DOI:10.1021/jf902074f

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Grain Zinc, Iron, and Copper Concentrations of Wheat Grown in Central Iran and Their Relationships with Soil and Climate Variables

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We performed a survey in central Iran to assess the variability in grain zinc (Zn), iron (Fe), and copper (Cu) concentrations of winter wheat and their relationships with soil and climate variables under field conditions. The goal was to identify factors that should be studied further to improve wheat cultivation in the study area with respect to the nutritional quality of this main Iranian staple crop. Soil and grain samples were collected from 137 randomly selected wheat fields in the provinces of Qom, Isfahan, and Fars. In general, soils were characterized by a high pH. Grain micronutrient concentrations ranged from 11.7 to 64.0 mg kg^{-1} (mean, 31.6 mg kg^{-1}) for Zn, from 21.1 to 96.6 mg kg^{-1} (mean, 42.7 mg kg^{-1}) for Fe, and from 2.4 to 9.3 mg kg^{-1} (mean, 5.5 mg kg^{-1}) for Cu. The grain concentrations of these three metals were positively correlated to each other. DTPA-extractable and total soil micronutrient concentrations alone were very poor predictors of grain micronutrient concentrations. Predictions were slightly improved when other soil and climate variables were taken into account (Zn, $R^2 = 0.26$; Fe, $R^2 = 0.08$; and Cu, $R^2 = 0.13$).

KEYWORDS: Wheat; micronutrients; soil properties; climate variables; regression analysis

INTRODUCTION

Zinc (Zn), iron (Fe), and copper (Cu) are essential micronutrients for plants as well as for humans. There are estimates that some 3 billion people worldwide are afflicted by Fe deficiency, and up to half of the population in developing countries are at risk of Zn deficiency (*1*, *2*). Zinc and Fe deficiencies are particularly known to be a common problem in populations that depend on cereals as the main staple food and with little or no access to animal products (*3*,*4*). Wheat is the most important staple food in many developing countries (*1*, *5*). Thus, the Zn and Fe concentrations of wheat grains are important nutritional factors in these countries.

Soil is the primary source of micronutrients for plants (*6*). Therefore, the transfer of micronutrients from soil to crop plants is important for both plant and human nutrition (*7*, *8*). The phytoavailability of soil micronutrients depends on soil properties such as total micronutrient concentrations, pH, calcium carbonate ($CaCO_3$) content, organic matter (OM) content, soil moisture conditions, and available phosphorus (*9*−*12*). Empirical relationships between soil micronutrient concentrations and plant uptake were found to differ widely among different regions in the world (*10*, *13*−*15*). Nan et al. (*15*) used a third-order polynomial to describe the relationship between the total soil Zn and the Zn concentration of spring wheat grains (with correlation coefficient, $r = 0.48$) grown on calcareous soils in northern China. Krauss et al. (*14*) applied a Freundlich type function to model Zn ($r = 0.69$) and Cu concentration ($r = 0.44$) of wheat grains from the ethylenediaminetetraacetic acid (EDTA)-extractable soil Zn and Cu concentrations of soils sampled in the northern and central parts of Slovakia.

Climate variables such as temperature, rainfall, and evaporation demand have also been reported to affect plant uptake of micronutrients (*16*). It has been indicated that micronutrient levels in wheat grains were controlled to a large extent by environmental factors and interactions between the genotypes and the environment (*5*, *17*). However, there is little information on micronutrient accumulation by wheat grains on calcareous soils in arid or semiarid climates under field conditions.

In this study, we performed a survey in three major wheat-growing provinces of Central Iran to investigate the variability of Zn, Fe, and Cu concentrations in wheat grains and to study their relationships with soil and climatic variables. The three provinces are Qom, Fars, and Isfahan. Together, they have a production area for wheat (*Triticum aestivum*) of more than 612000 ha. This region is characterized by a semiarid to arid climate and calcareous soils. Cereals, in particular wheat, are the main staple food of the population. Zn and Fe deficiencies in humans have been reported for some parts of this area (*3*). Although there are reports indicating that Zn is a limiting factor for crop production on some Iranian soils (*18*, *19*), micronutrient accumulation in wheat grains has never before been surveyed. Our objective was

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 Published on Web 11/02/2009

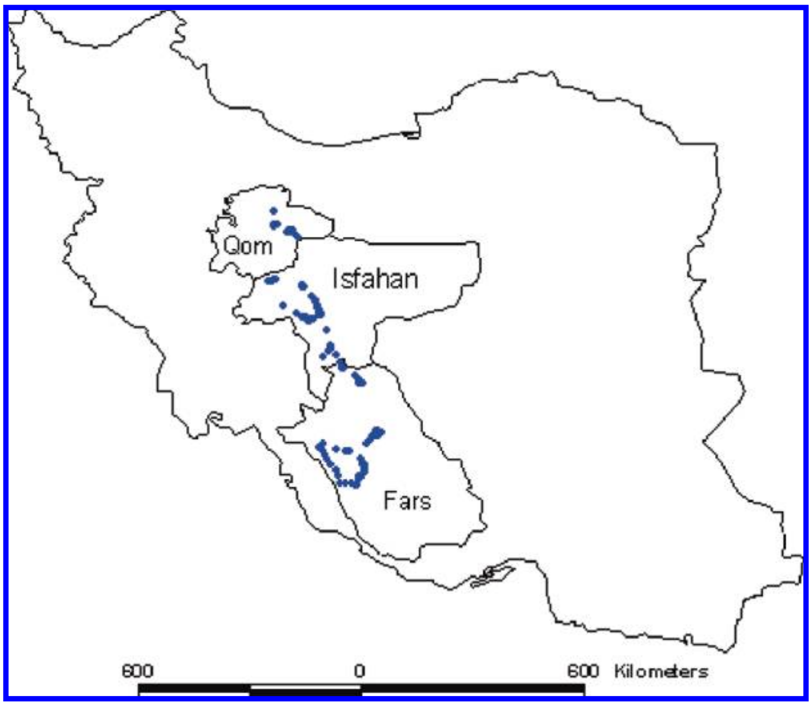

Figure 1. Location of the sampling sites.

therefore to study the relationship between the Zn, Fe, and Cu concentrations in grains of current wheat cultivars on the one hand and the soil micronutrients concentrations, further soil properties [such as pH, electrical conductivity (EC), available phosphorus (P), clay, sand, and $CaCO_3$], and climatic variables (annual mean rainfall, temperature, and potential evaporation) on the other. The goal was to obtain clues on factors that should be addressed in further in-depth studies to improve the management of wheat in the study area with respect to the nutritional quality of this main staple crop in Iran.

MATERIALS AND METHODS

Study Area. Winter wheat grain and soil samples were collected from 137 randomly selected fields in the three provinces: Qom, Fars, and Isfahan (from 28°51′ to 35°6′ N, latitude, and from 50°21′ to 53°4′ E, longitude) (**Figure 1**). Most of the sampled soils were classified as Aridisols, Inceptisols, and Entisols according to the U.S. soil taxonomy (1998) using land capability maps, obtained from the Soil and Water Institute of Iran. Quaternary deposits, conglomerates, marl, and limestones are the predominant parent materials of the soils in this region according to maps of the Geological Survey of Iran obtained from the National Cartographic Center of Iran. Rainfall averages are 125 mm/year in the area sampled in Qom, 338 mm/year in Fars, and 172 mm/year in Isfahan. Up to 80% of the total annual rainfall occurs in autumn and winter. Therefore, irrigation is a common practice in most parts of the study area.

Sampling and Analysis. Soil and grain samples were collected during harvest time in May and June, 2007. From each of the 137 fields, five soil samples were taken at different locations from 0 to 20 cm depth and mixed to make a composite sample. Similarly, wheat grain samples were taken from five plants growing at the locations from which the soil samples were taken and also combined to one composite sample per field. The coordinates of each sampling point were recorded using a portable global positioning system receiver. The composite soil samples were air-dried and passed through a 2 mm sieve. The grain samples were stored in brown paper bags and oven-dried at 75 °C. The soil samples were analyzed for pH in a water-saturated paste using a glass electrode. Soil EC was determined in saturation extracts. Oxidable organic carbon (OC) was measured using the method of Walkey and Black (*20*). Extraction by DTPA (diethylenetriamine-penta-acetate) according to Lindsay and Norvell (*21*) in combination with atomic absorption spectrophotometry (AAS) was used to characterize phytoavailable Zn, Fe, and Cu concentrations. The hydrometer method (*22*) was used to determine particle size distributions. Available P was analyzed by the Olsen method (*23*), $CaCO_3$ content was analyzed by dissolution with HCl, and back-titration with NaOH (*24*) and total nitrogen (N) by Kjeldhal digestion (*25*).

To determine the total concentrations of elements in soil and grain samples, the dried samples were milled; 4 g of each milled sample was well-mixed with 0.9 g of Licowax C Micropowder PM (Clariant, Switzerland), shaken for 8 min (17 times per second), and then pressed into a 32 mm pellet applying a pressure of 15 bar using a Specac (PT. NO. 3350) machine. The tablets were then analyzed using an energy dispersive X-ray fluorescence spectrometry (XRF), using a SPECTRO X-LAB 2000 instrument (SPECTRO Analytical Instroments, Germany), as described by Dittmar et al. (*26*) and Omote et al. (*27*). The accuracy of XRF analysis was controlled by analyzing certified reference samples. **Table 1** shows that all of the measurements were within the certified confidence limits of the reference samples.

Climate data were derived from rainfall, temperature, and potential evaporation contour line maps (1:250000). The maps were obtained from the Public Weather Service of the Iranian Meteorological Organization. The value of the line closest to the respective sampling point was ascribed to that sample using the ArcGIS 9.2 (*28*) software.

Statistical Analysis. Correlations between variables were characterized by Spearman rank correlation coefficients. Some soil, plant, and climate variables showed positively skewed frequency distributions (available P, DTPA-Zn, DTPA-Fe, and grain Fe, **Table 2**), and these variables were therefore transformed to logarithms for the regression analysis. To identify the factors that influence grain micronutrient concentrations, stepwise linear multiple regression was used. All statistical analyses were performed by means of the software SPSS 11.5 (*29*) and Excel 2007 for Windows.

RESULTS AND DISCUSSION

Descriptive Statistics. All sampled soils were characterized by pH values larger than 7 and a high $CaCO_3$ content (more than 360 g kg^{-1} in average), a medium to high salinity (EC ranged from 0.3 to 10.3 dS m^{-1}), and a medium to high clay content (ranging from 160 to 560 g kg^{-1}) (**Table 2**). The soil OC content was generally low (< 1%), ranging from 0.1 to 15.6 g kg^{-1}, which is typical for soils in semiarid regions (**Table 2**). Climatic annual

Table 1. Results of Chemical Analysis of the Reference Samples BCR No. 62 [Olive (*Olea europaea*) Leaves], CTA-VTL-2 (Virginia Tobacco Leaves), 950 (Sandy Soil from Tanzania), and 988 (Sandy Soil from the Netherlands)[a]

		certified value (mg kg^{-1})		measured value (mg kg^{-1})	
reference materials	element	mean	confidence interval	mean	standard deviation
		plant materials			
BCR No. 62[b]	Zn	16.0	±0.7	15.8	±0.3
	Cu	46.6	±1.8	45.7	±0.6
	Mn	57.0	±2.4	59.0	±0.5
CTA-VTL-2[c]	Zn	43.3	±2.1	43.6	±0.6
	Fe	1083	±33	1105	±16
	Cu	18.2	±0.9	19.0	±0.5
	Ca (g kg^{-1})	36.0	±1.5	36.9	±0.5
	P	2204	±78	2148	±32
		soil materials			
950[d]	Zn	62.2	±3.7	63.0	±2.5
	Fe (g kg^{-1})	38.3	±0.7	38.1	±0.7
	Cu	28.1	±2.7	27.5	±0.9
988[d]	Zn	13.0	±3	12.7	±0.6
	Fe (g kg^{-1})	6.32	±0.58	7.0	±0.2
	Cu	10.1	±1.8	10.5	±1.3

[a] Where other units are not stated, the values are given in mg kg^{-1}. Confidence intervals are uncertainty for 95% of the mean for plant standards and median of deviations for soil standards. [b] Certified by BCR (Community Bureau of Reference, the former reference materials program of the European Commission), the certificate has been revised under the responsibility of IRMM (Institute for Reference Materials and Measurements), Belgium, 2007. [c] Certified by the Institute of Nuclear Chemistry and Technology and Commission of Inorganic Trace Analysis of the Committee for Analytical Chemistry of the Polish Academy of Sciences, Poland, 1997. [d] Certified by the Wageningen Evaluating Programs for Analytical Laboratories, The Netherlands, 2001.

rainfall sums varied between 100 and 800 mm, the annual mean temperature was between 8 and 20 °C, and the annual cumulative potential evaporation was between 1500 and 3500 mm (**Table 2**).

The total soil micronutrient concentrations averages were all in ranges that are considered normal for soil background concentrations (*30*). DTPA-extractable soil metal concentrations were generally low, as is to be expected for calcareous soils. According to Lindsay and Norvell (*21*), the concentrations of DTPA-extractable Zn of 0.8 mg kg^{-1}, DTPA-extractable Fe of 4.5 mg kg^{-1}, and DTPA-extractable Cu of 0.2 mg kg^{-1} are considered as critical levels for normal crop growth. The DTPA-extractable concentrations were below the critical levels on 16% (Zn) and 19% (Fe) of the surveyed fields, respectively. DTPA-Cu exceeded the critical concentration in all instances. Our measurements can also be compared to the critical DTPA-extractable concentrations proposed by Agrawal (*31*) for winter wheat: 26% of our samples had DTPA-Fe < 5.0 mg kg^{-1}, and but only 3% of samples had DTPA-Cu < 0.78 mg kg^{-1}.

Similar to the total soil micronutrient concentrations, the ranges of the sampled grain micronutrient concentrations also compared well with the respective values reported from other countries (*30*). Nahapetian and Bassiri (*32*) studied mineral and phytate concentrations of different wheat varieties during 2 years in the province of Fars. The mean concentrations reported for seven varieties (Deihim, Derakhshan, Jolgeh, Koohrang, Navid, Ommid, and Roshan) ranged from 17 to 70 (Zn) and from 29 to 83 mg kg^{-1} (Fe), respectively.

Only 20% of our grain samples had a Zn concentration below 24 mg kg^{-1} dry matter. This critical value was determined for rain-fed wheat grown on alkaline soils in Pakistan as the minimum Zn concentration of grain required to produce 95% of the maximum grain yield (*33*). We did not find any critical values of Fe and Cu concentrations in wheat grains in the literature with which to compare our results.

Table 2. Descriptive Statistics of Variables Determined in This Study

parameters	unit	mean	median	min−max	standard deviation	skewness
pH		8.10	8.14	7.07−8.88	0.38	−0.54
EC	dS/m	3.83	2.34	0.31−10.29	3.31	0.86
available P	mg/kg	42.10	37.59	10.38−165.10	22.54	2.40
total N	g/kg	0.66	0.71	0.10−2.00	0.38	0.29
clay	g/kg	384	366	160−560	90	0.03
sand	g/kg	227	166	30−660	179	0.72
$CaCO_3$	g/kg	365	367	117−693	126	0.11
OC	g/kg	5.80	5.84	0.10−15.60	3.22	0.28
total Zn	mg/kg	75.2	73.0	20.7−148.8	19.9	0.46
total Fe	g/kg	32.0	31.9	17.0−56.0	5.8	0.48
total Cu	mg/kg	27.5	28.1	1.7−42.5	5.6	−0.69
DTPA-Zn	mg/kg	2.33	1.49	0.30−20.50	2.92	3.00
DTPA-Fe	mg/kg	8.80	7.18	2.40−46.00	6.44	3.10
DTPA-Cu	mg/kg	1.90	1.67	0.20−5.70	0.99	1.00
rain[a]	mm/year	243	200	100−800	128	1.00
temp[a]	°C	14.9	14.0	8.0−20.0	2.37	0.10
evap[a]	mm/year	2254	2000	1500−3500	437	1.00
grain Zn	mg/kg	31.6	30.7	11.7−64.0	9.48	0.31
grain Fe	mg/kg	42.7	38.9	21.1−96.6	14.76	1.90
grain Cu	mg/kg	5.5	5.6	2.4−9.3	1.18	0.07

[a] Rain is the climatic mean annual rainfall sum, temp is the climatic mean annual temperature, and evap is the climatic mean annual cumulative potential evaporation.

According to Cakmak (*2*), whole-grain Zn concentrations of 20−35 mg kg^{-1} (the average grain Zn concentration reported from various countries) are too low for humans that depend on cereal-based diets to meet their daily Zn demand of about 15 mg of Zn (*34*). Assuming a daily consumption of 400 g of whole-grain wheat, the grain Zn concentration should be increased by at least 10 mg kg^{-1} to satisfy this demand in such populations.

Wheat is the most important staple food in Iran. The average annual consumption ranges between 135 and 150 kg per person (*35*). Between 10 and 30% of the grain mass is lost during food processing (milling, baking, etc.). Thus, the effective annual wheat consumption is in the range of 94.5−135 kg per person or approximately 0.26−0.37 kg per day. Using the results of our survey, we estimated the average daily dietary intake (DI) of Zn, Fe, and Cu by consumption of wheat in the study area and compared the results with dietary reference intake (DRI) values recommended by the WHO (*36*). The DI (mg day^{-1} person^{-1}) was computed as DI = DIW × *C*, where DIW is the daily intake of wheat (kg day^{-1} person^{-1}) and *C* is the metal concentration (mg kg^{-1}) of the grains.

With a wheat grain Zn concentration of 31.6 mg kg^{-1} found in our survey, the daily Zn intake averages 8.2−11.7 mg per person. The Zn DRI is 11 mg day^{-1} person^{-1} for adult males and 8 mg day^{-1} person^{-1} for adult females. Thus, under the assumption that the rates of wheat intake are similar in both genders, wheat-based food could on average supply 75−100% of the daily Zn requirement in males and more than 100% in females. For Fe, the DRI is 8 mg day^{-1} person^{-1} for males and 18 mg day^{-1} person^{-1} for females, and thus, under the analogous assumptions as for Zn, wheat consumption would on average provide more than 100% of the required Fe in adult males and 61−86% in adult females. Similarly, with a DI of 1.4−2 mg day^{-1} person^{-1} Cu, it would also supply more than the 0.9 mg of Cu intake daily required by adult persons of both genders (*36*).

Table 3. Spearman Coefficients of Correlation (n = 137)

	pH	EC	available P	total N	clay	sand	$CaCO_3$	OC	total Zn	total Fe	total Cu	DTPA-Zn	DTPA-Fe	DTPA-Cu	rain	temp	evap	grain Zn	grain Fe	grain Cu
pH	1																			
EC	−0.14	1																		
available P	−0.07	0.21[b]	1																	
total N	−0.28[a]	−0.16	0.31[a]	1																
clay	−0.20[b]	0.1	0.16	0.52[a]	1															
sand	0.24[a]	0.15	−0.01	−0.61[a]	−0.70[a]	1														
$CaCO_3$	0.05	−0.13	−0.14	−0.20[b]	−0.23[a]	0.14	1													
OC	−0.19[b]	−0.22[a]	0.19[b]	0.78[a]	0.42[a]	−0.53[a]	−0.16	1												
total Zn	0.03	0.28[a]	0.29[a]	0.24[a]	0.22[a]	0.03	−0.59[a]	0.14	1											
total Fe	0.03	0.22[b]	0.11	−0.08	0.01	0.21[b]	−0.67[a]	−0.1	0.73[a]	1										
total Cu	−0.01	0.11	0.22[b]	0.31[a]	0.41[a]	−0.21[b]	−0.61[a]	0.23[a]	0.73[a]	0.66[a]	1									
DTPA-Zn	−0.06	0.14	0.33[a]	0.21[b]	−0.04	0.14	−0.08	0.14	0.51[a]	0.16	0.25[a]	1								
DTPA-Fe	−0.17[b]	−0.51[a]	0.05	0.59[a]	0.23[a]	−0.44[a]	0.09	0.52[a]	−0.10	−0.31[a]	0.04	0.13	1							
DTPA-Cu	−0.13	−0.39[a]	0.06	0.62[a]	0.47[a]	−0.58[a]	−0.22[b]	0.60[a]	0.11	−0.13	0.35[a]	0.11	0.71[a]	1						
rain	−0.20[b]	−0.64[a]	−0.27[a]	0.32[a]	0.04	−0.37[a]	0.29[a]	0.32[a]	−0.49[a]	−0.45[a]	−0.29[a]	−0.26[a]	0.56[a]	0.42[a]	1					
temp	−0.25[a]	−0.25[a]	−0.07	0.18[b]	0.01	−0.32[a]	−0.15	0.17[b]	−0.14	−0.06	0.04	−0.12	0.32[a]	0.40[a]	0.21[b]	1				
evap	−0.08	−0.15	−0.04	0.09	−0.14	−0.11	−0.16	0.07	0.06	0.14	−0.01	0.01	0.21[b]	0.23[a]	0.08	0.63[a]	1			
grain Zn	−0.13	0.02	−0.15	0.09	−0.04	−0.08	−0.08	−0.01	0.17[b]	0.02	−0.02	0.25[a]	−0.02	0.06	−0.11	0.31[a]	0.26[a]	1		
grain Fe	−0.13	0.05	−0.04	0.02	0.00	−0.14	−0.07	−0.09	0.19[b]	0.14	0.09	0.12	0.02	0.07	−0.08	0.15	0.20[b]	0.41[a]	1	
grain Cu	−0.15	0.14	−0.11	−0.02	0.01	−0.12	−0.01	−0.12	0.06	0.01	−0.04	−0.04	−0.12	−0.01	−0.11	0.23[a]	0.22[b]	0.64[a]	0.43[a]	1

[a] Correlation is significant at the 0.01 level. [b] Correlation is significant at the 0.05 level.

The results indicate that wheat is an important source of micronutrients for the human population of the study region. However, the actual supply of micronutrients to humans by wheat-based products will be much less than the above figures suggest, because the mineral-rich outer parts of wheat grains are usually removed by milling. Furthermore, the absorption of micronutrients by the human body depends on other food components. In particular, high contents of phytate reduce the absorption of Zn and Fe strongly (*7*). Considering these reservations, the above calculations are rather optimistic. Given that they relate to average conditions, they confirm the concerns of Iranian health authorities that substantial fractions of the population who do not have access to alternative dietary sources of these micronutrients are at risk of Zn and Fe deficiency.

Correlation between Soil Micronutrient Concentrations, Other Soil Properties, and Climate Variables. As **Table 3** shows, total soil Zn was positively correlated with EC, available P, total N, and clay content. Total Fe was positively correlated with EC and sand content. The total Cu concentration was positively correlated with available P, total N, clay, and OC. All three metals positively correlated with each other and negatively with the $CaCO_3$ content. The total Cu was negatively correlated with the sand content.

The positive correlation between total soil Zn and available P points to P fertilizers as an important source of Zn in these calcareous agricultural soils. Afyuni et al. (*37*) reported that superphosphate (simple and triple) fertilizers applied to Iranian soils contain high levels of Zn impurities (100−1100 mg kg^{-1}). The negative correlation between $CaCO_3$ and total Fe suggests that soils high in carbonate were low in Fe oxides and vice versa. Iron oxides are important for binding Zn, Cu, and P in soils (*30*). Positive correlations of total soil Zn, Fe, and Cu with the soil clay content have also been reported by Chahal et al. (*38*) and Katyal and Sharma (*39*).

The close correlation between the total soil contents of Zn, Fe, and Cu suggests that these micronutrients, to a substantial extent, come from the same sources, such as the parent material. Significant positive correlations among total soil micronutrients also have been reported in previous studies by Katyal and Sharma (*39*) and by Lombnæs and Singh (*13*). Katyal and Sharma (*39*) found a positive association among the micronutrients (Zn, Fe, Cu, and Mn) and concluded that similar factors governed their distribution in the investigated soils.

Significant negative correlations were found between total concentrations of Zn, Fe, and Cu and annual rainfall, for the entire data set (**Table 3**) as well as for the nonirrigated fields alone (−0.38*, −0.27, and −0.20 for Zn, Fe, and Cu, respectively). This means that the three metals are on average more abundant in soils of the drier parts of the study area independent of irrigation.

DTPA-extractable soil metal concentrations were significantly correlated with the respective total concentrations and with some other soil variables (**Table 3**). Positive correlation of DTPA-Zn was found with total N, available P, and OC content. DTPA-extractable Fe and Cu were negatively correlated with EC and sand content and positively with total N, clay, and OC content and with each other. DTPA-Cu correlated negatively with $CaCO_3$. Wei et al. (*40*) found that DTPA-Zn concentrations correlated positively with OM and were higher in P-fertilized than in unfertilized fields on calcareous soils of the Loess Plateau in China. They also reported a negative correlation of DTPA-Cu with OM, available P, and $CaCO_3$.

While total concentrations correlated among all three metals, the DTPA-extractable concentrations only correlated among Fe and Cu. This suggests that the availability of Fe and Cu was governed by similar factors, whereas those governing the availability of Zn were rather different. Positive correlations of DTPA-extractable Fe and Cu with OC as well as a negative correlation between DTPA-extractable Fe and pH were also reported by Katyal and Sharma (*39*). Cancela et al. (*41*) reported significant positive correlation of the OM content with DTPA-Fe and DTPA-Zn and concluded that the DTPA-extractable fractions of these metals were largely held by OM. Similarly, Wei et al. (*40*) reported positive correlations of DTPA-Fe with OM and available P.

DTPA-Zn was negatively correlated with rainfall. In contrast, DTPA-Fe and DTPA-Cu were positively correlated with rainfall, temperature, and evaporation (**Table 3**), indicating that leaching or accumulation by capillary rise of evaporating soil−water was not an important process for the variation in the availability of these two elements.

Table 4. Multiple Linear Regression Models for Grain Concentrations of Micronutrients

multiple regression models	model R^2	significance level
Zn		
eq 1: grain Zn = 31.752 + 1.533 (temp) + 7.444 log (DTPA-Zn) − 13.708 log (available P) − 1.25×10^{-2} (rain)	$R^2 = 0.26$	$P < 0.0001$
eq 2: log (grain Zn) = 1.437 + 2.473×10^{-2} (temp) + 0.120 log (DTPA-Zn) − 0.193 log (available P) − 2.02×10^{-4} (rain)	$R^2 = 0.29$	$P < 0.0001$
Fe		
eq 3: log (grain Fe) = 1.504 + 3.816×10^{-2} (total Fe) + 0.896 (total N)	$R^2 = 0.08$	$P < 0.05$
Cu		
eq 4: grain Cu = 2.75 + 0.164 (temp) + 0.081 (EC)	$R^2 = 0.13$	$P < 0.001$

Relationships of Grain Micronutrients and Soil and Climate Variables. The micronutrient concentrations of the grains were not significantly correlated with any of the measured soil variables, including the DTPA-extractable micronutrient concentrations, with the only exception that grain Zn showed weak correlations to the DTPA-extractable and total soil Zn concentrations (**Table 3**). Similar to our results, Oury et al. (*17*) reported that for six different genotypes of bread wheat that were grown at four different locations in France, the grain Zn concentration was higher at sites with high quantities of available Zn in soil but found no correlation between soil available Fe and grain Fe concentrations. Using log-transformed data from the northern and central parts of Slovakia, Krauss et al. (*14*) found a close relationship between EDTA-Zn and wheat grain Zn ($r = 0.69$) and a weaker relationship between EDTA-Cu and grain Cu ($r = 0.44$).

Thus, in agreement with previous studies (*13*, *42*), we concluded that DTPA-extractable and total soil micronutrients concentrations are not well-suited as predictors for the accumulation of Fe and Cu by wheat grains, whereas Zn accumulation by wheat grains can be predicted coarsely from the concentration of available Zn in soil.

We observed positive correlations of grain Zn, Fe, and Cu with temperature and potential evaporation (for Fe only significant with potential evaporation, **Table 3**). The effects of temperature and potential evaporation can be attributed to an increased transpirational water flow, which in turn results in an increased rate of passive solute transport through the soil−plant system. Kabata-Pendias and Pendias (*30*) confirmed that in general, a higher ambient temperature results in a larger uptake of trace elements by plants. They mentioned that the influence of climatic conditions on the rate of trace elements uptake may be partly an indirect impact due to the water flow phenomenon.

Multiple Regression Models Predicting Grain Micronutrients Concentrations. Grain micronutrients concentrations were related to soil and climate variables by multiple linear regression models. In more detail, we used total and DTPA-extractable soil micronutrient concentrations, other soil properties, and climate information as explanatory variables. The best-fit regression models, found in a stepwise procedure, are listed in **Table 4**. Regression eq 1 relates the grain Zn concentration to the concentrations of DTPA-extractable Zn and available P and temperature as well as rainfall and explains 26% of the observed variation in grain Zn concentration. The coefficient of determination slightly increased (to 29%) when the log-transformed grain Zn concentration was used as a response variable (eq 2). The antagonistic effect of available soil P (or P fertilization) on grain Zn accumulation agrees with results on Zn uptake by crops reported in other studies, as, for example, by Rashid and Ryan (*11*) and Ryan et al. (*43*).

Total soil Fe and N contents explained only 8% of the variance in grain Fe concentrations (eq 3, **Table 4**). The positive effect of N (which is added in form of urea to the agricultural soils of the study area annually), might be related to competition between NH_4^+ and other cations for exchange sites, resulting in the desorption of cationic (micro-) nutrient ions such as Fe as proposed by Mitchell et al. (*44*). Mitchell et al. (*44*) reported that durum grain Fe concentration of wheat increased with increasing application rate of N fertilizer.

Equation 4 shows that the grain Cu concentration was independent of the Cu status of the sampled soils. Furthermore, temperature and EC together explained only 13% of the variance. Krauss et al. (*14*) suggested that the reason for the poor predictions of Cu in comparison to plant Zn and Cd contents was internal regulation processes tending to keep the Cu concentration in the plant at a constant level, so that it depended only weakly on the metal concentration of the soil.

The rather poor coefficients of determination of the regression models indicate that factors other than those considered influence micronutrients accumulation by wheat grains. Such factors could include, for example, variation in plant physiological characteristics and genotypes, poorly understood micronutrients transfer within the plant from root to shoot and from shoot to grains, and field management factors (such as crop rotation and tillage systems, fertilization and plant residual management, or manure application). Unfortunately, such additional information was not available in this study. Various studies suggest that in particular the genotype is a very important factor in this respect (*5*, *17*, *32*). Furthermore, yield may have to be considered when predicting grain micronutrient accumulations from environmental variables. Negative correlations between grain yield and grain micronutrient concentrations indicate that increased grain biomass may result in decreased grain mineral accumulation due to a "dilution effect" (*5*, *17*, *45*, *46*).

There are some studies reporting significant relationships between wheat grain and soil-extractable metals [such as logarithmic relationship by Krauss et al. (*14*)] or between wheat grain and soil variables [such as multiple linear regression by François et al. (*47*)]. In contrast, Nan et al. (*42*) reported that for wheat grain Zn and Cu, no meaningful multiple regression models were found for any combination of the explanatory soil variables (pH, OM, available P, and total metal concentrations of soil). They suggested that under field conditions the uptake and allocation of grain Zn and Cu were largely affected by crop physiological characteristics.

Correlations among Grain Element Concentrations. The uptake and the translocation of micronutrients in the plants can also be affected by interactions among these elements (*15*). The grain concentrations of Zn, Fe, and Cu were mutually positively

Table 5. Spearman Correlation Coefficients among Wheat Grain Element Concentrations ($n = 137$)

	Zn	Fe	Cu	Mn	P	Ca
Zn	1.00					
Fe	0.41[a]	1.00				
Cu	0.62[a]	0.43[a]	1.00			
Mn	0.26[a]	0.33[a]	0.47[a]	1.00		
P	0.29[a]	0.01	0.19[b]	0.19[b]	1.00	
Ca	0.35[a]	0.59[a]	0.39[a]	0.31[a]	0.21[b]	1.00

[a] Correlation is significant at the 0.01 level. [b] Correlation is significant at the 0.05 level.

correlated (**Table 5**). These three micronutrients were also positively correlated with Mn and Ca. Furthermore, the grain Zn and Cu concentrations showed positive correlations to the grain P concentration. It seems that Zn, Fe, and Cu accumulation in the grains was controlled by common factors. There are many studies that have reported positive correlations between grain Zn and Fe concentrations for various wheat cultivars (*1*, *5*). Morgounov et al. (*5*) reported strong linear dependence of grain Fe on grain Zn concentration for winter ($R^2 = 0.63$, $P < 0.001$) and spring ($R^2 = 0.63$, $P < 0.001$) wheat cultivars, but the relationship was not so strong for the pooled spring and winter wheat data ($R^2 = 0.19$, $P < 0.001$). Oury et al. (*17*) reported a weak ($r = 0.30$, $p < 0.001$) correlation between grain Fe and Zn concentrations but a relatively strong correlation between grain Zn and Mg ($r = 0.67$, $p < 0.001$) in wheat. They suggested that these correlations may be due to some physiological coupling of the accumulation of these elements in wheat grains.

The grain P concentration ranged from 698 to 2866 mg kg^{-1} (mean, 1952 mg kg^{-1}) in this study. Approximately 75% of total P content in cereal grains is stored as phytic acid, particularly in the germ and aleuron layers (*48*, *49*). Phytic acid is a strong chelator of mineral cations such as Ca, Fe, and Zn (*49*). Therefore, the positive correlation between Zn and Cu with P may be the result of their association with phytate in cereal grains.

Conclusions. Our results indicate that the Zn concentration of the sampled wheat grains was mostly sufficient with respect to plant nutrition. Grain Fe and Cu concentrations did not correlate to the total or DTPA-extractable soil Fe and Cu concentrations. Only grain Zn was significantly correlated to the soil Zn concentration. Multiple regression models showed weak dependences of grain micronutrient concentrations on soil and climatic variables with low although significant R^2 values. This means that other factors than those considered here also had a strong influence on the uptake of Zn, Fe, and Cu by wheat on these soils.

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Received for review June 17, 2009. Revised manuscript received October 15, 2009. Accepted October 16, 2009. We thank the North South Centre of ETH Zurich for financial support, which allowed a stay of the main author at ETH as part of the project “Zn Fluxes from Soil into the Food Chain in Arid Agroecosystems”.