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Maize (*Zea mays*) reaction in response to rubber rag additive into the soil

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Abstract

The amount of used and worn out tires around the world reaches about 10 million tons. Rubber introduction to the environment in this shape is connected with its pollution, caused by tires' chemical composition, which, apart from synthetic and natural rubber, contain soot as a filler, plasticizers having some amounts of polycyclic aromatic hydrocarbons and helping substances: activators, accelerator of vulcanization, cross-link means and homogenizers. The most important activator is zinc oxide. The aim of the conducted studies was to determine the influence of rubber rag added to the subsoil on the amount of yield, physiological state and the amount of taken zinc by maize (*Zea mays*). It was found that the rubber rag introduced into the soil caused an increase in the yield of maize and the plants growing on the soil with the highest amount of rubber rag showing higher efficiency photosynthetic apparatus than others. The zinc content in the aboveground parts of maize increased with the increase of rubber rag in the soil.

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1. INTRODUCTION

The mass share of rubber elements in a passenger car, although it constitutes only 7% of the total mass of the car, fulfils a very important function. It seals the body (windows, doors), absorbs energy (shock absorbers, suspension elements) and ensures the flow of liquids and gases (hoses and pipes) [Lipski, Zaborowski, 2013]. It is estimated that every year around the world, about 1000 million used tires are produced as waste [Taheri et al., 2013], while in Poland alone, between 120,000 and 155,000 tonnes of tires are withdrawn from service every year [Lipski, Zaborowski, 2013].

Rubber from used tyres is associated with material that is useless and onerous for the environment, the most popular method of recovery, until recently, was storage (now prohibited by law). The dissemination and adoption of new environmental standards, formulated by European and national legislation, as well as those resulting from the increased environmental awareness, necessitates the search for effective methods of disposal of post-consumer tyres [Ołdakowska, 2015]. An example of the use of end-of-life tyres is their use as a substitute for natural aggregates in the production of normal concrete.

Crushed rubber, due to its good insulation, acoustic and thermal properties, as well as water resistance, is used

in the construction of roads, bridges, tunnels and can also be used for the production of playground surfaces, playgrounds and insulation materials. It was to be used as an additive to rubber compounds, from which new products are formed, for example, car mats, wipers, mats and so on, and can also be used as an additive to asphalt [Merkisz-Guranowska, 2005; Yung et al., 2013; Stevenson et al., 2008].

Due to the very large amount of rubber waste generated, it is very important to search for other, innovative and, at the same time, safe technologies for recycling these materials [Hrdlicka et al., 2010]. One such proposed method is the use of used rubbers as a harmless and effective source of Zn or other agricultural nutrients [Taheri et al., 2013; Khoshgoftarmanesh et al., 2012]. The tires contain 1–2% zinc, but their addition also provides sulphur and calcium to the soil, without the risk of cadmium contamination [Fahad et al., 2015]. As stated in the paper [Navizaga et al., 2017], the deficiency of micronutrients such as Zn causes a decrease in maize yields. Zinc has great influence on the basic life processes, such as nitrogen metabolism, photosynthesis or resistance to stress, both abiotic and biotic.

Zinc, due to its many physiological functions in plants, is considered an essential nutrient for them [Alloway, 2004].

However, since it is quite common in the environment and is also a component of many compounds emitted to the environment and waste substances used in agriculture, it can accumulate in the soil. The natural zinc content of the soil is determined mainly by the nature of the parent rock and the granulometric composition of the soil. According to the data given in the study [Kaniuczak, Pruszyński, 2015], the soil application rate for zinc for maize should be 4–10 kg Zn·ha⁻¹ of soil. In acidic soil, the zinc in the rubber abrasive becomes available to plants. An additional source of zinc in the soil may cause toxicity symptoms in plants, which means wilting, discoloration of the leaves and high concentration of this metal in the leaf tissue. Although zinc is important for plants, most of the soils may have its appropriate amount thanks to fertilization, and therefore, rubber abrasive used as a substrate supplement may affect plant growth [Taheri et al., 2013; Khoshgoftarmanesh et al., 2012; Fahad et al., 2015].

With the increasing use of information on the addition of materials from worn out tyres as well as from shredded tyres to the soil, difficult and extensive questions need to be asked: what is the impact of tyre material on the environment? Can arable crops grow and develop on soils contaminated with this material and would energy recovery be possible from plants used for these soils' recultivation? The research described by Taheri et al. [2013] show that both finely divided tires and ashes obtained after their combustion can be an effective source of zinc in maize cultivation. It is indicated in the results of laboratory researches, which were obtained after planting maize, which had been in plant pots, in the soil from wheat field, located in the Isfahan Province in the central Iran, enriched with waste from used tires. Additionally the zinc assimilation with the growth of maize and sunflower was increasing the use of selected bacteria strains, accelerating the degradation of tires in limestone soil with the addition of rubber waste [Khoshgoftarmanesh et al., 2012]. Also, the research was carried out in pot experiments.

The results of the work described by Fahad et al. [2015] also showed the effectiveness of using rubber waste in the form of ash as a source of Zn, increasing the efficiency of maize, while reducing the accumulation of Cd.

The aim of the research described in this paper was to determine the reaction of maize plants (yield, zinc phyto-availability and efficiency of the photosynthetic apparatus) growing on soil with the addition of rubber rag.

2. EXPERIMENTAL

2.1. Plant material and growth conditions

The vegetation experiment was carried out in pots with a capacity of 3 dm³. The research was conducted in April and May 2018. The experimental scheme was as follows: 0 - control and three more (I, II, III) with an increasing amount of rubber rubbing in the soil (Table 1). The rubber rag used in the experiment was characterized by a high content of zinc and iron (Table 2). It should be noted that

Table 1. The experimental scheme

Object	Rubber rag content in soil [g×kg ⁻¹ DM* soil]
0 - control	0
I	10
II	50
III	100

* DM - dry matter

Table 2. Heavy metals' content in rubber rag [μg×g⁻¹]

Ni	Cd	Fe	Cu	Mn	Zn	Pb
1.02	0.91	607.50	57.42	12.50	7386.51	12.50

the experiment was of a model character, carried out in laboratory conditions (in phytotron chambers) and not in the field. This was the reason why the selected doses of rubber abrasive were so different (from 10 to 100 g×kg⁻¹ DM soil). The high amounts of waste in object III of the experiment resulted from the hypothesis to introduce a potentially high amount of plant stress factor into the soil and be able to clearly observe the reaction of the plants.

Soil of the pH_{KCl} = 7.03 and granulometric composition of silty soil (clay content 8%), was used as a subsoil for experiment. The soils contained an average of 7.1 g×kg⁻¹ DM of organic carbon and 1.12 g×kg⁻¹ DM of nitrogen. The total zinc content of the soil was 92.64 μg·g⁻¹. The experiment was carried out in four replicates with three plants per pot. Uniform basic nutrition was applied for all objects: 0.3 g N, 0.3 g K, 0.075 g P and 0.05 Mg per 1 kg subsoil by water solutions of the following salts into the subsoil: NH₄NO₃, KCl, KH₂PO₄ and MgSO₄·7H₂O. The test plant was maize (*Zea mays*). Plants were grown for 40 days maintaining the temperature at the level of 23/15°C, at photoperiod 16/8 (day/night). During the vegetation period, the plants were watered with distilled water, maintaining soil moisture at the beginning of the experiment at 40% and then 50% of the capillary water capacity. The plants were harvested in phase 7–9 leaves (after 40 days of the vegetation) separating the aboveground parts and roots. The yield of the aboveground parts and roots was determined after drying them at 70°C.

2.2. Chlorophyll fluorescence

During plant vegetation period, disturbances of photosynthesis process were monitored by measurements of chlorophyll fluorescence parameters using fluorometer IMAGING-PAM in maxi version (Walz). Following parameters were determined: F₀ – zero fluorescence of

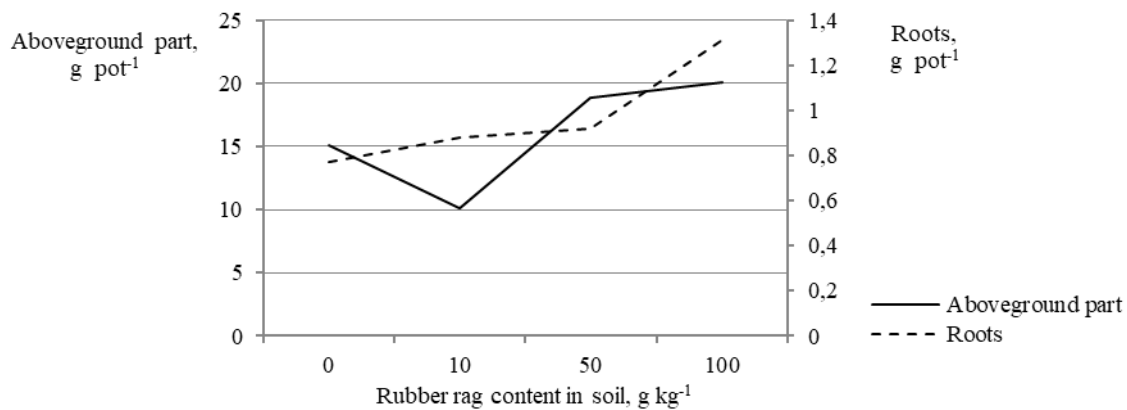


Figure 1. The yield of roots and aboveground parts of maize [g×pot⁻¹] grown on soil without (0) and with application of rubber rag (10, 50, 100 g×kg⁻¹ DM soil)

objects adapted to darkness; F_M – maximum fluorescence; F_V – variable fluorescence $F_V = F_M - F_0$; F_V/F_0 – maximum efficiency of water splitting at the donor side of PSII and F_V/F_M – maximum photochemical efficiency of PSII. All measurements were made in four replicates, while a single replicate consisted of 10 measurements.

2.3. Chemical analysis

After the completion of the pot experiment, the plant material was dried at 75°C and grinded. Zinc content in the aboveground parts of maize was determined by atomic absorption spectrometry AAS method (using Hitachi Z-2000 - Japan) after digestion in a microwave system in concentrated HNO_3 with addition 30% H_2O_2 in Berghoff apparatus.

2.4. Statistical analysis

The experiment was established by means of a randomized Split-plot method in 4 replications. The results were statistically processed using two-factor variance analysis (ANOVA) and the Tukey's and the Dunn's test using Statistica 10 software (StatSoft Inc.).

3. RESULTS AND DISCUSSION

3.1. Plant yield, zinc content

The obtained results of maize yield are shown in Fig. 1. The highest average yield of dry matter of aboveground parts (20.10 g·pot⁻¹) and roots (1.31 g·pot⁻¹) was obtained for plants grown on the substrate containing the highest amount of rubber rubbish (100 g·kg⁻¹ soil). Increased maize yield may be a positive effect of zinc contained in rubber abrasion. Zinc content in the dry matter of roots and aboveground parts of maize was differentiated according to the dose of rubber rag in the soil (Fig. 2). The plants growing in the soil with the highest rubber rag content, accumulated the highest amount of zinc both in the roots

and in the aboveground parts. It should be noted that, in the case of plants growing on soil with the highest amount of rubber rag (100 g×kg⁻¹ DM soil), the zinc content exceeded the limit value set for plants used for agriculture, according to Kabata-Pendias et al. [1993], 100 mg Zn·kg⁻¹ DM.

3.2. Chlorophyll content

Indicators of the chlorophyll content of maize leaves are shown in Figure 3. The highest content of chlorophyll (32.4 SPAD) is characteristic for plants grown on a control medium – without the addition of rubber rag to the soil. The lowest amount of chlorophyll (26.3 SPAD) was observed in plants grown on the substrate with the highest content of rubber rag. The chlorophyll content decreases as the amount of rubber rag in the soil increases.

3.3. Chlorophyll fluorescence parameters

Figures 4 and 5 show the values of fluorescence parameters of chlorophyll maize grown on subsoil with different content of rubber rag. The highest value of F_0 was observed in plants grown on a subsoil with the addition of 10 g of rubber rag per 1 kg of soil, while the lowest value of F_0 was observed in plants growing on a subsoil with the addition of 100 g of rubber rag per 1 kg of soil. The Dunn test confirmed the statistical significance of these differences ($p = 0.02$). Other values of chlorophyll fluorescence parameters in maize leaves also show variability due to the presence of increasing amounts of rubber rubbing in the soil.

The ANOVA procedure for the value of SPAD in maize leaves showed significant differences at a significance level of $p < 0.05$. The Tukey test confirmed that the plants cultivated on the substrate with the highest content of rubber rubbing (100 g·kg⁻¹ soil) have lower amount of chlorophyll than those cultivated on the control substrate ($p = 0.00$) and on the substrate with the addition of rubber rag in the amount of 10 g·kg⁻¹ soil ($p = 0.02$).

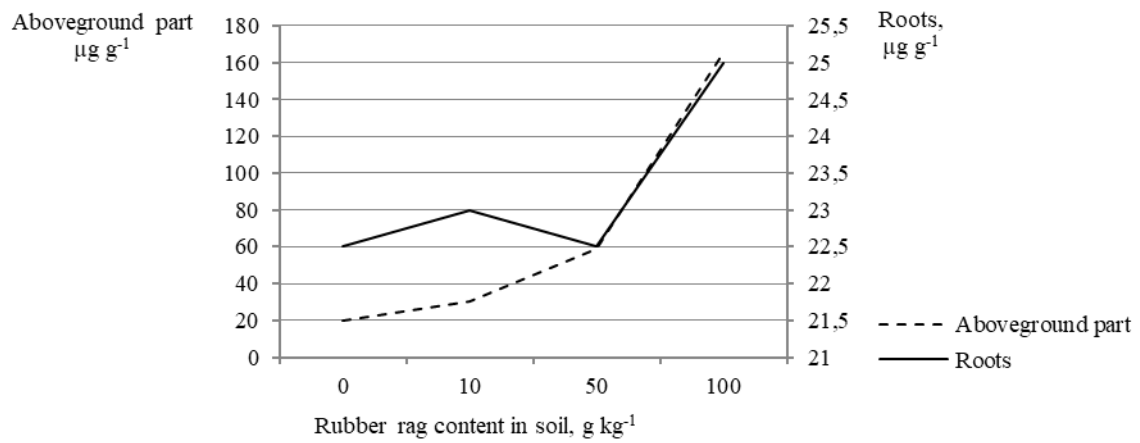


Figure 2. Zinc content [$\mu\text{g}\times\text{g}^{-1}$ DM] in roots and aboveground parts of maize grown on soil without (0) and with application of rubber rag (10, 50, 100 $\text{g}\times\text{kg}^{-1}$ DM soil)

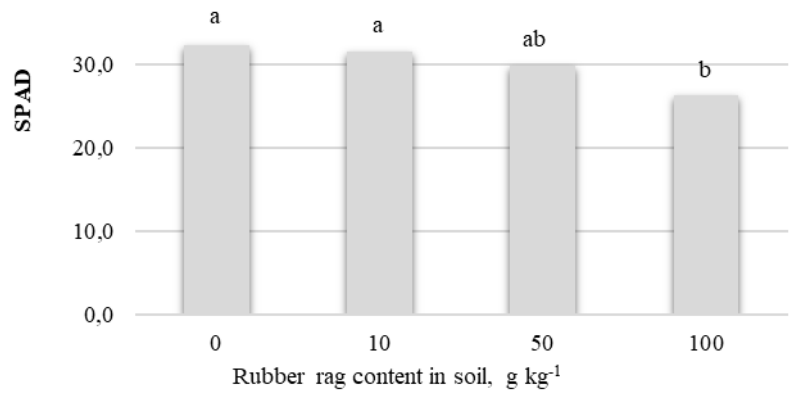


Figure 3. SPAD values in maize leaves grown on soil without (0) and with application of rubber rag (10, 50, 100 $\text{g}\times\text{kg}^{-1}$ DM soil); a, b – homogeneous groups (ANOVA and Tuckey's test $p < 0.05$)

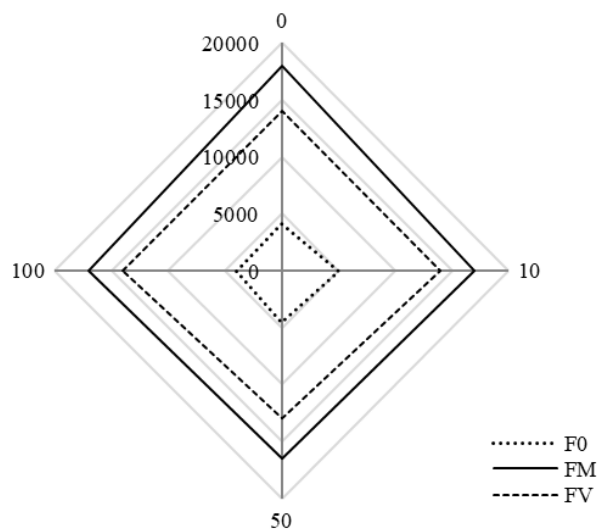


Figure 4. Radar charts of physiological features of maize leaves F_0 , F_M , F_V grown on soil without (0) and with application of rubber rag (10, 50, 100 $\text{g}\times\text{kg}^{-1}$ DM soil)

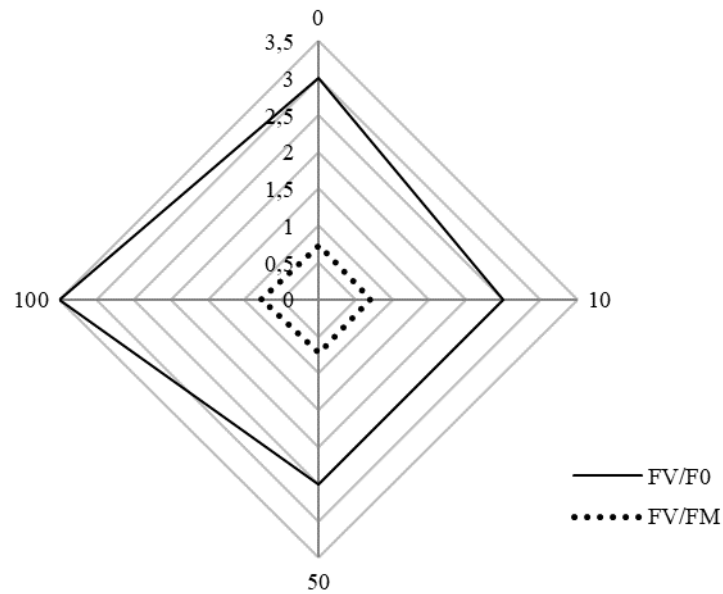


Figure 5. Radar charts of physiological features of maize leaves F_v/F_0 , F_v/F_M , grown on soil without (0) and with application of rubber rag (10, 50, 100 $\text{g}\cdot\text{kg}^{-1}$ DM)

The reduction of chlorophyll in maize leaves is likely to be influenced by heavy metals contained in the waste added to the soil (Table 2.). For example, studies conducted by Lagriffoul's et al. [1998] have shown that a dose of $1.7 \mu\text{M}$ Cd, reduces the chlorophyll content of maize. Jinhua et al. [2009] confirmed that increasing amounts of chromium in the substrate lower the content of chlorophyll in maize leaves in comparison to control plants.

The detection and analysis of chlorophyll fluorescence parameters can serve as a precise tool for testing the photosynthesis reaction under stress conditions, for example, the F_0 index (zero fluorescence) provides information on excitation energy losses during transmission from power antennas to the PSII reaction centre. Under the influence of stress factors, that is, high temperature [Murkowski 2002], salt stress or heavy metals, the value of the zero fluorescence parameter F_0 usually increases. In the experiment described in this paper, a decrease of F_0 parameter was observed in plants growing on the soil with the addition of the largest amounts of rubber rag. This suggests that the presence of high amounts of rubber rag in the soil did not cause plant stress.

The studies described in the paper by Kalaji, Rutkowska [2004] have shown that the values of the individual fluorescence parameters are correlated and a change in one will affect the other. As a result of the lost excitation energy in the photosynthetic dye molecules, the F_M (maximum fluorescence) and F_v (variable fluorescence) values decrease within the energetic antennas, so the F_0 parameter influences the F_M and F_v parameters. The value of the maximum fluorescence index F_M depends on many factors, including the type of saturation light and the content of chlorophyll in the test tissue. In the case of

plants exposed to stress factors, the F_M value is reduced. In the experiment described in this paper, the values of maximum F_M fluorescence were highest in plants grown on the control medium – without the addition of a rubber rag, and lowest on the medium with the addition of a rubber rag in the amount of $50 \text{ g}\cdot\text{kg}^{-1}$ of soil. Moreover, F_M values are characterized by slight fluctuations between plant groups growing on particular soils [Cetner et al., 2016; Sulkiewicz, Ciereszko, 2016; Kalaji et al., 2017; Staniak, Baca, 2018].

The most commonly used reliable photochemical activity index of a plant's photosynthetic apparatus is the F_v/F_M parameter, which should be 0.83 in stress-free plants [Kalaji, Łoboda, 2010; Wang et al., 2009]. A decrease in F_v/F_M indicates that the plant may have experienced stressful conditions that reduced the PSII function and reduced the efficiency of electron transport. The obtained results show that the value of F_v/F_M in maize leaves was the closest to 0.83 in plants cultivated on a substrate with the addition of the largest amount of rubber rag (Fig. 5). In other words, the plants growing on the control subsoil showed a lower efficiency of the photosynthetic apparatus than those grown on the subsoil with the highest amount of rubber rag.

The F_v/F_0 parameter is characterized by maximum efficiency of water splitting at the donor side of PSII and is highly sensitive to various stress factors [Pereira et al., 2000]. In the experiment, the highest F_v/F_0 values were observed again in plants growing on the substrate with the highest amount of rubber rag. This suggests that the addition of rubber rag to the soil has a stimulating effect on the physiological state of maize by improving the fluorescence values of chlorophyll.

4. CONCLUSIONS

The studies described were an attempt to check how maize plants react to the contact with a worn out car tyres. The introduction of material from shredded tyres into the soil has resulted in some substances and elements that have passed into the soil and been taken up by the plants being washed out even in such a short period of time. It turned out surprisingly that the maize yield increased due to the addition of waste material, which was probably caused by the increase in the available quantities of zinc and iron in the soil. This raises a difficult but interesting question: 'Is it possible to use used car tyres for the production of certain fertilizers'?

In conclusion, the growing amount of rubber rag in the soil stimulated the growth of roots and aboveground parts of maize and the addition of rubber rag to the soil caused a significant increase in the zinc content in roots and aboveground parts of plants. The content of chlorophyll in maize leaves decreased with the increase of rag content in soil. Plants growing on the soil with the addition of the largest amount of rubber rag showed higher efficiency of the photosynthetic apparatus than the others.

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