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# Effect of long-term fertilization of the permanent dry meadow on the zinc content in soil and meadow sward

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## Abstract

The study took place between 2012 and 2014 in Falenty near Warsaw, Poland, as part of a long-term scientific experiment (first began in 1987) using the randomized block method. All blocks were irrigated until 2008. In 2009 each block was divided into two areas: irrigated and non-irrigated. The study involved four levels of inorganic nitrogen fertilizer and two levels of mixed inorganic and organic fertilizer in the form of fermented cattle urine. The soil in all experimental plots was characterized by low levels of zinc, ranging from 7.6 to 16.7 mg Zn·kg<sup>-1</sup> dry matter. Much lower Zn content in both soil layers of all irrigated plots was associated with increased yields on these plots, regardless of the level and form of fertilizer. The content of Zn in soil and sward in 2014 year was significantly lower compared in 2012. Inadequate levels of zinc for ruminant nutrition were observed in the sward from all plots (15.4–28.8 mg·kg<sup>-1</sup> dry matter). The higher content of zinc was found in sward harvested from the plot, which was not fertilized with phosphorus. The long-term inorganic and fermented urine fertilization resulted in very low zinc content in the soil and meadow sward.

**Key words:** *fertilization, irrigation meadow, non-irrigation meadow, permanent meadow, soil, sward, zinc*

## INTRODUCTION

Zinc is an important trace element that stimulates the functioning of plants, animals and humans. It is involved in the formation of growth hormones and many enzymes participating in carbohydrate, protein and phosphorus metabolism. It also affects the permeability of cell membranes, controls the formation of ribosomes, participates in the synthesis of auxins and increases plant resistance to drought and disease. Due to its vital role, Zn deficiency generally causes impairments in physical development and fertility. Zn deficiency usually appears simultaneously in humans, livestock and crops as a consequence of low soil Zn concentrations [ALLOWAY 2009; HAFEEZ *et al.* 2013; PRASAD 2010]. Zinc fertilization significantly increased the yield and sulforaphane content in broccoli [ŚŁOSAR *et al.* 2017]. Zn superoxide dismutase (ZnSOD) is a dietary

factor, which contributes to the antioxidant defense system. Zinc administered orally together with Se and vitamin E to growing ram-lambs induced a decrease in cholesterol content in the blood and meat, and led to increased conjugated linoleic acid (CLA) isomers level in the meat and liver [GABRYSZUK *et al.* 2007]. Zinc is an element of high mobility in the soil habitat. Its availability to plants is determined by the pH and by the Ca:Zn ratio in soil solution [DOMAŃSKA 2009; STRACHEL *et al.* 2016]. Soil pH and soil Zn concentration affected arbuscular mycorrhizal fungi (AMF), which positively affected Zn concentration in various crop plant tissues [LEHMANN *et al.* 2014]. Total Zn content in soil, moisture, the presence of carbonates, microbial activity of the rhizosphere, and organic substance levels are all important for Zn uptake by plants [KABATA-PENDIAS 2010].

Appropriate levels of zinc in sward, a key fodder for farm animals, provide animals with essential amounts of this chemical element. Increasing the acidity of soil has significant impact on the solubility of zinc and thus its activity and bioavailability. The absorption of zinc by plants, especially in slightly acidic and acidic soils is high due to the solubility of the majority of its compounds [DOMAŃSKA 2009; KABATA-PENDIAS 2010]. The availability of this element for plants also depends on its overall content in soil, soil moisture level, organic compound levels and levels of other elements and macro-elements, principally phosphorus, nitrogen and copper [ALLOWAY 2008; LONERAGAN, WEBB 1993]. TILLER [1989] claims that soil properties are the main factor in the absorption of zinc by plants.

The aim of the study was to examine the effect of long-term inorganic and mixed organic (fermented urine) and inorganic fertilization, with and without irrigation, on the zinc content in meadow sward and in the soil.

## MATERIALS AND METHODS

The study took place between 2012 and 2014 in Fałenty near Warsaw, Poland, as part of a long-term scientific experiment, first began in 1987, using the randomized block design with four replication in proper dry meadow conditions on degraded black soil with a granulometric composition consistent with loamy soil. All blocks were irrigated until 2008. In 2009 each block was divided into two areas: irrigated and non-irrigated, resulting in plots of 27 m<sup>2</sup>. The sward on the non-irrigated plots received its water from the soil profile supplemented with atmospheric precipitation. On the irrigated plots water deficiencies were supplemented between May and September to maintain water content of 60–100% of field capacity (*FC*) in the upper soil layer. The water requirements of the soil were supplied in single doses of about 25 mm. The soil water content was monitored using readings from sensors (Em 50) at depths of 10–15, 25–30, and 40–45 cm. The study involved four levels of inorganic nitrogen fertilizer and two levels of mixed organic and inorganic fertilizer (Tab. 1). The phosphorus fertilization dose at N-180bis is zero because no phosphorus had been applied since 1997. The inorganic fertilizers (Tab. 1) applied were nitrogen as ammonium nitrate (34.5% N), phosphorus as triple superphosphate (20.1% P), and potassium as potassium oxide (47.3% K). The mixed organic and inorganic fertilizer was applied as fermented cattle urine, according with the potassium plant demands, while nitrogen and phosphorus levels

**Table 1.** Meadow fertilization scheme

Fertilization object		Fertilization dose (kg·ha <sup>-1</sup> )		
		N	P	K
Mineral	N-60	60	10.9	33.2
	N-120	120	21.8	66.4
	N-180	180	31.7	99.6
	N-180bis	180	0	99.6
	N-240	240	43.6	132.8
Organic-mineral	G1-180	180	31.7	99.6
	G2-240	240	43.6	132.8

Source: own elaboration.

were supplemented to standard dosages with superphosphate and ammonium nitrate. On G1-180 experimental plot the organic fertilizer dose applied was 17.1 m<sup>3</sup>·ha<sup>-1</sup>, this meaning 44.6 kg N·ha<sup>-1</sup>, 0.86 kg P·ha<sup>-1</sup> and 99.6 kg K·ha<sup>-1</sup>. Organic fertilizer dose on G2-240 plot was 22.8 m<sup>3</sup>·ha<sup>-1</sup>, representing 59.3 kg N, 1.14 kg P and 132.8 kg K. Before each application the liquid manure was analyzed regarding dry weight, nitrogen, phosphorus and potassium levels. Fertilization with a mixture of fermented urine, nitrogen and potassium was applied three times a year, once for every harvest. The phosphorus was applied only once, in the spring. In autumn 2011 the meadow was limed with calcium-magnesium carbonate at a dose of 4 Mg·ha<sup>-1</sup>. Calcium fertilizer dose was calculated on the basis of average soil pH (4.6 pH) and carbon content in the upper soil layer, which was 2.25–2.50%. Soil at depths of 0–10 and 10–20 cm was tested for pH in 1 M KCl (pH<sub>KCL</sub>), as well as for zinc content in autumn 2012 and 2014. The Zn content in the soil was determined by Atomic Absorption Spectrometer (ASA) (Thermo Scientific, USA), after mineralization in 10 cm<sup>3</sup> chloric (VII) acid 60%. The meadow was harvested three times a year and the dry matter (DM) of the fodder was determined. The sward samples from each harvest were tested for zinc content with an ASA (Thermo Scientific, USA), after mineralization in a mixture of acids (10 cm<sup>3</sup>): nitric (V), chloric (VII) and sulphuric (VI) acid (in ratio 8:2:1).

Data were processed using the analysis of variance and via Sidac's test of the IBM SPSS Statistics 24.0 using the dependent and the independent variables.

## RESULTS AND DISCUSSION

**Plant growth and yield.** Dry matter yields from irrigated and non-irrigated plots are presented in Table 2. The

**Table 2.** Dry matter yields (t·ha<sup>-1</sup>) from irrigated and non-irrigated objects

Year	Irrigation	Dry matter yield from object						
		N-60	N-120	N-180	N-180bis	N-240	G1-180	G2-240
2012	non-irrigated	2.96a	4.93b	6.42bcd	5.90bc	7.60d	5.22b	7.33cd
	irrigated	4.06a	6.74b	7.98bc	8.45cd	10.08d	7.83bc	9.39cd
2013	non-irrigated	6.15a	8.46abc	10.31bc	8.02ab	11.05c	9.46bc	10.64bc
	irrigated	8.81a	10.09ab	12.99c	12.20c	13.27c	11.78bc	12.89c
2014	non-irrigated	6.99a	9.95ab	11.53bc	11.00bc	13.50c	10.98bc	13.39c
	irrigated	8.06a	11.47b	13.47cd	12.40bc	14.97d	11.92bc	14.35d

Explanations: a, b, c, d = values in rows with different letters are significantly different ( $p \leq 0.05$ ).

Source: own study.

highest dry matter yields were obtained in 2014 year on the irrigated plots. The lowest yields were obtained in 2012 year on the non-irrigated plots. This was due to good conditions for the growth of *Dactylis glomerata* and *Arrhenatherum elatius*. Significant differences in crop yield were noticed in the most of plots in line with the different levels of fertilization. The lowest yields were obtained from plots with the lowest level of inorganic fertilization (N-60). Compared to this low level, a significant increase in crop yield was noticed in subsequent years in the most of the plots, irrigated and non-irrigated, with higher levels of organic and mixed organic and inorganic fertilizer. No significant difference in crop yield was observed in the plots deprived by phosphorus fertilizer (N-180bis) compared with the plots where this element was used alongside an identical dose of nitrogen in the mineral form (N-180) and mixed organic with inorganic fertilizer (G1-180). The greatest DM crops in 2012, 2013 and 2014 were obtained when were used the highest levels of inorganic fertilizer (N-240) and mixed organic with inorganic fertilizer (G2-240). Crops obtained from plots fertilized with ammonium nitrate and fermented urine indicate that these fertilizers improved yields similarly.

**Soil pH.** At the beginning of the study was noticed a variation in soil pH at depths of 0–10 cm that oscillate between 4.75 and 6.08. At 10–20 cm depths the pH ranged from 4.79 to 5.72. After the initial three years of the experiment was observed a clear upward trend in soil pH in all

fertilized plots, both at 0–10 cm level and at 10–20 cm level (Tab. 3), this being the result of the meadow liming in 2011. The lowest pH values were observed in plots fertilized with the highest levels of inorganic fertilizer (N-240), while the highest pH values occurred at the lowest levels (N-60). A similar soil pH was observed in irrigated plots compared to non-irrigated plots.

**Zinc in soil.** In all the experimental plots soil was characterized by low levels of total zinc, ranging from 10.0 to 16.7 mg Zn·(kg DM)<sup>-1</sup> at a depth of 0–10 cm and from 7.6 to 14.8 mg Zn·(kg DM)<sup>-1</sup> at a depth of 10–20 cm (Tab. 4). In the beginning of long-term experiments in 1987, average content of Zn in soil ranged between 31 and 52 mg·(kg DM)<sup>-1</sup>. The zinc levels observed were much lower than the average levels for loamy soil in Poland, which amounts to 52 mg Zn·(kg DM)<sup>-1</sup>. WIATER and ŁUKOWSKI [2014] demonstrated that the total average zinc in light soil was in a range from 19.4 to 52.5 g·kg<sup>-1</sup>. The lowest Zn content in the soil was determined in irrigated plots in 2014. The Zn content in 2014 was lower than in 2012. The content of Zn in all irrigated plots was lower in 2014, compared to non-irrigated plots. Also, the soil layer has influence on the content of Zn, respectively – in the layer 0–10 cm the content of Zn was higher in comparison with the layer 10–20 cm. Zinc is an element with high mobility in the soil environment. The total concentration and activity of zinc in the soil solution increased with increasing Zn content in the soil and rising soil acidity, while they

**Table 3.** Soil pH for particular fertilization objects

Irrigation	Soil layer (cm)	Year	pH for object						
			N-60	N-120	N-180	N-180bis	N-240	G1-180	G2-240
Non-irrigated	0–10	2012	5.82	5.51	5.44	5.19	4.75	5.53	5.42
		2013	6.00	5.81	5.67	5.11	4.61	6.26	5.93
		2014	5.90	5.86	5.58	5.54	4.72	6.01	6.02
	10–20	2012	5.72	5.60	5.34	5.10	4.79	5.44	5.18
		2013	5.97	5.78	5.67	5.43	4.84	6.06	5.73
		2014	6.38	6.37	6.30	6.29	5.86	6.45	6.37
Irrigated	0–10	2012	5.65	5.74	5.30	5.49	4.77	5.91	6.08
		2013	6.04	5.96	5.60	5.83	4.65	6.05	5.96
		2014	5.86	5.82	5.55	5.45	5.08	5.92	5.54
	10–20	2012	5.69	5.58	5.56	5.31	4.97	5.69	5.46
		2013	5.97	5.86	5.69	5.81	5.02	5.88	5.80
		2014	6.27	6.26	6.22	5.93	5.90	6.28	6.30

Explanations: objects as in Tab. 1.

Source: own study.

**Table 4.** Zinc content (mg·(kg DM)<sup>-1</sup>) in soil layers depending on the form and level of fertilization on irrigated and non-irrigated objects

Year	Irrigation	Soil layer (cm)	Zinc content in soil from object						
			N-60	N-120	N-180	N-180bis	N-240	G1-180	G2-240
2012	non-irrigated	0–10	15.0abA	13.9abA	15.6abA	12.9aA	13.6abA	16.7bA	16.2bA
		10–20	13.2abB	10.9aBC	12.6abAB	10.8aB	11.9abAB	14.1bA	13.4abAC
2012	irrigated	0–10	16.3bA	13.6abAC	15.5abA	13.8abA	11.9aAB	16.4bA	14.4abA
		10–20	14.5aB	12.1aACD	13.1aAB	11.5aA	12.6aAB	14.8aA	13.0aA
2014	non-irrigated	0–10	13.6aB	13.6aA	13.4aA	11.7aA	12.4aAB	14.1aA	13.9aA
		10–20	11.9aB	10.1aBD	11.1aBC	10.5aB	12.2aAB	10.8aB	10.9aBC
2014	irrigated	0–10	11.2abcB	11.4abcA	10.2abBC	10.0aBC	10.3abB	12.4cB	12.1bcAC
		10–20	9.9bC	9.0abBD	9.7bC	7.6aC	9.2abC	8.6abC	8.7abBD

Explanations: a, b, c, d = values in rows with different letters are significantly different ( $p \leq 0.05$ ), A, B, C, D = values in columns with different letters are significantly different ( $p \leq 0.05$ ).

Source: own study.

**Table 5.** Zinc content in the sward (average from 3 cuts) from 2012–2014 in mg·(kg DM)<sup>-1</sup>

Irrigation	Year	Zinc content in sward from objects						
		N-60	N-120	N-180	N-180bis	N-240	G1-180	G2-240
Non-irrigated	2012	23.7abA	23.0abA	22.7abA	22.9abA	21.4aA	24.8bA	23.8abA
	2013	24.2aA	23.9aA	22.6aA	24.4aA	23.4aA	24.4aA	22.2aA
	2014	17.5aB	16.9aB	16.2aBC	18.0aBC	17.6aBC	19.0aBC	18.2aBC
Irrigated	2012	23.2aA	22.3aA	22.9aA	26.0aA	23.3aA	23.7aA	23.8aA
	2013	22.7bcA	21.1abcA	20.4abB	23.9cB	18.7aB	21.0abcB	21.6abcB
	2014	15.6aB	17.0abB	15.9aC	17.4abC	15.7aC	17.0abC	17.7bC

Explanations: a, b, c = values in rows with different letters are significantly different ( $p \leq 0.05$ ), A, B, C = values in columns with different letters are significantly different ( $p \leq 0.05$ ).

Source: own study.

decreased with increasing the content of organic carbon and clay particles [RUTKOWSKA *et al.* 2015]. The total Zn content in the soil depended by liming and the absorption of Zn to the sward. Soil without plants had higher available Zn concentrations and lower pH than did the soil with plants. There were significant differences in Zn chemical fractions concentrations and proportions between the soils with and without plants at each growth stage [LIU *et al.* 2018]. At the start of the experiment the greatest zinc levels found at both tested layers were determined in the plot with the mixed organic and inorganic fertilizer (G1-180). Zinc contents in soil determined in 2012 in the plots that were not fertilized with phosphorus were lower compared to other plots at both of the tested soil depths. During the experimental period the zinc content of the soil declined both in irrigated and non-irrigated plots. After three years the lowest zinc levels were observed in an irrigated plot that was not fertilized with phosphorus. The highest zinc levels were observed in the non-irrigated and irrigated plots fertilized with mixed organic and inorganic fertilizer G1-180 and G2-240, which may be the result of zinc contained in fertilized urine – 220 mg·(kg DM)<sup>-1</sup>. The NPK plus farm yard manure treatment was found to be the most effective treatment in terms of content of Zn and Cu in soil [PRADHAN *et al.* 2015]. Much lower Zn content in both 0–10 and 10–20 cm layers of all irrigated plots was associated with increased yields on these plots. The present study showed that the increases in soil pH due to liming decreased the overall content of zinc in the soil. The availability of mobile zinc in soil increases in inverse ratio with soil pH [KABATA-PENDIAS, PENDIAS 1999].

**Zinc in meadow sward.** The zinc content in the meadow sward is presented in Table 5. In all plots the zinc content in the meadow sward in 2014 was significantly lower compared with the years 2013 and 2014. The lowest Zn content in sward was determined in all irrigated and non-irrigated plots in 2014 compared to 2012 year. This can be explained that the soil pH has increased after liming, which resulted in less absorption of Zn by the plants. The dry matter yields were higher and Zn content in soil was the smallest in 2014. The significantly higher Zn content in sward was found only in the 2012 year in the non-irrigated plots. All irrigated objects with fertilization above N-120 (Tab. 5) already in the second year after liming were significant lower zinc content in the meadow sward. Sward zinc content from the plot G1-180 was significantly higher than the content from the plot N-240. Average Zn content in the sward from the N-180bis plot (from three

cuts) was significantly higher than the N-180 and N-240 plots on the irrigated plots in 2013. In 2014 the average content of zinc in the sward was significantly higher in the plot G2-240 than the plots N-60 and N-180. Regarding the zinc content of the sward there was noticed a decline trend as the level of fertilization increased, which may have been caused by the greater crop yields obtained through the use of higher quantities of inorganic fertilizer, as well as the effect of dilution. There is a relationship between the use of zinc and phosphorus in the mineral nutrition of plants. As the level of phosphorus in soil increases the amount of zinc available to the plant decreases [ZHU *et al.* 2001], this being the reason why the high levels of phosphorus fertilizer can be a factor in inhibiting the absorption of zinc by plants [MARSCHNER 1998]. The regular application of P increased the P content of the upper soil layer, which caused a Zn deficiency [KREMPER *et al.* 2015]. P and Zn have an antagonistic relationship and inoculation could supply a sufficient quantity of P for plants, mainly in early crop growth stage [HUSSAIN *et al.* 2017]. This was confirmed by the greater quantity of zinc determined in the sward from plot N-180bis, which was deprived by phosphorous fertilizer, compared to an analogous plot where phosphorus was applied. The zinc content in the plots fertilized with fermented urine (G1-180 and G2-240) was greater than in analogous plots fertilized with the same amount of nitrogen in inorganic form but in most not significant. This could be the result of the increased supply of zinc provided from fermented urine, which is better absorbed by plants. A greater quantity of zinc was observed in the sward in most non-irrigated plots compared to irrigated plots. Inadequate levels of zinc were observed in the sward from all the plots. The U.S. National Research Council [NRC 2001] suggested that Zn requirements for dairy cattle are approximately 35 mg·kg<sup>-1</sup> feed DM. The feeding of ruminants on the grassland from this study could cause metabolic diseases. A study of macro- and micromineral contents in wrapped forages from farms in Sweden and Norway indicated a low Zn content – 23 mg·kg DM)<sup>-1</sup>, which was insufficient for horses [ZHAO, MULLER 2015].

## CONCLUSIONS

In conclusion, the application of fertilizer, both inorganic and fermented urine, determined a decrease of the zinc content in the soil to 10.0–16.7 mg Zn·(kg DM)<sup>-1</sup> at a depth of 0–10 cm and to 7.6–14.8 mg Zn·(kg DM)<sup>-1</sup> at

a depth of 10–20 cm, irrespective by their initial zinc levels and irrigation. Much lower Zn content in both soil layers of all irrigated plots was associated with increased yields on these plots, regardless of the level and form of fertilizer. The higher level of zinc in sward harvested was noticed in the plot not fertilized with phosphorus (17.4 and 18.0 mg·(kg DM)<sup>-1</sup>). The high level of phosphorus fertilizer can be a factor in inhibiting the absorption of zinc by plants. Long-term inorganic and fermented urine fertilization determinate very low zinc content in the soil and meadow sward. Farmers should use mixed inorganic and organic fertilizer in the form of fermented cattle urine because the yield and zinc content in the meadow sward were higher.

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