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Original Article

Comparative effectiveness of EDTA and citric acid assisted phytoremediation of Ni contaminated soil by using canola (*Brassica napus*)

Eficácia comparativa da fitorremediação assistida por EDTA e ácido cítrico de solo contaminado com Ni, usando canola (*Brassica napus*)

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Abstract

Phytoremediation is an ecofriendly technique to clean heavy metals from contaminated soil by the use of high biomass producing plant species. Chelators can help to improve this biological technique by increasing metal solubility. Therefore, a pot experiment was conducted to determine the effect of the chelators EDTA and citric acid (CA) in phytoremediation of Ni contaminated soil by using *Brassica napus* (canola). Two cultivars of *B. napus*, Con-II (tolerant) and Oscar (sensitive), were selected after screening and exposed to $NiSO_4$ at 30 ppm at the time of sowing. CA (10 mM) and EDTA (1.5 mM) were applied either alone or in combination with each other after two weeks of Ni treatments. Different parameters like morpho-physiological and biochemical data were recorded after 15 days of chelate application. The results highlighted the successful use of chelating agents (CA and EDTA) not only to ameliorate Ni stress but also to enhance Ni accumulation which is prerequisite for phytoremediation. The basal application of 10 mMCA and 1.5 mM EDTA concentration proved to be effective for the growth of plants. The combination of chelating agents failed to show any synergistic effects.

Keywords: *Brassica napus*, citric acid, EDTA, hyper-accumulator, nickel, phytoremediation.

Resumo

A fitorremediação é uma técnica ecologicamente correta para limpar metais pesados de solo contaminado pelo uso de espécies vegetais produtoras de alta biomassa. Os quelantes podem ajudar a melhorar esta técnica biológica aumentando a solubilidade do metal. Para tanto, foi realizado um experimento em vaso para determinar o efeito dos quelantes EDTA e ácido cítrico (AC) na fitorremediação de solo contaminado com Ni, utilizando *Brassica napus* (canola). Duas cultivares de *B. napus*, Con-II (tolerante) e Oscar (sensível) foram selecionadas após triagem e expostas a NiSO4 a 30 ppm no momento da semeadura. CA (10 mM) e EDTA (1,5 mM) foram aplicados sozinhos ou em combinação um com o outro após duas semanas de tratamentos com Ni. Diferentes parâmetros como dados morfofisiológicos e bioquímicos foram registrados após 15 dias de aplicação de quelato. Os resultados destacaram o uso bem-sucedido de agentes quelantes (CA e EDTA) não apenas para melhorar o estresse de Ni, mas também para aumentar o acúmulo de Ni, um pré-requisito para a fitorremediação. A aplicação basal da concentração de 10 mMCA e 1,5 mM de EDTA mostrou-se eficaz para o crescimento das plantas. A combinação de agentes quelantes não mostrou quaisquer efeitos sinérgicos.

Palavras-chave: *Brassica napus*, ácido cítrico, EDTA, hiperacumulador, níquel, fitorremediação.

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1. Introduction

Heavy metal pollution is a serious threat for plant growth (Bhat et al., 2019) and a major concern for environmental management due to rapid increase in anthropogenic activities like disposal of industrial waste, irrigation of crops with sewage water, use of synthetic fertilizer, pesticide, smelting and mining which are adding heavy metals to the environment (Liu et al., 2018). Hence, the gradual increase in heavy metal levels in soil ecosystems is a major environmental issue throughout the world (Singh and Kalamdhad, 2011). Additionally, the toxicity of heavy metals due to their non-degradable nature and accumulation pose health risks for living organisms (Mani and Kumar, 2014). In plants, the high concentration of heavy metals can cause metabolic disturbances and alter the levels of reactive oxygen species (ROS) which in turn disturb cellular redox homeostasis (Flora et al., 2008).

Among different heavy metals Ni is widely distributed in the environment and it is an essential element for plants from germination to yield (Sreekanth et al., 2013). In plant tissues, Ni concentrations from 0.19 to 0.85 mg kg^{-1} dry weight show toxic effects and decrease the content of micro and macronutrients (Yusuf et al., 2011). The high concentrations of Ni cause many toxic effects on plants such as inhibition of seed germination, low seedling growth, reduction of root growth due to inhibition of mitotic activity, suppression of photosynthesis due to the disrupted chloroplast structure, blocked chlorophyll synthesis and disordered electron transport, and CO_2 deficit caused by stomatal closure (Gajewska et al., 2006). Elevated concentrations of Ni (500 mg kg^{-1}) also decrease antioxidant enzyme activity and cause oxidative damage to plants (Georgiadou et al., 2018). Interaction of Ni with ligand enzymes may inhibit metabolic activities and Ni stress also causes chlorosis and necrosis in plants (Gajewska et al., 2006). In summary, these reports clearly show that Ni stress is considered a great threat to agriculture in major areas of the world (Viet et al., 2010).

Different remediation techniques are suggested in the literature to reduce the metal entry from the contaminated soil to the food chain (Park et al., 2012). Among biological techniques, phytoremediation is quite effective with high public acceptance and environmental friendly aspects (Bhat et al., 2019). Phytoremediation is a technique which uses green plants for the release, transfer, stabilization or degradation of pollutants from soil, water and air (Glass, 2000; Paz-Ferreiro et al., 2014). However, successful phytoremediation relies on heavy metal mobility and availability for plants. Different chelating agents have been used to enhance the metal solubility in the soil. (Tang et al., 2012). Upon chelation, heavy metals are easily up-taken by plant roots and transported to different parts of the plants there by causing removal from the contaminated soils. The addition of chelates like EDTA and citric acid (CA) therefore improved the plants ability to uptake and tolerate high metal concentrations (Liphadzi et al., 2005). However, EDTA is a stable entity and exists for a long time in the soil which can be a problem for the availability of different transition metal ions inside soil (Seth et al., 2012). Other than EDTA, various organic chelating agents like CA are not reported to show any of the adverse effects on soil and plants (Ogunleyel et al., 2016). Furthermore, CA significantly increased the solubility and uptake of metals by plants (Melo et al., 2008). By contrast, it has been reported that CA is only effective in low concentration, whereas at higher concentrations, it causes toxicity in plants (Turgut et al., 2004).

Certain plant species, known as hyper accumulators, show specific adaptation mechanisms to survive in highly polluted environments. Phylogenetically, many *Brassica* species are metal hyper accumulators and grow on contaminated soil for remediation purposes (Szczygłowska et al., 2011). Currently, *B. napus* has gained an importance as a potential crop and because of its high metals absorption ability, fast growth, high above ground biomass and rich oil content in seeds (Park et al., 2012).

The present study was conducted to evaluate the individual as well as the combined effect of EDTA and CA in enhancing the bioremediation properties of canola for Ni. Application of single chelating agents may be effective for pollutants removal but can cause toxic effects on the soil as well as on the plants if applied at higher concentration (Leštan et al., 2008). On the other hand, a combination of two different chelators may prove to be effective, both in term of pollutants removal and safety. This may be attributed to the alleviating effects of one chelator over the other, which we investigate in the present study.

2. Materials and Methods

An experiment was conducted in the Nuclear Institute for Agriculture and Biology (NIAB) for phytoremediation of Ni by canola. Seeds of B. napus varieties were obtained from Ayub Agricultural Research Institute, Faisalabad. Two cultivars of B. napus, Con-II (tolerant) and Oscar (sensitive), were selected after screening and exposed to 30 ppm NiSO4 at the time of sowing. CA (10 mM) and EDTA (1.5 mM) were applied through rooting either alone or in combination after two weeks of Ni treatments. After 15 days, vegetative samples were harvested for analysis to collect physiological and biochemical parameters.

2.1. Chlorophyll contents

Chlorophyll contents were determined as described in methods of Arnon (1949) and Davies (1976). 0.5 g of fresh leaves were chopped into small segments and extracted with 5 mL acetone (80%) at -10°C. The extract was centrifuged at 14000 x g for 5 min and the absorbance of the supernatant was recorded at 645, 652 and 663 nm using a spectrophotometer (IRMECO U2020). Chlorophyll a, b and total chlorophyll contents were calculated by using the following formulae (Equations 1-3):

$$Chlorophyll\ a = \begin{bmatrix} 12.7\left(OD_{663}\right) - \\ 2.69\ \left(OD_{645}\right) \end{bmatrix} \times V / 1000 \times W \qquad (1)$$

$$Chlorophyll\ b = \begin{bmatrix} 22.9\ (OD_{645}) - \\ 4.68\ (OD_{663}) \end{bmatrix} \times V / 1000 \times W \quad (2)$$

$$Total\ chlorophyll = \begin{bmatrix} 20.2\ (OD_{645}) + \\ 8.02\ (OD_{663}) \end{bmatrix} \times V / 1000 \times W \quad (3)$$

V= Volume
W= weight of the sample

2.2. Leaf water potential (-MPa)

In plants from each treatment, the third leaf from the top (i.e. the youngest, fully expanded leaf) was used to determine the leaf water potential (Ψw). Leaf water potential measurements were recorded from 8.00 to 10.00 am with a Scholander type pressure chamber (Arimad-2, ELE International, Tokyo, Japan).

2.3. Leaf osmotic potential (-MPa)

The same leaf that was used for water potential was frozen in a freezer below -20 °C for seven days, thawed and the cell sap extracted with the help of a disposable syringe. The extracted sap was directly used for the measurement of Ψs in an osmometer (Wescor5520, Wescor Inc., Logan, UT, USA).

2.4. Turgor pressure (MPa)

Turgor potential (Ψp) was calculated from the values of water potential (Ψw) and osmotic potential (Ψs) with the following formula (Equation 4);

$$\Psi p = \Psi w - \Psi s \quad (4)$$

2.5. Leaf gas exchange parameters

The fully expanded second leaf of plants from each treatment was used for the determination of net CO2 assimilation rate (A), stomatal conductance (gs) and transpiration rate (E), as measured by a portable infrared gas analyzer (Model Cl-340; Analytical Development Company, Hoddesdon, England). Leaf gas exchange measurements were recorded from 10.00 am to noon.

2.6. Analysis of ions

For determination of ions, we followed a method by Wolf (1982). The collected samples were dried in oven at 60°C for 72 hours and dried samples (0.5 g) were added to concentrated H2SO4 (5 mL) in digestion tubes. The plant material was incubated overnight at 25 °C. Then, 0.5 mL of H2O2 were added to the digestion tubes and the samples placed in a digestion block. The probes were heated at 350 oC until fumes were produced and left for another 30 minutes. The tubes were removed from the digestion block and allowed to cool at 25°C. Then, 0.5 mL H2O2 were added again and the tubes placed back in the digestion block. We repeated this step until the material become colorless. By using a volumetric flask, the volume of the extract was adjusted to 50 mL with distilled H2O. The transparent solution was used to determine potassium, Sodium and Nickle ions.

2.7. Total free amino acids

Total amino acids were determined following protocols by Hamilton and VanSlyke (1973). Fresh plant leaves were chopped and extracted in 4mL phosphate buffer with pH 7 at room temperature. One 1mL of extract was mixed with 1 mL of pyridine (10%) and 1ml of 2% ninhydrin solution in a test tube. The mixture was heated at 100°C in a water bath for 30 min. The final volume was raised up to 50 ml with autoclaved distilled water. Optical density was recorded at 570 nm using a spectrophotometer (IRMECO U2020). Total amino acid were calculated by using the formula (Equations 5-7).

$$Total\ amino\ acids = Graph\ reading\ of\ the\ sample \times dilution\ factor \quad (5)$$

$$Graph\ reading = Compare\ the\ optical\ density\ of\ the\ sample\ with\ graph\ of\ known\ concentration \quad (6)$$

$$Dilution\ factors = dilution\ of\ samples\ with\ distilled\ water \quad (7)$$

2.8. Total soluble protein contents

Total soluble proteins were determined using the method of Ali et al. (2022a).

Solution A, B, C (Lowry solution for protein determination) and folin phenol reagent was prepared and used for protein estimation.

Five milliliters phosphate buffer (0.2 M having pH 7.0) were used to chop and grind fresh leaf material (0.5 g). This ground material was centrifuged at 5000 × g for 5 min. 1 ml of the leaf extract was taken in a test tube. In each test tube, solution C (1 mL) was added and all reagents (Lowry solutions + sample) were mixed thoroughly. The solution was placed for ten minutes at 250C. Afterwards, 0.5 mL Folin-Phenol reagent (1:1 diluted) was poured into each test tube and then mixed gently. This mixture was incubated for half an hour at 25C0. Optical density was read at 620 nm using a spectrophotometer (Hitachi, 220, Japan).

2.9. Antioxidant enzymes extraction

Fresh leaves (0.5 g) were ground in phosphate buffer (8 mL; 50 mM, pH 7.8). The material was centrifuged at 4 °C on 15000 xg for 20 min. For estimation of enzymatic activities, the supernatant was used.

2.10. Determination of superoxide dismutase

A method by Zainab et al. (2021) was followed to determine the superoxide dismutase (SOD) activity. The ability of SOD to restrict the photochemical reduction of nitro blue tetrazolium was observed. The SOD reaction mixture consisted of 13 mM methionine, 50µM NBT, 1.3 µM riboflavin, 50mM phosphate buffer (pH 7.8), 75 mM EDTA, and 0.050 cm3 enzyme extract. The reaction mixture was placed under a fluorescent light (15 W lamp) for 15 min.

At 560 nm, the absorbance of the irradiated mixture was measured on a spectrophotometer (Hitachi U-2100, Tokyo, Japan). 1 unit SOD activity was the enzymatic concentration which induced 50% inhibition of photo-reduction of NBT (Nitro blue tetrazolium).

2.11. Determination of peroxidase (POD) and catalase (CAT) activities

We followed a protocols of Mehmood et al. (2021) and Maehly and Chance (1954) to measure the activities of catalase and peroxidase with some modification.

To prepare CAT reaction mixture, Five milliliter of 50mM phosphate buffer (pH 5.9 ___ 7.0), 40 ml H2O2 and (0.1 mL) enzyme extract from sample was used. H_2O_2 induced changes in reaction mixture and absorbance was read at 240 nm after every 20 s. One unit catalase activity was equal to 0.01 units per minute in absorbance change.

The peroxidase reaction mixture consisted of (0.1 mL) enzyme extract, 50 mM phosphate buffer (pH 5.0), 40 mM H2O2 and (20 mM) guaiacol. H2O2 induced absorbance changes in the reaction mixture which were read at 470 nm every 20s. One unit peroxidase activity was equal to 0.01 units per min in absorbance change.

2.12. Total soluble sugars determination

Total soluble sugars (TSS) were determined by the method of Ali et al. (2022b). Plant extracts were taken in test tubes, anthrone reagent (6 ml) was added and heated in a boiling water bath (10 min). The test tubes were ice-cooled for10 min and incubated for 20 min at25°C. Optical density (OD) of the plant extracts was recorded at 625 nm using a spectrophotometer (Hitatchi, 220, Japan). The TSS concentration was calculated from the standard curve developed by using the above method.

3. Results

3.1. Growth parameters and physiological data

The exposure of both canola cultivars to Ni stress decreased plant height, as well as fresh and dry weight (Figure 1a-1c). The application of CA and EDTA separately as well as in combination significantly affected these parameters (Figure 1a-1c). The increase in plant height, dry weight and fresh weight was highest when CA and EDTA were applied separately in both, Ni treated, and non-Ni treated plants. A decreasing trend was observed when EDTA and CA were used in combination with each other (Figure 1a-1c).

Figure 1. The role of EDTA and CA on (Figure 1a) plant height and (Figure 1b) shoot fresh weight at the vegetative stage of two canola cultivars (Con-II and Oscar, respectively) in control and Ni treatment and the role of EDTA and citric acid on (Figure 1c) dry weight and (Figure 1d) photosynthetic rate at vegetative stage for phytoremediation of Ni by using canola plant.

Ni contamination significantly affected physiological processes of the sample plants, i.e. net photosynthetic rate, transpiration rate and carbon dioxide concentration in both cultivars (Figures 1d-2a-2b). Application of CA and EDTA alone increased the activity of these physiological parameters. However, when both, EDTA and CA, were used in combination, these parameters decreased.

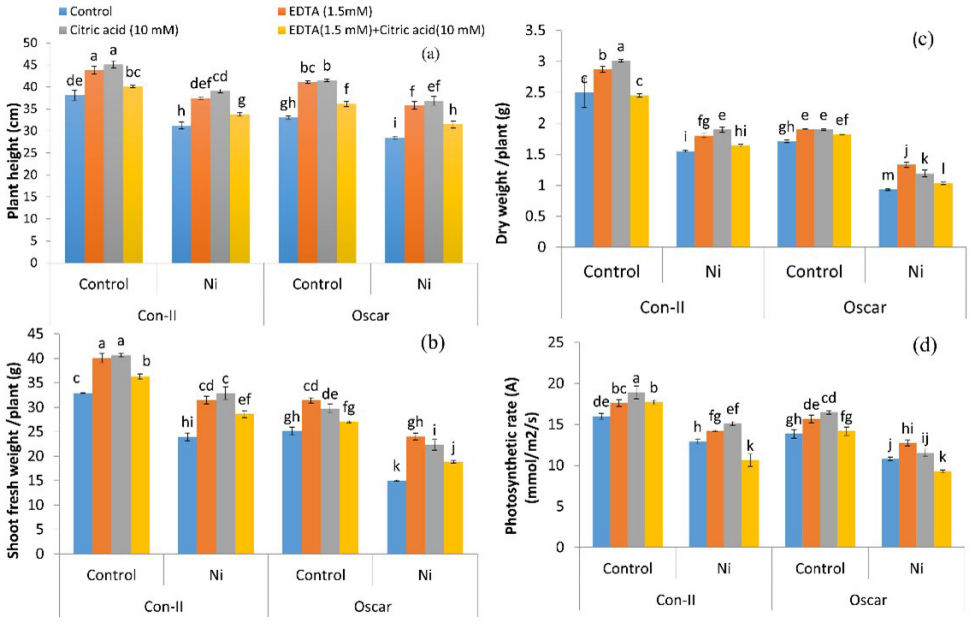

Figure 1. The role of EDTA and CA on (a) plant height and (b) shoot fresh weight at the vegetative stage of two canola cultivars (Con-II and Oscar, respectively) in control and Ni treatment and the role of EDTA and citric acid on (c) dry weight and (d) photosynthetic rate at vegetative stage for phytoremediation of Ni by using canola plant.

Leaf water potential (Ψw) and osmotic potential (Ψs) of *B. napus* cultivars (Con-II and Oscar) were significantly affected by Ni stress (Figure 2c-2d). Application of EDTA and CA separately enhanced water potential to some extent but a decreasing trend was recorded when both EDTA and CA were used in combination with each other.

Figure 2. The role of EDTA and citric acid on (Figure 2a) transpiration rate and (Figure 2b) Carbon dioxide concentration at vegetative stage for phytoremediation of Ni by using canola plant and the role of EDTA and CA on (Figure 2c) leaf water potential (Ψw) and (Figure 2d) leaf osmotic potential (Ψs) at vegetative stage for phytoremediation of Ni by using canola plant.

Leaf turgor potential (Ψw) and water use efficiency increased in Ni treated plants as compared to control (Figure 3a-3b). The application of CA and EDTA in combination further increased the turgor pressure (Ψp) and water use efficiency in both cultivars of canola. In Con-II (tolerant) leaf turgor pressure (Ψp) decreased when CA and EDTA were used in combination while in Oscar (sensitive), it increased by combined application of EDTA and CA. On the other hand, Ni stress significantly increased the concentration of potassium (K+) and sodium (Na+) in both cultivars (Figure 3c-3d). The application of CA and EDTA further increased the concentration of both K+ and Na+ in control and Ni treated plants (Figure 3c-3d). Moreover, results showed that the combined application of CA and EDTA reduced the K+ concentration in Con-II and Oscar. However, Oscar accumulated more Na+ as compared to Con-II (Figure 3d).

Figure 3. The role of EDTA and citric acid on (Figure 3a) leaf turgor potential and (Figure 3b) water use efficiency, (Figure 3c) potassium and (Figure 3d) sodium at vegetative stage for phytoremediation of Ni by using canola plant

Changes in the activities of antioxidants enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are presented in Figure 4a-4c. Antioxidants enzymes activities increased by Ni stress when compared to control plants grown without Ni. Moreover, application of CA and EDTA separately as well as in combination further enhanced the activities of CAT, SOD and POD in both cultivars of canola. This effect was more significant when CA and EDTA were applied separately (Figure 4a-4c).

Total free amino acid and total soluble sugars significantly increased under Ni stress as compared to control (Figure 4d and 4f). Application of CA and EDTA separately further increased the total free amino acid contents and soluble sugars in both cultivars of canola as compared to the respective plants treated with Ni only (Figure 4g). This effect was more pronounced in Con-II as compared to Oscar, however, a decreasing trend was observed in total free amino acid and total soluble sugar when EDTA and CA were used in combination with each other (Figure 4e-4g).

Figure 4. The role of EDTA and citric acid on (Figure 4a) SOD and (Figure 4b) CAT, (Figure 4c) POD, (Figure 4d) Total free amino acid, (Figure 4e) Total soluble proteins, (Figure 4f) Total soluble sugars at vegetative stage for phytoremediation of Ni by using canola plant and the role of EDTA and citric acid on Ni contents (mg/ pot) in

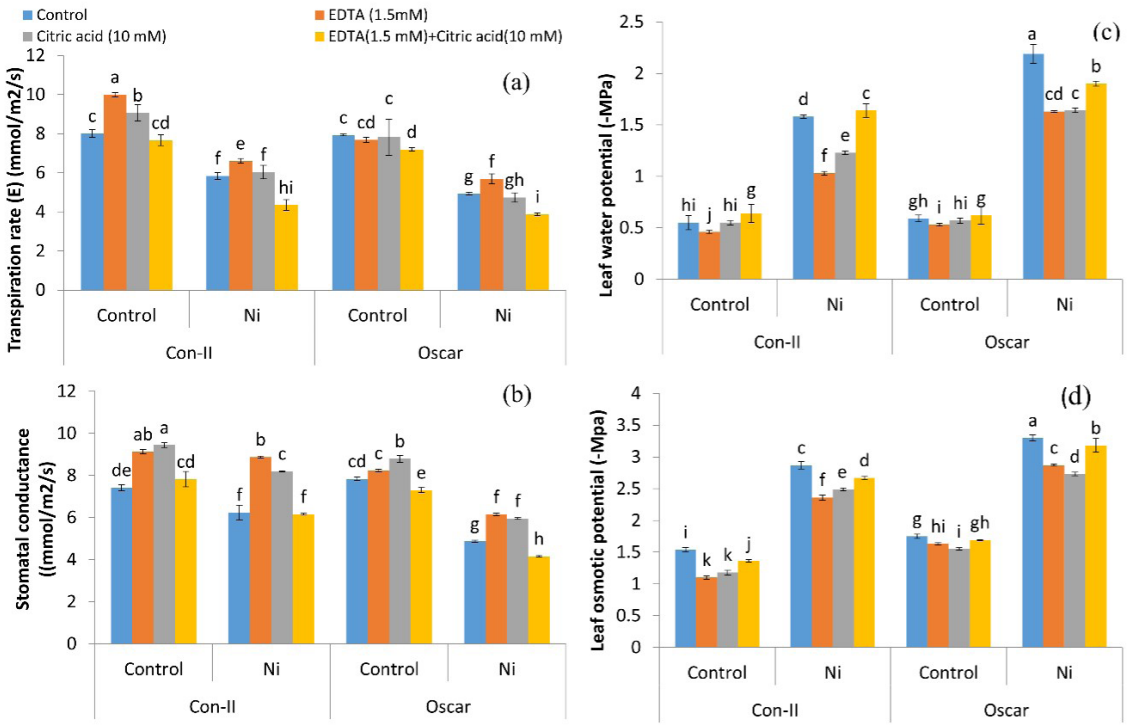

Figure 2. The role of EDTA and citric acid on (a) transpiration rate and (b) Stomatal conductance at vegetative stage for phytoremediation of Ni by using canola plant and the role of EDTA and CA on (c) leaf water potential (Ψw) and (d) leaf osmotic potential (Ψs) at vegetative stage for phytoremediation of Ni by using canola plant.

above ground biomass (Figure 4g) at vegetative stage for phytoremediation of Ni by using canola

Highest Ni contents (22.16 mg/pot) in plants of Con-II receiving EDTA followed by CA treatment (19.7 mg/ pot) and combined EDTA + CA treatment (17.3 mg/ pot) while Oscar cultivar showed significantly lower Ni accumulation in above ground parts (22 to 27%) compared to Con-II. Combined EDTA and CA treatment showed significantly lower Ni contents in both cultivars compared to their individual treatments (Figure 4g).

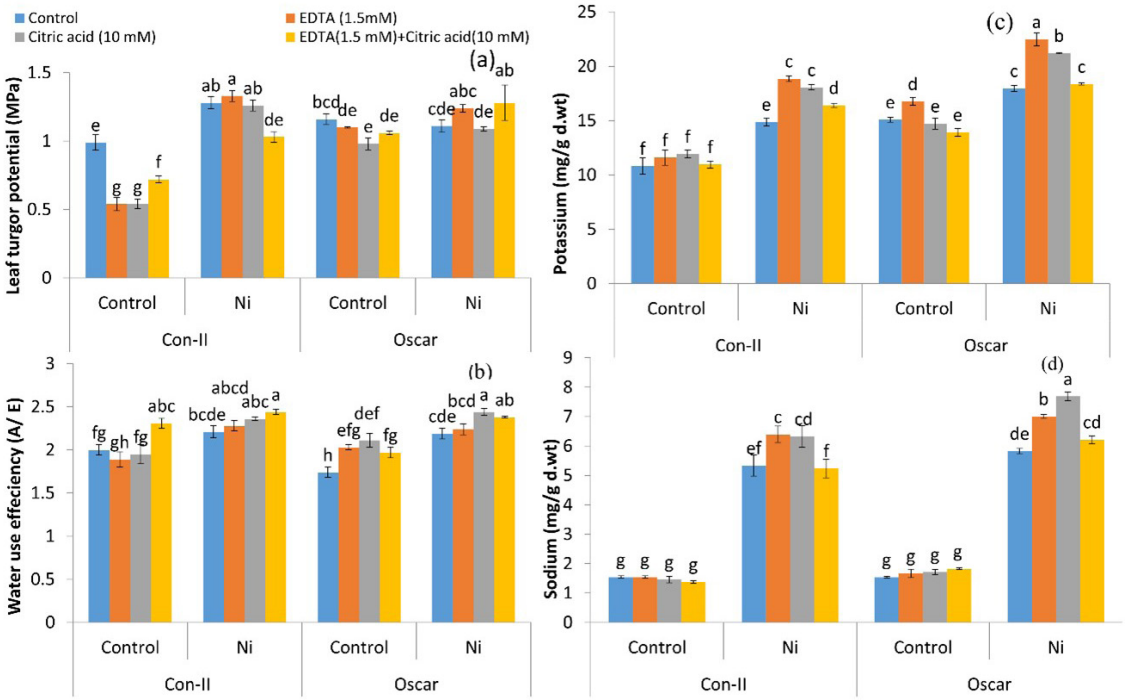

Figure 3. The role of EDTA and citric acid on (a) leaf turgor potential and (b) water use efficiency, (c) potassium and (d) sodium at vegetative stage for phytoremediation of Ni by using canola plant.

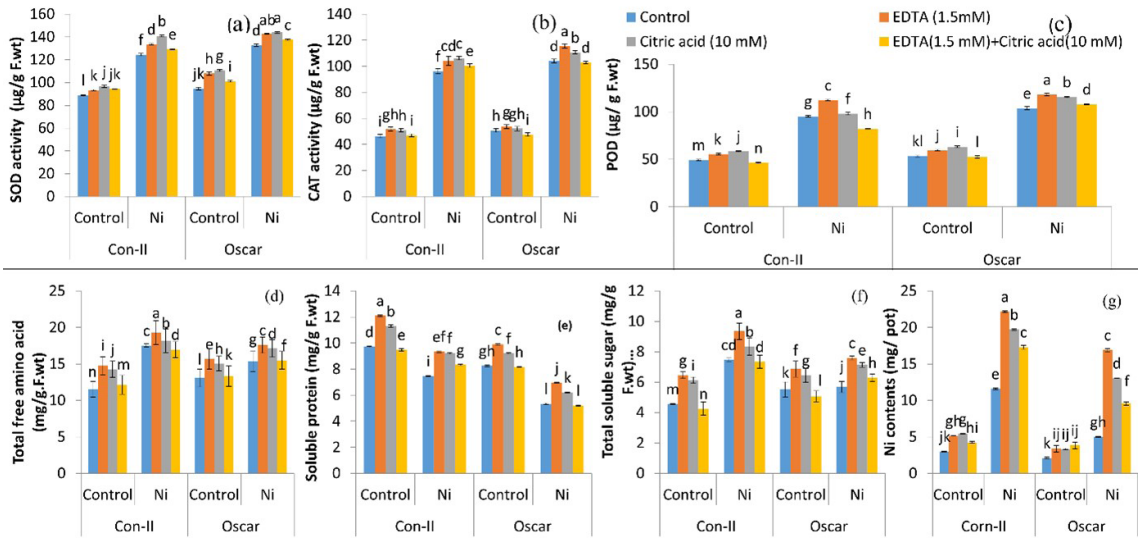

Figure 4. The role of EDTA and citric acid on (a) SOD and (b) CAT, (c) POD, (d) total free amino acid, (e) total soluble proteins, (f) total soluble sugars at vegetative stage for phytoremediation of Ni by using canola plant and the role of EDTA and citric acid on Ni contents (mg/ pot) in above ground biomass (g) at vegetative stage for phytoremediation of Ni by using canola.

4. Discussion

In this study, we evaluated the effects by which CA and EDTA application, separately as well as in combination with each other increase Ni tolerance in two *B. napus* cultivars (Con-II and Oscar). Addition of chelating agents such as CA and EDTA in contaminated soil could alleviate heavy metal toxicity in plants (Ruley et al., 2006). The present study highlights beneficial effects of CA and EDTA application in *B. napus* cultivars under Ni stress. Ni stress reduced the plant height, shoot fresh and dry weights in both cultivars of canola. The results are in agreement with earlier studies on other crops like sunflower, Brassica, *Zea mays* L., Marigold, barley etc. (Ali et al., 2011) which reported that Ni stress reduced the yield of the respective plants (Peralta et al., 2001; Ain et al., 2016).

Excessive amounts of Ni in the growth medium can be toxic leading to inhibition of plant growth, photosynthesis and membrane functioning (Rao and Sresty, 2000). Such growth inhibitory effects of Ni stress are believed to be due to inhibition in enzyme activities (Protease and alpha amylase), protein synthesis, carbohydrate metabolism and mobilization of food reserves (Maheshwari and Dubey, 2007). However the application of chelating agents like EDTA and CA improved plant tolerance to Ni and enhance the plant height, shoot fresh and dry weights in Iris halophila seedlings cultivated in Pb mine tailings as compared to those without application of chelating agents under Ni stress (Han et al., 2018). Beneficial effects of chelate-assisted increase in growth and biomass has also been reported in sudan grass and sweet sorghum (Mani and Kumar, 2014). In the present study, an increase in plant height, fresh and dry weight with the addition of EDTA and CA probably occurred due to Ni-chelation which reduced the toxic effects of Ni. On the other hand, a decreasing trend in these parameters was recorded when EDTA and CA were applied in combination with each other. This can be due to the fact that EDTA and CA increase the solubility of Ni in soil that ultimately increased Ni availability for plant leading to suppressed growth.

Nickel has been reported to induce a decline in plant transpiration rate and water contents (Sheoran et al., 1990). The presence of Ni in the growth medium increased the level of ABA which is known to induce stomata closure (Leymarie et al., 1998). In this study, Ni induced a decrease in moisture content and stomatal closure and reduced photosynthesis. Also the reduction in photosynthetic rate can be due to changes in the ultra-structure of chloroplasts, stomatal closure and chlorophyll synthesis disruption (Seregin and Ivanov, 2001). Here, the application of EDTA and CA significantly enhanced net photosynthetic rate (A), transpiration rate (E) and carbon dioxide concentration (Ci) under Ni stress reflecting the positive effect of CA and EDTA on gas exchange attributes. In a study by Ruley et al. (2006), Pb and its chelates with organic acid affected the growth due to hindered photosynthetic activity in *Sesbenia durmmandii* grown under Pb stress. It is well documented that heavy metals stress decreased the water contents of plant which is an indirect damage by heavy metal toxicity (Ashraf et al., 2011). Zaier et al. (2010) reported that water content of *B. juncea* drastically decreased under heavy metal stress, even in the species which were considered to be tolerant. However, in the present study, the application of CA and EDTA significantly enhanced the water content in *B. napus* under Ni stress.

Furthermore, the activity of SOD, POD and CAT significantly increased under Ni stress. Application of EDTA and CA further increased the activities of these enzymes in plants growing under Ni stress. This increase in the activity of antioxidants by EDTA application is attributed to protection mechanisms of the plants via scavenging ROS (Zhao et al., 2010). Our study also depicted that an excessive Ni concentration in the growth medium reduced soluble protein contents in plants which may be due to cellular injuries mediated by oxidative stress and leading to inhibition of protein synthesis in plants (Gupta et al., 2008). The EDTA and CA application resulted in an increase of total soluble protein content in *B. napus* which may add to increased antioxidant activities leading to better oxidative stress tolerance.

The decline in nutrients uptake as a result of Ni stress may be due to Ni induced metabolic disorders (Ahmad et al., 2007). Nutrients like K+ and Mg2+ are cofactors for a variety of metabolic processes and their deficiency may seriously affect several metabolic processes. Our results showed that K+ contents decreased and Na+ content increased under Ni stress but by the application of chelating agents K+ increase and Na+ decrease (Rahman et al., 2005)

In the present study, CA and EDTA significantly increased the total amino acid and total soluble sugar content in *B. napus* under Ni stress. Total soluble sugars and amino acids have protective roles in regulating osmotic potential and detoxification of ROS (Krämer, 2005).This protective role of total amino acid and total soluble sugar in *B. napus* plant has well been documented for Cd stress by Ali et al. (2015).

5. Conclusion

We highlighted the successful use of chelating agents (CA and EDTA) not only to ameliorate Ni stress but also to enhance Ni accumulation which is prerequisite for phytoremediation. Application of 10 mM CA and 1.5 mM EDTA separately to the rooting medium proved to be most effective in ameliorating the growth inhibition induced by Ni. The combination of chelating agents failed to show any synergistic effects.

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References

AHMAD, M.S.A., HUSSAIN, M., SADDIQ, R. and ALVI, A.K., 2007. Mungbean: a nickel indicator, accumulator or excluder? *Bulletin of Environmental Contamination and Toxicology*, vol. 78, no. 5, pp. 319-324. http://dx.doi.org/10.1007/s00128-007-9182-y. PMid:17619800.

AIN, Q., AKHTAR, J., AMJAD, M., HAQ, M. and SAQIB, Z., 2016. Effect of enhanced nickel levels on wheat plant growth and physiology under salt stress. *Communications in Soil Science and Plant Analysis*, vol. 47, no. 22, pp. 2538-2546. http://dx.doi.org/10.1080/00103624.2016.1254796.

ALI, B., WANG, X., SALEEM, M.H., AZEEM, M.A., AFRIDI, M.S., NADEEM, M., GHAZAL, M., BATOOL, T., QAYYUM, A., ALATAWI, A. and ALI, S., 2022a. Bacillus mycoides PM35 reinforces photosynthetic efficiency, antioxidant defense, expression of stress-responsive genes, and ameliorates the effects of salinity stress in maize. *Life*, vol. 12, no. 2, p. 219. http://dx.doi.org/10.3390/life12020219. PMid:35207506.

ALI, B., WANG, X., SALEEM, M.H., SUMAIRA, HAFEEZ, A., AFRIDI, M.S., KHAN, S., ZAIB-UN-NISA, ULLAH, I., AMARAL JÚNIOR, A.T., ALATAWI, A. and ALI, S., 2022b. PGPR-mediated salt tolerance in maize by modulating plant physiology, antioxidant defense, compatible solutes accumulation and bio-surfactant producing genes. *Plants*, vol. 11, no. 3, p. 345. http://dx.doi.org/10.3390/plants11030345. PMid:35161325.

ALI, S., CHAUDHARY, A., RIZWAN, M., ANWAR, H.T., ADREES, M., FARID, M., IRSHAD, M.K., HAYAT, T. and ANJUM, S.A., 2015. Alleviation of chromium toxicity by glycinebetaine is related to el- evated antioxidant enzymes and suppressed chromium uptake and oxidative stressin wheat (Triticum aestivumL.). *Environmental Science and Pollution Research International*, vol. 22, no. 14, pp. 10669-10678. http://dx.doi.org/10.1007/s11356-015-4193-4. PMid:25752628.

ALI, S., ZENG, F., CAI, S., QIU, B. and ZHANG, G., 2011. The interaction of salinity and chromium in the influence of barley growth and oxidative stress. *Plant, Soil and Environment*, vol. 57, no. 4, pp. 153-159. http://dx.doi.org/10.17221/335/2010-PSE.

ARNON, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, vol. 24, no. 1, pp. 1-15. http://dx.doi.org/10.1104/pp.24.1.1. PMid:16654194.

ASHRAF, M.Y., SADIQ, R., HUSSAIN, M., ASHRAF, M. and AHMAD, M.S.A., 2011. Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (Helianthus Annuus L.). *Biological Trace Element Research*, vol. 143, no. 3, pp. 1695-1703. http://dx.doi.org/10.1007/s12011-011-8955-7. PMid:21240566.

BHAT, J.A., SHIVARAJ, S.M., SINGH, P., NAVADAGI, D.B., TRIPATHI, D.K., DASH, P.K., SOLANKE, A.U., SONAH, H. and DESHMUKH, R., 2019. Role of silicon in mitigation of heavy metal stresses in crop plants. *Plants*, vol. 8, no. 3, p. 71. http://dx.doi.org/10.3390/plants8030071. PMid:30901942.

DAVIES, B.H., 1976. Carotenoids. In: T.W. GOODWIN, ed. *Chemistry and biochemistry of plant pigments*. London: Academic Press, vol. 2, pp. 38-165.

FLORA, S.J.S., MITTAL, M. and MEHTA, A., 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *The Indian Journal of Medical Research*, vol. 128, no. 4, pp. 501-523. PMid:19106443.

GAJEWSKA, E., SKLODOWSKA, M., SLABA, M. and MAZUR, J., 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll content in wheat shoots. *Biologia Plantarum*, vol. 50, no. 4, pp. 653-659. http://dx.doi.org/10.1007/s10535-006-0102-5.

GEORGIADOU, E.C., KOWALSKA, E., PATLA, K., KULBAT, K., SMOLIŃSKA, B., LESZCZYŃSKA, J. and FOTOPOULOS, V., 2018. Influence of heavy metals (Ni, Cu, and Zn) on nitro-oxidative stress responses, proteome regulation and allergen production in basil (Ocimum basilicum L.) plants. *Frontiers in Plant Science*, vol. 9, p. 862. http://dx.doi.org/10.3389/fpls.2018.00862. PMid:30026745.

GLASS, D.J., 2000. Phytoremediation's economic potential. In: I. RASKIN and B.D. ENSLEY, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York: John Wiley and Sons, pp. 15-31.

GUPTA, U.C., WU, K. and LIANG, S., 2008. Micronutrients in soils, crops, and livestock. *Earth Science Frontiers*, vol. 15, no. 5, pp. 110-125. http://dx.doi.org/10.1016/S1872-5791(09)60003-8.

HAMILTON, P.B. and VANSLYKE, P.D., 1973. Amino acid determination with ninhydrine. *Journal of Biochemistry*, vol. 150, pp. 231-232.

HAN, Y., ZHANG, L., GU, J., ZHAO, J. and FU, J., 2018. Citric acid and EDTA on the growth, photosynthetic properties and heavy metal accumulation of Iris halophila Pall. cultivated in Pb mine tailings. *International Biodeterioration & Biodegradation*, vol. 128, pp. 15-21. http://dx.doi.org/10.1016/j.ibiod.2016.05.011.

KRÄMER, U., 2005. Phytoremediation: novel approaches to cleaning up polluted soils. *Current Opinion in Biotechnology*, vol. 16, no. 2, pp. 133-141. http://dx.doi.org/10.1016/j.copbio.2005.02.006. PMid:15831377.

LEŠTAN, D., LUO, C.L. and LI, X.D., 2008. The use of chelating agents in the remediation of metal-contaminated soils: a review. *Environmental Pollution*, vol. 153, no. 1, pp. 3-13. http://dx.doi.org/10.1016/j.envpol.2007.11.015. PMid:18155817.

LEYMARIE, J., LASCÈVE, G. and VAVASSEUR, A., 1998. Interaction of stomatal responses to ABA and CO2 in Arabidopsis thaliana. *Australian Journal of Plant Physiology*, vol. 25, pp. 785-791.

LIPHADZI, M.S., KIRKHAM, M.B. and MUSIL, C.F., 2005. Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment. *South African Journal of Botany*, vol. 71, no. 1, pp. 24-37. http://dx.doi.org/10.1016/S0254-6299(15)30145-9.

LIU, L., LI, W., SONG, W. and GUO, M., 2018. Remediation techniques for heavy metal-contaminated soils: principles and applicability. *The Science of the Total Environment*, vol. 633, pp. 206-219. http://dx.doi.org/10.1016/j.scitotenv.2018.03.161. PMid:29573687.

MAEHLY, A.C. and CHANCE, B., 1954. The assay of catalases and peroxidases. *Methods of Biochemical Analysis*, vol. 1, pp. 357-424. PMid:13193536.

MAHESHWARI, R. and DUBEY, R.S., 2007. Nickel toxicity inhibits ribonuclease and protease activitiesin rice seedlings: protective effects of proline. *Plant Growth Regulation*, vol. 51, no. 3, pp. 231-243. http://dx.doi.org/10.1007/s10725-006-9163-x.

MANI, D. and KUMAR, C., 2014. Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. *International Journal of Environmental Science and Technology*, vol. 11, no. 3, pp. 843-872. http://dx.doi.org/10.1007/s13762-013-0299-8.

MEHMOOD, S., KHATOON, Z., AMNA, AHMAD, I., MUNEER, M.A., KAMRAN, M.A., ALI, J., ALI, B., CHAUDHARY, H.J. and MUNIS, M.F.H., 2021. Bacillus sp. PM31 harboring various plant growth-promoting activities regulates Fusarium dry rot and wilt tolerance in potato. *Archives of Agronomy and Soil Science*, vol. 67, pp. 1-15. http://dx.doi.org/10.1080/03650340.2021.1971654.

MELO, E.E.C., NASCIMENTO, C.W.A., ACCIOLY, A.M.A. and SANTOS, A.C.Q., 2008. Phytoextraction and fractionation of heavy metals in soil after multiple applications natural chelants. *Scientia Agrícola*, vol. 65, no. 1, pp. 61-68. http://dx.doi.org/10.1590/S0103-90162008000100009.

OGUNLEYEL, J.H., DAGARI, M.S., BALOGUN, O.L. and MAIGARI, A., 2016. Effect of oxalic acid on Cr+3 ion uptake, accumulation and oxidative stress by sorrel (Hibiscus sabdariffa L.) seedlings in hydroponic solution. *International Journal of Chemical, Material and Environmental Research*, vol. 3, pp. 49-55.

PARK, J., KIM, J.Y. and KIM, K.W., 2012. Phytoremediation of soil contaminated with heavy metals using Brassica napus. *Geosystem Engineering*, vol. 15, no. 1, pp. 10-18. http://dx.doi.org/10.1080/12269328.2012.674428.

PAZ-FERREIRO, J., LU, H., FU, S., MENDEZ, A. and GASCO, G., 2014. Use of phytoremediation and biochar to remediate heavy metal polluted soils: a review. *Solid Earth*, vol. 5, no. 1, pp. 65-75. http://dx.doi.org/10.5194/se-5-65-2014.

PERALTA, J.R., GARDEA-TORRESDEY, J.L., TIEMANN, K.J., GOMEZ, E., ARTEAGA, S., RASCON, E. and PARSONS, J.G., 2001. Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa). *Bulletin of Environmental Contamination and Toxicology*, vol. 66, no. 6, pp. 727-734. http://dx.doi.org/10.1007/s001280069. PMid:11353374.

RAHMAN, H., SABREEN, S., ALAM, S. and KAWAI, S., 2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrients solution. *Journal of Plant Nutrition*, vol. 28, no. 3, pp. 393-404. http://dx.doi.org/10.1081/PLN-200049149.

RAO, K.V.M. and SRESTY, T.V.S., 2000. Antioxidative parameters in the seedlings of pigeonpea (Cajanuscajan) (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science*, vol. 157, no. 1, pp. 113-128. http://dx.doi.org/10.1016/S0168-9452(00)00273-9. PMid:10940475.

RULEY, A.T., SHARMA, N.C., SAHI, S.V., SINGH, S.R. and SAJWAN, K.S., 2006. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in Sesbania drummondii grown in soil. *Environmental Pollution*, vol. 144, no. 1, pp. 11-18. http://dx.doi.org/10.1016/j.envpol.2006.01.016. PMid:16522347.

SEREGIN, I.V. and IVANOV, V.B., 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian Journal of Plant Physiology*, vol. 48, no. 4, pp. 523-544. http://dx.doi.org/10.1023/A:1016719901147.

SETH, C.S., REMANS, T., KEUNEN, T., JOZEFCZAK, M., GIELEN, H., OPDENAKKER, K., WEYENS, N., VANGRONSVELD, J. and CUYPERS, A., 2012. Phytoextraction of toxic metals: a central role for glutathione. *Plant, Cell & Environment*, vol. 35, no. 2, pp. 334-346. http://dx.doi.org/10.1111/j.1365-3040.2011.02338.x. PMid:21486307.

SHEORAN, I.S., SINGAL, H.R. and SINGH, R., 1990. Effects of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeon pea (Cajanus cajan L.). *Photosynthesis Research*, vol. 23, no. 3, pp. 345-351. http://dx.doi.org/10.1007/BF00034865. PMid:24419658.

SINGH, J. and KALAMDHAD, A.S., 2011. Effects of heavy metals on soil, plants, human health and aquatic life. *International Journal of Research in Chemistry and Environment*, vol. 1, no. 2, pp. 15-21.

SREEKANTH, T.V.M., NAGAJYOTHI, P.C., LEE, K.D. and PRASAD, T.N.V.K.V., 2013. Occurrence, physiological responses and toxicity of nickel in plants. *International Journal of Environmental Science and Technology*, vol. 10, no. 5, pp. 1129-1140. http://dx.doi.org/10.1007/s13762-013-0245-9.

SZCZYGŁOWSKA, M., PIEKARSKA, A., KONIECZKA, P. and NAMIESNIK, J., 2011. Use of brassica plants in the phytoremediation and biofumigation processes. *International Journal of Molecular Sciences*, vol. 12, no. 11, pp. 7760-7771. http://dx.doi.org/10.3390/ijms12117760. PMid:22174630.

TANG, J.H., ZHANG, Q.Q., ZHANG, Y.F. and XU, G., 2012. Diagnosis of the anomaly source of heavy metals in soil around the Minhe HaishiwanArea, Qinghai. *Earth and Environ.*, vol. 40, pp. 195-200.

TURGUT, C., PEPE, M.K. and CUTRIGHT, T.J., 2004. The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using Helianthus annuus. *Environmental Pollution*, vol. 131, no. 1, pp. 147-154. http://dx.doi.org/10.1016/j.envpol.2004.01.017. PMid:15210283.

VIET, H.N., FRONTASYEVA, M.V., THI, T.M.T., GILBERT, D. and BERNARD, N., 2010. Atmospheric heavy metal deposition in northern Vietnam: Hanoi and Thainguyen case study using the moss biomonitoring technique, INAA and AAS. *Environmental Science and Pollution Research International*, vol. 17, no. 5, pp. 1045-1052. http://dx.doi.org/10.1007/s11356-009-0258-6. PMid:19924458.

WOLF, B., 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Communications in Soil Science and Plant Analysis*, vol. 13, no. 12, pp. 1035-1059. http://dx.doi.org/10.1080/00103628209367332.

YUSUF, M., FARIDUDDIN, Q., HAYAT, S. and AHMAD, A., 2011. Nickel: an overview of uptake, essentiality and toxicity in plants. *Bulletin of Environmental Contamination and Toxicology*, vol. 86, pp. 1-17. http://dx.doi.org/10.1007/s00128-010-0171-1. PMid:21170705.

ZAIER, H., GHNAYA, T., LAKHDAR, A., BAIOUI, R., GHABRICHE, R., MNASRI, M., SGHAIR, S., LUTTS, S. and ABDELLY, C., 2010. Comparative study of Pb-phytoextraction potential in Sesuvium portulacastrum and Brassica juncea: tolerance and accumulation. *Journal of Hazardous Materials*, vol. 183, no. 1-3, pp. 609-615. http://dx.doi.org/10.1016/j.jhazmat.2010.07.068. PMid:20708335.

ZAINAB, N., AMNA, KHAN, A.A., AZEEM, M.A., ALI, B., WANG, T., SHI, F., ALGHANEM, S.M., MUNIS, M.F.H., HASHEM, M., ALAMRI, S., LATEF, A.A.H.A., ALI, O.M., SOLIMAN, M.H. and CHAUDHARY, H.J., 2021. PGPR-mediated plant growth attributes and metal extraction ability of Sesbania sesban L. in industrially contaminated soils. *Agronomy*, vol. 11, no. 9, p. 1820. http://dx.doi.org/10.3390/agronomy11091820.

ZHAO, Z., XI, M., JIANG, G., LIU, X., BAI, Z. and HUANG, Y., 2010. Effects of IDSA, EDDS and EDTA on heavy metals accumulation in hydroponically grown maize (Zeamays,L.). *Journal of Hazardous Materials*, vol. 181, no. 1-3, pp. 455-459. http://dx.doi.org/10.1016/j.jhazmat.2010.05.032. PMid:20627568.