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Nutrient Profiles and Bioavailability in Industrial Hemp (*Cannabis sativa* L.) Seeds from Diverse Provenances †

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† This paper is an extended version of our paper published in Islam, M.M.; Solaiman, Z.M.; Rengel, Z.; Abbott, L.K.; Storer, P.; Siddique, K.H.M. Dietary minerals in seeds of industrial hemp (*Cannabis sativa* L.) varieties differ with the origin of sources. In Proceedings of 2nd Australian Industrial Hemp Conference, Fremantle, Australia, 25–28 February 2020.

Abstract

Hemp (*Cannabis sativa* L.) seeds have been essential for human nutrition for millennia. The products and by-products of hemp seeds are gaining popularity nowadays as food, feed and medicine for their high nutritional and nutraceutical properties. In parallel, concerns about phytate, an antinutritional compound limiting nutrient bioavailability in hemp seeds and seed meal are rising. Hemp seeds contain an array of nutrients, but their bioavailability is mostly unknown. Here, we report nutrient and phytate concentrations and phytate contents in source seeds and multiplied seeds of seven industrial hemp varieties. We estimated the bioavailability of specific nutrients based on calculated molar ratios of phytate to minerals. Seed multiplication was carried out in a phytotron using a compost-based growth medium. Five macronutrients (P, K, Mg, S, Ca), four micronutrients (Fe, Mn, Zn, Cu) and Cr were measured in seeds using ICP-OES. Seed phytate was determined using a UV-visible spectrophotometer rapid colourimetric assay. The results revealed significant differences between seven industrial hemp varieties for most macro- and micronutrient concentrations (not Fe), phytate concentration and content and phytate-to-mineral molar ratios in both source and multiplied seeds. Multiplied hemp seeds had higher K, Mn and Zn and, lower Cr and phytate concentrations and lower phytate content than source seeds. Considering nutrient bioavailability, Ca and Fe are non-bioavailable, and Zn is bioavailable in hemp seeds. Ferimon has increased Zn bioavailability in source and multiplied seeds, indicating the variety's potential for seed production in Western Australia.

Keywords: industrial hemp; dietary nutrients; phytate; bioavailability; Western Australia

Academic Editor: Marko Vinceković

Received: 4 May 2025
Revised: 11 June 2025
Accepted: 23 June 2025
Published: 25 June 2025

Citation: Islam, M.M.; Siddique, K.H.M.; Solaiman, Z.M. Nutrient Profiles and Bioavailability in Industrial Hemp (*Cannabis sativa* L.) Seeds from Diverse Provenances. *Sustainability* **2025**, *17*, 5844. https://doi.org/10.3390/su17135844

1. Introduction

Industrial hemp (*Cannabis sativa* L.) seeds have been considered a vital source of human nutrition in ancient cultures for over a millennium [1,2]. Historically, hemp seeds have been used as a food source, either raw, cooked or roasted, and hemp seed oil has been used as food/medicine [2,3]. The hemp seeds are nuts comprising a protective outer shell known as 'hemp hull', a good source of minerals and dietary fibre and a soft inner kernel called 'hemp heart', rich in oil, proteins and vitamins. Overall, whole hemp seed contains 35.5% oil, 24.8% protein, 27.6% dietary fibre, 6.5% moisture, 5.6% ash and numerous vitamins and dietary nutrients [2].

Hemp seeds have been overlooked despite considerable evidence of their numerous health benefits. For many years, the modern world has considered hemp seeds a by-product of fibre production, and they are used mainly for poultry and other animal feed [1,4]. However, in recent years, the products and by-products of hemp seeds (e.g., oil, cake, seed meal, flour, protein powder) have regained their historical uses, with renewed popularity among consumers due to high nutritional and nutraceutical values [5–9]. However, numerous studies report that seed-based food products may contain antinutritional compounds such as phytate [10–15]. Hemp seeds are a rich source of essential macro- and micronutrients, including P, K, Mg, Ca, Fe, Mn, Zn and Cu, all of which play crucial roles in maintaining human health [2,6,16]. However, few studies report on the bioavailability of these nutrients [17]. There is literature available that compares phytate compositions (mainly content in oils and flours) in cereals, legumes and oilseed crops, including industrial hemp [14,18,19]. Still, variety-specific data on phytate concentrations and contents in hemp seeds are rare [10,13,17,20–22].

Phytate (*Myo*-inositol phosphate) is distributed widely in the plant kingdom, mainly in seeds as a storage form of excess inorganic phosphorus and minerals [14,18]. Phytate is frequently associated with nutrient deficiencies in susceptible human populations reliant on grain-based diets because it binds minerals strongly, primarily iron (Fe), calcium (Ca) and zinc (Zn), decreasing their bioavailability in the human digestive tract [11,13]. However, despite an antinutritional stigma of phytate in human and animal diets, it has a crucial role in plant metabolism [14]. During seed germination, phytate is hydrolysed by phytase, making phosphate and other nutrients available to developing seedlings [14,18]. Moreover, myo-inositol and its phosphate derivatives, namely, phytic acid may suppress some gastrointestinal cancers and may exert beneficial health effects including anti-inflammatory, anti-oxidant and anti-diabetic effects [23]. Therefore, it is essential to recognise the dual nature of this compound, which, despite its inhibitory effects on mineral absorption, also offers potential health benefits [24].

The phytate-to-mineral molar ratio (e.g., phytate/Ca, Fe or Zn) is widely used as a proxy for estimating mineral bioavailability in plant-based foods [25–31]. This ratio simplifies the complex interplay of factors affecting mineral absorption, assuming a linear relationship between phytate content and mineral uptake. However, it does not account for other dietary inhibitors like polyphenols, oxalates and fiber, which can also significantly impair the bioavailability of Ca, Fe and Zn [32]. Conversely, enhancers such as ascorbic acid (vitamin C), certain amino acids, and organic acids (e.g., citric acid) can promote mineral absorption, yet are not captured in the molar ratio calculation [33]. Additionally, food processing techniques such as soaking, fermentation, germination and thermal treatments can reduce phytate content and modify mineral bioavailability, further complicating the predictive accuracy of the molar ratio [11].

Other factors such as seed maturity at harvest, storage conditions, and post-harvest handling practices significantly influence nutrients and phytate composition. Seeds harvested before full physiological maturity may have lower concentrations of macro- and micronutrients due to incomplete nutrient translocation and deposition [34]. Conversely, over-mature seeds may experience nutrient degradation, particularly of heat- or oxidation-sensitive compounds such as certain vitamins and unsaturated fatty acids. During storage, nutrient levels—especially Fe, Zn and vitamin content—can decline due to oxidation, moisture fluctuations, and biochemical changes, while phytate content may remain stable or even increase due to residual metabolic activity in improperly dried seeds [35,36]. Moreover, post-harvest handling steps like delayed drying, exposure to high humidity, or mechanical damage play a crucial role in determining the phytate content and nutrient bioavailability of plant-based foods [32,37,38].

Source seeds obtained from multiple suppliers may vary significantly in nutritional composition due to differences in genetic background, seed quality, storage conditions, and the agro-ecological environments in which they were produced. Such variability can affect key nutritional parameters, including macro- and micronutrient concentrations, phytate content, and nutrient bioavailability [11,39]. In contrast, producing multiplied seeds under controlled environmental conditions helps standardise external factors such as soil fertility, irrigation, and nutrient management, thereby minimising environmental variability and allowing a clearer assessment of the seeds' genetic potential for nutrient accumulation [40].

Comparing the nutritional profiles between these source and multiplied seeds is therefore crucial for evaluating the consistency, reliability, and integrity of the seed material used in downstream applications such as biofortification trials, food security interventions, or nutritional breeding programs. Moreover, assessing phytate content and mineral bioavailability is essential, as high nutrient concentrations in source seeds may not translate into improved nutritional outcomes if anti-nutritional factors are also elevated [41,42]. Such comparative analysis supports the selection of high-quality seed stock and helps ensure that nutritional enhancements observed under controlled conditions are not confounded by prior environmental influences. However, such comparisons have not been made previously with hemp seeds.

This study aimed at characterising the nutrient status and bioavailability in the source and multiplied seeds of seven industrial hemp varieties in Western Australia. Previously, germination and early growth responses [43], as well as the macro- and micronutrient composition [44], of 14 industrial hemp varieties imported into Western Australia from overseas were reported. These 14 hemp varieties were grown from source seeds, and new seeds of seven varieties were produced. The macro- and micronutrient concentrations, phytate content, and phytate concentration in the source and freshly multiplied seeds were measured. Phytate-to-mineral molar ratios were calculated to estimate seed nutrient bioavailability. A null hypothesis was assumed that nutrient concentrations, phytate concentrations and contents, and nutrient bioavailability varied independently of source and multiplied hemp seeds.

2. Materials and Methods

2.1. Source Seeds

The source seeds of seven industrial hemp (*Cannabis sativa* L.) varieties were imported by various companies in Western Australia (Table 1). French monoecious varieties were obtained from the Western Australian Hemp Growers' Co-op Ltd. (HempGro, Carbunup River, Australia). Dioecious varieties originating in China were obtained from Premium Hemp Australia and the Department of Primary Industries and Regional Development. A widely adopted and locally grown Danish variety (Morpeth) was obtained from the Food, Fibre and Land International Group Pty Ltd. (Perth, Australia).

2.2. Seed Multiplication

Hemp seed multiplication was performed in a controlled-environment phytotron at the University of Western Australia (31.9789° S, 115.8181° E). The average temperature, relative humidity and light intensity during a 14/10 h light/dark period inside the growth room were 24.8/21.5 °C (SD ± 3.0/2.5 °C), 71.8/70.2% (SD ± 5.5/12.1%) and 5.9/0.0 W m^{-2} (SD ± 0 W m^{-2}). The experiment comprised a randomised complete block design with three replications. The source seeds were sown in 4.5 L pots filled with 3 kg of RICHGRO® UWA Plant Bio Mix M3 (Product Code: SSM2380) inside a plastic bag. The pots were hand-watered by weighing daily to maintain the water content of the potting mix at 70% field capacity. The pots were re-randomised within each block every 7 d to minimise

environmental effects. Plants were harvested at 70 d after sowing (=80% seed maturity to prevent seed shattering). Shoots with intact inflorescences were kept in plastic bags, immediately transferred to a cool room (4 °C) and kept for one week to attain full seed maturity. Shoots were kept in a drying room (25 °C) for 72 h to obtain optimum seed moisture content (<12%) for storage. Seeds were then collected from inflorescences and stored in screwcap plastic containers.

Table 1. Varietal information and seed weight of seven industrial hemp (*Cannabis sativa* L.) varieties in Western Australia. One-way ANOVA revealed significant differences ($p \leq 0.05$) between varieties for source and multiplied seed weights. Means within the same column followed by different letters differ significantly at $p \leq 0.05$. Chi-square (χ^2) *p*-values indicate difference between source and multiplied seeds.

Variety	Sex Type	Country of Origin	Supplier	Seed Weight (mg seed^{-1})		
				Source	Multiplied	
Ferimon	Monoecious	France	WA Hemp Growers' Co-op Ltd., Carbunup River, Australia	18.5 d	16.4 e	
Fedora 17	Monoecious	France	WA Hemp Growers' Co-op Ltd., Carbunup River, Australia	21.1 cd	15.2 e	
Santhica	Monoecious	France	WA Hemp Growers' Co-op Ltd., Carbunup River, Australia	19.2 d	19.7 d	
Morpeth	Monoecious	Denmark	Food, Fibre and Land International Group, Perth, Australia	21.2 cd	27.9 b	
Han NE	Dioecious	China	Premium Hemp Australia, Perth, Australia and DPIRD-WA *, South Perth, Australia	28.8 a	23.9 c	
Han FNQ	Dioecious	China	Premium Hemp Australia, Perth, Australia and DPIRD-WA, South Perth, Australia	24.0 bc	24.0 c	

Table 1. *Cont.*

Variety	Sex Type	Country of Origin	Supplier	Seed Weight (mg seed^{-1})		
				Source	Multiplied	
Han NW	Dioecious	China	Premium Hemp Australia, Perth, Australia and DPIRD-WA, South Perth, Australia	26.8 ab	46.1 a	
Mean				22.8	24.8	
p-value				<0.001	<0.001	
χ^2 *p*-value				0.0		

* DPIRD-WA = Department of Primary Industries and Regional Development, Western Australia.

2.3. Sample Preparation, Digestion and ICP-OES Analysis

Samples were digested following the method developed by Simmons [45]. Briefly, 0.2–0.25 g of ground seed powder was placed into 25-mL Erlenmeyer flasks. Digestion was performed by adding 6 mL of concentrated nitric acid (cHNO_3), and then heating the flasks on a frypan at 120 °C for approximately 20 min. The flasks were then allowed to cool for about 5 min. Digestion was continued by adding 1 mL of concentrated perchloric acid (c$HClO_4$) and heating the flasks at 150 °C until the solutions turned colourless and emitted white fumes. The flasks were allowed to cool before reheating at 170–180 °C for 10 min to dehydrate any silica present in the digest. The flasks were allowed to cool before warming up at 80 °C on a frypan and then adding 4 mL of Milli-Q® H_2O to dissolve any $KClO_4$ crystals. The warm solutions were transferred to 10 mL vials, each containing 50 µL of yttrium (Y) internal standard solution for calibration [Yttrium (Y) Pure Standard, PerkinElmer, Shelton, CT, USA] and the volume was made up to 10 mL with Milli-Q® H_2O. The samples were analysed using ICP-OES (PerkinElmer, Shelton, CT, USA) to measure five macronutrients (P, K, Mg, S, Ca), four micronutrients (Fe, Mn, Zn, Cu) and Cr. Sample concentrations were calculated, with errors corrected based on Simmons [46].

2.4. Phytate Extraction and Spectrophotometric Analysis

Seed phytate was determined using a rapid colourimetric assay originally suggested by Latta and Eskin [47] for different cereal, legume and oilseed crops and later adopted by Vaintraub and Lapteva [48] for crude seed extracts. The direct assay of phytic acid is detailed in Makkar, et al. [49]. Briefly, 0.2 g of ground seed powder was mixed with 20 mL of 0.65 M HCl in 50-mL centrifuge tubes, shaken for 2 h and centrifuged at 2000× *g* for 10 min (Eppendorf, Hamburg, Germany). The supernatant solutions were filtered through 0.45 and then 0.2 µm syringe filters, collected into 15-mL centrifuge tubes and stored at –20 °C. Following extraction, 1 mL of solution was diluted to 9 mL with Milli Q® water and mixed with 3 mL of Wade reagent (0.030% *w*/*v* $FeCl_3$ in 0.3% *v*/*v* sulfosalicylic acid) in a 3:1 ratio using a vortex mixer (Ratek Instruments, Boronia, Australia). The mixtures were then analysed on a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) with absorbance at 500 nm vs. a Wade reagent blank with 0.65 M HCl. Seed phytate concentration was calculated from a standard curve (Figure 1) of seven known concentrations (0, 20, 40, 60, 80, 100, 120 µg mL^{-1}) of commercial phytic acid (phytic acid dodecasodium salt, Sigma-Aldrich, St. Louis, MO, USA) with corrections for the dilution factor.

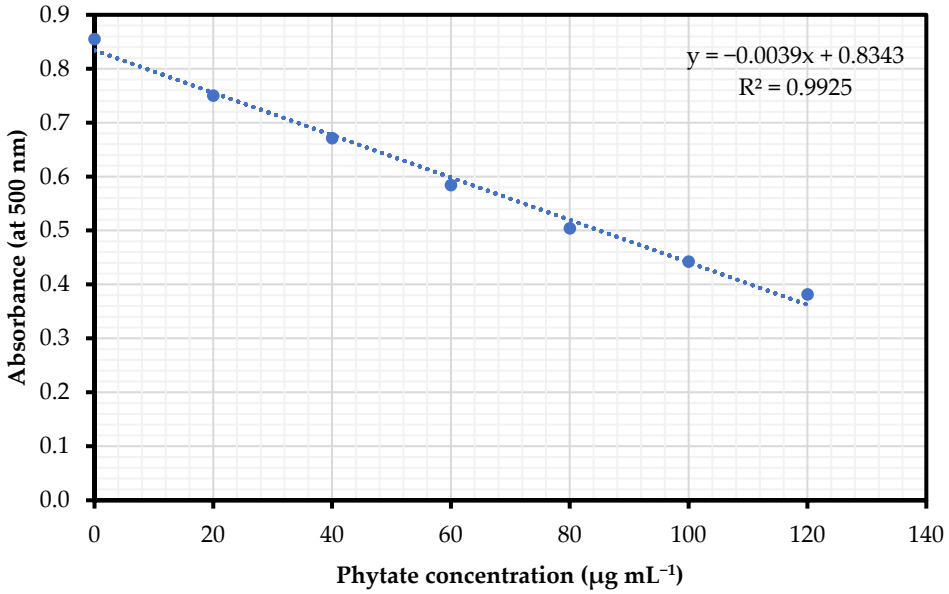

Figure 1. Standard calibration curve used to determine phytate concentrations in source and multiplied seeds of industrial hemp (*Cannabis sativa* L.) varieties. A linear regression model was used to fit the relationship between known phytate concentrations of commercial phytic acid and their corresponding absorbance values. The equation derived from the linear model (i.e., y = −0.0039x + 0.8343) was then used to calculate the phytate concentrations (x) of seed samples from their known absorbance values (y).

2.5. Molar Ratios and Mineral Bioavailability

The phytate/Ca,/Fe and/Zn molar ratios were calculated as millimoles of phytate content divided by millimoles of Ca, Fe or Zn content in hemp seeds, respectively. The (phytate × Ca)/Zn molar ratio was expressed as millimoles. The suggested critical values used to predict mineral bioavailability were phytate/Ca > 0.24, phytate/Fe > 1.0 [28], phytate/Zn > 15.0 [29–31] and (phytate × Ca)/Zn > 200 for the combined effect of phytate and Ca on Zn bioavailability [25–27].

2.6. Experimental Design and Statistical Analyses

Three replicated samples were analysed for macro- and micronutrients, phytate and molar ratios in source and multiplied seeds. Analysis of variance (ANOVA) was performed using Genstat® software version 19.1 (VSN International, Hemel Hempstead, UK). One-way ANOVA was used to determine differences in macro- and micronutrient concentrations and phytate concentration, content and molar ratios in seven industrial hemp varieties. Tukey's posthoc test was used for multiple comparisons and to estimate significant mean differences ($p \leq 0.05$). The Chi-square (χ^2) test for independence was performed between source and multiplied seeds for different parameters (i.e., macro- and micronutrient concentrations, phytate concentration and content and molar ratios) to measure their relationships, assuming a null hypothesis that the parameters varied independently of source and multiplied seeds.

3. Results

3.1. Macronutrient Concentrations in Source and Multiplied Seeds of Industrial Hemp

The macronutrient concentrations varied significantly in the source and multiplied seeds of seven industrial hemp varieties (Table 2). On average, source seeds were rich in P (9.1 g kg^{-1}) followed by K (7.3 g kg^{-1}), Mg (4.1 g kg^{-1}), S (2.6 g kg^{-1}) and Ca (1.2 g kg^{-1}). In contrast, multiplied seeds were rich in K (12.7 g kg^{-1}) followed by P (8.6 g kg^{-1}), Mg (3.9 g kg^{-1}), S (2.5 g kg^{-1}) and Ca (1.5 g kg^{-1}) (Table 2).

Table 2. Macronutrient concentrations in source (SS) and multiplied seeds (MS) of seven industrial hemp (*Cannabis sativa* L.) varieties. One-way ANOVA revealed significant differences ($p \leq 0.05$) between varieties for the macronutrient concentrations of SS and MS. Means within the same column followed by different letter(s) differ significantly at $p \leq 0.05$. Chi-square (χ^2) *p*-value indicates differences between SS and MS.

Variety	P (g kg^{-1})		K (g kg^{-1})		Mg (g kg^{-1})		S (g kg^{-1})		Ca (g kg^{-1})	
	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS
Ferimon	8.0 bc	11.6 a	6.5 b	11.5 c	3.8 b	4.9 a	2.6 bc	2.7 ab	1.1 c	1.3 d
Fedora 17	10.1 a	9.5 bc	7.6 a	9.5 d	4.3 ab	4.5 ab	2.3 cd	2.6 ab	1.1 c	1.5 cd
Santhica	9.1 abc	9.9 bc	7.5 a	12.3 c	4.1 ab	4.3 b	2.6 bc	2.4 b	1.3 a	1.4 cd
Morpeth	10.6 a	5.9 d	8.0 a	14.6 b	4.9 a	2.8 c	2.0 d	2.4 b	1.1 bc	1.6 bc
Han NE	8.9 abc	8.8 c	7.3 ab	12.1 c	3.9 b	4.4 b	2.9 ab	2.7 ab	1.2 ab	1.7 b
Han FNQ	9.6 ab	10.2 b	7.4 ab	12.1 c	4.3 ab	4.1 b	2.4 cd	2.7 a	1.1 bc	1.3 d
Han NW	7.5 c	4.4 e	6.5 b	16.8 a	3.7 b	2.5 c	3.1 a	1.8 c	1.2 bc	2.0 a
Mean	9.1	8.6	7.3	12.7	4.1	3.9	2.6	2.5	1.2	1.5
p-value	0.001	<0.001	<0.001	<0.001	0.010	<0.001	<0.001	<0.001	<0.001	<0.001
χ^2 *p*-value	0.69		0.00		1.00		1.00		1.00	

3.2. Micronutrient Concentrations in Source and Multiplied Seeds of Industrial Hemp

The micronutrient concentrations differed significantly in the source and multiplied seeds of seven hemp varieties except for Fe concentrations (p = 0.073) in source seeds (Table 3). On average, source seeds were rich in Fe (142.8 mg kg^{-1}) followed by Mn (101.9 mg kg^{-1}), Zn (62.3 mg kg^{-1}), Cu (12.9 mg kg^{-1}) and Cr (2.8 mg kg^{-1}). In contrast, multiplied seeds were rich in Mn (123.1 mg kg^{-1}) followed by Fe (121.9 mg kg^{-1}), Zn (101.3 mg kg^{-1}), Cu (9.8 mg kg^{-1}) and Cr (0.4 mg kg^{-1}) (Table 3).

Table 3. Micronutrient concentrations in source (SS) and multiplied seeds (MS) of seven industrial hemp (*Cannabis sativa* L.) varieties. One-way ANOVA revealed significant differences ($p \leq 0.05$) between varieties for the micronutrient concentrations of SS and MS, except Fe (p = 0.073). Means within the same column followed by different letters differ significantly at $p \leq 0.05$. Chi-square (χ^2) *p*-value indicates differences between SS and MS.

Variety	Fe (mg kg^{-1})		Mn (mg kg^{-1})		Zn (mg kg^{-1})		Cu (mg kg^{-1})		Cr (mg kg^{-1})	
	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS
Ferimon	143.1 a	162.1 a	153.2 a	160.0 a	81.2 a	149.0 a	16.1 a	13.9 a	2.0 d	0.4 ab
Fedora 17	146.6 a	158.3 a	112.3 b	162.4 a	69.7 b	101.0 d	14.5 b	13.7 a	2.4 c	0.3 b
Santhica	126.0 a	126.7 b	92.3 cd	113.0 c	69.4 b	127.7 b	13.4 bc	12.2 b	1.8 d	0.3 b
Morpeth	149.6 a	83.8 c	76.3 d	92.8 d	76.5 ab	85.2 e	12.8 c	5.4 e	2.2 cd	0.4 ab
Han NE	150.1 a	130.2 b	106.8 bc	128.3 bc	51.1 c	116.0 bc	10.9 d	9.0 c	3.9 a	0.5 a
Han FNQ	134.9 a	137.3 b	92.2 cd	141.7 b	39.7 d	112.2 cd	11.2 d	12.0 b	4.0 a	0.4 ab
Han NW	149.2 a	93.3 c	79.9 d	93.9 d	48.7 cd	59.5 f	11.5 d	7.0 d	3.5 b	0.5 a
Mean	142.8	121.9	101.9	123.1	62.3	101.3	12.9	9.8	2.8	0.4
p-value	0.073	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
χ^2 *p*-value	0.00		0.00		0.00		0.42		0.00	

3.3. Phytate Concentration and Content in Source and Multiplied Seeds of Industrial Hemp

Phytate concentrations and content varied significantly in the source and multiplied seeds of seven industrial hemp varieties (Table 4). Overall, source seeds contained 12.2 g kg^{-1} and 278.2 µg seed^{-1}, whereas, multiplied seeds contained 11.4 g kg^{-1} and 269.1 µg seed^{-1} for phytate concentration and content, respectively (Table 4).

Table 4. Phytate concentration (g kg^{-1}) and content (µg $seed^{-1}$) in source (SS) and multiplied seeds (MS) of seven industrial hemp (*Cannabis sativa* L.) varieties. One-way ANOVA revealed significant differences ($p \leq 0.05$) between varieties for phytate concentration and content in SS and MS. Means within the same column followed by different letter(s) differ significantly at $p \leq 0.05$. Chi-square (χ^2) *p*-value indicates differences between SS and MS.

Variety	Phytate Concentration (g kg^{-1})		Phytate Content (µg $seed^{-1}$)	
	SS	MS	SS	MS
Ferimon	11.7 e	13.4 a	217.2 c	220.2 d
Fedora 17	13.4 a	12.4 c	283.6 b	187.6 e
Santhica	11.6 f	12.7 b	223.2 c	251.2 cd
Morpeth	13.2 b	9.6 f	279.8 b	267.6 bc
Han NE	12.0 d	11.0 e	346.3 a	262.8 bc
Han FNQ	12.2 c	11.9 d	292.9 b	285.9 b
Han NW	11.4 g	8.8 g	304.3 b	408.2 a
Mean	12.2	11.4	278.2	269.1
p-value	<0.001	<0.001	<0.001	<0.001
χ^2 *p*-value	1.00		0.00	

3.4. Phytate-to-Mineral Molar Ratios in Source and Multiplied Seeds of Industrial Hemp

Phytate-to-mineral molar ratios indicated that source and multiplied seeds had comparatively higher phytate/Ca and phytate/Fe molar ratios than the suggested critical values (Table 5) but about a 4-fold lower (phytate × Ca)/Zn molar ratio than the suggested critical value (>200). The phytate/Zn molar ratio was higher in source seeds (20.6) and lower in multiplied seeds (11.0) than the critical value (>15) (Table 5).

Table 5. Phytate-to-mineral molar ratios in source (SS) and multiplied seeds (MS) of seven industrial hemp (*Cannabis sativa* L.) varieties. One-way ANOVA revealed significant differences ($p \leq 0.05$) between varieties for all molar ratios in SS and MS. Means within the same column followed by different letter(s) differ significantly at $p \leq 0.05$. Chi-square (χ^2) *p*-value indicates differences between SS and MS. SCV = suggested critical value.

Variety	[Phytate/Ca] SCV > 0.24		[Phytate/Fe] SCV > 1.0		[Phytate/Zn] SCV > 15		[Phytate × Ca/Zn] SCV > 200	
	SS	MS	SS	MS	SS	MS	SS	MS
Ferimon	0.7 bc	0.6 a	7.0 abc	7.0 d	14.4 e	8.9 e	37.9 d	29.4 d
Fedora 17	0.8 a	0.5 b	7.8 ab	6.6 d	19.2 cd	12.2 b	51.1 c	45.2 b
Santhica	0.5 d	0.6 ab	7.8 a	8.5 b	16.7 de	9.9 cde	55.7 c	34.4 cd
Morpeth	0.7 ab	0.4 c	7.5 ab	9.7 a	17.3 de	11.2 bc	48.8 c	44.2 b
Han NE	0.6 cd	0.4 c	6.8 bc	7.1 d	23.3 b	9.4 de	71.5 b	40.9 bc
Han FNQ	0.6 bc	0.6 ab	7.7 ab	7.4 cd	30.4 a	10.6 cd	86.9 a	33.3 cd
Han NW	0.6 cd	0.3 d	6.5 c	8.0 bc	23.3 bc	14.7 a	67.5 b	74.9 a
Mean	0.6	0.5	7.3	7.8	20.6	11.0	59.9	43.2
p-value	<0.001	<0.001	0.039	<0.001	<0.001	<0.001	<0.001	<0.001
χ^2 *p*-value	1.00		1.00		0.00		0.00	

4. Discussion

4.1. Changes in Macro- and Micronutrient Composition in Hemp Seeds

The macro- and micronutrient concentrations in seeds of seven industrial hemp varieties align with the findings reported elsewhere [2,4,17,19,50–54]. Overall, the source and multiplied seeds of the seven varieties had higher P, K and Mg concentrations than the

other macronutrients, as reported by others [17,50,55]. Notably, multiplied seeds had 74% increased K than source seeds (Table 2).

Hemp seeds could be a good source of P, K and Mg for human and animal nutrition with their higher P, K and Mg composition (per 100 g) than other specialty seeds (black cumin, chia, flax, perilla, pumpkin, quinoa and sesame) [55]. In particular, the Ferimon variety contained 45%, 76.9% and 28.9% more P, K and Mg in multiplied seeds than source seeds. Similarly, Lan et al. [50] reported higher P, K and Mg composition in four industrial hemp varieties (CRS-1, CFX-2, CFX-1 and Canda) grown in North Dakota, USA, with Canda outperforming most macro- and micronutrients in the 2017 harvest relative to 2015. Siano et al. [54] reported very high K, Mg and Ca concentrations relative to other macro elements in seeds and flour of the hemp cultivar, Fedora; in particular, hemp flour had higher K and Ca concentrations ($\geq$50.0%) than seeds.

The source and multiplied seeds of the seven hemp varieties had higher Fe, Mn, Zn and Cu concentrations than the other micronutrients, as reported by others [4,17,19,54]. Multiplied hemp seeds had elevated Mn (20.8%) and Zn (62.6%) concentrations than source seeds (Table 3).

Ferimon had high concentrations of all micronutrients (Fe, Mn, Zn and Cu) in both source and multiplied seeds, particularly 13.3% and 83.5% more Fe and Zn concentrations in multiplied seeds than source seeds. Ferimon also had smaller seed size with lower seed weight than other varieties (Table 1), resulting in higher seed count per unit of seed mass and higher seed surface-to-volume ratio [56], which might contribute to the higher nutrient concentrations than other varieties. Similar to Ferimon, higher Fe, Mn and Cu concentrations were also observed in multiplied seeds of Fedora 17 having smaller seed sizes with lower seed weights than other varieties.

All varieties had a significantly lower (85.7%) Cr concentrations in multiplied seeds than source seeds (Table 3). Seeds containing trace amounts of Cr may benefit from improved metabolic activity during germination and contribute to dietary Cr intake, which can be important for managing glucose tolerance and preventing type 2 diabetes [57] whereas High Cr levels can lead to reduced seed germination, lower seedling vigor, and oxidative stress [58] and can be fatal to animals and humans if consumed in high amounts [59]. Therefore, monitoring Cr levels in seeds is critical for both plant health and food safety.

The chi-square (χ^2) *p*-value indicated that P, Mg, S, Ca (Table 2) and Cu (Table 3) concentrations varied independently of source and multiplied seeds ($p \geq 0.05$) which supports the expected genetic model for nutrient inheritance. Conversely, for K (Table 2), Fe, Mn, Zn and Cr (Table 3) concentrations, a likely association or effect was present ($p < 0.05$) indicating possible gene interactions, environmental effects, or segregation distortion affecting their concentrations. In breeding programs, nutrient traits are sometimes segregated in progenies (e.g., F_2, backcross generations). This finding will help plant breeders to verify whether observed segregation ratios (e.g., high:medium:low nutrient categories) match expected Mendelian ratios [60,61].

4.2. Changes in Phytate Composition in Hemp Seeds

The multiplied seeds had lower average phytate concentrations and contents (by 6.7% and 3.3%, respectively) for the seven hemp varieties than the source seeds. Furthermore, phytate concentration in multiplied seeds of Chinese dioecious varieties decreased more (10.7%) than European monoecious varieties (3.8%); in contrast, total phytate contents decreased more in European varieties (7.7%) than Chinese varieties (1.4%). Russo and Remo [13] also reported differences in phytate composition between varieties from different origins; French monoecious varieties (Fedora 17, Felina 32, Ferimon) had significantly lower mean phytate contents (by 0.55 g kg^{-1} dry matter) than Italian dioecious varieties (Carmag-

nola, Carmagnola selezionata, Fibranova). Multiplied seeds of the Danish variety Morpeth had the most significant decrease (27.3%) in phytate concentration, and Fedora 17 had the most significant decrease (33.9%) in total phytate content compared with source seeds. The source seeds of Ferimon had the lowest phytate content, as reported elsewhere [13].

The chi-square test for independence indicated that phytate concentrations varied independently of source and multiplied seeds (χ^2 p-value = 1.00), whereas total phytate contents were variety-dependent and/or influenced by external factors (χ^2 p-value = 0.00). This revealed significant deviation from expected Mendelian segregation ratios supports the hypothesis that non-Mendelian factors, such as epistasis, quantitative trait loci (QTL) interactions, or preferential allele transmission, may influence phytate accumulation [61]. Moreover, environmental conditions such as soil phosphorus levels, temperature, and moisture during seed development can affect phytate biosynthesis, independent of genetic background [62]. Understanding these complex interactions is essential, especially in breeding programs aiming to reduce phytate content to improve mineral bioavailability, since high phytate can inhibit the absorption of critical micronutrients like iron and zinc [32].

4.3. Changes in Bioavailability of Minerals in Hemp Seeds

Mineral bioavailability can be defined as the portion of mineral intake absorbed into the blood system and used by the body for various physiological functions [63]. Plant-based diets usually have high phytate content and thus poor mineral bioavailability, frequently associated with micronutrient deficiencies (Ca, Fe, Zn) in susceptible human populations [11].

The source and multiplied hemp seeds had poor Ca and Fe bioavailability, with much higher mean phytate/Ca and phytate/Fe molar ratios than the suggested critical values. Low Ca and Fe bioaccessibility in hemp seeds was also predicted with lower Ca/P and higher phytate/Fe ratios than the recommendations [17]. However, the study also reported that the Ca/P molar ratio might not represent the competition regarding Ca absorption as P remains in hemp seeds, mainly in the phytate form [17].

The hemp varieties had relatively good Zn bioavailability in multiplied seeds, with lower mean phytate/Zn and (phytate × Ca)/Zn molar ratios than the suggested critical values. In contrast, a higher phytate/Zn molar ratio than the recommendation was also observed in hemp seeds, suggesting the Zn bioaccessibility to be compromised [17].

The Ca and Fe bioavailability differed independently of source and multiplied hemp seeds, as revealed by chi-square (χ^2) p-value ($p \geq 0.05$), whereas, Zn bioavailability is tightly linked with genetic background and/or other external factors ($p < 0.05$). These findings are especially important when differences in bioavailability are not aligned with seed origin, suggesting that internal genetic factors, rather than external production conditions, are playing a dominant role. For instance, phytate-mineral interactions, controlled by specific QTLs, can dramatically influence Fe and Zn bioavailability [32]. Furthermore, environmental conditions such as soil pH, nutrient availability, and temperature during seed development may also impact phytate levels and mineral complexation, thereby affecting mineral uptake in the human gut [37]. Recognising whether these traits are environmentally plastic or genetically fixed has significant implications for breeding nutrient-dense cultivars, ensuring that improved mineral bioavailability is heritable and consistent across seed production systems.

Overall, Ca and Fe are non-bioavailable, and Zn is bioavailable in hemp seeds with the variety Ferimon having increased Zn bioavailability in both source and multiplied seeds, indicating the potential of the variety for seed production in Western Australia for Zn biofortification.

4.4. Limitations and Future Scopes

Oil, protein, carbohydrate and other seed constituents relevant to hemp seed nutritive properties were not quantified in this study. As human nutrition was not the primary objective, the focus was placed on characterising the varieties based on the accumulation and bioavailability of selected macro- and micronutrients in source and multiplied seeds. Differences in seed nutrient parameters were subsequently tested to determine whether they varied independently of seed origin or were influenced by genetic and/or external environmental factors. Mineral bioavailability was estimated solely using phytate-to-mineral molar ratios, and other internal or external factors known to influence bioavailability were not taken into account. Only the bioavailability of Ca, Fe and Zn was assessed, based on established critical limits for the phytate/Ca,/Fe,/Zn and (phytate × Ca)/Zn molar ratios. Due to the absence of established thresholds for other macro- and micronutrients, their bioavailability was not evaluated. Future research should include a broader analysis of seed constituents and evaluate the bioavailability of a wider range of macro- and micronutrients, with an emphasis on the nutritive potential of hemp seed and its prospects as an oilseed or specialty crop for human consumption. The findings from this study can support agronomists, plant breeders and seed nutritionists in designing targeted prebreeding efforts and nutritional breeding programs, while also informing more effective biofortification strategies and comprehensive seed quality assessments.

5. Conclusions

Multiplied hemp seeds exhibited higher concentrations of K (+74%), Mn (+20.8%), and Zn (+62.6%), along with lower concentrations of Cr (−85.7%) and phytate (−6.7%), and a reduced total phytate content (−3.3%) compared to source seeds. Phytate concentration in the multiplied seeds of Chinese dioecious varieties showed a greater reduction (−10.7%) compared to European monoecious varieties (−3.8%). In contrast, the total phytate content decreased more in European varieties (−7.7%) than in the Chinese varieties (−1.4%). Most macronutrients (excluding K), Cu and phytate concentrations, as well as the bioavailability of Ca and Fe, varied independently of source and multiplied hemp seeds, whereas, most micronutrients (excluding Cu), K concentrations, phytate content, and Zn bioavailability were associated with genetic and/or external environmental influences. Overall, Ca and Fe were found to be non-bioavailable, while Zn was bioavailable in hemp seeds, with the variety Ferimon demonstrating enhanced Zn bioavailability in both source and multiplied seeds—highlighting its potential suitability for seed production in Western Australia.

Author Contributions: Conceptualization, M.M.I. and Z.M.S.; methodology, M.M.I., Z.M.S. and K.H.M.S.; software, M.M.I.; validation, Z.M.S. and K.H.M.S.; writing—original draft preparation, M.M.I.; writing—review and editing, Z.M.S., M.M.I. and K.H.M.S.; supervision, Z.M.S. and K.H.M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: Mohammad Moinul Islam thanks the Australian Government and The University of Western Australia for awarding him the International Research Training Program (RTP) Fees Offset Scholarship and University Postgraduate Award (UPA) to complete this research. All authors thank the WA Department of Primary Industries and Regional Development, Food, Fibre and Land International Group, Premium Hemp Australia and WA Hemp Growers' Co-op Ltd. for supplying the seeds of industrial hemp varieties.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SS	Source Seeds
MS	Multiplied Seeds
QTLs	Quantitative Trait Loci
SCV	Suggested Critical Value
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
DPIRD-WA	Department of Primary Industries and Regional Development, WA

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