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Comparative Analysis of the Effect of Cadmium and Nickel on Chlorophyll Content of Barnyard Millet (*Echinochloa frumentacea* Link)

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ABSTRACT

Background: Both cadmium (Cd) and nickel (Ni) are recognised environmental toxins that may have devastating effects on plant life, particularly on their photosynthetic pigments. Heavy metal toxicity is one of several abiotic stressors that is especially relevant to investigate because of its impact on crop species growing in close proximity to heavy industrial sites, especially in less developed nations.

Methods: The chlorophyll content in barnyard millet (*Echinochloa frumentacea*) was evaluated across five different Cd and Ni concentrations (50, 100, 150, 200 and 250 mg/kg of soil) by pot experiment. We measured and compared the influence of Cd and Ni on the chlorophyll content of the leaves of *E. frumentacea* seedlings at 15, 30 and 45 days of intervals.

Result: The results reveal that there was regularly a reduction attributable to Cd and Ni application in chlorophyll a, chlorophyll b and total chlorophyll content. Comparatively, the chlorophyll content was higher in Ni stress than Cd stress at all the concentrations ranging from 50 to 250 mg/kg of soil. Based on the results, it was concluded that the chlorophyll content declined progressively with increasing concentrations of Cd and Ni in all day intervals.

Key words: Barnyard millet, Cadmium, Chlorophyll, Heavy metal, Nickel.

INTRODUCTION

Due to industrialization and urbanisation on a worldwide scale, organic and inorganic wastes including pesticides, petroleum products, acids and heavy metals have polluted essential resources like soil, water and air, harming primary and secondary consumers and eventually, people (Ali *et al.*, 2019; Bhunia, 2017; Zeller and Feller, 1999). Human activities such as mining, manufacturing, agriculture, waste burning, manufacture of batteries and other metal goods are a major cause of environmental pollution (Nedelkoska and Doran, 2000). Heavy metals and other industrial pollutants pose a significant risk to agricultural operations because, when present in excess, they may become toxic and stunt the development of most plant species, often even killing them (Weiqiang *et al.*, 2005). Heavy metals including lead, nickel, cadmium, copper, cobalt, chromium and mercury have devastating effects on plant life and are major environmental pollutants that threaten agricultural ecologies and decrease crop yields (Sethy and Ghosh, 2013). They cause physiological changes in plants by acting as a stressor (Chin, 2010). Heavy metals disrupt the ultrastructure of chloroplasts and cause thylakoids to disassemble, hence preventing gas exchange and the manufacture of photosynthetic pigments (Yang *et al.*, 2020).

India is the one of the leading producers of millets and their grain has been a staple food for sustaining the livelihood of the millions of the poorest and rural people (IIMR, 2018). Barnyard millet is a common weed of temperate and warm regions mainly cultivated in China, Korea and Japan (Sood *et al.*, 2015). However, it is also extensively grown in India from Kashmir to Sikkim in the north and Tamil Nadu in the south and is commonly known as “sawa” (de Wet *et al.*, 1983). In India, the cultivation of Barnyard millet is mainly confined to Tamil Nadu, Andhra Pradesh, Karnataka and Uttar Pradesh. The grains of barnyard millet consist of a low amount of phytic acid and a high amount of iron and calcium (Sampath *et al.*, 1986). In Tamil Nadu, it is mainly cultivated in drylands and some hilly areas of Ramanathapuram, Madurai, Salem, Namakkal, Vilupuram, Dindugal, Coimbatore and Erode districts (Channappagoudar *et al.*, 2008).

Echinochloa frumentacea exists in the family Poaceae and it is commonly called as Indian barnyard millet (Sood *et al.*, 2015). Barnyard millet is an important nutritional food including proteins, crude fibers, low amount of fats and carbohydrates, vitamins and minerals when compared with

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other cereals, namely rice and wheat (Devi *et al*., 2014; Kumar and Parameshwaran, 1998; Veena *et al.*, 2005). It also consists of some antinutritional components namely phenolic acids, flavonoids and tannins (Kulkarni *et al.*, 1992), which serve as good natural antioxidants. As in presently increased diabetes mellitus, barnyard millet is a preferred food.

According to the WHO, (1996), the permissible limits of Cd and Ni for plants are 0.2 and 10 mg/kg, respectively. The regulatory limit of Cd in agricultural soil is 100 mg/kg soil (Salt *et al.*, 1995). But this threshold is continuously exceeding because of several human activities. Ni concentration in polluted soil may reach 20- to 30-fold (200-26,000 mg/kg) higher than the overall range (10-1000 mg/kg) found in natural soil (Izosimova, 2005).

In the present study, we compared the effect of different concentrations (50, 100, 150, 200 and 250 mg/kg of soil) of Cd and Ni on the content of chlorophyll a, chlorophyll b and the total chlorophyll of barnyard millet (*E. frumentacea*). The concentrations or doses of Cd and Ni were finalized based on the literature study (Dinu *et al.*, 2021; Oyedeji *et al.*, 2017; Patel *et al.*, 2005; Wójcik *et al.*, 2005).

MATERIALS AND METHODS

Experimental site

During the months of January and February of 2022, the tests were conducted at the nursery of the Department of Botany, Madras Christian College (Autonomous), Chennai, Tamil Nadu, India.

Pot experiment

These findings were obtained using sandy loam soil. Soil was tested for a wide range of physical properties, including its type, colour, pH, minerals, trace amounts of heavy metals, moisture level, hardness, permeability, salinity, temperature and water-holding capacity. Each pot was filled with 5 kg of soil and mixed with different concentrations (50, 100, 150, 200 and 250 mg/kg of soil) of Cd and Ni, separately, in the form of $CdCl_2$ anhydrous and $NiSO_4.6H_2O$. The soil without heavy metals (i.e., Cd and Ni) was treated as control. Fifteen seeds were put into each of the pots. The seeds are surface-sterilized with 0.1% mercuric chloride for 2 min before being washed and sown. The young plants were subjected to daily light and dark cycles seen in nature. The soil in each container was watered until it reached its field capacity. For each therapy, four replicas were maintained. The leaf samples were collected at regular intervals of 15, 30 and 45 days for chlorophyll analysis.

Extraction and estimation of chlorophyll content

We used a mortar and pestle to crush 1 g of fresh leaf samples from each Cd and Ni concentration in 10 ml of 80% acetone. Supernatant was obtained in a 100 ml beaker after centrifuging the acetone leaf extract at 5000 rpm for 5 min. At least four times, or until no colour remains, this procedure was repeated. Afterwards, 80 ml of 80% acetone were added to the leaf extract to bring the final volume to 100 ml. Light levels were kept low throughout the process to limit the photodegradation of the chlorophyll pigments. At 645 and 663 nm, using acetone at 80% as a blank solution, the spectrophotometer evaluated the extracted pigments' optical density (OD) or absorbance. The chlorophyll content was estimated by taking the mean of three replicate samples (Arnon, 1949; Sadasivam and Manickam, 2008).

"Estimation of chlorophyll was done using the following Arnon's equation.

$$\text{Chlorophyll a (mg/g)} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times V/1000 \times W$$

$$\text{Chlorophyll b (mg/g)} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times V/1000 \times W$$

$$\text{Total chlorophyll (mg/g)} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times V/1000 \times W$$

The quantity of chlorophyll present in the leaf tissue was expressed as mg/g of the fresh sample based on the formula

$$V/1000 \times W = 100/1000 \times 1 = 0.1$$

Where

V = Final volume of leaf extract in 80% acetone (100 ml).

W = Fresh weight of leaf tissue extracted in acetone (1 g).

A_{645} and A_{663} = Absorbance values at 645 nm and 663 nm, respectively".

Statistical analysis

The data were represented as mean values ± SE. Measurements were performed on the four replicas for each treatment. Using SPSS 22.0, we performed an ANOVA on the data.

RESULTS AND DISCUSSION

The data on the effect of different concentrations (50, 100, 150, 200 and 250 mg/kg of soil) of Cd and Ni on photosynthetic pigments of 15-, 30- and 45-day-old seedlings of barnyard millet (*E. frumentacea*) were represented in Tables 1-3. The chlorophyll a, chlorophyll b and total chlorophyll was not much affected by Ni and Cd at the concentration of 50 mg/kg of soil on 15-day-old seedlings. However, the chlorophyll content declined progressively with increasing concentrations of Ni and Cd, *i.e.*, 100 mg/kg and above.

On 15-day-old seedlings, the highest value of chlorophyll a and chlorophyll b were found in control (0.6023 and 0.3958 mg/g), followed by 50 mg/kg of Ni (0.5942 and 0.3927 mg/g) and Cd (0.5935 and 0.3465 mg/g), whereas the lowest value of chlorophyll a and chlorophyll b were observed in 250 mg/kg of Ni (0.4397 and 0.2695 mg/g) and Cd (0.3422 and 0.1587 mg/g), respectively. On 30-day-old seedlings, the maximum amount of chlorophyll a and chlorophyll b were observed in control (0.9136 and 0.7762 mg/g) and the minimum amount of chlorophyll a and chlorophyll b were found in 250 mg/kg of Ni (0.5317 and 0.4897 mg/g) and Cd (0.5077 and 0.3321 mg/g), respectively. The same pattern of effect was observed on 45-day-old seedlings, *i.e.*, the chlorophyll a and b were decreased in the highest concentration (250 mg/kg) of Ni

(0.8412 and 0.6583 mg/kg) and Cd (0.7572 and 0.6789 mg/kg), respectively.

Figs 1 and 2 show the comparison on the growth stage of barnyard millet with the influence of Cd and Ni. While Figs 3-5 show the total chlorophyll levels after accounting for the influence of Cd and Ni. The total chlorophyll content were decreased when the concentrations are increased from 50 to 250 mg/kg of soil. Measurements of chlorophyll

Table 1: Estimation of chlorophyll content with the effect of Cd and Ni on 15-day-old seedlings of *Echinochloa frumentacea*.

Concentrations of Cd and Ni (mg/kg of soil)	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)	
	Cd	Ni	Cd	Ni
Control	0.6023 ± 0.020	0.6023 ± 0.020	0.3958 ± 0.017	0.3958 ± 0.017
50	0.5935 ± 0.010	0.5942 ± 0.006	0.3465 ± 0.018	0.3927 ± 0.015
100	0.5063 ± 0.012	0.5414 ± 0.015	0.3129 ± 0.011	0.3579 ± 0.018
150	0.4483 ± 0.011	0.4872 ± 0.012	0.2379 ± 0.013	0.2892 ± 0.012
200	0.4123 ± 0.010	0.4397 ± 0.014	0.2336 ± 0.011	0.2695 ± 0.016
250	0.3422 ± 0.016	0.4037 ± 0.016	0.1587 ± 0.010	0.1623 ± 0.012

Table 2: Estimation of chlorophyll content with the effect of Cd and Ni on 30-day-old seedlings of *Echinochloa frumentacea*.

Concentrations of Cd and Ni (mg/kg of soil)	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)	
	Cd	Ni	Cd	Ni
Control	0.9136 ± 0.021	0.9136 ± 0.021	0.7762 ± 0.015	0.7762 ± 0.015
50	0.8283 ± 0.020	0.8404 ± 0.013	0.6543 ± 0.014	0.6542 ± 0.010
100	0.7961 ± 0.016	0.8069 ± 0.018	0.5873 ± 0.010	0.6293 ± 0.016
150	0.7114 ± 0.013	0.7146 ± 0.014	0.5221 ± 0.017	0.6086 ± 0.012
200	0.5874 ± 0.010	0.6558 ± 0.011	0.4605 ± 0.010	0.5734 ± 0.011
250	0.5077 ± 0.012	0.5317 ± 0.012	0.3321 ± 0.015	0.4897 ± 0.011

Table 3: Estimation of chlorophyll content with the effect of Cd and Ni on 45-day-old seedlings of *Echinochloa frumentacea*.

Concentrations of Cd and Ni (mg/kg of soil)	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)	
	Cd	Ni	Cd	Ni
Control	1.1946 ± 0.012	1.1946 ± 0.012	0.9181 ± 0.012	0.9181 ± 0.012
50	1.0607 ± 0.013	1.0813 ± 0.018	0.8272 ± 0.015	0.8897 ± 0.010
100	1.0037 ± 0.010	1.0261 ± 0.011	0.7846 ± 0.014	0.8115 ± 0.012
150	0.9913 ± 0.012	0.9887 ± 0.012	0.7564 ± 0.011	0.7267 ± 0.011
200	0.8523 ± 0.014	0.9056 ± 0.016	0.7397 ± 0.017	0.6894 ± 0.013
250	0.7572 ± 0.016	0.8412 ± 0.019	0.6789 ± 0.010	0.6583 ± 0.013

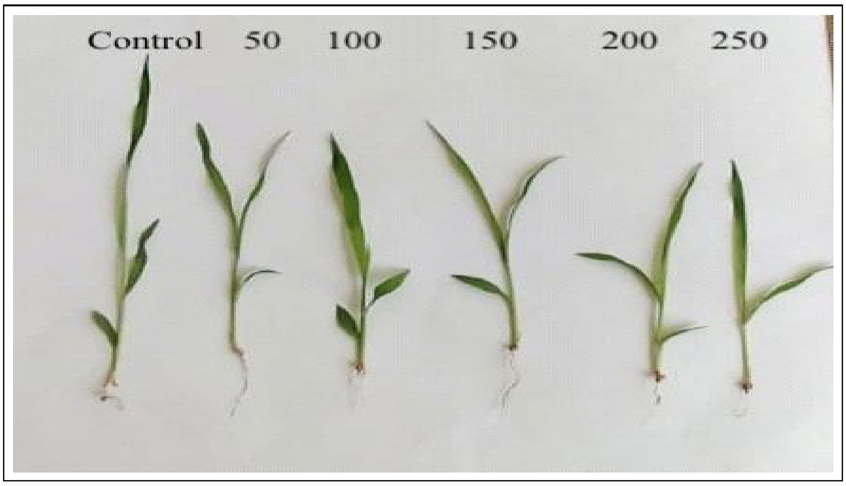

Fig 1: Seedling growth of barnyard millet with different concentrations of cadmium on the 10^{th} day.

concentration are useful for gauging the impact of environmental stress on plants since shifts in pigment content are associated with outward indications of plant disease and variations in photosynthetic output (Parekh *et al.*, 1990). Heavy metals have been shown to reduce chlorophyll levels in a variety of plant species, according to several studies. Oncel *et al.* (2000) reported that the total chlorophyll levels were drop by 50% in the *Triticum aestivum* cultivar Gerek 79 and by 70% in the cultivar Bolal 2973 after being treated with Cd and Pb. This is because heavy metals prevent the enzymes involved in chlorophyll production from doing their jobs, slowing down the body's metabolism. Cadmium has been connected to modifications in chlorophyll biosynthesis, similar to how it inhibits protochlorophyll reductase and aminolevulinic acid (ALA) production (Stobart *et al.*, 1985). The accumulation of Na^+, K^+ and Ca^{2+} in mung bean roots has also been related to Ni stress, as have alterations in photosynthetic pigments and reduced output (Ahmad *et al.*, 2007).

It has been hypothesised that Ni is toxic to most plant species because it blocks the production of essential enzymes like amylase, protease and ribonuclease, therefore delaying the growth and development of a wide variety of food plants (Ahmad and Ashraf, 2011). According to research, it prevents proteins and carbohydrates from being broken down and used by germinating seeds, resulting in shorter plants, shorter roots, less chlorophyll, less fresh weight and less enzyme carbonic anhydrase activity, as well as an increase in malondialdehyde concentration and electrolyte leakage (Ahmad and Ashraf, 2011; Ashraf *et al.*, 2011; Siddiqui *et al.*, 2011). Applications of Ni and NaCl to growing seeds of *Brassica nigra* considerably impair growth, leaf water potential, pigments and photosynthetic machinery due to increased electrolyte leakage, lipid

Fig 2: Seedling growth of barnyard millet with different concentrations of nickel on the 10th day.

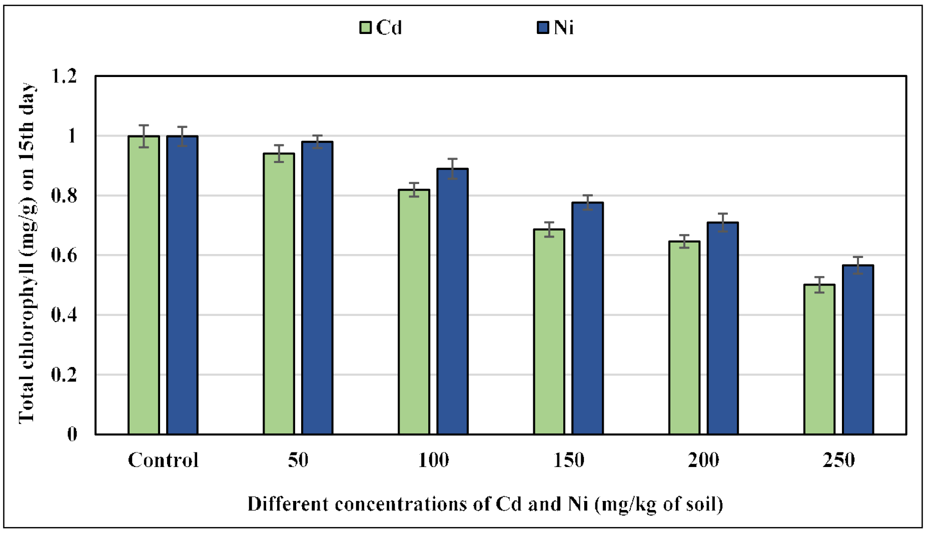

Fig 3: Total chlorophyll content with the effect of Cd and Ni on 15-day-old seedlings of *Echinochloa frumentacea*.

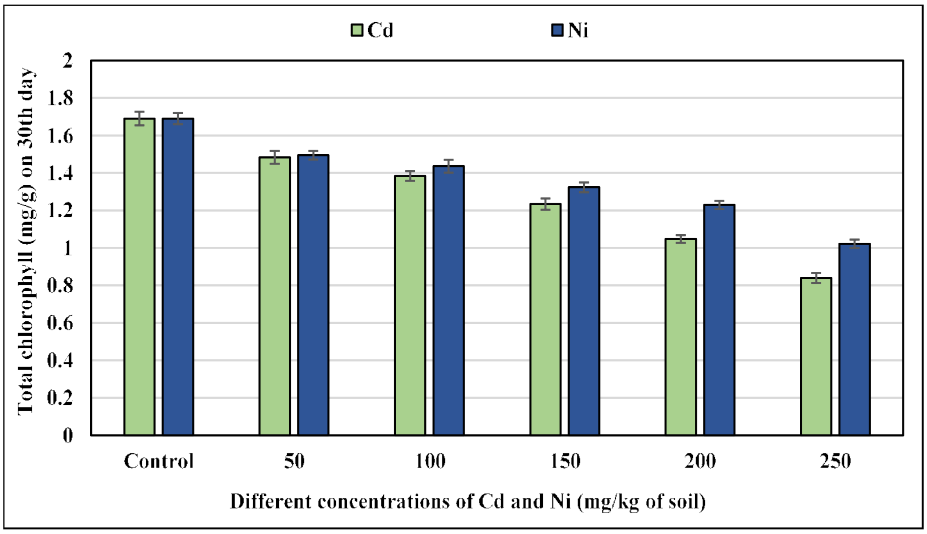

Fig 4: Total chlorophyll content with the effect of Cd and Ni on 30-day-old seedlings of *Echinochloa frumentacea*.

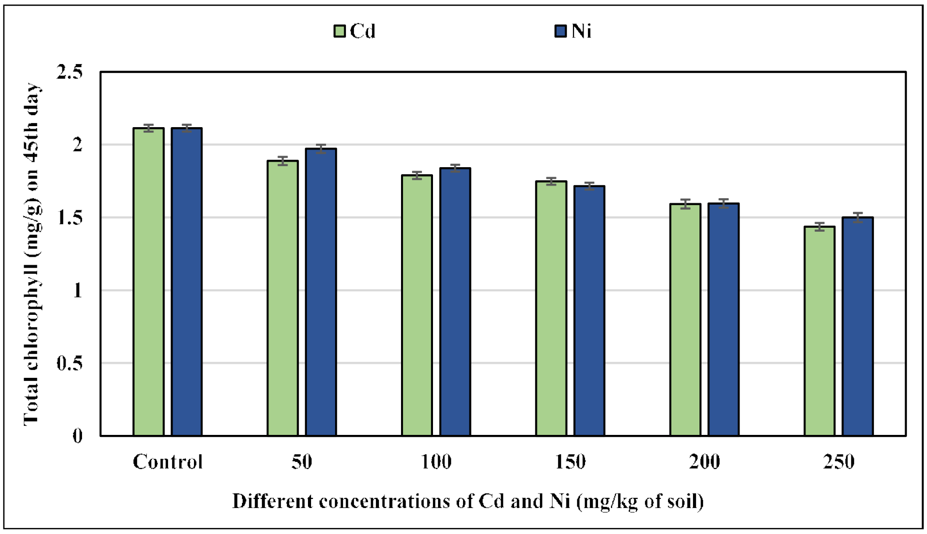

Fig 5: Total chlorophyll content with the effect of Cd and Ni on 45-day-old seedlings of *Echinochloa frumentacea*.

peroxidation, hydrogen peroxide concentration, antioxidative enzyme activity and proline levels (Yusuf *et al.*, 2012). Reductions in nitrate reductase activity, carbonic anhydrase activity and membrane stability have also been recorded (Yusuf *et al.*, 2012).

Apart from the inhibition of photosynthetic pigments, Cd has also been shown to cause delay in germination, induce membrane damage, impair food reserve mobilization by increased cotyledon/embryo ratios of total soluble sugars, glucose, fructose and amino acids (Rahoui *et al.*, 2010), mineral leakage leading to nutrient loss (Sfaxi-Bousbih *et al.*, 2010), accumulation in seeds and over-accumulation of lipid peroxidation products (Ahsan *et al.*, 2007; Smiri *et al.*, 2011) in seeds. It has been reported to reduce the percentage of germination, growth of embryo and distribution of biomass and inhibit the activities of alpha-amylase and invertases (Sfaxi-Bousbih *et al.*, 2010), reduce water content, shoot elongation and biomass (Ahsan *et al.*, 2007). Cd poisoning has been related to increased protein synthesis associated with defence and detoxification, antioxidant and germination functions (Ahsan *et al.*, 2007).

CONCLUSION

The present study clearly shows that chlorophyll a, b and total chlorophyll content all decreased when Ni and Cd concentrations increased. Plants exposed to Cd had the greatest influence on chlorophyll a, chlorophyll b and total chlorophyll across all time periods. At 50, 100, 150, 200 and 250 mg/kg of soil, cadmium was shown to be more hazardous than nickel. Seasonal and temporal change in chlorophyll concentration may be associated with heavy metal toxicity, however this hypothesis needs further research.

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Conflict of interest: None.

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