Final Report

Studying the Impact of Bio-solids Residuals and Effluent Constituents on Soil Fertility and Crop Production

Case Study for Madaba Wastewater Treatment Plant

(Maize)

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SUBMITTED TO

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Application of Treated Bio-solids to Land Irrigated with Effluent

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1. INTRODUCTION

The reuse of treated wastewater and bio-solids is a concept that is being increasingly accepted in most regions of the world, both in industrialized and developing countries, but one which will dramatically increase in global importance over the coming decades. The reason for this is as simple as it is stark: the number of people living in water-stressed and water-scarce countries is increasing at a rate much greater than that of the number of people in the world; hence, the need for water and food is continuously increasing. (Kretschmer *et al.*, 2002 & Mara, 2006).

In Jordan, reclaimed wastewater treated at domestic wastewater treatment plants is an important component of the national water budget. About (94) Million Cubic Meters (MCM) in the year 2003, 101 MCM in the year 2004 and 107 MCM in the year 2005 were treated and discharged into various water bodies or used directly or indirectly for irrigation and other uses. Reuse of treated effluent is expected to increase up to 262 MCM in the year 2020. (Uleimat, 2006).

Sewage sludge / bio-solids represents an increasing challenge all over the world. In Jordan, huge amounts of bio-solids are generated annually and discharged of into dumping sites. In other words, none of the bio-solids is being reused. Bio-solids contain organic matter and nutrients that are essential to crops. Potentially, bio-solids can be used as fertilizer to enhance soil fertility and crop production. In this respect, the reuse of bio-solids in agriculture can be both economically and environmentally accepted (Amin and Sherif, 2001).

The current project is a continuing activity to a previously funded project by the International Arid Lands Consortium (IALC) at the University of Arizona (UoA). The activity was executed by the Environmental Research Center (ERC) of the Royal Scientific Society (RSS) of Jordan over a period of two successive seasons. The previously funded project aimed at assessing the feasibility of utilizing generated dewatered bio-solids for improving soil fertility and crop production in Jordan. This project investigates attempts at evaluating the feasibility of the combined reuse of reclaimed wastewater and bio-solids for improving soil fertility and crop production in addition to studying the fate of pathogens when Type I bio-solids is rewetted with treated effluent.

The general objective of the project is to investigate the feasibility of using bio-solids and treated effluent for improving soil fertility and crop production. The specific measurable objectives are:

1. To evaluate the impacts of applying bio-solids and treated effluent on soil properties and on crop yield and quality based on field-pilot experiments.
2. To recommend appropriate bio-solids application procedures and loading rates for fodder crops irrigated with treated effluent.
3. To determine the fate of pathogens in bio-solids when re-wetted with treated effluent under irrigation of fodder crops.

In January of 2007 the USAID, under a cooperative agreement with IALC / University of Arizona, approved a request by RSS through BRDC / Jordan to finance this research project that is to be implemented throughout two phases at Madaba WWTP.

This is the final technical report that summarizes different activities and tasks executed throughout the first growing season of the project that was executed during the period Jan – August 2007.

2. LITRATURE REVIEW

Many field studies were carried out to investigate the reuse of reclaimed wastewater for irrigation purposes.

Panoras *et al* (2003) studied the effect of irrigation with effluent on field grown corn. The experimental layout was that of a split plot design with six replications, three water qualities and two irrigation methods were used. Potable water was used as the control. No significant differences in corn yield have been observed among the three water-quality treatments. However, significant differences have been observed in corn yield between the irrigation methods. Furrow irrigation has produced about 10% more corn yield than trickle irrigation.

Tamrabet *et al* (2004) carried out two experimentations under semi-controlled environment to investigate the effect of wastewater and sewage sludge applications on growth of barley and soil properties. For the first study, Wastewater applications were carried out according to three modalities; application with 100% wastewater, 100% plate water and 50%/50% wastewater to plate water. The second study was similar to the first one, except that sewage sludge doses applied were zero, 30 and 60t/ha. Results showed that the irrigation with wastewater and applications of sewage sludge contribute to the improvement of the plant yield with increases ranging from 100% to 250%. Irrigation with wastewater and particularly applications of sewage sludge improve effectively crop water use efficiency and reduces the evaporative part of irrigation water.

Rusan *et al* (2006) studied the long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. In their study, sites irrigated with wastewater for 10, 5, and 2 years and site not irrigated were sampled for soil and plant chemical analysis to evaluate its long term effect. Long term wastewater irrigation increased salts, organic matter and plant nutrients in the soil. Soil pH was not consistently affected. Soil Cu was not affected by wastewater application while Zn, Fe and Mn was not consistently affected. Wastewater irrigation had no significant effect on soil heavy metals (Pb and Cd) regardless of duration of wastewater irrigation. The barley biomass increased with added wastewater and nutrients provided with the wastewater. However, longer period of wastewater application (10 years) resulted in lower biomass production but remained higher than that of the control plants. Plant essential nutrients (Total-N, NO3, P, and K) were higher in plants grown in soils irrigated with wastewater. Plant Cu, Zn, Fe, Mn increased with 2 years of wastewater irrigation, then reduced with longer period. Plant Pb and Cd increased with wastewater irrigation and their levels were higher the longer the period of wastewater irrigation. Based on these results, it can be concluded that proper management of wastewater irrigation and periodic monitoring of soil and plant quality parameters are required to ensure successful, safe, long-term wastewater irrigation.

In addition, numerous investigations have been conducted in either greenhouses or in field-pilot experiments to asses impacts of bio-solids on soil fertility and crop production.

The Royal Scientific Society of Jordan carried out a research study over two successive seasons during 2004-2006 at Ar-ramtha Regional Center to investigate the role of bio-solids application in improving soil fertility and crop production in Jordan. The experiment was established using Factorial Randomized Completely Block Design (FRCBD) with four replications. Different bio-solids application rates were applied to designed plots grown with barley. Results showed increase in barley yield with increase in bio-solids application rate, also, increase in nutrients concentrations in both soil and plant. There were no clear evidence for heavy metals accumulation in both soil and plant.

Bozkurt and Yarilgac (2003) investigated the effects of various sewage sludge (bio-solids) rates and a single dose barnyard manure application on the fruit yield, growth, nutrition and heavy metal accumulation of apple trees. The experiment was conducted using a completely randomized design with four replicates in 2000 and 2001. Two years data showed that the addition of sewage sludge to soil significantly increased fruit yield, accumulative yield efficiency, shoot growth and leaf N, Mg, Fe, Mn and Zn concentrations. These increases were generally lower with barnyard manure applications. The sewage sludge and manure applications did not cause any significant increase in tree trunk girth and P, K, Ca, Ni, Cr and Cd concentrations in leaf samples. Leaf Fe, Mn and Zn concentrations increased at the highest sludge application rate. The two-year results of this study demonstrated that sewage sludge applied to apple trees did not cause toxicity in the leaves.

3. ROGRAMMED & EXECUTED ACTIVITIES

Table (1) below shows the schedule for implementing different activities for the first growing season of the project (Jan. to Oct. 2007). Following is a description of the activities executed throughout the period covered by this report.

Table (1): Implementation schedule for the first growing season of the project (Jan.– Oct. 2007).

Activity	Month									
	Jan 07	Feb 07	Mar 07	April 07	May 07	June 07	Jul 07	Aug 07	Sep 07	Oct 07
Site Investigation & Final Selection	X	X								
Land Preparation			X	X						
Plantation					X	X	X	X		
Soil Sampling & Analysis				X				X		
Plant Sampling & Analysis								X		
Evaluation and Reporting										X

3.1 Mobilization

RSS is conducting the project in a close cooperation with Water Authority of Jordan (WAJ). The field experiments were conducted on agricultural reuse land within the vicinity of Madaba WWTP. WAJ facilitated the use of the reuse land.

The project team consisted of the following staff members:

1. Dr. Bassam Hayek: PhD in Chemical Engineering. Director of the Environmental Research Center ERC / RSS. (Role: provide overall guidance, coordinate with various parties, and acted as a contact person with IALC).
2. Dr. Nisreen Al-Humoud: PhD in Microbiology. Researcher at ERC / RSS. (Role: Provide overall guidance on the execution of the project, supervising all technical aspects of the project and coordinated with various parties).
3. Eng. Asma Alsheraideh: M.Sc. in Civil Engineering / Water Resources & Environment. Researcher at ERC / RSS. (Role: Follow-up field-pilot experiments, supervise data collection and assessment and preparing progress and final technical reports).
4. Naser Budier: B.Sc. in Agricultural Science / Soil, Water and Environment. (Role: Follow-up day-to-day activities of the project conducting physical-chemical analysis of bio-solids / treated effluent samples, in addition to managing the sampling / harvesting process and participate in preparing progress and final technical reports).

The *ad hoc* committee that was formed during Ramtha project continued its meetings and supervisory role. The ad hoc committee comprised representatives of different stakeholders including governmental and non-governmental organizations as well as academic institutions. The committee met regularly to follow-up and for discussing the different aspects and to get updated results of various activities, and to provide suggestions and recommendations. The following list shows names of the *ad hoc* committee members:

1. Dr. Manar Fayyad: Director of the Water and Environment Research and Study Center, University of Jordan.
2. Dr. Maha Halalsheh: University of Jordan.
3. Dr. Sa'ad Al-Ayyash: Badia Research and Development Center BRDC.
4. Dr. Ziad Al-Ghazawi: Jordan University of Science and Technology.
5. Eng. Saleh Malkawi: Water Authority of Jordan WAJ / Ministry of Water and Irrigation MWI.

6. Eng. Khalil Jamjoum: National Center for Agricultural Research and Technology Transfer NCARTT / Ministry of Agriculture.
7. Eng. Husni Hamdan: Ministry of Environment.
8. Eng. Ahmad Ulimat: Directorate of Water Quality, WAJ / MWI.
9. Dr. Bassam Hayek: Director of ERC / RSS.
10. Dr. Nisreen Al-Humoud: ERC / RSS.
11 . Eng. Asma Alsheraideh: ERC / RSS.

3.2 Location:

Madaba wastewater treatment plant, where the project activities were carried out, started operation in (1988). The treatment system used to be of waste stabilization ponds type, and then later in (2002) it was changed to mechanical system (activated sludge). The treatment plant currently serves about (50,000) inhabitants. The influent to the treatment plant is 5,500m^3/d. The treated effluent amount is totally used to irrigate lands grown with forage crops within the vicinity of the treatment plant. Figure (1) shows a schematic flowchart for Madaba treatment plant.

3.3 Land Preparation and Plantation:

First of all, land was fenced for safety reasons, then it was cultivated two times and plots were established. Figure (2) shows the experimental layout.

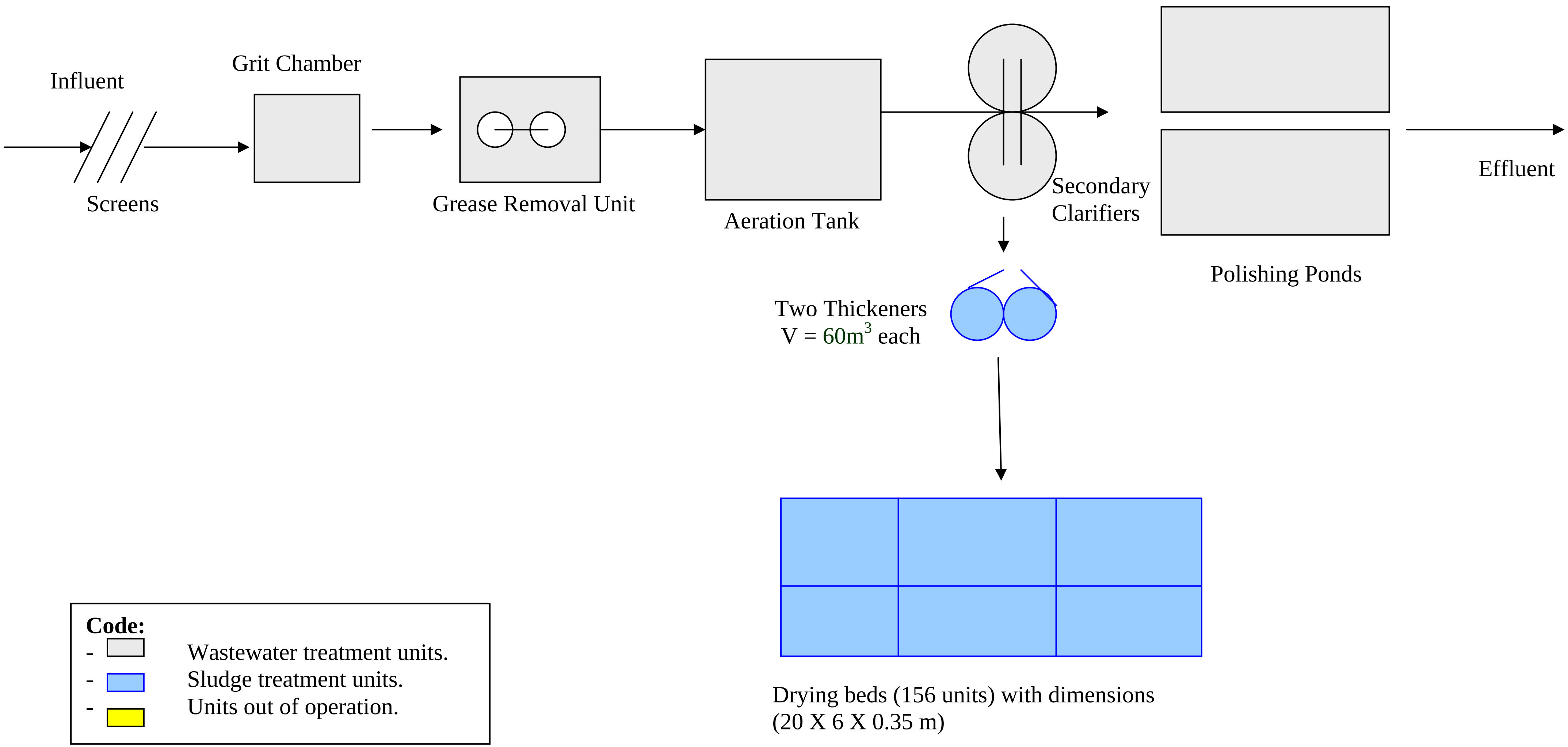


Figure (1): Madaba Wastewater Treatment Plant / Schematic Flowchart.

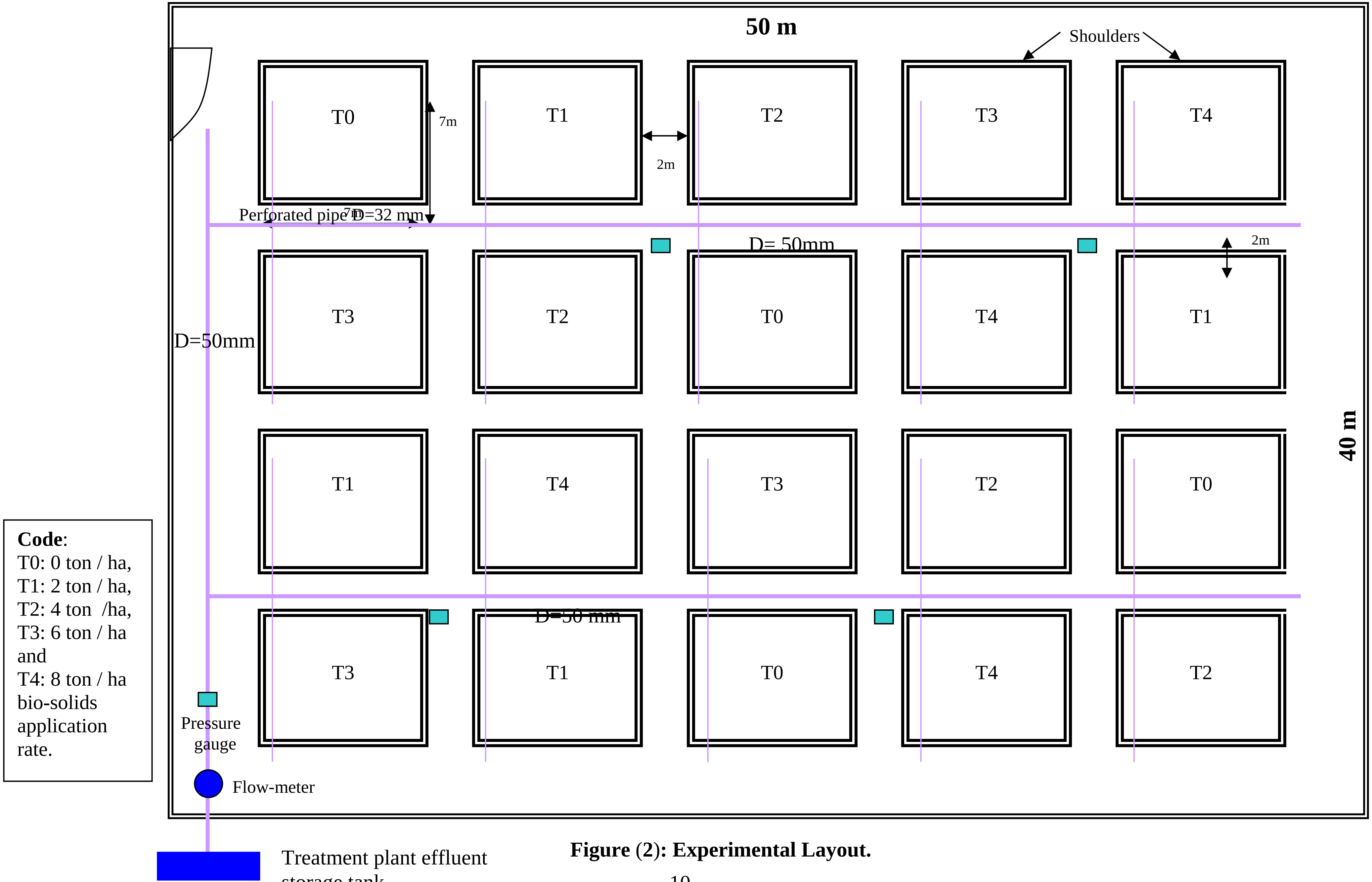


Figure (2): Experimental Layout.

The irrigation system shown in Figure (2) was laid out in a manner to insure equal distribution of irrigation water over the plots. The irrigation system received the treated effluent from the effluent storage tank at the treatment plant though pumping. A flow meter was installed at the main pipe of the system in order to measure quantities of the irrigation water. A main pipe, branches into two sub-mains (50 mm in diameter), was used. Perforated pipes (32 mm in diameter) were laid in front of each plot in order to evenly distribute the irrigation water on each plot. Five pressure gauges were distributed throughout the system in order to ensure constant pressure (minimum head-losses generated).

Dewatered bio-solids was obtained from Madaba treatment plant and was applied as such to each plot after land cultivation and establishment of layout. Five different bio-solids treatments were applied, control, 2, 4, 6 and 8 ton/ ha, as shown in the experimental layout. Bio-solids were incorporated uniformly with soil to a depth of (10 cm).

Miaze was sowed manually at a seeding rate of 1.1 kg/plot, crop was irrigated on a bi-weekly basis with a total amount of 500 m^3 of treated effluent over the whole season.

4. BASELINE DATA (RECLAIMED WASTEWATER, BIO-SOLIDS & SOIL)

4.1 Reclaimed Wastewater Quality

Three reclaimed wastewater samples were taken from the irrigation system during irrigation processes. Samples were physically, chemically and microbiologically analyzed as shown in Table (2). Results show variation in some parameter values between the samples, such as T.kj.N and nitrate. In addition, most micronutrients values were less than detection limits or relatively have low concentrations.

In general, it can be noticed that effluent analysis meets the requirements of the Jordanian Standard No. (893/2006) for irrigation of field crops.

Reclaimed wastewater samples were analyzed following the "Standard Methods for the Examination of Water & Wastewater", 20th edition,1998.

Table (2): Results of reclaimed wastewater analysis at Madaba WWTP.

Parameter	Unit	S1	S2	S3	Average	JS: 893/2006
pH	SU	8.15	7.80	8.00	7.98	6-9
BOD_5	mg/l	41	34	35	37	<300
COD	mg/l	91	71	82	81	<500
TSS	mg/l	74	35	30	46	<300
T.kj.N	mg/l	18.5	39.2	41	33	-
NO_3	mg/l	70.6	0.5	0.5	23.9	<70
B	mg/l	0.75	0.70	0.70	0.72	<1
Cd	mg/l	<0.005	<0.005	<0.005	<0.005	<0.01
Cr	mg/l	<0.02	<0.019	<0.019	<0.019	<0.1
Cu	mg/l	<0.02	<0.018	<0.018	<0.018	<0.2
Pb	mg/l	<0.09	<0.017	<0.017	<0.017	<5
Mo	mg/l	<0.04	<0.02	<0.02	<0.02	<0.01
Ni	mg/l	<0.04	<0.04	<0.04	<0.04	<0.2
Zn	mg/l	0.035	0.033	0.032	0.033	<5
Salmonella	MPN/l	-	N.D.	N.D.	N.D.	-
TFCC	MPN/100ml	-	2.40E+04	7.90E+03	1.60E+04	-
E. coli	MPN/100ml	-	2.40E+04	7.90E+03	1.60E+04	-
Nem. Eggs	egg/5l	-	N.D.	N.D.	N.D.	<=1 (MPN/l)

N.D. : Not Detected

4.2 Bio-solids Quality

Five dewatered bio-solids samples were collected from different drying beds at Madaba treatment plant to be analyzed. Table (3) shows analysis results of physical, chemical and microbial properties for the samples.

As can be noticed from the table, levels of trace elements in the five samples were relatively low when compared to the requirements of the US EPA Rule 503 and the Jordanian Standard (1145/2006) for utilizing bio-solids for agricultural land application. In addition, *fecal coliform* TFCC, *Salmonella* and Nematodes Eggs IPN values are not exceeding the requirements of the two standards for class A or type 1 bio-solids. Hence, applied bio-solids can be classified as class A according to US EPA Rule 503 or type 1 according to JS (1145/2006) assuming no viruses present in the biosolids.

Table (3): Results of bio-solids analysis at Madaba WWTP.

Parameter	Unit	S1	S2	S3	S4	S5	Average	JS: 1145/2006	US EPA	
									Ceiling Conc.*	Poll. Conc.**
TS	%	95.5	94.9	94.8	95.5	94.4	95.0	>90	-	-
T-N	%	5.3	4.6	4.6	4.8	5.2	4.9	-	-	-
T-P	%	1.30	1.44	1.31	1.23	1.33	1.32	-	-	-
K	mg/kg D.W.	52	67	61	69	32	56	-	-	-
Cd	mg/kg D.W.	12.0	16.0	16.7	12.0	17.1	14.8	<40	< 85	< 39
Cr	mg/kg D.W.	37.3	47.9	49.3	40.0	51.6	45.2	< 900	-	-
Cu	mg/kg D.W.	97.9	112.4	117.7	104.1	135.4	113.5	< 1500	< 4300	< 1500
Pb	mg/kg D.W.	115.9	165.5	177.2	123.9	192.7	155.0	< 300	< 840	< 300
Mo	mg/kg D.W.	23	22	21	23	23	22	< 75	< 75	-
Ni	mg/kg D.W.	22	25.2	24.2	19.7	26.7	23.6	< 300	< 420	< 420
Zn	mg/kg D.W.	1036	1058	1088	1062	1192	1087	< 2800	< 7500	< 2800
Salmonella	MPN/4 gm	0.23E+00	0.46E+00	<3.00E+00	0.84E+00	0.90E+00	1.09E+00	< 3/4 gm	< 3/4 gm	
TFCC	MPN/gm	<3.00E+00	<3.00E+00	1.59E+01	<3.00E+00	<3.00E+00	5.57E+00	< 1 X 10^3	< 1 X 10^3	
Nem. Eggs	Egg/gm	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 1/4 gm	< 1/4 gm	

D.W. : Dry Weight.
N.D. : Not Detected.
*: Maximum concentration of each pollutant that bio-solids can contain and still be land applied. Limits are applied as maximum, never to be exceeded values.
** : Land applier has no land application requirements relative to pollutants for bio-solids meeting these limits.

Bio-solids samples were analyzed following the "Standard Methods for the Examination of Water & Wastewater", 20th edition,1998. Other analytical methods were also applied, especially those of the US EPA. Figure(3) shows bio-solids application to the experimental plots.

Figure (3): Bio-solids application to the experimental plots.

4.3 Soil Quality

In order of collecting data about the soil quality at the experimental site prior bio-solids application, fifteen composite soil samples were collected at depth of 0-15 cm. Samples were analyzed in accordance to the Soil Science Society of America (1996) for general physical, chemical and microbial characteristics. Results are shown in Table (4).

The preliminary analyses of soil show that the soil is alkaline, pH ranges between 7.87-8.03, with relatively low organic matter content, Nitrogen, Potassium and phosphorus contents are relatively high, while trace elements levels are within normal low range. Microbiological analysis of soil show low values of *fecal coliform* TFCC, *Salmonella* and Nematodes Eggs IPN. Figure (4) shows the experimental site after one month of seeding.

Table (4): Soil quality at the experimental site prior to bio-solids application.

Parameter	Unit	To	T1	T2	T3	T4
Soil Texture	-	Clay	Clay	Clay	Clay	Clay
pH 1:1	SU	8.03	7.92	7.87	8.03	7.88
EC 1:1	dS/m	1.24	1.13	1.20	1.08	1.36
Organic matter	%	2.27	3.08	3.38	2.23	3.57
T-N	mg/kg D.W	1704	2026	2229	1676	2404
NH4-N	mg/kg D.W	40.67	39.43	40.15	40.73	46.67
NO3-N	mg/kg D.W	125.47	132.57	157.83	62.63	156.83
available-P	mg/kg D.W	796	993	1096	555	1258
available-K	mg/kg D.W	479.67	604.67	540.33	523.67	537.00
exchangeable-Na	mg/kg D.W	525.00	467.33	509.00	466.33	608.67
Cd	mg/kg D.W	0.10	0.13	0.14	0.08	0.15
Cr	mg/kg D.W	<0.05	<0.05	<0.05	<0.05	<0.05
Cu	mg/kg D.W	1.69	1.80	2.09	1.38	1.44
Pb	mg/kg D.W	0.83	0.98	1.11	0.71	0.91
Mo	mg/kg D.W	<0.05	<0.05	<0.05	<0.05	<0.05
Ni	mg/kg D.W	0.54	0.65	0.58	0.53	0.73
Zn	mg/kg D.W	9.92	14.86	14.38	9.66	12.87
Salmonella	MPN/gm	3.10E+01	N.D.	2.80E+01	3.67E+00	1.33E+00
TFCC	MNP/gm	0.30E+00	0.30E+00	2.52E+01	3.69E+01	3.69E+01
Nem. Eggs	egg/20gm	N.D.	N.D.	N.D.	N.D.	N.D.

D.W. : Dry Weight.
N.D. : Not Detected.

Figure (4): The experimental site after one month of seeding

5. MONITORING AND FIELD MEASUREMENTS

In order to assess impacts of bio-solids application and irrigation with effluent on plant and soil properties, many field measurements, laboratory analysis and monitoring activities were carried out. These are described below:

5.1 Sampling and Analysis

Plant samples from the above ground parts were collected from plots at harvesting stage. **Figure** (**5**) shows plant sampling.

Figure (5): Plant sampling.

Samples were transferred directly after collection to the laboratories in sterile sealed bags and then analyzed for *salmonella spp*., TFCC and Intestinal Pathogenic Nematodes eggs IPN following the World Health Organization WHO Technical Report No.778, 1989 and Manual of Food Quality Control, 1992. On the other hand, samples for chemical analysis were collected in clean paper bags and transferred directly to the labs, where they were dried at (65^{o}C) to stop enzymatic reaction, then samples were grinded using laboratory mill with (0.5 mm) sieve size to obtain suitable and homogeneous samples for laboratory analysis. Samples were then kept in sealed jars and analyzed for total nitrogen, total phosphorus, potassium and trace metals. Plant chemical analyses were carried out following (Soil and Plant Analysis, Laboratory Manual, Second Edition, John Ryan and others, ICARDA, 2001). More details on the analysis methods of plant are shown in Annex (2).

Soil was sampled prior to bio-solids application to get baseline data, as mentioned earlier, and after harvesting. Samples were collected randomly from different plots at a depth (0 - 15cm). Augers and shovels were used to collect samples from the field. Samples were kept in clean sterile labeled plastic bags, then transferred directly to the laboratories for analysis. Samples were analyzed for microbiological testing: *salmonella spp*., TFCC and Intestinal Pathogenic Nematodes eggs IPN (using Method of Soil Analysis,1994) without any pretreatment. For chemical analysis, samples were air-dried then sieved at (2 mm) sieve size. The following soil chemical tests were preformed: soil pH, Electrical Conductivity (EC), organic matter (OM), total nitrogen (T-N), nitrate (NO_3), total phosphorus, pottasium, borone and heavy metals.

Soil chemical analyses were carried out following "Methods of Soil Analysis, Part 3, Chemical Methods, D. L. Sparks and others, Published by Soil Science Society of America, Inc. and American Society of Agronomy, Inc. 1996". More details on the analysis methods of soil are shown in Annex (2).

Results of agronomic, chemical and microbiological analysis of plant and soil were subjected to analysis of variance (ANOVA) using MSTATC PROGRAM (Michigan State University). To determine the main effect of each factor, the LSD. 05 (Least Significant Difference at propability 0.05) was used to separate treatments mean.

Bio-solids composite samples were collected from drying beds at Madaba WWTP before application. Samples were analyzed for solids contents, macro and micro-nutrients, in addition to microbial aspects. Analyses were carried out following "Standard Methods for the Examination of Water & Wastewater,Online 2004". Other analytical methods were also applied, especially those of the US EPA.

5.2 Plant Analysis at Harvesting Stage

The following is a description of the results of crop measurements in addition to plant analysis carried out at harvesting stage.

5.2.1 Crop Measurements

The biological yield, which can be defined as the total above ground biomass produced by a plant, was measured for maize at harvesting. Table (5) and Figure (6) show the biological yield of maize. The minimum biological yield was obtained at 4 tons/ha bio-solids treatment (16.25 kg/m^2), while the maximum was obtained at 8 tons/ha bio-solids treatment (19.50 kg/m^2). The biological yield for maize grown by farmers next to the experimental site was within the range (10-12 kg/m^2) which is much less than that grown within the experimental site. Statistical analysis of the biological yield values showed no significant differences between various bio-solids treatment.

Table (5): Maize biological yield.

Bio-solids Treatment	**Biological Yield (kg/m^2)**
T0	16.75 A
T1	18.13 A
T2	16.25 A
T3	17.50 A
T4	19.50 A
LSD	4.58

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.

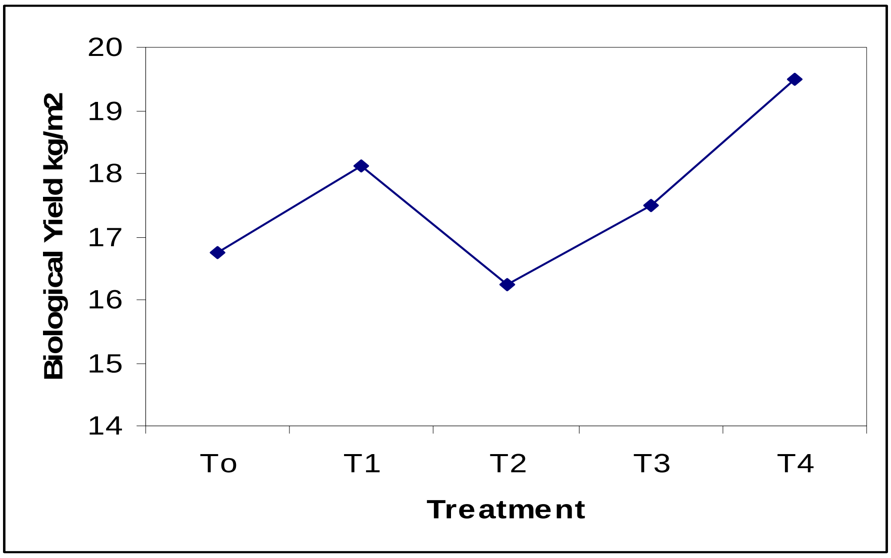


Figure (6): Biological yield of maize at different bio-solids treatment.

5.2.2 Plant Chemical Analysis

- **Effect of different treatments on macro-nutrients concentrations**

Table (6) shows macronutrients concentrations in maize at harvesting. Total nitrogen values show no significant differences between various bio-solids treatments. The maximum T-N concentration was obtained at control treatment, while the minimum concentration was obtained at 2ton/ha bio-solids treatment. Figure (7) shows T-N concentrations in plant.

Table (6): Macronutrients concentrations in plant at harvesting.

Treatment	T-N (mg/kg)	T-P (mg/kg)	K (mg/kg)
T0	19,740 A	2,499 AB	28,830 B
T1	17,610 A	2,303 B	29,850 AB
T2	19,610 A	2,181 B	33,100 AB
T3	18,830 A	1,994 B	42,180 A
T4	19,100 A	2,953 A	26,500 B
LSD	4,609	634	12,720

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.

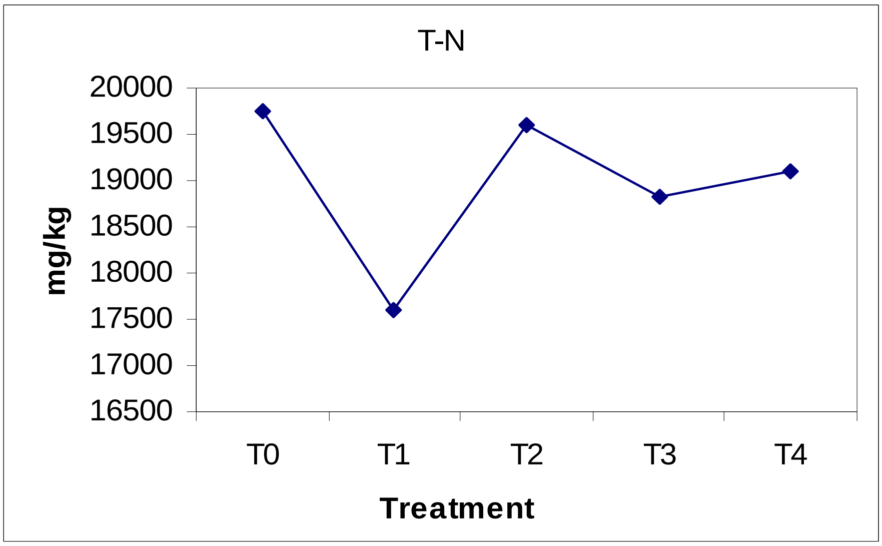


Figure (7): Total nitrogen concentration in plant.

The maximum phosphorus concentration was obtained at the maximum bio-solids treatment which was significantly different from other bio-solids treatments with the exception of the control treatment. There were no significant differences between the control treatment and other bio-solids treatments. The minimum T-P concentration was obtained at 6ton/ha treatment. Figure (8) shows phosphorus concentration in plant.

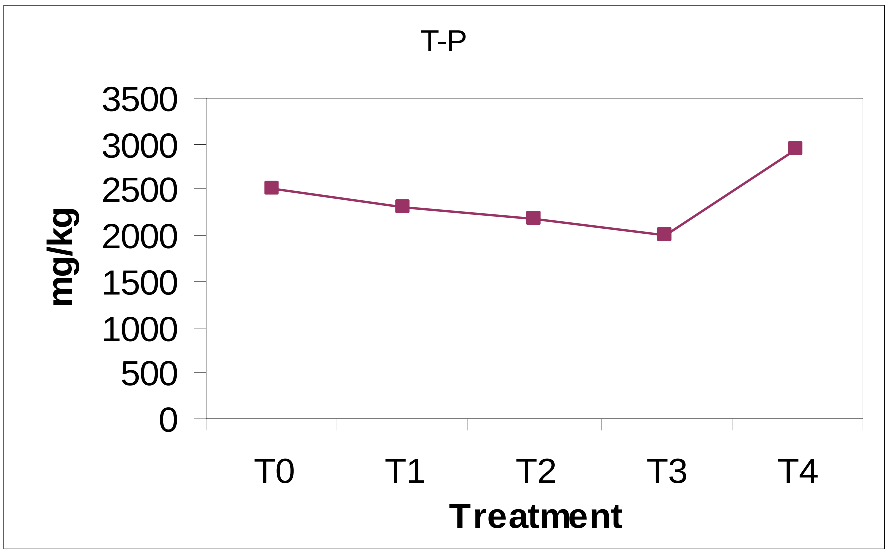


Figure (8): Phosphorus concentration in plant.

Figure (9) shows potassium concentration in plant. The maximum potassium concentration was obtained at 6ton/ha bio-solids treatment which is significantly higher than that for the 8ton/ha and the control treatments. There were no significant differences between the control treatment and the 2, 4 ,8 tons/ha treatments.

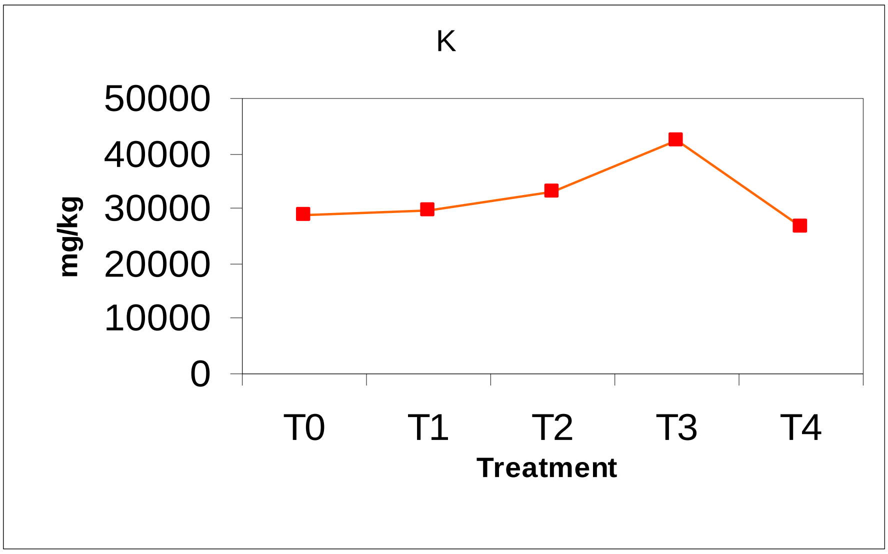


Figure (9): Potassium concentration in plant.

- **Effect of different treatments on micro-nutrients concentrations**

Table (7) shows micronutrients concentrations in plant at harvesting. The maximum cadmium concentration was obtained at 8ton/ha bio-solids treatments which is not significantly different from the control treatment. The minimum concentration was obtained at 4 tons/ha treatment. There are no significant differences between different treatments and the control. Figure (10) shows cadmium concentration in plant.

Table (7): Micronutrients concentrations in plant at harvesting.

Treatment	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mo (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
T0	0.34 AB	1.93 B	7.17 A	29.20 A	<1.0	<1.6	<0.5
T1	0.33 AB	3.00 A	7.47 A	26.33 A	<1.0	<1.6	<0.5
T2	0.30 B	2.30 AB	7.27 A	30.87 A	<1.0	<1.6	<0.5
T3	0.36 AB	2.17 B	6.57 A	27.17 A	<1.0	<1.6	<0.5
T4	0.39 A	3.00 A	7.96 A	37.70 A	<1.0	<1.6	<0.5
LSD	0.08	0.71	1.61	8.10	-	-	-

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.

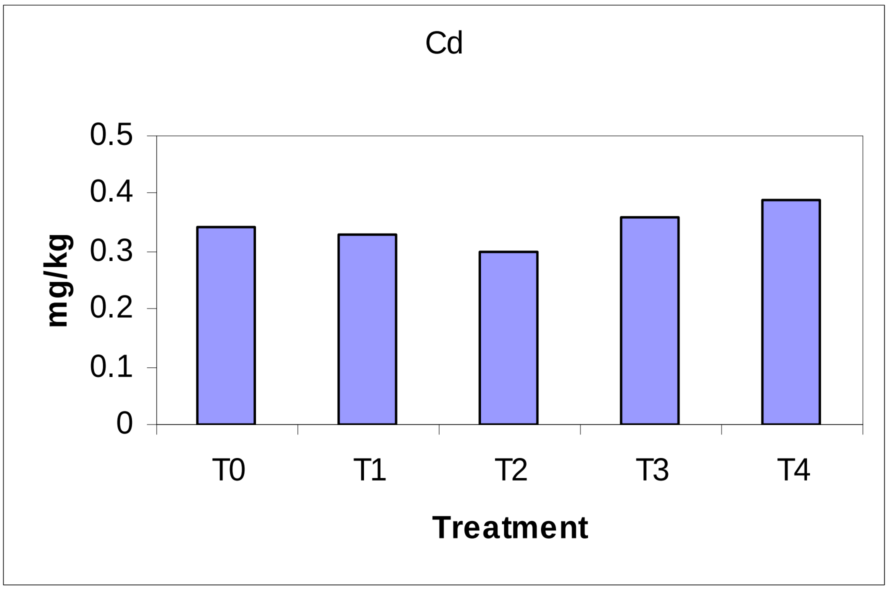


Figure (10): Cadmium concentration in plant

The maximum chromium concentrations were obtained at 2 tons/ha and 8 tons/ha bio-solids treatments, while the minimum concentration was obtained at the control treatment. There were no significant differences between 4 and 6 tons/ha treatments and the control treatment.

For copper and zinc concentrations in plants, there were no significant differences between various bio-solids treatments. Figure (11) shows zinc concentration in plant.

Molybdenum, nickel and lead concentrations were less than the detection limits.

In general, it could be concluded that plant characteristics were not significantly affected with bio-solids application, may be the reason is that the effect of irrigation with reclaimed wastewater was more predominant, especially that Madaba effluent has good nutrients concentrations and considerable amounts were used for irrigation processes.

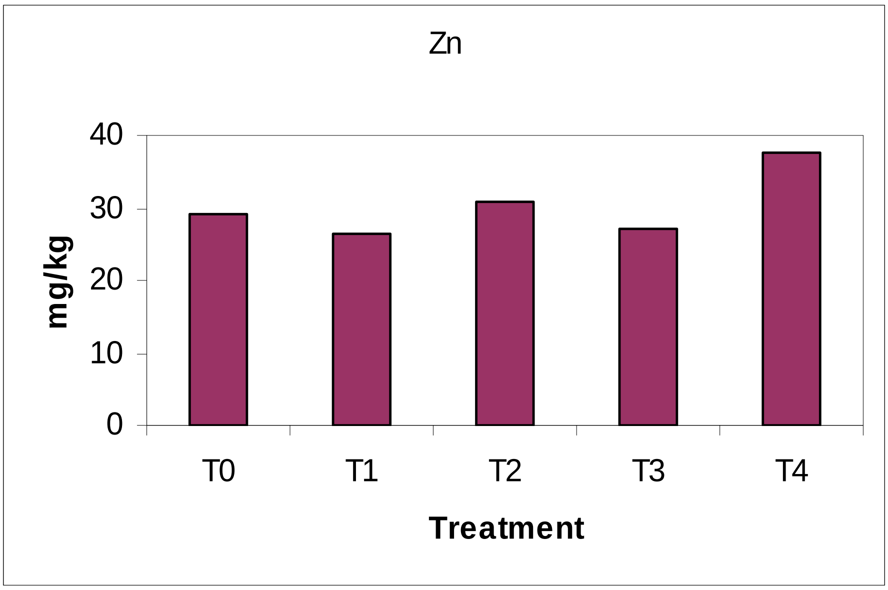


Figure (11): Zinc concentration in plant

5.2.3 Plant Microbiological Analysis

Plant microbiological analysis shown in Table (8) was carried out two weeks after the last irrigation. The results indicate that *Salmonella* and IPN were not detected. For TFCC, a maximum value of (3.67E+03 MPN/gm) was obtained at 6 tons/ha treatment and a minimum value of (2.33E+01 MPN/gm) was obtained a 2 tons/ha treatment. There are no significant differences in TFCC values between different bio-solids treatments.

Table (8): Microbiological analysis of Plant at harvesting stage.

Treatment	*Salmonella* /25gm	IPN eggs/50 gm	TFCC MPN/gm
T0	N.D	N.D	8.37E+02 A
T1	N.D	N.D	2.33E+01 A
T2	N.D	N.D	3.69E+01 A
T3	N.D	N.D	3.67E+03 A
T4	N.D	N.D	5.57E+02 A
LSD	-	-	5.25E+03

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.
N.D:Not Detected.

5.3 Soil Analysis at Harvesting Stage

5.3.1 Soil Chemical Analysis

Soil was sampled after harvesting and analyzed for pH, electrical conductivity EC, total nitrogen T-N, nitrate, ammonia, available phosphorus and organic matter. Soil properties at harvesting stage are presented in Table (9).

Table (9): The effect of different treatments on soil properties at harvesting stage.

Treatment	pH (SU)	EC (dS/m)	T-N (mg/kg)	NH_4 (mg/kg)	NO_3 (mg/kg)	Na (mg/kg)	P (mg/kg)	K (mg/kg)	O.M (%)	B (mg/kg)
T0	7.75 A	2.67 A	2064 AB	4.70 AB	244.0 A	387 A	562.2 AB	43.0 A	2.45 AB	<0.1
T1	7.86 A	2.35 A	1498 B	1.46 B	116.7 A	418 A	432.3 AB	50.0 A	1.89 B	<0.1
T2	7.69 A	3.06 A	2244 AB	7.60 AB	87.7 A	452 A	680.4 AB	37.7 A	2.85 AB	<0.1
T3	7.81 A	2.22 A	1635 B	8.23 AB	169.0 A	347 A	407.5 B	46.0 A	1.72 B	<0.1
T4	7.63 A	3.30 A	2668 A	10.09 A	180.7 A	429 A	795.5 A	37.7 A	3.40 A	<0.1
LSD	0.24	1.43	1033	8.24	183.5	110	373.1	20.0	1.36	-

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.

There were no significant differences in soil pH values between different bio-solids treatments, a maximum pH value of (7.86) was obtained at 2ton/ha treatment while the a minimum value of (7.63) was obtained at 8ton/ha treatment. Soil pH variation is shown in Figure (12).

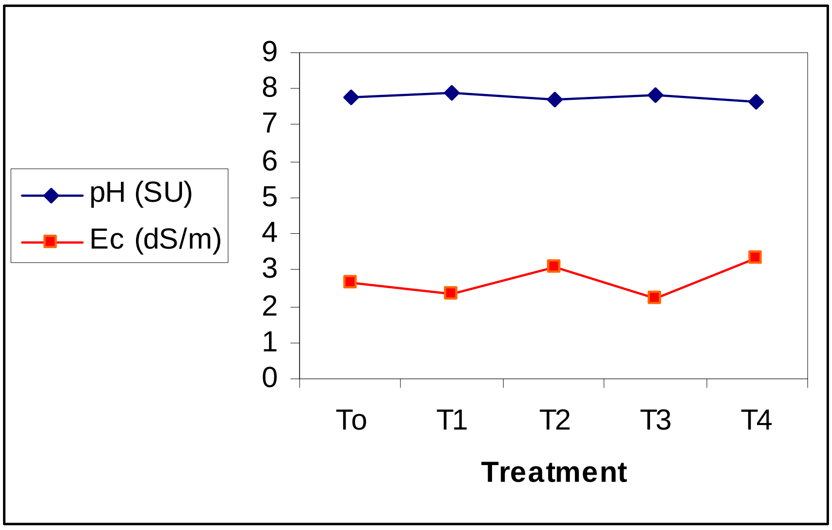


Figure (12): Soil pH and EC at harvesting stage.

Electrical conductivity EC values show no significant difference between various bio-solids treatments. The maximum EC value was obtained at 8ton/ha bio-solids treatment while the minimum value was obtained at 6ton/ha treatment. In addition, it's noted that EC values had increased for the different treatments when compared to soil baseline data shown in table (4). Figure (12) shows EC variation.

- **Effect of different treatments on macro-nutrients concentration in soil**

Figure (13) shows soil total nitrogen, nitrate and ammonia. Total nitrogen values in soil show a significant difference between 8ton/ha and 2&6 ton/ha bio-solids treatments. However, there's no significant difference between all treatments and the control treatment. A maximum nitrogen value of (2668 mg/kg) was obtained at 8 tons/ha bio-solids treatment while a minimum value of (1498 mg/kg) was obtained at 2 tons/ha treatment.

Nitrate values show no significant differences between different bio-solids treatments, a maximum nitrate value of (244 mg/kg) was obtained at control treatment while a minimum value of (87.7 mg/kg) was obtained at 4 tons/ha bio-solids treatment.

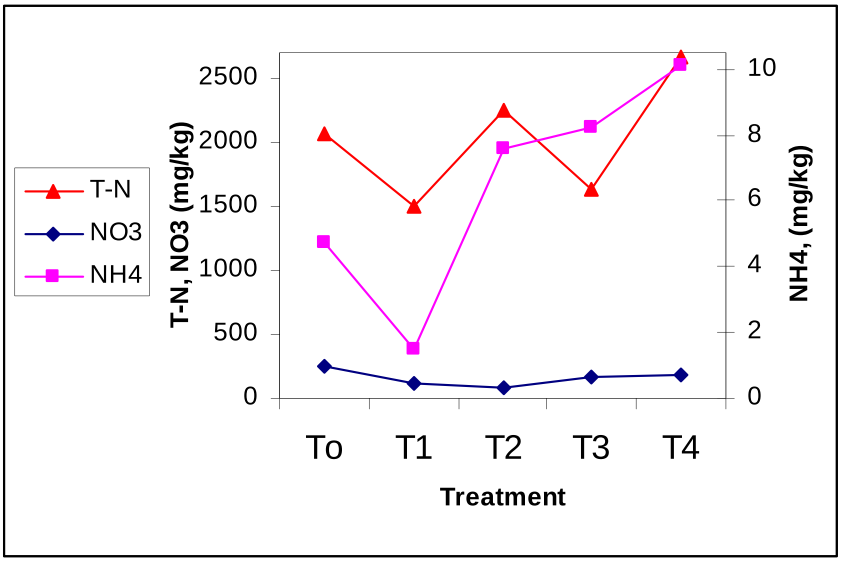


Figure (13): Soil T-N, NO_3, NH_4 at harvesting stage.

Ammonia concentrations in soil show significant difference between 8ton/ha and 2 tons/ha bio-solids treatments. However, there's no significant difference between these treatments and the other treatments or between various treatments and the control treatment.

Figure (14) shows soil phosphorus and organic matter. Phosphorus concentrations in soil show a significant difference between 6 tons/ha and 8 tons/ha treatments but there's no difference between these treatment and the other treatments or between various treatments and the control treatment.

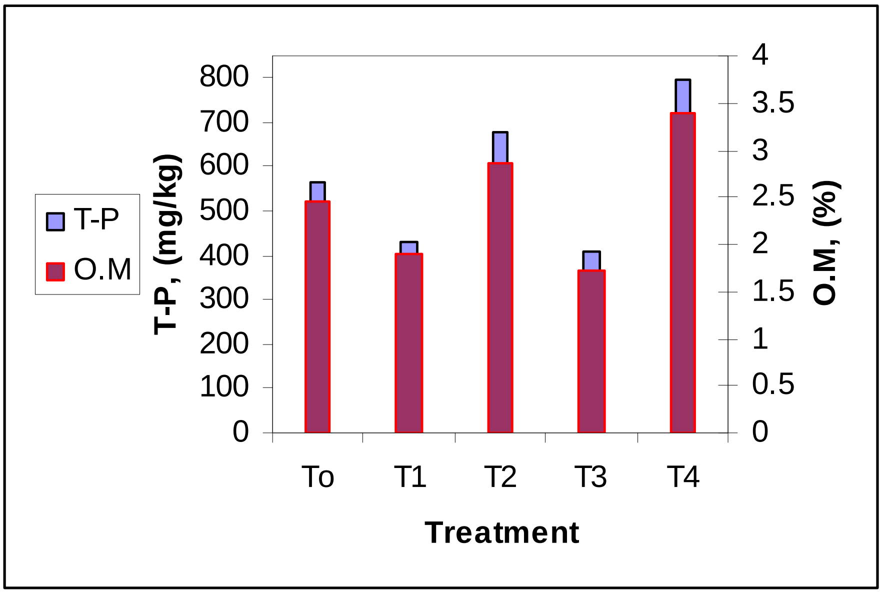


Figure (14): Soil T-P and O.M at harvesting stage.

Organic matter percentages show a significant difference between 8ton/ha treatment and the 2 and 6 tons/ha bio-solids treatments. The maximum organic matter percentage was obtained at 8 tons/ha treatment, while the minimum was obtained at 6 tons/ha treatment. There's no significant difference between different bio-solids treatment and the control treatment.

Boron concentration in soil was less than the detection limit for all treatments.

- **Effect of different treatments on micro-nutrients concentration in soil**

Table (10) shows micronutrients concentrations in soil at harvesting. The maximum cadmium concentration was obtained at 8ton/ha bio-solids treatment which is significantly different from the 2 and 6 tons/ha treatments. The cadmium concentration at control treatment was not significantly different from other bio-solids treatment. Figure (15) shows cadmium concentration in soil.

Table (10): Micronutrients concentrations in soil at harvesting.

Treatment	Cd (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Mo (mg/kg)
T0	0.147 AB	1.77 A	2.57 AB	1.00 A	11.57 AB	<0.05	<0.05
T1	0.087 B	1.53 A	2.27 AB	0.70 A	7.90 B	<0.05	<0.05
T2	0.153 AB	0.83 A	2.83 A	0.80 A	12.70 AB	<0.05	<0.05
T3	0.100 B	1.00 A	2.17 B	0.63 A	7.27 B	<0.05	<0.05
T4	0.180 A	1.13 A	2.60 AB	1.00 A	15.37 A	<0.05	<0.05
LSD	0.078	1.57	0.59	0.59	6.06	-	-

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.

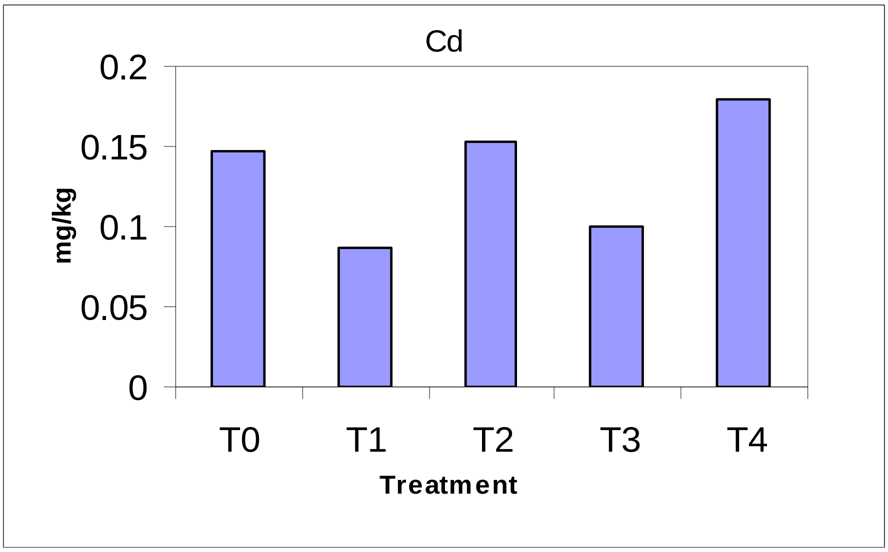
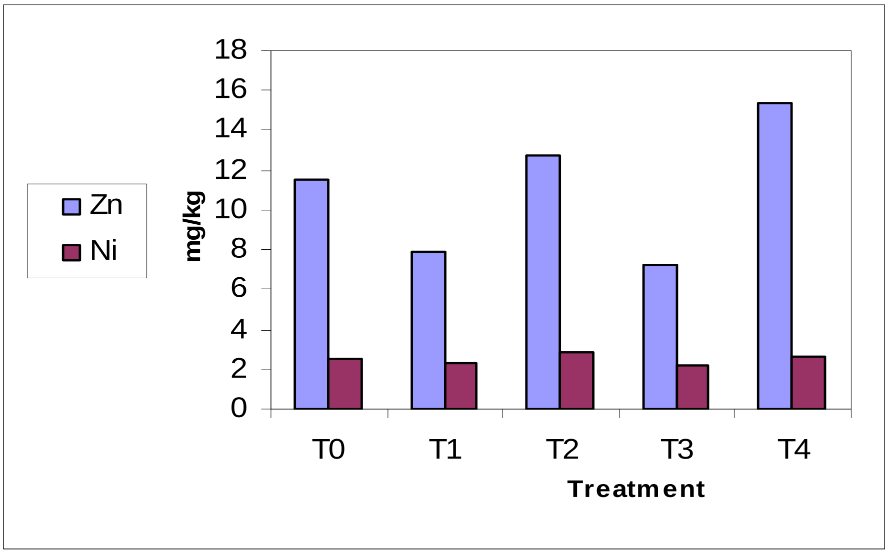


Figure (15): Cadmium concentration in soil.

There's no significant difference in nickel concentration between the control and other bio-solids treatments. The maximum concentration was obtained at 4 tons/ha, while the minimum at 6 tons/ha treatments. Nickel concentration in soil is shown in Figure (16).



T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.

Figure (16): Zinc and nickel concentrations in soil.

As shown in Figure (16), the maximum zinc concentration was obtained at 8ton/ha bio-solids treatment, which insignificantly differ form the control treatment. There are no significant differences between the control treatment and the different bio-solids treatments. For cupper and lead, there are no significant differences among different bio-solids treatments.

Chromium and molybdenum concentrations were less than the detection limits.
It could be concluded that soil characteristics were not significantly affected with bio-solids application for the same reason mentioned earlier in plant analysis.

5.3.2 Soil Microbiological Analysis

Table (11) show microbiological analysis of soil at harvesting (soil was sampled two weeks after the last irrigation process). The results indicate that *Salmonella* and IPN were not detected. For TFCC values, a maximum value of (1.00E+02 MPN/gm) was obtained at the maximum bio-solids application rate, while a significantly different minimum value of (1.350E+01 MPN/gm) was obtained at the control treatment.

There's no significant difference between the treatments 2, 4, and 6 tons/ha and the control or the 8 tons/ha bio-solids treatment.

Table (11): Microbiological analysis of soil at harvesting stage.

Treatment	*Salmonella* /20gm	IPN eggs/50 gm	TFCC MPN/gm
T0	N.D	N.D	1.350E+01 B
T1	N.D	N.D	3.32E+01 AB
T2	N.D	N.D	1.71E+01 AB
T3	N.D	N.D	2.43E+01 AB
T4	N.D	N.D	1.00E+02 A
LSD	-	-	7.86E+01

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha.
LSD: Least Significant Difference at 0.05 probability.
N.D:Not Detected.

6. CONCLUSIONS

- There were no significant differences in biological yield and plant total nitrogen content between different bio-solids treatments. In addition, phosphorus and potassium concentrations in plant were slightly affected with bio-solids addition and there were no significant differences between the maximum bio-solids application rate and the control treatment.
- Cadmium and chromium concentrations in plant were slightly affected with bio-solids application. For zinc and cupper concentrations, there were no significant differences between different treatments. Molybdenum, nickel and lead concentrations were less than the detection limits.
- Plant microbiological analysis showed that *Salmonella* and IPN were not detected, while there were no significant differences in TFCC values between different treatments.
- Soil pH, electrical conductivity EC, nitrate, sodium and potassium were not significantly affected by bio-solids application. Total nitrogen, ammonia, phosphorus and organic matter in soil were slightly affected with bio-solids

application. However, there were no significant differences between various treatments and the control treatment. Boron concentration in soil was less than the detection limit.

- Cadmium, nickel and zinc concentrations in soil were slightly affected with bio-solids application; there were no significant differences between various treatments and the control treatment. Cupper and lead were insignificantly affected with bio-solids application. Chromium and molybdenum concentrations were less than the detection limits.
- Soil microbiological analysis showed that *Salmonella* and IPN were not detected, TFCC values were slightly affected with bio-solids application, there were no significant difference between the control treatment and other treatments with the exception of the maximum bio-solids application rate.
- In general, it could be concluded that plant and soil characteristics were not significantly affected with bio-solids application, may be the reason is that the effect of irrigation with reclaimed wastewater was more predominant, especially that Madaba effluent has good nutrients concentrations and considerable amounts were used for irrigation processes.

7. LABORATORY TRAINING AND WORKSHOP

A two days workshop, during 30th July and 1st August 2007, and a capacity building program on Required Bio-solids Laboratory Training, during 1st - 6th August 2007, were carried out at the Royal Scientific Society RSS of Jordan. The main objective of the workshop was to review and update the analytical procedures in the field of bio-solids sampling and laboratories analyses. The workshop consisted of three main components: first, delivering lectures on bio-solids and relevant previous and current activities in this field; the second, dealing with RSS equipments and sampling issues; and the third, reviewing the operational procedures for bio-solids analysis in order of developing a manual for the analytical procedures of sludge and bio-solids.

The workshop and training were financially supported by the Sustainable Development of Dry Lands Project that is funded by the United States Agency for International Development (USAID) (Washington and Jordan / the office of

Water Resources and Environment -Jordan), under a cooperative agreement with the International Arid Lands Consortium (IALC) / University of Arizona.

8. INTERNATIONAL VISITS

Dr. Akrum Tamimi, the representative of IALC/University of Arizona and Bob Freitas from USAID visited the experimental sites at Madaba with RSS team in April 2007. Dr. Akrum, also, visited the sites two times in June and July in order to follow up the precedence in the experiments there. In addition, in August 2007, Dr. Chuck Gerba and Dr. Janick Artiola from University of Arizona visited the different bio-solids activities carried out by RSS at Madaba wastewater treatment plant; the land application experiment, the sludge treatment and bio-solids modeling experiments.

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ANNEX (1)

Photos Taken for the Site at Different Stages

P1: Land preparation

P2: The site after land preparation processes.

P3: Irrigation system installation

P4: Experimental plots and the irrigation system

P5: Irrigation processes

P6:The site after plantation.

P7: Maize harvesting

P8: General View of the experimental site

ANNEX (2)

Analytical Methods

Table (2.1): Methods of soil analysis.

Test	Preparation and Analysis Method	Reference
soil texture	Hydrometer	SSSA
soil pH	(1:1) soil to water mixture	SSSA
EC	(1:1) soil to water mixture	SSSA
Organic Matter	Walkley-Black Method	SSSA
Exchangeable and Soluble Na and K	extraction with ammonium acetate, flame photometer	SSSA
Nitrogen	TKN	SSSA
NH_4	extraction with 2M KCl , colorimetric	SSSA
NO_3	extraction with 0.01M KCl, Ion chromatography	
Available P	extraction with sodium bicarbonate solution, colorimetric	SSSA
As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, Se, Zn, Co	wet digestion, atomic absorption spectrometer	SSSA

SSSA : Methods of Soil Analysis, Part 3, Chemical Methods, D. L. Sparks and others, Published by Soil Science Society of America, Inc. and American Society of Agronomy, Inc. 1996.

ICARDA : Soil and Plant Analysis, Laboratory Manual, Second Edition, John Ryan and others, ICARDA, 2001.

Table (2.2): Methods of plant analysis.

Test	Method of analysis	Reference
Nitrogen	Total Kjeldahl Nitrogen	ICARDA
Total Phosphorus	Dry ashing, colorimetric	ICARDA
Potassium	Dry ashing, flame photometer	ICARDA
Nitrate	Extraction, colorimetric	ISO, 6635
As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, Se, Zn, and Co .	Wet digestion (HNO_3 & HCl), atomic absorption spectrometer	ICARDA

Table(2.3) :List of the names and models of analytical instruments used.

Parameter	Instrument used	Model
pH	pH/ Ion Meter	Metrohm 692
Electrical Conductivity	Conduct meter	Metrohm 712
Organic Matter	Titroprossor	Metrohm 682
Sodium and Potassium	Flame Photometer	Eppendorf Elex 6361
Nitrogen	Distillation Unit	Buchi B – 324
	Titration	Metrohm Titrino 719 S
Phosphorus	Spectrophotometer	Helios Gamma 9423 UVG 1702 E
	Photometer	Metrohm 662
Ammonia, and Nitrite	Photometer	Metrohm 662
Nitrate	Ion Chromatography	Dionex Ion Chromatography DX-300
As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, Se, Zn, and Co .	Atomic Absorption Spectrometer	Solar M6-Thermo Elemental