Review on Agriculture and Rural Development 2022 vol. 11 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2022.1-2.186-192

IMPACT OF HEAVY METAL POLLUTED WASTEWATER SEDIMENT ON ELEMENT CONTENT AND ENZYME ACTIVITY OF SUDAN GRASS

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ABSTRACT
Growth chamber pot experiment was set up with two varieties of Sudan grass. Plants were grown in slightly contaminated (control) topsoil of the former wastewater settling pond from Debrecen, which was amended with 10% (m/m) of wastewater sediment (P: 5125; Fe: 22756, Pb: 287; Cr: 1027; Zn: 888 mg/kg). The elemental composition of soil and shoots of plants and activities of some enzymes in leaves were measured. It can be concluded that the roots and shoots of plant individuals of both varieties consistently took up higher concentrations of each of the elements from the medium "enriched" with wastewater sediment. The elevated element concentrations were found in some cases in the roots, and in other cases in the leaves. The excessive accumulation of toxic elements was not observed. The activities of four enzymes were measured in the leaves of plants (glucose 6-phosphate dehydrogenase, G6PDH; isocitrate-dehydrogenase, ICDH; peroxidase, POX; catalase, CAT). The enzyme activities of POX increased meanwhile that of CAT decreased significantly, in spite of slight differences. As a trend, the specific activity for G6PDH increased by 26% (GK Csaba) and 36% (Akklimat), while values for ICDH became higher by 28% (GK Csaba) and 41.5% (Akklimat), for the two varieties studied, respectively.

Keywords: wastewater sediment, potentially toxic elements, antioxidative enzymes, Sudan grass

INTRODUCTION

Energy and mineral consumption by mankind is the main cause of trace element pollution in the biosphere. During the last century, soil and water resources were contaminated with metals and metalloids all over the world, as a consequence of industrial production, mining, smelting, traffic, disposal of wastes, etc. This becomes of environmental concern when metals (i.e.: Pb, Cd, Zn, Cu, Cr, Ni, Hg) in soils and waters enters to the food chain, and begins to affect human health (SIMON, 2014).

Heavy metal is a collective term for metals and metalloids, with density higher than 5 g/cm^3. One part of heavy metals is essential for plants and for animals, while the other part is generally toxic for living organisms (ASATI ET AL., 2016). Heavy metals can be accumulated in the air, in surface and subsurface waters, and in soils mainly in industrial regions. Heavy metals in the soils are mainly immobilized on soil particles at near neutral pH, this way their bioavailability is generally low. The main problem is that they are nonbiodegradable and can be accumulated in the food chain (SIMON, 2014, ASATI ET AL., 2016).

The presence of potentially toxic heavy metals can affect adversely the biochemical processes in cells, or physiological functions of organs. Heavy metals can be bound directly to biologically active molecules, to proteins modifying their activity and effectiveness. Some heavy metals can bind to sulfhydryl functional groups hampering catalytic function of some enzymes. Phytochelatins and metallothioneins are products of cells, which can bind to heavy metal cations and take part in temporary detoxification. Heavy metals can also exert their toxic effects indirectly by inducing reactive oxygen forms (ROS), resulting in oxidative stress (EMAMVERDIAN ET AL., 2015). There are well known adaptation mechanisms in plants for eliminating ROS elements during enhanced level of toxic heavy metals. This system includes non-enzymatic antioxidants (glutathione

(GSH), ascorbate (ASA), etc.) and special enzymes (catalase (CAT), superoxide dismutase (SOD), peroxidase (POD)) (MADHU and SADAGOPAN, 2020). The presence of high amounts of heavy metals in cells generally raise the activity of enzymes taking part to lower the level of pollutants and at elevated heavy metal concentrations the depots of low molecular weight non enzymatic antioxidants are depleted (GJORGIEVA ACKOVA, 2018).
The aim of our work was to investigate the impact of sewage sediment on the accumulation of toxic elements and on activity of certain enzymes in two varieties of Sudan grass.

MATERIALS AND METHODS

Growth chamber pot experiment was set up with two special varieties of Sudan grass named "GK Csaba" [hybrid of *Sorghum bicolor* (L.) Moench x *Sorghum* Sudanense (piper) Stapf.] and "Akklimat" (*Sorghum sudanense* (piper) Stapf.) (Cereal Research Non-Profit Ltd., Szeged, Hungary). Plants were grown in slightly contaminated soil (control – C, topsoil from the experimental field), without any heavy metal contamination, which was amended with 10% (m/m) wastewater sediment (WS). WS originated from Lovász-zug suburban area of Debrecen city, Hungary (47°29′07″ N, 21°35′46″ E), where formerly a sewage settling pond was operated as a secondary biological purification unit (TŐZSÉR ET AL., 2018). The control soil (C) studied in our pot experiment was used during the recultivation of settling ponds as a soil cover, its genetic type could not be determined. WS samples were collected from this recultivated sewage settling pond (geographical point EOV X: 240876 m; EOV Y: 842073 m), where WS was located under soil cover (C) in a 70-110 cm depth. Larger amounts were taken to the laboratory from topsoil (C) and from WS. Control soil samples were air dried, mixed, and passed through a 2-mm diameter sieve. WS samples were also air dried, shredded, thoroughly mixed, and passed through a 5-mm diameter sieve.

Samples were taken in 4 replicates from the C soil and from WS for basic properties analysis. Basic characteristics of the C soil were the followings: loamy texture; pH-H_2O 7.72; pH-KCl 7.30, total salt content (m/m%): 0.057; $CaCO_3$ (m/m%): 2.25; humus (m/m%): 2.27; NH_4-N (mg/kg): 37.0; NO_3-N (mg/kg): 10.9; P–1122, K–1859, Ca–17921, Mg–5055; Fe–11799; As–7.16, Cd–0.303, Cr–120, Cu–44.4, Mn–306, Ni–31.8, Pb–35.8, and Zn–176 mg/kg; as determined from cc. HNO_3–cc. H_2O_2 extract, followed the instructions of a Hungarian Standard MSZ 21470-50 (2006). WS basic characteristics were the followings: pH-H_2O 7.11; total salt content (m/m%): 1.80; dry matter (m/m%): 91.98; organic matter (m/m%): 26.9; P–5125, K–2963, Ca–29206, Mg–7331; Fe–22756; As–12.3, Cd–1.27, Cr–1027, Cu–198, Mn–514, Ni–49.5, Pb–287, and Zn–888 mg/kg; as determined from cc. HNO_3 – cc. H_2O_2 extract, followed the instructions of a Hungarian Standard MSZ 21470-50 (2006).
Plants were grown in a growth chamber under controlled environment: light (10000 lux till 8^{th} day → 17500 lux till 28^{th} day → 21500 lux till 44^{th} day), humidity (30-40%), temperature (24-26 ^{o}C during day, 18-19 ^{o}C during night). The evaporated water was supplied with distilled water in every second day (according to change of the constant weight of pots). Plant samples for elemental analysis and for enzymatic tests were collected at the end of the experiment (44^{th} day). The plant material was washed, dried (70 ^{0}C, 10 hours) and ground (<1-mm) before the elemental analysis. For enzymatic assays the first fully developed leaves from top were collected from 4 plants. Leaves were frozen and kept in a refrigerator at -80 ^{0}C until use.
Elemental analysis of growth media and plant samples was done in three phases of our experiment: (i) from control soil (C) and wastewater sediment (MWS) just before

preparing media for experiments; (ii) from the growing media (C, C+10% WS) for plants at the end of the experiment; (iii) from plant leaves at the end of the experiment. Soils were in all cases dried, homogenated, 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 Spectrometry (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.

For the enzymatic assay 0.5 g of plant material (leaves) was ground and extracted in a chilled mortar and pestle using 50 mM Tris-HCl buffer (pH: 7.0) (in 1:5 m/V rate), containing 5 mM $MgCl_2$, 1 mM EDTA, 10% glycerol and 1% β-mercaptoethanol. Protein determinations were based on the method of BRADFORD (1976) using Bio-Rad protein assay reagent.

The enzyme activities of G6PDH (glucose 6-phosphate dehydrogenase), ICDH (isocitrate dehydrogenase) and POX (guaiacol peroxidase) were determined by spectrophotometric methods of MOCKUOT ET AL., (1996) (Jasco UV-VIS 530), the activity of CAT (catalase) was measured by LUCK (1974).

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.

RESULTS

The high essential and toxic element containing wastewater sediment was mixed with topsoil of the experimental field (slightly contaminated control soil) at 10% (m/m) rate. The studied wastewater sediment contains much higher concentrations of all investigated elements compared to uncontaminated control topsoil.

Table 1. The essential plant nutrient content of the media used for pot experiments set up with Sudan grass. (University of Nyíregyháza, 2018.07.18.)

Treatment	Variety	Essential plant nutrients							
		P	K	Ca	Mg	Fe	Cu	Mn	Zn
		mg/kg							
Control	cv. GK Csaba	1409	2088	19951	5309	13993	47.7	319	162
Control+10% D-L	cv. GK Csaba	1547	2223	21213	5416	15109	67.5	344	290
Control	cv. Akklimat	1340	2089	20193	5163	14048	47.3	318	164
Control+10% D-L	cv. Akklimat	1545	2218	21133	5358	15179	70.0	338	290

Data are means of 3 replications.

Table 2. The toxic element content of the media used for pot experiments set up with Sudan grass. (University of Nyíregyháza, 2018.07.18.)

Treatment	Variety	Toxic elements					
		As	Ba	Cd	Cr	Ni	Pb
		mg/kg					
Control	cv. GK Csaba	8.00	118	0.272	149	25.2	29.9
Control+10% D-L	cv. GK Csaba	11.6	164	0.337	330	32.5	52.0
Control	cv. Akklimat	7.92	116	0.274	154	24.5	28.3
Control+10% D-L	cv. Akklimat	11.0	168	0.321	330	31.4	51.4

Data are means of 3 replications.

The elemental composition of the media used for our experiments can be seen in the *Table 1*. and *Table 2*. As data show, concentrations of all essential and toxic elements consequently enhanced after mixing.
The elemental composition of Sudan grass grown on uncontaminated and treated soils was also determined at the end of the experiment. We found that concentrations of all essential plant nutrients were higher in the plants grown on treated soil (in roots and shoots and, in both varieties) (*Table 3*.). The rate of increase was different in the root and, in the shoot: in roots, significant excess of essential macroelements was found (in case of K +30.3% (cv. GK Csaba) and, in case of Ca +60.0% (cv. Akklimat)), while in shoots the accumulation of macroelements (P, K, Ca, Mg) was no more than 20%.

Table 3. Concentrations of essential elements in plant organs (root and shoot) collected et the end of pot experiment. (University of Nyíregyháza, 2018.07.18.)

Treatment	Variety	Essential plant nutrients							
		P	K	Ca	Mg	Fe	Cu	Mn	Zn
		mg/kg							
		Root							
Control	cv. GK Csaba	1874[a]	11122[a]	4673[b]	2402[a]	355[a]	7.91[a]	9.01[a]	130[a]
Control+10% D-L	cv. GK Csaba	1987[b]	14500[b]	5473[d]	2944[c]	854[d]	11.6[c]	20.8[c]	237[c]
Control	cv. Akklimat	2053[c]	18753[c]	3087[a]	2729[b]	449[b]	10.4[b]	14.4[b]	139[b]
Control+10% D-L	cv. Akklimat	2319[d]	19684[d]	4941[c]	3163[d]	753[c]	14.5[d]	25.2[d]	251[d]
		Shoot							
Control	cv. GK Csaba	2046[a]	29240[a]	5768[a]	3254[a]	41.0[a]	4.08[a]	5.54[a]	44.3[a]
Control+10% D-L	cv. GK Csaba	2412[b]	32754[c]	6242[b]	3334[b]	45.3[b]	4.49[a]	6.47[b]	79.5[c]
Control	cv. Akklimat	2558[c]	32288[b]	6194[b]	3656[c]	47.4[c]	4.31[a]	6.47[b]	52.3[b]
Control+10% D-L	cv. Akklimat	2753[d]	35484[d]	6930[c]	3944[d]	52.7[d]	5.99[b]	7.54[c]	98.9[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 $P<0.05$.

Table 4. Concentrations of toxic elements in plant organs (root and shoot) collected et the end of pot experiment. (University of Nyíregyháza, 2018.07.18.)

Treatment	Variety	Toxic elements					
		As	Ba	Cd	Cr	Ni	Pb
		mg/kg					
		Root					
Control	cv. GK Csaba	0.69[a]	5.48[a]	0.356[a]	1.47[a]	2.41[a]	0.277[a]
Control+10% D-L	cv. GK Csaba	1.09[b]	7.29[b]	0.523[b]	3.30[b]	4.95[b]	0.478[b]
Control	cv. Akklimat	1.19[c]	7.71[c]	0.350[a]	1.67[a]	7.58[c]	0.473[b]
Control+10% D-L	cv. Akklimat	1.67[d]	15.7[d]	0.519[b]	4.51[c]	14.7[d]	0.884[c]
		Shoot					
Control	cv. GK Csaba	0.194[b]	2.56[a]	0.344[b]	0.170[a]	0.621[b]	0.084[a]
Control+10% D-L	cv. GK Csaba	0.274[d]	3.94[c]	0.414[d]	0.189[b]	0.746[c]	0.157[c]
Control	cv. Akklimat	0.163[a]	3.49[b]	0.257[a]	0.189[b]	0.468[a]	0.141[b]
Control+10% D-L	cv. Akklimat	0.251[c]	4.74[d]	0.358[c]	0.210[c]	0.835[d]	0.208[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.

The increase of essential microelements (Fe, Cu, Mn, Zn) in root and shoot was significant. The change is more emphasized in root where the concentrations are 39-140% higher comparing to the control values. In shoot the measured values were only moderately higher (10-18%) with the exceptions of Zn (+80-90%, in both varieties) and of Cu (+39%, in cv. Akklimat).

Concentrations of toxic elements of plant samples are shown in *Table 4.* Similar tendencies can be found than in case of essential macroelements, that is, the accumulation of toxic elements in root was much higher than in shoot. The enhancement of As and Cd as „moderate" (+40-60%), while in case of Ba, Cr, Ni and Zn the concentrations became much higher (+72-170%). In shoot the concentrations of toxic element increased typically with 15-50%, as a consequence of the treatment. We could find extreme values in case of Pb (73-87% in two varieties) and Ni (78%, cv. Akklimat) where values calculated were higher comparing to the control.

The enzyme activities of two antioxidant enzymes (POX, CAT) and, of two enzymes of carbohydrate metabolism (G6PDH, ICDH) were determined. Data are shown as specific activities in *Table 5*. The protein concentrations were unchanged in the presence of wastewater sediment. The activities of the enzymes of carbohydrate metabolism were not affected by soil treatment.

Table 5. The enzyme activities in leaves of Sudan grass varieties at the end of experiment. (University of Nyíregyháza, 2018.07.19.) 10% D-L - 10% (m/m) wastewater sediment from Debrecen-Lovászzug) (Abbreviations: G6PDH - glucose 6-phosphate dehydrogenase; ICDH - isocitrate dehydrogenase; POX - guaiacol peroxidase; CAT - catalase).

Treatment	Variety	Protein mg/ml	G6PDH	ICDH	POX	CAT
			ΔOD/min.mg protein			
Control	cv. GK Csaba	1.46	0.077^{a}	0.147^{a}	13.5^{ab}	1.06^{ab}
Control+10% D-L	cv. GK Csaba	1.45	0.097^{a}	0.188^{a}	13.8^{ab}	0.84^{a}
Control	cv. Akklimat	1.49	0.072^{a}	0.147^{a}	12.3^{a}	1.33^{b}
Control+ 10% D-L	cv. Akklimat	1.49	0.098^{a}	0.208^{a}	14.5^{b}	0.46^{a}

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.

In spite of about 30% difference between treated and untreated samples, the differences were not statistically confirmed. The activity of POX slightly increased in plants grown in treated soil in both varieties (cv. GK Csaba and Akklimat). The differences were statistically significant in spite of slight elevation of values. The activity of CAT dropped sharply during soil treatment. The decrease was significant in case of variety named Akklimat.

DISCUSSION

The effects of a heavy metal containing wastewater sediment on two varieties of Sudan grass was investigated in a pot experiment. Increased uptake of all essential macro-, and microelements, as well as potentially toxic elements was measured in the presence of polluted wastewater sediment. The accumulation of essential microelements (Fe, Cu, Mn, Zn) were more significant than the uptake of essential macroelements (K, Ca, Mg, P). Remarkable uptake was detectable in roots comparing to shoots in both Sudan grass varieties. Some toxic elements were accumulated in high rate mainly in roots: Ba - +103.3% (Akklimat), Cr - +125.5 and 170.0% (GK Csaba and Akklimat respectively), Pb - +72.6 and 86.9% (GK Csaba and Akklimat respectively). In shoot the increase was moderate (+10-50%) but Ni - +78.4% (Akklimat), Pb - +86.9% (GK Csaba). As a consequence of treatment with wastewater sediment the activities of all investigated enzymes changed. The increase of POX activity and the decrease of CAT activity were significant statistically. The change of activities of antioxidative enzymes under stress are not defined, it depends on many factors: species, type and intensity of stress, physiological status of the organism (KUSVURAN ET AL., 2016).

ACKNOWLEDGEMENTS

This research was supported in the frame of Hungarian GINOP 2.2.1-15-2017-00042 R&D Competitiveness and Excellence Cooperation by the „Genetic utilization of plants in the Pannon region" project. The authors appreciate the precise service of Dr. Tünde Pusztahelyi and her team from the Agricultural Instrument Center University of Debrecen, Debrecen, Hungary in the plant and soil analysis. Sudan grass seeds were provided by Dr. Andrea Palágyi from Cereal Research Non-Profit Ltd., Szeged, Hungary.

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