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INFLUENCE OF ZINC (Zn) ON GERMINATION OF WHEAT (*Triticum aestivum* L.)

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ABSTRACT
The paper presents the results of research of negative effects of zinc (Zn) on seed germination of wheat (Triticum aestivum L.*). Besides the impact on the percentage of germination, the toxic effect on some morphometric characteristics was followed, too. Ripe seeds of wheat were exposed in standard laboratory conditions to the influence of zinc, in form of zinc chloride ($ZnCl_2$), at different values of concentration. For each value of concentration it was determined the percentage of germinationed seeds, as well as the length of root and shoot. It as found the value of the concentration of $ZnCl_2$, which inhibited completely germination of wheat. The mean values of the length of root and shoot for each concentration were compared with values obtained for the control group of seeds, which were not treated with $ZnCl_2$. In addition to causing of inhibition of seed germination, the presence of zinc in the medium affects disorder of the physiological - biochemical processes during the growth and development of vegetative organs that it indicates the difference in the length of the root and shoot of treated seeds in relation to the control group of untreated seeds.*

Keywords: wheat, zinc, Zn, seed germination

Introduction
In normal conditions, heavy metals are environmental constituents, some of which being essential elements in numerous physiological processes in living organisms. Their presence in the soil, water and air originates from a great number of complex geochemical processes. The increased concentration of heavy metals in the environment is most often the result of human activities, such as industry, transportation, the use of chemicals in agriculture and urban waste (9, 19, 21). Heavy metals, when present in abnormal amounts, have harmful effects on living organisms. Plant and animal organisms are characterized by different sensitivity to the presence of heavy metals in the soil, water and air. Due to their harmful effects on living organisms, heavy metals are the most dangerous environmental pollutants (5, 15).

Due to its indispensable role in basic biochemical-physiological reactions, zinc is the element of essential importance. It provides activation of enzymes, synthesis of nucleotides as well as synthesis of photosynthetic pigments and production of chlorophyll. Zinc is indispensable in the metabolism of the plant hormone auxin**.** Due to its effect on enzyme activity, it is indispensable in the physiological functions of a cell membrane (1). Positive effect of zinc is also found to mitigate the toxicity of other heavy metals in a plant organism and negative effects of free radical activities (13).

Plants uptake zinc mostly through roots from ground solution and through organs above the ground from the atmosphere to a lesser extent. The uptake of zinc depends on numerous internal and exterior factors, primarily on the concentration, Ph value of soil substrate and the concentration of other heavy metals in the soil. The zinc uptake from the nutrient soil mostly retains in the root, but a portion is relocated into the parts above the ground and seed (16).

When present in nutrient substrate in high concentrations, zinc is very toxic and prevents normal growth and development of the plant. The mechanism of zinc action is still not quite clear, but it was proved that it induced numerous anatomic and morphological alterations in plants. Negative effect of high concentration of zinc on the growth and development of a plant is based on its genotoxicity and a disturbance of cell division (2, 12, 17, 20). A great number of authors investigated the effect of zinc on seed germination and growth and development of plants (3, 5, 7, 8, 12, 13, 14, 18, 22, 23). The results of their research showed that zinc reduced the rate of seed germination and inhibited the elongation of shoot and root in various plant species.

The aim of the present study was to examine the effect of high concentrations of zinc as $ZnCl_2$ on seed germination in wheat (*Triticum aestivum* L.) and to determine lethal concentration of this heavy metal in the process of germination. The dynamics of germination was followed up concomitantly with the measurements of root and shoot at different concentrations.

Materials and Methods

To investigate the effect of zinc (Zn) on seed germination, the species *Triticum aestivum* L., the sort Ana Morava was used. The zinc used in the treatment of seeds as $ZnCl_2$ was obtained from Acros Organics, Geel, Belgium.

For the zinc treatment of seeds, the initial solution of $ZnCl_2$ and distilled water at the concentration of 0.2 M was prepared. From the initial solution, 19 dilutions with descending gradients of concentration were prepared until the concentration of 0.1 M $ZnCl_2$. Thus, 20 samples of the following concentrations were obtained: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19 and 0.2 M. However, the medium for seeding of controls contained distilled water only.

The seeding was performed in Petri dishes with filter paper previously laid and soaked with 5 ml of the solution. For each concentration of $ZnCl_2$, 100 seeds were seeded. Petri dishes were kept under standard laboratory conditions (light/darkness – 16/8 h, temperature 22/18 ^oC and humidity 55/65%). The percentage of germinated seeds and the length of root and shoot were noted for each sample three and five days after seeding.

Results and Discussion

The analysis of the percentage of germination showed that the number of germinated seeds depended on the concentration of $ZnCl_2$ in the medium. In comparison to control sample without $ZnCl_2$, the number of germinated seeds was considerably reduced with the rise of $ZnCl_2$ concentration. After three days, the percentage of germination determined in control sample was 91%, while in the first sample with the lowest $ZnCl_2$ concentration (0.01 M) it was 80%. The percentage of germination in the sample containing 0.16 M solution was 3%. No germination was found in the samples with $ZnCl_2$ concentrations higher than 0.16 M (**Table 1**).

TABLE 1

Percentage of germination, root and shoot lenght after three days of incubation

Cocentration of $ZnCl_2$(M)	% of germination	Root lenght[a] (mm)	Shoot lenght[a] (mm)
0.20	0	0	0
0.19	0	0	0
0.18	0	0	0
0.17	0	0	0
0.16	3	0	0.09 ± 0.01
0.15	7	0.07 ± 0.01	0.19 ± 0.02
0.14	12	0.12 ± 0.04	0.27 ± 0.03
0.13	16	0.21 ± 0.01	0.30 ± 0.01
0.12	22	0.26 ± 0.09	0.43 ± 0.12
0.11	25	0.30 ± 0.18	0.72 ± 0.10
0.10	30	0.38 ± 0.06	0.80 ± 0.18
0.09	32	0.46 ± 0.02	0.89 ± 0.09
0.08	40	0.59 ± 0.07	1.40 ± 0.08
0.07	46	0.64 ± 0.02	1.76 ± 0.10
0.06	53	0.71 ± 0.11	2.10 ± 0.15
0.05	59	0.85 ± 0.18	4.10 ± 0.92
0.04	65	0.98 ± 0.12	5.60 ± 0.96
0.03	72	2.16 ± 1.10	7.48 ± 1.20
0.02	76	3.48 ± 0.60	9.70 ± 1.46
0.01	80	5.68 ± 1.00	11.6 ± 1.21
0	91	19.8 ± 2.10	26.7 ± 2.01

[a]Values expressed as mean ± standard deviation (n = 3)

The determination of the germination percentage after five days revealed the percentage of 95% in the control sample and 90% in the sample with the lowest concentration of $ZnCl_2$. With the increase of zinc concentration in the medium, the percentage of germination decreased, which was also found after the first measurement. After the second measurement, the lowest value of germination percentage amounting 9% was determined in the sample with 0.17 M $ZnCl_2$.

The imbibition process begins with the contact of seed with humid environment, quickly followed by activation of metabolic processes as the most important stage in the process of germination. The activation of metabolic processes provides numerous synthesis actions in the seed and accumulation of products necessary for mitotic divisions in further growth of the germinated seed (4, 6).

TABLE 2

Percentage of germination, root and shoot lenghtht after five days of incubation

Cocentration of $ZnCl_2$(M)	% of germination	Root lenght[a] (mm)	Shoot lenght[a] (mm)
0.20	0	0	0
0.19	0	0	0
0.18	0	0	0
0.17	9	0	0.08 ± 0.01
0.16	15	0.06 ±0.01	0.16 ± 0.05
0.15	19	0.19 ± 0.02	0.27 ± 0.01
0.14	22	0.26 ± 0.01	0.41 ± 0.02
0.13	27	0.34 ± 0.03	0.55 ± 0.07
0.12	30	0.50 ± 0.04	0.73 ± 0.11
0.11	31	0.59 ± 0.03	0.92 ± 0.09
0.10	37	0.65 ± 0.13	1.15 ± 0.48
0.09	40	0.73 ± 0.08	2.01 ± 0.90
0.08	49	0.85 ± 0.12	3.22 ± 1.00
0.07	52	0.99 ± 0.05	5.56 ± 0.98
0.06	64	1.52 ± 0.12	7.68 ± 1.10
0.05	68	1.88 ± 0.09	11.24 ± 0.96
0.04	71	2.12 ± 0.75	12.04 ± 1.05
0.03	79	2.68 ± 0.98	13.92 ± 1.14
0.02	88	4.84 ± 2.10	18.04 ± 2.01
0.01	90	7.48 ± 2.21	21.68 ± 1.85
0	95	46.1 ± 3.2	62.96 ± 1.92

[a]Values expressed as mean ± standard deviation (n=3)

The presence of heavy metals in the medium in which the seed germinates complicates normal course of these processes due to their fast penetration inside the seed together with water. Among physiological processes, respiration is the most sensitive to the presence of heavy metals. When Zn is present in cytoplasm and mitochondria, it inhibits Krebs cycle and transportation of electrons in the process of oxidative phosphorilation (10, 11, 18, 20, 24, 25). Due to negative effect of zinc on the main physiological process during germination, its presence in the germination medium largely reduces the percentage of germinated seeds; in higher concentrations, above 0.17 M, like in this case, it completely inhibits the germination process.

In addition to inhibition of germination, the results of the experiment show that $ZnCl_2$ exerts a powerful effect on further growth and development of germinated seeds. The analysis of the average length of root and shoot showed remarkable differences between the values of control group and the values of each group of treated seeds after both first (**Table 2**) and second measurement of root and shoot length. After the first measurement, the average length for the control seed group was 19.8 mm for the root and 26.7 mm for the shoot. The average length for the seeds that germinated in the medium with the lowest $ZnCl_2$ concentration, i.e. 0.01 M solution was 5.68 for root and 11.6 mm for the shoot. The average values of root and shoot lengths decreased with the increase of the zinc concentration in the medium. In the sample with 0.15 M solution of $ZnCl_2$, the average length of root was 0.07 mm, while in the samples with the $ZnCl_2$ solution concentration above 0.15 M, the root did not develop until the third day. In the sample with 0.16 M concentration, the seeds did not develop the root, but only the shoot with average lengths of 0.09 mm at this value of concentration. In the samples with higher concentrations, shoot did not develop, i.e., the seeds did not germinate.

The analysis of the second measurement showed that the length of the root and shoot in the sample with the lowest concentration was drastically reduced in comparison with the control group. Also, the values of root and shoot length decreased with the increase in $ZnCl_2$ concentration. After the fifth day, the highest concentration at which the root developed was 0.16 M $ZnCl_2$ solution with the average length of 0.06 mm, while at the concentration of 0.17 M, only shoot was developed with the average length of 0.08 mm, but its development stopped and it decomposed soon hereafter.

At the concentrations above 0.17 M, neither the root nor the shoot developed after the fifth day, the seeds lost their viability and changed the colour into dark – morose. In the samples with $ZnCl_2$ concentration above 0.17 M, the seeds did not germinate. The value of this concentration was the highest $ZnCl_2$ concentration at which the seeds of wheat germinated, while the value of 0.18 M $ZnCl_2$ concentration could be qualified as lethal zinc concentration for the germination of wheat (**Table 1**).

After the germination of seeds and formation of root and shoot, the stage of growth and development of the plant based on intense metabolic processes begins, controlled by plant hormones. In numerous studies, higher concentrations of heavy metals were found to have negative effects on the growth and development of plants, their anatomic and morphological composition as well as their yields. The disturbance of plant hormone metabolism and prevention of normal photosynthesis process, mineral nourishment, water route and transport of photosynthesis products are the mechanism by which heavy metals inhibit normal growth and development of a plant. The heavier growth and development were also the result of a disturbed anatomic structure of conducting root system, since the number and volume of conducting vessels is reduced in the plants exposed to heavy metals. The presence of heavy metals inhibits the normal division and elongation of cells, which is also one of the causes of disturbed growth and development of the plants exposed to heavy metals (17, 18, 20).

Conclusions

The percentage of seed germination of wheat *Triticum aestivum* L. depends on the $ZnCl_2$ concentration in the medium in which the seeds germinate. The seeds exposed to 0.17 M concentration of the solution have the lowest percentage of germination and develop shoot only. If the seeds are exposed to 0.18 M $ZnCl_2$ concentration, they completely lose the ability to germinate. The average length of the root and shoot of wheat decreases with the increase of $ZnCl_2$ in the medium due to violation of basic metabolic processes for growth and development.

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