INFLUENCE OF CADMIUM ON THE RESPIRATION INTENSITY OF *ZEA MAYS* SEEDLINGS

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Abstract. The respiration intensity of corn seedlings treated with different concentrations of cadmium chloride was ascertained at the beginning of the experiment for a duration of three consecutive days, followed then by a new assessment in the 10th day, and the respiration intensity at the roots and at the above ground parts of corn seedlings of each experimental kind were measured in the last day of the experiment. In order to test the cadmium effect, this element was administered as a cadmium chloride water solution ($CdCl_2$) of different concentrations. In this study it was found that the respiration intensity proportionally increases with the quantity of administered heavy metal. Comparing the results obtained subsequent to the determining of the respiration intensity of the root and above ground parts of corn seedlings it was found that the highest recorded values of the respiration intensity were obtained at the above ground parts.

Keywords: cadmium chloride water solution, respiration intensity, experimental variant, seedling, corn caryopses.

INTRODUCTION

Plants represent the feeding resources for man and most of animals, and their contamination with different pollutants determines the propagation of their elements along the trophic chain [1]. For this reason studying the accumulation of different toxic elements of plants is very important, measures being necessary to face out the destruction of the terrestrial and aquatic ecosystems.

Man, as product and part of Nature, in time has become a disturber of the natural ecosystems. The fundamental causes are the rapid growth of the population and industrial technologies, which, unfortunately, brought only a relatively small part of the world's population an excess of energy and goods, and all of that for the price of a stressed environment [2].

Urbanization and the industrial revolution put up severe issues caused by the accumulation of xenobiotics (Hg, Pb, Cd, SO_2, etc.) in air, water and soil, and at the end of the last century the attention was focused on the undesired climate change, as a consequence of pollution (acid rains, greenhouse effect, drought) [5].

The future development of industrial activity should be carried out according to the principle of durable development, protecting the environment, since "biologically correct means economically advantageous" [6].

Up to date, nature protection, environment quality improvement and species conservation must be a major preoccupation for the human kind, which has the duty of transferring the natural heritage less deteriorated to the next generations [3].

Generally, an important characteristic of heavy metals, notwithstanding if they are biologically essential or not, is that in excess they are highly phytotoxic. Although the relative toxicity of different metals can vary relative to the plant genotype and the experimental conditions, the most phytotoxic , in excessive quantities, are: Hg, Cu, Ni, Pb, Co and Cd [9].

Cadmium increases the activity of some hydrolytic enzymes and peroxidase, thus intensifying the respiration and concurring to the acceleration of senescence [8].

MATERIALS AND METHODS

The respiration intensity of corn seedlings treated with different concentrations of cadmium chloride was ascertained at the beginning of the experiment for a duration of three consecutive days, followed then by a new assessment in the 10th day, and the respiration intensity at the roots and at the above ground parts of corn seedlings of each experimental kind were measured in the last day of the experiment.

In order to test the cadmium effect, this element was administered as a cadmium chloride water solution ($CdCl_2$) of different concentrations.

The work was done using 120 corn seeds of the Dobrogean variety, which were kept in ethylic alcohol for one minute for disinfection, then they were washed in running water, and soaked for one hour with a solution of $CdCl_2$ of different concentrations, then they were put to germination.

The control was treated with running water. The soaked seeds were put on filter paper, each germinator comprising 10 caryopses.

The germinative conditions were ensured as the process took place at a constant temperature of $20^0C \pm 1^0C$, in natural light. The corn caryopses were daily wetted with corresponding solutions, respectively the control with running water. The schedule of the experimental variants was set up as it follows: S_1 (Control – running water); S_2 (concentration of $CdCl_2$ solution 1 mg/l); S_3 (concentration of $CdCl_2$ solution 5 mg/l); S_4 (concentration of $CdCl_2$ solution 10 mg/l); S_5 (concentration of $CdCl_2$ solution 100 mg/l); S_6 (concentration of $CdCl_2$ solution 200 mg/l).

In order to determine the respiration, the method based on determining the produced CO_2 quantity was used, being applied the confined atmosphere procedure (Boysen-Jensen).

In case of this procedure, the contact between the vegetal material and atmosphere takes place in a closed recipient, in which CO_2 - resulted from respiration – is fixed by a solution of $Ba(OH)_2$.

For determination, we used 7 jars, having a volume of 500 cm^3; in each jar we introduced 20 ml of a solution of $Ba(OH)_2$ 7%, and some drops of phenolphthalein 1%.

One of the jars was immediately closed, and it represented the blind sample. The vegetal material, previously measured by an analytical balance, was introduces in the other jars. The caryopses derived from the experimental variants were put into gauze baskets, which were then suspended by hooks that were fixed to the jar faucets. The jars were immediately closed, the starting time of the experiment was recorded, and then they were put into a dark room for one hour.

The jars were intermittently agitated during the determination, in order to break the crust of $BaCO_3$ formed at the surface of hydrate, after fixing CO_2. Periodic agitation facilitates permanent contact between the hydrate solution and CO_2 liberated by the vegetal material during respiration.

The vegetal material is taken out from the jars after one hour, and the remaining uncarbonated hydrate is then titrated with a solution of oxalic acid 2.8636‰, of which 1 ml corresponds to 1 cm^3 of fixed CO_2.

The remaining uncarbonated hydroxide from the jar without vegetal material is then titrated, too. Titration is made until the solution becomes almost uncoloured, of very pale pink.

During the experiment, the humidity and temperature of the germinators place are daily controlled, adjusting their values if it is necessary.

RESULTS

The results obtained following determining of corn seedlings respiration intensity, treated with a solution of $CdCl_2$, are shown in the tables 1 & 2.

Comparing the results obtained following the determining of the respiration of the roots and above ground parts, we can remark, from the Figure 1 that the highest recorded values of the respiration intensity were obtained at the above ground parts after 17 days since germination.

DISCUSSIONS

We analyzed every set of values, the standard being the respiration intensity of corn seedlings on solutions with different concentrations of $CdCl_2$: 1 mg/l $CdCl_2$, 5 mg/l $CdCl_2$, 10 mg/l $CdCl_2$, 100 mg/l $CdCl_2$ and 200 mg/l $CdCl_2$.

It can be noted that the corn seeds from the control sample recorded the highest value of respiration intensity, and the lowest value belongs to the S_6 variant (Table 1).

The respiration intensity of the corn seedlings after 3 days of germination do not show large differences among the results recorded at the variants S_3 and S_4.

In the 4[th] germination day it is noted that the respiration intensity is reduced for all the experimental variants; it can be noted a much more significant difference between the values recorded at the control and the S_2 variant.

The highest value of the respiration intensity was recorded at the control sample, and the smallest value at the S_6 variant

Table 1. The variation of the respiration intensity of *Zea mays* seedlings, after 3, 4, 5 and 10 days since germination, using 60 minutes for duration of determining.

Sample No.	Sample (with vegetal material)	Mass of vegetal material (g)				ml of $C_2O_4H_2$ used for titration				Respiration intensity (cm^3 CO_2/g/h)			
		3 days	4 days	5 days	10 days	3 days	4 days	5 days	10 days	3 days	4 days	5 days	10 days
1	Control	5.21	5.29	5.17	5.75	1.3	1.3	1.2	1.6	0.153	1.132	0.115	0.034
2	1 mg/l $CdCl_2$	4.66	5.18	4.73	5.80	1.4	1.4	1.4	1.5	0.150	0.115	0.084	0.051
3	5 mg/l $CdCl_2$	4.35	4.64	4.97	5.68	1.5	1.5	1.4	1.5	0.137	0.107	0.080	0.052
4	10 mg/l $CdCl_2$	4.57	5.29	4.71	5.33	1.5	1.5	1.5	1.5	0.131	0.094	0.063	0.056
5	100 mg/l $CdCl_2$	4.50	4.9	4.69	5.73	1.6	1.6	1.6	1.4	0.111	0.081	0.042	0.069
6	200 mg/l $CdCl_2$	4.54	5.3	5.28	5.67	1.8	1.8	1.7	1.3	0.066	0.037	0.018	0.088
7	Blind sample (without vegetal material)	N/A	N/A	N/A	N/A	2.1	2	1.8	1.8	N/A	N/A	N/A	N/A

Table 2. The variation of the respiration intensity of the root and the above ground parts of Zea mays seedlings, after 17 days since germination

Sample No.	Sample (with vegetal material)	Mass of vegetal material (g)		ml of $C_2O_4H_2$ used for titration		Respiration intensity (cm^3 CO_2/g/h)	
		roots	above ground parts	roots	above ground parts	roots	above ground parts
1	Control	2.02	4.02	1.9	1.5	0.049	0.124
2	1 mg/l $CdCl_2$	1.95	3.89	1.8	1.4	0.102	0.154
3	5 mg/l $CdCl_2$	1.80	3.76	1.7	1.3	0.166	0.186
4	10 mg/l $CdCl_2$	1.72	3.56	1.7	1.3	0.174	0.196
5	100 mg/l $CdCl_2$	N/A	4.15	N/A	1.1	N/A	0.216
6	200 mg/l $CdCl_2$	N/A	4.18	N/A	1	N/A	0.239
7	Blind sample (without vegetal material)	N/A	N/A	2	2	N/A	N/A

*The respiration intensity was determined for the whole parts of the corn seedlings for the variants S_5 and S_6.

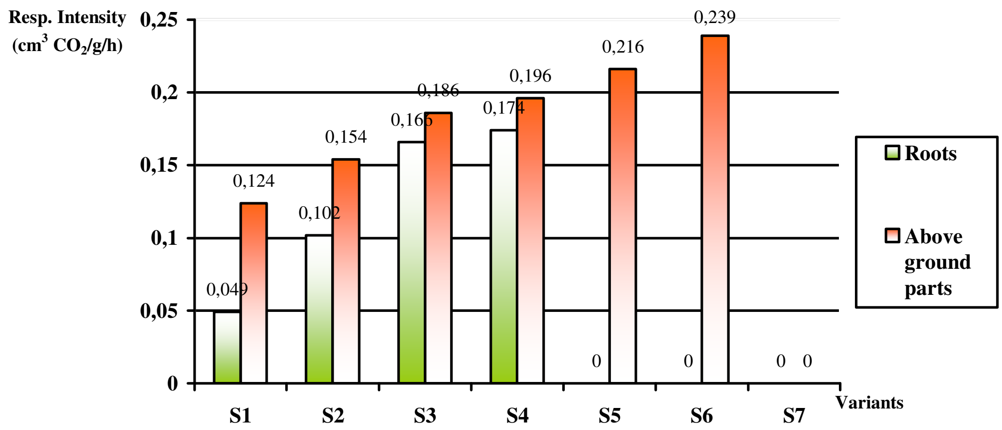

Figure 1. The variation of the respiration intensity of the above ground parts of *Zea mays* seedlings, after 17 days since germination

In the 5th germination day it was found that the difference between the respiration intensity of the control and that from the S_2 variant has increased. Diminishing of the respiration intensity can be observed, as there are not significant differences among the values recorded at the S_2 and S_3 variants. The maximum value was recorded at the control variant, and the minimum value at the S_6 variant.

In the 10th day of determining of respiration intensity, the highest value was recorded at the S_6 variant, and at the control the smallest value was recorded. No significant differences were recorded among the S_2, S_3 and S_4 samples.

We can note that the respiration intensity proportionally increases with the administered cadmium concentration.

The respiration intensity at the roots and at the above ground part of corn seedlings under cadmium treatment was assessed in the last day of the experimental stage. A small number of seedlings developed at the S_5 and S_6 variants that were treated with the highest cadmium concentrations; the development of the root system was highly affected, and at the end of the experiment the above ground part was also presenting a highly degree of chlorosis and dryness of the vegetative top. Owing to this fact, the determining of the respiration intensity could not been accomplished for the roots and the above ground parts at this variant; still, it was carried out for the whole plant, and the results were recorded within the table in which are shown the observations made for the above ground parts of seedlings, which were treated with different cadmium concentrations.

The Table 2 shows the results obtained following the determining of the respiration intensity of the roots of seedlings. In it is noted that the respiration intensity proportionally increases to the administered cadmium quantity; a significant increase was recorded at the S_3 and S_4 variants.

The same table also presents the respiration intensity of the above ground parts of corn seedlings, where the maximum value was recorded at the S_6 variant with the highest concentration, and the minimum value at the control.

The respiration intensity proportionally increases with the quantity of administered cadmium.

Comparing the results obtained following the determining of the respiration intensity of the roots and the above ground parts, it is noted that much higher values were recorded at the above ground parts.

Analyzing the results obtained subsequently of determining the respiration of corn seedlings, it was found that the highest values were recorded at the end of the experiment.

The influence of heavy metals on the physiological processes of plants was studied by many researchers [7], who observed the growth of the whole plant and the chlorophyll content, oxygen evolution, and chloroplast ultrastructure of leaf tissues. These were studied in maize plants grown on a culture medium either without cadmium (Cd) or supplied with increasing concentrations of the metal. The plants treated with high Cd concentrations showed symptoms of heavy metal toxicity, such as length reduction of both roots and shoots, leaf bleaching, ultrastructural alterations of chloroplasts and lowering of photosynthetic activity. Some symptoms appeared at 100 mg Cd, but the strong toxic effect of the metal was found only at 250 mg Cd.

Another study [10] was also carried out regarding the uptake of cadmium and the interaction with phytochelatins in wheat protoplasts. In order to investigate the role of phytochelatins in short-time uptake of cadmium into the cytosol of wheat protoplasts, a new method was applied, using fluorescence microscopy and the heavy metal-specific fluorescent dye, 5-nitrobenzothiazole coumarin, BTC-5N. The uptake of cadmium into protoplasts from 5 to 7 day old wheat seedlings was lower in protoplasts from seedlings raised in the presence of 1 mg $CdCl_2$, than in the absence. Presence of $CdCl_2$ in the cultivation medium increased the content of phytochelatins (PCs) in the protoplasts. When seedlings were raised in the presence of both cadmium and buthionine sulfoximine (BSO), an inhibitor of glutathione (GSH) synthesis, only little PC was found in the protoplasts. Pre-treatment with BSO alone did not affect the content of PC, but inhibited that of GSH. The inhibition of GSH was independent of pre-treatment with cadmium. Unidirectional flux analyses, using cadmium, showed approximately the same uptake pattern of cadmium as did the fluorescence experiments showing the cytosolic uptake of cadmium. Thus, the diminished uptake of cadmium into protoplasts from cadmium-pre-treated plants was not depending on PCs. Instead, it is likely that pre-treatment with cadmium causes a down-regulation of the short-term cadmium uptake, or an up-regulation of the cadmium extrusion. Moreover, since addition of cadmium to protoplasts from control plants caused a cytosol acidification, it is likely that a Cd^{2+}/H^{+}-antiport mechanism is involved in the extrusion of cadmium from these protoplasts.

Other studies carried out upon other heavy metals, such as lead, and how they influence certain physiological processes [4]. The exposure of detached leaves of pea, barley and maize to 5 mg lead nitrate for 24 h caused a reduction of their photosynthetic activity by 40–60%, whereas the respiratory rate was stimulated by 20–50%. Mitochondria isolated from lead-treated pea leaves oxidized substrates (glycine, succinate, malate) at higher rates than mitochondria from control leaves. Lead caused an increase in ATP content and the ATP/ADP ratio in pea and maize leaves. Rapid fractionation of barley protoplasts incubated at low and high CO_2 conditions indicated that the increased ATP/ADP ratio in lead-treated leaves resulted mainly from the production of mitochondrial ATP. The activity of NAD-malate dehydrogenase in the protoplasts from barley leaves treated with lead was 3-fold higher than in protoplasts from control leaves. The activities of photorespiratory enzymes were not affected. The presented data indicate that stimulation of respiration in leaves treated by lead is in a close relationship with activation of malate dehydrogenase and stimulation of the mitochondrial ATP production. Thus, respiration might fulfil a protective role during heavy metal exposure.

Coming back to our study, as about the determining of the respiration intensity, is was found that it proportionally increases with the administered quantity of heavy metal.

During the respiration processes, reserve organic substances decompose into small osmotic active molecules that can remake the osmotic balance of cells, due to the accumulation of metallic ions.

An enhancement of all vital functions takes place under the action of cadmium; plants treated with different concentrations of cadmium chloride try to counteract the cadmium toxicity by intensifying all metabolic processes.

Due to this fact, the highest values of the respiration intensity were recorded for the variants that were treated with high concentrations of cadmium.

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