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# EFFECT OF BATTERY AND PHARMACEUTICAL INDUSTRIAL EFFLUENTS ON PHOTOSYNTHESIS PIGMENTS IN RICE (*Oryza sativa* L.)

*Katepogu Raju and T. Damodharam

Department of Environmental Science, Sri Venkateswara University, Tirupati-517502 Andhra Pradesh, India.
*Corresponding author: E-Mai: envirosvu@gmail.com

**ABSTRACT:** Rice cultivar plants raised in pot culture containing the red soil amended with battery and pharmaceutical industrial effluents. The effluents were collected Karkambaddi and Renigunta industrial area, Tirupati, Andhra Pradesh, India. The aim of present investigation to evaluate the adverse effect of battery and pharmaceutical industrial effluents on photosynthetic pigments chlorophyll-a, chlorophyll-b, total chlorophyll, Carotinoids, total sugars and proteins contents of rice (*Oryza Sativa* L.).The biochemical constituents (chlorophyll 'a', chlorophyll 'b', total chlorophyll, carotinoids, total sugars, starch, amino acids, proline and protein) were analysed on 10, 20, and 30th day. The results showed that the application of battery industrial effluents at 10, 20th day as resulted significantly decreased ($P \leq 0.001$) in chlorophyll-a, chlorophyll-b and total chlorophyll compared with the control, but the industrial effluents effect at 30th day more significant decreases were observed compared with control, 10 and 20th day interval effect, the plant exhibited industrial effluents showed an 36-59.7% increase in proline levels. From the present investigation it can be concluded that the battery and pharmaceutical industrial effluents causes the decreases. The biochemical constituents (chlorophyll, carotene, total sugars, starch, amino acids and protein contents of leaves) of rice plants. But the proline content of rice plants increased with an increase in time of intervals. This leads to decreased the various growth and yield parameters such as length of the root and shoot, area of leaves and dry weight of root and shoot.
**Key words:** Battery industrial effluent, Carotenoid, Chlorophyll, Proline…etc

## INTRODUCTION:

Rice (*Oryza sativa* L.) is an important crop worldwide and is the staple food in the diet of more than one third of the world's population [12, 14]. Rice is also considered to be a model plant among monocots for biological research because of its small genome size [8]. The growth and yield of rice depends upon various factors, one of which is the soil quality. Soil pollution is a major cause of change in the quality of the soil. Industrial effluents are responsible for serious water [19] and soil pollution [14], which is considered as one of the major factors responsible for low productivity of crops. A considerable number of reports are available on the effect of different industrial effluents on different crops [6, 18]. Polluted water directly affects soil not only in industrial areas but also in agricultural fields and river beds, creating secondary sources of pollution [13]. Industrial waste has been a major cause in reducing soil fertility and causing great damage because effluent are being added to the neighbouring soil and water (frequently used for irrigation) continuously [10]. The harmful nature of industrial effluents in relation to plant growth and development is well recognized owing to the presence of toxic chemicals present in it. Agricultural production in many countries is being severely affected by the reckless discharge of these effluents to the water bodies near the industrial establishments which are the main source of irrigation. In addition to providing large quantities of water, some effluents contain considerable amount of essential nutrients, which may prove beneficial for plants [21, 20]. Polluted water directly affects soil not only in industrial area but also in agricultural fields and river beds, creating secondary sources of pollution [13].

## MATERIALS AND METHODS:

FC- Reagent, BSA, Trichloro acetic acid, Glacial acetic acid, Ninhydrin and other chemicals purchased from indigenous companies were of pure and used for the analysis in our research.

## Plant Materials and Incubation Conditions

Rice seeds were obtained from Nagarjuna Ranga Agricultural Regional Research Station Tirupati, Andhra Pradesh, India. The seeds are surface sterilized with 0.1% $Hgcl_2$ solution for 10 min and rinsed with double distilled water, shown in earth pots (30 cm x 25 cm, diameter and deep) containing red soil and battery industrial effluent. The $S_8$ and $S_9$ industrial effluent samples were collected near the Gajulamandyam, Renigunta and Karkambaddi, Tirupati. The pots were kept under natural photo radiation and each pot contained 20 seedlings. The pot culture of rice was analyzed the pigmental and biochemical changes of growth and yielding of three interval of 10, 20 and 30$^{th}$ day experimental period.

## Estimation of chlorophyll

The chlorophyll content was estimated according to the method of Arnon 1949[1]. About 1 gr of leaf sample was cut in to small pieces and homogenized in a pre-cooled mortar and pestle using 80% (V/V) acetone. A pinch of calcium carbonate was added while grinding. The extract was centrifuged at 3000 rpm for 15 min and made up to 25 ml with 80% (V/V) acetone. The clear solution was transferred to a colorimeter tube and the optical density was measured at 645 nm and 663 nm, against an 80% acetone blank in shimadzu Double Beam spectrophotometer (UV - 240).

The levels of chlorophyll 'a' and chlorophyll 'b' were determined using the equation given below:

Chlorophyll 'a' (μ/g/ml) = (12.7 x O.D. at 663 nm) – (2.69 x O.D. at 645 nm)
Chlorophyll 'b' (μ/g/ml) = (22.9 x O.D. at 645 nm) - (4.08 x O.D. at 663 nm)
Total chlorophyll (μ/g/ml) = (20.2 x O.D. at 645 nm) + (8.02 x O.D. at 663 nm)

The chlorophyll content was expressed as mg chlorophyll / gr fresh wt of the leaf.

## Carotenoid

Carotinoids were determined as per the method of Jensen and Jensen (1971) [11].one gr of freshly harvested leaf material made into small pieces was macerated with acetone in a mortar. The extract was centrifuged and residue thus obtained was again reextracted followed by centrifugation. The procedure was repeated until no more pigments were extracted. The supernatants were pooled up and the acetone was removed in vacuo. The residue after completed removal of acetone was dissolved in a small volume of 10% (UV) methanolic KOH solution (10 ml final solutions for each mg of pigment). The mixture was allowed to stand at room temperature for 2 hr and then transferred to a separating funnel. The reaction was stopped by the addition of 5% (W/V) aqueous NaCl solution. After separation, the aqueous phase was extracted thrice with petroleum ether and the combined petroleum to remove methanol. The ether extract was dried over anhydrous sodium sulphate and the petroleum ether was removed in vacuo. The residue was dissolved in a known volume of acetone and O.D. was measured at 445 nm in shimadzu double beam spectrophotometer (UV 240). The Carotinoids content was calculated using the following equation.

C = d.v.f.10/2500

Where C = Carotinoids in mg
d = Optical density at the middle main maximum.
v = Total volume in ml.
f = Dilution factor for the sample actually measured.

## Total sugars

100 mg plant material was weighed and homogenate with 10 ml 80% ethanol. It was centrifuged for 10 rpm for 10 min. Supernatant first was collected, while 10 ml 80% ethanol was added again to the residue, centrifuge and collect supernatant first and second was mixed with supernatants were mixed and discarded residue. The 1ml alcoholic aliquot, 1 ml 1N $H_2SO_4$ was added and heated at $49^0$ C in water bath for 30 min for hydrolysis of the mixture. 1-2 drop of methyl red indicator was added. 1N NaOH was added drop wise for the neutralization (colour was to yellow from pink). 1 ml Nelson Somogyi's reagent was added to it and the tube was kept in boiling water bath for 20 min. After cooling of the test tube, 1 ml arsenomolybdate was added and final volume was made up to 20 ml with DW. O.D. was noted at 540 nm. Blank was prepared in the same manner.

## Starch

Estimation of starch by anthrone method McCredy *et al*., (1950) [16]. Homogenize 0.1 to 0.5 gr of the sample in hot 80% ethanol to remove sugars. Centrifuge and retain residue. To the residue add 5.0 ml of water and 6.5 ml of 52% Perchloric acid. Extracted at $0^0$C for 20 min. centrifuge and save the supernatant. Repeat the extract using fresh Perchloric acid and centrifuge and pool the supernatants and make up to 100 ml. pipette out 0.1 or 0.2 ml of the supernatants and make up the volume to 1 ml with distilled water. Prepare the standards by taking 0.2 to 1 ml of the working standard and make up the volume to 1 ml in each tube with water and 4 ml Anthrone reagent add to each tube. Heat for 8 min in a boiling water bath. Cool rapidly and read the intensity of green to dark green color at 630 nm. Standard curve was prepared with known amounts of glucose equivalent present in the sample with 0.9.

**Amino acids**

The activity was measured by the method of Moore and Sand Stein, (1948) [17]. Weigh 500 mg of the plant sample and grind it in a pestle and mortar with a small quantity of acid washed sand. To this homogenate, add 5 to 10 ml of 80% ethanol. Filter or centrifuge. Save the filtrate or the supernatant. Repeat the extraction twice with the residue and pool all the supernatants. Reduce the volume if needed by evaporation and use the extract for the quantitative estimation of total free amino acids. If the tissue is tough, use boiling 80% ethanol for extraction. To 0.1ml of extract, add 1 ml of ninhydrin solution. Make up the volume to 2 ml with distilled water. Heat the tube in a boiling water bath for 20 min. add 5 ml of the diluents and mix the contents. After 15 min read the intensity of the purple colour against a reagent blank in systronic (UV-VIS, 118) double beam spectrophotometer 570 nm. The colour is stable for 1hr. Prepare the reagent blank as above by taking 0.1 ml of 80% ethanol instead of the extract. Dissolve 50 mg leucine in 50 ml of distilled water in a volumetric flask. Take 10 ml of this stock standard and dilute to 100 ml in another volumetric flask for working standard solution. A series of volume from 0.1 to 1 ml of this standard solution gives a concentration range 10µg to 100 µg. proceed as that of the sample and read the color.

**Proline**

Proline was estimated according to the procedure of Bates *et al* (1973) [5]. One gram of freshly harvested leaves were taken and washed thoroughly with tap and distilled water. Leaves were blotted dry and cut into small bits. The leaf bits were ground in 10 ml of 3% aqueous sulfosalic acid and filtered through whatman No.1 filter paper. For two ml of filtrate, 2 ml of acid ninhydrin reagent (1.25gr ninhydrin, 20 ml of glacial acetic acid, 20 ml of 6M Phosphoric acid) and 2 ml of glacial acetic acid were added and samples were kept in boiling water bath for one hr. After 1 hr heating the samples were kept in an ice bath to terminate the reaction. Each tube Four ml of toluene was added and mixed vigorously. The chromophore was aspirated from the aqueous phase and warmed the samples to room temperature. The samples were read at 520 nm in schimadzu UV- VIS Spectrophotometer. Proline concentration in the samples were computed based on a similarly prepared standard curve for proline.

**Protein**

Protein content was with Folin-Phenol method of Lowry *et al*., (1951) [15]. The protein was extracted from leaf tissue as per the method outlined by Key (1964) [17]. 100 mg of dry powder was accurately weighed and taken in a centrifuge tube directly to which 10 ml of 5% (W/V) TCA was added and left for 4 hr and was centrifuged, again washed with 10 ml of 5% (W/V) TCA to remove interfering aminoacids. The supernatant was discarded and the protein precipitate was dissolved in 2N NaoH and was left for 2 hr at room temperature. An aliquot of protein solution was taken in to a test tube to which 5 ml of alkaline copper solution was added. Shaken thoroughly, after 10 min, 0.5 ml of Folin-phenol reagent (commercially preparation was diluted to equal volume with distilled water) was added and shaken thoroughly. After 30 min the protein solution was scanned for absorbance at 660 nm in a schimadzu spectrophotometer (UV-240) using BSA as a standard.

## RESULTS AND DISCUSSION:

The total chlorophyll, including chlorophyll 'a', chlorophyll 'b' and Carotinoids (Fig.1) were decreased on 10$^{th}$ day in compared with S8 to S9 compared with control plants. The percentage changes for total chlorophyll including chlorophyll *a,* chlorophyll b and Carotinoids varied from 17.58% to 33.71%, 21.19% to 32.64%, 12.83% to 32.64% and 21.28% to 37.17% respectively. At the 20$^{th}$ day (Fig.2) the pigment contents of percentage changes were 33.62% to 33.95%, 40.11% to 45.95%, 32.64% to 35.38% and 38.17% to 41.34% respectively.

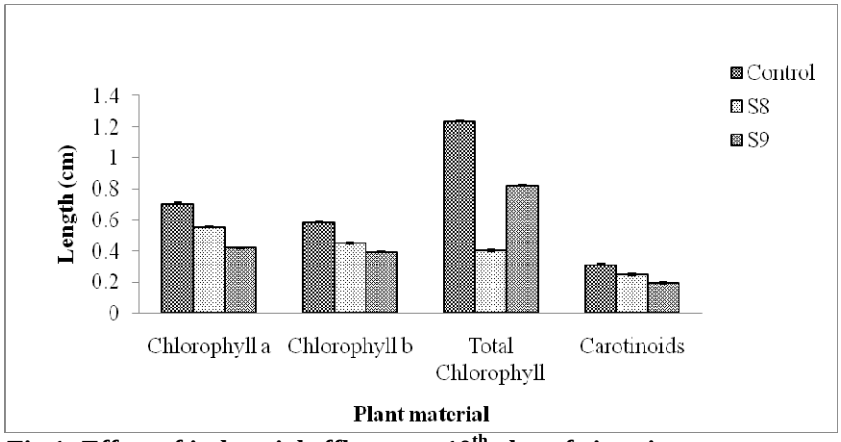


**Fig.1: Effect of industrial effluent on 10$^{th}$ day of rice pigment contents**

Values are six replicates of mean ± S.E.

$S_8$ = Pharmaceutical industrial effluent influenced rice plants.

$S_9$ Battery industrial effluent influenced rice plants.

At the 30th day (Fig.3) pigment contents of percentage were changed from 49.95% to 58.36%, 47.26% to 63.22%, 47.69% to 58.80% and 66.02% to 72.11% respectively. The biochemical contents (*Oryza sativa* L.) of total sugars, starch, amino acids, proline and protein levels were changed the variation of percentage in sample S8 to S9 on 10th day (Fig.4) from 12.57% to 27.97%, 8.98% to 24.97%, 0.71% to 19.17%, 26.53% to 36.28% and 3.39% to 18.36% respectively. At the 20th day the levels of bio chemical content variation (Fig.5) percentages from 19.87% to 39.43%, 21.95% to 31.98%, 29.60% to 38.27%, 15.95% to 38.13%, and 15.77% to 24.67% respectively.

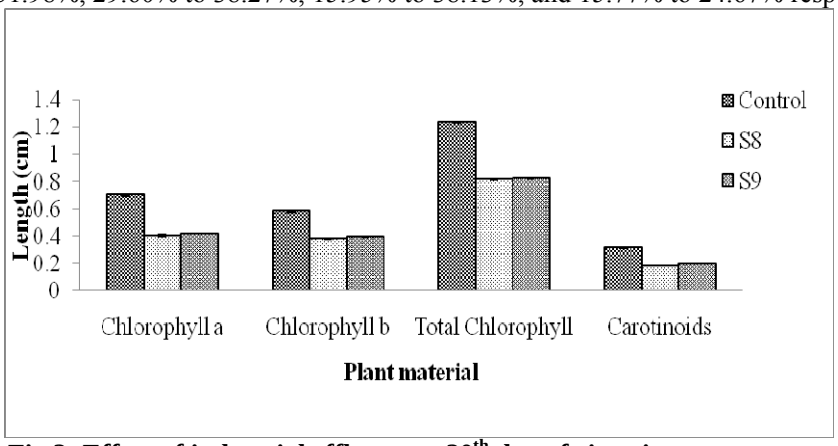


**Fig.2: Effect of industrial effluent on 20th day of rice pigment contents**

Values are six replicates of mean ± S.E

$S_8$ = Pharmaceutical industrial effluent influenced rice plants.

$S_9$ Battery industrial effluent influenced rice plants.

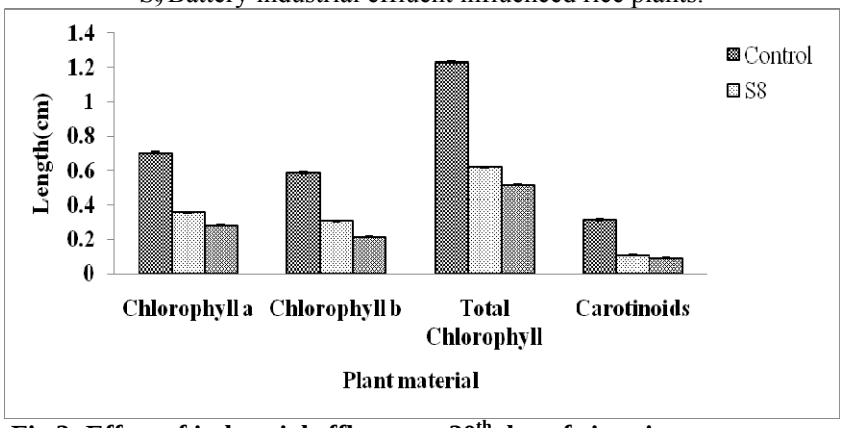


**Fig.3: Effect of industrial effluent on 30th day of rice pigment contents**

Values are six replicates of mean ± S.E

$S_8$ = Pharmaceutical industrial effluent influenced rice plants.

$S_9$ Battery industrial effluent influenced rice plants.

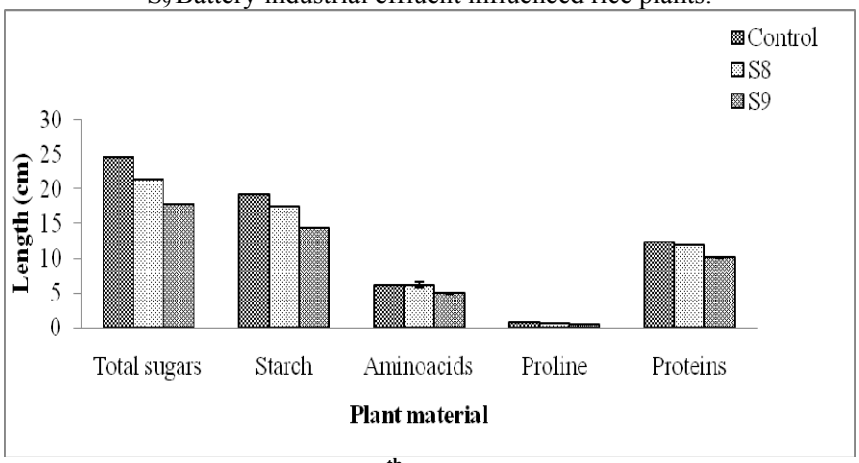


**Fig.4: Effect of industrial effluents on 10th day of Biochemical contents of *Oryza sativa* L.**

Values are six replicates of mean ± S.E

$S_8$ = Pharmaceutical industrial effluent influenced rice plants.

$S_9$ Battery industrial effluent influenced rice plants.

At 30th day (Fig.6) the bio chemical content variation percentages were changed from 28.73% to 47.06%, 31.87% to 41.69%, 21.45% to 52.97%, 28.00% to 41.10%, and 30.26% to 37.86% respectively. The total Sugars, Chlorophylls, Carotinoids and proline is seriously affected by heavy metals presented in the industrial effluent. Chlorophyll 'b' was reduced more as compared to Chlorophyll 'a'. The amount of proline increased in plants under stress caused by industrial effluents. Battery industry proved more toxic than pharmaceutical industry. The deleterious effects of these industrial effluents may be alleviated in plants.

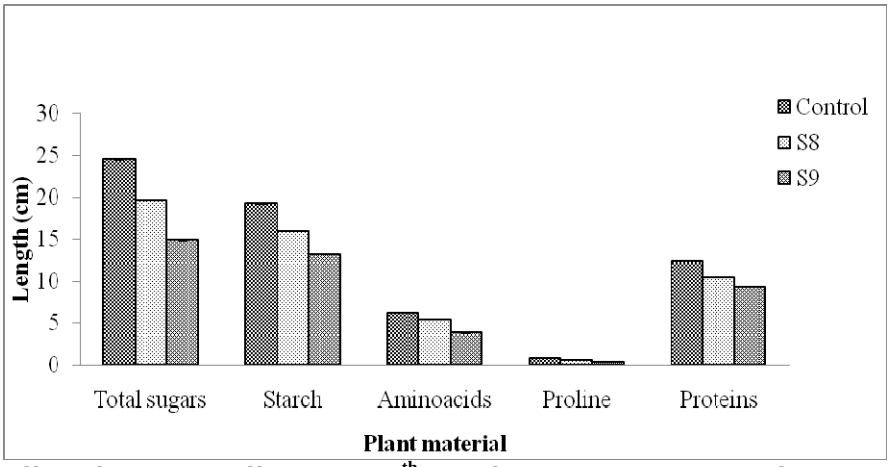


**Fig.5: Effect of industrial effluents on 20th day of Biochemical contents of *Oryza sativa* L.**

Values are six replicates of mean ± S.E

$S_8$ = Pharmaceutical industrial effluent influenced rice plants.

$S_9$ Battery industrial effluent influenced rice plants.

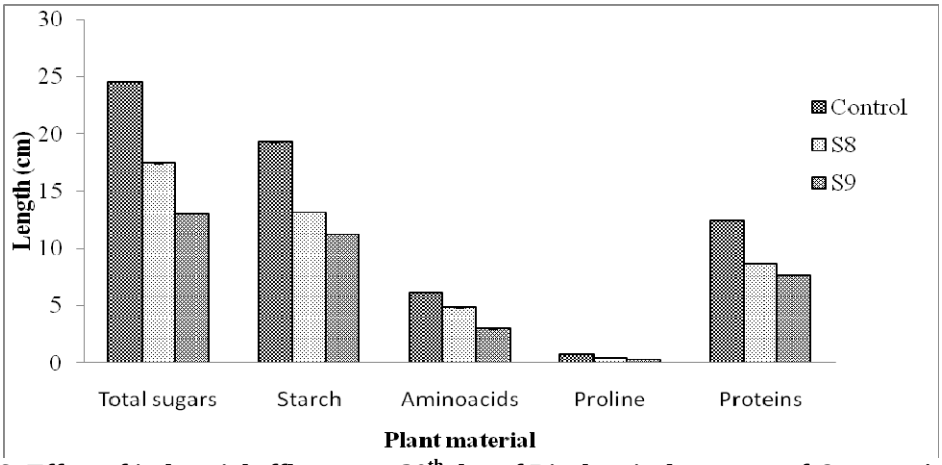


**Fig.6: Effect of industrial effluents on 30th day of Biochemical contents of *Oryza sativa* L.**

Values are six replicates of mean ± S.E

$S_8$ = Pharmaceutical industrial effluent influenced rice plants.

$S_9$ Battery industrial effluent influenced rice plants.

## CONCLUSION

Industrial effluents are being used for irrigation in dry areas. These effluents not only contain nutrients that enhance the growth of crop plants but also have toxic materials. In the present investigation, it is found that the application of diluted form of battery industrial wastewater was slightly impact of the growth of rice plant. Therefore, it is recommended that, industrial effluents are, after treated water to enhance the crop growth and yielding of the rice plant.

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