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SEED GERMINATION AND EARLY SEEDLING DEVELOPMENT OF MAIZE (*Zea mays* L.) UNDER THE STRESS OF DIFFERENT HEAVY METAL CONCENTRATIONS

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Abstract

Since the lifecycle of plants begins with the germination phase, evaluating this process under different environmental factors may reveal some parameters with great importance for the further development of plants. Also, it can represent a base for other studies. The purpose of this paper was the evaluation of germination and seedling growth of maize under different levels of heavy metal contaminations. The following heavy metals were used as mixtures: Cd, Cu, Ni, Pb, Co and Zn. Five mixtures with different concentrations of Cd (3 to 20 ppm), Cu (20 to 200 ppm), Ni (25 to 200 ppm), Pb (30 to 150 ppm), Co (20 to 100 ppm) and Zn (100 to 700 ppm) were used as variants, and distilled water as control variant. Seed germination percentage was determined in accordance to ISTA (International Seed Testing Association). A series of physiological indicators associated with germination and seedling growth were calculated. The germination was observed for ten days, until no changes occurred for three consecutive days. Several effects were observed at the end of the experiment regarding the seed germination and especially to seedling development. Although there were some differences on germination between control variant and high contaminated variants, the growth of seedlings showed a high variation as the concentration of heavy metals increased.

Key words: *Zea mays L., heavy metals, germination, seedling.*

INTRODUCTION

As the first stage of plant life, the germination plays a substantial role on plant development. This process is easily influenced by environmental factors, which most often have negative effects on germination parameters, reducing growth, development and production of plants.

Some of these factors have been studied in many works, each of them influencing germination parameters differently. Important changes were noted due to humidity stress (Shaban, 2013; Khodarahmpour, 2012), temperature (Farooqi & Lee, 2016; Wang et al., 2018; Bano et al., 2015), salt stress (Mirosavljević et al., 2013; Sozharajan & Natarajan, 2014; Turk & Eser, 2016) and heavy metals (Bashmakov et al., 2005; Sethy & Ghosh, 2013; Oladele et al., 2018). Maize (*Zea mays* L.) are known to be a good accumulator of heavy metals and lot of experiments was conducted to study the effects of these contaminants on growth and yield of maize (Aladesanmi et al., 2019; Ahmed & Hanan, 2015).

The capacity of plants to accumulate heavy metals and other contaminants can vary with the type of plant species, the nature of metal, but also other environmental factors or soil properties (Oladejo et al., 2017). The existence of heavy metals in soil can lead to their accumulation in different parts of plant with major consequences to health. The excessive amounts can determine physiological and biochemical toxic effects (Shahid et al., 2015). On a study in 2005, Bashmakov et al., observed that on high concentrations of heavy metals, the growth of shoots and roots was inhibited, followed by death of the majority of seedlings. Heavy metals can also determine other physiological responses like necrosis, browning of roots or chlorosis, but the effects may differ in function of environmental condition, plant type or metal amount (Pourrut et al., 2013).

The majority of metals are barely soluble and mobile in soils, with very low phytoavailability. Almost all heavy metals occur in soils as residual forms, due to strong binding with different ligands (Uzu et al., 2009).

Even the plants can easily accumulate metals that are dissolved as ionic compounds in the soil solution, also chelated or complexed metals can be absorbed due the complex structure of soils and complex absorption mechanisms of plants (Fairbrother et al., 2007). This would be the main different between laboratory tests and “in situ” growth of plants. Yet, the influence of increasing doses of heavy metals on the behavior of seeds and seedlings can be observed in laboratory, with very good remarks. This work aimed to observe the behavior of seeds and seedlings of maize (*Zea mays* L.) in the presence of increasing doses of soluble heavy metals (Zn, Cu, Ni, Co, Pb and Cd).

MATERIALS AND METHODS

In order to characterize the effects of heavy metals on seed germination and seedling growth, six soluble salts of heavy metals were used ($Cd(NO_3)_2 \cdot 4H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Pb(CH_3COO)_2 \cdot 3H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Zn(CH_3COO)_2 \cdot 2H_2O$), representing those heavy metals (Cd, Cu, Ni, Pb, Co and Zn). The salts were mixed together in five concentrations, representing five variants of treatments. A control variant represented by distilled water was used also, resulting a total of six variants. The concentrations for the five variants were chosen according the Romanian regulation (Order 756/1997), aiming to cover the whole interval from reference value to values over intervention threshold for sensitive use of soils, as described in the order mentioned above. The variants are showed in the Table 1.

Table 1. Variants of concentrations used in experiment

Metal	Variants (ppm)					
	M	C1	C2	C3	C4	C5
Ni	0	25	75	100	150	200
Cu	0	20	60	100	150	200
Cd	0	3	5	10	15	20
Zn	0	100	300	450	600	700
Pb	0	30	50	75	100	150
Co	0	20	30	50	75	100

The biological material was represented by maize seeds (DKC 3969 hybrid). The experiment was conducted using 20 centimetres Petri dishes, with two filter paper disks which have been soaked in heavy metal solutions. Each variant had four replicates (two per vessel), and each replicate used 25 seeds (Figure 1), resulting a total of 600 seeds. The germination took place at room temperature (22°C during daytime and 19°C during night), at natural light, excepting first 24 hours when the vessels were kept in dark conditions. The seeds were considered to have germinated after radicle emerged 2 mm through tegument. Germination test was ended when no seeds have germinated for 3 days consecutively. The germination period ended after 8 days, the seeds being monitorized for 10 days.

Figure 1. The arrangement of seeds in Petri dishes (two replicates: 2 x 25 seeds per vessel)

A series of physiological indicators were determined, as presented on the Table 2.

Table 2. List of determined physiological indicators

Indicator	Unit	Formula	Source
Final germination percentage	FGP (%)	(total number of germinated seeds / total number of planted seeds) x100	Ranal & Santana, 2006, according to ISTA, 1999
Length of radicle, shoot and total length of seedling	Lr, Ls, T	Metric measurements	-
Maximum germination value (“peak value”)	PV	FGP / the number of days required to reach the maximum germination value	Czabator, 1962
Seedling growth inhibition	SGI (%)	(Seedling length of control variant - Seedling length of tested variant) / length of control variant x100	Ali et al., 2015
Seedling vigor	PVI	PVI = seedling length x FGP	Pati & Chowdhury, 2015

RESULTS AND DISCUSSIONS

After eight days, the germination ended resulting the daily evolution presented in Figure 2. The last three days are identical, meaning that germination is over.

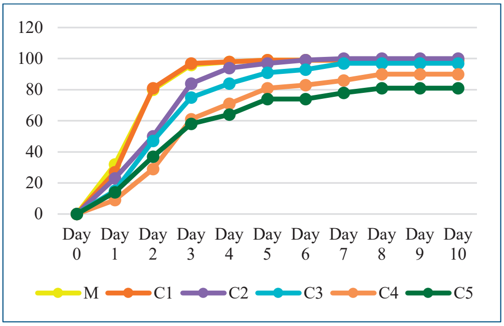

Figure 2. Germination dynamics by days

There were counted the seeds whose radicle reached a length of at least 1 mm. It can be observed that the contamination with heavy metals delayed the germination in concordance with the level of toxicity. The way how the germination influenced the others indicators is shown on the following calculations.

Final germination percentage (FGP) represents the total number of germinated seeds reported to total number of planted seeds (Figure 3).

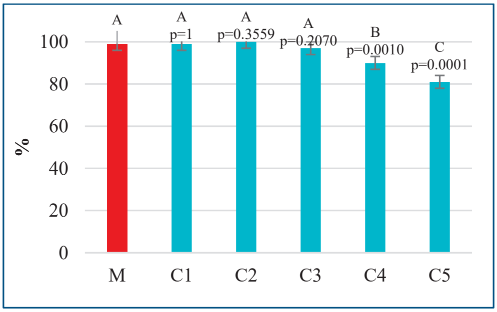

Figure 3. Final germination percentage (FGP) (means±SE)

Although the maximum FGP was obtained for C2 treatment, the difference between this variant and the control (M) in not significant (p-value > 0.05), according to ANOVA unifactorial test (α = 0.05). Actually, there are no significant difference between control M and C1, C2 and C3 variants. In the case of C4 variant, there is a significant difference beside control M, and moreover, between M and C5 variant is highly significant difference.

Seedling length was obtained by adding the length of radicle with the length of shoot (Figure 4).

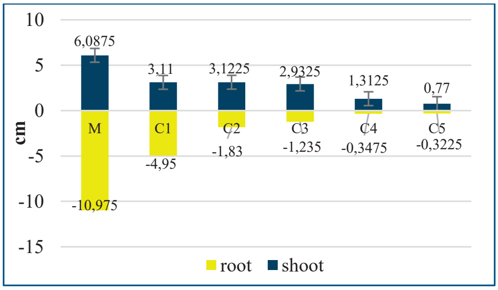

Figure 4. Seedling length (radicle + shoot) (means ± SE)

Although the FGP decreases from 99% to 81% with the increase of concentrations, the effect of heavy metals is not so relevant on germination as it is observed on seedling length (Figures 4 and 5).

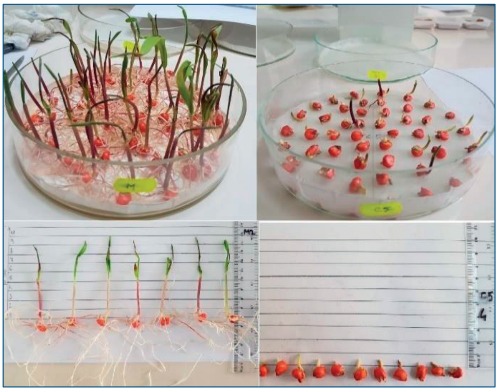

Figure 5. Differences between M and C5 seedling length

Running ANOVA test unifactorial test (α = 0.05), it is shown that there are highly significant differences between control variant and all treatments, from C1 to C5 (Table 3).

Table 3. ANOVA P-value comparing control variant with the rest of variants regarding seedling length

Variant	M	C1	C2	C3	C4	C5
P-value	A	D 4.95E-05	D 4.38E-06	D 3.73E-06	D 1.13E-06	D 7.28E-07

As can be seen, the length of seedlings dropped significantly from an average of 17.06 cm (M) to an average of 1.09 cm(C5). The root length followed the same trend dropping from average of 10.97 cm (M) to an average of 0.32 cm (C5).

Maximum germination value (PV) represents the mean daily germination of the most vigorous component of the seed lot. It has a high relevance in seed germination interpretations (Czabator, 1962). It is determined by reporting FGP to the number of days required to reach the maximum germination value.

For all tested variants, the germination started on Day 1 and ended differently, the latest germination being observed on C5 variant. The highest PV was reported for control variant, followed at slight difference by C1 variant. Running ANOVA unifactorial test (α=0.05), it was noted that there is no significant difference between M, C1 and C2 variants. For C3 and C4 variants, the values of PV differ significantly in in relation to M. Also, between C5 and M the ANOVA test showed a highly significant difference (Figure 6).

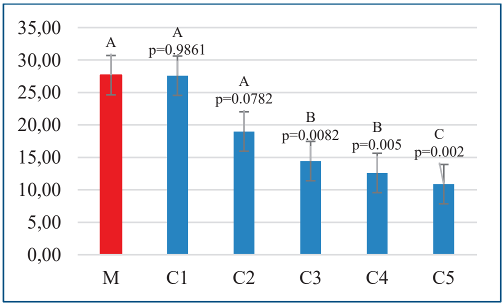

Figure 6. Maximum germination value (PV) (means±SE)

Seedling growth inhibition (SGI) indicator show how much a seedling growth is inhibits by a toxic factor. It is determined by the difference between control variant and tested variant length, reported to control length. Actually, the control variant has zero inhibition and as the length of a seedling decreases, the SGI indicator will increase.

As the control variant was not inhibited at all by heavy metal treatment (SGI = 0), the Figure 7 show the evolution of this indicator as for the rest of the variants.

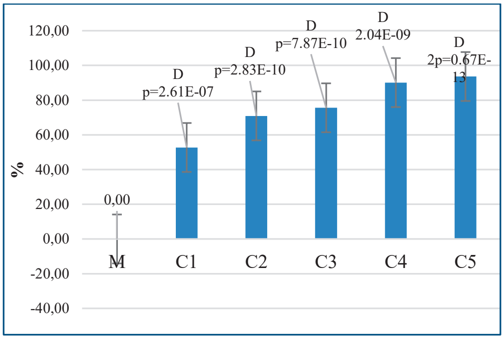

Figure 7. Seedling growth inhibition (SGI) (means±SE)

Given that for control variant the SGI is null, from statistical point of view (ANOVA unifactorial test α=0.05), between control and all other variants there are highly significant difference. As well, it can be observed that the seedling growth inhibition follows the trend of contamination level (Figures 7 and 8).

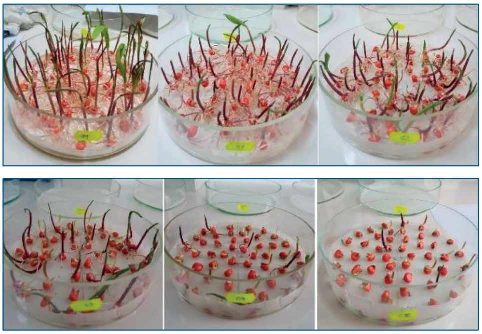

Figure 8. The seedling growth inhibition at the end of the experiment, from control variant (M) to highest used concentration (C5)

Seedling vigour (PVI) indicator is derived from final germination percent and seedling growth parameters (i.e. length) and is calculated as seedling length multiplied by FGP (Figure 9).

The ANOVA test unifactorial test (α = 0.05) reported that all variants differ significantly from control variant. This shows that seedling vigour is substantially reduced by any contamination with heavy metals.

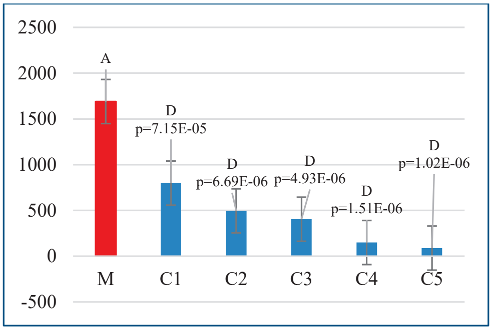

Figure 9. Seedling vigour (PVI) (means ± SE)

CONCLUSIONS

The results showed that the germination percent did not decrease significantly for the lower level of contamination, but increasing the concentration of heavy metals close to intervention threshold for sensitive soils the germination percent dropped significantly.

Regarding the seedling development, the high concentration of heavy metals inhibited the growth, especially the radicle evolution which was almost stopped. The radicle length was reduced by 17 times on highest heavy metal treatment. Although the shoot continued to grow slowly, it may be due to seed nutrient reserve which did not end in the supervised period.

The maximum germination value showed that the higher the contamination was, the harder the seed germinated. The highest level of contamination increased the germination time almost twice compared to non-contaminated seeds.

Considering the seedling growth inhibition, it can be noted that the first level of contamination reduced the length of seedling more than half of the control variant and the highest level of contamination, the length of seedling was reduced over 93%.

The same way, the seedling vigour is reduced by half at the first contamination and at the highest contamination is reduced about 20 times than the control variant.

Overall, even the germination indicators of some variants did not suffer significant modifications related to control variant, the indicators concerning the seedling growth are totally different beside the control variant, heavy metal contaminations affecting much more the early development than the germination.

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