

THE INFLUENCE OF SOME HEAVY METALS ON SEED GERMINATION AND SEEDLING GROWTH AT *CANNABIS SATIVA* L.

Anișoara STRATU¹, Laura FRENȚOAIE¹,
Naela COSTICĂ¹

¹ „Al. I. Cuza” University Facultatea of Biology, Iași
Phone : 40.232.201510

The paper presents the results of a study concerning the influence of different concentrations of Pb and Cd on some physiological and morphological indicators in the dynamics of the process of seed germination and in the incipient phases of seedling growth. The results underline the specific variations of the analysed indicators (water and dry substance content, content of total mineral elements, content of assimilating pigments, cellular liquid concentration, respiration intensity, lengths of vegetative organs of the seedling), depending on the nature of metals and their concentrations used for the seed treatments. At every investigated experimental variants, the water and total mineral elements contents increases during the analyzed period; the cellular liquid concentration decreases. Cd in concentration of 1ppm determine the intensification of respiration process, indicator for underlining the metabolic activity. Regardless to the concentration, Pb and Cd determine the increase of the content of assimilating pigments, more obvious in the case of a chlorophyll.

Key words: heavy metals, physiological and morphological indicators.

The specialty literature referring to the influence of heavy metals on the plants is numerous [1, 3, 4, 5, 6, 7, 8, 9, 10]. According to some authors quoted by Linger and collab. (2005), Cd is known as being one of the most phytotoxic heavy metals.

The paper continues the research regarding the effect of the treatment with heavy metals carried out in the previous years at species with phyto-remediation potential from the *Fabaceae*, *Poaceae* and *Brassicaceae* families [8, 10].

The paper presents the results of a study concerning the influence of the different concentrations of Pb and Cd on some physiological indicators in the dynamics of the process of seed germination and in the incipient phases of seedling growth in *Cannabis sativa* L.

MATERIAL AND METHOD

As vegetal material, we used seeds of *Canabis sativa*. We had five experimental variants: 1 - control – M (with distilled water) and 4 variants of treatment with heavy metals. They were used in form of acetate in the following concentrations: Pb (50ppm – Pb1, 200ppm – Pb2); Cd (1ppm – Cd1 ;10 ppm – Cd2). The duration of the treatment

was 3 hours. After the treatment, the seeds were put for germination in Petri plates, on filter paper, in laboratory conditions.

We analysed the following physiological indicators: at intervals of 1 - 13 days: the content of water and dry substance (gravimetric method) and intensity of the respiration (Warburg method); at intervals of 4 - 10 days: cellular liquid concentration (refractometrical method) and the content of total mineral elements (dry calcination at 450° C method) [2]. Moreover, at 10 days after the germination we determined the content of assimilating pigments (spectro-photometrical method). At 13 days from the experiment beginning, we measured the length of vegetative organs of seedlings.

RESULTS AND DISCUSSIONS

Hemp is an herbaceous, annual, unisexual-dioecious, cultivated, often subspontaneous plant, originating from Central Asia. It is widely spread, from the tropical areas to the cold ones. It is an important technical plant but it can be used in the human, veterinary medicine, as a fodder for the animals and fertilizer for the soil [3, 6, 7]. The seed is intergrown with the fruit and is known by the name of nutlet. The seeds of hemp contain high quantities of carbohydrates, lipids and reserve proteins that are used during germination as well.

The researches carried out on *Cannabis sativa* by Linger and collab.(2002, 2005), indicate the fact that this species has the ability to accumulate heavy metals (Ni, Pb, Cd) in the vegetative reproduction organs, being considered an ideal; candidate for the phytoremediation of contaminated soils. The results obtained are presented in figure 1-5.

The content of water and dry substance (fig.1). In all the experimental variants, the water content has an ascendant evolution during the analyzed period. This is in accordance with the cytophysiological and biochemical transformations characteristic to the germination and growth process of seedlings in the first stages.

In comparison with the control, in the treatment variants, the water content has in the majority of cases analyzed lower/higher values but similar to them.

In all the experimental variants, the content of dry substance decreases beginning with the first day and until the 10th. This reality is due to the fact that the reserve substances from the endosperm and cotyledons are hydrolyzed, mobilized and used as respiratory sublayer and for the synthesis of new substances necessary for nutrition and embryo growth.

The cellular liquid concentration (fig.2). By comparison with the witness, at the treatment variants the cellular liquid concentration values are inferior to it on the 10th day. We can ascertain an inverted correlation between the cellular liquid concentration and the water content.

The content of total mineral elements (fig.2). Both in the control and in the treatment variants, the content of total mineral elements registers a value increase during the analyzed period.

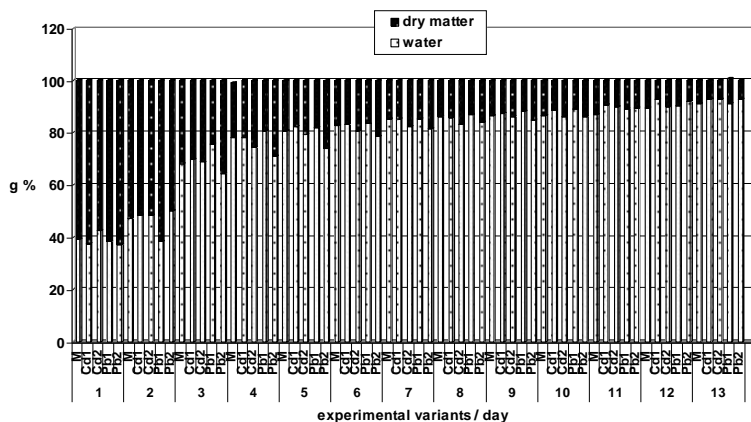


Figure 1 The content of water and dry matter

Between the control and the treatment variants, we ascertain small value variations, with the exception of Pb1 and Pb2 variants (day 10th: values with 23% and respectively 27% “control”). It is possible that Pb in the concentrations used for the treatment influence the mineral metabolism characteristic to germination.

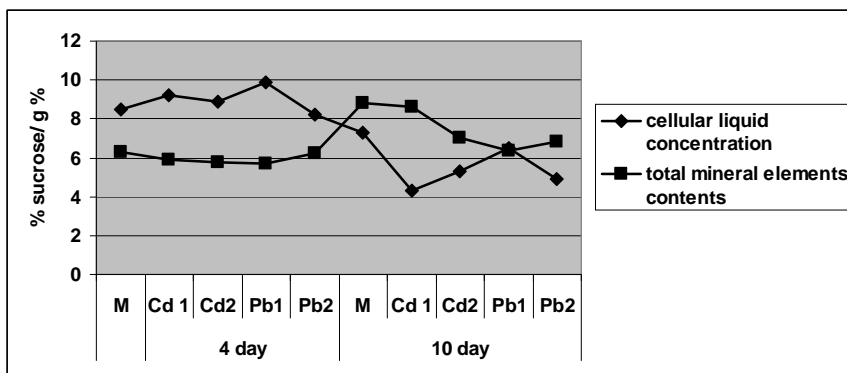


Figure 2 The cellular liquid concentration and the content of total mineral elements

The respiration intensity (fig.3). The respiration process generally presents a similar dynamics in all the experimental variants: its intensity grows beginning with the 2nd day, registers maximum values in the 5th day for the control and Pb2, the 4th day- for Cd1 and Cd2 and the 6th day for Pb1 and is maintained at moderate values in the rest of the analyzed interval.

In comparison with the control, the treatment carried out with Cd1 ppm determines a slight stimulation of the respiration process, achieving an increase with 54% in the 3rd day and with 36% in the 4th day since the experiment beginning.

In the variants treated with Pb the breathing intensity is maintained at smaller values compared to the control with the exception of Pb2 for the days 7,8,12 and 13.

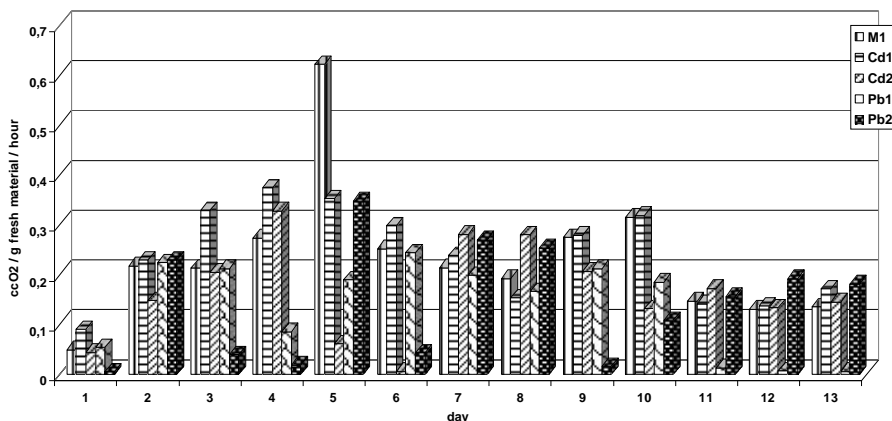


Figure 3 The respiration intensity

The content of assimilatory pigments (fig.4). From among the forms of analyzed assimilated pigments there prevails in the quantitative aspect in all experimental variants, chlorophyll a, followed in decreasing order by chlorophyll b and by carotenoidic pigments.

We ascertain an increase of the chlorophyll content **a** and **b** in all the treatment variants, more obvious in Cd2 (Cd in concentration of 10 ppm).

In all the treatment variants, the content of total assimilatory pigments has superior values compared to the control (1.7 times in Cd1; 2.43 times in Cd2; 1.80 times in Pb1; 1.54 times in Pb2), the highest values being registered in the variants treated with Cd2. The ratio $cl\ a / cl\ b$ has values comprised between 3.72 in the witness and 3.05- 3.59 in the variants treated with heavy metals, fact that denotes a specific biosynthesis rhythm of the two chlorophyll forms, in normal and modified conditions.

Kovascovics and collab. (2001) have ascertained in the salad plants grown on the soil polluted with Cd, a decrease of the content of chlorophyll pigments (respectively chlorophyll a) with 42% in the variants with 50 ppm Cd.

Linger and collab.(2005) show the fact that in *Cannabis sativa*, the treatment with Cd in a concentration of 72 mg/kg determines the chlorosis of leaves because of the oxidative stress, negatively influences the chlorophyll synthesis and the photosystem 2.

In *Medicago sativa*, the treatments with Pb (50ppm) and Cd (0.1 and 1ppm) determine the growth of the a and b chlorophyll content and total assimilatory pigments, compared with the control [8].

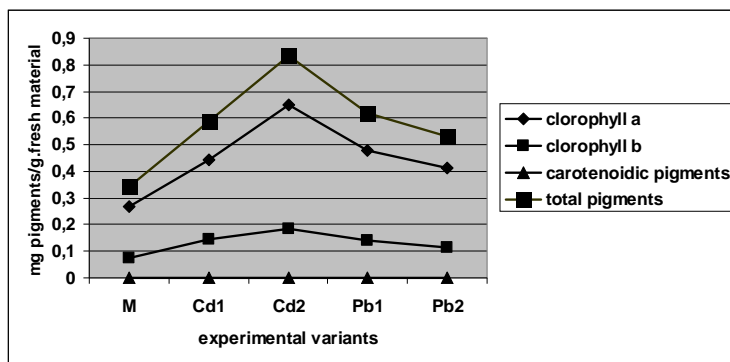


Figure 4 The content of assimilatory pigments

The measurements carried out regarding the length of vegetative organs from the seedlings of *Cannabis sativa*, have emphasized the following aspects.

In comparison with the control in the treatment variants, the root and stem length register higher values. The treatment with Cd determines a slight stimulation of the root length growth and the one with Cd 1 ppm and Pb slightly stimulate the growth of stem length. Regarding the length of leaf, we ascertain small value variations between the control and the treatment variants (fig. 5).

Linger and collab. (2005) show the fact that in *Cannabis sativa*, the treatment with Cd (in concentration of 17 mg/kg sol and 72 mg/kg sol) inhibit the growth of plants.

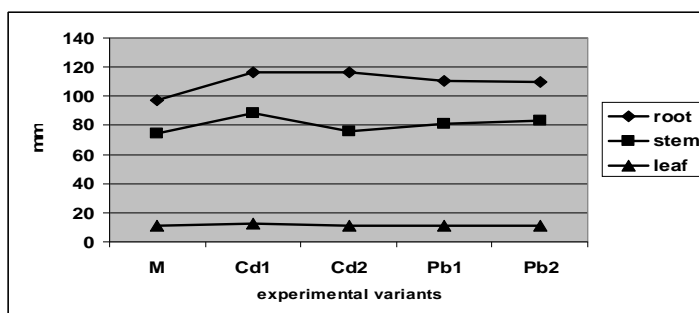


Figure 5 The length of vegetative organs of seedlings

CONCLUSIONS

The results obtained emphasize specific value variants of the indicators analyzed according to the nature of metals and their concentrations used for the treatment of seeds.

Cd in concentration of 1 ppm determines the intensification of respiration process, indicator for emphasizing the metabolic activities.

No matter the concentration, Pb and Cd determine the growth of the assimilator pigment content, more obvious in the case of a chlorophyll and specifically stimulate the growth of seedlings.

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