

**Soil, grain and water chemistry in relation to human selenium-responsive diseases in Enshi District, China.**

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**Abstract** - Selenium deficiency (Keshan Disease) and toxicity diseases in humans occur within 20 km of each other in Enshi District in China and have been linked to environmental levels of Se. Low concentrations of Se are associated with Jurassic siltstones and sandstones, whereas high concentrations occur in areas underlain by Permian carbonaceous strata. Although these broad relationships between Se in the environment and the human population have been established previously, not all villages underlain by the carbonaceous strata suffer Se toxicity problems and the precise controls on Se distribution and availability have not been quantified. In the present study soil, grain, drinking water and human hair samples are examined to determine the controls on Se availability in three Se environments in Enshi District. Five low-Se and Keshan Disease villages, five high-Se and no toxicity villages and five high-Se and toxicity villages were selected for the study. Results show that the majority of samples in the low-Se villages are deficient or marginal in Se and that Se availability to plants is inhibited by adsorption onto organic matter and Fe oxyhydroxides in soil. Therefore, remediation strategies involving the application of Se fertiliser direct to the soil may not increase plant Se levels as expected. In the high-Se villages, localised lithological variations result in considerable ranges in Se concentrations in all sample types. Deficient and excessive levels of Se are recorded samples from the same village. Selenium bioavailability in the high-Se toxicity villages is controlled by the total soil Se concentration and pH. A greater proportion of the Se is plant available in villages where the carbonaceous strata are interbedded with limestone. Villagers should be advised to avoid planting crops in these areas if possible.

**INTRODUCTION**

Selenium was identified as an essential trace element to humans and other animals in the late 1950's. It forms a vital constituent of the biologically important enzyme glutathione peroxidase (GSH-Px) which acts as an anti-oxidant preventing tissue degeneration. Selenium is also toxic to humans at high doses and the range between deficiency levels ( $< 11 \mu\text{g/g}$  per day) and toxic levels in susceptible people ( $> 900 \mu\text{g/g}$  per day is very narrow) (Yang and Xia, 1995).

In China, Se deficiency has been linked to an endemic degenerative heart disease known as Keshan Disease (KD) and an endemic osteoarthropathy (Kaschin-Beck Disease) which causes deformity of affected joints (Tan *et al.*, 1989). These diseases occur in a geographic belt stretching from Heilongjiang Province in north-east China to Yunnan Province in the south-west (Figure 1). Selenium toxicity (selenosis) resulting in hair and nail loss and nervous system disorders in the human population, has also been recorded in Enshi District, Hubei Province and in Ziyang County, Shanxi Province in China (Yang *et al.*, 1983).

It is estimated that the annual incidence rate of Se deficiency diseases in China is 9.3 per 100 000 (Ge Xiaoli, pers. commun.). In the period between 1959 and 1970 peak KD incidence rates exceeded 40 per 100 000 (approximately 8500 new cases per annum) with 1400 - 3000 deaths recorded each year. Incidence rates have since fallen to less than 5 per 100 000 with approximately 1000 new cases reported annually (Levander, 1986).

China possesses one of the best epidemiological databases in the world on Se-related diseases, which has been used in conjunction with geochemical data to demonstrate a significant geological control on human Se exposure (Tan, 1989).

Epidemiological studies concentrating on the dietary intake and Se status of the local population were carried out in the Enshi area during the 1970's and 1980's in response to increased incidence of KD in the late 1960's and human selenosis in the late 1950's and 1960's.

Between 1923 and 1988, 477 cases of human selenosis were reported, resulting in hair and nail loss and nervous disorders in the local population (Mao & Su, 1993). In Yu Tang Ba (HT4) village, Shuang He community, the population was evacuated after 19 of the population of 23 people were affected and all the livestock died from Se poisoning. No human cases of Se toxicity have been reported in recent years but animals commonly suffer hoof and hair loss as a result of the high environmental Se (Mao Dajun pers. commun.).

Previous epidemiological investigations also identified an area of Se deficiency and KD, in close proximity to the high Se area (Yang *et al.*, 1983). Keshan Disease mainly occurred in six communities in the north-west of Lichuan County. In total, 312 people have suffered KD in the County, an average incidence rate of 103 per 100 000. Among the 312 cases, 136 recovered, 163 died and 13 persons still suffer from the disease. Children between the ages of 3 - 8 accounted for 83.4% of the total cases (Mao & Su 1993) and 80% of the children affected by the disease died (Mao Dajun pers. commun.). As yet there is no explanation why children of the 3 - 8 year old age group were particularly affected. This is in contrast to other KD areas of China, such as Zhangjiakou, where it is mainly women of child bearing age that suffer from the

disease (Johnson et al. 1996). Following a peak in incidence rates in 1969 (106 cases), the number of cases has fallen. Current incidence rates for the disease are unknown as no medical investigations have been carried out in recent years. No occurrences of Kaschin-Beck Disease have been reported in the Enshi area.

Yang *et al.* (1983) compared levels of Se in soil, food and human Se-status indicators from the Enshi area with other regions of China and demonstrated that the endemic Se intoxication of humans in Enshi was related to the occurrence of Permian Se-enriched carbonaceous strata. These comprise interbedded carbonaceous shales (known locally as “stone coal”), carbonaceous siliceous rocks, limestones and dolostones of varying carbon contents (Zheng *et al.*, 1992) (Table 1). Selenium concentrations in soil, food and human samples from areas underlain by carbonaceous strata were up to 1000 times higher than in samples from Se-deficient areas where KD was prevalent. Although Yang *et al.* (1983) speculated that the water-soluble (plant available) portion of soil Se may be influenced by several factors, including total soil Se, organic matter, iron content and soil pH, the impact and relative importance of these geochemical factors was not quantified.

These studies did not provide a detailed evaluation of the relationships between environmental Se concentrations and human Se status at the village level. In particular, they did not elucidate why some villages underlain by carbonaceous strata had several cases of Se toxicosis whereas other villages sited in apparently similar geological and geochemical environments had no recorded cases of human or animal toxicosis.

The current study was designed to address these knowledge gaps and to use the new information on geochemical controls to identify strategies which may help to enhance the Se status of residents in Se-deficient areas and reduce the risk of Se toxicity to people living in the high-Se areas of Enshi District.

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## **METHODS**

### *Sampling Strategy*

During the present study, fifteen villages from three Se environments in Enshi District were investigated (Figure 2). Soil, grain, drinking water and human hair samples were collected from five Low-Se-Keshan-Disease villages (LK), five High-Se-No-toxicity (HN) villages, and five High-Se-Toxicity (HT) villages. This sampling strategy was designed to establish the relationship of the human Se status of communities (represented by hair samples) and their dietary Se intake (represented by staple grain and drinking water samples) to the local geochemical environment characterised by cultivated soils. Where possible, soil, grain and hair samples were collected from the same farm.

### *Soil Sampling*

Five composite near-surface (10 - 30 cm, below A<sub>0</sub>) soil samples were collected from each village. Each composite soil sample comprised four sub-samples collected in the same field. Using the assembled sectional soil auger, four holes were made at the corners of a square. Where possible, this square had dimensions 20 x 20 m in accordance with IGCP 259 recommendations (Darnley et al., 1995) but many of the terraced fields in the area were smaller than this and in some fields it was only possible to separate the sub-sample holes by 3 - 5 m. The sub-samples were homogenised on a plastic sheet and samples were stored in air-tight plastic containers to retain the moisture content. Sample sites were selected so as to cover all the topographic environments of arable land around each village and fields managed by different farmers. Duplicate samples were collected from 1 in every 10 sample sites.

### *Grain Sampling*

Five maize-corn samples were collected from different grain stores in each village, corresponding, wherever possible, to farms where soils had been collected. It is common practice in the HN and HT villages to dry the corn over carbonaceous-shale fires before and during storage. Smoke from the fires is thought to contaminate the corn with Se (Mao and Su, 1993). Duplicate samples were collected one in every ten samples.

### *Water Sampling*

In each village, a spring used as a supply for drinking water was sampled. In the villages of Shadi (HT1) and Huabei (HT2), the population had been advised not to drink the spring water due to the high Se content, but the springs were sampled anyway to determine the local hydrogeochemical environment. Duplicate samples were collected at 1 in every 5 springs. At each spring, the following suite of water samples was collected: (i) three 30 ml 0.45 µm filtered water samples for multi-element analysis; (ii) one 30 ml unfiltered water sample for pH and Eh determinations; and (iii) one 250 ml unfiltered water sample for bicarbonate and conductivity determinations.

Samples collected for multi-element and Se analysis were acidified by the addition of 0.3 ml ARISTAR grade nitric and hydrochloric acid, respectively, thus preventing adsorption of dissolved metals to the interior walls of the storage bottle and minimising post-sampling microbial activity. Eh, pH, bicarbonate and conductivity determinations were carried out at the end of each day's sampling.

### *Hair Sampling*

Approximately 10 g of hair was collected from the nape of the neck. Where possible, samples were taken from five individuals from different families corresponding to the farms where soil and grain samples had been collected. Individuals of a range of ages

were sampled, and age, health, disease and medication details were recorded (Fordyce *et al.*, 1998).

### *Analysis*

Soil samples were dried at a low temperature of 35 °C for 6 - 12 hours to avoid loss of Se through volatilisation. The soils were disaggregated to pass a 2 mm nylon sieve mesh and were ground in an agate vibrating-cup mill to < 150 µm. Total (hydrofluoric-nitric-perchloric digestion) and water-soluble Se in soils were determined by hydride generation Atomic Fluorescence Spectrometry (AFS). Partial (nitric-perchloric digestion) soil Ca, Cd, Cu, Fe, Mg, V and Zn concentrations were determined by ICP-AES (Fordyce *et al.*, 1998).

Soil pH was determined on a paste (of specific consistency) of the unprepared samples to which deionised water had been added (Fordyce *et al.*, 1998).

Soil loss on ignition (LOI) measurements were carried out on powders dried at 35 °C before being placed in a furnace and kept at 450 °C for a minimum of four hours.

Total organic carbon concentrations (TOC) were determined in fifteen selected soils by LECO CS125 Carbon Analyser. Dried samples were ground and accurately weighed into LECO crucibles and treated four times with hot 10% hydrochloric acid (HCl) to remove oxidised carbon. The samples were washed four times with deionised water and then dried in an oven at 60 °C prior to analysis. The instrument was calibrated with a standard LECO carbon steel ring of 0.815% carbon content. An internal claystone standard with a TOC content of 2.5% was included in the analysis.

Thirty selected soil samples underwent analysis for approximately 25 elements by ICP-AES and ICP-MS at the Institute of Rock and Mineral Analysis, Beijing.

Maize-corn samples were ground in a Waring blender for 2 minutes. The possible effects of Se contamination from carbonaceous-shale smoke during the grain-drying process employed in high-Se areas were assessed by washing one grain sample from the LK, HN and HT village groups prior to preparation and analysis. The samples were coned and quartered. One quarter was retained for reference, one quarter was washed twice with 1% HCl; one quarter was washed twice with deionised water, and one quarter remained unwashed prior to being processed along with the other samples.

The amounts of Se in the wash solutions from the LK villages, where grain is not dried over carbonaceous-shale fires, are similar to those from the HN and HT villages (Table 2). Furthermore, the amount of Se present in the HCl wash solutions is not consistently greater than the amount present in the deionised water wash solutions, as would be expected if the grain surfaces were contaminated with carbonaceous-shale smoke. The amount of Se in the wash solutions of the HN and HT samples as a proportion of the levels in grain is extremely small (< 0.005%) but the amount in wash solutions of the LK villages is greater (1 - 2.5%) (Table 3). This reflects the far lower levels of Se in grain samples from the LK villages and is not related to

contamination by carbonaceous- shale smoke. As a result of these determinations the grain samples were not washed prior to analysis.

Hair samples were washed twice in deionised water and allowed to dry on clean plain paper. Once dry, the hair was cut into 2 - 4 mm lengths with a pair of clean stainless-steel scissors and homogenised prior to analysis.

Total-Se determinations by AFS in grain and hair samples were based on a nitric-perchloric attack.

Following initial acidification, water samples underwent no further treatment prior to AFS analysis for Se. Chloride, NO<sub>3</sub> and SO<sub>4</sub> were determined in the unacidified samples by ion chromatography.

In all cases, data quality was assured by the inclusion of certified reference materials in the analytical runs and by selected repeat measurements (Table 4). The limits of detection for Se analysis by AFS in each sample type are given in Table 5.

### *Data Processing*

In order to carry out statistical analyses of the data, results for analytical and field duplicates were averaged, and rice-paddy soil results were removed from the datasets once a comparison between rice-paddy soils and maize-corn soils was completed. Spearman Rank non-parametric correlation coefficients were calculated for statistical analysis, as these are less sensitive to outlying values than product moment (Pearson) correlation coefficients. In addition to correlation coefficients, factor analysis was used to indicate relationships between variables.

LOI is used as an indicator of organic-matter content in soils, as a close correlation was noted between LOI and total organic-matter content (Figure 3). Assuming that TOC generally accounts for 58% of the organic matter in soils, the organic-matter content can be calculated by multiplying the TOC values by 100/58 (Rowell, 1994). LOI correlates closely ( $R^2= 0.862$ ) with TOC and organic matter in the soils but the LOI values are higher than the TOC and organic-matter values (Figure 3). At most, organic matter accounts for 63% of the LOI in the Enshi soils. The disparity between organic-matter content and LOI is probably due to the loss of water from clay minerals during ignition. Rowell (1994) reports that LOI can be used as an estimate of organic-matter content in sandy soils but may be up to twice the organic-matter content in heavy textured soils because clays and sesquioxides lose 'structural' water between temperatures of 100 and 500 °C.

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## **RESULTS AND DISCUSSION**

### *Soils*

It has been suggested (Yang *et al.*, 1983), that the incidence of selenosis in the 1960's was related in part to a drought which resulted in the failure of the rice crop. Villagers were forced to grow maize-corn on dry rice-paddy fields. In general maize-corn contains more Se than rice, however it is also suggested that the maize-corn accumulated more Se, probably as a result of the oxidation of  $\text{Se}^{2-}$  and  $\text{Se}^0$  to more bioavailable  $\text{Se}^{4+}$  and  $\text{Se}^{6+}$  under dry conditions (Yang *et al.* 1983). During the present study, ten rice-paddy soils were sampled to assess the chemical characteristics of soils under the waterlogged conditions necessary for rice cultivation. No consistent difference in soil chemistry between the rice paddies and other fields is apparent in the HN and HT villages, but in the LK villages the paddy soils generally have higher total Se and pH and contain lower Fe and Ca than the maize-corn soils (Figure 4).

Selenium availability in the environment is controlled by several factors. The main parameters affecting Se uptake from soils to the food chain include geology, pH and Eh conditions, Se speciation, organic matter and clay content, and the concentrations of Fe, P and sulphur (McNeal and Balistrieri, 1989).

The influence of geology on Se distribution in Enshi District, is evident from the results for total and water-soluble (bioavailable) Se in soil and for total Se in water, grain and hair, which indicate that Se levels in the HN and HT villages are 17 to 690 times those in the LK villages (Figure 5 and Table 6). These results confirm the findings of previous investigations demonstrating that low levels of Se are associated with Jurassic siltstones and sandstones which outcrop on the north-west of Enshi District whereas high environmental Se occurs in areas underlain by Permian carbonaceous strata. Selenium-deficient and Se-toxic environments occur within 20 km of each other and are fundamentally controlled by the underlying geology.

There are broad ranges in total and water-soluble Se concentrations in the HN and HT villages, in Luojiaba (HN1) and Bajiao (HN2) in particular (Figure 6), indicating the localised influence of geology within villages on soil Se levels.

In the LK villages, the stronger correlation between total Se and LOI ( $r = 0.611$ ) than between total Se and pH ( $r = -0.453$ ) and the lack of a significant (95% confidence level) correlation between total Se and Fe, suggest that organo-complexing of Se is a more important control on total-Se chemistry than Fe concentration or pH (Table 7). Soil pH is lowered as the organic-matter (and associated Se) content of soil increases, as expected; hence the negative relationship between total Se and soil pH (Table 7).

Soil water-soluble Se correlates negatively (95% confidence level) with Fe (Table 7). This is to be expected, as Se held in the form of Fe oxyhydroxides is less available for plant uptake (Mikkelsen *et al.*, 1989).

In the HN and HT villages, soil Se levels reflect the distribution of the carbonaceous strata. The highest Se levels are associated with high levels of other elements common in carbonaceous rocks, such as Cd, Cu, F, Hg, S, V and Zn, also with high levels of organic matter (Table 8). The association between high levels of Se in soil and organic matter is probably a reflection of bedrock geochemistry rather than the complexing of soil Se by secondary biologically derived organic matter.

Factor analysis was applied to the data for soils (Table 9). High factor loadings (> 0.500) indicate significant associations between variables and each factor (Factor 1, 2, 3) represents a linear combination of related variables. The importance of each factor is indicated by the proportion of variability, therefore a factor accounting for 30% of the variance is more important than a factor representing 15% of the variance. In both the HN and HT villages, there are strong associations between Cd, Cu, V, Zn, total Se and water-soluble Se (Factor 1 HN and HT, factor loadings > 0.500, Table 9). This association, which probably represents the geological control on soil trace element chemistry, accounts for approximately 50% of the variance and is therefore highly significant. This factor also demonstrates the relationship between the bioavailability of Se (indicated by water-soluble Se) and soil total-Se levels.

In both the HN and HT villages, the second factor indicates a strong relationship between pH and Ca as expected (Factor 2, HN and HT, factor loading > 0.500, Table 9). In the HT villages, the second factor grouping also includes soil water soluble Se indicating that soil pH is an important control on Se bioavailability (HT Factor 2, Table 9). In the HT villages, the negative association between water-soluble Se with Fe and LOI (HT Factor 3, Table 9) suggests that to some extent Se availability may be restricted by adsorption onto Fe oxyhydroxides and secondary biologically derived organic matter, but these results should be treated with caution due to the low significance of this factor (variance proportion = 15%) and the moderate factor loading for water-soluble Se (- 0.336, HT Factor 3, Table 9).

### *Water*

Differences in anion dominance between the LK and HN/HT villages reflect regional geological variations,  $\text{SO}_4$  and  $\text{HCO}_3$  anions being more prevalent in waters associated with the sulphide-rich carbonaceous shales and limestones of the HN and HT villages (Table 10). Selenium concentrations in water in the HN and HT villages are 46 to 194 times those in the LK villages (geometric mean results) (Table 6). The maximum Se concentration (275  $\mu\text{g/l}$ ) occurs in Huabei (HT2) which greatly exceeds the World Health Organisation (WHO) maximum recommended concentration of 10  $\mu\text{g/l}$  in drinking water (WHO, 1984). Villagers have been provided with an alternative water supply in this village.

### *Grain*

Concentrations of total-Se in grain (maize-corn) in the HN and HT villages are 99 to 690 times those in the LK villages (geometric mean values) (Table 6), indicating the fundamental control of geology at a regional level on concentrations of Se in staple food crops in the Enshi area.

The wide ranges in total and water-soluble soil Se content noted in Luojiaba (HN1) and Bajiao (HN2), are reflected in maize-corn total-Se levels for these villages, confirming that localised variations in soil chemistry within villages can significantly affect the amount of Se entering the food chain (Figure 6).



In the HT villages, levels of maize-corn total-Se in Xin Tang (HT3) and Yu Tang Ba (HT4) are higher than would be predicted from the total and water-soluble Se contents of soil (Figure 6). Levels of Cd, Cu, P, Se and V are lower in these two villages than in the rest of the HT villages, whereas soil Ca and pH are higher (Fordyce *et al.*, 1998). This suggests that limestone rather than carbonaceous shale dominates the soils collected in Xin Tang (HT3) and Yu Tang Ba (HT4). Although levels of total Se in soil are lower than in the other HT villages, levels of Se are high (2.7 - 8.9 µg/g) compared to most soils in China (< 0.125 - 0.4 µg/g, Tan, 1989). The combination of relatively high Se in soil associated with the carbonaceous strata and higher soil pH due to the presence of limestones probably accounts for the greater proportion of water-soluble Se in soil and greater uptake of Se by maize-corn in these villages (Figure 6).

### *Hair*

Total-Se concentrations in hair in the HN and HT villages are, respectively, 17 and 87 times (geometric mean values) the values in the LK villages, indicating the strong environmental control on human Se status in the Enshi area (Table 6).

Total-Se concentrations in hair vary considerably within individual HT villages, reaching a maximum of 141 µg/g in Shadi (HT1) (Figure 6). It was not always possible to collect grain samples from villagers whose hair had been sampled, particularly in the LK villages, and this may explain why trends in hair Se chemistry in these villages do not relate to trends in grain total Se (Figure 6).

Variations in hair total Se in the HN and HT villages correspond to variations in total Se in grain and water, with the exception of samples from Yu Tang Ba (HT4). The inhabitants of this village had only been resident for 3 months and did not consume grain from the village. The results for hair clearly define the lower Se status of this population supporting the link between dietary Se intake and hair Se levels (Figure 6).

Hair Se concentrations in Luojiaba (HN1) and Bajiao (HN2) are higher than in other HN villages, reflecting greater concentrations of Se in soil, grain and water samples in these two villages.

### *Deficient, Marginal and Excessive Levels of Selenium*

On the basis of previous investigations into human Se imbalances carried out in China, threshold levels in various sample types indicative of low and toxic Se diseases have been defined (Tan, 1989). Applying these levels to the Enshi area, most samples in all five LK villages are deficient or marginal in total Se in soil, water-soluble Se in soil and total Se in grain (Table 11). In contrast, most hair Se values in the LK villages are marginal, suggesting that dietary sources other than locally grown maize-corn may contribute significantly to human Se intake. The population in the LK villages may be at risk from Se deficiency if these other dietary sources of Se are not maintained and enhanced.

Most samples in nine out of ten HN and HT villages surpass the excessive threshold value for total Se in soil. Luojiaba (HN1), Bajiao (HN2), Shadi (HT1), Huabei (HT2) and Xin Tang (HT3) have a greater proportion of excessive water-soluble Se concentrations in soil than the other HN and HT villages (Table 11). The remaining HN villages are marginal or deficient in water-soluble Se in soil, and grain levels in these villages do not surpass the excessive threshold.

Local lithological changes result in considerable variations in Se concentrations in all sample types at the individual village level. Villages can contain both deficient and excessive levels of Se in soil and grain, making the assessment of Se toxicity risk difficult. The proportion of crops grown on relatively low or very high Se soils in an individual village may, in part, determine which individuals within villages are at risk.

The levels of Se found in hair, and therefore the potential selenosis risk, in Luojiaba (HN1) and Bajiao (HN2) are comparable to levels in the HT villages and yet, no selenosis has been reported in these villages. It is likely that other factors have prevented the occurrence of selenosis in these villages.

Yang *et al.* (1983) reported higher levels of Se in maize-corn than in rice and markedly greater concentrations in vegetables than in cereal crops grown in the Enshi high-Se area. Selenium levels in hair are, therefore, probably influenced by the proportions of vegetables, rice, maize-corn and other foods in the diet. The consumption of food from outside the high-Se area will also affect the Se status of the population.

Yang *et al.* (1983) indicated that the worst outbreaks of human selenosis occurred in Enshi District during the early 1960's and coincided with a period of drought and rice crop failure. It is suggested that the shortage of food, greater proportion of vegetables and maize-corn and the lesser proportion of protein in the diet at this time contributed to the incidence of the disease (Yang *et al.*, 1983). These factors may also explain why selenosis occurred in some villages with high environmental levels of Se and not in others.

## CONCLUSIONS

Geology is the main control on Se and other trace element distributions in soil in Enshi District. Most trace elements and other soil parameters (pH and organic matter) are higher over the carbonaceous and limestone rocks of the HN and HT villages than over the sandstone bedrock of the LK villages.

Selenium in soil in the LK villages is strongly adsorbed by organic matter, and complexing with Fe oxyhydroxides also restricts bioavailability. Most of the soil and grain samples in the LK villages are deficient in Se, whereas most hair samples have marginal Se concentrations. The present incidence of KD in the LK villages is unknown, but the results of this study suggest that the population may be at risk from Se deficiency disorders and measures may need to be taken to enhance Se levels in the diet of the local population.

In the LK villages it is recommended that a dietary survey be carried out to determine the range of Se intake of the local population. Should a survey confirm that the daily Se intake is below 40 µg/day (UK Lower Dietary Nutrient Reference Intake, MAFF, 1997) then remedial action involving the use of Se fertilisers to increase grain, vegetable and farm animal Se concentrations would be recommended as a precautionary measure.

The relative benefits of adding Se fertiliser to soil or applying direct to crops should be investigated, as the results of the present study and other investigations carried out in China (Johnson *et al.*, 1996) strongly suggest that soil Se in low-Se environments is fixed by organic matter and is therefore not readily bioavailable. As a result, the addition of Se fertiliser to soil may not increase crop Se levels.

In the HN and HT villages, Se levels in soil reflect the distribution of Se-rich carbonaceous strata. Selenium bioavailability (water-soluble Se) is dependent on total-Se levels in soil and on pH. Levels of Se in grain increase as the water-soluble Se content of the soil increases in the HN and HT villages. It is possible that, in some villages, higher Ca and pH, indicating a greater abundance of limestone bedrock, make Se more available for plant uptake.

Selenium concentrations can vary from deficient to toxic in the same village. Individuals at highest risk may be those with fields sited directly over carbonaceous strata or areas where limestones increase the availability of Se, whereas neighbours with adjacent fields on surrounding siliceous rocks are less at risk.

Despite the high levels of Se found in the population of the HN and HT villages, no incidences of selenosis have been reported in recent years in Enshi District. This suggests that the local population may have adapted to the high Se intake or that, although Se levels in soil, grain, water and hair samples give an indication of the potential risk of human Se toxicity problems, other factors may precipitate the onset of human selenosis. Previous investigations have shown that Se concentrations in various foodstuffs grown in the high-Se areas vary markedly and are greater in vegetables and maize-corn than in rice. Factors such as dietary composition and the consumption of foods from outside the high-Se areas will affect the Se status of the population as well as external pressures such as drought and food shortages. In the HN and HT villages, it is recommended that, where possible, villagers should avoid cultivating high-Se soils directly underlain by carbonaceous strata. In particular, villagers should be discouraged from growing vegetables adjacent to outcrops of the carbonaceous strata. However, it is recognised that population pressure may require all land in a village to be cultivated. The local population should also be encouraged to reduce the proportion of high-Se foods in the diet and to increase the percentage of produce from outside the high-Se areas.

## **ACKNOWLEDGEMENTS**

This study forms part of a British Government Department for International Development (DFID) Knowledge and Research (KAR) Project to determine methods

of predicting and remediating human Se imbalances (Project R6227). The authors extend grateful thanks for the excellent assistance provided during fieldwork and the completion of this study to: Prof Li Jiayi, Prof Huang Huaizeng, Liu Xiaoduan, Ms Ge Xiaoli, Prof Luo Daihong, Ms Zhang Qiling and Mrs Yin Pinjun, Institute of Rock and Mineral Analysis, Beijing; Dr Li, Prof Mao Dajun, Dr Su Hongcan and members of the Public Health Department and Enshi Local Government; Dr J. D. Appleton, Dr M. Cave, Mr M. Allen, Dr V. Hards, Mrs B. Vickers and Dr H. W. Haslam, British Geological Survey. Very grateful thanks are extended to all the residents of Enshi District who agreed to take part in this study. This paper is published with the permission of the Director of the British Geological Survey.

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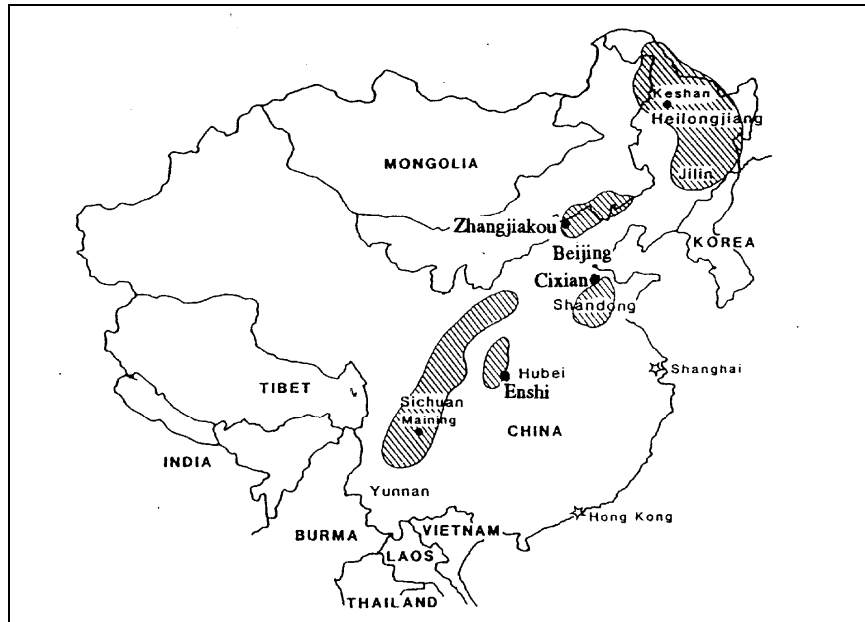


FIG. 1. Location of Enshi and the KD incidence belt (shaded) in China (modified after Crounce et al., 1983).



FIG 2. Sketch map of Enshi District showing the 15 villages sampled during the present study.

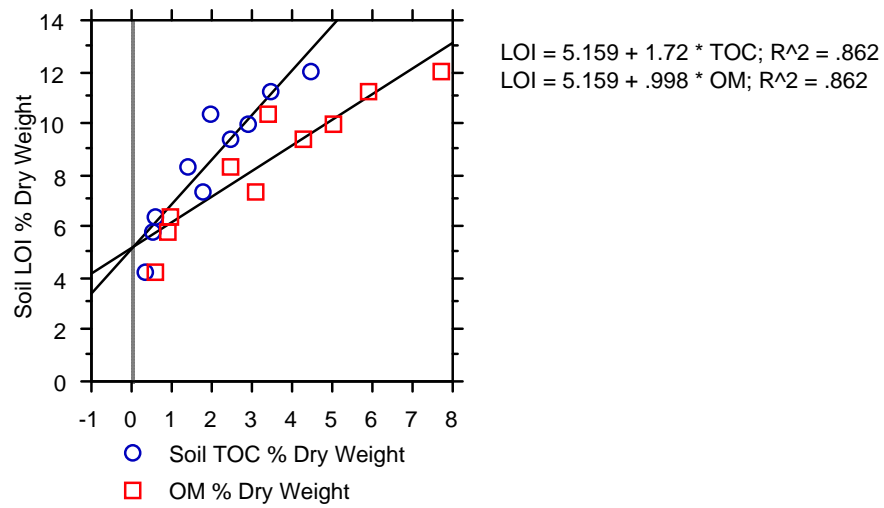


FIG 3. Comparisons between LOI, TOC and organic matter (OM) in Enshi soils.



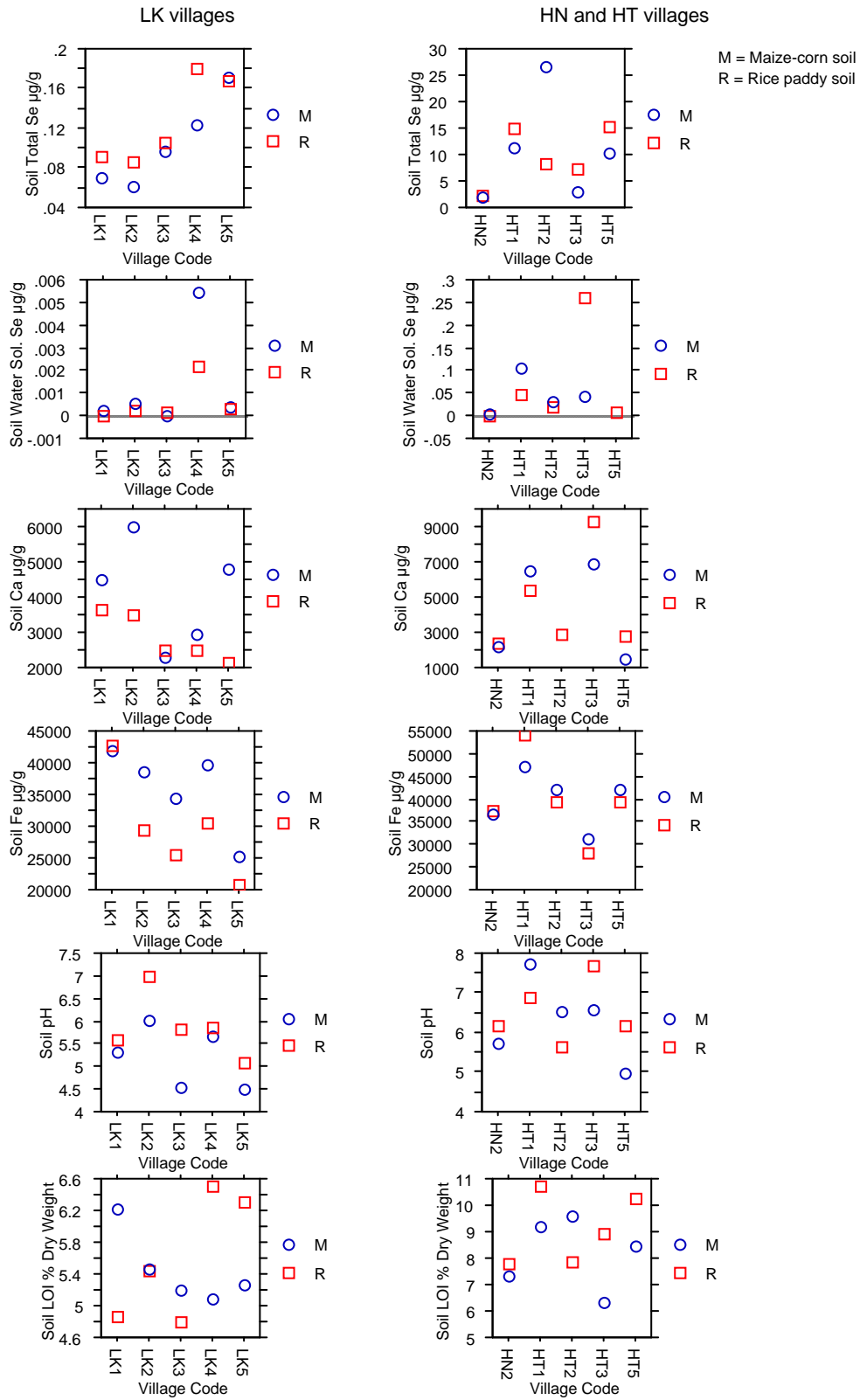
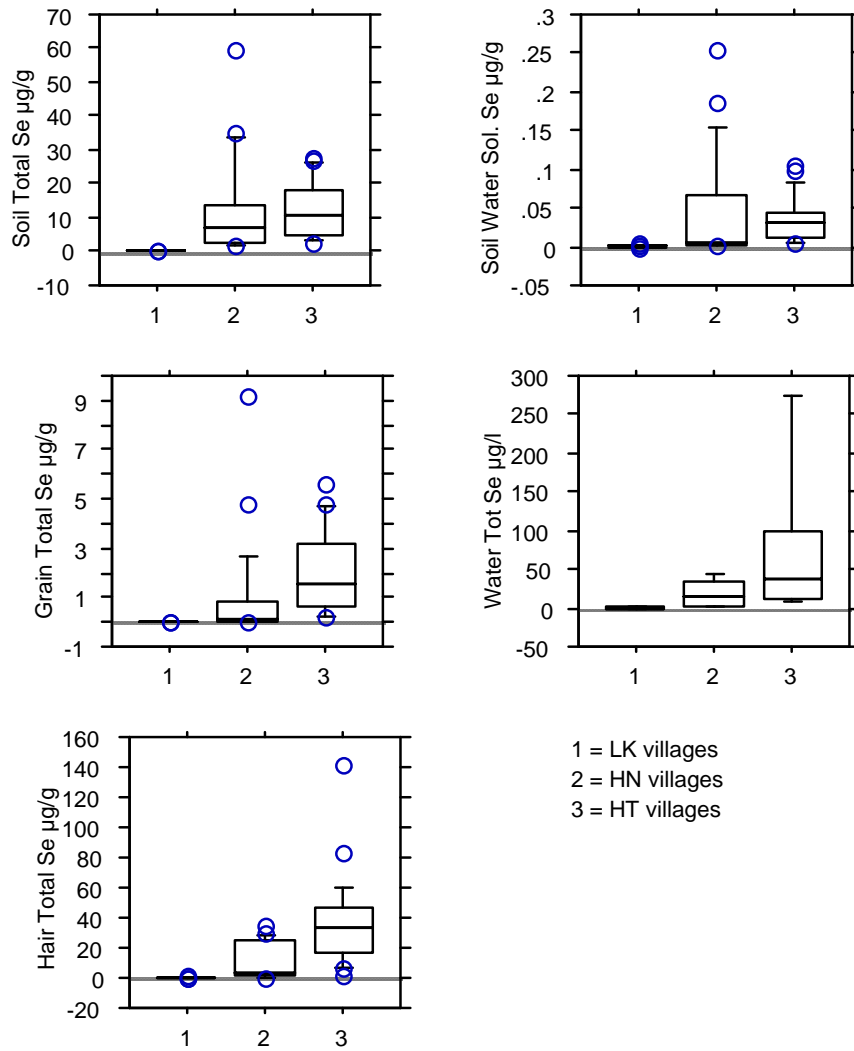


FIG 4. Comparisons between rice-paddy and maize-corn field soil samples.



Number of samples in each village group = 25

FIG 5. Box and whisker plots of the 10th, 25th, 50th, 75th and 90th percentiles of Se distributions in all sample types in the LK, HN and HT villages.



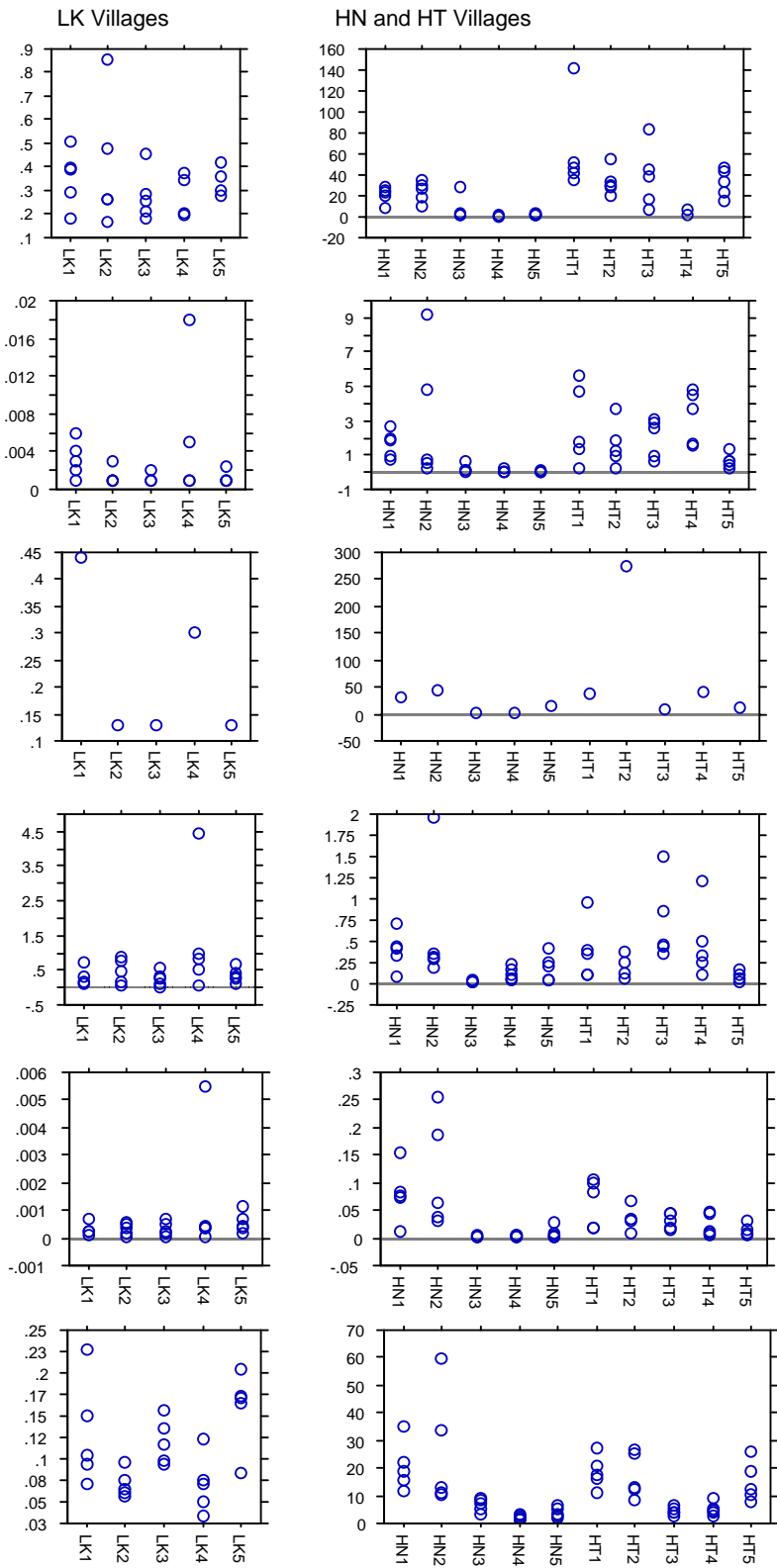


FIG 6. Selenium distributions in all sample types in the LK, HN and HT villages.

Table 1. Geological strata underlying villages sampled in Enshi District.

Geological Unit	Era	Rock Type
Q	Quaternary	Loess
K <sub>2</sub>	Cretaceous	Quartzose sandstone and conglomerate
J <sub>2x</sub>	Jurassic	Mudstone and feldspatic sandstone
J <sub>2s</sub>	Jurassic	Purple silty sandstone, calcareous argillaceous silty sandstone
T <sub>3</sub>	Triassic	Clay, sandstone, carbonaceous shale and coal layer
T <sub>2</sub> and T <sub>2b</sub>	Triassic	Limestone, marl and shale
T <sub>1j</sub>	Triassic	Dolostone, limestone and breccia
T <sub>1</sub>	Triassic	Dolostone, oolitic limestone and breccia
P <sub>2</sub>	Permian	Limestone, <u>carbonaceous shale</u> , calcareous mudstone and <u>carbonaceous siliceous rocks</u>
P <sub>1m</sub>	Permian	Limestone with flints, siliceous rocks and marl
P <sub>1q</sub>	Permian	Limestone with flints, quartzose sandstone, <u>carbonaceous shale and siliceous rocks</u>
P <sub>1</sub>	Permian	Limestone, shale, siltstone and pelite
C <sub>1+2</sub>	Carboniferous	Dolostone, limestone, dolomitic limestone and quartzose sandstone
D <sub>2+3</sub>	Devonian	Limestone, shale and quartzose sandstone and oolitic hematite
S <sub>2</sub>	Silurian	Quartzose fine sandstone, shale and bioclastic limestone
S <sub>1ln</sub> <sup>2</sup>	Silurian	Shale, sandy pelite and sandstone
S <sub>1ln</sub> <sup>1</sup>	Silurian	Yellow-green shale, sandstone and calcareous sandstone
S <sub>1l</sub>	Silurian	Shale, sandy shale, and pelitic sandstone
C-O	Cambro-ordovician	Shale, limestone, dolomitic limestone and breccia

Based on translation of selected geological units from the geology map of Enshi District. High Se-bearing strata are underlined.

Table 2. Comparison of Se concentration in washed and unwashed grain samples and in wash solutions from the LK, HN and HT villages.

Sample Number Village Code	EN92 LK5	EN98 HN2	EN95 HT4
Unwashed Grain Se ng/g	3	4831	3742
Water-washed Grain Se ng/g	5	5916	3706
HCl-washed Grain Se ng/g	4	5023	3630
Deionised-wash water Se ng/g	0.033	0.072	0.033
HCl-wash solution Se ng/g	0.051	0.017	0.134

Table 3. Weight of Se in grain and wash solutions and percentage of 'Se smoke contamination' in the LK, HN and HT villages.

Sample	Village Code	Sample Weight g	Weight of Se in sample ng	% 'Smoke Contamination' $\frac{\text{Weight of Se in wash}}{\text{Weight Se in grain}} \times 100$
EN92 Unwashed grain	LK5	25	75	
EN92 Water-washed grain		25	125	1.32
EN92 HCl-washed grain		25	100	2.55
EN92 Deionised water		50	1.65	
EN92 HCl-wash solution		50	2.55	
EN98 Unwashed grain	HN2	22	106282	
EN98 Water-washed grain		22	130152	0.00276
EN98 HCl-washed grain		22	110506	0.00076
EN98 Deionised water		50	3.60	
EN98 HCl-wash solution		50	0.85	
EN95 Unwashed grain	HT4	22	82324	
EN95 Water-washed grain		22	81532	0.00202
EN95 HCl-washed grain		22	79860	0.00838
EN95 Deionised water		50	1.65	
EN95 HCl-wash solution		50	6.70	

Table 4. Total Se concentrations in international reference materials determined by AFS.

Reference material	Average Se $\mu\text{g/g}^*$	Number of Analyses	Standard Deviation	Reference Value $\mu\text{g/g}^*$
NRCCRM Stream sediment GBW07312	0.251	6	0.007	0.25
WRC Aquacheck Soil Distribution 89	515	2		540
WRC Aquacheck Soil Distribution 97	855	2		880
NRCCRM Tea GBW08505	0.037	5	0.006	0.041
WRC Aquacheck Water Distribution 89	6.26	6	0.15	6.3
WRC Aquacheck Water Distribution 93	2.57	6	0.14	2.1
WRC Aquacheck Water Distribution 101	4.26	7	0.21	4.2
WRC Aquacheck Water Distribution 105	4.46	5	0.19	4.1
BCR Hair 397	1.837	15	0.392	2.00

\* Concentrations in water =  $\mu\text{g/l}$



Table 5. AFS limits of detection for Se in various sample types

Sample Type	Se LOD ng/g
Soil	15
Soil Water Soluble Se	0.04
Grain	2
Water	0.2
Hair	10

Table 6. Summary of Se determinations in all sample types in the LK, HN and HT villages.

Region	Sample Type	Minimum Se µg/g	Maximum Se µg/g	Geometric Mean Se µg/g	Number
LK	Soil, Total	.034	.288	.099	25
	Soil, Water-soluble	.00003	.005	.000288	25
	Grain	.001	.018	.002	24
	Water (µg/l)	.100	.440	.168	5
	Hair	.170	.853	.304	25
HN	Soil, Total	1.494	59.4	7.06	25
	Soil, Water-soluble	.001	.254	.01	25
	Grain	.017	9.175	.198	25
	Water (µg/l)	.460	44	7.7	5
	Hair	.566	34.6	5.24	23
HT	Soil, Total	2.736	27.5	9.46	25
	Soil, Water-soluble	.005	.107	.022	25
	Grain	.182	5.6	1.38	25
	Water (µg/l)	7.3	275	32.6	5
	Hair	1.832	141	26.4	23

Table 7. Spearman Rank correlation coefficients for selected soil parameters in the LK villages.

	Water-sol. Se	LOI	pH	Fe
Total Se	0.139	0.611	-0.453	-0.101
Water-sol. Se		-0.265	0.126	-0.388
LOI			-0.355	0.412
pH				0.007

n = 25  $r_{95\%} = 0.337$  (Koch and Link, 1970) sol. = soluble

Table 8. Spearman Rank correlation coefficients for selected soil parameters in the HN and HT villages.

	F*	S*	Hg*	LOI	pH	Water-sol. Se	Cd	Cu	Fe	V	Zn
Total Se	0.586	0.580	0.923	0.612	-0.171	0.660	0.845	0.868	0.350	0.910	0.817
F*		0.082	0.508	0.442	-0.016	0.440	0.337	0.547	0.405	0.163	0.354
S*			0.631	0.691	-0.300	0.189	0.646	0.633	0.396	0.607	0.720
Hg*				0.570	-0.075	0.544	0.834	0.833	0.359	0.795	0.927
LOI					-0.235	0.222	0.520	0.708	0.575	0.532	0.517
pH						0.159	-0.022	-0.326	-0.509	-0.068	-0.157
Water-sol. Se							0.686	0.477	0.126	0.548	0.408
Cd								0.781	0.331	0.807	0.854
Cu									0.642	0.765	0.805
Fe										0.228	0.366
V											0.825
Number	18	18	18	50	50	50	50	50	50	50	50
r10 95 % = 0.549    r25 95% = 0.337 (Koch and Link, 1970)    sol. = Soluble    *IRMA Analysis											

Table 9.

## Factor analysis results for selected soil parameters in the HN and HT villages.

		Factor	Factor	Factor
		1	2	3
Variance % HN		50	23	9
Variance % HT		54	19	15
HN Villages	Total Se	0.927	-0.026	0.113
	LOI	0.312	-0.377	0.694
	pH	-0.088	0.860	0.224
	Water-soluble Se	0.842	0.046	0.081
	Ca	0.002	0.574	0.744
	Cd	0.956	0.001	-0.041
	Cu	0.753	-0.326	0.226
	Fe	0.038	-0.839	0.119
	Mg	0.120	0.756	-0.113
	V	0.922	0.045	0.087
	Zn	0.963	0.099	-0.224
HT Villages	Total Se	1.117	-0.012	-0.210
	LOI	0.271	0.072	0.726
	pH	-0.037	0.872	0.073
	Water-soluble Se	0.841	0.953	-0.336
	Ca	-0.257	0.807	0.182
	Cd	0.882	0.276	0.162
	Cu	0.789	-0.036	0.209
	Fe	0.011	-0.065	0.909
	Mg	-0.892	-0.022	1.439
	V	1.376	-0.020	-0.679
	Zn	1.025	-0.077	-0.135

In each case for HN and HT villages, number of variables = 11, number of factors = 3, number of cases = 25, degrees of freedom = 65, factor extraction method = principal components. Data were log transformed to reduce the influence of outlying values prior to statistical analysis. Variance % values  $\leq 10$  should be treated with caution.

Table 10. Percentage dominance of major anions in LK, HN and HT waters.

Village Code	Sample Number	HCO <sub>3</sub> %	SO <sub>4</sub> %	Cl %	NO <sub>3</sub> %
LK1	5	21.7	12.5	36.5	29.4
LK2	85	92.6	5.5	1.6	0.3
LK3	73	40.4	6.7	15.8	37.1
LK4	6	15.5	37.7	17.8	29.0
LK5	63	65.2	28.9	2.4	3.6
HN1	18	66.2	28.6	2.3	2.8
HN2	94	53.4	41.2	1.2	4.2
HN3	74	54.1	45.4	0.1	0.3
HN4	52	94.0	3.9	0.4	1.7
HN5	68	60.2	33.5	0.5	5.8
HT1	19	5.7	90.3	1.0	3.1
HT2	72	30.0	64.3	2.3	3.4
HT3	1	69.1	8.8	1.3	20.7
HT4	84	79.3	20.0	0.2	0.5
HT5	93	54.2	41.1	0.5	4.2

Table 11. Percentage of deficient, marginal and excessive Se concentrations in soil, grain and hair samples in the 15 study villages.

Village Code	% of deficient samples				% of marginal samples				% of excessive samples			
	TSe Soil	WSe Soil	TSe Grain	TSe Hair	TSe Soil	WSe Soil	TSe Grain	TSe Hair	TSe Soil	WSe Soil	TSe Grain	TSe Hair
LK1	60	100	100	20	20			60				
LK2	100	100	100	20				60				
LK3	60	100	80	20	40			80				
LK4	100	80	100	20		20		80				
LK5	20	100	100		60			100				
HN1									100	80	100	100
HN2									100	100	60	100
HN3		80				20	20		100			40
HN4		60	20			20	60		40			
HN5		20	20				20		80	20		20
HT1									100	60	80	100
HT2									100	80	80	100
HT3									100	60	80	100
HT4						20			100	40	100	67
HT5						40			100	20	20	100
From Tan (1989)		Soil TSe µg/g			Soil WSe µg/g			Grain TSe µg/g			Hair TSe µg/g	
Deficient		< 0.125			<0.003			< 0.025			< 0.2	
Marginal		0.125 - 0.175			0.003 - 0.006			0.025 - 0.04			0.2 - 0.5	
Mod - High		0.175 - 3			0.006 - 0.02			0.04 - 1			0.5 - 3	
Excessive		≥ 3			≥ 0.02			≥ 1			≥ 3	

TSe = Total Se      WSe = Water-soluble Se