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THE POSSIBILITY OF USING 5-AMINOLEVULINIC ACID IN LEAD PHYTOEXTRACTION PROCESS

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Abstract. Studies conducted in the greenhouse involving a sunflower (*Helianthus annuus* L.) grown in medium containing 200 mg Pb · dm^{-3} which were designed to evaluate the impact of foliar nutrition with 5-aminolevulinic acid (5-ALA) on the chemical composition of sunflower in reference to possible use of this plant in the process of induced phytoextraction. The study revealed from 4.8% to 34.1% increase in aboveground matter of sunflower which was sprayed by 5-ALA solution at concentrations of 0.01-0.1 ppm in comparison with plants grown in a medium containing 200 mg Pb · dm^{-3} with no 5-ALA spraying. The sunflower leaves sprayed by 5-ALA solution contained from 3.78% to 27.1% more lead in comparison to plants not sprayed by this agent. As well as remarkable decrease in lead content from 17.4 to 33.4% was recorded in the roots of sunflower sprayed by 5-ALA solution. The lead content in plant shoots was independent from foliar application of 5-aminolevulinic acid.

Key words: *Helianthus annuus*, heavy metals, phytoremediation, induced phytoextraction, 5-ALA, lead

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Introduction

Regarding the phytoremediation process considered as the most effective way of purifying the soil from heavy metals, the induced phytoextraction seems to be the most promising (Salt *et al.* 1998). According to Małkowski (2011), this process uses commonly cultivated species producing sufficiently large vegetative matter (such as mustard, sunflower or maize) and they are subsequently stimulated to uptake heavy metals by adding particular agents to the soil or directly onto the plants. To induce phytoextraction, following root chelators are usually used: ethylenediaminetetraacetic acid (EDTA), N,N-ethylenediaminedisuccinic acid (EDDS), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), citric acid, malic acid, etc. (Małkowski 2011). There are few literature reports on the possibility of inducing the phytoextraction by applying the foliar inductor.

5-aminolevulinic acid (5-ALA) is a non-protein amino acid found in every living organism, playing the role of a chlorophyll precursor at plants (Jarosz 2012; Tanaka *et al.* 2005). Memon *et al.* (2009) showed greater photosynthetic and antioxidant activity of 5-aminolevulinic acid at its foliar application in the cultivation of Chinese cabbage. Numerous studies revealed that regular use of 5-ALA resulted in a significant increase in the uptake of nutrients and ballast ions by some test plants (den Hertog 2008; Smolen & Sady 2010). These interesting results gave grounds for believing that regular use of 5-aminolevulinic acid would increase the uptake of heavy metals from the soil by plants that are not typical hyper-accumulators.

Another factor that may have favored the phytoextraction of heavy metals by plants sprayed by 5-ALA is the phenomenon of transpiration intensification observed in some studies upon regular use of this non-protein amino acid. This thesis was confirmed by Żurek & Majtkowski (2009) proving that the main factor determining the efficiency of phytoextraction consists in a water quantity along with dissolved substances (including heavy metals) passing through a plant per unit of time.

The study aimed at evaluating the effect

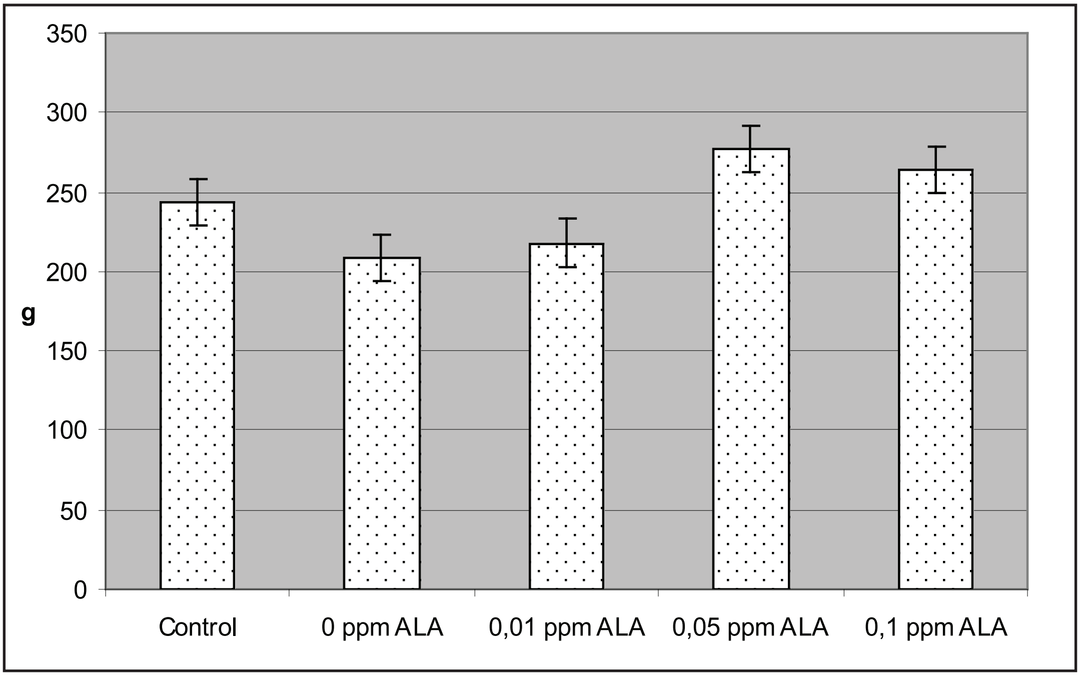

Fig. 1. The effect of spraying 5-aminolevulinic acid on fresh weight aboveground parts of sunflower.

of regular use of 5-aminolevulinic acid on the chemical composition of sunflower in terms of potential application of the compound during induced phytoextraction process.

Material and methods

The study was carried out in an experimental greenhouse of Department of Cultivation and Nutrition of Horticultural Plants, University of Life Sciences in Lublin, in the framework of project No. 225 "Support for Regional Cooperation Network" within "Human Capital" Operational Program 2007-2013. Sunflower (*Helianthus annuus* L.) considered by many authors as a species useful in the phytoextraction (Bosiacki & Wojciechowska 2012) as well as induced phytoextraction process (Kucharski *et al.* 1998), was the experimental crop. Plants were grown in 5 dm^3 capacity pots filled with the peat substrate neutralized to pH 6.5 in 8 replications (3 plants per each pot). The repetition consisted of a pot with three plants growing. Sunflower seeds were sown directly into the peat substrate. The experiment system included the following objects:

I – control: plants grown with no lead and no spraying with 5-ALA,

II – 200 mg Pb · dm^{-3} in substrate and no spraying with 5-ALA,

III – 200 mg Pb · dm^{-3} in substrate and spraying with 0.01 mg ALA · dm^{-3}

IV – 200 mg Pb · dm-3 in substrate and spraying with 0.05 mg ALA · dm^{-3}

V – 200 mg Pb · dm-3 in substrate and spraying with 0.1 mg ALA · dm^{-3}

Lead was applied to the substrate in two equally divided doses of 100 mg Pb · dm^{-3} in a form of lead nitrate $Pb(NO_3)_2$ after plant germination and in the stage of full forming the second leaf. Plants fertilization was carried out on the basis of their nutritional requirements using the multi-component fertilizer 'Nutrifol', as well as one- and two-component agents (calcium nitrate, ammonium nitrate, potassium sulfate, magnesium sulfate).

The plant spraying with 5-aminolevulinic acid solutions was made regularly every 7-10 days in accordance to the experimental pattern. Plants were sprayed by water in control series.

After the experiment complete, weight of

Table 1. The contents of nutrients, sodium and lead in sunflower leaves.

Treatment	N total	P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Pb
	% d.w.					mg · kg^{-1} d.w.					
Control	4,02	0,44	4,71	4,02	0,45	25,9	90,3	46,0	71,8	3,44	-
Pb + 0,00 ppm ALA	4,68	0,44	4,66	3,93	0,48	24,0	124,5	49,2	76,4	2,85	108,4
Pb + 0,01 ppm ALA	5,00	0,44	4,91	3,88	0,44	23,8	103,8	46,4	74,4	2,78	112,5
Pb + 0,05 ppm ALA	4,78	0,44	5,00	3,91	0,43	27,2	123,1	44,2	76,5	2,60	122,8
Pb + 0,1 ppm ALA	4,06	0,42	4,58	4,71	0,48	24,1	153,1	52,4	95,2	2,56	137,8
Mean	4,51	0,43	4,77	4,09	0,46	25,0	118,9	47,7	78,6	2,85	120,4
$NIR_{0,05}$	0,29	r.n.	r.n.	0,60	r.n.	r.n.	32,4	5,29	4,61	r.n.	5,99

aboveground parts of plants was determined and plant material samples were collected for subsequent analyses – leaves, stems, inflorescences, and roots separately. The plant material was dried in a drier (105°C) and samples were the subject to determinations for total Kjeldahl nitrogen (Tecator). After wet digestion in a mixture of nitric acid (65%) and perchloric acid in 3:1 ratio (v:v) performed in DK-20 digestion unit (Velp), following items were determined: phosphorus by means of colorimetry using ammonium vanadomolybdate, while K, Ca, Mg, Na, Fe, Zn, Cu, Mn, and Pb applying atomic absorption spectrometry on Perkin-Elmer device Analyst 300 (OSTROWSKA *et al.* 1991).

The statistical processing of results was performed applying variance analysis using average values and Tukey test for assessing the differences at a significance level of α = 0.05.

Results and discussion

Phytoextraction is undoubtedly the most effective and safest way to clean the soils from a variety of contaminants, mainly heavy metals (MAŁKOWSKI 2011). Numerous authors emphasize the exceptionally favorable economic aspect of phytoextraction, when compared it to traditional methods of removing the chemical contaminants from the soil, which are nowadays mainly the chemical or thermal methods and those based on the contaminants extraction. GHOSH & SINGH (2005) estimate the costs of conventional methods applied for decontaminating the soils from heavy metals from 10 to 1000 USD per 1 m^3 of soil. Such vast expenditures are usually the result of having to replace the top layer of soil from a land to be purified. The costs of soil remediation using plants are estimated to be approximately 0.05 USD per m^3, while the researchers emphasize that the effectiveness of this process, in comparison with conventional methods, is much higher (GHOSH & SINGH 2005; ROSADA 2007).

To achieve a sufficiently large weight of the aboveground parts of plants used in the process is the basis of induced phytoextraction efficiency. Here reported results showed a significant decrease in aboveground sunflower matter (14.7%) grown in objects containing 200 mg Pb · dm^{-3} without 5-ALA application, as compared to the control (Fig. 1), which is consistent with numerous literature reports. Accordingly to GRUCA-KRÓLIKOWSKA & WACŁAWSKI (2006), the main reason for the decline in productivity of plants growing on heavy metal contaminated areas is a significant decrease in the photosynthesis efficiency due to interference of chlorophyll biosynthesis. Here presented studies can be some confirmation of this thesis, because there was a significant increase in the plant matter ranging from 4.8 to 34.1% in objects regularly sprayed by 0.01-0.1 ppm 5-ALA solutions. As it is well known, 5-aminolevulinic acid is an essential element of biosynthesis porphobillinogen, the substrate for porphyrite arrangement synthesis.

In opinion of TYKSIŃSKI (2002), the reason for the mass decrease of plants growing on heavy metal contaminated areas may also consist

Table 2. The content of selected nutrients and lead in the shoots of sunflower.

Treatment	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Pb
	% d.w.			mg · kg^{-1} d.w.					
Control	4,16	1,42	0,34	371,1	23,2	29,2	84,6	3,15	-
Pb + 0,00 ppm ALA	4.40	1,46	0,30	363,8	50,6	36,1	80,1	3,81	62,3
Pb + 0,01 ppm ALA	4,36	1,13	0,28	412,6	57,1	26,9	73,8	3,32	52,1
Pb + 0,05 ppm ALA	4,61	1,20	0,31	307,4	43,3	28,3	76,8	2,61	53,7
Pb + 0,1 ppm ALA	4,82	1,17	0,28	427,4	38,2	23,5	84,9	2,53	69,9
Mean	4,48	1,27	0,30	374,8	42,1	26,5	79,1	3,06	59,5
$NIR_{0,05}$	r.n.	r.n.	r.n	101,6	9,5	6,2	r.n.	r.n.	r.n.

Table 3. The content of selected nutrients and lead in sunflower inflorescences.

Treatment	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Pb
	% d.w.			mg · kg^{-1} d.w.					
Control	3,11	1,31	0,19	19,7	28,2	29,7	36,4	5,04	-
Pb + 0,00 ppm ALA	3,21	1,26	0,19	16,5	32,1	39,3	40,3	4,60	32,8
Pb + 0,01 ppm ALA	3,10	1,15	0,18	16,8	33,9	35,4	35,1	4,02	37,8
Pb + 0,05 ppm ALA	3,05	1,07	0,17	16,5	39,9	33,5	40,5	5,23	38,2
Pb + 0,1 ppm ALA	3,15	1,24	0,19	17,6	27,3	34,1	35,9	4,62	38,0
Mean	3,11	1,22	0,18	17,9	32,2	34,2	37,5	4,69	36,7
$NIR_{0,05}$	r.n.	0,18	r.n.	r.n.	r.n.	r.n.	r.n.	r.n.	4,6

Table 4. The content of selected nutrients and lead in the roots of sunflower.

Treatment	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Pb
	% d.w.			mg · kg^{-1} d.w.					
Control	3,00	0,99	0,12	50.3	330,0	21,1	55,1	3,32	-
Pb + 0,00 ppm ALA	2,58	0,90	0,13	41,0	505,6	34,5	65,4	5,68	752,1
Pb + 0,01 ppm ALA	2,66	0,87	0,12	37,2	475,7	28,9	66,9	8,48	580,4
Pb + 0,05 ppm ALA	2,63	0,94	0,13	34,9	545,4	23,6	53,8	4,60	501,3
Pb + 0,1 ppm ALA	2,90	0,82	0,12	36,1	275,6	21,9	94,7	3,89	621,2
Mean	2,75	0,90	0,12	40,0	425,4	26,2	66,8	5,19	601,2
$NIR_{0,05}$	0,30	r.n.	r.n.	r.n.	173,6	r.n.	r.n.	r.n.	123,2

in any disturbances in the uptake, transport, and assimilation of macro and micronutrients. According to the author, negative impact of heavy metals on the uptake and transport of nutritional cations and anions results from a competition for a sorption site on a root surface or creation of unavailable complexes. Analysis of results obtained in present study reveals no such relationship (Tabs. 1-4).

Results of nutrients contents in particular parts of studied plants under the influence of regular spraying with 5-ALA solution seem to be very interesting (Tab. 1-4). Statistical analysis of these results showed significant differences in the contents of total nitrogen, calcium, iron, manganese, and zinc in leaves, while sodium, iron, and zinc in the shoots, calcium in inflorescences, as well as total nitrogen and iron in roots of sunflower. The literature data indicate that foliar use of 5-aminolevulinic acid causes a remarkable increase in the nutrients content at tested plants (Jarosz 2012; den Hertog 2008; Smolen & Sady 2010). Results achieved in this study show that the effect is highly dependent on the concentration of 5-aminolevulinic acid which is applied to the plants.

Analysis of results obtained in present study showed an increase in lead concentration in sunflower leaves sprayed by 5-ALA solution at 0.01-0.1 ppm concentration from 3.78% to 27.1% as compared to plants grown in objects containing 200 mg Pb · dm^{-3} substrate, while not sprayed by 5-ALA solution. A significant increase in the lead content of 15.2-16.5% was also observed in sunflower inflorescences sprayed by 5-ALA solution. It should be emphasized that there was a significant decrease in the lead content (17.4 to 33.4%) in roots of sunflower, the leaves of which were sprayed by 5-ALA solution of 0.01-0.1 ppm concentration as compared to plants not sprayed by this compound (Tab. 4). This is a very important result for assessing the effectiveness of phytoextraction inductor. As it is known, roots accumulate largest amounts of heavy metals (Gadapati & Macfie 2006; Kurtyka *et al.* 2008). In the case of lead, 93-96% of this element is not reutilized into aboveground parts and remains within roots (Gruca-Królikowska & Wacławek 2006). Accordingly to Małkowski (2011), the primary parameter to evaluate the suitability of a substance used to induced phytoextraction is its impact on a metal displacement from roots to the aboveground parts of a plant. Maximizing the heavy metal concentration in the aboveground parts of plants used in the phytoextraction process is one of the key factors determining its effectiveness and profitability (Luo *et al.* 2005).

The main factor determining the efficiency of phytoextraction seems to be the choice of appropriate plants. Use common hyper-accumulators that accumulate 1-2% of heavy metals in their dry matter is marginal due to the negligible mass produced by these plants (Żurek & Majtkowski 2009). The solution may consist in the use of induced phytoextraction applying plants that produce a sufficiently large vegetative mass and certain substances that stimulate the intake of heavy metals.

Achieved results indicate great usefulness of 5-aminolevulinic acid as an inducer of lead phytoextraction from the sunflower root environment. However, this issue requires further studies, particularly with regard to the applicability of this non-protein amino acid in the cultivation of other plants and its interactions with a chelator used for roots.

Conclusions

1. A significant decrease (14.7%) in the aboveground mass of sunflower grown in substrate supplemented with 200 mg Pb · dm_{-3} as compared to plants grown in lead-free substrate was observed.
2. Spraying plants grown in substrate containing 200 mg Pb · dm^{-3} with 0.01-0.1 ppm 5-ALA solution resulted in an increase in aboveground sunflower weight from 4.8% up to 34.1%.
3. The sunflower leaves sprayed by 0.01-0.1 ppm 5-ALA solution revealed from 3.78% to 27.1% more lead in comparison with the plants not sprayed by this agent.
4. A significant decrease in lead content (from 17.4 to 33.4%) was recorded in roots of sunflower sprayed by 0,01-0,1 ppm 5-ALA solution.

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