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Assessment of impact of nickel stress on the accumulation, pigments and protein content of water hyacinth (*Pontederia crassipes* L.)

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Abstract
Pontederia crassipes L. plants were collected and exposed to different concentrations (Control, 0.01, 0.1, 1.0 and 10.0 ppm) of Ni by using $NiSO_4$ in medium in the laboratory. The effect of Ni^{2+} concentrations on visible symptoms such as toxicity, pigments (Chlorophyll- a, b and total) contents were estimated. Plants exposed to high nickel (1.0 and 10.0 ppm) showed visible toxicity symptoms such as wilting, chlorosis in young leaves, browning of roots noticed after 6 days of treatment. Nickel was accumulated more in stem (651.1µg/g dry weight) than leaves (115.23 µg/g dry weight) at 6 days of treatment. Nickel exposure decreased Chlorophyll-a, b and total chlorophyll contents. Body water content decreased at high nickel (1.0 and 10.0 ppm). Increased nickel concentrations increases antioxidants such as proline content. On the other hand carotenoids and protein contents at 1.0 ppm of nickel were decreased. The low level of nickel stimulates photosynthetic pigments and antioxidative parameters.

Keywords: *Pontederia crassipes*, water hyacinth, nickel stress, antioxidative, phytoremediation

Introduction
Pontederia crassipes (Formerly *Eichhornia crassipes*), also known as common water hyacinth is an aquatic plant which is naturalized throughout the world, and often invasive outside in every geographic condition. The plant is with broad, thick, glossy, ovate leaves, floating by means of buoyant bulb-like nodules at its base above the water surface. They have long, spongy, bulbous stalks. The roots are feathery, freely hanging in nature. The heavy metals are generally a group of toxicants those cannot be broken down to nontoxic forms which persist for a longer time (Jabeen *et al*., 2009) [10]. Such metals of densities greater than 5g cm^{-3} are known as heavy metals which usually cause pollution resulting in toxicity, pollution, etc. Some of these heavy metals are important micronutrients for the plants and Nickel(Ni) is one such metal (Satpathy *et al*., 2021) [22] Translocation of such toxic metals to foliage and even to the edible fruits poses serious health concern (Singh *et al*., 2010a). Nickel acts as a co-factor of enzymes and is beneficial for animals in trace quantities but its higher concentrations bring toxic effects to plant growth. High nickel concentration in plants lowers the rate of metabolic activities and decrease water and nutrient uptake capability in plants (Gajewaska *et al*., 2006) [7].
In a condition of heavy metal stress reactive oxygen species (ROS) such as O^{2-}, O. and OH. are generated in plants (Pflugmacher,2004) [19] which causes the oxidative damage to proteins, lipids and DNA when produced in excess (Apel and Hirt, 2004) [1]. ROS also affect the antioxidative defence system in plant cells. Therefore, to scavenge ROS and to avoid oxidative damage, plants do have enzymatic and non-enzymatic antioxidants (Halliwell, 1987). Plants subjected to high concentration of nickel accelerate generation of ROS (Baccouch *et al*., 1998) [2].
Contamination of water by toxic heavy metals has become a global problem and the hydrophytes possess tremendous potential to absorb heavy metals. *Pontederia crassipes* L. has a great potential to bio accumulate and is a bio indicator of various heavy metals (Sinha *et al*., 2005) [25]. Such plants are important in phytoremedial strategies, with variable tolerance. The present study was aimed to find out tolerance capacity of *P. crassipes* with respect to accumulation of nickel and its effect on photosynthetic pigments and antioxidative components at various nickel-exposure levels. This study may be helpful in finding better phytoremedial strategies and biological indication of nickel toxicity.

Materials and Methods

Water hyacinth plant was collected from the village pond located in the vicinity of District Hospital, Nayagarh, Odisha and were brought to the laboratory for further culture in a large cement tub. Out of the tub, the healthy plants were selected and acclimatized in 10% Hoagland's solution for one week in the laboratory with a photoperiod of 16 hr light and 8 hr dark with a regulated temperature of 25±2 ^{0}C. The small healthy plants of uniform size and equal weight (15-17g) were selected.

The selected plants from culture were exposed to different concentrations (Control, 0.01, 0.10, 1.0 and 10.0 ppm) of Ni prepared by dissolving $NiSO_4$ (Hi media) in 10% Hoagland's solution with a control. A single plant was kept in 250ml beaker each filled with 200ml solution of each strength.

The nutrient solution was prepared with the mentioned composition: 4mM $Ca(NO_3)_2$, 4mM KNO_3, 2mM $MgSO_4$, 2µM $MnSO_4$, 0.4mM $(NH_4)2SO_4$, 0.3µM $CuSO_4$, 30µM NaCl, 0.1µM Na_2MoO_4, 0.43µM KH_2PO_4, 10µM H_3BO_3 and 20µM $FeSO_4$.

Observations were made after 6 days with visible symptoms of nickel toxicity on the leaves. The blotted leaves were used for biochemical estimations (chlorophyll, carotenoids, protein, proline, etc.) and oven dried leaves and roots were used to estimate tissue concentrations of nickel.

The fresh and blotted leaves were used for the determination of chlorophylls (a, b and total), carotenoids and protein contents. Chlorophyll and carotenoid contents were determined by the method of Porra *et al*. (1989) [20] and Duxbury and Yentsch (1956) [5], respectively. Protein content in the leaves was measured using the method of Lowry *et al*. (1951) [29]. Proline concentration was determined using the methods of Bates *et al*. (1973) [3]. Fresh leaves (300 mg) were homogenized in 10 ml 30% aqueous sulphosalicylic acid for determination of proline.

Analysis of heavy metals were done from the harvested plants which were made to wash with distilled water and the leaves and roots were separated mechanically. The parts were kept in an oven for drying at 80 ^{0}C for 48 hours. Dried plant tissue (1g) were digested in HNO_3 (70%) and $HClO_4$ (70%) (10:1 v/v). Perkin–Elmer (700) atomic absorption spectrophotometer equipped with an air - acetylene flame atomizer was used to estimate the Nickel.

Data obtained were analysed statistically as per the standards for mean (n=5) values and test of significance for each results were done (p=<0.05) and least significance difference (LSD) were calculated.

Table 1: Effect of Nickel on the Chlorophyll and protein content on a 6 day treated leaf.

Conditions (Fresh weight)	Control	Concentration of Nickel (in ppm)				
		0.01	0.1	1.0	10.0	LSD p<0.05
Chl-a(mg/g)	1.25	1.43	1.04	0.98	0.53	0.41
Chl-b(mg/g)	0.43	0.52	0.47	0.37	0.33	0.08
Total(mg/g)	1.18	1.92	1.48	1.38	0.86	0.49
Chl-a: Chl-b(mg/g)	2.90	2.75	2.21	2.64	1.60	0.67
Carotenoids (mg/g)	0.60	0.63	0.66	0.56	0.40	0.11
Proteins(µg/g)	377.68	27.15	466.80	244.40	111.15	182.18
Proline (µg/g)	0.73	1.11	1.47	2.30	2.81	1.09

Table 2: Assessment of accumulation of Nickel (after 6 days of treatment) in the roots and leaves (dry weight).

Plant part (mg/l)	Control	Concentration of Nickel (in ppm)				
		0.01	0.1	1.0	10.0	LSD p<0.05
Roots(µg/g)	NIL	21.42	33.40	150.21	651.1	434.26
Leaves(µg/g)	NIL	9.92	14.61	32.26	115.23	68.16

Results and Discussion

The explants were given exposures to different nickel (Ni^{2+})concentrations (*viz*. Control, 0.01, 0.1, 1.0 and 10 ppm) accumulated high content of nickel at higher concentrations(Table:2). Tissue concentration of Ni^{2+} in both leaves and root was dose dependent. The data revealed that the accumulation of maximum Nickel was observed in the root (651.1 µg/g dry weight) than the leaves when exposed to 10.0 ppm nickel, which showed low translocation of nickel towards aerial parts of the plants. The roots of plants act as a barrier against heavy metal translocation possibly as a result of potential tolerance mechanism (Ernst *et al*., 1992) [6]. Uptake in root and translocation in aerial parts may be supported with low translocation factor which showed great potential for phyto-stabilization of nickel in root. The heavy metal accumulation in root was more than the shoot in radish and spinach (Pandey, 2006) [16]. Vajpayee *et al*. (2001) [27] also reported high accumulation of heavy metal (Cr) in root of an aquatic plant (*Vallisneria spiralis* L.) than the shoot. Therefore, these findings have relevance towards the different levels of influence of nickel which affect the uptake and translocation of nickel.

There were some visible symptoms observed in the plant material after getting exposures to different concentrations of Nickel ranging from 1.0 to 10ppm. The visible symptoms might be due to nickel toxicity which resulted in the bleaching of leaf margins towards the base, root tip browning, chlorosis of young leaves, etc. These symptoms arising due to toxicity corroborated with the findings of Bisht *et al*. (1976) [4]. Kabata-Pendias and Pendias (1992) [11] could also observed the toxicity symptoms in tissues while working on Nickel stress, The severity of symptoms were less with less nickel and move up with increased exposure *i.e.* 10ppm. The toxicity symptoms were not marked in the plants with exposure of 0.01 and 0.1 ppm of nickel. Pandey and Gautam(2009) [17] observed that the presence of nickel ions decrease the permeability of the cell membrane, checks the root development and becomes a reason for chlorosis, necrosis.

The study was conducted (Table: 1) to find out the effect of different concentrations of nickel on pigments such as Chl-a, Chl-b and total Chlorophyll content on the leaves of the test plant. There was an induction of Chl-a and Chl-b

noticed with the treatment of low nickel at 0.01ppm whereas much reduction of such pigments noticed with high nickel exposure i.e. 10ppm. Rahman *et al*. (2005) [21] while working on barley observed the increase in pigment contents when treated with lower dose of nickel. Nickel at higher doses was found to be inhibitorier towards the Chl-a compared to Chl-b which signifies that the Chl-a synthesis is more nickel sensitive. Similarly the ratio of Chl-a/Chl-b decreases with the increased nickel concentration. Such observations were found support of Pandey and Sharma (2002) [18] where similar reduction of Chl-a than Chl-b in the nickel treated cabbage leaves. Similarly, Kumar *et al.* (2022) [12] while working on sweet potato observed that with the increased concentration (i.e. 60mg/l) of nickel, the synthesis of Chl-a, Chl-b, Carotenoids decreases compared to the control. But, all the pigments found to enhance their synthesis when treated with 15mg/l of nickel than control. Vajpayee *et al.* (2000) [26] interpreted such nickel toxicity leading to reduction of pigments was due to the utilization of α - aminolevulinic acid. Similarly, there were reports stating that nickel inhibits the biosynthesis of Chl-b by creating nutrient imbalance, replacement of Mg^{++} ions (Gautam and Pandey, 2008; Molas, 2002) [8, 14]. The reduction of proteins in the leaf explants were observed at higher concentrations of nickel (1.0 to 10.0 ppm) but increase in proteins observed at low concentrations (0.01 to 0.1ppm). Such reduction of protein content was due to the activity of nitrate reductase (Vajpayee *et al*., 2000) [26]. Carotenoid levels increase in the leaves with increased dose of nickel (upto 0.1ppm) but decreased at high nickel levels (upto 10ppm). The relative water content of the leaves of *P. crassipes* did not show any significant change under the low exposure of nickel i.e. upto 0.1ppm but gradually show drastic reduction when treated with higher nickel concentration of upto 10ppm. Panda and Patra (2000) [15] while studying the role of nickel interpreted that such types of change of water content might be due to toxic condition created by nickel which further results in wilting and plasmolysis of plants cells. This change of water content was said to be due to the production of reactive oxygen species (ROS) which has the capacity to damage membrane integrity and thus causing leakage of cell sap through lipid peroxidation (Pandey and Gautam, 2009) [17]. The carotenoids were reportedly play a vital role to protect the cells from stress and bear the capability to quench ROS (Sen and Mukherjee, 2009) [23]. The leaf proline concentration was observed to increase under increased concentration of nickel. It was known in stress physiology that proline is one of the most abundant metabolites formed during the water stress in a plant. Similar findings were reported by Pandey and Sharma (2002) [18] where accumulation of proline was observed under a condition of heavy metal stress in cabbage leaves.

Conclusion

The test plant *Pontederia crassipes* can be recommended as a good phytoremediation plant in the areas where nickel stress is more prevalent.

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