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Impact of Tetracycline on Population Dynamics of Soil Microorganisms

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ABSTRACT

The field studies were conducted to determine the effects of three antibiotics tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC) at three different concentrations (50, 100 and 200 mg kg^{-1} soil) on total bacterial population present in two alluvial soils of Aligarh, one irrigated with tube well water and other by sewage water at four depths (0-15; 15-30; 30-45 and 45-60 cm) for a period of 91 days. Nutrient agar medium was used for the count of bacterial population. The results obtained from studies revealed that in presence of studied antibiotics significant reduction in the number of bacterial population occurs up to 21-35 days of application in tube well water irrigated soil and 35-42 days of application in sewage water irrigated soil and thereafter population of bacteria increased. The bactericidal activity of antibiotics was in the order CTC> OTC>TC. The results also showed that with increase of antibiotics concentration and soil depth bacterial population decreased appreciably. A comparison of results of both studies revealed that bacterial population in sewage irrigated soil was higher than tube well water irrigated soil. The % reduction of bacterial population in presence of antibiotics was lesser in sewage irrigated soil than tube well water irrigated soil, suggesting that soil irrigated by sewage water might have antibiotics resistant bacteria. Soil organic matter was significantly correlated with soil bacterial population.

Key words: *Antibiotics, Tetracycline, Sewage water, Microorganisms, Bacteria.*

INTRODUCTION

Maintenance of good soil quality is of prime importance for sustainable agriculture. Soil biology is a significant component of soil quality and microorganisms play vital roles in soil fertility and primary production through organic matter decomposition and nutrient cycling[1, 2]. When some stress factors such as temperature, extreme pH or chemical pollution are imposed on a natural environment, soil biota can be affected as well as the micro-organisms regulate the ecological processes Pharmaceuticals are used in humans, animals and aquatic farming for disease control and for maintenance of health. Certain activities such as disposal of expired medicine in the sewage system, excretions of unmetabolized pharmaceuticals from humans and animals, discharge of wastewater and surface runoffs to receiving water[3, 4], land application of biosolids and manure or disposal of biosolids at landfill can result in the dispersion of these compounds in the environment[5, 6]**.** Tetracyclines are broad spectrum antibiotics widely used as growth promoters in modern animal husbandry. Tetracycline administered in humans and animals undergo minimal or no metabolism and are excreted in urine and manure in an either unaltered or as metabolites some of which are still bioactive[7], which makes them potentially hazardous to bacteria and other organisms in the environment.

Tetracyclines might affect microorganisms by reducing their numbers, biochemical activity, diversity and changing the microbial community structure[8-9] Soil micro flora is the first biota that undergoes direct and indirect impacts of toxic substances introduced to soil. Due to its fast response to contaminants, ability size and recycling of elements soil micro flora is suitable to act as a "biomarker" reflecting the negative effects of antibiotics treatment and is commonly used in ecotoxicological tests to evaluate the influence of chemicals on soil system[10, 11.] This study was conducted to evaluate the effect of different rates of tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC) on the population of soil bacteria at the depth of 0-15 cm; 15-30 cm; 30-45 cm and 45-60 cm from agricultural field irrigated by sewage effluent.

MATERIALS AND METHODS

Experiments were conducted in two plots of 2x2 m size marked by wooden pegs during the months of September-December 2012 at Aligarh (27°54'30"N, 78°4'26" E). One of the plots was irrigated by tube well water and the other by sewage water. The experimental sites were having no history of antibiotics usage. The experimental area belongs to semi arid climate with an average annual temperature of 24.6°C and annual rainfall of 600 mm. The soils were classified as alluvial typic ustochrept, with soil (1:2.5) pH 8.2 and 8.1; organic carbon 4.8 and 5.9 g kg^{-1}; soil ammonium- nitrogen 11.2 mg and 7.6 kg^{-1}, nitrate-nitrogen 65.2 and 68.9 mg kg^{-1}, soil P 8.6 and 8.9 mg kg^{-1}, soil K 106 and 95 mg kg^{-1}respectively for tube well water and sewage water irrigated soils respectively (Table 1).

After removing the vegetation cover the soil was treated with 50, 100 and 200 mg kg^{-1} soil with three antibiotics separately. The different concentration of antibiotics was prepared in water and applied to soil in each plot using Knapsack sprayer. In control only water was applied. The experiments were conducted in triplicate.

Core sampling method was used in which soil samples were collected from four spots at the depth of 0-15, 15-30, 30-45 and 45-60 cm from each plot weekly for 13 weeks to determine the bacterial population. After thoroughly mixing the soil samples of each plot, the soil samples were placed in sterile polythene bags separately and transferred to laboratory for microbial analysis. All the soil samples were stored at 4°C.

The bacteria were determined in each soil sample using dilution plate technique[12]. The composition of media was as: Nutrient agar : (peptic digest of animal tissue 5g ; sodium chloride 5 g ; beef extract 1.5 g ; agar 15g). The plates were inoculated with 0.1mL of soil suspensions and cultured at 28± 2°C for seven to ten days.

RESULTS AND DISCUSSION

The data of physico-chemical properties (Table 1) denoted that the soil irrigated by sewage water has higher organic matter content than tube well water irrigated soil, while pH of tube well water irrigated soil was slightly higher than sewage irrigated soil. The data also denoted that in both the studied soils the organic matter content decreased with increase in soil depth.

The results indicated that bacterial population was affected by antibiotics. The observed effects were correlated with antibiotics type, its doses and time of exposure.

An examination of Table 2 denote that the bacterial population in tube well water irrigated soil in presence of all the three concentrations of antibiotics at all the studied depths decreased significantly with increase in time of application up to 21-35 days (21 days for TC; 35 days for CTC and 28 days for OTC) the decrease in bacterial population in top soil (0-15 cm) was maximum in CTC treated soil @ 200mg kg^{-1} after 35 days of application ($10x10^6$ from $104x10^6$). The bacterial population increased thereafter. The increase of bacterial population after 21-35 days of application of antibiotics might be due to dissipation of antibiotics and/ or due to development of antibiotics resistant bacteria. These phenomena could be expected as a result of utilization of applied antibiotics as a source of carbon and other nutrient elements by soil bacteria. The results also showed that bacterial population decrease with increase in antibiotics concentration. The bactericidal activity of antibiotics was in the order CTC> OTC>TC. The results also showed that with increase of soil depth the bacterial population in control soil and % decrease of bacterial population in antibiotics treated soils decreased. The decrease in bacterial population with depth may be correlated with soil organic matter and lesser leaching of antibiotics due to their high adsorption in soil.

The results of Table 3 denote that bacterial population in sewage water irrigated soil was higher than tube well water irrigated soil and % decrease in bacterial population was lower than tube well water irrigated soil at all the concentrations for three studied antibiotics, indicating a positive correlation in between bacterial population and organic matter and presence of antibiotics resistant bacteria in sewage water irrigated soil. The results also showed that bacterial population decreased up to 35-42 (35 for TC and OTC and 42 days for CTC) of application. The bacterial population also decreased with increase in antibiotics concentration or soil depth.

Table 1: Physicochemical Properties of the sewage effluent irrigated soil at different soil depths

PARAMETERS	TUBE WELL WATER IRRIGATED SOIL				SEWAGE EFFLUENT IRRIGATED SOIL			
Depths(cm)	0-15	15-30	30-45	45-60	0-15	15-30	30-45	45-60
pH	8.2	8.2	8.4	8.5	8.1	8.1	8.2	8.2
Organic matter (%)	4.78	4.52	3.74	2.28	5.92	5.33	3.85	2.74
Ammonium- nitrogen (mg $kg^{-1)}$	11.2	9.8	6.6	5.2	7.6	5.4	4.4	3.8
Nitrate-nitrogen(mg $kg^{-1)}$	65.2	60.5	50.4	34.2	68.9	63.4	52.4	33.4
Soil P (mg $kg^{-1)}$	8.6	7.8	5.9	4.7	8.9	7.5	7.0	5.1
Soil K (mg $kg^{-1)}$	106	92	78	54	95	84	63	42

Table 2: Effect of three antibiotics on bacterial population in soil ($x10^6$ g^{-1} soil) in tube well water irrigated soil at different depths

0-15 cm

Days after treatment	Control	TC			CTC			OTC		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	127	106	95	74	102	90	66	104	93	70
7	116	71	40	24	63	32	19	66	36	21
14	120	59	25	19	52	21	15	55	23	17
21	110	44	17	12	38	13	10	41	15	12
28	115	65	32	24	30	11	9	35	13	11
35	104	75	45	33	26	10	8	58	24	21
42	110	86	63	42	45	25	15	78	42	34
49	118	97	71	47	74	48	27	91	59	42
56	116	104	76	53	89	61	42	97	66	48
63	109	111	88	65	101	72	55	106	82	60
70	118	118	97	77	110	88	68	114	92	66
77	108	123	108	89	115	95	80	120	100	84
84	116	125	112	108	118	104	95	115	110	100
91	120	122	118	116	120	110	112	125	115	108

LSD for days = 4.2; LSD for days = 4.4 (TC); LSD for days = 4.0; LSD for doses = 4.9 (CTC)
LSD for doses = 4.1; LSD for doses = 4.7 (OTC)

15-30 cm

Days after treatment	Control	TC			CTC			OTC		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	110	94	84	78	88	80	72	92	83	75
7	106	62	48	36	53	38	25	60	42	30
14	110	52	38	28	44	31	20	50	33	24
21	105	47	28	19	40	20	15	39	23	17
28	109	60	40	28	35	17	13	35	20	14
35	104	70	48	38	32	15	12	52	36	25
42	110	78	56	47	54	27	21	68	50	36
49	102	87	66	50	74	40	34	82	57	45
56	106	94	72	64	82	54	45	92	68	52
63	101	96	80	72	91	68	52	98	76	65
70	108	98	91	83	98	84	64	94	89	72
77	108	103	105	95	105	90	73	100	96	88
84	102	95	102	98	108	96	85	105	100	94
91	110	102	98	101	100	100	94	99	101	100

LSD for days = 4.4; LSD for days = 3.9 (TC); LSD for days = 3.8; LSD for doses = 4.4 (CTC)
LSD for doses = 4.7; LSD for doses = 3.9 (OTC)

30-45 cm

Days after treatment	Control	TC			CTC			OTC		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	90	76	65	50	60	45	32	68	54	44
7	92	62	48	34	50	38	26	58	30	23
14	91	44	34	26	34	28	19	40	30	21
21	88	30	26	20	25	20	15	26	25	17
28	92	41	34	26	22	18	13	23	20	15
35	94	54	49	38	20	16	12	42	35	28
42	90	66	58	44	36	29	21	61	48	34
49	98	77	69	48	48	42	35	69	56	45
56	96	84	78	57	64	56	46	78	69	54
63	90	88	84	67	74	72	54	84	79	62
70	88	88	83	72	82	81	64	87	82	70
77	92	93	88	85	85	86	78	82	85	78
84	96	95	89	92	88	84	90	95	88	84
91	94	96	92	90	90	88	90	92	90	90

LSD for days =3.1; LSD for days = 3.3 (TC); LSD for days = 3.2; LSD for doses = 3.5 (CTC)
LSD for doses = 3.2 LSD for doses = 3.7 (OTC)

45-60 cm

Days after treatment	Control	TC			CTC			OTC		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	66	52	40	34	42	32	28	48	36	32
7	62	42	33	26	33	26	19	37	30	23
14	64	35	26	21	26	20	16	32	24	19
21	60	30	22	15	20	16	13	26	19	14
28	58	38	30	26	18	24	12	24	16	12
35	62	44	36	32	16	21	11	36	28	21
42	60	48	42	38	28	19	20	44	39	30
49	62	56	48	45	38	34	32	52	46	40
56	58	58	53	50	52	43	42	54	50	48
63	58	62	57	54	58	54	48	56	52	51
70	62	58	52	56	56	58	52	54	50	53
77	64	56	55	59	55	55	54	58	56	54
84	62	63	62	58	58	64	55	62	60	58
91	64	62	62	60	59	62	58	60	60	58

LSD for doses= 2.7; LSD for days = 3.1(TC); LSD for days = 2.9; LSD for doses = 3.2 (CTC)
LSD for doses = 2.8; LSD for doses = 3.0 (OTC)

Table 3: Effect of three antibiotics on bacterial population in soil (x10^6 g^{-1} soil) in sewage water irrigated soil at different depths

0-15 cm

Days after treatment	**Control**	**TC**			**CTC**			**OTC**		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	175	156	132	111	142	110	95	150	120	102
7	176	138	112	98	114	92	75	124	101	88
14	170	116	84	69	98	70	54	106	76	62
21	166	100	68	42	78	58	30	88	63	36
28	175	88	60	34	65	50	26	77	54	30
35	174	75	55	31	49	42	24	62	48	27
42	180	84	73	54	45	35	20	80	66	51
49	178	115	89	77	69	58	36	98	84	73
56	166	134	102	98	94	82	64	118	97	93
63	169	151	132	115	118	104	88	136	126	111
70	178	168	150	128	140	128	110	144	140	136
77	168	173	158	145	155	135	130	150	148	142
84	176	165	152	158	158	144	135	160	152	150
91	170	172	168	166	160	156	148	165	155	148

LSD for days =4.8; LSD for days = 5.2 (TC); LSD for days = 4.2; LSD for doses = 5.0 (CTC)
LSD for doses = 4.7; LSD for doses = 5.1(OTC)

15-30 cm

Days after treatment	**Control**	**TC**			**CTC**			**OTC**		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	150	136	120	100	112	96	82	125	108	92
7	156	118	100	78	94	74	55	105	88	68
14	152	106	80	52	84	56	36	96	72	45
21	155	90	65	32	62	44	22	77	54	26
28	149	78	50	24	50	30	16	64	42	20
35	154	68	41	21	40	24	14	54	33	18
42	150	84	64	34	36	20	12	75	51	32
49	152	105	80	62	64	40	21	91	66	60
56	146	124	95	88	95	63	39	111	88	80
63	151	136	118	105	114	88	68	128	104	100
70	148	140	126	118	126	105	88	134	116	112
77	148	143	138	125	132	121	116	138	125	120
84	152	145	132	134	138	132	130	140	132	134
91	150	142	148	146	144	140	138	145	138	140

LSD for days =4.7; LSD for days = 5.3 (TC); LSD for days = 4.2; LSD for doses = 4.9 (CTC)
LSD for doses = 4.5; LSD for doses = 4.7 (OTC)

30-45 cm

Days after treatment	Control	TC			CTC			OTC		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	110	70	60	44	52	40	30	62	50	38
7	110	56	44	30	40	32	24	48	37	27
14	108	40	32	22	30	22	18	36	28	20
21	105	30	22	18	22	16	15	27	20	15
28	109	25	19	16	18	13	12	22	17	13
35	104	23	17	15	15	12	11	19	15	12
42	110	40	38	26	13	10	10	37	32	24
49	108	58	59	38	25	21	19	52	45	36
56	106	74	70	55	45	31	28	68	59	52
63	101	88	82	70	64	54	38	80	73	64
70	108	92	85	75	80	74	64	83	80	70
77	108	95	88	82	86	80	78	86	84	80
84	108	95	90	88	88	84	84	90	88	86
91	112	104	98	96	96	94	100	98	95	94

LSD for days =4.1; LSD for days = 4.4 (TC); LSD for days = 3.9; LSD for doses = 4.5 (CTC)
LSD for doses = 3.8; LSD for doses = 4.4 (OTC)

45-60 cm

Days after treatment	Control	TC			CTC			OTC		
		50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil	50 mg kg^{-1} soil	100 mg kg^{-1} soil	200 mg kg^{-1} soil
1	90	66	56	40	50	40	30	60	48	37
7	94	52	40	28	45	32	24	48	36	26
14	91	44	34	24	32	24	18	39	30	21
21	92	32	26	18	24	18	16	28	23	16
28	90	28	22	16	22	15	14	25	19	14
35	94	26	20	14	20	14	13	23	17	12
42	92	46	38	26	18	13	12	40	34	25
49	98	64	56	40	48	22	36	58	52	38
56	94	80	68	60	66	38	48	72	64	54
63	90	84	76	68	78	60	56	80	72	62
70	92	88	80	75	82	76	64	84	80	70
77	92	86	84	80	84	82	74	82	82	78
84	94	95	92	92	88	86	86	88	88	84
91	96	94	92	94	92	92	90	90	88	86

LSD for days =3.7; LSD for days = 3.9 (TC); LSD for days =3.5; LSD for doses = 3.8 (CTC)
LSD for doses = 3.8; LSD for doses = 4.0 (OTC)

CONCLUSIONS

From the results it might be concluded that all the studied antibiotics have a significant effect on bacterial population. Up to 21-35 days of application the population of bacteria decreased in tube well water irrigated soil while up to 35-42 days of application in sewage water irrigated soil. The bacterial population in both the soils decreased with increase in antibiotics concentration or soil depth. The effect of antibiotics on bacterial population was in the order CTC> OTC> TC. The higher toxicity of antibiotics at the higher rate of application showed that the toxicity of antibiotics is dose dependent.

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