I.J.S.N., VOL. 3(2) 2012: 259-262 ISSN 2229 – 6441

INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

AN ESTIMATION OF MICROBIAL COUNT INCLUDING NITROGEN FIXING BACTERIA IN AGRICULTURAL FIELDS OF ALIGARH DISTRICT IRRIGATED WITH UNTREATED SEWAGE WATER

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ABSTRACT
In the peri-urban areas of India sewage water is a valuable resource for agricultural production. This study pertains to the investigation of the impact of domestic and industrial sewage water irrigation on chemical and biological characteristics in alluvial soils of Aligarh district. Ten fields were selected which were irrigated by only sewage water (SI), eight selected fields were partially irrigated by sewage water (PSI) and for the three additional selected fields the source of irrigation was tube well water. The results denoted that the soil organic matter and CEC increased with sewage irrigation and duration of irrigation. The data of study indicated that there was a significant increase in the bacterial and fungal population and decrease in actinomycetes population in sewage irrigated soils as compared to control. The population density of bacteria and fungi in sewage irrigated soils increased with the duration of sewage irrigation. Soil organic matter was significantly positively correlated with soil bacterial and fungal population, and negatively correlated with actinomycetes. The results also showed that the number of a symbiotic nitrogen fixing bacteria decreased in presene of sewage water, denoting that mineralization of nitrogen decreased in presence of sewage water, while sewage water had no appreciable effect on sulfur-oxidizing bacteria. The total nitrogen content and total heavy metals concentration in SI were 2-2.4 times than control, while in PSI it was 1.5-1.6 times. The concentration of DTPA extractable heavy metals showed no difference between partial sewage irrigated and sewage irrigated soils, which may be due to deposition of heavy metals in crops grown on the soils.

KEY WORDS: sewage irrigation, alluvial soil, Microbial population, heavy metals.

INTRODUCTION
Maintenance of good soil quality is of prime importance for sustainable agriculture. Heavy metals constantly interplay with human life environmentally, industrially and biologically (Doran and Zeiss, 2000). The toxicity resulting from the continuous and excessive exposure of heavy metals poses a grave risk factor to human health. Accumulation of toxic metals in human beings causes toxic effects like inhibition of haemoglobin formation, sterility, hypertension, kidney damage and mental retardation (Muller *et al.,* 2007). In India, there is a gradual decline in freshwater availability for agricultural fields so sewage and other industrial effluents are being used for irrigation of agricultural fields particularly in periurban areas. The indiscriminate disposal of industrial and sewage effluents on agricultural lands is becoming a major source of heavy metal contamination in irrigated soils and ground water (Elgala *et al*., 2003; Patel *et al.,* 2004). The uptake of heavy metals from contaminated soils by plants comprises a major path for such elements to enter the human and animal food chain (Ghafoor *et al.,* 2004). Plant uptake of metals from sewage treated soils is related to the soil exchange capacity, pH of soil as well as the sewage contamination (Adhikari and Gupta, 2002). 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. 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 these micro-organisms regulate the ecological processes. In general, an increase of metal concentration adversely affects soil microbial properties *e.g*. respiration rate, enzyme activity, which appears to be very useful indicators of soil pollutions (Pawloska and Charvat, 2004). Microorganisms including bacteria, fungi, actinomycetes serve as indicator for studying the harmful effects of metals at the cellular level. These microorganisms can also be used to remove toxic metals from contaminated sites as they can efficiently accumulate heavy metals and radionuclides from their external environment (Ali and Wainwright, 1995). Present study enumerates of general soil microbes as well as some specific microbes including sulfur oxidizing, nitrogen fixing of different soils of Aligarh irrigated by sewage water/ partially irrigated by sewage water.

MATERIALS AND METHODS
Soil samples (0-25 cm) from ten sewage irrigated, eight partially sewage irrigated and three ground water irrigated were collected bimonthly from different agricultural lands of Aligarh districts (27°53′N 78°35′E) (from each site 5-7 samples were collected and were bulked) from the month of June 2010. The collected soil samples were brought to the laboratory in sterile polythene bags and stored at 4^{o}C for further analysis.

The collected soil samples were air-dried, grounded and sieved through 2 mm sieve. The soil characteristics like soil moisture, pH, electric conductivity, organic matter, nitrogen and phosphorous were determined by usual methods (Bansal, 1982).Total and DTPA extractable heavy metals namely Cu, Cd, Zn, Cr, Pb and Ni were determined by atomic absorption Spectrophotometer (Lindsay and Norvell, 1978). The results are given in Tables 1 and 2.

Isolation of bacteria, fungus, Actinomycetes was made in each soil sample using dilution plate techniques; dilution was up to 10^{-7} for bacteria, 10^{-3} for fungus and 10^{-3} for actinomycetes (Cuppucino and Sherman, 1983). The results are given in Table 3.

Asymbiotic nitrogen fixing bacteria were estimated as: Nitrogen-Free Glucose broth medium was used for enrichment and nitrogen-Free Glucose agar (Benson) for isolation. Medium containes 1.0 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.05 g of $FeSO_4$ $.7H_2O$, 0.1 g of $CaCl_2$. $2H_2O$ and 0.001 g of Na_2MoO_4 $.2H_2O$ $L^{-1}$and Glucose solution (10.0 g glucose dissolved in 100 mL water) pH was adjusted to 7.2. Both solutions were sterilized separately at 121°C at 15 lbs pressure for 15 minutes. Enrichment cultivation was carried out with 250-mL Erlenmeyer flask containing 10 g of fresh soil and 100 mL of Nitrogen-Free Glucose broth medium for seven days at 30°C under oxic conditions without shaking to avoid disruption of biofilms. Now a loopful biofilm samples were homogenized and diluted up to 10^{-7} and 0.1 ml of the diluted samples were spread over the agar plates of the same medium containing agar and incubated at 30°C for a few (6-7) days. The results are given in Table 3.

MPN counts of sulfur-oxidizing bacteria: The MPN technique (Alexander, 1982) was used to estimate the abundance of sulfur-oxidizing bacteria (SOB) in the different samples. For enrichment 10 g of fresh soil was added in100 mL of thiosulfate mineral salts medium (thiosulfate MSM) and incubated for 20 days at 30°C in dark conditions. The medium composition in 1L of distilled water is 2.0 g KNO_3, 1.0 g NH_4Cl, 2.0 g KH_2PO_4, 2.0 g $NaHCO_3$, 0.8 g $MgSO_4.7H_2O$, 5.0 g $Na_2S_2O_3.5H_2O$ and 1.0 mL trace element solution with the pH adjusted to 6 with 1N KOH. The trace element solution contained in 1L of distilled water: 50 g Na_2-EDTA, 7.34 g $CaCl_2.2H_2O$, 5.0 g $FeSO_4.7H_2O$, 2.5 g $MnCl_2.4H_2O$, 2.2 g $ZnSO_4.7H_2O$, 0.5 g $(NH4)_6Mo_7O_{24}.4H_2O$, 0.2 g $CaSO_4.5H_2O$ and 11.0 g NaOH. After incubation samples were serially diluted to appropriate range for SOB. Between every dilution step, the samples were vigorously shaken on a vortex apparatus to dislodge the bacteria from the sediment particles. From each dilution, five replicate tubes containing growth medium were inoculated and incubated for 4 weeks. Growth was determined by pH reduction test (pH 6 to 3.5 pH reductions). The results are given in Table 3.

TABLE 1. Physicochemical properties of collected soil samples (June, 2010 to April, 2011)

S.N.	Name of sample sites	pH	Organic Matter (%)	CEC Cmol kg^{-1}	Total Nitrogen (%)	Total Phosphorous (%)
	TAPPAL (PSI)	8.23 (8.15-8.32)	2.91(2.84-2.96)	12.5	0.13 (0.12-0.14)	0.052(0.05-0.054)
2	KHAIR (SI)	7.82 (7.7-7.9)	4.26(4.11-4.35)	15.2	0.16 (0.15-0.175)	0.054(0.052-0.056)
3	LODHA (SI)	7.83 (7.75-7.92)	3.88(3.80-3.98)	14.1	0.164 (.155-0.172)	0.050(0.049-0.052)
4	MANDRAK (PSI)	7.93 (7.84- 8.12)	3.03(2.97-3.08)	12.7	0.128 (0.122-0.136)	0.055(0.053-0.057)
5	IGLAS (PSI)	7.81 (7.75-7.91)	2.63(2.5-2.70)	11.8	0.134 (0.128-0.140)	0.052(0.05-0.054)
6	SASANI (PSI)	7.75 (7.74-7.82)	2.95(2.88-3.04)	12.9	0.126 (0.122-0.30)	0.048 (0.047-0.050)
7	GONDA (SI)	7.88 (7.82-8.04)	4.27(4.11-4.42)	15.8	0.158 (0.152-0.166)	0.051(0.05-0.053)
8	KHAIRYA (SI)	8.08 (8.04-8.22)	4.44(4.20-4.64)	16.1	0.154 (0.150-0.160)	0.056 (0.054-0.059)
9	MULLAPADA (SI)	7.96 (7.84- 8.15)	5.16(4.91-5.30)	16.4	0.164 (0.162-0.174)	0.050 (0.049-0.052)
10	DHANIPUR (PSI)	7.84 (7.75-7.94)	3.06(2.98-3.14)	13.0	0.132 (0.128-0.136)	0.048 (0.047-0.050)
11	GANGARI (SI)	8.06 (7.95-8.24)	3.64(3.56-3.72)	14.5	0.164 (0.160-0.172)	0.052(0.05-0.055)
12	KASHIMPUR (G)	8.58 (8.55-8.64)	2.02 (2.0-2.05)	10.2	0.072 (0.070-0.074)	0.032 (0.030-0.035)
13	ATROULI (G)	8.40 (8.35-8.45)	2.11(2.06-2.18)	9.8	0.076 (0.074-0.080)	0.034 (0.032-0.038)
14	KALINADI-1ST (SI)	7.80 (7.72- 7.90)	5.25(5.12-5.34)	16.4	0.168 (0.162-0.174)	0.058 (0.056-0.062)
15	KALINADI-2ND (SI)	7.52 (7.45-7.62)	4.62(4.51-4.73)	15.7	0.158 (0.154-0.168)	0.056 (0.054-0.0590
16	JALALI (PSI)	8.12(8.05-8.22)	3.00(2.94-3.08)	14.0	0.132 (0.128-0.136)	0.048 (0.046-0.051)
17	AKRABAD (PSI)	7.79(7.72-7.94)	2.84(2.80-2.90)	13.4	0.136 (0.132-0.140)	0.049 (0.046-0.053)
18	GABHANA (PSI)	7.52(7.42-7.60)	3.04 (3.0-3.10)	13.0	0.126 (0.124-0.132)	0.052 (0.050-0.055)
19	KILA (SI)	8.36(8.25-8.52)	5.62(5.42-5.82)	16.8	0.166 (0.162-0.172)	0.062 (0.058-0.064)
20	CHANDAUS (SI)	7.58 (7.52-7.70)	3.06(3.03-3.12)	13.0	0.156 (0.152-0.164)	0.051 (0.050-0.051)
21	PANATHI (G)	8.28 (8.25-8.32)	1.82(1.78-1.86)	10.1	0.078 (0.074-0.080)	0.028 (0.027-0.0300

SI = Sewage irrigated soil; PSI = Partial sewage irrigated soil; G =Ground water irrigated soil

TABLE 2. Concentration (mg kg^{-1}) of heavy metals in collected soil samples of Aligarh district (June10-April, 11)

S.N.		Zn		Cu		Cr		Pb		Cd		Ni	
		T	E	T	E	T	E	T	E	T	E	T	E
1	TAPPAL (PSI)	68(62-74)	44 (42-48)	29 (27-32)	12 (11-13)	48 (45-54)	28 (26-30)	40 (38-44)	21 (20-23)	0.34 (0.32-0.36)	0.20(0.19-0.22)	16 (14-18)	9.5 (9-11)
2	KHAIR (SI)	97 (94-104)	48 (44-52)	37(35-40)	22 (20-25)	62 (57-64)	34 (32-38)	54 (52-58)	24 (22-26)	0.42 (0.4-0.44)	0.24 (0.22-0.26)	21 (20-24)	11 (10-13)
3	LODHA (SI)	95 (92-100)	47 (45-49)	34 (32-39)	21 (20-24)	60 (59-62)	32 (30-35)	55 (52-58)	24 (22-27)	0.40 (0.38-0.42)	0.23 (0.21-0.25)	20 (19-22)	11 (10-12)
4	MANDRAK (PSI)	71 (68-75)	46 (43-48)	27 (25-30)	12.5 (12-13.5)	46 (44-49)	30 (28-33)	38 (36-40)	20 (19-22)	0.31 (0.30-0.33)	0.20 (0.18-0.22)	15 (14-17)	9 (8.5-10.5)
5	IGLAS (PSI)	64 (61-69)	40 (38-41)	25 (23- 27)	14 (13-16)	45 (44-48)	29 (28-31)	39 (36-41)	22 (20-25)	0.30 (0.28-0.32)	0.19 (0.18-0.20)	15 (13-17)	9 (8-10)
6	SASANI (PSI)	60 (56-64)	39 (37-41)	24 (23-26)	12 (11-13)	44 (42-48)	29 (28-31)	36 (33-38)	21 (20-23)	0.32 (0.30-0.35)	0.19 (0.18-0.21)	16 (15-18)	10 (9-11)
7	GONDA (SI)	102 (96-110)	51 (48-54)	38 (36-41)	24 (23-27)	64 (62-68)	37 (35-39)	58 (54-62)	25 (23-28)	0.44 (0.42-0.48)	0.25 (0.23-0.28)	22 (20-25)	12.5 (11.5-14)
8	KHAIRYA (SI)	96 (92-102)	49 (45-52)	37 (35-40)	23 (22-26)	61 (58-63)	35 (33-38)	55 (52-59)	24 (22-26)	0.42 (0.40-0.44)	0.24 (0.23-0.27)	21.5 (20-23)	12 (11-13)
9	MULLAPADA (SI)	92 (90-96)	47 (45-50)	36 (34-38)	21 (20-23)	64 (62-68)	37 (35-40)	52 (50-55)	25 (23-28)	0.45 (0.42-0.48)	0.25 (0.23-0.27)	23 (22-26)	13 (11-14)
10	DHANIPUR (PSI)	72 (70-78)	38 (36-41)	25 (23-28)	14 (13-17)	49 (45-55)	32 (31-35)	40 (38-43)	20 (19-22)	0.32 (0.30-0.35)	0.19 (0.18-0.21)	17 (16-19)	9 (8.5-10)
11	GANGARI (SI)	94 (92-98)	48 (46-51)	36 (33-39)	22 (21-24)	62 (60-64)	38 (36-40)	57 (55-60)	26 (24-29)	0.42 (0.40-0.44)	0.25 (0.23-0.27)	22 (21-25)	12.5 (11.5-13)
12	KASHIMPUR (G)	41 (38-45)	24 (23-25)	17 (16-18)	9.8 (9.2-10.6)	19 (18-20)	12 (11-13)	12 (11-13)	13 (12-15)	0.14 (0.13-0.15)	0.08 (0.07-0.09)	10 (9-12)	6.5 (6-7)
13	ATROULI (G)	37 (34-41)	22 (21-24)	16 (15-17)	9.3 (9.0-9.8)	18 (16-21)	11(10-13)	11(10-12)	14 (13-16)	0.15 (0.14-0.17)	0.09 (0.08-0.10)	9.5 (9-11)	6.5 (6-7.5)
14	KALINADI-1ST (SI)	105 (100-110)	52 (50-56)	39 (37-42)	25 (23-29)	65 (61-70)	37 (32-39)	60 (55-64)	27 (24-29)	0.46 (0.42-0.50)	0.26 (0.23-0.28)	26 (23-28)	13 (12-14)
15	KALINADI-2ND (SI)	100 (96-110)	50 (49-52)	38 (36-40)	24.5 (24-26)	63 (60-67)	37 (34-39)	58 (55-62)	26 (24-28)	0.45 (0.42-0.48)	0.26 (0.22-0.30)	25 (24-28)	12.5 (12-14)
16	JALALI (PSI)	62 (58-64)	37 (35-41)	26 (24-29)	16 (15-18)	42 (41-44)	27 (25-28)	35 (33-38)	19 (18-21)	0.28 (0.27-0.30)	0.18 (0.17-0.21)	15 (14-17)	9.5 (9-11)
17	AKRABAD (PSI)	58 (54-62)	35 (33-37)	22 (21-24)	16 (15-17)	40 (38-44)	25 (24-27)	34 (62-37)	22 (21-25)	0.32 (0.30-0.35)	0.18 (0.16-0.20)	16 (14-17)	10 (9.5-11)
18	GABHANA (PSI)	62 (60-66)	39 (37-42)	26 (24-29)	17 (16-19)	42 (40-44)	28 (26-30)	36 (34-39)	21 (20-23)	0.33 (0.32-0.36)	0.20 (0.19-0.22)	18 (17-20)	11 (10-12)
19	KILA (SI)	116 (110-126)	59 (55-64)	40 (39-43)	26 (24-29)	69 (66-74)	49 (45-55)	60 (54-66)	27 (24-30)	0.48 (0.44-0.52)	0.30 (0.26-0.35)	24 (21-27)	13 (11-15)
20	CHANDAUS (SI)	96 (92-100)	55 (53-57)	37 (36-39)	23 (21-25)	59 (55-62)	40 (37-42)	52 (50-55)	24 (22-27)	0.40 (0.38-0.43)	0.19 (0.18-0.21)	16 (15-18)	9 (8.5-10)
21	PANATHI (G)	40 (38-43)	23 (22-25)	17 (16-19)	9.2 (8.8-9.5)	18 (17-20)	11 (10-13)	12 (11-13)	13 (12-15)	0.14 (0.13-0.16)	0.12 (0.11-0.13)	9.5 (9-11)	5.5 (5-6)

SI = Sewage irrigated soil; PSI = Partial sewage irrigated soil; G =Ground water irrigated soil, T= Total; E= DTPA- extractable

RESULTS AND DISCUSSION

The results showed that there was no obvious change in soil pH values in sewage irrigation, partial sewage irrigation and ground water irrigation (Table-1). Soil organic matter was much higher in sewage irrigated soils than partial sewage irrigated soils or ground water irrigated soils. The CEC, an important index of soil holding cation nutrients capacity, significantly increased in sewage irrigated soils than in partial or ground water irrigated soils (Table 1). The total nitrogen content was 0.16±0.05% in sewage irrigated soils and 0.13± 0.03% in partial sewage irrigated soils, which were much higher than 0.07±0.03% in ground water irrigated soils. There was no distinct difference in the total P content in sewage irrigated soils and partial sewage irrigated soils but was much higher in sewage irrigated soils than ground water irrigated soils.

The average concentration of total heavy metals and DTPA extractable heavy metals viz. Cu, Mn, Zn, Cr, Cd, Pb and Ni are recorded in Table 2. The results showed that the concentrations of total heavy metals in sewage irrigated soils were about 2-2.4 times higher than that of ground water irrigated soils , while in partial sewage irrigated soils it was1.6-1.8 times. The results also showed that concentration of total heavy metals increased with time in sewage irrigated and partial sewage irrigated soils. The lowest concentration of heavy metals in the top layer was in the month of September- October which may be due to leaching of metals from top layer to lower depths. The concentration of DTPA extractable heavy metals showed no difference between partial sewage irrigated and sewage irrigated soils, which may be due to deposition of heavy metals in crops grown on the soils. There was a significant positive correlation between total heavy metal concentration, DTPA- extractable metal concentration, and soil-organic matter.

The data on bacterial, fungal and actinomycetes populations of the soils (Table 3) showed that number of bacterial and fungal colonies increased significantly with sewage water irrigation, maximum in sewage irrigated soils followed by partial sewage irrigated soils and least in ground water irrigated soils (Zhang *et al.,* 2008; Corstanje and Reddy, 2006). The fungal and bacterial population slightly increased with time. The increase in bacterial or fungal population may be due to mineralization of organic matter (Deshmukh *et al.,* 2010). The minimum population of actinomycetes was in sewage water irrigated soils (Tables 3). Soil organic matter was significantly positively correlated with soil bacterial and fungal population, and negatively correlated with actinomycetes The results also showed (Table 3) that number of asymbiotic nitrogen fixing bacteria decreased with increase in bacterial population, denoting that mineralization of nitrogen decreased in presence of sewage. Sulfur-oxidizing bacteria ranges from 0.01 $\times 10^6$ to 5.28 $\times 10^6$ cells of per g of soil, only exception was Khairya contains 11.55×10^6. Besides this some soils do not show specific growth and pH reduction was not in permissible level.

TABLE 3: Microbial population of collected soil samples (June, 2010 to April, 2011)

S.N.	Name of sample sites	Total bacteria count CFU$\times 10^7$ / of dry soil	Total fungal count CFU$\times 10^3$ /of dry soil	Total actinomycetes CFU$\times 10^3$ /of dry soil	Total nitrogen fixing bacteria CFU $\times 10^5$/mL	Sulfur oxidizing bacteria MPN x 10^6 / g soil
	TAPPAL (PSI)	74 (58-78)	12 (10-14)	11 (9-15)	76 (65-85)	0.04 (ND-0.06)
2	KHAIR (SI)	168 (130-195)	22 (20-26)	9 (6-11)	80 (78-90)	0.94 (0.8-1.15)
3	LODHA (SI)	152 (140-187)	21 (19-23)	13 (12-15)	21 (9-33)	0.02 (ND-0.03)
4	MANDRAK (PSI)	68 (60-80)	13 (11-15)	14 (11-16)	13 (9-17)	0.02 (0.01-0.03)
5	IGLAS (PSI)	72 (66-84)	12 (10-14)	13 (11-15)	11 (4-20)	0.01 (ND-0.02)
6	SASANI (PSI)	84 (74-100)	14 (13-17)	32 (30-36)	14 (4-18)	ND
7	GONDA (SI)	150 (125-175)	24 (22-26)	20 (19-22)	35 (20-50)	0.25 (0.20-0.30)
8	KHAIRYA (SI)	178 (170-195)	25 (22-28)	23 (21-26)	33 (24-37)	2.12 (1.38-3.12)
9	MULLAPADA (SI)	172 (121-204)	27 (24-29)	38 (27-44)	27 (24-30	4.21 (3.24-5.25)
10	DHANIPUR (PSI)	84 (54-110)	14 (12-16)	22 (20-24)	104 (90-111)	ND
11	GANGARI (SI)	154 (125-184)	23 (21-27)	34 (27-42)	23 (15-26)	0.04 (0.02-0.07)
12	KASHIMPUR (G)	100 (90-120)	10 (9-11)	26 (22-33)	48 (43-53)	ND
13	ATROULI (G)	120 (98-142)	12 (10-14)	12 (11-13)	20 (18-25)	ND
14	KALINADI-1ST (SI)	248 (220-285)	29 (24-32)	16 (14-20)	16 (12-19)	0.46 (0.44-0.48)
15	KALINADI-2ND (SI)	260 (235-295)	28 (24-30)	14 (13-19)	26 (14-29)	0.52 (0.46-0.56)
16	JALALI (PSI)	125 (110-1400	15 (12-18)	17 (15-18)	21 (18-24)	0.04 (0.03-0.07)
17	AKRABAD (PSI)	105 (95-125)	13 (11-15)	22 (21-24)	15 (12-19)	0.42 (0.34-0.52)
18	GABHANA (PSI)	122 (115-135)	14 (12-18)	14 (12-16)	8 (6-11)	ND
19	KILA (SI)	275 (245-300)	32 (30-35)	42 (33-56)	13 (10-15)	2.42 (2.12-2.77)
20	CHANDAUS (SI)	165 (140-185)	29 (28-32)	23 (14-33)	25 (21-28)	ND
21	PANATHI (G)	95 (85-108)	10 (9-12)	19 (12-24)	36 (30-40)	ND

SI = Sewage irrigated soil; PSI = Partial sewage irrigated soil; G =Ground water irrigated soil, ND= Non detectable

CONCLUSION

The application of sewage water for irrigation increases soil organic matter and CEC which in turn enhanced bacterial and fungal population. The application of sewage water also increased total heavy metal concentration and nitrogen content in soils. The DTPA extractable metal concentration in SI and PSI soils were almost same suggesting that the heavy metals are deposited in crops grown on the soils,

ACKNOWLEDGEMENT
The authors express their sincere thanks to The Council of Science and Technology, U.P., Lucknow for financial assistance**.**

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