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Changes in Plant Nutrients, and Microbial Biomass in Different Soil Depths After Long-Term Surface Application of Secondary Treated Wastewater

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Abstract **- Long-term effects of surface application of secondary treated wastewater on plant nutrients dynamics, the cycling of C and N within the system through the determination of microbial biomass, and associated health hazards were studied in different soil locations. Sites that have been irrigated with wastewater for the last 1, 4, 10, and 17 years were identified and used as sampling locations for this study. Two other sites that have not been irrigated with wastewater were sampled as a control. Soil samples were taken from several sites within each location, and at the following depths: 0-20, 20-40, and 40-60 cm. Results obtained indicated that microbial biomass C and N were increased significantly with increasing application period of treated wastewater. Barley plant tissues analysis showed that plant nutrients content was significantly higher in sites which received wastewater for a long period than other sites. No significances in accumulation of lead (Pb) in barley plant tissues were observed with sites received wastewater for different periods. The bacteriological analysis showed that the total bacterial count of surface soil (0-20 cm) was higher in sites irrigated with wastewater for the last 10 and 17 years. The total coliforms ranged from $0.92x10^2$ cfu/g soil to $3.3x10^2$ cfu/g soil, while fecal coliform were less and detected only in top soils at sites irrigated with wastewater for the last 10 and 17 years.**

Keywords **– Barley, Coliform, Irrigation, Nutrients**

I. Introduction

Soil serves an important ecological function in sustaining a diverse and dynamic microbiological community, cycling of element and being the ultimate receptor of wastes. In many arid and semi-arid countries, treated wastewater (TWW) is considered an additional source of water that is required for landscaping and crop production [4,14] . This was attributed to limited water resources, increased population, and consumption of water, increased awareness and interest in environmental and health issues and the safe disposal of wastewater [3, 15]. There are several wastewater treatment plants established and under operation in Jordan [2]. The amount of wastewater discharged in Jordan is expected to increase up to 150 million cubic meter (MGM) by the year 2015 [7] and most of this water undergone secondary treatment [11]. This large amount of wastewater is available source of irrigation water and fertility materials and is seriously considered for agricultural use. Wastewater may contains a wide spectrum of pathogens and sometimes heavy metals and organic compounds that are hazardous to the soil environment [23]. Reference [12] recommended for disinfection of TWW to ensure further inactivation of microorganisms, and special care should be taken during handling and using TWW [5]. Accordingly, the application of wastewater to the soil also has effects on microbial biomass and activity because it influences the soil characteristics. The objectives of this study were to evaluate the long-term effects of surface application of secondary treated wastewater on plant nutrients dynamics, the cycling of C and N within the system through the determination of microbial biomass, and the health hazards that may be associated with the use and handling of wastewater.

II. Materials and Methods

A. Sampling

Soil samples were collected from the area around Ramtha Wastewater Treatment Plant (33.28^0N and 35.68^0E), north of Jordan. The plant was established in 1987 where municipal wastewater is treated with biological stabilized ponds. The soil of the sampling area was classified as a fine carbonatic, thermic deep family of xerochreptic calciorthid, received an average winter rainfall between 300 to 350 mm per year, mainly between November and April. Soil temperature regime ranges from mesic to thermic class. The treated wastewater has been used to irrigate several forage crops using a drip irrigation system without application of any fertilizer or pesticide. There are sites that have been irrigated with wastewater for many years. In this study, sites that have been irrigated with wastewater for the last 1, 4, 10, and 17 years were identified and used as sampling sites. Each site was randomly divided to three locations and an area of 1000 square meters was used for sampling in each location. In addition, another two sites that have not been irrigated with wastewater (irrigated with groundwater and rain water) for more than 17 years were sampled as a control. Soil samples were taken from each 1000 square meters area (three at least), and at the following depths: 0 –20 cm, 20 – 40 cm, and 40 – 60 cm by Auger. A composed soil sample was made for each soil depth. Soil samples were divided into two portions, samples were stored in plastic packs at 4 ^{0}C for microbiological studies and the other samples were air-dried, ground, and then sieved to pass through a 2- mm sieve screen for physical and chemical analysis. Samples of water used for irrigation were analyzed at two different periods. One square meter of barley (*Hordeum vulgare* L.) plant samples were taken from sampling locations (three at least) before the milk

stage, oven dried (70 ^{0}C) for 48 hours, ground, and then sieved to pass a 0.5 mm sieve screen for analysis.

B. Soil, Water and Plant Analysis

Soil samples were analyzed for soil pH and for electrical conductivity (EC) in the 1 : 5 (soil : water) suspension [20] , pH and EC of irrigation water samples were also measured, soil organic matter was determined by Walkley-Black method [16], calcium carbonate equivalent values were determined by acid neutralization method [20], available phosphorus was determined by extraction with sodium bicarbonate [17], exchangeable potassium was determined by extraction with ammonium acetate [21], cation exchange capacity (CEC) [18], Total plant nitrogen was determined according to [18], Lead (Pb) was determined by using atomic absorption spectroscopy, PerkinElmer AAnalyst 300, and soil texture by hydrometer [8].

C. Microbiological Analysis

Soil microbial biomass C and N were determined using the Chloroform Fumigation Extraction (CFE) technique as outlined by [22]. Soil samples were fumigated under vacuum in desiccators for 24 hours in the dark. After fumigation, the chloroform was removed by repeated evacuation of the chamber. The un-fumigated soil is also incubated in the dark at 25^{0}C for 24 hours. Soil samples were then adjusted to 50% water holding capacity (WHC), and the fumigated samples were inoculated with 1:10 soil: water suspension. After the end of a 10 day incubation period, microbial biomass-N was measured by extraction of soil samples by KCl and filtered through a filter paper and ammonium and nitrate concentrations in the soil extract were measured by the distillation method as described by Keeney and Nelson (1982). Microbial biomass N was calculated by substitution of NH_4 -N and NO_3 –N in un-fumigated soil from fumigated soil after a 10 day incubation divided by the fraction of the killed biomass mineralized (k_n = 0.57). Microbial biomass C was measured by transferred soil samples to gas-tight-jars containing NaOH to collect CO_2. Microbial biomass C was calculated by the substitution of CO_2 evolved in un-fumigated soil from fumigated soil divided by the fraction of biomass carbon mineralized (kc = 0.45). To enumerate the total aerobic heterotrophic bacteria, serial soil dilution was performed by weighing 10 g moist soil and dispersing it by magnetic agitation for 30 min. in 90 ml of sterile water. Plating in duplicates of successive dilution of 10^{-3}, 10^{-4}, and 10^{-5} was then carried out at a rate of 1.0 ml on PGY agar (peptone-glycose-yeast extract agar) plates [13]. The plates were incubated at 25^{o}C for 72 hours and the total aerobic microflora were counted as colony forming units per 1 g soil (CFU g^{-1}soil). The total coliforms were enumerated according to the multiple tube fermentation tests, in which Lauryl tryptose broth tubes (presumptive test) were inoculated from serial soil dilution 10^{-1}, 10^{-2}, and 10^{-3} for each soil sample. The tubes were incubated at 35^{0}C for 48 hours and the most probable number (MPN) was calculated for the samples. The fecal coliforms were assayed on fecal coliforms EC broth, which were incubated at 44^{o}C for 24 hours. The total coliforms and fecal coliforms were enumerated as described by the Standard Methods for the Examination of Water and Wastewater (1995).

D. Statistical Analysis

The data were subjected to an analysis of variance (ANOVA) in completely randomized design (CRD) with factorial arrangement of treatment using JMP IN version 4 procedure 2001 by SAS Institute Inc. Comparison of treatment means was done using Turkey-Kramer test (<0.05).

III. Results and Discussion

A. Soil Analysis

The soils were characterized as a highly calcareous and clayed textured throughout the soil profile, the clay content ranged between 58% to 61% and the sand content was between 5% to 7%, the salts content as measured by electrical conductivity were low, and the cation exchange capacity (CEC) were relatively high (31-35 Cmol/Kg), (Table 1).

B. Total Bacterial counts

Distribution of heterotrophic aerobic bacteria in soil samples irrigated with TWW for different periods showed that soils which received TWW for the long periods, 10 and 17 years, have significantly higher count than sites which received TWW for the last 1and 4 years, at soil depths 0-20 cm and 20-40 cm, whereas no significant differences were found in bacterial count at the soil depth 40-60 cm. Results also indicated that the total heterotrophic aerobic bacterial counts at soil surface (0 – 20 cm) were higher in soils irrigated with TWW compared with other soil depths. For example, the counts were 5.2 x 106 CFU/ g soil and 5.8 x 106 CFU/ g soil in soils which received TWW for the last 10 years and 17 years, respectively. While the counts were 2.3 x 106 CFU/ g soil and 3.5 x 106 CFU/g soil in sites which received TWW for the last 1 and 4 years, respectively (Table 2).

The bacterial count (CFU) represents the whole community of bacteria and may involve bacteria that are more resistance to water pollution. Soil microbial populations can actually benefit from the addition of nutrients, organic materials and microbes present in wastewater effluents. Results of soil samples analysis showed significant increases ($p<0.05$) of soil organic matter, total nitrogen and available phosphorus in sites irrigated with TWW for the last 10 and 17 years in top soil (0-20 cm). No significant increases in soil organic matter content were observed at the soil depths 20-40 cm and 40-60 cm. This organic carbon accumulation may serves as a source of energy for heterotrophic bacteria and attributed to the possible decomposition and oxidation of the soil carbon by the introduced microorganisms through TWW.

TABLE 1

COMPARISON OF SOME CHEMICAL PROPERTIES OF SOILS IRRIGATED WITH AND WITHOUT SECONDARY TREATED WASTEWATER (GROUND WATER, GW AND RAIN WATER, RW) FOR DIFFERENT PERIODS

Depth (cm)	Period (years)	pH	EC (dS/m)	CEC ($Cmol_e$/Kg)	$CaCO_3$ (%)	OM (%)	P (ppm)	N (ppm)
0 -20	1	7.7b	0.5a	33.5bc	11.6a	0.95c	4.9d	520c
	4	7.9b	0.5a	35.6a	12.0a	1.06bc	18.1c	790b
	10	7.9ab	0.6a	34.5abc	12.3a	1.23ab	40.5b	930a
	17	8.0a	0.6a	35.2ab	12.5a	1.28a	51.6a	1010a
	Gw	7.9ab	0.5a	33.0c	11.7a	0.72d	20.5c	410d
	Rw	7.8ab	0.4a	34.0abc	10.9a	0.61d	3.6d	270e
20-40	1	7.8b	0.5a	32.5c	15.3a	0.75a	4.4c	560b
	4	8.0ab	0.6a	33.9bc	14.2ab	0.70a	20.3b	700a
	10	8.2a	0.6a	33.7bc	13.2bc	0.71a	29.5a	620b
	17	8.0ab	0.7a	34.2b	15.2a	0.70a	30.2a	750a
	Gw	8.0ab	0.5a	34.0ab	13.2bc	0.50b	18.2b	412c
	Rw	7.8b	0.5a	36.3a	12.2c	0.50b	3.1c	300d
40-60	1	7.8b	0.5b	33.7b	14.6ab	0.49a	2.5d	415b
	4	8.2a	0.6ab	31.4c	15.5a	0.50a	19.5b	400b
	10	8.1a	0.8a	33.4b	15.1ab	0.50a	25.8a	505a
	17	8.1a	0.8a	34.1b	15.6a	0.50a	20.1b	490a
	Gw	8.1a	0.7a	36.2a	13.8ab	0.32a	10.5c	308c
	Rw	7.8b	0.5b	36.0a	14.3b	0.30a	3.2d	275c

Mean within a column of each soil depth followed by the same letter are not significantly different at $p<0.05$ according to Tukey-Kramer HSD test.

TABLE 2

DISTRIBUTION OF HETEROTROPHIC AEROBIC BACTERIAL COUNTS (CFU/ G SOIL X 10^6) IN SOILS IRRIGATED WITH AND WITHOUT SECONDARY TREATED WASTEWATER (GROUND WATER, GW AND RAIN WATER, RW) FOR DIFFERENT PERIODS

Soil depth	Period of TWW irrigation, years				Conventional irrigation	
(cm)	1	4	10	17	Gw	Rw
0 – 20	2.3c	3.5bc	5.2a	5.8a	4.7ab	2.2c
20 – 40	1.2bc	2.1b	4.1a	3.8a	3.6a	1.0c
40 – 60	1.0a	1.2a	1.4a	1.3a	1.5a	0.8a

Mean within a row of each soil depth followed by the same letter are not significantly different at $p<0.05$ according to Tukey-Kramer HSD test.

TABLE 3

CHARACTERISTICS OF WATER USED FOR IRRIGATED AT TWO DIFFERENT PERIODS (AVERAGE OF THREE REPLICATES)

Parameter	Unit	Winter period			Summer period		
		Treated Wastewater	Ground-water	Rain Water	Treated Wastewater	Ground-water	Rain Water
pH		7.4	7.5	6.8	7.3	7.2	-[1]
Electrical Conductivity (EC)	dSm^{-1}	2.28	1.54	0.12	2.75	1.70	-
Total Dissolved Salts (TDS)	ppm	1459	996	115	1760	1049	-
Nitrate (NO_3^-),	ppm	32.2	10.0	-	45.6	1.8	-
Phosphorus (P)	ppm	26.4	3.8	-	27.6	-	-
Potassium(K)	ppm	31.3	17.4	-	30.6	16.2	-

1, Non detectable

The Roman numerals used to number the sections are The average of microbial counts in soils irrigated with groundwater was 4.8 x 10^{-6} CFU/ g soil at soil surface (0 - 20 cm) and was significantly higher than that of soils irrigated with rain water, 2.2x10^{-6} CFU/g soil, whereas no significant differences were found compared with sites which received TWW for the last 4, 10, and17 years (Table 2). These differences in microbial counts may be attributed to the characteristics of water used for irrigation.

Results of water samples analysis (Table 3) showed that groundwater contains nutrients such as N, P, and K compared with rain water which is free of nutrients. Reference [12] reported that the total aerobic bacterial counts of surface soil were similar in all irrigated plots with and without TWW,

suggesting that the use of TWW did not stimulate or inhibit these microfloras. While other researchers reported an increase in the soil organic carbon and stimulate heterotrophs for their maintenance and growth [13, 25].

C. Microbial Biomass C and N

The microbial biomass as C is always larger than the microbial biomass as N, because microbes consists of approximately 50% C and about 5% N, and the amount of nitrogen as biomass tends to vary for different types of microbes [1]. Both biomass C and N showed significant differences between sites which received TWW for different period of irrigation. Sites irrigated with TWW for the last 10 years and 17 years have microbial biomass at soil surface (0 – 20 cm) 200.3 mg C/ kg and 290.7 mg C/ kg soil, respectively. While sites irrigated with wastewater for the last 1 year and 4 years have microbial biomass 80.6 mg C/ kg and 100.2 mg C/ kg soil, respectively. Generally, microbial biomass as C and N were decreased with the soil depths (Tables 4 and 5).

TABLE 4

DISTRIBUTION OF MICROBIAL BIOMASS CARBON (MG C/KG SOIL) IN SOILS IRRIGATED WITH AND WITHOUT SECONDARY TREATED WASTEWATER (GROUND WATER, GW AND RAIN WATER, RW) FOR DIFFERENT PERIODS

Soil depth	Period of TWW irrigation, years				Conventional irrigation	
(cm)	1	4	10	17	Gw	Rw
0 – 20	80.6c	100.2c	200.3b	290.7a	300.1a	80.4c
20 – 40	55.1c	77.5b	105.6a	118.2a	80.1b	45.7c
40 – 60	50.2ab	45.6ab	55.1a	55.3a	56.0a	43.4b

Mean within a row of each soil depth followed by the same letter are not significantly different at $p<0.05$ according to Tukey-Kramer HSD test

TABLE 5

DISTRIBUTION OF MICROBIAL BIOMASS NITROGEN (MG N/KG SOIL) IN SOILS IRRIGATED WITH AND WITHOUT SECONDARY TREATED WASTEWATER (GROUND WATER, GW AND RAIN WATER, RW) FOR DIFFERENT PERIODS

Soil depth	Period of TWW irrigation, years				Conventional irrigation	
(cm)	1	4	10	17	Gw	Rw
0 – 20	10.8c	12.1c	27.7b	32.3ab	36.1a	11.7c
20 – 40	7.1ab	5.3bcd	8.0a	6.7abc	4.1cd	3.6d
40 – 60	2.2a	2.1a	1.5a	1.4a	2.0a	1.1a

Mean within a row of each soil depth followed by the same letter are not significantly different at $p<0.05$ according to Tukey-Kramer HSD test.

Soils contain naturally occurring bacteria, fungi, actinomycetes, and protozoa which make their habitat on soil surfaces and are responsible for many biological transformations of nutrients in wastewater. Actually microbial populations may benefit from addition of carbonaceous substrates, nutrients, and microbes present in wastewater effluents. Addition of these substrates in TWW may maintain elevated microbial biomass. Increased microbial biomass as C and N in surface soils has also been reported by other researchers [24, 9]. Soils with high microbial biomass may have more microbial activities, more biochemical transformation of elements in soil. In order to find the relationship between soil biomass and fertility, concentrations of some plant nutrients were evaluated in different sites irrigated with TWW and for barley plants (Table 6). Results of barley plant tissues analysis showed that N, P, and K concentrations were significantly higher in sites irrigated with TWW for the long periods than other sites. These findings support the results of the direct relationships between soil biomass and soil fertility reported by [6]. On the other hand, results indicated that there were no significant differences in accumulation of Lead (Pb) in barley plant tissues with sites received TWW for different periods. This may be due to the possibility of reducing soil contaminants by continuous cultivation of sites with forage crops.

TABLE 6

AVERAGE CONCENTRATION OF SOME NUTRIENTS FOR BARLY PLANTS IN DIFFERENT LOCATIONS

Period, year	N,%	P,%	K,%	Pb, ppm
1	1.02a	0.15a	2.1a	5.81a
4	1.07a	0.19ab	3.3b	6.12ab
10	1.40b	0.23b	4.3c	6.08ab
17	1.31b	0.30c	4.8c	5.92a
GW	0.76a	0.13a	2.6a	6.05ab
RW	0.31c	0.08d	1.1d	3.21c

Mean within a column followed by the same letter are not significantly different at p<0.05 according to Tukey-Kramer HSD test.

D. Total Coliforms and Fecal Coliforms

Results of total coliform counts in sites irrigated with TWW showed that sites received TWW for the last 10 years and 17 years were 2.6 x 102 and 3.3 x 102 CFU/ g soil, respectively. While site received TWW for the last 1 year was 0.92 x 102 CFU/ g soil. However, in general the counts tended to be higher at soil surface (0 – 20 cm) that at soil depth 20 – 40 cm and non detectable counts were observed at soil depth 40 – 60 cm (Table 6). On the other hand, sites irrigated with conventional water (ground water and rain water), results showed that the total coliform counts in sites irrigated with groundwater were higher than sites received TWW at all periods of application. For example, the total coliform count at the soil depth 0 – 20 cm was 4.6 x 102 CFU/ g soil in site irrigated with groundwater, whereas the count was 3.3 x 102 CFU/ g soil in site irrigated with TWW for the last 17 years. However, non detectable counts were found with using rain water for irrigation (Table 7).

Results of fecal coliform counts, as indicated in Table 8, showed that the higher count was found in the site irrigated with TWW for the last 17 years and to a less extend in sites irrigated with groundwater and with TWW for the last 10 years. Other sites, however, showed non detectable fecal coliforms. The fecal coliform counts were lower or even not present in soil samples irrigated with TWW for the last 1 and 4 years. Fecal coliform counts were detected only at the soil surface 0 – 20 cm (Table 8). Results of groundwater analysis

indicated that the groundwater was free of fecal coliforms. The fecal coliforms detected in soil irrigated with groundwater could be introduced by other sources. Compared with WHO (1989) recommended guidelines for wastewater use in agriculture, the results obtained in this study indicated that there is no human health impact. Reference [10] and other researchers reported the use of fecal coliforms as an indicator of pollution is a better indication of pollution than total coliforms since total coliforms may include strains that are not of fecal origin.

TABLE 7

TOTAL COLIFORM COUNTS (CFU/ G SOIL X 10^2) IN SOILS IRRIGATED WITH AND WITHOUT SECONDARY TREATED WASTEWATER (GROUND WATER, GW AND RAIN WATER, RW) FOR DIFFERENT PERIODS

Soil depth	Period of TWW irrigation, years				Conventional irrigation	
(cm)	1	4	10	17	Gw	Rw
0 – 20	0.92	1.1	2.6	3.3	1.6	*
20 – 40	*	0.3	0.6	0.7	0.7	*
40 – 60	*	*	*	*	*	*

* Non detectable

TABLE 8

TOTAL FECAL COLIFORM COUNTS(CFU/ G SOIL X 10^2) IN SOILS IRRIGATED WITH AND WITHOUT SECONDARY TREATED WASTEWATER (GROUND WATER, GW AND RAIN WATER, RW) FOR DIFFERENT PERIODS

Soil depth	Period of TWW irrigation, years				Conventional irrigation	
(cm)	1	4	10	17	Gw	Rw
0 – 20	*	*	0.2	0.8	0.2	*
20 – 40	*	*	*	*	*	*
40 – 60	*	*	*	*	*	*

* Non detectable

IV. CONCLUSION

This study has shown that the soil microbial population can actually benefit from the addition of nutrients, organic materials and microbial present in wastewater effluent used for irrigation. The effect was greatest in the top 0-20 cm soil depth and declined sharply below that depth. The changes in microbial population microbial biomass and the availability of plant nutrients coincided with the continuous surface application of wastewater resulted in accumulation of organic materials at the top soil depth. Microbial biomass C and N were increased significantly with increasing application period of treated wastewater.

The enhanced bacterial counts and microbial biomass in soil samples is related directly to the long term application of TWW. Enhancement of microbial counts and activities related to increased nutrient cycling, through biochemical transformation, and were, therefore, indicative of improved soil fertility resulting from long term irrigation of soil with TWW. No significances in accumulation of lead in barley plant tissues were observed with sites which received treated wastewater. Additionally, with regard to the health problem, surface drip irrigation reduce the contact between the effluent and the workers or the aerial parts of the plants. Thus, the prompt recovery of the limited water resources in the region would be very important. Disinfection of TWW is recommended to insure against further inactivation of microorganisms.

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