The cup plant (Silphium perfoliatum L.) – a viable solution for bioremediating soils polluted with heavy metals

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Abstract

Heavy metal pollution, manifested by the accumulation, toxicity and persistence in soil, water, air, and living organisms, is a major environmental problem that requires energetic resolution. Mining tailing areas contain metal minerals such as Cu, Zn, Pb, Cr and Cd in high concentrations that pollute the environment and pose threats to human health. Phytoremediation represents a sustainable, long-term, and relatively inexpensive strategy, thus proving to be convenient for stabilizing and improving the environment in former heavy metal-polluted mining sites. This study presents the bioremediation potential of Silphium perfoliatum L. plants, in the vegetative stages of leaf rosette formation, grown on soil polluted with heavy metals from mining dumps in Moldova-Noua, in the Western part of Romania. The bioaccumulation factor (BAF), translocation factor (TF), metal uptake (MU) and removal efficiency (RE) of Cu, Zn, Cr and Pb by S. perfoliatum plants were determined in a potted experiment in controlled environmental conditions. The reference quantities of heavy metals have been determined in the studied soil sample. The experiment followed the dynamics of the translocation and accumulation of heavy metals in the soil, in the various organs of the silphium plants, during the formation of the leaf rosette (13-18 BBCH). The determination of the amount of heavy metals in soil and plants was achieved by the method of digestion with hydrochloric and nitric acid 3/1 (v/v) quantified by atomic absorption spectroscopy (AAS). The obtained experimental results demonstrate that the substrate has a high heavy metal content being at the alert threshold for Zn (260.01 mg kg⁻¹ in substrate compared with alert threshold 300 mg kg⁻¹) and at intervention thresholds for other metals (Cu -234.66 mg kg⁻¹/200 mg kg⁻¹; 299.08 mg kg⁻¹/300 mg kg⁻¹ and Pb-175.18 mg kg⁻¹/100 mg kg⁻¹). The average concentration of the metals determined in dynamics in the dry biomass of plants varied between roots, petioles, and laminas. The root is the main accumulator for Cu and Cr (Cu – 37.32 mg kg⁻¹ – 13 BBCH to 43.89 mg kg⁻¹ – 15 BBCH and 80.71 mg kg⁻¹ – 18 BBCH; Cr – 57.43 mg kg⁻¹ – 13 BBCH to 93.36 mg kg⁻¹ – 18 BBCH), and for Zn and Pb the lamina seems to carry the same function. Preliminary results show that Silphium perfoliatum may be a viable alternative in the bioremediation and treatment of heavy metal-contaminated area.
**Keywords:** bioaccumulation factor; contaminated areas; cup plant; phytoextraction; removal efficiency; translocation factor

**Introduction**

Soil pollution with heavy metals is an environmental problem that manifests itself globally. The presence of heavy metals in soils is mainly due to anthropogenic activities such as mining, steel casting, medical waste, chaotic application of pesticides and fertilizers, use of wastewater for irrigation, burning of leaded gasoline, coal, etc. (Alloway *et al.*, 2000; Yoon *et al.*, 2006; Cao *et al.*, 2009; Huang *et al.*, 2017; Zhang *et al.*, 2017, 2018 a).

Heavy metals have negative effects on the health of humans and animals mainly due to the tendency to bio-accumulate in the food chain and their long-term persistence in the environment (Yoon *et al.*, 2006; Azimi *et al.*, 2017; Shen *et al.*, 2019; Sall *et al.*, 2020).

With the rapid development of mining activities, landscape changes as well as environmental pollution have become even more serious. The intensive mineral extraction has generated a large amount of waste accumulated in waste and dumps (Ma *et al.*, 2015, Demková *et al.*, 2017). Without proper management, the abandoned mines and accumulated residues are the source of heavy metal pollution, which is washed away and can contaminate all environmental components (Liakopoulos *et al.*, 2010; Li *et al.*, 2014, Yang *et al.*, 2018).

Soil contamination with heavy metals because of mining operations is present in Europe on fairly large areas, with an undesirable impact on the environment in particular and the quality of life in general. This is also true for Romania, where mining has been a core economic branch providing more than 1 million jobs in the 14 mining areas located in 16 counties. After 2000, the mining production has dropped dramatically, leaving behind depopulated areas, abandoned mines, waste dumps and tailings ponds with high heavy metal content. Some of the shutdown mines have become, over time, true ‘environmental bombs’ because the funds allocated for closing, rehabilitating the surfaces, and restoring them to the natural/economic circuit have been insufficient ([https://energyindustryreview.com/metals-mining/mining-industry-left-without-miners-lamp/](https://energyindustryreview.com/metals-mining/mining-industry-left-without-miners-lamp/)).

Decanting ponds as well as waste dumps through low stability, high content of metal ions and flotation agents produce disastrous and long-term negative effects on air quality, soils, surface water and groundwater (Hudson-Edwards *et al.*, 2011).

These mining decanting areas contain metal minerals in quite large quantities, the best known of which, acting as pollutants are; arsenic (As), lead (Pb), mercury (Hg), chromium (Cr), zinc (Zn), cadmium (Cd), copper (Cu), and nickel (Ni) (Hansda *et al.*, 2014; Pratush *et al.*, 2018).

In recent decades, numerous technologies have been tested and applied to reduce the large concentrations of heavy metals in the environment (e.g. chemical precipitation and coagulation, ion exchange, reverse osmosis, membrane filtration, soil washing, etc.) but most of these methods are not practical because of the environmental danger, high costs and low efficiency (Gavrilnescu, 2004; Olguín and Sánchez- Galván, 2012; Wang and Sun, 2013; Martin-Lara *et al.*, 2014; Hanif and Bhatti, 2015; Choinska-Pulita *et al.*, 2018).

The growing need to remediate contaminated, safe and low-cost sites has led to the search for solutions through the development of green, cost-effective and environmentally friendly biotechnology, such as phytoremediation, which relies on the potential of some plant species to extract, fix and detoxify metal pollutants (Nowack *et al.*, 2006; Ali *et al.*, 2013; Pandey *et al.*, 2015; Rezania *et al.*, 2016).

Heavy metals present in high concentrations in the surface layer of soil directly influence the growth and development of plants in the affected areas (Zhang *et al.*, 2018b). Like other stressors, heavy metal toxicity induces oxidative stress and modulates the expression of genes involved in stress tolerance (Shri *et al.*, 2009; Tripathi *et al.*, 2012; Dubey *et al.*, 2018).

Considering the acute toxicity of some heavy metals, it is necessary to develop quickly cheap, efficient, sustainable and sustainable methods that ensure the accumulation, dissipation, immobilization and degradation of pollutants by the use of plant organisms. These processes are based on phytoremediation
techniques (phytoextraction, rhizofiltration, phytovolatilisation, phytostabilization, hydraulic control, rhizodegradation and phytodegradation of heavy metals).

Thus, the identification of plant species with the ability to tolerate (and concentrate in their biomass) the high levels of these heavy metals becomes an important factor in phytoremediation studies (Yu et al., 2013; Singh et al., 2015).

To date, more than 500 species of plants capable of accumulating heavy metals have been identified (Baker and Brooks, 1989; Kramer, 2010; Hemen, 2011). Most of these are extremely specific to a particular type of metal, have low accumulation capacity, slow growth speed and thorough care technologies for multiplication (Gleba et al., 1999), thus making them unsustainable for large-scale use.

The presence of several types of heavy metals in the same soil determines their interaction with combined ecological effects, complicating the phytoremediation process (Fang et al., 2012). The identification of plant species with high biomass accumulation intensity, together with the ability to tolerate and accumulate several types of metals, is an essential feature of research into phytoremediation of soils polluted with heavy metals (Yu et al., 2015).

There are numerous studies in the literature showing different plant species with the ability to accumulate heavy metals from polluted soils. Starting with Solanum nigrum (Wei et al., 2010), Linum usitatissimum (Bjelkova et al., 2011), Pinus silvestris (Ostrowska et al., 2006), Ricinus communis (Zhi-Xin et al., 2007) to grasses (Zhang et al., 2010) and brassicacea (Palmer et al., 2001) accumulating cadmium (Cd) la Allicium fistulosum, Pteris cretica (Cho et al., 2009), Ricinus communis (Zhi-Xin et al., 2007), Phragmites australis (Weis and Weis, 2004) and Amorpha fruticosa (Shi et al., 2011) as lead accumulators (Pb), or Brassica Rapa (Meers et al., 2005) and Vetiveria zizanoides (Andra et al., 2009) zinc accumulators (Zn) there are numerous species whose bioremediation potential is still insufficiently known (Chirakarra et al., 2016).

Among these species, presented as a potential hyperaccumulator, is Silphium perfoliatum (the cup plant). It is known as the perennial herbaceous plant, with high capacity to accumulate biomass and high tolerance to abiotic stressors, extremely versatile and adaptable to extreme environmental conditions (Jucor and Sumalan, 2018). Figas et al. (2015) state that there are some studies confirming the potential of use of the species for the rehabilitation of degraded land, by phytoremediation (Klimont, 2007; Majtkowski, 2010). It can also be used as a potential species for the phytostabilization of cadmium in soil (Zhang et al., 2010). We note that the most important use of silphium biomass is as a source of renewable energy through the production of biogas.

Silphium perfoliatum L. (cup plant; rosinweed, silfie) is a multiannual herbaceous plant of the genus Silphium family Asteraceae, native to the North American prairies. In the first year it forms a rosette of leaves with a diameter of 60-70 cm that covers the soil almost completely. In the 2nd year the plant forms multiple stems, floriferous, 1.2 to 3 m with 8 to 14 pairs of leaves arranged alternately at intervals of 10-18 cm. On the island of the alternating leaves is concreted, forming a cup that the stem seems to pierce, hence the alternative colloquial name: the cup plant.

The objective of our study was to evaluate the adaptability and phytoremediation capacity of S. perfoliatum L. plants grown on soils polluted with heavy metals, from the dumps of the former mines in Moldova-Noua in the Western part of Romania. The distribution of heavy metals extracted in the root, petiole and the leaf tongue were analyzed, quantified, and compared with respect to bioaccumulation (BAF), translocation (TF) factors, as well as the metal uptake (MU) and removal efficiency (RE), of heavy metals.

**Materials and Methods**

**Description of the study site**

Moldova Noua is a settlement in Romania on the territory of which there is an old, abandoned copper mine. The remaining mining residues cause important environmental problems due to pollution.
Soil samples were collected (0-30 cm depth) from a mining area situated in Moldova Noua (44° 44’ 15.00” N/ 21° 40’ 09” E) in the southwest of Romania. The soil used for testing originates from the agricultural land in the immediate vicinity of mining tailing sites resulting from the exploitation of copper. 100 kg of soil were collected from 10 different points located on an area of 5000 sq meters. The collected soil was packed and transported, then it was homogenized and prepared for the initial analyses.

**Soil samples pretreatment**

The soil samples prepared for analysis were ground fresh repeatedly, after which they were air-dried under a sterile and moisture-free condition for further processing. The samples were sieved through a 2-mm mesh screen and separated manually in representative quantities (3g) for the initial analyses of heavy metal content. This preparation and storage of soil samples for future chemical analyses were carried out according to ISO 11464/2006 ([https://standards.iteh.ai/catalog/standards/iso/63199580-e1a0-4537-a141-ab923c298e27/iso-11464-2006](https://standards.iteh.ai/catalog/standards/iso/63199580-e1a0-4537-a141-ab923c298e27/iso-11464-2006)).

The pot culture experiment was conducted in the laboratory and greenhouse of the Plant Physiology Department, Banat’s University of Agricultural Sciences and Veterinary Medicine “King Michael I of Romania” from Timisoara and soil and plant tissue analyses were made in the Laboratory of Environmental Analysis, Research Institute of Renewable Energies, Politehnica University of Timisoara, in spring-summer 2020.

**Determination of heavy metal concentration in soil samples**

The soil samples homogenized through coning and quartering were dried at 75 °C for 48 hours and afterwards they were ground to obtain a fine powder. The soil dried and sifted samples were treated with a solution of hydrochloric acid (HCl) and nitric acid (HNO₃) in a ratio of 3:1 (v/v). The resulting solution was then cooled, filtered, and diluted with 25 ml of distilled water. The digested liquid was filtered through paper Whatman No. 0.5, and the total heavy metal content of the filtrate was analysed by AAS technique ([https://www.iso.org/standard/24010.html](https://www.iso.org/standard/24010.html)).

**Biological material**

The *Silphium perfoliatum* L. of the Asteraceae family used for this study is a high-biomass, tolerant to abiotic stress. The nomenclature of the cup plant species conforms to the online resource, IPNI (2020). The seeds used for growing the plants origin from the collection owned by the Plant Physiology Department, Banat’s University of Agricultural Sciences and Veterinary Medicine “King Michael I of Romania” from Timisoara.

**Experimental procedures**

Before being germinated, the seeds were layered by treatment with low temperatures (3-4 °C for 4 weeks) to interrupt the longing, then were sterilized in 10% NaOCl solution for 20 min to prevent the apparition of fungi, and then washed several times with distilled water. The seeds were soaked for 24 hours in distilled water for initiating the pre germination process and then they were directly sown in pots with 8 cm on substrate Bio Plantella Start (black peat – 50%; humanic white peat – 50% organic fattening – 4 kg cm⁻²; N – 1300-1800 mg kg⁻¹; P₂O₅ – 150-250 mg kg⁻¹; K₂O – 200-300 mg kg⁻¹, pH 6.2 – 6.8), produced by Unichem DOO -Slovenia. The pots were placed, until the appearance of the first leaf, in the growth chamber model WiseCube WTH E305 (Witeg Labortechnik GmbH, Germany) at the constant temperature of 20 °C, air relative humidity – 80%, 12/12 light/dark; the soil humidity has been kept at optimal values by daily watering.

After the seedlings had risen, 120 of them were moved to a greenhouse (26±3 /18±3 °C, day/night; 65 ± 5% air relative humidity and between 10 to 16 hours of light), and at the formation of the second leaf (12 BBCH) ([Meier et al., 2009](https://www.iso.org/standard/24010.html)), 60 plants, uniform in terms of growth and development stage, were transplanted into 2-litre capacity pots filled with soil polluted with heavy metals, collected from the mining area Moldova.
To avoid the loss of mineral elements by leaching, trays were placed under each plant pot and the collected solution was reintroduced into the circuit.

After 10 days of transplantation, with the formation of the third leaf (13 BBCH) analyses were carried out on the content of heavy metals in soil and plants (root, petiole, and leaf limb). Also, the fresh mass of the plant organs and the amount of dry matter were determined, by drying in the oven (Universal Oven UmUF 75m plus, Memmert GmbH & Co. KG, Germany) at 105 °C for 2 h and then continuously at 80 °C until achieving a constant dry weight. The following determinations and analyses were made in developmental stages 13 and 15 BBCH, i.e. at 3 and 5 leaves. Throughout the experiment the plants were tended and watered to maintain an optimal level of soil humidity (approx. 70% from field capacity).

The dried plants organs (roots, petioles, and laminas) were calcined to remove their organic part, for the purpose of their subsequent analysis.

The weighed plant material, placed in the molten pot, was heated to carbonization, 550-600 °C, for 5-6 hours, after which the melting pot was allowed to cool in the desiccator, and then the melting pot was weighed again with the resulting ash after carbonization of the plant. The difference between the initial mass of the plant and the mass remaining after calcination represents the loss at calcination.

The dry soil and ash samples resulted from the calcination of the plants samples were extracted with an acid mixture (aqua regia) made by combining concentrated hydrochloric acid and nitric acid in a volumetric ratio 3:1.

Samples of approximately 3 g of soil or ash to the nearest 0.001 g were placed into a 250 mL reaction flask and moistened with approximately 0.5 - 1 mL of water. A quantity of 21 mL of concentrated hydrochloric acid was added, followed by 7 mL of concentrated nitric acid. The sample was allowed 16 h at room temperature to permit slow oxidation of the organic matter in the soil. Afterwards, a slow raise of the temperature of the reaction mixture was allowed, until the reflux conditions were reached and maintained for 2 h, ensuring that the condensation area was less than 1/3 of the height of the refrigerant, and then allowed to cool. The contents of the absorption bottle were placed into the reaction flask through the refrigerant, rinsing both the absorption bottle and the refrigerant with a further 10 mL of nitric acid. The insoluble residue in the reaction flask was let to settle. The relatively free supernatant of the sediments obtained by decanting through a filter paper collecting the filtrate was carefully passed in a 100 mL volumetric flask. All the initial extract from the reaction flask was passed through the filter paper, and then the insoluble residue on the filter paper was washed with a minimum of nitric acid and collect this filtrate with the first.

The metal ion content in the filtrate were determined by AAS method (Varian SpectrAA 280FS, Agilent Technologies Inc. USA) according with ISO 11047 / 1998 (https://www.iso.org/obp/ui/#iso:std:iso:11047:ed-1:v1:en).

These determinations were repeated in the phenophases of 5 (15 BBCH) and 8 leaves (18 BBCH), June-September 2020, respectively. The number of repetitions was 5 for each parameter determined and at each time.

In order to establish the potential of S. perfoliatum plantlets for decontamination of heavy metals polluted soils, different phytoremediation indexes like bioaccumulation factor (BAF), translocation factor (TF), metal uptake (MU) and removal efficiency (RE), were calculated using the formulas (Gupta et al., 2008; Anning et al., 2013; Stanislawska-Glubiak et al., 2015; Anning and Akoto, 2018):

\[
BAF = \frac{\text{heavy metal concentration in plant}}{\text{heavy metal concentration in soil}}
\]

\[
TF = \frac{\text{heavy metal concentration in leaf}}{\text{heavy metal concentration in root}}
\]

\[
MU = \frac{\text{heavy metal concentration in leaf or root} \times \text{dry weight of leaf or root}}{\text{mg kg}^{-1} \text{dw}}
\]

\[
RE = \frac{\text{(initial heavy metal concentration in soil} – \text{final heavy metal concentration in soil)}\times 100}{\text{initial heavy metal concentration in soil}}
\]

2099
Statistical procedures
The data for all analyses and determination were statistically processed using ANOVA, and the means were compared using the least significant difference (LSD) test (Ciulca, 2006). The significance of differences was expressed based on letters, being considered as significant ($p<0.05$) the differences between means marked with different letters.

Results

Heavy metals concentration in the experimental substrate
Mean concentrations of Cu, Zn, Cr and Pb in the soil collected from the mining area of Moldova Noua, used as a growth substrate for *S. perfoliatum* during the formation of the rosette of leaves (13-18 BBCH) are relatively high, exceeding normal values, being in alert or intervention thresholds for sensitive soil use types according to Romanian legislation (Order 756/1997, Environmental Pollution Assessment Regulation) (Table 1). Chromium was the most abundant heavy metal with concentrations of 299.08 mg kg$^{-1}$, being practically at the level of the intervention threshold of 300 mg kg$^{-1}$ specific to sensitive types of use. High concentrations of Pb of 175.18 mg kg$^{-1}$ were encountered, well above the threshold for intervention of 100 mg kg$^{-1}$ and of Cu 234.66 mg kg$^{-1}$, over to the intervention threshold (200 mg kg$^{-1}$), while the Zn concentration is below the alert threshold (300 mg kg$^{-1}$).

| Heavy metal | Determined in soil (mg kg$^{-1}$) | Reference thresholds (mg kg$^{-1}$) |
|-------------|----------------------------------|-----------------------------------|
| Cu          | 234.66±3.08                      | 20 100 200                         |
| Zn          | 260.01±3.40                      | 100 300 600                        |
| Cr          | 299.08±2.39                      | 30 100 300                         |
| Pb          | 175.18±1.34                      | 20 50 100                          |

Values are means (mg kg$^{-1}$) of five replicate samples with standard errors

Metal accumulation and distribution in plants
The analysis of Cu accumulations in the root system of *S. perfoliatum* seedlings in vegetative growth stages reveals an insignificant variation of this element from 37.38 to 43.89 mg kg$^{-1}$ fresh weight (fw) in the first two analysed phenophases, for a significant 83.89% increase in Cu concentration in the period from 15 BBCH to 18 BBCH (Table 2). At the level of petiole during the three stages the Cu content showed a small and insignificant variation from 18.18 to 19.62 mg kg$^{-1}$fw (15 BBCH), after which it is reduced to 16.18 mg kg$^{-1}$fw in the 8 leaves stages. In the case of the foliar limb (lamina), during the experiment there is a significant increase in Cu content from one stage to another, from 12.6 mg kg$^{-1}$fw (13 BBCH) to 32.08 mg kg$^{-1}$fw (15 BBCH) and 61.69 mg kg$^{-1}$fw (18 BBCH) respectively. The rate of accumulation of Cu in the lamina is almost double from one phase of determination to another.

At the first determination (13 BBCH), the root had a significantly higher Cu content than the other two organs. The petiole also had a significantly higher concentration than the lamina. And in the 15 BBCH stage, there is a significantly higher content of Cu at the root level, associated with significantly equal values in the other organs. In the last stage, against the background of the high content of the root a significant increase in the Cu content of the lamina can be encountered by comparison to the petiole.
### Table 2. Heavy metals concentrations in different organs of *S. perfoliatum* for different growth stages

| Metal | Plant organ | Growth stage | 13 BBCH | 15 BBCH | 18 BBCH | LSD 5% |
|-------|-------------|--------------|---------|---------|---------|--------|
|       |             | 13 BBCH      | 15 BBCH | 18 BBCH |         |        |
|       |             | 13 BBCH      | 15 BBCH | 18 BBCH |         |        |
| Cu    | Root        | y 37.38±0.32 a | y 43.89±0.50 a | x 80.71±5.48 a | 11.06 |
|       | Petiole     | x 18.88±0.76 b | x 19.62±6.92 b | x 16.18±0.71 c | 13.98 |
|       | Lamina      | z 12.60±0.44 c | y 32.08±1.10 ab | x 61.69±0.99 b | 3.08  |
|       | LSD 5%      | 1.87         | 14.04    | 11.21   |        |
| Zn    | Root        | y 53.66±3.89 a | xy 62.83±0.53 a | x 73.10±6.53 b | 15.23 |
|       | Petiole     | y 15.53±0.43 b | x 24.71±4.20 b | x 35.46±2.58 c | 9.90  |
|       | Lamina      | z 12.66±1.42 b | y 54.90±0.74 a | x 88.88±1.51 a | 4.40  |
|       | LSD 5%      | 8.32         | 8.60     | 14.35   |        |
| Cr    | Root        | z 57.43±4.03 a | y 84.82±0.75 a | x 93.36±9.82 a | 21.26 |
|       | Petiole     | z 30.61±0.50 b | y 57.15±4.40 b | x 70.57±3.06 b | 10.76 |
|       | Lamina      | z 20.04±2.91 c | y 49.32±1.74 b | x 73.89±0.53 ab | 6.86  |
|       | LSD 5%      | 9.98         | 9.58     | 20.58   |        |
| Pb    | Root        | y 33.16±2.43 a | x 41.29±0.15 a | x 53.13±1.83 b | 6.09  |
|       | Petiole     | z 15.45±0.23 b | y 38.78±0.57 b | x 42.49±1.50 b | 3.23  |
|       | Lamina      | z 18.47±0.28 b | y 33.18±0.50 c | x 53.70±0.61 a | 3.67  |
|       | LSD 5%      | 4.92         | 1.55     | 4.87    |        |

Values are means (mg kg⁻¹) of tree replicate samples with standard errors. Means with different letters are significant at p<0.05. Letters a, b, c, were used for vertical comparisons and x, y, z, for horizontal comparisons.

Zn accumulation in the root system of plants recorded positive, but insignificant variations from one stage to another, between 53.66 and 73.10 mg kg⁻¹fw. Thus, only in the stage of 8 leaves the amount of this element was significantly higher by 36.3% compared to the initial determinations. The Zn content in the petiole showed a gradual increase of 9-10 mg kg⁻¹fw from one determination to another, associated with a significant difference only between 13 and 15 BBCH. At the lamina level, the amount of Zn was between 12.66 and 88.88 mg kg⁻¹fw, associated with significantly high variations between successive determinations. In the first stage (13 BBCH) the amount of Zn in the roots was significantly higher, with more than 38.13 mg kg⁻¹fw than in the petiole and lamina, which showed relatively equal levels. For 15 BBCH stage, a higher accumulation of Zn was observed in the root and lamina, against the background of significant deviations from the quantity in the petiole. In the last determination, there is a clear differentiation between the three organs, so that the Zn content in the lamina was significantly higher than that in the root, while in the petioles the lowest value was recorded.

With regard to the Cr content of the dry biomass of the *S. perfoliatum* plants there is a significant variation of this element from one stage to another, against the background of an amplitude between 35.93 mg kg⁻¹fw for roots and 53.85 mg kg⁻¹fw for lamina. In the first determination, the root system had a significantly higher Zn content than on the petiole and lamina. The petiole also had a significantly higher concentration than the lamina. In the 15 BBCH stage, a significantly higher Zn content associated with close values in the other organs is found at the root level. In 18 BBCH, against the background of the high content of Zn from the roots, a significant deviation was observed only compared to petiole, i.e. significantly equal values in the two components of the leaves.

The concentration of Pb in the roots registered a significant variation of 24.5% only between the first two stages, associated with close values in 15 and 18 BBCH. The content of Pb in petiole showed a gradual and significant increase from one phenophase to another, more intense between 13 and 15 BBCH. In the case of lamina, the concentration of this heavy metal was between 18.47 and 53.70 mg kg⁻¹fw, associated with significantly high variations from one stage to another. At the first determination the Pb amount in the roots was significantly higher by 14.69-17.71 mg kg⁻¹fw compared to the amount petioles and lamina which showed
significantly equal levels. In 15 BBCH stage, there is a significant differentiation between the three organs, so that the content of Pb in the roots was superior to that of petiole, while at the level of the lamina was recorded the lowest value. For the last determination, a higher accumulation of Pb is observed in the lamina, against the background of significant deviations from the amount of Pn in the petiole and in the root.

Phytoremediation indexes (indexes for metal enrichment in plants)

One of the important objectives of the research on phytoremediation of soils polluted with heavy metals is to assess the ability of plants to retrieve substrate elements and also to verify that the elements are transferred and accumulated in the various aerial organs of plants (Buscaroli, 2017).

Bioaccumulation (BAF) and translocation factor (TF)

The bioaccumulation factor is an important phytoremediation index of soils polluted with heavy metals by calculating the existing ratio between the concentration of heavy metals in the plant and the concentration of heavy metals in the soil at the same time of determination.

The data analysis concerning BAF values (Table 3) shows that the root system of S. perfoliatum plants exhibits a significantly superior accumulation capacity of Cu compared to the other analysed components, while the lamina showed a higher BAF than petiole. As for Zn, the lamina has a higher bioaccumulation capacity, followed by the root and the petiole.

| Metal | Plant organ | LSD 5% |
|-------|-------------|--------|
|       | Root        | Petiole | Lamina | Whole plant |
| Cu    | 5.17±0.35 a | 1.04±0.05 b | 3.95±0.06 a | 10.17±0.34 a | 0.72 |
| Zn    | 2.08±0.19 c | 1.01±0.07 b | 2.53±0.04 c | 5.63±0.10 c | 0.41 |
| Cr    | 3.22±0.34 b | 2.43±0.11 a | 2.55±0.02 c | 8.20±0.36 b | 0.69 |
| Pb    | 2.45±0.10 bc| 2.30±0.08 a | 2.91±0.03 b | 7.66±0.12 b | 0.26 |
| LSD 5%| 0.87        | 0.26    | 0.14    | 0.85        |

Values are means of three replicate samples with standard errors. Means with different letters are significant at p<0.05. Letters a, b, c, were used for vertical comparisons and x, y, z, for horizontal comparisons.

Plant roots showed the highest BAF in Cu (5.17), followed by Cr (3.22), Pb (2.45) and Zn (2.08). In the petiole, the BAF values calculated for Cr and Pb were significantly higher compared to Cu and Zn. The lamina recorded a high value of BAF for Cu (3.95), against the background of lower values for Zn (2.53) and Cr (2.55). At the plant level, a significantly higher accumulation capacity of Cu is observed, against the background of significantly equal values for Pb and Cr, respectively a lower value for Zn.

The translocation factor (TF) frequently appears in the literature as an indicator of phytoremediation of polluted soils. In general, this index refers to the ratio of accumulation of pollutants in the different above-ground parts of plants compared to the accumulation in the root system (Bose and Bhattacharyya, 2008).

The analysis of the experimental results of TF in Cu (Table 4) shows that the migration of this element did not show significant variations from one stage to another. The translocation of Zn from the root to the leaves is significantly and progressively increasing from one determination to another, so that at the end of the experiment only 36.63% of the amount of Zn/plant was present in the root. The same upward trend is observed for TF of Cr and Pb, so that in the end 38.75% of the total quantity of Cr and 31.85 % of the Pb/plant was accumulated at the root level.

At the first determination (13 BBCH) there is a higher translocation of Pb (1.0331), followed by Cr (0.8837) and Cu (0.8425), respectively a reduced migration to Zn (0.5261) at the determination made at 15 BBCH TF Pb is significantly higher than the other elements, which recorded very close values. In the determinations carried out in 18 BBCH stage it was found that TF of Pb was more intense than in the other elements, against the background of a lower migration of Cu (Table 4).
Table 4. Translocation factor (TF) of heavy metals for studied growth stages in *S. perfoliatum*

| Metal | Growth stage | 13 BBCH | 15 BBCH | 18 BBCH | LSD 5% |
|-------|--------------|---------|---------|---------|--------|
| Cu    | 15 BBCH      | x 0.8425±0.0347 b | x 1.1813±0.152 b | x 0.9739±0.0683 c | 0.34   |
| Zn    | 15 BBCH      | z 0.5261±0.0068 c | y 1.266±0.0695 b | x 1.7346±0.189 b | 0.41   |
| Cr    | 15 BBCH      | z 0.8837±0.0176 b | y 1.255±0.0307 b | x 1.5792±0.1543 b | 0.32   |
| Pb    | 15 BBCH      | z 1.0331±0.0695 a | y 1.7432±0.0308 a | x 2.1398±0.1109 a | 0.27   |
| LSD5% |              | 0.13     | 0.28    | 0.45    |        |

Values are means of tree replicate samples with standard errors. Means with different letters are significant at p<0.05. Letters a, b, c, were used for vertical comparisons and x, y, z, for horizontal comparisons.

Metal uptake (MU) and removal efficiency (RE)

The analysis of metal uptake (MU) data shows that in relation to the content of Cu in the root system of *S. perfoliatum* plants there is an insignificant variation from 8.21 to 10.8 mg kg⁻¹ dw in the first two determinations, so that subsequently during the development of plants from 15 to 18 BBCH there will be a significant increase of 12.09 mg kg⁻¹ dw (Table 5). In the case of petiole, the Cu content showed a small and insignificant variation from 3.82 to 4.49 mg kg⁻¹ dw, and in the lamina recorded a significant increase from one determination to another, with 158.7% between 13 and 15 BBCH and 92.4% between 15 and 18 BBCH respectively.

Table 5. Metal uptake in *S. perfoliatum* organs for different stages of development

| Metal | Plant organ | Growth stage | 13 BBCH | 15 BBCH | 18 BBCH | LSD 5% |
|-------|-------------|--------------|---------|---------|---------|--------|
| Cu    | Root        | y 8.21±0.07 a | y 10.80±0.12 a | x 22.89±1.55 a | 3.11   |
|       | Petiole     | x 3.82±0.15 b | x 4.49±1.58 b | x 3.79±0.17 c | 3.20   |
|       | Lamina      | z 3.15±0.11 c | y 8.15±0.28 b | x 15.68±0.25 b | 0.78   |
|       | LSD 5%      | 0.40         | 3.22    | 3.16    |        |
| Zn    | Root        | y 11.53±0.84 a | y 15.46±0.13 a | x 20.73±1.85 a | 4.07   |
|       | Petiole     | z 3.14±0.09 b | y 5.66±0.96 b | x 8.30±0.60 b | 2.28   |
|       | Lamina      | z 3.16±0.35 b | y 13.95±0.19 a | x 22.60±0.38 a | 1.12   |
|       | LSD 5%      | 1.83         | 1.98    | 3.97    |        |
| Cr    | Root        | z 12.34±0.87 a | y 20.86±0.18 a | x 24.05±0.21 a | 1.82   |
|       | Petiole     | z 6.19±0.10 b | y 13.08±1.01 b | x 16.51±0.72 c | 2.48   |
|       | Lamina      | z 5.00±0.73 b | y 12.53±0.44 b | x 18.78±0.13 b | 1.72   |
|       | LSD 5%      | 2.27         | 2.23    | 1.52    |        |
| Pb    | Root        | z 7.13±0.52 a | y 10.16±0.04 a | x 12.80±0.52 a | 1.47   |
|       | Petiole     | z 3.12±0.05 c | y 8.88±0.13 b | x 9.94±0.35 b | 0.76   |
|       | Lamina      | z 4.61±0.07 b | y 8.43±0.13 c | x 13.65±0.15 a | 0.42   |
|       | LSD 5%      | 1.06         | 0.38    | 1.29    |        |

Values are means (mg) of tree replicate samples with standard errors. Means with different letters are significant at p<0.05. Letters a, b, c, were used for vertical comparisons and x, y, z, for horizontal comparisons.

The Zn uptake by the root system recorded positive but insignificant variations between 11.53 and 15.46 mg kg⁻¹ dw at the first determinations. Only in the last determination the Zn concentration was significantly higher by 79.8% compared to the initial value. The assimilation of Zn into petiole showed a progressive and significant increase of 2.5-2.6 mg kg⁻¹ dw from one determination to another. In lamina the Zn uptake recorded values between 3.16 and 22.6 mg kg⁻¹ dw, associated with significantly high variations from one growth stage to another. In the first determination the absorption of Zn from the roots was significantly higher by over 8.37 mg kg⁻¹ dw compared to petiole and lamina which showed significantly equal levels. For stage 15
BBCH, a higher accumulation of Zn is observed in the root and lamina, against the background of significant deviations from the quantity of petiole. In the last determination, it is found that the Zn content in the lamina and root was significantly higher by over 12.43 mg kg\(^{-1}\) dw compared to that of petiole.

Analyses of Cr absorption data, relative to the mass of the dried substance in the analysed vegetable parts, show a significant variation of this element from one phenophase to another, against the background of amplitude of between 10.32 mg kg\(^{-1}\) dw for petiole and 13.78 mg kg\(^{-1}\) dw for lamina. In 13 BBCH stage, the root took significantly higher quantities by 6.15-7.34 mg kg\(^{-1}\) dw compared to the petiole and the lamina. And in the other two determinations, the root demonstrates a high absorption capacity for Cr.

The Pb concentration in the root recorded a significant positive variation of 2.64-3.03 mg kg\(^{-1}\) dw from one phenophase to another. The Pb content of petiole had a progressive and significant increase from one determination to another, more intense between 13 and 15 BBCH. In the case of lamina, the Pb accumulation was between 4.61 and 13.65 mg kg\(^{-1}\) dw, associated with significantly high variations in determination dynamics. In the first determination (13 BBCH) the absorption and accumulation of Pb from the root was significantly higher by 2.52-4.01 mg kg\(^{-1}\) dw compared to the leaf parts that showed significantly different levels, (higher in lamina). In 15 BBCH stage, there is a significant differentiation between roots and leaves, so that the Pb content in the root was higher than that of petiole, while at the laminate level the lowest value was recorded. At the last stage of the determination, a higher accumulation of Pb in the root and lamina is observed, against the background of significant deviations of 2.84-3.71 mg kg\(^{-1}\) dw compared to the absorption of petiole.

The *Silphium* plants grown on substrate with heavy metals in leaf rosette-forming stages (13-18 BBCH) recorded an efficiency for the removal of Cu from the substrate between 37.52% in the initial phase and 93.35% in the last one, against the background of significant variations from one stage to another (Figure 1). Compared to the Zn content of the soil, there is a significant reduction in this element from one stage to another, associated with an efficiency ranging from 35.26% to 86.5%. The reduction of Cr in soil content was between 43.13% for 13 BBCH and 90.30% at 18 BBCH, with a significant increase in the absorption of this metal during experiment. Compared to the initial quantity of Pb in the soil, there is a significant decrease in this element from one determination to another, associated with an efficiency ranging from 43.68 to 89.47%.

![Figure 1. Removal efficiency of heavy metals by *S. perfoliatum* plants in different growth stages](image-url)

* Error bars represent the standard error. In case of each metal, bars with different letters are significant at p<0.05

Therefore, the obtained results attest to the high ability of the *S. perfoliatum* plants to take over and store important quantities of Zn, Cu, Cr and Pb from polluted soils close to mining explorations. The elimination efficiency is high for all studied metals, ranging from 86.5% (Zn) to 93.35% (Zn), and is in line
with other studies (Anning and Akoto, 2018), which recommend this plant species as a potential alternative suitable for phytoremediation.

Discussion

Mining activity and its effects on soil contamination with heavy metals

Mining is widely perceived as an industrial activity that causes contamination of the environment with heavy metals (Pan et al., 2016; Pu et al., 2019). Moldova Noua is recognized for its complex polymetallic ores, copper extraction and processing (Harmanescu et al., 2011).

The Order 756/1997 (Environmental Pollution Assessment Regulation) stipulates that the "sensitive use of land is represented by its use for residential and recreational areas, for agricultural purposes, as protected areas or sanitary areas with restriction regime, as well as the areas of land provided for such uses in the future".

Framing the heavy metal concentration of the soil in the different reference thresholds is essential to deciding what measures are required for improvement. Thus, in the situations where the concentrations of pollutants in the soil are below the alert values for sensitive use of land it is not necessary to establish special measures. If the concentrations of one or more pollutants exceed the alert thresholds, but are below the intervention thresholds, it is considered that there is a potential impact on the soil. In such situations, measures must be ordered to prevent further soil pollution and to further monitor potential sources of pollution. If the concentrations of one or more pollutants exceed the intervention thresholds, these are considered to have an impact on the soil. In these situations, the use of the affected area for sensitive uses is not allowed and remedial measures will be taken (Order 756/1997, Environmental Pollution Assessment Regulation).

In this research, concentrations above the normal limits of Cr, Pb, Cu and Zn were determined in the agricultural soil located in the immediate vicinity of the abandoned mining site, thus demonstrating their negative impact on the environment.

Inefficient ore processing procedures have resulted in abundant accumulation of heavy metal residues in waste dumps and settling basins. Mining residues are exposed to the environment and rarely reused, leading to the mobilization and dispersion of heavy metals, sometimes causing serious pollution of soil, water and air (Zhu et al., 2018), with direct damaging effects on human health and ecosystems (Ali et al., 2013; Anning and Akoto, 2018).

Given the need for local people to cultivate agricultural land in the affected areas, these effects can be particularly problematic, requiring immediate and effective environmental remedial measures, a viable alternative being the phytoremediation (Ehsan et al., 2014; Huang et al., 2017; Wang et al., 2017; Areco et al., 2018; Feng et al., 2018)

Metal accumulation and distribution in plants

Although the scientific literature contains numerous researches on the use of species of the Asteraceae family as viable alternatives for the phytoremediation of soils polluted with heavy metals (Jadia and Fulekar, 2009; Nouri et al., 2009; Nikolic and Švetovic, 2015; Sharma et al., 2015; Sosa et al., 2016; Francis, 2017; Fu et al., 2017; Xiao et al., 2018), the species Silphium perfoliatum has been little researched from this point of view (Klimont, 2007; Majtkowski, 2010; Zhang et al., 2010).

The concept of phytoremediation was inspired by the discovery of hyperaccumulator plant species, most of which belong to the families Brassicaceae, Poaceae, Papilionaceae, Caryophyllaceae, and Asteraceae (Gawronski and Gawronska, 2007).

Silphium perfoliatum L. is a perennial herbaceous species (approx. 15 years), cultivated sporadically in Europe, especially as raw material for biogas production (Gansberger et al., 2015; Haag et al., 2015; Von Cossel et al., 2020) and as an ornamental plant (Titei, 2014; Jucor and Sumalan, 2018). The plant has several clear cultivation advantages, namely: it grows quickly, produces biomass in large quantities, shows high tolerance to
environmental stress conditions and is relatively easy to grow without special technological investments. Most of these characteristics are referred to as mandatory attributes for plants used in the phytoremediation of soils polluted with heavy metals, and the requirements achieved so far have demonstrated the high capacity of the phytostabilization of cadmium by the genotypes of *S. perfoliatum*.

The dynamics of heavy metal accumulation detected in fresh and dry biomass at *S. perfoliatum* (Tables 2 and 5) were higher in the roots compared to aerial components (petioles and lamina) in the early stages of vegetative growth (15 BBCH). The total amount of uptake of metals was dependent on the metal concentration in the soil, but also on the degree of development of the root system. Studies undertaken by Xiao *et al.* (2018), on the species of the *Asteraceae* family confirm that the accumulation of metals in plants has been mainly related to the total amount of soil, as well as their availability. The values of accumulation and concentration of heavy metals in plants of *S. perfoliatum* during the formation of leaf rosette are below the limits of hyperaccumulation mainly for two reasons: the quantities of metal existing in the soil and the stage of plant development.

Some of the previous research has shown that there are reduced correlations between the absorption of metals from plants and their concentrations in the soil (Keller *et al.*, 1998; Greger, 1999). Keller *et al.* (1998) considered that the absorption of metals, both by roots and leaves, was not linearly correlated with the increase in the external concentration of metals. However, numerous studies show a linear, positive link between the concentration of metals in the soil and their absorption into plants (Sagner *et al.*, 1998; Robinson *et al.*, 2003; Boyd, 2010). An essential element of these approaches must be related to the plant species used in the tests, it being known that some species grown on heavy metal-rich substrate behave as hyperaccumulators and others, on the contrary, manifest themselves as displaying hyper tolerance.

The classification of a plant species as a heavy metal hyperaccumulator is based on the criterion of absorption and concentration of the element(s) in the foliar apparatus. Verbruggen *et al.* (2009) show that, depending on the level of toxicity of the metal, the values usually vary between 100 and 10,000 mg kg\(^{-1}\) dry weight, thus for Cd ≥ 100; Cu; Pb; Ni ≥ 1000; Zn; Mn ≥ 10,000.

Studies carried out by Fitzgerald *et al.* (2003) in conjunction with the presented results show that the level of accumulation of heavy metals in plants is in a direct relationship with their concentration in the soil and that the root system of plants is their main storage organ, which shows the level of availability of substrate elements and limited mobility within the plants. However, the results showed that the leaf limb is also the main storage constituent, along with the root for Zn and Pb (Tables 2 and 5). The superior ability of aerial organs to accumulate higher amounts of heavy metals compared to the root system was also highlighted by studies carried out by Nouri *et al.* (2009) on different species of the *Asteraceae* family grown on contaminated land.

In addition to plant metal content, BCF and TF values are more relevant to reflect the accumulation of these elements in plant organisms and their translocation characteristics (Xiao *et al.*, 2017).

Plant species with hyperaccumulation capacity must have a super unitary bioaccumulation factor (BAF) and translocation factor (TF) (Cluis, 2004; Wei and Zhou, 2004; Laghlimi *et al.*, 2015). Thus, the obtained results indicate that *S. perfoliatum* presents features specific to plants with the potential for bioaccumulation of heavy metals in soils polluted with Cu, Zn, Cr and Pb (Table 3). Pehoiu *et al.* (2019 and 2020) also report high levels of accumulation of these heavy metals, specific to the Moldova Noua mining area, for two spontaneous species of the *Asteraceae* family (*Taraxacum officinale* and *Plantago major*).

TF is the ratio of heavy metals concentrated in the aerial parts to those in the roots, indicating the mobility of these components inside the plant. The presented data (Table 4) indicate that the metal uptake showed high TF values, in particular for the range between 13 and 15 BBCH. The highest (super unit) values of TF were recorded for Pb>Zn>Cr.

Several previous studies show that BAF and TF values vary quite a lot from one species to another and even within the same species (Rascio and Navari-Izzo, 2011). Furthermore, there is no clear evidence that plants with high levels of BAF have high TFs and vice versa. However, in the presented results, the metals
showed high values of BAF in the roots and lower in the aerial parts, which is consistent with some previous research (Lu et al., 2013; Nikolic and Svetovic, 2015; Singh et al., 2017).

The plants of *S. perfoliatum* showed very high values of removal efficiency, of more than 85%, for the four analyzed heavy metals. These results provide further evidence of the inherent affinities of some plant species for heavy metals and thus explain their widespread use in phytoremediation studies (Truong, 1999; Anning et al., 2013).

The existence of positive links between RE and BAF, TF show that the accumulation and translocation of metals in *S. perfoliatum* plants caused the soil decontamination. A high RE may be influenced in addition to plant characteristics (species, stage of development, metabolic activity, etc.) by the amount of soil used for testing, as well as by the duration of the experiments. This is important because, according to research carried out by Sharma and Pandey (2014) and cited by Anning and Akoto (2018), the permanent removal of heavy metals from polluted soils is the main argument for which phytoextraction is considered to be the most sustainable and effective phytoremediation technique.

### Conclusions

Former mining exploitation in the area of Moldova Noua is a pollution factor with heavy metals (Cu; Zn; Cr and Pb) for the agricultural land in the vicinity, with concentrations at alert limits for Zn and Cu, and within intervention limits for Pb and Cr.

The *S. perfoliatum* plants grown on soils with heavy metals at the initial stages of leaf rosette formation have a good accumulation capacity of the four heavy metals, without showing symptoms of toxicity, but the values recorded are well below the hyperaccumulation limits, mainly due to the concentration and limited volume of soil, as well as the stage of plant development.

However, the high values determined by the calculation of the main phytoremediation indices (BAF, TF and RE) show the capacity of the *S. perfoliatum* plants for the early accumulation at the level of the roots of the four heavy metals, so that after a few, important quantities of Zn and Pb were translocated and accumulated into the lamina. The high RE values positively correlated with BAF and TF demonstrate the high decontamination capacity of heavy metal-polluted soils within this species. However, further research is needed to determine the phytoremediation capacity of *S. perfoliatum* plants in phenophases of formation and growth of stems and vegetative apparatus when the plants accumulate the greatest amount of aerial biomass.

### Authors’ Contributions

Conceptualization (RLS; CM; SIC; AK); Data curation (NLJ; MG; RMS) Formal analysis (RLS; MCB; DK); Funding acquisition (CM; AK; DK); Investigation (CM; NLJ; RMS; MG); Methodology (RLS; SIC; AK; MG); Project administration (CM, AK; DK); Resources (SR; CS; MCB) Software (CS; RMS); Supervision (RLS; CM; AK); Validation (MG; MCB); Visualization (SIC; RLS); Writing - original draft (RLS; SIC; RMS); Writing - review and editing (RLS; CM; SIC; RMS; MCB). All authors read and approved the final manuscript.

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2107
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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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