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Germination and Early Seedling Growth in Sweet Sorghum Exposed to Heavy Metal Stress under Seed Priming Pretreatments

Priming Ön Uygulamaları Altında Ağır Metal Stresine Maruz Kalan Tatlı Sorgumda Çimlenme ve Erken Fide Gelişimi

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Abstract: This study was conducted under laboratory conditions to determine the effects of priming pretreatments (GA_3, KNO_3 and H_2O) on germination and seedling development of sweet sorghum exposed to different levels (0, 75, 150 and 300 mg l^{-1}) of lead, cadmium and nickel stress. The results of the research revealed that, in general, heavy metals negatively affected both germination and seedling growth properties. However, the severity of the negative effect showed significant differences depending on the kind and dose of the heavy metal. It has been determined that the negative effects of cadmium and nickel were higher than lead. Among the priming pre-treatments, it was determined that in general, pre-application with H_2O was sufficient, and there was no need for other priming agents. However, it has been determined that heavy metals significantly reduce root and seedling growth even at low doses. This situation showed that even if germination occurred, healthy plant growth would not occur at heavy metal doses above 150 mg l^{-1}.

Keywords: heavy metal, germination, seed priming, seedling growth

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Öz: Bu çalışma, laboratuvar koşullarında farklı düzeylerinde (0, 75, 150 ve 300 mg l^{-1}) kurşun, kadmiyum ve nikel stresine maruz bırakılan tatlı sorgumun çimlenmesi ve erken fide gelişimi üzerine tohum priming ön uygulamalarının (GA_3, KNO_3 ve H_2O) etkilerini belirlemek amacıyla yürütülmüştür. Araştırma sonuçları genel olarak ağır metallerin hem çimlenme hem de fide özelliklerini olumsuz yönde etkilediğini ortaya koymuştur. Bununla birlikte ağır metalin türüne ve dozuna bağlı olarak olumsuz etkinin şiddeti önemli farklılıklar göstermiştir. Kadmiyum ve nikelin olumsuz etkisinin kurşundan daha yüksek olduğu belirlenmiştir. Priming ön muamelelerinden genel olarak H_2O ile ön uygulamanın yeterli olduğu, ayrıca bir uyarıcı ön uygulamasına ihtiyaç olmadığı belirlenmiştir. Ayrıca, ağır metallerin düşük dozlardan itibaren kök ve fide gelişimini çok ciddi derecede azalttığı tespit edilmiştir. Bu durum çimlenme gerçekleşse bile 150 mg l^{-1} üzerinde ağır metal düzeylerinde sağlıklı bitki gelişiminin olmayacağını göstermiştir.

Anahtar Kelimeler: Ağır metal, çimlenme, tohum uyarıcı, fide gelişimi

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INTRODUCTION

Sweet sorghum is a member of the *Poaceae* family and is an annual energy crop that uses C_4 photosynthesis. Sorghum species have a great potential in terms of being an alternative to corn and other cultivated plants both in the utilization of dry lands and in seasons when water is limited in irrigated agricultural areas (Akinseye et al., 2020). Sorghum is a multi-purpose plant that can be used as a bioenergy source, sugar and paper, as well as human and animal nutrition (Almodares et al. 2008). Since its potential for non-food use is high and monocotyledonous plants are generally more tolerant to heavy metal stress (Jadia and Fulekar, 2008), sweet sorghum is a potential plant in the evaluation and reclamation of areas contaminated with heavy metals.

Among the heavy metals, approximately 20 elements attract ecological attention (Fe, Mn, Zn, Cu, V, Mo, Co, Ni, Cr, Pb, Be, Cd, Tl, Sb, Se, Sn, Ag, As, Hg, Al) and some of them can be micronutrients (Fe, Cu, Zn, Mn, Mo, Ni) for plants and animals and are not toxic as long as they do not exceed a certain limit (Okcu et al., 2009). Human activities have caused high levels of heavy metal accumulation in rural soils (Wierzbicka and Obidzinska, 1998). Heavy metals are important abiotic stress factors that can cause stress in plants. Stress affects the physiology of plants, changes their genetic potential, restricts their productivity and causes large amounts of product losses by causing their death (Saharan et al., 2022).

Lead (Pb), which is among the heavy metals, is one of the most dangerous heavy metals due to its high level in the environment in certain areas. While lead accumulated in the soil enters plants through their root systems, lead released into the air from dust and automotive exhaust accumulates directly in the above-ground parts of the plant (Wierzbicka and Obidzinska, 1998). The results of research conducted on different plant species have shown that lead negatively affects the germination and seedling characteristics of plants (Heidari and Sarani, 2011; Shafiq et al., 2008). In their study investigating the responses of different plant species to lead toxicity, Wierzbicka and Obidzinska (1998) reported that the response of the species was different, and lead uptake was delayed in species with an impermeable seed coat layer.

Cadmium (Cd) is a highly toxic metal that has come to the fore today with its various uses and its important role in environmental pollution. The main reason why cadmium has been on the agenda as a pollutant recently is that it is toxic even at very low doses and has a long biological half-life (Okcu et al., 2009). Cd enters agricultural soils mostly through anthropogenic activities such as the use of phosphate fertilizers, industrial activities and the application of sewage sludge (Abbas et al., 2018). Many researchers have emphasized the toxic effect of cadmium on plants (Haider et al., 2021; Rizwan et al., 2016). The results of research conducted with different plant species to determine the effects of cadmium on plant germination and seedling development have revealed that plants are negatively affected by increasing cadmium doses, but the tolerance thresholds of species to cadmium differed (Aydinalp and Marinova, 2009; Houshmandfar and Moragebi, 2011; Kabir et al., 2008; Shafiq et al., 2008; Smiri, 2011).

While low doses of nickel (Ni) are necessary for the plant, it is known that its amount increases in the ecological environment with increasing human activities, chemical fertilizers, chemical pesticides and residential and industrial wastes (Ceritoğlu et al., 2023) and as a result, excessive concentrations have negative effects on plants. (Ahmad et al., 2023; Sethy and Ghosh, 2013). High doses of nickel have a toxic effect on the growth and development of plants, starting from the germination stage (Razaq and Kadhim, 2018). Indeed, Akıncı and Akıncı (2011), and Aydinalp and Marinova (2009) observed the effects of nickel on germination and early seedling stages in different plant species.

Seed germination is one of the most delicate stages of the plant life cycle. Both the seed during the germination phase and the seedling formed after germination are extremely sensitive to abiotic stress factors such as heavy metals, and if damaged, the life cycle of the plant may end before it begins. In the presence of stress conditions, pre-planting seed priming treatments can provide successful results in ensuring germination occurs without any problems. Priming is the general name for commercially accepted seed practices that promote uniform germination, emergence and growth. Agents such as KNO_3, PEG (Mavi et al. 2010) and GA_3 (Kaur et al., 2023) are used in seed priming applications.

In the light of this information, one of the targets of this study is to determine the effect of some priming pre-treatments on germination and seedling development of sweet sorghum under certain heavy metal stress with different doses.

MATERIAL AND METHOD

The study was carried out as a laboratory study at Hatay Mustafa Kemal University, Faculty of Agriculture. Erduşmuş cultivar of sweet sorghum (*Sorghum bicolor* var. *saccharatum* (L.) Mohlenbr.) was used as plant material in the study. In the research, three different concentrations (75, 150, 300 mg l^{-1}) of three heavy metals (Lead (Pb), Cadmium (Cd) and Nickel (Ni)) and distilled water as control application were taken into consideration. In addition, H_2O, 2% KNO_3 and 500 ppm GA_3 were used as priming agents to determine the effect of priming applications on germination of seeds exposed to heavy metals.

Priming was applied by keeping the seeds of the species that underwent surface sterilization (10 minutes with 1% sodium hypochlorite solution) before planting the seeds, in KNO_3 and GA_3 solutions prepared in the specified doses at 25 °C for 12 hours. In addition, a group without a priming agent was prepared for planting being kept in distilled water (Hydropriming) under the same conditions as priming treatments for 12 hours after sterilization as control treatment.

The seeds, to which the necessary pre-treatments were applied, were placed on filter paper placed in two layers in petri dishes with a diameter of 9 cm, with 25 seeds in each petri dish. The 10 ml of the stock solution of all heavy metals prepared at doses of 75, 150, 300 mg l^{-1} (previously prepared as a 1l stock solution) and distilled water as control treatment were added to the petri dishes. The petri dishes, the edges of which were covered with parafilm to prevent water loss, were placed in a climate cabinet set at a temperature of 25± 1 °C and 16/8 h light/dark condition. In the experiment, the germination-related characteristics examined were recorded every day until the 10^{th} day. On the tenth day, seedling measurements were made on 10 plants that germinated in each petri dish.

Germination rate (GR) (1) (Akıncı and Çalışkan, 2010), germination index (GI) (2) (Korkmaz et al., 2023), and mean germination time (MGT) (3) (Fedeli et al., 2023) values were calculated according to the following formulas to interpret germination characteristics:

$$GR = \left(\frac{\text{number of seeds germinated}}{\text{total number of seeds sown}}\right) \times 100 \quad (1)$$

$$GI = \sum\left(\frac{\text{Gt}}{\text{Tt}}\right) \quad (2)$$

Where Gt is the number of seeds germinated on t^{he} day, and Tt is the number of days up to t^{he} day.

$$\text{MGT} = \Sigma(\text{n} \times \text{d})/\text{N} \quad (3)$$

Where n is the number of newly germinated seeds on each day, d is the day of counting and and N is the total number of seeds germinated at the end of the experiment.

The research was established and carried out according to the factorial arrangement of a completely randomized design with four replications. The first factor was the kind of heavy metal (Pb, Cd and Ni), the second was the doses of heavy metal (0, 75, 150, and 300 mg l^{-1}), and the third was the priming treatment (KNO_3, GA_3 and hydropriming). All data obtained from the present experiments were subjected to an analysis of variance with the use of MSTAT-C statistical software. Duncan multiple range test was used to determine statistical differences among mean values ($p \leq 0.05$).

RESULTS AND DISCUSSION

Germination Rate

The results of the analysis of variance applied to the germination rate values determined under different metal types, metal doses and priming treatments in sweet sorghum were given in Table 1. As shown in Table 1, the effects of the experimental factors individually and their double and triple interactions were found to be statistically significant at a 1% significance level.

Table 1. Mean squares of the combined analysis of variance for investigated characters.
Çizelge 1. İncelenen özelliklerin varyans analizi sonucu belirlenen kareler ortalamaları.

Source of variation	d.f	Mean Squares				
		GR	MGT	GI	Root Length	Shoot Length
Heavy Metal Type (A)	2	8420.111**	0.114	319.475**	289.418**	15720.924**
Heavy Metal Level (B)	3	11964.852**	11.338**	983.784**	40792.096**	32581.233**
A X B	6	1192.852**	0.137	50.901**	144.513**	2617.114**
Priming (C)	2	4364.111**	0.706*	222.213**	359.609**	3787.45**
A X C	4	409.444**	2.357**	50.209**	31.045	420.583**
B X C	6	363.519**	0.721**	29.953**	390.423**	820.529**
A X B X C	12	185.519**	0.427*	2.267	43.179	174.508**
Residual	108	41.926	0.218	4.605	40.859	42.528

* Significant at the 0.05 probability level, ** Significant at the 0.01 probability level.

According to the results of the research, it was determined that germination rates differed significantly depending on the heavy metal type, and the germination rate of sweet sorghum under cadmium stress (54.3%) was significantly lower than the germination rates determined in plants under nickel (74.4%) and lead (79.3%) treatments (Table 2). Especially the presence of cadmium in the germination medium had a more negative effect on the germination of sweet sorghum seeds. Similarly, Shafiq et al. (2008) reported that the negative effects of cadmium on seed germination of *Leucaena leucocephala* were greater than for lead. Çalışkan (2009) reported that the negative effects of cadmium are higher than nickel in line with our findings, while some researchers have expressed opposite results (Akar and Atış, 2018; Akar and Atış, 2019; Houshmandfar and Moraghebi, 2011). This can be explained by the different response of different plants to heavy metal species. Heidari and Sarani (2011) reported that the germination rate of mustard decreased significantly with increasing lead and cadmium levels.

Germination rates showed a significant difference depending on the increase in heavy metal levels. The germination rates were 90.6%, 75.0%, 64.8% and 47.1% for increasing heavy metal levels (0, 75, 150 and 300 mg l^{-1}, respectively) (Table 2). A continuous decrease was observed with increasing levels and each heavy metal level was in a separate statistical group. It can be said that germination was suppressed due to increasing heavy metal levels. At the highest dose of 300 mg l^{-1}, the average germination rate decreased by 48% compared to the control. Heidari and Sarani (2011) reported that the germination rate of mustard decreased significantly with increasing lead and cadmium levels. It has also been reported by other researchers that the presence of heavy metals in the environment and increasing doses increase the negative effect on seed germination (Akar and Atis, 2018; Ertekin and Bilgen, 2021; Kabir et al., 2008). Furthermore, Wierzbicka and Obidzinska (1998), who studied the effects of lead on the germination of different plant species, reported that germination results were not dependent on concentration but on the absolute amount of lead per unit of seed mass. In hydropriming pre-treatments, the germination rate was higher than the seeds pretreated with GA_3 and KNO_3. While the average germination rate of the seeds kept in distilled water was 77.4%, the mean germination rates of GA_3 and KNO_3 pre-treatments were 71.8% and 58.8%, respectively (Table 2). This indicates that seed priming agents present in the environment together with heavy metals may also have a negative effect (Akar and Atış, 2018; Akar and Atış, 2019).

Table 2. Sweet sorghum germination rate values determined in different heavy metal types, heavy metal levels and priming treatments (%).

Çizelge 2. Farklı ağır metal tipi, ağır metal düzeyi ve priming uygulamaları altında tatlı sorgumun çimlenme oranı değerleri (%).

		Heavy Metal Levels	Heavy Metal Types: Lead	Cadmium	Nickel	MEAN (Priming)
Priming	H_2O	**0 mg l^{-1}**	94.0 abc[ⱡ]	95.0 ab	96.0 ab	**77.4 A***
		75 mg l^{-1}	94.0 abc	72.0 ghı	98.0 a	
		150 mg l^{-1}	80.0 f-ı	44.0 n	86.0 b-f	
		300 mg l^{-1}	73.0 ghı	27.0 op	70.0 ıj	
	GA_3	**0 mg l^{-1}**	94.0 abc	92.0 a-d	91.0 a-e	**71.8 B**
		75 mg l^{-1}	82.0 d-g	50.0 lmn	81.0 e-h	
		150 mg l^{-1}	82.0 d-g	60.0 k	81.0 e-h	
		300 mg l^{-1}	59.0 kl	43.0 n	47.0 mn	
	KNO_3	**0 mg l^{-1}**	81.0 e-h	88.0 a-f	84.0 c-f	**58.8 C**
		75 mg l^{-1}	80.0 f-ı	47.0 mn	71.0 hı	
		150 mg l^{-1}	72.0 ghı	23.0 p	55.0 klm	
		300 mg l^{-1}	61.0 jk	11.0 q	33.0 o	
MEAN (Heavy Metal Types)			**79.3 A[+]**	**54.3 C**	**74.4 B**	

MEAN (Heavy Metal Levels)	0 mg l^{-1}	75 mg l^{-1}	150 mg l^{-1}	300 mg l^{-1}
	90.6 A[++]	**75.0 B**	**64.8 C**	**47.1 D**

(*) Means with different capital letters in the same column are statistically different from each other.
(+) Means with different capital letters in the same line are statistically different from each other.
(ⱡ) Means with different lowercase letters in the same row and column are statistically different from each other.
(++) Means with different capital letters in the same line are statistically different from each other.

The results of variance analysis revealed that the heavy metal type × heavy metal level interaction was statistically significant (Table 1). This was due to the different effects of different heavy metal types on germination at different heavy metal levels. While the highest germination rate value was determined in the control treatment, increasing doses generally caused a decrease in germination. While 75 mg l^{-1} lead application did not cause a statistically significant decrease compared to the control, the same dose of the other two heavy metal types caused a statistically significant decrease in germination rate (Figure 1a). As seen in the figure, the increase in cadmium level caused more serious decreases in germination rate. As a matter of fact, the lowest germination rate value was obtained in 300 mg l^{-1} cadmium treatment with 27.0% and the second lowest value was obtained in 150 mg l^{-1} cadmium treatment with 42.3%. The germination rates determined for all heavy metal levels, except 300 mg l^{-1} level, in lead and cadmium treatments were statistically similar. However, a lower germination rate was determined in nickel application at 300 mg l^{-1} dose compared to lead application at the same level. The effect of each heavy metal on different plant species varies. Indeed, Akar and Atis (2018) examined the effect of heavy metals on the germination of perennial ryegrass seeds and found that contrary to our findings, the negative effects of nickel started at lower doses than cadmium.

The effect of heavy metal type × priming interaction on germination rate was found significant (Table 1). In general, H_2O pre-treatment was more effective in lead and nickel polluted germination environments. In cadmium contaminated mediums, a higher germination rate was obtained in H_2O and GA_3 pre-treatments compared to KNO_3. The highest germination rate (87.5%) was determined in nickel pretreated with H_2O, followed by lead pretreated with H_2O and both treatments were statistically similar. The lowest value (42.3%) was determined in cadmium pretreated with KNO_3 (Figure 1b).

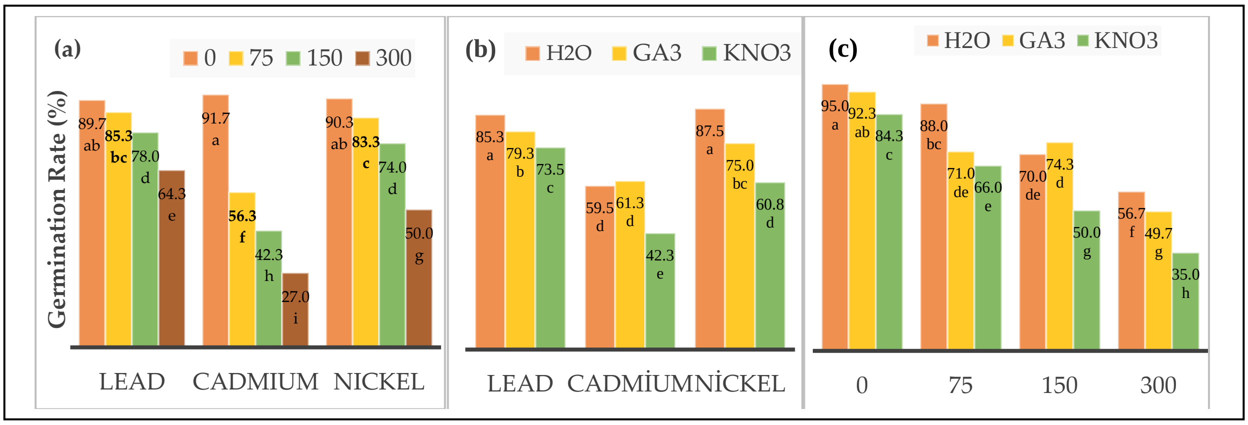

Figure 1. Effect of (a) heavy metal type × heavy metal level interaction, (b) heavy metal type × priming interaction, (c) heavy metal level × priming interaction on germination rate

Şekil 1. Çimlenme oranı üzerine (a) ağır metal tipi × ağır metal düzeyi, (b) ağır metal tipi × priming, (c) ağır metal düzeyi × priming interaksiyonlarının etkisi

Germination rate values were significantly affected by the metal dose × priming interaction (Table 1). At all heavy metal levels, the KNO_3 priming application lagged behind the other two applications in preventing the decline of germination rate. While no significant change occurred between 75 and 150 mg l^{-1} levels in GA_3 priming treatment, the germination rate decreased significantly with increasing heavy metal dose in other priming treatments. At 300 mg l^{-1} heavy metal level, the highest germination rate was found in H_2O pre-treatment (Figure 1c).

Analysis of variance results (Table 1) showed that the effect of metal type × metal level × priming triple interaction on germination rate was statistically significant. Germination rate values ranged between 11.0% and 98% among all treatments (Table 2). In seeds exposed to cadmium, the germination rate generally showed a more significant decrease with increasing levels. However, at the highest dose of 300 mg l^{-1} cadmium, the germination rate of the GA_3 priming treatment was 43%, which was better than the other two priming treatments. For other heavy metal types, it can be said that H_2O and GA_3 priming applications had similar effects against increasing heavy metal levels. As a result, the germination rate decreased significantly with increasing heavy metal levels. However, the response of sweet sorghum to heavy metal types was different. Especially cadmium had a more severe negative effect on the germination of sweet sorghum. In priming treatments, H_2O and GA_3 gave more favorable results, while KNO_3 did not contribute to the maintenance of germination against heavy metals.

Mean Germination Time

The effects of metal level, metal type x priming and metal level x priming binary interactions on the mean germination time of sweet sorghum were significant at a 1% level, while the effects of priming and triple interaction were significant at a 5% level. The effects of metal type and metal type × metal level binary interactions on mean germination time were statistically insignificant (Table 1). The mean germination times for control, 75, 150 and 300 mg l^{-1} treatments were 1.56, 1.89, 2.52 and 2.79 days, respectively (Table 3). Each heavy metal level was in a statistically different group from each other. The mean germination time of sweet sorghum was prolonged in parallel with the increasing heavy metal content depending on the heavy metal level. It was also reported by other researchers that germination time was prolonged due to increasing heavy metal concentration (Akıncı and Akıncı, 2011; Cokkizgin and Cokkizgin, 2010; Çalışkan, 2009). In contrast to this finding, Aygün et al. (2022) reported that the germination time of quinoa was shortened with increasing heavy metal concentration and the response of genotypes was different in terms of germination time. This showed that the responses of plant species were variable in terms of germination time under heavy metal stress.

Table 3. Sweet sorghum mean germination time values determined in different heavy metal types, metal levels and priming treatments (day).

Çizelge 3. Farklı ağır metal tipi, ağır metal düzeyi ve priming uygulamaları altında tatlı sorgumun ortalama çimlenme süresi değerleri (%).

		Heavy Metal Levels	Heavy Metal Types: Lead	Cadmium	Nickel	MEAN (Priming)
Priming	H_2O	0 mg l-1	1.91 g-o[ǂ]	1.61 j-o	1.25 no	2.26 A[*]
		75 mg l-1	2.00 f-n	1.72 h-o	1.16 o	
		150 mg l-1	3.36 a	2.90 a-d	2.24 c-k	
		300 mg l-1	3.22 ab	3.35 a	2.43 c-i	
	GA_3	0 mg l-1	1.43 l-o	1.30 mno	1.49 k-o	2.05 B
		75 mg l-1	1.72 h-o	1.70 h-o	2.36 c-j	
		150 mg l-1	1.71 h-o	2.47 b-h	2.25 c-k	
		300 mg l-1	1.94 g-o	3.21 ab	3.01 abc	
	KNO_3	0 mg l-1	1.65 i-o	1.69 h-o	1.76 g-o	2.25 A
		75 mg l-1	1.86 g-o	2.32 c-j	2.14 d-l	
		150 mg l-1	2.78 a-f	2.42 c-i	2.53 b-g	
		300 mg l-1	2.84 a-e	2.08 e-m	3.00 abc	
MEAN (Heavy Metal Types)			2.20	2.23	2.13	

MEAN (Heavy Metal Levels)	0 mg l-1	75 mg l-1	150 mg l-1	300 mg l-1
	1.56 D[+]	1.89 C	2.52 B	2.79 A

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(+) Means with different capital letters in the same line are statistically different from each other.
(ǂ) Means with different lowercase letters in the same row and column are statistically different from each other.

The mean germination time of sweet sorghum showed significant differences depending on the priming treatments. The mean germination times for H_2O, GA_3 and KNO_3 were calculated as 2.26, 2.05 and 2.25 days, respectively (Table 3). GA_3 pre-treatment provided statistically faster germination than the other two priming treatments. Akar and Atış (2019) determined that GA_3 provided faster germination in red fescue, while Akar and Atış (2018) reported that priming treatments did not significantly affect the germination time of tall fescue under heavy metal stress.

The mean germination time varied between 1.70 days and 2.62 days depending on the heavy metal type × priming interaction (Figure 2a). The fact that the seeds reacted differently to different heavy metal types in different priming treatments in terms of mean germination time caused the interaction to be significant. It can be said that GA_3 pre-treatment can provide faster germination in fields contaminated with lead, while H_2O pre-treatment can provide faster germination in fields contaminated with nickel. KNO_3 pre-treatment did not cause earlier germination regardless of heavy metal type. Galhaut et al. (2014) reported that germination time in heavy metal contaminated fields varied depending on the priming agent used and priming duration.

The mean germination times varied between 1.41 days and 3.00 days depending on the heavy metal level x priming interaction (Figure 2b). The fastest germination was obtained in the control treatment pre-treated with GA_3. However, this value was not statistically different from the other priming pre-treatments. GA_3 priming pre-treatment resulted in faster germination than other priming pre-treatments at a heavy metal dose of 150 mg l^{-1}. When the heavy metal dose increased to 300 mg l^{-1}, there were no significant differences among the pre-treatments.

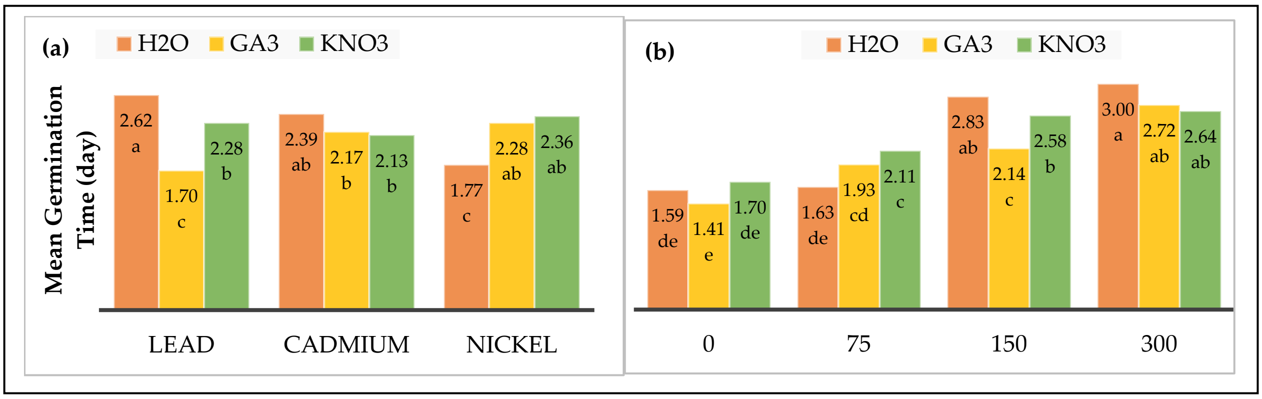

Figure 2. Effect of (a) heavy metal type × priming interaction, (b) heavy metal level × priming interaction, on mean germination time.

Şekil 2. Ortalama çimlenme süresi üzerine (a) ağır metal tipi × priming, (b) ağır metal düzeyi × primingi interaksiyonlarının etkisi.

According to the averages of heavy metal level x heavy metal type x priming interaction, the mean germination time varied between 1.16 days and 3.36 days (Table 3). The germination speed of lead treated seeds was generally maintained with GA_3 pretreatment despite the increasing heavy metal level. In cadmium and nickel treatments, mean germination time was prolonged with increasing level for all priming treatments. GA_3 and H_2O treatments generally prevented the mean germination time of seeds exposed to 75 mg l^{-1} nickel and cadmium from prolonging compared to the control, except for the nickel GA_3 treatment.

Germination Index

The effect of experimental factors and their binary interactions on germination index was found to be statistically significant at a 1% level of significance, while the effect of triple interactions was found to be insignificant (Table 1). According to the results of the research, germination index values differed significantly depending on the heavy metal type, and the germination indices of sweet sorghum under lead (15.4) and nickel (14.9) stress were significantly higher than the germination index determined in plants under cadmium stress (10.7) (Table 4). This indicates that the presence of cadmium in the germination medium has a more negative effect on germination. It was also reported by other researchers that the germination index decreased under heavy metal stress and the effect level of each heavy metal was different (Akar and Atış, 2018; Ertekin and Bilgen, 2021; Ertekin et al., 2020). As a matter of fact, Çalışkan (2009) reported that this negative effect varies according to different ionic properties of heavy metals and tolerance characteristics of plant species. In addition, the researcher emphasized that it is possible to rank the degree of impact of the heavy metals used as Cd>Cr>Ni>Pb starting from toxic. Germination index values of sweet sorghum showed significant differences depending on heavy metal levels. Germination index values varied between 7.7 and 19.9 depending on heavy metal doses (Table 4). Seeds exposed to heavy metals germinated significantly slower and less than the control. The germination index values of heavy metal treated seeds decreased significantly for each increasing level. Our findings that increasing heavy metal dose decreased the germination index support the results of some other researchers (Akar and Atış, 2018; Akar and Atis, 2018; Akıncı and Akıncı, 2011).

Priming treatments caused significant differences in germination index. While the germination index was 15.3 and 14.5 in H_2O and GA_3 treatments, respectively, these two values were statistically similar. KNO_3 treatment had a lower germination index value (11.2) compared to other pretreatments (Table 4). This indicates that H_2O and GA_3 treatments gave better results in terms of promoting germination of sweet sorghum. Similarly, Espanany et al (2016) reported that the effect of different priming treatments varied in the presence of heavy metals and salicylic acid pretreatment improved germination characteristics in black cumin under cadmium stress. In similar studies carried out in different plants under heavy metal stress, it was reported that priming applications generally positively affected germination (Kumar et al., 2016; Moulick et al., 2016). In parallel with our findings, Sneideris et al. (2015) found that priming with hormones improved germination characteristics, but the values obtained in these treatments were not statistically different from hydropriming (H_2O). Akar and Atış (2018) found that the germination index of tall fescue under heavy metal stress was negatively affected by GA_3 and KNO_3 priming treatments. This indicates that plant species under heavy metal stress react differently to priming agents.

Table 4. Sweet sorghum germination index values determined in different heavy metal types, metal levels and priming treatments.

Çizelge 4. Farklı ağır metal tipi, ağır metal düzeyi ve priming uygulamaları altında tatlı sorgumun çimlenme indeksi değerleri.

		Heavy Metal Levels	Heavy Metal Types: Lead	Cadmium	Nickel	MEAN (Priming)
Priming	H_2O	0 mg l^{-1}	19.4	20.8	22.8	15.3 A*
		75 mg l^{-1}	18.9	15.2	23.6	
		150 mg l^{-1}	12.0	7.0	16.3	
		300 mg l^{-1}	11.1	3.9	12.2	
	GA_3	0 mg l^{-1}	21.2	21.4	20.5	14.5 A
		75 mg l^{-1}	17.6	10.6	14.9	
		150 mg l^{-1}	17.2	10.5	15.2	
		300 mg l^{-1}	11.7	6.2	7.6	
	KNO_3	0 mg l^{-1}	17.3	18.5	17.4	11.2 B
		75 mg l^{-1}	16.5	8.6	13.6	
		150 mg l^{-1}	12.2	4.0	9.6	
		300 mg l^{-1}	10.2	1.9	4.9	
MEAN (Heavy Metal Types)			15.4 A+	10.7 B	14.9 A	

MEAN (Heavy Metal Levels)	0 mg l^{-1}	75 mg l^{-1}	150 mg l^{-1}	300 mg l^{-1}
	19.9 A++	15.5 B	11.6 C	7.7 D

(*) Means with different capital letters in the same column are statistically different from each other.
(+) Means with different capital letters in the same line are statistically different from each other.
(++) Means with different capital letters in the same line are statistically different from each other.

Sweet sorghum germination index values determined as a results of heavy metal type × heavy metal level interaction were given in Figure 3a. As seen in the figure, germination index values varied between 4.0 and 20.2. Seeds exposed to cadmium had lower germination index values than seeds exposed to other heavy metals. Also, the germination index value was not different from the control treatment in 75 mg l^{-1} lead treatment.

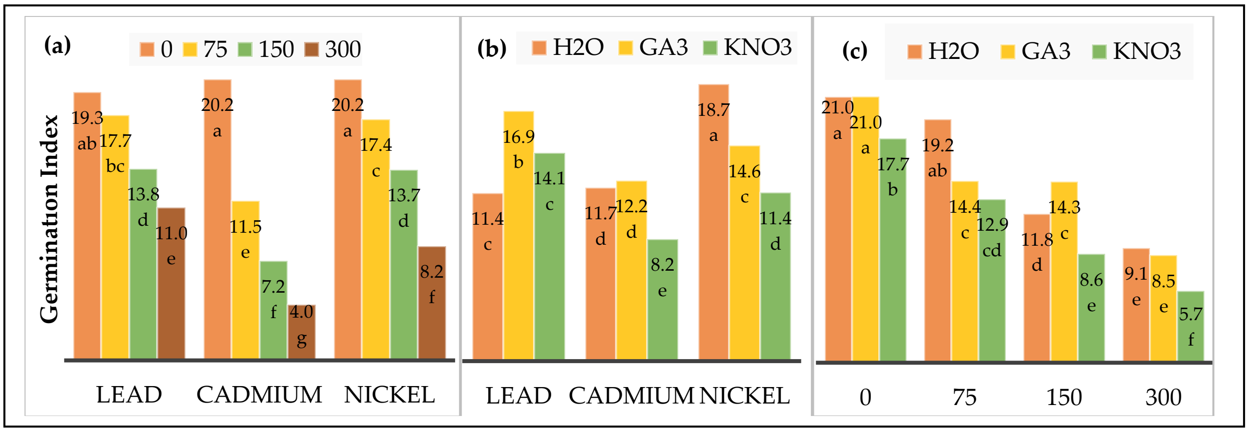

Figure 3. Effect of (a) heavy metal type × heavy metal level interaction, (b) heavy metal type × priming interaction, (c) heavy metal level × priming interaction on germination index.

Şekil 3. Çimlenme indeksi üzerine (a) ağır metal tipi × ağır metal düzeyi, (b) ağır metal tipi × priming, (c) ağır metal düzeyi × priming interaksiyonlarının etkisi.

Germination index values varied between 8.2 and 18.7 according to the heavy metal type × priming interaction. While H_2O was the best priming treatment for sweet sorghum under nickel stress, the highest value for lead and cadmium was obtained in the GA_3 priming treatment. KNO_3 was the pretreatment with the lowest germination index value for all heavy metal types (Figure 3b).

Germination index values varied between 5.7 and 21.0 depending on the heavy metal dose x priming interaction (Figure 3c). It can be said that the germination index value decreased with increasing heavy metal levels. However, the fact that the control and 75 mg l^{-1} heavy metal level treatments subjected to H_2O pretreatment and 75 and 150 mg l^{-1} heavy metal level treatments subjected to GA_3 pretreatment were statistically in the same group may have caused the interaction to be significant. At 75 mg l^{-1} heavy metal level, H_2O pretreatment gave better results, while at 150 mg l^{-1} level GA_3 pretreatment gave better results.

Root Length

The results of the analysis of variance applied to the root length values of sweet sorghum showed that the effects of heavy metal type, metal levels and priming treatments on root length were significant at a 1% level of significance. In addition, heavy metal type × metal level and heavy metal level × priming interactions were also significant at a 1% level (Table 1). The average root lengths determined for lead, cadmium and nickel were 22.01, 18.43 and 17.31 mm respectively, while the values determined for cadmium and nickel were statistically similar. The mean value determined for lead was statistically higher than the others (Table 5).

Table 5. Sweet sorghum root length values determined in different heavy metal types, metal levels and priming treatments (mm).

Çizelge 5. Farklı ağır metal tipi, ağır metal düzeyi ve priming uygulamaları altında tatlı sorgumun kök uzunluğu değerleri (mm).

			Heavy Metal Types			MEAN
		Heavy Metal Levels	**Lead**	**Cadmium**	**Nickel**	**(Priming)**
Priming	**H_2O**	**0 mg l^{-1}**	90.75	83.20	72.55	**22.29 A***
		75 mg l^{-1}	13.70	1.00	1.00	
		150 mg l^{-1}	1.00	1.00	1.00	
		300 mg l^{-1}	1.00	0.25	1.00	
	GA_3	**0 mg l^{-1}**	63.15	67.55	68.73	**18.48 B**
		75 mg l^{-1}	11.75	1.00	1.00	
		150 mg l^{-1}	4.78	1.00	1.00	
		300 mg l^{-1}	1.00	0.25	0.50	
	KNO_3	**0 mg l^{-1}**	60.08	62.98	57.93	**16.98 B**
		75 mg l^{-1}	14.90	1.00	1.00	
		150 mg l^{-1}	1.00	1.88	1.00	
		300 mg l^{-1}	1.00	0.00	1.00	
MEAN (Heavy Metal Types)			**22.01 A+**	**18.43 B**	**17.31 B**	
MEAN (Heavy Metal Levels)		0 mg l^{-1}	75 mg l^{-1}	150 mg l^{-1}	300 mg l^{-1}	
		69.66 A++	**5.15 B**	**1.52 C**	**0.67 C**	

(*) Means with different capital letters in the same column are statistically different from each other.
(+) Means with different capital letters in the same line are statistically different from each other.
(++) Means with different capital letters in the same line are statistically different from each other.

Ertekin et al. (2020) reported similar results for sorghum. The degree of effect of heavy metals on the root length of plants varies. Indeed, Aydinalp and Marinova (2009) reported that low doses (5 and 10 ppm) of chromium, copper, nickel, and zinc increased the root length of alfalfa compared to the control. They reported that the doses of heavy metals, except nickel, above 20 ppm significantly reduced root length and the degree of effect varied according to the metal type. Akar and Atis (2018) reported that the negative effect of nickel on the root length of perennial ryegrass was higher than cadmium, while Akar and Atış

(2018) reported that the root lengths of tall fescue under nickel and cadmium stress were similar. This shows that the effect of heavy metals varies according to plant species.

Increasing heavy metal levels caused a significant decrease in root length. Increasing the heavy metal level to 75 mg l^{-1} caused a 13.5-fold decrease in root length compared to the control and increasing the heavy metal level to 150 mg l^{-1} caused a 45.8-fold decrease (Table 5). Some researchers reported previously that root length decreased with increasing cadmium, nickel, and lead doses in different plants (Ertekin et al., 2020; Mami et al., 2011; Shafiq et al., 2008; Shao et al., 2011). The 300 mg l^{-1} level caused root growth to decrease below 1 mm on average. This shows that even if germination starts and takes place, heavy metal stress even at low doses can severely inhibit root growth.

Root length values were 22.29, 18.48 and 16.98 mm for H_2O, GA_3 and KNO_3, respectively, depending on priming treatments. Priming with H_2O generally resulted in higher root length (Table 5). In contrast to our findings, Akar and Atis (2018) reported that KNO_3 promoted root elongation in perennial ryegrass under heavy metal stress. Akar and Atış (2019) reported that the mean root length values of red fescue under heavy metal stress were similar for KNO_3, GA_3 and hydropriming pretreatments. This indicates that the effect of priming agents under abiotic stress conditions varies according to plant species.

The mean root length values varied between 1.00 mm and 71.33 mm depending on the heavy metal type × heavy metal level interaction (Figure 4a). While higher root lengths were generally found in control treatments, heavy metal treatments even at low levels caused a significant decrease in root length. However, 75 mg l^{-1} lead treatment showed higher root length than cadmium and nickel treatments at the same level. Increasing the heavy metal dose to 150 and 300 mg l^{-1} caused similar and lower root lengths regardless of the heavy metal type. This showed that cadmium and nickel had a higher toxic effect on root development than lead (Ertekin et al., 2020).

As shown in Figure 4b, root lengths varied between 0.58 mm and 82.17 mm depending on the heavy metal level × priming interaction. Control treatments generally gave higher root length values. Root lengths did not show a significant difference among heavy metal treatments, but the values measured in all heavy metal treatments were significantly lower than the control treatments. However, the significant difference in root lengths within the control treatments depending on the pre-treatment treatments caused the interaction to be significant. Indeed, higher root length was measured in H_2O pretreatment compared to GA_3 and KNO_3 pre-treatments. This indicates that the use of seed priming agents is not necessary in terms of root length in sugar sorghum. However, Galhaut et al (2014) found that the effect of priming agents on seedling growth in heavy metal contaminated areas depends on soil properties.

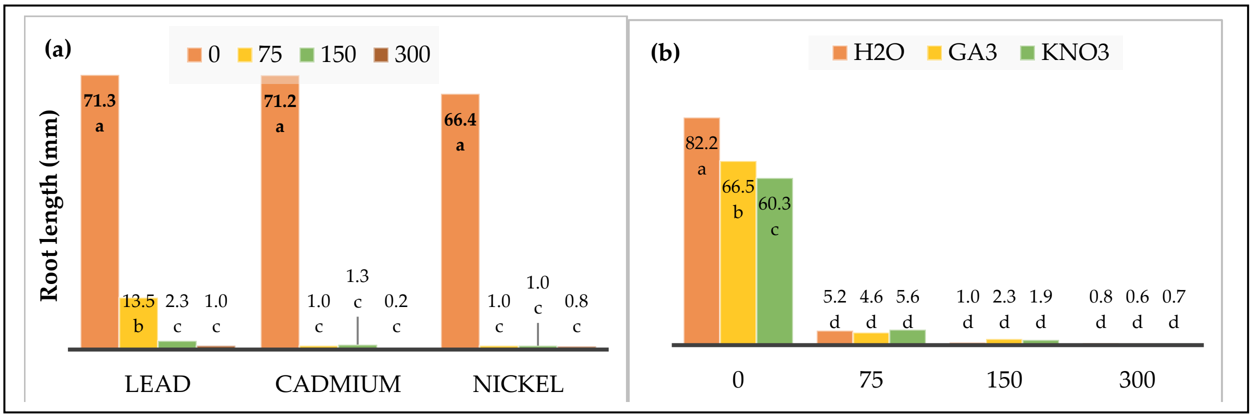

Figure 4. Effect of (a) heavy metal type × heavy metal level interaction, (b) heavy metal level × priming interaction on root length.

Şekil 4. Kök uzunluğu üzerine (a) ağır metal tipi × ağır metal düzeyi, (b) ağır metal düzeyi × priming interaksiyonlarının etkisi.

Shoot Length

As shown in Table 1, the effect of the experimental factors individually and the effects of double and triple interactions on shoot length were found statistically significant at a 1% level. According to the results of the research, it was determined that shoot lengths differed significantly depending on the heavy metal type, and the shoot length of sweet sorghum under lead stress (56.38 mm) was significantly higher than the shoot length values determined in seedlings under cadmium (24.46 mm) and nickel (25.65 mm) treatments (Table 6). Especially the presence of cadmium and nickel in the germination medium had a more negative effect on shoot length. Ertekin et al. (2020) reported similar results for cadmium, nickel and lead in terms of shoot length of sorghum.

Table 6. Sweet sorghum shoot length values determined in different heavy metal types, metal levels and priming treatments (mm).

Çizelge 6. Farklı ağır metal tipi, ağır metal düzeyi ve priming uygulamaları altında tatlı sorgumun sürgün uzunluğu değerleri (mm).

			Heavy Metal Types			MEAN (Priming)
		Heavy Metal Levels	**Lead**	**Cadmium**	**Nickel**	
Priming	**H_2O**	**0 mg l^{-1}**	67.68 c[ǂ]	64.75 c	63.38 c	**30.11 B[*]**
		75 mg l^{-1}	60.95 c	13.58e-h	17.30 ef	
		150 mg l^{-1}	42.58 d	6.40ghi	6.28 ghi	
		300 mg l^{-1}	15.18 efg	1.25 i	1.96 i	
	GA_3	**0 mg l^{-1}**	96.38 a	105.68 a	105.55 a	**45.75 A**
		75 mg l^{-1}	78.30b	13.20 e-h	13.63 e-h	
		150 mg l^{-1}	79.13 b	6.62 ghi	8.27 f-i	
		300 mg l^{-1}	39.54 d	1.13 i	1.58 i	
	KNO_3	**0 mg l^{-1}**	67.80 c	70.38 bc	65.15 c	**30.63 B**
		75 mg l^{-1}	60.25 c	7.64 f-i	19.47e	
		150 mg l^{-1}	45.66 d	2.88hi	3.62 hi	
		300 mg l^{-1}	23.15 e	0.00 i	1.63 i	
MEAN (Heavy Metal Types)			**56.38 A[+]**	**24.46 B**	**25.65 B**	
MEAN (Heavy Metal Levels)		0 mg l^{-1}	75 mg l^{-1}	150 mg l^{-1}		300 mg l^{-1}
		78.53 A[++]	**31.59 B**	**22.38 C**		**9.49 D**

([*]) Means with different capital letters in the same column are statistically different from each other.
([+]) Means with different capital letters in the same line are statistically different from each other.
([ǂ]) Means with different lowercase letters in the same row and column are statistically different from each other.
([++]) Means with different capital letters in the same line are statistically different from each other.

Shoot lengths differed significantly with increasing heavy metal levels. Shoot lengths were 78.53, 31.59, 22.38 and 9.49 mm for increasing levels, respectively (Table 6). A continuous decrease was observed with increasing heavy metal levels and each level was in a separate statistical group. It can be said that seedling development was suppressed due to increasing heavy metal levels. The results of some previous studies supported our findings (Akar and Atış, 2018; Kabir et al., 2008; Shao et al., 2011). The mean shoot length of sweet sorghum was significantly different depending on the priming treatments. The mean shoot lengths for H_2O, GA_3 and KNO_3 were 30.11, 45.75 and 30.63 mm, respectively. GA_3 treatment had statistically higher shoot length than the other two treatments (Table 6). In general, it can be said that GA_3 promotes shoot growth. Similarly, some previous research results showed that GA_3 promoted shoot growth under heavy metal stress. (Akar and Atış, 2019; Akar and Atis, 2018). In another study conducted on cumin, it was reported that different priming agents had positive effects on stem length in the presence of high doses of cadmium (Espanany et al., 2016).

The mean seedling length values varied between 80.27 mm and 0.79 mm depending on the heavy metal type × metal level interaction (Figure 5a). The seeds exposed to nickel had higher shoot length values than the seeds exposed to lead and cadmium, although it decreased with increasing heavy metal levels. The 75 mg l^{-1} cadmium treatment had lower shoot length values than the same level of nickel and lead treatments. At 150 and 300 mg l^{-1} levels, the shoot length values determined in cadmium and nickel treatments were statistically indistinguishable. Aydinalp and Marinova (2009) determined that the negative effects of different heavy metals on shoot growth differed depending on the concentration of the heavy metal. They reported that some heavy metals increased shoot length at low doses, while some heavy metals had serious negative effects even at low doses.

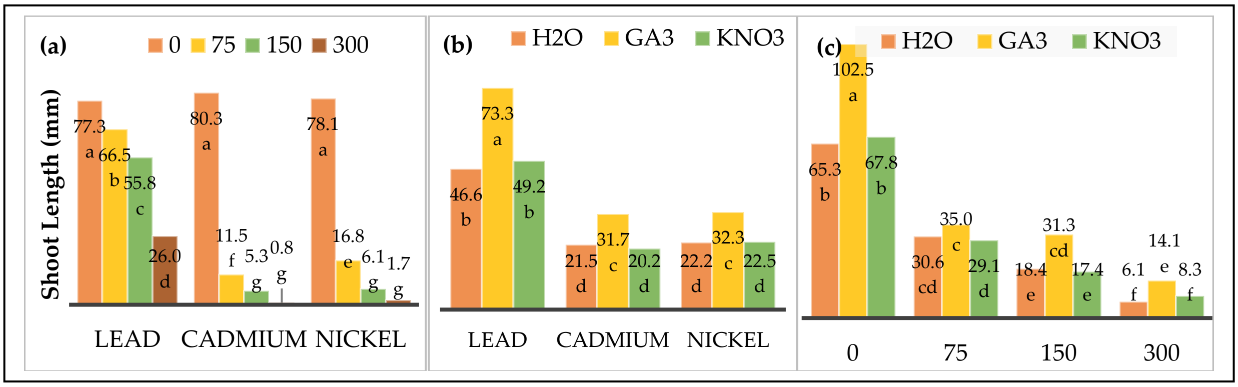

Figure 5. Effect of (a) heavy metal type × heavy metal level interaction, (b) heavy metal type × priming interaction, (c) heavy metal level × priming interaction on shoot length.
Şekil 5. Sürgün uzunluğu üzerine (a) ağır metal tipi × ağır metal düzeyi, (b) ağır metal tipi × priming, (c) ağır metal düzeyi × priming interaksiyonlarının etkisi.

The results of the analysis of variance showed that the heavy metal type × priming interaction had a significant effect on mean shoot length (Table 1). Seeds responded differently to different priming treatments in terms of mean shoot length, which caused the interaction to be significant. The mean shoot length varied between 20.22 mm and 73.34 mm depending on the heavy metal type x priming interaction (Figure 5b). It can be said that a better shoot growth can be achieved with GA_3 pretreatment in areas contaminated with heavy metals used.

The mean shoot lengths varied between 102.53 and 6.13 mm depending on the heavy metal level × priming interaction (Figure 5c). The highest shoot length was obtained in the control treatment pretreated with GA_3, while this value was statistically higher in the other priming treatments than in the control treatment. The shoot length values decreased with increasing heavy metal levels. In general, higher shoot length values were determined in GA_3 pretreatment. Similarly, Akar and Atis (2018) found that GA_3 positively affected shoot growth in the presence of heavy metals in their study on perennial ryegrass.

Analysis of variance results (Table 1) showed that the effect of heavy metal type x heavy metal dose x priming triple interaction on shoot length was statistically significant. Shoot length values ranged from 105.68 mm to 0.00 mm among all treatments (Table 6). In seeds exposed to cadmium and nickel, shoot length generally decreased more severely with increasing heavy metal levels. At 150 and 300 mg l^{-1} levels of cadmium and nickel, shoot growth almost completely stopped.

CONCLUSION

The results of the study showed that heavy metals in the germination medium of sweet sorghum were a significant suppressor of germination and seedling growth. However, it was determined that the negative effect varied depending on the type of heavy metal present in the germination medium. In general, it can be said that the limiting effect of lead on both germination and seedling growth characteristics is less than cadmium and nickel. In terms of priming applications, it can be said that pre-treatment with H_2O was generally sufficient, while the other two priming agents did not provide the expected benefit in terms of both germination and seedling growth. Although it is possible to mention a partial benefit of GA_3 pretreatment in terms of root development and KNO_3 pretreatment in terms of seedling development, no significant benefit was determined for both traits compared to H_2O pretreatment. As a result, it was determined that even at low levels of the heavy metals used, germination and seedling growth were limited, especially in areas contaminated with cadmium, sweet sorghum could not grow even at low doses. In areas contaminated with lead, it was observed that germination and seedling growth of sweet sorghum could be realized up to a dose of 150 mg l^{-1}. It was determined that pre-treatment with H_2O would be sufficient in terms of germination and seedling growth, and we can say that the priming agents of GA_3 and KNO_3 used are not necessary. However, it was evaluated that it may be useful to try other priming agents that may provide more effective benefits.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

İ. A.; Planning the research, providing the experiment material, writing the manuscript, **H. Ç.**; Conducting laboratory studies, making measurements and observations, **İ. E.**; Planning of laboratory studies, data processing, statistical analysis

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