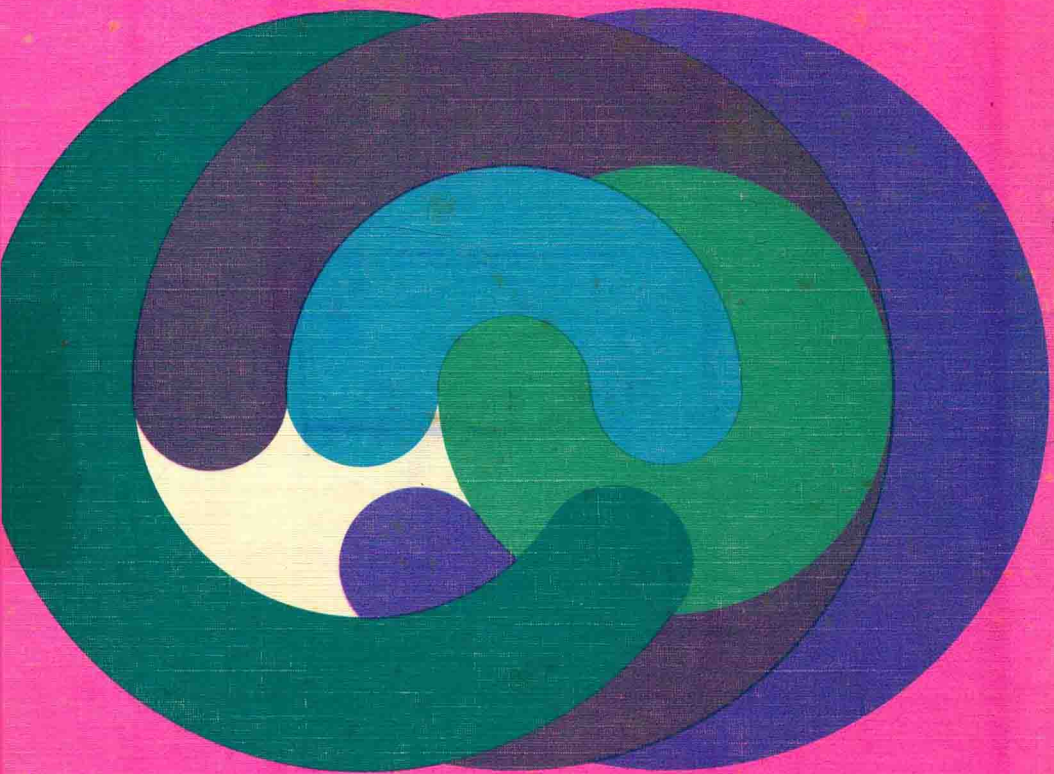
Fundamental aspects of pollution control and environmental science 6

MICROBIOLOGICAL ASPECTS OF POLLUTION CONTROL

SECOND EDITION

R.K. DART and R.J. STRETTON



Fundamental Aspects of Pollution Control and Environmental Science 6

MICROBIOLOGICAL ASPECTS OF POLLUTION CONTROL

SECOND EDITION

A completely revised edition of volume 2 of the series

R.K. DART and R.J. STRETTON

University of Technology
Loughborough (Great Britain)

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
Amsterdam — Oxford — New York 1980

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
335 Jan van Galenstraat
P.O. Box 211, Amsterdam, The Netherlands

Distributors for the United States and Canada:

ELSEVIER NORTH-HOLLAND INC.
52 Vanderbilt Avenue
New York, N.Y. 10017

ISBN: 0-444-41611-0 (series)
ISBN: 0-444-41918-7 (vol. 6)

Printed in The Netherlands

PREFACE TO SECOND EDITION

The past three years have seen no spectacular advances in the use of micro-organisms in pollution control. There has been consolidation and an increase in the number of examples of the role of micro-organisms in man made and natural problems.

In this edition new information has been included in each chapter and most have been substantially rewritten. We have again attempted to make each chapter self contained with an adequate bibliography of primary references.

We still consider that the systems for dealing with effluent are designed by engineers with little or no consideration given to the fact that micro-organisms are the essential components of the processes. Our emphasis has remained on the microbiological aspects of the problems and possible solutions.

Our grateful thanks are due to Mrs. M. Critchlow for typing this edition.

R. K. Dart
R. J. Stretton

June 1980

INTRODUCTION

This book has been written in an attempt to explain the use of micro-organisms in removing pollutants from the environment, and the ways in which they can be utilized to recycle essential materials.

However, micro-organisms can play a role, both advantageous and disadvantageous, to man and his activities. They may be pollutants themselves and cause disease, or they may cause pollution if their presence is encouraged.

In many industrial and engineering processes, it is desirable that engineers have some understanding of micro-organisms, so that full use may be made of their potential and any adverse effects avoided. In some cases the engineer may not immediately recognize the process he has designed because our emphasis has been on the microbiological aspects of the subject and not the processing aspects.

Because many engineers will be unfamiliar with some of the subject areas, we have tried to refer to primary sources of information and to treat each chapter as a self-contained review. This has meant some slight repetition, but as little as possible.

The book covers a wide range of subjects and includes practical aspects such as sewage treatment, and also aspects which at the present moment are mainly theoretical, for example the genetic aspects of bio-degradation of pesticides.

The most recent types of pollution are the production of new materials which cannot be broken down by natural reactions e.g. detergents and chlorinated hydrocarbons. An understanding of the biochemical capabilities of micro-organisms can help in the production of compounds which are both effective and bio-degradable e.g. synthetic detergents with biodegradable straight chain alkyl groups instead of non-biodegradable branched chain compounds.

A knowledge of the way in which diseases are spread has led to certain public health and hygiene measures being taken which have doubled the life expectancy in the United Kingdom since 1800. This has, however, meant a loss in other areas e.g. the correct treatment of human excreta required with increased urbanization has resulted in a net loss of calcium and iron from the soil to the rivers and sea.

However, if the biochemical capabilities of micro-organisms are maximized, the waste can often be disposed of by utilization, e.g. paper can be hydrolysed to give sugars, which in turn can be fermented to ethanol by yeasts. Similarly, petroleum waste can be utilized to give yeasts for use as single cell protein for cattle feed. We have tried where appropriate to consider possible new ideas, even if these are only at the level of theoretical consideration.

R.K. Dart
R.J. Stretton

CONTENTS

		Page
	PREFACE TO THE SECOND EDITION	V
	INTRODUCTION	VII
1.	MICROBIAL PRODUCTION OF POLLUTANTS	1
2.	AIR POLLUTION AND MICRO-ORGANISMS	32
3.	HEALTH HAZARDS ARISING FROM WATER-BORNE PATHOGENS	54
4.	WATER TESTING	75
5.	SEWAGE TREATMENT	118
6.	DISINFECTION AND RECYCLING OF WATER	158
7.	EUTROPHICATION	169
8.	THERMAL POLLUTION	185
9.	THE SULPHUR CYCLE AND WASTE RECOVERY	192
10.	OIL POLLUTION	202
11.	BIODEGRADATION	216
	INDEX	257

MICROBIAL PRODUCTION OF POLLUTANTS

In the technologically advanced countries water pollution rarely means contamination with potentially pathogenic bacteria, viruses, protozoa or metazoa, but a supply which contains unwanted chemicals. However, micro-organisms may still cause pollution, to a modest or serious level, and this can come from the growth of free living hetero- or auto-trophic micro-organisms which produce undesirable or toxic metabolites. The production of chemical pollutants affects the quality of water, or changes conditions in the soil or atmosphere. Similarly, food for human or animal consumption can become contaminated with toxic microbial metabolites, and made unfit for consumption.

Metabolism of Metals

i) Mercury

The chief raw material for the commercial production of mercury is cinnabar (mercuric sulphide) from which the metal is obtained by heating and condensation, in a state pure enough for most purposes. Mercury occurs naturally in the sea, at levels of 0.1-0.27 µg/l in the Pacific, the concentration increasing slightly with depth. The oceans hold about 2×10^8 metric tons of mercury. In ocean waters, mercury is thought to exist as the complex anion, $HgCl_4^{2-}$; in this form it does not appear to collect in bottom deposits, as it does in fresh waters. Lower values of 0.01-0.02 µg/l have been reported for the Solent and English Channel.

There is little data available on unpolluted fresh waters, but the concentration resulting from weathering of rocks is less than 0.1 µg/l, unless there are mercury deposits in the area. River water may contain 0.01-1.4 µg/l, sewage 0.07-2.2 µg/l, sewage effluents 0.2-1.3 µg/l and sewage sludges 0.03-0.75 µg/kg dry weight. The level of mercury detected in fresh water may not be a good indication of the contamination level, because mercury is concentrated in bottom deposits, where it may remain available for many years for uptake by aquatic organisms.

Mercury can also enter surface waters in waste discharged from industrial processes, where it is used mainly as the metal in electrical apparatus, and as cathodes in the manufacture of chlorine and caustic soda. It is also used in the manufacture of vinyl chloride and urethane plastics. Paper and pulp industries use large quantities of phenyl mercuric nitrate which adheres to

particles discharged into waterways and is deposited as sediment. The St. Claire River system has received about 20,000 lb. of mercury from industrial activity over a twenty year period [1]. Mercury compounds are also used in paints and as agricultural biocides. There may also be contamination of surface waters as a result of atmospheric pollution following the burning of fossil fuels, and the scrubbing of mercury vapour and methyl mercury from the air by rain. Human activities add about 12,500 tons of mercury per year to the system through mining and industrial activities.

Concern over the environmental behaviour of mercury started following an incident in the Minamata Bay area of Japan. During the period from 1953 to 1960, 116 people were poisoned irreversibly and 43 died from eating fish and shell-fish contaminated with mercury, which came from a vinyl chloride producing factory. There was a similar incident, involving vinyl chloride production in Niigata, where 120 people were poisoned and there were 5 deaths following the consumption of fish. In Sweden during the mid-1960's, mercury discharged in industrial waste either as inorganic compounds, or as phenyl mercury, accumulated as methyl mercury in fish in certain lakes and the resulting high concentrations caused the authorities to prohibit the sale of fish. There was also a decrease in the bird population in Sweden, which was associated with the use of methyl mercury dicyandiamide as a fungicide.

After the demonstration that mercury in fish was present predominantly in the form of methyl mercury, it was shown that unidentified micro-organisms in the natural organic sediment of lakes could methylate mercury. The net result of the process could be mono- or di-methyl mercury, the rate of biological methylation being correlated to the microbiological activity of the sediment. There have been several hypotheses [2] advanced for the mechanism of methylation, but the process is not completely understood. Using a cell-free extract of a methanogenic bacterium, non-enzymatic methylation of mercury has been shown, with methylcobalamin as the methyl donor, in the presence of ATP and a mild reducing agent.

Mercury can be methylated in a neutral aqueous solution by a purely non-biological reaction, where the methyl donor is methylcobalamin, the reaction being fast and almost quantitative, under both aerobic and anaerobic conditions (Fig. 1 and 2). However, it has been demonstrated that microbial activity is required for methyl mercury synthesis under natural conditions, unless other methyl metal compounds, e.g. methyl tin, are added. Bacteria have been isolated from mucous material in fish [3] which will methylate mercury, and methylation in the gills may account for the presence of methyl mercury in deep sea fish.

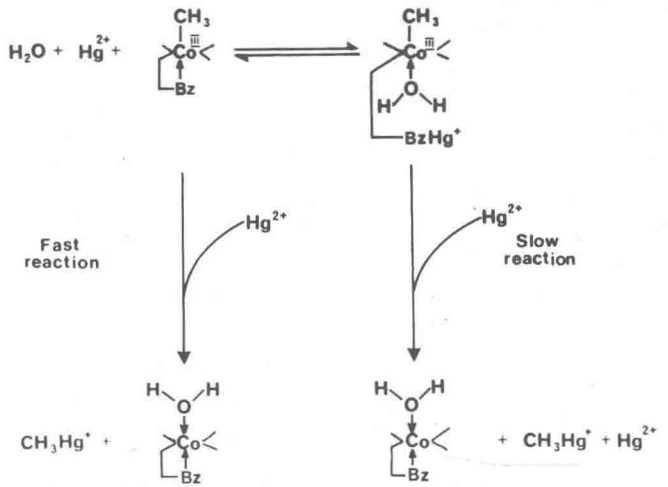


Fig. 1. Proposed aerobic methylation of mercury by methyl corrinoid under non-enzymatic conditions.

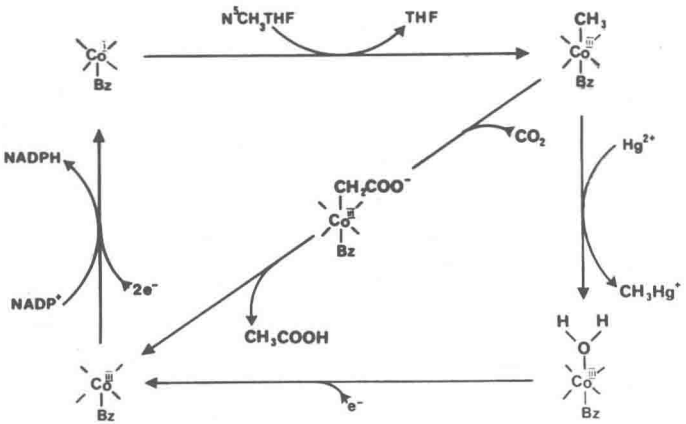


Fig. 2. Possible anaerobic mechanism of mercury methylation in methylcobalamin-acetate synthetase system. (THF = the coenzyme tetrahydrofolic acid).

Pseudomonas species from soil can methylate mercury under aerobic conditions [4]. Mercury tolerant mutants of Neurospora crassa can methylate mercury under aerobic conditions [5] when an excess of cysteine or homocysteine is present and methylation may be an incorrect synthesis of the amino acid, methionine. In addition, when mercuric chloride was added to five bacterial species and three fungal cultures, methyl mercury was produced under aerobic conditions [6].

The microbial conversion of inorganic mercury compounds into methyl mercury can take place in the soil under anaerobic conditions. For example, Clostridium cochlearium has a high capacity for methylation of inorganic mercury in the presence of cysteine and vitamin B_{12}. The methyl mercury compounds formed were decomposed by a mercury-resistant Pseudomonas [7].

Although the ability of micro-organisms to methylate mercury has been shown to exist under both aerobic and anaerobic conditions, the ecological importance of these observations is difficult to determine, as methyl-cobalamin is known to be unstable in natural environments. In addition, trans-methylating activity is inhibited in vitro by cellular proteins and thiol groups. Anaerobic methylation may not be of ecological significance because mercury is hardly ever present in nature without hydrogen sulphide also being present, therefore mercuric sulphide is likely to be formed. This may explain the absence of methyl mercury in anaerobic mud in certain experiments [8]. The ability of micro-organisms to demethylate mercury may also complicate experiments.

Anaerobic sediments treated with ionic mercury release elemental mercury. Four strains of bacteria capable of converting the methyl mercury cation to methane have been isolated from lake sediment and E. coli can convert $HgCl_2$ to elemental mercury [9]. Elemental mercury vapour and benzene were products of phenyl mercuric acetate degradation by cultures of mercury resistant Pseudomonas sp. [10].

Hamdy and Noyes [11] showed that a strain of Enterobacter aerogenes was resistant to 1,200 µg Hg^{2+}/ml and could produce methyl mercury from mercuric chloride, but could not produce voltatile elemental mercury. The amount of methyl mercury produced was decreased if DL-homocysteine was present in the growth medium. The production of methyl mercury was postulated as a detoxification mechanism.

Methyl mercury may be produced by a totally abiotic mechanism without the involvement of micro-organisms or their metabolic products. Up to 3% per day of mercuric acetate can be converted to methyl mercury by ultraviolet radiation [11a]. This rate of conversion exceeds that of microbial activity. However, the process is inhibited by the addition of mercuric chloride and acetic acid instead of mercuric acetate, and this could preclude the photochemical methylation of mercury in sea water.

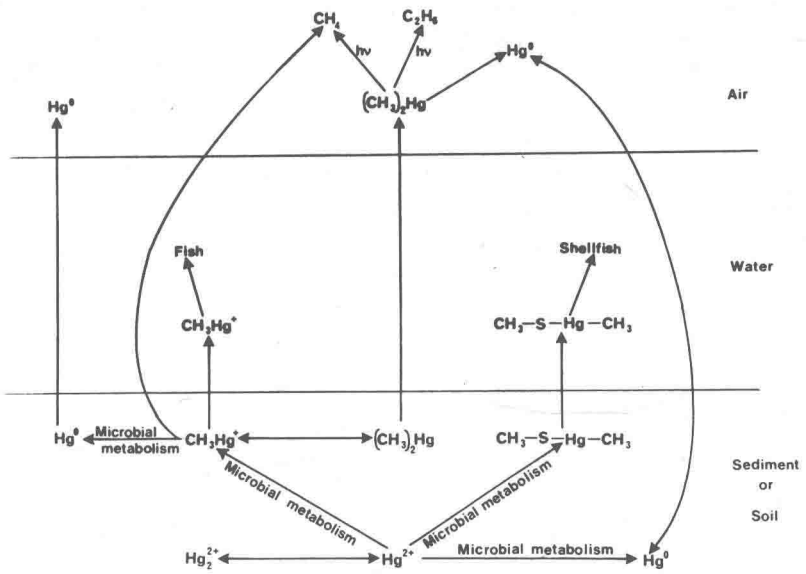


Fig. 3. Mercury cycle.

Methyl mercury is used as a denaturing agent in studies of nucleic acid structure and exposure to the levels produced may be unacceptably high [12]. This may constitute a hazard to people examining the biochemistry of mercury transformations in the laboratory.

Microbial resistance to mercury and organomercurial compounds

The earliest reports of the presence of bacteria resistant to mercury and organomercurial compounds came from Japan, when resistant bacteria were isolated from soil polluted with organomercurial compounds [13]. It was also found that many of the antibiotic resistance plasmids in both Gram-negative and Gram-positive bacteria have the genes to determine resistance to Hg^{2+} [14] and occasionally to organomercurials. Among hospital isolates of enteric bacteria the frequency of mercury resistance has ranged from 25% to nearly 60% [15]. The resistance to mercury is inducible and resistance to any one of the organo-mercurial compounds confers resistance to all. Plasmids confer resistance to

phenylmercuric acetate, ethyl mercuric thiosalicylate, methylmercuric chloride, ethyl mercuric chloride, p-hydroxymercuribenzoate, mercurochrome, and fluorescein mercuric acetate [16]. The basic mechanism of resistance appears to be conversion of Hg^{2+} to Hg^0 [17]:-

$$CH_3Hg^+ \xrightarrow{\text{lyase}} CH_4\uparrow + Hg^{2+} \xrightarrow{FAD} RSHg^+ \text{ or } RSHgSR \longrightarrow Hg^0$$

Pseudomonas sp. may be of possible use in removing mercury by volatalisation from effluents before they are discharged into bodies of water.

ii) Arsenic

Arsenic has a long history as a poison, being toxic to humans and animals with a central nervous system, to most higher plants and certain lower organisms. The inorganic ion arsenite (3^+) is more toxic than arsenate (5^+), and the volatile timethylarsine, $(CH_3)_3As$, is also toxic to humans. There have been cases of poisoning from drinking water containing 0.2 p.p.m. arsenic, whilst in the U.S.A. the recommended maximum for drinking water is 0.01 p.p.m. and the maximum permitted level 0.05 p.p.m.

Pattison [18] showed that detergent formulations which contain phosphate may have 70-80 p.p.m. arsenic, and the wash water into which these are introduced may have a level of 0.15 p.p.m. arsenic. Arsenite was used for the control of aquatic vegetation and organic arsenicals are still used as herbicides. Lead and calcium arsenates were commonly used as insecticides before 1960. Unlike mercury there is no evidence that arsenic bioaccumulates.

The microbial transformation of arsenic compounds was noticed in human poisoning cases, which occurred in rooms papered with wallpapers using arsenic containing pigments. Growth of fungi resulted in production of volatile trimethylarsine. Challenger [19] showed that Scopulariopsis brevis could produce trimethylarsine from compounds containing trivalent arsenic. In the B_{12}-dependent synthesis of dimethyl arsine and dimethyl selenide the inorganic salts may function as electrophiles (Fig. 4). This concept has been critically reviewed by Wood et al [20].

Various micro-organisms are capable of synthesising trimethylarsine from industrial and agricultural arsenic containing sludge [21]. Braman and Foreback [22] showed that methylated arsenic compounds were found in most biological materials. The rate of biological formation is probably high enough to compensate for oxidation of alkyl arsenes to arsenious acid. However, arsenious acid could in turn be an intermediate in the formation of methyl arsine compounds.

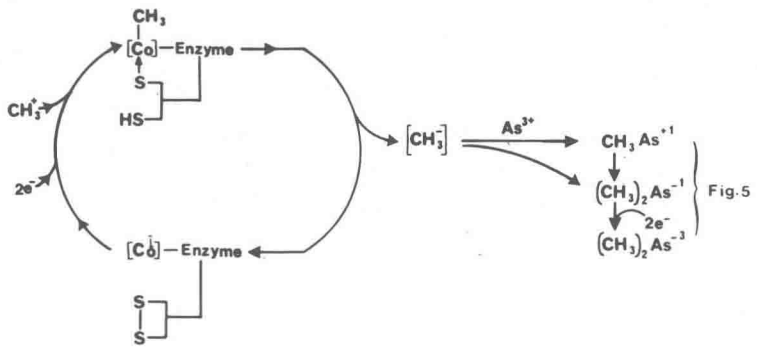
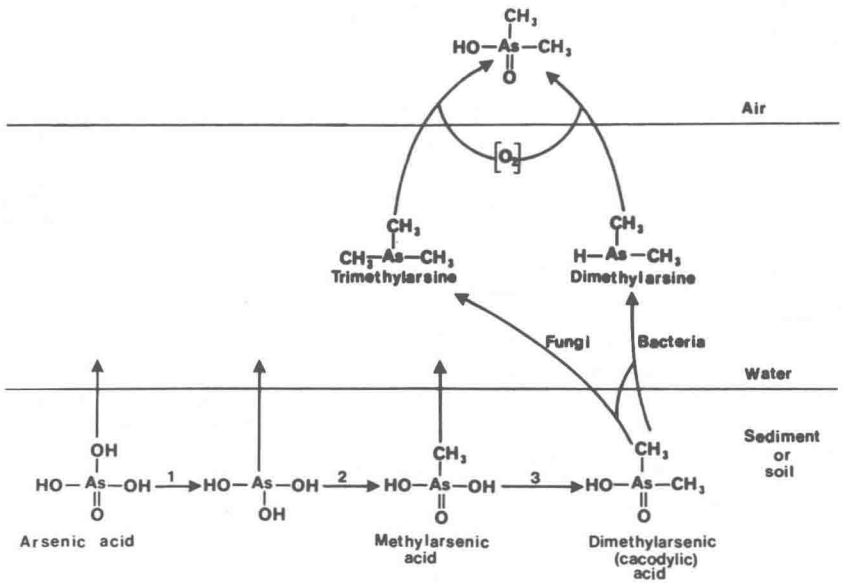


Fig. 4. Proposed mechanism for the thiol-promoted electrophilic attack on the Co-C bond which may be cleaved by the electrophiles (i) H^+ giving methane, (ii) CO_2 giving acetic acid, (iii) arsenic salts giving alkyl arsenic compounds and (iv) selenium compounds giving alkyl selenium compounds.



Stages 1, 2 and 3 carried out by microbial metabolism.

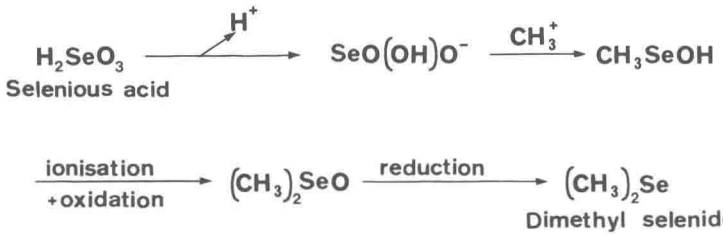
Fig. 5. Arsenic cycle.

The organisms capable of carrying out the transformations of arsenic are not uncommon, for example, Penicillium sp. can generate trimethylarsine from the pesticides monomethylarsenate and dimethylarsenite. The conversion of arsenate to dimethylarsine can be carried out in soil by Methanobacterium and possibly by Desulfovibrio [23]. Also arsenate reduction to the more toxic arsenite can be carried out by Chlorella, Micrococcus and the yeast Pichia guillermondii [24].

iii) Selenium and Tellurium

The oxoanions selenium and tellurium are close relatives of sulphur and are quite toxic to most micro-organisms. Several selenium compounds are toxic, but it is also an essential element for several mammals and possibly man, although there is a small margin of safety. Organic selenium compounds can be converted microbiologically to inorganic products, whilst photosynthetic purple bacteria will oxidize elemental selenium to selenate.

A biological mechanism for the methylation by fungi was proposed early this century, namely:



Biosynthesis of volatile dimethylselenide is a major metabolic pathway for detoxifying selenite in animals, e.g. the rat.

Tellurium is also toxic, but is not required as a trace element by any animal. Tellurite is the most toxic of the two common anions. Tellurium was found to methylate in the presence of different Penicillium strains and the volatile metabolite was identified as dimethyltelluride [25]. The practical significance in environmental contamination of these observations has yet to be determined, and it is not known whether micro-organisms do modify the element in natural ecosystems.

iv) Lead

Lead can be methylated by micro-organisms to give $(CH_3)_4Pb$ [24]. Tetramethyl lead is considerably more toxic to algae than either trimethyl lead acetate or inorganic lead. The ecological importance of organic lead compounds is not established.

v) Transalkylation

Using Pseudomonas, Nelson et al. [27] showed the formation of methyl tin compounds and, in later work, methyl tin and methyl mercury compounds occurred together. The formation of methyl mercury may not be a direct biological methylation, but could be a transalkylation from biologically formed methyl tin compounds [28]. Similarly, methylation of selenium compounds was carried out by Penicillium strains isolated from sewage and added tellurium compounds were also methylated, but only in the presence of selenium [29].

In order for a metal to be of ecological importance in exerting a toxic effect, the metabolized metal must have a tendency for complex formation compared with its original form. The metabolized form should also be soluble in both water and lipid systems, whereas the original compound is usually only soluble in one or the other. The toxic form will also exhibit a changed valency state and volatility compared with the original substrate.

Metabolism of Nitrogen Compounds

i) Ammonia

Robinson and Robbins [30] suggested that the major nitrogenous compound released into the atmosphere is ammonia and almost all of this comes from biological sources, produced mainly by the heterotrophic activity of microorganisms on land and in the sea. The nitrogen produced as ammonia from biological activity is eight times greater than that released as oxides of nitrogen from all sources combined. Ammonia is not only an atmospheric pollutant but its production below ground level can adversely affect plant roots.

Atmospheric ammonia can be absorbed by lakes, rivers, etc. and so give rise to another pollution problem by enriching surface waters as ammonium or nitrate ions (following denitrification), and then be used by algae as a nutrient giving a bloom (see chapter on eutrophication). The cost of treating water supplies can increase because of the reduction in the disinfecting power of chlorine by ammonia. Abeliovich and Azov [31] have shown that ammonia at 2.0 mmol at pH 8.0 is toxic to algae in sewage oxidation ponds.

Ammonia is produced during the decomposition of organic material in soil and the microbial hydrolysis of urea. The rate of loss of ammonia is governed by the type of soil, climatic conditions, the presence of vegetation and the application of nitrogenous fertilizers.

Ammonia formation can be appreciable, and volatilisation occurs when a field is treated with organic nitrogen compounds which can be broken down microbiologically. This is particularly true in the case of urea (a common fertilizer), as there are many urease containing heterotrophs in soil, and the concurrent rise in alkalinity favours volatilisation. MacRae and Ancajas [32] have shown that

if urea is applied directly to the soil surface, there is no time for the ammonia to react with the soil and the loss is very pronounced. This is more marked than if urea is introduced below the surface, when up to 70% of the urea-N can be lost to the atmosphere as ammonia.

There is a high local concentration of nitrogenous material in areas where cattle are intensively reared; the level of ammonia being twentyfold higher compared with distant sites. The significant part arises from manure undergoing decomposition, and especially urine of which 90% may be converted to ammonia and volatilised during a week [33]. Hutchinson and Viets [34] showed that sufficient ammonia was absorbed in a lake 2 km away from a large cattle rearing area, to raise the level above that required for algal bloom formation.

The gas can also be evolved during the decomposition of plant remains in soil, and the breakdown of sewage and other organic material in water during the reduction of nitrate.

ii) Nitrate

Nitrate is the end product of microbial breakdown of organic nitrogen in aerated environments in soil and water. The organic substrates are attacked and the nitrogen is released as ammonium salts. Where oxygen is present and the pH is not too low, the nitrifiers oxidize the ammonium ion to nitrate. This may be leached out by percolating water and could be carried to wells or to surface waters which ultimately may be used for drinking. Because of the increased use of synthetic fertilizers in agriculture, combined with the growth of urban areas and the associated development of industrial centres, there has been an increase in the amount of nitrogenous material for microbial attack concentrated in a smaller area. The levels of nitrate in drinking water have risen to approach toxic levels in several areas. There was concern in East Anglia during the very hot and dry summer of 1976 that the level in drinking water could reach danger level for infants and two water bottling plants were opened to provide supplies for infants at risk.

If the level of nitrate in water rises above 22 p.p.m. [35] there is a risk of methaemoglobinaemia, a disease of infants (particularly those less than six months old) and livestock. A total of 2,000 human cases, many of which were fatal, have been linked with drinking water polluted with nitrate [36]. The World Health Organization have recommended that water for human consumption should contain no more than 10 p.p.m. nitrate-N. In Illinois, where water has been sampled regularly since 1945, the streams contain in excess of 0.3 p.p.m. nitrate-N and in the reservoirs which they feed, the concentration may exceed 10 p.p.m. The area concerned is one in which fertilizers have been widely used [37].