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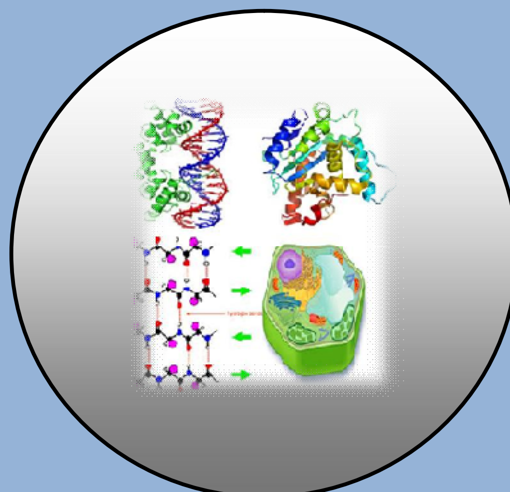

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RESEARCH PAPER

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Comparative Toxicity of Electroplating Industry Effluent on *Allium cepa* L. and *Vigna radiata* L. Plants

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ABSTRACT

Effect of electroplating industry effluents was studied on growth and metabolism of two economically important plants namely Allium cepa and Vigna radiata. Plant growth and certain metabolic parameters such as concentrations of total chlorophyll, carotenoid, proteins and sugars were found to be decreased while the activities of two antioxidant iron enzymes i.e. catalase and peroxidase were increased at increasing concentrations of effluent. A value of lipid peroxidation (MDA) was found to be on higher side at higher dose of the said industry effluent.
Key words: Electroplating industry effluent, plant growth, catalase, peroxidase, sugars, chlorophyll, carotenoid, protein and MDA.

INTRODUCTION

Although industrialization in many ways has improved our standard of living and given us lot of comforts but these are at the cost of serious encroachment in our natural environment. There are number of industries including electroplating which used to cause lot of pollution through their industrial effluents. According to Sumathi and Umagowrie (2012) industrialization although is a boon for developing countries including India but its effect on environment is equally dangerous. The industrial effluents are highly ecotoxic which causes ecological imbalances in the environment. Electroplating industry waste water is reported to contain Cr, Ni, Fe and Hg. Mercury can be a cause of damage to the central nervous system and chromosomes (Binupriya et al., 2006). Heavy metal pollution is considered to be a worldwide problem (Unikanan et al., 2011). They have also reported that chromium is one of the most toxic environmental pollutants of aquatic bodies. Chromium metal adversely affects the growth of *Hibiscus esculentis* and causes reduction in seed germination and seedling growth. Toxicity of chromium also adversely affect chlorophyll content (Amin et al., 2013).

Heavy metal pollution in soil and water is becoming a serious problem for agriculture and human health.

These aspects in view, an experiment was carried out to investigate the effect of electroplating industry effluent on the growth and certain important biochemical parameters in some economically important plants viz. onion and moong.

MATERIAL AND METHODS

Experiment was carried out in clay pot using normal soil. The soil for the experiment was collected from outskirts of Lucknow. These were allowed to dry in shade then powdered in agate mortar, finally sieved through 1 mm sieve before analyzing the different chemical characteristics of the soil. The central drainage holes were provided in these pots for leaching purpose was covered with an inverted watch glass over a pod of clean glass wool. The soil was thoroughly washed with water filled into these pots. Repeated flushing with distilled water was carried out to maintain the soil pH. Electroplating industry effluents were used to have values of pH 7.5 to 8.0, DO 6.3, BOD 20.66, COD 161.66, TDS at 180^{o}C mg/l 1545 and this industry effluent also contains certain amounts of Cl^{-}, Fe^{-}, PO^{4}, Pb, Zn, Ni, Fe and Cr. Electroplating industry effluent were collected from the Everready Flash factory, Lucknow, India. This effluent was taken in the percentage concentrations of 100, 75, 50 and 25. Deionised and distilled water was used for culture work. However, glass distilled water (GDW) was used for preparation of nutrient stocks and for all metabolic and analytical work. The composition of the nutrient solution was same as given by Hewitt (1966). However, different doses of industrial effluents were superimposed in the nutrient medium except control. Following parameters were observed and measured during the experiment:

a. Plant growth

Plant height was measured in centimeters from upper soil level upto the tip of the shoot at maturity.

b. Metabolic parameters

The concentration of total chlorophyll was estimated by the method of Arnon (1949). Concentration of carotenoid was estimated by the method of Duxbury and Yentsch (1956). Protein concentration was measured by the method of Lowry et al. (1951). Total sugar concentration was measured by the method of Dubias et al. (1956). Catalase activity was measured by the method of Euler and Josephson (1927). Peroxidase activity was measured by the method of Luck (1963). Level of lipid peroxidation was estimated in terms of malondialdehyde (MDA), a product of lipid peroxidation in plant samples by the method of Heath and Packer (1968).

RESULTS AND DISCUSSION

Physico chemical analysis of electroplating industry effluents revealed that this effluent which was more or less liquid of light green colour with pH of 7.683 and values of DO, BOD, COD, TDS to be 5.36, 35.66, 486.66, 3805, respectively and values of Cl^{-}, F^{-} and PO_4 to be 21.00, 0.30 and 2840.00 mg/l, respectively. This effluent also contained heavy metals such as Pb, Zn, Ni, Fe and Cr in the concentrations of 28.50, 130.00, 1980.00, 793.33 and 213.33 g/l, respectively.

A. *Allium cepa* L.

a. Plant growth

With increasing concentrations of electroplating industry effluents (25, 50, 75 and 100%) plant height increased significantly at 28.46, 36.88 and 43.27% accordingly. It was maximum in the control viz. 49.92 cms and reduces to 28.32 cms in 100% effluent concentrations. Similarly, significant decrease was observed in the total fresh weight and the total dry weight with increasing effluent concentrations (Table 1).

b. Metabolic activities

i. Total chlorophyll

Total chlorophyll content in leaves of *Allium cepa* treated with electroplating industry effluent progressively decreased in dose dependent manner with respect to their control with increasing concentration of pesticide effluent ($p<0.001$) (Table 1). Its value is noted at 1.390 mg/g for control reducing by 12.95, 24.94, 28.78 and 32.13% for 25, 50, 75 and 100% effluent concentration, respectively. At 100% effluent concentration, its value was 0.943 mg/g.

ii. Carotenoid

In control, the carotenoid content was 0.440 mg/g, which was decreased to 0.359, 0.296, 0.281 and 0.273 mg/g for 25, 50, 75 and 100% of pesticide industry effluent concentration. This decrease was significant at $p<0.001$ (Table 1).

iii. Sugar content

Sugar concentration decreased significantly ($p<0.001$). It was 46.299 µg/g fw for control and decreased by 61.49%, noted 17.367 µg/g fw for 100% effluent concentration (Table 1).

iv. Protein

A value of 147.013 µg/g fw is observed in the control. This value was reduced to 127.63 (13.18%), 96.149 (34.60%), 81.108 (44.83%) and 59.707 (59.39%) for 25, 50, 75 and 100%, respectively of pesticide industry effluent concentrations ($p<0.001$) (Table 2).

c. Enzyme activity

i. Catalase

Its activity was continuously and significantly increased with increasing effluent concentrations. It became 86.61, 100, 132.22 and 157.32% of the control value for 25, 50, 75 and 100% effluent concentrations, respectively (Table 2).

ii. Peroxidase

Peroxidase activity increased drastically and significantly ($p<0.001$) with increasing concentrations of pesticide effluents by percentage of 12.62, 28.72, 69.83 and 98.048% at 25, 50, 75 and 100% concentrations, respectively as compared to the control (Table 2).

iii. Lipid peroxidation

MDA content was found to be increased in *Allium cepa* at increasing doses of effluent concentrations ($p<0.001$). MDA content was 43.143 and 40.311 µM/g fw at 75 and 100% effluent concentration, respectively as compared to 25.403 for the control plant (Table 2).

B. *Vigna radiata* L.

a. Plant growth

Plant height of control plants were observed to be 35.36 cms which was increased for 25% concentration by 2.92% but reduced at the rate of 13.82 and 25.87% at 50 and 100% electroplating industry effluent concentrations, respectively.

Significant decrease was observed in the total fresh weight of the electroplating industry treated plants with increasing effluent concentrations. Value for total fresh weight was 2.90 gms which was reduced to 2.49, 1.58 and 0.65 in dose dependent manner. The total dry weight, however, increased at 25 and 50% concentrations but was decreased at 100% effluent concentration by 59.60% reduction (Table 3). Reduced plant growth due to supply of industrial effluent containing toxic heavy metals might be resulted due to the negative effect of metal containing effluent on zinc availability on plants resulting into reduction in the synthesis of growth hormone i.e. auxin via the synthesis of tryptophan which is a precursor of auxin. Similarly root and shoot lengths were also adversely affected by the electroplating effluents. Similar results were reported by Sahu and Arora (2008).

b. Metabolic activities

i. Total chlorophyll

Total chlorophyll content in leaves of *Vigna radiata* treated with electroplating industry effluent progressively decreased in dose dependent manner with respect to their control with increasing concentration of effluent. Inhibition of total chlorophyll at the rate of 22.55, 41.76 and 48.30% were at 25, 50, 75 and 100% effluent treated plants, respectively (Table 3).

ii. Carotenoid

The carotenoid content was decreased by 17.26, 36.99 and 36.50% at 25, 50, 75 and 100% effluent concentration, respectively. This decrease was significant at $p<0.001$ (Table 3).

iii. Sugar content

Sugar concentration of the plant decreased significantly ($p<0.01$). It was 77.440 g/g fw for control and decreased by 24.83, 40.14 and 52.46% at 25, 50 and 100% effluent concentration, respectively (Table 3).

iv. Protein

It was found to increase slightly for 25% effluent concentration (by 1.112%) but decreased profoundly by 32.41% at 100% concentration as compared to the control (Table 4).

c. Enzyme activity

i. Catalase

The activity was increased at increasing effluent concentrations in dose dependant manner. It increased by 103.33% of the control at 100 and 71.33% at 75% concentration of the effluent (Table 4).

ii. Peroxidase

Peroxidase activity was increased significantly with increasing concentrations of the effluents. Maximum increase was again noted in the higher concentrations. The values were 64.8, 85.8 and 104.667 ∆OD/g fw for 25, 50 and 100% concentrations and 58.467 ∆OD/mg proteins for the control (Table 4).

iii. Lipid peroxidation

MDA content was found to be increased moderately at progressive increase in effluent concentrations (Table 4).

Reduction in total chlorophyll content may be due to inhibition of δ-amino levulenic acid dehydrates a key enzyme in chlorophyll biosynthesis or chlorophyll degradation due to increase chlorophyllase activity (Gupta et al., 2011).

Table 1. Effect of various concentrations of electroplating factory effluent on plant height, total chlorophyll, carotenoid and sugar concentration of *Allium cepa* L.

Concentrations (%)	Plant height (cm)	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Sugar concentration (%)
Control	32.417± 1.294	1.390± 0.066	0.440± 0.020	46.299± 0.896
25	26.417**± 0.300 (-18.51%)	1.210*± 0.030 (-12.95%)	0.359**± 0.030 (-18.41%)	37.580**± 0.785 (-18.83%)
50	20.663**± 0.332 (-36.26%)	1.043**± 0.038 (-24.94%)	0.296**± 0.003 (-32.73%)	28.067**± 0.283 (-39.38%)
75	18.567**± 0.233 (-42.73%)	0.990**± 0.012 (-28.78%)	0.281**± 0.001 (-36.21%)	19.706**± 0.094 (-57.44%)
100	17.010**± 0.124 (-47.53%)	0.943**± 0.037 (-32.13%)	0.273**± 0.002 (-38.03%)	17.367**± 0.317 (-62.49%)

Values are mean of triplicates ±S.E. ANOVA ($p<0.01$)
**Significant ($p<0.01$) compare to control, *Significant ($p<0.05$) compare to control.

Table 2. Effect of various concentrations of electroplating factory effluent on protein content and different enzymes content in *Allium cepa* L.

Concentrations (%)	Protein (μg/g fw)	Catalase activity (μ moles H_2O_2 decomposed/mg fw)	Peroxidase activity (∆OD/mg protein)	MDA (μM/g fw)
Control	147.013± 15.516	79.667± 0.333	7.700± 0.300	25.403± 0.350
25	127.630**± 1.362 (-13.18%)	148.667**± 1.333 (+86.61%)	15.667**± 0.067 (+103.46%)	28.610**± 0.309 (+12.62%)
50	96.149**± 2.526 (-34.60%)	156.333**± 0.667 (+100%)	16.700**± 0.100 (+116.88%)	32.700**± 0.308 (+28.72%)
75	81.108**± 2.568 (-44.83%)	185.000**± 2.887 (+132.22%)	19.466**± 0.733 (+152.81%)	43.143**± 0.382 (+69.83%)
100	59.707**± 3.464 (-59.39%)	205.000**± 7.638 (-157.32%)	47.467**± 0.581 (+516.45%)	50.311**± 0.357 (+98.048%)

Values are mean of triplicates ±S.E. ANOVA ($p<0.01$)
**Significant ($p<0.01$) compare to control, *Significant ($p<0.05$) compare to control.

Carotenoid is an important non enzymic antioxidant which protects chlorophyll in stress condition (Sinha and Pandey, 2004). It is involved in scavenging of reactive oxygen species leading to protection of plant cells from oxidative damage (Blokhina et al., 2003; Gratao et al., 2005). It showed stimulation, inhibition as well as non significant change with increasing effluent concentrations. Decrease at higher concentrations may be due to toxicity of effluent (Gupta et al., 2011).

A decrease in total sugar content was observed with increasing effluent concentrations. The reason for reduced sugar (carbohydrate) content could be retarded growth and reduced chlorophyll content in the plants. Changes in pigment concentration (due to decrease in leaf area and number of leaves) by effluent treatment decreases photosynthesis, affecting the carbohydrate content (Malla and Mohanty, 2005). This finding is in conformity with the findings of present study which conforms to the present study results.

Protein and lipid content reduction could be due to disturbance in the nitrogen metabolism of the plants. Nitrogen cycle is responsible for synthesis of proteins in plants. Toxic metals adversely affect the enzymes proteases. The results are in conformity with that obtained from a study conducted by Azmat et al. (2009). Reduction in protein content may be due to degradation by proteases.

Table 3. Effect of various concentrations of electroplating factory effluent on plant height, total chlorophyll, carotenoid and sugar concentration of *Vigna radiata* L.

Concentrations (%)	**Plant height (cm)**	**Total chlorophyll (mg/g)**	**Carotenoid (mg/g)**	**Sugar concentration (%)**
Control	35.363± 0.319	1.499± 0.011	0.365± 0.000	70.440± 0.440
25	36.397**± 0.206 (+2.92%)	1.161*± 0.088 (-22.55%)	0.302**± 0.001 (-17.26%)	52.947**± 4.240 (-24.83%)
50	30.478**± 0.290 (-13.82%)	0.873**± 0.000 (-41.76%)	0.230**± 0.002 (-36.99%)	42.168**± 0.219 (-40.14%)
100	26.213**± 0.149 (-25.87%)	0.724**± 0.002 (-48.30%)	0.182**± 0.000 (-36.50%)	33.49**± 2.796 (-52.46%)

Values are mean of triplicates ± S.E. ANOVA ($p<0.01$)

**Significant ($p<0.01$) compare to control, *Significant ($p<0.05$) compare to control.

In the current study, catalase and peroxidase activity increased with increasing effluent concentrations. This might be due to the fact that catalase is an iron enzyme and the analysis of the effluents showed that they contain excess amount of iron which might have increased catalase and peroxidase activity. CAT is a peroxisomal haemoprotein that catalyses the removal of H_2O_2. It protects tissue from oxidative stresses of highly reactive hydroxyl radical (Baskaran et al., 2009).

Activities of peroxidase expression have been shown in several plant systems to be altered by stress chemicals and infection (Herbette et al., 2003). It has been observed that peroxidase induction is a general response of higher plants to the uptaking of toxic amounts of metals in roots and leaves of various species after application of toxic doses of Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} (Sinha et al., 2008).
Lipid peroxidation, which is considered an indication of oxidative stress in plants, can be induced via reactive oxygen species (ROS) that are generated as a result of heavy metal toxicity in plants. MDA is the decomposition product of polyunsaturated fatty acids of biomembranes and its increase shows the extent of membrane lipid peroxidation (Blokhina et al., 2003). Oxidative damage to biomembrane due to disruption of membrane lipids of *Lemna minor* treated with effluent samples have been reported.

Table 4. Effect of various concentrations of electroplating factory effluent on protein content and different enzymes content in *Vigna radiata* L.

Concentrations (%)	Protein (μg/g fw)	Catalase activity (μ moles H_2O_2 decomposed/mg fw)	Peroxidase activity (ΔOD/mg protein)	MDA (μM/g fw)
Control	85.552 ± 1.584	100.000 ± 5.000	58.467 ± 2.133	67.605 ± 2.025
25	86.503 ± 0.060 (+1.112%)	155.000** ± 0.000 (+55.00%)	64.800 ± 1.000 (+10.83%)	74.633 ± 0.333 (+10.40%)
50	71.558* ± 0.699 (-16.36%)	171.333** ± 0.667 (+100%)	85.800** ± 4.600 (+46.75%)	77.223 ± 0.068 (+14.24%)
100	57.827** ± 5.545 (-32.41%)	203.333** ± 1.667 (+103.33%)	104.667** ± 5.467 (+79.02%)	91.748** ± 2.408 (+35.710%)

Values are mean of triplicates ± S.E. ANOVA ($p<0.01$)
**Significant ($p<0.01$) compare to control, *Significant ($p<0.05$) compare to control.

As already reported effluent used in this study contained fair amount of Pb which is reported to cause increase in lipid peroxidation (Mostafa Lamhamdi et al., 2011). Pinho and Ladeiro (2012) focused on the uptake and interaction of Pb by plants and how it can be introduced in food chain. They have also emphasized on the oxidative stress caused by lead regarding the effects produced in plant physiological and biochemical processes.
The effluents used in this study also contains high amount of heavy metals such as Pb and Ni, which interferes with the normal metabolism of plants specially nitrogen and iron. Pb and Ni in excess amount might be responsible for replacement of essential nutrient like Zn, Fe and N which might be a cause of reduced values of total chlorophyll, carotenoid and the abnormal values of catalase, peroxidase and MDA activities.

However, such abnormal values were found to be dose dependent and thus lower concentrations of electroplating industry effluent were found to be less toxic and thus less damaging for both grown and metabolic parameters of two test plant systems. As far as comparative assessment of electroplating industry effluents among two plants systems are concerned, *Vigna radiata* was found to be more resistant and protective as compared to *Allium cepa* which was found to be more sucsceptible to effluent of electroplating industry effluent with regard to growth and metabolic parameters.

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