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STUDY OF HEAVY METALS EFFECTS ON *IN VITRO* CULTURES OF *SEDUM TELEPHIUM* SSP.*MAXIMUM* L.

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Abstract: *In this study we aim by using plant biotechnology to study the effect of heavy metals Cd and Pb on the regeneration capacity of Sedum telephium ssp. maximum L. plant species that grows spontaneously in our country and can also be cultivated for ornamental purposes. The results obtained at different concentrations of each metal will be compared to establish, from a morphological point of view, the existence of a dose-response relationship. According to the rules of the USA Energy Department, the hyperaccumulator plants have to fulfill the following characteristics: rapid growth and large biomass; pest and disease resistance; inedible for humans and animals; easy to harvest; very branched root system; accumulation of different types of heavy metals. Following preliminary results (in vitro and ex vitro) we considered that this species fulfils the above conditions.*

Keywords: *heavy metals, Sedum, in vitro, phytoremediation.*

1. Introduction

Plant tissue culture is a convenient laboratory instrument for phytoremediation studies. The most used forms of tissue culture are cell suspensions, calluses and organogenesis [1]. Once established, these *in vitro* cultures can be propagated indefinitely and are available on request [2]. Whole plants however, are cultivated in soil or grown in hydroponics, the systems have a limited life span and each individual plant has to be replaced and re-established after each experiment. Thus, the time for investigations can be substantially reduced using tissue culture instead of whole plants [3]. Therefore, in this study we aim by using plant biotechnology to study the effect of heavy metals Cd and Pb on the regeneration capacity of *Sedum telephium* ssp. *maximum* L. Sedum gender belongs to the Crassulaceae family [4]; [5] and consists of almost 400 species with succulent leaves. *Sedum telephium ssp. maximum* (L.) Krock is frequently spread in the Romanian flora as a spontaneous species, as well as an ornamentally cultivated species. More than that, the Romanian traditional medicine considers that this plant might have therapeutic (vulnerary, antiseptic, wounds) effects. In the middle of the sixteenth century, Hieronymus Bock reported that extracts of *Sedum telephium ssp. maximum* were used in the Rhine valley to treat internal injuries like lung ulcers [6]. Now today, medical researchers isolate the active ingredients from those traditional medicine plants and test their efficacy [13]. In the early 1990's, some researchers in Munich have identified two polysaccharides in *Sedum telephium ssp. maximum* that were anti-inflammatory [7]. A few years later, some italian scientists observed the ways in which the polysaccharides and flavonols operated on

cells during wound healing [14]. The vegetable from the *in vitro* culture is part of modern biotechnology industry that focuses on various areas, including a special interest in plant biotechnology presents, that *in vitro* cultivation of physiotherapeutic interest [8]. In general, many herbs micro propagated *in vitro* were used as starting material in the popular culture media that are filled with bioreactors, and the biomass collected from a number of days in vitro culture pass extraction and condition the compounds of pharmaceutical interest [9].

2. Materials and methods

2.1 Plant Material, composition of the growth medium and the vitro culture

The growth substrate used for all vitro culture experiments was made from agarised *Murashige - Skoog* (1962) (MS) base medium [10] consisting of macroelements, Fe EDTA and microelements, mineral blend according to the original formulation with the addition of 100 mg/l m-inositol, 30 g/l sucrose and 10 g/l agar-agar [11]. There was no addition of growth regulators (cytokinins or auxins) to this base medium. The added heavy metals were $CdSO_4$ and $PbCl_2$. The growth medium variants made during the experiments and presented in the order in which they were performed as well as the concentration of the heavy metals added to the culture medium are shown in table 1.

Prior to the sterilisation of the growth medium it's pH was adjusted to 5.5 with hydrochloric acid or sodium hydroxide depending on the basicity or acidity of the final medium [12].

15 ml of medium were introduced in culture containers made of colorless and thermoresistant glass, 8 cm high and 4 cm in diameter.

For autoclaving the containers used in all experiments, after portioning the growth medium, were coverd in aluminium foil. The sterilisation of the containers with the growth media was performed in an autoclave for 30 minutes at 121°C and 1 atm [15].

The plant material used to initiate the vitro cultures was represented by 2 cm long side shoots with 1-2 nodes plus and apical bud, taken from *Sedum telephium* ssp. *maximum* L plantlets regenerated from the zygotic embryos of seeds germinated for 30 days on *Murashige - Skoog* (1962) growth medium without growth regulators.

The seeds from which the explants germinated were sterilised in a 0.1 % sodium hypochlorite solution diluted with sterile water (1:2) with 2 – 3 drops of Tween 20 added to 150 ml disinfectant solution [15].

The containers with the inocula were transfered in the growth chamber, placed on shelves and exposed to a temperature between $23^0C \pm 2^0C$ during the light period and $20^0C \pm 2^0C$ during dark and a photoperiod of 16 h light/24 h. The light intensity was 1700 lux (Osram white fluorescent tubes; wavelength 590nm; dimensions: Lx590 mm Øx26 mm) [15].

2.2 Statistical Methods applied in the interpretation of the results

The results were expressed in averages ± standard error. To evaluate the statistically significant differences between the treatments, the averages were compared by variance analysis (ANOVA). The data was tested for the normality and homogeneity of variance using the Levene test. When the results were statistically significant, a multiple comparison post-hoc Tukey ($p \leq 0.05$) test was used. The software used for statistical analysis was IBM SPSS v20.

Table 1.

General scheme for the organisation of vitro culture experiments

Nr. crt.	Experiment type	Experimental variants code	Compoziţion of *MURASHIGE – SKOOG* (1962) growth media and the type of heavy metal	Heavy metal concentration	Vitro culture duration
1.	**Germination of** *Sedum telephium* ssp. *maximum* L. seeds in aseptic conditions on *Murashige - Skoog* (1962) culture medium without growth regulators.	-	*Murashige – Skoog* (1962) Base medium No growth regulators	-	30 days
2.	**Initiation of** *Sedum telephium* ssp. *maximum* L. vitro cultures from apical cuttings from regenerated plantlets from zygotic embryos on the 30th day of vitro germination on *Murashige - Skoog* (1962) culture medium.	**V_0** (control)	*Murashige – Skoog* (1962) Base medium No heavy metals	-	30 days
		V_1	*Murashige – Skoog* (1962) Base medium with **$CdSO_4$**	50 ppm	
		V_2	*Murashige – Skoog* (1962) Base medium with **$PbCl_2$**	50 ppm	
		V_3	*Murashige – Skoog* (1962) Base medium with **$CdSO_4$**	25 ppm	
		V_4	*Murashige – Skoog* (1962) Base medium with **$PbCl_2$**	25 ppm	

3. Results and discussion

The cultivated vitro plantlets were monitored every 7 days for 4 weeks. The biometrization of the qualitative and quantitative characters of vitro plantlets from this study consisted in making the following observations:

• measurements on the aerial system (vegetative) of plants made with a ruler (in cm)
- average stalklet length;
- number of branches at the base;
- average length of the branches;
- number of leaflets;
- average length of the leaflets;
- average width of the leaflets.

• measurements on the root system of plants made with a ruler (in cm):
- number of rootlets;
- total length of the rootlets.

The observations made during the 4 weeks of *in vitro* cultivation on the *Murashige - Skoog* (1962) (MS) growth medium allowed us to assert that the development of *Sedum* plantlets was positively influenced by the presence of the heavy metals Pb ($PbCl_2$) and Cd ($CdSO_4$) in the growth medium. The average length of the stalklet at 28 days was significantly greater compared to the control (V_0) especially for the plantlets grown on *Murashige - Skoog* (1962) (MS) growth medium supplemented with Pb, variant V_4 (Fig. 1). Regarding plant growth through formation of lateral branches, the plants grown on V_4 – $PbCl_2$ variant, the concentration of 25 ppm has recorded higher values compared to the V3 – $CdSO_4$ variant of the same concentration with both variants having significantly smaller branches compared to the control. However, after 28 days the

vitro plantlets grown on *Murashige - Skoog* (1962) (MS) medium supplemented with 25 ppm $CdSO_4$ (V_3) had a greater number of grown leaflets compared to the V_4 – $PbCl_2$ variant but significanlty smaller when compared to V_0 (Fig. 5).

The average length of the leaflets for V_4 variant has reached the highest value compared to the rest of the experimental variants followed by V_2, being able to notice the fact that the addition of 25 ppm $PbCl_2$ to the growth medium has stimulated the formation and development of the leaves.

Thus, it can be observed that the accumulation of metal stimulates the growth in length of the plantlets, the length and width of the leaves and especially the rhisogenesis (Figs. 2, 3, 5 and 8).

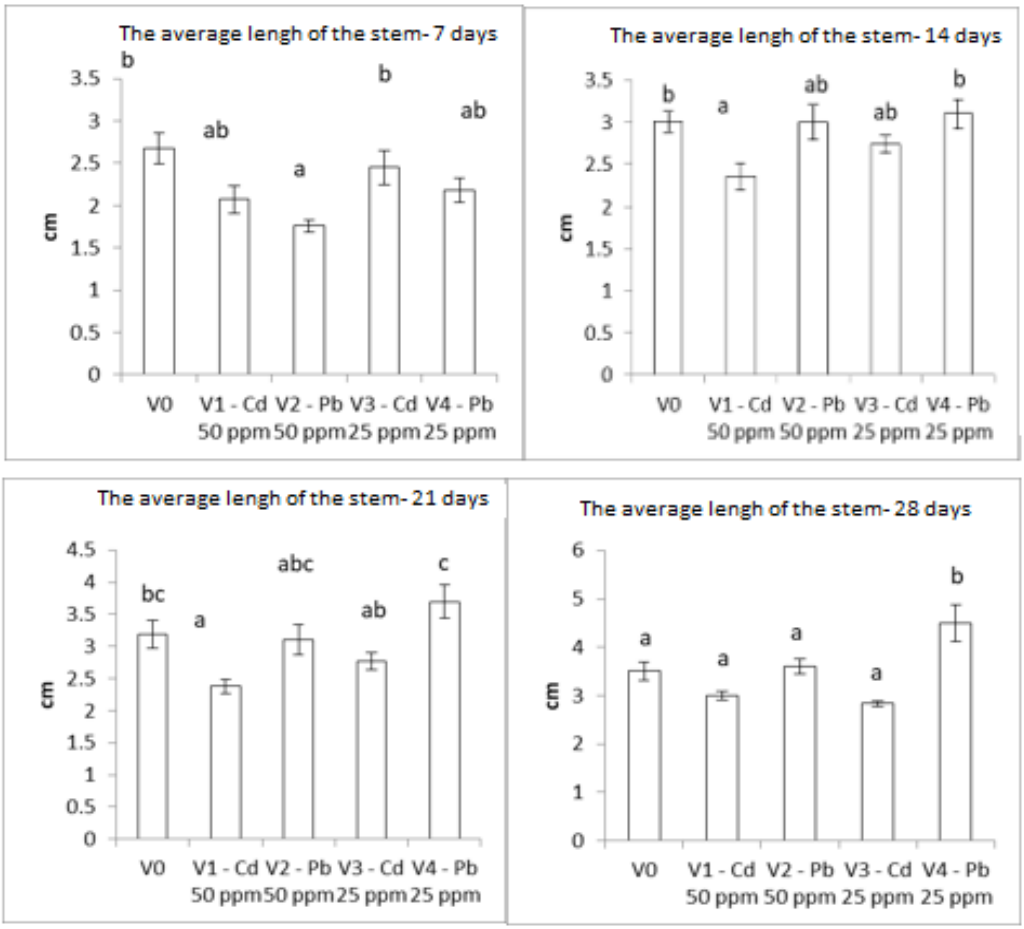


Fig. 1. Graphic representation of the average values corresponding to the average length of the stalklet of the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium with no added growth regulators (V_0 – control) and of those grown on ***Murashige – Skoog*** **(**1962) base medium supplemented with $CdSO_4$ and $PbCl_2$. Note: the values represent the average ± standard error (n = 10). The different letters represent significant differences ($p<0,05$).

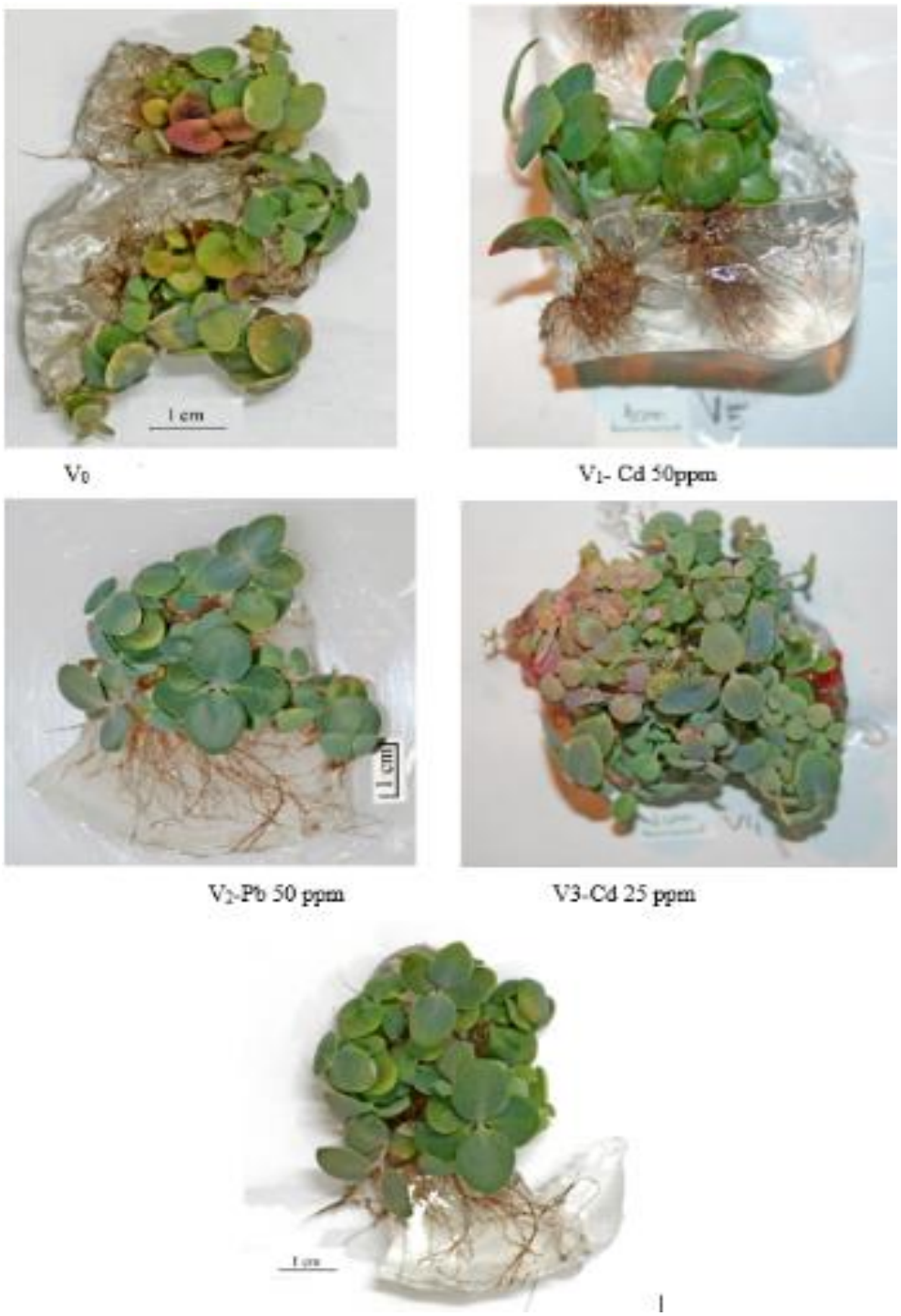
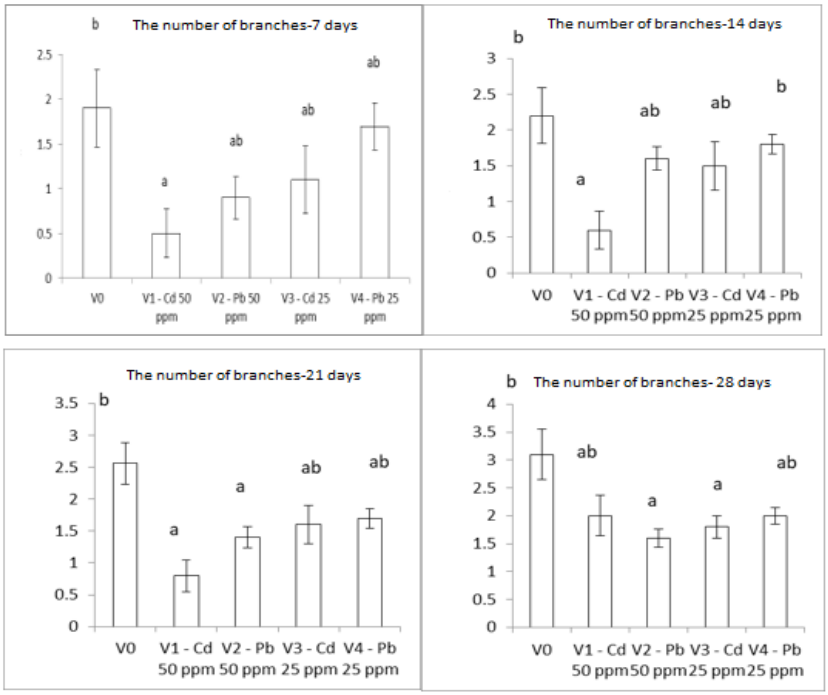
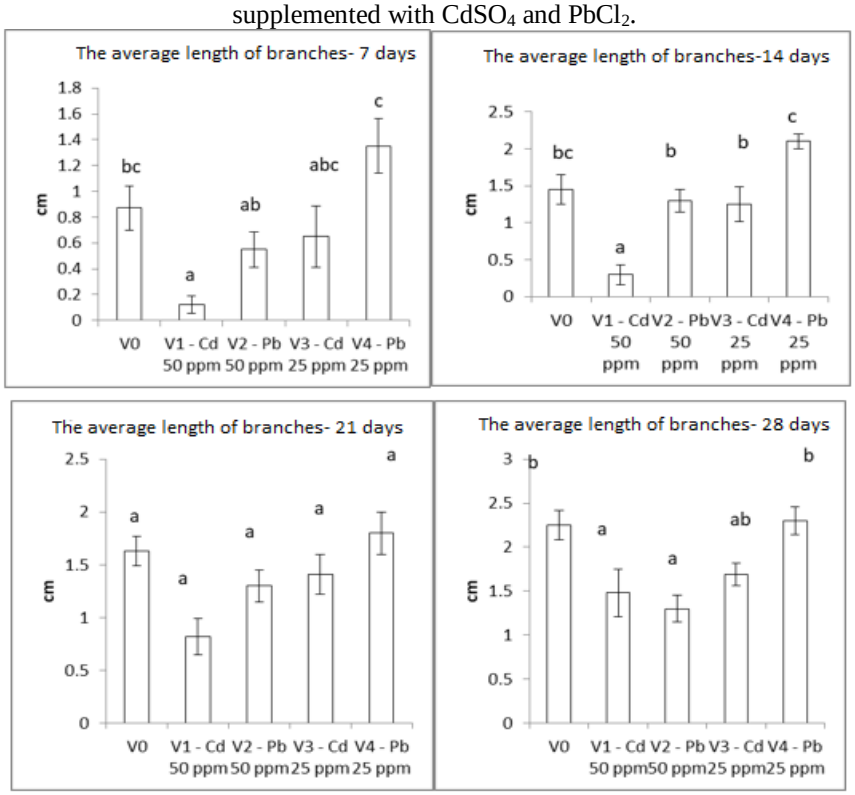


Fig. 2. Morphological aspects after 28 days of *in vitro* culture of plantlets grown on culture media without heavy metals (V_0 – control) and of those grown on media with heavy metals (V_1 – V_4).



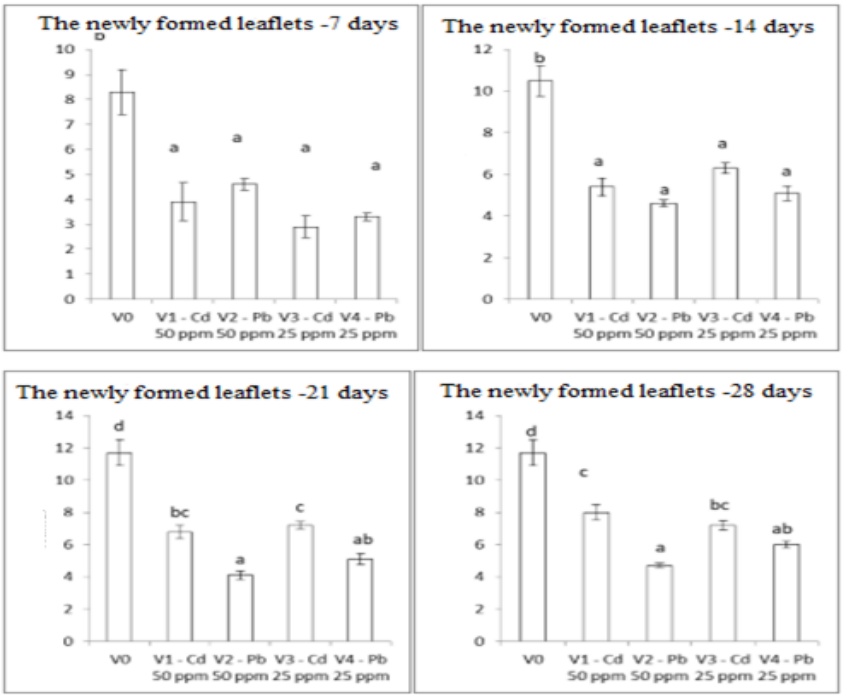
Note: the values represent the average ± standard error (n = 10). The different letters represent significant differences (p<0.05).

Fig. 3. Graphic representation of the average values corresponding to the number of branches for the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium without growth regulators (V_0 – control) and of those grown on ***Murashige - Skoog*** (1962) (MS) base medium supplemented with $CdSO_4$ and $PbCl_2$.



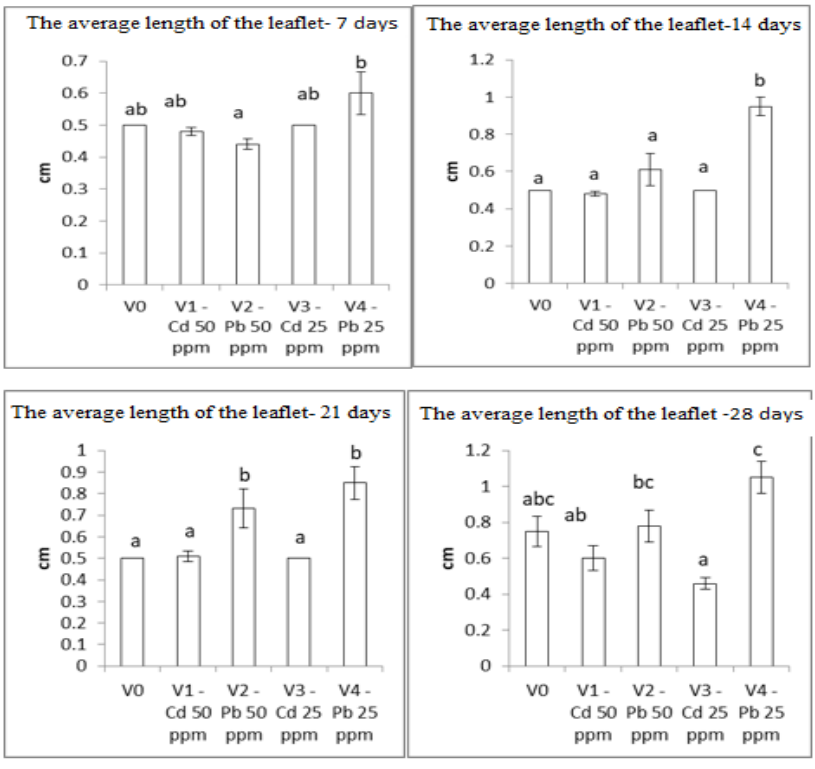
Note: the values represent the average ± standard error (n = 10). The different letters represent significant differences (p<0.05).

Fig. 4. Graphic representation of the average values corresponding to the average length of branches for the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium without growth regulators (V_0 – control) and of those grown on ***Murashige - Skoog*** (1962) (MS) base medium supplemented with $CdSO_4$ and $PbCl_2$.



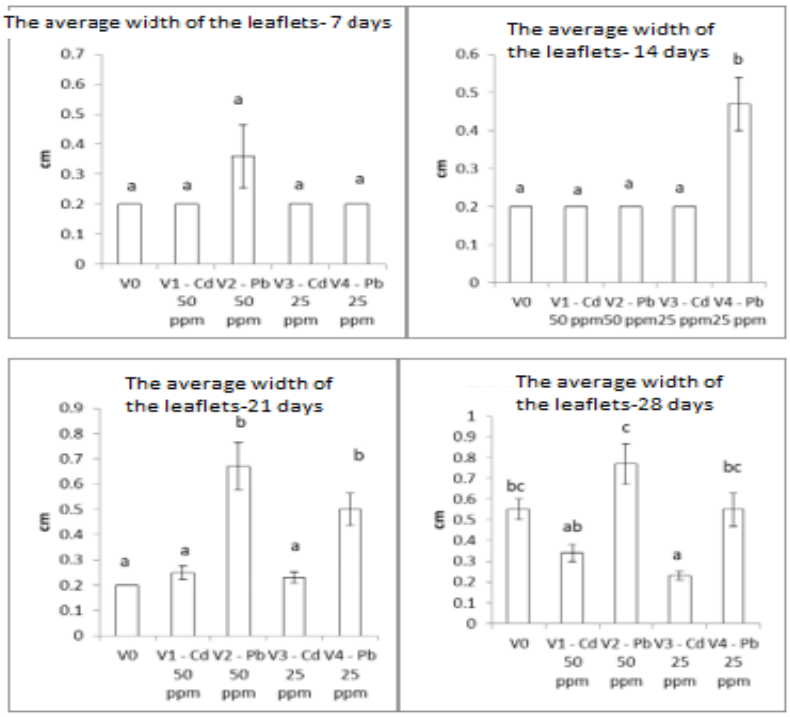
Note: the values represent the average ± standard error (n = 10). The different letters represent significant differences (p<0.05).

Fig. 5. Graphic representation of the average values corresponding to the newly formed leaflets for the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium without growth regulators (V_0 – control) and of those grown on ***Murashige - Skoog*** (1962) (MS) base medium supplemented with $CdSO_4$ and $PbCl_2$.



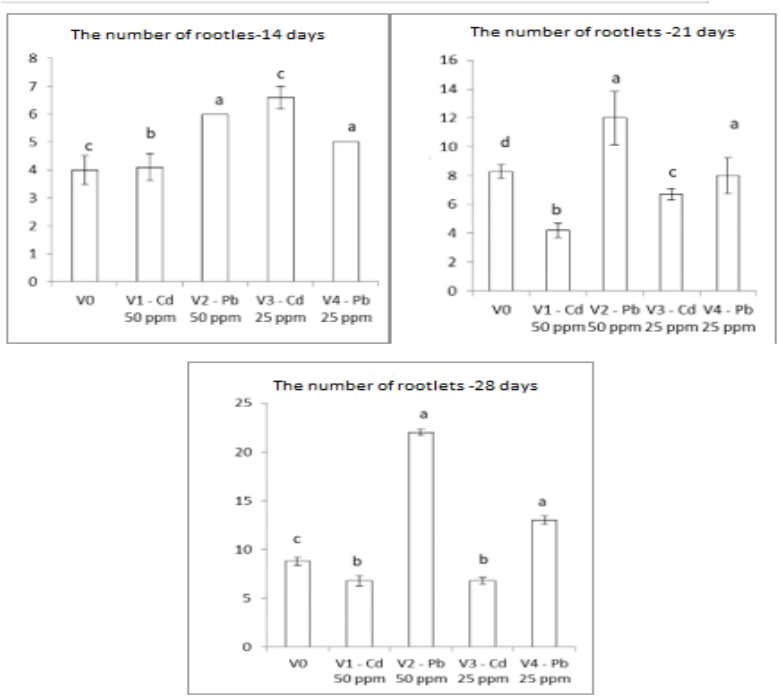
The values represent the average ± standard error (n = 10). The different letters represent significant differences (p<0,05).

Fig. 6. Graphic representation of the average values corresponding to the average length of the leaflet for the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium without growth regulators (V_0 – control) and of those grown on ***Murashige - Skoog*** (1962) (MS) base medium supplemented with $CdSO_4$ and $PbCl_2$.



Note: the values represent the average ± standard error (n = 10). The different letters represent significant differences (p<0,05).

Fig.7. Graphic representation of the average values corresponding to the average width of the leaflets for the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium without growth regulators (V_0 – control) and of those grown on ***Murashige - Skoog*** (1962) (MS) base medium supplemented with $CdSO_4$ and $PbCl_2$.



Note: the values represent the average ± standard error (n = 10). The different letters represent significant differences (p<0,05).

Fig.8. Graphic representation of the average values corresponding to the number of rootlets for the *Sedum telephium* ssp. *maximum* L. vitro-cultures grown for 7 – 28 days on ***Murashige – Skoog*** **(**1962) base medium without growth regulators (V_0 – control) and of those grown on ***Murashige - Skoog*** (1962) (MS) base medium supplemented with $CdSO_4$ and $PbCl_2$.

4. Conclusions

We consider that our results are in agreement with the data presented in the literature stating that heavy metals can induce the reduction of certain morphological features in cultivated plants such as, in our case, the reduction of the number of leaflets and branches. However, the growth was not slowed down, but on the contrary, the growth medium supplemented with the higher concentration of $PbCl_2$ (V_4) has stimulated the plant biomass and the vitro plantlets have not been affected morphologically. This means that the plant has the capacity to tolerate the heavy metal concentrations added to the growth medium and also to accumulate them.

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