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Impact of Cigarette Butts on Plant Germination Based on *Sinapis alba* L. and *Hordeum vulgare* L. Seeds

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ABSTRACT

Cigarette butts (CBs) have only recently begun to be considered environmentally harmful waste. CBs are common waste in the environment, that can cause air, soil, and water pollution and pose a threat to the living. CBs should be treated as toxic and hazardous waste due to its slow decomposition and accumulation of many toxic substances. There is a lack of research on the adaptation of CBs to the environment and what impact they have on vegetation. Therefore, the present work aimed to understand the toxicity of smoked CBs. Leachates of various concentrations were assessed with ecotoxicological tests. The effect of CBs on germination and development of plants at their early stage of growth was determined. Seeds of *Sinapis alba* L. and *Hordeum vulgare* L. were used in the CBs toxicity test. Two-way ANOVA was conducted to compare the main effects on plants. From the results, it is evident that low concentrations of toxic substances contained in CBs have a positive effect on plants; however, *Hordeum vulgare* L. expresses higher toxic resistance than *Sinapis alba* L. It has been proven that high concentrations of CBs in a water solution have negative effects on seed germination and radical growth. This research shows that varied plant species can cope with different levels of contamination by hazardous elements. CBs are an important source of contamination for the environment and the hazardous elements that are released from them when inappropriately disposed of can impair the development of plants and accumulate in them.

Keywords: personal litter; cigarette butts; ecotoxicological tests; adverse effects.

INTRODUCTION

Nowadays, the world is struggling with the increase of pollution in the air, soil, and water environment. The most disruptive wastes to the environment are those on a micro-scale. An example of such waste involves CBs, the abandonment of which in publicly accessible areas causes them to be washed away by rainwater urban runoff, as well as flood events and transferred to other ecosystems, such as aquatic environments (Akhbarizadeh et al., 2021). Cigarette butts (CBs) have only recently begun to be considered as environmentally harmful waste. ISO 3308 (2012) defines the length of a CB as "the length of unburned cigarette remaining at the time of cessation of smoking". CBs are common waste in the environment (Torkashvand et al., 2020) that can cause air, soil and water pollution as well as pose a threat to living organisms (Araújo & Costa, 2021; Chevalier et al., 2018). To verify the negative effect of CBs, it is initially necessary to verify the mechanism of movement of the hazardous substances inside them. It has been shown that Mn is likely to be released over a longer period time than Ni, Pb, and Zn, which are released after a few days (Moerman & Potts, 2011). It has also been proven that the longer the waste remains in a freshwater environment (pH~7), the greater the toxic contamination becomes. The levels of metals leached from CBs vary with the type of water, and it has been noted that with seawater, the maximum level is noticeable almost immediately, while with freshwater, it takes several days (Akhbarizadeh et.al., 2021).

Metals from CBs can be released into the environment in dissolved form and as nanoparticles (NP). The amount of NP-bound metals leached from CBs is almost twice higher than the number of metals in the dissolved fraction (Chevalier et al., 2018). These studies suggest a new pathway for metal leaching, which also highlights the urgency of solving the problem of NP removal. To date, no effective way has been found to treat the water from NP, which is why CBs contamination of water is so dangerous. The soils contaminated by heavy metals (HM) are also a serious problem, as they cause impairment of plant growth parameters such as germination index, root length, and shoot biomass (Jaskulak et al., 2019). Excess of Ni decreases plant metabolism, inhibits photosynthesis and transpiration, as well as leads to ultrastructural changes and oxidative stress (Chen et al., 2009). In various studies (Massimi, 2018; Vasić et al., 2020), it has been shown that the germination index is most affected by Cd, followed by Ni, and finally by Pb. In the environment, a small dose of a toxic substance can have a positive effect on the early developmental stage of plants, so-called hormesis (Kudryasheva & Rozhko, 2015); nevertheless, as the concentration of toxic compounds increases, the plant response changes, and the high dose of the toxic compound begins to have an inhibitory or toxic effect.

CBs are poorly degradable and take around 720 days to decompose under grassland conditions (Qamar et al., 2020). Moreover, some people treat CBs as toxic (Novotny et al., 2009) and hazardous waste due to its slow decomposition and accumulation of many toxic substances (Barnes, 2011). However, in most countries, CBs management consists of placing them with the mixed fraction in landfills, while in other countries they are incinerated and cause air pollution with toxic fumes (Rahman et al., 2020).

Studies show that 76–84% of smokers throw CBs into the environment instead of the trash, resulting in more than 4.5 trillion CBs being thrown into the environment each year (Araújo & Costa, 2019; Barnes, 2011). Kurmus & Mohajerani (2020) estimated that one in three CBs ends up in the environment. The Berlin study found that the average density of CB in cities, parks and, public green spaces was 2.7 CB per square meter, while the highest amount was found close to train stations with an average of 48.8 CB per square meter (Green et al., 2014). The problem regarding the appropriate management of CBs has not been yet solved worldwide. One idea involving the sustainable use of CBs is their use in construction (Kurmus & Mohajerani, 2020, Morales-Segura, 2020) and infrastructure (Marinello et al., 2020; Rahman et al., 2020) as a component of fired bricks (Mohajerani et al., 2016), which improves the properties of bricks (Marinello et al., 2020). CBs are also used in environmental engineering in the treatment of water from oil, specifically their use to separate various oils from water under emergency conditions (Ifelebuegu et al., 2018; Marinello et al., 2020; Xiong et al., 2018). Researchers have also tested the use of CBs in the metallurgical industry as an anti-corrosion material (Vahidhabanu et al., 2014), medical industry for the production of nanocrystalline cellulose (Ogundare et al., 2017), nanosynethesis pesticides for mosquitoes and tools for malaria vector management (Murugan et al., 2018). Moving towards the waste management, the research carried out by d'Heni Teixeira et al. (2017) has proposed recycling CBs in cellulose pulp in paper production. However, it should be remembered that the study is currently in its preliminary phase and, for the time being, CBs represent a nuisance of municipal waste that is constantly increasing (Wojciechowski & Iwaszczuk, 2021) and the CBs economy is becoming a pressing issue (Rahman et al., 2020).

In light of the scientific studies published so far, one can see an attempt to implement interesting technologies in connection with the processing of CBs. However, there is a lack of research on adaptation of CBs in the environment and what impact they have on vegetation. Overall, the present work aims to understand the toxicity of smoked CBs. For this, leachates of differing concentrations were assessed with a battery of ecotoxicological tests. The effect of CBs on germination and development of plants at their early stage of growth was determined. To compare the main effects of plant species, a number of toxic CBs, and their interaction on plant two-way ANOVA was conducted. Three hypotheses were tested: (hi) plant species (Sp) has a significant impact on ripening speed of the plant; (hii) the presence of CBs has a significant impact on root length growth of the plant; (hiii) there is an interaction between Sp and CBs.

MATERIALS AND METHODS

Cigarette butt collection and preparation

Unflavored filtered cigarettes of the same brand and type were purchased. CBs were obtained by smoking cigarettes until approximately 1 cm above the filter. The paper surrounding filter was left intact. All CBs were then dried at 40 °C overnight.

Leachate preparation and Toxicity tests

CBs were used to prepare concentrated leachate. All leachates were tested with a selected ecotoxicological test. The prepared water solution as germination medium contained 100 ml of water with different concentrations of CBs additive (3CBs, 5CBs, 10CBs). The solutions were "matured" at different times: 1 day, 7 days, and 21 days (Fig. 1). A seed germination toxicity test of *Sinapis alba* L. and *Hordeum vulgare* L. was performed on 9 cm diameter Petri dishes (PDs). Ten plant seeds were placed on each PD at equal intervals. According to Hernandez & Potts (2018), CBs can leach toxic compounds when exposed to an aqueous medium, which is a universal solvent (Ali et al., 2019); therefore, water was used in the preparation of the solution. After 24 h, 7 days, and 21 days, CBs were filtered off and 5 ml of the solution per PD was divided on a previously laid perforated tracing paper. The perforation of the tracing paper allows the roots to develop properly. The solution was not mixed before filtering off the CBs to simulate natural conditions. According to Moerman & Potts (2011), shaking is probably responsible for flushing out higher concentrations of toxic substances from cigarettes.

The PD test was performed in 3 replicates for each solution and control. The blank (control) sample contained 5 ml of tap water. PDs were covered with a plastic lid to avoid evaporation. The slides were incubated in the dark for 72 hours at 20±2 °C. After 3 days, root lengths were measured and growth rates were calculated such as seed germination (SG), relative seed germination (RSG), relative radical growth (RRG), and seed germination index (GI). The following formulas 1–4 were used for the calculations (Luo et al., 2018):

$$SG = \frac{Number\ of\ germinated\ seeds}{Number\ of\ total\ seeds} \times 100 \quad (1)$$

$$RSG = \frac{Number\ of\ germinated\ seeds\ (sample)}{Number\ of\ germinated\ seeds\ (control)} \times 100 \quad (2)$$

$$RRG = \frac{Total\ radical\ length\ of\ germinated\ seeds\ (sample)}{Total\ radical\ length\ of\ germinated\ seeds\ (control)} \times 100 \quad (3)$$

$$GI = RSG \times RRG \times 100 \quad (4)$$

The most important parameter is GI, which is calculated based on the length of the seedlings and the percentage of seed germination in the sample (CBs additive) compared to the control sample (tap water). Percentage and germination rate rises with an increasing GI value.

Figure 1. Experimental set up (a) after 0h, (b) after 24h

Figure 2. Seeds of Sinapis alba L. (a) and Hordeum vulgare L. (b)

Description of tested plants

Seeds of *Sinapis alba* L. and *Hordeum vulgare* L. were used in the CB toxicity test. *Sinapis alba* L. is an ideal plant for toxicity testing due to its sensitivity to a wide range of chemicals (Adamcová et al., 2019; Vaverková et al., 2020; Zloch et al., 2018) and fast growth (Maxianová et al., 2021). Its seeds are spherical (Fig. 2a) and odorless with a diameter of about 2.5 mm (Adamcová et al., 2019). In contrast, *Hordeum vulgare* L. is a versatile plant species that adapts to adverse climatic conditions, biotic and abiotic stresses (Kebede et al., 2019) and is often used in germination tests (Adamcová et al., 2019). The seeds of *Hordeum vulgare* L. are oblong (Fig. 2b), while their size can be divided into small (<0.25 cm), medium (0.25–0.275 cm), and large (>0.275) (Massimi, 2018).

Statistical analysis

Multiple physiological measurements for individual scales under different levels of treatment (3, 5 and 10CB) for statistical calculations were combined as "with CBs" and "without CBs". Mean values based on three measurements in each subgroup were calculated, giving a number of cases of n=12. Statistica 12 software was used for statistical analysis. Initially, the Shapiro-Wilk Normality Test was performed for each data set. Then, the homogeneity of the groups was verified using Levene's test, which assumes that when $p>0.05$, then the data is homogeneous. The next step was a two-way analysis of variance to identify significant effects of CBs, species, and interaction between groups. In turn, the post hoc Tukey HSD test further identified significant differences between treatment groups at a significance level of $p<0.05$. Data sets on CB seed timing (24 h, 7 days, and 3 weeks) were treated separately.

RESULTS AND DISCUSSION

Comparison of growth parameters

Seeds of *Sinapis alba* L. and *Hordeum vulgare* L. were tested for abiotic stress caused by supplementation with CB solution (3CBs, 5CBs, and, 10CBs concentrations). Growth parameters (SG, RSG, RRG, and GI) after 72 h for *Sinapis alba* L. and *Hordeum vulgare* L. were calculated and presented in bar graphs (Fig. 3, Fig. 4).

The bar groups were divided into 4 parts, i.e., control, 3CBs solution, 5CBs solution, and 10CBs solution in 3 color ranges (lightest color – solution formed after 24 h, dark grey – solution after 7 days and black – solution after 3 weeks).

SG for *Sinapis alba* L. in the control oscillated between 93% and 87%. Surprisingly, SG was higher in the solution with 3CBs soaked for 7 days, and 3 weeks (Fig. 3a). Nevertheless, some CB compounds can positively influence plant germination at low doses (Rascio & Navari-Izzo, 2011; Tiwari & Lata, 2018) and be toxic in a higher doses of CBs. The lowest SG were recorded for the 10CBs solution soaked for 24 h (53%), 7 days (47%), and 3 weeks (13%) respectively. These findings confirm that the highest concentration of CBs in the solution had the most inhibitory effect on the SG of *Sinapis alba*

L. and the factor of time further decreased the SG. The same was the case in a study (Drab et al., 2011) wherewith increasing concentrations of the solutions containing heavy metals, the number of germinated seeds of *Sinapis alba* L. gradually decreased.

In the case of RSG, the assumption of a positive effect of CBs solution for 3CBs variant is evident in Figure 3b where the value of RSG is higher than 100% for the variants of 3CBs soaked for 7 days and 3 weeks. The same RSG (RSG = 100%) was achieved for the control sample. In

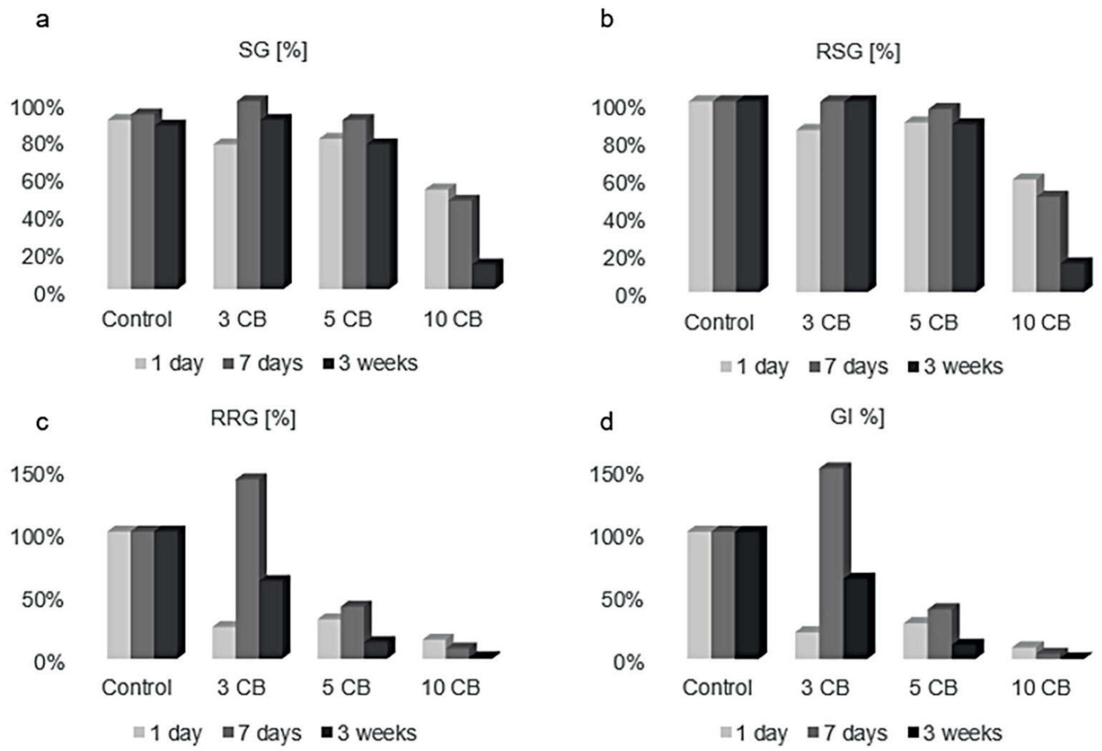

Figure 3. SG (a), RSG (b), RRG (c) and GI (d) for *Sinapis alba* L.

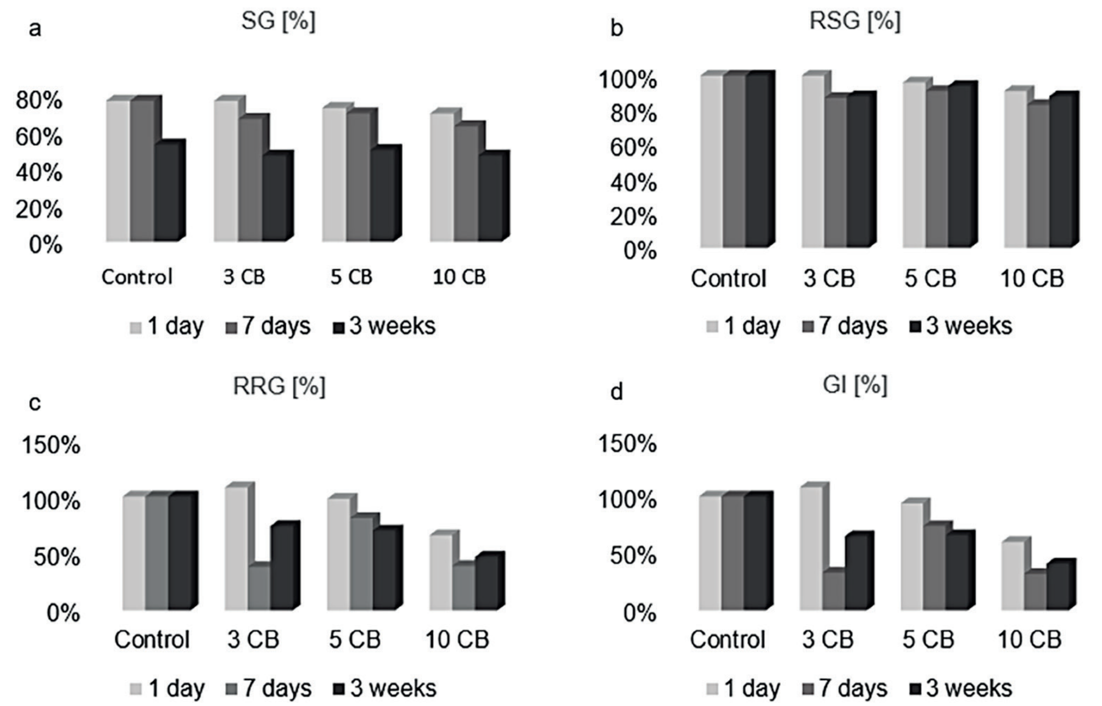

Figure 4. SG (a), RSG (b), RRG (c) and GI (d) for *Hordeum vulgare* L.

contrast, the most pronounced effect on germination of *Sinapis alba* L. seeds was seen in the 10CBs solution, where 24 h soaking of CBs in the aqueous solution reduced germination to 59%, 7 days soaking of CBs to 50%, and after 3 weeks of soaking to 15%. For RRG (Fig. 3c), the relative radical gain for the 3CBs solution soaked for 24 h was surprisingly low compared to other values. This may be due to the factors not necessarily related to the CB substances (e.g., PD contamination). The value of RRG, as well as the value of GI in the solution with 3CBs soaked for 7 days, was higher than in the control, which, as mentioned earlier, may indicate the positive effect of low doses of leachable compounds on radical growth and germination index of *Sinapis alba* L. In all other cases, the root length of *Sinapis alba* L. and GI decreased with increasing CBs in the solution.

The second tested species, *Hordeum vulgare* L. (Fig.4), owing to its high-stress tolerance, did not record significant changes and impediments to germination with increasing CBs concentration in the solutions (Fig. 4a). El Rasafi et al. (2020) demonstrated that the stress caused by some HM does not significantly affect the germination of *Hordeum vulgare* L. plants compared to control samples. SG in this study study was the highest in the control (24 h and 7day solution) and the solution with 3CBs soaked for 24 h and was 77% in all cases. The lowest SG values (47%) were observed in 3CBs and 10CBs solution soaked for 3 weeks. In the case of RSG, which reflects the number of germinated seeds in comparison with the control, the positive effect of low concentrations of CBs was noticed only in the case of soaking CBs for 24 h. In other cases, the parameter decreased with increasing CBs concentration and soaking time. On the other hand, the RRG index representing the total average root length compared to the control was the highest (108%) in the solution with 3CBs soaked for 24 h (Fig. 4c) making it also higher than in the control (100%) and at the same time the lowest in the case of 7 days soaking time (38%). The positive effect of some heavy metals (Ni and Cu) at low concentrations (10 mg l^{-1}) was observed in the study of El Rasafi et al, (2020) where root lengths of the *Hordeum vulgare* L. plants exposed to selected HM were studied. Moura et al. (2010) in their study demonstrated that Cu, Cd, and As can have positive effects on lignin biosynthesis in some plant species.

The increase in lignin, on the other hand, clearly reflects the protective response of organisms to increased HM concentrations (Ali et al., 2006), so that cell wall plasticity is maintained (Gall et al., 2015). This may be the reason for the increase in plant biomass (El Rasafi et al., 2020). In other cases, the RRG values decreased with the increasing amount of CB. Similar trends were observed for GI, which may suggest that in the case of *Hordeum vulgare* L. rapid root growth significantly influences the conducted experiment.

Comparison of root length depending on the amount of dissolved CBs in water solution

The results from testing the effect of CBs on the root length of *Sinapis alba* L. and *Hordeum vulgare* L. were presented in Figure 5.

In the case of CBs soaking for 24 h (Fig. 6), it was noticed that higher dispersion and higher values related to root length were adopted by *Hordeum vulgare* L. Average root lengths in the case of *Hordeum vulgare* L. oscillated around 30 mm for 0CBs, 3CBs, 5CBs, while for 10CBs they dropped to 19 mm. In the case of *Sinapis alba* L., a clear negative effect of the CBs content on the mean root length can be seen. The roots respond to abiotic stress, in this case to CBs contamination, more than the shoots, as they are in direct contact with the contamination (Kul, 2020). No addition of CBs to the aqueous solution gives an average length of 14 mm, while its addition causes a noticeable decrease (3CBs = 4 mm, 5CBs = 5 mm, 10CBs = 3 mm).

In the case of 7 days soaking time, a greater dispersion of values adopted by *Hordeum vulgare* L. can be noticed (Fig. 7). The highest average root length values were recorded in the absence of CBs addition (47 mm), while the lowest average (23 mm) was recorded in the case of 10CBs. *Sinapis alba* L. after exposure to 7 days solution responded best to the solution of 3CBs and took the highest mean of 31 mm. On the other hand, it responded negatively to increased CBs concentration and gradually decreased the adopted mean to 9 mm for 5CBs and 4 mm for 10CBs.

The results from the longest solution maturation time, i.e., 3 weeks, are shown in Figure 8. In this case, the dispersion of results for *Hordeum vulgare* L. was not noticed as much as in the previous trials. The exception here was the 10CBs solution, where the dispersion of results for *Hordeum vulgare* L. was the greatest. The 3 weeks solution

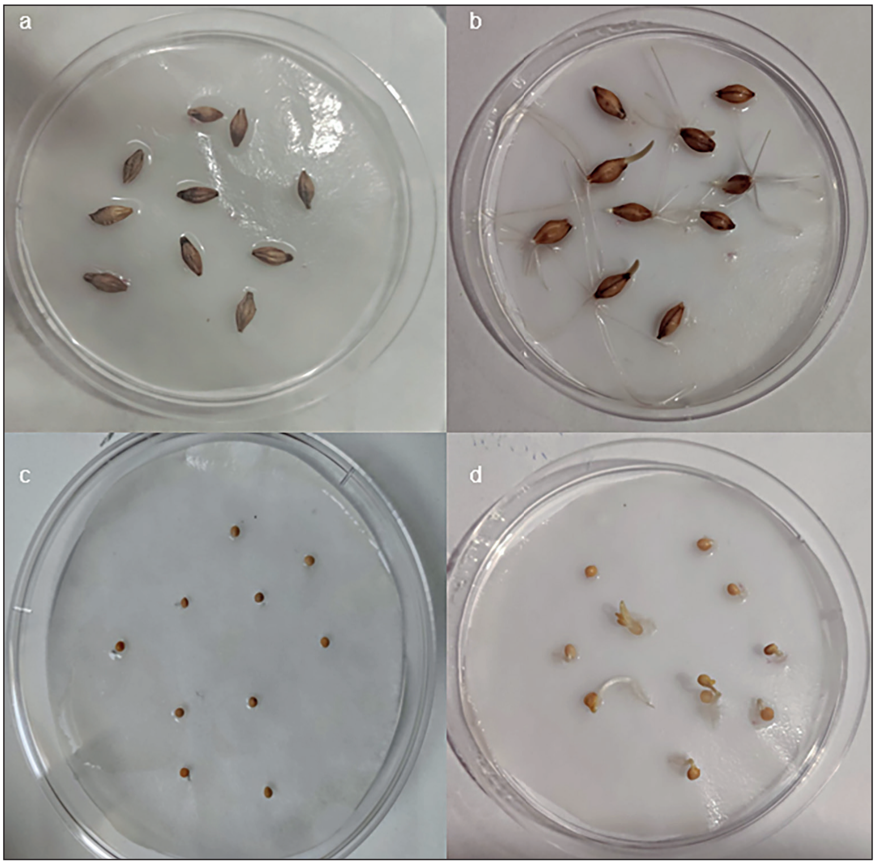

Figure 5. PD test for Hordeum vulgare L.(a) beginning of the experiment, (b) after 72h and Sinapis alba L.(c) beginning of the experiment, (d) after 72h

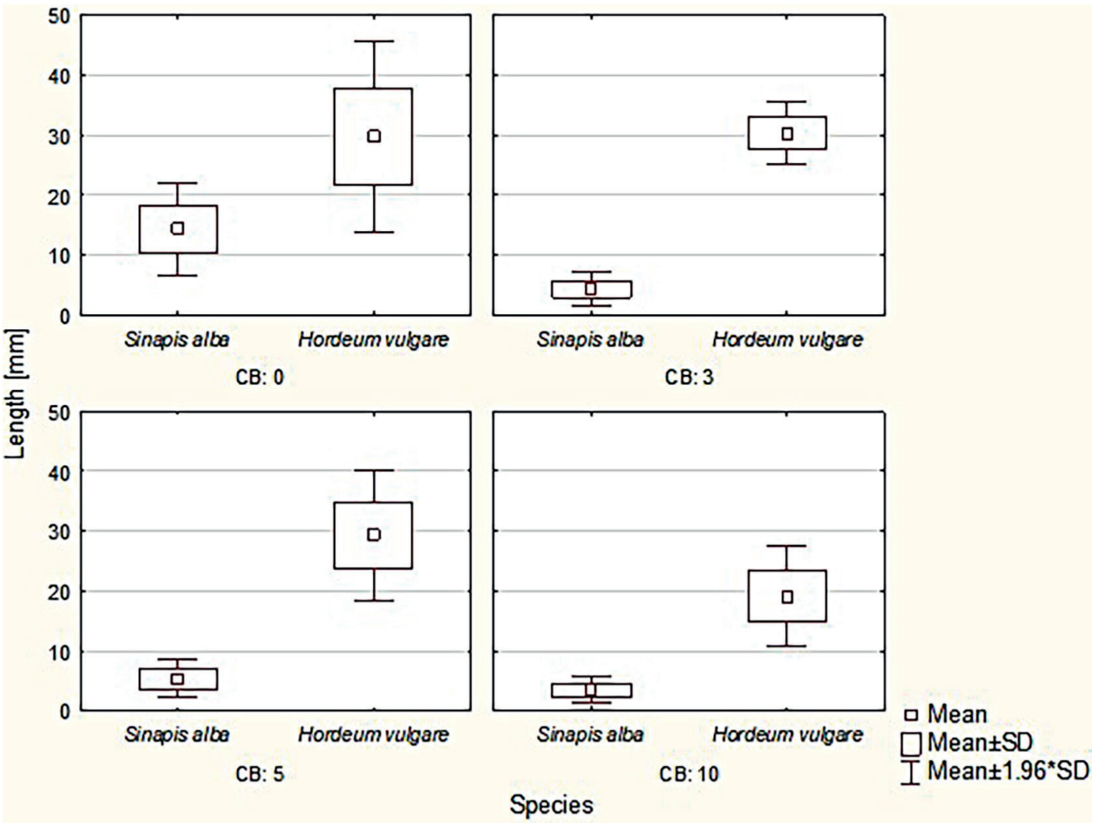

Figure 6. Length of plant in dependence on the amount of CBs dissolved in water after 24 hours

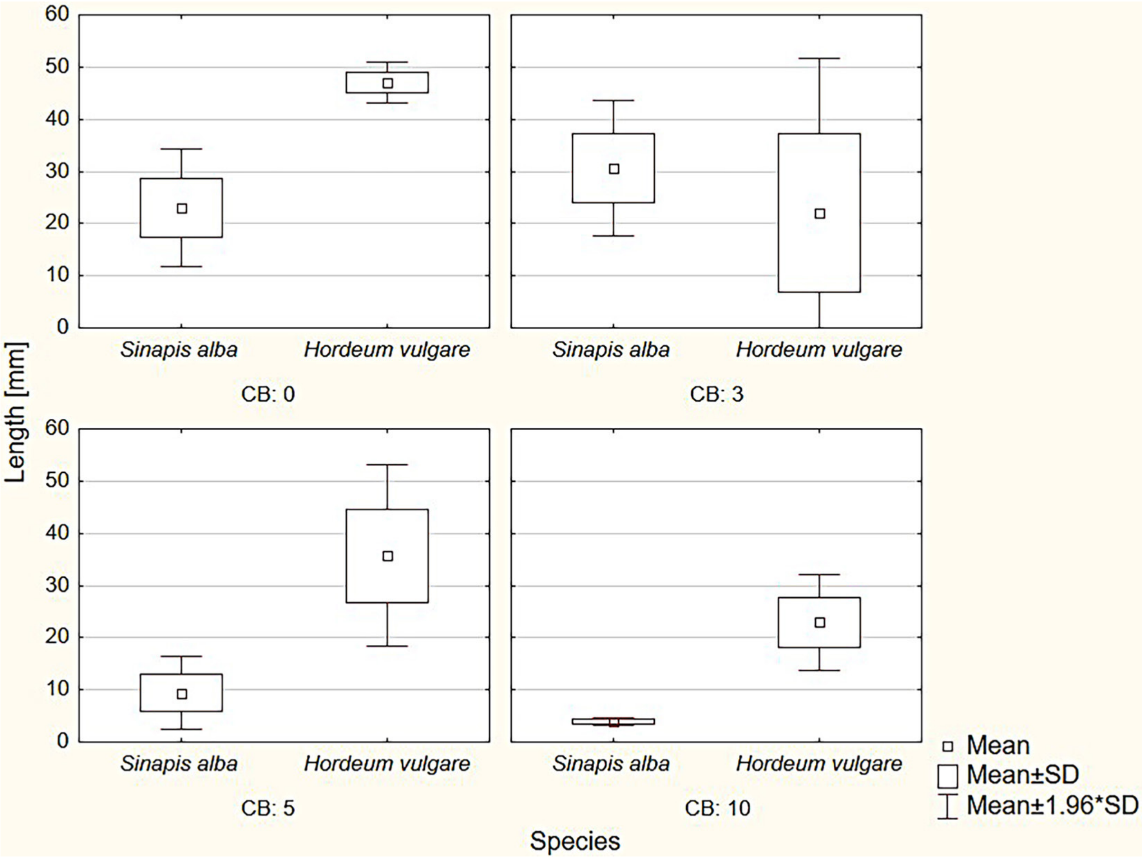

Figure 7. Length of plant in dependence on the amount of CBs dissolved in water after 7 days

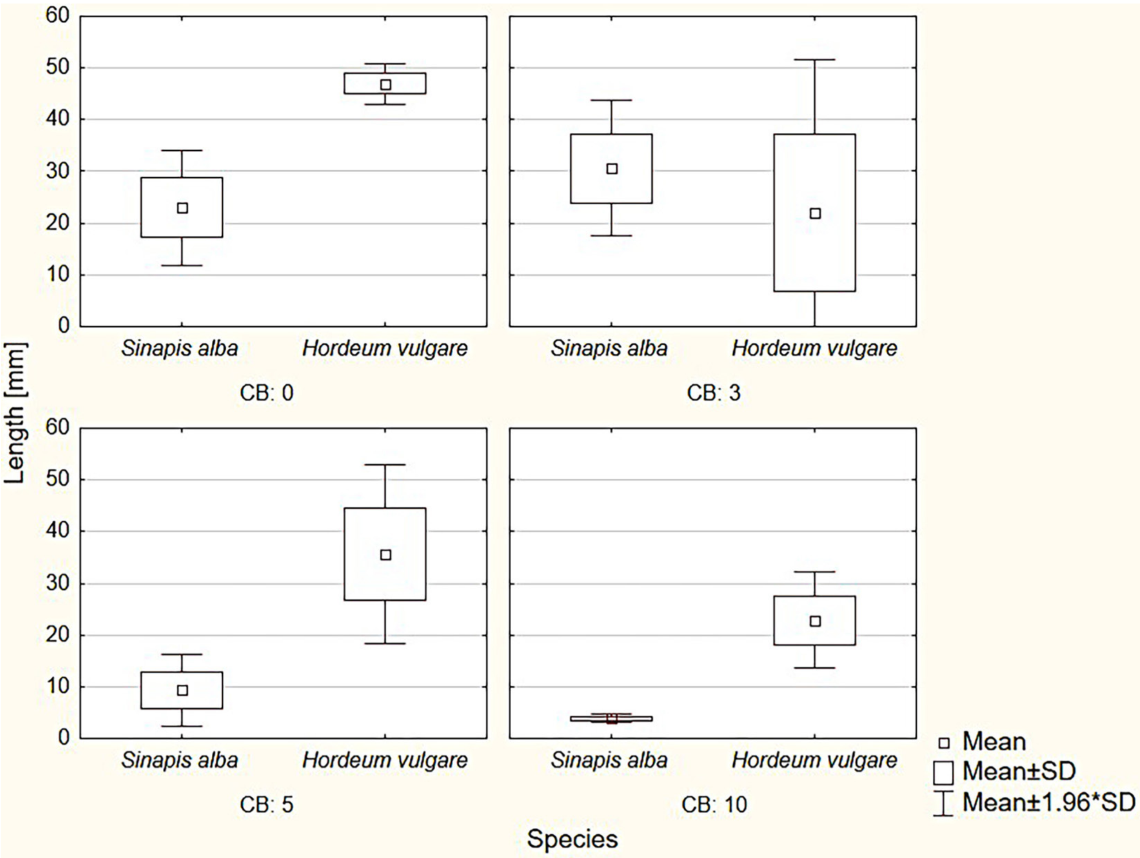

Figure 8. Length of a plant according on amount of CBs dissolved in water after 3 weeks

maturation time meant that regardless of plant species, average root length decreased with increasing CBs concentration in solution. For *Sinapis alba* L., the lengths recorded were as follows: 30 mm for 0CBs, 17 mm for 3CBs, 4 mm for 5CBs, and less than 2 mm for 10CBs. In contrast, for *Hordeum vulgare* L. the following lengths were recorded: 24 mm for 0CBs, 20 mm for 3CBs, 18 mm for 5CBs, and 13 mm for 10CBs.

Species-dependent changes in plant length in response to increasing CBs concentration in water solution

Plant root length is often dependent on abiotic stress, especially when the plant encounters toxic compounds that affect physiological development (Koevoets et al., 2016; Kul et al., 2020). The effect of plant species (*Sinapis alba* L. and *Hordeum vulgare* L.) and amount of CBs on plant growth was tested at three temporal periods, i.e., after 24 h, after 7 days, and after 3 weeks of CBs soaking in water. For the two-way ANOVA test, the assumptions that the samples had normal distribution were initially verified ($p>0.05$). A Shapiro-Wilk test performed on small samples ($n = 12$) showed that the data for 24 h (p-value = 0.471), 7 days (p-value = 0.459), and 3 weeks (p-value = 0.646) represent a normal distribution. Table 1 shows the results from Levene's test suggesting homogeneity of the data.

Two-way analysis of variance showed that for the temporal period of 7 days, plant species and CBs had a statistically significant ($p<0.05$) effect on plant length (Table 2) so in this case, hi and hii were accepted. In other cases, hypothesis hi (significant effect of plant species on root length) was accepted in the case of 24 hours of CBs soaking. While hii (significant effect of CBs concentration) on plant length was accepted in the case of 3 weeks.

On the other hand, no interaction was observed between species and CBs on plant growth. Thus, hiii involving the interaction between CBs and plant species can be rejected.

To find significant differences between the groups at the significance level of $p<0.05$, the Tukey HSD post hoc test was performed for 24 h, 7 days, and 3 weeks. For 24 h significant difference ($p=0.005312$) can be seen between the length of *Sinapis alba* L. with CBs and *Hordeum vulgare* L. with CBs. Significant differences can also be found for *Sinapis alba* L. without CBs + *Hordeum vulgare* L. without CBs ($p=0.03622$). Tukey's test after 7 days led to the conclusion that only for *Hordeum vulgare* L. without CBs and *Sinapis alba* L. with CBs there was a significant difference ($p=0.012548$). On the other hand, for the data after 3 weeks, post hoc test showed that the results obtained for *Sinapis alba* L. with and without CBs were significantly different ($p=0.012409$). The studies analyzing the toxicity of CBs leachate indicate that lethal and sublethal responses are induced at different dilutions and quantities of CBs per liter of stock solution. This is supported by the study of Register (2000), which demonstrated that the exposure of freshwater organisms such as *Daphnia Magna* to the leachate obtained from 2 and 4 CBs per 1 liter of water after 48 h corresponds to 100% mortality of the

Table 1. Levene's test for homogeneity of variances

Homogeneity of Variances (LEVENE'S TEST)				
Parameter	Factor	p-value		
		24 hours	7 days	3 weeks
Length [mm]	Sp	0.986	0.488	0.051
	CB	0.277	0.600	0.518
	Sp x CB	0.206	0.052	0.118

Table 2. Two-way ANOVA p – values

Two-way ANOVA				
Parameter	Factor	p value		
		24 hours	7 days	3 weeks
Length [mm]	Sp	<0.001	0.008	0.649
	CB	0.065	0.040	0.004
	Sp x CB	1.083	0.397	0.077

organisms. In contrast, Osuala et al. (2017) analyzed the effects of smoked and unsmoked CBs on juveniles of Nile tilapia and showed that cigarettes produced Lethal Concentration 50 (96 h) between 1 and 2 CBs per liter of water.

CONCLUSIONS

Low concentrations of toxic substances contained in CBs have a positive effect on SG, RSG, RRG, and GI, causing the phenomenon of hormesis; the inhibition of SG of *Sinapis alba* L. was growing with increasing concentration and time of soaking of CBs in the water solution. These factors affected the SG of *Hordeum vulgare* L. to a lesser extent, because this plant species is more stress-tolerant. It has been proven that high concentrations of CBs in water solution have negative effects on SG and RRG. *Hordeum vulgare* L. exhibits higher toxic resistance than *Sinapis alba* L. In this study, the factor of the soaking length of CBs in the water solution did not affect the root length of plants; however, it affected the SG. This research shows that varied plant species can cope with different levels of contamination by hazardous elements. CBs are an important source of contamination for the environment and hazardous elements that are released from them when disposed of inappropriately can impair the development of plants and accumulate in them. It has been statistically proven that after 7 day period of soaking, plant species and CBs presence have a statistically significant ($p<0.05$) effects on plant growth.

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