Advances in
MICROBIAL ECOLOGY

Volume 6

Edited by
K. C. Marshall

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Preface

This volume of *Advances in Microbial Ecology* marks a change in the editorship of the series. The Editorial Board wishes to take this opportunity to express its gratitude to Martin Alexander, the founding editor and editor of the first five volumes, for his enterprise in establishing the series and in ensuring that *Advances* has become an outstanding focal point for the identification of new developments in the rapidly expanding field of microbial ecology. With the publication of this volume, we welcome Howard Slater to the Editorial Board.

The policies of the Editorial Board remain the same as before. Most contributions to *Advances in Microbial Ecology* will be solicited by the Board. However, individuals are encouraged to submit outlines of unsolicited contributions to the Board for consideration for inclusion in the series. *Advances* is designed to serve an international audience and to provide critical reviews on basic and applied aspects of microbial ecology.

Contributions in the present volume are predominantly concerned with the ecology of aquatic microorganisms, but encompass a variety of approaches to this area. The exception is the chapter by J. W. Doran on the role of microorganisms in the cycling of selenium. G-Y. Rhee discusses the effects of environmental factors on phytoplankton growth. The factors limiting the productivity of freshwater microbial ecosystems are considered by H. W. Paerl. Problems in assessing biomass and metabolic activity of heterotrophic bacteria is the subject of the review by F. B. van Es and L.-A. Meyer-Reil. In considering nutrient-deficient marine habitats, R. Y. Morita presents evidence for mechanisms related to the survival of heterotrophic bacteria in such extreme conditions. M. Fletcher and K. C. Marshall discuss the role of solid surfaces in the ecology of aquatic bacteria.

K. C. Marshall, Editor
M. Alexander
T. Rosswall
J. H. Slater

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Microorganisms and the Biological Cycling of Selenium

JOHN W. DORAN

1. Introduction

Most studies on the microbial transformations of elements have emphasized nutrient cycling within the biosphere or the economics of agricultural or industrial processes. Cyclic transformations within the biosphere between soluble, insoluble, and gaseous forms of carbon, nitrogen, hydrogen, oxygen, and sulfur are well known. Recently, attention has been focused on the role of microorganisms in the production and degradation of chemicals containing toxic elements (Alexander, 1973; Wood, 1974). Measures to increase animal and food crop production or disposal of waste materials can result in the introduction of elements in amounts harmful to terrestrial and aquatic ecosystems. Many elements and their compounds vary widely in both toxicity and mobility. Consequently, their safe disposal or effective recycling requires an understanding of their potential toxicities and possible transformations in the environment.

Chemically, selenium (Se) is similar to sulfur (S), and both elements are placed in group VIA of the periodic table along with oxygen, tellurium, and polonium. The inorganic forms of Se (Table I) are structurally similar to their S analogs. Selenium and S exist in oxidation states of $+6$, $+4$, 0, and -2, and both have the six-electron system of valence orbitals. Because of its low reduction potential, free selenide is very unstable and is readily oxidized to elemental Se in the presence of O_2; thus, very little exists in soils with pH values below 9–10. The only stable selenides are those bound to metals or in organic

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Table I. Inorganic Forms of Selenium and Their Reduction Potentials[a]

Form	Oxidation state	E_0'	Aqueous form
Selenate	+6	+0.44	SeO_4^{2-}
Selenite	+4	+0.21	SeO_3^{2-}
Elemental selenium	0	−0.73	Se^0
Selenide	−2		HSe^-

[a]Reduction potentials (E_0') at pH 7.5 and unit activity for Se species were calculated from values given by Pourbaix (1966).

combination. Like elemental S, elemental Se occurs in three allotropic forms: "metallic," crystalline, and amorphous. Biological oxidation or reduction of inorganic Se can often be detected by the orange-red color characteristic of the freshly precipitated amorphous form of elemental Se. Except for elemental Se, which is particulate and insoluble, the other forms of inorganic Se, shown in Table I, are anionic and water soluble at pH 7.5.

Selenium is of considerable biological interest because it is required by animals, but it can also be highly toxic. The toxicity of Se was first recognized in the 1930s, when it was established that certain plants growing on high-Se calcareous soils accumulated the element in amounts which could be harmful to animals eating them (Franke, 1934). Moxon (1937) stated that "alkali disease," which plagued many stockmen in the western United States, was a form of Se toxicity. Research reports from other countries have indicated that hazards to the health of humans and domestic animals can result from production of food crops containing toxic Se levels (Lakin, 1972; Shapiro, 1973).

The toxicity of Se compounds varies greatly, but soluble, inorganic forms of Se are generally considered the most toxic of all Se compounds. Selenite, a powerful oxidizing agent, readily denatures sulfhydryl enzymes and oxidizes sulfhydryl groups to form disulfide and unstable selenotrisulfides (RS-Se-SR). The toxicity of selenate is less than that of selenite and is apparently manifested only after it has first been reduced to selenite (Oehme, 1972). Hydrogen selenide, an inhibitor of terminal cytochrome oxidase, is 100 times more toxic than hydrogen cyanide and is considered the most toxic Se compound (Martin, 1973; Painter, 1941). One of the major mechanisms of Se toxicity may be its substitution for S in many proteins and a resultant instability of the Se-substituted compounds (Shrift, 1972).

The toxicity of Se was considered its only role in animal nutrition, until Schwarz and Foltz (1957) discovered that certain Se compounds could replace a dietary factor that prevented severe necrotic degeneration of the liver in rats. Selenium has since been shown to be essential in preventing animal diseases involving tissue integrity and function (Muth et al., 1967; Scott, 1973), to be

a necessary component of several bacterial and mammalian enzyme systems (Stadtman, 1980), and is required for the growth of at least two bacteria (Dürre et al., 1981; Jones and Stadtman, 1977). Although research data are limited, dietary Se has also been implicated in detoxification of heavy metals and prevention of some forms of cancer (Frost, 1972). Many soils do not supply Se to crop plants in amounts adequate to support proper animal nutrition. Today, Se deficiencies in domestic animals are often corrected by injection or supplementation of animal feeds with selenite and selenate. Attempts to eliminate Se deficiencies by prophylactic use of Se compounds were blocked for many years by federal law because inorganic Se had been labeled a carcinogen (Frost, 1960).

2. Selenium in the Environment

2.1. Biogeochemistry

Selenium is considered one of the least plentiful but most toxic elements in the earth's crust (Frost, 1972). The distributions of Se in terrestrial, aquatic, anthropogenic, and extraterrestrial substances are given in Table II. Since Se and S are closely related, their biogeochemistry is similar. The selenide ion substitutes for the sulfide ion, and Se is often concentrated in the major sulfide minerals and pyritic coal deposits (Lakin, 1972). The average Se content of most soils ranges from 0.1 to 2 ppm, but soils derived from parent material formed during the Cretaceous period can contain more than 100 ppm Se. Crop plants grown on high-Se soil can accumulate concentrations of Se (>4 ppm) that are toxic to livestock and humans; however, in many parts of the world, the Se content of vegetation is less than 0.05 ppm and is not sufficient for proper animal nutrition. In the United States, the majority of forage and crop plants contain less than 0.1 ppm Se (Kubota et al., 1967).

The Se content of both fresh and salt waters is much lower than terrestrial materials and usually ranges from 0.05 to 4 ppb. A cursory examination of the data in Table II indicates the marked propensity for food-chain magnification of Se in aquatic ecosystems. Sandholm et al. (1973) reported that some zooplankton can absorb Se as selenite, and phytoplankton can actively concentrate certain organic Se compounds, such as selenomethionine. The same authors commented that fish concentrate little inorganic or organic Se directly from water, but accumulate Se when feeding on Se-containing phytoplankton and zooplankton. Lindström and Rhode (1978) demonstrated a selenium requirement for growth of the dinoflagellate Peridinium cinctum fa. westii. Optimal growth of this photosynthetic alga occurred at a selenite concentration in lake water as low as 0.05 ppm.

Any meaningful discussion of the distribution and biological availability

Table II. Distribution of Selenium in Natural and Anthropogenic Materials

Material	Selenium concentration (ppm Se)	Reference
Terrestrial		
Earth's crust	0.09	Lakin (1972)
Limestone	0.1–14	Rosenfeld and Beath (1964)
Shales and phosphate rocks	<1–55	Lakin (1972)
Crude oil	0.06–0.39	Pillay et al. (1969)
Coal	0.5–11	Pillay et al. (1969)
Soils		
Nonseleniferous	<0.1–2.0	Swaine (1955)
Seleniferous	2–200	Natl. Acad. Sci. (1971)
Vegetation		
Primary selenium accumulators	51–4474	Rosenfeld and Beath (1964)
Grains (seleniferous areas)	0.1–30	Rosenfeld and Beath (1964)
Crop plants (low-Se areas)	<0.05	Allaway (1973)
Fruits and vegetables	<0.01	Allaway (1973)
Blood		
Animals with Se poisoning	1–27	Rosenfeld and Beath (1964)
Human	0.1–0.34	Allaway (1973)
Aquatic		
Ocean waters	0.0001–0.004	Schutz and Turekian (1965)
River waters	0.0001–0.0004	Kharkar et al. (1968)
Aquatic plants	0.02–0.14	Sandholm et al. (1973)
Plankton	1.1–2.4	Sandholm et al. (1973)
Fish	0.5–6.2	Lakin (1972)
Anthropogenic		
Petroleum products	0.15–1.65	Lakin (1972)
Fly ash	1.2–16.5	Gutenmann et al. (1976)
Sewage sludge	1.8–4.8	Furr et al. (1980)
Certain paper products	1.6–19	Lakin (1972)
Extraterrestrial		
Lunar basalts	0.14–0.25	Lakin (1972)
Meteorites	3–15	Rosenfeld and Beath (1964)

of Se in the environment must be directed towards specific compounds or chemical forms of the element (Allaway, 1973). The total Se content of soils is of little use in determining the availability of Se to plants or microorganisms because the chemical forms of Se present in soil vary widely in availability. The forms of Se present in soil include (1) metal selenides, (2) elemental Se, (3) selenite, (4) selenate, and (5) organic Se. The pyritic, heavy metal selenide, and elemental forms of Se are essentially insoluble or very slowly soluble and of limited biological availability. Selenite and selenate, both water-soluble anions, are potentially available for biological uptake. Selenate occurs in appreciable quantities only in the highly oxidizing soils of arid regions (Geering et

al., 1968). Selenite is readily sorbed by iron hydroxide complexes in soils and is less available in acid than basic soils (Cary *et al.*, 1967). Thus, acidic lateritic soils in Hawaii and Puerto Rico, containing up to 30 ppm total Se, usually do not produce vegetation containing Se levels toxic to animals (Bisbjerg, 1972). As in soil, free selenite in water is readily adsorbed by iron hydroxides and manganese oxides. From 30 to 50% of this "sorbed" Se, which is carried in the suspended load of streams, can be desorbed when these waters mix with salt water (Kharkar *et al.*, 1968).

Olson and Moxon (1939) presented data that indicated up to 40% of the total Se of some soils is in the organic form. These same authors also stated that the availability of Se in soils seems to be dependent upon the amount of water-soluble Se, which, in turn, is correlated with the organic fraction of soil Se. Thus, the cycling of Se in soils is apparently related to the mineralization of Se-containing organic matter and the continuous release of soluble Se.

2.2. Mobilization of Selenium

Selenium is mobilized in the environment through natural processes of weathering, disposal of wastes, ore processing, and gaseous emissions to the atmosphere. Significant quantities of Se are currently moving through the food chain in the United States (Allaway, 1973), and the disposal of animal and domestic wastes may represent a significant source of Se to aquatic and ter-restrial ecosystems. The Se contained in such wastes originates from dietary amendments or injections, food and feeds, water, industrial wastes, and from commercial products, like some Se-containing medicated shampoos. The Se contained in sewage sludge is apparently in a form available for biological uptake. Furr *et al.* (1976, 1980) found that the Se contents of eight vegetable crops grown on sludge-amended soils were 2–11 times higher than those from nonamended soil.

Like S, Se is emitted to the atmosphere via volcanic activity and fossil fuel burning. However, unlike S, which is present predominantly as SO_2, the major Se form in these gaseous emissions, as determined from thermodynamic cal-culations and chemical characteristics, is elemental Se (Andren *et al.*, 1975; Suzuoki, 1965). The quantity of Se discharged to the earth's atmosphere from the burning of coal represents 6–11% of the Se mobilized through weathering processes and river flow (Bertine and Goldberg, 1971; Andren *et al.*, 1975). Fly ash, the residue produced at steam generation power plants during the burning of coal, can contain relatively high Se concentrations. Andren *et al.* (1975) estimated that 1.5–2.3 times as much Se is mobilized through disposal of fly ash and slag wastes than by natural weathering and erosion of crustal materials. The addition of fly ash to soil or aquatic habitats can result in increased Se contents in the plants, animals, and other organisms indigenous

to these environments. Gutenmann *et al.* (1976) reported that cabbage plants grown on soils containing 10% fly ash absorbed Se (up to 3.7 ppm) in direct proportion to the Se content of the fly ash. Also, sweetclover plants growing directly on fly ash disposal piles contained over 200 ppm Se. Since most of the Se in fly ash is in the elemental form (Andren *et al.*, 1975), the finding that plants grown on fly-ash-amended soils can contain toxic Se concentrations indicates that the Se originally present was transformed to a more available form. The release of fly ash to the atmosphere through burning of fossil fuels has also been implicated in the food-chain magnification of Se in aquatic ecosystems. Copeland (1970) reported that Se levels in zooplankton in Lake Michigan increased with proximity to metropolitan areas. Selenium levels in zooplankton in most areas of the lake averaged 1 to 2 ppm, but increased to maxima of 3 and 6 ppm in areas directly downwind from Milwaukee and Chicago, respectively.
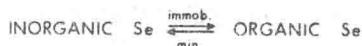
3. Microbial Transformations of Selenium

Selenium appears to be cycled predominantly via biological pathways (Shrift, 1973). The inorganic forms of Se are converted to reduced organic forms by plants, animals, and microorganisms. Microorganisms bring about the decomposition of biological residues and, thus, function as catalysts essential to Se recycling. The major microbial transformations of Se can be divided into three categories: oxidation and reduction, immobilization and mineralization, and methylation. These Se transformations have been categorized to facilitate discussion and, as shown in Fig. 1, are not mutually exclusive because

OXIDATION AND REDUCTION

$$Se^{2-} \rightleftharpoons Se^{\circ} \rightleftharpoons SeO_3^{2-} \rightleftharpoons SeO_4^{2-}$$
selenide   elemental   selenite   selenate

IMMOBILIZATION AND MINERALIZATION

$$INORGANIC \ Se \underset{min.}{\overset{immob.}{\rightleftharpoons}} ORGANIC \ Se$$

METHYLATION

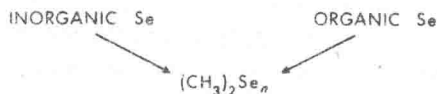INORGANIC Se   ORGANIC Se

$(CH_3)_2 Se_n$

Figure 1. Microbial transformations of selenium.

some overlap occurs. Many microorganisms have the capacity to reduce oxidized compounds, whereas a smaller number have been demonstrated to carry out the oxidation of inorganic Se. Because Se becomes incorporated into the cells of organisms in forms unavailable for uptake and metabolism by most plants and animals, the microbial mineralization of organic forms can regulate Se availability. The microbial methylation of Se represents both reduction and immobilization of Se and may be an important source of atmospheric Se.

3.1. Oxidation and Reduction of Inorganic Selenium

Because of the amount of Se that is assimilated into organic materials in plants, animals, and microorganisms, obviously a mechanism for recycling to the more-available oxidized forms of inorganic Se must exist. The energy available from the oxidation of inorganic Se would be sufficient to serve as an energy source for microorganisms; however, unlike S, the microbial oxidation of inorganic Se has received only limited investigation. Two microorganisms, an aerobic soil bacterium (Lipman and Waksman, 1923) and a purple sulfur bacterium (Saposnikov, 1937), were reported to have used the oxidation of elemental Se to selenic acid (H_2SeO_4) as a sole source of energy. Torma and Habashi (1972) reported that a strain of *Thiobacillus ferroxidans* derived its energy from the oxidation of copper selenide, with the resultant products being cupric ions and elemental Se. Limited research indicates that microorganisms are active in the oxidation of elemental Se and selenite in soils (Bisbjerg, 1972; Geering et al., 1968). Recently, a strain of *Bacillus megaterium*, a heterotrophic bacterium isolated from soil, was found to oxidize elemental Se to selenite in laboratory cultures (Sarathchandra and Watkinson, 1981). Unfortunately, these brief accounts of microbial oxidation of Se were not followed by more extensive studies.
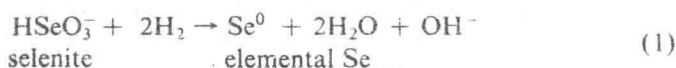
The microbial reduction of oxidized, inorganic Se compounds usually results in incorporation of selenide into organic Se compounds or the formation of elemental Se. The immobilization of Se will be discussed in the next section, and only transformations to more reduced inorganic forms will be discussed here. Many fungi, actinomycetes, and bacteria are capable of reducing inorganic Se salts. The reduced Se usually appears as red intracellular deposits, and several reports indicate that amorphous elemental Se is the final product in microbial reduction of selenite and selenate (Falcone and Nickerson, 1963; Levine, 1925; Zalokar, 1953). The reduction of selenite to elemental Se is reportedly a detoxification mechanism which enables *Salmonella* to tolerate higher concentrations of selenite than other microorganisms (McCready et al., 1966).

Levine (1925) studied the reducing properties of several microorganisms and concluded that the reduction of selenate and selenite is an intracellular
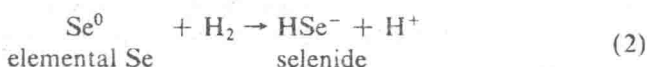
phenomenon and a function of growth. Fewer microorganisms could reduce selenate, and the formation of elemental Se was slower from selenate than from selenite. Thus, it was concluded the reduction of selenate is a two-step process in which selenite is an intermediate product. McCready *et al.* (1966) presented data indicating the presence of an intermediate Se compound in the reduction of selenite to elemental Se by *Salmonella heidelberg.* This intermediate Se form was reported to be an inorganic compound containing Se in the divalent state and was more toxic to the organism than selenite, its tetravalent precursor.

The reduction of both selenate and selenite is enzymatic and metabolically driven. Zalokar (1953) found that only living cells of *Neurospora crassa* could reduce selenite, and the reduction was inhibited by compounds that were known poisons of respiratory enzymes. Woolfolk and Whiteley (1962) studied the reduction of selenite by cell-free extracts of *Micrococcus lactilyticus* incubated in hydrogen atmosphere. Measurements of hydrogen uptake (manometric) and rate of formation of elemental Se and selenide suggested that the reduction involves two steps (equations 1 and 2):

$$HSeO_3^- + 2H_2 \rightarrow Se^0 + 2H_2O + OH^- \tag{1}$$
$$\text{selenite} \qquad\qquad \text{elemental Se}$$

and

$$Se^0 + H_2 \rightarrow HSe^- + H^+ \tag{2}$$
$$\text{elemental Se} \qquad \text{selenide}$$

The overall reduction of selenite to selenide was quantitative and involved a transfer of six electrons. The same authors demonstrated that hydrogenase-containing extracts of *Desulfovibrio desulfuricans* and *Clostridium pasteurianum* also utilized hydrogen to catalyze the reduction of selenite. Nickerson and Falcone (1963) also found that a specific dehydrogenase was involved in the reduction of selenite by yeast, and a NADPH-dependent reduction of selenite to elemental Se in the presence of glutathione reductase (isolated from yeast) has also been demonstrated (Ganther, 1974). Less information is available on the enzymatic reduction of selenate. In bacteria and yeasts, the enzyme responsible for the first step in the assimilative reduction of sulfate is also active on selenate. Wilson and Bandurski (1958) demonstrated that the ATP sulfurylase isolated from yeast also forms adenosine-5'-phosphoselenate (APSe) when incubated with selenate.

In soil, the reduction of oxidized forms of inorganic Se is often related to microbial activity. Some fungi and a large proportion of actinomycetes and bacteria isolated from soil can reduce selenate and selenite to elemental Se