EFFECTS OF FERTILIZATION AND IRRIGATION LEVELS ON PHYTOREMEDIATION PROPERTIES OF PERENNIAL RYEGRASS

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Abstract

The experiment was set up in the greenhouse of Faculty of Agriculture, University of Belgrade with the aim of investigating potential of perennial ryegrass (Lolium perennial L.) to grow on substrates of exceptionally unfavorable characteristics. Tailings, characterized by very poor physical and chemical properties and deficiency in nutrients were used as substrate. The research examined effects of three different fertilizers and two irrigation levels on plant growth and assimilation of Cu and Cd. There were statistically highly significant differences in plant height between plants treated with different fertilizers. Plants treated with organic fertilizer were tallest at 45.8 cm, while the plants treated with mineral fertilizer were shortest at 22.5 cm. Amounts of the assimilated metals varied between different type of fertilizers. The highest content of Cu, about 75 mg kg^{-1} was recorded in plants treated with mineral fertilizer and irrigated moderately while the lowest concentrations of about 30 mg kg^{-1} were measured in the same plants after the first cutting. There were no differences among plants treated with organic fertilizers in Cu amounts, under two irrigation levels. The second cutting showed a small increase in the assimilated amounts of Cu. Highest Cd concentration of 2.7 mg kg^{-1} was recorded in the plants treated with combination of both organic and mineral fertilizers.

Key words: *fertilization, irrigation, nutrients, perennial ryegrass, plant growth*

INTRODUCTION

Due to increasing industrialization and expansion of urban areas there is an increase in the concentration of some harmful substances in the environment in the last few decades. Harmful substances are defined as elements and compounds which in the increased concentrations lead to altered physical, chemical or biological properties of an ecosystem (Saleem et al., 2020). Pollutants include numerous simple and complex compounds both inorganic and organic. Regarding ecotoxicological importance principal soil pollutants are aromatic hydrocarbons and persistent organic pollutants while heavy metals are the main inorganic pollutants. Human activities such as mining, smelting, overuse of synthetic pesticides and fertilizers, burning of fossil fuels directly increase the amounts of pollutants in the biosphere (Yildirim & Sasmaz, 2017). Tailings are anthropogenic substrates left over after extracting valuable metals from the ore. They are characterized by the increased concentrations of metals and adverse physical and mechanical properties. Deposition of tailings on top of soil increases the amounts of heavy metals and changes the natural properties of soil, physical as well as chemical. The resulting soils are degraded, nutrient poor with scarce vegetation and vulnerable to erosion (Shi et al., 2011; Jakovljević et al., 2019). Due to water and wind erosion tailings are also a source from which the pollution is spreading to the surrounding ecosystems. Due to all of the above-mentioned reasons it is very important to ameliorate contaminated soil. Phytoremediation is effective and cheap method of soil melioration. It involves using plants that are able to grow on degraded soils and at some rate absorb heavy metals and other pollutants (Ghori et al., 2016). Plant species suitable for phytoremediation have strong root systems and produce large quantities of aboveground biomass. According to the work of Kumar Patra et al. (2021) grasses (fam. Poaceae) have favorable morphological traits and are tolerant to increased concentrations of soil pollutants. These characteristics make them suitable for soil amelioration. In the last few decades perennial ryegrass (*Lolium perenne* L.) is one of the most often used species in phytoremediation because of its fast early growth and tolerance to increased concentrations of heavy metals in substrate (Masu et al., 2018). Lambrechts et al. (2014) described perennial ryegrass as a hyperaccumulator of cadmium (Cd), lead (Pb) and selenium (Se).

The aim of this research was to investigate potential of perennial ryegrass (*Lolium perenne* L.) to grow on substrate of exceptionally unfavorable properties such are tailings. The goal was also to examine effects of three different fertilizer treatments and two irrigation levels on plant growth and absorption of metals (Cu and Cd).

MATERIALS AND METHODS

The experiment was set up in the greenhouse of Faculty of Agriculture, University of Belgrade in Zemun. Seed material of perennial ryegrass (*Lolium perenne* L.), variety K-11 used in the experiment was obtained from the Institute for forage crops in Kruševac. Seeds were sown in pots with the height of 10 cm and diameter of 12 cm previously filled with substrate. Chemical properties of tailings used as substrate are shown in Table 1.

Table 1. Chemical properties of tailings (Andrejić et al., 2022)

Parameter	Content
pH in H_2O	6.85
pH in KCl	6.59
N (%)	0.005 ± 0.001
K_2O (mg/100g soil)	8.49 ± 0.04
P_2O_5 (mg/100g soil)	1.08 ± 0.006
Organic C (%)	3.71 ± 0.25*
Pb (mg/kg)	655 ± 0.86
Zn (mg/kg)	1217 ± 49.3
Cu (mg/kg)	443 ± 2.30
Cd (mg/kg)	7.33 ± 0.63
Mn (mg/kg)	884 ± 50.9
Ni (mg/kg)	217 ± 8.47

*It should be noted that the increased content of the organic carbon doesn't originate from the humic substances but from the xanthates, organic compounds used to enhance ore extraction (Shen et al., 2016).

Field water capacity (FWC) value of the substrate was determined using Porous plate before setting up the experiment. FWC is the maximal amount of water that can be added during irrigation (Belić et al., 2014).

The experiment was set up in triplicates and it examined effects of different kinds of fertilizer treatments:

- Mineral NPK fertilizer of the 20:20:20 formulation
- Organic fertilizer made from dried and pelleted chicken and cow manure of the 4:4:4 formulation
- Mixture of the mineral NPK 20:20:20 and organic 4:4:4

and irrigation levels:

- 75% FWC
- 50% FWC

Each pot was filled with 1300 g of air-dry tailings amended by fertilizer. The amount of fertilizer was added according to the recommended nutrient dosages per 1 ha of soil. It was calculated as mass percent and 2% of pure nutrients were added to each pot. There were 18 pots in total, divided in 3 equal sets, 6 pots each. First set was amended with 25 g of organic fertilizer, second set was amended with 5 g of mineral NPK fertilizer and the third set was amended with the mixture consisting of 12.5 g of organic and 2.5 g of mineral NPK fertilizer. Substrate was then wetted and 0.67 g of seeds were sown in each pot. Seeds were covered with 1 cm of sieved tailings. Irrigation was introduced after sprouting. One half was irrigated with optimal amount of water (75% FWC) while the other half was irrigated with suboptimal amount (50% FWC). Plants were watered each 4 days by supplementing amount of used up water. The used up amount of water was determined by measuring the pot mass right after watering and again 4 days later, immediately before the next watering. The plant height was also monitored. Aboveground biomass was cut after the plants reached the height of about 20 cm. The plants were cut to the height of 5 cm which simulated mowing in the field. After cutting, the fresh biomass was weighted and oven dried at 60°C to a constant weight. The plant material was analyzed in order to determine the copper and cadmium content. The amounts of metals were established from the extract obtained via wet digestion with cc HNO_3 (USEPA 3052, 1996). Sample absorbances were measured by atomic absorption spectrometry (Shimadzu AA-7000) and concentrations were calculated from the standard curve which was constructed using series of standard solutions of known concentrations.

Data analysis was conducted via two-way analysis of variance (ANOVA) in Statistica 10 software. Two categorical variables were fertilization and irrigation.

Plant height and biomass were calculated cumulatively as a sum of two cuts. The element amounts in plant material are shown in Table 3 as arithmetic means ± standard deviation of triplicates from two cuts.

RESULTS AND DISCUSSION

Plants amended with different fertilizers showed significant differences in height. Plants treated with organic fertilizer were the tallest at 45.8 cm while those treated with mineral fertilizer were significantly shorter at 22.5 cm. The height of the plants treated with fertilizer mixture was 23.8 cm. Biomass weight showed similar trends. Plants treated with organic fertilizer yielded best aboveground biomass of 4.1 g, while plants treated with mineral fertilizer had the poorest yield of 1.95 g. There were not detected effects on aboveground biomass yield by irrigation rates. Also, interaction of applied treatments (fertilizer and irrigation) affected investigated parameters (Table 2). The results are in accordance with the research of Fiorentino et al. (2018) who found that adding organic matter during phytoremediation of degraded areas significantly improve plant growth as well as increase the microbial activity in the substrate.

Table 2. Effects of the fertilizer type and irrigation levels on the aboveground biomass yield of perennial ryegrass

Fertilizer (A)	Plant height (cm)	Biomass (g)
O.	45.8	4.11
M.	22.5	1.95
O.+M.	23.8	3.28
Irrigation (B)		
50% FWC	35.4	2.96
75% FWC	35.8	3.43
ANOVA		
A	**	**
B	NS	NS
A×B	*	*

Legend: NS - not statistically significant; *statistically significant; **statistically highly significant; O - organic fertilizer; M - mineral fertilizer; O+M combination of fertilizers/fertilizer mixture; FWC - field water capacity

Fertilization treatments showed very significant effect on Cu and Cd concentrations in aboveground plant parts. Amounts of absorbed Cu were larger than the amounts of Cd which reflects their concentrations in the substrate. Plants treated with mineral fertilizer had considerably lower concentrations of Cu in comparison to other two treatments after the first cut. Cu content was even 50% lower in plants treated with mineral fertilizer than in the plants treated with the organic fertilizer. In the second cut the amount of absorbed Cu doubled in plants treated with mineral fertilizer, while the Cu plant content in other treatments increased only slightly (Table 3). Although different irrigation levels had no significant influence on the intake of Cu, it should be noted that plants watered at 50% FWC had slightly (3-8 mg kg^{-1}) lower amounts of Cu in their aboveground organs. The exception to this were the plants treated with mineral fertilizer whose Cu contents were 75.5 mg kg^{-1} when irrigated at 50% FWC while the plants watered at 75% FWC had 72.5 mg kg^{-1} Cu in the second cut. Given that the usual concentration in non-accumulator plants is between 5 and 30 mg kg^{-1} (Mitić et al., 2013) our results show that perennial ryegrass can absorb increased concentrations of copper and affect the reduction of its content in the soil, which is in accordance with the researches of Radziemska et al. (2018).

Table 3. Aboveground contents of accumulated metals

	I swath		II swath	
75% FWC	Cu	Cd	Cu	Cd
O	61.1 ± 2.08	1.09 ± 0.01	68.2 ± 9.97	1.99 ± 0.21
M	33.6 ± 3.66	1.19 ± 0.09	72.5 ± 12.1	1.84 ± 0.05
O+M	51.1 ± 4.17	1.34 ± 0.28	55.7 ± 9.73	2.66 ± 0.54
50% FWC				
O	58.0 ± 7.03	1.24 ± 0.40	60.3 ± 5.15	1.85 ± 0.07
M	30.5 ± 5.14	1.61 ± 0.04	75.5 ± 5.96	1.89 ± 0.13
O+M	46.1 ± 3.25	1.40 ± 0.56	52.2 ± 2.33	2.41 ± 0.11

Legend: O - organic fertilizer; M - mineral fertilizer; O+M combination of fertilizers/fertilizer mixture; FWC - field water capacity

Small differences in Cd intake were noticed between the plants under different fertilization regimes in the first cutting. In the second cutting plants treated with the mixture of fertilizers had significantly higher Cd concentrations. Plants treated with organic fertilizer and irrigated at 75% FWC had the lowest Cd levels of 1.09 mg kg^{-1}. Meanwhile the highest Cd levels were recorded in plants treated with fertilizer mixture in the second cut. The Cd concentrations were 2.7 mg kg^{-1} in plants watered at 75% FWC and 2.4 mg kg^{-1} at 50% FWC. Cadmium amounts in the aboveground plant biomass were higher than usual and above permited concentrations of 0.1 to 0.3 mg kg^{-1} (Official gazette of RS, 28/2011). However it is documented that perennial ryegrass can absorb larger amounts of Cd without toxicity symptoms such are leaf chlorosis, decreased photosynthic rate and water uptake, respiratory inhibition etc. (Mitić et al., 2013).

CONCLUSIONS

Perennial ryegrass has the ability to grow in substrates with elevated heavy metal contents without showing symptoms of toxicity in aboveground plant organs. Adding organic fertilizer drastically improves plant growth and increases aboveground biomass, while addition of mineral fertilizer does not give satisfactory results regarding these parameters. Different irrigation levels did not have significant effects on the plant height and weight. Amounts of Cu in aboveground plant parts were at toxic levels to most plants. Concentrations of Cu were from 30 mg kg^{-1} in plants treated with mineral fertilizer and irrigated at 50% FWC in the first cut to 75 mg kg^{-1} in plants with the same treatment but in the second cut. Cadmium content in aboveground plant organs was also higher than usual. The largest amount, 2.7 mg kg^{-1} was recorded in plants treated with fertilizer mixture and optimally irrigated (at 75% FWC). Based on the results of this study it can be concluded that the perennial ryegrass is a suitable candidate species to be used in the phytoremediation of the contaminated areas. This experiment also showed capability of perennial ryegrass to accumulate certain heavy metals therfore decreasing their concentrations in the substrate.

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REFERENCES

Andrejić, G., Brajević, S., Simić, A., Dželetović, Ž., Aleksić, U., Sokolović, D. (2022). Accumulation of heavy metals in root and shoot of red fescue grown at the flotation tailings dump. *Agrores 64.*

Belić, M., Nešić, Lj, Ćirić, V. (2014). Practicum in pedology. University of Novi Sad, Faculty of Agriculture

Fiorentino, N., Mori, M., Cenvinzo, V., Giuseppe-Duri, L., Gioia, L., Visconti, D., Fagnano, M. (2018). Assisted phytoremediation for restoring soil fertility in contaminated and degraded land. *Italian Journal of Agronomy 13.* 34-44.

Ghori, Z., Iftikar, H., Bhatti, M.F., Sharma, I., Kazi, A.G., Ahmad, P. (2016). Phytoextraction: the use of plants to remove heavy metals from soil in: *Plant metal interaction. Amsterdam: Elsevier* 385-409

Jakovljević, K., Mišljenović, T., Savović, J., Ranković, D., Ranđelović, D., Mihailović, N., Jovanović, S. (2019). Accumulation of trace elements in *Tussilago farfara* colonizing post-flotation tailing sites in Serbia. *Environmental Science and Pollution Research 27.* 4089-4103.

Lambrechts, T., Leaqueue, G., Lobet, G., Godin, B., Bielders, C.L., Lutts, S. (2014). Comparative analysis of Cd and Zn impact on root distribution and morphology of *Lolium perenne* and *Trifolium repens*: Implications for phytostabilization. *Plants and Soil 376*. 229-234.

Masu, S., Cojocariu, L., Grecu, E., Morariu, F., Bordean, D.M., Horablaga, M., Nita, L., Nita, S. (2018). *Lolium perenne* - a phytoremediation option in case of total petroleum hydrocarbons polluted soils. *Revista de chimie 69.* 110-114.

Mitić, V., Stankov-Jovanović, V., Ilić, M., Jovanović, S., Nikolić-Mandić, S. (2013). Impact of the wild fire on the content of lead, zinc, copper and cadmium in soil plant material. *Zaštita materijala 54.* 75-82.

Official gazette of RS (2011). Rule book of permissible concentrations of dangerous and hazardous materials in soil and in water for irrigation and methods for analysis, 28.

Radziemska, M., Bilgin, A., Vaverková, M. D. (2018). Application of Mineral-Based Amendments for Enhancing Phytostabilization in Lolium perenne L. Cultivation. *CLEAN–Soil, Air, Water, 46*. 1-10.

Saleem, M.H., Kamran, M., Zhou, Y., Parveen, A., Rehman, M., Ahnar, S., Malik, Z., Mustafa, A., Anjum, R.M.A., Wang, B. (2020). Appraising growth, oxidative stress and copper phytoextraction potential of flax (*Linum usitatissimum* L.) grown in soil differentially spiked with copper. *Journal of Environmental Management* 257. 1-10.

Shen, Y., Nagaraj, D.R., Farinato, R., Somasundaram, P. (2016). Study of xanthate decomposition in aqueus solutions. *Mineral engineering 93.* 10-15.

Shi, X., Zhang, X., Chen, G., Chen, Y., Wang, L., Shan, X. (2011). Seedling growth and metal accumulation of selected woody species in copper and lead/zinc mine tailings. *Journal of Environmental Science 23.* 266-274.

USEPA Method 3051. (1998). Microwave assisted acid digestion of sediments, sludges and oils. − In: Test Methods for Evaluating Solid Waste, SW-846. *Environmental Protection Agency, Washington*, DC.

Yildirim, D., Sasmaz, A. (2017). Phytoremediation of As, Ag and Pb in contaminated soil using terestrial plants grown on Gumuskoy wining area (Kutahya Turkey). *Journal of Geochemical Exploration 182.* 228-234.