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Impact of Long Term Application of Municipal Solid Waste on Physicochemical and Microbial Parameters and Heavy Metal Distribution in Soils in Accordance to Its Agricultural Uses

Rinku Dhanker, Suman Chaudhary, Tanvi Bhatia, Sneh Goyal

Abstract**—**Municipal Solid Waste (MSW), being a rich source of organic materials, can be used for agricultural applications as an important source of nutrients for soil and plants. This is also an alternative beneficial management practice for MSW generated in developing countries. In the present study, MSW treated soil samples from last four to six years at farmer's field in Rohtak and Gurgaon states (Haryana, India) were collected. The samples were analyzed for all-important agricultural parameters and compared with the control untreated soil samples. The treated soil at farmer's field showed increase in total N by 48 to 68%, P by 45.7 to 51.3%, and K by 60 to 67% compared to untreated soil samples. Application of sewage sludge at different sites led to increase in microbial biomass C by 60 to 68% compared to untreated soil. There was significant increase in total Cu, Cr, Ni, Fe, Pb, and Zn in all sewage sludge amended soil samples; however, concentration of all the metals were still below the current permitted (EU) limits. To study the adverse effect of heavy metals accumulation on various soil microbial activities, the sewage sludge samples (from wastewater treatment plant at Gurgaon) were artificially contaminated with heavy metal concentration above the EU limits. They were then applied to soil samples with different rates (0.5 to 4.0%) and incubated for 90 days under laboratory conditions. The samples were drawn at different intervals and analyzed for various parameters like pH, EC, total N, P, K, microbial biomass C, carbon mineralization, and diethylenetriaminepentaacetic acid (DTPA) exactable heavy metals. The results were compared to the uncontaminated sewage sludge. The increasing level of sewage sludge from 0.5 to 4% led to build of organic C and total N, P and K content at the early stages of incubation. But, organic C was decreased after 90 days because of decomposition of organic matter. Biomass production was significantly increased in both contaminated and uncontaminated sewage soil samples, but also led to slight increases in metal accumulation and their bioavailability in soil. The maximum metal concentrations were found in treatment with 4% of contaminated sewage sludge amendment.

Keywords**—**Heavy metals, municipal sewage sludge, sustainable agriculture, soil fertility, quality.

I. Introduction

MSW is generated as the by-products of wastewater treatment plants. It constitutes of water, putrescible (organic) fraction, and non-putrescible materials recovered from wastewater originating from various sources (domestic sewage, industries), storm water run-off from roads and demolition sites, via physical, biological, and/or chemical treatments [1]. It is also referred as biosolids. With the breakneck rates of urbanization that has been occurring all around the world, the need of a dynamic and influential system for the management of solid waste is more crucial than ever before. Majority of countries do not have any proper techniques for managing this sewage sludge. The municipal waste generated is ultimately released into the surrounding environment [2]. In the developing world, most of the urbanization has been reported in Asia. This urbanization, with the increasing population, has been followed by dynamic economic growth, which has put more strain on management systems of MSW so to accommodate the transitions in the quality and quantity in order to secure the endurance of the environment [3]. It has been estimated that, by 2025, cities in Asian countries will generate 1.8 million tons of MSW per day [4]. Application of MSW in agricultural field, as the source for various plant nutrients and as a soil conditioner, is the only most cost-effective disposal practice because of its benefits over the other means such as incineration or landfilling. This enhances the chemical, physical, and biological parameters of impoverished soils by providing organic matter [5], [6].

One of the reviews coordinated in 2000 revealed that of total cultivated land 70% to 80% was insufficient in appealing supplements. Furthermore, from the survey, it was also observed that, due to the insufficient utilization of organic or bio fertilizers and exploitation of chemical fertilizers, the quality of soil was declined, which led to the low cultivation level and reduction in water retention power of soil [7]. Therefore, the application of sewage sludge in agricultural field is not only the best option for disposal of MSW but has great potential in improving the soil quality for better plant productivity. Although application of sewage sludge may be beneficial for soil and plant productivity, but there are other concerns like possible environmental pollution hazards causing by the heavy metal which may be present in the waste. So, at the time of application, this has to be considered [8]-[10]. Haphazard utilization of biosolids for improving agricultural yields without bothering about possible harmful effects may cause a major problem [11]. There are several conditions which determine the consequences of heavy metal in the soil and the plants. Like at neutral or slightly alkaline pH of soil, the metal mobility of sewage sludge in the soil will be less. And, at acidic conditions, it will increase, and there

Rinku Dhanker from Chaudhary Charan Singh Haryana Agricultural University, India (e-mail: rinku1989d@gmail.com).

are chances of accumulation or uptake of heavy metals in plants growing in that area [12]. Utilization of heavy metal contaminated waste by such plants may increase the risk of accumulation heavy metal contents in the food web [10], [13], [14]. Thus, heavy metal pollution has emerged as the main hurdle for the application of MSW in agricultural field. So, we carried out an experiment with the following objectives: (1) to study the impact of repeated application of sewage sludge for five to six years at farmer's field (2) And to determine the effect of uncontaminated and metal contaminated sewage sludge at different levels on physiochemical and microbiological properties of sandy loam soil.

II. Material and Methods

A. Sampling and Pretreatment

Soil samples treated and not treated with sewage sludge from last four to six years were collected from Gurgaon and Rohtak sites (Table I). The soil samples were sieved through 2-mm sieve and stored at 4 °C. For the determination of microbial biomass and microbial activities, the samples were moistened to 60% water holding capacity and incubated at 30° C for 10 days to permit uniform rewetting and to allow microbial activity to equilibrate after the initial disturbances. Sub samples of each soil were air dried and ground for chemical analysis.

TABLE I
Data of Soil Samples Collected

Place	No. of Addition	Tons ha^{-1} $year^{-1}$	Crop in sewage sludge	
			Untreated	Treated
Madina (Rohtak)	5	40	Flower	Flower
Bijwasan (Gurgaon)	6	30	Flower	Wheat, Vegetable Mustard,

The important agricultural parameters such as pH value, electrical conductivity, organic and microbial biomass carbon [15], [16], total and available nitrogen (TN) [17], [18], total and available phosphorus (TP) [19], [20], total potassium (K), ammoniacal and nitrate nitrogen, heavy metals and DTPA extractable metal [21], [22], enzymes activities [23]-[25] of soil samples were determined using standard analytical methods. The values obtained for these physicochemical parameters are given in Tables II-IV.

B. Sludge Sampling

At farmer's field, application of sewage sludge led to buildup of organic matter that sustains microbial biomass and activities. The heavy metal content of the sewage sludge at different sites was below the EU limits indicating that the amount of metals present may not exert any adverse effect on soil microbial activity. Therefore, to examine the effect of metals on soil processes, municipal sewage sludge sample was collected from the wastewater treatment plants of Gurgaon, and the artificially contaminated sludge doubled the EU limits. These sewage sludge samples were applied to a soil with a sandy texture and low organic matter content. The soil samples to be amended with sewage sludge were taken from fields of Haryana Agriculture University. The soil samples were incubated with different rates of uncontaminated and contaminated sewage sludge under laboratory conditions (Table V). The moisture content was adjusted to 60% water holding capacity throughout the incubation period and incubated at 30 °C for 90 days. Subsamples for evaluating different parameters were removed at 0, 15, 30, 60, and 90 days.

C. Chemical Analysis

The pH and electrical conductivity of the treatments were measured in a 1:5 sample:water extract after shaking for 30 min. Organic carbon (OC) was determined by oxidation of dichromate and titration with ferrous ammonium sulphate [15]. Total nitrogen in all samples was determined by Kjeldahl's method [16]. The contents of total macronutrients and heavy metals in the samples were determined following digestion with perchloric and nitric acids and then analyzed in an atomic absorption spectrophotometer (Perkin Elmer HGA500, USA).

D. Biological and Biochemical Analysis

Biomass C was estimated by method described by Vance et al. [16] by fumigating the samples with ethanol-free chloroform and extraction with 0.5 M K2SO4, after 24h incubation at room temperature.

TABLE II
Chemical Properties of Soils Treated and Untreated with Sewage Sludge

Sewage Sludge	pH (1:2.5)		EC (dSm^{-1})		Organic C (%)		Total N (%)		C/N ratio		Total P (%)		Total K (%)		Available N (mg kg^{-1})		Available P (mg kg^{-1})	
	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am
Sonepat	7.64	7.65	0.86	1.04	0.43	0.67	0.059	0.071	7.3	9.4	0.042	0.085	0.032	0.096	26	42	5.4	10.2
Faridabad	7.85	8.14	0.98	1.15	0.41	0.56	0.058	0.061	7.1	9.2	0.038	0.070	0.048	0.092	24	46	6.2	8.1

Un: Untreated, Am: Amended Or Treated

TABLE III
Microbial Biomass c, Dehydrogenase, Alkaline Phosphatase, and Urease Activities in Soil Amended or Unamended with Sewage Sludge

Sewage Sludge	Biomass C (mg kg^{-1} soil)		Dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$)		Alkaline Phosphatase Activity (µg $PNPg^{-1}$ soil h^{-1})		Urease Activity (µg NH_4^+ -N released g^{-1} soil h^{-1})	
	Un	Am	Un	Am	Un	Am	Un	Am
Sonepat	158	489	60	96	22	51	73	122
Faridabad	163	501	77	88	35	76	86	208

TABLE IV
CONTENT OF HEAVY METALS IN SOILS AMENDED OR UNAMENDED WITH SEWAGE SLUDGE

Treatments	(mg kg^{-1}soil)													
	Cu		Cr		Cd		Ni		Fe		Pb		Zn	
	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am	Un	Am
Sonepat	32	36	6	9	1.6	2.0	24	34	2385	2420	26	29	150	206
Faridabad	26	35	19	34	2.0	3.0	32	38	2378	2392	29	32	171	173

Dehydrogenase activity was determined by method of Casida et al. [23], by reducing 2,3,5 triphenyl tetrazolium chloride (TTC) to triphenyl formazan. The activity was measured at 485 nm on spectrophotometer after 24h of incubation of 5 gm of samples with 3% TTC and extracting with methanol.

Urease activity was determined by the method of Bremner and Mulvaney [25]. The method involved the determination of ammonia liberated on the incubation of toluene treated samples with urea in a buffered system.

TABLE V
DIFFERENT TREATMENTS OF SOIL AND SEWAGE SAMPLES

Treatment No	Treatments	Application Rate, %
T1	Soil	-
T2	Soil+ uncontaminated sewage sludge	0.5
T3	Soil+ uncontaminated sewage sludge	1.0
T4	Soil+ uncontaminated sewage sludge	2.0
T5	Soil+ uncontaminated sewage sludge	4.0
T6	Soil+ metal contaminated sewage sludge	0.5
T7	Soil+ metal contaminated sewage sludge	1.0
T8	Soil+ metal contaminated sewage sludge	2.0
T9	Soil+ metal contaminated sewage sludge	4.0

Alkaline phosphatase activity was determined by using *p*-nitrophenyl phosphate disodium (PNPP, 0.025 M) as substrates [24]. This assay is based on the release and detection of *p*-nitrophenol (PNP). 4 ml of 0.1 M maleate buffer (pH 11), 0.2 ml of toluene and 1 ml of p- nitrophenyl phosphate solution were added to 1 g of sample and incubated at 37 °C for 1h. Then, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added, and the mixture was centrifuged for 5 min. The PNP concentration was then determined by measuring absorbance in spectrophotometer at 420 nm [24].

III. RESULT AND DISCUSSION

Comparative assessment of physico-chemical variables at untreated and sewage sludge treated soils for last few years has been given in Table II-IV. The pH of soil varied from 7.65 to 8.15. A non-significant change in soil pH was observed after 3-6 yrs application of sewage sludge. However, a significant change in electrical conductivity was observed with sewage sludge amendment. It increased from 0.86 to 1.04 and 0.98 to 1.15 dSm^{-1}. Sewage sludge application led to increase in organic carbon content by 26.8 to 35.8% in comparison with untreated soil. The amount of total N increased from 0.059 to 0.071% and 0.058 to 0.061% in Rohtak and Gurgaon soils, respectively. It increased by 48 to 68%. The C:N ratio varied from 7.1 to 8.8 in untreated soils of different sites and it varied from 9.2 to 10.3 with sewage sludge amendment. Soil treated with sewage sludge had more C:N ratio as compared to untreated soil.

Sewage sludge amended soils had higher amounts of total phosphorus content in comparison with untreated soils. It increased from 0.042 to 0.085% and 0.038 to 0.070% in Rohtak and Gurgaon soil samples. Concentration of total P increased by 45.7 to 51.3% with the application of sewage sludge at different sites. The potassium content varied from 0.032 to 0.048% in untreated and from 0.092 to 0.096 in sewage sludge amended soil. It leads to increase in K content from 60 to 67% at different sites (Table II).

Mineral form of nitrogen varied from 24 to 26 mg kg^{-1} soil in untreated and from 42 to 46 mg kg^{-1} in sewage sludge treated soil samples. Total amount of nitrogen was more in Rohtak soils. However, available form of N was more in Gurgaon soils showing that Gurgaon sludge had more mineralizable form of N than Rohtak soils. The available P was highest in Rohtak soils treated with sewage sludge.

Application of sewage sludge increased microbial biomass C from 158 to 489 and 163 to 512 mg kg^{-1} soil at Rohtak and Gurgaon sites (Table III) as compared to untreated soil.

Dehydrogenase activity was more in soils receiving sewage sludge. It increased from 60 to 96 and 77 to 88 µg TPF g^{-1} soil 24 h^{-1} with the application of sewage sludge at Rohtak and Gurgaon sites.

The untreated sewage soils had lower alkaline phosphatase activity than amended soils. It increased from 22 to 51 and 35 to 76 µg PNP g^{-1} soil h^{-1} with the application of sewage sludge. Urease activity was highest in Gurgaon soil amended with sewage sludge and it was 207.5 µg NH_4+-N released per gram soil per hour.

The concentration of heavy metals in the soil increased with the application of sewage sludge in all the soil samples (Table IV). There was significant increase in total Cu, Cr, Ni, Fe, Pb, and Zn in all the soils with sewage sludge amendment, but all the metals were still below the current permitted (EU) limits.

A. Effect of Heavy Metal Contaminated Sewage Sludge on Microbial Activities in Soil

The problem of fractions heavy metal in sewage sludge needs to be taken care of at the time of its application in agricultural field. If heavy metals are present in high concentration then its repeated use year after year may lead to the accumulation of toxic heavy metals in the amended soil, which will have phytotoxic effects on various crops. And, when humans or animals would consume these commodities, it can cause several health hazards. So, it is very important to determine the heavy metal concentration of sewage sludge before it is being practiced in fields. In the present study, with the long-term application of MSW at farmer's field, it was found that there was no harmful impact on the soil physiochemical and microbiological properties.

So, to assess the harmful impacts of heavy metals, sewage sludge was artificially contaminated with heavy metals.

B. Soil pH

The soil pH was increased from 7.20 to 7.42 and 7.20 to 7.49, in the initial days of incubation with the application of metal contaminated and uncontaminated sewage sludge (Table VI). However, after 90 days of incubation, it found to be declined from 7.30 to 7.15, 7.42 to 7.49, 7.41 to 7.00, and 7.41 to 6.99 with 0.5, 1.0, 2.0, and 4.0% levels of uncontaminated sewage sludge application, respectively. Similarly, due to the application of heavy metal contaminated sewage sludge, the soil pH declined from 7.20 to 7.04 with different concentrations of sewage sludge. With the increase level of sewage sludge, the pH declined. This could be possible because the sewage sludge is rich in organic matter; it will increase the organic matter of soil, and produces CO2, carbonic acid and humic acids which play an important role in improving soil properties. These acids in calcareous soils will lower the soil pH [26], [27].

TABLE VI
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON PH AND EC

Treatments	pH* (1:2.5 ratio) Incubation period (Days)				EC** (dS m^{-1}) Incubation period (Days)			
	0		90		0		90	
	UC	C	UC	C	UC	C	UC	C
Soil+ Sludge (0%)	7.20		7.18		0.42		0.41	
(0.5%)	7.30	7.35	7.15	7.20	0.44	0.57	0.70	0.71
(1.0%)	7.42	7.49	7.05	7.10	0.64	0.68	0.89	1.01
(2.0%)	7.41	7.43	7.00	7.05	0.76	0.79	1.30	1.38
(4.0%)	7.41	7.44	6.99	7.04	1.10	1.20	1.82	2.00

C. Electrical Conductivity

From the Table VI, it can be observed that with increasing levels of sludge a significant increase in EC was observed after 90 days of incubation. It increased by 358.53% in case of uncontaminated sewage sludge and 414.63% in case of contaminated sewage sludge after 90 days of incubation. Maximum EC was found in soil amended with metal contaminated sewage due the presence of more soluble salts. After 90 days of incubation, EC in metal contaminated sewage sludge increased by 1.5, 3.9, 5.9 and 10.9% at 0.5, 1.0, 2.0 and 4.0% levels in comparison with uncontaminated sewage sludge (Table VI), respectively. The soil EC increased during 90 days of incubation due to the acids produced during decomposition, which caused dissolution of sparingly soluble salts [28].

D. Organic Carbon

With increasing levels of uncontaminated and metal contaminated sewage sludge the organic C increased from 0.44 to 1.15% at the start of incubation experiment (Table VIII). But, there was decline in organic C after 90 days of incubation. It decreased from 0.62 to 0.55, 0.83 to 0.77, 0.97 to 0.89 and 1.15 to 1.06% in uncontaminated sewage sludge amended soil, while in metal contaminated sewage sludge applied soil it declined from 0.62 to 0.55, 0.83 to 0.78, 0.97 to 0.91 and 1.15 to 1.08% at 0.5, 1.0, 2.0 and 4.0% levels, respectively. There was no significant difference between the organic C of metal contaminated and uncontaminated sewage sludge amended soil. But, with increasing sewage percentage organic C, total N, P and K increased gradually at the early stages of incubation due to presence of organic matter in the form of sewage sludge. However, after 90 days of incubation, organic C decreased sewage sludge amended soils due decomposition of organic matter. Kandpal et al. have reported that the metal spiking, with time decrease the oxidizable organic C content of sewage sludge under laboratory experiment [27]. In the present experiment soil amended with metal contaminated sewage sludge by 4% level had more organic C than uncontaminated sewage sludge at the corresponding level. More concentration of organic C in metal contaminated sewage sludge amended soils was due to lower rate of C mineralization of the organic matter. Abaye et al. and Kao et al. have also reported that soils amended with metal contaminated sewage sludge led to more accumulation of soil organic matter than uncontaminated sewage sludge [29], [30].

E. Total N, P and K

No significant difference in concentration of total N, P and K was observed between soils receiving uncontaminated or metal contaminated sewage sludge at different levels. But with increasing concentration of sewage sludge their concentration increased gradually. Their amount was more in soil amended with 4% of sewage sludge. Total N increased from 0.050 to 0.0128% after 90 days of incubation. Total P increased from 0.039 to 0.066% and total K 0.16 to 0.19% (Table VIII). With increasing amount of sewage sludge, soil microbial biomass increases, which serves as the portion of the soil responsible for the nutrient cycling and the regulation of transformation of organic matter into mineralized form of nutrients (N, P and K), which are continually assimilated during the growth of microorganisms [3], [31].

F. DTPA Extractable Heavy Metals

The DTPA extractable Ni, Cd, Cr, and Pb increased significantly with increasing levels of sewage sludge in soil (Table VII) The concentration of DTPA-extractable heavy metals (Ni, Cr, and Cd) increased with increasing incubation time up to 30 days, but thereafter a decline was observed. However, the concentration of Pb declined after 15 days. DTPA extractable Cd increased with increasing levels of both uncontaminated and metal contaminated sewage sludge. The availability of heavy metals in the initial days was less due to the formation of metallorganic complexes of heavy metals with the organic matter of sewage sludge, which reduced the availability of heavy metals in the soil system. But, on further incubation, sewage sludge decomposed slowly and released heavy metals. Due to the production of organic acids, pH of the soil also decreased, which increased the availability of heavy metals [32], [33]. Similar results have also been reported by Chand and Singha [34], [35]. The DTPA-Cd decreased with time and increased with increasing levels of both type of sewage sludge.

TABLE VII
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON DTPA-EXTRACTABLE METALS

	Treatments	Incubation period (Days) 0		15		30		60		90	
		UC	C	UC	C	UC	C	UC	C	UC	C
Ni (mg kg^{-1} soil)	Soil+ Sludge (0%)	1.02		1.084		1.040		1.018		1.003	
	(0.5%)	1.036	1.078	1.175	2.370	2.748	2.790	2.227	2.107	1.43	3.346
	(1.0%)	1.070	1.132	1.310	3.439	2.819	3.730	2.515	3.013	2.061	4.343
	(2.0%)	1.132	1.700	1.719	3.447	3.410	4.246	3.038	4.168	2.325	4.490
	(4.0%)	1.430	2.137	3.178	5.320	4.539	5.577	3.950	4.427	3.440	5.189
Pb (mg kg-1 soil)	(0%)	1.609		1.710		1.651		1.479		1.310	
	(0.5%)	1.725	2.260	2.987	7.069	2.538	5.800	2.209	3.759	2.010	2.719
	(1.0%)	1.970	3.128	3.322	8.175	2.580	6.178	2.310	5.309	2.109	4.468
	(2.0%)	2.119	5.229	3.859	11.910	3.729	9.178	3.559	8.329	3.300	7.680
	(4.0%)	2.919	7.979	5.938	27.679	4.682	21.319	4.145	18.957	3.610	15.437
Cr (mg kg-1 soil)	(0%)	1.134		1.569		2.199		1.684		1.300	
	(0.5%)	1.180	1.240	1.680	3.265	2.486	3.500	1.964	2.510	1.605	2.040
	(1.0%)	1.339	1.580	2.180	3.510	3.917	3.965	2.860	3.425	2.556	3.140
	(2.0%)	1.757	1.926	2.491	4.186	4.047	4.400	2.936	4.170	2.627	4.015
	(4.0%)	2.180	2.570	3.295	4.468	4.626	5.117	3.614	4.497	3.128	4.220
Cd (mg kg^{-1} soil)	(0%)	0		0		0		0		0	
	(0.5%)	0.004	0.010	0.016	0.055	0.019	0.065	0.016	0.054	0.015	0.026
	(1.0%)	0.026	0.040	0.057	0.086	0.070	0.100	0.054	0.070	0.033	0.060
	(2.0%)	0.040	0.060	0.104	0.175	0.150	0.210	0.077	0.200	0.062	0.161
	(4.0%)	0.105	0.126	0.170	0.480	0.275	0.515	0.190	0.440	0.158	0.360

TABLE VIII
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON ORGANIC CARBON, TOTAL N, P AND K UNDER LABORATORY INCUBATION

Treatments	**Organic C* (%) Incubation period (Days)** 0		90		**Nitrogen** (%) Incubation period (Days)** 0		90		**Phosphorus*** (%) Incubation period (Days)** 0		90		**Potassium (%)** Incubation period (Days)** 0		90	
	UC	C	UC	C	UC	C	UC	C	UC	C	UC	C	UC	C	UC	C
Soil+ Sludge (0%)	0.44		0.39		0.049		0.050		0.038		0.039		0.14		0.16	
(0.5%)	0.62	0.62	0.55	0.55	0.057	0.057	0.057	0.056	0.040	0.040	0.040	0.041	0.15	0.15	0.16	0.16
(1.0%)	0.83	0.83	0.77	0.78	0.070	0.070	0.071	0.070	0.039	0.038	0.043	0.043	0.15	0.15	0.17	0.17
(2.0%)	0.97	0.97	0.89	0.91	0.094	0.094	0.096	0.095	0.046	0.047	0.045	0.044	0.17	0.17	0.18	0.17
(4.0%)	1.15	1.15	1.06	1.08	0.130	0.130	0.129	0.128	0.062	0.061	0.065	0.066	0.20	0.19	0.19	0.18

G. Microbial Biomass Carbon

The increasing levels of sewage sludge increased the microbial biomass carbon significantly under laboratory incubations. In all the treatments, there was increase in microbial biomass carbon after 15 days of incubation followed by a gradual decline upto 90 days. With increasing levels of uncontaminated sewage sludge, it increased from 140 to 541 mg kg^{-1} over the control (Table IX). With the increasing concentration of sewage sludge, microbial biomass increased gradually after 15 days of incubation. A decline in microbial biomass C was more in metal contaminated sewage sludge amended soils in comparison with uncontaminated sewage sludge. Microbial biomass is directly influenced by organic matter content of soil [36], [37], [30]. Organic C is one of the important parameters in the enrichment of soil microbial biomass C. Since most microbial populations are heterotrophic, the soil microbial activities are also dependent upon C as a substrate [38], [39]. Metals inhibited the microbial biomass C in metal contaminated sewage sludge amended soil by 5.6 to 19.0% at different levels. Increase in microbial biomass C was due to availability of organic matter present in sewage sludge. Similar results were also observed by [40]-[42]. Sewage sludge acts as a source of nutrients for multiplication of soil microflora as reflected in the increase in soil microbial biomass at early stages (15 days) of incubation. But, long-term incubation (upto 90 days) resulted into decline in soil microbial biomass due to exhaustion of easily available carbon pool and inhibition by the presence of toxic elements. Due to the release of heavy metals by decomposition of organic matter the decline in microbial biomass C in metal contaminated sewage sludge was observed. Barajas has also reported that concentration of microbial biomass C in metal contaminated sewage sludge amended soils was about half of that found in soils amended with uncontaminated sewage sludge or chemical fertilizers [43].

H. Enzyme Activities

Dehydrogenase activity increased significantly with increasing levels of uncontaminated sewage sludge upto 15 days. But, after 30 days, it declined with increasing levels of uncontaminated or metal contaminated sewage sludge. Soil dehydrogenase activity increased significantly from 65 to 113 µg TPF g^{-1} soil 24 h^{-1} with the increasing levels of uncontaminated sewage sludge application after 15 days of incubation (Table X). The dehydrogenase activity increased from 65 to 80 µg TPF g^{-1} soil 24 h^{-1} at 0.5% level of metal contaminated sewage sludge after 15 days of incubation. However, with increasing levels of metal contaminated sewage sludge it decreased significantly. Dehydrogenase enzyme activity increased by 27 to 45% and 6 to 21% with, increasing amount of uncontaminated and metal contaminated sewage sludge. With the application of metal contaminated sewage sludge at different levels (0.5, 1.0, 2.0, and 4.0%) dehydrogenase activity decreased by 2.1, 13.6, 16.7, and 22.8% after 90 days of incubation. The activity of dehydrogenase enzyme depends on the metabolic state of the soil biota. This suggests that this enzyme could be a good indicator of soil microbial activity in semiarid areas [44]. Other authors have also reported that the activity of dehydrogenase enzyme was inhibited by the toxic impact of heavy metals present in the municipal sewage sludge, particularly Pb [45] and Cu [46].

Changes in soil alkaline phosphatase enzyme activities were also observed between different treatments, which play an important role in the mineralization of organic phosphorus,

Alkaline phosphatase activity increased from 19 to 39, 48, 60, and 69 µg $PNPg^{-1}$ soil h^{-1} with increasing levels of uncontaminated sewage sludge application significantly. However, application of metal contaminated sewage sludge led to increase in phosphatase activity from 19 to 39 µg $PNPg^{-1}$ soil h^{-1} at the same levels over the control. Maximum alkaline phosphatase activity was observed after 15 days of incubation in all the treatment and thereafter it declined on further incubation (Table X). After 30 and 60 days of incubation alkaline phosphatase activity varied from 25 to 50 and 26 to 53 µg $PNPg^{-1}$ soil h^{-1}, respectively, in the soil amended with different levels of uncontaminated sewage sludge. However, in the metal contaminated sewage sludge amended soil, it ranged from 24 to 41 and 28 to 40 µg $PNPg^{-1}$ soil h^{-1} at the same levels. Metal contaminated sewage sludge application led to decrease in alkaline phosphatase activity by 13.8, 19.1, 21.7, and 24.3% compared to uncontaminated sewage sludge at all the levels after 90 days of incubation.

Activity phosphatase enzyme can be inhibited not only by heavy metals [47] that are added with MSW into the soil, but also by inorganic phosphate, that is responsible for a feedback inhibition of the enzyme [48]. This is why available P is found in the soils amended with organic waste [44].

I .Effect of Heavy Metal Contaminated Sewage Sludge on C and N Mineralization

The amount of CO_2-C evolved from the soils amended with uncontaminated or metal contaminated sewage sludge addition is shown in Table XI. Addition of uncontaminated sewage sludge and metal contaminated sewage sludge led to a significant increase in CO_2-C evolution. In all the treatments, about 50% of the added organic C in the form of sewage sludge was evolved as CO_2-C in first 15 days of incubation indicating that mineralization rate was faster during early incubation period (Table XI). Increased C mineralization in soils with sewage sludge is due to organic matter buildup in sewage sludge amended soils as compared to control soils. Sewage sludge supplied additional source of labile C and other nutrients to soil for microbial growth and activity [49]. Griffiths et al. found that metal contamination which doubled the EU limits decreased the C mineralization [50]. However, over the entire incubation period, total amounts of CO2-C evolved varied among treatments. Addition of sewage sludge contaminated with metals (Pb, Cr, Ni, and Cd) led to less CO2 evolution in comparison with uncontaminated sewage sludge. There were more declines in CO_2 evolution with the addition of high levels of metal contaminated sewage sludge. A significant difference in CO_2-C evolution was observed in all the treatments throughout the 90 days of incubation. 65.6, 64.4, 62, and 60% of added C in the form of uncontaminated sewage sludge got mineralized after 90 days of incubation at 0.5, 1.0, 2.0, and 4.0% levels of sewage sludge application respectively. The addition of metal contaminated sewage sludge at 0.5, 1.0, 2.0, and 4.0% levels the C mineralization was 56.1, 53, 49.6, and 42.0%, respectively after 90 days of incubations. With the addition of metals contaminated sewage sludge at different levels the rate of C mineralization was affected, and this effect was more pronounced at 2 and 4% level of sewage sludge application.

The amount of ammoniacal nitrogen increased significantly with increasing levels of uncontaminated or metal contaminated sewage sludge application (Table XII). Application of uncontaminated and metal contaminated sewage sludge to soil at the 4% level increased the amount of NH_4+-N by 86.4% and 83.1% in comparison to control after 60 days of incubation. It increased with increasing incubation period significantly, but after 90 days it declined. Metal contaminated sewage sludge amended soils had 19% lesser amount of ammoniacal N in comparison with uncontaminated sewage sludge after 60 days of incubation.

Amount of nitrate nitrogen initially increased from 37.2 to 55.6 mg kg^{-1} soil in uncontaminated and from 37.2 to 56.4 mg kg^{-1} soil in metal contaminated sewage sludge amended soil at different levels (Table XII). It increased up to 60 days of incubation, and then, it declined. The highest value of NO_3--N (114.2 mg kg^{-1} soil) was observed at 2% level of uncontaminated sewage sludge application after 60 days of incubation. However, after 30 days of incubation, the amount of NO_3--N was less at 4% level of sewage sludge application in both uncontaminated and metal contaminated sewage sludge.

TABLE IX
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON MICROBIAL BIOMASS C UNDER LABORATORY INCUBATION

Treatments	Microbial biomass C (mg kg^{-1} soil) Incubation period (Days)							
	15		30		60		90	
	UC	C	UC	C	UC	C	UC	C
Soil+ Sludge (0%)	140		131		127		122	
(0.5%)	249	250	231	229	224	210	216	204
(1.0%)	312	310	280	271	277	254	251	241
(2.0%)	446	443	404	396	400	370	299	249
(4.0%)	541	540	510	487	501	454	375	355

TABLE X
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITY

	Treatments	(µg TPF g^{-1} soil $24h^{-1}$) Incubation period (Days)							
		15		30		60		90	
		UC	C	UC	C	UC	C	UC	C
Dehydrogenase activity	Soil+ Sludge (0%)	65		62		56		47	
	(0.5%)	86	80	80	60	71	50	57	55
	(1.0%)	97	75	71	49	64	46	56	49
	(2.0%)	100	72	70	47	60	45	55	44
	(4.0%)	113	65	69	45	49	43	48	37
Alkaline Phosphatase Activity	(0%)	19		24		28		22	
	(0.5%)	39	25	38	30	39	30	30	23
	(1.0%)	48	28	39	30	40	33	33	30
	(2.0%)	60	30	40	36	43	31	41	31
	(4.0%)	69	39	49	41	51	40	50	36

TABLE XI
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON C-MINERALIZATION UNDER LABORATORY INCUBATIONS

Treatments	CO_2 – C (mg kg^{-1} soil) Incubation period (Days)										Total C mineralized (mg kg^{-1} soil)		% of added C mineralized	
	0-5		5-15		15-30		30-60		60-90					
	UC	C	UC	C	UC	C	UC	C	UC	C	UC	C	UC	C
Soil+ Sludge (0%)	10.7		15.4		12.6		11.4		9.5		59.6		-	
(0.5%)	19.5	18.0	17.2	17.1	14.3	14.0	12.7	12.6	10.6	10.4	74.3	72.1	64.7	55.1
(1.0%)	23.8	22.7	20.7	19.3	16.7	16.5	14.3	13.9	12.9	11.0	88.4	83.4	63.4	52.4
(2.0%)	30.4	28.1	26.9	24.3	22.1	20.0	19.5	16.3	16.2	14.6	115.1	103.3	61.1	48.1
(4.0%)	45.8	35.3	37.7	30.2	34.3	27.5	27.2	21.8	22.8	19.3	167.8	134.1	59.6	41.0

TABLE XII
EFFECT OF UNCONTAMINATED (UC) AND METAL CONTAMINATED (C) SEWAGE SLUDGE ON NH_4^+ -AND NO_3^- - N UNDER LABORATORY INCUBATIONS

	Treatments	Incubation period (Days)									
		0		15		30		60		90	
		UC	C	UC	C	UC	C	UC	C	UC	C
NH_4^+ - N (mg kg^{-1}soil)	Soil+ Sludge (0%)	4.2		4.9		5.3		4.4		3.8	
	(0.5%)	4.5	4.4	7.2	6.2	8.6	7.3	9.2	8.6	8.8	7.8
	(1.0%)	6.9	6.3	9.3	8.9	12.4	11.9	13.6	11.2	11.6	10.8
	(2.0%)	8.2	6.8	12.6	11.2	14.3	13.4	16.4	13.4	14.2	12.1
	(4.0%)	11.9	7.2	23.8	23.1	26.1	27.2	32.3	26.1	26.5	23.4
NO_3^- - N (mg kg^{-1}soil)	(0%)	36.4		43.0		47.9		50.2		45.0	
	(0.5%)	46.4	48.2	49.5	50.9	60.5	56.8	84.6	57.0	79.2	50.6
	(1.0%)	51.4	54.5	57.2	60.5	81.6	79.1	88.8	61.5	76.1	63.8
	(2.0%)	53.6	54.7	63.9	67.7	101.6	92.8	113.2	70.3	101.6	87.1
	(4.0%)	54.6	55.4	68.0	71.4	95.3	72.9	106.1	67.3	97.8	84.9

Potentially mineralizable nitrogen was higher in soil amended with sewage sludge, which was due to a larger mineralizable N pool in organic matter that was build up from continuous use of sewage sludge. With the addition of different levels of sewage sludge, the amount of organic matter increased under laboratory incubation. Organic matter decomposed slowly and released the N content in soil which was indicated by increase in NH_4^+-N and NO_3^--N. The amount of NO_3^--N was less initially because NH^+_4 form of N was less and it increased as incubation progressed because of conversion of NH_4^+ form of N to NO_3^- form of N by activity of nitrifying bacteria. Mineral N (NH_4^++NO_3^--N) was higher in uncontaminated sewage sludge amended soil but was less in soils amended with metal contaminated sewage sludge. The reduction in (NH_4^++NO_3^--N) N contents in presence of metal contaminated sewage sludge could be attributed to increased mineralization of uncontaminated sewage sludge followed by denitrification losses [30]. Wong et al. observed that nitrogen mineralization became greater in sewage sludge amended soils than in unamended soils [51].

IV. Conclusion

Analysis of soils from the farmer's fields amended with 30 to 90 tons of sewage sludge showed the buildup of organic C, nitrogen, phosphorus, microbial biomass, dehydrogenase, alkaline phosphatase, and urease activities. Accumulation of heavy metals in sludge amended soils was below the permissible EC limits. Application of uncontaminated and metal contaminated sewage sludge at different rates 0.5 to 4.0% (corresponding to 10, 20, 40, and 80 t ha^{-1}) resulted in increase in soil organic C, total N, P, K contents significantly over control. Soil organic C declined gradually with incubation time under laboratory conditions. No significant difference in total N, P, and K was observed after 90 days of incubation as compared to zero day.

Available forms of N increased upto 60 days, and P increased up to 30 days, and then, both declined on further incubation. The DTPA extractable forms of heavy metals (Pb) increased up to 15 and (Ni, Cr and Cd) 30 days of incubation and then declined on further incubation. Microbial biomass C, soil dehydrogenase and alkaline phosphatase activities, and C and N mineralization increased significantly on soil amendment at different levels of uncontaminated sewage sludge. However, soils amended with metal contaminated sewage sludge had lower amount of microbial biomass, enzyme activities, and C and N mineralization in comparison with uncontaminated sewage sludge at different levels.

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