# Response of *Chelidonium majus in vitro* cultures and their alkaloid content under heavy metal stress

BalkanBio'2021

**Iva Doycheva[1], Stefan Philipov[2]**

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

## Introduction

Heavy metal contamination is becoming an increasing environmental problem worldwide. Heavy metals (HM) retard plant growth, yield and affect the secondary metabolism which results in fluctuations in plant metabolites, such as the alkaloids. The aim of the study was to investigate the influence of HM on the growth of *in vitro* cultivated plants of *Chelidonium majus* L. (Fig. 1) and their alkaloid content.



**Fig. 1.** *Chelidonium majus* L.

## Materials and methods

The plants were cultivated on a medium B5 with double the amount of macrosalts (2B5), 0.5 g/l activated charcoal and supplemented with different concentrations of HM – $Cd^{2+}$ (0.5; 1 mg/l); $Pb^{2+}$ (100; 250 mg/l); $Zn^{2+}$ (100; 250 mg/l). Plants were obtained *in vitro* from seeds or were propagated through adventitious shooting (AS). The raw alkaloid mixtures (RAM) were obtained by usual chemical procedure.

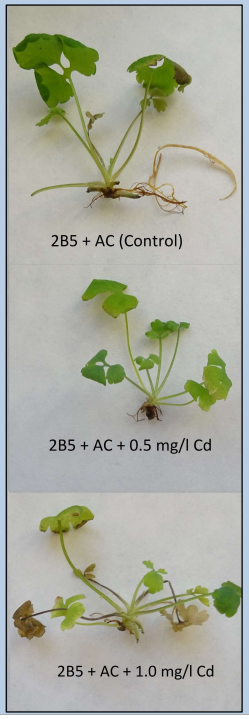


**Fig. 2.** Plants propagated through AS and cultured on 2B5 supplemented with $Cd^{2+}$.

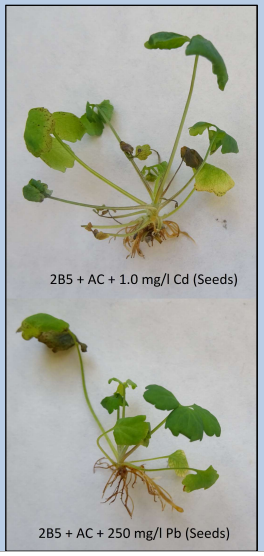


**Fig. 3.** Plants obtained from seeds and cultured on 2B5 supplemented with $Cd^{2+}$ or $Pb^{2+}$.

## Results and discussion

Plants cultivated at the lower concentration of $Cd^{2+}$ formed roots and developed normally with a gradual reduction in their growth and rooting as the concentration of the supplemented HM increased (Fig. 2). There were not any differences in the development of the plants cultivated on $Cd^{2+}$ according to their type of origin (Fig. 2; 3). Epinasty and browning of the leaves were observed among the plants cultivated on the medium supplemented with $Pb^{2+}$. These side effects got worse as the concentration of the HM increased, and were also accompanied by a strong reduction in the rooting (Fig. 4). The plants which were obtained from seeds developed normally and did not show any of the signs of the ones which were propagated through AS (Fig. 3; 4). None of the plants cultivated on a medium supplemented with $Zn^{2+}$ survived, regardless of their origin and HM concentration.

A phytochemical study showed that the plants cultivated at the highest concentrations of $Cd^{2+}$ and $Pb^{2+}$ and also obtained through AS had the highest RAM. However, the plants cultivated at the same concentrations but obtained from seeds had lower RAM similar to that of the plants cultivated at lower concentrations of HM and propagated through AS (Fig. 5).

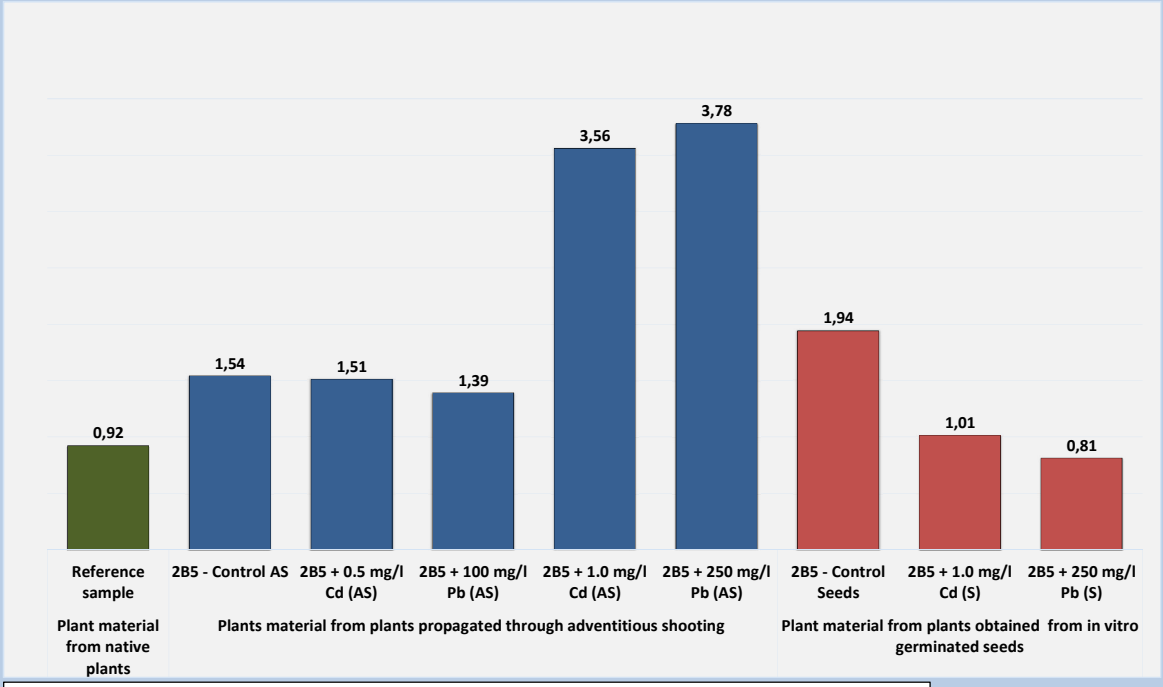


**Fig. 5.** RAM (%) in native plant sample and *in vitro* cultivated plant samples obtained through AS or seed germination.

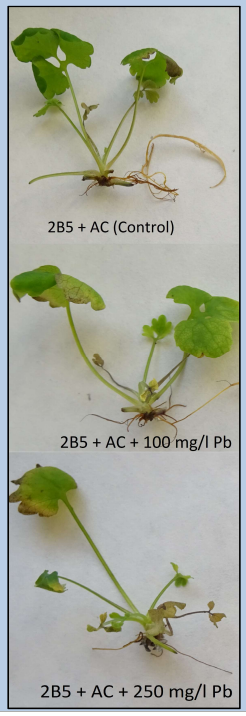


**Fig. 4.** Plants obtained through AS and cultured on 2B5 supplemented with different concentrations of $Pb^{2+}$.

## Conclusions

In conclusion, *Ch. majus* was able to cope with the influence of $Cd^{2+}$ and $Pb^{2+}$ in the culture medium, but not with that of $Zn^{2+}$. Quantity of RAM was influenced by the HM concentration and the origin of the plants.

**Acknowledgements:** This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Project КП-06-М26/4 from 01.12.2018).

For contacts: idoycheva@gmail.com