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Review

Agronomic Biofortification of Zinc in Rice for Diminishing Malnutrition in South Asia

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Abstract: Zinc (Zn) is increasingly recognized as an essential trace element in the human diet that mediates a plethora of health conditions, including immune responses to infectious diseases. Interestingly, the geographical distribution of human dietary Zn deficiency overlaps with soil Zn deficiency. In South Asia, Zn malnutrition is high due to excessive consumption of rice with low Zn content. Interventions such as dietary diversification, food fortification, supplementation, and biofortification are followed to address Zn malnutrition. Among these, Zn biofortification of rice is the most encouraging, cost-effective, and sustainable for South Asia. Biofortification through conventional breeding and transgenic approaches has been achieved in cereals; however, if the soil is deficient in Zn, then these approaches are not advantageous. Therefore, in this article, we review strategies for enhancing the Zn concentration of rice through agronomic biofortification such as timing, dose, and method of Zn fertilizer application, and how nitrogen and phosphorus application as well as crop establishment methods influence Zn concentration in rice. We also propose data-driven Zn recommendations to anticipate crop responses to Zn fertilization and targeted policies that support agronomic biofortification in regions where crop responses to Zn fertilizer are high.

Keywords: rice; Zn deficiency; agronomic biofortification; crop management; grain zinc; Zn sulfate; Zn-coated urea; Zn application; data-driven recommendations; digital soil mapping; policy options

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1. The Actuality of the Zinc Deficiency Problem

Zinc (Zn) deficiency in humans has emerged as a pressing global concern and requires immediate attention [1,2]. Zn plays a crucial role in the maintenance of immune health, bone mineralization, the growth of body tissues, sperm production and fertility, cell division, and protein and DNA synthesis [3–6]. In addition, Zn has strong antiviral and antibacterial effects on the human body, and it is involved in improving immune function against various viruses, including coronavirus [6]. Hence, adequate Zn intake is imperative for maintaining good health in humans. In general, micronutrient deficiencies in humans are not always acutely visible (hidden hunger), but they affect physical and mental development, the immune system, and human potential to a great extent [7]. Approximately 17.3% of the global population and 30% of South Asians suffer from inadequate Zn intake [2]. An additional 175 million people globally, including 63 million in South Asia, are expected to become Zn deficient by 2050 [8]. Moreover, about 16 million of the global disability-adjusted life years are caused by Zn deficiency [9,10]. Climate change is also expected to further worsen the situation by increasing the number of Zn-deficient people. Studies have shown that carbon dioxide emissions resulted in poor uptake of Zn from the soil by plants [11] and decreased the Zn concentration in cereal grains [12].

The rate of Zn deficiency remained the same over the years between 1995–2005 in South Asia (Figure 1) [2]. Hence, it is essential to address the Zn deficiency problem to alleviate malnutrition in South Asia. The fundamental cause of Zn malnutrition in South Asia is the consumption of rice and wheat, which are low in Zn content, especially when grown in Zn-deficient soils. The rice-wheat system covers about 24 million hectares in India, China, Pakistan, Nepal, and Bangladesh, and Zn deficiency is widespread in the rice-wheat areas of all these countries [13]. Additionally, in South Asia, rice provides more than 55% of the daily calorie intake in rural areas, where Zn deficiency is high [14,15]. For instance, most people in rural areas of India rely on rice as their major source of energy because of widespread poverty, high food prices, and cultural preferences [16,17]. The EAT-Lancet Commission recommended that about 33% of the total daily calorie intake should come from cereals. However, the average Indian household obtains almost half (47%) of its total calories from cereals and 70% in the case of Indian rural households [18]. Similarly, in Bangladesh, rice alone provides 49–69% of the dietary Zn to children and women [19]. Moreover, the Zn content in white or polished rice should be 28–30 mg kg^{-1} to meet the Zn requirement, but it is lower (13–18 mg Zn kg^{-1}) because of many factors, including genetics, low uptake of Zn from the soil, and paddy grain processing [20–22]. As a result, the consumption of excess polished rice coupled with a low concentration of Zn has led to malnutrition in rice-eating communities in South Asia [20,22,23]. Interestingly, there is a strong relationship between the health of the soil, plants, and humans [20,22]. The geographical distribution of Zn deficiency in the human population overlaps with the distribution of Zn-deficient soil and the low socioeconomic status of the population [24]. For example, the link between Zn-deficient soil and Zn-deficient humans is especially prevalent in India, which relies on rice as the main source of calorie intake [25,26]. Forty-eight million soil samples collected and analyzed under the Soil Health Card (SHC) scheme in India revealed that 37% of the soils are Zn deficient (Figure 2). Similarly, 34%, 32%, 29%, 16%, and 5% of Indian soils are deficient in iron, boron, sulfur, manganese, and copper, respectively (Figure 2). Furthermore, soil Zn deficiency varies across states, with 66% deficiency in Bihar, 58% in Karnataka, 51% in Maharashtra, 32% in Andhra Pradesh, 30% in Uttar Pradesh, and 25% in Haryana (SHC scheme). The main reason for soil Zn deficiency is the intensification of cropping systems by rice-rice or rice-wheat and the lack of application of organic inputs over the past four decades. Overall these practices have resulted in soil Zn deficiency across India [27].

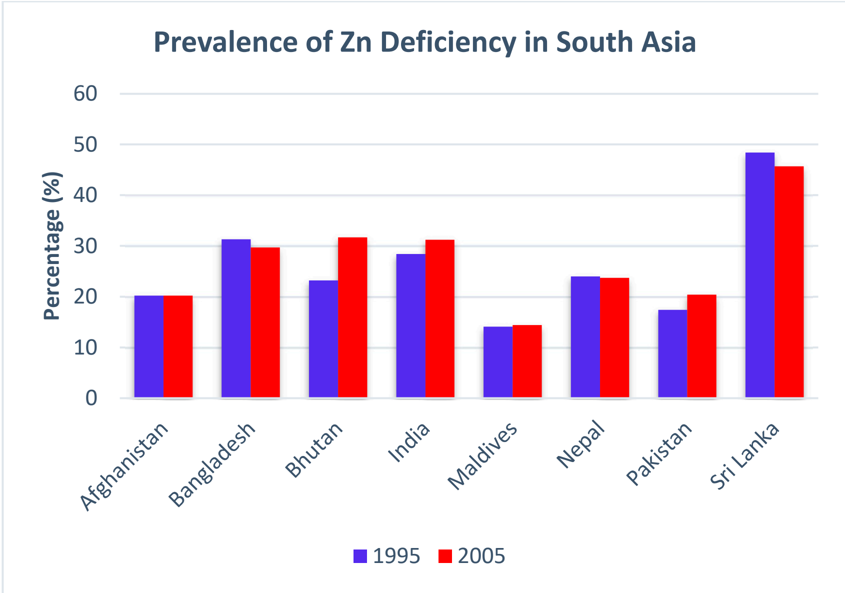


Figure 1. Prevalence of Zn deficiency in South Asia. This figure shows the percentage of Zn deficiency in the human population for 1995 and 2005 in the South Asian countries Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka. The data show that there has been no improvement in Zn deficiency status in the human population over the years. Zn deficiency either remained static or increased. Source: https://ourworldindata.org/micronutrient-deficiency, accessed on 22 April 2022.

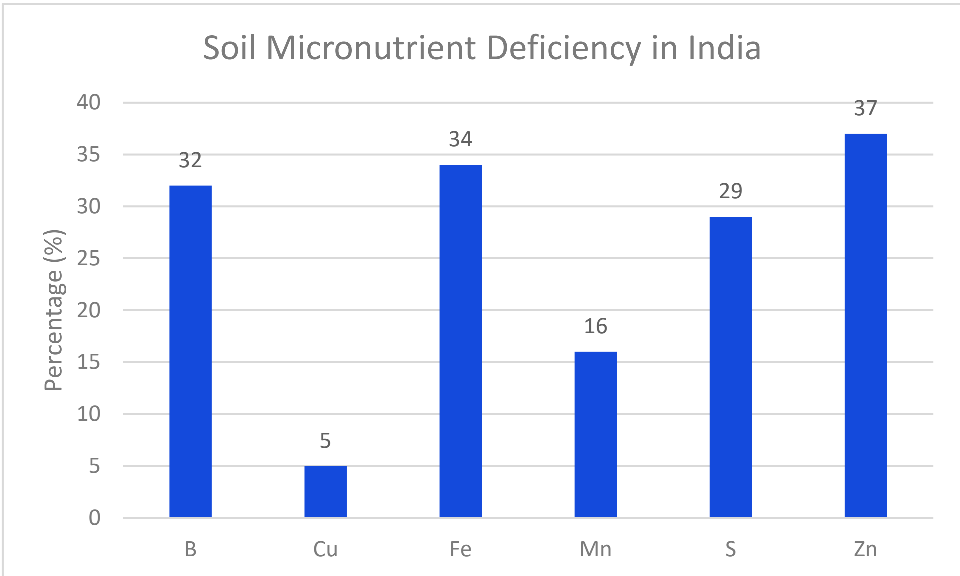


Figure 2. Prevalence of soil Zn deficiency in India. This figure shows the percentage of soils deficient in micronutrients such as boron (B), copper (Cu), iron (Fe), manganese (Mn), sulfur (S), and zinc (Zn) in India based on 48 million soil samples. Of the samples analyzed, Zn deficiency is predominant in Indian soils. Source: https://www.soilhealth.dac.gov.in/, accessed on 22 April 2022 [28].

Zn deficiency in humans is being addressed by food fortification by adding Zn to foods such as milk, bread, flour, and salt; supplementation such as oral use of Zn tablets, capsules, etc.; and dietary diversification by consuming foods with the highest concentration of Zn such as animal meat, fish, eggs, pulses, and nuts. Nonetheless, these interventions require infrastructure, purchasing power, access to markets, and health care centers, and have been more successful among urban populations than among the rural poor [29].

Therefore, biofortification of rice with Zn is an alternative strategy for alleviating Zn malnutrition in rural South Asia [12,30]. Zn biofortification is the process of increasing the Zn concentration during plant growth in a staple food [31]. This can be achieved by two main approaches: (1) conventional breeding [32,33] or genetic biofortification/transgenic lines [34] and (2) agronomic biofortification [35]. Many articles have highlighted the importance of biofortification in cereals to alleviate malnutrition [12,30]. However, in this review, first, we focused on Zn biofortification through genetic and agronomic strategies and the limitations associated with these strategies (Section 2). Next, we proposed the way forward with data-driven Zn recommendations using machine learning techniques to address soil and crop management variability at scale based on our ongoing project through Cereal Systems Initiative for South Asia (CSISA, Section 3), and finally, the last part discusses the policy options for Zn biofortification in India (Section 4).

2. Zn Biofortification

Zn concentrations in rice can be substantially increased by two approaches: (1) conventional breeding and genetic/transgenic methods and (2) agronomic biofortification [36]. Ultimately, both approaches complement each other in increasing Zn concentration in any staple crop for alleviating malnutrition in humans.

2.1. Conventional Breeding and Genetic/Transgenic Zn Biofortification

Zn biofortification in rice can be done by conventional breeding, marker-driven molecular breeding, or genetic engineering [37]. Conventional breeding is a seed-based approach in which the germplasm is enriched with specific nutrients, micronutrients, proteins, and amino acids [38], whereas, in the transgenic-based approach, the gene involved in a particular trait is identified and used to enhance nutrient and micronutrient concentrations [39]. Biofortification through conventional breeding is accomplished by using the genetic variations that are present in the gene pool in staple crops and their wild relatives [38]. In this method, desirable traits such as high yield and nutritional value are obtained by crossing parental lines over generations [40]. However, modern improved varieties and hybrids that are bred for higher grain yield have less Zn concentration (13 to 18 mg kg^{-1}) because of dilution or the genetic effect [41–43]. This Zn content is comparatively less than the targeted concentration of 28 to 30 mg kg^{-1} in polished rice grains, which is essential to reach 30% of the human estimated average requirement [31].

Owing to their eco-friendly and sustainable nature, various international and national organizations have established several breeding programs to increase the nutritional value of rice to alleviate malnutrition in developing countries (www.harvestplus.org, accessed on 22 April 2022). For instance, the HarvestPlus program has defined a target Zn concentration for brown and polished rice as 30 mg kg^{-1} and 28 mg kg^{-1}, respectively [12]. To achieve the desired concentration of Zn in the edible portion of rice, various old varieties were screened for increased Zn concentration in rice grain through conventional plant breeding techniques [44]. The International Rice Research Institute (IRRI, Philippines) carried out germplasm screening in rice varieties and observed large variations in Zn concentration among these varieties. Of the 1138 brown rice samples screened, Zn concentration varied from 13.5 to 58.4 mg kg^{-1} [45]. HarvestPlus developed the world's first Zn-enriched rice varieties such as BRRI dhan62, BRRI dhan72, and BRRI dhan64 (25 mg Zn kg^{-1}) in 2013, and these were released by the Bangladesh Rice Research Institute (BRRI) [40]. Recently, BRRI 84, Zn-fortified rice (27.6 mg Zn kg^{-1}) suitable for the dry season, was also released for cultivation in Bangladesh, and it took 13 years to develop this variety [46]. In addition, under conventional breeding, Zn-enriched rice varieties such as DRR 45, CGZR-1, Zn-Rice, and Swarna Shakti Dhan containing 23 to 27 mg Zn kg^{-1} were released from India [40].

Although conventional breeding has proven to be successful, cost-effective, and sustainable in the long run, the major drawback of using this method is that it depends on alleles available in the gene pool. If genetic variability is limited, then this method is not advantageous. Similarly, scaling of these biofortified varieties becomes a problem because of the lack of access, the trade-off between yield and micronutrient concentration in grain, and low acceptance among farmers [42,43]. For instance, the adoption rate (1.96%) for biofortified Zn rice varieties was very low in Bangladesh in 2018 [47,48]. Moreover, conventional breeding is time-consuming [46], requires ample resources, and depends on environmental factors such as soil type, pH, and temperature. To overcome the limitations of this approach, several transgenic lines have been established [49,50].

If genetic diversity is unavailable or limited in crops, then the plant breeding approach is not achievable. Alternatively, biofortification by genetic transformation can be followed to enhance the nutritional value of staple crops [39]. In this transgenic-based approach, the gene responsible for a particular trait is identified, engineered, and then used for targeting crops [51]. Unlike conventional breeding methods, transgenic lines are not dependent on gene pools. In addition, these are sustainable and cost-effective in the long run. Golden Rice rich in beta-carotene [49,50] and high iron rice [52,53] are examples of biofortified rice using genetic engineering. Studies have shown that Zn concentration in rice grains can be increased by 6–8 mg kg^{-1} by conventional breeding [40], whereas it can be increased by 15–30 mg kg^{-1} by transgenic rice lines [54]. Similarly, it was shown that, under iron and Zn deficiencies, both iron and Zn content were increased in transgenic rice by overexpressing *OsIRT1* [34]. Researchers also developed transgenic rice lines containing an enhanced expression of the barley *IDS3* gene that produced polished rice with high iron (1.4-fold) and Zn (1.35-fold) concentrations [55]. Despite the advantages, deregulation of transgenic crops for cultivation is still a major challenge [56]. In addition, under a poor supply of soil zinc, both the transgenic lines and the improved cultivars obtained from conventional breeding cannot produce the desired results [57]. Hence, agronomic approaches could be a potential option, cost-effective, and readily accessible for farmers to adopt with their preferred varieties.

2.2. *Agronomic Biofortification*

Agronomic biofortification is necessary not only for genetically inefficient cultivars but also for biofortified cultivars obtained by conventional and genetic methods. Although improved cultivars are available, grain Zn concentration depends on environmental factors such as temperature, soil type, soil pH, and availability of micronutrients in the soil [57,58]. Hence, agronomic biofortification is necessary to attain the desired results, and it is the cheapest method to reduce Zn deficiency in humans [59]. Agronomic biofortification is the process of enriching micronutrients in edible portions of the crops through fertilizer application and crop management practices. This method is potentially inexpensive and extremely effective for helping populations with widespread micronutrient deficiency [60]. The following section discusses the agronomic options to enhance grain Zn concentration.

2.2.1. Zn Fertilizer Application

In South Asia, Zn deficiency is the second most yield-limiting nutrient deficiency after nitrogen in lowland rice [61]. Zn deficiency has intensified because of the greater mining of Zn from the soil, and therefore, crop response to Zn application increased over the years from 63% of rice fields in 1985–2000 to 80% of rice fields across India in 2011–2016 [62]. Most importantly, the initial soil Zn content and its availability influence the Zn concentration of rice grains. The low soil Zn availability may be one piece of the "South Asian puzzle" of persistently low Zn nutrition despite growing income levels [63]. For instance, studies were conducted showing that rice grain Zn content ranged from 19.7 to 26.9 mg kg^{-1} under different soil Zn status [64]. Hence, Zn application is more critical in low-Zn soil than in high-Zn soil, and the response of grain Zn concentration to Zn application is higher in low-Zn soil than in high-Zn soil [65,66]. The following subsections discuss the method, form/dose, and timing of Zn fertilizer applications that influence rice yield and grain Zn concentration.

Method of Zn Fertilizer Application

Basal application of 5–25 kg Zn ha^{-1} as Zn sulfate incorporated in the soil before the last puddling or after transplanting is the most common practice in rice cultivation [67]. Rice yield gain increased with soil application, whereas elevated Zn concentration was seen with foliar application [21,68,69] (Table 1). In addition to this, the foliar application can avoid the problem of Zn fixation and the antagonistic effect of other nutrients in the soil. For instance, the presence of phytic acid in rice grain inhibits Zn bioavailability for human consumption [12]. However, the foliar Zn application has the benefit of reducing the phytic acid content of the grain by inhibiting the conversion of inorganic phosphorus to phytic acid [32,70] and, therefore, increases Zn bioavailability up to 65% in polished rice [71,72]. Furthermore, it was shown previously that the combined application of iron and Zn through foliar application enhanced wheat productivity along with quality grains [73]. Additionally, various studies have shown that a combination of soil and foliar application has resulted in both high yield and high grain Zn concentrations compared to soil or foliar application alone (Table 1). The extra labor and application costs involved in the foliar spray of Zn fertilizer can be avoided by spraying Zn sulfate along with pesticide solution, which is a common practice in rice fields. Ram et al. [74] evaluated the foliar application of Zn sulfate in combination with commonly used fungicides and insecticides to rice at 31 site-years in seven countries (India, China, Pakistan, Thailand, Turkey, Brazil, and Zambia) and showed that the grain Zn concentration in brown rice can be increased by 5 mg kg^{-1} with foliar Zn application alone or by 4.5 mg kg^{-1} with the combined application of Zn and pesticides. This indicates that Zn sulfate is compatible with commonly used pesticides, thereby diminishing labor and application costs.

Table 1. The gain in yield and Zn concentration in rice by Zn fertilizer application over control (without Zn).

S.No	Source of Zn Fertilizer	Amount of Zn Fertilizer (kg Zn ha^{-1})	Method of Zn Application	Yield in t ha^{-1}	Yield Gain in %	Zn Content in mg kg^{-1}	Gain in Zn Content in %	Reference
1	Control	0	No Zn	3.87		27.1		Shivay et al. [65]
	ZEU	1.3	0.5% ZEU at 10 DAT and PI	4.23 (0.36)	9.3	28.4 (1.3)	5	
	ZEU	2.6	1.0% ZEU at 10 DAT and PI	4.39 (0.52)	13	32.6 (5.5)	20	
	ZEU	3.9	1.5% ZEU at 10 DAT and PI	4.48 (0.61)	16	35.5 (8.4)	32	
	ZEU	5.2	2.0% ZEU at 10 DAT and PI	4.60 (0.73)	18	39.0 (12)	43	
	ZEU	6.5	2.5% ZEU at 10 DAT and PI	4.70 (0.83)	21	41.2 (14)	51	
	ZEU	7.8	3.0% ZEU at 10 DAT and PI	4.75 (0.88)	23	42.3 (15.2)	56	
	ZEU	9.1	3.5% ZEU at 10 DAT and PI	4.78 (0.91)	24	43.5 (16.4)	60	
2	Control	0	No zinc	6.70 (0)	NA	16.1 (0)	NA	Phattarakul et al. [21]
	Zn sulfate	10	Soil application	7.00 (0.3)	4.48	16.2 (0.1)	0.62	
	Zn sulfate	1	0.5% foliar application	6.90 (0.2)	2.99	17.7 (1.6)	9.94	
	Zn sulfate	11	Soil + foliar application	7.00 (0.3)	4.48	18.4 (2.3)	14.29	
3	Control	0	No zinc	3.58	NA	20.0	NA	Shivay et al. [68] (Aligarh, Uttar Pradesh site)
	Zn sulfate	5	Soil application	3.93 (0.35)	9.78	21.3 (1.3)	6.5	
	Zn sulfate	1	0.5% foliar application	3.80 (0.22)	6.15	22.0 (2)	10	
	Zn sulfate	6	Soil + foliar application	4.52 (0.94)	26.26	25.0 (5)	25	
	ZEU	2.6	1.0% ZEU at 10 DAT and PI (soil)	4.10 (0.52)	14.53	23.8 (3.8)	19	

Table 1. *Cont.*

S.No	Source of Zn Fertilizer	Amount of Zn Fertilizer (kg Zn ha^{-1})	Method of Zn Application	Yield in t ha^{-1}	Yield Gain in %	Zn Content in mg kg^{-1}	Gain in Zn Content in %	Reference
4	Control	0	No zinc	3.92 (0)	NA	26.1 (0)	NA	Prasad et al. [35]
	Zn sulfate	5.3	Soil application	5.20 (1.28)	32.65	40.3 (14.2)	54.41	
	Zn sulfate	1.2	0.2% foliar application	4.99 (1.07)	27.30	28.8 (2.7)	10.34	
	ZEU	2.6	1.0% ZEU at 10 DAT and PI	4.69 (0.77)	19.64	34.1 (8)	30.65	
	ZEU	5.2	2.0% ZEU at 10 DAT and PI	5.27 (1.35)	34.44	42.1 (16)	61.30	
5	Control	0	No zinc	3.00	0	17.6	0	Veni et al. [75]
	Zn sulfate	5	Soil application	3.60 (0.6)	20	20.2 (2.6)	14.77	
	Zn sulfate	1	0.5% foliar application	3.50 (0.5)	16.67	17.8 (0.2)	1.14	
	Zn sulfate	6	Soil + 0.5% foliar application	3.60 (0.6)	20	21.9 (4.3)	24.43	
6	Control	0	No zinc	3.36	NA	NA	NA	Pooniya and Shivay. [76]
	Zn sulfate	5	2.0% ZEU (Zn sulfate coating with urea)	3.79 (0.43)	12.80	NA	NA	
	Zn sulfate	5	Soil application	3.67 (0.31)	9.23	NA	NA	
	Zn sulfate	1	0.5% foliar application	3.60 (0.24)	7.14	NA	NA	
7	Control	0	No zinc	3.50	0	Faisalabad site	NA	Farooq et al. [77]
	Zn sulfate	10	Soil application	4.60 (1.1)	31.43		NA	
	Zn sulfate	1	0.5% foliar application	4.50 (1.0)	28.57		NA	
	Control	0	No zinc	3.40	0	Sialkot site	NA	
	Zn sulfate	10	Soil application	4.70 (1.3)	38.24		NA	
	Zn sulfate	1	0.5% foliar application	4.90 (1.5)	44.12		NA	
8	Control	0	NA	2.76	NA	23.25 (0)	NA	Bana et al. [78]
	ZEU	1.3	2% ZEU through ZnO	NA	NA	25.15 (1.90)	8.17	
	ZEU	1.3	2% ZEU through $ZnSO_4 \cdot 7H_2O$	NA	NA	27.41 (4.16)	17.89	
	ZEU	2.6	4% ZEU through ZnO	NA	NA	27.78 (4.53)	19.48	
	ZEU	2.6	4% ZEU through $ZnSO_4 \cdot 7H_2O$	NA	NA	29.59 (6.34)	27.27	
9	Control	0	Uncoated PU	3.90 (0)	NA	30.10 (0)	NA	Shivay and Prasad. [79]
	ZEU	1.3	0.5% Zn (ZnO)-coated PU	4.25 (0.35)	8.97	33.30 (3.20)	10.63	
	ZEU	2.6	1.0% Zn (ZnO)-coated PU	4.50 (0.6)	15.38	38.40 (8.30)	27.57	
	ZEU	3.9	1.5% Zn (ZnO)-coated PU	4.78 (0.88)	22.56	41.80 (11.70)	38.87	
	ZEU	5.2	2.0% Zn (ZnO)-coated PU	5.15 (1.25)	32.05	45.00 (14.90)	49.50	
10	Control	0	NA	3.00 (0)	NA	21.98 (0)	NA	Meena et al. [80]
	$ZnSO_4$	0.3%	0.3% at anthesis—foliar spray	4.82 (1.82)	60.67	23.03 (1.05)	4.78	
	$ZnSO_4$	0.3%	0.3% at early milking—foliar spray	4.44 (1.44)	48.00	28.84 (6.86)	31.21	
	$ZnSO_4$	0.3%	0.3% at dough stage—foliar spray	4.25 (1.35)	41.67	36.75 (14.77)	67.20	

Table 1. *Cont.*

S.No	Source of Zn Fertilizer	Amount of Zn Fertilizer (kg Zn ha^{-1})	Method of Zn Application	Yield in t ha^{-1}	Yield Gain in %	Zn Content in mg kg^{-1}	Gain in Zn Content in %	Reference
11	Control	0	NA	6.74 (0)	NA	17.00 (0)	NA	Jat et al. [81]
	ZEU	5.0	2.0% ZEU ($ZnSO_4.7H_2O$) soil application	7.53 (0.79)	11.72	23.00 (6.00)	35.29	
	ZEU	5.0	2.0% ZEU (ZnO) soil application	7.30 (0.56)	8.31	20.00 (3.00)	17.65	
	$ZnSO_4$	5.0	5.0 kg Zn/ha ($ZnSO_4.7H_2O$) soil application	7.17 (0.43)	6.38	21.10 (4.10)	24.12	
	ZnO	5.0	5.0 kg Zn/ha (ZnO) soil application	7.04 (0.3)	4.45	19.20 (2.20)	12.94	

Note: Actual gain in yield and Zn content are given in parentheses. This table shows the yield gain and Zn concentration gain based on the source and amount of the fertilizer used and the methods used to apply the Zn fertilizer to the rice crop. ZEU–Zn-enriched urea; DAT- days after transplanting; PI–panicle initiation; ZnSO4–Zn Sulfate; ZnO–Zn Oxide; ZnCU–zinc-coated urea; PU–prilled urea.

Recently, Prom-u-thai et al. [82] evaluated the effect of Zn and other micronutrients (iron, iodine, selenium) in Brazil, China, India, Pakistan, and Thailand in high-Zn soils and observed increased grain Zn content but no yield gains. The average increase in Zn concentration was 1.4 and 5.7 mg kg^{-1} under foliar application of Zn alone and 0.4 and 6.1 mg kg^{-1} under the cocktail spray of micronutrients in India and Pakistan, respectively (Figure 3). In summary, the results indicate that, when applied together with three other micronutrients, leaf absorption and transportation of Zn in the grain were not seriously affected by the other micronutrients present in the same spray solution [82].

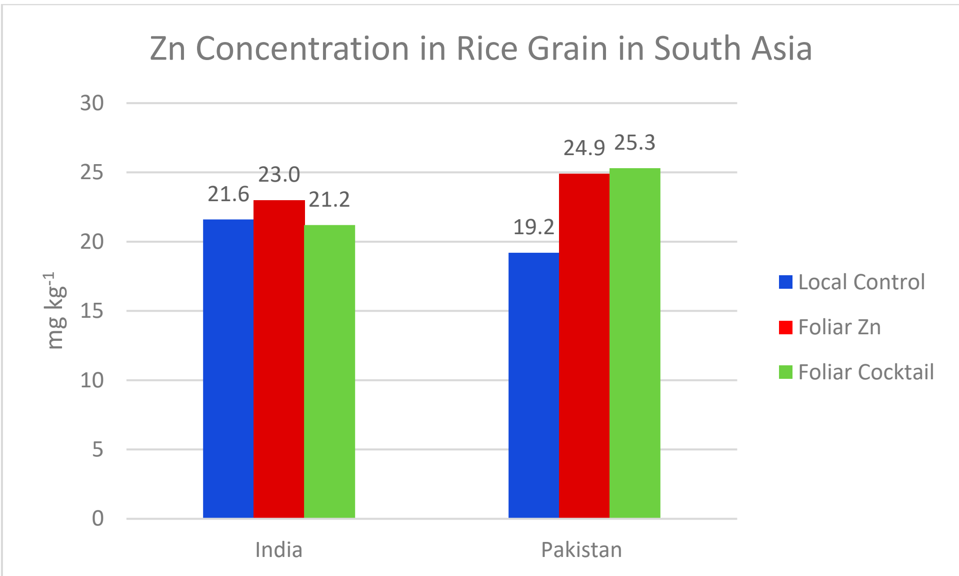


Figure 3. Zn concentrations in brown rice with different foliar treatments in India and Pakistan (adapted from Prom-u thai et al. [82]. The figure shows the Zn concentration in mg kg^{-1} in India and Pakistan by adopting different foliar treatments. Local control means without Zn application; foliar Zn means foliar spray 0.5% $ZnSO_4.7H_2O$ applied twice, once at panicle initiation and the other at the early grain milking stage. Foliar cocktail means foliar spray of micronutrients (Zinc, Iodine, iron, and Selenium) in a cocktail solution containing 0.5% $ZnSO_4.7H_2O$ + 0.05% KIO_3 + 0.02% Fe-EDTA + 0.001% Na_2SeO_4.

Form and Dose of Zn Fertilizer

The Zn application method depends on the type of fertilizer used. Zn sulfate is the most common source of Zn for rice among many different types of Zn fertilizer [35], and it performed better by improving yield and enhancing Zn content in rice than Zn oxide (Table 1) [81]. The various forms and doses of Zn fertilizer required for high grain yield and high Zn content from several studies are summarized in Table 1. The table clearly shows that the form and the dose of the Zn fertilizer influence grain yield and Zn content. Despite the increasing grain yield and Zn content, the lack of awareness, lack of Zn fertilizer availability, and high cost of these various forms/sources remain major constraints to Zn application. For example, the recommended rate of Zn sulfate is 25 kg ha^{-1} and it costs about USD 25, whereas farmers can purchase 290 kg of urea, the most widely used fertilizer in India [65], for the same price. As a result, farmers often fail to apply Zn fertilizer in rice cultivation. Zn application (1.3–9.1 kg Zn ha^{-1}) along with urea as Zn-coated urea/Zn-enriched urea (ZEU) at active tillering and panicle initiation stages increased grain yield by 0.36–0.91 t ha^{-1} and grain Zn concentration by 1.3–16.4 mg kg^{-1} in rice compared to the control (Table 1) [65]. Therefore, enriching urea with Zn during the manufacturing process could increase its availability and thus result in wider Zn application. Additionally, ZEU is relatively cheaper and easier to scale up without any additional labor for application, and it is easy to incentivize the product by supporting manufacturers for coating urea with Zn. Nevertheless, availability and access to ZEU are a concern that needs to be addressed. Hence, the government should encourage the production of ZEU and distribute it to regions deficient in soil Zn.

Timing of Zn Application

Along with the method of application and type of fertilizer used, the timing of application is also crucial in rice cultivation. Primarily, Zn fertilizer can be applied as a basal dose at the time of last puddling or planting and as a foliar spray during the vegetative, flowering, and grain-filling stages. However, the timing (crop stage) of application and the number of times applied are imperative for loading Zn into the grain [21,83,84]. For instance, two applications, the first as a basal dose by soil application followed by a second as a foliar spray at the grain-filling stage, resulted in higher grain yield and Zn loading into the grain than a single application (soil or foliar only) (Table 1). Furthermore, a foliar spray of 0.3% Zn sulfate at the dough stage significantly increased grain Zn content, whereas a foliar spray of 0.3% Zn sulfate at anthesis significantly increased grain yield (Table 1) [80]. Other studies have also shown that the foliar application of Zn at the flowering and grain-filling stages is more effective for increasing grain Zn concentration in conventional puddled rice systems under deficient soil Zn [21,66]. In brief, Zn fertilizer application through ZEU at active tillering and panicle initiation or Zn sulfate foliar spray at flowering and grain-filling stages led to higher loading of Zn into the grain and has the potential to reach the targeted concentration of 28 mg Zn kg^{-1}.

2.2.2. Influence of Phosphorus and Nitrogen on Zn Content in Rice Grain

Nitrogen plays a vital role in root Zn uptake, distribution, and accumulation in edible parts. Therefore, nitrogen management is essential for Zn biofortification [85,86]. Nitrogen and Zn applications have a positive relationship in increasing grain yield, grain Zn concentration, and uptake and translocation of nitrogen in rice plants [64]. Moreover, better management of nitrogen enhances grain Zn concentration under sufficient soil Zn, while it decreases grain Zn concentration under low-Zn soil because of the dilution effect [54,87]. In contrast to nitrogen, the application of phosphorus decreases the availability of Zn in soil solution and increases Zn fixation in the soil [88]. These effects were more pronounced in flooded regions than in non-flooded regions. Phosphorus interacts with Zn in soil and decreases Zn translocation from roots to shoots [89,90], and this interaction leads to imbalanced phosphorus: Zn ratio which has a negative effect on yield and Zn biofortification. As a result, foliar application of Zn is one of the best strategies for overcoming the antagonistic effect of phosphorus in the soil [91,92].

2.2.3. Alternative Crop Establishment Method in Influencing Rice Zn Content

Direct-seeded rice (DSR) has emerged as a resource- and energy-efficient, economically viable, and environmentally sustainable alternative to puddled transplanted rice (PTR) [93,94]. DSR saves labor (40%), costs (30%), irrigation water (20–50%), and energy (1500 MJ/ha/season), and it diminishes methane emissions by 30–98% and overall global warming potential by 20–44%, despite increasing nitrous oxide emissions by 17%. In addition, DSR increases net income by USD 100–120 ha^{-1} vis-à-vis PTR [93,95]. Because of its benefits, currently, about 30% of global rice cultivation is under DSR or aerobic rice. However, soil Zn availability and Zn loading into the grain under aerobic conditions are major concerns and are often lower than in PTR because of reduced soil moisture conditions, low rates of dissolution and diffusion of Zn, the low mass flow of Zn from soil to plants, and lower transpiration rate [96–99]. Therefore, the use of 4R principles for Zn fertilizer (right method, right form, right dose, and right time) for Zn biofortification under DSR systems is important to avoid further harm to Zn nutrition. On the other hand, Zn availability under DSR could be increased if the soil were wet throughout the season [77,100]. Future research should focus on Zn availability and dynamics under aerobic DSR in the view of Zn biofortification considering the increasing area under DSR in South Asia.

3. The Way Forward: Data-Driven Zn Recommendations for Biofortification at Scale

Despite the introduction of new cultivars and transgenic lines, soil Zn availability is crucial for increasing rice grain-Zn content. Hence, the effect of moving from low-Zn to high-Zn soils through Zn fertilization is substantial in connecting the health of the soil, plants, and humans. Widespread Zn fertilization could therefore increase Zn concentrations in cereals, thereby ultimately increasing the Zn status of humans consuming those fortified crops. For example, the effect of fertilizer interventions in improving child health was equivalent to Zn supplementation for 6 months in children in Thailand [101]. Zn availability in paddy soils depends on many factors, such as the pH of the soil, type of soil, and application of phosphorus fertilizer in high-Zn soils. Of these factors, the most important one affecting Zn availability is soil pH. Zn availability decreases in soil solution with increasing soil pH or alkalinity [102]. The low availability of Zn at high pH is due to Zn adsorption onto clay minerals and metal oxides [103]. Similarly, Zn deficiency has been frequently noticed in rice grown under calcareous soils of the Indo-Gangetic plains mainly due to high pH and bicarbonate content [104,105]. Also, phosphorus application at planting is a common practice in rice cultivation, and it affects Zn uptake by increasing its binding in the root cells [106,107]. Other factors that affect soil Zn availability are low soil organic matter, eroded soil, soil temperature, soil Zn content, and antagonistic effects of other nutrients such as calcium, iron, sodium, magnesium, and copper [86,102,108,109]. Thus, soil properties have an important effect on rice production and agronomic approaches play a pivotal role in addressing soil nutrient availability. For this reason, soil maps were generated by conventional methods to provide valuable information for fertilizer recommendations. However, the data provided are limited in these soil maps. Below, we discuss the limitations of conventional soil maps and the emergence of digital soil mapping (DSM), and how this can be used for targeted fertilizer application in rice production in South Asia.

3.1. Conventional Soil Maps and Limitations

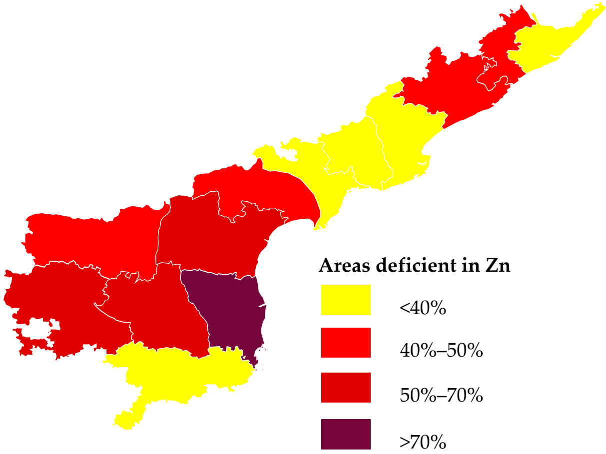
Conventional soil maps were prepared by soil surveyors using soil survey methods, so this survey relies solely on expert judgments, landscape features, and aerial photographs [110]. Most importantly, soil maps developed by this method lack spatial distribution of soil and accuracy in soil attributes, making it difficult to optimize crop inputs [111]. In addition, data collection is quite expensive and laborious since new samples must be collected from new surveys to get a soil map of a particular region [112]. Although these soil maps are devoid of high-resolution data, they have been used for decades in decision-making for sustainable crop management by maintaining soil health and minimizing fertilizer wastage [113]. In conventional methods, generalized soil information at the district scale is often used to guide extension [111]. However, the information obtained is not quantitative, and using these maps might result in the over- or under-application of nutrients, thus resulting in low yield and quality. Studies have shown that nitrogen fertilization in rice cultivation has increased dramatically in China [114,115]. Nevertheless, this increase did not correlate with an increase in rice grain yield, thus showing a decline in fertilizer use efficiency [114,115].

A comprehensive district-level map for 19 different states released by the Indian Institute of Soil Science, Bhopal, in 2014 showed that the soil is deficient in three important macronutrients: nitrogen, phosphorus, and potassium [116]. As a result, the external application of these fertilizers is imperative in rice production in India. However, studies have shown that imbalances in fertilizer usage occur in rice cultivation in India [117]. Similarly, using conventional soil maps for agronomic practices can lead to low Zn use efficiency, resulting in no gain in yield and grain Zn content [118,119]. Moreover, blanket Zn application in high-Zn soils has sometimes led to a decrease in yield and grain Zn because of the antagonistic effects with phosphorus and other nutrients [88,90]. Furthermore, soil information derived from conventional methods is fragmented, not easily shareable, and hence not accessible for decision-making [120]. Therefore, we propose steps to address soil Zn variability through machine learning methods to improve grain yield and grain Zn concentration at scale (Figure 4).

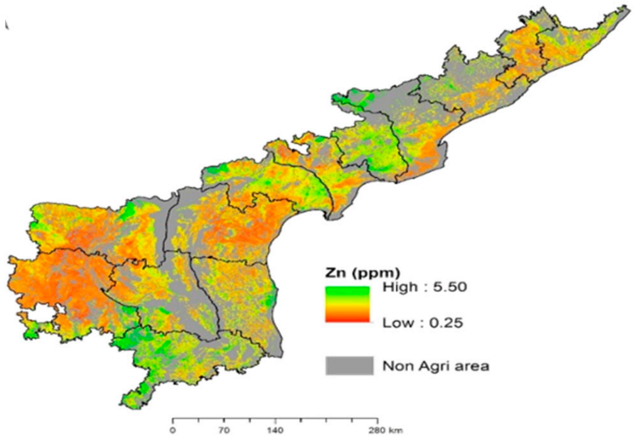
3.2. Digital Soil Mapping (DSM) for Fertilizer Recommendations at Scale

Computational and information technology have opened ways to improve soil maps obtained by conventional methods [121]. In this approach, soil data can be collected and shared, and this is essential for guiding data-driven nutrient management practices for sustainable crop production [122]. DSM is the computer-assisted prediction and production of digital maps of soil types and soil properties [123]. In DSM, soil properties are predicted over the entire area at fine resolution (from 1 km to 30 m) with machine learning and geostatistics with soil data collected at sampling points coupled with environmental covariates such as satellite imagery and digital elevation models [124,125]. Previous studies have shown that average rice yields were significantly higher when geographic information system-based soil maps were used for nutrient management over conventional practices in India [122]. Additionally, a digital soil map of Nepal was recently developed and released by the Nepal Agricultural Research Council, and it provides fruitful site-specific nutrient recommendations for agricultural crops in Nepal [126,127]. Likewise, studies are being conducted to generate digital soil maps in Bangladesh [128] and Sri Lanka [129].

Based on the importance of DSM as a decision-making tool in crop production, a first-generation digital soil map for Zn for Andhra Pradesh state in India from ~2.9 million soil data points (obtained from the Indian SHC scheme) combined with 300+ covariates was developed as shown in Figure 4. Figure 4A shows the conventional soil map for Zn in the Andhra Pradesh region, and Figure 4B shows the digital soil map of Zn predicted, using machine learning methods. The information generated by DSM is quantitative and is extremely resourceful compared to the conventional soil maps used in fertilizer decision-making in India. The prevailing recommendation logic in India suggests that the soil is Zn deficient if it has less than 0.6 parts per million of extractable Zn. However, DSM alone is not enough for fertilizer recommendations because the crop response to Zn fertilizer application is affected by several soil and crop management factors [130]. Hence, all the parameters should be taken into consideration for Zn management in sustainable rice production.



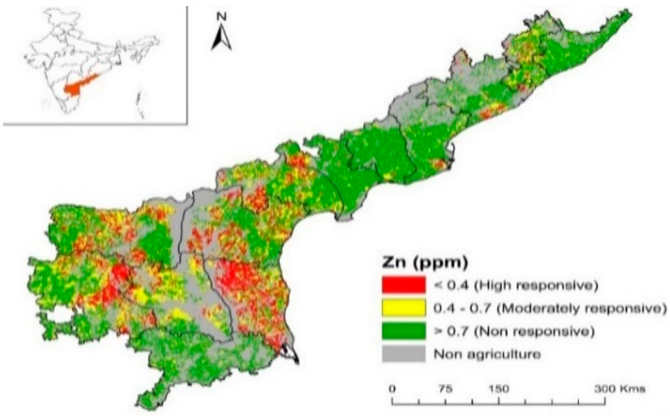
(**A**) Conventional method



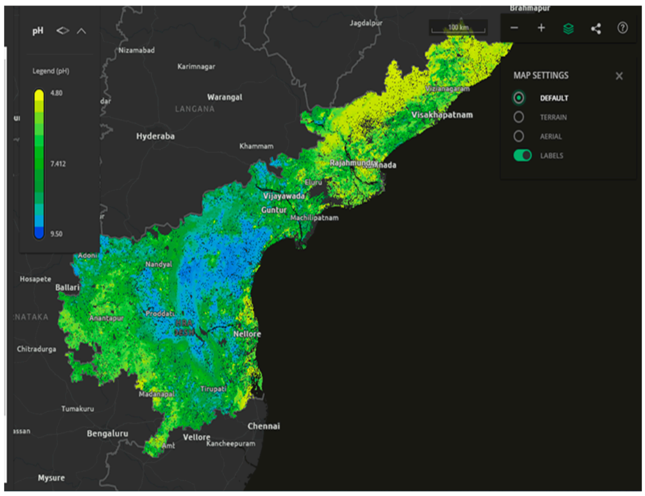
(**B**) Step 1. Zn digital soil mapping (DSM)

(**C**) Step 2. Zn response trials across soil gradients

(**D**) Step 3. Digitally enabled field monitoring to adaptively improve recommendations



(**E**) Step 4. Zn management zones based on crop response



(**F**) Step 5. Location intelligence to guide action

Figure 4. (**A**–**F**): **Data-driven Zn recommendations for biofortification at scale:** (**A**) Soil map obtained by conventional methods in Andhra Pradesh. The map shows the regions that are deficient in Zn. (**B**) Map obtained through DSM. This map shows the fine resolution of the Zn concentration for the same area in 4A obtained by machine learning techniques. Ppm–parts per million. (**C**) Zn response trials across soil gradients in Andhra Pradesh based on DSM. (**D**) Digitally enabled field monitoring to add and access more data and improve Zn recommendations. (**E**) Zn management zones based on

crop responses to Zn trials conducted. (**F**) Map showing the location intelligence for decision-making by different stakeholders, including farmers, fertilizer players, the extension system, and policymakers.

3.3. Data-Driven Spatial Nutrient Recommendations

Generating digital soil maps alone will not be sufficient in decision-making in agriculture. Zn nutrient response trials based on the digital soil map and storing the results digitally are equally important for precision rice farming. Studies have shown that variations exist in crop responses to specific nutrients in cereal-growing areas in sub-Saharan Africa [131]. Additionally, on the same farm in Western Kenya, soil fertility gradients influenced the response of a maize crop to fertilizer [132]. Hence, Zn response trials based on different soil Zn concentrations are important for farmers and policymakers. The crop response to fertilizer application across the different soil Zn concentrations is required for yield and nutritional profiling of rice at scale. The data on crop response to Zn fertilizer application across soil gradients (low-, medium-, and high-Zn soils) based on DSM provide the "gold standard" for guiding site-specific crop management. Consequently, to provide new insights into the association between soil variability and crop response to Zn fertilizer in Andhra Pradesh, the Krishi Vigyan Kendra (farm science center) worked across nine districts to pair on-farm trials (+/− Zn) in low-, medium-, and high-Zn soil based on first-generation digital soil maps [133] (Figure 4C, Step 2, Panneerselvam et al., Manuscript in preparation).

The digital data collection on the crop, soil, and management practices from the crop response trials conducted will help farmers to better understand the intersection of site factors, crop management, nutrient use efficiency, yield, and grain Zn concentration outcomes (Figure 4D, Step 3, Panneerselvam et al., Manuscript in preparation). Data obtained from these trials have been recorded and incorporated into the already generated digital soil maps. Consequently, these data will help in refining site-specific Zn recommendations based not only on initial soil Zn and crop response trials but also on other factors, such as crop management, soil properties, climate, and socioeconomic conditions.

3.4. Spatially Referenced Information on Soil and Crop Management for Government Programming

Deriving soil management zones is crucial for fertilizer use efficiency and diminishing the environmental burden caused by over-fertilization. Landscape information plays a pivotal role in fertilizer responses in crops [117,134]. Thus, soil Zn management zones (Figure 4E) were derived based on DSM (step 1) and the crop response to Zn fertilizer application across the different gradients of soil Zn concentration (step 2). As a result, soil Zn management zones will provide important information to farmers on whether to apply Zn fertilizer or not at site-specific levels. Finally, spatially referenced information on soil and crop management recommendations based on machine learning with geospatial crop response assessment and characterization of production practices was developed (Figure 4F) (Panneerselvam et al., Manuscript in preparation). Consequently, this information will be readily available to farmers and can be used to improve fertilizer use efficiency at the farm level, decrease the subsidy burden, and, most importantly, diminish environmental pollution.

This spatial intelligence will help both farmers and policymakers to prioritize micronutrient subsidies for high-response zones and the fertilizer industry or input dealers to target fertilizer in high-response zones. Spatial intelligence will also help fertilizer manufacturers produce custom-blended fertilizer or ZEU and position fertilizer for which a high response is expected. Moreover, spatial intelligence will provide useful information for the state extension system to send location-specific agro-advisories related to fertilizer management to farmers. Dashboards linking spatial intelligence with the government portal will serve as soil/crop monitoring, management, and education tools for policymakers for better decision-making in rice production in India.

4. Policy Options for Scaling Zn Biofortification of Rice

At present, governments in some states of India (such as Andhra Pradesh, Karnataka, and Telangana) are trying to promote the use of Zn and other micronutrients such as boron by selling them directly to farmers at highly subsidized prices or even free of cost at government outlets. However, the current subsidy on micronutrients has three major problems. First, the distribution of highly subsidized Zn is fiscally unsustainable for cash-strapped state governments. Thus, it forces strict rationing that often leads to its suboptimal allocation. For example, a recent study in Andhra Pradesh showed that an adequate amount of the free Zn is distributed to traditionally prosperous irrigated districts in contrast to regions where Zn deficiency is high [135]. Second, distributing subsidized or free Zn by the government crowds out commercial demand. The subsidy lowers farmers' willingness to pay for Zn through reference dependence or anchoring to a level that is not viable for private firms. This crowding-out effect may have a huge impact on decreasing the overall use of Zn. Finally, the direct distribution of Zn by government agencies imposes a significant administrative burden on them, tying up human resources that would be much better used to spread awareness among farmers about the need to apply micronutrients in areas where their deficiency is high.

Given these limitations of the current subsidy system, it should be redesigned to promote a vibrant commercial sector for Zn by providing subsidies in the form of cash transfers to farmers in regions with soil deficiency based on the data generated by DSM and soil Zn management zones (Figure 4F). Direct cash transfers might ensure more effective targeting of subsidized Zn at a lower cost to the state and incentivize fertilizer companies to expand the market for micronutrients by spreading awareness among farmers and competing among themselves to decrease costs and improve quality. We propose that encouraging fertilizer companies to sell ZEU in areas with high levels of Zn deficiency in the soil based on digital soil maps may be the most cost-effective and scalable strategy to promote Zn application. However, strict control on retail prices of urea discourages the production and sale of ZEU in India. Fertilizer companies sell urea at the price set by the Government of India and collect the difference between the price and the cost as a subsidy from the government. Hence, they cannot collect additional subsidies earmarked for Zn for ZEU because only designated Zn fertilizers qualify for micronutrient subsidies. To address this concern, state and central governments should change the subsidy system to add ZEU to the list of fertilizers eligible for Zn subsidies when this fertilizer is sold in areas identified to be deficient by digital soil maps. Currently, such area-specific targeting of subsidies is logistically possible because of the existence of a system in which retail sales of fertilizer are electronically recorded and verified using Aadhar-linked biometric verification. The system that was created to implement the direct benefit transfer of nitrogen, phosphorus, and potassium fertilizers can also be used to create a market for ZEU. In addition, low awareness of the benefits of the application of micronutrients is a major reason for their reduced usage by farmers in India [136]. Raising awareness among farmers about soil micronutrients can change their price elasticity of demand and make price subsidies more effective [137]. There may be a significant complementarity between subsidies and information. State and central governments should therefore complement micronutrient subsidies with an awareness campaign on the benefits of Zn application to increase grain Zn content in alleviating Zn malnutrition in India.

5. Conclusions

Zn-enriched varieties with 28 mg kg^{-1} Zn content have been bred through conventional breeding and genetic approaches. However, this had only a little impact on malnutrition due to the non-availability of Zn-enriched varieties for wider cultivation and failure to perform in low-Zn soils. Hence, agronomic strategies such as Zn fertilizer application play a pivotal role in obtaining high grain-Zn content in rice, complementing the genetic approaches. For that reason, we have proposed data-driven Zn recommendations for rice based on digital soil maps, crop management practices, and geospatial crop response with the use of machine learning data-mining techniques in the Andhra Pradesh region. This method will help in generating site-specific Zn nutrient recommendations and enables the government for targeted policies by implementing effective subsidy programs in regions with soil deficiency based on DSM and soil Zn management zones. Several studies are underway to generate data on DSM and crop and management practices in various regions in India (SHC scheme, India) and Nepal. As a result, these data can be used for decision-making in Zn fertilizer application for rice cultivation in these regions, thereby increasing grain Zn content to alleviate Zn malnutrition. Future research should investigate the effect of the combination of genetic and agronomic approaches using DSM on grain yield and Zn content in rice and the residual effect on succeeding crops such as wheat.

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Abbreviations

DSM—digital soil mapping, ZEU—zinc-enriched urea/zinc-coated urea, SHC—soil health card.

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