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On the Nature of Some Selenium Losses from Soils and Waters

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ON THE NATURE OF SOME SELENIUM LOSSES FROM SOILS AND WATERS

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Selenium is of interest to the animal nutritionist and to the biochemist from two standpoints. In some areas of the world it occurs in soils in excesses, and the plants growing in these soils contain levels of the element that are toxic to livestock that consume the plants. On the other hand, selenium appears to be essential to the well-being of certain animals, at least under some feeding regimens, and in a number of areas the soils produce feeds that are deficient in the element (48).

In either case, an understanding of the cycling of selenium in nature is essential to the explanation of why certain soils produce plants deficient in the element while others produce plants which contain an excess. Such an explanation should be helpful, not only in the matter of mapping these areas, but also in terms of managing the lands for optimum production. Reviews by Allaway et al. (2) and by Olson (41) have dealt with the cycling of selenium in nature and have pointed to the need for further investigation of certain phases of it. The possible role of micro-organisms, for instance, has not been given adequate consideration.

In part, having no adequate analytical method has contributed to the deficiency of information on this matter,

the methods used until recently lacking the sensitivity and perhaps the accuracy required for this type of work. Recent advances in methods for the analysis of biological materials for selenium have opened the possibilities for further work, and it was the purpose of the investigations discussed here to study and adapt the newer methods of use in preliminary studies to evaluate the possible role of microorganisms in cycling selenium in soils and waters. The radioisotope, Se^{75}, while extremely helpful in certain types of studies, cannot be substituted for the many forms of selenium that occur naturally in soils.

REVIEW OF LITERATURE

Methods of Analysis. A large number of methods for selenium analysis have been reported in the literature. Of these, however, only a few have been found satisfactory and have been used to any great extent for the analysis of biological materials, soils, or waters.

The first extensively used method was that of Robinson et al. (47). This method involved a Kjeldahl digestion in a system which allowed for the trapping of volatile selenium in a bromine-hydrobromic acid solution, distillation of the selenium as the tetrabromide, its precipitation by reduction, and quantitation by comparing the red turbidity with that of standards by visual means.

Klein (30) improved on the above method by using a HNO_3-H_2SO_4 mixture containing mercury for digestion and by the use of the Norris and Fay (39) method of titration for quantitation. His method eliminated some of the problems with bromine fumes encountered in the method of Robinson et al. and gave improved accuracy and sensitivity. It was used for many years in studies on selenium toxicity, although it lacked the sensitivity for certain types of work. Especially following the work of Schwarz et al. (50) on the use of selenium in the cure of exudative diathesis and subsequent studies on selenium as a possible essential nutrient, the development of more sensitive methods of

analysis was greatly stimulated.

Present chemical methods are based upon the reaction of selenious acid with aromatic o-diamines, the two most commonly used being 3,3'- diaminobenzidine (DAB) and 2,3-diaminonaphthalene (DAN). The piazselenols formed have a bright yellow color and also fluoresce on irradiation with ultraviolet light. They can be measured spectrophotometrically or fluorometrically.

Reaction of selenious acid with an excess of DAB to form 3',4'-diaminophenylpiazselenol has been adapted for spectrophotometric (12, 16, 17, 25, 26) and fluorometric (44, 53) determinations of submicrogram quantities of selenium. For the fluorometric determination, which is the more sensitive, DAB has two disadvantages. First, its piazselenol has a comparatively low fluorescence. Secondly, DAB contains two pairs of o-diamine groups, only one of which reacts with selenium under the conditions used in the analysis. The resulting piazselenol is still comparatively basic and can be extracted from aqueous solution into organic solvent only when the pH of the water solution is 7 or above (45). Extraction into an organic solvent is an essential step in the method, and increasing the pH to the level required complicates the procedure, especially when certain metal ions are present.

The use of DAN for a spectrophotometric selenium

analysis was reported by Milazzo et al. (37) and for fluorometric analysis by Parker and Harvey (45) in 1962. The work of these two groups indicated no interference by a number of ions. Cukor et al. (15) used a DAN method for the analysis of plant materials following destruction of the organic matter by oxygen flask combustion. An isotope dilution technique was used for correcting for incomplete yields. Only Cr^{+3}, Sb^{+3}, and Sn^{+4} interfered.

Allaway and Cary (1) also used oxygen flask combustion for preparing samples for fluorometric analysis of selenium in biological materials by a DAN method. They incorporated the coprecipitation of selenium with a large amount of arsenic described by Cousins (14) as a means of overcoming interferences. A more recent method using DAN was reported by Watkinson (54) in 1966. Either an oxygen bomb or a wet digestion with nitric acid and perchloric acid was used for destroying the organic matter. This method incorporates many of the advantages of methods previously described and it was adapted for use in the studies described here because of this and of its relative simplicity, its sensitivity, and its apparent versatility. A somewhat modified version of it will be described later.

Wet digestion of samples for the removal of organic matter has been investigated. Apparently, H_2SO_4-HNO_3 digestion can result in significant losses of selenium while

HNO_3-$HClO_4$ digestion does not (23). The latter mixture has been most frequently used in recent work on selenium analysis. Cummins et al. (16, 17), however, used a sodium molybdate-sulfuric acid mixture with good results.

Metallic ions can interfere in either the DAB or DAN methods of selenium analysis. Fluoride, phosphate, oxalate (26) or ethylenediaminetetraacetic acid (EDTA) additions (12), coprecipitation of selenium with arsenic (1), extraction of the selenium complex with dithiol (13,53), and cation exchange resin treatment (35) have all been used to prevent the interference. Of these methods, the use of EDTA is most common. Hydroxylamine has been used to prevent oxidation of selenite to selenate or of the DAN in the reaction mixture (1).

Radioactivation has recently been used for the nondestructive analysis of biological materials for selenium (5, 40, 52, 55). Since facilities for this type of analysis are not widely available, this method is not commonly used. It has, however, been used for the analysis of samples for the purpose of comparison in the development of methods. The method is very sensitive, and coupled with the isolation of the radioactive selenium with added carrier it eliminates interferences, at least in most biological samples. Its use on soils has not as yet been studied in any detail.

Soil Studies. Olson (41) has reviewed the selenium cycle in nature. He points out the need for research to establish the possible role of microorganisms in altering the chemical form of the element in soils, thereby making it more soluble and available to plants and subject to leaching, or causing its loss by volatilization.

A number of studies with biological systems have pointed to the probability that soil microorganisms may play an important role in the selenium cycle in soils. For instance, although Knight and Beath (31) doubted that bacterial action had any appreciable effect in converting the highly insoluble forms of selenium in soils to a soluble form, Lipman and Waksman (34) had as early as 1923 found that a bacillus species could convert elemental selenium to selenic acid much like Thiobacillus thioxidans oxidizes elemental sulfur to sulfuric acid. In addition, they presented some evidence that on adding elemental selenium to soil it was oxidized to a soluble form through bacterial action. Subsequently, Sapozhnikov (49) found elemental selenium to be oxidized to selenic acid during the photosynthetic reduction of carbon dioxide by a purple bacterium.

The production of volatile from inorganic selenium compounds was first reported by Hofmeister (24) during the late 19th century. Dogs injected with selenite had a garlic odor to their breath and he suggested that this was due to

the formation and exhalation of dimethyl selenide. This phenomenon in animals has been quite thoroughly investigated (29, 46. See also 48) and the work of McConnell et al. (36) established that dimethyl selenide is a major, if not the only, form of the element exhaled. The work of Challenger and his associates (4, 10, 11, 18) has demonstrated the ability of certain fungi to methylate selenium, forming volatile compounds. Scopulariopsis brevicaulis, Aspergillus niger, and Penicillium notatum were all found capable of doing this. Gasio (21) also found that Penicillium brevicaule volatilized selenium, producing a mercaptan-like odor. The work of Ganje and Whitehead (20) illustrates the probability that the production of volatile selenium compounds by microorganisms in soil may be of considerable importance in the cycling of selenium in nature. Using inorganic salts of Se^{75}, they found that almost no selenium was volatilized over a two month period from sterilized mixtures containing Pierre shale, vermiculite, Vienna loam, and organic matter. On the other hand, considerable selenium was volatilized from similar mixtures which had not been sterilized. The conditions under which they ran their experiments resulted in considerable mold growth in the unsterilized mixtures (Personal communication, E. I. Whitehead) and molds may well have been the cause of the volatilization.

In 1925, Levine (33) reported the reduction of selenate to elemental selenium by bacteria. Zalokar (57) reported the deposition of elemental selenium inside the mycelium of Neurospora species grown on a medium containing selenite. Falcone et al. (19) found that yeast reduced selenite to elemental selenium at pH 7. In addition to this type of reduction, the incorporation of inorganic selenium into selenium analogs of sulfur amino acids by microorganisms (51) must be considered as possibly important in changing the chemical form of selenium within soils.

Selenium in Waters. While there have been isolated reports of waters of relatively high selenium contents, in most instances the values reported have been very low. Some of the higher values that have been reported are shown in Table I. Usually, however, the selenium contents of river waters (9, 32, 56), the oceans (9, 22, 27, 28), and well waters (Unpublished data, South Dakota Agricultural Experiment Station) have been found to be less than 0.05 ppm. Stream and dam waters from eight locations on a seleniferous ranch in South Dakota were recently found to contain less than 0.005 ppm of selenium in all cases (Analyzed by O. E. Olson).

Byers (8) and Byers et al. (9) explain the low selenium content of waters as the result of precipitation with iron

TABLE I. SOME REPORTS OF WATERS OF HIGH SELENIUM CONTENTS

Kind of Water	State	Selenium Content (ppm)	Reference
Irrigation drainage water	South Dakota	1.2	6
Irrigation drainage water	Colorado	2.68	56
Irrigation drainage water	Colorado	1.98	56
Irrigation drainage water	Colorado	1.05	56
Irrigation drainage water	Colorado	0.70	56
Irrigation drainage water	Colorado	0.63	56
Irrigation drainage water	Colorado	0.32	56
River water below irrigated area	Colorado	0.22	56
River water below irrigated area	New Mexico	0.4	9
Shallow well water	Nevada	0.6	7
Spring water	Wyoming	1.6	3
Intermittent spring water	South Dakota	0.4	38

hydroxide. These workers found that the presence of iron oxide in clay reduced the solubility of selenate and especially of selenite in clay soils. On adding ferric chloride to a selenite solution at the proper pH, a basic ferric selenite of variable composition precipitated. It was concluded that the removal of selenium from natural waters by precipitation in this form was quite likely to occur. On the other hand, the removal of selenium by microbial activity offers another explanation, at least under certain conditions, and this must be considered.

MATERIALS

The chemicals and solutions used in the analytical work were as follows:

Concentrated HNO_3 (Fisher ACS nitric acid, redistilled from glass).

10% HCl: 10 ml concentrated hydrochloric acid (Mallinckrodt AR, ACS) made to 100 ml with water.

0.1 N HCl: 8.5 ml concentrated hydrochloric acid (Mallinckrodt AR, ACS) made to 1000 ml with water.

5 N NH_4OH: 34 ml concentrated ammonium hydroxide (Mallinckrodt AR, ACS) made to 100 ml with water.

Cresol red indicator: 0.01 g o-cresolsulfonphthalein (Eastman Kodak Company) dissolved in 10 ml water containing 1 drop 50% NaOH solution and then made to 50 ml with water.

EDTA-NH_2OH solution: 5 N NH_4OH added to 3.65 g ethylenediaminetetraacetic acid (Mallinckrodt AR, ACS) in 25 ml water until solution accomplished. 12.5 g hydroxylamine hydrochloride (Mallinckrodt AR, ACS) added and mixture made to 500 ml with water.

Decalin (Eastman Kodak Company decahydronaphthalene, BP 54-56°C at 5 mm Hg, less than 0.01%

tetralin).

0.1% DAN: Prepared under semi-darkened conditions. A 0.1% solution of 2,3-diaminonaphthalene (K & K Laboratories, Inc.) in 0.1 N HC1 extracted twice with decalin in a separatory funnel. Filtered through filter paper wet with 0.1 N HC1 to remove suspended decalin. Prepared fresh for each set of determinations.

Standard selenite solution: Stock solution of sodium selenite (prepared and analyzed by A. W. Halverson) in 0.1 N HC1 prepared to contain 10 mg Se/ml diluted with 0.1 N HC1 to contain 0.1 ug Se/ml.

The wheat samples used in this work were obtained from various sources. Samples #1 through #6 came from a known seleniferous area. Sample #7 was obtained from an area of Ohio known to be deficient in selenium, sample #8 from a non-seleniferous area of South Dakota.

Three soil samples were used in the studies described. They were obtained at a single location known to produce vegetation of high selenium content. Soil M92 was the top foot of soil, M93 the second foot, and M94 the third foot. The analysis of these soils is given later. Bentonite samples were obtained from the American Colloid Company

(Skokie, Illinois).

Vermiculite from an unknown source was used in some of the studies. It was ground to pass a 0.0787-inch opening screen and was found on analysis to contain less than 0.1 ppm of selenium.

The oxygen and nitrogen gases were obtained from the Air Reduction Products Company. The water used in all studies had been demineralized and then distilled and stored in glass.

Other materials used in certain phases of the work will be described later.

EXPERIMENTAL

Study of Methods. After some preliminary studies, the method adopted for use in these investigations was essentially that of Watkinson (54). It was, however, modified to allow for the analysis of samples of high selenium contents, to insure a blank of consistently low value, to provide for the elimination of oxidation of selenite and of DAN during their reaction, and for use on a variety of materials. With its modifications, the basic method was as follows:

> An appropriate weight or volume of sample was introduced into a 30 ml micro-Kjeldahl flask having a ground flass fitting on its neck. Following the addition of 5 ml of concentrated HNO_3 and two glass beads, the mixture was allowed to stand over night. A 6-inch air condenser with a ground glass fitting was attached and the sample was heated with the flask vertical on a micro-digestion rack. Heating was done slowly at first and then more strongly until HNO_3 condensed in the lower half of the air condenser. The heat was removed and when vigorous action had subsided a 2.0 ml portion of 70% $HClO_4$ was added through the air condenser while rotating it to allow the acid to wash down the materials from the neck. After refluxing again for 15-20 minutes, the air condenser was removed and the neck of

the flask was placed into the manifold of the digestion rack. The flasks were then heated with vigorous flames until the fumes of $HC1O_4$ were visible and for 15 minutes thereafter. If charring occurred during any phase of the digestion, the heating was stopped and 5.0 ml of concentrated HNO_3 was added before the digestion was continued.

After the flasks had cooled, 1.0 ml of water and 2.0 ml of 10% HC1 were added. The flasks were then heated in a boiling water bath for 30 minutes. This reduced any selenate that may have formed from the selenite.

One blank (2.0 ml of 10% HC1) and one standard (3.0 ml of the diluted standard plus 2.0 ml of 10% HC1) were prepared in micro-Kjeldahl flasks. To these and to the digested samples were added 5.0 ml of the EDTA-NH_2OH solution and one drop of the cresol red indicator. Enough 5N NH_4OH solution was added to change the color of the indicator from pink to orange (pH of about 1) and the lights were turned out, all work from here on being conducted in semi-darkness. After adding 5.0 ml of the DAN solution and enough 0.1N HC1 to bring the liquid level to the neck of the flask, the solutions were shaken well and placed in a covered water bath at 50°C for 20 minutes. During this period, 125 ml

separatory funnels with teflon stopcocks and polyethylene stoppers were placed in a funnel rack and 10.0 ml of decalin was added to each. At the end of the heating period, the micro-Kjeldahl flasks were removed from the bath and placed in cold water for about 5 minutes. Their contents were then transferred with minimum washing into the separatory funnels. The funnels were shaken for 1 minute and after a few minutes the bottom layer of liquid was withdrawn from each and discarded. The decalin solution was washed twice by shaking with 25 ml of 0.1N HC1, shaking for about 30 seconds each time and discarding the lower phase.

The washed decalin solution was transferred to conical 15 ml centrifuge tubes and centrifuged for a few minutes to separate the decalin and water solutions. The decalin layer was transferred to a 5 ml fluorometer tube. One additional tube containing only decalin was also prepared.

The samples were read in a Turner model 110 fluorometer equipped with a GE F4T4 lamp, a 7-60 filter (peaks at 360 mu) for the incident light, and a #58 filter (peaks at 525 mu) for the fluoresced light. Using the smallest aperature (1X), the instrument was set at zero using the pure decalin. The blank was then read against this to determine whether or not it was

excessively fluorescent. A reading of about 3 (equivalent to about 0.015 ug of selenium) was normally obtained. Readings below 5 were not considered excessive. The instrument was then reset at zero using the blank in place of the decalin, and the standard and samples were then read. The response to graded standards was found to be constant, and a factor derived from the concentration and reading of the standard was used in calculating the results.

For samples containing levels of selenium too high to read using the above method, dilution of the digested sample to a definite volume and analysis of an aliquot of this was found satisfactory. On the other hand, partial digestion in HNO_3, making the partial digest to a definite volume with HNO_3 and digesting a portion of this was also found satisfactory.

The reagent blank determined by running through the entire procedure with no sample added was found to be extremely small (less than 0.01 ug selenium). For most of the samples analyzed in this study it was, therefore, disregarded.

Soil samples gave serious problems from the standpoint of bumping during digestion when glass beads were used. Therefore, a porous porcelain material previously cleaned with hot concentrated HNO_3 and then broken into small chips

was used. This was satisfactory for soils, but it did not prevent bumping during the digestion of very fine materials such as bentonite. Another method of digestion was devised and will be described later.

In the analysis of water samples, the digestion was accomplished on samples of up to 10 ml volume without the use of air condensers. Both the HNO_3 and $HClO_4$ were added at the outset of the digestion, and the excess water and HNO_3 were removed at a vigorous boil. The same was true of HNO_3 solutions used to trap volatile selenium.

To determine the water soluble selenium content of soils, a 5.0 g sample of finely ground soil was weighed into a 50 ml heavy walled centrifuge tube having a neck to take a rubber stopper. After adding 35.0 ml of water, the tube was closed with a rubber stopper and mechanically shaken for 6 hours. The tube was then centrifuged for 15 minutes at 9000 rpm in a Sorvall centrifuge. A 5.0 ml sample of the clear liquid was withdrawn and analyzed by the method used for water samples.

Other deviations in procedure will be described during the discussion of results.

Soil Studies. These studies were made for the purpose of determining the rate of evolution of volatile selenium from soils and the factors affecting it. The apparatus

used is shown in schematic form in Figure 1. Air was supplied to the apparatus by a vibrating pump at about 15 inches of water pressure. Using the Hoke valve to regulate its flow, the air was bubbled through about 2 inches of water at a rate of about 60 bubbles per minute, and then over the soil surface and finally through about 4 inches of redistilled concentrated HNO_3. Others (29, 42, 46) had used a more dilute nitric acid containing mercury or a mercuric chloride solution to trap volatile selenium compounds from rats. In certain of the studies, a second HNO_3 trap containing 1 mg of mercury per ml was used. Since less than 1% of the evolved selenium was ever found in the second trap, its use was discontinued.

In preparing the soils in the erlenmeyer flasks, water additions were made slowly with mixing, in order to give uniform wetting. In some experiments, vermiculite was added to improve porosity, and either corn starch or finely ground wheat #7 was added as a source of organic matter. In one experiment, components of the mixture were sterilized prior to mixing them together. The soils were in this case autoclaved at 121°C and 18 psi for 3 hours, allowed to cool for 5 hours, and then autoclaved as before. The wheat and water were autoclaved under similar conditions for 30 minutes.

At various times during an experiment, the tube of HNO_3 was removed and replaced with a fresh tube. The amount of

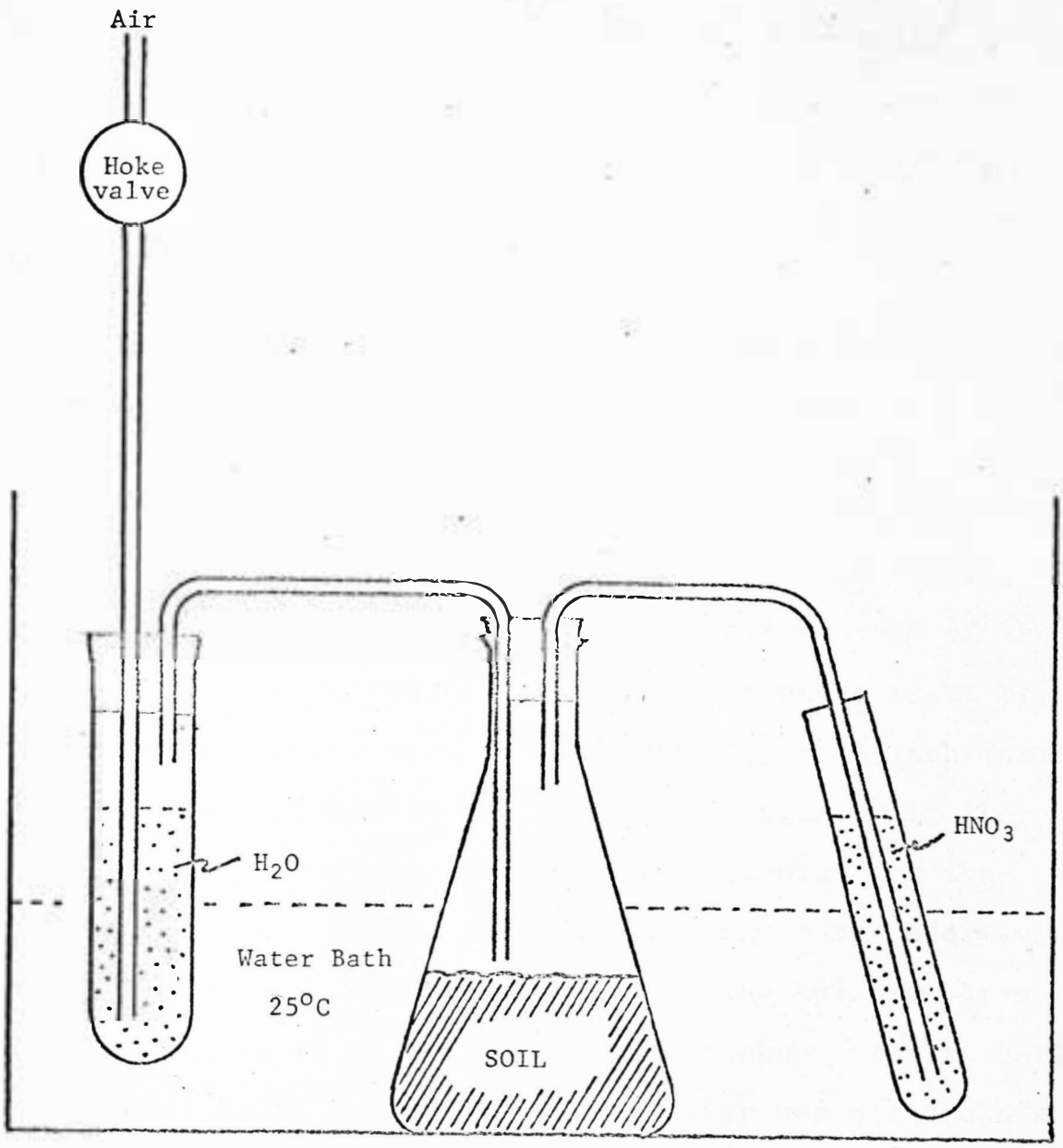

FIGURE 1. EQUIPMENT USED TO STUDY VOLATILIZATION OF SELENIUM FROM SOILS

evolved selenium trapped in the HNO_3 was determined by analyzing the entire sample by the method already described after adding 2 ml of 70% $HClO_4$ and two glass beads and heating 15 minutes beyond the appearance of $HClO_4$ fumes without the use of an air condenser.

Further detail concerning the various experiments will be given during the discussion of the results.

Water Studies. Investigations with waters were made for the purpose of determining whether selenium-bearing waters might on standing decrease in their content of the element and what the magnitude of this decrease might be. All of these studies were carried out in 2 x 15-inch test tubes. In some experiments the soluble selenium in the soils added was used as the source of selenium for the waters. In these cases, the soils and any other additives were added to the test tube, and after the addition of a definite volume of water the tube was shaken intermittently for about 2 hours. A sample of the water was withdrawn as soon as the soil particles had settled and analyzed for selenium to obtain an initial value. In other studies, a selenate solution was used. The tubes were left to stand at room temperature covered with watch glasses, and samples were withdrawn at various intervals for selenium analysis.

Where gases were bubbled through the water to provide

an atmosphere of nitrogen or of oxygen, the samples of water for analysis were removed and centrifuged at 9000 rpm for 30 minutes in a Sorvall model RC-2 refrigerated centrifuge. The clear supernatant liquid was used for analysis. Corrections for moisture loss were made in calculating the results of these experiments.

RESULTS AND DISCUSSION

Study of Methods. Preliminary to the work with waters and soils, two methods of analysis were used to determine the selenium content of 8 wheat samples in order to evaluate their suitability. The wheat samples had been analyzed by a number of other methods and their selenium contents varied over a wide range. The selenium contents as determined by the various methods are given in Table II. For samples containing over 2.0 ppm of selenium, either the method of Cummins et al. (17) or of Watkinson (54) was found satisfactory. The Watkinson method, however, proved considerably superior in analyzing for the very small amounts of selenium, and for this reason it was selected for these studies.

The Watkinson method had been designed for use on plant and animal tissues. In using it on some soils, it appeared that iron might be giving some interference. It was further felt that on occasion soils might contain enough chloride or manganese to give interference. Therefore a study was conducted to determine whether iron, manganese, or halides might interfere by causing a selenium loss during the digestion process. A known amount of $Na_2Se^{75}O_3$ was added along with 0.2g of wheat #7 to digestion flasks, salts as shown in Table III were added and digestion was accomplished in the usual manner. After digestion, the solutions were diluted

TABLE II. SELENIUM CONTENT OF WHEAT DETERMINED BY VARIOUS METHODS

Sample	Methods Used and Selenium Contents (ppm)					
	Klein[a]	Cheng[b]	Allaway and Cary[c]	Neutron Activation[d]	Cummins et al.[e]	Watkinson[f]
#1	61.7	70.4	-- [g]	70.4	60.8	57.5
#2	18.8	21.3	-- [g]	21.6	19.7	20.8
#3	3.7	2.8	2.64	2.86	2.8	2.13
#4	35.0	39.4	-- [g]	34.9	33.7	34.2
#5	13.7	14.8	-- [g]	-- [g]	14.1	15.0
#6	24.1	26.3	-- [g]	-- [g]	22.2	23.8
#7	0.2	-- [g]	0.085	0.022	0.0	0.024
#8	0.8	-- [g]	0.65	0.60	0.9	0.58

[a] Determined as described by Klein (30). Analyst, O. E. Olson.

[b] Determined by a method based upon the method of Cheng (12) and involving HNO_3-$HClO_4$ digestion similar to that described by Watkinson (53) and colorimetric estimation following treatment with 3,3'-diaminobenzidine. Analyst, D. Rehfeld.

[c] Determined by method of Allaway and Cary (1) in their laboratory.

[d] Determined by General Dynamics, General Atomics Division. Samples irradiated at a thermal neutron flux of $1.8 \times 10^{12} n/cm^2$-sec and counted after two months in a 3" × 3" well-type NaI(Tl) detector coupled to a 400 channel pulse-height analyzer.

[e] Determined by method of Cummins et al. (17). Analyzed by author.

[f] Determined by method of Watkinson (54) with modifications as discussed in the text. Analyses by author.

[g] No determination made.

TABLE III. RECOVERY OF SE^{75} IN DIGESTION MIXTURES IN THE PRESENCE OF VARIOUS SALTS

Compound Added	Element Studied	Level of Element in Digestion Mixture (mg)	CPM[a]	Se^{75} Recovery (%)
Standard[b]	None	--	5962	--
None	None	--	5819	97.6
None[c]	None	--	5848	98.1
$Fe(NO_3)_3$[d]	Fe	1	5857	98.2
$Fe(NO_3)_3$	Fe	10	5874	98.5
$Fe(NO_3)_3$	Fe	50	5860	98.3
KBr	Br	1	5885	98.7
KBr	Br	10	5892	98.3
KBr	Br	50	5840	98.0
NaCl	Cl	1	5831	97.8
NaCl	Cl	10	5848	98.1
NaCl	Cl	50	5891	98.8
NaI	I	1	5865	98.4
NaI	I	10	5840	98.0
NaI	I	50	5865	98.4
$KMnO_4$	Mn	1	5874	98.5
$KMnO_4$	Mn	10	5882	98.7
$KMnO_4$	Mn	50	5883	98.7

[a] Average counts per minute of aliquots of two digests counted in duplicate.

[b] Average counts of standard stock solution without digestion.

[c] No wheat added.

[d] Iron solution was made by dissolving 1 g of iron powder (Baker's Analyzed, CP) in concentrated HNO_3, and aliquots were taken to make the above levels.

to definite volume and aliquots were counted in a Packard Auto-Gamma Spectrometer. The study is summarized in Table III. The results indicate no loss of selenium as the result of salt additions, even at the highest level.

In attempting to analyze soils by the method described earlier, it was found that excessive bumping during digestion made the method unsuitable. In most samples, addition of boiling chips, made by crushing and extracting with hot HNO_3 a porous alundum filter crucible, prevented the bumping. However, in samples containing a high proportion of colloidal material, excessive bumping occurred even when these chips were used. Therefore, a process based on extracting the selenium with hot HNO_3 was studied. This method was used on three soil samples that could be analyzed by the usual method as well, and the study is summarized in Table IV. Apparently, two extractions with hot HNO_3 as described in the table remove essentially all of the selenium. Therefore, for highly colloidal soils where bumping during digestion is a problem this method can be substituted for the usual procedure. However, it is cumbersome and it is possible that on some soils the extraction of selenium may not be complete. It cannot be considered as highly reliable, but it should give a reasonably good approximation of the selenium content.

The analysis of some soils gave highly variable results

TABLE IV. ANALYSIS OF SOILS BY TWO METHODS

Soil	Analysis by HNO_3 Extraction[a]			Direct Analysis[b]
	1st Extraction (µg Se/g)	2nd Extraction (µg Se/g)	Total Extracted (µg Se/g)	(µg Se/g)
M92	5.1	1.1	6.2[c]	6.6
M93	5.9	1.0	6.9	6.9
M94	7.6	1.5	9.1	9.1

[a] A 2.0 g sample of soil was heated with occasional stirring on a steam bath with 20 ml concentrated HNO_3. After about 20 hours, the mixture was filtered with suction through a sintered glass filter and the residue was washed with HNO_3 to 100 ml. Aliquots of 10 ml were used for analysis. The second extraction was made on the residue in a similar manner, except that after heating the mixture was made to 25 ml without filtering, and 10 ml was used for analysis after allowing the soil to settle.

[b] A 0.1 g sample of soil analyzed as discussed in the text, using porous porcelain chips instead of glass beads to prevent bumping.

[c] A third extraction of this soil yielded 0.16 µg Se/g of soil.

in some instances. In these instances, the iron contents were always high. A study of the effect of iron on the analysis is summarized in Table V. This study showed that 2.0 mg or more of iron in the mixture analyzed gave a solution which fluoresced and thus could give rise to high selenium values. While the iron sample used in this study might have contained some selenium, the increase in fluorescence with increasing iron content was far from linear, so the values obtained could not have been the result of the selenium content. In other studies not reported here, it was found that the iron effect was somewhat variable. This variability may have been in part the result of difficulties in adjusting the pH of the solutions during the analysis because of the yellow color formed on the addition of EDTA. The yellow color made reading the color change of the indicator difficult. In addition, where large amounts of iron were present, iron hydroxide would on occasion precipitate or the decalin extract would have a very yellow color. The iron effects noted could not be overcome by increased additions of EDTA.

Allaway and Cary (1) used the coprecipitation of selenium with an excess of arsenic to separate selenium from other interfering elements. In his earlier method, Watkinson (53) used a zinc dithiol separation followed by redigestion for the same purpose. Either of these methods

TABLE V. INTERFERENCE OF IRON WITH SELENIUM DETERMINATION

Fe Content of Mixture Analyzed[a] (mg)	Apparent Selenium Content[b] (µg)
0.5	0.00
1.0	0.00
2.0	0.01
3.0	0.02
4.0	0.05
5.0	0.05
6.0	0.09
8.0	0.44
10.0	Greater than 0.46

[a] A solution of iron powder in HNO_3 equivalent to 10 times the amount of iron shown was digested and reduced with HCl in the usual manner. The digest was made to 100 ml with water, and 10 ml was analyzed.

[b] No selenium was added to any of the mixtures.

is time-consuming, and it was therefore decided that the use of ion exchange resins as suggested by Lott et al. (35) should be studied. One such study is illustrated in Table VI. In this study, a definite amount of selenium as selenite was digested with increasing amounts of added iron. When a portion of the diluted digest was run in the usual way, 5.0 mg of iron in the portion analyzed caused a slight decrease in the selenium value and 10.0 mg of iron made analysis impossible because of the precipitation of iron hydroxide. Another portion run through an AG 50W-X8 (Bio-Rad) column showed again an effect of iron opposite that found in the previous experiment. Comparing the results in the second and third columns of this table, it appears obvious that iron may not only contribute to errors by contributing to the fluorescence but also by in some way reducing the response from the added selenite. Assuming that iron adsorbed on the resin could in turn adsorb selenite, one might expect the results obtained in column three of the table. Another possible explanation appeared to be that the excessive levels of iron interfered with the reduction of selenate to selenite during heating with HC1. On treatment of the ion exchange effluent with HC1 at a concentration essentially equivalent to that normally used for the reduction, however, no increase in selenium content was noted (fourth column of table). On the other hand, when a portion

TABLE VI. ION EXCHANGE RESIN FOR IRON REMOVAL IN SELENIUM ANALYSIS

Fe in Sample[a] (mg)	Results of Selenium Analysis (µg/Sample)			
	Without Ion Exchange Treatment[b]	Following Ion Exchange Treatment[c]		
		Without Further Treatment[d]	Reduced With HCl[e]	Concentrated and Reduced with HCl[f]
0.0[g]	0.00	0.00		
0.0	0.31	0.31	0.30	
0.5	0.31	0.30	0.30	0.31
1.0	0.32	0.30	0.30	0.31
2.0	0.31	0.28	0.29	0.31
5.0	0.27	0.22	0.22	0.31
10.0	-- [h]	0.08	0.08	0.30

[a] A solution of iron powder in HNO_3 equivalent to 10 times the amount of iron shown was digested with 3.0 µg Se and then reduced with HCl in the usual manner. The digest was made to 100 ml with water.

[b] 10.0 ml of the diluted digest analyzed in the usual manner.

[c] 80 ml of the diluted digest run through a 15 × 70 mm column of AG 50W-X8 (H^+ form) at about 5 ml/min. Last 40 ml of effluent collected for use in subsequent analyses.

[d] 10.0 ml of column effluent analyzed without further treatment.

[e] 1.0 ml concentrated HCl added to 10.0 ml column effluent. Heated in boiling water bath 30 min and then analyzed.

[f] 10.0 ml of the column effluent and 2.0 ml 70% $HClO_4$ concentrated to $HClO_4$ fumes. Analyzed following reduction with HCl in usual manner.

[g] No selenium added.

[h] A heavy red precipitate of iron hydroxide formed making analysis impossible.

of the effluent was reduced in volume in the presence of perchloric acid and the reduction was then accomplished in the usual way, all of the added selenium was accounted for (5th column). It appears that the explanation for this lies in the need for HCl to be present in unionized form before it is an effective reductant. Reducing the volume in the presence of $HClO_4$ provided the conditions for this.

The results in Table VI indicate that treatment with ion exchange resin is an effective means of overcoming iron interference provided it is followed by reduction of the selenate selenium prior to treatment with DAN. Ion exchange treatment would, of course, remove the interference from other cations in the analysis of soils. Its use is probably necessary only in those soils of low selenium content (less than 1.0 ppm). When the selenium content is higher, the size of the sample can be small enough so that the amount of iron present is probably below that which would cause significant interference.

In order to further study the suitability of ion exchange resin for the removal of cationic interferences, several bentonites of variable iron content were extracted as described in Table VII. The extracts from these bentonites were made to a definite volume and aliquots were analyzed for selenium with and without known selenium additions, using ion exchange treatment followed by the

TABLE VII. USE OF ION EXCHANGE RESIN IN ANALYSIS OF BENTONITES

Sample	Fe in Solution Analyzed[a]	Selenium Content			
		Without Ion Exchange Treatment	With Ion Exchange Treatment[b]		
			No Added Se	1 ppm Added[c]	% Recovery
	(mg)	(ppm)	(ppm)	(ppm)	
#4	2.18	1.13	0.95	1.93	98
#10	0.99	0.86	0.58	1.41	83
#13	0.57	0.47	0.41	1.45	104
#15	5.52	0.93	0.18	1.08	90
#20	0.74	0.29	0.09	1.03	92
#25	0.26	0.97	0.90	1.87	97

[a] Determined colorimetrically by tripyridine method.

[b] As described in Table VI.

[c] Equivalent of 1 ppm Se added as selenite prior to digestion of acid extract of the bentonites.

concentration and reduction suggested as necessary by the data in Table VI. The amount of iron in each of the aliquots analyzed was also determined, and an aliquot of each extract was also analyzed without ion exchange resin treatment. The results are shown in Table VII. The results of the analyses without ion exchange treatment are all higher than those where ion exchange treatment was used. While there is good correlation between the level of iron in the sample analyzed and the difference in values obtained before and after ion exchange treatment ($r = 0.92$), in most samples the level of iron was less than that which would be expected to cause interference based on the data in Table VI. It is possible that cations other than iron which are capable of causing interference in the method are present in the bentonite. These would, of course, probably be taken care of by the ion exchange treatment. However, the results of the recovery trials are more variable than would be expected and are on the average low (average recovery 94%). Therefore, it must be concluded that, while ion exchange treatment no doubt is helpful in overcoming interference from iron, as it was used it lacks somewhat in the reliability that is demanded where highly accurate results are required.

The water soluble selenium in soils is apparently there entirely in an inorganic form(s) (43). Analysis of solutions containing inorganic selenium in known amounts

gave excellent results when amounts of up to 10 ml of the solution were digested with HNO_3 and $HClO_4$ as described under the "Experimental" section. Waters and water extracts of soils were therefore analyzed by the method as it is described, the digestion procedure actually serving largely as a means of concentration of the sample.

In general, the general method as described, with modifications as indicated for certain types of samples was considered as adequate for the type of work reported here. It cannot be considered satisfactory, however, for all types of soil or rock samples, especially those of very low selenium content.

Soil Studies. The soils used in these studies have already been described. Their analysis for total selenium is reported in Table IV. Their water soluble selenium contents were found to be as follows: M92, 0.083 ppm; M93, 1.0 ppm; and M94, 2.6 ppm. Their moisture contents, determined by drying at 105°C for 16 hours, were as follows: M92, 1.90%; M93, 2.48%; and M94, 2.76%.

In the first experiment, 150 g samples of each were weighed into duplicate flasks and moistened with 30 ml of water. The flasks were then attached to the apparatus used for aeration and collection of volatile selenium and analyses of the HNO_3 in the trap were made at various intervals.

After 10 days all flasks had evolved about the same amount of selenium, the rate of evolution being very slow. At this time, therefore, 7.5 g of wheat #7 (0.022 ppm Se) was mixed with one of the duplicate samples of each soil, and the flasks were again attached to the apparatus. The results of this study are shown in Figure 2.

A small amount of selenium was evolved from all of the flasks during the first 14 days. Previously, analysis of large air samples had been performed, and the results obtained indicated that the selenium measured in this experiment came from the soils and was not a contaminant of the air. After the addition of the wheat, mold growth was noticed on the 13th day, and the amount of mold increased with time. The wheat addition did not cause any noticeable increase in selenium evolution from soil M92, which had a very low water soluble selenium content. It did, however, increase selenium evolution from the other two soils, the increases being related to the water soluble selenium contents of the soils. This study indicated that the evolution of volatile selenium from soils probably occurs under natural conditions at a very slow rate. Organic matter additions to the soils probably increase this rate of evolution by supporting the growth of molds capable of transforming soluble selenium to a volatile form.

A second experiment was run to determine what effect

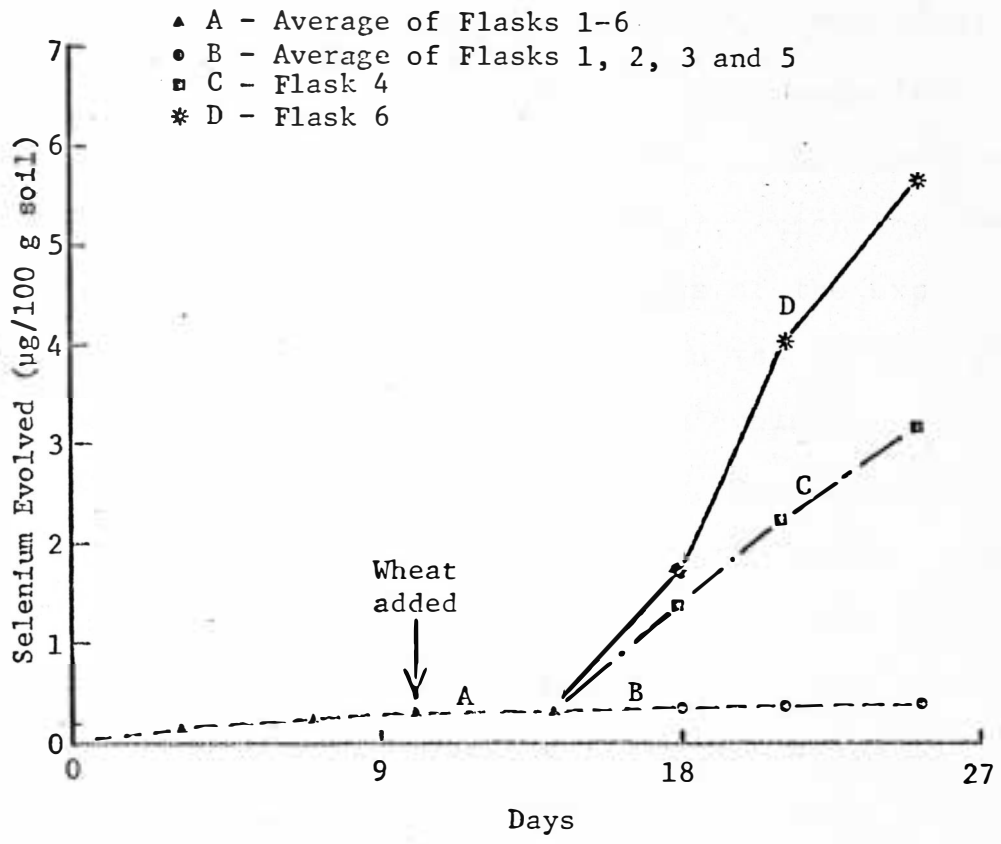

FIGURE 2. EFFECT OF WATER-SOLUBLE SELENIUM CONTENT AND OF WHEAT ADDITIONS ON EVOLUTION OF SELENIUM FROM SOILS

The various treatments were as follows:

Flask 1. 150 g soil M92 + 30 ml water.

Flask 2. Same as flask 1 with 7.5 g wheat added at 10 days.

Flask 3. 150 g soil M93 + 30 ml water.

Flask 4. Same as flask 3 with 7.5 g wheat added at 10 days.

Flask 5. 150 g soil M94 + 30 ml water.

Flask 6. Same as flask 5 with 7.5 g wheat added at 10 days.

the use of corn starch in place of wheat and of the level of corn starch would have on selenium evolution. The conditions of this experiment and the results are summarized in Figure 3. Here, the addition of corn starch increased selenium evolution except at the highest level, which appeared to have no effect during the first 13 days of the experiment. After the addition of more water on the 13th day, there was an increase in rate of selenium evolution in most of the flasks where starch had been added, indicating that higher moisture contents would be more satisfactory. However, even at the higher moisture levels the rates were all slow as compared to those obtained in the previous experiment where wheat had been added. No mold was noted in any of the flasks at any time during the experiment.

The results of an experiment to determine the effect of moisture level on the evolution of selenium from soil are shown in Table VIII. The data for moisture contents at the beginning and end of the experiment indicate that the system maintained moisture content throughout the experiment. The calculated values were slightly lower than the values determined at the conclusion of the experiment. There may, therefore, have been a tendency for the mixtures to accumulate some moisture during the aeration. Early in the experiment, the rate of evolution of selenium was highest at about the 24% moisture level, but as the experiment

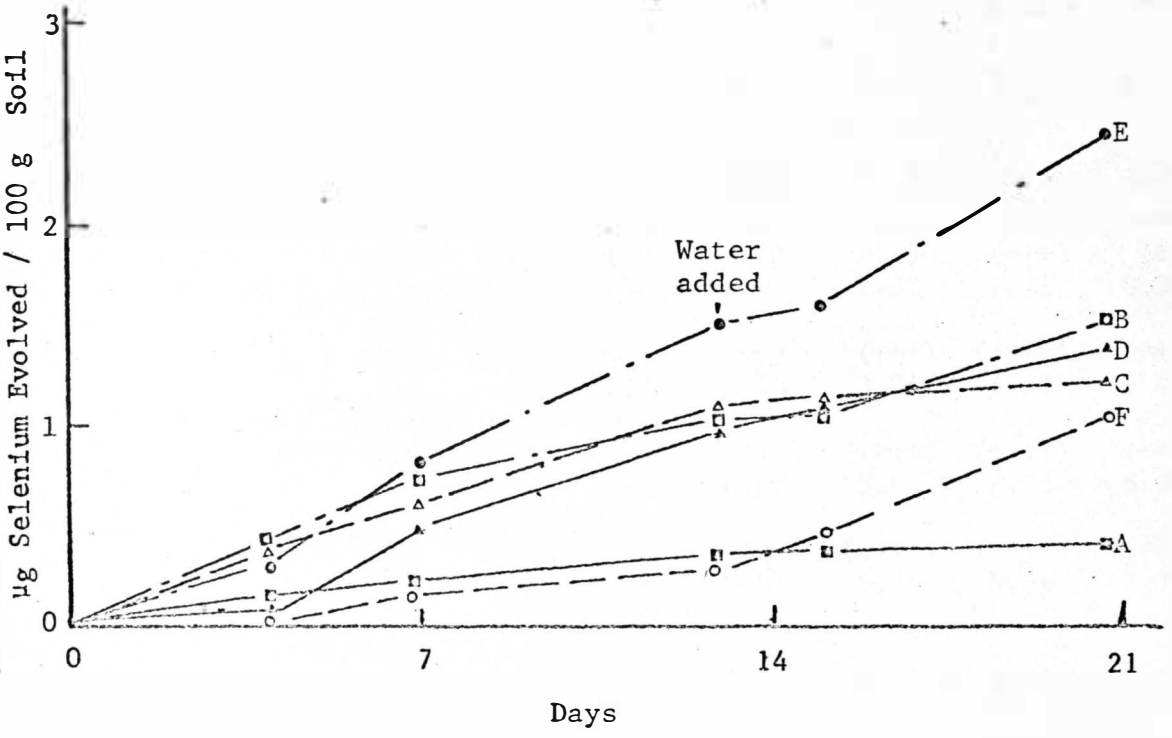

FIGURE 3. EFFECT OF ADDED STARCH ON SELENIUM EVOLUTION FROM SOIL M94

The various treatments were as follows:

A. 90 g soil M94 + 10 g vermiculite + water to give 25% moisture content.
B. Same as A with 0.5 g corn starch added.
C. Same as A with 1.0 g corn starch added.
D. Same as A with 2.0 g corn starch added.
E. Same as A with 4.0 g corn starch added.
F. Same as A with 8.0 g corn starch added.

On the 13th day, 10 ml of water was mixed with the contents of each flask.

TABLE VIII. EFFECT OF MOISTURE CONTENT ON EVOLUTION OF SELENIUM FROM SOIL AND ON ITS WATER SOLUBLE SELENIUM CONTENT

		Flask # 1	Flask # 2	Flask # 3	Flask # 4	Flask # 5	Flask # 6
Moisture content % (calculated)[a]		11.2	14.4	18.3	21.5	24.4	27.1
Moisture content % (determined)[b]		12.3	15.2	19.3	22.9	24.4	28.3
Mold growth and selenium evolved (µg Se/flask)[c]	6 days	(-) 0.06	(-) 0.36	(-) 1.97	(+) 2.25	(+++) 2.71	(±) 0.20
	8 days	(-) 0.29	(-) 1.17	(++) 3.18	(+++) 3.24	(++++) 4.37	(++) 1.40
	12 days	(±) 0.95	(+++) 3.43	(+++) 6.63	(++++) 5.85	(++++) 8.28	(++) 5.36
	15 days	(+) 1.51	(+++) 5.07	(++++) 10.05	(++++) 8.25	(++++) 9.36	(+++) 7.70
	19 days	2.06	6.01	12.13	8.76	9.74	9.10
Water soluble selenium at 19 days (µg/flask)		214	181	133	143	85	154
Total of water soluble and evolved selenium at 19 days (µg/flask)		216	187	145	152	95	163
Water soluble selenium not accounted for at 19 days (µg/flask)[d]		15	44	86	79	136	68

[a] Calculated from moisture content of components of mixture and added water. In addition to the added water each flask contained 90 g soil M94, 10 g vermiculite and 5 g wheat #7.

[b] Determined at conclusion of experiment by drying aliquots at 105°C for 16 hours.

[c] Mold growth expressed as - (none) through ++++ (very heavy). No mold observations were recorded for the 19th day. Values for evolved selenium are cumulative.

[d] Initial water soluble selenium content for each flask was 231 µg.

progressed the data indicated a rather broad optimum moisture content between about 18 to 25%. Visible mold growth appeared to correlate with the amount of selenium evolved. At the conclusion of the experiment, the water soluble selenium remaining in the soils was determined. This had decreased about in proportion to the amount of selenium volatilized, but the amount of decrease could not be accounted for by the selenium evolved. It is possible that some form(s) of volatile selenium was not trapped by the HNO_3. However, it appears more likely that the difference was the result of incorporation of some of the soluble selenium into the mold mycelium or into the protoplasm of other microorganisms.

The differences in rates of selenium evolution between soils to which wheat had been added and soils to which starch had been added raised a question as to whether the wheat might be the source of a microorganism which actively volatilized selenium. An experiment was designed to determine this. It is summarized in Figure 4. The data in this Figure show that when the soil was sterilized the rate of selenium evolution was very slow. With soil which had not been sterilized, the rate of evolution was rapid when either sterile or unsterilized wheat was added. It appears that sterilizing the wheat caused even greater evolution, but the significance of this difference cannot be determined because

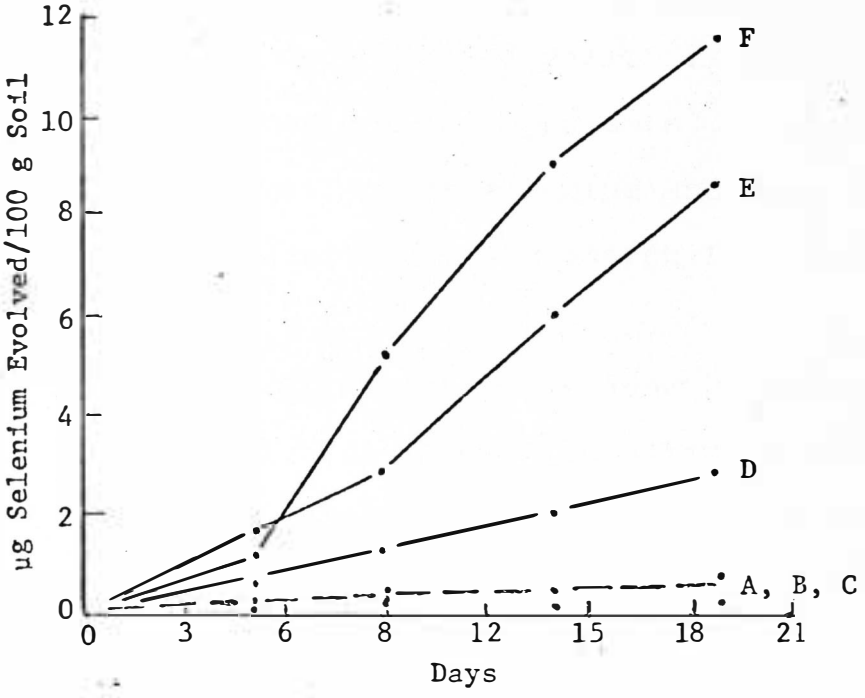

FIGURE 4. EFFECT OF STERILIZATION ON THE EVOLUTION OF SELENIUM FROM SOIL M94

The various treatments were as follows:

A. 90 g sterile soil + 5 g sterile wheat.
B. 90 g sterile soil + 5 g unsterilized starch.
C. 90 g sterile soil + 5 g unsterilized wheat.
D. 90 g unsterilized soil + 5 g unsterilized starch.
E. 90 g unsterilized soil + 5 g unsterilized wheat.
F. 90 g unsterilized soil + 5 g sterile wheat.

All flasks also contained 10 g unsterilized vermiculite and 30 ml sterile water.

only single flasks were used for each treatment. Again, the starch caused lesser response than the wheat in selenium evolution with the unsterilized soil. It appears that the soil is the source of a microorganism(s) which is largely responsible for volatilizing selenium and that wheat is a better growth stimulator than is starch for this microorganism(s).

While much work remains to be done on this problem, it appears that the microbial volatilization of selenium from soils may have played some role in the natural cycling of selenium. While Olson et al. (43) pointed to leaching as the cause for the greater concentration of soluble selenium in surface soils than in the deeper horizons, the matter of volatilization must be considered. Even though the rate of evolution was slow at low moisture contents and where rather large amounts of organic matter had not been added, it could through geological time have been great enough to cause the removal of a considerable amount of the soluble selenium from the surface soils. The accumulation of soluble selenium in the lower horizons, while no doubt in part the result of leaching and redeposition, may also be the result of lack of biological activity in these lower horizons.

Water Studies. As already pointed out, several dam and stream waters in seleniferous areas have been found to contain very low levels of selenium. Especially in the case

of dam waters, it might be expected that selenium would accumulate. Dam water is, however, somewhat subject to stagnation, and it is possible that biological activity could remove the selenium. A preliminary experiment to test this was set up as described under "Experimental". Seleniferous soil was used as a source of selenium, the soil being shaken with a definite volume of water to dissolve the soluble selenium. To provide organic matter, corn starch at two levels was added as indicated in Figure 5. The data as summarized in this figure show that on standing at room temperature the soluble selenium disappeared from the water at a rather rapid rate. Less than a third of that present at the outset of the experiment remained at 20 days. Starch additions increased the rate of disappearance. It was observed during this experiment that in the tubes with the higher level of added starch there was at about 6 days a very black layer about 1/8 inch thick on the surface of the settled soil. The black layer thickened with time, and gas pockets were noted in the soil matrix. Similar but less marked conditions were noted in the tubes containing the lower level of added starch. In the tubes with no added starch, only a few black spots were seen in the soil matrix. The significance of these observations is not known.

There are several possible causes for the disappearance of the selenium from the water. Some may have been

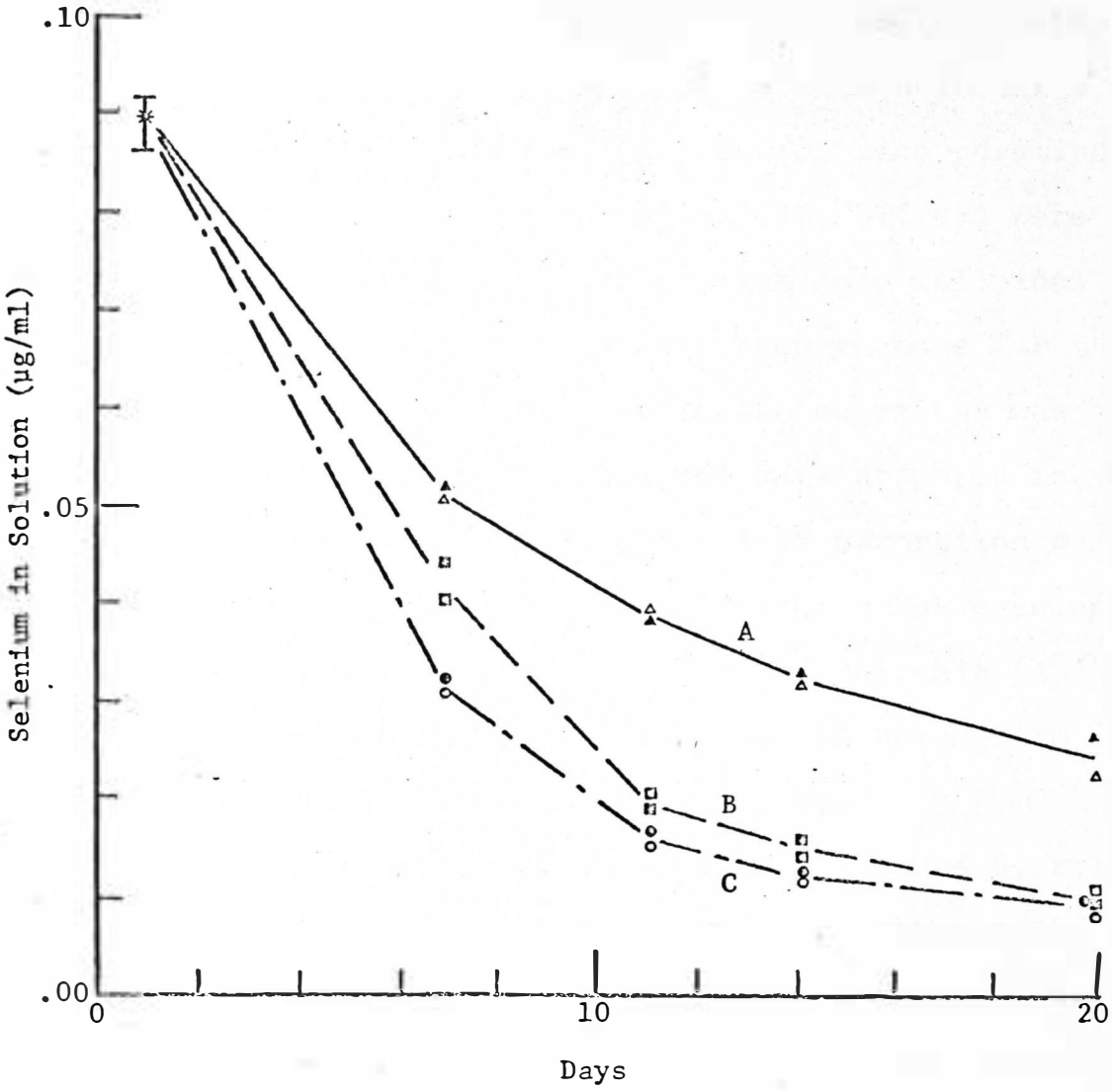

FIGURE 5. DISAPPEARANCE OF SELENIUM FROM WATER IN THE PRESENCE OF ADDED SOIL AND CORN STARCH

Soil-starch-water mixtures as described below were allowed to stand at room temperature without agitation, the clear liquid being analyzed for selenium at the intervals shown. The results of analysis of duplicate tubes are indicated, except that the average and range of values are indicated for the first day.

A. 450 ml water + 70 g soil M92 + 30 g soil M93.

B. 450 ml water + 70 g soil M92 + 30 g soil M93 + 0.5 g corn starch.

C. 450 ml water + 70 g soil M92 + 30 g soil M93 + 2.0 g corn starch.

volatilized into the atmosphere as in the case of soils. It is more likely, however, that precipitation in the elemental form as noted by Levine (33) and/or incorporation into the protoplasm of microorganisms (19, 51, 57) were responsible. Adsorption of the selenium onto colloidal material (8, 9) seems hardly to have been a cause for the disappearance, since the selenium in the solutions was derived from the soils and should not have appeared in the waters at the outset of the experiment if adsorption were a factor. It is, however, possible that the soluble selenium which probably was present in the form of selenate (43) was reduced by microbial action to selenite, in which form it would be more likely to be adsorbed (8, 9).

In a second experiment, summarized in Figure 6, solutions containing selenite or selenate were shaken with soil M92, which had a rather low soluble selenium content. Its soluble selenium did, however, contribute to the selenium content of the water to the extent of 0.02 ug Se/ml. Taking this into account, almost all of the selenite was probably immediately adsorbed, and the curve for the selenite more than likely represents what happened to the selenium from the soil. As before, the selenium disappeared from the solutions. Starch addition at 23 days stimulated the disappearance, suggesting microbial involvement. It is interesting that once the concentration fell to about 0.02 ug/ml the

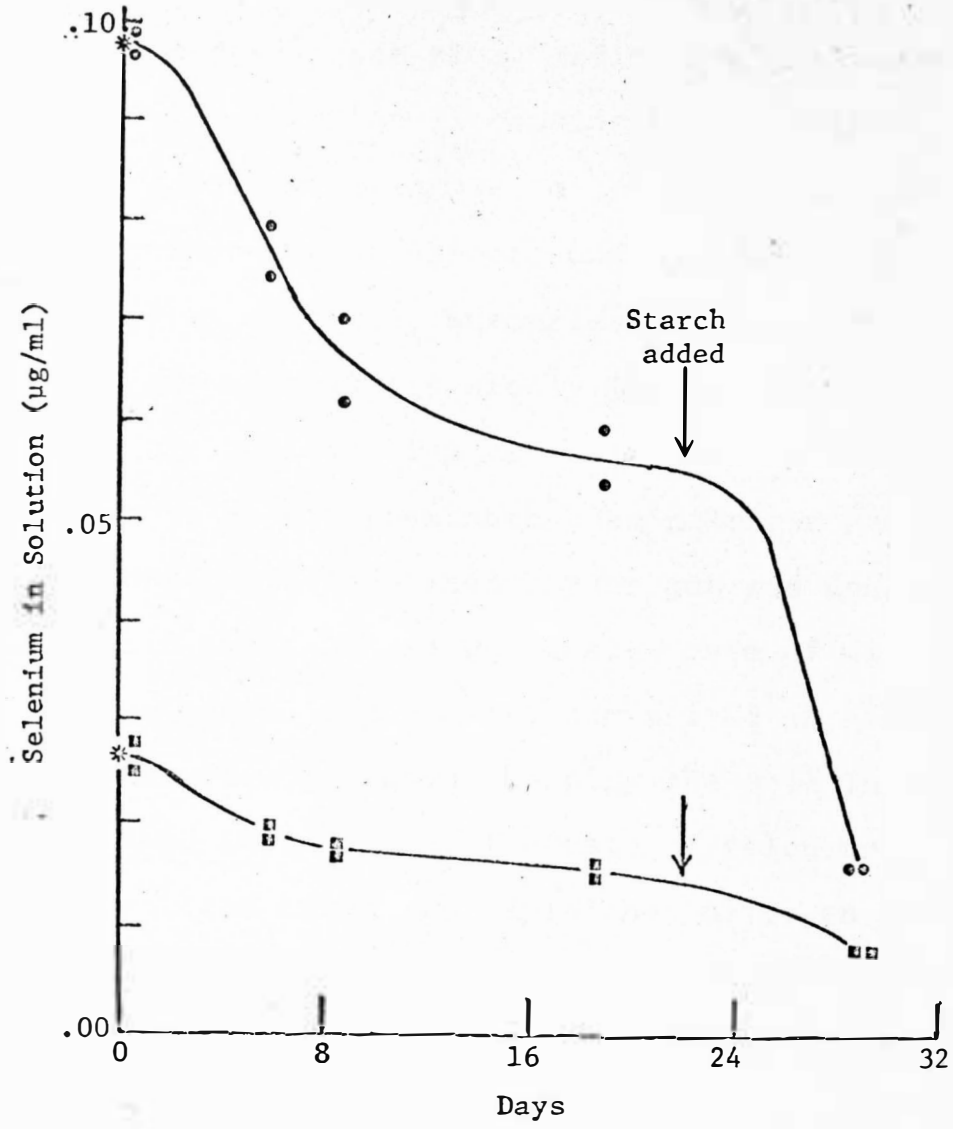

FIGURE 6. DISAPPEARANCE OF SELENATE AND OF SELENITE FROM SOIL-WATER MIXTURES AND EFFECT OF STARCH ADDITIONS THEREON

Solutions of K_2SeO_4 or of Na_2SeO_3 were mixed with soil as described below and allowed to stand at room temperature without agitation, the clear liquid being analyzed at intervals as indicated. The results of analysis of duplicate tubes are shown for all but the sample taken at two hours.

●——○ 450 ml K_2SeO_4 solution (0.084 µg Se/ml) + 100 g soil M92.
▪——▫ 450 ml Na_2SeO_3 solution (0.080 µg Se/ml) + 100 g soil M92.
* Average initial value on samples taken at two hours.

The 100 g of soil M92 contributed 0.02 µg Se/ml to the solutions. On the 23rd day, 5.0 g corn starch was added to each tube with a minimum of mixing.

rate of its disappearance was slow, indicating that once the selenium level falls this far it remains fairly stable.

Two experiments were designed to determine the rates of selenium disappearance under aerobic and anaerobic conditions. In the first of these, summarized in Figure 7, oxygen or nitrogen gas was bubbled slowly through a mixture of water and soil, the soil serving as the source of soluble selenium. The rate of disappearance when nitrogen gas was used was much more rapid than when oxygen gas was used, although even with oxygen there was a slow rate of disappearance. In the second experiment, summarized in Figure 8, a fast rate of gas flow was used, keeping the soil in continuous suspension. Here again, the rate of selenium disappearance from the water was rapid when nitrogen was used. In the case of oxygen, the disappearance was completely prevented. While this points to the reduction of selenium as necessary for its removal, it does not explain the mode of action, since most of the possible means of selenium removal as discussed previously here would require this reduction.

Finally, an experiment was undertaken to determine whether the addition of iron hydroxide to the medium might increase the rate of disappearance of selenium present as selenate from water under anaerobic conditions. If such were the case, it would suggest that the selenate was first

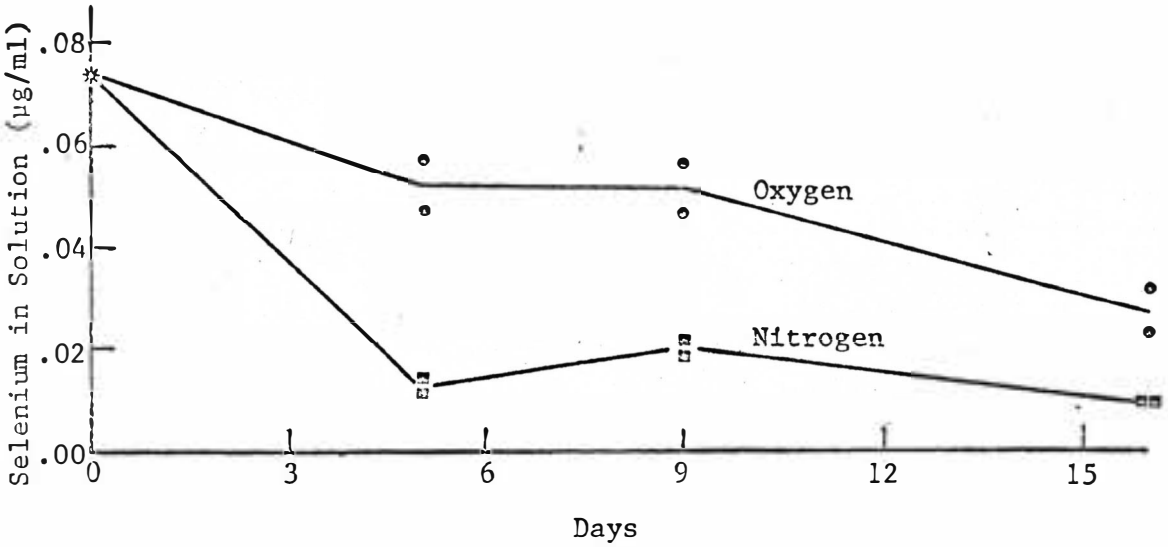

FIGURE 7. EFFECT OF AERATION OF SOIL IN WATER SUSPENSIONS WITH OXYGEN OR WITH NITROGEN ON THE REMOVAL OF SELENIUM FROM SOLUTION
(First Experiment)

60 g soil M92 and 20 g soil M93 in 350 ml water aerated with oxygen or nitrogen at such rate as to only slightly disturb the settled soil. The results of analysis of duplicate tubes are shown except that the average value for the initial sample, as indicated by the asterisk, is shown.

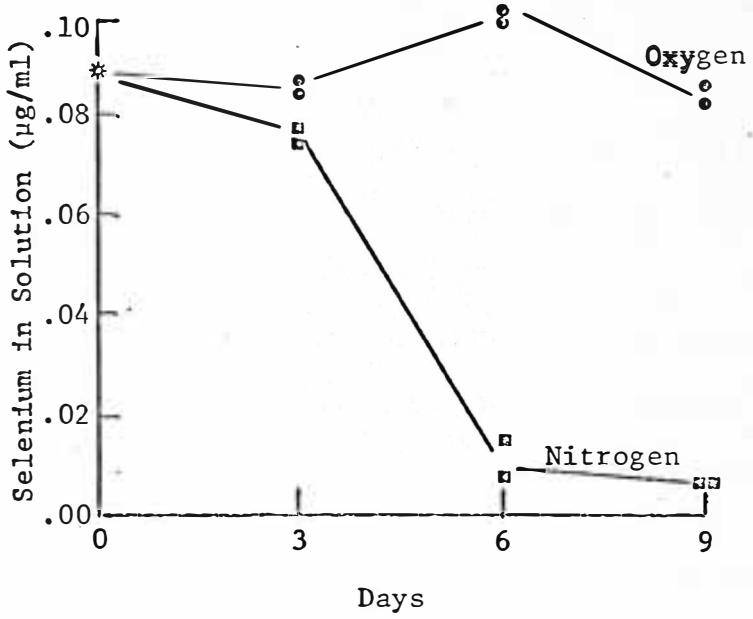

FIGURE 8. EFFECT OF AERATION OF SOIL IN WATER SUSPENSIONS WITH OXYGEN OR WITH NITROGEN ON THE REMOVAL OF SELENIUM FROM SOLUTION
(Second Experiment)

Suspensions of 5.0 g soil M92 and 2 g corn starch in 450 ml of K_2SeO_4 solution (0.84 µg Se/ml) aerated with oxygen or with nitrogen at such rate as to keep the soil in continuous suspension. The values shown have been corrected for moisture loss, and the results from duplicate tubes are indicated except that the initial value represents an average and is shown by an asterisk.

being reduced to selenite which was then removed from solution by adsorption on the iron hydroxide. The iron hydroxide was freshly prepared from a solution of $Fe(NH_4)(SO_4)_2$ by precipitation with NH_4OH and washing exhaustively with water. When added along with a small amount of soil as inoculum and starch as a carbohydrate source to a solution of selenate, it caused no greater rate of disappearance of selenium from the water than that which occurred when only the soil and starch were added (Figure 9). Either the reduction to selenite with subsequent adsorption on iron hydroxide is of little consequence in the process of removal of selenium from water, or the soil colloids themselves were capable of adsorbing all of the selenite formed. The latter seems improbable because of the small amount of soil used.

The experiments with water give no conclusive evidence as to the mechanism by which selenium is removed from water under the conditions used. The data suggest that microorganisms are involved and that these microorganisms are anaerobes. Whatever the mechanism, the rapid rate of removal of selenium under conditions similar to what might exist in dams and stagnant pools lying in intermittent streams is consistent with the low selenium contents found under natural conditions in these waters. From the standpoint of the selenium cycle in nature, therefore, the removal of soluble selenium from stagnant or semi-stagnant waters and deposition in the sediments appears important.

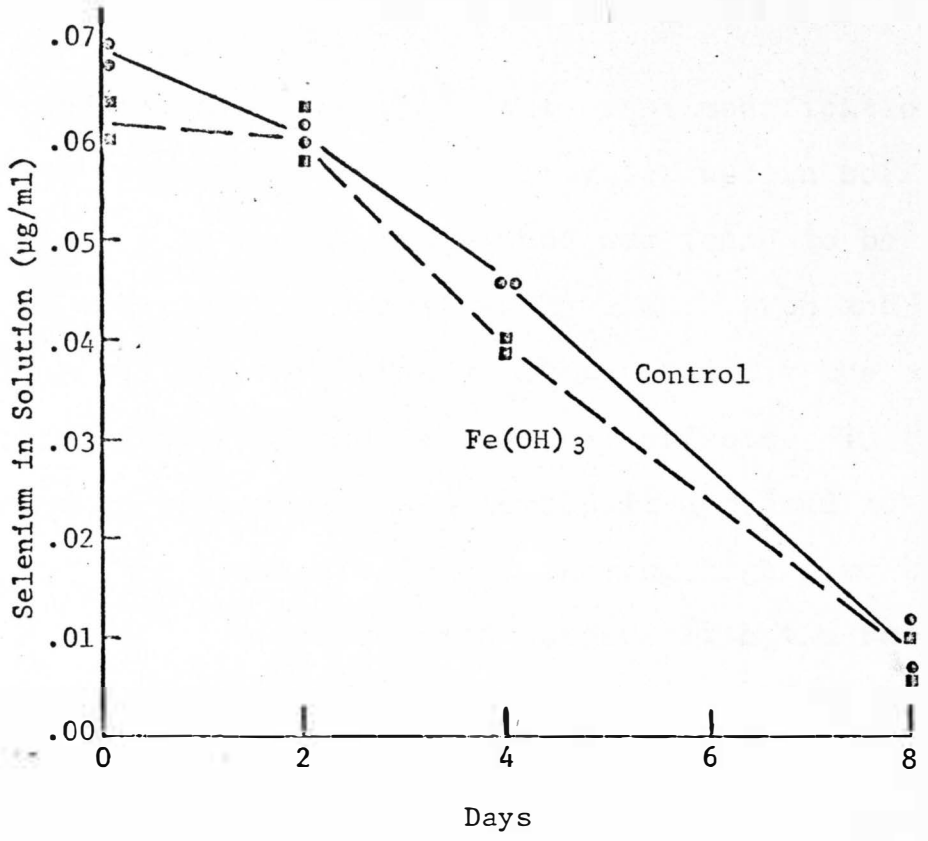

FIGURE 9. DISAPPEARANCE OF SELENIUM FROM SOIL IN WATER SUSPENSIONS IN THE PRESENCE AND ABSENCE OF ADDED IRON HYDROXIDE

Control. Suspension of 5 g soil M92 and 2 g corn starch in 500 ml of K_2SeO_4 solution (0.068 µg Se/ml) aerated briskly with nitrogen.

$Fe(OH)_3$. Same as control with iron hydroxide equivalent to 0.115 mg Fe added.

Results of analysis of duplicate tubes are shown.

SUMMARY

The method of Watkinson (54), with some modifications, was studied to determine its suitability for use in soil and water analysis. With soils, the method was found to be satisfactory, except where excessive levels of iron and possibly other cations were present in the soils. The interference of iron appeared to have two effects. In the first place, when present at high levels it appeared to give fluorescence in the decalin extract, causing high results. On the other hand, it appeared to interfere with the reduction of selenate to selenite during the course of the analysis, causing low results. Its removal with a cation exchange resin followed by reduction of the selenate gave good results. However, using this method with bentonites of varying iron content, the results of recovery experiments indicated that other cations than iron may have been giving some interference. The results obtained with the use of cation exchange resin cannot, therefore, be considered as highly reliable, although they are probably more accurate than those obtained on samples of high iron content without such treatment.

The method was found reliable for water samples of up to 10 ml volume without previous concentration of the solution. It was used in the determination of water-soluble

selenium in soils.

Selenium was found to be volatilized from soils at a very slow rate. The addition of starch and especially of wheat was found to increase selenium evolution when an appropriate amount of moisture was present. The optimum moisture content appeared to lie between about 18 and 25%. The amount of selenium evolved increased as the water-soluble selenium content of the soil increased. It also appeared to correlate with the amount of mold growth on the soil. The rate of selenium evolution from sterilized soils was very slow even with wheat or starch additions. The results of this work suggest that a mold(s), normally present in the soils used, is largely responsible for volatilizing selenium from these soils. They further suggest that under natural conditions the rate of evolution of selenium from soils is slow, but that in terms of geological time it is probable that this process has been of importance in the removal of selenium from surface soils.

Selenium was found to disappear from water in the presence of added soil at a relatively rapid rate. In the matter of about three weeks, the selenium contents of waters were reduced to about one-third or less of their original values when conditions were anaerobic. Under aerobic conditions, removal was prevented. The mode of action of the removal was not determined, but the data strongly suggest

the involvement of microorganisms.

It would appear from these experiments that additional study of the role of microorganisms in the genesis of both selenium-deficient soils and those producing toxic vegetation would improve our understanding of their formation and our ability to solve the nutritional problems they cause.

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