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Zinc lability and solubility in soils of Ethiopia—an isotopic dilution study

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ABSTRACT

Zinc (Zn) deficiency is a widespread nutritional problem in human populations, especially in sub-Saharan Africa (SSA). The Zn concentration of crops consumed depends in part on the Zn status of soil. Improved understanding of factors controlling the phyto-availability of Zn in soils can contribute to potential agronomic interventions to tackle Zn deficiency, although there are major knowledge gaps for many soil types in SSA.

Soil samples (n = 475) were collected from a large part of the Amhara Region of Ethiopia where there is widespread Zn deficiency. Zinc status was quantified by measuring several fractions: pseudo-total (Aqua-Regia digestion; Zn_{Tot}), available (DTPA-extractable; Zn_{DTPA}), soluble (dissolved in 0.01 M $Ca(NO_3)$; Zn_{Soln}) and isotopically exchangeable Zn using the enriched stable Zn isotope ^{70}Zn (Zn_E). Soil geochemical properties were assessed for their influence on Zn lability and solubility.

Zn_{Tot} ranged from 14.1 to 291 mg kg^{-1} (median = 100 mg kg^{-1}) whereas Zn_{DTPA} in the majority of soil samples was less than 0.5 mg kg^{-1} indicating widespread phytoavailable Zn deficiency in these soils. The labile fraction of Zn in soil (Zn_E as $\%Zn_{Tot}$) was low, with median and mean values of 4.7% and 8.0 % respectively. Labile Zn partitioning between the solid and the solution phases of soil was highly pH-dependent where 94% of the variation in the partitioning coefficient of ^{70}Zn was explained by soil pH. Similarly, 86% of the variation in Zn_{Soln} was explained by soil pH.

Zinc distribution between adsorbed Zn_E and Zn_{Soln} was pH controlled. Notably, Zn isotopic exchangeability increased with soil pH. This contrasts with literature on contaminated and urban soils and may arise from covarying factors such as contrasting soil clay mineralogy across the pH range of

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the soils used in the current study. These results could be used to improve agronomic interventions to tackle Zn deficiency in SSA.

1. Introduction

Zinc deficiency is a widespread nutritional disorder affecting ~17% of the global population, and rising to 25% of the population in countries within sub-Saharan Africa (SSA) (Kumssa et al., 2015; Wessells and Brown, 2012). Several interlinked causes contribute to the prevalence of Zn deficiency issues in SSA, including lack of access to animal source foods. This can lead to inadequate Zn intake if the diet is heavily reliant on staple crops which are inherently low in mineral micronutrients (Joy et al., 2014; Kumssa et al., 2015). Soil degradation and a lack of access to micronutrient fertilizers can contribute to the production of staple crops with poor nutritional quality (Kihara et al., 2020). Three-quarters of the arable land in SSA is reported to be depleted in plant nutrients and low in fertility (Toenniessen et al., 2008).

Phyto-availability of Zn in soil is largely controlled by a dynamic equilibrium between the solid phase and pore water and the absorption mechanisms of plant roots (Groenenberg et al., 2010; Menzies et al., 2007; Peng et al., 2020). Traditionally, chemical extraction procedures used to estimate an assumed 'phyto-available' pool of soil Zn have included reagents which vary widely in extraction power such as water, neutral salt solutions, dilute strong acids and chelating agents such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA; Kim et al., 2015). However, these approaches cannot fully characterise both the 'quantity' of potentially available Zn in the soil solid phase and its 'intensity' in the soil solution phase—both of which contribute to the phyto-availability of Zn over the course of a growing season. Isotopic dilution assays may provide a more mechanistically-based characterization of the geochemically reactive fraction of Zn in soils which buffers the free ion activity in the soil solution phase (Guzman-Rangel et al., 2020; Hamon et al., 2008; Young et al., 2005). This approach has been extensively used to study contaminated soils (Degryse et al., 2011; Izquierdo et al., 2013; Mossa et al., 2020; Nolan et al., 2005) but its application to Zn in agricultural soils generally, and especially in the soils of SSA countries, is very limited.

The aim of the present study was to investigate the status of Zn lability in soils from a large part of the Amhara Region of Ethiopia which represent a diverse range of soil types from SSA (Gashu et al., 2020), in which Zn deficiency is thought to be widespread (Hengl et al., 2017). The study used several assays of soil Zn status including an isotopic dilution assay, employing enriched ^{70}Zn, to examine the soil properties that control Zn phyto-availability. The primary objectives were: (i) to determine isotopically exchangeable Zn in soils from the Amhara Region; (ii) to compare different assays of Zn status; (iii) to examine the influence of soil properties on Zn partitioning between the solid and solution phases of these soils.

2. Material and methods

2.1. Soil sampling

Field sampling is described in detail in Gashu et al., (2020). Briefly, topsoil was collected from a target of 475 locations in the Amhara Region of Ethiopia according to a geospatial design intended to explore spatial variation in soil and crop properties. The sample frame was constrained to sites where the probability that the land was in agricultural use was ≥0.9. At each sampling location, five sub-samples of topsoil were collected from a 100 m^2 circular plot using a Dutch auger with a flight length of 150 mm and a diameter of 50 mm. Plant material was removed and the five sub-samples were combined, oven-dried at 40 °C for 24–48 hours, sieved to <2 mm and homogenised prior to analysis.

2.2. Geochemical analysis

Soluble trace and major metallic elements (M_{Soln}) were determined in the solution phase of soil suspensions in 0.01 M $Ca(NO_3)_2$ (1:10 soil:solution ratio) following equilibration for four days on an end-over-end shaker. The pH of this soil suspension (pH_{Ca}) was determined then solutions were isolated by centrifugation and filtration (<0.22 µm) prior to elemental analysis by ICP-MS (iCapQ; Thermo Fisher Scientific, Bremen, Germany). Total carbon content was determined by dry combustion (Tiessen et al., 1981) using a Leco TruMac CN Combustion analyser and inorganic C was measured using an Inorganic Carbon Analyser- Skalar Primacs (Skalar Analytical BV, Breda, Netherlands). Dissolved organic carbon (DOC) was estimated by measuring non-purgeable organic carbon using a Shimadzu TOC-VWP analyzer (Shimadzu Corporation, Kyoto, Japan). Estimates of amorphous and poorly crystalline oxides were obtained following extraction with a mixture of ammonium oxalate and oxalic acid at a 1:100 of soil:solution suspension (Schwertmann, 1964). Samples were shaken in the dark at 20 °C for 4 hours on a reciprocal shaker (120 rpm), then filtered (Whatman No 42), diluted and acidified to 5% HNO_3, and analysed using inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin Elmer Life and Analytical, Shelton, USA). The effective cation exchange capacity (eCEC) was determined using the cobalt hexamine (Cohex) method (ISO 23470; 2018). DTPA-extractable zinc (Zn_{DTPA}) was solubilized by shaking 5 g soil with 10 mL of 0.005 M DTPA, 0.1 M triethanolamine (TEA) and 0.01 M $CaCl_2$ at pH = 7.3 for 2 h on an end-over-end shaker (Lindsay and Norvell, 1978). The soil suspensions were then centrifuged and filtered (<0.22 µm) prior to analysis using ICP-MS (iCAP-Q; Thermo Fisher Scientific, Bremen, Germany). The pseudo-total Zn concentration in soil (Zn_{Tot}) was determined after digesting finely ground soil sample with aqua-regia (Crosland et al., 1995) and analysis using ICP-OES.

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2.3. Isotopic dilution assays

To determine the concentration of isotopically exchangeable Zn, 2.0 g of soil was equilibrated with 20 mL of 0.01 M $Ca(NO_3)_2$ on an end-over-end shaker for 24 h. This was followed by addition of 0.4 mL solution with a ^{70}Zn concentration of 11.9 mg L^{-1} of ^{70}Zn which provided an amount of ^{70}Zn equivalent to 2.3% of the average Zn_{Tot} (104 mg kg^{-1}) concentration in soil. The isotopic tracer solution was prepared from a stock solution enriched with ^{70}Zn (250 mg L^{-1}; isotopic abundance (IA) = 95.47%). To avoid acidifying the soil suspension, the pH of the spiking solution was adjusted to pH 4.0–4.5 using an ammonium acetate buffer immediately before use. After spiking with the isotope, samples were re-equilibrated for a further 3 days, then centrifuged (3500 rpm for 15 min), filtered (< 0.22 µm), and acidified to 2% HNO_3 prior to isotopic analysis using ICP-MS (iCAP-Q; Thermo Fisher Scientific, Bremen, Germany). The instrument was operated in 'collision cell mode' using He with kinetic energy discrimination (KED). Rhodium (^{103}Rh; 10 µg L^{-1}) was used as an internal standard to correct for instrumental drift. The ICP-MS was calibrated for individual Zn isotopes (^{66}Zn and ^{70}Zn) using a multi-isotope Zn standard (CLMS2; SPEX CertiPrep). In practice, it was found that the measurement of ^{70}Zn in the supernatant solution of the soil suspensions required two corrections due to significant, and variable, concentrations of soil-derived $^{70}Ge^{+}$ and (plasma-generated) doubly-charged $^{140}Ce^{++}$ (m/z = 70). The correction for ^{70}Ge (IA = 69.9%) was achieved by determining the intensity (count per second (CPS)) of ^{72}Ge in samples and using the measured CPS ratio 72/70 for Ge standards to infer the proportion of the intensity at m/z 70 arising from ^{70}Ge. The universal isotopic ratio $^{72}Ge/^{70}Ge$ is 1.34; the measured intensity ratio in a Ge standard (including error due to mass discrimination) was typically 1.53. The correction for doubly-charged $^{140}Ce^{++}$ was implemented by running Ce standards, which typically produced a 70/140 intensity ratio of 0.025, and measuring m/z 140 on samples. The Ce standards were analysed in three concentrations of NaCl (0, 1, and 10 mg L^{-1}) to confirm minimal change in the generation of doubly-charged Ce in the plasma with alkali cation concentration. The correction for ^{70}Ge produced a change in Zn_E that ranged from 0.027 mg kg^{-1} to 0.976 mg kg^{-1} (median = 0.253 mg kg^{-1}) while for $^{140}Ce^{++}$ the change was 0.024 mg kg^{-1} to 0.973 mg kg^{-1} (median = 0.747 mg kg^{-1}).

The E-value of Zn (Zn_E; mg kg^{-1}) was calculated from Eq. 1:

$$Zn_E = Zn_{Soln}\left(Kd_{Lab} + \frac{L}{S}\right) \qquad (1)$$

Where Zn_{Soln} is the measured concentration of Zn of an equilibrated soil suspension; $\frac{L}{S}$ the liquid to solid ratio (L kg^{-1}); Kd_{Lab} is the distribution coefficient (L kg^{-1}) of the added ^{70}Zn isotope spike between a weight of soil (S; kg) and volume of liquid (L) and is calculated as shown in Eq. 2.

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$$Kd_{Lab} = \frac{^{70}Zn_{Ads}}{^{70}Zn_{Soln}} \qquad (2)$$

The variable $^{70}Zn_{ads}$ is the adsorbed ^{70}Zn spike (µmol kg^{-1}) and was calculated as the difference between the total ^{70}Zn added to the soil suspension and the amount of ^{70}Zn spike remaining in the solution after equilibration; $^{70}Zn_{Soln}$ is the measured concentration (µmol L^{-1}) of ^{70}Zn spike in solution after equilibration. Crucially, the value of $^{70}Zn_{Soln}$ was corrected for the presence of native ^{70}Zn in solution which was estimated from the measured concentration of ^{66}Zn and the known isotopic ratio ^{70}Zn:^{66}Zn; this was implemented after all analytical corrections (^{70}Ge and $^{140}Ce^{++}$) and calculation of the Zn isotope concentrations (µmol L^{-1}) from isotopic calibration. The measured ^{70}Zn was overwhelmingly (97.7% ± 2.20%) dominated by the added spike. Therefore, any deviation, in the native soil Zn of individual samples, from the expected isotopic ratio of ^{70}Zn:^{66}Zn would incur a negligible error.

2.4. Geochemical modelling using WHAM7

The geochemical model WHAM7 (Tipping, 1994) was used to speciate Zn in the solution phase of the 0.01 M $Ca(NO_3)_2$ suspensions. Inputs to the model included cation and anion concentrations, colloidal (dissolved) fulvic acid, pH, temperature and partial pressure of CO_2. Colloidal fulvic acid (FA) was estimated from NPOC assuming (i) a carbon content in FA of 50% and (ii) that 65% was 'active' (Lofts et al., 2008). Partial pressure of CO_2 (PCO_2) and the temperature were set to 0.004 atm and 25°C respectively. WHAM7 was also used to predict the labile pool of Zn (Zn_E) within the soil particulate phases. This required inclusion of suspended particulates, calculated from 2 g solid in 20 mL of electrolyte, and included Fe, Al and Mn oxides (estimated by oxalate extraction) and particulate humic acid which was estimated from soil organic C assuming 50% is 'active' humic acid (Buekers et al., 2008; Marzouk et al., 2013b).

2.5. Data analysis

Data analysis was carried out using R (version 4.0.2) software (R Core Team, 2020). Measured soil properties were related to Zn lability (Zn_E) and the labile distribution coefficient of ^{70}Zn (Kd_{Lab}) using standard least squares regression. Soil variables used in the regression were: soil pH (measured in the $Ca(NO_3)_2$ suspensions), organic C (%), sum of the concentration of Al, Fe and Mn in the oxalate extractions (mol kg^{-1}), dissolved organic C (mg L^{-1}) and the effective cation exchange capacity (eCEC; cmol$_C$ kg^{-1}). All data were checked for normality using the Shapiro-Wilk normality test and log-transformed when necessary.

3. Results and Discussion

3.1. General soil properties

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Most measured soil properties varied widely (Table 1). Soil pH ranged from 4.2–7.5 with ca. 70% soils having pH values below 6.0. The organic carbon (C_{Org}) also varied widely with a median value of 1.72% (Table 1). There was a 10-fold variation in eCEC, potentially indicating a large range of Zn binding strength within the studied soils.

Table 1. Selected properties for soil samples (n = 465).

	Minimum	Maximum	Median	Mean	Std.dev.
pH_{Ca}	4.2	7.5	5.5	5.6	0.8
Total N (%)	0.03	0.67	0.15	0.17	0.10
Total C (%)	0.27	8.40	1.70	1.90	1.20
C_{org} (%)	0.27	8.36	1.70	1.90	1.20
eCEC (cmolc kg^{-1})	4.96	55.8	33.5	32.2	12.6
Ca (cmolc kg^{-1})	3.12	42.8	21.0	20.5	9.39
K (cmolc kg^{-1})	0.05	4.03	0.49	0.61	0.48
Mg (cmolc kg^{-1})	0.91	21.20	6.97	7.35	4.06
Na (cmolc kg^{-1})	0.03	0.64	0.08	0.09	0.06
AlOx (mg kg^{-1})	969	18800	3600	4010	1940
FeOx (mg kg^{-1})	1030	3220	9500	9740	4980
MnOx (mg kg^{-1})	153	4690	1430	1500	686
Olsen P (mg kg^{-1})	1.40	178	10.6	17.6	21.6
Zn_{Tot} (mg kg^{-1})	14.1	291	100	102	24.3
Zn_{DTPA} (mg kg^{-1})	0.01	5.25	0.69	0.89	0.73
Zn_{E} (mg kg^{-1})	0.44	57.7	4.82	7.93	8.66
Zn_{soln} (mg kg^{-1})	0.007	0.789	0.032	0.106	0.141

To further illustrate the general status of Zn in the soils, histograms of different indices of Zn lability and solubility are presented in Fig. 1. Total concentration of Zn in soil (Zn_{Tot}) ranged from 14.1 to 291 mg kg^{-1}. The median value was 100 mg kg^{-1} (Fig. 1A and Table 1), which is at the top of the range suggested for uncontaminated soils: 10—100 mg kg^{-1} (Mertens and Smolders, 2013). Values of Zn_{E} ranged from 0.44 to 57.7 mg kg^{-1} (median: 4.82 mg kg^{-1}) (Table 1 and Fig 1B). The labile fraction (Zn_{E} as % of Zn_{Tot}) ranged from 0.75% to 69.7% with median and mean values of 4.66% and 8.00% respectively. These values (%Zn_{E}) are lower than those reported for both contaminated and uncontaminated soils (Degryse et al., 2011; Izquierdo et al., 2013; Marzouk et al., 2013b). The distribution of Zn_{DTPA} concentrations were positively skewed (Fig. 1C) with a variation of 0.01–5.25 mg kg^{-1} (median = 0.69 mg kg^{-1}). Only 31.4% of samples had Zn_{DTPA} less than 0.5 mg kg^{-1}, indicating that they are potentially Zn deficient (Mertens and Smolders, 2013). Values of Zn_{DTPA} as % of Zn_{Tot} ranged from 0.013% to 3.82% with a median and mean of 0.690% and 0.871% respectively. There was a

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significant positive correlation (r = 0.25) between C_{Org} and $\%Zn_{DTPA}$ possibly indicating that Zn bound to soil organic matter is in a form accessible to DTPA extraction.

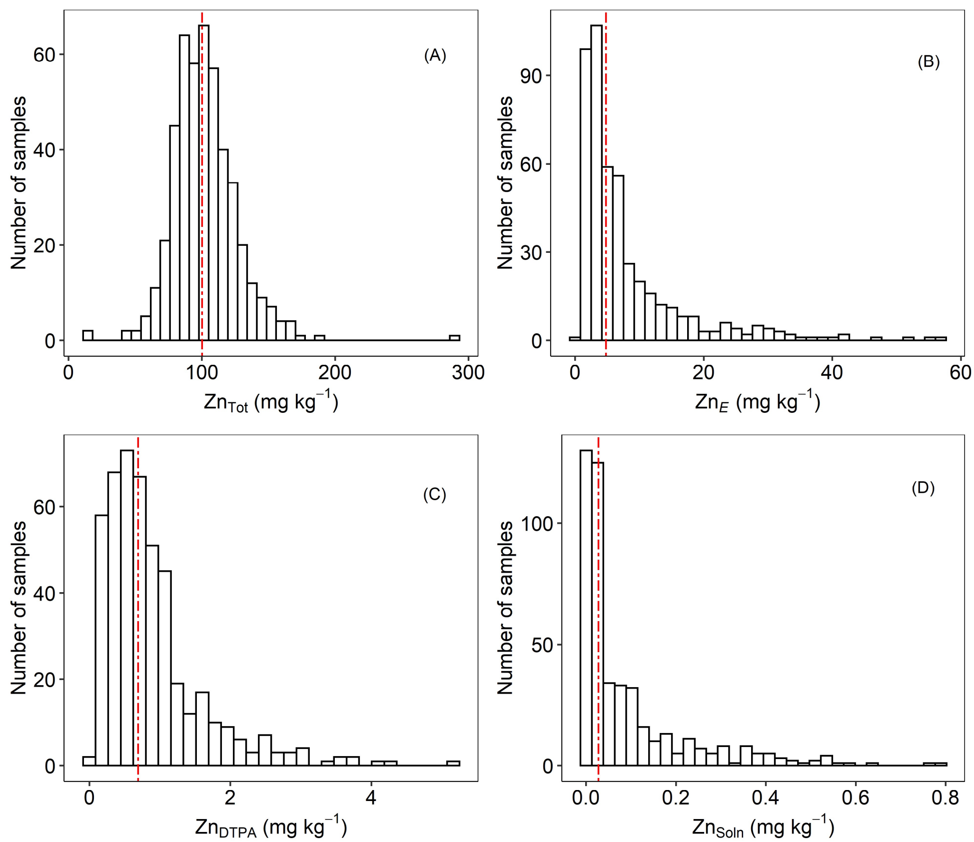

Figure 1. Frequency distributions of (A) Zn_{Tot}; (B) Zn_E; (C) Zn_{DTPA}; (D) Zn_{Soln} concentrations in agricultural topsoil samples from the Amhara region, Ethiopia. Vertical dashed lines represent median values.

The Zn concentration in 0.01 M $Ca(NO_3)_2$, Zn_{Soln}, varied by more than two orders of magnitude (0.001–0.789 mg kg^{-1}). Values of Zn_{Soln} showed a unimodal and positively skewed distribution (Fig 1D), indicating predominately small concentrations (<0.1 mg kg^{-1} in 72% of soils studied). A maximum of 0.96% of Zn_{Tot} was extracted in $Ca(NO_3)_2$ (median = 0.027%).

To evaluate the correlation between soil variables, principal component analysis (PCA) was employed (Fig. 2). The first two principal components (PCA 1 and 2) explained 58.7 % of the variation in the datasets; 41.1% was explained by PCA 1. PCA 1 was strongly correlated with Kd_{Lab} and soil properties

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that are likely to affect Kd_{Lab}, such as pH and eCEC. PCA 2 correlated with Zn_{DTPA}, C_{org}, and mineral oxides (Fig. 2). PCA analysis also shows that Zn_{Soln} and Zn_E react in opposite ways.

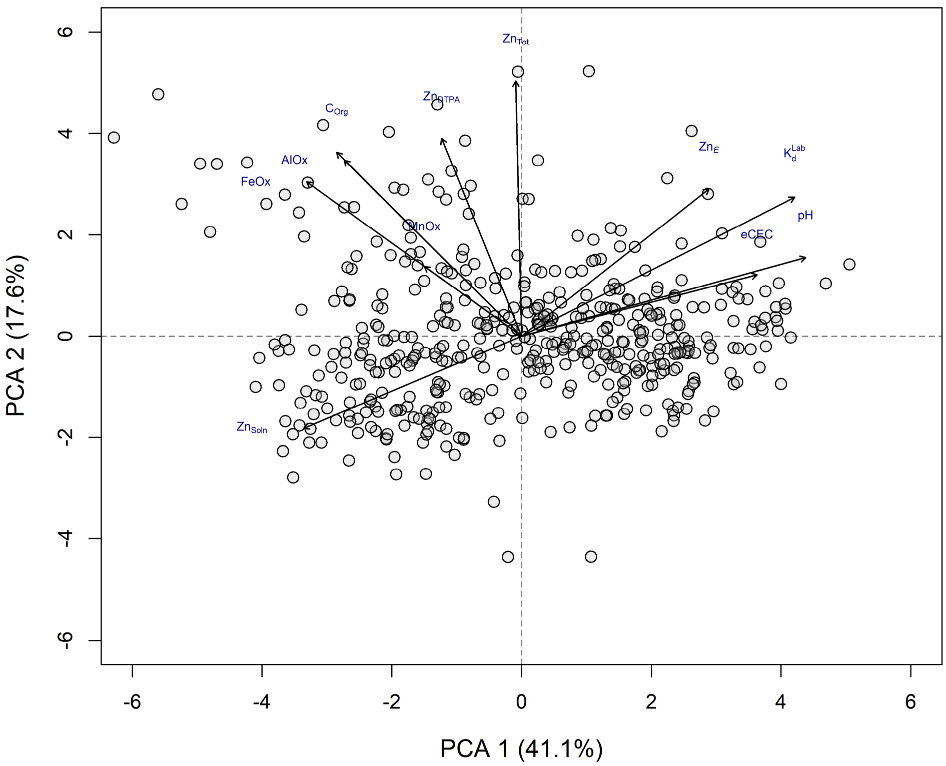

Figure 2. Principal component analysis of main soil variables and Zn indices.

3.2. Isotopically exchangeable Zn

3.2.1. Method assessment and validation

In principle, *E*-value determination is based on the premise that an added isotope is reversibly adsorbed and is in a dynamic equilibrium between the solid and solution phases (Hamon et al., 2008; Young et al., 2005). Therefore, the reliability of the determined *E*-value rests on an accurate measurement of the distribution coefficient of the added ^{70}Zn (Kd_{Lab}) and Zn concentration in the soil solution Zn_{Soln} (Eq. 1). For an accurate measurement of Kd_{Lab}, the added ^{70}Zn must produce a change in the isotopic ratio ($^{70}Zn/^{66}Zn$) that can be reliably quantified while still reflecting the native Zn equilibrium in the soil. As illustrated in Fig. 3A, there was a clear distinction between the natural isotopic ratio (0.02) and measured ratios, with a minimum $^{70}Zn/^{66}Zn$ ratio of 0.15 which is almost 8 times the natural ratio. At the same time, the amount of the added isotope was small compared to

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Zn_{Tot} and amounted to 2.1% of Zn_{Tot} on average (< 5% in 94% of the samples). To confirm the consistency of Zn_{Soln}, an inter-laboratory comparison was undertaken. Figure 3B shows the results of Zn_{Soln} measurements produced by two different laboratories (University of Nottingham and Rothamsted Research) but using different equilibrating electrolytes (0.01 M $Ca(NO_3)_2$ and 0.01 M $CaCl_2$) and different instruments. All data were within one order of magnitude from the line of equality with close agreement (r = 0.96) across the range of the Zn_{Soln} concentrations.

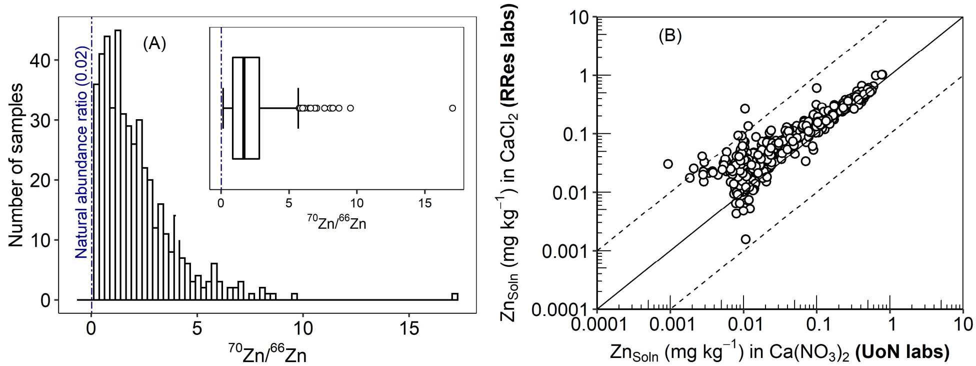

Figure 3. (A) Histogram of the isotopic ratio ($^{70}Zn/^{66}Zn$) in the spiked soils; (B) an inter-laboratory comparison of Zn_{Soln} extracted in 0.01 M $Ca(NO_3)_2$ at the University of Nottingham (x-axis) and in 0.01 $CaCl_2$ at Rothamsted Research (y-axis). The dashed lines and the solid line in B represent 1 log unit interval and the line of equality respectively.

3.2.2. Soil factors determining Zn lability (Zn_E)

Previous studies, mainly on contaminated soils, have reported that the labile fraction of metals tends to decline with rising pH in response to increased adsorption strength (Degryse et al., 2004; Tye et al., 2003); data in the current study showed the opposite trend (Fig. 4A-B). However, with contaminated soils the behaviour of trace metals often partly reflects the properties of the source of metal (Mao et al., 2014; Marzouk et al., 2013b). For example, contamination with calcareous materials in the case of soils contaminated with mine spoil produces co-variation of total Zn concentration with pH. Furthermore, there is usually a restricted pH range in the case of urban soils and temperate agricultural soils. The current study deals with soils that have comparatively low $\%Zn_E$ and Zn_{Tot} and a wide range of pH values (c. 4.0 – 7.5) which are likely to include substantial changes in geocolloidal mineralogy (e.g. oxide-based vs alumina-silicate clays) (Fig. 4C). Thus the trend depicted in Fig. 4A probably reflects a combination of different factors. For example, in soils with higher pH values it is possible that Zn adsorption is on surfaces which are more likely to retain Zn in a *labile* form — e.g. humic acid and 2:1 alumino-silicate clays. Similarly, there may be greater Zn fixation under acid conditions because of the greater incidence of oxide-rich mineralogy in highly weathered soils with a

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low pH (Fig 4C). A significant negative correlation (r = -0.26; $p < 10^{-8}$) between Zn_E and the sum of the concentration of mineral oxides in soil may support that hypothesis. Furthermore, solution phase speciation (from WHAM7) suggested that the proportion of Zn bound to dissolved organic carbon increased with pH (Fig. 5A). At very low Zn concentrations in solution (Zn_{Soln} c. 1 µg L^{-1} above pH 6.5) it is possible that the fulvic-bound Zn was sufficiently strongly bound to be *non-labile* – i.e. excluded from isotopic equilibrium with the added ^{70}Zn. In the calculation of *E*-value (Eq. 1) this would (erroneously) inflate the apparent Zn_E. A similar outcome would occur if there were significant amounts of non-labile Zn held in particulate form as part of the measurement of Zn_{Soln} at higher pH values. Non-labile particulate metal was first demonstrated by Lombi et al., (2003) who used chelating resin in *E*-values measurements (E_r) to quantify the fraction of the colloidal metal that was not isotopically exchangeable. They reported that E_r values were generally less than E values based on equilibrated solution measurements (E) and that the ratio E/E_r increased with pH. Use of the resin method has produced variable results. Marzouk et al., (2013b) also reported metals associated with sub-micrometre colloidal particles in the solution phase, based on resin phase measurements. They found this association to be positively correlated with soil humus content and pH. However, for their dataset, they found that the presence of nano-particulate non-labile Zn in solution produced, on average, less than 2% difference in the determination of E-values (E vs E_r). Mao et al., (2017) also investigated the presence of non-labile metal fractions of Ni, Cu, Zn, Cd, and Pb in suspended colloidal particles. They also found an average of only 2% difference between E_r and E for all five metals and the difference was only significant for Cu with an increased presence of non-labile colloidal particles at high pH – probably organically bound Cu.

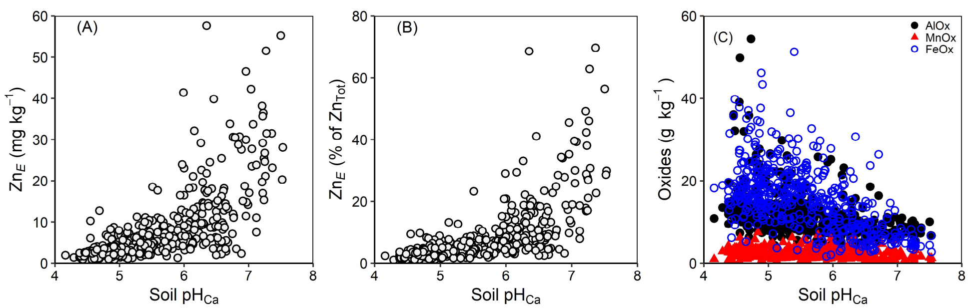

Figure 4. Concentration of (A) Zn_E in soil; (B) Zn_E as % of Zn_{Tot}; and (C) mineral oxides in soil, as a function of soil pH_{Ca}.

If the trend in Zn_E with soil pH (Fig. 4A-B) was affected by interferences from particulate materials in the soil solution then the source of the error would either be in Kd_{Lab} or Zn_{Soln}—the two variables in the calculation of Zn_E (Eq. 1). However, the presence of non-labile Zn within particulate matter in the

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isolated soil suspension supernatant would not contribute to an error in Kd_{Lab}. This is because, by definition, the labile spiked isotope is excluded from mixing with the particulate Zn phase. However, the measurement of ^{66}Zn would include Zn in solution and any particulate-bound Zn (< 0.22 µm), with which the ^{70}Zn would not have mixed. Thus it is the determination of Zn_{Soln} (Eq. 1) that may produce an error in Zn_E. Tavakkoli et al., (2013) investigated the possible occurance of non-isotopically exchangable Zn in sub-micron sized colloids in filtered soil extraction at high soil pH. They found no non-exchangeable Zn when filtering to <0.1 µm to remove particles but gradually increasing proportions of isotopically non-exchangable Zn where solutions had been filtered using progressively larger filter pore sizes (0.22, 0.45, and 0.7 µm). In the present study soil extraction solutions were filtered to <0.22 µm.

The possible presence of non-labile nanoparticulate Zn in the soil solution was investigated using a resin (Chelex-100) purfication step (Marzouk et al., 2013b) in the determination of Zn_E. A comparison was made of $^{70}Zn/^{66}Zn$ ratios in the centrifuged, filtered solution and in a resin extraction of that solution. No evidence of non-labile nanoparticulate Zn below pH 5.5 was found; the isotopic ratios $^{70}Zn/^{66}Zn$ in the solution and resin phases were equal. Unfortunately, at higher soil pH (>5.5), our investigation was confounded due to resin Zn contamination that compromised the measurement of low soluble Zn concentrations in soils with high pH. However, considering the magnitude of the trend depicted in Fig. 4A, the majority of Zn in the filtered soil solution would have to be present as non-labile particulate matter for the trend shown to be due to non-labile particulate Zn contributing to Zn_{Soln}. This seems unlikely and so we therefore suggest that the increase in Zn_E values with soil pH in the soils studied is probably a genuine trend.

The WHAM7 predictions of *labile* Zn distribution among different soil surfaces are presented in Fig. 5B. At low soil pH, the WHAM7 model predicted the sorption to be overwhelmingly onto Mn oxide and humic acids, whereas at intermediate and high pH, humic acid-bound Zn became dominant. WHAM7 predicts a negligible role for Fe oxide in adsorbing Zn but at pH > 6.5 sorption onto Al oxides was important. The fractionation suggested by the WHAM7 model relates only to labile Zn and does not predict the location of the 'fixed' (non-labile) Zn in the soils. The speciation of Zn in the soil solution, as calculated by WHAM, is presented in Fig. 5A. It was predicted that the free Zn ion activity (Zn^{2+}) constituted 36.1% to 99.2% (median = 77.1%) of the total Zn_{Soln} and was highly correlated with pH. At pH < 5.5, the majority of Zn_{Soln} was present as the free Zn^{2+} ion (> 61%). This percentage decreased to an average of 49% at soil pH > 7. Previous studies have also shown minimal complexation of Zn in the soil solution at pH < 6.5 (Catlett et al., 2002; Rutkowska et al., 2015). The proportion of the total Zn present as dissolved fulvic acid complexes ranged from 0.81% to 61.2.% (median 22.7%). The proportion of FA-complexed Zn increased with increasing soil pH (Fig. 5A); at pH > 7 an average

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of 47.7% of Zn_{Soln} was apparently complexed to FA. The only inorganic Zn complexes were carbonates and these accounted for < 4% at soil pH > 7.

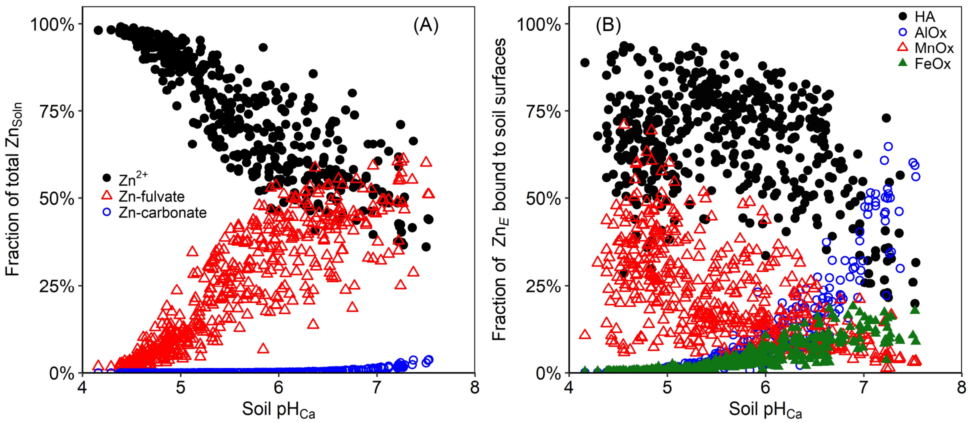

Figure 5. (A) Zn speciation in the *solution* phase of Ethiopian soil suspensions as predicted by WHAM7 (free divalent ions = black circles; FA complexes = red triangles; carbonate complexes = blue circles). (B) Zn fractionation in the *solid phase* as predicted by WHAM7 (HA complexes = black circles; Al oxide bound = blue circles; Mn oxide bound = red triangles; Fe oxide bound = green triangles).

3.3. Zinc solubility

The partition coefficient in the current study (Kd_{Lab}) represents the distribution of the added ^{70}Zn spike between the isotopically exchangeable Zn on the solid phase and in the solution phase (Eq. 2). Values of Kd_{lab} varied by more than 3 orders of magnitude—ranging from 15.4 to 42600 L kg^{-1}. As shown in Fig. 6A, values of Kd_{Lab} were highly pH-dependent, in agreement with increased adsorption strength of cationic trace metals onto soil surfaces with pH. Regression analysis of soil properties (eCEC, C_{Org}, Zn_{Tot}, mineral oxides) against Kd_{Lab} is presented in Table 2. Only significant variables were retained in regression equations and the variables were checked for multicollinearity using variance inflation factors (VIF). Values of VIF for all variables were less than 3. While all variables in Table 2 were significant in the regression analysis, they accounted for a very small proportion of the variation (< 4%) in the data. The majority of the variation in the data (90%) was explained solely by soil pH (Table 2 and Fig. 6A). Despite the fact that soil organic matter is known to be an important sorbent for trace elements (Degryse et al., 2011), C_{Org} had a negligible influence on Kd_{Lab}.

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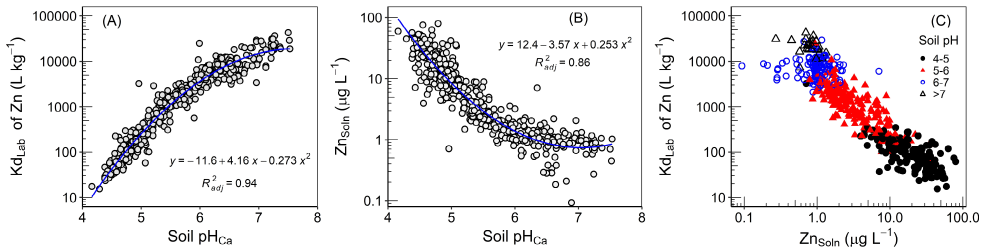

Figure 6. Values of (A) Kd_{Lab} and (B) Zn_{Soln} as a function of soil pH; (C) relationship between K_d^{lab} and n_{Soln}

As seen for Kd_{Lab}, Zn_{Soln} was also mainly controlled by soil pH; 77% of the variation in Zn_{Soln} was explained solely by soil pH (Fig 6B). There was also a weak, but significant, correlation between Zn_{Soln} and soil C_{Org} (r = 0.23, $\rho < 5.8 \times 10^{-7}$); some influence on metal adsorption strength would be expected (Fan et al., 2016). However, the limited effect of soil organic matter may be due to a dual influence on Zn solubility. Soil humus will contribute to Zn adsorption within the soil solid phase but also produce greater DOC (r = 0.47 between C_{org} and DOC) which will promote dissolved organo-complexation of Zn.

Table 2. Regression equations for $Log_{10}(Kd_{Lab})$

Regression equation	R^2_{adj}	*p*
$Log_{10}(Kd_{Lab}) = 1.0 \times pH_{Ca} + 0.3 \times Zn_{Tot} + 0.3 \times C_{org} + 0.5 \times eCEC + 0.9 \times oxides - 4.0$	0.93	$< 2.2 \times 10^{-16}$
$Log_{10}(Kd_{Lab}) = 1.0 \times pH_{Ca} - 2.8$	0.90	$< 2.2 \times 10^{-16}$

The concentration of Zn in soil solutions is largely determined by the combined influence of soil properties which affect the strength of adsorption and the total Zn concentration in soil. Thus, the relationship between Kd_{Lab} and Zn_{Soln} (Fig. 6C) demonstrates the much greater importance of soil characteristics over the influence of Zn_{Tot} in the Amhara soils. In considering the relationship in Fig 6C, it should be emphasised that Kd_{Lab} and Zn_{Soln} are completely independent of each other. The value of Kd_{Lab} is the distribution coefficient of the added ^{70}Zn isotope and Zn_{Soln} is determined from measured values of ^{66}Zn; this negates the common, and justified, criticism of such relations in which Zn_{Soln} is the denominator of the Kd which would tend to produce a declining trend with Zn_{Soln}. Therefore, the very strong capacity-intensity dependence of the studied soils genuinely reflects control by soil properties over Zn solubility. In particular, for the soils studied, soil pH alone virtually controls the strength of Zn adsorption and Zn_{Soln} (Fig. 6A&B), despite considerable variation in Zn_{Tot} (14.1 – 291 mg kg^{-1}; Table 1).

3.4. Multi-surface modelling of soluble Zn concentration

It is widely recognised that while the total concentration of an element in soil is important, it is the chemical speciation that plays a key role in determining availability to plants. Despite that, direct

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measurement of the chemical forms of an element is limited. Therefore, geochemical modelling offers a feasible alternative and has been widely applied to soil (Bonten et al., 2008; Cui and Weng, 2015; Klinkert and Comans, 2020). An important consideration when using geochemical models is the choice of the 'reactive' pool of metals, which is in equilibrium with the soil solution, as an input variable. It has been well established that the total concentration of metals does not reflect the reactive fraction in soil (Kelepertzis and Argyraki, 2015; Peng et al., 2018; Rodrigues et al., 2013). Extractions with 0.43 M nitric acid (HNO_3) and EDTA have been frequently used to approximate the geochemically reactive pool of metals in soil (Groenenberg et al., 2017; Liu et al., 2019; Ren et al., 2017). However, the isotopic dilution method is recognized to be conceptually the most robust and mechanistically based method that reliably quantifies the reactive pool of metals in soil (Groenenberg et al., 2017; Hamon et al., 2008; Peng et al., 2018). To assess the capability of WHAM7 to predict Zn solubility, the concentrations in the solution phase (Zn_{Soln}) were compared with the outputs from fractionation of Zn across the whole soil-solution system, using either Zn_{Tot}, Zn_{E}, or Zn_{DTPA} concentrations as the fraction of Zn controlling Zn solubility. Results of these simulations are presented in Fig. 7.

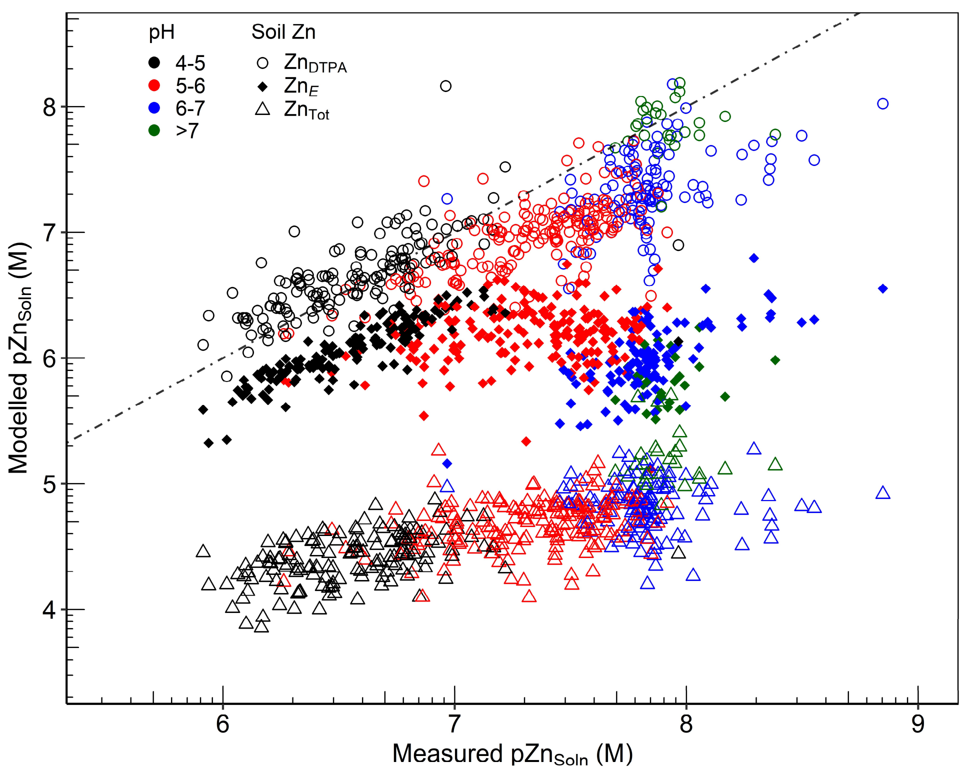

Figure 7. Comparison between observed pZn_{Soln} (X-axis) and modelled pZn_{Soln} (Y-axis) using either Zn_{DTPA}, Zn_{E} or Zn_{Tot} as WHAM inputs. The dashed line represents the line of equality.

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Figure 7 clearly illustrates that using Zn_E, substantially improves the prediction of Zn solubility compared to using Zn_{Tot}, particularly at low soil pH. This reinforces the conclusion that the geochemically reactive metal pool, rather than the total soil Zn concentration, is the most relevant representation of Zn availability in soil. At high pH (>7.5), the model predicts higher Zn_{Soln} than observed concentrations. This may be partly due to limitations in binding surfaces considered in WHAM7. At pH >7, adsorption on calcium carbonate or phosphate minerals may occur which is not accounted for in WHAM7. This was reported by Peng et al., (2018) who excluded data at pH >7 from their results, when using WHAM7 to predict the solid-solution partition and speciation of heavy metals, in response to a lack of consideration of precipitation on carbonates. Izquierdo et al., (2013) listed the failure to include binding to carbonate surfaces as a possible source of error in predicting metal concentration in the soil solution from WHAM7. Mao et al., (2017) also attributed the overestimation of metal concentrations in the soil solution to the exclusion of phases such as calcite and hydroxyapatite as binding phases in WHAM7. Additionally, overestimation of *E*-values at high pH due to the presence of (non-labile) Zn which has not isotopically mixed with the added ^{70}Zn spike would also explain the poorer performance of WHAM7 in predicting Zn solubility at high pH.

When Zn_{DTPA} was used as input, prediction of Zn solubility by WHAM7 was apparently improved over that achieved by using Zn_E (Fig. 7), particularly at high pH. This may confirm the possible over-estimation of Zn_E, as discussed above. Alternatively, it may reflect counteracting errors between (i) the inadequacy of DTPA (0.005 M) as an extractant which would decrease modelled Zn_{Soln} and (ii) the underestimation of Zn binding in WHAM7 at high pH which would raise the estimate of Zn_{Soln}. It is recognised, for example, that 0.005 M DTPA extracts less Zn from soil than 0.05 M EDTA and also underestimates Zn_E (Marzouk et al. 2013a). To assess whether the binding capacity of the DTPA used was limited, the mole ratios of cations to DTPA in the extracted solutions (excluding alkali/alkali-earth cations) were calculated. The average ratio was only 0.17 ± 0.08, suggesting that the DTPA extractant was probably not capacity-limited. These results suggest, broadly, that both Zn_{DTPA} and Zn_E may be reasonable estimates of the 'labile' pool of Zn in soil. DTPA appears to provide a better estimate of Zn_{Soln} using a current geochemical model, especially at high pH. Alternatively, the isotopic dilution method, measured in neutral 0.01 M $Ca(NO_3)_2$, probably better reflects variation with pH in labile Zn Kd value, and possibly in the true labile pool of Zn in soil, compared with DTPA, which is buffered at pH 7.3.

3.5. Free Zn^{2+} activity in soil solution

The free ion activity is considered a key factor controlling plant uptake, although other factors will affect buffering and diffusion rates in the soil (Degryse et al., 2012). The concentration of Zn^{2+} activity in the soil solution is effectively an integration of soil properties that govern sorption processes. Data

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presented in Fig 8 show that the activity of Zn^{2+} is highly pH-dependent; 81% of variation in the free Zn^{2+} activity was accounted for solely by soil pH in the Ethiopian Amhara soils. The concentration of free Zn^{2+} activity varied over 3 orders of magnitude. The range of the free Zn^{2+} activity is probably a product of the counteracting effects of Zn_E and Zn_{Soln} variation with pH; Zn_E increases with pH while Zn_{Soln} falls as pH rises as discussed above (Fig 4B and 6B).

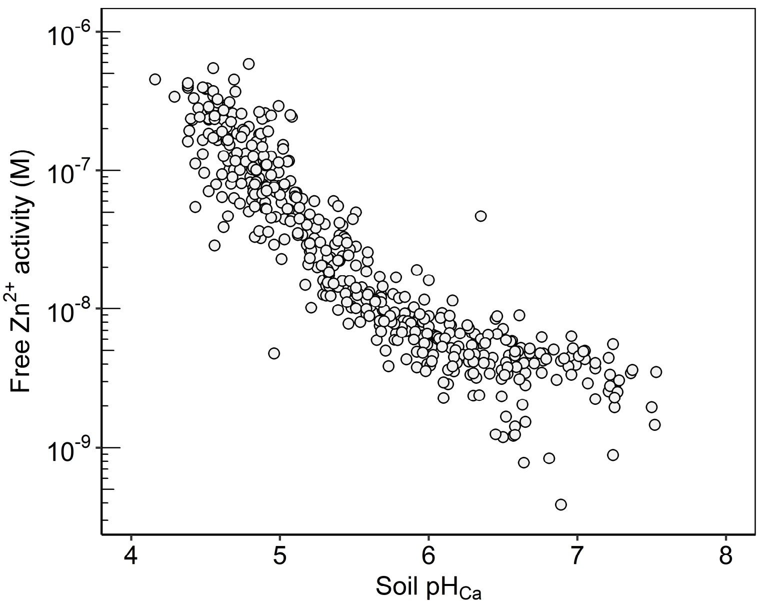

Figure 8. Concentration the free Zn^{2+} activity in the soil solution as a function of soil pH

4. Conclusions

Combining isotopic dilution method with geochemical speciation modelling in this study provides useful insights into the intrinsic reactivity of Zn in soils at a regional scale and elucidates the key variables determining Zn phyto-availability. The results demonstrate that intrinsic soil properties, rather than the variation in Zn_{Tot} concentration, determine the adsorption strength (Kd) of labile Zn and dictate Zn solubility. In the Amhara dataset soil pH was the key determining factor. The traditional DTPA extraction method provided a better estimate of Zn_{Soln}, predicted from a geochemical modelling approach, when compared with Zn_E as a model input variable. However, reasons for this remain unresolved and may reflect shortcomings in either approach and in model prediction at higher pH values.

These findings may have practical implications for agronomic interventions to improve crop Zn concentrations for they provide a tool for differentiating between soils in terms of the strength with

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which they adsorb Zn. This is an important consideration for a site-specific strategy to ensure a more effective agronomic biofortification of staple crops with Zn fertilizers (Joy et al., 2015; Manzeke et al., 2014, 2020; Zia et al., 2020). For instance, these results indicate that in soils with pH >6.5 foliar fertilisers are most appropriate because Zn is strongly adsorbed, while in soils with low pH applying fertilizers to the soil might be feasible. Furthermore, these findings can be used to identify areas where the use of soil management practices, such as organic matter incorporation, could increase Zn availability in soil—thus improving the Zn concentration of staple crops (Manzeke et al., 2019; Wood et al., 2018).

Data availability

Data used in this study is available for the corresponding author upon a reasonable request as the lab data will be published as part of a national datasets.

Author contribution

Conceptualization of the study for this manuscript was done by AWM, MRB, SPM, and SDY, with input from EHB, GD, and SJD. Data curation and investigation: AWM. Analysis, methodology, and visualization for the manuscript was performed by AWM with substantial input from SJD, MRB, SDY, EHB and feedback from all authors. AWM wrote the initial draft and all authors were involved in the review and editing of the manuscript.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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