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EFFECTS OF TRACE ELEMENTS ON ARYLSULFATASE ACTIVITY IN SOILS[1]

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ABSTRACT

Studies to evaluate the effects on arylsulfatase activity of 21 trace elements showed that all elements inhibited the activity of this enzyme in soils. Results showed that the relative effectiveness of trace elements in inhibition of arylsulfatase activity depends on the soil. When the trace elements were compared by using 25 μmole/g soil, the average inhibition of arylsulfatase in the four soils studied showed that Ag(I), Hg(II), B(III), V(IV), and Mo(VI) were the most effective inhibitors (average inhibition>50 percent) and that Fe(II) was the least effective (average inhibition <10 percent). Other trace elements that inhibited arylsulfatase in soils were: Cu(I), Cd(II), Sn(II), Zn(II), Cu(II), Ba(II), Mn(II), Ni(II), Pb(II), Co(II), Cr(III), Al(III), Fe(III), As(III), Se(IV), Ti(IV), As(V), and W(VI). Among these elements, Cu(II) and Fe(III) were more effective than Cu(I) and Fe(II), respectively. But As(V) was somewhat less effective than As(III).

Generally, the inhibitory effect of the trace elements studied decreased when the amount of trace element added per gram of soil decreased by 10-fold (from 25 μmoles to 2.5 μmoles). The inhibition by MoO_4^{2-}, WO_4^{2-}, AsO_4^{3-}, and PO_4^{3-} showed competitive kinetics. Related anions, such as NO_2^-, NO_3^-, Cl^-, SO_4^{2-}, did not inhibit arylsulfatase activity in soils.

INTRODUCTION

Arylsulfatases (arylsulfate sulfohydrolases, EC 3.1.6.1) are the enzymes that catalyze the hydrolysis of an arylsulfate anion by fission of the O—S bond (Spencer 1958). The reaction ($R\text{-}OSO_3^- + H_2O = R\text{-}OH + H^+ + SO_4^{2-}$) is irreversible, and there is no evidence that any sulfate acceptor other than water can be utilized, nor that any metal ion is required for their catalytic function. These enzymes have been detected in plants, animals, and microorganisms (Nicholls and Roy 1971). The presence in soils and marine sediments of arylsulfatase activity also has been reported (Tabatabai and Bremner 1970a,b; Cooper 1972; Chandramohan, Devendran, and Natarajan 1974). Arylsulfatase is believed responsible for S cycling in soils. The suggested role of this enzyme in S mineralization is largely derived from studies showing that, on the average, about 50 percent of the total S in soils is reduced to H_2S by HI and is converted to inorganic SO_4^{2-} with hot alkali (Freney 1961; Tabatabai and Bremner 1972a; Neptune, Tabatabai, and Hanway 1975). These findings indicated that this fraction of S in surface soils is present in the form of ester sulfates (organic sulfates) and suggested that sulfatases may play an important role in the processes whereby organic soil S is mineralized and made available for plant growth.

Recent concerns about environmental pollution of soils by heavy metals and other trace elements[2] have led several research centers to evaluate the toxic effects of trace elements on the biochemical processes in soils because several of these elements are added to soils as fertilizers, as impurities in fertilizers, or as components of municipal and industrial wastes (Berrow and Webber 1972). In addition, several of these elements (e.g., Pb and V) are present in fuel oils and gasoline and are emitted into the atmosphere upon burning and deposited on soils, especially near urban areas (Lagerwerff 1972; Tyler 1974, 1976).

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[2] The term *trace element* is used here to refer to elements that are, when present in sufficient concentrations, toxic to living systems.

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Although extensive research has been done on the chemistry of trace elements in soils and on the toxicity of the various elements to biological systems (Chaney 1973), little information is available about the relative effects of trace elements on the biochemical processes in soils. To our knowledge no studies have been conducted on the effects of trace elements on the enzymatic processes responsible for S cycling in soils. Because of this lack of information, this study was conducted to evaluate the relative effects of equimolar concentrations of trace elements on arylsulfatase activity in soils. An additional objective was to determine the mode of inhibition of this enzyme by several anions.

MATERIALS AND METHODS

The soils used (see Table 1) were surface soils (0–15 cm) selected to obtain a range in pH (6.2–7.6), organic matter content (2.73–4.70 percent organic C), texture (26–34 percent clay, 34–45 percent silt, and 21–39 percent sand), and arylsulfatase activity (77–190 μg of *p*-nitrophenol released/g soil/h). Before use, each soil was air dried and crushed to pass a 2-mm screen. The methods used for determination of pH, organic C, total N, total S, and texture were those reported by Tabatabai and Bremner (1972a). The analyses for organic C, total N, and total S were performed on subsamples that had been ground to pass a 100-mesh sieve. The other analyses were performed on the coarser, <2mm, soil samples.

The trace elements used were Fisher certified reagent-grade chemicals. Of these, Ag(I), Cd(II), Zn(II), Cu(II), Co(II), Fe(II), Ti(IV), and V(V) were added as the sulfate; Cu(I), Hg(II), Sn(II), Ba(II), Mn(II), Ni(II), Cr(III), Al(III), and Fe(III) were added as the chloride; Pb(II) was added as the nitrate; and As(III), B(III), Se(IV), As(V), Mo(VI), and W(VI) were added as $NaAsO_2$, $Na_2B_4O_7$, H_2SeO_3, Na_2HAsO_4, H_2MoO_4, and Na_2WO_4, respectively.

In testing the effects of trace elements on arylsulfatase activity, 1 g soil in a 50-ml Erlenmeyer flask was treated with 1 ml of solution containing either 2.5 or 25 μmole of trace element. This solution was added dropwise to moisten the whole soil sample. After a 30-min equilibration, arylsulfatase activity was assayed by the method of Tabatabai and Bremner (1970). This method involves colorimetric determination of the *p*-nitrophenol released by arylsulfatase when soil is incubated with 0.25 ml toluene, 4 ml 0.5 *M* acetate buffer, pH 5.8, (we used 3 ml of this buffer because the soil sample was treated with 1 ml of trace-element solution), and 1 ml of 5 m*M* buffered potassium *p*-nitrophenyl sulfate solution at 37°C for 1 h. The results of arylsulfatase activity with trace-element-treated soils were compared with those obtained with 1 g of soil treated with 1 ml of water. Percentage inhibition of arylsulfatase activity by each trace element was calculated from $(1 - B/A)100$, where A is arylsulfatase activity of untreated soil, and B is arylsulfatase activity of trace-element-amended soil.

In the study of determination of the mode of inhibition of arylsulfatase by MoO_4^{2-}, WO_4^{2-}, AsO_4^{3-}, and PO_4^{3-}, the 1 g soil was treated with 1 ml of solution containing 25 μmole of trace element as described, but the enzyme was assayed by adding 1 ml of buffered 5, 6.65, 10, 20, or 40 m*M* potassium *p*-nitrophenyl sulfate solution (1, 1.33, 2, 4, or 8 m*M* on soil solution basis). The results obtained were plotted in the form of the Lineweaver-Burk plot; plotting $1/V$ versus $1/S$. All results reported are averages of duplicate determinations calculated on an oven-dry basis, moisture being determined from loss in weight after drying at 105°C for 24 h.

RESULTS AND DISCUSSION

Table 2 shows that the effects of trace elements on arylsulfatase activity differed considerably among the four soils studied; Ag(I),

TABLE 1

Properties of soils used

Soil		pH	Organic C, %	Total N, %	Total S, %	Clay, %	Sand, %	Arylsulfatase activity[a]
Type	Subgroup							
Nicollet cl	Aquic Hapludoll	6.2	2.73	0.226	0.033	29	34	77
Harps cl	Typic Calciaquoll	7.6	3.24	0.274	0.047	30	31	142
Webster 1	Typic Haplaquoll	6.5	2.91	0.247	0.033	26	39	150
Okoboji 1	Cumulic Haplaquoll	7.0	5.32	0.418	0.068	34	21	190

[a] Micrograms of *p*-nitrophenol released per gram of soil per hour.

TABLE 2

Effects of trace elements on arylsulfatase activity in soils

Trace element		Percentage inhibition of arylsulfatase activity in soil specified[a]				
Element	Oxidation state	Nicollet	Harps	Webster	Okoboji	Average
Ag	I	94	99(80)	97(53)	95	96
Cu		17	7(0)	18(0)	3	14
Hg	II	96	97(34)	97(39)	86	94
Cd		27	23(7)	55(5)	42	37
Sn		60	11(9)	45(6)	32	37
Zn		20	36(11)	33(10)	17	27
Cu		22	26(19)	32(6)	17	24
Ba		24	20(13)	14(8)	13	18
Mn		20	12(10)	4(2)	10	12
Ni		26	2(0)	17(3)	4	12
Pb		10	4(0)	19(9)	11	11
Co		10	14(11)	10(9)	8	11
Fe		2	2(1)	6(4)	10	5
B	III	70	65(8)	72(31)	60	67
Cr		54	35(18)	43(20)	32	41
Al		43	42(7)	34(8)	24	36
Fe		59	8(3)	45(8)	23	34
As		17	17(9)	15(5)	18	17
V	IV	87	88(32)	90(76)	85	88
Se		32	26(14)	42(21)	26	32
Ti		33	9(3)	31(5)	10	21
As	V	2	13(6)	17(5)	7	10
Mo	VI	63	60(26)	79(40)	14	54
W		23	25(2)	38(14)	14	25
	LSD ($P < 0.05$)	3.4	2.9(2.4)	3.7(2.0)	3.0	
	($P < 0.01$)	4.6	3.8(3.3)	4.9(2.7)	4.0	

[a] Twenty-five μmole trace element/g soil. Figures in parentheses indicate percentage inhibition of arylsulfatase activity by using 2.5 μmole trace element/g soil.

Hg(II), B(III), V(IV), and Mo(VI) were, however, the most effective (average inhibition > 50 percent) inhibitors of this enzyme in soil. The average inhibition observed with the four soils by using 25 μmoles of trace element/g soil/h ranged from 54 percent with Mo to 96 percent with Ag. Also, Cu(II) and Fe(III) were stronger inhibitors than were Cu(I) and Fe(II), respectively. But As(V) was somewhat less effective than As(III). The inhibition by Cu and Fe is important because Cu(I) and Fe(II) are readily oxidized to Cu(II) and Fe(III) in soils under aerobic conditions. Reduction of the amount of trace element added per gram of soil by 10-fold (from 25 μmoles to 2.5 μmoles) decreased markedly the degree of inhibition of arylsulfatase by trace elements (Table 2). Other elements that inhibited arylsulfatase in soils were: Cu(II), Sn(II), Zn(II), Ba(II), Mn(II), Ni(II), Pb(II), Co(II), Cr(III), Al(III), Se(IV), Ti(IV), and As(V). Of the trace elements studied, only Fe(II) inhibited this enzyme in soils by < 10 percent when added at 25 μmoles/g soil.

The pH values of the trace element solutions varied considerably. They ranged from 2.1 for the Sn(II) solution to 9.6 for the As(III) and B(III) solutions. Tests indicated, however, that, with use of 0.5 *M* acetate buffer (pH 5.8), the pH of the soil-buffer mixture ranged from 5.7 with Webster and Nicollet soils to 5.9 with Harps and Okoboji soils. The deviation in pH values resulting from addition of trace elements in the presence of acetate buffer did not exceed ± 0.1 pH unit.

Metal ions may inhibit enzyme reactions by complexing the substrate, by combining with the protein-active groups of the enzymes, or by reacting with the enzyme-substrate complex. The mode of inhibition is dependent on the type of substrate used. To our knowledge, no information is available on the inhibition of arylsulfatase in soils by metal ions. The information available

about inhibition of other enzymes by metal ions, however, indicates that the inhibition is non-competitive in nature. Metal ions are assumed to inactivate enzymes by reacting with —SH groups, a reaction analogous to the formation of a metal sulfide (Shaw and Raval 1961). Sulfhydral groups in enzymes may serve as integral parts of the catalytically active sites or as groups involved in maintaining the correct structural relationship of the enzyme protein.

Experiments with 25 μmoles of NaCl and K_2SO_4/g soil indicated that the K^+, Na^+, Cl^-, and SO_4^{2-} associated with the trace elements studied did not have any effect on arylsulfatase activity in soils. Related anions, such as NO_2^- and NO_3^-, were not inhibitory. Orthophosphate added as (KH_2PO_4) at 25 μmoles/g soil, however, inhibited this enzyme in soils. Because of the similarity in the ionic structure of MoO_4^{2-}, WO_4^{2-}, AsO_4^{3-}, and PO_4^{3-}, we studied the mode of inhibition of arylsulfatase by these anions by determining the effect of substrate concentration on the initial velocity of the enzyme reaction in soils in the presence and absence of the anions indicated. The results obtained are plotted in a double reciprocal plot in Figs. 1 and 2. The results reported indicate that all four of these anions are competitive inhibitors of arylsulfatase in soils. The results reported in Figs. 1 and 2 also explain the variation in K_m values reported for

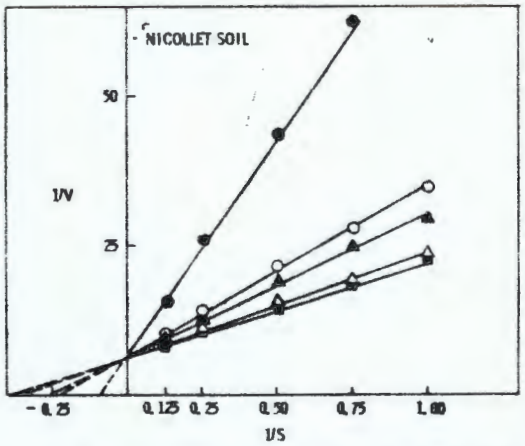

FIG. 1. Lineweaver-Burk plots of arylsulfatase in Nicollet soil in the presence and absence of selected anions (25 μmole/g soil). S and V are expressed in mM and mg of p-nitrophenol released/g soil/h at 37°C, respectively. With the anions added in parentheses, the treatments were: ■ (none); ● (MoO_4^{2-}); ○ (PO_4^{3-}); ▲ (WO_4^{2-}); △ (AsO_4^{3-}).

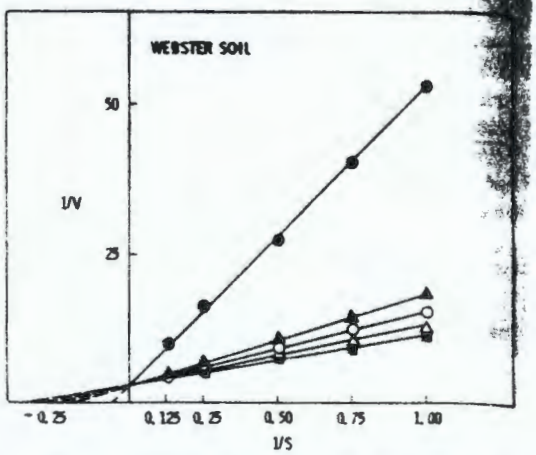

FIG. 2. Lineweaver-Burk plots of arylsulfatase in Webster soil in the presence and absence of selected anions (25 μmole/g soil). S and V are expressed in mM and mg of p-nitrophenol released/g soil/h at 37°C, respectively. With the anion added in parentheses, the treatments were: ■ (none); ● (MoO_4^{2-}); ○ (PO_4^{3-}); ▲ (WO_4^{2-}); △ (AsO_4^{3-}).

TABLE 3

Effects of selected anions on K_m values of arylsulfatase in soils

Anion added[a]	K_m values (mM) of arylsulfatase in soil specified	
	Nicollet	Webster
None	2.63	3.13
MoO_4^{2-}	11.11	20.00
WO_4^{2-}	3.85	5.88
AsO_4^{3-}	2.86	3.85
PO_4^{3-}	4.35	4.76

[a] Twenty-five μmole/g soil.

arylsulfatase in soils (Tabatabai and Bremner 1971) and explain the observed decrease in activity of this enzyme when Canadian soils were incubated under aerobic conditions for various times (Kowalenko and Lowe 1975). The variation of the K_m values of arylsulfatase in soils is clearly partly due to the variation in concentration of the soluble native orthophosphate in soils, which show competitive kinetics with respect to the substrate, potassium p-nitrophenyl sulfate, for arylsulfatase in soils; competitive inhibitors alter the K_m constants but not the V_{max}. The apparent K_m constants of arylsulfatase in the presence of inhibitors are larger than the K_m constant in the absence of inhibitors (Table 3).

The inhibition of arylsulfatase by orthophos-

phate ions is of particular importance because this enzyme has been implicated in hydrolysis of soil organic sulfates and because, besides its addition to soils as a fertilizer, orthophosphate is the end product of P mineralization in soils. Kowalenko and Lowe (1975) reported that arylsulfatase activity decreased markedly in four Canadian soils incubated at 30°C for 28 weeks and that the greatest rates of decrease were associated with the first week of incubation. For example, they reported that Prest soil, which had the highest initial activity, dropped from 190 to 9 μg *p*-nitrophenol released/g soil/h during the first 4 weeks of incubation. This observed decrease in arylsulfatase activity, the general lack of relationship between arylsulfatase activity and S mineralization, and the slow release of inorganic SO_4^{2-} during incubation of soils (Kowalenko and Lowe 1975; Tabatabai and Bremner 1972b) are perhaps due to inhibition of this enzyme by soluble native orthophosphate in soils.

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