

1 **Influence of long-term mineral fertilization on metal contents and properties of**
2 **soil samples taken from different locations in Hesse, Germany**

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8 **Abstract** Essential and non-essential metals occur in soils as a result of weathering,
9 industrial processes, fertilization and atmospheric deposition. Badly adapted
10 cultivation of agricultural soils (declining pH-value, application of unsuitable
11 fertilizers) can enhance the mobility of metals and by the way increase their
12 concentrations in agricultural products. **As the enrichment of metals in soils occurs**
13 **long time periods, monitoring of the long-term impact of fertilization is necessary to**
14 **assess metal accumulation in agricultural soils.** The main objective of this study was
15 to test the effects of different mineral fertilizer variations on soil properties (pH, C_{org}
16 and CEC) and pseudo total and mobile metal contents of soils after 14 years of
17 fertilizer application and to determine residual effects of the fertilization 8 years after
18 cessation of fertilizer treatment. Soil samples were taken from a field experiment
19 which was carried out at four different locations 210, 260, 360, and 620 m above sea
20 level, in Hesse, Germany. During the study, a significant decrease in soil pH and an
21 evident increase in soil carbon content and cation exchange capacity with fertilization
22 were determined. The CEC of the soils was closely related to their organic C
23 contents. Moreover, pseudo and mobile metal (Cd, Cu, Mn, Pb, Zn) contents in the
24 soils increased due to application of 14 years mineral fertilizer treatments (N, P, NP,
25 and NPK) when compared to control plots. 8 years after termination of the

26 fertilization in the soil samples taken from soil profiles of the fertilized plots (NPK) for
27 monitoring the residual effects of the fertilizer application, a decrease of 82.6 %, 54.2
28 %, 48.5 %, 74.4 %, and 56.9 %, respectively, in pseudo total Cd, Cu, Mn, Pb, and Zn
29 contents was determined.

30

31 **1. Introduction**

32 The world population continues to increase at an alarming rate. As a result, new
33 farmland in previously non-arable locations will be called upon to help support this
34 growing population and more fertilizers will have to be utilized to increase food
35 production (Hagen and Howard, 2011). There are concerns about whether
36 continuous use of such fertilizers over a long period of time will cause an
37 accumulation of metals to high levels, thereby increasing risk to environmental and
38 human health (Huang et al., 2004). Fertilizers and soil amendments can contain
39 significant amounts of potentially hazardous trace elements of geologic or man-made
40 origin. The risk of soil and environmental pollution through the application of these
41 materials to agricultural lands has therefore raised some concern (Raven et al.,
42 1997). Inorganic fertilizers contain elevated quantities of metals like Cd, Pb, As, and
43 other trace elements of environmental relevance (Ajayi et. al, 2012, Nicholson et al.,
44 2003).

45 Metals can be toxic to humans and plants, therefore a long-term application of
46 inorganic fertilizers, organic waste and pesticides to soils, requires a detailed risk
47 assessment of heavy metal accumulation in agricultural lands
48 (Papafilippaki et al., 2007). Metals required by plants include Mn, Fe, Cu, Zn, Mo,
49 and, possibly, Ni. The phytotoxicity of such relatively common metals as Cd, Cu, Hg,
50 and Ni is substantially greater than that of Pb and Zn (Raskin et. al., 1994).

51 Cadmium concentrations in soils in many countries are increasing due to inadvertent
52 additions in fertilizer, biosolids, soil amendments and additions from the atmosphere
53 (McLaughlin et al., 1999). The management of phosphate (P) fertilizer application,
54 both in the short- and long-term, can influence the potential accumulation of Cd in
55 foods. The Cd added to agricultural systems in P fertilizers accumulates over time if
56 application rates are in excess of Cd removal as an effect of long-term P fertilizer
57 application (Grant, 2011). Nicholson et al. (2003) predicated that P fertilizers in
58 particular, are an important source of metals, particularly for Zn, Cu and Cd, entering
59 agricultural soils. Increase of Cu and Zn in soils is associated mainly with NPK
60 fertilizers (Kabata - Pendias, 2011). High application rates of nitrogen fertilizer to
61 agricultural soils resulted in increased accumulation of some heavy metals such as
62 Cd and Pb in agricultural products (Zhou, 2003).

63 In this study we analyzed soils from long-term fertilizer experiments in Hesse,
64 Germany. We investigated the long-term effects of different NPK fertilizer regimes on
65 soil properties, and soil metal contents. Therefore, the aim of this paper was to
66 answer the following question: how do (1) soil organic carbon content and cation
67 exchange capacity, (2) soil pH and (3) pseudo and mobile concentrations of metals
68 differ between fertilized and control plots after 14 years of fertilizer application and
69 after 8 years of fertilization termination in Hesse, Germany?

70

71 **2. Materials and Methods**

72 **2.1 Study area and fertilizer treatments**

73 This study was carried out at four different locations in different altitudes in Hesse,
74 Germany. The region has a climate with a relative strongly maritime influence.
75 Different doses of nitrogen, phosphorus and potassium fertilization has been

76 undertaken since 1986 until 2002 in a Latin rectangle design, including the control
77 treatment that did not receive any fertilizers to verify metal input due to fertilizer
78 treatments. Some basic physicochemical characteristics of the non-fertilized (control)
79 plots in the studied areas are given in Table 1.

80 The size of the field trial was 45x54 m and divided in 81 plots each of 30 m² size.
81 The top soil (0-30 cm) was sampled in April, August, and November in three
82 replications in the year 2000 (14 years after fertilization application) and in July 2011
83 from soil profiles (8 years after fertilization termination). According to the WRB
84 (World Reference Base for Soil Resources) classification (2014), we determined the
85 soil types as Eutric Cambisol (210 m a.s.l), Skeletic Cambisol (260m and 360 m
86 a.s.l) and Gleyic Fluvisol (620 m a.s.l). *Lolium-Cynosuareturnum* was the cultivation crop
87 during the study.

88 Calciumammoniumnitrate (CAN) (74% NH₄NO₃, 26% CaCO₃) as N fertilizer, hyper
89 phosphate (26% P₂O₅) and superphosphate (16-22% P₂O₅) as P fertilizer, and
90 magnesia kainit (11% K₂O, 5% MgO, 20% Na, 4% S) and K₂SO₄ as K fertilization
91 were used in various levels which refer to different application rates (Table 2). N
92 fertilization was used in the same proportions in middle of April, May, June, and July;
93 P and K fertilizers were applied in the middle of April as hyper phosphate and
94 magnesia kainit and in the middle of June as superphosphate and K₂SO₄.

95

96 **2.2 Analytical Procedures**

97 Soil samples were air dried, crushed, homogenized, and sieved through 2 mm
98 screen for the analysis of physical and chemical characteristics and stored in clean
99 HNO₃ treated plastic bottles.

100 The characteristics of soil such as soil pH (DIN ISO 10390), soil carbonate content
101 (DIN ISO 10693), particle size distribution (DIN ISO 11277) were determined for the
102 samples collected. Moreover, total contents of carbon (C_t) were examined on finely
103 ground samples by a CNS analyzer with a Thermal Conductivity Detector
104 (VarioEL III Elementar, Germany). Inorganic carbon (C_i) was calculated from the
105 carbonate content by multiplying the factor 0.1199. The amounts of organic C (C_{org})
106 resulted from the difference between C_t and C_i . Effective cation exchange capacity
107 (CEC_{eff}) was examined with NH_4Cl extraction according to Trüby and Aldinger (1989)
108 and exchangeable cations were determined with AAS (AA240FS, Varian, USA).
109 Pseudo total metal contents of soil samples were determined by modified USEPA
110 Method 3051A (USEPA) with microwave assisted extraction-aqua regia (MAE-AR).
111 The modified program for MAE-AR (0.3 g of soil, 6 ml HCl and 2 ml HNO_3) is
112 described in Öztan and Düring (2012). Mobile fraction of metals was measured after
113 the extraction of soils with ammonium nitrate (NH_4NO_3) (20 g soil, 50 ml 1M NH_4NO_3 ,
114 shaken for 120 min) (DIN 19730, 2009). Metal concentrations in soil extracts were
115 determined by inductively coupled plasma-optical emission spectrometer (ICP-OES;
116 Agilent 720ES) which provides rapid multi-elemental analysis.
117 The reagents used were all of analytical-reagent grade certified for the impurities.
118 Distilled and deionized water, purified with a Milli-Q plus system (Merck Millipore,
119 Darmstadt, Germany), was used for the experiments. For quality assurance, two
120 certified reference materials (CRMs), “7001” (light sandy soil) and “7004” (loam),
121 from Analytika Co. Ltd, Prague, Czech Republic, were analyzed repeatedly during
122 analysis of the sample set. To designate the amount of possible cross
123 contamination, blank values were determined within each sample series.

124

125 **2.3 Statistics**

126 For data analysis SPSS 19.0 for Windows was used. Before testing for differences in
127 analyte concentrations, each element across samples was tested for normality by
128 examination of histograms and residual plots. All data were subjected to analysis of
129 variance (ANOVA) to determine the main effect of each fertilizer variation on soil
130 metal content and soil properties at the $p < 0.05$ probability level.

131

132 **3. Results and Discussion**

133 **The results show that long-term N, P, and K fertilizer treatments have significant**
134 effects on soil properties. During the study, significant decrease in soil pH was
135 detected due to fertilization (Table 3). Decrease in pH of the surface layer in the
136 fertilizer might be attributed to the nitrification and acidification processes stimulated
137 by continuous application of fertilizers as well as by H^+ released by roots (Liang et al.
138 2012). Where N fertilizer was applied the pH slightly decreased with respect to the
139 initial value. Significant differences were determined between the control plots and
140 N, P, NP, and NPK applications in soil pH level in April. However a decrease in soil
141 pH was found when compared to control plots in soil August and November pH
142 degrees, no significant differences were seen for N and P treatments in soil-August
143 and for P application in soil-November pH level (Table 3). Continued use of
144 ammonia-based fertilizers can induce soil acidity (Schwab et al. 1990). Results from
145 the study were in agreement with the findings of Tsadilas and colleagues (2005) who
146 reported that the application of ammonium fertilizer significantly decreased soil pH
147 more than the nitrate treatments. Results from a pot fertilizer experiment which
148 showed that application of NH_4Cl lowered soil pH from 4.51 to 4.07 was presented
149 by Liu et al. (2007). The major mechanism of soil acidification by N fertilization is

150 related to hydrogen ion (H^+) release through nitrification of NH_4^+ and subsequent
151 leaching of NO_3^- . The most important acid forming reaction by fertilizers is microbial
152 oxidation of ammoniacal fertilizers (Barak et al. 1997).

153 **Significant** differences in soil organic carbon (C_{org}) content and effective cation
154 exchange capacity (CEC) which was caused by fertilization treatments can be seen
155 in Fig. 1 and 2. Significant increase in C_{org} content was determined in April, August
156 and November soil samples. All fertilizer treatments show significant differences
157 when compared to the control plots. In April and August soil samples, no significant
158 differences were determined on C_{org} content between N and P treatments.
159 Differences between N and P variations are evident in November C_{org} content. C_{org}
160 level with P fertilizer was almost two times higher than C_{org} content in N treatment
161 (Fig. 1). Addition of fertilizers to soil influences the chemical composition of soil
162 solution. Increased plant biomass produced by fertilizers results in increased returns
163 of organic material to the soil in the form of decaying roots, litter and crop residues
164 (Haynes and Naidu, 1998). Our results are similar with the findings of
165 Raun and co-workers (1998) and with the study of Halvorson et. al. (1999) who
166 demonstrated a rise in C_{org} with the applied increasing N ratios. Messiga and
167 colleagues (2013) although found no significant **effect** for total carbon content in 0 to
168 5 cm soil layer by N application, they noted a tendency for greater total carbon
169 concentration with increasing N application. Liu et al. (2005) reported that C_{org} in
170 surface soil (0-15 cm) layer was 7.7%, higher in mineral fertilizer treatment than
171 without fertilizer application. Similar results obtained in a long-term experiment
172 showed that low nitrogen rates poorly increased C_{org} content compared to the control
173 (0.14 vs 0.03 $Mg\ ha^{-1}year^{-1}$), while the medium and high rates increased it by 0.45
174 and 0.49 $Mg\ ha^{-1}year^{-1}$, respectively (Mazzoncini et al., 2011). After 14 years of

175 fertilizer application, there was an increasing trend to C_{org} in April in NPK treatment
176 (6.02 %) contrasting to the control soil content of 0.43%. This is in accordance with
177 the results of Xie et al. (2011) who found in a pot experimental study the highest C_{org}
178 content was in the NPK (80 N mg kg⁻¹, 35 P mg kg⁻¹, and 60 K mg kg⁻¹) treatment,
179 which was significantly higher than that in the NK (80 N mg kg⁻¹, and 60 K mg kg⁻¹)
180 treatment. Gong and co-workers (2009) determined an increase in C by long-term
181 applications of manures and mineral fertilizers - both alone and in combination - and
182 concluded that application of mineral fertilizer may have stimulated microbial activity
183 and enhanced decomposition. More soil organic matter was decomposed and more
184 available nutrients could then be provided for better crop growth, resulting in
185 increased crop residues (root debris and secretion) being returned to the soil.

186 The CEC is a very important soil property for nutrient retention and supply and acts
187 as a bridge between soil and plant (Caravaca et al. 1999). N, P, NP, and NPK
188 treatments significantly increased CEC in April, August, and November soil samples
189 when compared to non-fertilized plots (Fig. 2). Cakmak and colleagues (2010)
190 reported that 40 years application of P fertilizers significantly decreased pH and
191 increased CEC of the soil. Thus, phosphate fertilizer applications not only increase
192 Cd concentration of soils but also may change their chemical speciation and thus
193 bioavailability. As the unfertilized soil in our study has not received any kind of
194 fertilizer during 14 years of investigation, the differences observed here may
195 therefore be judged conservative estimates of the effect of soil organic matter on
196 CEC. This is in accordance with the results of Schjonning et al. (1994) who found
197 11 % higher CEC than in the control plots. Nitrogen application as CAN fertilizer only
198 or in combination with NPK complex fertilizer rises the soil CEC value. This can be
199 explained by soil colloid retention of applied Ca^{2+} , NH_4^+ , and K^+ ions

200 (Radulov et al.,2011) and by organic carbon content of soil which is the most
201 important factor affects soil CEC (Rashidi and Seilsepour, 2008). Positive linear
202 relationships were determined between C_{org} and CEC (Fig. 3). The CEC of the soils
203 were closely related to their organic C contents (Caravaca et al. 1999). In contrast to
204 observations in other recent studies (Bationo et. al, 2007), a significant negative
205 linearity between soil pH and organic carbon content was found (Fig. 3). There are
206 two possible explanations for these adverse observations. Either organic matter
207 accumulation does not necessarily result in pH decreases or other mechanisms
208 causing pH change are more dominant (Ritchie and Dolling, 1985). In particular,
209 accumulation of undecomposed soil organic matter rich in organates, and inputs of
210 symbiotically fixed N and ammonium- based fertilizers with consequent nitrate
211 leaching are involved in the accelerated acidification of agricultural soils (Bolan and
212 Hedley, 2003). Acidification from soil organic matter accumulation or the direct
213 effects of fertilizers on the soil chemistry can, however, be of significant importance
214 (de Klein et al., 1997). Results from our study were in agreement with the findings of
215 Cakmak et al., (2007) who also reported a negative correlation between soil pH and
216 SOC content. Schwab and colleagues (1990) determined a decrease in soil pH and
217 an increase in soil organic matter content due to applied high N rate.

218 The main effect of each fertilizer variation on soil pseudo total and mobile
219 (NH_4NO_3 -extractable) metal (Cd, Cu, Mn, Pb, and Zn) contents can be seen in Fig. 4
220 and Fig. 5. Though total concentrations of metals in soil are used to characterize its
221 base-line elemental composition (parent geological material), pseudo total metal
222 concentration analysis which is performed by an estimation with aqua regia, allows
223 to assess soil pollution and to ascertain heavy metal and other pollutant contents in
224 the soil (Ure, 1990). Pseudo total metal contents in soils increased due to application

225 of long-term chemical fertilizer treatments when compared to control plots (Fig. 4).
226 Applications of N, P, NP, and NPK fertilizers increased soil April, August, and
227 November Cd levels compared to non-fertilized soil, but no significant differences
228 were found between P and NP fertilizer variations for pseudo total Cd concentrations
229 (Fig. 4). Significant differences between P and NP applications were obtained for
230 mobile Cd content (Fig. 5). Trace metal enrichment in soils through mineral fertilizer
231 applications is well documented for Cd. Loganathan and colleagues (1997) reported
232 that in pasture systems in New Zealand, ten years of P fertilizer application caused a
233 marked increase in surface soil pseudo total Cd concentration. Identical findings
234 reported Gray et. al, (1999) who specified a significant increase in total Cd
235 concentration in soils under pasture with application of superphosphate fertilizer over
236 a period of 44 years in New Zealand.

237 No significant differences were determined between N, P, and NP fertilizer variations
238 for pseudo total soil Cu concentrations in April and November, but August Cu level
239 increased significantly with NP application (Fig. 4). Same effects can be seen by
240 mobile (NH_4NO_3 -extractable) Cu content (Fig. 5). Thomas and colleagues (2012)
241 reported that total Zn, Cu and Cd concentrations show an increase in the soil
242 concentration with an increase in levels of phosphate fertilizer.

243 Although NP fertilizer applications show significant differences to N and P treatments
244 for soil-April Mn concentration, no significant differences were obtained by addition of
245 NP when compared to N and P fertilization variations for August and November Mn
246 levels (Fig. 4). Soil mobile Mn concentration increased due to fertilizer application.
247 Significant differences were observed between NPK and NP treatments for April and
248 August, but no significant differences were determined for November Mn
249 concentrations (Fig. 5).

250 April Pb concentrations increased in N and P fertilizer variations, however no
251 significant effects were found in Pb contents when compared to control plots (Fig. 4).
252 No significant differences were noted on soil August and November Pb mobile
253 concentrations among NP and NPK treatments (Fig. 5), whereas distinct significant
254 differences were obtained for pseudo total contents (Fig. 4). A recent research
255 showed that the amount of hot acid-extractable Pb in the 0- to 30-cm soil interval
256 increased significantly ($p < 0.05$) in accordance with the amount of added P fertilizers
257 (Cakmak et al., 2010). Our results agree with the findings of Atafar and co-workers
258 (2010) who reported that total Cd, Pb, and As concentrations increased in the
259 cultivated soils due to fertilizer application.

260 Addition of fertilizer at all rates increased soil Zn level when compared to control
261 plots. Soil pseudo total Zn concentration increased from 63.1 mg kg⁻¹ in control
262 treatment to 151.5 mg kg⁻¹, 242.5 mg kg⁻¹ and 185.4 mg kg⁻¹, respectively, in April,
263 August, and November Zn concentrations in NPK application. The highest Zn
264 concentration in soils in August recorded in NPK treatment was probably related to
265 lower soil pH (4.64). Based on the results, Zn content in soils increased significantly
266 by N and P applications, but no significant increase was determined between N and
267 P treatments (Fig. 4). Significant differences between NP and P treatments were
268 found in pseudo total soil August Zn concentration (Fig. 4), however, no significant
269 differences were noted for mobile Zn level for the same fertilizer variations (Fig. 5).

270 Soils have a distinct influence on human health (Brevik and Sauer, 2014), hence soil
271 contaminants constitute a known global problem, and more knowledge is required
272 regarding their behavior, and their pathways to humans (Abrahams, 2002). Although
273 many grassland fertilizer experiments have been performed worldwide, information
274 about residual effects of fertilizer applications on grassland ecosystem functioning is

275 still rare (Hejzman and Schnellberg, 2009). Fertilization is one of the major paths for
276 metal input to agricultural soils, therefore monitoring of the long term impact of
277 fertilization residual effects is necessary. On this account, in July 2011 mixed soil
278 samples from soil profiles of the fertilized plots (NPK) in the study areas were taken
279 and analyzed for pseudo total and mobile metal concentration and for soil properties
280 such as pH, C_{org} and CEC. Soil pseudo and mobile metal contents (Cd, Cu, Mn, Pb,
281 Zn) and soil pH, C_{org} and CEC of the soil profiles are summarized in Table 4 and
282 detailed distribution of the metals in soil profiles are given in Appendix 1.

283 After 8 years of finalization of the experimental fertilization design an element
284 specific decrease in pseudo total Cd, Cu, Mn, Pb, and Zn concentration in soil
285 samples was determined. Compared to the pseudo total contents of the soil samples
286 taken in August 2000, Cd concentrations evidently decreased in the whole soil
287 profile. In 2011 Cd contents in the upper soil layers decreased down to the value in
288 the control plots determined in August-2000. Cd content in the studied soils in
289 August 2000 was in the range of 0.06 -0.19 mg kg⁻¹ with a mean value of 0.10 ± 0.05
290 mg kg⁻¹ in plots without fertilization, and in NPK treated plots Cd content was
291 between 0.64 - 0.78 mg kg⁻¹ (Fig. 4). Nevertheless, in July 2011 Cd concentrations in
292 treated plots were between 0.08 and 0.19 mg kg⁻¹ with a mean value of 0.12 mg kg⁻¹
293 (Table 4). Decrease in soil Cd concentration could be due to its mobility and its
294 uptake by plants. Soil Cd is mostly adsorbed at exchange sites and its plant
295 availability is closely related to pH and soil organic matter content (Puschenreiter
296 and Horak, 2000).

297 Furthermore, though a decrease in Cu, Mn, Pb, and Zn contents was determined in
298 the first 30 cm of the soil profiles in July 2011, an evident decrease such as in Cd
299 concentration was not observed. Detected pseudo total Cu, Mn, Pb, and Zn

300 concentrations in August-2000 in NPK amended plots were 72.3 mg kg⁻¹,
301 2704.4 mg kg⁻¹, 72.8 mg kg⁻¹, and 242.5 mg kg⁻¹, and in non-fertilized plots
302 (control-treatment) were 11.4 mg kg⁻¹, 624.4 mg kg⁻¹, 11.4 mg kg⁻¹, and 63.3 mg kg⁻¹,
303 respectively (Fig. 4). High contents of Cu, Mn, Pb, and Zn from fertilizers residual
304 effects still persisted in the top soil layer of the field trial in July 2011. In the soils that
305 received the maximum dose of fertilization (NPK) for 14 years and followed by no
306 additional input for 8 years, Cu, Mn, Pb, and Zn contents were still around two times
307 higher than those in the control plots in August 2000 (App. 1).

308 These metals do not show this mobility in soils like Cd, so i.e Pb can be regarded
309 more or less as permanent in soils (Alloway, 1990). Moreover, the absorption of Pb
310 by root is passive and thus the rate of its uptake from soils is rather low (Kabata-
311 Pendias and Mukherjee, 2007).

312 Increase of metal contents in soil due to fertilization should be considered not only as
313 a matter of soil contamination but also a potential risk for human health. The
314 precaution values for soils were chosen as criteria in terms of correlations between
315 metal contamination and human health. Precaution values according to BBodSchV
316 (1999) for Cd, Pb, Cu, and Zn are 1 mg kg⁻¹, 70 mg kg⁻¹, 40 mg kg⁻¹, and 150
317 mg kg⁻¹, respectively. Though, in the samples taken from 2000, Cd, Cu, and Pb
318 contents mostly were below the precaution levels, Zn concentration (151.5 mg kg⁻¹ in
319 NPK treated plots) just met or exceeded the precaution values. The metal
320 concentrations determined in the samples taken from 2011 all fell below the
321 precaution values. However, Pb showed some residual effect as it could be
322 determined still in higher concentrations in the upper soil layer.

323 In the soil samples taken in August 2000, significant increase in C_{org} and CEC
324 content was determined in April, August and November due to N, P, NP, and NPK

325 treatments when compared to non-fertilized plots (Fig. 1 and 2). Rasool and
326 colleagues reported that, N₁₀₀P₅₀K₅₀ increased soil organic matter content by 21%
327 (Rasool et. al., 2008). After finalization of the fertilization design an appreciable
328 decrease in July 2011 soil C_{org} and CEC was detected (Table 4). Grassland suffered
329 throughout centuries from nutrient depletion through removal of organic material as
330 dung and forage, and subsequent transfer to agricultural land or high productive
331 lowland grassland (Hejcman and Schnellberg, 2009).

332

333 **4. Conclusions**

334 Based on the results from the present study, the main conclusion is that 14 years of
335 mineral fertilization has a significant effect on soil metal contents and soil properties
336 such as pH, C_{org} and CEC_{eff}, and therefore, has a great impact on metal availability.
337 Continued long-term fertilizer use increased soil metal content, soil organic C, CEC
338 and decreased soil pH level. Another important aspect of this study was the
339 establishment of soil metal concentrations 8 years after cessation of the fertilizer
340 application. Soluble metals were taken up by plants and thus leaving lower amounts
341 in the soil, probably due to decreased pH values in the long-term fertilized soils. Cd
342 concentrations in 2011 evidently decreased down to the value in the control plots
343 determined in August 2000. Despite decrement in metal contents, Cu, Mn, Pb, and
344 Zn still remained in the top soil profile around two times higher than the control soils
345 taken 14 years after fertilization application. Moreover, in terms of human health,
346 metal concentrations determined in the samples taken in 2011 all fell below the
347 precaution values according to BBodSchV (1999).
348 This research has thrown up some questions in need of further investigation.
349 Applications of fertilizers not only provide plant nutrients, but also change the

350 availability of metals and their uptake into the plants. As plants represent the first
351 compartment of the terrestrial food chain, future research should therefore
352 concentrate on the investigation of the impacts of various soil factors on metal
353 transfer into the plants.

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520 Table 1: Basic physicochemical properties of soil samples (control plots) (mean
 521 values of 4 locations)

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Properties			April	August	November	
Aqua regia extractable metals mg kg ⁻¹	pH [CaCl ₂]	min	5.10	5.03	5.10	
		max	5.57	5.82	5.45	
		mean±sd	5.32±0.18	5.28±0.32	5.27±0.15	
	Clay [%]	min	24.1	30.0	27.8	
		max	48.4	43.0	43.1	
		mean±sd	32.9±7.9	34.9±4.4	34.8±4.5	
	C _{org} [%]	min	0.39	0.39	0.40	
		max	0.52	0.51	0.49	
		mean±sd	0.43±0.05	0.42±0.03	0.42±0.03	
	CEC _{eff.} [cmolc kg ⁻¹]	min	9.21	9.23	9.22	
		max	14.2	17.3	17.7	
		mean±sd	11.9±1.83	13.6±2.43	13.5±2.51	
		Cd	min	0.059	0.057	0.056
			max	0.190	0.190	0.190
			mean±sd	0.097±0.04	0.093±0.05	0.095±0.04
Cu		min	8.92	9.81	8.99	
		max	12.0	18.1	15.9	
		mean±sd	10.3±0.73	11.4±2.22	11.7±2.08	
Mn		min	538.9	532.2	533.6	
		max	786.0	782.2	785.3	
		mean±sd	627.3±98.6	624.4±99.9	631.0±97.5	
Pb		min	8.01	8.12	8.12	
		max	19.1	19.4	19.2	
		mean±sd	11.3±4.62	11.4±4.61	11.3±4.54	
Zn		min	41.1	42.0	41.0	
		max	87.4	87.3	86.6	
		mean±sd	63.5±17.9	63.3±17.6	63.1±17.9	

523 CEC_{eff.}: Cation Exchange Capacity-effective, C_{org.}: Soil organic carbon

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531 Table 2: Fertilization variations of the study sites

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Fertilizer Variations	kg ha ⁻¹		
	N	P₂O₅	K₂O
Control	0	0	0
N	320	0	0
P	0	120	0
NP	320	120	0
NPK	320	120	160

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551 Table 3: Effect of fertilizers on soil pH (mean values of 4 locations)

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Fertilizer	Value	pH (April)	pH (August)	pH (November)
Control	min	5.10	5.03	5.10
	max	5.57	5.82	5.45
	mean±sd	5.32±0.18 ^a	5.28±0.32 ^a	5.27±0.15 ^a
N	min	4.00	4.99	4.07
	max	5.07	5.16	5.18
	mean±sd	4.76±0.43 ^b	5.07±0.05 ^{ab}	4.78±0.44 ^b
P	min	4.53	5.00	4.90
	max	5.16	5.26	5.07
	mean±sd	4.96±0.25 ^b	5.06±0.07 ^{ab}	5.00±0.05 ^{abc}
NP	min	4.52	4.66	4.49
	max	5.07	5.05	5.09
	mean±sd	4.80±0.24 ^b	4.88±0.13 ^b	4.90±0.23 ^b
NPK	min	4.20	4.19	4.35
	max	5.08	5.02	5.09
	mean±sd	4.78±0.33 ^b	4.64±0.35 ^c	4.71±0.32 ^b

553 (Mean values followed by different superscript letters between fertilizer variations differ at 5 % probability)

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567 Table 4: Soil pseudo and mobile metal contents (Cd, Cu, Mn, Pb, Zn) and soil pH,
 568 C_{org} and CEC of the soil profiles in July 2011 in the 4 study areas

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Properties		min	max	mean
	pH [CaCl ₂]	4.03	5.98	5.14
	C _{org} [%]	0.31	4.58	2.09
	CEC _{eff.} [cmolc kg ⁻¹]	20.4	23.8	21.3
Aqua regia extractable metals mg kg ⁻¹	Cd	0.08	0.19	0.12
	Cu	20.7	54.9	33.1
	Mn	993.0	1867.5	1390.2
	Pb	6.66	38.9	18.6
	Zn	87.1	146.3	104.5
NH ₄ NO ₃ extractable metals mg kg ⁻¹	Cd	0.00	0.08	0.02
	Cu	0.03	0.22	0.11
	Mn	1.20	48.2	21.7
	Pb	0.00	0.19	0.02
	Zn	0.01	1.45	0.50

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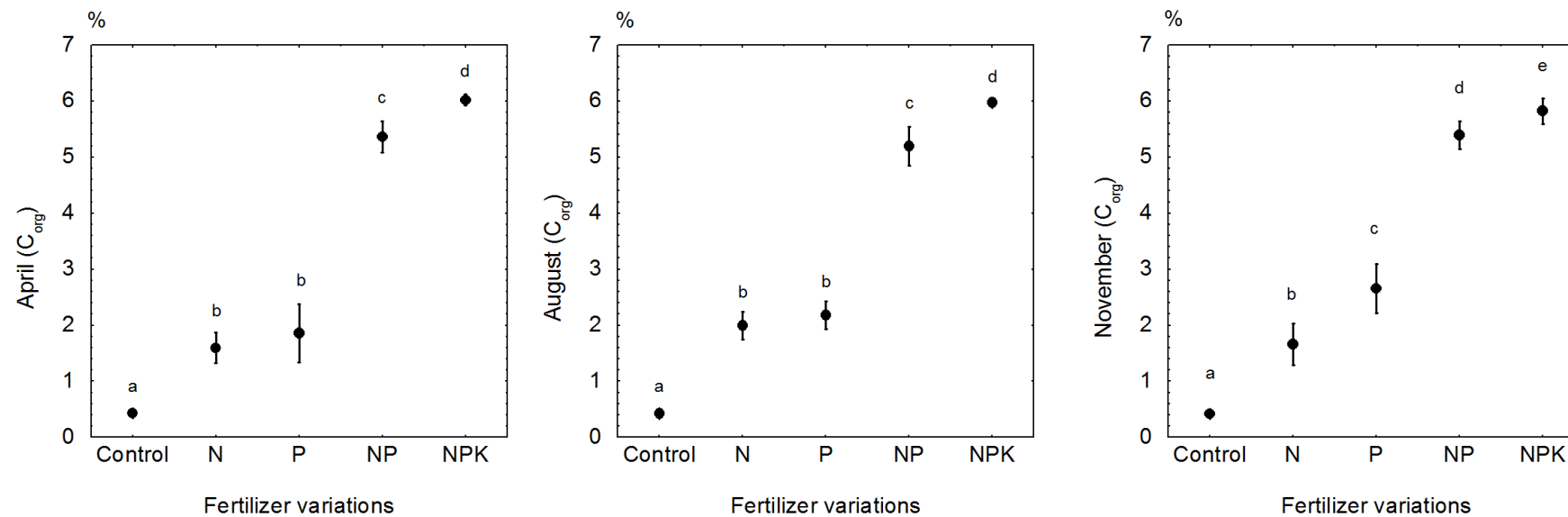
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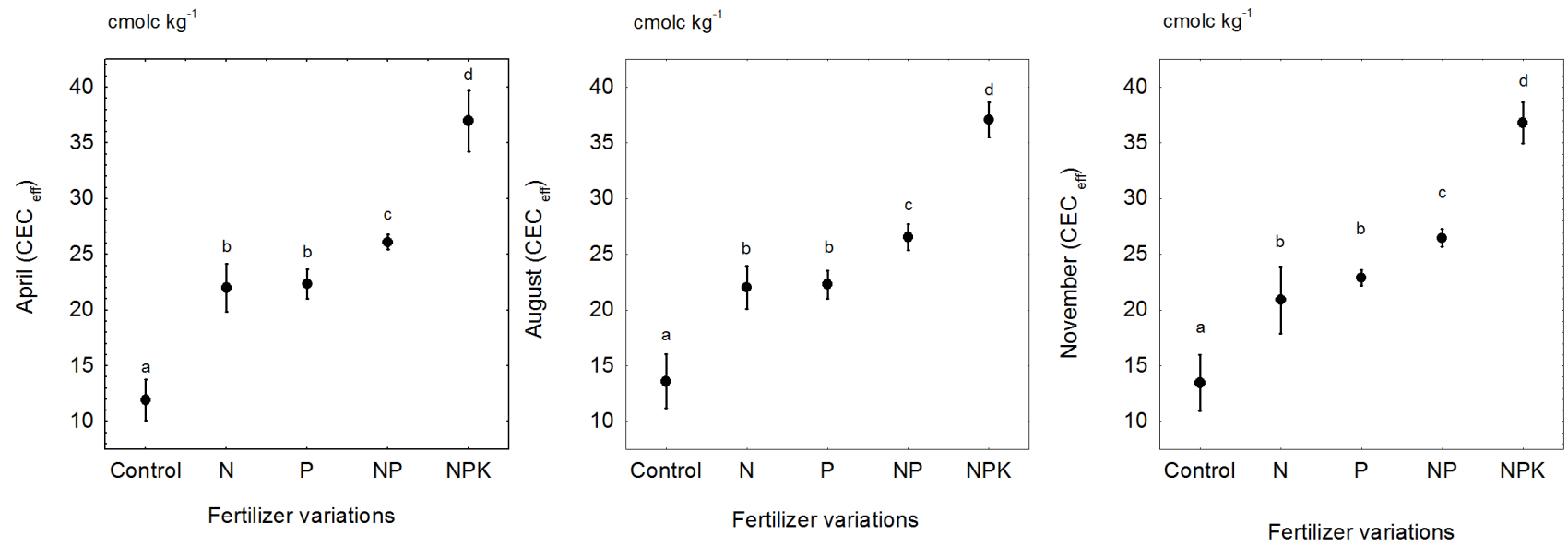
584 Fig. 1: Effect of fertilizers on soil organic carbon content (mean values of 4 study areas-Error bars indicate standard deviation)

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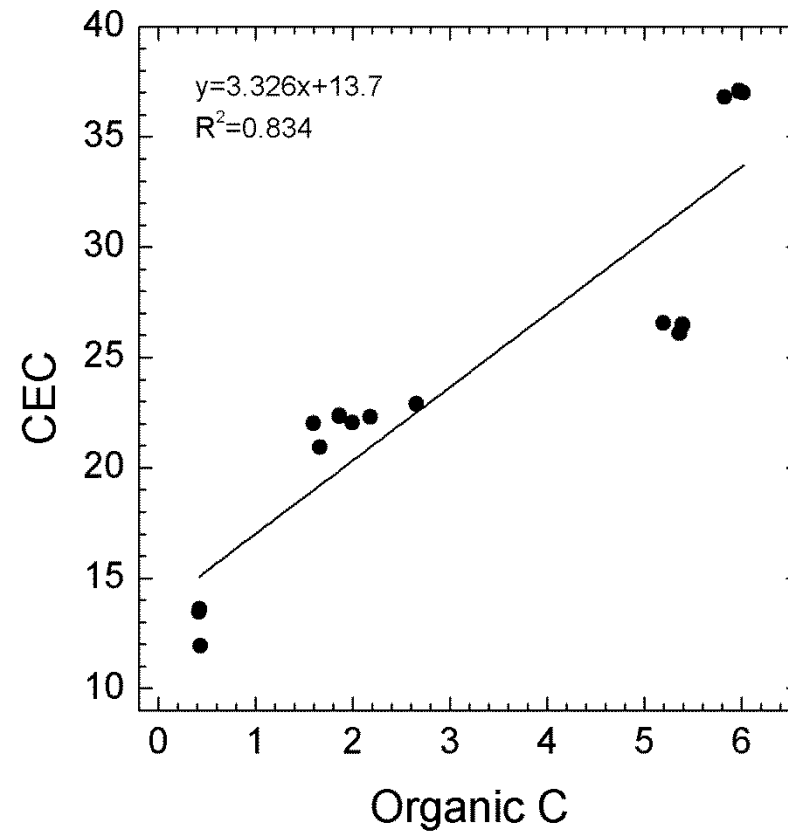
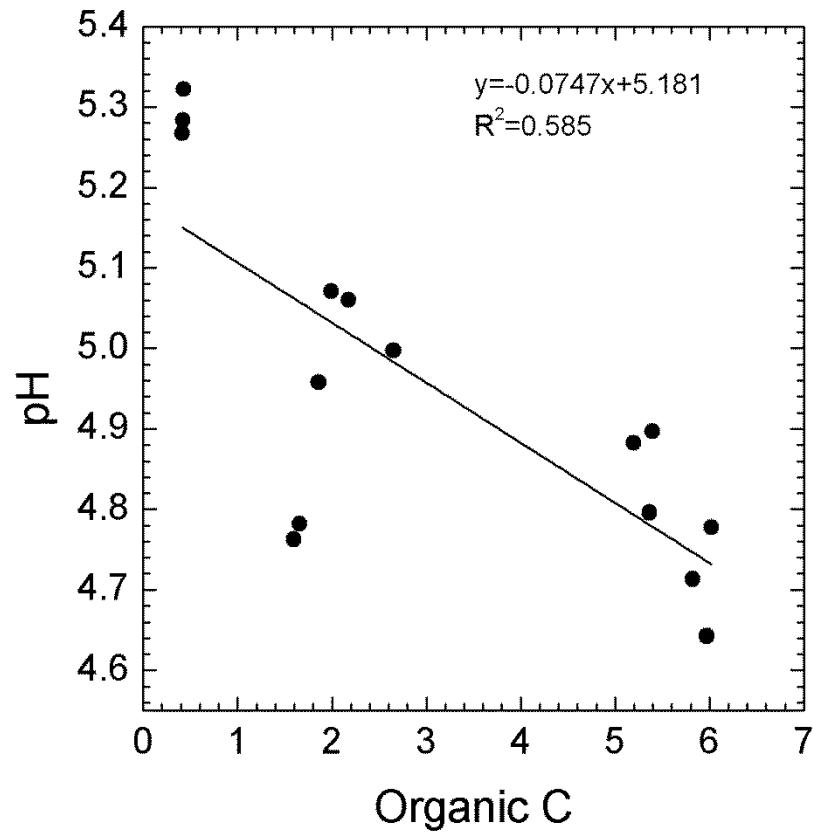
590 Fig. 2: Effect of fertilizers on soil effective cation exchange capacity (mean values of 4 study areas-Error bars indicate standard
591 deviation)

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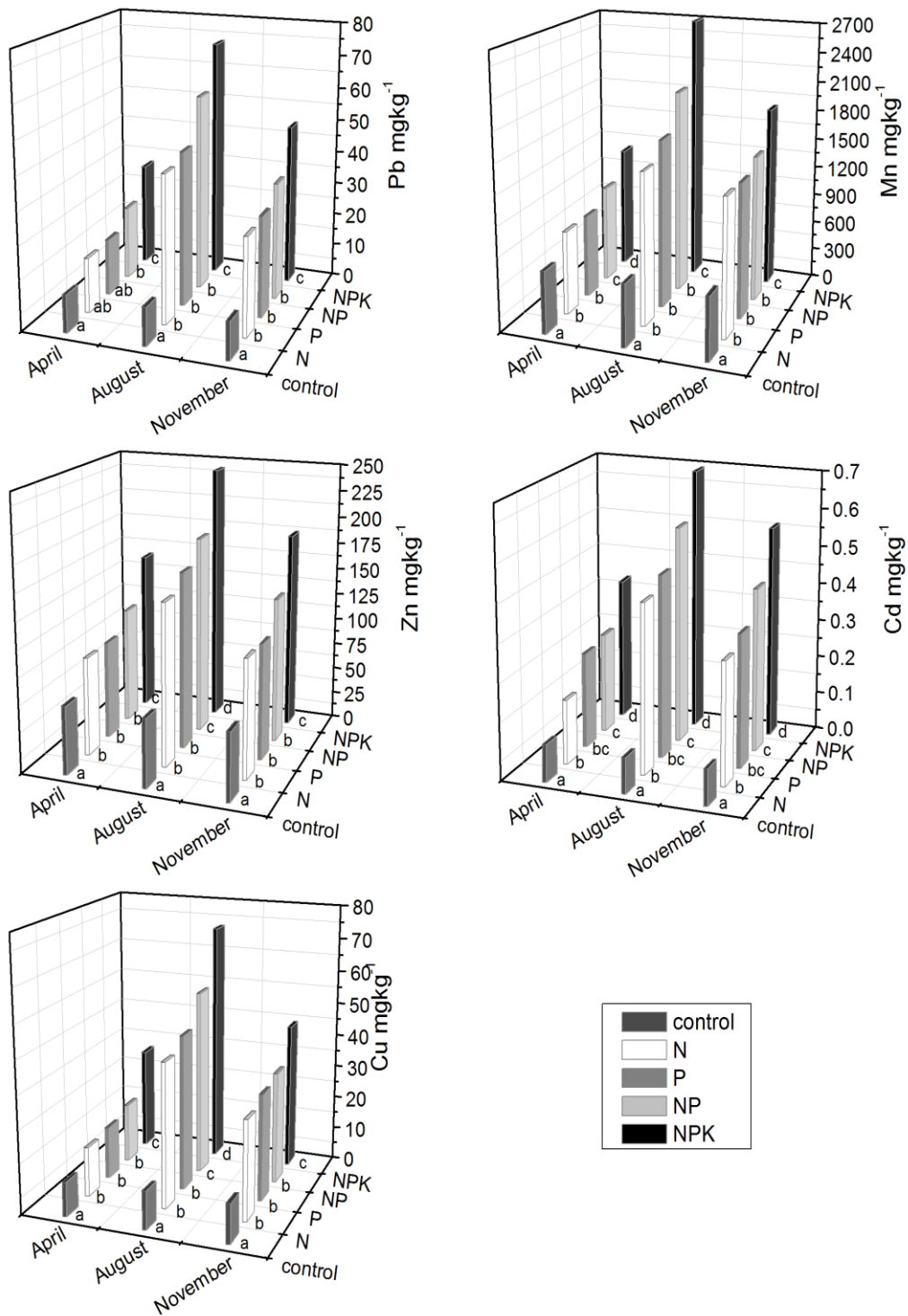
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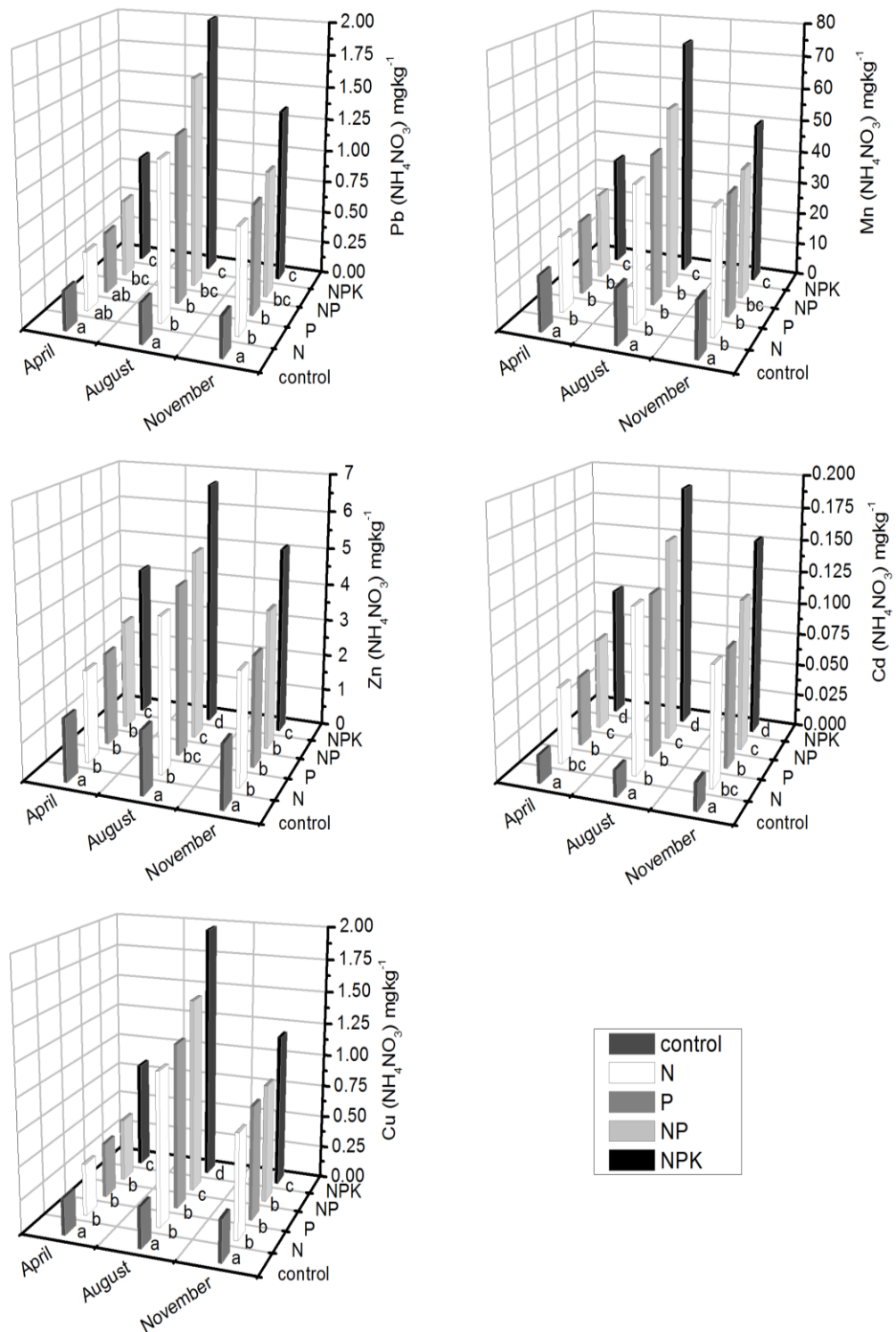
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597 Fig. 3: Relationship between organic C and pH and soil CEC_{eff} using mean values of all treatments (mean values of 4 study areas)

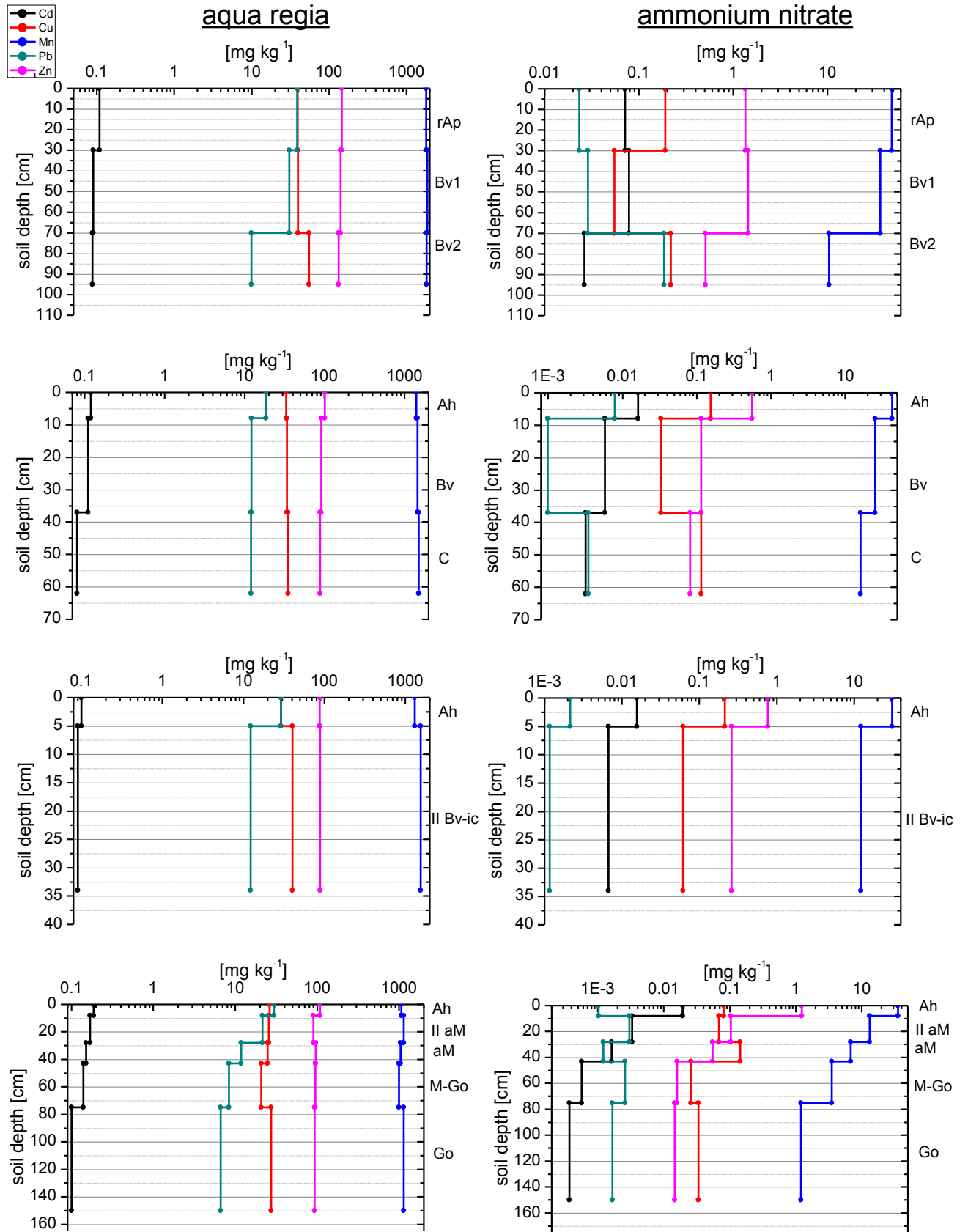


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599 Fig. 4: Effect of fertilizer treatments on pseudo total (aqua regia) metal contents in
 600 soil samples (Mean values followed by different superscript letters for each month between the
 601 fertilizer variations differ at 5 % probability)



604 Fig. 5: Effect of fertilizer treatments on mobile (NH₄NO₃) metal contents in soil
 605 samples (Mean values followed by different superscript letters for each month between the fertilizer
 606 variations differ at 5 % probability)



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609 Appendix. 1: Soil pseudo and mobile metal contents (Cd, Cu, Mn, Pb, Zn) of the soil
 610 profiles in 2011

Responses to the referees

Dear Referees,

Thank you for giving us the opportunity to rearrange and correct our manuscript and we are thankful to your comments that helped us to improve the research article.

Here are the details of our responses to the suggestions:

Point-by-point summary (Referee-1)

Page 240, line 16-17: Fertilization.....

The sentence is rewritten. Please see lines 12-14 in the manuscript (in yellow highlighted).

Page 244, lines 6-8

The sentence is deleted. Please see “Section 2.3 Statistics” in the corrected manuscript (please see lines 125-130).

Page 244, line 10

The word “evidently” is removed from the manuscript. Please see “line 133” in the corrected manuscript (sentence is in yellow highlighted)

Page 245, line 3

The word “conspicuous” is changed to “significant” in the manuscript. Please see “line 153” in the corrected manuscript (in yellow highlighted).

Page 245, line 16

The word “affection” is changed to “effect” in the manuscript. Please see “line 167” in the corrected manuscript (in yellow highlighted).

Page 247, lines 8-11

The sentence is rewritten. Please see “lines 218-220” in the corrected manuscript (in yellow highlighted).

Conclusion

The “Conclusion” section of the manuscript is rewritten (please see lines 333-353) (in yellow highlighted).

Figures

Figure 1, 2, 4 and 5 is revised (please see figures in the attached file).

Fertilizer metal content

This article focused on the relation of the metal contents in soil and soil properties and the effects of fertilizer treatments on them. Different doses of nitrogen, phosphorus and potassium fertilization were applied. Control treatments did not receive any fertilizers to verify metal input due to fertilizer treatments. Considerable increments in soil pseudo total and mobile metal content were detected in fertilized plots. On the other hand, results suggested that application of mineral fertilizers accelerated soil acidification which has an impact on metal mobility. Due to this long-term experimental design which was started in 1986 and ended in 2002, the fertilizers used at the beginning of this study are not accessible anymore. Although, the metal concentrations in the fertilizers could not be analyzed, the effects of 14 years of mineral fertilization on metal content of the soils can clearly be seen by the comparison of control plots (non-fertilized 14 years long) with the fertilized plots. The non-fertilized plots served as control data to assess the metal input into the soils due to the mineral fertilization design. In any case, among different literature data (Jiao, 2012; Lambert, 2007; Grant, 2011), metal contamination on account of application of long-term fertilizers is most acquainted. Overuse of fertilizers can provide soil contamination; it increases the concentration of a particular element or substance of environmental interest above its natural occurrence in the soil. According to USEPA (1999), among the fertilizers used, the phosphate shows the highest levels of toxic metals (especially Cd), followed by nitrogen and potassium fertilizers.

References:

USEPA – United States Environmental Protection Agency. Background Report on Fertilizer Use, Contaminants and Regulations. Columbus: USEPA; 1999.

Jiao, W., Chen, W., Chang, A. C. and Page, A. L. 2012. Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. *Environmental Pollution* 168 (2012) 44-53.

Lambert, R., Grant, C. and Sauvé, S. (2007). Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. *Science of the Total Environment* 378 (2007) 293–305.

Grant, C. A. (2011). Influence of phosphate fertilizer on cadmium in agricultural soils and crops. *Pedologist* (2011) 143-155.

Point-by-point summary (Referee-2)

1- Experimental design

The explanations about the experimental design are rewritten. Please see “Section 2.1 Study area and fertilizer treatments” of the manuscript (please see yellow highlighted lines 73-79, 81, 83-86).

2- Pseudo total

The description of “pseudo total metal content” is given in the manuscript. (please see lines 220-224 in the revised manuscript- in yellow highlighted).

3- Ni as essential plant nutrient

It was a citation from “Raskin, I., Kumar, P. B. A. N., Dushenkov, S., and Salt, D. E.: Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 5: 285-290, 1994.”

Some more recent references about the essentiality of Ni could also be found in the literature:

- Chen, C., Huang, D. and Liu, J.: Functions and Toxicity of Nickel in Plants: Recent Advances and Future Prospects. *Clean* 2009, 37 (4-5), 304-313.
- López, M. A. and Magnitskiy, S.: Nickel: The last of the essential micronutrients. *Agronomía Colombiana* 29 (1), 49-56, 2011.

- Yusuf, M., Fariduddin, Q., Hayat, S. and Ahmad, A.: Nickel: An Overview of Uptake, Essentiality and Toxicity in Plants. Bull. Environ. Contam. Toxicol. (2011) 86:1-17. DOI 10.1007/s00128-010-0171-1.

4- Material and Methods (Soil Classification)

Soil classification according to WRB 2014 is added to the manuscript. Please see “section 2.1 Study area and fertilizer treatments” of the manuscript (please see lines 83-86) (in yellow highlighted).

The reference is also added to the list. [IUSS Working Group WRB. 2014. World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome] (please see lines 422-424).

5- Results and Discussion: the meaning of “considerable”

The word “considerable” is changed to “significant” in the manuscript. Please see “line 133” in the corrected manuscript (in yellow highlighted).

6- “Results” section of the manuscript is revised.

A new sentence is added (please see lines 270-272) and between lines 283-293 and 297-322 are rewritten (all is in yellow highlighted).

The reference “Golia, E. E., Dimirkou, A., and Mitsios, I. K.: Influence of Some Soil Parameters on Heavy Metals Accumulation by Vegetables Grown in Agricultural Soils of Different Soil Orders. Bull Environ Contam. Toxicol. (2008) 81:80–84, 2008. doi 10.1007/s00128-008-9416-7” is removed from the manuscript.

Moreover, five new references are added:

- Kabata- Pendias, A. and Mukherjee, A. B.: Trace elements from soil to human. Springer-Verlag Berlin Heidelberg, 2007 (lines 427-428).

- Alloway, B.J.: Heavy metals in soils (Alloway B.J., ed). Blackie, Glasgow and London, 1990” (lines 364-365).
- Abrahams, P. W.: Soils: their implications to human health. The Science of the Total Environment, 291, 1 32, 2002 (lines 356-357).
- BBodSchV (Bundes-Bodenschutz- und Altlastenverordnung) Bundesgesetzblatt, Bundesanzeigerverlagsgesellschaft mbH, Köln, Germany, 1999 (lines 372-373).
- Brevik, E. C. and Sauer, T. J.: The past, present, and future of soils and human health studies. SOIL Discuss., 1, 51–80, 2014, doi:10.5194/soild-1-51-2014 (lines 377-378).

Point-by-point summary for the other changes that was made during the interactive Discussion

The authors are thankful to Mr. Eric Brevik to his comments about the human health that helped us to improve the research article.

Here are the details of our responses to the suggestions:

Lines 270-272 and 312-322 (manuscript-pdf)

The “precaution values” are added the manuscript. And moreover, three new references are added to the manuscript (in yellow highlighted)

1-Abrahams, P. W.: Soils: their implications to human health. The Science of the Total Environment, 291, 1 32, 2002 (lines 356-357).

2-BBodSchV (Bundes-Bodenschutz- und Altlastenverordnung) Bundesgesetzblatt, Bundesanzeigerverlagsgesellschaft mbH, Köln, Germany, 1999 (lines 372-373).

3-Brevik, E. C. and Sauer, T. J.: The past, present, and future of soils and human health studies. SOIL Discuss., 1, 51–80, 2014, doi:10.5194/soild-1-51-2014 (lines 377-378).