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Abstract

Contamination of sunflower seeds from soil Cd is an important issue for food and feed because this species strongly accumulates this metal. The present work reports that seeds from three sunflower varieties (ES Biba, Extrasol, Vellox) cultivated in the field in a calcareous agricultural soil having a moderately high Cd content (1 mg Cd/kg) had Cd contents of 0.84, 0.88, and 0.76 mg Cd/kg, respectively, all exceeding the regulation limit of 0.5 mg Cd/kg seeds for human food. On average, for the three varieties, washing seeds did not affect their total Cd contents but slightly increased the Cd in the kernels at the expense of that in hulls. Despite the Cd content of the whole seeds did not differ between the varieties, the Cd fraction in the edible kernel differed significantly between varieties from 78 to 87% of the total seed Cd. The results of this study suggest that i) the size of the kernel, relatively to that of the hull may affect the dilution of Cd in kernel tissues, ii) there might be genetic variability for the capacity of transfer of Cd from the hull to the kernel. This opens the perspective to increase food safety by selecting sunflower genotypes that retain more Cd into the hull and transfer less of it to the edible kernel.

Keywords: Agricultural soil contamination, ES Biba, Extrasol, Genetic variability, Oil seed, Regulation limit, Vellox

Introduction

Sunflower is an important food crop, the seeds of which are consumed by humans or extracted for oil production whereas the meals (press cakes) return to animals as a valuable source of proteins and fibres (Adeleke and Babalola, 2020; de Oliveira and Egea, 2021). Unfortunately, sunflower also strongly transfers cadmium (Cd) from soil to seeds, much more than wheat or rice (Li et al., 1997). Apart from polluted soils like those near mine sites that pose serious health risks (Chen et al., 2022), diffuse contamination of agricultural soils by Cd derived from soil formation or from agricultural inputs and atmospheric fallouts (Sterckeman et al., 2018) is also a concern because it may result in harvests that do not comply with the regulation for contaminants in food products (Nguyen et al., 2021). The Cd content of sunflower seeds for human consumption excluding oil production is regulated at 0.5 mg Cd/kg (EC 2023/915). Generally, only the kernels (almonds) are consumed as food and the study of Linero et al. (2018) suggests that Cd may be more concentrated in the kernels compared to the hulls but this result should be confirmed because it was obtained in hydroponics. On the other hand, little Cd is found in oil (Petraru et al, 2021; Zehra et al., 2020) and most of the contaminant remains in the meals (Angelova et al., 2004; Yang et al., 2017). If the latter are used for animal feed, they should not contain more that 1 mg Cd/kg (Directive 2002/32/EC). Furthermore, if Cd is actually more concentrated in the kernels, de-hulling the seeds to increase the protein content of meals for feed could increase their level of Cd. There are also some prospects to valorise sunflower meals from pressed kernels as a source of proteins and other nutrients for humans (Adeleke and Babalola, 2020) but this may be seriously hampered by the fact that the meal fraction derived from kernels is expected to particularly concentrate Cd compared to the whole seed.

Like in many other species, in sunflower, there is some genetic variability in the potential accumulation of Cd (Laporte et al., 2015; Li et al, 1997) but it is unknown if it also affects the partitioning of Cd between the hull and the kernel, which would be of great interest for selecting genotypes that transfer less Cd to the kernels in the perspective of preserving food/feed safety. Therefore, the goals of this work were 1) to confirm that Cd was more concentrated in kernels compared to hulls in field-grown plants and to evaluate the consequences for the Cd content in meals, 2) to test the hypothesis that there were some differences between varieties for the partitioning of Cd between the hulls and the kernels as a possible perspective for breeding.

Materials and Methods

Sunflower seeds were collected in a field trial comparing three oil seed sunflower varieties, ES Biba, Extrasol and Vellox, cultivated on a calcareous loam in the Charentes-Maritimes county in France. The trial consisted in a randomized 3 blocks design where each block was divided into 3 subplots randomly assigned to the 3 varieties to compare them. Therefore, there were 3 replicates for each variety. Sowing (72 000 plants/ha) was performed in mid-April 2013 and plants were harvested at maturity the first week of September. Plants were fertilized in the last week of May with ternary NPK 22-13-12 fertilizer (77 kg N, 46 kg P_2O_5 and 42 kg K_2O per hectare). The soil had the following characteristics: pH (water) 8.6, $CaCO_3$ 30 g/kg, clay 27%, loam 58%, sand 15%, CEC (Metson following the normalized method NF X31-130) 19.5 cmol$^+$/kg, organic carbon 1.09%, aqua regia Cd content 0.99 mg Cd /kg. Plants looked healthy during their whole growth and did not suffer from any symptoms of Cd toxicity, which is consistent with the moderate soil Cd content and with the fact that for these ranges of soil Cd, sunflower is known to have some tolerance (Zehra et al., 2020). A representative sample of 1.4 kg seeds was collected at maturity for each block x variety subplot and used for the study. In order to check that seed Cd was not significantly affected by some dust deposited onto the hull during harvest, half of each sample was washed three times with distilled water whereas the other half was kept unwashed. Washed and unwashed subsamples were all split into two additional batches, one was used to separate kernels from hulls by a de-huller at 2500-3000 rounds/min (pilot de-hulling equipment Techmachine) and the other was left intact to study the whole seeds. The water content of the samples was determined after drying at 103°C until constant weight. The oil content of the seeds for the different varieties was determined by nuclear magnetic resonance (NMR) following the international standard method ISO 10565 (ISO 10565:1998). The Cd contents of seeds, kernels and hulls were determined by atomic absorption spectroscopy after sample digestion in HNO_3-HCl mixture. The analyses were performed by the Cofrac quality-certified laboratory Capinov (https://www.capinov.fr). The limit of quantification of Cd was 0.01 mg/kg. The Cd content of meals were calculated based on the Cd contents of hulls and kernels and on the oil content of seeds, assuming that Cd in oil was 10% of the whole seed Cd (median calculated from the work of Zehra et al., 2020 for 40 sunflower genotypes). All Cd contents were expressed relatively to the dry weight except the Cd contents of the whole seeds in Table 1, which were based on a residual water content of 9%, which is the standard humidity for trading (Incograin, 2021).

Data were analysed by a mixed-effect model with the block as random effect whereas the variety, the seed parts (kernels or hulls) and the washing treatments were set as fixed effects. Normality of the residuals was checked and when present, heteroscedasticity was corrected by the *varPower()* function of the *nlme* package (version 3.1-162) for the R language (R version 4.2.2). Treatments were compared by using Tukey post-hoc tests at $p<0.05$ adjusted for multiplicity (*emmeans* R package, version 1.8.4-1) with three replicates corresponding to the blocks for each variety.

Results and discussion

The Cd content in sunflower seeds, recalculated from the Cd contents in hulls and kernels, yielded on average 97+/- 0.08% of that directly measured on the whole seeds (not shown), indicating that de-hulling had a negligible effect on the relative proportions and Cd content of hulls and kernels.

Weak effect of seed washing on the Cd content of hulls and kernels

The Cd content of the whole seeds (Table 1) was not significantly affected by washing ($p=0.71$), nor by the variety ($p=0.58$; interaction varieties x washing $p=0.79$). Therefore, some possible dust deposited onto the hull did not significantly affect the Cd content of seeds, which fully derived from the transfer from soil to plant organs and finally to seeds. However, a weak significant effect of washing was found for kernels and hulls. Washing decreased the Cd content of the hulls by 27% ($p=0.09$) whereas it increased that of kernels by 18% ($p=0.04$), regardless of the variety (Fig. 1). Our interpretation is that hull dessication during seed maturation likely disrupted the internal pericarp cells, which also contain Cd (Pessôa et al., 2017), making the Cd more mobile during the washing and therefore, part of the hull Cd from the inner hull might have diffuse towards and into the kernels as they become soaked. So, washing sunflower seeds to remove part of the Cd contained onto or in the hulls may not be a good option since according to our results, it might increase Cd in kernels, which are the main edible parts of the seeds.

High Cd contents in whole seeds despite alkaline soil conditions

The Cd contents of seeds at the trading standard water content of 9% were all below the current regulatory limit of 1 mg/kg for feed (Directive 2002/32/EC), but above the current regulation limit of 0.5 mg Cd/kg for human food (EC 2023/915; Table 1). This contamination is not representative of the average level of Cd in sunflower oil seeds in France, the mean of which being 0.354 mg Cd/kg (Terre Inovia, National Survey Plan on Oilseeds food safety, France). The high contamination observed here is linked to the soil used in this study, which had a Cd content of 0.99 mg Cd/kg soil. This is high compared to most of the French agricultural soils, the median of which being 0.2 mg Cd/kg (90^{th} percentile at 0.61 mg Cd/kg). This high Cd content is frequent in the county of Charentes-Maritimes and originated from the Cd-rich calcareous substratum on which the soils developed. Literature reports that there is a strong control of Cd bioavailability by pH (McLaughlin et al., 2021). In general, this is true but as shown by our results, high soil pH like the one here (pH 8.6) does not guarantee that the crop will little accumulate Cd, especially if the total Cd content of soils is high. The most likely reason is that calcareous soils are unfavourable for the bioavailability of important micronutrients like Fe, Zn and therefore, plant roots are able to modify soil conditions in the rhizosphere to increase the bioavailability of micronutrients by acidification and by the release of chelating compounds (Vélez-Bermúdez and Schmidt, 2023). However, this is likely also effective for toxic cations like Cd, which become more bioavailable for root absorption.

Kernels are more concentrated in Cd than hulls

As hypothesized, kernels were more concentrated in Cd compared to hulls (Fig. 2) and the ratio depended on the varieties: x2.6 for ES Biba, x1.3 for Extrasol and x1.5 for Vellox. This observation has important consequences for the food/feed chain. Indeed, the Cd concentrations in kernels for ES Biba, Extrasol and Vellox were 1.1, 0.93 and 0.85 mg Cd/kg DM, respectively, much above the regulation limit of 0.5 mg Cd/kg for human food (EC 2023/915). The European Food Safety Authority recommended a maximum dietary intake of Cd of 2.5 µg Cd/kg body weight/week (Efsa, 2011). For a person of 70 kg, this limit would be reached by consuming 159 g of ES Biba kernels from our study in one week, or 23 g per day, which is plausible.

The three sunflower varieties used in our study are seed oil ones. The Cd content of oil were not determined here but literature shows that the fraction of seed Cd found in oil is low, most of the Cd remaining in the meals, which are therefore more concentrated compared to the whole seeds before oil extraction (Angelova et al., 2004; Llorent-Martínez et al., 2019; Petraru et al., 2021; Yang et al., 2017). From the work of Zehra et al., (2020) we calculated that on average, for 40 genotypes grown in two soils, 10% of the whole seed Cd was found in oil (median absolute deviation: 0.06%). This fraction was used to estimate the Cd content of the meals after oil extraction for our three sunflower varieties from the Cd in kernels, hulls and from the oil contents (Table 2). The calculated Cd contents in the meals derived from the whole seed (hulls + free-oil kernel residues) exceed the regulation limit for feed of 1 mg Cd /kg (Directive 2002/32/EC). Moreover, because of the higher content of Cd in kernels compared to hulls, the partial de-hulling, which is performed to increase the protein content of meals for feed, results in an increase in the Cd content of meals (Table 2). The prospects of using kernel meals obtained after oil extraction as a source of proteins for humans is also strongly hampered by the fact that kernels concentrate Cd. Here, we calculated Cd contents in kernel meals of 2.6 to 3.4 mg Cd/kg DM (Table 2), which is above the limits of 1 mg/kg for food supplements for humans (EC 2023/915).

Variation in kernel Cd contents depending on varieties

The Cd contents of kernels significantly differed between varieties, in particular, ES Biba kernels were 1.3 times more concentrated in Cd compared to Vellox (Fig. 2). These differences might be in part explained by the efficiency of Cd transfer from the hull to the kernel because the fraction of the seed Cd recovered in kernels was significantly the highest for ES Biba (Table 2). During seed filling, the pericarp, which is a maternal tissue and which will become the hull, is not symplastically connected to the filial tissues of the kernel (Patrick and Offler, 2001, Zhang et al., 2007). The nutrients, and likely Cd, are excreted in the pericarp apoplast from which they are absorbed by the kernel cells. So, both the excretion and the absorption can differ between varieties, resulting in varietal differences in the efficiency of Cd transfer from the hull to the kernel.

On the other hand, ES Biba had the lowest kernel:hull biomass ratio whereas Vellox had the highest (Table 2). Hence, smaller kernel size relatively to that of the hull means that the Cd transferred from the hull dilutes less in the kernel, which is therefore more concentrated as illustrated by ES Biba.

This might be an additional explanation to the varietal differences in the content of Cd in kernels observed in our study. The variations in the kernel:hull biomass ratios cannot be explained by some geometry reasons linked to the size of the seeds, which predicts that small seeds should have relatively smaller kernel:hull biomass ratios and conversely. Indeed, compared to ES Biba, Vellox had a lower individual seed weight but a larger kernel:hull biomass ratio (Table 2). These differences in the kernel:hull biomass ratio between varieties might reflect the fact that in sunflower, the kernel variably fills the hull at maturity (Lindström et al., 2007). The space corresponds to former tissues of the internal pericarp that decayed during the final stage of seed growth, whereas the external pericarp remains and becomes the hull (Lindström and Hernández, 2015).

Previous studies have reported a genetic variability in the Cd contents of sunflower kernels/seeds (for example, Li et al., 1997; Zehra et al., 2020) and selection of low Cd accumulating cultivars can be used to preserve food safety regarding Cd. However, the ‘low-Cd’ cultivars must also perform well regarding yield and other food values such as the contents in proteins and oil and the quality of the latter. The exact mechanisms underlying the variable capacity of sunflower genotypes to accumulate Cd in seeds are poorly understood. There is some variability in Cd root uptake and sequestration (Laporte et al., 2015) and there might be a limitation of Cd supply to the seed itself: poor root uptake during seed maturation due to the decline of the root system and/or to the least Cd bioavailability soil, competition between seeds due to transport limitations (Steer et al., 1988), for example. Here, our study suggests that cultivars maximizing the size of the kernels inside the hulls might be of interest to dilute Cd in the kernels. A high kernel:hull dry weight ratio is also valuable since it contributes to increase the fraction of the seed that is the most used for food. A high ratio is also among many others, a favourable component for the individual seed weight and therefore for yield. So, the kernel:hull ratio merits further investigations to better understand its genetic and environmental controls because it has important effects on the agronomic performances and on the food value and safety of sunflower harvests.

Implications for food safety

The fact that most of the Cd of sunflower seeds is contained in kernels (around 80% in this study) questions the European regulation for Cd in foodstuff (EC 2023/915), which sets the maximum Cd content for sunflower seeds (0.5 mg Cd/kg) without clearly indicating if the limit is for the whole seeds or for only the kernels, which are generally the only part consumed by humans. Indeed, on one hand, the article 2 of the regulation states that the limits are for the edible part of the product which suggests that the Cd limit for sunflower seeds is only for the kernels, but on the other hand, whereas for tree nuts, it is clearly stated that the limit is for the product after removal of the shells, for oilseeds, this detail is not mentioned. Considering the potential importance of sunflower kernels to the dietary intake of Cd for humans, in our opinion, the regulation should be less ambiguous. Also, the genetic variability for Cd content in sunflower seeds already reported in literature (for example Li et al., 1997) should be reconsidered to examine the variability for Cd in kernels. As it was done for durum wheat (Maccaferri et al., 2019), the physiological and genetic controls of Cd transfer to the kernels should be investigated to help breeding/selecting varieties that accumulate less Cd in kernels.

Conclusions

The present work showed that contrary to our hypothesis 1, washing seeds did not remove significant amount of Cd but, worst, it slightly increased the Cd in kernels from the hull. As assumed by hypothesis 2, kernels were actually much richer in Cd than hull. Despite the Cd content of sunflower whole seeds did not differ between the three varieties, there were significant differences regarding the Cd content of kernels, as stated by our hypothesis 3. The results of this study suggests two directions to further explore in order to understand these differences and in the end, to improve the safety of sunflower seeds regarding Cd: i) the size of the kernel relatively to that of the hull, which may favour the dilution of Cd in the kernel tissues, ii) the genetic variability of the capacity of the pericarp to excrete Cd into the apoplast and that of the kernel to absorb the metal from this space. As also shown by this work, there is an important challenge to guarantee a low Cd content in the edible fraction of sunflower seeds, particularly in the prospect of using the meals obtained after oil extraction for valorizing their proteins and other nutrients in human food products.

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Statements and Declarations

Author contributions

Christophe NGUYEN: Conceptualization; Data curation; Formal analysis, Writing original draft

Jean-Philippe LOISON: Performing experiment, Review & editing

Céline MOTARD: Review & editing

Sylvie DAUGUET: Conceptualization; Data curation; Formal analysis; Methodology; Project administration and supervision; review & editing.

Ethics declarations

Ethical approval

There is no ethical issues in this research. No Human participants nor Animals were involved.

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Consent to participate

All authors agree to participate to this research.

Consent to Publish

All authors agreed with the content of the present manuscript and all gave their explicit consent to submit it for publication in Environmental Science & Pollution Research. All authors have the consent from their responsible institute for this submission.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data availability

Detailed data are available upon request to the corresponding author.

Table 1 : Cd contents (mg Cd /kg DM at 9% residual water content) of the whole seeds of three sunflower varieties grown in the field in a calcareous soil. Seeds were washed or not prior Cd determinations (see materials and methods for the washing details). There were no significant effect of the variety nor of the washing on the Cd contents of whole seeds. Standard errors given between parentheses (n=3).

	Cd contents (mg Cd /kg DM at 9% residual water content)	
Varieties	No seed washing	Seed washing
ES Biba	0.84 (0.11)	0.88 (0.12)
Extrasol	0.88 (0.13)	0.83 (0.11)
Vellox	0.76 (0.11)	0.70 (0.11)

Table 2: Some characteristics of seeds and their Cd contents (mg Cd /kg dry matter) in three field-grown sunflower varieties. For a given line varieties with different letters differed significantly at $p<0.05$ (Tukey test). Standard errors given between parentheses (n=3).

	Varieties		
	ES Biba	Extrasol	Vellox
Mean dry matter of individual seeds (mg/seed)	56.1	58.7	54.5
Whole seed oil content (% of dry matter)	54.1	53.1	57.5
Kernel:hull dry matter ratio	2.52 (0.10) a	2.71 (0.11) ab	2.88 (0.11) b
Fraction of Cd in kernels (%)	86.6 (2.5) a	78.1 (3.3) b	82.0 (2.7) b
Calculated Cd content of meals from whole seeds (mg/kg dry matter) [1, 4]	1.74 (0.22) a	1.65 (0.23) a	1.55 (0.21) a
Calculated Cd content of meals from de-hulled seeds (67% hulls removed) (mg/kg dry matter) [2, 4]	2.43 (0.22) a	2.05 (0.23) b	2.17 (0.22) b
Calculated Cd content of meals from kernels (mg/kg dry matter) [3, 4]	3.41 (0.32) a	2.61 (0.32) b	2.84 (0.32) b

[1] Calculated from the quantity of Cd in hulls and in kernels

[2] Calculated from the quantity of Cd in kernels and in 1/3 of the weight of hulls corresponding to meal of high protein grade with 67% of hulls removed.

[3] Calculated from the quantity of Cd in kernels only, for producing kernel meals for possible use as food

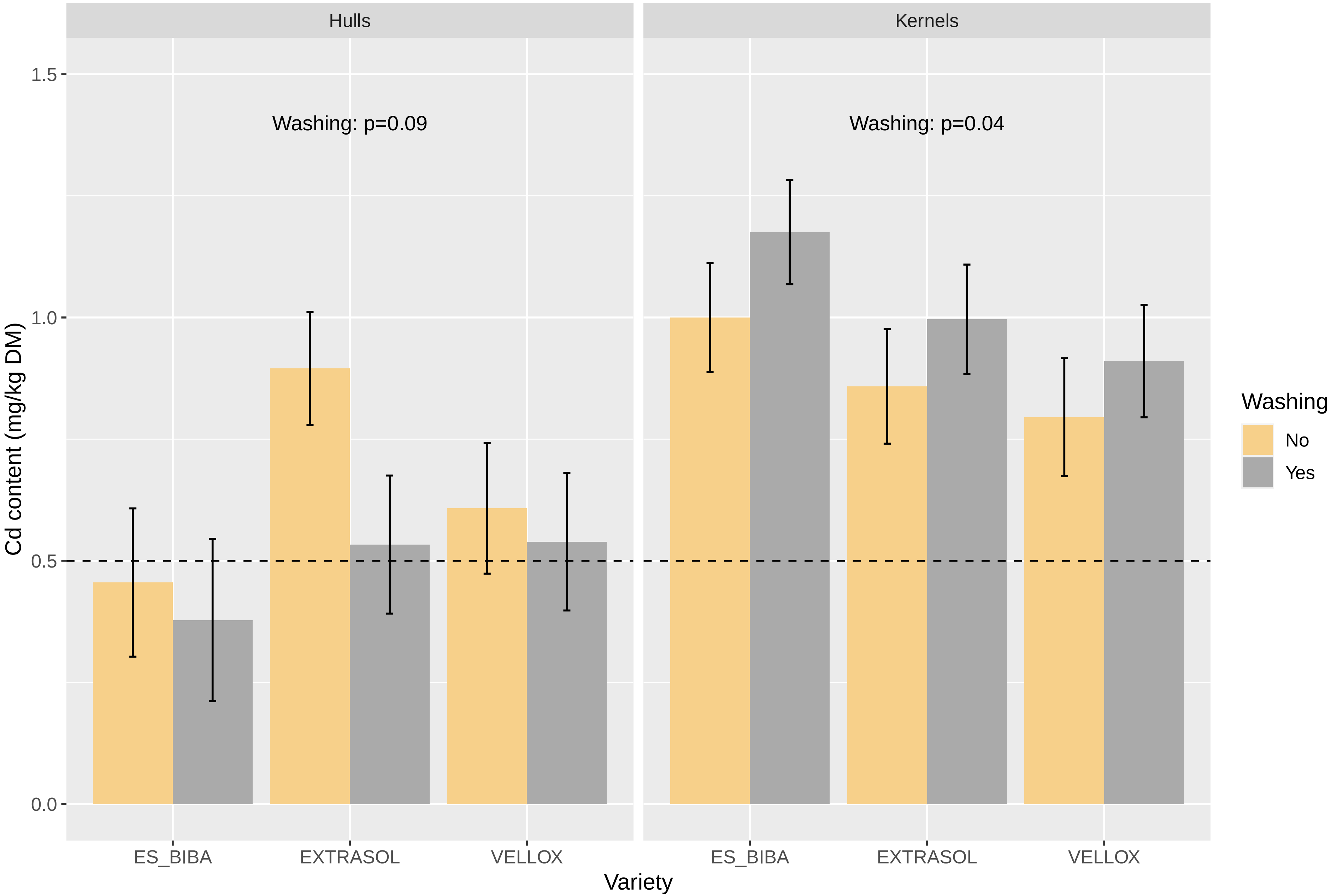
[4] After extraction of the whole oil and assuming that oil contains 10% of the whole seed Cd (Zehra et al., 2020).

Figure captions

Figure 1: Effect of washing sunflower seeds on the Cd content (mg Cd /kg dry matter) of hulls and kernels in three field grown sunflower varieties cultivated on a calcareous soil (0.99 mg total Cd/kg soil). The horizontal dashed line indicates the regulation limit for human food (EC 2023/915).Vertical segments indicate +/- one standard error (n=3).

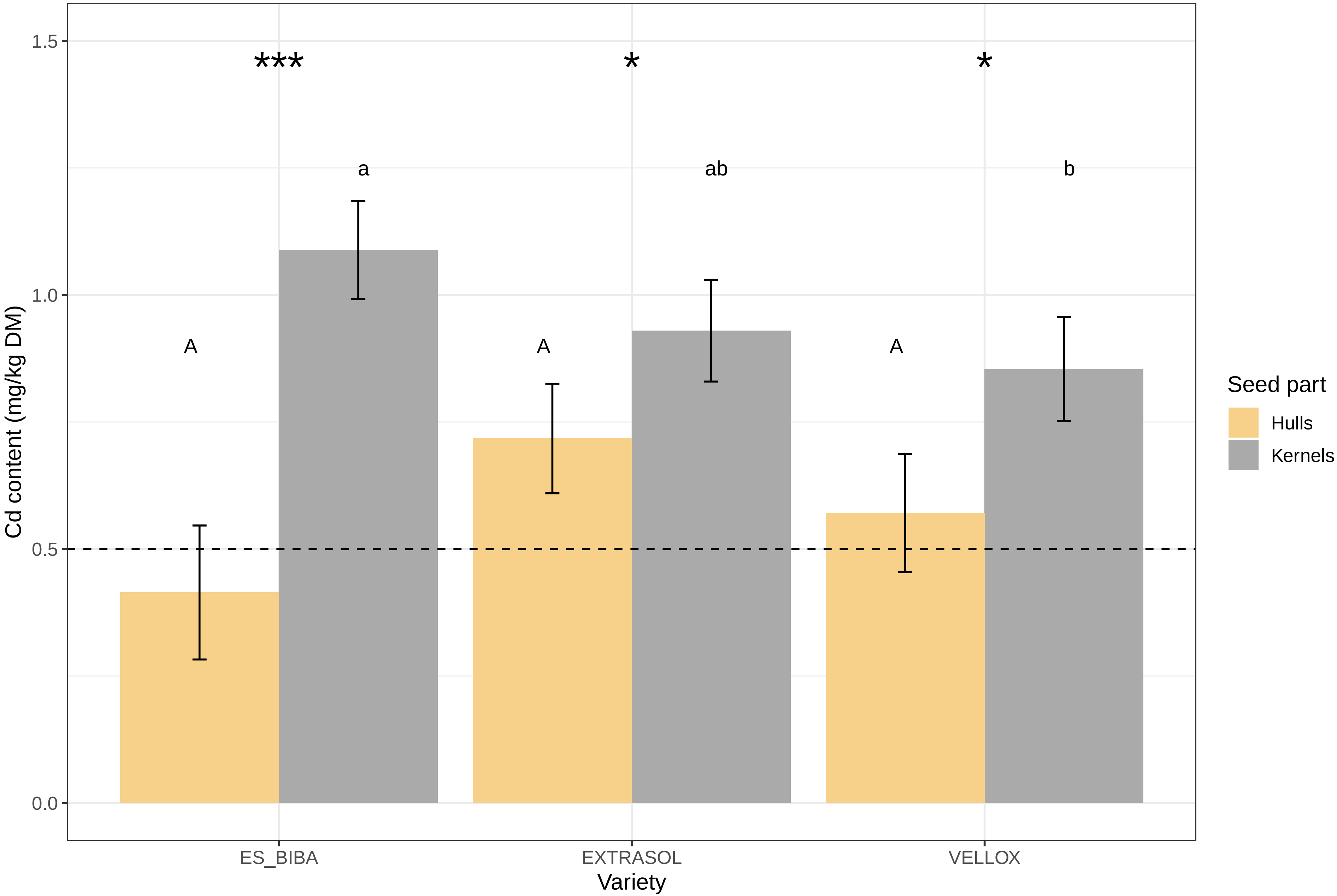
Figure 2: Cd contents (mg Cd /kg dry matter) of hulls and kernels in three field-grown sunflower varieties cultivated on a calcareous soil (0.99 mg total Cd/kg soil). The horizontal dashed line indicates the regulation limit for human food (EC 2023/915). Stars indicate significant differences between the hulls and the kernels for each variety (*** $p<0.001$, * $p<0.05$). Lowercase letters are mean groupings for the comparison of Cd content in kernels between varieties (Tukey tests at $p<0.05$). Uppercase are the same comparisons but for hulls . Vertical segments indicate +/- one standard error (n=3).

Figure 1



Hulls
Kernels
Washing: p=0.09
Washing: p=0.04
1.5
1.0
0.5
0.0
Cd content (mg/kg DM)
ES_BIBA
EXTRASOL
VELLOX
ES_BIBA
EXTRASOL
VELLOX
Variety
Washing
No
Yes

Figure 2



1.5
1.0
0.5
0.0
Cd content (mg/kg DM)
***
*
*
a
ab
b
A
A
A
ES_BIBA
EXTRASOL
VELLOX
Variety
Seed part
Hulls
Kernels