

Remediation of Animals, Plant and Insect toxic metal, Cadmium through Hyperaccumulator Plant, *Solanum nigrum*

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ABSTRACT

Cadmium (Cd) is highly toxic to animal, plant and agricultural important insects. Hyperaccumulator plant, *Solanum nigrum* L. was exposed to different concentrations of (Cd) such as 10 mg/kg and 50 mg/kg of soil. The results revealed that concentration of Cd reduced plant growth attributes such as shoot length and root length in a dose-dependent manner as compared to control. The reduction of growth attributes was significant ($p < 0.05$) at 50 mg/kg of soil as compared to 10 mg/kg of soil and control. Application of Cd also reduced the chlorophyll contents, fresh and dry biomass significantly ($p < 0.05$) as compared to control. The assessment of Cd accumulation in shoot and roots through inductively coupled plasma mass spectrometry (ICPMS) analysis showed that a higher concentration of Cd was accumulated in roots as compared to shoot. The study concluded that *S. nigrum* has potential to be used in Cd-contaminated marginal land for phytoremediation.

KEY WORDS: PHYTOREMEDIATION, CADMIUM DETOXIFICATION, SOLANUM NIGRUM, GROWTH ATTRIBUTES.

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INTRODUCTION

The presence of heavy metals in the environment has become a major threat to plants, animals and human life due to their tendency of bioaccumulation and toxicity in living organisms (Bahadir et al., 2007). Larger agricultural soil has been contaminated with heavy metals due to mining activities, industrial discharges and the application of agrochemicals and lime products. Contamination of soil with heavy metals enhances plant uptake leading to accumulation in various parts of the plants (Bahadir et al., 2007; Ullah et al., 2019). From soil to plant, transport of heavy metals depends on total amount of heavy metals, ionic ratios of elements in soil solution and rate of transfer of element from solid to liquid stages and to plant roots (Buendía-González et al., 2010). Severe heavy metal contamination in the soil may cause a variety of problems, including low crop yield and toxicity in plants, animals, and humans. A trace amount of few heavy metals such as Cu and Zn are essential for plant growth and natural development because they are used as co-factors for many enzymes (Ullah et al., 2015).

However, high concentrations of both essential and unnecessary heavy metals in the soil can lead to toxic symptoms and growth inhibition in most of the plants (Dahmani-Muller et al., 2000). At the cellular level, excessive amounts of toxic heavy metal ions stimulate many stress responses and damage various cell components such as cell membranes, proteins and nucleic acid (Fidalgo et al., 2011; Chauvin et al., 2017).

Plant treatment refers to the natural ability of certain plants called hyper-accumulator that accumulate the pollutants and either immobilize them or turn them harmless to the soil, water or air. Contaminants such as minerals, pesticides, solvents, explosives, crude oil, and derivatives have been detoxified by plant treatment known as phytoremediation (Buendía-González et al., 2010). *Solanum nigrum* has been successful in accumulating highly toxic pollutants at pollutant sites (Fidalgo et al., 2011). Phytoremediation technique is a method of treatment that takes advantage of the ability of hyper-accumulator plants to accumulate heavy metals and toxic compounds from the environment and metabolize them in their tissues (Garbisu and Alkorta, 2001).

S. nigrum is a fast-growing plant that produces high biomass even in soil contaminated with heavy metals. Thus, this plant may be effective in plant filtration or plant stability of wastewater contaminated with heavy metals. To date this type of plant has been used in studies of the effects of heavy metals such as cadmium hyper-accumulator (Ullah et al., 2013; Xia et al., 2016). The aim of this study is to know the absorption of heavy metals by plants and their effect on the content hyperaccumulator plants. *S. nigrum* was selected because it accumulates heavy metals in higher concentration and known as Cd hyperaccumulator plant.

MATERIALS AND METHODS

Seed germination and pot experiment:Seeds of

Table 1. Growth attributes of *Solanum nigrum* L. with different concentrations of Cd

| Cd Treatment (mg/kg) | Shoot length (cm) | Root Length (cm) | Chlorophyll contents (SPAD) |
|----------------------|---------------------------|---------------------------|-----------------------------|
| 0 | 10.26 ± 2.02 ^a | 12.33 ± 0.54 ^a | 22.11 ± 2.23 ^a |
| 10 | 9.40 ± 1.38 ^a | 11.00 ± 1.43 ^a | 18.21 ± 1.67 ^b |
| 50 | 5.38 ± 0.57 ^b | 6.25 ± 0.56 ^b | 12.56 ± 2.11 ^c |

Mean ± SD values are presented in each column different letters represent the significant difference ($p < 0.05$) as analyzed by Duncan's multiple range test.

S. nigrum L. were collected, surface-sterilized and germinated according to the procedure of Wei et al., (2009). The germinated seeds were grown commercial soil and were put in a growth chamber at 25 ± 2 °C. Two different Cd concentrations: 10 mg/kg and 50 mg/kg of soil were used as treatments and 0 mg/kg was (without Cd) was used as control. The experiment lasted for 4 weeks. Three replications were used and ten plants per replication were used in the experiment.

Measurement of plant growth attributes: After 4 weeks, the experiment was harvested and plant growth attributes of Cd treated and control plant was determined. The total length of the plants (Cd treated and control) was measured as root length and shoot lengths. The chlorophyll contents of the treated and control plants were also determined using chlorophyll meter (SPAD- 502, Minolta, Japan).

Fresh and dry biomass assessment: The plants were cut off and roots and shoot were separated. Fresh weight of roots and shoots of the Cd treated and control plants were measured. The shoots and roots were dried separately in paper bags at 100°C for 10 minutes and dried at a constant weight at 70°C.

Assessment of Cd in plant shoots and roots: The oven-dried plant samples (Cd treated and control) were crushed into powder and the 100 mg of

crushed samples were digested in a solution of HNO₃-HClO₄. The concentration of Cd in the digested samples was determined by double plasma spectral analysis (ICP, Optima 79000DV, PerkinElmer, USA).

RESULTS AND DISCUSSION

Assessment of plant growth attributes: The results revealed that the application of Cd negatively affected the growth characteristics of *S. nigrum* compared to the control (0 mg/kg of Cd). Plant growth attributes of Cd treated plants were not significant ($p < 0.05$) different at 10 mg/kg as compared to control; however, when the concentration reached from 10 mg/kg to 50 mg/kg the root lengths and shoot length were significantly decreased as compared to control (Table 1). The magnitude of the growth reduction compared to the control was evident at higher concentrations of cadmium. The length of root and shoot were significantly affected in a dose-dependent manner compared to the length of the control plants. Previous studies have shown that bacterial endophytes isolated from *S. nigrum* were not only capable of detoxifying Cd but also promoting growth of *S. nigrum* Khan et al. (2015). Based on previous findings, we designed a study to isolate bacterial endophytes from *S. nigrum* and to determine their effects on plants. *S. nigrum* was selected for the experiment because of its tolerance for heavy metals and comparatively short life cycle (Jabeen et al., 2009).

Measurement of chlorophyll content: The contents of chlorophyll present in the plants exposed to Cd from 10 mg/kg to 50 mg/kg were measured. The results revealed (Table 1) that plants there was a significant reduction in chlorophyll contents in plants exposed to Cd concentrations as compared to the control plants. In addition, a significant difference in contents was measured in plants exposed to 10 mg/kg and 50 mg/kg of Cd as compared to the control. The plant growth inhibition by Cd was presumably due to the damage of chlorophylls contents and other photosynthetic pigments by toxic effects of Cd Ullah et al., (2013) suggested that chlorophyll degradation was a major reason for growth reduction in plants exposed to under Cd stress.

Table 2. Concentration of Cd in shoot and roots of *Solanum nigrum* treated with different concentrations of Cd of mg/kg of soil.

| Cd Treatment (mg/kg) | Fresh weight | Dry weight |
|----------------------|-------------------------------|-------------------------------|
| 0 | 60.54 \pm 3.22 ^a | 22.68 \pm 1.45 ^a |
| 10 | 40.33 \pm 1.53 ^b | 12.19 \pm 0.66 ^b |
| 50 | 15.83 \pm 1.45 ^c | 9.25 \pm 0.56 ^c |

Mean \pm SD values are presented in each column different letters represent the significant difference ($p < 0.05$) as analyzed by Duncan's multiple range test.

Table 3. Fresh and dry biomass of *Solanum nigrum* after treatment with different concentrations of Cd (mg/kg of soil).Method

| Cd Treatment (mg/kg) | Cd Concentration mg/kg of Plant DW* | |
|----------------------|-------------------------------------|--------------------|
| | Shoot | Root |
| 0 | ND* | ND |
| 10 | 120.37 \pm 3.54 | 289.23 \pm 8.34 |
| 50 | 251.82 \pm 5.47 | 487.23 \pm 14.42 |

ND* = represents not detected and DW* = represents dry weight. The values are expressed as the mean \pm SD and different letters represent significant differences ($p < 0.05$).

Fresh and dry biomass assessment: Fresh and dry biomass of the *S. nigrum* plants exposed to the Cd was greatly affected as compared to controlled plants. The fresh weight of the plant exposed to Cd showed a gradual and significant reduction with gradual increase in Cd concentrations. Similarly, the dry weight of the plants was significantly decreased with increased concentration of Cd in dose dependent manner as compared to control (Table 2). Fidalgo et al., (2011) reported similar results; their report revealed that the Cd concentration greatly reduced the biomass of *S. nigrum*, with increased concentration of Cd. In addition, inhibition of growth in *S. nigrum* plants was assessed by Wan et al., (2012) and fresh and dry biomass were monitored under increasing concentrations of Cd. The inhibition of plant growth under higher Cd concentration was assumed to be mainly due to the effect of heavy metals on the contents of photosynthetic pigments, which are responsible for photosynthesis (Liu et al., 2009; Kurzbaum et al., 2014).

Cadmium accumulation in shoot and root of the *S. nigrum*: The concentration of Cd in shoots and roots of the plant significantly increased in a dose-dependent manner. The Cd concentrations and accumulations in different parts of the plants grown in different Cd concentrations revealed that Cd accumulation in a Cd-exposed plant was significantly higher than that in the control (Table 3). Moreover, the Cd contents increased

from 120.37 to 251.82 mg/kg in the shoots and 289.23 to 487.23 mg/kg in the roots. A higher concentration of Cd was accumulated in the roots of *S. nigrum* as compared to shoots. Same Cd behavior of Cd accumulation was reported by Wei et al. (2013), their report showed that an increase in Cd in the soil was associated with higher amounts of Cd accumulating in roots of the *S. nigrum*. The studies conducted by Malandrino et al., (2006), Wan et al., (2012). John et al., (2008) showed that Cd accumulation was higher in the roots of plant as compared to shoots. Similarly Wan et al., (2012) reported that the level Cd accumulated in the roots was many-fold higher as compared to the shoots of the *S. nigrum*.

CONCLUSION

Heavy metal such as Cd is highly toxic to plants and animals including human being and Cd contamination has been a major issue in recent decades. Phytoremediation through Cd hyperaccumulator plant *S. nigrum* is an economic and most important technique used to eliminate the Cd from soil. In the present study, *S. nigrum* plant was used in Cd contaminated soil. The study showed the *S. nigrum* successfully accumulated Cd in roots and shoot and eliminated the Cd from the soil.

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