Concentrations of 55 Major and Trace Elements in Danish Agricultural Crops Measured by Inductively Coupled Plasma Mass Spectrometry. 2. Pea (*Pisum sativum* Ping Pong)

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The multielement (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Re, Rh, Sb, Sc, Se, Si, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr) levels in peas (*Pisum sativum* Ping Pong) are presented in this paper. The peas were collected from 10 background areas, on Funen and mid-Jutland, Denmark, with intensive cultivation with use of fertilizer and pesticides. High-resolution inductively coupled plasma mass spectrometry (HR-ICPMS) was used for the analyses of the samples. The contents of the elements are generally at the same levels or even lower than those reported from other surveys. The results provide useful biological, nutritional, and background level information on peas.

Keywords: *Pea (Pisum sativum Ping Pong); HR-ICPMS; major elements; trace elements; multi-elements analysis*

INTRODUCTION

A few extensive studies on the concentrations of elements in peas (*Pisum sativum*) have been reported from different countries: e.g., Fuchs et al. (1976) for Zn, Pb, and Cd, from Sweden; Hansen and Andersen (1982) and Levnedsmiddelstyrelsen (1995) [Danish National Food Agency] for Cu, Ni, Zn, Se, Cd, and Pb, from Denmark; and Varo et al. (1980) for K, Ca, Mg, P, S, Fe, Cu, Mn, Mo, Co, Ni, Cr, Se, Rb, Al, B, Br, Hg, As, Cd, and Pb, from Finland. In these, all peas are collected at the retail marked level (market basket studies). In other words, no special care was taken in the selection of sampling sites, in the process of sampling, in packaging, and in shipment to avoid contamination from external sources. Before analysis, they were prepared as normal household practice (scrubbed, peeled, washed, etc.). The analytical results from these studies, therefore, do not reliably represent the background levels (free from external contamination) of these elements.

The Danish Food Technology and Development Program (FØTEK) financed this study to carry out a comprehensive survey of the background levels of as many elements in selected Danish agricultural crops as possible within the analytical techniques, time frame, and resources available. These crops provide a major portion of the elements in human diets. An attempt was made to include different types of vegetables (leafy, root, legume) and various grains as this study continues. A previous study (Bibak et al., 1998) reported on the background concentrations of 63 major and trace elements from a large sampling and carefully controlled analytical program for onions. The results for peas that represent legume plants are reported here.

Table 1. Quality Control Measurement of NIST 1567a Wheat Flour: Mean Values and 95% Confidence Intervals Reported in μg/g, Dry Weight

element	certfd	measd
Ca	191 ± 4	150 ± 2
P	1340 ± 60	1660 ± 7
Al	5.7 ± 1.3	4.0 ± 0.5
Cd	0.026 ± 0.002	0.031 ± 0.01
Cu	2.1 ± 0.2	1.9 ± 0.2
Fe	14.1 ± 0.5	12.7 ± 0.6
Mn	9.4 ± 0.9	8.1 ± 0.2
Mo	0.48 ± 0.03	0.44 ± 0.05
Se	1.1 ± 0.2	1.7 ± 0.4
Zn	11.6 ± 0.4	15.5 ± 2.0
Co[a]	0.006	0.017 ± 0.002
Pb[a]	<0.020	0.019 ±.005
U[a]	0.0003	0.0009 ± 0.0001
V[a]	0.011	0.012 ± 0.002

[a] Noncertified values.

The 55 elements measured in this study are those for which a routine HR-ICPMS method could be applied. The number of elements possible to measure depends on the composition of the sample matrix and on the concentration of the individual elements and may therefore vary from matrix to matrix. These data will be used to relate the trace element levels to the agricultural production method and to gain information on the levels of many trace elements in crops in order to evaluate their role in the food chain.

MATERIALS AND METHODS

Site Selection. Pea samples were collected from 10 production areas on Funen and mid-Jutland in Denmark. The criterion for site selection was to use as wide a variation in soil properties as possible with use of intensive cultivation,

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Table 2. Elements in Peas (μg/kg, Fresh Weight)

element	n	mean	median	min	max	BEC[c]	DL[a]
Ag	84	0.59	0.45	0.116	2.8	0.097	0.00071
Al	85	89	72	32	270	7.7	0.097
Au	84	0.71	0.66	0.119	3.0	0.21	0.002
B	93	12200	12200	1120	26000	460	0.22
Ba	94	200	164	30	690	0.44	0.036
Be	86	0.139	0.099	b > c[b]	1.07	0.028	0.00032
Bi	91	0.089	0.061	0.0095	0.37	0.0124	0.00017
Ca	92	230000	210000	89000	480000	1600[d]	0.65
Cd	95	12.1	11.3	5.0	30	0.75	0.0047
Ce	77	b > c	b > c	b > c	1.53	4.1	0.038
Co	93	5.5	4.6	0.57	17	0.62	0.007
Cr	86	3.3	3.1	0.45	6.7	0.68	0.028
Cu	95	1390	1370	770	2100	0.99	0.03
Dy	96	0.0116	0.0043	b > c	0.092	0.0108	0.0007
Er	96	0.0048	0.004	b > c	0.051	0.034	0.0007
Eu	95	0.37	0.27	b > c	2.2	0.065	0.0009
Fe	96	10200	9800	5100	21000	14.1	0.21
Ga	95	0.44	0.40	0.090	0.98	0.0108	0.00029
Gd	93	0.055	0.047	0.0082	0.163	0.0175	0.0022
Ge	93	79	78	39	125	0.56	0.013
Hf	84	0.30	0.183	b > c	2.0	0.056	0.0019
Ho	95	0.00144	0.0000184	b > c	0.0177	0.00142	0.0001
Ir	84	b > c	b > c	b > c	0.088	0.025	0.00047
La	95	0.22	0.184	0.050	0.66	0.0092	0.019
Lu	94	b > c	b > c	b > c	0.0060	0.0069	0.00019
Mn	96	2200	2100	1260	4200	1.54	0.01
Mo	96	250	160	26	1500	10.7	0.3
Nb	78	1.16	0.97	0.178	3.3	0.49	0.0059
Nd	96	0.139	0.105	0.0189	0.52	0.0046	0.011
P	94	1290000	1300000	800000	1790000	2200[d]	0.24
Pb	91	5.6	5.1	0.44	18.0	0.53	0.023
Pd	57	5.0	3.9	0.57	14.0	0.65	0.016
Pr	96	0.029	0.021	b > c	0.177	0.078	0.0041
Pt	86	0.080	0.040	b > c	1.38	0.088	0.0009
Re	92	0.0102	0.0057	b > c	0.23	0.0113	0.00051
Rh	95	0.44	0.36	0.060	1.82	0.0156	0.0051
Sb	95	0.95	0.82	0.20	3.2	0.091	0.0011
Sc	57	2.2	2.1	0.65	5.9	0.0161	0.0031
Se	93	80	72	23	159	27	0.092
Si	85	8700	7000	2500	27000	0.00053	0.42
Sm	94	0.029	0.023	b > c	0.121	0.56	0.0002
Sr	93	490	470	179	1120	0.149	0.12
Ta	83	0.98	0.82	0.0179	4.0	0.007	
Tb	93	0.0031	0.00189	b > c	0.022	0.81	0.0006
Te	96	1.64	1.53	0.103	4.7	0.0178	0.0031
Th	76	0.32	0.28	0.0102	1.4	66	0.00048
Ti	96	1030	1010	420	1920	0.053	8.8
Tl	95	0.34	0.28	b > c	1.53	0.00002	0.00021
Tm	96	0.0025	0.00191	b > c	0.0108	0.0063	0.00008
U	91	0.045	0.034	b > c	0.171	0.031	0.00018
V	94	0.31	0.28	0.080	0.79	0.0088	0.0039
Y	96	0.24	0.22	0.090	0.66	0.0047	0.0048
Yb	95	0.00114	b > c	b > c	0.028	174	0.00022
Zn	95	7900	7600	4200	16300	0.132	5.4
Zr	75	2.2	2.0	0.42	5.3	0.132	0.0049

[a] DL = detection limit; 3 times the standard deviation of 10 replicates of the blank analyzed as a sample. [b] b > c = element concentration below the mean of 10 blanks. [c] BEC = background equivalent concentration = mean of concentrations of the elements in 10 replicates. Blank × individual dilution factor (approximately 90). [d] Dilution factor (approximately 3400).

with use of fertilizer and pesticides, and with use of the same sorts of peas so big differences in the site areas occur. Three farms had fertilizer application, one had farmyard manure (cattle manure) application, and the rest had not soil fertility product applications in the year of cultivation. The limitations imposed on site selection to minimize the effects of contamination caused by human activity are described in Bibak et al. (1998).

Peas. A total of 20 undamaged closed pea pods from 10 healthy pea plants (2 pea pods per plant) were nipped evenly across each site. All samples were collected with Nitrilite gloves (Nitrilite, powder free, Ansell Edmont) and were packaged in poly(ethylene terephthalate) (PET) bags for shipments.

Table 3. Some Properties of the Surface Soil (0−25 Cm) at 10 Sites [Soil Texture: Sand (0.063−2.0 mm), Silt (0.002−0.063 mm), and Clay (<0.002 mm)]

	field texture of surface, %				
sites	sand	silt	clay	pH ($CaCl_2$)	org C, %
1	65.5	22.8	9.9	5.1	1.06
2	62.1	27.2	7.5	5.7	1.89
3	45.6	35.9	16.0	4.8	1.41
4	66.2	21.2	10.4	5.6	1.02
5	55.7	27.6	14.7	6.9	1.13
6	67.8	19.9	10.6	5.4	1.00
7	79.9	9.7	6.7	6.5	2.20
8	62.7	25.5	10.7	6.5	1.41
9	81.3	10.2	7.3	7.1	0.69
10	72.0	15.6	10.6	6.2	1.04

Sample Preparation. Peas were refrigerated in the laboratory to avoid decomposition. Laboratory modification, special equipment for sample preparation, digestion, laboratory ware cleaning procedures, and deioinzed water (DW) and double-deionized water (DDW) supplies are described in Bibak et al. (1998). All sample preparations after the cleaning procedures were carried out in class 1000 environment. Disposable latex gloves (Gammex, sterile and powder free, Ansell Edmont) and full laboratory dress (Tyvek) were worn throughout the procedure.

The 20 pea pods collected from a field were composited for analysis as follows. Two frozen pea pods from one pea plant representing one sample for analysis were opened manually by applying finger pressure to the sides of pods. Then four pea seeds were freed by plastic tweezers, two nearest the stalk and two at the middle of the pod from the first one and two farthest from the stalk and two at the middle of the pod from the second one. These eight seeds were put directly into a Teflon digestion vessel (perfluoroalkoxy, CEM Co., Matthews, NC) and were mashed by a Teflon stave for acceleration of digestion.

Soils. A composite sample was collected at each site after the peas were harvested as follows. Ten cores (7.5 cm diameter and 25 cm deep) were taken from each site and mixed. Samples were air-dried and coarse materials crushed and then sieved (2 mm) to remove stones and debris. In this material, the texture, pH, and total C were determined as described by Bibak et al. (1998).

Multielement Determination. The homogenized samples were digested with HNO_3 (Merck p.a., subboiled in clean room class 1000) in a microwave oven (MDS 2000, CEM Co.) equipped with 12 closed Teflon PFA (perfluoroalkoxy) digestion vessels (CEM Co.) and were used for the determination of the 55 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Re, Rh, Sb, Sc, Se, Si, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr) by high-resolution inductively coupled plasma mass spectrometry (HR-ICPMS) in the digests of the peas. The microwave oven was programmed to run at increasing pressures of 40, 85, 100, 120, and 150 psi in five steps. The pressure was held constant for 3, 3, 5, 15, and 5 min during each of the five steps. The clear and residue-free digest was then cooled to room temperature and transferred quantitatively to a 50-mL polyethylene flask, and double-deionized water was added to a mass of approximately 30 g (weighed to the nearest 0.0001 g). These sample solutions were stored at 5 °C until analysis.

Quality control procedures include the analysis of blanks and certified standard reference material (NIST 1567a wheat flour).

Instrumental parameters for the HR-ICPMS analysis, quantification, quality control procedures, and the data processing have been described by Bibak et al. (1998).

The results were analyzed statistically using the simple correlation coefficient (r) in the Statgraphics statistical software package (Statgraphics Plus, 1995).

Table 4. Simple Correlation Coefficients (*r*) between Soil Properties and the Elemental Concentrations (μg/kg) in Peas

element	clay, %	pH	org C, %
Ag	−0.0279	0.1103	0.5353[a]
Al	0.2517	−0.1175	−0.4466[b]
Au	0.2649[a]	0.0240	0.2747[a]
B	−0.1781	0.2333[a]	−0.2120
Ba	−0.1578	0.2544	−0.4803[c]
Be	−0.0724	−0.0147	−0.2536
Bi	0.0941	−0.0069	0.0595
Ca	−0.2708[a]	0.3679[b]	0.2606[a]
Cd	0.6385[c]	−0.3379	0.0258
Ce	−0.1151	0.3320	−0.0293
Co	0.2267	−0.0283	−0.6817[c]
Cr	−0.0916	0.02204	−0.2343
Cu	0.0235	−0.0678	0.0377
Dy	0.0256	−0.0610	−0.0099
Er	0.1755	0.3527[b]	−0.1573
Eu	0.1857	−0.3693[a]	−0.2666
Fe	0.2110	−0.6365[c]	−0.3864[b]
Ga	0.5179[c]	−0.3943[b]	0.0196
Gd	−0.2036	0.4145[c]	−0.0188
Ge	0.1996	−0.1367	0.1259
Hf	0.0107	0.1311	0.2347
Ho	−0.1955	0.0016	−0.1693
Ir	−0.0735	0.1604	0.1944
La	0.5002[b]	−0.3192	0.2043
Lu	−0.1346	0.2496[a]	0.1311
Mn	−0.2074	0.2255	−0.4740[c]
Mo	−0.4683[c]	0.4578[c]	0.5700[c]
Nb	0.3474[b]	−0.0773	0.0079
Nd	0.6098[c]	−0.4265[a]	0.0758
P	0.0061	−0.2223	0.7090[c]
Pb	−0.1072	−0.4367[c]	0.2948[a]
Pd	0.0751	0.0309	0.7905[c]
Pr	−0.1347	0.3570[a]	−0.4487[b]
Pt	0.1565	0.1532	0.1480
Re	−0.1036	0.3064[a]	−0.1495
Rh	−0.4294[a]	0.4479[b]	0.7229[c]
Sb	0.3098	−0.1589	−0.2148
Sc	−0.2433	0.1903	0.3930[b]
Se	0.2385	−0.4221[c]	0.3028[a]
Si	−0.1753	0.2827[a]	−0.1661
Sm	0.3068	−0.1292	0.2417
Sr	−0.2181	0.0362	0.3475[b]
Ta	0.0637	0.0695	−0.0203
Tb	0.1632	−0.0996	−0.2495[a]
Te	−0.3393	0.1626	0.4389[a]
Th	−0.4135[c]	0.2041	0.4561[c]
Ti	−0.4465[b]	0.1193	0.3572[a]
Tl	−0.1924	−0.0367	−0.2161
Tm	0.2240	0.1336	−0.1031
U	0.0494	0.2097	0.1836
V	−0.2035	0.1504	−0.2420
Y	0.3864[b]	−0.5723[c]	0.0643
Yb	−0.1378	−0.1363	−0.3011[a]
Zn	0.4814[c]	−0.7098[b]	−0.0551
Zr	0.3030[a]	0.0953	0.0599

[a] Significance at $P < 0.05$. [b] Significance at $P < 0.01$. [c] Significance at $P < 0.001$.

RESULTS AND DISCUSSION

The elemental mean and 95% confidence interval (μg/g, dry weight) of 10 replicate analyses of the NIST 1567a wheat flour reference material are shown in Table 1. For the 14 elements, 10 certified (Ca, P, Al, Cd, Cu, Fe, Mn, Mo, Se, and Zn) and 4 noncertified (Co, Pb, U, and V), listed there, an acceptable order of accuracy was obtained using HR-ICPMS taking into account the 95% confidence interval and the multielement method. Comparison of the data between the certified and the measured validates the sample digestion method used and provides a degree of confidence in results obtained by the multielemental HR-ICPMS analytical method. In general, precision levels of <15% were obtained for most elements when the concentration of the element considered was well above the detection limit. When the concentration of an element was close to the detection limit, it was somewhat higher.

The concentrations of the 55 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Re, Rh, Sb, Sc, Se, Si, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr) in the peas taken from 10 background areas and measured by HR-ICPMS are shown in Table 2. The values are expressed in μg/kg fresh weight. From the data, material outliers were omitted. For some elements, it was necessary to remove one to five estimations after spiking on high levels due to tailing. Therefore, the number of samples (*n*) in Table 2 are different. The detection limits (μg/kg) are calculated as 3 times the standard deviation of 10 replicates of the blank determination.

The statistical data shown in Table 2, such as the means and medians, show that great variation exists between the minimum and maximum values. For most elements, the mean exceeds the median (Table 2) because a few sites have very high values. Some elements were present at levels below the mean of 10 consecutive blank determinations; these are listed as b > c in Table 2. In these cases, the negative values (c − b) are included in the statistical calculations. The background equivalent concentrations (μg/kg) in Table 2 are calculated from the mean of the concentrations of the elements in 10 replicates of blanks times the dilution factor of the pea samples.

The compositions of the soils differ greatly (Table 3). Thus, the organic carbon and clay contents range from 0.69% C to 2.2% C and from 6.7% clay to 16.0% clay, respectively. Accordingly, the pH varies from 4.8 to 7.1. Because only one genotype of pea was grown in the range of soils, the variation in concentrations of the elements are probably related to the soil properties and agricultural practices. These variations in properties, therefore, may be responsible for the variations in contents of the elements in the peas, since the soil composition or soil parameters (pH, clay contents, and organic matter) may influence the elemental distribution between soil and soil solution and different soils may show different affinities for elements at comparable soil solution composition. Correlations between these parameters and the elemental concentrations of the peas can be helpful tools to improve our understanding of the role of soil parameters on the availability of the elements to the crop.

The results of the correlation analysis are shown in Table 4. A simple correlation between the clay contents and concentrations of the elements was significantly positive ($P < 0.05$) with Au and Zr; ($P < 0.01$) La, Nb, and Y; and ($P < 0.001$) Cd, Ga, Nd, and Zn and significantly negative ($P < 0.05$) with Ca and Rh; ($P < 0.01$) Ti; and ($P < 0.001$) Mo and Th (Table 4). The correlation between the elemental concentration and pH was significantly positive ($P < 0.05$) with B, Lu, Pr, Re, and Si; ($P < 0.01$) Ca, Er, and Rh; and ($P < 0.001$) Gd and Mo and significantly negative ($P < 0.05$) with Eu and Nd; ($P < 0.01$) Ga and Zn; and ($P < 0.001$) Fe, Pb, Se, and Y (Table 4). Organic carbon correlated significantly positive ($P < 0.05$) with Ag, Au, Ca, Pb, Se, Te, and Ti; ($P < 0.01$) Sc and Sr; and ($P < 0.001$) Mo, P, Pd, Rh, and Th and significantly negative ($P < 0.05$) with

Table 5. Comparison of Mean Values (μg/kg, Fresh Weight) Reported in Various Countries, Studies, and This Study

element	Finland[a]	Denmark 1977–1980[b]	Denmark 1988–1992[c]	Sweden[d]	general[e]	this study
Al	2000		1000			89
Si	4000					8700
P	1300000					1290000
Ca	310000					230000
Cr	20		14.0			3.3
Mn	3800					2200
Fe	20000					10200
Co	5.0					5.5
Cu	2200	1100	3000			1390
Zn	13000	7100		8000		7900
Mo	200					250
Cd	30	6.0	3.0	4.0	3.0–30	12.1
Pb	30	36	< 4.0	14.0	8.0–14.0	5.6

[a] Vero et al., 1980. [b] Hansen and Andersen, 1982. [c] Levnedsmiddelstyrelsen, 1995. [d] Fuchs et al., 1976. [e] Tahvonen, 1996.

Tb and Yb; (P < 0.01) Al, Fe, and Pr; and (P < 0.001) Ba, Co, and Mn (Table 4). These are in good agreement with many investigations showing the importance of clay minerals, showing that the pH and organic matter are the main factors influencing the solubility, and showing the availability of trace elements in arable soils (Bibak, 1994; McKenna et al., 1993; Alloway, 1995; Öborn et al., 1995; Miner et al., 1997; Jinadasa et al., 1997).

Table 5 compares the data for Al, Si, P, Ca, Cr, Mn, Fe, Co, Cu, Zn, Mo, Cd, and Pb from this study with other surveys conducted in different countries or reported by other scientists. It is difficult to compare the results for all the elements mentioned above on the bases of two values. In general, our values are lower than those reported from other studies. The reason could be that we have measured the background values. A variety of factors such as sampling, transport, storage, sample pretreatment, decomposition, and analytical methods may be responsible for these differences. But the contents of elements, such as Cu, Zn, Cd, and Pb, are comparable to the lowest values in Table 5. This agrees well with many investigations on the Pb content in vegetable, fruits, and berries reported in recent quality-controlled studies; the contents have been rather low compared to previously reported data (Tahvonen, 1996). Since analytical quality control activities are not yet in general use or standardized, it is often difficult to compare the results from different studies (Tahvonen, 1996).

The methodology used in this study may be efficiently employed to study a wide range of elements in agricultural crops. The results are important in providing nutritional information on the background levels of trace elements in peas. The variation in contents of some elements in peas is due to the variation in soil properties.

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