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THE NICKEL CONCENTRATION IN RAW MILK OBTAINED FROM COWS GRAZING SERPENTINE PASTURES

SUMMARY

The purpose of this paper is to determine the Nickel level found in the raw milk obtained from the cows grazing in the serpentine pastures with much higher nickel concentration than the normal pastures. The average value of Ni concentration in the examined flora samples comprised of 16 different plant species resulted to be 566 mg kg⁻¹. A total of 12 samples of the raw milk were taken from a herd consisting of 60 cows, which had been grazing for six months in serpentine and non-serpentine pastures. Obtained results have revealed much higher concentration of nickel in the raw milk of cows fed on the serpentine pastures than those grazing in the non-serpentine pastures or those kept inside the barn during the winter.

In conclusion, serpentine soil of Mushtisht is a potentially toxic contaminated source to surrounding environment due to the high content of total and exchangeable nickel.

INTRODUCTION

Ni as a mineral does not have a direct toxic effect. However, when certain chemical compounds derived from Ni (carbonyls and other chemical species) penetrate the respiratory tract in high quantities; they can potentially cause lung cancer. In addition, the chronic consumption of high quantities of Ni can lead to myocardial, brain, liver and kidney degeneration (DENKHAUS and SALNIKOW, 2002).

The gastrointestinal absorption of nickel varies greatly depending on its chemical form and can be increased during an iron deficiency. Nickel can cross the placental barrier and be excreted in milk (WHO, 2005).

Land plant tissue contains much more Nickel than that of animal tissues (ALLEN, 1989). High concentration of Nickel is sometimes found in processed foods. This is free Nickel, picked up from the stainless steel used in the manufacture of equipment and containers. In general, cooking in stainless utensils releases negligible amount of nickel; however, cooking acidic food in these utensils may increase the Nickel content.

The US National Research Council (NIELSEN, 1980) has not set up any official recommendations for dietary intake Nickel, although suggestions have been made by several authors, such as Nielsen (NIELSEN, 1980) who has proposed a recommended daily nickel intake of $75\mu\text{g d}^{-1}$.

Nickel produces more cases of allergic contact dermatitis than all other metals together. The hands are the most commonly affected sites for systemic Nickel dermatitis. However, other body areas may be affected as well. Therefore; a good knowledge of the presence of nickel in food is helpful for the management of nickel allergy. Cows' milk, which is an essential part of human diet, fortunately has relatively low concentration of Nickel; it is about 0.03 ppm of Nickel (DARA, 2006).

Thus, given the fact that Kosovo has a considerable area of agricultural land and grasslands where we provide food from, for the animals, through this study we aimed to determine: (i) The Ni quantity in serpentine soil and consequently in plants that grow in this soil and the use of these pastures by cattle and (ii) the possibility of the Ni entry into the food chain.

MATERIALS AND METHODS

The serpentine region where the study was conducted lies in the south-eastern part of Kosovo with geographical grid of $42^{\circ}17.38' \text{ N}$; $20^{\circ}52.15' \text{ E}$ and altitude of 595 m above the sea level with Mushtisht being the closest settlement to this location. This serpentine region covers an area of 21 hectares containing several kinds of low herbaceous plants known to accumulate or hyper-accumulate Nickel, serving also as pastures for the village livestock.

The sampling was conducted in compliance with national guidelines of "Food and veterinary agency" of Republic of Kosovo and the Law of animal welfare (Law No.02/L-10). This approval was issued in written, prior to sampling being done.

In 2014, we collected three soil samples in the superficial soil horizon H 0-30 cm. The samples were taken in three different sites along the diagonal of the zone, so as to represent the whole studied area. These samples were then dried at the room temperature, ground to 2 mm size and sent to the lab for determination of Mg, Ca, Fe and heavy metals (Tab. 1).

The entire flora present therein was also sampled at that time of sampling. Species and family of the plants were determined (TUTIN *et al.*, 1993) and their chemical composition as well. For each plant sample, 5 -7 individual plants were randomly collected (not less than three individuals per plant). Plants with scarce presence (less than 3 individuals) were not considered. The individuals of a same plant species were then mixed altogether to form a composite plant sample.

Based on the information obtained from analyses of the soil and plants performed in the first year of study, (Table 1) in the second year we proceeded with the sampling of cow's milk. The whole village owns a herd comprised of 60 cows that are taken care by a shepherd hired by the local villagers. The cows graze on these pastures throughout the day from dawn to dusk during the period from April to October passing every day through these serpentine pastures. In the shepherd's words, the cows nevertheless don't seem to enjoy much the grass.

Sampling was conducted into two stages: we completed the first stage in the end of June 2016 when we took 6 samples of milk from 6 different cows, which represents 10% of this herd and this milk straight from the udder was then analysed for its nickel content. At the same time we took fresh milk samples from three other cows that were grazing on other common pastures (from non-serpentine regions). The second stage of sampling took place in January 2017, when six more samples were taken from the same cows to see whether the Ni quantity had diminished when cows were no longer grazing on those pastures due to the cold winter time, being kept inside the barns and fed on dried feed originating from the non-serpentine regions. It should be mentioned that the milk was poured straight from the udder into the PVC flask with the purpose of eliminating any contact of milk with utensils galvanized with nickel. The samples were then placed into a portative fridge in order to secure the temperature of 4°C and sent to the lab for determination of their Ni quantities.

Soil and plant analyses

The total concentrations of heavy metal in soil samples were analyzed by using US EPA Method 3050B as follows: 10 mL of the solution 1:1 HNO₃ was added to 1.0 g soil aliquots in Erlenmeyer flasks. The solution was heated to 95 ± 10°C for 15 min. After cooling off, 5 more mL of HNO₃ was added and the solution was heated again for 30 min. After cooling, another 2.0 ml of water and 3.0 ml 30% H₂O₂ were added and solutions were heated at 95 ± 5 °C. After digestion, the solution was cooled and again one mL of 30% H₂O₂ was added. Heating continued until the remaining volume was of about 5 ml and after cooling off, the solution was diluted with deionized water to reach the volume of 50 ml. Element concentrations in the final solutions was

performed on the 4200 MP-AES-microwave plasma-atomic emission spectrometer (Agilent order.de@agilent.com).

Before drying, all plant samples were carefully washed, with tap and deionized water, before they spread on the paper sheets and air-dried at room temperature, for weeks. Then all aerial part (shoots, leaves and flowers) of each species were ground into fine powder, put in polythene bags, labeled and sent to Laboratory for analyzing. Plant samples are analyzed in the following parameters: Zn, Cd, Mn, Ni, Cu, Pb, Cr, Co, Ca, and Mg.

Total concentration of heavy metal in plants' samples were analyzed by using Method 999.11

(A.O.A.C 17th edition, 2000). 5-10 g of air dried plant sample is placed in a quartz beaker. Then gently heat on a hot plate. Continue heating until enough water is driven off for partial carbonization to occur. Then place the beaker in an electrical furnace, and increase the heat at a rate of 100°C per 1 hour. Then heat at 500°C for several hours to conduct ashing. If ashing is incomplete, wet with 2~5 ml of a 50% magnesium nitrate solution or nitric acid (1+1). After drying, continue ashing. Add 2~4 ml of water to the ash, and after drying, add 5 ml of hydrochloric acid to dissolve the salts. Use water to prepare fixed volumes of measurement solution.

Reading is performed on the 4200 MP-AES (microwave plasma-atomic emission spectrometer).

Milk's Sample Analyses

For the analysis of the samples, the method of AOAC 99.11 was followed.

Pre-treatment- Milk was homogenized using non-contaminating equipment.

Drying- Milk samples (50 g) were weighed in crucibles and, once the sample was dried at 100°C.

Ashing- The sample was incinerated in a furnace applying the following mineralization stages: 90-250°C (ramp time 1h, hold time 1h), 460°C (ramp 2 h, holdtime 8 h) and 460-1000°C (ramp time 2 h). The ash was extracted with 2M-HNO₃ (2 mL), dried on a thermostatic hot plate and replaced in the furnace for a further 1 h at 460°C. The resulting white ash was recovered using 2M-HNO₃ (5 mL) and 0.1 M-HNO₃ (20 mL) in a 25 mL volumetric flask and stored in propylene flasks under refrigeration (4°C). The determinations were performed with a Perkin-Elmer model 2100 atomic absorption spectrophotometer connected with a Perkin Elmer HGA-700 graphite furnace and M-2 100 Multielement Program Software. Argon as internal and external gas, a hollow cathode lamp for nickel, a deuterium lamp as a background corrector and graphite pyrolytically-coated tubes with L'vov platform was

employed. In order to optimize the analytical signal, diverse tests with different lamp intensities (18-25 mA), temperature ranges 800-1550°C for pre-atomization and 2000-2500°C for atomization) A study of standard additions was carried out to prevent nickel losses and to corroborate the linear calibration of the apparatus.

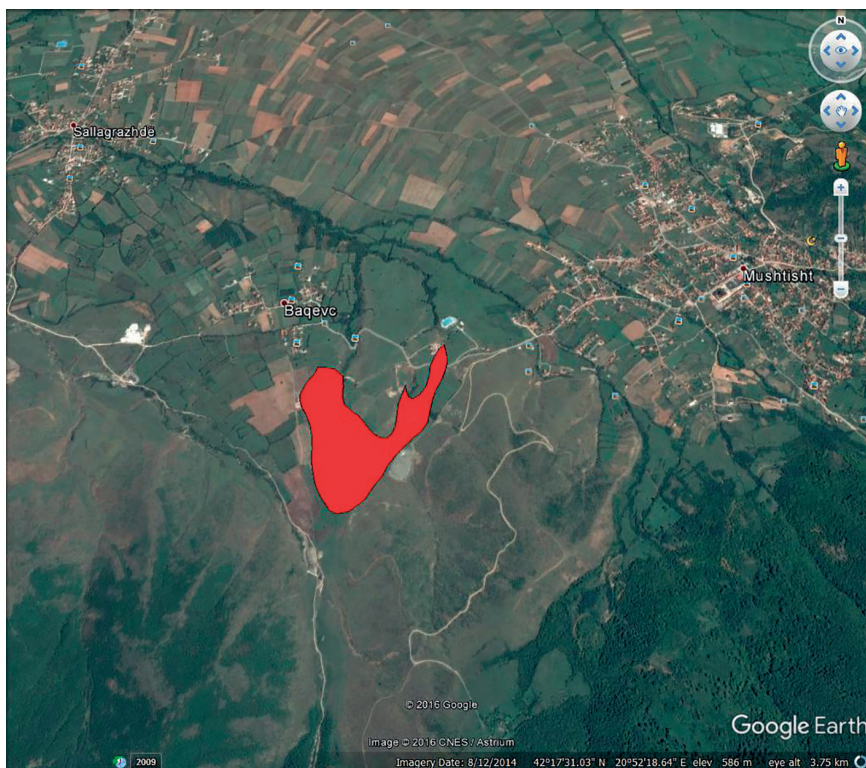


Fig. 1. Serpentine site in Mushtisht.

RESULTS AND DISCUSSION

Concerning the concentrations of major elements (Fe, Ca, and Mg) in soil samples (Table 1), the soils of Mushtisht, Kosovo showed typical ultramafic characteristics. The concentration of total Fe and Mg was high respectively 5.9% and 17.7, while the concentration of total Ca was low (0.15%). The ultramafic soils at of Mushtisht were characterized by elevated levels of heavy metals elements such as Mn, Ni, Co and Cr that are directly derived from the ultramafic bedrock (BROOKS, 1987 and, BANI *et al.*, 2014).

Tab. 1. Heavy metals content in soil of serpentine site of Mushtisht.

Soil	Zn	Cd	Co	Cu	Ni	Pb	Mn	Cr	Ni Exchangeable
Mushtisht H-A	34.8±5.4	69.6±2.6	60±8	5.9±1.04	2428±112	8.8±.8	660±36	783±36	68.7±2.5

Tab. 2. Heavy metals content in flora of serpentine site of Mushtisht.

Species	Zn mgkg ⁻¹	Cd mgkg ⁻¹	Mn mgkg ⁻¹	Ni mgkg ⁻¹	Cu mgkg ⁻¹	Pb mgkg ⁻¹	Mo mgkg ⁻¹	Cr mgkg ⁻¹	Co mgkg ⁻¹
<i>Alyssum markgrafii</i>	34.8	2.7	40.8	7117	13.9	4.5	< 1	8.8	< 1
<i>Anthylus aurea</i>	21.4	0.1	51.0	78	8.8	3.6	< 1	30.1	< 1
<i>Avenochloa pubescens</i>	27.4	0.0	63.8	144	3.7	4.3	< 1	52.8	< 1
<i>Consolida regalis</i>	26.3	0.2	28.3	39	6.4	3.5	< 1	12.5	< 1
<i>Dorycnium pentaphyllum</i>	37.0	0.1	78.4	136	5.5	4.2	< 1	52.5	< 1
<i>Ehium rubrum</i>	16.3	0.3	128	118	5.9	3.9	< 1	64.6	< 1
<i>Gallium verum</i>	33.7	0.4	27.6	23.1	7.4	3.3	< 1	3.1	< 1
<i>Juniperus communis</i>	9.4	0.2	30.4	36.5	2.8	3.7	< 1	8.9	< 1
<i>Linum tauricum</i>	28.9	0.2	35.1	69.6	6.0	3.6	< 1	25.0	< 1
<i>Lolium multiflorum</i>	23.8	0.4	61.8	94.8	3.3	4.0	1.3	76.3	< 1
<i>Medicago falcata</i>	26.9	0.4	70.2	38.8	3.8	4.1	< 1	15.3	< 1
<i>Potentilla recta</i>	28.7	0.4	91.1	82.6	4.7	4.3	< 1	100	< 1
<i>Rumex acetosella</i>	16.1	0.6	44.9	50.9	4.3	4.2	< 1	74.8	< 1
<i>Sanguisorba minor scop.</i>	23.3	0.4	94.9	114	5.2	3.8	< 1	94.5	< 1
<i>Sedum ochroleucum</i>	12.3	0.4	20.2	28.4	5.0	3.8	< 1	25.6	< 1
<i>Thymus pulegioides</i>	35.6	0.5	148	241	4.7	3.5	< 1	206	< 1

Tab. 3. Presence of Nickel in samples of raw milk.

Sample	Ni in milk during grazing season (mg.kg ⁻¹)	Ni in milk during non-grazing season(mg.kg ⁻¹)	Ni in milk in non-serpentine pastures(mg.kg ⁻¹)
Sample # 1	1.756	0.521	0.686
Sample # 2	2.663	0.786	0.234
Sample # 3	3.163	1.362	0.213
Sample # 4	2.325	0.745	
Sample # 5	1.884	0.532	
Sample # 6	2.522	0.862	
Average	2.39	0.80	0.38

The mean total Cr value was 783 mg kg⁻¹. Total concentrations of Ni in the soils were high in the range of serpentine soils reported by GHADERIAN *et al.*, 2007 (500–8000 mg kg⁻¹). Also available Nickel concentrations in the soil was high about 68.7 mg kg⁻¹ presented a potential risk for food chain (EVERHART *et al.*, 2006).

As shown by the Table 2, the plant *Alyssum markgrafii*, which is known as hyper-accumulative plant of Ni, had the highest content of nickel reach-

ing an amount of 7117 mg. kg⁻¹, while the *Galium verum* had the lowest Ni content of only 23.1 mg kg⁻¹. In some species in our sites, Ni content was elevated whilst samples of the non-serpentine substrata, they contain the expected very low Ni concentrations (REEVES, 1992.)

The average Ni content found in 16 species of plants picked from this area, amounts to 566 mg kg⁻¹, which is considered too high in order to be used as grazing pasture for the cattle.

The Table 3 shows the Ni concentration in the raw cow milk taken in 12 samples during the two-stage sampling. As it can be seen from the table, the Ni content found in the fresh cow milk is much higher during the grazing period outnumbering for many times the amount of Ni found in the normal cow milk. This Ni content varies in its amount from 1.76 to 3.16 mg kg⁻¹, whereas the average amount of Ni in those six samples reaches a value of 2.39 mg kg⁻¹. In the second stage of sampling conducted six months later, when the cows stayed inside the barn, the Ni content was much lower varying from 0.52 to 1.36 mg kg⁻¹ with the average value at only 0.80 mg kg⁻¹.

The Table 3 also gives the Ni values in the three raw milk samples from the non-serpentine pastures, whose findings indicate that the Ni level in these samples is considerably lower than the level found in the samples from the serpentine pastures. These values vary from 0.21 to 0.69 mg kg⁻¹, with an average of 0.38 mg kg⁻¹, which is generally higher than findings of other authors, as indicated by the Table 4 (AMARO *et al.*, 1998). Fig. 2 gives the average Ni concentration in all the three groups of samples and as it can be seen from the diagram the lowest average value is found in the sample group picked from the non-serpentine pastures.

The data were tested by test-paired, where $t = 15.48$ at $p < 0.01$ showed that the difference between the milk content during the grazing season in the serpentine area with the nickel in milk during non-grazing season is statistically significant. The correlation coefficient was very high, $R = + 0.945$, which shows that the cows that have grazed in the serpentine areas continue to subsequently have nickel consequences in the organism. Also the test-paired where $t = 6.14$ at $p < 0.01$ indicates that there is statistically significant difference between the milk content during the grazing season on the serpentine and nickel content in milk in non-serpentine pastures.

Given the recommended daily intake of 75 µg d⁻¹ according to Nielsen (AMARO *et al.*, 1998) and the Ni average concentration in the raw cow milk coming from the Mushtisht serpentine site, it results that we achieve this recommended daily intake by consuming an amount of only 31.5 g of such milk. This would mean that if we have a glass of 250 ml of such milk daily we would overcome the recommended daily intake of Ni for 8 times, without including the Ni intake from other foods. And it exceeds around 150 times according to World Health Organization (WHO, 2005).

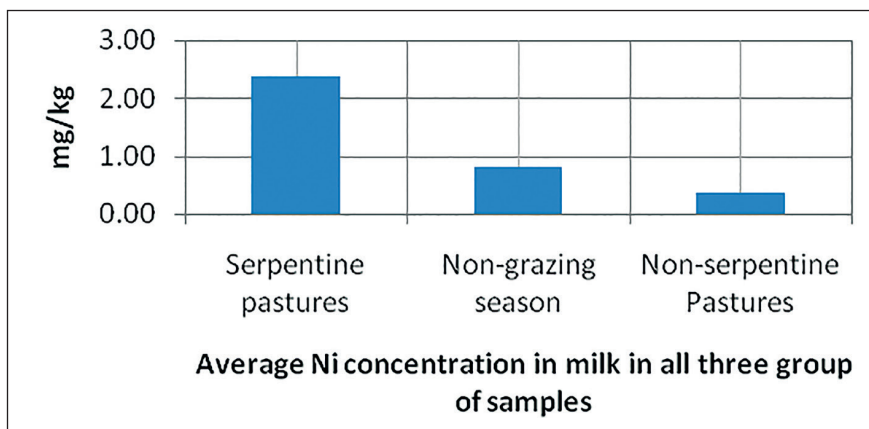


Fig. 2. Average Ni concentration in cow's raw milk in all three groups of samples.

Tab. 4. Nickel content ($\mu\text{g}/\text{kg}$) in raw cow milk from other authors.

Milk type	Mean \pm S.D.	Range	Reference
COW MILK	94 \pm 42	28-180	FRANCO <i>et al.</i> , 1981
	20.8 \pm 6.8		PERTOLDI <i>et al.</i> , 1984
	18.9 \pm 1.7	16-81	GABRIELLI and PERTOLDI, 1984
	60 \pm 9		FISCHBACH and POTTER, 1986
	25		ALEGRIA <i>et al.</i> , 1988
		65-129	GARCIA <i>et al.</i> , 1990
	4-60	SOUCI <i>et al.</i> ,1994	

CONCLUSION

Serpentine soil of Mushtisht is a potentially toxic contaminated source to surrounding environment due to the high content of total and exchangeable Nickel.

Present data suggest that the plant species collected at the site exhibited different concentrations in metals. Most plants of this serpentine area showed slightly elevated Ni concentrations in comparison with those on other soil types and possible Ni hyperaccumulator plants were individuated. The present preliminary data showed that Nickel concentration was higher in milk during grazing season in serpentine area than during non-grazing season or

in the raw milk samples from the non-serpentine pastures. Our first results from this study area will guide us for a more detailed study in the future to confirm the dangerous Ni presence in a food source for humans.

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