Journal of Science and Sustainable Development (JSSD), 2020, 8(2), 19-33
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ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: **https://doi.org/10.20372/au.jssd.8.2.2020.0146**

**RESEARCH PAPER**

# Assessing Crop Response to Zinc Fertilization: a Meta-Analysis

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## Abstract

*Several studies on effects of Zinc fertilization on cereals yield and their Zinc content on soils with various soil properties and using different Zn fertilization strategies have been conducted. Nevertheless, studies that summarized the studies in the form of meta-analysis as to what extent the crop Zn content and grain yield could be improved as result of Zn fertilization were limited. Hence, the objective of this study was to evaluate the response of cereal crops to Zinc fertilization across selected soil properties and Zinc fertilization startegies from large number of published studies through a meta-analysis. Fourty-two field experiments, from 27 peer-reviewed published articles were included in the analysis. MetaWin v 2.1 was used for the analysis and effect sizes estimated by using the natural logarithm of response ratio method. Cumulative analyses of each study showed a positive and significant effect of Zn fertilization over no Zn fertilization on Zn content and grain yield of agricultural crops. The average Zn content and grain yield of crops across studies is found to be 12.6; 20.4 mg/kg, and 3526; 4370kg/ha, respectively for no Zn vs Zn applied treatments; corresponding to approx. 62% and 24% increase in Zn content and grain yield as result of Zn application. In general, in Zn deficient areas, Zn biofortification through Zn fertilization of the soils can be used to improve crop Zn content and grain yield significantly. Moreover, other factors such as application methods, soil pH, SOM, and P should be managed integratively for successful Zinc biofortification.*

**Keywords:** Zinc, biofortification, cereals, fertilizer, meta-analysis, yield

## Introduction

Zinc (Zn) is a micronutrient which is very useful for healthy growth and reproduction of plants and nutrition of human being. Zn plays key role as structural constituent or regulatory co-factor in various enzymes of biochemical pathways involved in carbohydrate metabolism, photosynthesis and conversion of sugars to starch by plants (Ahmad et al., 2012). Zn is also required for the regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants, such as high light intensity and high temperatures (Cakmak, 2000). It is also vital for metabolism of proteins and auxins, maintaining integrity of biological membranes and those related to infection by certain pathogens (Alloway, 2004). Moreover, adequate supply of this micronutrient can also increase agricultural productivity through increased crop yields (Hossain et al., 2008). However, Zn deficiency in soils of many parts around the world is one of the major challenges in crop production.

Zn deficiency is the most widespread micronutrient deficiency in the world, and it’s estimated that about 50% of agricultural soils used for growing cereals in the world have low levels of available Zn (Alloway, 2004). This is a common feature in tropical and temperate climates, in particular for acid and alkaline soils (Cakmak et al., 1996) because of low Zn availability and high Zn fixation under such conditions (Donner et al., 2010). Zn deficiency can cause heavy yield losses. For example, heavy yield loss was reported in Bangladesh, China, Turkey, India, Iraq, Pakistan (Alloway, 2004), and Africa (Kang and Osiname, 1985). Moreover, it is also known to cause human health problems in regions where crops are grown on Zn deficient soils and are their staple food (Cakmak et al., 2010).

Several soil factors can cause deficiency of total Zn content and Zn availability to plant uptake. Weathered parent material, nature of clay minerals, alkaline pH, soil organic matter (SOM), sandy texture, calcareousness, intensive cultivation, agronomic practices such phosphorus application, soil available Zn and nutrients (potassium and Iron) (Huang et al., 2019) are considered to be the major factors associated with the occurrence of Zn deficiency (Alloway, 2009). Genetic biofortification (crop improvement for Zn efficiency) and agronomic management (fertilizer application) of Zn are the two most commonly used strategies to overcome such Zn deficiency problems (Cakmak, 2008). While the genetic biofortification is likely to be the most cost-effective strategy and its research and development is underway (Noulas et al., 2018), using Zn fertilization strategy is a promising intervention to improve Zn contents in grains or diets.

Several studies on effects of Zn fertilization on crops (cereals) yield and their Zn content on soils with various soil properties and using different Zn fertilization strategies have been conducted. Nevertheless, studies that quantitatively estimated, or summarized the studies in the form of meta-analysis, to what extent the crop Zn content and grain yield could be improved as result of Zn fertilization are limited. Meta-analysis, which focuses on contrasting and combining results from different studies, is very useful to estimate the average response of agricultural crops to Zn fertilization across a large number of studies varying cropping systems, climatic conditions, agro-ecosystem properties and fertilizer strategies. It's also important to test whether the response is significantly affected by aforementioned issues. Moreover, it's also resource and time efficient. Information (quantitative), obtained from meta-analysis is very useful for researchers and policy makers. Hence, the objective of this study was to evaluate the response of cereal crops to Zinc fertilization across selected soil properties and Zinc fertilization startegies from large number of published studies through a meta-analysis.

## Materials and Methods

### Data collection/literature search

Scientific databases were searched in May 2019 using Scopus, Web of science, CAB-abstract and Google Scholar and the keywords "Zinc" in combination with "fertilizer," "fertilization," "availability," "uptake," "Africa," "Asia," over the period 1960 to 2019. In addition, a more general (without keyword "fertilizer") and crop focused search was done using the keywords "zinc" in combination with the crops "wheat," "cereal, "maize," or "rice." Following the general search, articles were screened based on certain criteria: field experiments (no pot/greenhouse, aqueous) experiments conducted in areas with comparable soil properties such as Zn content, soil pH and soil organic matter (SOM) or tropics and sub-tropics in general; studies that focused on effect of Zn fertilizer rates, Zn form and application methods on the crop Zn uptake, content and grain yield; studies that showed replication of treatments, and experiments that reported a measure of variance such as standard deviation (SD), standard error (SE) or at least ANOVA tables, LSD were retained for further analysis.

However, in an attempt to obtain sufficient data that would allow to use the meta-analysis approach, studies which did not report the SD or SE values were also included by using an arbitrary SD value based on coefficient of variation which is 1.25 times the average CV (CVav) in the other studies (taking into account crop specific differences). Then, the SD was calculated as: SD = 1.25 * CVav * mean. When data were not presented as tables, a freeware digitizing software for data extraction from graphs (GetData Graph Digitizer version 2.22) was used.

Mean crop responses in grain yield and Zn concentration of experimental and control groups with their SD and replicates (n), from the screened studies were collected. When only SE was reported, SD was calculated as SD= SE* sqrt (N). Data were subdivided into various subgroups related to factors that could affect the concentration in or uptake of Zn by cereal crops. The factors included were: location (country), basic fertilization (with N, P, and K ), soil characteristics (Zn content, clay content, pH and soil organic matter), fertilizer properties (Zn species, application form [liquid, granular, foliar]), fertilizer rate, and crop properties (crop species, crop variables [grain yield and Zn content], crop part). The response variables used were grain yield (kg ha-1) and Zn content (mg Kg-1) of the agricultural crops, and the analysis was conducted for these variables separately.

For the independent variables such as soil Zn and soil pH, data was collected only from studies that used the frequently used extraction techniques: DTPA-extractable Zn and pH-H2O, respectively. Furthermore, the soil Zn data was grouped into three (<0.5, 0.5-1.5 and >1.5mg kg-1) based on literatures (Alloway, 2009). Similarly, pH was categorized based on USDA soil pH classification into <6.5 (acidic), 6.5 to 7.3 (Neutral) and >7.4 (Alkaline). Overview of the publications used for this meta-analysis study is provided below (table 1).

Table 1. Overview of the publications used for the meta-analysis.

| S/N | Reference | Crop | Soil pH | Soil Zn (mg kg$^{-1}$) | SOM (%) | P (kg ha$^{-1}$) | Zn Application method | Continent |
|---|---|---|---|---|---|---|---|---|
| 1. | Abunyewa and Mercer-qurshie, 2004 | Maize | 5.1 | 1.64 | 1.72 | 137 | Soil | Africa |
| 2. | Bereket et al., 2011 | Teff | 7.53 | 0.68 | 1.89 | 90 | Soil | Africa |
| 3. | Bharti et al., 2013 | Wheat | 8.1 | - | 0.6 | | Soil, Seed + Foliar | Asia |
| 4. | Biljon et al., 2013 | Maize | 4.1-6.1* | 2-5.3* | 0.34 | 45 | Soil | Africa |
| 5. | Chiezey, 2014 | Maize | 5-5.4* | 0.9-1.9* | 0.34 | 59 | Soil | Africa |
| 6. | Dwivedi and Srivasta, 2014 | Rice + Wheat | 7.01 | 0.57 | 1.77 | - | Soil | Asia |
| 7. | El-Attar et al., 1982 | Wheat | 8.1 | 3.49 | 1.7 | 60 | Soil | Africa |
| 8. | Ezik et al., 2008 | Wheat | 7.8 | 0.1 | 2.1 | 68 | Soil | Asia |
| 9. | Guo et al., 2016 | Rice | 5.6-7.7* | 0.6-2.3* | - | 75 | Soil, Foliar, Soil +Foliar | Asia |
| 10. | Gupta et al., 1991 | Wheat | 8.3 | 0.42 | 0.55 | 60 | Soil | Asia |
| 11. | Hossain etal 2008 | Maize | 8.2 | 0.58 | 1.44 | 63 | Soil | Asia |
| 12. | Kalayci et al., 1999 | Wheat | 7.6 | 0.1 | 2.6 | 68 | | |
| 13. | Mao et al., 2014 | Wheat | 8.12-8.21* | 0.73-0.78* | 1.21-1.36* | 100 | Foliar | Asia |
| 14. | Mathur and Lal, 1991 | Wheat | 8 | 0.42 | 0.27 | 40-60 | Soil | Asia |
| 15. | Mehla, 1999 | Rice | 8.3 | 0.85 | 0.55 | 60 | Soil | Asia |
| 16. | Nayyar and Takkar, 1980 | Rice | 10.4 | 0.56 | 1.33 | - | Soil | Asia |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17. | Rafique et al., 2015 | Pea | 8.1-8.3* | 0.28-0.42* | 3.4-4.9* | 100 | Soil | Asia |
| 18. | Sankhyan and Sharma, 1997 | Maize | 5.4 | 0.68 | 1.33 | 39-78 | Soil | Asia |
| 19. | Sharma and Katyal, 1986 | Wheat | 8.4 | 0.3 | 0.77 | 57 | Soil | Asia |
| 20. | Sharma et al., 1982 | Rice | 9 | 0.4 | 0.3 | 57 | Soil, Seed, Foliar | Asia |
| 21. | Singh and Abrol, 1986 | Rice | 10.45 | 0.38 | 0.33 | - | Soil | Asia |
| 22. | Tariq et al., 2002 | Maize | 8 | 0.25 | 1.38 | 90 | Soil | Asia |
| 23. | Yerokun and Chirwa, 2014 | Maize | 7.2 | 0.8 | 2 | - | Soil, Foliar | Africa |
| 24. | Yilmaz et al., 2008 | Wheat | 7.8 | 0.1 | 2 | 68 | Soil, Foliar, Seed, Soil + Foliar | Asia |
| 25. | Yoshida et al., 2012 | Rice | 7.9 | - | 1.68 | 67 | Soil, Seed, Foliar | Asia |
| 26. | Zhang et al., 2012 | Wheat | 5.7-8* | 0.4-1.59* | - | 100 | Soil | Asia |
| 27. | Zhao et al., 2014 | Wheat | 7.98 | 0.6 | 1.38 | 120 | Soil, Foliar, Soil +Foliar | Asia |

## Meta-analysis

Meta-analysis is an analytical technique designed to summarize the results of multiple studies. A meta-analysis (performed using MetaWin programme) can be used to estimate the average response of agricultural crops to Zn fertilization across a large number of studies varying in cropping systems, climatic conditions, agro-ecosystem properties, fertilizer strategies (timing, dose, Zn species), and to test whether the response is significantly affected by aforementioned issues. Background information on meta-analysis can be found in the study of (Gurevitch and Hedges, 1999; Rosenberg et al., 2000; Gurevitch and Hedges, 2001). The current meta-analysis focuses on the averaged effects across the groupings involved. Based on this general meta-analysis, it is possible to identify the most important factors controlling the efficiency of Zn fertilizers quantitatively. This analysis helps to identify the relevant agro-ecosystem properties affecting the efficiency of Zn fertilizers as an agronomic fortification strategy.

## Effect size calculation

Standardized mean difference, Pearson’s correlation coefficient, and the log response ratio are the effect size metrics used in soil science and ecological meta-analyses studies (Gurevitch and Hedges, 1999). In this this study, the commonly used natural log of response ratio was used as it estimates the proportionate change due to experimental manipulation (Zn fertilization in this study), and it was calculated by using treatment mean and control mean, their standard deviations (SD), variance and sample size (N) with MetaWin v2.1 software (Gurevitch and Hedges, 1999; Rosenberg et al., 2000; Gurevitch and Hedges, 2001). Finally, cumulative effect size is calculated for each study which represents the overall magnitude of the effect present in the studies, and this value is considered to be significantly different from zero if its confidence limits do not bracket zero (i.e. the effect size is significant at P=0.05) (Rosenberg et al., 2000).

## Data exploration

The funnel plot and Normal quantile functions of the MetaWin software were used for exploring the distribution of data and detecting potential publication bias. Scatterplots of effect size vs. sample size or variance, respectively, were checked to test for potential publication bias. Publication bias was tested by using Rank correlation test of Kendall’s Tau and Spearman rank order. The Fail-Safe number was calculated by using Rosenthal's Fail-Safe (Alpha =0.05) and Orwin's Fail-Safe method. Finally, the meta-analysis, based on random-effects model, was conducted by using MetaWin version 2.1.

# Result and Discussion

## Dataset description

A total of 90 studies published between 1960 and 2019 were identified and collected, of which 27 studies included reliable and quantitative data for this meta-analysis. A total of 1, 032 studies or observations (n = 1032) from 42 field experiments published in 27 peer reviewed papers where the effect of Zn fertilization was tested in comparison with an unfertilized control have been collected.

Most of these experiments were performed in zinc deficient arable ecosystems in the Asian continent including India, China, Turkey, Pakistan, and Bangladesh. These regions are shown to be Zn-deficient by soil analysis with 70% of the arable land is Zn deficient in India (Singh et al., 2008), 49% in China (Zou et al., 2008), 14% in Turkey (Cakmak, 2008), and 15% in Pakistan (Yoshida and Akira, 2012). About 89% of the observations were derived from experiments conducted in Asia, and the remaining 8% and 3% from Africa and South America, respectively. All of the experiments were conducted on cereal crops (wheat, maize, rice, barley and Teff), of which wheat comprises 76.5%, rice 15%, maize 8%, barley and teff 0.5% of observations. Most of the Zn concentration were reported for grain and shoot. Afterwards, the data was split into grain yield and Zn concentration so as to study Zn fertilization effect on the two factors separately.

About 60% of observations had initial soil Zn content of <0.5 mg kg-1 indicating that majority of the studies were conducted on Zn deficient soils with Zn concentration lower than widely accepted critical Zn concentration of 0.5 mg kg-1 (Sims and Johnson, 1991); 23% had 0.5-1 mg kg-1 and only 7% had >1 mg kg-1. The soil pH ranged from 4.1 to 10.5 of which 8% have pH values below 5.5, 69% with pH values between 7.0 and 8.0, and about 22.5% with pH values above 8 indicating that the experiments were conducted on Alkaline/calcareous soils. About 10% of the soil organic matter (SOM) had < 0.5%, 25% had 0.5 to 1.4%, 35% had 1.5 to 2.5%, 25% had >2.5% soil organic matter content, and the remaining 5% didn't include information on SOM. Summary of basic statistics for some soil properties where the studies were conducted is provided in Table 2.

The most common Zn fertilizers used in these experiments are based on Zinc sulphate (ZnSO4.7H2O) (88%) followed by Zinc oxide (ZnO) 4.5% of the observations. The fertilizer dose ranges from 0.44 to 120 kg ha-1 (assuming a soil density of 1400 kg m-3 and a soil layer of 10 cm for up-scaling). Application dose of Zn fertilizers 15-25 kg ha-1 comprises the majority of the observations (62%), whereas <15 kg ha-1 and >25 kg ha-1 comprises 28.5% and 9% of the observations, respectively. Majority of the studies used Nitrogen fertilizers and Phosphorus fertilizers optimum for the crops and the region.

Table 2. Dataset description (basic statistics) of some soil properties where the experiments were conducted

| **Soil variables** | **Range** | **Median** | **SD** | **Most common** |
|---|---|---|---|---|
| **DTPA-Zn (mg kg$^{-1}$)** | 0.1-5.3 | 0.12 | 0.56 | <0.5 |
| **Soil pH (pH-$H_2O$)** | 4.1-10.4 | 7.8 | 1.03 | 7-8 |
| **SOM (%)** | 0.27-2.6 | 2.1 | 0.82 | 1.5-2.5 |

Based on the suggested data exploration and publication bias testing methods no obvious bias could be detected (Plots provided in Appendix). Even though there is publication bias the high number of Fail-Safe number (see supplementary materials), compared to the number of observations in this study, gives us confidence that the results are reliable (Ros et al., 2016). A threshold of >5*n+10, where n is the number of observations included in the current study, was set by Gurevitch and Hedges (Gurevitch and Hedges, 2001) indicating that fail-safe numbers above the threshold, results are reliable even when there is significant publication bias.

## Crop responses: Zinc content and grain yield

The study showed a positive and significant effect of Zn fertilization over no Zn fertilization. The cumulative analyses of each study (the middle point of each horizontal line) where the response ratio (plotted on X-axis) and its confidence interval remained above zero (zero being no effect of Zn fertilization. Note that this value is supposed to be 1 but changed to 0 because we used natural logarithm of response ratio (ln R) (fig.1).

The average Zn content and grain yield of crops across studies is found to be 12.6; 20.4 mg/kg, and 3526; 4370kg/ha, respectively for no Zn vs Zn applied treatments. This corresponds to approx. 62% and 24% increase in Zn content and grain yield as result of Zn application.

The independent variables (Zn fertilization and soil properties) considered influence the response variables i.e. Zn concentration in the plant parts and also grain yield (herein after referred together as ‘crop responses’) with an overall effect size of 0.35.

Zn fertilization: Zn fertilization had an effect size of 0.48 and 0.22 for Zn concentration and grain yield which means that Zn fertilization has improved the Zn concentration and grain yield of crops grown in Zn fertilized soils by 62% and 25% compared to their counterparts grown on Zn unfertilized soils (Fig.2A, 2B). The Zn concentration is also shown to be more responsive to Zn fertilization than grain yield as it had higher effect size. Application of optimum Zn rate i.e. 15-25 kg ha-1 resulted in highest effect size for both dependent variables (fig.3B; fig.4A). Increasing Zn application rate (up to 25 Kg ha-1) increased crop zinc content; however, application rate of >25 kg Zn ha-1 didn’t result in any better performance.

Alloway (2004) suggested that optimum fertilizer regimes that will restore yield (due to Zn deficiency) and also enrich grains with Zn need to be investigated thoroughly. This meta-analysis, based on the analyzed papers, shows that Zn fertilization of 15-25 kg ha-1 on soils with low Zn and alkaline soils would result in highest Zn content and grain yield of crops.

Zn fertilization in soils with low Zn content is also shown to restore yield loss due to Zn deficiency of soils. Application of Zn fertilizers in Zn deficient soils is crucial to avoid stagnant yield and increase crop productivity. It was indicated that a reduction in yield by approx. 28% for maize when Zn was omitted from the fertilizer treatments (Ahmad et al., 2012). The current meta-analysis also showed the possibility of increasing crop yield by applying Zn fertilizers. Moreover, Zn application could also help increase Zn concentration in roots, leaves and stems through application of Zn-fertilizers. For example, root Zn concentration could be increased with 5 to 50%, and leaf concentration up to 70% (Ahmad et al., 2012).

The current meta-analysis confirmed these observations where Zn fertilizer application could increase crop Zn content (averaged over grains, leaves, stems, straw) by 62%. Increasing the Zn concentration in the shoot and grains of crops in Zn deficient areas is very important because limited (dietary) Zn intake increases the incidence of various diseases in children aged < 5 years (WHO, 2005) especially in developing countries where cereals are staple food.

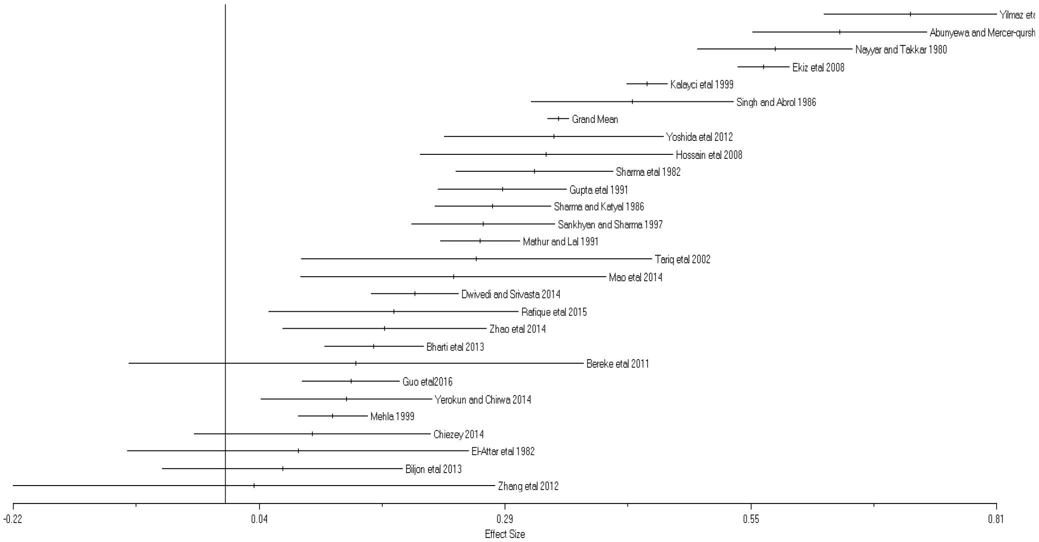

Figure 1. Forest plot of the cumulative effect size and confidence limits for each study (cumulative summary analyses). The cumulative effect size represents the overall magnitude of the effect present in the studies; this value is considered to be significantly different from zero if its confidence limits do not bracket zero (i.e. the effect size is significant at P=0.05). The vertical line (X=0) indicates the point of No response.

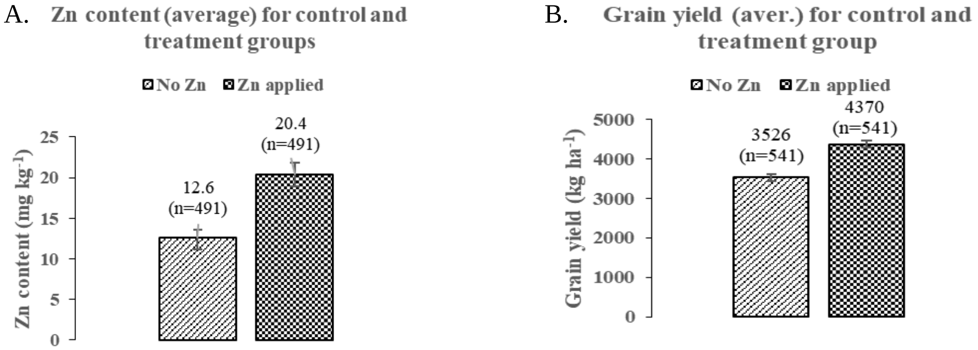

Figure 2. Average response of crops to Zn fertilization interms of Zn content (A) and grain yield (B). Error bars indicate 95% confidence interval.

In cases where soil Zn application is used to ameliorate Zn deficiency problems or as a biofortification strategy it's important to conduct periodic soil or plant analysis for Zn content. This is because after the use of zinc fertilisers, there is normally a period of several years that the residual effect of the applied zinc adsorbed in the soil is still providing an adequate supply to successive crops (Alloway, 2004). Therefore, in areas where Zn is applied (in to soil) continuously, soil analysis may help to implement cost effective Zn fertilization and also to make sure that Zn doesn't accumulate in undesirably high concentrations in the soil.

Soil Zn: Soil Zn influenced the response of crops to Zn fertilization with the highest effect size for low soil Zn content (<0.5 mg kg-1) (fig 3C; fig.4B). Crop responses increased as the soil Zn content decreased indicating the importance of Zn fertilization of low soil Zn for Zn biofortification of crops. In other words, Zn fertilizer application on soils with low soil Zn content as low as 0.5 mg kg-1 may increase the Zn concentration and grain yield of crops by as much as 48% and 22%, respectively. However, this may depend on the crop species as crops differ in their relative sensitivity to Zn deficiency. For instance, maize and rice are categorized as highly sensitive whereas wheat has low sensitivity (although this is also intra-specific) to Zn deficiency (Alloway, 2004).

The slightly high crop response in Zn rich soils (soil Zn > 1.5) was unexpected and might be related to the limited number of observations (n=36) and hence, higher uncertainty for this category.

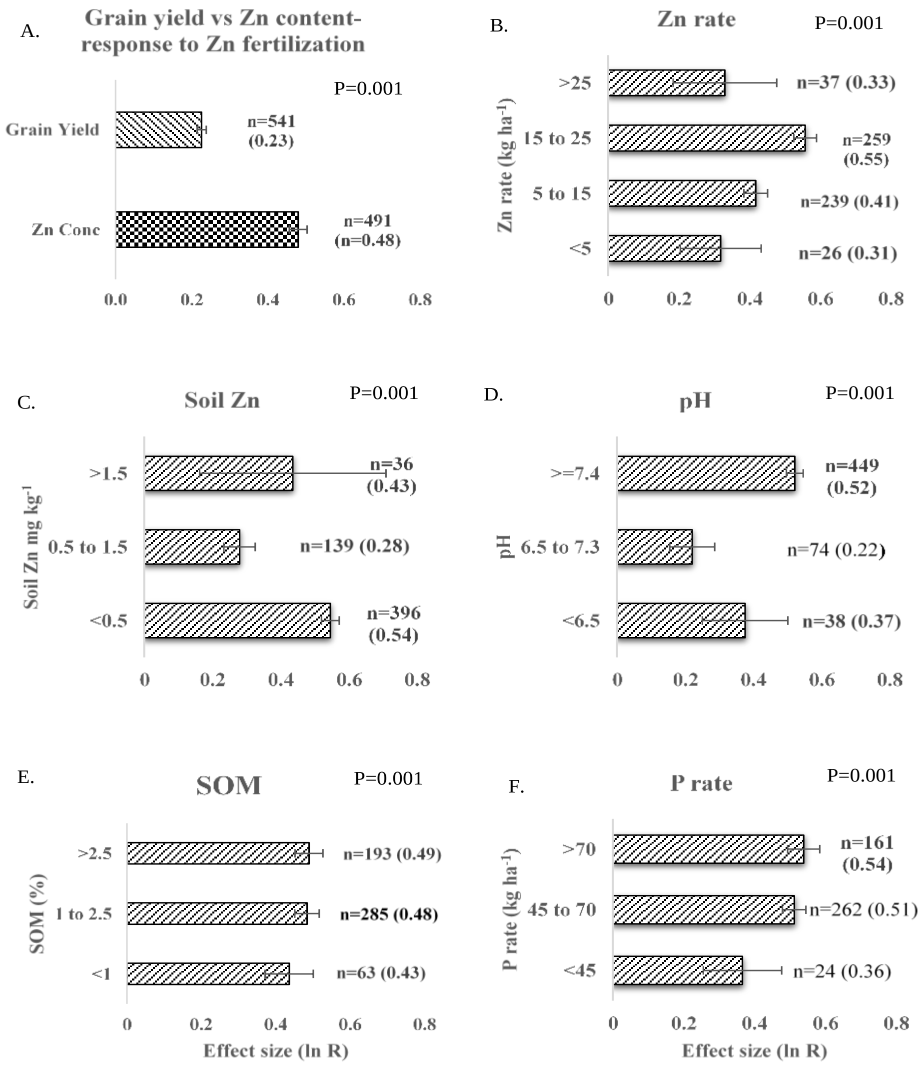

Figure 3. Averaged effect of: Zn fertilization on grain Zn concentration vs grain yield (A), rates of Zn fertilization (B), soil factors (C to E) and P application (F), on the crops' Zn concentration response to Zn fertilization. n=number of studies; numbers in brackets are the effect sizes; Error bars indicate 95% confidence intervals.

Soil pH: The study also showed that the pH level of the soil could influence the response of crops to zinc fertilization with the highest effect size observed for alkaline, calcareous soils than acidic or neutral soils (fig.3D; fig.4C). Zn concentration and grain yield can be influenced by Zn uptake of the crops and Zn availability in the soil which in turn is controlled by many soil factors including soil pH. Soil pH plays a decisive role in reducing Zn availability to plant roots by stimulating absorption to soil particles (e.g. clay minerals and Fe/Al oxides) (Alloway, 2009). Zn solubility decreases as pH increases which again results in reduced Zn uptake. In general, Zn fertilization in alkaline soils resulted in more crop response which might be due to reduced availability of Zn for uptake is compensated by Zn applied from external source. This implies that application of optimum Zn rates in alkaline/calcareous soils would improve the Zn content of the crops.

Soil Organic matter (SOM): Crop response to Zn fertilization generally increased as the SOM content increased with highest effect size (0.48) for 1-2.5% SOM category (fig.3E; fig.4D). This implies that soils of at least 1% organic matter content are needed to achieve sufficient crop response to Zn application. Improved crop responses with increased SOM can be explained by the fact that efficient uptake of Zn from soil solution by plant roots depends on the growth condition of the crop. Soils with good organic matter content may promote better growing condition for crops, and consequently uptake and content would be higher. Moreover, improved SOM content through long-term application of organic matter is reported to reduce concentration of toxic heavy metal Cadmium but not Zn in crops, typically wheat (Gruter et al., 2019). However, Alloway (2009) indicated that relatively higher soil organic matter content (>3%) would also be associated with Zn deficiency in crops. Actually, the current work also confirms this where Zn concentration didn't increase (or even slightly lower) at SOM >2.5% compared with 1 to 2.5% SOM range.

Phosphate fertilization: P application didn't result in significantly reduced Zn content of the crops although higher applications (>70kg ha-1) didn't result in significantly higher Zn contents either (fig.3F; 4E). P typically is known to counteract the Zn availability in the soil and uptake by the crops, but this occurs when the P is applied in over dose and continuously during successive (e.g. four) cropping seasons causing elevated soil P concentrations (measured as Olsen-P) (Chen et al., 2017). The same authors indicated that increase in Zn with P application can be related to a general deficiency in plant nutrients e.g. P in the soil. In case of stresses because of P, an increase in P at first may cause the plant to grow better, have a better root system and be more effective at liberating Zn uptake. A plant will do strong efforts to maintain minimum Zn for its physiological requirements, and eventually be limited in biomass production because of low Zn. Therefore, the current study is not in contrast with previous finding. On the other hand, P-induced reduction in crop Zn contents may be attributed to a combination of several processes, including reduced plant-availability of Zn in the rhizosphere, reduction in Zn uptake per unit of root weight, decreasing mycorrhizal colonization, diminished root-to-shoot translocation of Zn, and yield-induced dilution effect (Chen et al., 2017).

The increase in Zn concentration, which is likely due to improved uptake, with P application could explain increase in yield with P application (fig.4E). Phosphorus may stimulate Zn uptake predominantly by enhancing Zn concentration in soil solution and by increasing metabolic Zn absorption by plant roots.

Zn uptake increased with P (P2O5) application, or in other words, P application didn't influence (negatively) the Zn uptake of crops.

Application Method: Applying Zn fertilizers in soil at sowing followed by foliar application (spray) i.e. Soil + Foliar application had the highest effect size for both crop Zn content and grain yield (fig. 5A, B). The 'soil + foliar' method is a multiple Zn fertilizer application where Zn is applied at the beginning or sowing of seeds (to the soil) and later during the vegetative growth stage through spray on the leaves (foliar). The higher crop response with this method might be because zinc is immobile in plants (Ahmad et al., 2012), and its deficiency could occur at any of the vegetative, flowering or seed development stage. Therefore, continuous supply of Zn might have helped the crops to overcome deficiency during various crop growth stages. Moreover, foliar spray is also best to supply Zn when crop root activity is limited at any of the growth stages. In general, frequent supply of Zn throughout the crop growing season would help to better manage Zn deficiency in crops.

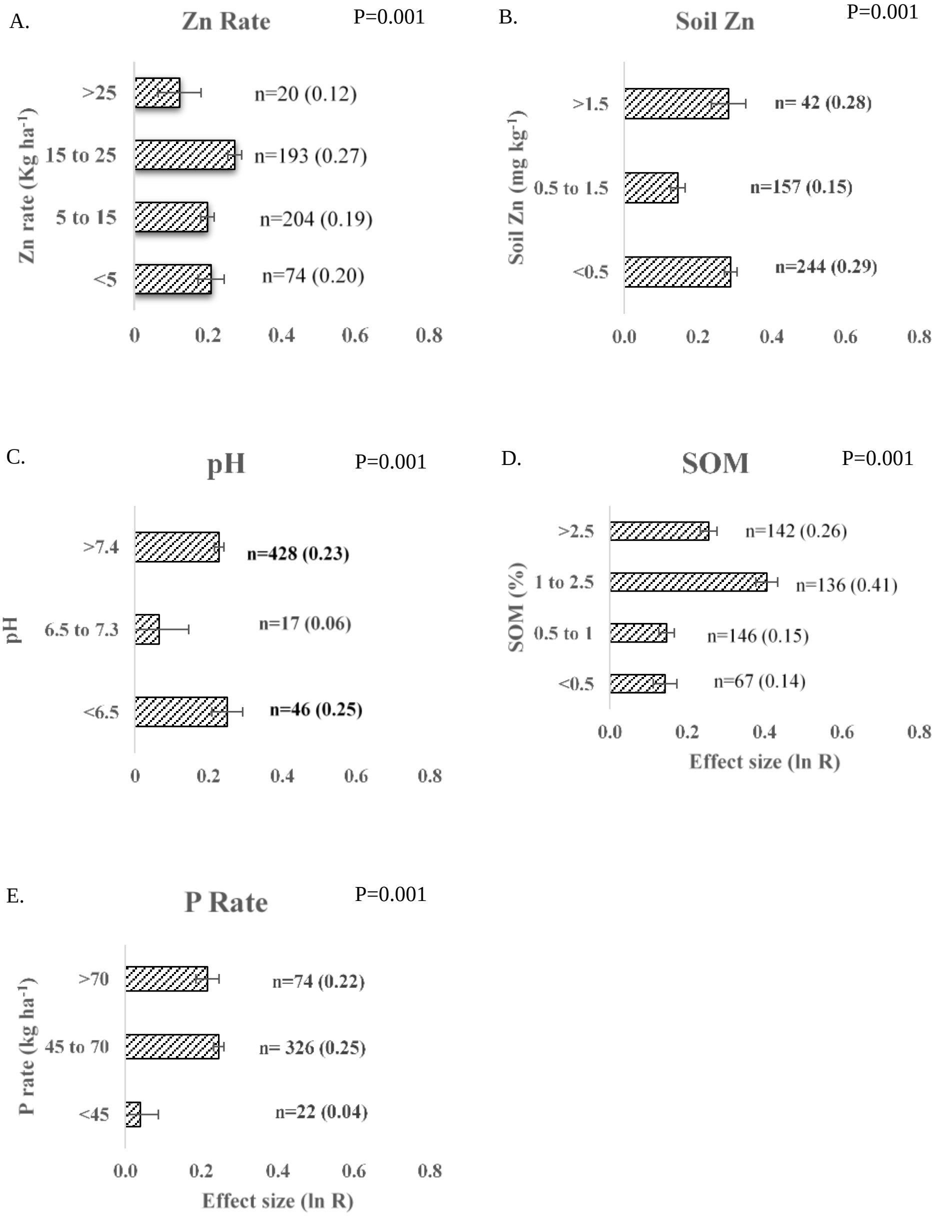

Figure 4. Averaged effect of rates Zn fertilization (A), soil factors (B to D) and P application (E), on the crops' yield response to Zn fertilization. n=number of studies; numbers in brackets are the effect sizes; Error bars indicate 95% confidence intervals.

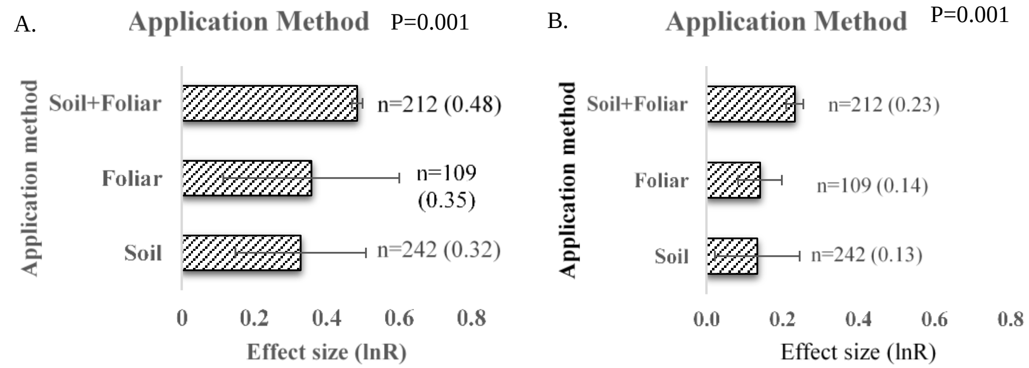

Figure 5. Averaged effect of Zn application methods on crops' response to Zn fertilization interms of Zn content (A), grain yield (B). n=number of studies; numbers in brackets are the effect sizes; Error bars indicate 95% confidence intervals.

This study showed the high influence of Zn fertilization and soil characteristics, and hence relevance of their consideration for tackling the Zn deficiency problems. However, since Zn deficiency can also be caused and aggravated by other factors it would be more effective to implement integrated strategies by combining one or more of the following interventions recommended in previous studies: Zn biofortified or Zn efficient crops, Zn fertilization, using more effective application method, choosing appropriate cropping systems that doesn't deplete already low soil Zn and agronomic management of fertilizers (N & P) and soil properties (Huang et al., 2019; Cakmak, 2009; Chaundry et al., 1977; Guo et al., 2019; Graham et al., 2011; Vitousek et al., 2009).

# Conclusion

This meta-analysis study showed that Zn fertilization had positive and significant impact, over control (no Zn fertilization), interms of improving crop Zn content and grain yield. All the independent variables considered had high and comparable effect sizes towards improving crop Zn content with Zn fertilizer application, Soil Zn, soil pH, SOM and P application had high and relevant influence on the dependent variables: Zn content of the crop and total grain yield. This implies that all of them are important parameters to consider for effective Zn deficiency management in Zn deficient areas. Zn doses of 15 to 25 kg ha-1 is shown to produce the highest effect size of crop response to Zn fertilization and might be sufficient in Zn deficient areas to improve both Zn content and grain yield of arable crops. Zn doses higher than 25 kg ha-1 doesn't (significantly) increase crop response anymore.

## Authors' contribution

This work was carried out in collaboration between both authors. Author Zewdneh Zana Zate designed the study, extracted data, performed the statistical analysis, wrote the protocol and the first draft of the manuscript, and finalized the revised version. Author Bikila Olika Fufa helped in the literature search and provided inputs for overall improvement of the study. Both authors read and approved the final manuscript.

## Acknowledgement

I acknowledge the contribution of Gerard Ros (PhD), Wageningen University and Research; and Philip Tack (PhD), University of Gent in providing some literatures and general comments about the study.

## Conflict of Interest

The authors indicate that there is no conflict of interest.

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