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ORIGINAL ARTICLE

The toxic effects of nickel and cadmium on germination, seedling growth and biochemical contents of *Rauwolfia serpentina* Benth. ex Kruz

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Effects of different concentrations of nickel sulphate and cadmium nitrate on germination, root and shoot growth, dry weight and changes in contents of total sugar, protein and pigments of this plant was studied. The study shows that the lower concentration of nickel has no effect or beneficial effect but application of higher level of nickel has an adverse effect. Cadmium reduced the percentage of germination, root and shoot length and dry weight of root and shoot. The content of pigments, total sugar and protein were also affected by cadmium nitrate and their contents were decreased.

Key words: Germination and growth, heavy metals, medicinal plant, nickel and cadmium toxicity, Rauwolfia serpentina Benth. ex Kruz

The heavy metal pollution is one of the causes for the risk of medical use of medicinal plant raw materials and preparations on their basis (Mironov, 2013). Hazardous metals are widely distributed in the Earth's crust and are classified among serious pollutants within the natural environment. Recently, the presence of hazardous metals in the ecosystem has become a global concern due to the perilous effects they pose to humans and other organisms when allowable limits are exceeded, persistence and potential bioaccumulation. These metals are capable of decreasing crop production due to the risk of bioaccumulation and bio magnifications in the food chain (Wuana and Okieimen, 2011).

Rauwolfia serpentina L. Benth. Ex Kurz. is an evergreen, woody, perennial, erect under shrub grows up to a height of 60 cm, usually 30 to 40 cm. The plant possesses tuberous root with pale brown cork. Leaves are in the whorls of three, elliptic to lanceolate or obovate, bright green above, pale green below, tip acute or acuminate, base tapering and petioles long. Flowers are bisexual, white in colour. They occur in many flowered irregular cymose cymes. Fruits are drupes 0.5 to 0.7 cm across and purplish black in colour.

The roots, leaves and juice are of medicinal importance and have attracted the attention of practitioners of indigenous system of medicine, as it contains a large number of secondary metabolites (Mittal *et al*., 2012; Poonam *et al*., 2013). The roots of *R. serpentina* are used as a remedy for curing hypertension, mental agitation, gastrointestinal disorders, excitement, epilepsy, anxiety (Meena *et al*., 2009; Poonam *et al*., 2013). The other diseases such as pneumonia, malaria, body aches, eczema, burns, menstrual disorders, scabies, skin cancers, asthma, respiratory problems, eye inflammation, spleen diseases and fever can also be cured using *R. serpentina* (Rai, 2004; Itoh *et al*., 2005; Mohanta *et al*., 2006; Bhattarai *et al*., 2009; Dey and De, 2010; Azmi and Qureshi, 2012).

Certain workers studied the effect of nickel on plant growth (Das *et al*., 1978; Welch, 1981; Brown *et al*., 2007; Ahmad and Ashraf, 2011; Güleryüz *et al*., 2016). Several workers contributed on the effect of cadmium on seed germination, seedlings growth and nutrition (Blum *et al*., 1997; Djukic *et al*., 2013; Konate *et al*., 2018). This study aims at determining the effect of different concentrations levels of two heavy metals nickel sulphate and cadmium nitrate on seed germination, growth and biochemical content of *R. serpentina*.

MATERIALS AND METHODS

Seed sample and treatment

Seed samples were collected from widely grown plant in the campus of Veer Narmad South Gujarat University, Surat, India. Cleaned and healthy seeds were selected and sterilized with 0.2% $HgCl_2$ for the germination study.

Experimental procedure

Ten seeds were placed in petri dishes lined with filter paper. The various concentrations of nickel sulphate and cadmium nitrate solution (10, 25, 50, 75, 100, 150 and 200 ppm) were prepared and used for the seed germination and growth studies. The seed germinated with distilled water was treated as control. The germination percentage, seedling growth, dry matter yield and biochemical contents were studied ten days after showing. For the study of dry weight, the root and shoot were cut into small pieces and placed separately in brown bags after weighing and kept in oven at 80 °C for a period of 8 days for drying. The dry weight of these organs was recorded.

The biochemical estimation of total sugar was studied by Nelson (1944) method in which the amount of sugar was calculated from optical density obtained by spectrophotometer. Similarly, the protein content was determined from optical density following the procedure of Lowry *et al*. (1951). For the pigment content determination acetone extract of fresh leaves was used and the amount of chlorophyll a, chlorophyll b and carotenoid was calculated by using the formulae given by Machalachalan and Zalik (1963) and Duxbury and Yentsch (1956).

Statistical analysis

All the experiments were repeated twice and ten replicates were maintained for each experiment. The values are given as mean and standard error (SE) from five replicates.

RESULTS AND DISCUSSION

Effect of nickel and cadmium on germination

Present study showed no effect of lower concentration of nickel on seed germination of *R. serpentina*. The percentage of germination of seeds in the control as well as in the 10 and 25 mg/l of nickel treatment was indicating no effect of nickel on germination at lower concentration. The treatment of higher concentrations of nickel had an adverse effect on seed germination (Fig. 1A). Similarly, negative effect of higher concentration of nickel on seed germination and seedling growth was also reported by Akar and Atis (2018) in ray grass plant. The reduction in seed germination by higher concentration of nickel was explained by Hall (2000). According to him the higher nickel concentration reduces all energy requiring cellular process during seed germination causing its reduction. Likewise, when different concentration of cadmium was used, it also reduced germination of *R. serpentina* seeds (Fig. 1B). It was observed that more reduction in seed germination was at higher level of cadmium in the treatment. Similar reduction in seed germination after cadmium treatment was found in four species of wheat by Ahmad *et al*. (2012) as well as in durum wheat by Yourtchi and Bayat (2013). The reason behind this is might be the toxic effects of ions on germination process (Talebi *et al*., 2014).

Effect of nickel and cadmium on root and shoot length

The treatment of lower concentration of nickel increase the root length, when the concentration of nickel was increased from 50 mg/l onwards it reduced the growth of root (Fig. 2A). In corroboration with present results, in wheat cultivates also the root length has been increased at lower concentration of nickel which reduced at higher concentration (Shweti and Verma, 2018). Similar effects of nickel have been also reported in maize, bean and lettuce (Antonkiewicz *et al*., 2018). According to Ouzounidou *et al*. (2006) this reduction of plant growth by higher concentration of nickel is due to reduction of supply of additional ions required for plant growth. The present study indicated that lower concentration (10 mg/l) of nickel has no effect on shoot growth but when concentration is increase from 25 to 200 mg/l showed adverse effect on shoot length. Thus according to this work the nickel has toxic effect on both root and shoot growth. The toxicity of nickel is found much more on root growth as comparison to shoot growth. Similar toxicity of nickel was also reported in maize plant by Seregin *et al*. (2003). The higher concentration of metals results in photo toxicity through changes in permeability of cell membrane of essential ions and replacement of essential ions (Patra *et al*., 2004). Similarly, shoot and root elongation was also greatly inhibited by cadmium treatment, the degree of inhibition was found to increase as the concentration of cadmium was increased in the treatment (Fig. 2B). Similar results are also reported recently in *Rheum emodi* where increase in cadmium concentration decreases shoot and root length (Shukla and Thapliyal, 2021). However, the inhibition was more pronounced in root growth than shoot growth. The study of Blum *et al*. (1997) and John *et al*. (2009) on heavy metal toxicity indicated that the radical is most sensitive part to cadmium treatment and has more effects on its growth than shoot.

Effect of nickel and cadmium on root and shoot dry weight

The treatment of 10 and 25 mg/l of nickel increases the dry weight of shoot and root and it has been recorded that the higher concentrations decreased the shoot and root dry weight. The maximum decrease in dry weight of both the organs was observed at 200 mg/l (Fig. 3A). The reduction of dry weight by the treatment of higher level of nickel was also found in other plants. They are Cowpea (Mujeeb *et al*., 2019), Soyben (Malan and Farrant, 1998) and Maize (Baccouch *et al*., 2001). The cause of this reduction in dry weight was explained by Chen *et al*. (2009). According to them, the toxic effect of nickel on germination and plant growth is due to its interference of nickel with others essential metal ions and induction of oxidative stress. The dry weight of root and shoot was also affected by another heavy metal i.e. cadmium. The reduction of shoot dry weight is 5.00% and root dry weight is 9.09% at 10 mg/l in treatment which gradually decreases by increasing cadmium concentration in the treatment (Fig. 3B). Cadmium stress negatively affected dry weight of shoots and roots which is previously reported in many studies in which decline in biomass production due to disruptions in the processes of respiration, photosynthesis, and nitrogen metabolism owing to the toxic concentrations of cadmium (Påhlsson, 1989; Larsson *et al*., 1998; Haag-kerwer *et al*., 1999).

Effect of nickel and cadmium on chlorophyll A and B

In the present investigation it was found that the photosynthetic pigments are also affected by treatment with both metals. A gradual reduction of Chlorophyll A and Chlorophyll B was observed after nickel concentration was increased from 10 to 200 mg/l. Neverthless, the content of chlorophyll B was more sensitive to nickel than Chlorophyll A, as it showed more decrease over control than that of chlorophyll A (Fig. 4A). The reduction in chlorophyll content is due to inhibition of enzymes for chlorophyll biosynthesis by nickel (Kaveriammal and Subramani, 2013). Chlorophyll content showed a decreasing trend with progressive increase in cadmium concentration too (Fig. 4B). The similar phenomenon was reported earlier in sunflower (Zengin and Munzuroglu, 2006) and almond (Nada *et al*., 2007). The synthesis and level of chlorophyll decrease was also observed in other plants like cress and lettuce (Czuba and Ormond, 1974), *Hordeum vulgare* (Stiborova *et al*., 1986) and *Zea mays* (Ferretti *et al*., 1993) under the influence of the cadmium stress. The decline in chlorophyll content in plants exposed to cadmium stress is due to inhibition of important enzymes associated with chlorophyll biosynthesis (Padmaja *et al*., 1990; Van Assche and Clijsters, 1990) and impairment in the supply of Mg^+, Fe^+ and Zn^+ (Van Assche and Clijsters, 1990; Küpper *et al*., 1996).

Effect of nickel and cadmium on carotenoid

When carotenoid content was analyzed in *R. serpentina*, it was noted that it was decreased gradually as the concentration of nickel was increased (Fig. 5A). Previously, Latif (2010) also reported decrease carotenoid content in *Raphanus stavius* after nickel treatment. Similarly, reduction in carotenoid content is also documented in *Cicer aritinum* (Batool *et al*., 2018), carrot, onion and potato (Flemotomou *et al*., 2011) and tomato (Pandey and Sharma, 2003) after nickel treatment. Pandey and Sharma (2003) explained that this is due to reduction in activities of Fe enzymes, catalase and peroxidase. When *R. serpentina* was treated with cadmium, a same result was recorded i.e. gradual decrease in carotenoid content as concentration of cadmium increased (Fig. 5B). The decrease in carotenoid content by the high concentration of cadmium was well documented in *Phaseolus vulgaris* (Bhardwaj *et al*., 2009) and *Pisum stavium* (Deswal and Laura, 2018). Bhardwaj *et al*. (2009) suggested that the decline in the level of carotenoid was due to the metal interference with pigment metabolism.

Effect of nickel and cadmium on protein

Another biochemical parameter which was taken into consideration was protein content as Deswal and Lora (2018) investigated that amount of protein decreases with the increasing concentration of heavy metals like cadmium, nickel and lead in the seedling of *P. sativum*. In this study also it was seen that the protein content in the seedlings reduced by increasing the nickel level (Fig. 6A). This is also documented by Latif (2010) in radish plants. According to Palma *et al*. (2002) the decrease in the protein content may be due to enhance protein degradation as a result of increase protease activity by higher concentration of nickel. Similarly cadmium also had an adverse effect on protein content as its various concentrations reduced the amount of protein (Fig. 6B). The decrease in protein content by cadmium treatment was also well studied in *Tagetas eracta* (Shah *et al*., 2017), sugarcane (Jain and Srivastava, 2006), *P. vulgaris* (Bhardwaj *et al*., 2009) and *Rheum emodi* (Shukla and Thapliyal, 2021). The reduction in protein by cadmium is due to enhance protein hydrolysis by the influence of cadmium (Melnichuk *et al*., 1982).

Effect of nickel and cadmium on total sugar

At last, total sugar content in seedlings was estimated and it was also affected by nickel concentration and it gradually decreased by increasing the level of nickel (Fig. 7A). Reduction in sugar content due to higher concentration of nickel was also observed in *Arachis hypogaea* (Ezhilvannan *et al*., 2011), raddish (Espen *et al*., 1997) and *Abrus precatorious*

(Vyas, 2017). Rabie *et al*. (1992) reported decrease in carbohydrates with respect to the high levels of nickel in Corn and Broad bean and suggested that this was due to role of nickel on enzymatic reaction related to the carbohydrate catabolism. Whereas results with another heavy metal indicated that the exposure of *R. serpentina* seeds at lower concentration of cadmium (10 and 25 mg/l) increased the content of total sugar; but at higher concentrations its proportion was reduced (Fig. 7B). This is in line with earlier reports in *T. eracta* (Shah *et al*., 2017) and *Lemna polyrrizha* (John *et al*., 2008) where treatment with lower concentration of cadmium increased the total sugar content and reduced at higher concentration. On the contrary, lower as well as higher concentration of cadmium cause adverse effect in *P. vulgaris* (Bhardwaj *et al*., 2009).

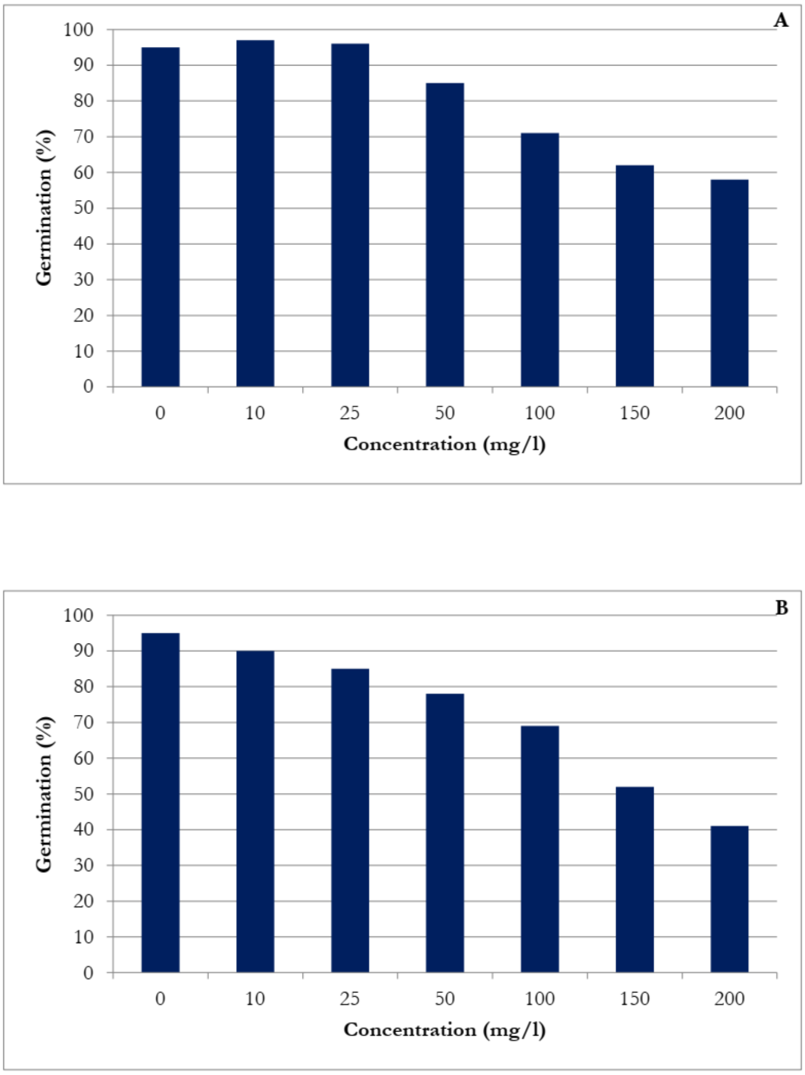

Figure 1. Effect of heavy metals on on seed germination of *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10).

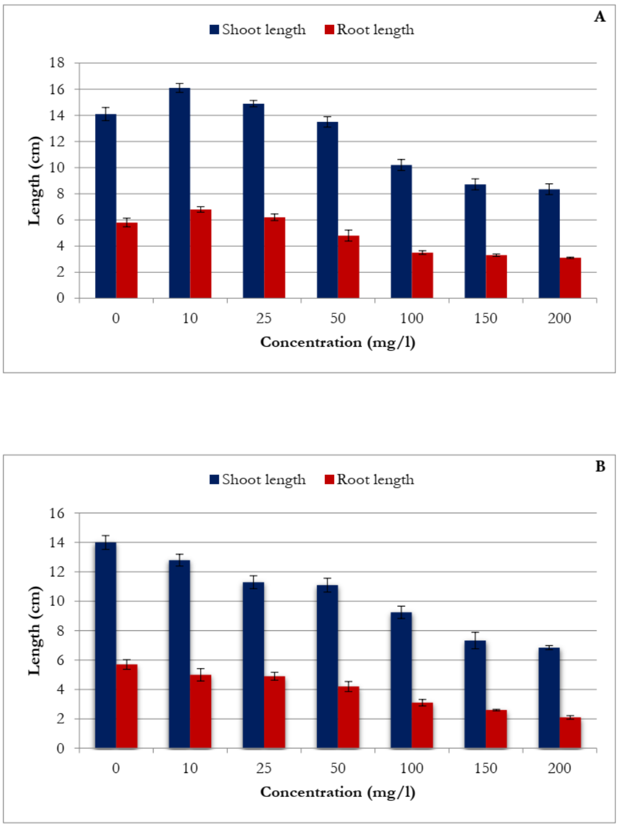

Figure 2. Effect of heavy metals on shoot and root length of *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10) and error bar as standard error.

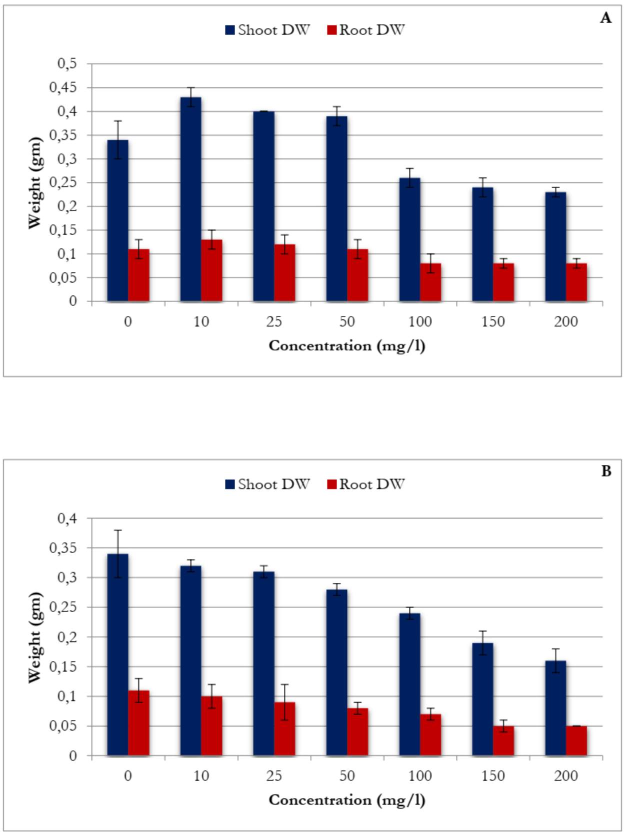

Figure 3. Effect of heavy metals on shoot and root dry weights of *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10) and error bar as standard error.

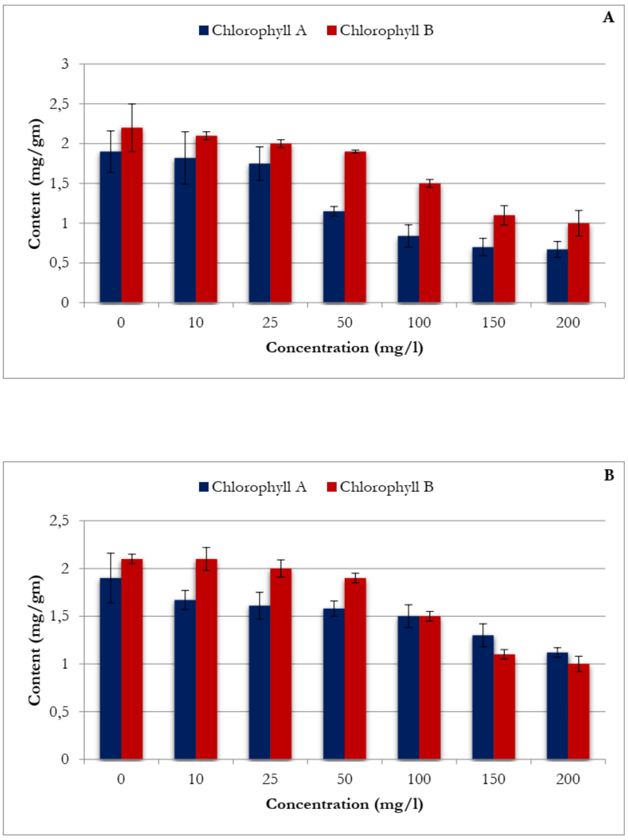

Figure 4. Effect of heavy metals on chlorophyll A and B content in *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10) and error bar as standard error.

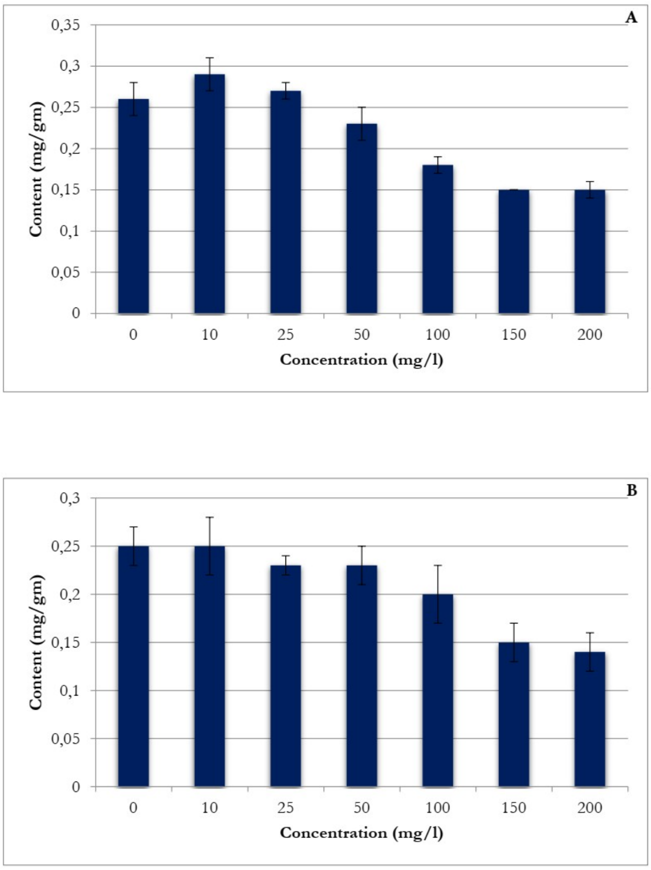

Figure 5. Effect of heavy metals on carotenoid content in *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10) and error bar as standard error.

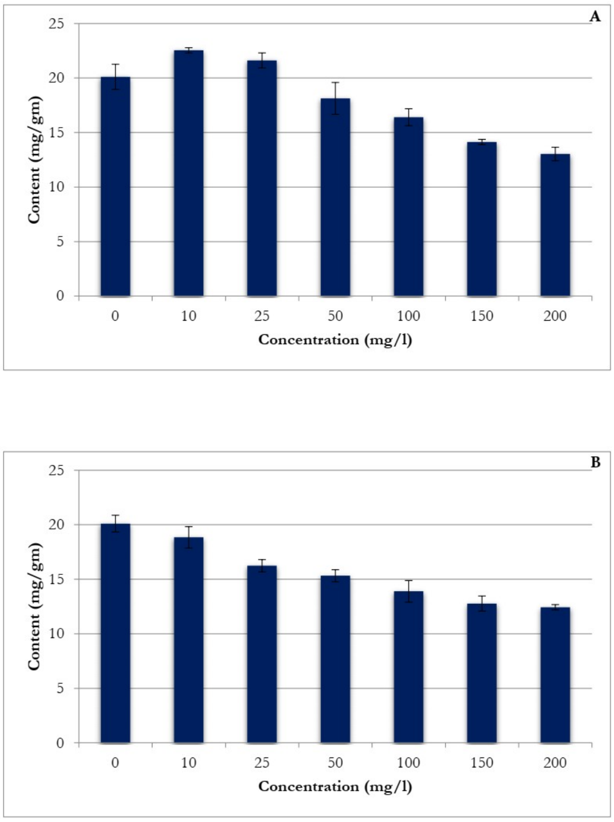

Figure 6. Effect of heavy metals on protein content in *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10) and error bar as standard error.

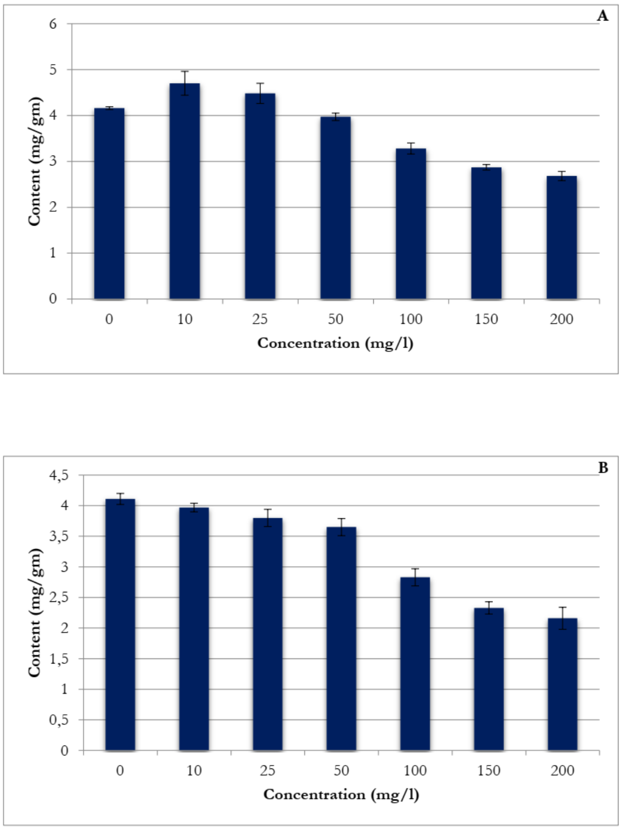

Figure 7. Effect of heavy metals on total sugar content in *R. serpentina*. (A) Nickel and (B) cadmium. Each bar shows the mean values (n = 10) and error bar as standard error.

CONCLUSION

The present study has been carried out on effect of different concentrations of two heavy metals nickel and cadmium on different parameters like germination, growth, pigments and nutrients like proteins and total sugar in medicinal plant *R. serpentina*. The study showed that nickel has beneficial effect on *R. serpentina* at lower concentration, but had an adverse effect at higher concentration. Whereas cadmium proved to have an undesirable effect on growth and development, however lower concentration of cadmium had no adverse effect on sugar content. From this study it can be concluded that the cadmium has comparatively more adverse effect than nickel on growth and development of *R. serpentina*.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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