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THE EFFECTS OF PRIMING PRETREATMENTS ON GERMINATION AND SEEDLING GROWTH IN PERENNIAL RYEGRASS EXPOSED TO HEAVY METAL STRESS

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ABSTRACT

This research was conducted to determine the effects of priming treatments (GA_3, KNO_3 and Hydropriming) on germination and seedling growth in perennial ryegrass contaminated with different doses (0, 75, 150, and 300 mg l^{-1}) of cadmium and nickel. The study was conducted under laboratory conditions. Perennial ryegrass cv. Integra was used as the plant material. The results showed that both germination and seedling growth properties were adversely affected by heavy metals. The adverse effects of nickel were more pronounced than those of cadmium. The GA_3 seed priming pretreatment had positive effects on both germination properties and seedling growth. As a result, GA_3 can be used as a seed priming agent if the perennial ryegrass is to be grown in areas contaminated with nickel and cadmium.

KEYWORDS:
Heavy Metal, Priming, Germination, Perennial Ryegrass, Nickel, Cadmium.

INTRODUCTION

Healthy seed germination plays a critical role in ensuring desired plant density and successful production [1, 2]. The success of germination and emergence depends on the interaction between the environmental conditions in the seed bed and the seed-quality [1]. Many of the biotic and abiotic stress factors present in the seed bed prevents the desired plant density from being achieved. Heavy metals are the major abiotic stress factors that cause stress on the plant. Much of the research investigating the effects of heavy metals on plants has been carried out on grown or already germinated seedlings in order to determine the tolerance of heavy metals on plants and the amounts that can be taken up by plants [3]. However, it is a fact that plants that fail to germinate cannot become seedlings and mature plants. For this reason, it is very important to know the effect of heavy metals existing in the germination environment of a plant species on germination and seedling-forming ability.

Cadmium (Cd) is a highly toxic metal that has come to the fore because its various uses and its important role in environmental pollution. The main reason that cadmium has recently been on the agenda as a pollutant is that it is toxic, even at very low doses and the biological half-life is long [4, 5, 6]. Plants are adversely affected by increasing cadmium doses during whole growth period [7], but the cadmium tolerance thresholds of the various plant species are different [3, 8, 9, 10, 11, 12]. For nickel (Ni), low doses are required for the plant [13], but high concentrations are known to have adverse effects [14, 15].

Necessary precautions must be taken in the presence of these stress conditions. Priming treatments can provide successful germination results in the presence of stress conditions. Priming is the generic name for commercially accepted seed practices that promote uniform germination and emergence. Agents such as KNO_3, PEG [16], $CuSO_4$ [17], and GA_3 [18] can be used for priming treatments.

Lawn plants are of great importance in terms of environmental protection and pollution reduction. The plants in this group have characteristics of strong regeneration, rapid growth and development, and frequent cutting during the year. It is possible to reduce the heavy metals found in the environment, especially through cutting, which is done many times during the year [19]. Perennial ryegrass (*Lolium perenne* L.) is one of the most preferred plant species for this purpose [20, 21, 22, 23].

In this study, we aimed to determine the effect on the germination properties of perennial ryegrass of different nickel and cadmium doses and to determine the effect of KNO_3 and GA_3 as priming material and hydropriming as control in order to encourage germination under cadmium and nickel stress conditions.

MATERIALS AND METHODS

Plant materials and treatment condition. The research was conducted in laboratory conditions in the Field Crops Department of the Mustafa Kemal University Faculty of Agriculture. In the experiment, the seeds of perennial ryegrass (*Lolium perenne* L. cv. Integra) were used as plant material. In the experiment, Cd was used in the form of cadmium sulfate hydrate ($3CdSO_4.8H_2O$) and Ni in the form of nickel (ii) sulfate hexahydrate ($NiSO_4.6H_2O$). Potassium nitrate (KNO_3) and gibberellic acid (GA_3) were used as priming agents.

Methods. In the study, three different doses of cadmium and nickel (75, 150, 300 mg l^{-1}) and distilled water as the control treatment were used as seed germination mediums. To determine the effect of the priming treatments on germination under heavy metal stress, 2% KNO_3 and 500 ppm GA_3 were used as priming agents. The priming treatments were carried out by soaking seeds in KNO_3 and GA_3 solutions prepared at the indicated doses for two days (48 hours) at 25 ° C after applying surface sterilization to the seeds. In addition, a group of non-priming seeds were prepared for planting in soaked distilled water (hydropriming) under the same conditions as the 48-hour priming treatments after sterilization. The seeds to which the required pretreatment had been applied were planted in petri dishes of 9 cm diameter containing in two layers of filter paper. Twenty-five seeds were placed in each petri dish. 10 ml of the stock solution prepared in doses of 75, 150, 300 mg l^{-1} of both heavy metals (previously prepared as 1L stock solution) and pure water was used as a control treatment. The petri dishes were placed in a climate chamber set at a temperature of 25 ± 1 ° C and 16/8 h light/dark conditions. The petri dishes were placed in a growth container set at a temperature of 25 ± 1 °C and 16 h light/8 h dark conditions. Observations were carried out only until the 14th day due to the risk of infection although observations were planned to be continued until the 21st day. Germination data were recorded daily until the 14th day. The seedling growing measurements were made on the 14th day for 10 seedlings per petri dish. Germination percentage (GP) [24], germination index (GI) [25], and mean germination time (MGT) [26] values were calculated according to the following formulas to interpret germination characteristics:

GP(%)= (number of seeds germinated / total number of seeds sown) × 100;

$MGT= \Sigma(n.D)/\Sigma n$

where n is the number of newly germinated seeds on each day, and D is the day of counting;

$GI = \sum(Gt/Tt)$

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

The experimental design was three factorial, arranged in a completely randomized design with four replications. The first factor was the kind of heavy metal (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). An analysis of variance was performed using the MSTAT-C software package. Significant differences among the mean values were grouped by the Duncan test ($P < 0.05$).

RESULTS AND DISCUSSION

Responses to the kind of heavy metal. The effects of the kinds of heavy metal were statistically significant in terms of germination percentage (GP), mean germination time (MGT), and root length while nonsignificant in term of germination index (GI) and shoot length (Table 1). The GP (83.83%) of perennial ryegrass under nickel stress was significantly lower than the GP (88.92%) of perennial ryegrass under cadmium stress (Table 2). Similarly, some previous researchers reported that the fatal effects of nickel on plant seedlings were greater than for cadmium [27, 28] while Caliskan [29] reported that cadmium was more toxic than nickel for plant seedling. This situation showed that the response of plant species to different heavy metal kinds is different, and this reaction is very important in terms of the success of plant growth. The germination index value determined for plants under cadmium stress was 5.21 while the mean germination index value for plants under nickel stress was found to be 5.31, but this difference was statistically insignificant (Table 1, 2). Although the effects of cadmium and nickel on the germination index of perennial ryegrass are not related to each other, it can be said that adverse effects on germination are due to increasing doses of both metals. As a matter of fact, Caliskan [29] reported that the adverse effects of heavy metals on germination could vary depending on the kind of metal and its dose.

The mean germination time value was 4.31 days under cadmium stress while the mean germination time value under nickel stress was 5.27 days (Table 1). This situation showed that germination of perennial ryegrass under nickel stress was more delayed than cadmium stress conditions. Heavy metals delay the germination of plants, but this effect may vary depending on the plant species, the kind of heavy metal and the dose of heavy metal that was applied [29]. The delay of germination increases the likelihood of the seed being exposed to adverse environmental conditions (drought, insects, frost, excessive water, etc.) and lessens the emergence of homogenous seedlings and the achievement of adequate plant density. The root length value (12.43 mm) recorded under nickel stress was significantly lower than in the root length value (17.02 mm) recorded under cadmium stress. Our results indicated that the adverse effects of nickel on the root length of perennial ryegrass is greater than that of cadmium during the germination period [27, 28]. In the study conducted by Peralta-Videa et al. [28] with different kinds of heavy metals, it was determined that the most accumulated heavy metal in alfalfa was nickel. This result is explained by the fact that nickel has a

TABLE 1
Mean squares of the combined analysis of variance for investigated characters

Source of variation	d.f	Mean Squares				
		GP (%)	GI	MGT (day)	Root Length	Shoot Length
Heavy Metal Kind (A)	1	504.167**	0.254	22.042**	506.231**	0.970
Heavy Metal Dose (B)	3	697.056**	5.324**	12.103**	1734.256**	333.795**
A X B	3	183.278*	0.467	8.511**	68.599**	12.435
Priming (C)	2	9.500	1.681**	5.066**	118.906**	3382.331**
A X C	2	16.167	0.172	0.250	1.719	80.452*
B X C	6	222.389**	1.283**	0.930	64.042**	27.926
A X B X C	6	9.944	0.523	1.311	18.320	15.709
Residual	72	48.611	0.279	16.453	11.675	25.132

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

TABLE 2
Effect of heavy metal kind, heavy metal dose and priming treatment on germination percentage (GP), mean germination time (MGT), germination index (GI), root length and shoot length

	GP (%)	GI	MGT(day)	Root Length (mm)	Shoot Length (mm)
Heavy Metal Kind					
Cadmium	88.92 a*	5.21	4.31 b	17.02 a	49.53
Nickel	83.83 b	5.31	5.27 a	12.43 b	49.33
Heavy Metal Dose					
0 mg l^{-1}	92.67 a	5.62 a	4.21 b	27.38 a	53.89 a
75 mg l^{-1}	89.33 a	5.58 a	5.08 a	12.01 b	50.90 b
150 mg l^{-1}	83.83 b	5.25 b	5.08 a	9.84 c	47.46 c
300 mg l^{-1}	80.67 b	4.60 c	4.80 a	9.68 c	45.46 c
Priming					
Hydropriming	87.00	5.34 a	4.67 b	14.15 b	40.81 c
GA_3	86.88	5.44 a	4.59 b	13.16 b	60.81 a
KNO_3	86.00	5.00 b	5.11 a	16.88 b	46.66 b

*Means followed by the same letters in each column are not significantly different at P= 0.05 probability level

more dominant influence on some plants than cadmium. The effects of kinds of heavy metals were statistically insignificant in terms of shoot length (Table 1). The shoot length determined for plants under cadmium stress was 49.53 while the shoot length for plants under nickel stress was 49.33. The results of the study indicated that the effects of nickel and cadmium were different for the underground parts of perennial ryegrass but similar for its aboveground parts.

Responses to heavy metal doses. Germination percentages varied significantly depending on the increase in heavy metal doses (Table 1). Germination percentages were determined as 92.67%, 89.33%, 83.83%, and 80.67% depending on the increasing doses (respectively for 0, 75, 150, and 300 mg l^{-1}). A continuous decrease in the percentage of germination due to increased heavy metal doses was observed while germination percentages determined at control (0 mg l^{-1}) and at a dose of 75 mg l^{-1} were statistically similar. The germination percentages determined at doses of 150 and 300 mg l^{-1} were not statistically different from each other (Table 2). Other researchers reported that the presence of heavy metals in the growing medium adversely affected germi-nation, and the increased heavy metal doses increased the negative effect on germination of plants [9, 11, 29]. The germination index values decreased significantly due to increased metal doses. The germination index values were determined as 5.62, 5.58, 5.25, and 4.60 for heavy metal doses of 0, 75, 150, and 300 mg l^{-1}, respectively. The increase of the heavy metal dose to 75 mg l^{-1} did not cause a significant decrease in the germination index value compared to the control. The increase of the heavy metal dose to 150 mg l^{-1} did cause a significant decrease in the germination index value compared to the control and 75 mg l^{-1}. The germination index value determined at 300 mg l^{-1} was significantly lower than all other doses (Table 2). Also, other researchers determined that significant reductions in the germination index occurred due to increased heavy metal doses [29, 30]. The mean germination times varied between 4.21 days and 5.08 days depending on the heavy metal doses. Seeds exposed to heavy metals germinated considerably more slowly than those in the control group. The mean germination times of heavy metal applied seeds (75, 150, and 300 mg l^{-1}) were statistically similar. This result indicated that heavy metals were an abiotic stress factor that delay germination [29, 30]. The effects of heavy metal doses were statistically significant in terms of root length (Table 1). Root lengths ranged from 9.68 mm to 27.38 mm depending on the metal doses (Table 2). Root length in the control group was statistically higher than in all the other groups treated with heavy metal. Generally, the root length decreased with increasing doses. While the root length measured at a 75 mg l^{-1} metal dose was statistically higher than the 150 mg l^{-1} and 300 mg l^{-1} doses, root lengths measured at doses of 150 and 300 mg l^{-1} were not statistically different from each other. Similar to our findings, the negative effects of nickel and cadmium on root growth have been reported by other researchers [8, 14, 30, 31]. Shoot lengths were significantly decreased depending on increasing heavy metal doses (Table 1). Shoot lengths were determined as 53.89, 50.90, 47.46, and 45.46 mm for heavy metal doses of 0, 75, 150, and 300 mg l-1, respectively. Shoot lengths of seeds exposed to heavy metals were significantly lower than for the control group. While the root length recorded at a 75 mg l^{-1} metal dose was statistically higher than the 150 mg l^{-1} and 300 mg l^{-1} doses, shoot lengths determined at doses of 150 and 300 mg l^{-1} were statistically similar to each other. While the findings of some previous researchers supported our results [9, 14, 28, 32], Kalaycioglu [33] reported that cadmium did not adversely affect the plant height of the common nettle (*Urtica dioica* L.). This finding demonstrates that the effect of heavy metals may vary according to the plant species.

Responses to seed priming pre-treatments. The effect of seed priming pre-treatments on germination percentages was statistically insignificant (Table 1). The germination percentages were 87.00%, 86.88%, and 86.00% for hydropriming, GA_3 and KNO_3, respectively (Table 2). Germination index values varied between 5.00 and 5.44, depending on seed priming pretreatments. The highest germination index value was identified with GA_3 treatment. The germination index value calculated for the KNO_3 treatment was statistically lower compared to hydropriming and GA_3 treatments while the germination index values of hydropriming and GA_3 treatments were statistically similar. The results of the study showed that KNO_3 treatment did not achieve the expected positive effect on the germination of perennial ryegrass under heavy metal stress. GA_3 could be preferred as a priming agent that contribute to the germination of perennial ryegrass under heavy metal stress. Espanany et al. [34] reported that the effect of priming pretreatments on different seeds varied in the presence of heavy metals (especially with high doses of heavy metals). Also, a dose of seed priming agent can play a decisive role in the effect on germination [33]. A GA_3 treatment provided the fastest mean germination time of 4.59 days while the hydropriming treatment provided 4.67 days -- a mean germination time very close to that with the GA_3 treatment. The mean germination time recorded with the KNO_3 treatment was statistically slower than that recorded with GA_3 and hydropriming treatments (Table1, 2). This result indicated that KNO_3 pretreatment did not provide the expected benefits in terms of the germination time of perennial ryegrass under cadmium and nickel stress. The effects of seed priming pretreatments on germination times and other germination properties may vary according to the different plant species and germination conditions. Galhaut et al. [36] reported that, while seed priming pretreatments provided significant advantages in soils contaminated with heavy metals, it did not provide an important benefit in uncontaminated soils, and the effects of the priming agents used were different from each other. Thus, for a successful germination and seedling growth, it is important to know the interaction between plant species, seed priming agents, and environmental conditions. The effects of seed priming treatments were significant in terms of root length (Table 1). Depending on the priming pre-treatments, root lengths ranged between 13.16 mm and 16.88 mm. While the longest root length value was determined with the KNO_3 pretreatment, the value determined in this application was found to be statistically higher than with the other two pretreatment applications (Table 2). This situation indicated that KNO_3 treatment promoted root growth in germinated seeds of perennial ryegrass although it did not show sufficient effect in initiating germination. The effect of seed priming pre-treatments on shoot length was statistically significant (Table 1). The shoot lengths were 40.81, 60.81 and 46.66 mm for hydropriming, GA_3 treatment and KNO_3 treatment, respectively. The seedling length determined in the GA_3 pretreatment was in the first rank with 60.81 mm, and this value was statistically higher than with other priming pretreatments. Our results show that GA_3 is a good seed priming agent especially in terms of seedling growth. Similarly, some other researchers have reported that the effect of different seed priming agents varies in the presence of heavy metals [34, 37].

Interaction effects. The factors of the interactions that were statistically significant in this section were interpreted. In this study, the effect of heavy metal kind x heavy metal dose and heavy metal dose x priming interactions on germination percentage, the effect of heavy metal dose x priming interaction on the germination index, the effect of heavy metal kind x heavy metal dose interaction on the mean germination time, the effect of heavy metal kind x heavy metal dose and heavy metal dose x priming interactions on root length, and the effect of heavy metal kind x priming interactions on shoot length were statistically significant (Table 1).

When the effect of metal kind x metal dose binary interaction on germination percentage was evaluated, differences among germination percentages calculated for cadmium doses of 0, 75, and 150 mg

l^{-1} were statistically insignificant, but the germination percentage of seeds exposed to cadmium stress significantly decreased with a dose of 300 mg l^{-1} (Figure 1a).

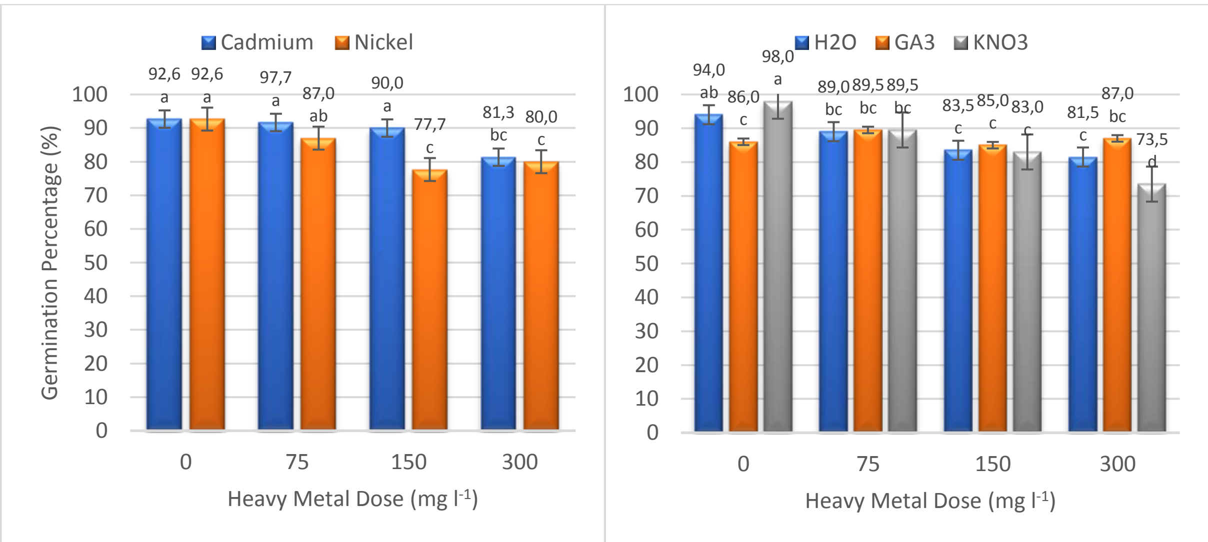
This result indicated that the breaking point was 300 mg l^{-1} in terms of germination percentage in perennial ryegrass exposed to cadmium stress. In nickel treatment, the germination percentages determined at doses of 150 and 300 mg l^{-1} were considerably lower than those of 0 and 75 mg l^{-1} (Figure 1a). Toxicity began at lower doses of nickel compared to cadmium in terms of germination of perennial ryegrass. The variance analysis results showed that the effect of metal dose x priming binary interaction on germination percentage had significance at a 1% probability level. (Table 1). The highest germination percentage was determined in the control (without heavy metal) application of the KNO_3 pretreatment. The value determined in this application was similar to the control application of the hydropriming pretreatment while it was statistically higher than the germination percentage of the control group pretreated with GA_3. The germination percentages of seeds pretreated with GA_3 were statistically similar in the control group and all heavy metal dose groups (Figure 1b). Although the highest germination percentage was obtained in the control application of seeds pretreated with KNO_3, the germination percentage of seeds pretreated with KNO_3 decreased significantly due to the increase in the heavy metal dose. This situation indicated that heavy metals could have entered into a negative interaction with KNO_3. Our results indicated that, although KNO_3 was an effective priming agent in conditions without heavy metals, GA_3 could be preferred as a priming agent that contribute to the germination of perennial ryegrass under heavy metal stress.

The effect of heavy metal dose x priming interaction was statistically significant in terms of germination index. In control and 75 mg l^{-1} heavy metal treatments, all three priming pretreatments had similar germination index values. The increase in the heavy metal dose to 150 mg l^{-1} resulted in a germination index lower than that with the other seed priming pretreatments in the KNO_3 pretreatment. A similar situation was observed with a heavy metal dose of 300 mg l^{-1} (Figure 2). In terms of the germination index, the effect of KNO_3 on high heavy metal doses was found to be negative in contrast to the expected results.

The mean germination times (days) determined as the result of the metal kind x metal doses interaction are given in Figure 3. As shown in Figure 3, the mean germination times of seeds under cadmium stress did not show any significant difference from that of the control to the highest cadmium dose.

The time of germination of nickel exposed seeds was considerably longer than that of the control. Specifically, doses of nickel of 75 mg l^{-1} and 150 mg l^{-1} resulted in a significant delay in the germination period. Our results showed that cadmium did not have any effect on the germination time of perennial ryegrass although nickel did have such an effect.

The root lengths were significantly affected by both heavy metal kind × heavy metal dose and heavy metal dose × priming interactions (Table 1). The root lengths (mm) determined as the result of the heavy metal kind x heavy metal dose interaction are given in Figure 4a. Generally, the root lengths of plants under cadmium stress are longer than those under nickel stress while root lengths significantly decreased due to increasing doses in both kinds of metals. This decrease was more pronounced with nickel treatments.



(a) **FIGURE 1** (b)

Effect of (a) heavy metal kind × heavy metal dose interaction, (b) heavy metal dose × priming interaction on germination percentage

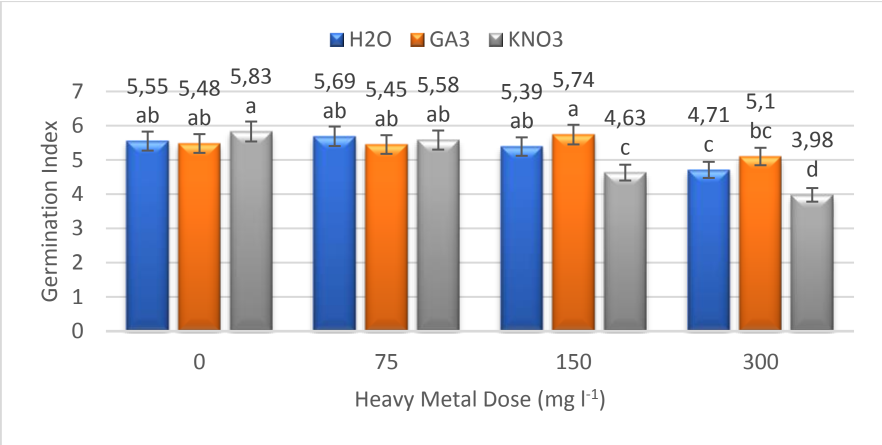


FIGURE 2
Effect of heavy metal dose × priming interaction on germination index

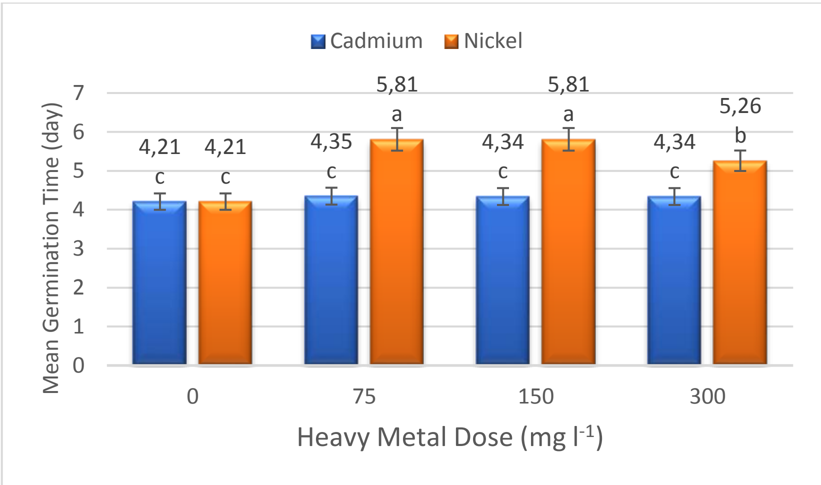
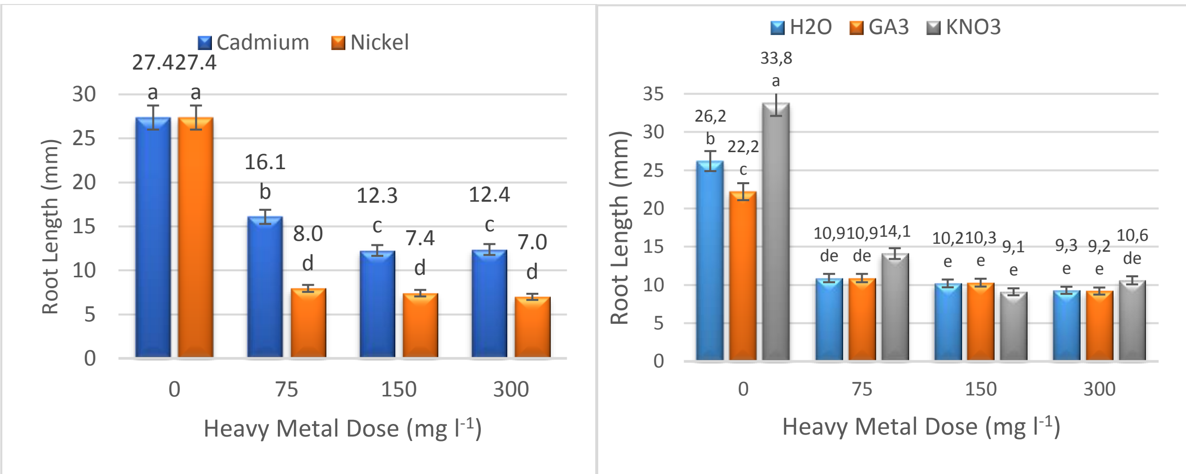


FIGURE 3
Effect of heavy metal kind × heavy metal dose interaction on mean germination time



(a) **(b)**

FIGURE 4
Effect of (a) heavy metal kind × heavy metal dose interaction, (b) heavy metal dose × priming interaction on root length

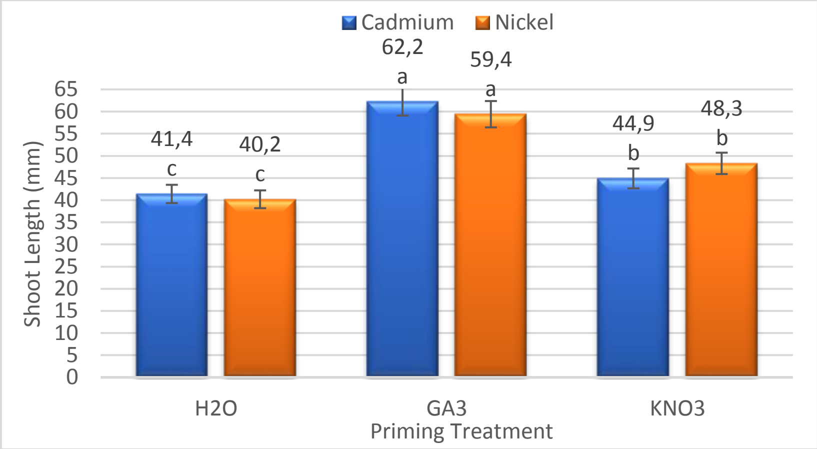


FIGURE 5
Effect of heavy metal kind × priming treatment interaction on shoot length

When the effect of metal dose x priming binary interaction on root length was evaluated, the root length values ranged between 33.8 mm and 9.1 mm in all treatments (Figure 4b). Higher root lengths were observed in the control treatment than all heavy metal treatment. The decrease in root lengths depending on the increase in heavy metal doses. In the 75 mg l^{-1} heavy metal treatment, the root length with the KNO_3 priming pretreatment was higher compared to 75, 150, and 300 ml l^{-1} heavy metal doses in all priming pretreatment (except the KNO_3 pretreatment in the 300 mg l^{-1} heavy metal dose). This demonstrated that KNO_3 has a limited benefit in the low heavy metal doses, but seed priming pretreatments had no effect on root growth at doses of heavy metal above 75 mg l^{-1}. Espanany et al. [33] reported that there was a significant increase in the root length of black cumin seeds under cadmium stress with KNO_3 pre-treatment but that the salicylic acid pretreatment was more effective in removing the negative effects of cadmium on the seedling growth of the plant. Some researchers reported previously that root length was positively affected by seed priming pretreatments [37, 38, 39, 40].

The effects of heavy metal kind x priming interactions on shoot length were statistically significant (Table 1). Seedling lengths determined with GA_3 seed priming pretreatment for both nickel stress and cadmium stress were statistically higher than with other seed priming pretreatments (Figure 5). This result suggested that GA3 as a priming agent should be preferred for reducing heavy metal stress in terms of seedling growth for perennial ryegrass.

CONCLUSION

The results of the research showed that both nickel and cadmium had adverse effects on germination and early seedling growth of perennial ryegrass. The adverse effects of nickel were more pronounced than those of cadmium. The level of adverse effects had also increased due to increased heavy metal doses. The KNO_3 pretreatment had a limited benefit only in terms of root development under heavy metal stress while the GA_3 pretreatment had positive effects on both germination properties and seedling growth. As a consequence of all this, GA_3 can be used as a seed priming agent if the perennial ryegrass is to be grown in areas contaminated with nickel and cadmium.

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The authors declare no conflicts of interest.

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