

## THE AIM OF THE STUDY

❖ In the present study, the suitability of *Chlorella spp.* microalgae for mine drainage (MD) treatment was investigated. The efficiency of heavy metals removal was also evaluated. The toxicity of MD before and after treatment with *Chlorella spp.* on seed germination was assessed using tomatoes, onions, mustard, beans, sunflowers, wheat and corn seeds.

## MATERIALS AND METHODS



Fig. 1 Cultivation of *Chlorella spp.* in MD

❖ *Chlorella spp.* was inoculated at 1:15 (v/v) ratio in mine drainage (MD) when the exponential phase of growth was reached.

❖ The cultivation in MD was carried out for 15 days at a temperature of  $25 \pm 1^\circ\text{C}$ , under artificial illumination of 1200 lux for 16/8 h day/night cycle, at laboratory scale.

❖ The pH, conductivity and metals content (Al, Cr, Fe, Ni, Pb Cu, Zn) were determined every 2 days during 15 days of growth.

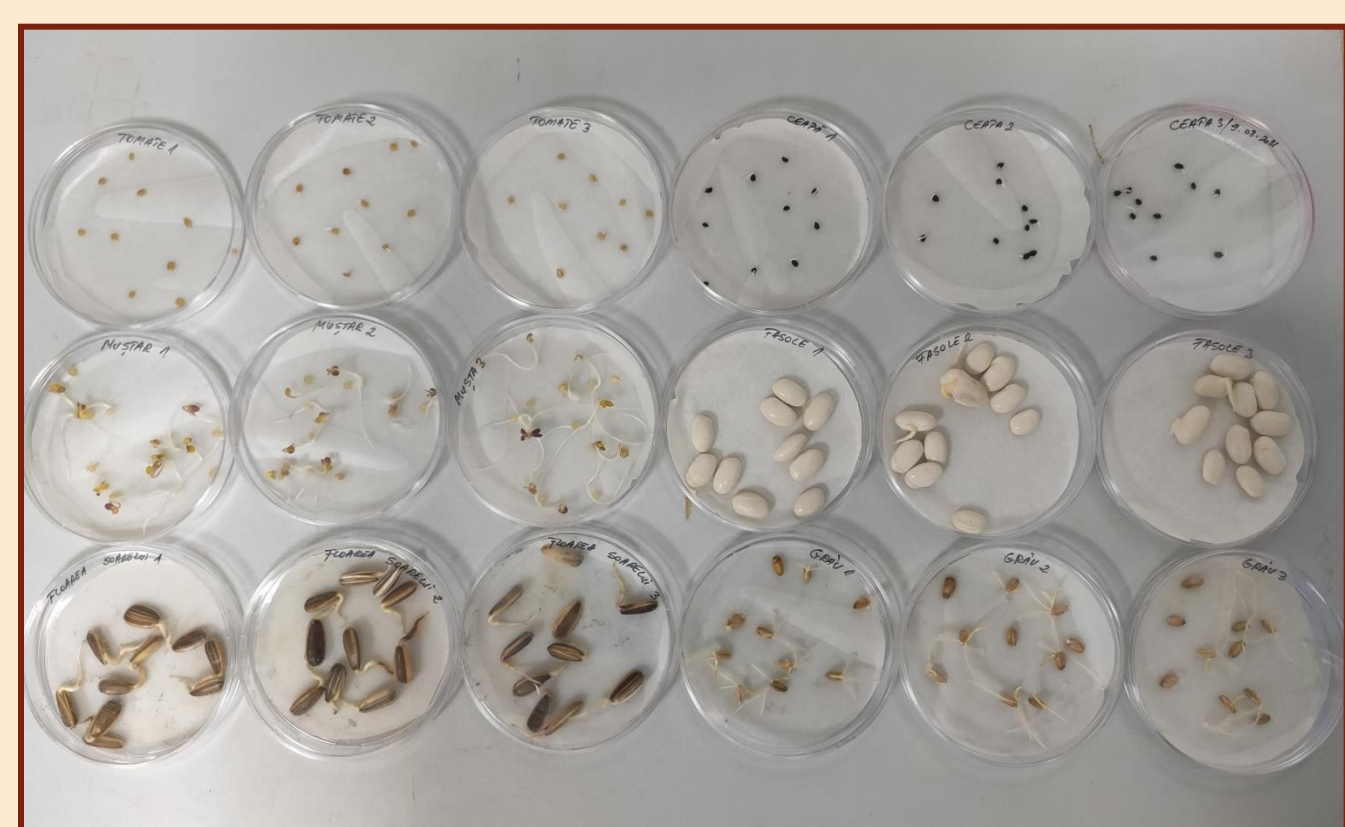


Fig. 2 Phytotoxicity effect of MD on seeds before and after treatment with *Chlorella spp.*

❖ The phytotoxicity effect of MD was studied on tomatoe, onion, mustard, bean, sunflower, wheat and corn seeds before and after treatment with *Chlorella spp.*

❖ Relative seed germination index (RSG), relative root growth index (RRG) and germination index (GI) were calculated after 3 and 6 days.

## RESULTS AND DISCUSSIONS

### Effect of MD treatment with *Chlorella spp.* on physico-chemical parameters and metals removal during cultivation

#### pH and conductivity

Day	pH	Conductivity ( $\mu\text{S/cm}$ )
MD	8.8	186
Inoculation	9.3	591
1	10.6	658
3	10.6	658
5	10.4	660
7	10.4	667
9	10.3	611
11	10.3	605
13	9.9	548
15	9.3	533

❖ The pH of MD used as growth medium for *Chlorella spp.* was slightly alkaline.

❖ The pH value during MD treatment increased up to 10.6 (day 1 of treatment) and remained almost constant until day 13; and further slowly decreased.

❖ The conductivity value remained constant during the first 7 days of treatment and further slowly decreased.

#### Metals removal

- ❖ The average removal efficiencies of metals ranged from 69.1% to 100% (Table 1).
- ❖ The highest removal efficiencies were observed for Pb, Ni, Cr and Fe, with 90.0%, 93.1%, 97.4% and 100%, respectively.
- ❖ The removal efficiencies values remain constant after the day 13 of treatment.

Table 1 Metal removal efficiency under MD conditions after *Chlorella spp.* treatment

Metals (%)	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
Al	59.9	81.2	81.2	81.8	86.1	86.9	88.2	88.3
Cr	89.5	89.5	89.5	90.5	92.1	97.4	97.4	97.4
Fe	12.6	97.1	100	100	100	100	100	100
Ni	58.6	79.3	86.2	86.2	89.7	89.7	93.1	93.1
Cu	21.1	61.4	64.6	66.7	68.4	70.1	74.4	75.4
Zn	6.21	45.7	50.6	54.3	55.6	64.2	70.4	69.1
Pb	56.7	76.7	76.7	76.7	82.0	86.7	90.0	90.0

## RESULTS AND DISCUSSIONS

### Effect of MD and *Chlorella spp.* treatment on the germination parameters

- ❖ The germination traits of seeds (RSG, RRG, GI) presented an upward trend starting with the 3<sup>rd</sup> day of MD treatment with *Chlorella spp.*
- ❖ After 6 days of germination, the value of RSG increased for onion, wheat, bean and tomatoe seeds, ranging from 17% (onion) to 104% (tomatoe) (Fig.3).
- ❖ RRG increased as well for all tested seeds, ranging from 13% (onion) to 62% (tomatoe), with the exception of mustard, which has decreased in value with 60% when MD was treated with *Chlorella spp.* (Fig.4).
- ❖ High values up to 156%, 162% and 230% for beans, wheat and tomatoes, respectively were obtained for GI (Fig.5).

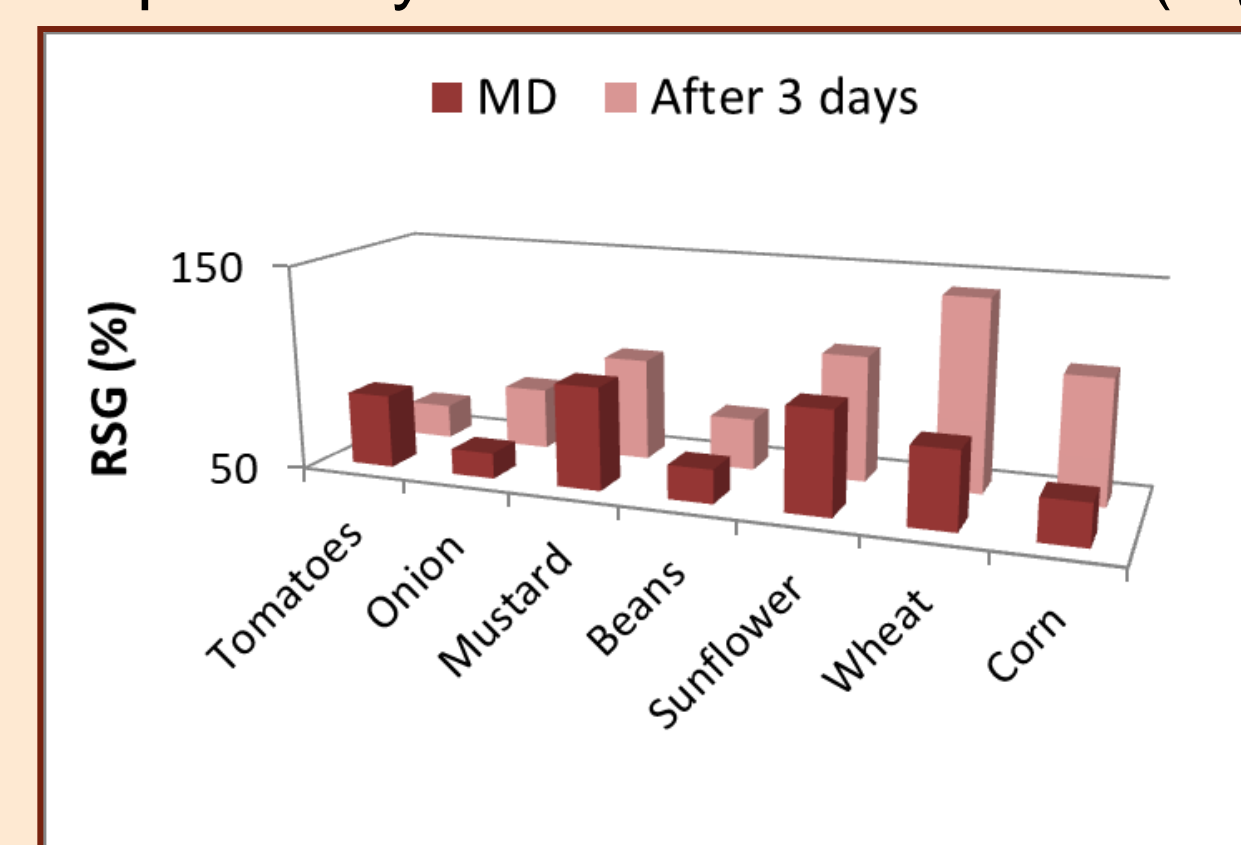


Fig. 3 Relative seed germination – (RSG) (%) under MD conditions and after 3 and 6 days of treatment with *Chlorella spp.*

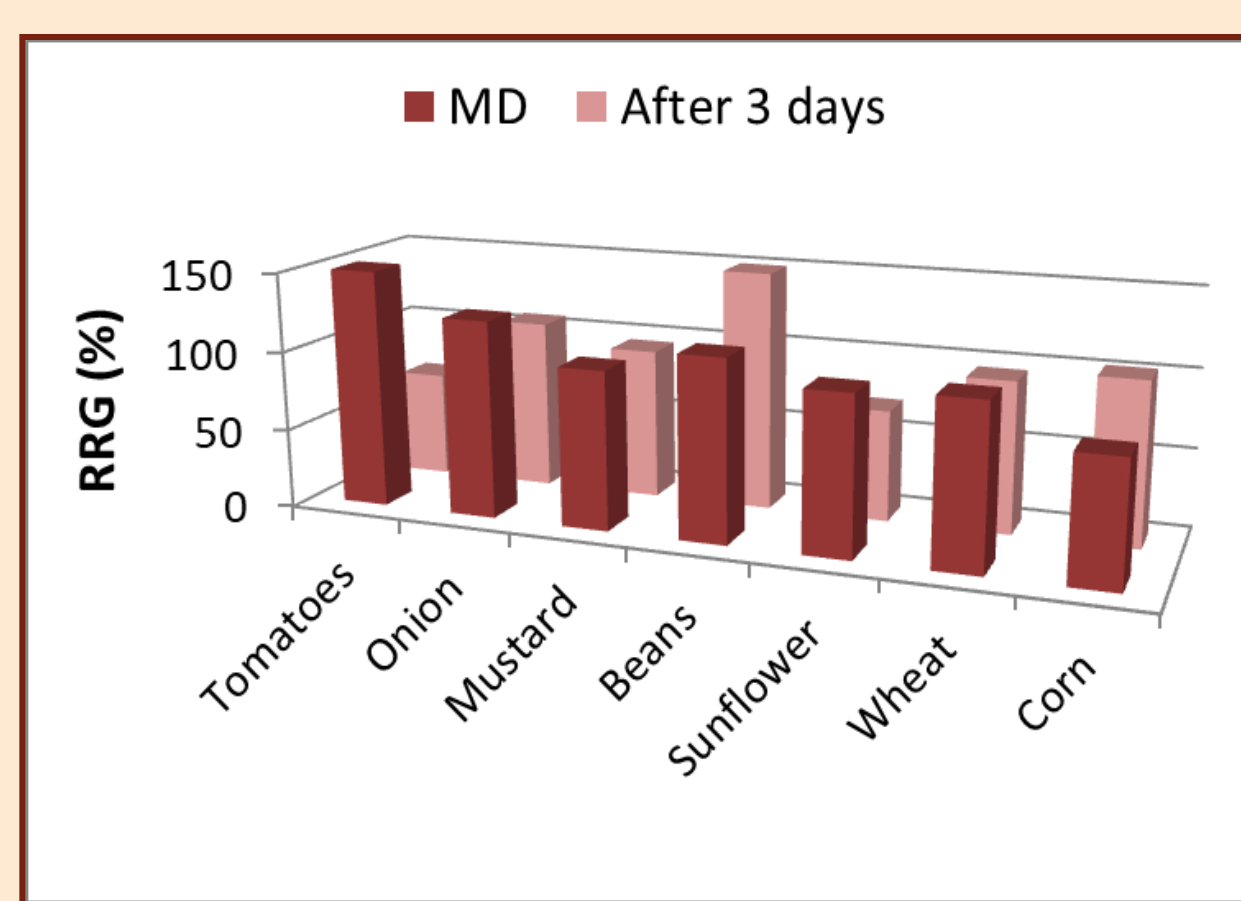


Fig. 4 Relative root growth (RRG) (%) under MD conditions and after 3 and 6 days of treatment with *Chlorella spp.*

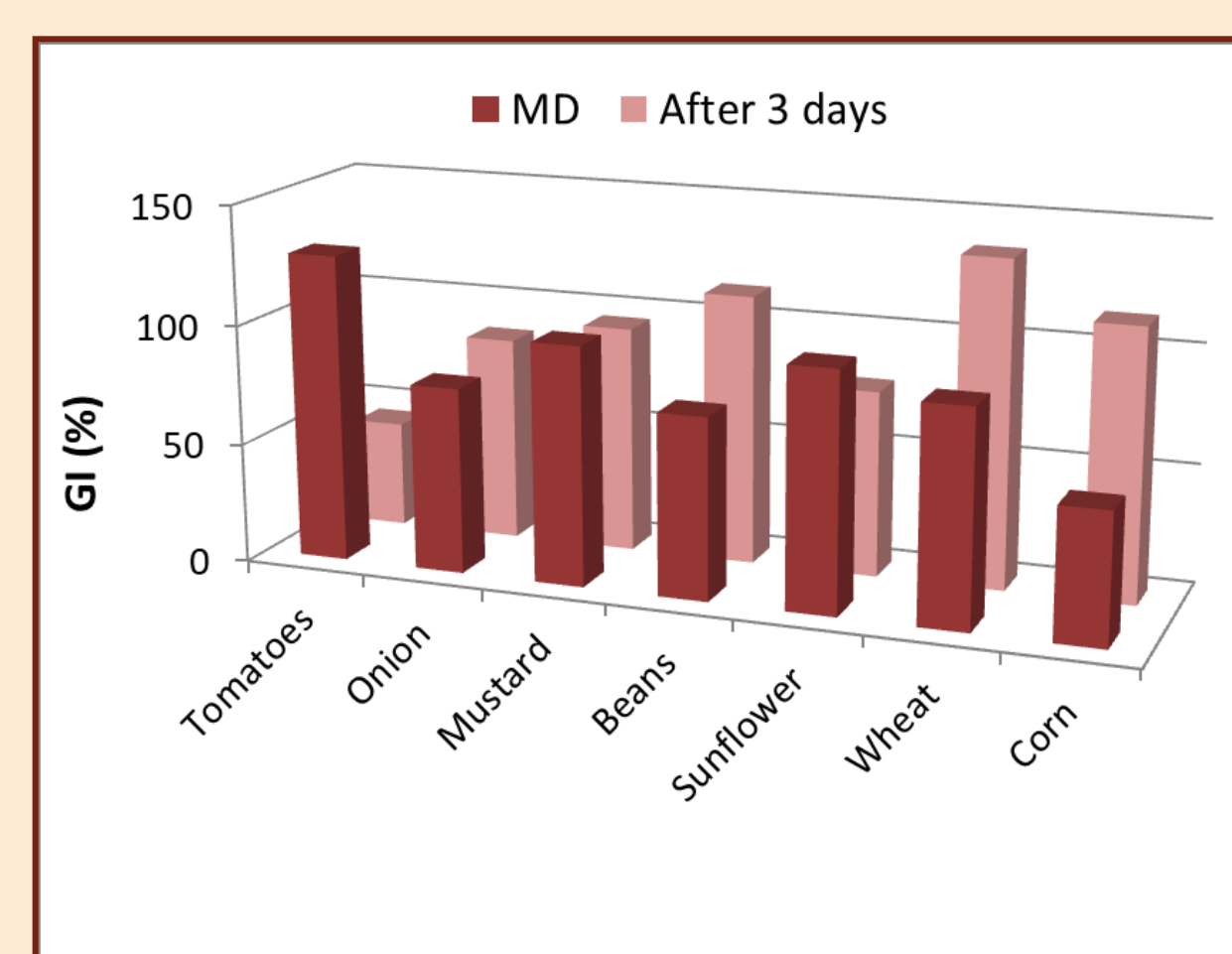


Fig. 5 Germination index – GI (%) under MD conditions and after 3 and 6 days of treatment with *Chlorella spp.*

## CONCLUSIONS

- ❖ The treatment of mine drainage (MD) with *Chlorella spp.* microalgae proved to be efficient, considering both, physical-chemical and toxicological analysis. The results showed high efficiencies for metals removal up to 90.0%, 93.1%, 97.4% and 100% for Pb, Ni, Cr and Fe, respectively.
- ❖ Toxicological studies on seeds revealed high reduction of toxicity after treatment with *Chlorella spp.* microalgae. The RSG, RRG and GI values increased after MD treatment with *Chlorella spp.*

## REFERENCES

- ❖ Bwapwa J K, Jaiyeola A T, Chetty R, 2017. Bioremediation of acid mine drainage using algae strains: A review. South African Journal of Chemical Engineering 24, 62-70
- ❖ Batista dos Santos K, de Almeida V, O, Weiler J, Homrich Schneider I, A, 2020. Removal of Pollutants from an AMD from a Coal Mine by Neutralization/Precipitation Followed by "In Vivo" Biosorption Step with the Microalgae *Scenedesmus sp.*, Minerals, 10, 711
- ❖ Chen X, Zhang R, Xing Y, Jiang B, Li B, Xu X, et al., 2021. The efficacy of different seed priming agents for promoting sorghum germination under salt stress. PLoS ONE 16(1):e0245505
- ❖ Şentürk, T., & Yıldız, Ş., 2016. Adsorbent effect of *Chlorella vulgaris* and *Scenedesmus sp.* (Chlorophyta) for the removal of some heavy metals and nutrients, Turkish Journal of Biochemistry, 41(2)

## ACKNOWLEDGEMENTS

This work was funded by the Romanian National Core Program, project no. PN 19-18.01.01 (contract no. 18N/08.02.2019)