

EFFECTS OF SEWAGE SLUDGE APPLICATIONS TO AGRICULTURAL SOIL ON THE BIOCHEMICAL PARAMETERS OF FABA BEAN (*FABA SATIVA* BERNH.), WHEAT (*TRITICUM AESTIVUM* L.), SPINACH (*SPINACIA OLERACEA* L.) AND CUCUMBER (*CUCUMIS SATIVUS* L.) CROPS

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Abstract. This study aimed to assess the sewage sludge (SS) amendments to agricultural soil on the biochemical composition of four plants (faba bean, wheat, spinach and cucumber). The applied SS rates were 0, 10, 20, 30, 40 and 50 g/kg soil. Plant leaves 45 days after SS treatments were used in biochemical parameter analyses. Starch decreased in spinach from 179.34 to 41.38 mg/g DW with SS treatments and increased in faba bean, wheat and cucumber at 10, 20, 30 and 40 g/kg rates. Water soluble carbohydrates and total non-structural carbohydrates declined in the four plants with SS treatments. Total lipids increased in faba bean and spinach up to 41.94 and 7.89 mg/g FW, respectively, at the SS amendment of 50 g/kg, while in wheat and cucumber the increase was detected only at 40 and 50 g/kg rates. In spinach, the accumulation of proline started at SS treatment of 10 g/kg, while, for wheat and cucumber the increase of proline was started at 20 g/kg SS. The highest proline accumulation was 0.96 mg/g FW in spinach at 50 g/kg SS rate. SS did not affect phenol content in faba bean and spinach with any treatment, whereas, the phenol declined in wheat and cucumber.

Keywords: carbohydrates, economic plants, environmental pollution, phenol, proline

Introduction

Application of sewage sludge (SS) in the agricultural sector as soil fertilizers is in several countries (Singh and Agrawal, 2008 and 2010). The municipal SS is a source of macro- and micro-nutrients for crops cultivated in SS amended agricultural soil (Ahmed et al., 2010; Abdul Khaliq et al., 2017). Soil amendment with SS at different rates has improved the growth and yield of several crops including faba bean (*Faba sativa* Bernh.), wheat (*Triticum aestivum* L.), spinach (*Spinacia oleracea* L.) and cucumber (*Cucumis sativus* L.) (Eid et al., 2017a, b, 2018, 2019). Bioaccumulation and translocation of certain heavy metals in tissues of crops cultivated in soil amended with sewage sludge were analyzed in several plants (Kabata-Pendias, 2011; Bouriou et al., 2014; Eid et al., 2020a, b).

Chemical constituents of plants grown in soils amended with SS play a vital role in plant physiology as biochemical responses to abiotic stresses (Sharma et al., 2018). Carbohydrates are major components in plant tissues since they are related to the primary metabolism. Carbohydrates content has been monitored in several crops when cultivated in SS amended soils (Han et al., 2004). Elevation of nitrogen, phosphorus and potassium content and other macro-nutrients in plants grown in agricultural soil supplemented with SS has been reported in several studies (Hue, 1988; Bouriou et al., 2014; Kępka et al., 2016). Proline content has been investigated widely as protectant to environmental toxicity stress in many crops (Boudjabi et al., 2015). Phenolic compounds in plants such as *Beta vulgaris* and *Albizia lebbek* were reported in response irrigation with municipal waste water and heavy metal toxicity (Tripathi and Tripathi, 1999; Singh and Agrawal, 2010). Biochemical composition analyses of the four economic plants under investigation is essential since leaves of spinach, seeds of faba bean, grains of wheat and fruits of cucumber are used for human consumption and the hay produced from wheat and faba bean is used for animal feeding.

The objective of the current investigation is to determine the effect of SS application to agricultural soils on the content of starch, water soluble carbohydrates (WSC), total non-structural carbohydrates (TNC), total lipids, proline and phenol in leaves of faba bean, wheat, spinach and cucumber plants.

Materials and methods

Soil-sewage sludge treatments

Four plant species, namely, faba bean (*Faba sativa*), wheat (*Triticum aestivum*), spinach (*Spinacia oleracea*) and cucumber (*Cucumis sativus*) were used to assess the effects of soil amendments with SS on biochemical composition of leaves of the tested crops. The rates of soil-sewage sludge amendments were zero (control), 10, 20, 30, 40, and 50 g SS/kg soil. The detailed analysis the physic-chemical properties of the applied SS used in the current study was previously performed; salinity 2.07; pH 6.38; organic matter 65.1%; the heavy metals concentrations were Cd (1.17 mg/kg), Co (27.9 mg/kg), Cr (179.1 mg/kg), Cu (162.6 mg/kg), Fe (25.4 mg/g), Mn (595.7 mg/kg), Ni (138.7 mg/kg), Pb (671.2 mg/kg) and Zn (667.6 mg/kg) (Eid et al., 2017a, b, 2018, 2019).

Plant materials and growth conditions

The broad bean, wheat, spinach (Superdane 7 Star, F1 Hybrid, Denmark) and cucumber seeds were obtained from local markets in Abha City. The seeds were planted

in plastic pots filled with 4 kg of each respective SS-soil mixture. Three biological replicates were used. The broad bean and wheat were grown for 80 days, while, spinach and cucumber were grown for 50 days. *Figure 1* shows the experimental setup of the four plants grown in pots represent the rates of sewage sludge-soil amendment rates. The greenhouse conditions were natural day/night regime and the plants were watered when needed (Eid et al., 2017a, b, 2018, 2019).

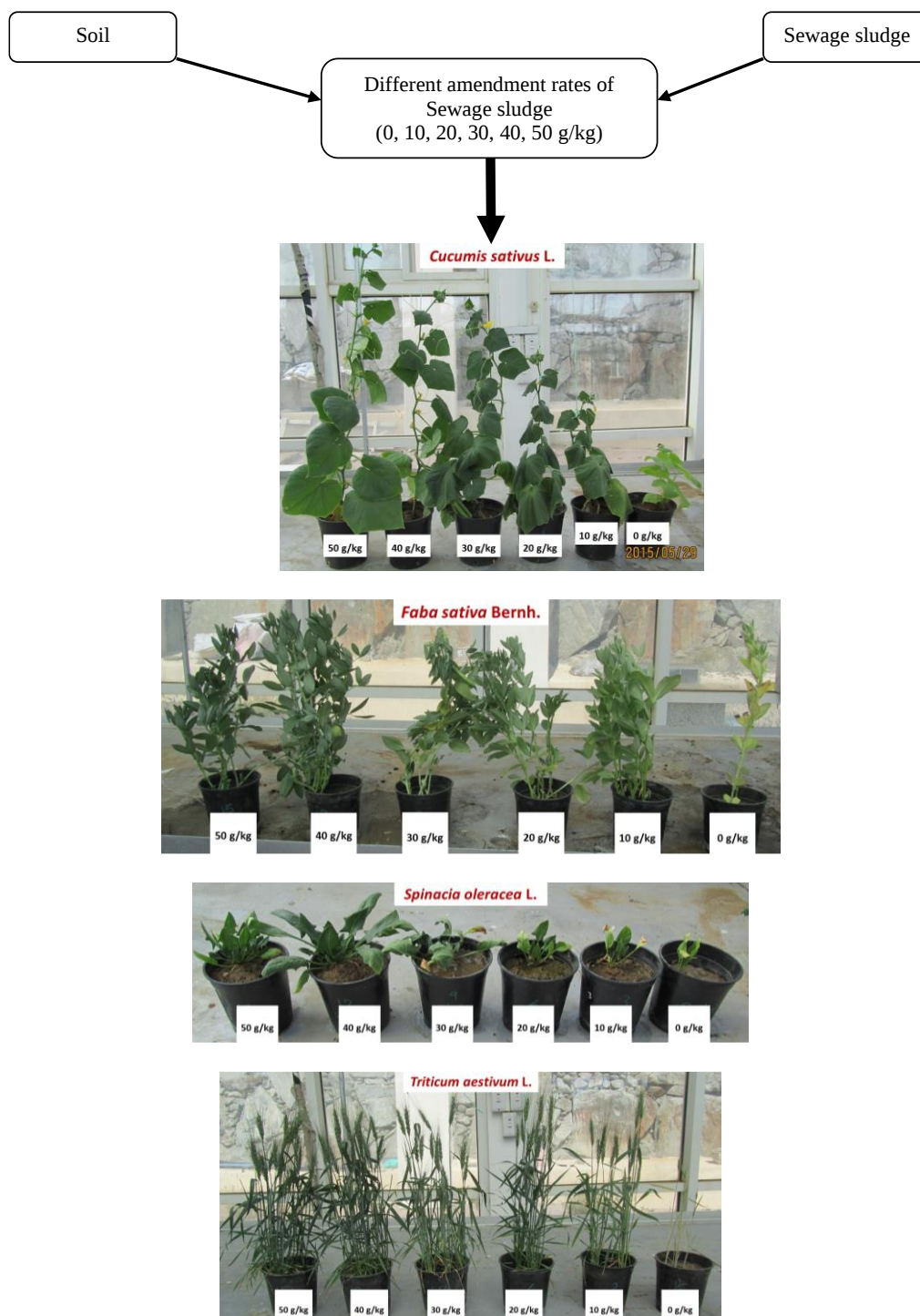


Figure 1. A demonstration of the experiment steps with the four plants in pots represents the treatments of sewage sludge-soil amendment rates

Biochemical analyses

Fully expanded fresh leaves from all the replicates for four species were sampled manually at 45 days, first washed under the running water and some kept in freezer at -20°C for further estimation of total lipids, proline and phenol. Some leaves were dried in the oven at a temperature of 60°C . Dried leaf materials were ground using a metal-free plastic mill into particles less than 0.4 mm for carbohydrate analysis. Total lipids concentration was measured following the method of Byreddy et al. (2016). Proline concentration was measured by the method of Bates et al. (1973). Total phenol concentration was measured following the method of Bray and Thorpe (1954). Water-soluble carbohydrates (WSCs) were extracted from 0.25 g of ground material with hot distilled water, while total non-structural carbohydrates (TNCs) were extracted from another 0.25 g of ground material using diluted H_2SO_4 following the method of Smith et al. (1964). WSC and TNC concentrations (mg/g DW) in the extracted solutions were measured by spectrophotometry using the phenol- H_2SO_4 colorimetric method as described in Granéli et al. (1992), where the standard was glucose. The starch concentration was calculated according to Equation 1:

$$\text{Starch (mg/g DW)} = 0.9 \times (\text{TNC conc. (mg/g DW)} - \text{WSC conc. (mg/g DW)}) \quad (\text{Eq.1})$$

where TNC is the total non-structural carbohydrates and WSC is the water-soluble carbohydrates

Data analyses

Because absorption, accumulation and tolerance to heavy metals vary between different crops and at different levels of SS amendments (see Eid et al., 2017b) and hence heavy metal effects on the concentrations of total lipids, proline, phenol, WSC, TNC, and starch concentrations may vary between these different crops. Therefore, significant differences in the total lipids, proline, phenol, WSC, TNC, and starch concentrations between the spinach, cucumber, faba bean and wheat plants grown in soils amended with different rates of SS were evaluated using a two-way analysis of variance (ANOVA). The data were examined for their homogeneity of variance and normality of distribution, and when necessary, the data were log-transformed before a two-way ANOVA was performed. Significant difference between means among the five SS treatments were identified using the Tukey's HSD test at $p < 0.05$. Statistica 7.1 was used to process all of the statistical analyses (Statsoft, 2007).

Results and discussion

Application of sewage sludge to the agricultural soil has been reported to increase the growth and biomass of several crops. The growth of the four plants used in the current study was promoted when grown in soil amended with SS (Eid et al., 2017a, b, 2018, 2019). The biochemical parameters of leaves from the treated plants were determined. Figure 2 illustrates the effect of SS treatments on starch content (mg/g DW) in faba bean, wheat, spinach and cucumber leaves. Spinach showed the highest content of starch as compared to faba bean, wheat and cucumber, however, the treatment with SS decreased the starch concentration significantly in spinach leaves with all SS amendment treatments from 179.34 to 41.38 mg/g DW. For faba bean there

was an increase in starch concentration at all SS treatments with obvious significant increase at amendments rates of 10 and 20 g/kg with concentrations of 73.07 and 89.56 mg/g DW, respectively. Starch concentration in leaves of wheat and cucumber increased at treatment rates of 10, 20, 30 and 40 g/kg with the highest concentrations at SS treatment of 20 g/kg with 100.42 mg/g DW of wheat and 95.86 mg/g DW of cucumber then starch concentrations were declined significantly at the highest SS treatment rate of 50 g/kg. Stimulation of carbohydrates as response to SS application has been reported in bean (Zeid and Abou El Ghate, 2007). The enhancement of starch synthesis in plants treated with sewage sludge could be a result of increasing photosynthetic pigments, i.e. chlorophyll, with the organic matter, macro- and micro-nutrients in SS which result in increasing of the starch biosynthesis (El-Maghraby and Gomaa, 1992).

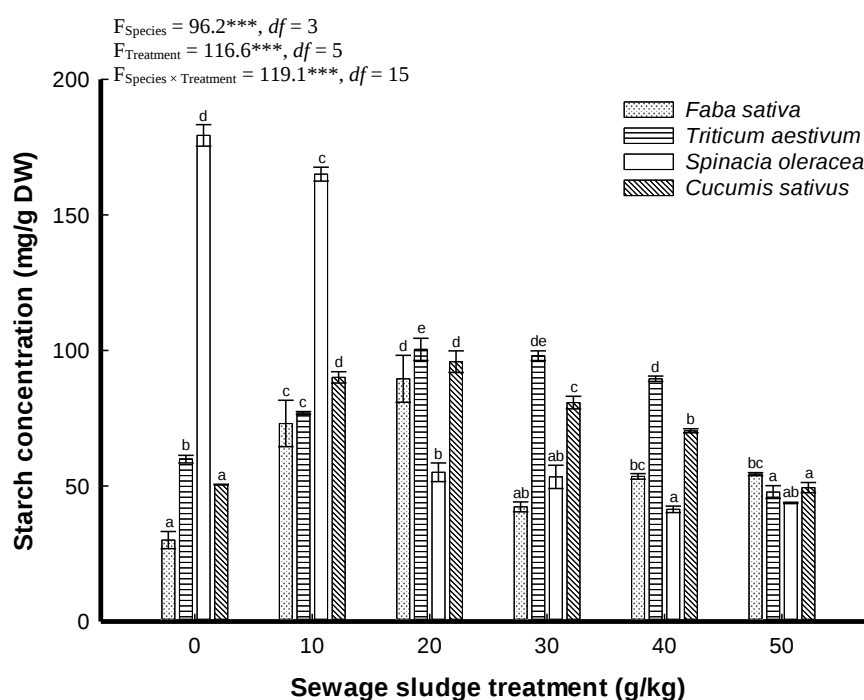


Figure 2. Starch concentration (mean $\pm$ standard error) in leaves of the four plant species *Faba sativa*, *Triticum aestivum*, *Spinacia oleracea* and *Cucumis sativus* grown in soil amended with different sewage sludge treatment rates. Means (for each plant species) with different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$, df: degrees of freedom

The concentrations of water soluble carbohydrates (Fig. 3) and total non-structural carbohydrates (Fig. 4) were inhibited in the four plant leaves due to soil amendment with SS at all treatment rates. It has been reported that total organic carbon was increased in response to SS treatments (Abdul Khaliq et al., 2017). Soluble sugar contents in sunflower plant grown in SS amended soil were increased significantly and suggested to play role in defense mechanisms that triggered by heavy metals in the SS (Belhaj et al., 2016). In maize plants, sugars content, proline and antioxidant enzymes activity were increased when the plant irrigated with sewage water (Abdel Latef and Sallam, 2015).

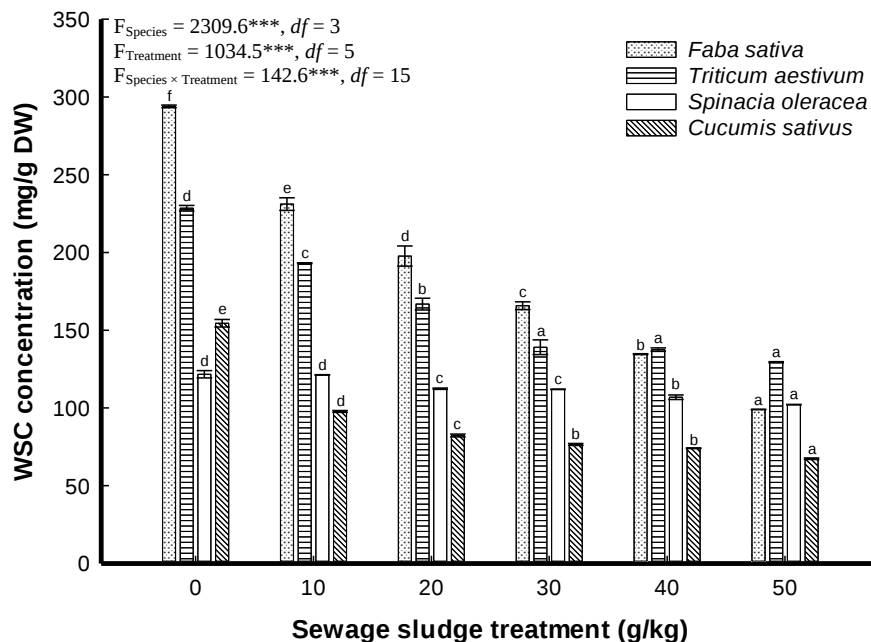


Figure 3. Water soluble carbohydrates (WSC) concentration (mean $\pm$ standard error) in leaves of the four plant species *Faba sativa*, *Triticum aestivum*, *Spinacia oleracea* and *Cucumis sativus* grown in soil amended with different sewage sludge treatment rates. Means (for each plant species) with different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$, df : degrees of freedom

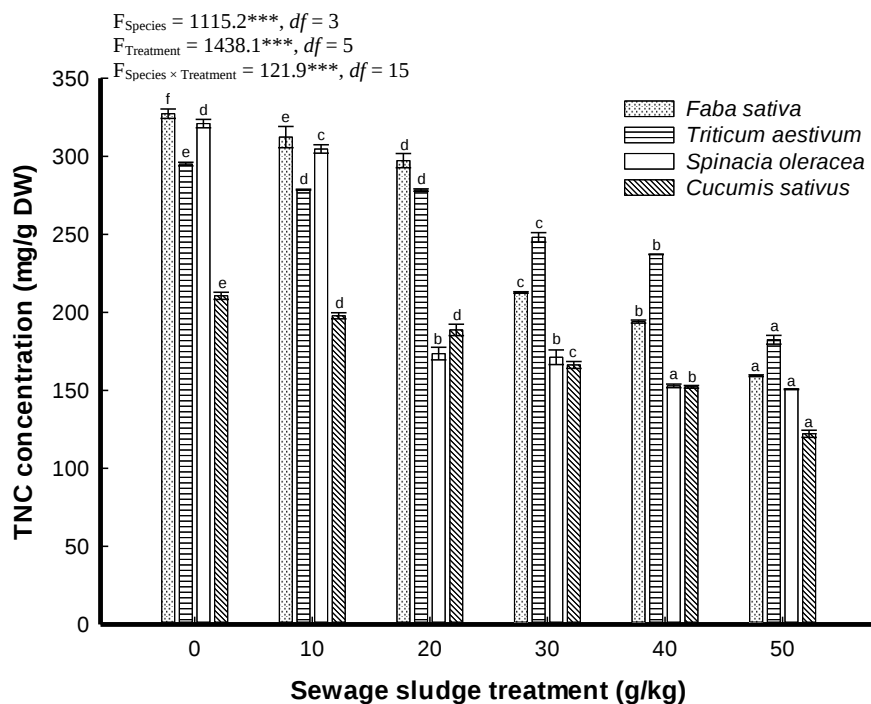


Figure 4. Total non-structural carbohydrates (TNC) concentration (mean $\pm$ standard error) in leaves of the four plant species *Faba sativa*, *Triticum aestivum*, *Spinacia oleracea* and *Cucumis sativus* grown in soil amended with different sewage sludge treatment rates. Means (for each plant species) with different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$, df : degrees of freedom

Total lipids concentrations in leaves of the four analysed plants are demonstrated in Figure 5. In both faba bean and spinach, the increase of total lipids was gradual and the significant levels were detected on SS treatment rates of 20 g/kg and above. For the wheat and cucumber, the significant increase of total lipids content in leaves was observed only at high SS treatment rates of 40 and 50 g/kg. Increasing of lipid peroxidation and proline content has been detected in tomato seedling grown in soil amended with SS (Elloumi et al., 2016). Several studies reported detoxification processes after exposure to SS which indicated using lipid peroxide assays in plant tissues and associate that with uptake of heavy metals from SS (Wyrwicka and Urbaniak, 2016).

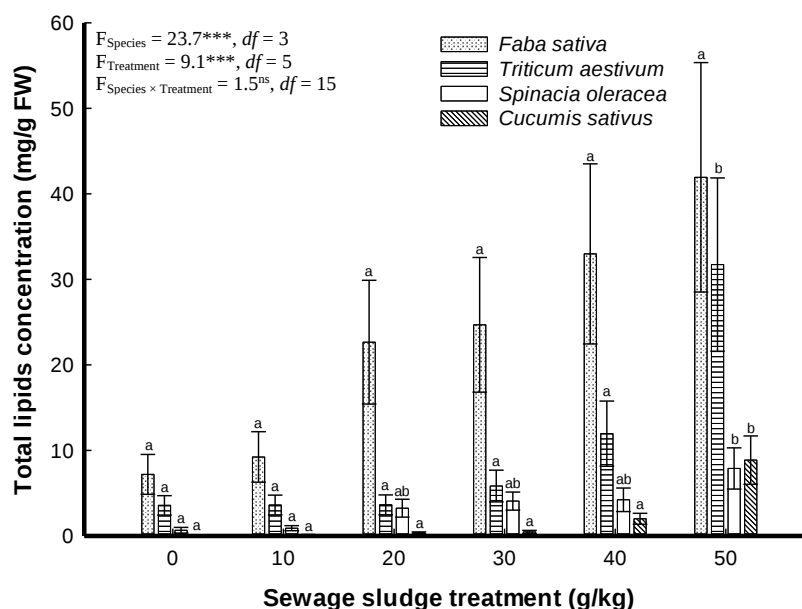


Figure 5. Total lipids concentration (mean $\pm$ standard error) in leaves of the four plant species *Faba sativa*, *Triticum aestivum*, *Spinacia oleracea* and *Cucumis sativus* grown in soil amended with different sewage sludge treatment rates. Means (for each plant species) with different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$), df: degrees of freedom

The amino acid proline is known to be accumulated in plant tissues as response of abiotic stresses on several crops (John et al., 2009). Proline concentration in leaves of the four plants was assessed (Fig. 6). The proline concentration was in the same level in control plants. In faba bean, the increase of proline was slight at all SS treatment rates. The application of SS amendment rates stimulated the biosynthesis and accumulation of proline in leaves of wheat, spinach and cucumber plants. In spinach, the significant increase level of proline started at SS treatment of 10 g/kg with concentration of 0.39 mg/g FW to 0.96 mg/g FW at SS treatment 50 g/kg, while, in case of wheat and cucumber the significant increase of proline was detected at 20, 30, 40 and 50 g/kg SS treatment rates. It has been reported that proline may play a role in protection of enzymes, cellular organelles and adjust the osmotic pressure of plant cells under environmental stress conditions (Dhir et al., 2004; Tantrey and Agnihotri, 2010). The stress-protective role of proline was documented in wheat plants treated with cadmium (Asgharipour et al., 2011). Proline was accumulated in wheat plants cultivated in desert reclaimed soil amended with SS (Mazen et al., 2010).

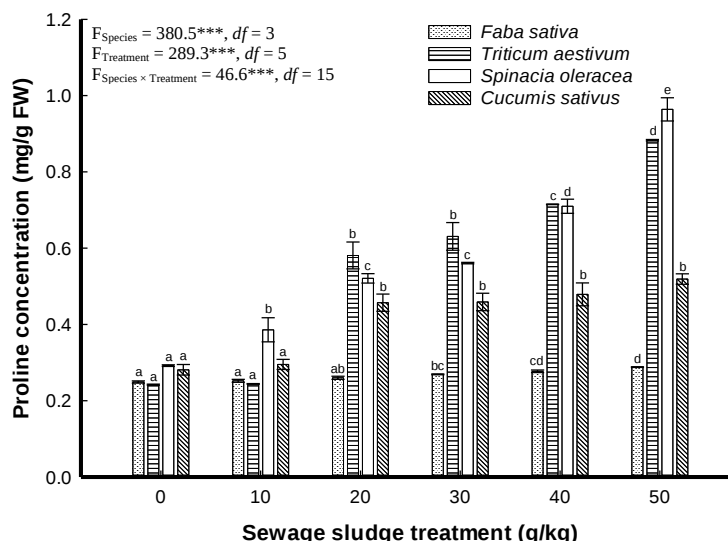


Figure 6. Proline concentration (mean $\pm$ standard error) in leaves of the four plant species *Faba sativa*, *Triticum aestivum*, *Spinacia oleracea* and *Cucumis sativus* grown in soil amended with different sewage sludge treatment rates. Means (for each plant species) with different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$, df: degrees of freedom

Figure 7 presents the relationship between SS treatment rates and phenol concentrations in leaves of faba bean, wheat, spinach and cucumber. SS amendment did not affect the phenol content in faba bean and spinach at all treatment rates. On the other hand, phenol concentration in leaves of wheat and cucumber was declined with SS treatment. The decline was at rates of 40 and 50 g/kg in wheat and was at all SS treatment rates in case of cucumber plant.

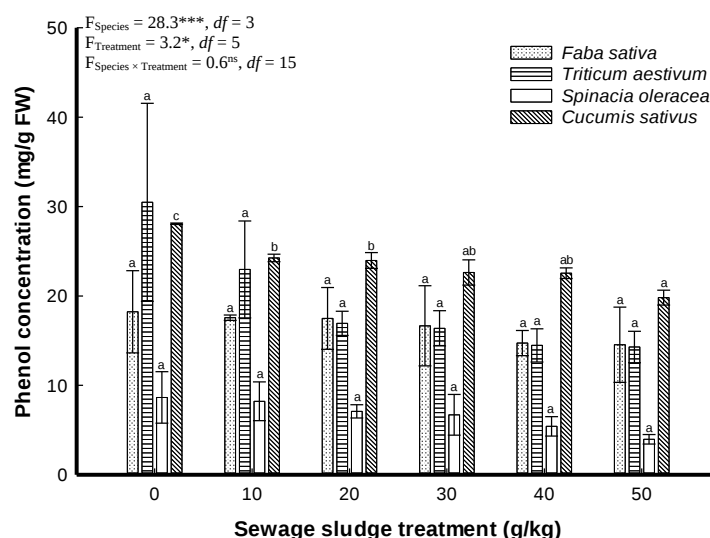


Figure 7. Phenol concentration (mean $\pm$ standard error) in leaves of the four plant species *Faba sativa*, *Triticum aestivum*, *Spinacia oleracea* and *Cucumis sativus* grown in soil amended with different sewage sludge treatment rates. Means (for each plant species) with different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$, *: $p < 0.05$, ns: not significant (i.e., $p > 0.05$), df: degrees of freedom

Phenolic compounds have been known in plant defense mechanisms against abiotic stresses such as heavy metal toxicity and to biotic stresses such as phytopathogenic fungi and bacteria in several plants (Singh and Agrawal, 2010; Daayf et al., 2012; El-Bebany et al., 2013). Thus, diminishing phenol content in plants grown in soil amended with SS may inhibit their resistance to pathogens.

Conclusions

The current investigation assessed the biochemical parameters in four crops (faba bean, wheat, spinach and cucumber) cultivated in agriculture soil amended with SS. The results revealed that the response at the biochemical level is differing according to the plant species and SS application rate. In spinach, starch was decreased significantly with SS treatments, whereas, increased in faba bean, wheat and cucumber at 10, 20, 30 and 40 g/kg SS amendment rates. The concentrations of water soluble carbohydrates and total non-structural carbohydrates were declined in all the tested plants as response to SS amendments. Total lipids were increased in all plants depend on the SS application rates. The increase of total lipids started at 20 g/kg SS treatment in faba bean and spinach, whereas, at 40 and 50 g/kg SS amendments in wheat and cucumber. The amino acid proline accumulated significantly in spinach, wheat and cucumber at all SS treatment rates, while the changes were not significant in faba bean. There were no significant differences in phenol content in faba bean and spinach. The phenol content was decreased in wheat and cucumber leaves with SS treatment. Application of SS to agricultural soils alters the chemical composition of crops. The application of SS at low amendment rates from 10 to 40 g/kg could be useful, whereas, the highest amendment rate, i.e. 50 g/kg is not recommended because of the negative effects on the biochemical composition of the plant, in addition to the possible accumulation of heavy metals in plant materials that may be used for human food or animal feed. The SS amendments could be oriented according to the crop consuming purposes. Future experiments on successive applications of SS in agricultural sites are needed to explore the long-term effects of SS on crops.

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