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Comparative Effects of Organic and Conventional Cropping Systems on Trace Elements Contents in Vegetable Brassicaceae: Risk Assessment

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Abstract: Genotypes selected from 3 plant species (Brassica rapa, Eruca vesicaria and Sinapis alba) belonging to the Brassicaceae family were chosen to compare the concentrations of 9 inorganic elements (Cd, Co, Cr, Cu, Fe, Ni, Mn, Pb and Zn) in these varieties, that were grown under both conventional and organic conditions during two agricultural seasons (2018/2019 and 2019/2020) on two different experimental farms (Farm I and Farm II). We found that, together with agriculture practices, the inorganic element concentrations in Brassicas depended on many other factors, including soil characteristics. However, there were no conclusive results indicating a lower heavy metal content or a higher nutritionally beneficial trace elements content in vegetables grown under organic agriculture. Finally, a probabilistic assessment (@Risk) derived from the consumption of 150–200 g of these vegetables showed that organic Brassicas fulfill in comparison with the conventional ones, similar Dietary Reference Intakes (DRI) percentages for Co, Cr, Cu, Fe, Mn and Zn. Regarding heavy metals (Cd, Ni and Pb), we only found slight differences (mainly in the case of Pb) in the Tolerable Intakes (TI) between both cropping systems.

Keywords: organic farming; conventional farming; trace elements; heavy metals; risk assessment

1. Introduction

Nowadays, the maintenance of a good health status via appropriate dietary habits has become of great social concern, but we also have to bear in mind what has been called the “health trilemma”, which tells us that food, health and the environment are closely linked, should also be borne in mind, in order to establish a balance between them for a healthier life on a more sustainable planet.

Currently, the consumption of plant-based foods with nutraceutical properties is one of the crucial factors for the welfare and promotion of health, preventing various pathologies like cancer, cardiovascular and neurodegenerative diseases [1,2]. Vegetable species belonging to the Brassicaceae (formerly Cruciferae) family are considered as being one of the first cultivated and domesticated plant groups and is appreciated for constituting a good source of minerals and trace elements [3] and for their health-promoting phytochemicals such as glucosinolates [4]. Plant species from this family include nutritionally important human and animal foodstuffs such as broccoli, turnip, cabbage, cauliflower, rapeseed, mustard, rocket, and is one of the ten most economically important plant families in the world [5,6].
In addition, in recent years, developed countries have been showing a greater interest in organic agriculture, with an increase of around 250% in the last 10 years [7]. This type of agriculture is based on the non-use of synthetic fertilizers or pesticides, and, instead, fertilization of the land with composted material, rich in organic matter, derived from the biodegradation of plant and animal sources [8].

Some authors have pointed out that organic foods contain higher concentrations of nutritionally beneficial trace elements and lower concentrations of harmful heavy metals [9]. In fact, vegetables can uptake and retain these inorganic elements from the surrounding environment through their roots and leaves [10]. However, the data existing in the bibliography on this topic are inconclusive, and it is difficult to make a valid comparison between both vegetable groups due to the limited availability of well-controlled or paired studies [11].

Therefore, for all the above reasons, the objectives of this research were (a) to compare the concentrations of nine inorganic elements (Co, Cr, Cu, Cd, Fe, Ni, Mn, Pb and Zn), well known both for their nutritional and toxicologic role in 3 species of Brassicaceae (Brassica rapa, Eruca vesicaria and Sinapis alba) grown under both conventional and organic conditions during two agricultural seasons (2018/2019 and 2019/2020) on two different experimental farms; (b) to make a probabilistic estimation with computer software (@Risk) of the contributions of the inorganic elements present in these vegetables to the recommended intakes or to the toxicologic limits established for them. This was to find out whether organically-grown Brassicas have a greater nutritional value than the conventionally-grown ones.

The novelty of this work lies in the fact that few long-term studies have been made comparing vegetables grown under organic and conventional conditions during two agriculture seasons.

2. Material and Methods

2.1. Plant Material

Genotypes selected from 3 plant species belonging to the Brassicaceae family were chosen based on previous studies showing their differences in their glucosinolates profile and trace elements concentration: Brassica rapa L. (turnip greens and top greens), Eruca vesicaria L. (rocket) and Sinapis alba L. (white mustard). Turnip greens are the young leaves harvested in the vegetative growth period, and turnip tops are the fructiferous stems with flower buds and the surrounding leaves that are consumed before opening and while still green. These plant species are well adapted to Mediterranean environmental conditions and have been obtained by the Plant Breeding Group at the Institute for Sustainable Agriculture (IAS-CSIC) after several generations of breeding for seed yield and glucosinolate content.

This material was sown and cultivated during two seasons, 2018–2019 and 2019–2020, in two Farms (I and II) in Southern Spain (see Figure 1).

The Farm I land (37°51’ N, 4°48’ W) is located in Córdoba, next to the Guadalquivir River, in a position of the first terrace (altitude of 106 m), with a deep soil (Typic Xerofluvent) of sandy-loam texture with high pH (around 8), intermediate organic matter content (1.6%), and high carbonate content (17%). The experimental plot size for conventional cultivation of Brassicaceae species on Farm I was 25 x 25 m. The climate is typically continental Mediterranean (Csa in Köppen’s climate classification), with relatively cold winters, intensely hot dry summers and mean annual precipitations of 650 mm. On this Farm, the three Brassicaceae species were only grown under conventional conditions with herbicides and mineral fertilization being applied. In pre-sowing, an herbicide with trifluralin as its active matter was used at a dose of 1.5 L/ha. Moreover, before sowing, a basic dressing with 8-15-15 bottom fertilizer was applied at a rate of 600 kg/ha. A top dressing (cover fertilization) with 300 kg/ha of Ammonium Nitrate was applied after the winter stop at the resumption of vegetative growth.
Farm II is located in the municipal district of Alcalá la Real (Jaén) (37°27′ N 3°55′ W, Spain) in the Sub-Baetic zone, next to the Velillos River (altitude 920 m) with a moderately stony structure and clay loam texture (Xerofluvent-Fluvisol calcareous) with high pH (8.2), high organic matter content (3%), and high carbonate content (16%). The experimental plot size for conventional and ecological cultivation of Brassicaceae species in Farm II were 25 × 25 m each. Both experimental plots were close together and separated only by a 2-m-wide border. The climate is typically continental Mediterranean (Csa in Köppen’s climate classification), with short summers, very hot, arid and mostly cloudless, winters are long, very cold and partially cloudy and mean annual precipitations of 650 mm. In this Farm, the three Brassicaceae species were grown both under conventional and organic conditions.

The conventional cultivation conditions on Farm II were similar to those of the Farm I. In organic cultivation, neither herbicides nor mineral fertilizers were applied. Instead, only treatment with a mixture of goat and sheep manure was applied at a rate of 3 kg/m².

When plants from the different species reached their optimal moment of consumption (from 3 to 5 months after sowing), leaf samples from individual plants of each species were harvested, pooled, and processed for chemical analysis. The number of analyzed plants throughout the two-years duration of the study were turnip greens (Brassica rapa) (n = 60), turnip tops (Brassica rapa) (n = 85), Eruca vesicaria (n = 18), and Sinapis alba (n = 12). The higher number of harvested Brassica rapa samples is due to turnip greens and turnip tops having good commercial prospects, and their consumption, both fresh and processed, has considerably increased in the last years. Furthermore, Sinapis alba and Eruca vesicaria are currently minority crops consumed only in salads, although their consumption may increase in the future due to their special composition in glucosinolates with medicinal properties (Sinalbine in Sinapis alba and Glucorafanine in Eruca vesicaria).

Plants were thoroughly washed with tap water to remove dirt and dust, and they were finally rinsed with deionized water. Then, they were stored at −80 °C until freeze-drying, which was done in Telstar® model Cryodos-50 equipment (Telstar, Terrasa, Spain).
The freeze-dried samples were ground in a Janke and Kunkel Model A10 mill (IKA-Labortechnik, Staufen, Germany) for about 20 s, and stored in a desiccator until their analysis.

2.2. Materials and Reagents

All the reagents were of an analytical-reagent grade. Ultrapure water (18 MΩ/SCF) prepared with a Milli-Q Reference Water Purification (Millipore, Madrid, Spain) was used throughout the experiments. All the glassware and plastic containers were soaked in 50% nitric acid overnight, then in 20% hydrochloric acid for an additional night and rinsed three times with de-ionized water prior to use. Hyperpure nitric acid (65%) and hydrochloric acid (35%) were obtained from Panreac (Barcelona, Spain). Hydrogen peroxide (33%) was acquired from Sigma Aldrich (St. Louis, MO, USA).

Standard solutions for measuring the elements Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were prepared immediately before use by dilution with distilled deionized water of 1000 mg/L standard solutions (Certipur-Merck, Darmstadt, Germany).

2.3. Trace Element Determination

To determine the trace element content (Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) of Brassicaceae species, 0.5 g of freeze-dried sample was weighed in a porcelain crucible. Samples were incinerated in a muffle furnace at 460 °C for 15 h. The ash was bleached after cooling by adding 200 µL of hyperpure HNO₃ and 1 mL of deionized water, drying this on thermostatic hotplates, and maintaining it in a muffle furnace at 460 °C for 1 h more. Ash recovery was performed with 100 µL of hyperpure HNO₃, making up to 10 mL with deionized water.

To Cd analysis, in order to avoid Cd volatilization, 0.5 g of freeze-dried sample was placed in a Teflon vessel. Then, 3 mL of hyperpure HNO₃ and 2 mL of H₂O₂ were added to each vessel and kept for 10 min at room temperature. After sealing the vessels hermetically, they were placed in a microwave oven (Multiwave GO, Anton Paar, Germany) and digested following the instrumental parameters indicated by the manufacturer. Every sample was diluted up to a volume of 20 mL with ultrapure water.

Elemental analyses for Fe (\(\lambda = 248.3\) nm; Slit width = 0.2 nm), Mn (\(\lambda = 279.5\) nm; Slit width = 0.2 nm) and Zn (\(\lambda = 213.9\) nm; Slit width = 0.7 nm) were performed by flame absorption atomic spectroscopy (FAAS) with a Varian Spectra AA-50B model, equipped with standard air-acetylene flame, and single-element hollow cathode lamps. Finally, electrothermal atomic absorption spectroscopy (ET-AAS) was used for the determination of Cd, Co, Cr, Cu, Ni and Pb (Agilent Technologies model 240Z AA with a graphite furnace and autosampler). This equipment was certified by an equipment qualification report (EQR; Agilent Technologies). In this latter, analytical methodology was developed following the instrumental parameters indicated by the manufacturer with slight modifications (Table 1).

For Cd and Pb analysis, a chemical modifier (200 mL solution) was prepared containing a mixture of 0.1% Paladium matrix modifier 10 g/L (Merck, Spain) plus 0.06% Magnesium nitrate hexahydrate in 10 mL HNO₃ hyperpure solution (69%). For each measurement, 15 µL of sample and 5 µL of modifier solution were injected. The accuracy and precision of the different analytical techniques used in determining trace element concentrations were validated by recovery experiments using Certified Reference Materials (Table 2).
Table 1. Instrumental conditions for Cd, Co, Cr, Ni and Pb analysis by ET-AAS in Brassicaceae samples.

| Step          | T (°C) | t (s) | T (°C) | t (s) | T (°C) | t (s) | T (°C) | t (s) | T (°C) | t (s) | T (°C) | t (s) | Argon Flow (L/min) |
|---------------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|-------------------|
| Drying        | 85     | 5     | 85     | 5     | 85     | 5     | 85     | 5     | 85     | 5     | 85     | 5     | 0.3               |
|              | 95     | 40    | 95     | 40    | 95     | 40    | 95     | 40    | 95     | 40    | 95     | 40    | 0.3               |
|              | 120    | 10    | 120    | 8     | 120    | 20    | 120    | 10    | 120    | 10    | 120    | 10    | 0.3               |
| Pyrolysis     | 300    | 5     | 750    | 5     | 1000   | 5     | 800    | 5     | 800    | 5     | 500    | 10    | 0.3               |
|              | 300    | 3     | 750    | 3     | 1000   | 3     | 800    | 3     | 800    | 3     | 500    | 7     | 0                 |
| Atomization   | 1800   | 2.8   | 2300   | 2.8   | 2600   | 2.8   | 2300   | 2.8   | 2400   | 2.8   | 2300   | 5     | 0.3               |
| Cleaning      | 1800   | 2     | 2300   | 2     | 2600   | 2     | 2300   | 2     | 2400   | 2     | 2400   | 4     | 0.3               |
|               |        |       |        |       |        |       |        |       |        |       |        |       |                   |

Table 2. Analysis of certified references materials (mean ± standard deviation), limit of detection and limit of quantification.

| Element | Certified References Material (mg kg\(^{-1}\)) | LOD (µg/g) | LOQ (µg/g) | Certified Found | Recovery (%) | Certified Found | Recovery (%) |
|---------|-----------------------------------------------|------------|------------|-----------------|--------------|-----------------|--------------|
| Cd      | White Cabbage BCR-679 | 0.005     | 0.016     | 1.66 ± 0.07 | 1.57 ± 0.01 | 95   | 0.07 * | 0.063 ± 0.020 | 90   |
| Co      | Peach Leaves NIST-1547 | 0.023     | 0.077     | 0.6 ± 0.1    | 0.61 ± 0.03 | 102  |          |              |      |
| Cr      |                                              | 0.016     | 0.052     | 2.89 ± 0.12  | 3.13 ± 0.25 | 108  |          |              |      |
| Cu      |                                              | 0.078     | 0.260     | 55.0 ± 2.5   | 56.2 ± 3.0  | 102  |          |              |      |
| Mn      |                                              | 0.138     | 0.459     | 13.3 ± 0.5   | 13.2 ± 0.4  | 99   |          |              |      |
| Fe      |                                              | 0.056     | 0.187     | 27.0 ± 0.8   | 25.6 ± 0.9  | 95   |          |              |      |
| Ni      |                                              | 0.048     | 0.150     | 79.7 ± 2.7   | 78.2 ± 3.0  | 98   |          |              |      |
| Pb      |                                              | 1.20      | 4.84      | 150–200 g    | 150–200 g    | 102  |          |              |      |
| Zn      |                                              |           |           |                |              |      |          |              |      |

* Indicative value.

2.4. Statistical Analyses and Risk Assessment

The IBM SPSS 25 statistical software package was used for statistical analysis. The data were expressed as mean and standard deviation. Data were analyzed using ANOVA tests. Significant differences were considered when \( p < 0.05 \).

A probabilistic model was developed to estimate the intake level for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn derived from feeding with Brassica vegetables. It should be pointed out that in developing the model, only the concentrations of trace elements for the *Brassica rapa* species were considered as it is the one most consumed in Spain, Portugal and Southern Italy, being part of very traditional recipes. Nowadays, turnip greens and turnip tops have good commercial prospects, and their consumption, both fresh and processed, has considerably increased in the last years. *Eruca vesicaria* and *Sinapis alba* have only eaten in small amounts in some salads, and they have a slightly spicy flavor like mustard greens.

This model followed a probabilistic approach in which variables were described by probability distributions, and they were fitted to concentration data obtained in this study for each element. Additionally, to estimate the intake level, serving size was considered assuming 15–20 g per day (around 150–200 g of fresh matter) of *Brassica rapa* (turnip greens or turnip tops). Daily intake was defined by a uniform distribution in the probabilistic model, meaning that all values in that range had the same probability to occur.

The probability distributions describing the trace element concentration data were fitted using @Risk v7.5 (Palisade, Newfield, NY, USA). The simulation ran with 10,000 iterations per element. The goodness of fit assessed how well the fitted distribution described the data; in this section, the Akaike Information Criterion (AIC) and Chi-square statistical tests were used. Additionally, the visual analysis was considered to assess the fit of the probability distributions to intake data. Data obtained through this probabilistic model was...
3. Results and Discussion

3.1. Trace Element Contents in Brassicaceae Species: Conventional Versus Organic

The concentrations of Cu, Mn, and Zn for organic turnip greens (Brassica rapa) grown on Farm II were 7.4; 100.4 and 24.4 µg/g d.w (see Table 3). The concentrations of these trace elements in ones conventionally grown on the same farm were practically the same, with values of 7.5; 110.8 and 22.9 µg/g d.w. Conversely, there were statistically significant differences for Zn (p < 0.05) and Cu, Mn (p < 0.01) between the turnip greens grown on Farm I (conventional system) and those grown on Farm II (both conventional and organic systems). The highest concentrations of Cu and Zn (11.3; 38.9 µg/g d.w) and the lowest one Mn (56.0 µg/g d.w) found in turnip greens grown on Farm I compared to those analyzed on Farm II, demonstrate that, together with farming systems, the trace element concentrations in foodstuffs depend on many other factors, including soil characteristics, pollution from anthropogenic sources, genetic factors, seasonal influences and interactions between the elements [16].

Table 3. Total trace elements concentration (dry matter) in Brassicaceae species analyzed (mean ± standard deviation).

|                | Co (µg/g) | Cr (µg/g) | Cu (µg/g) | Fe (µg/g) | Mn (µg/g) | Zn (µg/g) |
|----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| **Farm I**     |           |           |           |           |           |           |
| Brassica rapa  | 0.23 ± 0.10| 2.41 ± 1.50| 11.3 ± 3.8| 223 ± 133| 56.0 ± 20.1| 38.9 ± 23.1|
| (turnip greens)|           |           |           |           |           |           |
| Brassica rapa  | 0.14 ± 0.04| 0.87 ± 0.59| 8.06 ± 3.80| 71 ± 22| 24.2 ± 9.9| 29.9 ± 9.1|
| (turnip tops)  |           |           |           |           |           |           |
| Eruca vesicaria| 0.39 ± 0.08| 7.17 ± 0.40| 8.19 ± 1.73| 539 ± 324| 34.8 ± 8.6| 37.1 ± 5.0|
| **Farm II**    |           |           |           |           |           |           |
| Brassica rapa  | 0.28 ± 0.12| 2.06 ± 0.98| 7.54 ± 1.55| 194 ± 75| 110.8 ± 60.6| 22.9 ± 4.7|
| (turnip greens)|           |           |           |           |           |           |
| Brassica rapa  | 0.16 ± 0.08| 0.52 ± 0.21| 4.56 ± 2.27| 60 ± 14| 25.5 ± 13.3| 25.1 ± 6.2|
| (turnip tops)  |           |           |           |           |           |           |
| Eruca vesicaria| 0.26 ± 0.09| 3.29 ± 1.72| 9.33 ± 1.30| 413 ± 175| 33.2 ± 8.0| 34.2 ± 4.5|
| Sinapis alba   | <LOQ      | 0.51 ± 0.03| 5.57 ± 0.24| 153 ± 32| 18.7 ± 0.7| 25.9 ± 3.5|
| **Organic**    |           |           |           |           |           |           |
| Brassica rapa  | 0.22 ± 0.06| 1.20 ± 0.66| 7.39 ± 1.79| 105 ± 63| 100.4 ± 38.9| 24.4 ± 3.3|
| (turnip greens)|           |           |           |           |           |           |
| Brassica rapa  | 0.19 ± 0.06| 0.48 ± 0.24| 9.04 ± 6.33| 72 ± 43| 24.8 ± 6.6| 31.2 ± 5.9|
| (turnip tops)  |           |           |           |           |           |           |
| Eruca vesicaria| 0.44 ± 0.11| 5.17 ± 2.25| 10.2 ± 1.1| 700 ± 207| 481 ± 9.8| 33.0 ± 3.0|
| Sinapis alba   | <LOQ      | 0.26 ± 0.08| 5.97 ± 1.56| 68 ± 11| 17.8 ± 2.4| 23.8 ± 3.2|

The Cu, Mn and Zn in turnip tops grown under the three experimental conditions were Farm I, conventional, 8.1; 24.2 and 29.9 µg/g d.w respectively; Farm II, conventional 4.6; 25.5 and 25.1 µg/g d.w.; Farm II, organic, 9.0; 24.8 and 31.2 µg/g d.w. The concentrations in the turnip tops of the three trace elements were lower than those analyzed in the corresponding turnip greens. This could be explained by considering that turnip greens are the vegetative Brassica rapa leaves, whereas the turnip tops are the fructiferous stems with flower buds and the surrounding leaves. Previous studies have indicated that metals tend to accumulate preferentially in roots rather than in storage organs or fruits [17,18]. Moreover, unlike what happens to the turnip greens, statistically significant differences were found for Cu (p < 0.05) and Zn (p < 0.01) between the conventional and organic turnip tops grown on Farm II, the highest concentrations being found in the organic ones. These results are in agreement with those reported by Kelly and Bateman [9] for similar studies made with other vegetables species (tomatoes and lettuces).

Cu, Mn and Zn concentrations in Sinapis alba were 6.0; 17.8 and 23.8 µg/g d.w for the organic cropping system and 5.6; 18.7 and 25.9 µg/g d.w for the conventional one. Similarly, concentrations for these trace elements in Eruca vesicaria were 10.2; 48.1 and 33.0 µg/g d.w for the organic system and 9.3; 33.2 and 34.2 µg/g d.w for the conventional one. Zn
concentrations found in these *Brassicaceae* species are in agreement with those reported by Câmara–Martos et al. [3] in a previous study (*Sinapis alba* 20.8 µg/g d.w.; *Eruca vesicaria* 23.5 µg/g d.w.). However, our work again failed to find statistically significant differences in the concentrations of these trace elements between organic and conventional agriculture.

The results appearing in the bibliography do not show any clear trend for these trace elements either. Krejcova et al. [19], in conventionally-grown carrots, have shown a higher content of Mn and Cu but a lower content of Zn than in organic ones. Hadayat et al. [18], for organic lettuce, potato and carrot reported higher Cu contents than those in the same conventional vegetables. However, for conventional lettuce and carrot, higher Zn concentrations than those in the organic ones, were also found.

On the other hand, although in our study no differences were found in Mn concentrations between conventionally-grown *Brassicas* and organic ones, some authors have demonstrated that a lower mean concentration of Mn in organic crops is a common pattern. This could be due to the high concentrations of arbuscular mycorrhizal fungi in organic soils [9,20]. Although, this aspect has not been completely clarified. Other studies have shown that Mn is used as an additive to livestock feed supplements and, in turn, that this trace element would be present in the manure used in organic farming [9].

Co concentrations in turnip greens (*Brassica rapa*) were very similar in the three studied conditions (Farm I conventional 0.23 µg/g d.w; Farm II conventional 0.28 µg/g d.w and Farm II organic 0.22 µg/g d.w, with no significant differences between them (Table 3). Regarding Cr contents in turnip greens, in organic plants harvested on Farm II, lower concentrations (1.20 µg/g d.w) (*p* < 0.01) than in conventional ones of Farm I (2.41 µg/g d.w) and Farm II (2.06 µg/g d.w) were found. These results are in agreement with those reported by Krejcova et al. [19], who also found higher Cr concentration in conventional carrots (0.059 µg/g) than in organic carrots (0.046 µg/g). We have presumed that the main chemical form in which Cr is found in *Brassicaceae* vegetables, would be Cr (III). This chemical form is considered as being a beneficial element for human health, and according to several previous research works [21,22], it is the main chemical form (unlike Cr(VI) in which Cr is found in waters and foods. Therefore, according to their Co and Cr content, organic turnip greens would not have a higher nutritional value than conventional grown ones.

For the same reason as that already mentioned for the previous trace elements, Co and Cr concentrations in turnip tops (*Brassica rapa*) also decreased with respect to the corresponding turnip greens (*Brassica rapa*) (Farm I, conventional, 0.14 and 0.87 µg/g d.w.; Farm II, conventional, 0.16 and 0.52 µg/g d.w.; Farm II, organic, 0.19 and 0.48 µg/g d.w.) Again we find differences in Cr concentrations between turnip tops grown on the two Farms but not between the cropping systems (conventional versus organic). Thus, there were significant statistical differences for Cr contents (*p* < 0.05) between conventional turnip tops grown on Farm I and conventional turnip tops grown on Farm II. We also found statistically significant differences for Cr contents (*p* < 0.01) between conventional turnip tops grown on Farm I and organic ones grown on Farm II. Therefore, this again indicates that the total inorganic element content in vegetables does not only depend on the farming system [16].

*Sinapis alba* showed higher Cr concentrations (*p* < 0.01) for conventionally produced vegetables (0.51 µg/g d.w.) than organically ones (0.26 µg/g d.w.). Co contents for this *Brassicaceae* species were below the quantification limit (LOQ < 0.07 µg/g). On the other hand, Co and Cr contents in *Eruca vesicaria* were Farm I, conventional, 0.39 and 7.17 µg/g d.w; Farm II, conventional, 0.26 and 3.29 µg/g d.w, and, Farm II, organic 0.44 and 5.17 µg/g d.w. Cr contents in *Eruca vesicaria* are slightly higher with those reported in a previous study (2.59 µg/g) [3]. In addition, there were no statistically significant differences for both elements between organic and conventional *Eruca vesicaria*. Previous studies have reported that there is no clear trend in this matter. Thus, Hadayat et al. [18] gave higher Co contents in conventional potato, onion, tomato and carrot versus organic ones, whereas Cr contents were higher for organic onion, carrot and potato.
Regarding Fe, we found statistically significant differences \((p < 0.01)\) between organic turnip greens \((Brassica rapa)\) \((0.10 \text{ mg/g d.w.})\) and conventional ones \((0.19 \text{ µg/g d.w.})\) from Farm II; and between organic Sinapis alba \((0.07 \text{ mg/g d.w.})\) and conventional one \((0.15 \text{ mg/g d.w.})\) from Farm II (Table 3). Similarly, Krejčova et al. [19] have also reported higher Fe content for conventional carrots \((5.24 \text{ µg/g})\) versus organic ones \((4.96 \text{ µg/g})\). Nevertheless, for the rest of the vegetable Brassicaceae studied, the differences between conventional and organic cultivation were scant. In relation to this latter aspect, Kelly and Bateman [9] observed only minor variations in Fe concentrations between tomatoes and lettuces cultivated on organic and conventional farms.

While the above trace elements have a clear nutritional role, Pb and Cd are considered to be heavy metals that have harmful effects on the environment and human health. With respect to Ni, although the nutritional and/or toxicologic role of Ni in humans is unclear, in animal models, severe Ni deficiency can affect vision, Fe metabolism, and Na homeostasis [23]. However, high concentrations of this element can also affect vital processes in plants and induce toxic effects at morphologic, physiologic and biochemical levels [24].

For this latter element, we found statistically significant differences \((p < 0.05)\) for Ni content in turnip tops \((Brassica rapa)\) grown by organic agriculture \((0.85 \text{ µg/g d.w.})\) on Farm II and turnip tops grown under conventional agriculture \((1.02 \text{ µg/g d.w.})\) on the same Farm, with the lowest concentrations in organic ones (see Table 4). Similarly, Krejčova et al. [19] have also reported a lower Ni content in organic carrots \((0.79 \text{ µg/g})\) than in conventional ones \((1.58 \text{ µg/g})\). Other vegetables such as tomato and onion have also shown lower Ni contents when they are grown by organic systems. Conversely, other vegetable foodstuffs such as organic wheat (semolina samples) have shown higher Ni content than conventional wheat.

Table 4. Total heavy metals concentration (dry matter) in Brassicaceae species analyzed (mean ± standard deviation).

|                  | Cd (µg/g) | Ni (µg/g) | Pb (µg/g) |
|------------------|-----------|-----------|-----------|
| **Conventional** |           |           |           |
| Farm I           |           |           |           |
| Brassica rapa (turnip greens) | 0.28 ± 0.16 | 1.56 ± 1.11 | 0.99 ± 0.80 |
| Brassica rapa (turnip tops)    | 0.12 ± 0.03 | 0.81 ± 0.32 | 0.90 ± 0.88 |
| Eruca vesicaria   | 0.72 ± 0.10 | 3.99 ± 1.47 | 3.40 ± 0.61 |
| Farm II           |           |           |           |
| Brassica rapa (turnip greens) | 0.19 ± 0.06 | 1.39 ± 0.91 | 0.62 ± 0.37 |
| Brassica rapa (turnip tops)    | 0.13 ± 0.03 | 1.02 ± 0.21 | <LOQ       |
| Eruca vesicaria   | 0.48 ± 0.05 | 1.76 ± 0.48 | 0.55 ± 0.07 |
| Sinapis alba      | 0.23 ± 0.01 | 0.21 ± 0.03 | 0.16 ± 0.02 |
| **Organic**       |           |           |           |
| Brassica rapa (turnip greens) | 0.18 ± 0.05 | 1.02 ± 0.46 | 0.33 ± 0.23 |
| Brassica rapa (turnip tops)    | 0.12 ± 0.05 | 0.85 ± 0.22 | <LOQ       |
| Eruca vesicaria   | 0.54 ± 0.04 | 2.55 ± 0.71 | 0.91 ± 0.39 |
| Sinapis alba      | 0.07 ± 0.01 | <LOQ       | 0.45 ± 0.31 |

Ni values for conventional turnip tops grown on Farm I were \((0.81 \text{ µg/g d.w.})\) and Farm II \((1.02 \text{ µg/g d.w.})\) with statistically significant differences \((p < 0.05)\) between them. Trace element content in organic Sinapis alba was below the quantification limit \((\text{LOQ} < 0.18 \text{ µg/g})\) whereas Ni values for Eruca vesicaria ranged between \(1.76–3.99 \text{ µg/g d.w.}\) without significant differences between conventional and organic system. These results are in agreement with those found in a previous study [3] for this latter Brassica specie \((1.12 \text{ µg/g})\).
Regarding Pb, there were statistically significant differences \((p < 0.05)\) between organic turnip greens \((Brassica rapa)\) \((0.33 \mu g/g \text{ d.w.})\) and conventional ones \((0.62 \mu g/g \text{ d.w.})\) from Farm II \((\text{Table 4})\). Nevertheless, for the rest of the \(Brassicaceae\) studied, the differences between conventional and organic cultivation were scant. The influence of the soil in which the plants are grown has also been demonstrated. Thus, we found statistically significant differences \((p < 0.01)\) for \(Eruca vesicaria\) between conventional plants grown on Farm I \((3.40 \mu g/g \text{ d.w.})\) and plants grown on Farm II \((0.55 \mu g/g \text{ d.w.})\). Furthermore, while for organic and conventional turnip tops grown on Farm II, Pb concentrations were below the quantification limit \((\text{LOQ} < 0.160 \mu g/g)\), concentrations in conventional turnip tops grown on Farm I reached mean values of \(0.90 \mu g/g \text{ d.w.}\).

There are no conclusive results regarding a lower Pb content in vegetables grown through organic agriculture. Thus, Hadayat et al. \([18]\) found lower Pb concentrations in organic tomato, lettuce, onion and carrot but not in potato. Krejcová et al. \([19]\) reported higher Pb contents in conventional carrots \((0.064 \mu g/g)\) versus organic ones \((0.043 \mu g/g)\). However, Zaccone et al. \([16]\) found higher contents of this heavy metal in organic wheat \((94 \mu g/g)\) versus that in conventionally grown wheat \((82 \mu g/g)\). Finally, Karavoltsos et al. \([25]\) have indicated that, although the majority of organic vegetables may have lower Pb content, organic agriculture such as does not necessarily reduce the content of this heavy metal in organically cultivated products.

Another heavy metal whose consumption is aimed to reduce with the development of organic agriculture is Cd. Nevertheless, in the present study, we only found statistically significant differences for Cd levels \((p < 0.01)\) between organic \((0.07 \mu g/g \text{ d.w.})\) and conventional \((0.23 \mu g/g \text{ d.w.})\) \((\text{Table 4})\). Sinapis alba grown on Farm II. For the rest of the \(Brassicaceae\) cultivated on Farm II, Cd values were very similar both in organic and conventional plants. Comparing our results with those in a previous study, Hadayat et al. \((2018)\) reported lower Cd concentrations in organic tomato, onion, carrot and potato but not in lettuce. Câmara–Martos et al. \([26]\) for infant foods, such as weaning jars, made with organic vegetable ingredients, it was also found that Cd concentrations were considerably lower than those reported in weaning formulas which were not categorized as organic. Krejcová et al. \([19]\) showed slightly higher mean Cd concentrations in conventional carrots \((0.066 \mu g/g)\) versus organic ones \((0.060 \mu g/g)\), and Hoefkens et al. \([11]\) indicated significant higher or lower concentrations and even non-significant differences in Cd concentrations, depending on the food matrix.

A factor that again influenced the Cd concentrations in \(Brassicaceae\) was the soil in which they were grown. Thus, we found statistically significant differences in Cd levels \((p < 0.05)\) for conventional \(Eruca vesicaria\) grown on Farm I \((0.72 \mu g/g \text{ d.w.})\) and those conventionally grown on Farm II \((0.48 \mu g/g \text{ d.w.})\).

According to Karavoltsos et al. \([25]\), organic agriculture could eventually lead to the production of foodstuffs with a lower heavy metal content, although organic agriculture as such is not able to secure low metal contents in its products. Our results show that this final content is also influenced by other factors such as soil, vegetable variety or even the presence of these elements in the air and in irrigation waters.

### 3.2. Probabilistic Assessment: Conventional Versus Organic

As already mentioned in the Material and methods section, a probabilistic model approach was developed to estimate the intake level of trace elements, which were derived from the consumption of 15–20 g (around 150–200 g of fresh matter) of these \(Brassicas\). It should be pointed out that in developing the model, only the concentrations of trace elements for the \(Brassica rapa\) species (turnip greens and turnip top) were considered as they are the ones most consumed.

Dietary reference intakes (DRI) for the Spanish population were considered \([12]\). There is not DRI for Co; however, this element represents approximately a 4.3% of vitamin B12. Considering a DRI for vitamin B12 between 2–2.4 \(\mu g\), this corresponds to around 0.10 \(\mu g/\text{day}\) \([27]\). In the case of Ni \([14]\) and Cd \([13]\), considering that they are heavy metals,
the tolerable intake (TI) of 2.8 \( \mu g/kg \) body weight-day and 2.5 \( \mu g/kg \) body weight-week (0.36 \( \mu g/kg \) body weight-day) was used. For Pb a benchmark dose (BMDL\(_{01}\)) for cardiovascular effects (1.50 \( \mu g/kg \) body weight-day) was considered [15]. It should also be noted that the present statistical tool was completed using the variability of inorganic elements present in plants as well as the variability of the Brassica vegetable ingested. Both aspects determine the total intake of the inorganic elements.

Thus, the results obtained from the simulation of the probabilistic model with conventional Brassicas, indicated values for Co, Cr, Cu, Fe, Mn and Zn of 2.58 \( \mu g \), 17.41 \( \mu g \), 0.14 mg, 1.74 mg, 0.55 mg and 0.56 mg, respectively, for 50th percentile (Figure 2). This shows that the intake of trace elements of at least half of the population consuming these conventional Brassicas will not be lower than these values. That intake fulfills Co DRI and complies with the following percentages of DRI for studied elements: Cr 69.6%, Cu 12.7%, Fe: 9.7%, Mn 30.6% and Zn 8.0%. When the same simulation of the probabilistic model is developed with concentrations belonging to organic Brassicas, the values obtained for 50th percentile were 3.61 \( \mu g \), 9.60 \( \mu g \), 0.12 mg, 1.20 mg, 0.65 mg and 0.49 mg for Co, Cr, Cu, Fe, Mn and Zn, respectively (Figure 3), which satisfy similarly to the conventional Brassicas the DRI percentages for these trace elements.

Regarding heavy metals, we have considered intakes for 95th percentile as being the most unfavorable situation. Thus, Ni, Pb and Cd intakes for 95th percentile with conventional Brassicas were 39.00, 47.34 and 6.82 \( \mu g \), respectively (Figure 4), whereas intakes with organic Brassicas were 25.70, 15.55 and 4.56 \( \mu g \) for Ni, Pb and Cd, respectively (Figure 5). According to these values, organic Brassicas led to a decrease in the intake of these three elements metals. However, when these results were expressed as percentages of TI for these elements, we only found slight differences (mainly in the case of Pb) between both agriculture systems. The TI percentages for a mean body weight of 70 kg per person were 19.9, 45.1 and 27.1% for Ni, Pb and Cd, respectively, with conventional Brassicas, and 13.1, 14.8 and 18.1 % for organic Brassicas. These results indicate that Cd, Ni and Pb contents in vegetable Brassica rapa harvested under both conventional and organic farming conditions are below the accepted safety limits and do not represent any toxicologic risk.
It should be pointed out that in developing the model, only the concentrations of trace elements for the *Brassica rapa* species (turnip greens and turnip top) were considered as they are the one most consumed. Dietary reference intakes (DRI) for the Spanish population were considered [12]. There is not DRI for Co; however, this element represents approximately a 4.3% of vitamin B12. Considering a DRI for vitamin B12 between 2–2.4 µg, this corresponds to around 0.10 µg/day [27]. In the case of Ni [14] and Cd [13], considering that they are heavy metals, the tolerable intake (TI) of 2.8 µg/kg body weight·day and 2.5 µg/kg body weight·week (0.36 µg/kg body weight·day) was used. For Pb a benchmark dose (BMDL01) for cardiovascular effects (1.50 µg/kg body weight·day) was considered [15]. It should also be noted that the present statistical tool was completed using the variability of inorganic elements present in plants as well as the variability of the *Brassica* vegetable ingested. Both aspects determine the total intake of the inorganic elements.

Thus, the results obtained from the simulation of the probabilistic model with conventional *Brassicas*, indicated values for Co, Cr, Cu, Fe, Mn and Zn of 2.58 µg, 17.41 µg, 0.14 mg, 1.74 mg, 0.55 mg and 0.56 mg, respectively, for 50th percentile (Figure 2). This shows that the intake of trace elements of at least half of the population consuming these conventional *Brassicas* will not be lower than these values. That intake fulfills Co DRI and complies with the following percentages of DRI for studied elements: Cr 69.6%, Cu 12.7%, Fe: 9.7%, Mn 30.6% and Zn 8.0%. When the same simulation of the probabilistic model is developed with concentrations belonging to organic *Brassicas*, the values obtained for 50th percentile were 3.61 µg, 9.60 µg, 0.12 mg, 1.20 mg, 0.65 mg and 0.49 mg for Co, Cr, Cu, Fe, Mn and Zn, respectively (Figure 3), which satisfy similarly to the conventional *Brassicas* the DRI percentages for these trace elements.

**Figure 2.** Simulated data and fitted probabilistic distribution for Co, Cr, Cu, Fe, Mn and Zn in conventional *Brassicas*. 
Regarding heavy metals, we have considered intakes for 95th percentile as being the most unfavorable situation. Thus, Ni, Pb and Cd intakes for 95th percentile with conventional Brassicas were 39.00, 47.34 and 6.82 µg, respectively (Figure 4), whereas intakes with organic Brassicas were 25.70, 15.55 and 4.56 µg for Ni, Pb and Cd, respectively (Figure 5). According to these values, organic Brassicas led to a decrease in the intake of these three elements metals. However, when these results were expressed as percentages of TI for these elements, we only found slight differences (mainly in the case of Pb) between both agriculture systems. The TI percentages for a mean body weight of 70 kg per person were 19.9, 45.1 and 27.1% for Ni, Pb and Cd, respectively, with conventional Brassicas, and 13.1, 14.8 and 18.1 % for organic Brassicas. These results indicate that Cd, Ni and Pb contents in vegetable Brassica rapa harvested under both conventional and organic farming conditions are below the accepted safety limits and do not represent any toxicologic risk.

Figure 3. Simulated data and fitted probabilistic distribution for Co, Cr, Cu, Fe, Mn and Zn in organic Brassicas.
Regarding heavy metals, we have considered intakes for 95th percentile as being the most unfavorable situation. Thus, Ni, Pb and Cd intakes for 95th percentile with conventional Brassicas were 39.00, 47.34 and 6.82 µg, respectively (Figure 4), whereas intakes with organic Brassicas were 25.70, 15.55 and 4.56 µg for Ni, Pb and Cd, respectively (Figure 5). According to these values, organic Brassicas led to a decrease in the intake of these three elements metals. However, when these results were expressed as percentages of TI for these elements, we only found slight differences (mainly in the case of Pb) between both agriculture systems. The TI percentages for a mean body weight of 70 kg per person were 19.9, 45.1 and 27.1% for Ni, Pb and Cd, respectively, with conventional Brassicas, and 13.1, 14.8 and 18.1 % for organic Brassicas. These results indicate that Cd, Ni and Pb contents in vegetable Brassica rapa harvested under both conventional and organic farming conditions are below the accepted safety limits and do not represent any toxicologic risk.

**Figure 4.** Simulated data and fitted probabilistic distribution for Cd, Ni and Pb in conventional Brassicas.

**Figure 5.** Simulated data and fitted probabilistic distribution for Cd, Ni and Pb in organic Brassicas.
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