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SEED GERMINATION OF SELECTED PLANTS UNDER THE INFLUENCE OF HEAVY METALS

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In the paper has been presented the influence of selected heavy metals salts solutions: $CuSO_4$, $Pb(NO_3)_2$, $ZnCl_2$ and $Cd(NO_3)_2$ on the germination of seeds of the higher plants. The solutions with the various concentrations of abovementioned salts have been tested on seeds of the following plants: rye *(Secale cereale* L.*)*, common wheat *(Triticum aestivum* L.*)*, rape *(Brassica napus oleifera* L.*)* and white mustard *(Sinapis alba* L.*)*. The pot experiment was carried out in laboratory conditions. Each variant of the experiment has been made in three replications. After the 7 days of germination, number of the germinated seeds was counted. There was no significant variation of the impact of the solutions tested on seeds used in the experiment. The influence of $CuSO_4$ was the highest on seed germination in all the plants except rye. Higher concentrations of the salts caused reduced germination of most from the tested plants.

Keywords: cadmium, copper, germination, heavy metals, lead, seeds, zinc

1. INTRODUCTION

The group of heavy metals are: Cd, Cr, Cu, Pb, Hg, Ni, Zn [30], although some authors [22] include in this group also Se, some others eligible there elements with a density of 4.5 g cm^{-3} [5] and still others [3] ones heavier than 6 g cm^{-3}. Despite the widespread use of the term "heavy metals" including a number of elements mentioned above, the definition of "heavy metals" is not uniform and depends on the criterion for division [8].

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The presence of heavy metals in the environment can be natural (background) or anthropogenic. The primary source of metals such as copper, cadmium, zinc and lead in soil is the weathering of rocks [12]. As a result of anthropogenic activity, elements enter the environment by artificial means such as being released into the environment from municipal wastes, pesticides, industrial and automotive emissions.

The content of heavy metals in different soils varies considerably – depending on the type of soil and the country of origin of samples, due to the different contents of these elements in parent rocks. Kabata-Pendias & Pendias [11] reported that the average copper content in the upper layers of the profiles are 13 – 24 mg kg^{-1}, lead from 22 to 44 mg kg^{-1}, zinc 45 – 100 mg kg^{-1} , cadmium 0,37 – 0,78 mg kg^{-1}.

The ranges and means of the total concentration of selected heavy metals are summarized in Table 1.

Table 1. The ranges and means of the total concentration of selected heavy metals in top soil, in mg·dm^{-3} [11]

	Podzols		Cambisols		Rendzinas		Kastanozems & chernozems		Histosols	
	a)	b)	a)	b)	a)	b)	a)	b)	a)	b)
Cu	1-70	13	4-100	23	6,8-70	23	6,5-140	24	1-113	16
Pb	2,3-70	22	1,5-70	28	10-50	26	8-70	23	1,5-176	44
Zn	3,5-220	45	9-362	60	10-570	100	20-770	65	5-250	50
Cd	0,01-2,7	0,37	0,08-1,61	0,45	0,38-0,84	0,62	0,18-0,71	0,44	0,19-2,2	0,78

[a] range value, [b] mean value

Data about the natural content of copper, lead, cadmium and zinc (background) in various environment elements is collected in Table 2.

Table 2. Background content of selected heavy metals in various environments [5]

	Soils, mg·dm^{-3}	Marine waters, μg·dm^{-3}	Inland waters, μg·dm^{-3}	Atmosphere, ng·dm^{-3}	Plants, mg·dm^{-3} d.m.
Cu	5,0-15,0	0,01-0,02	0,003	≤ 4,0	5,0-30,0
Pb	20,0-40,0	0,01-0,04	0,1-0,2	0,1-1,0	0,5-7,0
Zn	10,0-125,0	0,03-0,14	5,0-70,0	10,0	25,0-150,0
Cd	0,05-0,7	0,01	0,02-0,1	0,003-0,6	0,05-0,5

The presence of heavy metals in the environment is a serious ecological problem, because these elements can enter food chains and the biological cycle [16]. They can eventually affecting animals and humans and causing metabolic disorders and damages to internal organs, including liver, kidneys and brain [7, 31].

Some of these elements (such as Cu, Zn) are essential for living organisms, because they are presented in the enzymes composition catalyzing important life processes [3]. Heavy metals are relatively easily extracted from the soil solution by plants and strongly affect their metabolism. In extreme cases they may cause their deadly poisoning [9, 10, 17].

Heavy metals can be defined as a stress factor, which may lead to disturbances of a number of functions of organisms [1, 2, 16, 19, 21, 24, 25, 26]. The results presented by a number of authors [1, 14, 27, 30], clearly indicate a negative impact of heavy metals on seed germination. This limitation impact depends mainly on heavy metals, the concentration applied and plant species [1, 14, 15, 27].

The aim of this study was to investigate the impact of various concentrations of 4 salts of heavy metals, such as $CuSO_4$, $Pb(NO_3)_2$, $ZnCl_2$, $Cd(NO_3)_2$ on the strength of seed germination of rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), rape (*Brassica napus oleifera* L.) and white mustard (*Sinapis alba* L.).

2. MATERIAL AND METHODS

The experiment was carried out in laboratory conditions. All the tests were carried out according to the Polish standard [18]. Experiment was made as a random blocks method with three replications.

The experiment was constructed by means of the method of random blocks with three replications.

The experiment was split into 2 parts. In the first part we made a preliminary test, where all the seeds were treated with varied concentrations of solutions of heavy metals. After 7 days the number of germinated seeds was counted. This number was compared with seeds of plants treated with distilledH_2O. All the results from this part of the experiment were used in the second part – 20 seeds of selected plants were put in Petri dishes and treated with each solution. Concentrations of the solutions tested are presented in table 3.

Table 3. Concentrations of the tested solutions

Salt	Concentration ($mg \cdot dm^{-3}$)					
$Cd(NO_3)_2$	0	4	8	12	16	20
$CuSO_4$	0	4	8	12	16	20
$Pb(NO_3)_2$	0	10	20	50	100	150
$ZnCl_2$	0	20	40	60	80	100

The strength of seed germination was evaluated after 7 days of incubation. The results obtained were analysed statistically using the Statsoft Statistica 8 software. LSD values calculated this way are presented in Table 4.

Table 4a. Mean values of germinated seeds of *Secale cereale* L. and *Tritcum aestivum* L.

Tested solutions	Rye			Common wheat		
	Concentration	Quantity		Concentration	Quantity	
	$mg \cdot dm^{-3}$	Pieces	%	$mg \cdot dm^{-3}$	Pieces	%
H_2O	Mean value	16,0	80,0	Mean value	18,0	90,0
$Cd(NO_3)_2$	4	13,7	68,5	4	14,3	71,5
	8	8,7	43,5	8	12,0	60,0
	12	8,3	41,5	12	12,0	60,0
	16	5,3	26,5	16	11,0	55,0
	20	2,7	13,5	20	10,3	51,5
	Mean value	7,7	38,5	Mean value	11,9	59,5
$CuSO_4$	4	10,7	53,5	4	13,7	68,5
	8	9,7	48,5	8	10,7	53,5
	12	9,3	46,5	12	9,7	48,5
	16	7,3	36,5	16	8,3	41,5
	20	7,0	35,0	20	8,0	40,0
	Mean value	8,8	44,0	Mean value	10,1	50,5
$Pb(NO_3)_2$	10	10,7	53,5	10	16,3	81,5
	20	8,0	40,0	20	13,7	68,5
	50	8,3	41,5	50	10,3	51,5
	100	4,3	21,5	100	10,0	50,0
	150	3,0	15,0	150	9,0	45,0
	Mean value	6,9	34,5	Mean value	11,9	59,5
$ZnCl_2$	20	13,0	65,0	20	16,7	83,5
	40	10,0	50,0	40	14,3	71,5
	60	9,3	46,5	60	14,3	71,5
	80	6,3	31,5	80	10,0	50,0
	100	4,0	20,0	100	8,3	41,5
	Mean value	8,5	42,5	Mean value	12,7	63,5
	$LSD_{0,01}$ for concentration = 3,3 pieces			$LSD_{0,01}$ for salt = 2,2 pieces $LSD_{0,01}$ for concentration = 3,2 pieces $LSD_{0,05}$ for interaction = 6,4 pieces		

Tab. 4b. Mean values of germinated seeds of *Brassica napus oleifera* L. and *Sinapis alba* L.

Tested solutions	Rape			White mustard		
	Concentration	Quantity		Concentration	Quantity	
	$mg \cdot dm^{-3}$	Pieces	%	$mg \cdot dm^{-3}$	Pieces.	%
H_2O	Mean value	18,0	90,0	Mean value	19,0	95,0
$Cd(NO_3)_2$	4	16,7	83,5	4	16,7	83,5
	8	16,0	80,0	8	13,7	68,5
	12	10,0	50,0	12	11,0	55,0
	16	9,7	48,5	16	9,7	48,5
	20	9,0	45,0	20	8,0	40,0
	Mean value	12,3	61,5	Mean value	11,8	59,0
$CuSO_4$	4	14,7	73,5	4	15,7	75,5
	8	13,0	65,0	8	12,7	63,5
	12	12,0	60,0	12	6,0	30,0
	16	6,0	30,0	16	5,0	25,0
	20	4,7	23,5	20	2,7	13,5
	Mean value	10,1	50,5	Mean value	8,4	42,0
$Pb(NO_3)_2$	10	16,7	83,5	10	17,0	85,0
	20	16,7	83,5	20	15,7	78,5
	50	14,0	70,0	50	12,0	60,0
	100	8,0	40,0	100	11,7	58,5
	150	7,7	38,5	150	9,3	46,5
	Mean value	12,6	63,0	Mean value	13,1	65,5
$ZnCl_2$	20	15,0	75,0	20	17,3	86,5
	40	13,7	68,5	40	16,7	83,5
	60	9,0	45,0	60	16,0	80,0
	80	9,0	45,0	80	13,0	65,0
	100	5,0	25,0	100	9,0	45,0
	Mean value	10,3	51,5	Mean value	14,4	72,0
	$LSD_{0,01}$ for metals = 1,7 pieces $LSD_{0,01}$ for concentrations= 1,9 pieces $LSD_{0,01}$ for interactions = 4,6 pieces			$LSD_{0,01}$ for metals = 2,4 pieces $LSD_{0,01}$ for concentrations = 2,9 pieces $LSD_{0,01}$ for interactions = 5,7 pieces		

3. RESULTS

The salts of heavy metals used in these studies had a negative impact on the germination of rye seeds (Table 4). There was no significant difference between the effects of various metal salts on the strength of the germination of rye. $Pb(NO_3)_2$ had the most decreasing effect (mean 65.5%). $CuSO_4$ and $ZnCl_2$ had the least decreasing effect (56% and 57.5%).

Increasing concentrations of salt solutions significantly reduced the strength of germination of rye seeds. Salts with a high concentration of selected metals had the most negative impact on the feature under discussion. The greatest reduction in the strength of germination was found in the case of $Cd(NO_3)_2$ from 68.5% under the influence 4 mg·cm^{-3} to 13.5% under the influence of the concentration of 20 mg·cm^{-3}.

The heavy metal salts applied significantly diversified the strength of wheat seed germination – LSD = 2.2 pieces. The least harmful salt was $ZnCl_2$ – the average germination was 63.5%. CuSO4 was found to be the most toxic one, the average value of the features analysed was 50.5%.

Increasing concentrations of heavy metals have resulted the important decrease of germination strength of wheat seeds. LSD for the concentrations of the solutions was 3.2 pieces, $p \leq 0,01$. The lowest concentrations of all the solutions of salts of heavy metals had the least harmful influence on the feature under discussion. The greatest influence was found when the highest concentrations of salts were used.

It should be emphasized that the slightest variation of the strength of germination of wheat seeds under the influence of increasing concentrations occurred in the case of copper sulphate, while the largest decreases in the strength of germination was found under the influence of increasing concentrations of zinc chloride.

The metal salts used in this experiment significantly varied the strength of the germination of rape seeds (Table 4), LSD was 1.7 pieces, $p \leq 0,01$. The lowest number of germinated seeds was found when a solution of copper sulphate was used, on average 50.5%. The highest number of germinated seeds was obtained after the use of lead nitrate – an average of 63.0%.

Each time, an increase in the concentrations of the salts tested induced a reduction in the strength of germination of rape seeds. These differences were proven statistically, LSD was 1.9 pieces, $p \leq 0,01$. A reduction in the strength of germination of rape seeds under the influence of increasing concentrations of all the salts was relatively high and exceeded 40%, in the case of the application of copper sulphate and zinc chloride we reached 50%.

All the solutions of the salts tested significantly varied the strength of germination of mustard seeds (Table 4), LSD = 2.4 pieces, $p \leq 0.01$. The germination of seeds of this plant decreased under the influence of copper sulphate – an average of 42%. When zinc chloride was used, the germination of mustard seeds had an average value of 72%.

Increasing concentrations of all the salts under research in each case, significantly reduced the strength of germination of mustard seeds. LSD was 29 pieces, $p \leq 0.01$. The highest negative effect of increasing concentrations was found when copper sulphate was applied. The results were 78.5% under the influence of 4 mg·dm^{-3} and 13.5% under the influence of 20 mg·dm^{-3}. A reduction in the strength of germination under the influence of increasing concentrations of the other salts was similar and amounted to about 40%.

4. DISCUSSION

The presence of heavy metals in the environment is a stress factor for seeds that disturb an osmotic balance [4; 20]. The mechanism of this action is not clearly defined.

According to Ahsan et al. and Qurehi et al. [1, 2, 19] under the influence of stress caused by metals quantitative and qualitative changes of proteins occur in plants. Some authors [21] found that the presence of this stress may be associated with impaired basic steps in the metabolism of carbohydrates and aminoacids. According to Zhang et al. [32], stress is the result of changes in the activity of autooxidating enzymes and peroxidating lipids, whereas according to Taulavuori et al. [25] stress is the result of increased active oxygen in plants.

Consequences of stress caused by heavy metals are a number of important disorders of metabolic processes, including disturbances in seed germination [1, 2, 6, 16, 24] .

The results presented in this article indicate that the salts of heavy metals varied the strength of germination of the seeds used in the experiment. There were not any clear differences in the germination of cereal seeds in comparison to seeds of plants from the cabbage family – there was even a slight predominance of the strength of germination of seeds of *Brassiceae* over crop seeds.

Experiments carried out by Wierzbicka & Obidzińska [28] also showed small differences in the strength of seed germination of 34 plant species. The abovementioned authors investigated the permeability of lead through the seed coat and consequently the impact on seed germination. However, the results obtained in the work quoted do not show explicitly that there is a link between them.

In our studies zinc chloride proved to be the least harmful to the germination of seeds. Under the influence of this salt the impact on the germination of seeds of the plants selected was the least negative. The results obtained were confirmed by the studies of Matthews et al. [15], Peralta-Vedea et al. [17] and Somowa & Pechurkin [23], which showed a high tolerance of plants to zinc salts.

Copper sulphate caused a significant reduction in the strength of germination seeds of all the plants tested except winter rye. These results confirmed the results obtained by Ahsan et al. [1], which clearly demonstrated the oxidative stress of copper. It eventually showed itself as a significant reduction in the germination of seeds of rice. However, studies conducted by Liu et al. in 2009 [13] showed that low concentrations of copper accelerate the rate of seed germination. High concentrations of this element clearly inhibited this process.

The increasing concentrations of all the salts of heavy metal solutions applied in these studies clearly diminished the strength of germination of seeds of the plants under research. The lowest concentrations of salts were the least harmful to the germination of seeds. With increasing concentrations of the solutions the strength of germination of the seeds decreased. The results obtained fully confirm the results of [6, 13, 17, 21, 27, 32].

5. CONCULSIONS

Some conclusions may be drawn from the results of this research:

- The germination of selected seeds was the lowest under the influence of copper sulphate – excluding rye seeds. Zinc chloride reduced the strength of germination of seeds of common wheat and white mustard. Seeds of rape showed the least negative effect of $Pb(NO_3)_2$, and the seeds of rye showed the advantage of $CuSO_4$.
- Increasing concentrations of all the solutions of heavy metals decreased the number of germinated seeds of the plants tested. The highest concentrations of salt solutions had the most negative influence on the feature under examination.

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WPŁYW WYBRANYCH SOLI METALI CIĘŻKICH NA SIŁĘ KIEŁKOWANIA NASION NIEKTÓRYCH ROŚLIN

S t r e s z c z e n i e

Praca przedstawia wpływ roztworów soli metali ciężkich: $CuSO_4$, $Pb(NO_3)_2$, $ZnCl_2$ i $Cd(NO_3)_2$ na siłę kiełkowania nasion roślin. Stosowano roztwory o różnym stężeniu wymienionych soli na nasiona żyta ozimego (*Secale cereale L.*), pszenicy ozimej (*Triticum aestivum L.*), rzepaku ozimego (*Brassica napus oleifera L.*) i gorczycy białej (*Sinapis alba L.*).

Doświadczenie prowadzono w warunkach laboratoryjnych. Każdy wariant doświadczenia powtórzono 3 razy. Po upływie 7 dni liczono ilości wykiełkowanych nasion.

Nie wykazano większego zróżnicowania wpływu stosowanych soli metali na kiełkowanie nasion poszczególnych gatunków roślin. $CuSO_4$ u wszystkich roślin za wyjątkiem żyta ozimego najbardziej ograniczył wschody nasion. Wzrastające stężenia roztworów soli metali ciężkich ograniczały kiełkowanie nasion u wszystkich badanych roślin.