Faculty of Graduate Studies

Master Program in Water and Environmental Sciences

The Impact of Sewage Sludge as a Soil Amendment to Saline Soil on the Growth and Development of Processing Tomatoes

تأثير إضافة الحماة إلى التربة المالحة على نمو وتطور نباتات البندورة التصنيعية

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Alhamdulillah at the beginning

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Father who wait me, you are always, my pillar, my joy and my guiding light. brothers and sister are highly appreciated and acknowledged.

My love wife suha , I extend my thanks and express my gratitude for your support .
My sons Motaz , Mohamed ,Qasem .

To who , I lost the dearest thing in my life , burned itself to light the way to us,......
My Mother, I'll miss you terrible! I wished you to be here today ...!

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Abstract :

Salinization of greenhouse soils in Palestine, in particular in the Jericho district, is a serious problem facing protected agriculture, mainly due to the excessive use of chemical fertilizers and due also to the inferior quality of irrigation water.

The possibility of using sewage sludge, a byproduct of municipal wastewater treatment processes, as a soil amendment to alleviate salinity stress of saline soils is highly attractive. Sewage sludge is rich in organic compounds and plant nutrients, and it can be considered as a valuable source of N, P, and K that are essential plant nutrients. Accordingly, the reuse of sludge may substitute substantially part of the needed plant nutrients. However, there is a need for ecotoxicological evaluation of the usage of sludge. It is well known that urban sludge is usually non-toxic, whereas sludge from industrial zone might be very toxic. Accordingly, the aim of this study is to assess the impact of amending alkaline calcareous saline soil with sewage sludge on the growth and development of processing tomatoes.

Sewage sludge was obtained from "Al-Bireh Wastewater Treatment Plant" after processing according to the standard procedure, whereas saline soil was obtained from Azubidat area . The processed sludge was incorporated at various ratios and the experimental material was the processing tomato.

Results show that amending sludge to saline soil at ratios of 1: 10 and 1: 6 had positive effects in improving soil conditions and also on plant growth and development . The most important parameter is the consumption quality of tomatoes, which include heavy metals content of fruits, and the degree of contamination of the fruits with pathogens. The main finding is that no contamination was observed, and the concentration of heavy metals is lower than levels considered toxic for humans.

In conclusion, addition of sewage sludge to saline soil alleviates partially the negative impact of salinity stress, in particular at mix ratios of 1: 10 and 1: 6 (sludge: saline soil).

الملخص:

ملوحة التربة داخل الدفيئات البلاستيكية هي مشكلة تواجه القطاع الزراعي في فلسطين وخاصة في منطقة أريحا حيث يرجع سبب تملح التربة إلى الاستخدام المفرط للأسمدة الكيماوية وكذلك استخدام مياه ري ذات نوعية رديئة تحتوي على نسبة أملاح عالية

هناك إمكانية لاستخدام حمأة الصرف الصحي (وهي التي تنتج عن محطات معالجة المياه العادمة) كمحسن للتربة حيث تحتوي هذه الحمأة على العناصر الغذائية الضرورية لنمو وتطور النباتات وكذلك تحتوي على المركبات العضوية . توجد هناك حاجة لدراسة وتقييم كفاءة إعادة استخدام الحمأة , حيث أن الحمأة الناتجة من محطات معالجة المياه الناتجة عن الاستخدام المنزلي غير ضارة بالنباتات , أما الحمأة الناتجة عن محطات معالجة مياه الصرف الصناعي فهي تعتبر سامه للنباتات ومن الممكن ان تسبب أضرار كبيرة للنبات

الهدف الأساسي لهذه الدراسة هو تقييم تأثير الحماة على التربة المالحة من خلال دراسة نمو وتطور نباتات البندورة التي تستخدم للتصنيع .

تم الحصول على الحمأة من محطة معالجة المياه العادمة في مدينة البيرة وتم التعامل مع الحمأة حسب المواصفات والتعليمات الفلسطينية , بينما تم احضار التربة المالحة من غور الأردن تحديدا من منطقة الزبيدات وتم خلط التربة المالحة بالحمأه بنسب مختلفة وتم زراعة نباتات البندورة التصنيعية .

النتائج أظهرت ان هناك تحسن بشكل ايجابي على نمو وتطور نباتات البندورة في المعاملتين 1:10 و 1:6 (تربة : حمأة) وكان العامل المهم هو عدم وجود تاثير سلبي على جودة الثمار حيث نتائج فحوصات العناصر الثقيلة والفحص البيولوجي أظهرت قابلية هذه الثمار للاستهلاك البشري .

من خلال هذه الدراسة نستنتج أن إضافة حمأة الصرف الصحي للتربة المالحة تعمل على تخفيف الأضرار الناتجة عن الإجهاد الملحي وخاصة عند خلطها بنسبة 1:10 ونسبة 1:6 (تربة :حمأة) .

Abbreviations:

Acronym	**Definition**
$\mu S.cm^{-1}$	Micro-Siemens per Centimeter
CD	Concentration in the digest (mg N/I)
CEC	Cation Exchange Capacity
$dS.m^{-1}$	decisiemens per meter
EC	Electrical Conductivity
FAO	Food and Agriculture Organization
FC	Faecal Coliform
MOA	Ministry of Agriculture
OM	Organic Matter
PPM	Parts per Million
PWA	Palestinian Water Authority
TC	Total Coliform
TDS	Total Dissolved Salts
TN	Total Nitrogen
TOC	Total Organic Carbon
TpW	Tap Water
TWW	Treated Wastewater
WHO	World Health Organization
WW	Wastewater
WWTP	Wastewater Treatment Plant
$Mmohs.cm^{-1}$	millimohs per Centimeter

Chapter one

Introduction :

Soil salinity is a growing threat to agriculture in several countries in the World. At the same time the need to produce more food is increasing to meet the needs for growing population. Accordingly, the need to increase cultivated areas necessitates the expansion into marginal areas, which usually suffer from salinity problem (Qadir and Oster, 2004).

Saline soil is the soil that contains high amounts of Na. which will affect negatively plant growth and development. There are two main sets of reasons for the spread of salinity in the soil; the natural factors and anthropogenic factors. The natural factors include weathering of minerals from rocks that contain naturally large proportion of salts. In terrains where water accumulates in low places leading to poor drainage and low permeability soil water, high evaporation rates lead to the accumulation of salts on the surface of the soil. As for anthropogenic factors, the interventions by humans represent the major factors affecting the degree of salinity of soils. In this respect, poor management in irrigation projects in terms of not giving the proper amounts of irrigation and the unequal distribution of water in the field, problems of irrigation network and the high level of the ground water, and use of saline water in irrigation dictate the degree of the negative impact of salinity in plant growth and development. These ions influence directly the electrical condition, which is considered as the most reliable measurement of salinity level. In this respect, scientists consider soils with electrical conductivity of 4 $dS.m^{-1}$ or more as saline soils. The following classification is common among horticulturists.

Soil salinity (EC in $ds.m^{-1}$)	Impact on plants
Less than 2	No damage occurs to the plants
From 2 – 4	Sensitive plants show injiuries
4 – 8	Most plants will be affected
8 – 16	Only resistant plants can survive
More than 16	Only plants which are highly resistant to salts can survvie

Table 1: the impact of soil salinity (as EC unit; $ds.m^{-1}$) on plants

In Palestine, soil salinity is one of the main obstacles facing the agriculture sector, especially in the region of Jordan valley, which is considered as the main agricultural region in Palestine. The major factors that led to salinization problem in Jordan valley are the evapotranspiration rates, excessive use of fertilizers, and inferior quality of irrigation water. To overcome this problem, even partially, there are a set of agricultural practices that are adopted by farmers, which include increasing the amount of irrigation water and the use of organic fertilizer to improve soil physical properties. In this sense, the use of sewage sludge can improve the properties of the soil, since sludge is rich in organic matter and nutrients that are needed by plant. Moreover, using sewage sludge in agriculture is one of the best methods for final disposal of these wastes, rather than burning or placing such wastes in landfills. Samara (2008) reported that biosolids are available in liquid or solid forms and the type of processes used to reduce pathogens determines the type of material available. Utilizable sludge forms are: (1) Liquid sludge that have total solids content of less than 8 percent, (2) Dewatered sludge that is a form of liquid sludge, but was subjected to partial drying resulting in a solid content of around 25%, (3) Biosolids compost that is a relatively stable humus-like material, and (4) Advanced alkaline stabilized sludge that results from treatment process using lime or hydrated lime.

However, the usage of sewage sludge is risky since sewerage system transports industrial wastes and storm-water runoff from roads and other paved areas, which are frequently discharged into sewers. Accordingly biosolids may contain, in addition to organic wastes, traces of many pollutants (e.g. heavy metals) that can be toxic to plants, animals and even to humans (Dean & Sues, 1985). On the other side, some heavy metals in sewage sludge are micro-nutrient that are essential for plant growth (e.g. copper, and zinc) and subsequently beneficial to crops. However, like most elements, excess amounts of these elements may present problems for plant growth. In this respect, heavy metals that are essential for plant or animal nutrition have a very limited availability range, and become toxic to plants, animals and humans at define concentrations.

In addition to that, another hazard is related to pathogens, since sludge may contain pathogenic bacteria, viruses and protozoa along with other parasites.

Concerning the agricultural use of sewage sludge, its usage is possible, if certain precautions are handled well to eliminate possible contamination for both soil and plant products. In this sense, tomato plants are an excellent model to test the influence of amending sewage sludge to saline soils. Tomato (*Lycopersicon esculentum* Mill.) is produced globally, locally it is very important, and its fruits are processed easily for various industrial products. Taking into account that tomato requires high temperatures, abundance of sunlight, high levels of nutrients, and high amount of water, it is of value to test the possibility of sewage sludge as a resource rather than a waste.

Accordingly, the aim of this study is to assess the impact of amending alkaline calcareous saline soil with sludge on the growth and development of processing tomatoes. Studies dealing with amending saline soils with sludge are very rare in Palestine, and using sewage sludge may alleviate, although partially, salinity stress.

Chapter two :Literature Review

2.1 Definitions

Soil salinity is usually described and characterized in terms of concentration of dissolved salts, in particular sodium (Na) ions. However, the level of salinity is given usually in electrical conductivity (EC) values, and scientists consider soils as having salinity problem, even mild, when the EC is more than 4 $ds.m^{-1}$. When the EC value is from 4 to 8, the soil is called slightly saline, whereas values from 8 to 16 represent moderate salinity level. Soils with EC values of 16 or more are severly saline, and considered as very hostile for most plants (Munns, 2005). It is worth to mention that soil slinization is one of the major problem that adversely affects agricultural activities worldwide, in particular in arid regions. In Palestine, particullay in Jordan valley, salinization is becoming a serious problem facing agriculture, and the major cause for this problem is the excesive use of chemical fertilizers. Taking into account the prevailing low rainfall (<450 mm per year) and the high evaporation rates (> 900 mm per year) (Schofield et al, 2001), the severity of salinization may reach lethal levels for cultivated plants. In this respect, it is also well noticed that in other regions, like most Mediterranean countries, the widespread use of water that contains relatively high levels of dissolved salts is the rule, mainly due to the scarcity of other water resources. This led to the accumulation of salts in the top soil, and imposes abiotic stress on cultivated plants (Pereira et al 2002). It is well known that severe abioctic stress resulted in lower productivity, although quality may be improved by mild to moderate stresses (Wang et al 2003).

In addition to the inferior quality of water, the irrigation method and discharge rates have also great impact on the salinization of soil (Hanson & May 2004). In this respect, it is well documented that flood irrigation transfers salts generally below the root zone (Letey et al 2011), whereas drip irrigation leads to the accumulation of salts on the topsoil (Assouline et al 2006). Furthermore, the fertigation techniques may have also a negative impact, in particular when water of inferior quality is used (Ju et al 2007).

Concerning other causes of salinity, which are mostly human-induced processes, the excessive usage of synthetic chemical fertilizers is the major one (Darwisha et al 2005).

2.2 Impacts of salinity

The direct impact of salinity is related to high osmotic potential of the soil solution, which has a negative influence on plants that grow in soils with high salts concentration (Dodd & Donovan 1999), since plants have to use more energy to absorb water. Further, plants under extreme salinity conditions may be unable to absorb water and wilt, even when the surrounding soil is saturated with water molecules (Marcelis & Hooijdonk, 1999). On the other hand, plants grow in saline soils generally have higher levels of Na and Cl ions, which negatively affect the absorption of mineral nutrients, especially N, Ca, and K (Ghoulam et al., 2002). In this respect, accumulation of salts in the soil, especially Na^+, affects adversely the physical conditions of the soil with respect to aggregate formation, water infiltration, and water-holding capacity (Lax et al., 1994). That results in stunted growth of plants, small leaves, marginal necrosis of leaves, and even fruit distortions (Cicek, & Çakirlar 2002).

Despite the above-mentioned responses of plants to salinity stress, plants differ widely in their tolerance to abiotic stresses (Ashraf , & Harris *2004*). In this respect, sensitive plant species start to suffer in soils with slight salinity levels, whereas other plant species may tolerate much higher salinity levels. The ability of plant species to tolerate abiotic stresses, including salinity stress, relies mainly on the ability of plant tissue to synthesize compatible solutes (Chen et al, 2007), to detoxify excessive Na^+ ions (Munns and Tester, 2008), and/or to compartmentalize these ions in vacuoles (Yeo, 1998).

Although salinity stress imposes severe damage to plants, the control of soil salinity is not difficult, giving that proper cultural practices can be implemented. In this respect, good drainage is of outmost importance to avoid soil salinization, whether natural soil salinity or man-made (Christen & Skehan, 2001). Coupled with good drainage, the disruption of hardpans by deep tillage is crucial in soils that have high salt levels (Huffmana et al 2000). Another technique is the application of excessive amounts of low-salt water to the soil (Wana et al, 2007), although such technique is hard to adopt in Palestine, due to severe shortage in water resources. Other useful cultural practices include also the selection of tolerant plant species and varieties, and amending soils with natural or synthetic organic materials.

Referring back to soil salinity, the scope of this problem is noticed in Jordan valley and Gaza strip (Dudeen, 2000) due to very low precipitation rates and high temperatures during summer. While other areas of the West Bank are affected by salinity, mainly due to improper cultural practices carried out by farmers, in particular the excessive application of synthetic fertilizers, but, fortunately, in these areas the rainfall is working to reduce the impact of soil salinization. However, the salinization problem may still remain in these areas inside the greenhouses (Dudeen, 2000).

2.3 Controlling soil salinity

As mentioned above, managing saline soils is possible, and maintaining soil drainage and providing good irrigation management are the key factors that can help to control soil salinity. In addition, selection of tolerant plant species and genotypes is a good alternative (Yamaguchi & Blumwald, 2005), although that has to be coupled with proper cultural practices that aim to prevent any increase in salinity level.

However, for long-term salinity control, the proper management practices are the most appropriate approach. It is important here to take into consideration that there are separate cultural practices that can have a profound effect upon germination, early seedling growth and ultimately on yield of crop, even under saline conditions (Qadir & Oster 2004). Actually, the short-term cultural practices, which help in counteracting salinity stress, became more important, as the quality of irrigation water deteriorates rapidly in Palestine. Such practices include land smoothing for better water distribution, better timing of irrigations to prevent crusting and water stress, better placement of seeds to avoid zones that are likely to be highly salinized, and proper selection of starting materials (Ben-Gal, 2008). In addition, the proper selection of chemical fertilizers, and addition of manures and compost, which are coupled with better scheduling of fertilizer application, will greatly reduce the severity of salinity stress (Lakhdar et al, 2009). Additionally, another cultural practice received more attention in the last years, and this technique is soil amendment with natural or synthetic compounds to alleviate salinity stress (Walker & Bernal, 2008; Chartzoulaki et al, 2010).

2.4 Soil amendments

In this respect, soil amendments are materials added to soils to improve its physical and chemical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure. The final goal is to provide better environment for root growth and development, and subsequently plant productivity and quality of plant products (Shokohifard et al, 1989). For soil amendments there are two types, namely organic and inorganic. Organic amendments such as sphagnum peat, wood chips, grass clippings, straw, manure, compost, solid waste, wood ashes and sludge arose from living materials, or their dead bodies, or their secretions (Bulluck, et al 2002). On the other hand, inorganic amendments such as vermiculite, perlite, gravel, and sand are extracted, mined, or synthesized by human beings. Soil amendments usually alter both physical and chemical properties of affected soils, and the major effects are on soil structure, water holding capacity, and cation exchange capacity (Walker and Bernal, 2008).

In addition to that, organic amendments increase soil organic matter content and may increase soil aeration, water infiltration, soil contents of several plant nutrients (Ismail 2013). Bulluck et al (2002) reported that compost additions to the soil add organic compounds, which bind soil particles and improve its structure, porosity, water retention, and oxygen supply. Furthermore, organic material tends to increase microbial activities of soils, which may lead in lowering soil pH through production of organic acids during decomposition (Chorum & Regasamy, 1997). In this respect, organic matter provides an energy source for the microbial population in the soil, which can promote stable aggregation of soil particles (Brown et al, 2000). Taking into account that our soils are alkaline, the addition of amendments might be more advantageous than amending acidic soils due to acidic nature of these amendments. Another important issue is the leaching of sodium. Tejada et al. (2006) reported steady decline in the sodium content, which was accompanied by a marked increase in plant cover and soil porosity, upon the addition of compost and poultry manure to saline-sodic soil. In another experiment, El-Shakweer et al. (1998) found that the application of compost and poultry manure led to an accelerated sodium leaching that resulted in a reduced electrical conductivity of soil. Researchers also reported improvements in water-holding capacity and soil aggregation stability.

Accordingly, the benefits of amending soils with organic materials for remediation of salt-affected soils are great to handle these soils (Suriyan & Chalermpol, 2011).

Moreover, Walker and Bernal (2008) found that the addition of composted olive mill waste and poultry manure to saline soils increased the cation exchange capacity of treated soils.

2.5 Municipal sewage sludge amendment

Another new option for soil amendments to alleviate soil salinity is the possibility of using sewage sludge, which is a byproduct of municipal wastewater treatment processes. Sewage sludge is rich in organic matter and plant nutrients, mainly of N, P, and K, which are considered as essential plant nutrients (Shahnaz et al., 2011), and can substitute large part of the needed plant nutrients. However, the usage of sewage sludge is risky, and there is a need for ecotoxicological evaluation, before its application to saline soils. In this respect, it is well known that urban sludge is usually non-toxic, whereas sludge from industrial zones may be very toxic (Da-Luz et al., 2009). In this sense, Liang et al (2003) reported that adding sludge to the soil is the most preferred method of disposal. In that, it is also the most economical way to reduce the amount of sewage sludge. Moreover, this approach provides an opportunity to recycle plant nutrients and organic material for the benefit of soils and crop production (Laturnus and Arnold, 2007, Singh & Agrawal, 2008). At the same time, the method of sludge disposal has to be acceptable in terms of human and environmental safety, social acceptance, and the cost. These restrictions are highly dependent on the economical situation, the local culture and traditions, and specific topography and land availability (EPA, 1998)

2.6 Sludge as a valuable source of nutrients

Referring back to the above mentioned beneficial effects of sludge, as soil amendment, various studies addressed this approach and showed clearly the potential positive effects. It is of value to mention here that such positive effects vary depending on properties and characteristics of the added sludge (Mitchell et al., 1978; Gupta et al., 1977). Tejada et al (2006) also reported that the soil microbial biomass and some enzymatic activities (e.g. urease, alkaline phosphatase and β-glucosidase, which are linked to C, N, P and S cycles) increased upon the addition of sludge.

Another important aspect is the soil pH, which may reach very high values in Palestine (Dudeen2001). Silviera and Sommers (1977) and Tsadilas et al. (1995) reported that amending municipal sewage sludge to soil led to higher soil pH values, which may compromise its usage in certain districts in Palestine. However, other researchers reported significant decreases in soil pH in sludge-amended soils (Epstein et al., 1976; LaHann, 1976). Changes in soil pH is coupled highly to the calcium carbonate content of sludge, and mostly also to the acid production capacity during the decomposition of sludge (Silviera & Sommers 1977).

Another important issue in using sludge as soil amendment is the capacity of soils to adsorb cations. It is worth to mention here that increasing the cation exchange capacity (CEC) of soils results in additional binding sites that may retain more essential plant nutrients in the rooting zone (Soon, 1981; Kladivko & Nelson, 1979; Mitchell et al., 1978; Epstein et al , 1976). Taking into account that 50 % of the sludge content is actually organic material, it is easy to predict that such materials will improve the physical condition of soils, in particular the CEC, in addition to lower bulk density, better aggregate stability, better water holding capacity, and greater water infiltration (Khaleel et al., 1981; Kladivko & Nelson, 1979 ; Kelling et al., 1977; Epstein, 1975; Mitchell et al., 1978; Gupta et al., 1977). Furthermore, it is valuable to know that digested or secondary sludge has higher nutrient content than primary sludge, since most of volatile organic matter were given off as CH_4 or CO_2 during the digestion process (Hue and Ranjith ,1994**).**

Concerning the impact of amending sewage sludge to soils on plant growth and development, Morera et al. (2002) reported an increase in the dry weight of sunflower plants (*Helianthus annus* L.) grown in sludge amended soil. Moreover, the yield of maize and barley was reported to be enhanced as a result of sludge application (Hernandez et al., 1991). Mazen et al. (2010) found also that adding sludge to desert soil lowered soil pH and resulted in a better soil texture, although metals were accumulated at higher rates in root tissues. In this respect, Smith (1992) noticed that sewage sludge, which contains high levels of toxic metals, may limit their application to soils due to food chain contamination. Koenig et al. (1998) reported that incorporation of sludge produced yields similar to those obtained with inorganic fertilizer. They found also that sludge application to grass hay gave yields that were intermediate between nitrogen fertilizer applied at 16.7 kg/du and an untreated (unfertilized) control. With grass hay, sludge addition resulted in significantly higher levels of calcium, magnesium, phosphorus, iron, copper, manganese, and zinc compared to ammonium nitrate fertilizer. In another experiment, with dry beans (*Phaseolus vulgaris* L), Togay et al. (2008) found that grain yield increased significantly following applications of sludge; the increase is believed to be the result of minerals enrichment of alkaline fine textured soils, which are suitable for dry bean production and known to be deficient in nutrients for grain production. In Palestine, Samara (2008) reported also a positive impact on the growth and development of Egyption clover following the addition of stabilized sewage sludge to alkaline soil.

2.7 Negative impacts of sludge

Despite all positive effects of sewage sludge, which are mentioned above, it is of outmost importance to handle its amendment in soils with caution, in order to avoid possible negative effects of its addition. Sewage sludge may contain some potential hazardous compounds and elements (e.g. heavy metals) which should be assessed and avoided before any amending of sludge to soil. These hazardous substances may have a negative impact on humans and environment as well. It is well known that heavy metals are non-degradable and, above certain levels, are toxic to humans and environment, since these minerals will accumulate along the food chain (Dudka & Miller, 1999). In this respect, McBride et al. (2003) showed that adding sludge to soil increased Cd, Ni, Cu and Zn concentrations significantly in the edible portions of cultivated crops. Furthermore, Li et al. (2005) reported that the concentrations of Cd and Zn increased in alfalfa upon the addition of sludge. Consequently, addition of sewage sludge that is contaminated with heavy metals increased the likelihood of contamination of the environment (Selivanovskaya & Latypova, 2003; Singh & Agrawal., 2007). On the other hand, Ian et al. (2008) indicated that, in long-term, the land application of biosolids (class B) enhanced microbial activity and had no adverse effect on the soil microbial community, although certain bacteria (e.g. nitrogen fixing bacteria) may be more sensitive to sludge addition, and their population may decrease drastically with higher doses of sludge addition (Tripathi, 2011).

Chapter three

Materials and Methods:

3.1 Treatments and experimental design:

The experiment was conducted at BZU campus during the 2012 growing season .

Sewage sludge was obtained from Al-Bireh Wastewater Treatment Plant(AWWTP), wich is located east of Ramallah and treats approximately $1.25x10^6$ m ^{3} year-1 of raw municipal wastewater. The treatment at the WWTP consists of oxidation ditches, secondary clarifiers and a UV-disinfection system for pathogen removal. Saline soil was obtained from Jericho district (Alzubaidat area) and the EC of the soil is about 7.4 ds/m The soil consider alkaline soil with a pH of the soil is 7.6 . The processed sludge was incorporated at the following weight ratios (soil: sludge): 4:1, 6:1, and 10:1. In addition to that there were two controls, namely: untreated saline soil (negative control), and natural soil (positive control). Consequently, there were five treatments with six replicates per treatment. The experimental design was completely randomized design as in the following figure

.

CONTROL +	T1
CONTROL -	T2
01:10	T3
01:06	T4
01:04	T5

T5R6	T4R6		T5R5	T2R6		T5 R2	
T3R6	T3R5		T4R5	T2R5		T1R1	
T4R4	T5R4		T2R2	T1R4		T2R4	
T5R3	T4R3		T3R2	T3R4		T1R2	
T1R6	T2R3		T1R5	T3R1		T3R3	
T5R1	T4R1		T2R1	T4R2		T1R3	

Fig 1: Experimental design of the exesperiment.

The sludge: soil mix was filled in 40 liters pots, stabilized for four weeks, and fresh water was added at frequent intervals. Chemical fertilizers were added to the plants according to a standard program as recommended by Ministry of Agriculture. Processing tomato transplants were obtained from a certified nursery, planted on May 2012 in the green house , and irrigated, fertilized, and protected from pests following the recommendations of the Extension Service of the Ministry of Agriculture. The drip irrigation net work was installed, and each plant had one dripper with a discharge rate of 8 liters per hour. At the beginning, all plants were irrigated with freshwater three times per week until plants reached a height of 15 cm. After that, each plant was fertilized with 2 grams of ammonium sulphate fertilizer (21% N) until the cluster number per plant reached three. Following that, each plant was fertilized with 2 grams of 14: 7: 28 (N: P: K) complete fertilizer at weekly intervals, and the quantity of irrigated water was increased proportionally to plant growth and climatic conditions to reach 3.5 liters of water daily by the end of experiment

3.2 Parameters

To assess the impact of treatments, the following parameters were assessed:

3.2.1 Soil: sludge mix analysis:

Soil samples were collected at several times before planting to assess properties of the soil before planting, and by the end of growing season to assess the impact of treatments. Collected samples were analyzed for the following parameters: (a) pH, (b) EC of the soil solution, (c) Cation Exchange Capacity, (d) Total N, and (e) Minerals levels.

3.2.1.1 Total nitrogen determination

Nitrogen determinations were conducted using Kjeldahl method according to ICARDA Manual . 0.15-0.20 g of dried plant tissue or 0.45-0.5 g of soil were weighed out into a clean. dry digestion tube, and 3.5 ml of concentrated H_2SO_4 and one metal catalyst digestion tablet were added into each tube. Further, tubes were placed in a block digester and heated at 160°C for 20 minutes. After that, temperature was raised to

380°C for 240 minutes. Later, samples were removed and allowed to cool. Subsequently, tubes were filled to 50 ml (deionised water). and 7 ml of the digested solution were transferred to tubes, and ammonium concentrations were determined by Flame Ionization Analyzer (FIA). The nitrogen contents are calculated using the following formula:

ppm N = 50/Ws **X** CD (for soil sample)

% N = 50/Ws **X** CD/10,000 (for plant sample).

Ws = Weight of sample (g), and CD = concentration in the digest (mg N/L)

Throughout the analyses laboratory reagent blanks were also analyzed using the same procedure.

3.2.1.2 Cation exchange capacity (CEC)

CEC was assessed according to FAO procedure (2003). In brief, 5 g of soil were transferred to a 50-ml centrifuge tube, and 25 ml of 1.0M sodium acetate solution were added to each tube and shaked for 5 minutes. Following centrifugation at 2000 rpm for 5 minutes (or until the supernatant liquid is clear), the liquid phase was decanted. The extraction was repeated three more times. In addition to that, and to determine the sodium concentration by flame photometry, a series of Na standard solutions in the range of 0–10 meq/litre of Na were prepared, and the concentrations of unknown sample extracts were calculated accordingly. Concentrations were calculated according to the following formula :

Ammonium acetate extractable Na (exchangeable Na in meq./100 g soil)=

$$\frac{\text{Na conc.of extract in meq/litre (Y) 100}}{\text{Wt. of soil in g (5)}} \times \frac{\text{Vol. of extract in ml (100)}}{1000}$$

This displaced Na is actually a measure of the CEC of the soil. Therefore, the values of me Na/100 g soil is actually values for meq-exchangeable cations (Ca, Mg, Na and K)/100 g soil

3.2.1.3 Soil pH

The procedure for measuring soil pH was that of FAO (2003). The pH meter was calibrated using two buffer solutions, one with a neutral pH (7.0), and the second was selected to correspond to the range of pH in the soil.

For soil samples, 10.0 g of soil sample were placed into a 50-ml or 100-ml beaker, and 20 ml of *0.01M* $CaCl_2$ solution were added. The soil was allowed to absorb the $CaCl_2$ solution without stirring, and then samples were stirred for 10 seconds using a glass rod. Following that, the suspensions were stirred for 30 minutes, and pH values were recorded on the calibrated pH meter.

3.2.1.4 Electrical conductivity

The procedure of FAO (2003) for soil electrical conductivity measuring was adopted. 40 gm of soil were added to 80 ml distilled water, mixed well for one hour. After that the paste of soil was placed on filter paper, and the electrode was inserted to soil paste. The electrode was calibrated by washing it with distilled water, and rinsing with KCl solution. Following that the electrode was dipped in the KCl solution. The conductivity meter was adjusted to 1.412 $mS.cm^{-1}$ reading.

3.2.1.5 Soil organic matter

The procedure used is that of FAO, (2003). For each sample, 1.0 g of the prepared soil sample was placed in a 500-ml conical flask, and 10 ml of 0.1667M $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 containing Ag_2SO_4 were added. Solutions were mixed thoroughly, and reactions were allowed to complete in 30 minutes. After that, the reaction mixtures were diluted with 200 ml of water, 10 ml of H_3PO_4, 10 ml of NaF solution and 2 ml of diphenylamine indicator. Finally, the solution was titrated with 0.5 M $FeSO_4$ solution to a brilliant green colour. In addition to that, blanks, without soil samples, were simultaneously measured.

The percentage of organic C is given by the following formula:

$$\frac{10\,(S - T)\ \mathbf{X}\ 0.003}{s} \times \frac{100}{\text{Wt. of soil}}$$

Since 1 g of soil is used, this equation can be simplifies to:

$$\frac{3(S - T)}{S}$$

S = millilitres of $FeSO_4$ solution required for blank; T= millilitres of $FeSO_4$ solution required for soil sample; 0.003 = weight of C (1 000 ml 0.1667M $K_2Cr_2O_7$ = 3 g C. Thus, 1 ml 0.1667M $K_2Cr_2O_7$ = 0.003 g C).

Taking into account that organic C recovery is around 77 percent, the actual amount of organic C (Y) will be:

$$\frac{\text{Percent value of organic carbon obtained} \times 100}{77}$$

Or: percentage value of organic C × 1.3.

Percent OM = Y × 1.724 (organic matter contains 58 percent organic C, hence 100/58 = 1.724).

3.2.1.6 Potassium :

The amount of potassium in the soil was determined using flame photometer according to ICARDA manual (2001). 5 gm of air- dried soil were placed in a beaker, and 100 ml of distilled water were added. Samples were shaken well for one hour, filtered after that, and measured using flame photometer. Actual values were calculated according to following formula:

$$\text{Soluble K (ppm)} = \text{ppm k (from the curve)} \times \frac{A}{Wt}$$

Where: A: dilution factor, and Wt: weight of soil

3.2.1.7 Phosphorous (PO_4):

Phosphorus determination was conducted according to Olsen's method for alkali soils (Olsen et al., 1954). 2.5 g of soil were added to 50 ml of the bicarbonate extractant, and 1 g of activated carbon. Samples were shaken for 30 minutes, and filtered after that. Readings were taken using spectrophotometer at 660 nm, and calculated as:

$$P(kg/ha) = \frac{A}{1000000} \mathbf{X} \frac{50}{5} \mathbf{X} \frac{2000000}{5}$$

where:

weight of the soil taken = 5 g; volume of the extract = 50 ml; volume of the extract taken for estimation = 5 ml; amount of P observed in the sample on the standard curve = A (μg); weight of 1 ha of soil down to a depth of 22 cm is taken as 2 million kg.

3.2.1.8 Calcium and Magnesium:

Ca and Mg determinations were conducted according to FAO Manual (FAO 2008). Exchangeable cations are usually determined in a neutral normal ammonium acetate extract of soil. Extraction is carried out by shaking the soil–extractant mixture, followed by filtration or centrifugation. Calcium and Mg are determined either by the EDTA titration method or by using an AAS after the removal of ammonium acetate and OM .

3.2.2 Plant Growth and Development:

To assess the influence of treatments on plant growth and development, the following parameters were assessed:

3.2.2 .1 Plant height

The height of the plants at the end of the season was recorded

3.2.2 .2Date of flowering

Dates of flowering were identified for each treatment and these dates have the relationship between the plant's ability to grow in saline soils and between the plant's ability to reproduce. Readings were taken during the flowering season.

3.2.2 .3 Number of leaves, clusters, fruits and branches

The aim of this measure is to determine the direct effect of soil salinity on the treatment plants, where these measurements were taken at the end of the season.

3.2.2 .4 Weights of fruits

Fruits were measured following harvest , each alone . The aim here to study the effect of salinity on the size of the fruit

3.2.2.5 Salts injuries

These measurements are designed to find out burns resulting from the salinity on edges of the leaves, as well as yellowing of leaves caused by salinity. Furthermore, the failure rate in retaining flowers were assessed on a scale of 1-10; 1 indicates the worst and 10 refers to the natural rate.

3.2.2.6 Visual fruits appearance :

This measurement is selected to assess the external shape of fruits. The shape is an important quality criterion, and it is very important in the marketing of agricultural products. This measure was determined on a range of 1-10, where 1 is the worst and 10 is the best.

3.2.2 .7 Total area of leaves:

At the end of the season, samples were taken from lower and central leaves, and leaf area was determined using the leaf area meter (Li-COR) instrument.

3.2.2 .8 The Chlorophyll content:

The chlorophyll contents were determined according to Sadasivam and Manickam (1996). Leaves were washed with distilled water, frozen in liquid nitrogen, and stored at -20°C until the analysis. Shortly, chlorophyll a, b and total chlorophyll were extracted with 80% acetone and the absorbance of the extract was recorded at 645, 663 and 652 nm using a spectrophotometer. The chlorophyll content was determined as $mg.g^{-1}$ fresh weight.

3.2.3 Assessment of Fruit Samples:

Processing Tomatoes were harvested once at the end of the season. Samples were analyzed for the (1) Degree of contamination of the fruits with pathogens, and (2) Heavy metals content of fruits.

3.2.3.1 Contamination of the fruits with pathogens

Tests were conducted at the Birzeit University, Biology Department laboratories according to FDA Bacteriological Analytical Manual procedure (2001). For total and fecal coliforms, 50g of fresh unwashed pieces of tomato were added to 200 ml of 0.1% peptone water, and homogenized and shaken. Samples were diluted 1:5 (50:200). Further, serial dilutions were prepared, and after that 0.1ml from each dilluent was distributed into violet red bile lactose agar for total coliforms and 0.1ml into EMB agar for fecal coliforms. Petri dishes were incubated at 35 ºC (for total coliforms plates) and at 44.5 ºC (fecal coliforms) for 24 hours. Violet red colonies (as total coliforms) and green metallic (as fecal coliforms) were recorded. Degree of contamination was calculated according to the following formula:

Number of colonies **X** 5 **X** 10 (the dilution factor)

Where: 5: first dilution, and 10 to convert 0.1ml to 1ml

For *Salmonella*, samples were prepared by adding 25 g of the product to 225 ml peptone water. Mixtures were blended in a stomacher for one minute at medium speed, and incubated further for 16 hours at 37 **º**C. After that *Salmonella* was isolated by adding 10 ml of culture (peptone water) to 100 ml of selenite cystine medium, and incubated for 24 hours at 37 **º**C.

3.2.3.2 Heavy metals content of fruits:

Unwashed tomato fruits was dried on 60 ºC on oven, and grinded to fine powder using grinding mill. 0.25g of powder was digested using 3 ml of 1:1 nitric: perchloric acid on sand bath at 150 ºC using scintillation vials. Reagent blank and reference material were digested using the same conditions as the samples. The digestion is completed when the digestion solution is clear. Scintillation vials were filled with double distilled water to 25 ml. Further, cadmium, selenium, arsenic, zinc, copper, nickel, cobalt, manganese, chromium, vanadium, lead, and thallium trace metals were measured by ICP/MS (Agilent technologies 7500 series) found at Alquds University (Malassa et al 2013). Results were calculated in ppb and further to microgram per gram dry weight.

3.3 Statistical analysis

All obtained data were subjected to analysis of variance (ANOVA) at p L 0.05, and mean separation was conducted using Duncan's Multiple Range Test (DMRT) using costal software.

Chapter four

Results

Results obtained from the research is divided into three sections. The first section is the soil and the changes that have occurred to the soil as a result of sludge addition. For that, soil salinity, soil pH, soil cation exchange capacity, organic matter content, and levels of total nitrogen, calcium, magnesium, potassium, and phosphorus were measured, The second section of results is about impact of addition sludge to saline soil on the growth and development of plants. This impact was evaluated through measuring the length of plants, dates of flowering, number of leaves, branches, number of clusters and number of fruits, weights of fruits, as well as the damage caused by salinity in terms of yellowing of leaves, burns on the edges of the leaves and the failure to fruits set. Furthermore, the general appearance for plants and leaf area were evaluated. As for section three, the suitability of tomato fruits for human consumption was assessed. For that, levels of heavy metals and the extent of bacterial contamination of fruits were assessed.

4.1: Soil analysis

The aim of such analyses is to assess changes on soil to determine the sustainability of sludge amendment.

4.1.1 Electrical conductivity:

Taking into account that saline soils exert severe stress on plants, any reduction in electrical conductivity, which reflects salinity level, will be of value for Palestinian farmers. The electrical conductivity of the soil before starting the experiment was too high and after the addition of the sludge into the soil there were real significant differences between the soil which was not amended with sludge and between soil that was amended at 1:6, 1:10 ratios. However, there are no significant differences between other treatments. (Figure 1)

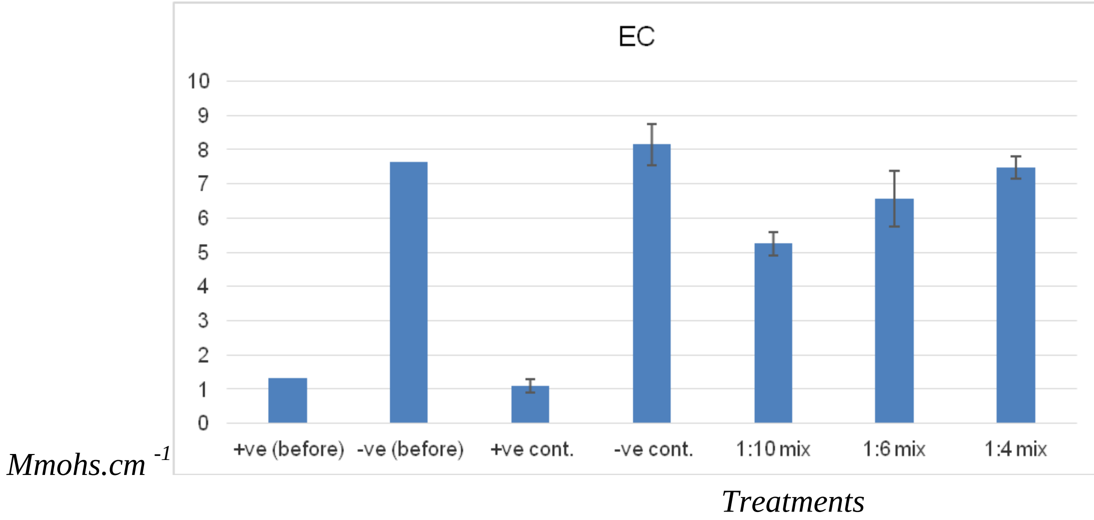


Fig1: Influence of sludge treatments on the electrical conductivity (EC) level(mmoh.cm^{-1} of the soil planted with tomato plants .

4.1.2 pH

pH reflects how acid or alkaline a substance is, and soil acidity or alkalinity (soil pH) is important, since it influences how easily plants can take up nutrients from the soil. Accordingly, this measurement was conducted to assess, although indirectly, the impact of sludge amendment on nutrients availability; nutrients are more available at the soil pH range of 6.5 – 7.

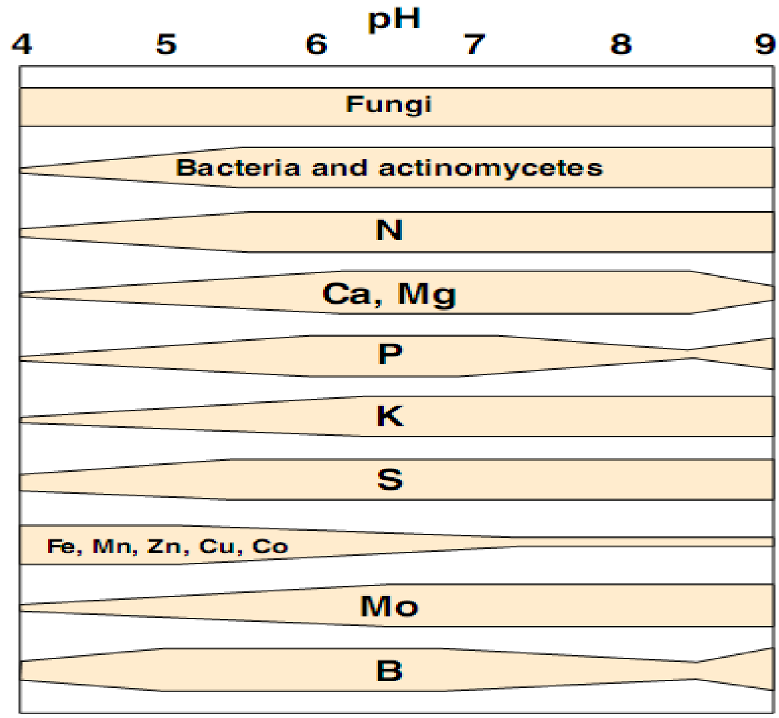


Fig 2: Chart of the effect of soil pH on nutrient availability, spark(2003)

Results (figure 3) show that amending soil with sludge had slight effects, and the major change occurred with control soil (+ve control) after cultivation of plants.

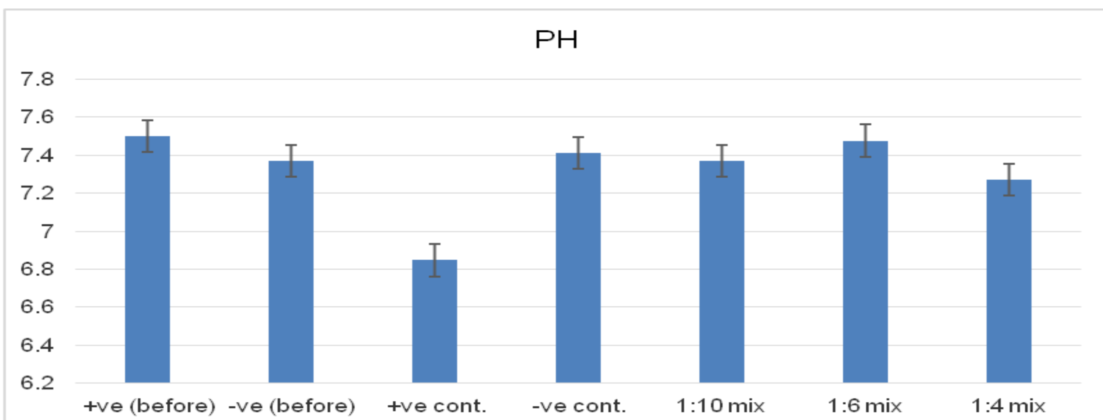


Fig 3: Influence of sludge treatments on pH level of the soil planted with tomato plants.

4.1.3 Calcium (Ca):

Calcium is one of the necessary plant nutrients, and its deficiency causes loss in production, and with tomatoes its deficiency may lead to blossom end rot. In the current experiment the amount of calcium was not affected by addition of sludge, and the only difference is between positive control and other treatments (figure 4).

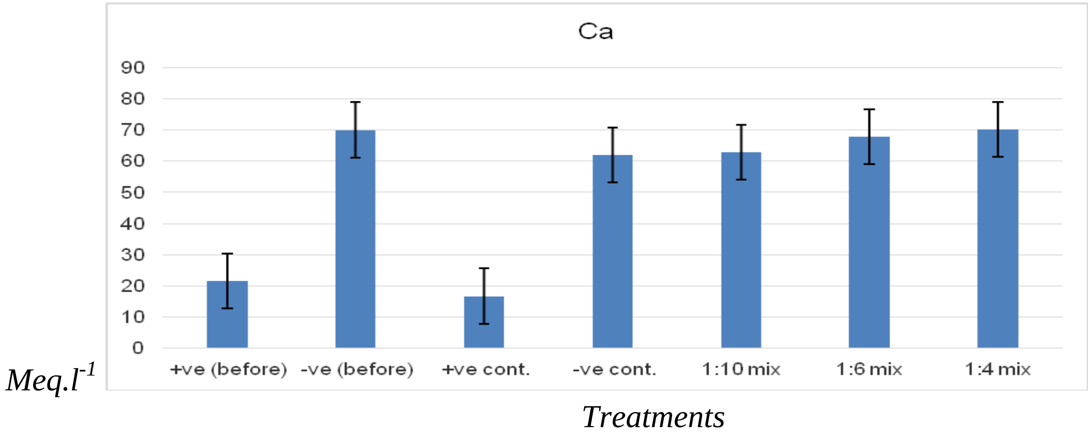


Fig 4: Influence of sludge treatments on the Ca level (Meq/l) of the soil planted with tomato plants

4.1.4 Magnesium (Mg):

Magnesium is also necessary for plant growth and development, as Mg is part of the chlorophyll molecule. Magnesium deficiency, like any deficiency, leads to reduction in yield, and it also leads to higher susceptibility of cultivated plants to diseases. Results (figure 5) showed that there were no significant differences between treatments as a result of amending sludge to saline soil.

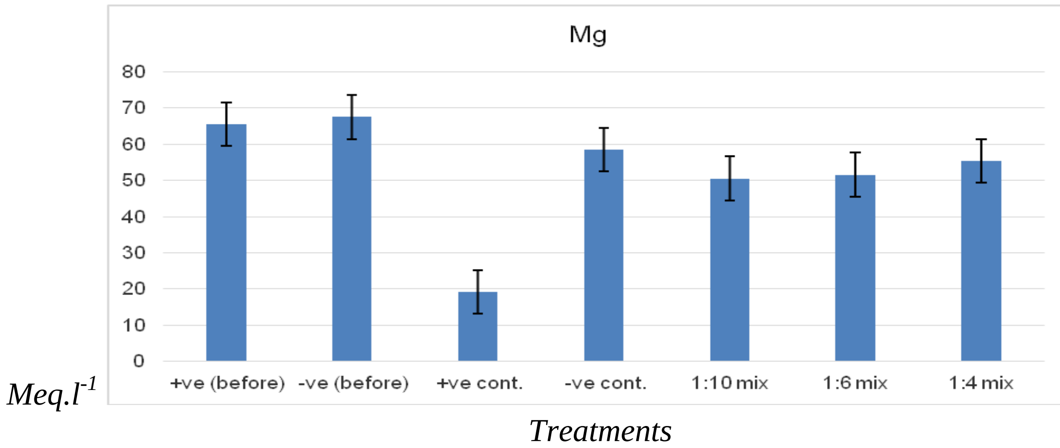


Fig 5: Influence of sludge treatments on the Mg level (Meq/l) of the soil planted with tomato plants

4.1.5 Cation exchange capacity (CEC)

Cation-exchange capacity is the degree to which a soil can adsorb and exchange cations, and this term is used to measure the fertility and nutrient retention capacity of soils. Results (figure 6) show that there were no significant differences between treatments upon sludge amendment.

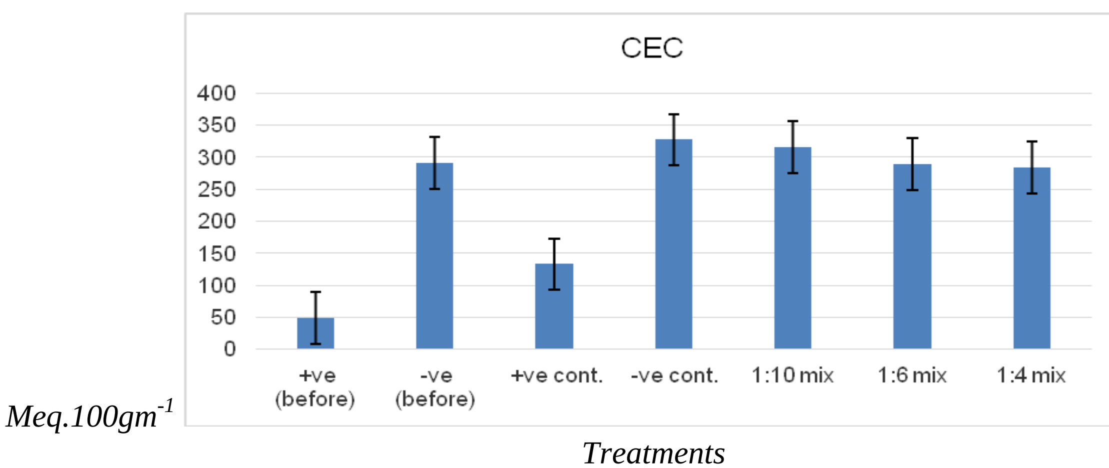


Fig 6: Influence of sludge treatments on the cation exchange capacity (CEC) level of the soil planted with tomato plants .

4.1.6 Potassium (K):

Potassium is another essential nutrient for plants, which is required in large quantities for the proper growth and production. In this sense, potassium is regarded as the "nutrient quality", as it affects the shape, size, color, and taste of plants. Results (figure 7) show that there were no significant differences in the amount of potassium in the soil following soil amendment with sludge.

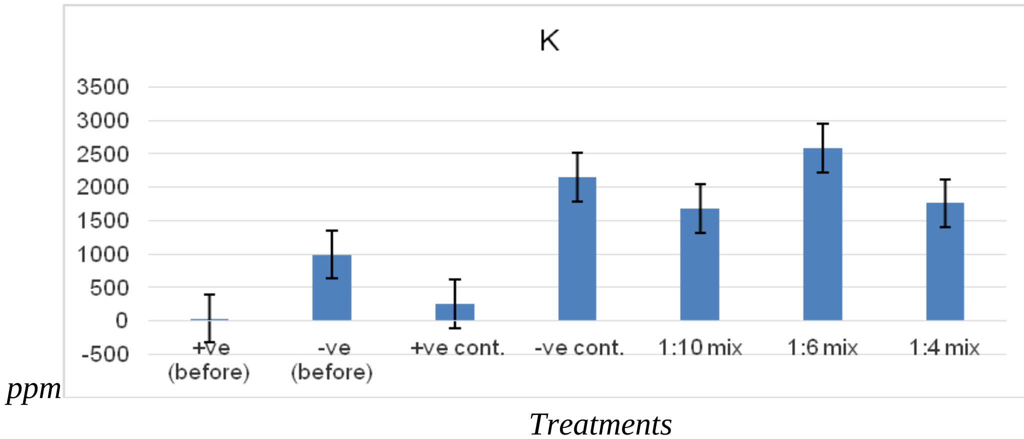


Fig 7: Influence of sludge treatments on the potassium (K) level of the soil planted with tomato plants(ppm).

4.1.7 Phosphorous (PO_4):

Phosphorus is an important nutrient for plants, in particular for the activity and spreading of the roots. Phosphorus is also a macronutrient that is needed in large quantities. Results (figure 8) clearly show that amending saline soil with sludge increased significantly the P-content of these soils, in particular with 1:10 treatment.

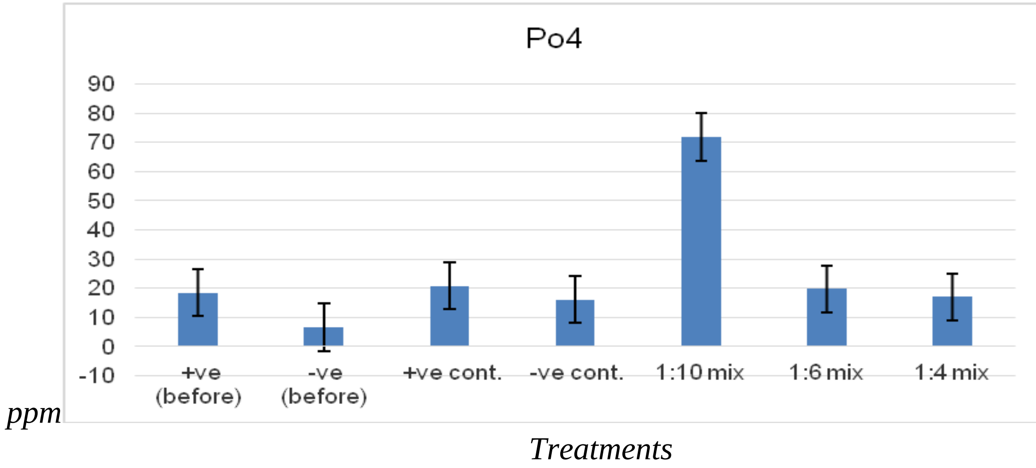


Fig 8: Influence of sludge treatments on the phosphorous (PO4) level of the soil planted with tomato plants (ppm).

4.1.8 Organic matter (O.M):

The presence of organic matter in the soil is important, and it improves substantially the physical and chemical properties of the soil. In this study, amending saline soil with sludge led to significant increase in OM content with 1:4 ratio (figure 9). However, it is clear that sludge-amended soils maintained during cultivation significantly their OM content compared to control soils .

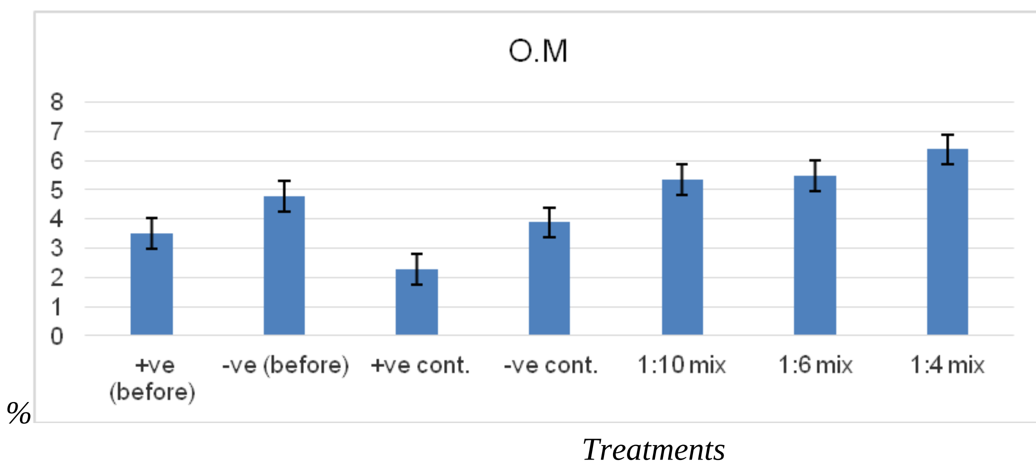


Fig 9: Influence of sludge treatments on the organic matter (O.M) content of the soil planted with tomatoes plants as %.

4.1.9 Total Nitrogen (T.N):

Nitrogen is the most needed nutrient for plants, mainly due to its existence in chlorophyll molecule; N-deficient plants exhibit yellowing of old leaves. As mentioned above, N was determined as total nitrogen, which reflects all forms of nitrogen that are available in the soil. In this study, results show that sludge amendments resulted in significantly higher levels of N in soil, in particular with 1:4 ratio (figure 10).

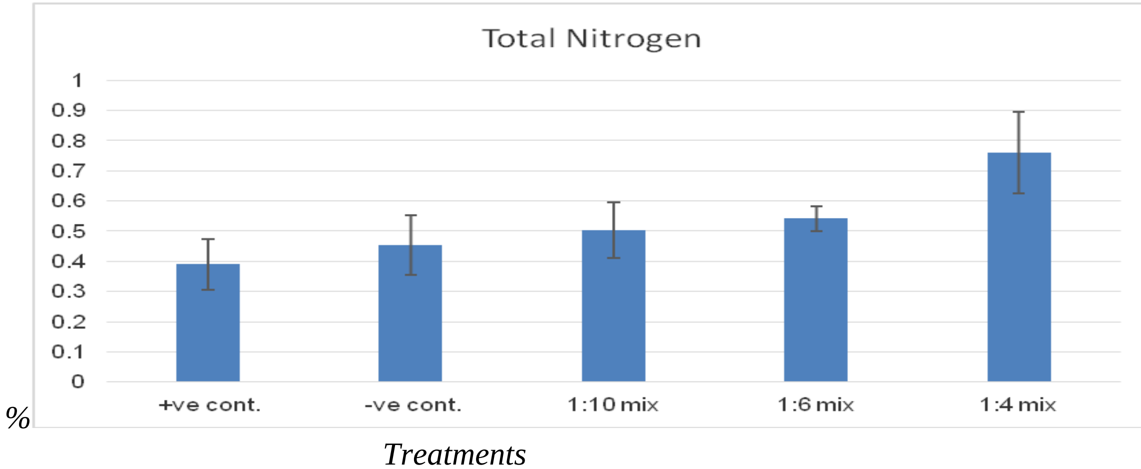
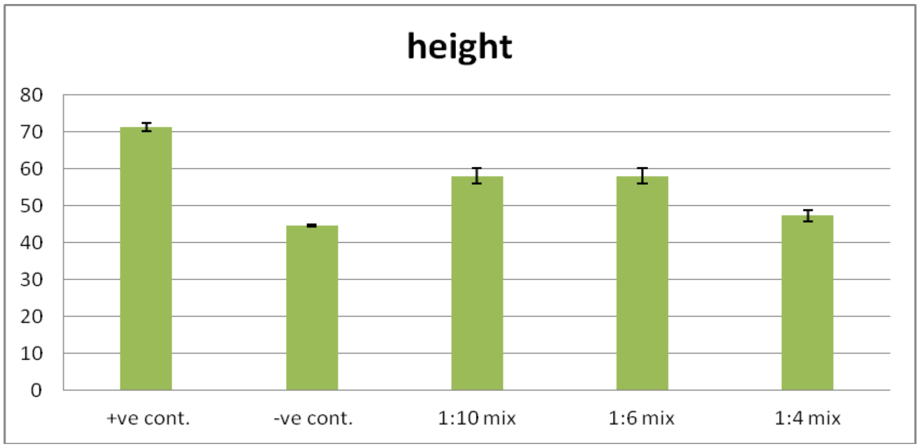


Fig 10: Influence of sludge treatments on the total nitrogen content % of the soil planted with tomato plants .

4.2 Vegetative growth

4.2.1 Plant height

Comparing the results of treated soils with natural soil clearly indicates the influence of adding sludge on saline soils. In respect to plant length (figure 11), it is obvious that addition of sludge to saline soil at ratios 1: 10 and a ratio 1: 6 were better than 1: 4 ratio. Soil with the highest salt content (-ve control) gave significantly the shortest plants. Accordingly, adding sludge to saline soil appears to alleviate, although partially, the negative impact of salinity stress on vegetative growth.



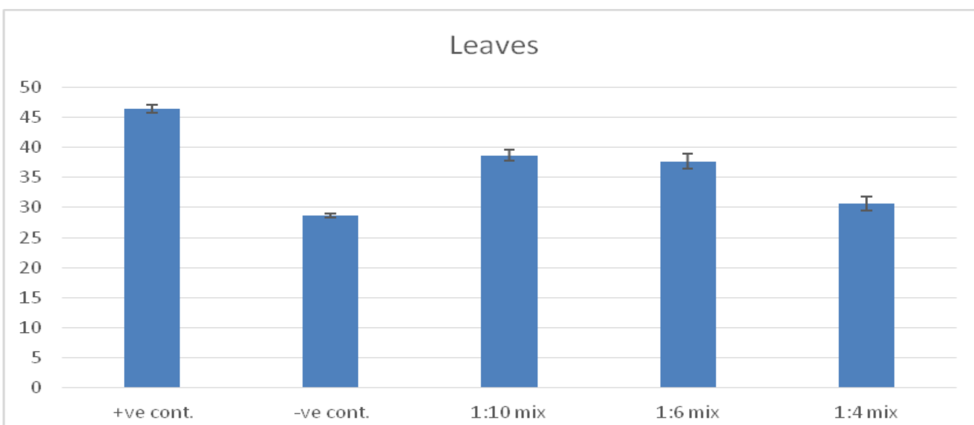
Cm. plant^{-1}

Treatments

Fig 11: Influence of sludge treatments on height of tomato plants .

4.2.2 Number of plant leaves

similarity to plant height parameter the saline soil gave significantly the lowest number of leaves, whereas the natural soil (+ve control) gave the highest number of leaves (figure12). Amending soil at higher ratios (1:10 and 1:6) were better than lower ratio (1:4).



Leaf.plant^{-1}

Treatments

Fig 12: Influence of sludge treatments on number of leaves of tomato plants .

4.2.3 Number of clusters

Figure 13 illustrates the effects of sludge addition to saline soil on number of floral clusters. Once again the addition ratios 1:10 and 1:6 alleviate significantly, although partially, the negative impact of salinity stress on the reproductive growth of tomato plants.

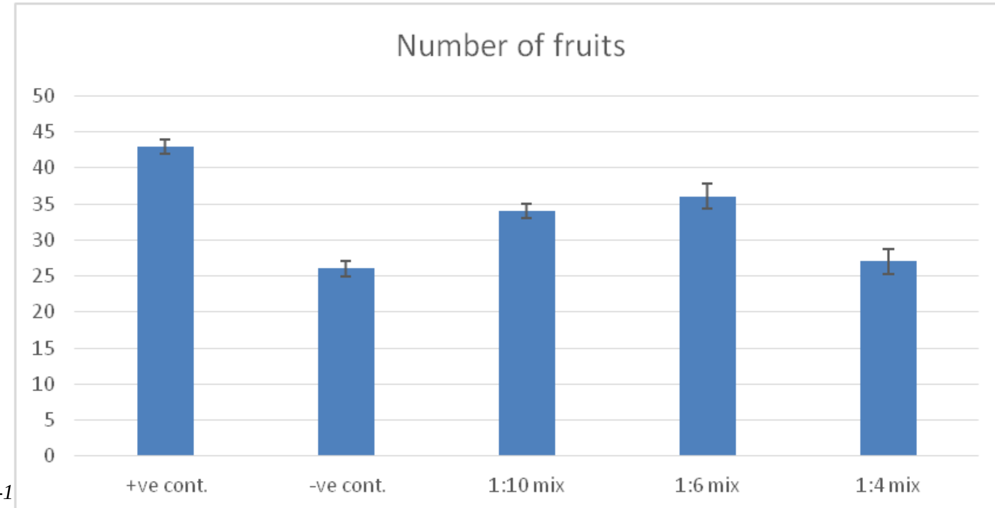
Cluster.plant^{-1}

Treatments

Fig 13: Influence of sludge treatments on number of clusters of tomato plants (cluster.plant^{-1}).

4.2.4 Number of fruits:

The positive impact of soil amendment with sludge on number of clusters resulted in an increased number of fruits by the amendment ratios of 1:10 and 1:6 compared to both saline (-ve control) and 1 : 4 ratio. But fruit number was significantly the highest in the + ve control. (figure 14).



Fruit.plant^{-1}

Treatments

Fig 14: Influence of sludge treatments on number of fruits of tomato plants (fruit/plant).

4.2.5 Number of branches:

Figure 15 shows the influence of treatments on number of branches. Plants grown under non-saline condition gave significantly the highest number of branches, whereas plants grown under saline condition (-ve control) had the lowest number of branches. Soil amendment with sludge at 1:10 and 1:6 ratios were superior over 1:4 ratio.

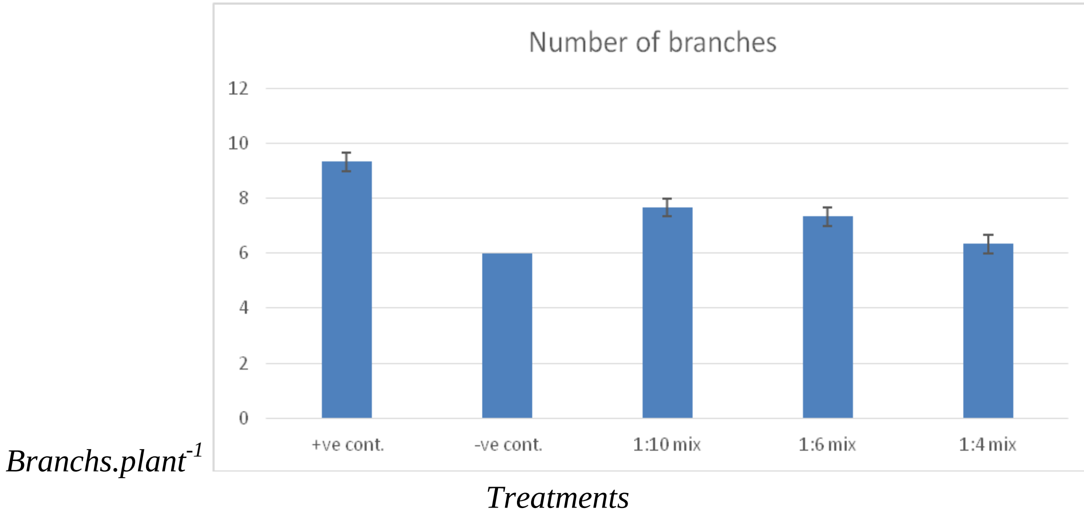


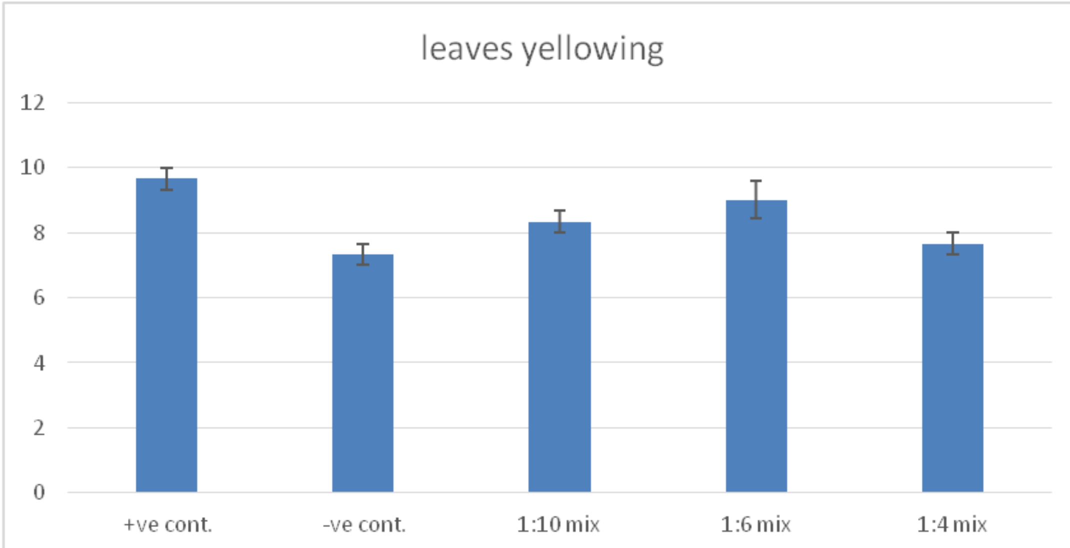
Fig 15: Influence of sludge treatments on branches of tomato plants(branch/plant).

4.2.6 Salt injuries

4.2.6.1 Leaves yellowing :

Yellowing of leaves caused by salinity is an indicator of the extent of damage to plants from soil salinity. Results (figure 16) show the extent of injury caused by salinity stress (-ve control), and that of treatments. Addition of sludge at ratios of 1: 10 and 1: 6 resulted in slightly less yellowing of leaves than the –ve control and 1:4 treatments; there were no significant differences between plants growing in a ratio 1:4 and the negative control (saline soil) treatments..

scale



Treatments

Fig 16: Influence of sludge treatments on yellowing of leaves of tomato plants.
(Scale: 1 more affected , 10 natural leaves)

4.2.6.2 Leaf edges Burns

One of the obvious salinity symptoms is the burning of edges of the leaves, which reflects the presence of high level of salts in soil. Such burns may extend and resulted in complete damage of leaves. Results (figure 17) show significant differences between plants from saline soil and plants from soils amended with sludge at a ratios 1: 10 , 1: 6 and 1: 4. It is interesting to indicate that there is no significant difference between 1: 10 and +ve control treatments.

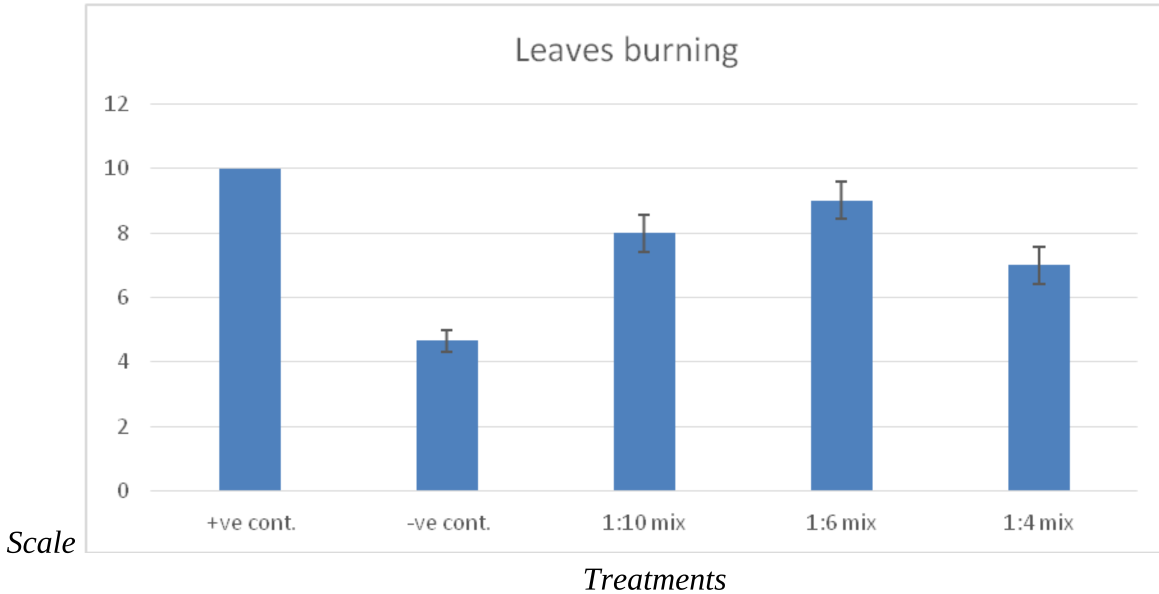


Fig 17: Influence of sludge treatments on marginal (edge) burning of leaves of tomato plants.

(Scale: 1 more affected , 10 natural leaves)

4.2.6.3 Fail of fruit set

Failed fruit set in plants is a direct consequence of salinity stress, since the stage of fruit sset is a very sensitive stage by flowering plants. Results (figure 18) clearly show that plants that were planted in saline soils amended with sludge by 1: 10 and 1: 6 had better fruits set than plants from 1: 4 amended soil. The greatest failure level was recorded by the –ve control treatment, followed by 1:4 treatment. It is worth to mention that all of these treatments had fruit set level ehat is lower than the control.

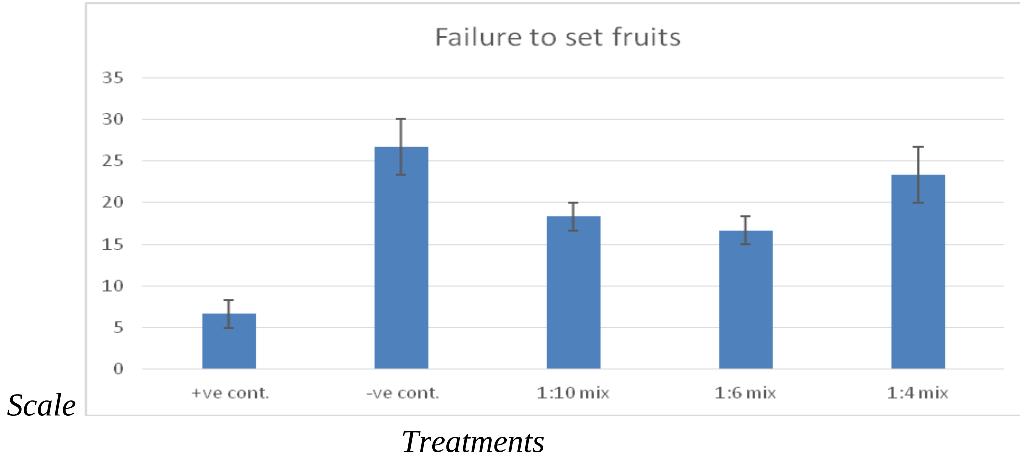


Fig 18: Influence of sludge treatments on failure to set fruits by tomato plants.

(Scale: 1 more affected , 10 natural)

4.2.6.4 Visual appearance

The influence of salinity on plant growth and development can be also assessed through the visual appearance of plants. Results show that plants not subjected to salinity stress (+ve control) looked healthier, whereas those subjected to severe salinity stress (-ve control) looked retarded in growth and development (figure 19). Amending soil with sludge at ratios 1:10 and 1:6 resulted significantly in better appearance than –ve control , although such an improvement was a partial one. Such an improvement was not recorded by 1:4 treatment.

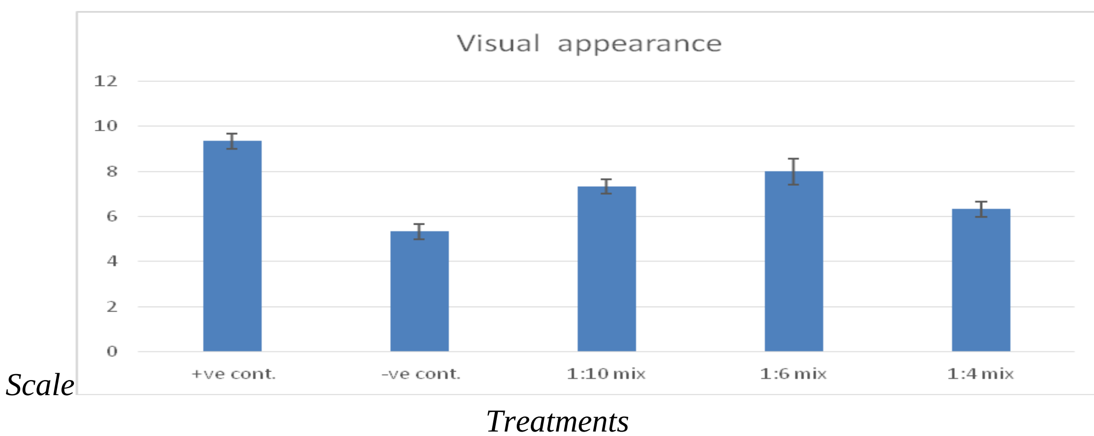


Fig 19: Influence of sludge treatments on the visual appearance of tomato plants.

(Scale: 1 more affected , 10 natural)

4.2.7 Fruit weight

The size of the fruit and thus the yield is the most important criteria to assess for studies related to presence of salts, since high soil salinity levels lead to smaller fruit size. Results (figure 20) show that fruits obtained from plants subjected to severe salt stress level (-ve control) were significantly smaller than all other treatments. Amending soil with sludge significantly improved the fruit weight, to the point that 1:6 amendment ratio resulted in fruits equivalent to the +ve control plants

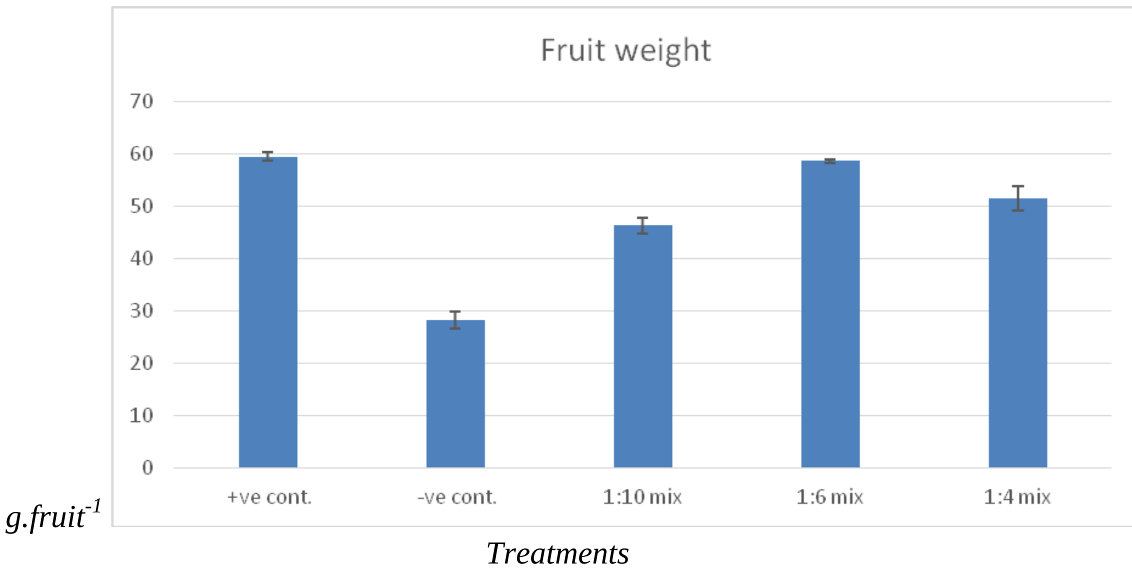


Fig 20: Influence of sludge treatments on fruits weight of tomato plants(g.fruit^{-1}).

4.2.8 Leaf area

consequence of salinity stress include also the size of the leaves, and smaller leaves are usual with severe stress. Results (figure 21) show clear significant differences. Plants subjected to severe stress had significantly much smaller leaves than unstressed plants (+ve control). In addition, it is obvious that amending soils with sludge (in particular at 1:10 ratio) resulted in larger leaves (compared to –ve control), to the point that it was close to that of unstressed plants.

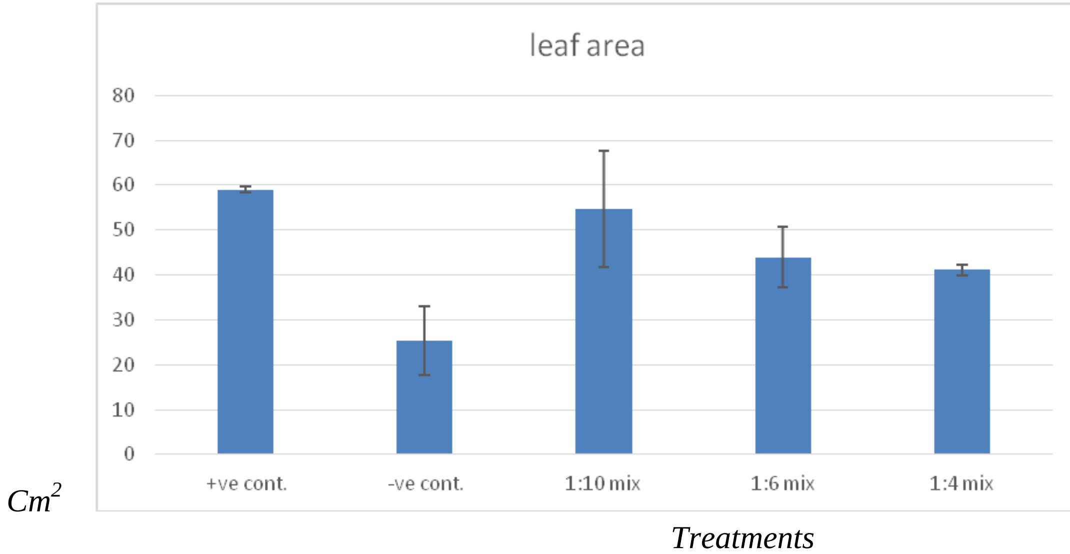


Fig 21: Influence of sludge treatments on the leaf area of tomato plants (cm^2) .

4.2.9 Chlorophyll content

Chlorophylls are the most important pigments in plants due to their role in photosynthesis, and low chlorophyll content leads to the yellowing of leaves, and this leads to low productivity in plants. As mentioned above, salinity stress led to yellowing of leaves, and accordingly the chlorophyll content of leaves was measured. Results (figure 22) show, however that there are no significant differences in respect to total chlorophyll between treated plants, but significant differences in respect to chlorophyll b between the +ve and –ve controls were recorded.

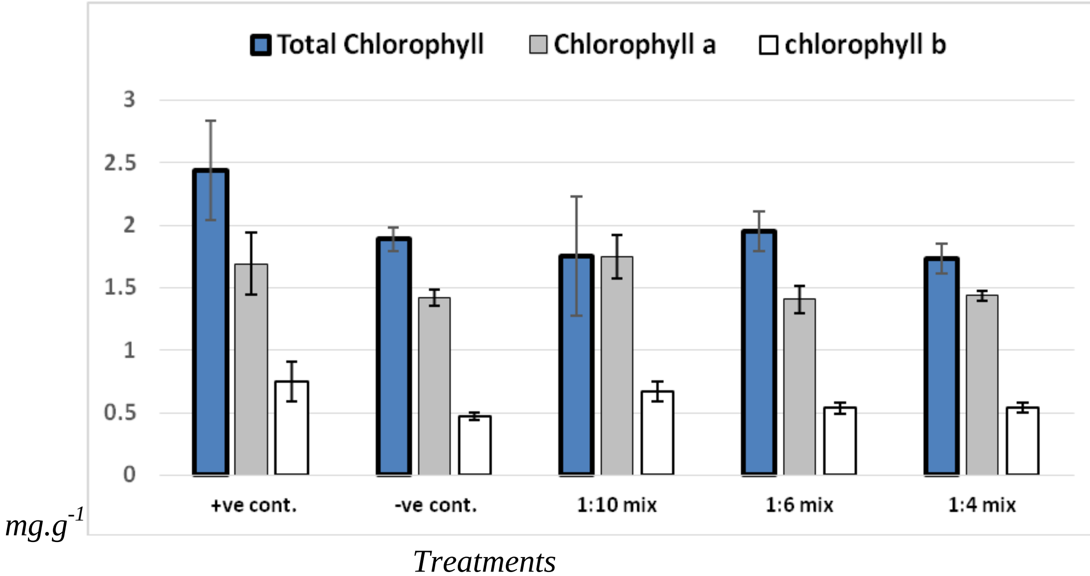


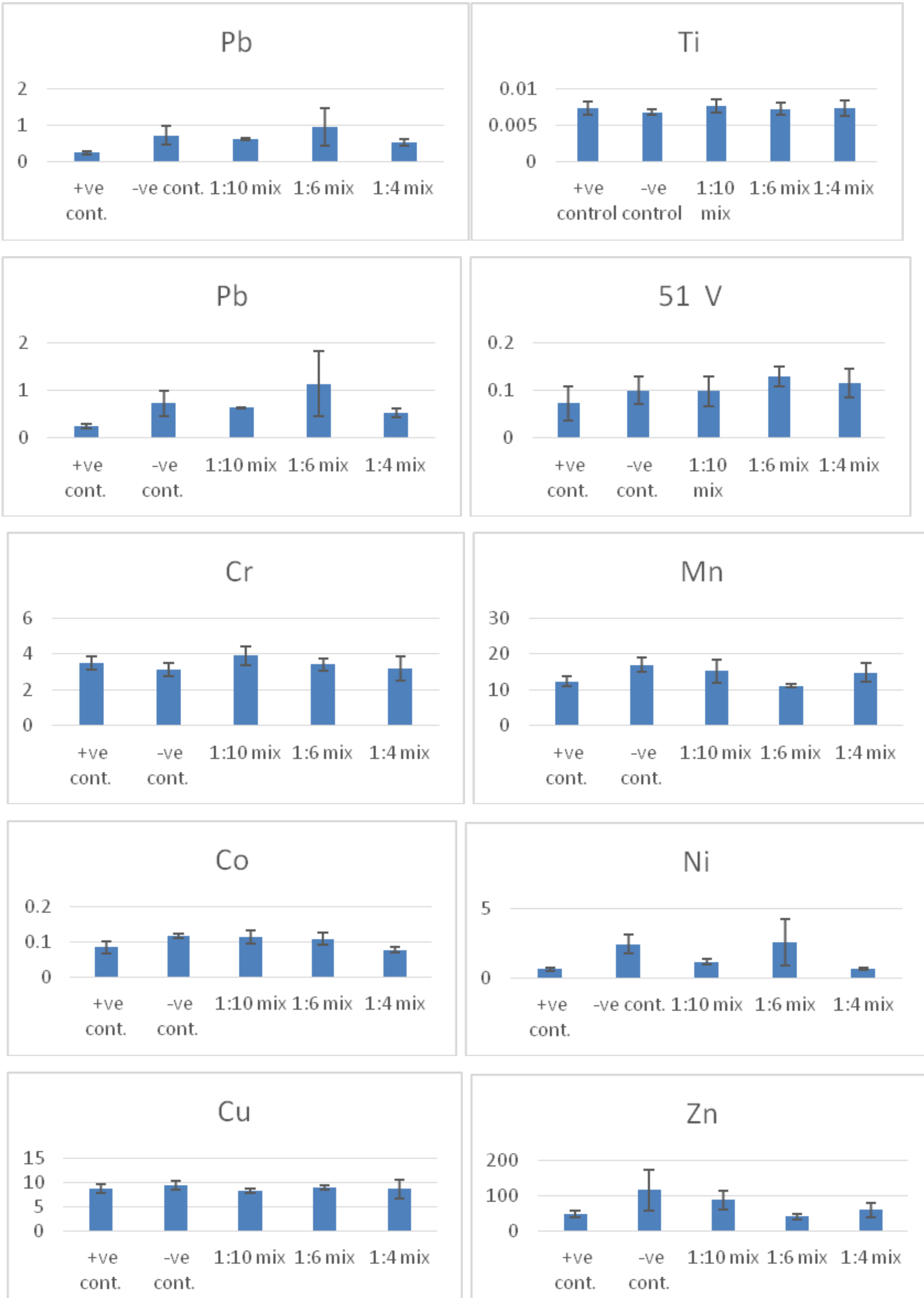
Fig 22: Influence of sludge treatments on the chlorophyll level of tomato plants (mg chlorophyll.g^{-1}).

4.3: Suitability of the product for human consumption

The aim of these assessments is to determine the suitability of the products for human consumption. Assessment included heavy metals content of fruits and the degree of contamination of the fruits with pathogens.

4.3.1 Heavy metals content of fruits:

Taking into account that sludge may contain heavy metals, which can move to plants and accumulate in the fruits,it is crucial to run this test. The following metals were tested: cadmium, selenium, arsenic, zinc, copper, nickel, cobalt, manganese, chromium, vanadium, lead, and thallium. Results (figure 24) show that fruits from sludge-amended soils are not contaminated with heavy metals. These results may allow for future use in agriculture if such trend persist in future studies, since the major obstacle of using sludge as a soil amendment is the concern about heavy metals contamination.


Pb
2
1
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Ti
0.01
0.005
0
+ve control
-ve control
1:10 mix
1:6 mix
1:4 mix
Pb
2
1
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
51 V
0.2
0.1
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Cr
6
4
2
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Mn
30
20
10
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Co
0.2
0.1
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Ni
5
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Cu
15
10
5
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix
Zn
200
100
0
+ve cont.
-ve cont.
1:10 mix
1:6 mix
1:4 mix

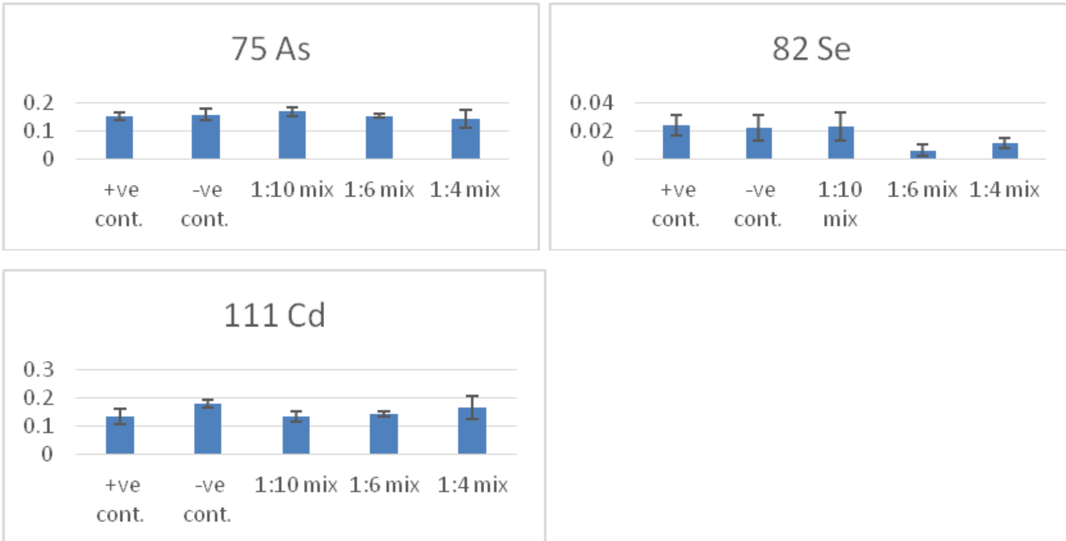


Fig 23: Influence of sludge treatments on heavy metals (cadmium, selenium, arsenic, zinc, copper, nickel, cobalt, manganese, chromium, vanadium, lead, and thallium) concentrations of fruits (µ.g $^{-1}$) .

4.3.2: Degree of contamination of fruits with pathogens.

The contamination of fruits with various bacteria was investigated, and no significant differences are recorded between treatments (figure 24). Parameters examined were total Coliform, fecal Coliform, *and Salmonella*

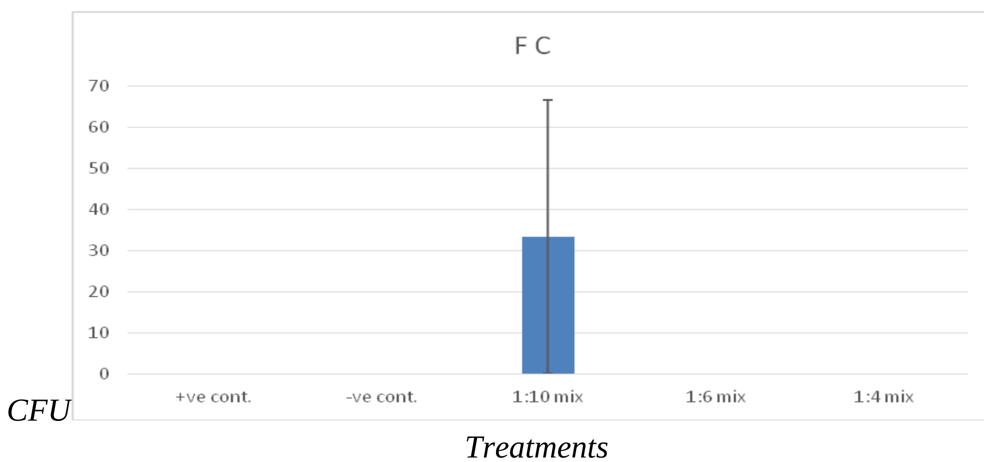


Fig 24: Influence of sludge treatments on bacterial contamination (CFU) of tomato fruits from the soils planted with tomato plants.

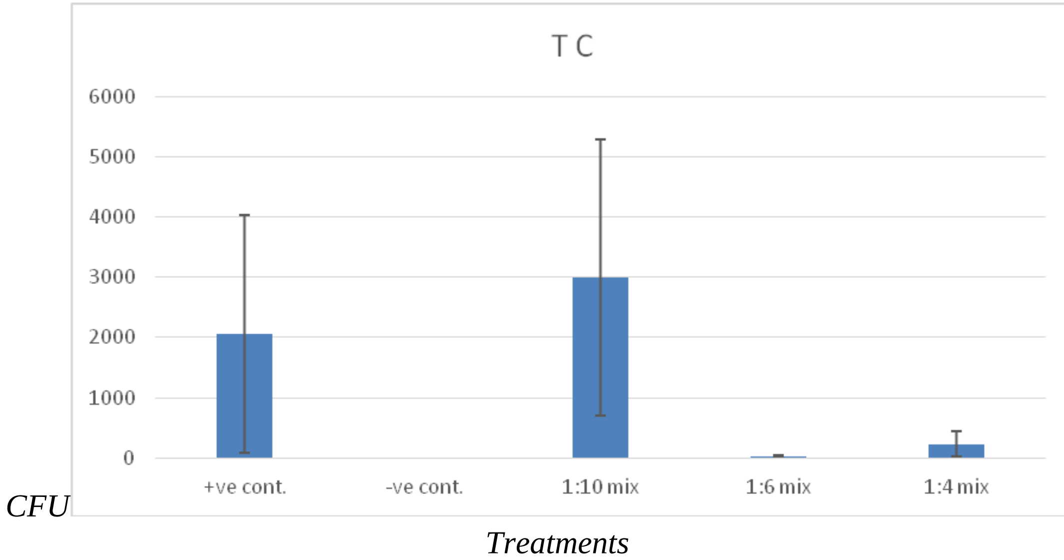


Fig 25: Influence of sludge treatments on bacterial contamination (CFU)of tomato fruits from the soils planted with tomato plants .

Chapter five

Discussion

Results obtained from this research are divided into three sections. The first section addresses the effect of amending saline soils with sludge on the characteristics and components of the soil itself. The second section handles the effect of amending sludge on the vegetative growth of tomato plants, and the third section discusses the effect of amending sludge on fruits and ability of these fruits for human consumption.

First Section: Effect of amending sludge on soil

Before starting the experiment samples were taken from natural soil, as well as saline soils. Also by the end of experiment samples were taken from both soils and from saline soil-sludge mixture and subjected to analyses. As presented above, amending sludge to saline soil at 1: 10 or 1: 6 ratios decreased significantly the salinity level. It is clear here that such effect is due to sludge with its high organic matter content that has high capacity to adsorb salts. In agreement with this study, Samara (2008) reported that the salinity level of the sludge obtained from Albireh WWTP is 3.43 $ds.m^{-1}$. Accordingly, it is assumed that such sludge decreases soil salinity, and these results are compatible with Lakhdar et al. (2010). However, Casado-Vela et al. (2007) reported that there was no significant difference in soil pH values upon the addition of sludge to the soil. This is attributed mostly to well known phenomenon called soil buffering, despite the fact that the sludge pH is low (6.55), as reported by Samara (2008). In addition to that, the calcium level in the soil was not affected negatively by amending sludge to saline soil, which is very positive for such soil, as Ca plays a role in stabilizing soil structure. In contrast, the calcium level in the control soil was lower than all treatments, which can be attributed to higher Na levels in such soils. Moreover, the Mg levels were not affected negatively due to sludge amendment, except in the positive control, in which Mg decreased significantly after planting the tomato plants. Furthermore, no significant differences between saline soil and mix treatments in respect to potassium levels were detected. However, in the positive control a real significant difference was reported, which can be attributed to lower K level in the sludge. Similar trend was also evident for phosphorous. On the other hand, the cation exchange capacity in saline soil was higher than natural soil, which might be attributed to the soil texture differences, as soils with higher clay levels are characterized by higher cation exchange capacity. In addition, the addition of organic matter increases CEC. In this sense, it is possible to predict that the buffering action of the saline soil is too high and that the mineral content of sludge was also high enough to compensate for the dilution effect. Accordingly, the slight changes in cation exchange capacity might be attributed also to the soil texture and the lack of high proportion of clay in the soil (Cavallaro, 1993). In addition to that the significant increase in organic matter upon sludge amendment may add more to the buffering action of the sludge-soil mix. In this sense, it is well known that organic matter plays a major role in maintain soil structure, and its role in supplying plant nutrients is minor (Waters and Oades, 1991).

Concerning K-levels in saline soil treatments, which were more than that of natural soil, it is possible to predict that higher growth rates of plants cultivated in natural soil led to higher rates of absorption for this essential element. As an indication for that, these plants gave qualitatively better fruits. Hence, the reported promotion of growth of processing tomatoes can be attributed to improvements in soil properties, in particular the lower electrical conductivity values, and higher organic matter levels.

Based on the previous results and reports, the impact of sewage sludge on soil properties depends highly on the nature of soil and cultural practices, in particular irrigation and fertilizers application rates. In this sense, it was reported that the major limitation is the increased soil salinity (Roca-Pérez et al. 2009). In addition, it was reported that the levels of the extractable metals tend to be higher in the arable, lower organic matter soil, and that the inoculation with mycorrhizal fungi led to higher Cd and Zn concentrations (Oudeh et al. 2002). On the other hand, the complexation of metals (e.g. Cu) by the organic matter found on the sewage sludge may led to a reduced soil sorption and an enhanced mobility, and consequently lower toxicity as reported by Ashworth and Alloway (2007).

The impact of amending sludge to saline soil is reflected finally on the quality of fruits, since absorption of metals, in particular heavy metals, takes place on soil. Taking into account that the transpiration rates of reproductive parts (e.g. fruits) are much lower than leaves, it is logical that these parts had lower levels of these metals. In addition, it is well known that various elements (e.g. Zn) adsorbed strongly onto organic matter and clay particles of the soil, which render such elements partially immobile (Antoniadis, 1998). Taking into account that the presence of copper dramatically reduced the competitive adsorption of Zn (Pérez-Novo et al., 2008), the higher Cu content of fruits is logical. Similarly, Ni is adsorbed strongly on the organic matter (Leeper, 1978), and the cultivated tomato plants may absorb accordingly limited amount of this element also. However, the most worrying metal is the lead (Pb), as it is considered to be the most serious pollutant of all heavy metals (Antoniadis, 1998). The results of the current study show that sludge amendment tend to increase Pb content in fruits, although slightly and not significantly. In this sense it is crucial to improve the content of soils with organic matter, since organic matter is a very important sink for Pb (Kabata-Pendias and Pendias, 1992).

Another important factor is the soil pH. In this sense, it is well accepted that lower pH values result in more solubility of cationic forms of metals in soil solution These heavy metals became accordingly more readily available to plants (Gray et al., 1998; Salam and Helmke, 1998; Oliver et al., 1998). In the current study, amending sludge in saline soils failed to lower the soil pH, and most probably affects negatively the solubility of such metals. Such development is welcomed as it results in lower absorption of metals by the cultivated plants. However, it is questionable if such development is sustainable over years, as continuous amendment of sludge over years may leads to accumulation of heavy metals to unacceptable levels.

Consequently, the lower content of tomato fruits in heavy metals, which are highly desirable, might be attributed to both strong adsorption of metals to soil particles and lower transpiration rates of fruits. In this sense, Bose and Bhattacharyya (2008) reported, by wheat, that the translocation factor from shoot to grain is smaller than that of root to shoot, which had an implication that the heavy metal accumulation was proportionally lesser in grains.

Second Section: Effect of amending saline soil with sludge on the vegetative growth of tomato plants:

Several criteria were selected to assess the effect of sludge amendment on vegetative growth of processing tomatoes. Taking into account that tomato plants do not tolerate high salinity levels, and will be moderately to severely affected at salinity levels of more than 2.5 $mS.cm^{-1}$ (Shalhevet & Yaron, 1973), it is clear that all treatments in this study, except positive control, encounter high degree of salinity stress; that was reflected by injuries following exposure to salinity stress. Such injuries may further led to the recorded changes in plant height, number of leaves, number of branches, number of clusters and also the productivity of plants..

In this respect, previous studies showed clearly that amending saline soils with organic compounds improved their properties (Ju et al 2007, Lakhdar et al 2009, Walker et al 2008); this is compatible with our study that amendment ratios of 1: 10 and 1: 6 improved significantly the vegetative growth. However, amending soil at ratio of 1:4 (sludge: soil) gave no significant difference in comparison to the negative control treatment, since the degree of soil salinity after addition of sludge was still high (7.47 $mS.cm^{-1}$ vs. 8.14 $mS.cm^{-1}$ before addition). The reason of not decreasing the soil salinity after amendment at 1:4 ratio is related directly to the sludge salinity level. Such an effect is reflected by the productivity of tomato plants, which increased in sludge amended soil, in particular with 1: 10 and 1:6 ratios (fig 13, and 14). This increase in production was primarily due to the increase in vegetative growth of the plants which was improved by adding the sludge to the saline soil at the ratios 1:10 and 1: 6. Similar results were reported by Navas et al. (1998) and Christie et al. (2001).

Third Section: Effect of amending sludge on fruits and suitability of these fruits for human consumption

The results of heavy metal analysis in fruits clearly show that there are no significant differences between treatments. These results are in agreement with that of Rapheal and Sunday (2011) who reported lower contamination levels according to safe limits of heavy metals issued by WHO, FAO, and EU Standards. Results of our study are also below these standards. However, the risk is that such heavy metals may accumulate upon long-term usage of sludge over many years (McGrath,et al 1995), and a monitoring program has to be developed to tackle this risk. In this sense, Samara (2008) reported that the maximum concentration limits of heavy metals found in sludge obtained from Al Bireh WWTP are below the ceiling concentrations for the addressed metals, except Nickel; these regulations are standardized by various organizations and governments, including EU and EPA. In addition to that, the degree of contamination by bacteria reported here clearly show that proper processing and handling of sludge may eliminate any risk for final consumers; results show no kind of contamination for the tested *Salmonella*, fecal and total coliform. Reasons beyond that are the exposure of sludge to solar drying before usage, and the adoption of cultivation in pots; both are assumed to eliminate pathogens in the amended sludge. Similar results were obtained with Ögleni& Özdemir (2010).

Chapter six

Conclusions and recommendations :

6.1 Conclusion:

According to the findings of this study, sludge might be considered as a suitable soil amendment for saline soils, since it improved soil properties. Moreover, amending sludge in soil is a valuable environment-friendly disposal technique. However, further investigations are needed for a solid recommendation.

6.2 Recommendations:

(1) Further research studies are needed to explain sludge effect in alleviating the stress and on soil properties, including flora and fauna.

(2) Studies should be done on the economic benefits expected from using of sludge on saline soils

(3) More detailed long-term studies are necessary to monitor heavy metal accumulation in treated soils and of cultivated plants and their products.

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