Bio-solids Application for Improving Soil Fertility and Crop Production in Jordan

Final Technical Report

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List of Abbreviations

APHA	:	American Public Health Association.
AWWA	:	American Water Works Association.
BRDP	:	Badia Research and Development Program.
CEC	:	Cation Exchange Capacity.
CFR	:	Code of Federal Regulations.
CFU	:	Colony Forming Unit.
CPLRs	:	Cumulative Pollutant Loading Rates.
CRMs	:	Certified Reference Materials.
DWTPs	:	Domestic Wastewater Treatment Plants.
EC	:	Electrical Conductivity.
ERC	:	Environmental Research Center.
IALC	:	International Arid Lands Consortium.
IPN	:	Intestinal Pathogenic Nematodes.
JUST	:	Jordan University of Science and Technology.
MPN	:	Most Probable Number.
MoU	:	Memorandum of Understanding.
MWI	:	Ministry of Water and Irrigation.
NCARTT	:	National Center for Agricultural Research and Technology Transfer.
OM	:	Organic Matter.
RBCs	:	Rotating Biological Contactors.
RSS	:	Royal Scientific Society.
RRC	:	Ramtha Regional Center.
SSSA	:	Soil Science Society of America.
TFCC	:	Total Fecal Coliform Count.
UKAS	:	United Kingdom Accreditation Services.
USAID	:	United States Agency for International Development.
US EPA	:	United States Environmental Protection Agency.
WAJ	:	Water Authority of Jordan.
WHO	:	World Health Organization.

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Special thanks are also expressed to the following: the National Center for Agricultural Research and Technology Transfer, members of the *ad hoc* committee for their valuable contribution, staff of the Water Authority of Jordan for their appreciated assistance, staff of the Environmental Research Center / Royal Scientific Society for their efforts in sampling and analysis of soil, plant, and bio-solids, and whoever contributed in the execution and completion of this project.

EXECUTIVE SUMMARY

This project has been implemented throughout two successive growing seasons (2004/2005 and 2005 / 2006), it aimed at investigating the feasibility of utilizing bio-solids for improving soil fertility and crop production in Jordan. Field experiments location was identified at National Center for Agricultural Research and Technology Transfer NCARTT / Ramtha Regional Center. Dewatered bio-solids was obtained from Wadi Hassan treatment plant. This report covers the second growing season (2005 / 2006).

During the second growing season, treatments were designed to study the accumulative and residual effects of different bio-solids application rates. As previously mentioned, in the first growing season, six different bio-solids treatments, (0, 2, 4, 6 and 8 T/ha) in addition to one chemical fertilizer treatment were applied manually to the sub-plots. For the second season, these treatments were applied to certain sub-plots, these are designated as accumulative sub-plots, while the other sub-plots were left without any bio-solids application, these are designated as residual sub-plots.

Crop (barley) measurements were conducted at tillering and harvesting stages. Accumulative bio-solids application during the second season insignificantly increased barley biological weight and straw yield and significantly decreased grain yield compared to residual treatments. Nitrogen and protein content in grain significantly increased with accumulative bio-solids application with a percentage of (11%) both, while, phosphorus content in straw significantly increased with a percentage of (29%). There was a significant increase in copper concentration in straw at different treatments, the reason for that is the high copper concentration in the applied bio-solids. In addition, chromium concentration in grain significantly increased at (4 ton/ha) rate. Similar results were obtained by referenced research and studies*. In addition, copper concentration in grain showed a significant increase with the accumulative application of chemical fertilizer, the reason for that may be the impurity of the chemical fertilizer. No other significant impacts on micro-nutrients levels in barley were observed.

The accumulative application of bio-solids significantly increased nitrogen and nitrate concentrations at (8 ton/ha) bio-solids application rate with a percentage of (42%) for the soil upper layer. Phosphorus concentration also increased significantly with a percentage of (49%) for the application rate (6 ton/ha). No significant differences in macro-nutrients concentrations were found for soil sub-layer at different treatments. Micro-nutrients concentration in soil at the upper-

* Johnson and Vancey (1998), Christie *et al* (2002) and Mullen *et al* (2005).

layer and sub-layer were insignificantly affected with accumulative bio-solids and chemical fertilizer application.

In order to ensure laboratory data quality, a quality assurance program had been implemented and applied throughout the project execution period.

In parallel, other activities were undertaken in direct relation to the project. These were the modification of the Jordanian standard No.(1145/1996) for bio-solids reuse in agriculture (which will be published formally soon), and a training workshop and a seminar about risk assessment of bio-solids used for agricultural purposes were held at Royal Scientific Society (RSS) during the period Dec. 13-15, 2005. Professors from University of Arizona delivered the training.

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Bio-solids Application for Improving Soil Fertility and Crop Production in Jordan-Second Growing Season

1. INTRODUCTION

Wastewater management is a continuing problem in many countries in the world. The problem is relatively more acute in Jordan because of the need to conserve and reuse water resources. The recent changes in regulations concerning municipal wastewater management in the country had resulted in significant increase in reclaimed water as well as bio-solids quantities. Although reclaimed water reuse plans have been set since the eighties of the last century, still there are no definite policies and solid regulations for utilizing generated bio-solids for beneficial usages. As a result, accumulated quantities at treatment plants is being dealt with an uncontrolled manner that may cause negative impacts on public health and the environment.

The majority of municipal wastewater treatment plants MWTPs in Jordan are of secondary type, achieving nutrient and pathogen reduction utilizing conventional and modified activated sludge processes that generate relatively huge amounts of bio-solids. Bio-solids generated at MWTPs are usually thickened, dewatered using drying beds, then disposed of at adjacent dumping sites and landfills, while anaerobic lagoons are occasionally de-sludged for operational purposes, and bio-solids are inadequately stored in nearby areas. In other words, none of bio-solids are currently being reused or recycled. Obviously, these current practices cannot be continued indefinitely. Adverse impacts include potential operational problems such as leachate management (especially in rainy seasons) and gas hazards. Key stakeholders in Jordan are seeking sustainable methods of treating and recycling bio-solids in ways that minimize potential risks to the public and environment. Guidelines for bio-solids land application, currently the most widely–employed reuse options in many countries, need to be developed under Jordanian arid and semi-arid conditions.

In June 2003 the United States Agency for International Development USAID (Washington and Jordan/ the office of Water Resources and Environment Office-Jordan) under a Cooperative Agreement with the International Arid Lands Consortium IALC / University of Arizona approved a request by the Royal Scientific Society RSS of Jordan through the Badia Research and Development Center BRDC / Jordan to contribute in financing a one-year research project (Phase I). Phase (I) aimed at assessing the quality of bio-solids generated at some DWTPs in Jordan (these are Wadi Hassan, Wadi Mousa and Jordan University for Science and Technology JUST treatment plants), as well as reviewing relevant local regulations and international guidelines.

The current project is considered as a continuing activity, or Phase (II) activity, and aims at investigating the feasibility of utilizing bio-solids for improving soil fertility and crop production in Jordan. The specific measurable objectives are:

1. To evaluate impacts of bio-solids application on soil properties and crop yield and quality based on field-pilot experiments.
2. To recommend appropriate bio-solids application procedures and rates that are suitable to local conditions.
3. To work through a collaborative model with the concerned governmental organizations and academic institutions.

In October 2004 the United States Agency for International Development USAID (Washington & Jordan / Water Resources & Environment Office - Jordan), and through a cooperative agreement with the International Arid Lands Consortium IALC / University of Arizona represented by the Badia Research & Development Center BRDC / Jordan, approved a request by the Royal Scientific Society RSS of Jordan to contribute in financing this project. A one-year contract was signed by BRDC and RSS in November 2004. Upon the end of the contract, both parties mutually agreed to extend the work for another year starting Nov. 2005, and another one-year contract was signed by both parties; the second year of the project aimed at assessing the accumulative and residual effects of bio-solids application on soil and plant.

This is the final technical report that summarizes different activities and tasks executed throughout the second growing season of Phase (II) of the project. Before going into details of the second growing season activities, it is worth to summarize the major findings of the first growing season of the project (details are shown in the final technical report that was issued by RSS and submitted to IALC / University of Arizona in Sep. 2005).

2. MAJOR ACTIVITIES EXECUTED DURING THE FIRST GROWING SEASON

2.1 Site Selection

Field experiments location was identified at NCARTT / Ramtha Regional Center RRC. Researchers from RSS and NCARTT met several times and visited the research station in Ramtha during Oct. 2004 to decide on the site where the field-pilot experiments were conducted. Several sites were investigated, one of these were selected for field experiments. This site was a fallow one that hadn't been exposed to previous experiments, accessible to staff and vehicles (for land preparation and plantation and for supplemental irrigation if needed).

2.2 Baseline Data of Soil and Bio-solids

Prior to applying bio-solids and seeding, composite soil samples were collected at three depths and analyzed chemically and microbiologically. Results showed that soil is alkaline, pH ranges between (8.38 - 8.52 SU) with relatively low organic matter content, while trace elements levels are within normal low range. In addition, several dewatered bio-solids samples were collected from Wadi Hassan treatment plant and analyzed just prior to application and field experiments. Bio-solids was classified as class B and pollutants levels in bio-solids were well below the ceiling concentrations specified in the US EPA 40 CFR Part 503.

2.3 Land Preparation and Plantation

Land was cultivated two times, one before plant (barley) seeding aimed at soil aerating and the another cultivation, which was after seeding and bio-solids application, aimed at preparing the seeds for planting in addition to mixing of applied bio-solids with soil. Six different treatments (0, 2, 4, 6, 8 ton/ha of bio-solids and a fertilizer treatment) were designed using Factorial Randomized Completely Block Design (FRCBD) with four replications, the total number of plots was (48) with a total of (8) plots for each treatment.

2.4 Monitoring and Field Measurements

In order to asses the impacts of bio-solids application on plant and soil properties, many field measurements, laboratory analysis and monitoring activities were carried out.

-Plant Analysis

Agronomic, chemical and microbiological analysis were carried out for barley at tillering and harvesting stages. A significant increase in biological yield, number of tillers and number of fertile tillers was observed for different bio-solids application rates over the control. Nitrogen and potassium contents increased in barley for different bio-solids treatments while no significant impacts on micronutrients levels were observed. Microbiological analysis showed that Intestinal Pathogenic Nematodes (IPN), TFCC and E. *coli* were not detected in most of the plant samples with the exception of low levels of TFCC and E. *coli* detected at harvesting stage .

-Soil Analysis

Chemical and microbiological analysis of soil were carried out after harvesting at depths (0-15 cm) and (15-30 cm). It was observed that bio-solids application significantly increased organic matter content of the upper layer as well as the sub-layer over the control. Nitrogen levels increased significantly over the control at the maximum bio-solids application rate for the two layers. Phosphorus and potassium concentrations increased only in the soil upper layer and for the highest application rate. Bio-solids application at all rates increased chromium, copper and zinc levels for both soil layers. Nickel concentrations increased significantly in the soil upper layer at the highest application rate, while lead levels significantly increased in the soil sub-layer at the highest application rate. Soil microbiological analysis showed that IPN, TFCC and *Salmonella* were not detected for both soil layers.

2.5 Pathogen Reduction Utilizing Solar Disinfection

A weather station was built near-by a drying bed within the premises of Wadi Hassan plant in August 2005 in order to investigate potentials of bio-solids pathogen reduction in correlation to environmental factors while being dewatered in the drying beds. Three cycles of experiments were carried out: summer, winter and spring. Based on bio-solids microbiological properties, class (A) bio-solids was obtained for the summer and spring cycles, while class (B) bio-solids was obtained for the winter cycle.

3. PROGRAMMED AND EXECUTED ACTIVITIES DURING THE SECOND GROWING SEASON

Table (1) shows the implementation schedule of the second growing season of Phase (II) of the project (Oct. 2005 – Oct. 2006). Following is a description of the major programmed activities carried out during the second growing season.

3.1 Mobilization

Based on the project's term of reference, RSS is conducting the project in close cooperation with the National Center for Agricultural Research and Technology Transfer NCARTT. NCARTT is a local research center involved in applied research activities in the agricultural field. Both parties signed a Memorandum of Understanding MoU upon which NCARTT is offering a piece of land as a research site within the premises of a research station in the northern part of Jordan (in Ramtha city).

The following staff are directly involved in different activities since the commencement of the second year of the project:

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 act as a contact person with IALC).
2. Eng. Wa’el Suleiman: M.Sc. in Water & Environmental Engineering. Researcher at ERC / RSS. (Role: supervise different activities, and participate in preparing progress and final technical reports).
3. Eng. Asma Alsheraideh: M.Sc. in Civil Engineering / Water Resources & Environment. Researcher at ERC / RSS. (Role: follow-up day-to-day work, and participate in preparing progress and final technical reports).
4. Naser Budier: B.Sc. in Agricultural Science / Soil, Water and Environment. (Role: follow-up field-pilot experiments with NCARTT

staff, perform physical and chemical analysis and participate in preparing progress and final technical reports).

In addition, Eng. Loai Al-Quraan and Eng. Said El-Zuriqi, researchers at NCARTT Ramtha station, follow-up field-pilot experiments with RSS staff.

The *ad hoc* committee that was formed during the first year of the project continue its meetings. It comprises representatives of different stakeholders including governmental and non-governmental organizations as well as academic institutions. The committee meet regularly to follow-up and discuss different aspects and updated results of various activities, and to firm-up 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. Sa'ad Al-Ayyash: Badia Research and Development Center BRDC.
3. Dr. Ziad Al-Ghazawi: Jordan University of Science and Technology.
4. Eng. Saleh Malkawi: Water Authority of Jordan WAJ / Ministry of Water and Irrigation MWI.
5. Eng. Khalil Jamjoum: National Center for Agricultural Research and Technology Transfer NCARTT / Ministry of Agriculture.
6. Eng. Husni Hamdan: Ministry of Environment.
7. Eng. Ahmad Ulimat: Directorate of Water Quality, WAJ / MWI.
8. Dr. Bassam Hayek: Director of ERC / RSS.
9. Eng. Wa’el Suleiman: ERC / RSS.
10. Eng. Asma Alsheraideh: ERC / RSS.

Table (1): Implementation schedule of the second growing season of Phase II of the project (Oct. 2005 – Oct. 2006).

Activity	Month												
	Oct. 05	Nov. 05	Dec. 05	Jan. 06	Feb. 06	Mar. 06	Apr. 06	May 06	Jun. 06	Jul. 06	Aug. 06	Sept. 06	Oct. 06
Mobilization and Literature Review													
Bio-solids Sampling & Analysis													
Land Preparation													
Plantation													
Soil Sampling & Analysis													
Plant Sampling & Analysis													
Evaluation and Reporting													

3.2 Literature Review

Many field studies were carried out to investigate the accumulative effects of bio-solids application on soil and plant.

Shober *et al* (2003) studied the effects of long-term commercial-scale application of bio-solids on soils and crop tissues sampled from (18) production farms throughout Pennsylvania. Biosolids application rates ranged from (5 to 159 Mg /ha). Soil cores and crop tissue samples from corn, soybean, alfalfa and sorghum were collected for three years from each farm. Samples were tested for nutrients, trace elements, and other variables. Bio-solids-treated fields had more post–growing season soil NO_3 and Ca and less soil K than control fields and there was some evidence that soil P concentrations were higher in treated fields. The soil concentrations of Cu, Cr, Hg, Mo, Mn, Pb, and Zn were higher in bio-solids-treated fields than in control fields. There were no differences in the concentrations of measured nutrients or trace elements in the crop tissues grown on treated or control fields at any time during the study.

Johnson and Vance (1998) evaluated the effect of five years of bio-solids (sewage sludge) applications at a semi-arid site in southwestern Wyoming by determining element (Cd, Cu, Ni, Pb, Se, and Zn) contents in soils and different grass species. Although general trend suggested land application of bio-solids increased trace element contents in the upper soil layers, only total Cu, Ni, and Pb were found to be significantly different between sites; Cd levels were below detection limit in all soils. Fifteen grass species, planted during the first year of biosolid application, were also analyzed for trace element contents. Significant differences were found with plant Cu, Se, and Zn concentrations; Cd levels were below the detection limit. Results of plant trace element concentrations indicated some of the wheatgrass species accumulated trace elements levels greater than the other grasses.

Christie *et al* (2002) applied dewatered bio-solids at four rates and inorganic P or K fertilizer to seven consecutive annual spring barley crops on two contrasting soils. One of the soils, a basaltic clay, was low in P and the other, a shale clay loam, was low in K. All bio-solids and fertilizer treatments gave higher yields than controls. Grain and straw Zn and Cu were higher in bio-solids treatments than fertilizer and control treatments, but the magnitude of differences were small.

Gaskin *et al* (2003) studied the long-term application of biosolids on soil and plant, (As, Cd, Cu, Pb, Hg, Mo, Ni, Se, and Zn) concentrations in soil and Bermuda grass forage from ten fields in Georgia were evaluated, the following categories of biosolids application were determined: six or more years (>6YR), less than six years (<6YR), and no applications. The study indicated that toxic levels of metals have not accumulated in the soils due to long-term biosolids application. Overall forage quality from the bio-solids-amended fields was similar to that of commercially fertilized fields.

The effect of long-term bio-solids application on tissue molybdenum and tissue copper of winter wheat forage was studied by Mullen *et al* (2005). Two nitrogen sources, anaerobically digested bio-solids and ammonium nitrate were applied annually from (1993–2001) to continuous winter wheat. Application of biosolids did not significantly alter soil pH, but increased soil Cu and soil Mo. Forage uptake of Mo and Cu showed temporal variation between years but increased with bio-solids application rate.

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 at the end of the study. 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 rate. The two-year results of this study demonstrated that sewage sludge applied to apple trees did not cause toxicity in the leaves.

3.3 Land Preparation and Plantation

As mentioned earlier, field experiments location was identified at Ramtha research station / NCARTT. The site is located 70 km to the north of Amman and 5 km away from Wadi Hassan treatment plant (where bio-solids were obtained), $32^{o}30$ north latitude and $35^{o}59$ east longitude with an altitude of 590 m above sea level (Figure(1)). The climate in the area is characterized by cold winter and hot summer with an average annual rainfall of (221 mm) for the period of (1998-2006), the average temperature ranges from (5^{o}C) in January to (35 ^{o}C) in August. Generally, rainfall starts in early November and ends in early April. Maximum rainfall occurs during January-February.

Two shallow cultivations (10-12 cm depth) perpendicular to each other were carried out using chisel plow (duck foot model). The experiments have been established during the first year of the project using Factorial Randomized Completely Block Design (FRCBD) method with four replications. Experiment plots of 4m X 6m were established. Field layout of the experiments is shown in Figure(2).

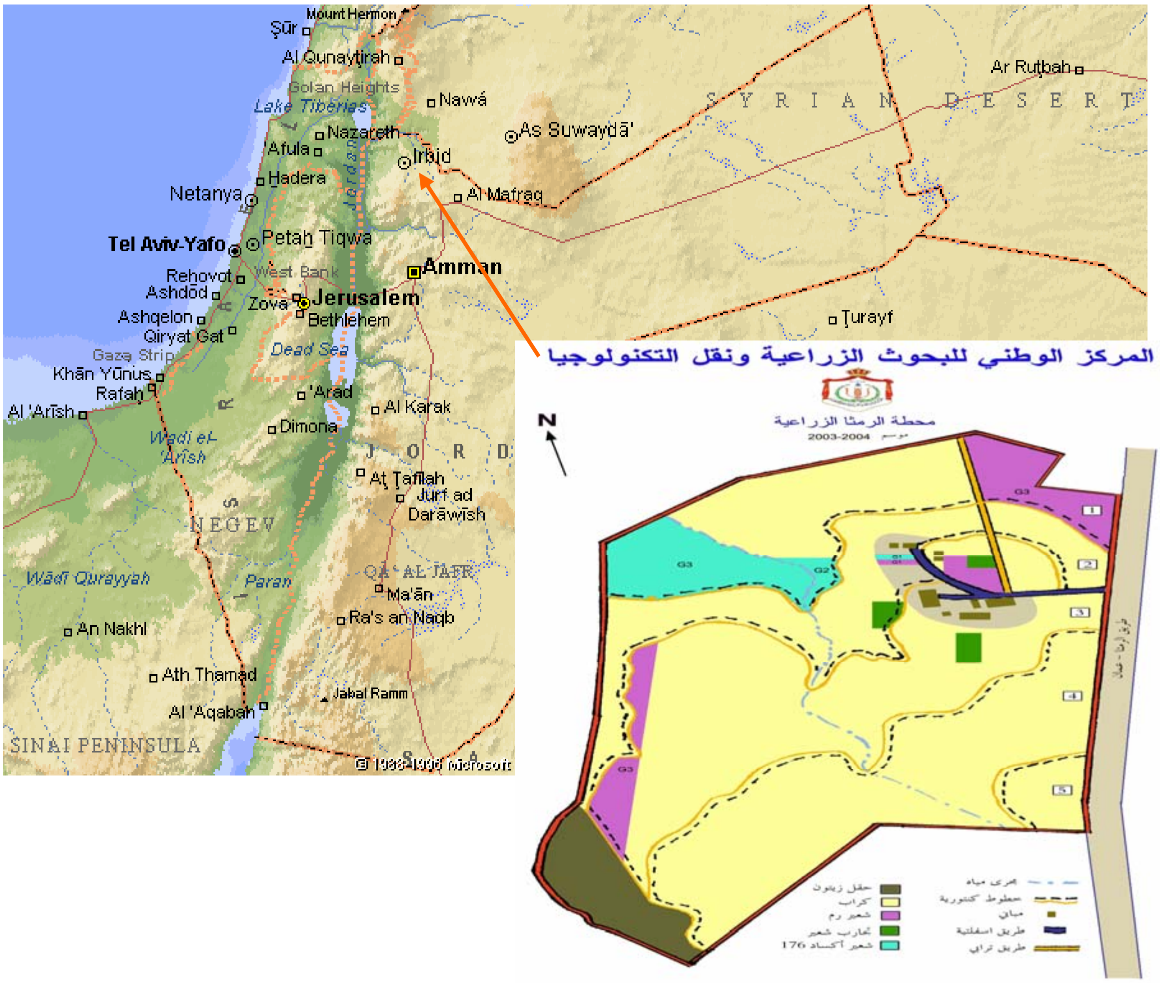
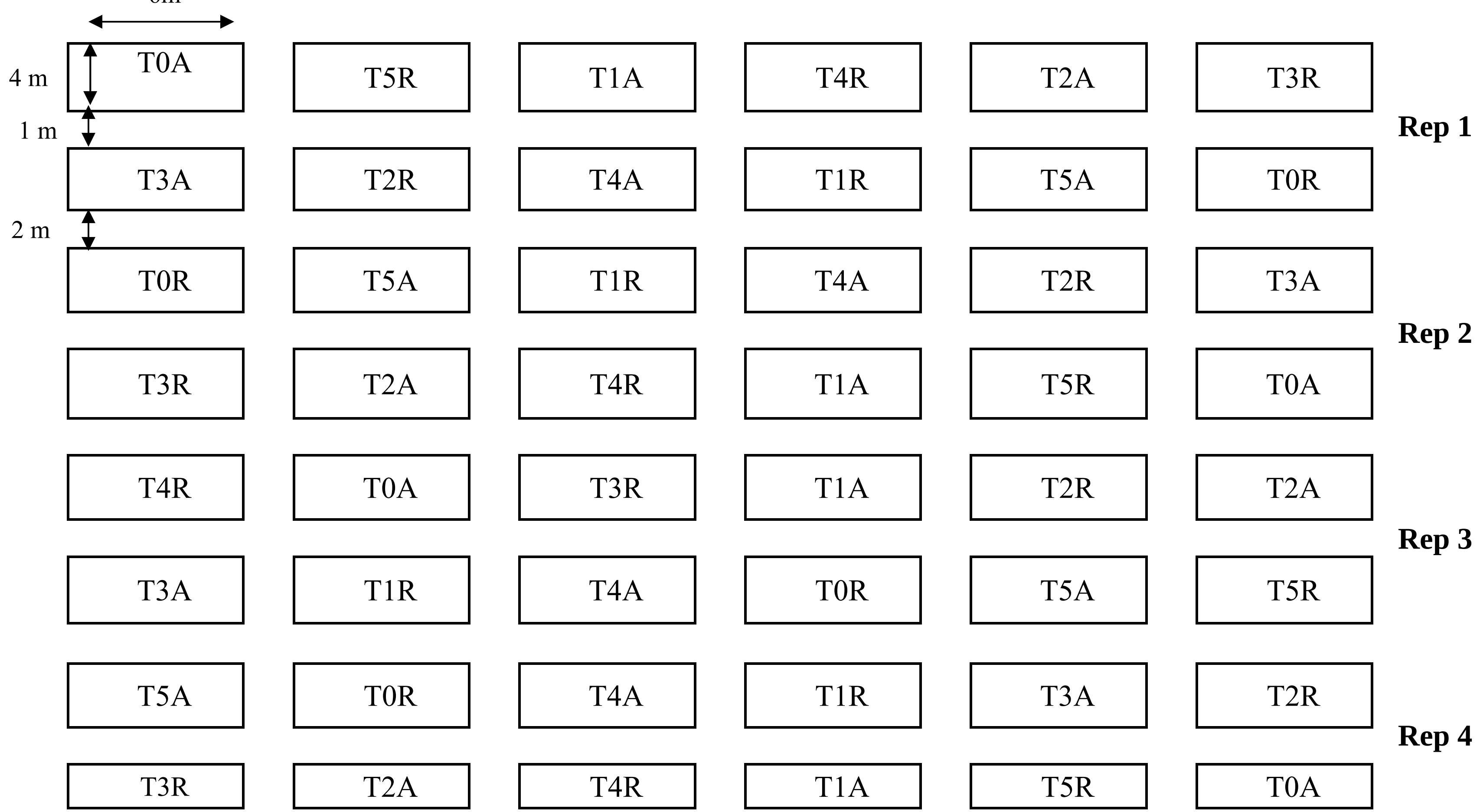


Figure (1): Map of Jordan showing the research site.

Dewatered bio-solids was obtained from Wadi Hassan treatment plant. As in the first season, six different treatments, zero sludge application as a control, 2, 4, 6 and 8 T/ha in addition to one chemical fertilizer treatment (di-ammonium phosphate DAP) that represents the recommended fertilizer rate, were applied manually but to certain sub-plots, these are designated as accumulative sub-plots and signed by (A) letter. The other sub-plots were left without any bio-solids application, these are designated as residual sub-plots and signed by (R) letter. Bio-solids were incorporated uniformly with soil to a depth of (8 cm). Barley was sowed using sowing machinery with a seeding rate of 100 kg/ha. Figure (3) shows the experimental site during land preparation process.



T = different treatments, A = accumulative, R = residual

Figure (2): Experimental layout.

Figure (3): The experimental site during land preparation process.

4. BASELINE DATA (SOIL & BIO-SOLIDS)

4.1 Soil Quality

In order to collect data about the soil quality at the experimental site prior to bio-solids application for the second year, twelve composite soil samples were collected at depths (0-15cm & 15-30cm). 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 (2).

The preliminary analyses showed that the soil is alkaline, pH ranges between 7.9 - 8.1, with relatively low organic matter content, while trace elements levels are within normal low range. Table (2) shows also slight changes in Organic Matter, Electrical Conductivity EC, Cation Exchange Capacity CEC, macro-nutrients and some micro-nutrients concentrations when compared to soil baseline data carried out at the beginning of the first growing season.

Table (2): Soil quality at the experimental site prior to application / second year

Parameter	Unit	T0		T1		T2		T3		T4		T5	
		0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
pH 1:1	SU	8.1	8.0	8.0	8.0	8.0	8.0	8.0	8.1	8.0	8.1	7.9	8.0
EC 1:1	dS/m	0.696	0.526	0.493	0.552	0.547	0.622	0.660	0.642	0.681	0.594	0.618	0.644
CEC	cmol/kg	38	39	43	37	36	37	37	37	38	37	38	33
Organic matter	%	1.95	1.86	2.01	2.02	2.19	2.09	2.03	2.02	2.18	2.53	2.39	2.11
T.kj.N	mg/kg D.W.	867	866	1013	969	1021	1017	1016	961	981	933	972	944
NH4-N	mg/kg D.W.	22.0	30.5	20.7	25.8	25.2	26.9	25.8	20.7	32.8	29.0	35.0	37.7
NO3-N	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
available-P	mg/kg D.W.	8.7	8.0	13.6	12.9	9.7	12.1	12.6	14.7	14.2	12.8	13.8	38.2
available-K	mg/kg D.W.	44	46	52	42	42	42	36	26	32	36	26	28
exchangeable-Na	cmol/kg D.W.	64.0	49.6	56.5	62.6	85.0	70.0	69.6	76.5	82.6	87.8	90.0	100.0
exchangeable -Mg	cmol/kg D.W.	8162	8236	8003	7880	8081	7721	7842	7687	7899	7859	7828	7553
exchangeable -Ca	cmol/kg D.W.	8477	8440	8547	8406	8498	8244	8171	8061	8115	8172	8164	8052
As	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cd	mg/kg D.W.	0.835	0.470	0.574	0.625	0.490	0.500	0.428	0.506	0.522	0.733	0.481	0.481
Cr	mg/kg D.W.	20.30	21.20	23.10	23.00	20.60	21.10	18.20	23.40	22.34	N.D.	7.73	7.30
Cu	mg/kg D.W.	8.35	2.56	2.30	2.20	1.93	3.28	1.90	4.28	2.24	23.62	8.46	39.70
Pb	mg/kg D.W.	6.00	6.30	6.63	7.14	5.68	6.30	5.01	6.58	6.21	14.65	9.51	8.71
Hg	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Mo	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ni	mg/kg D.W.	21.10	21.00	22.70	22.35	20.31	21.50	19.43	23.42	21.92	48.40	19.33	19.33
Se	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Zn	mg/kg D.W.	6.53	8.20	7.62	6.49	5.83	6.36	6.21	8.46	6.84	57.4	21.1	21.0
Co	mg/kg D.W.	35.0	33.42	29.80	30.50	26.60	30.50	25.50	34.46	27.14	31.30	28.73	30.50
Salmonella	cell/gm	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TFCC	MNP/gm	0.23	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Nem. Eggs	cell/gm	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

T0: control, T1: 2 ton/ha, T2: 4 ton/ha, T3: 6 ton/ha, T4: 8 ton/ha, T5: chemical fertilizer.
D.W: Dry Weight.
N.D: Not Detected.

4.2 Bio-solids Quality

Seven dewatered bio-solids composite samples were collected from different drying beds at Wadi Hassan treatment plant to be analyzed physically, chemically and microbiologically before application. Bio-solids samples were analyzed following the "Standard Methods for the Examination of Water & Wastewater", Online, 2004. Other analytical methods were also applied, especially those of the US EPA.

Table (3) below shows the analysis results, which indicate the following:

- Pollutants levels are well below the ceiling concentrations specified in the US EPA 40 CFR Part 503, and are also below the maximum allowable limits in the Jordanian Standard (JS: 1145/1996).
- Geometric means of TFCC as well as results of other pathogenic micro-organisms (*Salmonella spp.* and Intestinal Pathogenic Nematodes Eggs) show that bio-solids used for field-pilot experiments can be classified as class (B) bio-solids according to the US EPA rule, and as bio-solids treated to level (I) based on the Jordanian Standard (JS: 1145/1996).

5. MONITORING AND FIELD MEASUREMENTS

In order to assess impacts of bio-solids application 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 were collected from plots at different growth stages (tillering and harvesting stages) as shown in Figure (4).

Table (3): Characteristics of bio-solids generated at Wadi Hassan treatment plant

Parameter	Unit	S1	S2	S3	S4	S5	S6	S7	Average	JS: 1145/1996	US EPA	
											Ceiling Conc.*	Poll. Conc.**
TS	%	90	91	90	91	91	92	91	91	-	-	-
TVS of TS	%	66	65	66	66	67	65	66	66	-	-	-
T.kj.N	%	5.8	5.7	5.8	5.6	5.7	5.7	5.6	5.7	-	-	-
NH4-N	%	0.040	0.042	0.045	0.043	0.056	0.064	0.054	0.049	-	-	-
T-P	%	0.34	0.39	0.34	0.48	0.46	0.35	0.39	0.38	-	-	-
K	mg/kg D.W.	3088	3485	4245	2111	3253	2439	1234	2836	-	-	-
As	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 75	< 75	< 41
Cd	mg/kg D.W.	2.00	2.04	1.81	2.13	1.86	1.73	1.30	1.83	< 85	< 85	< 39
Cr	mg/kg D.W.	21.3	27.4	18.1	16.3	20.6	21.9	12.4	19.7	< 3000	-	-
Cu	mg/kg D.W.	103.1	103.6	94.8	78.9	101.0	100.5	74.5	93.8	< 4300	< 4300	< 1500
Pb	mg/kg D.W.	41.2	41.8	37.2	41.8	38.9	38.4	28.1	38.2	< 840	< 840	< 300
Hg	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 57	< 57	< 17
Mo	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 75	< 75	-
Ni	mg/kg D.W.	28.4	31.6	27.3	34.2	28.3	29.1	21.3	28.6	< 420	< 420	< 420
Se	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 100	< 100	< 36
Zn	mg/kg D.W.	744	741	680	576	722	716	547	675	< 7500	< 7500	< 2800
Co	mg/kg D.W.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 150	-	-
Salmonella	MPN/4 gm	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 3/4 gm	< 3/4 gm	
TFCC	MPN/gm	>1.10E+03	>1.10E+03	>1.10E+03	>1.10E+03	>1.10E+03	>1.10E+03	>1.10E+03	>1.10E+03	$< 1 \times 10^3$	$< 1 \times 10^3$	
Nem. Eggs	Egg/gm	N.D.	N.D.	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.

Figure (4): Plant sampling.

The above-ground barley was manually collected from the sampling plots, and at harvesting stage grain and straw were separated. Samples were transferred directly after collection to the laboratories in sterile sealed bags and then analyzed for *salmonella spp*., TFCC, and E. *coli* 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^oC) 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, protein content, nitrate, total phosphorus, total potassium, total sodium, total magnesium, boron, and trace metals. Plant chemical analyses were carried out following (Soil and Plant Analysis, Laboratory Manual, Second Edition, John Ryan and others, ICARDA, 2001) and (International standard ISO 6635, Fruits, Vegetables and Derived Products –Determination of Nitrite and Nitrate Content Molecular Absorption Spectrometric Method, 1st edition, 1984). 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, at tillering stage and after harvesting. Samples were collected randomly from different plots at two depths (0 - 15cm and 15 - 30cm) with the exception of tillering stage was at one depth (0 - 15cm) by qualified staff from RSS and RRC. 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*. and TFCC (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), Cation Exchange Capacity (CEC), organic matter (OM), total kjeldahl nitrogen (TKN), ammonia (NH_4), nitrate (NO_3) some macro and micro-nutrients.

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" and "Soil and Plant Analysis, Laboratory Manual, Second Edition, John Ryan and others, ICARDA, 2001". 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 Wadi Hassan plant 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 Tillering Stage

Barley was analyzed at tillering stage for different chemical and microbiological properties. Following is a brief description of the analysis results.

5.2.1 Chemical Analysis

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

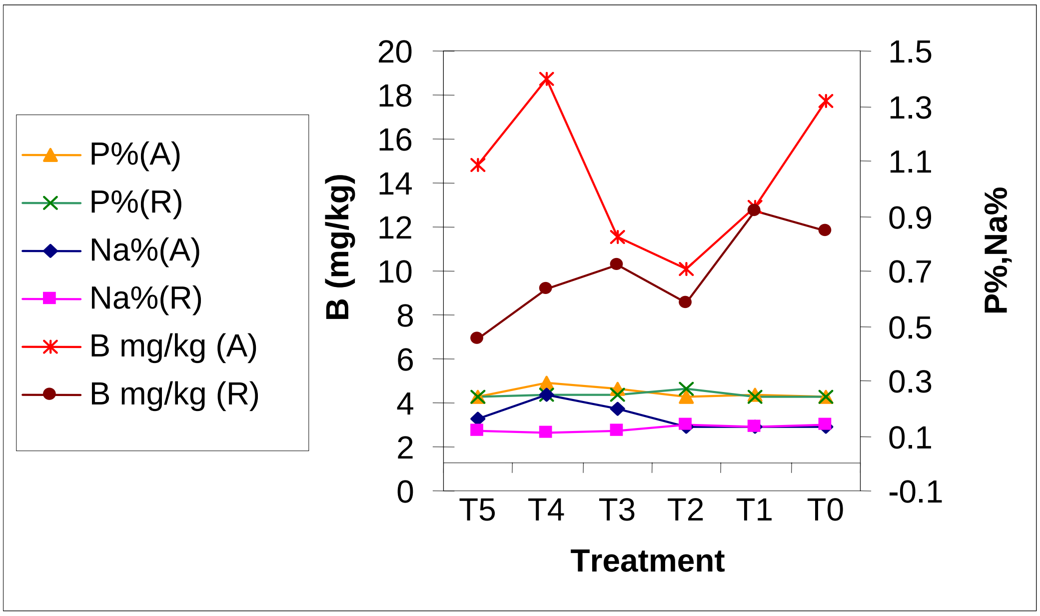
The effects of different treatments on macro-nutrients concentration in barley at tillering stage are shown in Table (4) and Figure (5).

Table (4): The effect of different treatments on macro-nutrients concentrations in barley at tillering stage.

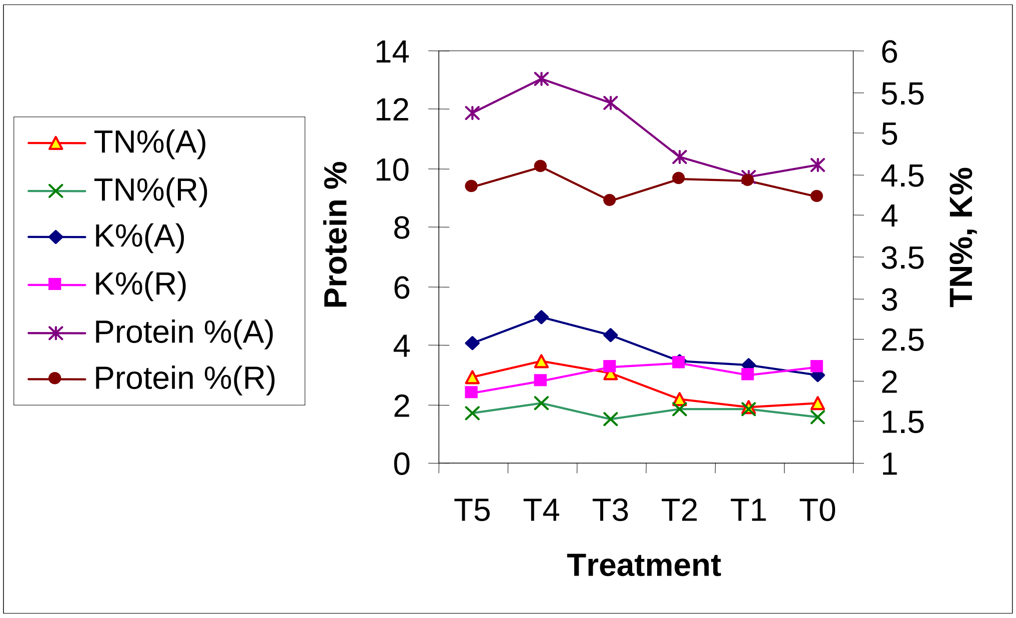
Treatment	TN (%)	Protien (%)	P (%)	Ca (%)	Na (%)	K (%)	Mg (%)	NO_3 (mg/kg DW)	B (mg/kg DW)
T0A	1.73 AB	10.10 AB	0.24 C	0.26 A	0.13 DE	2.08 CD	0.17 CD	66.33 AB	17.69 AB
T0R	1.55 B	9.04 B	0.24 C	0.25 A	0.14 D	2.16 BCD	0.17 BCD	74.67 A	11.80 ABC
T1A	1.67 AB	9.75 AB	0.25 BC	0.20 A	0.13 DE	2.19 BCD	0.17 BCD	67.67 AB	12.90 ABC
T1R	1.65 AB	9.61 AB	0.24 C	0.23 A	0.13 DE	2.08 CD	0.17 BCD	60.67 AB	12.71 ABC
T2A	1.78AB	10.39 AB	0.24 C	0.23 A	0.13 DE	2.24 BCD	0.18 BCD	61.00 AB	10.11 ABC
T2R	1.65 AB	9.63 AB	0.27 AB	0.28 A	0.14 D	2.22 BCD	0.17 CD	62.33 AB	8.54 BC
T3A	2.10AB	12.25 AB	0.27 A	0.26 A	0.20 B	2.55 AB	0.19 A	54.33 AB	11.55 ABC
T3R	1.53 B	8.92 B	0.25 BC	0.26 A	0.12 EF	2.17 BCD	0.18 ABC	48.33 AB	10.30 ABC
T4A	2.23 A	13.02 A	0.29 A	0.23 A	0.25 A	2.78 A	0.20 A	43.33 AB	18.70 A
T4R	1.73 AB	10.08 AB	0.25 C	0.23 A	0.11 F	2.00 D	0.18 ABC	52.67 AB	9.19 BC
T5A	2.04 AB	11.90 AB	0.24 C	0.23 A	0.16 C	2.46 ABC	0.19 AB	55.00 AB	14.85 ABC
T5R	1.61 AB	9.36 AB	0.24 C	0.22 A	0.12 DEF	1.86 D	0.16 D	42.00 B	6.87 C
LSD	0.55	3.184	0.017	0.076	0.017	0.386	0.017	27.79	9.28

T0 : control,T1 : 2ton/ha,T2 : 4ton/ha,T3 : 6ton/ha,T4 : 8ton/ha,T5 : chemical fertilizer, A : accumulative and R : residual.
LSD: Least Significant Difference at 0.05 probability.
D.W.: Dry Weight.

A: P, Na and B



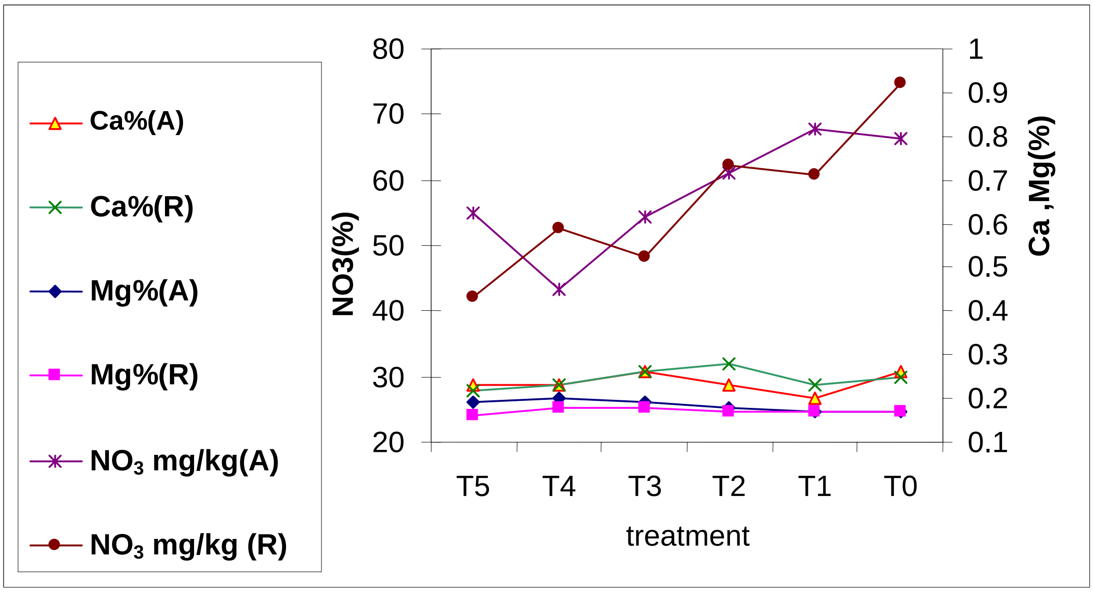
B: TN, K and Protein



* T0= control, T1=2 ton/ha, T2=4 ton/ha, T3=6 ton/ha, T4=8 ton/ha, T5= fertilizer, A=accumulative and R=residual.

Figure (5): The effect of different treatments on macro-nutrients concentrations in barley at tillering stage.

C: Ca, Mg and NO_3



* To= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= fertilizer, A= accumulative and R= residual.

Cont. Figure (5) :Effect of different treatments on macro-nutrients concentrations in barley at tillering stage.

The trend shown in Figure (5) indicates a general increase in macro-nutrients concentrations in barley at accumulative plots over residuals. The addition of bio-solids to accumulative plots during the second season resulted in an increase of plant nitrogen content, the maximum TN% was at the maximum bio-solids application rate, there were no significant differences between the different bio-solids treatments, the control, and the chemical fertilizer treatment. Figure (5) shows that TN% for accumulative plots were higher than that for residuals at all bio-solids treatments, the minimum nitrogen content was obtained for control plots, barley nitrogen content for accumulative plots at the maximum bio-solids application rate was significantly higher than that of the control plots.

The accumulative addition of bio-solids resulted in an increase of protein concentration in barley, it was increased from (10.10%) at control treatment to (13.02%) at the maximum application rate, as for nitrogen content there were no significant differences between the other bio-solids treatments, the control and the fertilizer treatment. Figure(5) shows an increase of protein concentration for accumulative plots over residuals for all treatments, barley protein content for accumulative plots at the maximum bio-solids application rate was significantly higher than that of the control plots.

Phosphorus concentration was significantly affected by accumulative bio-solids application during the second growing season. There were significant differences between different bio-solids treatments and the control. Phosphorus content increased from (0.24 %) in the control treatment to (0.29 %) in the accumulative (8 ton/ha) bio-solids treatment. Figure (5) shows that P content at the accumulative maximum bio-solids application rate and at 6 ton/ha rate was significantly higher than that for residual treatment, but there were no significant differences between the other residual and accumulative treatments.

Potassium levels in plant were also affected with bio-solids application during the second season. Figure (5) shows that plots with accumulative bio-solids application have higher K concentrations than residuals. There was a significant difference between the accumulative and residual treatments at the maximum bio-solids application rate and at 6 ton/ha rate, but no differences were found between the different accumulative and residual cases for the other treatments.

Magnesium concentrations were also affected by accumulative bio-solids application. It increased from (0.17 %) in the control case to (0.20 %) in the case of accumulative (8ton/ha) application rate which was also higher than that of residual (8ton/ha) case (0.18 %). There were no significant differences between the accumulative and residual bio-solids treatments and chemical fertilizer treatment.

Sodium concentration was significantly increased with accumulative bio-solids application at (6ton/ha) and (8ton/ha) over residual cases of the two treatments or that of the control case; Na concentration increased from (0.13 %) in the control case to (0.25 %) at accumulative (8ton/ha) treatment, there was a significant difference between this value and Na level at accumulative chemical fertilizer case (0.16 %).

Calcium and nitrate concentrations were slightly affected by bio-solid application, there were no significant differences between different treatments.

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

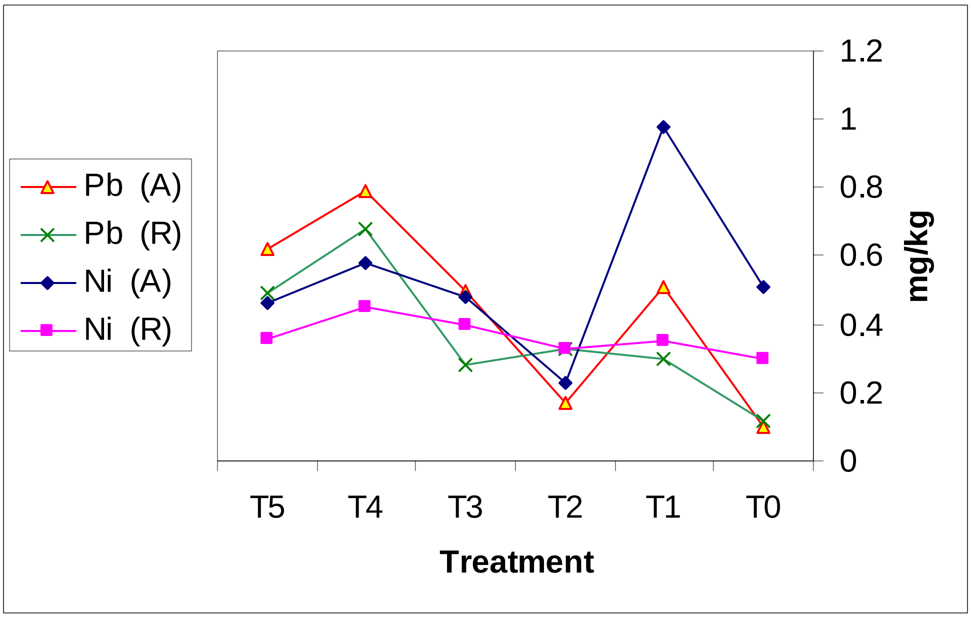
Table(5) and Figure (6) show some micro-nutrients concentrations and their trends in barley at tillering stage.

Table (5): The effect of different treatments on micro-nutrients concentrations in barley at tillering stage.

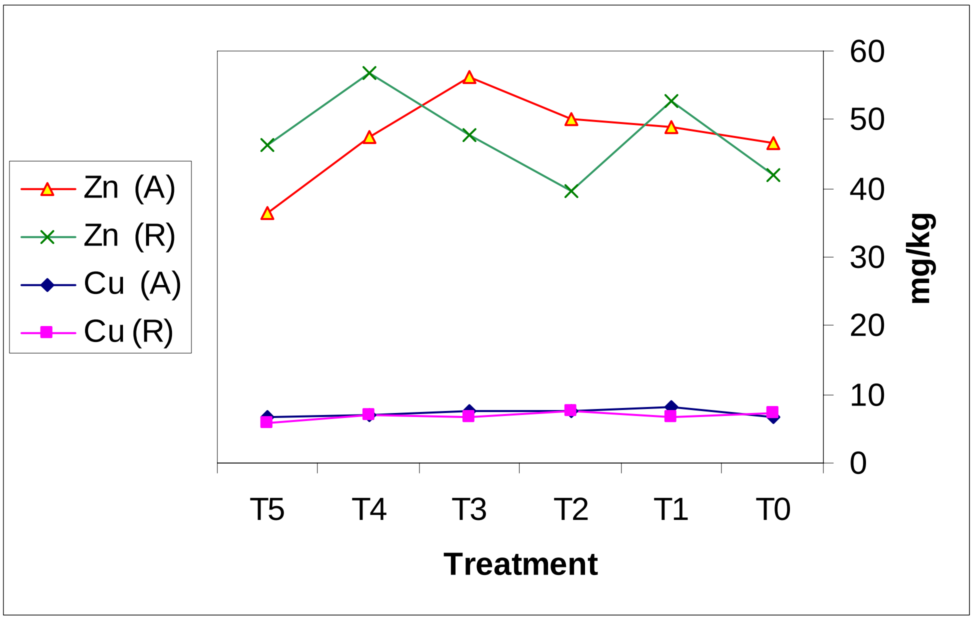
Treatment	Pb (mg/kg DW)	Zn (mg/kg DW)	Cu (mg/kg DW)	Ni (mg/kg DW)
T0A	0.10 C	46.72 A	6.64 A	0.51 AB
T0R	0.12 C	41.82 A	7.14 A	0.30 B
T1A	0.51 ABC	49.06 A	8.09 A	0.98 A
T1R	0.30 ABC	52.68 A	6.57 A	0.35 B
T2A	0.17 BC	50.08 A	7.71 A	0.23 B
T2R	0.33 ABC	39.53 A	7.44 A	0.33 B
T3A	0.50 ABC	56.31 A	7.50 A	0.48 AB
T3R	0.28 ABC	47.63 A	6.57 A	0.40 B
T4A	0.79 A	47.43 A	6.98 A	0.58 AB
T4R	0.68 AB	56.69 A	6.85 A	0.45 AB
T5A	0.62 ABC	36.44 A	6.69 A	0.46 AB
T5R	0.49 ABC	46.20 A	5.72 A	0.36 B
LSD	0.54	23.72	3.11	0.54

T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= fertilizer, A=accumulative and R=residual.
D.W: Dry Weight.
LSD: Least Significant Difference at 0.05 probability.

A: Pb and Ni



B: Zn and Cu



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= fertilizer, A= accumulative and R= residual.

Figure (6): Micro-nutrients concentration trends in barley at tillering stage.

Figure (6) and Table (5) show a general increase in lead concentration due to accumulative bio-solid application, it increased significantly from (0.10 mg/kg) for the control case to (0.79 mg/kg) at the accumulative (8 ton/ha) bio-solids application rate, there were no significant differences between accumulative and residual cases of the other treatments.

Zinc and copper levels were slightly affected with accumulative bio-solids application, there were no significant differences between different treatments. Nickel levels were also slightly affected with accumulative bio-solids application as shown in figure(6), table(5) shows that there are no significant differences between residual and accumulative cases except at (2 ton/ha) bio-solids application rate.

It should be noted that analysis of dewatered bio-solids utilized for the field experiments indicated that micro-nutrients content in bio-solids was relatively low, pollutants levels were well below the maximum allowable limits in the US EPA and Jordanian regulations. This could be the main reason for the slight impacts of bio-solids application on micro-nutrients levels in barley.

5.2.2 -Microbiological Analysis

Table (6) shows the microbiological analysis of barley at tillering stage. Results indicate that *Salmonella*, TFCC, and *Escherichia coli* (*E-coli*) were not detected in all plant samples.

Table (6): Microbiological analysis of barley at tillering stage.

Treatment	TFCC (MPN/g)	*E. coli* (MPN/g)	*Salmonella spp.* (Presence or absence /100g)
T0A	<0.03	<0.03	N.D.
T0R	<0.03	<0.03	N.D.
T1A	<0.03	<0.03	N.D.
T1R	<0.03	<0.03	N.D.
T2A	<0.03	<0.03	N.D.
T2R	<0.03	<0.03	N.D.
T3A	<0.03	<0.03	N.D.
T3R	<0.03	<0.03	N.D.
T4A	<0.03	<0.03	N.D.
T4R	<0.03	<0.03	N.D.
T5A	<0.03	<0.03	N.D.
T5R	<0.03	<0.03	N.D.

T0: control,T1: 2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
N.D.: Not Detected.

5.3 Plant Analysis at Harvesting Stage

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

5.3.1 Crop Measurements

One of the major activities carried out during this stage was crop measurements. The following parameters were determined: biological yield, grain yield, straw yield, kernel weight and plant height (see figure(7)). Table (7) and Figure (8) illustrates the effects of bio-solids application on these parameters.

Figure (7): Crop measurements at harvesting stage.

- **Biological yield**

Biological yield of cereal crops can be defined as the total above ground biomass (total dry matter) produced by a plant. It is the outcome of photosynthesis and respiration during the growing season (Shakhatreh,1998). Table (7) and Figure (8) show that biological yield was affected with accumulative bio-solids addition during the second season, minimum yield was obtained at control case while the maximum was obtained at accumulative maximum bio-solids application rate. Although Figure (8) shows that biological yields for accumulative treatments were higher than that of residuals, Table (7) illustrates that they are insignificantly different. In general, biological yields during the second season were lower than that of the first growing season as a result of low precipitation rate, consequently low plant growing rate.

Table (7): The effect of different treatments on barley growth parameters at harvesting stage

Treatment	Plant Height (cm)	Biological Yield (ton/ha)	Grain Yield (ton/ha)	Straw Yield (ton/ha)	Weight of 1000 Grain (gm)
T0A	22.5 DE	1.72 E	0.26 DE	1.46 E	33.25 A
T0R	20.0 E	1.91 DE	0.23 DE	1.67 DE	33.25 A
T1A	24.3 BCD	2.41 AB	0.16 E	2.25 A	32.50 AB
T1R	21.8 DE	2.30 ABC	0.29 BCD	2.00 ABCD	32.50 AB
T2A	25.5 ABCD	2.30 ABC	0.14 E	2.15 AB	33.25 A
T2R	23.3 DE	2.11 ABCD	0.28 CD	1.83 BCD	34.00 A
T3A	28.5 A	2.36 ABC	0.35 ABC	2.00 ABCD	32.00 AB
T3R	23.8 CDE	2.01 CDE	0.36 ABC	1.64 DE	32.75 A
T4A	28.0 AB	2.48 A	0.39 A	2.08 ABC	30.00 B
T4R	23.3 DE	2.22 ABCD	0.38 AB	1.84 BCD	31.75 AB
T5A	27.5 ABC	2.11 ABCD	0.34 ABC	1.76 CDE	32.50 AB
T5R	23.3 DE	2.04 BCDE	0.29 BCD	1.74 CDE	32.275 A
LSD	4.0	0.38	0.10	0.36	2.70

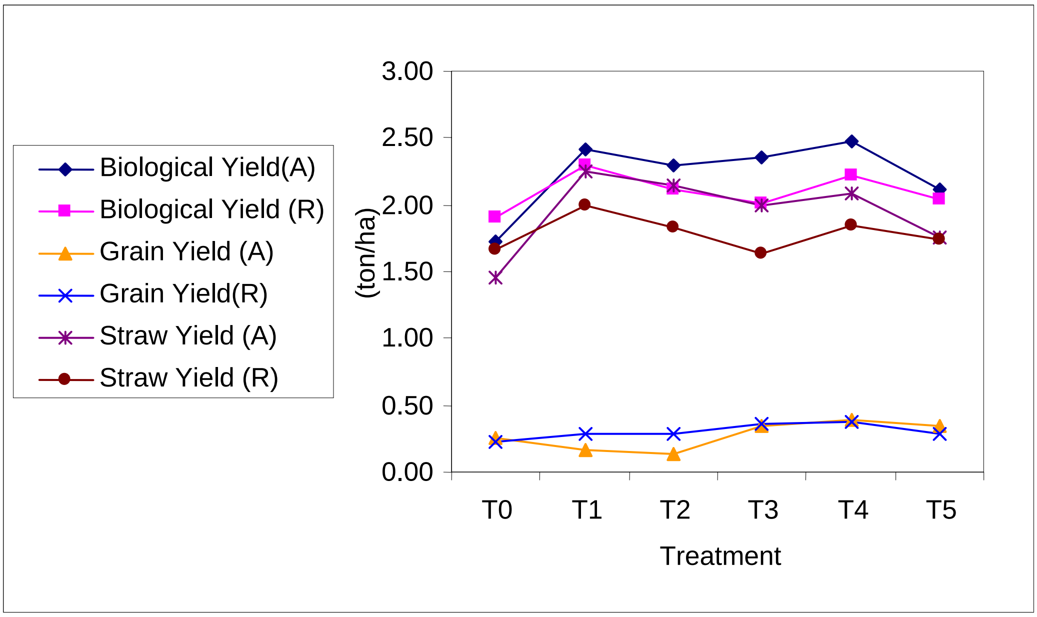
T0: control,T1: 2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.

- **Grain yield**

Grain yield significantly decreased at accumulative (2 & 4 ton/ha) bio-solids application rate compared to residual values and it was insignificantly different from that of control case. For (6&8ton/ha) bio-solids treatments and fertilizer case, accumulative treatments results were insignificantly different from residual treatments.

- **Straw yield**

The analysis of variance shows significant increase in straw yield at accumulative bio-solids treatments over control. However, they are insignificantly different from each other or from residual and chemical fertilizer treatments. Minimum straw yield was obtained at control case while the maximum was obtained at accumulative (2ton/ha) treatment.



* T0= control, T1=2 ton/ha, T2=4 ton/ha, T3=6 ton/ha, T4=8 ton/ha, T5= fertilizer, A=accumulative and R=residual.

Figure (8): Biological yield, grain yield and straw yield of barley at harvesting stage.

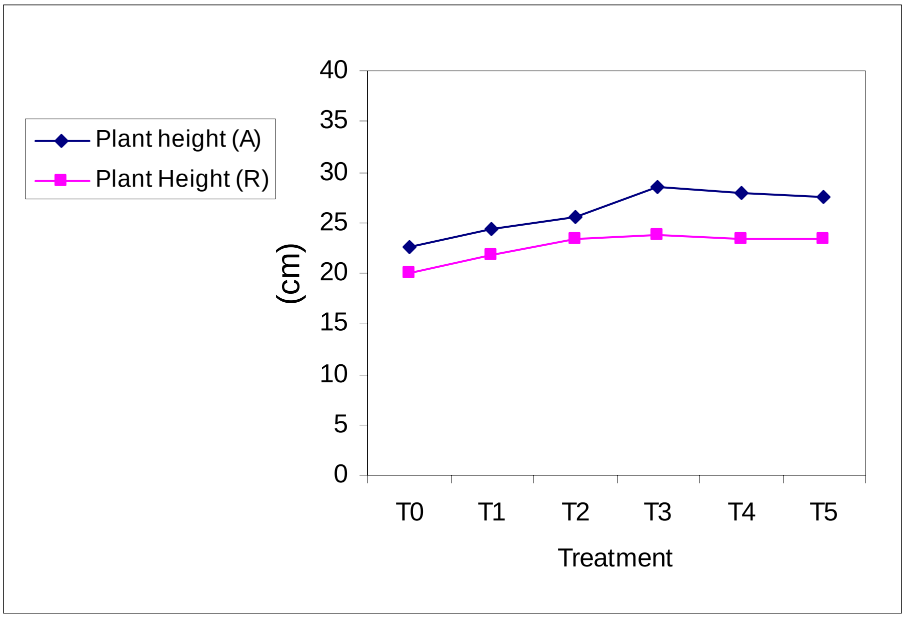
- **Thousand kernel weight**

Kernel weight was slightly affected with accumulative bio-solids application, the minimum value was obtained at accumulative (8 ton/ha) application rate, which was significantly different from control case, kernel weights for other treatments were insignificantly different from each other.

- **Plant height**

Plant height increased significantly with accumulative bio-solids application at (6 & 8ton/ha) rates and for fertilizer case over control or residual treatments. Minimum value was obtained at control case while the maximum was obtained at accumulative (6 ton/ha) bio-solids application rate. Plant height for accumulative (2 & 4ton/ha) application rates were higher but insignificantly different from values of residual treatments (Figure (9)).

Amin and Sherif (2001) obtained similar results, the two-weeks height of maize plant increased significantly with sludge application by a percentage of (18%) over the control and chemical fertilizer. There were no differences between the control and fertilizer treatments, while the five-weeks plant height was significantly indifferent between all treatments.



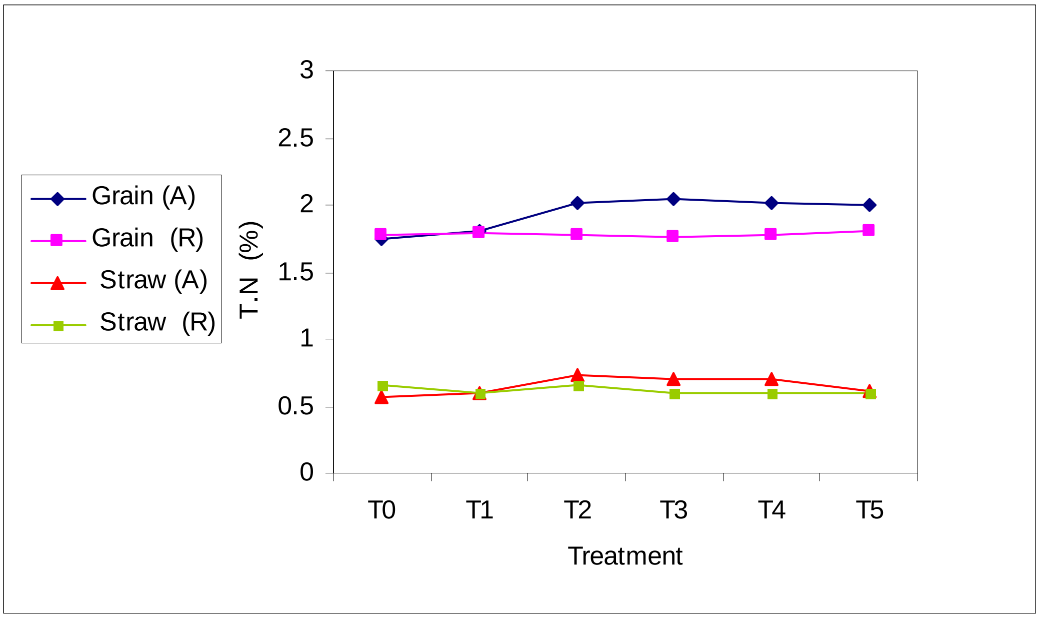
* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= fertilizer, A= accumulative and R= residual.

Figure (9): Plant height variation.

5.3.2 Plant Chemical Analysis

Effect of different treatments on macro-nutrients concentration in barley straw and grain.

Table (8) shows the effect of different treatments on macro-nutrients concentration in barley straw and grain. Accumulative application of bio-solids has resulted in an increase in total nitrogen concentration in grain, the minimum concentration was obtained at control case while the maximum was obtained at accumulative (6 ton/ha) application rate. Figure (10) shows total nitrogen concentration in grain and straw at harvesting. As shown in Figure (10) and in Table(8) that nitrogen concentration in grain was significantly higher for accumulative bio-solids and chemical fertilizer treatments than residual treatments; there were no significant differences between control case and residual treatments or between different accumulative treatments. Nitrogen concentration in straw was slightly affected with bio-solids application in the second season. As shown in Table(8) that there were no significant differences between different treatments.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (10):Nitrogen concentration in straw and grain at harvesting stage.

Accumulative bio-solids application during the second season had resulted in a significant increase in protein concentration in grain over residual treatments; minimum concentration was obtained for control case while the maximum was obtained at accumulative (6 ton/ha) bio-solids application rate. There were no significant differences between control and residual bio-solids and chemical fertilizer treatments or between different accumulative bio-solids and chemical fertilizer treatments. There were no significant differences between protein concentration in straw among different bio-solids, control or chemical fertilizer treatments.

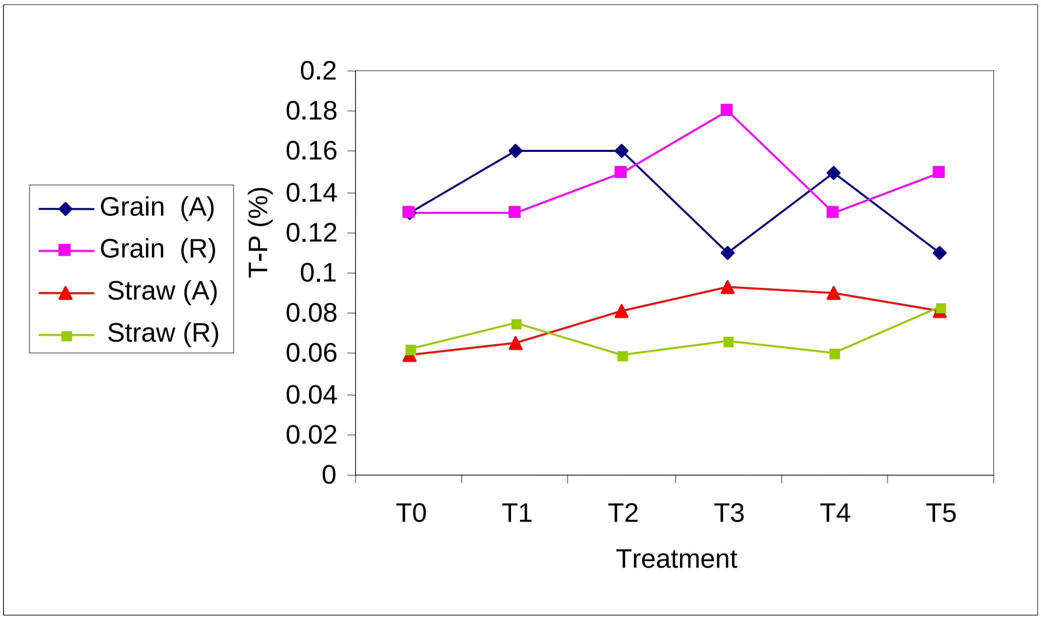
Nitrate concentration in grain was slightly affected with accumulative bio-solids application, minimum concentration was obtained for accumulative (2 & 4 ton/ha) bio-solids application rate while the maximum value, which was significantly different, was obtained for accumulative fertilizer treatment, there were no significant differences between different bio-solids treatments (either accumulative or residual) and the control case.

Phosphorus concentration in grain was slightly affected with bio-solids application during the second season; the minimum concentration was obtained at accumulative (6 ton/ha) bio-solids application rate and at accumulative chemical fertilizer treatment while the maximum was obtained at residual (6 ton/ha) rate. There were no significant differences between different bio-solids treatments (either accumulative or residual) and the control or between chemical fertilizer treatment and control. Figure (11) shows phosphorus concentration in straw and grain.

Table (8) : The effect of different treatments on macro-nutrients concentrations in barley straw and grain at harvesting stage.

Treatment	TN (%)		Protein (%)		T-P (%)		Ca (%)		Mg (%)		Na (%)		K (%)		NO_3 (mg/kg)		B (mg/kg)	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T0A	1.75 B	0.57 A	10.22 B	3.34 A	0.13 ABC	0.059 E	0.064 A	0.409 A	0.145 AB	0.213 C	0.029 AB	0.109 A	0.582 A	0.880 B	28.0 AB	-	4.55 A	3.12 BC
T0R	1.78 B	0.66 A	10.39 B	3.90 A	0.13 ABC	0.062 DE	0.095 A	0.326 AB	0.169 AB	0.266 ABC	0.024 B	0.110 A	0.544 A	0.952 AB	26.3 AB	-	4.72 A	3.49 ABC
T1A	1.81 B	0.60A	10.55 B	3.49 A	0.16 AB	0.065 D	0.159 A	0.385 AB	0.187 A	0.242 ABC	0.025 B	0.096 A	0.593 A	0.961 AB	18.7 B	-	5.76 A	3.77 AB
T1R	1.79 B	0.59 A	10.44 B	3.44 A	0.13 ABC	0.075 C	0.119 A	0.415 A	0.164 AB	0.243 ABC	0.030 AB	0.093 A	0.364 B	0.829 B	23.0 AB	-	5.54 A	3.65 AB
T2A	2.01 A	0.73 A	11.70 A	4.27 A	0.16 AB	0.081 B	0.164 A	0.282 AB	0.139 AB	0.241 ABC	0.026 B	0.108 A	0.599 A	1.105 AB	18.3 B	-	5.59 A	3.91 AB
T2R	1.77 B	0.65 A	10.34 B	3.81 A	0.15 ABC	0.059 E	0.207 A	0.405 A	0.173 AB	0.223 BC	0.030 AB	0.117 A	0.597 A	0.878 B	29.0 AB	-	4.48 A	2.79 C
T3A	2.05 A	0.70 A	11.97 A	4.07 A	0.11 C	0.093 A	0.080 A	0.308 AB	0.148 AB	0.313 AB	0.032 AB	0.123 A	0.617 A	1.111 AB	23.0 AB	-	4.62 A	4.02 A
T3R	1.76 B	0.59 A	10.22 B	3.43 A	0.18 A	0.066 D	0.162 A	0.305 AB	0.167 AB	0.258 ABC	0.032 AB	0.091 A	0.611 A	0.792 B	25.3 AB	-	4.83 A	3.34 ABC
T4A	2.02 A	0.70 A	11.80 A	4.11 A	0.15 ABC	0.090 A	0.085 A	0.242 B	0.143 AB	0.272 ABC	0.031 AB	0.127 A	0.597 A	1.275 A	23.7 AB	-	5.45 A	3.27 ABC
T4R	1.77 B	0.59 A	10.29 B	3.44 A	0.13 ABC	0.060 E	0.288 A	0.392 A	0.189 A	0.261 ABC	0.039 A	0.094 A	0.603 A	0.975 AB	29.0 AB	-	5.43 A	3.14 BC
T5A	2.00 A	0.61 A	11.67 A	3.56 A	0.11 BC	0.081 B	0.099 A	0.292 AB	0.181 AB	0.315 A	0.030 AB	0.131 A	0.598 A	1.051 AB	32.7 A	-	5.24 A	3.51 ABC
T5R	1.80 B	0.59 A	10.47 B	3.44 A	0.15 ABC	0.083 B	0.104 A	0.410 A	0.113 B	0.232 ABC	0.024 B	0.117 A	0.595 A	1.000 AB	26.0 AB	-	4.52 A	3.28 ABC
LSD	0.18	0.16	1.06	0.99	0.05	0.005	0.230	0.146	0.073	0.092	0.012	0.041	0.136	0.359	14.0	-	2.06	0.84

T0: control,T1: 2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.
(-) : Results excluded.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

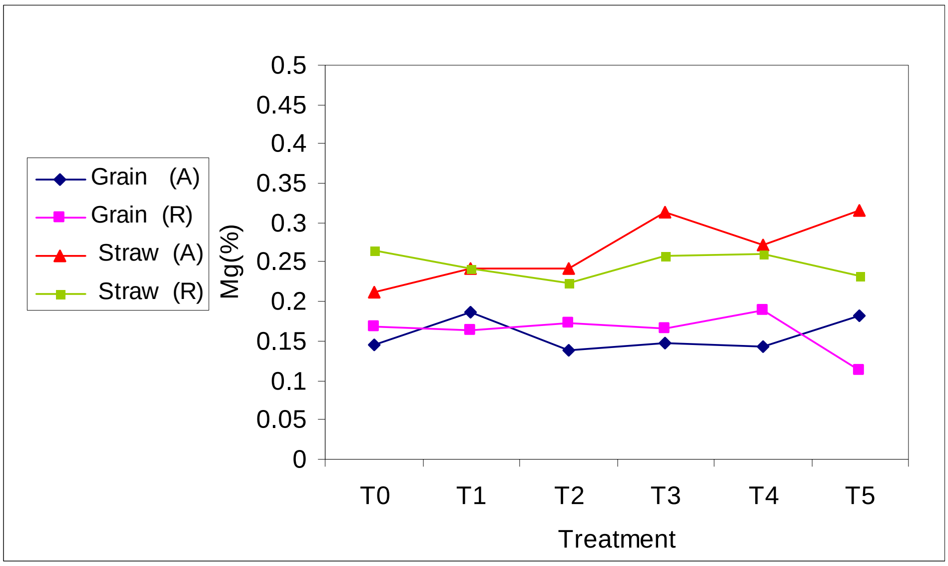
Figure (11): Phosphorus concentration in straw and grain at harvesting stage.

Phosphorus concentration in straw was significantly affected with accumulative bio-solids application, the maximum concentration was obtained at accumulative (6 & 8 ton/ha) bio-solids application rate while the minimum concentration was obtained at control case; different accumulative bio-solids treatments were significantly higher than residual treatments (except at 2 ton/ha rate) as shown in Figure(11). There was no significant difference between accumulative and residual chemical fertilizer treatments or between control treatment and residual (4 & 8 ton/ha) treatments.

There were no significant differences in calcium concentration in grain among different treatments. In straw, calcium concentration was slightly affected with bio-solids application. There were no significant differences between accumulative and residual treatments with the exception of (8 ton/ha) rate, also there were no significant differences between different bio-solids treatments and control case and between chemical fertilizer treatments and control case.

Magnesium concentration in grain was slightly affected with bio-solids application; the minimum concentration was obtained at residual chemical fertilizer treatment while the maximum concentration was obtained at residual (8ton/ha) bio-solids application rate. There were no significant differences between different bio-solids application rates either accumulative or residual or between different bio-solids treatments and control treatment.

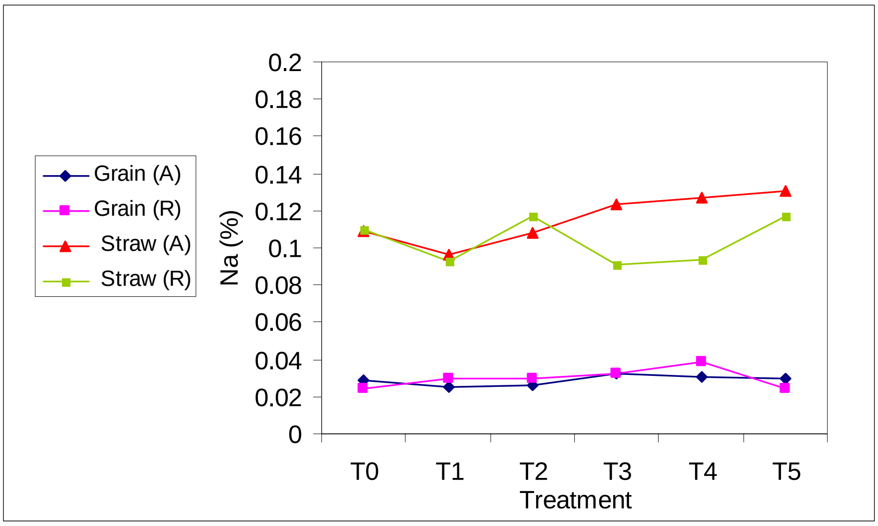
Magnesium concentration in straw was also slightly affected with bio-solids application during the second growing season; the minimum concentration was obtained for the control case while the maximum concentration was obtained at accumulative chemical fertilizer treatment. However, there were no significant differences between different bio-solids treatments either accumulative or residual, or between bio-solids treatments and control case and between accumulative chemical fertilizer treatment and different bio-solids treatments. Figure (12) shows magnesium concentration in straw and grain for different treatments.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (12): Magnesium concentration in straw and grain at harvesting stage.

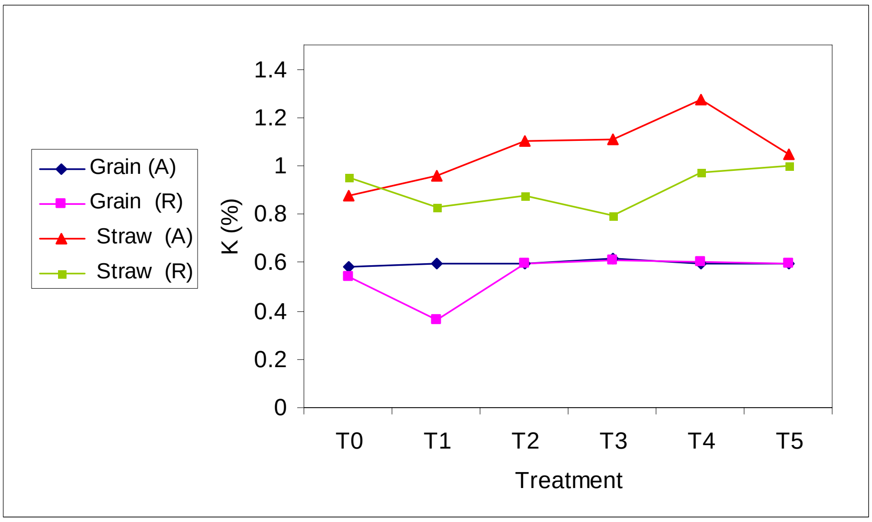
Sodium concentration in grain was also slightly affected with bio-solids application; minimum concentration was obtained for control treatment and residual chemical fertilizer treatment while the maximum concentration was obtained at residual (8 ton/ha) bio-solids treatment. There were no significant differences between different bio-solids treatments either accumulative or residual, between bio-solids treatments and control case and between accumulative and residual chemical fertilizer treatments. In straw, there were no significant differences in sodium concentration between different treatments. Figure (13) shows sodium concentration in straw and grain at harvesting.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (13): Sodium concentration in straw and grain at harvesting stage.

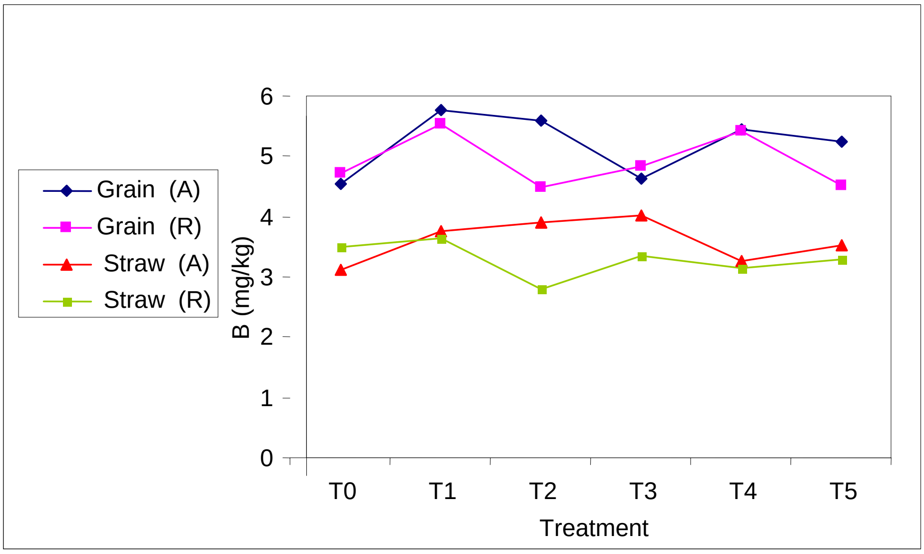
Potassium concentration results in grain show no significant differences between different treatments. In straw, slight differences were found; the maximum concentration was obtained at accumulative (8 ton/ha) bio-solids treatment while the minimum was obtained at residual (6 ton/ha) rate, which is not significantly different from control. There were no significant differences between accumulative and residual bio-solids treatments or between different treatments and control. Potassium concentration in straw and grain is shown in Figure (14).



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (14): Potassium concentration in straw and grain at harvesting stage.

Boron concentration results in grain show no significant differences between all treatments. In straw, it was slightly affected with bio-solids application; the maximum concentration was obtained at accumulative (6 ton/ha) bio-solids treatment while the minimum was found at residual (4 ton/ha) treatment which is not significantly different from control. There were no significant differences found between residual and accumulative treatments or between different treatments and control treatment. Boron concentration in straw and grain is shown in Figure(15).



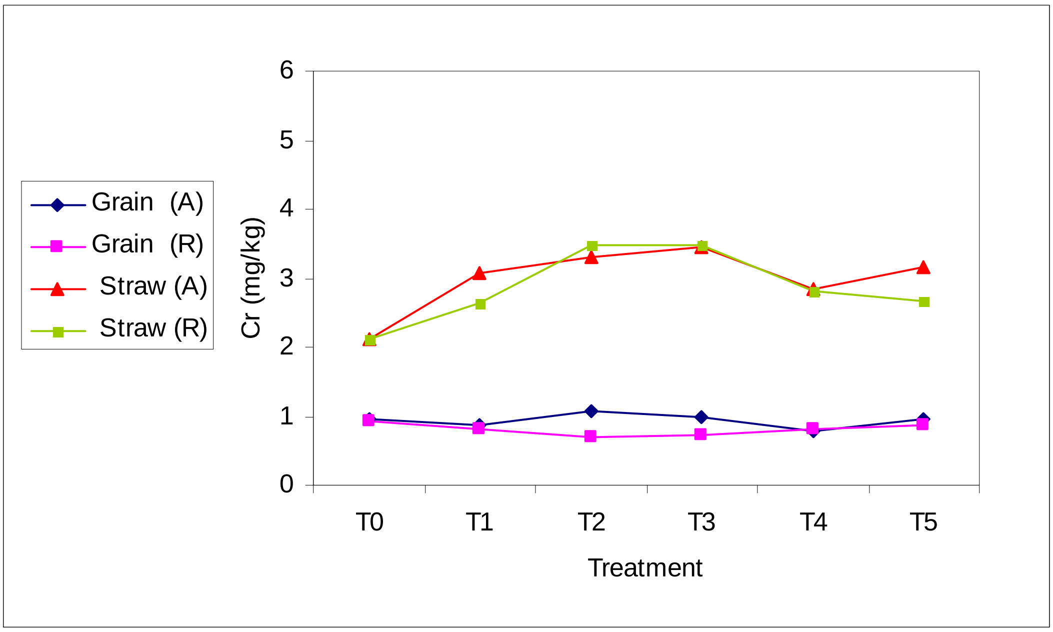
* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (15): Boron concentration in straw and grain at harvesting stage.

Effect of different treatments on micro-nutrients concentration in barley straw and grain.

The effect of bio-solids application on micro-element concentrations in barley straw and grain is presented in Table (9). Arsenic concentration in grain was slightly affected with bio-solids application during the second season, the maximum concentration was obtained at residual (8 ton/ha) bio-solids application rate while the minimum concentration was found at control case. There were no significant differences found between accumulative and residual treatments except at maximum bio-solids application rate and fertilizer treatments. In straw, arsenic concentration was found to be maximum at accumulative (6 ton/ha) bio-solids application rate which was significantly different from control unlike other treatments. Also, no significant differences were found between accumulative and residual treatments.

Chromium concentration results in grain show no significant differences between accumulative and residual treatments except at (4 ton/ha) bio-solids treatment where the maximum chromium concentration was found at the accumulative treatment of this application rate; no significant differences were found between different treatments and control treatment. Chromium concentration results in straw show no significant differences between different bio-solids and chemical fertilizer treatments; accumulative (2 & 4& 6 ton/ha) application rates and accumulative chemical fertilizer treatments were significantly different from control case. Figure (16) shows chromium concentration in straw and grain.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

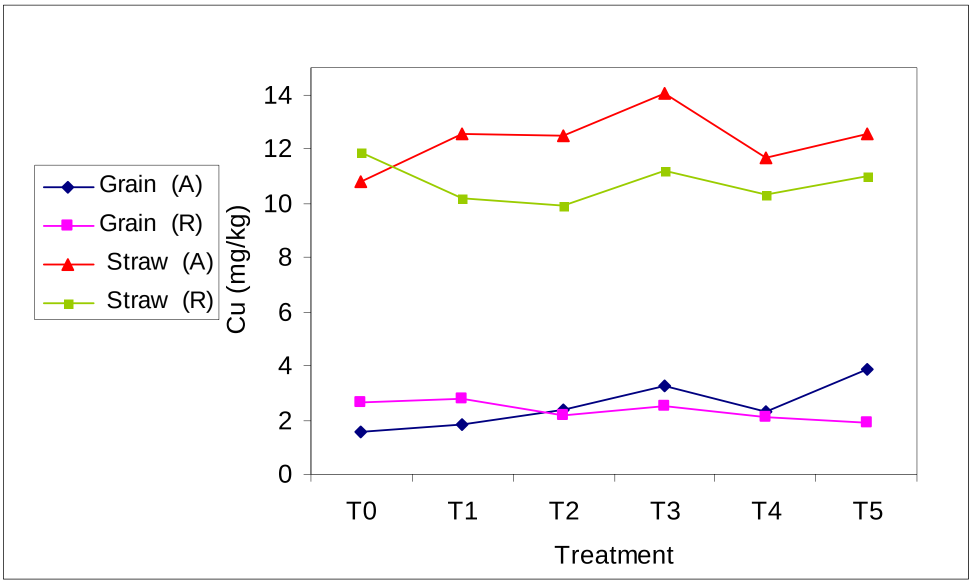
Figure (16): Chromium concentration in straw and grain at harvesting stage.

There were no significant differences in copper concentration results in grain between accumulative and residual treatments with the exception of chemical fertilizer treatment, where accumulative treatment was significantly higher than residual treatment; different treatments were not significantly different from control case. In straw, copper concentrations for accumulative treatments were significantly higher than residuals. The maximum concentration was obtained at accumulative (6 ton/ha) bio-solids treatment while the minimum was found at residual (4 ton/ha) rate, which is not significantly different from control treatment. Copper concentration in straw and grain is shown in Figure (17).

Table (9): The effect of different treatments on micro-nutrients concentrations in barley straw and grain at harvesting stage.

T	Se (mg/kg)		Cd (mg/kg)		Pb (mg/kg)		As (mg/kg)		Cr (mg/kg)		Cu (mg/kg)		Hg (mg/kg)		Mo (mg/kg)		Ni (mg/kg)		Zn (mg/kg)	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T0A	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.16 DE	0.14 B	0.96 AB	2.13 B	1.59 B	10.80 DE	<0.04	<0.04	3.31 A	6.27 C	2.56 AB	1.74 A	58.29 A	15.44 AB
T0R	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.15 E	0.15 B	0.93 AB	2.13 B	2.62 AB	11.85 BCD	<0.04	<0.04	2.91 AB	6.55 BC	2.51 ABC	1.21 A	65.84 A	15.00 AB
T1A	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.17 BC	0.14 B	0.88 AB	3.06 A	1.82 B	12.53 BC	<0.04	<0.04	2.02 AB	7.38 ABC	2.12 ABCD	1.49 A	67.66 A	17.65 AB
T1R	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.17 BCD	0.15 B	0.80 AB	2.65 AB	2.75 AB	10.17 E	<0.04	<0.04	1.37 B	7.47 ABC	1.94 ABCD	1.76 A	62.05 A	14.05 B
T2A	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.18 BC	0.18 AB	1.06 A	3.29 A	2.36 AB	12.49 BC	0.18	<0.04	2.13 AB	7.95 AB	2.59 A	1.61 A	67.01 A	22.35 AB
T2R	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.18B	0.16 AB	0.70 B	3.49 A	2.14 AB	9.90 E	<0.04	<0.04	2.02 AB	7.70A BC	1.78 D	2.77 A	54.26 A	21.46 AB
T3A	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.18 B	0.21 A	1.00 AB	3.45 A	3.28 AB	14.06 A	0.22	<0.04	2.82 AB	8.19 A	2.32 ABCD	2.11 A	59.31 A	18.88 AB
T3R	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.18 BC	00.16 AB	0.73 AB	3.47 A	2.53 AB	11.22 CDE	<0.04	<0.04	1.42 B	7.37 ABC	1.88 CD	2.46 A	54.10 A	16.11 AB
T4A	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.16 E	0.15 B	0.77 AB	2.83 AB	2.33 AB	11.67 BCD	0.23	<0.04	2.41 AB	7.72 AB	2.30 ABCD	1.70 A	53.56 A	22.88 A
T4R	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.21 A	0.15 B	0.81 AB	2.81 AB	2.10 AB	10.29 E	<0.04	<0.04	2.52 AB	7.30 AB	2.09 ABCD	1.64 A	54.32 A	15.20 AB
T5A	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.19 B	0.19 AB	0.97 AB	3.17 A	3.87 A	12.57 B	<0.04	<0.04	1.74 B	7.75 AB	1.933 BCD	1.71 A	61.07 A	18.42 AB
T5R	<0.05	<0.05	<0.004	<0.004	<0.025	<0.025	0.16 CDE	0.18 AB	0.87 AB	2.67 AB	1.93 B	11.02 DE	<0.04	<0.04	1.66 B	6.97 ABC	1.82 D	1.31 A	54.22 A	17.31 AB
LSD	-	-	-	-	-	-	0.02	0.05	0.32	0.85	1.83	1.33	0.10	-	1.56	1.45	0.65	1.60	18.06	8.85

T0: control,T1: 2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

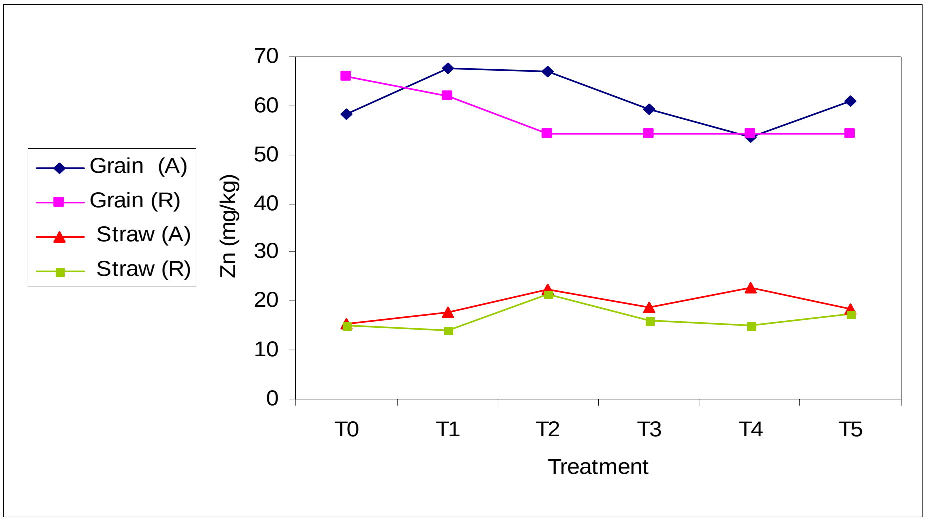
Figure (17): Copper concentration in straw and grain at harvesting stage.

Mercury concentration was below detection limit in grain at different treatments except at accumulative (4 & 6 & 8 ton/ha) bio-solids treatments where its concentration increases with the increase in bio-solids application rate. In straw, mercury concentration was below detection limit for all treatments.

Molybdenum concentration in grain was slightly affected with bio-solids application; no significant differences were found between accumulative and residual treatments or between different bio-solids treatments, fertilizer treatments and control. Molybdenum concentration in straw was also slightly affected with bio-solids application; the maximum concentration was found at accumulative(6 ton/ha) bio-solids treatment, which is significantly different from control. There were no significant differences between accumulative and residual treatments.

Nickel concentration in grain was slightly affected with bio-solids application; no significant differences were found between accumulative and residual treatments except at (4 ton/ha) bio-solids treatment where the accumulative treatment was significantly higher than residual. In straw, no significant differences were found between different treatments.

Zinc concentration in grain was insignificantly different for various treatments. In straw, slight differences were found; the maximum zinc concentration was obtained at accumulative (8 ton/ha) bio-solids treatment while the minimum was found at residual (2 ton/ha)treatment; there were no significant differences between accumulative and residual treatments or between different treatments and control case. Zinc concentration in straw and grain is shown in figure (18).



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (18): Zinc concentration in straw and grain at harvesting stage.

As for the other micro-elements (Se, Cd, Pb), analysis results were below detection limits for both barley straw and grain as shown in Table (9).

5.3.3 Plant Microbiological Analysis

Table (10) shows the microbiological analysis of barley at harvesting stage. The results indicate that *Salmonella* and IPN were not detected and levels of TFCC and *E.coli* were less than their detection limits.

Table (10): Microbiological analysis of barley at harvesting stage*.

Treatment	*Salmonella* /20gm	IPN eggs/50 gm	TFCC MPN/gm	*Escherichia coli* MPN/gm
T0A	N.D	N.D	<0.03	<0.03
T0R	N.D	N.D	<0.03	<0.03
T1A	N.D	N.D	<0.03	<0.03
T1R	N.D	N.D	<0.03	<0.03
T2A	N.D	N.D	<0.03	<0.03
T2R	N.D	N.D	<0.03	<0.03
T3A	N.D	N.D	<0.03	<0.03
T3R	N.D	N.D	<0.03	<0.03
T4A	N.D	N.D	<0.03	<0.03
T4R	N.D	N.D	<0.03	<0.03
T5A	N.D	N.D	<0.03	<0.03
T5R	N.D	N.D	<0.03	<0.03

T0: control,T1: 2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R:residual.
N.D: not detected.

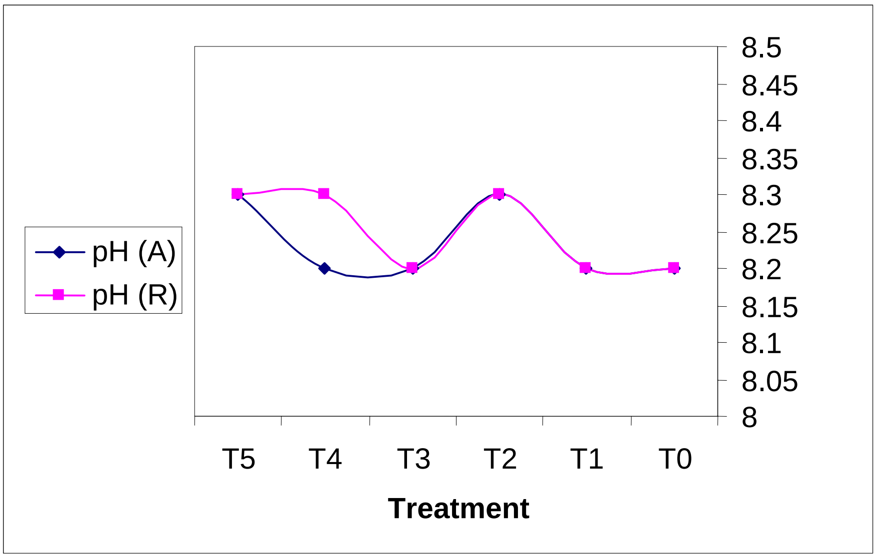
5.4 Soil Analysis

Soil samples were collected and analyzed prior to bio-solids application upon tillering stage and at harvesting stage to assess impacts of bio-solids application on soil properties. At tillering stage, soil samples were collected at depth (0-15cm) from three replications; all samples were chemically analyzed while samples from only one replication were microbiologically analyzed. At harvesting stage, soil samples were taken at two depths (0-15 cm) and (15-30 cm) from three replications and analyzed chemically and microbiologically. Soil baseline quality was discussed earlier, while results of soil analysis upon tillering stage and at harvesting stage will be discussed below.

5.4.1 Soil Analysis at tillering stage

Soil samples were chemically analyzed for electrical conductivity (EC), organic matter (OM), pH, available P, available K, available Na, available Mg, available Ca and trace metals. The effects of different treatments on soil properties are presented in Table (11).

Soil pH values show no significant differences between different treatments; pH value increased from (8.2) at control to (8.3) at (4 ton/ha) then decreased at (6 ton/ha) then increased again at residual (8 ton/ha) bio-solid treatment to a value of (8.3). Soil pH variation is shown in Figure(19).

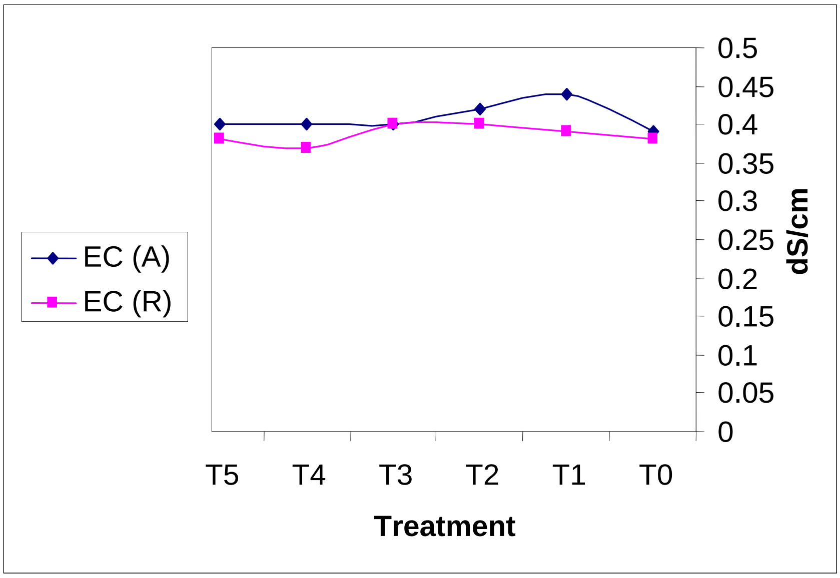


* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (19): Soil pH variation at tillering stage.

Electrical conductivity values show no significant differences between the different treatments (neither accumulative nor residual); EC values ranged from

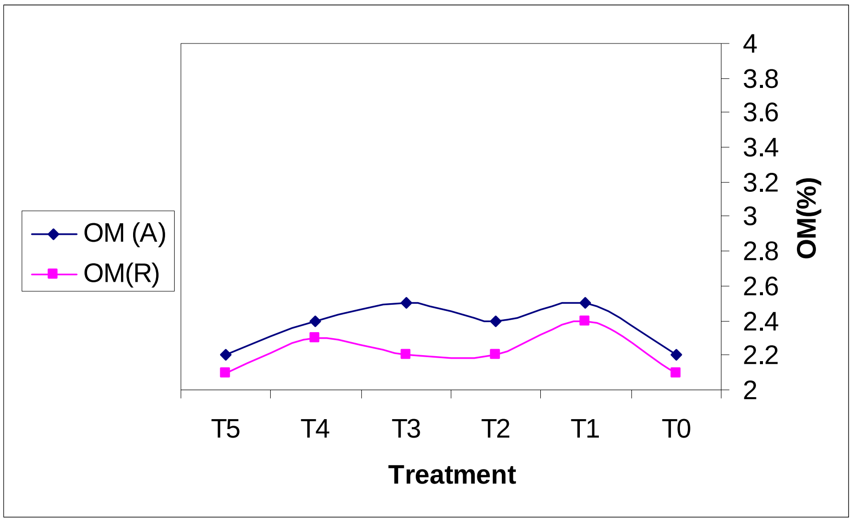
0.37 to 0.44 ds/cm. Figure (20) shows electrical conductivity values of soil for the different treatments.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (20): Soil EC at tillering stage.

Organic matter concentrations show no significant differences between the different treatments or between residual and accumulative treatments; OM values ranged from (2.1%) at control and residual chemical fertilizer treatments to (2.5%) at accumulative (2 and 6 ton/ha). Figure (21) shows OM values for the different treatments.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (21): Soil organic matter at tillering stage.

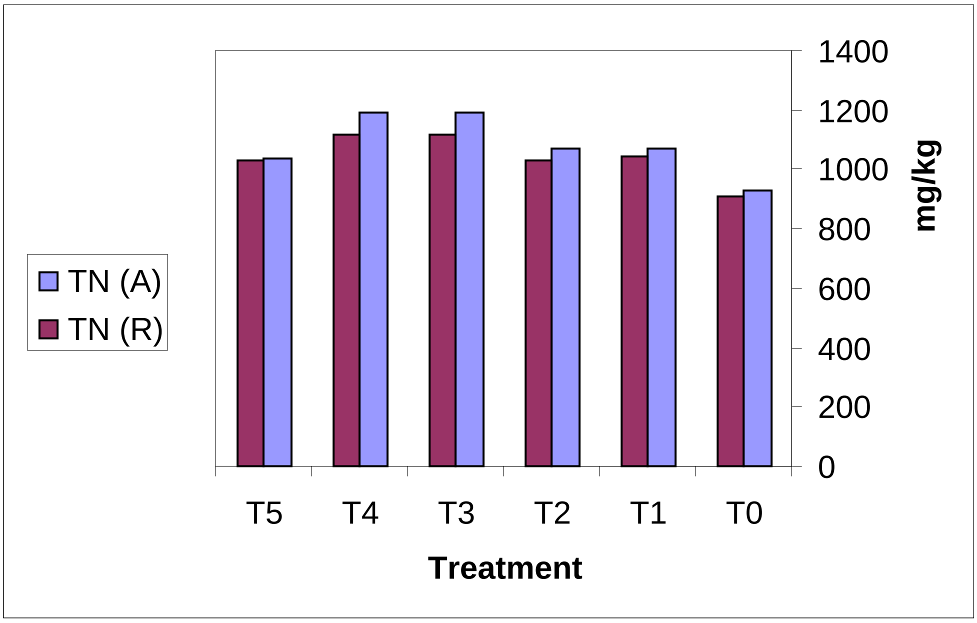
Table (11): The effect of different treatments on soil properties at tillering stage.

Treatment	pH (SU)	EC (ds/cm)	P (mg/kg)	TN (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	OM (%)	B (mg/kg)
T0A	8.2 AB	0.39 AB	13.6 B	925.7 A	8318.5 A	710.7 A	112.7 AB	598.9 AB	2.2 A	7.90 A
T0R	8.2 AB	0.38 AB	14.3 AB	910.8 A	8153.5 A	785.2 A	111.3 AB	558.6 B	2.1 A	6.24 B
T1A	8.2 AB	0.44 A	17.8 AB	1069.3 A	8249.5 A	696.7 A	124.4 AB	699.4 AB	2.5 A	4.00 CD
T1R	8.2 AB	0.39 AB	16.3 AB	1043.5 A	8300.0 A	790.9 A	120.6 AB	676.3 AB	2.4 A	3.65 D
T2A	8.3 A	0.42 AB	17.8 AB	1068.1 A	8000.7 A	848.3 A	132.6 A	706.0 AB	2.4 A	4.48 CD
T2R	8.3 A	0.40 AB	16.3 AB	1030.0 A	8067.9 A	795.0 A	122.3 AB	666.8 AB	2.2 A	3.63 D
T3A	8.2 AB	0.40 AB	22.6 AB	1189.9 A	8013.9 A	789.0 A	113.6 AB	724.8 AB	2.5 A	5.22 BC
T3R	8.2 AB	0.40 AB	20.4 AB	1115.7 A	8097.9 A	773.7 A	98.2 B	718.0 AB	2.2 A	4.96 BCD
T4A	8.2 AB	0.40 AB	25.8 A	1193.7 A	8023.8 A	835.8 A	121.9 AB	757.5 A	2.4 A	4.11 CD
T4R	8.3 A	0.37 AB	19.4 AB	1114.9 A	8041.1 A	809.0 A	116.1 AB	684.0 AB	2.3 A	4.32 CD
T5A	8.3 A	0.40 AB	20.5 AB	1035.5 A	8080.5 A	808.7 A	117.7 AB	637.4 AB	2.2 A	3.72 D
T5R	8.3 A	0.38 AB	17.7 AB	1027.5 A	7824.7 A	854.8 A	128.3 A	632.4 AB	2.1 A	4.10 CD
LSD	0.05	0.05	10.14	270	504.9	150.4	23.61	168	0.5	1.41

T0: control,T1: 2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.

Effect of different treatments on macro-nutrients concentration in soil

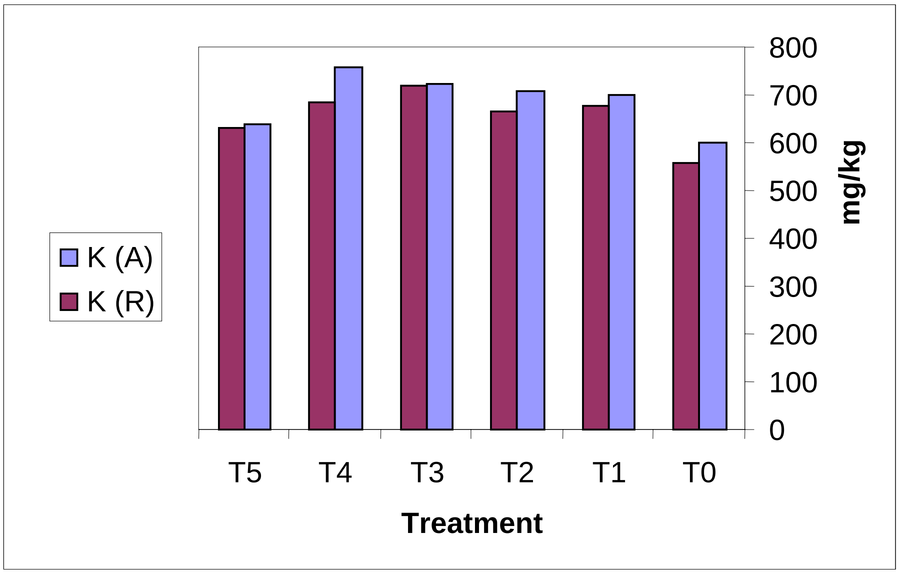
Nitrogen concentration in soil for the different treatments is shown in Figure (22). It could be noted that accumulative treatments had higher nitrogen levels than residuals. Nitrogen level ranged from (910.8 mg/kg) at control case to (1193.7 mg/kg) at accumulative case of the maximum bio-solids application rate. In general, there were no significant differences in nitrogen levels in soil between the different treatments.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (22): Nitrogen concentration in soil at tillering stage.

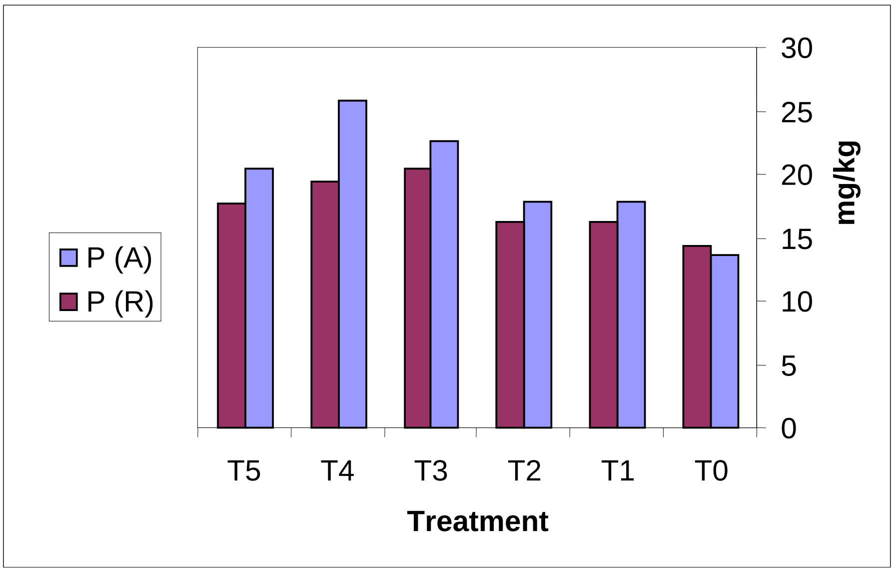
Figure(23) shows that potassium concentration increases with the increase in bio-solid application rate and that accumulative treatments have higher concentrations than residuals. Maximum potassium concentration was obtained at accumulative maximum bio-solids application rate treatment and the minimum was obtained at control case.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (23): Potassium concentration in soil at tillering stage.

Phosphorus concentration in soil increased with the increase in bio-solids application rate as shown in Figure (24); it is also shown that accumulative treatments have higher concentrations than residuals. A maximum phosphorus concentration of (25.8 mg/kg) was obtained at accumulative (8 ton/ha) treatment while a minimum concentration of (13.6 mg/kg) was obtained for the control treatment. There was a significant difference between phosphorus concentration for at accumulative maximum bio-solids application rate treatment and the control but no significant differences were found between the other treatments or between the accumulative and residual treatments.

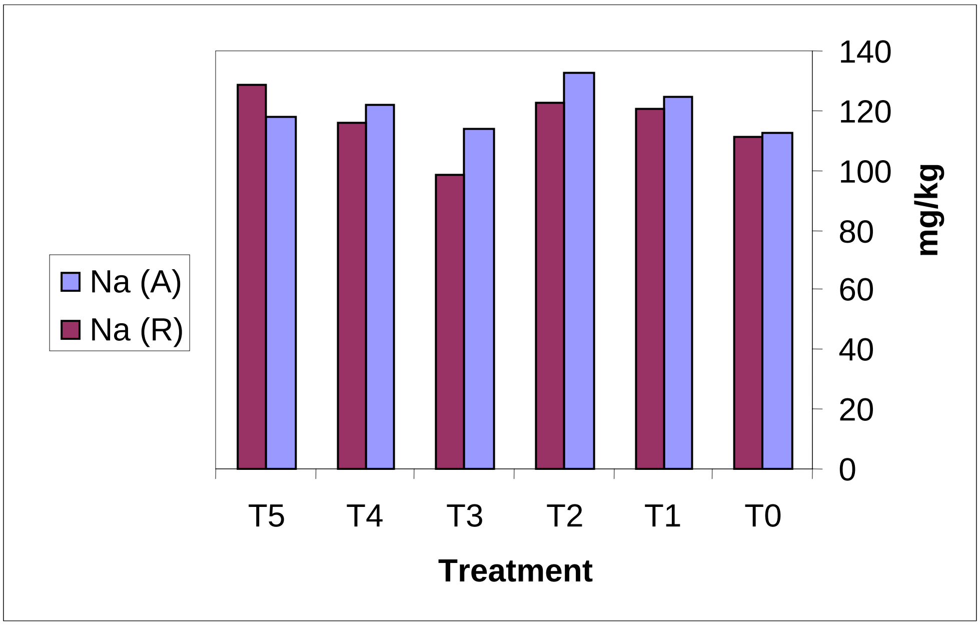


* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (24): Phosphorus concentration in soil at tillering stage.

As shown in Figure (25), the maximum concentration of sodium in soil was obtained at accumulative (4 ton/ha) bio-solids treatment while the minimum value was obtained for the residual (6 ton/ha) treatment; there were no significant differences between the other treatments or between the accumulative and residual treatments.

Magnesium and calcium concentrations in soil show no significant differences between different treatments as shown in Table (11). Boron concentration was slightly affected with accumulative bio-solids application; there were no significant differences between accumulative and residual treatments.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (25): Sodium concentration in soil at tillering stage.

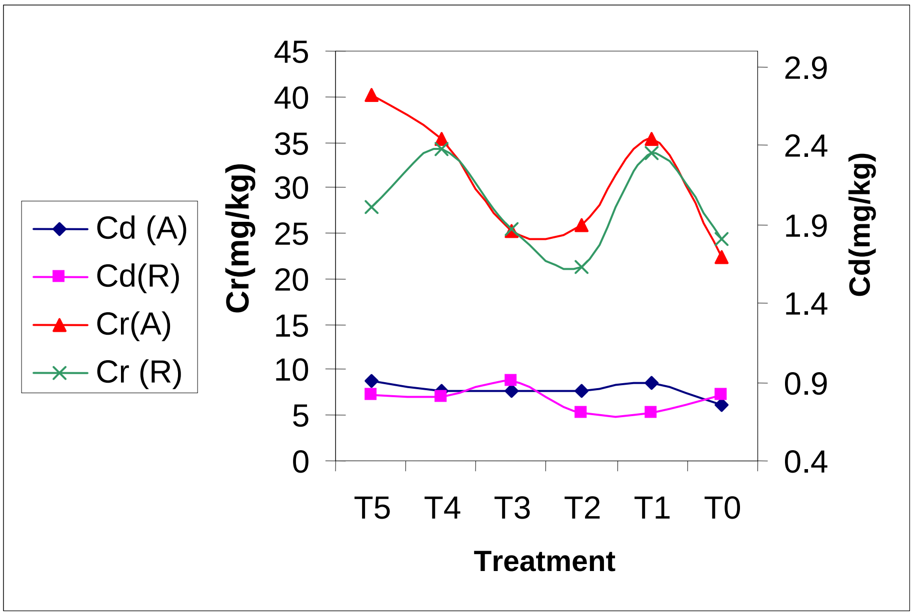
Effect of different treatments on micro-nutrients concentration in soil

Table (12) shows micro-nutrients concentrations in soil. Results show that cobalt concentration was not detected in soil while arsenic and lead concentrations were not significantly affected with bio-solids application. Cadmium concentration was slightly affected with accumulative bio-solids application as shown in Figure (26). A maximum concentration of (0.91mg/kg) was obtained at residual (6 ton/ha) bio-solids treatment while the minimum value was found at residual (2 & 4ton/ha) treatments. There were no significant differences between other treatments or between the accumulative and residual treatments.

Table (12): Effect of different treatments on micro-nutrients in soil at tillering stage.

Treatment	As (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	Mo (mg/kg)	Ni (mg/kg)	Se (mg/kg)	Zn (mg/kg)	Co (mg/kg)
T0A	4.85 A	0.76 AB	22.35 E	11.75 AB	6.75 A	2.58 AB	1.17 C	19.04 B	0.54 DE	11.19 ABCD	N.D.
T0R	4.66 A	0.82 AB	24.33 E	12.23 AB	6.75 A	3.08 A	1.28 BC	19.65 B	0.47 E	13.93 ABC	N.D.
T1A	5.82 A	0.89 AB	35.33 A	12.00 AB	7.52 A	3.23 AB	1.63 BC	40.27 A	0.63 CDE	17.11 A	N.D.
T1R	3.78 A	0.70 B	33.84 ABC	10.82 B	6.23 A	1.81 AB	1.41 BC	27.38 B	0.48 E	14.38 AB	N.D.
T2A	5.70 A	0.85 AB	25.90 BCDE	13.10 A	7.40 A	2.93 AB	2.56 A	21.65 B	0.77 BCDE	11.14 ABCD	N.D.
T2R	4.71 A	0.70 B	21.30 E	9.81 B	5.88 A	1.53 B	1.23 C	19.21 B	0.55 CDE	7.68 D	N D.
T3A	5.70 A	0.85 AB	25.22 DE	11.49 AB	7.20 A	2.66 AB	1.32 BC	20.95 B	0.98 ABCD	15.07 ABCD	N.D.
T3R	5.30 A	0.91 A	25.51 CDE	11.14 AB	7.07 A	2.04 AB	1.72 ABC	24.17 B	1.29 A	9.15 BCD	N.D.
T4A	5.42 A	0.85 AB	35.25 A	11.08 AB	6.77 A	2.30 AB	1.11 C	22.04 B	1.12 AB	9.51 D	N.D.
T4R	4.98 A	0.80 AB	34.30 AB	11.10 AB	6.13 A	1.70 AB	1.47 BC	26.23 B	1.03 ABC	9.26 BCD	N.D.
T5A	6.32 A	0.91 A	40.16 ABCD	11.42 AB	7.53 A	2.07 AB	1.89 AB	28.32 B	1.25 A	8.13 D	N.D.
T5R	5.96 A	0.82 AB	27.87 ABCDE	10.60 AB	5.89 A	2.18 AB	1.49 BC	24.17 B	1.36 A	8.17 CD	N.D.
LSD	1.70	0.1417	8.53	2.25	2.04	1.46	0.65	10.2	0.47	6.13	-

T0: control,T1 :2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.



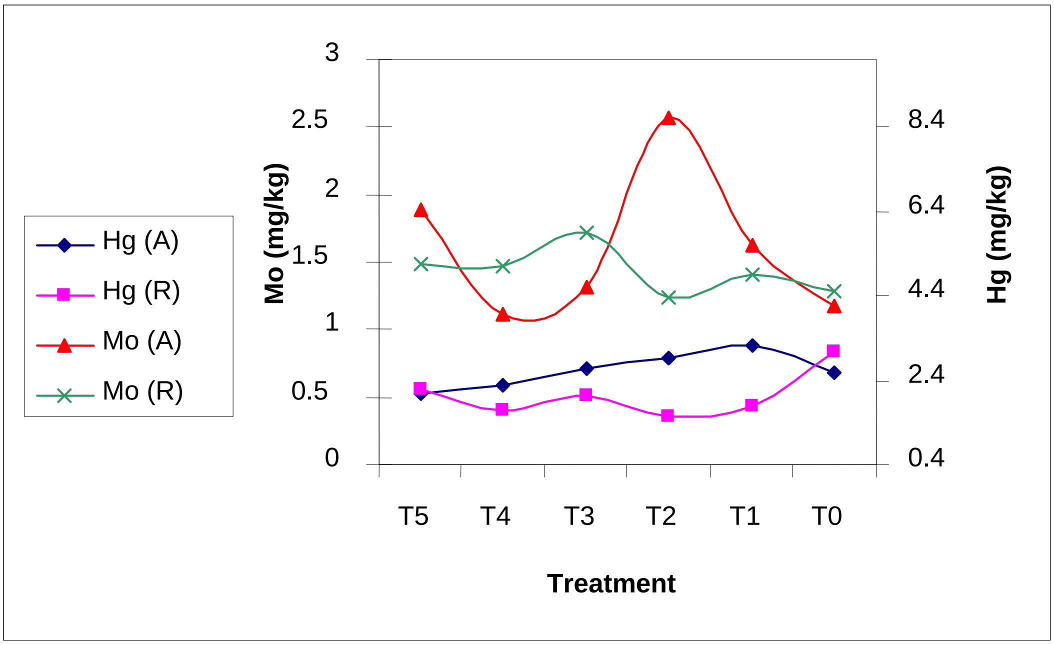
* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (26): Cadmium and chromium concentrations in soil at tillering stage.

It's noted that the chromium concentration which is shown in Figure (26) is slightly affected with accumulative bio-solids application; maximum concentrations were found at accumulative (2 & 8 ton/ha) treatments while minimum value was found at residual (4 ton/ha) bio-solids treatment. There were no significant differences between the accumulative and residual treatments.

Copper concentration in soil was slightly affected with accumulative bio-solids application, there were no significant differences between residual and accumulative treatments with the exception of (4 ton/ha) bio-solids treatment. Also, no significant differences were found between different treatments and control treatment.

Mercury concentration in soil was slightly affected with accumulative bio-solids application, there were no significant differences between different treatments neither accumulative nor residual. Molybdenum concentration in soil was also slightly affected with bio-solids application; maximum value was found at accumulative (2 ton/ha) bio-solids application rate while minimum value was found at accumulative (8 ton/ha) application rate. There were no significant differences between the accumulative and residual treatments with the exception of (4 ton/ha) treatment. Mercury and molybdenum concentrations in soil are shown in figure(27).

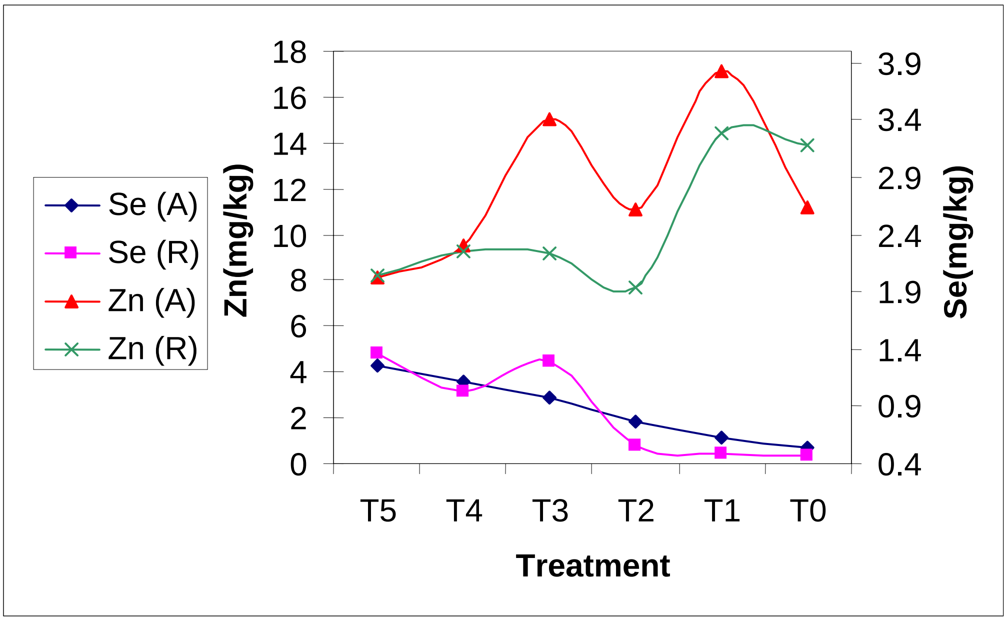


* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (27): Mercury and molybdenum concentrations in soil at tillering stage.

Nickel concentration results in soil show no significant differences between different treatments neither accumulative nor residual with the exception of accumulative (2 ton/ha) treatment, which was significantly higher than other treatments.

Selenium and zinc concentrations in soil were slightly affected with bio-solids application, almost there were no significant differences between different treatments neither accumulative nor residual. Selenium and zinc concentrations are shown in Figure(28).



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (28): Selenium and zinc concentrations in soil at tillering stage.

Soil Microbiological Analysis

Table (13) shows soil microbial properties at tillering stage, it can be noticed that TFCC and *Salmonella* were not detected for all treatments.

Table (13): Microbiological analysis of soil at tillering stage.

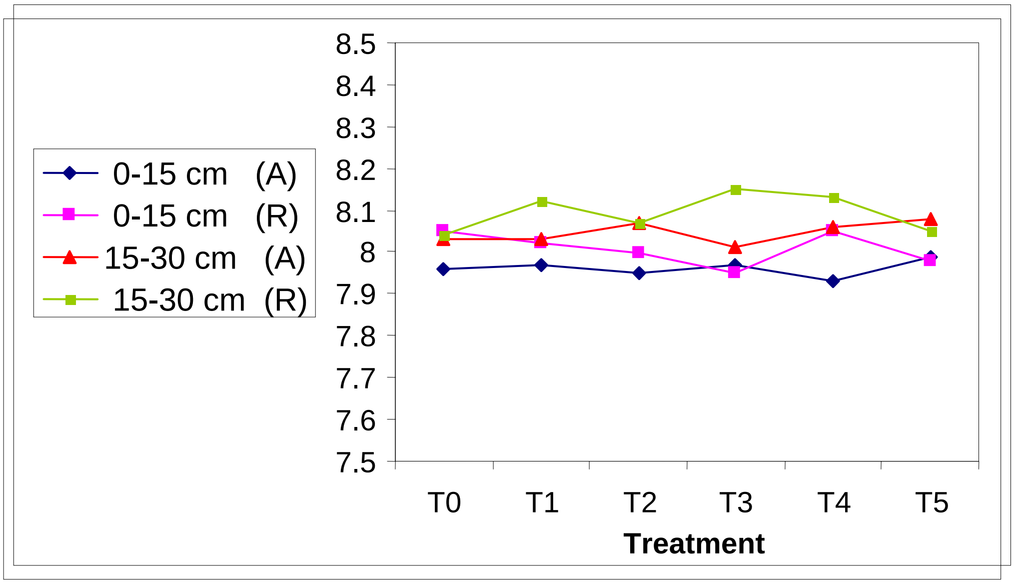
Treatment	TFCC (MPN/g)	*Salmonella spp.* (Presence or absence /25g)
T0A	<0.3	N.D.
T0R	<0.3	N.D.
T1A	<0.3	N.D.
T1R	<0.3	N.D.
T2A	<0.3	N.D.
T2R	<0.3	N.D.
T3A	<0.3	N.D.
T3R	<0.3	N.D.
T4A	<0.3	N.D.
T4R	<0.3	N.D.
T5A	<0.3	N.D.
T5R	<0.3	N.D.

* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual
N.D.: not detected.

5.4.2 Soil Analysis at Harvesting Stage

Soil was analyzed at depths (0-15cm) and (15-30cm). The chemical analysis of soil samples included: electrical conductivity (EC), organic matter (OM), pH, cation exchange capacity CEC, total nitrogen, available P, available K, available Na, available Mg, available Ca, nitrate, nitrite, boron and trace metals. The effects of different treatments on soil properties at the two depths are presented in Table (14).

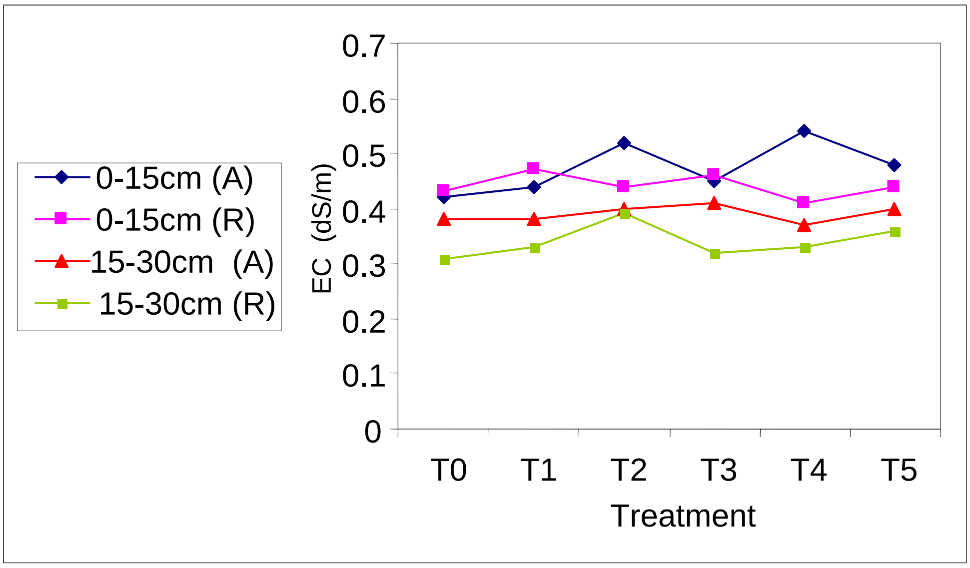
Soil pH results for the two depths show no significant differences between different treatments neither accumulative nor residuals. Soil pH at harvesting stage is shown in Figure (29).



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (29): pH of soil at harvesting stage.

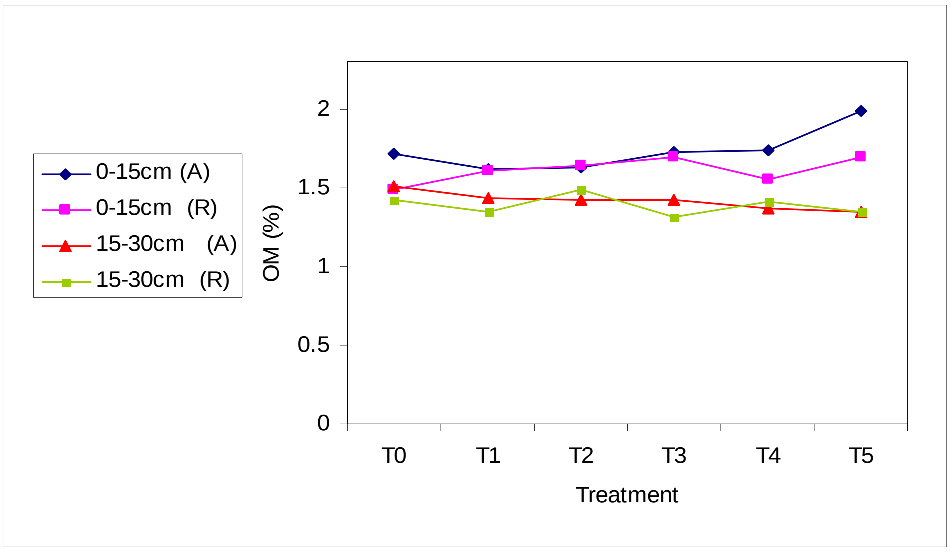
Soil EC was slightly affected with bio-solids application at the two depths. At depth (0-15cm), the maximum EC was obtained at accumulative (8 ton/ha) bio-solids treatment while the minimum was found at residual (8 ton/ha) rate. There were no significant differences between residual and accumulative cases or between different treatments and control treatment. At (15-30cm) depth, there were no significant differences at different treatments. EC of soil at harvesting stage is shown in Figure (30).



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual.

Figure (30): EC of soil at harvesting stage.

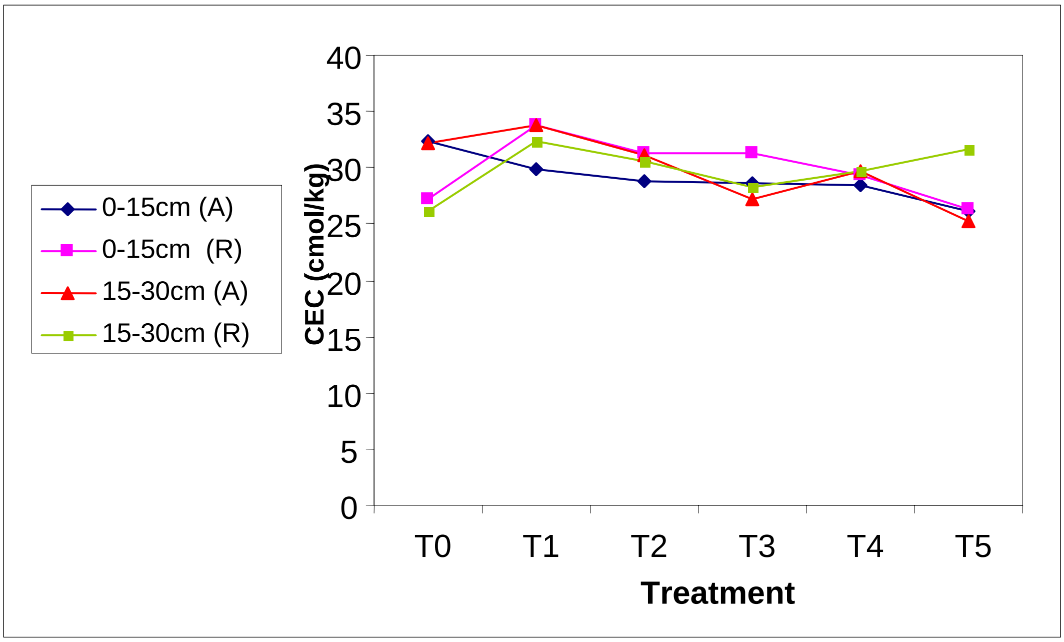
Organic matter of soil at the two depths was slightly affected with bio-solids application. At depth (0-15cm), maximum OM content was obtained at accumulative chemical fertilizer treatment, which was not significantly different from accumulative (6 & 8 ton/ha) bio-solids treatments No significant differences were found between accumulative and residual treatments. At depth (15-30cm), there were no significant differences between different treatments. Figure (31) shows OM of soil at harvesting stage.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (31):Organic matter of soil at harvesting stage.

Cation exchange capacity of soil at the two depths was not significantly affected with bio-solids application. Figure (32) shows CEC of soil at harvesting stage.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (32):CEC of soil at harvesting stage.

<u>Effect of different treatments on macro-nutrients concentration in soil</u>

Macro-nutrients concentration in soil at harvesting stage is shown in Table (14). Total nitrogen content of soil at depth (0-15cm) was significantly increased at accumulative (8 ton/ha) bio-solids treatment, other bio-solids and chemical fertilizer treatments were not significantly different from each other and from control treatment. At depth (15-30cm), there were no significant differences in T-N content between different treatments. T-N content of soil at harvesting stage is shown in Figure (33).

Phosphorus content of soil at depth (0–15 cm) was significantly affected with bio-solids application at accumulative (6 ton/ha) bio-solids application rate which is significantly different from residual and control treatments. For other treatments there were no significant differences between accumulative, residual and control treatments. At depth (15-30cm), there were no significant differences between different treatments. T-P concentration in soil at harvesting stage is shown in Figure (34).

Table (14): The effect of different treatments on soil properties at harvesting stage.**

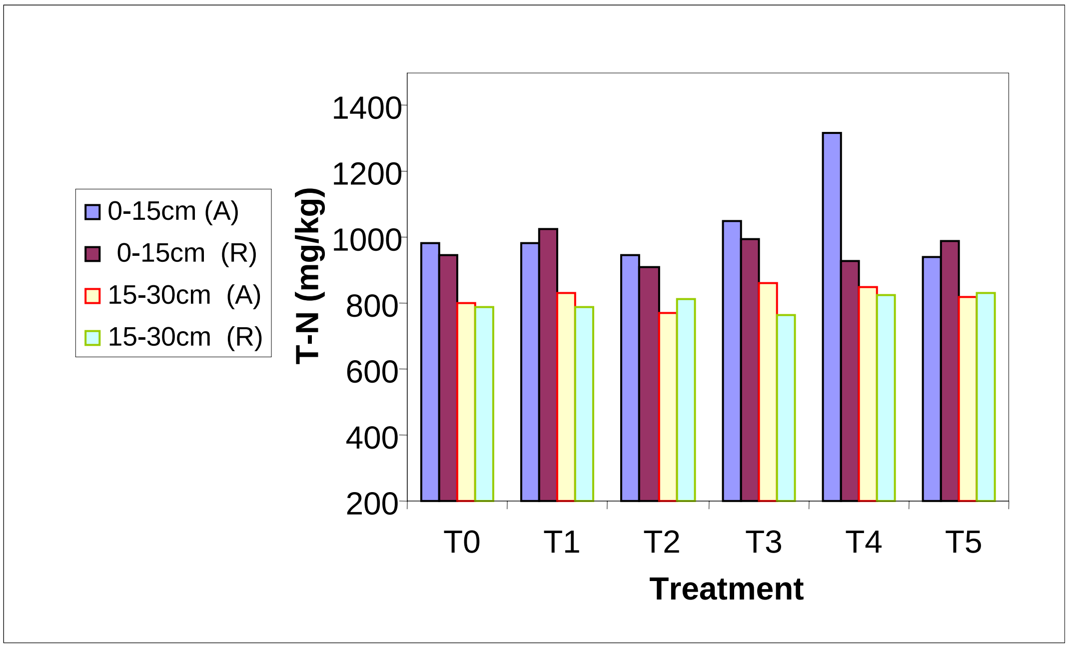
Treatment*	pH (SU)		EC (dS/m)		OM (%)		CEC (cmol/kg)		T.N.(mg/kg)		T-P (mg/kg)	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
T0A	7.96 A	8.03 A	0.42 BC	0.38 A	1.71 AB	1.51 A	32.3 A	32.23 A	983 B	799 A	19.40 B	12.33 A
T0R	8.05 A	8.04 A	0.43 BC	0.31 A	1.49 B	1.42 AB	27.27 A	26.17 A	950 B	790 A	16.00 B	10.10 A
T1A	7.97 A	8.03 A	0.44 ABC	0.38 A	1.62 B	1.43 AB	29.87 A	33.73 A	981 B	829 A	24.30 AB	12.97 A
T1R	8.02 A	8.12 A	0.47 ABC	0.33 A	1.61 B	1.35 AB	33.73 A	32.30 A	1027 B	791 A	22.23 AB	20.43 A
T2A	7.95 A	8.07 A	0.52 AB	0.40 A	1.63 B	1.42 AB	28.83 A	31.03 A	949 B	770 A	24.10 AB	14.43 A
T2R	8.00 A	8.07 A	0.44 BC	0.39 A	1.64 B	1.49 AB	31.37 A	30.53 A	909 B	816 A	17.30 B	9.93 A
T3A	7.97 A	8.01 A	0.45 ABC	0.41 A	1.72 AB	1.42 AB	28.67 A	27.23 A	1052 B	864 A	31.90 A	19.27 A
T3R	7.95 A	8.15 A	0.46 ABC	0.32 A	1.69 AB	1.31 B	31.30 A	28.20 A	995 B	763 A	21.40 B	13.77 A
T4A	7.93 A	8.06 A	0.54 A	0.37 A	1.74 AB	1.37 AB	28.47 A	29.77 A	1319 A	847 A	25.63 AB	15.03 A
T4R	8.05 A	8.13 A	0.41 C	0.33 A	1.55 B	1.41 AB	29.33 A	29.77 A	926 B	825 A	18.10 B	13.20 A
T5A	7.99 A	8.08 A	0.48 ABC	0.4 1A	1.99 A	1.34 AB	26.07 A	25.17 A	941 B	822 A	15.93 B	14.23 A
T5R	7.98 A	8.05 A	0.44 BC	0.36 A	1.69 AB	1.35 AB	26.27 A	31.60 A	988 B	834 A	23.47 AB	19.37 A
LSD	0.13	0.174	0.10	0.11	0.35	0.20	10.59	9.29	211	131.4	9.73	10.77

T0: control,T1 :2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.

Cont. Table (14): The effect of different treatments on soil properties at harvesting stage.

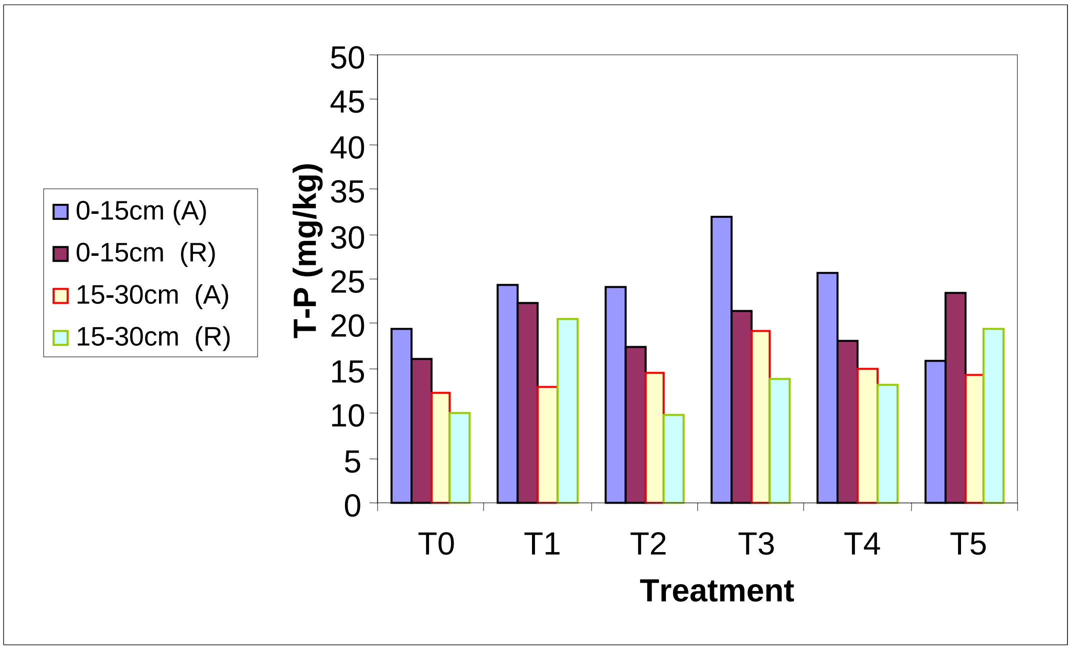
Treatment	NH_4 (mg/kg)		NO3 (mg/kg)		K (mg/kg)		Na (mg/kg)		Mg (mg/kg)		B (mg/kg)	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
T0A	2.27 B	2.24 BC	15.15 BC	8.67 BCDE	803 A	599 A	136 B	138 A	1113 A	1072AB	2.54 ABC	2.59 A
T0R	2.61 AB	2.51 BC	15.15 BC	5.48 BCDE	734 A	582 A	140 B	147 A	1195 A	1218 A	2.14 BC	2.16 A
T1A	2.28 B	2.17 C	17.61 BC	14.17 ABCD	737 A	627 A	154 AB	149 A	1225 A	1017AB	2.27 BC	2.45 A
T1R	2.46 B	2.52 ABC	10.92 C	6.02 BCDE	733 A	581 A	148 AB	143 A	704 A	1223 A	2.27 BC	2.09 A
T2A	2.49 AB	2.49 ABC	30.30 ABC	17.36 ABC	765 A	630 A	171 A	168 A	1199 A	994 AB	2.39 ABC	2.40 A
T2R	2.40 B	2.30 BC	6.69 C	2.10 DE	625 A	516 A	158 AB	154 A	796 A	852 B	1.86 C	1.98 A
T3A	2.37 B	2.60 A	23.60 ABC	18.42 AB	748 A	650 A	139 B	134 A	1298 A	891 AB	2.66 AB	2.52 A
T3R	2.42 B	2.38 ABC	10.92 C	1.04 E	844 A	765 A	144 B	160 A	793 A	866 B	1.98 BC	2.02 A
T4A	2.73 AB	2.50 ABC	45.09 A	26.21 A	705 A	594 A	146 AB	150 A	763 A	786 B	3.09 A	2.32 A
T4R	2.41 B	2.52 ABC	9.51 C	5.12 CDE	680 A	662 A	139 B	145 A	799 A	876 B	2.60 ABC	2.30 A
T5A	3.25 A	2.50 ABC	39.10 AB	15.59 ABC	764 A	555 A	140 B	163 A	869 A	858 B	2.13 BC	1.94 A
T5R	2.42 B	2.27 BC	10.92 C	18.07 ABC	651 A	507 A	148 AB	147 A	711 A	895 AB	2.49 ABC	2.43 A
LSD	0.78	0.40	24.73	12.97	273	260	27	46	699	334	0.79	0.86

T0: control,T1 :2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (33):T-N of soil at harvesting stage.

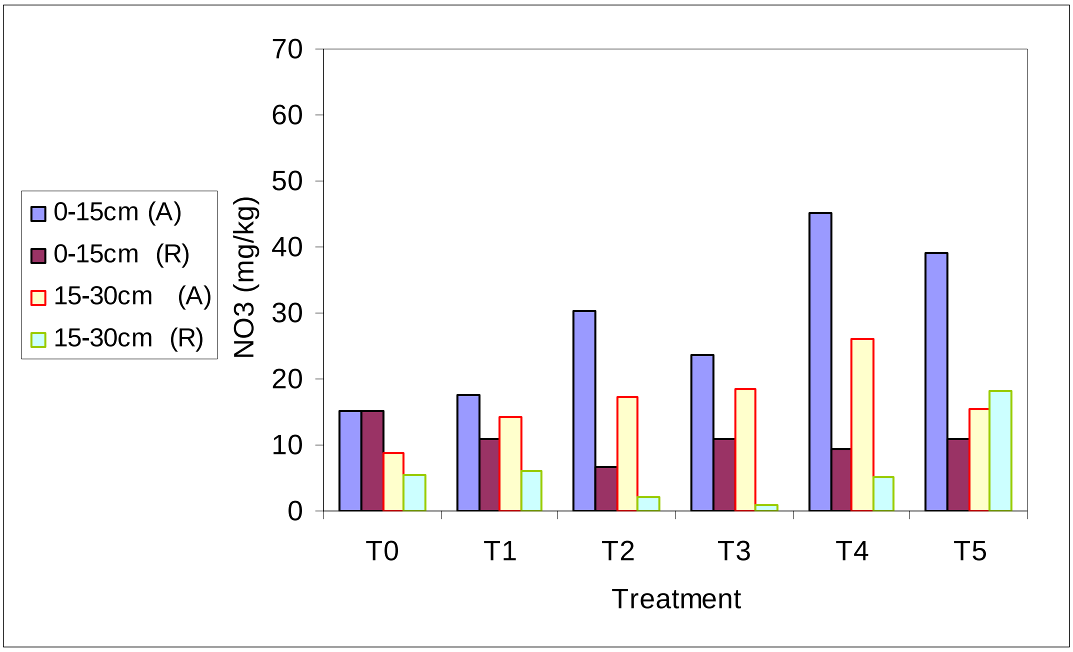


* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (34): T-P of soil at harvesting stage.

Ammonium concentration in soil at depth (0-15cm) was significantly increased at accumulative chemical fertilizer treatment than residual case. For other treatments, there were no significant differences between accumulative and residual treatments or between different treatments and control case. At depth (15-30cm), significant increase in NH_4 concentration was obtained at accumulative (6 ton/ha) bio-solids application rate. There were no significant differences between accumulative and residual cases for various treatments.

Nitrate concentration of soil at depth (0-15cm) was significantly increased with bio-solids application at accumulative (8 ton/ha) bio-solids application rate and also at accumulative chemical fertilizer treatment. Nitrate concentration for other treatments were not significantly different from control. Nitrate concentration at depth (15-30cm) was significantly increased with bio-solids application at accumulative (8 ,6 , 4 ton/ha) bio-solids treatments, other treatments were not significantly different from control treatment. Figure (35) shows nitrate concentration in soil at harvesting stage.



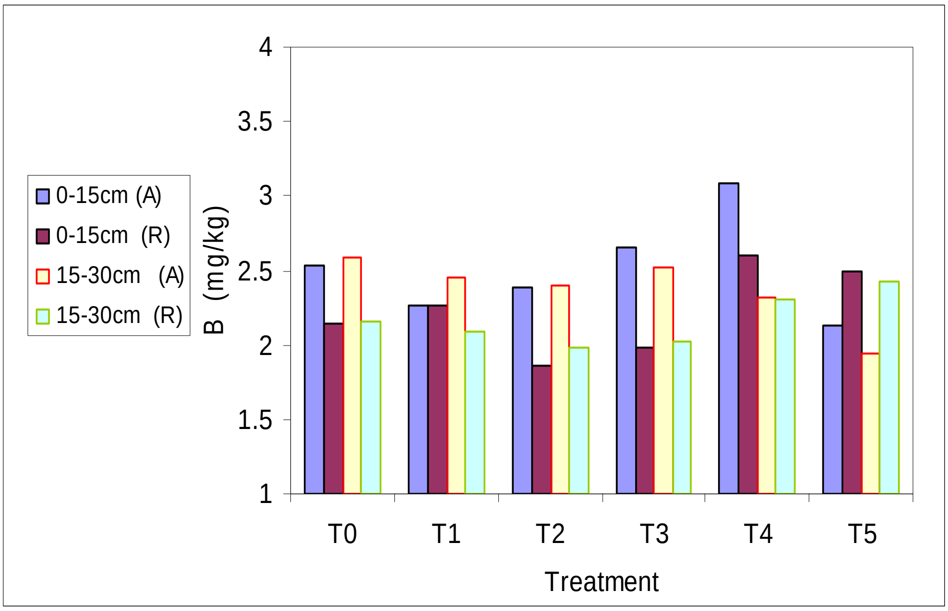
* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (35): Nitrate of soil at harvesting stage.

Potassium concentration of soil was not significantly different among the different treatments. Sodium concentration at depth (0-15cm) was slightly affected with bio-solids application, the maximum concentration was obtained at accumulative (4 ton/ha) bio-solids treatment while the minimum concentration was obtained at control treatment. There were no significant differences between accumulative and residual treatments. At depth (15-30cm), there were no significant differences obtained among different treatments.

Magnesium concentration in soil at depth (0-15cm) was not significantly different among different treatments. At depth (15-30cm), magnesium concentration was slightly affected with bio-solids application. There were no significant differences between accumulative and residual treatments or between different treatments and control treatment.

Boron concentration in soil was slightly affected with bio-solids application at depth (0-15cm); the maximum concentration was obtained at accumulative (8 ton/ha) bio-solids treatment. There were no significant differences between accumulative and residual treatments or between different treatments and control treatment. At depth (15-30 cm), no significant difference was obtaine in boron concentration among different treatments. Boron concentration in soil at harvesting stage is shown in Figure (36).



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (36): Boron concentration of soil at harvesting stage.

Effect of different treatments on micro-nutrients concentration in soil

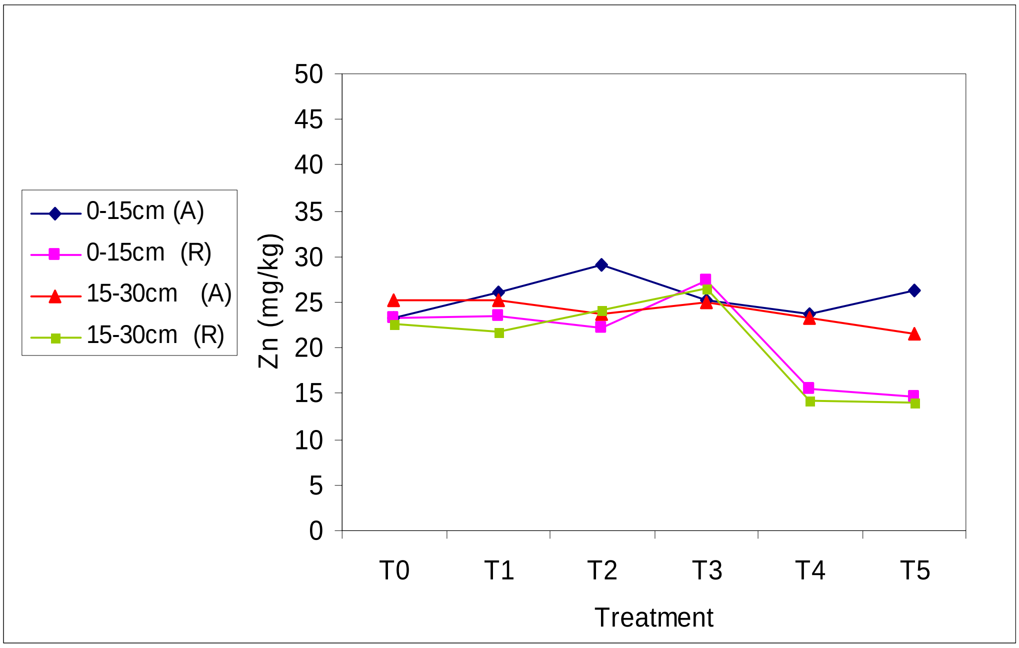
Table (15) shows micro-nutrients concentrations in soil at depths (0-15cm) and (15-30cm). Results show that selenium concentration in soil at depth (0-15cm) was slightly affected with bio-solids application; there were no significant differences between accumulative and residual treatments or between different treatments and control treatment with the exception of residual fertilizer treatment. At depth (15-30cm), no significant differences were found between different treatments.

Cadmium concentration in soil at depth (0-15cm) was not significantly affected with bio-solids application. At depth (15-30cm), slight effects in cadmium concentration were found, the maximum concentration was obtained at accumulative (8 ton/ha) bio-solids treatment, which was not significantly different from control treatment. No significant differences were found between accumulative and residual treatments with the exception of accumulative and residual (8 ton/ha) and all treatments were insignificantly different from control treatment.

Lead concentration in soil at depth (0-15cm) was not significantly affected with bio-solids application. At the (15-30cm) depth, slight effects were found; however there were no significant differences between accumulative and residual treatments or between different treatments and control treatment.

Mercury concentration at depth (0-15cm) was slightly affected with bio-solids application; the maximum concentration was obtained at accumulative (6 ton/ha) bio-solids application rate, which was not significantly different from control. There were no significant differences between accumulative and residual treatments or between different treatments and control treatment. At depth (15-30cm), no significant differences were obtained between different treatments.

Zinc concentration in soil was slightly affected with bio-solids application at the two studied depths. There were no significant differences between different treatments and control treatment. Figure(37) shows zinc concentration of soil at harvesting stage.



* T0= control, T1= 2 ton/ha, T2= 4 ton/ha, T3= 6 ton/ha, T4= 8 ton/ha, T5= chemical fertilizer, A= accumulative & R= residual

Figure (37): Zinc concentration of soil at harvesting stage.

For other micronutrients concentrations (As, Cr, Cu, Mo and Ni), no significant differences were found between different treatments at the two depths.

Table (15): The effect of different treatments on micro-nutrients in soil at harvesting stage.

T	Se (mg/kg)		Cd(mg/kg)		Pb(mg/kg)		As(mg/kg)		Cr(mg/kg)		Cu(mg/kg)		Hg(mg/kg)		Mo(mg/kg)		Ni(mg/kg)		Zn(mg/kg)	
	0-15	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**
T0A	0.65 B	0.66 A	0.77 A	0.81 AB	21.76 A	27.37 A	3.84 A	3.84 A	21.53 A	22.12 A	7.26 A	7.77 A	0.73 AB	0.76 A	2.01 A	2.37 A	15.71 A	16.12 A	23.35 ABC	25.22 A
T0R	0.65 B	0.70 A	0.91 A	0.73 B	18.87 A	21.89 AB	3.00 A	3.62 A	18.29 A	19.63 A	7.48 A	7.62 A	0.76 AB	0.58 A	2.22 A	2.87 A	14.77 A	15.97 A	23.21 ABC	22.59 AB
T1A	0.67 AB	0.76 A	0.82 A	0.76 B	22.74 A	22.91 AB	2.70 A	3.49 A	19.65 A	22.38 A	8.14 A	7.71 A	0.55 B	0.72 A	1.95 A	2.95 A	15.74 A	12.26 A	26.07 ABC	25.17 A
T1R	0.65 B	0.73 A	0.86 A	0.86 AB	23.33 A	23.30 AB	4.03 A	4.22 A	21.35 A	22.71 A	7.65 A	8.07 A	0.60 AB	0.67 A	2.55 A	2.50 A	15.71 A	16.46 A	23.40 ABC	21.87 AB
T2A	0.65 B	0.66 A	0.86 A	0.69 B	21.71 A	24.20 AB	3.31 A	3.39 A	21.35 A	22.21 A	7.74 A	7.70 A	0.61 AB	0.67 A	2.09 A	2.64 A	15.63 A	16.20 A	29.16 ABC	23.64 AB
T2R	0.65 B	0.66 A	0.69 A	1.08 AB	20.80 A	22.27 AB	3.72 A	3.85 A	20.52 A	21.42 A	7.44 A	8.22 A	0.59 AB	0.72 A	3.38 A	3.45 A	15.22 A	16.03 A	22.16 ABC	24.06 AB
T3A	0.65 B	0.71 A	1.08 A	0.72 B	24.99 A	23.24 AB	4.43 A	4.18 A	22.58 A	22.67 A	7.87 A	8.11 A	1.20 A	0.34 A	2.69 A	2.65 A	16.39 A	16.76 A	25.28 ABC	25.05 A
T3R	0.73 AB	0.68 A	0.8 A	0.99 AB	23.13 A	13.59 B	4.23 A	2.76 A	21.73 A	14.31 A	8.35 A	7.53 A	0.71 AB	0.88 A	3.44 A	2.69 A	16.40 A	15.75 A	27.47 AB	26.54 A
T4A	0.72 AB	0.72 A	1.00 A	2.07 A	22.98 A	18.00 AB	3.33 A	3.05 A	16.89 A	17.04 A	6.13 A	6.38 A	0.78 AB	0.73 A	2.44 A	2.37 A	14.55 A	16.19 A	23.80 ABC	23.19 AB
T4R	0.74 AB	0.76 A	0.72 A	0.80 B	16.46 A	16.87 AB	4.06 A	4.01 A	18.74 A	17.99 A	6.56 A	6.23 A	0.81 AB	0.67 A	2.29 A	1.78 A	14.30 A	14.05 A	15.58 BC	14.23 B
T5A	0.67 AB	0.69 A	0.76 A	0.92 AB	21.08 A	21.77 AB	3.23 A	3.20 A	19.39 A	19.63 A	8.74 A	7.17 A	0.55 B	0.63 A	2.37 A	2.34 A	15.45 A	15.33 A	26.19 ABC	21.47 AB
T5R	0.81 A	0.78 A	0.93 A	0.89 AB	15.94 A	16.19 AB	3.75 A	3.96 A	17.45 A	17.61 A	6.55 A	6.42 A	0.65 AB	0.78 A	1.94 A	2.36 A	13.37 A	13.66 A	14.74 C	14.08 B
LSD	0.14	0.14	7.17	1.26	11.76	12.40	1.88	2.11	7.98	10.55	2.80	2.48	0.61	0.45	1.76	1.91	3.86	4.94	12.56	10.62

T0: control,T1 :2 ton/ha,T2: 4 ton/ha,T3: 6 ton/ha,T4: 8 ton/ha,T5: chemical fertilizer, A: accumulative and R: residual.
LSD: Least Significant Difference at 0.05 probability.

6 Conclusions of Second Season Application

- Accumulative bio-solids application during the second season insignificantly increased barley biological weight and straw yield compared to residual treatments. On the other hand, grain yield was significantly decreased for accumulative treatments compared to residuals.
- The accumulative addition of bio-solids significantly increased nitrogen and protein content in grain with a percentage of (11%) both, and phosphorus content in straw with a percentage of (29%). Nitrate, calcium, magnesium, sodium, potassium and boron concentrations increased insignificantly in both straw and grain.
- Accumulative bio-solids application significantly increased copper concentration in straw at different treatments, the reason for that is the high copper concentration in the applied bio-solids. In addition, chromium concentration in grain significantly increased at (4 ton/ha) rate. Similar results were also obtained by Johnson and Vancey (1998) and Mullen *et al* (2005). Accumulative application of chemical fertilizer resulted in an increase in copper concentration in grain, may be the reason for that is the impurity of the chemical fertilizer. No other significant impacts on micro-nutrients levels in barley were observed.
- Accumulative bio-solids application did not result in significant change of pH, organic matter content, EC and CEC in the upper layer (0-15 cm) and sub-layer (15-30 cm).
- Nitrogen levels increased significantly with a percentage of (42%) at accumulative (8 ton/ha) bio-solids application rate for the soil upper layer. Phosphorus concentration increased significantly with a percentage of (49%) in the soil upper layer for the application rate (6 ton/ha). Ammonium concentration in the upper layer increased with accumulative application of chemical fertilizer. Nitrate concentration in the upper layer was significantly increased for accumulative (8ton/ha) bio-solids application rate over residual. No significant differences in macro-nutrients concentrations were found for soil sub-layer at different treatments.
- Micro-nutrients concentration in soil at the upper layer and sub-layer were insignificantly affected with accumulative bio-solids and chemical fertilizer application.
- Microbiological analysis showed no detected limits for all treated samples.

7 QUALITY ASSURANCE SCHEMES

ERC laboratories are certified according to ISO 9001:2000 system, and most of the analysis are accredited locally according to ISO 17025 system. Furthermore, most of the analysis at the microbiology laboratory in addition to some chemical analyses are internationally accredited by the United Kingdom Accreditation Services UKAS.

The quality policy of ERC ensures maintaining well performed applied research and specialized technical consultations, testing and services based on approved national and international standards, achieving customer satisfaction and ensuring compliance with ISO 9000 and ISO 17025 requirements. ERC seeks to upgrade and complement its technical capabilities and services to meet the increasing needs of clients and is committed to upgrade technical and administrative staff capabilities.

A quality assurance program had been implemented and applied, throughout the project execution period, in a proper way to ensure laboratory data quality. This includes the following:

- External calibration was conducted for balances, incubators, ovens, furnaces and refrigerators.
- Internal calibration was carried out periodically for tests and instruments using externally supplied certified standard solutions. Linearity verification was done as well.
- Purchasing of high quality laboratory grade reagents and chemicals, including Class A volumetric glassware. Grade A reagent water is prepared in the laboratories using nano-pure water producing apparatus.
- Trueness was checked for by analyzing matrix certified reference materials (CRMs); results are shown in Table (16). Precision was checked by occasionally running duplicate analyses. Digestion and distillation efficiencies were always checked for by using in-house spiked standards.
- Newly hired analysts are not allowed to produce data unless they demonstrate their ability to perform analyses by comparing their parallel analysis to experienced analysts.
- Sampling, sample preservation, storage, sample preparation, and analyses were performed in accordance with well recognized analysis standards such as the “Standard Methods for the Examination of Water & Wastewater” and “Soil Science Society of America SSSA”.
- Raw data and final results were always inspected and checked.
- Internal audits on quality assurance/quality control programs were conducted on regular bases.

Table (16): Analysis results of certified reference materials at ERC labs.

Parameter	Unit	Results obtained at ERC labs.	Certified Value	Performance acceptable limits
Soil Sample				
T.kj.N	%	0.110	0.109	0.106 - 0.112
Organic Matter	%	1.90	1.82	1.73 – 1.91
Available Phosphorus	mg/Kg	23	21.2	17.6 - 24.8
Plant Sample				
T-N	%	2.94	3.03	2.88-3.18
T-P	%	0.207	0.216	0.206-0.220
Ca	mg/kg	4.99	5.05	4.96-5.14
K	mg/kg	2.74	2.70	2.65-2.74
Cu	mg/kg	5.56	4.7	4.56-4.84
Ni	mg/kg	1.32	1.59	1.583-1.597

* Plant CRM :1573a Tomato leaves ,NIST.
*Soil CRM: NCS DC85101(GBW07412),China National Analysis Center for Iron and Steel.

8 MODIFICATION OF THE JORDANIAN STANDARD NO.(JS:1145/1996)

With regard to the modification of the Jordanian Standard No. (JS: 1145/1996), the *ad hoc* committee prepared and approved a modified version of the standard taking into consideration the major findings of this research project. This proposed standard was presented and discussed throughout several meetings with the National Domestic Wastewater Management (NDWM) Committee (a committee headed by the secretary general of WAJ/MWI and formulated from decision makers at different governmental and research institutions). The proposed standard was slightly modified and approved by NDWM committee. In April, 2006 WAJ sent the standard to the Jordan Institution of Standards and Metrology JISM for final approval and dissemination. JISM formulated a committee from different governmental and research institutions in order to get the approval on the standard, the formulated committee signed on the approval of the standard and it's now in it's way of display in the formal press.

9 SCIENTIFIC WORKSHOP

A workshop about risk assessment of bio-solids used for agricultural purposes was held at Royal Scientific Society (RSS) during the period Dec. 13-15,2005. Within the activities of the workshop, researchers from University of Arizona (Dr. Chuck Gerba, Dr. Chris Choi and Dr. Janick Artiola) presented the experiment of Arizona state in reusing bio-solids after treatment for planting some crops such as cotton, barley and others. Many representatives from governmental and non-governmental institutions and universities participated in the workshop in addition to representatives of international donor institutions.

A scientific seminar was also held for successive two days after the end of the workshop to discuss modifying the Jordanian standard for bio-solids reuse in agriculture (No. 1145/1996); many institutions participated in the process including Jordan Institution for Standards and Metrology (JISM), Ministry of Water and Irrigation (MWI) and Ministry of Environment in addition to some governmental universities.

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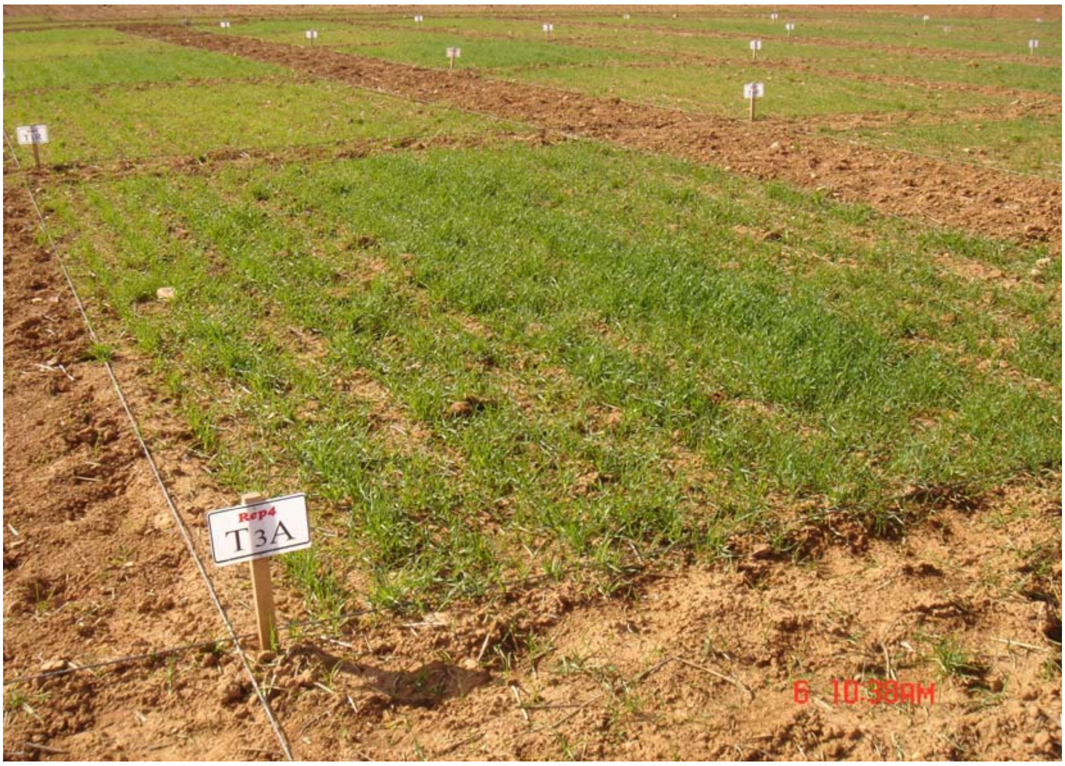
Annexes

Annex (1)

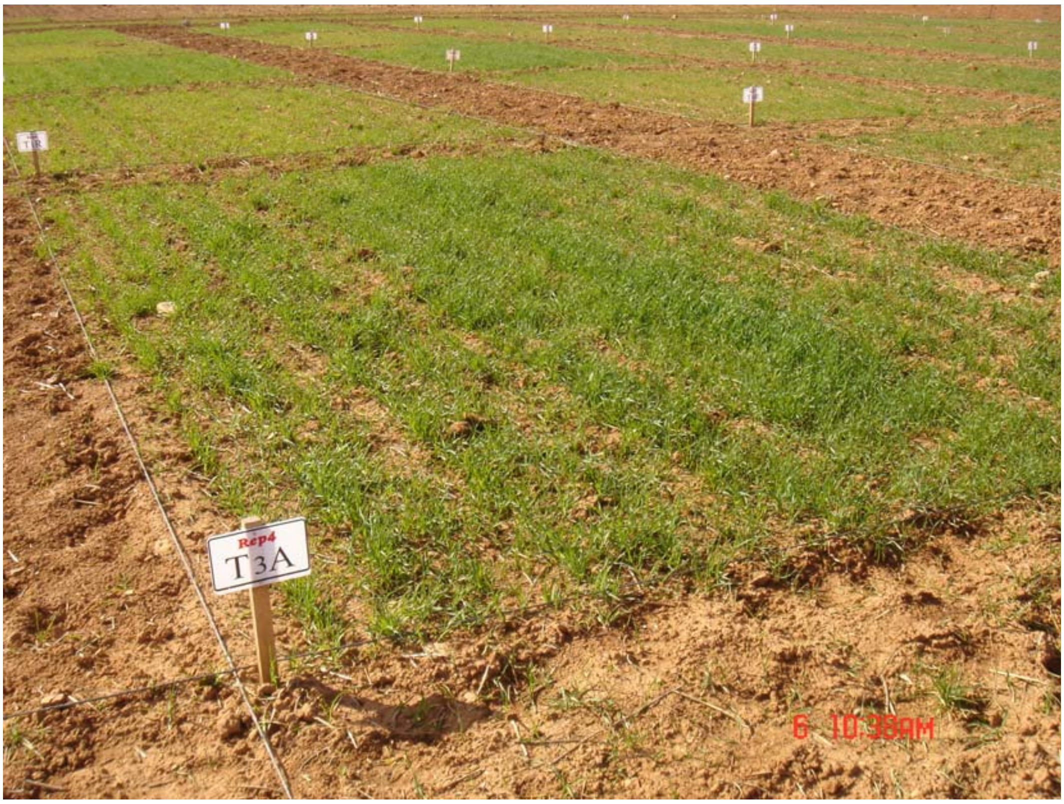
Photos Taken for the Site at Different Stages

P1: Experimental site at the beginning of the second season.

P2: Plant sampling at tillering stage.



P3: Comparison between T0R and T3A plots.



P4: Comparison between T3R and T3A plots.

P5: Harvesting stage.

P6: Plant Analysis.

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
CEC	saturated with sodium acetate then extraction by ammonium acetate, flame photometer	ICARDA
Exchangeable and Soluble Ca and Mg	extraction with ammonium acetate, EDTA titration	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

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Table (2.2): Methods of plant analysis.

Test	Method of analysis	Reference
Nitrogen & Protein	Total Kjeldahl Nitrogen	ICARDA
Total Phosphorus	Dry ashing, colorimetric	ICARDA
Sodium & Potassium	Dry ashing, flame photometer	ICARDA
Calcium & Magnesium	Dry ashing, EDTA titration	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