The effect of simultaneous magnesium application on the biological effects of titanium

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ABSTRACT

Most of the works published since the beginning of the 20th century have brought interesting results about beneficial effects of titanium (Ti) on plants, but much less is known about its phytotoxic effects at higher doses. Here we demonstrate the influence of Mg treatment on the phytotoxic effects of Ti. Mg, Ti + Mg and two different concentrations of Ti leaf sprays were applied on oats (*Avena sativa* L. cv. Zlaťák) grown on three different soil types (Fluvisol, Luvisol and Chernozem). Physiological parameters of oats as well as some essential elements contents were analyzed. The foliar applications of Ti caused significant toxic manifestations on oats at ≥ 10 mg/kg concentrations. Mg partially ammeliorates these toxic effects if applied together with Ti. The effect was strong on Fluvisol, but weaker on Chernozem and Luvisol. Ti effects are more significant the further the soil is from the nutritional optimum of the plants. This is most evident in the case of Fluvisol, which is deficient in Mg, Fe, Mn, and Zn. It seems that Ti possesses a generally equalizing effect on the elements content in the plant.

Keywords: titanium; magnesium; iron; plants; *Avena sativa* L.; nutrition; hormesis; toxicity

Empirical observations considering the effects of titanium (Ti) compounds on plants have been reported since the beginning of the 20th century (see Kužel et al. 2003b for review). Most of the works published during that period have brought interesting results about beneficial effects of Ti, but much less is known about its phytotoxic effects at higher doses. Therefore, this poorly explored area of plant physiology remains the challenge for a more thoroughgoing research.

Nearly all experiments studying phytotoxicity of Ti were carried out in hydroponic solutions. Early references were published by Pais et al. (1969a, b), investigating the effects of various trace elements on the development of sweet pepper (*Capsicum annuum* L.) and tomatoes (*Lycopersicon lycopersicum* L.) in hydroponic cultures; they discovered that although Ti stimulates the plants at concentrations lower than 1 mg/kg, it is phytotoxic at higher doses. Hara et al. (1976) studied the influence of titanium on cabbage plants (*Brassica capitata* L.) in hydroponic culture and they observed a decrease of the plants' yield already at 0.4 mg/kg Ti. Wallace et al. (1977) found a significant yield decrease of bush beans (*Phaseolus vulgaris* L.) at 10^{-4} mol/dm^3 Ti (~ 4.8 mg/kg Ti) applied as $TiCl_3$ into nutrient solution. A similar toxicity limit for Ti(IV) ascorbate was documented by Maroti et al. (1984) in tobacco (*Nicotiana tabacum* L.) callus culture: 5 mg/kg of Ti inhibited the growth, 10 mg/kg led to necrosis. Some phytotoxic impact on oats (*Avena sativa* L.) was observed for the same compound at 18 mg/kg of Ti in nutrient solution (Hrubý et al. 2002).

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Table 1. Beneficial and phytotoxic concentrations of Ti sprays for different plants

	Kužel et al. (2003a)	Huang et al. (1993)
Ti chemical form	citrate	ascorbate or citrate
Beneficial concentration	10 mg/kg	2–50 mg/kg
Phytotoxic concentration	50 mg/kg	> 100 mg/kg
Plant	oats	wheat

The hydroponic methods stated above enable an exact definition of experimental conditions; however, the obtained data are hardly comparable with experiments where Ti is applied in form of foliar spray, as widely used in agriculture. Generally, the effect of Ti is considerably weaker if it is applied on leaves than if added into the nutrient solution (Kužel et al. 2003a). Surprisingly, experiments that examine toxic levels of Ti in form of foliar sprays are practically missing. The only available data are from the studies performed by Huang et al. (1993) and Kužel et al. (2003a) and are stated in Table 1. Phytotoxic effects of Ti leaf sprays were compared to their beneficial effects at lower concentrations.

In our recent work (Kužel et al. 2003a) we observed an increased beneficial stimulation by Ti in plants oversupplied by Mg. We demonstrate here that this phenomenon might be generally useful not only at the beneficial levels of Ti, but generally for an amelioration of its phytotoxicity.

MATERIAL AND METHODS

Plants culture

Oat plants (*Avena sativa* L. cv. Zlaťák) were grown in pots (20 plants per pot) filled with 5 kg of three different soil types (Luvisol, Chernozem and Fluvisol; see Table 2 for soil chemical composition before fertilization). Four replications of each experimental treatment were made. All treatments were fertilized by NPK in following doses: 0.2 g N (NH_4NO_3), 0.03 g P and 0.08 g K per kg of soil using KH_2PO_4 as fertilizer.

The following leaf sprays (30 ml per pot) were used: (i) deionized H_2O; (ii) Mg sulphate solu-

Table 2. Chemical analysis of soils; all localities are situated in the Czech Republic

	Locality/Soil type					
	Přerov/Fluvisol		Suchdol/Chernozem		Červený Újezd/Luvisol	
	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*
C_{ox} (%)	0.68	0.085	1.39	0.032	1.71	0.099
pH_{KCl}	5.7	0.07	7.2	0.07	6.8	0.00
Ca	1212	29	8793	15	3328	32
K	217	8	174	8	316	5
Mg	60.5	3.4	161	0	164	3
P	353	37	155	5	188	22
Cr	14.4	0.0	65.4	0.2	68.7	2.0
Cu	9.91	0.80	27.9	1.7	31.7	0.6
Fe	6970	1780	22110	2115	27960	505
Mn	249	57	605	32	831	11
Zn	51.5	5.4	112	5	85.5	4.8

Element contents are stated in mg/kg except for C_{ox} (stated in mass %); $\bar{x}$ – average value of analyzed parameter, *s* – standard deviation

tion (2000 mg/kg Mg); (iii) Ti-citrate solution (10 mg/kg Ti) [Recently it was shown by Collins et al. (2005) that the reaction of $TiCl_4$ with at least three equivalents of citric acid (in pH range 3–8) produces 3:1 citrate/titanium complexes with successive deprotonation of carboxylates as the pH increases. In this range and under these conditions, hydroxo- or oxo-metal species are believed not to be present in solution; thus 3:1 citrate/titanium complex could be expected as the active form in this experiment.]; (iv) Ti-citrate solution (50 mg/kg Ti); (v) Mg sulphate + Ti-citrate mixed solution (2000 mg/kg Mg, 10 mg/kg Ti). The sprays were adjusted to the pH = 5. Citric acid control was not included because the typical citrate content in plant tissues is about several orders of magnitude higher than the citrate content in spray solution. Thus, we supposed that such low concentrations of citrate could not significantly influence the metabolism of the plants (Tlustoš et al. 2005).

Oat was sown on April 25, the first foliar treatment was applied on June 7, and the second on June 21. Oat was harvested in milky ripe stage two weeks after the second foliar application.

Soil and plant analysis

Samples of three different soil types were taken from Přerov (Fluvisol), Suchdol (Chernozem) and Červený Újezd (Luvisol), all in the Czech Republic. The amounts of available nutrients (P, K, Mg, Ca) were determined in air-dried soil samples using Mehlich III extraction solution (Mehlich 1984) and AAS spectrometer (Varian SpectrAA 300) in the case of K, Mg, and Ca, and spectrophotometry (Specol 210) in the case of P. The pH-value was determined in 0.2 mol/dm^3 KCl suspension (1:2.5 w/v) by glass ISE (Zbíral 2001). C_{ox} was determined spectrophotometrically by oxidation of the organic matter by $K_2Cr_2O_7$ in acidic environment (Sims and Haby 1971). The total element contents in soils (Cr, Cu, Fe, Mn, Zn) were determined in digests obtained by a two-step decomposition by dry ashing in a mixture of oxidizing gases and the ash was decomposed in a mixture of HNO_3 + HF, evaporated and dissolved in diluted *aqua regia* as described by Száková et al. 2000 (see Table 2 for data).

Following parameters of the plants were determined in plants washed with distilled water:

- Aboveground fresh weight (AFW), in grams per pot.
- Aboveground dry weight (ADW); dried in oven (10 hours at 105°C), in grams per pot.
- The content of chlorophylls (CH) in flag leaf, by spectrophotometry (Carl Zeiss, Spekol 220 spectrophotometer) after extraction (according to Porra et al. 1989) of homogenized raw material with ethanol (with the addition of $CaCO_3$), in mg/g of raw leaf biomass.
- The Ti (TiC), Mg (MgC), Fe (FeC), Zn (ZnC) and Mn (MnC) contents in top dry mass, in mg/kg.

The contents of the elements in the plant material after washing at *aqua regia* were determined in digest using dry ashing procedure (Mader et al. 1998). Flame and flameless atomic absorption spectrometry (Varian SpectrAA 300) were used for the determination of these elements in digests. The quality of analyses was controlled by reference materials.

Statistical analysis of data

Data were filtered *via* Q-test at level $\alpha = 0.05$, maximum 1 value per variant was excluded if distant. One-way ANOVA was used to test differences among the variants and it was computed in Microcal ORIGIN program, version 5.0.

RESULTS

The results are summarized in Table 3; trends are marked T (Trend number). The strength of the influence (how much the factor influences the parameter) is stated in text as the ratio of maximal and minimal value in the trend. Unless stated otherwise, the effects of Ti alone and Mg alone are stated against blank (deionized water treatment) and the effect of Ti in combination with Mg is stated against the Mg leaf spray alone to distinguish the effects of Ti from the effects of Mg in the combined treatments. Only data necessary for the demonstration of the trends are given.

Fluvisol. In both concentrations used Ti alone causes a considerable decrease of both AFW (1.38-times, T1) and ADW (1.39-times, T2), but it increases both AFW (1.25-times, T1) and ADW (1.17-times, T2) if in combination with Mg; Mg alone decreases AFW (1.22-times, T1) and ADW (1.21-times, T2). Ti alone at the lower concentration increases CH (1.12-times, T3) and decreases it at the higher concentration (1.20-times, T3); Mg alone strongly increases CH (1.92-times, T3). Ti alone does not significantly influence FeC, but strongly increases it in combination with Mg

Table 3. The effect of single and combined Ti and Mg foliar application on changes of investigated parameters (see Material and Methods for abbreviations and measurement units of the followed parameters)

Soil type	Trend	Parameter	A	*SD*(A)	B	*SD*(B)	C	*SD*(C)	D	*SD*(D)	E	*SD*(E)	ANOVA		
													F	*p*	α
Fluvisol	T1	AFW	151.6	11.8	145.3	7.2	109.9	2.3	124.1	0.8	155.3	22.8	7.64	2.14E-03	0.01
	T2	ADW	48.2	3.6	44.3	2.0	34.8	0.5	39.9	2.7	46.6	5.9	7.92	1.49E-03	0.01
	T3	CH	0.123	0.007	0.138	0.016	0.102	0.012	0.235	0.047	0.207	0.010	23.29	2.74E-06	0.01
	T4	FeC	62	15	58	8	79	18	69	4	152	19	30.59	4.70E-07	0.01
	T5	MgC	1222	102	1412	104	1300	100	1400	125	1556	83	5.95	4.50E-03	0.01
	T6	MnC	208	30	186	53	35	2	28	1	81	67	15.44	4.93E-05	0.01
	T7	TiC	2.25	0.58	2.89	0.81	2.60	0.60	2.48	0.18	4.62	2.12	2.91	6.07E-02	–
	T8	ZnC	74.0	1.7	63.8	15.9	74.4	45.4	54.3	4.0	63.5	24.9	0.34	8.47E-01	–
Luvisol	T9	AFW	96.1	6.1	95.3	4.1	101.1	6.8	127.5	7.6	125.6	8.5	22.45	3.45E-06	0.01
	T10	ADW	30.8	1.4	30.8	0.2	31.3	1.3	41.0	0.1	40.9	1.6	73.71	8.42E-09	0.01
	T11	CH	0.096	0.008	0.104	0.009	0.118	0.014	0.232	0.033	0.236	0.038	33.86	2.40E-07	0.01
	T12	FeC	100	18	73	9	105	24	100	31	106	30	1.27	3.27E-01	–
	T13	MgC	1357	94	1326	90	1422	81	1587	59	1495	67	7.17	1.96E-03	0.01
	T14	MnC	39.7	1.4	39.4	8.3	40.3	1.4	44.0	7.0	44.8	1.5	0.91	4.87E-01	–
	T15	TiC	3.22	1.13	2.69	0.78	2.64	0.09	2.35	0.75	3.05	0.66	0.77	5.63E-01	–
	T16	ZnC	54	13	57	20	46	16	42	10	72	47	0.89	4.96E-01	–
Chernozem	T17	AFW	141.4	12.9	136.1	13.3	136.2	1.3	158.6	5.4	153.4	3.8	4.83	1.17E-02	0.05
	T18	ADW	46.0	5.1	44.2	3.6	44.7	1.0	49.7	0.9	49.5	1.9	3.09	4.84E-02	0.05
	T19	CH	0.118	0.011	0.115	0.009	0.121	0.005	0.233	0.015	0.212	0.013	108.05	7.21E-11	0.01
	T20	FeC	112	26	108	39	88	24	86	17	178	26	7.52	1.57E-03	0.01
	T21	MgC	1365	48	1414	39	1467	21	1644	102	1597	114	10.31	3.21E-04	0.01
	T22	MnC	29.5	2.3	25.4	0.8	23.1	1.7	30.4	3.0	29.5	1.2	10.67	2.68E-04	0.01
	T23	TiC	2.25	0.54	2.69	0.69	2.84	0.81	2.93	0.90	4.38	1.04	3.90	2.29E-02	0.05
	T24	ZnC	71.0	9.7	64.3	19.3	68.9	23.1	40.6	10.3	47.9	9.9	3.04	5.06E-02	–

Ti and Mg concentrations: A – no Ti, no Mg; B – medium Ti concentration (10 mg/kg), no Mg; C – high Ti concentration (50 mg/kg), no Mg; D – no Ti, Mg concentration 2000 mg/kg; E – medium Ti concentration (10 mg/kg), Mg concentration 2000 mg/kg; *SD* – standard deviations respective to the stated values

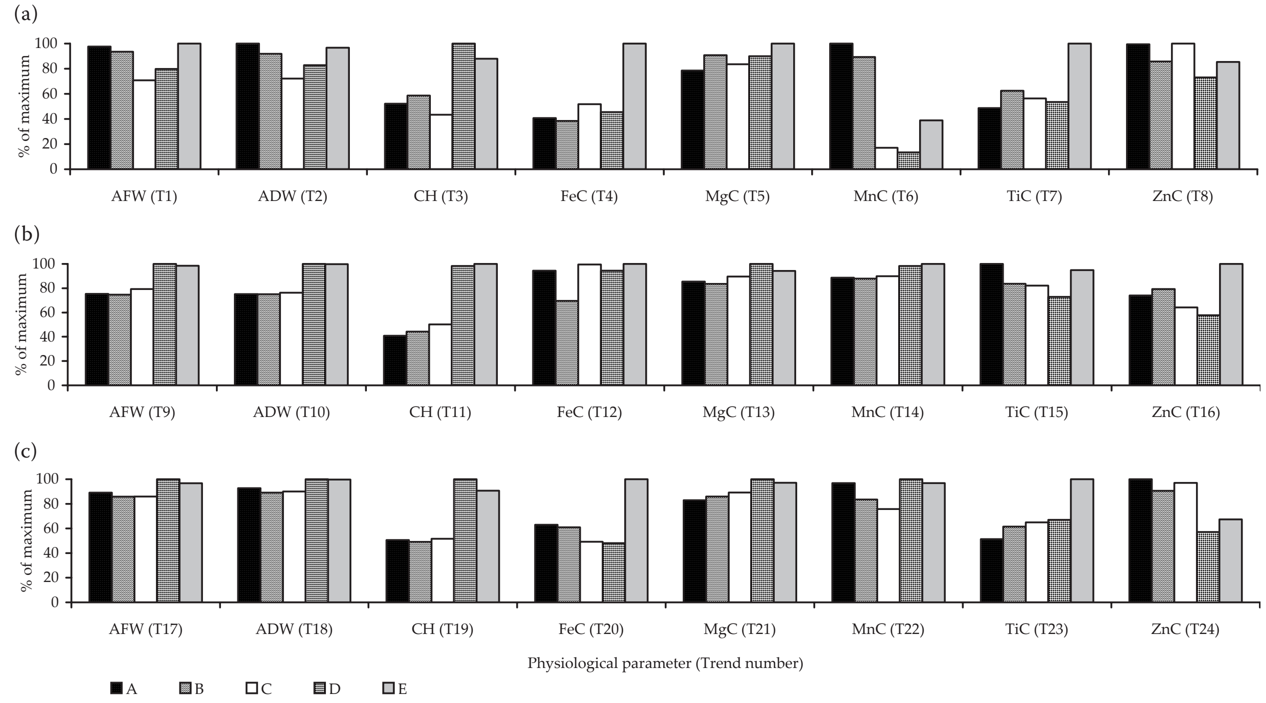

Figure 1. The effects of the treatments on investigated parameters divided according to soils: (a) Fluvisol; (b) Luvisol; (c) Chernozem; the values are stated in percents of the maximal value in the particular trend

Ti and Mg concentrations: A– no Ti, no Mg; B– medium Ti concentration (10 mg/kg), no Mg; C– high Ti concentration (50 mg/kg), no Mg; D – no Ti, Mg concentration 2000 mg/kg; E– medium Ti concentration (10 mg/kg), Mg concentration 2000 mg/kg

(2.20-times, T4); Mg alone slightly increases FeC (1.12-times, T4). Ti alone (1.16-times, T5) as well as in combination with Mg (1.11-times, T5) increases MgC; MgC is increased by the foliar absorption by Mg alone as well (1.15-times, T5). MnC is strongly (5.88-times, T6) decreased by Ti alone at the higher Ti concentration, but is significantly increased if in combination with Mg even at the medium Ti concentration (2.89-times); Mg alone extremely decreases MnC (7.44-times, T6). Mg increases the resorption of Ti from leaves (1.60-times, T7). ZnC is not significantly influenced by Ti alone, but it is increased by Ti in combination with Mg (1.17-times, T8); Mg alone decreases ZnC (1.36-times, T8). The results are summarized in Figure 1a.

Luvisol. Ti alone does not significantly influence AFW (T9) and ADW (T10), but Mg alone has a significant positive effect on both these parameters [1.33-times increase of AFW (T9) and 1.33-times increase of ADW (T10)]. CH is increased by Ti alone (1.23-times, T11), but the effect of Mg is much stronger (2.41-times increase, T11); the combination Mg + Ti is not significantly different from Mg alone in CH (T11). The effect of Ti on FeC (T12) and MgC (T13) is not distinguished even in combination with Mg, but Mg alone increases MgC (1.17-times, T13). MnC (T14) is not significantly influenced by any treatment used. Mg increases the resorption of Ti from leaves if sprayed in combination with Ti (1.30-times, T15), but Mg alone decreases the uptake of Ti from soil. ZnC is not influenced by Ti alone, but is significantly increased by the combination Ti + Mg against Mg alone (1.73-times, T16); Mg alone decreases ZnC (1.28-times, T16). The results are summarized in Figure 1b.

Chernozem. Ti does not have a significant effect on AFW (T17) and ADW (T18), but both these parameters are increased by Mg alone [AFW – 1.12-times (T17) and ADW – 1.08-times (T18)]. CH is not significantly influenced by Ti alone, but is decreased by the combination of Ti + Mg against Mg alone (1.10-times, T19); Mg alone significantly increases CH (1.98-times, T19). FeC is decreased by Ti alone (1.28-times, T20), but Ti + Mg strongly increases FeC (2.08-times, T20) against Mg alone; Mg alone decreases FeC (1.31-times, T20). MgC is increased by Ti application (1.08-times, T21), but the effect of the application of Mg alone is much stronger (1.21-times, T21). MnC is considerably lowered by Ti alone (1.28-times, T22), but the effect practically disappears in the case of simultaneous application of Mg (T22); Mg alone does not show a significant effect on MnC (T22). Mg alone considerably stimulates the uptake of Ti from soil (1.62-times, T23). ZnC is not almost influenced by Ti alone (T24); Mg alone lowered ZnC (1.75-times, T24). The results are summarized in Figure 1c.

DISCUSSION

The results from our experiment are strongly dependent on soil properties, but we can generalize some trends. Both the concentrations of Ti in leaf sprays used in this experiment were already phytotoxic. The typical beneficial manifestations of Ti were not observable except for some effects on Fluvisol, see below. The effects connected with the detoxification of Ti (changes in element and chlorophyll contents) were the only significant. The toxicity limit of Ti could be quite sharp, as demonstrated by Maroti et al. (1984); they observed strong beneficial effects of Ti on tobacco callus at the concentration 2 mg/kg Ti in nutrient medium, but the inhibition of growth followed by necrosis was observed at concentrations higher than 5 mg/kg. We used another way of application of Ti compounds on plants in this experiment – a leaf spray. As the concentration 10 mg/kg Ti showed already some manifestations of Ti toxicity, it is the lowest phytotoxic concentration of Ti leaf spray described up today (compare with Table 1).

If we consider that Ti(IV) is present in plants in the form of phosphate complexes (Gryzhankova and Boichenko 1975, Gryzhankova et al. 1975), we could explain this primary toxicity on molecular basis as a destabilization of biologically active phosphate esters/normally stabilized by Mg(II) ions in the cell/by Ti(IV). The importance of high affinity of Ti(IV) to phosphate groups for its biological activity was referred to by Guo et al. (2001). We can thus expect protective effects of higher Mg concentrations against phytotoxicity of Ti on the basis of competitive antagonism. This effect was really observed – we found that the additional Mg ameliorated the toxic effects of Ti.

Similarly to Kužel et al. (2003a), the effect of Ti (in both concentrations used), as compared to the control with no application, is radically different from the effect of (Ti + Mg), especially in the essential elements contents in plant (as FeC); redistribution of mineral nutrients during seed-filling stage within shoot was not considered in this study because whole shoots were analyzed. Another effect is a significant increase of CH by a single Mg application (more than twice on average), while MgC is increased considerably less. This would suggest that although most of the Mg present in leaf is metabolically inactive (in phytate depo etc.), the additional Mg from the leaf spray is biologically much more available. This effect cannot be explained by non-uniform senescence of the variants because there were not observed any signs of senescence in any variant in time of harvest.

However, the trends greatly differ in the strength of the particular effect if we look at the effect of soil the plants grow in. It was observed that the effects of treatments are relatively strong on Fluvisol, but weak on Chernozem and Luvisol. It thus generally confirms that Ti effects are most evident under conditions of limited and insufficient supply of the essential elements (see data in Table 2 for Fluvisol that is deficient in Mg, Fe, Mn and Zn). Ti is beneficial in this case to the elements contents and some physiological parameters. The fact that Ti is more beneficial on a poor soil is already known (Lopez-Moreno et al. 1996); these authors assume that the crucial effect is the lack of phosphorus, but we have observed a rather reversed effect. The effect is the most evident in our experiment on Fluvisol, which is rich in phosphate (353 mg P per kg, see Table 2) that decreases the bioavailability of the cationic nutrients complexed by it for the plant. The soil pH value also seems to play some role, due to its influence on the mobility of the ionic nutrients in soil (Ti is the most efficient on more acidic Fluvisol).

The effect observed in this paper should be considered more as phytotoxicity than stimulation. However, we could explain the general phenomenon of Ti-plant interaction as follows: we suppose that Ti competitively replaces some essential elements from their natural binding sites and causes apparent essential elements (especially Fe and Mg) deficiency in the plant. This results in a complicated defense reaction that may increase the health status of the plant more than the toxic effects of Ti decrease it [increased uptake of essential elements *via* the divalent ion transporter (known to be activated under the Fe starvation), release of complexing organic acids and subsequent increase of mobility and availability of other elements, activation of some enzymes, etc.]. Whether the effect of Ti on the particular parameter is "positive" or "negative" is dose-responsive and depends on the strength of the plant defense reaction versus Ti toxic effect on the particular parameter (see Hrubý et al. 2002 and Kužel et al. 2003a for details). This effect is usually called *hormesis* and is known for a range of elements, mainly heavy metals (e.g. As, Cd, Pb, Hg, Se and Zn) (Calabrese and Baldwin 2003).

The above stated complex defensive reaction results in equalizing effect of Ti on the elements contents in the plant. This means that if the blank level of the element in the plant is rather low, it is increased by Ti and *vice versa* to a certain, probably optimal, concentration. This could be advantageous for the amelioration of the toxic effects of heavy metals by Ti. Protective effects of Ti on plants intoxicated by heavy metals (cadmium) were described in (Lesko et al. 2002) and we observed here similar results for manganese that is phytotoxic in higher concentrations; Ti application caused a significant decrease in MnC.

Besides, statistically significant correlations were observed between TiC and the other parameters followed in this study: AFW (0.37), ADW (0.33), FeC (0.64), MgC (0.45); the number in parentheses is the particular correlation coefficient between the stated parameter and TiC for all the variants and replicates. The correlation was statistically significant at level $\alpha = 0.01$ in all the cases stated above. The correlation of TiC with the other investigated parameters is not statistically significant at level $\alpha = 0.05$.

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