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# Investigation of the impacts of the by-product of sewage treatment on some characteristics of maize in the early growth stage

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## *SUMMARY*

*The use of sewage sludge on arable land has been widespread for many years. This by-product, treated as waste, can provide valuable nutrients to the soil, but the applied amount of sewage sludge to arable land is limited. The possibility of application of sewage sludge is essentially determined by the composition of the sludge. The goal of the experiment was to demonstrate that the physiological, morphological, and biochemical parameters of maize (Zea mays L. cv. Armagnac) linearly change with increasing concentrations of sewage sludge (25%, 50%, and 75% as m/m%). The experiment was set up in a glasshouse. The following parameters were investigated: plant height, relative chlorophyll content, photosynthetic pigments (chlorophyll-a, chlorophyll-b, carotenoids), and leaf proline and malondialdehyde (MDA) content, and PS II quantum efficiency in the 3-leaf stages of the plants. Sewage sludge applied in lower doses had a beneficial effect on the initial growth of maize. The relative chlorophyll content was significantly higher in all treatments compared to the control. There was no significant difference in the maximum quantum efficiency of PS II reaction centers among the treatments. In this experiment, different concentrations of sewage sludge treatments had different impacts on the MDA and proline content of maize leaves. The proline content was significantly higher in all treatments, while the MDA content did not change significantly compared to the control.*

***Keywords:*** *chlorophyll; malondialdehyde; nutrients; proline; PS II*

## *INTRODUCTION*

With the professional use of wastewater, treated sludge, sewage sludge, and composted sludge, negative consequences on soil, ground, and surface water, as well as the health of people, plants, and animals can be avoided. The agricultural use of sewage sludge and sewage sludge compost needs an agricultural use permit, which is authorized by the district office of the county seat (hereinafter referred to as soil protection authority) acting in the area of protection of the county government officials acting in the area of soil protection, which can be authorized for a maximum period of five years. In Hungary, the use and treatment of wastewater, sewage sludge, and sewage sludge composts are regulated by Government Decree 50/2001 (IV.3.). The regulation aims to avoid several environmental damages.

The continuously growing human population requires more food that needs sufficient nutrient application. The favorable effects of fertilizers on crops are well-documented. The application of sewage sludge (SS) can decrease the cost of nitrogen and phosphorus fertilizers and may improve crop yield (Petersen et al., 2003). So, new plant nutrient supply materials are needed for plant nutrition. Several researchers have investigated the impacts of sewage sludge on crops (Lamastra et al., 2018; Burducea et al., 2019; Tóth and Moloi, 2019; Ekane et al., 2021; Buta et al., 2021). More and more studies deal with the amount of nutrients and heavy metals that can be absorbed by plants from sewage sludge (Li et al., 2019). Microelements (like B, Fe, Mn, Cu, Zn, and Mo) are crucial for plants and they are existing in significant amounts in sewage sludge, but these elements can be toxic in excessive amounts. Thomas et al. (2006) found that the relative intensity of growth and the net assimilation index of sunflowers were higher when sewage sludge treatment was used compared to commercial fertilizer treatment. Plants treated with sewage sludge increased the mass of accumulated dry matter, as well as the amount of $NO_3$-N, Ca, total N, Mg, and K. A significant change was also observed when the impacts of sewage sludge were examined on morphological characteristics, e.g. leaf area. The heavy metal content of sewage sludge is a controversial topic. Inevitably, heavy metals do not get into the wastewater, and through it into its sludge, from there into the soil, and then into the food chain. Sewage sludge can increase the heavy metal content of the soil when applied to fields (Speir et al., 2003). No harmful accumulation of heavy metals was found in some experiments. However, as a result of sludge loading with a higher heavy metal content, a significant increase was observed for almost all heavy metals in the upper cultivated layer of the soil (Kádár and Morvai, 2008). Reason of the high economic and environmental costs of incineration and landfilling operations, the use of sewage sludge on fields may be a favored alternative (Singh and Agrawal, 2008). Singh and Sinha (2005) stated that in the application of sewage sludge 30 and 60 days after the sowing, the concentration of carotenoid and total chlorophyll contents in mustard leaf were higher compared to the control, non-sewage sludge-treated plants. However, these pigments decreased after 90 days of sowing. High heavy metal concentrations can create an accumulation of proline in plants because of the heavy metal content of the sludge. Lipid peroxidation and proline content increased significantly in mustard treated with sewage

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sludge (Singh and Sinha, 2005). Burducea et al. (2019) proved that the utilization of SS on eroded soil increases the maximum quantum yield of photosystem II *F*v/*F*m in basil leaves compared to the basil grown on eroded soil without sewage sludge application. In addition, they found that the content of chlorophyll-a and carotenoids increased in plants treated with SS.

This study aimed to examine the effects of the increasing concentration of SS and prove that the application of the increasing concentration of SS increases the measured parameters in maize treated with sewage sludge compared to the control, non-sewage sludge-treated maize.

## *MATERIALS AND METHODS*

The examination was conducted in the glasshouse of the Institute of Horticulture, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. The test plant was maize (*Zea mays* L. cv. Armagnac). Four treatments (0%, 25%, 50%, and 75% sewage sludge based on mass) were applied with seven plants per treatment (one plant per pot) in three replicates. Maize plants were planted in PVC tubes. The volume of the experimental pots was 750 ml, each pot contained 500 g soil as the control or soil and sewage sludge mixtures as the three applied treatments. Three seeds per tube were sown, and one week after planting only well-developed maize plants were separated. Temperatures for growth varied between 32 °C (daytime) and 25 °C (night), the humidity was kept above 40% (45–55%) during the experiment.

The dewatered sewage sludge came from a sewage treatment plant in Debrecen, a large city in Hungary. The main parameters of both the soil and the sewage sludge were determined in an accredited laboratory (*Tables 1–2*).

The used soil originated from Karcagpuszta, Hungary (N 47.25154; E 20.83351). The soil type is poor-quality saline soil (K3).

*Table 1.* **The element contents of the sewage sludge used**

| **Parameter** | **Element content (mg kg$^{-1}$ dry material)** | **Element content concerning the permitted limit (%)** |
|---|---|---|
| Cadmium (Cd) | 0.85 | 8.5 |
| Cobalt (Co) | 4.44 | 8.88 |
| Chromium (Cr) | 50.43 | 5 |
| Copper (Cu) | 181.04 | 18.1 |
| Mercury (Hg) | 0.63 | 6.3 |
| Molybdenum (Mo) | 6.3 | 31.5 |
| Nickel (Ni) | 22.14 | 11 |
| Lead (Pb) | 19.2 | 2.5 |
| Selenium (Se) | 1.5 | 1.5 |
| Zinc (Zn) | 1500.6 | 60 |

*Table 2.* **The main parameters of the soil used**

| **Title** | **Value** |
|---|---|
| pH value (KCl) | 6.3 |
| Gold-bonded number [$K_A$] | 50 |
| Total water soluble salinity [m/m%] | 0.06 |
| Carbonic acid lime [m/m%] | 0.21 |
| Humus content [m/m%] | 2.9 |
| (nitrate+nitrite)-N (KCl soluble) [mg kg$^{-1}$] | 4.2 |
| Phosphorus pentoxide (AL soluble) [mg kg$^{-1}$] | 211 |
| Potassium oxide (AL soluble) [mg kg$^{-1}$] | 419 |
| Sodium (AL soluble) [mg kg$^{-1}$] | 445 |
| Magnesium (KCl-soluble) [mg kg$^{-1}$] | 647 |
| Sulphate-sulphur (KCl-soluble) [mg kg$^{-1}$] | 7.9 |
| Zinc (EDTA-Na2 soluble) [mg kg$^{-1}$] | 0.9 |
| Copper (EDTA-Na2 soluble) [mg kg$^{-1}$] | 9.0 |
| Manganese (EDTA-Na2 soluble) [mg kg$^{-1}$] | 201 |

Plant height was measured with a metal ruler between the soil surface and the origin of the most developed leaf.

Chlorophyll Meter SPAD-502 Plus (Minolta, Japan) was used to determine the relative chlorophyll content of the 2nd and 3rd leaves of plants at the 3-leaf stage, five times per leaf.

The photosynthetic pigments were determined by the method of Moran and Porath (1980) and Wellburn (1994).

The photochemical efficiency PS II system was measured using the pulse-modulated chlorophyll fluorescence induction procedure (Murchie and Lawson, 2013) using an OS5p+ pulse-modulated portable chlorophyll fluorometer (Opti-Sciences, Hudson, USA). The leaves were dark-adapted for 20 min before the measurements.

The proline content was determined based on Carillo and Gibon (2011). Leaf samples (0.1 g) were rubbed with 2 mL of 70% ethanol and placed on ice. Subsequently, they were centrifuged for 10 min at maximum speed and 500 mL of supernatant was placed in clean Eppendorf tubes to which 500 mL of 60% glacial acetic acid and 1% ninhydrin mixture, and 500 mL of 20% ethanol were added. The mixtures were then vortexed and heated for 20 min at 95 °C in a water bath (Bandelin Sonorex Digitec DT 255 H, German). The mixtures were then centrifuged at 10 000 rpm for 10 minutes, and placed on ice. The absorbance was measured at 520 nm using a Nicolet Evolution 300 LC spectrophotometer (England). Finally, the proline content was calculated using the pre-fitted standard curve.

The extent of lipid peroxidation was measured by the amount of malondialdehyde (MDA) produced according to Heath and Packer (1968). The plant sample (0.1 g) was rubbed with 1 mL of 0.25% (w/v) thiobarbituric acid (TBA) and 1 mL of 10 % trichloroacetic acid (TCA) in liquid nitrogen. The extracts were centrifuged at 10800 rpm for 25 min at 4

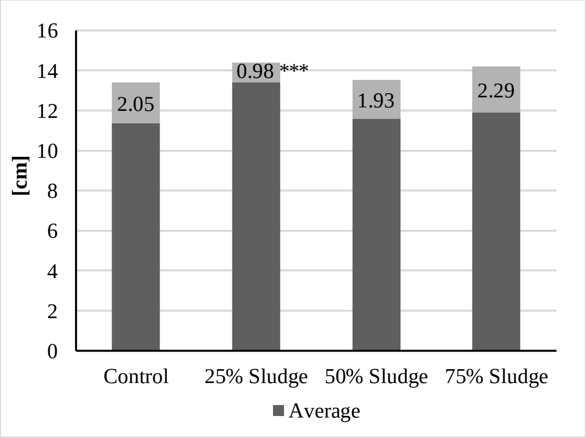
°C. Then, 0.3 mL of supernatant was pipetted into clean Eppendorf tubes containing a mixture of 0.5% (w/v) thiobarbituric acid (TBA) and 20% trichloroacetic acid (TCA). These were heated in a water bath (Bandelin Sonorex Digitec DT 255 H, German) at 95 °C for 30 min. 532 and 600 nm were used to measure the absorbance of the extracts (Nicolet Evolution 300 LC spectrophotometer, England). The used extinction coefficient for the MDA calculation was 155 $mM^{-1}$ $cm^{-1}$.

All of the measurements were done 23 days after sowing in the 3-leaf stage.

## *RESULTS AND DISCUSSION*

In terms of plant height (*Figure 1*), the 25% sewage sludge treatment resulted in plants that were 15% taller than the control plants. For the other treatments, there were no statistically significant differences. This means that the application of a lower dose had a beneficial effect at the initial growth stage. Lobo and Filho (2009) also proved the positive impact of sewage sludge application on sunflower height. In addition, the shoot length of mung beans also was higher when sewage sludge was applied (Singh and Agrawal, 2010). The usage of sewage sludge increased the plant height of the golden rod. The plant height was linearly increased with an increase in sewage sludge application rates and it was the highest in the 100% sewage sludge treatment (Solanki et al., 2016).

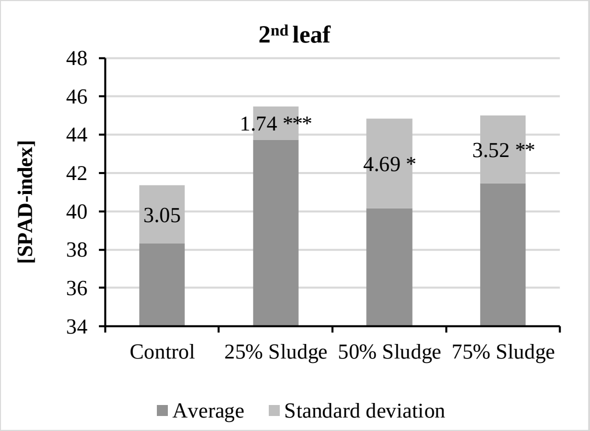
*Figure 1.* **Height of maize under different concentrations (25%; 50%; and 75%) of sewage sludge treatments in the 3-leaf stages (23-day-old plants)**



Significant difference relative to the control: *** $p < 0.001$

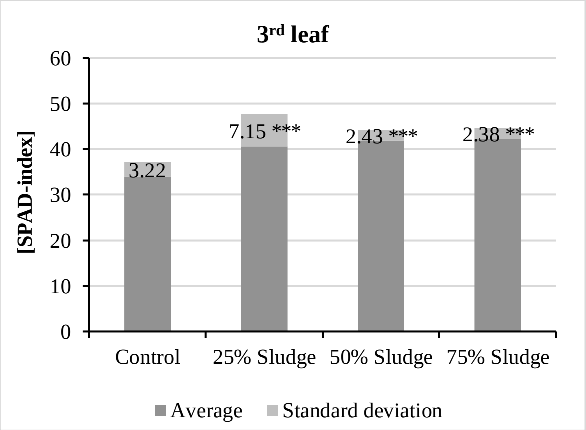
The relative chlorophyll content in the second and third leaves of the plants was measured. It can be seen (*Figures 2* and *3*) that all treatments had significantly higher values than the control, non-treated plants.

*Figure 2.* **The impact of different sewage sludge treatments (25%; 50%; and 75%) on the relative chlorophyll content (SPAD-index) in the second leaf of maize in the 3-leaf stages (23-day old plants)**



The significant difference compared to the control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

*Figure 3.* **The impact of different sewage sludge treatments (25%; 50%; and 75%) on the relative chlorophyll content (SPAD-index) in the third leaf of maize in the 3-leaf stages (23-day old plants)**



Significant difference relative to the control: *** $p < 0.001$

We also measured the amount of photosynthetic pigments. It can be seen (*Table 3*) that the 50% sewage sludge treatment had a significantly higher value relative to the control, whereas 25% and 75% sludge treatments had no effects.

Plants planted in 50% SS had significantly higher levels of photosynthetic pigments compared to the control. The increase was 26% for chlorophyll-a, 81% for chlorophyll-b, and 26% for carotenoids. Furthermore, the highest treatment effect (75% sludge) was around the control except for chlorophyll-b, where it was almost 5% lower (*Table 3*). The application of sludge (vermicomposted) caused a significant increase in the chlorophyll content of beans relative to the control soil (Belmeskine et al., 2020). The chlorophyll content in sugar beet leaf increased when 10% and 25% concentrations of SS treatment were applied, while the

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50% concentration did not induce an increase (Yilmaz and Temizgül, 2012).

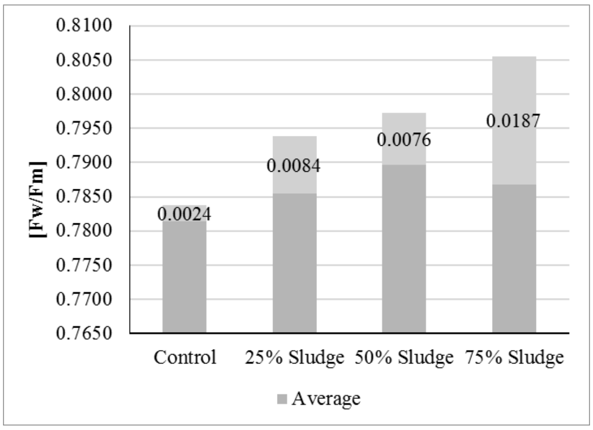
*Table 3.* **The impact of different sewage sludge treatments (25%; 50%; and 75%) on the photosynthetic pigments content (mg $g^{-1}$) of the third leaf of maize in the 3-leaf stages (23-day-old plants)**

| Treatment | Chlorophyll-*a* | Chlorophyll-*b* | Carotenoids |
|---|---|---|---|
| Control | 16.75 ±1.80 | 5.27±1.28 | 8.18±0.87 |
| 25% Sludge | 17.93±3.74 | 5.97±2.25 | 8.52±1.49 |
| 50% Sludge | 21.14±1.49* | 9.55±1.90* | 10.35±0.30* |
| 75% Sludge | 17.32±1.59 | 5.05±1.25 | 8.19±0.63 |

Significant difference relative to the control: * $p < 0.05$

None of the applied treatments had effects on the maximum quantum efficiency of the PS II reaction center (*Figure 4*) among the treatments.

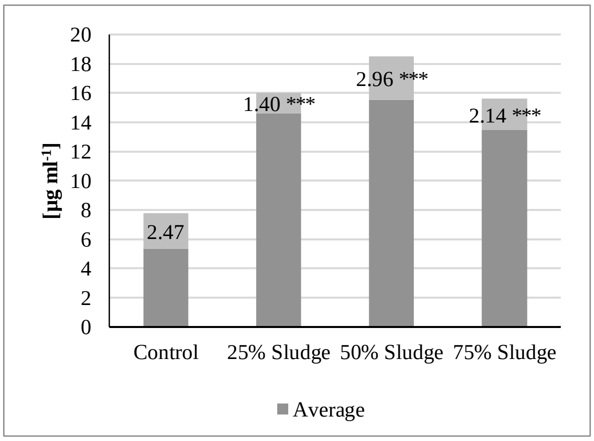
*Figure 4.* **The impact of different sewage sludge treatments (25%; 50%; and 75%) on the maximum quantum efficiency ($F_V/F_m$) of the PS II reaction center in the third leaf of maize in the 3-leaf stages (23-day-old plants)**



Malondialdehyde is the outcome of lipid peroxidation. It can be found in cells naturally and the increased amount of MDA indicated the oxidative stress condition. The extent of lipid peroxidation and the amount of MDA are influenced by various stress factors (Prakash et al., 2018). Proline is a so-called stress amino acid that accumulates in response to physiological stress (Hayat et al., 2012). It can be seen (*Figure 5*) that the proline was notably higher in 25%, 50%, and 75% of sludge treatments relative to the non-treated plants.

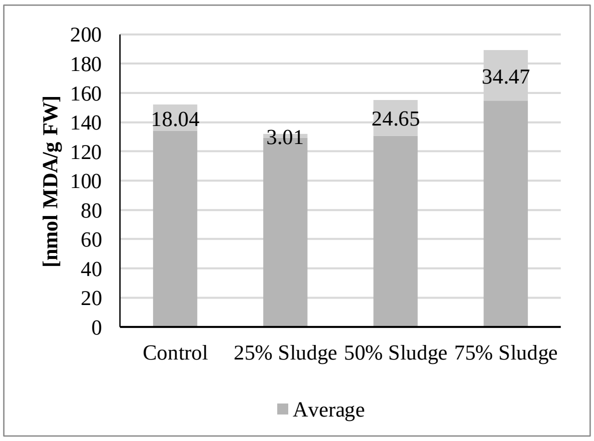
However, the amount of MDA did not change significantly compared to the values measured in control plants (*Figure 6*). It can be concluded that the applied sewage sludge treatments stressed the plants but did not affect all parameters. The MDA content of lettuce significantly increased when the solid by-product of wastewater treatment (2.5%, 5%, and 7.5%) was used in comparison with the control (Fedeli et al., 2023). However, they did not find any significant difference between the 2.5 and 5% treatments. Proline content was also notably higher in the 7.5% SS-treated plants, while no significant difference was observed between 2.5 and 5% SS treatments. There was no significant difference among the three (2.5%, 5%, and 7.5%) treatments when the maximum leaf conductance was measured (Elloumi et al., 2016). Spormann et al. (2023) stated that the elevated proline content is an indicator of stress sensitivity with no clear correlation with higher antioxidant activity. Du et al. (2014) observed a slightly positive correlation between MDA and proline content. On the other hand, Ozden et al. (2009) stated that the higher proline content may reduce the MDA content which can reduce the unwanted impacts of stress.

*Figure 5.* **The impact of different sewage sludge treatments (25%; 50%; and 75%) on the proline content in the third leaf of maize in the 3-leaf stages (23-day-old plants)**



The significant difference compared to the control: *** $p < 0.001$

*Figure 6.* **The impact of different sewage sludge treatments (25%; 50%; and 75%) on the malondialdehyde content (MDA) in the third leaf of maize in the 3-leaf stages (23-day-old plants)**



## *CONCLUSIONS*

The results showed that the lower dose sewage sludge treatment (25% Sludge) had positive effects on the initial growth of the plants. In addition, all the applied treatments increased the relative chlorophyll content of maize but did not affect the PS II reaction center. However, the proline content also indicates that all treatments stressed the plants, though the nature and extent of the stress are not clear yet. In addition, the amount of MDA did not change significantly compared to the values measured in control plants. This indicates that there is no correlation between MDA and proline content in this experiment.

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