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STERILIZATION OF SOILS WITH
SODIUM ARSENITE

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ARSENIC FIXATION IN RELATION TO THE STERILIZATION OF SOILS WITH SODIUM ARSENITE^{1,2}

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INTRODUCTION

DATA FROM GREENHOUSE EXPERIMENTS on the toxicity of arsenic to oats in 80 California soils are reported by Crafts and Rosenfels (7)⁵ in another paper of this issue. In most of the soils tested, texture predominated as a determiner of toxicity; that is, toxicity was greatest in light and least in heavy soils. The few exceptions to this general rule are explained by the content of iron compounds of the soils (as indicated by their reddish color) or by the properties of the soil colloids. A similar relation between toxicity and soil texture has been noted by Cooper, *et al.* (4 and 5), Albert and Arndt (2), and Albert (1) working with South Carolina soils, and Reed and Sturgis (11), working with Louisiana soils.

The total arsenic content of a soil has not proved to be a satisfactory criterion of toxicity. As Vandecaveye, Horner, and Keaton (15) have shown, arsenic toxicity to barley is more closely correlated with the fraction soluble in 0.1 *N* ammonium acetate solution than with the fraction soluble in hot concentrated HNO₃. The results of Reed and Sturgis (11) show that the total arsenic content of the soil does not determine toxicity to rice. They indicate that toxicity is more closely correlated with arsenic soluble in 0.05 *N* HCl than with that soluble in water. According to Albert and Arndt (2), arsenic soluble in a collodion-bag dialysate is a reliable index of toxicity, whereas total arsenic is not. Greaves (9) has found no correlation between total and water-soluble arsenic in orchard soils.

Judging from other researches, not concerned directly with toxicity to plant growth, soils vary widely with respect to capacity for arsenic fixa-

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⁵ Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

tion⁶ and retention against leaching. In the work of Schulz and Thompson (13), sodium arsenite was readily leached from a Wisconsin forest soil. McGeorge (10), on the other hand, showed by lysimeter experiments that sodium arsenite was strongly held despite excessive leaching by certain Hawaiian soils; he also found that soils varied in their capacity to fix soluble sodium arsenite. According to Dratschew (8) also, soils vary in capacity for arsenic adsorption. Stewart (14) records further evidence of variation between soils, showing that water extracts of various soils differ markedly in their capacity for dissolving lead arsenate.

The general principle may therefore be advanced that toxicity is directly related to the water-soluble, the dilute-acid-soluble, or some similar fraction of the total arsenic. This holds true whether one is dealing with the native arsenic in an untreated soil or with the arsenic applied as a soluble or an insoluble salt. Applied to the relation between toxicity and textural grade, this principle leads one to suppose that in heavy soils less arsenic is water-soluble for a given application, than in light soils. Since the ability of some soils to fix arsenic is greater than that of others, one may explain the relation of toxicity to textural grade by assuming a greater fixation of soluble arsenic in heavy soils than in light. In the present work, this assumption was submitted to experimental test; that is, an attempt was made to determine whether or not the toxicity of sodium arsenite in California soils, measured by greenhouse tests already reported (7), could be explained by arsenic fixation.

METHODS

The greenhouse technique in the determination of arsenic toxicity has been described in detail in the previous paper of this issue (7, p. 181). Results obtained by the method outlined are also given for 80 California soils (7, table 3) as yield of tops, in grams fresh weight, of oat plants corresponding to various applications of sodium arsenite to the soil expressed as p.p.m. As_2O_3 on the basis of air-dry soil.

⁶ In this paper the word *fixation* denotes the process of rendering soluble arsenic insoluble by contact with the soil. *Fixed arsenic* is *arsenic insoluble in water*. Both adsorption and chemical precipitation are included in this definition, the emphasis being placed entirely upon the extent of insolubility rather than the manner of its accomplishment.

This limited definition is necessary because the word *fixation* has had at least three different uses: first, it has been used to mean loss of availability to plants; second, loss of solubility; and third, retention against leaching. The present paper is concerned solely with the second of these meanings, and the previous paper (7) with the first. The term could therefore have been used in connection with either or both studies. To avoid ambiguity, the word was not used at all in the previous paper (7), and the specific definition given above adhered to in the present paper. This is purely a matter of convenience, and no claim is made that the "best" or most suitable use of the term has been attained.

Thirty-three of the 80 soils were chosen for the present study, a wide range of textural grades being selected. Toxicity curves were plotted for these 33 soils, the application of sodium arsenite expressed as p.p.m. As_2O_3 on the basis of oven-dry soil being plotted on the horizontal axis against the yield expressed as a percentage of the check, that is, of the yield without arsenic. The hygroscopic moisture content, which varied from about 0.5 to 8.0 per cent, was thus eliminated as a variable, and the curves all originated at the same height on the vertical axis. The data given in columns 4 and 5 of table 1 (p. 209) and in column 4 of table 2, (p. 213) were taken from the 33 curves thus produced.⁷

Arsenic fixation was measured in a series of standard laboratory runs conducted rigidly as follows:

Sodium arsenite stock solution was applied to samples of air-dry or moist soil equivalent to 100 grams of oven-dry soil. This solution, identical with the one used in the greenhouse tests, contained 5.00 grams As_2O_3 and 1.25 grams NaOH per liter. Enough water was added to make a total of 100 ml, including soil moisture and water added as sodium arsenite solution. Two samples of each of 6 different soils were handled in each run. One sample of each pair received a uniform application of arsenic; the other an application varying from soil to soil in a manner that will be explained.

The 12 soil and water mixtures thus prepared were agitated mechanically for 18 hours in 1-pint wide-mouthed jars by continuous rotation on the parallel shafts of a machine, and a portion of each was then filtered. Usually enough filtrate could be obtained under gravity, but sometimes filtration by suction was necessary. Some filtrates, especially those of sandy soils, were turbid. These were returned to the filter as often as necessary to get a clear solution. Occasionally some very fine suspended material remained despite these precautions.

The 1:1 extracts thus prepared were analyzed singly by the Gutzeit method (3, p. 306) within 2 hours of filtration,⁸ without preliminary acid digestion or other treatment. An attempt was made to choose an aliquot for analysis that would yield approximately 0.015 mg As_2O_3 , since this amount could be most accurately determined.

⁷ These toxicity curves have not been published. Four examples of this type of curve are given in figure 5 (p. 226) of this paper, but the points plotted represent the means of 3 or 4 greenhouse tests. The toxicity data of tables 1 and 2 were taken from curves plotted from greenhouse results obtained simultaneously on 20 or 40 soils, each curve being derived from a triplicated test series on the soil concerned.

⁸ According to tests, the arsenic content of extracts decreased markedly in some cases during periods of several days to a month. All extracts were therefore analyzed immediately after filtration. If the aliquot was poorly chosen, a new extract was prepared.

The directions for the Gutzeit method given by the Association of Official Agricultural Chemists (3, p. 306) were followed in detail with one exception: instead of maintaining the Gutzeit generator-bottle units at a constant temperature between 20° and 25° C for 1½ hours after addition of the zinc, the generator bottles were placed in an ice bath 10 minutes before the zinc was added and were held in it for 20 minutes after.⁹ They were then removed and let stand at room temperature 1 hour before the stains were measured. Standard stains for 0.005, 0.010, 0.015, 0.020, and 0.025 mg As_2O_3 were prepared in duplicate for each run from the same Hanford-Pratt sensitized sheet used for the 12 soil-extract analyses. The paper sheets were sensitized by soaking 1 hour in 4 per cent mercuric bromide solution in 95 per cent alcohol.

All operations in the standard run described above were carried out in 2 days. The soil and water mixtures were customarily prepared in the pint jars in the afternoon and then agitated overnight. The filtrations were made the following morning, and the Gutzeit analyses completed the same day.

To calculate the percentage fixed, the difference between the concentration of arsenic applied and that found soluble was expressed as a percentage of the concentration applied. This value will be called the "apparent fixation," or "percentage fixed as determined."

Errors affecting the fixation values presented in this paper are as follows:

a) The inherent standard deviation of single analyses for arsenic by the Gutzeit method. This was determined by analyzing two different sodium arsenite stock solutions, of the composition given above, eighteen times each, and was found to be approximately ± 7 per cent for aliquots yielding 0.015 mg As_2O_3 .

b) The effect of magnitude of fixation upon the accuracy of the fixation percentage. Since fixation was determined by difference in arsenic concentration of a solution before and after contact with the soil, the values for percentage fixed are subject to a sliding scale of error even though the standard deviation of the analysis itself remains constant. At a determined fixation of 0 per cent (the arsenic remaining 100 per cent soluble), the true fixation may lie anywhere between + 7 or - 7 per cent; at 50 per cent fixed between 46.5 and 53.5; while at 100 per cent fixed the error is zero.

c) The effect of the soil extract upon the arsenic analysis. By attempting the recovery of 0.015 mg As_2O_3 in the presence of 1 ml or less¹⁰ of 1:1 extract of several different soils, it was found that soil extract caused high results. Since the amount soluble as determined is thus too high, the percentage fixed as determined is too low and must be given an upward correction to compensate for this source of error.

⁹ The use of an ice bath was recommended by the California State Department of Agriculture Division of Chemistry.

¹⁰ In the routine analytical work the aliquots taken were restricted to no more than 1 ml and were sometimes as small as 0.06. All aliquots less than 1 ml were secured by measuring relatively large volumes of diluted extracts.

From this discussion it should be apparent that all values for percentage fixed determined by the method above outlined are subject to an uncertainty which diminishes in the direction of high fixation. The fixation values tend to be too low because of the presence of soil extract.

Figure 1 shows an attempt to illustrate graphically the range within which the corrected fixation values are expected to lie.

In this diagram, the 33 soils are grouped into two classes. One class includes the light soils which required 0.1 ml or less of extract¹¹ for an analysis. The tests on effect of extract show that the maximum error produced by any soil in this class was about + 9 per cent and the minimum + 3 per cent. An error of + 9 per cent combined

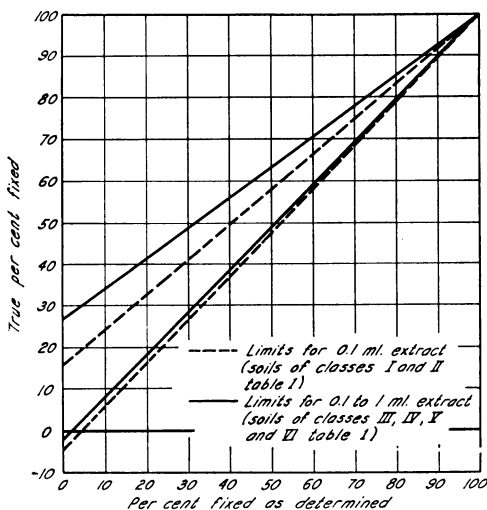


Fig. 1.—Apparent fixation, or percentage fixed as determined, plotted against the limits within which the true percentage fixed is expected to lie.

with + 7 per cent standard deviation gives a total of + 16 per cent. Negative corrections of 16 per cent in the *soluble* arsenic to compensate for this error would result in the series of *fixation* values given by the upper dotted line in figure 1. If the + 9 per cent error coincided with the negative value of the standard deviation, the net effect would be a + 2 per cent error. However, a lower limit based on corrections of —2 per cent is not plotted in figure 1 because only the limits for the entire group of soils are desired. Instead, the minimum error for the group of + 3 per cent is combined with a —7 per cent standard deviation for a net negative error of 4 per cent. Corrections of + 4 per cent in the soluble arsenic result in corresponding negative corrections in the fixation values; these are indicated by the lower dotted line in figure 1.

¹¹ As will become evident, the volume required depends on three factors: (a) application of arsenic; (b) time interval; (c) fixation. In the tests on effect of extract, the volumes chosen were those necessary to provide about 0.015 mg As_2O_3 with an application of 300 p.p.m. As_2O_3 and a time interval of 18 hours, these specifications applying to the data of table 1 (p. 209).

The limits indicated by solid lines apply to the balance of the soils, which require more than 0.1 and up to 1.0 ml of extract for an analysis. The maximum error in soluble arsenic due to extract in this group was + 20 per cent, the minimum + 5 per cent.

Statistical odds cannot be given for the expectation that the true values will lie between these limits. For soils midway between the extremes, with respect to effect of extract, the odds would be very high, and for soils near or at the extremes the odds would be lower.

RESULTS

Toxicity-Fixation Interrelation at 300 P.P.M. As_2O_3 .—Table 1 presents data on toxicity, arsenic fixation, and soil moisture content at field capacity in 33 California soils. Of these soils, all but one, Yolo sand, are included in the group of 80 described in the preceding paper (7). Yolo sand is an infertile sand of practically no agricultural value deposited by the flood waters of Putah Creek.

The field capacities of the soils are given in column 3 of table 1.

The data on toxicity appearing in columns 4 and 5 were taken from the unpublished toxicity curves described in the section on "Methods." Column 4 gives for each soil the approximate application reducing the yield 95 per cent in the greenhouse test. Column 5 gives the area, in square centimeters, under each toxicity curve, measured with a planimeter. These areas are, of course, significant only on a relative basis, the absolute values being determined arbitrarily by the scales adopted in plotting the curves.

The data on arsenic fixation are given in columns 6 and 7. It was originally intended to compare the soils, as to percentage fixed, at a uniform application of 300 p.p.m. As_2O_3 . The Gutzeit analyses of the sodium arsenite stock solution varied from run to run, however, and the actual applications were varied in an attempt to compensate for the supposed changes in concentration of the stock solution. It was learned later that these changes in concentration were not real, but were embraced by the inherent standard deviation of the analysis. As has been explained, the limits of error for the percentage fixed graphically presented in figure 1 include an allowance for the standard deviation.

In experiments referred to throughout the paper as the "first series," actual applications of sodium arsenite varied from 275 to 315 p.p.m. As_2O_3 . To permit a valid comparison on the basis of 300 p.p.m. applied, slight corrections were made, where necessary, by interpolation. As previously stated, different applications of arsenic were made to two samples of each soil. Each was plotted against the corresponding percentage fixed, and a straight line drawn between these points. The fixation percentage

TABLE 1
SOIL TYPE, ARSENIC TOXICITY, AND ARSENIC FIXATION AT 18 HOURS,
IN 33 CALIFORNIA SOILS

Soil characteristics			Greenhouse results		Fixation measurements at an application equivalent to 300 p.p.m. As_2O_3	
Soil grouping based upon toxicity and fixation of arsenic	Soil type, soil series, and textural grade	Field capacity (H_2O) on basis of dry soil	Application causing 95 per cent reduction in yield (As_2O_3 basis)	Area under curve	Apparent fixation	Limits of expected deviation
1	2	3	4	5	6	7
		percent	p.p.m.	sq. cm.	percent	per cent
CLASS I, toxicity limits 50 to 150 p.p.m. (col. 4); fixation limits 20 to 45 per cent (col. 6)	(Greenfield coarse sandy loam	17.4	95	5.5	22.4	19.0-35.0
	Fresno sandy loam	17.6	65	5.3	27.5	24.5-39.0
	Hanford sandy loam	27.0	140	9.7	39.3	37.0-49.0
	Hanford fine sandy loam	17.8	140	10.7	42.3	40.0-51.5
	Average for class I	19.9	110	7.8	32.9	30.0-43.5
CLASS II, indeterminate between I and III	(Delano fine sandy loam	18.4	250	16.0	35.9	33.0-46.0
	Rocklin sandy loam	21.0	450	22.0	48.8	46.5-57.0
	Rositas fine sand	17.4	100	8.9	55.6	53.5-63.0
CLASS III, toxicity limits 250 to 450 p.p.m. (col. 4); fixation limits 60 to 85 per cent (col. 6)	(Sierra sandy loam*	17.1	275	21.5	43.6	42.5-58.5
	Chino silty clay loam	25.5	250	22.9	64.3	63.5-74.0
	Columbia fine sandy loam	23.1	250	20.3	65.7	65.0-75.0
	Yolo fine sandy loam	19.8	525†	31.6	67.3	66.5-76.0
	Madera loam	15.6	425	25.0	70.3	69.5-78.5
	Yolo silt loam	23.5	300	22.3	71.8	71.0-79.5
	Yolo sand	18.2	325	29.3	72.1	71.5-79.5
	Yolo loam	25.4	300	26.2	74.4	74.0-81.5
	Yolo clay loam	35.9	350	27.0	80.5	80.0-86.0
	Yolo adobe clay‡	36.1	375	24.3	86.0	85.5-90.0
	Average for class III	24.0	337	25.0	72.5§	72.0-80.0
CLASS IV, indeterminate between III and V	(Fresno light clay	32.9	550	21.1	60.2	59.5-71.0
	Arbuckle clay loam	23.3	500	34.7	77.8	77.0-83.5
	Stockton adobe clay	42.4	700	34.7	80.3	80.0-85.5
	Montezuma adobe clay	36.8	425	40.6	86.0	85.5-90.0
CLASS V, toxicity limits 500 to 700 p.p.m. (col. 4); fixation limits 85 to 93 per cent (col. 6)	(Madera clay	32.5	600	38.9	85.1	84.5-89.0
	Sites adobe clay	28.9	625	36.9	86.9	86.5-90.5
	Yolo clay¶	34.0	900¶	80.8	87.5	87.0-91.0
	Sacramento clay loam	40.0	575	35.3	88.5	88.0-91.5
	Egbert loam	40.9	525	36.1	90.1	90.0-93.0
	Panoche adobe clay	34.2	600	44.2	90.9	90.5-93.0
	Imperial clay	35.4	650	45.4	92.5	92.5-94.5
	Average for class V	35.1	639	45.4	88.8	88.5-92.0
CLASS VI, toxicity limits 800 to 1,300 p.p.m. (col. 4); fixation limits 93 to 99 per cent (col. 6)	(Sierra gravelly loam	15.8	825	64.9	91.4	91.0-93.5
	Merced adobe clay	62.3	1,100	68.8	94.0	94.0-95.5
	Anita adobe clay	36.3	900	56.9	94.9	94.5-96.0
	Dublin adobe clay	46.2	850	77.8	95.5	95.5-96.5
	Aiken clay loam	29.2	1,300	95.0	98.6	98.5-99.0
	Average for class VI	38.0	995	72.7	94.9	94.5-96.0

* Shows a very low rate of fixation; at the end of 7 weeks is about equal to Yolo adobe clay.

† Repeat greenhouse test gave 275 p.p.m. as the application producing 95 per cent yield reduction.

‡ Shows a very high rate of fixation and consequently has too high a relative position at 18 hours.

§ Does not include Sierra sandy loam.

¶ This soil might belong in class VI. A different greenhouse test, however, gave 650 p.p.m. as the application producing 95 per cent yield reduction.

|| Being in the same series as Sierra sandy loam, this soil has probably a low rate of fixation. If so, it would have too low a relative position at 18 hours.

at 300 p.p.m. was taken from this straight-line graph. Column 6 gives the percentages fixed, corrected to a uniform application of 300 p.p.m.; column 7, the limits of deviation within which the true values of these fixations may be expected to lie, these limits having been taken from figure 1. As previously indicated, two sets of limits are included in figure 1. One set applies to the fixation data for the soils in classes I and II, table 1. These soils, which fix the least arsenic, permit Gutzeit analyses on 0.1 ml or less of 1:1 extract at 18 hours with approximately 300 p.p.m. As_2O_3 applied. The other set of limits applies to the soils in the other four classes, which furnish 1:1 extracts such that 0.1 to 1 ml is required for an analysis with the same time and application.

The correlation of textural grade with fixing power, toxicity, and field capacity is indicated by the grouping of soils into classes given in column 1. The soils in class I—all light soils—exhibit the greatest toxicity, the lowest fixation, and the lowest field capacity. One can best see these facts by comparing class averages. Although the soils of class II do not fit into either I or III as delimited, they are obviously nearer these classes than any other. They have therefore been designated as “indeterminate” between I and III. The soils of class III illustrate the fact that textural grade is not the only factor involved in arsenic toxicity. This class includes the soils of intermediate toxicity, fixing power, and field capacity, yet includes 4 sandy soils and 1 adobe clay, 3 of which belong to the Yolo series. As the preceding paper (7) indicates, the Yolo series is a poor one for present purposes of illustration. This series is of recent alluvial origin from original sedimentary sources, and all types display a high capacity for arsenic fixation.

At this point, attention may be drawn to the importance of the time factor. As will become evident, soils do not all display the same rate of fixation. Thus both Sierra sandy loam and Yolo adobe clay are indeterminate in ultimate fixing power, but the former fixes much less arsenic than the latter at 18 hours.

Class IV is again indeterminate between adjacent classes. Class V includes what might be called medium-heavy soils, although there is little, in terms of soil type, to distinguish it from class VI, which includes the soils of lowest toxicity, highest fixation, and highest field capacity. Three outstanding exceptions appear in the last two classes. Egbert loam is an organic soil. Sierra gravelly loam and Aiken clay loam are both red soils and, as has frequently been observed, possess arsenic- and phosphorus-fixing capacities beyond expectation in terms of textural grade.

The sharply delimited classes given in table 1 represent, however, only one of many possible groupings. Broader class limits would eliminate

some of the seven indeterminate soils; but one need not suppose that fixation and toxicity should be correlated in every instance when one considers that, up to the attainment of fixation equilibrium in time, other sets of fixation figures at other time intervals would doubtless give different relative as well as absolute results. Furthermore, as will become evident (p. 225), the rate of change of percentage fixation with change in application, with a fixed time interval, differs somewhat for different soils. Thus, had the soils been compared as to fixing power at some application other than 300 p.p.m., somewhat different relative as well as absolute results would probably have been obtained.

There is also the question as to which measure of toxicity is best. In the preceding paper (7), the relative toxicity of arsenic in 80 soils was made evident by arranging the yield data in a regular order according to textural grade of the soil. The toxicity curves, previously described, in which percentage yield is plotted against application (on the basis of dry soil), provide a means of more accurate comparison. At least four methods, however, may be used to obtain a measure of toxicity from the curves. One may (1) determine the applications producing some given percentage reduction in yield; (2) measure the areas under the curves as expressing an average toxicity over the entire effective range of applications; (3) determine the percentage reductions in yield caused by some given application; or (4) measure the slopes of the curves at some fixed place. The class limits set in column 1 of table 1 were determined on the basis of the application causing a 95 per cent yield reduction. This criterion of toxicity was used because it is the most practical in terms of soil sterilization. Though a yield reduction of 100 per cent is, of course, the ideal, the application for this point cannot well be determined accurately because of the increasingly gentle slope of the curves as they approach the base line.

The area under the curve is apparently an inverse measure of average toxicity over the entire range of applications; and although this average toxicity might be of considerable theoretical interest in some cases, it has less practical importance than a determination of the application necessary to sterilize the soil.

Methods 3 and 4, mentioned above, were also tried. A determination of the yield reductions resulting from an application of 200 p.p.m., for example, provides a set of figures ranging from 100 per cent in some of the soils in class I to as low as 5 per cent in one of the soils in class VI. The determination of the slopes of the curves does not provide a generally satisfactory basis for estimating toxicity because the general trend of a curve may not be correctly indicated by a particular segment of it.

In most cases the two measures of toxicity given (table 1, cols. 4 and 5) substantially agree. In some cases they do not, however. Thus, with Fresno light clay and in similar cases, a disproportionately large application is required to accomplish the last 10 or 15 per cent of yield reduction necessary to the total of 95.

Finally, one must realize that the greenhouse runs could not all be made at the same time and that if the tests were repeated on all 33 soils, differences in relative results might occur. Repeated runs on several soils are reported in the previous paper (7).

Toxicity-Fixation Interrelation at Approximately 50 Per Cent Yield Reduction.—Besides the first series of applications at approximately 300 p.p.m., another set, hereafter called the "second series," was made in connection with the fixation studies. The actual applications are tabulated in column 3 of table 2, and the reduction in yield corresponding to each appears in column 4. The reductions recorded vary between 40 and 60 per cent. In seven cases where the reductions were beyond these limits, the data are omitted.

Column 5 gives the apparent fixation, or the percentage fixed as determined, at each of the recorded applications; column 6 the limits of deviation within which the true values are expected to lie. These limits (taken from fig. 1) were originally determined for applications of about 300 p.p.m. The applications in the second series were usually, however, less than this, and the average aliquot of extract necessary for an analysis was 0.74 ml as opposed to 0.33 ml for the first series of applications of 300 p.p.m. Despite this circumstance, the limits of deviation for applications of about 300 p.p.m. provide a generally satisfactory basis for evaluating the fixation data of table 2 because the limits were set to allow for extreme effects of extract. To illustrate, an upper limit of + 20 per cent was set for the error due to extract in all classes except I and II. The extracts of many of the soils in these classes, nevertheless, would not have produced so great an error even with much larger aliquots than were actually used.

An independent consideration of the relation between toxicity and fixation is afforded by the data of column 7. Here the concentrations of soluble arsenic at 100 per cent moisture, time interval 18 hours, are recorded for each soil at a series of applications giving approximately the same biological result (40 to 60 per cent reduction of yield). The class averages in this column reveal no clearly significant difference between any one class and another, which suggests that when fixation is allowed for, all soils are alike with respect to the concentration of soluble arsenic required for a given degree of toxicity. In interpreting these data, one

TABLE 2
ARSENIC FIXATION AT APPLICATIONS CAUSING YIELD REDUCTIONS
OF APPROXIMATELY 50 PER CENT

Soil grouping	Soil type	Applica- tions of arsenic (As ₂ O ₃ basis), on basis of dry soil	Reduction in yield	Arsenic fixation measurements		Soluble arsenic (As ₂ O ₃ basis) in 1:1 extract, limits of deviation
				As de- termined	Limits of expected deviation	
1	2	3	4	5	6	7
		<i>p.p.m.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>p.p.m.</i>
Class I	Fresno sandy loam.....	25	41	40.0	37.5-49.5	15.6-12.6
	Hanford sandy loam.....	45	48	47.8	45.5-56.0	24.5-19.8
	Hanford fine sandy loam.....	57	48	56.9	55.0-64.0	25.6-20.5
	Average for class I.....	42	46	48.2	46.0-56.5	22.7-18.3
Class II	Delano fine sandy loam.....	95	58	52.8	51.0-60.5	46.5-37.5
	Rocklin sandy loam.....	72	41	64.7	63.0-70.5	26.6-21.2
	Rositas fine sand.....	55	50	55.5	53.5-63.0	25.6-20.3
Class III	Sierra sandy loam.....	115	46	56.5	55.5-68.0	51.2-36.8
	Chino silty clay loam.....	120	40	72.0	71.5-79.5	34.2-24.6
	Madera loam.....	155	53	79.0	78.5-85.0	33.3-23.2
	Yolo silt loam.....	105	40	78.4	78.0-84.5	23.1-16.3
	Yolo sand.....	137	40	82.5	82.0-87.5	24.7-17.1
	Yolo loam.....	137	40	82.0	81.5-87.0	25.3-17.8
	Average for class III.....	128	43	78.8*	78.0-84.5	28.2-19.8
Class IV	Fresno light clay.....	65	53	62.8	62.0-72.5	24.7-17.9
	Arbuckle clay loam.....	250	60	80.0	79.5-85.5	51.2-36.2
	Stockton adobe clay.....	130	45	89.5	89.0-92.5	14.3- 9.7
	Montezuma adobe clay.....	237	54	87.7	87.0-91.0	30.8-21.3
Class V	Madera clay.....	145	52	91.2	91.0-93.5	13.0- 9.4
	Sites adobe clay.....	155	45	92.7	92.5-94.5	11.6- 8.5
	Yolo clay.....	427	45	84.6	84.0-89.0	68.3-47.0
	Egbert loam.....	152	45	92.7	92.5-94.5	11.4- 8.4
	Panoche adobe clay.....	227	53	91.8	91.5-94.0	19.3-13.6
	Imperial clay.....	210	42	95.5	95.5-96.5	9.4- 7.3
	Average for class V.....	219	47	91.4	91.0-93.5	19.7-14.2
Class VI	Sierra gravelly loam.....	295	40	91.5	91.0-94.0	26.5-17.7
	Merced adobe clay.....	325	45	93.2	93.0-95.0	22.7-16.2
	Anita adobe clay.....	277	51	95.5	95.5-96.5	12.5- 9.7
	Dublin adobe clay.....	502	53	94.0	94.0-95.5	30.1-22.6
	Average for class VI.....	350	47	93.5	93.5-95.5	22.7-15.7

* Does not include Sierra sandy loam.

must again consider differences in rates of fixation; and since the toxic effect of the arsenic is being registered over a period of a month, one could not well specify a time interval that would be best from the biological standpoint. The effect of differences in moisture content at field capacity will be considered in a later section (see footnote 17, p. 225). At this point, suffice it to state that such differences apparently have less effect than might be expected.

The Time Factor in Fixation.—Table 3 gives the results of fixation measurements at approximately 7 weeks on samples of 9 of the 33 soils listed in table 1. Measurements on 4 of the same 9 samples at approximately 16 weeks, made in connection with another experiment (see fig. 3, p. 219), are also included. The soil samples used originated in standard greenhouse tests described on page 217 in connection with table 4. In the series from which the samples were obtained, each application of arsenic to the soil was replicated four times, and each can was watered daily. The cans were let stand in the greenhouse without watering for 2 days

TABLE 3
ARSENIC FIXATION AT 18 HOURS AND APPROXIMATELY 7 AND 16 WEEKS, AT AN APPLICATION EQUIVALENT TO 340 P.P.M. As_2O_3

Soil	Apparent fixation, 18 hours	Approximate 7-week interval		Approximate 16-week interval	
		Actual period	Apparent fixation*	Actual period	Apparent fixation
	<i>per cent</i>	<i>days</i>	<i>per cent</i>	<i>days</i>	<i>per cent</i>
Fresno sandy loam.....	25.7	46	72.4	105	74.5
Yolo adobe clay.....	85.0	54	89.4	116	90.4
Sierra sandy loam.....	40.8	54	87.9	119	93.3
Yolo clay.....	86.6	54	98.5	111	98.9
Greenfield coarse sandy loam.....	19.5	46	73.2
Delano fine sandy loam.....	32.5	46	72.4
Sacramento clay loam.....	87.6	49	99.8
Egbert loam.....	89.7	49	99.9
Sierra gravelly loam.....	91.0	54	99.4

* The original data on soluble arsenic, from which these fixation percentages were computed, are given in table 5 for 4 soils. In table 5 the application of 340 p.p.m. is arbitrarily called "treatment 1."

after the oat plants were harvested at the end of the standard 30-day growth period. The contents of the 4 cans were then combined, thoroughly mixed, and stored in a sealed Mason jar. The application of sodium arsenite at a rate equivalent to 340¹² p.p.m. As_2O_3 was taken in all 9 cases. As moisture determinations showed, the sandy soils had fallen to about 50 per cent of field capacity, and the others had decreased to about 85 per cent. Fixation measurements were made according to the standard procedure previously outlined in the section on "Methods," the moist equivalent of 100 grams of dry soil being taken for each extraction. For comparison, the fixation percentages at 18 hours are also included. These were corrected to the basis of 340 p.p.m. As_2O_3 applied by the method previously outlined (p. 208) for correction to 300 p.p.m.

¹² Cultures were established in the greenhouse tests containing 0, 15, 40, 80, 140, 220, 340, 490, 680, and 920 p.p.m. As_2O_3 , on the basis of oven-dry soil.

The errors in the measurement of arsenic fixation were somewhat greater than in the tests reported in tables 1 and 2. Limits of deviation for the fixation percentages cannot be given because the limits set forth in figure 1 apply only to aliquots of extract yielding approximately 0.015 mg As_2O_3 . Because of the positive error arising from the presence of soil extract, all aliquots were restricted to a total of 1 ml regardless of concentration. As fixation increases with time, the amount of arsenic in the 1-ml aliquot often falls considerably below 0.015 mg As_2O_3 , especially with heavy soils and small applications. In these cases the limits of deviation, if determined, would be somewhat farther apart than those given in figure 1.

Another possible source of error is the absorption of arsenic by the oat plants. Crafts and Kennedy (6) have shown, however, that the lethal concentration of arsenic in morning-glory is approximately 0.02 per cent As_2O_3 on the dry basis, in the tops, and 0.0003 in the roots (6, p. 339). With the higher of these figures as a basis of approximate calculation, and the highest yield recorded in the present cultures of oat plants (8.6 grams fresh weight), roughly 0.3 mg As_2O_3 would be absorbed if the root system weighed half as much as the tops, and the average content of dry matter were 10 per cent of the fresh weight. Since most of the cultures contained the moist equivalent of 500 grams of dry soil, about 0.6 p.p.m. As_2O_3 would be absorbed. The decrease in concentration of soluble arsenic would be less than this, however, due to replacement of part of the absorbed arsenic from the solid phase. Even though the arsenic tolerance of oats might be much greater than that of morning-glory, arsenic absorption would evidently not cause errors beyond the limits of error of the method itself.

Table 3 thus shows that arsenic fixation in Sierra sandy loam is approximately the same as in Yolo adobe clay at 7 and 16 weeks, although widely different at 18 hours. Yolo adobe clay shows a very slight increase in fixation after 18 hours, whereas Sierra sandy loam has apparently not accomplished all its ultimate fixation at the end of 54 days. Of the four soils studied at the longest interval, only Sierra sandy loam increased significantly in fixation after about 7 weeks.

Besides the results in table 3 other data on the effect of time are shown graphically in figure 2. In these experiments, the air-dry samples of soil were given enough total water to make 1:1 extracts at the start of the tests, as in the procedure for the 18-hour fixation tests. The arsenic application was equivalent to 250 p.p.m. As_2O_3 on the basis of oven-dry soil. The jars containing the arsenic-treated soils at 100 per cent moisture were let stand, with occasional shaking, at laboratory temperatures for

the intervals indicated, one jar for each point on each curve. The extracts were analyzed singly by the Gutzeit procedure as outlined. Comments in the preceding paragraph concerning limits of deviation for the fixation percentages apply here as well. The experiment was performed during June, July, and August, 1937. For comparison, the approximate 7-week

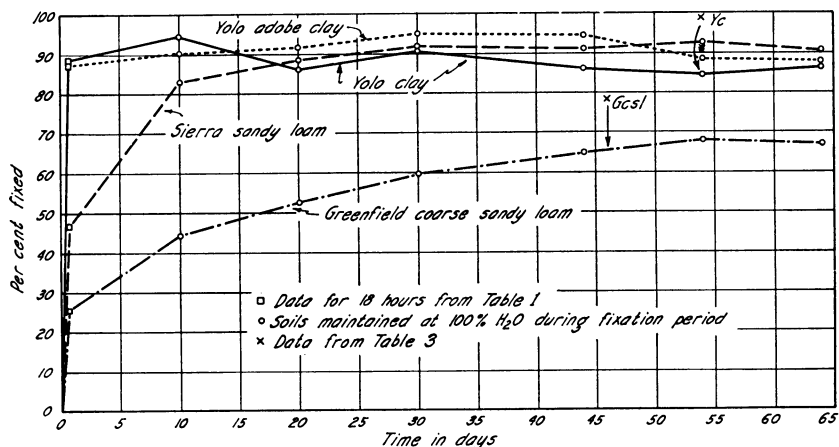


Fig. 2.—Time curves for Greenfield coarse sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay. All fixation percentages based upon an arsenic application of 250 p.p.m. As_2O_3 on the basis of dry soil.

fixation figures (table 3), corrected to a basis of 250 p.p.m. As_2O_3 applied, are plotted. Also the 18-hour figures, similarly corrected, are included as the first point on each curve.

These curves show that pronounced differences in rates of fixation do not appear after the first 10 days, even though differences in absolute level of fixation may exist throughout.

Whereas the 54-day points from table 3 for Sierra sandy loam and Yolo adobe clay lie fairly close to the curves, the 46-day point for the Greenfield coarse sandy loam appears to be significantly high, and the 54-day point for Yolo clay is unquestionably high at 99.2 per cent as opposed to 84.6 per cent on the curve. The points from table 3 represent fixation in soils held at field capacity or less, while the other points in figure 2 represent soils held at 100 per cent moisture. Apparently Greenfield coarse sandy loam and Yolo clay fix less arsenic from the 1:1 mixture than when held at a lower moisture content.

Relation of Moisture Content to Toxicity and to Arsenic Concentration.—Table 4 gives the results of standard greenhouse toxicity deter-

minations on 8 of the 9 soils¹³ listed in table 3, along with the results obtained when the cans of soil were watered daily so as to maintain more nearly a constant percentage of moisture at field capacity. The tests were performed during February and March, 1937, after the original greenhouse work on arsenic toxicity in 80 California soils had been completed. They were intended to check the greenhouse results in the 9 soils selected, and also to measure the effect of the less-frequent watering practiced in the standard greenhouse runs. In the standard series, each application was duplicated, and the cans were watered whenever the plants showed

TABLE 4
GREENHOUSE DETERMINATIONS OF ARSENIC TOXICITY UNDER DAILY
AND UNDER LESS-FREQUENT WATERING

Soils	Arsenic application (As ₂ O ₃ basis) giving 95 per cent yield reduction	
	Watered daily	Watered 5 times during 30-day growth period
	<i>p.p.m.</i>	<i>p.p.m.</i>
Fresno sandy loam.....	135	135
Greenfield coarse sandy loam.....	200	200
Sierra sandy loam.....	200	150
Delano fine sandy loam.....	250	250
Yolo adobe clay.....	335	335
Yolo clay.....	650	800
Sacramento clay loam.....	900	900
Egbert loam.....	1,000	1,000

signs of wilting. This amounted to five times during the 30-day growth period. In the other series, each application was made in quadruplicate, and each can was brought to field capacity by weight each day.¹⁴ As in the toxicity tests reported in table 1, curves were plotted relating the yield of tops, expressed as a percentage of the check, to the application of sodium arsenite as p.p.m. As₂O₃ on the basis of oven-dry soil. From these curves the applications causing a 95 per cent reduction in yield were determined as given in table 4.

Table 4 shows that in 6 cases out of 8 the results (toxicities) were identical under the two treatments, while in one case (Sierra sandy loam),

¹³ Unfavorable physical condition of the soil made it impossible to get a uniform original stand of oats on Sierra gravelly loam. The results for this soil were therefore omitted as unreliable.

¹⁴ The soil samples from two different applications for each soil in the series watered daily were saved at the end of the run, and utilized for the fixation measurements reported in table 3 at approximately 7 and 16 weeks.

the toxicity was higher with less frequent watering, and in another (Yolo clay) it was lower. Although the moisture content obviously fluctuated to some extent even in the cans watered daily, the fluctuation was much less than in the series watered only five times, wherein the moisture content undoubtedly fell to a point near the permanent wilting percentage between waterings. The fact that the toxicity was substantially unaffected by this marked decrease in moisture content suggests that the concentration of arsenic failed to increase as the moisture content diminished.

An attempt was made to secure experimental evidence on this point by determining the effect of varying the amount of water in the extract upon the concentration of soluble arsenic. Four soils of contrasting type were studied—Fresno sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay. The samples employed were the same ones used in the fixation tests at approximately 7 weeks reported in table 3. The extraction ratio tests were performed about 16 weeks after the arsenic was applied. During the period between these experiments, the soils were kept in sealed Mason jars at room temperatures. The tests were performed according to standard procedure, the moist equivalent of 100 grams of dry soil being taken for each extraction, and the extract being agitated 18 hours before being filtered and analyzed. The soil samples used were those originally given an application equivalent to 340 p.p.m. As_2O_3 (on the basis of oven-dry soil). Each experimental determination of concentration is plotted as a point in figure 3. The results with an extraction ratio of 1:1 (100 per cent water) were used to calculate the fixation percentages given in table 3 for approximately 16 weeks.

The curves of figure 3 were calculated in each case from the two experimental points marked by arrow; the adsorption equation used will be described in the next section, headed "Calculations." Each curve was extended to the moisture content at field capacity, but the lowest moisture contents reached experimentally were about twice field capacity.

For purposes of discussion, the following statement is given of the four general types of behavior which may occur when the moisture content of a soil is decreased.

1. The concentration of the given ion may rise in inverse proportion to the decreasing moisture content as, for example, with nitrate.
2. The concentration may rise but to a smaller extent than in 1. This is typical of ions which are adsorbed.
3. The concentration may remain practically constant. This is typically true with slightly soluble salts which provide a saturated solution over a wide range of moisture contents.

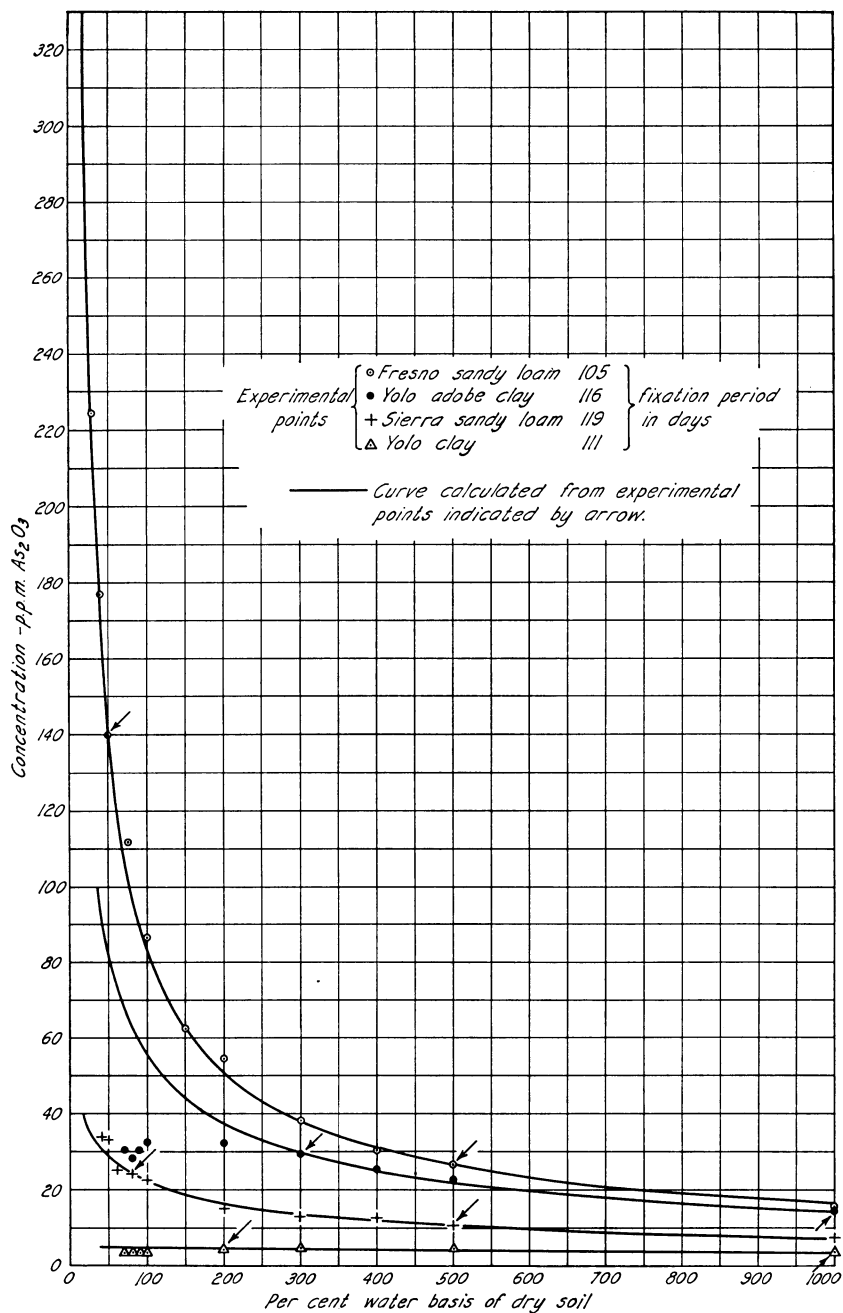


Fig. 3.—Concentration of soluble arsenic at various moisture contents for Fresno sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay. Application of sodium arsenite equivalent to 340 p.p.m. As_2O_3 on the basis of dry soil.

4. The concentration may decrease. This would happen in a mixture of slightly and readily soluble salts containing a common ion. For example, with calcium phosphate and calcium nitrate, a decrease in moisture content would increase the calcium ion concentration, and this in turn would result in a decrease in phosphate ion concentration owing to the operation of the solubility-product principle.

Behavior 1 is evidently not involved in the present study, but the other types are :

Fresno sandy loam and Sierra sandy loam constitute examples of behavior 2 over the range of moisture contents studied, as judged by the fact that the experimental points fall on or near the adsorption curves (fig. 3).

From 1,000 per cent to about 300 per cent water, Yolo adobe clay follows the adsorption curve but thereafter tends to follow first behavior 3 and then 4.

Yolo clay follows the adsorption curve fairly closely as plotted (fig. 3), but if a larger vertical scale were used, it would be seen that behavior 4 occurs between 200 per cent and 100 per cent water, and that behavior 3 apparently occurs between 100 per cent and 70 per cent. With this soil, however, the distinction between 2 and 3 is virtually nonexistent because the adsorption curve itself is practically a straight line with a very slight slope.

Returning to the toxicity data of table 4 : clearly, with soils displaying behavior 3 or 4, no increase in toxicity would be expected as a result of a decrease in moisture. True, in table 4 the decreases in moisture content were in the range of field capacity or less, but if one may judge from the trends of the points in figure 3 for Yolo adobe clay and Yolo clay, an increase in arsenic concentration would not occur as the moisture content decreased. In the cases of Fresno and Sierra sandy loams, the application of 340 p.p.m. As_2O_3 is respectively beyond and at the upper limit of the sublethal range of applications. This is brought out in the toxicity curves of figure 5 (p. 226).

As will be shown in the next section in considering figure 4, the increase in concentration with decreasing moisture is far less than in figure 3 over the sublethal range of concentrations even though the behavior is dominantly one of adsorption. This principle is well illustrated by Yolo clay. With this soil, an application of 340 p.p.m. causes a reduction in yield of 63 per cent (fig. 5), which places this application in the sublethal range. The adsorption curve is so nearly flat that there would be no appreciable increase in concentration with a decrease in moisture even though adsorption were the dominant process.

CALCULATIONS

Curves relating the percentage yield to the concentration of arsenic in the soil solution would theoretically be alike for soils of different texture if fixation were the dominant factor in toxicity. Experimental data secured were not sufficient to permit an estimate of the concentration of soluble arsenic at each of the 9 applications used in the greenhouse tests. Given experimental measurements of concentration at 2 different applications, however, it is possible to calculate concentrations at other applications. The object of this section of the paper is to demonstrate these calculations, and to present curves of percentage yield against concentration of soluble arsenic resulting from them.

The equation¹⁵ used was,

$$\log C = -\frac{3}{2} \log (F-x) + \log K,$$

wherein C signifies the concentration of soluble arsenic (p.p.m. As_2O_3), x the amount of arsenic in mg As_2O_3 fixed by 1,000 grams of dry soil, and F and K are constants. In the method of calculation used, F was first calculated from the following relations:

$$\log C_1 = -\frac{3}{2} \log (F-x_1) + \log K \text{ and}$$

$$\log C_2 = -\frac{3}{2} \log (F-x_2) + \log K. \text{ Subtracting:}$$

$$\log C_1 - \log C_2 = -\frac{3}{2} [\log (F-x_1) - \log (F-x_2)] \text{ and}$$

$$\log \frac{C_1}{C_2} = -\frac{3}{2} \log \frac{F-x_1}{F-x_2} \quad \text{and}$$

$$-\frac{2}{3} \log \frac{C_1}{C_2} = \log \frac{F-x_1}{F-x_2}. \text{ Therefore}$$

$$\log \frac{F-x_1}{F-x_2} = \frac{2}{3} (\log C_2 - \log C_1).$$

One can easily calculate the numerical value of the quantity, $\frac{2}{3} (\log C_2 - \log C_1)$, from the values of C obtained at the two different applications of arsenic made, letting C_2 be greater than C_1 . If the antilog of $\frac{2}{3} (\log C_2 - \log C_1)$ be set equal to a , then

$$a (F-x_2) = F-x_1,$$

and from this,

$$F = \frac{ax_2 - x_1}{a-1}.$$

Also,

$$F = x_2 + \frac{x_2 - x_1}{a-1},$$

and the latter expression is more convenient to use.

Having measurements of C at 2 different applications, it is then possible to locate 2 points on, and hence determine, the straight-line curve of $\log C$ against $\log (F-x)$.

¹⁵ The writers extend thanks to Dr. Herbert S. Zuckerman, who suggested this equation and assisted with the calculations.

If 1:1 extracts are used, x is equal to $A-C$, where A is the application as mg As_2O_3 per 1,000 grams of dry soil. Once the curve is plotted, as many C , x pairs as desired may be obtained from it. If it is desired to determine A corresponding to various values of C , the general formula is:

$$A = x + \frac{CW}{1,000},$$

wherein W signifies the volume of water in ml associated with 1,000 grams of dry soil. Thus, in a 1:1 extract, W is equal to 1,000 and A is equal to $x + C$. Curves of A against C at any other moisture content may be easily obtained by substituting the desired value of W .

If measurements of C are available at 2 different moisture contents, A held constant as in figure 3, it is again possible to plot the straight-line curve of $\log C$ against $\log (F-x)$. In this case

$$x = A - \frac{CW}{1,000}.$$

W corresponding to various values of C may be determined from the formula,

$$W = \frac{1,000 (A-x)}{C}.$$

This is the method which was employed in constructing the adsorption curves of figure 3, the experimental C , W pairs used being marked by arrow. These points were chosen by inspection. The shape of the curves would be little affected by the particular pairs chosen except in the case of Yolo adobe clay. The determinations in the latter case reveal a change in trend below 300 per cent water; and the points marked by arrow were therefore chosen to fit the determinations down to this percentage.

Figure 4 gives the calculated curves¹⁶ of C against A for the same four soils that were used in figure 3. All curves represent C at 100 per cent moisture ($W = 1,000$) except two of the curves for Fresno sandy loam, which were calculated to field capacity (17.6 per cent) and to the lowest extraction ratio reached experimentally in figure 3 (30 per cent). The calculations were based on the fixation data for approximately 7 weeks given in full for the 4 soils concerned in table 5. This table includes the original data from which the fixation percentages in table 3 at 340 p.p.m. were calculated. Applications listed under treatment 2 varied from soil to soil, being usually chosen as near as possible to the applications listed in table 2 (p. 213); the samples for these determinations were handled as described on page 214 in connection with table 3.

The justification for calculating these curves lies in the fact that the

¹⁶ The sharp break in the curves at values of A of about 5 p.p.m. is due to the fact that, according to the equation, there is a low value of C for each soil at which x becomes equal to zero. At values of C below this point, x becomes negative. Since a negative x has no reality significance, the curves were continued to the origin as straight lines from the point at which $x = 0$. It was not determined experimentally whether this part of the curves is correct, but if incorrect, the effect would be to make the values of C slightly too high at low values of A .

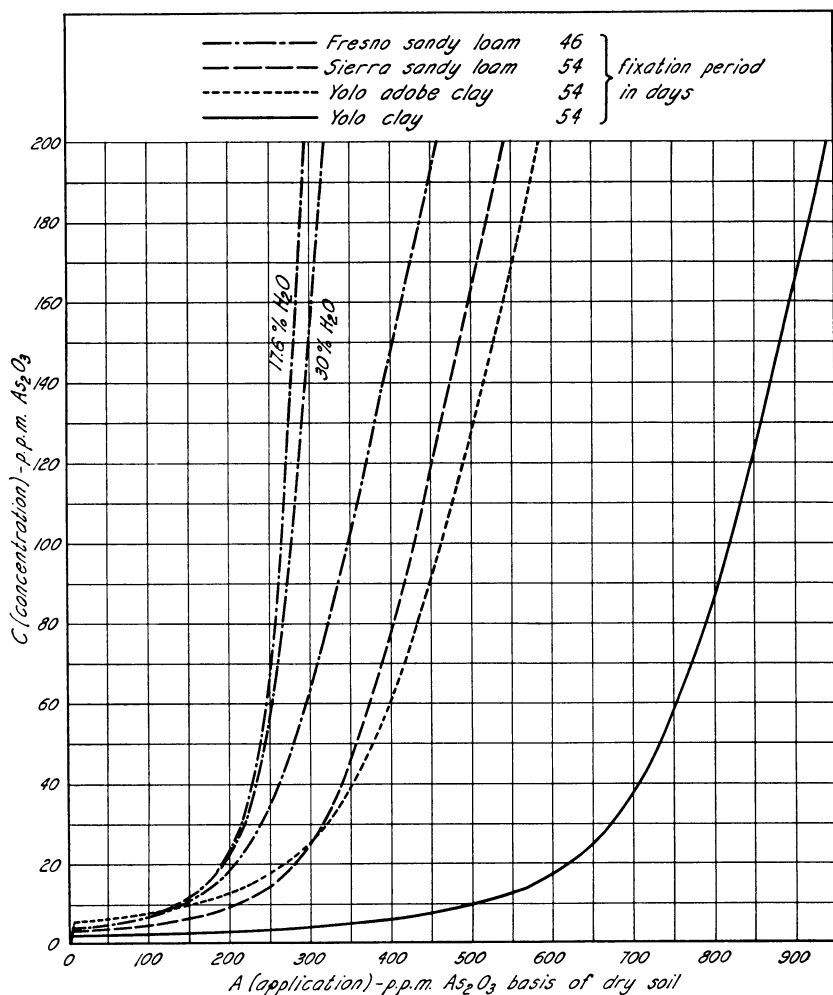


Fig. 4.— A (application) plotted against C (concentration) at 100 per cent water except as noted. Calculations based on fixation data at approximately 7 weeks given in table 5.

equation used was satisfactory in figure 3 wherever behavior 2 (adsorption) was involved. The fundamental relation is the change of x with C , and this can be tested either by varying A , the application, or W , the water content.

Regarding the time interval of approximately 7 weeks, it is clear that no interval could be defended as theoretically best, since the final greenhouse yields are related to the net effect of arsenic over a period of 30

days. The 7-week interval was chosen because, as figure 2 has indicated, a period of at least 10 days is preferable to 18 hours, and the 7-week interval was the next available. The latter, as is also evident from figure 2, gives about the same results as would be obtained at 30 days.

The values of C were calculated to 100 per cent water because this procedure was thought to give the best general approximation of the concentrations in the soil solution. Three points are involved: (a) with behavior 3 dominant, 100 per cent is best because the experimental measurements were made at this moisture content (1:1 extracts used); (b)

TABLE 5
VALUES OF C (CONCENTRATION) AND A (APPLICATION)
AT APPROXIMATELY 7 WEEKS

Soil	Actual length of period	Application (As_2O_3 basis)		Concentration of As_2O_3 (C) 1:1 extract
		Treatment No.	Rate (A) on basis of dry soil	
Fresno sandy loam.....	days 46	{ 1	p.p.m. 340	p.p.m. 94.0
		{ 2	40	4.8
Sierra sandy loam.....	54	{ 1	340	41.0
		{ 2	140	6.2
Yolo adobe clay.....	54	{ 1	340	36.0
		{ 2	140	9.5
Yolo clay.....	54	{ 1	340	5.1
		{ 2	680	31.6

with behavior 4, the moisture content at which the experimental determinations were made is again the best, since the use of a lower value would involve a calculated increase in place of an actual decrease in concentration. If the decrease is not great, the values at 100 per cent water may be a close approximation to those in the soil solution. Yolo adobe clay is the only soil of the 4 studied in which behavior 4 might be of practical moment, and in this soil (fig. 3), the trend of the experimental points below 100 per cent water, while apparently downward, appears somewhat erratic; (c) with behavior 2, the increase in concentration in going below 100 per cent water is negligible over most of the range of sublethal applications. This point is well illustrated by Fresno sandy loam. The standard toxicity curve for this soil in figure 5 shows that only 3 per cent of the check yield is obtained at an application equivalent to 140 p.p.m. As_2O_3 . Figure 4 shows that with A equal to 140 p.p.m., C is

equal to 10 p.p.m. at 100 per cent water, and 11 p.p.m. at both 30.0 and 17.6 per cent, the curves for the last-mentioned moisture contents having merged at a value for A of about 175 p.p.m. At all sublethal applications below 140 p.p.m., it evidently makes no practical difference which curve is taken. At an application of 220 p.p.m., however, there is still a 1 per cent yield (fig. 5), which places this application roughly at the upper end of the sublethal range, and at this point C increases from 24 to 35 in going from 100 per cent to field capacity. Thus, at the very lowest percentage yields, the values of C at 100 per cent water may be somewhat too low with soils showing behavior 2.¹⁷

Thus the curves of figure 4 apparently represent an approximation of the relation between the application, A , and the concentration, C , in the soil solution at the end of the greenhouse growth period. They may therefore be used to replot the toxicity curves of figure 5 on the basis of C , as above qualified, instead of A . Figure 6 gives the curves on this basis, the value of C corresponding to A for each percentage yield being taken from figure 4. Figures 5 and 6 are plotted on the same scale, and it is at once evident that the outstanding differences between the curves in figure 5 are absent in figure 6. This suggests that differences in fixation are largely responsible for the original differences between soils of different textural grades.

Although, as above noted, fixation appears to be the dominant factor in toxicity, attention may be drawn to the fact that the curves of figure 6 are not identical, a considerable divergence appearing between them below yields of approximately 30 per cent. This suggests the possibility that the concentration of water-soluble arsenic is not the only factor which may influence toxicity. If part of the insoluble or fixed arsenic were, for example, available in the case of the two sandy loams, the divergence between the curves would be largely accounted for. Part of the insoluble nutrient elements are of course known to be available in the soil, and it may be that because of replacement, less intense fixation, or other causes, some of the insoluble arsenic in certain soils may be effective biologically.

As mentioned on page 211, a comparison of soils as to fixing power at any given application is subject to the criticism that at other applications other relative as well as absolute results might prevail. This is illustrated in figure 4 by the crossing of the curves for Sierra sandy loam and Yolo

¹⁷ Points a , b , and c above indicate that the concentrations given in column 7 of table 2 at 100 per cent water represent an approximation of the concentrations in the soil solution. Roughly equal toxicities are thus associated with roughly equal average concentrations for four different classes of soils, suggesting that the soils would be alike in field and greenhouse toxicity if it were not for differences in fixing power.

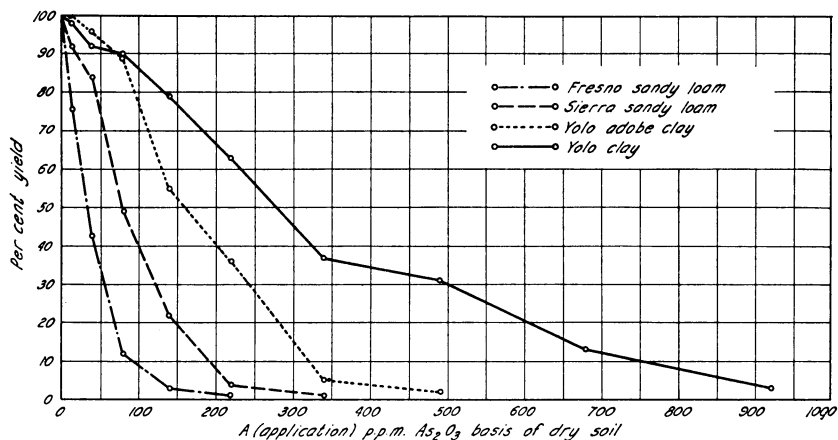


Fig. 5.—Percentage yields plotted against applications of arsenic to the soil for Fresno sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay, each point representing the mean of 3 or 4 greenhouse runs.

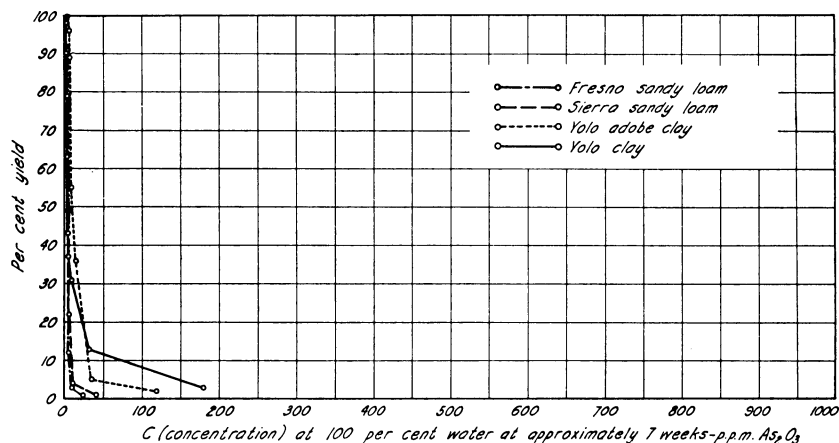


Fig. 6.—Same percentage yields as in figure 5 plotted against the calculated concentrations of arsenic at 100 per cent water taken from figure 4.

adobe clay at an application of about 305 p.p.m. Below this application, Sierra sandy loam would show the greatest percentage fixation, while above it the relation would be reversed, as shown in table 3 at approximately 7 weeks and an application of 340 p.p.m. Reversals in rank would undoubtedly occur in some cases in table 1 if comparisons were made at an application other than 300 p.p.m. Apparently, however, these reversals would involve minor shifts in position. Some of the present inconsistencies might be resolved and others accentuated, but apparently the broad relation between toxicity and fixation would be unaffected.

GENERAL DISCUSSION

The data presented in this paper lead to several general conclusions. First, different soils fix different percentages of a given application of arsenic. Second, the light soils of relatively low moisture-holding capacity, in general, fix least, and the heavy soils of high moisture-holding capacity fix most arsenic. Several exceptions are noted. Thus the red soils appear to have a high fixation capacity, apart from textural grade, because of their iron content, whether this be a chemical or physical or mixed effect. The one organic soil tested proved to have a high fixing power, though of medium textural grade. Third, as above qualified, light soils require less arsenic to sterilize them than do heavy soils. In a general sense, therefore, toxicity is inversely related to fixing power. Fourth, moisture content at field capacity is not important in determining toxicity. Thus the light soils display, on the whole, the greatest toxicity because they fix least arsenic, not because they hold little water. Fifth, considerable differences appear in the rates at which different soils fix arsenic.

With these broad conclusions in mind, one sees that the concentration of water-soluble arsenic serves as a general indicator of toxicity. As mentioned in the Introduction, other investigators (15, 11, 2) have suggested that the 0.1 N ammonium-acetate-soluble, the dilute-acid-soluble, and the dialyzable arsenic are each closely correlated with toxicity. Also (p. 225), as the present authors have indicated, part of the fixed or insoluble arsenic may be effective in causing toxicity. Judging from these observations, in the field of arsenic toxicity, as in the study of mineral nutrition, the question of "availability" is complex; in the present state of knowledge, one cannot state which fractions of the total arsenic should be considered strictly responsible for toxicity effects. The replacement of adsorbed phosphate by other anions occurs, as shown by Scarseth (12) and others; and Cooper, *et al.* (4) have observed in the field that arsenic toxicity is greatly increased by treatment with soluble phosphates. All that can be said is that toxicity is more closely correlated with water-soluble arsenic than with total arsenic, and that other fractions, more closely related to water-soluble than total, may also correlate with toxicity.

The data bearing on the effect of the amount of water upon concentration indicate that one should not use an unnecessarily large amount of water in making a soil extract for purposes of determining arsenic solubility if the results are to be reported on the basis of dry soil. Data at any convenient extraction ratio may serve certain purposes of comparison

within the limits of a given investigation, but the results obtained with 1:10 extracts by Reed and Sturgis (11), for example, are not comparable with those obtained with 1:5 extracts by Vandecaveye, Horner, and Keaton (15), or with those obtained with 1:1 extracts reported here, even though all are given on the basis of dry soil.

With behavior 2, 3, or 4, as described on pages 218–20, an erroneously high result will be obtained by multiplying the observed concentration by a factor equivalent to the extraction ratio, and the higher the ratio the greater the error.

SUMMARY

With certain exceptions, notably the red soils, light soils fix the lowest, and heavy soils the highest, percentage of a given application of sodium arsenite.

Light soils require the least and heavy soils the most arsenic to sterilize them. Toxicity is thus inversely proportional, in general, to arsenic fixation.

Arsenic fixation does not occur at a uniform rate in all soils, one soil showing an increase in fixation after 7 weeks, another showing very little after 18 hours.

Decreasing the moisture content of a soil below field capacity has no effect upon toxicity. This is apparently because, within the range of sub-lethal applications, the concentration of arsenic remains about the same in some soils and in others decreases.

Moisture content at field capacity is not an important factor in arsenic toxicity.

Curves of concentration, C , against application, A , may be constructed from two experimental determinations of concentration by the use of the equation

$$\log C = -\frac{3}{2} \log (F-x) + \log K.$$

Curves relating the percentage yield in the greenhouse tests to the concentration of soluble arsenic, plotted from the curves of C against A , were much more alike than the standard toxicity curves relating percentage yield to application of arsenic on the basis of dry soil—a fact suggesting that arsenic toxicity can be largely explained in terms of fixation.

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