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PHYTOTOXICITY EFFECTS OF LEAD ON SEEDS GERMINATION AND SEEDLING GROWTH OF `WIZARD ROSE` AND `WIZARD JADE` COLEUS VARIETIES

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Abstract

The study presents the comparative analysis on the impact of different concentrations of Pb(II) (25 to 300 mg/kg) on germination, seedling rate, velocity, tolerance index, toxicity index and vigour index at Coleus blumei 'Wizard Rose' and 'Wizard Jade'. The experience was conducted in 6 variants with 3 replicates, each replicate having 10 seeds. The humidity of the substrate was performed with water for the control sample (C) and with $PbCl_2$ solutions for the other 5 variants. At high concentrations of lead (300mg/l), a significant decrease in the germination percentage can be seen of approximately 20% in the case of the 'Wizard Rose' coleus and 50% in the case of the 'Wizard Jade' coleus. The results obtained in the study show that lead had the highest inhibitory effect on germination, seedling rate, velocity, and plant growth at the coleus 'Wizard Jade'. The degree tolerance of Coleus blumei varieties to the stress caused by the lead decreases with the increase in the concentration of Pb(II), coleus 'Wizard Rose' highlighting a better tolerance to Pb(II) compared to coleus 'Wizard Jade'.

Key words*: Coleus blumei, germination, toxicity, velocity.*

INTRODUCTION

At present, various activities such as industrialization and urbanization contribute to soil contamination with various heavy metals, Hadi & Aziz, 2015. In recent decades, worldwide soil pollution with heavy metals is one of the major problems for both environment and human health (Singh et al., 2011, Bihola et al., 2020). Among the potential heavy metals that are neither essential elements nor have any role in the process of plant cell metabolism, lead (Pb) is a highly toxic and persistent environmental pollutant derived from various sources (Sharma & Dubey, 2005), easily absorbed and accumulated in different parts of a plant, Hadi & Aziz, 2015. Lead is one of the most prevalent heavy metal contaminants in soil that can be retained for a long period of time ranging from 150-5000 years (Prasad et al., 1999). Although lead has no biological purpose, it can disrupt the morphology, physiology and biochemistry of a plant (Fahr et al., 2013; Dabgar et al., 2023). The effects of heavy metals on seed germination differ from species to species due to differences that occur in seed structure, especially in seed layers that not only have a different chemical composition but also a wide range of anatomical shapes that do not exist in any other organ or plant tissue (Yuan et al., 2013; Islam et al., 2008). In general seed germination is dependent on the type of metal and its concentration. Over the years, numerous studies have been conducted focusing on the effect of heavy metals on seed germination and seedling growth (Hatamzadeh et al., 2012; Fatarna et al., 2017). Seed germination represent the starting point in the life cycle of a plant and is considered the earliest stage of a plant's exposure to heavy metal stress, so detection of seed germination and seedling growth index is a good method to evaluate the capacity of plants to heavy metal toxicity (Bae et al., 2016; Taghizadeh & Solgi, 2017). Pb inhibits seed germination and delays seedling growth, decreases germination percentage, germination index, root and shoot length, tolerance index and dry matter content of roots and sprouts (Mishra et al., 2006; Hadi & Aziz, 2015; Dabgar et al., 2023; Stratu & Costică, 2013). The results of studies presented in the literature indicate lower concentrations of heavy metals (between 3 and 33 ppm) with partial inhibitory effect on seed germination in *Picea abies* L. while higher concentrations (100 ppm) of all metals cause partial inhibition in some species (Hagiu Zaleschi et al., 2022; Malkowski et al., 2002; Jeliazkova, 2000) while in others total inhibition. High Pb concentrations inhibit plant growth development which leads to reduction of fresh biomass, tolerance index in roots, sprouts and leaves (Cozma et al., 2019). Similar results have been obtained in other studies at increased Pb concentrations: root, sprouts and leaf growth; fresh and dry biomass is much reduced in *Lavandula angustifolia* (Hagiu Zaleschi et al., 2022), in *Zea mays* (Cimrin et al., 2007), in *Ipomoea aquatica* (Göthberg et al., 2004), in *Ageratum houstonianum* Mill (Stratu & Costică, 2013) in mung bean genotypes (Hassan & Mansoor, 2014).

MATERIALS AND METHODS

For the experiment, the biological material used was professional seeds of *Coleus blumei* 'Wizard Rose' and 'Wizard Jade' purchased from Anthesis International S.R.L.

The experiment was carried out at the Horticultural Research Centre and at the Greenhouses of the ICAM Research Centre of "Ion Ionescu de la Brad" Iasi University of Life Sciences, Romania. The methodology has been developed in line with the OECD guidelines for testing of chemicals.

The experiment was organized in six variants (in the first variant the substrate was uncontaminated and was considered the control variant V_1-control, and in the other five variants the substrate was contaminated with Pb(II) ($PbCl_2$): V_2 - 25 mg/kg, V_3 - 50 mg/kg, V_4 - 100 mg/kg, V_5 - 250 mg/kg, V_6 - 300 mg/kg) of three replicates, each variant having 10 seeds. Substrate contamination was carried out before filling the containers, in order to obtain the established concentration of lead, the amount of $PbCl_2$ required to contaminate the substrate was calculated for each variant.

After lead application, the substrate was allowed to stand for 3 days for efficient homogenization. Sowing was carried out in 2 kg containers in substrate consisting of two parts peat and one part garden soil. Germination testing was carried out under greenhouse conditions at temperature: 22ºC ± 10ºC; humidity: 70% ± 25% and photoperiod: minimum light of 16 hours. For each pot watering was done with tap water applying the same amount of water per container. Measurements were carried out daily at the same time until three successive measurements showed the same values. Measurements were made on the influence of lead on germination percentage, seedling rate, velocity, toxicity index, tolerance index, and vigour index.

RESULTS AND DISCUSSIONS

Germination monitoring was carried out daily at the same time until three consecutive measurements showed the same values. In each experimental variant, the germination process was carried out by averaging the three replicates. The results obtained in each experimental variant contaminated with lead were compared with the control sample. The seeds of 'Wizard Rose' and 'Wizard Jade' used in the experiment started to germinate 7 days after sowing, a process that took place over 8 days from the start (Table 1).

Table 1. Influence of lead on the seed germination of the 'Wizard Rose' and 'Wizard Jade'

Variant		Sowing date	Germination onset date	End of germination date	Total germination *
V_1 (control)	'Wizard Rose'	8.04.2021	14.04	20.04	98
	'Wizard Jade'		14.04	21.04	96
V_2	'Wizard Rose'	8.04.2021	14.04	20.04	96
	'Wizard Jade'		14.04	21.04	88
V_3	'Wizard Rose'	8.04.2021	14.04	21.04	92
	'Wizard Jade'		15.04	21.04	80
V_4	'Wizard Rose'	8.04.2021	15.04	21.04	84
	'Wizard Jade'		15.04	21.04	78
V_5	'Wizard Rose'	8.04.2021	15.04	21.04	80
	'Wizard Jade'		15.04	21.04	60
V_6	'Wizard Rose'	8.04.2021	15.04	21.04	78
	'Wizard Jade'		15.04	21.04	48

The results on the influence of Pb(II) on germination, seedling rate, velocity, toxicity index, tolerance index and vigour index in the two coleus varieties are shown in figures 1-8. From the analysis of the data on the influence of Pb(II) on germination rate in 'Wizard Rose' and 'Wizard Jade' it is evident that both germination rate, relative germination index and vigour index decrease with increasing heavy metal concentrations from 250 mg/kg to 300 mg/kg.

Regarding the germination degree of the seeds of the two coleus cultivars, it can be observed that 'Wizard Jade' is more affected by lead toxicity in the germination substrate compared to 'Wizard Rose' in all experimental variants (Figure 1). In both varieties, a decrease in germination percentage with increasing Pb(II) concentrations in the substrate is evident compared to the control, by 20% in 'Wizard Rose' and 50% in 'Wizard Jade'. In 'Wizard Rose' the value of this indicator decreases from 98% for the control sample to 78% for 300 mg/kg Pb(II). In the case of 'Wizard Jade', at the same concentration value for Pb(II), the germination rate decreases compared to the control and registers in the case of the contaminated variant with the highest concentration the value of 48%.

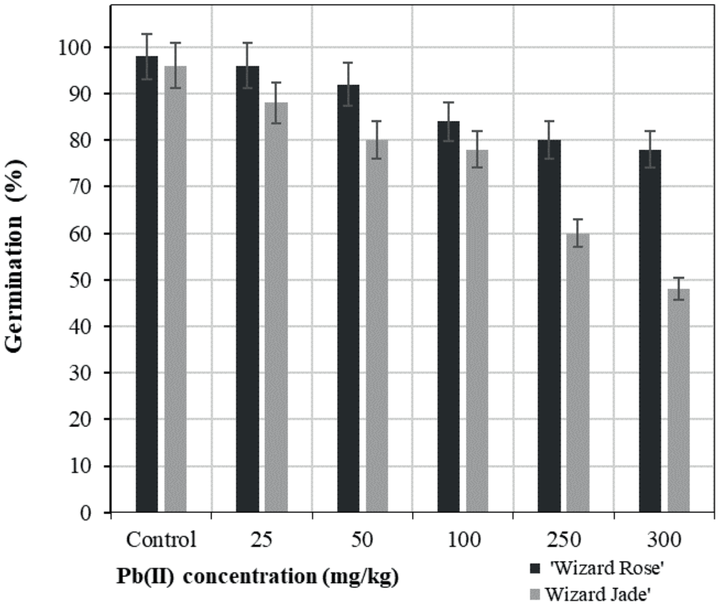

Figure 1. Influence of Pb(II) stress on the germination (%)'Wizard Rose' and 'Wizard Jade' cultivars

The effect of lead on germination was evidenced by the analysis of the main germination and sprouting indicators: sprouting dynamics, sprouting rate and sprouting speed.

The results of the dynamics of plant sprouting in the two coleus varieties are presented in Table 2. For both species, sprouting started 7 days after sowing and took place over six days in the control variants and eight days in the other experimental variants. In 'Wizard Rose' under lead-contaminated conditions the earliest sprouting occurred 7 days after sowing in the variants with lead concentrations of 25 mg/kg soil and 50 mg/kg soil. In the variants where lead was present at concentrations of 100 mg/kg soil (V_4), 250 mg/kg soil (V_5) and 300 mg/kg soil (V_6) sprouting started 8 days after sowing and took place over 8 days. Compared to the control, the increase of lead concentrations in the substrate resulted in a minor delay in plant sprouting (1 day).

In the case of 'Wizard Jade', the sprouting started 7 days after sowing in the control variant and the variant with the lowest Pb(II) concentration and 8 days after sowing in the other experimental variants. It is worth noting that in the case of the other variety, in the variants with the highest lead concentrations (V_5, V_6) the sprouting occurred within 8 days.

Table 2. The dynamic emergence of 'Wizard Rose' and 'Wizard Jade' cultivars

Variant		Date/sprouted plants (%)							
		14.04	15.04	16.04	17.04	18.04	19.04	20.0	21.04
V_1 (control)	'Wizard Rose'	25	49	82	83	90	98	98	98
	'Wizard Jade'	26	36	75	76	80	93	95	96
V_2	'Wizard Rose'	25	44	70	82	87	90	96	96
	'Wizard Jade'	25	39	48	57	60	68	76	88
V_3	'Wizard Rose'	20	25	46	55	67	76	88	92
	'Wizard Jade'	0	18	32	49	62	68	77	80
V_4	'Wizard Rose'	0	15	31	48	60	68	81	84
	'Wizard Jade'	0	16	37	44	58	69	75	78
V_5	'Wizard Rose'	0	10	31	45	65	78	79	80
	'Wizard Jade'	0	12	28	33	40	48	53	60
V_6	'Wizard Rose'	0	10	19	49	56	66	73	78
	'Wizard Jade'	0	5	10	23	33	41	45	48

The dynamics of the germination rate in the two coleus varieties (Table 3) show some variations determined, in particular, by the concentration of lead in the substrate. In both varieties, in the control variants the absence of lead in the substrate resulted in maximum values of germination rate in the first three days, 33% in cultivar 'Wizard Rose' and 39% in cultivar 'Wizard Jade'.

Seeds sown in substrate contaminated with 25 mg/kg Pb(II) soil had a higher germination rate during the first three days after the start of sprouting, (10-39% in 'Wizard Rose' and 19-26% in 'Wizard Jade') and on the eleventh day the lowest rate in both cultivars (5% in 'Wizard Rose' and 3% in 'Wizard Jade'). Variants with substrate contaminated with Pb(II) in concentrations of 250 mg/kg soil and 300 mg/kg soil (V_5-V_6) had high germination rate values on the first days, with maximum values the tenth day after germination onset, after which they were decreasing. In both varieties, lead contamination resulted in a lower germination rate compared to the control, indicating slower and more inuniform germination. In 'Wizard Rose' maximum values were recorded on the first day after the onset of sprouting in variants V_2-V_3, on the second day in variants V_4-V_5 and on the third day after the onset of sprouting in variant V_6. In 'Wizard Jade' maximum values were recorded on the first day after the onset of sprouting in variants V_1, V_2, on the second day in variants V_3-V_4 and on the third day after the onset of sprouting in variants V_5, V_6. The highest germination rate was observed in the control variants three days after the onset of sprouting (33% 'Wizard Rose', 39% 'Wizard Jade'). In the case of these variants, where the total percentage of germinated seeds was above 96%, on the penultimate day of measurements the germination rate dropped suddenly to low values.

Germination velocity (Table 4) was unfavourably influenced by increasing Pb(II) concentration in the substrate. The velocity showed different values from variety to variety, from variant to variant, and during the sprouting period. In both varieties, the control variants, with seeds sown on substrate uncontaminated with Pb(II), the highest values of velocity (3.67% in 'Wizard Rose 'and 4.33% in 'Wizard Jade') were recorded nine days after the end of the experiment respectively the third day after the onset of sprouting. The evolution of the two varieties in the case of seeds contaminated with Pb(II) (V_2-V_6) was characterized both by larger differences determined by the resistance of the variety to Pb(II) , and by fluctuations from day to day, with alternating maxima and minima.

Thus, germination in 'Wizard Rose' in the control variant was more rapid in the first three days, with maximum velocity showing values between 3.00% and 3.67%, and low velocity in the last days, with values reaching 0.67%. In 'Wizard Jade', the velocity results also showed maximum values in the first three days and a more pronounced decrease in values compared to the other variety nine days after sowing. Increasing the concentration of Pb(II) in the germination substrate to 300 mg/kg substrate (V_6 variants) resulted in a decrease of the velocity, which at the beginning of sprouting was 1.25 in cultivar 'Wizard Rose' and 0.63% in cultivar 'Wizard Jade', and towards the end of the period 0.36 in cultivar 'Wizard Rose' and 0.21% in cultivar 'Wizard Jade'.

Table 3. The sprouting rate of the 'Wizard Rose' and 'Wizard Jade' cultivars under exposure to different doses of $PbCl_2$

Variant		**Date/ sprouting rate %**							
		14.04	**15.04**	**16.04**	**17.04**	**18.04**	**19.04**	**20.04**	**21.04**
V_1 (control)	*'Wizard Rose'*	25	24	33	1	7	8	0	0
	'Wizard Jade'	26	10	39	1	4	13	2	1
V_2	*'Wizard Rose'*	25	19	26	12	5	3	6	0
	'Wizard Jade'	25	14	9	9	3	8	8	12
V_3	*'Wizard Rose'*	20	5	21	9	12	9	12	4
	'Wizard Jade'	0	18	14	17	13	6	9	3
V_4	*'Wizard Rose'*	0	15	16	17	12	8	13	3
	'Wizard Jade'	0	16	21	7	14	11	6	3
V_5	*'Wizard Rose'*	0	10	21	14	20	13	1	1
	'Wizard Jade'	0	12	16	5	7	8	5	7
V_6	*'Wizard Rose'*	0	10	9	30	7	10	7	5
	'Wizard Jade'	0	5	5	13	10	8	4	3

Table 4. The velocity of the 'Wizard Rose' and 'Wizard Jade' cultivars under exposure to different doses of $PbCl_2$

Variant		**Date/ velocity (%)**							
		14.04	**15.04**	**16.04**	**17.04**	**18.04**	**19.04**	**20.04**	**21.04**
V_1 (control)	'Wizard Rose'	3.57	3.00	3.67	0.1	0.64	0.67	0.00	0.00
	'Wizard Jade'	3.71	1.25	4.33	0.1	0.36	1.08	0.15	0.07
V_2	'Wizard Rose'	3.57	2.38	2.89	1.2	0.45	0.25	0.46	0.00
	'Wizard Jade'	3.57	1.75	1.00	0.9	0.27	0.67	0.62	0.86
V_3	'Wizard Rose'	2.86	0.63	2.33	0.9	1.09	0.75	0.92	0.29
	'Wizard Jade'	0.00	2.25	1.56	1.7	1.18	0.50	0.69	0.21
V_4	'Wizard Rose'	0.00	1.88	1.78	1.7	1.09	0.67	1.00	0.21
	'Wizard Jade'	0.00	2.00	2.33	0.7	1.27	0.92	0.46	0.21
V_5	'Wizard Rose'	0.00	1.25	2.33	1.4	1.82	1.08	0.08	0.07
	'Wizard Jade'	0.00	1.50	1.78	0.5	0.64	0.67	0.38	0.50
V_6	'Wizard Rose'	0.00	1.25	1.00	3	0.64	0.83	0.54	0.36
	'Wizard Jade'	0.00	0.63	0.56	1.3	0.91	0.67	0.31	0.21

Influence of Pb(II) on germ development in 'Wizard Rose' and 'Wizard Jade' cultivars. Coleus seedlings in the growing pots were collected and measured on each component: radicle, hypocotyl and leaves, the results obtained representing the average of each measured seedling component. Figures 4-5 show the influence of Pb(II) stress on radicle, hypocotyl and leaf development in 'Wizard Rose' and 'Wizard Jade'.

Analysing each component separately, it can be seen that the presence of Pb(II) affected the radicle most in both varieties. In 'Wizard Rose' the radicle length starts at 3.20 cm at 25 mg/kg Pb(II) and reaches a length of 1.72 cm at 300 mg/kg Pb(II) (Figure 2).

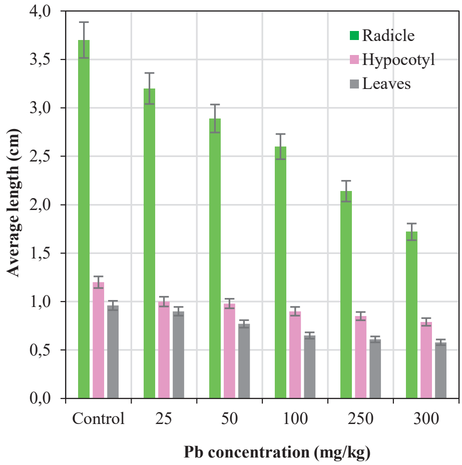

Figure 2. Influence of Pb(II) stress on the development of seedlings 'Wizard Rose' cultivar

In the case of Coleus 'Wizard Jade' a radicle length of 2.80 cm was recorded at 25 mg/kg Pb(II) concentration and reduced to 1.43 cm at 300 mg/kg Pb(II) concentration (Figure 3).

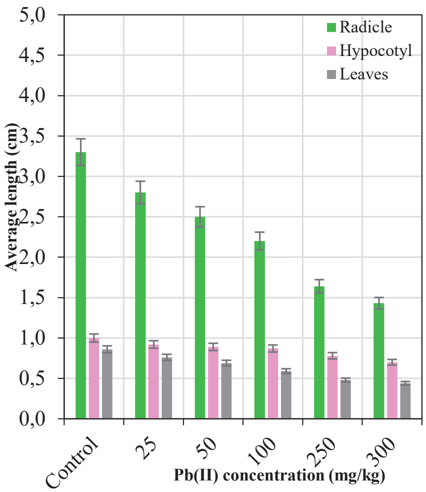

Figure 3. Influence of Pb(II) stress on the development of seedlings 'Wizard Jade' cultivar (cm)

Comparing with the control sample where the radicle length is 3.7 cm in 'Wizard Rose' and 3.3 cm in 'Wizard Jade', in both varieties the increase in Pb(II) concentration causes a reduction in radicle growth (radicle length decreases by 53.51% in 'Wizard Rose' and 56.66% in 'Wizard Jade'). The same trend of decreasing values due to increasing Pb(II) concentration is observed for hypocotyl length and leaf length. It can be seen that at the concentration of 300 mg/kg Pb(II), hypocotyl length decreases compared to the control sample with 34.17% in 'Wizard Rose' and 30.00% in 'Wizard Jade'. In terms of leaf size, leaf length decreases compared to the control sample by 39.58% in 'Wizard Rose' and 48.84% in 'Wizard Jade'

Degree of toxicity of Pb(II) to the radicle, hypocotyl and leaves of 'Wizard Rose' and 'Wizard Jade'.

Another indicator reflecting the inhibitory effect of lead for the two coleus varieties is the toxicity index (%). Thus, in Figures 4-5, the degree of toxicity of Pb(II) to the radicle, hypocotyl and leaves of 'Wizard Rose' and 'Wizard Jade' is represented. In both cultivars, in the case of Pb(II) contamination of samples (Figures 4-5), the degree of toxicity increases with the concentration of metal in solution and follows the order radicle>leaf>hypocotyl. In 'Wizard Rose', the toxicity degree values for radicle, hypocotyl and leaves show higher values with increasing Pb(II) concentration in the substrate. For the concentration of 300 mg/kg Pb(II) substrate, the toxicity index value was 53.51% in the radicle, 34.17% in the hypocotyl and 39.58% in the leaves.

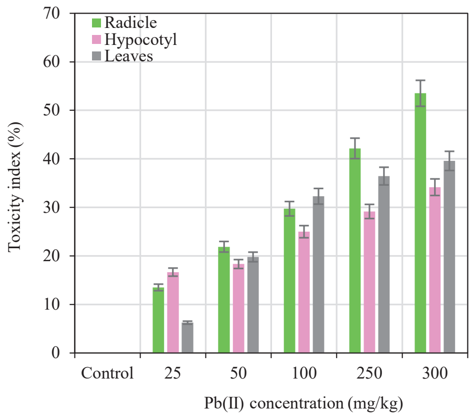

Figure 4. Influence of Pb(II) stress on the toxicity index 'Wizard Rose' cultivar (%)

In 'Wizard Rose', the toxicity degree values for radicle, hypocotyl and leaves show higher values with increasing Pb(II) concentration in the substrate. For the concentration of 300 mg/kg Pb(II) substrate, the toxicity index value was 53.51% in the radicle, 34.17% in the hypocotyl and 39.58% in the leaves. The toxic effect of lead induced in 'Wizard Jade' not only a much lower germination percentage but also much higher toxicity index values compared to 'Wizard Rose' cultivar. For the concentration of 300 mg/kg Pb(II) substrate, the toxicity index value was 56.67% in the radicle, 30.00% in the hypocotyl and 48.84% in the leaves.

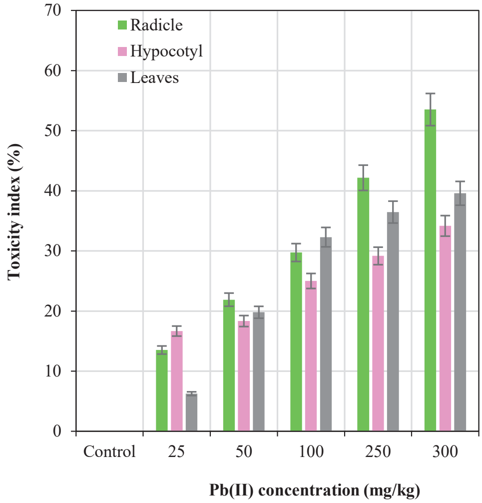

Figure 5. Influence of Pb(II) stress on the toxicity index 'Wizard Jade' cultivar (%)

These results are in agreement with the results of other studies confirming that the presence of Pb(II) in concentrations of 25-300 mg/L decreases radicle length: in *Lavandula angustifolia* L. (Hagiu Zalenschi et al., 2022), in *Brassica napus* L. (Rosca et al., 2021), in *Miscanthus* (Hsu et al., 1992).

Degree of tolerance of Coleus 'Wizard Rose' and Coleus 'Wizard Jade' varieties to Pb(II) stress

Figures 6-7 show the degree of tolerance of 'Wizard Rose' and 'Wizard Jade' to Pb(II) stress for radicle, hypocotyl and leaves. The tolerance of 'Wizard Rose' and 'Wizard Jade' to Pb(II) stress for radicle, hypocotyl and leaves is shown in the two graphs. Also for this indicator, the radicle is the most affected component of the seedling compared to the other components. With increasing Pb(II) concentration from 50 mg/kg to 300 mg/kg, the order of tolerance of seedling components is hypocotyl>leaf>root (Figures 6-7).

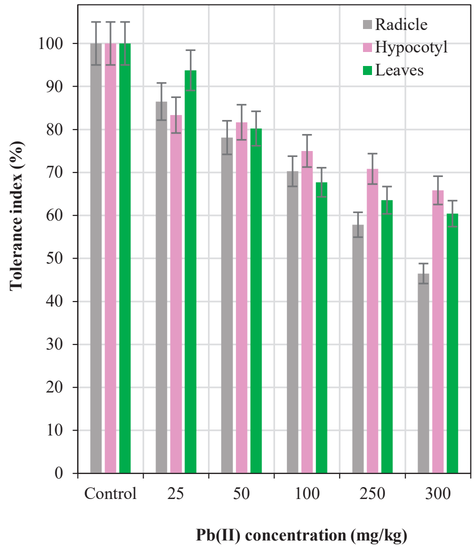

Figure 6. Influence of Pb(II) stress on the tolerance index 'Wizard Rose' cultivar (%)

At the radicle level, with increasing Pb(II) concentration from 25 mg/kg to 300 mg/kg the tolerance index decreases, with values ranging from 86.49% to 46.49 in Coleus 'Wizard Rose' and from 84.85% to 43.33 in Coleus 'Wizard Jade'.

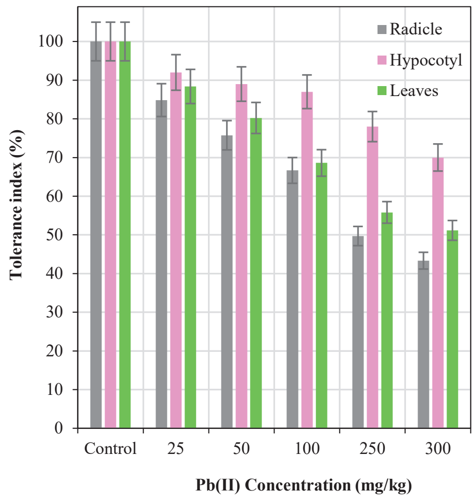

Figure 7. Influence of Pb(II) stress on the tolerance index 'Wizard Jade' cultivar (%)

In hypocotyl and leaves the decrease of the tolerance index is evident with the increase of Pb(II) concentration in the substrate, with very low values at the concentration of 300 mg/kg

(65.83% in hypocotyl and 60.42% in leaves in 'Wizard Rose' and 70% in hypocotyl and 51.16% in leaves in 'Wizard Jade').

Similar results were obtained by the study of the germination characteristics of *Lavandula angustifolia* under Pb(II) stress (Hagiu Zalenschi et al., 2022).

Vigour index of 'Wizard Rose' and 'Wizard Jade' varieties to Pb(II) stress.

Concerning the vigour of the seedlings of the two coleus varieties, the results show in all Pb(II)-contaminated variants of the two varieties a decrease in vigour index with increasing concentration. The vigour index values ranged from 5.00 at 25 mg/kg Pb(II) to 3.22 mg/kg at 300 mg/kg Pb(II) in 'Wizard Rose' (Figure 8).

In 'Wizard Jade' the seedling vigour index decreased with increasing concentration from 25 to 300 mg/kg, from 4.34 to 2.89 (Figure 8).

Our results confirm the results of other studies that lead accumulation in the soil and the plants inhibits germination of seeds and retards growth of seedlings, decreases germination percent, germination index, root/shoot length and tolerance index (Nas & Ali, 2018; Qu et al., 2021).

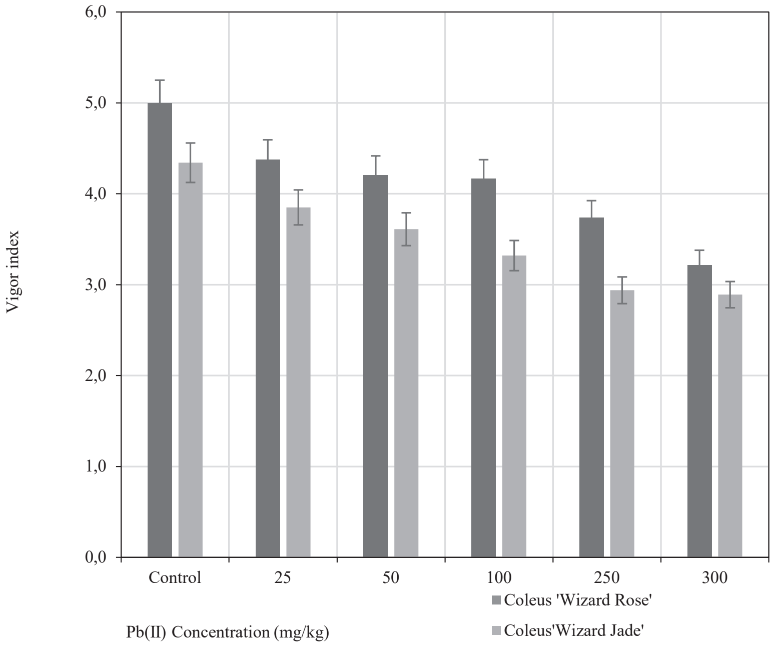

Figure 8**.** Influence of Pb(II) stress on the vigor index 'Wizard Rose' and 'Wizard Jade' cultivars

CONCLUSIONS

The germination percentage of seeds of the two coleus varieties under stress conditions caused by the presence of Pb(II) in concentrations of 300 mg/kg shows a significant decrease of 20% in the case of 'Wizard Rose' and 50% in the case of 'Wizard Jade'.

The seedling sprouting dynamics of the two coleus varieties started 7 days after sowing and lasted for six days in the control and eight days in the other experimental variants, suggesting that seedling sprouting was not significantly influenced by lead toxicity.

The dynamics of germination rate in the two coleus varieties indicate some variations determined, in particular, by the concentration of lead in the substrate, both varieties in the lead-contaminated variants had a lower germination rate than the control.

The velocity of germination rate was unfavourably influenced by the increase of Pb(II) concentration in the substrate with different values between varieties and variants and during the sprouting period.

The toxicity index for the two varieties follows the order: 'Wizard Jade'> 'Wizard Rose', increases with the metal concentration in the substrate and at the seedling level for both varieties the order is radicle > leaves > hypocotyl.

The tolerance index of the two varieties decreases with increasing metal concentration in the substrate and at the seedling level for both varieties the order is hypocotyl>leaf>radicle. The following tolerance scale was obtained for the varieties studied: 'Wizard Rose'> 'Wizard Jade'.

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