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The heavy metal ions (Cu^{2+}, Zn^{2+}, Cd^{+}) toxic compounds influence on triticale plants growth

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Abstract. The presence of the heavy metals toxic compounds ($CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ and $3CdSO_4 \cdot 8H_2O$) in water and soil can be observed by their negative effects on the germination and growth process for different vegetable (barley, oat, maize) who are used for human and animal consumption. This paper it aims the determination of germination and growth inhibition negative effects for triticale plants in the heavy metals ions presence by ecotoxicological laboratory tests. The triticale plants was chosen for their different characteristics to the other grasses respectively: a very good resistance for a wide range of diseases, an accelerated growth and a very good tolerance for aluminum ions presents in acid soils. The determinations were conducted step by step, first, we put the triticale grains in contact with the heavy metal solutions with different concentration then for 3 days we noticed the triticale germination inhibition effects and finally we noticed the growth inhibition process for triticale plants respectively in 7^{th} and 9^{th} day from the start of the experiment. At the end of the tests we can conclude that the triticale roots have a very great sensibility to a $CuSO_4$ solutions compared to the effects for their stalks. A positive effect for triticale stalks we can see for low $CuSO_4$ solution concentrations thus for 5 mg Cu/l the growth is 19,44%. A positive effect for triticale roots it can see for low $ZnSO_4$ solution concentrations so for 5 – 15 mg Zn/l the growth is 24,4%. In the presence of the $CdSO_4$ solution all the processes are inhibited (germination and growth for triticale plants) even for a low concentrations for this toxic.

1. Introduction

The concept of ecotoxicity refers to the processes that disrupt the ecosystems's structure and functions, as a consequence of direct or indirect action of some agents of pollution, manifested through reversible and irreversible ecological imbalances [1].

In the current context, regarding the residual industrial pollution with heavy metal ions of soils in mine sites, particularly mining waste dumps contaminated with heavy metals, for their bioremediation have been carried out a series of tests on the subject of a variety of graminaceous mixtures for grassing (wheat, oat, rye) in order to lead to the installation of vegetation on the polluted fields as well as to extract the heavy metal ions that are in the soil.

The tests proposed in this paper have been focused on using as a working material the triticale seeds obtained artificially by hybridizing wheat and rye.

The resulted hybrid presents a series of biological and economical characteristics that recommend it: greater resistance to low temperatures; rapid growth rhythm, vigorous plants and a high berry and mass production; genetic resistance to a large range of diseases, tolerance for aluminum ions toxicity

that determines the cultivation on acid soils without the necessity of liming; triticales spring more rapidly as a result of a greater water absorption capacity and of a more intense activity of the alpha-amylase hydrolyser enzyme; the triticale cultures do not require the use of herbicides.

2. Ecotoxicity of the heavy metal ions

Prolonged exposure of certain plants to high concentrations of heavy metals originating from the atmosphere or directly form the soil constitutes the premise of their accumulation in quantities which can affect plant metabolism and can lead to visible changes of their structure.

Through transfer via the food-chain, these plants which bio-accumulate heavy metal ions represent a potential hazard for the consumers' health.

2.1. Copper toxicity

Copper is an essential element for the growth and development of plants, and its absorption in superior plants is achieved mainly as Cu^{2+}. Copper has redox properties and is a structural and catalytic component for the protein and enzyme molecules involved in the metabolic processes.

The toxicity of copper resides in its ability to form free radicals which cause an oxidative stress. Copper is assimilated by plants as divalent ions or as chelates, and can penetrate the plant both through its roots and its leaves. The visible effects of the toxicity of copper upon terrestrial plants are chlorosis, necrosis, plant growth diminution, or the drying of plants.

The soil PH directly influences the organic compounds formed by copper in the soil, as well as the plant absorption process, i.e. soils with an alkaline PH bond copper more easily than acidic soils. Copper deficiency in crops appears when the soil is unable to supply an adequate amount of the element in a form that is absorbable by plants. Copper deficiency causes similar symptoms in barley, oats, rye and wheat [2].

In case of excess, copper can become extremely toxic, which manifests itself as chloroses, necroses, leaf discolorations. At cellular level, toxicity can be the consequence of copper bonding to the sulfides groups of proteins, thus preventing the activity of enzymes and proteins.

Plants that are sensitive to copper excess are the clover, the alfalfa, the poppy, the potato, and the strawberry [3].

During tests with triticale one could notice the positive influence of copper ions in small quantities, i.e. 5 mg Cu/L, upon the germination and growth of the triticale plants. As one uses experimental variants with higher concentrations of copper ions, the germination of the triticale seeds is slowed down or even inhibited completely [4].

Copper in a concentration of 6.25 mg/L has a negative effect upon the growth of the roots (12% growth diminution) and the stem (16% growth diminution) of the alfalfa seedlings after 14 days. As regards the Lolium perenne plants, copper in concentrations lower than 6.25 mg/L does not inhibit the growth of either roots or stems. At concentrations of 100 mg Cu/L the inhibition of root growth is almost complete (84 %) [5].

For duckweed, several studies indicated that copper excess interferes with respiration, photosynthesis, synthesis of pigments and enzymatic activities. Copper toxicity for duckweed has the value of CL_{50} equal to 1.51 mg/L [6].

2.2. Zinc toxicity

One noticed that for the alfalfa, zinc, even in low and moderate concentrations (between 6.25 and 50 mg/L) reduces the growth of the plant, in comparison to the control sample, by about 20%; only in high concentrations (100 mg/L) does zinc strongly inhibit growth (i.e. 40% diminution of the root growth and 30% of the stem growth) [5].

Copper and zinc in low concentrations have positive effect upon the germination and growth of plants.

2.3. Cadmium toxicity

Cadmium pollutes the soils through atmospheric precipitation and deposits in areas in the neighbourhood of metallurgical plants, as well as through employment in agriculture as chemical fertilizers in the group of super-phosphates. Cadmium in the soil is considered to be bio-available; it can be easily absorbed by plants through radicular absorption.

Cadmium negatively influences the growth of alfalfa even at 0.9 mg/L, but in Lolium there is a slightly positive effect. The toxicity tests for cadmium in grains highlighted the fact that at a concentration of 62.5 mg Cd/L the growth of roots is practically stopped (a decrease by 89%), and the growth of seedlings is also drastically affected (a decrease by 60%) [7].

The accumulation of cadmium in plants follows predominantly this pattern: roots> stems> leaves > seeds. Cadmium is an inhibitor of photosynthesis, and the effects which occur are mainly linked to the diminution of chlorophyll, stomatal closure (in plants such as the clover, beans, soy, alfalfa); it induces the activity of peroxidation in roots and stems (fact proven in rice); in is involved in the generation of oxidative stress in adult peas plants; it accumulates in the mitochondria, and inhibits certain enzymes in the respiratory system of the plants [5].

The toxicity of cadmium for plants is most often clearly identified, yet it can also be the result of the interaction of the main toxic ions with other ions, as well as other environmental factors. The various degrees of cadmium tolerance in various plants definitely involve differences in the structure and functioning of the cellular membrane [8].

3. Materials and manner of work

3.1. Required materials

Ecotoxicological laboratory tests for triticale were carried out in various types of laboratory dishes and glasswear, which had been cleaned and sterilized accordingly.

The laboratory devices and materials used for the tests are:

- ✓ Solutions for toxic medium:
- copper sulfate solution ($CuSO_4 \cdot 5H_2O$) at 5% concentration, reactive p.a.;
- zinc sulfate solution ($ZnSO_4 \cdot 7H_2O$) at 5% concentration, p.a;
- cadmium sulfate solution ($3CdSO_4 \cdot 8H_2O$) at 1% concentration;
- ✓ Triticale seeds;
- ✓ Petri dishes;
- ✓ Laboratory glasswear (graded cylinders, glass flat-bottom flasks, and Erlenmeyer glasses);
- ✓ Clean plastic cups and support sieves for germinated triticale seeds;
- ✓ Automate droppers;
- ✓ Tap water;
- ✓ Filter paper;
- ✓ Analytical balances.

3.2. *Manner of work*

The experimental tests were carried out in stages, by exposing the triticale seeds to toxic solutions (copper sulfate, zinc sulfate and cadmium sulfate) at various concentrations and observing the germination process and subsequently the plant growth over a period of 7 to 9 days.

For the control sample only tap water was used. For each variant, three repetitions were carried out (marked A, B and C).

The preparation of the experimental toxic concentrations started with the following stock solutions:

- ✓ S_1 - solution in concentration 5000 mg Cu/L using chemically pure reactive copper sulfate ($CuSO_4 \cdot 5H_2O$);
- ✓ S_2 - solution in concentration 5000 mg Zn/L using chemically pure reactive zinc sulfate ($ZnSO_4 \cdot 7H_2O$);

✓ S_3 - solution in concentration 1000 mg Cd/L using chemically pure reactive cadmium sulfate ($3CdSO_4 \cdot 8H_2O$).

The test solutions were prepared by diluting the stock solutions with tap water. Thus, the following toxic concentrations were used in the experiments, as illustrated in Table 1.

Table 1. Toxic concentrations of test solutions

No. crt.	**Toxic ($CuSO_4 \cdot 5H_2O$)** S_1 = 5000 mg Cu/L	**Toxic ($ZnSO_4 \cdot 7H_2O$)** S_2 = 5000 mg Zn/L	**Toxic ($3CdSO_4 \cdot 8H_2O$)** S_3 = 1000 mg Cd/L
1.	C_0(M) = 0 mg Cu/L	C_0(M) = 0 mg Zn/L	C_0(M) = 0 mg Cd/L
2.	C_1=5 mg Cu/L	C_1=5 mg Zn/L	C_1=1 mg Cd/L
3.	C_2=15 mg Cu/L	C_2=15 mg Zn/L	C_2=2 mg Cd/L
4.	C_3=45 mg Cu/L	C_3=45 mg Zn/L	C_3=4 mg Cd/L
5.	C_4=135 mg Cu/L	C_4=135 mg Zn/L	C_4= 8 mg Cd/L
6.	C_5= 405 mg Cu/L	C_5= 405 mg Zn/L	C_5= 16 mg Cd/L

Tests were carried out in two stages: the germination stage of triticale seeds in toxic medium and the triticale seedling growth stage after germination.

Germination tests – the tests were carried out in Petri dishes, properly sterilized, 12 cm in diameter, in which two layers of regular filter paper were placed, which were then properly soaked in the toxic solution, according to the work variant. Over the filter paper, 5 seeds of triticale were placed in each Petri dish and were left to germinate for 3 days. The Petri dishes were kept closed under a lid at room temperature ($18 \div 20^0$C) (Figure 1).

After 3 days, the number of germinated triticale seeds was recorded and measurements were carried out in order to gauge the average length of the roots, as well as the average length of the stems. Moreover, particular observations were recorded for each sample (root ramifications, occurrence of fungi, stem coloration, plant vigor etc).

Tests for growth inhibition – after the 3 days germination period, the triticale seeds were moved from the Petri dishes into 150 ml plastic cups on the support sieves. The triticale roots were positioned in such a way that they hung through the net of the sieves directly into the culture medium (Figure 2). The samples were kept in the laboratory at constant temperature, and fresh toxic solution was added to them when necessary.

The plants were kept for 9 days in these solutions, in the laboratory in natural light at room temperature (Figure 3). After 7 days of exposure and 9 days after the beginning of the tests, new measurements were carried out to gauge the length of the roots and the stems, and observations regarding the vigor of plants, the root ramification etc. were also recorded.

Figure 1. Sample preparation for germination of triticale [9]

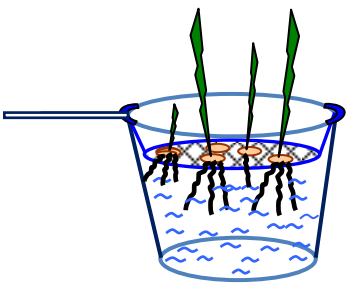

Figure 2. Sketch positioning triticale roots, growth stage

Figure 3. Triticale samples to 9 days [9]

4. Test results and discussions

4.1. Test results in the presence of the toxic ($CuSO_4 \cdot 5H_2O$)

The data of the research regarding the germination and development of triticale plants in the presence of the toxic $CuSO_4 \cdot 5H_2O$ at various concentrations are centralized in Table 2.

Table 2. Data on germination and development of triticale in the presence of toxic $CuSO_4 \cdot 5H_2O$

No days	Parameters	U.M.	Concentration of toxic ($CuSO_4 \cdot 5H_2O$)					
			Control sample	C_1 = 5 mg Cu/L	C_2 = 15 mg Cu/L	C_3 = 45 mg Cu/L	C_4 = 135 mg Cu/L	C_5 = 405 mg Cu/L
3 days	The average length of roots, L_{MR}	(mm)	18.8	17	11,2	7,93	5,06	1,5
		(%)	100	90,42	59,57	42,18	26,91	7,96
	The average length stems, L_{MT}	(mm)	7,2	8,6	7,13	6,6	6,11	2,72
		(%)	100	119,41	99,03	91,66	84,86	37,77
7 days	The average length of roots , L_{MR}	(mm)	21,18	18,53	11,73	8,14	5,25	0,33
		(%)	100	87,48	55,38	38,43	24,78	1,55
	The average length stems, L_{MT}	(mm)	36,53	36,45	25,4	20,75	16,22	3,83
		(%)	100	99,78	69,53	56,8	44,4	10,48
9 days	The average length of roots , L_{MR}	(mm)	23,08	15,13	12,0	7,73	4,91	0,33
		(%)	100	65,55	51,99	33,49	21,27	1,43
	The average length stems, L_{MT}	(mm)	63,56	46,45	41,86	31,33	25,73	2,97
		(%)	100	73,08	65,85	49,29	40,48	4,67

Laboratory tests highlighted interdependence between the concentration of the toxic and the accentuation of the inhibition process both during the germination and the development stage of triticale plants, and the results are presented in Figures 4 and 5.

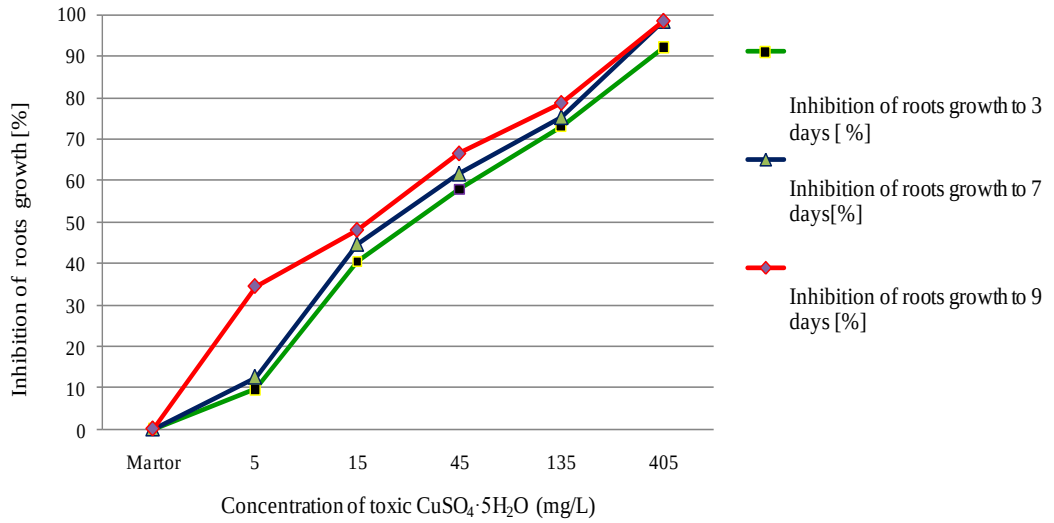

Figure 4. Variation of inhibition of the triticale roots growth in the presence of toxic $CuSO_4 \cdot 5H_2O$

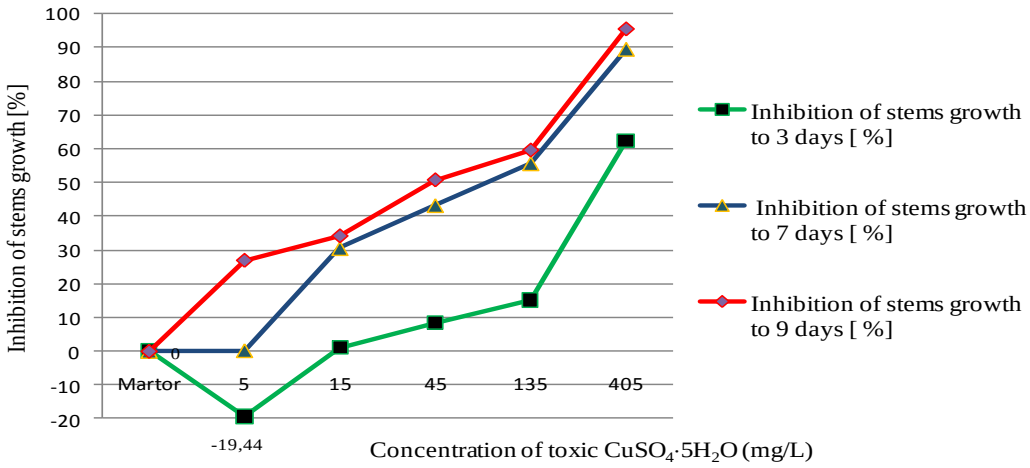

Figure 5. Variation of inhibition of the triticale stems growth in the presence of toxic $CuSO_4 \cdot 5H_2O$

After analyzing the data from the germination and development stages of the triticale exposed to various concentrations of copper sulfate for 3, 7 and 9 days respectively, the following observations were made:

✓ for the experimental variants C_1=5 mg Cu/L and C_2=15 mg Cu/L, all triticale seeds germinated, all having 2-3 well-developed root ramifications;

✓ after 3 days of exposure at a concentration of 5 mg Cu/L a positive evolution of the stem growth by 19.44 % was recorded as compared to the control sample;

✓ for the experimental variants C_3=45 mg Cu/L and C_4=135 mg Cu/L germination is slowed down, roots and stems have limited sizes, and the inhibition of the growth and development of the plants is between 66.51 and 78.72 % for the roots and 50.71-59.51% for the stems.

✓ for the experimental variant C_5= 405 mg Cu/L the germination process (after 3 days) is inhibited by 92.03 %, and after 9 days in contact with the toxic medium, the growth of the roots is inhibited by 98.57% and that of the stems by 95.33%.

4.2. Test results in the presence of the toxic ($ZnSO_4 \cdot 7H_2O$)

The data of the research regarding the germination and development of triticale plants in the presence of the toxic $ZnSO_4 \cdot 7H_2O$ at different concentrations are centralized in table no. 3, and the inhibition of the growth and development of the triticale plants in Figures 6 and 7.

Table 3. Data on germination and development of triticale in the presence of toxic $ZnSO_4 \cdot 7H_2O$

No days	Parameters	U.M.	Concentration of toxic ($ZnSO_4 \cdot 7H_2O$)					
			Control sample	C_1 = 5 mgZn/L	C_2 = 15 mg Zn/L	C_3 = 45 mg Zn/L	C_4 = 135 mg Zn/L	C_5 = 405 mg Zn/L
3 days	The average length of roots, L_{MR}	(mm)	10,53	13,1	12,81	8,93	5,1	3,3
		(%)	100	124,4	121,65	84,8	48,43	31,33
	The average length stems, L_{MT}	(mm)	8,53	9,06	7,45	8,73	6,26	4,7
		(%)	100	106,21	102,34	87,33	73,38	55,09
7 days	The average length of roots, L_{MR}	(mm)	30,47	14,16	13,3	10,9	5	4,09
		(%)	100	46,47	43,64	35,77	16,4	13,32
	The average length stems,L_{MT}	(mm)	59,3	47,68	37,86	35,86	27,2	23,2
		(%)	100	80,4	63,84	60,47	45,86	39,12
9 days	The average length of roots, L_{MR}	(mm)	35,46	13,86	13,8	11,86	4,45	3,7
		(%)	100	39,08	37,16	33,44	12,54	10,43
	The average length stems , L_{MT}	(mm)	86	70,39	63,26	32,45	28,8	23,4
		(%)	100	81,84	73,55	37,73	33,48	27,2

The data analysis highlights the following aspects:

- During the first stage of the process, the germination stage of the triticale seeds, the presence of the toxic $ZnSO_4 \cdot 7H_2O$ for the experimental variants C_1=5 mg Zn/L and C_2=15 mg Zn/L, show a positive influence upon the growth of the triticale roots (24.4% and 21.65%) as compared to the control sample;
- Also during the germination stage of the triticale, the presence of the toxic in the concentrations C_1=5 mg Zn/L and C_2=15 mg Zn/L leads to a growth of the triticale stems by 6.21% and 2.34% as compared to the control sample.

The increase in the concentration of the toxic and in the exposure time from 7 to 9 days shows an inhibition of the growth of the stems with values between 18.16% at the concentration C_1=5 mg Zn/L and 72.8% at a concentration of the toxic C_5=405 mg Zn/L, as well as the inhibition of the growth of the roots with values between 60.82% at the concentration C_1=5 mg Zn/L and 89.57% at a concentration of the toxic C_5=405 mg Zn/L.

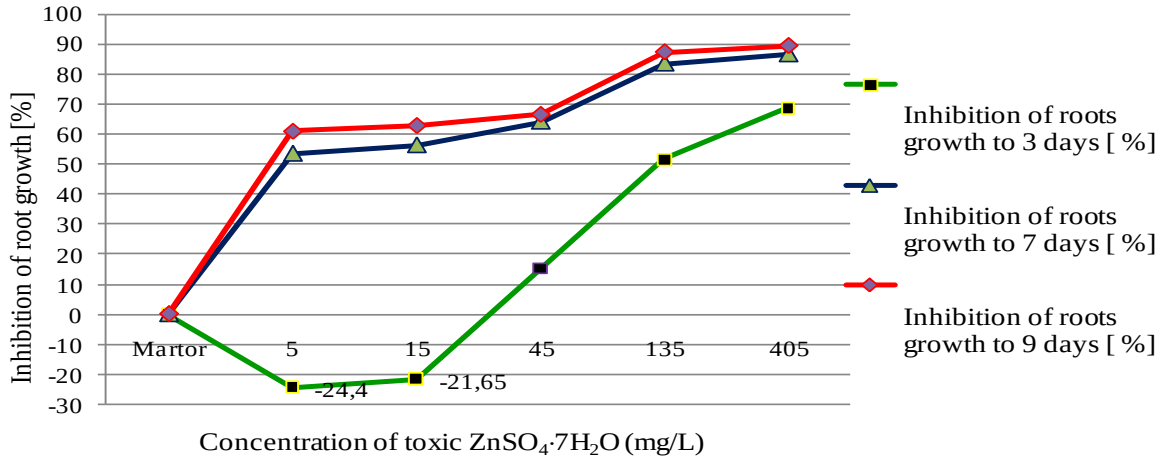

Figure 6. Variation of inhibition of the triticale roots growth in the presence of toxic $ZnSO_4 \cdot 7\ H_2O$

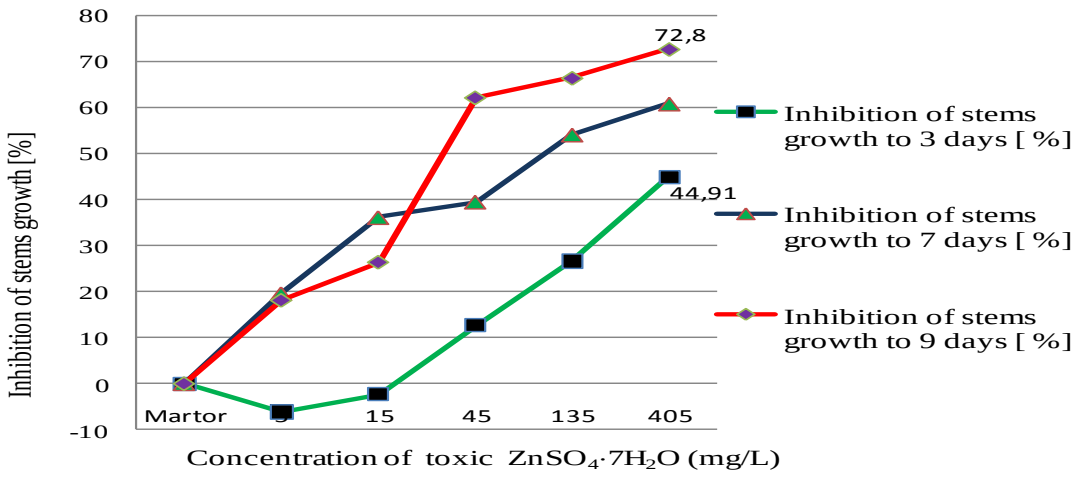

Figure 7. Variation of inhibition of the triticale stems growth in the presence of toxic $ZnSO_4 \cdot 7H_2O$

4.3 Test results in the presence of toxic $3CdSO_4 \cdot 8H_2O$

Inhibition of the growth and development of triticale plants in the presence of toxic $3CdSO_4 \cdot 8H_2O$ at various concentrations can be observe in Figures 8 and 9.

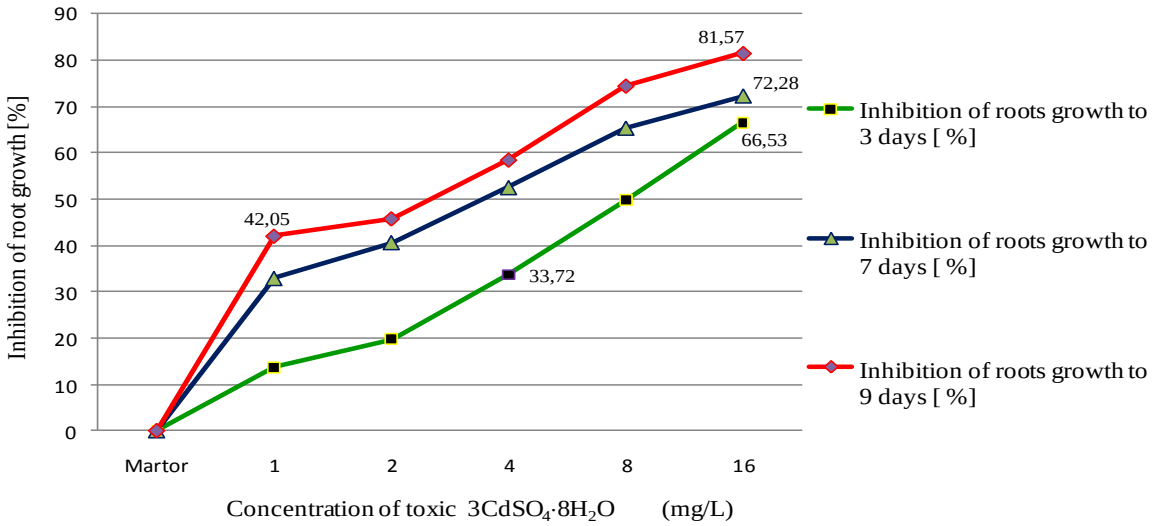

Figure 8. Variation of inhibition of the triticale roots growth in the presence of toxic $3CdSO_4 \cdot 8H_2O$

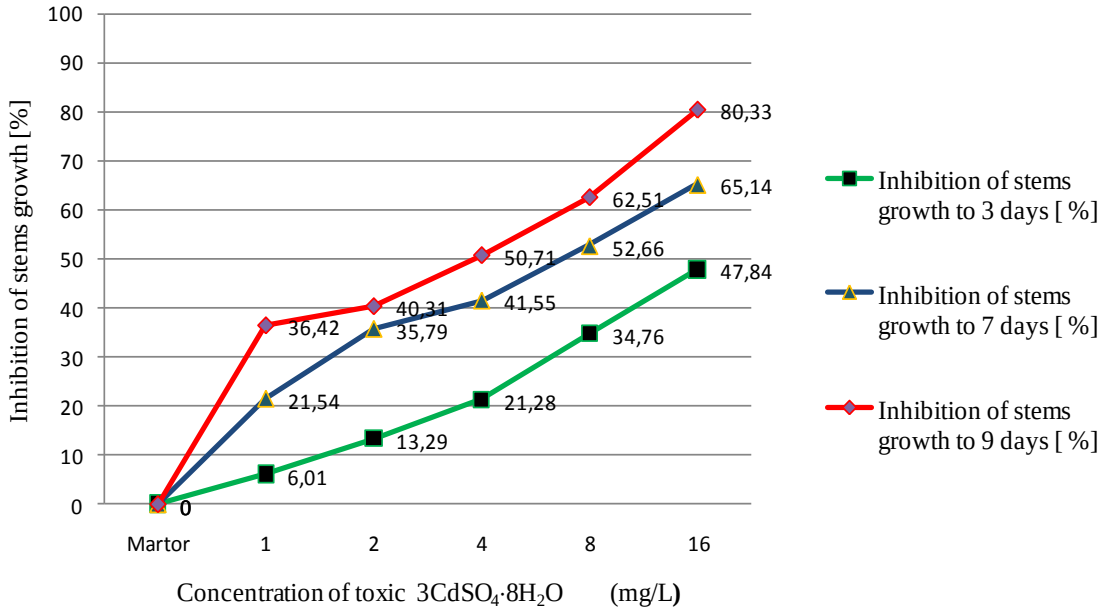

Figure 9. Variation of the inhibition of the triticale stems growth in the presence of toxic $3CdSO_4 \cdot 8H_2O$

After analyzing the test results, one can state the following:

✓ The triticale roots are more sensitive to the effects of cadmium than the stems, one noticing an accentuated inhibition of root growth since the first days of tests (at concentrations of 4 mg Cd/L root inhibition is 33,72%);

✓ Concentrations of 16 mg Cd/L lead to a major inhibition of roots growth, starting from the germination stage by 66.53% and reaching 81.57% inhibition after 9 days of tests;

✓ The triticale stems are less sensitive in comparison with the roots, tests showing the inhibition of their growth by 47.84% after 3 days of tests, at a concentration of 16 mg Cd/L.

✓ The occurrence of toxic cadmium in the environment negatively influences the germination and growth of triticale seedlings even at low concentrations and at the increase of the time spent in contact with the toxic element there appear necroses on the brims of the leaves and the triticale plants dry wither.

5. Conclusions

Another important aspect to be observed is the fact that, as compared to their stalks, the triticale roots are more sensitive to the presence of copper ions as toxic compound. Basically, this is the confirmation of the absorption process of ionic copper in soil and water, especially in the plant roots through radicular absorption.

The reduced copper concentrations (5 mg Cu/L) had a positive influence on triticale stems growth (by 19,44% in relation to the control sample) on a short duration exposure (3 days), while the 5mg Zn/L and 15 mg Zn/L concentrations highlighted a positive influence on roots as well as stalks.

A long exposure to any of the three types of used toxic compounds influences the triticale plants in a negative way, so that after 9 days of exposure the tests show a major inhibition of the growth and evolution of new plants.

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