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Trifolium pratense and the Heavy Metal Content in Various Urban Areas

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Abstract: Effective biomonitoring strategies are essential for identifying and assessing the sources and levels of contamination of heavy metal pollutants in urban areas, given their negative impacts on human health and the environment. This study aimed to assess the potential of common weed, *Trifolium pratense* as a bioindicator of heavy metal contamination in various land uses in urban areas, with a focus on Cd, Cu, Cr, Ni, and Pb. The results have shown that Cr and Ni had high bioconcentration factor (BCF) values in most sites, in comparison with Cu, Cd and Pb. Contamination factor (CF) values varied across all sites. The industrial area and old town sites had the highest translocation factor (TF) values for Cr and Ni, indicating greater transport of these metals from roots to aerial parts of plants. Differences between heavy metals (HMs) according to land use were observed; especially, Pb and Cu were more concentrated in soils than other heavy metals in industrial areas. Overall, these findings suggest that *Trifolium pratense* is a promising bioindicator for heavy metal contamination in various land uses in urban areas, making it a potentially valuable tool for monitoring heavy metal pollution in cities of the northern hemisphere.

Keywords: biomonitoring; red clover; plant uptake; soil pollution; aerial pollution

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1. Introduction

Urban environments are recognized as significant sources of hazardous and noxious pollutants that can contaminate the air, water, and soil [1]. Atmospheric pollution is considered one of the most pressing environmental problems affecting urban regions [2]. Human activities, such as industrial, municipal, and commercial operations, generate a diverse range of toxic pollutants that can negatively impact the health and well-being of living organisms [3,4].

Among the pollutants emitted through various human activities, industries, heating, and transportation in urban environments, heavy metals are a significant source of concern and a major pollutant [1,5]. Conventionally defined as elements with an atomic density greater than 5 g cm^{-3} and atomic numbers >20 [6], heavy metals are known for their ability to bioaccumulate, inherent toxicity, and long-lasting presence in various environments, making them commonly referred to as environmental pollutants [7]. Once heavy metals persist in the environment, their removal is challenging [8]. Unfortunately, due to the increasing number of pollution sources, low air quality, and inappropriate urban planning, human exposure to heavy metals has risen dramatically, especially in urban and industrial areas [9]. In the environment, accumulated heavy metals can be transferred to humans by contaminated water, inhaling polluted air, and consuming plants grown in contaminated soil [10]. So, the problem of heavy metals is widespread [11]. For humans, heavy metal exposure is a significant contributor to various health problems, including developmental retardation, immune system dysfunction, several types of cancer, endocrine disruption, neurological effects, kidney damage, and other disorders [8]. Furthermore, the concentration of heavy metals in plants can have negative impacts on plant growth, yield, and the environment [12]. For example, chromium (Cr) is a known carcinogenic metal that can accumulate in plant tissues, resulting in decreased growth and yield [13,14]. Cadmium (Cd) is a toxic heavy metal that can cause leaf chlorosis, stunted growth, and reduced root length [15]. Copper (Cu) is an essential micronutrient for plant growth but can be toxic at high concentrations, causing oxidative stress and decreased photosynthesis [16]. Nickel (Ni) is a heavy metal that can accumulate in plant tissues, causing oxidative damage and reduced growth [17]. Zinc (Zn) is an essential micronutrient that is toxic at high concentrations, causing chlorosis and reduced growth [12]. Lead (Pb) is a toxic heavy metal that can cause decreased photosynthesis and chlorosis, as well as neurological and developmental effects in humans and animals [18,19]. Therefore, their concentrations must be monitored and analyzed to assess and mitigate environmental heavy metal pollution [7]. Consequently, over the years, a large number of studies have analyzed the accumulation of heavy metals in different components, such as soils [20,21], water [20,22], sediments [22], and the tissues of living organisms [23]. Additionally, a good deal of research over the years has shown that a suitable choice to indicate the accumulation of heavy metals in different environments was to use plants, known as bioindicators [24,25].

In order to monitor the state of the environment, bioindicators are an adequate tool [26]. Bioindicators include species, communities, and biological processes used to assess the environment's quality. Due to their moderate tolerance to environmental variability, bioindicators effectively indicate the environment's condition [24,27]. Furthermore, an effective bioindicator plant for heavy metal pollution should possess the ability to efficiently accumulate heavy metals in its tissues, thereby serving as a sensitive detector of environmental pollution. In addition, the plant should have a wide distribution range, allowing for its use as a bioindicator across multiple geographic areas, and exhibit a moderate tolerance to environmental variability to grow in a variety of soil types and conditions. These criteria are essential in selecting appropriate bioindicator plants for monitoring heavy metal pollution in urban areas, ensuring that reliable and comprehensive data can be obtained to assess the environmental quality and potential risks associated with pollution [24,27,28].

Therefore, worldwide, in different environments, analyses of the heavy metal content in potential bioindicators have been conducted, such as lichens, mosses, and vascular plants [25,29]. Among wild plants, *Trifolium pratense* L. has received attention as a possible bioindicator plant for heavy metals [5]. *Trifolium pratense* L. (Red Clover) is a wild plant belonging to the legume (*Leguminosae*) family [30]. Due to its ability to accumulate heavy metals and other contaminants from the soil, it has been proposed as a potential bioindicator plant [5,31–34]. Therefore, several studies have investigated the use of red clover as a bioindicator of soil and air pollution (e.g., [5,35–37]). Overall, the use of *Trifolium pratense* L. as a bioindicator plant has shown promising results, although more research is needed to fully understand its potential and limitations.

The main aim of the present study was to evaluate *T. pratense* as a heavy metal bioindicator in urban areas of the representative city concerning land use. *Trifolium pratense* L. was selected as a potential bioindicator plant for heavy metal contamination in urban areas due to its widespread distribution, high biomass production, and ability to accumulate heavy metals in its tissues.

Moreover, the research was carried out to assess the concentration and translocation of the metals Cd, Cu, Cr, Ni, Pb, and Zn in the organs of *T. pratense* plants depending on the soil content. The heavy metals investigated in this study were chosen due to their prevalence as common pollutants in urban areas [38,39].

2. Materials and Methods

2.1. Study Area

Our study was conducted in Poznań, located in western Poland (52°24′30′′ N, 16°56′03′′ E). With an area of approximately 261.91 km^2 and a population of around 530,000 inhabitants, Poznań ranks as the fifth-largest city in Poland in terms of population and the eighth largest in terms of area [40]. It is known for its temperate continental climate, which is characterized by warm summers and cold winters [41]. In Poznań, transport and built-up areas cover 43.5% of the total urban area. Industrial sites are predominantly located near the major roads in the western, eastern, and southeastern areas of Poznań, as well as in the surrounding areas. The surrounding areas of the city are home to the city's developing automotive industry and agro-food sector. The most common soil textures are sand and sandy loam, with an average pH of 6.5–8.0 [42].

2.2. Experimental Materials

Experimental materials were one-year-old specimens of *Trifolium pratense* L. collected from 8 different research sites: individual houses area, area near the lake (15 m from shore), near a river (16 m from the riverbank), a high-density residential area, an industrial area, a park, an old town, and agricultural land (Table 1, Figure 1). Only plants in identical vegetative stages that showed no evident damage (such as discoloration, insect or disease indications), were gathered. Due to the key role of soil as the main factor modulating the physiological response of plants, and thus directly affecting the process of heavy metal accumulation, soil samples were collected in the same places where *T. pratense* grew.

Table 1. Sample sites.

No.	Description of Sample Sites	Code
1	individual houses	POZ01
2	area near the lake	POZ02
3	area near the river	POZ03
4	high-density residential area	POZ04
5	industrial area	POZ05
6	Park	POZ06
7	old town	POZ07
8	agricultural land	POZ08

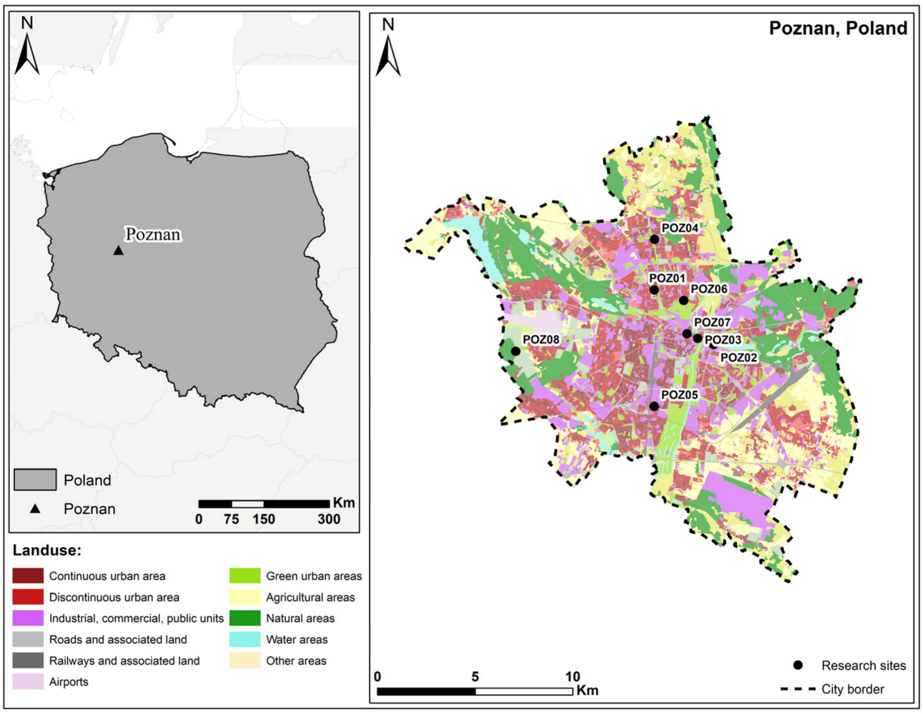

Figure 1. Location of research sites in Poznan (POZ01-POZ08) (source: own study based on Urban Atlas 2018).

2.3. Soil and Plants Sampling

Trifolium pratense L. and soil samples from particular localizations were collected between 5 and 11 May 2022. Soil materials and plant samples were taken from sample sites (Table 1), each one covered square-shaped areas with a 20 to 50 m^2 range. Nine specimens of *T. pratense* were collected for each square-shaped area, from each rooting zone, and 0.5 kg of soil samples were collected in a layer of 0–20 cm depth. Separate plastic boxes were used to store the gathered samples of soil and plants before being delivered to the lab. We confirm that all methods, including the collection of plant material, were carried out in accordance with relevant guidelines and regulations. Voucher specimens were deposited in the herbarium of the Department of Ecology and Environmental Protection, Poznań University of Life Sciences.

2.4. Sample Preparation and Digestion Procedure

In the laboratory, the plant samples were first purified with deionized water using Milli-Q Advantage A10 Water Purification Systems, Merck Millipore (Merck, Darmstadt, Germany), and separated into leaves and roots. The soil samples were sieved (2 mm). To achieve constant dry weight, the plant and soil samples were dried at 40 ± 3 °C in an electric oven (TC 100, SalvisLAB, Rotkreuz, Switzerland) for 120 h. Digestion of the crushed in an agate mortar sample was carried out in the CEM Mars 5 Xpress microwave mineralization system (CEM, Matthews, NC, USA). From each plant or soil sample, 0.3000 ± 0.0001 g was added to a 55 mL vessel containing 8 mL of concentrated (65%) HNO_3 Suprapur® (Merck, Darmstadt, Germany) and 1 mL of H_2O_2 for ultratrace analysis (Merck, Darmstadt, Germany). The program of digestion included three steps: ramp to temperature to 180°C—20 min; hold temperature at 180 °C—20 min; cool to room temperature—20 min. After the digestion steps using Qualitative Filter Papers (Grade 595: 4–7 µm Whatman, Kent, UK), the solutions were filtered, placed in flasks and made up to a final volume of 15.0 mL with deionized water. Reagents blank solutions were prepared in the same way as samples. The pH and electrical conductivity of the soil samples were measured using a pH meter and a conductometer, specifically the WTW Multi 3630 IDS EETF model. For pH measurement, a 1 N KCl solution with a proportion of 1:2.5 (10 g soil/25 mL KCl) was used. For electrical conductivity measurement, a proportion of 1:5 (10 g soil/50 mL demineralized H_2O) was used.

2.5. Heavy Metal Determination

The determination of cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and lead (Pb) in plant samples was carried out using an inductively coupled plasma mass spectrometer (ICP-MS). The instrument used was the Agilent 7700x (Agilent, Santa Clara, CA, USA), which is equipped with a collision/ reaction cell (Octopole Reaction System, ORS), and was operated in no-gas and helium modes. The sample was introduced into argon (Linde Gas, Cracow, Poland) plasma via a MicroMist concentric nebulizer, quartz Scott double pass spray chamber and a quartz torch with a quartz injector. The operating conditions for the inductively coupled plasma mass spectrometry (ICP-MS) were optimized daily using the Tuning Solution (Agilent). The instrumental parameters were as follows: 1550 W for radiofrequency (RF) power, 15 L min^{-1} for plasma gas flow rate, 0.98 L min^{-1} for nebulizer gas flow rate, and 0.9 L min^{-1} for auxiliary gas flow rate. The ORS mode with helium gas (Linde Gas, Poland) was used in order to eliminate spectral interferences. To reduce non-spectral interferences, a 10 µg L^{-1} solution of 103 Rh was used as an internal standard. High-purity argon (99.999%) was used as a nebulizer, auxiliary, and plasma gas for the ICP-MS (Messer, Chorzów, Poland). An external calibration curve was prepared by preparing a set of 5 standard solutions in the concentration ranges of 0.05–50 µg L^{-1} for Cd, Cr, Ni, and Pb, and 0.05–100.0 µg L^{-1} for Cu.0–5 µg L^{-1}. Calibration solutions were prepared by diluting a 10 mg L^{-1} multielement stock solution in 5% HNO_3 (Multi-Element Calibration Standard 3, PerkinElmer, Waltham, MA, USA) [43].

2.6. Quality Assurance

To assess the precision and trueness of the analytical procedure-certified reference material (CRM), Trace Elements in Spinach Leaves (1515, NIST, Gaithersburg, MD, USA) and the method of standard additions were used [44]. The linearity of the calibration curves was calculated as a coefficient of correlation R, which was greater than 0.9996 for all analytes. The LOD was calculated according to the equation LOD = 3.3 SBL, where SBL is the standard deviation of repeated blank measurements. The LOD values were as follows: Cd 0.003 $\mu g\ g^{-1}$, Cr 0.006 $\mu g\ g^{-1}$, Cu 0.008 $\mu g\ g^{-1}$, Ni 0.006 $\mu g\ g^{-1}$, and Pb 0.006 $\mu g\ g^{-1}$. The LOQ was calculated as three times the LOD value. Precision values were calculated as the coefficient of variation (CV) (%), which ranged from 0.8% to 2.5% for all elements. Trueness was evaluated as recovery (%), which ranged from 97% to 103%, respectively. The results of the Student's t-test confirmed that there were no significant differences between the measured concentration ± standard deviation and the certified concentration ± standard uncertainty.

2.7. Ratios of Accumulation, Translocation and Contamination

Pollution of plants and soils with heavy metals was assessed by the bioconcentration factor (BCF), the translocation factor (TF), and the contamination factor (CF). The BCF reflects a plant's ability to accumulate and translocate heavy metals, and is calculated as the ratio between the concentrations of heavy metals in plants and in soils [45]. The heavy metal bioconcentration factor (BCF) in plants was calculated with the formula (1), which expresses the ratio between the concentration of trace elements in root samples and the concentration of trace elements in soil samples [46]:

$$\text{BCF} = \text{heavy metal concentration in roots (mg kg}^{-1}\text{ DW)/heavy metal concentration in the soil (mg kg}^{-1}\text{ DW)} \quad (1)$$

To assess the ability of heavy metals to move from plant roots to other organs, we calculated the translocation factor (TF), which is a measure of a compound's capacity for translocation. The TF can be used to evaluate the extent to which heavy metals are transferred from one plant organ to another [47]. With the following formula (2), according to Yu and Zhou [48], the translocation factor (TF) was calculated as the ratio between elements' concentration in leaves and their concentration in the roots:

$$\text{TF} = \text{heavy metal content in leaves (mg kg}^{-1}\text{ DW)/heavy metal content in roots (mg kg}^{-1}\text{ DW)} \quad (2)$$

The contamination factor (CF) is a useful single index for monitoring heavy metal contamination [49]. It provides an effective means of quantifying the extent to which heavy metals have contaminated a given area. The CF for heavy metals can be calculated using the following formula (3):

$$\text{CF} = \text{C}^{\text{i}}/\text{Cn}^{\text{i}} \quad (3)$$

where C^{i} is the mean accumulation of the element in the soil, and Cn^{i} is the reference level for the element. The value of contamination factor allows classification of the degree of pollution in following way:

- CF < 1—LCF—low contamination factor,
- 1 ≤ CF < 3—MCF—moderate contamination factory,
- 3 ≤ CF < 6—CCF—considerable contamination factor,
- CF ≥ —VHCF—very high contamination factor [49].

According to [50] the reference levels for heavy metals are: Cd—0.41 mg kg^{-1}; Cr—59.5 mg kg^{-1}; Ni—29 mg kg^{-1}; Cu—38.9 mg kg^{-1}; Pb—27 mg kg^{-1}.

2.8. Statistical Analyses

Statistical analyses were carried out using statistical software (R Core, 2014) and Statistica 13.1. A descriptive statistical analysis was performed to assess the concentrations of heavy metals in the examined plant species from different samples and also the concentration of defense system and physiological parameters. To assess the significance of differences between heavy metal levels in plant species, one-way analysis of variance (ANOVA) and post hoc Scheffé test were used. Data were visualized using heatmaps to compare the concentration of a particular group of elements in plants and soils at specific research sites, with two-dimensional variables (research sites, element) represented by colors. Specifically, darker colors correspond to higher concentrations, while lighter colors represent lower value. In addition, to discover distinctions and similarities among sites, sample types, and element accumulations, cluster analysis was also carried out.

3. Results

3.1. Soils Characteristics and Concentration of Heavy Metals in Soils

In research sites of Poznań, the soil pH ranged from 6.372 (high-density residential area) to 7.506 (industrial area), while the electrical conductivity (EC) ranged from 0.05 mS cm^{-1} (high-density residential area) to 0.221 mS cm^{-1} (park) (Table 2).

Table 2. Soil parameter at research sites (3 replications for each site).

Research Site	pH	EC [mS cm^{-1}]
Individual houses (POZ01)	6.886 ± 0.012	0.102 ± 0.002
Area near the lake (POZ02)	7.209 ± 0.013	0.101 ± 0.001
Area near the river (POZ03)	6.414 ± 0.031	0.088 ± 0.004
High-density residential area (POZ04)	6.372 ± 0.034	0.050 ± 0.003
Industrial area (POZ05)	7.506 ± 0.025	0.147 ± 0.004
Park (POZ06)	7.314 ± 0.032	0.221 ± 0.003
Old town (POZ07)	7.033 ± 0.023	0.096 ± 0.002
Agricultural land (POZ08)	7.353 ± 0.014	0.106 ± 0.004

The concentrations of various metals (Cd, Cu, Cr, Ni, and Pb) in soil samples were found to vary across different areas. Specifically, the highest concentration of Cr was observed near the river (POZ03), while the lowest concentration occurred in the high-density residential area (POZ04). For Cd, the highest concentration was found in the park soils (POZ06), while the lowest concentration was observed in the high-density residential area (POZ04). The lowest concentrations of Ni, Cu, and Pb were also observed in the high-density residential area (POZ04). On the other hand, their highest concentrations were observed in the industrial area (POZ05) (Table 3).

In the graph (Figure 2), the standardized results of heavy metal concentration in soils concerning land use were presented. Heavy metals concentration formed two main groups. The first group includes the industrial area (POZ05) and park (POZ06), and the second main group includes other research sites. From the intensity of the heatmap (the darker the color, the higher uptake of the heavy metal), it can be noted that soils from the industrial area (POZ05) and from the park (POZ06) compared with other research sites revealed an elevated level of all heavy metals (Cr, Cu, Ni, Cd, and Pb). However, from the intensity of the heatmap color in the soils of the industrial area (POZ05), these soils are noted to have a higher concentration of Pb, Ni, and Cu. Furthermore, in the park (POZ06), the soil is noted to have a higher concentration of Cd and Cu. Moreover, it can be noted that soils near the river (POZ03) revealed an elevated level of Cd and Cr (Figure 2).

3.2. Content of Heavy Metals in Plant Roots

Concerning roots, the highest concentration of Cr and Ni was noted in agricultural land (POZ08), and their lowest concentration was observed in the industrial area (POZ05). Cu and Cd's highest concentration was observed in the park (POZ06). On the other hand, for Pb, the highest concentration was observed in roots of agricultural land, and the lowest concentration was observed near the lake (POZ02) (Table 3).

Table 3. The HMs content (mg kg^{-1} DW) in soil and plants (mean ± SD), where different letters (a–f) indicate means are significantly different ($p < 0.05$) in the same row according to the post hoc Scheffé test.

HMs		**POZ01**	**POZ02**	**POZ03**	**POZ04**	**POZ05**	**POZ06**	**POZ07**	**POZ08**
soil	Cr	14.2 ± 1.22 ^{d}	8.69 ± 0.574 ^{de}	47.3 ± 4.62 ^{a}	5.714 ± 0.403 ^{e}	38.3 ± 4.75 ^{b}	21.4 ± 1.26 ^{c}	8.797 ± 0.158 ^{de}	8.487 ± 0.669 ^{de}
	Ni	10.9 ± 0.823 ^{c}	5.43 ± 0.391 ^{d}	10.7 ± 1.10 ^{c}	4.04 ± 0.285 ^{d}	22.3 ± 2.81 ^{a}	13.9 ± 0.778 ^{b}	5.70 ± 0.097 ^{d}	6.30 ± 0.567 ^{d}
	Cu	15.9 ± 1.25 ^{bc}	10.1 ± 0.652 ^{c}	21.3 ± 1.78 ^{b}	6.38 ± 0.274 ^{c}	78.5 ± 9.74 ^{a}	72.6 ± 4.22 ^{a}	20.2 ± 0.273 ^{b}	12.7 ± 1.13 ^{bc}
	Cd	0.321 ± 0.030 ^{de}	0.386 ± 0.010 ^{d}	0.971 ± 0.021 ^{b}	0.143 ± 0.010 ^{f}	0.853 ± 0.025 ^{c}	1.12 ± 0.123 ^{a}	0.322 ± 0.009 ^{de}	0.268 ± 0.003 ^{d}
	Pb	13.6 ± 1.08 ^{e}	12.0 ± 0.805 ^{e}	39.5 ± 3.33 ^{bc}	7.99 ± 0.445 ^{e}	90.4 ± 14.6 ^{a}	49.1 ± 3.3 ^{b}	27.7 ± 0.458 ^{cd}	15.4 ± 1.22 ^{de}
roots	Cr	32.5 ± 0.771 ^{b}	38.3 ± 2.25 ^{b}	35.9 ± 0.037 ^{b}	54.8 ± 2.24 ^{a}	7.10 ± 0.314 ^{d}	20.8 ± 0.717 ^{c}	18.3 ± 0.648 ^{c}	60.6 ± 4.71 ^{a}
	Ni	9.97 ± 0.322 ^{b}	11.2 ± 0.617 ^{b}	10.4 ± 0.383 ^{b}	15.6 ± 0.779 ^{a}	3.78 ± 0.081 ^{d}	6.5 ± 0.226 ^{c}	5.6 ± 0.179 ^{c}	16.8 ± 1.22 ^{a}
	Cu	6.47 ± 0.081 ^{f}	7.71 ± 0.482 ^{ef}	10.5 ± 0.485 ^{d}	6.98 ± 0.230 ^{ef}	19.5 ± 0.746 ^{b}	24.3 ± 1.04 ^{a}	15.1 ± 0.663 ^{c}	8.56 ± 0.688 ^{e}
	Cd	0.084 ± 0.003 ^{f}	0.336 ± 0.006 ^{c}	0.463 ± 0.035 ^{a}	0.178 ± 0.008 ^{e}	0.404 ± 0.008 ^{b}	0.498 ± 0.023 ^{a}	0.257 ± 0.006 ^{d}	0.156 ± 0.006 ^{e}
	Pb	1.06 ± 0.020 ^{f}	1.00 ± 0.055 ^{f}	3.25 ± 0.121 ^{c}	1.45 ± 0.081 ^{e}	8.65 ± 0.210 ^{a}	4.74 ± 0.169 ^{b}	4.66 ± 0.186 ^{b}	2.04 ± 0.166 ^{d}
leaves	Cr	3.48 ± 0.039 ^{e}	7.49 ± 0.382 ^{d}	15.46 ± 0.327 ^{c}	15.8 ± 1.047 ^{c}	52.4 ± 3.20 ^{a}	9.53 ± 0.273 ^{d}	35.8 ± 1.25 ^{b}	36.9 ± 1.38 ^{b}
	Ni	1.33 ± 0.037 ^{e}	2.71 ± 0.156 ^{d}	5.03 ± 0.170 ^{c}	4.92 ± 0.253 ^{c}	15.3 ± 0.777 ^{a}	3.06 ± 0.144 ^{d}	11.2 ± 0.613 ^{b}	10.9 ± 0.326 ^{b}
	Cu	6.28 ± 0.111 ^{e}	6.40 ± 0.358 ^{e}	7.49 ± 0.173 ^{d}	6.54 ± 0.466 ^{e}	11.2 ± 0.659 ^{b}	13.0 ± 0.377 ^{a}	8.58 ± 0.301 ^{c}	6.60 ± 0.277 ^{de}
	Cd	0.008 ± 0.003 ^{e}	0.012 ± 0.001 ^{e}	0.061 ± 0.007 ^{b}	0.032 ± 0.003 ^{d}	0.109 ± 0.003 ^{a}	0.044 ± 0.006 ^{c}	0.040 ± 0.002 ^{cd}	0.111 ± 0.004 ^{a}
	Pb	0.176 ± 0.004 ^{e}	0.340 ± 0.017 ^{de}	0.955 ± 0.025 ^{c}	0.416 ± 0.036 ^{d}	3.60 ± 0.232 ^{a}	1.04 ± 0.028 ^{c}	2.48 ± 0.109 ^{b}	1.06 ± 0.040 ^{c}

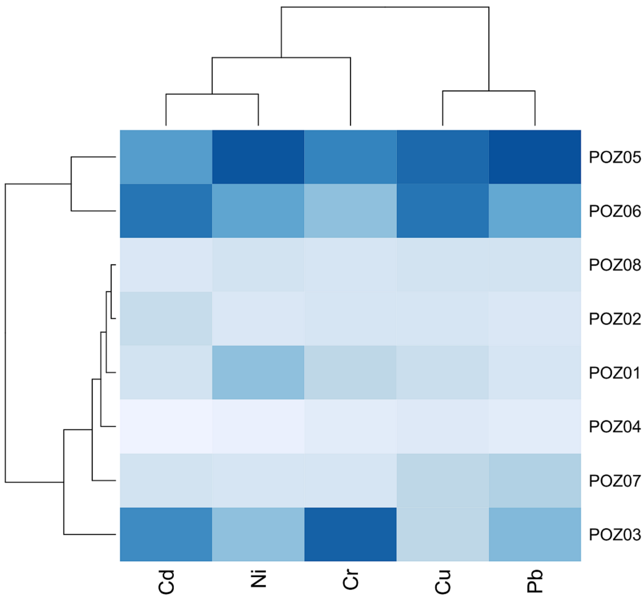

Figure 2. Heatmap and cluster analysis of heavy metal (Cd, Cu, Cr, Ni and Pb) concentration in soils from all research sites.

The standardized results of the analysis of heavy metals concentration in roots in relation to land use revealed the grouping of heavy metals into two main groups (Figure 3). The first main group included two subgroups; the first one includes a high-density housing area (POZ04) and agricultural land (POZ08), while the second subgroup includes individual houses (POZ01), near the lake (POZ02), and near the river (POZ03). The second main group included two subgroups; the first included the industrial area (POZ05), the second one—the park (POZ06) and the old town (POZ07). From the intensity of heatmap color (the more the intensity of the color is dark, the more the uptake of the heavy metal is high), it can be noted that the highest Cr and Ni concentration is noted in the agricultural land (POZ08) and the high-density housing area (POZ04). Pb and Cu's highest concentration was noted in the industrial area (POZ05), park (POZ06), and the old town (POZ07). At the same time, Cd's highest concentration was observed in the park (POZ06), near the river (POZ03), and in the industrial area (POZ05) (Figure 3).

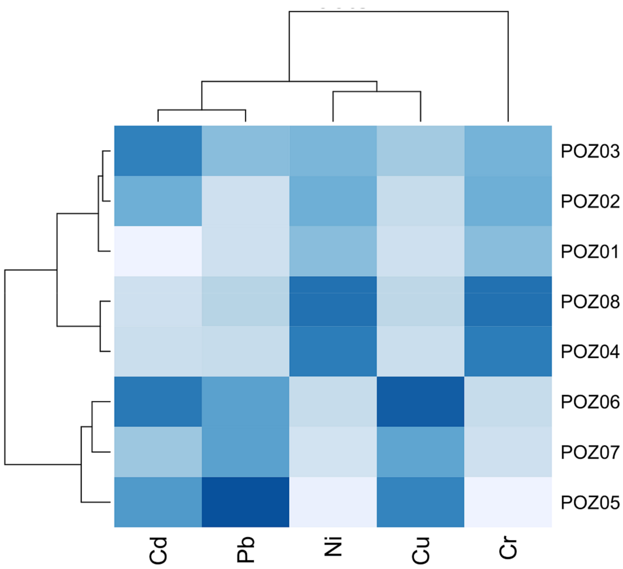

Figure 3. Heatmap and cluster analysis of heavy metal (Cd, Cu, Cr, Ni and Pb) concentration in roots from all research sites.

3.3. Content of Heavy Metals in Plant Leaves

In leaves, the highest concentration of Cr, Ni, and Pb was observed in the industrial area (POZ05), and their lowest concentration was observed in the individual houses (POZ01). For Cu, the highest concentration was observed in the park (POZ06), and the lowest concentration was observed in the agricultural land (POZ08). On the other hand, concerning Cd, the highest concentration was observed in agricultural land (POZ08), and the lowest concentration was observed in individual houses (POZ01) (Table 3).

The standardized results of analyzed heavy metal concentrations in *T. pretense* leaves in relation to land use revealed the grouping of heavy metals into two main groups. The first group includes the industrial area (POZ05), the old town (POZ07), and the agricultural land (POZ08), and the second group includes other research sites. From the intensity of the heatmap color (the darker the color, the higher the uptake of the heavy metal), it can be noted that Pb, Ni, and Cr were revealed to be present in higher concentrations in the leaves of the industrial area (POZ05). In addition, it can be noted that Cu revealed a higher concentration in the industrial area (POZ05), but the concentration is lower than in the park leaves (POZ06). In addition, Cr and Ni revealed higher concentrations in the old town (POZ07) and the agricultural land (POZ08) (Figure 4).

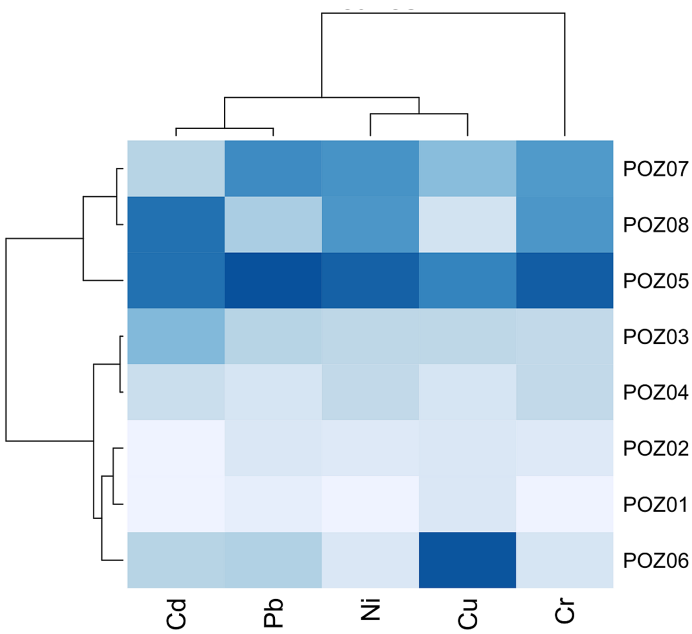

Figure 4. Heatmap and cluster analysis of heavy metal (Cd, Cu, Cr, Ni and Pb) concentration in leaves from all research sites.

3.4. Bioconcentration (BCF), Translocation (TF) and Contamination Factor (CF)

The bioconcentration factor (BCF) of chromium (Cr) in comparison with other heavy metals (Ni, Cu, Cd, and Pb) exceeded a value of 1, in most of the research sites except those near the river (POZ03), industrial area (POZ05), and park (POZ06). The nickel (Ni) bioconcentration factor (BCF) exceeded a value of 1 near the lake (POZ02), in the high-density residential area (POZ04), and on the agricultural land (POZ08). In contrast, the bioconcentration factor of Cu and Cd only exceeded a value of 1 in the high-density residential area (POZ04). On the other hand, the bioconcentration factor of Pb did not exceed the value of 1 in any research site. While concerning the translocation factor (TF), Cr and Ni recorded the highest translocation factor. Both elements exceeded the value of 1 in the industrial area (POZ05) and the old town (POZ07). Whereas other heavy metals did not exceed the value of 1 in any research site. Moreover, for the contamination factor (CF), Cr, Ni, Cu, Cd, and Pb showed different contamination values at all research sites. Cr and Ni, all research sites, were recorded to have a low contamination factor (LCF). In the case of

Cu, the medium contamination factor (MCF) was recorded in the industrial area (POZ05) and the park (POZ06), whereas other research sites recorded a low contamination factor (LCF). In addition, as for the Cd, a medium contamination factor (MCF) was recorded in the river (POZ03), industrial area (POZ05), and the park (POZ06), while a low contamination factor (LCF) was recorded in other research sites. Additionally, in the case of Pb in the industrial area (POZ05) a considerable contamination factor (CCF) was recorded, while in the river (POZ03), park (POZ06), and the old town (POZ07) a medium contamination factor (MCF) was noted, whereas other research sites recorded a low contamination factor (LCF) (Table 4).

Table 4. The bioconcentration, translocation and contamination factor of Cd, Cu, Cr, Ni and Pb in research sites.

Research Sites	BCF Where: The Highlighted Values Mean Concentration in Roots Biomass					TF Where: The Highlighted Values Mean Effective Metals Translocation within the Plant					CF Where: The Highlighted Values Mean Moderate or Considerable Contamination Factors				
	Cr	**Ni**	**Cu**	**Cd**	**Pb**	**Cr**	**Ni**	**Cu**	**Cd**	**Pb**	**Cr**	**Ni**	**Cu**	**Cd**	**Pb**
POZ01	**2.291**	0.919	0.412	0.259	0.078	0.107	0.135	0.972	0.088	0.167	0.238	0.371	0.404	0.793	0.500
POZ02	**4.448**	**2.085**	0.765	0.87	0.084	0.196	0.241	0.829	0.038	0.339	0.144	0.185	0.257	0.942	0.439
POZ03	0.779	0.976	0.492	0.48	0.082	0.422	0.488	0.718	0.133	0.297	0.731	0.365	0.543	**2.374**	**1.451**
POZ04	**9.679**	**3.907**	**1.104**	**1.26**	0.184	0.288	0.312	0.931	0.176	0.284	0.095	0.138	0.162	0.347	0.293
POZ05	0.186	0.171	0.249	0.477	0.095	**7.428**	**4.038**	0.572	0.269	0.418	0.638	0.762	**2.003**	**2.069**	**3.369**
POZ06	0.973	0.473	0.337	0.442	0.097	0.46	0.465	0.535	0.09	0.222	0.357	0.474	**1.853**	**2.734**	**1.807**
POZ07	**2.077**	0.985	0.748	0.797	0.169	**1.956**	**1.997**	0.569	0.156	0.532	0.148	0.197	0.519	0.787	**1.027**
POZ08	**7.004**	2.609	0.668	0.59	0.13	0.618	0.666	0.777	0.712	0.53	0.143	0.218	0.327	0.652	0.573

4. Discussion

In the presented study, as a potential bioindicator for heavy metals in an urban area, *Trifolium pratense* was used. Based on the obtained data, the spatial distribution of heavy metals (Cd, Cu, Cr, Ni and Pb) in the analyzed research sites was irregular. These differences in heavy metal concentrations in urban areas can result from many factors. Besides parameters, such as the type and number of vehicles and traffic that affect air pollution in urban areas [1], heavy metal concentrations can also be different due to other human activities and the natural background (land use propose, green areas, construction zones, industrial sites, roadside), demographic factors, soil type, and its parameters [25]. In addition, topography and meteorological conditions will determine their dispersion and transformation. Therefore, there is considerable diversity in the content of metals in urban soils in various locations [51]. In the presented study, the research sites were divided into two groups based on the content of heavy metals in the soil (first: industrial area and park; second: individual houses, near the lake, near the river, high-density housing area, the old town, and agricultural land).

The soils of the industrial area (POZ05) were characterized by the highest concentration of Pb (90.4 mg kg^{-1}) and Cu (78.5 mg kg^{-1}). In addition, Ni (22.2 mg kg^{-1}) in this research site was higher than in other research sites. Lead (Pb), which is one of the most common elements [52], has many applications in various fields, such as industrial, agricultural, and household uses [53]. The high concentration of Pb in the soils of the industrial area (POZ05) can be attributed to its widespread use in various industries, including mining, smelting, and battery manufacturing. Similarly to Pb, metals such as Cu, Cd, and Zn can originate from sewage sludge, landfills, vehicle transport, geochemical processes, and industries [52]. Furthermore, the high concentrations of Cu and Ni in the soils of the industrial area can be attributed to the discharge of industrial effluents, as well as anthropogenic activities such as vehicular emissions [53]. The high concentrations of Pb, Cu, and Ni found in the soil of the industrial area suggest that anthropogenic activities in the area have significantly contributed to heavy metal pollution and the relation between land use and heavy metals.

The plant's ability to accumulate and tolerate heavy metals makes it a promising tool for monitoring selected heavy metals in the soil [5] of urban areas. That land use may have a relationship with the amounts of heavy metals is also concluded by Degórska [54], Lisiak-Zielińska et al. [25], and Adamu and Nganje [55]. Degórska [54] also found a significant relationship between the content of heavy metals in soil and land use in her investigation. Urban environments, which have experienced industrialization and urbanization, have shown a considerable increase in heavy metals such as Pb, Cd, Cu, and Zn on streets. Lisiak-Zielińska et al. [25] utilized *Taraxacum officinale* as a bioindicator of heavy metals in their study. The results showed that the heavy metal content in soil was primarily related to land use type, as determined by the geochemical background. Similarly, Adamu and Nganje [55] concluded that the elevation of heavy metal content in the surface soils of Benue State was related to urbanization and poor land planning and use. These findings underscore the importance of effective land management and planning in mitigating heavy metal pollution and preserving soil quality.

Furthermore, concerning Cd and Cr, the soils of the park (POZ06) were noted to have the highest Cd (1.13 mg kg^{-1}) concentration, and those near the river (POZ03) were noted to have the highest Cr (35.9 mg kg^{-1}) concentration. The cement-soil road bases in this area were probably the cause of the increased amounts of this element because chromium is mainly leached from the cement–ground foundation. The concentration of Cd can be related to the fact that these two areas are quite popular for recreation. Tourism activities, including the intensification of traffic and other related human activities, have been shown to contribute to the pollution of cadmium (Cd). As a result, it is clear that the occurrence and cycling of Cd in tourism environments are common phenomena [56]. The study conducted by Lisiak-Zielińska et al. [25] in Poznan also found similar results, where *Taraxacum officinale* accumulated higher amounts of Cd in areas near the lake, which is a popular recreational site. The increased traffic and other human activities associated with tourism were identified as contributing factors.

The roots of plants in the agricultural land (POZ08) were found to have higher concentrations of Cr and Ni. Heavy metals from human activities can significantly influence agricultural soils, leading to the bioconcentration of Cr and Ni in the roots of plants. The bioconcentration factor (BCF) for Cr and Ni in agricultural soil was found to exceed the value of 1, indicating that these elements were transported from the soil to the plant roots. The metals taken from the soil are initially stored in the plant roots. These metals can then be translocated to the aboveground plant parts through the xylem sap, which is driven by the plant's respiration power [57]. These findings are supported by other studies [58,59], which demonstrate that chromium can be permanently bound by living cells in plant roots, leading to low chromium content in aboveground parts of the plants. Similarly to this study, extreme concentrations of Cr, Ni, and Cu have been found in agricultural soils in Qatar [60] and other soils in different land-use types, that are affected by human activities, including roadside, urban and industrial areas [61].The higher concentrations of Cr and Ni found in the roots of plants in the agricultural land (POZ08) could be attributed to the application of fertilizers, which can contain heavy metals such as chromium and nickel. Several studies have reported the occurrence of heavy metals in fertilizers, and their potential impacts on soil quality and crop productivity [62–64].

The highest values of the translocation factor for Cr and Ni were observed in the industrial area (POZ05) and the old town (POZ07), which could be linked to the presence of manufacturing plants and intensive traffic in these areas. Previous studies have also reported similar findings, highlighting the role of industrial activities and traffic in the emission of heavy metals into the environment and their subsequent accumulation in plants [14,65]. The main sources of Ni can also be related to the use of cadmium–nickel batteries and local industry, which have been shown to contribute to the contamination of soil and water with heavy metals [66]. A similar correlation between the translocation factor and land use was also noted by Angelova and Ivanov [67], who found higher values of this index in areas with heavy traffic. Cadmium and copper were found to accumulate mainly in the roots. In the present study, the highest concentration of Cu and Cd was found in the roots of plants in the park (POZ06), which is located in close proximity to the highway and has undergone urbanization.

Furthermore, in the leaves, the industrial area (POZ05) showed a high concentration of Cr, Ni, and Pb, compared to other research sites, indicating poor air quality and proximity to pollution sources [68]. The concentration of heavy metals in leaves can be attributed to their uptake through the stomata or the adsorption of atmospheric deposition [14,69]. The elevated concentrations of Cr, Ni, and Pb detected in the leaves of the industrial area (POZ05) are in agreement with the emissions from industrial activities and transportation, recognized as significant contributors to heavy metal pollution in urban areas. Additionally, the close proximity of the industrial area to other potential sources of heavy metals in the surrounding environment may also have contributed to the observed levels of heavy metals in the leaves. These findings are consistent with the results of Farooq et al. [70], who conducted a study on heavy metal pollution in the vicinity of industrial areas. Their study showed that the concentration of heavy metals was significantly higher in the leaves of various vegetables grown in areas close to industrial zones compared to areas further away, which provides further support for the idea that industrial activities and transportation are significant sources of heavy metal pollution in urban areas. In contrast, the leaves of agricultural land (POZ08) showed a higher concentration of Cd, which may be related to agricultural activities, especially the use of fertilizers. The occurrence of cadmium in soil is predominantly influenced by the application of phosphate fertilizers [71]. The deposition of Cd in crops can pose health risks to humans; hence, it is crucial to monitor and control its levels in food crops [72].

5. Conclusions

Our research revealed that the order of heavy metal concentration in soil was Pb > Cu > Cr > Ni > Cd. Specifically, the industrial area had the highest contamination levels of Pb, Ni, and Cu. In roots and leaves, the order of heavy metal concentration was Pb > Cu > Cr > Ni > Cd, where the roots of *T. pratense* in agricultural land had the highest concentration of Cr, Ni, and Cu. In addition, the leaves of *T. pratense* had the highest concentration of Cr, Ni, and Pb in the industrial area. Furthermore, the consistent pattern of heavy metal concentration observed in the roots and leaves of *T. pratense* may be due to the specific physiological and biochemical mechanisms involved in heavy metal uptake, translocation, and concentration within the plant. Our study demonstrated the potential of *T. pratense* as a bioindicator of heavy metal contamination in urban areas. However, further research is necessary to validate the effectiveness of *T. pratense* as a bioindicator in controlled conditions as well as in other urban areas and throughout various seasons.

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