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LAND APPLICATION OF SEWAGE SLUDGE: PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE OF THE *RIO GRANDE* TOMATO

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ABSTRACT

Treated wastewater produces sewage sludge as a by-product that may have beneficial implications on agricultural practices. The effects of sludge amendments on growth, morphological and biochemical characteristics of the agro-industrial Tomato cultivar *Rio Grande* were observed. A pot culture experiment was carried out during 4 months (16 February 2019 to 18 June 2019), at an experimental green house in the locality of Salah Soufi, Guelma, north-eastern Algeria. There were significant differences between soil and sewage sludge samples. Total nitrogen (20.58 mg/kg) was more available in sewage sludge than in soil (N = 2.09 ± 0.3 mg/kg). In contrast, sludge contained less phosphorus and organic matter than soil. The observation of the morphological characteristics of the plants showed significant variations between the treatments. The germination rates for treated soils decreased significantly to reach 50%, compared to the control. Growth patterns (dry and fresh weight of leaves and roots) changed significantly ($P < 0.05$) between the treatments. Chlorophyll contents of plants in treated soils were more than twice as high as those of the control, but started decreasing at 75% amendment rates.

Keywords: Biosolids, environment, plant yields, valorisation, wastewater.

INTRODUCTION

When wastewater from different sources is treated at a wastewater treatment facility, it goes through several processes, one of which include separation of sewage sludge from the effluent (Grobelak et al., 2019). Unfortunately, the quantity of this waste matter has been increasing significantly year after year, posing a major threat to the environment and to the health worldwide. For that reason, managed properly, use of sewage sludge is an inevitable step in environmental conservation plans (Grobelak et al., 2019). The main disposal strategy for sewage sludge management is reuse, mainly for agricultural or landscaping purposes. There are many strategies to reuse sewage sludge but also many restrictions on the use of the given management method (Martinez et al., 2003). Several studies have indicated that crop production has benefitted from land application of sewage sludge (Kacprzak et al., 2017; Rulkens, 2007).

The use of sludge as a soil amendment is widely encouraged among farmers due to its high content of organic matter and plant nutrients (Bot and Benites, 2005). In Algeria, many researchers suggested that sewage sludge is a powerful way to increase crop yields and enhance soil fertilization. Studies suggest that this waste matter could help revitalize soils in arid zones (Lassoued et al., 2013; Ati, 2010). However, potentially present agrochemicals in sewage sludge could be harmful to terrestrial ecosystems as they impose severe constraints on crop growth and productivity (what is known as the environmental stresses) (Lamastra et al., 2018).

Singh and Agrawal (2009; 2010) observed an increase in total chlorophyll contents in leaves of *B. juncea* grown in tannery sludge amendment; increments in protein content and accumulation of proline in plants due to sludge amendments were also reported. The behaviour of these compatible compounds, coupled with morphological structure of plants is a useful indicator of the degree of plant resistance to such environmental stresses (Juan et al., 2005).

This study was conducted to demonstrate the utility of sewage sludge for agricultural purposes. The main focus of this work was to assess potential benefits and risks of sewage sludge application on land fertility and eventual effects on plant production, by recording physiological and biochemical variations of tomato cultivars (Rio Grande) grown on different sewage sludge amendment rates in a mini pot culture experiment.

MATERIALS AND METHOD

Study Region

The pot culture experiment was carried out during 4 months (from 16 February 2019 to June 18 2019), at an experimental green house built in the locality of Salah soufi, Guelma, situated in north-eastern Algeria, about 65 kilometres from the Mediterranean coast. It is a large agricultural land at 290 m (above sea level.). The climate is sub-humid with an average precipitation of 600 mm/year and a temperature of 18.5 °C. It is a fertile area due to Seybouse river and to a big dam ensuring irrigation of a vast perimeter of 9650 ha (Figure 1) (Kachi et al., 2016). The sludge samples used in this experiment were obtained from the sewage treatment plant of Guelma City.

Figure 1. Map of Guelma city, Algeria

Plant Material and Transplanting

Seeds of tomato cultivars (Rio Grande) used in this study were obtained from a local farmer, and were sown on 16 February, 2019 in separate pots (thirty seeds were planted in the pot) having a mixture of soil and sewage sludge. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. There were three treatments consisting of T1 (25 % sewage sludge), T2 (50 % sewage sludge), T3 (75 % sewage sludge) and T0 (Control, no sewage sludge) (Figure 2).

Figure 2. Layout of the experiment

Biochemical and Physiological Determinations

i. Physiological Parameters

The data was recorded on the specific parameters (seed germination, length, fresh weight, dry weight and water content) during the course of experiment. Seed germination rate was the number of seeds germinated as per total number of seeds planted from day of germination expressed as a percentage. Lengths of aerial and root parts (LAP and LRP) were recorded. The fresh weight (FW) was recorded immediately after the plant (aerial and root parts) was harvested and the dry weight (DW) was recorded after drying the plant (aerial and root parts) at 80 ^{0}C. Water content (WC) was calculated from the values of the fresh and dry weight recorded previously, applying the following formula:

$$WC (\%) = (FW-DW)/FW * 100.$$

ii. Biochemical Parameters

At the fourth month after sowing, plants were carefully removed from their pots, washed with tap water to remove any attached soil particles, and rinsed with deionized water and analysed at the laboratory of Centre for Biotechnology Research, Constantine, Algeria. Chlorophyll content expressed as mg/g was estimated according to the method of McKinney (1941). Protein, proline, and sugar content were measured by the method of Bradford (1976); Troll and Lindsley (1955); Dubois et al. (1956) respectively.

iii. Soil and sludge characteristics

Soil and sewage sludge samples were air-dried at room temperature and sieved through a 2 mm mesh (to remove as much plants material and stones as possible). Conductivity of soil (soil: distilled water, 1:5 w/v) and pH (soil: distilled water, 1:2.5 w/v) were measured by using the glass-electrode method, organic matter (OM, %) and carbon content (C, %) were determined using wet oxidation method (Walkey and Black, 1934), total nitrogen (TN) was measured using the kjeldahl method described by Pansu and Gautheyrou (2006). The available phosphorus (P, mg/kg) was determined by the method of Olsen et al. (1954).

Data Analyses

The data was statistically analysed according to the PASW Statistics 18 for Windows. Mean values per treatment were compared by ANOVA; where significant differences ($P < 0.05$) were indicated, a post hoc t-test (LSD: Least significant difference) was performed.

RESULTS

Sewage Sludge and Soil Characteristics

The pH of sewage sludge coming from the sewage treatment plant of Guelma City, was slightly acidic (6.76±0.06), and showed a high electrical conductance (2091±5.88 µS/cm) as compared to the agricultural farm soil. Total Nitrogen (20.58 mg/kg) seemed to be more available in sewage sludge than in the studied soil (Table 1).

Plant Growth Patterns

Seed germination rate (Relative growth rate) responses were significantly different ($P < 0.05$) for Tomato cultivar seedlings raised in the various soil treatments (Table 2). Germination rates of our seedlings in the treated soil decreased significantly at approximately half the value against those planted in the control. Also, growth patterns changed significantly ($P < 0.05$) among treatments. This fact was specially shown by changes in dry weight and fresh weight of leaves, where these two physiological parameters were higher in the treated soil, but did not vary between the different amendments rates.

Table 1: Physico-chemical characteristics of sewage sludge and soil (Mean ±SD)

	Electrical conductivity (µS/cm)	pH	Organic matter (%)	Phosphorus (mg/kg)	Azote (mg/kg)
Sludge	2091±5.88	6.76±0.06	3.06 ±0.0 4	3.93±0.32	20.58±0
Soil	272.66 ± 24.85	7.4 ± 0.08	10. 52± 0.32	12.62 ±0.43	2.09 ± 0.3

Table 2: Results of the morphological parameters

Treatment	Growth rate (%)	Dry weight (g)		Fresh weight (g)		Water content (%)	
		Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	100 ± 0*	3.73 ±1.08*	0.68 ±0.19	16.9 ±2.17*	3.75 ±1.15	78.43 ±3.4	81.75 ± 0.48
T1 (%)	53± 3.33*	7.91 ± 2.01*	0.86 ±0.36	34.14 ± 10.83	5.03 ± 2.11	76.2 ± 2.98	82.64 ± 0.55
T2 (50%)	57.78 ± 8.38*	6.31 ± 2.31*	0.98 ± 0.55	36.02 ± 11.41	5.2 ±2.83	82.69 ± 1.25*	81.04 ± 0.94
T3 (75%)	51.11 ± 13.47*	7.35 ±1.85*	0.71 ±0.3	32.98 ±6.41	4.5 ±2.36	78.53 ±1.71	83.23 ±3.14

Biochemical Determinations

i. Chlorophyll Content

Chlorophyll content differed significantly among the seedlings grown in the different sewage sludge amendments rates ($P < 0.05$). Chlorophyll values in the leaves of plants in treated soil were more than twice as high as those measured in seedlings raised in the control. It was noted that chlorophyll content started decreasing at 75 % (T3) amendment rate when compared to the precedent (T2) treatments (Figure 3).

ii. Patterns of Protein, Proline and Sugar Allocations

One-way ANOVA was used to determine that concentrations of these three elements in the tissues of Rio Grande leaves showed significant differences among control and treated soil ($P < 0.05$). In particular, seedlings grown in treated soil (50 % and 75 % amendment rates) had remarkably higher contents in their leaves compared with those not treated with sludge. In contrast, sugar content was clearly highest in the control (Figure 4).

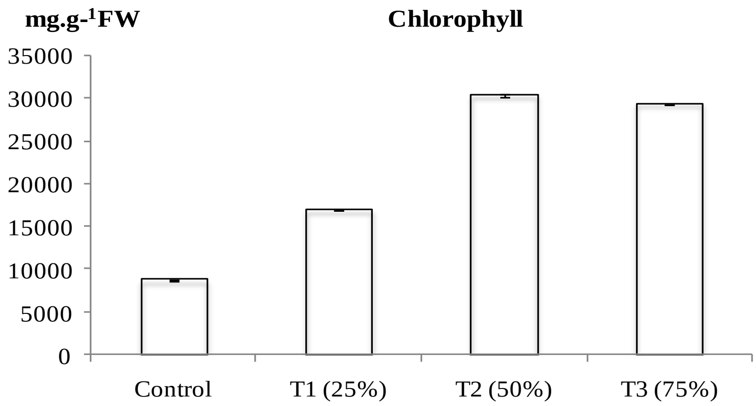

Figure 3: Chlorophyll values in leaves of Tomato *Rio Grande* grown in the different sewage sludge amendments rates

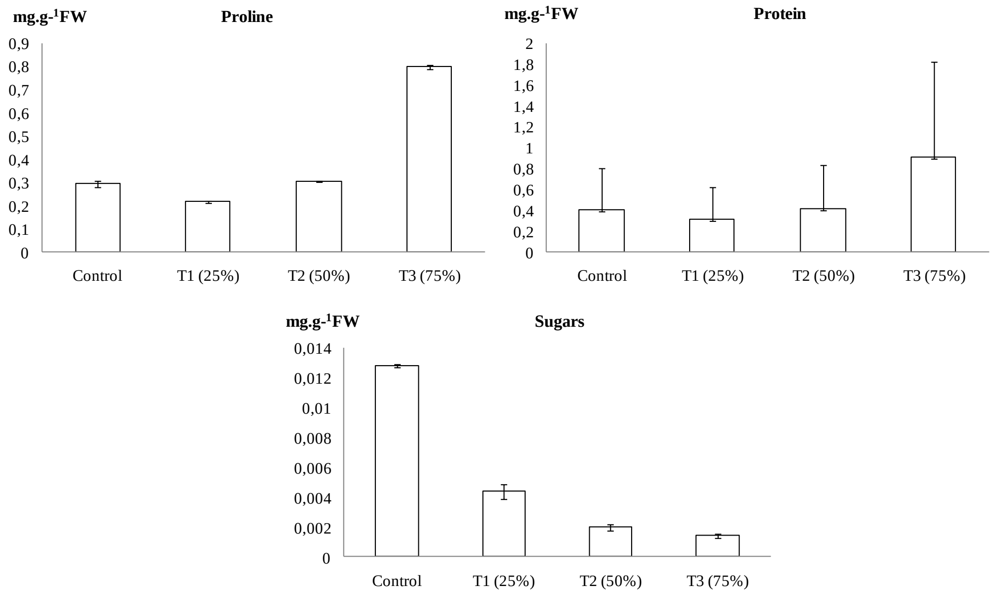

Figure 4: Patterns of protein, proline and sugar allocations in Tomato Rio Grande grown in the different sewage sludge amendments rates

DISCUSSION

The use of sludge as soil amendment is widely encouraged. Also, increase in soil nutrients due to sewage sludge application has been reported by previous research (Usman et al., 2012). Several studies around the globe have previously reported a significantly positive effect of compost on seed germination rates of tomato (Arancon et al., 2012; Atiyeh et al., 2003) and similar results have been reported for other plant species (Singh and Agrawal, 2010). In contrast, our results showed a significantly negative effect, where the highest germination rate was observed with control while, the lowest percentage of seed germination was observed with the highest amendment rate. This could possibly be related to differences in sewage sludge characteristics.

The compost used in this experiment seems to be detrimental to germination rates. The high electrical conductivity (i.e. high salinity) coupled with the acidic character of the sludge were possibly the main factors negatively affecting seed germination (EC and pH values were not conformed to standard values recommended agriculture) (Begum et al., 2010). Despite their negative effects on seed germination, it should be noted that the used sludge, produced more vigorous seedlings when compared with the control (in terms of dry weight and fresh weight of plant leaves and roots). The enhanced quality of seedlings grown under composted soils was mainly due to improvement in photosynthesis which leads to a high conversion efficiency of intercepted light into biomass (higher contents of chlorophyll and protein were recorded in their leaves).

In general, factors that influence leaf area development and duration as well as the conversion efficiency of intercepted light into biomass, are critical for plant growth (Cannell et al., 1988). One factor that is of central importance in this context is deficiency/availability of plant nutrients. The most important benefit of sewage sludge application in agricultural soils is the increased availability of these nutrients (Singh and Agrawal, 2010).

The results demonstrate that plant protein accumulation is closely related to the applied dosage of sludge. It is possible that the nutrient levels (nitrogen as a major element that is included in the composition of proteins) in the composted soil in this study were sufficient to improve growth of seedlings and overcome some physiological damage. Many authors have shown that sludge can be a source of nitrogen and its use improves the amount of nitrate in the soil (Courtney and Mullen, 2008). Also, the fertilizer value of sludge (nitrogen and phosphorus) is comparable to commercialized mineral fertilizers as demonstrated by many researchers (Bousselhaj, 1996; Antolínet al., 2005; Rashid et al., 2017).

The obtained results in term of tomato growth under composted soil provided encouraging results in crop production. However, changes in some important plant component behaviour especially sugars and proline foreshadow a negative impact. Lower level of reducing sugar content in leaf samples obtained from the composted soils as compared to the control in this study, illustrated hardening conditions. In this case, sugar concentration decreased because of the increased respiration rate and decreased rate of carbon fixation as sulfites reacted with the aldehydes and ketones of carbohydrates and reduced the carbohydrate content as was also observed by Seyyednejad and Koochak (2013); Stambulska et al. (2018). Many researchers observed that the more resistant plant species show accumulation of reducing sugars while less stress-resistant plants show less accumulation of sugars (Kameli andLöselδ, 1993; Keller and Ludlow, 1993).

The second crucial stress indicator also showed a negative correlation with increasing amendment rates. The amino acid proline is accumulated in plant tissues in response to a variety of stresses (Kocheva and Georgiev, 2008). It is a strong reductant and protects cellular components against SO_2 and OH accumulation and protects the enzymes of the Calvin cycle (Krishnaveni and Kumar, 2018). When the plants were given a salt source (sludge compost), proline accumulated in the tissue at the highest rate, these findings are in congruence with those of Inal (2002). It is well-known that in salt stress conditions, proline accumulation in plants increases for osmo-regulation (Günes et al., 1996). This biochemical substance is the universal osmolyte and has a defensive action against the stress condition as an adaptation during any kind of environmental stress (Akshita et al., 2018).

CONCLUSIONS

The results of the present investigation report that sewage sludge can be considered as a good fertilizer as it increases organic carbon, total nitrogen and available phosphorus in soil. Plants grown at different treatment levels showed a positive correlation with nutrient availability in soil by enhancing growth characteristics and biomass yield. Unfortunately, a decrease in the reducing sugar content and increase in the proline content are a major concern due to risk to crop production health. This may suggest that amendment of sewage sludge in soil may be a good option for agricultural practices. However, a lower sewage sludge use rate is more advisable to safely avoid high environmental stress levels and risk to crop yield.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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