

Research Article

Received: 23 November 2018

Revised: 26 February 2019

Accepted article published: 13 March 2019

Published online in Wiley Online Library: 17 April 2019

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9681

Zinc application in conjunction with urea as a fertilization strategy for improving both nitrogen use efficiency and the zinc biofortification of barley

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Abstract

BACKGROUND: Intensive cropping systems have caused widespread Zn deficiency, low nutritional quality of cereals and environmental problems. The aim of the microplot field experiment reported in this paper was to assess the option of using Zn in conjunction with urea fertilization in order to reduce N rate and to maintain the yield level and grain quality but minimizing environmental risks. Barley (*Hordeum vulgare* L.) was cultivated in a calcareous soil under semi-realistic conditions. Combinations of four Zn levels, applied by spraying aqueous solutions of ZnSO₄, and three N levels, applied by spreading granular urea, were tested.

RESULTS: Zn and N showed a synergistic effect, increasing yield and Zn content in all plant parts and protein content in grain. A low Zn dosage of 5 kg ha⁻¹ was sufficient to significantly increase the amount of bioavailable Zn in soil and significantly raise its concentration in plant material and also the protein content in grain. The remobilization of Zn from leaf tissue to grain was dependent on the availability of Zn and was only crucial when its bioavailability was low.

CONCLUSIONS: A Zn dosage of 5 kg ha⁻¹ enhanced the agronomic efficiency of N by 15.5 kg grain kg⁻¹ N. The Zn applied to the soil permitted a reduction in the rate of N with only a small decrease in barley grain yield and nutritional value. However, due to the interannual variability in rainfall, which is characteristic of Mediterranean climates, further studies will be necessary to confirm and extend these results.

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Keywords: agronomic efficiency of nitrogen; calcareous soil; field conditions; nutritional quality; Zn availability

Title:

Zinc application in conjunction with urea as a fertilization strategy for improving both nitrogen use efficiency and Zn biofortification of barley

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Number of text pages: 22

Number of tables: 5

Number of figures: 5

1 **Zinc application in conjunction with urea as a fertilization strategy for improving both**
2 **nitrogen use efficiency and Zn biofortification of barley**

3 ABSTRACT: Intensive cropping systems have caused widespread Zn deficiency, a low
4 nutritional quality of cereals and environmental problems. The aim of this microplot field
5 experiment was to evaluate the possibility of use Zn in conjunction with urea in order to reduce
6 N rate for minimizing the environmental impact of fertilization but maintaining yields and
7 quality of grain. A barley crop (*Hordeum vulgare* L.) was developed in a calcareous soil under
8 **semi-realistic conditions**. Combinations of four Zn levels as ZnSO₄ and three N levels as urea
9 were tested. Zinc and N showed a synergistic effect by increasing yield and Zn content in all
10 barley parts, and protein content in grain. The remobilization of Zn from leaf tissue to grain was
11 dependent on Zn availability, and was only crucial under low availability. An application rate of
12 5 kg Zn ha⁻¹ was sufficient to increase the availability of Zn in soil and produce significant
13 increases in both plant Zn concentration and grain protein content. This rate produced an
14 increase in the agronomic efficiency of nitrogen of 15.5 kg grain kg⁻¹ N, and its application to
15 the soil allowed a reduction in the rate of N with a small decrease in barley grain yield and
16 nutritional value. Nevertheless, due to the interannual variability of rainfall, characteristic of the
17 Mediterranean climate, further studies are necessary to confirm and extend these results.

18 **KEYWORDS: agronomic efficiency of nitrogen; calcareous soil; field conditions; nutritional**
19 **quality; Zn availability**

20

21 INTRODUCTION

22 Nitrogen is one of the most yields-limiting nutrients in most crop production systems in the
23 world. Applying N has a major effect on crop biomass production, yield and quality, ¹ but
24 excessive application rates can cause a reduction of these as well as serious environmental
25 problems. Losses of N by volatilization and denitrification contribute to climate change, and
26 leaching of nitrates impact surface and ground water pollution. ² High N rates have been
27 applied for decades worldwide. The current nitrate concentrations in rivers across Europe are
28 often sufficient to promote eutrophication in many of Europe's coastal waters. In addition,
29 approximately 13% of groundwater monitoring stations across Europe recorded in 2009
30 exceeded the 50 mg NO₃⁻/L limit. ³ Currently around one third of groundwater bodies in
31 Europe exceeded the nitrate guideline value established by "Nitrates Directive 91/676/EEC" ⁴
32 which aims to reduce and prevent water pollution caused by nitrates loss from agricultural
33 sources. In year 2011, the regional government of "Castilla-La Mancha" (region located in
34 central Spain), following this EU directive, published the guidelines for N fertilization of
35 crops in the areas considered vulnerable to nitrate contamination. The maximum authorized N
36 rates for rainfed barley crop is between 50 and 60 kg N ha⁻¹ year⁻¹. ⁵ Therefore, N fertilizer
37 application rates should be reduced to minimize environmental problems. However, supply of
38 N below optimum level significantly decreases crude protein and Zn concentrations in wheat
39 and barley grains. ⁶

40 Barley (*Hordeum vulgare* L.) is widely grown in semiarid Mediterranean areas. Both the
41 low water requirements and the low cost of cultivation make barley an interesting crop for
42 farmers. As a result, this cereal is the largest crop grown, on the basis of surface area, in Spain
43 ⁷ and the fourth largest crop grown worldwide ⁸. Barley grain and straw are widely used for
44 animal fodder, and barley grain is also considered a raw material for some industries and is
45 consumed as unleavened bread. Barley is the main cereal used in the manufacture of animal

46 feed in Spain. The total amount consumed is in the order of 3.5 million Mg year⁻¹, which
47 represents about one-third of all cereals.⁹ An important limiting factor for barley production
48 is the poor fertility status of soils in these areas, coupled with imbalanced nutrition. Intensive
49 cropping systems in these areas based on continued use of NPK fertilizers without
50 micronutrient fertilization have caused, in addition to ground water pollution, widespread Zn
51 deficiency, mainly in light-textured soils.¹⁰ According to Alloway¹¹, Zn deficiency is major
52 problem particularly in alkaline-calcareous soils. Plants grown under Zn-deficient soil
53 conditions show a high susceptibility to environmental stress factors such as drought and
54 pathogenic infections. Among plants, cereals are generally the most susceptible to Zn
55 deficiency¹¹. Zinc deficiency produces a severe reduction in yield, but also in the Zn content
56 of cereal grain and hence in its nutritional quality. Barley is considered medium sensitive to
57 Zn deficiency¹² and, in the case of acute deficiency, its response to Zn application tends to be
58 very marked with yield increases of 48% compared with controls which only receive NPK
59 fertilizer.¹³ Although synthetic Zn chelates are highly efficient at correcting Zn deficiency in
60 calcareous soils,¹⁴⁻¹⁶ their use is limited due to their high cost. Zinc sulfate is the most
61 common Zn fertilizer applied to cereals crops because of its high water solubility and low cost
62¹⁷, and rates of 5-10 kg Zn ha⁻¹ soil as ZnSO₄ are adequate to correct Zn deficiency.¹¹ In
63 addition, the application of Zn to soil can be considered a tool for the agronomic
64 biofortification of crops.¹⁸ Therefore, increasing the Zn concentration in grain is a challenge
65 with significant implications in barley production and the nutritional quality of grain.¹⁹

66 As one of the major agricultural practices in crop production, N fertilization can have
67 significant effects on Zn accumulation in plants, possibly through alteration of soil
68 characteristics in the rhizosphere and/or the uptake and translocation of Zn in plants.²⁰ The
69 increase in grain Zn could be related to the increase in N compounds involved in the transport
70 of Zn in plants as nicotianamine, histidine, mugineic acid and proteins.²¹ Zebarth et al.²² and

71 De Brouwere and Smolders²³ showed that N fertilization increased Zn accumulation in wheat
72 and barley grain, whereas Brennan²⁴ found that N fertilizer application decreased the Zn
73 concentration of wheat grain grown on a range of soils in southwestern Australia. Recent
74 studies have shown that Zn micronutrient plays an important role in the utilization of N by
75 wheat plant.²⁵⁻²⁹ Khanda and Dixit³⁰ also found in rice under lowland conditions that the
76 maximum grain yield and nutrient uptake were observed with combined application to soil of
77 N and Zn. Therefore, Zn management could be an effective agronomic tool to reduce N
78 fertilizer application rate.

79 The selection of the appropriate source of N is very important to improve Zn availability
80 and to reduce N losses. In soils with high pH, the formation of Zn-NH₃ complexes increases
81 the solubility of Zn;³¹ urea or ammonium-based fertilizers could therefore be more effective
82 than nitrate-based fertilizers in order to maintain Zn in available forms.³² Urea is the most
83 common source of N fertilizer because of its low cost and high N content. However, its rapid
84 hydrolysis promotes NH₃ volatilization when it is applied to the soil surface without
85 incorporation, reducing its efficiency and contributing to environmental pollution. A wide
86 variety of compounds that include ZnSO₄ have been shown to inhibit the effects of urease
87 activity and to retard urea hydrolysis. Recently, Guimarães et al.³³ based on a greenhouse
88 study, showed that the use of ZnSO₄ in conjunction with urea significantly increased both the
89 total N uptake and the efficiency of urea N fertilization in a forage crop. However, they did
90 not study the efficiency of Zn applied or the concentration of Zn in the crop.

91 The main goal of this research was to evaluate in a calcareous soil the use of Zn (as
92 ZnSO₄) in conjunction with urea in order to reduce N rate for minimizing the environmental
93 impact of fertilization but maintaining yields and achieving an optimal concentration of
94 protein and Zn in barley grains. This goal is particularly important to adjust N fertilization to
95 the maximum authorized N rates in sensitive areas to nitrate contamination. The specific

96 objectives were to study the effect of combined ZnSO₄ and urea N fertilization on: (i) soil Zn
97 status, (ii) partitioning of Zn among different vegetative and grain tissues in barley crop (iii)
98 crop yields and the accumulation of both Zn and N in grain, and (iv) the agronomic
99 efficiencies of N and Zn.

100 MATERIALS AND METHODS

101 **Soil Characteristics.** An experiment was conducted at an agricultural field located in
102 Alcalá de Henares (Madrid) (40°32'N, 3°17'W). This area is close to the “Alcarria-
103 Guadalajara” sensitive area to nitrate contamination of “Castilla-La Mancha”.⁵ Soil samples
104 of the Ap horizon (0-28 cm) were air-dried, sieved (< 2 mm) and then analyzed according to
105 the Spanish official methodology.³⁴ The soil was classified as Calcic Haploxerept.³⁵ The pH
106 and electrical conductivity (EC) were measured at a 1:2.5 soil-water ratio; organic matter and
107 total nitrogen content were determined using the Walkley-Black and Kjeldahl methods,
108 respectively. Total and free CaCO₃ contents were determined by volumetric method; and
109 available phosphorus was evaluated using sodium bicarbonate.³⁶ The cation exchange
110 capacity (CEC) was assessed using both sodium acetate and ammonium acetate extraction
111 procedures. Water-holding capacity (WHC) at 33 kPa was determined in a Richard’s
112 membrane-plate extractor. Soil texture was analyzed using a Bouyoucos densimeter. Table 1
113 shows the main physico-chemical properties of the soil.

114 << Table 1 >>

115 **Field experiment.** Barley (*Hordeum vulgare* L., cv. Quench) was selected as the test plant.
116 A randomized complete block design with a factorial arrangement structure was used with
117 three replicate microplots (7.5 m² plot⁻¹). A rate of 175 kg barley ha⁻¹ was sown by direct
118 drilling in December of 2013. Basal fertilization included 90 kg P₂O₅ ha⁻¹ and 70 kg K₂O ha⁻¹
119 (as aqueous solutions of KH₂PO₄ and H₃PO₄). In February of 2014, fertilizer treatments of Zn
120 and N were applied to obtain all possible combinations of four Zn levels (control = 0, 5, 10

121 and 15 kg ha⁻¹) and three N levels (30, 60 and 90 kg ha⁻¹). In Spain, the average optimum N
122 rate can vary greatly because barley grain yield in rain-fed conditions is estimated to vary
123 between an average of 2000 kg ha⁻¹ in arid regions and an average of 5300 kg ha⁻¹ in humid
124 regions. A mineral fertilization of about 100 kg N ha⁻¹ is traditional in the study area (37).
125 Hence, the N applications rates were lower than the traditional rate. Zinc treatments were
126 applied by spraying aqueous solutions of ZnSO₄ in several passes, and nitrogen was applied
127 by spreading granular urea (46% N). In addition, control treatments without Zn or N
128 application were performed. The long-term means annual temperature and rainfall are 14.5 °C
129 and 402.7 mm, respectively.³⁸

130 Harvesting took place in June 2014, after maturity of barley grains. Barley biomass of six
131 0.5 m-long rows was collected randomly from each plot. The different plant parts (root, stem,
132 leaf and grain) were separated, and roots were washed in deionized water and subsequently
133 dried. The grain of the remaining plants in each plot were harvested by using a plot combine
134 (Wintersteiger Classic). All plant parts were dried to a constant weight in a forced-draft oven
135 at 60 °C, weighed and stored for later analysis. Soil samples were collected by shaking off soil
136 adhering to roots of the collected plants (approximately 10 cm depth). Soil samples were
137 homogenized, air-dried, sieved (< 2 mm), and stored for further analysis.

138 **Plant and soil analysis.** Total Zn in plant parts (root, stem, leaf and grain) was determined
139 by wet digestion in Sample Preparation Block System (SPB PROBE Perkin Elmer) with
140 “Teflon tubes” at a maximum temperature of 150 °C during 2 hours using 1.5 g of dried
141 samples, 10 mL of HCl (37%) and 10 mL of HNO₃ (65%). Grain N concentration was
142 determined using the Dumas method. Protein concentration was calculated as N concentration
143 multiplied by 5.83.³⁹

144 Total Zn in soil was determined as total Zn in plant ,but changing 10 mL of HCl (37%) for
145 5 mL of HF (48%) and 5 mL of water, and increasing digestion time to 3.5 h. Potentially

146 available Zn was extracted with DTPA 5 mM + CaCl₂ 10 mM + triethanolamine (TEA) 0.1 M
147 adjusted to pH 7.3 (40). Soil Zn was fractionated into: water soluble plus exchangeable
148 (WSEX), carbonate bound (CAR), easily reducible metal oxide bound (RMO), amorphous
149 mineral colloid bound (AMC), crystalline Fe oxide bound (CFeO), organic material and
150 sulfide bound (OM), and residual (RES) fractions using a modified version of the procedure
151 developed by Leleyter and Probst.⁴¹ Soil samples (2.5 g) were sequentially extracted with 25
152 mL of each reagent (Table 2). After each extraction, the suspension was centrifuged at 5000 ×
153 g (relative centrifuge force) for 15 min. and the supernatant solution was vacuum-filtered
154 through a 0.45 μm cellulose acetate paper.

155 << Table 2 >>

156 All samples were extracted and analyzed. The Zn concentration was determined by AAS or
157 by graphite furnace atomic absorption spectrophotometry (GF-AAS). The “Perkin-Elmer
158 Pure” standard checks were used for the Quality Assurance System (certified by NIST-SRM).
159 Standard solutions of Zn were prepared for each extraction in a background solution of the
160 extracting agents.

161 **Statistical Analysis.** Correlation analyses and other statistical studies were performed with
162 Statgraphics Plus software, version 5.1 (Manugistic Inc. Rockville, MD). Multifactor analysis
163 of variance was carried out to determine the main effects and interactions of the different
164 parameters. Multiple comparisons of variables were made using the least significant
165 difference (LSD) test for separation of means. To establish statistical significance, a
166 probability level of $P \leq 0.05$ was chosen.

167 RESULTS

168 **Soil Zn Status.** The ANOVA of the effects of Zn application rate, N application rate and
169 their interactions revealed that total Zn, potential available Zn (DTPA-extractable Zn) and Zn
170 associated to different fractions at the time of the barley harvest only depended on Zn

171 application rate and there was no significant interaction between Zn and N (Table 3). The
172 values of DTPA-extractable Zn of soils without Zn addition (Zn_0 ; Table 3) and of initial soil
173 (see Table 1) showed that Zn soil content was low and nearly at the limit of sufficiency (1.0
174 mg Zn kg⁻¹ soil).⁴⁰ However, Zn treated soils (even at the lower Zn application rate)
175 presented values of potential available Zn, considerably above the levels of sufficiency.

176 << Table 3 >>

177 The lowest values of Zn concentrations in the more labile fractions of soil, which are the
178 most important for crop nutrition, were obtained with the Zn_0 treatment (see Table 3). The
179 added Zn was redistributed in the different soil fractions with significant increases mainly in
180 the first three fractions (WSEX, CAR and RMO). The applications of 5 kg Zn ha⁻¹ produced
181 Zn concentrations in WSEX, CAR and RMO fractions of 3.3, 3.1 and 2.0 times the
182 concentration in the Zn_0 treatment, respectively. The rate 10 kg Zn ha⁻¹ soil produced Zn
183 concentrations of 6.4, 4.7 and 2.7 times the concentration in the Zn_0 treatment for the same
184 fractions, and the highest Zn rate applied (15 kg Zn ha⁻¹) produced Zn concentrations 12.7,
185 10.3 and 2.8 times the concentration in the Zn_0 treatment for the same fractions, respectively.

186 **Plant Response to Zinc and Nitrogen Fertilization.** The Zn application rate significantly
187 affected Zn concentrations in root, stem, leaf and grain of barley, dry matter yield (DM) of all
188 plant parts, as well as the protein concentration in grain. Nitrogen application rates also
189 significantly affected these parameters except for root Zn concentration and root yield. The
190 interaction between Zn and N had significant effects on root, stem and leaf yield and also on
191 root Zn concentration and grain protein content (Table 4).

192 << Table 4 >>

193 With respect to Zn concentration (Figure 1a), the highest values in root were obtained with
194 the combined treatments $N_{30}Zn_{15}$, $N_{60}Zn_{15}$, $N_{90}Zn_{10}$ and $N_{60}Zn_{10}$ which were not significantly
195 different between them with an increase nearly 2.5 times the concentration of the $N_{30}Zn_0$

196 treatment. The lowest values were found in the N₃₀Zn₀, N₆₀Zn₀ and N₉₀Zn₀ treatments (all of
197 them without Zn application). The 5 and 10 kg Zn ha⁻¹ application rates produced significant
198 increases in stem Zn concentration (Figure 1b): 1.28 and 1.78 times the concentration in Zn₀
199 treatments, respectively. However, continued Zn supply, from 10 to 15 kg Zn ha⁻¹ soil did not
200 produce a significant increase in stem Zn concentration. Similar effects were observed in leaf
201 Zn concentration, however it is interesting to note that the increase produced by the 5 kg Zn
202 ha⁻¹ application rate was higher, 3.33 times the concentration of Zn₀ treatments, which had a
203 mean value of 6.43 mg Zn kg⁻¹ DM (Figure 1c). Grain Zn concentration increased
204 significantly with the increase in the rate of Zn. The increases with respect to the Zn₀
205 treatments were 4.13, 5.44 and 8.56 mg Zn kg⁻¹ for the 5, 10 and 15 kg Zn ha⁻¹ application
206 rates, respectively (Figure 1d).

207 << Figure 1 >>

208 Concerning the effect of N fertilization, the increase in the N application rate produced
209 increases in the Zn concentrations in stem, leaf and grain of barley (see Figure 1). The Zn
210 concentration increased significantly from 30 to 90 kg N ha⁻¹ for stem, leaf and grain.
211 However, from 30 to 60 kg N ha⁻¹ the increase in Zn concentration was only significant in
212 grain.

213 << Table 5 >>

214 The highest root yield was obtained with the combined treatment N₆₀Zn₁₅ followed by the
215 N₉₀Zn₁₀, which were not significantly different between them (Table 5). The highest stem
216 yield was obtained with the N₉₀Zn₁₀ and N₉₀Zn₅ treatments, while the highest leaf yield was
217 observed with the N₉₀Zn₁₀ followed by the N₉₀Zn₅ and the N₆₀Zn₁₅ treatments. In contrast, the
218 lowest DM yield of root, stem and leaf was obtained with the N₉₀Zn₀, N₆₀Zn₀ and N₆₀Zn₀
219 treatments, respectively (all of them without Zn addition).

220 << Figure 2 >>

221 As shown in Figure 2, grain protein concentration reached its maximum with the combined
222 treatment N₉₀Zn₁₅ (with the highest N and Zn rates) followed by the N₉₀Zn₁₀ (which was not
223 significantly different) and the N₉₀Zn₅ treatments, and the lowest value with N₃₀Zn₀, N₃₀Zn₁₅,
224 and N₆₀Zn₀ treatments.

225 Zinc and N rates significantly influenced grain yield, but their interaction was not
226 significant (see Table 4). The grain yield showed a clear response to the 5 kg Zn ha⁻¹
227 application rate (Zn₅), resulting in an increase of 20% in grain yield with respect to the
228 treatments without Zn application (Zn₀). However, the other two Zn application rates (10 and
229 15 kg Zn ha⁻¹) did not produce any significant increase in yield with respect to the 5 kg Zn ha⁻¹
230 application rate (Figure 3). Furthermore, the N application rate produced increases in the
231 grain yield of barley, although this increase was only significant when raising the rate from 30
232 to 90 kg N ha⁻¹, obtaining an increase of 13%. As indicated in the Materials and Methods
233 section, a control without either N or Zn (N₀Zn₀) was performed. The results of this treatment
234 were: 2022 ± 189 kg grain ha⁻¹, 17.23 ± 0.58 mg Zn kg⁻¹ grain and 5.66 ± 0.18% of protein in
235 grain.

236 << Figure 3 >>

237 DISCUSSION

238 The application of the lower rate of Zn sulfate (5 kg Zn ha⁻¹) to the soil was sufficient to
239 increase the availability of Zn in soil above the level sufficiency indicated in the literature for
240 this type of soil.⁴⁰ Furthermore, the residual Zn produced by this Zn treatment was
241 sufficiently available that latter crops could be grown without further micronutrient additions.
242 According to various authors, Zn fertilizers could have a residual effect of several years.⁴²⁻⁴³

243 The increase in both Zn associated to the most labile fractions and DTPA-extractable Zn of
244 soil, *i.e.* in potential availability of Zn in soil due to Zn supply, not only produced increases in
245 the concentrations of Zn in all parts of plants but also in their yields. For example, the

246 correlation factors (r) between grain Zn concentration and Zn-DTPA-TEA, Zn-WSEX and
247 Zn-CAR were 0.884, 0.868 and 0.883 ($P \leq 0,001$), respectively, and 0.702, 0.664 and 0.659 (P
248 < 0.05), respectively for grain yield.

249 The Zn concentrations in barley grain for the control treatments without either N or Zn
250 (N_0Zn_0 ; 17.23 ± 0.58 mg Zn kg^{-1}) and for treatments with N but without Zn (20.10 mg Zn kg^{-1} ;
251 see Figure 1d) were lower than the value of 23 mg Zn kg^{-1} indicated by the CVB ⁴⁴ for
252 barley grain grown in the Netherlands. However, treatments with 5, 10 and 15 kg Zn ha^{-1}
253 (combined with 30, 60 and 90 kg N ha^{-1}) produced grain Zn concentrations ranging from 24
254 to 29 mg Zn kg^{-1} . The results also revealed that N fertilization was effective in enhancing Zn
255 concentration in all barley parts especially in grain (see Figure 1). Similar results were
256 reported by different authors ^{25,28,45-46} for wheat crops. According to numerous authors ^{47,26,17}
257 N is a critical player in the uptake and accumulation of Zn in plants, since important N
258 compounds as nicotidamine, mugineic acid and some proteins are responsible for root Zn
259 uptake, transport and hence mobility of Zn in plant. Moreover, N fertilization was also
260 effective in enhancing grain protein concentration, which is very important for the nutritional
261 quality of grain. For a production with the highest protein content, combined treatments with
262 90 kg N ha^{-1} and 10 or 15 kg Zn ha^{-1} should be selected.

263 As expected, root, stem and leaf Zn concentrations increased with Zn application up to an
264 application rate of 10 kg Zn ha^{-1} . In contrast, these Zn concentrations were not significantly
265 affected by an additional increase in the Zn application rate from 10 to 15 kg Zn ha^{-1} .
266 Interestingly, the concentration of Zn in the leaf at harvest time for the Zn_0 treatment was low
267 in comparison with the treatments in which Zn was applied (see Figure 1c). This can be
268 explained by the remobilization of Zn from leaves to grain in conditions of restricted Zn
269 availability, since the control soil was close to the limit of sufficiency in Zn whereas treated
270 Zn soils were considerably above the levels of sufficiency. The values of the translocation

271 factor from leaves to grain of barley calculated as Zn concentration in the grain divided by Zn
272 concentration in the leaves, which were 3.16 (*b*), 1.14 (*a*), 1.10 (*a*) and 1.15 (*a*) (statistical
273 letters are after numbers) for Zn₀, Zn₅, Zn₁₀, and Zn₁₅, respectively, showed this very clearly.
274 Under conditions of sufficient Zn availability (Zn₅, Zn₁₀, and Zn₁₅) the remobilization of Zn
275 from other plant parts (as leaf) to grain is not crucial. Therefore, the remobilization of Zn
276 from leaf tissues to grain was dependent on Zn availability in soil. In an experiment of
277 biofortification of durum wheat with Zn, Kutman et al.²⁷ reported that when the root uptake
278 of Zn is restricted due to drought or low availability of this nutrient, remobilization of the
279 previously absorbed and stored Zn from source tissues (leaves, stems) may be a major
280 contributing factor to the grain Zn accumulation.

281 The results of our experiment also indicated that N and Zn fertilization promoted the
282 uptake of Zn by barley grain, which is interesting from the point of view of biofortification.
283 Consequently, the combined N-Zn fertilization could be considered an effective
284 biofortification strategy of barley grain with Zn.

285 The effect of adequate rates of N fertilizer on crop yield is generally accepted. In our study,
286 a high N application rate was required to obtain the highest root, stem and leaf yields.
287 However, the lowest yields were obtained without Zn fertilization. Thus, a minimum Zn
288 availability to plant is necessary to improve root, stem and leaf yields through N fertilization.
289 This suggests that the Zn micronutrient plays an important role in the utilization of N by
290 barley plant. Similar conclusions were reached by Kutman et al.²⁷ for a wheat crop.
291 Therefore, an adequate availability of Zn is crucial for ensuring that crops utilize N fertilizer
292 inputs efficiently. In the present experiment, the increase in grain yield of barley due to the
293 increase in the rate of N from 30 to 90 kg ha⁻¹ was of 473 kg grain ha⁻¹ (see Figure 3).

294 In our experiment, the grain yield obtained with treatments where Zn and N were applied
295 was superior to the mean value of two-row barley grain yield in Spain in the year 2014, under

rain-fed conditions (2608 kg ha⁻¹).⁷ Figure 3 shows the significant effect of an adequate Zn supply on barley grain yield. The lower application rate of Zn (5 kg Zn ha⁻¹) was sufficient to increase the grain yield by more than 20% with respect to the control (Zn₀). This effect could be due to the fact that Zn acts as a co-factor of numerous enzymes involved in the metabolism of carbohydrates, proteins and auxins, in membrane integrity and in flowering and seed production.¹¹ Different authors^{19,48-50} have reported increases in yield of different crops as wheat, maize, safflower and rice with Zn fertilization. Arora and Singh,⁵¹ in a field experiment with barley as test plant, obtained the highest grain and straw yield with the application of 7.5 kg Zn ha⁻¹ and 90 kg N ha⁻¹, but these yields were not significantly different with the application of 7.5 kg Zn ha⁻¹ and 60 kg N ha⁻¹. The efficiency of N fertilization is a very important factor since improved efficiency can result in a reduction of input costs and of the negative impact of excessive N on the environment. In relation to this, the value of the agronomic efficiency of N (A.E.N.) was calculated as (Grain Yield of Treatment, kg ha⁻¹ – Grain Yield of Control, kg ha⁻¹) / (Quantity of N applied, kg ha⁻¹).⁵² The ANOVA of the effects of Zn rate, N rate and their interactions showed that the A.E.N depended on Zn (P < 0.001) and N (P < 0.0001) rates, but there was no significant interaction between them. Figure 4 shows the effect of both Zn and N fertilization on the A.E.N. It is interesting to note that the application of 5 kg Zn ha⁻¹ produced a significant increase in the A.E.N. (1.67 times) with respect to the control (Zn₀), and that additional amounts of Zn did not significantly increased this parameter. With respect to the rate of N, the highest A.E.N. was obtained with the lower rate, and there were no significant differences between the other N rates. Tilman et al.⁵³ indicated that the highest efficiency of N fertilizer is achieved with the first increments of added nitrogen, and that efficiency declines at higher levels of addition. Anbessa et al.⁵⁴ reported that the mean A.E.N. values of three spring barley cultivars in Canada during the period 1994 to 1996 were 25.2 and 15.0 kg grain kg⁻¹ N for the rates 50 and 100 kg N ha⁻¹,

321 respectively, but without Zn fertilization. In our experiment with combined N-Zn fertilization,
322 the results obtained were higher. So, for example, for the lowest application rate of Zn (5 kg
323 Zn ha⁻¹) the A.E.N. values were 61.0, 32.4 and 23.1 kg grain kg⁻¹ N for the rates 30, 60 and 90
324 kg N ha⁻¹, respectively, showing the importance of the Zn supply. This low Zn rate produced
325 an increase in the A.E.N. of 15.5 kg grain kg⁻¹ N (the mean value for the 30, 60 and 90 kg N
326 ha⁻¹), and its application to soil allows reduction of the rate of N to 60 kg N ha⁻¹ or even less
327 with a small decrease in yield and the nutritional value of barley grain.

328 Due to the rapid hydrolysis of the amide N by reaction with the enzyme urease, it is
329 possible to find significant losses of urea N through NH₃ volatilization and NO₃⁻ leaching,
330 resulting in a decrease in the urea N use efficiency.^{55,56} One effective way of mitigating these
331 problems is to use compounds that inhibit soil urease activity and retard urea hydrolysis. In
332 this respect, the observed increase in the A.E.N. in the present experiment could be partially
333 explained by the inhibitory effects of ZnSO₄ (applied to the soil in conjunction with urea
334 fertilizer) on urease activity. Guimarães et al.³³ also showed that the use of ZnSO₄ in
335 conjunction with urea significantly increased both the total N uptake and the efficiency of
336 urea N fertilization in a forage crop. Although they found that Zn was effective for inhibiting
337 urease activity, they suggested that the most likely benefits of applying Zn in conjunction
338 with urea could be attributed to both supply an essential micronutrient and the synergistic
339 interaction of these two nutrients. According to Marschner,¹⁷ this interaction could be due to
340 the occurrence of Zn as a constituent of carbonic anhydrase and other enzymes required for
341 protein synthesis and carbohydrate metabolism.

342 << Figure 4 >>

343 Another interesting parameter considered in this study was the agronomic efficiency of Zn
344 (A.E.Zn), which shows the capacity of yield increase per kg of applied Zn [A.E.Zn = (Grain
345 Yield Treatment, kg ha⁻¹ – Grain Yield of Control, kg ha⁻¹) / (Quantity of Zn applied, kg ha⁻¹

346 ¹]. The ANOVA of the effects Zn rate, N rate and their interactions showed that the A.E.Zn
347 depended on Zn ($P < 0.01$) and N ($P < 0.05$) rates, though there was no a significant
348 interaction between them. The highest A.E.Zn was obtained with the lower rate of Zn (5 kg
349 ha^{-1}), though it was not significantly different from the 10 kg Zn ha^{-1} rate (Figure 5). Although
350 the A.E.Zn decreased with the rate of N, this decrease was only significant from 60 to 90 kg
351 N ha^{-1} . If the combined fertilization N-Zn is considered, the best treatment was N₃₀Zn₅ (30 kg
352 N ha^{-1} and 5 kg Zn ha^{-1}) with an A.E.Zn value of 178.6 kg grain kg^{-1} Zn. For barley crop,
353 Genc et al. ⁵⁷ also reported that the highest AEZn was obtained with the lower Zn application
354 rate.

355 << Figure 5 >>

356 In conclusion, in soils with low Zn availability, Zn fertilization produces significant
357 increases in Zn concentration and dry matter yield of all barley parts, and also in protein
358 content of grain. In addition, N fertilization also produces increases in the same parameters.
359 All of this demonstrates the synergistic effect of Zn and N fertilization. In our experiment, the
360 5 kg Zn ha^{-1} application rate was sufficient to increase the availability of Zn in the soil and
361 hence to produce the aforementioned increments in plant parameters. The application of 5 kg
362 Zn ha^{-1} also allowed to reduce the rate of N to 60 or even 30 kg N ha^{-1} with only small
363 decreases in barley grain yield and nutritional quality, and hence to adjust N fertilization to
364 the maximum authorized N rates in sensitive areas to nitrate contamination.

365 Our results also confirm that the remobilization of Zn from leaf tissue to grain is dependent
366 on soil Zn availability to plants, with this being crucial only under low Zn availability.

367 The A.E.N. also shows that 5 kg Zn ha^{-1} is the optimum rate, since the highest increase in
368 this parameter (15.5 kg grain kg^{-1} N) was obtained with this Zn rate.

369 Moreover, the highest A.E.Zn (51.4 kg grain kg^{-1} Zn; mean value for the 30, 60 and 90 kg
370 N ha^{-1}) was again obtained with the same rate (5 kg Zn ha^{-1}).

371 All of the conclusions shown the potential for using Zn in conjunction with urea in
372 calcareous Mediterranean soils in order to obtain both higher barley yields and greater
373 nutritional quality. Other advantages would include reducing input costs and environmental
374 problems resulting from excessive N applications, such as greenhouse gas emissions and
375 nitrate leaching losses. However, due to the interannual variability of rainfall, characteristic of
376 the Mediterranean climate, further studies are necessary to confirm and extend these results.

377 **ACKNOWLEDGEMENTS**

378 This study was financially supported by the Community of Madrid (Agrisost Project,
379 S2013/ABI-2717).

380 **LITERATURE CITED**

- 381 (1) Fageria, N.K. Nitrogen harvest index and its association with crop yields. *J. Plant Nutr.* **2014**, *7(6)*, 795-
382 810. doi:10.1111/j.1469-8137.2010.03488.x.
- 383 (2) Cameron, K. C.; Di, H. J.; Moir, J. L. Nitrogen losses from the soil/plant system: a review. *Ann Appl Biol.*
384 **2013**, *162*, 145–173. doi:10.1111/aab.12014
- 385 (3) EUROSTAT. [http://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-](http://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_nitrate_pollution_of_water)
386 [_nitrate_pollution_of_water](http://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_nitrate_pollution_of_water). **2016**. Accessed 24 February 2017
- 387 (4) Nitrates Directive 91/676/EEC. Council Directive 91/676/EEC of 12 December 1991 concerning the
388 protection of waters against pollution caused by nitrates from agricultural sources.
389 <http://data.europa.eu/eli/dir/1991/676/oj>
- 390 (5) DOCM. Diario Oficial de Castilla la Mancha. Orden de 07/02/2011, de la Consejería de Agricultura y
391 Medio Ambiente. **2011**.
- 392 (6) Erbs, M.; Manderscheid, R.; Jansen, G.; Seddig, S.; Pacholski, A.; Weigel, H-J. Effects of free-air CO₂
393 enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley
394 grown in a crop rotation. *Agr Ecosyst Environ.* **2010**, *136*, 59–68. doi:10.1016/j.agee.2009.11.009
- 395 (7) MAGRAMA. Anuario de estadística. Ministerio de Agricultura, Alimentación y Medio Ambiente, 2014.
396 <http://www.magrama.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/default.aspx>
- 397 (8) FAO. Food and Agriculture Organization of the United Nations. FAOSTAT Statistics Database.
398 <http://www.fao.org/faostat/en/> **2017**. Accessed 24 February 2017.

- 399 (9) FEDNA. Fundación Española para el Desarrollo de la Nutrición Animal.
400 http://www.fundacionfedna.org/ingredientes_para_piensos/cebada-dos-carreras-nacional-96-pb. Accessed
401 December 2016.
- 402 (10) Kumar, V.; Ahlawat, V. S.; Antil, R. S. Interactions of nitrogen and zinc in pearl millet: 1. Effect of
403 nitrogen and zinc levels on dry matter yield and concentration and uptake of nitrogen and zinc in pearl
404 millet. *Soil Sci.* **1985**, *139*, 351–356.
- 405 (11) Alloway, B. J. *Zinc in soils and crop nutrition*. International Zinc Association, Brussels, Belgium. 2008.
406 Available via web. <http://www.zinc.org/publications/>. Accessed 24 February 2017.
- 407 (12) Martens, D. C.; Westerman, D. T. Fertilizer Applications for Correcting Micronutrient Deficiencies, in:
408 Mortvedt, J.J. et al. (Eds.) *Micronutrients in Agriculture* (2nd edn.), Book Series No.4, Soil Science Society
409 of America, Madison, WI, USA, **1991**; pp 549–592.
- 410 (13) Singh, M. V. Evaluation of micronutrient status of difference agroecological zones of India. *Fert. News*.
411 **2001**, *46(2)*, 25–42.
- 412 (14) Gonzalez, D.; Obrador, A; Alvarez, J. M. Behavior of zinc from six organic fertilizers applied to a navy
413 bean crop grown in a calcareous soil. *J. Agric. Food Chem.* **2007**, *55*, 7084–7092.
- 414 (15) Gonzalez, D.; Novillo, J.; Rico, M. I.; Alvarez, J. M. Leaching and efficiency of six organic zinc fertilizers
415 applied to navy bean crop grown in a weakly acidic soil of Spain. *J. Agric. Food Chem.* **2008**, *56*, 3214–
416 3221.
- 417 (16) Obrador, A.; Novillo, J.; Alvarez, J. M. Mobility and availability to plants of two zinc sources applied to a
418 calcareous soil. *Soil Sci. Soc. Am. J.* **2003**, *67*, 564–572.
- 419 (17) Marschner, H. *Mineral Nutrition of Higher Plants*, 3rd ed. Academic Press, London. **2012**.
- 420 (18) Khoshgoftarmanesh, A. H.; Schulin, R.; Chaney, R. L.; Daneshbakhsh, B.; Afyuni, M. Micronutrient-
421 efficient genotypes for crop yield and nutritional quality in sustainable agriculture: A review. *Agron.*
422 *Sustain. Dev.* **2010**, *30*, 83–107. doi:10.1051/agro/2009017.
- 423 (19) Guo, J. X.; Feng, X. M.; Hu, X. Y.; Tian, G. L.; Ling, N.; Wang, J. H.; Shen, Q. R.; Guo, S. W. Effects of
424 soil zinc availability, nitrogen fertilizer rate and zinc fertilizer application method on zinc biofortification of
425 rice. *J. Agr. Sci.* **2016**, *154*, 584–597. doi:10.1017/S0021859615000441.
- 426 (20) Gao, X.; Grant, C.A. Interactive effect of N fertilization and tillage management on Zn biofortification in
427 durum wheat (*Triticum durum*). *Can. J. Plant Sci.* **2011**, *91*, 951–960.

- 428 (21) Gupta, N.; Ram, H.; Kumar, B. Mechanism of Zinc absorption in plants: uptake, transport, translocation
429 and accumulation. *Rev. Environ. Sci. Biotechnol.* **2016**, *15*, 89–109. doi:10.1007/s11157-016-9390-1.
- 430 (22) Zebarth, B. J.; Warren, C. J.; Sheard, R. W. Influence of the rate of nitrogen fertilization in the mineral
431 content of winter wheat in Ontario. *J. Agric. Food Chem.* **1992**, *40*, 1528–1530.
- 432 (23) De Brouwere, K.; Smolders, E. Yield response of crops amended with sewage sludge in the field is more
433 affected by sludge properties than by final soil metal concentration. *Eur. J. Soil Sci.* **2006**, *57*, 858–867.
- 434 (24) Brennan, R. F. Availability of previous and current applications of zinc fertilizer using single
435 superphosphate for the grain production of wheat on soils of South Western Australia *J Plant Nutr.* **1996**,
436 *19*, 1099–1115.
- 437 (25) Cakmak, I.; Kalayci, M.; Kaya, Y. et al. Biofortification and localization of zinc in wheat grain. *J. Agric.*
438 *Food Chem.* **2010**, *58*, 9092–9102.
- 439 (26) Erenoglu, E. B.; Kutman, U. B.; Ceylan, Y.; Yildiz, B.; Cakmak, I. Improved nitrogen nutrition enhances
440 root uptake, root-to-shoot translocation and remobilization of zinc (⁶⁵Zn) in wheat. *New Phytol.* **2011**, *189*,
441 438–448. doi:10.1080/01904167.2014.881855.
- 442 (27) Kutman, U. B.; Yildiz, B.; Ozturk, L.; Cakmak, I. Biofortification of durum wheat with zinc through soil
443 and foliar applications of nitrogen. *Cereal Chem.* **2010**, *87(1)*, 1–9.
- 444 (28) Kutman, U. B.; Yildiz, B.; Cakmak, I. Improved nitrogen status enhances zinc and iron concentrations both
445 in the whole grain and the endosperm fraction of wheat. *J. Cereal Sci.* **2011**, *53*, 118–125.
- 446 (29) Kutman, U. B.; Kutman, B. Y.; Ceylan, Y.; Ova, E. A.; Cakmak, I. Contributions of root uptake and
447 remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and
448 nitrogen nutrition. *Plant Soil.* **2012**, *361*, 177–187.
- 449 (30) Khanda, C. M.; Dixit, L. Effect of zinc and nitrogen fertilization on yield and nutrient uptake of summer
450 rice. *Indian J. Agron.* **1996**, *41(3)*, 368–372.
- 451 (31) Lindsay, W. L. Inorganic phase equilibria of micronutrients in soils. In *Micronutrients in Agriculture*, 2nd
452 ed., J.J. Mortvedt, et al. (Eds.) Book Series No.4, Soil Science Society of America, Madison, WI, USA,
453 **1972**, pp 41–57.
- 454 (32) Rehman, H.; Aziz, T.; Farooq, M. et al. Zinc nutrition in rice production systems: a review. *Plant Soil.*
455 **2012**, *361*, 203–226. doi:10.1007/s11104-012-1346-9
- 456 (33) Guimarães, G. G. F.; Mulvaney, R.L.; Cantarutti, R. B.; Teixeira, B. C.; Vergütz, L. Value of copper, zinc,
457 and oxidized charcoal for increasing forage efficiency of urea N uptake. *Agr. Ecosyst. Environ.* **2016**, *224*,

- 458 157–165. doi:10.1016/j.agee.2016.03.036
- 459 (34) MAPA Métodos oficiales de análisis, Vol. III. Ministerio de Agricultura, Pesca y Alimentación, Madrid,
460 Spain. **1994**.
- 461 (35) Soil Survey Staff. Keys to soil taxonomy. 11th ed. USDA (ed) - Natural Resources Conservation Service,
462 Washington, DC, USA. **2010**.
- 463 (36) Olsen, S. R.; Cole, C. V.; Watanabe, F. S.; Dean, L. A. Estimation of available phosphorous in soils by
464 extraction with sodium bicarbonate; Circ. 939; USDA: Washington, DC. **1954**.
- 465 (37) Lopez-Bellido, L.; Aso, J. B. et al. Abonado de los cereales de invierno: trigo y cebada. In: *Guía práctica*
466 *de la fertilización racional de los cultivos en España, parte II*. Ministerio de Medio Ambiente y Medio
467 Rural y Marino; **2009**, Available at:
468 [www.mapama.gob.es/es/agricultura/.../02_FERTILIZACIÓN\(BAJA\)_tcm7-207770.pdf](http://www.mapama.gob.es/es/agricultura/.../02_FERTILIZACIÓN(BAJA)_tcm7-207770.pdf)
- 469 (38) Fernández-Getino, A. P.; Tenorio, J. L.; Santín-Montanyá, M. I. Overview on soil fertility management for
470 cereals crops in semi-arid conditions of Spain. *Arch Agron Soil Sci.* **2015**, *61*(2), 139-151.
- 471 (39) Merrill, A. L.; Watt, B. K. Energy value of foods: basis and derivation. Agriculture Handbook No. 74.
472 Washington, DC, ARS United States Department of Agriculture. **1973**.
- 473 (40) Lindsay, W. L.; Norvell, W. A. Development of a DTPA soil test for zinc, iron, manganese, and copper.
474 *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428.
- 475 (41) Leleyter, L.; Probst, J. L. A New Sequential Extraction Procedure for the Speciation of Particulate Trace
476 Elements in River Sediments. *Int. J. Environ. An. Ch.* **1999**, *73*(2), 109–128.
- 477 (42) Almendros, P.; Gonzalez, D.; Alvarez, J. M. Long-term bioavailability effects of synthesized zinc chelates
478 fertilizers on the yield and quality of a flax (*Linum usitatissimum* L.) crop. *Plant Soil.* **2013**, *368*, 251–265.
479 doi:10.1007/s11104-012-1502-2.
- 480 (43) Brennan, R.F.; Bolland, D. A. Residual values of soil–applied zinc fertilizer for early vegetative growth of
481 six crop species. *Aust J Exp Agr.* **2006**, *46*, 1341–1347.
- 482 (44) CVB. Feed Tables. Produktschap Diervoeding, The Netherlands. Wageningen Academic Publishers, The
483 Netherlands. **2007**.
- 484 (45) Martín-Ortiz, D.; Hernández-Apaolaza, L.; Gárate, A. Efficiency of a NPK fertilizer with adhered zinc
485 lignosulfonate as a zinc source for maize (*zea mays* l.). *J. Agric. Food Chem.* 2009, *57* (19), 9071–9078.
486 doi: 10.1021/jf9017965.

- 487 (46) Xue, Y-F.; TEagling, T.; He, J.; Zou, Ch-Q.; McGrath, S. P.; Shewry, P. R.; Zhao F-J. Effects of nitrogen
488 on the distribution and chemical speciation of iron and zinc in pearling fractions of wheat grain. *J. Agric.*
489 *Food Chem.* **2014** 62 (20), 4738–4746. doi: 10.1021/jf500273x.
- 490 (47) Curie, C.; Cassin, G.; Couch, D. et al. Metal movement within the plant: contribution of nicotianamine and
491 yellow stripe 1-like transporters. *Ann Bot.* **2009**, 103, 1–11.
- 492 (48) Aytac, Z.; Gulmezoglu, N.; Sirel, Z.; Tolay, I.; Torun, A. A. The effect of zinc on yield, yield components
493 and micronutrient concentrations in the seeds of safflower genotypes (*Carthamus tinctorius* L.). *Not. Bot*
494 *Horti Agrobi.* **2014**, 42, 202–208.
- 495 (49) Singh, D.; Yadav, S.; Nautiyal, N. Evaluation of growth responses in wheat as affected by the application
496 of zinc and boron to a soil deficient in available zinc and boron. *Comm. Soil Sci. Plant. Anal.* **2014**, 45,
497 765–776. doi:10.1080/00103624.2013.858729
- 498 (50) Tariq, A.; Anjum, S. A.; Randhawa, M. A.; Ullah, E.; Naeem, M.; Qamar, R.; Ashraf, U.; Nadeem, M.
499 Influence of zinc nutrition on growth and yield behaviour of maize (*Zea mays* L.) hybrids. *Am. J. Plant Sci.*
500 **2014**, 5, 2646–2654. doi:10.4236/ajps.2014.518279
- 501 (51) Arora, S.; Singh, M. Interaction effect of zinc and nitrogen on growth and yield of barley (*Hordeum*
502 *vulgare* L.) on Typic Ustipsamments. *Asian J Plant Sci.* **2004**, 3, 101–103. doi:10.3923/ajps.2004.101.103
- 503 (52) Baligar, V. C.; Fageria, N. K.; He, Z. L. Nutrient use efficiency in plants. *Commun Soil Sci Plant Anal.*
504 **2001**, 32, 921–950.
- 505 (53) Tilman, D.; Cassman, K. G.; Matson, P. A.; Naylor, R.; Polasky, S. Agricultural sustainability and
506 intensive production practices. *Nature.* **2002**, 418, 671–677.
- 507 (54) Anbessa, Y.; Juskiw, P. Review: Strategies to increase nitrogen use efficiency of spring barley. *Can J Plant*
508 *Sci.* **2012**, 92, 617–625. doi:10.4141/cjps2011-207.
- 509 (55) Nannipieri, P.; Falchini, L.; Landi, L.; Benedetti, A. et al. Nitrogen uptake by crops, soil distribution and
510 recovery of urea-N in a sorghum-wheat rotation in different soils under Mediterranean conditions. *Plant*
511 *Soil.* **1999**, 208, 43–56.
- 512 (56) Blennerhassett, J. D.; Quin, B. F.; Zaman, M.; Ramakrishnan, C. The potential for increasing nitrogen
513 responses using Agrotain treated urea. *Proceed NZ Grassl Assoc.* **2006**, 68, 297–301.
- 514 (57) Genc, Y.; McDonald, G. K.; Graham, R. D. Critical deficiency concentration of zinc in barley genotypes
515 differing in zinc efficiency and its relations to growth responses. *J. Plant Nutr.* **2002**, 25, 545–560.
- 516

Figure 1. Effect of Zn (0, 5, 10, 15 kg ha⁻¹) and N (30, 60, 90 kg ha⁻¹) treatments on plant parts Zn concentrations (mg kg⁻¹) of barley (*Hordeum vulgare* L.) grown under field conditions. Vertical bar at each of the data points represents the standard deviation from the means. Different letters represent statistical differences at P < 0.05 (LSD test).

“1.5 column fitting image”

Figure 2. Effect of Zn (0, 5, 10, 15 kg ha⁻¹) and N (30, 60, 90 kg ha⁻¹) treatments on grain protein concentrations (%) of barley (*Hordeum vulgare* L.) grown under field conditions. Vertical bar at each of the data points represents the standard deviation from the means. Different letters represent statistical differences at P < 0.05 (LSD test).

“single column fitting image”

Figure 3. Effect of Zn (0, 5, 10, 15 kg ha⁻¹) and N (30, 60, 90 kg ha⁻¹) treatments on grain yield (kg ha⁻¹) of barley (*Hordeum vulgare* L.) grown under field conditions. Vertical bar at each of the data points represents the standard deviation from the means. Statistical differences at P < 0.05 (LSD test) are represented by different letters.

“single column fitting image”

Figure 4. Effect of Zn (0, 5, 10, 15 kg ha⁻¹) and N (30, 60, 90 kg ha⁻¹) treatments on agronomic efficiency of N (kg grain kg⁻¹ N) of barley (*Hordeum vulgare* L.) grown under field conditions. Vertical bar at each of the data points represents the standard deviation from the means. Different letters represent statistical differences at P < 0.05 (LSD test).

“single column fitting image”

Figure 5. Effect of Zn (0, 5, 10, 15 kg ha⁻¹) and N (30, 60, 90 kg ha⁻¹) treatments on agronomic efficiency of Zn (kg grain kg⁻¹ N) of barley (*Hordeum vulgare* L.) grown under field conditions. Vertical bar at each of the data points represents the standard deviation from the means. Different letters represent statistical differences at P < 0.05 (LSD test).

“single column fitting image”

“Figures: our preference for color is online only”

Table 1. Selected properties of the original soils (n = 3).

Soil property ^a	Value
pH _w (1:2.5; w/v)	8.2 ± 0.1 ^b
EC (μS cm ⁻¹)	188.1 ± 1.8
Organic matter (g kg ⁻¹)	12.6 ± 0.4
N (g kg ⁻¹)	1.0 ± 0.2
Total CaCO ₃ (g kg ⁻¹)	88.7 ± 4.2
Free CaCO ₃ (g kg ⁻¹)	34.3 ± 2.5
Extractable P (mg kg ⁻¹)	30.9 ± 2.1
CEC (cmol _c kg ⁻¹)	25.7 ± 0.5
Total Zn (mg kg ⁻¹)	51.7 ± 0.7
DTPA-extractable Zn (mg kg ⁻¹)	0.98 ± 0.13
WHC (33 kPa; w/w) (g H ₂ O 100 g ⁻¹ soil)	25.1 ± 0.3
Clay (g kg ⁻¹)	150 ± 7
Silt (g kg ⁻¹)	540 ± 11
Sand (g kg ⁻¹)	310 ± 6
Texture (USDA)	clay loam

^a WHC, water-holding capacity; EC, electrical conductivity; CEC, cation exchange capacity.

^b Standard deviation.

Table 2. Sequential extraction procedure of Zn from soils.

Step	Fraction	Extracting solution	Shaking conditions
1	Water soluble plus exchangeable (WSEX)	NH ₄ Ac 1.0 M, pH=7.0	2 h, 120 rpm
2	Carbonate bound (CAR)	NaOAc 1.0 M. pH = 4.5	5 h, 120 rpm
3	Easily reducible oxide bound (RMO)	NH ₂ OH·HCl 0.1M in HNO ₃ , pH = 2.0	0.5 h, 120 rpm
4	Amorphous minerals colloids bound (AMC)	(NH ₄) ₂ C ₂ O ₄ 0.2M + H ₂ C ₂ O ₄ 0.2 M, pH = 3,0	(darkness), 120 rpm
5	Crystalline Fe oxide bound (CFeO)	(NH ₄) ₂ C ₂ O ₄ 0.2 M + H ₂ C ₂ O ₄ 0.2 M, pH = 3.0 in ascorbic acid 0.1 M	2 h, 120 rpm
6	Organic material and sulfide bound (OM)	Digestion: 8 mL H ₂ O ₂ 30% pH = 2.0 + 3 mL HNO ₃ 0.02 M Extraction: 25 mL NH ₄ OAc 3.2 M in HNO ₃ 20%	almost to dryness, 85°C 15 min, 120 rpm
7	Residual (RES)	Differences between the total Zn and the sum of the previous fractions	-

Developed by Leleyter and Probst (1999).

Table 3. Effect of fertilizer treatment on total Zn, DTPA-TEA extractable Zn and Zn fractions in soil (mg kg⁻¹) at the time of barley harvest.

Treatment	Total Zn	DTPA-TEA	WSEX	CAR	RMO	AMC	CFeO	OM	RES
Zn ₀	52.4 a	1.06 a	0.16 a	1.01 a	0.74 a	3.32 a	1.11 a	0.99 a	45.0 a
Zn ₅	60.1 b	6.82 b	0.53 b	3.11 b	1.49 b	7.57 ab	1.86 b	1.11 ab	44.4 a
Zn ₁₀	68.6 c	12.2 c	1.03 c	4.74 c	1.97 c	8.10 ab	2.49 b	0.94 a	46.5 a
Zn ₁₅	82.0 d	21.2 d	2.03 d	10.4 d	2.05 d	11.0 b	4.05 c	1.23 b	54.2 b
Zn rate (a)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0,05	<0,0001	<0,05	<0,01
N rate (b)	ns	ns	ns	ns	ns	ns	ns	ns	ns
a x b	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data are the mean value for nine experimental replications since there is no interaction between N and Zn rates (and each Zn rate include three N rates). Values within a column were compared using LSD multiple range test at the 95% level. Homogeneous groups are denoted with the same letter. Fractions are: water soluble plus exchangeable (WSEX), carbonate bound (CAR), easily reducible oxide bound (RMO), amorphous mineral colloid bound (AMC), crystalline Fe oxide bound (CFeO), organic material and sulfide bound (OM) and residual (RES).

Table 4. Analysis of variance (ANOVA) of the effects of Zn rate, N rate and their interactions on reported traits of barley (*Hordeum vulgare* L.) grown in field conditions.

Source of variation	df	Root Zn conc.	Stem Zn conc.	Leaf Zn conc.	Grain Zn conc.	Grain Protein conc.	Root yield ^a	Stem yield	Leaf yield	Grain yield
Zn rate (a)	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	<0,0001	<0,001	<0,0001	<0,001
N rate (b)	2	ns	<0.05	<0.001	<0.0001	<0.0001	ns	<0,0001	<0,0001	<0,05
a x b	6	<0.001	ns	ns	ns	<0.01	<0.05	<0,01	<0,0001	ns

^a dry matter (DM)

Table 5. Effect of nitrogen and zinc treatments on root, stem and leaf DM yield (kg ha⁻¹) of barley (*Hordeum vulgare* L.).

Treatment	Root yield ^a	Stem yield	Leaf yield
N ₃₀ Zn ₀	540 a-c	1882 ab	1683 ab
N ₆₀ Zn ₀	448 ab	1565 a	1382 a
N ₉₀ Zn ₀	442 a	2254 b	2266 c-e
N ₃₀ Zn ₅	597 bc	2185 b	1956 bc
N ₆₀ Zn ₅	671 cd	2803 cd	2446 de
N ₉₀ Zn ₅	750 de	3640 e	3464 f
N ₃₀ Zn ₁₀	609 cd	2152 b	1908 bc
N ₆₀ Zn ₁₀	674 cd	2358 bc	2065 b-d
N ₉₀ Zn ₁₀	866 ef	4010 e	4033 g
N ₃₀ Zn ₁₅	680 cd	2186 b	1807 ab
N ₆₀ Zn ₁₅	915 f	3011 d	3168 f
N ₉₀ Zn ₁₅	753 de	2868 d	2706 e

^a dry matter (DM)

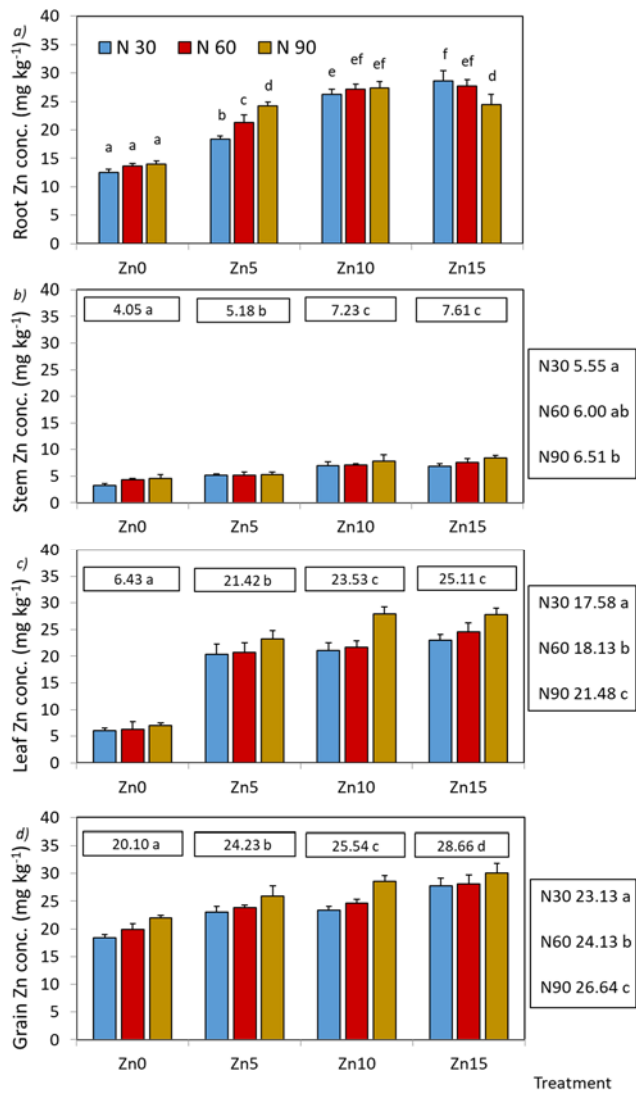


Figure 1

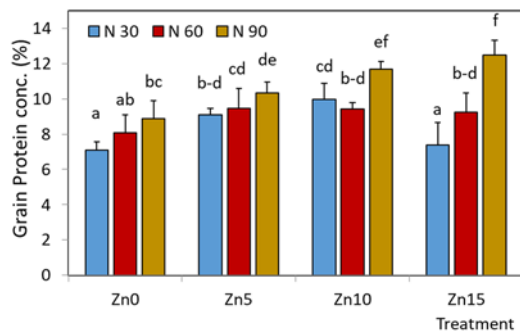


Figure 2

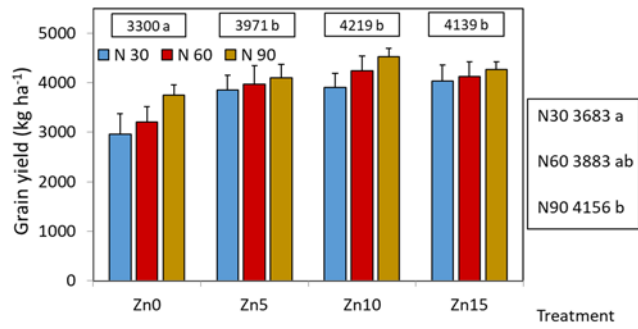


Figure 3

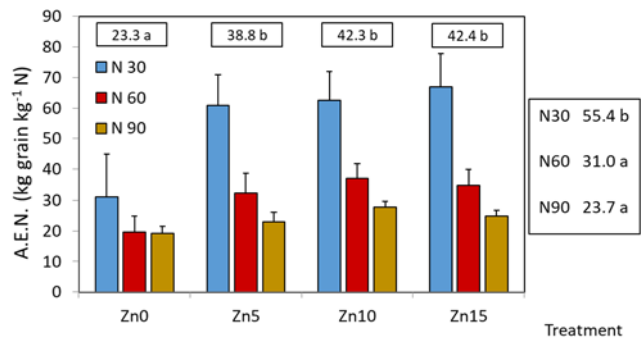


Figure 4

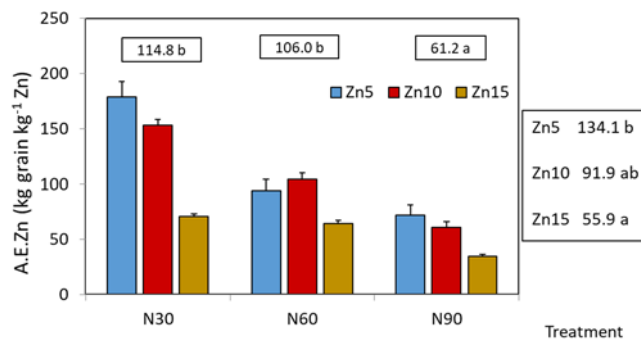


Figure 5

Table of contents graphics (TOC)



Barley



↑ Grain yield
Nutritional quality
(Zn, Protein)

