Agronomic biofortification of rice and wheat with zinc: A metanalytical study

Biofortificação agronômica de arroz e trigo com zinco: Um estudo metanalítico

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Elisangela Sordi

ORCID: https://orcid.org/0000-0003-3464-8326 University of Passo Fundo, Brazil E-mail: elisangelasordi@hotmail.com Jaqueline Huzar Novakowiski ORCID: https://orcid.org/0000-0003-1037-2396 University of Passo Fundo, Brazil E-mail: jaquelinehuzar@upf.br **Renata Rebesquini** ORCID: https://orcid.org/0000-0001-9659-0022 University of Passo Fundo, Brazil E-mail: renatarebesquini@hotmail.com Tatiana Benedetti ORCID: https://orcid.org0000-0003-3066-8805 University of Passo Fundo, Brazil E-mail: benedettitatiana@hotmail.com **Ivan Ricardo Carvalho** ORCID: https://orcid.org/0000-0001-7947-4900 University Regional Northwestern of the State of Rio Grande do Sul, Brazil E-mail: carvalho.irc@gmail.com **Francine Lautenchleger** ORCID: https://orcid.org/0000-0003-0219-6062 University of the Midwest, Brazil E-mail: francine.lau@gmail.com Edson Campanhola Bortoluzzi ORCID: https://orcid.org/0000-0002-0967-0057 University of Passo Fundo, Brazil E-mail: edsonb@upf.br

Abstract

A diet based on cereals may lack essential mineral elements, among them zinc. The provision of this element in diets can be via supplements, food fortifiers or agronomic biofortification (AB), a practice adopted on a farmer scale. It was carried out different studies in countries with specific local conditions. The meta-analysis allows combining quantitative results from different studies, providing a synthesis of results with high reliability. The objective of this work was to analyze the response of rice (*Oryza* spp.) and wheat (*Triticum* spp.) to fertilization with zinc in terms of grain yield and accumulation of this nutrient in the grain. We carried out a systematic review where 16 scientific articles from the last five years were selected, and 179 studies fitted the established criteria. The effect size for Zn application via leaf or soil in rice and wheat compared to the control was calculated using the natural logarithm (lnR) between the ratio of the treatment group and the control group for both variables. Agronomic biofortification with Zn increases grain yield (7%) and zinc content in grains (53%). These results depend on plant species and the fertilization way (via the soil or foliar spray). Agronomic Biofortification may be a valuable strategy to combat malnutrition and guarantee food sovereignty.

Keywords: Productivity; Zinc content in grains; Food sovereignty; Nutrients; Hunger.

Resumo

Uma dieta baseada em cereais pode resultar em carência de elementos minerais essenciais, entre eles o zinco (Zn) A disponibilização desse elemento nas dietas pode ser por meio de suplementos, fortificantes alimentares ou por meio da biofortificação agronômica (AB), prática que pode ser adotada por agricultores. Diferentes estudos são realizados em países com condições locais específicas e a metanálise permite combinar resultados quantitativos de diferentes estudos, proporcionando uma síntese de resultados com alta confiabilidade. O objetivo deste trabalho foi analisar a resposta do arroz (*Oryza* spp.) E do trigo (*Triticum* spp.) À fertilização com zinco em relação à produtividade de grãos e acúmulo desse nutriente no grão. Foi realizada uma revisão sistemática e selecionados 16 artigos científicos dos últimos 5 anos. Destes, 179 estudos foram obtidos. O tamanho do efeito da aplicação de Zn via folha e/ou solo em arroz e trigo em relação ao controle sem aplicação foi calculado por meio do logaritmo natural (lnR) entre a razão do grupo tratamento e do grupo controle para ambas as variáveis. A biofortificação agronômica com Zn aumenta o

rendimento de grãos (7%) e o teor de zinco nos grãos (53%), dependendo da espécie da planta e da forma de fertilização (via solo ou por pulverização foliar). A biofortificação agronômica pode ser uma estratégia para combater a desnutrição e garantir a soberania alimentar.

Palavras-chave: Produtividade; Conteúdo de Zinco nos grãos; Soberania alimentar; Nutrientes; Fome.

Resumen

Una dieta a base de cereales puede resultar en una falta de elementos minerales esenciales, incluido el zinc (Zn). La disponibilidad de este elemento en las dietas puede ser a través de suplementos, fortificantes alimentarios o mediante biofortificación agronómica (AB), práctica que puede ser adoptada por agricultores. Se realizan diferentes estudios en países con condiciones locales específicas y el metaanálisis permite combinar resultados cuantitativos de diferentes estudios, proporcionando una síntesis de resultados con alta confiabilidad. El objetivo de este trabajo fue analizar la respuesta del arroz (*Oryza* spp.) Y el trigo (*Triticum* spp.) A la fertilización con zinc en relación al rendimiento de grano y la acumulación de este nutriente en el grano. Se realizó una revisión sistemática y se seleccionaron 16 artículos científicos de los últimos 5 años. De estos, se obtuvieron 179 estudios. El tamaño del efecto de la aplicación de Zn vía hoja y/o suelo en arroz y trigo en relación al control sin aplicación se calculó utilizando el logaritmo natural (lnR) entre la razón del grupo de tratamiento y el grupo de control para ambas variables. La biofortificación con Zn aumenta el rendimiento de grano (7%) y el contenido de zinc en los granos (53%), dependiendo de la especie de la planta y la forma de fertilización (vía suelo o por aspersión foliar). La biofortificación es una estrategia para combatir la desnutrición y otorga soberanía alimentaria.

Palabras clave: Productividad; Contenido de zinc in grano; Soberanía alimentaria; Nutrientes; Hambre.

1. Introduction

Two-thirds of the world's population lack essential mineral elements (White & Broadley, 2009), causing "hidden hunger" when referring to vitamins and micronutrients (Stein et al., 2007), most commonly linked to iron, A vitamin and iodine deficiencies. However, it can also be associated with zinc, calcium and selenium. The origins of these deficiencies range from poor diet, with little quantity and diversity of food (Allen et al., 2006), to factors related to plant growth, such as the adoption of more intensive cultivation practices. The lack of use of organic fertilizers and the unbalanced plant nutrition (Kumar et al., 2016), the access to food, the consumption of safe and healthy food and distribution of wealth, whose consequence is the lack of food security (Bliska et al., 2009). In this sense, it is necessary to encourage local food production that does not come from transgenic and is free of pesticides, preferably from agroecological crops (Silva, 2020).

Among the elements, zinc (Zn) stands out, an essential element for animals, humans and plants (Hafeez et al., 2013). In living organisms, this element is present in more than 300 enzymes, and many of them need this element to perform their function. Among them can be highlighted hydrolases transfers and oxidoreductases. Thus, these enzymes are required to metabolize proteins, carbohydrates, lipids, nucleic acids, and the command of genetic transcription (Okigami, 1996). Its deficiency affects mainly women and children (Who, 2002). It can interfere in growth and cause diarrhea and pneumonia in children under five, contributing significantly to the increase in infant mortality worldwide (Stein et al., 2005), mainly where the diet is based on cereals (Montoya et al., 2020).

Supplement the diet with medications or fortify the food when ready can cause an unpleasant taste when added to certain foods (Allen et al., 2006; Walker & Black, 2014). The other possibility is still biofortification, which can increase the soil levels of any element resulting in increases in plant grains without changing the food taste. The plants may translocate a surplus of minerals from the soil to seeds through agronomic practices, more precisely, for the edible part of the plant (Boius, 2018). The accumulation of zinc in grains occurs in different physiological ways. The main form is absorption by the roots, translocation to tillers and the remobilization of this element inside plants (Erenoglu et al., 2011).

However, in soil, only a tiny amount of this element remains available to vegetables in the form that it can be absorbed. Among the factors that affect this availability, there are the reactions that occur in soil, which fix elements to the soil constituents (i.e., organic matter and minerals), phosphates levels that trace competition between elements (He et al., 2021) can be highlighted (Brasil Sobrinho et al., 1979). Thus, the application of Zn may also be necessary for crops to grow healthier and

increase yields. We can apply the Zn via the soil or foliar spraying (Hafeez et al., 2013).

The main world food crops, such as rice and wheat, are part of this process (Li et al., 2016; Mangueze et al., 2018) since rice feeds more than half of the world population and is a staple food in most of Asia (Bashir et al., 2013).

On the other hand, malnutrition mainly affects countries in which cereals are part of the essential diet (Montoya et al., 2020) and represents a humanitarian challenge (Rashid et al., 2019), as cereal grains have minimal concentrations of Zn in the grains when grown on Zn-deficient soils (Zaman et al., 2018). In this context, numerous studies indicate the possibility of raising nutrient concentrations and ensure a high yield using agronomic fortification (AB) (Souza et al., 2014). With biofortification, higher productivity of crops combined with a higher zinc content in the grain is expected (Chattha et al., 2017). However, a bibliographic review analyzing the effectiveness of AB of Zn on the main crop is still scarce. In this sense, the use of meta-analysis may improve AB practice's understanding. In addition, we seek to answer the following question: When we add Zn in rice and wheat grains, through AB practice, what occurs whit productivity and zinc content in grain?

The meta-analysis technique summarizes several independent studies and statistically combines the effect size and modelling the effect sizes with the characteristics studied (Cheung & Vijayakumar, 2016). This approach combines quantitative results from different studies (i.e. regions, grow, technics, crops, elements supply), providing a synthesis of results with high reliability (Zeffa et al., 2020).

We hypothesize that the application of Zn via soil or leaf influences the accumulation of this mineral in the grain without compromising grain productivity. Therefore, the aim of this meta-analysis was (1) to analyze the productivity response of rice and wheat to fertilization with Zn; (2) estimate Zn accumulation in the grains of the cereals.

2. Methodology

Classification of the research (meta-analysis)

Regarding the objectives, the research is characterized as descriptive-exploratory since it uses the existing literature to describe the effect of zinc biofortification in wheat and rice. For the technical procedures, we considered bibliographic. As a source of information, we used scientific articles (Silveira & Córdova, 2009).

Data selection and collection

A systematic review from scientific articles was carried out (Figure 1). In the Science Direct database, we used "biofortification AND zinc" as the search term. We carried out this search on May 15, 2020, in manuscripts published from 2015 to 2020. This period concentrates a high number of articles with the studied variables and the necessary dispersion measures. Additionally, on May 16, 2020, the search was completed in ResearchGate, where we found more than 60 articles.



Figure 1 - Flowchart of the study selection process.



Reference	Author	Journal
1	Mangueze et al.	Journal of Cereal Science, 82, 34–41, 2018
2	Wang et al.	Field Crops Research, 184, 58–64, 2015
3	Biswas et al.	Current Plant Biology, 16, 22–26, 2018
4	Jaksomsak et al.	Journal of Cereal Science, 79, 6-12, 2018
5	Li et al.	Field Crops Research, 187, 135–14, 2016
6	Liu et al.	Environmental Pollution, 257, 1-8, 2020
7	Amanullah & Inamullah.	SpringerPlus, 5, 1-9, 2016
8	Singh & Shivay	Biological Agriculture & Horticulture, 29, 271–287, 2013
9	Nahar et al.	Asian Soil Research Journal, 3, 1-6, 2020
10	Das et al.	Acta Agrobotanica, 73, 1-13, 2020
11	Chattha et al.	Frontiers in Plant Science, 8, 1-8, 2017
12	Biswas et al.	International Journal of Plant & Soil Science, 8, 203-217, 2015
13	Ramzan et al.	International Journal of Plant Production, 1-10, 2020
14	Gomez-Coronado et al.	Plant Soil, 401, 331-346, 2016
15	Montoya et al.	Journal of Plant Nutrition and Soil Science, 1-11, 2020
16	Zou et al. et al.	Journal of Agricultural and Food Chemistry, 67, 8096-8106, 2019

Table 1	- List	of work	s used	in the	meta-analy	vsis.
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Source: Authors.

Effect measurement and meta-analysis

We obtained the effect measure from the natural logarithm (lnR) of the ratio between the productivity of the treatment with application of Zn and the control without Zn application (Hedges et al., 1999; Hou et al., 2020; Lajeunesse, 2011;

Lehmann & Rillig, 2015). We used the following equation:

$$lnR = \ln(\frac{\bar{x}_{Trat}}{\bar{x}_{Contr}})$$
Equation 1

Where: \bar{x}_{Treat} the mean of the treatment response variable with the application of the micronutrient zinc and \bar{x}_{contr} the mean of the control treatment response variable without applying the nutrient.

The studies reported different dispersion measures, including coefficient of variation (CV), standard deviation (SD), least significant difference (LSD) and mean standard error (SE). The dispersion measures were standardized for the standard deviation. It is a great challenge to carry out a meta-analysis, as many studies do not present these values (Lajeunesse, 2011), so it is necessary to convert other dispersion measures (Higgins et al., 2008; Hou et al., 2020; Lehmann & Rillig, 2015; Silva et al., 2017; Tupich et al., 2017).

We performed the meta-analysis using a random-effects model (Viechtbauer, 2010) as it allows the true effect to varying from study to study (Borenstein et al., 2009), and the restricted maximum likelihood estimate (REML) was used to estimate the variation between studies (Gao & Carmel, 2020).

We realized the meta-analysis in the metafor package (Viechtbauer, 2010) in the RStudio program (R Core Team, 2020). For a more practical interpretation of the final result, we transformed the lnR value into a response percentage (%) (Dai et al., 2020; Hou et al., 2020; Zeffa et al., 2018):

$$R = (e^{\ln RR} x 100) - 100$$
 Equation 2

Heterogeneity

We assessed the heterogeneity with several tests, including the Q test (Cochran, 1954), which assesses the heterogeneity of the collective effect estimates and the individual effects differ significantly from the common effect (Cohen et al., 2015). Another simple way to assess heterogeneity is through the I2 index, which was used to verify the variation caused by real changes between studies and assess the heterogeneity between effect sizes (Borenstein et al., 2009) and its significance (Dai et al., 2020).

Publication bias

The verification of the existence of publication bias refers to the fact that research that presents statistically significant results is more likely to be published than works with null or non-significant results. This fact may compromise the quality of the meta-analysis and other studies that depend on published data. Besides, there is a risk of emphasizing any significant differences whose result does not represent the real effect of the studied treatment (Easterbrook et al., 1991)

We performed the funnel plot to verify the publication bias and the robustness of the data (Borenstein et al., 2009).

Effect moderators

We carried out the classification of the studies in effect moderators according to the crop and the mode of zinc application. We created subgroups to make it possible to expand the knowledge about the meta-analysis (Lehmann & Rillig, 2015; Tupich et al., 2017). The criteria used to allocate to groups were: a) studies with zinc application in wheat via soil; b) studies with zinc application in wheat via foliar fertilization; d) studies with zinc application in rice via soil; b) studies with zinc application in rice via foliar fertilization; e) studies with zinc application in wheat via fertilization; e) studies with zinc application in the soil and the leaves.

3. Results and Discussion

Descriptive analysis

We analyzed 161 studies for the variable productivity, while the zinc content in the grain we had 175 studies. The studies come from several countries (Figure 2 a); however, in some publication, the experiments were conducted in more than one country. Most of the experiments were conducted in countries on the Asian continent (Figure 2b), approximately 70%. The Asian studies are essential because rice and wheat have been cultivated in the continent for over 1000 years (Amanullah & Inamullah, 2016), and zinc is one of the main limitations of rice productivity (Quijano-Guerta et al. 2002).

In addition, in Asia, rice and wheat are stapled foods, and a considered portion of the population ingests inadequate amounts of Zn, in which biofortification has a fundamental role in saving lives, prevent morbidities and assist the adequate allocation of health sector resources for malnutrition (Stein et al., 2007). However, studies in a broader approach should be made, such as verifying agricultural, environmental, economic, and social aspects (Gunaratna et al., 2010).

Figure 2 - Frequency of publications (A) and the number of studies used for the meta-analysis (B) for the country.







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Source: Authors.

Effect measure and meta-analysis

Heterogeneity

The values of the meta-analytical estimate (Chart 1) are fundamental to determine heterogeneity, as the I2 test demonstrated heterogeneity with an amplitude of up to 99.99%. These studies show high heterogeneity between the effect measures (Zeffa et al., 2018). However, only the subgroups zinc application via leaf and soil for the productivity variable did not show heterogeneity (0%).

Thus, the description of the extent of heterogeneity between studies is of paramount importance because this may interfere with the study's conclusions (Higgins & Thompson, 2002). As this heterogeneity was high, random-effects models were used for the meta-analysis as preconized by Zeffa et al. (2020). However, values equal to 0% reveal homogeneity between the studies. Higher values portray the amplitude of the heterogeneity, where high values may indicate an inconsistency between the results of the investigations (Martinez, 2007). I2 values are considered: low with 25%, moderate with 50% and high with 75% heterogeneity (Higgins et al., 2003).

The Q statistic evaluates the null hypothesis in which all studies are examining the same effect. However, when this is significant, it reveals that the studies do not have a common effect size (Quintana, 2015). The total number of studies shows the number of effects as independent samples, most of them often indicate the number of studies, and the p-value indicates statistical significance (Afshardoost & Eshaghi, 2020).

Chart 1 -	- Meta-analytic	estimate,	probability	value,	I2 index	and Q	value	of studies	s using	zinc	biofortification	to ¢	evaluate
productivi	ity and content of	of the elen	nent in the g	rain.									

Variable	Outcome/analysis	No of	Heterogeneit	I ² % (95%		
		studies	Q	df	Р	confidence
						interval)
	All studies	161	12011.56	160	0.01	98.57
Productivity		8	0.46	7	1.00	0
(t/ha)	Application via leaf Oryza sp.					
	Application via soil <i>Oryza</i> sp.	5	0.67	4	0.96	0
	Application via leaf Triticum sp.	62	1644.53	61	< 0.01	92.24
	Application via soil <i>Triticum</i> sp.	59	4148.2	58	< 0.01	98.75%
	Application via soil and leaf <i>Triticum</i> sp.	27	491.86	26	< 0.01	98.60
	All studies	175	507276.74	174	< 0.01	99.99%
Zinc content		8	74.22	7	<0.01	91.99%
in the grain	Application via leaf Oryza sp.					
	Application via soil Oryza sp.	8	33.65	7	< 0.01	73.41%
	Application via leaf <i>Triticum</i> sp.	66	1436.81	67	< 0.01	98.26%
	Application via soil and leaf <i>Triticum</i> sp.	63	338805.51	62	< 0.01	99.99%
	Application via soil <i>Triticum</i> sp.	25	218.66	24	<0.01	88.18%

Source: Authors.

Publication bias

In general, data dispersion was relatively symmetrical (Figure 3), which shows a weak publication bias (Zeffa et al.,

2018) for the productivity variable and zinc content in the grain. Another critical point is that these results may significantly impact the meta-analysis (Dai et al., 2020).



Figure 3 - Funnel plot for productivity (A) and zinc content in grains (B).

Source: Authors.

Publication bias indicates the phenomenon by which studies are more likely to be published when they have more substantial effect sizes. A funnel chart is a graphical tool used to check for possible publication bias in meta-analyses (Quintana, 2015). The funnel graph shows the relationship between the residuals of the effect measures on the horizontal axis and the average standard deviation on the vertical axis. In addition, a vertical line shows an estimate according to the model used and an apparent confidence interval of ± 1.96 of standard error in the vertical (Viechtbauer, 2010).

Effect moderators

The application of zinc affects productivity and zinc content in the grain (Figure 4). It observed an increase in the average productivity in wheat of 11% when the zinc was applied via soil. However, when the nutrient was applied via leaf, it did not affect or increase productivity by only 2%. When this application was via soil and leaf, the amplitude of productivity varies widely, reducing productivity by 35% or increasing up to 75%. In rice, the productivity increased when the zinc was applied via soil by 41%. However, they presented no effect when applied via leaf (Figure 4A). In the confidence interval, the lower and upper limits show the range within which the real effect is found (Afshardoost & Eshaghi, 2020).

For zinc content in the grain, the behaviour of the moderating variables was different. In the wheat, there was an increase of 25% of Zn content in the grain when fertilizers containing Zn were applied to the soil, whereas when applied via leaf, it was 77%. When zinc applied in both ways, we observed a wide range of effect, from a reduction of 61% until an increase of 11 times. On the other hand, in rice plants, when Zn was applied via leaf, it showed an increase of only 3%. When it applied via soil, extensive results were observed (Figure 4B).

Figure 4 - Forest plot for the meta-analysis of the effect of zinc application on productivity (A) and zinc content in the grain (B), with corresponding 95% confidence intervals in studies with moderating variables and based on a random-effects model.



Forest plot Estimate [95% CI] WS 15.27% 0.23 [0.16, 0.31] WL 20.57% 0.57 [0.50, 0.63] RS 64.02% 0.07 [0.03, 0.10] RL 0.11% 0.22 [-0.70, 1.13] WSL 0.03% 0.77 [-0.95, 2.49] 3 -1 0 1 2 Effect size

WS: application of zinc carried out via soil in wheat; WL: application of zinc performed via leaf in wheat; RS: application of zinc carried out via soil in rice; RL: application of zinc performed via leaf in rice; RSL: application of zinc carried out via soil and leaf on wheat. Source: Authors

In addition to the crop and type of application, the dose applied also can influences the analyzed variables, generating different results, beneficial or not (Biswas et al., 2015). We can highlight other factors with strong influence on the results of biofortification, among them: genetic material, time of application, number of applications, application stage, environmental conditions, management practices and soil fertility (Nakandalage et al., 2016; Rao et al., 2020; Rashid et al., 2019; Saha et al., 2017). Approximately 50% of the soils cultivated with wheat in the world have low available Zn for plants (Cakmak & Kutman, 2018).

Zinc is an essential element for all biological systems in animals and plants. The low availability of zinc can also cause a decrease in yield and zinc content (Chattha et al., 2017). The increase in productivity due to Zn fertilization may occur because of the increase in chlorophyll content, with a consequent beneficial effect on photosynthesis, in the synthesis of substances that act on growth, metabolites, and metabolic activities, causing improvement in plant growth and development (Kumar & Bohra, 2014). In addition, zinc acts in the biosynthesis of indolacetic acid and in the initiation of propagating parts (Das et al., 2020).

Other factors as the increase in the number of reproductive tillers can be highlighted. This element has a crucial role in the biochemical, physiological, and metabolic processes. These processes accelerate the partition of the assimilation of zinc into the flowers, increase of leaf area, number of grains per ear, and the mass of thousand grain (Jalal et al., 2020; Ramzan et al., 2020).

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The AB is one of the most promising practices to increase the concentration of micronutrients in grains (Ramzan et al., 2020). The AB has emerged as one of the fundamental tools to combat micronutrient malnutrition worldwide (Singh & Prasad, 2014). Using this approach, the concentration of zinc in grain can increase by 17% compared to the control treatments, even when low Zn doses are applied (4.5 kg Zn/ha) (Das et al., 2020). Another benefit of biofortification with zinc is reducing Cadmium concentration and the associated health risks of this heavy metal due to the competition between these elements (Cakmak & Kutman, 2018).

A study to verify the use of different wheat lines found that when cultivated with additional Zn supplies, 50% of them showed an increase in grain production of up to 100% and the concentration of this element in the grains. The application of this fertilization was beneficial for all strains, providing an average increase of 84% (Souza et al., 2014). In addition, the combined use with another element such as selenium can be beneficial since it is also essential for humans, does not cause harmful interference in productivity and contributes synergistically to the increase of selenium, zinc and iron in the grains (Souza et al., 2014). The competition among elements should be considered in the AB approach because it may affect other essential nutrients, such as P (He et al., 2021).

In addition, studies must be carried out to check the bioavailability of zinc in the food after its preparation. In addition to assessing how much of the element will be available for use in the metabolic processes. This disponibility is determined by the amount absorbed by the gastrointestinal tract. However, this absorption is fundamental in the bioavailability of zinc (King, 2002). However, some compounds such as phytate and phenolic compounds may interfere and limit the absorption of zinc (Cakmak & Kutman, 2018) and depend on the magnitude of industrial processing (Saha et al., 2017).

Human health and the hungry problems will not be solved with an isolated technic or one alone approach, such as AB. These issues are systemic and chronic and involve many political, social, and local factors. From an agronomic perspective, enrichment of food grain with nutrients through adequate soil fertilization is a relatively easy way, which can alleviate human issues.

4. Conclusion

Our meta-analysis highlights the positive effect of zinc biofortification on rice and wheat crops. This technique can increase the concentration of this element in food products and improve its supply. Furthermore, it can increase grain productivity by 7% and zinc content in grains by 53%, which can be considered a strategy to combat human malnutrition and food sovereignty.

Nutrient management is significant for increasing crop productivity in cereal-based systems. However, further studies are needed to adjust the dose to achieve adequate accumulation of Zn and to avoid overuse, which can cause a significant risk to both health due to food consumption and soil environment.

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