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*Full Length Research Paper*

# Assessment of heavy metal content in soil and grasslands in national park of the lake plateau of the N. P. "Durmitor" Montenegro

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**Investigations were conducted in the N.P. Durmitor area of Montenegro where anthropogenic activities are negatively impacting forests and grasslands. According to meteorological data, this area has been polluted with heavy metals via aero deposition. The aim of this paper was to present an assessment which describes the content of heavy metals in the top soil layer (0 to 20 cm) and the corresponding vegetation cover. Linear regression was used to explain the relationship between heavy metals in the soils and plants. Statistically significant correlation coefficients were determined between content of heavy metals (Zn, Cu, Pb and Cd) in the soil and plants. Since a statistical significant correlation existed, it was possible to suggest that, increasing the content of heavy metals in the soil will influence an increase in the content of heavy metals in the vegetation cover. Also, transfer factor (TF) values were used to assess the concentrations of elements in plants taken from the soil. The highest transfer factors were obtained for Zn and Cu followed by Cd, with Pb been the least. According to values of TF in the studied conditions, the content of Zn, Cu and Cd were greater in the mobile fractions than in the non-mobile fractions, but the content of Pb was not mobile and available for plants. Thus, we were able to conclude that, soil properties and the form of heavy metals in the soil influence the uptake of heavy metals from the soil by the vegetation cover.**

**Key words:** Soil, grassland vegetation, heavy metals, relationship, transfer factor.

## INTRODUCTION

The N.P. Durmitor region is a rare and authentic work of nature, situated in the north-western Montenegro (Figure 1). It was proclaimed the National Park in 1952. The Park occupies a large area of the Durmitor massif covering a total of 39,000 hectares, with the canyons belonging to the rivers Tara, Draga, Susica and also the upper part of the river Komarnica canyon. Since 1980, the Park and the Tara Canyon have been under the protection of UNESCO. According to previous researches (UNESCO, 2005), the flora of NP Durmitor consists of 700 species of vascular plants. This great species richness makes Durmitor one of the most important refugia of arctic-tertiary flora. Numerous endemic, relic and endemic-relic species are the best examples including: *Verbascum durmitoreum, Gentiana levicalix, Edraianthus glisicii, Edraianthus sutjeskae, Valeriana braun-blanquetii, Daphne malyana, Carum carvi, Saxifraga prenja, Trifolium durmitoreum, Oxytropis dinarica, Silene graminea, Plantago durmitorea* and *Viola zoysii*. There are 37 taxa endemic to the area and 6 specific to Durmitor (Figure 2). The vegetation zones range from deciduous valley forests, Mediterranean conifer forests, sub-alpine *Fagetum subalpinum* and *Pinetum mughi* forests, subalpine heath and peat bogs, to alpine meadows. The dominant species include *Pinus sylvestris*, *Pinus resinosa, Pinus mugo, Abies alba, Fagus sylvatica, Betula alba, Juniperus communis* and *Pinus heldreichii*. The Park contains one of the last virgin forests of tall black pine *Pinus nigra* ssp. *illyrica* in Europe, growing in soils where beech woodland would be most commonly expected to develop (UNESCO, 2005).

In the Durmitor region, the pressure of anthropogenic

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**Figure 1.** Study area of Mountain Durmitor in Montenegro.

**Figure 2.** Grassland of the Lake Plateau at 1350 m.

activities (urbanization and exploitation of nature) have exerted a greater negative impact on the highly complex and important forests and grassland ecosystems than on water quality resources, soil resources and loss of biodiversity via the collection, use and trade of comercially important species. Overall, the mountain and sub mountain meadows and pastures have been significantly negatively impacted in the course of the 20th century.

Inputs of trace elements in terrestrial ecosystems via anthropogenic activities have been increasing since the last century. Different types of pollutants have entered into the ecosystems via dry and wet deposition (He, 2005). The Durmitor area has undergone extensive management of grasslands and pollution through agricultural fertilization which is also considered to be an overall negative impact. The wet deposition of pollutants mainly impacts terrestrial ecosystems in an indirect manner through the soil; however, there is also a direct impact of aero pollutants on the vegetation cover. From 1990 to 2001 the deposition of lead decreased by 2.5 times, while cadmium and quicksilver concentrations decreased by 1.5 times. The greatest deposition of heavy metals in South Eastern Europe is greater by a few orders of magnitude than those found in Scandinavia (Kadović and

**Figure 3.** Typical soil profile of Rendzic Leptosol on morainic deposits.

Knežević, 2004). The amount of essential elements in the soil, if found to be low, is considered deficient, while a higher amount is considered toxic. For non-essential metals a gradation of deficiency is not referenced, and are only designated as either optimal or toxic (Adriano, 2001). Knowing the concentration of metals in a soil profile is valuable for many scientific disciplines, but it is most important to understand the relationship between the total concentration of metals and their biological effect (Herbert and Yin, 1998). The availability of heavy metals to plants, primarily depends on adsorption – desorption reactions between the solid phase and the soil solution (Leslie, 2002). The movement of heavy metals through the soil is directly caused by adsorption processes (Vogeler, 2001) and chemical speciation in the soil is important for their activity and is dependent on several factors including the initial concentration, the presence of other ions such as chloride and the degree of organic complexation (Lumsdon et al., 1995). Overall, the impact of aeropollution increases the concentration of metals in the soil, which results in a greater concentration of heavy metals that become available to plants (Påhlsson, 1989).

Heavy metals, which by wet and dry deposition reach terrestrial ecosystems, accumulate in the surface layers. Heavy metals in the soil become mobile, through dilution and also through biological and chemical degradation of organometallic complexes (Kabata-Pendias and Pendias, 2000).

The quantity of heavy metals that can be found in the soil depends on the plant species, soil properties, soil pH and on the properties of the elements of interest (Kashem and Singh, 2002). Likewise, root exudates have an important role in mobilizing heavy metals because they form complexes with stable forms which become available to plants (Vrbničanin et al., 2004). subsequently, the addition of elements like N and P change the solubility of Cd and Zn making them mobile and available for plant uptake (Kashem and Singh, 2002).

In addition to the indirect uptake of heavy metals from the soil, heavy metals are available to plants through their foliage by way of aerodeposition, although, this mechanism has not yet been explained in detail (De Vries and Bakker, 1998). Kadović and Knežević (2002) suggested that, prior to the incorporation of heavy metals on the surface of photosynthetic organs, the transformation process of migrating forms of elements in the ecosystem results in the transfer of a significant portion of metals in soluble substances.

The aim of this paper was to present the content of heavy metals in the surface soil layers (0 to 10 and 10 to 20 cm) and their vegetation cover and relationship between the content of heavy metals in the soil and plants. Also, values of the transfer factor are used to assess the concentrations of elements in plants taken from soil.

## MATERIALS AND METHODS

### Description of the locality

The total area of the Lake Plateau is 5105 ha. The alpine grasslands are the most dominant ecosystems on the Plateau, occupying 3671 ha or 72% of the forests (non grasslands) and 1431 ha or 28% of the total area.

Climate conditions were typically alpine with cold mean annual temperatures. According to the isotherm map, the mean annual temperature (MAT) varies between 4 to 6°C, with an average of 5°C. Annual precipitation is 1,458 mm and during the growing season the precipitation was 618 mm or 42.3% (Anđelić, 2001).

The unique climate and vegetation types, together with possible negative impacts of human disturbance, make the plateau an interesting region for investigating spatial patterns and environmental controls of SOC storage in high-altitude ecosystems. In this study, we conducted two field sampling campaigns during the summer (July) of 2009, to investigate the soils and vegetation.

### Soil and biomass survey

The site was represented by rendzic leptosol on morainic deposits. On this substrate, the soils were weakly to moderately skeletal and the percentage of skeleton increased with depth. The soil textual class was sandy loam. The dominant fractions were fine sand and silt, the percentage of colloid clay accounted for maximally 15%. Soil pH ranged from weak acid to weak alkaline, in the most samples and based on the pH in the water, they belonged to the class of neutral soils (Fuštić and Đuretić, 2000).

In order to estimate the storage and patterns of HM in alpine grasslands, we sampled 16 soil profiles on the Lake Plateau of Mountain Durmitor (Figure 3).

At each sampling site, soil pits were excavated to collect the samples for analyses of physical and chemical properties. For each pit, soil samples were collected at the depths of 0 to 10 and 10 to 20 cm. Bulk density samples were obtained for each layer using a standard container with 100 $cm^3$ in volume (55.50 mm in diameter and 41.40 mm in height) and weighed to the nearest 0.1 g. Soil moisture was measured gravimetrically after 24 h of desiccation at 105°C. Bulk density was calculated as the ratio of the oven-dry soil mass to the container volume. Soil samples for C analysis were air-dried, sieved (2 mm mesh), handpicked to remove fine roots and then ground in a ball mill. SOC was measured using Tyurin method (for mineral layers). The soil granulometric fractions were separated using the combined method of sieving by 0.2 mm mesh sieves and by the pyrophosphate pipette B-method, after the removal of organic matter and calcium carbonates. Additionally, all plants in three plots (0.50 × 0.50 m) at each site were harvested to measure the aboveground biomass (AGB). Biomass samples were oven-dried at 65°C to a constant weight, and weighed to the nearest 0.1 g.

The heavy metal content was determined in mixed grasses, leguminoses and other herbaceous plants. For dominant plant species analysis of heavy metals content was performed. Total heavy metal content was determined by AAS on Varian AA-10. The conservation and preparation of samples was performed by extraction with HCl, $HNO_3$ and $H_2O_2$ (3:1:2).

#### Floristic survey

Floristic research was performed in 2009. All plants were collected, identified and vouchers were stored at the herbarium of the Department of Landscape Architecture and Horticulture, Faculty of Forestry, University of Belgrade, Serbia. The plant material was identified using relevant literature (Josifović, 1970-1977; Tutin et al., 1964-1980; Sarić and Diklić, 1986; Sarić, 1992).

#### Statistical analyses and calculations

Ordinary least squares (OLS) regression analyses were conducted to evaluate the relationships between HM content as the dependent variable and the heavy metals content in soil as independent variables. Correlation coefficients were calculated with Statistical software (Statgraphics Plus) according to the least squares method. Transfer factor (TF) was calculated for the heavy metals according to the formula:

TF = $Cp$ (mg $kg^{-1}$ dry wt) / $Cts$ (mg $kg^{-1}$ dry wt),

Where, $Cp$ is the plant metal content and $Cts$ is the total metal content in the soil.

This term was adopted from the discipline of radio ecology and was defined by Freitag (1986, cit. Puschenreiter and Horak, 2000). Transfer factors (TF) of heavy metals from soil to vegetation are one simple way to explain human exposure to metals through the food chain (Prabu, 2009). Values of TF are used for many assessment models to predict the concentrations of elements in plants taken from soil (Chojnacka et al., 2005). According to this theory, the concentration of some elements in the plants has linear relationships with some concentrations in the soil. Transfer factors (TF) of heavy metals from soil to vegetation can only be evaluated when a linear relationship is observed between the soil and plant concentrations (Chojnacka et al., 2005).

## RESULTS

The study soil is typologically classified as Rendzic Leptosol on morainic deposits of various depths and the morphological composition of pits was designated as A-C. Soil depths were measured to 50 cm. Limestone fragments were found in the pit.

The soil textural class was defined as loam; a granular soil structure and physical characteristics were considered favorable. The reaction of the soil was moderately acidic to acidic. The soil was rich in humus and the humus content was found to decrease with depth favorably. Also, the contents of the total nitrogen and phosphorus were high.

Table 1 shows the basic chemical properties of the soil layers of 0 to10, 10 to 20 cm and their statistically basic values and Table 2 shows the heavy metals content (Zn, Cu, Pb i Cd) of the soil layers (0 to 10 and 10 to 20 cm) with statistically basic values.

The measured content of Zn in the soil ranged from 20.9 to 354.6 mg/kg, Cu content was from 5.5 to 51.7 mg/kg, Pb content was from 13 to 41.5 mg/kg and Cd was from 0.16 to 2.9 mg/kg. The contents of Zn and Cu in the soil were balanced or in some cases higher at a depth of 10 to 20 cm than at the 0 to 10 cm layer, while the contents of Pb and Cd decreased with depth.

### Species composition

The grasslands were comprised of varying numbers of plant species of different morphological, biological and production characteristics. The floristic mixture of the study grasslands consisted of the species of the families Gramineae, Leguminosae, Compositae, Rosaceae, Caryophyllaceae, Cyperaceae, Juncaceae, etc.

The percentage of individual grassland components was: 29.2% grasses, 15.4% leguminoses and 55.4% of other herbaceous plants. Above ground biomass (AGB) varied markedly across the 16 sampling sites. AGB for alpine meadows ranged from 76.20 to 173.68 g/$m^2$, with an average of 114.08 g/$m^2$.

In the mixture of herbaceous plants, Zn content ranged from 24.74 to 46.37 mg/kg, Cu content was 5 to 13.5 mg/kg, Pb content was 1.9 mg/kg and Cd content was 0.63 mg/kg (Table 3).

In Figure 4 (a, b and d) the linear regression equation (Y = a + b * X) is shown of certain elements and their content as a function of the content of elements in the soil.

Statistical parameter regression analyses for the studied conditions are shown in Table 4.

Since the P-values for Zn and Cd were less than 0.01, there were statistically significant relationships between variables at the 99% confidence intervals. Since the P-values for Pb and Cu were less than 0.05, there was a statistically significant relationship between variables at the 95% confidence interval.

The R-squared statistic indicated that, the model as fitted explains 40.09% of the variability in content of the meadow for Zn (30.70%), Cu (34.06%) Pb and Cd (41.64%). The correlation coefficients equaled 0.63 (Zn), 0.58 (Cu), 0.58 (Pb) and 0.64 (Cd), indicating moderately strong relationships between the variables. The content of heavy metals in the plants increased with the

**Table 1.** pH, content of organic carbon, total nitrogen and phosphorus and C: N in the soils of the Lake Plateau, Mountain Durmitor (n= 16).

| Depth (cm) | Value | pH | | C | TN | TP | C:N |
|---|---|---|---|---|---|---|---|
| | | $H_20$ | $CaCl_2$ | g/kg | | | |
| 0-10 | Min | 4.95 | 3.94 | 32.4 | 2.8 | 0.2 | 8.0 |
| | Max | 7.50 | 6.48 | 74.6 | 8.2 | 3.3 | 12.5 |
| | Mean | 6.10 | 5.31 | 54.5 | 5.4 | 2.0 | 10.32 |
| | S.D. | 0.72 | 0.80 | 1.50 | 1.80 | 0.9 | 1.20 |
| 10-20 | Min | 5.00 | 4.17 | 15.6 | 1.5 | 0.0 | 7.71 |
| | Max | 7.27 | 6.70 | 61.0 | 6.5 | 4.0 | 11.37 |
| | Mean | 6.08 | 5.63 | 38.6 | 4.3 | 1.9 | 9.15 |
| | S.D. | 0.88 | 0.76 | 1.33 | 1.46 | 0.85 | 1.08 |

**Table 2.** Heavy metal content in soils of the Lake Plateau Mountain Durmitor (n= 16).

| Depth (cm) | Value | Zn | Cu | Pb | Cd |
|---|---|---|---|---|---|
| | | mg/kg | | | |
| 0-10 | Minimum | 25.99 | 5.50 | 13.50 | 0.16 |
| | Maximum | 250.45 | 44.60 | 41.49 | 2.895 |
| | Mean | 100.77 | 23.37 | 27.97 | 1.38 |
| | S.D. | 55.26 | 8.86 | 7.34 | 0.93 |
| 10-20 | Minimum | 20.99 | 5.50 | 16.99 | 0.16 |
| | Maximum | 353.65 | 51.70 | 32.99 | 2.895 |
| | Mean | 104.74 | 23.55 | 23.66 | 1.24 |
| | S.D. | 79.78 | 12.42 | 6.21 | 0.97 |

**Table 3.** Content of heavy metals and value of transfer factor in meadow vegetation (mixed) of the Lake Plateau, Mountain Durmitor (n= 16) and value of transfer factor.

| Mixed plant | Zn | Cu | Pb | Cd | Zn | Cu | Pb | Cd |
|---|---|---|---|---|---|---|---|---|
| | $mg/kg^{-1}$ | | | | TF | | | |
| Minimum | 24.74 | 5.00 | 0.00 | 0.00 | 0.13 | 0.17 | 0.00 | 0.00 |
| Maximum | 46.37 | 13.47 | 1.90 | 0.63 | 1.97 | 1.36 | 0.07 | 2.31 |
| Mean | 31.74 | 8.25 | 0.83 | 0.35 | 0.45 | 0.43 | 0.03 | 0.37 |
| S.D. | 6.22 | 1.90 | 0.68 | 0.37 | 0.42 | 0.27 | 0.02 | 0.54 |

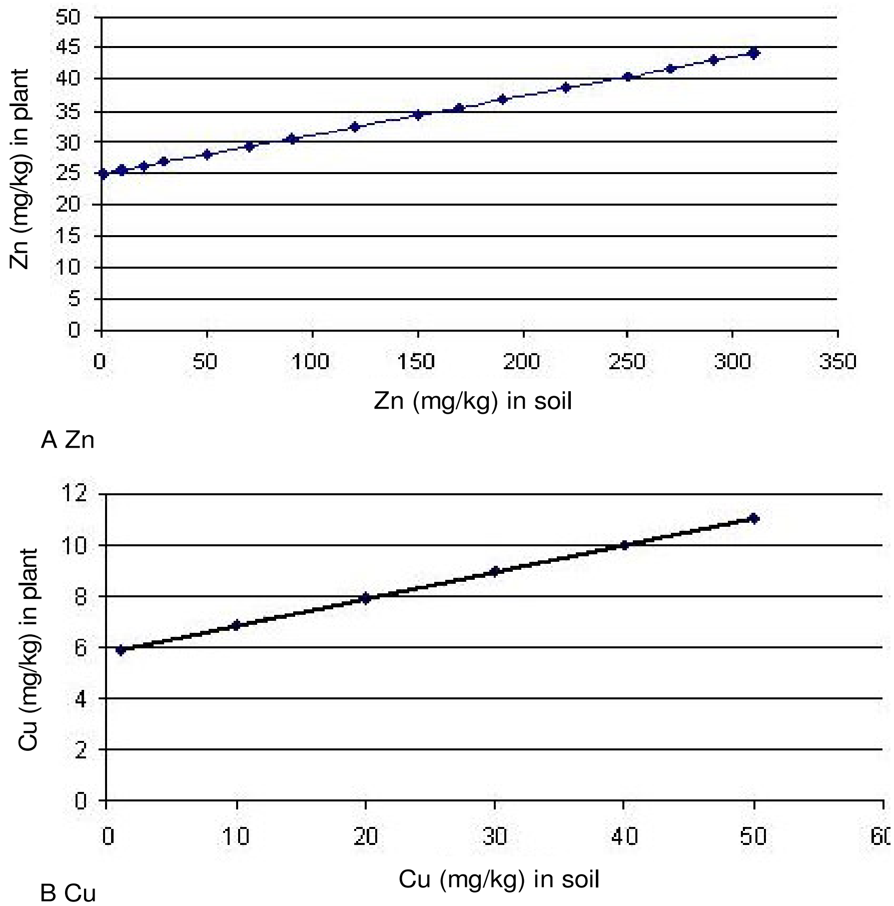
increasing content of heavy metals in the soil and with regard to linearity dependence (Table 4). The highest transfer factors were obtained for Zn and Cu followed by Cd, with Pb been the least (Table 4)

## DISSCUSION

Heavy metals occur in the soil as exchangeable and adsorbed and are found on soil colloids. They are specifically-adsorbed, bound in various chemical compounds (oxides, carbonates, phosphates, sulphides) and are structurally bound in silicates (primary and secondary minerals) (Adriano, 2001). Different factors affect the bonding of heavy metals to the soil and it is difficult to estimate the heavy metal load in the soil. The load of heavy metals in the soil can be known to some extent, by monitoring their content in the soil.

According to meteorological data in 2001, the total deposition of Pb was 1.2 to 1.6 $kg/km^2/god$ and total deposition of Cd was 25 to 38 $g/km^2/god$ in the Durmitor area (Kadovic and Knezevic, 2004). This concentrations of deposited Cd are considered very high for European standards. The loads certainly contributed to the increased accumulation of mainly Cd and Zn in the soil.

The measured average content of Zn, Cu and Pb in the soils were below critical concentrations according to Witeru (1992) [Zn (170 mg/kg) Cu (60 mg/kg), Pb (100 to




A Zn

B Cu

**Figure 4**

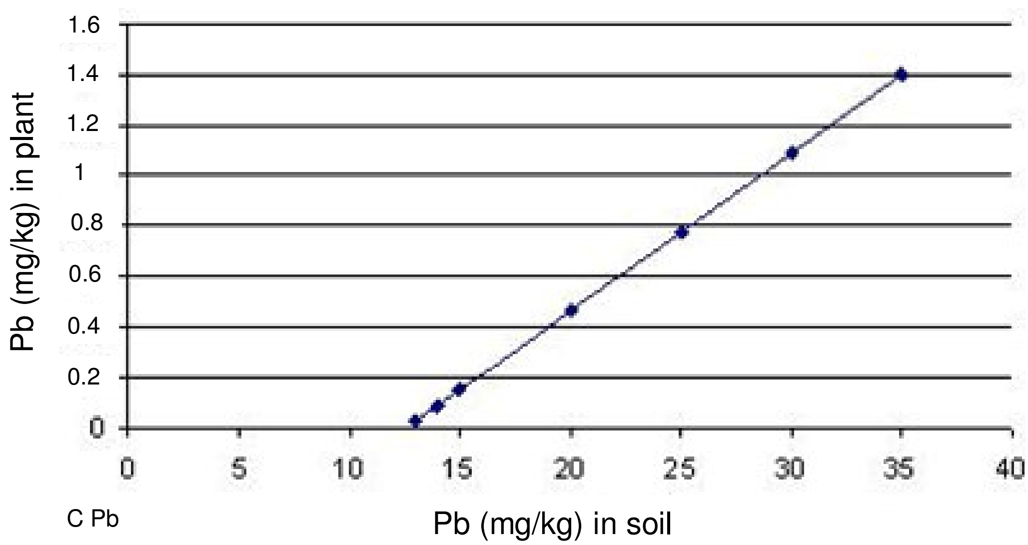
400 mg/kg)] and below the critical concentrations for multi-funcional uses according to De Vries and Bakker (1998) [(Zn (50 to 200 mg/kg), Cu (30 to 70 mg/kg) and Pb (32 to100 mg/kg)], but the concentrations of Cd were lower than the critical concentrations according to Kabata - Pendias and Pendias (2000) (3 to 8 mg/kg). However, maximum Zn concentrations in the soils were much higher than the critical limits and maximum concentrations of Cd in the soil were greater than the critical concentrations for multifuncional uses (Cd, 0.3 to 2.0 mg/kg).

The measured minimums and maximums and also the average values of Zn and Cu, like the maximum values of Cd in the grassland sample mixes were greater than the upper concentration limits for toxicity according to de Vries and Bakker (1998) [Cu (2.0) Zn (10.0) Pb (3.0) and Cd, (0.5)] and in comparison to Kabata-Pendias and Pendias (2000) [Zn (12 to 47 mg/kg), Cu (1 to10 mg/kg)], indicating that these values do not exceed 20 mg/kg; Pb, 0.1 to 10 mg/kg), while the concentrations for lead were significantly below these concentration limits.

High content of Cd was measured in dry weight of *Cynosurus cristatus*. The concentration of Cu, according to Kabat-Pendias and Pendias (2000), exceeded the allowable limits for the species *Lotus corniculatus, Trifolium montanum* and *Trifolium pratense* and was slightly increased in *Sanguisorba minor*. The concentration of Pb, according to Kabat-Pendias and Pendias (2000), was within the boundary with the exception of *Agrostis tenuis,* which exceeded the specified limits. In most locations and in plant species Cd was not detected, but it was significant that in all the mixtures of plant species, the concentrations of Cd were detected. The high content of Cd was measured in the dry weight of *C. cristatus,* also. Average Zn content in the grass was 3 times higher and for Cu it was up to 4 times higher than the phito-toxic concentration limits, while the average contents of Pb and Cd were lower.

The chemical composition of plants in general reflects the elementary compositions of the environment in which they grow. The common content of trace elements in plants in different unpolluted soils are characterized by very wide variations. If trace elements are found in high concentrations, they have a toxic effect, regardless of the plant necessity.

Between the different plant species there were differences in the uptake of heavy metals, which mainly depends on their genetic characteristics, the influence of



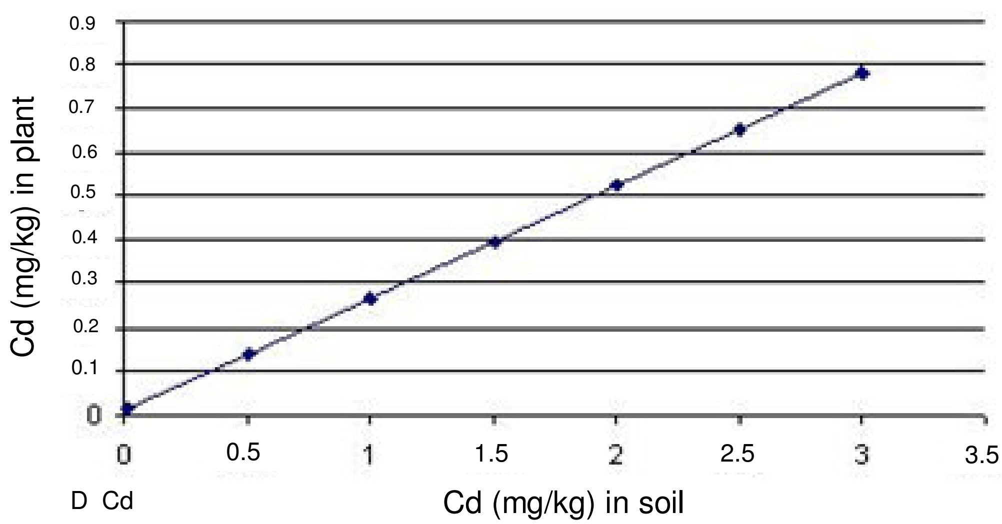




**Figure 4.** Linear regression functions of heavy metal content in soils and plants.

**Table 4.** Statistical parameters regression analyses, linear models.

| Element | Jednačina regresije | r | $R^2$ (%) | P -value | Sx |
|---|---|---|---|---|---|
| Zn | $Zn_m$=24.95 + 0.062 $Zn_s$ | 0.63 | 40.09 | 0.008 | 5.40 |
| Cu | $Cu_m$= 5.80 + 0.104 $Cu_s$ | 0.58 | 30.70 | 0.026 | 1.84 |
| Pb | $Pb_m$= -0.78+0.062 $Pb_s$ | 0.58 | 34.06 | 0.018 | 0.59 |
| Cd | $Cd_m$ = 0.01+0.257 $Cd_s$ | 0.64 | 41.64 | 0.007 | 0.30 |

$Zn_m$. $Cu_m$. $Pb_m$. $Cd_m$, content of elements in meadow; $S_x$, standard error estimation; p, confidence level; $R^2$, determination coefficient; r, correlation coefficient.

the surface of the root system, its capacity for adsorption of ions and the shape and velocity of evapotranspiration (Kadović and Knežević, 2002; Ogundiran and Osibanjo, 2008). However, differences in the uptake of heavy metals exist between plants of the same species, according to the climate and moisture regime.

The statistical analysis showed that, there was a highly statistically significant correlation coefficient between the content of heavy metals in the mixtures of grass and soil. Plants incoporated the heavy metals from the soil solution, but plants also adopted some forms of heavy metals from the atmospheric deposition through their photosynthetic organs (Kadović and Knežević, 2002).

Linearity dependence was found between the total heavy metal content in the soil and in the plant for all the elements studied. This may suggest that, plant absorption is controlled by the content of heavy metals in the soil solution and also by the content that is bioavailable in the soil. If the transfer factor value is higher, it can be supposed that more elements would be accumulated by the plants. According to Freitag (1986) and Puschenreiter and Horak (2000) all TF values were below 1. Puschenreiter and Horak (2000) reported that, in plants, the micronutrients Cu and Zn are much higher concentrated in grain than in straw, but Cd content is always higher in straw than in grain. We analyzed mixed plants material but the transfer factor values increased in same order as in Puschenreiter and Horak (2000) who reported a TF for grain (Cd<Cu<Zn). A sequence of decreasing TF values: Zn> Cu> Cr> Ni> Mn> Pb> Cd> As> Hg can be generalized for plants in the investigations conducted by Chojnacka et al. (2005).

Soil properties and the chemical form of heavy metals in soil are influenced by the uptake of heavy metals. The TF values under the study conditions suggested that the content of Zn, Cu and Cd was greater in the mobile fractions, but the content of Pb was not mobile and therefore unavailable for plants. On the other hand, linear regression showed dependence between heavy metal content in plants and soils.

## Conclusions

The research showed that, the average concentration of heavy metals in the soil were below the critical concentrations, but the measured maximum concentrations of Zn and Cd were above the critical concentration of multifunctional land use. The content of heavy metals in mixed grasses were within the critical concentrations limits, with the exception of Cd content. The content of Cd was above the critical concentrations.

The concentration of Cu, according to Kabat-Pendias and Pendias (2000), exceeded the limits for the species *L. corniculatus, T. Montanum* and *T. pratense* and were slightly above the limits for *S. minor*. The concentration of Pb were within the boundaries with the exception of *A. tenuis* and a high content of Cd was measured in the dry weight of *C. cristatus*.

Linearity dependence was found between the total heavy metal content, the soils and plants for all the elements studied. This may suggest that, plant absorption is controlled by the content of heavy metals in the soil solution and also by the content of heavy metals that are bioavailable. Soil properties and the form of heavy metals in the soil influence the uptake of heavy metals by plants. The TF values under the study conditions suggested that the content of Zn, Cu and Cd were more in the mobile fractions, but the content of Pb was not mobile and therefore, unavailable for plants.

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