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Effect of Partial Organic Fertilizer Substitution on Heavy Metal Accumulation in Wheat Grains and Associated Health Risks

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Abstract: The partial substitution of chemical fertilizers with organic manure is an important strategy for improving agricultural sustainability. However, its effect on heavy metal (HM) pollution and its potential risk to human health remain unclear. Therefore, a field study was conducted to evaluate the effects of partial organic fertilizer substitution (0, 10%, 20%, 30%, and 40%) on health risks posed by HMs, including Zn, Cu, Ni, Cd, Pb, As, and Cr. The results showed that organic fertilizer substitution significantly increased Cu, Cd, Pb, and As accumulations in the soil. The Zn, Cu, Pb, and As contents were significantly higher in grains grown under organic fertilizer substitution (40%) conditions. The HM contents in the soil and grains were below the safety threshold limits in all treatments. Furthermore, the health risk caused by the exposure to As, Cu, and Zn accounted for 86% of the hazard index (HI) value. The HI value was significantly greater at a substitution ratio of 40% than in the no-nitrogen fertilizer treatment. Ingestion of the wheat grains grown at substitution ratios $\geq$ 30%) increased the carcinogenic risk of As and the total carcinogenic risk. In conclusion, organic fertilizer substitution at high ratios significantly increased the non-carcinogenic and carcinogenic risks associated with ingesting wheat grain. The optimal organic fertilizer substitution ratio (20%) maintained crop yields and improved soil fertility without increasing the non-carcinogenic or carcinogenic risks to human health. These findings highlight the importance of understanding the impacts of optimal organic fertilizer management in wheat growing systems.

Keywords: heavy metal; health risk assessment; wheat grain; organic fertilizer substitution

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1. Introduction

Heavy metals (HMs), such as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), arsenic (As), and chromium (Cr), are commonly detected in farmland soils and agricultural crops. This widespread pollution poses health risks due to the HM toxicity, bioaccumulation potential, and persistence in the natural environment [1,2]. The long-term excessive intake of these HMs can damage human skeletal, nervous, circulatory, enzymatic, endocrine, and immune systems [3]. The HMs in farmland soils originated from natural sources; however, some anthropogenic activities such as smelting, fossil fuel combustion, wastewater irrigation and excessive application of fertilizers can cause soil HM pollution [4]. Ingestion is the primary pathway for human exposure to HMs (in addition to dermal contact and inhalation), accounting for more than 90% of the intake [5]. Wheat (*Triticum aestivum* L.) is a major staple cereal crop, providing an essential source of calories and other important nutrients for millions of people worldwide [6,7]. China is the largest producer and consumer of wheat globally. Therefore, the safety and stability of wheat grain supplies directly affect food security and human health in China and many other regions worldwide. Therefore, it is imperative to investigate the accumulation of pollutants, such as HMs, in wheat.

Organic fertilizer substitution is a promising long-term approach to sustainable agriculture, providing various economic and environmental benefits, such as enhanced crop yield sustainability and improved soil physicochemical properties and soil quality [8–11]. However, organic fertilizers derived from livestock and poultry manure contain high contents of harmful elements and are a main source of HM contamination in the soil [12]. Wang et al. [13] showed that the average contents of Cd, Cr, Cu, Pb, Zn, Ni, As, and Hg in a commercially available organic fertilizer used in agricultural areas in North China were 0.21, 45.42, 69.22, 87.40, 274.58, 16.50, 3.21, and 0.33 mg kg^{-1}, respectively, much higher than the contents in comparable inorganic fertilizers [14]. Couto et al. [15] demonstrated that the Cr, Ni, and Zn contents in a Typic Hapludalf soil increased significantly after 10 years of pig slurry manure applications in southern Brazil. Wang et al. [16] showed that although pig manure fertilizer applications significantly increased the biomass of peanut (*Arachis hypogaea* L.) crops, it increased the levels of soil contamination with Cu, Zn, and Cd. Therefore, the inappropriate or excessive application of organic fertilizers is likely to exacerbate the risk of HM pollution in agricultural soils [17,18].

The uptake of HMs by crops is closely related to the state of HMs in soil [19,20]. The accumulation of HMs in soil can increase the HM contents in the edible parts of crops, increasing the risk to human health [21]. Furthermore, the presence of HMs in cereal grains, even at doses considered safe, has been shown to cause deleterious effects when the grains are ingested for long periods [22]. Human health risk assessment (HHRA) is an effective and widely used method to estimate pollutant exposure health risks in human populations [23,24]. The non-carcinogenic and carcinogenic risks of human exposure to HMs in cereal grains have been assessed, and the effect of the exposure time has been determined, providing useful risk information for decision makers [25,26]. Therefore, the optimal organic fertilizer substitution ratio requires careful consideration in agroecosystems to avoid unnecessary human health risks due to HM accumulation in soils and crops while increasing the sustainability and stability of agricultural systems.

A field study was performed to assess organic fertilizer substitution in wheat grain crops and evaluate the health risk posed by the consumption of HMs (Zn, Cu, Ni, Cd, Pb, As, and Cr) in wheat grains and soils. The objectives of this study were to (1) evaluate the effects of organic fertilizer substitution on the contents of HMs in wheat grains and soils and (2) assess the potential health risks to local consumers of wheat grains potentially enriched in HMs due to organic fertilizer substitution at different ratios. The findings of this study provide guidance for the strategic management of organic fertilizer application in wheat production on North China Plain, balancing the need for agricultural productivity, sustainability, and food safety.

2. Materials and Methods

2.1. Site Description and Experimental Design

The field study started in 2016 in Jiyang County (Shandong Province, China; 36°58′ N, 116°59′ E). The experimental site has a cool-to-warm temperate monsoon climate. The mean temperature over the last 2 years was 14.0 °C and annual precipitation was 602.3 mm. The soil was calcareous and moist, with the following initial soil characteristics: pH 8.7, 1:2.5 *w/v* ratio of soil: water, organic matter content of 14.2 g kg^{-1}, Olsen phosphorus content of 8.8 mg kg^{-1}, and available potassium content of 94.5 mg kg^{-1}. The contents of total Zn, Cu, Ni, Cd, Pb, As and Cr were 58.2, 20.1, 29.1, 0.22, 17.0, 10.9 and 54.1 mg kg^{-1}, respectively.

The field study was conducted in a rotation system with wheat production in winter and maize production in summer. The same winter wheat cultivar (Luyuan 502) and summer maize cultivar (Denghai 618) were used throughout the experimental period. The experiment consisted of six treatments, with each treatment applied to triplicate plots: (1) no N fertilizer application (CK); (2) chemical fertilizer (MF); (3) organic fertilizer (OF) substitution 10% of chemical fertilizer (10% OF); (4) organic fertilizer substitution 20% of chemical fertilizer (20% OF); (5) organic fertilizer substitution 30% of chemical fertilizer (30% OF); (6) organic fertilizer substitution 40% of chemical fertilizer (40% OF). The area of each plot was 40 m^2. Except for the CK treatment, the total amounts of N, P, and K applied to the soils in each treatment were 195 kg ha^{-1}, 45.9 kg ha^{-1}, and 62.2 kg ha^{-1}, respectively. The organic fertilizer made of cow manure and crop straw in the experiment contains 1.30% N, 0.68% P, 1.01% K, and 55.9% organic matter. The amount of organic fertilizer used to substitute chemical fertilizer was calculated according to N. The application rates of the chemical phosphorus (as superphosphate) and potassium fertilizers (as potassium sulfate) were determined by subtracting the phosphorus and potassium input of the organic fertilizers. In this study, 50% N fertilizer (including organic N and urea N), phosphate and potassium fertilizers were applied basally. Additionally, 97.5 kg N ha^{-1} (50% N, urea) was applied as urea at the jointing stage of wheat production. In the maize growing season, equal amounts of N (225 kg ha^{-1}), P (52.4 kg ha^{-1}), and K (112.0 kg ha^{-1}) were applied to all plots, except for the CK treatment. The phosphate and potassium fertilizers were basally applied, and the ratio of basal to top-dressing in the N fertilizer application was 5:5. The HM contents in the commercially available organic and chemical fertilizers used in this field study are listed in Table 1.

Table 1. Heavy metals are contained in organic fertilizer and chemical fertilizers.

Samples	**Contents of Heavy Metals (mg kg^{-1})**						
	Zn	**Cu**	**Ni**	**Cd**	**Pb**	**As**	**Cr**
Organic fertilizer	284.50	26.38	25.2	0.67	26.9	13.68	21.72
Urea	0.05	0.06	0.46	0.05	0.06	0.03	0.52
superphosphate	0.45	0.20	21.7	0.21	12.4	15.0	12.1
potassium sulfate	0.21	0.05	0.38	0.02	0.72	0.22	1.25

2.2. Sampling and Analyses

During the wheat harvest in 2021 and 2022, the plants were manually removed from a 4 m^2 area (2.0 m × 2.0 m) in the center of each plot to determine the yield per plot. Wheat shoot samples were collected randomly from two adjacent rows (0.5 m length) in each plot to analyze the HM content in the grains. The wheat shoot samples were obtained from an area with uniform plant density, growth height, and growth stages to reduce sampling variation. The samples were separated into grains and straw. The grain samples were carefully washed with deionized water, dried at 60–65 °C until a constant weight was achieved, and ground to a powder using a stainless-steel grinder for HM analysis. The samples were digested with HNO_3–H_2O_2 using a microwave-accelerated reaction system (CEM, Matthews, NC, USA). The concentrations of Zn, Cu, Ni, Cd, Pb, As, and Cr in the digested grain solutions were determined via inductively coupled plasma mass spectroscopy (ICPMS) (ICAP RQ, Thermo, Waltham, MA, USA). The IPE126 was used as the quality control reference material (Wageningen University, the Netherlands) for HM analysis, and the recoveries of HMs were 93.6–108.5%.

Five soil cores (20 cm depth) were collected in an X pattern from each plot using a stainless-steel auger. The cores were combined to create one homogenous composite sample. The composite samples were air-dried and sieved through a 100-mesh screen to obtain a homogenous sample for further analysis. The soil samples were subjected to microwave-accelerated acid digestion (HNO_3-HCl digestion method) as described by Micó et al. [27], and the HMs concentrations in the digested solutions were determined through the use of ICPMS using NST2 (IGGE, Beijing, China) as the standard soil material for quality control. The recoveries of HMs in NST2 were 91.2–106.7%.

2.3. Calculations

2.3.1. Health Risk Assessment

The United States Environmental Protection Agency (USEPA) guidelines (2006) [28] were used to assess the non-carcinogenic human health risk from the consumption of wheat grains based on the threshold hazard quotient (THQ), which was calculated using Equation (1). The hazard index (HI) was calculated following Equation (2) to assess the total non-carcinogenic risk of all HMs with ingesting the grain. THQ or HI values ≤ 1 indicate that the exposed population is not likely to suffer any health risks, while values > 1 indicate that the exposed population has been subjected to an adverse health risk [28].

$$\mathrm{THQ} = \frac{\mathrm{C_{grain}} \times \mathrm{D} \times \mathrm{EF} \times \mathrm{ED_{total}}}{\mathrm{RfD} \times \mathrm{Bw} \times \mathrm{ATn}} \tag{1}$$

$$\mathrm{HI} = \mathrm{THQ1} + \mathrm{THQ2} + \mathrm{THQ3} + \ldots + \mathrm{THQn} \tag{2}$$

where C_{grain} is the HM content in grain; D is the daily intake of wheat grains, which was 94.47 g day^{-1} for children and 159.9 g day^{-1} for adults [29]; EF is the exposure frequency (350 days/year); ED_{total} is exposure period duration, which was 6 years and 30 years for children and adults, respectively; RfD is the reference dose (mg kg^{-1} day^{-1}) of Zn (0.3), Cu (0.04), Ni (0.02), Cd (0.001), Pb (0.004), As (0.0003), and Cr (1.5) [29]; Bw indicates the average body weight of children (18.6 kg) and adults (61.6 kg) [30]; and ATn refers to the duration of the exposure to HMs, which was calculate as $ED_{total} \times 365$ days/year.

2.3.2. Carcinogenic Risk

The threshold cancer risk (TCR) was calculated to assess the carcinogenic risk to the population due to food intake over a lifetime [28]. The TCR of carcinogenic Cd, Pb, and As was calculated following Equation (3):

$$\mathrm{TCR} = \frac{\mathrm{C_{grain}} \times \mathrm{D} \times \mathrm{SF} \times \mathrm{EF} \times \mathrm{ED_{total}}}{\mathrm{Bw} \times \mathrm{ATc}} \tag{3}$$

where ATc is the average time for carcinogens (70 × 365 days); SF is the cancer slope factor, with values of 6.1, 8.5×10^{-3}, and 1.5 µg g^{-1} day^{-1} for Cd, Pb, and As, respectively [28].

2.4. Statistical Analysis

Excel 2010 (Microsoft, Redmond, DC, USA) was used for the calculations. Two-way analysis of variance (ANOVA) was performed using SAS v.8.0 (SAS Institute, Cary, NC, USA) to determine the effects of organic fertilizer substitution on the HM content in wheat grains, and one-way ANOVA was used to determine the health risk assessment parameters. Duncan's test was used to compare means.

3. Results

3.1. Wheat Yield Affect by Organic Fertilizer Sunstitution

The grain yields of wheat among all treatments were 2.8–7.8 Mg ha^{-1} in 2021 and 4.3–9.6 Mg ha^{-1} in 2022 (Figure 1). The wheat yield in the CK treatment was significantly lower than in the other treatments. However, there were no significant differences in wheat yield between the treatments with organic fertilizer substitution at different ratios in each year.

3.2. Contents of HMs in Soils and Grains

In 2021, the average contents of Zn, Ni, and Cr were 29.06, 0.34, and 0.87 mg kg^{-1}, respectively. They were significantly higher than those (22.1, 0.3, and 0.7 mg kg^{-1}) in 2022 (Table 2). However, no significant differences were observed in the contents of Cu, Cd, Pb, and As between the two years. The HM contents of Zn, Cu, Pb, and As were significantly influenced by the organic fertilizer substitution (Table 2). The grain Zn contents in the CK and 40% OF treatments were significantly higher than those in the MF and 10% OF treatments in both 2021 and 2022. The grain Cu, Pb, and As contents were the highest in the 40% OF treatment in both years, with levels significantly higher than in the CK and 10% OF treatments. The contents of all HMs were not affected by the organic substitution × year interaction.

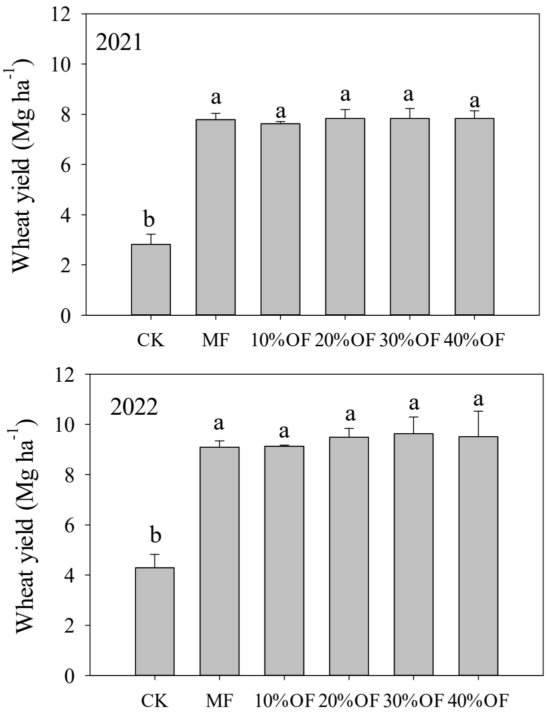

Figure 1. Effect of organic fertilizer substitution on wheat grain yield in 2021 and 2022. The same lowercase letters are not significantly different at $p < 0.05$.

Table 2. Effect of organic fertilizer substitution on heavy metal contents in wheat grains. The values are the means ± SD of triplicate samples and are not significantly different at $p < 0.05$ when followed by the same lowercase letter in each year.

Treatments	Content of Heavy Metals in Wheat Grain						
	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Ni (μg kg^{-1})	Cd (μg kg^{-1})	Pb (μg kg^{-1})	As (μg kg^{-1})	Cr (μg kg^{-1})
2021							
CK	31.5 ± 2.1 a	2.9 ± 0.1 b	318.8 ± 76.9 a	8.5 ± 1.0 a	51.9 ± 4.5 b	16.8 ± 1.9 c	838.1 ± 222.3 a
MF	26.6 ± 1.0 b	3.0 ± 0.1 b	348.6 ± 134.2 a	8.0 ± 1.0 a	51.6 ± 2.0 b	24.7 ± 1.5 bc	853.2 ± 211.7 a
10% OF	26.1 ± 1.3 b	3.4 ± 0.2 a	369.3 ± 48.3 a	8.2 ± 0.4 a	52.1 ± 10.0 b	24.0 ± 3.6 bc	887.9 ± 125.5 a
20% OF	29.1 ± 1.5 ab	3.3 ± 0.2 a	321.0 ± 57.6 a	8.6 ± 0.4 a	55.2 ± 12.8 ab	23.8 ± 3.0 bc	901.5 ± 78.4 a
30% OF	29.8 ± 3.7 ab	3.4 ± 0.2 a	362.3 ± 62.9 a	8.5 ± 0.6 a	61.7 ± 3.1 ab	30.9 ± 4.0 ab	893.1 ± 145.4 a
40% OF	31.3 ± 2.7 a	3.3 ± 0.1 a	337.0 ± 84.5 a	9.1 ± 1.9 a	67.8 ± 5.9 a	36.4 ± 9.5 a	871.1 ± 288.5 a
2022							
CK	24.3 ± 3.4 a	3.0 ± 0.2 c	238.1 ± 12.8 a	8.9 ± 0.7 a	55.9 ± 9.7 b	20.6 ± 1.5 c	653.7 ± 110.2 a
MF	19.6 ± 1.8 b	3.0 ± 0.1 c	265.1 ± 23.0 a	9.3 ± 3.2 a	61.0 ± 12.1 ab	24.9 ± 2.5 bc	634.7 ± 104.8 a
10% OF	20.3 ± 1.1 b	3.2 ± 0.1 bc	258.4 ± 16.8 a	8.3 ± 1.4 a	59.8 ± 2.7 ab	26.1 ± 3.6 abc	718.3 ± 111.5 a
20% OF	21.5 ± 1.2 ab	3.3 ± 0.0 ab	246.4 ± 38.6 a	8.4 ± 4.4 a	60.8 ± 3.4 ab	25.7 ± 4.3 abc	647.1 ± 58.3 a
30% OF	22.4 ± 2.3 ab	3.3 ± 0.1 ab	270.6 ± 6.7 a	9.1 ± 2.7 a	63.0 ± 4.7 ab	28.9 ± 4.7 ab	702.3 ± 79.9 a
40% OF	24.5 ± 0.9 a	3.4 ± 0.1 a	256.3 ± 42.8 a	8.7 ± 1.9 a	70.2 ± 2.1 a	31.8 ± 2.1 a	667.9 ± 261.2 a
Source of variation							
Treatment (T)	**	***	ns	ns	**	***	ns
Year (Y)	***	ns	***	ns	*	ns	**
T × Y	ns	ns	ns	ns	ns	ns	ns

*, **, ***, and ns indicate significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels and no significant difference, respectively. The limits of detection for Zn, Cu, Ni, Cd, Pb, As, and Cr were 0.5, 0.2, 0.2, 0.002, 0.02, 0.002 and 0.05 mg kg^{-1}, respectively.

The mean soil contents of Zn, Cu, Ni, Cd, Pb, As, and Cr in the two years were 58.5, 22.1, 29.3, 0.23, 18.6, 11.3, and 54.1 mg kg^{-1}, respectively (Figure 2). The total contents of Cu, Cd, and Pb were the highest in the soil in the 40% OF treatment, with contents significantly higher than in the CK. The As content in the 40% OF treatment was the highest and was significantly higher than in any other treatment. There were no significant differences in the contents of Zn, Ni, and Cr in soils between the treatments.

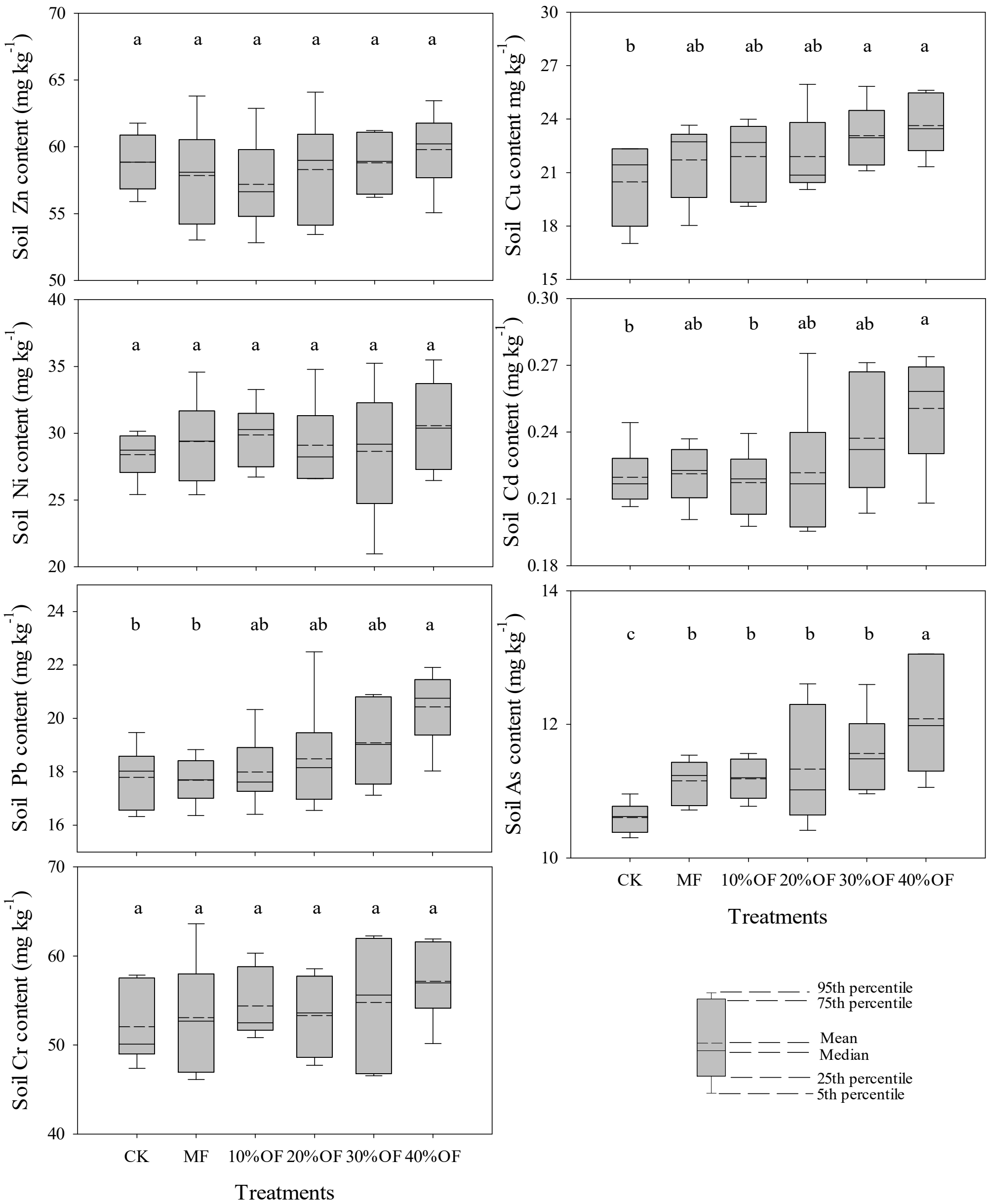

Figure 2. Contents of Zn, Cu, Cd, Pb, As, and Cr in soils for different organic fertilizer substitution ratios. The same lowercase letters are not significantly different at $p < 0.05$. The limits of detection for soil Zn, Cu, Ni, Cd, Pb, As, and Cr were 1, 0.6, 1, 0.09, 2, 0.4 and 2 mg kg^{-1}, respectively.

3.3. Human Health Risk Assessment

The THQ values of all HMs were below 1, and the value of individual HMs was larger for children than for adults (Table 3). The THQ values of the HMs in the wheat grains had the same ranking for children and adults: Zn > As > Cu > Ni > Pb > Cd > Cr. The THQ values of Zn, Cu, Pb, and As were significantly affected by organic fertilizer substitution. The THQ for Zn was significantly higher in the CK and 40% OF treatment groups than in the MF and 10% OF treatment groups. Increasing the organic fertilizer substitution ratio significantly increased the THQ values for Cu, Pb, and As in children and adults. The THQ values for Cu, Pb, and As in the 40% OF treatment group were 1.14-, 1.31-, and 2.13-fold higher than those in the CK group, respectively. The HI values of all HMs ranged from 1.28 to 1.62 for children and from 0.66 to 0.83 for adults. Only the HI of the HMs in the 40% OF treatment was significantly higher than that in the CK, MF, 10% OF, and 20% OF treatments.

Table 3. Effect of organic fertilizer substitution on the threshold hazard quotient and hazard index due to consumption of wheat grains contaminated by heavy metals for children and adults. The values are the means ± SD of 2 crop years and are not significantly different at $p < 0.05$ when followed by the same lowercase letter.

Treatments	Threshold Hazard Quotient in Grain							Hazard Index
	Zn	Cu	Ni ($\times 10^{-2}$)	Cd ($\times 10^{-2}$)	Pb ($\times 10^{-2}$)	As	Cr ($\times 10^{-3}$)	
Children								
CK	0.45 ± 0.04 a	0.36 ± 0.01 b	6.78 ± 0.99 a	4.24 ± 0.29 a	6.56 ± 0.84 c	0.30 ± 0.01 c	2.42 ± 0.28 a	1.28 ± 0.01 c
MF	0.37 ± 0.02 b	0.36 ± 0.01 b	7.47 ± 1.78 a	4.22 ± 0.80 a	6.85 ± 0.86 bc	0.40 ± 0.03 bc	2.42 ± 0.50 a	1.33 ± 0.07 c
10% OF	0.38 ± 0.02 b	0.40 ± 0.02 a	7.64 ± 0.39 a	4.02 ± 0.45 a	6.81 ± 0.61 bc	0.41 ± 0.06 bc	2.61 ± 0.10 a	1.37 ± 0.06 bc
20% OF	0.41 ± 0.00 ab	0.40 ± 0.01 a	6.91 ± 0.50 a	4.15 ± 1.10 a	7.07 ± 0.92 bc	0.40 ± 0.04 bc	2.51 ± 0.17 a	1.39 ± 0.04 bc
30% OF	0.42 ± 0.04 ab	0.40 ± 0.01 a	7.71 ± 0.79 a	4.29 ± 0.74 a	7.59 ± 0.23 ab	0.49 ± 0.07 ab	2.59 ± 0.23 a	1.51 ± 0.11 ab
40% OF	0.45 ± 0.02 a	0.41 ± 0.00 a	7.22 ± 1.52 a	4.32 ± 0.94 a	8.40 ± 0.44 a	0.55 ± 0.09 a	2.50 ± 0.89 a	1.62 ± 0.13 a
Adults								
CK	0.23 ± 0.02 a	0.18 ± 0.01 b	3.47 ± 0.51 a	2.17 ± 0.15 a	3.35 ± 0.43 c	0.15 ± 0.01 c	1.24 ± 0.14 a	0.66 ± 0.00 c
MF	0.19 ± 0.01 b	0.19 ± 0.00 b	3.82 ± 0.91 a	2.16 ± 0.41 a	3.50 ± 0.44 bc	0.21 ± 0.01 bc	1.23 ± 0.26 a	0.68 ± 0.03 c
10% OF	0.19 ± 0.01 b	0.20 ± 0.01 a	3.91 ± 0.20 a	2.05 ± 0.23 a	3.48 ± 0.31 bc	0.21 ± 0.03 bc	1.33 ± 0.05 a	0.70 ± 0.03 bc
20% OF	0.21 ± 0.00 ab	0.20 ± 0.01 a	3.53 ± 0.26 a	2.12 ± 0.56 a	3.61 ± 0.47 bc	0.21 ± 0.02 bc	1.28 ± 0.10 a	0.71 ± 0.02 bc
30% OF	0.22 ± 0.02 ab	0.21 ± 0.01 a	3.94 ± 0.40 a	2.19 ± 0.38 a	3.88 ± 0.12 ab	0.25 ± 0.03 ab	1.32 ± 0.12 a	0.77 ± 0.06 ab
40% OF	0.23 ± 0.01 a	0.21 ± 0.00 a	3.69 ± 0.78 a	2.21 ± 0.48 a	4.29 ± 0.22 a	0.28 ± 0.05 a	1.28 ± 0.46 a	0.83 ± 0.06 a

The THQ value indicated that As, Zn, and Cu contributed the most to the HI, accounting for nearly 86% of the risk for children and adults (Figure 3). The contribution of Zn to the HI was significantly higher in the CK group than in the other treatments. The relative contribution of As to the HI increased with the increasing organic fertilizer substitution ratio. In contrast, the contribution of Cu increased and decreased.

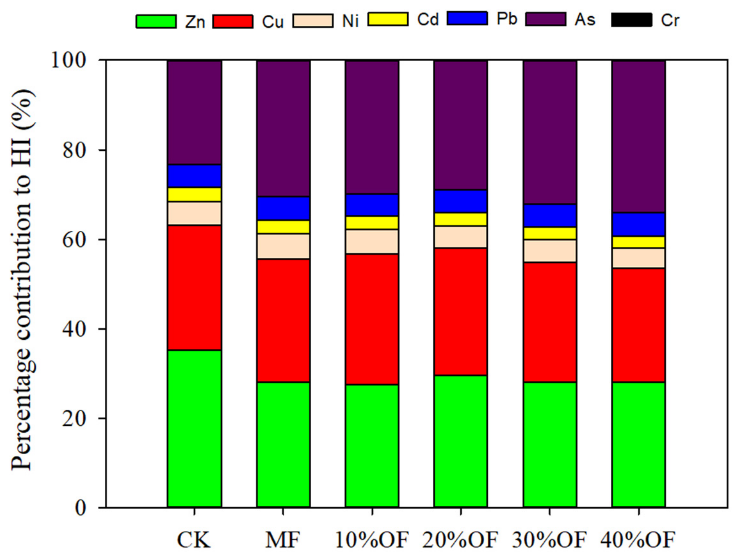

Figure 3. Percentage contributions of heavy metals in wheat grain to the hazard index (HI) for different organic fertilizer substitution ratios.

The TCR values of Cd, Pb, and As were generally greater for adults than for children (Table 4). The mean TCR values for Cd, Pb, and As were 2.20×10^{-5}, 2.10×10^{-7}, and 1.64 $\times$ 10^{-5} for children and 5.62 $\times$ 10^{-5}, 5.37 $\times$ 10^{-7}, and 4.18 $\times$ 10^{-5} for adults, respectively. Organic fertilizer substitution increased the TCR values for Pb and As for children and adults. The TCR values exhibited the largest and most significant increase in the 40% OF treatment compared to the CK group (28.1% and 87.5%, respectively). The total threshold cancer risk (TTCR) of children and adults increased as the substitution ratio increased. The TTCR was significantly higher in the 30% OF and 40% OF treatments than in the CK group.

Table 4. Effect of organic fertilizer substitution on the threshold cancer risk due to the consumption of wheat grains contaminated with heavy metals for children and adults. The values are the means $\pm$ SD of 2 crop years and are not significantly different at p < 0.05 when followed by the same lowercase letter.

Treatments	Threshold Cancer Risk							
	Cd ($\times 10^{-5}$)		Pb ($\times 10^{-7}$)		As ($\times 10^{-5}$)		Total ($\times 10^{-5}$)	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
CK	2.22 $\pm$ 0.15 a	5.67 $\pm$ 0.38 a	1.91 $\pm$ 0.25 c	4.88 $\pm$ 0.63 b	1.14 $\pm$ 0.04 c	2.91 $\pm$ 0.11 c	3.38 $\pm$ 0.16 b	8.63 $\pm$ 0.10 b
MF	2.21 $\pm$ 0.42 a	5.64 $\pm$ 1.07 a	2.00 $\pm$ 0.25 bc	5.10 $\pm$ 0.64 bc	1.55 $\pm$ 0.10 bc	3.97 $\pm$ 0.27 bc	3.78 $\pm$ 0.34 ab	9.66 $\pm$ 0.86 ab
10% OF	2.10 $\pm$ 0.23 a	5.37 $\pm$ 0.60 a	1.99 $\pm$ 0.18 bc	5.07 $\pm$ 0.45 bc	1.57 $\pm$ 0.21 bc	4.01 $\pm$ 0.55 bc	3.69 $\pm$ 0.36 ab	9.43 $\pm$ 0.91 ab
20% OF	2.17 $\pm$ 0.57 a	5.54 $\pm$ 1.46 a	2.06 $\pm$ 0.27 bc	5.26 $\pm$ 0.68 bc	1.55 $\pm$ 0.14 bc	3.96 $\pm$ 0.36 bc	3.74 $\pm$ 0.68 ab	9.5 $\pm$ 1.75 ab
30% OF	2.24 $\pm$ 0.39 a	5.73 $\pm$ 0.99 a	2.21 $\pm$ 0.01 ab	5.65 $\pm$ 0.17 ab	1.87 $\pm$ 0.26 ab	4.78 $\pm$ 0.68 ab	4.14 $\pm$ 0.57 a	10.57 $\pm$ 1.46 a
40% OF	2.26 $\pm$ 0.49 a	5.78 $\pm$ 1.26 a	2.45 $\pm$ 0.13 a	6.26 $\pm$ 0.32 a	2.14 $\pm$ 0.36 a	5.46 $\pm$ 0.92 a	4.42 $\pm$ 0.85 a	11.30 $\pm$ 2.16 a

4. Discussion

4.1. Contents of HMs in Soils and Wheat Grains Following Organic Fertilizer Substitution

Due to the high contents of HMs in organic fertilizers, long-term organic fertilizer substitution will likely increase HM pollution in agricultural soils [31,32]. Commercially available organic fertilizers used in this study have been found to contain more HMs than chemical fertilizers, with higher average contents of Ni, Cd, and As [13]. However, in this study, the total contents of Zn, Cu, Ni, Cd, Pb, As, and Cr in the treated soil were below the Chinese national soil standard limits of 300, 100, 190, 0.6, 170, 25, and 250 mg kg^{-1}, respectively [33]. This finding indicates that even high ratios of organic fertilizer substitution may not cause direct HM pollution in farmlands in the North China Plain. However, in agreement with previous reports [15,34,35], this study found that the addition of organic fertilizer increased Cu, Cd, As, and Pb accumulations in soils; these HMs are classified as priority hazardous HMs to human health. Therefore, the accumulation of Cu, Cd, As, and Pb in agricultural soils and crops may cause serious adverse effects on environmental and human health.

In this study, the contents of Zn, Cu, Ni, Cd, Pb, As, and Cr in wheat grains were below the standard threshold limits of 50, 20, 1000, 0.1, 0.2, 0.5, and 1.0 mg kg^{-1}, respectively [36]. This result indicates that the addition of organic fertilizers for multiple years is not likely to present a direct health risk associated with wheat grain consumption. The wheat grain HM contents in this study were similar to those in wheat grown in calcareous soils in Northwest China [37] but lower than those in two organic fertilizer experiments conducted in the North China Plain [38,39]. The variable results may be due to the dose of organic fertilizer supplementation. It was typically higher (>30 mg ha^{-1}) than the dose used in this study. Furthermore, the different sources of organic fertilizer material and the background HM contents in the initial soil may have contributed to the discrepancies in the results. In this study, high ratios of organic substitution increased the contents of Cu, Pb and As in wheat grains, consistent with the results of Zaccone et al. [40], who found that semolina samples grown using organic fertilizer contained higher Pb contents (94 mg kg $^{-1}$) than those grown using inorganic fertilizer (82 mg kg $^{-1}$). In this study, the grain Zn content was the highest in the CK group, which was attributed to the concentration effect, as reported previously [41]. Although a high ratio of organic fertilizer substitution (40%) had no effect on the total soil Zn content, the grain Zn content was significantly increased, unlike the

Pb and As contents. In addition to the release of endogenous Zn contained in the organic fertilizer material, small molecules of organic chelating substances are released during manure degradation. They combine with Zn and reduce the surface potential of Zn^{2+} in the soil solution, reducing its capacity to be adsorbed by soil colloids [42]. This increases the mobility and uptake of Zn from soils to plant roots, subsequently increasing Zn uptake by wheat grains [43]. In contrast, the accumulation of Cd in soils did not increase the Cd contents in the grains, which can be attributed to the antagonistic effect between Zn and Cd in plant transport systems [44].

4.2. Human Health Risk Assessment for HMs Following Organic Fertilizer Application

The human health risk was determined based on the HM contents and the dietary habits of the exposed population [45]. The THQ and HI values in this study were higher for children than for adults, similar to the results of previous studies [15,46,47], suggesting that children are subjected to a higher health risk from HMs in wheat grains than adults. The THQs of the HMs for children and adults were <1, indicating that the ingestion of wheat grain did not present a non-carcinogenic risk due to the HM contents, even at the highest organic fertilizer substitution ratio. The HMs As and Zn contributed the most to the HI for the exposed population, consistent with the results of Chen et al. [46], who reported that As and Zn had the highest THQ values following long-term application of chemical fertilizer in Hebei Province (China). In this study, the THQ values of Zn and As were significantly higher after the addition of organic fertilizer. Therefore, ingesting wheat grains that have received long-term high doses of organic fertilizer is likely to pose a potential threat to human health. Couto et al. [15] found that the THQ of Zn increased to >1 for children after the application of organic fertilizer to typical Hapludalf soils in southern Brazil in a ten-year period. Therefore, considerable attention should be paid to the health risks caused by As and Zn as a result of wheat consumption, especially in children and vulnerable members of the exposed population. However, Zn is an essential element for humans, and wheat grains should contain at least 40 mg kg^{-1} Zn to meet human nutritional requirements [48]. Therefore, the total Zn intake of local populations should be considered when assessing the health risk posed by Zn ingestion [37]. In this study, organic fertilizer substitution increased the non-carcinogenic risk posed by Pb, similar to previously reported results showing that the THQ of Pb increased after wastewater irrigation in suburban areas in the south Cairo Province (Egypt) [49].

The average HI for children was 1.42 in this study, higher than previously reported HI values for the main wheat-producing regions of China [37]. This result was attributed to the differences between wheat cultivars and initial soil conditions. The non-carcinogenic risk to children was greater than 1 in this study, indicating that the long-term ingestion of wheat grains in this area presents a potential health risk for children. These results were similar to those of previous studies in the North China Plain [38,46]. No significant differences were observed in the HI values among all treatments, except for the 40% OF treatment, indicating that excess organic fertilizer addition presents a non-carcinogenic health risk. The optimal ratio of organic fertilizer substitution was $\leq$30%. However, our risk assessment method has limitations. First, the health risk of consuming wheat flour rather than wheat grains should be determined. The HM contents in wheat flour are only 30% of that in raw grains [50]. Second, accurate health risk assessments should be based on the bioavailability of HMs rather than their contents in grains [51]. Therefore, since the THQ and HI values were based on HM contents in this study, we may have overestimated the HM exposure risk.

Cadmium, As, and Pb have been the focus of much previous research because they are classified as carcinogenic to humans when ingested [52,53]. The acceptable carcinogenic risk posed by individual HMs in wheat grain ranges from 1×10^{-6} to 1×10^{-4} [26]. In this study, adults had a higher carcinogenic risk than children for ingesting wheat grains, similar to the results of previous studies [38,46,52]. The TCR values of Cd, Pb, and As in this study were all within the safe range, indicating that the ingestion of wheat grown under these conditions (organic fertilizer substitution) does not increase the lifetime carcinogenic risk for children or adults. Cadmium and As generally have higher carcinogenic risk levels than Pb [46,52]; therefore, the carcinogenic risk posed by Cd and As ingestion should be the focus in populations with high levels of wheat grain consumption. In this study, a high proportion of organic fertilizer substitution significantly increased the carcinogenic risk posed by As and Pb. Although the carcinogenic risk from Pb increased due to organic fertilizer substitution, the TCR value for Pb in wheat grains was less than the critical value of 1×10^{-6}. Therefore, the increase in human carcinogenic risk caused by organic fertilizer application was primarily attributed to the TCR of As. The TTCR was significantly higher at substitution ratios of 30% and 40% than in the CK group, indicating that excess organic fertilizer addition presents a carcinogenic health risk. The TTCE was not significantly higher at ratios of 10% and 20% than in the CK group, suggesting that moderate application of organic fertilizer may not increase the carcinogenic risk.

4.3. Organic Fertilizer Management in Wheat Production

The substitution of chemical fertilizers with organic fertilizers is widely used in intensive crop farming systems in China due to its synergistic improvement in crop yields, sustainability, and soil fertility [54–56]. However, due to the slow release of nutrients and inefficiency of organic fertilizers, the complete replacement of chemical fertilizers with organic fertilizers can often lead to an immediate lack of nutrient availability, reducing crop yields. A meta-analysis study showed that the substitution of chemical fertilizer with manure did not reduce wheat yields when the substitution ratio was 43% or less [57]. In this study, no significant difference was observed between chemical and organic fertilizer application treatments, suggesting that organic fertilizer substitution could maintain high wheat yields, even at the highest ratio of 40% (Figure 1). In addition, soil fertility improved with organic fertilizer substitution. Soil organic matter content was 3.5–12.0% higher in the organic fertilizer substitution treatments than in the chemical fertilizer treatment (Table S1). The impact of organic fertilizer substitution on HMs in the soil–crop system was quantified based on the health risks to adults and children. Organic substitution at a high ratio significantly increased the total non-carcinogenic health risk (40%) and total carcinogenic risk ($\geq$30%) due to the consumption of wheat grains. In contrast, substitution ratios of 20% or less caused no significant increase in health risk compared to the CK and MF treatments. Therefore, organic fertilizer substitution at a ratio of 20% was optimal in this study, providing a balance between crop yield, soil fertility, and human health risk.

5. Conclusions

The results showed that long-term partial substitution of chemical fertilizers with commercial organic fertilizers did not lead to direct pollution of Zn, Cu, Ni, Cd, Pb, As, and Cr in soil, although different HMs exhibited different accumulation trends. Increasing the organic fertilizer substitution ratio increased the contents of Zn, Cu, Pb, and As in wheat grains. Further analysis indicated that organic fertilizer substitutions at ratios of $\geq$30% significantly increased the total non-carcinogenic and carcinogenic risks of wheat grain. The increase in human carcinogenic risk caused by organic fertilizer substitution was primarily attributed to As accumulation in grain, which requires further attention. Organic fertilizer substitution at a ratio of 20% was optimal for wheat production in this system, maintaining crop yields and improving soil fertility without causing an additional health risk to human health. The results of this study provide valuable guidance for using organic fertilizers in agriculture from a human health risk perspective, helping to improve the safety, sustainability, and economics of crop production in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13122930/s1, Table S1. Heavy metals concentrations in organic fertilizer and chemical fertilizers. Figure S1. Effect of organic fertilizer substitution on soil organic matter. Values are means of triplicate samples and are not significantly different at $p < 0.05$ when followed by the same lowercase letters.

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