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EFFECT OF ZINC AND IRON SUPPLY AND INOCULATION WITH *AZOSPIRILLUM* ON GROWTH OF MAIZE AND WHEAT SEEDLINGS

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Abstract: The aim of this study was to determine the effect of different zinc and iron concentrations in culture medium on growth and development of maize and wheat seedlings in terms of their inoculation with bacteria of *Azospirillum* genus. Maize and wheat *in vitro* cultures were inoculated, respectively, by strains of *Azospirillum lipoferum* and *Azospirillum brasilense* strains. The experimental factor was the supplementation of the culture medium with zinc (25, 200 and 600 $mg \cdot kg^{-1}$ of the medium) and iron (25, 200 and 600 $mg \cdot kg^{-1}$ of the medium). Counts of bacteria from the *Azospirillum* genus were analysed and plant seedling growth and development as well as the content of chlorophyll in plant leaf blades were monitored.

Zinc turned out to reduce strongly numbers of bacteria of the *Azospirillum* genus. *Azospirillum brasilense* turned out to be particularly sensitive to elevated levels of this chemical element in the environment. The negative influence of increased quantities of zinc on cereal seedlings became apparent only after the application of the highest concentrations of this metal in the medium (600 $mg \cdot kg^{-1}$), while quantities which did not exceed 200 $mg \cdot kg^{-1}$ exerted a stimulation effect on the mass of maize and wheat seedlings.

Iron added to the culture medium in quantities which did not exceed 200 $mg \cdot kg^{-1}$ did not reduce numbers of bacteria of the *Azospirillum* genus; on the contrary, they stimulated their growth. However, at higher concentrations, this metal turned out to exert a strong negative impact on the chlorophyll content in leaf blades as well as on the mass of maize and wheat seedlings.

The inoculation with bacteria of the *Azospirillum* genus exerted a positive influence on the mass increase of maize and wheat seedlings and increased chlorophyll concentrations in leaf blades. At the same time, it contributed significantly to limiting or even levelling out the toxic impact of zinc and iron during the initial phases of plant growth and development.

INTRODUCTION

Some chemical elements, even at very low concentrations in soil environment, strongly influence the course of biological processes preconditioning, among others, growth and development of plants and, at the same time, modifying significantly soil microbiological life. There is no doubt that some metals, e.g. zinc and iron included in the group of heavy metals, belong in this group. These elements, on the one hand, are necessary for the life of organisms but, on the other, when they occur in excessive quantities, they may be strongly toxic. This statement is particularly true with regard to zinc, although excess of iron can also indirectly affect organisms, for example, by intake modification of other constituents.

Zinc acts as an activator and is a constituent of various oxidoreductive enzymes and facilitates the synthesis of proteins, carbohydrates and vitamins. When zinc occurs in excessive amounts, it blocks enzyme functional groups and causes displacement from cells of other metals indispensible for proper metabolism [19]. Also iron belongs to those chemical elements essential for appropriate functioning of all living organisms. Like many other elements, it regulates enzymatic activities of microorganisms, including nitrogenase complex which participates in atmospheric nitrogen fixation [10]. Excessive accumulation of heavy metals in farmland can result, among others, in the reduction of both quantities and diversity of microorganisms and, in this way, contributes to decreased soil fertility [2].

Uptake and accumulation of trace elements by cells of microorganisms may lead to their death when they do not excrete special metal-binding metabolites [14]. However, they have at their disposal a number of adaptive mechanisms associated with their physiological properties which allow their survival and adaptation to the environment with elevated concentrations of heavy metals [5]. Badura [1] estimated approximate sensitivity of living organisms to metal ions and concluded that plants exhibited the strongest response to zinc, animals – moderate and bacteria – the smallest.

Toxic effect of chemical elements depends on a variety of different factors including: type of soil, its physical and chemical properties, form of the element; it also depends on the species of plants and interactions taking place between them and soil microorganisms [1, 11, 15, 21]. That is why it is important to recognize the impact of both shortage as well as excess of chemical elements on plants and soil microflora and to elaborate effective methods of their possible removal from the soil environment, including bioremediation.

One of the groups of endophytic microorganisms exhibiting a number of positive impacts on plants which remain within the sphere of interest of both agricultural science and practice include bacteria of the *Azospirillum* genus [3, 16]. Determination of the response of bacteria of the *Azospirillum* genus to elevated concentrations of metals in soil environments is one of the steps on the road to a comprehensive characterization of the ecological amplitude of these microorganisms and to the recognition of their impact on plants in diverse, at times extreme, conditions.

The objective of the performed investigations was to gain a better understanding of the functioning of bacteria of the *Azospirillum* genus and the association system they develop with plants, in the presence of various zinc and iron concentrations, in the medium with growing maize and wheat seedlings inoculated with these bacteria.

MATERIAL AND METHODS

Two *in vitro* laboratory experiments were carried out on maize (*Zea mays*) plants of Claudia cultivar and on wheat (*Triticum vulgare*) plants of Kris cultivar. Experimental plants were cultivated in controlled conditions at the temperature of about 22^{o}C during the day and 18^{o}C at night in test tubes on slants with SHM (Schenk & Hildebrandt Medium) mineral-vitamin medium commonly used for the cultivation of monocotyledonous plants.

Maize plants were inoculated with a strain of *Azospirillum lipoferum* Br 17 obtained from the Division of Microbiology of IUNG in Puławy (experiment I), whereas wheat plants – with *Azospirillum brasilense* SP T60 isolated from wheat roots in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH w Braunschweig, Germany (experiment II). Different concentrations of zinc and iron in the medium were treated as experimental factors with three experimental levels of each metal. In the case of zinc, these levels amounted to: 25, 200 and 600 mg·kg^{-1} of the medium (in the form of $ZnSO_4 \cdot 7\ H_2O$) and for iron – 50, 200 and 800 mg·kg^{-1} of the medium (in the form of $FeSO_4 \cdot 7H_2O$). The control comprised plants of maize and wheat growing on the medium without additional quantities of metals. Each combination comprised 20 test tubes with seedlings.

Seeds of maize and wheat used in the experiment were subjected to sterilization; first, they were washed for 5 minutes with 70% alcohol solution, next – for 2 minutes in 5% hydrogen peroxide solution and, finally, with sterile water (5 times, for two minutes each). The same method of seed sterilization was applied, among others, by Chebotar *et al.* [4]. Seeds prepared in the way described above were pregerminated and placed in test tubes on slants. After 24 hours, they were inoculated with the appropriate strain of *Azospirillum* bacteria earlier incubated for 24 h at the temperature of 28°C in a liquid DAS medium [6]. The number of *Azospirillum* cells in 1 ml suspension ranged from 10^8 to 10^9 cfu. The suspension was introduced into each combination in the amount of 0.1 ml.

The effectiveness of the association system 'plants – *Azospirillum*' functioning in conditions of elevated zinc and iron concentrations in the substrate was estimated in the course of three weeks after plant inoculation (after 7, 14 and 21 days). The assessment was carried out on the basis of numbers of bacteria in plant roots – method of poured plates using DAS medium [6], concentrations of chlorophyll dyes in leaf blades of experimental seedlings (on the basis of N-tester indications, i.e. on the basis of the so called "leaf greenness index" expressed by the difference between the absorption of 650 nm and 940 nm light penetrating through the leaf blade [7], seedling mass (shoot with roots following their removal from the test tube, washing off the medium and filtering off water).

Research results were subjected to statistical analysis with the assistance of the STATPAK software. The significance of differences between the examined objects in the analysis of variance was assessed by the F-Snedecor test calculating the least significance differences. The obtained results were elaborated using the t-Student test assessing their significance at the levels of $\alpha = 0.05$ and $\alpha = 0.01$.

RESULTS AND DISCUSSION

The increased metal concentrations in the experimental medium exerted a significant influence on numbers of both species of bacteria of the *Azospirillum* genus.

The impact of zinc and iron on numbers of *Azospirillum lipoferum* used to inoculate maize seedlings and *Azospirillum brasilense* employed to treat wheat seedlings is presented in Table 1. Zinc added to the applied medium turned out to be a very toxic element for both species of bacteria of the *Azospirillum* genus. The observed negative effect of this chemical element on the numbers of bacterial populations of *Azospirillum lipoferum* and *Azospirillum brasilense* was found to increase together with the increase of its concentration in the medium. *Azospirillum brasilense* bacteria living in wheat roots were found to be particularly sensitive to elevated zinc concentrations in the growth medium. Zinc exerted a significantly negative influence on the number of these bacteria even at the lowest rate i.e., 25 mg·kg^{-1}. This led to reduction of their populations by about 70% as compared to the control treatments. Higher concentrations of this element (200 and 600 mg·kg^{-1} of the medium) inhibited very strongly growth and development of these bacteria (Tab. 1). The above dependence was further corroborated by a high, negative correlation between the amount of zinc in the medium and numbers of bacteria of the *Azospirillum* genus.

A slightly different response of bacteria of the *Azospirillum* genus was recorded when iron concentrations in the medium were elevated (Tab. 1). The two lower concentrations of this element in the medium (50 and 200 mg·kg^{-1}) stimulated numbers of *Azospirillum*. This was particularly noticeable in the case of *Azospirillum lipoferum* where, in the combination with the lowest dose of iron (50 mg·kg^{-1} of the medium), a significant, over twofold increase in numbers of these bacteria was registered. It was only in the combination with the highest of the applied iron concentrations in the medium (800 mg·kg^{-1}) that the number of *Azospirillum* declined considerably and was several times lower than in the control.

Table 1. Number of bacteria of the *Azospirillum* genus in 1 g of roots of maize and wheat seedlings

Treatments	Number of bacteria (n·10^{6}cfu)	
	Azospirillum lipoferum	***Azospirillum brasilense***
Control	**311.0**	**222.1**
Zn25	187.7	69.2
Zn200	135.3	39.7
Zn600	39.4	13.1
Mean value Zn	**186.4**	**85.8**
r- correlation coefficient	**-0.89**	**-0.68**
$LSD_{\alpha = 0.01}$	**80.10**	**50.44**
Control	**311.0**	**222.1**
Fe50	638.4	310.1
Fe200	301.7	247.4
Fe800	61.1	43.7
Mean value Fe	**328.1**	**205.6**
r- correlation coefficient	**-0.78**	**-0.92**
$LSD_{\alpha = 0.01}$	**127.74**	**59.58**

It is clear, then, that zinc turned out to be a considerably more toxic element than iron as confirmed also by other investigations. Investigations carried out by Januszek [8] indicate that zinc exerts a more toxic effect on soil microorganisms and enzymes than other trace elements. *In vitro* experiments carried out by Zborowska *et al.* [20] the aim of which was to determine the effect of zinc on the development of microorganisms in artificial media revealed that the response of bacteria to growing concentrations of zinc in the medium measured by the degree of cell proliferation varied and depended on the extent of contamination of the medium by this metal. It exerted a toxic effect even at its lowest dose (5 mg Zn^{+2}×dm^{-3}) and its inhibitory impact on microorganisms increased together with the growing contamination of the medium with this element. Furthermore, it was also proved that zinc was more toxic for bacteria than fungi and actinomycetes. Experiments conducted by Wyszkowska and Kucharski [17, 18] showed that zinc disturbed a biological balance. This chemical element exerted a negative effect on numbers of oligotrophic, copiotrophic and cellulolytic bacteria of the *Azospirillum* genus. On the other hand, cellulolytic bacteria were found to develop better in environments rich in zinc. Also actinomycetes and fungi developed well in such conditions with the latter exhibiting 2.5 times increase of their populations in the presence of zinc. This phenomenon can be attributed to differences in enzymatic systems specific for individual species of soil microbes [13].

Iron which, at lower concentrations, stimulated numbers of bacteria of the *Azospirillum* genus was, by no means, neutral for them at its higher concentrations as could have been surmised on the basis of the scarce literature available on this subject. It is worth emphasizing that the sensitivity of microorganisms to the presence of metals in the environment still requires investigations. Under laboratory conditions, the concentration of a specific ion can lead to their death, whereas in the soil environment, this effect is not fully explicit; the presence of metals can even stimulate their growth [1].

The impact of the examined metals on plants was more varied and ambiguous than on bacteria. Tables 2 and 3 present the response of maize and wheat seedlings to elevated concentrations of zinc and iron on the basis of the concentration of chlorophyll dyes in leaf blades. It turned out that zinc exerted a significantly negative effect on the concentration of chlorophyll dyes in maize leaf blades already at its lowest concentration (25 $mg \cdot kg^{-1}$ of the medium) decreasing their content by about 18% (Tab. 2) and the picture of this negative impact of zinc became more apparent together with the increase of zinc concentrations to 72.3%. On the other hand, in the case of wheat (Tab. 3), the lowest zinc concentrations failed to exert a negative influence of chlorophyll concentrations, or even increased them slightly. A clearly negative impact of zinc concentrations was observed only at higher levels of this element.

Table 2. Concentration of chlorophyll dyes (SPAD) in maize leaf blades

Treatments	SPAD Value		
	Non - inoculated	Inoculated	Mean value
Control	260.5	461.7	361.1
Zn25	211.5	385.2	298.4
Zn200	217.6	221.6	219.6
Zn600	98.5	102.9	100.7
Mean value	**197.03**	**292.9**	**244.9**
r- correlation coefficient	**-0.94**	**-0.93**	**-0.96**
$LSD_{\alpha = 0.01}$ (inoculation x Zn)	**107.92**		
$LSD_{\alpha = 0.01}$ (Zn)	**76.31**		
$LSD_{\alpha = 0.01}$ (inoculation)	**53.96**		
Control	**260.5**	**461.7**	**361.1**
Fe50	253.0	526.6	389.8
Fe200	122.1	249.5	185.8
Fe800	34.1	96.4	65.3
Mean value	**167.42**	**333.55**	**250.48**
r- correlation coefficient	**-0.92**	**-0.90**	**-0.91**
$LSD_{\alpha = 0.01}$ (inoculation x Fe)	**122.55**		
$LSD_{\alpha = 0.01}$ (Fe)	**86.65**		
$LSD_{\alpha = 0.01}$ (inoculation)	**61.27**		

The observed stimulatory effect of zinc on chlorophyll dye concentrations in wheat can be attributed to the properties of these dyes which involve possibilities of an easy exchange of Mg^{+2} ions into ions of other divalent metals. The result of such reactions is color change and the introduction of Zn^{+2} ions into a chlorophyll molecule caused increased stability of the natural green color [12].

When analyzing the effect of iron on experimental plants, it should be stated that the lowest concentration of this metal in the medium (50 mg·kg^{-1}) exerted a stimulating impact on the content of chlorophyll dyes in leaf blades, both of maize and wheat seedlings. The two consecutive levels of iron concentrations reduced the concentration of plant chlorophyll dyes. What was interesting was that, in combinations with the highest of the applied iron concentrations (800 mg·kg^{-1} of the medium), the content of chlorophyll dyes in leaf blades of both plant species was considerably, even several times, lower than in the control. It was exceptionally surprising that chlorophyll content was even lower than at the highest zinc concentration (Tabs. 2 and 3).

Table 3. Concentration of chlorophyll dyes (SPAD) in wheat leaf blades

Treatments	SPAD Value		
	Non-inoculated	**Inoculated**	**Mean value**
Control	**153.0**	**433.0**	**293.0**
Zn25	272.8	398.3	**335.0**
Zn200	226.3	329.8	**278.0**
Zn600	39.5	125.5	**82.5**
Mean value	**172.9**	**324.1**	**231.8**
r- correlation coefficient	**-0.79**	**-0.99**	**-0.97**
$LSD_{\alpha = 0.01}$ (inoculation x Zn)	**131.67**		
$LSD_{\alpha = 0.01}$ (Zn)	**55.22**		
$LSD_{\alpha = 0.01}$ (inoculation)	**87.61**		
Control	**153.0**	**433.0**	**293.0**
Fe50	169.2	529.4	**349.3**
Fe200	100.6	316.0	**208.3**
Fe800	14.1	20.3	**17.3**
Mean value	**109.3**	**324.7**	**217.0**
r- correlation coefficient	**-0.97**	**-0.96**	**-0.96**
$LSD_{\alpha = 0.01}$ (inoculation x Fe)	**115.43**		
$LSD_{\alpha = 0.01}$ (Fe)	**79.12**		
$LSD_{\alpha = 0.01}$ (inoculation)	**64.17**		

Another parameter analyzed in the course of the discussed investigations was the mass of maize and wheat seedlings (the overground part and roots). When analyzing the impact of the applied metals on the weight of the overground parts (shoots) of seedlings, it can be noticed that the two lower concentrations of both elements did not affect plants negatively, on the contrary they even stimulated their growth and it was only the highest doses of zinc and iron which clearly checked the growth of shoots in comparison with the control (Tabs. 4 and 5).

Table 4. Shoot mass (g) of maize seedlings

Treatments	**Mass (g)**		
	Non - inoculated	**Inoculated**	**Mean value**
Control	**0.90**	**1.13**	**1.02**
Zn25	0.57	1.00	**1.01**
Zn200	0.93	1.17	**0.78**
Zn600	0.30	0.73	**1.05**
Mean value	**0.68**	**1.01**	**0.52**
r- correlation coefficient	**-0.70**	**-0.81**	**-0.75**
$LSD_{\alpha = 0.01}$ (inoculation x Zn)	ns		
$LSD_{\alpha = 0.01}$ (Zn)	0.082		
$LSD_{\alpha = 0.01}$ (inoculation)	0.073		
Control	**0.90**	**1.13**	**1.02**
Fe50	0.80	1.10	**0.95**
Fe200	0.60	0.80	**0.70**
Fe800	0.10	0.13	**0.12**
Mean value	**0.60**	**0.79**	**0.70**
r- correlation coefficient	**-0.99**	**-0.99**	**-0.99**
$LSD_{\alpha = 0.01}$ (inoculation x Fe)	ns		
$LSD_{\alpha = 0.01}$ (Fe)	0.065		
$LSD_{\alpha = 0.01}$ (inoculation)	0.098		

ns – not significant

Table 5. Shoot mass (g) of wheat seedlings

Treatments	**Mass (g)**		
	Non - inoculated	**Inoculated**	**Mean value**
Control	**0.23**	**0.37**	**0.30**
Zn25	0.37	0.56	**0.47**
Zn200	0.29	0.32	**0.30**
Zn600	0.24	0.24	**0.24**
Mean value	**0.28**	**0.37**	**0.33**
r- correlation coefficient	**-0.37**	**-0.74**	**-0.65**
$LSD_{\alpha = 0.01}$ (inoculation x Zn)	**ns**		
$LSD_{\alpha = 0.01}$ (Zn)	**0.112**		
$LSD_{\alpha = 0.01}$ (inoculation)	**0.080**		
Control	**0.23**	**0.37**	**0.30**
Fe50	0.27	0.52	**0.39**
Fe200	0.21	0.26	**0.24**
Fe800	0.09	0.12	**0.11**
Mean value	**0.20**	**0.32**	**0.25**
r- correlation coefficient	**-0.95**	**-0.85**	**-0.89**
$LSD_{\alpha = 0.01}$ (inoculation x Fe)	**ns**		
$LSD_{\alpha = 0.01}$ (Fe)	**0.089**		
$LSD_{\alpha = 0.01}$ (inoculation	**0.063**		

ns – not significant

A similar picture of the effect of the applied metals on seedling development emerged from the analyses of maize (Tab. 6) and wheat (Tab. 7) roots.

Table 6. Root mass (g) of maize seedlings

Treatments	Mass (g)		
	Non - inoculated	**Inoculated**	**Mean value**
Control	**0.47**	**0.73**	**0.60**
Zn25	0.33	0.37	**0.35**
Zn200	0.70	1.10	**0.90**
Zn600	0.13	0.97	**0.50**
Mean value	**0.41**	**0.79**	**0.59**
r- correlation coefficient	**-0.54**	**0.58**	**0.02**
$LSD_{\alpha = 0.01}$ (inoculation x Zn)	**ns**		
$LSD_{\alpha = 0.01}$ (Zn)	**0.327**		
$LSD_{\alpha = 0.01}$ (inoculation)	**0.231**		
Control	**0.47**	**0.73**	**0.60**
Fe50	0.33	0.37	**0.35**
Fe200	0.63	1.13	**0.88**
Fe800	0.13	0.08	**0.11**
Mean value	**0.39**	**0.58**	**0.49**
r- correlation coefficient	**-0.69**	**-0.59**	**-0.62**
$LSD_{\alpha = 0.01}$ (inoculation Fe)	**ns**		
$LSD_{\alpha = 0.01}$ (Fe)	**0.416**		
$LSD_{\alpha = 0.01}$ (inoculation)	**ns**		

ns – not significant

Table 7. Root mass (g) of wheat seedlings

Treatments	Mass (g)		
	Non - inoculated	**Inoculated**	**Mean value**
Control	**0.08**	**0.49**	**0.29**
Zn25	0.21	0.32	**0.26**
Zn200	0.14	0.21	**0.17**
Zn600	0.29	0.09	**0.19**
Mean value	**0.18**	**0.28**	**0.23**
r- correlation coefficient	**0.78**	**-0.88**	**-0.73**
$LSD_{\alpha = 0.01}$ (inoculation x Zn)	**0.292**		
$LSD_{\alpha = 0.01}$ (Zn)	**ns**		
$LSD_{\alpha = 0.01}$ (inoculation)	**ns**		
Control	**0.08**	**0.49**	**0.29**
Fe50	0.11	0.41	**0.26**
Fe200	0.03	0.21	**0.12**
Fe800	0.02	0.03	**0.23**
Mean value	**0.06**	**0.28**	**0.22**
r- correlation coefficient	**-0.75**	**-0.93**	**-0.18**
$LSD_{\alpha = 0.01}$ (inoculation x Fe)	**ns**		
$LSD_{\alpha = 0.01}$ (Fe)	**0.189**		
$LSD_{\alpha = 0.01}$ (inoculation	**0.134**		

ns – not significant

Within the framework of the analyses and observations performed, an attempt was also made to assess nitrogenase activity in individual experimental combinations. Since no activity was recorded, this thread of studies has been confined to this information, although it was not altogether abandoned. Despite the lack of nitrogenase activity, a distinct influence of the inoculation with the bacteria of the *Azospirillum* genus was noticeable in all experimental combinations on both increased concentrations of chlorophyll dyes (Tabs. 2 and 3) and on higher mass of maize and wheat seedlings (Tabs. 4–7). The only exceptions included: a/ the highest zinc concentration leveled out the effect of inoculation with regard to the mass of wheat seedlings and b/ the highest iron concentration – with regard to the root mass of maize seedlings. Therefore, it can be said that inoculation of maize and wheat seedlings with bacteria of the *Azospirillum* genus limited strongly or even leveled out the toxic impact of zinc and iron during the initial phases of plant growth and development and the performed statistical analysis corroborated the significance of this influence.

Although it is known that the excess of iron inactivates manganese or causes a change in leaf color, this metal is considered to be the least toxic against all living organisms from among all heavy metals [12]. However, the research results obtained in the described trial indicate a comparable and, at times, stronger (in comparison with zinc) negative impact of iron on the examined plants, especially at the highest of the applied concentrations of these chemical elements in the medium. It is surprising in view of the fact that this metal cannot be found in Tables among chemical elements harmful or toxic for plants [9] and its concentrations in Polish soils ranges from of 30–645 mg Fe kg^{-1} soil, i.e. similar to the concentrations used in these experiments. In natural conditions, 5–10% of total iron would be directly available, i.e. would enter into direct interaction with *Azospirillum* as well as maize and wheat seedlings. However, according to the main concept of the investigations performed, the authors introduced Fe into soil in a form directly available both for *Azospirillum* as well as for maize and wheat seedlings with the aim to trace the direct interaction in the result of which, iron would modify nitrogenase activity, content of chlorophyll dyes, seedling mass, etc.

Therefore, it can be said that the research results presented in this article supplement our knowledge regarding the impact of Fe on *Azospirillum* and maize and wheat seedlings. In addition, they also indicate that it is necessary to be cautious in the assessment of the harmful impact of this element on the selected flora or fauna on the basis of its total content in soil.

CONCLUSIONS

Zinc turned out to reduce strongly bacteria of the *Azospirillum* genus. *Azospirillum brasilense* turned out to be particularly sensitive to elevated quantities of this chemical element in the environment.

Negative impact of increased zinc quantities on cereal seedlings became apparent only at the highest concentrations of this metal in the medium (600 $mg \cdot kg^{-1}$). Quantities which did not exceed 200 $mg \cdot kg^{-1}$ were found to stimulate the mass of maize and wheat seedlings.

Iron added to the experimental medium in quantities not exceeding 200 $mg \cdot kg^{-1}$ did not reduce the number of bacteria from the *Azospirillum* genus, on the contrary, they even increased their populations. The highest of the applied concentrations of this element in the medium (800 mg·kg^{-1}) reduced numbers of *Azospirillum* bacteria very strongly.

Higher concentrations of iron in the medium exerted a strong negative impact on chlorophyll content in leaf blades as well as on the mass of maize and wheat seedlings. In some cases, this influence was even stronger than that of zinc.

The inoculation with bacteria of the *Azospirillum* genus affected positively concentrations of chlorophyll dyes and the biomass of maize and wheat seedlings. At the same time, it strongly limited the toxic impact of zinc and iron during the initial phases of plant.

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WPŁYW DODATKU CYNKU I ŻELAZA ORAZ INOKULACJI *AZOSPIRILLUM* NA WZROST SIEWEK KUKURYDZY I PSZENICY

Celem niniejszej pracy jest określenie wpływu różnych stężeń cynku i żelaza w podłożu na wzrost i rozwój siewek kukurydzy i pszenicy w warunkach ich inokulacji bakteriami z rodzaju *Azospirillum*. Hodowle *in vitro* kukurydzy i pszenicy, inokulowano, odpowiednio szczepami bakterii *Azospirillum lipoferum* i *Azospirillum brasilense*. Czynnikiem doświadczalnym było wzbogacenie podłoża cynkiem (25, 200 i 600 $mg \cdot kg^{-1}$ podłoża) i żelazem (50, 200 i 800 $mg \cdot kg^{-1}$ podłoża). Analizowano liczebność bakterii z rodzaju *Azospirillum* oraz monitorowano wzrost i rozwój siewek roślin, a także zawartość chlorofilu w ich blaszkach liściowych.

Cynk okazał się pierwiastkiem silnie ograniczającym liczebność bakterii z rodzaju *Azospirillum*. Szczególnie wrażliwe na zwiększoną ilość tego pierwiastka w środowisku okazało się *Azospirillum brasilense*. Negatywne oddziaływanie zwiększonych ilości cynku na siewki zbóż było zauważalne dopiero przy najwyższym z zastosowanych stężeń tego metalu w podłożu (600 $mg \cdot kg^{-1}$). Ilości nieprzekraczające 200 $mg \cdot kg^{-1}$ oddziaływały stymulująco na masę siewek kukurydzy i pszenicy.

Żelazo dodane do podłoża w ilościach nieprzekraczających 200 $mg \cdot kg^{-1}$ nie ograniczało liczebności bakterii z rodzaju *Azospirillum*, a nawet wpływało na jej wzrost. Przy wyższych stężeniach okazało się jednak pierwiastkiem, który miał silny, negatywny wpływ na zawartość chlorofilu w blaszkach liściowych oraz na masę siewek kukurydzy i pszenicy. Oddziaływanie to było w niektórych przypadkach silniejsze niż cynku. Inokulacja bakteriami z rodzaju *Azospirillum* wpłynęła pozytywnie na zwiększenie masy siewek kukurydzy i pszenicy oraz na wzrost koncentracji barwników chlorofilowych w ich blaszkach liściowych. Zabieg ten przyczynił się także do ograniczenia, a nawet zniwelowania toksycznego oddziaływania cynku i żelaza w początkowych fazach wzrostu i rozwoju roślin.