RESEARCH PAPER

EVALUATING ZINC NUTRITION IN PERENNIAL RYEGRASS GROWN IN AN ANDISOL

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ABSTRACT. Zinc is an essential nutrient for humans, animals, and plants. Zinc uptake by crops is dictated by zinc availability in the soil, which in turn may be dictated, at least in part, by soil mineralogy. Little is known about the phytoavailability of Zn in Andisols, which are important agricultural soils in volcanic regions, such as Japan, New Zealand, and southern Chile. In this study, we assessed the vegetative growth response of perennial ryegrass (*Lolium perenne*, L.) to Zn fertilization in an Andisol from southern Chile. Ryegrass was grown in a greenhouse pot experiment with twelve rates of Zn application from 0 to 6075 mg Zn/kg soil. After 63 days, shoot length, specific leaf area, and biomass were measured. Foliar Zn concentrations were measured and correlated with plant-available Zn as measured by a diethylenetriaminepentaacetic acid (DTPA)-soil extraction (DTPA-Zn hereafter). Zinc toxicity to ryegrass was assessed using the Toxicity Relationship Analysis Program. This study demonstrated that a DTPA-Zn level of 1 mg Zn/kg soil was not limiting for ryegrass growth. Although Zn fertilization did not improve ryegrass growth in the studied Andisol, this study still has practical implications. Zinc deficiency in humans is a global problem and increasing Zn in staple food and forage crops may require Zn fertilization. This study suggests that Andisols can be fertilized with high doses of Zn without a risk of causing Zn toxicity to crops. However, a DTPA-Zn level of >489 mg Zn/kg soil decreased shoot length, indicating a toxicity response.

KEYWORDS: Zinc, ryegrass, soil, Andisols, DTPA

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INTRODUCTION

Zinc is an essential nutrient for humans, animals, and plants. Specifically, Zn is a vital component of many enzymes involved in the metabolic growth and development of plant tissue. Zinc is particularly important for nitrogen metabolism and protein synthesis (Brown et al. 1993).

Assessing the potential for Zn deficiency in crops rests on correlating method-dependent extractable Zn levels to plant growth responses in various soils. The critical limit for Zn is the soil-extractable Zn level below which a particular crop will respond to Zn application. For instance, DTPA-extractable Zn (hereafter, DTPA-Zn) was correlated with corn yields in 77 near-neutral and calcareous soils from Colorado, USA (Lindsay and Norvell 1978). The critical level for corn was 0.8 mg/kg DTPA-Zn (Alley et al. 1972, Lindsay and Norvell 1978). Similarly, the critical level for corn was 0.9 mg/kg NH_4HCO_3-DTPA-extractable Zn in 44 Colorado soils (Havlin and Soltanpour 1981). In soils of the Atlantic Coastal Plain, Midwest, and California (USA), the critical levels for soybean and corn were in the range of 0.5-0.6 mg/kg DTPA-Zn (Brown et al. 1971, Makarim and Cox 1983). The critical level of Zn was 0.69 mg/kg DTPA-Zn for peas grown in Indian paddy soils (Indira Sarangthem et al. 2018), and is generally between 0.6 and 1.0 mg/kg in Indian soils depending on the crop (Katyal 1993, Katyal and Sharma 1991).

Salazar et al. (2021) developed a model for recommending the application of Zn fertilizer in the Mediterranean region of central Chile. However, little is known about Zn phytoavailability in Andisols (i.e., soils derived from volcanic ash) (Hue 2004). Andisols contain a preponderance of short-range order minerals, such as allophone and imogolite, which are known to limit phosphorus availability to plants (Velásquez et al. 2016, Vistoso et al. 2020). Andisols are geographically prominent in agricultural regions of southern Chile, Colombia, Iceland, Indonesia, Japan, central Mexico, New Zealand, and the pacific northwest of the United States. Andisols are important for cereal crops and livestock grazing in southern Chile.

In this study, we hypothesized that Zn may be a crop-limiting nutrient in Andisols. Accordingly, the objective of this research was to evaluate the vegetative growth response of perennial ryegrass *(Lolium Perenne*, L.) to Zn fertilization in an Andisol of southern Chile. Perennial ryegrass was chosen for its global use as a forage crop.

MATERIALS AND METHODS

Ryegrass growth bioassay

Soil from a 2 m^2 area was excavated completely from the land surface to a depth of 20 cm from a permanent grassland field at the Austral Farming Experimental Station (EEAA) of the Universidad Austral de Chile (39° 47' S, 73° 14' W). The soil is classified as a Duric Hapludand (Bravo et al. 2020). The sample was sieved though a 5 mm mesh, homogenized and dried at 40°C for 48 hours. In order to determine the general physicochemical characteristics of the soil (Table 1), a 1 kg subsample was taken and sieved through a 2 mm mesh. Soil texture and total metal concentrations were determined by standard methods (Sadzawka et al. 2015).

Perennial ryegrass (*Lolium perenne* L.), variety Nui, was used for the Zn nutrition assessment bioassay. Twelve different Zn^{2+} application rates with four replicates were used: 0 mg kg^{-1}, 12 mg kg^{-1}, 28 mg kg^{-1}, 40 mg kg^{-1}, 61 mg kg^{-1}, 121 mg kg^{-1}, 202 mg kg^{-1}, 405 mg kg^{-1}, 810 mg kg^{-1}, 2025 mg kg^{-1}, 4050 mg kg^{-1}, and 6075 mg kg^{-1} (Supplementary Table 1). One week prior to the bioassay, the different Zn dose treatments were fertilized using $ZnSO_4$, then incubated at field capacity and room temperature, and homogenized daily.

A mass of 450 g of soil was placed in a plastic container measuring 9 cm x 9 cm x 9.5 cm (width x length x height; 770 cm^3) for each replicate. The containers were placed in the greenhouse using a fully randomized design. Fifty seeds were sown per container and were thinned out on day 7 to leave 25 plants in each container. The total length of the test period was 63 days, including the germination period. Plant-available Zn was determined for the initial soil and at the end of the 63-day greenhouse experiment for each treatment using a DTPA extracting solution (0.005 M DTPA + 0.005 M $CaCl_2$ + 0.1 M TEA, pH 7.3) (Lindsay and Norvell 1978).

Each container was watered and fertilized with a Hoagland nutrient solution without Zn and diluted 5 times more than the standard nutrient solution: 1.40 mM K^+; 3.80 mM NO_3^-; 0.40 mM PO_4^{3-}; 0.011 mM Na^+; 1.40 mM Ca^{2+}; 0.40 mM Mg^{2+}; 0.41 mM SO_4^{2-}; 0.0056 mM Fe^{2+}; 0.0018 mM Mn^{2+}; 0.0091 mM BO_3^{3-}; 0.0001 mM Cu^{2+}; 2.73 x 10^{-5} mM MoO_4^{2-}.

Plant responses

Upon completion of the biotesting period, the shoot length of five representative plants was recorded. Shoot lengths were measured from the root neck to the distal ends of the last leaf. The biomass of shoots was determined with a precision balance (Radwag WTC 2000, Radom, Poland) after drying the shoots in an oven at 70°C for 48 hours. In order to compare with field conditions, shoot dry biomass is expressed in ton ha^{-1}, calculated by projecting the surface of the container used in the bioassay (63.6 cm^2). Specific leaf area in each container was measured using an electronic leaf area meter (LI 3100, Licor Inc., Lincoln NE, USA). Specific leaf area is expressed in m^2 kg^{-1} dry weight. Foliar concentrations of Zn were measured at the end of the testing period using standard methods (Kalra 1998).

Statistical analysis

Simple linear and non-linear regressions were carried out between the biological responses and DTPA-Zn and foliar Zn concentrations. Normal distribution and homogeneity of residuals were verified (Kutner et al. 2004). Linear and polynomial regressions between plant and soil variables were performed using the software GraphPad Prism 8. Statistical analyses were carried out using Statgraphics Centurion 18. DTPA-Zn effective concentrations (EC_x) were determined by the Toxicity Relationship Analysis Program (TRAP) version 1.22 (US EPA 2013). For the determination of the EC_x values, the 0 mg Zn kg^{-1} rate was used as a control (i.e., the unamended soil that contained 1.0 DTPA-Zn).

RESULTS AND DISCUSSION

Zinc availability and uptake response to Zn application

The DTPA-Zn level of 1 mg/kg in the studied Andisol falls within the typical range of 0.5-1.5 mg/kg for soils of southern Chile (Table 1; Figure 1). Similarly, DTPA-Zn levels in agricultural Andisols of the Ethiopian Rift Valley were in the range of 0.9-3.5 mg/kg (Baissa et al. 2007). Our results showed that applying additional Zn to the studied Andisol increased the plant available Zn (DTPA-Zn) and foliar uptake of Zn in ryegrass. Specifically, foliar Zn increased linearly up to 1.6 x 10^3 mg/kg with increasing Zn application rate up to 2.0 x 10^3 mg/kg and increased exponentially up to 3.3 x 10^4 mg/kg with a further increase in Zn application rate up to 6.1 x 10^3 mg/kg (Figure 2). DTPA-Zn increased up to 4.9 x 10^2 mg/kg with increasing Zn application rate up to 2.0 x 10^3 mg/kg (Figure 3). Foliar Zn increased linearly to 1.8 x 10^2 mg/kg as DTPA-Zn increased up to 4.9 x 10^1 mg/kg, and then tapered with increasing DTPA-Zn levels up to 2.5 x 10^2 mg/kg (Figure 4). As DTPA-Zn increased to 4.9 x 10^2 mg/kg and beyond, foliar Zn increased exponentially (Figure 4; Supplementary Figure 1). Similarly, DTPA-Zn strongly correlated with Zn uptake by ryegrass in Zn-contaminated soils with a range of DTPA-Zn of 10-3500 mg/kg (Singh et al. 1996).

Plant growth response to Zn availability and uptake

DTPA-Zn level in the range of 0-5 x 10^2 mg/kg did not impact shoot dry biomass, specific leaf area, and shoot length for ryegrass (Figure 5). Similarly, shoot dry biomass, specific leaf area, and shoot length for ryegrass were independent of foliar Zn concentrations (Figure 6). Thus, a DTPA-Zn level of 1 mg/kg was sufficient for growth of ryegrass in an Andisol of southern Chile. This is the first assessment of Zn nutrition for ryegrass grown in Andisols of which we are aware.

Perennial ryegrass is sensitive to excessive Zn levels (Grigorita et al. 2020). As DTPA-Zn levels increased above 5 x 10^2 mg/kg, shoot length in ryegrass decreased,

Fig. 1. General physicochemical properties of the studied soil

Soil property	Unit	Value
Organic matter	%	15
Texture		Silt loam
Total Cu	mg kg^{-1}	53
Total Zn	mg kg^{-1}	86
Total Pb	mg kg^{-1}	28
Total Cd	mg kg^{-1}	1.4
Total Cr	mg kg^{-1}	15
DTPA-Zn	mg kg^{-1}	1.0

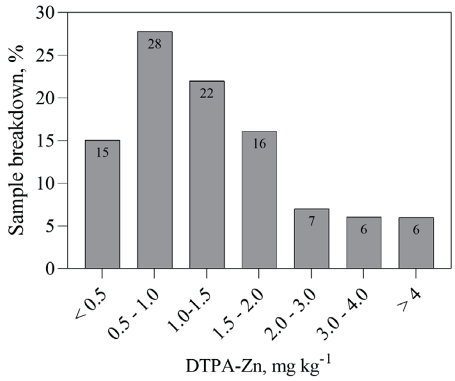

Fig. 1. Frequency histogram for the sample breakdown of DTPA-extractable Zn (DTPA-Zn) in soils from southern Chile. The data were obtained from the soil service laboratory of the Instituto de Ingeniería Agraria y Suelos, Universidad Austral de Chile (n = 1714)

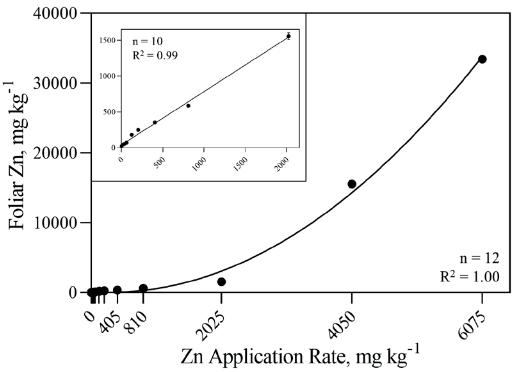

Fig. 2. Foliar Zn concentration as a function of the Zn application rate (n = 12). A third order polynomial function is fitted to this relationship. The inserted figure shows a linear regression model when we used the first 10 of 12 Zn dose treatments

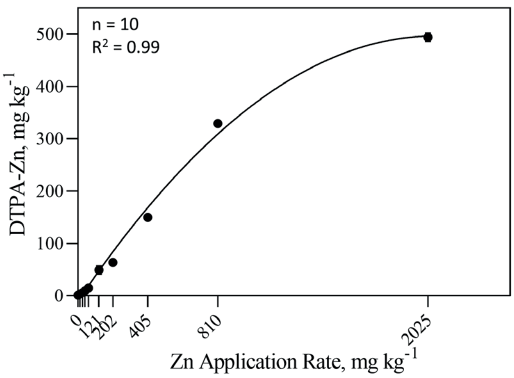

Fig. 3. DTPA-extractable Zn (DTPA-Zn) concentration as a function of the Zn application rate (n = 10). A second order polynomial function is fitted to this relationship

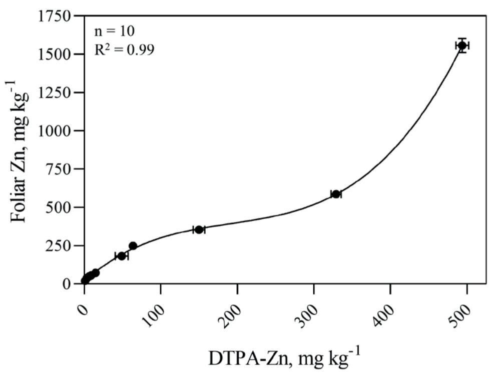

Fig. 4. Foliar Zn concentration as a function of DTPA-extractable Zn (DTPA-Zn) concentration (n = 10). A third order polynomial function is fitted to this relationship. Bars show the standard error of the means

exhibiting a toxicity response (Figure 7). For instance, at a DTPA-Zn level of 6.2 x 10^2 mg/kg, the shoot length of ryegrass decreased by 50% relative to control treatments (Figure 7; Supplementary Table 2). Thus, Zn application rates of 810 mg/kg Zn^{2+} (2000 mg/kg $ZnSO_4$) induced toxicity in ryegrass.

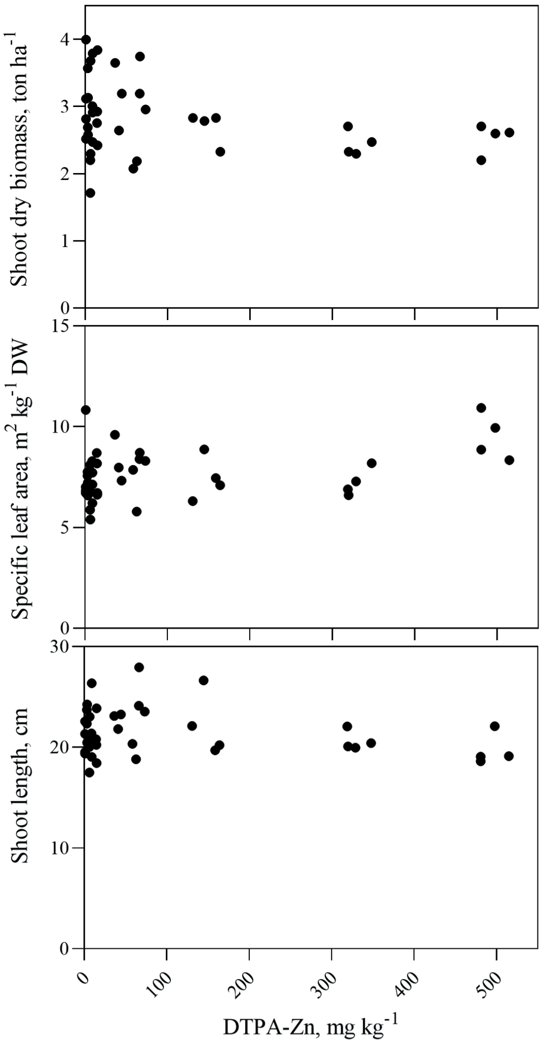

Fig. 5. Ryegrass responses as a function of DTPA-extractable Zn (DTPA-Zn) concentration (n = 10). Biological responses were independent of DTPA-Zn concentration not showing a linear or non-linear regression model

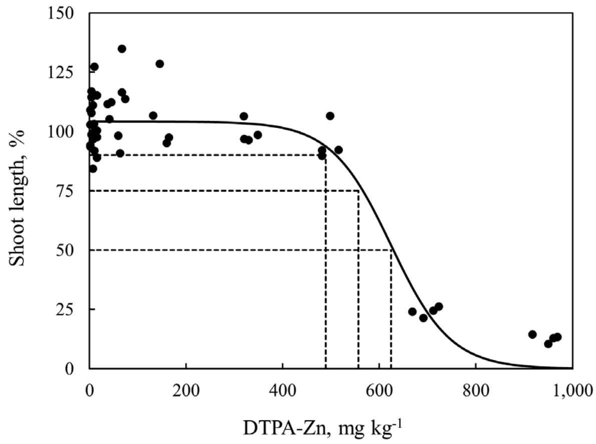

Fig. 7. Ryegrass shoot length as a function of DTPA-extractable Zn (DTPA-Zn) concentration. The y-axis represents shoot length expressed as a percentage respect to the control experiments. A logistic sigmoid regression analysis was used to fit the data (Toxicity Relationship Analysis Program, US Environmental Protection Agency). EC_{10}, EC_{25}, and EC_{50} are indicated

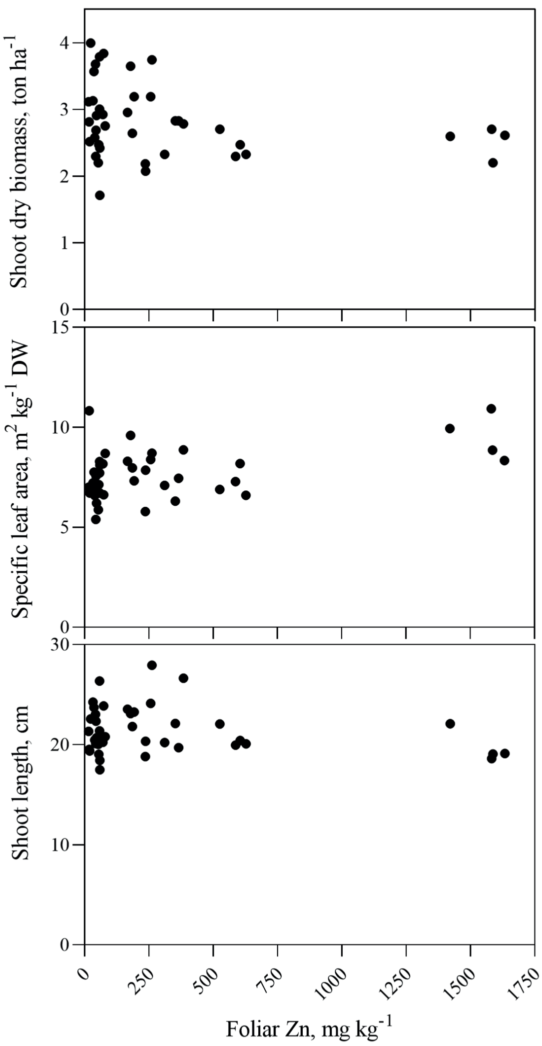

Fig. 6. Ryegrass responses as a function of foliar Zn concentration (n = 10). Biological responses were independent of foliar Zn concentration not showing a linear or non-linear regression model

CONCLUSION, PRACTICAL IMPLICATION AND FUTURE STUDY NEEDS

In this study, we hypothesized that zinc may be a crop-limiting nutrient in Andisols. However, a DTPA-Zn level of 1 mg Zn/kg soil was not limiting for ryegrass growth.

Although zinc fertilization did not improve ryegrass growth in the studied Andisol, this study still has practical implications because zinc deficiency in humans is a global problem. Indeed, approximately one-fifth to one-third of the world's human population has insufficient dietary Zn (Hotz and Brown 2004, Stein 2010). Thus, increasing Zn in staple food and forage crops may require Zn fertilization (Cakmak et al. 2017). This study suggests that Andisols can be fertilized with high doses of Zn without a risk of causing Zn toxicity to crops. However, a very high dose of Zn fertilization (>2000 mg Zn/kg soil, equal to a DTPA-Zn level of >489 mg Zn/kg soil) decreased shoot length, indicating a toxicity response.

Zinc fertilization should be studied in Andisols with DTPA-Zn levels lower than 1 mg Zn/kg soil. Likewise, studies involving other crops are warranted.

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