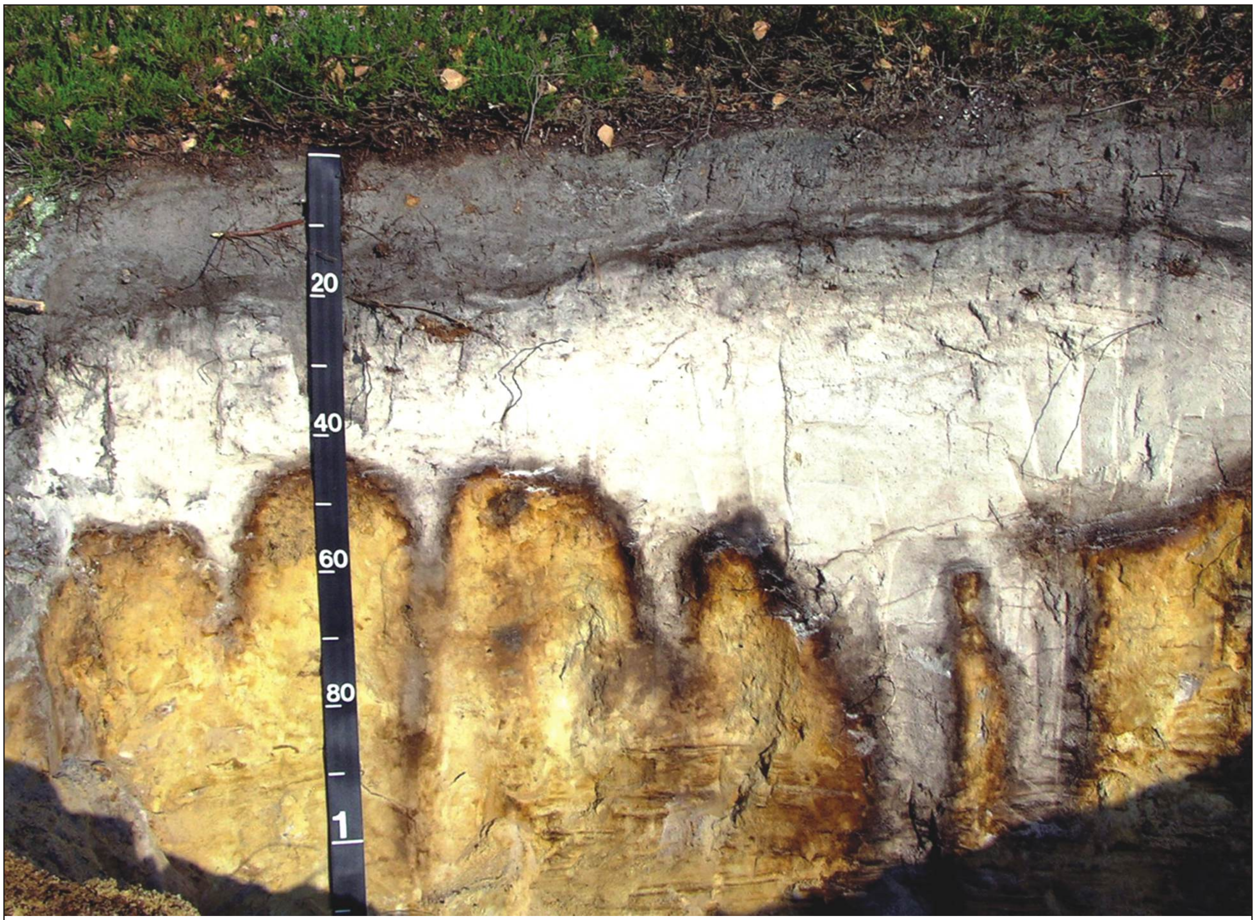

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Enzyme Activities of Laterite Soils as Influenced by Trace Element Contamination

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Abstract: The current study aimed to find out the effect of Cu^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Mo^{6+} and Al^{3+} at 25 and 75 µmole concentrations on acid phosphatase, alkaline phosphatase, aryl sulfatase and urease activities in the tea soils of south India. Soil samples, collected from four different zones, were contaminated with equimolar concentrations (25 and 75 µmole) of the above mentioned trace elements. In the absence of contamination, acid phosphatase activity was higher than alkaline phosphatase activity. Similarly aryl sulfatase activity and urease activity were higher in the soils of Munnar and Nilgiris, respectively. The acid and alkaline phosphatase activities were inhibited effectively by Cu in the soils of Anamallais and Munnar and by manganese in the case of Nilgiris. Zinc inhibited aryl sulfatase activity to a very large extent in the soils of Munnar and Vandiperiyar and molybdenum in the case of Nilgiris. Inhibition of aryl sulfatase activity was as high as 79% due to addition of copper. Urease activity was the highest in Nilgiris followed by Vandiperiyar and its inhibition was up to 81% due to copper contamination. Zinc was an effective inhibitor in Anamallais and Munnar, whereas Al and Mn played significant roles in the soils of Nilgiris and Vandiperiyar, respectively.

Key words: Acid phosphatase, alkaline phosphatase, aryl sulfatase, trace elements, urease

Introduction

Trace elements are inevitable for the growth of plants. However, when the concentration of such trace elements goes higher either by external application or by changes in soil pH the contamination takes place. The negative effect of contamination depends on the percentage weight of their concentration as well as on the physical and chemical soil characteristics like texture, organic matter, redox potential and pH. Once these elements enter the soils, they remain there for a long time without being destroyed by microorganism, whereas other polluting molecules of organic origin can be microbially degraded (Mule and Melis, 2000). Although several studies have been carried out to determine the toxic effect of trace elements on plant growth (Liang and Tabatabai, 1978; Chang and Broadbent, 1981; Kupermal and Carreiro, 1997), information relating to enzyme activities is limited in general and particularly absent in the case of tea soils. There are several ways to measure the adverse effect of trace elements on soil (Brookes, 1995), among which assaying of enzyme activity has been established as one of the important methods (Tabatabai and Bremner, 1970; Vig *et al*., 2003). In the present study efforts have been made to determine the influence of copper, manganese, iron, zinc,

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molybdenum and aluminium on the activities of the various hydrolyzing enzymes like phosphatases, urease and aryl sulfatase.

Materials and Methods

Study Site

The study site is situated in south India, which was classified into four zones depending on the elevation and rainfall pattern. The Anamallais is located on the western face of Western ghats at elevation ranging from 1000 to 1400 m above Mean Sea Level (MSL). Munnar is at an elevation of 1500-2000 m above MSL with 3000 mm annual rainfall. Nilgiris is situated on the eastern face of Western ghats at an elevation ranging between 1700 and 2000 m above MSL and receiving an annual rainfall of 1500 mm. The other study center, Vandiperiyar, is in Kerala state at an elevation of 1000 m above MSL with 2000 mm annual rainfall. The mean minimum temperatures of the study site varied between 4 and 10°C, while the maximum temperatures ranged 16-32°C. The soil characteristics of the experimental sites are given in Table 1.

Soil Sampling

An incubation experiment was performed to study the influence of trace elements Cu^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Mo^{6+} and Al^{3+} on acid phosphatase, alkaline phosphatase, urease and aryl sulfatase. From each zone 15 to 20 soil samples were taken, using sampling auger (Eijkelkamp, ME 52C09) during February 2005. The individual soil samples collected from each zone were mixed together by hand on a polythene sheet (Klose and Tabatabai, 2000). The bulk quantity of the sample was reduced by quartering method to make one composite sample.

Assay Methods

Phosphatases Activity

Dried soil from each zone was placed in 36 plastic containers, each containing one gram soil. The solutions containing the trace elements of Cu, Mn, Fe, Zn, Mo and Al were added so as to incorporate 25 and 75 μmole of trace elements per gram of soil. The treatments were replicated thrice. The phosphatase activities were assayed using the method of Tabatabai (1982) and modified by Schinner *et al*. (1996). Assay involved quantitative colorimetric determination of p- nitro phenol released by the incubation mixture at 37°C for 1 h.

Urease Activity

To five grams of soil placed in plastic containers, metal solutions were added to make the soil contaminated with 25 and 75 μmole of trace element per gram. Urease was assayed by the method of Tabatabai (1982), which involved the estimation of ammoniacal nitrogen released by incubation at 37°C for 2 h.

Table 1: Physico- chemical properties of tea soils

	Anamallais	Munnar	Nilgiris	Vandiperiyar
pH	4.40	4.70	4.10	4.40
EC (dS m^{-1})	0.15	0.28	0.28	0.11
OM (g kg^{-1})	68.00	73.00	77.00	55.00
K (mg kg^{-1})	217.00	226.00	458.00	249.00
P (mg kg^{-1})	26.00	81.00	410.00	290.00
Clay (g kg^{-1})	190.00	170.00	200.00	270.00
Silt (g kg^{-1})	100.00	90.00	160.00	140.00
Sand (g kg^{-1})	710.00	740.00	640.00	590.00

Aryl Sulfatase Activity

To one gram of soil placed in a plastic container metal solutions were added to contaminate them with 25 and 75 μmole of trace elements per gram of soil. Aryl sulfatase activity was estimated using the substrate p-nitro phenyl sulfate (Across chemical, AR grade), which involves the determination of p-nitro phenol released due to incubation at 37°C for an hour (Dick *et al*., 1996). A parallel set of soil samples was prepared and incubated at 32°C for 24 h after adding metal solutions to make the soil contaminated with 25 and 75 μmole of trace elements. Statistical analysis was carried out using the software SPSS 7.5 for Windows.

Results and Discussion

Acid and Alkaline Phosphatase Activity

In general a significant inhibition on the activity of the enzymes was noticed due to application of trace elements. It is obvious that the enzymes are primarily sensitive to pH. Addition of trace elements to the soil could alter the soil pH, thereby affecting the enzyme activities (Speir *et al*., 1999). To check this we had estimated the pH of the soil seperatively after adding the specific quantum of trace elements. It was found that there was no appreciable change in the pH of the soil as observed by various scientists (Haanstra *et al*., 1991; Pennamen *et al*., 1998; Kelly *et al*., 1999). Hence it is understood that the variation in activity was not due to variation in soil pH, when trace elements were added.

Among these four soils both acid and alkaline phosphatase activities were the highest in those of Nilgiris and Munnar. Addition of trace elements drastically reduced the activity in all cases. It is inferred from Table 2 that acid phosphatase activity was higher by 1.5 times than alkaline phosphatase activity. These results were similar to those obtained by Juma and Tabatabai (1977) in acid soil. The higher activity in the soils of Nilgiris and Munnar could probably be due to the presence of higher organic carbon. Although there are no major differences in the cultivation practices adopted in the tea gardens of south India, the activity varied from zone to zone, which could probably be due to the differences in the physico- chemical properties of these soils. Inhibition in the activity was found to be dependent on the concentration of trace element added. The suppression in enzyme activities observed due to the addition of 75 μmole of trace element was much higher than that caused by lower concentration (25 μmole). In a few cases the reduction was almost double due to higher concentration. While, Cu produced an increase in the percentage of inhibition of the acid phosphatase activities in Anamallais and Munnar, Mn and Al caused a high percentage inhibition in the soils of Nilgiris.

The acid phosphatase activities in the soils of Vandiperiyar were inhibited appreciably by Zn than by the other elements. Even at lower concentration, Zn inhibited the alkaline phosphatase activity in the soils of Anamallais and Vandiperiyar and at higher concentrations in Nilgiris. The order of inhibiting power of trace elements on acid phosphatase activity was different from alkaline phosphatase activity (Table 2 and 3).

Aryl Sulfatase Activity

The aryl sulfatase activity of tea soils of south India was estimated for the first time. The activity varied between 9 and 42 μg of p-nitro phenol formed/g soil/h under controlled conditions. Similar range of activity was earlier reported by Tabatabai and Bremner (1970) in Iowa state soils. The activity recorded in the soils of Munnar was three times higher than that of Nilgiris and Vandiperiyar and two times higher than that of Anamallais. Table 4 showed highest percentage of inhibition due to higher doses of trace elements. The activity was least affected by Zn, Mo and Al in the Anamallais,

Table 2: Effect of trace elements on acid phosphatase activity in tea soils of south India

	Acid phosphatase activity (µg of p-nitro phenol released g^{-1} soil h^{-1})							
	Anamallais		Munnar		Nilgiris		Vandiperiyar	
Trace element	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM
Control	582^{d}	582^{c}	523^{c}	523^{e}	823^{e}	823^{d}	536^{f}	536^{d}
Cu	371^{b}	278^{a}	317^{a}	149^{a}	504^{c}	384^{b}	281^{b}	276^{b}
	(36)	(52)	(39)	(72)	(39)	(53)	(48)	(49)
Mn	397^{bc}	350^{b}	427^{b}	259^{b}	284^{a}	278^{a}	397^{cd}	342^{c}
	(32)	(40)	(18)	(50)	(65)	(66)	(26)	(36)
Fe	365^{ab}	358^{b}	445^{b}	452^{c}	557^{d}	534^{c}	426^{de}	347^{c}
	(37)	(38)	(15)	(14)	(32)	(35)	(21)	(35)
Zn	384^{b}	354^{b}	481^{c}	342^{c}	514^{cd}	489^{c}	225^{a}	204^{a}
	(34)	(39)	(8)	(35)	(38)	(41)	(58)	(62)
Mo	434^{c}	341^{b}	474^{bc}	386^{c}	358^{b}	352^{b}	461^{e}	286^{b}
	(25)	(41)	(9)	(26)	(57)	(57)	(14)	(47)
Al	324^{a}	286^{a}	365^{a}	363^{c}	296^{a}	285^{a}	371^{c}	368^{c}
	(44)	(51)	(30)	(31)	(64)	(65)	(31)	(31)
SEM	22.1	24.4	26.8	28.1	21.9	31.6	20.8	23.1
p = 0.05	46.2	50.8	55.9	58.6	45.6	65.9	43.4	48.1

Figures followed by the same letter (s) in a vertical column are not significantly different at 5% level. Figures in parentheses indicate percentage inhibition of acid phosphatase activity

Table 3: Effect of trace elements on alkaline phosphatase activity in tea soils of south India

	Alkaline phosphatase activity (µg of p- nitro phenol released g^{-1} soil h^{-1})							
	Anamallais		Munnar		Nilgiris		Vandiperiyar	
Trace element	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM
Control	403^{e}	403^{d}	523^{e}	523^{f}	545^{e}	545^{e}	390^{d}	390^{e}
Cu	243^{cd}	106^{a}	229^{a}	221^{b}	288^{b}	203^{b}	221^{b}	210^{c}
	(40)	(74)	(56)	(57)	(47)	(63)	(43)	(46)
Mn	247^{cd}	153^{abc}	260^{a}	169^{a}	223^{a}	200^{b}	288^{c}	269^{d}
	(39)	(62)	(50)	(67)	(59)	(63)	(26)	(31)
Fe	187^{ab}	181^{bc}	281^{ab}	274^{c}	269^{ab}	373^{d}	175^{a}	103^{a}
	(54)	(55)	(46)	(47)	(51)	(32)	(55)	(74)
Zn	153^{a}	147^{ab}	337^{bc}	329^{d}	369^{cd}	82^{a}	164^{a}	155^{b}
	(62)	(64)	(35)	(37)	(32)	(85)	(58)	(60)
Mo	281^{d}	183^{bc}	344^{c}	297^{c}	318^{bc}	300^{c}	274^{c}	251^{d}
	(30)	(55)	(34)	(43)	(42)	(45)	(30)	(36)
Al	211^{bc}	197^{c}	424^{d}	407^{e}	371^{d}	122^{a}	163^{a}	98^{a}
	(48)	(51)	(19)	(22)	(32)	(78)	(58)	(75)
SEM	19.8	22.7	27.0	14.3	24.5	28.6	20.4	15.2
LSD (0.05)	41.3	47.3	56.3	29.8	51.1	59.7	42.5	31.8

Figures followed by the same letter (s) in a vertical column are not significantly different at 5% level. Figures in parentheses indicate percentage inhibition of alkaline phosphatase activity

by Al in Nilgiris and by Cu in Vandiperiyar. The concentration of trace elements had positive influence on inhibiting nature. Among the various elements added Cu caused maximum inhibition (79%) when compared to control in the soils of Anamallais. The activity of this enzyme could be inhibited even up to 90% by using 300 mg of Cu per kg of soil (Kandeler *et al*., 1996). Zinc inhibited aryl sulfatase activity to a maximum extent in the soils of Munnar and Vandiperiyar, whereas it was due to Mo and Al in the case of Nilgiris.

Table 4: Effect of trace elements on aryl sulphatase activity in tea soils of south India

	Aryl sulphatase activity (µg of p- nitro phenol released g^{-1} soil h^{-1})							
	Anamallais		Munnar		Nilgiris		Vandiperiyar	
Trace element	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM
Control	15^{e}	15^{e}	33^{c}	33^{d}	10^{d}	10^{c}	11^{e}	11^{d}
Cu	3^{a}	3^{a}	21^{b}	15^{b}	$7b^{c}$	7^{b}	9^{d}	6^{c}
	(80)	(80)	(36)	(55)	(30)	(30)	(18)	(45)
Mn	8^{b}	6^{b}	21^{b}	20^{c}	7^{bc}	6^{b}	6^{c}	5^{b}
	(47)	(60)	(36)	(39)	(30)	(40)	(45)	(55)
Fe	10^{c}	8^{c}	20^{b}	11^{a}	5^{ab}	4^{a}	8^{d}	4^{ab}
	(33)	(47)	(39)	(67)	(50)	(60)	(27)	(64)
Zn	12^{d}	10^{d}	14^{a}	13^{ab}	6^{abc}	6^{b}	3^{a}	5^{ab}
	(20)	(33)	(58)	(61)	(40)	(40)	(73)	(55)
Mo	12^{d}	11^{d}	17^{ab}	11^{a}	4^{a}	3^{a}	$5b^{c}$	3^{a}
	(20)	(27)	(48)	(67)	(60)	(70)	(55)	(73)
Al	12^{d}	10^{d}	21^{b}	19^{c}	8^{cd}	3^{a}	4^{ab}	4^{ab}
	(20)	(33)	(36)	(42)	(20)	(70)	(64)	(64)
SEM	0.6	0.8	2.5	1.8	1.0	0.8	0.7	0.6
LSD (0.05)	1.2	1.7	5.2	3.7	2.0	1.6	1.5	1.3

Figures followed by the same letters in a vertical column are not significantly different at 5% level. Figures in parentheses indicate percentage inhibition of aryl sulphatase activity

Table 5: Effect of trace elements on urease activity in tea soils of south India

	Urease activity (ammoniacal nitrogen formed g^{-1} soil $2h^{-1}$)							
	Anamallais		Munnar		Nilgiris		Vandiperiyar	
Trace element	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM	25 µM	75 µM
Control	32^{d}	32^{e}	27^{d}	27^{d}	52^{c}	52^{c}	46^{e}	46^{c}
Cu	27^{c}	26^{d}	17^{b}	14^{b}	42^{b}	41^{b}	24^{d}	18^{b}
	(16)	(19)	(37)	(48)	(19)	(21)	(48)	(60)
Mn	16^{a}	15^{b}	17^{b}	14^{b}	42^{b}	39^{ab}	12^{a}	9^{a}
	(50)	(53)	(37)	(52)	(19)	(25)	(74)	(80)
Fe	21^{b}	18^{c}	16^{b}	13^{ab}	45^{b}	39^{ab}	19^{c}	16^{b}
	(34)	(44)	(41)	(52)	(13)	(25)	(59)	(65)
Zn	16^{a}	13^{ab}	12^{a}	11^{a}	42^{b}	41^{b}	15^{b}	10^{a}
	(51)	(59)	(56)	(59)	(19)	(21)	(67)	(78)
Mo	22^{b}	14^{ab}	21^{c}	19^{c}	38^{a}	39^{ab}	14^{ab}	10^{a}
	(31)	(56)	(22)	(30)	(27)	(25)	(70)	(78)
Al	14^{a}	12^{a}	20^{c}	18^{c}	38^{a}	36^{a}	20^{c}	16^{b}
	(56)	(63)	(26)	(33)	(27)	(31)	(57)	(65)
SEM	1.0	1.0	1.0	1.1	1.5	1.6	1.0	1.3
LSD (0.05)	2.0	2.1	2.0	2.3	3.2	3.3	2.0	2.7

Figures followed by the same letters in a vertical column are not significantly different at 5% level. Figures in parentheses indicate percentage inhibition of urease activity

Urease Activity

Activity of urease was the highest in Nilgiris followed by Vandiperiyar, when no trace elements were added. The soils collected from the Nilgiris were richer in organic matter, which could be contributed to the higher urease activity. The activity observed at Munnar was nearly half of that observed at Nilgiris (Table 5). This study assumes significance because 90% of the annual nitrogenous fertilisers are applied in the form of urea, which needs urease for its conversion into plant available form. Hence it is postulated that the urea hydrolysis would be better and faster in Nilgiris followed by Vandiperiyar. The maximum inhibition (81%) was noted due to addition of copper at 75 µmole concentration in the soils of Vandiperiyar. The higher percentage of inhibition (94%) has already been reported by Eivazi and Tabatabai (1977) in Harbs soils when 5 μ mole of trace element was added/g of soil. Lowest inhibition of urease was found due to the addition of iron in the soils of Nilgiris. It had been reported that the content of iron is higher in the case of Nilgiris (Venkatesan *et al*., 2003), which could be one of the reasons why urease was not effectively inhibited by addition of iron.

Zinc was the effective inhibitor in Anamallais and Munnar soils whereas it was Al and Mn in the soils of Nilgiris and Vandiperiyar. At analyzing percentages inhibition of urease induced by trace elements, the urease activity was generally lower in the soils of Nilgiris when compared to other soils studied. The percentage of inhibition varied between 13 and 29 in the soils of Nilgiris, which could be due to higher organic matter status. Similar kind of postulation was already made by Chandler *et al*. (1997).

Conclusions

Tea plants receive zinc and copper through foliar applications, which are now proved to inhibit various enzyme activities in tea soils. Hence care should be taken to avoid spillage while spraying. Since tea soils are richer in iron and aluminium, care should be taken to reduce their availability by frequent liming operations.

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