ASSESSMENT OF HEAVY METALS ACCUMULATION IN PERENNIAL LEGUMES (WHITE CLOVER AND RED CLOVER) IN THE COPȘA MICĂ AREA, ROMANIA

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Abstract

The impact of heavy metals on perennial legumes, just like on other plant species, can be detrimental and has several ecological and agricultural implications. The ability of perennial legumes to fix atmospheric nitrogen through symbiotic interactions with nitrogen-fixing bacteria in their root nodules makes them a significant group of plants.

The present study aims to evaluate the total concentration of heavy metals (cadmium, lead, copper, and zinc) in red and white clover plants harvested from grasslands located in heavily polluted areas (Copșa Mică, Axente Sever, Valea Viilor, Micăsasa and Târnava). White clover (Trifolium repens L.*) recorded Cd content values between 0.06 - 3.53 mg/kg dry weight, Cu had values between 4.09 - 33.4 mg/kg dry weight, Pb content was between 0.18 - 4.77 mg/kg dry weight and Zn had values between 38 - 186 mg/kg dry weight. Regarding red clover (Trifolium pratense* L.*) the heavy metal content values were as follows: for Cd (0.08 - 3.38 mg/ kg d.w), for Cu (6.03 - 13.5 mg/kg d.w), for Pb (0.13 – 2.34 mg/kg d.w), for Zn (33.1 - 169 mg/kg d.w).*

Our results show that clover species harvested from grasslands in these geographical areas have high levels of heavy metals due to metallurgical activities. Grazing in these areas affected by heavy metal pollution can pose serious health problems for animals and later for humans through consumption of their products.

Keywords: *accumulation, clover, heavy metals, perennial legumes, pollution*

INTRODUCTION

Clover is a perennial legume species belonging to the *Fabaceae* family that plays an important role in animal feed due to its nutritive qualities as well as in improving soil fertility by fixing atmospheric nitrogen (Rahimi-Ashtiani et al., 2015). White clover (*T. repens* L.) is often found in temperate grasslands but can be grown successfully in other climatic conditions. It is also an important species used in animal feed due to its high protein content helping to improve animal performance (Chu et al., 2022; Sawicka et al., 2023). Due to its traits of strong seedling vigor, quick development, and tolerance to acidic and humid conditions, red clover (*T. pratense* L.) is a significant forage legume that is widely grown in most temperate countries. Because of its high nutrient content, which is partly due to symbiosis with nitrogen-fixing bacteria of the genus *Rhizobium*, it is also utilized as a green manure crop (Sato et al., 2005).

According to Marshall et al. (2017) and Dhamala et al. (2017) cited by McKenna et al. (2018), red clover is an important forage legume both in animal feed due to its high protein content and in the biological fixation of nitrogen in the soil in high quantities.

The development of the global economy has led to increased pollution of the environment with various contaminants, in particular, heavy metal pollution, which

presents a high risk of contamination due to its ability to persist for a long time in nature compared to other pollutants (Han et al., 2002). Pollutants in the environment are spread through various pathways, many of which eventually enter the animal and human food chain (Rajaganapathy et al., 2011).

Cui et al. (2004) cited by Sun et al. (2013), reported that heavy metal contamination is a major concern due to the potential damage it poses to food safety, human health, and soil ecosystems. Heidar et al. (2021) demonstrated that the presence of Cd in plants has numerous negative effects leading to disruption of normal plant growth and development.

According to Lux et al. (2011), numerous studies have focused on the toxicity of heavy metals on shoots, although the main pathway for heavy metals to enter the plant is through the root.

Deribachew et al. (2015) indicated that plants acquire heavy metals from polluted soils and water through their root systems as well as through aerial parts that are exposed to the air. Heavy metals are mainly concentrated in the roots of plants, and a significantly lower content is found in the biomass of aerial parts, while the concentration of heavy metals increases significantly with the altitude of the meadows (Tomaškin et al., 2013).

According to Bidar et al. (2007), the heavy metals accumulated in *T. repens* had higher concentrations in the roots than in the shoots. Ratko et al. (2011) reported that the absorption of the toxic elements by plants from the soil is influenced by soil properties and the form of heavy metals. Zeng et al. (2007) reported that Pb is a toxic contaminant in soil, harming the physiological and biochemical processes of plants.

This work aims to evaluate the total amount of heavy metals in clover species harvested from natural meadows in one of the most polluted areas in Romania, Copșa Mică, due to metallurgical activities that have taken place since the 1930s.

MATERIALS AND METHODS

This study was carried out in 2023 in one of the most polluted areas in Romania, Copșa Mică.

Figure 1. Copșa Mică area (original photo)

In this study, 29 samples of both white and red clover (*T. repens* L. and *T. pratense* L.) plants were collected from natural meadows heavily affected by pollution to determine the total content of heavy metals (Cd, Pb, Cu, and Zn). The studied localities were: Copșa Mică, Axente Sever, Micăsasa, Târnava, and Valea Viilor (Figure 2).

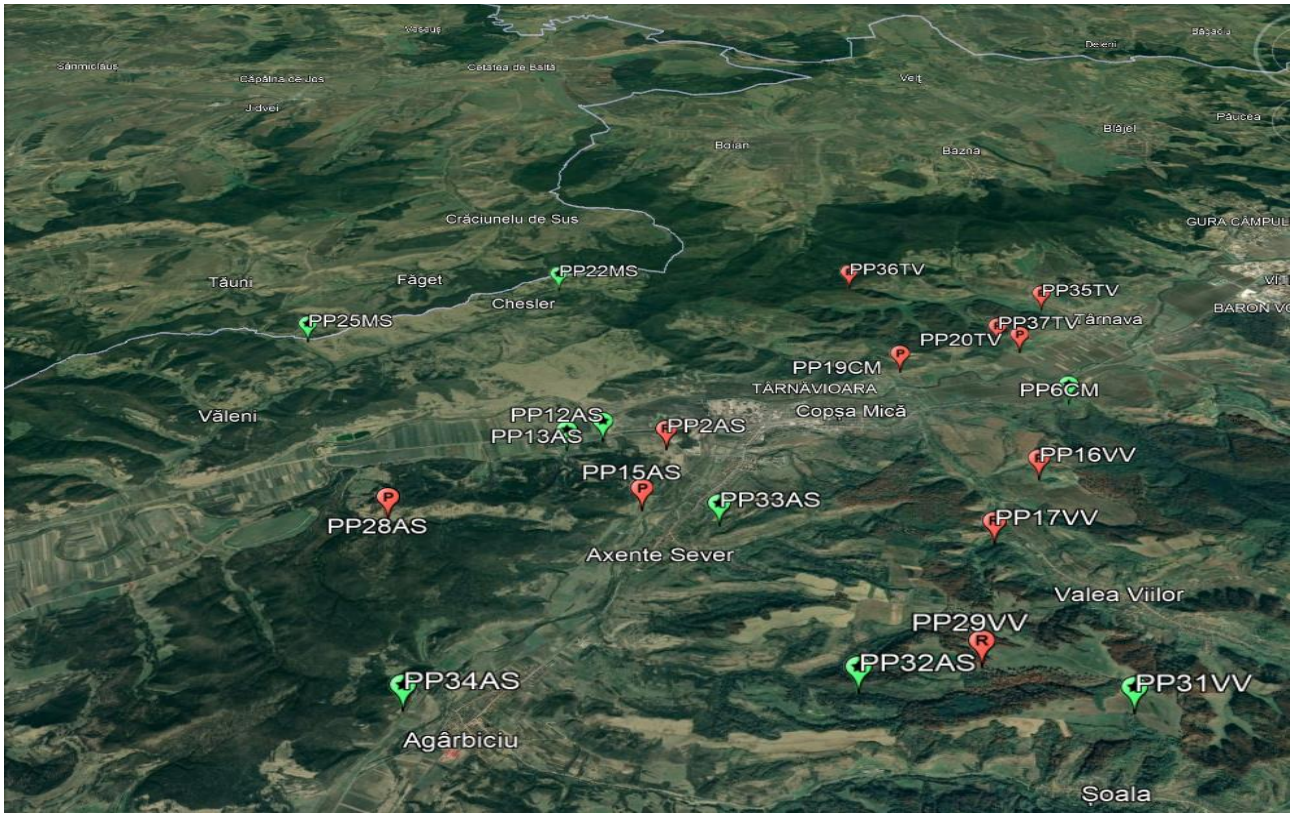

Figure 2. Harvest points in the Copșa Mică area (P red mark = *Trifolium pratense* L.; R red mark = *Trifolium repens* L.; green mark = *Trifolium pratense* L. and *Trifolium repens* L.) (Source: https://earth.google.com)

The samples collected were oven dried then milled and treated with nitric acid in a microwave digestion system. To determine the content of heavy metals the atomic absorption spectrometry was used (Flame GBC 932AA or Graphite furnace GBC SavanatAAZ). The statistical processing of the data was done using Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

Analyzing the results obtained for white clover (*T. repens* L.) regarding the total contents of heavy metals (Table 1), it can be seen that the highest value was recorded in the case of Cu content with a mean concentration of 8.16 mg/kg d.w. The lowest value was recorded for the Cd content, with an average concentration in the plant of 1.02 mg/kg d.w., which exceeds the permissible Cd content in feed materials of vegetable origin according to Commission Directive 2005/87/EC. Regarding the accumulation of Pb by the white clover species, a total mean value of 1.56 mg/kg d.w can be observed. In the case of Pb content it does not exceed the limit allowed by Commission Directive 2005/87/EC. At the same time, the Zn content had a value of 99 mg/kg d.w. Regarding the minimum contents of the heavy metal, they had values as follows: 0.06 mg/kg d.w Cd, 0.18 mg/kg d.w Pb, 4.09 mg /kg d.w and 38

mg/kg d.w Zn. Analyzing the maximum contents of Cd, Cu, Pb and Zn, they recorded values of 3.53 mg/kg d.w, 33.4 mg/kg d.w, 4.77 mg/kg d.w, respectively 186 mg/kg d.w. Bidar et al. (2007) reported higher contents of heavy metals in the roots compared to the shoots of white clover grown on polluted soils.

Table 1. White clover (*T. repens* L.) heavy metal content (n=12)

Variable	Min.value (mg/kg d.w)	Max.value (mg/kg d.w)	Arithmetic mean (mg/kg d.w)
Cd white clover	0.06	3.53	1.02
Cu white clover	4.09	33.4	8.16
Pb white clover	0.18	4.77	1.56
Zn white clover	38	186	99

According to Lambrechts et al. (2014) *Trifolium repens* shoots have a higher tolerance to heavy metal pollution which proves that it is a suitable crop plant for phytoremediation of contaminated soils. Moreover, their study shows that heavy metal impact toxicity varies on the pollutant and plant species. Following the content of heavy metals in the red clover plant (*T. pratense* L.) (Table 2) it is observed that the lowest value was obtained in the case of Cd content which recorded a mean value of 0.68 mg/kg d.w. At the same time, the highest content was recorded for Cu with a concentration of 8.56 mg/kg d.w. In terms for Pb and Zn contents, they obtained a mean value of 0.75 mg/kg d.w and 74 mg/kg d.w respectively. The concentration of Cd in the plant ranged from 0.08 mg/kg d.w to 3.38 mg/kg while Pb ranged from 0.13 mg/kg to 2.34 mg/kg d.w. Cu and Zn content ranged from 6.03 mg/kg d.w to 13.5 mg/kg d.w and 33.1 mg/kg - 169 mg/kg d.w respectively.

Table 2. Red clover (*T. pratense* L.) heavy metal content (n=17)

Variable	Min.value (mg/kg d.w)	Max.value (mg/kg d.w)	Arithmetic mean (mg/kg d.w)
Cd red clover	0.08	3.38	0.68
Cu red clover	6.03	13.5	8.56
Pb red clover	0.13	2.34	0.75
Zn red clover	33.1	169	74

Satarug & Moore (2004) reported that long-term exposure to cadmium causes kidney disease leading to kidney dysfunction. Zn and Cu are vital micronutrients, but in high quantities, can have harmful effects for plants, animals and humans. High amounts of Cu can lead to the appearance of diseases for people such as Alzheimer, Parkinson and occipital horn syndrome (Desai & Kaler 2008).

CONCLUSIONS

In this study white clover (*T. repens* L.) and red clover (T. *pratense* L.) were harvested from one of the most polluted areas, Copșa Mică, and analyzed to evaluate the heavy metal total contents. The concentrations of heavy metals in both white and red clover plants were in the following order: Zn>Cu>Pb>Cd. It can be observed that white clover plants accumulated more Zn (99 mg/kg d.w) than

red clover (74 mg/kg d.w), however, this element had the highest value in both species compared to the other heavy metals studied. The lowest heavy metal content in both plant species was obtained for Cd, with values of 1.02 mg/kg d.w and 0.68 mg/kg d.w respectively. Cu content had higher concentrations in red clover plants compared to white clover, while for Pb, white clover species had higher concentrations (1.56 mg/kg d.w). The Cd and Pb content in red clover species did not exceed the limit permitted by Commission Directive 2005/87/EC for heavy metal concentrations in feed materials of plant origin, whereas white clover exceeded the permitted limit for Cd content.

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