Assessing the residual benefit of soil-applied zinc on grain zinc nutritional quality of maize grown under contrasting soil types in Malawi

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Summary

A proper understanding of the residual value of zinc (Zn) is necessary for sustainable biofortification of food crops. This study aimed to establish the extent to which application of Zn at the national rate, plus two experimentally elevated rates, in one year provided any benefit to plant yield and nutritional quality in the following growing season. Residual effects of soil-applied Zn on grain Zn concentration and uptake were estimated by an experiment in which maize was grown in successive seasons at two agricultural research stations in Malawi, with Zn applied to the soil in the first season but not the second. At each site two common soil types were used: Lixisols and Vertisols. The study used three Zn fertilizer rates of 1, 30 and 90 kg Zn ha$^{–1}$ applied to the soil in the previous cropping season, arranged in a randomized complete block design (RCBD) with 10 replications at each experimental site. At harvest, maize grain yield and Zn concentration in grain and stover were measured; Zn uptake by maize grain and stover were determined and Zn harvest index was calculated. Effects on grain yield and Zn uptake by the crop were assessed in relation to residual Zn fertilizer and soil type. Maize grain yield on plots in the second season where 30 kg Zn ha^{-1} had been applied exceeded that on second season plots where 1 kg Zn ha^{-1} had been applied by 25%. The grain Zn concentration and Zn uptake in the second season after fertilizer application were larger by 13% and 30% respectively on the plots which had received 30 kg Zn ha^{-1} than those which had received 1 kg Zn ha^{-1}. There was no evidence that applying Zn at 90 kg Zn ha^{-1} resulted in larger crop yield, grain Zn concentration, or Zn uptake the second year after application than was seen in plots the second year after application of 30 kg Zn ha^{-1}. The magnitude of the benefits attributed to residual effects of soil-applied Zn did not depend on soil type. Conclusively, the residual effects of 30 kg ha^{-1} of soil-applied Zn in the preceding season benefited the subsequent maize compared to the national recommendation of 1 kg Zn ha^{-1}. The benefits of larger applications of Zn than the current national recommendations should be considered across at least two seasons and for different crops.

Key words: Grain zinc concentration, Residual zinc, Soil type, Zinc deficiency, Zinc fertilizers

Introduction

Zinc (Zn) deficiency is widespread, with high prevalence rate among women of reproductive age and children of under 5 years, especially in the developing countries (Kahlon *et al.*, 2018). It is estimated that nearly 1 billion people worldwide suffer from Zn malnutrition (Vaid *et al.*, 2019). The deficiency of Zn in humans is associated with multiple health problems that include immune system impairments, retarded physical growth and brain development among children under 5 years of age, and poor birth outcomes in women (Gibson, 2012; Krebs *et al.*, 2014; Terrin *et al.*, 2015). Various interventions such as application of Zn fertilizers are suggested to be possible means of alleviating Zn deficiency in humans through increasing the concentration of Zn in the edible parts of the crops, a process termed agronomic biofortification or agro-fortification (Gregory *et al.*, 2017; Miller and Welch, 2013; Wang *et al.*, 2016; White and Broadley, 2009). This is achieved either through sole or co-application of foliar and soil Zn fertilizers (Boldrin *et al.*, 2013; Esfandiari *et al.*, 2016; Liu *et al.*, 2017; Manzeke *et al.*, 2014).

In Malawi, Zn-enriched fertilizers are recommended for basal application in maize cropping system at the rate of 92 kg N ha^{-1}, 10 kg P_2O_5 ha^{-1}, 5 kg K_2O ha^{-1} and 6 kg S ha^{-1} in NPKS fertilizers applied immediately after seedling emergence (MoAFS, 2018). It is reported that the effectiveness and efficiency of soil-applied Zn in improving grain Zn nutritional quality of staple crops is influenced by fertilizer form, soil and environmental factors such as pH, moisture, temperature, organic matter and clay content (Azouzi *et al.*, 2015; Botoman *et al.*, 2022a; Kim *et al.*, 2015). These factors also determine whether the nutrient will be available to the succeeding crop (Brennan, 2005). Previous studies have reported that only a small fraction of Zn applied to the soil under field conditions is taken up by crops with a recovery rate ranging from 0.5 to 5% of the annually applied Zn depending on soil type, fertilizer types and application rates (Rico *et al.*, 1996; Zhao *et al.*, 2011; Abid *et al.*, 2013). This means that a considerable amount of applied Zn remains in the soil, some of which may be available to crops in subsequent seasons (Boawn, 1974; Brennan and Bolland, 2007; Mari *et al.*, 2015).

A pragmatic way to assess the residual benefit of nutrients is by growing a second crop in the subsequent year and determining their nutrient uptake (Chilimba *et al.*, 2012). This approach provides a direct measure between the original amount of fertilizer nutrient applied and the crop uptake. Measuring the amount of residual nutrient in the soil through chemical extraction is another option for predicting the benefit to a subsequent crop (Boawn, 1974), however, this approach can be ambiguous as it may over or under estimate plant available nutrients (Chilimba *et al.*, 2012). This is partly due to chemical transformations of the nutrients in the soil. Trace metals such as Zn exist in soil adsorbed within different chemical pools (fractions) which affects their bioavailability for crop uptake (Singh *et al.*, 2021). These operationally defined fractions include water soluble and exchangeable Zn, organic matter-bound Zn, carbonate-bound Zn, iron and manganese oxide-bound Zn and residual Zn (Tessier *et al.*, 1979). Other studies further indicate that the availability of Zn for crop uptake varies between soil types due to various underlying soil physico-chemical properties (Kim *et al.*, 2015; Soltani *et al.*, 2015; Tazisong *et al.*, 2004).

In the current study, the focus was to assess the residual benefit of soil-applied Zn under contrasting soil types by growing a second maize crop in the subsequent cropping season following application of Zn fertilizer. Our previous experiments in Malawi have shown that agronomic biofortification is a viable way of improving the Zn nutritional quality of maize in the first season of application (Botoman et al., 2020, 2022b). These results showed that Zn fertilizer application rates of 1, 30 and 90 kg Zn ha^{-1} yielded average maize grain Zn concentrations of 26.5, 30.3 and 31.2 mg kg^{-1}, respectively (Botoman *et al.*, 2022b). Following large Zn application rates in the previous study (Botoman *et al.*, 2020), we could examine residual benefit to a subsequent crop. In the present study, field experiments were conducted in the 2020-21 growing season to assess the residual benefit of soil Zn fertilization on maize grain Zn quality. The current study sought to address the following hypothesis: soil residual Zn fertilization can increase Zn concentration in maize grain thereby improving the Zn nutritional quality of maize. The study was important to assess the residual value of Zn fertilizer given that there may be widespread future use of Zn biofortification.

Materials and methods

The design of the original experiment

The original study was conducted at Chitedze, Chitala and Ngabu Agricultural Research Stations in Lilongwe, Salima and Chikwawa Districts, respectively, during the 2019-20 cropping season (Botoman *et al.*, 2020). Since larger Zn application rates were considered in the original study, this experiment was conducted to examine residual effects in a second cropping season at the same locations. Subsequent trials were however not successful at Ngabu Agricultural Research Station due to drought and failure of trial establishment.

Measurements of residual availability of zinc in soil

The residual benefit of soil-applied Zn to subsequent crops has previously been noted (Boawn, 1974; Brennan and Bolland, 2007; Grewal and Graham, 1999; Mari *et al.*, 2015). Measurement of residual Zn in the soil prior to another crop being planted can determine the extent of its availability for the next crop. Samples were analyzed as described by Botoman *et al.*, (2020). Soil samples from the depth of 0–20 cm were collected at the final harvest in 2020 from all the plots at Chitedze

Research Station. The samples were collected at ten points along the summit of one of the peripheral ridges, which were selected at random from each net plot, using a Dutch soil auger with a flight length of 15 cm and a diameter of 3.5 cm, and the 10 samples from each plot were bulked. The samples were air-dried, sieved (<2 mm) and homogenized before determination of extractable Zn as a measure of plant-available Zn using the diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell, 1978). The extraction procedure was undertaken on duplicate subsamples from each plot, using 5 g of soil extracted with 10 mL of 0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$ at pH = 7.3 shaken for 2 h on an end-over-end shaker. Thereafter, the samples were centrifuged at 3000 rpm for 15 minutes and the supernatant filtered through <0.22 µm syringe filters prior to analysis using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Trial establishment and management

The field trials were conducted at Chitedze and Chitala Agricultural Research Stations on two contrasting soil types; Lixisols and Vertisols. The original experiment was laid out in a randomized complete block design (RCBD) with each Zn fertilizer treatment (1, 30 and 90 kg Zn ha^{-1} applied to the soil as $ZnSO_4.7H_2O$) replicated 10 times for each soil type at the experimental sites (Botoman et al., 2020, 2022b). The recommended planting pattern was followed as described in the Guide to Agricultural Production and Natural Resource Management of the Ministry of Agriculture (MoAFS, 2018). The residual benefit of Zn to the maize crop was assessed by growing the crop in the subsequent cropping season (2020-2021) on the same plots and ridges without ploughing or any added Zn. Good agronomic practices were followed except for avoiding creating new ridges. The SC 403 maize variety, locally known as Kanyani, was used. General information about the maize variety and climatic conditions of the sites is provided in Botoman et al. (2020). Kanyani is a F_1 hybrid variety widely grown in Malawi, can mature in ~90 days and adapts to a wide range of environmental conditions. Critical nutrients including nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) were adequately applied as straights to avoid extra Zn coming in following the guidelines outlined in the Guide to Agricultural Production and Natural Resource Management of the Ministry of Agriculture (MoAFS, 2018).

Data collection and sample laboratory analyses

Maize was sown in December 2020 when effective rains started and harvested in April 2021 at Chitala, and in May 2021 at Chitedze. At harvest, grain and stover samples were collected. Grain yield (kg) and dry weight of stover (kg) was recorded from the net plots and used to calculate Zn uptake and harvest index of the crop. The Zn harvest index is a ratio between Zn accumulated in the grain to the sum of the Zn accumulated in the grain and stover (Fageria, 2014), expressed as a percentage. Daily rainfall (mm) was also recorded using rain gauges stationed in each of the research stations where the experiment was conducted (Fig. S1). Generally, rainfall was adequate at both Research Stations and additional irrigation was not used given that rain-fed agriculture is the common practice in Malawi.

Grain and stover samples were prepared and Zn concentrations determined as described by Botoman *et al.*, (2020). A total of 12 digestions for Wheat flour Certified Reference Material, (SRM 1567b, NIST, Gaithersburg, MD, US; Zn concentration = 11.61 mg kg^{-1}) and 12 operational blank digestions were used to determine the accuracy of the analyses and the limit of detection (LOD). The measured recovery of Zn was 105%.

Statistical data analysis

Data analyses were conducted using the linear and non-linear mixed effects (nlme) package for the R platform (Pinheiro *et al.*, 2021). The analysis of data was done after validating the assumptions of normal distribution of residuals and homogeneity of variances by checking the model plots. After estimation of the model parameters, histograms were plotted of the random effects estimates at each level, the marginal residuals were plotted against the fitted values (Fig. S2–S7) and summary statistics (Tables S1–S6) were computed. The outputs for maize grain yields, grain and stover Zn concentrations and uptake met these assumptions. For harvest index, these assumptions were not valid and data were transformed using a natural log. A linear mixed model (LMM) was used with a random effects structure to reflect how the fertilizer rate was randomized among plots within sets of blocks all within one sub-site of a single soil type. A fixed effects model was used comprising main effects of fertilizer rate, soil type and their interaction. Further, the main effect of fertilizer rate was partitioned into linear and non-linear components with an appropriate choice of orthogonal polynomials, and the soil type by fertilizer rate interaction was similarly partitioned into contrasts between the linear and non-linear responses to Zn applicate rate on the different soils. The output of the analysis tested the hypothesis concerning the differences between soil types and Zn fertilizer rates with respect to the response variable.

Results

Residual availability of zinc in soil after harvest in the first growing season

The residual Zn availability, at the end of the growing season in the year of application, typically increased with an increase in applied Zn fertilizer rate (Fig 1). There were no significant differences in the concentration of DTPA-extractable Zn between the application rates of 1 and 30 kg ha^{-1}. However, the differences were significant when the rate was increased to 90 kg ha^{-1}. There were also no observable toxic effects of Zn on the maize crops.

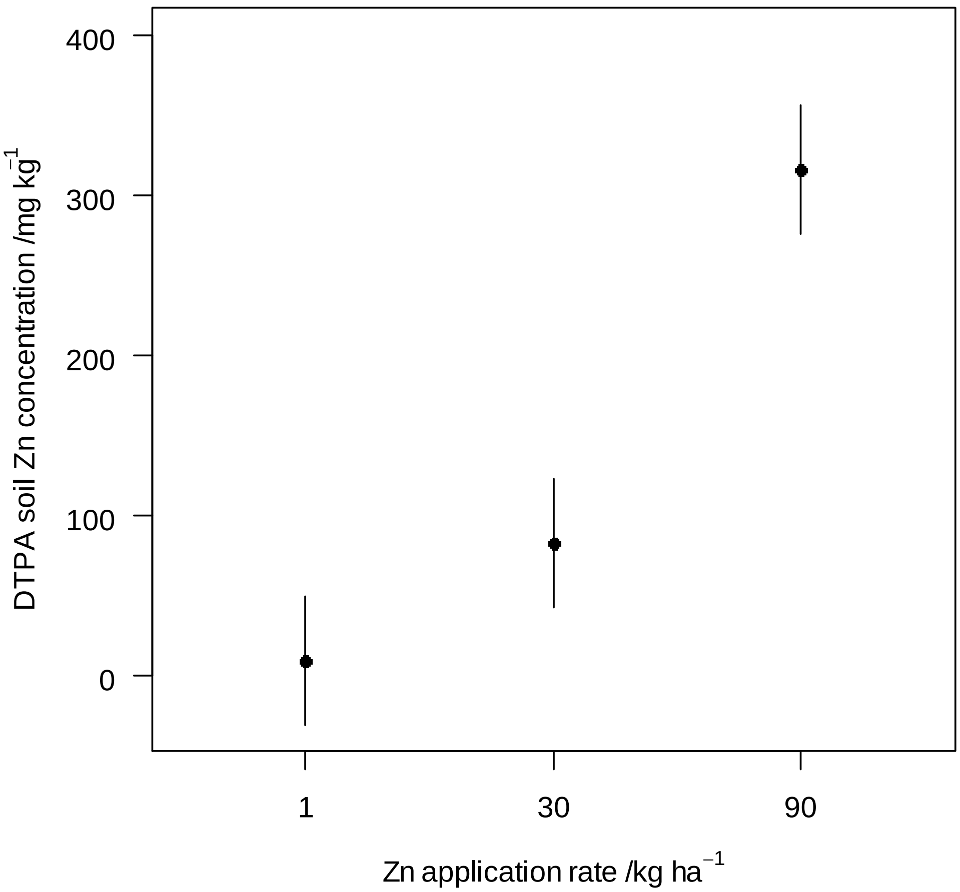

Fig. 1. Residual DTPA-extractable Zn concentration measured at the end of the growing season in which the fertilizer was applied (1, 30 and 90 kg ha^{-1}) for the experimental sub-sites at Chitedze Agricultural Research Station. The error bars show the standard error of the mean (±SEM).

Effect of soil type and residual Zn fertilizer on maize grain yields

The maize grain yields obtained over all experimental sites are presented in Fig. 2a, error bars show the standard error. Some of the soil-applied Zn appeared to remain in an available form to the succeeding maize crop resulting in a positive grain yield response. Soil type is not replicated within sites, and so we can make inferences only about an additive soil effect over all the sites. A LMM framework was used to fit the data as proposed by Botoman *et al.*, (2020). The main effect of Zn fertilizer rate was partitioned into linear and non-linear components. A positive response of maize grain yield to residual Zn for each Zn fertilizer rate was observed at each site. The mean grain yield increased by ~1500 kg ha^{-1} in response to residual Zn from the 30 kg ha^{-1} Zn fertilizer rate relative to the 1 kg Zn ha^{-1} rate (approximately 25% higher). However, no further significant increases in yield was observed when the Zn application rate was increased to 90 kg ha^{-1} .

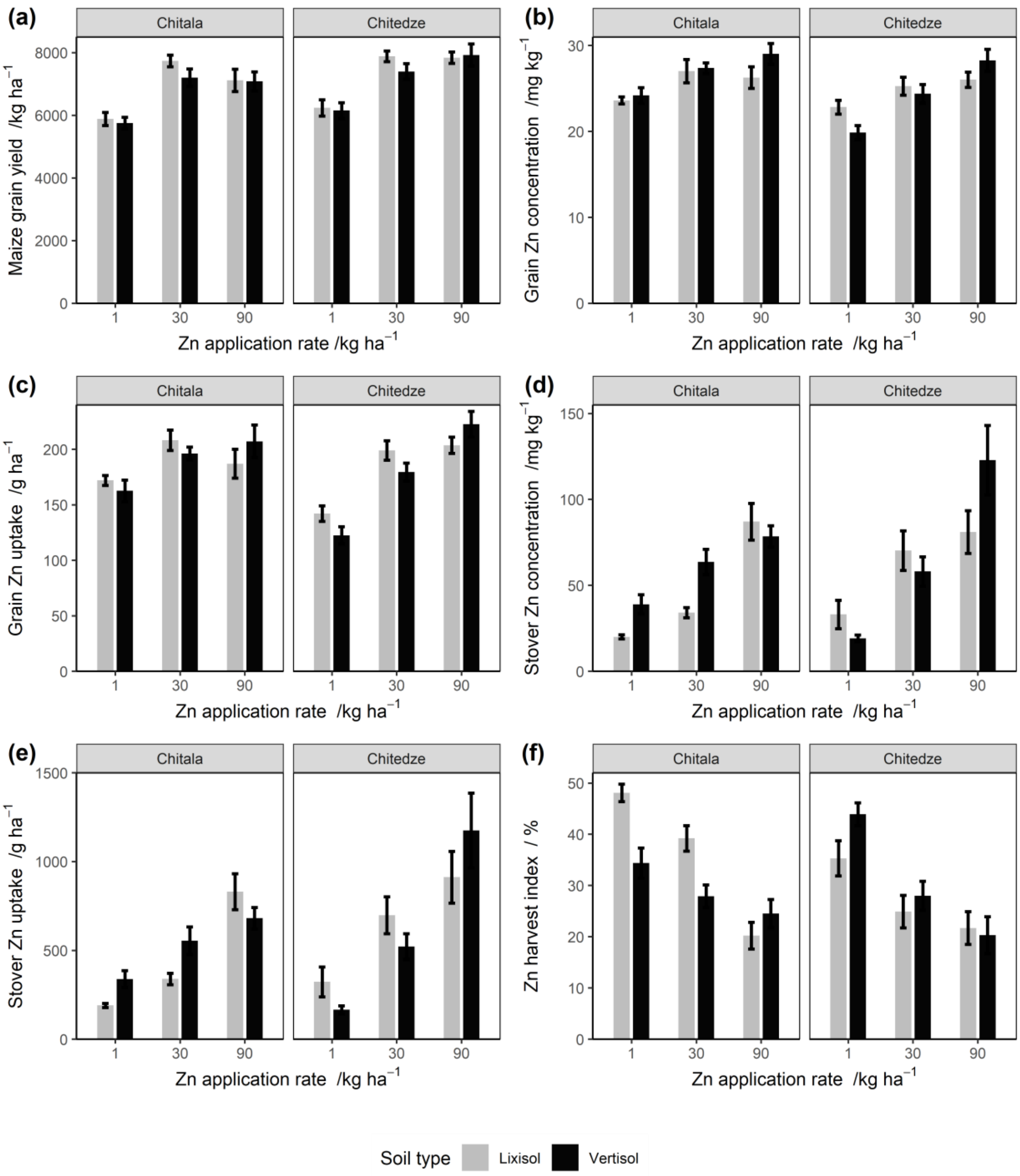

Fig. 2. Effects of residual Zn fertilizer rate and soil type on (a) maize grain yield, (b) grain Zn concentration, (c) grain Zn uptake, (d) stover Zn concentration, (e) stover Zn uptake, and (d) Zn harvest index at the experimental sites during the 2020-21 cropping season. The error bars show the standard error of the mean (±SEM).

The analysis of variance (ANOVA) for the maize grain yield is shown in Table 1. There is strong evidence for an effect of residual Zn in soil for both linear and non-linear components. The linear component ($p < 0.05$) represents the positive effect of residual Zn on grain yield, while the non-linear component ($p < 0.05$) shows the diminishing marginal returns of 90 kg ha^{-1} rate, relative to the response at 30 kg ha^{-1}. However, there was no significant differences among the soil types ($p > 0.05$). Furthermore, an interaction of linear response of Zn fertilizer rate with soil type ($p > 0.05$) and the non-linear response with soil type ($p > 0.05$) was not significant. This, therefore, suggests that maize grain yield response to Zn fertilizer rate did not depend on soil type.

Table 1. ANOVA output table for maize grain yield, grain Zn concentrations, grain Zn uptake, stover Zn concentrations, stover Zn uptake and natural log of Zn harvest index at Chitala and Chitedze agricultural research stations

			Grain yield		Grain Zn conc.		Grain Zn uptake		Stover Zn conc.		Stover Zn uptake		Zn HI	
Factor	Num DF	Den DF	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Soil type	1	1	1.3271	0.4551	0.1587	0.7586	0.3681	0.6528	2.56236	0.3555	0.16819	0.7522	0.085	0.8194
Zn lin	1	76	70.7532	<.0001	38.5574	<.0001	53.4562	<.0001	81.4031	<.0001	80.6168	<.0001	71.68	<.0001
Zn rem	1	76	47.6878	<.0001	8.4122	0.0049	21.2399	<.0001	1.497	0.2249	1.01045	0.318	1.922	0.1697
Soil type ● Zn lin	1	76	0.0474	0.8283	7.1444	0.0092	7.5350	0.0075	0.9747	0.3266	0.1837	0.6694	0.526	0.4705
Soil type ● Zn rem	1	76	0.9745	0.3267	0.0473	0.8283	1.0339	0.3125	0.01479	0.9035	0.00125	0.9719	0.465	0.4972

A dot,●, denotes interaction; Zn lin =linear effect of Zn application rate and Zn rem = non-linear effect of Zn application rate; Num DF = Numerator degrees of freedom, Den DF = Denominator degrees of freedom

Effect of residual soil Zn on maize grain Zn concentration and uptake

The grain Zn concentrations and uptake for each fertilizer rate at all experimental sites are presented in Fig. 2b and 2c, along with the standard errors calculated for each treatment level. The uptake of residual Zn from soil was clearly observed in the subsequent maize crop. As observed for grain yield, positive responses of grain Zn concentration and uptake to residual Zn fertilizer were apparent. The overall mean grain Zn concentrations at 1, 30 and 90 kg ha^{-1} Zn fertilizer rate were 22.6, 26.1 and 27.4 mg kg^{-1} respectively, with their standard errors, for the three Zn fertilizer rates as estimated in the LMM. Similarly, maize grain Zn uptake at 1, 30 and 90 kg ha^{-1} Zn fertilizer rate were 149, 195 and 205 g ha^{-1} respectively. The estimated additional grain Zn concentration and uptake arising from residual soil Zn following the 30 kg ha^{-1} were ~3.5 mg kg^{-1} (approximately 13% higher than for 1 kg ha^{-1}) and ~45 g ha^{-1} (approximately 30% higher than for 1 kg ha^{-1}), respectively; no further significant increases were observed when the Zn fertilizer rate was increased to 90 kg ha^{-1}.

The ANOVA for maize grain Zn concentration and Zn grain uptake are presented in Table 1. There was a significant response of maize grain Zn concentration and uptake to Zn fertilizer rate for the linear ($p < 0.05$) and non-linear ($p < 0.05$) components of the response. Over all sites, there was no evidence for differences in grain Zn concentration ($p > 0.05$) and grain Zn uptake ($p > 0.05$) between soil types. The linear response was noticed when the Zn fertilizer rate was increased from 1 to 30 kg ha^{-1} whereas increasing Zn application from 30 to 90 kg ha^{-1} resulted in a non-linear response. Thus, increasing the Zn fertilizer rate from 1 to 30 kg ha^{-1} results in a proportional increase in maize grain Zn concentration and uptake from residual Zn in the subsequent growing season, while an increase from 30 to 90 kg ha^{-1} results in a proportionally smaller increase in grain Zn concentration and uptake. Furthermore, the interaction of soil type and linear response for grain Zn concentration ($p < 0.05$) and grain Zn uptake (($p < 0.05$) was significant. For both response variables, no significant differences ($p > 0.05$) in grain Zn concentration and ($p > 0.05$) in grain Zn uptake were observed with the interaction of non-linear response and soil types. This suggests that maize grain Zn concentration and uptake depended on soil type when the rate was increased from 1 to 30 kg ha^{-1} while from 30 to 90 kg ha^{-1}, soil type did not have any effect over all sites.

Effect of residual soil Zn on maize stover Zn concentration and uptake

The results on the effect of residual available Zn for each Zn fertilizer rate on stover Zn concentration and uptake at all experimental sites are shown in Fig. 2d and 2e. When the main effect of Zn fertilizer rate was partitioned into linear and non-linear components, a positive response of stover Zn concentration and uptake to Zn fertilizer rate was observed at all sites. The stover Zn concentrations at applications of 1, 30 and 90 kg ha^{-1} were 27.8, 56.5 and 92.3 mg kg^{-1}, respectively while the stover Zn uptake at these rates were 254, 528 and 900 g ha^{-1}, respectively. Thus, over all sites, increasing the Zn fertilizer rate from 1 to 90 kg ha^{-1} resulted in a linear correlation between the fertilizer rate and stover Zn concentration and stover Zn uptake.

The ANOVA output for stover Zn concentration and uptake are presented in Table 1. There was a significant response of stover Zn concentration and uptake to Zn fertilizer rate for the linear ($p < 0.05$) response. However, the non-linear response was not statistically significant for both stover Zn concentration ($p > 0.05$) and stover Zn uptake ($p > 0.05$). Similarly, over all sites, there was no evidence for differences in stover Zn concentration ($p > 0.05$) and stover Zn uptake ($p > 0.05$) between soil types. There was no significant interaction of the linear response ($p > 0.05$) and non-linear component of the response ($p > 0.05$) with soil type in stover Zn concentration over all sites. Similarly, there was no evidence for an effect of the interaction of linear ($p > 0.05$) and non-linear responses ($p > 0.05$) with soil type in stover Zn uptake. This suggests that stover Zn concentration and uptake did not depend on soil type when the rate was increased from 1 to 90 kg ha^{-1}.

Effect of soil type and residual Zn fertilizer on Zn harvest index

The mean Zn harvest indices (Zn_{HI}) for each fertilizer rate at all sites are presented in Fig. 2f, accompanied by their standard errors estimated for each treatment level. The effects of soil type, Zn fertilizer rate and their interaction on Zn_{HI} were analyzed using the LMM. Prior to analysis, Zn_{HI} was tested for normality of the residuals and the outputs showed a skewed distribution and, therefore the response variable was transformed to natural logarithm values. After the transformation, the assumption of a normal distribution and homogeneity of variances of the residuals were valid. Generally, mean Zn_{HI} decreased for all the soil types in response to the increase of Zn fertilizer rate (Fig. 3). Note that no statistical inference about soil type at each site could be made since soil type was not replicated within each experimental site. There was no observed effect in Zn_{HI} between soil types when the rate was increased from 1 to 30 kg ha^{-1} while at 90 kg ha^{-1} the decrease was statistically different. The observed variations in Zn_{HI} response to Zn fertilizer rate over all sites might be due to differences in soil physical and chemical behaviour.

Table 1 shows the ANOVA output for the natural log of Zn harvest index. There was strong evidence for an effect on Zn_{HI} of Zn fertilizer rate for the linear ($p < 0.05$) component of the response. However, there was no evidence for an effect on Zn_{HI} of Zn fertilizer rate for the non-linear ($p > 0.05$) component of the response. Normally, when the rate was increased from 1 to 90 kg ha^{-1}, there was a negative response for both linear and non-linear components. The observed reduction in Zn_{HI} with Zn fertilizer rate shows that Zn partitioning efficiency to the grain was negatively affected by the physiological response of the crop to Zn availability in the soil through reduction of Zn uptake by the crop roots. Over all sites, there were no differences in Zn_{HI} reduction as the Zn fertilizer rate was increased from 1 to 30 kg ha^{-1} while noticeable differences were observed as the rate was increased from 30 to 90 kg ha^{-1}.

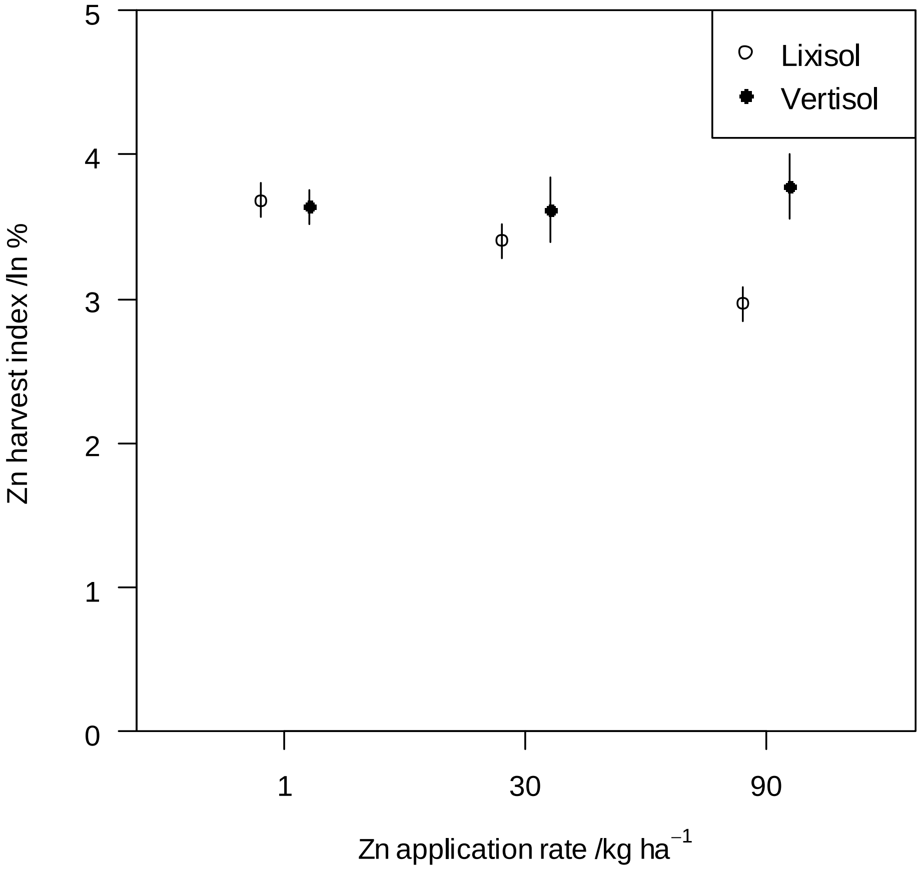

Fig. 3. Mean Zn harvest index of maize in response to residual Zn fertilizer during the 2020-21 cropping season. The error bars show the standard error of the mean (±SEM).

Discussion

Residual Zn fertilizer improved maize grain yields and economic returns

Increased maize grain yield from residual available Zn in soil has been reported previously (Soleimani, 2012). For example, Mari *et al.*, (2015) observed that maize grain yield increases were essentially consistent during the second year of maize cropping in Brazil when Zn fertilizer application rates of 2, 4 and 8 kg Zn ha^{-1} were used. These findings are supported by (Boawn, 1974) who reported pronounced residual effects in terms of plant Zn uptake and increased plant extractable Zn up to 6 years after Zn application when 5.6, 11.2, 16.8 and 22.4 kg ha^{-1} application rates were used. Similarly, the findings of the present study show the positive effect of residual Zn fertilizer on maize grain yields. At a fertilizer rate of 30 kg ha^{-1}, maize grain yields increased by 25% over the national recommendation rate of 1 kg ha^{-1}. This translates to about 1500 kg ha^{-1} of additional grain produced when the Zn fertilizer rate was increased from 1 to 30 kg ha^{-1}.

The residual effect of Zn fertilizer offers potential economic and food security benefits to the farmer. In this study, a minimum annual benefit (minimum additional income for the farmer) of about MK330,000 ha^{-1} (~\$330 ha^{-1}) was estimated, which is much higher than the annual benefit reported previously (Botoman *et al.*, 2022b). Note that the return on yield was calculated using the minimum farm gate maize price of MK220 kg^{-1} by the government. However, the annual benefit might be higher than estimated in the current study as the price of maize varies with location and period of the year. In our previous study, ~660 kg ha^{-1} additional grain was obtained when the Zn fertilizer rate was increased from 1 to 30 kg ha^{-1} (Botoman *et al.*, 2022b). This indicates that more annual benefit can be realized in the subsequent season from the residual available Zn.

Residual Zn fertilizer could help improve the food security situation of farmers. Maize grain yields obtained at a Zn application rate of 1 kg ha^{-1} were less than the yields obtained at 30 kg ha^{-1} in the subsequent cropping season. This suggests that the residual plant-available Zn is very low in the subsequent cropping season following application of 1 kg ha^{-1} in the previous season. The formation of Zn complexes with organic matter and adsorption of the element on Fe and Mn oxides and aluminosilicate clays might explain why application of 1 kg ha^{-1} does not result in a residual yield benefit (Catlett *et al.*, 2002; Hernandez-Soriano and Jimenez-Lopez, 2012; Rutkowska *et al.*, 2015). The effect of Zn adsorption in soil is likely to have a smaller effect on Zn availability at a Zn fertilizer rate of 30 kg ha^{-1}.

Residual effect of Zn fertilizer on maize crop Zn uptake

When the initial Zn fertilizer rate was 30 kg ha^{-1}, grain Zn concentration in the residual year was 13% greater than when Zn fertilizer rate was 1 kg ha^{-1}. This difference in grain Zn concentration is similar to the difference of 15% grain Zn concentration between these two treatments in the first year of application, as reported in our previous study (Botoman *et al.*, 2022b). Similarly, grain Zn uptake was greater by 30% when the initial Zn fertilizer rate was 30 kg ha^{-1}, compared to 1 kg ha^{-1} This was slightly higher than the differences in grain Zn uptake reported previously of 23%.

Data reported in the current study indicate that residual Zn fertilizer also increases stover Zn concentration and uptake. Increasing Zn fertilizer rate from 1 to 90 kg ha^{-1} resulted in a proportional increase of stover Zn concentration and uptake. This increase might benefit livestock farmers as ruminants could be fed with high Zn feedstock to improve the Zn nutritional status of the animals. Further, increased stover Zn concentration and uptake might benefit farmers who practice conservation agriculture (CA) encompassing residue incorporation to improve the Zn soil fertility status of their farms. In the medium and long term, this might reduce Zn fertilizer related costs, thus improving net economic returns to farmers.

Residual Zn fertilizer affected maize grain Zn partitioning efficiency

Zinc harvest index (Zn_{HI}), which shows the grain Zn partitioning efficiency of the crop, was estimated. Increasing Zn fertilizer application rates resulted in significant decreases in Zn_{HI}. The grain Zn loading efficiencies at 1, 30 and 90 kg ha^{-1} were 40%, 30% and 22%, respectively. The decrease of 10% in grain Zn loading efficiency when the Zn fertilizer application rate was increased from 1 to 30 kg ha^{-1}, which is consistent with the first season (Botoman *et al.*, 2022b). However, when the rate was increased from 30 to 90 kg ha^{-1}, the loading efficiency decreased to 22% compared to 30% in the previous study. This observation might be attributed to reduction in residual available Zn at lower application rates due to the effect of Zn^{2+} ion interaction with soil geocolloids. The residual Zn is likely to have been subjected to losses by leaching and fixation into unavailable forms. The findings in this study are consistent with those reported by Liu *et al.*, (2019) where the Zn_{HI} of maize grown in China under field conditions decreased from 74% to 52% when Zn fertilizer rates were increased from 2.3 to 34.1 kg ha^{-1}. The unusual delivery of Zn to the root xylem could be a possible cause for the observation where it is reported that xylem loading and unloading of Zn is suppressed by high levels of available Zn (Curie *et al.*, 2009; Palmer and Guerinot, 2009).

Conclusions

This study was designed to assess the effect of residual Zn fertilizer on improvement in grain Zn nutritional quality of maize grown under two contrasting soil types in Malawi. The results showed that both grain yield and Zn uptake of maize significantly increased with initial Zn fertilizer rate. Further, the results revealed that the response of maize to residual Zn fertilizer remained essentially unchanged in the subsequent cropping season. The response of maize to residual Zn fertilizer did not depend on soil type. The increase in grain Zn concentration from the residual Zn fertilizer could help reduce Zn deficiency among the rural populations of developing countries such as Malawi. This implies that farmers may not need to apply Zn fertilizer every cropping season. In the medium and long term, this might reduce Zn fertilizer related costs, thus improving net economic returns to farmers.

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Declaration of Conflict of Interest

Authors have no conflict of interest to declare regarding the reported work.

Data availability statement

Data associated with this article are available in the online Supplementary Materials.

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Supplementary information

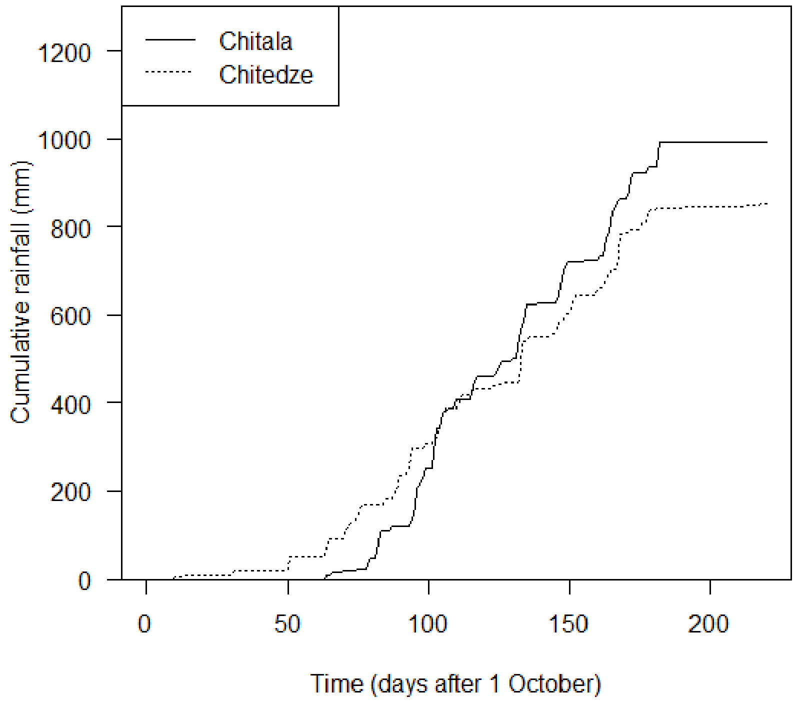

Fig. S1. Rainfall distribution (mm) at Chitala and Chitedze Agricultural Research Stations during the 2020-21 cropping season.

Exploratory analysis of model residuals

Table S1. Summary statistics of data on maize grain yield (kg ha^{-1})

```
                  Mean  Median  Quartile.1  Quartile.3  Variance      SD  Skewness
Experiment-level     0  -22.00     -499.25      504.70  665397.2  815.72     -0.02
Site-level           0  -39.18     -487.87      528.79  616361.2  785.09      0.01
Subsite-level        0  -39.18     -487.87      528.79  616361.2  785.09      0.01
Block-level          0  -22.53     -418.02      473.10  425469.5  652.28     -0.07
                 Octile skewness  Kurtosis  No. outliers
Experiment-level            0.05     -0.09             0
Site-level                  0.08     -0.33             0
Subsite-level               0.08     -0.33             0
Block-level                 0.10     -0.50             0
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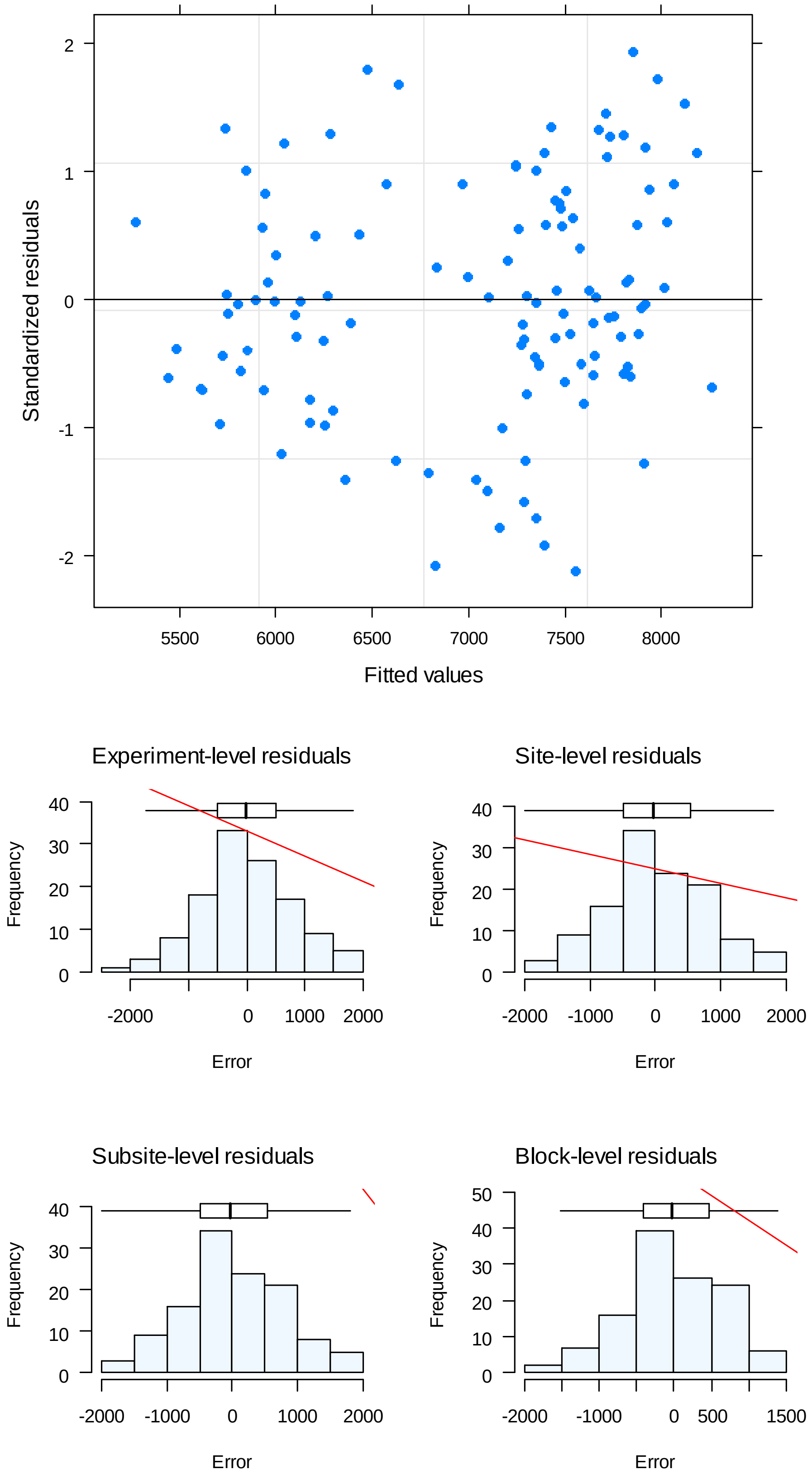

Fig. S2. Residuals against fitted values and histogram for the residuals of the random effects for maize grain yield

Table S2. Summary statistics of data on maize grain Zn concentration (mg kg^{-1})

```
                     Mean Median Quartile.1 Quartile.3 Variance   SD Skewness

Experiment-level     0  -0.24      -2.03        1.60     10.68 3.27      0.26

Site-level           0  -0.49      -1.78        1.60      9.90 3.15      0.28

Subsite-level        0  -0.43      -1.75        1.61      9.71 3.12      0.32

Block-level          0  -0.43      -1.75        1.61      9.71 3.12      0.32

                  Octile skewness Kurtosis No. outliers

Experiment-level             0.18      0.03            0

Site-level                   0.25     -0.01            0

Subsite-level                0.17      0.00            0

Block-level                  0.17      0.00            0
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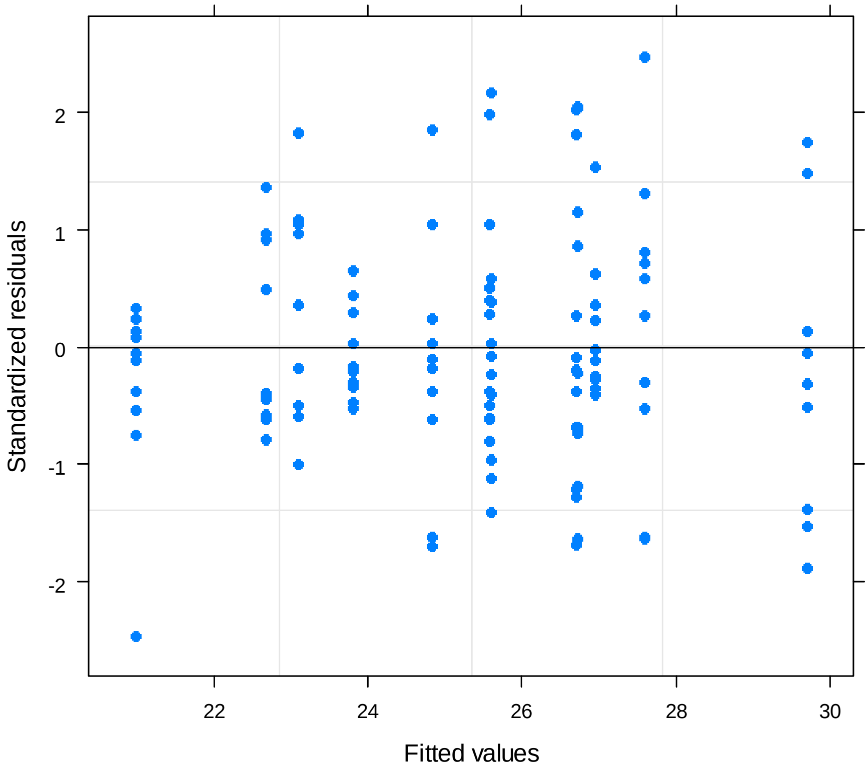


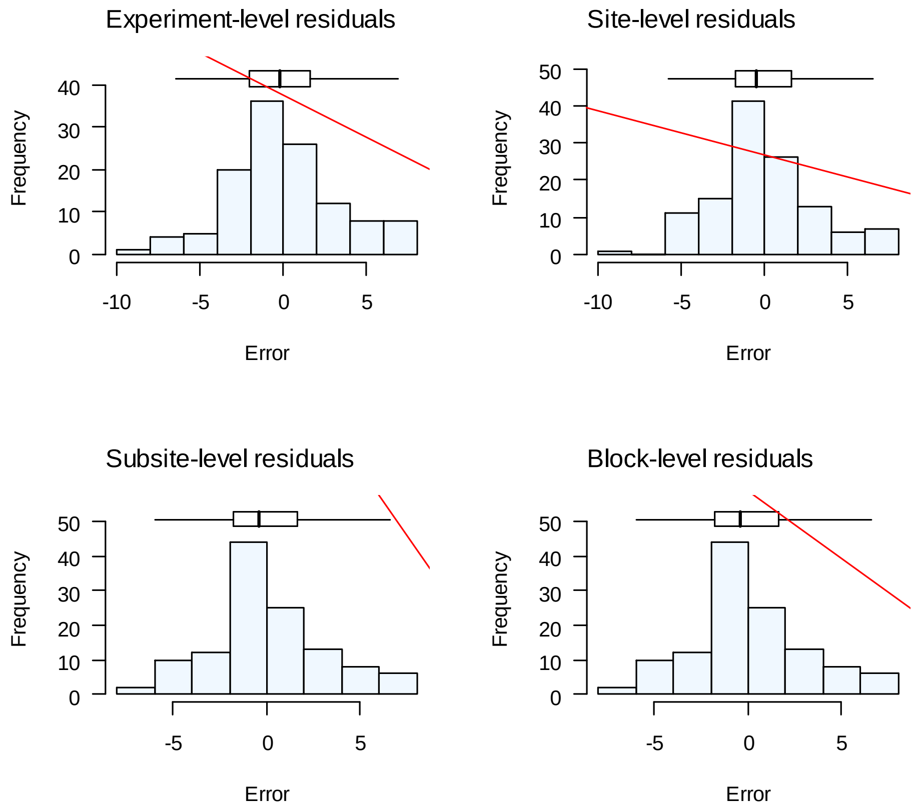


Fig. S3. Residuals against fitted values and histogram for the residuals of the random effects for concentration of Zn in grain

Table S3. Summary statistics of data on maize grain Zn uptake (g ha^{-1})

	Mean	Median	Quartile.1	Quartile.3	Variance	SD	Skewness	Octile skewness	Kurtosis	No. outliers
Experiment-level	0	-1.25	-21.14	23.21	943.92	30.72	0.12	0.08	0.01	0
Site-level	0	-2.94	-20.98	21.78	918.00	30.30	0.07	0.13	0.16	0
Subsite-level	0	-2.94	-20.98	21.78	918.00	30.30	0.07	0.13	0.16	0
Block-level	0	-0.83	-20.38	20.40	870.37	29.50	0.06	0.06	0.18	0

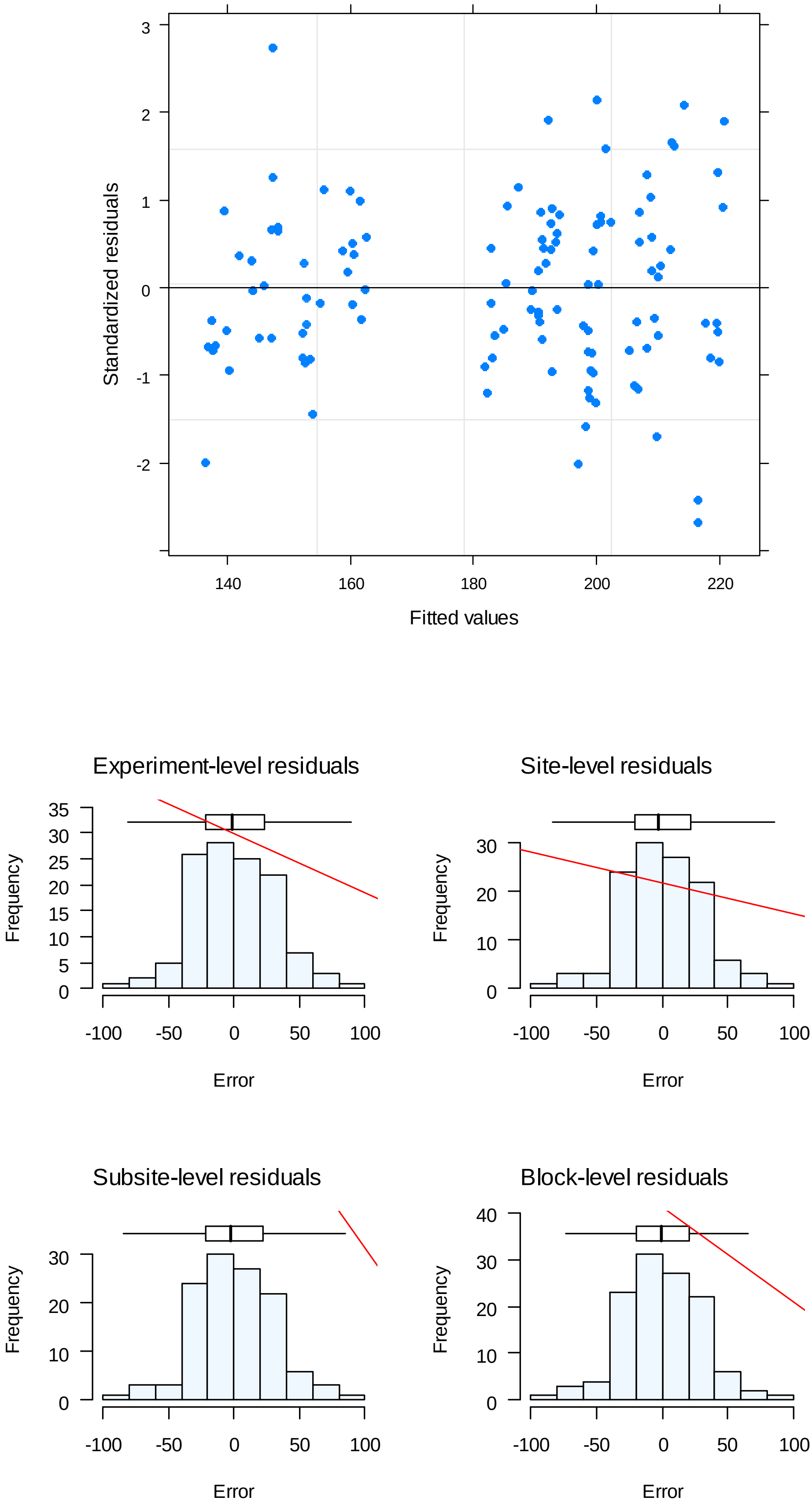


Fig. S4. Residuals against fitted values and histogram for the residuals of the random effects for grain Zn uptake

Table S4. Summary statistics of data on maize stover Zn concentration (mg kg^{-1})

```
                 Mean Median Quartile.1 Quartile.3 Variance    SD Skewness

Experiment-level    0  -6.17     -14.95       8.83   985.68 31.40     1.19

Site-level          0  -4.56     -14.91       7.37   961.35 31.01     1.08

Subsite-level       0  -4.56     -14.91       7.37   961.35 31.01     1.08

Block-level         0  -4.56     -14.91       7.37   961.35 31.01     1.08

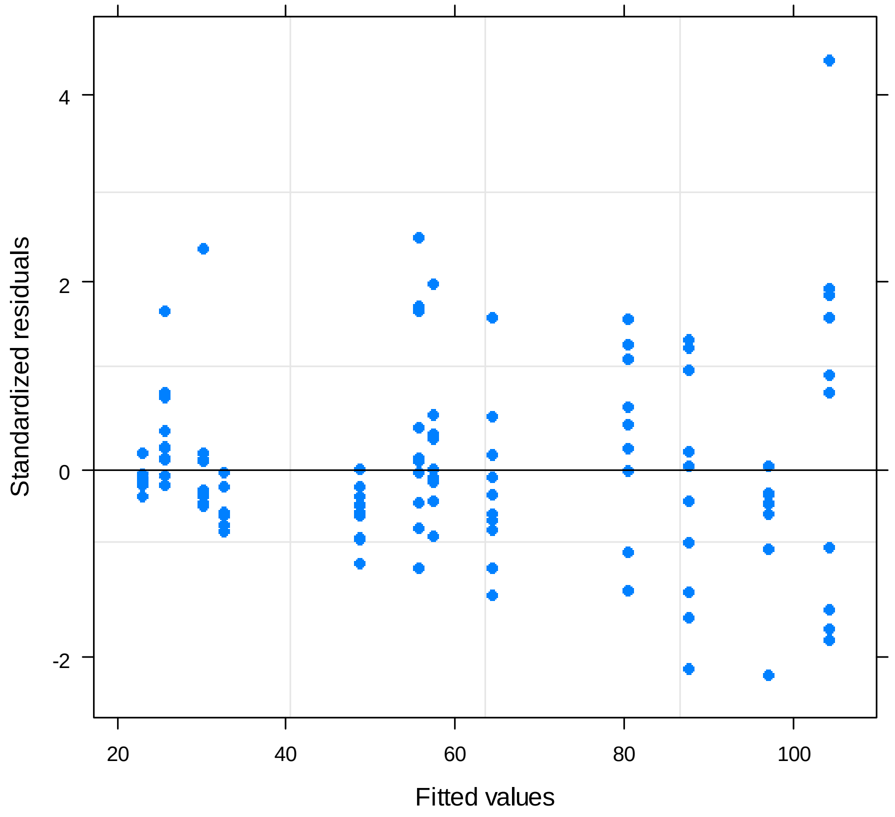
                Octile skewness Kurtosis No. outliers

Experiment-level           0.30     3.08            2

Site-level                 0.32     2.85            3

Subsite-level              0.32     2.85            3

Block-level                0.32     2.85            3
```



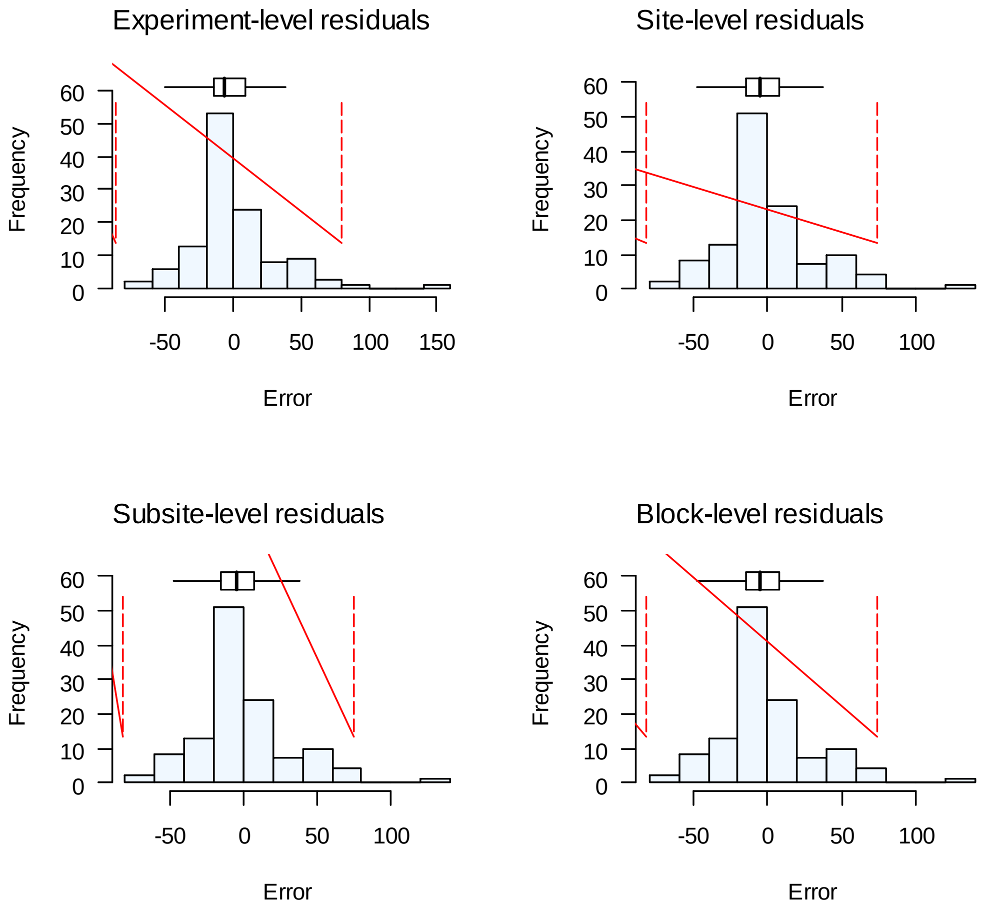


Fig. S5. Residuals against fitted values and histogram for the residuals of the random effects for concentration of Zn in stover

Table S5. Summary statistics of data on maize stover Zn uptake (g ha^{-1})

```
                   Mean Median Quartile.1 Quartile.3 Variance      SD Skewness

Experiment-level     0 -63.95    -153.71      109.66 102998.85 320.93     1.24

Site-level           0 -25.37    -158.23      99.71   97956.81  312.98     1.04

Subsite-level        0 -25.37    -158.23      99.71   97956.81  312.98     1.04

Block-level          0 -25.37    -158.23      99.71   97956.81  312.98     1.04

                 Octile skewness Kurtosis No. outliers

Experiment-level            0.32     3.15            1

Site-level                  0.17     2.84            1

Subsite-level               0.17     2.84            1

Block-level                 0.17     2.84            1
```

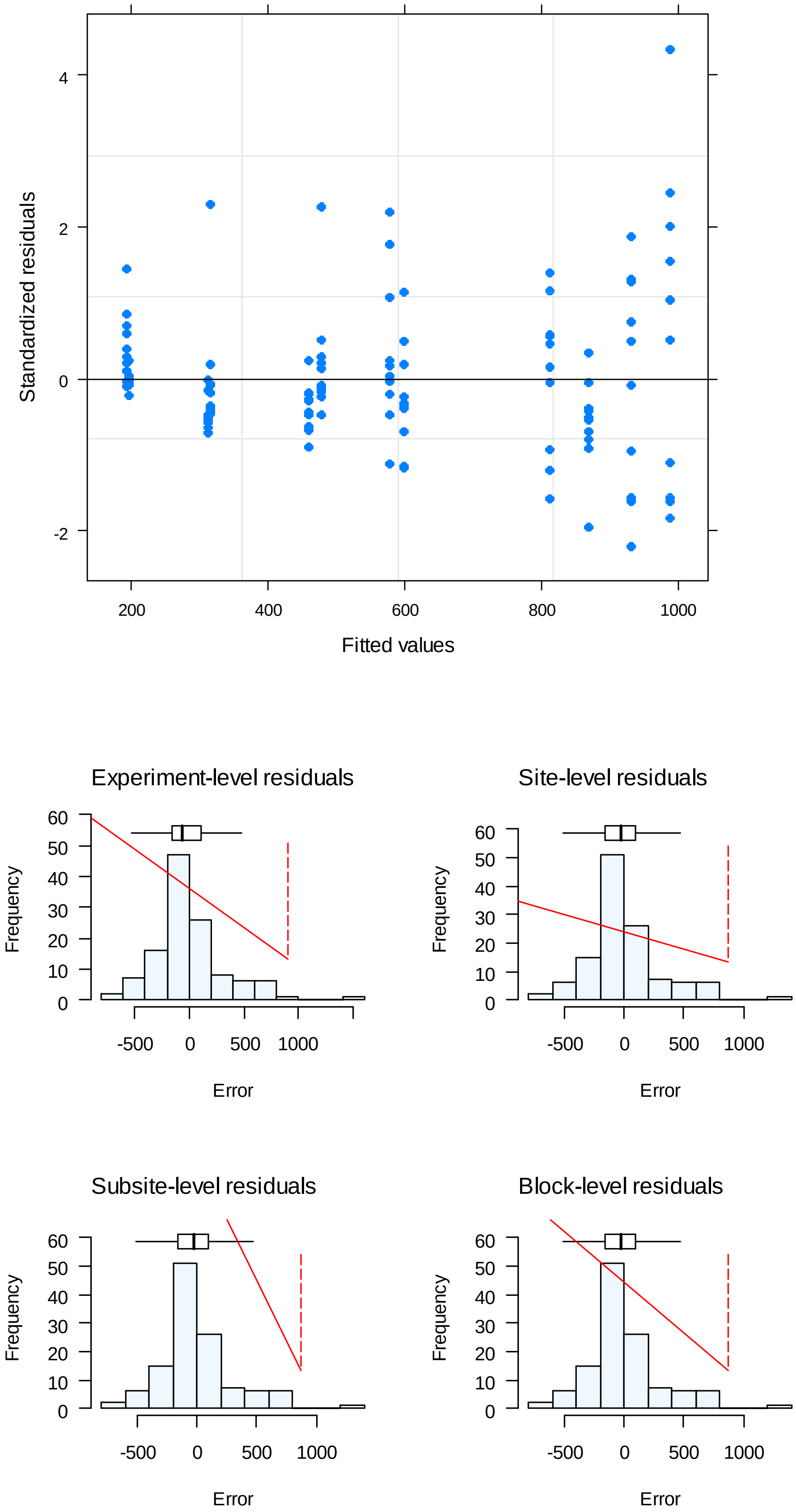


Fig. S6. Residuals against fitted values and histogram for the residuals of the random effects for stover Zn uptake

Table S6. Summary statistics of data on Zn harvest index (ln %)

```
                  Mean Median Quartile.1 Quartile.3 Variance   SD Skewness

Experiment-level     0   0.06      -0.26       0.24     0.13 0.35    -0.34

Site-level           0   0.06      -0.25       0.24     0.12 0.35    -0.32

Subsite-level        0   0.01      -0.22       0.23     0.12 0.34    -0.20

Block-level          0   0.01      -0.22       0.23     0.12 0.34    -0.20

                 Octile skewness Kurtosis No. outliers

Experiment-level           -0.17     0.15            0

Site-level                 -0.18     0.14            0

Subsite-level              -0.08     0.19            0

Block-level                -0.08     0.19            0
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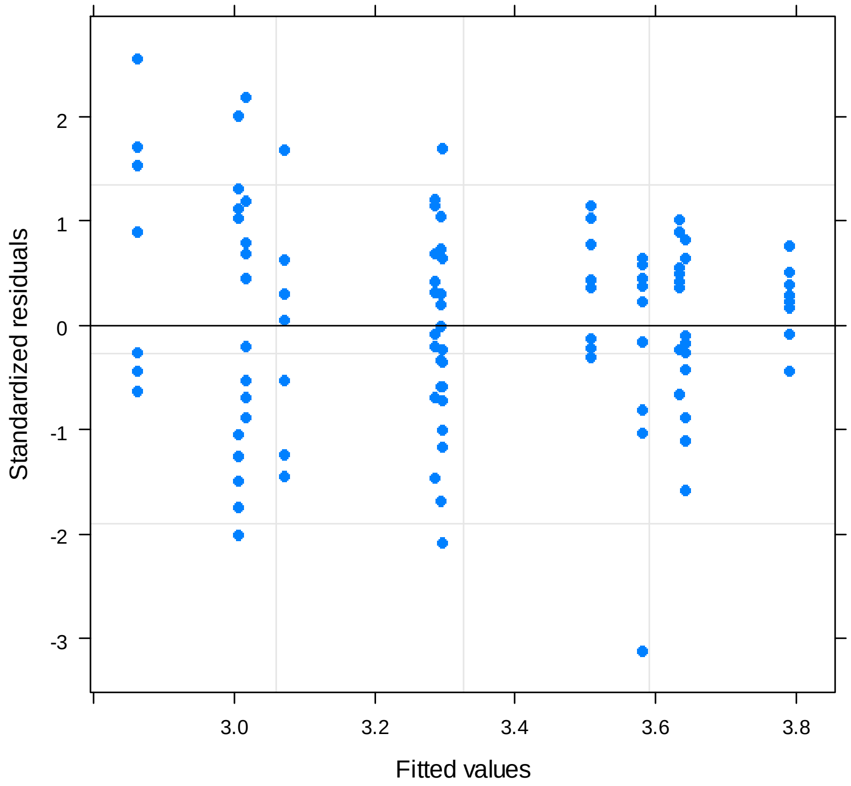

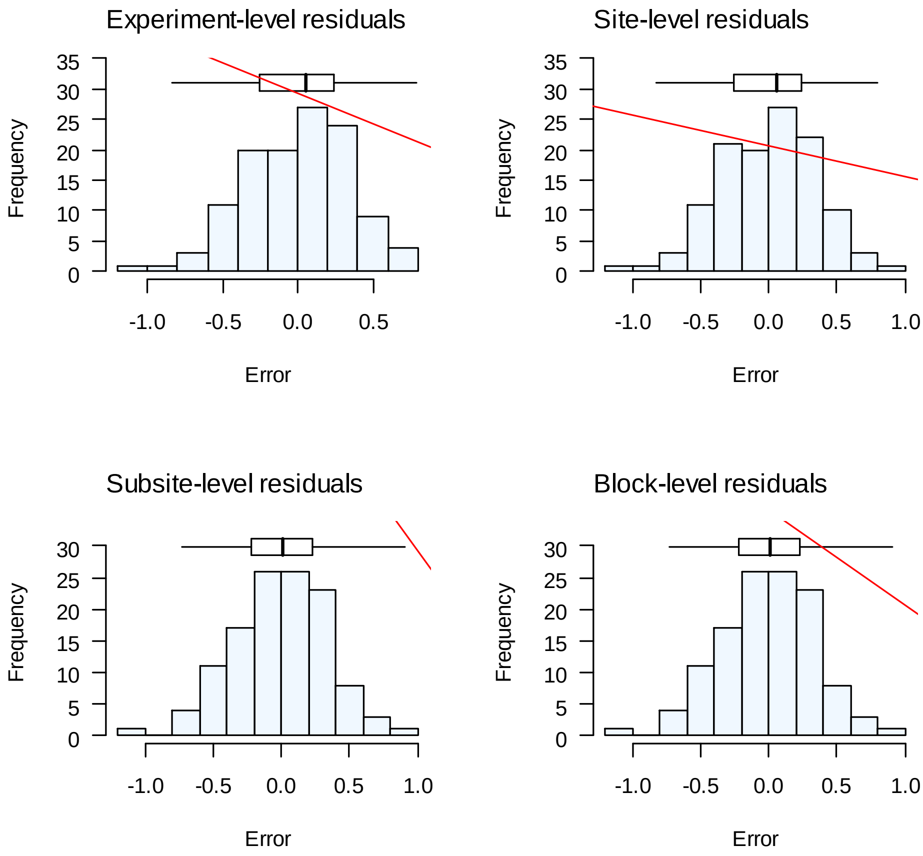

Fig. S7. Residuals against fitted values and histogram for the residuals of the random effects for Zn harvest index