EFFECTS OF HIGH NUTRIENT AND HEAVY METAL CONTAINING SOILS ON SOME CHEMICAL AND BIOCHEMICAL FEATURES OF SUDAN GRASS

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Abstract: In this research we studied the effects of different soil amendments on the elemental composition and the activities of some enzymes of a Sudan grass variety (GK Csaba). In the pot experiment we used a soil with low heavy metal content as a plant grow media, as a control (C) we used the same soils „enriched" with a 20% wastewater sediment (WS) (originated from a former wastewater settling plant), a 10% compost (made of sewage sludge and green waste, SG), or their mix (C + 10% SG + 20% WS). The compost contained much more essential plant nutrients, while the wastewater-sediment was rich in toxic heavy metals compared to control soil. In this study, we determined the elemental composition of soil and organs of the plants (roots, leaves) and activities of some enzymes in leaves. Higher concentrations of all essential plant nutrient were detected in both roots and leaves of the treated plants. The concentration of copper and zinc were significantly higher (2 and 3 times higher, respectively) in plants grown in WS containing media (C+20% WS and C+10% SG + 20% WS). The concentrations of toxic heavy metals were higher in roots compared to leaves. As an exception, the cadmium concentration was nearly equal in the roots and the leaves, showing that this element's accumulation is not limited to the roots, but was also transported into the leaves. The activities of the examined enzymes (glucose-6 phosphate dehydrogenase, G6PDH; isocitrate-dehydrogenase, ICDH; peroxidase, POX) did not show significant changes in presence of WS and SG.

Keywords: Sudan grass, heavy metals, wastewater sediment, compost, enzyme activities

1. Introduction

In the past century, the world's economy has grown at an unprecedented rate to support the ever-growing population of the Earth. The technologies used require the use of many organic and inorganic chemicals that were rarely used before (Bhujam & Islam 2017). The preparation of the necessary raw materials, the production process, and later the wear and tear of the manufactured product all result environmental pollution (Jacob et al. 2018). The largest sources of heavy metals and petroleum hydrocarbons released into the natural ecosystems are mining, electroplating, smelting, fertilizer, pesticides, tanneries, paper and electronic industries (Taiwo et al. 2016).

Based on their physiological effects, heavy metals can be divided into two groups: on the one hand, essential heavy metals, which are essential for the biochemical processes of living organisms (Fe, Cu, Mn, Zn) (Asati et al. 2016), and on the other hand, the toxic heavy metals having only well-known negative physiological effects (Cd, Pb, Hg). Of the heavy metals, arsenic, aluminium, cadmium, lead, chromium, mercury, zinc and nickel are the heavy metals that pose the greatest threat to the environment (Xiang et al. 2021, Rajendran et al. 2022). Heavy metals entering the environment can be accumulated in the soil and in the sediments of natural waters, bound to their organic compounds. Roots of crops and other plants pick up these elements along with water and pass on to plants and then plants to animals (Simon et al. 2022, Karimyan et al. 2020).

The heavy metals as nonbiodegradable pollutants can be accumulated in the food chain. During evolution, organisms have developed specific defence mechanisms against the harmful effects of heavy metals (Emamverdian et al. 2015). One type of detoxification process is non-enzymatic, in which simple metabolites (ascorbate, glutathione), or proteins

(phytochelatins, metallothioneins) bind heavy metals that pose a threat to the active elements of metabolism (Briffa et al. 2020). Another option for removing reactive oxygen forms (ROS) that appear as an indirect effect of heavy metals is offered by special enzymes (catalase - CAT), peroxidase - POX, superoxide dismutase - SOD) (Mahdu & Sadagopan 2020). The aim of our work was to investigate the impact of some soil amendments (wastewater sediment, sewage sludge – green waste compost and their mix) on the accumulation of toxic elements and on activity of certain enzymes in a Sudan grass variety.

2. Materials and methods

Growth chamber pot experiment was set up with a special variety of Sudan grass named "GK Csaba" [hybrid of *Sorghum bicolor* (L.) Moench x *Sorghum* Sudanense (piper) Stapf.] (Cereal Research Non-Profit Ltd., Szeged, Hungary). Plants were grown in a slightly contaminated soil (control (C), topsoil of the experimental field, with low heavy metal content), which was amended on one hand with mainly heavy metal polluted wastewater sediment (WS, 20% (m/m)) originated from the former wastewater settling plant of Debrecen, Hungary), found in 70-110 cm depth; (details in Vincze et al. 2022), on the second hand with 10% (m/m) high macroelement-containing compost made of sewage sludge and green waste (AKSD waste-processing plant, Debrecen, Hungary (SG)), on the third hand with their mix (20% WS + 10% SG).

Plants were grown in a growth chamber under controlled environment for 44 days. Details of setting up the experiment in Vincze et al. (2022).

Elemental analysis of growth media and plant samples was done in three phases of our experiment: (i) from control soil (C), wastewater sediment (WS) and compost made of sewage sludge + green waste (SG) just before preparing media for experiments; (ii) from the growing media (C, C+20% WS, C+10% SG, C+20% WS+10 % SG); (iii) from plant organs at the end of the experiment. Soils were in all cases dried, homogenized, and sieved (< 2 mm) From 25 subsamples composite samples were formed with a total weight of 100 grams, and a small portion (5 grams) was used for analytic procedure. The elemental composition of soil and plant samples was determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) technique (model iCAP 7000, ThermoFischer Scientific, USA) following a digestion of samples in cc. HNO_3−cc. H_2O_2 mixture according to the Hungarian Standard (MSZ 21470-50, 2006). All measurements were done in 3 replicates.

The details of methods used for preparation of plant extracts, to determine the enzymatic activities (G6PDH - glucose 6-phosphate dehydrogenase, ICDH - isocitrate dehydrogenase, MDH - malate dehydrogenase and POX - guaiacol peroxidase) and the protein content can be found in Vincze et al. (2022) (Mocqout et al. 1996).

Statistical analysis of experimental data was conducted with SPSS 26.0 software using analysis of a variance (ANOVA) followed by treatment comparison using Tukey's b-test.

3. Results

The elemental compositions of the soil and the soil amendments are summarized in the *Table 1* and *Table 2*. According to the date the compost (SG) contains very high amount of some essential plant nutrients (P, K, Ca, Fe and Mn) compared to both the control soil (C) and wastewater sediment (WS), respectively. On the other hand, the highest toxic element (As, Cr and Pb) as well as Cu and Zn content can be determined in the wastewater sediment (WS) compared to both control soil (C) and compost (SG).

Table 1: The essential plant nutrient content of the soil and the soil amendments used for pot experiments set up with Sudan grass. (**C**ontrol soil - C; **W**astewater **S**ediment - WS from Debrecen-Lovászzug; Compost made of **S**ewage sludge and **G**reen-waste - SG). (University of Nyíregyháza, 2018.10.30.)

	Essential plant nutrients							
	P	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**
	mg/kg							
Control soil	1122	1859	17921	5055	11799	306	44.4	176
Wastewater sediment	5125	2963	29206	7331	22756	514	189	888
Compost	20507	6972	42410	8475	31364	963	112	605

Source: Author's own editing

Table 2: The toxic element content of the soil and the soil amendments used for pot experiments set up with Sudan grass. (**C**ontrol soil - C; **W**astewater **S**ediment - WS from Debrecen-Lovászzug; Compost made of **S**ewage sludge and **G**reen-waste - SG). (University of Nyíregyháza, 2018.10.30.)

	Toxic elements				
	As	**Cd**	**Cr**	**Ni**	**Pb**
	mg/kg				
Control soil	7,16	0,303	120	31,8	35,8
Wastewater sediment	12,25	1,27	1027	49,5	287
Compost	6,66	0,722	55,00	35,8	23,3

Source: Author's own editing

As it can be seen in the *Table 3* and *Table 4*, the application of the wastewater sediment as soil amendment increased mainly the heavy metal content (toxic metals in C+20% WS or C+10% SG+20% WS), while the use of the compost as a soil amendment, elevated mainly the concentrations of essential macroelements (P and K) in the media used for plant grow.

Table 3: The essential plant nutrient content of the media used for pot experiments set up with Sudan grass. (University of Nyíregyháza, 2018.10.30.)

Treatments	Essential plant nutrients							
	P	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**
	mg/kg							
Control soil	1374[a]	2266[a]	19922[a]	5300[a]	13987[a]	317[b]	46,1[a]	161[a]
C+10% SG	3167[c]	2760[c]	22118[b]	5571[b]	15388[b]	350[c]	53,9[b]	200[b]
C+20% WS	1916[b]	2330[b]	22808[c]	5636[c]	16151[c]	311[a]	98,2[c]	519[c]
C+10% SG+20% WS	3848[d]	2819[d]	25123[d]	5968[d]	17746[d]	348[c]	104,6[d]	574[d]

Data are means of 3 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically significant at P<0.05. Source: Author's own editing

Comparing, the concentrations of essential elements, measured in the roots and leaves (*Table 5*) it can be concluded that the values of P, K, Ca and Mg are significantly higher in leaves, while in contrast those of Fe, Mn, Cu and Zn are higher in the roots. The concentrations of the elements in plant organs (leaves and roots) developed in accordance with the same trends.

The higher the concentrations of an element in the soil the greatest accumulation of that element both in roots and leaves.

Table 4: The toxic element content of the media used for pot experiments set up with Sudan grass. (University of Nyíregyháza, 2018.10.30.)

Treatments	Toxic elements				
	As	Cd	Cr	Ni	Pb
	mg/kg				
Control soil	7,81[a]	0,270[a]	149[b]	23,9[a]	29,8[b]
C+10% SG	8,12[b]	0,334[b]	141[a]	24,7[b]	28,9[a]
C+20% WS	12,1[c]	0,479[c]	382[c]	45,8[c]	90,0[c]
C+10% SG+20% WS	12,6[d]	0,532[d]	377[c]	47,3[d]	89,7[c]

Data are means of 3 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically significant at P<0.05. Source: Author's own editing

Higher element concentrations were determined in the case of all applied treatments compared to control. The changes in Ca, Mg and Mn concentrations were least of all sensitive to treatments: the increase for these elements did not exceed 20%. In contrast, the average increase in case of P, Cu and Zn exceeded 40%. In the case of Cu there were exceptionally high values detected in both roots and leaves (90 - 100% greater) compared to the control while the concentrations of Zn were more than 200% higher in leaves compared to control.

Table 5: Concentrations of essential elements in plant organs (root and leaves) collected at the end of pot experiment. (University of Nyíregyháza, 2018.10.29.)

Treatments	Essential plant nutrients							
	P	K	Ca	Mg	Fe	Mn	Cu	Zn
	mg/kg							
	Root							
Control soil	1705[a]	10131[a]	4623[a]	2442[a]	366[a]	10,2[a]	8,19[a]	141[a]
C+10% SG	2533[c]	13684[c]	4995[b]	2570[b]	401[a]	11,8[c]	9,34[b]	163[b]
C+20% WS	2340[b]	10127[a]	5014[b]	2488[a]	454[b]	12,7[d]	17,0[c]	242[d]
C+10% SG+20% WS	2634[d]	12496[b]	5799[c]	2702[c]	465[b]	11,2[b]	18,2[d]	206[c]
	Leaves							
Control soil	1987[a]	26631[a]	5844[a]	3244[a]	41,7[a]	5,71[b]	4,22[a]	45,2[a]
C+10% SG	2279[b]	29293[c]	6427[b]	3328[b]	47,1[b]	6,30[c]	4,84[b]	55,1[b]
C+20% WS	2797[c]	27278[b]	6622[c]	3396[c]	51,9[c]	5,48[a]	8,17[c]	140,7[c]
C+10% SG+20% WS	3025[d]	33241[d]	6998[d]	3558[d]	54,3[d]	6,28[c]	8,54[d]	145,2[d]

Data are means of 3 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically significant at P<0.05. Source: Author's own editing

Regarding toxic elements, the trend of "higher heavy metal concentration in the soil, higher metal content in plant organs" prevailed (*Table 6*). The greatest amounts of heavy metals were detected in presence of 20% WS. The accumulation of heavy metals was concentration dependent: the presence of WS in the medium elevated strongly the heavy metal content and the highest concentrations were measured in these experiments. In the case of As and Cd, the concentration-dependent increase varied between 5-75% (both of

plant organs), while in the case of Cr, Ni and Pb, the increase exceeded 100% and even 200% (Pb) both in the roots and leaves.

Table 6: Concentrations of toxic elements in plant organs (root and leaves) collected at the end of pot experiment. (University of Nyíregyháza, 2018.10.29.)

Treatments	Toxic elements				
	As	Cd	Cr	Ni	Pb
	mg/kg				
Root					
Control soil	0,665[a]	0,338[a]	1,40[a]	2,53[a]	0,297[a]
C+10% SG	0,691[b]	0,388[b]	1,26[a]	2,55[a]	0,290[a]
C+20% WS	0,952[c]	0,574[c]	3,74[b]	4,97[b]	0,902[b]
C+10% SG+20% WS	1,050[d]	0,596[d]	3,83[b]	5,02[b]	1,033[c]
Leaves					
Control soil	0,199[a]	0,337[a]	0,177[a]	0,631[a]	0,096[a]
C+10% SG	0,206[a]	0,388[b]	0,169[a]	0,683[b]	0,097[a]
C+20% WS	0,299[b]	0,549[c]	0,447[c]	1,20[c]	0,297[c]
C+10% SG+20% WS	0,310[c]	0,589[d]	0,427[b]	1,30[d]	0,281[b]

Data are means of 3 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically significant at P<0.05. Source: Author's own editing

The protein content showed a significant 20% increase in presence of compost (*Table 7*). The MDH had the least sensitivity to the treatments applied, it produced only slight changes. The activities of enzymes taken part in the carbohydrate metabolism (G6PDH, ICDH) changed parallel to protein content: the greater amount of protein the higher the enzyme activities measured. The enzyme activity of POX was higher in presence of high heavy metal containing wastewater sediment indicating more intensive stress.

Table 7: The enzyme activities in leaves of Sudan grass variety at the end of experiment. (University of Nyíregyháza, 2018.07.19.) (Abbreviations: G6PDH - glucose 6-phosphate dehydrogenase; ICDH - isocitrate dehydrogenase; MDH - malate dehydrogenase; POX - guaiacol peroxidase).

Treatments	Protein mg/ml	Enzyme activities (ΔOD/min.mg protein)			
		G6PDH	ICDH	MDH	POX
Control soil	0,83[a]	0,405[b]	0,72[b]	14,6[a]	24,8[b]
C+10% SG	1,02[b]	0,582[c]	1,21[c]	12,7[a]	18,5[a]
C+20% WS	0,84[a]	0,314[a]	0,58[a]	13,5[a]	28,9[c]
C+10% SG+20% WS	1,01[b]	0,372[ab]	0,71[b]	12,4[a]	30,0[c]

Data are means of 3 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically significant at P<0.05. Source: Author's own editing

4. Discussion

The effects of a heavy metal containing wastewater sediment and a compost made of sewage sludge and green waste on a variety of Sudan grass (GK Csaba) was investigated in a pot experiment. The uptake of element proved concentration dependent, that is, the higher elemental concentrations in the soil the greater amounts of elements in plant organs. Of the essential elements the P (+15-55%), the Cu (+15-120%) and the Zn (15-220%) showed the

highest accumulation in plant roots and leaves. The accumulation of toxic heavy metals was extremely high: the concentrations of Cr (10-170%), Ni (10-110) and Pb (5-250%) far exceeded the values measured in control plants. Of the enzymes taking part in the carbohydrate metabolism G6PDH and ICDH showed elevated activities in presence of compost (SG). In the same time the activity of POX was elevated in presence of the heavy metal rich wastewater sediment, as it was expected during greater stress.

Acknowledgements

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