Effect of nickel-stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L.

**Abstract**

Water lettuce plants were exposed to various concentrations (0, 0.01, 0.1, 1.0 and 10.0 ppm) of nickel as nickel sulphate in nutrient medium. The effect of graded nickel (Ni^{2+}) concentrations on visible symptoms of toxicity, pigments (chlorophyll a, b and total) and antioxidative attributes were evaluated. Plants exposed to high nickel (1.0 and 10.0 ppm) showed visible toxicity symptoms, such as wilting, chlorosis in young leaves, browning of root tips and broken off roots, observed at 6 days after treatment. Nickel was accumulated more in root (863.3 µg g^{-1} dry weight) than leaves (116.2 µg g^{-1} dry weight) at 6 days of treatment. Nickel exposure decreased chlorophyll a, b and total chlorophyll contents. Relative water content decreased at high nickel (1.0 and 10.0 ppm). Antioxidants, such as proline content and peroxidase activity increased with increase in nickel concentrations, whereas, other carotenoids and protein contents at 1.0 ppm and activity of catalase at 10 ppm of nickel were decreased. The low level of nickel stimulates photosynthetic pigments and antioxidative attributes. The study may be helpful in phytoremedial strategies and biological indication of nickel toxicity in aquatic plants.

**Key words**

Nickel-stresses, Antioxidative responses, *Pistia stratiotes* L., Phytoremediation

**Introduction**

Heavy metals accumulate and biomagnify into the food chain, leading to serious ecological and health problems (Pandey, 2006). Most of them are toxic at low concentration. They do not biodegrade and require removal from water and soil system. Nickel is widely used in silver refineries, alloy, pigments electroplating, zinc based casting and storage batteries. Nickel is an essential micronutrient for some higher plants (Gerendas et al., 1999). It acts as a co-factor of enzymes and is beneficial for animals in trace quantities, but its higher concentrations pose toxic effects in plant growth. High nickel levels in plants reduce the rate of metabolic activities and decrease water and nutrient uptake in plants (Gajewaska et al., 2006).

Plants generate reactive oxygen species (ROS) such as \( ^{1}O_{2}^{*} \), O and OH under heavy metal stress conditions (Pflugmacher, 2004) and over production of these reactive oxygen species (ROS) in plants causes oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). ROS also affect the antioxidative defence system in plant cells. Therefore, to scavenge ROS and to avoid oxidative damage, plants possess enzymatic and non-enzymatic antioxidants (Halliwell, 1987). Plants subjected to high concentration of nickel accelerate generation of ROS (Baccouch et al., 1998).

Heavy metal contamination in the aquatic environment is a global problem. Aquatic plants possess immense potential to accumulate heavy metals. *Pistia stratiotes* L. a common water lettuce belongs to family *Araceae*, and widely grows as a, free-floating macrophyte in natural freshwater bodies. It has a great potential to bioaccumulate and is a bioindicator of various heavy metals (Sinha et al., 2005). Such plants are important in phytoremedial strategies, with variable tolerance. The present study was aimed to find out tolerance capacity of *P. stratiotes* with respect to accumulation of nickel, and its effect on photosynthetic pigments and antioxidative components at various nickel-exposure levels. It will be helpful in evolving phytoremedial strategies and biological indication of nickel toxicity.
Material and Methods

Water lettuce plant (Pistia stratiotes L.) obtained from a pond located in the premises of National Botanical Research Institute (NBRI), Lucknow, and were maintained in large hydroponic tubs. Healthy plants were acclimatized in 10% Hoagland’s solution for one week in the laboratory at 16 hr L – 8 hr D dark and 25 ± 2°C. The plants of uniform size and equal weight (5-8 g) were selected.

They were taken out and exposed to different concentrations (0, 0.01, 0.1, 1.0 and 10.0 ppm) of nickel prepared by dissolving NiSO₄ (Merck) in 10% Hoagland’s solution with control set containing only 10% Hoagland’s solution. The nutrient solution consisted of 4 mM Ca(NO₃)₂, 4H₂O, 2 mM MgSO₄, 7H₂O, 4 mM KNO₃, 0.4 mM (NH₄)₂SO₄, 2 µM MnSO₄, 4H₂O, 0.3 µM CuSO₄, 5H₂O, 0.8 µM ZnSO₄, 7H₂O, 30 µM NaCl, 0.1 µM Na₂MoO₄, 0.43 µM KH₂PO₄, 10 µM H₂BO₃ and 20 µM FeSO₄·7H₂O. One plant was kept in 250 ml beaker filled with 200 ml solution of each strength. Plants were harvested after 6 days with visible symptoms of nickel toxicity on the leaves. The blotted leaves were used for biochemical estimations (chlorophyll, carotenoids, protein, proline, peroxidase and catalase), 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) and Duxbury and Yentsch (1956), respectively. Protein content in the leaves was measured using the method of Lowry et al. (1951). Proline concentration was determined using the methods of Bates et al. (1973). Fresh leaves (300 mg) were homogenized in 10 ml 30% aqueous sulphosalicylic acid for determination of proline. Peroxidase activity was assayed by the modified method of Luck (1963). Catalase activity was measured according to the method of Euler and Josephson (1927).

Heavy metals were analyzed in harvested plants which were thoroughly washed with distilled water, the leaves and roots were separated manually, dried in an oven at 80°C for 48 h. Dried plant tissue (1 g) were digested in HNO₃ (70%) and HClO₄ (70%) (10:1 v/v). Nickel was estimated by using Perkin – Elmer (700) atomic absorption spectrophotometer equipped with an air – acetylene flame atomizer.

Data presented was statistically analyzed (Panse and Sukhatme,1961) for mean (n=5) values. Significance of treatment effects were tested by Least significant difference (p<0.05).

Results and Discussion

Pistia stratiotes plants were exposed to various nickel (Ni²⁺) concentrations (0, 0.01, 0.1, 1.0 and 10 ppm) accumulated high content of nickel at higher concentrations (Table 2). Tissue concentration of Ni²⁺ in both leaves and root was dose dependent. The maximum nickel accumulation was observed in the root (863.3 µg g⁻¹ dry weight) than the leaves at 10.0 ppm nickel – exposure, which showed low translocation of nickel towards aerial parts of 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). Uptake in root and translocation in aerial parts may be supported with low translocation factor (Fig. 1) which showed great potential for phytostabilization of nickel in root. The heavy metal accumulation in root was more than the shoot in radish and spinach (Pandey, 2006). Vajpayee et al. (2001) also reported high accumulation of heavy metal (Cr) in root of an aquatic plant (Vallisneria spiralis L.) than the shoot. Thus, these findings indicate that various levels of nickel influence uptake and translocation of nickel.

Nickel also induced visible symptoms of toxicity in P. stratiotes subjected to exposure of higher concentrations (1.0 and 10.0 ppm). Some of the prominent symptoms were bleaching of leaf margins towards the base, chlorosis in young leaves, browning of root tips and broken off roots. These symptoms highly resemble with the nickel toxicity in crop plants (Bisht et al., 1976). The toxicity symptoms in plants could be attributed to high accumulation of nickel (Table 2) in tissues (Kabata-Pendas and Pendas, 1992). The severity of symptoms was less in plants exposed to 1.0 ppm nickel. It may also result in alteration in metabolism, loss of water and adverse effects on chlorophyll synthetic mechanism (Tripathi et al., 1981; Baccouch et al., 1998). Toxicity symptoms did not appear in plants exposed to low levels (0.01 and 0.1 ppm) of nickel. It has been reported that, nickel ions decrease the permeability of the cell membrane, inhibit root system development and cause necrosis and chlorosis (Pandey and Gautam, 2009).

The pigments, such as chlorophylls (a, b and total) (Table 1) content in P. stratiotes leaves were reduced following exposure to above 0.1 ppm nickel, whereas they increased at 0.01 ppm nickel. The increase in pigment contents at low doses of nickel have also been reported earlier (Rahman et al., 2005). Low nickel (0.01 ppm) induced chlorophyll a and b. Maximum reduction in chlorophyll a and b was observed at 10.0 ppm exposure. Inhibitory effect of high level of nickel was more marked on chlorophyll a than chlorophyll b. It is indicative of more nickel sensitivity of chlorophyll a. Chlorophyll a/b ratio also decreased with increase in nickel concentrations. These findings exhibit resemblance with those Pandey and Sharma (2002), who reported that concentration of chlorophyll a was more reduced than that of chlorophyll b in leaves of nickel treated cabbage. Vajpayee et al. (2001) also reported greater inhibition in chlorophyll a than b following exposure to heavy metal (Cr) in submerged aquatic plants. The reduction in pigment contents due to nickel – toxicity could be attributed to α – aminolevulinic acid (ALA) utilization (Vajpayee et al., 2000). In addition, nickel also inhibits chlorophyll biosynthesis by creating nutrient imbalances, replacement of Mg²⁺ ions (Molas, 2002; Gautam and Pandey, 2008). Low concentrations (0.01 to 0.1 ppm) of nickel increased the protein content in P. stratiotes leaves, which decreased at higher concentrations (1.0 and 10.0 ppm). Reduction in protein content in plants could be attributed to effect on nitrate reductase activity (Vajpayee et al., 2000).
Effect of nickel stresses on water lettuce

Relative water contents in *P. stratiotes* leaves did not change significantly up to 0.1 ppm nickel concentration in nutrient medium, while gradually decreased above this with increase in nickel concentrations (1.0 and 10.0 ppm). The decrease in water content might be due to the nickel toxicity causing wilting and plasmolysis in plant cells (Panda and Patra, 2000). Also, loss of water may be due to the production of reactive oxygen species (ROS) which cause damage of membranes and leakage of cell saps through lipid peroxidation (Pandey and Gautam, 2009).

Carotenoid contents in *P. stratiotes* leaves increased with increase in nickel concentration up to 0.1 ppm, which further decreased at high nickel levels (1.0 and 10 ppm). Carotenoids (beta carotene and xanthophylls) play an important role in protecting cells against the stress and have ability to quench ROS (Sen and Mukherjee, 2009). Proline content in leaves increased with increase in nickel concentrations. Proline is one of the most widespread metabolites produced in plant tissues under water stress conditions. Pandey and Sharma (2002) reported, accumulation of proline in the cabbage leaves due to excess heavy metals. Metal ions block the electron flow in photosystem II, which leads to the formation of excited chlorophyll that in turn causes production of free oxy-radicals (Kato and Shimizi, 1985).

The antioxidants (catalase, peroxidase activities and carotenoids content) showed variable responses to different nickel concentrations (Table 1). The activity of peroxidase (POX) increased with increase in nickel-stress. Catalase (CAT) activity also showed increasing trend with increased nickel concentrations up to 1.0 ppm, which decreased at 10 ppm nickel exposure. Under metal – stress conditions, including excess nickel exposure, an imbalance between generation and removal of ROS arise in plant tissues (Grataq et al., 2005). POX and CAT activities are essential components of plant antioxidant defence system. Elevation in POX activity in nickel treated *P. stratiotes*, suggests its role in the detoxification of H$_2$O$_2$ (Dey et al., 2009). The enhance activity of POX in excess nickel treated plants might result either in peroxidative damage of the thylakoid membrane or lower auxin and protein contents in tissues (Sandman and Boger, 1980). The activities of CAT and POX protect the metabolism in plant cells (Pandey, 2008).

It may thus be suggested that *Pistia stratiotes* have a great potential to phytoremedial strategies and acts as a bioindicator of nickel in aquatic system.

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References


