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Effect of Pesticide Residues on Microbiological and Biochemical Soil Indicators in Tea Gardens of Darjeeling Hills, India

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Abstract: The aim of the present work was directed to investigate the status of microbiological and biochemical soil indicators in relation to both the residue level of major pesticide groups (e.g., organochlorine and organophosphorus) commonly used and the tea garden management practices generally followed per year in tea gardens of Darjeeling Hills, India. The soils were collected from conventional and organically managed tea gardens during summer (April) and winter (November). The soils collected from the adjacent forests served as the control. The results showed that all the parameters (e.g., microbial biomass carbon (MBC), soil respiration (basal (BSR) and substrate induced (SIR)), fluorescein diacetate hydrolyzing activity (FDHA) and β-glucosidase activity) were higher in organically managed tea garden and control than the conventionally managed gardens both in summer and winter. The pesticide residues were exclusive to the conventionally managed tea gardens recording comparatively higher values in winter. Canonical correlation analysis (CCA) revealed that 90% of the total variation was associated with the negative impact of certain pesticides (e.g., α-endosulfan and endosulfan sulfate) on MBC and SIR during summer. The CCA data from winter revealed that 86% of the total variation was associated with the negative impact of chlorpyriphos, β–endosulfan and ethion on β–glucosidase activity.

Key words: Tea soil, Pesticide residues, Microbial biomass carbon, Soil respiration, Fluorescein diacetate hydrolyzing activity, β-_glucosidase activity

INTRODUCTION

Tea is an important beverage used all over the world and has great impact on the economy of several tea producing countries. The specific input management practices particularly that of pesticides application associated with the cultivation makes it a unique plantation crop in the perspective of soil biology and fertility. Tea (*Camellia sinensis* L.) produced in Darjeeling Hill, in India, often referred to as the champagne of India, holds the place of pride in the world. It is the icon of tea exports of India. Maintaining quality commensurate with sustained productivity is of vital importance to the Darjeeling tea industry. Trends show a decline in crop productivity, which might be related to natural ageing of the tea bushes and loss of soil quality as well. Presence of pesticide residues in Darjeeling tea is also on record [1].

The tea plantations of Darjeeling Hill region of West Bengal, India are traditionally tea-growing belts where both conventional and organically managed tea cultivations exist. Conventional tea gardens largely depend on chemical inputs, such as fertilizers and pesticides for boosting production. In contrast, organic tea gardens use wide varieties of manures and composts as plant nutrient supplements and bio-pesticides exclusively to combat pests, avoiding the chemical pesticides.

Microbial and biochemical parameters of soil are choice indicators of soil quality evaluations [2], because of their early responses to soil disturbances than those of the physical and chemical parameters. Such information for the tea soils are limited [3] and there is a complete lack of information for Darjeeling Hill region. Due to the

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limitations of a single method, wide ranges of parameters are suggested to assess soil quality. Some baseline parameters like microbial biomass carbon, soil respiration (both basal and substrate induced), β-glucosidase activity and fluorescein diacetate hydrolyzing activity are considered as classical indicators for describing soil environments [3].

The aim of this work was thus to develop an understanding of the limited information available with regard to the soil quality parameters in conventionally and organically managed tea garden soils in different seasons. Concurrently, we also tried to estimate the pesticide residues in tea garden soils and establish the canonical relationship of these pesticide residues on the soil quality parameters during different seasons.

MATERIALS AND METHODS

Soil: In this study, soils were collected from five different tea gardens in the Darjeeling Hill region receiving different management practices plus two adjacent forests consisting of evergreen trees served as control. The sampling locations are described in (Table 1). The age of the tea bushes ranged from 8 to12 years. The organic tea gardens received 120-180 t/ha/year well-decomposed cow dung manure and leaf litter. Manual weeding and spraying of bio-pesticides are the intercultural operations done in the organic tea gardens. On the other hand, conventional tea gardens received 120:90:170 kg/ha/year N, P and K respectively. The fertilizers urea and muriate of potash to supply N and K, respectively, while single superphosphate and diammonium phosphate fertilizers were used to supply P. A variety of chemical insecticides and herbicides were used to control pests in the conventional tea gardens.

Soil samples were collected from each tea garden by dividing into six transects with an area of 100 x 50 m. Surface soils (0-15 cm depth) from three portions of each transect were collected and composited in to one sample during summer (April) and winter (November) of 2006. The sampling times were synchronized during the first flush period in the summer (March-May) and dormancy period of tea in the winter (November-February). In case of the controls, six samples were collected from each of the forests. Altogether 42 samples from the Darjeeling Hill region were collected and brought to the laboratory in properly sealed sterile polythene bags.

Soil Sample Preparation: Visible plant debris and fauna were removed from the collected soil samples by hand and the soils were gently sieved (< 2 mm fraction) and stored in sealed polythene boxes at 4°C before microbial and biochemical analysis. A portion of the soil samples was air-dried and the same was used for physico-chemical analyses. Another portion of the collected soils was stabilized at 25°C for seven days, after adjusting to 60% of its water holding capacity, before the determination of microbial and biochemical parameters. The results are expressed on an oven dry weight (105°C, 24 hour) basis of soil.

Determination of Pesticide Residues in Soil: The following pesticides commonly used in tea cultivation were selected for the study: organophosphorus (e.g. ethion and chlorpyriphos) and organochlorines (e.g. heptachlor, dicofol, α-endosulfan, β-endosulfan and endosulfan sulfate). Analytical standards of these pesticides were obtained from ACCU standard, USA (purity > 98%). Standard stock solutions (50 mg/L) were

Table 1: A list of the sampling locations in the present study

Sampling site	Management practice	Location	
		Latitude	Longitude
Makaibari Forest Land	Control	26° 48' 55" N	88° 15' 31" E
Margaret's Hope Forest Land	Control	26° 57' 47" N	88° 15' 58 " E
Makaibari Tea Garden	Organic	26° 50' 48" N	88° 15' 52" E
Ambootia Tea Garden	Organic	27° 02' 36" N	88° 15' 47" E
Mullotaar Tea Garden	Organic	26° 42' 21" N	88° 15' 30" E
Margaret's Hope Tea Garden	Conventional	26° 59' 22" N	88° 15' 44 " E
Tindharia Tea Garden	Conventional	26° 39' 37" N	88° 16' 54 " E

prepared in n-hexane. All other solvents and chemicals were of analytical grade from E-Merck (Merck India, Mumbai, India).

Organophosphorus and organochlorine pesticides were detected by a gas-liquid chromatograph Hewlett-Packard 6890 series, equipped with an auto sampler, nitrogen-phosphorus detector (NPD) and electron capture detector (ECD). Pesticide residues in air-dried soil (50 g) were determined by extraction with acetone (200 mL) in Soxhlet apparatus for 24 hours. After centrifugation and concentration with rotary vacuum evaporator at 40°C, the extracts were combined and partitioned with dichloromethane (100 + 50 + 50 ml). The aqueous layer was discarded and the organic layer was passed through anhydrous sodium sulfate. The filtrate was centrifuged and concentrated with rotary vacuum evaporator at 40°C. A chromatographic column was washed with n-hexane and then packed with anhydrous sodium sulfate, silica gel and florisil (5 g each). The sample extract was added to it and the solvent was allowed to settle and collected drop wise in a conical flask. The sample extract was eluted off from the column with 200 ml hexane-ethyl acetate (8:2; v/v).The eluate was centrifuged, concentrated to 5 ml with rotary vacuum evaporator at 40°C and the volume was made up by n-hexane (10 ml).

Organophosphorus pesticides were detected with NPD fitted with fused silica semi capillary (HP-5) column (30 m x 0.32 mm x 0.25 µm). The operating conditions were: injector temperature 160°C, detector temperature 300°C, initial oven temperature 160°C for 1 min, raised to 250°C at 10°C/min and then held at 250°C for 4 min, again raised to 270°C at 10°C/ min and finally held at 270°C for 4 min. The carrier gas was nitrogen at 10 ml/min and the injection volume was 3µl. Organochlorine pesticides were detected with ECD fitted with fused silica semi capillary (HP-5) column (30 m x 0.32 mm x 0.25 µm). The operating conditions were: injector temperature 190°C, detector temperature 300°C, initial oven temperature 190°C for 1 min, raised to 200°C at 10°C/min and then held at 200°C for 1 min, again raised to 270°C at 10°C/min and finally held at 270°C for 5 min. The carrier gas was nitrogen at 10 ml/min and the injection volume was 1µl.

Quantification of pesticides was accomplished using standard curves prepared by diluting the stock solutions of the standard pesticides in n-hexane to levels at which good linearity were achieved in the range of 0.5 to 2.0 ppm with a coefficient of variation between 0.9990 to 0.9997. Overall limit of sensitivity or detection limit for soil was 0.01 ppm. The recoveries for each of the pesticides were calculated from the addition of respective standards (obtained from Accu standard, USA) to the control soils. The average recoveries of the pesticides were 88 to 90.3%. Determinations of the various pesticide residues were carried out as per the recovery study. The retention times of ethion and chlorpyriphos were 9.9 and 7.3 min. and that of heptachlor, dicofol, α-endosulfan, β endosulfan and endosulfan sulfate were 3.2, 3.8, 5.0, 5.8 and 6.4 min. respectively.

Determination of Physico-Chemical Properties of Soil: The pH (1:2.5 H_2O), cation exchange capacity, organic C, Total N, available P, available K were determined by standard methods [4].

Microbiological Assays: The MBC of soil was determined by fumigation-extraction method [5] followed by determination of the K_2SO_4 extractable C (6) of fumigated and unfumigated soils. MBC was estimated using the following relationship: MBC = 2.64 E_C, where E_C is the difference of K_2SO_4 extractable C between the fumigated and unfumigated soils [6]. Basal and substrate (glucose) induced respirations, FDHA and β-glucosidase activity of the soil samples were determined by standard methods [7-9].

Statistical Analysis: Analysis of variance (ANOVA) and Duncan's Multiple Range Test were done using STATISTICA 7.0 statistical software package (Statsoft, USA). Canonical correlation analysis (CCA) was frequently applied as a valuable technique to identify associations and their significance between different dependent variables [10-12]. In this study, CCA was employed for assessing the relationship between the predictor set of variables as well as pesticide residues and soil microbiological and biochemical parameters as dependent set. Such analysis reveals the maximum correlation coefficient between linear combinations of predictor set and that of dependent set.

RESULTS AND DISCUSSION

Physico-Chemical Parameters: The studied physicochemical properties had statistical variation between the soils collected from five tea gardens plus two control sites (Table 2). The textural class of the soils varied from sandy loam, loam to silty loam with 35-70% sand, 19 to 50% silt and 11 to 15% clay (Data not shown in the table).

Table 2: Physico-chemical properties of soils (± SD) collected from Darjeeling hill region during April and November

	pH (1:2.5) H_2O		CEC (cmol (p+)/kg)		Organic Carbon (g/kg)		Total N (g/kg)		Available P (kg/ha)		Available K (kg/ha)	
	Month		Month		Month		Month		Month		Month	
Sampling Site	April	November	April	November	April	November	April	November	April	November	April	November
Makaibari Forest Land	4.52 e	4.52 e	13.00 b	13.00 b	44.00 a	42.00 b	3.00 c,d	3.20 b	42.00 b	38.00 c	311.00 b	287.00 b
	(±0.11)	(±0.11)	(±0.08)	(±0.10)	(±1.79)	(±1.55)	(±0.24)	(±0.42)	(±3.74)	(±2.97)	(±2.76)	(±6.03)
Margarets Hope Forest Land	5.20 c	5.20 c	11.17 d	11.00 d	32.50 b	31.00 c	2.40 e	2.80 b	38.00 b	36.00 c	223.00 c	238.00 c
	(±0.05)	(±0.04)	(±0.08)	(±0.12)	(±2.35)	(±1.41)	(±0.15)	(±0.24)	(±4.00)	(±2.37)	(±2.90)	(±4.73)
Ambootia Tea Garden	4.95 d	4.96 d	12.00 c	12.00 c	45.50 a	46.00 a	2.50 e	2.30 c	41.00 b	47.00 a	228.00 c	235.00 c
	(±0.07)	(±0.06)	(±0.28)	(±0.37)	(±1.16)	(±1.26)	(±0.29	(±0.24)	(±4.00)	(±3.79)	(±4.98)	(±6.87)
Makabari Tea Garden	5.60 a	5.60 a	14.00 a	13.67 a	34.00 b	31.50 c	2.70 d,e	3.00 b	31.00 c	50.00 a	139.00 d	157.00 d
	(±0.09)	(±0.10)	(±0.11)	(±0.20)	(±2.17)	(±2.45)	(±0.19)	(±0.24)	(±3.69)	(±3.29)	(±4.43)	(±5.10)
Mullotaar Tea Garden	4.90 d	4.91 d	11.83 c	11.83 c	33.50 b	31.50 c	4.30 a	3.80 a	48.00 a	43.00 b	344.33 a	322.00 a
	(±0.13)	(±0.12)	(±0.18)	(±0.30)	(±0.81)	(±0.65)	(±0.32)	(±0.32)	(±3.46)	(±4.10)	(±7.01)	(±4.82)
Margarets Hope Tea Garden	5.37 b	5.37 b	14.00 a	14.00 a	28.00 c	29.50 d	3.30 b,c	3.60 a	25.00 d	30.00 d	351.00 a	282.00 b
	(±0.07)	(±0.07)	(±0.11)	(±0.05)	(±1.09)	(±1.15)	(±0.44)	(±0.47)	(±3.69)	(±3.41)	(±6.07)	(±5.51)
Tindharia Tea Garden	5.20 c	5.20 c	12.00 c	12.00 c	26.50 c	28.50 d	3.50 b	3.00 b	32.00 c	36.00 c	132.00 d	152.00 d
	(±0.07)	(±0.07)	(±0.14)	(±0.14)	(±1.01)	(±1.31)	(±0.24)	(±0.24)	(±4.52)	(±3.58)	(±4.05)	(±5.73)
Mean	5.11	5.11	12.57	12.50	34.90	34.30	3.10	3.10	36.71	40.00	246.90	239.00

(a,b...) alphabets denote Duncan's Multiple Range test results

Table 3: Microbiological and biochemical properties of soils (± SD) collected from Darjeeling hill region during April and November

	Microbial biomass carbon (µg/g soil)		Basal soil respiration (µg CO_2–C/g soil/h)		Substrate induced soil respiration (µg CO_2–C/g soil/h)		Fluorescein diacetate hydrolyzing activity (µg fluorescein/g soil/h)		β-glucosidase Activity (µg pnp/g soil/h)	
	Month		Month		Month		Month		Month	
Sampling Site	April	November	April	November	April	November	April	November	April	November
Makaibari Forest Land	1269a	753b	4.60a	2.95b	8.60b	5.76c	220a	129a	107b	57a
	(±12.46)	(±9.30)	(±0.10)	(±0.06)	(±0.13)	(±0.09)	(±6.03)	(±5.22)	(±3.46)	(±3.69)
Margarets Hope Forest Land	724e	669c	2.75d	1.93e	6.84d	5.95b	175b	109c	82d	45b
	(±7.62)	(±6.00)	(±0.75)	(±0.04)	(±0.07)	(±0.04)	(±5.29)	(±3.94)	(±3.02)	(±3.22)
Ambootia Tea Garden	997b	770a	3.45b	3.25a	8.89a	7.10a	181b	120b	108b	55a
	(±5.22)	(±9.65)	(±0.18)	(±0.07)	(±0.07)	(±0.24)	(±4.94)	(±5.69)	(±2.90)	(±3.95)
Makabari Tea Garden	744d	563b	2.95c	2.75c	6.20e	5.00d	141c	103d	90c	41c
	(±6.72)	(±10.64)	(±0.09)	(±0.09)	(±0.08)	(±0.24)	(±5.40)	(±2.83)	(±5.48)	(±3.58)
Mullotaar Tea Garden	906c	610d	2.70d	2.18d	7.90c	4.35e	139c,d	129a	123a	55a
	(±6.16)	(±10.33)	(±0.15)	(±0.06)	(±0.13)	(±0.07)	(±6.32)	(±6.51)	(±3.79)	(±3.90)
Margarets Hope Tea Garden	532g	341g	2.63d,e	1.72f	5.75f	3.48d	133e	85e	56f	34d
	(±8.34)	(±10.53)	(±0.09)	(±0.08)	(±0.09)	(±0.09)	(±4.94)	(±3.10)	(±4.43)	(±4.43)
Tindharia Tea Garden	671f	438f	2.50e	1.50g	6.75d	3.13g	109f	81e	74e	33d
	(±6.69)	(±6.32)	(±0.13)	(±0.07)	(±0.10)	(±0.03)	(±4.73)	(±3.74)	(±3.58)	(±3.58)
Mean	835	592	3.08	2.33	7.28	4.97	157	108	91	46

(a,b...) alphabets denote Duncan's Multiple Range test results

The pH, CEC, organic carbon and total N contents averaged over soils collected from 5 tea gardens and two control sites were more or less similar during the month of April and November (Table 2). The average available P content of soil during November was higher than in April. However, the soil contained higher average available K in April than November. The soils under study were acidic in nature and there was significant variation between them. Soils from different tea gardens and the control sites also varied in respect of CEC, organic carbon, total N and available P and K. Organic carbon and available phosphorus contents of the organically managed tea garden, i.e. Makaibari, Ambootia and Mullootaar (as certified by Institute for Marketecology, Switzerland) were higher than those of the conventional gardens. On the contrary, total N and available potassium showed higher values in the conventional gardens (Table 2).

Microbiological and Biochemical Parameters: The MBC (µg/g) of the soils under study ranged from 532 to 1269 during April and 341 to 770 during November (Table 3). The mean MBC of soils during April (835) was higher than

Table 4: Pesticide residues of soils (ppb) (±SD) collected from Darjeeling hill region during April and November

	Ethion		Chlorpyriphos		Heptachlor		Dicofol		α- Endosulfan		β- Endosulfan		Endosulfan sulfate	
	Month		Month		Month		Month		Month		Month		Month	
Sampling Site	April	Nov.	April	Nov.	April	Nov.	April	Nov.	April	Nov.	April	Nov.	April	Nov.
Makaibari Forest Land	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Margarets Hope Forest Land	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Ambootia Tea Garden	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Makabari Tea Garden	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mullotaar Tea Garden	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Margarets Hope Tea Garden	21.20	28.50	25.20	257.50	82.10	160.70	BDL	310.50	22.00	35.20	21.00	38.90	27.40	45.00
	(±0.49)	(±0.63)	(±0.60)	(±1.76)	(±0.46)	(±0.49)	BDL	(±0.40)	(±0.48)	(±0.96)	(±0.56)	(±0.81)	(±0.83)	(±0.63)
Tindharia Tea Garden	28.70	67.00	24.30	98.00	78.90	149.40	BDL	64.90	30.50	31.80	28.00	31.80	32.10	64.90
	(±0.45)	(±4.80)	(±0.82)	(±1.59)	(±1.19)	(±0.58)	BDL	(±0.61)	(±0.73)	(±1.31)	(±0.93)	(±0.65)	(±0.42)	(±0.82)
Mean	24.95	47.75	24.75	177.75	80.50	155.05	BDL	187.70	26.25	33.00	24.50	35.35	29.75	54.95

that observed in November (592). In the Darjeeling Hill region the ambient temperature during winter stays around 6 to 15°C, which is much lower than that in summer (19 to 30°C). Strong positive correlation between mean annual temperature and MBC has been suggested [13, 14]. Several reports are available on the effect of season on microbial biomass. These temporal variations might have been driven by environmental factors (temperature and moisture) and crop growth [15]. Measures of MBC showed higher degrees of seasonality having summer maxima and winter minima [16]. Along with the effect of low temperature, higher amount of pesticide residues in soil (Table 4) during the month of November might be another contributing factor in lowering the MBC contents of the soils. Data indicated that there were large differences in MBC between the soils collected from different tea gardens. The organically managed tea garden soils and the control soils maintained higher MBC than those of the conventional tea garden soils (Table 3), which could be related to higher organic carbon in the former group of soils [17].

The basal soil respirations (μg CO_2-C/g soil/h) varied from 2.50 to 4.60 during April and 1.50 to 3.25 during November with the mean value being higher during April (3.08) than November (2.33) (Table 3). The same trend also occurred for substrate-induced respiration with higher mean values in April (7.28) than in November (4.97) with the values ranging between 5.75 to 8.89 in April and 3.13 to 7.10 in November. The basal soil respiration was studied as a measure of overall, potential microbial activity [18]. Furthermore, the substrate-induced soil respiration was also determined to measure the total physiologically active part of the soil microflora [19]. Both the methods are commonly applied to characterize the microbiological status of soils [20]. Data indicated that the organically managed gardens and the control sites showed higher microbial activity with higher physiologically active microorganisms than the conventional gardens. Higher mean values of soil respiration (basal and substrate) during April might be ascribed to relatively higher MBC content and lower amount of pesticide residues during this period.

The FDHA (μg fluorescein/g soil/h) assay is an important tool in measuring overall microbial activity as this assay is mediated simultaneously by the non-specific proteases, lipases and esterases. The mean values of FDHA were higher in April than in November with the range varying from 109 to 220 in April and 81 to 129 in November. The β-glucosidase is an important enzyme in terrestrial carbon cycle in producing glucose, which contributes important energy source for microbial biomass [21]. β-glucosidase (μg *p*-nitrophenol/g soil/h) activity followed the trend of FDHA, with higher values in April than in November and the values ranged from 56 to 123 in April and 33 to 57 in November. Data revealed that the studied enzymatic activities were higher in organically managed gardens as well as the control sites than the conventional gardens. This could be ascribed to higher organic carbon in the former group of soils. Soil enzyme activities are highly positively correlated with soil organic matter [22]. Higher enzyme activities of soil during April seemed to be related to higher soil microbial biomass during this period, as microbial biomass is the most potential source of soil enzymes.

Table 5: Canonical correlation results of the soils collected from Darjeeling hill region during April and November

	Canonical Weights (Independent set) April				Canonical Weights (Independent set) November Variable			
	Root 1	Root 2	Root 3	Root 4	Root 1	Root 2	Root 3	Root 4
Ethion	-0.105	-1.093	21.685	27.558	1.422	0.006	-9.778	-9.831
Chlorpyriphos	-1.362	6.783	-23.248	1.891	7.737	-8.236	-66.399	-14.378
α endosulfan	1.583	-4.493	14.877	-8.120	-0.004	0.931	-0.856	14.803
β endosulfan	-0.983	1.563	-6.106	-18.835	3.880	1.615	-34.856	38.045
Endosulfan Sulfate	1.030	-1.758	8.587	24.925	0.057	-0.664	-1.884	-2.459
Dicofol	0.000	0.000	0.000	0.000	-4.610	7.239	43.403	-7.277
Heptachlor	-1.196	-1.044	-16.211	-28.144	-9.268	-0.265	69.658	-23.527
Canonical Weights (Dependent set)								
Variable	Root 1	Root 2	Root 3	Root 4	Root 1	Root 2	Root 3	Root 4
Microbial Biomass Carbon	-1.126	4.260	-3.427	-3.168	0.135	-3.316	-0.416	0.282
Basal Soil Respiration	0.178	-2.470	3.316	0.404	0.038	0.055	0.034	1.295
Substrate Induced Respiration	-0.514	-0.224	0.676	2.031	0.395	2.039	0.167	-0.116
Fluorescein diacetate hydrolyzing Activity	1.170	-0.657	-1.520	0.399	1.179	1.201	-2.523	-1.144
β -glucosidase Activity	1.435	-1.153	1.627	0.559	-0.704	0.180	3.102	-0.168
Eigenvalues (Canoni)								
Variance	Root 1	Root 2	Root 3	Root 4	Root 1	Root 2	Root 3	Root 4
Value	0.904	0.452	0.087	0.001	0.858	0.684	0.204	0.005
Variance Extracted (Proportions) (Independent Set)								
Factor	Root 1	Root 2	Root 3	Root 4	Root 1	Root 2	Root 3	Root 4
Variance Extracted	0.976	0.016	0.003	0.002	0.830	0.152	0.015	0.001
Redundancy	0.883	0.007	0.000	0.000	0.712	0.104	0.003	0.000
Variance Extracted (Proportions) (Dependent Set)								
Factor	Root 1	Root 2	Root 3	Root 4	Root 1	Root 2	Root 3	Root 4
Variance Extracted	0.398	0.161	0.050	0.070	0.740	0.018	0.086	0.097
Redundancy	0.360	0.073	0.004	0.000	0.636	0.012	0.017	0.001

Pesticide Residues: The distribution of organophosphorus and organochlorine pesticide residues in the studied soils are presented in (Table 4). The two conventional tea gardens had variable levels of pesticide residues (both OP and OC) in the soils and the mean values were higher in November than April. None of the organically managed gardens responded positively to the presence of these xenobiotics.

Canonical Correlation Analysis: To study the effect of pesticides on soil microbiological and biochemical parameters during both the seasons, we employed canonical analysis technique. The results are given in (Table 5). Resultant canonical weights of both sets of variable along with redundancy study and "accounted for variances" were described.

During both the seasons the first two pairs of canonical variables were highly correlated. In April, for the first variate we observed that the dependent set (microbial and biochemical parameters) and independent set (pesticide residues) share about 90% of their variance, while in November sharing was about 86%. Inspection of the canonical weights depicted that during April the first pair of canonical variates is dominated by α-endosulfan followed by endosulfan sulfate of the independent set and is negatively correlated with MBC and SIR of the dependent set. The first pair was also dominated by chlorpyriphos, heptachlor and β-endosulfan but was negatively associated with β glucosidase and FDHA of the dependent set.

In November, the scenario was a bit different, where the first pair of canonical variates was dominated by chlorpyriphos, β-endosulfan and ethion of the independent set and had negative impact on β-glucosidase of the dependent set. The first pair was also dominated by heptachlor, dicofol of the independent set and was negatively correlated with FDHA of the dependent set. For the second variate during April the first pair shared about 45% of the variance and was dominated by chlorpyriphos, β-endosulfan had negative impact on BSR, β glucosidase followed by FDHA of the dependent set. These pair was also dominated by α-endosulfan, endosulfan sulfate, ethion, heptachlor but were negatively correlated with MBC. As that of the first variate, the scenario was different in November for the second pair of canonical variates. The second pair shares about 68% of their variance. Here the independent set was dominated by dicofol, β-endosulfan, α-endosulfan and were negatively correlated with MBC. This pair was also dominated by chlorpyriphos, endosulfan sulfate and heptachlor of the independent set and was negatively associated with SIR, FDHA of the dependent set.

From the variance study we observed that 83% of the variability in the independent set variables and 74% of the variability in the dependent set were accounted for by the first set of canonical variates during November, while in April 98% of the variability in the independent set variables and 40% of the variability in the dependent set are accounted for by the first set.

During April, the redundancy study shows that 89% of the independent set variability can be explained by the dependent set and 43% of the dependent set variability can be explained by the independent set. In November, redundancy study revealed that 82% of the independent set variability can be explained by the dependent set and 65% of the dependent set variability can be explained by the independent set.

CONCLUSIONS

Sustainable crop production and soil quality are intimately related. That the management system for tea cultivation has a strong impact on soil quality is evident in this study. Data indicated that soil quality is better preserved in organically managed gardens. Pesticide residues could not be detected in organically managed tea gardens. However, these were found in the conventional tea gardens recording higher amount in winter dormancy period. These residues, albeit in ppb, exerted strong negative impact on microbiological and biochemical parameters, which are important attributes of soil quality. From the point of view of sustainable tea production and quality, as also environmental benefits organically produced tea is preferable.

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REFERENCES

1. Bishnu, A., K. Chakrabarti, A. Chakraborty and T. Saha, 2009. Pesticide residue level in tea ecosystems of Hill and Dooars regions of West Bengal, India. Environ. Monit. Assess., 149(1-4): 457-464.
2. Winding, A., H.R. Kerstin and M. Rutgers, 2005. The use of microorganisms in ecological soil classification and assessment concepts. Ecological and Environmental Safety, 62(2): 230-248.
3. Han, W., J.K. Sarah and P.C. Brookes, 2007. Soil Microbial biomass and activity in Chinese tea gardens of varying stand age and productivity. Soil Biol. Biochem., 39(7): 1468-1478.
4. Jackson, M.L., 1967. Soil chemical analysis. Prentice Hall of India, New Delhi, India.
5. Joergensen, R.G., 1995. Microbial Biomass. In: Methods in Applied Soil Microbiology and Biochemistry. Alef, K. and P. Nannipieri (Eds.). Academic Press, London, pp: 382-386.
6. Vance, E.D., P.C. Brookes and D.S. Jenkinson, 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem., 19(6): 703-707.
7. Alef, K., 1995. Estimation of soil respiration. In: Methods in Applied Soil Microbiology and Biochemistry. Alef, K. and P. Nannipieri (Eds.). Academic Press, London, pp: 215-216.
8. Schnürer, J. and T. Rosswall, 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol, 43(6): 1256-1261.
9. Alef, K. and P. Nannipieri, 1995. β-glucosidase activity. In: Methods in Applied soil microbiology and Biochemistry. Alef, K. and P. Nannipieri (Eds.).Academic Press, Harcourt Brace and Company, London, pp: 350-352.
10. Dillon, W.R. and M. Goldstein, 1984. Multivariate Analysis-Methods and Applications. John Wiley & Sons Inc, New York, pp: 337-358.
11. Tan, Z.X., R. Lal, N.E. Smeck, F.G. Calhoun, R.M. Gehring and B. Parkinson, 2003. Identifying associations among soil and site variables using canonical correlation analysis. Soil Science, 168(5): 376-382.
12. Braimoh, A.K., A. Stein and L.G. VlekPaul, 2005. Identification and mapping of associations among soil variables. Soil Science, 170(2): 137-148.

13. Piao, S.C., G.S. Liu, Y.Y. Wu and W.B. Xu, 2001. Relationships of soil microbial biomass carbon and organic carbon with environmental parameters in mountainous soils of southwest china. Biol. Fertil. Soils, 33(4): 347-350.
14. Bijayalaxmi-Devi, N. and P.S. Yadava, 2006. Seasonal dynamics in soil microbial biomass C, N and P in a mixed-oak forest ecosystem of Manipur, North-east India. Appl. Soil Ecology, 31(3): 220-227.
15. Debosz, K., P.H. Ramussen and A.E. Pederson, 1999. Temporal variations in microbial biomass carbon and cellulolytic enzyme activity in arable soils: effects of organic matter. Appl. Soil Ecology, 13(3): 209-218.
16. Bardgett, R.D., R.D. Lovell, J. Hobbs and S.C. Jarvis, 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grassland. Soil Biol. Biochem., 31(7): 1021-1030.
17. Jenkinson, D.S. and J.N. Ladd, 1981. Microbial biomass in soil measurement and turnover. In: Soil Biochemistry. Paul, E.A. and J.N. Ladd, (Eds.). Marcel Dekker Inc, N.Y., pp: 415-471.
18. Gray, T.R.G., 1990. Methods for studying the microbial ecology of soil. In: Methods in microbiology. Grigorova, R. and J.R. Norris (Eds.). Academic Press, London, pp: 309-342.
19. Anderson, J.P.E. and K.H. Domsch, 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem., 10: 215-221.
20. Stenberg, B.M., M. Pell and L. Torsteensson, 1998. Integrated evaluation of variation in biological, chemical and physical soil properties. Ambio, 27(1): 9-15.
21. Tabatabai, M.A., 1994. Soil enzymes. In: Methods of Soil Analysis. Part 2. Microbial and Biochemical properties. (Weaver, R.W., J.S. Angel and P.S. Bottomley (Eds.). Soil Science Society of America, Madison, W.I., USA, pp: 775-783.
22. Gianfreda, L. and J.M. Bollag, 1996. Influence of natural and anthropogenic factors on enzyme activity in soil. In: Soil Biochemistry. Stotzky, G. and J.M. Bollag (Eds.). Marcel-Dekker Inc, N.Y., USA, pp: 123-193.