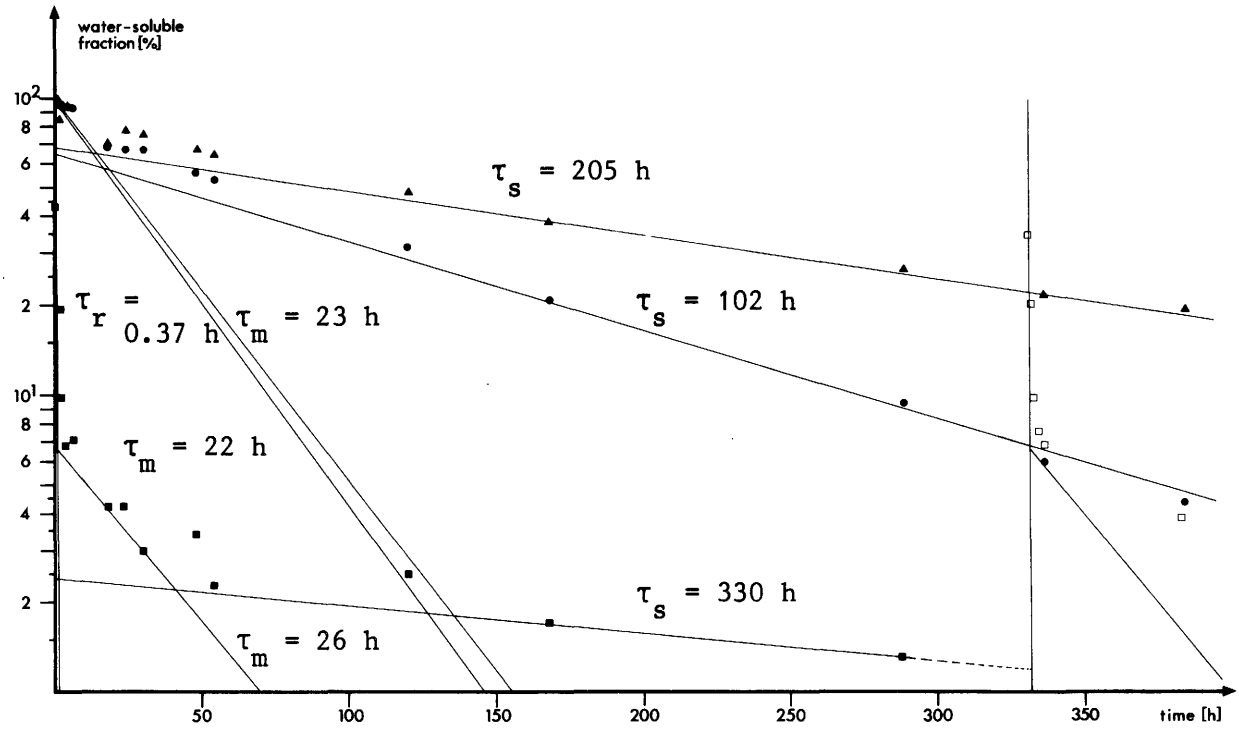

FIGURE 2 Immobilization of I-125 in non-treated (■), sterilized (▲), and sterilized/re-inoculated soil (●)

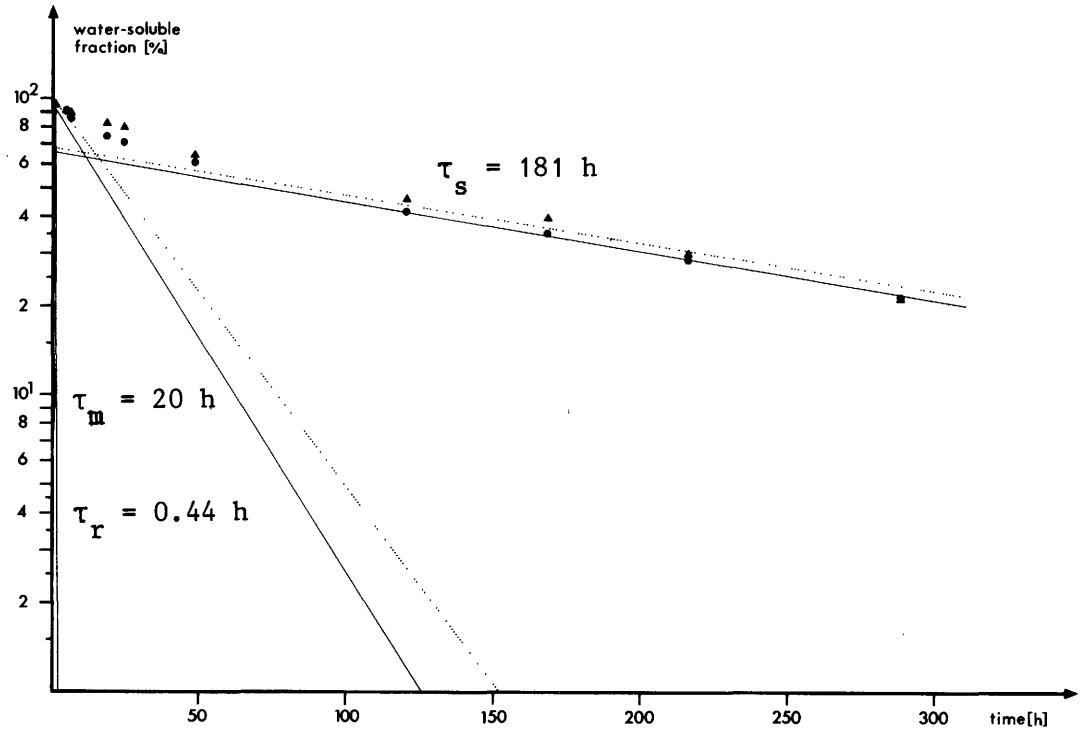

FIGURE 3 Immobilization of I-125 in soil sterilized and re-inoculated with soil bacteria (●) comparison to sterile soil (▲)

In case of the non-treated soil a very rapid immobilization process (half-period 0.37 h ≙ 22 min) takes place, resulting in a more than 90 % fixation of the applied iodine within the first few hours of the experiment. The following decrease of the extractability can be described by two exponential terms characterized by half-periods of 26 h and 330 h. Only 4.2 % and 2.4 %, respectively, are involved in these processes.

A second administration of the same amount of iodine onto the columns after 330 h resulted in a curve with nearly the same shape indicating that the first 'rapid' compartment has not yet been saturated.

In case of the soil sterilized by autoclaving a 'rapid' compartment does not become evident. Only a 'medium' compartment with a half-period of 23 h and a 'slow' compartment with a half-period of 205 h can be observed (fig. 2). It is clear that in this case these two compartments must be clearly governed by physico-chemical mechanisms. The sterility was tested several times during the experiment. Gamma-spectrometric measurement of the columns gave no indication of an iodine loss by volatilization.

In order to compare sterilized soil and soil subjected to identical physical treatment, but with microbiological activity, autoclaved soil was re-inoculated with an extract of soil organisms and incubated for 5 days before application of the iodine. An examination of the microflora grown on the re-inoculated soil gave a high concentration of organisms, however, only a small amount of bacteria and actinomycetes settled on the autoclaved soil, the overwhelming majority consisting of soil fungi. The resulting curve (fig. 2) shows similarity with the 'sterile' curve. About 30 % is exponentially immobilized with a half-period of 22 h, and more than 60 % is fixed in a 'slow' compartment, but the process is clearly accelerated (half-period 102 h). However, a regression analysis gave a much better fit, when a 'rapid' compartment of 3 % is included in the calculations.

This finding led to the assumption that the rapid immobilization compartment represents uptake or bioabsorption processes, preferably caused by bacteria or actinomycetes. Therefore, in a further experiment (fig. 3) sterilized soil columns were tested which had been re-inoculated with a mixture of soil bacteria obtained by suspending the colonies of an agar count plate. This procedure allowed the transfer and

growth of only a few species which are usually found in the soil used in the experiments. Regression analysis of the data set shows in fact an enlargement of the first compartment (8 %, half-period 0.44 h = 26 min). A 'medium' compartment with a half-period of 20 h is also found. The 'slow' compartment is characterized in this case by a half-period of 181 h.

The variations of the half-periods observable in the third compartment cannot be interpreted easily with the results obtained. It is assumed that an abiotic process is responsible for this behaviour, as it is the case in the soil sterilized by autoclaving. Microbial activities, however, may cause a change in the chemical properties (e.g. pH or redox-potential) resulting in the observed variations of the slopes.

3.2 Treatment by chemical agents

Preliminary results obtained by particular, more selective treatments of the microflora (ß-propiolacton, polymyxin B, glucose), had shown also significant alterations in absorption kinetics. However, in this case the interactions proper of iodine with the additives has to be taken into account. Figure 4 shows the results of a control test. The desinfectant ß-propiolacton was administered to sterilized soil at different concentrations.

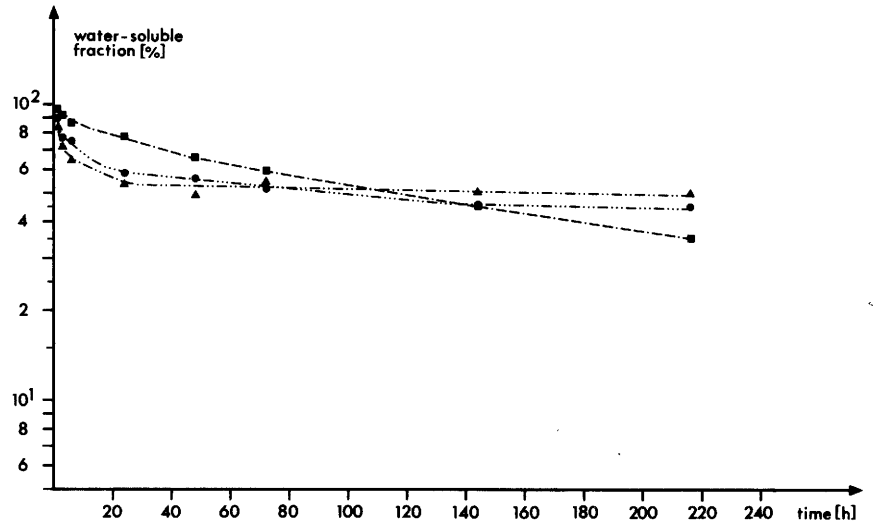

FIGURE 4 Immobilization of I-125: solution of ß-propiolacton (400 µl per column) administered at different concentrations to sterilized soil; 0 % (■), 1 % (●), 2 % (▲).

4 CONCLUSIONS

The immobilization of iodine applied as sodium iodide to humic soil can be described adequately by a sum of three exponential functions representing three processes which obey first order kinetics. Due to a special treatment of the soil the 'slow' compartment undergoes the most variations. Possible microbiological influences on the chemical properties of the soil may be the reason for the fluctuation of the immobilization rates of that probably abiotic process.

The 'medium' compartment shows only slight variations after treatment and is governed probably only by physico-chemical processes.

All results obtained indicate that the 'rapid' compartment represents a biological uptake or bioabsorption process. Certain soil microorganisms (preferably bacteria or actinomycetes) seem to be responsible for this considerable fixation capacity. Further experimental work is needed.

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ACKNOWLEDGEMENT

We thank Mrs. Annette Riedl for assistance. This work was supported by the Commission of the European Communities, Contract BIO-B-484-82-D.

Session : III/A

Paper by : S. Strack

Comment by : A.J. Francis

Text of comment
or author's answer :

Have you isolated pure or mixed culture of organisms to demonstrate that they are responsible for fixation of radioiodine?

No, up to now we have investigated the behaviour only with cultures associated with soil material.

Session : III/A

Paper by : S. Strack

Comment by : Frissel

Text of comment
or author's answer :

In one of your experiments you inoculated the soil upon sterilization. Because by sterilization all (micro) organisms are killed and thus become available as substrate for microorganisms I had expected a higher microbial activity then in the non sterilized soil.
Nevertheless, the I adsorption was lower. Do you have an explanation ?

In the re-inoculated soil after sterilization we observed a considerable microbial activity, however, it is impossible to reestablish a microflora composed as the natural one which has existed before sterilization.
And we found in fact that the majority of organisms consist of soil fungi. So we think, that the organisms or society of organisms mainly responsable for the rapid immobilization has been only limited re-settled on the sterilized soil.

Session : III/A

Paper by : S. Strack

Comment by : Bittel

Text of comment
or author's answer :

L'utilisation de I-125 donne-t-elle une image fidèle de ce qui se passe avec I-129?
Ne pourrait-on pas essayer de travailler sur l'iode stable naturel du sol (grâce aux méthodes modernes de l'analyse chimique. activation, catalyse oxydante de I).

We do not suspect that a possible isotopic effect can play a significant role in these experiments. On the other hand the advantages of an utilisation of I-125 are rather important, due to the detection method i comparison to the stable iodine and due to radioprotection in comparison to the long-lived I-129.

Session : III/A

Paper by : Strack

Comment by : A. Saas

Text of comment
or author's answer :

Have you determined carbon content of your extractable phase after irradiation ? But normally after irradiation you have a carbon lossess at this organic carbon fixed iodine. This can be explained by chromatography of soluble phase.
We are aware that some alterations of carbon compounds will occur resulting in a possibly higher water solubility. The comparison of γ-radiation with chloroform-fumigation indicates only a slight influence on the extractability of iodide.

Normally, the equilibrium and incorporation time for iodine in soil is 120-150 days (determined by Hoffmann in Oak Ridge by Saas, 1976 in Cadarache). In this case is necessary by extractibility with water, KI, NaOH at different other solutions to prove the evolution of iodine content in different phases?
Have you extracted iodine in your soil in different cases (normal, sterilized, after fumigation)?

Our extraction solution was destilled water in any cases.

We used also only water for extraction. Under our experimental conditions, we observed in fact that a steady state is not established after 16 days, but that a "slow" conversion process is still going on.

INVESTIGATIONS ON THE INFLUENCE OF MICROORGANISMS ON THE TRANSLOCATION OF RADIO-IODINE IN SOIL.

J. BORS, R. MARTENS[+] AND W. KÜHN

Niedersächsisches Institut für Radioökologie, Hannover

[+]Institut für Bodenbiologie, Bundesforschungsanstalt für Landwirtschaft, Braunschweig - Völkenrode

ABSTRACT

In order to assess to what extent living soil microorganisms participate in adsorption and translocation processes of radioiodine, sodium iodide was applied in adsorption experiments with sterile and non-sterile soils and was extracted after different incubation times. Furthermore, the role of living microorganisms in adsorption processes was verified in experiments, in which the microbial biomass in the soil had been killed by fumigation with chloroform and in which the iodide liberated by the mineralization of the dead organisms became additionally extractable. Besides the role of living constituents of soils the effect of tributylphosphate as an antropogenic complexing agent was tested also.

The experiments showed that iodine is adsorbed in the two investigated soils (Chernozem and Podsol) already a few minutes after application and that immediately after application the extractability of iodine is already different for sterile and insterile soils. Fixation in insterile soils is more pronounced, and the extraction yield is reduced to about 1% within 14 days. With sterile soils fixation is much less, and the extractability is still above 10% after this time. Furthermore, fumigations (4 th and 11 th day) significantly raise the extractability indicating that at least part of the iodine is bound to biologically active microorganisms. First results indicate that tributylphosphate influences the adsorption behaviour of iodine in the soil.

INTRODUCTION

Microorganisms are indispensable agents in maintaining the circulation of matter in the biosphere; they have the capability to accumulate numerous organic and inorganic compounds from the environment in their cells. On the other hand microbes are able to detoxify many potential pollutants, preventing their accumulation.[1-4]

In order to assess to what extent living soil microorganisms participate in adsorption and translocation processes of radioiodine, sodium iodide was applied in adsorption experiments with sterile and non-sterile soils and was extracted after different incubation times. Furthermore, the role of living microorganisms in adsorbtion processes was verified in experiments, in which the microbial biomass in the soil had been killed by fumigation with chloroform and in which the iodide liberated by the mineralization of the dead organisms became additionally extractable.[5] The fumigation with chloroform causes a flush of decomposition which can be used as a

measure of the amount of soil biomass. This method was introduced by Jenkinson and Powlson [4] and is frequently used in soil biology.

Besides the living and non-living constituents of a soil the presence of anthropogenic organic complexing agents can influence the adsorption of radio-nuclides.[6-8] It is well known that, for instance, tributylphosphate together with iodic-compounds is emitted by resprocessing plants. The aim of running experiments was to find out, if tributylphosphate can indeed reduce the adsorption of iodide by complex reactions. Should this be the case, investigations are planned to study the stability of these complexes against microbial attack in soil.

MATERIALS AND METHODS

Subsamples of 100g of two different soils, a chernozem and a podsol, were either untreated or were sterilized by gamma-radiation. Some properties of the two soils are shown in Table I.

TABLE I Characteristics of soils

Location	Soil type and Texture	Sand [%]	Silt [%]	Clay [%]	C_{org} [%]	pH (n-KCl)
Didderse	Podsol Loamy sand	84,7	10,0	5,3	1,4	6,5
Jerxheim	Chernozem Clay silt	4,6	71,9	23,5	2,4	7,5

Sufficient soil samples of each group were treated with 6 ml of an aqueous solution of 10 µCi corresponding to 8 pg of sodium iodide-125 to allow extractions of five parallels after six different incubation times at 22°C. Four and eleven days after the addition of the sodium iodide, parallels of the in-

sterile soil samples were fumigated with chloroform for 24 hours, in order to kill most of the microbial biomass. After an additional incubation of two days the soil samples were analysed. The amount of extractable radioiodide in all samples was estimated by stirring the soil with 200 ml water for five minutes. After filtration of the suspensions aliquots of the extracts were assayed by gamma-spectroscopy.

For testing the influence of tributylphosphate on the adsorbtion of iodide 200 or 300 µl of a 0.05 molar solution of the phosphate in ethanol was added to the aqueous sodium-iodide solution. This mixture was applied to both non-irradiated soils resulting in a 10^{-3} molar concentration of tributylphosphate in the soil water. After mixing, the soils were extracted immediately by the method described above.

RESULTS AND DISCUSSION

The results of the adsorption experiments are shown in Figure 1. Immediately after the application and mixing, a great proportion of the applied iodide was already absorbed by both soils, and a distinct difference between the sterile and insterile soil samples could be established throughout the whole experimental period. Fixation in insterile soils was more pronounced and the extraction yield was reduced to about 1% after 14 days. With irradiated, sterile soils the extractability was still above 10% after this time. Fumigations of the insterile soils 4 and 11 days after the application of iodide raised the extractibility significantly. These results support the assumption that the iodide was partly absorbed by the living biomass in the soils.

A surprising result of these experiments was that the insterile sandy podsol adsorbed more iodide than the chernozem soil with its much higher content of organic carbon, microbial biomass and clay. These soil constituents are known to be responsible for the adsorption capacity of soils.

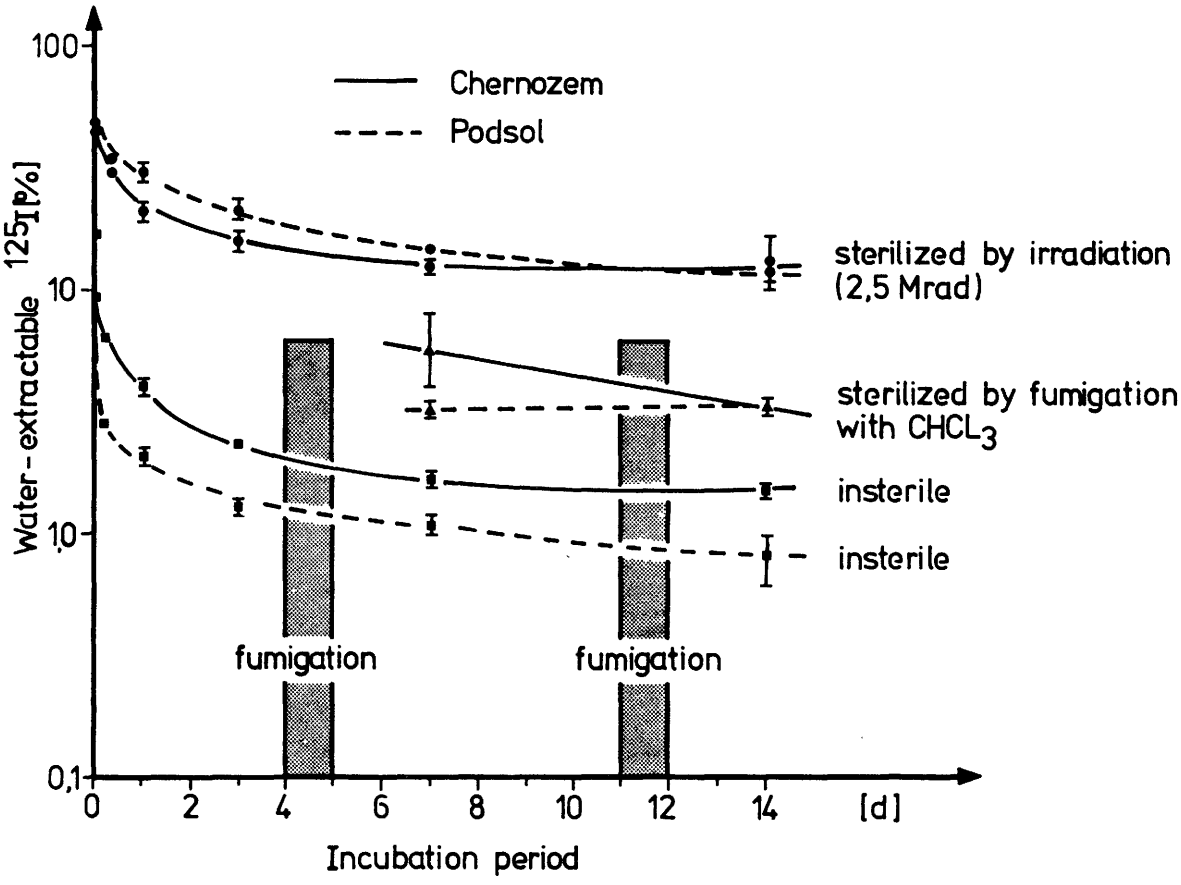

FIGURE 1 Extraction yield of ^{125}I from chernozem and podsol.

A order to test the result that these soil components are obviously not the main factors for the adsorption of iodide, ten other soils were investigated in corresponding experiments where the iodide was extracted immediately after the application. As can be seen from Table II, no correlation could be established between the above mentioned soil factors and the adsorption of iodide. For instance, a parabrown earth with an organic carbon content of 9.4% showed a relatively high recovery of iodide. From another parabrown earth with an organic carbon content of 1% only 8.6% could be extracted. The redzina with an extremely high clay content of 46% and a high organic carbon content of 7.8% released more iodide than

TABLE II Extraction yield of ^{125}I from various soil types with different properties. Extraction with 200 ml H_2O immediately after addition of ^{125}I-solution with 10 µCi activity per 100 g d.w. of soil.

Soil types	Soil characteristics			^{125}I extracted
	Clay [%]	C_{org} [%]	pH	[%]
Para brown earth+	12,7	1,2	6,6	12,1
Para brown earth	12,9	9,4	3,0	25,4
Para brown earth	11,6	1,0	3,8	8,6
Rendzina	46,2	7,8	6,0	16,6
Chernozem	18,4	2,0	6,0	14,9
Brown earth	4,6	1,4	4,2	27,4
Brown earth	6,1	2,1	5,0	21,7
Brown earth	1,9	1,2	5,7	24,5
Brown earth	2,5	2,0	5,2	23,0

+ "Luvisol" (FAO-Classification)

other soils with lower contents of clay and carbon. These results indicate that the iodide-ion will have other binding sites in soils that those known for organic molecules and cations. Further investigations are needed to elucidate the soil factors responsible for the adsorption behaviour of iodide.

The results of the first experiments with tributylphosphate reveals the general problem of application in laboratory experiments, which should model the environmental pollution (Table III). While under practial conditions contaminating chemicals often reach the soil as an aerosol yielding a good dispersion on and in the soil, in laboratory experiments additional solvents are often needed to apply and to scatter compounds

TABLE III Effect of tributylphosphate (TBP) on the extraction yield of ^{125}I from Chernozem and Podsol. ^{125}I activity added: 9,12 µCi/100 g d.w. of soil = 100%. TBP concentration = 10^{-3} M.

Soil	Treatment	^{125}I extracted [µCi]	rel. yield [%]
Chernozem	$^{125}I + H_2O$	3610±160	40
	$^{125}I + H_2O$ + Ethanol	2203±116	24
	$^{125}I + H_2O$ + Ethanol + TBP	2534±177	28
Podsol	$^{125}I + H_2O$	2102±71	21
	$^{125}I + H_2O$ + Ethanol	1181±20	12
	$^{125}I + H_2O$ + Ethanol + TBP	1992±135	20

with a low water-solubility. In our case ethanol was used to make tributylphosphate applicable to the soils together with the iodide. In either soils the ethanol alone had a remarkable influence on the adsorption of iodide. The amount of extractable iodide was reduced to nearly one half in the presence of the alcohol. Tributylphosphate could more or less compensate this effect indicating its influence on the adsorption behaviour of iodide. For further investigations an experimental set up has to be employed, which allows an application similar to that under practical conditions.

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Session : III/A

Paper by : Martens

Comment by : Strack

Text of comment
or author's answer :

You observed in your experiments an increase of the water extractable iodine after fumigation with chloroform and you take it as an indication that the nuclide has been associated with living microorganisms. Did you make the control by fumigation of the sterilized soil, in order to eliminate a possible effect on the extraction by the chloroform itself ?

An additional slide shows that after the sterilization with chloroform less iodide could be extracted from the two soils than after γ-radiation. This indicates that besides the microorganisms also other soil constituents were effected by γ-sterilization. As opposed to this the inert chloroform is supposed to affect only the microorganisms.

Influence of pH and redox-potential on the extractability of plutonium from soil.

R.M.J. Pennders, J.F. Stoutjesdijk and M.J. Frissel.
National Institute for Public Health, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

Summary

The chemical availability of Pu in soils may be influenced by microbial growth. Therefore an experiment has been performed with a suspension of sediment, containing ^{239}Pu and ^{240}Pu, in a diluted salt solution which is representative for the solution present in Dutch soils wetted by rain.
The pH and the *Eh* could be controlled by the amount of air and CO_2 gas supplied to the system. The amount of extractable Pu appeared to be highly dependent on the amount of CO_2: most Pu was extracted when the CO_2 concentration was low. After an equilibrium period glucose has been added to decrease the *Eh* and to initiate microbial growth, with the production of organic compounds. This resulted in a higher extractability of Pu.

ANAEROBIC MICROBIAL TRANSFORMATIONS OF RADIOACTIVE WASTES IN SUBSURFACE ENVIRONMENTS

A. J. FRANCIS

Department of Applied Science, Brookhaven National Laboratory
Upton, New York 11973, USA

ABSTRACT

Radioactive wastes disposed of in subsurface environments contain a variety of radionuclides and organic compounds. Microorganisms play a major role in the transformation of organic and inorganic constituents of the waste and are partly responsible for the problems encountered at the waste disposal sites. These include microbial degradation of waste forms resulting in trench cover subsidence, migration of radionuclides, and production of radioactive gases such as $^{14}CO_2$, $^{14}CH_4$, HT, and CH_3T. Microbial processes involved in solubilization, mobilization, and immobilization of toxic metals under aerobic and anaerobic conditions are reviewed. Complexing agents and several organic acids produced by microbial action affect mobilization of radionuclides and heavy metals from the wastes. Microorganisms play a significant role in the transformation and cycling of tritium in the environment by (i) oxidation of tritium and tritiated methane under aerobic conditions and (ii) production of tritium and tritiated methane from wastes containing tritiated water and organic compounds under anaerobic conditions.

1 INTRODUCTION

A major concern in the disposal of nuclear and nonnuclear wastes is the contamination of surface and ground waters by waste leachates containing radionuclides, toxic metals, and organic compounds. These contaminants may initially exist in soluble form or they may be formed after disposal as the result of physical, chemical, and microbiological processes. Microorganisms, which are ubiquitous throughout nature, have long been recognized for their ability to bring about transformation of organic and inorganic compounds. To date, we have little information on the microbial processes, particularly anaerobic microbial processes, which influence the fate and long-term transport of toxic metals and radionuclides in the subsurface environments. The form in which a metal occurs (e.g., ionic, inorganic complex, organometallic complex) strongly influences its toxicity, bioavailability, and mobility in the environment. Toxic metals in the environment may be more insidious than pollution by organic chemicals because metals, unlike organic chemicals, cannot be degraded to innocuous products such as carbon dioxide and water. In this paper the microbial processes involved in the mobilization and immobilization of toxic metals from energy wastes and the anaerobic microbial transformation of radioactive wastes in subsurface environments are discussed.

2 MICROBIAL MOBILIZATION AND IMMOBILIZATION OF TOXIC METALS

Mobilization and immobilization of toxic metals in the environment are brought about by direct or indirect action of the microorganisms. For example, various microbial processes may bring about (i) changes in pH and Eh (oxidation-reduction reactions) which affect the valence or ionic state of the radionuclides and enhance their mobility in the environment by retarding the soil-binding characteristics; (ii) chelation, solubilization, and leaching of certain elements by microbial metabolites or decomposition products; (iii) bioaccumulation and biosorption by microorganisms; (iv) biomethylation; and (v) production of gaseous compounds such as CO_2, H_2, CH_4, and H_2S. These biochemically transformed radionuclides, toxic metals, and organic compounds are indeed transported in the environment and thus increase their bioavailability to man. Several of the microbial processes involved in the mobilization and immobilization of toxic metals and radionuclides under aerobic and anaerobic conditions have been summarized in Figure 1.

2.1 Solubilization of Metals by Autotrophic Microbial Activity

Microorganisms are known to solubilize various metals from ores and in soil by production of mineral acids, organic acids, and oxidizing agents. Much of the information on the microbial contribution to leaching of metals comes from ore leaching, however. The responsible microorganisms, the chemical and the biochemical mechanisms involved in the microbial leaching or biomining of metals, have been studied intensively during the past several years.[1-4] Consequently, such microbiological processes are now being exploited on a commercial scale for extraction of copper and uranium from ores and to recover strategic metals from wastes. Since the waste materials contain iron and sulfur, either in the oxidized or reduced form, these elements are important in driving the overall chemical and biochemical reactions at the disposal environment. In aerobic environments, solubilization and leaching of metals are principally brought about by the activities of autotrophic bacteria. Oxidizing conditions promote the oxidation of reduced forms of sulfur to sulfuric acid which, in turn, play a dominant role in the mobilization of acid-soluble metals. Further, acid pH drastically retards

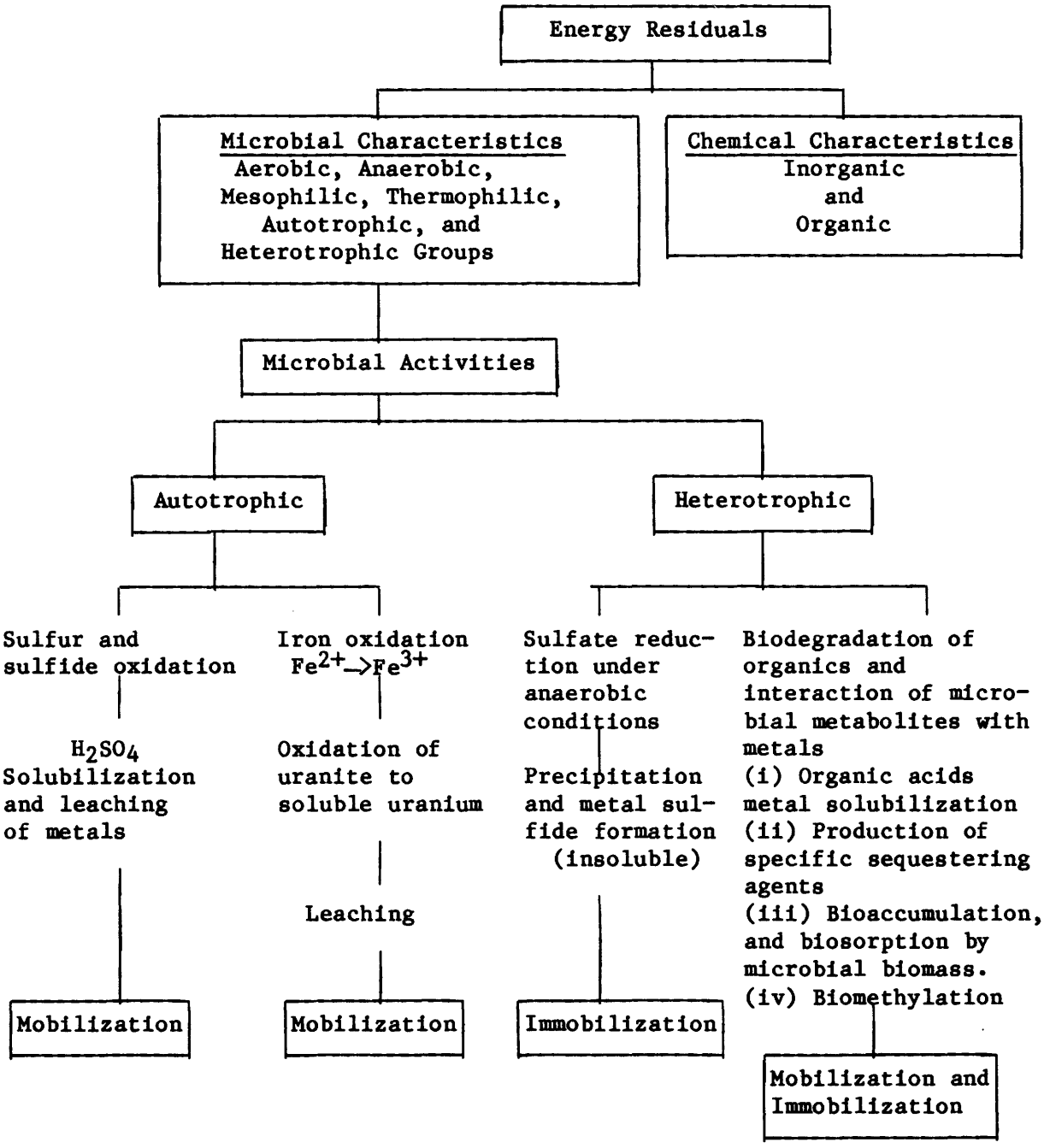

FIGURE 1 Microbial transformation of toxic metals.

the capacity of the soil or clay liner of a landfill to attenuate the movement of heavy metals from the landfill. For example, solubilization of radionuclides from radioactive wastes, uranium, and possibly thorium from mill tailings and coal wastes by sulfur- and iron-oxidizing bacteria could be significant.

2.2 Mobilization and Immobilization of Metals by Heterotrophic Microbial Activity

The presence of organic materials in the waste creates great concern because they are responsible for most of the problems encountered at burial sites. Many of the organic compounds are capable of forming stable complexes with

radionuclides or of increasing the solubilization and leaching of the buried radionuclides. Heterotrophic microbial utilization of the organic constituents of the waste under aerobic or anaerobic conditions may affect the transformations and transport of radionuclides in subsurface environments. These include oxidation-reduction reactions, production of organic acids, synthesis of specific and nonspecific sequestering agents, bioaccumulation of radionuclides, biomethylation, and production of radioactive gaseous products such as tritiated and ^{14}C methane.

In anaerobic environments, naturally occurring organic materials in soils can be degraded by microorganisms to simple organic acids, alcohols, aldehydes, ketones, esters, and gases such as H_2, H_2S, CO_2, and CH_4. Several aerobic and anaerobic bacteria, denitrifiers, sulfate reducers, and methanogens were found in leachate samples collected from low-level radioactive waste disposal sites.[5] The denitrifiers are responsible for the reduction of nitrate and nitrite in the presence of an adequate supply of available organic compounds under anaerobic conditions. The end products of biological denitrification are N_2O and N_2. Sulfate-reducing bacteria convert the sulfate to sulfide in anoxic environments containing sulfate. These bacteria are active in the corrosion of iron and aluminum alloys, desulfurization of oil, and deposition of mineral sulfides. The formation of metal sulfides by microbial sulfate reduction may determine the mobility of the metal, which, in several metal sulfides, is controlled by the solubility of the respective sulfides. In general, most of the metal sulfides exhibit low solubility in an aqueous solution.

2.3 Anaerobic Microbial Degradation of Organic Compounds in Radioactive Waste Leachate

Water leachate samples collected from low-level radioactive wastes contained radioactive materials such as ^{14}C, HT, ^{60}Co, ^{90}Sr, $^{134,137}Cs$, ^{241}Am, $^{238,239,240}Pu$ and organic compounds consisting of straight- and branched-chain aliphatic acids, aromatic acids, alcohols, aldehydes, ketones, amines, aromatic hydrocarbons, ethers, and phenols.[6] These organic compounds and the microbial degradation products can influence the oxidation reduction states of radionuclides, react with radionuclides to form complexes, and influence the solubility and leachability of radionuclides. The ability of indigenous microflora of the radioactive waste to degrade the organic compounds under anaerobic conditions was examined.[7] Leachate samples collected under anoxic conditions from the radioactive waste disposal site were incubated anaerobically in sterilized stoppered 120-ml serum bottles in an atmosphere of 85% N_2, 10% CO_2, and 5% H_2 at 28°C. Acidified control samples were incubated under identical conditions. After 30 days of incubation, samples were acidified and extracted with glass-distilled methylene chloride. The methylene chloride extracts were then concentrated and analyzed by gas chromatography and mass spectrometry.[7] The changes in concentration of several organic constituents due to anaerobic microbial action are shown in Table I. Concentrations of several organic acids increased, probably because of breakdown of complex organic materials in the leachate. Addition of nitrogen in the form of NH_4NO_3 and $(NH_4)_2SO_4$ to leachate enhanced the degradation of several compounds. Several of the low-molecular-weight organic acids are formed as the result of the breakdown of complex organic materials and are further metabolized by microorganisms; hence these compounds are in a dynamic state, being both synthesized and destroyed. The formation and accumulation of organic acids from anaerobic decomposition of organic compounds in the waste may indeed influence the mobility of radionuclides from the disposal environments.

TABLE I. Anaerobic degradation of organic compounds present in low-level radioactive waste leachate sample.[7]

Compound	Initial concentration mg/ℓ	Percent change Addition None	Percent change Addition Nitrogen
Acetic acid[a]	NQ	+ 0	- 8
Benzene[a]	NQ	- 8	- 17
Benzoic acid	17.0	0	- 17
C_8 acid[a]	NQ	- 42	- 48
Cresol	6.5	- 7	- 44
Cyclohexanol	1.2	+ 33	+ 10
Decanoic acid	0.2	+ 66	+ 1
2-Ethylhexanoic acid	62.6	+ 20	+ 5
3-Ethyl-1-hexanol	13.2	+ 4	- 5
Hexanoic acid	78.2	+ 11	- 4
2-Methylbutanoic acid	113	+ 3	- 12
2-Methylhexanoic acid	14.0	+ 18	+ 3
2-Methylpentanoic acid	20.2	+ 9	- 5
3-Methylpentanoic acid	2.6	+ 9	- 6
2-Methylpropionic acid	36.0	- 3	- 21
Nonanoic acid	4.2	+ 52	+ 22
Octanoic acid	22.6	+ 33	+ 6
p-Dioxane[a]	NQ	- 4	- 13
Phenol	5.8	+ 13	- 16
Phenylacetic acid	13.6	0	- 16
Phenylhexanoic acid[a]	NQ	+ 1	- 17
Phenylpropionic acid	10.6	+ 1	- 15
Tributyl phosphate	0.7	0	0
Tripropylene glycol methyl ester[a]	NQ	0	- 6
Toluene	13.3	- 17	- 20
Toluic acid	2.6	+ 2	- 14
Valeric acid	79.0	+ 4	- 14

[a]Percent change in concentration was determined on the basis of the ratio of the compound to the internal standard.
NQ - Not quantified.

2.4 Microbial Production of Organic Acids and Chelating Agents

Chelation and solubilization of metals are brought about by the activities of microorganisms in nature. Several mechanisms for microbial solubilization of insoluble metals have been proposed, including organic acid production,[8] formation of chelating agents,[9] and metabolism of the metal-associated anion.[10]

2.4.1 Organic acids. Microbially generated dicarboxylic acids, polyhydroxy acids, 2-ketogluctonic acid and phenolic compounds, such as protocatechuic acid and salicylic acid, are effective chelating agents of heavy metals and are known to accelerate the movement of metals in soils.[8,11] Increased solubilization of heavy metal sulfides by heterotrophic bacteria under anaerobic incubations of soil or sludge[12,13] and aerobically in culture media as a result of an undescribed solubilizing agent have been reported.[14] Bolter et al.[15] found that organic acids from decaying leaf litter in soil

increased the solubility of heavy metals deposited from smelters. Complexation of cadmium by organic components of sanitary landfill leachates was attributed to low and high molecular-weight compounds representing simple carboxylic acids and compounds containing hydroxyl groups.[16]

Heterotrophic organisms, bacteria and fungi, are able to release metals from various materials including copper-nickel concentrates, low-grade copper ore, uranium from granites, manganese ore, and potassium from leucite. Leaching by heterotrophic organisms is entirely due to the chemical reaction of excreted microbial metabolites with the materials. In many cases, a combination effect is important, for example when the organism secretes organic acids which may have the dual effect of increasing metal dissolution by lowering pH and increasing the load of soluble metal by complexation. Heterotrophic leaching could occur in an acid environment (pH 2-4) because of organic acid production or in an alkaline environment (pH 6-9) with no acid production.

2.4.2 Chelating agents. Chelating agents are produced by microorganisms that require iron or other essential metals for growth. Much is known about the chemistry, the biochemistry, the type of microorganisms, and the rate of production of the complexing agents (siderophores) which chelate iron and transport iron into the cell. Chelating agents enhance the dissolution of the metals with which they complex and thus increase their mobility and perhaps bioavailability. As chemical and biochemical similarities have been observed between Pu (IV) and Fe (III) and between Th (IV) and Pu (IV), the iron sequestering agents could play an important role in the complexation of Pu and other metals and thus increase their bioavailability. Dissolution of plutonium dioxide was enhanced in the presence of Desferol, a polyhydroxamate chelate produced by microorganisms.[17] This clearly indicates the potential of Pu and other metals present in the waste for complexation by microbially produced chelating agents. Wildung and Garland[18] found that microorganisms grown in the presence of Pu produced complexing agents of higher molecular weight than that of DTPA. Many of the cultures tested were capable of transporting Pu into the cell, and the role of complexing agents with such a transport has been suggested but not identified.[18,19] Pseudomonas aeruginosa (known to bioaccumulate Pu and U) produced Th and U complexing agents in culture medium.[20] In addition to phenolic and hydroxamate functional groups such as found in siderophore-type compounds, new natural products unrelated to siderophores appear to be present in the culture medium.[21] Many of the studies to date on microbial chelation of toxic metals are concerned with aerobic organisms and we have little information on anaerobic microbial production of complexing agents which could play a significant role in the mobilization of radionuclides in subsurface environments. The significance of microbially synthesized chelating agents in the mobilization of toxic metals in the environment is poorly understood and warrants further study.

3 MICROBIAL PRODUCTION OF RADIOACTIVE GASES

Microorganisms may play a significant role in the generation of radioactive gases directly through their metabolic activity or they may indirectly enhance the release of trapped gases such as radon from the radioactive decay of radium from the environment. Much attention has been given to methanogenic bacteria because of anoxic conditions that prevail in the trenches and the release of tritiated methane. Although much is known about the microbial metabolism of ^{14}C compounds and production of $^{14}CO_2$ and $^{14}CH_4$, very little is known about the microbial generation of tritiated methane.

Radioactive gaseous compounds such as CH_3T, HTO, HT, other tritiated hydrocarbons, ^{85}Kr, ^{222}Rn, $^{14}CO_2$, $^{14}CH_4$, and other ^{14}C-hydrocarbons have been detected seeping from burial trenches at West Valley, N.Y.[22] Of these, tritiated methane is among the most abundant; it has been estimated that one-tenth to two curies per year of CH_3T are released to the environment from various trenches at the West Valley disposal site.[22]

3.1 Microbial Transformation of Tritium

Water leachate samples from low-level radioactive waste disposal sites were collected anoxically and analyzed for methane bacteria by the most probable number technique; the results showed a population range of 20 to 230 per 100 ml of leachate. The ability of methanogens to produce tritiated methane from trench leachate containing tritium and other radionuclides and from synthetic media spiked with tritiated water was investigated. For this purpose, a mixed methanogenic bacterial culture was isolated from the leachate sample. Leachate aliquots of 30 ml each were transferred to sterile stoppered 60-ml serum bottles filled either with 85% N_2, 10% CO_2, and 5% H_2 or with 80% H_2 and 20% CO_2. They were incubated (a) with 10% formaldehyde to prevent bacterial growth (control) and (b) inoculated with mixed methanogenic culture. Total methane production by the control and inoculated samples is shown in Table II. The gas samples were analyzed for the presence of ^{14}C and tritium activity in the methane fraction. The samples incubated under $H_2 + CO_2$ produced more methane with higher $^{14}CH_4$ and CH_3T activity than the samples incubated under $N_2 + CO_2 + H_2$ (Table II).

TABLE II Microbial production of $^{14}CH_4$ and CH_3T from radioactive waste leachate samples.[23]

	Methane Produced (nmol)	Total Activity (pCi)	
		$^{14}CH_4$	CH_3T
Control	980	0.5	0.03
Inoculated ($N_2 + CO_2 + H_2$)	18,000	0.59	1.0
Inoculated ($CO_2 + H_2$)	68,000	12	57

Furthermore, significant quantities of tritiated methane were produced from synthetic media containing 2 mCi of tritium as tritiated water.[23] The levels of tritium used in this study had no apparent effect on methanogenesis, and the production of CH_3T increased proportionally with the increase in concentration of HTO added to the medium (Figure 2).

The low-molecular-weight ^{14}C- and tritium-containing hydrocarbons are transformed by methane bacteria to $^{14}CH_4$ and CH_3T, respectively. In addition, the methane bacteria are able to metabolize HTO in the presence of other carbon sources and produce tritiated methane. Soil microorganisms are capable of oxidation of tritium (HT) to tritiated water (HTO), and tritiated methane can be oxidized under aerobic conditions by methane-oxidizing bacteria and thus tritium can be recycled in the terrestrial environment.

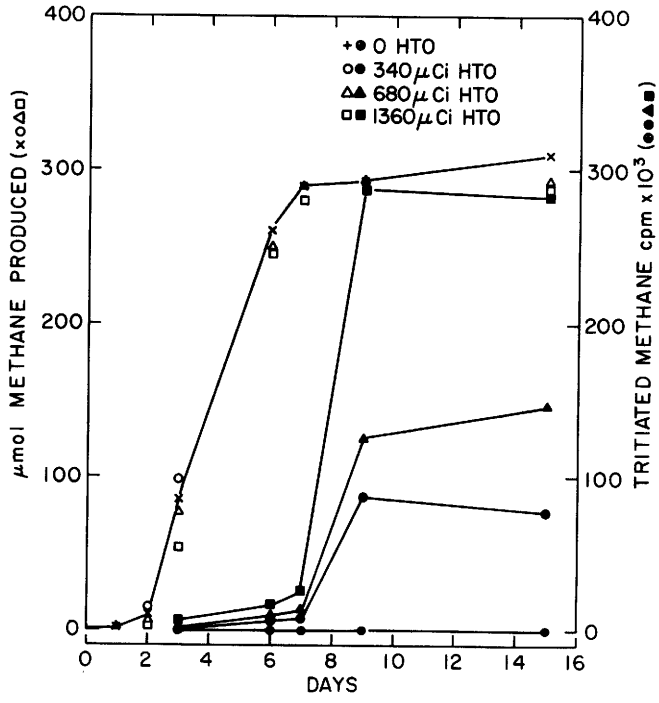

FIGURE 2 Effect of addition of tritiated water on methanogenesis and production of tritiated methane by mixed methanogenic culture.

4 ACKNOWLEDGMENTS

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Session : III/B

Paper by : A.J.Francis

Comment by : I.Mc.Kinley

Text of comment

or author's answer :

Your model assumes that all of the biomass is involved in complexations of radionuclides and hence mobilization. In fact you may have a situation where lot of microbial biomass and no consequence on mobilization. Where as a few organisms which may be responsible for mobilization by oxidation - reduction reactions production of specific sequestering agents etc. Although we do not have much information on biochemical transformations of radionuclides .I feel it is important to be considered in the model.

I agree completely the calculation of biomass was introduced to investigate the possibility of completely discounting microbial activity due to minimal content of organic carbon. This was subsequently found to be impossible to justify in a conservative manner.

MICROORGANISMS AND MICROBIAL ACTIVITY IN GROUNDWATER FROM GEOLOGICAL ENVIRONMENTS OF RELEVANCE TO NUCLEAR WASTE DISPOSAL.

N. CHRISTOFI,[a] JAMES C. PHILP,[a] and JULIA M. WEST[b]

a. Department of Biological Sciences, Napier College, Edinburgh EH10 5DT, U.K.

b. British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK.

ABSTRACT

The final stage in the nuclear fuel cycle is the disposal of radioactive waste. One of the options currently considered and in which research is being carried out is the disposal of intermediate and high level waste in repositories deep underground. Although extensive geochemical and hydrogeological research has been carried out to determine the suitability of deep geological strata as environments affording long term containment of nuclear waste, little work has been carried out on the function of native or introduced microorganisms in such habitats.

During the past year various test sites for nuclear waste disposal have been examined for the presence of microorganisms. These have included the examination of groundwaters in drilled boreholes at Altnabreac and Harwell and in mines in Cornwall, U.K. In addition various test mines in the Federal Republic of Germany, Belgium and Sweden have been investigated.

A wide variety of microorganisms have been demonstrated and some of the species identified could well effect waste containment materials. Data are presented on the range of organisms found and the possible role of microorganisms in nuclear waste containment and ultimate release of radionuclides to the 'biosphere' discussed.

1. GEOLOGICAL SITES AND THE IMPORTANCE OF MICROORGANISMS

The disposal of high-, intermediate- and low-level nuclear waste in deep geological environments is an option currently considered.

Geological formations envisaged include granite, salt and clay and the depth of the formations used may depend on the type of radioactive waste. Hydrogeological and geochemical feasibility studies have been underway for a number of years and more recently pilot studies have been carried out on geomicrobiological aspects[1,2,3].

Microorganisms, whether naturally present in deep groundwater or introduced to such waters during excavation and related processes, could effect nuclear waste isolation if they are able to grow within (near-field) or in the vicinity of (far-field) a waste repository. Microbial activity within a repository may enhance the release of contained radionuclides by direct deterioration of isolation materials[4] used, whereas activity in groundwater away from a repository can modify the chemistry of the water and indirectly affect the function of a repository e.g. enhancement of deterioration of materials due to the generation of acidic biproducts or enhancement of radionuclide mobility through radionuclide speciation changes. In addition, any released radionuclides, whether through biological or non-biological processes, could be concentrated on or within motile microorganisms. Mobility of microorganisms could then facilitate the transport of radionuclides through the surrounding geological formation e.g. granite, and therefore enhance its release to the 'biosphere'.

Success of microorganisms in the near- or far-field of a waste repository could also have beneficial effects. These include the growth of microorganisms within pores or fissures in backfill/buffer materials and host crystalline rock or other formation, and the blocking of movement of radionuclide-containing groundwater.

2. GEOMICROBIOLOGY OF RELEVANT MINES

Between July and December, 1983, water samples were removed from various experimental mines in Europe for the determination of microorganisms and microbial activity. Samples were taken from the South Crofty tin mine, U.K; the Konrad iron-ore mine, FRG; the Asse salt-mine, FRG; the Mol Boom-clay mine, Belgium and the Stripa iron-ore mine, Sweden. Water was either that flowing along gallery streams (South Crofty, Konrad), percolating through gallery walls (Konrad), running into the relevant stratum via a vertical shaft from shallower formations (South Crofty, Mol) or present in gallery pools (Asse, Konrad).

In these studies isolation techniques were used to delineate groups of organisms likely to influence the containment of nuclear waste and included both autotrophic and heterotrophic microorganisms[2]. It was not intended to differentiate between possible native, or introduced microorganisms, although identification of the dominant heterotrophic bacteria isolated from South Crofty indicated that they were of surface soil origin. The major genera included *Bacillus*, *Arthrobacter*, *Flavobacterium* and members of the corynebacteria[5].

All the samples examined contained culturable microorganisms with lowest populations in the highly saline Asse waters. The organisms demonstrated in the mines included sulphur-oxidising bacteria (*Thiobacillus* sp.), nitrifying bacteria, denitrifying bacteria, iron-oxidising bacteria (*Gallionella* *Crenothrix*) and sulphate-reducing bacteria.

The groups demonstrated can all theoretically affect nuclear waste isolation. Fungi were also present as were photosynthetic bacteria (cyanobacteria) growing in pool water in the Stripa mine, deriving energy from artificial gallery lighting.

3. MICROBIAL ACTIVITY IN MINE WATERS

Mine-water samples were tested for ability to support heterotrophic microbial populations present. Heterotrophic activity was determined by carbon dioxide evolution (measured using gas chromatography) in natural unammended waters, or waters ammended with organic carbon substrates. A number of substrates were utilised[5]. Yeast extract appeared to have the most stimulating effect.

In Figure 1 data are presented on heterotrophic activity in some of the samples from Konrad, Asse, Mol and Stripa. It is seen from these assays that heterotrophic microorganisms require organic carbon addition for activity indicating that the mine waters are organic carbon-limited. Low heterotrophic activity was however monitored in two of the water samples from Konrad and Asse without organic carbon addition (data not presented). In these samples organic substrates were present and it is interesting to note that activity was arrested when exogenous carbon was added. This possibly indicates the presence of oligotrophic populations adapted to growth in low nutrient environments. Indeed chemical analyses of the Asse water revealed the presence of unidentified low molecular weight organic carbon substances.

4. FACTORS AFFECTING MICROBIAL ACTIVITY IN A REAL NUCLEAR WASTE REPOSITORY

Microbial activity in a fully engineered nuclear-waste repository[4] will rely on a number of factors. These include availability of nutrient and energy substrates, adequate electron acceptors and donors, trace metals and the ability of microorganisms to withstand the changes in temperature, pressure and radiation.

Native microbial populations, if they exist in deep geological strata, will have adapted to growth in low nutrient environments subject to high pressure and may only need to overcome temperature and radiation variations. Introduced microorganisms, particularly heterotrophic forms will require an input of e.g. organic carbon which may arise from clay (backfill) isolation materials[6]. It may be however that organic carbon in clays is of high molecular weight and not available to important heterotrophs[5]. Mineralisation of high molecular weight substances to available low molecular weight substrates will be more important in the presence of oxygen. After backfilling of waste, oxygen in air will be trapped in a repository and a significant increase in the concentration of labile organic compounds will be determined by the amount trapped and whether this oxygen is solely used for mineralisation. It may be that non-biological processes will remove the oxygen e.g. chemical iron-oxidation.

Availability of oxygen may also affect organic carbon levels through autotrophic activity. In the presence of inorganic carbon (e.g. CO_2) and oxygen autotrophic microorganisms such as thiobacilli and nitrifiers can increase the organic carbon reserves which may then be available to important heterotrophic organisms[5]. Release of radionuclides into groundwater and radiolysis will generate oxidising species facilitating the breakdown of high molecular weight organic compounds. Oxygen may also be

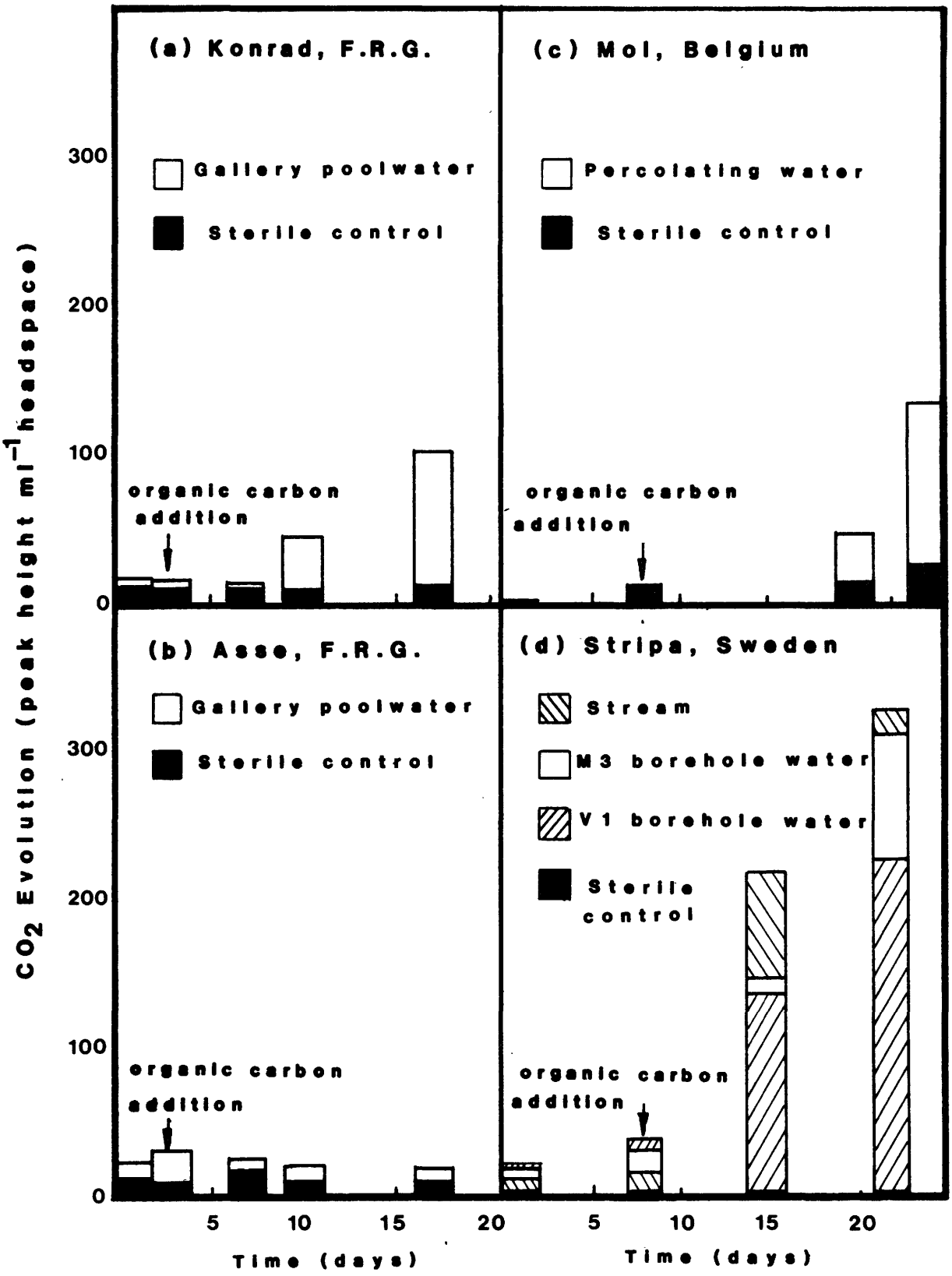

FIGURE 1. Heterotrophic activity (CO_2 evolution) in natural unammended or organic carbon ammended mine waters

available through radiolysis for autotrophic and heterotrophic populations[5].

Temperature and radiation will vary depending on the waste form. For example, organisms in the near-field will be more subject to extreme temperature and radiation fluxes in a high-level waste repository than organisms in the far-field. High temperature and radiation may inhibit microbial activity in the near-field but activity in the far field may still occur under other favourable conditions, leading e.g. to changes in groundwater chemistry affecting the near-field.

5. CONCLUSIONS

Microorganisms do exist in waters in geological sites of relevance to nuclear-waste disposal. Populations consist of organisms capable of growth in nutrient-poor environments but the heterotrophic microbiota in the majority of waters require exogenous input of organic carbon.

It is important that research is carried out to determine whether microorganisms will be active in an ideal waste repository and whether they will affect nuclear-waste isolation. This will involve studies under various conditions of e.g. temperature, pressure and radiation expected in a repository, utilising proposed isolation materials.

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Session : III/B

Paper by : Cristofi

Comment by : Förstel (Jülich)

Text of comment
or author's answer :

There are different kinds of water in the Asse deposit. What is the origin of your sample(s) ?

Two water samples were abstracted from Asse and both were from pools within galleries. The smaller pool contained water contaminated by mine workers and drilling / pump fluids. We were assured that the larger pool contained groundwater untouched for 25 years.
Small microbial populations were present in the waters.

MODELLING MICROBIAL CONTAMINATION OF A DEEP GEOLOGICAL REPOSITORY FOR HLW.

IAN G. MCKINLEY[a], FRITZ VAN DORP[b] and JULIA M. WEST[c]

a. Eidg. Institut für Reaktorforschung, 5303 Würenlingen, Switzerland.

b. Nationale Genossenschaft für die Lagerung radioaktiver Abfälle, Parkstrasse 23, 5401 Baden, Switzerland.

c. British Geological Survey, Keyworth, Notts, NG12 5GG, U.K.

ABSTRACT

Recently the possibility of microbial contamination of deep geological repositories for high level nuclear waste (HLW) has been considered in several publications[1-3]. No estimates, however, of either the likely biomass or the level of metabolic activity in such an environment have been reported. In this paper a simple model to assess inherent geochemical constraints on these parameters due to limited supply of nutrients and free energy is discussed. For the reference case, based on current Swiss concepts, of vitrified waste encapsulated in an iron container which is surrounded by bentonite, the limiting factor is likely to be "available" carbon which limits the maximum biomass to $\sim 10^4$ g (dry) in 26 m^3 of backfill per canister. At such a level of contamination, significant influence on canister corrosion, nuclide solubility and subsequent migration cannot be precluded.

INTRODUCTION

A general characteristic of many potential host-rock formations is a very low groundwater flow rate and thus, if present, microbial populations are expected to be severely limited by the rate of supply of nutrients and energy sources. The emplacement of a large number of materials such as the waste itself, metal canisters and overpacks, bentonite and concrete backfills etc. will, however, completely alter groundwater chemistry with additional perturbations from the effects of radiogenic temperature increases and radiation fields. In this paper the geochemistry of the HLW "near-field" will be modelled in a fairly simple manner to allow contraints on possible population size to be evaluated at least semi-quantitatively.

GEOCHEMISTRY OF THE "NEAR FIELD"

A reference case disposal scenario is defined based on current Swiss proposals. High level waste is assumed to be immobilised in borosilicate glass, encapsulated in a 30 cm thick Fe canister and emplaced into 3 m diameter horizontal drifts which are subsequently infilled with compacted bentonite backfill. The repository is assumed to be sited in the crystalline (granitoid) basement in northern Switzerland at a depth of ∿1 km. The geochemistry is thus defined by the initial inventories involved, the nucleogenic temperature and radiation fields, the chemistry and inflow rate of groundwater and the hydrothermal reactions between this groundwater and the waste package[4].

The chemical environment in the near field will be dynamic, continously evolving with time, but three distinct episodes might be recognised:

i) Post emplacement, unsaturated backfill.

When emplaced the backfill will be dry (water content ∿7-10%) with most of the integranular porosity filled with air. The bentonite will then be subject to fluxes of water from the surrounding rock and heat from the canister which, due to coupling of these processes, results in a complex wetting/heating profile.

ii) Saturated backfill, canister intact.

After a period of time the backfill will reach equilibrium saturation, the initial oxygen in the entrapped air will be displaced or used up in corrosion processes and a "steady state" thermal profile from the canister to the drift wall will be established which will alter only very slowly as the thermal output of the canister decays.

iii) Post canister failure.

Although in a low-flow environment the corrosion rate would probably be very slow, eventually the canister will fail and groundwater will contact the waste-glass matrix. The near-field chemistry will then be altered due to both hydrothermal reactions with the glass and α-radiolysis.

Given that the near-field environment is not sufficently hostile to prevent microbial life, the maximum extent of such contamination must be assessed. For this purpose three constraints on the steady state biomass and level of metabolic activity which could be supported in the near field

are considered - free organic (or "available" inorganic) carbon, other important nutrients (N,P,S) and chemical energy.

Considering the sources of nutrients in the original trapped air, backfill, waste matrix and groundwater[4], a summary of the inventories of these species can be prepared[5] (Table 1a).

Chemolithotrophic organisms, which obtain energy from chemical reactions in the absence of light and build organic compounds from inorganic substrates, would form the base for the "food chain" in such an environment. Within the near field, the major chemical process which could be harnessed as an energy source would be the oxidation of the iron canister. Such oxidation can proceed in either oxic or anoxic conditions. Although the maximum energy which could be produced from the corrosion of the entire canister is $\sim 1.8 \times 10^{10}$ cal, the rate of supply of this energy would subject to kinetic constraints.

As a reference case, only oxic corrosion will be considered a suitable energy source for microbial metabolism. Combining models of the various sources of oxygen with thermodynamic data for iron corrosion yields[5]:

a) 4.8×10^{7} cal energy from the oxidation of 2.8×10^{4} g of Fe by the entrapped air

b) continous supply of 60 cal/year from oxidation of 3.5×10^{-2} g Fe by inflowing groundwater

c) after canister failure, a further 4.8×10^{3} cal/y from oxidation of 2.8 g Fe by radiolytically produced oxidants.

TABLE 1 Summary of inventory calculations

a) Inventory (g)	C		N	P	S
	org.	inorg.			
Trapped Air	-	0.9	2.6×10^{4}	-	-
Backfill	1.1×10^{4}	1.7×10^{5}	2.2×10^{4}	2.5×10^{4}	1.1×10^{4}
Waste Matrix	-	-	-	5.3×10^{2}	-
Groundwater					
- annual	4.6×10^{-3}	0.6	1.1×10^{-2}	2×10^{-4}	0.42
- integral over 10^{6} y	4.6×10^{3}	6×10^{5}	1.1×10^{4}	2×10^{4}	4.2×10^{5}

b) Ratio

Initial inventory					
Org C	1	15.5	2.0	2.1	1.0
Initial inventory					
total C		1	0.12	0.12	0.06
Annual Supply					
Org C	1	130	2.4	0.43	9.1
Annual Supply					
total C		1	0.018	3.3×10^{-4}	0.7

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nb $C_{160} H_{280} / _{80} N_{30} P_2 S = CH_{1.25} O_{0.5} N_{0.19} P_{0.0125} S_{0.0063}$

EVALUATION OF THE CONSTRAINTS ON MICROBIAL CONTAMINATION

The geochemical data presented can now be used to evaluate the maximum possible biomass which can be supported in the near field. Very simplistically the "average composition" of a mixed culture of microorganisms might be taken as

$$C_{160} H_{280} O_{80} N_{30} P_2 S.$$

Comparing the ratio of carbon to particular nutrients in this formula with those in summed initial inventories (trapped air + backfill content) it is immediately apparent (Table 1b) that initially limits on microbial growth will be set by available carbon. If only "organic" carbon can be utilised, the maximum initial biomass (dry) would be $\sim 2.20 \times 10^4$ g. If inorganic "carbonate" carbon could be utilised the biomass could be increased by up to an order of magnitude but the energy requirements would be prohibitive (comparable to that produced by oxidation of the entire canister)[5].

Considering only oxic corrosion mechanisms, and utilisation of the "organic" carbon inventory, the initial post-closure period would provide $\sim 4.8 \times 10^7$ cals/2.2×10^4 g = 2.2×10^3 cal/g organic dry weight. The additional supply of dissolved oxygen would provide only 2.7×10^{-3} cal/g/year which would allow only an extremely low level of metabolic activity. Following canister failure a further 200 cal/g/year may be available which could considerably enhance activity levels.

From the viewpoint of repository safety assessment, a number of possible consequences of microbial activity require evaluation:

i) Catalysis of corrosion.

For the "realistic"scenario for the development of microbial populations, the biomass (dry) of the initial population maximum would be $\sim 2.2 \times 10^{4}$ g and, even though such biomass would be distributed throughout ~ 26 m^3, as Fe corrosion is energy producing such catalysis could be very important.

ii) Physical disruption.

One obvious physical effect which has been tacitly assumed in (i) above is the disruption of the "protective" or "passivating" layers, which generally control corrosion kinetics, by the microbes. A potentially more serious effect, corruption of the physical properties of the bentonite backfill, seems unlikely for the reference scenario considered[5].

iii) Radionuclide uptake.

Microorganisms generally show the ability to greatly concentrate trace elements from solution by either active uptake into the organism or surface absorption onto outer membranes. In the reference case microbial mobility in the bentonite would be very limited[4] and hence any such uptake would tend to be "favourable", introducing further retardation into the system.

iv) Alteration of groundwater chemistry.

Although gross changes in groundwater chemistry are not expected, microenvironments (eg. with very low pH) may be formed by particular organisms which could greatly alter canister corrosion rate and nuclide solubility. Possibly more important than such localised effects would be organic biproducts which could reach concentrations[5] of 60 mg/l which would greatly influence expected actinide solubilities[4].

In summary it can be concluded that based on this approach the possibility of significant microbial contamination cannot be excluded although the extent of contamination may well be limited by "available" carbon. Potentially important consequences would include radionuclide transport on mobile organisms and enhanced solubility of particular nuclides by organic biproducts. Greatest uncertainties in this treatment arise from lack of data on the proportion of the total carbon inventory of backfill which is accessible to organisms and limiting kinetics and efficiency of energy in-

put from Fe corrosion. As this paper is intended primarily to allow focusing of research in this potentially vast field it would thus seem that resolution of these uncertainties are the most urgent requirement at present.

ACKNOWLEDGEMENTS

The preparation of this paper was greatly aided by comments and constructive criticism from N. Christofi, H. Grogan and J. Jiskra.

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SESSION IV : Microflora in higher trophic levels

Chairman	F.AZAM
Co-Chairman	M.FRISSEL

The role of micro-organisms in the digestive tract of domestic animals and man, and its consequences for the absorption of radionuclides.

J. van den Hoek

Laboratory of Animal Physiology, Haarweg 10,
Wageningen, The Netherlands

Introduction

The digestive tract of animals and man is essentally a long tube, running from mouth to anus. Specialization has occurred along the length of the tube for the different processes of intake, digestion, absorption and elimination to take place in this order. The structure of the digestive tube is also essentially the same everywhere but certain tissues are better developed in certain parts of the gut according to their functions.

For the purpose of our discussion, we shall not give attention to the processes of intake and the passage of food from the mouth to the stomach. It is here that our considerations will beginn.

As is well-known, specialization has taken place amongst animal species with respect to the kind of food which they eat. This allows a classification of animals on the basis of their eating habits. Carnivores obtian most of their food by eating other animals. They have one "simple" stomach, and are known as monogastric animals. Plant eating or herbivorous animals fall into two categories. The first group possesses three so-called fore-stomachs of which the rumen is the most important organ. This part of the digestive tract is situated before the actual or true stomach, and it is specialized in extensive microbial fermentation of the vegetable diet prior to digestion by alimentary enzymes further down in the gut. The ruminants or polygastric animals such as cow, sheep and goat, belong to this group. The second group, of which horse and rabbit are representatives, has only one stomach like the carnivores, and microbial fermentation takes place in the posterior part of the digestive tract. Finally,

there are omnivorous animals which feed on both plants and animals and which have also simple stomachs. There is considerable microbial breakdown of plant material in the large intestine. Man and pig belong to this category.

As a result of the adaptations which have taken place in relation to diet, there are important differences in the length of the digestive tract, carnivores have a much shorter alimentary canal of smaller relative capacity than have herbivores.

In the following sections, the importance of microbial action on digestion for monogastric and polygastric animals will be discussed. On this basis, preliminary conclusions can be drawn on the consequences for the absorption of radionuclides.

Microbial digestion in monogastric animals

Carnivorous animals

The most important characteristic of the stomach for our considerations is its acidity. This is the result of secretion of hydrochloric acid by certain specialized glands in the gastric mucosa, leading to a strongly acid p_H of about 1-2. The vast majority of microorganisms which are washed down with the saliva from the oral cavity into the stomach, are destroyed by gastric acid, Only the acid-resistant species can survive, and there is little, if any, microbial action on food molecules.

The passage of the food components from the stomach into the small intestine brings about a change in p_H from strongly acid to near-neutrality. This environment is favourable for microbial growth, and this is reflected by the increase in number of microbes towards the end of the small intestine. In man, the number of microbes per ml increases from $0\text{-}10^3$ in stomach and duodenum to $10^3\text{-}10^9$ in the latter part of the small intestine and to $10^{10}\text{-}10^{12}$ in colon and feces.

Since digestion and absorption of food molecules takes place almost entirely in the small intestine under the influence of enzymatic action, it can be readily understood that the effect of microorganisms on digestion in carnivores with their relatively short alimentary canal is quite limited. The most important physiological process which takes place in the large intestine, is the absorption of water. Digestive and absorptive processes are practically completed in the small intestine. For this reason, the action of microbes on the food residue in the large intestine will be small.

Omnivorous animals

The animals of this group feed on both plants and animals. The pig is usually considered to be omnivorous and man also falls in this category. The digestion of omnivorous animals is mainly enzymic in nature like that of the carnivores.

The environment in the stomach is strongly acid and this leads to a very important reduction in number of microorganisms which have come down the esophagus with the food. The microbial population is restored to some extent in the small intestine and reaches its greatest density in the large intestine. True fermentation of the food is limited, and since the absorptive capacity of the mucosal wall of the large intestine is limited, microbial action on food in the omnivorous animal may be considered to be of only minor importance.

Herbivorous animals

Monogastric herbivores

The horse and the rabbit are examples of herbivorous animals with a simple stomach. Microbial fermentation of plant material takes place in a manner which is comparable to that in ruminants and which also leads to the formation of volatile fatty acids besides other products. Some fermentation occurs in an area of the stomach (saccus caecus) where the gastric juice cannot penetrate very easily but this is of little quantitative importance. The greater part of the plant material will undergo fermentation in the various parts of the cecum and colon. However, absorption in this part of the gastro-intestinal tract is low, and is limited to water, electrolytes, some volatile fatty acids and other simple compounds.

It is of interest for our considerations that plant material has a relatively low energy content. This means that herbivores must consume a large quantity of food to satisfy their energy requirement. They will therefore graze large surfaces and this will lead to a relatively greater uptake of radionuclides, deposited on or taken up by plants.

Polygastric herbivores

The animals of this plant eating group have the unique feature of possessing 4 stomachs, three of which serve as a fermentation vat for the cellulose containing vegetable material in their diet. And of these three so-called fore-stomachs, the rumen is by far the most important one. The fourth stomach resembles the stomach of the monogastric animal in that there is considerable secretion of acid gastric juice. This leads to a strongly acid environment with the concomitant result that the numbers of protozoa and other microbes which are passed on with the food from the fore-stomachs, are greatly reduced.

Fermentation of the plant material in the rumen and reticulum of the grazing polygastric herbivores results in extensive chemical changes of the ingested food. The soluble products of fermentation are largely absorbed and the gaseous products are erupted. The material leaving the rumen consists of a mixture of food residues, microbes and some soluble fermentation products. Once this mixture reaches the abomasum, a situation is encountered which resembles that in monogastric animals.

The result of fermentation of carbohydrates is that only the structural components of plant tissues, namely cellulose and the hemicelluloses survive to some extent, depending principally on the degree of lignification of the material. Simple soluble sugars are rapidly fermented, starches a little less rapidly. Very little of the structural components of vegetable material which survive ruminal fermentation, will be broken down later on in the alimentary canal.

A considerable fraction of the dietary proteins undergo proteolysis in the rumen under formation of widely different concentrations of amino acids and of ammonia, depending on the nature of the original protein. A substantial amount of the amino acids formed after proteolysis by ruminal microbes is used for the formation of microbial proteins.

Lipids also undergo hydrolysis in the rumen to glycerol and fatty acids, due to the action of rumen microorganisms. Hydrolysis of phospholipids occurs in a similar way. Many unsaturated fatty acids of the food are hydrogenated.

The question now arises as to what are the consequences of the differences in microbial action on food components in mammals for the absorption of radionuclides. As far as I know, investigations desigend specifically to study these aspects, have never been carried out. It seems most logical, therefore, to discuss these consequences in relation to the characteristics of digestion, that is for carnivores, omnivores and herbivores separately.

Carnivorous animals

It follows from the foregoing discussion that the influence of microorganisms on the absorption of radionuclides from the food is negligible in this group of animals. Radionuclides contained in the food, will be liberated and absorbed in the small intestine as a result of the enzymatic breakdown of the food molecules without interference of microbes. Their numbers are small in the small intestine and increase only in the large intestine but absorption in this part of the digestive system is limited to water and some electrolytes.

Omnivorous animals

As has been pointed out, the influence of microorganisms on digestion of the food of omnivores, is of minor importance only. In fact, a situation exists which is very similar to the situation in carnivores. The biological availability of radionuclides contained in the animal's food, depends mainly on whether or not the digestive enzymes, produced in the digestive tract, are capable of breaking down the food molecules. The contribution of microorganisms to this process is quite limited.

Herbivorous animals

As has been pointed out, the fermentation of vegetable material by micro-organisms is a very important feature of the digestive processes in herbivores. And this is particularly true for ruminants. The effect of microbial action will be that cell wall polysaccharides belonging to the group of hemicelluloses, will be broken down. In this way, radionuclides could become available for absorption which might leave the organism undigested otherwise. Obviously, if such an effect exists, it will be much stronger in ruminants than in monogastric herbivores because microbial action is limited to the large intestine in the latter group where absorption is small. Another aspect is that only radionuclides capable of penetrating inside the plant cell are affected in this way. To these radionuclides belong ^{3}H, ^{14}C, ^{35}S. Maximum release of radionuclides situated inside the plant cell, will take place when the lignification of the plant structures is minimal, and this is the case in young plants, usually in the spring. At the end of the growing season, and in the winter time, chances are highest that radionuclides, contained inside the cell, will pass the digestive tract of the herbivore unaffected. The cellulose content of grasses may range from 15 to 35 percent of the dry matter, the quantity increasing with the age of the plant. The hemicelluloses form as much as 14 to 25 per cent of the dry matter of grasses and the lignin content may vary from 2 to 12 per cent. In all cases, the higher percentages are found in older plant material. The conclusion can be that a notably lower biological availability of ^{3}H and ^{14}C in older grasses and plants is a real possibility, and more so for ruminants than for monogastric herbivores. In other words, the transfer of ^{3}H and ^{14}C to the animal from its diet may be significantly lower at the end of the growing season. But these possibilities, though realistic are theoretical and speculative because investigations to study such aspects have never been carried out.

The situation with respect to other radionuclides is even more uncertain. It is unlikely at first sight that microbial activity in the digestive tract will influence the biological availability of the radionuclides from the radium and other heavy element series nor that of the strontium isotopes. Cesium has physiological characteristics similar to those of potassium, and K is mainly present inside cells. It is therefore possible that the reasoning, followed for ^{3}H and ^{14}C, also applies to cesium. It is interesting, in this respect, that the transfer of cesium under "natural" conditions and using fall-out cesium was always lower than under laboratory conditions when the soluble CsCl was usually administered to the animal. A seasonal effect was found for the transfer of isotopes of iodine. However, the question remains open as to what this would be due.

Summarizing our considerations, we can conclude that possible variations in the bio-availability of radio-nuclides as a result of the role of micro-organisms on digestion, has not been investigated so far. On the basis of our knowledge on digestive physiology, it seems unlikely that such variations will be found in carnivorous and omnivorous animals. However, variations are possible in herbivorous animals; if they exist, they will be more prominent in ruminants than in monogastric herbivores. Their quantitative importance remains questionable and it will be limited to radionuclides which enter the interior of the plant cells such as ^{3}H, ^{14}C, ^{35}S and possibly the isotopes of iodine, cesium and some other radionuclides.

ABSENCE OF AN ENHANCEMENT OF GASTROINTESTINAL UPTAKE OF PLUTONIUM COMPLEXED BY POLYETHER IONOPHORES

Robert A. Bulman and Tracey E. Johnson

National Radiological Protection Board, Chilton, Didcot, Oxon OX11 ORQ, UK.

ABSTRACT

The interaction of Pu(IV) with ionophores of microbial origin is of interest because some ionophores are added to animal foodstuffs. The formation of partially lipophilic Pu(IV) complexes with the ionophores could increase the gastrointestinal (GIT) uptake of Pu(IV). In this study only ionomycin, a β-diketone, not yet used as an additive in animal feedstuffs complexed the element. In the hamster this ionophore did not enhance the GIT-uptake of the ^{239}Pu.

INTRODUCTION

The intensive rearing of cattle and poultry has necessitated the introduction of complex feedstuffs which contain antibiotics and other additives. The inclusion of these additives promotes growth and thus lowers production costs. Currently, several naturally occurring ionophores - complexing agents which facilitate the transport of monovalent cations and, to a lesser extent, divalent cations across cell membranes - are used as feedstuff additives[1-4]. At least one synthetic complexing agent, caprylhydroxamic acid, has been the subject of patent rights as a growth promoting agent for pigs[5].

Ionophores are normally of microbial origin and are generally typified by their polyether moieties[6]. The β-diketone is a rare moiety in natural products and is present in ionomycin, an ionophore which complexes Ca(II)[7] and might also be expected to complex the radiocation Pu(IV). It has previously been reported from these laboratories that a synthetic β-diketone - containing ionophore, trivially known as FOD, appears to elevate the GIT-uptake of ^{239}Pu(IV)[8]. In this study we have screened several ionophores for ability to complex ^{239}Pu(IV).

MATERIALS AND METHODS

The ionophores salinomycin (I) X-14547 A (II), ICI 139603 (III) and ionomycin (IV) were generous gifts from Hoeschst UK Ltd., Dr J. W. Westley of Hoffman-La Roche Inc.; Dr M. Doyle of ICI Pharmaceutical Division and Dr G. R. A. Hunt, Polytechnic of Wales, respectively. Lasalocid A (V) was obtained from Sigma Chemical Co. Ltd.

The ionophores were dissolved in dimethylsulphoxide:methanol (50:50, v/v). For administration to animals dimethylsulphoxide was the sole solvent. ^{239}Pu(IV) nitrate in 0.01 M HNO_3 was ultrafiltered through filters with a pore diameter of 0.025 μm (Milipore (UK) Ltd.) to minimize on the presence of polymeric forms of ^{239}Pu(IV). Small volumes (10 μl) of this solution was added to the organic solution and an equal volume of 0.01 M of NaOH solution added. Complexation of ^{239}Pu(IV) by the ionophores was determined by a previously described method[8]. Essentially

this procedure involves passing the solution of $^{239}Pu(IV)$ nitrate and the ionophore through Sephadex LH 20 (10 x 150 mm) with dimethylsulphoxide and methanol (50:50, v/v) as the eluent. In the absence of an effective complexing agent the radioactivity remains on the column. Solutions were administered to the hamster (1210 Bq per animal) by placing the solution on the tongue. Typically, 50 µl solutions were administered. The animals were maintained for 7d, and examined as previously described[9]. Radiochemical analyses of an established procedure were used[10]. Golden Syrian hamsters (3 months old, DSN strain, Intersimian Ltd., Abingdon) were not fasted and food and water were readily available throughout the experiment.

RESULTS AND DISCUSSION

Salinomycin and ICI 139603, two monovalent ionophores used as animal feedstuff additives, did not facilitate the transport of $^{239}Pu(IV)$ through Sephadex LH-20. As these ionophores did not complex the radiocation under these conditions they were excluded from this further study. For the same reason the divalent cation ionophores X-14547A and lasalocid A, an anti-coccidial agent used in feedstuffs, were also excluded. These four ionophores were also shown to be ineffective complexing agents for $^{241}Am(III)$. It is worth noting that lasalocid A formed a complex with Fe(III), as judged by the formation of a purple coloured complex characteristic of the interaction of Fe(III) with a phenolic group.

Although ionomycin formed a complex with $^{239}Pu(IV)$, as judged by a recovery of 50% of $^{239}Pu(IV)$ from Sephadex LH-20, it did not appear to enhance the GIT-uptake of $^{239}Pu(IV)$ in hamsters (Table 1). The values obtained for the GIT-uptake of $^{239}Pu(IV)$ in this study are similar to those obtained for the nitrate (0.002%) in hamsters in these laboratories[9].

In general, the naturally occurring complexing agents are characterized by a high cationic-specificity, as typified by that displayed by the catechol-based and hydroxamate-based ferric specific

TABLE 1. Retention of ^{239}Pu(IV) in the hamster[a] at 7d.

	% ± SEM
Carcass[b]	0.003 ± 0.001
Liver	0.0003 ± 0.0001
Retention[c]	0.003 ± 0.001

[a]mean of animals; [b]'carcass' - head, lower limbs, tail, pelt, intestines, kidneys and liver all removed; [c]'carcass' + liver.

complexing agents of microbial origin[11]. With the exception of the ferric-specific complexing agents (siderophores) the naturally occurring ionophores exhibit only a weak selectivity for Pu(IV), ionomycin, in fact, being the only non-siderophore ionophore yet shown to complex Pu(IV). Of note are other studies from these laboratories which have shown that rhodotorulic acid, a siderophore, increases the retention of ^{239}Pu a mere 3-fold over that of citrate[12].

CONCLUSION

It must be presumed that the polyether ionophores currently used as animal feedstuff additives will not increase the GIT-uptake of Pu(IV). Nevertheless some caution should be exercised before other lipophilic complexing agents are introduced as additives for animal feedstuffs before they have been tested for ability to enhance the GIT-uptake of toxic and radiotoxic elements. Ionomycin, the only ionophore shown in the study to complex Pu(IV) did not enhance the GIT-uptake of ^{239}Pu administered as its nitrate.

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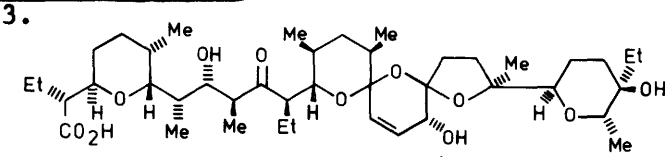
(I)



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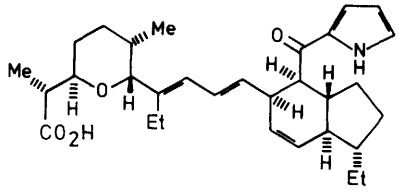

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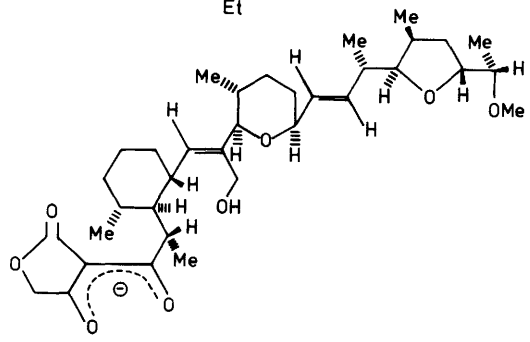

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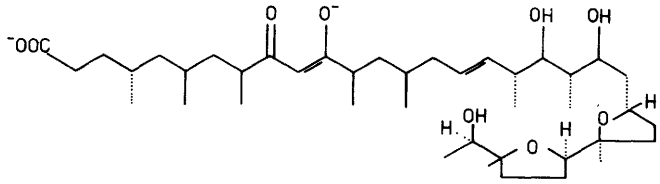

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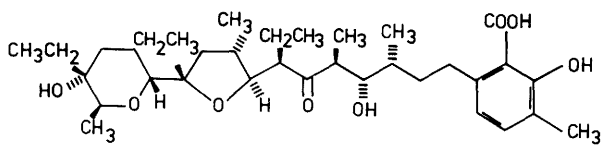

Session : IV

Paper by : R.A.Bulman

Comment by : S.Bonotto

Text of comment
or author's answer :

Might complexing agents of microbial origin have practical uses in nuclear industry and also in tumour therapy ?

It is possible that complexing agents of microbial origin might find some application in the nuclear fuel processing industry. However, I am not familiar enough with this section of the nuclear industry to speak with authority on it.

Desferroxamine has been examined in several laboratories for ability to remove 239-Pu from experimental animals. Neither it nor a lipophilic derivative, and other microbial complexing agents, are of any real value for enhancing the clearance of Pu-239 from animals.
(Health Physics , 37, 729 - 734 (1974)).

At least two groups are engaged in making new complexing agents which might enhance 239-Pu clearance. The approach is termed "biomimetic", basically they are making stable version of enterobactin, a catechol-containing molecule which is not stable to weaks acids.

SESSION V : Panel Discussion

Moderators : A.J.FRANCIS, P.O.AGNEDAL

General aspects of the session I/A

"Marine and estuarine microorganisms"

Chairman : E.K.Duursma

Co-Chairman : N.S.Fisher

Report presented by F.Azam

The common theme running through all the papers was the importance - either documented or potential - of surface area of suspended particulates in consideration of radionuclide accumulation in marine microorganisms. Particular attention was paid towards adsorption phenomena is regulating microbial association with radionuclides.

The role of bacteriaplankton in marine food webs has, until recently, been underestimated owing to methodological difficulties. With the advent of fluorescence microscopy, reliable estimates of bacterial abundance are now being made. Approximately 20% of water column biomass and 80% of suspended biosurfaces in the sea can be attributed to the bacteria. Bacteriovores, particularly small ciliates and flagellates, may assimilate radionuclides which associate with the bacteria, and given the enormous surface area of bacteriaplankton in seawater, there is a substantial potential to accumulate radionuclides.

The results of a series of laboratory experiments with a group of gamma-emitting radionuclides (Mn, Tc, Ag, Cd, Hg, Po, Pb, Np, Pu, Am, Cm, Cf) and six planktonic algae species showed that a number of reproducible phenomena can be detected. The bioconcentration of the nuclides conformed with Freundlich adsorption isotherms, with dead and living algae accumulating radionuclides similarly. Thus, this "uptake" process appears to be a passive phenomena. There were great differences between reactivities of metals and only moderate differences for any given metal among algal species.
Concentration factors (calculated at equilibrium) correlated significantly with solubility products for the corresponding metal hydroxides, so it appears that the reactivity of metals for any phytoplankton can be predicted to within half an order of magnitude.

Surface adsorption phenomena can be visualized by autoradiographic techniques, which can distinguish between homogenous distributions on biological surfaces and "hot spots" on particles of different kinds.
Results obtained with various types of samples form the Irish Sea demonstrate that the fact of surface-bound radionuclides is determined through many steps in the transfer from phase to phase, either biologically or abiotically. Results point to the importance of abiotic processes in particular, and the presence of alpha-tracks is most evidently associated with particles, living and non-living, with sizes of 10 to 30 um.

A series of laboratory experiments was conducted to measure the accumulation of Zn and Hg by marine phytoplankton and subsequent transfer to herbivores (mussels and brine shrimps). No evidence of biomagnification up the food chain was obtained. Moreover, there was no observed interaction between Zn and Hg in uptake by the phytoplankton.

There is a lack of knowledge about the surface phenomena described as "black boxes". What it is new is to define the chemical entities responsible for the surface interaction, this will be one of the priority of research in this area.

Summary of Session I /B

"Marine and estuarine microorganisms"

Chairman : E.Hamilton

Co-Chairman : M.Zhou

For purposes of direct application it is the radiation dose (to marine ecosystem)which is of paramount importance to radiological protection, together with transport of radionuclides along or through marine food-chain to man. The only paper dealing with this topic (Polikarpov et al.) was delivered in title only ; it most purports to identify radiation effects on marine biota. Uptake and loss of radionuclides by marine and freshwater organisms continue to be reported based upon laboratory experiments with little verification of this validity, or value, under natural conditions. Ruthenium complexes are not taken up by bacteria but more readily become associated with the inorganic components of sediment. The complexity in the composition of marine seston , as it contribute to marine foodchain is identified, especially seasonal variability in estuaries and near surface waters.

In spite of more than 100 years of intensive research into marine ecosystems it is surprising that in recent years so many major discussions have been made ; for example the abundance and productivity of micro-organisms in the shelf seas feeding process of zooplankton (continuous feeding v specialized feeding) and the geographical distribution of some species, in very large numbers, even larger areas of the seas. Despite attempts to identify one or a few constituents as dominant factors (e.g. humic acids, clay mineral, faecal pellets) which can account for the distinction and behaviour of radionuclides in the sea it is becoming increasingly obvious that there is a need to consider that marine ecosystem characteristics, using simple indices of performance in conjunction with simple modelling. However, there is also a need for special studies using a wide range of technical expertise which brings together knowledge from many disciplines, and where the barriers surrounding individual disciplines are lowered.

With the exception of Tc and Pm the performance of all radionuclides should be capable of interpretation by using stable element analogues. When this does not occur then attention should be focussed upon the physical and chemical composition of the source term which may not be natural. Therefore, today as no effects of any significance are found which indicate that ionising radiation is harmful to marine ecosystems, radionuclides'as used in radioecology' are important components whereby the rates of transfer, and rates at which processes take place in the sea may be identified by use of the radioactive label. However, until the significance of chronic low dose exposure, is fully undescribed, continued research in this field has to be sustained. Progress in the effects of ionizing radiation, and the manner in which radionuclides become distributed in marine and estuarine ecosystems, can only be made by improvements in fundamental marine research. It is premature to place too much reliance upon what is known today.

This workshop has identified gaps in our knowledge in the role played by microorganisms in relation to the distribution of radionuclides in the marine environment.

An important point, shown is this meeting, is the extrime difficulty to extrapolate results from one species of animal obtained in laboratory to the environment where several species of are present and so many processes taking place. In term of radioecology and radioprotection, effects must be sustained for looking for effects in real marine ecosystems. Actually, the tendency is to move from laboratory tanks to large volume enclosed in the sea, and that is a considerable improvement.

Summary of Session II

"Fresh-water microorganisms"

Chairman : J.Pieri

Co-Chairman : T.H.Sibley

Five papers were presented in the session on freshwater microorganisms that considered diverse aspects of the role of microorganisms in aquatic ecosystems. The invited paper by Mr.Balvay presented an overview of planktonic food chain dynamics in lake ecosystems. It is important to remember the trophic interactions that can occur in natural communities since they indicate significant relationships between species where transfer of radionuclides from one species to another is likely to occur. This is a subject that is quite well studied, relative to other areas of radioecology, and has limited controversy at the moment. However, the recent discoveries concerning the quantity and distribution of bacteria in marine systems (summarized by Dr.Azam at this meeting) have completely revised our understanding of planktonic interactions in the oceans. Similar discoveries and reevaluations should be expected for freshwater lakes.

Bourdon et al. described the use of on algal culture technique to investigate the presence and behavior of radioisotopes, especially 3-H and 14-C in effluents from nuclear installations.
A question arose regarding the need for an additional monitoring tool. It was noted that some isotopes (eg.54-Fe, 57-Co and 186-Re) detected in the algae after culturing were not measured by conventional monitoring programs. Moreover, this method provides a comparison between conventional laboratory uptake experiments and accumulation from the complex effluents of reactors. Therefore, it can be used to evaluate the appropriateness of laboratory experiments for extrapolation to natural situations. Isotopes of antimony and cerium do not accumulate in the algae and this is not a more sensitive method than other monitoring procedures for those elements.

The presence of microorganisms in the cooling systems of nuclear reactors was discussed by Mergeay et al. When the reactor was shut down several strains of aerobic microorganisms were isolated including radioresistant bacteria and red yeast . When the reactor was operating only red yeast were isolated by plating techniques. During the discussion it was mentioned that microscopic techniques probably would have detected additional microorganisms and the population estimates would be several orders of magnitude higher. It was agreed that radioresistant microorganisms are abundant in the cooling systems of nuclear reactors but their significance to proper operation is currently unknown.

In heterogeneous environments it is often difficult to specify the principal source for radionuclide accumulation by aquatic organisms. The paper by Lambrechts and Foulquier considered the role of sediments and microorganisms (the phytoplankton species Chlorella sp.) in accumulation of 60-Co and 137-Cs by selected freshwater species. This paper generated much discussion concerning the appropriate experimental methodology for evaluating transfer between trophic levels. For example, should animals be allowed to void theis guts of radioactive sediments before measurements of

description of the radioactivity in soils.

Dr.Förstel reported on the HT/HTO-conversion in the soil on the basis of a laboratory experiment. The biochemical conversion is an important aspect, as portions of the tritium releases from nuclear installations seem to be in the form of HT.
The first experiment showed that microorganisms are reponsible for the conversion to HTO. For the assessment of the impact of tritium to man further investigations should be performed concerning the conversion also into organically bound tritium (OBT) in dependance of relevant soil parameters and microbial action. This work deserves special emphasis in view of fusion reactors.

Both, Dr.Martens and Dr.Strack, showed experimentally that soil microorganisms may accumulate iodine. Sterile and non-sterile soils act very different in this respect. For sterile soils the extractability of iodine is much higher. Their findings are of great importance in case of accidental releases. The results also show that the curve shapes and the mathematical descriptions of the increased extractability from sterile soils are equal for different types of sterilizations, namely for autoclaving, for fumigation by chloroform and for irradiation. The third type may play a role in the case of greater accidents when irradiation of the soil may occur. The investigations must be continued also for other elements and with regard to organic complexing agents released from reprocessing plants. There is a need to define the terminology used uptake, fixation and assimilation for example.

General aspects of the session III/A

"Terrestrial Soil Microorganisms"

Chairman : W.Kühn

Co-Chairman : S.Strack

As is well known, the soil is a very complicated system. This system has important influences on the uptake of radioactive elements into plants and on the recharge of groundwater reservoirs. Consequently, the soil may effect the entrance of radioactivity into the food chain in two ways :

firstly in view of the resulting concentrations of radioactivity in plants and secondly by way of the translocation of radioactivity towards potential drinking water reservoirs.
The main structures of those two ways have been investigated in function of the well known parameters for many years (transfer coefficients or concentration factors, Kd-values and retention factors and their interrelations).

Speaking about the influence of microorganisms on the behaviour of radionuclides : the investigations on this problem are dealing in my opinion with a sort of fine structures of the translocation mechanisms of radioactivity in the soil. Till now we did not consider this effect, and all our regulatory guides do not involve its influence.

If we accept the Kd-values, and we do so, as one of the most complex factors describing the sum of many single phenomena in the behaviour of radionuclides in soils, we should remember that it also includes the role of microorganisms as one of the many parameters. Therefore, investigations on the relation between Kd-values and soil microorganisms should be promoted.

During the workshop some information was given on the valences of Pu and other transuranium elements, but very little was said about the geometric form and size of the complex compounds and about the atomic radii of the high cationic or anionic valences.
As shape and size of the molecules and in particular of organic molecules play an important role, this should be considered in connection with the microorganisms, as it concerns basic research on surface adsorption.

In session III/A four lectures were given.

Dr.Frissel explained some types of existing models for the growth and disappearance of microorganisms in soils. The models give an understanding of the microbiological activity. The action of microorganisms is manifested by the change of redox potentials of the soil, by the production of CO_2 and by the production of organic complexing agents. All three effects are responsible for the changes in the migration and accumulation of radioactive substances in the soil.

The models are theoretically tested, and they may be useful in explaining the role of microorganisms. In our case the application of such models has the aim to describe the distribution of radioactivity in the soil. In order to experimentally verify them for this purpose, they should be experimentally applied in a more conventional way for general use, i.e. for cases of normal plant nutrition and afterwards for the theoretical

uptake are made ? Perhaps not, if we are considering questions of radiological protection and the actual transfer that occurs when one animal eats another. However, if we are investigating metabolism and bioaccumulation, then we must be careful to assess the actual assimilation within body tissues. The need to present details of experimental methodology and to use terminology precisely was very evident from this discussion. It would be even more valuable to have consistent terminology among different authors.

Vandecasteele et al. studied some interactions between technetium and nitrogen fixing organisms. Their studies of specific enzyme systems and competition for molybdenum binding sites is at a more biochemical or molecular level than the other papers. An important observation in their research is that TcO_4^-, unlike the metals that were discussed in the marine sessions, clearly enters the cell ; only 5% of 99-Tc was associated with the membrane fraction during cellular fractionation procedures. Application of these biochemical techniques should help us to better understand the cellular distribution of other isotopes as well.

Compared to the discussions in the marine sessions, there was little consideration of radionuclide adsorption on the surfaces of microorganisms. This should not be considered to indicate that adsorption is better understood in freshwater environments nor that adsorption on microorganism surfaces is less important.
On the contrary, so little research has been conducted to date that adsorption to biological surfaces may be even less well understood in freshwater systems. Certainly, we seldom have sufficient data to distinguish between adsorption and absorption.

In freshwater environments the chemical characteristics are more variable both among different environments and with a given ecosystem at different times. Therefore, the importance of adsorption is also likely to be more variable. To evaluate the significance of adsorption to microorganism surfaces we will need to consider such chemical parameters as pH, redox potential and concentration of dissolved ligands in addition to the concentration of microorganisms and other suspended particulate matter.

Summary of Session III/B

"Terrestrial Soil Microorganisms"

Chairman : A.Cremers

Co-Chairman : P.Bovard

As you know this session was devoted to four papers : the paper of Dr.Bulman had a objective somewhat different of the three other papers which reflect the concern both in the area of shallow land burial and high level waste disposal in deep underground depositories, so the possible microbial effects on the fate of radionuclides.

Such concern is mainly motivated by the possibility of microbial oxidation of low molecular weight soluble organic compounds which may be generated in aerobic an anaerobic conditions and which have complexing properties. The results enhance the degree of mobilization of radionuclides.

These three papers have illustrated various aspects of this problem area; the evidence presented obviously more convincing is in the case of shallow burial, whereas for deep geological disposal the results are more of qualitative nature due to the difficulties of making a reliable inventories of native microbial populations and avoiding contamination in geological samplings. A quantitative conclusion will be somewhate hazardous and premature at this stage.
One should make a very clear distinction between these two cases (shallow land burial and geological high level waste disposal in deep underground repositories) : these two systems may differ in two main respects.
The first one is the presence and ample supply of degradable energy source in shallow land burial which appears as a key condition for this mobilization process to occur, and this has been demonstrated in the paper presented by Dr.Francis.

The second difference is the rate of groundwater flow which is generally quite very low in deep underground repository formation. Groundwater flow would automatically exclude such depository considered as a possibility. If these processes could occur in deep geological formation one should attempt to quantify the possible effects . Perhaps a word of caution is useful in order to avoid to deduce from such discussion that metal-complex naturally formed with microbial exudate may be generated in sedimentary deposit and live a life of their own. There are some doubts into this, and perhaps a route to quantify these effects may be to express these phenomena as a competitive effects between a pool of low molecular weight and a second pool of high molecular complexes which are insoluble, so essentially an approach could be that one could evaluate the relative importance of these two pools (immobile organic pools, mobile organic pools) and in such a case the enhance diffusional mobility could perhaps be define in term of a modified distribution coefficient between insoluble sinks and soluble low organic molecules.

To conclude, there is no doubt as to shallow land burial, these things have been unambigously shown; in the case of deep geological waste disposal the question remains completely open.

GENERAL DISCUSSION

Comment by C.Myttenaere

Radioecologists use "magic words" to mask ignorance of the problems they are in charge with. They always use the word "Chelating Agent", "Kd-value", and now we have found another magic word "microorganism". I think that if we want to give a valid interpretation we must go deeper in the research and that applies particularly to microbiology. The Panel's Chairman view is that more basic research is needed.

Floor Discussion (Summarized)

- Förstel : Microbiologists tend to work with well defined microorganisms and here we are speaking about populations of microorganisms and very often methods do not exist for example to measure microbial population in the environment. That is a methodological gap which has been revealed here.
- Pieri ask to the floor how many true bacteriologists are present here? He gave example of what have been achieved in medicine in this field.
- Frissel said he was a little disappointed by this meeting, asking where is the place were most of microorganisms are? He replied that this place is the upper five centimeters of the soil. Unfortunately there was no report on this topic and in particular on any accumulation process. We are still missing an important part of our information.
- The Chairman does not agree with this view that there is no or very little microorganisms in sub-surface environment, that has been completely changed in recent literature : people finding a lot of microorganisms (oligotrophic .. 10^4 - 10^5).
- Frissel added that unfortunately there was no report on the rhizosphere
- Kühn said he was happy to see a meeting devoted to the role of microorganisms, and he is not desappointed because a workshop can not cover all the field. Nevertheless he felt that some information concerning the value of Pu was given but nothing about the stecheometric form and sizes of the complexes.
- Bonotto noted that in natural environment, sterile conditions do not exist, there are also interactions between species. Even with cell culture, one can have contamination by mycoplasma. Also virus can be present, so the situation is a complicated one. What can be retained is that there are interaction between microbes and more higher organized species. So, as pointed out by Dr.Hamilton, field experiments are very useful but if we want to understand what happen in nature, we must isolate species and perform laboratory experiments. In conclusion both are necessary.
- Cristofi refered to a publication in "Nature"; two years ago, of a article written by soil scientists (Scotland) in which hydrolixic acid

produced by soil microorganisms in upper layer of soil is not able to stabilize Pu bound to soil particle.

- Strack agreed that there is still work to do in order to get more transparency on the society of microorganisms present in the upper layer of the soil.
- (Unnamed intervenant): After three days of meeting the feeling is that the methodology per se is something that should be discussed more intensively and perhaps if there is a future meeting on the subject, a session to specifically address this topic.
- The Chairman'feeling is that in the last decade the methods improved quite a bit but the problem is to define the system.
- Data on technics in the marine field were mentioned and it appears that there are for example a few instruments available at the moment for doing total size particles analysiss which represents a major advance.
- According to Pieri, the problem of counting is a routine problem for this kind of research.
- (Unnamed intervenant): One thing which has been general about this meeting is that it covered radioactive input from a number of sources implicity in consideration with ocean, near surface and deep disposal so in the whole range of environments and source-terms considered.
  The near surface environment is well-defined and specially near surface trench disposal, the problems are of microbiological and hydrological nature. But for deep disposal it is very much an open question to know if there is a microbiological influence at all. As Frissel stated the number of microorganisms found in deep formations is very small, but a growing field of geomicrobiology has shown that geochemical processes, the formation of ore bodies which were thought to be completely dominated by inorganic chemistry, a lot of redox boundaries which were found are caused by microorganisms, so microorganisms could affect very slowly in deep geological formation but is there some way,for the particular cases of high level waste disposal at depth of 1.5km,to suggest to close this question?Because the last thing would be a so nice way of doing calculations showing that microbial effects will be insignificant in this environment from millions years. This shall be great because one could say "forget high waste disposal and move on more difficult problems like low and intermediate level wastes".
- Chairman's comment that we need to consider the scenario to that in time (one thousand or two thousand years) high level waste will become intermediate and low level wastes, and that microbial action on mobilization will be on this low activity waste. The microbial action will not acting on the high level waste itself. We must also take into account that in high level waste repositories microorganisms from the surface are involved and that we really disturb the system.
- Pieri asks if one had an idea of the kind of microbes found at the five to eight hundred meters depth and deeper?
- Answer is that to find this type of information, mines (some were closed 500 years ago) are suitable for this kind of investigation. A technical discussion on the question of species and the numeration of bacteriae followed this statement.(Method of sampling, temperature, pressure etc...)

- West J.: I think Dr.Pieri is talking about resident microbs which are actually present in geological formation. What we want to do is to look at microbs which has been introduced in the mine.

- Cremers :
To distinguish biological and physical processes is what is still coming on. But when we look at the present conference and previous one dealing with radionuclides accumulation, it is under steady-state conditions but in fact one must look more closely at the rate process, which is what we are deeling with. There is little information provided in the past and present conference on this subject.

- Chairman notes that this comment goes very well with Dr.Kühn'remarks : one must define the oxidation state of the elements, always, because you may have different states in aerobic an anaerobic media and the complexes are never defined and this is also importante because the matter of stability is important. You may have complexes which are not assimilated, so I think that people measure some overall effect but with no exact definition of what they are studiyng and for the rate processes it is the same thing if you can define on what you are working, so perhaps you can measure a rate process.

- Cremers : that should be a very important point in the future years :to try to characterize exactly what we are dealing with and doing that to try to define stabilities of these complexes. And eventually demonstrate if they are relevant or not.

- Kühn: We should divide in different things. One is the deep layer disposal radioactive waste ; I was told in my country and abroad (Sweden) that if the containers are very dense and that there will be no leakage for thousands of years, why to speak about microorganisms ?
Second thing is, in what we are primarily interested, is the upper soil layer where are acting complexes compounds, microorganisms, kerosene, tributyl-phosphate etc.. on the translocation of radionuclides.

- (Unnamed intervenant)
Simple comment about the waste canister which can be safe for thousands of years : locally design has to be developed before the end of the century and also a number of countries (Sweden, Switzerland) have to do a safety assessment on possible consequences of geological disposal of high level wastes and if it is impossible to show with a reasonable justification that this disposal is safe, there is a possibility to see a shut down of the reactors. The total cost involves billions of dollars thus there is a very strong incentive to investigate some of these properties and the requirement is to have simple models and the time scale is for the end of this year or certainly end of the decade !

- Chairman : We want information on the interaction microorganisms and radionuclides in terms of complexation and we need to do something about it.

- Wirth : I agree that we must do calculation about the importance of microorganisms for high level waste, but why it is not clearly said" I think from our calculation there is no problem"?

- Wirth comes back to Dr.Myttenaere statement about the number of microbiologists present in this meeting; the answer reflects the typical situation of the radioecologists in general. For example, at the meeting on nutrition or transfer of radionuclides to plant how many scientists

of this field are really present ?
Or if you are talking on meteorological dispersion, how many meteorologists are present ? The field is so wide in radioecology, you can not be a specialist in each field. I have the feeling that we do not understand what we are doing.

- Myttenaere agree fully with this statement and says that is the reason to create teams of scientists, multidisciplinary teams, in order to study these different problems. One of the aim of the CCE programme is to give opportunity to people to meet and to discuss.

- Agnedal : when I started radioecology about thirty years ago, a meeting for discussing radioecology was composed of almost phycists, chemists and health physicists. But since last ten years, it has developed what we can called "pure" radioecologists, I think that the present meeting will give you opportunity to start a new area of radioecology to come up in the microbiology; this potential curve will grow very quickly in the future for this type of work. Why it has not be done before is because it is a such difficult problem, and we have to invite the microbiologists now to join the field of radioecology and to give us more knowledge about this area. I feel that this panel has been very useful for all of us and that there will be more "white boxes" in the future than "Black Boxes". I thank all participants in this panel discussion.

Session : V. Panel discussion.

Comment : S.Bonotto

Text of comment
or author's answer :

I would emphasize the importance of a better knowledge of ecology, because in nature a complex relationship exists between microorganisms and more organized higher species. To understand the behaviour of radionuclides in the environment and the role of microorganisms, both field and laboratory investigations are useful.
Sterile conditions do not exist in the environment. On the other hand, investigations in the laboratory under sterile conditions, necessary to reveal the specific role of certain microbes.

Session : V. Panel Discussion

Comment by : Förstel

Text of comment
or author's answer :

I. There seem to be difficulties in the studies of the role of microorganisms, which they play in radioecological problems. These barriers may have too reasons :

   1. Microbiology tends to work with single well-defined species on even special genetic types. The results concentrate more and more on biochemical details.

   2. Necessary for these ecological studies are general statements, which necessitate especially adapted methods (e.g.for the total living biomass in the soil, for the biomass of bacteria or fungi). These methods do not exist or are not generally accepted in all cases, which are needed for radioecological considerations.

II. Unfortunately the paper about the role of microorganisms in the root-soil interface has not been presented. The plant roots are an important factor, if one considers the uptake and transport of nuclides in the soil. Plant roots actively can change their environmental conditions, as one can see easily by pH-changes of soil around roots; they change by secretion and absorption of substances. These substances may serve as a substrate for soil organisms, too. Another important part are symbiotic organisms incorporated into roots an attached to the roots.

Concerning the distinction of non-biological and biological processes, it is very difficult to distinguish between "dead" and "living" compounds. Biological processes are biochemical events, that means at least chemical reactions. There is (perhaps) no general difference, and therefore nearly no possiblity to distinguish them in a complex system as,for example soil.

Session : V Panel discussion

Paper by :

Comment by : Bors

Text of comment
or author's answer :

Everybody who investigate the influence of microorganisms on the translocation processes in aquatic or terrestrial system is faced with the problem how to separate biological and nonbiological part of the processes. Nearly all chemical transfer can run without microorganisms too, but much slowlier of course. On the other hand, eliminating microbial growth by biocidal treatment we may produce side effect which may influence the results. May be somebody have a suggestion how to solve this experimentally problem.

CLOSING SESSION

Ladies and Gentlemen,

I am going to speak as a Health Physicist and not as a scientist. This Workshop comes at its end and what are the conclusions ?
The organisms have two roles :

- one direct role as an element of food chain as macroorganism;
- an indirect role as factor of evolution of local conditions : pH, rH, solubility and availability.

The discussions let appear methodological difficulties for the experimental studies, as example : isolation, identification, variety and complexity of microorganisms.
For the future, you have various perspectives for your studies or experiments : Mechanism of transfer through the food chain,effects of the microorganisms on the environmental conditions and conditioned material (e.g.bitume), effect of sewage plant treatment on the transfer of radioactivity to Man. Your experiment field is large but an effort remains to be done in the methods of validity and justification of studies.

Maintenant je vais parler en français car lorsque la politique veut que lorsqu'on s'adresse à la Communauté on parle français, tout au moins en France.

Je vais commencer par remercier mon vieux camarade Myttenaere, sans lequel ce workshop n'aurait pas pu avoir lieu. Je sais toute l'activité qu'il a menée et toutes les difficultés qu'il a du résoudre avec Kirchmann pour que vous soyez tous ici et que tout se passe bien.
Car ce n'est pas simple de réunir 80 personnes et de les faires manger, dormir et en plus travailler !

Je demanderai à Monsieur Myttenaere d'être notre interprête auprès de ses autorités et de les remercier de nous avoir permis de nous réunir ici.

Ensuite je continuerai en français puisque c'est une des langues utilisées en Belgique; je m'adresserai à l'Institut Royal des Sciences Naturelles de Belgique et j'excuserai auprès de vous le Professeur Misonne, Directeur de l'Institut, qui n'a pas pu venir et je demanderai à Monsieur Van der Ben qui a été aussi une des chevilles ouvrières de ce Colloque d'être notre interprête d'abord auprès de son Directeur et de toutes les personnes qui l'ont aidé dans cette phase "expérimentale" qui a précédé ce Colloque et en particulier le Service Educatif dirigé par Monsieur Quintart et qui nous a fait connaître ce Musée. J'ai appris quant à moi que c'est ici qu'il y avait la première collection au monde d'iguanodons, une baleine extraordinaire et autres curiosités.

Monsieur Van der Ben,je vous remercie de ce que vous avez fait ainsi

que les projectionnistes et Monsieur Pitcher qui a servi de courroie de transmission entre le Service de l'Institut et vous-mêmes (diapositives, audiovisuel etc..). Ensuite je vais avoir une opération assez agréable à faire : offrir un bouquet de fleurs à un certain nombre de dames qui nous ont aidés et c'est une manière de les remercier de notre part.

Nous avons d'abord Madame Dubois, chargée de régler vos problèmes de logement et de voyage. Je suis maire dans ma commune et quand je remets un bouquet de fleurs, j'embrasse (applaudissements).

Les personnes suivantes seront Mesdames Arkosi et De Porter qui ont assuré le secrétariat de cette réunion (applaudissements).

Ensuite Madame Van Lommel et Madame Tuaux qui se sont chargées des pauses café (applaudissements).

Enfin les trois dernières personnes qui méritent nos remerciements sont d'abord, Mademoiselle Henrot,traductrice émérite, qui a aussi, avec Monsieur A.Itschert, collationné les fruits de nos discussions. (applaudissements).
Ensuite les deux secrétaires, la première étant Madame Robertz, fidèle et sympathique secrétaire de Monsieur Myttenaere qui a participé comme son patron et peut-être même plus travailler que lui!

Enfin, j'ai gardé pour la bonne bouche, Madame Bonnyns que je connais depuis déjà pas mal de temps qui non seulement a travaillé pour ce colloque mais qui depuis la création de l'Union Internationale des Radioécologistes en assure le secrétariat et qu'il me soit permis aujourd'hui de la remercier tout particulièrement (applaudissements).

Maintenant je vous souhaite à tous de bien rentrer chez vous, vous allez avoir un week-end assez prolongé puisque nous allons avoir la fête du Travail et I declare this Workshop Closed.

P.BOVARD

Président Honoraire
de l'U.I.R.

LIST OF PARTICIPANTS

Belgium

ARAPIS G.,
BIERKENS J.,
BINET J.,
BONOTTO S.,
BOUQUEGNEAU J.M.,
BOURDEAU Ph.
CREMERS A.,
DE BRABANDERE J.,
DE KEIJSER S.,
DESCY J.P.,
HENRION P.,
JANSSEN J.,
KIRCHMANN R.,
LAMBOTTE J.M.,
LECLERE R.,
MAES A.,
MANIA B.,
MERGEAY M.,
MOUREAU Z.,
NUYTS G.,
PIGNOLET L.,
SOMBRE L.,
STALMANS M.,
VAN BAELEN J.,
VAN BRUWAENE R.,
VANDECASTEELE C.,
VAN DER BEN D.,
VANDERBORGHT O.,
VAN ELEMWIJT F.,
VANGENECHTEN J.,
VERTHE C.,

Federal Republic of Germany

BORS J.,
FÖRSTEL H.,
KISTNER G.,
KÜHN W.,
MARTENS R.,
STRACK S.,
WIRTH E.,

France

ABDALLAH M.,
AIT N.,
BALVAY G.,
BITTEL R.,
BOVARD P.,
GUARY J.C.,
LAMBRECHTS A.,
PIERI J.,
SAAS A.,

Italy

BENCO C.,
PERONI C.,
ROSSI L.,

Japan

AMANO M.,
WATABE T.,

Monaco

FISHER N.,

People's Republic of China

ZHOU M.,

Sweden

AGNEDAL P.O.,
CARLSON L.,
ERIKSSON A.,

Switzerland

Mc.KINLEY

The Netherlands

DUURSMA E.,
FRISSEL M.,
NIENHUIS P.,
TACKX M.,

United Kingdom

BULMAN R.A.,
CAWSE P.,
CRISTOFI N.,
HAMILTON E.,
HUGHES M.,
PHILP J.,
POOLE R.,
POWELL B.,
WEST J.,
WHEATON R.,

U.S.A.

AZAM F.,
FRANCIS A.J.,
SIBLEY T.,

Commission of the European Communities

GERBER G.,
HAIJTINK B.,
MYTTENAERE C.,
WISTUBA C.,

International Union of Radioecologists

AGNEDAL P.O.,
BOVARD P.,
KIRCHMANN R.,
MYTTENAERE C.,