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GEOCHEMISTRY OF BULGARIAN SOILS IN VILLAGES AFFECTED AND NOT AFFECTED BY BALKAN ENDEMIC NEPHROPATHY: A PILOT STUDY*

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Abstract. Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial disease whose mosaic-like distribution throughout the Balkans has not changed significantly since its initial description. In this study, we explored the hypothesis that the occurrence of BEN is linked with the environmental geochemistry of villages. Soil samples were collected from BEN and non-BEN villages in the Vratza region of Bulgaria. Samples were digested in nitric acid and analyzed for 22 elements by hexapole, inductively coupled plasma, and mass spectrometry. Selected results are that: 1) absolute concentrations from both types of villages were not enriched above "background" concentrations; and 2) copper, molybdenum, lead, and cadmium concentrations were higher in BEN than in non-BEN soils, while selenium concentrations were lower. Although geochemical differences between BEN and non-BEN villages were found, not all differences were statistically significant, in part due to a limited number of samples.

Key words:
Balkan endemic nephropathy (BEN), Metals, Environment, ICP-MS, Health

INTRODUCTION

Balkan endemic nephropathy (BEN), first identified in the 1950s [1], affects people living in the high rainfall and humid regions of the alluvial plains along tributaries of the Danube River in Bosnia, Croatia, Serbia, Bulgaria, and Romania. BEN is a slowly progressing, familial,

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chronic, non-inflammatory, bilateral, primarily tubulointerstial, distinct nephritis, becoming overt usually in the fourth or fifth decade of life, and eventually leading to renal failure and death. The overall area of the BEN occurrence is about 20,000 km^2 and its geographic loci have not changed over time. The clusters of BEN cases predominately occur in rural areas in close (one to two km) neighborhood of non-endemic villages.

Hypotheses to explain the occurrence of BEN include exposure to low levels of environmental toxicants such as Pb, Cd, As, polycyclic aromatic hydrocarbons and Ochratoxin A, elemental deficiencies in the diet such as Se, genetic susceptibility, and exposure to slow acting viruses [2–6]. Environmental toxicants and trace elements can be absorbed from food and water [7,8], and concentrations in soil or geologic factors [9] may influence their intake [10–12]. Although various hypotheses have been proposed to explain the distribution of BEN, its etiology still remains unclear [8]. BEN could also be related to a combination of these factors [13].

These various hypotheses indicate that the geochemistry of the environment (e.g., soils, water, food) may play a role in the cause and distribution of BEN. To explore if the geochemistry of the environment might be linked to the distribution of BEN, a pilot study was conducted in which soil samples were collected from BEN and non-BEN villages in the Vratza region in Bulgaria. The multi-element capabilities of hexapole inductively coupled plasma mass spectrometry were used to determine the geochemical patterns [14] in village soils. If the geochemistry of the environment is related to the distribution of BEN, then there should be elemental distributions that are distinguishable in the environment between BEN and non-BEN villages.

STUDY AREA

The study area is the Vratza region of northwest Bulgaria, lying just north of the Stara Planina or the Balkan Mountain Range. The endemic area covers approximately 2,500 km^2 with over 40 affected villages. The topography of the region is characterized as low mountainous terrain with elevations between 200 and 600 meters. This is in contrast to the Danube River plane with elevations around less than 200 m, typical of the endemic areas in Bosnia, Croatia, and Serbia. The climate is defined as moderate continental. The geology is characterized by Lower Cretaceous marls, sandstone siltstones, glauconite; Miocene clays, sandstones, limestones; and Quaternary alluvial deposits.

MATERIALS AND METHODS

Soil samples were taken from three BEN-endemic villages (eight total households) and three non-endemic villages (fifteen total households). Non-garden soils were collected at each household at depths of 0–2 cm (shallow) and 10–12 cm (deep). Since the results of the analysis from both depths were similar, only the results of the 10–12 cm were presented. Sediments were lyophilized after collection and metals extracted via microwave, nitric acid digestion [15]. A standard reference material (SRM 2704, Buffalo River sediment) was also digested and analyzed for the quality control purposes. Extraction fluids were filtered through a 0.4um Nuclepore filter that had been acid washed.

Metal concentrations in the extraction fluids were quantified using inductively coupled plasma mass spectroscopy with hexapole technology (Micromass Platform). This instrumentation allows for the simultaneous determination of elements such as ^{75}As, ^{54}Fe, ^{80}Se, and ^{40}Ca with other elements typically analyzed for by more standard inductively coupled mass spectrometry methods. Indium-115 and bismuth-209 were used as internal standards. Elemental concentrations that were in the high mg/L range were quantified using flame atomic absorption spectroscopy (Perkin-Elmer Model 5100PC). Other elements analyzed included ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{39}K, ^{138}Ba, ^{44}Ca, ^{24}Mg, ^{51}V, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{114}Cd, ^{208}Pb, ^{27}Al, ^{45}Sc, ^{47}Ti, ^{96}Mo and ^{202}Hg.

RESULTS

The results indicate that, in general, elemental concentrations in the soils are similar and have the same abundance

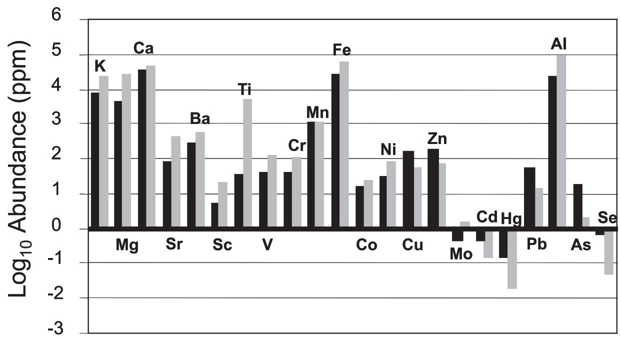

Fig. 1. Average elemental concentrations in soils from BEN villages (black) compared to average crustal concentrations (gray).

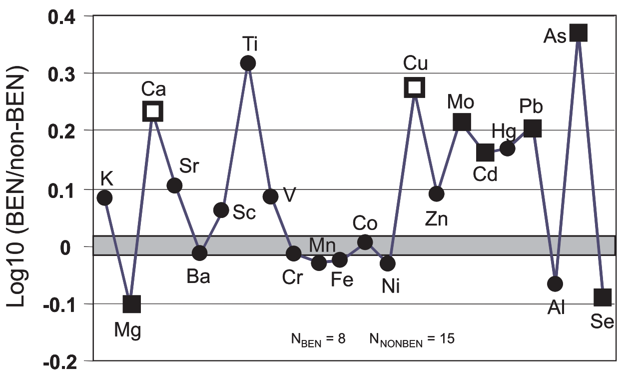

Fig. 2. Log_{10} ratio of elemental concentrations in soils from BEN villages to non-BEN villages. Solid squares are ratios significantly different at 95%CI, open squares at 90%CI.

pattern to average crustal concentrations [16] (Fig. 1). Patterns for the deep soils in both BEN and non-BEN villages are similar, therefore only concentrations of the elements in the soils from the BEN villages are shown in Fig. 1. Although some elements (e.g., Cd, Pb, As) in the soils have slightly higher concentrations than average crustal concentrations, these results indicate that for the elements studied, the soils have relatively normal concentrations.

The geochemical patterns of the deep soils from BEN and non-BEN villages are compared in Fig. 2. The elements are arranged in the figure from left to right in the following order: alkali and alkaline earths, first row transition elements, selected second row transition elements, heavy metals, and metalloids. To construct this figure, the averages were first calculated for the concentrations of the elements in the soils from the two types of villages. The ratios of the BEN averages to the non-BEN averages were calculated. To be able to display all the data on one graph, these ratios were converted to log_{10} values. Thus, if the ratio was greater than 0 the concentrations were higher in the BEN villages. Finally, Student's t-test was performed using a 2-tailed test at the 95% and 90% confidence limits. The computer program StatMost^{tm} was used for these calculations.

The results showed that BEN soils are apparently higher in K, Ca, Sr, Sc, Ti, V, Cu, Zn, Mo, Cd, Hg, Pb, and As, and lower in Mg, Ba, Cr, Mn, Fe, Co, Ni, Al and Se than the soils from non-BEN villages. The differences are significant at the 95% confidence interval for Mg, Mo, Cd, Pb, As, and Se and at the 90% confidence interval for Ca and Cu.

DISCUSSION

The results of this pilot study indicate that differences may exist and that some of the geochemical differences in the soils from BEN and non-BEN villages (e.g., Pb, Cd, As, and Se) are consistent with the hypotheses presented earlier. Interpreting these differences as the explanation of the distribution of BEN is not justified at this time for a variety of reasons. One is the limited number of samples, which makes the observations in this pilot study tentative. Another reason is the uncertainty as to what extent the observed elemental soil concentrations reflect the intake by the local population. Clearly, the soils and aquifer media are important sources of elements, but elemental mobility and bioavailability depends on the environmental conditions, such as oxidation-reduction state, pH, and general chemistry of the groundwater as well as types of soil phases sequestering the elements [14]. Finally, the ability of specific elements to cause BEN has not as yet been clarified, although some are known to be nephrotoxic [2,17].

Other multi-element studies of geochemical patterns between BEN and non-BEN environments have been inconclusive. These include studies of both groundwater [7] and soils (C. Tatu personal communication, 2000). In our study, although the concentrations for the measured elements approximate the average crustal concentrations, some discernible differences between BEN and non-BEN villages were observed. Clearly, more exploration is needed of the environmental geochemistry and BEN, which is most likely a multi-factorial disease [13].

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