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

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

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

1. Introduction

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

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

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

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

2. Materials and Methods

2.1 Study area and fertilizer treatments

This study was carried out at four different locations in different altitudes in Hesse, Germany. The region has a climate with a relative strongly maritime influence. Different doses of nitrogen, phosphorus and potassium fertilization has been undertaken since 1986 until 2002 in a Latin rectangle design, including the control treatment that did not receive any fertilizers to verify metal input due to fertilizer treatments. Some basic physicochemical characteristics of the non-fertilized (control) plots in the studied areas are given in Table 1.

The size of the field trial was 45x54 m and divided in 81 plots each of 30 m^2 size. The top soil (0-30 cm) was sampled in April, August, and November in three replications in the year 2000 (14 years after fertilization application) and in July 2011 from soil profiles (8 years after fertilization termination). According to the WRB (World Reference Base for Soil Resources) classification (2014), we determined the soil types as Eutric Cambisol (210 m a.s.l), Skeletic Cambisol (260m and 360 m a.s.l) and Gleyic Fluvisol (620 m a.s.l). *Lolio-Cynosuretum* was the cultivation crop during the study.

Calciumammoniumnitrate (CAN) (74% NH_4NO_3, 26% $CaCO_3$) as N fertilizer, hyper phosphate (26% P_2O_5) and superphosphate (16-22% P_2O_5) as P fertilizer, and magnesia kainit (11% K_2O, 5% MgO, 20% Na, 4% S) and K_2SO_4 as K fertilization were used in various levels which refer to different application rates (Table 2). N fertilization was used in the same proportions in middle of April, May, June, and July; P and K fertilizers were applied in the middle of April as hyper phosphate and magnesia kainit and in the middle of June as superphosphate and K_2SO_4.

2.2 Analytical Procedures

Soil samples were air dried, crushed, homogenized, and sieved through 2 mm screen for the analysis of physical and chemical characteristics and stored in clean HNO_3 treated plastic bottles.

The characteristics of soil such as soil pH (DIN ISO 10390), soil carbonate content (DIN ISO 10693), particle size distribution (DIN ISO 11277) were determined for the samples collected. Moreover, total contents of carbon (C_t) were examined on finely ground samples by a CNS analyzer with a Thermal Conductivity Detector (VarioEL III Elementar, Germany). Inorganic carbon (C_i) was calculated from the carbonate content by multiplying the factor 0.1199. The amounts of organic C (C_{org}) resulted from the difference between C_t and C_i. Effective cation exchange capacity (CEC_{eff}) was examined with NH_4Cl extraction according to Trüby and Aldinger (1989) and exchangeable cations were determined with AAS (AA240FS, Varian, USA).

Pseudo total metal contents of soil samples were determined by modified USEPA Method 3051A (USEPA) with microwave assisted extraction-aqua regia (MAE-AR). The modified program for MAE-AR (0.3 g of soil, 6 ml HCl and 2 ml HNO_3) is described in Öztan and Düring (2012). Mobile fraction of metals was measured after the extraction of soils with ammonium nitrate (NH_4NO_3) (20 g soil, 50 ml 1M NH_4NO_3, shaken for 120 min) (DIN 19730, 2009). Metal concentrations in soil extracts were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES; Agilent 720ES) which provides rapid multi-elemental analysis.

The reagents used were all of analytical-reagent grade certified for the impurities. Distilled and deionized water, purified with a Milli-Q plus system (Merck Millipore, Darmstadt, Germany), was used for the experiments. For quality assurance, two certified reference materials (CRMs), “7001” (light sandy soil) and “7004” (loam), from Analytika Co. Ltd, Prague, Czech Republic, were analyzed repeatedly during analysis of the sample set. To designate the amount of possible cross contamination, blank values were determined within each sample series.

2.3 Statistics

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

3. Results and Discussion

The results show that long-term N, P, and K fertilizer treatments have significant effects on soil properties. During the study, significant decrease in soil pH was detected due to fertilization (Table 3). Decrease in pH of the surface layer in the fertilizer might be attributed to the nitrification and acidification processes stimulated by continuous application of fertilizers as well as by H^+ released by roots (Liang et al. 2012). Where N fertilizer was applied the pH slightly decreased with respect to the initial value. Significant differences were determined between the control plots and N, P, NP, and NPK applications in soil pH level in April. However a decrease in soil pH was found when compared to control plots in soil August and November pH degrees, no significant differences were seen for N and P treatments in soil-August and for P application in soil-November pH level (Table 3). Continued use of ammonia-based fertilizers can induce soil acidity (Schwab et al. 1990). Results from the study were in agreement with the findings of Tsadilas and colleagues (2005) who reported that the application of ammonium fertilizer significantly decreased soil pH more than the nitrate treatments. Results from a pot fertilizer experiment which showed that application of NH_4Cl lowered soil pH from 4.51 to 4.07 was presented by Liu et al. (2007). The major mechanism of soil acidification by N fertilization is related to hydrogen ion (H^+) release through nitrification of NH_4^+ and subsequent leaching of NO_3^-. The most important acid forming reaction by fertilizers is microbial oxidation of ammoniacal fertilizers (Barak et al. 1997).

Significant differences in soil organic carbon (C_{org}) content and effective cation exchange capacity (CEC) which was caused by fertilization treatments can be seen in Fig. 1 and 2. Significant increase in C_{org} content was determined in April, August and November soil samples. All fertilizer treatments show significant differences when compared to the control plots. In April and August soil samples, no significant differences were determined on C_{org} content between N and P treatments. Differences between N and P variations are evident in November C_{org} content. C_{org} level with P fertilizer was almost two times higher than C_{org} content in N treatment (Fig. 1). Addition of fertilizers to soil influences the chemical composition of soil solution. Increased plant biomass produced by fertilizers results in increased returns of organic material to the soil in the form of decaying roots, litter and crop residues (Haynes and Naidu, 1998). Our results are similar with the findings of Raun and co-workers (1998) and with the study of Halvorson et. al. (1999) who demonstrated a rise in C_{org} with the applied increasing N ratios. Messiga and colleagues (2013) although found no significant effect for total carbon content in 0 to 5 cm soil layer by N application, they noted a tendency for greater total carbon concentration with increasing N application. Liu et al. (2005) reported that C_{org} in surface soil (0-15 cm) layer was 7.7%, higher in mineral fertilizer treatment than without fertilizer application. Similar results obtained in a long-term experiment showed that low nitrogen rates poorly increased C_{org} content compared to the control (0.14 vs 0.03 Mg $ha^{1}year^{-1}$), while the medium and high rates increased it by 0.45 and 0.49 Mg ha^{1} $year^{1}$, respectively (Mazzoncini et al., 2011). After 14 years of fertilizer application, there was an increasing trend to C_{org} in April in NPK treatment (6.02 %) contrasting to the control soil content of 0.43%. This is in accordance with the results of Xie et al. (2011) who found in a pot experimental study the highest C_{org} content was in the NPK (80 N mg kg^{-1}, 35 P mg kg^{-1}, and 60 K mg kg^{-1}) treatment, which was significantly higher than that in the NK (80 N mg kg^{-1}, and 60 K mg kg^{-1}) treatment. Gong and co-workers (2009) determined an increase in C by long-term applications of manures and mineral fertilizers - both alone and in combination - and concluded that application of mineral fertilizer may have stimulated microbial activity and enhanced decomposition. More soil organic matter was decomposed and more available nutrients could then be provided for better crop growth, resulting in increased crop residues (root debris and secretion) being returned to the soil.

The CEC is a very important soil property for nutrient retention and supply and acts as a bridge between soil and plant (Caravaca et al. 1999). N, P, NP, and NPK treatments significantly increased CEC in April, August, and November soil samples when compared to non-fertilized plots (Fig. 2). Cakmak and colleagues (2010) reported that 40 years application of P fertilizers significantly decreased pH and increased CEC of the soil. Thus, phosphate fertilizer applications not only increase Cd concentration of soils but also may change their chemical speciation and thus bioavailability. As the unfertilized soil in our study has not received any kind of fertilizer during 14 years of investigation, the differences observed here may therefore be judged conservative estimates of the effect of soil organic matter on CEC. This is in accordance with the results of Schjonning et al. (1994) who found 11 % higher CEC than in the control plots. Nitrogen application as CAN fertilizer only or in combination with NPK complex fertilizer rises the soil CEC value. This can be explained by soil colloid retention of applied Ca^{2+}, NH^{+}_{4}, and K^{+} ions

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

The main effect of each fertilizer variation on soil pseudo total and mobile (NH_4NO_3-extractable) metal (Cd, Cu, Mn, Pb, and Zn) contents can be seen in Fig. 4 and Fig. 5. Though total concentrations of metals in soil are used to characterize its base-line elemental composition (parent geological material), pseudo total metal concentration analysis which is performed by an estimation with aqua regia, allows to assess soil pollution and to ascertain heavy metal and other pollutant contents in the soil (Ure, 1990). Pseudo total metal contents in soils increased due to application of long-term chemical fertilizer treatments when compared to control plots (Fig. 4). Applications of N, P, NP, and NPK fertilizers increased soil April, August, and November Cd levels compared to non-fertilized soil, but no significant differences were found between P and NP fertilizer variations for pseudo total Cd concentrations (Fig. 4). Significant differences between P and NP applications were obtained for mobile Cd content (Fig. 5). Trace metal enrichment in soils through mineral fertilizer applications is well documented for Cd. Loganathan and colleagues (1997) reported that in pasture systems in New Zealand, ten years of P fertilizer application caused a marked increase in surface soil pseudo total Cd concentration. Identical findings reported Gray et. al, (1999) who specified a significant increase in total Cd concentration in soils under pasture with application of superphosphate fertilizer over a period of 44 years in New Zealand.

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

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

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

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

Soils have a distinct influence on human health (Brevik and Sauer, 2014), hence soil contaminants constitute a known global problem, and more knowledge is required regarding their behavior, and their pathways to humans (Abrahams, 2002). Although many grassland fertilizer experiments have been performed worldwide, information about residual effects of fertilizer applications on grassland ecosystem functioning is still rare (Hejcman and Schnellberg, 2009). Fertilization is one of the major paths for metal input to agricultural soils, therefore monitoring of the long term impact of fertilization residual effects is necessary. On this account, in July 2011 mixed soil samples from soil profiles of the fertilized plots (NPK) in the study areas were taken and analyzed for pseudo total and mobile metal concentration and for soil properties such as pH, C_{org} and CEC. Soil pseudo and mobile metal contents (Cd, Cu, Mn, Pb, Zn) and soil pH, C_{org} and CEC of the soil profiles are summarized in Table 4 and detailed distribution of the metals in soil profiles are given in Appendix 1.

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

Furthermore, though a decrease in Cu, Mn, Pb, and Zn contents was determined in the first 30 cm of the soil profiles in July 2011, an evident decrease such as in Cd concentration was not observed. Detected pseudo total Cu, Mn, Pb, and Zn concentrations in August-2000 in NPK amended plots were 72.3 mg kg^{-1}, 2704.4 mg kg^{-1}, 72.8 mg kg^{-1}, and 242.5 mg kg^{-1}, and in non-fertilized plots (control-treatment) were 11.4 mg kg^{-1}, 624.4 mg kg^{-1}, 11.4 mg kg^{-1}, and 63.3 mg kg^{-1}, respectively (Fig. 4). High contents of Cu, Mn, Pb, and Zn from fertilizers residual effects still persisted in the top soil layer of the field trial in July 2011. In the soils that received the maximum dose of fertilization (NPK) for 14 years and followed by no additional input for 8 years, Cu, Mn, Pb, and Zn contents were still around two times higher than those in the control plots in August 2000 (App. 1).

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

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

In the soil samples taken in August 2000, significant increase in C_{org} and CEC content was determined in April, August and November due to N, P, NP, and NPK treatments when compared to non-fertilized plots (Fig. 1 and 2). Rasool and colleagues reported that, $N_{100}P_{50}K_{50}$ increased soil organic matter content by 21% (Rasool et. al., 2008). After finalization of the fertilization design an appreciable decrease in July 2011 soil C_{org} and CEC was detected (Table 4). Grassland suffered throughout centuries from nutrient depletion through removal of organic material as dung and forage, and subsequent transfer to agricultural land or high productive lowland grassland (Hejcman and Schnellberg, 2009).

4. Conclusions

Based on the results from the present study, the main conclusion is that 14 years of mineral fertilization has a significant effect on soil metal contents and soil properties such as pH, C_{org} and CEC_{eff}, and therefore, has a great impact on metal availability. Continued long-term fertilizer use increased soil metal content, soil organic C, CEC and decreased soil pH level. Another important aspect of this study was the establishment of soil metal concentrations 8 years after cessation of the fertilizer application. Soluble metals were taken up by plants and thus leaving lower amounts in the soil, probably due to decreased pH values in the long-term fertilized soils. Cd concentrations in 2011 evidently decreased down to the value in the control plots determined in August 2000. Despite decrement in metal contents, Cu, Mn, Pb, and Zn still remained in the top soil profile around two times higher than the control soils taken 14 years after fertilization application. Moreover, in terms of human health, metal concentrations determined in the samples taken in 2011 all fell below the precaution values according to BBodSchV (1999).

This research has thrown up some questions in need of further investigation. Applications of fertilizers not only provide plant nutrients, but also change the availability of metals and their uptake into the plants. As plants represent the first compartment of the terrestrial food chain, future research should therefore concentrate on the investigation of the impacts of various soil factors on metal transfer into the plants.

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

Properties			April	August	November
	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
Aqua regia extractable metals mg kg^{-1}	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

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

Table 2: Fertilization variations of the study sites

		kg ha^{-1}	
Fertilizer Variations	**N**	**P_2O_5**	**K_2O**
Control	0	0	0
N	320	0	0
P	0	120	0
NP	320	120	0
NPK	320	120	160

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

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}

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

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

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_4NO_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

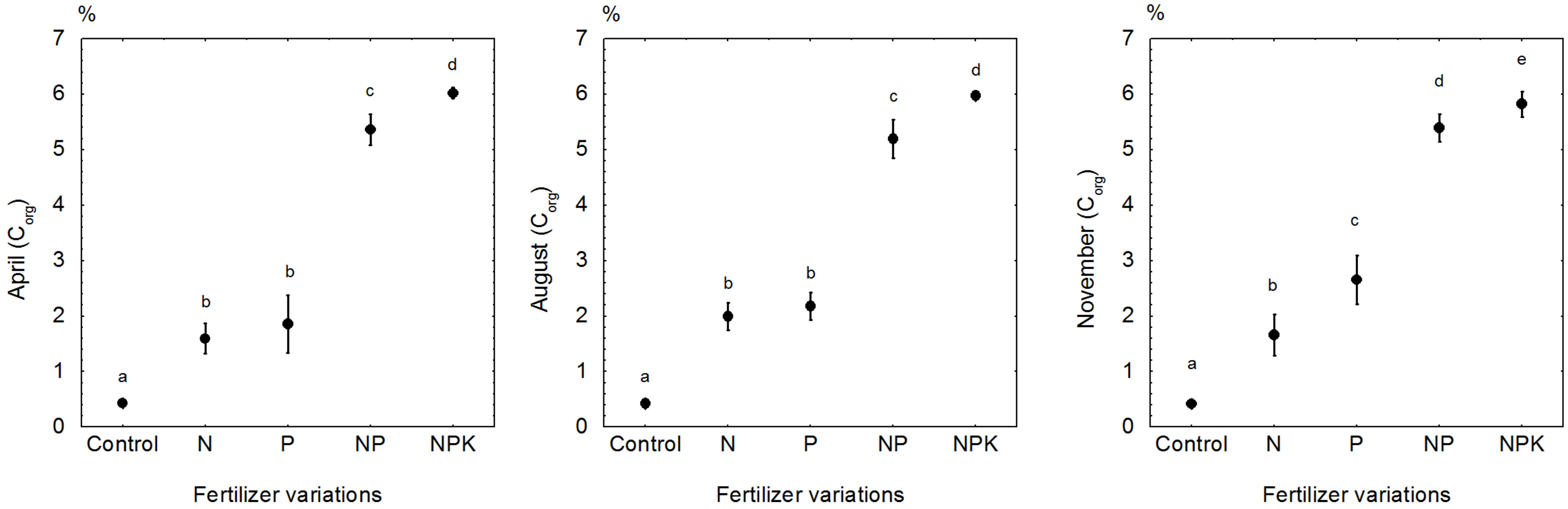


Fig. 1: Effect of fertilizers on soil organic carbon content (mean values of 4 study areas-Error bars indicate standard deviation)

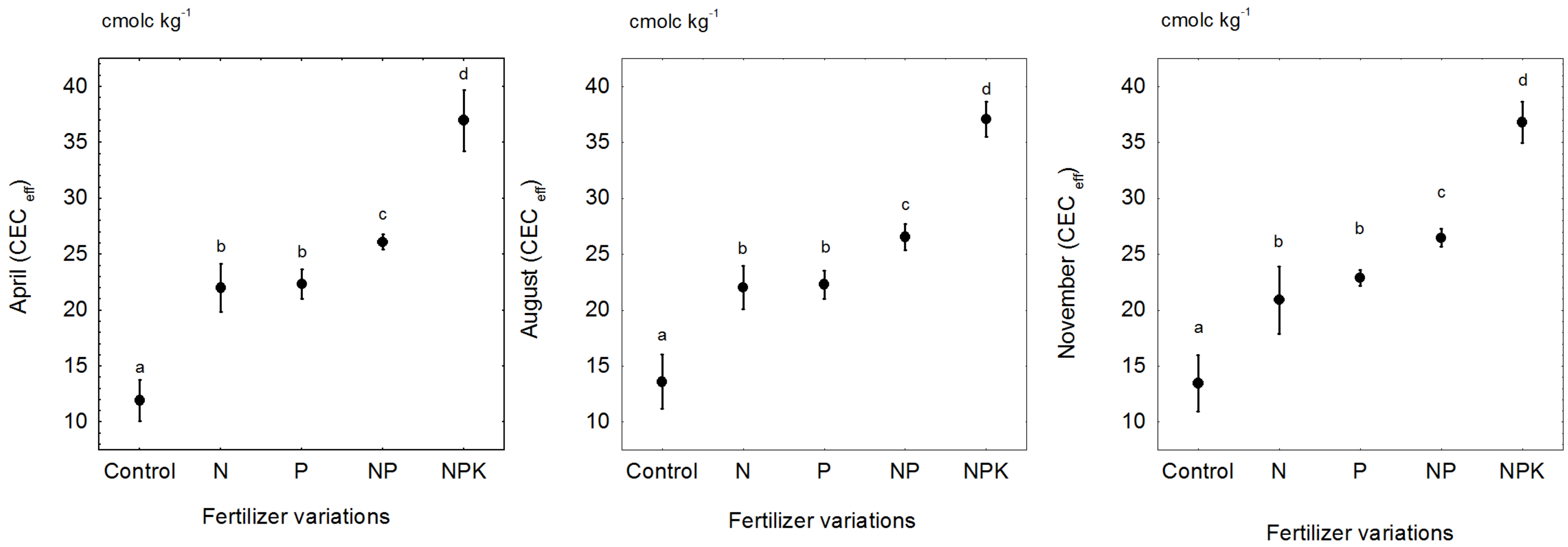


Fig. 2: Effect of fertilizers on soil effective cation exchange capacity (mean values of 4 study areas-Error bars indicate standard deviation)

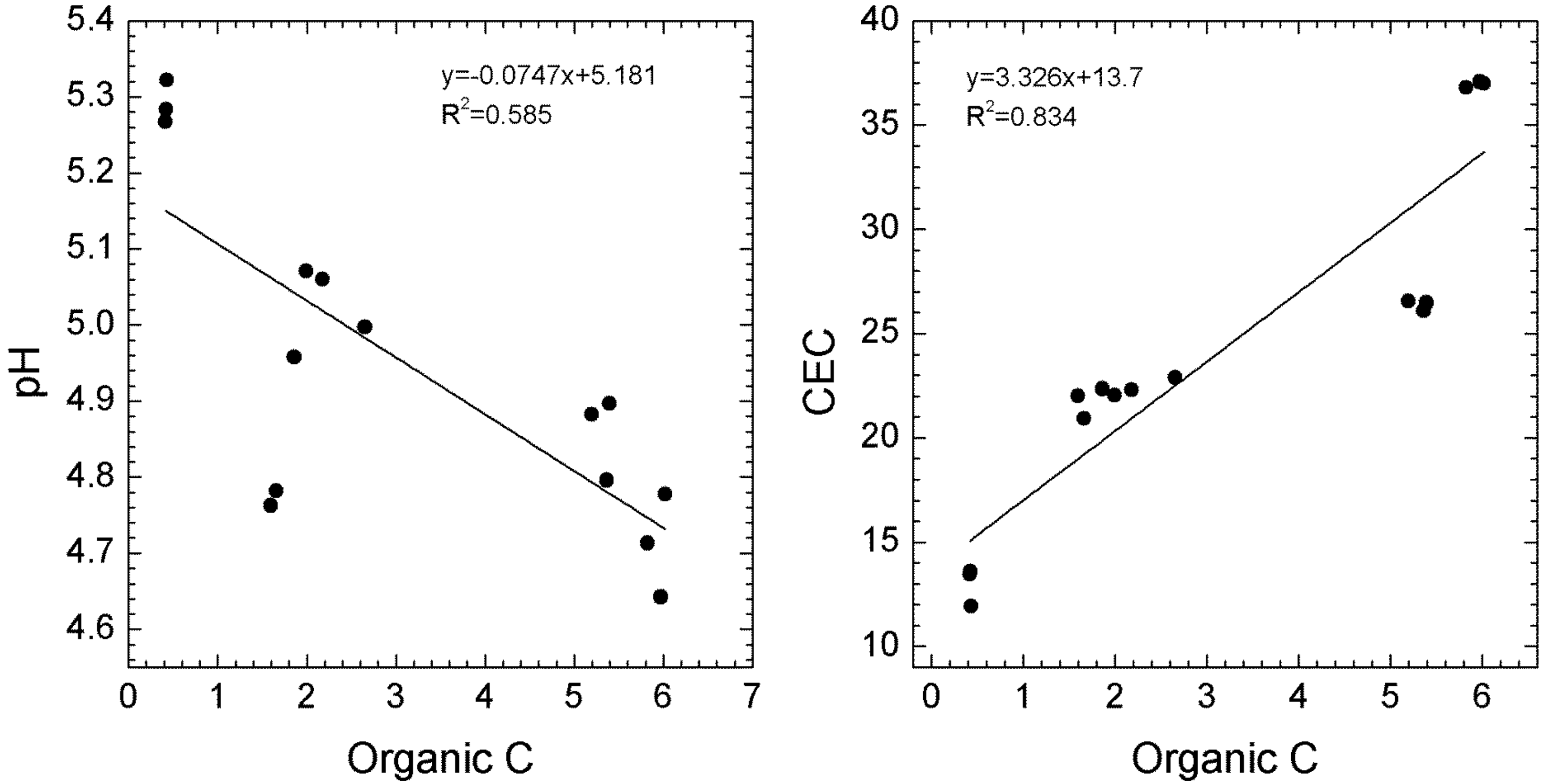


Fig. 3: Relationship between organic C and pH and soil CEC_{eff} using mean values of all treatments (mean values of 4 study areas)

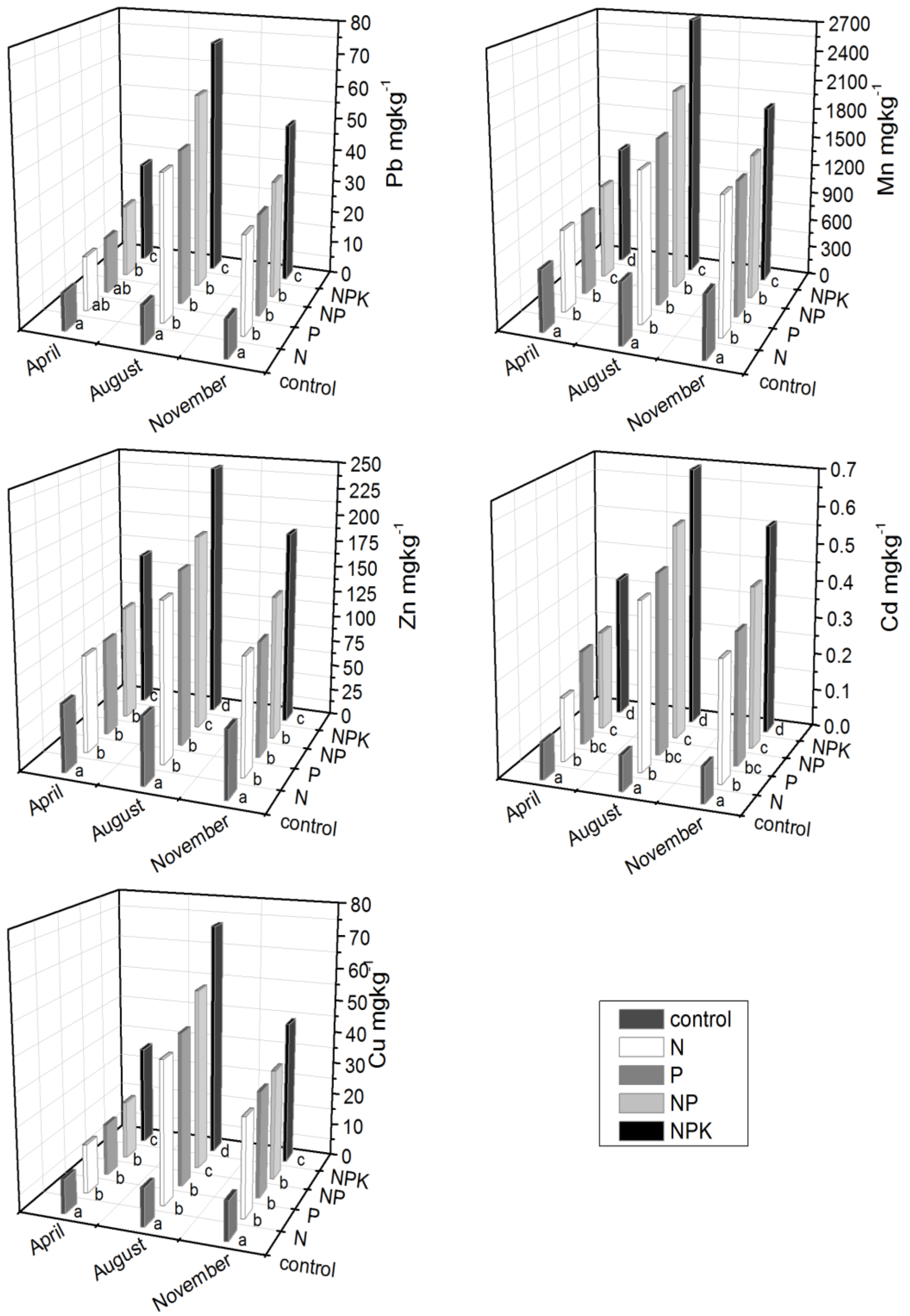


Fig. 4: Effect of fertilizer treatments on pseudo total (aqua regia) metal contents in soil samples (Mean values followed by different superscript letters for each month between the fertilizer variations differ at 5 % probability)

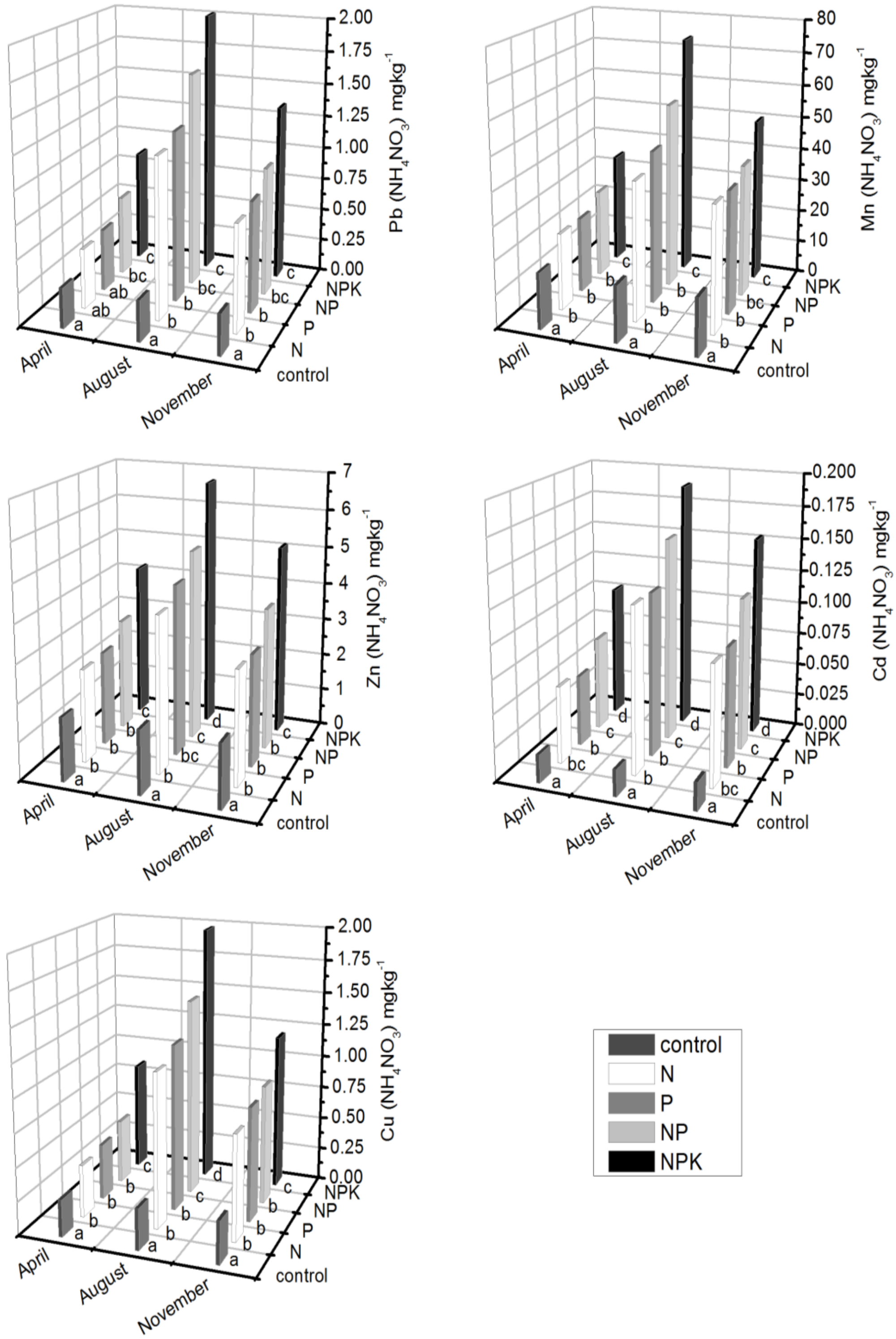
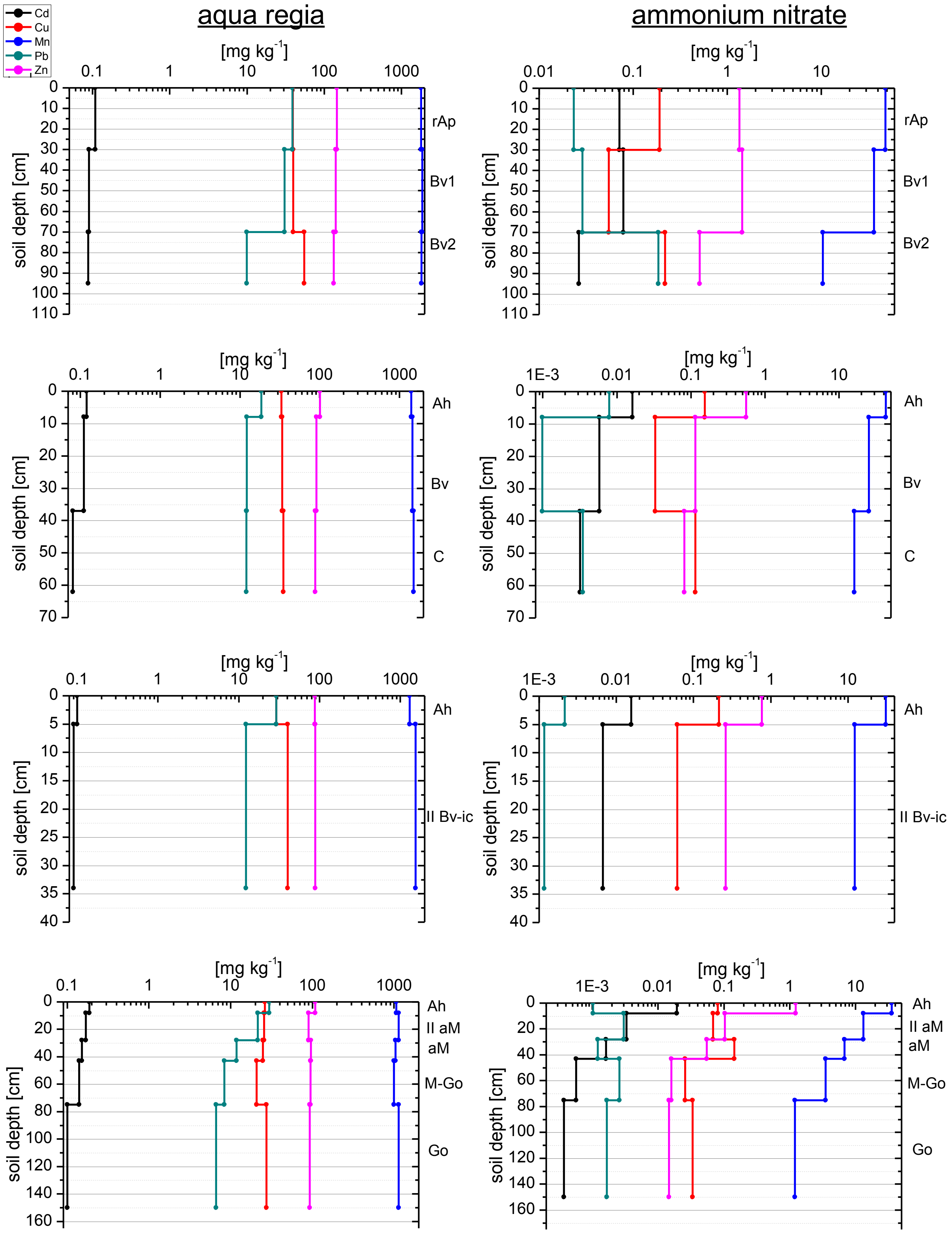


Fig. 5: Effect of fertilizer treatments on mobile (NH_4NO_3) metal contents in soil samples (Mean values followed by different superscript letters for each month between the fertilizer variations differ at 5 % probability)



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