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CADMIUM AND LEAD IN BRITISH WHEAT AND BARLEY: SURVEY RESULTS AND FACTORS AFFECTING THEIR CONCENTRATION IN GRAIN

by

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Abstract

An investigation of cadmium and lead uptake into British wheat and barley is reported. The results of cadmium and lead analyses performed on samples from the 1998 Cereals Quality Survey, and representative paired soil and grain samples collected from the 1998-2000 harvests are reported. Concentrations of cadmium and lead in the vast majority of samples were below the newly-introduced European Commission Regulation specifying the maximum permissible contaminant levels in foodstuffs. In general, wheat had higher grain concentrations of cadmium than barley, and both species had low concentrations of lead. In the paired soil and grain samples, total soil cadmium and soil pH were found to be the significant factors influencing grain cadmium concentrations. Significant varietal differences in cadmium uptake were observed for both wheat and barley, with certain cultivars having higher concentrations in both the field and pot experiment samples.

The effects of previous sludge applications on wheat and barley grain cadmium concentrations were investigated at two sites with different soil textures. Grain cadmium concentrations approached the maximum permissible contaminant levels at different total soil cadmium concentrations at the two sites, indicating that caution is required in using soil total metal concentration data in isolation for evaluating the potential for grain to exceed the European Commission levels. However, soil pH and total soil cadmium concentration when used together explained a large amount of the variation occurring in grain cadmium concentrations at the two sites.

The effects of fertiliser rate and application forms, and the addition of various soil amendments on cadmium and lead uptake in wheat and barley, were also investigated using pot experiments under controlled environment conditions. The applications of lime and an oxide-rich soil amendment were shown to reduce the concentration of cadmium in grain grown on a non-calcareous soil. Samples from a field experimental site also showed that higher application rates of ammonium nitrate fertiliser could lead to higher grain cadmium concentrations.

Practical implications and recommendations based on these results are discussed. In addition, a short comment on analytical quality control issues with regard to submission of samples for cadmium and/or lead analysis to commercial laboratories is provided.

Introduction

In recent years, there has been increasing awareness and concern over heavy metal contamination of soils and the potential effects this may be having on the food chain. High concentrations of heavy metals in agricultural soils can occur naturally, or *via* the application of metal-contaminated sewage sludges, animal manures, fertilisers and atmospheric deposition (Ryan *et al.*, 1982; Alloway and Steinnes, 1999). With regard to human health, the cadmium (Cd) and lead (Pb) concentrations of agricultural produce are of particular importance, as the consumption of agricultural foodstuffs is thought to contribute significantly to the dietary intake of these metals.

The entry of cadmium into the food chain is of concern as it can cause chronic health problems in humans such as bone disease, lung oedema, renal dysfunction, liver damage, anaemia, hypertension and has recently been associated with brittle bones (Nordberg, 1974; Nath *et al.*, 1984; Staessen *et al.*, 1999). Due to this, cadmium is one of a very small group of metals for which the FAO/WHO (1978) have set a limit for the provisional daily intake by humans (70 μg cadmium/day). For example, in the 1980's the US adult population was reported to receive about 20% of the FAO/WHO (1978) allowable daily intake of cadmium from the consumption of grain and cereal products (Wagner *et al.*, 1984). In contrast, during the same period in the early 1980's, grain and cereal products accounted for about 30 to 40% of the daily allowable cadmium intake in the European Community (Hutton, 1982). Similarly, lead is a known physiological and neurological toxin that, together with cadmium, bioaccumulates within the body. Lead is particularly hazardous for children, where it can induce reductions in cognitive development and intellectual performance. In adults, excessive exposure to lead may cause a variety of conditions including renal dysfunction, increased blood pressure, cardiovascular disease and the inducement of a number of trace element deficiencies (Ferguson, 1990).

Agricultural management practices that directly affect cadmium and lead concentrations in the soil and soil solution may influence cadmium and lead accumulation by crops. Specifically, the addition of sludge or fertiliser having high cadmium and lead concentrations to agricultural land may cause significant increases in the uptake of these metals by crops (Grant *et al.*, 1999). Recent research suggests that inputs of *ca*. 40 tonnes of cadmium and 767 tonnes of lead annually enter agricultural soils in England and Wales (Alloway *et al.*, 2000). In that work, approximately 52% of cadmium and 76% of lead was from atmospheric deposition, with a further 5% and 15% of cadmium and lead coming from sewage sludge applications. However, the total amounts of atmospheric deposition for the whole country can be misleading, as they are based on small amounts per unit area multiplied by a very large land area. McGrath (2000) has shown that the effect of concentrated sources such as sewage sludge and industrial wastes applied to specific areas of land are by far the most important inputs for those particular areas. Those areas that receive applications either currently or in the past, therefore present the greatest risk at the field level of exceeding food regulation limits now or in the future.

The impacts on grain metal concentrations of long-term anthropogenic inputs to soils are still not clear. A number of investigators have shown that the long-term application of cadmium-containing fertilisers may increase cadmium uptake by crops (Andersson and Siman, 1991; Nicholson *et al.*, 1994). In contrast, similar studies have indicated that, often despite an increase in soil cadmium content with P fertiliser application, cadmium concentration in crops has not increased with long term fertiliser use (Mordvedt, 1987; Jones and Johnston, 1989; He and Singh, 1993). In general, it appears that the rate and total loading of contaminant inputs, the soil type and the crop variety are important factors in the relationship between added metal and plant uptake.

The presence of high concentrations of contaminant metals in agricultural produce can have limitations on its sale in the international community, and there are a growing number of examples where this has affected exports. Durum wheat shipped from the US to Finland and Switzerland, peanuts to Australia and flax to Germany have been rejected because of their cadmium concentrations. Sunflower kernels from the US shipped to Germany have come under extreme limitations due to cadmium, which in turn forced the industry to change where the crop was grown to minimise cadmium in the kernels. Previous international grain cadmium and lead limits of 0.1 mg/kg fresh weight have led the US agricultural community to seriously evaluate and quantify the concentrations of cadmium and lead in their agricultural products. However, evidence on cadmium and lead concentrations in cereals has previously been difficult to obtain, as this issue can be a sensitive one. A survey conducted by Rothamsted in 1993 indicated that about 4% of UK wheat grain samples exceeded 0.1 mg/kg cadmium (Chaudri *et al.*, 1995). Little work has been done on grain cadmium and lead in the UK recently, but the Maltsters Association of Great Britain reported values for lead in barley grain of up to 0.9 mg/kg, with as many as 38% of 65 samples taken from grain stores exceeding 0.1 mg/kg (Farmers Weekly, 16 May 1997).

In consultation with the constituent member states, the European Union has recently introduced legislation defining the maximum permissible levels for cadmium and lead concentrations in a range of foodstuffs, including wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) grain (European Commission, 2001*a*). For cereals excluding wheat grain, bran, germ and rice, the maximum permitted cadmium concentration is 0.1 mg/kg (fresh weight), while the respective limit for the aforementioned exceptions at 0.2 mg/kg (fresh weight). For lead, the corresponding limit for all cereals is 0.2 mg/kg (fresh weight).

An important point from the regulation is that "Food ingredients used for the production of compound foodstuffs should comply with the maximum levels set in this Regulation prior to addition to the said compound foodstuff in order to avoid dilution". This would therefore appear to preclude the possibility of dilution of non-conforming grain batches with grain batches having lower metal concentrations. It is also noted that the regulation stipulates the Commission will review the maximum levels of heavy metals every five years, before 5 April 2003 for the first time, with an overall objective of ensuring a high level of consumer health protection. Elsewhere it is stated that these reviews will take account of the advance of scientific and technical knowledge and improvements in manufacturing or agricultural processes, with the objective of steadily decreasing the maximum permitted levels of cadmium and lead in food.

Project Objectives:

As UK soils may potentially be more contaminated with cadmium and lead than those in the US and Canada (McGrath and Loveland, 1992), it was important that good information was obtained on the range of cadmium and lead concentrations in British grain in order to:

(1) anticipate any threats to farming practices and viability of exports,
(2) understand the factors which lead to high cadmium and lead concentrations, and
(3) assess possible strategies for reducing these contaminants to acceptable levels in situations where high crop metal uptake occurs.

This project was initiated in 1998, partially as a response to the concerns of the milling and malting industries that, based on their earlier commissioned research, significant amounts of UK grain may exceed the proposed EU food quality standards circulating in draft form at that time. The specific objectives of the project were:

(1) To assess the geographical distribution of cadmium and lead concentrations in grain samples and relate these to soil properties.

(2) To quantify the effects of variety, nitrogen fertiliser form and rate, and soil amendments on the cadmium and lead uptake into cereal grains.

(3) To measure to what extent soil or dust contamination may be responsible for observed high concentrations of metals in grain samples.

(1) To measure the effects of past sewage sludge applications on grain cadmium and lead, using existing field experiments.

Materials and Methods

1998 Cereals Quality Survey

250 wheat and 233 barley samples from throughout the UK were collected during the 1998 HGCA Cereals Quality Survey. Crop variety and sampling location (by county) were recorded. For analyses by location, counties were grouped into the geographical regions used in Cereals Quality Survey publications (South Eastern, South Western, Midlands and Western, Wales, Northern, Scotland, Northern Ireland and the East). Grain samples were analysed for cadmium and lead as described below.

National Survey of Milling Wheat and Malting Barley Crops

Sampling strategy and rationale

Paired soil and crop samples from milling wheat and malting barley crops were collected across the main cereal growing areas in England and Wales by ADAS staff shortly before harvest. There was a target of *ca*. 40 sampling sites in harvest year 1998 and *ca*. 110 sites in both the 1999 and 2000 harvest years. In practice, a total of 44, 116 and 120 paired soil and crop samples were successfully collected in each respective year. The corresponding target number of sites in Scotland, which were sampled by SAC staff, were 10 in 1998 and 40 in each of the subsequent two harvest years. Within each year the aim was to collect equal numbers of wheat and barley crop samples, including both autumn- and spring-sown crops.

In the 1998 survey, sites were selected at ADAS Research and Crop Centres and on commercial farms, to represent a range of soil types throughout England with typical soil concentrations of cadmium and lead. In 1999 and 2000, the site selection criteria were targeted to include as many sites as possible with high soil concentrations of cadmium and/or lead in the sampling programme. Potential sites were identified according to knowledge of:

- naturally high background levels (due to geochemical factors);
- potential contamination from past sewage sludge/industrial or other waste applications;
- proximity to major roads or motorways;
- effects of past mining activities.

Data from the National Soils Inventory (McGrath and Loveland, 1992) were also used to identify localised areas in England and Wales where systematic grid sampling and analysis of top soils had shown above average soil concentrations of cadmium and lead. Where these areas were suitable for cereal cropping, attempts were made to locate specific sites for sampling. This approach proved moderately successful in identifying such sites, with approximately 20% and 30% of the sites sampled having respective soil cadmium and lead concentrations above the median soil values of 0.7 and 40 mg/kg in England and Wales, respectively (McGrath and Loveland, 1992). Information recorded at the sampling site included details of site and sampling location, soil type, history of manure and/or sewage sludge applications in last 10 years, fertiliser inputs, crop species and variety, drilling and harvest dates, and proximity of the sampling location to any major roads or industrial sites.

Sampling Method

At each site, a 1m^2 quadrat was placed at random in the field or plot to be sampled. A 0-15 cm top soil sample (*ca.*1 kg in weight and comprised of 15 cores) was obtained by manual coring within the quadrat area. A crop sample was collected at the same time by hand-cutting the crop near ground level from all of the marked quadrat area. The whole-crop sample was subsequently threshed and the resulting grain sample retained for analysis.

Grain and Top Soil Analyses

Soil samples were air dried and mechanically ground through a 2-mm sieve. The sample was then split into two sub-samples prior to analysis by ADAS for pH (in water), sodium bicarbonate-extractable P, ammonium nitrate-extractable K and Mg (Anon, 1986), organic matter (by loss on ignition based on the method described by Ball, 1964) and for cadmium and lead analysis by IACR-Rothamsted (details given below).

Each grain sample obtained from threshing was oven dried overnight at 100°C ±2ºC to 100% dry matter content, prior to laboratory analysis for cadmium and lead concentrations.

Historic Sewage Sludge Experimental Sites

Historic sewage sludge experimental sites at ADAS Gleadthorpe and at ADAS Rosemaund were sampled to provide further site-specific information on the effects of soil parameters on cadmium and lead concentrations in the grain of cereal crops. Grain and soil samples were taken from each experimental plot at Gleadthorpe, and from selected treatments at Rosemaund in both the 1999 and 2000 harvest years.

At Gleadthorpe, a total of twenty two sludge treatments were established in 1982 and 1986 to obtain a range of soil heavy metal concentrations (Table 1). At Rosemaund, untreated controls and nine sludge treatments were established between 1968 and 1971 that had varying amounts of cadmium and lead. (Table 1). There were two or four replicates per treatment at Gleadthorpe and Rosemaund, respectively.

Table 1. Top soil characteristics in samples taken in 2000 from the Gleadthorpe and Rosemaund sewage sludge sites.

Site	Texture	Clay	pH*		Organic matter (%)		Cadmium	Lead
		(%)	Mean	Range	Mean	Range	(mg/kg)	(mg/kg)
Gleadthorpe	Loamy sand	6	6.1	5.5-6.5	3.16	2.17-4.58	0.14-1.07	17-89
Rosemaund	Sandy loam	14	6.6	5.7-7.1	4.20	3.47-4.68	0.04-2.70	21-48

* Soil pHs of samples taken in 1999 were 5.3-6.6 for Gleadthorpe and 6.2-7.2 for Rosemaund.

Sampling procedures

Crop samples, comprising approximately 100 tillers per treatment, were taken shortly before harvest to determine Dry Matter Harvest Index per treatment after threshing of the whole crop samples to separate the grain from the straw plus chaff. Grain yield per plot was measured by hand cutting the crop at harvest and a cleaned sub-sample (*ca*. 500 g) of the threshed grain was taken per plot to determine dry matter content and to provide a dried sub-sample for heavy metal analysis.

Top soil samples (0-15 cm, *ca*. 500 grams from 10 cores per plot) were taken manually shortly after harvest, air-dried and mechanically ground through a 2 mm sieve. The sample was then split into two sub-samples prior to analysis for various soil parameters at the ADAS and Rothamsted laboratories as described above.

Grain Washing Experiments

To establish whether high concentrations of cadmium or lead measured in the grain occurred as a result of plant uptake or were due to external factors such as surface contamination prior to the samples being received for analysis, a series of grain washing experiments were performed. Sub samples of whole grain (5 g) were rinsed for 2 mins with 10 mL of either (1) ultra-pure water, (2) 0.05 M of the chelating reagent ethylenediaminetetraacetic acid (EDTA) or (3) 1% HNO_3. The grain samples were subsequently rinsed with ultra-pure water, before being dried (80^{o}C for 24 hrs) and microwave digested (see below) without being ground. The 10 mL washing solutions (which contained particulate matter washed off the grain samples) were evaporated to approximately 1 mL volume, before being digested using the *aqua-regia* soil digest method described below. The grain and washing digest solutions were analysed for cadmium and lead by graphite furnace atomic absorption, while marker elements (e.g. titanium) which indicate the presence of soil/dust contamination in plant samples were analysed by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

Wheat and Barley Cultivar Pot Experiment

A pot experiment was performed to investigate the effect of wheat and barley cultivars on the uptake of cadmium and lead. The soil used for this experiment was collected from Buxton in Derbyshire and had previously been identified as having relatively high cadmium concentrations. Table 2 shows the locations and selected soil properties of this and the other soils used for pot experiments in this work.

Table 2. Selected properties of soils used for pot experiments.

Soil	County	pH	Organic C (%)	Total N (%)	Cadmium mg/kg	Lead mg/kg	Zn mg/kg
Buxton	Derbys.	5.90	6.30	0.55	1.62	134	230
ADAS Bridgets	Hants.	8.14	2.94	0.30	1.51	25	83
Leatherhead	Surrey	8.16	1.69	0.19	0.94	34	79

The cultivars selected for use were Consort, Hereward, Malacca, Rialto, Riband and Soissons (winter wheats) and Chariot, Fanfare, Gleam, Optic and Riviera (winter and spring barley). Four replicates were grown for each cultivar. Soil (sieved <5 mm, 1 kg dry weight) was weighed into each pot and adjusted to a moisture content of approx. 70% water holding capacity (WHC) using deionised water. The winter wheat and winter barley cultivars were germinated in vermiculite and vernalised at 6^{o}C for 6 weeks before being transplanted into the pots (4 seedlings per pot). Pots were watered with deionised water in the saucers and arranged in a glasshouse with controlled environmental conditions: day/night duration 16/8 hrs and day/night temperatures 20/16^{o}C. Each pot received a basal nutrient solution dressing of 78 mg P (KH_2PO_4), 100 mg K (KH_2PO_4), 32 mg Mg ($MgSO_4.7H_2O$), 43 mg S ($MgSO_4.7H_2O$), 3 mg Mn ($MnCl_2.4H_2O$), 0.7 mg B (H_3BO_3) and 0.3 mg Mo ($Na_2MoO_4.2H_2O$). This was applied in two equal applications, before stem elongation and a second application before anthesis. Nitrogen was added as NH_4NO_3, with each pot receiving 400 mg N applied in two equal applications together with the nutrient solution applications.

Plants were harvested at maturity by cutting the stems at soil level. Grain was threshed mechanically and fresh grain yields measured. After threshing, the grain was ground to <0.5 mm using a Reitch ultra-centrifugal stainless steel mill and dried at 80°C prior to analysis and digestion.

Fertiliser Form and Soil Amendment Pot Experiments

A second pot experiment was established to investigate the effect of N fertiliser form and the application of various soil amendments on the uptake of cadmium and lead by selected winter wheat (Rialto) and winter barley (Regina) cultivars. The soil used for these experiments was the Buxton Derbyshire soil as used in the cultivar experiment described above.

Three nitrogen fertiliser and three soil amendment treatments for each cultivar were used:

(1) NH_4NO_3
(2) NH_4^+
(3) NO_3^-
(4) 6% moist peat
(5) 1% lime
(6) 2% red mud

Red mud is an oxide-rich industrial by-product that has the capacity to bind metals in the soil, hence making them less available for plant uptake. The peat, lime and red mud treatments were incorporated into the soil by mechanical mixing. Each treatment was replicated fourfold for each of the wheat and barley cultivars. Sieved soil (<5 mm, 1 kg dry weight) was weighed into each pot and adjusted to approx. 70% water holding capacity (WHC) using deionised water. Seeds were germinated in vermiculite and vernalised at 6°C for 6 weeks before being transplanted into pots (4 seedlings per pot). Pots were watered with deionised water in the saucers and arranged in a glasshouse with controlled environmental conditions: day/night duration 16/8 hrs and day/night temperatures 20/16°C.

Each pot received a basal nutrient solution dressing as described above, which was again applied in two equal applications, before stem elongation and the second application before anthesis. Nitrogen was added as NH_4NO_3 for the NH_4NO_3 treatments and for the three soil amendment treatments,

$(NH_4)_2SO_4$ for the NH_4^+ treatments, and $Ca(NO_3)_2.4H_2O$ for the NO_3^- treatments, respectively. Each pot received 600 mg N per pot applied in three equal applications, the first two applications being applied with the nutrient solution, and the final application 14 days after the second nutrient application.

Plants were harvested at maturity by cutting the stems at soil level. Prior to analysis stems were washed with deionised water and dried (80^{o}C for 24 hrs) before grinding in a stainless steel hammer mill (Glen Creston Ltd. Middx.). Grain was threshed mechanically and fresh grain yields measured. After threshing the grain was ground to <0.5 mm using a Reitch ultracentrifugal stainless steel mill and dried at 80^{o}C prior to analysis and digestion. After harvest, Rhizon moisture samplers (Rhizosphere Research Products, Wageningen, Holland) were used to extract soil pore water in the soil amendment pot experiment following the method of Knight *et al.* (1998). Cadmium concentrations in the pore water were determined using a Perkin-Elmer 4100ZL graphite furnace atomic absorption spectrophotometer (GFAAS) (Perkin-Elmer, Norwalk, CT) with Zeeman background correction. A palladium matrix modifier was used for cadmium analysis. Organic carbon in the pore water solution was determined using the non-purgeable organic carbon (NPOC) analysis function of a TOC 200 instrument (UV Developments, Cambs.).

Fertiliser Rate Field Experiment Samples

To investigate the effect of fertiliser application rate on grain uptake, samples previously archived at Rothamsted were reanalysed for cadmium and lead. These samples were harvested in 1995 from a field experimental site located at ADAS Bridgets, which had investigated the effect of sulphur and nitrogen fertiliser application rates on the sulphur content of wheat grain. Winter wheat (Cv. Hereward, a premium breadmaking cultivar) was sown during the 1994 autumn period and grown in an experiment with 12 treatments which consisted of factorial combinations of 2 N rates, 180 and 230 kg/ha, and six S rates of 0, 20, 40, 60, 80 and 100 kg/ha. All treatments were replicated in three plots (*ca.* 40 m^2) in a randomised block design. Nitrogen was applied as ammonium nitrate in two dressings during March and April, and S applied as gypsum (18% S) in March. Herbicides, fungicides and insecticides were applied according to good commercial practices and grain was harvested in August 1995. Further experimental details are given in Zhao *et al*. (1999).

Zinc Soil Amendment Pot Experiment

A third pot experiment was established to investigate the effect of zinc (Zn) applications to soil on the uptake of cadmium and lead into grain. Selected soil properties of the Bridgets and Leatherhead calcareous soils used for this experiment are given in Table 2.

Four Zn treatments (0 (control), 2, 5 and 10 mg Zn/kg soil) were established, with 4 replicates of each treatment per soil. Zinc was added to the soil as a $ZnSO_4$ solution, and was incorporated into the soil using a mechanical mixer. Sieved soil (<5 mm, 1.2 kg dry weight) was weighed into each pot and adjusted to approx. 70% water holding capacity (WHC) using deionised water. Winter wheat (Rialto) was germinated and vernalised as described above; 4 seedlings per pot were transplanted. Each pot received two equal applications of basal nutrient solution as described above. Nitrogen was applied as NH_4NO_3, with each pot receiving 600 mg N per pot in three equal applications. Pots were watered with deionised water into saucers and plants grown under the same controlled environmental conditions previously described. Plants were harvested at maturity, and stems and grain prepared for analysis as described above for the fertiliser and soil amendment pot experiments.

Chemical Analyses

Grain

Samples of grain, as received, were ground to <0.5 mm (Reitch ultracentrifugal stainless steel mill) and oven dried at 80^{o}C before analysis. Subsamples (*ca.* 1 g) of the dried and ground grain were digested in XP1500plus teflon® PFA microwave liners (CEM Corp, Matthews, NC) using 3 mL of Primar ultrapure concentrated nitric acid (70% w/v) (Fisher Scientific), 2 mL of Primar 30% w/v hydrogen peroxide (Fisher Scientific) and 7 mL ultra-pure (18 MΩ specific resistance) water (ELGA Maxima, High Wycombe, U.K.). A CEM model Mars X microwave (CEM Corp.) equipped with a 12 sample carousel was used for sample digestion. The in-built CEM system software was used to control the digestion conditions of the microwave (Table 3) using a control vessel constantly monitored for pressure and temperature control. After completion of the heating process the vessels were cooled and made up to volume (25 mL) with ultra-pure H_2O prior to analysis. Cadmium and lead concentrations in the digest solutions were determined by GFAAS using a palladium matrix modifier.

Table 3. Closed-vessel microwave digestion program (CEM Mars X).

Stage	Max power (W)	Temperature (^oC)	Ramp (min)	Hold (min)	Max pressure (psi)
1	1200	115	12	1	450
2	1200	175	8	10	450

The performance of the microwave digestion system was compared with that of an established nitric-perchloric acid open tube digestion method (Zhao *et al.*, 1994) using a heating block. The microwave digestion protocol gave acceptably consistent and reliable results for the elements studied when compared to the traditional technique (Adams *et al.*, 2001). The inclusion and analysis of an Internationally Certified Reference Material in each batch of samples for microwave digest also maintained quality assurance throughout the analytical process. The following standard reference materials relevant to the project were used during various periods for quality control: Community Bureau of Reference (BCR) CRM191 brown bread, National Institute of Standards and Technology (NIST) SRM1567a wheat flour, NIST RM8433 corn bran and NIST RM8436 durum wheat flour. A sample blank was also included in each microwave digest batch. Sample replicates were regularly analysed every *ca.* 15 samples. All glassware and microwave vessels were acid-washed and thoroughly rinsed with de-ionised and ultra-pure water before use. Microwave vessels were also regularly cleaned by microwaving capped vessels containing 20 mL of 50% nitric acid solution for 10 min at 175^{o}C.

The digestion and analytical method used in this work for grain analysis conform to the European Commission Directive (European Commission, 2001*b*) on sampling and analysis methods that accompanies the European Commission Regulation specifying the maximum permissible contaminant levels in food stuffs. Although the maximum permissible concentration limits in the latter regulation are specified with respect to fresh weight concentrations, in this work all grain concentrations are expressed on a dry-weight mg/kg basis. Assuming a standardised 85% dry-matter content, the effective European Commission limits for cadmium and lead in cereals on a dry weight basis are shown in Table 4.

Table 4. Effective maximum permissible concentration limits on a dry weight basis for cadmium and lead in cereals (85% dry-matter content assumed). All concentrations are in mg/kg.

Crop	Fresh weight limit		Effective dry-weight limit	
	Cadmium	Lead	Cadmium	Lead
Cereals (excluding wheat, bran, germ and rice)	0.100	0.200	0.118	0.235
Wheat, bran, germ and rice	0.200	0.200	0.235	0.235

Soil

Soil pH was determined using a glass electrode in a soil:water ratio of 1:2.5, and total soil N and C by Dumas combustion (Leco CNS 2000). Water holding capacity was determined using the soak and drain method of Parent and Caron (1993). Soil chloride was extracted with a 1:5 soil:water ratio following the method of Rayment and Higginson (1992), and determined by ion chromatography (Dionex DX500) fitted with an IonPac AS9-SC column and AG9 guard column. Soil Al, Fe, Mn and P oxides were extracted using a mixture of ammonium oxalate and oxalic acids (0.114/0.086 M) following the procedure of Janssen *et al.* (1997), before determination by inductively-coupled plasma atomic emission spectrometry ICP-AES (Fisons ARL Accuris).

Soils were air dried, sieved to <2.0 mm and finely ground to <150 µm in an agate ball mill, before analysis for cadmium and lead. Sub samples of 0.25 g were digested with aqua regia (4:1 v/v concentrated HCl-HNO_3), following the method of McGrath and Cunliffe (1985) and using the heating programme given in Table 5. Cadmium concentrations in the digest solutions were determined by GFAAS, as described above. Lead concentrations were measured by ICP-AES. Soil cadmium and lead concentrations are expressed on a dry weight basis (mg/kg). Quality control for soil analysis was ensured by the digestion of replicate samples of a BCR Certified Reference Light Sandy Soil CRM142R in each digestion batch of 54 samples. Two blanks were also included in each digestion batch and every 10th sample was digested in duplicate to verify the repeatability of the analysis procedure.

Table 5. Open-tube heating block program for *aqua regia* soil digestion.

Stage	Ramp rate ($^{o}C\ h^{-1}$)	Dwell temp. (^{o}C)	Dwell time (h)
1	60	25	2
2	120	60	3
3	120	105	1
4	120	125	2

Statistical Analyses

Statistical analyses, including multi-linear regressions and analysis of variance (ANOVA) were performed using Genstat 5 for Windows (NAG, 1998). Where appropriate, variates that showed skewed distributions were log-transformed prior to statistical testing to achieve normality. For samples having cadmium or lead concentrations below the respective analytical detection limits (i.e. <0.003 mg/kg dry weight for cadmium and <0.020 mg/kg dry weight for lead), concentrations equal to half the value of the detection limit for that element were used in the subsequent statistical analyses.

Results and Discussion

Quality Control Sample Results

As previously discussed in the Materials and Methods section, sample repeats, blanks and Internationally Certified Reference Materials were routinely analysed in each batch of samples. The inclusion and analysis of reference materials, and/or well-characterised in-house reference samples is an important aspect of laboratory quality assurance. The standards used should ideally be of a similar matrix to the samples requiring analysis i.e. wheat flour standards were used extensively in this work for analysis alongside the unknown wheat samples, whereas a standard reference soil was analysed alongside the soil samples. Furthermore, the reference samples should also ideally contain the required elements at similar concentrations to those being measured in the samples.

Laboratory participation in ring-test schemes is also a valuable method to establish the validity of analytical protocols. In this work, cadmium and lead were analysed in unknown samples provided quarterly by the International Plant Exchange analysis scheme co-ordinated by Wageningen University, the Netherlands. This allowed an independent check that the sample concentrations determined by the Rothamsted laboratory agreed with those determined by other laboratories worldwide.

If requested, commercial (accredited) laboratories should be able to produce evidence of their analytical performance and accuracy of a given analysis. It might for instance, also be of relevance to request the analytical results of any standard reference material analysed by the laboratory at the same time as any submitted samples, to confirm that the determined concentration falls within the stated certified concentration range. If selected samples are analysed by a laboratory in duplicate or triplicate within an analysis batch it may also be pertinent to request that the individual replicate results are reported together with the final (mean) values. This will allow the analytical reproducibility to be inspected. If the standard deviation obtained from the individual determinations is large compared to the mean value, the results should be regarded with caution. Different types of analyses will have intrinsically different variabilities, but assuming the concentrations being measured are not near the stated detection limit of a technique, then the standard deviation calculated from the individual replicate values should generally not exceed about 15% of the mean value. Laboratories may also be able to provide a measure of their performance in ring tests that they participate in. Usually this is in the form of a test statistic (e.g. Z-score) that measures how closely concentrations determined in that particular laboratory agree with those determined by all laboratories in the scheme. Although certain commercial laboratories may on occasion be reluctant to release such details, the view can be taken that a paying customer is entitled to information of this nature, as it has a direct bearing on the confidence that can subsequently be given to the reported results.

Results of certified reference samples that were analysed during the various sections of this work are presented in Table 6. The summarised quality control data shows that the digestion and analysis procedures gave reliable and reproducible results for the certified reference materials analysed. The analytical results were therefore deemed to be accurate.

1998 Cereals Quality Survey

Barley

The mean concentrations of cadmium and lead in barley were 0.022 and 0.038 mg/kg, respectively (Table 7). Differences in cadmium and lead concentrations according to the region of crop growth were not statistically significant. However, significant differences were observed (P<0.001) between the mean cadmium concentrations of the twelve barley varieties for which 5 or more samples were analysed (Table 7). Alexis (malting) and Fighter (feed) had the lowest mean cadmium concentrations (0.009 mg/kg) and Melanie (malting) the highest (0.042 mg/kg). For lead, varietal mean concentrations (for samples having n ≥ 5) varied from 0.024 mg/kg (for malting variety Optic) to 0.050 mg/kg (Melanie), but the differences were not significant.

The frequency distributions of cadmium and lead concentrations in barley (Fig. 1) confirm that the vast majority of samples were within the permitted concentration limits. Of particular note was the large number of samples (*ca*. 30%) that had lead concentrations lower than the detection limit of the analytical method (<0.020 mg/kg dry weight). Of the 233 barley samples, only 13 had cadmium concentrations higher than half the maximum permitted concentration, and 80% of samples had concentrations lower than one quarter of the cadmium limit. Only one sample (from West Sussex) exceeded the effective 0.118 mg/kg dry weight concentration limit for cadmium. A similar situation was observed for lead, with only four samples being over half the effective 0.235 mg/kg limit and 84% lying below one quarter of the limit. Two samples (<1% of the total) exceeded the limit for lead (samples from Isle of Wight and N. Ireland).

Table 6. Summarised quality control data showing mean cadmium and lead concentrations (mg/kg) and standard deviations (sd) determined in certified reference materials analysed for method validation and process control purposes.

Sample Type and Experiment		Cadmium			Lead			No.
		certified [A]	mean	sd	certified [A]	Mean [B]	sd	
Grain samples:	National Survey of Milling Wheat	0.026 ± 0.002 [D]	0.026	0.002	<0.015 [D]	<DL	-	41
	and Malting Barley [C]	0.0284 ± 0.0014 [E]	0.030	0.003	0.187 ± 0.014 [E]	0.187	0.018	7
Soil samples:	National Survey of Milling Wheat and Malting Barley [C]	0.25 ± 0.01 [F]	0.249	0.02	25.7 ± 1.6 [F]			23
Grain samples:	Grain Washing Experiments	0.026 ± 0.002 [D]	0.028	0.001	<0.015 [D]	<DL	-	
Grain samples:	Cultivar Pot Experiment	0.026 ± 0.002 [D]	0.028	0.001	<0.015 [D]	<DL	-	7
Grain samples:	Soil Amendment Pot Experiment	0.026 ± 0.002 [D]	0.025	0.003	<0.015 [D]	<DL	-	11
Grain samples:	Fertiliser Rate Field Experiment	0.026 ± 0.002 [D]	0.028	0.001	<0.015 [D]	<DL	-	4

[A] Uncertainties are expressed as 95% confidence intervals.

[B] Detection limit for lead <0.020 mg/kg.

[C] Including samples from the Historic Sewage Sludge Experimental Sites.

[D] National Institute of Standards and Technology (NIST) SRM1567a wheat flour.

[E] Community Bureau of Reference (BCR) CRM191 brown bread.

[F] BCR Certified Reference Light Sandy Soil CRM142R.

Table 7. Mean and standard deviation (σ) of barley grain cadmium and lead concentrations (dry weight) for crop varieties having 5 or more samples.

Variety	Cadmium (σ) (mg/kg)		Lead (σ) (mg/kg)		No. of Samples
Alexis	0.009	(*0.005*)	0.027	(*0.017*)	12
Angora	0.030	(*0.022*)	0.040	(*0.020*)	15
Chariot	0.019	(*0.016*)	0.036	(*0.017*)	17
Fanfare	0.015	(*0.013*)	0.039	(*0.037*)	28
Fighter	0.009	(*0.001*)	0.026	(*0.018*)	5
Gleam	0.015	(*0.013*)	0.043	(*0.024*)	24
Halcyon	0.018	(*0.021*)	0.031	(*0.027*)	26
Intro	0.021	(*0.014*)	0.030	(*0.020*)	26
Melanie	0.042	(*0.029*)	0.050	(*0.036*)	6
Optic	0.014	(*0.008*)	0.024	(*0.012*)	18
Regina	0.027	(*0.018*)	0.046	(*0.077*)	40
Riviera	0.026	(*0.006*)	0.043	(*0.032*)	6
Grand mean (all samples)	0.022	(*0.031*)	0.039	(*0.046*)	233

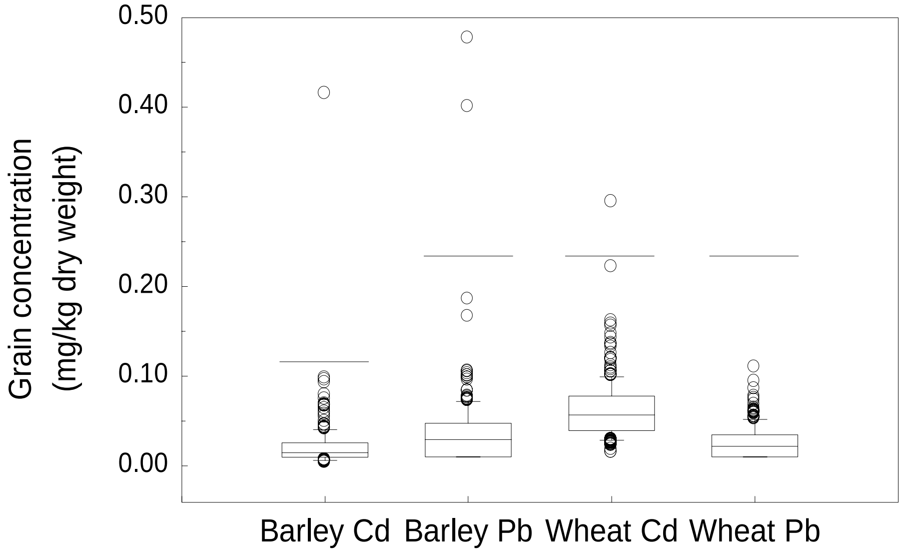


Figure 1. The distribution of concentration ranges for cadmium and lead in barley (n = 233) and wheat (n = 250) samples collected as part of the 1998 Cereals Quality Survey. The boxes represent the 25th to 75th percentiles and the horizontal solid line within indicates the median concentrations. The ends of the T bars mark the 10th and 90th percentiles, and the circles represent outlying points. The horizontal bars represent the effective European Commission concentration limits on a dry matter basis.

Wheat

The distribution of cadmium and lead wheat grain concentrations is shown in Fig. 1. Means for cadmium and lead concentrations in wheat were 0.063 and 0.025 mg/kg, respectively (Table 8). Differences between regions with respect to cadmium and lead concentrations were not statistically significant.

Table 8. Mean and standard deviation (σ) of wheat grain cadmium and lead concentrations (dry weight) for crop varieties having 5 or more samples.

Variety	Cadmium (σ) (mg/kg)		Lead (σ) (mg/kg)		No. of samples
Brigadier	0.065	(*0.030*)	0.020	(*0.015*)	26
Charger	0.069	(*0.016*)	0.013	(*0.007*)	11
Consort	0.053	(*0.025*)	0.025	(*0.014*)	28
Hereward	0.062	(*0.047*)	0.028	(*0.019*)	45
Hussar	0.074	(*0.041*)	0.025	(*0.019*)	22
Reaper	0.055	(*0.032*)	0.031	(*0.025*)	27
Rialto	0.074	(*0.029*)	0.031	(*0.022*)	29
Riband	0.060	(*0.027*)	0.022	(*0.015*)	62
Grand mean (all samples)	0.063	(*0.029*)	0.025	(*0.018*)	250

Significant varietal differences for cadmium were observed (P<0.05), although the differences in mean concentrations between varieties were not large. Of the eight wheat varieties, Rialto (breadmaking) and Hussar (feed) had the highest mean cadmium concentrations (0.074 mg/kg), and Consort (biscuit wheat) the lowest (0.053 mg/kg). Significant varietal differences in mean lead concentrations were also observed (P<0.05). As was observed for barley, a large number of wheat samples (*ca*. 50%) had concentrations below the detection limit. Only one sample (0.4% of the total number) (from S. Wales) had a cadmium concentration greater than the effective 0.235 mg/kg dry weight legislative limit, although a second sample from Dorset approached this limit. Of the remaining samples, 94% had cadmium concentrations of less than half the limit. All wheat samples had lead concentrations of less than half the maximum permitted concentration.

The concentration distribution plots for the wheat and barley samples (Fig. 1) indicate that on average, the concentration of cadmium in wheat tends to be higher than that observed in barley. In contrast, the distributions of lead concentrations in wheat and barley were similar. This difference in cadmium uptake between these species has been reflected in the Commission regulation, where wheat grain is listed as an exception to the general cereals classification and given a higher maximum permissible concentration limit of 0.2 mg/kg (fresh-weight) instead of the general cereals 0.1 mg/kg fresh-weight limit. Nevertheless, it is clear on the basis of this 1998 survey data that should the cadmium wheat limit be re-evaluated and adjusted downward, the majority of UK grain would still be able to meet the lower limits. For example, a limit of 0.15 mg/kg (fresh-weight) would have caused only one additional sample to breach the limit (the Dorset sample previously mentioned that approached the 0.2 mg/kg fresh-weight cadmium limit). Should the cadmium limit be reduced further still to the level of the general cereals limit (0.1 mg cadmium/kg fresh weight) then a further 12 samples would have breached the limit, or 5.6% of the total number of wheat samples collected in the 1998 Cereals Quality Survey.

In a previous study, Chaudri *et al.* (1995) investigated the cadmium content of British wheat grain in samples collected for the HGCA wheat quality assessments in 1982, 1992 and 1993. Mean wheat cadmium concentrations in samples from 1992 and 1993 were similar (0.042 and 0.038 mg/kg dry weight respectively), but were slightly lower than the mean concentration in the 1982 samples (0.052 mg/kg). The mean cadmium concentration of wheat determined in this study was 0.063 mg/kg (Table 8), slightly higher than the determined values from the previous years. The observed increase in mean cadmium concentration was a little unexpected given that mean wheat cadmium concentrations in the UK might have been expected to decline as a result of 1) decreased cadmium fallout from the atmosphere due to controls on industrial emissions, 2) growth dilution due to increased wheat yields, 3) use of P fertilisers lower in cadmium (Chaudri *et al.*, 1995). However, a number of factors such as variable growth and climatic conditions, and changes in the cultivars grown over the 16-year period may have caused the observed annual variations in cadmium uptake in wheat grain.

In conclusion however, it is clear from the results of samples collected during the 1998 Cereals Quality Survey, that the vast majority of UK wheat and barley samples will be able to meet the present maximum concentration limits specified in the European Commission Regulation.

The significant differences in grain cadmium concentration between cultivars, shown in Tables 7 and 8, should be interpreted with caution. This is because climatic, geographical and/or soil variations between the field sampling sites may have been responsible for, or contributed toward the differences in cadmium uptake, and not the crop variety *per se*. The situation is further complicated due to the relatively small (and variable) number of samples for each variety and geographical combination in the

Cereals Quality Survey data. As the variations within each classification are unlikely to be normally distributed, interpretation of statistically significant results should be attempted with caution. For these reasons, it is difficult to assess the true significance of differences from field data alone. Cultivar differences should be investigated under controlled conditions (see later).

National Survey of Paired Crop and Soil Samples

The two primary aims of the wheat and barley survey research were:

(1) to continue monitoring concentrations of cadmium and lead in grain samples from around Britain to establish the likelihood that the maximum permitted concentrations specified in the European Commission regulation would be exceeded, and
(2) to investigate the relationships between plant, land management and selected soil factors that could be used to predict grain cadmium concentrations.

A number of previous studies have investigated the relationships between various soil characteristics and uptake of cadmium in plants (e.g. Krishnamurti *et al.*, 1995; Mench *et al.*, 1997; Smolders *et al.*, 1998; Gray *et al.*, 1999; McLaughlin *et al.*, 1999). However, many such studies have used contaminated soils and/or soils spiked with either sewage sludge or inorganic metal salts in glasshouse experiments that do not translate to actual field conditions. Studies such as those reported here which use paired soil and grain samples from a large number of field sites that encompass a wide variety of soil types, geographic and climatic variations etc. are few.

Wheat grain cadmium

A wide range of wheat grain cadmium concentrations was found over the three harvest periods sampled (1998-2000), with values ranging from 0.010 mg/kg to 0.620 mg/kg dry weight. Median and mean grain cadmium concentrations were 0.056 mg/kg and 0.077 mg/kg, respectively, for the three-year period. The annual distributions of cadmium concentrations in wheat grain were similar (Fig. 2). Likewise, there was no statistical difference between the mean grain cadmium concentrations over the three years (P>0.05). The median cadmium concentrations for each of the three years (0.051, 0.059 and 0.056 mg/kg for the years 1998, 1999 and 2000, respectively) compare well with the median concentration (0.057 mg/kg) determined in samples from the 1998 Cereals Quality Survey discussed above. As was observed in the Cereals Quality Survey work, significant differences (P<0.001) were again observed between wheat varieties where the number of varieties sampled over the three year period was ≥ 5. Mean cadmium concentrations in milling wheat varieties are shown in Table 9.

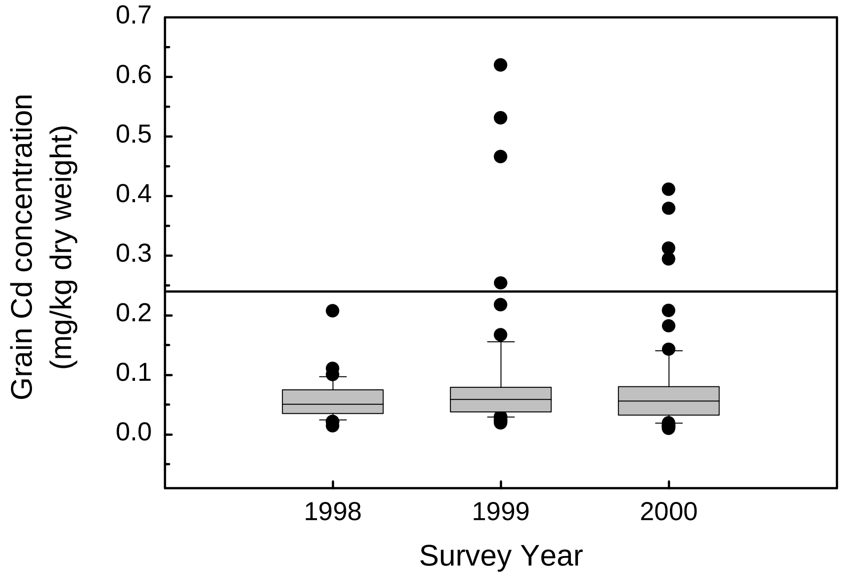


Figure 2. The distribution of concentration ranges for cadmium in wheat sampled in 1998 (n = 34), 1999 (n = 61) and 2000 (n = 67). The boxes represent the 25th to 75th percentiles and the horizontal solid lines within indicate the median concentration. The ends of the T bars mark the 10th and 90th percentiles, and the circles represent outlying points. The horizontal line represents the effective European Commission concentration limit for cadmium in wheat of 0.235 mg/kg on a dry matter basis.

Table 9. Mean and standard deviation (σ) of wheat grain cadmium concentrations dry weight in wheat varieties having 5 or more samples.

Variety	Cadmium (σ) mg/kg		No. of Samples
Abbot	0.067	*(0.047)*	22
Charger	0.040	*(0.027)*	5
Consort	0.203	*(0.168)*	10
Hereward	0.049	*(0.037)*	34
Malacca	0.047	*(0.035)*	9
Rialto	0.117	*(0.141)*	25
Soissons	0.079	*(0.069)*	25
Spark	0.041	*(0.022)*	7

Excluding the variety Consort, where the mean cadmium concentration of 10 samples was skewed by several having very high concentrations due to very high soil cadmium concentrations (see discussion and Table 10 below), the variety having the highest mean cadmium concentration was Rialto, followed by Soissons and Abbot. It is interesting to note that Rialto also had the highest mean cadmium concentration of the varieties sampled in the 1998 Cereals Quality Survey (see earlier), although neither Soissons nor Abbot had sufficient sample numbers in the 1998 Survey for a mean value to be calculated.

As was observed in the 1998 Cereals Quality Survey, the vast majority of samples in this survey work had concentrations below the European Commission limits. However, as shown in Fig. 2 and observed for the Quality Survey samples, a small number of grain samples (8 of 162 samples or 4.9% of the wheat samples collected over the three year period) contained cadmium at concentrations that would have exceeded these limits. Selected details of these samples are given in Table 10. Five of the sites have very high soil cadmium concentrations (> 5 mg/kg) which accounts for the high grain cadmium concentrations observed at these sites. For the purposes of comparison, the mean soil cadmium concentration for arable/ley grassland in England and Wales is 0.3 mg/kg (Webb *et al.* 2001).

Table 10. Selected details for winter wheat samples having dry weight cadmium concentrations higher than the effective limit of 0.235 mg/kg.

Sample	County	Variety	Soil pH	Soil cadmium (mg/kg)	Grain cadmium (mg/kg)
1	Essex	Rialto	7.5	7.90	0.620
2	Staffs.	Consort	6.6	33.86	0.531
3	Essex	Rialto	7.6	17.36	0.466
4	Mid Glam.	Soissons	6.9	1.92	0.426
5	Staffs.	Consort	6.9	9.87	0.411
6	Oxfords.	Consort	6.3	5.36	0.313
7	Dorset	Rialto	5.5	0.946	0.294
8	Hants.	Abbot	6.8	0.840	0.254

Reasons for the elevated concentrations at the three remaining sites are less clear, although several factors are likely to be responsible. For example, sample 7 (Dorset) had a relatively low soil pH for an arable soil, and a reasonably high, although by no means exceptional, soil cadmium concentration. However, as cadmium availability increases as pH decreases, the two factors combined increase the risk that a crop will take up higher cadmium concentrations than might otherwise be expected.

Similarly, the choice of cultivar may also have had a contributory effect at the three sites (nos. 4, 7 and 8) with elevated soil cadmium levels. Information on the relative rates of cadmium uptake is available for the three cultivars (Abbot, Rialto, and Soissons) grown at these sites. As previously discussed, the same three varieties exhibited the highest mean grain cadmium concentrations of the varieties sampled as part of this survey work, and Rialto had the highest mean cadmium concentration of wheat samples in the 1998 Cereals Quality Survey. Rialto and Soissons also had the highest amounts of cadmium uptake in the pot experiment conducted as part of this project (see results later). It therefore appears that on soils having elevated cadmium contents, the choice of variety may be important in minimising the potential risk of exceeding the permitted wheat grain cadmium concentration limit.

Given this, however, it must be clearly stated (as is mentioned in the Materials and Methods), that for the sampling years 1999 and 2000 a particular effort was made to sample some locations that had soil characteristics that would potentially lead to relatively higher metal concentrations in the grain. It is therefore likely that the percentage (4.9%) of wheat samples identified in this part of the research work as exceeding the maximum permitted limits would be an overestimate of the true situation. Indeed, samples from the 1998 Cereals Quality Survey were collected in a non-targeted manner, and as previously discussed, results indicate that only 0.4% of samples exceeded the current permitted cadmium limit for wheat.

Soil factors influencing wheat grain cadmium

Multiple linear regression analysis was used to identify significant soil variables that could be used to fit a model describing grain cadmium uptake based on the experimental data collected. Soil and grain metal concentrations were log-transformed prior to regression to ensure residual values were normally distributed. As there was no statistical difference between wheat grain cadmium concentrations over the three harvest years sampled, data from all years ($n = 162$) was pooled for subsequent modelling purposes.

Soil pH is usually regarded as an important variable that controls the extent of cadmium availability in soils. Consequently, to reduce the extent of cadmium uptake by crops, the addition of lime is often used as a soil management practice to reduce crop concentrations (Alloway, 1990; Grant *et al.*, 1998). However, a simple regression between soil pH and wheat grain cadmium did not show evidence of a particularly strong relationship (Fig 3*a*), with only 6% of variation in grain cadmium concentrations being explained by soil pH alone, even though pH was a significant variable in the regression ($P<0.001$). Similarly, the soil cadmium concentration is also expected to be of importance when considering the amount of cadmium that is available for plant uptake (Grant *et al.*, 1998). A simple regression between wheat grain cadmium and total soil cadmium concentration over the three harvest years sampled indicated that for wheat, the soil cadmium concentration was a more significant variable than soil pH, and explained 32% of the variation ($P<0.001$) in grain cadmium content (Fig 3*b*). A similar regression between wheat grain cadmium and logarithmically transformed values of soil cadmium concentrations explained 39% of the variation ($P<0.001$) in grain cadmium (figure not shown).

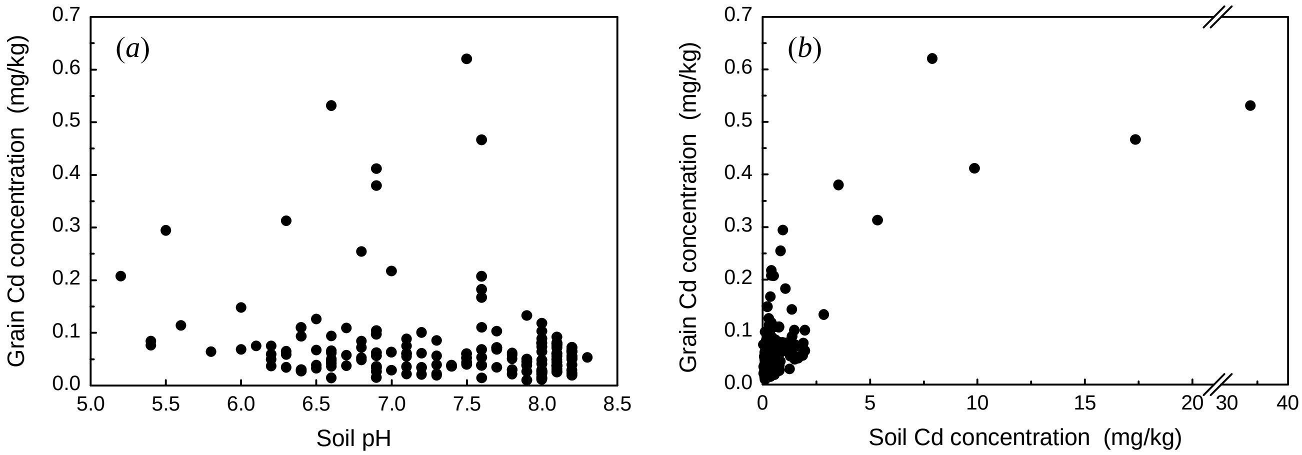


Figure 3. Relationships between milling wheat grain cadmium concentrations and (*a*) soil pH and (*b*) soil cadmium concentration.

However, combination of the two variables soil pH and soil cadmium concentration together, resulted in a highly significant model ($P<0.001$) that explained 49% of the variation in grain concentrations. Figure 4 shows the fit between the observed and fitted grain cadmium values obtained for wheat samples from this regression model where log grain cadmium (mg/kg) = 0.282 + 0.44 log soil cadmium (mg/kg) – 0.1834 soil pH.

Inclusion of other factors into the fitted model was also examined. Interestingly, no significant correlation was apparent between either grain cadmium or lead concentrations and sites having a history of manure or sludge applications to soil. At such sites soil metal levels might be expected to have increased, causing on average a greater amount of cadmium and/or lead to be taken up into grain. However, as the manure and sludge applications were applied in different years, at varying rates and metal loadings to soils that would have contained different background concentrations of cadmium and lead in the first instance, it perhaps is not too surprising that no clear effects were apparent.

The organic matter content of a soil has been shown in studies to affect cadmium uptake in crop species through organic binding of cadmium, thereby making it less available for plant uptake (Wenzel *et al.*, 1996; Grant *et al.*, 1999). Incorporation of loss on ignition (LOI) data, a measure of the organic matter content of a soil, into the model indicated it was a significant explanatory variable ($P<0.05$), although it added only 1% to the total percentage variance explained by the model containing soil pH and cadmium concentrations alone. Due to the small relative improvement obtained, it was not included in further

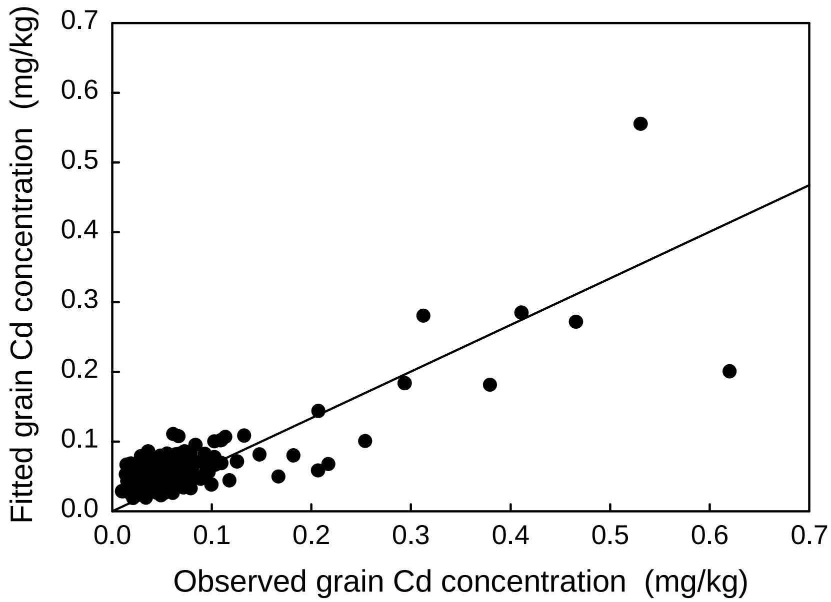


Figure 4. Relationship between observed and fitted milling wheat cadmium concentrations calculated from the regression model including soil total cadmium concentration and pH as variables.

models. Inclusion of Al, Fe and Mn oxide terms (as determined in soils by the ammonium oxalate-oxalic acid extraction procedure of Janssen *et al.*, (1997)) into the regression model together with soil pH and soil cadmium concentration, resulted in a 5% improvement to the model fit, compared with the 49% of variance explained by the model containing the two latter soil parameters alone. However, as none of the oxide terms were themselves significant parameters in the regression equation, and therefore as their inclusion into the model could not be justified statistically, they were excluded from subsequent model fitting.

Cultivar effects on wheat cadmium concentrations were found to be significant ($P<0.05$) by ANOVA and incorporation of a cultivar term into the model again resulted in an improved fit to the observed data, increasing the percentage variation accounted for to 56%. The cultivar term was a significant variable in the regression ($P<0.05$). Together with total soil Cd and organic matter, Wenzel *et al.* (1996) also found that a cultivar term was a significant variable in regression models explaining cadmium uptake in wheat grown on seven soil types. Observed and predicted grain concentrations for this model are shown in Fig. 5. A visual inspection of the two regression models displayed in Figs. 4 and 5 shows few differences.

Several studies modelling the uptake of cadmium into crops have identified soil chloride as an important variable in regression modelling. Chloride forms relatively strong complexes with cadmium, and as a result mobilises soil cadmium, hence increasing its availability for uptake by plants (Bingham *et al.*, 1984; McLaughlin *et al.*, 1994; Smolders *et al.*, 1998; McLaughlin *et al.*, 1999). Chloride levels were determined in selected soils from the 2000 harvest year, that had been sampled at both coastal and inland locations. Although chloride levels were marginally higher in the coastal soils than in the inland, all samples had low chloride levels (<5 mg/kg), which is two orders of magnitude below the concentration where chloride was determined to affect plant uptake in the two McLaughlin papers cited above. These two papers dealt with crops grown in soils containing high chloride concentrations or were irrigated with saline waters in Australia, where soil concentrations reached >1500 mg/kg. Chloride levels in British soils are therefore not expected to be a significant factor influencing cadmium uptake in grain.

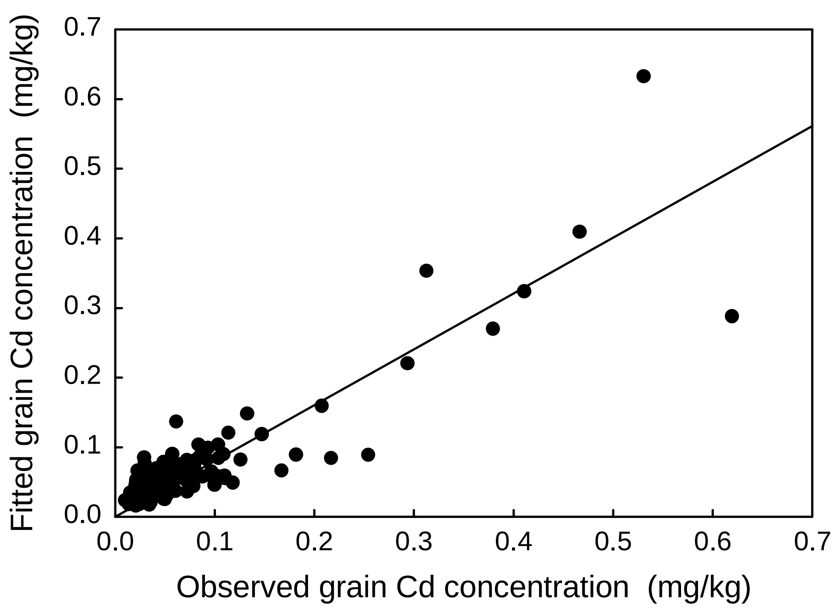


Figure 5. Relationship between the observed and fitted milling wheat cadmium concentrations calculated from a regression model including soil total cadmium concentration, soil pH and cultivar terms.

Wheat grain lead

As observed for cadmium, a range of wheat grain lead concentrations was also determined from the three harvest periods sampled (1998-2000), with concentrations ranging from less than the analytical detection limit (<0.020 mg/kg) to 0.194 mg/kg (n = 162). A large proportion of the wheat samples (*ca.* 50% of the total) contained concentrations of lead that were below the detection limit of the analytical technique used (<0.02 mg/kg). Over the three year period, median and mean grain lead concentrations were 0.015 mg/kg and 0.027 mg/kg, respectively. Although the distribution of lead concentrations in wheat was slightly higher in samples collected in the first year of the study (1998) (Fig. 6), this may be an artefact of the small number of samples collected in the first year (n = 34).

The mean lead concentrations in the years 1998-2000 sampled (0.049, 0.023 and 0.019 mg/kg, respectively) compare with a mean concentration of 0.025 mg/kg in samples from the 1998 Cereals Quality Survey. Unlike cadmium, differences in mean lead concentrations between varieties were not significant, although the large number of samples having concentrations below the detection limit reduced the effectiveness of this statistical analysis.

Only one sample from Nottinghamshire collected in the 2000 harvest year approached the effective dry weight concentration limit for lead in wheat grain of 0.235 mg/kg. However, as can be seen from the concentration distributions plotted in Fig. 6, this sample is very much a single outlying point. Excluding this single sample, no other wheat sample had lead concentrations higher than half the permitted concentration limit.

Together with the results from the 1998 Cereals Quality Survey, for which again no wheat samples exceeded the lead concentration limit, it therefore appears clear that the British wheat and milling industries can easily meet the required European Commission lead concentration limit.

Development of a statistically significant model for lead uptake in wheat grain was not successful, which was largely due to the very low lead concentrations determined in the majority of the samples. This consequently meant that for potential explanatory variables such as soil pH, soil lead concentration etc. there was no matching magnitude or resolution of range in grain lead concentrations.

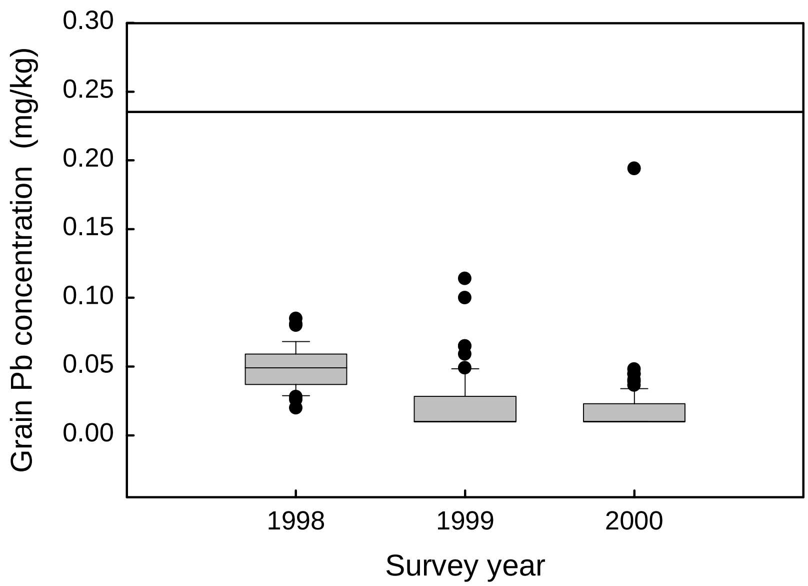


Figure 6. The distribution of concentration ranges for lead in wheat sampled in 1998 (n = 34), 1999 (n = 61) and 2000 (n = 67). The boxes represent the 25th to 75th percentiles and the horizontal solid lines within indicate the median concentration. The ends of the T bars mark the 10th and 90th percentiles, and the circles represent outlying points. The horizontal line represents the effective European Commission concentration limit for lead in wheat of 0.235 mg/kg on a dry matter basis.

Barley grain cadmium

The distribution of cadmium concentrations in combined spring and winter varieties of barley collected over the 1998-2000 harvest periods is shown in Fig. 7. Concentrations of cadmium varied from less than the analytical detection limit (<0.003 mg/kg) to 0.100 mg/kg, with median and mean cadmium concentrations of 0.014 mg/kg and 0.019 mg/kg respectively, over the three years. The mean value of 0.015 mg/kg is similar to that determined for cadmium concentrations in barley samples from the 1998 Cereals Quality Survey (0.022 mg/kg). Of the 213 barley samples collected over the three year period (and bearing in mind that sampling featured some selection towards situations where high metal concentrations might have been expected), no samples exceeded the effective dry weight limit for cadmium in barley of 0.118 mg/kg.

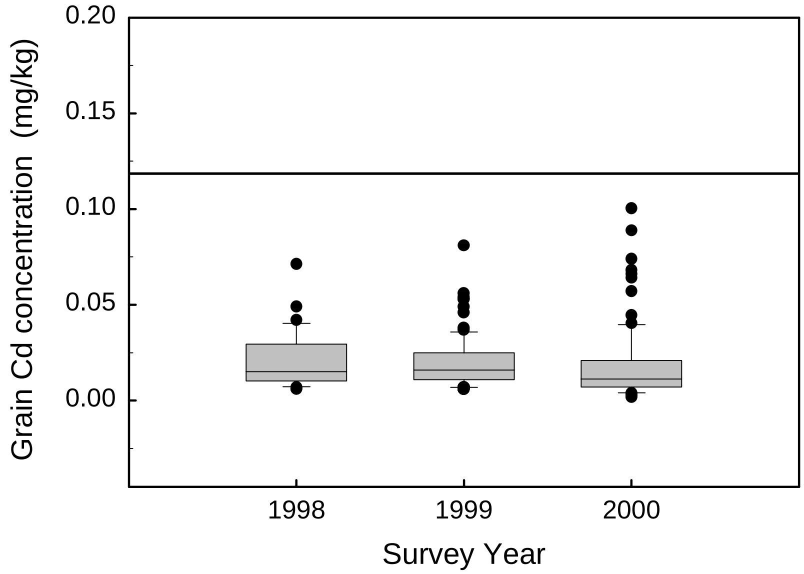


Figure 7. The distribution of concentration ranges for cadmium in barley sampled in 1998 (n = 27), 1999 (n = 95), and 2000 (n = 93). The boxes represent the 25th to 75th percentiles and the horizontal solid lines within indicate the median concentration. The ends of the T bars mark the 10th and 90th percentiles, and the circles represent outlying points. The horizontal line represents the effective European Commission concentration limit for cadmium in barley of 0.118 mg/kg on a dry matter basis.

Analysis of the barley cadmium results by ANOVA showed that there was a significant difference between the mean concentrations of cadmium in spring (n = 125) and winter (n = 88) varieties ($P<0.001$), with mean concentrations of 0.012 and 0.018 mg/kg, respectively. Due to the intrinsic differences between the spring and winter varieties, subsequent statistical testing was performed separately for each crop type.

(i) Winter barley cadmium

Analysis of the winter barley samples (n = 88) showed no significant differences in mean cadmium concentrations by year. However, for the three winter barley varieties which made up the bulk of the samples (and for which the number of samples collected was greater than 5), significant differences in mean cadmium concentrations existed ($P<0.001$). Fanfare and Halcyon varieties had low mean cadmium concentrations of 0.012 and 0.011 mg/kg respectively, while the mean concentration in Regina samples was more than double that of the other two varieties (0.029 mg/kg).

Winter barley cadmium regression modelling

In contrast to wheat for which soil cadmium concentration was the most significant single variable explaining grain cadmium concentrations, for winter barley soil pH was the most significant variable ($P<0.001$) and was the single soil factor that explained most of the percentage variance (20%) observed in grain cadmium concentrations (Fig. 8). Although incorporation of a log soil cadmium concentration term ($P<0.05$) into the regression model did improve the fit of the model to a small extent (by increasing the percentage variance accounted for to 25%), neither the inclusion of LOI (as an organic matter estimate) or soil oxide data showed these to be significant parameters (for all $P>0.05$). However, the inclusion of a cultivar term into the regression did substantially improve the goodness of fit to 47%. This reflects the large percentage difference observed between mean varietal grain cadmium concentrations discussed above. Figure 9 shows the fit obtained between the observed and predicted grain cadmium concentrations in winter barley for this final regression model.

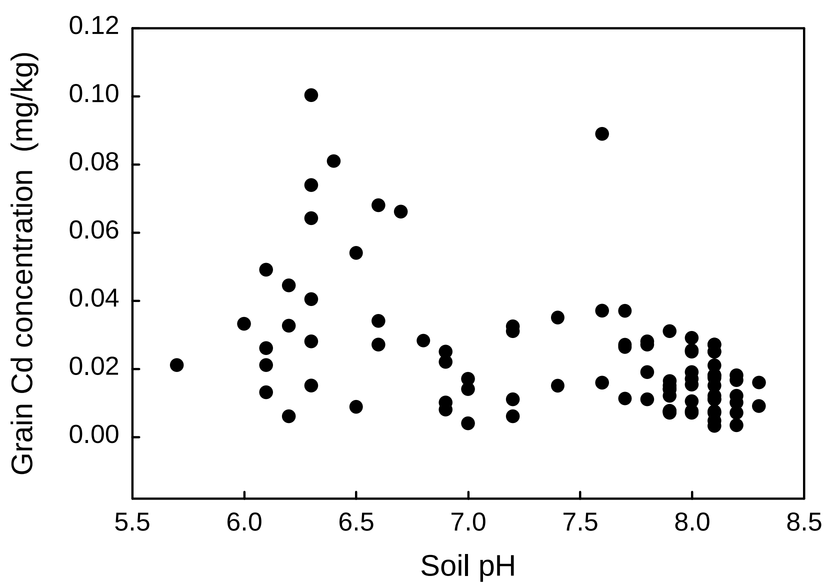


Figure 8. Relationship between malting barley grain cadmium concentrations and soil pH.

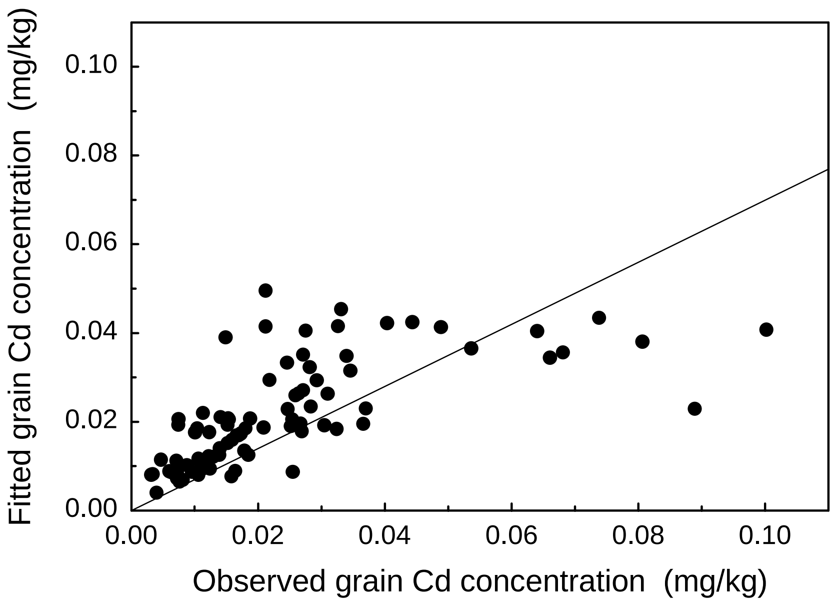


Figure 9. Relationship between observed and predicted barley cadmium concentrations calculated from a regression model including soil pH, soil cadmium concentration and cultivar terms.

(ii) Spring barley cadmium

As was observed for winter barley, spring malting barley varieties (n = 107) also showed significant differences (P<0.001) in mean cadmium concentrations for the varieties having n>5. Although the mean concentrations were uniformly low, grain from Chariot varieties had the highest mean cadmium content (0.017 mg/kg) and Prisma the lowest (0.008 mg/kg).

Attempts to model the uptake of cadmium into spring barley grain were unsuccessful, as no combination of likely explanatory variables resulted in a regression model that gave a high degree of fit. As was the case for the modelling of lead concentrations in wheat grain, much of the reason for this is likely to be due to the generally low and narrow range of concentrations determined in the spring barley.

Barley lead

Lead concentrations in barley grain collected from the three harvest years 1998-2000 were low, and no samples exceeded the effective 0.235 mg/kg dry weight limit. As for wheat, the vast majority of samples had concentrations of less than one half of this limit, and a substantial number of samples (42%) had lead concentrations lower than the analytical detection limits (<0.020 mg/kg). Concentrations ranged from this detection limit to 0.131 mg/kg, with a median and mean lead concentration of 0.030 mg/kg and 0.036 mg/kg respectively, for the three years (spring and winter varieties combined, n = 183). The mean value of 0.036 mg/kg compares with a mean lead concentration of 0.039 mg/kg in barley from the 1998 Cereals Quality Survey.

Samples of spring barley collected from Scotland in the 2000 harvest year are not included with these results, as analytical evidence pointed towards these samples having been contaminated at some point prior to lead analysis. For comparative purposes, the distribution of lead concentrations in the 2000 Scottish samples is shown in Fig. 10, together with the distributions of lead in the England and Wales samples of that year, and those from the previous years. Clearly, the lead concentrations in the Scottish samples from 2000 are anomalously high. Grain washing experiments to determine whether the high concentrations were naturally occurring, or were indicative of potential contamination by soil or dust were performed. A substantial amount of lead was removed from the grain by a surface washing process, which is indicative of surface contamination (see discussion below). As these samples were processed and analysed in a similar manner to previous samples collected, it was not possible to identify a precise source for the contamination. Nevertheless, despite the contamination of these samples, it is still relevant to note that of the 40 samples, only 10 exceeded the 0.235 limit for lead in barley.

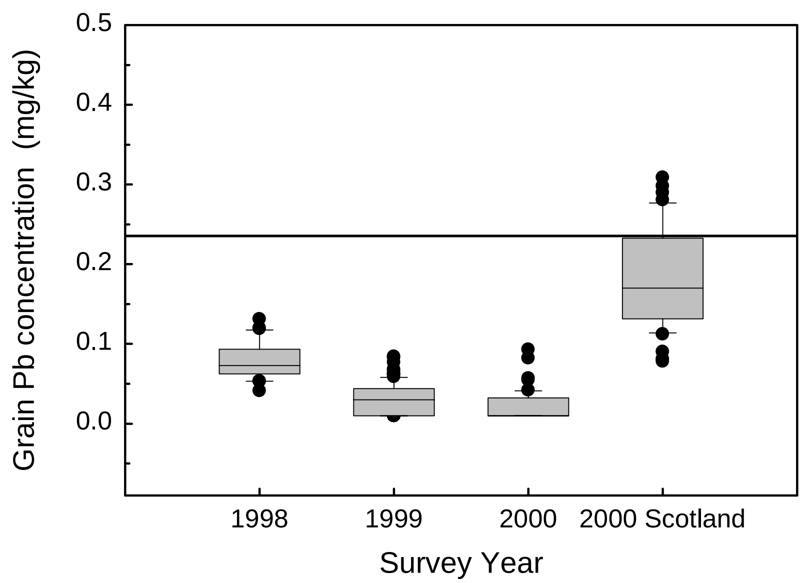


Figure 10. The distribution of concentration ranges for lead in barley sampled in 1998 (n = 27), 1999 (n = 95), 2000 (England and Wales n = 53) and 2000 Scotland (n = 40). The boxes represent the 25th to 75th percentiles and the horizontal solid lines within indicate the median concentration. The ends of the T bars mark the 10th and 90th percentiles, and the circles represent outlying points. The horizontal line represents the effective European Commission concentration limit for lead in barley of 0.235 mg/kg on a dry matter basis.

As was observed when investigating regression models for the lead concentrations in wheat grain, attempts to develop a significant regression model for lead in barley (excluding the 40 Scottish samples from 2000) were unsuccessful. As before, the low and narrow concentration range of lead in the barley grain was the primary reason for this.

Historic Sewage Sludge Experiments

The historic sewage sludge experiments at ADAS Gleadthorpe and Rosemaund provide a unique resource for investigating and quantifying the effects of previous sludge applications on metal uptake into crops. In 1999, both experimental sites were planted in winter wheat (Cv. Hereward) and for harvest year 2000 winter barley (Cv. Optic) was grown. In 1999, strong relationships between total soil and wheat grain cadmium concentrations were observed at the two sites (Fig. 11). The regressions shown in the figures explain a high amount of the variance in grain cadmium concentrations, 83% and 79% at Gleadthorpe and Rosemaund, respectively. These fits are better than those obtained for the paired soil and crop samples collected from around Britain, which is largely due to the individual treatment plots at each site sharing the same soil type, similar local environmental and climatic conditions, and that the range of soil cadmium concentrations was relatively wide.

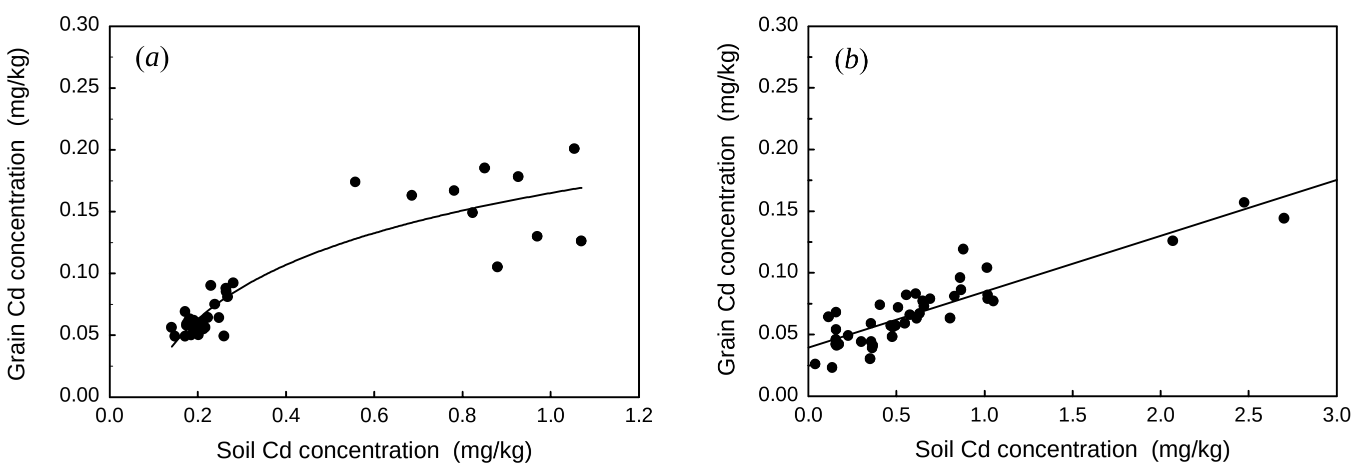


Figure 11. Cadmium uptake by winter wheat (Cv. Hereward) at (*a*) Gleadthorpe and (*b*) Rosemaund sewage sludge experimental sites in 1999. The regression lines fitted have R^2 coefficients of 0.83 and 0.79 respectively.

It is noticeable at the Gleadthorpe site that several points at high soil cadmium concentration had grain cadmium concentrations lower than the fitted regression line. Interestingly, the samples from these plots correspond to the high Zn treatments. Given these plots are of similar soil pH to the remainder at the site, it is likely that the higher soil Zn concentrations caused reduced plant uptakes of cadmium through metal competition effects. A pot experiment investigating the effects on grain cadmium uptake caused by Zn additions to soil was later established and is described below.

It is also important to note the differences in grain cadmium concentrations that were obtained at a given total soil cadmium concentration at the two sites. At Gleadthorpe, wheat grain cadmium reached as high as 0.20 mg/kg at a soil concentration of approx. 1.0 mg/kg, whereas at Rosemaund grain cadmium concentrations reached only 0.15 mg/kg at a soil cadmium concentration of around 2.50 mg/kg. The differences in cadmium availability at the two sites are due primarily to the different soil properties at the two sites, and specifically to the lower soil pH at Gleadthorpe. Incorporating both soil pH and soil cadmium concentration into a regression model (the same two soil variables that were the most significant parameters in the wheat survey regression model) shows that grain from both sites follows the same relationship, with 80% of the percentage variance in grain cadmium concentrations accounted for (Fig. 12). The high degree of fit observed will be assisted by the fact that the same wheat cultivar (Cv. Hereward) was grown at both sites.

These results also have implications for the addition of cadmium-containing sludge to agricultural land. At present, in order to protect agricultural soils from accumulating high concentrations of cadmium via the addition of sludges, the UK forbids sludge application onto soils having soil cadmium concentrations of greater than 3 mg/kg. On the basis of the soil cadmium analyses at Gleadthorpe (maximum soil concentration of *ca*. 1.0 mg/kg), additional sludge could legally be applied. However, should such an application significantly increase the existing soil cadmium concentration, the relationship shown in Fig. 11 suggests that the relatively high availability of cadmium at the soil pHs present at the site (5.3 - 6.6, Table 1) could easily lead to grain being produced that would exceed the maximum permitted grain concentration of cadmium.

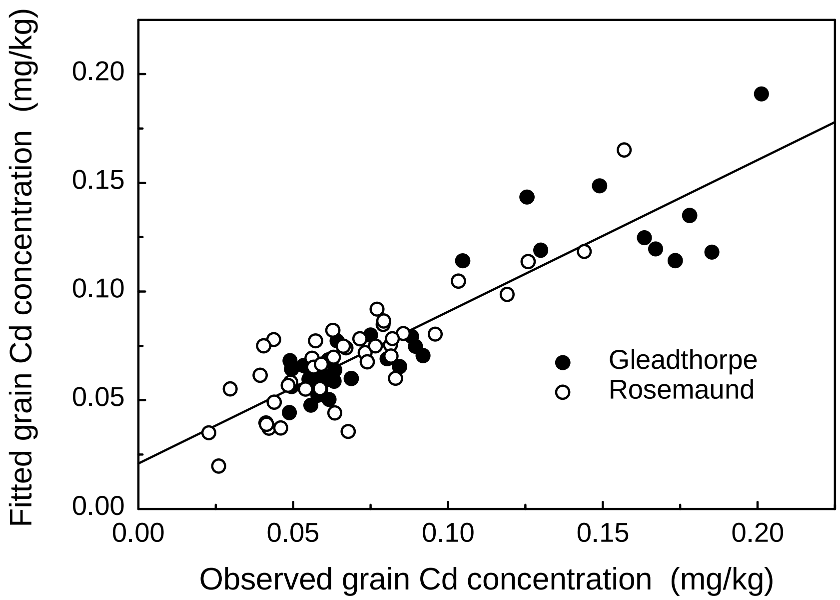


Figure 12. Relationship between observed and predicted wheat cadmium concentrations calculated from a regression model including soil pH and soil total cadmium concentration as explanatory variables. The fitted line has R^2 = 0.80. The regression model used to calculate the predicted values was log grain cadmium (mg/kg) = 0.392 + 0.4235 log soil cadmium (mg/kg) – 0.2116 soil pH.

The current draft European Commission Working Document on Sludge (European Commission, 2000) proposes revisions to the current soil metal limits that must be met before sludge may be applied to soils. It has proposed maximum permitted soil cadmium concentrations for sludge applications that depend on soil pH, so that a lower soil metal concentration is necessary for soils of lower pH. Whereas the previous directive (86/278/EEC) effectively allowed member states to select national limits for soil cadmium in the range of 1-3 mg/kg for soils with a pH of 5.0 or above, the latest (third) draft of the working document specifies limit values of cadmium in soil of 0.5 mg/kg (5≤pH<6), 1.0 mg/kg (6≤pH<7) and 1.5 mg/kg for soil having pH ≥7.

Barley grown at the two experimental sites had lower concentrations of cadmium than wheat, although good correlations between grain and soil cadmium concentrations were again observed (Fig. 13). Cadmium uptake at Gleadthorpe was once again higher than at Rosemaund, reflecting the lower soil pHs at the former site. As was the case for wheat grown in the previous year, the Gleadthorpe crop was at most risk of exceeding the permitted cadmium limit for barley (effective dry weight limit of 0.118

mg/kg) if further additions of cadmium-containing sludge were made, even though total soil cadmium concentrations at this site are lower. Combining results from the two sites in a regression model incorporating soil pH and cadmium concentration resulted in an acceptable fit between observed and fitted grain concentrations (Fig. 14) which explained 62% of the variance in barley grain cadmium concentrations, although the fit at higher grain concentrations was poorer and there appeared to be some divergence between the data from the two sites.

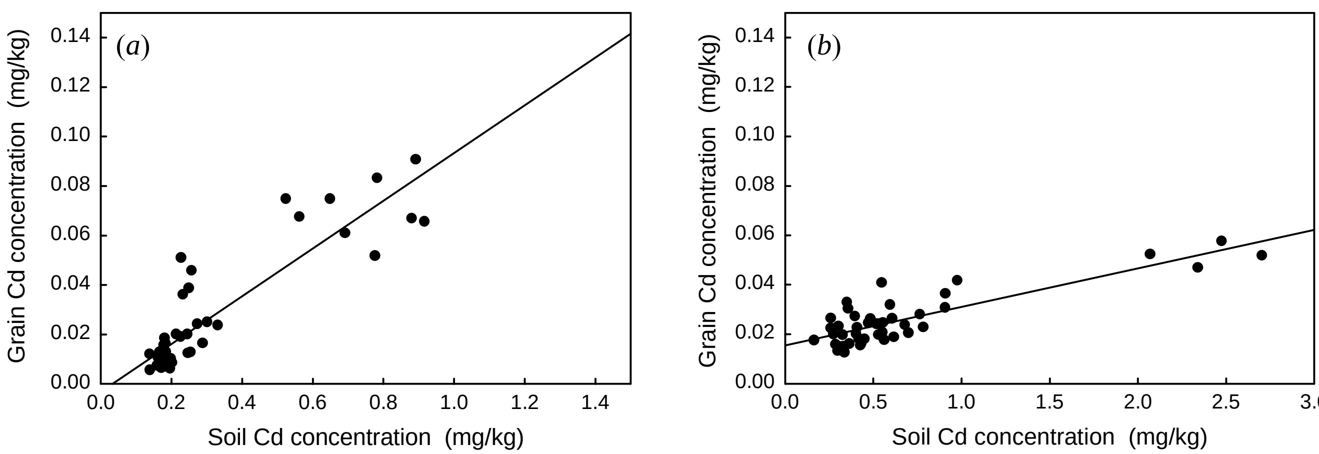


Figure 13. Cadmium uptake by malting barley (Cv. Optic) at (*a*) Gleadthorpe and (*b*) Rosemaund sewage sludge experimental sites in 2000. The regression lines fitted have R^2 coefficients of 0.80 and 0.71 respectively.

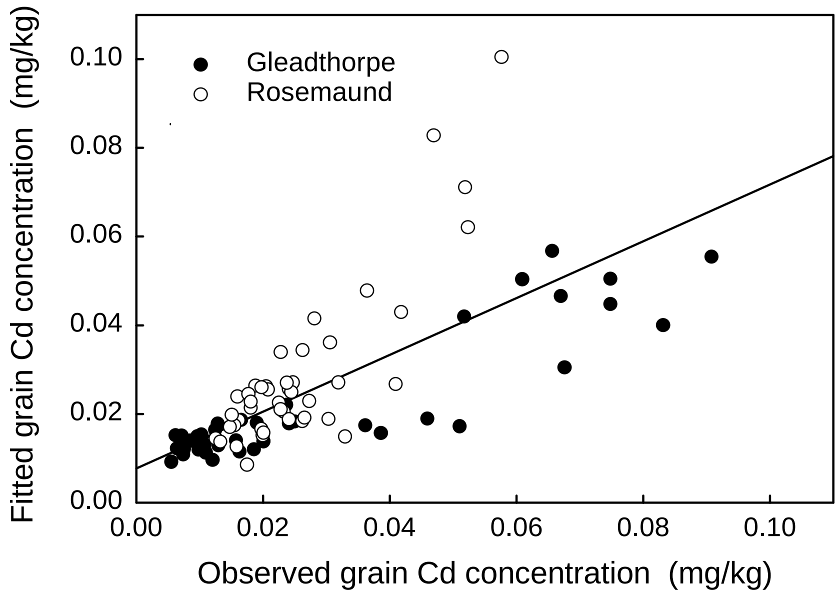


Figure 14. Relationship between observed and predicted barley cadmium concentrations calculated from a regression model including soil pH and soil cadmium concentration as explanatory variables (log grain cadmium (mg/kg) = 0.200 + 0.8205 log soil cadmium (mg/kg) – 0.2397 soil pH. The fitted regression explains 62% of the variance in grain cadmium concentrations.

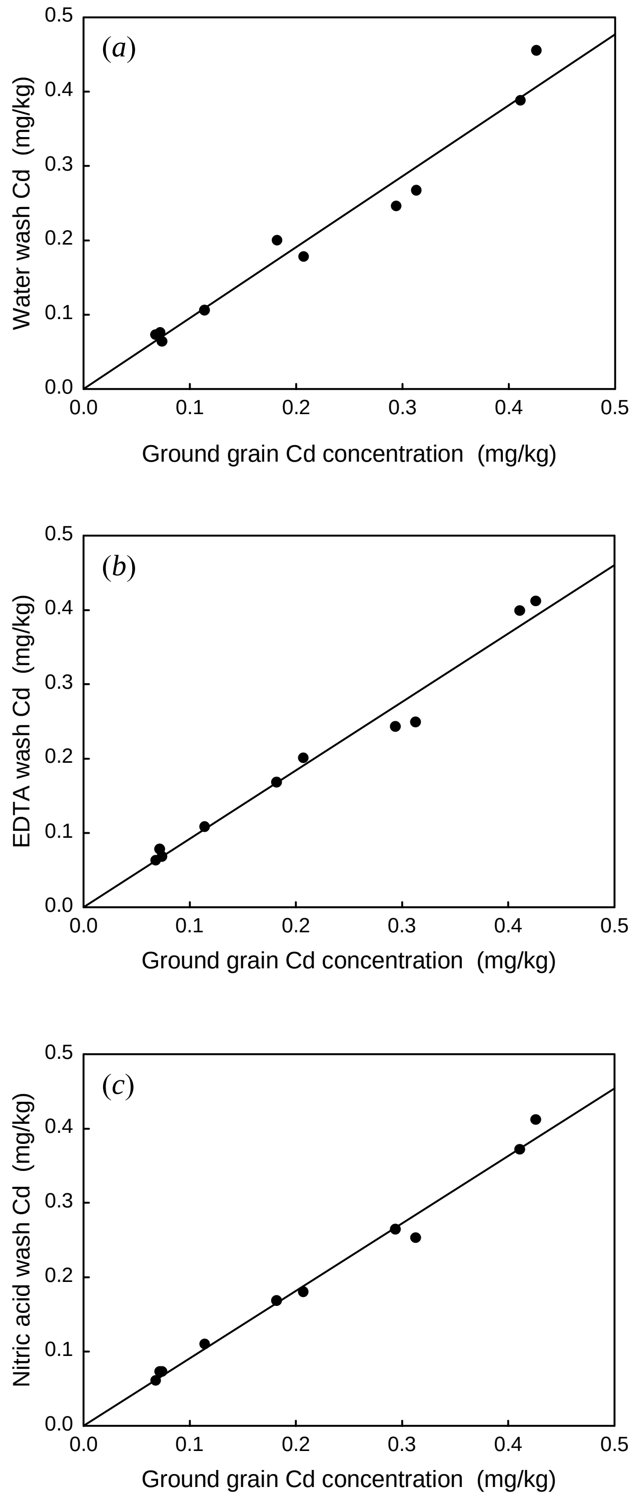
Concentrations of lead in wheat and barley were generally lower than Cd at the two sites over the two year period, although concentrations at Gleadthorpe were on average higher than those observed at Rosemaund. Concentrations ranged from <0.02 mg/kg to 0.211 mg/kg at the two sites. No significant relationship was found between grain lead concentrations and soil pH, total soil lead concentration or other possible predictive variables (LOI, soil P etc.). Soil lead concentrations at the two sites were relatively low, ranging from 17-89 mg/kg at Gleadthorpe and from 21-48 mg/kg at Rosemaund (Table 1). Soils in England and Wales have a median lead concentration of 40 mg/kg (McGrath and Loveland, 1992). Although there was a range of soil lead concentrations at both sites, the generally low uptake of lead by cereal crops (as evidenced by concentrations observed in the various survey samples) meant grain samples from these two sites were similarly low.

Grain Washing Experiments

A series of grain washing experiments were performed on selected representative wheat and barley samples collected from the 2000 harvest year as part of the National Survey of Paired Crop and Soil Samples. Grain samples having a range of cadmium concentrations (both high and average values), and Scottish barley samples (having high grain lead concentrations), were used to establish whether the concentrations of cadmium or lead originally quantified in the ground grain occurred as a result of plant uptake, or were due to external factors such as surface contamination prior to the samples being received for analysis. It was expected that where surface contamination was responsible for the elevated concentrations of cadmium or lead measured, then one or other of the chosen extractants would be able to remove a fraction of the contamination from the surface. Subsequent digestion of contaminated grain would therefore result in lower values than originally determined, while high concentrations would be determined in the wash solution itself.

Ten winter barley and wheat samples were washed with different extractants (water, the chelating reagent EDTA, or HNO_3) before cadmium, lead and titanium were determined by GFAAS and ICP-AES in digests of the whole grain and in the wash solutions. Results of the grain digests following washing with the extractants are shown in Fig. 15, and are compared with the concentrations determined in the original ground grain samples.

There was excellent agreement between cadmium concentrations determined in the original ground grain and in the washed grain samples, showing that only negligible amounts of cadmium were removed from the grain samples by washing. The slope of fitted lines between the data sets decreased as the aggressiveness of the reagent increased i.e. nitric acid was able to extract slightly more cadmium off the grain surface than the other reagents. Cadmium concentrations in the wash solutions were consequently low, with the majority of samples having concentrations that accounted for <0.01 mg/kg of cadmium removed from the whole grain. For several samples the washing reagents appeared to remove a small amount of cadmium from the grain, which accounts for the observed slopes being slightly lower than the theoretical value of unity. However, the reductions in grain concentration were not more than about 10% of the total concentration determined in the ground grain. Concentration differences between the washed and ground grain samples may also have arisen in this case from sample inhomogeneity occurring as a result of analysing whole, as opposed to ground grain. It therefore seems clear from these



results that the high cadmium concentrations measured in grain in this work are likely to be caused by actual plant uptake, and not by external factors such as surface contamination by soil or dust.

Figure 15. Relationships between cadmium measured in ground grain and in grain washed with an extractant. Slopes and R^2 coefficients respectively, for the three graphs are (*a*) 0.95 and 0.97; (*b*) 0.92 and 0.98 and (*c*) 0.91 and 0.99.

Representative samples of Scottish barley from the 2000 harvest, for which lead concentrations were high, were also subjected to the same washing process as described above. The results were very much in contrast to those for cadmium, as large changes in grain lead concentrations were observed before and after washing with the extractant solutions. Analysis of the wash solutions for these samples also contained high lead concentrations, confirming that for every sample, a substantial amount of lead was removed from the grain surface by washing.

Plotting the lead values determined in grain after the washing protocol against the concentrations originally determined in the ground grain (Fig. 16) resulted in relatively poor fits compared to the equivalent plots for the cadmium samples. Such behaviour could again be regarded as indicative of non-systematic lead surface contamination.

ICP-AES analysis of the grain digest and wash solutions confirmed the presence of elevated lead concentrations in the wash solutions themselves. Titanium (Ti) concentrations were also determined; Ti is commonly used as a marker to indicate the presence of soil or dust in crop samples as it is a non-essential element for plants, and therefore not taken up by crops despite significant concentrations being present in soil. However, there was no indication of elevated Ti levels in the wash solutions, and therefore it is unlikely that the surface contamination of the Scottish barley samples was due to soil.

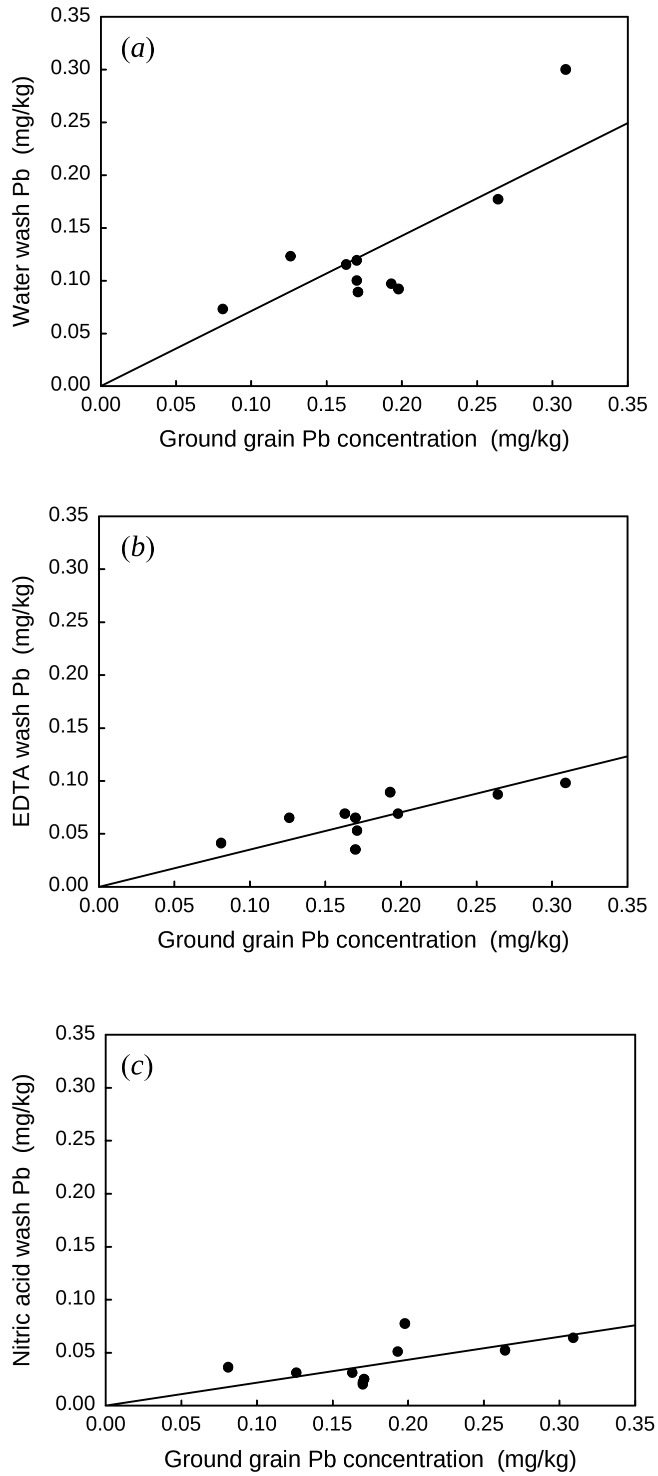
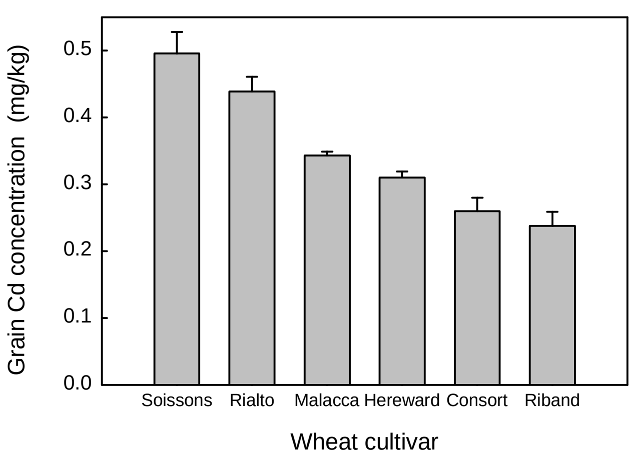


Figure 16. Relationships between lead measured in ground grain and in grain washed with an extractant. Slopes and R^2 coefficients respectively, for the three graphs are (*a*) 0.71 and 0.66; (*b*) 0.35 and 0.46 and (*c*) 0.22 and 0.31.

Wheat and Barley Cultivar Pot Experiments

To investigate whether the significant differences in grain cadmium concentrations determined for wheat and barley cultivars in the 1998 Cereals Quality Survey and the 1998-2000 milling wheat and malting barley survey were real, or due to soil or climatic factors, pot experiments were performed under controlled environmental conditions. These involved the growth of a range of widely grown (and NIAB recommended) cultivars in a single soil type (Buxton, Derbyshire soil, Table 2) under controlled glasshouse conditions. The cultivars selected were Consort, Hereward, Malacca, Rialto, Riband and Soissons (winter wheat), and Chariot, Fanfare, Gleam, Optic and Riviera (winter and spring barley).

Significant ($P<0.001$) differences in cadmium uptake were determined in both the winter wheat and the spring and winter malting barley crops (Fig. 17). Ranking of the wheat cultivars, in terms of grain cadmium concentrations, showed surprisingly good agreement with the results from the 1998 Cereals Quality Survey and the 1998-2000 wheat and barley survey. The two cultivars having the highest grain concentrations in the pot experiments (Soissons and Rialto) also had the highest concentrations in the survey studies. Similarly, Hereward was middle-ranked for both the surveys and in the pot experiment, while Consort and Riband that had the lowest grain cadmium concentrations in the pot experiment both had low concentrations in the 1998 Cereals Quality Survey.



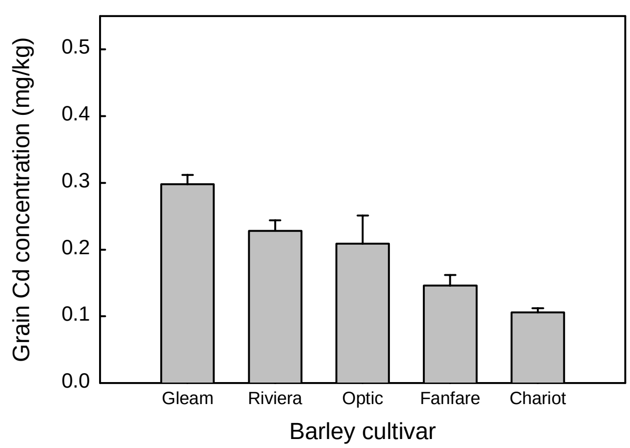


Figure 17. Cadmium concentrations in wheat and barley cultivars grown in soil from Buxton, Derbyshire. The error bars represent the standard error of the mean.

The magnitudes of differences in grain cadmium concentrations ranged from approx. 2-fold in the wheat crops, to 3-fold in the barley cultivars. However, whether the size of these differences between cultivars observed in the pot experiment translates to field conditions (i.e. on a given soil type at a single field site) is not known. Nevertheless, it is clear that the choice of certain cultivars (e.g. Rialto and Soissons) will increase the risk that a crop takes up a higher amount of cadmium than might otherwise occur with an alternative cultivar. Choice of cultivar therefore, may provide growers with a useful management tool should crops be deemed at risk of exceeding the European Commission cadmium concentration limit.

Lead concentrations were again low with a number falling below the detection limit, and consequently no significant differences were observed between cultivars.

Fertiliser Form and Soil Amendment Pot Experiments

One of the aims of this research project was to quantify the effects of nitrogen fertiliser form and of soil amendments on cadmium and lead uptake into wheat and barley grain. The cultivars Rialto (winter wheat) and Regina (winter barley) were selected for use in this pot experiment for the following reasons:

(1) both had been identified in results from the 1998 Cereals Quality Survey and from the 1998-2000 survey work as cultivars with above average grain cadmium concentrations
(2) both were widely grown in the UK, and
(3) at the time, both were fully recommended NIAB varieties.

The soil used in these experiments was the same Buxton, Derbyshire soil used in the cultivar pot experiments (Table 2). In addition to the three N fertiliser treatments, NH_4NO_3 (used as a control treatment), NH_4^+ applied as $(NH_4)_2SO_4$ and NO_3^- applied as $Ca(NO_3)_2.4H_2O$, the effects of three soil amendments (lime, peat and oxide-rich red mud) on grain cadmium uptake were also investigated.

Cadmium and lead concentrations were measured in the grain and straw of both wheat and barley. Mean results of each experimental treatment are shown in Appendices A and B for Rialto (wheat) and Regina (w. barley), respectively. Results of the grain cadmium analyses are shown for Rialto (Fig. 18*a*) and Regina (Fig. 18*b*).

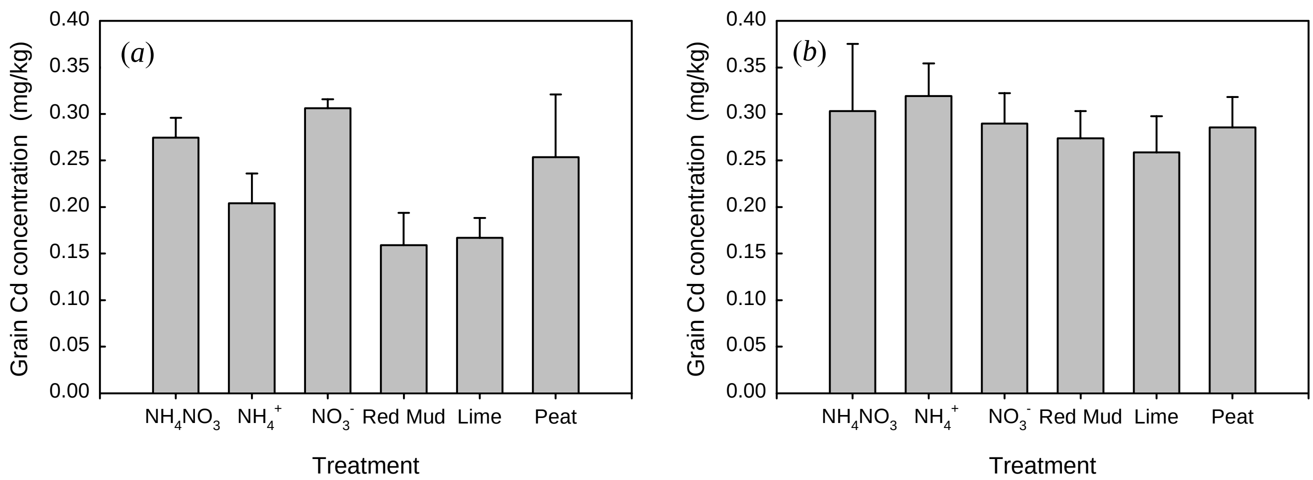


Figure 18. Cadmium uptake into (*a*) Rialto wheat and (*b*) Regina barley grown in soil from Buxton, Derbyshire to which the fertiliser and soil amendment treatments had been added. Error bars show the standard deviation of each treatment mean.

Significant treatment differences (P<0.001) in grain and straw cadmium concentrations were observed for Rialto, and although similar trends for the equivalent treatments were observed in Regina grain, these differences were not statistically significant. The yields of both grain and straw for Rialto were similar on all treatments (Appendices A and B), although the NH_4NO_3 and peat treatments for Regina had decreased grain yields compared to other treatments (P<0.05). The Regina crop also had a low mean grain Harvest Index, which may have contributed towards the non-significance of the treatment differences.

For Rialto, application of red mud and lime amendments to the soil caused a reduction in grain cadmium concentrations compared with other treatments, and in soil pore water cadmium concentrations compared with the control. As previously discussed, lime application to soil is a conventional means of reducing crop cadmium uptakes in situations where crop cadmium concentrations are of concern. In arable soils of relatively low pH, such as the Buxton soil used in these experiments (pH 5.9), it appears to be an effective management option. The application of the oxide-rich red mud also resulted in lower grain cadmium concentrations. Although red mud is an industrial by-product, it is not yet available for purchase by farmers, and so this result is perhaps of future interest if cadmium reduction strategies need to be developed for specific areas.

In contrast, application of peat to soil (which might have been expected to reduce cadmium availability via organic matter complexation) had no significant effect on cadmium concentrations in either grain or straw when compared to the control (NH_4NO_3) treatment. Total organic carbon measurements in the soil pore water solution collected after plant harvest were not significantly higher in the peat treatment compared with the other treatment, although microbial degradation of the extra soluble organic matter may have occurred by the time the soil pore water was extracted.

It might have been expected that the NH_4^+ fertiliser treatment would have increased grain Cd concentrations as a result of increased cadmium availability, caused by the soil acidification. The reasons for the decrease in Rialto cadmium concentrations in both grain and straw for this treatment relative to the control (NH_4NO_3) treatment are unclear, especially as soil pore water cadmium concentrations from the Rialto NH_4^+ treatments were the highest of any Rialto treatment (Appendix A). In the case of Regina, the NH_4^+ treatment did show elevated cadmium concentrations in the grain and straw, but these were not significant.

Lead concentrations in both the Rialto and Regina grain were low, with around 50% of the individual sample replicates having concentrations below the detection limit (0.020 mg/kg). Due to these uniformly low concentrations no significant differences between treatment means were able to be determined.

Fertiliser Rate Field Experiment Samples

Cadmium concentrations determined in the archived field experiment grain samples from ADAS Bridgets are shown in Fig. 19. Changes in grain cadmium concentrations were not significant with increasing rates of sulphur fertilisation at either of the two nitrogen fertilisation rates. However there were significantly higher grain cadmium concentrations ($P<0.001$) at the higher of the two nitrogen (NH_4NO_3) fertiliser application rates (230 kg/ha) when compared to the lower nitrogen application rate of 180 kg/ha, regardless of the rate of sulphur application.

Ammonium nitrate applications to soil will cause soil acidification and decreased soil pH (although not to the same extent as purely ammonium based fertilisers per unit of N applied), which along with enhanced nitrogen uptake at the higher rate of N, may have led to the increased cadmium grain concentrations. In addition, higher N supply increases the ionic strength of the soil solution, and can result in the release of more Cd from the soil solid phase, albeit for a limited time. The substantial number of samples that had lead concentrations lower than the detection limit (92%) meant that it was not possible to identify any trends in grain lead concentrations with respect to the different fertiliser application rates.

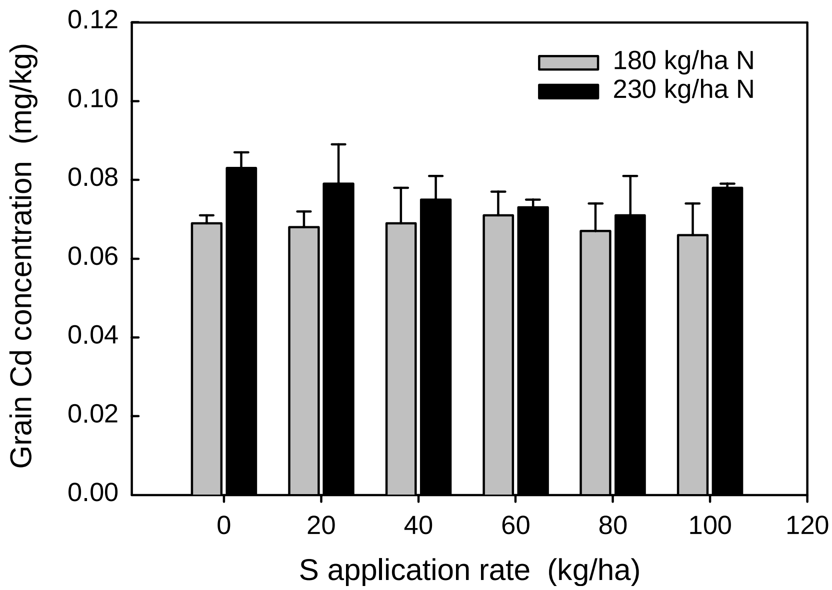


Figure 19. Effect of fertiliser application rates on grain cadmium concentrations in wheat grown at an ADAS Bridgets experimental field site.

Zinc Soil Amendment Pot Experiment

A third pot experiment was established to investigate the effect of zinc (Zn) applications to soil on the uptake of cadmium and lead into grain. This was performed largely as a result of the observations from the ADAS Gleadthorpe historic sewage sludge experimental site that the presence of increased soil concentrations of Zn tended to result in a concomitant reduction in grain cadmium. The two calcareous soils used for this experiment had previously been sampled from locations on the North (Leatherhead) and South Downs (ADAS Bridgets), and winter wheat (Rialto) was planted in both. Together with a control, the experimental treatments consisted of increasing soil concentrations by 2, 5 and 10 mg Zn/kg soil. The added Zn was only a small percentage of that already occurring in the soils as background concentrations (Table 2), but realistic in terms of additions that might be added in a field situation.

Appendices C and D give the mean experimental results for in the Bridgets and Leatherhead soils, respectively. Reductions were observed in grain cadmium concentrations in both soils (Fig. 20), with grain concentrations reducing with increasing soil Zn concentration, although these differences were not significant. However, significant reductions ($P<0.05$) were observed with increasing soil Zn concentrations for Cd uptake into straw for both soils.

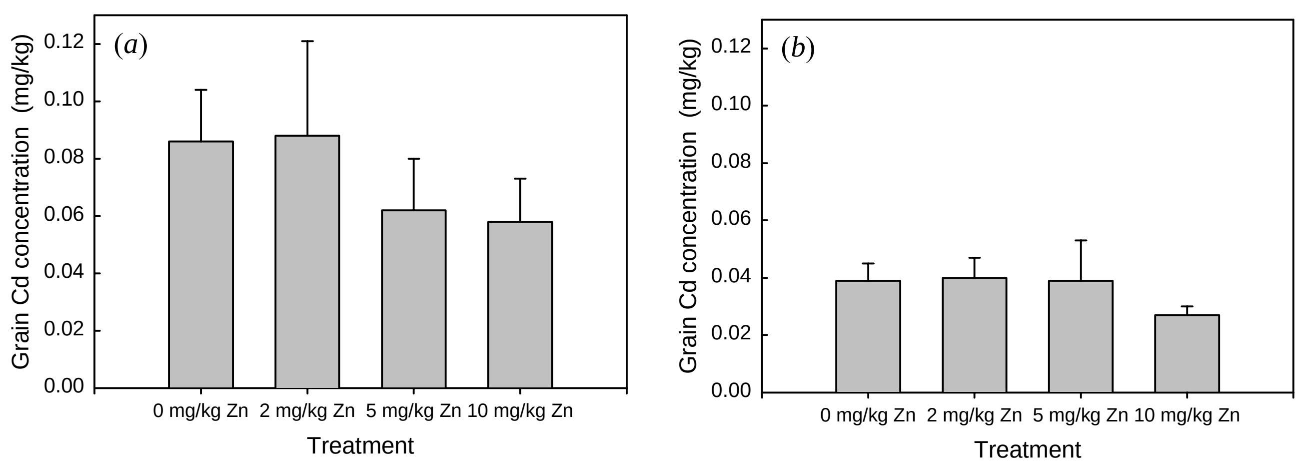


Figure 20. Cadmium concentrations in Rialto wheat grain grown in (*a*) Bridgets soil and (*b*) Leatherhead soil spiked with various amounts of Zn. Error bars show the standard deviation of each treatment mean.

No effect on crop yield with increasing Zn concentration was observed, implying that the wheat grown in both soils was not Zn deficient and that the lower grain cadmium concentrations did not occur as a result of growth dilution. In a study investigating the cadmium uptake of wheat grain grown in five different soil series, Mench *et al.* (1997) reported that the highest grain cadmium concentrations occurred in plants grown on a soil that led to marginal zinc deficiencies occurring in the shoots.

As with the previous pot experiments, lead concentrations in grain were low and no significant treatment effects were apparent. Although lead concentrations in straw were higher than those observed in the grain, standard deviations from the individual replicates were high, and there were no consistent relationships in the two soils between increasing soil Zn concentrations and straw lead content.

Given that the reduction in plant cadmium uptake did not appear to be caused by growth dilution effects, the mechanism behind the observed reduction in plant uptake with increasing soil Zn concentrations is likely be one of competitive exclusion occurring during root uptake.

Cadmium concentrations in the soil pore water solutions collected from these pots were very low (Appendices C and D), with a number of samples having concentrations below the analytical detection limit of the GFAAS instrument used. It was therefore not possible to demonstrate whether soil solution cadmium concentrations had changed with the increased concentrations of soil Zn.

Summary and Recommendations

This report describes research that investigated cadmium and lead uptake into British wheat and barley. Included in the research were:

1) an investigation of the geographical distribution of cadmium and lead concentrations in grain samples and how these related to soil properties,
2) a quantification of the effects of variety, nitrogen fertiliser form and rate, and of soil amendments on the cadmium and lead uptake into cereal grain,
3) measurements of the effects of past sewage sludge applications on grain cadmium and lead, using existing field experimental sites, and
4) measurements of the extent to which soil or dust contamination may have been responsible for observed high concentrations of metals in grain samples.

Information from this project was used by the UK Food Standards Agency for the European Commission negotiations on the cadmium and lead limits in cereal grain.

Cadmium and lead analyses were performed on grain samples from the 1998 Cereals Quality Survey and on representative paired soil and grain samples collected in a National Survey of Wheat and Barley from the 1998-2000 harvests. Results indicate that in general, wheat had higher grain concentrations of cadmium than barley, although both species had similarly low concentrations of lead. Concentrations of cadmium and lead in the vast majority of samples were below the newly-introduced European Commission limits specifying the maximum permissible contaminant levels in foodstuffs (cadmium in barley grain 0.1 mg/kg and wheat grain 0.2 mg/kg; lead in both barley and wheat grain 0.2 mg/kg fresh weight). Therefore, the majority of growers will not need to take any specific action to ensure that their grain complies with these limits.

The effects of previous sludge applications to land on cadmium concentrations in wheat and barley grain were also investigated at two field sites (ADAS Gleadthorpe and Rosemaund) with contrasting soil types. As for the survey investigations, barley grown at both sites had lower concentrations of cadmium than wheat grown the previous season. However, both the wheat and barley crops grown at Gleadthorpe had higher grain cadmium concentrations than at Rosemaund, despite total soil cadmium concentrations being lower at the former site. The variations in grain concentrations were primarily caused by differences in soil pH between the two sites, with the lower pHs at Gleadthorpe resulting in higher cadmium availability. This resulted in wheat grain concentrations approaching the maximum permissible contaminant levels at different total soil cadmium concentrations and indicates the caution required in evaluating the potential for a crop to exceed the European Commission levels. However, soil pH and total soil cadmium concentration when used together explained a large amount of the variation in grain cadmium concentrations. The proposed changes described in the European Commission draft Working Document on Sewage Sludge limit sludge applications to agricultural land based on both soil metal concentrations and soil pH.

Significant differences in grain cadmium concentrations were observed between wheat and barley cultivars from samples collected in the field and from pot experiments grown under controlled conditions. The choice of certain cultivars (e.g. the wheat cultivars Rialto and Soissons) may increase the risk that grain will take up relatively higher amounts of cadmium than might otherwise occur with an alternative cultivar.

Experiments also showed significant differences in grain cadmium concentrations with different nitrogen fertiliser forms and application rates, and with the application of certain soil amendments in pot experiments. Lime and an oxide-rich material (red mud), reduced cadmium concentrations in grain compared with the control. In contrast, a higher application rate of ammonium nitrate fertiliser (230 vs 180 kg/ha N) in a wheat field experiment resulted in higher grain concentrations of cadmium, which may have been due to increased soil acidification and enhanced Cd uptake in association with the increased nitrogen rate. However, no differences in grain cadmium were observed with different application rates of S fertiliser in the same experiment.

In summary, barley grown in Britain under typical field conditions and management regimes is unlikely to exceed the European Commission limits for either cadmium or lead. However for wheat, it is possible that in certain situations a combination of factors could lead to grain approaching or exceeding the permissible cadmium limits. Elevated grain wheat cadmium concentrations may occur where wheat is grown on soil that contains relatively high soil cadmium concentrations (0.84 - 33.86 mg/kg in the paired soil and grain sample survey), with Cd uptake further enhanced by low soil pH conditions.

Lime application is a feasible management strategy to reduce crop cadmium uptake where elevated grain concentrations are of concern, although the effects of any lime addition on trace element availability, in particular manganese, clearly needs to be borne in mind.

The choice of wheat cultivar can influence the grain cadmium concentration. For example, in this work the wheat cultivars Rialto and Soissons were identified as two varieties that may take up relatively higher amounts of cadmium. However, the continual development and breeding of new cultivars means any cultivar recommendations given here are likely to be outdated within a few years. As only a small percentage of UK field sites appear likely to exceed the Cd grain limit values it is also unlikely to be cost effective to routinely screen new wheat cultivars for cadmium uptake during their development. Liming is therefore likely to be the primary management tool available to farmers to reduce high grain cadmium concentrations.

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Appendices

Appendix A. Treatment means and standard deviations (sd) from the Fertiliser and Soil Amendment Pot Experiments for winter wheat (Cv. Rialto).

Characteristic		Treatment					
		NH_4NO_3	NH_4^+	NO_3^-	Red mud	Lime	Peat
Grain yield (g)	mean	18.3	17.7	16.8	20.5	18.4	18.6
	sd	3.4	2.3	1.7	0.5	1.6	1.1
Straw yield (g)	mean	8.2	9.0	9.7	9.0	8.7	10.3
	sd	0.6	0.5	1.8	0.8	0.8	0.8
Grain Cd (mg/kg)	mean	0.275	0.204	0.306	0.159	0.167	0.253
	sd	0.021	0.032	0.010	0.034	0.021	0.067
Grain Pb (mg/kg)	mean	0.030	0.021	0.022	0.010	0.025	0.014
	sd	0.040	0.022	0.014	0.000	0.021	0.009
Straw Cd (mg/kg)	mean	0.712	0.284	0.591	0.270	0.479	0.637
	sd	0.161	0.035	0.084	0.029	0.040	0.155
Straw Pb (mg/kg)	mean	0.086	0.111	0.160	0.044	0.052	0.058
	sd	0.027	0.029	0.033	0.029	0.057	0.038
Porewater Cd (μg/L)	mean	8.9	16.6	4.5	0.7	0.5	12.6
	sd	1.5	7.1	0.7	0.3	0.2	5.0
Porewater soluble	mean	88.4	134.0	82.4	91.3	103.5	95.8
organic C (mg/L)	sd	23.6	65.6	16.2	30.4	46.0	43.7

Appendix B. Treatment means and standard deviations (sd) from the Fertiliser and Soil Amendment Pot Experiments for winter barley (Cv. Regina).

Characteristic		Treatment					
		NH_4NO_3	NH_4^+	NO_3^-	Red mud	Lime	Peat
Grain yield (g)	mean	3.3	6.1	6.7	8.1	6.2	4.3
	sd	0.6	2.1	1.9	1.7	2.8	2.0
Straw yield (g)	mean	15.2	17.1	13.7	20.4	18.7	21.3
	sd	2.6	1.8	3.2	5.5	3.1	2.4
Grain Cd (mg/kg)	mean	0.303	0.319	0.290	0.274	0.259	0.285
	sd	0.072	0.035	0.032	0.029	0.039	0.033
Grain Pb (mg/kg)	mean	0.040	0.034	0.018	0.030	0.018	0.019
	sd	0.021	0.017	0.016	0.014	0.010	0.011
Straw Cd (mg/kg)	mean	0.535	0.755	0.711	0.700	0.603	0.846
	sd	0.180	0.118	0.148	0.062	0.125	0.139
Straw Pb (mg/kg)	mean	0.164	0.212	0.133	0.081	0.031	0.193
	sd	0.095	0.024	0.034	0.066	0.030	0.030
Porewater Cd(µg/L)	mean	5.7	16.9	2.2	0.9	0.5	6.2
	sd	2.4	2.8	0.9	0.2	0.2	1.7
Porewater soluble	mean	84.9	141.3	73.9	112.8	173.6	87.3
organic C (mg/L)	sd	6.0	73.5	18.2	52.6	48.6	32.6

Appendix C. Treatment means and standard deviations (sd) from the Zinc Soil Amendment Pot Experiments for winter wheat (Cv. Rialto) grown in Bridgets soil.

Characteristic		**Treatment**			
		0 ppm Zn	2 ppm Zn	5 ppm Zn	10 ppm Zn
Grain yield (g)	mean	12.4	11.9	10.6	10.8
	sd	3.1	2.6	2.5	2.1
Straw yield (g)	mean	14.3	13.6	13.5	13.9
	sd	0.6	1.8	2.1	2.0
Grain Cd (mg/kg)	mean	0.086	0.088	0.062	0.058
	sd	0.018	0.033	0.018	0.015
Grain Pb (mg/kg)	mean	0.036	0.041	0.015	0.024
	sd	0.028	0.046	0.013	0.027
Straw Cd(mg/kg)	mean	0.355	0.213	0.128	0.179
	sd	0.177	0.068	0.045	0.078
Straw Pb (mg/kg)	mean	0.129	0.076	0.101	0.026
	sd	0.072	0.037	0.064	0.032
Porewater pH	mean	8.05	8.08	7.97	8.07
	sd	0.15	0.25	0.08	0.15
Porewater Cd (μg/L)	mean	0.064	0.146	0.066	0.108
	sd	0.012	0.077	0.025	0.103
Porewater soluble	mean	13.5	15.2	13.8	12.4
organic C (mg/kg)	sd	5.7	2.2	7.8	5.1

Appendix D. Treatment means and standard deviations (sd) from the Zinc Soil Amendment Pot Experiments for winter wheat (Cv. Rialto) grown in Leatherhead soil.

Characteristic		**Treatment**			
		0 ppm Zn	2 ppm Zn	5 ppm Zn	10 ppm Zn
Grain yield (g)	mean	7.1	10.6	10.8	9.8
	sd	1.0	3.7	5.7	3.9
Straw yield (g)	mean	11.3	10.4	11.5	12.0
	sd	1.5	1.7	1.4	2.1
Grain Cd (mg/kg)	mean	0.039	0.040	0.039	0.027
	sd	0.006	0.007	0.014	0.003
Grain Pb (mg/kg)	mean	0.018	0.022	0.017	0.029
	sd	0.016	0.010	0.011	0.015
Straw Cd (mg/kg)	mean	0.203	0.171	0.111	0.130
	sd	0.032	0.046	0.024	0.068
Straw Pb (mg/kg)	mean	0.204	0.107	0.119	0.153
	sd	0.186	0.029	0.038	0.051
Porewater pH	mean	7.99	8.13	8.06	8.10
	sd	0.04	0.17	0.15	0.11
Porewater Cd (μg/L)	mean	0.126	0.065	0.089	0.083
	sd	0.039	0.013	0.032	0.037
Porewater soluble	mean	14.0	17.0	17.4	18.7
organic C (mg/kg)	sd	4.3	12.7	5.1	5.3