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Original research

Effects of chromium, nickel and their combined applications on *Triticum aestivum* L. in germination stage

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Abstract: In the present study, effects of Cr and Ni and their combinations on seed germination and seedling growth in bread wheat cultivars (Basribey-95 and Guadalupe) were determined. The seed germination did not significantly change in 25 and 50 µM Cr, Ni and Cr+Ni applications. However, wheat seed germinations were significantly inhibited by 100 µM Cr, 100 µM Ni and 100 µM Cr + 100 µM Ni applications of both varieties, exception of 100 µM in Guadalupe. Root and shoot developments of seedlings were adversely affected by high concentrations and combinations. Concentration-dependent decreases in protein contents of roots and shoots were found. Contrary to this, increases in proline contents were determined. Cr, Ni and Cr+Ni applications induced oxidative stress in roots and shoots as demonstrated by quantitatively with malondialdehyde and by qualitatively with a histochemical method.

Keywords: Bread wheat, Chromium, Nickel, Seed germination, Physiological effect

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Introduction

Heavy metals are often used for metals and metaloids such as Ni, Cr, Cd, Cu, Pb, As and Hg which are often associated with pollution and toxicity problems. Heavy metals are normally found in living organisms, water, sediments and in the earth because of the presence of rocks and ore in the earth (Alloway and Ayres, 1993). Because they can persist for long period in environment due to their non degradable nature, heavy metal pollution has increased beyond the recommended limit and is detrimental to all life forms (Watanabe, 1997; Tak et al., 2013; Gaur et al., 2014).

Chromium and its compounds are extensively distributed in environment. Among the factors that enable it to spread to the environment are alloying, animal hides, plating, tanning of inhibition of water corrosion, textile dyes and pigments, ceramic glazes, mordants, refractory bricks, and pressure-treated lumber (Avudainayagam et al., 2003). Thus, Cr contamination in the environment has become a major area of concern (Salunkhe et al., 1998). It is not a necessary element for plant metabolism. Chromium accumulation by plants causes high toxicity and structural changes in terms of growth and reduction in biomass accumulation. It interferes with the photosynthesis system and respiration processes, and water and nutrients uptake mechanisms. It causes oxidative stress with the destruction of membrane lipids and DNA damage (Singh et al., 2003; Dogan and Gultekin, 2017).

Nickel is a naturally occurring element that can exist in various mineral forms. On the other hand, the majority of anthropogenic activities, especially those related to raw materials, are used in the metallurgy and galvanic industries (Salt et al., 2000). For many decades, nickel was regarded as a potentially toxic heavy metal. It is now

considered a possible essential metal for plants. However, it can be toxic at elevated levels (Pais and Jones, 1997).

Many studies have shown the effects of individual stress factors such as heavy metals. Since many heavy metals are present in the environment, it is useful to take into account their interactions and toxic effects in plants. The aim of the present study was to evaluate the effect of Cr, Ni and their combinations on the germination of *T. aestivum* L. cv Basribey-95 and Guadalupe seeds and their physiological impact on the seedlings.

Material and methods

Wheat seeds and treatment: For experiment, *Triticum aestivum* L. cv Basribey-95 and Guadalupe seeds were used. The seeds were treated with 0, 25, 50 and 100 µM Cr as $K_2Cr_2O_7$ (Riedel-de Haen), 0, 25, 50 and 100 µM Ni as $NiCl_2$ (Merck) and 0 µM Cr + 0 µM Ni, 25 µM Cr + 25 µM Ni, 50 µM Cr + 50 µM Ni and 100 µM Cr + 100 µM Ni. For each treatment or untreated seeds, four replicates of 20 seeds were placed on two filter layers in 120 mm sterile petri dishes. Before experiment, the wheat seeds were sterilized with 5% NaClO solution for 15 minutes and then washed three times with sterile distilled water. The filter paper was moistened with distilled water for controls (without metal treatment) or aqueous solutions of Ni and Cr concentrations and their combinations. In order to maintain wet of the petri dishes, their solutions were added periodically during the experiment. The petri dishes were incubated in a growth chamber (Snijders Scientific, Netherlands) at 24±1°C. The seeds were considered to be germinated at the emergence of radicle. The number of germinated seeds was noted daily. After 7 days, seedlings were harvested from the roots of seedlings washed three times with deionized water to remove any elements adhering to the root surface. The root and shoot lengths were measured using ruler. Roots and shoots were separated and dried at 80 °C in order to determine dry weight and element contents. Chemicals used in the study were analytical grade.

Physiological analyses: Determination of proline accumulation in the samples was done according to Bates et al. (1973). Fresh plant material was homogenized using 3% sulphosalicylic acid. The homogenization was centrifuged at 5000 rpm for 10 minutes. 2 ml of the supernatant was mixed with 2 ml of acidninhydrin and 2 ml of glacial acetic acid in the test tube. The mixture was heated to 100 °C for 1 hour in a water bath. At the end of this period, tubes were taken into an ice bath and the reaction was terminated. The reaction mixture was extracted with 4 ml of toluene and then vortexed for 15-20 seconds. The samples were read in a spectrophotometer (CINTRA 202, Australia) at a wavelength of 520 nm. *L*-proline was used as standard.

Lipid peroxidation levels of the root and shoots were determined according to Zhou (2001). Fresh seedling parts were homogenized using 10% trichloroacetic acid. The homogenized samples were centrifuged at 10000 rpm for 20 minutes Then, 2 ml supernatant and 2 ml of thiobarbutyric acid were mixed and heated at 95 °C for 30 min. At the end of this period, the samples were quickly cooled in an ice bath. The absorbances were read in the spectrophotometer at 532, 600 and 450 nm.

Protein content was determined according to Lowry et al. (1951). To determine the content, fresh samples were homogenized in 5 ml of 0.1 M phosphorous buffer (pH 7) and centrifuged at 12000 rpm for 10 minutes. Protein contents of the seedling parts were then calculated using calibration curve of bovine serum albumin.

For determination of Cr and Ni concentrations in the roots and shoots, the samples were dried and then pulverized. The samples were dissolved using 14 M HNO_3 on hot plate. The residues were dissolved in 1 M HCl. After mineralization, Cr and Ni concentrations were determined using an atomic absorption spectrometer (Perkin Elmer AAnalyst 400, USA).

Histochemical analysis: The Schiff 's reagent was used for histochemical detection of lipid peroxidation level in roots (Pompella et al., 1987). The wheat seedling roots were incubated in Schiff's reagent for 60 minutes. The roots were then rinsed with 0.5% $K_2S_20_5$ (w/v) (prepared in 0.05 M HCl) until the root color became light red.

Data analysis: Final germination percentages were calculated as follows:

$$Final\ germination\ (\%) = \frac{Number\ of\ germinated\ seeds}{20} x100$$

All analyses were carried out triplicate. Data were analyzed by SPSS 11.0. The significance of differences between mean values were determined by Least Significant Difference (LSD) at the 0.05 level of confidence.

Results and Discussion

Many environmental factors have negative effects on seed germination. One of these factors is undoubtedly heavy metals. Seed germination is the first physiological process affected by heavy metals. The effects of Cr, Ni and their combinations on the germination of Basribey-95 and Guadalupe seeds are given in Fig 1. The highest germination rates were found in the control groups. All three applications were generally determined to cause a reduction in the germination of the seeds. Final germination rates of 100 μM Cr, 100 μM Ni and 100 μM Cr + 100 μM Ni applications were 81.21%, 91.25% and 88.75% ($p<0.05$) for Basribey 95 and for Guadalupe 85.0% ($p<0.05$) 93.75% ($p>0.05$) and 88.75% ($p<0.05$), respectively. Previous studies have reported that Cr and Ni concentrations are negatively affected by seed germination (Akinci and Akinci, 2010; Akinci and Akinci, 2011). Germination rates of Cr application were generally lower than those of Ni and combination of Cr+Ni when considering applications. The least adverse effects on germination were determined in Ni applications. Hevay metals may have a negative effect on seed germination, especially as a result of amylase activity and reduced embryonic sugar transport. Reductions in germination of the seeds may be due to this reasons.

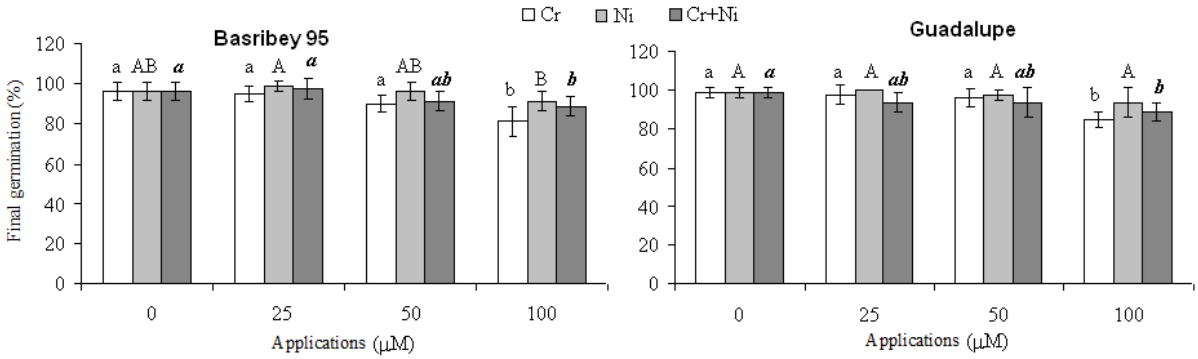

Figure 1. Effects of different Cr and Ni concentrations and their combinations on seed germination. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

Chromium contents of root and shoots tissues were found to increase with the applied Cr and Cr+Ni concentrations (Fig. 2). It has been determined that these increases in the Cr+Ni combination are generally low compared to singly Cr applications. Similar to the finding in the Cr applications, Ni contents of both wheat varieties increased with increasing Ni concentrations (Fig. 3). These increases were found to be lower in the Cr+Ni combinations than in singly Ni applications. When compared to seedling parts, it was determined that roots accumulate metal at higher concentrations compared to shoots. In roots and shoots of both wheat varieties, Cr accumulated more than Ni. This may indicate that seedlins have different affinity to heavy metal uptake. In addition, Guadalope generally accumulates more metal than Basribey-95. This shows that the wheat varieties have species-specific heavy metal accumulation. According to our results, accumulation of Cr and Ni changed according to the metal concentration, type of metal, metal-metal interaction, wheat varieties and the seedling organs.

It is known that growth and development are the basic processes of life and propagation plants. There are some studies showing that early growth stages of plant seedlings are very important indicators in determining the toxicity effects of heavy metals such as Cr and Ni (Scoccianti et al., 2006; Kanwar et al., 2012). Furthermore, the negative effects of Cr and Ni on the growth and development of plants have been reported by other researchers as well (Szárazová et al., 2008; Fargašová et al., 2011). The root and shoot lengths Basribey-95 and Guadalupe seedlings are given in Fig 4. The highest root and shoot lengths were determined in control plants. The lowest lengths were obtained at the highest concentrations. According to this, the root lengths of Basribey-95 and Guadalupe in 100 μM Cr concentration were measured as 3.03 and 3.25 cm, respectively ($p <0.05$). Similarly, the shoot lengths of Basribey-95 and Guadalupe in 100 μM Ni concentration were measured as 5.68 and 6.10 cm, respectively ($p<0.05$). According to our findings, the applications negatively affected the root and shoot development of both bread wheat varieties. On the other hand, adverse effect of Cr on

growth and development of the seedlings is greater than Ni. Chromium compounds are highly toxic to plants and adversely affect their growth and development. Inhibition of growth in the seedlings can be due to inhibition of cell division by inducing chromosomal aberrations (Liu et al., 1993).

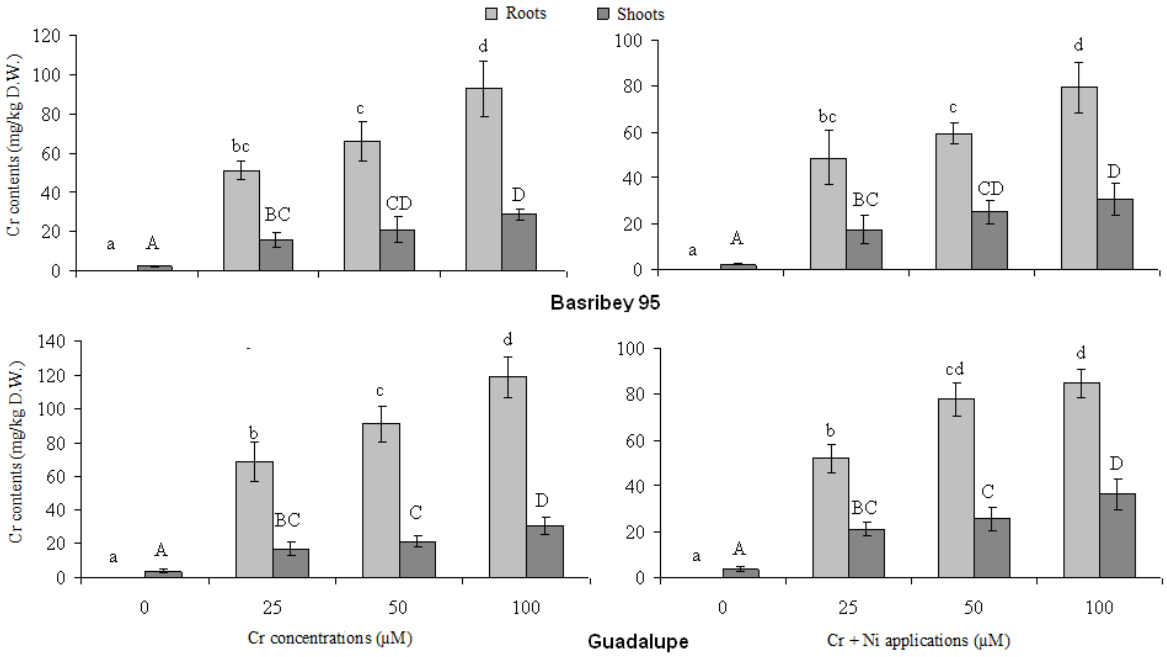

Figure 2. Chromium accumulations of root and shoot tissues of the seedlings at different Cr concentrations and Cr+Ni combinations. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

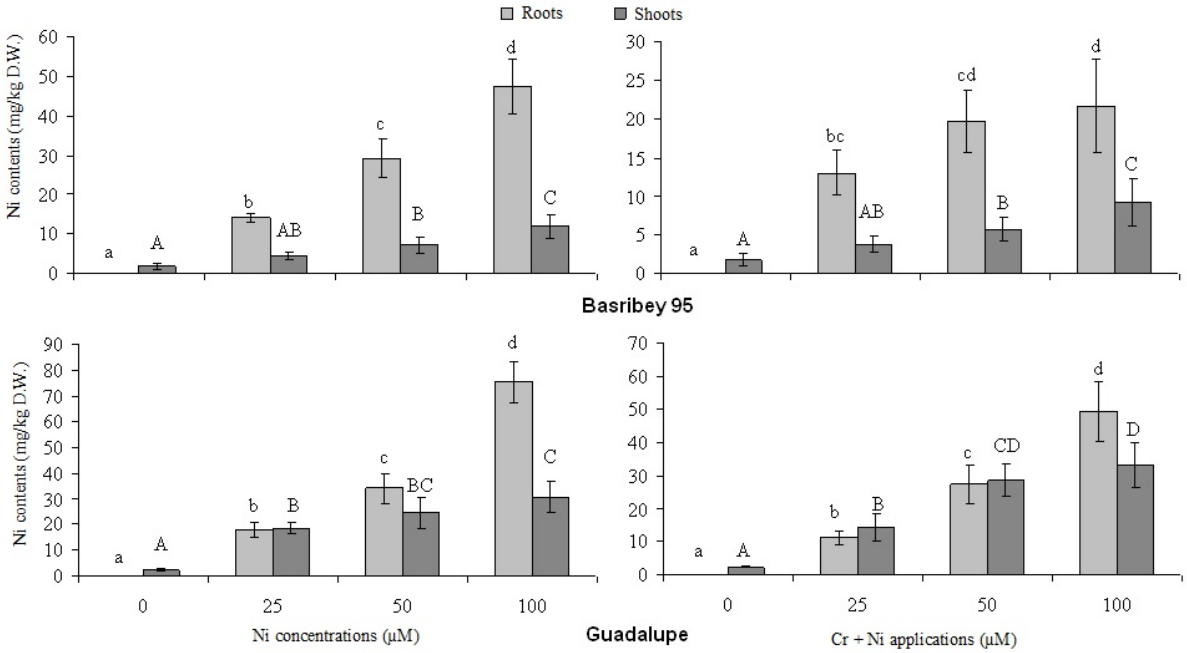

Figure 3. Nickel accumulations of root and shoot tissues of the seedlings at different Ni concentrations and Cr+Ni combinations. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

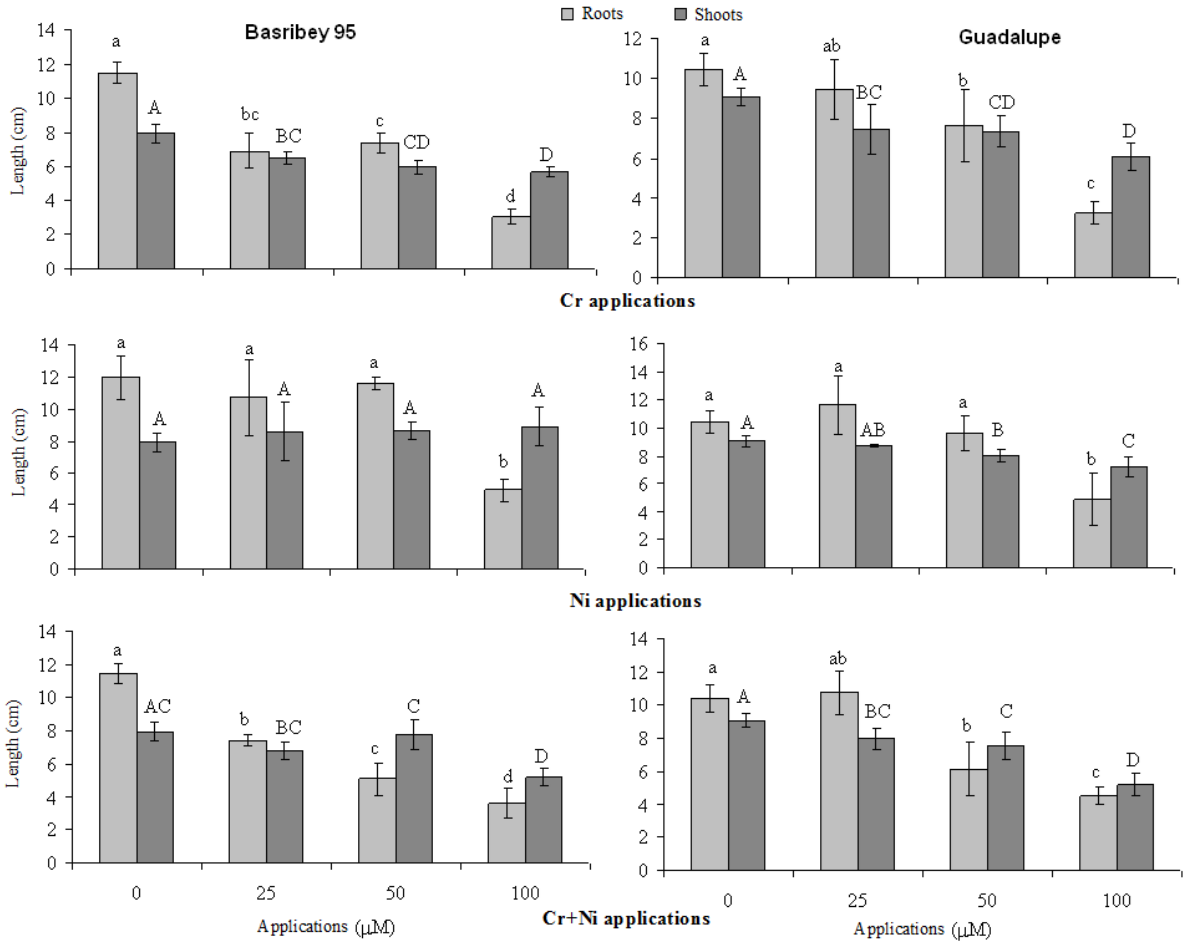

Figure 4. Root and shoot lengths of the seedlings at different Cr and Ni concentrations and their combinations. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

The protein contents of root and shoot of Basribey-95 and Guadolope decreased under the influence of Cr, Ni, Cr+Ni concentrations (Fig. 5). These reductions were particularly high at higher concentrations ($p<0.05$). For example, protein contents in 100 µM Cr, 100 µM Ni and 100 µM Cr plus 100 µM Ni applications in Basribey-95 shoots were decreased by 49.6%, 38.5% and 43.1% ($p<0.05$), respectively. Similarly, the contents in 100 µM Cr, 100 µM Ni and 100 µM Cr plus 100 µM Ni applications in Guadalupe roots were decreased by 28.8%, 19.3% and 32.0% ($p<0.05$), respectively. The increase in the amount of the heavy metals that accumulate in the seedling tissues with the applied concentration may explain the reason for these decreases in protein quantities. This is also supported by the fact that roots accumulate at higher concentrations of the metals and cause a decrease in the amount of protein. In addition, heavy metal stress may be caused by proteolytic degradation caused by inhibition of protein synthesis, or by oxidative stress-induced reactive oxygen species (ROS) (Nagoor, 1999; Dogan et al., 2009).

According to our findings, applied concentrations of Cr, Ni and Cr+Ni resulted in an increase in proline contents in the root and shoot tissues (Fig. 6). In Basribey-95, the highest increases for roots were determined as 56.8%, 43.2% and 154.0% ($p<0.05$) in 100 µM Cr, 100 µM Ni and 100 µM Cr+100 µM Ni, respectively. The contents in shoot of Basribey-95 were increased by 18.6% ($p>0.05$), 44.2% and 90.7% ($p<0.05$), respectively, at 25, 50 and 100 µM Cr concentrations. Nickel application did not show significant change at concentrations of 25 and 50 µM in Basribey-95 ($p>0.05$). In the combinations of 25+25, 50+50 and 100 +100 (µM+ µM), proline quantities of shoots were increased by 2.3%, 32.6% and 93.0%, respectively, compared to the control. The proline levels in 100 µM Cr, 100 µM Ni and 100 µM Cr plus 100 µM Ni applications in Guadalupe roots were increased by 212.2%, 109.1% and 297.0% ($p<0.05$), respectively. Similar results were found in shoots. When proline

quantities of root and shoot of both species were taken into account, the minimum increases were found in Ni applications. The maximum contents were found in Cr and Cr+Ni applications. The functional significance of proline accumulation under heavy metal toxicity might include, next to water balance maintenance, scavenging of hydroxyl radicals (Chen and Goldsbrough, 1994) as well as metal chelation in cytoplasm (Kocsy et al., 2000). Considering the above reasons, Cr and Ni toxicity may explain the increase of proline quantities in the root and shoot tissues.

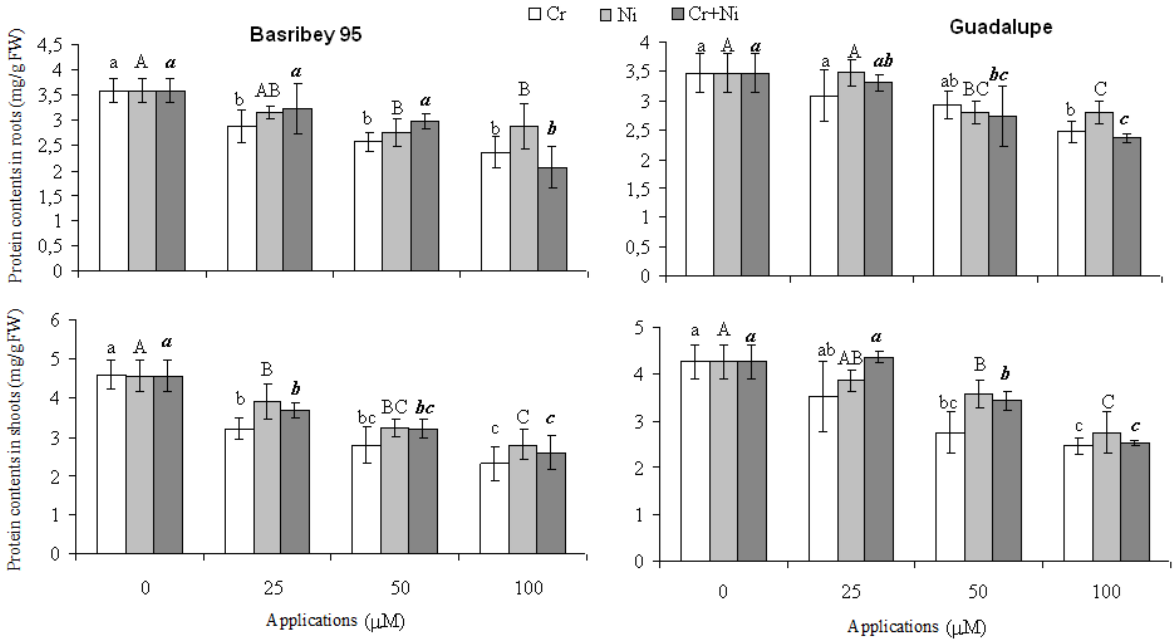

Figure 5. Effects of different Cr and Ni concentrations and their combinations on protein contents. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

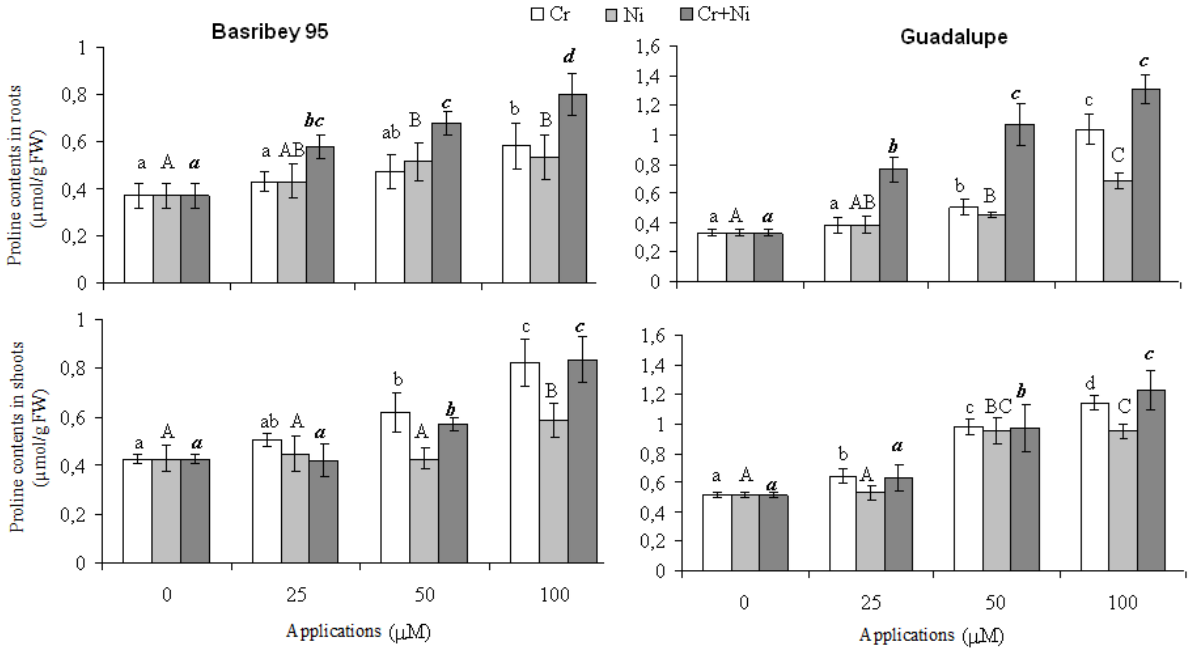

Figure 6. Effects of different Cr and Ni concentrations and their combinations on proline contents. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

MDA contents of both wheat varieties generally showed an increase in the effect of metal applications (Fig. 7). The contents of Basrive-95 in 100 μM Cr, 100 μM Ni and 100 μM Cr+100 μM Ni were increased for roots as 100.0%, 61.9% and 123.1% ($p < 0.05$) and for shoots as 163.7%, 138.4% and 149.1% ($p<0.05$), respectively, compared to their controls. Similar to Basribey-95, when all concentrations and combinations were taken into consideration, it was determined that the maximum increases in roots of Guadalupe were significant at high applications ($p<0.05$). MDA contents of shoots of Guadalupe were also found to increase by 116.7%, 98.7% and 186.7% ($p < 0.05$), for Cr, Ni and Cr + Ni at the highest concentrations, respectively. Beside detection of quantitative lipid peroxidation, lipid peroxidation in wheat roots were also qualitatively determined (Fig. 8). Accordingly, it was determined that roots of Basribey-95 at 100 μM Cr concentration and 50 μM Cr +50 μM Ni and 100 μM Cr+100 μM Ni combinations stained. When Guadalupe roots were taken into account, it was determined that only 100 μM Cr and 100 μM Cr+100 μM Ni combination stained. Membrane lipids are frequently qualitatively and quantitatively modified under biotic and abiotic stress conditions (Kuiper, 1985). Especially, these increases in the amount of MDA were found to be higher in Cr and Cr+Ni applications. Our histochemical method also supported the explanation of the peroxidation of lipids at high concentrations of Cr and Cr+Ni applications. The staining of the root tips of wheat varieties also show this situation.

It has been determined that the metals and combinations applied are influenced by germination of bread wheat seeds as well as by some biochemical processes. Seed germination showed significant changes in Cr, Ni and Cr+Ni applications, especially at high concentrations and combinations. Root and shoot developments of both varieties were affected negatively as well. Protein contents were decreased due to oxidative stress by high concentrations. Concentration dependant enhancement in lipid peroxidation in the roots and shoot was assumed to be resulted from provoked oxidative stress, especially with Cr and Cr+Ni applications. Increase in proline contents in roots and shoots showed that the seedlings accumulated proline under Cr and Ni stress.

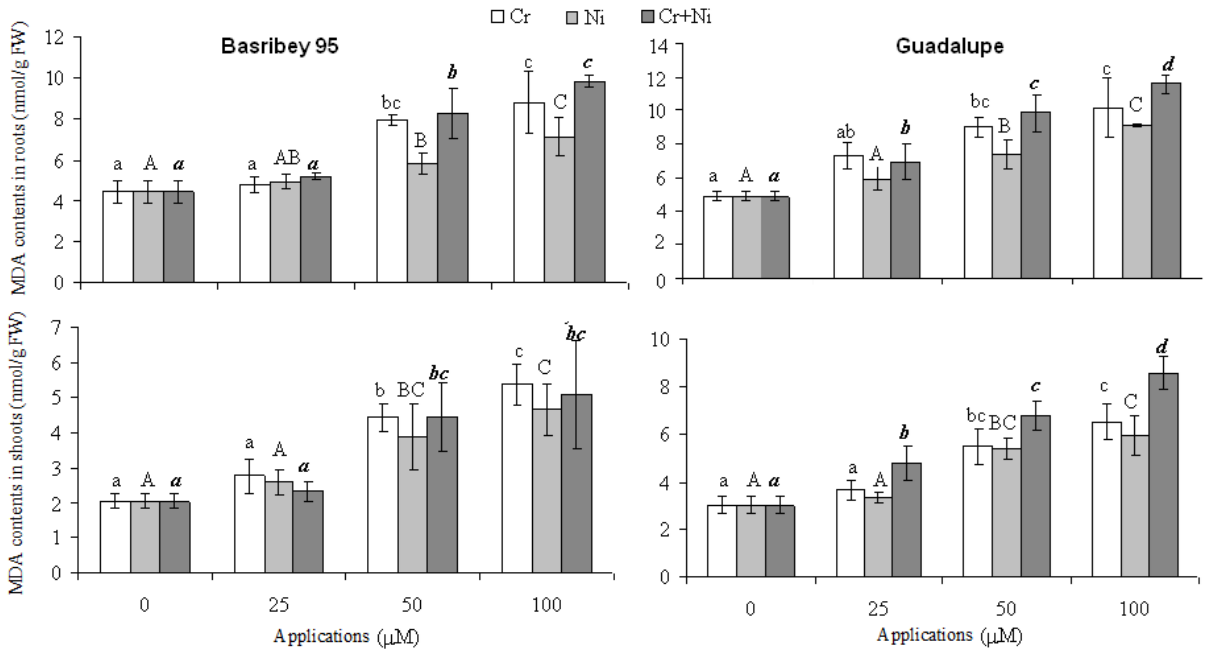

Figure 7. Effects of different Cr and Ni concentrations and their combinations on MDA contents. Error bars represent the standard deviation of mean. Means with different letters are significantly different from one another according to the LSD test ($p<0.05$).

Figure 8. Histochemical detection of lipid peroxidation caused by different Cr and Ni concentrations and their combinations in seedling roots.

References

Akinci I.E., Akinci S. 2010. Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.). African Journal of Biotechnology, 9: 4589-4594.

Akinci S., Akinci İ.E. 2011. Nikelin ıspanakta (*Spinacia oleracea*) çimlenme ve bazı fide büyüme parametreleri üzerine etkisi. Ekoloji, 20: 69-76.

Alloway B.J., Ayres D.C. 1993. Chemical Principles of Environmental Pollution, Blackie Academic and Professional, Glasgow, U.K.

Avudainayagam S., Megharaj M., Owens G., Kookana R.S., Chittleborough D., Naidu R. 2003. Chemistry of chromium in soils with emphasis on tannery waste sites. Reviews of Environmental Contamination and Toxicology, 178: 53-91.

Bates, L.S., Waldren, R.P., Teare, D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-297.

Chen J., Goldsbrough P.B. 1994. Increased activity of glutamylcysteine synthetase in tomato cells selected for cadmium tolerance. Plant Physiology, 106: 233-239.

Dogan M., Demirors Saygideger S., Colak U. 2009. Effect of lead toxicity on aquatic macropyhte *Elodea canadensis* Michx. Bulletin of Environmental Contamination and Toxicology, 83: 249-254.

Dogan M., Gultekin E. 2017. Effects of single and combined applications of chromium and clarithromycin on wheat seedlings. Fresenius Environmental Bulletin, 26: 1154-1162.

Fargašová A., Šmelková M., Matúš M. 2011. *Sinapis alba* reactions on the stress induced by chromium and nickel. Fresenius Environmental Bulletin, 20: 3374-3380.

Gaur N., Flora G., Yadav M., Tiwari A. 2014. A review with recent advancements on bioremediation-based abolition of heavy metals. Environ. Sci. Process. Impacts, 16: 180-193.

Kanwar M.K., Bhardwaj R., Arora P., Chowdhary S.P., Sharma P., Kumar S. 2012. Plant steroid hormones produced under Ni stress are involved in the rugulation of metal uptake and oxidative stress in *Brassica juncea* L. Chemosphere, 86: 41-49.

Kocsy G., Von Ballmoos P., Suter M., Ruegsegger A., Galli U., Szalai G., Galiba G., Brunold C. 2000. Inhibition of

glutathione synthesis reduces chilling tolerance in maize. Planta, 211: 528-536.

Kuiper P.J.C. 1985. Lipid Metabolism of Higher Plants as a Factor in Environmental Adaptation. In: Siegenthaler, P.A. and Eichenberger, W. (Eds) Structure, Function and Metabolism of Plant Lipids. Elsevier, Amsterdam, pp, 525-530.

Liu D.H., Jaing W.S., Li M.X. 1993. Effect of chromium on root growth and cell division of *Allium cepa*. Israel J. Plant Sci., 42: 235-243.

Lowry O.H., Rosebrought N.J., Farr A.L., Randall R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Nagoor S. 1999. Physiological and biochemical responses of cereal seedlings to graded levels of heavy metals. II. Effects on protein metabolism in maize seedlings. Adv. Plant Sci., 12: 425-433.

Pais I., Jones J.B. Jr. 1997. The Handbook of Trace Elements, Pp. 102-125, St. Lucie Press, Boca Raton, Fla, USA.

Pompella A., Maellaro E., Casini A.F., Comporti M. 1987. Histochemical detection of lipid peroxidation in the liver of bromobenzene-poisoned mice. Am. J. Pothol., 129: 295-301.

Salt D.E., Kato N, Kramer U., Smith R.D., Raskin I. 2000. The role of root exudates in nickel hyperaccumulation and tolerance in accumulator and nonaccumulator species of *Thlaspi*, N. Terry, G. Banuelos (Eds.), Phytoremediation of contaminated soil and water, CRS Press LLC, London, pp. 189-200.

Salunkhe P.B., Dhakephalkar P.K., Paknikar K.M. 1998. Bioremediation of hexavalent chromium in soil microcosms. Biotechnol. Lett., 20: 749-751.

Scoccianti V., Crinelli R., Tirillini B., Mancinelli V., Speranza A. 2006. Uptake and toxicity of Cr (III) in celery seedlings. Chemosphere, 64: 1695-1703.

Singh H.P., Mahajan P., Kaur S., Batish D.R., Kohli R.K. 2003. Chromium toxicity and tolerance in plants. Environmental Chemistry Letters, 11: 229-254.

Szárazová K., Fargašová A., Hiller E., Velická Z. Pastierová J. 2008. Phytotoxic effects and translocation of Cr and Ni in washing wastewaters from cutlery production line to mustard (*Sinapis alba* L.) seedlings. Fresenius Environmental Bulletin, 17: 58-65.

Tak H.I., Ahmad F., Babalola O.O. 2013. Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. Reviews of Environmental Contamination and Toxicology, 223: 33-52.

Watanabe M.A. 1997. Phytoremediation on the brink of commercialization. Environ. Sci. Technol. A, 31: 182-186.

Zhou Q. 2001. The measurement of malondialdehyde in plants. In: Zhou Q. (Ed.): Methods in Plant Physiology. China Agricultural Press, Beijing, pp.173-174.