# *Carduus nutans* L and the Effect on the Heavy Metals and Microenvironment Biota

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*The aim of the study was to identify the effect of Carduus nutans L on the heavy metals and microenvironment biota. Ten plants of Carduus nutans L were collected togheter with the rhizosphere soil and as reference the soil was collectued from an abandonated pasture where no vegetation was growing. The metal concentrations in the filtrate were determined by flame atomic absorption spectrophotometry and the total number of bacteria and actinomycetes from the average soil samples was established. Based on the mathematical models we managed to identify the effects of the heavy metals present in the Carduus nutans L rhizosphere soil samples on the development and presence of bacteria and actinomycetes colonies. We can state that iron and manganese influence the CFU of bacteria and actinomycetes and these in their turn have an impact on the zinc concentration in soil which will influence total chromium content and copper concentrations.*

*Keywords: spatial interpolation, bacteria and actinomycetes colonies, fingerprinting map*

*Carduus nutans* L (musk thistle*)* is an invasive species on degraded pastures in Europe [34]. On pastures, this species competes with native fodder plants and hinders the circulation of animals. It appears isolated or in groups depending on several factors, such as light, temperature, soil moisture, the variability of available resources, microorganisms in the soil [2, 6, 9, 10, 43]. In Romania, toxic weeds, in special *Carduus nutans* L., was considered useless, but currently, it's esteemed due it's therapeutically properties in traditional medicine [34], nutraceutical content and phytoremediation of soils, due to its capacity to accumulate high quantities of heavy metals [20]. The function of hyperaccumulation depends not only on the plant, but also on the microenvironment, in special on the interaction of the plant roots with rhizosphere microbiota and the concentrations of bioavailable metals in the soil [23, 33]. The microenvironment formed by rhizosphere, microbiota and plant roots form unique communities that present potential for detoxication processes where the unique properties of the host plant are of high importance remediate potential soil contamination [42]. The rhizosphere is an active and dynamic area of the soil under the influence of plant roots, where the root exudates are available for the growth of diverse microbiota [14]. Several scientists have published studies which indicate that plants have a strong effect on the bacterial community in the rhizosphere [17, 37]. Other researchers, such as Garbeva, P. V. et al, 2008 and Lundberg D. S. et al, 2012 have noticed that the soil plays an important part in the rhizosphere [11, 22]. The presence of metals in the soil is another factor that disturbs the microorganisms living there [12; 40] but it also influences the properties of soil and the growth and development of plants [5].

The transfer of metals from soil to plant tissues is studied using the index called Transfer Factor (TF). Higher TF values (≥1) indicate higher absorption of metal from soil by the plant and higher suitability of the plant for phyto-extraction and phytoremediation [25].

## Exprerimental part

### *Material and method*

The experiments were performed at the Didactic and Experimental Station belonging to Banat University of Agricultural Sciences and Veterinary Medicine King Mihai I of Romania from Timisoara.


Fig. 1. The location of experimental site

The experimental site, where 10 plants of *Carduus nutans* L were collected with rhizosphere soil around, is rectangular and is defined by the following stereographic coordinates: X =205306.421, Y = 483434.791, X = 205319.438, Y = 483416.482, X = 205534.175, Y = 483581.582, X = 205511.95, Y = 483604.76. The dimensions of experimental site are: length = 270.57 m, average width = 25 m and the surface = 7666.79 m$^2$.

### *Sample collection and preparation*

The reference soil was collected from a pasture that had been abandoned five years before, and had belonged to SDE Timisoara. All soils were sampled using hand driven stainless steel augers. The collected soil samples were dried out two days and sieved and the impurities removed. The soil samples were analyzed using the procedures described by Bordean D.M. et al, 2012 [3, 4].

From each plant three samples of *Carduus nutans* L leaves were collected, separated, and rinsed in distilled water to wash off potential air pollutants. The plant leaves were oven dried at 105°C to constant weight and prepared for analysis as described by Nica D.V, et al, 2013 [28].

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*Isolating the bacteria and actinomycetes and establishing the CFU/g soil*

The bacteria were isolated on Topping growth medium, while for actinomycetes the medium used was Gause; the method employed was that of incorporation into the medium [44]. The total number of bacteria and actinomycetes was established taking soil humidity into consideration, after 48 h of incubation (for bacteria) and 7 days of incubation, respectively (for actinomycetes). The optimal growth temperature for both microbial groups was 28° C [38].

*Heavy metal analysis*

Measurements of HM (Fe, Mn, Cu, $Cr_{tot,}$ Cd, Ni, Pb, Zn) concentrations in soil and *Carduus nutans* L leaves were performed in the Environmental Research Test Laboratory (Banat's University of Agricultural Sciences and Veterinary Medicine King Mihai the Ist of Romania, from Timisoara, Romania).

For *soil analysis*, metals were passed out to solution by wet extraction/proceeding using 5 g of dried and ground soil per each sample. Each sample was treated with mineral acids ($HNO_3$ 0.5N) at 1:10 soil/nitric acid solution ratio for 24 h , centrifuged at 1500 rpm and transferred in sterile polyethylene tubes. Each sample volume was bringing up to 50 mL with $HNO_3$ 0.5N.

The heavy metals content in *Carduus nutans* L leaves was carried out in $HNO_3$ solution resulted by ash digestion [4, 18]. Each sample solution was prepared with dilute HNO3 (0.5N), as described in earlier papers [4, 18].

All samples were weighed on an analytical balance to the nearest 0.1 mg. The digestion solutions ($HNO_3$ 0.5 N) were prepared from nitric acid (65%, $\rho = 1.39/cm^3$). The metal concentrations in the filtrate were determined by flame atomic absorption spectrophotometry and they were expressed as miligram per kilogram dry weight ($mgkg^{-1}$ d.w.). Double distilled water (spectro-scopic pure) was used for the preparation of reagents and standards. All chemicals were trace metal grade (Suprapur) [28].

The metal concentrations in the filtrate were determined by flame atomic absorption spectrophotometry with high resolution continuum source (Model ContrAA 300, Analytik Jena, Germany)

*Determination of Transfer Factor (TF)*

The heavy metal transfer coefficient ($FT_{HM}$) was calculated by dividing the *Carduus nutans* L heavy metals concentrations by the total heavy metals concentration in the soil and is presented in equation 1.

$$F_{HM} = HMplant / HMsoil \quad (1)$$

where: HMplant = metal concentration in plant tissue [$mgkg^{-1}$ dry weight] and HMsoil = metal concentration in soil [$mgkg^{-1}$ dry weight] [25].

***Statistical analyses*** were performed by using PAST software package [15].

Spatial interpolation using Kriging as algorithm provides the advantage that the spatial variation of a continuous attribute is modeled as a stochastic surface or random field [46].

The *Neighbor-Joining* algorithm is a clustering technique, which attempts to approximate the least squares tree, relying strongly on the addition, to join clusters that are not only close to one another, but are also far from the rest [16].

**Results and discussions**

The heavy metal analysis performed on the average samples of rhizosphere soils, reference soil and *Carduus nutans* plants provide the information regarding their fingerprint map which is presentend in figure 2. The map presents the spatial interpolation of heavy metals content *Carduus nutans* L rhizosphere soil (CnS) compared to the reference soil and *Carduus nutans* L plants (CnP), based on Kriging algorithm using as semivariogram plot the cubic model and cross validation jackknife.

The TF value for total chromium (≥1) [25] highlighted by figure 3 indicates that chromium presents higher absorption from soil by *Carduus nutans* L and shows that this plant is suitable for phyto-extraction of chromium.

The high concentration in plant even if in soil the chromium total content is lower than the normal content for Romania [30 $mgKg^{-1}$] shows that this species presents a very high capability of absorption of chromium and for phytoremediation of soils contaminated with chromium [45].

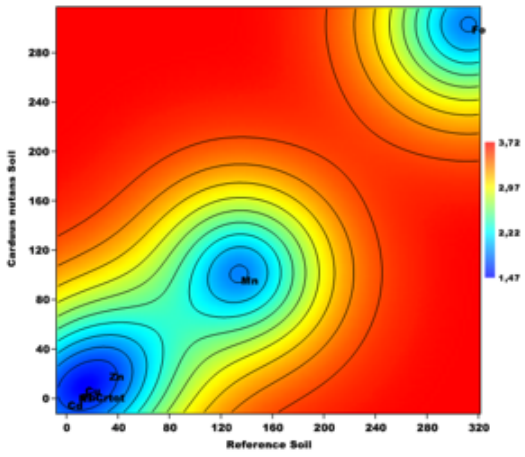
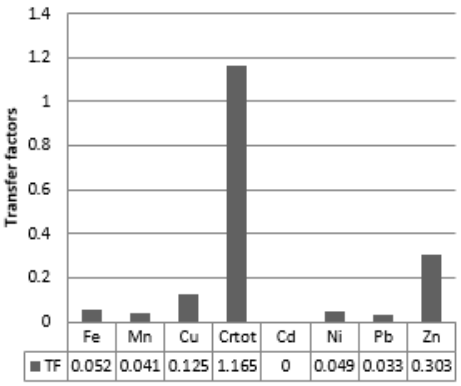



Fig. 2 Spatial interpolation of heavy metals content in soil and *Carduus nutans* L plants compared to the reference soil and *Carduus nutans* L plants compared to the reference soil




Legend: R= reference soil sample from rhizosphere, CnS = *Carduus nutans* L soil from rhizosphere, CnP = *Carduus nutans* L Plant sample

Fig. 3. Transfer factors (TF) of heavy metals plant/soil

Using the *Neighbor-Joining* algorithm based on Euclidian distances, we manage to obtain the least squares tree (fig. 4) which permits to identify the root and effects of the heavy metals present in the reference rhizosphere soil and the soil samples from the *Carduus nutans* L soil rhizosphere on the development and presence of colonies of bacteria and actinomycetes. Based on figure 4 we can state that iron and manganese influence the CFU of bacteria and actinomycetes and these in their turn have an impact on the zinc concentration in soil which will influence total chromium content and copper concentrations.

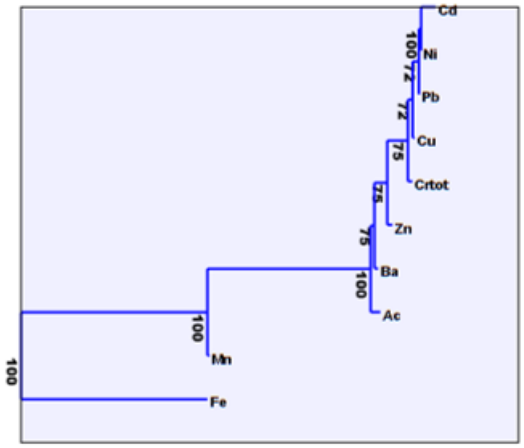



Fig. 4 Graphical representation of Neighbor joining clustering method

Legend: Ba = Bacteria (CFU/g soil) x$10^7$, Ac = Actinomycete (CFU/g soil) x$10^6$, (Fe, Mn, Cu $Cr_{tot}$ Cd Ni Pb Zn) = analysed heavy metal content

The bacteria and actinomycetes in the rhizosphere (fig. 4) outnumber the two microbial groups in the control variant. The rhizosphere is dominated by the bacteria, rather than by the actinomycetes. Acording to the results obtained by some authors, root exudates have an impact on microorganisms [13; 41].

The effect of metals on microorganisms depends on their concentration. Copper and chrome are among the microelements with an important role in biochemical reactions. When they appear in high concentration, they become toxic [7].

Nogueira M. A. et al., 2007 [29], state that the excess manganese in the soil is toxic for plants, but they add that this effect can nevertheless be lessened in the plants by mycorrhizae.The same authors prove that the balance between the Mn-reducing and MN-oxidizing microorganisms in the rhizosphere of these plants can be overthrown, with direct effects on the quantity of Mn extracted. Steve P. McGrath et al. (1995) [24] highlighted that after a longer time, metals (Zn, Cu, Ni, Cd , Cr, Pb), even when appearing in low concentrations, are toxic for the microbes in the soil. Some metals are indispensable to microorganisms, having a beneficial role up to certain concentrations [19]. Others can have a toxic effect [12] and their accumulation in the animal body can cause serious illnesses. The very high concentrations of Cu and Zn reduce the microbial biomass. Bacteria are among the microbial groups whose numbers were cut [31].

Rajendran P. Et al. (2003) [32], mention that microorganisms have special mechanisms for detoxing their environment. The studies published by some researchers highlight the fact that microorganisms (bacteria and fungi) are involved in the oxidation of Mn and they improve the reaction speed, which can lead to reduced contamination by metals [1; 26; 39].

There are various microbial species involved in the bioremediation of the natural medium, by metal reduction (ex. chromate and ferric iron) [21].

The reduction of the heavy metal concentration in the medium can be dome either by enzymatic or by non-enzymatic mechanisms [27].

Based on the generalized linear model (fig. 5) of the heavy metal concentration from reference soil without vegetation, we can predict the heavy metal concentration in the rhizosphere soil samples where *Carduus nutans* L plants are growing (2).

$$Y = 0.93838 \cdot x - 0.063843 \quad (2)$$

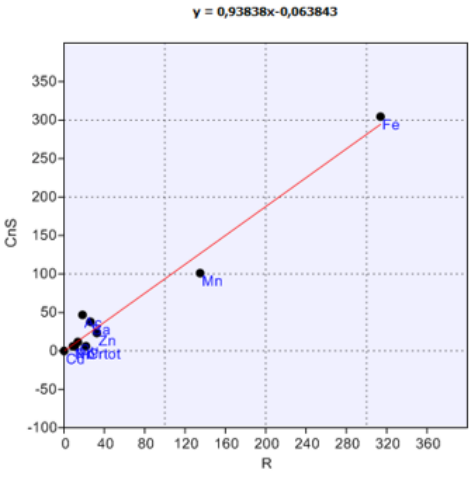



Fig. 5. Generalized linear model of CnS (*Carduus nutans* soil from rhizosphere) on R (reference rhizosphere soil) dependence

where x = reference rhizosphere soil heavy metal concentrations and y =heavy metal concentrations in *Carduus nutans* L rhizosphere soil.

Through some organic compounds of microbial nature, as well as through the microorganism-root interaction, the characteristics of some metals can be changed, the toxicity can be modified ($Cr^{6+}$), the bioavailability can be made different, and their absorption can be facilitated (Fe, Cd) [8; 36], but also the mobility of some metals in the medium can be limited ($Pb^{2+}$, $Cd^{2+}$) [30].

Salazar M. J. et al. (2012) [35], noticed the accumulation and increased concentration of metals in the soil cultivated with soybean. The authors consider that metal accumulation is due to anthropic pollution sources placed near the cultivated field.

## Conclusions

The microorganisms under analysis dominate the rhizosphere, as compared to the control variant and are influenced by plants, soil, and by the presence of metals, as well. Microorganisms, plants, as well as other edaphic and environmental factors can influence the toxicity of metals having a number of mechanisms through which they can play a role in the detoxification of soils.

Using mathematical models we manage to identify the effects of the heavy metals present in the rhizosphere reference soil and the *Carduus nutans* L rhizosphere soil samples on the development and presence of bacteria and actinomycetes colonies. We can state that iron and manganese influence the CFU of bacteria and actinomycetes and these in their turn have an impact on the zinc concentration in soil which will influence total chromium content and copper concentrations.

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