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Effect of Organic and Conventional Crop Rotation, Fertilization, and Crop Protection Practices on Metal Contents in Wheat (*Triticum aestivum*)

Julia Cooper,[†] Roy Sanderson,[‡] Ismail Cakmak,[§] Levent Ozturk,[§] Peter Shotton,[†] Andrew Carmichael,[†] Reza Sadrabadi Haghighi,[||] Catherine Tetard-Jones,[†] Nikos Volakakis,[†] Mick Eyre,[†] and Carlo Leifert*,[†]

[†]Nafferton Ecological Farming Group, School of Agriculture, Food and Rural Development (AFRD), Newcastle University, Stocksfield, Northumberland, NE42 7XD, U.K.

[‡]School of Biology, Ridley Building, Newcastle University, NE1 7RU, U.K.

[§]Faculty of Engineering and Natural Science, Sabanci University, 34956 Istanbul, Turkey

[||]Agronomy Department, Islamic Azad University—Mashhad Branch, P.O. Box 91735−413 Mashhad, Iran

ABSTRACT: The effects of organic versus conventional crop management practices (crop rotation, crop protection, and fertility management strategies) on wheat yields and grain metal (Al, Cd, Cu, Ni, Pb, and Zn) concentrations were investigated in a long-term field trial. The interactions between crop management practices and the season that the crop was grown were investigated using univariate and redundancy analysis approaches. Grain yields were highest where conventional fertility management and crop protection practices were used, but growing wheat after a previous crop of grass/clover was shown to partially compensate for yield reductions due to the use of organic fertility management. All metals except for Pb were significantly affected by crop management practices and the year that the wheat was grown. Grain Cd and Cu levels were higher on average when conventional fertility management practices were used. Al and Cu were higher on average when conventional crop protection practices were used. The results demonstrate that there is potential to manage metal concentrations in the diet by adopting specific crop management practices shown to affect crop uptake of metals.

KEYWORDS: Metals, organic farming, cadmium, copper, aluminum, zinc, lead, nickel

INTRODUCTION

Demand for foods produced using organic and other low-input crop production methods has increased rapidly over the last 20 years.[1,2] Organic farming standards prohibit the use of chemosynthetic pesticides and water-soluble mineral nitrogen, phosphorus, and potassium fertilizers and restrict the use of other mineral fertilizers. Fertilization therefore relies more on organic fertilizers, especially green and animal manures. The use of animal manures and copper (Cu) fungicides (which is permitted under organic farming practice) has resulted in concerns about higher loads of nutritionally undesirable metals such as cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni) as well as aluminum (Al) in organic crops.[3,4] Some metals (e.g., Cu) are essential micronutrients for plants and humans at low concentrations but are toxic at higher exposure and dietary intake levels.[5,6]

Crop-based foods account for over 80% of Cd and Pb intake.[7,8] The metal content of crops is reported to be affected by a range of factors, including soil type,[9−11] climatic conditions,[11,12] crop species, and variety choice.[5,13−16]

The use of mineral phosphorus (P) and sulfur (S) based fertilizers and pesticides has been linked to increased levels of Cd in food crops. Similarly the use of Cu fertilizers and fungicides has been linked to an increased Cu content in both soils and food crops.[12,17−19] In a group of Swedish studies mineral P fertilizer use was linked not only to an increase in Cd concentrations in soils[20] and cereal grain[21,22] but also to increased Cd concentrations in kidneys and other tissues of pigs and dairy cows.[23,24] This has led to stricter monitoring of Cd concentrations in P-fertilizers in many countries[23,24] and efforts to breed crop varieties/cultivars with low metal uptake efficiency (so-called pollution-safe cultivars or PSCs) in order to reduce metal intake via major food crops.[16]

A range of survey and field experiment based studies have compared the cadmium content (and in some cases the content of other metals) in organic and conventional food products. The majority of studies reported significantly higher or trends toward higher Cd levels in conventionally produced crops. This included studies on wheat, oats, onions, tomatoes, lettuce, peppers, peach, lentils, and peas.[5,14,18,25] In contrast, a survey-based study by Jorhem and Slanina[11] reported inconsistent results for wheat, and a field experimental study by Rossi et al.[19] found significantly higher Cd levels in organically produced tomato.

There are also some survey and experimental studies in which the Pb content in organic and conventional crops was compared. Rossi et al.[19] reported 4 times higher Pb concentrations in conventional compared to organic wheat produced in Italy,

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Table 1. Sequence of Crops in the Nafferton Factorial Systems Comparison Experiments in the Organic Rotation (ORG) and Conventional Rotation (CON)[a]

	rotation	2002	2003	2004	2005	2006	2007	2008
expt 1	ORG	G/C[b]	winter wheat[c]	potato/veg[d]	beans	potato/veg	spring barley	G/C
	CON	G/C	winter wheat	**winter wheat**	winter barley	potato/veg	**winter wheat**	winter barley
expt 2	ORG	G/C	G/C	**winter wheat**	potato/veg	beans	potato/veg	spring barley
	CON	G/C	G/C	**winter wheat**	**winter wheat**	winter barley	potato/veg	**winter wheat**
expt 3	ORG	G/C	G/C	potato	G/C	G/C	**winter wheat**	potato/veg
	CON	G/C	G/C	potato/veg	grass	grass	**winter wheat**	**winter wheat**
expt 4	ORG	G/C	potato/veg	spring barley	G/C	G/C	G/C	**winter wheat**
	CON	G/C	potato	**winter wheat**	winter barley	G/C	G/C	**winter wheat**

[a] Results for winter wheat crops in bold type are reported in this paper. [b] G/C = grass/clover ley. [c] For cereals, beans, and grass/clover the whole area (12 m × 24 m) of fertilization sub-subplots was planted with the same crop. [d] In years when potatoes/vegetables (potatoes/veg) were grown, half the area of fertilization sub-subplots (6 m × 24 m) was planted with potatoes and the other half with four different vegetables (cabbages, onions, lettuce, and carrots) with each vegetable grown on a 6 m × 6 m area.

and Karavoltsos et al.[25] reported 60% higher Pb levels in conventional foodstuffs (which included both crop and livestock products) collected from a Greek market. In farm surveys in Denmark and Sweden no significant differences in Pb concentrations could be detected between organic and conventional wheat, onions, and peas.[12] In contrast, in a field trial, Rossi et al.[19] found higher Pb levels in tomatoes grown under organic compared to conventional farming conditions.

Copper and aluminum levels in organic and conventional crops have only been compared in a few published studies, and there are to our knowledge no studies in which nickel (Ni) concentrations were compared. Ryan et al.[26] studied grain mineral concentrations in grain grown on paired organic and conventional farms. They found that conventionally grown grain had lower Zn and Cu levels than the organically grown grain. In contrast, Mäder et al.[27] reported higher Cu levels in wheat that was grown using conventional fertilizers, compared to systems that used manure. In other crops, a trend toward higher levels of Cu in organic onions, but lower levels in organic peas, was found in a farm survey by Gunderson et al.[12] Rossi et al.[19] reported no significant differences between organically and conventionally produced tomato, although Cu-fungicides were used in the organic, but not the conventional, crop protection protocol. For Al, Gundersen et al.[12] reported about 90% higher Al concentrations in conventionally produced onions, but no significant differences for peas.

Many of these comparative studies may be criticized for not controlling confounding factors such as soil type, climatic conditions, and variety and also for not repeating experiments over several growing seasons. In addition, these studies were designed as systems comparisons and therefore did not provide information on the effect of individual production system components (e.g., rotational design, fertilization regimes, and crop protection practices) on the metal concentrations of crops.

The objective of the study presented here was to identify the effect of and interactions between (i) rotational position/precrop, (ii) fertilization regimes, and (iii) crop protection methods used in organic, low-input, and conventional production systems on metal (Cd, Pb, Cu, Ni, Zn, and Al) concentrations in wheat, the main cereal used for human consumption in Europe. The study also assessed crop yield to identify potential "dilution effects" of higher yields on metal concentrations and compared results from four growing seasons to explore the effects of contrasting climatic conditions on the measured metals.

■ MATERIALS AND METHODS

Site and Climatic Conditions. The experiments presented were carried out within the Nafferton factorial systems comparison (NFSC) trial at the University of Newcastle's Nafferton Experimental Farm, Northumberland, U.K. (54:59:09 N; 1: 43:56 W). The soil in the 6 ha field used for the experiment is a uniform sandy loam soil of the Stagnogley type with a mean organic matter content of 3.3%.

The NFSC trial was established in 2001 and is a series of four long-term factorial experiments, all replicated four times in the field, designed to identify the effects of crop production systems on (i) soil physical, chemical, and biological characteristics, (ii) biodiversity, (iii) crop yield and quality, and (iv) sustainability related parameters. The experiment was designed to minimize confounding factors (e.g., soil type, climatic conditions, tillage systems) and allow the effects of principal production system components (rotational design, fertilization regime, and crop protection method) and interactions between these three factors to be identified.

The data presented was generated in the wheat harvest years 2004, 2005, 2007, and 2008. Table 1 shows the sequence of crops grown in each experiment and indicates which winter wheat crops are the focus of this study. Weather patterns differed considerably between the four experimental years and are summarized in Table 2.

Field Trial Design. In each of the four NFSC experiments the effects of crop rotation (identified as precrop, PC, in our design), crop protection (CP), and fertility management (FM) are studied using a split−split plot design.

There are two crop rotation main plots in each experiment (each 12 m × 96 m) which follow either (a) a diverse rotation (rich in legume and potato/vegetable crops as recommended by organic farming standards/principles, ORG rotation) or (b) a nondiverse rotation (cereal crop-dominated rotation typical for conventional systems, CON rotation) (Table 1). Each rotation main plot is divided into two crop protection subplots (12 m × 48 m) in which crop protection is carried out according to either (a) conventional farming practice (CC; Red Tractor Farm Assured Combinable Crops standard[28]) or (b) organic crop protection standards (OC; Soil Association organic farming standards[29]). Each of the crop protection subplots is divided into two fertility management sub-subplots (12 m × 24 m) in which fertilization is either carried out according to (a) conventional farming practice (CF; Red Tractor Farm Assured Combinable Crops standard[28]) or (b) organic farming standards (OF; Soil Association organic farming standards[29]). The arrangement of crop protection subplots and fertilization sub-subplots within experiments is randomized, and 10 m unplanted separation strips are established between crop protection subplots and 5 m unplanted separation strips between fertilization sub-subplots.

All four NFSC experiments follow this design, but each starting at a different stage in the crop rotation, so that a diversity of crops can be studied in the NFSC in each year, and as mentioned before each experiment is replicated four times in the field.

Table 2. Climatic Conditions during the 2004, 2005, 2007, and 2008 Winter Wheat Harvest Years

year	parameter	April	May	June	July	Aug	Sept
2004	precipitation (mm)	58	20	86	88	149	19
	mean irradiation (kW m^2)	0.11	0.18	0.18	0.16	0.12	0.10
	mean relative humidity (%)	85	84	84	84	79	67
	mean air temperature (°C)	8.2	10.3	13.7	13.9	15.2	13.0
	mean soil temperature (°C)	8.8	12.6	15.6	15.5	16.7	13.8
2005	precipitation (mm)	92	28	55	70	26	54
	mean irradiation (kW m^2)	0.13	0.17	0.17	0.16	0.15	0.11
	mean relative humidity (%)	81	78	81	81	81	83
	mean air temperature (°C)	7.3	9.6	13.7	14.6	14.5	13.5
	mean soil temperature (°C)	8.0	10.7	14.6	16.2	15.6	14.4
2007	precipitation (mm)	13	51	118	69	36	23
	mean irradiation (kW m^2)	0.15	0.17	0.13	0.17	0.14	0.10
	mean relative humidity (%)	77	77	83	80	78	78
	mean air temperature (°C)	9.3	10.0	12.9	13.9	14.0	12.3
	mean soil temperature (°C)	10.5	12.9	14.9	15.7	15.5	13.3
2008	precipitation (mm)	72	7	57	101	107	158
	mean irradiation (kW m^2)	0.12	0.17	0.17	0.15	0.12	0.08
	mean relative humidity (%)	77	72	71	72	71	67
	mean air temperature (°C)	6.2	10.7	12.6	14.7	14.7	12.1
	mean soil temperature (°C)	6.8	12.2	14.6	15.9	15.5	13.1

Crop Management Practices Used. Wheat crops were sown using a commercial drill (3 m Lely combination drill; Lely U.K. Ltd., St. Neots, U.K.) in late October/early November (see Table 3 for the exact sowing dates). The wheat (*Triticum aestivum*) cultivar Malacca was used in all experiments. Wheat seeds used in conventional crop protection subplots (CC) were supplied by Horizon Seeds (Eye, U.K.) and were produced under conventional seed production conditions and treated with standard seed pesticide treatments. Wheat seeds used in organic crop protection subplots (OC) were also supplied by Horizon Seeds, (Eye, UK) and produced to organic seed production standards (Soil Association, Bristol, U.K.) with no standard seed pesticide treatments applied.

The crop protection treatments used in the CC plots in different years are shown in Table 3 and are the same as those used in commercial conventional winter wheat crops at Nafferton farm. In the OC plots only mechanical weed control treatments are used; an Einbock tined weeder was used twice (in March and April) in the 2004, 2005, and 2007 harvest years, but only once in 2008, due to the wet weather conditions in April 2008.

The fertilization treatments used in the experimental plots are the same as those used in commercial conventional or organic wheat fields at Nafferton farm. In the OF sub-subplots no fertilizer inputs are applied to the wheat. Depending on the experiment, some OF sub-subplots received compost in the years prior to wheat. These were plots that had been planted to potatoes in 2003 immediately prior to wheat (experiment 4, CON rotation), in 2004 3 years prior to wheat (experiment 3, ORG and CON rotation), and in 2006 immediately prior to wheat (experiment 1, ORG rotation). These potato plots received 1.5−2.5 t dry compost ha^{-1} which was equivalent to 170 kg total N ha^{-1}. Compost typically contained 67.3 mg Cu kg^{-1},

Table 3. Previous Crops, Planting, and Harvesting Dates, Moisture Content at Harvest, and Conventional Crop Protection Regimes Used for Wheat Crops in the Four Harvest Years Included in the Study

	harvest year			
	2004	2005	2007	2008
previous crops	grass/clover, winter wheat, or potato	winter wheat	grass/clover, grass, or potato	grass/clover, winter wheat, or potato
planting date	07−10−2003	14−10−2004	04−10−2006	11−10−2007
harvesting date	01−09−2004	23−08−2005	23−05−2007	29−08−2008
grain moisture content at harvest (%)	13−18	17−21	13−18	16−20
		Conventional Crop Protection Regimes		
weed control (applied in Nov)	isoproturon (2 L ha^{-1}), mecoprop-P (1 L ha^{-1}), and diflufenican + isoproturan (1 L ha^{-1})	isoproturon (3 L ha^{-1}), mecoprop-P (1 L ha^{-1}), and pendimethalin (1.5 L ha^{-1})	isoproturon (2.5 L ha^{-1}), mecoprop-P (0.5 L ha^{-1}), and pendimethalin (2.5 L ha^{-1})	isoproturon (2.5 L ha^{-1}), mecoprop-P (1 L ha^{-1}), and pendimethalin (2.5 L ha^{-1})
disease control				
first tank mix (applied in April)	epoxiconazole (0.5 L ha^{-1}), chlorothalonil (1 L ha^{-1}), and chlormequat (2.3 L ha^{-1})	epoxiconazole (0.5 L ha^{-1}), chlorothalonil (1 L ha^{-1}), fenpropimorph (0.2 L ha^{-1}), and chlormequat (2.3 L ha^{-1})	epoxiconazole (0.5 L ha^{-1}), chlorothalonil (1 L ha^{-1}), proquinazid (0.125 L ha^{-1}), and chlormequat (2.3 L ha^{-1})	epoxiconazole (0.5 L ha^{-1}), chlorothalonil (1 L ha^{-1}) proquinazid (0.125 L ha^{-1}), and chlormequat (2.3 L ha^{-1})
second tank mix (applied in May)	epoxiconazole (0.5 L ha^{-1}), chlorothalonil (1 L ha^{-1}), and fenpropidin (0.25 L ha^{-1})	epoxiconazole (0.75 L ha^{-1}), chlorothalonil (0.75 L ha^{-1}), fenpropidin (0.15 L ha^{-1}), and trifloxystrobin (0.25 L ha^{-1})	epoxiconazole (0.5 L ha^{-1}), chlorothalonil (0.4 L ha^{-1}), fenpropidin (0.25 L ha^{-1}), and azoxystrobin (0.6 L ha^{-1})	epoxiconazole (0.6 L ha^{-1}), chlorothalonil (1 L ha^{-1}), and pyraclostrobin (0.3 L ha^{-1})
pest control	no input	no input	no input	no input

219 mg Zn kg^{-1}, 2.89 mg Pb kg^{-1}, 3.11 mg Ni kg^{-1}, and 0.312 mg Cd kg^{-1}. In the CF sub-subplots ammonium nitrate (Yara U.K. Ltd.) equivalent to 180 kg N ha^{-1} (50 kg N ha^{-1} in mid-March and 130 kg N ha^{-1} in mid-April) was applied to the first wheat crops (wheat grown after precrops other than wheat). In second wheat crops (wheat grown after a wheat precrop) ammonium nitrate equivalent to 210 kg N ha^{-1} (80 kg N ha^{-1} in mid-March and 130 kg N ha^{-1} in mid-April) and superphosphate and potassium chloride (equivalent to 64 kg P_2O_5 ha^{-1} and 96 kg K_2O ha^{-1}; 0:20:30 Carrs Fertiliser Co., U.K.) were applied. Typical metal contents of the ammonium nitrate were 0.26 mg Cu kg^{-1}, 0.42 mg Zn kg^{-1}, <0.10 mg Pb kg^{-1}, 1.85 mg Ni kg^{-1}, and <0.10 mg Cd kg^{-1}, and of the 0:20:30 were 5.3 mg Cu kg^{-1}, 69.0 mg Zn kg^{-1}, 1.36 mg Pb kg^{-1}, 5.27 mg Ni kg^{-1}, and 10.1 mg Cd kg^{-1}.

Crops were harvested using a plot combine harvester (Claas Dominator 38; Claas U.K. Ltd., Bury St. Edmunds, U.K.), and grain samples were dried (hot air drying using an electric motor fed through a 3 m × 1.5 m × 0.70 m wooden box with a meshed surface for grain sacks to rest on) and then cleaned (Lainchbury HC1/7W grain cleaner, Blair Engineering, Blairgowrie, U.K.) immediately after harvest.

Metal Analyses in Wheat Grain Samples. *Sample Preparation.* Wheat grain material of approximately 20 ± 3 g was dried at 40 °C for 6 h and milled using a vibrating agate cup mill (Pulverisette 9, Fritsch GmbH, Idar-Oberstein, Germany) at 700 rpm for 5 min to afford finely pulverized whole-grain flour with a final fineness of 10−20 μm. The standard reference material SRM 1567a wheat flour was used as received from the National Institute of Standards and Technology, Gaithersburg, MD, U.S.A.

Digestion and Metal Analyses. Flour samples were subjected to acid digestion in a closed-vessel microwave reaction system (MarsExpress; CEM Corp., Matthews, NC, U.S.A.). About 200 ± 5 mg of finely pulverized whole-grain flour sample was weighed into 55 mL Teflon vessels with 1 mL of 30% H_2O_2 (Merck 107209, Perhydrol) and 5 mL of 65% HNO_3 (Merck 100456, GR for analysis) added. Self-pressure-controlled caps were screwed on using the constant-torque motorized wrench of the microwave reaction system. The 1 h digestion program consisted of the following four steps: step 1, ramp to 180 °C in 15 min; step 2, hold at 180 °C for 10 min; step 3 ramp to 205 °C in 15 min; step 4 hold at 205 °C for 20 min. At the end of the digestion, samples were cooled to room temperature and filtered through Whatman grade 589/3 Blue Ribbon quantitative filter paper (i.e., particle retention <2 μm). The filtrates were collected in plastic graduated tubes and diluted with Milli-Q water (resistivity: 18.2 MΩ·cm at 25 °C; dispensed through a 0.22 μm membrane filter) to yield 20 mL of sample volume. Metals in digestates were analyzed with an inductively coupled argon plasma optical emission spectrometer equipped with a CCD detector (Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia). Analytical quality was checked against the certified values of the quality check sample (i.e., SRM 1567a) which was included in every batch of 40 samples.

Statistical Analyses. Mixed-effects models[30] were used to analyze the data in a series of analyses to produce ANOVA *P*-values for main effects and all interactions using the nlme (nonlinear mixed effects) package in R (R Development Core Team 2009). Where 4 years of data were available, three-factor analyses with year, crop protection, and fertility management as fixed effects were carried out. Data from individual years with more than one previous crop (2004, 2007, 2008) were used in a model with previous crop, crop protection, and fertility management as fixed effects. In 2005, since there was only one previous crop, the model only included crop protection and fertility management as fixed effects. This reduced model was also used where previous crop did not have a significant effect.[31] The hierarchical nature of the split−split plot design was reflected in the random error structures that were specified as block/year/precrop/crop protection. Where analysis at a given level of a factor was carried out, that factor was removed from the random error term. The normality of the residuals of all models was tested using QQ-plots. In all years cadmium and lead data were cube root transformed to meet the criteria of normal data distribution. Differences between the four crop management strategies (FM × CP interaction means) were tested using Tukey contrasts in the general linear hypothesis testing (glht) function of the multcomp package in R. A linear mixed-effects model was used for the Tukey contrasts containing a treatment main effect with four levels with the random error term specified as described above.

The relationships between wheat grain metal concentrations and a number of environmental factors, as well as agronomic factors, were investigated using redundancy analysis (RDA). Redundancy analysis is a constrained ordination process that seeks combinations of explanatory variables (in this case environmental or agronomic factors) that best explain variations in the dependent variables (e.g., metal concentrations). The environmental factors were the amount of precipitation, the mean daily relative humidity, air temperature, soil temperature, and radiation during the harvest year. Agronomic factors were organic and conventional fertilization regimes, organic and conventional crop protection, and crop yield. Separate RDAs were carried out using the agronomic factors and then environmental factors. In both cases the response variables were the grain metal levels (Al, Cd, Cu, Ni, Pb, Zn). RDAs were carried out using the CANOCO package.[32] Automatic forward selection of the environmental and agronomic factors within the RDAs was used and their significance in explaining additional variance calculated using Monte Carlo permutation tests.

RESULTS

Effects of Crop Management Practices. *Yields.* All three main factors affected grain yield significantly (Table 4). Yields were highest in the 2004 season and lowest in 2007. On average the use of conventional fertility management increased grain yields relative to organic fertility management by 24%, but there was also a year × FM interaction, with fertility management having no effect on yield in 2004, but higher yields under conventional fertility management in the other three years (Table 5).

There was a significant main effect for crop protection in all four seasons, and although the relative effect of using pesticides differed between harvest years, on average conventional crop protection resulted in 35% higher yields.

There was also a significant CP × FM interaction for grain yield over all seasons which is illustrated in Figure 1. This shows that the yield increase from conventional fertility management was lower when organic crop protection was used (only 13% increase) compared to when conventional fertility management was used in combination with conventional crop protection (yield increase of 24%).

The effect of different precrops could only be studied when wheat was grown after different precrops in the same season. In two harvest years (2004 and 2008) wheat was grown after potato, grass clover, and winter wheat, but in 2007 it was grown after potato, grass/clover, and pure rye grass (Table 5).

Precrop significantly affected yield only in 2007 with yields highest after a precrop of grass/clover. There was also a significant interaction between precrop and fertility management (Figure 2) with a precrop of grass/clover resulting in the highest yields under organic fertility management, while a precrop of potatoes resulted in the highest yields under conventional fertility management. Figure 3 illustrates the interaction between precrop and fertility management for grain yields in 2008. In this harvest year precrop only significantly affected yields under organic fertility management, when a precrop

Table 4. Main Effect Means ± SE and ANOVA *P*-Values for the Effects of Harvest Year, Crop Protection, and Fertility Management on the Grain Yield and Grain Aluminum (Al), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn) Contents of Wheat

factors	grain yield (t ha^{-1})	Al (mg kg^{-1} DW)	Cd (μg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Ni (μg kg^{-1} DW)	Pb (μg kg^{-1} DW)	Zn (mg kg^{-1} DW)
Harvest Year							
2004	6.7 ± 0.3	2.67 ± 0.22	11.1 ± 1.5	5.00 ± 0.11	≤40	52.6 ± 14.7	22.2 ± 0.5
2005	6.3 ± 0.4	3.89 ± 0.35	38.7 ± 2.2	4.36 ± 0.11	81.8 ± 12.7	17.1 ± 5.1	18.9 ± 0.6
2007	5.7 ± 0.2	2.80 ± 0.16	68.0 ± 5.8	4.93 ± 0.11	88.4 ± 3.5	84.3 ± 3.7	23.5 ± 0.4
2008	5.9 ± 0.2	2.35 ± 0.20	36.0 ± 2.2	4.35 ± 0.10	≤40	89.7 ± 11.6	19.8 ± 0.5
Crop Protection (CP)							
organic	5.2 ± 0.1	2.92 ± 0.19	38.6 ± 3.7	4.84 ± 0.09	51.8 ± 4.3	72.4 ± 8.2	22.5 ± 0.4
conventional	7.0 ± 0.2	2.48 ± 0.11	37.7 ± 3.1	4.54 ± 0.08	49.8 ± 4.1	72.4 ± 9.0	20.3 ± 0.4
Fertility Management (FM)							
organic	5.4 ± 0.1	2.73 ± 0.16	28.1 ± 3.4	4.42 ± 0.07	56.0 ± 4.8	73.0 ± 9.1	19.2 ± 0.3
conventional	6.7 ± 0.2	2.66 ± 0.15	48.3 ± 3.1	4.95 ± 0.09	45.7 ± 3.4	71.8 ± 8.2	23.5 ± 0.4
ANOVA *P*-Values							
year	0.001	0.162	0.002	0.028	<0.001	0.249	0.028
crop protection	<0.001	0.043	0.758	<0.001	0.612	0.997	<0.001
fertility management	<0.001	0.687	<0.001	<0.001	0.009	0.906	<0.001
year × CP	0.002	0.226	0.678	0.219	0.371	0.981	<0.001
year × FM	<0.001	0.012	0.017	0.002	0.169	0.957	0.019
CP × FM	<0.001[a]	0.758	0.912	0.197	0.816	0.869	0.069
year × CP × FM	0.143	0.047	0.884	0.391	0.038	0.862	0.053

[a] See Figure 1 for details of the interaction.

Table 5. Main Effect Means ± SE and ANOVA *P*-Values from an Experiment into the Effects of Harvest Year, Precrop, Crop Protection, and Fertility Management on Wheat Yield, Cd, Cu, and Zn (Only the Results for Metals Where Precrop Was Involved in Significant Main Effect or Interactions Are Included)[a]

	yield (t ha^{-1})				Cd (μg kg^{-1})		Cu (mg kg^{-1})	(Zn mg kg^{-1})
factors	2004	2005	2007	2008	2004	2008	2007	2007
Precrop (PC)								
potato	6.6 ± 0.3		5.9 ± 0.4	5.5 ± 0.4	13.9 ± 3.2	28.6 ± 4.4	4.17 ± 0.09	22.1 ± 0.9
grass/clover	6.9 ± 0.3		6.0 ± 0.2	6.0 ± 0.2	10.8 ± 1.7	41.5 ± 2.8	5.41 ± 0.16	24.2 ± 0.6
grass			5.1 ± 0.3				5.21 ± 0.15	24.2 ± 0.4
winter wheat	6.4 ± 0.7	6.3 ± 0.4		6.0 ± 0.5	≤10	32.6 ± 4.1		
Crop Protection (CP)								
organic	5.7 ± 0.2	5.7 ± 1.2	5.1 ± 0.2	4.8 ± 0.1	≤10	38.0 ± 3.2	5.08 ± 0.17	24.1 ± 0.6
conventional	7.6 ± 0.2	7.1 ± 1.3	6.3 ± 0.3	7.0 ± 0.3	13.3 ± 2.5	34.0 ± 2.9	4.78 ± 0.14	22.9 ± 0.5
Fertility Management (FM)								
organic	6.6 ± 0.2	5.3 ± 1.0	4.7 ± 0.2	5.1 ± 0.2	≤10	22.2 ± 1.6	4.57 ± 0.12	21.7 ± 0.5
conventional	6.7 ± 0.3	7.4 ± 1.0	6.6 ± 0.2	6.7 ± 0.3	14.5 ± 2.5	49.9 ± 1.9	5.29 ± 0.16	25.3 ± 0.4
ANOVA *P*-Values								
precrop	0.235		<0.001	0.118	0.428	<0.001	<0.001	0.021
crop protection	<0.001	0.006	<0.001	<0.001	0.197	0.087	0.012	0.016
fertility management	0.928	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
PC × CP	0.772		0.399	0.065	0.139	0.794	0.613	0.574
PC × FM	0.141		<0.001	<0.001	0.186	0.004[b]	0.186	<0.001
CP × FM	0.019	0.974	<0.001	<0.001	0.990	0.540	0.037[c]	0.007
PC × CP × FM	0.538		0.821	0.055	0.043[d]	0.813	0.7491	0.282

[a] In 2005 wheat was only grown in the conventional rotation after a first crop of wheat; therefore, no analysis of precrops effects is possible in this year. [b] See Figure 4 for interactions between previous crop and fertilization in the 2007/08 season for Cd. [c] See Figure 5 for interactions between crop protection and fertilization in the 2006/07 season for Cu. [d] Cd concentrations were generally very low in 2004, and for several treatments Cd levels were below the detection level preventing further statistical analysis of this three-way interaction.

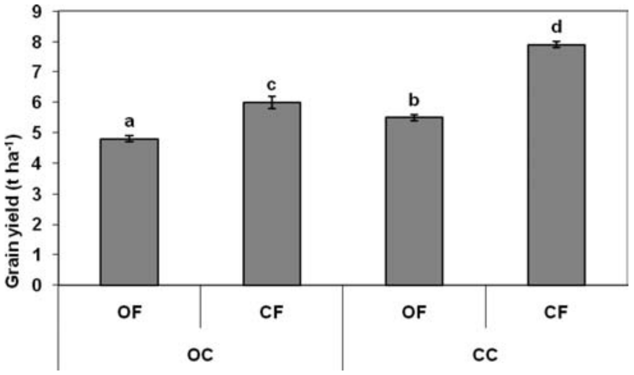

Figure 1. Wheat yields under organic crop protection (OC) or conventional crop protection (CC) and organic fertility management (OF) or conventional fertility management (CF) averaged over four harvest years. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$). Standard errors of the mean (error bars) were calculated from all four field plot replicates.

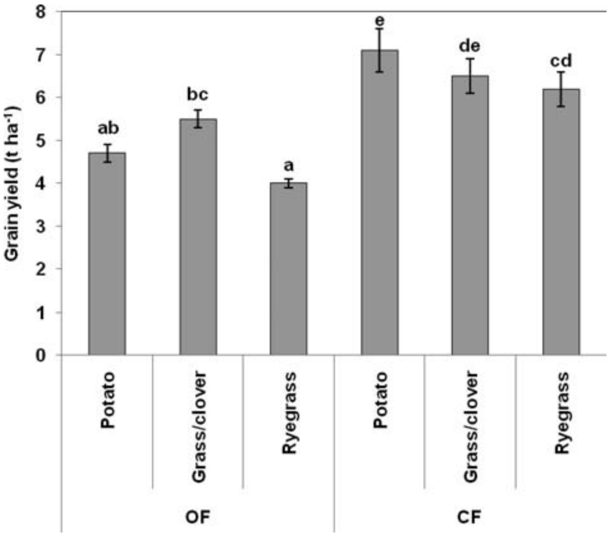

Figure 2. Effects of fertility management (OF = organic fertility management; CF = conventional fertility management) and previous crop on wheat grain yield in the 2007 harvest year. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$). Standard errors of the mean (error bars) were calculated from all four field plot replicates.

of grass/clover resulted in higher yields than a precrop of potatoes.

Metals. Over all years conventional crop protection resulted in significantly lower Al (15%), Cu (6%), and Zn (10%) concentrations in wheat compared to organic crop protection, but had no effect on Cd, Ni, or Pb (Table 4). In addition there was a significant main effect of fertility management on the concentrations of Cd, Cu, Ni, and Zn with organic fertilization regimes resulting in 42% lower Cd, 11% lower Cu, and 18% lower Zn concentrations, but 23% higher Ni concentrations (Table 4).

For Al, Cu, Zn, and Cd there were significant two-way interactions between year and fertilization regime (Table 4). In 2004 conventional fertility management increased grain Al concentrations but decreased concentrations in 2007. Fertility management had no effect on grain Al in the 2005 and 2008 harvest year. Grain Cu concentrations were higher under conventional fertility management in all harvest years except for 2005 when FM had no effect. In 2007 only there was a significant CP × FM interaction for grain Cu concentrations with the combination of organic crop protection and conventional fertility management resulting in the highest grain Cu concentrations. Conventional fertility management always resulted in significantly higher levels of grain Cd; however, in 2004 the magnitude of that difference was relatively low, due to the much lower overall average levels in that year (data for individual years not shown).

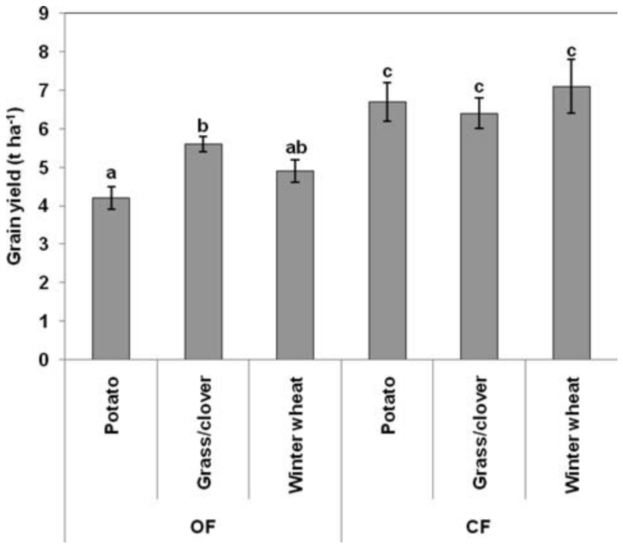

Figure 3. Effects of fertility management (OF = organic fertility management; CF = conventional fertility management) and previous crop on wheat grain yield in the 2008 harvest year. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$). Standard errors of the mean (error bars) were calculated from all four field plot replicates.

Grain Zn concentrations were also higher under conventional fertility management in each year, but in the last 2 years, there were significant CP × FM interactions with the highest concentrations occurring when conventional fertility management was used in combination with organic crop protection (data for individual years not shown).

As reported for Cu and Zn, in 2004 significantly higher grain Al concentrations were recorded when conventional fertility management was used in combination with organic crop protection, but the difference between fertilization treatments was not significant under conventional crop protection. In 2007 the opposite result was found for grain aluminum concentrations. In the 2007 harvest year organic fertilization resulted in significantly higher Ni concentrations when used in combination with organic crop protection regimes, but the difference between fertilization treatments was not significant when used in combination with conventional crop protection regimes. In the three other harvest years, no significant differences in grain Ni concentrations could be detected between treatments (individual results not shown).

The results of a more detailed analysis that included the effects of precrop are shown in Table 5. Only the results for the metals where precrop had a significant main effect or where there were significant PC interaction terms are shown. In 2004 precrop had no effect on wheat Cd levels, but concentrations were

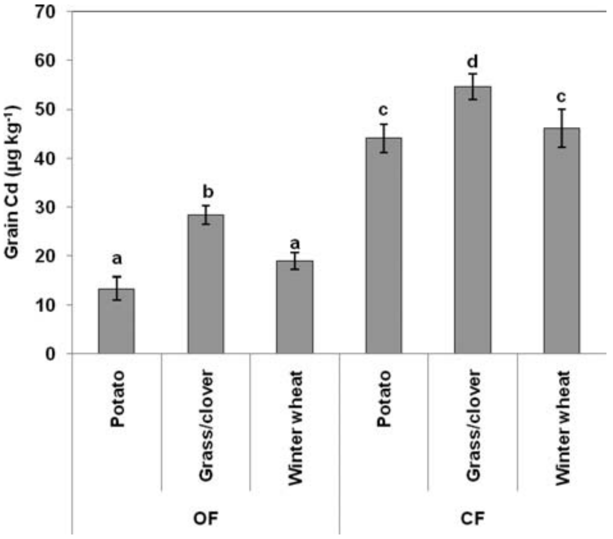

Figure 4. Effects of fertility management (OF = organic fertility management; CF = conventional fertility management) and previous crop on wheat grain Cd concentrations in the 2008 harvest year. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$). Standard errors of the mean (error bars) were calculated from all four field plot replicates.

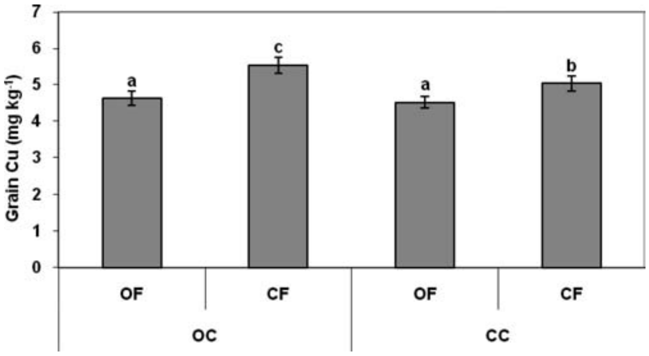

Figure 5. Effects of fertility management (OF = organic fertility management; CF = conventional fertility management) and crop protection (OC = organic crop protection; CC = conventional crop protection) on wheat grain Cu concentrations in the 2007 harvest year. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$). Standard errors of the mean (error bars) were calculated from all four field plot replicates.

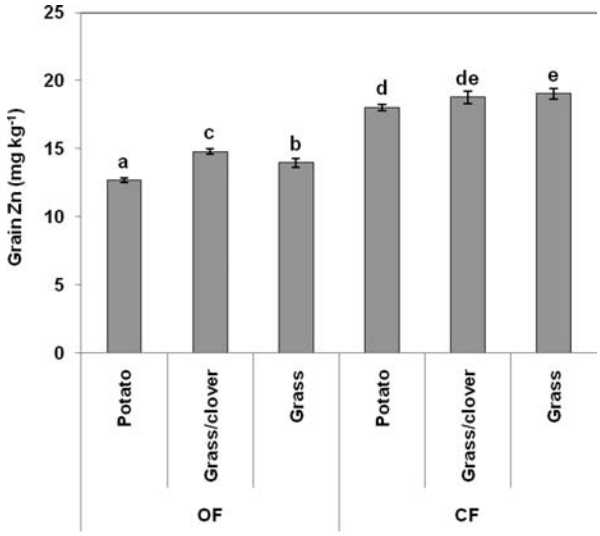

Figure 6. Effects of fertility management (OF = organic fertility management; CF = conventional fertility management) and previous crop on wheat grain Zn concentrations in the 2007 harvest year. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$). Standard errors of the mean (error bars) were calculated from all four field plot replicates.

significantly higher when conventional fertility management was used, and a similar CF effect was found in 2008. On average in the 2008 harvest year Cd concentrations in wheat grain were highest after a precrop of grass/clover, with intermediate levels following wheat and lowest levels after potatoes. Although the PC × FM interaction term in 2007 was significant, this is not apparent in Figure 4 which shows that a precrop of grass/clover always resulted in higher Cd levels than the other two precrops, regardless of the fertility management regime.

In the 2007 harvest year Cu concentrations were highest after a precrop of grass/clover, followed by intermediate levels for the rye grass precrop and lowest concentrations after a precrop of potatoes (Table 5). Organic crop protection resulted in higher grain Cu concentrations, and conventional fertility management also increased grain Cu concentrations; however, there was a significant CP × FM interaction (Figure 5) which shows that Cu concentrations were highest when conventional fertility management was used in combination with organic crop protection.

The results for wheat Zn levels in 2007 were similar to Cu results. Zinc levels after a precrop of potatoes were lower than after the pure grass or grass/clover precrop. Organic crop protection increased Zn levels and organic fertility management resulted in lower Zn concentrations in the grain. As reported for the combined year analysis in Table 4, in 2007 highest Zn concentrations occurred when conventional fertility management was used in combination with organic crop protection (CP × FM $P = 0.007$). There was also a significant PC × FM interaction, illustrated in Figure 6, which shows that under conventional fertility management grain Zn concentrations were less sensitive to the precrop than under organic fertility management.

The biplot (Figure 7) showing the relationship of the agronomic variables to the grain metals showed a strong association along the positive axis 2 with yield, with axis 1 associated with fertility management. The biplot indicates that crop protection management was also associated with axis 2 but with less influence than yield. Axis 1 explained only 5.3% of the variation and axis 2 a further 1.3%. Nickel, and to a lesser extent Cd and Zn, had negative relationships with increasing yield, while Cu, Al and Pb were relatively unaffected. Higher concentrations of Pb, Cu, and Zn, along the negative axis 1, were associated with conventional fertility management, but the other three metals were not related to either fertility variable. Most additional variance was explained by yield ($F = 4.8$, $P = 0.010$), less by organic fertility ($F = 2.9$, $P = 0.058$), and a significant amount by conventional crop protection ($F = 4.4$, $P = 0.018$).

Effect of Harvest Year and Environmental Conditions. Harvest year had a significant effect on the crop yield and concentrations of all metals except for Al and Pb. Harvest year also frequently interacted with crop management factors.

The relationship of the environmental variables to grain metals is shown as a biplot derived from the RDA (Figure 8).

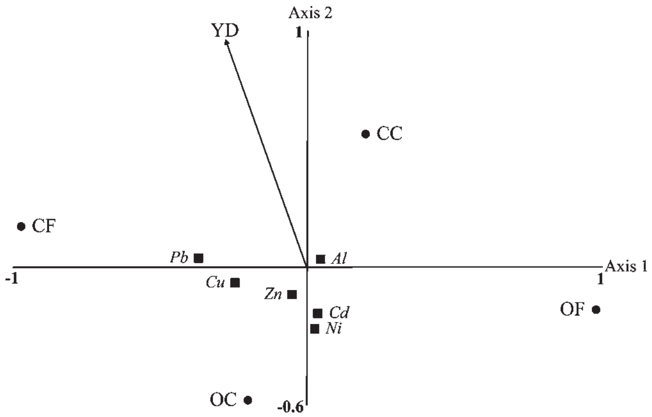

Figure 7. Biplot derived from the redundancy analysis showing the relationship between the heavy metals (Al, Cd, Cu, Ni, Pb, Zn) and agronomic factors (categorical variables, organic and conventional crop protection, OC, CC; organic and conventional fertility management, OF, CF indicated with a dot; continuous variable, yield, YD, indicated with an arrow).

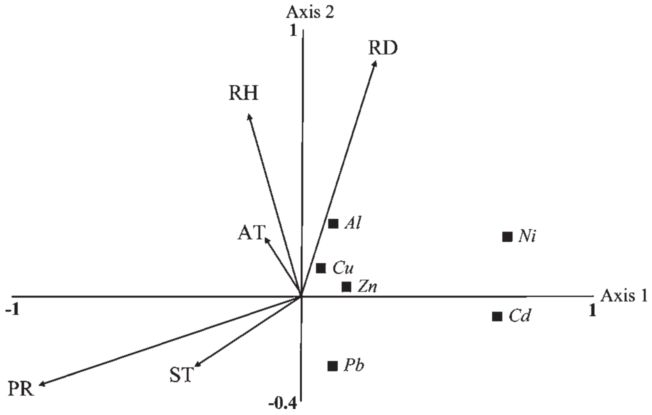

Figure 8. Biplot derived from the redundancy analysis showing the relationship between the heavy metals (Al, Cd, Cu, Ni, Pb, Zn) and environmental (AT, air temperature; ST, soil temperature; PR, precipitation; RH, relative humidity; RD, radiation) variables.

Eigenvalues indicated that axis 1 explained 18.0% of the variation and axis 2 a further 5.4%. There were strong associations along the negative axis 1 with precipitation and soil temperature and, to a lesser extent, air temperature and relative humidity. Increasing radiation and relative humidity was spread along the positive axis 2, generally opposite precipitation and soil temperature. The biplot indicates that the greater the precipitation and soil temperature, the less Ni and Cd in the grain, with similar, but far weaker, trends with Al, Pb, and Zn. Aluminum and Pb showed positive and negative relationships, respectively, to relative humidity and radiation with less influence of these variables on Ni, Cd, and Zn. None of the environmental variables had a strong effect on Cu. Precipitation ($F = 32.4$, $P = 0.002$), radiation ($F = 10.7$, $P = 0.006$), and relative humidity ($F = 6.4$, $P = 0.010$) explained significant amounts of additional variance.

Discussion. The metals cadmium (Cd) and lead (Pb) have no useful function in humans, are toxic at very low levels, and are known to accumulate in the body.[8] For example, Cd is known to accumulate in the liver (half-life 5−10 years) and especially the kidneys (half-life 10−30 to 100 years) of humans.[33] Lead absorption in humans was linked to reduced cognitive development in children and increased blood pressure and cardiovascular disease in adults, while cadmium may cause kidney dysfunction, skeletal damage, and affect reproductive health and can currently not be excluded as a human carcinogen.[34] The EC scientific committee for food therefore recommended that efforts should be made to further reduce dietary exposure to Cd and Pb, and this led to the adoption of European legislation which sets maximum residue levels for Cd (100 μg kg^{-1} wet weight) and Pb (200 μg kg^{-1} wet weight) in foodstuffs.[35]

In this context, the relatively large increase in Cd concentrations in wheat grain observed when conventional fertilization regimes were used in this experiment is of particular concern, since wheat is known to contribute significantly to the total dry matter intake of western European and North American diets.[36] Previous studies suggest that the increase in Cd content in conventionally fertilized wheat crops was most likely due to the Cd inputs associated with superphosphate applications in conventional fertilization regimes.[12,17−19] The Cd concentration in the 0:20:30 fertilizer used in the Nafferton experiments was 10.1 mg kg^{-1}, which is on the lower end of the range reported for P-fertilizers used in previous studies (5 and 64 mg kg^{-1}) that have linked P-fertilizer use to Cd levels in soils, groundwater, and crop plants.[37−42] This suggests that there is a risk of significantly higher levels of Cd contamination if P-fertilizers with a higher Cd content are used.

Other factors that affect root uptake and grain accumulation of Cd in crop plants include soil salinity,[43,44] application of Cl-containing fertilizers,[45,46] and Zn deficiency in soils and plants.[47,48] Our results indicate that an additional factor affecting Cd uptake in crop plants is the use of conventional fertilizer. It is therefore advisable in cropping systems where there is already an increased risk of Cd uptake (e.g., salty or Zn-deficient soils) to implement organic fertilization practices to reduce this risk.

The regular organic matter inputs (composted cow manure) used for vegetable and potato precrops in experimental plots under organic fertilization regimes may also have contributed to the differences in grain metal concentrations between fertilization regimes. For example, Cd availability in soil and subsequent plant uptake may have been reduced by higher concentrations of organic compounds in soils under organic fertilization regimes, which can lead to increased chelation of metals and reductions in plant availability of metals in acidic soils.[39]

An additional factor which may have increased Cd availability in the conventional fertility plots is soil pH. Results from the 2007 soils indicated significantly lower pH values in CF plots compared to OF plots (6.1 vs 6.3, $P < 0.0001$). The availability of most metals in soils increases as pH decreases.[49] This may explain the increased levels of grain Cu as well as Cd where conventional fertilizers were used.

The lower Al and Cu concentrations in grain from plots under conventional crop protection and the lower Ni levels in wheat grain from conventionally fertilized plots may have been the result of a "dilution effect" since higher yields/biomass production were associated with conventional management practices. This effect has been described previously for micronutrients in wheat and other crops[50] and is illustrated for our experiment in the RDA in Figure 8 which shows a negative correlation between yield and grain metal concentration.

The RDA indicated that some of the variation in grain metal contents could be explained by climatic factors. This confirms results from previous studies.[11,12] In our study in seasons with relatively higher precipitation levels and air and soil temperatures, wheat grain had lower concentrations of Ni and Cd (and to a lesser extent Al). Also relatively higher radiation and relative humidity levels correlated with lower Pb but an increase in Al concentrations in wheat grain. The effects of environment on wheat metal concentrations will be further tested/confirmed by including data sets from additional harvest years in the RDA in the future. Clearly these investigations should include plant physiological studies (e.g., quantification of xylem transport rates for metals in plants) which could explain associations between environmental parameters and metal loads. For example, poor xylem transport may be one explanation for the negative association between Pb concentrations in grain and high humidity, which is known to negatively affect phloem immobile elements such as Ca.[51] Such studies may also support the identification of cultivars with low metal uptake capacity and their use as pollution safe cultivars.[16]

This study has demonstrated a strong relationship between crop management practices, in particular fertility management, and grain metal concentrations. The increased levels of grain Cd under conventional fertility management are of particular concern, although all values measured in this study were still below the residue levels proscribed by the EU. Seasonal variations in metal levels were high and frequently interacted with crop management practices to produce differing results in each year. As more data is collected from this long-term trial, it will be possible to further elucidate the relationships among crop metal contents, crop management practices, and environmental variables.

AUTHOR INFORMATION

Corresponding Author

*Phone: +44 1661 830 222. Fax: +44 1661 831 006. E-mail: c.leifert@ncl.ac.uk.

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