Original Research Article

Accumulation and Translocation of Heavy Metals in Plant Organs of White Clover (*Trifolium repens* L.) as Dependent on Soil Content

ABSTRACT

Soil contamination with heavy metals is becoming an increasingly global issue, as elevated levels of heavy metals exceeding regulatory limits are diminishing agricultural productivity and consequently endangering human health and the environment. This study aimed to assess the seasonal dynamics, accumulation, and translocation of heavy metals (As, Cd, Cr, Cu, Fe, Pb, Ni, and Zn) in the organs of white clover (*Trifolium repens* L.) depending on soil content. The heavy metal content in soil and white clover samples was determined using atomic absorption spectrophotometry. The results revealed that the analyzed soil was significantly contaminated with Cu and Zn in the industrial zone (GS) and with Pb near the urban landfill (D3). The content of heavy metals in white clover varied depending on the location and season. It is evident that white clover accumulated certain metals in its roots and leaves during spring, summer, and autumn, including Pb, Cr, Cu, Fe, Ni, and Zn. The analysis results indicated that Fe was the predominant element in white clover roots, while Zn and Fe were most abundant in the leaves. Values of BCF >1 and TF >1 for Zn suggest that white clover is a potential accumulator of this heavy metal.

Keywords: heavy metals, Trifolium repens, accumulation, translocation

1. INTRODUCTION

Environmental pollution by heavy metals has become a significant global issue in modern society, characterized by cumulative effects [1,2,3]. Besides natural processes, nearly all human activities have the potential to contaminate soil with heavy metals, such as the use of phosphate fertilizers in agriculture [4]sewage sludge [5] mining and metal smelting [6], pesticide application [7], galvanization, and fossil fuel combustion[8].The uptake of heavy metals from contaminated soil by plants growing in these areas introduces these metals into the food chain, posing a significant threat to human and animal health [9,10,11]. Concentrations of heavy metals in the environment are increasing year by year, underscoring the importance of decontaminating soil polluted with heavy metals [12,13]. It has been demonstrated that plants have the ability to remove various pollutants from soil and are particularly effective at phytoremediation of heavy metals [14,15, 16].
White clover (*Trifolium repens* L.) is a facultative metallophyte that exhibits substantial accumulation of heavy metals [17,18]. It is frequently mentioned in the literature as a suitable plant model for phytoremediation purposes [19,20,21,22,23,24,]. The primary objective of this research is to determine the concentration, seasonal dynamics, and accumulation and translocation of heavy metals (As, Cd, Cr, Cu, Fe, Pb, Ni, and Zn) in the organs of white clover (*Trifolium repens* L.) as influenced by soil content.

2. MATERIAL AND METHODS

The content and dynamics of heavy metals (As, Cd, Cr, Cu, Fe, Pb, Ni, and Zn) in soil and white clover (*Trifolium repens* L.) were analyzed at six sites (five exposed to pollution and one control site) within the municipality of Gračanica (Bosnia and Herzegovina). A description of the sites is provided in Table 1.

Table 1. Sample sites

No	Locality name	Description of Sample Sites
1	L	agricultural land
2	SP	immediate vicinity of the main traffic route
3	GS	industrial zone of the city
4	D1	area near the city landfill
5	D2	area near the city landfill
6	D3	area near the city landfill

2.1. Sampling

Soil samples were collected from suitable pedological plots measuring 9 x 9 meters. An average sample (approximately 500 g) was collected from multiple individual samples using a chrome-plated probe and handheld plastic tools. Soil samples were taken from a depth of 0-25 cm, corresponding to the plough layer, consistently from the same experimental plot. Multiple sub-samples were collected at each sampling location to create a composite sample.

Plant material was manually collected from a 5-10 m² area, amounting to approximately 500 g from each plant species population. The collected plant material was separated into plant organs (roots and leaves). Sampling was performed three times during the growing season.

2.2. Sample Preparation

Soil samples were air-dried at room temperature and crushed to particles smaller than 1 mm in size, with plant roots removed beforehand. Prepared samples were stored in containers with airtight lids and kept in desiccators until chemical analysis.

Each part of the plant was thoroughly rinsed with tap water and then with distilled water to remove dust and soil particles. For chemical analysis of plant material, 1 g of plant material was placed in a 25 ml measuring flask and digested in nitric acid (HNO_3, p.a.) and hydrochloric acid (HCl, p.a.) with heating. After sample digestion (clarification of the flask contents), the flask was filled with perchloric acid ($HClO_4$, p.a.), and the sample was heated until the appearance of perchloric fumes and their rise to the flask neck. The flask contents were cooled

to room temperature and topped up with distilled water to the mark, with mixing of the contents. This prepared the sample solution for measuring the concentration of heavy metals.

2.3. Analytical Methods

The content of heavy metals (As, Cd, Cr, Cu, Fe, Pb, Ni, and Zn) in soil samples and *Trifolium repens* was determined using Optical Emission Spectrometry (OES) on a PerkinElmer Optima 2100 DV instrument. Metals were extracted according to ISO 11466 and ISO 11047 standards.

2.4. Determination of Accumulation and Translocation

To establish the relationship between the concentration of heavy metals in plant tissues (roots, leaves) and soil, and the ability to accumulate each investigated heavy metal, the bioaccumulation factor (BCF) was calculated as follows [25]:

$$\mathbf{BCF} = C_{plant} / C_{soil}$$

The Translocation Factor (TF) indicates the plant's efficiency in translocating accumulated heavy metals from roots to shoots. It is the ratio of the concentration of the metal in the shoots (stems or leaves) to that in the roots. It is calculated as follows [26,27]:

$$\textbf{TF} = C_{shoots} / C_{roots}$$

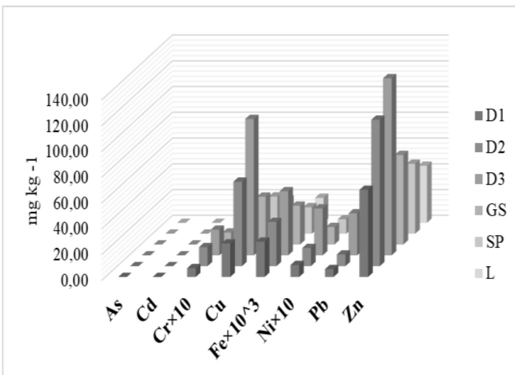
2.5. Statistical Data Analysis

Statistical analysis of the obtained data was conducted using SPSS 20. for Windows.

3. RESULTS AND DISCUSSION

3.1. Analysis of Heavy Metal Content in Soil

Seasonal values of heavy metal content (As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn) in the soil are presented in Figure 1.



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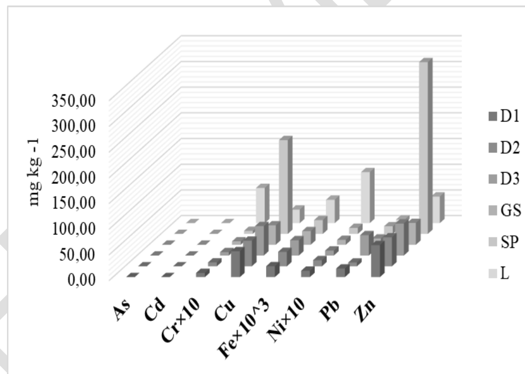

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Fig. 1.Heavy Metal Content in Soil at the Investigated Sites: a) Spring, b) Fall

Values of As and Cd in soil at all analyzed sites during the seasons (spring, autumn) were below the detection limit. The highest values of Cr, Cu, Fe, Ni, Pb, and Zn in spring were recorded at site D3, and the lowest at site L. In autumn, the highest values for Cr, Fe, and Ni were observed at site L. The highest concentrations of Cu and Zn were detected at site GS. The highest values of Pb were found at site D3.

3.2. Analysis of Heavy Metal Content in White Clover (*Trifolium repens* L.)

Figure 2 presents the results of heavy metal content (As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn) in the roots and leaves of white clover during the spring, summer, and autumn.

The content of As in the roots of white clover was below the detection level at all investigated sites, while the presence of As was detected in the leaves at site L. The concentrations in the leaves were within normal values according to [28] and [29], indicating that foliar absorption likely contributed to the presence of this element in the above-ground part of white clover.

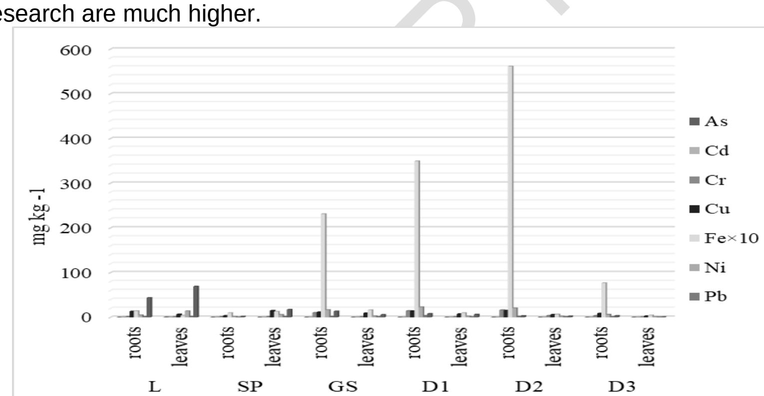
The highest concentration of Cd in the roots of white clover was recorded at site L (0.27 mg kg^{-1}), while in the leaves, it was highest at site GS (0.30 mg kg^{-1}). According to [30], normal Cd levels in plants range from 0.1 to 2.4 mg kg^{-1}. The results show that the concentration of Cd in white clover is within the normal range for plants.

White clover roots contained Cr at all sites except in autumn at site SP. The leaves of white clover contained Cr at all sites except in autumn at site L and in summer at site D1. It is evident that significantly higher concentrations of Cr were found in the roots compared to the leaves on most sites. In comparison to other heavy metals, the mobility of Cr in plant roots is low. Greater accumulation of Cr in the roots may be attributed to the sequestration of Cr in cell vacuoles of the roots as a protective mechanism that provides a certain natural tolerance of plants to Cr toxicity [31].

Measured amounts of Cu in the roots and above-ground parts of white clover collected during spring, summer, and autumn at most sites fell within normal values for plants according to [28]. However, in the roots of white clover at site GS and D1, as well as in the roots and above-ground parts at site D3, Cu values exceeded toxic levels [32]. Most plants primarily accumulate Cu in the roots, while its content in the leaves can vary significantly between species [33].

The Fe content in the roots of white clover at most analyzed sites was generally above normal values reported in the literature [30]. The highest Fe concentration in the roots of white clover was recorded in spring at site D2, reaching 5613.33 mg kg^{-1}, which can be considered a critical threshold of toxicity [32]. The values of Fe in white clover leaves during the season remained within normal limits. Iron concentrations were significantly higher in the roots compared to the leaves at all analyzed sites, with no pronounced seasonal differences.

In our research, Ni was detected in the roots and leaves of white clover at almost all sites throughout the seasons. According to [28], the critical toxicity threshold for Ni is usually > 10 mg kg^{-1} for sensitive plant species and > 50 mg kg^{-1} for more resistant plant species, while [29] defined a range of 10-100 mg kg^{-1}. Toxic concentrations of Ni were detected in the roots of white clover at several research sites, with the highest value at site GS in autumn (34.30 mg kg^{-1}) and in the leaves at site D3 in summer (23.86 mg kg^{-1}). Comparing our results with the study by [16], it is evident that the Ni values in white clover in our research are much higher.



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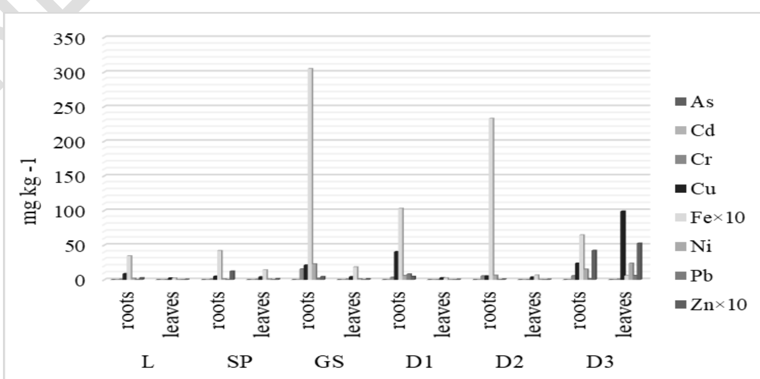

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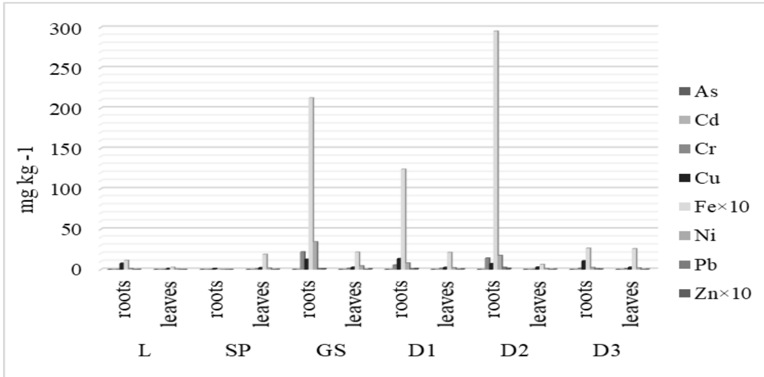

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Fig. 2.Seasonal Dynamics of Heavy Metal Content in White Clover: a) Spring; b) Summer; c) Autumn

The maximum concentration of Pb in the roots of white clover was recorded in summer at site D1 (8.20 mg kg^{-1}), and in the leaves at site D3 (6.06 mg kg^{-1}). Toxic concentrations of lead were not recorded in our research [34]. [35] found that white clover in fields near lead smelters accumulated higher levels of metals in the roots than in the above-ground parts, which is in line with the results of our research. The results of our research showed the presence of Zn in the roots and leaves of white clover at all investigated sites throughout the seasons. Toxic concentrations of Zn in the roots and leaves of white clover were recorded at sites D3 and L [29] (Fig.2)

Table 2 presents measures of central tendency and dispersion of the content of examined metals in white clover.

Table 2. Measures of Central Tendency and Dispersion of the Content of Examined Metals in White Clover (*Trifolium repens* L.)

	Whole plant				Plant organ			
					root		leaf	
	AS	SD	Min	Max	AS	SD	AS	SD
As	0.01	0.04	0.00	0.27	0.00	0.00	0.01	0.06
Cd	0.07	0.11	0.00	0.30	0.06	0.10	0.08	0.11
Cr	3.86	5.65	0,00	21.90	6.81	6.82	0.90	0.86
Cu	11.23	16.94	1.16	99.06	12.49	9.06	9.98	22.48
Fe	804.53	1288.14	2.90	5613.3	1497.31	1547.31	111.75	75.59
Ni	7.11	8.80	0.00	34.30	10.53	9.94	3.69	5.99
Pb	1.12	1.73	0.00	8.20	1.48	1.95	0.76	1.45
Zn	85.74	164.60	0.50	690.66	81.21	132.84	90.28	195.17

The average concentration of examined metals in white clover varies and is ranked as follows: Fe > Zn > Cu > Ni > Cr > Pb > Cd > As. The average concentration of Cd in white clover leaves is 0.08 ± 0.11 mg kg^{-1}, and in the roots, it is 0.01 ± 0.10 mg kg$^{-1.}$ The average concentration of Cr in white clover leaves is 0.90 ± 0.86 mg kg^{-1}, and in the roots, it is 6.81 ± 6.82 mg kg^{-1} (Table 2).

Table 3 presents the results of the influence of site and season on the variability of heavy metal content in white clover (*Trifolium repens* L.).

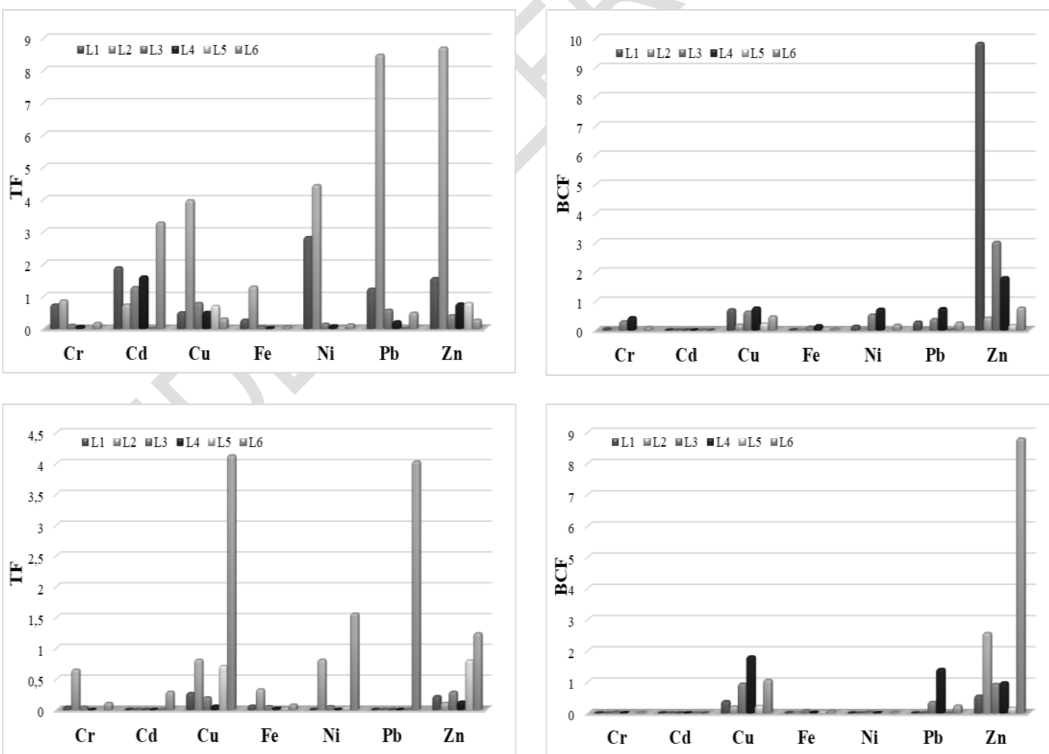
Table 3.Influence of Site and Season (Spring, Summer, Autumn) on the Variability of Heavy Metal Content in White Clover (*Trifolium repens* L.)

Metals	Sites		Season		Site-Season	
	F	p	F	p	F	p
As	1.00	.446	1.00	.387	1.00	.479
Cd	1.24	.329	54.61	**.000**	2.60	**.037**
Cr	1.68	.189	.28	.754	.29	.972
Cu	1.40	.269	2.32	.127	1.50	.215
Fe	1.44	.256	.33	.722	.18	.996
Ni	1.26	.321	.19	.827	.89	.555
Pb	1.07	.407	1.25	.310	.82	.608
Zn	10.16	**.000**	18.69	**.000**	17.65	**.000**

Season has a statistically significant effect on the content of Cd in white clover ($p \leq 0.001$). The content of Zn is statistically significantly influenced by both site ($p \leq 0.001$) and season ($p \leq 0.001$), with season having a greater impact. Neither site nor season has an effect on the remaining heavy metal content in white clover.

3.2. Analysis of bioconcentration and translocation factors

Figure 3 presents the seasonal dynamics of BCF (Bioaccumulation Factor) and TF (Translocation Factor) for Cr, Cd, Cu, Fe, Ni, Pb, and Zn in white clover (*Trifolium repens* L.). The results indicate that the BCF values for Cr are less than 1 at all analyzed sites, indicating a higher concentration of these elements in the roots and lower in the leaves. These findings are supported by other studies [36,37], which show that living cells in plant roots can permanently bind Cr, resulting in low Cr content in aboveground plant parts. Based on the calculated BCF, it is clear that white clover cannot be considered an accumulator for Cr, Fe, and Ni (BCF < 1), but rather an excluder.



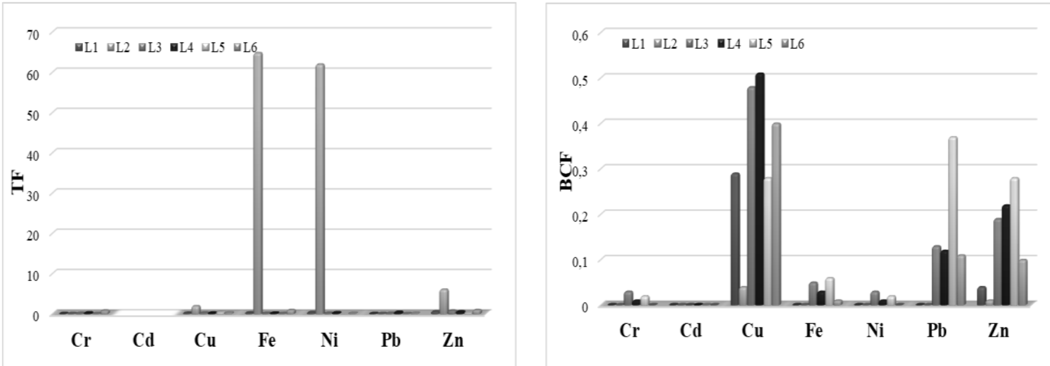


Fig.3. **Seasonal Dynamics of TF and BCF for Cr, Cd, Cu, Fe, Ni, Pb, and Zn in *Trifolium pratense* L. at the Investigated Sites**

TF values can indicate the movement and distribution of heavy metals in the plant [38]. Hyperaccumulating plants are characterized by TF > 1[39]. During the spring, at most analyzed sites, TF values > 1 were determined for Cd, indicating a higher Cd concentration in aboveground parts compared to the roots. [40] reported an antagonistic effect of Zn on Cd absorption, which fully corresponds to our results, as the Cd concentration in plants was practically negligible, unlike the high Zn concentration.

For most analyzed sites, calculated BCF and TF values for Cu are less than 1, indicating that this species contains higher Cu concentrations in the roots compared to the leaves. The higher amount of Cu in the roots of the analyzed plant species can be explained by a stronger binding capacity of Cu to the cell wall of the roots (60-93%), thereby reducing its further transport to the leaves [33].

TF values for Fe less than 1 were found at most investigated sites, except for site SP where a TF value > 1 (64.84) was determined, confirming the dominant influence of the site on which the plant grows. TF values > 1 were observed for Ni at several urban sites. TF > 1 represents efficient metal translocation from roots to shoots [41]. At site L, BCF > 1 and TF > 1 were determined for Zn, indicating that white clover accumulates Zn in its leaves.

4. CONCLUSION

Our research has shown that the soil is significantly contaminated with Cu and Zn in the industrial zone (GS), and with Pb near the city landfill (D3). Soil enrichment with metals in the studied area follows this order: Cu > Pb > Zn > Ni > Cr.

The content of heavy metals in white clover varied depending on the location and season. The highest concentrations of heavy metals (Cr, Cu, Fe, Ni, Pb) were found in the roots and ranged from 6.81±6.82 mg kg-1 for Cr to 1497.31±1547.31 mg kg-1 for Fe. Concentrations of heavy metals (As, Cd, and Zn) were higher in the leaves of white clover and ranged from 0.01±0.06 mg kg-1 (As) to 90.28 mg kg-1 (Zn). Based on the obtained BCF values < 1 for As, Cd, Cr, Fe, and Ni, we can conclude that white clover cannot be considered an accumulator of these metals but rather an excluder. TF values > 1 for Ni and Pb were determined at several urban sites, indicating efficient translocation of these metals from the roots to the shoots. BCF values > 1 and TF values > 1 for Zn suggest that white clover is a potential accumulator of this heavy metal. The research results have demonstrated an increasing need for further investigation of the phytoaccumulation potential of plant species growing on contaminated soil in order to develop modern strategies, methods, and approaches for environmental remediation.

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