Research Article

Effect of Nickel on the morphology and Phytochemical characters of *Hibiscus esculentus* Linn.

S. SUDEEPTHA*, SHEELA. D.
Department of Botany, St. Teresa's College, Ernakulam, Kerala, India.

ABSTRACT

The accumulation of heavy metal- Nickel was studied by treating the chemical Nickel nitrate in four different concentrations- 100µM, 500 µM, 1mM and 5mM on the plant *Hibiscus esculentus* L. The studies showed presence of Nickel in leaves and fruits of plants in various concentrations at end of 40 days.

Key words: Heavy metal, Nickel nitrate, *Hibiscus esculentus* L.

INTRODUCTION

Development is the term recently added by man to cover up all his technological activities, which means to introduce significant changes in environment and mould it according to his need. To fulfill his basic needs for food, cloth and shelter, man has started exploiting nature, which resulted in ecological destruction [1-4]. Heavy metal pollution is gaining importance day by day due to its obvious impact on human health through food chain. Crops get saturated with heavy metals and other such chemicals which are harmful to man and other organisms [5-7].

The species of *Hibiscus esculentus* L, native of tropical Africa is now grown in tropics and subtropical regions. Present study aims at determining the effect of heavy metal Nickel on morphology and phytochemical characters of plant and also to analyze the amount of heavy metal accumulation in leaves and fruits, at the end of 40 days using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

MATERIALS AND METHODS

Before starting the experiment, seeds of the plant "*Hibiscus esculentus* Linn." were collected from Agricultural Technology centre of Kerala Agricultural University, Thrissur, Kerala.

Surface sterilized seeds were soaked for 24 hours in various concentrations (100µM, 500µM, 1mM and 5mM) of Nickel nitrate. For control, distilled water was used. Seeds were placed on filter paper in sterilized Petri dishes for germination and moistened with 15 ml of different concentrations of Nickel nitrate. 10 seeds were taken in triplicate at room temperature. After 4 days the data on percentage was documented and length of radicle and hypocotyl was recorded.

For field studies, seeds were allowed to grow in potting mixture taken in polyethylene bags. For preparing potting mixture soil was mixed with dung and sand in the ratio 3:2:1. Then the bags were irrigated daily with different concentrations of Nickel nitrate (100µM, 500µM, 1mM and 5mM). For control distilled water was used for irrigation.

Length of shoot, length of root, number of secondary roots, width of shoot, number of leaves, length of petiole and number of fruits were recorded at 15th, 30th and 40th day. For each treatment 3 replicates were maintained. Foliar features like stomatal index, palisade ratio, vein islet number and vein termination number were studied using microscope. In Phytochemical analysis, test for tannins, saponins, flavonoids, steroids and alkaloids were done. Estimation of carbohydrates was also carried out using Anthrone method. ICP-AES was performed to know the accumulation of heavy metal Nickel in the leaves and

* **Address for correspondence:**
S. Sudeeptha, Department of Botany, St. Teresa's College, Ernakulam, Kerala, India
Telephone: +91-9142304126, E-mail address: sheelaajayaghosh@gmail.com
Received: 05/03/2013, Revised: 15/05/2013, Accepted: 03.07.13

fruits of the plant at the end of 40 days. Samples of dry soil of each treatment were collected for soil analysis.

Table 1: Effects of different concentrations of Nickel nitrate on germination of *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	Length of Radicle (cms)	Length of hypocotyl (cms)	Germination percentage (%)
Control	0.9	3.5	100
100 µM	1	2.5	84.6
500 µM	0.8	2.5	92.3
1 mM	0.5	1.8	69.2
5 mM	0.2	0.5	46.1

Table 2: Effects of Nickel nitrate on Growth parameters at different concentrations in *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	No: of leaves				Root length (cms)				Shoot length (cms)				No: of Secondary roots			
	8th day	15th day	30th day	40th day	8th day	15th day	30th day	40th day	8th day	15th day	30th day	40th day	8th day	15th day	30th day	40th day
Control	2	2	8	9	0.9	2.5	28	36	3.5	15.5	39	52	1	2	21	30
100 µM	2	2	8	10	1	2	25	29	2.5	15	38	53	-	2	25	34
500 µM	2	2	7	8	0.8	2.6	20	25	2.5	16.6	39	59	1	3	13	22
1 mM	1	1	6	8	0.5	1.8	15	19.7	1.7	12	26	38	-	1	8	9
5 mM	1	1	7	8	0.2	4.8	16	20	0.5	7.4	25.5	40	-	1	9	11

Table 3: Effects of Nickel nitrate on width of shoot, length of petiole and number of fruits at different concentrations in *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	Width of shoot (cms)			Length of petiole (cms)			Number of fruits		
	15th day	30th day	40th day	15th day	30th day	40th day	15th day	30th day	40th day
Control	0.4	0.8	1.9	10.9	12.5	13.5	-	2	4
100 µM	0.7	1	2	11.5	14	15	1	5	8
500 µM	0.9	1.6	2.6	13	15.7	16.6	1	3	7
1 mM	0.1	0.5	0.9	9	11.2	12	-	1	2
5 mM	0.2	1	1.1	8.6	10	10.8	-	1	1

Table 4: Effects of Nickel nitrate on stomatal index, palisade ratio, vein islet number and vein termination number at different concentrations in *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	Stomatal index				Palisade ratio				Vein islet number				Vein termination number			
	8th day	15th day	30th day	40th day	8th day	15th day	30th day	40th day	8th day	15th day	30th day	40th day	8th day	15th day	30th day	40th day
Control	32	33	34.2	35	12	12.2	12.4	12.5	0.30	0.34	0.35	0.36	2.99	3.30	3.33	3.54
100 µM	32	32	35	35.6	13	13	14	13.5	0.32	0.33	0.38	0.39	2.90	3.66	3.66	3.79
500 µM	34	34.3	35.6	36.8	14	14.8	15	15	0.38	0.38	0.40	0.45	3.11	3.80	3.85	4.12
1 mM	25	25	28.4	28	9	9.9	10	10.2	0.20	0.23	0.23	0.25	2.77	2.82	2.82	3.32
5 mM	20	20.1	24	24	7	8	8.3	8.8	0.16	0.21	0.20	0.23	2.19	2.22	2.23	2.85

Table 5: Preliminary Phytochemical screening of extracts of different concentrations in *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	Tannins	Saponins	Flavonoids	Steroids	Alkaloids	Carbohydrates
Control	-	-	+	+	+	+
100 µM	+	-	+	-	+	+
500 µM	+	-	++	-	++	++
1 mM	-	-	+	-	+	+
5 mM	-	-	+	-	+	+

High precipitation: ++, Moderate precipitation: +, Absent: -

Table 6: Estimation of carbohydrates in different concentrations in *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	Carbohydrates concentration (µg/mL)
Control	0.36
100 µM	0.31
500 µM	0.28
1 mM	0.27
5 mM	0.19

Table 7: ICP-AES studies in different concentrations in *Hibiscus esculentus* L.

Concentration of Nickel nitrate (M)	Nickel concentration in leaf (ppm)	Nickel Concentration in fruits(ppm)
Control	0	0
100 µM	116.82	123.79
500 µM	83.30	92.14
1 mM	62.45	71.26
5 mM	63.70	73.81

RESULTS AND DISCUSSION

In the present study, it was evident that as the concentration of Nickel nitrate is increased, there is a decrease in growth and development of plant. Control showed highest value in radicle and hypocotyl length, while 5mM concentration of chemical treated plant showed the lowest (Table 1). At higher concentrations there is inhibition of seed germination. Control showed highest germination percentage and 5mM concentration of chemical treated seeds the lowest germination percentage (Table 1). Similar results were documented by [8-11]. Heavy metal toxicity decreases germination percentage and retards growth of seedling in several plants. In *Lupinus* sps. lead reduced number of germinating seeds and caused shortening of hypocotyls as well as radicle [12]. High concentration of lead (1mM) caused 14 to 30 % decreased germination in rice [13].

Field studies revealed that root length, shoot length, number of secondary roots, width of shoot, number of leaves, length of petiole and number of fruits showed higher values for 100µM and 500µM concentrations of Nickel nitrate treated plants. Lowest was shown by plants treated with 5mM concentration of chemical treated plants (Table 2 & 3). The inhibitory effect at higher concentrations might be due to excess of nitrogen, which happens to be injurious to plant growth, affecting water absorption and other metabolic process in plants [6]. A significant inhibitory effect at higher concentrations of heavy metals (Cd, Pb and Mn) on the morphological growth was reported by [7,9,10].

Foliar features like stomatal index, palisade ratio, vein islet number and vein termination revealed higher values for 100µM and 500µM concentrations of Nickel nitrate treated plants. Lowest value was for 5mM concentration of chemical treated plants (Table 4). Polluted environment has decreasing effect on stomatal frequency. When there is decrease in stomata number, gaseous exchange in plant gets reduced. This affects photosynthesis and respiration, which in turn affects productivity and then growth declines. These observations are in agreement with those of [5].

In phytochemical analysis, tannins were present only in plants treated with 100µM and 500µM concentrations of Nickel nitrate. Saponins were absent in all plants including control. Flavonoids were seen in plants treated with 500µM concentration of Nickel nitrate. Steroid was present only in control plants. Alkaloids and

carbohydrates were present in all plants including control and plants treated with 500µM concentration of Nickel nitrate (Table 5). At higher concentration, Nickel nitrate causes some alternation in metabolic pathway which clearly stunts the growth and development of plants. Studies of [2] also supported these findings.

Estimation of carbohydrate revealed that the concentration of carbohydrate is high in control and gradually decreased as the concentration of strontium chloride increases and then gets remained stable as the concentration reached 500µM (Table 6). From ICP-AES studies, it is found that at 100µM concentration of Nickel nitrate treated plants, Nickel was accumulated in leaves and fruits at toxic levels followed by those treated with 500µM concentrations of Nickel nitrate. Plants treated with 1mM and 5mM concentrations of Nickel nitrate showed somewhat toxic accumulation of Nickel in their leaves and fruits (Table 7). In plants treated with 100µM concentration of Nickel nitrate, due to higher growth rate and development, absorption of chemical maybe more compared to rest. So it showed toxic level accumulation of Nickel (Table 7). Many scientists have worked out the effect on accumulation of heavy metal. According to [3, 14], lower concentrations of heavy metal adversely affect the rate of accumulation.

CONCLUSION

Even if lower concentrations of Nickel nitrate are used for enhancing the growth of the plants, it is harmful to humans as well as animals. It is imperative to have proper understanding of plant response and pollutant concentration relationship with environmental conditions so as to preserve our nature and natural resources. In the present study, the Nickel accumulation in leaves and fruits were found to be at toxic level which is harmful to humans. Since *Hibiscus esculentus* is used as a vegetable, it is important to know the response of the plant to the heavy metal at different concentrations.

REFERENCES

1. Baker A.J.M. and Brooks R.R. Terrestrial higher plants which hyper accumulate metallic elements- Are view of their contribution, ecology and phytochemistry.1989; Biorecovery.1:81-126
2. Dutta Mitta, Sen Ali and Bhattacharya Sima. Effect of heavy metals Cobalt, Mercury and Cadmium on Malvacean family members, 1988; Env.Eco.6 (2): 410-416 (10).
3. Goswami Mamta. Effect of fertilizer factory effluent on soil and crop plant parts of *Vigna radiata* L, 1993; Proc. Acad. Environ.Bio.2 (1): 11-15 (8).
4. Jacobson and Hill. Effect of application of urea on plants, 1989; TMH publishers: pg no: 23-75.
5. Jha Arabinda Kumar. Environmental modification of leaf surface traits as induced by pollutants from fossil fuel firing, 1988; Env. Eco.6 (3): 781-785 (3).
6. Karunyal Samuel, Renuga G and Palwal Kailash. Effect of tannery effluents on seed germination, leaf area, biomass and mineral content of some plants, 1994; Bio resource Techno. 4 (3): 215-218 (8).
7. Malone C, Miller R and Koeppe D. Root growth in corn and soya beans: effects of cadmium and lead in lateral root initiation, 1977; J. Environ. Qual.25: 562-566.
8. Rakesh R Kumar and Jain VK. Effect of fungicide (Bavistin) on seed germination, Seedling growth and chlorophyll development in *Pisum sativum* L, 1991; Ind. J. Bot. Soc, 70: 407-408.
9. Shua W. S, Yeb Z. H, lana C. Y, Zhanga, Z. Q. Wongb M. H. Lead ,zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*, 2002; Environ. Pol., 120: 445- 453.
10. Tracy J E. Manganese toxicity in avocado (*Persea americana* Mill) and *California avocado*, 1991; Society Yearbook. 75: 147- 158.
11. Unnithan RV and Remani K N. Organic pollution in Cochin Backwaters, 2009; Ind. J. Mar. Sci, 4: 39-42.
12. Vassilev A. Yordav I. and Tsonev T. Physiological responses of two barly cultivars to soil pollution with Cadmium, 1998; Environ. Pollution. 100:1- 7.
13. Verma S and Dubey R. S. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. 2003; Plant Sci., 164: 645- 655.
14. Warren J M. An environmental view of toxicology and pesticide regulation. 1981; A C S. Sympo. Ser, American Chem. Soc, Washington D. C, 473-480.