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Concentration of trace and major elements in mountainous grasslands of Bosnia and Herzegovina in relation to soil properties and plant species

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Abstract

Many grazing animals are solely or mostly dependent for their dietary needs and essential minerals on the forage available, either in its natural state or conserved as hay or silage. A soil and plant survey was carried out in May 2014, incorporating 100 sampling points, in the area of Manjača and Vlašić Mountains in Bosnia and Herzegovina. Main investigated soil types in the area were Cambisol, Fluvisol, and Leptosol, while botanical composition consisted of wide range of species, mainly of the families *Poaceae*, *Leguminosae*, *Plantaginaceae*, *Scrophulariaceae*, *Asteraceae, Fabaceae, Polygonaceae, Violaeeae, Lamiaceae, Euphorbiaceae,*. In total sixty one different species of legumes, herbs and grasses were identified, of which some were categorized as worthless and harmful as animal feed.

This study was conducted to investigate the nutrient and trace element status of soil and herbage plants in the sampling area, and to examine the concentrations observed for their potential influence on animal performance. Soil parameters, such are texture, trace element concentrations, pH, SOC, and plant type were considered as a factors affecting trace element concentrations in the forage plants. The soil pH varied from strongly acidic to moderately alkaline. Percentage of SOC varied from 0.5 % to 12.3 %. Soil texture analysis showed that most of the soil samples were high in silt content.

The average concentrations of sodium, phosphorus, zinc, selenium, copper, cobalt, and boron were low in both soil and herbage plants. Plant potassium, calcium, magnesium, molybdenum, and manganese concentrations were sufficiently high to meet the requirements of animals, while iron concentrations were even elevated in same sampled areas. High levels of molybdenum have been found in both soil and plants, which may be plant toxic, however, effect on animal is not determined. In conclusion, imbalances observed in natural pastures of Manjača and Vlašić area, caused by low soil trace element status, and other soil and plant properties, could impair animal performance in the studied area.

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Jasmina Simic

Ås, August 2015

I am glad to declare that this thesis is my own work and it has not been submitted for a degree at any other institution.

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Ås, Norway

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List of symbols and abbreviations

B	Boron
B&H	Bosnia and Herzegovina
Ca	Calcium
Cd	Cadmium
Co	Cobalt
CEC	Cation exchange capacity
Cu	Copper
CRM	Certified Reference Material
DW	Dry weight
FAO	Food and Agricultural Organization
Fe	Iron
ICP MS	Inductively coupled plasma mass spectrometry
ICP OES	Inductively coupled plasma optical emission spectrometry
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NRC	National Research Council
P	Phosphorous
R^2	Coefficient of determination
Se	Selenium
SOM	Soil organic matter
SD	Standard deviation
SRM	Standard Reference Material
TE	Trace element
TF	Transfer factor
Zn	Zinc

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1. Introduction

Of the total area of B&H (51.000 km^2), 50.3% is arable land and the remaining is under forests (Custović, 2005). In the total structure of agricultural land in B&H, natural grasslands and meadows have the share of 56% (FAO, 2009). Surfaces under natural pastures and meadows are dominant land use system in mountainous regions. Hills (300 – 700 m) account for 45% of total arable land, which is categorized as medium quality and mostly used for extensive livestock production. Mountainous areas (>700 m) account for 35% of arable land, which due to steep slopes and low fertility levels are used only for grazing (FAO, 2009). Bosnia and Herzegovina has heterogenic soils because of a great diversity of geological base, climate, vegetation, and paedo-fauna (Manojlovic and Singh, 2012). Only 14% is the best quality soil (classes I to III) in B&H (Dugoročni Program Razvoja, 1986).

Meadows and pastures of mountainous and hilly regions in B&H are mostly on shallow soil, botanically comprised of mixture of grasses (*Lolium* and *Festuca* spp.), legumes (*Trifolium* spp.) with less productive species, and are not subject to any improved management practices (FAO, 1999). The domestic livestock and dairy production is not sufficient to serve domestic needs, despite favorable conditions in B&H. Poor animal nutrition is among the main reasons for low productivity in livestock and dairy sector. According to latest statistical data (Agency for Statistics of B&H, 2014) animal population in B&H is 447.000 cattle, 1.020.000 sheep, 530.000 pigs and 69.000 goats, which are less than half as compared before 1991 (FAO, 1999).

Some inorganic minerals are essential for normal growth, development, and reproduction of animals. Those elements required in gram quantities are referred to as macro minerals, while those required in milligram or microgram amounts are referred to as the trace minerals (NRC, 2001). Most of the trace elements are found in soils and plants in varying quantities and proportions. Therefore, a relationship between nutrient deficiencies in soils, fodder crops, animals, food, and human nutrition exists. Trace element concentration in soils and forages influence mineral status of grazing livestock (Espinoza *et al.*, 1991). There is an indication of direct linkage between iodine, selenium and zinc concentration in soils with their deficiencies in humans (Bevis, 2015). In China, 60% of the rural population suffers from zinc deficiency, connected to zinc deficiency in the soil (Ma *et al.,* 2012). Increase in iron content of food plants can contribute to reduction of human iron deficiency, whereas there is little information supporting direct relationship between soil iron status and human iron deficiency (Nubé and Voortman, 2011). Factors other than low iron content of crops are probably more important in causing iron deficiency in humans.

The presence of antinutritional components such as phytate in food and feed has been connected with reduced zinc and iron absorption (Walter *et al*., 2002).

Concerning animal nutritional requirements, some species of forage plants can contain excess of some trace elements and deficiency of others (Juknevičius and Sabiene, 2007). The trace element concentration of forage mixture is influenced both by the differences in trace element concentrations between legumes and grasses, and their species composition in the mixture (Høgh-Jensen and Søegaard, 2012). Ability of plants to take up minerals from soil solution depends on many factors, such as soil pH, total trace element concentration in soil, organic matter, CEC, redox potential, climatic conditions, plant type and maturity, interaction of different elements, chelates (Havlin *et al*., 2005).

Some trace elements can be essential for growth and development of plants but not for animals and vice versa (Suttle, 2010; Fisher, 2004). Selenium in plants has beneficial effect as antioxidant but it is not essential for plant growth and development (Germ *et al*., 2007; Kabata-Pendias, 2011) while animals can develop deficiency symptoms. The same is true for iodine and cobalt that are essential for animals but not required by plants (Suttle, 2010). Cobalt is indirectly essential to legumes since it is required by the *Rhizobium* for the synthesis of leghemoglobin (Weisany *et al.,* 2013; Kabata-Pendias, 2011; Taiz and Zeiger, 2010). However, since Co is not essential for plant growth, no critical concentrations have been listed. In some cases, additional application of Mg is necessary to meet animal requirements (lactating cows), while plants do not exhibit any improvement after the application. Potassium and manganese are essential for animals and plants, however, even in deficient soils forage concentration is generally adequate to meet the requirements of grazing livestock (Underwood and Suttle, 1999). Iron, zinc, calcium and magnesium are essential both for plants and animals, however, even if the concentration in the plant tissue is not low, deficiency in animals may occur due to presence of phytic acid (Bohn *et al*., 2008).

In certain quantities, trace elements are essential or beneficial for plants and animals; however, there is a risk of toxicity if they are present in excessive concentrations. The deficiency and toxicity range may be species specific for plants (McGrath *et al*., 2001) and animals (Suttle, 2010). Even by providing high quality forages from mineral content viewpoint; unbalanced animal feeding can reduce productivity and develop deficiency symptoms in animals (Juknevičius and Sabiene, 2007).

Deficient concentration of trace elements in soils, forages, and animals have been reported in several areas of Balkan region (Jug *et al*., 2008, Manojlović and Singh, 2012, Muratović *et al*., 2005, Maksimović and Djujić, 1997), while trace element contamination of both soil and plants have also been reported by other researchers (Manojlovic and Singh, 2012, Murtić *et al.,* 2014). The challenge in overcoming the problem of low productivity and quality in livestock and dairy production is the lack of data on the nutritional status of the pastures and soils in the region. Analysis of soils and forages for mineral composition is important for understanding the main limiting factors of livestock and dairy production, as well as mineral deficiency problems in animals (Suttle, 2010). Parent material and micronutrient concentrations in the soil are mostly reflected in the trace element concentrations of plants (FAO, 1982). In the regions with poor soil nutritional status, variation in trace element content among forage species may be used to increase the overall micronutrient status of the pasture. There is insufficient data on soil characteristic, pasture quality, and nutrient deficiencies in livestock from these areas.

Research hypothesis:

(H1) Examined soil parameters (total trace element concentration in soil, soil texture and type, pH, soil organic carbon, altitude, and plant type) influence the trace element concentration of the pasture plants.

(H2) Trace element concentration in the pasture plants do not meet the animal requirements in the area.

1.1. Objectives

Considering the importance of the mountain Vlašić and Manjača for livestock and dairy production in B&H, the main objective of the study was to investigate the concentration of trace elements in soils and pasture plants and to relate them with animal requirements. Sub objectives were to:

I. Investigate the concentration of trace elements in soils and plants;
II. Assess the relationship between soil parameters and the trace element status in pasture plants;
III. Find out if the dominant plant species affects the overall trace element concentration in pastures;
IV. Determine if, and to what degree, trace element concentration in pasture plants meet animal requirements in the area.

2. Review of literature

2.1. Soil characteristics and deficiency problems

Crop production is dependent on the phytoavailability of sufficient quantities of the essential mineral elements required for plant growth and development. However, it has been estimated that 60% of the presently cultivated soils globally have severe mineral problems, either toxicities of Al, Mn and Na, or deficiencies of N, P, K, S, Fe and Zn (Cakmak, 2009). Deficiency in any one of essential elements restricts plant growth and reduces crop yields.

The primary deficiency of micronutrients in soil occurs mostly in extremely degraded or sandy soils. Secondary micronutrient deficiency can be caused by many soil and climatic factors that reduce the ability of plants to utilize micronutrients (FAO, 1982). In order to find appropriate strategy for overcoming the mineral deficiency, it is necessary to know the main factor causing it.

There is no extensive research on trace element concentration in the soil and their relationship with plant-animal system in Bosnia and Herzegovina, especially in the area of Vlašić and Manjača mountains. Soil acidity and low levels of plant available phosphorus are limiting factors for field crops in some parts of B&H. According to Marković *et al*, (2011) 60 percent of the tested soil samples in the area of northern Bosnia (Gradiška area) had pH lower than 4.5, and were low in plant available phosphorous, while only 10 percent of soil samples were low in plant available K.

In the study of Ljubojević *et al.,* (2014) the total concentration of heavy metals in soils (silt loam) was: Fe 4.9 mg kg^{-1}, Zn 64.3 mg kg^{-1}, total Cu 30.8 mg kg^{-1}, and pH 4,6. According to Savić (1964), the highest concentration of molybdenum in grassland soils in Bosnia was 0.76 – 1.03 mg kg^{-1} in brown calcerous, 0.52 – 0.74 mg kg^{-1} in pseudogley, 0.35 – 0.53 mg kg^{-1} in red brown alluvial soil, and 0.17 – 0.51 mg kg^{-1} in podzol. In Western Serbia molybdenum is deficient in acid soils which especially affects legumes in forage production (Vuckovic, 1999). Hydromorphic pseudoglay soils of B&H have total molybdenum concentration range from 0.35 to 1 mg kg^{-1} (Aubert and Pinta, 1970). Hydromorphic soils are often P deficient due to more acid reactions and relatively heavier texture, which results in stronger P fixation (Vukadinović *et al.,* 1988). Vucković (1999) discussed inherently low soil P concentration in Balkan region as limiting factor for forage production.

Comprehensive study on selenium status in soils, water, cereal crops, food and human tissue in Serbia sowed serious defficiencies in many parts of the country (Maksimović and Djujić, 1997). A study on Se soil concentration in ex Jugoslavia showed wide variations (39-44 µg kg^{-1}) which can indicate deficient levels in many regions (Jović, 1996). Some epidemiologic studies suggest that seleniium deficiencies and heavy metal toxcisities might be among main etiological factor of endemic nephropathy in rural areas of Balkan region (Komatina, 2004; Jonge and Vanrenterghem, 2007).

Copper concentration in forage plants ranged from 8.90 to 11.3 mg kg-1 DM in Kupres area (Muratović, 1997). Excessive amount of zinc and especially copper was reported by Murtić *et al*., (2014) in Goražde area, B&H. Copper concentration was up to several times higher compared to other elements (80 mg kg^{-1} in topsoil 0 – 30 cm, 72 mg kg^{-1} in subsurface soil 30 – 60 cm) while zinc concentration was 218 mg kg^{-1} in topsoil 231 in subsurface soil 30 – 60 cm. These findings can be contributed to application of mineral fertilizers and metal based pesticides in cultivated areas, but the same might not be expected in natural pastures.

In Western B&H, there are large masses of ultrabasic rocks and serpentines. These soils are considerably higher in Mg, Fe, Cr, Ni and Co but poorer in other biologically important microelements (Maksimović, 1975). In northeast B&H, there are breakthroughs of tertiary igneous rocks that are associated with high levels of Fe, Zn and other minerals (Midzić and Silajdzić, 2005). However, Zn and Fe deficiencies were causing plant chlorosis in east part of Croatia due to high soil pH value (Jug *et al*., 2008).

Plants grown in alkaline soils contain less of important trace elements, such Zn, Mn, and Fe (Juknevičius and Sabiene, 2007). In the study of medicinal plants in B&H (Saletović *et al*., 2011), it was found that the concentration of Zn, Cu and Mn ranged from 14.2 to 103.4 mg kg^{-1}, 2.8 to 15.4 mg kg^{-1}, and 14,3 to 500 mg kg^{-1}, respectively . These concentrations varied depending on the type of plant and locality.

Muratović *et al*., (2005) determined Cu deficiency in pasture and sheep's blood but not in soil and forage crops on natural pastures in Nišići Plateau. Muratović *et al*., (2006) found that Se concentration in sheep blood serum, plants, soil and wool in B&H ranged from 0.86 to 2.59 mol l^{-1}, 0.032 – 0.784 mg kg^{-1} DM, 0.396–1.134 mg kg^{-1} DM, and 0.022–0.499 mg kg^{-1} DM, respectively; while in Croatia it ranged from 0.0443 to 1.52 ɳmol l^{-1}, 0.006 to 0.057 mg kg^{-1}, 0.065 to 0.975 mg kg-1, 0.003 to 0.059 mg kg^{-1}, respectively.

2.2. Relationship of trace elements in soil and other soil parameters

Soil properties influence solubility of trace elements and are important indicators of their availability. The concentration of trace elements in soil can be an indicator of surplus or deficiencies for plant nutrition, animal and human health (Haluschak, 1998; Boila *et al*., 1984, 1985; Kruger *et al*., 1985; Gupta, 1986). However, total concentration may not be the best indicator of trace element bioavailability due to numerous factors influencing the absorption, such as pH, sorption-desorption reactions, chemical complexation with inorganic and organic ligands, redox biotic and abiotic reactions, organic and inorganic ligands, humic and fulvic acid, root exudates, microbial metabolites, and other nutrients (Violante *et al*., 2010). The large number of these factors and their considerable spatial and temporal variability in field conditions makes it difficult to predict trace element deficiencies or potential phytotoxicity.

Table 2.2.1. Potentially useful diagnostic categories of micronutrients in soil (Fisher, 2008)

Element (mg kg^{-1})	Very low	Low	Average	High	Very high
Fe	<5	5–10	10–15	15–25	25–50
Cu	<0,3	0,3–0,8	0,8–1,2	1,2–2,5	2,5–10,0
Zn	<0,6	0,6–1,0	1,0–3,0	3,0–8,0	8,0–20,0

Generally, plants are able to accumulate more minerals in light, slightly acid soils. Soil pH of 6.5 is considered the optimum for a soil with balanced trace element levels for plants. Manganese and zinc contents of plants decrease greatly with rising pH, while the Mo contents increase, and deficiencies of both Mn and Mo can therefore hardly exist in same soil (FAO, 1982). In lower pH values P availability decreases because P ions react with Fe and Al, while in alkaline soils they reacts with Ca (Vucković, 1999). In forages grown on alkaline soils, excess of selenium and deficiency of iron, copper, zinc, boron, and manganese may be found (Huston, 2006). Low pH can lead to deficiency of Se in animals grazing from plants low in shoots and seeds Se concentration, even though the total concentration of Se in soil might not indicate deficiency problem.

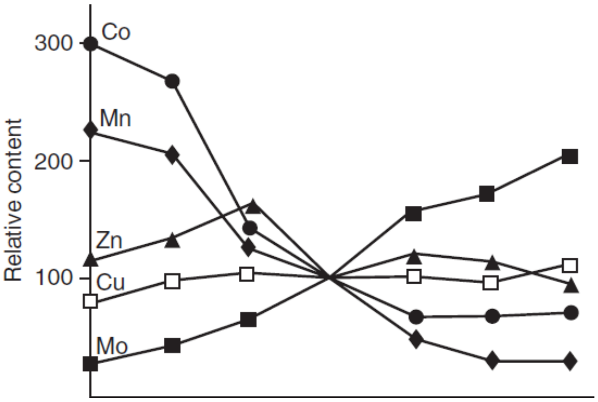

Figure 2.2.1. An example of the influence of soil pH on the concentration of selected microelement in ryegrass, (Suttle, 2010)

The solubility of heavy metals in soil is related to redox potential (Patrick *et al.,* 1990). "In most soils there is observed a positive correlation between the content of the clay fraction and amounts of trace elements, in particular cations" (Kabata-Pendias and Mukherjee, 2007). However, trace elements are also more available in sandy soils than in clayey, as sand particles have a much lower surface area and contain fewer cation exchange sites comparing to clay particles (Ansari *et al*., 2015). Clay retains more B, but in contrast, plant B uptake is higher on sandy soils (Havlin, *et al.*, 2005). Sandy soils have lowest amount of molybdenum (Huston, 2006). Broad study on European soils (Gawlik and Bidoglio, 2006) indicated that increased levels of heavy metals can be observed when soil texture is getting fine, but the opposite trends were observed as well.

Soil organic matter is important for transfer of trace elements from soil to plant because it mostly binds minerals in plant unavailable forms in soil solution and after mineralization processes it releases them in plant available forms (Stevensen and Ardakani, 1972). "From 98 to 99% of Cu, 84 to 99% of Mn, and 75% of Zn are carried on organic complexes within the soil" (Barry and Merfield, 2008). Soluble Cu is most commonly highly complexed with soil organic compounds comparing to other micronutrients (Havlin *et al*., 2005).

Adsorption of trace elements by roots is controlled by the concentration of other elements in soil solution (Taiz and Zeiger, 2010). Synergistic or antagonistic effect between soil trace elements should be considered while determining their bioavailability. High Zn, Fe, and P concentration in soil can inhibit Cu absorption by plant root system (Havlin *et al.*, 2005). High levels of soluble P in soil solution can enhance plant uptake of Mo, while available S and Cu can have opposite effect (Bergmann, 1992; Haque, 2012). However, Komljenović *et al*., (2006) found lower levels of Mo in leaf and grain because of ameliorative P fertilization of acid soils in Potkozarje area of Bosnia and Herzegovina. Low Zn concentration in soil will result in poor pasture growth due to underutilized nitrogen among plants that are Zn deficient (MacNaeidhe, 2001).

In assessing bioavailability of trace elements in soil-plant system we should consider not only trace elements and soil properties, but plant species as well (Zhang and Shan, 2000), because plant species have different affinity in accumulating different trace elements.

Form in which the mineral is found in soil can influence mobility, efficiency of uptake and metabolism in plant system. Selenite and selenate are major two forms of Se in alkaline soil solution (Mayland *et al.,* 1991). Selenate ions are rapidly absorbed into plant xylem sup compared to selenite. However, selenium in the form of selenite in plant is more efficiently metabolized into organic compounds and transported to upper parts of the plant (Mayland *et al.,* 1991).

High rainfall can affect deficiency of Se in plants and animals. Firstly due to leaching of Se, and secondly the dilution of Se in fresh weight of forage crops (Underwood and Suttle, 1999). Selenium deficiencies may occur in areas with higher rainfall (more than 600 mm) and in forages grown on light sandy soils with less than 0,50 mg kg^{-1} per plant dry matter (Vuckovic, 1999). Wet weather increases Mn in soil solution while dry condition can promote oxidation to plant unavailable forms; however, wet conditions are usually connected to Mn deficiency in oats (Havlin *et al.,* 2005). Weather conditions during early spring that contribute to Zn plant deficiency are low insolation, low temperature, and excessive moisture (Havlin *et al.,* 2005).

2.3. Nutrient and element requirements for animal feed

Animals receive high portion of required minerals through forage plants. Important minerals like Ca and P are required in large amounts by the animal body, but mostly their deficiency is not a problem because they are present in high quantities in many feeds. Some other minerals such are Fe, Mg, K, Na, Cl, N, and S are also required in higher amounts by animals and are considered to be macronutrients. Other minerals, like Mn, Zn, Cu, Co, Mo, and Se are required in small amounts, thus they are called micronutrients or trace minerals. Certain trace elements are essential to plants and animals to support health, growth, and reproduction (Roberts *et al*., 2000). If these nutrients are not present in adequate amounts, animals can show deficiency symptoms. Low Mg concentration in forage crops, particularly grasses, may cause grass tetany (hypomagnesaemia) which is an abnormally low level of blood Mg (Havlin *et al.* 2005).

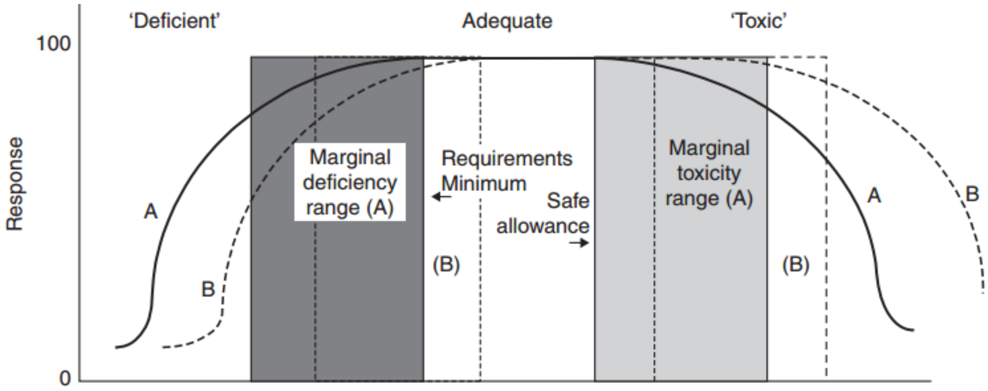

Figure 2.3.1. Dietary mineral concentration (Source: Suttle, 2010)

The main reason for mineral deficiencies in grazing livestock is that the soils are inherently low in plant available minerals. Low mineral concentration in soils and forage can be directly related to mineral deficiency symptoms in animals, poor growth, and reproductive problems even if the forage supply is adequate (McDowell, 1997). Nutrient deficiencies affect more health and growth rate of ruminant animals than related forage crops. For example, supply of Ca and Mg can be sufficient for normal plant growth, but not to meet animal requirements. Additional difficulty is that animal deficiency symptoms appear only when the deficiency is severe (Whitehead, 2000).

Indicator of mineral uptake of livestock can be mineral concentration of the vegetative parts and seeds of plants from grazed pastures. Quality evaluation of feed concerning total mineral concentration does not provide enough information because total element concentration in feed is often not available for animal in the gut. Although total trace element concentration in forage plants corresponds to the nutritional values, imbalance may occur due to different interactions between plants and animal organism (Marschner, 1995; Juknevičius and Sabiene, 2007). For minerals like sodium and potassium, absorption is almost complete under all circumstances, but for copper and manganese, most of the ingested mineral can remain unabsorbed (Suttle, 2010; NRC, 2001). This may vary in regards to plant species and the age of the animal. The form in which mineral can be found in forage plant is important. For example, cereals are high in phosphorus in the form of phytate which can be unavailable for pigs and poultry (Underwood and Suttle, 1999). Nevertheless, total mineral concentration in forage plants can be used as indicator of the forage quality.

Recent research suggests that mineral availability in forages decreases with plant maturity because it gets more associated to indigestible fiber fraction. The concentration of P, Co, Cu, Fe, K, Mg, Mn, Mo, and Zn in forage plants decline with maturity (Suttle, 2010). Therefore, undiversified feeding leads to metabolic disorders and decreased production. This problem is emphasized in areas where livestock production depends mostly on natural pastures.

Table 2.3.1. Micronutrient recommendations for ruminants (mg kg^{-1} of dietary dry matter), (Fisher, 2008)

Element	Young calf	Growing bullock	Cows	Lambs	Sheep
Fe	40	35	30	30	40
Cu	1,2	15	15	5	7,0
Co	0,11	0,11	0,10	0,1-0,2	0,1-0,2
Se	0,1	0,1	0,1	0,1	0,1
Mn	25	25	40	25	40
Zn	50	40	40	40	40
B	5	5	5	5	5

Animals have different requirements for trace elements in different development stages. Estimation of zinc requirement in sheep in early growth stages is 27 mg kg^{-1} DM, in adult 10,8-17,2 mg kg^{-1} DM and in period of lactation 11,6-17,9 mg kg^{-1} DM (Suttle, 2010). Some results show that soil ingestion in animals can occur because of essential mineral deficiencies, such as Cu, Co, Mn, and Se (Suttle, 2010; Marta López-Alonso, 2012) because soil represent more concentrated source.

2.4. Trace element concentration in pasture plants and differences between species

Plants are main sources of mineral elements for grazing animals on natural grasslands and as such represent important factor in providing quality food source. Trace element deficiencies in plant can result in poor animal diet, deficiency symptoms, and diseases. Concentrations of trace minerals in plants vary from part to part and with maturity (Suttle, 2010). There are differences in the major mineral concentration of different plants species grown under the same soil conditions as well (Beeson, 1941).

Factors effecting plant ability to accumulate minerals are complex and depend on plants root system, synergetic and antagonistic interactions between the elements, rainfall amount and intensity, soil N status and pH (Marschner, 1995). Genotype differences in absorption of trace elements from soil can be related to absorption rates, larger plant root mass, increased solubility of trace elements due to root exudates effect on pH or redox potential, efficient transport to above ground plant system or lower trace element requirements (Havlin *et al*., 2005). Among annual crops, beans, lupine, and soybean utilize better insoluble P forms (Vucković, 1999). Perennial plants due to deeper root system and those that exudates more H^+ ions are more efficient in using insoluble phosphorus forms (Al, Fe and Ca phosphates) (Havlin *et al*., 2005). Legumes generally show greater capacity to absorb phosphorus compared to grasses (Caradus, 1980), and their decline of phosphorus availability with advanced maturity is less in relation to grasses (Coates *et al*., 1990).

Table 2.4.1. Potentially useful diagnostic categories of micronutrient in grass (Fisher, 2008)

Element (mg/kg DM)	Very low	Low	Average	High	Very high
Fe	<50	50–100	100–150	150–250	250–500
Cu	<5	5–8	8–10	10–12	12–15
Co	<0,05	0,05–0,10	0,10–0,15	0,15–0,20	0,20–0,40
Se	<0,01	0,01–0,10	0,10–0,15	0,15–1,50	>1,5
Mn	<25	25–50	50–100	100–150	150–300
Zn	<15	15–25	25–50	50–75	75–150

Some species have coping mechanisms to tolerate poor Fe soil concentration. Grass root system is able to exudates amino acids called phytosiderophores with high affinity for Fe, that enables efficient Fe transport to root surface and absorption by root cells (Havlin *et al*., 2005). Within grasses in same development stage grown on the same soil type significant differences in the concentrations of cobalt, copper, and manganese have been demonstrated (Underwood and Suttle, 1999).

Legumes are richer sources of all minerals than grasses, with the exception of manganese and silicon (Huston, 2006). Most pasture herbs are higher in trace element concentration comparing to grasses as well. There is higher calcium and magnesium concentration of clovers and other legumes than in grasses (Underwood, 1956; Juknevičius and Sabienė, 2007). Rough stalked meadow grass (*Poa trivialis*) and clover have high trace element concentrations and should be included in grass seed mixtures (Marta López-Alonso, 2012). Forage crops seams to contain somewhat higher Se concentration compared to cereals (Johnsson *et al.,* 1997). Yarrow (*A. millefolium* L.) accumulate higher concentrations of Cd (1.5 mg kg^{-1}) than alfalfa, grass and other crops (0.25 – 0.5 mg kg^{-1}) grown at the same location (Jakovljević and Antić-Mladenović, 2000). Rye absorbs twice as much Cu as wheat under same conditions. Varietal differences in tolerance to low Cu can be as large as those among crop species (Havlin *et al*., 2005). However, these differences decrease when the soil is low in available minerals (Suttle, 2010).

Some plants have special requirements for trace elements. Legumes are especially sensitive to cobalt and molybdenum deficiency (Vucković, 1999). Although, this cobalt requirement can be connected to nitrogen fixing bacterium rather than legumes (Hopkins and Hüner, 2009). Grasses are less dependent on B for normal cell wall expansion comparing to dicot (Havlin *et al*., 2005).

2.5. Soil physical properties in relation to trace elements and forage production

In spite of changes during weathering, both soil texture and trace element concentration is strongly related to soils parent material. From agronomic perspective, suitable soils for forage production contain 70-80% sand, 20-30% clay (Vucković, 1999). The soil strength around the root influences the pressure that a root must exhibit to penetrate the soil. Clay soils are less favorable for good plant growth because of high bulk density. Tap-rooted, perennial legumes *Stylosanthes hamata* is more efficient in creating their own root macropores than others species are (Lesturgez *et al.*, 2004).

Sandy soils have intensive drainage and plant roots are not able to absorb enough water or nutrients, they are low in organic matter and this makes them poor source of trace elements. Clay content has a vital role in soil fertility since clay mineral surfaces serve as sites for nutrient storage.

Soil mechanical properties have a significant impact on botanical composition and quality and productivity of natural pastures. The percentage of legumes decreases, while the percentage of grass increases with rising percentage of soil with particle size <0.01 mm or < 0,002 mm (Vuckovic, 1999). Sometime, hay yield and quality significantly decrease with rising percentage of soil with particle size < 0.01 mm or < 0,002mm (Ivanek, 1988).

3. Methodology

3.1. Description of the area

Sampling was performed in two mountain areas in central and northern B&H because of their importance for livestock and diary production. Vlašić Mountain in central Bosnia (altitude 1,933) is historically sheep farming area that can be categorized as non-certified organic or nomadic production. Sheep are farmed outdoors for most of the year, housed in winter when grazing is unavailable. Vlašić Mountain is known for locally produced autochthonous cheese manufactured from sheep milk named Travnički or Vlašićki cheese. Problems in this area are insufficient production of fodder crops, degraded pastures of low productivity with low nutritional value, soil erosion, long and harsh winters, and poor road communication. Conserved forage is mostly kept as low quality hay outdoors. It is still common practice for sheep to migrate from mountain to hilly areas for grazing during winter.

Manjača Mountain in northern B&H (altitude 1 239 m) is mostly dairy farming area with low milk yields. Extreme water erosion processes took place in both sampling areas due to steep slopes and rainfall in April and May 2014 (Figure 3.2).

Botanical compositions of natural grasslands in B&H are rich in species, because they vary from calcareous to neutral substrates, from wet to dry weather, and deep to shallow soils. According to the literature and some recent inventories, B&H grassland in hilly and mountain areas include species rich *Festuco-Brometalia* grasslands with some rare and endangered species (FAO, 2009).

3.2. Climatic conditions

The amount of rainfall differs between various parts of the country. The central part of the country, including study area, is characterized with continental mountain climate. The main characteristics are harsh winters with average temperature in January ranging from -7.4˚C (1964) to 6.2˚C (2007), with absolute minimum temperature -23°C (2000). Summers are warm with average temperatures in July ranging from 18.2 °C (1961) to 25.2 °C (2012). The average annual sum of precipitation is 1043 mm (1961-2014), with abundant snowfall, especially at higher altitudes. There are no meteorological stations situated near the sampled area, so the following graphs refer to nearest meteorological stations in Banja Luka, approximately 23 to 65 km distance from the sampling area.

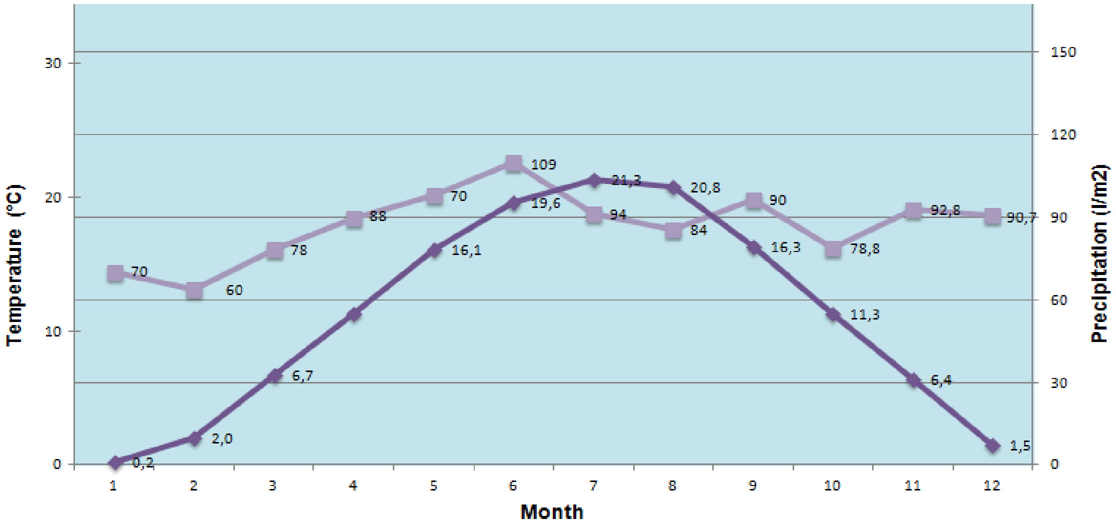

Figure 3.2.1. Climate diagram for period 1961–2014 (Walter and Lieth, 1960)

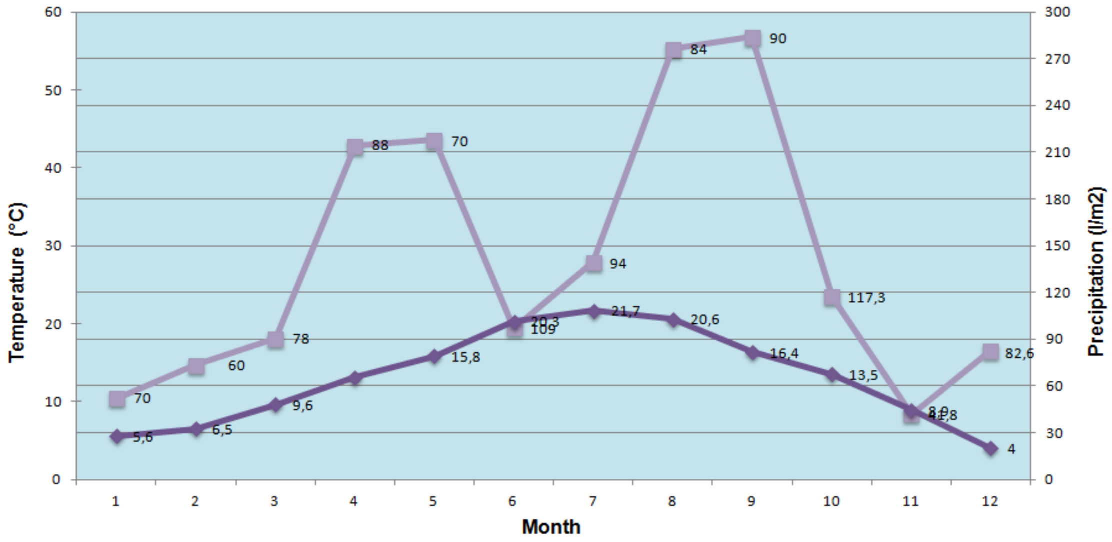

Figure 3.2.2. Climate diagram for 2014 (Walter and Lieth, 1960)

3.3. Sampling procedure

Plant and soil samples were collected in May of 2014 from 100 sampling points (Figure 3.3.1 and 3.3.2). Sampling points were randomized by throwing 50 x 50 cm square quadrate at preselected locations of natural pastures. Hundred soil samples were collected from 0-20 cm depth (hereafter called surface soil) and 20 samples (every fifth sample) from 20-40 cm depth (hereafter called subsurface soil) at different altitudes. Of the total 120 soil samples, 60 soil samples (surface and subsurface soil) and 50 related plant samples were taken from Manjača Mountain (Figure 3.3.1), and 60 soil samples (surface and subsurface soil) with related 50 plant samples were taken from Vlašić Mountain (Figure 3.3.2). The samples collected were all from natural pastures from three main different soil types (Fluvisols, Cambisols, and Leptosols) (FAO, 2006). Maps of the sampled area were created in ESRI ArcGIS version 10.

Soil samples were placed in paper bags and dried at 40°C for three days, then passed through a 2 mm mesh prior to the determination of pH, soil organic carbon content and soil texture. Plant samples were cut in pre-flowering stage with hand clipper over the same area from which the soil was sampled. The inventory of plant species was taken together with determination of most dominant plant species in the sample-square. Coordinates for every sampling site were taken with GPS type GARMIN eTrex Vista HCx.

Figure 3.3.1. Sampled area on Manjača Mountain with marked sampling sites and soil types (1:60.000)

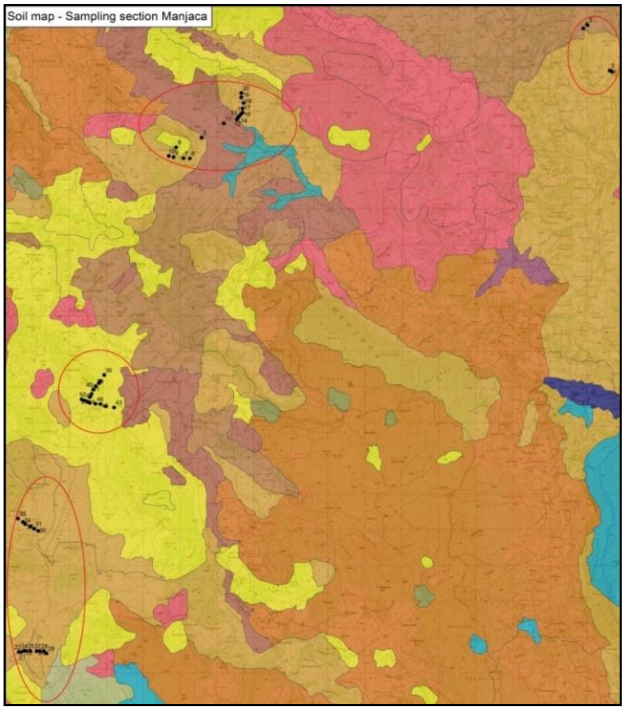

Figure 3.3.2. Sampled area on Vlašić Mountain with marked sampling sites and soil types (1:70.000)

Legend

■ Calcaric Cambisols
■ Calcaric Fluvisols
■ Cambic Podzols
■ Chromic Luvisols
■ Dystric Cambisols
■ Dystric Fluvisols
■ Dystric Leptosols
■ Dystric Podzoluvisols
■ Eutric Cambisols
■ Eutric Fluvisols
■ Eutric Leptosols
■ Eutric Vertisols
■ Humic Cambisols
■ Lithic Leptosols
■ River
■ Stagnic Luvisols
■ Vertic Cambisols
■ Vertic Luvisols
• Sampling locations

3.4. Sample analysis

3.4.1. Soil sample analysis

Soil sample analysis was performed for pH, humus content, organic carbon, soil texture, and the concentration of macro, micro, and trace elements (B, Na, Mg, P, K, Ca, Mn, Fe, Co, Cu, Zn, Mo, Se, Cd) as described below.

Soil pH was determined electrometrically in soil-to-water ratio of 1:2.5 suspension. Ten grams of air-dry soil was mixed with 25 ml of distillated H_2O and after 30 minutes pH was measured with pH meter (pHM240 pH/ion meter–Radiometer).

Humus content was determined with colorimetric method after wet combustion of the samples with potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4), (Resulović, 1969). Color intensity depends on the humus content in the substrate, darker colors indicating higher levels of humus.

Organic carbon content was derived from the total humus content (58% of humus is organic carbon and 5-7% is total nitrogen content).

Soil texture or determination of particle size content was performed by the international pipette method with sodium-pyrophosphate as dispersing agent (Piper, 1966).

For trace element analysis soil samples were pulverized with a mortar and dried at 105 °C for 48 hours to achieve constant weight. Samples were weighted to approximately 0.25 g and five ml of ultrapure concentrated nitric acid (HNO_3) was added prior to two-hour digestion in ultra clave microwave reactor (MLS-MILESTONE, ultraCLAVE III) at maximum 250°C and 160-bar pressure. The digested samples were transferred to vessel and diluted to achieve 50 ml in volume by adding double deionizer water. In total 80 soil samples, 3 Standard Reference Material (SRM) and method blanks (5 ml HNO_3 solution) were digested and diluted for total analysis of trace elements with ICP MS.

3.4.2. Plant sample analysis

Plant biomass was mixed and dried at 40°C for 3 days, afterwards finely grinded in a mill and dried at 55°C for 48 hours. Samples were weighted to approximately 0.25 g and five ml of ultrapure concentrated nitric acid HNO_3 was added prior to two-hour digestion in ultra clave microwave reactor (MLS-MILESTONE, ultraCLAVE III) at maximum 250°C and 160-bar pressure. The digested samples were transferred to vessel and diluted to achieve 50 ml in volume by adding double deionizer water. In total 80 plant samples, 3 Standard Reference Material (SRM) and method blanks (5 ml HNO_3 solution) were digested and diluted for total analysis of trace elements with ICP MS.

3.4.3. Quality assurance and method validation

The accuracy of the measurement for soil samples was obtained from certified reference material (CRMs) corresponding to two main soil types in the studied area (CRM 73324, CRM 2709a). Accuracy and reproducibility of the results were also controlled by analyzing some of the elements using both ICP-MS and ICP-OES. Selenium was analyzed with Te in 20% ethanol as online internal standard. Repeated measurements were to monitor the instrumental drift during the analysis.

To ensure the accuracy of the selected method for plant samples, the analyses of the apple (CRM 1515) and tea leaves (NCS ZC 73014) as certified reference material (CRMs) were carried out. Plant samples were analyzed on Agilent 8800QQQ with Sc, Ge, In, Rh and Bi as internal standards. Selenium analyzed with Te in 20% ethanol as online internal standard. Repeated measurements were to monitor the instrumental drift during the analysis.

3.5. Statistical analysis and calculations

A linear regression and fitted line plots were used to demonstrate response variable (trace element concentration in plant samples), and the predictor variable (trace element concentration in the soil). The model approach further used was stepwise regression, applying actual plant trace element concentrations as the response and total soil trace element concentrations, together with soil physic-chemical properties as the predictors. Non-numerical, categorical predictor (in statistical terms dummy variable) was introduced to evaluate the dominant plant species affecting the levels of trace elements in the plant samples.

Correlation matrix for all the investigated parameters was created. For all statistical operations software Minitab 17 was used. The transfer factor (TF) was obtained by dividing the element concentration in the plant over its concentration in soil was also calculated.

4. Results

4.1. Soil physical characteristics

The soil samples from tree main soil types in the studied area were collected at different altitudes (Table 4.1.1). From hundred and twenty soil samples, sixty nine were Cambisols, four Fluvisols, and forty seven Leptosol. Concerning the particle size distribution, percentage of sand ranged from 5.4 % to 51.5 %, percentage of silt from 35.9 % to 79 %, while clay from 4.9 % to 49.9 %. The soil pH varied from strongly acidic (4.7) to moderately alkaline (7.8). Percentage of organic carbon varied significantly among the samples, ranging from 0.5 % to 12.3 %.

Table 4.1.1. Distribution of samples at different altitudes and soil groups

Elevation range (m alt.)	Depth of soil (cm)	Soil type	No of samples
55 – 1180	0 – 20	Cambisols	57
599 - 716		Fluvisols	3
792 - 1183		Leptosols	40
Total number of samples			100
519 - 1160	20 – 40	Cambisols	12
599		Fluvisols	1
835 - 1168		Leptosols	7
Total number of samples			20

Table 4.1.2. Summary statistics for the principal soil characteristics in different soil types

Soil type	Soil texture						pH		Organic C content (%)		No of samples
	Sand (%) (2-0.06 mm)		Silt (%) (0.06-0.002 mm)		Clay (%) (<0.002 mm)						
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Cambisol	6-52	16	36-73	61	5-50	23	4.7-7.8	5.8	0.5-12.3	3.3	68
Fluvisol	5-25	11	52-75	66	16-27	23	4.9-7.6	6.7	1.6-2.3	4.8	4
Leptosol	9-34	16	36-79	60	13-50	24	4.8-7.4	5.6	0.8-10.1	3.7	48

Based on the USDA classification, 81 samples belong to texture category silty loam, twenty to silty clay loam, six to silty clay, while the remaining samples belong to other textural categories. There was no significant difference in particle size distribution between different soil categories, while pH and organic C were slightly higher in Fluvisols than in Cambisols and Leptosols (Table 4.1.2).

4.2. Soil and plant chemical characteristic

Table 4.2.1. Summary statistics for the trace elements concentration in soil and plant samples (all values are in mg kg^{-1} unless otherwise indicated)

Element	Soil			Plant		
	Mean	SD	Range	Mean	SD	Range
B	35.9	11.3	11 – 57	14.3	5.2	4.2 – 30
Na	630	121	360 – 970	34,6	58	7.4 – 350
Mg (g/kg)	9.9	5.3	4.3 – 35	2.2	0.6	1.1 – 3.9
P (g/kg)	0.8	0.3	0.4 – 2.2	2.2	0.7	1.1 – 3.7
K (g/kg)	10.2	2.3	4.2 – 15	22,9	6,1	10 – 40
Ca (g/kg)	7.5	10.8	1.8 – 56	7.1	2.3	2.3 – 14
Mn (g/kg)	1.6	5.5	0.6 – 3.2	0.16	0.1	0.04 – 0.5
Fe (g/kg)	40	5.6	26 – 54	0.2	0.3	0.05 – 2.1
Co	22.6	6.1	9.9 – 34	0.1	0.2	0.02 – 1.3
Cu	34.3	13.1	14 – 71	6.6	1.3	4.5 – 12
Zn	121	22.8	75 – 210	31.7	7.3	19 – 60
Mo (µg/kg)	420	350	85 – 1800	440	600	32 - 3100
Se (µg/kg)	390	80	190 – 640	23.8	11.2	9.1 – 72
Cd	0.69	0.5	0.2 – 2.1	0.2	0.1	0.03 – 0.8

Table 4.2.2. Summary statistics of the trace element concentration in soil in different soil types (all values are in mg kg^{-1} unless otherwise indicated)

Element	Leptosols			Cambisols		
	Mean	SD	Range	Mean	SD	Range
B	41	10	19–57	32.7	11	11–54
Na (g/kg)	0.6	1.1	0.44–0.97	0.6	0.1	0.36–0.97
Mg (g/kg)	10.4	4.7	6,4–29	9.6	5.8	4.3–35
P	812	238	500–1700	780	365	440–2200
K (g/kg)	10.8	2.1	5.2–15	9.7	2.3	4.2–14
Ca (g/kg)	6.9	9.5	2–47	7.6	11.5	1.8–56
Mn (g/kg)	1.8	0.5	0.8–3.2	1.5	0.4	0.6–2.3
Fe (g/kg)	41	4.3	29–52	40	6.5	26–54
Co	24	6.7	13–34	21.5	5.5	9.9–33
Cu	39	15.4	19–71	31	9.7	14–50
Zn	121	17.9	98–180	122	26.3	75–210
Mo (µg/kg)	600	500	100–1800	300	200	100–900
Se (µg/kg)	400	100	300–600	400	100	200–600
Cd	0.7	0.5	0.2–2	0.7	0.5	0.2–2.1

Table 4.2.3. Summary statistics of the trace element concentration in plants in different soil types (all values are in mg kg^{-1} unless otherwise indicated)

Element	Leptosols			Cambisols		
	Mean	SD	Range	Mean	SD	Range
B	15.4	4.9	6.3 - 27	13.5	5.5	4.2 – 30
Na	25.8	22.9	11 - 110	35	64,5	7.4 – 350
Mg (g/kg)	2.2	0.5	1.1 – 3.4	2.1	0.7	1.1 – 3.9
P (g/kg)	2.2	0.6	1.1 – 3.5	2.2	0.7	1.1 – 3.6
K (g/kg)	23.1	5.9	12 – 33	22.5	6.3	10 – 40
Ca (g/kg)	7	1.5	4.8 – 11	6.9	2.6	2.3 – 12
Mn (g/kg)	0.2	0.1	0.04 – 0.5	0.2	0.1	0.04 – 0.4
Fe (g/kg)	0.2	0.1	0.07 – 0.5	0.2	0.2	0.05 – 1.3
Co	0.1	0.1	0.03 – 0.4	0.1	0.1	0.02 – 0.6
Cu	6.59	0.9	4.9 – 8.2	6.5	1.48	4.5 – 12
Zn	33	7	24 – 50	31	7.36	19 – 60
Mo (µg/kg)	450	580	30 - 2200	420	560	40 – 3100
Se (µg/kg)	30	10	10 – 70	20	10	10 – 40
Cd	0.2	0.1	0.06 – 0.6	0.2	0.14	0.03 – 0.8

In this study, the soil-to-plant transfer factor (TF) for investigated trace elements in forage samples consumed by animals were calculated (Table 4.2.4) and the data showed that the TF values varied between different altitudes in some trace elements.

Table 4.2.4. Transfer factor of trace elements at different altitudes of the sampling area

Trace element	Altitude			
	400 – 600	600 - 800	800 – 1000	1000 – 1200
B	0.42	0.40	0.50	0.38
Na	0.06	0.06	0.09	0.03
Mg	0.26	0.27	0.25	0.24
P	3.40	2.89	2.85	3.85
K	2.36	2.33	2.46	2.08
Ca	2.17	1.72	1.36	2.65
Mn	0.01	0.16	0.10	0.08
Fe	0.01	0.01	0.01	0.01
Co	0.01	0.01	0.01	0.00
Cu	0.21	0.25	0.24	0.15
Zn	0.28	0.30	0.26	0.28
Mo	1.25	1.37	1.97	0.19
Se	0.07	0.05	0.07	0.07
Cd	0.50	0.63	0.36	0.68

4.3. Factors affecting trace element concentrations in plants

Fitted line plots (Figures 4.3.1 – 4.3.14) with the use of the regression model showed relatively low coefficient of determination values (R^2). Low R^2 in this study indicate that only small percentage of the plant TE concentration can be explained by the total TE concentration in the soil, and that other environmental factors might had stronger effect.

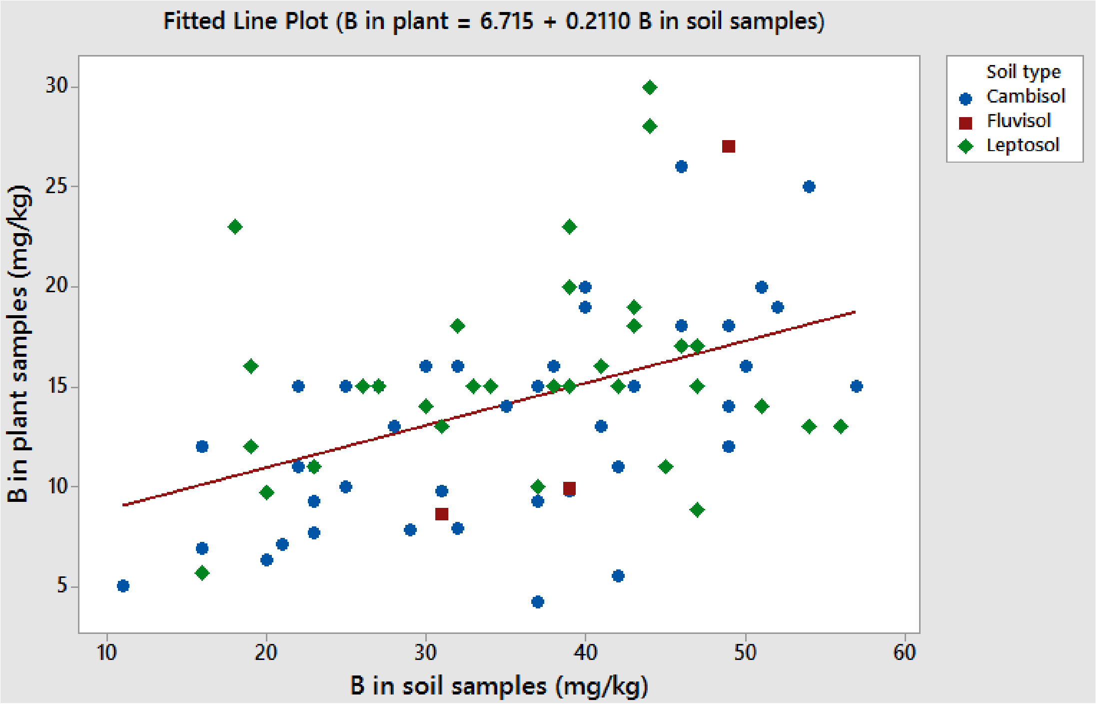

Figure 4.3.1. Fitted line plot of B concentrations in plant samples grown in different soil types

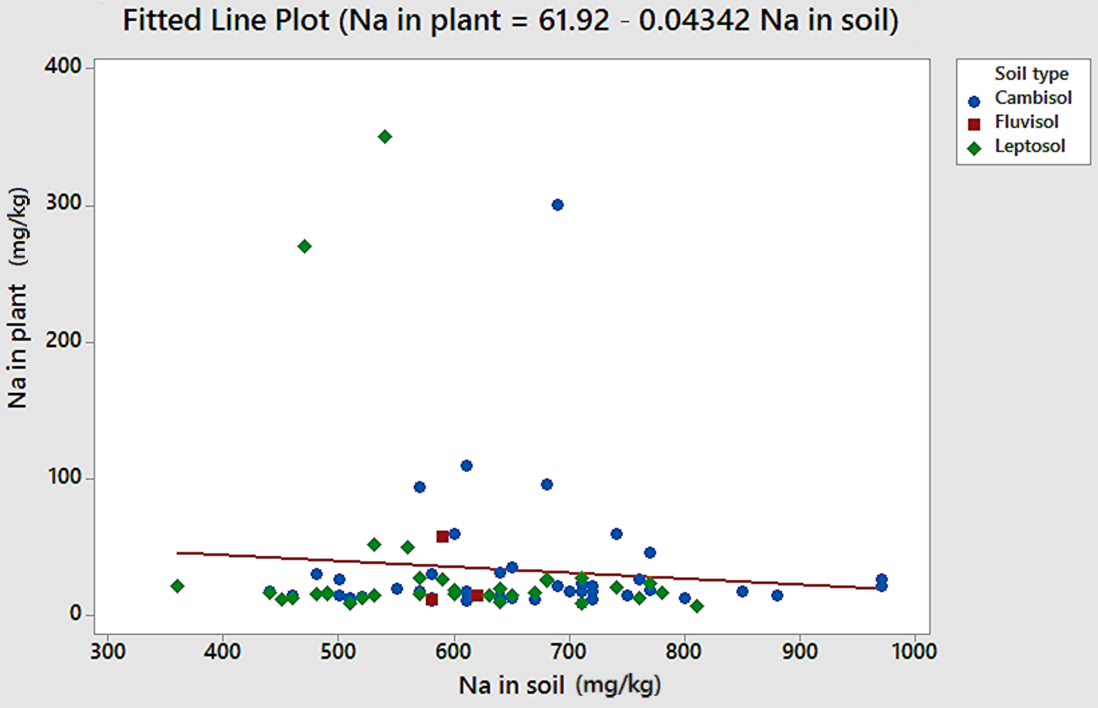

Figure 4.3.2. Fitted line plot of Na concentrations in plant samples grown in different soil types

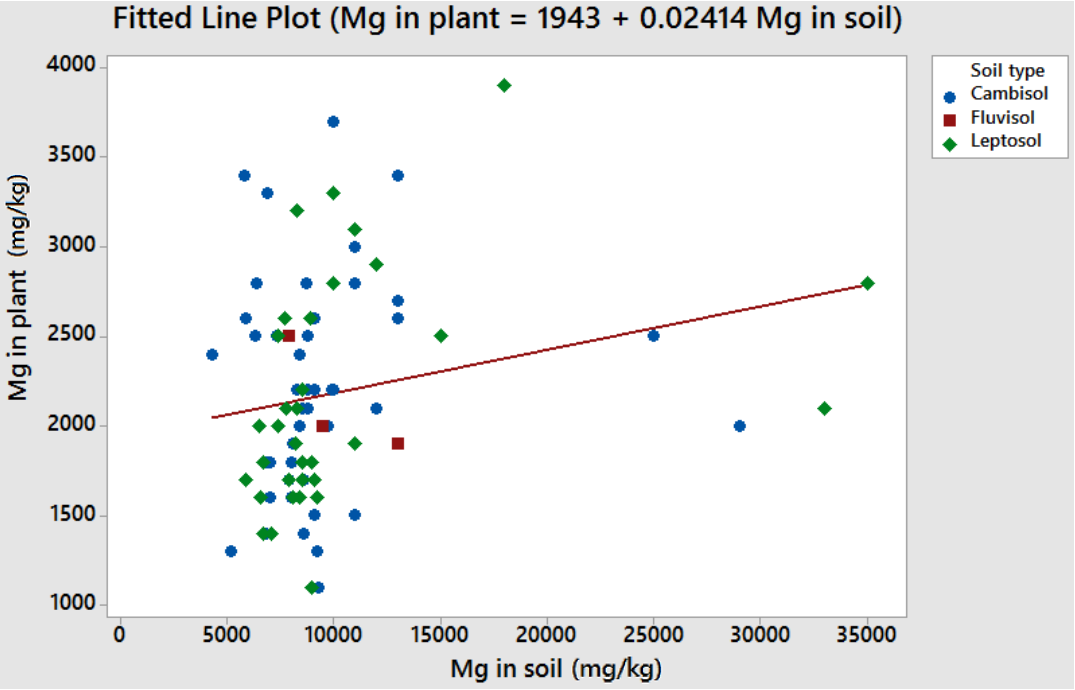

Figure 4.3.3. Fitted line plot of Mg concentrations in plant samples grown in different soil types

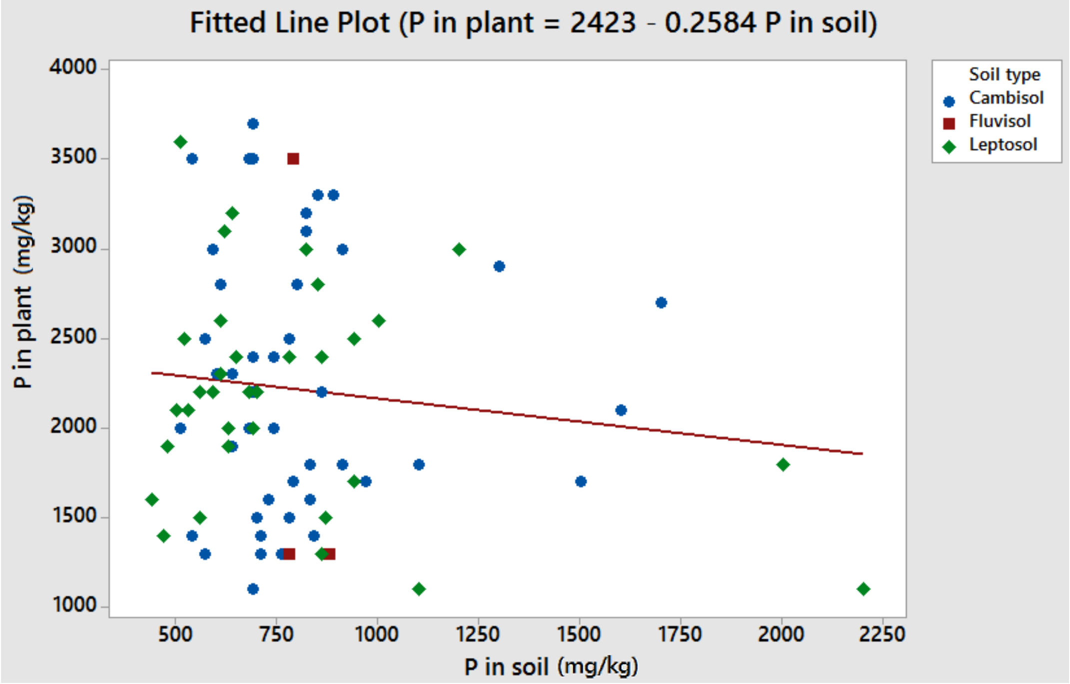

Figure 4.3.4. Fitted line plot of P concentrations in plant samples grown in different soil types

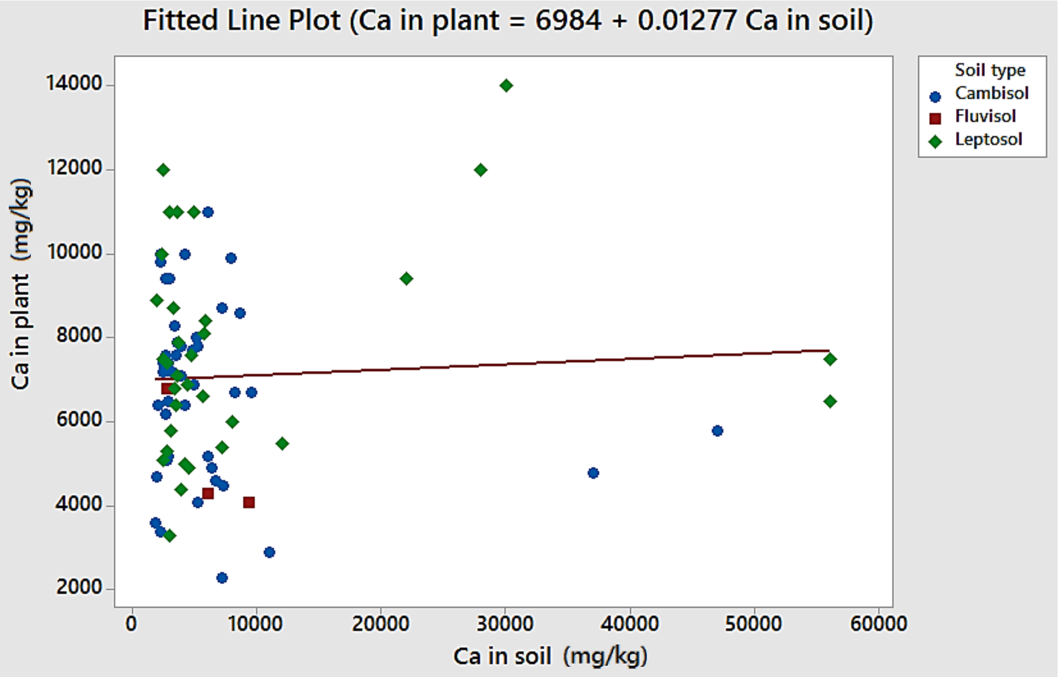

Figure 4.3.5. Fitted line plot of Ca concentrations in plant samples grown in different soil types

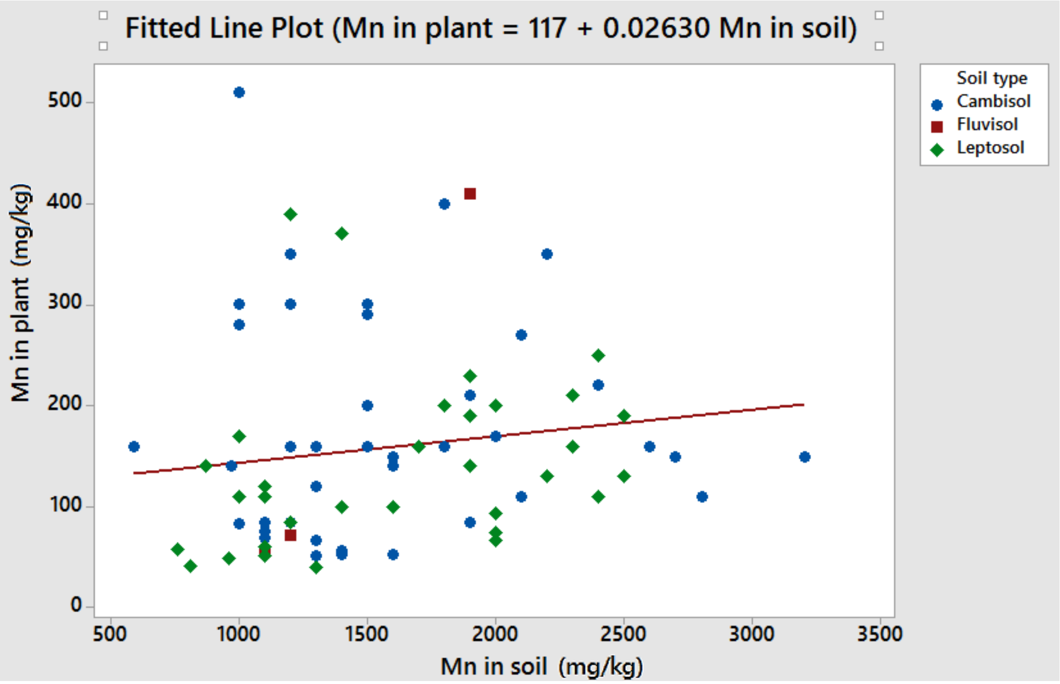

Figure 4.3.6. Fitted line plot of Mn concentrations in plant samples grown in different soil types

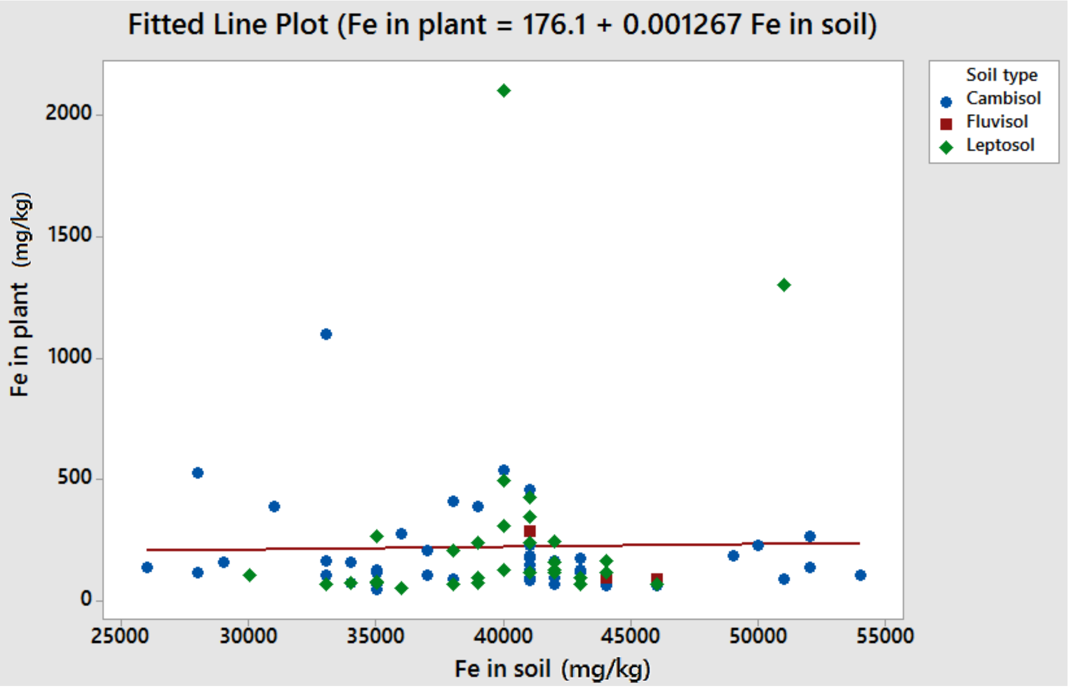

Figure 4.3.7. Fitted line plot of Fe concentrations in plant samples grown in different soil types

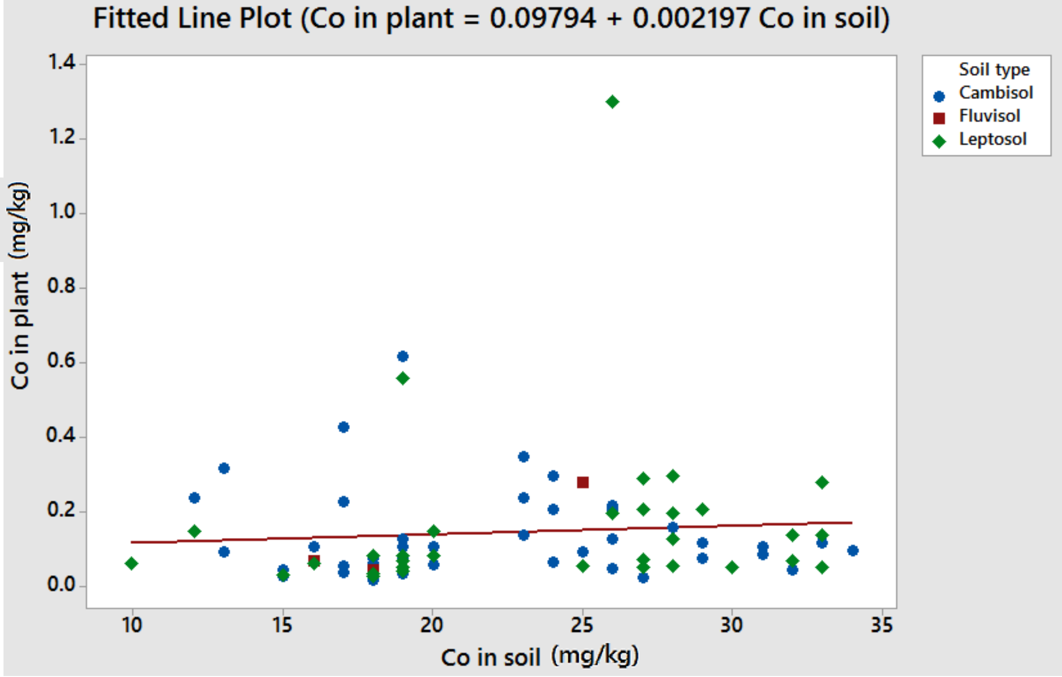

Figure 4.3.8. Fitted line plot of Co concentrations in plant samples grown in different soil types

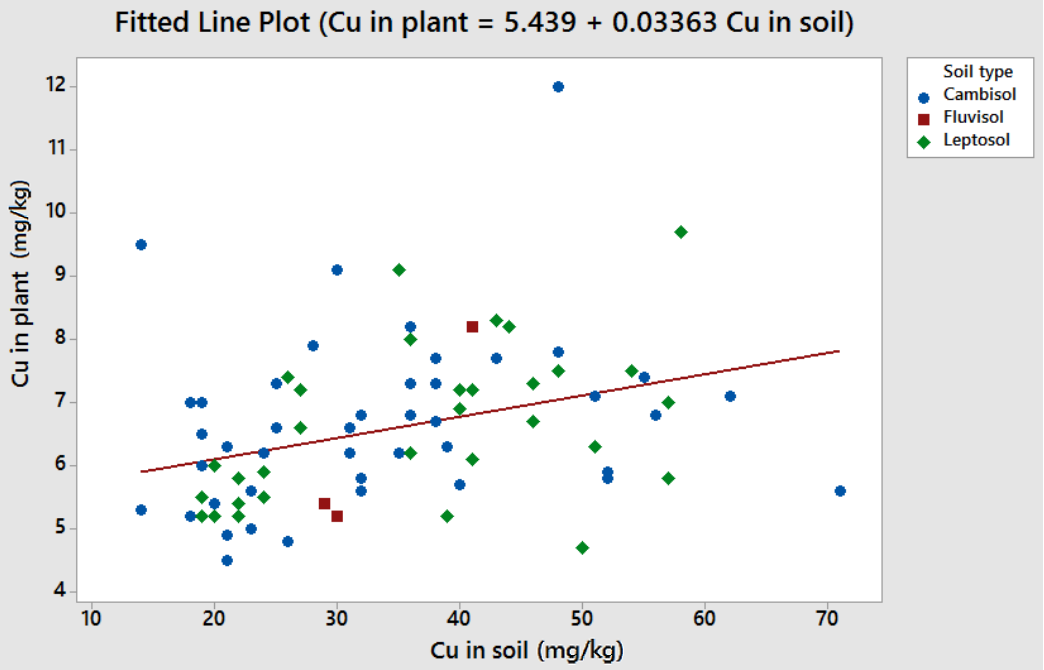

Figure 4.3.9. Fitted line plot of Cu concentrations in plant samples grown in different soil types

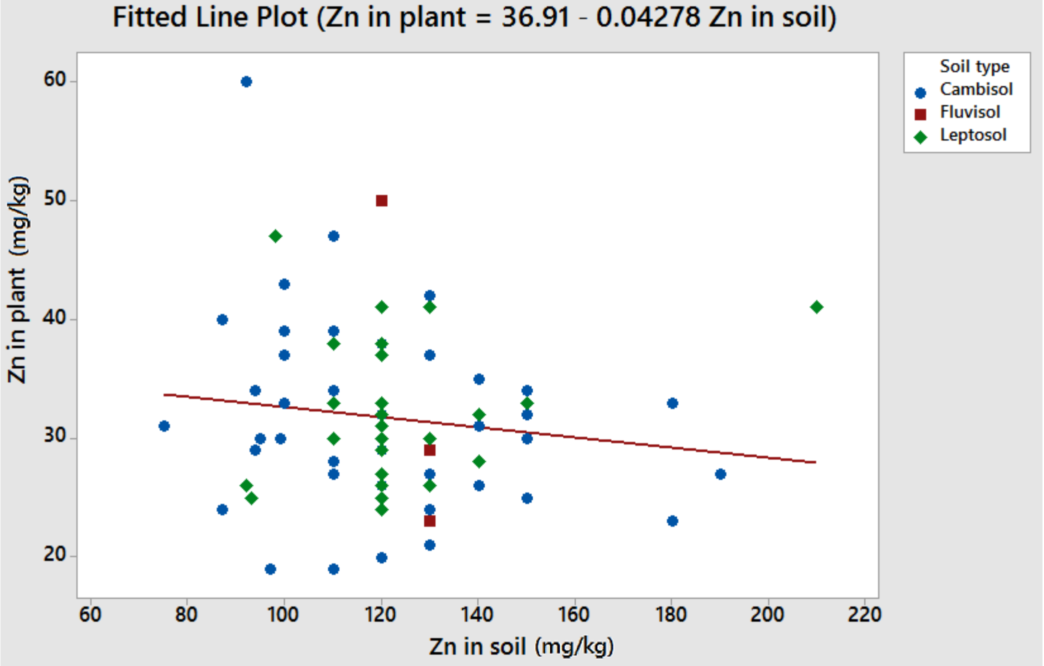

Figure 4.3.10. Fitted line plot of Zn concentrations in plant samples grown in different soil types

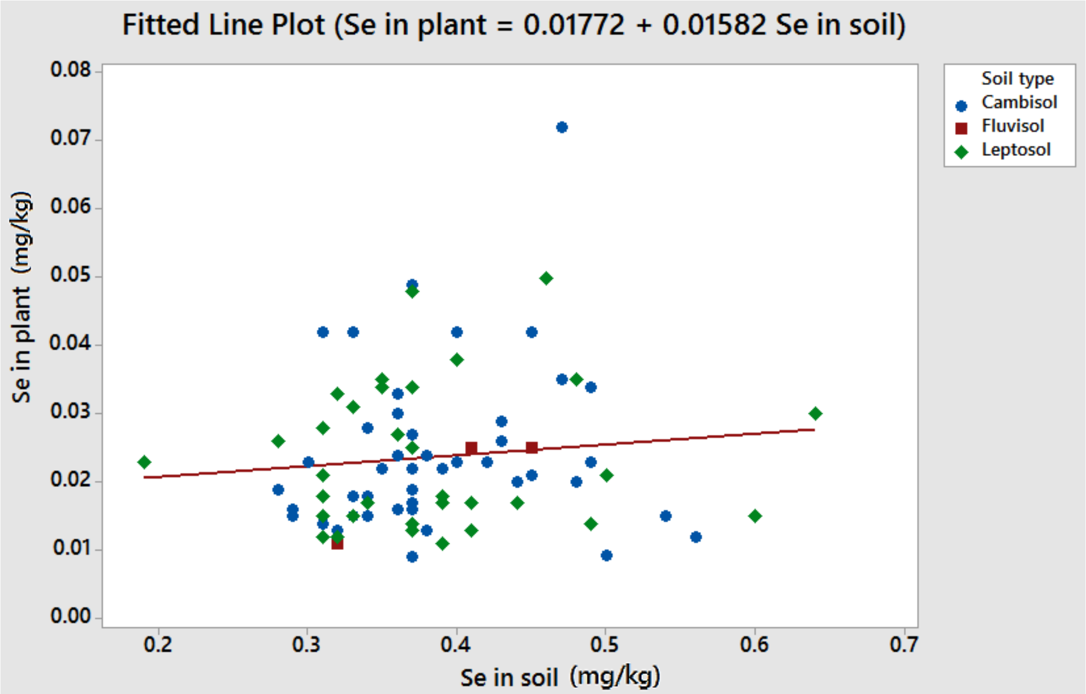

Figure 4.3.11. Fitted line plot of Se concentrations in plant samples grown in different soil types

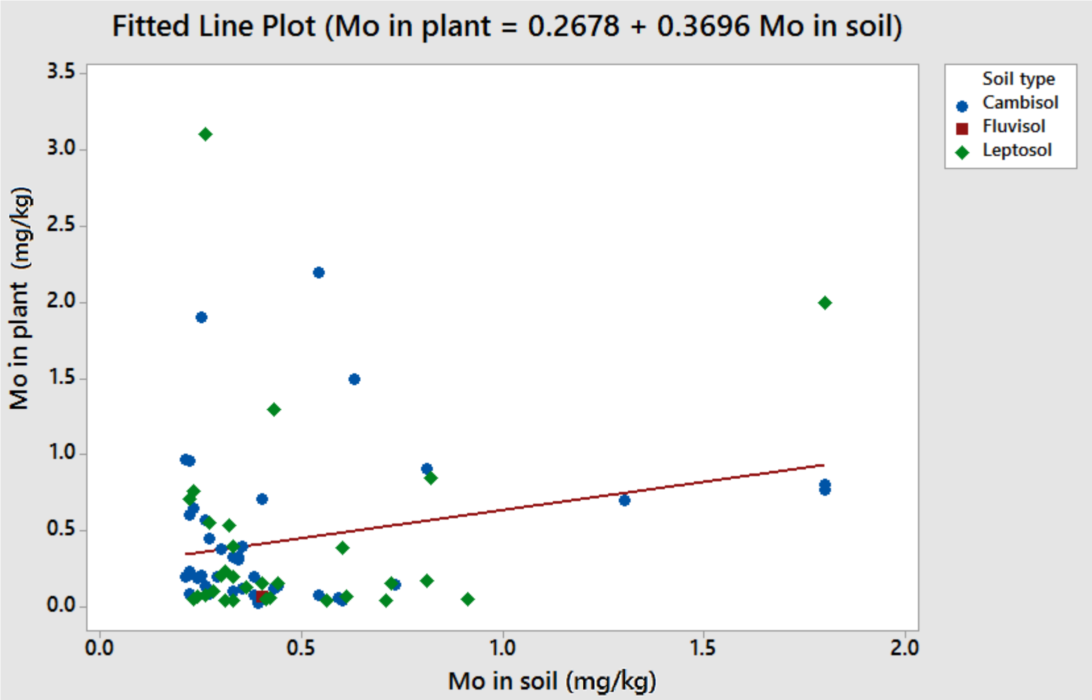

Figure 4.3.12. Fitted line plot of Mo concentrations in plant samples grown in different soil types

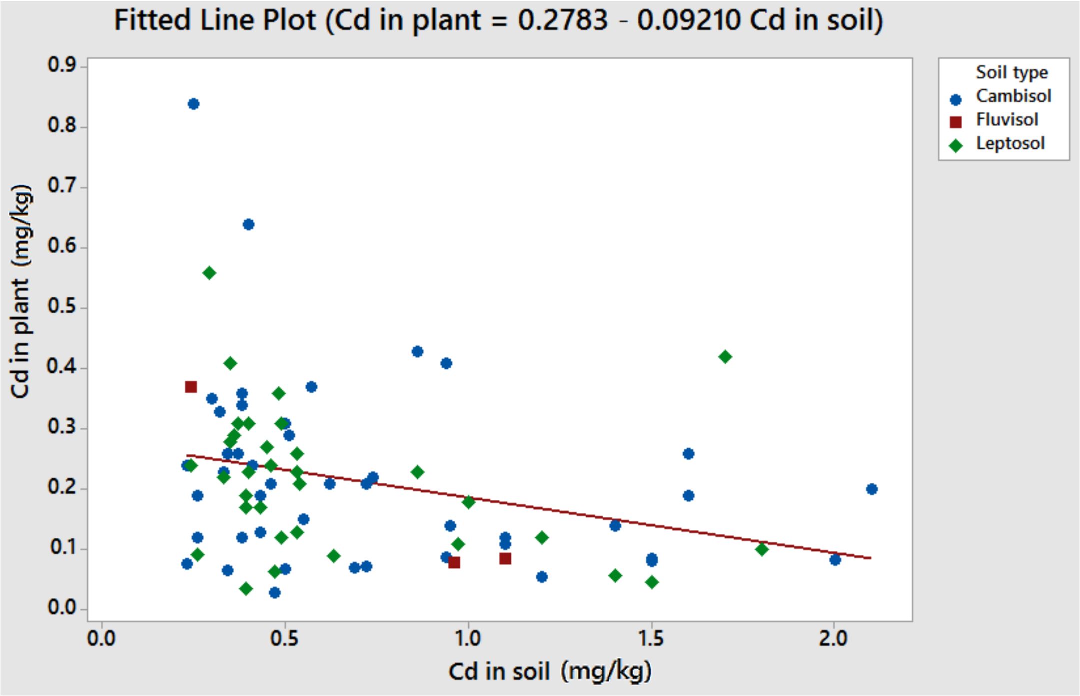

Figure 4.3.13. Fitted line plot of Cd concentrations in plant samples grown in different soil types

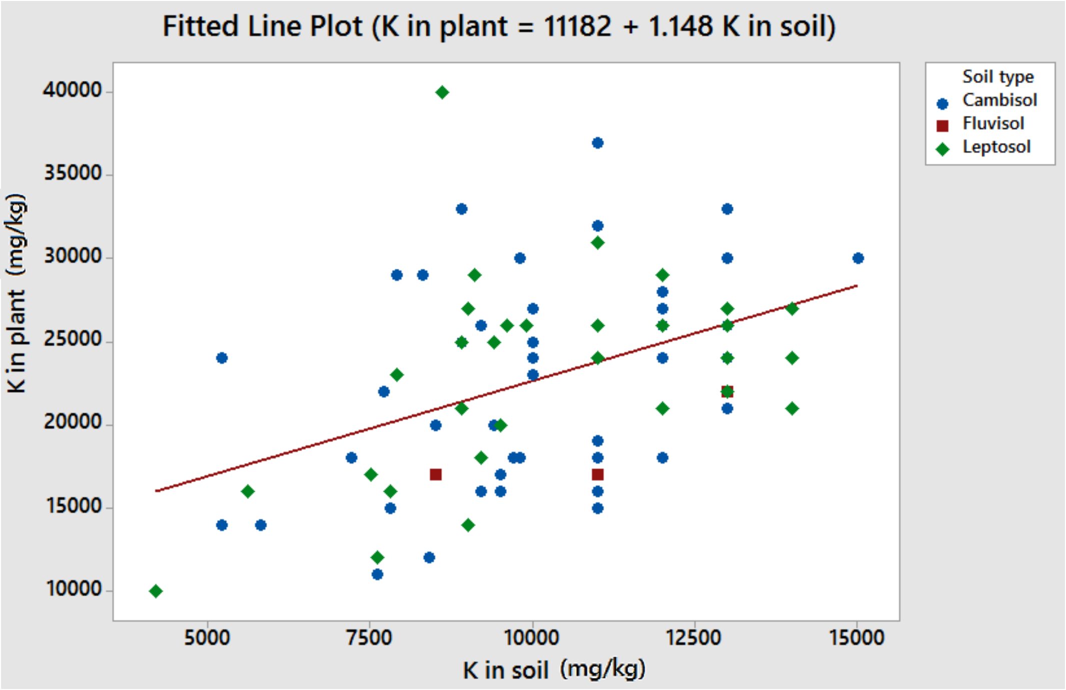

Figure 4.3.14. Fitted line plot of K concentrations in plant samples grown in different soil types

Stepwise regression where multiple environmental factors were introduced to the model (Table 4.3.1) had higher R^2 values indicating which individual factor contributed to the plant TE concentration in addition to the soil TE concertation.

Table 4.3.1. Regression equation[b] of the trace element concentration in aboveground plant system and the best predicting factors

TE in plant	Intercept	pH in H2O	SOC	Clay content	Sand content	Co Soil	Na Soil	Mg Soil	K Soil	P Soil	Mn Soil
Zn	72,3***	-4,52*	-1,948**	0,169^{ns}	0,382*	-1,303*	-4,1^{ns}	0,388^{ns}	0,256^{ns}	3,78^{ns}	2,87^{ns}
Na	0,108^{ns}	-0,006^{ns}	-0,004 ^{ns}	0,001^{ns}	0,003*	-0,003^{ns}	-0,019^{ns}	-0,02***	0,002^{ns}	0,045^{ns}	-0,001^{ns}
Se	0,007^{ns}	0,002^{ns}	0,000^{ns}	0,000^{ns}	0,001*	0,000^{ns}	0,021^{ns}	-0,000^{ns}	-0,001^{ns}	-0,00^{ns}	-0,007^{ns}
K	18,5^{ns}	-0,87^{ns}	-0,815^{ns}	-0,04^{ns}	-0,183^{ns}	0,847*	-10,98^{ns}	-0,147^{ns}	1,06*	5,74^{ns}	-6,34^{ns}
P	3,70**	-0,039^{ns}	-0,167*	-0,013^{ns}	-0,014^{ns}	0,009^{ns}	-1,393^{ns}	-0,032^{ns}	0,095^{ns}	1,131**	-0,142^{ns}
Mo	-0,619^{ns}	0,027^{ns}	-0,037^{ns}	0,01^{ns}	0,014^{ns}	0,065*	-0,443^{ns}	-0,043^{ns}	-0,037^{ns}	0,413^{ns}	-0,327^{ns}
Mn	583**	-63,4**	3,28^{ns}	-1,28^{ns}	0,24ns	2,98^{ns}	24^{ns}	2,54^{ns}	-7,10^{ns}	-33,7^{ns}	-27,0^{ns}
Mg	0,91^{ns}	0,078^{ns}	-0,095^{ns}	0,008^{ns}	0,008^{ns}	0,001^{ns}	-0,371^{ns}	0,039^{ns}	-0,054^{ns}	0,552^{ns}	-0,001^{ns}
Ca	1,69^{ns}	0,370^{ns}	-0,394*	-0,037^{ns}	0,009^{ns}	0,136^{ns}	-1,07^{ns}	-0,292*	0,008^{ns}	1,81^{ns}	-1,44^{ns}
Cd	0,985***	-0,096**	-0,014^{ns}	-0,00^{ns}	0,004^{ns}	-0,002^{ns}	-0,042^{ns}	0,005^{ns}	-0,011^{ns}	0,132^{ns}	-0,053^{ns}
Fe	-0,489^{ns}	0,143*	-0,033^{ns}	-0,002^{ns}	0,004^{np}	-0,019^{ns}	-0,233^{ns}	-0,044*	0,018^{ns}	0,043^{ns}	-0,162^{ns}
Cu	7,68**	-0,28^{ns}	-0,273*	0,032^{ns}	0,059*	-0,044^{ns}	-0,99^{ns}	-0,089^{ns}	-0,004^{ns}	2,287*	-1,202^{ns}
Co	0,092^{ns}	0,051^{ns}	-0,018^{ns}	-0,002^{ns}	-0,001^{ns}	-0,016^{ns}	-0,029^{ns}	-0,039**	0,017^{ns}	0,048^{ns}	-0,095^{ns}
B	16,5^{ns}	-0,37^{ns}	-0,593^{ns}	-0,02^{ns}	0,019^{ns}	-0,162^{ns}	-9,79^{ns}	-0,138^{ns}	0,697^{ns}	5,29^{ns}	-0,91^{ns}

[a] Levels of significance: *p<0.1, **p<0.01, ***p<0.0001, ns: not significant; R^2 = coefficient of determination;

[b] The general equation: TEcp = a + bpH + cOC + dClay + eSand + fCo + gNa + hMg + iK + jP + kMn + lFe + mCa + nCu + oZn + pMo + qSe + rB + sCd + tHerb. + uLeg.; TE trace element in plants; a, b, c, d, e, f, g, h, I, j, k, l, m, n, o, p, q, r, s, t, and u are the coefficients with positive or negative sign, SOC is the soil organic carbon

Table 4.3.1: Continuation of the table

TE in plant	Fe Soil	Ca Soil	Cu Soil	Zn Soil	Mo Soil	Se Soil	B Soil	Cd soil	Plant type		R^2 (%)
									Herbaceous	Legumes	
Zn	0,02^{ns}	0,04^{ns}	0,128^{ns}	-0,037^{ns}	1,98^{ns}	0,1^{ns}	0,079^{ns}	-6,51*	4^{ns}	-1,31^{ns}	42,89
Na	0,003*	0,01***	0,000^{ns}	-0,000^{ns}	0,009^{ns}	-0,039^{ns}	0,001^{ns}	-0,022^{ns}	0,009^{ns}	0,015^{ns}	64,31
Se	-0,001^{ns}	-0,000^{ns}	0,001*	0,000^{ns}	0,002^{ns}	0,022^{ns}	-0,000^{ns}	-0,001^{ns}	0,003^{ns}	0,001^{ns}	25,91
K	-0,127^{ns}	0,073*	-0,077^{ns}	0,042^{ns}	-0,50^{ns}	8,2^{ns}	-0,039^{ns}	0,36^{ns}	-0,42^{ns}	1,72^{ns}	48,90
P	0,001^{ns}	0,009^{ns}	-0,006^{ns}	-0,001^{ns}	-0,02^{ns}	-1,71^{ns}	-0,001^{ns}	0,166^{ns}	0,200^{ns}	0,216^{ns}	50,15
Mo	0,033*	0,038*	-0,024*	0,001^{ns}	0,598***	-1,996*	-0,008^{ns}	0,370*	-0,171^{ns}	0,102^{ns}	71,25
Mn	-4,66^{ns}	0,80^{ns}	1,58^{ns}	0,514^{ns}	34,6^{ns}	153^{ns}	-0,65^{ns}	-94,4*	57,9*	3,4^{ns}	52,31
Mg	0,045*	-0,02^{ns}	-0,027*	0,001^{ns}	0,615**	-0,54^{ns}	0,002^{ns}	-0,163^{ns}	-0,089^{ns}	-0,079^{ns}	41,11
Ca	0,101^{ns}	0,152^{ns}	0,014^{ns}	-0,002^{ns}	1,077^{ns}	-1,34^{ns}	0,044^{ns}	-0,22^{ns}	0,003^{ns}	-0,520^{ns}	44,15
Cd	-0,002^{ns}	-0,004^{ns}	0,003^{ns}	0,001^{ns}	0,149**	-0,454^{ns}	0,000^{ns}	-0,041^{ns}	0,016^{ns}	-0,067*	55,44
Fe	0,004^{ns}	0,021*	0,009^{ns}	0,003*	0,104^{ns}	0,783^{ns}	-0,001^{ns}	-0,422**	0,101^{ns}	-0,022^{ns}	51,85
Cu	0,016^{ns}	0,033^{ns}	0,066*	0,003^{ns}	0,463^{ns}	-1,91^{ns}	0,001^{ns}	-0,898^{ns}	0,574^{ns}	0,212^{ns}	40,72
Co	0,003^{ns}	0,02**	0,006*	0,001^{ns}	0,044^{ns}	0,269^{ns}	0,001^{ns}	-0,203*	0,064^{ns}	-0,009^{ns}	54,23
B	0,3^{ns}	0,085^{ns}	-0,109^{ns}	-0,019^{ns}	3,14^{ns}	-20,9^{ns}	0,145*	-1,14^{ns}	-0,98^{ns}	-2,70*	38,36

[a] Levels of significance: *p<0.1, **p<0.01, ***p<0.0001, ns: not significant; R^2 = coefficient of determination;

[b] The general equation: TEcp = a + bpH + cOC + dClay + eSand + fCo + gNa + hMg + iK + jP + kMn + lFe + mCa + nCu + oZn + pMo + qSe + rB + sCd + tHerb. + uLeg.; TE trace element in plants; a, b, c, d, e, f, g, h, I, j, k, l, m, n, o, p, q, r, s, t, and u are the coefficients with positive or negative sign, SOC is the soil organic carbon

4.4. Plant species distribution

The natural pastures and grasslands were comprised of varying numbers of plant species of different morphological, biological and production characteristics (Figure 4.4.1 and 4.4.2). The floristic mixture of the studied grasslands consisted of the species of the families *Poaceae*, *Leguminosae*, *Plantaginaceae*, *Scrophulariaceae*, *Asteraceae, Fabaceae, Polygonaceae, Violaeeae, Lamiaceae, Euphorbiaceae,* etc. The percentage of individual grassland components was: 32.8% grasses, 30.4% leguminoses and 36.8% of other herbaceous plants.

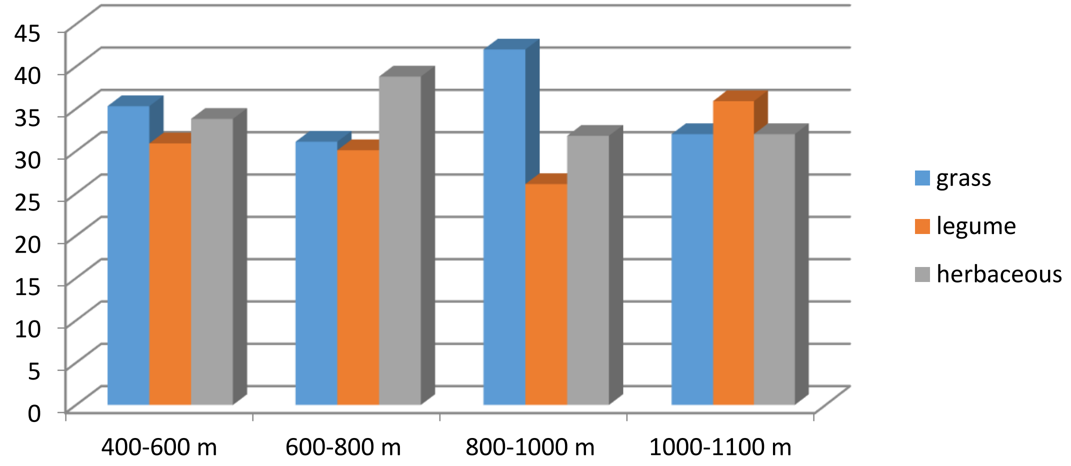

Figure 4.4.1: Frequency distribution of plants in different altitudes in the sampled area

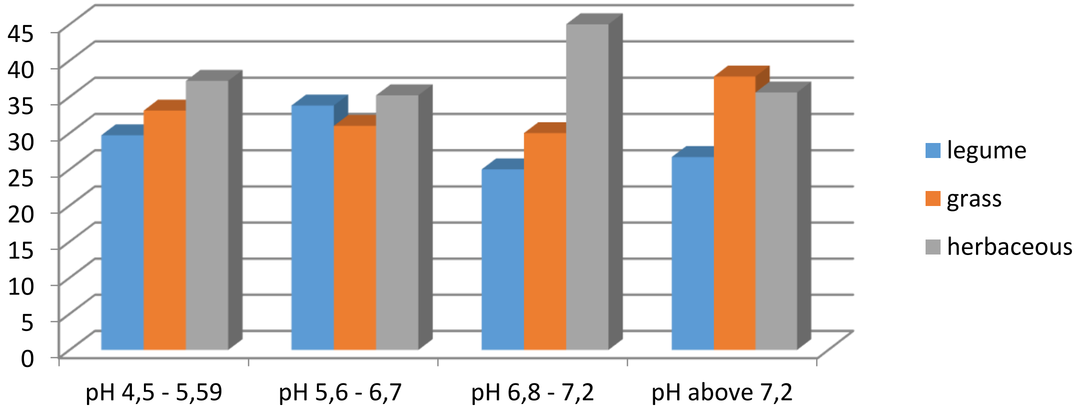

Figure 4.4.2. Frequency distribution of plants in different soil pH in the sampled area

5. Discussion

Many grazing animals are dependent for their nutrients and essential minerals on the available forage, either in its natural state or conserved as hay or silage. Trace element concentration in soils and forages influence mineral status of grazing livestock (Espinoza *et al*., 1991). In order to avoid serious forage and livestock production restrictions it is important to timely diagnose and identify reasons for mineral deficiency in forage and animals.

In this study the soil pH varied from strongly acidic (4,7) to moderately alkaline (7.8), while Marković *et al.,* (2011) reported that 60 percent of soil samples in northern Bosnia had pH lower than 4.5. Predić *et al*., (2014) reported that 26 % of the soils analyzed (n=1876, 2320 ha) were unsuitable for agricultural production due to strong acidity (16 %) or alkalinity (11 %) in the Republic of Srpska (northern and eastern B&H).

Soil mechanical properties have a significant impact on botanical composition, quality and productivity of natural pastures. Soil texture effects the vegetation composition sometimes as an indirect factor, through soil water permeability and the ability to provide nutrients supply (Rainer, 1990). Presence and domination of *Avena* spp. and *Taeniatherum asperum* in grassland can be directly related to high clay content, while domination by *Erodium botrys* to moderate clay content (Huenneke and Mooney, 1989). Vucković (1999) discussed an optimal soil for forage production contains 70-80% sand, 20-30% clay in agro-ecological conditions of Balkan region. The percentage of legumes decreases, while the percentage of grass increases with rising percentage of soil particle size <0.01 mm or < 0,002 mm (Vucković, 1999). In this study, most of the soil samples were very high in silt content, which leads to the conclusion that textural soil characteristics may not be optimal for forage production. However, Coffin (1994) reported that fastest pasture recovery after intensive grazing is achieved on soils with the highest silt content, due to easier seedling establishment, while slowest in soil with low silt content, and either high or low water holding capacity.

Factors influencing botanical distribution and the dominance in the grassland are important in meeting animal nutrient requirements considering that the plant species differ considerably in their abilities to absorb trace elements. Diversity in grasslands improve biomass production and provide more efficient nutrient use in livestock production (Dybzinski *et al*., 2008). The botanical composition of the studied grasslands consisted of wide range of species, in total 61 different species were identified, of which some could be categorized as worthless and even harmful. The weed species with high abundance was *Euphorbia cyparissias*, while with less abundance were *Ranunculus repens*, *Rhinanthus rumelicus*, *Chamaespartium sagittale*.

5.1 Concentration of important minerals in soil and forages

Pasture sampling is the easiest and most reliable mean of diagnosing Na deficiency in dairy cows (O'Connor, 2010). The average concentrations of Na in the soil samples was 0,63 g kg^{-1}, while range varied from 0,36 g kg^{-1} to 0,97 g kg^{-1} (Table 4.2.1). The average concentrations of Na in the plant samples was 34,6 mg kg^{-1}, while the range varied from 7,4 mg kg^{-1} to 350 mg kg^{-1} (Table 4.2.1). There was no difference between Na concentrations in different soil types (Table 4.2.2), however, plants grown on Cambisols had higher Na concentration (Table 4.2.3). Results from this study indicate that plant Na concentration is not sufficient to meet animal requirements in the range from 0,6-0,8 g kg^{-1} (Morris 1980, NRC, 1996), especially considering that lactating cows have 1 g kg^{-1} higher requirements (Edmeades and O'Connor, 2003, NRC, 1996).

The average concentrations of K in the soil samples was 10,2 g kg^{-1}, while range varied from 4,2 g kg^{-1} to 15 g kg^{-1} (Table 4.2.1). The average concentrations of K in the plant samples was 23 g kg^{-1}, while range varied from 10 g kg^{-1} to 40 g kg^{-1} (Table 4.2.1). Animal potassium requirement ranges from 3 g kg^{-1} DM for sheep to 8,4 g kg^{-1} DM for young calf (Suttle, 2010). The excess of K in forage is not a problem due to high margin of 60 g kg^{-1} that can lead to animal appetite decline (Suttle, 2010), reduction of magnesium absorption (NRC, 1996) and other adverse effects. In addition, in this study higher concentration of K in forage can provide slightly higher requirements for lactating cow, approximately 1,5 g K per day extra for each liter of milk produced (NRC, 1996). Others also found elevated K concentrations, exceeding the limits of nutritional value, in many forage plant species on cultivated pastures (Juknevičius and Sabienė, 2007).

The average concentrations of Ca in the soil samples was 7,5 g kg^{-1}, while range varied from 1,8 g kg^{-1} to 56 g kg^{-1} (Table 4.2.1). The average concentrations of Ca in the plant samples was 7,1 g kg−1, while range varied from 2,3 g kg^{-1} to 14 g kg^{-1} (Table 4.2.1). Calcium dietary requirements for sheep and cattle are 3-4 g kg^{-1} (NRC, 1984). These results indicate that forage plants in this study were above animal Ca dietary requirements. However, in regards to Ca forage concentration, ratio Ca:P is of importance. Only Ca:P range from 1:1 to 7:1 is satisfactory, otherwise growth and feed efficiency decreases (Underwood and Suttle, 1999).

The average concentrations of Mg in the soil samples was 9,9 g kg^{-1}, while range varied from 4,3 g kg^{-1} to 35 g kg^{-1} (Table 4.2.1). The average Mg concentration in Leptosols was slightly higher (Table 4.2.2). The average concentrations of Mg in the plant samples was 2,2 g kg^{-1}, while the range varied from 1,1 g kg^{-1} to 3,9 g kg^{-1} (Table 4.2.1). Magnesium dietary requirements for sheep and cattle are 1,2 g kg-1, 1,9 g kg-1, respectively, while higher values than 2 g kg-1 are recommended if K and N are high in diet (NRC, 2001). These results indicate that the forages in this study meet animal Mg requirements. The average plant Mg concentration was slightly higher in plants grown on Leptosols (Table 4.2.3).

The average concentrations of P in the soil samples was 0,8 g kg^{-1}, while range varied from 0,44 g kg^{-1} to 22 g kg^{-1} (Table 4.2.1). According to Marković *et al*, (2011) 60 percent of the tested soil samples in northern B&H were deficient in plant available P. Similar conclusion had Vuckovic (1999), discussing that inherently low soil P concentration in Balkan region is limiting factor of forage production. In this study, the average P concentrations in the plant samples was 2,2 g kg^{-1}, ranging from 1,1 g kg^{-1} to 3,7 g kg^{-1} (Table 4.2.1). Sheep dietary requirements for P are 2,5 g kg^{-1} and for cattle 3 g kg^{-1} (NRC, 1984), which leads to a conclusion that plant samples were slightly deficient with the respect to nutritional requirement of animals. In relation to soil type, the average soil P concentration was only slightly higher in Leptosols (Table 4.2.2), while the average P concentration in plants did not show major differences (Table 4.2.3).

5.2. Concentration of trace elements in soil and forages

The average concentrations of Zn in the soil samples was 121 mg kg^{-1}, while range varied from 75 mg kg^{-1} to 210 mg kg^{-1} (Table 4.2.1). Kabata-Pendias (2011) reported the world mean for soil Zn concentration of 64 mg kg^{-1}, and for Cambisols 60 mg kg^{-1}. In this study, the mean Zn concentration did not differ in relation to soil type. The mean plant Zn concentration of 32 mg kg^{-1} (Table 4.2.1) in this study was in accordance to other reported research for grass and clover ranging from 25 to 47 mg kg^{-1} (Kabata-Pendias and Mukherjee, 2007). Zinc deficiency in plants is observed when the plant concentration is less than 10 to 20 mg kg^{-1}, while toxic effects are found when the concentration exceeds 300–400 mg kg^{-1} (Vitosh *et al*., 1994; Havlin, 2005). Therefore, the problem of Zn deficiency in plants, was not found. However, while referring to Fisher (2008) recommendations for Zn ruminant uptake ranging from 30 to 40 mg kg^{-1}, it could be concluded that forages from some pastures, could be insufficient to meet animal Zn requirements. The average Zn concentration was slightly higher in forages grown on Leptosols (Table 4.2.3).

The average concentrations of Se in the soil samples was 390 µg kg^{-1}, while range varied from 190 µg kg^{-1} to 640 µg kg^{-1} (Table 4.2.1). Soil Se concentrations did not vary in relation to soil type (Table 4.2.2). These results are close to reported mean values of Se in deficient soils in Serbia, ranging from 0,04 to 0,44 mg kg^{-1} (Maksimović and Djujić, 1998; Jović, 1996). The world mean soil Se concentration is 0,33 mg kg^{-1}, but the values range widely from 0,005 to 3,5 mg kg^{-1} (Kabata-Pendias and Mukherjee, 2007). Forage Se concentration above 100 µg kg^{-1} meets the requirement of most animals (Kabata-Pendias and Mukherjee, 2007; Fisher, 2008). In this study the average Se concentrations in forages was 23,8 µg kg^{-1}, ranging from 9,1 µg kg^{-1} to 72 µg kg^{-1} (Table 4.2.1), which is considerably lower than recommended values for animal diet. In addition, lower absorption of selenium in ruminants, related to reduction of selenite in the rumen, should be considered (NRC, 1996). Selenium is not essential trace elements for plants, thus no critical plant growth concentrations are stated.

The average concentrations of Mo in the soil was 420 µg kg^{-1}, ranging from 90 µg kg^{-1} to 1800 µg kg^{-1} (Table 4.2.1), which is close to the results published by Savić (1964) for Mo concentration in grassland soils in B&H. Results of this study were lower than the world mean Mo soil concentration of 1,1 mg kg^{-1} (range 0,9–1,8 mg kg^{-1}), for loamy Cambisols 2,8 mg kg^{-1} and sandy Podzols 1,3 mg kg^{-1} reported by Kabata-Pendias (2011). The average soil Mo concentrations was two times higher in Leptosols than Cambisols (Table 4.2.2), while the difference in average plant Mo concentration was smaller (Table 4.2.3). Molybdenum concertation of forages is of a special importance for animal nutrition. The average concentrations of Mo in the plant samples was 0,4 mg kg^{-1}, while range varied from 0,03 mg kg^{-1} to 3,1 mg kg^{-1} (Table 4.2.1). In the range from 0,1 to 0,5 mg kg^{-1} Mo in plants meet the requirements, while higher concentration are toxic for most species (Kabata-Pendias and Mukherjee, 2007). In this study, the mean Mo concertation in plant samples was 0,4 mg kg^{-1}, however, standard deviation and range indicate that many samples exceeded optimal concentrations and could be plant toxic. Animal requirements for molybdenum have not been established (NRC, 2001). However, Cu animal uptake can be excessive when dietary Mo and S are low (Underwood and Suttle, 1999). “Dietary molybdenum concentrations >10 mg kg^{-1} present a major obstacle to absorption of copper” (NRC, 2001).

The average concentrations of Mn in the soil samples was 1,6 g kg^{-1}, while range varied from 0,6 g kg^{-1} to 3,2 g kg^{-1} (Table 4.2.1). The world mean soil Mn concentrations range widely, from 10 to 9 000 mg kg^{-1} (Kabata-Pendias and Mukherjee, 2007), while for Cambisols was reported as 0,5 g kg^{-1} (Kabata-Pendias, 2011). Average Soil Mn concentration was only slightly higher in Leptosols than in Cambisols (Table 4.2.2), while there were no differences in the average plant Mn concentration between two soil types (Table 4.2.3). The average concentrations of Mn in the plant samples was 159 mg kg^{-1}, ranging from 40 mg kg^{-1} to 510 mg kg^{-1} (Table 4.2.1), which meets animal requirements in all growth and development stages (Fisher, 2008; Suttle, 2010; Kabata-Pendias and Mukherjee, 2007). Kabata-Pendias and Mukherjee (2007) reported mean range for Mn concentration in grasses 71–127 mg kg^{-1}, and clovers 25–89 mg kg^{-1} from different countries.

The average concentrations of Cd in the soil samples was 690 µg kg^{-1}, while range varied from 230 µg kg^{-1} to 2100 µg kg^{-1} (Table 4.2.1). Average soil Cd concentrations did not vary in relation to soil type (Table 4.2.2). According to Kabata-Pendias and Mukherjee (2007), the world mean soil Cd concentration is estimated at 0,5 mg kg^{-1}, ranging between 0,06 and 1,1 mg kg^{-1}, while the mean Cd concertation in Cambisols is 0,45 mg kg^{-1}. The concentrations of Cd in the plant samples was 0,21 mg kg^{-1}, while range varied from 0,03 mg kg^{-1} to 0,84 mg kg^{-1} (Table 4.2.1). Average plant Cd concentrations did not vary in relation to soil type (Table 4.2.3). These results are in accordance with the Cd range for grasses from 50–320 µg kg^{-1} and clovers from 20 to 350 µg kg^{-1} reported by Kabata-Pendias and Pendias (2001). Forages and crops grown on normal soils usually contain <1 mg Cd kg^{-1} DM (Underwood and Suttle, 1999). Plants have no metabolic requirement for Cd, therefore, availability can cause toxicities in plants and animals. The main concern related to Cd intake is transfer into the food chain. The major risk of toxicity occurring in grazing livestock is via the ingestion of soils enriched with cadmium from inorganic (superphosphate) or organic (sewage sludge) fertilizers (Suttle, 2010). However, this study was performed on natural pastures where no fertilizers were used and therefore Cd toxicity should not be a problem.

Areas of Fe deficiency in soils are common, however, in most cases this deficiency is related to a low concentration of soluble Fe species and not the total amount of Fe in the soil. The average total concentrations of Fe in the soil samples was 39,8 g kg^{-1}, while range varied from 26 g kg^{-1} to 54 g kg^{-1} (Table 4.2.1). The estimated range of the Fe concentration in the world soils is between 1 and 100 g kg^{-1} (Kabata-Pendias and Mukherjee, 2007). The average concentrations of Fe in the plant samples was 200 mg kg^{-1}, while range varied from 0,05 mg kg^{-1} to 2100 mg kg^{-1} (Table 4.2.1). According to Fisher (2008) recommendation for ruminant dietary intake of Fe ranges between 35–40 mg kg^{-1}, which indicates that the studied area has elevated values in regards to ruminant diet. However, there is a high tolerance towards dietary iron in all animal species (Suttle, 2010) so toxicity should not be a problem.

The average concentrations of Cu in the soil samples was 34.3 mg kg^{-1}, while range varied from 14 mg kg^{-1} to 71 mg kg^{-1} (Table 4.2.1). Average soil Cu concentrations was higher in Leptosols (Table 4.2.2), while there were no differences in the average Cu plant concentrations between two soil type (Table 4.2.3). The world mean total Cu concertation in different soil types are reported to range between 20 and 30 mg kg^{-1} according to Alloway (1995), or between 8 in acid sandy soils, and 80 mg kg^{-1}, in heavy loamy soils (Kabata-Pendias and Pendias, 2001). According to Kabata-Pendias (2010) normal concentration of Cu in plant leaves ranges from 0,5 to 30 mg kg^{-1}. In current study, the average concentrations of Cu in the plant samples was 6,6 mg kg^{-1}, while range varied from mg 4,5 mg kg^{-1} to 12 mg kg^{-1} (Table 4.2.1). Results agree with those published by Muratović (1997) for Cu concentration in forage plants ranging from 8,9 to 11,3 mg kg^{-1} in Kupres area, B&H. Although results from this study were in the range for meeting calves and lambs dietary Cu requirements, large percentage of analyzed samples provides deficient to marginal supply to growing bullocks and cows of 15 mg Cu kg^{-1}, according to Fisher (2008). The deficiency of Cu in livestock occur mainly in grazing conditions (Khan *et al*., 2011). Kassaye *et al*., (2012) found that fodder species *Cynodon aethiopicus*, *Acacia tortilis* and *Opuntia ficus-indicus* had low Cu levels in regards to animal requirements. Considering that the animals grazing on these pastures are mostly dependent on forages for meeting dietary requirements, Cu deficiency may present a problem.

The average concentrations of Co in the soil samples was 22,6 mg kg^{-1}, while range varied from 9,9 mg kg^{-1} to 34 mg kg^{-1} (Table 4.2.1). The world mean soil Co concentration is between 4,5 and 12 mg kg^{-1}, being the highest in heavy loamy soils, and the lowest for light sandy and organic soils (Kabata-Pendias and Mukherjee, 2007). Soil Co concentrations was slightly higher in Leptosols (Table 4.2.2), while there were no differences in the average plant Co concentration between two soil types (Table 4.2.3). Pasture soils worldwide are known to have too low a Co content for sheep or cattle. Grasses grown on soils with the Co content less than 5 mg kg^{-1} may be Co deficient for the normal growth of animals (Kabata-Pendias, 2007). The mean concentrations of Co in the plant samples was 0,15 mg kg^{-1} (Table 4.2.1), which meets ruminant dietary requirements (Fisher, 2008). However, standard deviation and wide range indicate that many plant samples were low in Co concentration and this could be a problem in some areas. Kabata-Pendias (2011) found that clovers from different countries meet critical values for ruminant diet, around 0,08-0,1 mg kg^{-1} of Co. Cobalt is not essential trace elements for plants, thus no critical plant growth concentrations are stated.

The average total concentrations of B in the soil samples was 36 mg kg^{-1}, while range varied from 11 mg kg^{-1} to 57 mg kg^{-1} (Table 4.2.1). Slightly higher B concentration was found in Leptosols than in Cambisols (Table 4.2.2). The estimated world B concentration in arable soils very in broad range from 10 to 100 mg kg^{-1}, while for Cambisols mean concentration is 40 mg kg^{-1} (Kabata-Pendias and Mukherjee, 2007). The average concentrations of B in the plant samples was 14,3 mg kg^{-1}, while range varied from 4,2 mg kg^{-1} to 30 mg kg^{-1} (Table 4.2.3), which is not enough to meet ruminant dietary requirements of 35 – 40 mg kg^{-1} (Fisher, 2008; Suttle, 2010). Average plant B concentration was higher in Leptosols (Table 4.2.3).

5.2 Relationship between minerals and trace elements in grasses and the soil chemical properties, elevation and plant type

5.2.1 Important minerals

The total Na concentration alone was a weak predictor of plant Na concentration and gave low R^2 values of the regression equation (Figure 4.3.2). In this study, two best predicting variables for plant Na concentration was the soil Mg concentration having highly significant negative relationship, and soil Ca concentration having very significant positive relationship (Table 4.3.1). Pessarakli (1999) discussed that in saline soil conditions may cause an increase in membrane permeability, which may result in excessive uptake of Na at the cost of K and Ca. Others found that K fertilization dramatically reduces forage Na content (Underwood, 1981), which was not confirmed with this study. In addition, sand content and soil Fe concentration had significantly positive relationship with plant Na concentration (Table 4.3.1). Sandy soils have low CEC (Havlin, 2005) which can increase availability of Na cations in the soil solution. However, Acosta *et al*., (2011) found that Na in soil did not show any trend of accumulation in any specific size fraction.

The total K concentration alone was a weak predictor of plant K concentration and gave low R^2 values of the regression equation (Figure 4.3.14). However, when accounted other factors, regression equation improved and showed that best predicting variables, in fact, were soil K, Co, and Ca concentration having statistically significant positive relationship (Table 4.3.1). Other authors also found strong relationship between soil and plant K concentration (Gagnon *et al*., 2005; Suttle, 2010). Havlin *et al.,* (2005) indicated that loams and silt loams can have limited K availability comparing to coarse textured soils, whereas in this study soil texture did not show significant influence. The contents of K in soil did not show any trend of accumulation in any specific size fractions (Acosta *et al*., 2011). Others have suggested Ca, Mg and K have to be balanced in the soil not to cause plant deficiency (Havlin, 2005; Vitosh *et al.,* 1994; Suttle, 2010). In calcareous soils, the proportion of Ca to other exchangeable cations may lead to K deficiency in plants (Marschner, 1995). Optimal levels of calcium improve uptake of potassium (Mengel and Kirkby, 2001).

Total Ca soil concentration was a weak predictor of the plant Ca concentration (Figure 4.3.5). The regression model accounting all the investigated factors showed that best predicting variables were soil organic C and Mg concentration, both being in significant negative relationship (Table 4.3.1). Antagonistic relationship between Ca and Mg plant uptake were reported (Vitosh *et al.*, 1994). Gagnon *et al*., (2002) found the forage K/(Ca+Mg) ratio significantly correlated with soil K and Mg concentration, while plant Mg and Ca concentrations showed low correlations and inconsistent trends with soil K, Mg, and Ca contents. Falkengren-Grerup *et al.,* (1995) found that there are highly significant negative correlation between soil Ca concertation and the growth of plants typical for acidic soils. In the study of Juknevičius and Sabienė (2007) legumes accumulated over twice as much Ca comparing to grasses, however, this was not found in current study. The regression equation did not find any relationship between soil texture and plant Ca concentration (Table 4.3.1). This is in accordance with Acosta *et al.,* (2011) that found no distinct trends of Ca accumulation in specific soil fractions.

Total soil Mg concentration alone was a weak predictor of the plant Mn concentration and gave low R^2 values of the regression equation (Figure 4.3.3). When considered all the investigated factors the best predicting variable was soil Mo concentration having very significant positive relationship (Table 4.3.1). Soil Fe concentration had also significant positive relationship, while soil Cu concentration had significant negative relationship to plant Mg concentration (Table 4.3.1). in contrast, Fageria *et al.,* (2010) reported the opposite, that uptake of Mg decreased in nutrient solution with the increasing Fe concentration. Kabata-Pendias (2011) also reported antagonistic relationship between Mg and Fe. None of the other tested parameters seem to explain the soil characteristics being important for plant Mg concentration in this region. Other authors discussed the difficulty to relate soil properties to occurrence of grass tetany, metabolic disease in ruminant livestock caused by Mg deficiency (Robinson *et al*., 1989). A mixed sward of *Lolium perenne* and *Trifolium repens* had consistently higher concentrations of calcium, magnesium and potassium than a pure sward of *L. perenne* (Underwood and Suttle). The legumes accumulated more Mg compared to the grasses (Juknevičius and Sabienė, 2007), however this study did not found significant difference between Mg concentrations in forage samples with different dominating species.

The total P concentration in the soil was a weak predictor of plant P concentration and gave low R^2 values for the regression equation (Figure 4.3.4). When regression equation accounted other factors, R^2 values improved significantly and it showed that the best predicting variable was, in fact, soil P concentration (Table 4.3.1). Organic C had significant negative relationship with plant P concentration in this study, while other authors suggest the opposite relationship due to P forming plant available organophosphate complexes, replacement of P anions on adsorption sites, mineralization processes, and organic compound immobilization of Fe/Al oxides (Havlin, 2005).

5.2.2 Trace elements

The total Zn concentration in soil was not strong predictor of plant Zn concentration and alone gave low R^2 values of the regression equation (Figure 4.3.10). This indicates that many other factors affected plant Zn absorption. Regression equation that included other investigated factors showed very strong negative relationship between soil organic C content and plant Zn concertation, being the strongest predictor variable in the regression model (Table 4.3.1). Havlin *et al.,* (2005) found that soil Zn forms stable complexes with soluble and insoluble high molecular weight organic compounds that can contribute or limit Zn plant availability. This regression equation showed that sand content had positive relationship with plant Zn concertation (Table 4.3.1). Acosta *et al.,* (2011) investigated the concentration of Zn in various particle size classes, showing the highest Zn concentration in clay fraction. Kabata-Pendias and Mukherjee (2007) discussed two mechanisms of Zn sorption, one in acid soil reaction related to cation exchange sites, and other in alkaline soil reaction mostly connected to organic ligands. According to the regression equation, a negative relationship was found with the soil pH and both soil Co and Cd content. Zinc and cadmium antagonistic relationship is reported in other research as well (Kabata-Pendias, 2001; Chaney and Hornick, 1977; Underwood and Suttle, 1999; Cherif *et al.,* 2011). However, Kabata-Pendias (2011) discussed Zn–Cd interactions being both antagonistic and synergistic in the uptake–transport processes. Nan *et al.* (2002) concluded that Cd–Zn interaction is synergistic under field condition. In contrast to this study, Davies (1992) found that raising soil pH increases accumulation of Zn in radish (*Raphanus sativus* L.). Others disused soil conditions connected to Zn deficiencies being acid, sandy soils, poor in Zn, neutral, basic or calcareous soils, fine textured, high available P, high organic matter content, and eroded soils (Havlin *et al.,* 2005; Vitosh *et al.,* 1994). It has been suggested that legumes, especially red clover, are higher in Zn concentration, (Sherrell and Smith, 2012), however, results from this study did not indicate that dominant plant species had significant relationship to sample Zn concentration (Table 4.3.1). Kabata-Pendias and Krakowiak (1998) reported that Zn transfer factor (TF) varied significantly between different species of pasture plants grown in same environmental conditions ranging from 0,09 in bromgrass (*Bromus unioloides*) to 6,8 in dandelion (*Taraxacum officinale*).

Selenium plant uptake depend on climate, soil parameters, and plant capacity to accumulate. The total Se concentration in the soil was not strong predictor of plant Se uptake and alone gave low R^2 values of the regression equation (Figure 4.3.11). When other factors were introduced into the regression equation, R^2 values improved significantly, however, still leaving a lot of plant Se variation unexplained. The regression model showed that the best predicting variables were soil sand content and Cu concentration having significant positive relationship (Table 4.3.1). Kabata-Pendias (2011) suggested antagonistic reaction between Cu and Se. Positive relationship of sand content and plant Se concentration could be explained by increased rainfall during prolonged period prior to the sampling dates (Figure 3.2.2), which increased Se availability in soil solution. However, sandy soils often have lower total Se concentration but higher availability, comparing to clay soils (Whitehead, 2000; Carlson *et al.* 1991). Selenium addition to soil can stimulate the accumulation Cu in wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) (Landberg and Greger, 1994). A close relationship has been observed between Se plant concentration and organic C content (Kabata-Pendias and Mukherjee, 2007), plant species, and total soil selenium content (NRC, 1996), however, this was not found in current study.

Some authors reported a linear relationship between Mo content of herbage and total concentration in soil (Kabata-Pendias, 2011). However, the total Mo concentration in soil was a weak predictor of the plant Mo concentration and alone gave low R^2 values for the regression equation in current study (Figure 4.3.12). After introducing other investigated factors R^2 value increased significantly, and the regression equation explained Mo plant variation more than for all the other investigated elements. The best predicting variable was Mo soil concentration (Table 4.3.1), which is in agreement with other research (Goldberg and Forster, 1998; Sherrell and Smith, 2012). According to the regression model soil Cu and Se concentration had significant antagonistic effect on plant Mo uptake, while soil Fe, Ca, Cd and Co concentration showed positive relationship (Table 4.3.1). Similarly Kabata-Pendias (2011) found the Mo–Cu antagonism in plants is strongly related to N metabolism. However same author discussed Mo-Fe relationship to be antagonistic due to low Mo availability in Fe rich soil, while the opposite was found in current study. Copper and molybdenum are known antagonists, and soils high in Fe oxides absorb Mo strongly (Havlin, 2005; Kabata-Pendias and Mukherjee, 2007, FAO 1982), which might explain the relationships shown in the regression table. In addition, Zakikhani *et al.*, (2014) found that Mo soil application significantly negatively affected shoot Fe concentration, while in this study soil Fe concentration negatively influenced plant Mo concentration. Underwood and Suttle (1999) reported statistically significant higher Mo concentration in clover tops from calcareous sand (10,1 mg kg^{-1}) than from deep sands (1,1 mg kg^{-1}). Although Mo plant uptake usually increases as soil pH rises (Suttle 2010; Mengel and Kirkby, 2001), in this study regression model did not show significant relationship between these two variables (Table 4.3.1). Kassaye *et al*., (2012) found that leguminous plants such as *A. tortilis*, *Senna didymobotrya* and *Sesbania sesban* accumulate Mo because of its importance in nitrogen fixation, however, dominant plant species seemed not to have significant relationship with the plant Mo concentration. Among grass and legume species differences in Mo concentration have been observed (Whitehead, 2000), which may explains why the regression equation did not indicate significant relationship between mixed plant samples composed of several plant species, with one being dominant.

Total Mn concentration in soil was a weak predictor of the plant Mn concentration and gave low R^2 values of the regression equation (Figure 4.3.6). The regression equation accounting other factors explained more of the Mn plant variation, with best predicting variable being soil pH with highly significantly negative relationship (Table 4.3.1). This was in accordance with other authors (Kabata-Pendias and Mukherjee, 2007; FAO, 1982, Singh and Mishra, 1987). Soil Cd concentration showed significant positive relationship with plant Mn concentration. In current study, Cd might block Zn which is known antagonist of Mn (Kabata-Pendias, 2010), indirectly influencing positively the plant Mn concentration. Opposite relationship found Peng *et al.* (2008), that Mn reduces the Cd concentrations in all organs of a plant (*Phytoacca americana* L.). Similarly stated Kabata-Pendias (2011) reporting of either antagonistic or synergistic effects of Mn on the uptake of Cd. Particle size distribution did not show relationship with plant Mn concentration, which is in accordance with Acosta *et al*., (2011) that found Mn did not show any trend of accumulation in any specific soil size fraction. According to other authors (Kabata-Pendias and Mukherjee, 2007), organic matter and clay content has positive association to Mn uptake, however, it was not confirmed in current study. Plant samples with predominantly herbaceous plants had significantly higher Mn concertation comparing to samples with predominantly legume plants (Table 4.3.1). Kabata-Pendias and Krakowiak (1998) reported that Mn transfer factor varied significantly between different species of pasture plants grown in same environmental conditions, ranging from 0,06 in bromgrass (*Bromus unioloides*) to 8,43 in dandelion (*Taraxacum officinale*). Juknevičius and Sabienė (2007) reported Mn accumulation differences in different species, where legumes absorbed sufficient levels, while grasses were deficient, whit the exception of *Dactylis glomerata*.

The total Cd soil concentration was a weak predictor of the plant Cd concentration (Figure 4.3.13). In regression model, accounting all investigated factors showed the best predicting variables were soil pH with very significant negative relationship, and soil Mo concentration, with very significant positive relationship (Table 4.3.1). The relationship between soil pH and uptake of Cd by plant is in accordance with other research (FAO, 1982, Singh and Mishra, 1987; Underwood and Suttle, 1999). Basta *et al*., (1993) demonstrated the correlation between organic matter and the adsorption of Cd, directly connecting with pH as a controlling variable of metal complexation by organic matter. Highly significant positive influence of Mo to plant Cd concentration is most likely due to different synergistic and antagonistic relationships in soil solution. Acosta *et al.,* (2011) investigated the concentration of Cd in various particle size classes, showing the highest Cd concentration in clay fraction, however this research did not find relationship between particle size distribution and plant Cd concentration. In addition, regression model indicated that samples with predominantly legume plants had significantly lower Cd concertation compared to samples with predominantly herbaceous plants (Table 4.3.1). This is in accordance with other research that confirmed Cd concentration in plants grown on contaminated soil will vary in different plant species (Underwood and Suttle, 1999). Kabata-Pendias and Krakowiak (1998) reported that Cd transfer factor varied significantly between different species of pasture plants grown in same environmental conditions ranging from 0,11 in bromgrass (*Bromus unioloides*) to 3,42 in dandelion (*Taraxacum officinale*).

As for previous trace elements, total Fe soil concentration was a weak predictor of the plant Fe concentration (Figure 4.3.7). The regression model that accounted all the investigated factors showed the best predicting variable was soil Cd concentration having very significant negative relationship (Table 4.3.1). It has been reported that Cd uptake can decrease the uptake of other metals (Adhikari *et al.,* 2006), and that plants accumulate Cd in Fe deficient soils (Wuana and Okieimen, 2011). However, Kabata-Pendias (2011) reported synergistic relationship between Fe and Cd resulting from the destruction of physiological barriers in under the stress conditions. While Meda *et al.,* (2007) found the Fe-Cd interaction observed in phytosiderophores is complex and contributes to the Cd tolerance of a plant. Antagonistic relationship has been reported between Fe and Co, Mn, Mo, and Zn (Kabata-Pendias and Pendias, 2001), however, results in this study indicate different interactions. Significant positive relationship was found between plant Fe concentration and soil pH, Ca, and Zn concentration, while significant negative relationship with soil Mg concentration (Table 4.3.1). Strong positive correlations between the plant contents of Fe and Zn has been reported in previous studies (FAO, 1982). The concentration of Fe in tobacco plants was influenced positively by Ca treatment (Lopez-Lefebre *et al*., 2001). However, Kabata-Pendias (2011) discussed complex Fe–Ca interactions, in both plant and soil, stating that Ca may suppress Fe availability, and thus may lead to Fe chlorosis in plants on calcareous soils. In this study, increase in soil pH and Ca concentration positively affected Fe plant uptake, however Havlin *et al*., (2005) discussed possibilities of soil $CaCO_3$ interacting with other soil conditions that lead to Fe deficiency indirectly. Excessive amounts of calcium reduces uptake of iron (Mengel and Kirkby, 2001). Kabata-Pendias (2011) found that the concertation of available Fe was high for loamy and alkaline soils. The regression equation did not find any relationship between sand and clay soil content with plant Fe concentration (Table 4.3.1). This is in accordance with Acosta *et al.,* (2011) that found no distinct trends of Fe accumulation in specific soil fractions. Kabata-Pendias and Krakowiak (1998) reported that Fe transfer factor varied significantly between different species of pasture plants grown in same environmental conditions ranging from 0,49 in bromgrass (*Bromus unioloides*) to 2,72 in dandelion (*Taraxacum officinale*). However, the regression equation did not find relationship between the dominant plant species and the concentration of Fe in plant samples.

The total Cu soil concentration was a weak predictor of the plant Cu concentration (Figure 4.3.9). This is in accordance with previous studies where no correlation was found between copper concentration in plant foliage and soil (Burton *et al*. 1984, Davies 1992). The regression model accounting all the investigated factors showed the best predicting variable were soil Cu concentration, P concentration, and sand content having significant positive relationship (Table 4.3.1). Opposite relationship was found for organic C content and plant Cu concentration. Copper forms complexes with the soil organic matter (Lato *et al.,* 2012; Havlin *et al.,* 2005), which explains negative relationship found in this study. High soils P levels reduce mycorrhizal absorption of Cu, while excess of Cu reduces availability of P (Kabata-Pendias, 2011). Others also found that high P concentration in soil solution can depress Cu absorption via root (Havlin *et al.,* 2005), however the opposite relationship was found in this study. It has been suggested that Cu and Fe plant uptake have similar pathways which can lead to ion antagonism (Schulze *et al.,* 2005). However, in this study regression equation did not suggest this kind of antagonistic relationship. Similar relationship was described for Cu and Mo both for plant and animals uptake (Hooda, 2010; Khan *et al.,* 2011), however, the ratio of Cu:Mo is important in that regard (Underwood and Suttle, 1999) and interrelation only exists in presence of sulphate. Acosta *et al.,* (2011) investigated the concentration of Cu in various particle size classes, showing the highest Cu concentration in clay fraction. This study showed that sand content had positive relationship with plant Cu concertation, which indicates that Cu is more plant available in sandy soils. Kabata-Pendias and Krakowiak (1998) reported that Cu transfer factor varied significantly between different species of pasture plants grown in same environmental conditions ranging from 0,52 in bromgrass (*Bromus unioloides*) to 2,6 in dandelion (*Taraxacum officinale*). In addition, Khan *et al*., (2011) found that the soil–plant Cu TF was higher in legumes compared to other forage species, however the dominant plant type did not seem to have significant relationship to Cu plant concentration in this study (Table 4.3.1).

The Co uptake by plants is positively correlated to Co mobile fractions in soil (Kabata-Pendias, 2010). However, the total Co soil concentration was very weak predictor of the plant Co concentration in this study (Figure 4.3.8). The regression equation accounting all the investigated factors showed the best predicting variable was soil Ca concentration with very significant positive relationship (Table 4.3.1), which is not in accordance to antagonistic interaction between Ca and Co reported by Kabata-Pendias (2001). Very significant antagonistic relationship was found between soil Mg and plant Co concentration (Table 4.3.1), which was confirmed by Kabata-Pendias (2001). Soil Cu concentration had significant positive relationship with plant Co concertation, while soil Cd concentration had opposite relationship (Table 4.3.1). In contrast, antagonistic interactions between Co and Cu was observed in sludge (Begona Osuna *et al.,* 2004). Although other research indicates that soil texture and SOM are two of the most important factors affecting Co plant uptake (Li *et al*., 2004; Kabata-Pendias and Mukherjee, 2007) this connection was not found in the current study. Concentration of Co in *Lolium* was related to soil pH in the study of Ervio and Sippola (1993), while Kabata-Pendias stated that alkaline or calcareous soils contribute to Co deficiency, however, this was not shown in the current study. Acosta *et al.,* (2011) investigated the concentration of Co in various particle size classes, showing the highest Co concentration in clay fraction, while the regression equation in the current study did not find relationship between particle size distribution and plant Co concentration (Table 4.3.1).

The regression model indicated that only soil B concertation had significant effect on plant B concertation and that samples with predominantly legumes had significantly lower B concertation in DM compared to samples with predominantly herbaceous plants (Table 4.3.1). In this study pH did not show significant relationship with plant B concertation while many authors stated that B availability decreases at higher pH (Kabata-Pendias and Mukherjee, 2007, Vitosh *et al*., 1994), especially between 6,3 to 6,5 (Havlin *et al*., 2005). Clay retains more B than sand does, while plant uptake is higher in sandy soils (Havlin *et al*., 2005; Fleming, 1980; Vitosh *et al*., 1994). However, soil texture did not show significant effect on the plant B concertation in this study.

5.2.3 Transfer factor

Soil to plant transfer is one of the most important components of human exposure to metals through food chain. Elements that showed variation of TF in relation to sampling altitude were Na, P, Mo, Mn, Ca, Cu, and B; while remaining elements did not show differences in TF depending on altitude (Table 4.2.4).

Elements with high TF values (1–2) were P, K, Ca, and Mo. Elements with medium TF values (0,1–0,9) were Zn, Mn, Mg, Cd, Cu, and B. The remaining elements had TF values lower than 0,09. According to Kassaye *et al*., (2012), the transfer factor >1 indicates enrichment of this element in plants. The difference in TF values between altitudes may be related to soil properties and differences in climatic conditions. Kabata-Pendias and Krakowiak (1998) reported that Zn, Mn, Cd, Fe, and Cu transfer factor varied significantly between different species of pasture plants grown in same environmental conditions. Jolly *et al.*, (2013) found the TF values for K, Ca, Mn, Fe, Co, Cu, Zn, Se, and Cd for various vegetables varied greatly between plant species and locations.

In addition to other factors in this study, variations of TF at different altitudes can be due to differences in plant botanical composition, since certain changes were observed (Figure 4.4.1), or due to differences in weather conditions. King *et al.*, (2014) found evidence for significant loss of Mo in the early stages of weathering on drier soils, but that losses are minimal and even gains are apparent in wetter soils, probably due to OM retention.

Considering that the Na TF is lower at the highest sampling altitudes the deficiency could be a problem. Manganese TF was very low at the lowest sampling altitudes, which might not be a problem considering that the average Mn plant concentration in the study was enough to meet animal requirements. Potassium transfer factor was in the slight decline with rising altitude which might be due to harsher weather conditions and runoff.

The average Co and Cu plant concentration was optimal considering animal diet, however, both high standard deviation and extremely low TF at the highest sampling altitudes may indicate that those plant samples were too low to meet animal requirements in both minerals.

6. Conclusion

In conclusion, the results of this study indicate that the average concentrations of sodium, phosphorus, zinc, selenium, copper, cobalt, and boron were low in both soil and plants.

Plant potassium, calcium, magnesium, molybdenum, manganese, and iron concentrations were sufficiently high to meet the requirements of animals, while iron concentrations were even elevated in same sampled areas. Molybdenum soil and plant concentration was higher in some sampling areas, which may create imbalance in Cu nutrition in animal diet.

Plant concentration of zinc, manganese, cadmium, and iron were largely explained by soil pH. Soil organic carbon explained variations of zinc, phosphorous, calcium, and copper. Total concentration of element in soil explained only potassium, phosphorous, molybdenum, boron, while other investigated trace elements could be more explained by the interactions with other elements in soil and plants. Soil texture explained variation in plant zinc, sodium, selenium, and copper, while changes in dominant plant type explained only variations in manganese, cadmium and boron concentrations.

Differences in the transfer factor at different altitudes may be due to observed shifts in botanical composition of grasslands plant species in addition to other factors.

Some trace elements essential for animal nutrition were in the marginal quantities, and therefore small changes in concentration due to seasonal or other soil and plant factors may create deficiency problems in grazing animals in this area.

7. References

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Annex 1

Table 1: Correlation matrix of all the tested variables

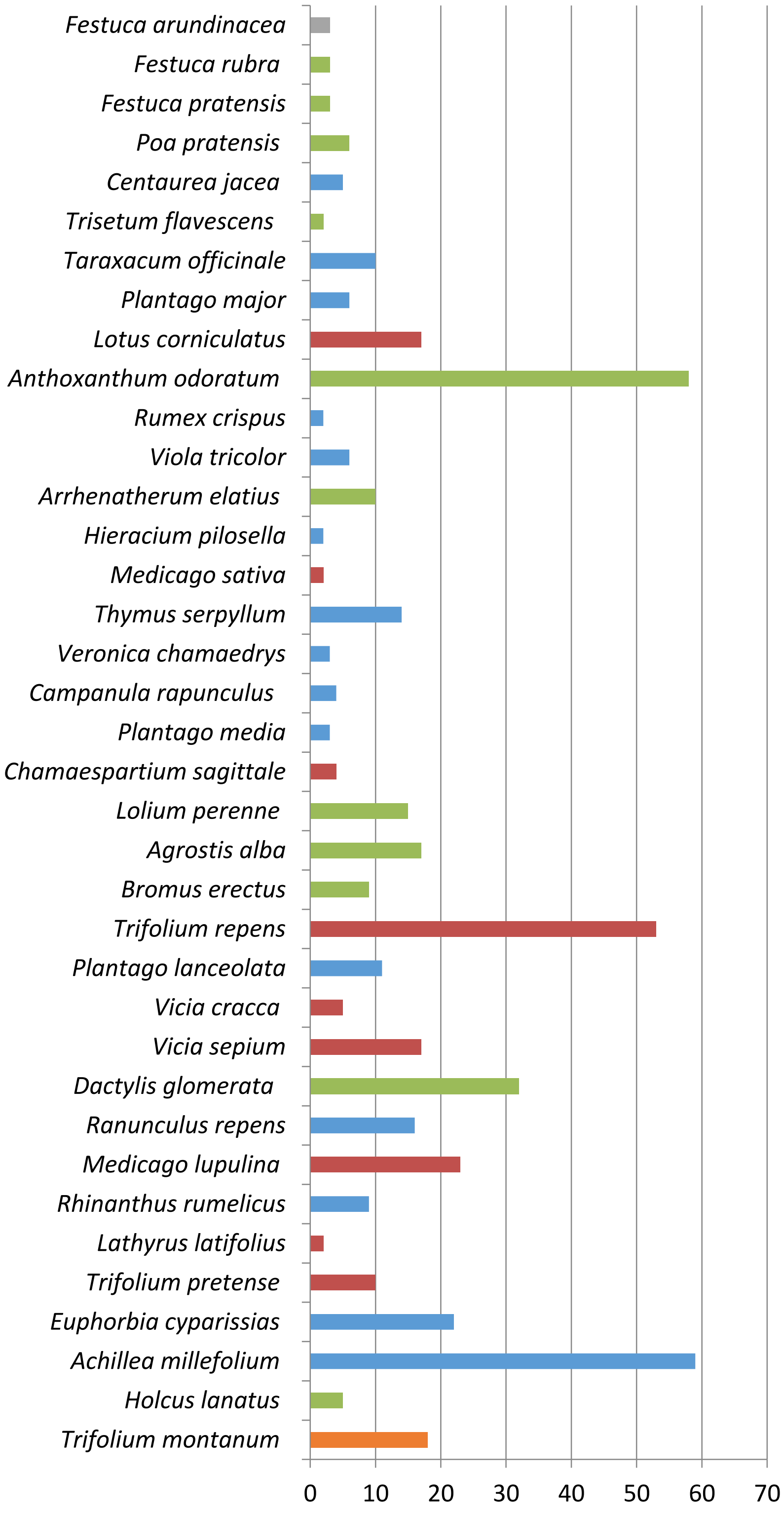
	B_{plant}	Na_{plant}	Mg_{plant}	P_{plant}	Ca_{plant}	Mn_{plant}	Fe_{plant}	Co_{plant}	Cu_{plant}	Zn_{plant}	Se_{plant}	Mo_{plant}	Cd_{plant}	K_{plant}	B_{soil}
Na_{plant}	0.1^{ns}														
Mg_{plant}	0.56^{***}	0.12^{ns}													
P_{plant}	0.34^{**}	0.36^{**}	0.21^{ns}												
Ca_{plant}	0.62^{***}	0.38^{***}	0.28^{*}	0.52^{***}											
Mn_{plant}	-0.03^{ns}	0.05^{ns}	0.00^{ns}	0.26^{*}	-0.04^{ns}										
Fe_{plant}	0.19^{ns}	0.53^{***}	0.19^{ns}	0.19^{ns}	0.49^{***}	0.00^{ns}									
Co_{plant}	0.21^{ns}	0.59^{***}	0.16^{ns}	0.26^{*}	0.53^{***}	0.13^{ns}	0.93^{***}								
Cu_{plant}	0.43^{***}	0.54^{***}	0.29^{**}	0.59^{***}	0.62^{***}	0.19^{ns}	0.46^{***}	0.53^{***}							
Zn_{plant}	0.39^{***}	0.30^{**}	0.38^{**}	0.33^{**}	0.29^{**}	0.46^{***}	0.21^{ns}	0.28^{**}	0.59^{***}						
Se_{plant}	-0.13^{ns}	0.16^{ns}	-0.06^{ns}	-0.33^{**}	0.11^{ns}	-0.02^{ns}	0.29^{**}	0.25^{*}	-0.05^{ns}	0.14^{ns}					
Mo_{plant}	-0.04^{ns}	0.41^{***}	0.45^{***}	0.07^{ns}	0.04^{ns}	-0.27^{*}	0.11^{ns}	0.10^{ns}	0.14^{ns}	-0.02^{ns}	0.00^{ns}				
Cd_{plant}	0.33^{**}	0.18^{ns}	0.22^{ns}	0.35^{**}	0.13^{ns}	0.49^{***}	0.01^{ns}	0.03^{ns}	0.37^{***}	0.58^{***}	-0.08^{ns}	-0.16^{ns}			
K_{plant}	0.21^{ns}	0.13^{ns}	0.08^{ns}	0.74^{***}	0.43^{***}	0.20^{ns}	0.15^{ns}	0.14^{ns}	0.46^{***}	0.07^{ns}	-0.41^{***}	-0.07^{ns}	0.15^{ns}		
B_{soil}	0.45^{***}	-0.08^{ns}	0.03^{ns}	0.09^{ns}	0.29^{**}	-0.12^{ns}	-0.05^{ns}	-0.04^{ns}	0.13^{ns}	0.13^{ns}	-0.19^{ns}	-0.22^{ns}	0.06^{ns}	0.15^{ns}	
Na_{soil}	-0.08^{ns}	-0.09^{ns}	0.08^{ns}	0.02^{ns}	-0.16^{ns}	0.25^{*}	-0.12^{ns}	-0.05^{ns}	-0.11^{ns}	0.01^{ns}	-0.09^{ns}	-0.17^{ns}	0.08^{ns}	0.02^{ns}	-0.28^{*}
Mg_{soil}	0.01^{ns}	-0.12^{ns}	0.21^{ns}	-0.26^{*}	-0.06^{ns}	-0.38^{**}	-0.05^{ns}	-0.08^{ns}	-0.15^{ns}	-0.03^{ns}	0.02^{ns}	0.45^{***}	-0.33^{**}	-0.29^{**}	0.15^{ns}
P_{soil}	-0.02^{ns}	-0.07^{ns}	0.23^{*}	-0.12^{ns}	-0.12^{ns}	-0.26^{*}	-0.13^{ns}	-0.14^{ns}	-0.09^{ns}	-0.03^{ns}	0.06^{ns}	0.33^{*}	-0.11^{ns}	-0.23^{*}	0.07^{ns}
Ca_{soil}	-0.06^{ns}	0.18^{ns}	0.10^{ns}	-0.20^{ns}	0.06^{ns}	-0.36^{**}	0.15^{ns}	0.15^{ns}	-0.06^{ns}	-0.06^{ns}	0.12^{ns}	0.56^{***}	-0.37^{**}	-0.27^{*}	-0.02^{ns}
Mn_{soil}	0.15^{ns}	0.02^{ns}	-0.30^{**}	0.36^{**}	0.34^{**}	0.14^{ns}	0.00^{ns}	-0.02^{ns}	0.21^{ns}	-0.02^{ns}	-0.13^{ns}	-0.31^{**}	0.14^{ns}	0.40^{***}	0.39^{***}
Fe_{soil}	0.23^{*}	-0.13^{ns}	0.10^{ns}	-0.09^{ns}	0.18^{ns}	-0.28^{*}	0.02^{ns}	-0.04^{ns}	0.08^{ns}	-0.13^{ns}	-0.16^{ns}	-0.06^{ns}	-0.20^{ns}	0.07^{ns}	0.46^{***}
Co_{soil}	0.19^{ns}	0.10^{ns}	-0.29^{**}	0.41^{***}	0.42^{***}	0.20^{ns}	0.09^{ns}	0.08^{ns}	0.29^{*}	-0.02^{ns}	-0.17^{ns}	-0.30^{*}	0.14^{ns}	0.53^{***}	0.37^{**}
Cu_{soil}	0.20^{ns}	0.09^{ns}	-0.34^{**}	0.28^{*}	0.41^{***}	0.04^{ns}	0.18^{ns}	0.16^{ns}	0.34^{*}	-0.02^{ns}	-0.07^{ns}	-0.31^{**}	0.05^{ns}	0.37^{**}	0.49^{***}
Zn_{soil}	-0.07^{ns}	-0.15^{ns}	0.14^{ns}	-0.11^{ns}	-0.03^{ns}	-0.26^{*}	0.11^{ns}	-0.01^{ns}	0.04^{ns}	-0.13^{ns}	0.10^{ns}	0.24^{*}	-0.11^{ns}	-0.06^{ns}	-0.01^{ns}
Se_{soil}	-0.09^{ns}	-0.22^{ns}	0.23^{*}	-0.40^{***}	-0.22^{ns}	-0.24^{*}	-0.17^{ns}	-0.22^{*}	-0.24^{*}	-0.04^{ns}	0.11^{ns}	0.14^{ns}	-0.15^{ns}	-0.34^{**}	0.14^{ns}
Mo_{soil}	0.31^{ns}	-0.07^{ns}	0.32^{**}	0.09^{ns}	0.13^{ns}	0.04^{ns}	-0.02^{ns}	-0.03^{ns}	0.04^{ns}	0.12^{ns}	-0.09^{ns}	0.20^{ns}	0.32^{**}	0.07^{ns}	0.24^{*}
Cd_{soil}	-0.14^{ns}	-0.20^{ns}	0.20^{ns}	-0.35^{**}	-0.24^{*}	-0.47^{***}	-0.22^{ns}	-0.25^{*}	-0.30^{**}	-0.23^{*}	0.11^{ns}	0.40^{***}	-0.30^{**}	-0.41^{***}	-0.03^{ns}
K_{soil}	0.37^{**}	-0.06^{ns}	-0.15^{ns}	0.34^{**}	0.30^{**}	0.12^{ns}	0.03^{ns}	0.04^{ns}	0.22^{ns}	0.06^{ns}	-0.26^{*}	-0.39^{***}	0.16^{ns}	0.43^{***}	0.51^{***}
pH	-0.06^{ns}	0.19^{ns}	0.16^{ns}	-0.23^{*}	0.06^{ns}	-0.59^{***}	0.27^{*}	0.22^{ns}	-0.10^{ns}	-0.30^{**}	0.14^{ns}	0.58^{***}	-0.57^{***}	-0.29^{*}	-0.08^{ns}
OC	-0.13^{ns}	-0.19^{ns}	0.16^{ns}	-0.54^{***}	-0.39^{***}	-0.38^{***}	-0.26^{*}	-0.28^{*}	-0.36^{**}	-0.20^{ns}	0.15^{ns}	0.23^{*}	-0.30^{**}	-0.55^{***}	0.00^{ns}
Clay	0.05^{ns}	-0.11^{ns}	0.12^{ns}	-0.18^{ns}	-0.01^{ns}	-0.32^{**}	-0.01^{ns}	-0.10^{ns}	0.04^{ns}	-0.18^{ns}	-0.04^{ns}	0.13^{ns}	-0.25^{*}	0.04^{ns}	0.12^{ns}
Sand	-0.05^{ns}	0.30^{**}	0.04^{ns}	-0.26^{*}	-0.03^{ns}	-0.15^{ns}	0.13^{ns}	0.10^{ns}	-0.01^{ns}	0.10^{ns}	0.31^{**}	0.30^{**}	-0.09^{ns}	-0.37^{**}	-0.04^{ns}

Table 1: Correlation matrix of all the tested variables (continuation of the table)

	Na$_{soil}$	*Mg*$_{soil}$	*P*$_{soil}$	*Ca*$_{soil}$	*Mn*$_{soil}$	*Fe*$_{soil}$	*Co*$_{soil}$	*Cu*$_{soil}$	*Zn*$_{soil}$	*Se*$_{soil}$	*Mo*$_{soil}$	*Cd soil*	*K soil*	*pH*	*OC*	*Clay*
Na$_{plant}$																
Mg$_{plant}$																
P$_{plant}$																
Ca$_{plant}$																
Mn$_{plant}$																
Fe$_{plant}$																
Co$_{plant}$																
Cu$_{plant}$																
Zn$_{plant}$																
Se$_{plant}$																
Mo$_{plant}$																
Cd$_{plant}$																
K$_{plant}$																
B$_{soil}$																
Na$_{soil}$																
Mg$_{soil}$	-0.35^{**}															
P$_{soil}$	-0.14^{ns}	0.67^{***}														
Ca$_{soil}$	-0.41^{***}	0.90^{***}	0.60^{***}													
Mn$_{soil}$	-0.20^{ns}	-0.20^{ns}	-0.13^{ns}	-0.19^{ns}												
Fe$_{soil}$	0.00^{ns}	0.04^{ns}	-0.10^{ns}	-0.12^{ns}	0.22^{ns}											
Co$_{soil}$	-0.10^{ns}	-0.38^{**}	-0.42^{***}	-0.33^{**}	0.90^{***}	0.29^{**}										
Cu$_{soil}$	-0.34^{**}	-0.16^{ns}	-0.26^{*}	-0.14^{ns}	0.84^{***}	0.46^{***}	0.84^{***}									
Zn$_{soil}$	-0.20^{ns}	0.34^{**}	0.43^{***}	0.30^{**}	-0.05^{ns}	0.27^{*}	-0.21^{ns}	0.03^{ns}								
Se$_{soil}$	-0.17^{ns}	0.51^{***}	0.63^{***}	0.36^{**}	-0.28^{**}	0.17^{ns}	-0.49^{***}	-0.27^{*}	0.36^{**}							
Mo$_{soil}$	0.02^{ns}	0.02^{ns}	0.10^{ns}	-0.08^{ns}	0.06^{ns}	0.00^{ns}	0.01^{ns}	0.00^{ns}	-0.10^{ns}	0.15^{ns}						
Cd$_{soil}$	-0.29^{**}	0.63^{***}	0.63^{***}	0.55^{***}	-0.35^{**}	0.10^{ns}	-0.59^{***}	-0.31^{**}	0.53^{***}	0.69^{***}	0.07^{ns}					
K$_{soil}$	0.20^{ns}	-0.34^{**}	-0.34^{**}	-0.46^{***}	0.62^{***}	0.47^{***}	0.71^{***}	0.65^{***}	-0.19^{ns}	-0.35^{**}	0.26^{*}	-0.47^{***}				
pH	-0.29^{**}	0.63^{***}	0.37^{**}	0.74^{***}	-0.24^{**}	0.04^{ns}	-0.32^{**}	-0.14^{ns}	0.29^{**}	0.20^{ns}	-0.06^{ns}	0.49^{***}	-0.33^{**}			
OC	-0.14^{ns}	0.58^{***}	0.64^{***}	0.45^{***}	-0.45^{***}	0.06^{ns}	-0.67^{***}	-0.41^{***}	0.27^{*}	0.70^{***}	0.02^{ns}	0.74^{***}	-0.42^{***}	0.40^{***}		
Clay	-0.11^{ns}	-0.02^{ns}	-0.18^{ns}	-0.08^{ns}	0.07^{ns}	0.41^{***}	0.19^{ns}	0.19^{ns}	0.10^{ns}	-0.07^{ns}	-0.08^{ns}	-0.01^{ns}	0.11^{ns}	0.21^{ns}	-0.02^{ns}	
Sand	-0.26^{*}	0.44^{***}	0.39^{***}	0.52^{***}	-0.22^{ns}	-0.17^{ns}	-0.37^{**}	-0.22^{ns}	0.16^{ns}	0.35^{**}	-0.03^{ns}	0.36^{**}	-0.32^{**}	0.36^{**}	0.49^{***}	-0.40^{***}

Annex 2

Figure 1. Frequency of the plant species in the area



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