Effects of Sewage Sludge Amendments on the Growth and Physiology of Sweet Basil

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Abstract: Currently, wastewater treatment plants produce large amounts of sewage sludge. Due to the rich content of organic matter and minerals, sewage sludge can be used as soil amendments for eroded soils. The aim of this work was to assess sewage sludge (SS) in combination with an eroded soil (ES) collected from the North Eastern Romania as growth substrate for sweet basil, and their effect on basil growth and physiology. The experiment was conducted in a greenhouse under controlled environment conditions. The tested substrates were: (1) eroded soil, ES; (2) mixture of eroded soil (15%) + sewage sludge, ES + SS (85%); and (3) sewage sludge, SS (100%). Three types of parameters were studied: morphological traits, physiological, and biochemical parameters. The maximum quantum yield of Photosystem II Fv/Fm was reduced in basil leaves grown on eroded soil (0.80) and was close to the normal value in ES + SS (0.83). Chlorophyll a and the carotenoids content were higher for plants grown on SS and significantly higher for those grown in ES + SS compared with the one of plants grown on ES. The fresh biomass yield and height of basil increased with 44% and 34.5% under ES + SS over ES. Total phenolic content was higher in plants grown on ES (7.34 mg/g dry weight Gallic acid equivalent), which also led to an increased antioxidant activity (44.4%) evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Fourier-Transform Infrared (FT-IR) (4000–400 cm⁻¹) spectra of basil did not show significant qualitative differences among the plants from different treatments. The results of this study demonstrated that SS application led to the improvement of the basil morpho-physiological parameters, allowing the growth of basil on ES + SS.

Keywords: basil; assimilatory pigments; chlorophyll fluorescence; phenolic contents; FT-IR profile

1. Introduction

Sweet basil (Ocimum basilicum L.) is one of the most popular medicinal and aromatic crops that are grown all over the world [1,2]. Basil herbage is known for its proven antimicrobial, insecticidal, antioxidant, and anti-inflammatory in vitro activities [1,2]. Sweet basil synthesizes and accumulates...
essential oils and phenolic compounds, and has shown large intraspecific variability with numerous phenotypes and chemotypes [3,4]. The production of secondary metabolites compounds, such as phenolics or tocopherols is influenced by environmental factors (salinity, moisture, temperature, soil, light) [5,6]. These compounds determine the color of the leaves and flowers, have a role in pollination by attracting pollinators, while also offering a protection against pests and ultraviolet light [7,8]. Basil requires a well-drained soil, rich in nitrogen (N) and phosphorus (P) but also in other micro and macronutrients. Proper plant nutrition ensures high biomass yields with a high content of bioactive compounds [9–11]. Even though the global market demand for horticultural plants is high and increasing, the suitable cultivation land areas may decrease in the future because of soil erosion [12,13].

Soil erosion represents the loss of the top part of the soil and it is a major concern nowadays because it affects yield of agricultural crops, biodiversity, and may cause landslides. At the European Union (EU) level, moderate to high soil erosion affects an area of 140,373 km² of agricultural land, which represents 12.7% of the total EU arable land [12,13]. Soil erosion is caused by wind, rain, substrate characteristics, and also by anthropogenic activities. Soils with low level of organic matter are prone to soil erosion. A suitable method for improving the physico-chemical proprieties of these soils is the use of amendments, which contain a high concentration of organic matter, such as biosolids, compost, vegetal waste, etc. [14].

Sewage sludge (SS) results from wastewater treatment processes. Several studies reported that addition of SS to eroded soil (ES) could improve soil physical and chemical characteristics by altering the bulk density of the soil, aeration and stabilization of ES [15–18]. Large amounts, approximately 10 million tons of dry solids (d.s.) of SS are produced in the EU and 7 million tons of d.s. in the USA every year [19]. The Sewage Sludge Directive 86/278/EEC encouraged the use of SS in agriculture, as it is the most viable method for reusing this “waste material”, because it contains high amounts of macronutrients and organic matter of up to 50–60% d.s. Furthermore, there is an increased interest in biological (organic) farming due to the adverse effects of conventional fertilizer to the environment [20]. Most scientific papers show positive effects of SS application on plant yields, due to the macro nutrient content of the material [21–24]. There are also reports on some negative effects of SS, due to the presence of heavy metals, pesticides, detergents or pathogens such as bacteria, viruses, worm eggs, etc., in biosolids, concerning about the potential transfer of the these elements to edible plant parts, and also esthetic alteration of environment such as bad odor [25–28].

Current legislation on waste water treatment aims to protect human health and the environment, therefore the SS quality was improved compared to that from several decades ago [29,30]. However, in some EU countries, such as Hungary, Greece, and Romania, the SS utilization in agriculture is relatively low, limited to 9.5%, 11.8%, and 4.1%, respectively, of the total amounts of this product [31].

According to previous studies, the cultivation of basil on substrates containing SS has led to increased yield, while the essential oil was free of contaminants [32]. The primary objective of this study was to assess sewage sludge, in combination with an eroded soil, as growth substrate for sweet basil, and their effect on growth and physiology. The secondary objective was to evaluate the macromolecular profile of basil plants in different substrates assessed by the Fourier-Transform Infrared (FT-IR) technique.

2. Materials and Methods

2.1. Plant Material

The experiment involved cultivating sweet basil (Ocimum basilicum L.) plants in a greenhouse in various substrates. Basil seeds of green leaved cultivar ‘Aromat de Buzau’ were obtained from the Agricultural Research and Development Station at Buzau, Romania.
2.2. Experimental Design

For the growth of basil plants, 4 L (15 cm height × 18 cm upper and lower diameter) plastic pots were used. The treatments were: ES—eroded soil (100%); ES + SS—eroded soil + sewage sludge (85% + 15%, v/v); and SS—sewage sludge (100%). The percentages in the mixture were selected based on preliminary studies in which different dosages of SS and ES were applied [33]. The soil (loamy chernozem) used in this study originated from a farmland from the North Eastern region of Romania (46°20’29.0” N 27°41’44.6” E Vaslui), highly eroded by agricultural practices and environmental conditions. In this study, ES was selected, because in Romania, erosion affects 6.3 million ha of land [34]. The SS was obtained from the Municipal wastewater treatment plant in Iasi, Romania after the product was platform dried for two years. Romania produces high amount of sewage sludge, 283 thousand tons dry solids, of which only 35 thousand tons are used in agriculture [35]. In this study, standard soil (potted soil from the market) was not used, because this growth medium has already been tested in previous research [33]. The physico-chemical composition of the growth substrates are shown in Table 1. Heavy metals concentration in SS was within their respective permissible concentrations for use in agriculture [33]. The concentration of heavy metals did not exceed the maximum allowable values for agricultural use [33].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eroded Soil</th>
<th>Sewage Sludge</th>
<th>Eroded Soil + Sewage Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>2.53</td>
<td>2.07</td>
<td>2.5</td>
</tr>
<tr>
<td>Ni</td>
<td>7.56</td>
<td>9.38</td>
<td>8.4</td>
</tr>
<tr>
<td>Cu</td>
<td>4.67</td>
<td>32.07</td>
<td>20</td>
</tr>
<tr>
<td>Zn</td>
<td>13.6</td>
<td>952.1</td>
<td>502</td>
</tr>
<tr>
<td>As</td>
<td>6.17</td>
<td>7.09</td>
<td>6.2</td>
</tr>
<tr>
<td>Cd</td>
<td>0.06</td>
<td>0.55</td>
<td>0.12</td>
</tr>
<tr>
<td>Hg</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.32</td>
<td>1.5</td>
<td>0.65</td>
</tr>
<tr>
<td>Al</td>
<td>246</td>
<td>271</td>
<td>255</td>
</tr>
<tr>
<td>Cr</td>
<td>20.3</td>
<td>27.0</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Elemental analysis of substrates was performed using inductively coupled plasma mass spectrometry (ICP-MS): A sample of 0.1–0.4 g of substrate were mixed with sulfuric acid 96% (3 mL), nitric acid 60% (1 mL), and hydrofluoric acid 48% (1 mL). A nitrogen pressurized (40 bar) microwave digester Ultrawave, manufactured by Milestone SRL, Sorisole (BG) Italy), was used for samples digestion. Gradient temperature 50 to 240 °C was applied, then samples were diluted with 40 mL mixture of 1% H$_2$SO$_4$, 1% HNO$_3$, and 1% HF in ultrapure water. ICP-MS 7700s (Agilent Technologies, Santa Clara, CA, U.S.A.) with Ar (0.7 L/min) as gas carrier and integration time 0.1 s/channel or 0.3 s per m/z was used for elemental analyses of the solutions. Individual elemental concentrations were calculated based on the natural isotopic abundance of each element.

Substrates were sieved through a 0.2 cm mesh size sieve. For each treatment, three pots were filled with the corresponding substrates, and were seeded using 10 basil seeds. The plants were thinned down to four per pot after a week. The experiment was set up in triplicate. Cultivation conditions...
were 22 °C (night) and 25 °C (day), artificial light was supplied by 4800 K fluorescent tubes (14 h light: 10 h dark), and the atmospheric humidity was relatively constant, at around 50%. Plants were irrigated twice a week with distilled water (50 mL/L. The growth of basil lasted for three months, until the plants reached the full flowering stage.

2.3. Morphological Traits

In order to evaluate the effects of the ES, SS, and ES + SS on basil growth, morphological, physiological, and biochemical measurements were performed. For each treatment, 12 individual plants were assessed for stem height (cm), fresh weight (g), and root length (cm).

2.4. Physiological Parameters

Chlorophyll fluorescence (Fv/Fm and ΦPSII) was measured with a portable fluorometer FMS2 (Hansa Tech Ltd. King’s Lynn, Norfolk, UK). Fv/Fm was measured following a 20 min dark adaptation of leaves using provided clips. ΦPSII was measured at normal light regime.

2.5. Biochemical Parameters

Assimilatory pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted from 0.1 g of leaves in acetone (80%). Pigment contents were calculated using equations described in [36], using the optical density of leaf extracts recorded at 470 nm, 646 nm, and 663 nm.

For total phenolic, total flavonoids and antioxidant activity, a 5% (w/v) leaf extract in absolute ethanol was prepared.

The total phenolic content: A sample of 0.1 mL of extract was mixed with Folin Ciocalteu reagent (Merck, Darmstadt KGaA, Germany). After 5 min incubation Na2CO3 7.5% was added. After 90 min incubation, results were calculated using the optical density recorded at 760 nm and expressed, according to a calibration curve, as gallic acid equivalents (GAE) per gram of fresh weight [37].

Total flavonoid content: A sample of 0.25 mL extract was mixed with 0.1 mL NaNO2. After 5 min incubation, 0.15 mL AlCl3 was added. After 6 min incubation, 0.5 NaOH was added and results were calculated using the optical density recorded at 510 nm and expressed, according to a calibration curve, as quercetin equivalents per gram of dry weight [37].

Antioxidant activity: A sample of 0.1 mL extract was mixed with 2.9 mL of 60 µM DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma, Germany). After 180 min incubation, the optical density was recorded at 515 nm. The results were calculated as inhibition percentage of a blank composed of DPPH and extract solvent (ethanol) [37].

All spectrophotometric measurements were performed with a Shimadzu UV mini 1240 (Kyoto, Japan) spectrophotometer. The length of the light path glass cuvettes used was 1 cm.

2.6. Fourier-Transform Infrared (FT-IR) Spectroscopy Assay

Infrared spectra were recorded in transmission mode using a Bruker Vertex 70 FT-IR (Billerica, MA, USA) spectrometer, in the range of 4000-400 cm⁻¹, taking 32 scans with 2 cm⁻¹ resolution of each sample. Samples from fresh basil leaves were prepared initially as a fine powder. In order to record FT-IR data, the samples were compressed at 10 t/cm² to form pellets with a diameter of 13 mm.

2.7. Statistical Analyses

Data were analyzed by ANOVA followed by a post hoc test Tukey (p < 0.05). Normality and homogeneity of variances were tested before Tukey test. The program used was IBM SPSS v20 (Armonk, NY: IBM Corp.), the results were expressed as means and standard errors.
3. Results

3.1. Morphological Traits

Morphological traits of the basil plants depending on the treatments are presented in Table 2. The overall growth of basil was stimulated by the SS treatments. Plant height and fresh yield were significantly increased by the SS amendments. The height of plants was increased by 34.5% when cultivated on 15% SS compared with the plants cultivated on ES and SS. Fresh yield per plant was also increased by 44.2% in the case of basil plants cultivated on the amended substrates compared with plants in ES. Root length was not significantly affected by the treatments (Table 2).

Table 2. Morphological traits of basil plants grown on different substrates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eroded Soil</th>
<th>Eroded Soil + Sewage Sludge</th>
<th>Sewage Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>28.56 ± 0.53 a</td>
<td>38.64 ± 0.55 b</td>
<td>28.64 ± 0.96 a</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>8.75 ± 0.18 a</td>
<td>11 ± 0.1 b</td>
<td>8.03 ± 0.53 a</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>5.83 ± 1.09 a</td>
<td>7.33 ± 0.33 a</td>
<td>4.33 ± 0.6 a</td>
</tr>
</tbody>
</table>

Results are means ± standard errors. Values with the same lower-case letters are not statistically different at $p < 0.05$ according to Tukey’s test.

3.2. Physiological Parameters

Regarding the chlorophyll fluorescence, the maximum yield of PSII ($F_v/F_m$) was significantly increased in the ES + SS treatment, while the actual yield of PSII ($\Phi_{PS2}$) was not statistically significant for this parameter (Table 3).

Table 3. Chlorophyll fluorescence parameters of basil leaves grown on different substrates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eroded Soil</th>
<th>Eroded Soil + Sewage Sludge</th>
<th>Sewage Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_{PS2}$</td>
<td>0.83 ± 0 a</td>
<td>0.82 ± 0 a</td>
<td>0.84 ± 0 a</td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>0.80 ± 0 a</td>
<td>0.83 ± 0 b</td>
<td>0.82 ± 0 a</td>
</tr>
</tbody>
</table>

Results are means ± standard errors. Values with the same lower-case letters are not statistically different at $p < 0.05$ according to Tukey’s test.

3.3. Biochemical Parameters

Basil leaves chlorophyll a content was not significantly affected by the treatments, while chlorophyll b and carotenoids were significantly increased in basil leaves cultivated on SS and SS amended substrates compared with basil cultivated on ES (Table 4).

Table 4. Assimilatory pigments contents in basil leaves grown on different substrates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eroded Soil</th>
<th>Eroded Soil + Sewage Sludge</th>
<th>Sewage Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorophyll a</td>
<td>0.84 ± 0.06 a</td>
<td>0.91 ± 0.05 a</td>
<td>1.02 ± 0.04 a</td>
</tr>
<tr>
<td>chlorophyll b</td>
<td>0.44 ± 0.01 a</td>
<td>0.51 ± 0.02 b</td>
<td>0.61 ± 0.01 c</td>
</tr>
<tr>
<td>carotenoids</td>
<td>0.18 ± 0.02 a</td>
<td>0.19 ± 0.01 ab</td>
<td>0.27 ± 0.02 c</td>
</tr>
</tbody>
</table>

Results are means ± standard errors. Values with the same lower-case letters are not statistically different at $p < 0.05$ according to Tukey’s test.

In this experiment, the synthesis of phenolic compounds expressed as total phenolic content and flavonoid content varied depending on the growth substrate (Table 5). The amount of these compounds was significantly higher under the ES treatment. Furthermore, the antioxidant capacity of basil plants cultivated on ES, determined by the capacity of leaves extracts to scavenge DPPH free radicals was considerably higher compared to plants cultivated on SS (Table 5).
FT-IR analyses (Figure 1) revealed a biochemical profile of basil leaves with various functional groups which can be assigned as shown in Table 6. The broad band between 3620–3080 cm\(^{-1}\) is associated to hydrogen-bonded to oxygen -OH stretching vibrations of alcohols and phenols type and N-H bond of amino group. Furthermore, the strong absorption peaks from 2918 cm\(^{-1}\) and 2853 cm\(^{-1}\) are attributed to aliphatic \(\nu\)(C-H) from methyl CH\(_3\) and methylene CH\(_2\) functional groups [38]. In the “fingerprint” region, the dominant peak around 1737 cm\(^{-1}\) and its shoulder around 1610 cm\(^{-1}\) are assigned to presence of carbonyl C=O stretches of carboxylic acid type and, respectively, the \(\nu\)(C-O) in carboxyl coupled to the \(\nu\)(N-H) of the primary amine (-NH\(_2\)). The absorption \(\nu\)(C-N) of aromatic amine group absorb at 1379 cm\(^{-1}\) [39]. Furthermore, the infrared peak around 1063 cm\(^{-1}\) might be characteristic to C-C stretching of alcohols.

**Table 5.** Phenolic contents and radical scavenging activity of extracts of basil leaves grown on different growth substrates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eroded Soil</th>
<th>Eroded Soil + Sewage Sludge</th>
<th>Sewage Sludge</th>
<th>Control ASCORBIC Acid (1mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols (GAE/g f.w.)</td>
<td>7.34 ± 0.03 b</td>
<td>7.18 ± 0.11 b</td>
<td>5.17 ± 0.16 a</td>
<td>-</td>
</tr>
<tr>
<td>Total flavonoids (mg quercetin eq/g f.w.)</td>
<td>14.41 ± 0.23 c</td>
<td>7.04 ± 0.11 b</td>
<td>5.07 ± 0.25 a</td>
<td>-</td>
</tr>
<tr>
<td>DPPH radical Scavenging activity (%)</td>
<td>44.37 ± 0.47 b</td>
<td>44.02 ± 0.51 b</td>
<td>25.98 ± 1.22 a</td>
<td>91.97 ± 0.16</td>
</tr>
</tbody>
</table>

Results are means ± standard errors. Values with the same lower-case letters are not statistically different at \(p < 0.05\) according to Tukey’s test.

**Figure 1.** Fourier-Transform Infrared (FT-IR) spectra of sweet basil (Ocimum basilicum L.) cv. “Aromat de Buzau” leaves grown on different growth substrates.

**Table 6.** Values of absorbance peaks and the chemical groups assignment obtained from the infrared spectra of basil leaves grown on different substrates.

<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Chemical Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3620–3080</td>
<td>-OH stretching vibrations from alcohols and phenols,</td>
</tr>
<tr>
<td></td>
<td>N-H bond of amino type</td>
</tr>
<tr>
<td>2918, 2853</td>
<td>To aliphatic (\nu)(C-H) from methyl and methylene functional groups</td>
</tr>
<tr>
<td>1737</td>
<td>Stretching vibrations of carbonyl C=O</td>
</tr>
<tr>
<td>1610</td>
<td>N-H bending of the primary amine</td>
</tr>
<tr>
<td>1379</td>
<td>C-N stretching of aromatic amine</td>
</tr>
<tr>
<td>1063</td>
<td>C-C stretching of alcohols</td>
</tr>
</tbody>
</table>
4. Discussion

This study assessed the possibility for cultivating sweet basil on SS amended ES, and evaluated the growth and physiology depending on the treatments. The plant height and yields were increased by the application of SS. The increase may be explained by the much higher contents of nutrients and increased biological activities of SS in the SS treatments compared to the ES. The total N, P, and K contents were 71, 127, and 22 times higher, respectively, in the SS treatment compared with the ES. Furthermore, the organic matter content was 21 times higher in the SS treatment compared with the one in the ES. It is known that higher content of organic matter provide higher amounts of macronutrients and also a higher bioavailability of these nutrients [40,41]. Moreover, the addition of municipal SS resulting from the waste water treatment facilities contributes to the enrichment of microbes in soil that produce enzymes (dehydrogenases, phosphatases, peroxidases, etc.) [42]. According to previous research [43], long term application of biosolids led to a significant negative correlation between soil pH and the microorganisms biomass (bacteria and fungi), and positive correlation between soil NO$_3^-$ and microbial biomass. Another study reported that microorganisms biomass was not modified by application of a fertilizer made from sewage sludge ash [44]. Other researchers [45] found that microorganisms' biomass was not affected considerably by successive application of sewage sludge; however, basal respiration and the fluorescein diacetate hydrolytic activity increased with the sludge dosage increase. Furthermore, these soil enzymes increase the availability of mineral nutrients and the concentration of low molecular mass organics [46]. Vegetation plays an essential role in soil rehabilitation, leading to increases in organic carbon, N, and other macro- and micronutrients as a result of accumulation and formation of organic matter and humus resulting from the above ground biomass, litter, and roots [41].

Similar results were obtained for other plant species under SS treatments, with significant positive influences on response variables [23,47], but also for basil [48]. High concentrations of nutrients in the growth substrate stimulate development of new organs (stems, leaves, flowers) in basil plants but also induce leaves senescence [49]. Some species may be sensitive to the toxic effects of sludge amendments, with reductions in root lengths and other morphological traits [27,50], but such effects were not reported for basil grown on the analyzed sludge. Moreover, the contents of heavy metals in the tested SS do not exceed the limits for agricultural use stated in the EU legislation and the country specific regulations [51]. However, the maximum basil growth was observed on ES + SS and not for SS alone. This may be explained by the non-optimal physical characteristics of the SS. The higher fresh biomass obtained for plants cultivated on SS amended substrate may be attributed to an intense development of new organs (stems, leaves, flowers) [52]. In addition, it may suggest a better absorption of water through the radicular system because of the low density of the substrate.

The increase in assimilatory pigments content in basil leaves grown on SS and SS-amended substrates may be attributed to the presence of essential metal ions and the high concentration of available N and P in SS, elements required for chlorophyll biosynthesis [53]. Although some species may display reduction in chlorophyll contents grown on SS, this may be due to metal toxicity at elevated concentrations [25]. A positive correlation between N levels and chlorophyll amounts of leaves has been shown for several species [26,54] and occurs due to the fact that N is included in the structure of chlorophyll molecules [55]. Carotenoids play an important role in the antioxidant activity [56], acting as free radical quenchers in the thylakoid lumen of chloroplasts. The elevated levels recorded for the SS treatment may be an indication of stress on basil plants cultivated on SS, probably due to high amounts of copper (Cu) and zinc (Zn). For example, Zn concentration can reach up to 911 mg/kg in leaves of basil grown on biosolids [33].

The positive effects of SS on plant growth have been reported, suggesting the use of SS as organic soil amendment and fertilizer. However, concerns about possible toxicity of SS have also been expressed [57,58]. One parameter that reflects possible toxic influences on plant physiology is chlorophyll fluorescence, which may be used to assess the efficiency of the photosystem II and therefore of the photosynthetic capacity of plants [59]. Parameters such as $F_v/F_m$ and $\Phi_{PSII}$ (maximum
quantum yield of PSII and actual quantum yield of PSII) were reported to vary with the application of SS amendments [60], showing likely toxic effects.

On the other hand, the effect of SS amendment was also shown to exert beneficial effects on plant physiology [61]. Differential plant physiological responses observed through chlorophyll fluorescence measurements may be species-specific [62]. The results from this study showed that, at the level of photosystem II, the effects of SS were minimal, further underlining the lack of toxicity of tested SS on basil.

The second objective was related to the macromolecular profile of basil plants in different substrates assessed by FT-IR technique. The macromolecular composition of the basil extracts appeared unaltered, indicating that no major effects on metabolic processes occurred as a result of SS application. Quantitative changes manifested through the increased synthesis of phenolic compounds in ES-grown plants can be considered a stress response to the presence of certain limiting factors [62], especially taking into account the low nutrient content of this substrate. According to the growth/defense balance, plants supplied with adequate N amounts use carbon (C) mainly for primary metabolites, while secondary metabolites with protective role are produced under N limitation [63]. Although the phenolic contents were decreased in plants grown on SS alone, the FT-IR profile did not show differences between treatments. This indicates that SS amendment induced only quantitative changes in basil plants and not qualitative ones, with positive effects on plant growth. Similarly, previous research has shown a positive correlation between organic and inorganic N from SS and shoot biomass of ryegrass *Lolium perenne* L. [64]. The addition of 15% SS to ES may represent a feasible alternative for SS reuse as important amounts of SS can be thus disposed with positive effects on soil properties and plant yields.

5. Conclusions

This study indicated that the tested, air dried municipal solid SS can be used for growing sweet basil with positive effects on plant growth and physiology when grown on ES. The SS exerts stimulatory effects on phenolics production, while the investigated physiological parameters were not affected. The results showed that selected SS from the Romanian wastewater treatment plants can be used for the fertilization of ES and for the production of high-value crops such as sweet basil. Further testing may be conducted to optimize the SS application rates in order to improve ES properties for basil crop fertilization and for other high-value crops.


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**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES</td>
<td>eroded soil</td>
</tr>
<tr>
<td>ES + SS</td>
<td>mixture of eroded soil (15%) + sewage sludge, (85%)</td>
</tr>
</tbody>
</table>

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