EFFECTS OF URBAN SLUDGE APPLICATION ON PHOTOSYNTHETIC AND GROWTH CHARACTERISTICS OF ALFALFA SEEDLINGS

WANG, T. E.[1†] – JIA, S. S.[1†] – WANG, Y.[1] – ZHAO, G. Y.[2*] – HAN, X.[3*]

[1]*School of Geography and Tourism, Harbin University, Harbin 150086, China*

[2]*Forest and Grassland Bureau Forest and Grassland Work Station of Mishan City, Mishan 158300, China*

[3]*Institute of Natural Resources and Ecology. HAS, Harbin 150086, China*

[†]*These authors contributed equally to this work.*

[*]*Corresponding author*
e-mail: hanxubest9@163.com; lyjzgy@126.com

(Received 24th Jul 2024; accepted 3rd Dec 2024)

Abstract. This study used urban sewage-treated sludge and soil from rocky desertified areas in Harbin, China, as experimental materials. Through indoor pot experiments, different proportions of sludge were applied to rocky desertified soil, and Alfalfa (*Medicago sativa*) was planted to explore the effects of sludge addition on the photosynthesis and growth of alfalfa seedlings during the seedling stage. The study aims to provide a scientific basis for the resource utilization of urban sludge in Harbin. The results show that as the proportion of added sludge increases, the organic matter, total nitrogen, total phosphorus, available nitrogen, and available phosphorus in the soil significantly increase. However, the content of heavy metals also increases, especially when the sludge addition ratio reaches 60% to 75%, the cadmium (Cd) and zinc (Zn) content in the soil exceeds the "Soil Environmental Quality Risk Control Standard for Soil Pollution of Agricultural Land (Trial) (GB 15618-2018)" limits. At a sludge addition ratio of 15% to 45%, the transpiration rate, stomatal conductance, net photosynthetic rate, and water use efficiency of alfalfa increase with growth time. However, at a sludge addition ratio of 60% to 75%, the growth rates of these indices significantly decrease. This study also establishes a foundation for future long-term monitoring to assess the sustained impacts of sludge application on soil quality and alfalfa growth.
Keywords: *soil fertility, sludge application', heavy metal accumulation, ecological impact, alfalfa growth*

Introduction

Urban sludge, as a solid waste produced in the process of sewage treatment, has continuously increased in output with the development of urbanization, becoming an increasingly serious environmental issue (Li et al., 2018; Sun et al., 2019; Zou et al., 2021). Sludge is rich in organic matter, nitrogen, phosphorus, and other nutrients, which have a significant effect on improving soil fertility (Rillig et al., 2017; Horton et al., 2017). Currently, China is facing exacerbated issues of soil rocky desertification, desertification, and salinization, with a large area of medium and low-yield soil; therefore, the use of urban sludge for soil improvement has great potential (Huang et al., 2019; Zhang et al., 2020). The rational use of sludge in rocky desertification areas can not only improve soil quality but also has remarkable practical significance (Wright et al., 2013; Boots et al., 2019; Xie et al., 2020).

Photosynthesis is the process by which plants absorb light energy, fix carbon dioxide, and produce carbohydrates. It is the basis of plant physiology and life activities, closely

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

related to plant growth, development, and survival, and is an important indicator of plant growth status. More than 90% of the dry matter in plants comes from the photosynthetic assimilation of leaves, and the interaction between plants and environmental factors and their adaptability to the environment can be reflected through photosynthetic characteristics, leaf traits, and their relationships. Therefore, the photosynthetic characteristics of plants can not only reflect the growth status of plants but also to some extent reflect soil quality (de Souza Machadoet al., 2018; Zhou et al., 2019).

Alfalfa (*Medicago sativa*) was selected as a model plant due to its broad adaptability to environmental stresses. Additionally, alfalfa has a high biomass and nutritional value, making it an ideal plant for assessing soil improvement effects. The seedling stage was chosen for measurement as it is highly sensitive to soil conditions, providing a direct reflection of soil fertility changes. Alfalfa (*Medicago sativa*), known as the "king of forage," is renowned for its high yield and rich nutrition, playing a significant role in livestock production and ecological agriculture construction in China. However, there are few studies on the improvement of soil fertility in rocky desertification areas and the promotion of plant photosynthetic characteristics and growth by urban sludge. Therefore, this study analyzed the effects of different proportions of urban sludge addition on soil nutrients and alfalfa photosynthetic characteristics in rocky desertification areas, exploring the feasibility of urban sludge in improving soil quality and promoting plant growth, aiming to provide a reference for the resource utilization of urban sludge.

Materials and methods

Test materials and treatment

Soil samples were collected from suburban areas of Harbin, Heilongjiang Province (*Table 1*) specifically from loam soil at a depth of 20 to 40 cm. The primary vegetation includes *Rosa roxburghii* and *Clematis*. Using a five-point sampling method, soil was collected from the top 0-20 cm layer.

Table 1. *Soil sampling information*

Parameter	Details
Location	Suburban areas of Harbin, Heilongjiang Province
Soil Type	Loam
Sampling Depth	20 to 40 cm
Primary Vegetation	*Rosa roxburghii*, *Clematis*
Sampling Method	Five-point sampling method
Soil Layer Collected	Top 0-20 cm layer
Organic Matter Content	7.48-10.77 $g \cdot kg^{-1}$
Alkaline Nitrogen Level	7.72 $g \cdot kg^{-1}$
Available Phosphorus Content	2.23 $g \cdot kg^{-1}$
Total Potassium Content	1.94 to 131.67 $mg \cdot kg^{-1}$

The sludge samples, obtained from the dewatered sludge of Harbin Urban Sewage Treatment Plant, had a moisture content of 60% and a pH of 7.9. After collecting, the soil

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

and sludge were air-dried at approximately 25°C, ground, and sieved through a 2 mm mesh for consistency in the particle size before further experimental analysis.

Plant Growth Conditions: The indoor pot experiments were conducted in a controlled greenhouse at Harbin University, maintaining temperatures between 20°C and 30°C to support optimal alfalfa growth. Each pot contained a soil-sludge mixture of 2.5 kg. Alfalfa seeds were sterilized with a 5% hypochlorous acid solution, rinsed with distilled water, and then soaked in warm water for 24 hours before planting. In each pot, six seeds were initially sown, and after germination, three uniformly growing seedlings were selected for further growth.

The plants were watered as needed to maintain the soil moisture at approximately 70% of the field capacity. This consistent hydration was achieved by monitoring soil weight daily to adjust the water level, minimizing stress on the plants.

Photosynthetic parameters-transpiration rate, stomatal conductance, net photosynthetic rate, and intercellular CO_2 concentration-were measured using a Ciras-3 photosynthesis meter (PP Systems) on days 10, 20, 30, 40, 50, and 60. Measurements focused on the penultimate leaf of each alfalfa plant to ensure consistent data.

Harvesting and Growth Measurement: After 60 days, the plants were harvested to record plant height, stem diameter, and biomass. Measurements were repeated five times per treatment group for statistical reliability, ensuring that results reflected a range of sludge proportions and their effects on alfalfa seedling growth.

Determination parameters and methods

The sludge application rates were chosen to explore the effects of both agronomically relevant concentrations (15-45%) and higher concentrations (60-75%) to assess any potential threshold effects on soil fertility and plant growth. The air-dried sludge was uniformly mixed with soil at mass ratios of 0% (control group, CK), 15% (T1), 30% (T2), 45% (T3), 60% (T4), and 75% (T5). These mixtures were placed in plastic pots with a diameter of 18 cm and a height of 12 cm, with a total mass of 2.5 kg per pot. They were kept at room temperature for 30 days without any disturbance to maintain equilibrium. Each treatment was repeated five times, ensuring that the soil moisture content was maintained at approximately 70% of the field capacity during the equilibrium period. Field capacity was determined using the gravimetric method, which involved saturating the soil, allowing it to drain, and then measuring the water retained in the soil.

After the equilibrium period, 100 grams of soil were taken from each pot, and soils from three pots with the same treatment were mixed into one sample. Subsequently, the pH value, organic matter content, total nitrogen, alkaline nitrogen, total phosphorus, available phosphorus, available potassium content, and total amounts of copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) in the soil were measured. The remaining soil was used for pot experiments with alfalfa (*Medicago sativa*). The soil nutrient contents were measured using an atomic absorption spectrophotometer (PerkinElmer Analyst 400)

The pot experiments were conducted in the experimental greenhouse of Harbin University, where the temperature was maintained at 20 to 30°C. Alfalfa seeds were first sterilized with a 5% hypochlorous acid solution and then rinsed with distilled water. Plastic pots with a diameter of 15 cm and a height of 12 cm were used in this experiment, with a total volume of 2.5 kg of the soil-sludge mixture per pot. After soaking the seeds in warm water for 24 hours, six seeds were sown in each pot. After the seedlings emerged, three seedlings with consistent growth were selected for transplantation in each pot. On the 7th, 14th, 21st, 28th, 35th, and 42nd days after emergence, a photosynthesis meter

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

(Licor-6800, USA)) was used to measure the transpiration rate, stomatal conductance, net photosynthetic rate, and intercellular CO_2 concentration of alfalfa leaves. The plants were harvested after 60 days of growth, and their biomass, plant height, and stem diameter were measured. Each treatment was replicated five times.

Photosynthetic gas exchange parameters were measured

The penultimate leaf of *Alfalfa* (*Medicago sativa*) seedlings was selected. The CO_2 concentration was fixed at 400μl·L^{-1} in a CO_2 cylinder. The light intensity PFD was set to 1000 μmol·m^{-2}·s^{-1} with the light source built in the instrument. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), intercellular CO_2 concentration (C_i) and Water use efficiency, WUE was calculated as the ratio of net photosynthetic rate (P_n) to transpiration rate (T_r), providing insights into the plant's ability to use water effectively, *Alfalfa* (*Medicago sativa*) leaves under different treatments were measured. Repeat 5 times.

The determination of the physicochemical properties, the specific methods are as follows

The pH was determined using the potentiometric method; The organic matter content was measured by the potassium dichromate oxidation method; The total nitrogen content was determined using the Kjeldahl method; The alkaline nitrogen content was measured by the alkaline hydrolysis diffusion method; The total phosphorus content was determined by the sodium hydroxide fusion method, followed by the molybdenum-antimony colorimetric method;The available phosphorus content was determined by extraction with $NaHCO_3$ (sodium bicarbonate) and subsequent measurement by the molybdenum-antimony colorimetric method; The total potassium content was determined by sodium hydroxide fusion followed by flame photometry; The available potassium content was determined by extraction with ammonium acetate and measured by flame photometry; The total amounts of heavy metals (such as copper Cu, cadmium Cd, lead Pb, zinc Zn, chromium Cr, and nickel Ni) were determined by digestion with HCl (hydrochloric acid), HNO_3 (nitric acid), and $HClO_4$ (perchloric acid) and measured by atomic absorption spectrophotometry; These determination methods ensure the accuracy and scientific validity of the analysis, in compliance with current soil analysis standards. The applied analytical methods were based on standard soil analysis protocols (Ahmed et al., 2009; Cao et al., 2021).

Data and analysis

Excel and SPSS (24.0) software were used for statistical analysis of the measured data. The data in the figure were the mean ± standard deviation (SE) of three repetitions. One-way ANOVA and least significant difference (LSD) were used to compare the differences among different data groups.

Results and analysis

Effects of sludge addition on soil pH and nutrient content

According to preliminary experimental results of this study (Wu et al., 2019), mixing urban sludge with rocky desertified soil in different proportions can significantly improve soil fertility. Specifically, the pH value of the mixed substrate ranged from 7.48 to 7.70,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

significantly lower than that of the control soil. The organic matter content increased by 1.30 to 2.52 times compared to the control group as the proportion of sludge addition increased. Total nitrogen content increased by 1.19 to 2.69 times, and alkaline nitrogen content increased by 2.58 to 4.44 times. Total phosphorus content increased by 1.07 to 2.08 times, and available phosphorus content increased by 1.11 to 2.23 times. However, the total potassium content did not significantly increase with the increase in the proportion of sludge addition. Because the available potassium content in the sludge was lower than that in the control soil, the available potassium content in the mixed substrate decreased by 7.69% to 34.62% compared to the control group (*Table 2*).

Table 2. *Effects of different proportions of municipal sewage sludge on soil nutrient content*

Parameter	CK	T1	T2	T3	T4	T5
pH	7.70 ± 0.09a	7.55 ± 0.77b	7.50 ± 0.65b	7.48 ± 0.64b	7.50 ± 0.60b	7.53 ± 0.59b
Organic matter content ($g \cdot kg^{-1}$)	16.50 ± 0.30d	21.50 ± 0.34c	23.50 ± 0.28c	31.20 ± 0.49b	24.50 ± 0.55b	41.50 ± 0.59a
Total N content ($g \cdot kg^{-1}$)	0.78 ± 0.03e	0.93 ± 0.05c	1.19 ± 0.05c	1.85 ± 0.05c	2.05 ± 0.06b	2.10 ± 0.05a
Alkaline hydrolysis N content ($mg \cdot kg^{-1}$)	225.00 ± 14.00e	580.00 ± 17.00c	760.00 ± 26.00e	690.00 ± 28.00d	875.00 ± 35.00b	1000.00 ± 54.00a
Total P content ($g \cdot kg^{-1}$)	13.50 ± 0.10d	14.50 ± 0.70d	18.50 ± 0.70d	21.20 ± 0.60c	24.50 ± 0.80b	28.10 ± 1.00a
Available P content ($mg \cdot kg^{-1}$)	9.00 ± 0.30e	10.20 ± 0.35d	12.00 ± 0.40d	15.10 ± 0.50c	18.00 ± 0.55b	20.10 ± 0.60a
Total K content ($g \cdot kg^{-1}$)	18.30 ± 0.30e	20.50 ± 0.40d	23.00 ± 0.40d	26.40 ± 0.50c	31.20 ± 0.60b	35.10 ± 0.62a
Available K content ($mg \cdot kg^{-1}$)	130.00 ± 6.00a	120.00 ± 5.00a	115.00 ± 5.00a	100.00 ± 6.00a	85.00 ± 4.00d	92.00 ± 5.00a

The air-dried sludge was uniformly mixed with soil at mass ratios of 0% (control group, CK),15% (T1), 30% (T2), 45% (T3),60% (T4), and 75% (T5). Note: The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences ($P < 0.05$)

Effects of sludge addition on soil heavy metal content

Preliminary experimental results of this study (Wright et al., 2013) that the content of heavy metal elements in the mixed substrate significantly increased after the addition of urban sludge. Specifically, after adding 15% to 75% sludge, the total copper (Cu) content in the mixed substrate increased by 3.82% to 32.82%. The total zinc (Zn) content increased by 7.14% to 78.57%. The total cadmium (Cd) content increased by 1.22 to 2.56 times. The total lead (Pb) content increased by 4.23% to 49.30%. Notably, when 75% sludge was added, the total zinc content in the mixed substrate exceeded the standards of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

"Soil Environmental Quality Agricultural Land Soil Pollution Risk Control Standards (Trial) (GB 15618-2018)" by 25.00%. When adding60% to 75% sludge, the proportion of total cadmium content exceeding the standard ranged from 6.45% to 30.00% (*Table 3*).

Table 3. *Effects of different proportions of municipal sewage sludge on soil heavy metal content (mg·kg⁻¹)*

Treatment	Cu Content (mg·kg⁻¹)	Zn Content (mg·kg⁻¹)	Cd Content (mg·kg⁻¹)	Pb Content (mg·kg⁻¹)
CK	65.50 ± 2.40c	140.00 ± 3.20c	0.50 ± 0.05f	35.50 ± 1.20c
T1	68.00 ± 1.85c	170.00 ± 8.60c	0.61 ± 0.04e	37.00 ± 0.85c
T2	69.00 ± 1.90c	180.00 ± 14.00c	0.70 ± 0.03d	39.00 ± 2.50bc
T3	72.00 ± 2.80bc	150.00 ± 9.00c	0.63 ± 0.04d	40.00 ± 2.20bc
T4	78.00 ± 3.70b	210.00 ± 11.00b	0.65 ± 0.07e	42.00 ± 2.80b
T5	87.00 ± 5.50a	250.00 ± 7.00a	0.78 ± 0.04d	53.00 ± 1.80a
Soil quality standard	100	300	0.600	170

Note: The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences ($P < 0.05$)

Effects of sludge addition on the photosynthetic physiology of alfalfa (Medicago sativa)

By analyzing the data from *Figures 1 to 5*, it can be seen that with the addition of 15% to 60% sludge, the transpiration rate of alfalfa (*Medicago sativa*) showed an increasing trend with growth time. However, when the sludge proportion increased to 60% to 75%, the growth rate of transpiration was significantly lower than that of the control group (P<0.05). Specifically, on the 10th day after emergence, the transpiration rate of alfalfa in the treatment group with 15% to 60% sludge addition was 11.1% to 127.3% higher than that of the control group. In contrast, in the treatment group with 60% to 75% sludge addition, the transpiration rate was 4.5% to 29.1% lower than that of the control group. By the 50th day after emergence, the transpiration rate of alfalfa in the treatment group with 15% to 60% sludge addition was still 6.7% to 120.0% higher than that of the control group; in the treatment group with 60% to 75% sludge addition, the transpiration rate was only 10.0% to 20.0% lower than that of the control group (*Figure 1*).

Starting from the 20th day after emergence, the stomatal conductance of alfalfa (*Medicago sativa*) showed an increasing and then decreasing trend with changes in the sludge addition ratio. Specifically, on the 20th day after emergence, when 15% to 60% sludge was added, the stomatal conductance of alfalfa (*Medicago sativa*) was 1.05 to 1.57 times higher than that of the control group. In contrast, when 60% to 75% sludge was added, the stomatal conductance was 4.3% to 25.0% lower than that of the control group. By the 50th day after emergence, the stomatal conductance of alfalfa in the treatment group with 15% to 60% sludge addition was 1.20 to 1.75 times higher than that of the control group. In the treatment group with60% to 75% sludge addition, the stomatal conductance was 7.7% to 16.7% lower than that of the control group, indicating that a high proportion of sludge addition would inhibit the stomatal conductance of alfalfa (*Medicago sativa*), as shown in *Figure 2*.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

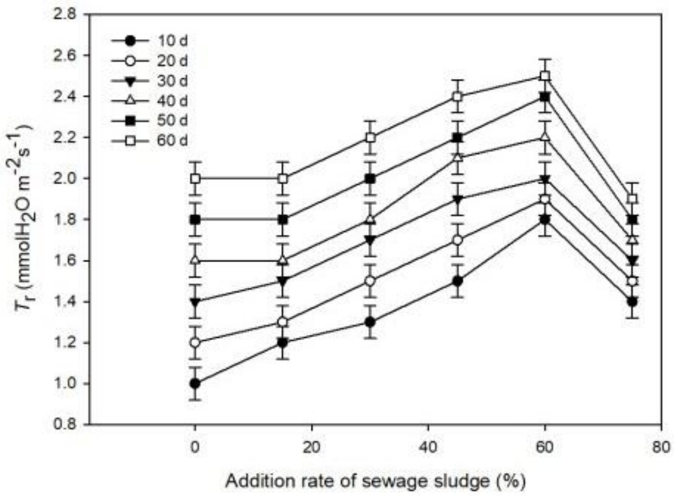

Figure 1. *Effect of sewage sludge addition on the transpiration rate of alfalfa (Medicago sativa). The air-dried sludge was uniformly mixed with soil at mass ratios of 0% (control group, CK), 15% (T1), 30% (T2), 45% (T3), 60% (T4), and 75% (T5). Note: The data in the picture are the mean and standard error (SE)*

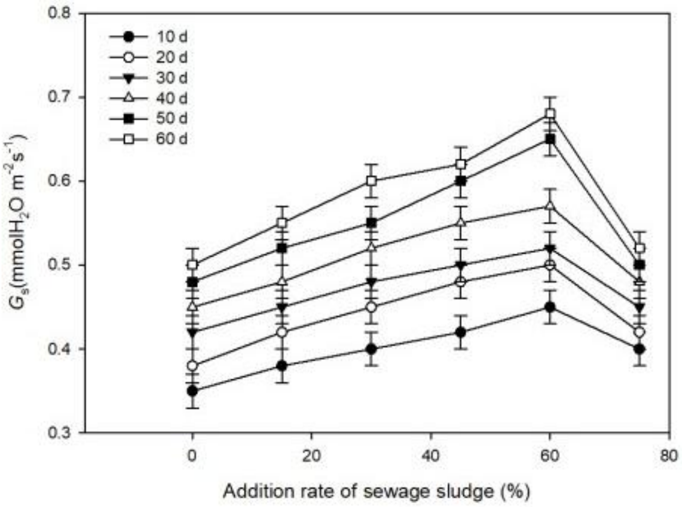

Figure 2. *Effect of sewage sludge addition on the stomatal conductance of alfalfa (Medicago sativa). Note: The data in the picture are the mean and standard error (SE)*

Additionally, starting from the 20th day after emergence, the net photosynthetic rate of alfalfa (*Medicago sativa*) also showed an increasing and then decreasing trend with the increase in the sludge addition ratio. In the T2 and T3 treatment groups, the net photosynthetic rate was significantly higher than that of the control group ($P<0.05$). On the 20th day after emergence, when 15% to 60% sludge was added, the net photosynthetic rate of alfalfa (*Medicago sativa*) was 8.6% to 52.9% higher than that of the control group. However, in the treatment with 60% to 75% sludge addition, the net photosynthetic rate was 3.4% to 11.6% lower than that of the control group. By the 50th day after emergence, the net photosynthetic rate of alfalfa in the treatment group with 15% to 60% sludge addition was 10.0% to 37.5% higher than that of the control group. In contrast, in the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

treatment with 60% to 75% sludge addition, the net photosynthetic rate was 7.0% to 25.0% lower than that of the control group, indicating that a high proportion of sludge addition would inhibit the net photosynthetic rate of alfalfa (*Medicago sativa*), as shown in *Figure 3*.

The graph shows the effect of different sewage sludge addition rates on the intercellular CO_2 concentration (C_i) of alfalfa (*Medicago sativa*) over various days after emergence (10, 20, 30, 40, 50, and 60 days). The C_i values generally increased with the addition rate of sewage sludge up to 60%, peaking at this level for most of the observed days. Specifically, at 10 days after emergence, the C_i values ranged from approximately 360 to 400 μmol CO_2 m^{-2} s^{-1} across different sludge addition rates. As the growth period extended to 20 days, the C_i values increased more noticeably, reaching about 370 to 420 μmol CO_2 m^{-2} s^{-1}. This increasing trend continued with each subsequent measurement, with C_i values reaching their maximum around 40 to 60 days after emergence. At 60 days, the C_i values for the 60% sludge addition rate reached the highest observed values, between 410 and 450 μmol CO_2 m^{-2} s^{-1}. However, beyond the 60% sludge addition rate, the C_i values began to decline slightly, indicating that excessive sludge addition might negatively impact the intercellular CO_2 concentration in alfalfa.

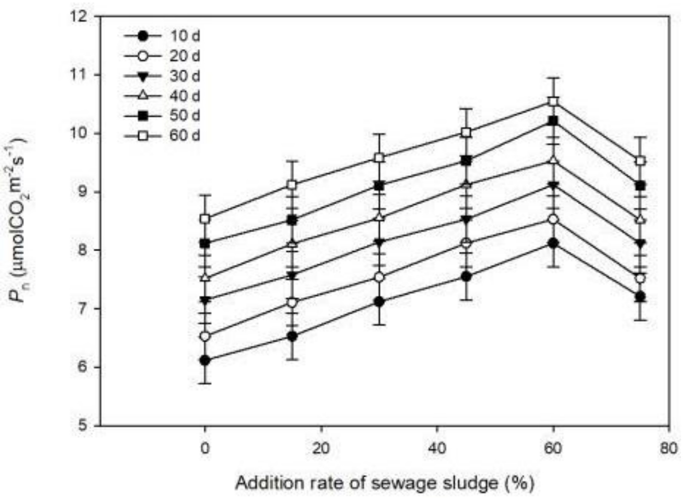

Figure 3. *Effect of Sewage Sludge Addition on the net photosynthetic rate of Alfalfa (Medicago sativa). Note: The data in the picture are the mean and standard error (SE)*

The intercellular CO_2 concentration of alfalfa (*Medicago sativa*) mainly changes with growth time, and its changes are not significant under different sludge addition ratios, as shown in *Figure 4*. Starting from the 30th day after emergence, the water use efficiency of alfalfa (*Medicago sativa*) tends to stabilize and shows an increasing and then decreasing trend with the increase in the sludge addition ratio. Specifically, on the 30th day after emergence, the water use efficiency of alfalfa (*Medicago sativa*) in the treatment group with 15% to 60% sludge addition was 10.4% to 41.7% higher than that of the control group. In contrast, in the treatment group with60% to 75% sludge addition, the water use efficiency was 6.7% to 17.1% lower than that of the control group. By the 50th day after emergence, the water use efficiency of alfalfa (*Medicago sativa*) in the treatment group with 15% to 60% sludge addition was 8.3% to 50.0% higher than that of the control group. In the treatment group with 60% to 75% sludge addition, the water use efficiency

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

was 4.0% to 13.3% lower than that of the control group, as shown in *Figure 5*. These results indicate that although an appropriate amount of sludge addition can improve the water use efficiency of alfalfa (*Medicago sativa*), an excessive sludge ratio will have an adverse effect on its water use efficiency baol (Ahmed et al., 2009; Arena et al., 2017). Therefore, when using urban sludge for soil improvement, it is necessary to precisely control the addition ratio to optimize the water use efficiency of plants (Andrady, 2011; Akdogan et al., 2021).

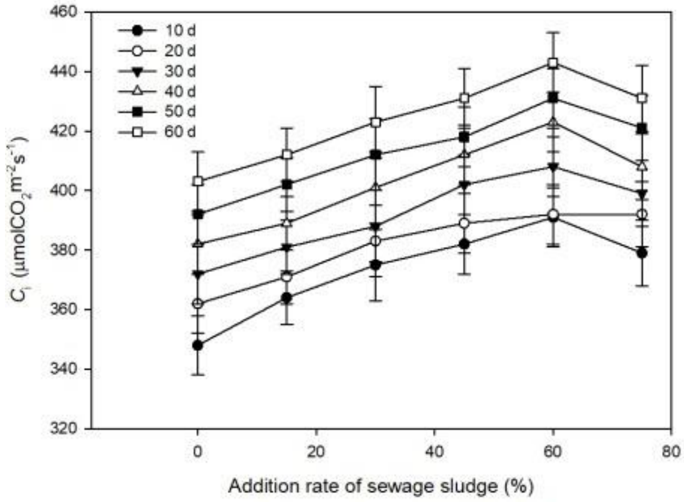

Figure 4. *Effect of sewage sludge addition on the intercellular CO_2 concentration of alfalfa (Medicago sativa). Note: The data in the picture are the mean and standard error (SE)*

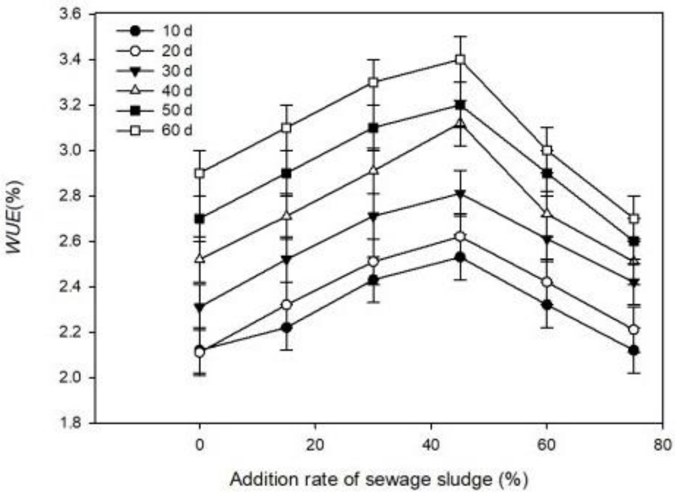

Figure 5. *Effect of sewage sludge addition on the water use efficiency of alfalfa (Medicago sativa). Note: The data in the picture are the mean and standard error (SE)*

Effects of sludge addition on the growth of alfalfa (Medicago sativa)

According to the data in *Figures 6-8*, we can observe that, except for treatment group T5, an appropriate amount of sludge addition significantly increased the average plant height, stem diameter, and biomass of alfalfa (*Medicago sativa*). Specifically, when the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

sludge addition ratio was between 15% and 60%, the plant height of alfalfa was 28.6% to 43.8% higher than that of the control group, the average stem diameter was 100.0% to 200.0% higher than that of the control group, and the dry matter biomass increased by 77.8% to 222.2% compared to the control group. However, when the sludge addition ratio was between 70% and 80%, the plant height, stem diameter, and dry matter biomass of alfalfa (*Medicago sativa*) all began to decrease.

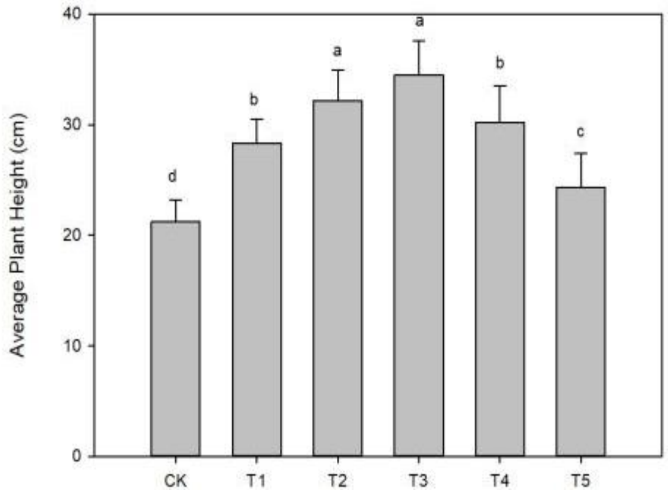

Figure 6. *Effect of sewage sludge addition on the average plant height of alfalfa (Medicago sativa). The air-dried sludge was uniformly mixed with soil at mass ratios of 0% (control group, CK),15% (T1),30% (T2), 45% (T3),60% (T4), and 75% (T5). Note: The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P ＜0.05)*

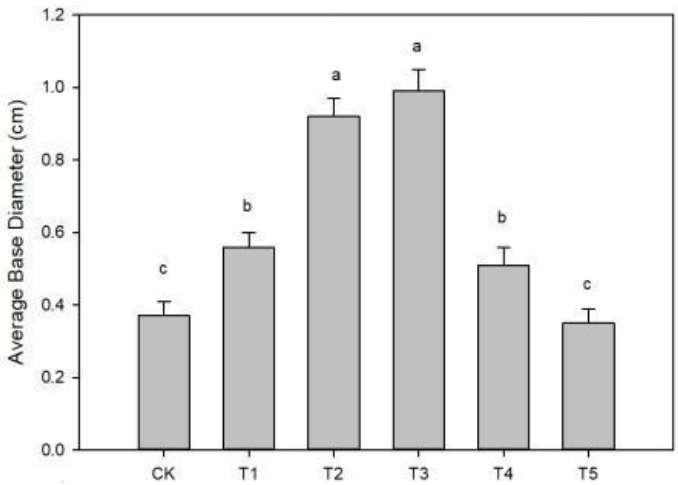

Figure 7. *Effect of sewage sludge addition on the average base diameter of alfalfa (Medicago sativa). The air-dried sludge was uniformly mixed with soil at mass ratios of 0% (control group, CK),15% (T1),30% (T2), 45% (T3),60% (T4), and 75% (T5). Note: The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P ＜0.05)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

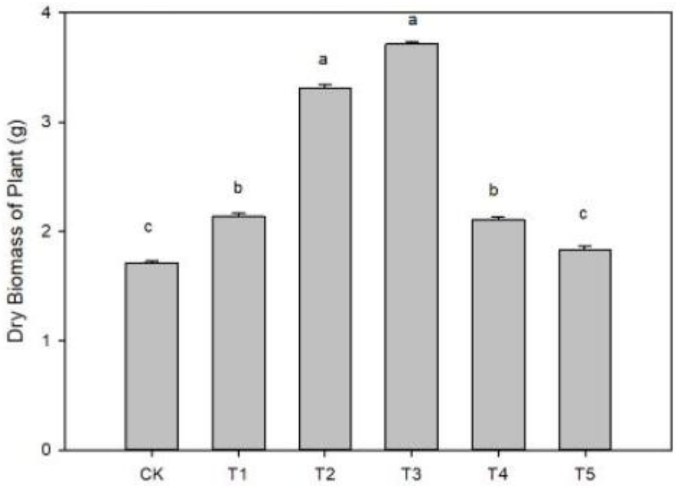

Figure 8. *Effect of sewage sludge addition on the dry biomass of alfalfa (Medicago sativa). The air-dried sludge was uniformly mixed with soil at mass ratios of 0% (control group, CK),15% (T1),30% (T2), 45% (T3),60% (T4), and 75% (T5). Note: The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P ＜0.05)*

Discussion

Effects of sludge addition on soil nutrients

Changes in soil pH not only affect and constrain a series of physical and chemical changes in the soil but also significantly influence the quantity and types of soil microorganisms (Barnes et al., 2009; Song et al., 2021; Sun et al., 2021). It is an important indicator of the basic physical and chemical properties of soil and directly affects plant growth. This study found that the pH of the mixed substrate decreased after the addition of sludge. This may be due to the production of a certain amount of organic acids during the decomposition of organic matter in the sludge maturation process (Thompson et al., 2012; Wu et al., 2019), similar to the results in sandy soil when sludge was added (Zhang et al., 2021; Ziajahromi et al., 2021).

Soil nutrient content is an important indicator of soil fertility levels, with organic matter, nitrogen, and phosphorus content reflecting the potential nutrients in the soil. These nutrients directly affect the soil's aggregate structure, physical properties, and water and nutrient retention capacities, determining the soil's resistance and buffering capacity (Souza Machado et al., 2019; Huang et al., 2021). Major nutrients required for plant growth, such as nitrogen and phosphorus, participate in various metabolic activities within plants and are key limiting factors affecting plant growth. The content of available nutrients in the soil is an important indicator of soil nutrient supply and a direct source of nutrients for plants. Among them, the content of alkali-hydrolyzable nitrogen is positively correlated with soil nitrogen surplus and is an important indicator reflecting the nitrogen supply in the soil (Qi et al., 2020; Wang et al., 2020), while available phosphorus is the portion of phosphorus in the soil that can be directly absorbed and utilized by plants, which is significant for assessing the phosphorus supply status of the soil.

Urban sludge is rich in organic matter, nitrogen, phosphorus, and other nutrients. When added to the soil, the contents of organic matter, total nitrogen, total phosphorus, alkali-hydrolyzable nitrogen, and available phosphorus in each treatment significantly increased,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

consistent with the findings. These findings emphasize the potential application value of urban sludge in soil improvement and plant growth promotion (Lebreton et al., 2017; Jiang et al., 2021).

Effects of sludge addition on soil heavy metal content

In addition to being rich in organic matter, nitrogen, and phosphorus, urban sludge also contains a certain amount of heavy metals. Its addition to the soil inevitably increases the heavy metal content in the mixed substrate. Excessive heavy metal content in the soil can lead to soil pollution, thereby affecting plant growth. Previous experimental studies have shown that adding sludge to the soil significantly increases the total copper (Cu), total zinc (Zn), total cadmium (Cd), and total lead (Pb) content in the mixed substrate. When adding 15% to 75% sludge, the total copper content in the mixed substrate increased by 3.82% to 32.82%, and the total zinc content increased by 7.14% to 78.57%; the total cadmium (Cd) content increased by 22.0% to 56.0%, and the total lead content increased by 4.23% to 49.30%. Particularly, when adding 60% to 75% sludge, the total cadmium content exceeded the "Soil Environmental Quality Agricultural Land Soil Pollution Risk Control Standard (Trial) (GB 15618-2018)" by 30.0%; and when adding 80% sludge, the total zinc content in the mixed substrate exceeded the standard by 25.0%. These results indicate that with the increase in the proportion of sludge applied, the heavy metal content in the mixed soil shows an increasing trend. High proportions of sludge addition may lead to heavy metal pollution (Li et al., 2020; Liu et al., 2020; Ma et al., 2021). Therefore, although the addition of urban sludge to the soil can improve soil fertility, its application should be controlled to avoid potential environmental risks.

Effects of sludge addition on photosynthetic characteristics and growth of plants at seedling stage

Photosynthesis in plants is the basis of their physiological processes and life activities, closely related to environmental factors, and is an important indicator of plant growth status (Galloway et al., 2014; Souza Machado et al., 2019; Huang et al., 2021; Cao et al., 2021). Stomatal conductance, representing the degree of stomatal opening in plant leaves, directly affects the plant's ability to absorb carbon dioxide, determining intercellular CO_2 concentration and transpiration rate, thereby influencing photosynthetic rate. This study found that as the growth period of alfalfa (*Medicago sativa*) seedlings increased, the trends of transpiration rate, stomatal conductance, net photosynthetic rate, intercellular CO_2 concentration, and water use efficiency tended to stabilize. With the addition of 15% to 45% sludge, the transpiration rate and stomatal conductance of alfalfa showed an increasing trend over time, but when the sludge proportion increased to 60% to 75%, the growth rate of these indicators was lower than that of the untreated group.

After a certain period post-emergence, the net photosynthetic rate of alfalfa initially increased with the sludge addition ratio but then decreased, with high proportions of sludge addition inhibiting the net photosynthetic rate. Water is an important reactant in photosynthesis, and the efficiency of water use determines the carbohydrate yield. Combined with net photosynthetic rate, it can reflect the strength of photosynthesis and the plant's adaptability to the substrate. After a certain period post-emergence, the water use efficiency of alfalfa also showed an initial increase followed by a decrease with the sludge addition ratio.

Considering the growth status of plants during the experiment, abnormal growth was observed in alfalfa treated with 60% and 75% sludge. Some studies also indicate that

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

appropriate proportions of urban sludge application can promote plant growth, whereas high proportions inhibit growth (Li et al., 2020; Wang et al., 2021). This indicates that the inhibition of photosynthetic characteristics in alfalfa with high proportions of sludge addition may be due to excessive heavy metals in the substrate.

Conclusion

The results of this study indicate that urban sludge, rich in organic matter, nitrogen, and phosphorus, can significantly enhance the nutrient content of rocky desertification soil. However, while improving soil fertility, it also leads to an increase in heavy metal content such as copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb). Particularly, when 60% sludge is added to the soil, the total cadmium content exceeds the limit set by the "Soil Environmental Quality Agricultural Land Soil Pollution Risk Management Standard (Trial) (GB 15618-2018)"; and with 75% sludge addition, both total zinc and total cadmium contents exceed the standard.

As the growth of alfalfa (*Medicago sativa*) progresses post-emergence, its photosynthetic characteristics initially increase and then decrease with the rise in sludge addition ratio. Under the conditions of 15% to 45% sludge addition, the transpiration rate, stomatal conductance, net photosynthetic rate, and water use efficiency of alfalfa (*Medicago sativa*) all show an upward trend over time. However, under 60% to 75% sludge addition conditions, the growth rates of these indicators decline. High proportions of sludge addition inhibit the photosynthetic characteristics of alfalfa (*Medicago sativa*), likely due to the excessive levels of heavy metals such as zinc and cadmium in the mixed substrate. Therefore, when using urban sludge to improve the fertility of rocky desertification soil, it is necessary to control the application ratio, suggesting not to exceed60% to avoid potential soil pollution problems caused by heavy metal input.

Based on the results of this experiment, we plan to conduct long-term monitoring of alfalfa growth in sludge-amended soils to further assess the prolonged effects of sludge application on soil fertility and ecosystem health. Through continuous monitoring of soil properties and plant growth indices, we aim to reveal the potential impacts of sludge application on soil quality and plant performance over extended time scales, thereby providing a more comprehensive scientific basis for the resourceful utilization of urban sludge

REFERENCES

[1] Ahmed, C. B., Rouina, B. B., Sensoy, S., Boukhris, M., Abdallah, F. B. (2009): Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. – Environmental & Experimental Botany 67(2): 345-352.

[2] Akdogan, Z., Yildiz, B., Kaya, T., Uzun, H. (2021): The effects of microplastics on soil organisms: A review. – Environmental Pollution 270: 116012.

[3] Andrady, A. L. (2011): Microplastics in the marine environment. – Marine Pollution Bulletin 62(8): 1596-1605.

[4] Arena, C., Figlioli, F., Sorrentino, M. C., Izzo, L. G., Capozzi, F., Giordano, S., Sapgnuolo, V. (2017): Ultrastructural, protein and photosynthetic alterations induced by Pb and Cd in *Cynara cardunculus* L. and its potential for phytoremediation. – Ecotoxicology and Environmental Safety 145: 83-89.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

[5] Barnes, D. K. A., Galgani, F., Thompson, R. C., Barlaz, M. (2009): Accumulation and fragmentation of plastic debris in global environments. – Philosophical Transactions of the Royal Society B: Biological Sciences 364(1526): 1985-1998.
[6] Boots, B., Russell, C. W., Green, D. S. (2019): Effects of microplastics in soil ecosystems: Above and below ground. – Environmental Science & Technology 53(19): 11496-11506.
[7] Cao, B., Chen, M., Zhang, H., Lin, Y., Yang, S. (2021): Microplastics in soils: A review of environmental behavior, ecological effects, and implications for soil remediation. – Environmental Pollution 288: 117753.
[8] Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T. S. (2011): Microplastics as contaminants in the marine environment: A review. – Marine Pollution Bulletin 62(12): 2588-2597.
[9] Galloway, T. S., Cole, M., Lindeque, P., Fileman, E., Goodhead, R. (2014): Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota. – Environmental Science & Technology 48(3): 11503-11512.
[10] Horton, A. A., Walton, A., Spurgeon, D. J., Lahive, E., Svendsen, C. (2017): Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. – Science of the Total Environment 586: 127-141.
[11] Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W., Qin, X. (2019): LDPE microplastic films alter microbial community composition and enzymatic activities in soil. – Environmental Pollution 254: 112983.
[12] Huang, Y., Zong, Y., Wu, X., Zhao, Y., Ba, H., Zhu, J. (2021): Differential toxicity of polystyrene microplastic particles of varying sizes to nematode *Caenorhabditis elegans*. – Scientific Reports 11(1): 1-12.
[13] Jiang, Q., Wang, Z., Li, X., Zhang, J., Zhao, Y. (2021): Effects of microplastics on soil microbial community structure and functions: A review. – Environmental Pollution 272: 115984.
[14] Lebreton, L. C. M., van der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., Reisser, J. (2017): River plastic emissions to the world's oceans. – Nature Communications 8(1): 1-10.
[15] Li, Z., Wang, J., Gao, P. (2018): Ecotoxicity and risk assessment of microplastics to terrestrial plants: A review. – Ecotoxicology and Environmental Safety 156: 447-454.
[16] Li, C., Wang, H., Liu, S., Zhang, X., Tang, M. (2020): Microplastics in terrestrial ecosystems: A review of sources, fate, effects, and potential solutions. – Science of the Total Environment 717: 137164.
[17] Li, W., Zhou, Y., Zhang, T., Chen, X., Dong, J. (2020): Interactions between microplastics and plants: A review. – Science of the Total Environment 713: 136443.
[18] Liu, J., Li, H., Zhou, Q., Sun, Y., Cao, J. (2020): Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection and quantification. – Science of the Total Environment 747: 141314.
[19] Ma, Q., Zhang, W., Wu, H., Sun, L., Huang, X. (2021): Impacts of microplastics on nutrient cycling in aquatic ecosystems: A review. – Science of the Total Environment 777: 146133.
[20] Qi, R., Jones, D. L., Li, Z., Liu, Q., Yan, C. (2020): Behavior of microplastics and plastic film residues in the soil environment: A critical review. – Science of the Total Environment 703: 134722.
[21] Rillig, M. C., Ziersch, L., Hempel, S. (2017): Microplastic transport in soil by earthworms. – Scientific Reports 7(1): 1-6.
[22] Song, Y., Li, J., Xu, Y., Wei, J., Li, C. (2021): Effects of microplastics on soil physicochemical properties: A review. – Environmental Pollution 272: 115869.
[23] de Souza Machado, A. A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M. C. (2018): Microplastics as an emerging threat to terrestrial ecosystems. – Global Change Biology 24(4): 1405-1416.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867

[24] de Souza Machado, A. A., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M. C. (2019): Impacts of microplastics on the soil biophysical environment. – Environmental Science & Technology 52(17): 9656-9665.
[25] Sun, J., Dai, X., Wang, Q., van Loosdrecht, M. C., Ni, B. J. (2019): Microplastics in wastewater treatment plants: Detection, occurrence, and removal. – Water Research 152: 21-37.
[26] Sun, H., Wang, J., Jin, Z., Zhou, Y., Li, F. (2021): Effects of microplastics on soil fauna: A review. – Environmental Pollution 268: 115947.
[27] Thompson, R. C. (2012): Microplastics in the marine environment: Sources, consequences, and solutions. – Marine Nature Conservation in Europe 9: 185-200.
[28] Wang, W., Ge, J., Yu, X., Li, H. (2020): Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. – Science of the Total Environment 708: 134841.
[29] Wang, L., Zhao, X., Li, Y., Yu, W., Zhang, D. (2021): Microplastics in water and soil: Occurrence, analytical methods, and ecotoxicological effects. – Ecotoxicology and Environmental Safety 232: 113281.
[30] Wright, S. L., Thompson, R. C., Galloway, T. S. (2013): The physical impacts of microplastics on marine organisms: A review. – Environmental Pollution 178: 483-492.
[31] Wu, T., Zhang, L., Yang, G., Liu, Y., Zhou, Q. (2019): Microplastics in sediments: A review of environmental sources, occurrence, and effects. – Science of the Total Environment 672: 713-726.
[32] Xie, S., Guo, X., Liu, L., Zhang, M., Chen, J. (2020): Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. – Science of the Total Environment 703: 134699.
[33] Zhang, H., Chen, Q., Yang, Y., Zhao, X., Xu, B. (2020): Microplastics in terrestrial ecosystems: Characteristics, ecological effects, and implications. – Science of the Total Environment 732: 139233.
[34] Zhang, X., Liu, H., Li, R., Guo, L., Chen, Y. (2021): Microplastics in the soil-plant system: Occurrence, distribution, uptake, and toxicity-a review. – Science of the Total Environment 789: 147990.
[35] Zhou, J., Wang, H., Cui, C., Yao, Y., Li, Y. (2018): The effect of microplastics on soil physicochemical properties: A comprehensive review. – Environmental Pollution 242: 827-835.
[36] Ziajahromi, S., Neale, P. A., Rintoul, L., Leusch, F. D. L. (2021): Microplastics as vectors of heavy metal contamination: A systematic review. – Environmental Pollution 289: 117925.
[37] Zou, X., Zhang, Y., Yang, J., Chen, H., Wang, Y. (2021): Effects of microplastics on nutrient concentration and physiological responses of crops: A review. – Science of the Total Environment 778: 146316.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_28532867