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*Full Length Research Paper*

# Physiological studies of some weeds grown under heavy metal and industrial effluent discharge zone of fertilizer factory

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**The present study deals with the characterization of effluent released from Brahmaputra valley Fertilizer complex, Namrup, Assam, India and distribution of heavy metals in water near to effluent discharge channel. The extent of damage caused by the effluents on physiological and biochemical properties of plants was investigated. Chlorophyll pigment, foliar protein and sugar concentration were measured in *Amaranthus spinosus* L., *Cassia obtusifolia* L. *Croton bonplandianum* Baill. *Leonurus sibiricus* L. during the growing season and compared with control. Results indicated that effluents contained toxic elements and the observed weeds were found to be susceptible while *C. bonplandianum* shows tolerance to some extent. Present study suggests that although weeds are often tolerant to extreme hostile environment but some time contaminated soil threatens their normal metabolisms. This leads to some new dimensions towards further research.**

**Key words:** Chlorophyll, effluent, heavy metal, protein, sugar, weed.

## INTRODUCTION

The discharge of heavy metals into natural ecosystems has become a matter of concern in India over the last few decades. These pollutants are introduced into the aquatic systems significantly as a result of various industrial operations. Industrial effluent discharges eventually polluting the environment to a great extent and in most cases exert various bio-chemical and physiological effects on plants. It is necessary for the soil to have nutrients rich and shortage of essential and trace elements result abnormalities in plants growth (Sarma and Sarma, 2007).

The potential toxic heavy metals such as vanadium, arsenic, chromium, etc. have already been identified and they cause health hazards in animal and plant lives (Bryan, 1976). The weed plants, being constantly exposed to the industrial effluent discharge, absorb, to water or organic acids exudates by root system, through the roots from the contaminated soil. The major accumulate and integrate undesirable chemical, soluble components of toxic chemicals are heavy metals and other non-biodegradable components, sometimes lethal to weed plants. In addition, a lot of available data also highlights heavy metal impact on agriculture as well as on natural ecosystems (Alloway, 1995). The present study is on the metabolic disorder and biochemical response of weed plants towards the growing load of effluent and heavy metal contaminated soils at Namrup, Assam.

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## MATERIALS AND METHODS

### Study site

The field site is located at Namrup, in South western Assam (27° 10′ N and 95° 21′ 30″ E), within the Bengal and Assam plain ecoregion at an elevation of 123 m above sea level on the bank of the river Dilli in Dibrugarh district is the potential source of pollution

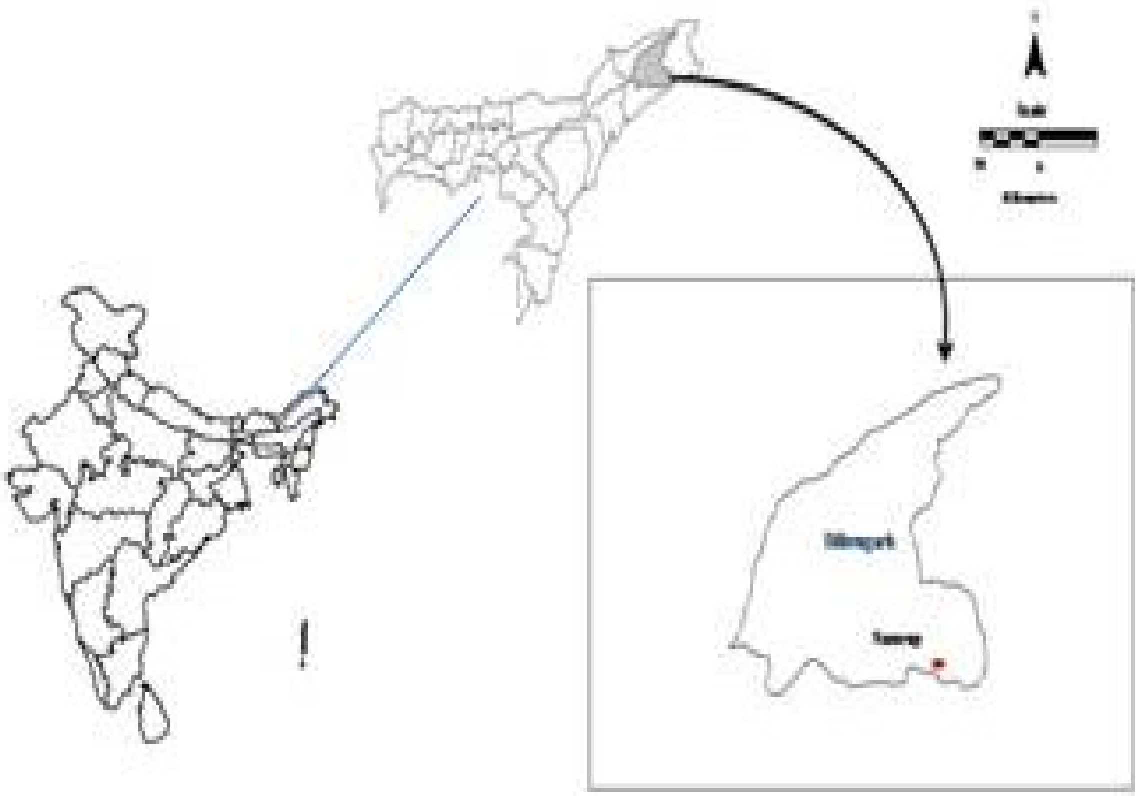

**Figure 1.** Locational setup of study area, Namrup, Assam, India.

due to the disposal of raw waste water in the study site (Figure 1). Namrup Fertilizer Complex renamed as Brahmaputra Valley Fertilizer Complex (BVFCL), the premier nitrogenous fertilizer production unit located on the striking distance from Dilli River and the study site disposes the raw wastewater and the sludge produced by wastewater treatment plants in to the water of Dilli River. This region is characterized by a hot sub-humid (moist) to humid (inclusion of perhumid) climate with alluviaum dried soil and growing periods of approximately 210+ days. Mean daily temperatures range from $23 \pm 2$ °C in January to $32 \pm 3$°C in July, and precipitation averages 2332 mm year$^{-1}$ (Statistical hand book in Assam 2007).

### Plant species

The weed species viz. *Amaranthus spinosus* L. *Cassia obtusifolia* L. *Croton bonplandianum* Baill. and *Leonurus sibiricus* L. were found to be growing on the area where effluents were dumped and these were collected and processed for biochemical analysis. The results obtained were later compared with same species grown on non polluted site, 5 km away from the original site of study.

### Biochemical parameters

Chlorophyll content was measured from tender shoots of experimental weed plants collected from the site and chlorophyll a (Chl a), b (Chl b), and a:b (Chl a:b) were measured at 645, 652, and 663 nm with three replication each using B and L Spectronic 20 (Arnon, 1949).

Foliar protein analysis was measured from mature weed plants collected from experimental site with three replication each. The foliage was removed and ground and protein percentage were measured (Lowry et al., 1951).

Foliar sugar percentage was determined from randomly selected shoots of each weed and total soluble sugar was obtained using Anthrone method (Thimmaiah, 2004).

### Effluent analysis

Effluent samples were collected in each month throughout the year (January –December, 2006) at the out fall of BVFC from two sampling site (PI and P II) of Dilli River. Control sample was collected from the Dilli river basin ~1km away from the sampling site. The collected samples were analyzed for physicochemical properties by standard methods of APHA (Lenore et al., 1998).

### Statistical analysis

All the data were subjected to analysis of variance (ANOVA). Correlations between concentrations of protein, sugar and chlorophyll in all the examined weeds grown in polluted and unpolluted site were analyzed. The ANOVA procedure of the Statistical Analysis System (SAS) program was used in variance analysis (SPSS 12 for windows).

**Table 1.** Photosynthetic pigment (mg $g^{-1}$ fresh weight), protein and total soluble sugar percentage of weed plants grown under effluent contaminated soil (Average of three determinations).

| Species | Population | Habit | Injury Symptoms (Morphological) | Chl a (mg/g) | Chl b (mg/g) | Chl a:b | Protein (%) | Total (%) soluble sugar |
|---|---|---|---|---|---|---|---|---|
| *Amaranthus spinosus* L. | Site I | Herbs | Interveinal necrosis | 0.820 | 0.465 | 1.760 | 3.20 | 4.80 |
| | Site II | | | 1.278 | 0.492 | 2.590 | 4.20 | 6.10 |
| | Mean | | | 1.049 | 0.478 | 2.175 | 3.70 | 5.45 |
| | SD | | | 0.320 | 0.010 | 0.580 | 0.70 | 0.65 |
| | Site I | Herbs | | 1.152 | 0.429 | 2.680 | 3.20 | 5.60 |
| *Cassia obtusifolia* L. | Site II | | Small chlorotic spots | 1.605 | 0.810 | 1.980 | 8.70 | 9.40 |
| | Mean | | | 1.378 | 0.619 | 2.330 | 5.95 | 7.50 |
| | SD | | | 0.320 | 0.260 | 0.700 | 3.88 | 2.68 |
| *Croton bonplandianum* Baill. | Site I | Herbs | No apparent injury | 0.603 | 0.237 | 2.540 | 5.00 | 8.20 |
| | Site II | | | 0.830 | 0.372 | 2.230 | 5.10 | 8.60 |
| | Mean | | | 0.716 | 0.304 | 2.385 | 5.05 | 8.40 |
| | SD | | | 0.110 | 0.090 | 0.210 | 0.07 | 0.28 |
| *Leonurus sibiricus* L. | Site I | Herbs | Marginal necrosis | 1.389 | 0.583 | 2.380 | 2.00 | 1.92 |
| | Site II | | | 1.454 | 0.646 | 2.250 | 3.80 | 6.10 |
| | Mean | | | 1.421 | 0.614 | 2.315 | 2.90 | 4.01 |
| | SD | | | 0.040 | 0.040 | 0.090 | 1.27 | 2.95 |
| All species | Mean | | | 1.141 | 0.504 | 2.301 | 4.40 | 6.34 |
| | SD | | | 0.350 | 0.170 | 0.310 | 2.01 | 2.40 |
| | r value | | | 0.153 | 0.062 | 0.180 | 0.195 | 0.169 |
| ANOVA | P Value | | | 0.717 | 0.883 | 0.669 | 0.642 | 0.687 |

Polluted for 'Site I' and unpolluted for 'Site II'.

## RESULTS AND DISCUSSION

In the present study, the four weeds species, depending upon plant habit, were broadly divided into herbs and shrubs." The test parameters namely, chlorophylls, foliar proteins and sugars of the examined weeds were found significantly decreased as compared to species grown at non polluted site (Table 1). The chlorophyll a content (mg $g^{-1}$ Fr.wt) of *Amaranthus spinosus* L. *Cassia obtusifolia* L. *Croton bonplandianum* Baill. and *Leonurus sibiricus* L. from contaminated site was 0.820 ± 0.093, 1.152 ± 0.099, 0.603 ± 0.014, 1.389 ± 0.046 respectively.

The chlorophyll content of the plants was observed to have decreased, in general in contaminated site and this decrease might be attributed to the presence of heavy metals in the study site. The heavy metals in particular alter metabolic pathways of the plants (Clijsters and Van Assche, 1985) and this may be the reason while examined weeds sample contain reduced amount of chlorophyll.

Other than chlorophyll formation, magnesium is also necessary to maintain the structure of ribosome and ribonucleoprotein bodies and is essential for protein synthesis. The presence of excessive nitrogen and sodium in the effluent (Table 2) may play a role for inhibiting the uptake of other elements like Mg (Thabaraj, 1964). The foliar protein content (%) of *A. spinosus* L., *C. obtusifolia* L., *C. bonplandianum* Baill., and *L. sibiricus* L. from contaminated site was 2 ± 0.46, 3.2 ± 0.54, 5.0 ± 0.23, 3.2 ± 0.85 respectively and except *C. bonplandianum* Baill., in all other weeds the protein synthesis mechanism is found to be hampered resulting in the reduce production of protein (Table 1).

Phosphate is essential for normal translocation of Carbohydrates in plants and as well as for the transformation of starch into sugar, but the excess of other elements in contaminated soil prevents the plants to uptake the adequate amount of phosphate resulting in the interruption of normal metabolism. In the present study, soluble sugar content (%) of *A. spinosus* L., *C.*

**Table 2.** Physicochemical properties of raw effluents at PI and PII sampling points and Dilli river water. In each sampling point 12 (twelve) samples observed in each sampling point.

| Parameter | Raw effluent | | | Dill water body contaminated with effluent | | | IS standard for inland surface water |
|---|---|---|---|---|---|---|---|
| | PI mean | PII mean | Average mean | Max | Min | Mean | |
| DO | 4.61 | 4.46 | 4.535 | 7.83 | 7.73 | 7.28 | - |
| PH | 10.108 | 10.058 | 10.083 | 7.92 | 7.9 | 7.91 | 5.5 - 9.0 |
| EC | 2224.66 | 2320.75 | 2272.705 | 21.6 | 10.1 | 15.63 | - |
| SS | 167.33 | 161 | 164.165 | 32 | 28 | 30 | - |
| Turbidity | 138 | 142.166 | 140.083 | 45 | 31 | 38 | - |
| BOD | 28.83 | 28.33 | 28.58 | 2 | 3 | 2.5 | - |
| COD | 135.16 | 134.66 | 134.91 | 25 | 30 | 27.5 | 250 |
| Odour | | Ammonical | | | Mild Ammonical | | |
| Colour | | Brown | | | Colourless | | |
| Ammonical Nitrogen | 160.83 | 157 | 158.915 | 60 | 48 | 54 | - |
| Total Kjeldahl Nitrogen (TKJ) | 183.84 | 177.65 | 180.745 | 82 | 65 | 73.5 | - |
| Nitrate Nitrogen | 13.66 | 13.25 | 13.455 | 3 | 2 | 2.5 | - |
| Nitrite Nitrogen | 7.483 | 8.03 | 7.7565 | 3 | 1 | 2 | - |
| Oil and grease (mg/100 ml) | 2.65 | 2.598 | 2.624 | 0.5 | 0.25 | 0.375 | 10 |
| Cyanide (CN) | BDL | BDL | | BDL | BDL | | |
| Vanadium (V) | 16.58 | 18.44 | 17.51 | 4 | 2 | 3 | - |
| Arsenic (As) | BDL | BDL | | BDL | BDL | | - |
| Chromium (Cr) | 0.133 | 0.134 | 0.1335 | 0.83 | 0.68 | 0.755 | 2 |
| Fe | 8.53 | 8.19 | 8.36 | 0.13 | 0.12 | 0.125 | 3 |
| Cu | 0.79 | 0.65 | 0.72 | 0.1 | 0.09 | 0.095 | 3 |
| Mn | 0.72 | 0.61 | 0.665 | 0.43 | 0.32 | 0.375 | - |

Parameters are expressed in mgl $^{-1}$ except pH, Turbidity and Conductivity expressed in NTU and mho/cm respectively.

*obtusifolia* L., *C. bonplandianum* Baill., and *L. sibiricus* L. from contaminated site was 1.92 ± 0.46, 5.6 ± 0.54, 8.2 ± 0.23, 4.8 ± 0.22 respectively. Reduction of sugar content may be the result of the minimum uptake of phosphate.

The concentration of different forms of nitrogen give a useful indication of the level of micronutrients in the water and hence their ability to support plant growth. On the contrary, a high content of $NH_3$-N, $NO_3$-N, and $NO_2$-N in the liquid effluents may be toxic to plants. In the present study the ammonical nitrogen values ranged from 135 to 187 mgl$^{-1}$, nitrates values ranged from 9 to 18 mgl$^{-1}$, nitrite values ranged from 5.60 to 9.40 mgl$^{-1}$ and total Kjeldahl nitrogen (TKJ) values ranged from 162 to 198.40 mgl$^{-1}$ at both the sampling points (PI and PII), were recorded which are detrimental for plant metabolisms and found to have inhibition of biochemical content in the examined weeds.

The present findings show that waste water released from Namrup Fertilizer complex contains certain toxic chemicals which are not recycled or reused except in case of few parameters mention above (Table 2). Such effluents are causing detrimental effect on the environment in general and there is every possibility of contamination of the perennial water source of Dilli River itself. The fluctuations in physicochemical parameters were attributed to seasonal variations, varying effluent volumes, increased production capacity of the fertilizer plant, and the type of fertilizer minerals produced (Ekundayo, 1995).

Vanadium in its pentoxide form is known to have produced a variety of toxic effect (Altamirano et al., 1996; Zhong et al., 1994) in animals but in case of plants, specific data appears to be scanty. In the present analysis, the presence of excessive amount of vanadium was recorded and the concentration ranged between 12.45 to 23.45 mgl$^{-1}$ at both PI and PII sampling points and might be toxic for test plants.

The uptake of chromium by plants is passive and uptake and transport of chromium in plants seem to be associated, among others, with iron, as suggested by constant Cr/Fe ratios (Kabata-Pendias and Pendias, 1992). For chromium, toxicity threshold values have been established by various authors and presented data (Table 2) enforce the conclusion that any physiological disturbances may not be due to excessive Cr concentration in the study site.

Oil spreads on water to form a thin layer on the surface and tend to prevent the diffusion of oxygen leading to a fall in the level of dissolved oxygen. The oil, being lighter than water, remains suspended and tends to drift towards the bank due to currents and winds. Thus the banks are spoiled by the oil and it reduces photosynthetic activities on weed plants by reflecting the sunlight. In the present study, oil and grease ranged from 2.650 - 2.598 $mgl^{-1}$ at both PI and PII sampling points and they must have been toxic for photosynthesis.

By examining the overall performance, the present study reveals the metabolic efficiency of plants exposed to liquid effluents to be are adversely affected, leading to decreased growth in terms of various morphological and biochemical parameters (Table 1). The visible symptoms of toxicity of pollution vary from plant to plant and even within the same species. The common and nonspecific symptoms as chlorosis, interveinal chlorosis, necrosis, and stunted growth were exhibited by the plant species examined in the vicinity of the fertilizer factory.

The study indicates that despite the weed possessing relatively sound defense mechanism, the industrial effluents and heavy metals sometime interfere in their normal metabolism and as a result essential foliar contents like chlorophyll, protein and sugar are considerably decreased. In summary, it is apparent that the soil contaminated by effluents and heavy metals responds negatively to the growth of weeds particularly in industrial environment although C. *bonplandianum* Baill shows tolerance to a certain extent.

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