Final report to Ekhagastiftelsen

Project 2016-88 Trends of cadmium concentrations in organic and conventional wheat from a long-term field experiment

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Introduction

Cadmium (Cd) exposure and health effects

For the non-smoking, non-occupationally exposed population, food is the predominant source of Cd exposure, with cereals, vegetables, and fish being the primary sources for adults. Cd is toxic to the kidney, can cause bone demineralization (and fractures), and is a human carcinogen. Current dietary intakes in Europe are close to the tolerable weekly intake 2,5 µg Cd/kg body weight per week: The mean dietary exposure is estimated at 2,3 µg Cd/kg bw per week. For vegetarians, the mean exposure is estimated at 5,4 µg/kg bw per week [1]. A high intake of wholegrain products is expected to further contribute to Cd exposure.

There is a risk that the overall positive health effects of a diet rich in wholegrain and vegetables in part may be compromised by a high Cd exposure from these foods. For Sweden, the annual socioeconomic costs for bone fractures due to Cd exposure from food are estimated at 4,2 billion SEK. Limiting the Cd exposure from food is therefore expected to give rise to substantial socioeconomic gains [2].

One way of decreasing the Cd exposure via food is to use agricultural management techniques that promote a low Cd content of crops.

Determinants of crop Cd concentration

Cd is present naturally in soils in varying concentration. The influx of Cd to soils is predominantly from fertilizer, although atmospheric deposition also plays a role. The crop Cd concentration is dependent on the type of crop, the type of fertilizer and its Cd content, the soil Cd concentration and pH, liming, soil organic matter, and soil type. There are both short-term (direct uptake) and long-term effects (via accumulation in soil) of Cd application from fertilizers on crop Cd concentration [3].

Cd content in fertilisers

The Cd content in mineral and organic fertilizers is variable. However, there appear to be substantial systematic differences. In fertilizer samples from a large German fertilizer collection, different types of mineral P fertilizers had mean Cd concentrations ranging from 56-133 mg Cd/kg P [4], while organic fertilizers ranged between 14 and 37 mg Cd/kg P [5]. For comparison, the average Cd concentration of mineral fertilizers marketed in the EU is estimated to be approximately 75 mg Cd/kg P [6, 7].

Cd content in organic and conventional crops

There are indications that organic crops, specifically cereal crops, have a lower cadmium (Cd) content compared to conventional crops. One meta-analysis of 21 comparative studies reports such a difference [8], while another meta-analysis does not report this difference [9], but two large high-quality studies indicating differences have been missed (apparently by mistake) in the latter [10, 11]. We have been in touch with authors of both meta-analyses in order to discuss this matter. One author has supplied additional data where several inconsistencies have been addressed [12]; a statistically significant lower Cd concentration in crops, specifically cereal crops, was still reported. However, so far no single study comparing the effect of long-term (>10 years) organic vs. conventional management has been published.

The rationale for a lower Cd content in organic crops is primarily a lower Cd content in organic compared to mineral fertilizers, and secondly a higher soil organic matter content in organically managed soils [13, 14].

Hypothesis

Studies included in the meta-analyses above have only addressed short-term effects of conventional vs. organic crop management, because no long-term field trials, or farm-pairing studies reporting the history of farms, have been published. Time trends of crop Cd concentration have not been reported. This surprising knowledge gap has recently been identified by us [15]. Therefore, published studies are likely to underestimate the effect of farm management on crop Cd concentration, specifically in the long term.

Hypothesis:

(1) In a long-term farming experiment, the time trend of Cd concentration has a higher slope for conventional compared to organic wheat.

I.e., Cd concentrations are higher in conventional wheat than in organic wheat, and this difference is increasing over time.

Materials and methods

The DOK trial

The DOK trial, situated in Switzerland, is the most significant long-term experiment comparing organic and conventional farming practices in the world. Ongoing since 1978, it compares eight production systems in a seven-year crop rotation, with 12 replicate plots per system [16].

Samples

For this study, we chose winter wheat samples grown twice during the 7-year rotations in the DOK trial. A total of 130 wheat samples as well as the corresponding 130 soil samples are available per production system. For this project, we select the systems O2 (organic management at regular fertilizer level, manure and slurry from organic farms) and M (conventional management with mineral fertilizer, the legal limit for Cd is 115 mg Cd/kg P in Switzerland).

These systems are chosen as most relevant systems, which best represent contemporary organic and conventional production in Europe. Furthermore, for soil samples, we also add system N (no fertilization) as a comparison.

Additional data

Existing data on soil organic matter and soil pH are of relevance to this project.

Measurements of cadmium and other minerals

The elemental composition of wheat grains was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) (Cd and Mo) and ICP-optical emission spectroscopy (ICP-OES) (Mg, P, S, K, Ca, Mn, Fe, Cu, Zn) following the description by Laursen et al. (2011) and Hansen et al. (2013). In summary, 100 mg of dry and powdered tissue was digested in vessels containing 2.5 mL of 70 % ultrapure HNO_3 and 1 mL of 30 % H_2O_2 in a pressurized microwave oven (UltraWAVE, Single Reactor Chamber System, Milestone Srl, Sorisole, Italy). The analysis was conducted using an Agilent Technologies 7900 ICP-MS or Agilent Technologies 5100 ICP-OES coupled to a CETAC ASX-520 auto sampler. Blanks and certified reference materials from NIST (National Institute of Standards and Technology, MD, USA) and NACIS (National Analysis Center for Iron and Steel, China) were included in the sequence. The NIST references were 1515 (apple leaves) and 1567b (wheat flour) and the NACIS reference was zc73013 (spinach leaves). Elements present above the limit of detection and with accuracy >90% were accepted.

Data analysis

Data analysis is primarily performed with linear mixed-effects models, performed in RStudio using R version 3.6.1 [17] and the R package nlme version 3.1-140 [18].

Two linear mixed models were constructed: The initial model to describe the overall observations of wheat Cd content in relation to the production system and the wheat cultivar. Here, the production System and the Cultivar were specified as main effects, while the field Block and field Plot (nested in Block) were specified as random effects; on these latter units, repeated (longitudinal) measures were taken. In the second model, Year, System, Cultivar, Soil pH and C_{org} (soil organic matter) were specified as main effects, and an interaction of System * Year was used to test for time trends in Cd concentration that differ between production systems. Again, Block and Plot were specified as random effects.

The structure of random effects was initially evaluated by a likelihood ratio test of a suggested model and a simplification. The structure of the variance-covariance matrix was determined using knowledge of the study design, Akaikes information criterion (AIC), as well as the Bayesian information criterion (BIC): Due to an expected autoregressive element in the data and varying time periods between consecutive wheat cultivation years, a first-order continuous autoregressive (CAR(1)) covariance structure with constant variances is *a priori* expected to best describe the data. A covariate representing the chronological time (number of years) since the first available sample (in 1992) was included in the model for fitting the CAR(1) covariance structure. This structure was compared to the alternative structures compound symmetry (CS), first-order autoregressive (AR(1)), and unstructured (UN). *A priori*, the variance was assumed to be homogenous. As alternatives, heterogenous variances with respect to Year, Cultivar, and Year*Cultivar were considered. All combinations of covariance and variance structures were considered.

An alternative structure was chosen if it led to a decrease in AIC by at least 2 units compared to the a priori expected model, while a substantial increase in BIC with increased model complexity was interpreted as a sign of overfitting, and such models were avoided [19-21]. If this rationale for model selection did not produce an unequivocally favoured model, the most favoured model was chosen as the main model, and alternative models were separately evaluated with respect to the effect size and hypothesis test of the factor of interest (i.e. the Year*system interaction term), and any substantial deviations from the main model were reported.

Model assumptions were evaluated as follows: fitted values were plotted against standardized residuals and the plot was visually evaluated for extreme values (outliers) or data structures which the model does not account for. Normal distribution of residuals (error terms and random effects) was evaluated by visual inspection of qq-plots and Shapiro-Wilks test. The absence of a correlation of random effects and error terms was evaluated by plotting and visual inspection as well as by calculating and testing Pearson's correlation. If indicated, simple transformations were attempted (inverse, square root, log transformation, inverse of square root). Deviations from the assumptions were reported. The source of the deviation was investigated and where possible addressed in a sensitivity analysis (e.g. exclusion of outliers); results from the modified model were compared to the original model, and any major deviations reported. If no major deviations were observed, only the results from the main model representing the entire study population were reported.

The final specification of the linear mixed models used in the analysis was

```
wheat.fit <- lme(log10(Cd) ~ system * cultivar,
        random=~1|block,
        correlation=corAR1(form=~1|block),
        weights=varIdent(form=~1|cultivar),
        data=Cddata)
```

and

```
wheat.fit.2 <- lme(log10(Cd) ~ system + cultivar + pH + Corg + year + system:year,
        random=~1|block,
        correlation=corAR1(form=~1|block),
        weights=varIdent(form=~1|cultivar),
        na.action=na.exclude,
        data=Cddata)
       method="REML", data=dataset)
```

Results

Analysis of plant nutrients indicates that no mineral or other deficiency was present and that adequate plant nutrition was achieved in all samples (details not shown).

Figure 1 illustrates wheat kernel Cd concentrations during the course of the experiment. Across the entire range of years with available samples, and across all varieties, organic wheat samples apparently contain less Cd.

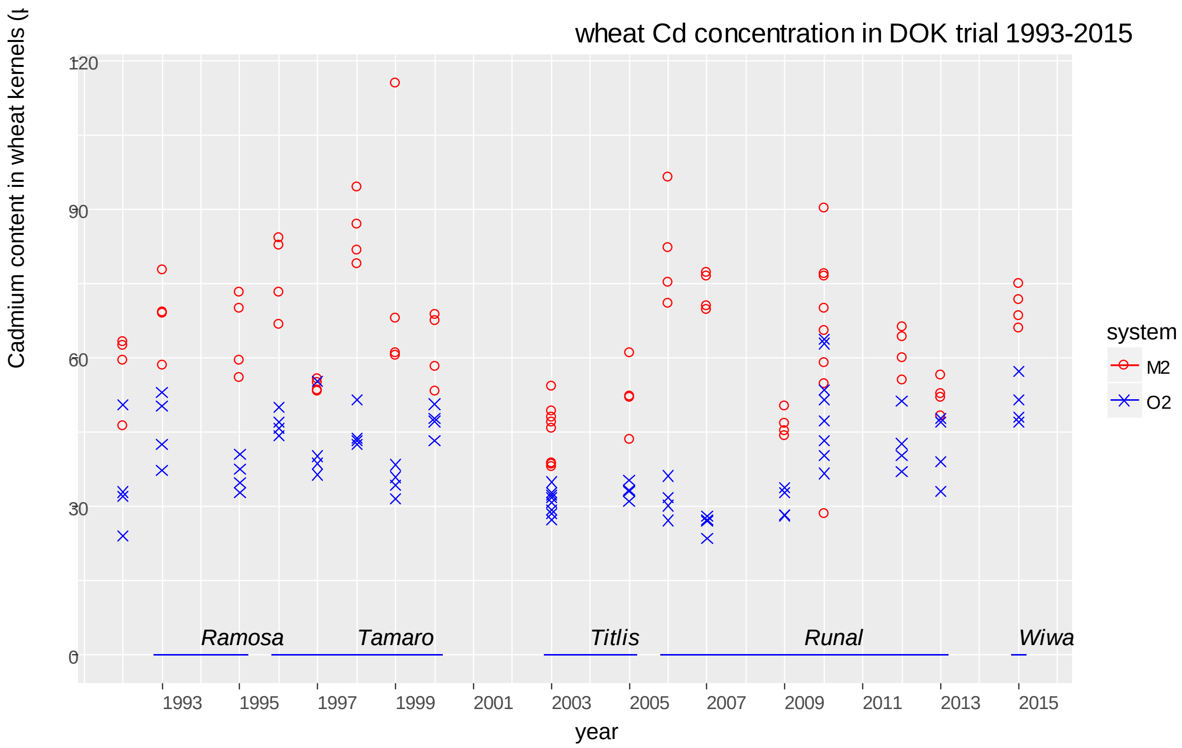

Figure 1. Cadmium content of wheat kernels over time, by production system. The wheat variety used in the respective year is indicated. M2 – minerally fertilized; O2 – fertilized with farmyard manure

Table 1 shows overall means of wheat kernel Cd content across all samples in the present study.

	M2	O2
mean	63,5	39,7
stddev	15,2	9,3

Table 1. Overall means and standard deviations of wheat cadmium concentration by production system, in µg/kg. M2 – minerally fertilized; O2 – fertilized with farmyard manure

A 60% higher cadmium content in the minerally-fertilised (conventional, M2) system, compared to the manure-fertilised (organic, O2) system is apparent.

	M2	O2
Titlis	47,4	31,7
Ramosa	63,8	39,0
Runal	63,8	39,0
Tamaro	71,1	43,4
Wiwa	70,4	51,0

Table 2. Means of wheat Cd concentration by System and Cultivar, in µg/kg.

An initial linear mixed model was constructed to formally test these observations. Initial observations indicated a log-normal distribution; Cd concentrations in µg/kg were therefore used as their natural logarithms. After testing, only the Block was included as a random effect. The covariance structure CAR(1) was not compatible with this random effects structure, because several measurements in a single year within one block occur in two of the years. The selected covariance structure was AR(1). The alternative simpler model CS did not improve model, as evaluated by AIC. A high shift in BIC (+52 units) of UN compared to AR(1) indicated overfitting. Inclusion of variance heterogeneity with respect to the variety did improve the model substantially and was therefore implemented. Random effects and error terms were normally distributed. There was no indication of a correlation of error terms and random effects (r=-0.13, p=0.11).

The resulting model demonstrates that the main effect “System” was statistically significant at $p=3.2*10^{-5}$. Back-transformed means were 47.5 and 31.0 µg Cd/kg for M2 and O2 wheat, respectively, maintaining the relative difference in Cd concentration between wheat from different production systems as shown in Table 1. Also, the cultivar had an overall significant effect on wheat kernel Cd concentrations.

When included into the same model, a System x Cultivar interaction did not have an apparent effect on wheat Cd concentrations (p=0.14), confirming that the shift in wheat Cd between production systems is independent of the cultivar used, among the five cultivars tested.

The model indicates that a major fraction of variation is unexplained.

Therefore, and to test if time trends exist, a second linear mixed model was constructed. Again, only the Block was retained as a random factor. The covariance structure AR(1) with a variance heterogeneity with respect to the cultivar was selected based on AIC and BIC. Random effects showed a slight deviation from normal distribution, which could not be remedied by transformation. Error terms were normally distributed, and there was no correlation between error terms and random effects (r=-0.13, p=0.11).

Tests of the resulting model show a significant main effect of the production system on wheat Cd concentrations ($p=4.7*10^{-11}$), where the difference between systems is essentially unchanged from the initial analysis above. Also, the main effect of the cultivar is confirmed. The pH is negatively correlated to log_{10}(Cd) concentrations in wheat. In units of log_{10}(Cd) expressed in µg Cd/kg, the effect per unit of pH decrease is +0.075 ($p=9.7*10^{-4}$), for example corresponding to a change from 50 to 59 µg Cd/kg under otherwise constant conditions.

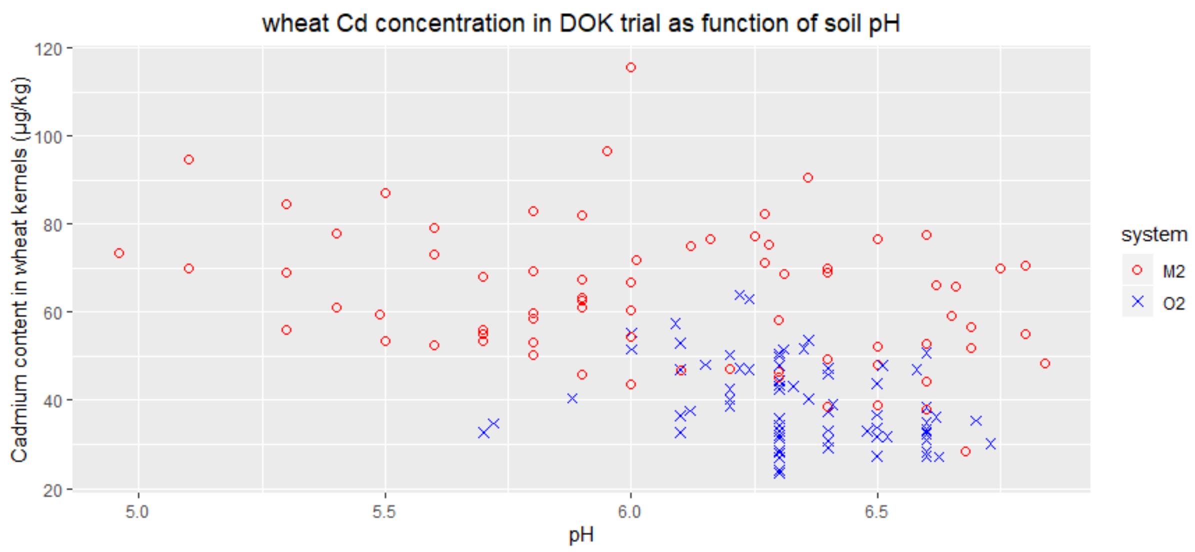

Figure 2 wheat Cd concentration in DOK trial as function of soil pH

This relationship is further explored in Figure 2 and Figure 3. The pH range within the DOK trial is wider for M2 than for O2 soils; namely, lower pH values occur in the M2 soil samples. A temporal decreasing trend in pH values is indicated for M2 soils between 1977 and 1999, which is reversed by liming in 1999 and again in 2005. A corresponding trend is not apparent in animal-manure fertilized O2 soils.

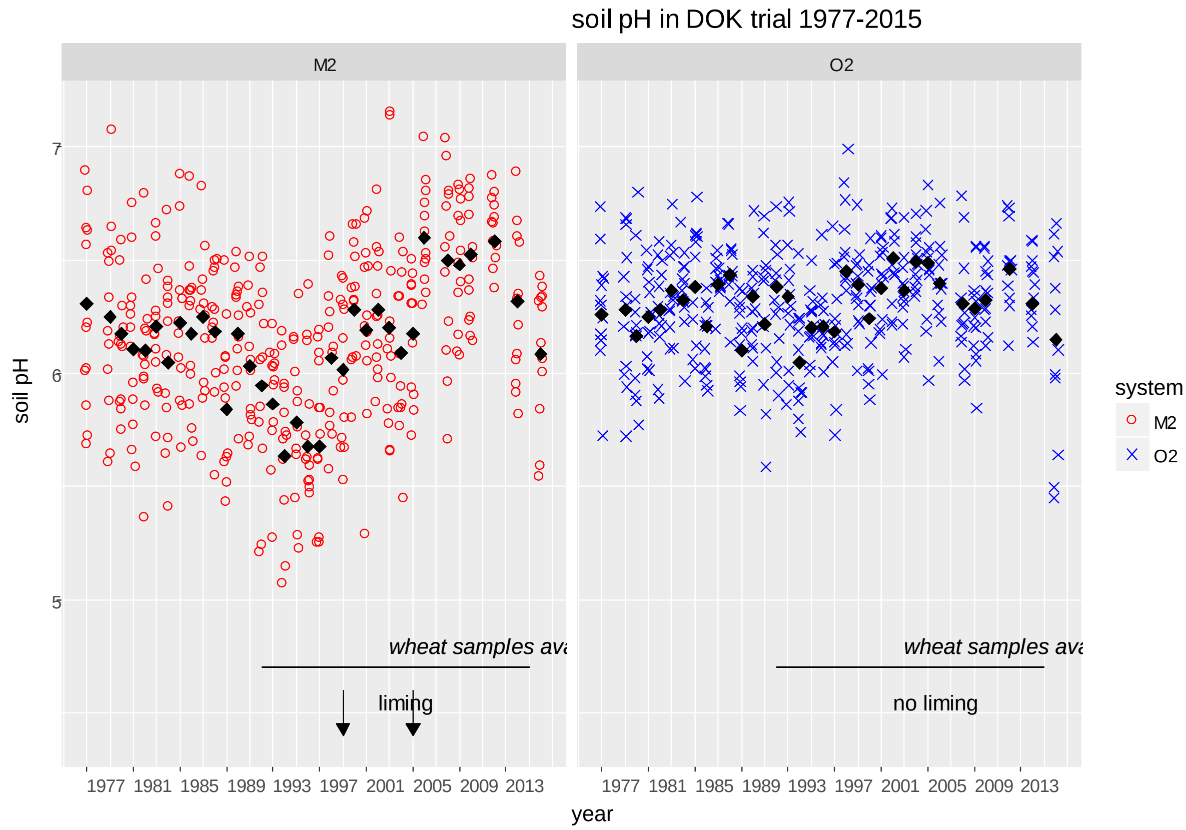

Figure 3. DOK trial soil pH data over time, all field plots. Liming in the M2 system is indicated by arrows. Diamond symbols represent medians for that year

	mean soil Corg (%)		
system	**1977-1990**	**1991-2002**	**2003-2014**
M2	1,54	1,33	1,14
O2	1,47	1,37	1,24

Table 3. Soil organic matter, expressed as % organic carbon

No effect of soil organic matter, the production year, nor the interaction of production system and year was indicated within this study. Accordingly, the null hypothesis, that there is no time trend in the difference in wheat Cd between production system, could not be rejected. Instead, we found a stable difference in wheat Cd between production systems over time

Discussion

To our knowledge, this is the first study which addresses long-term time trends in crop Cd concentrations under organic compared to conventional production methods. We assumed that a heterogeneity in study results investigating crop Cd contents in relation to production system, as discussed in the introduction, might be explained by the fact that some studies of this matter might have been too short in order for such an effect to manifest.

The DOK trial was established in 1977, but wheat samples were only available from 1992-2015 for the present study. For this period, we find a stable difference of approximately 50% high Cd levels in conventional compared to organic wheat. We conclude that within the DOK trial, 15 years of M2 vs O2 management are sufficiently long for differences in crop Cd concentrations to develop and to stabilize. What we cannot conclude based on this study is how many (or how few) years are actually needed for this stabilization.

Other findings include a negative association of soil pH and wheat Cd; increased uptake of Cd at lower pH is well described in the literature. Also, an effect of soil organic matter, know to counteract Cd uptake, has not been observed; possibly, the range of soil organic matter (0.78 – 2.28 % expressed as organic C) was too narrow for such an effect to be measurable.

Further work

We initially allocated the limited resources towards analyses of cadmium on wheat, as well as to analyses of additional plant nutrients in wheat, the latter with the purpose of demonstrating adequate plant nutrition. We decided however at a later stage that top soil Cd measurements would still be informative as they might constitute an important predictor of wheat Cd concentrations in the present study. Therefore, we initiated soil Cd measurements corresponding to each data point in this study, as well as additional soil samples from the same field plots during the initial phase (0-15 years) of the experiment. These measurements have been performed (November 2019) and are currently (December 2019) under data pretreatment. A manuscript based on this study is under preparation, which will also include the soil Cd data.

Conclusion and final word

We conclude that in a long-term field trial, the cadmium content of wheat from a conventional system with mineral fertilizer was increased by approximately 50% compared to wheat from an organic system fertilized with farmyard manure. This difference in cadmium content was stable during the time span between 15 and 38 years from the start of the experiment, and independent of the wheat cultivar. Due to a lack of wheat samples from the time span 0-15 years, we cannot conclude on the dynamics of the developing difference in wheat Cd content due to production system.

Among existing studies of cadmium content in conventional and organic crops, the present study is, as far as we can tell, the only one with a dedicated longitudinal design and analysis and also the only one to include several important soil properties as covariates. This project might therefore add to existing knowledge and supports meta-analyses that have identified a lower cadmium content in organic compared to conventional crops.

We would like to thank Ekhagastiftelsen for providing funding for this project.

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