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Research Article

COMPARATIVE ANALYSIS OF EFFECT OF HEAVY METALS (CD &PB) ON CHLOROPHYLL CONTENT OF RICE (*oryzasativa L*), VARIETY IR-36 FROM LOWER GANGETIC BASIN, WB, INDIA

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ABSTRACT

Plants can't move away and are therefore continuously confronted with un-favourable environmental conditions (i.e. different kind of biotic & abiotic stress). Effect or impact of heavy metals (i.e. heavy metal stress) on agricultural crops is a growing concern of recent times. Out of different morphometric & physiological effects, chlorosis (lack of synthesis or accumulation of adequate amount of Chlorophyll) is one of the visible symptom of heavy metal stress. IR-36 a very popular and high yielding rice variety to Indian farmers, artificially treated with successive grades of Cd & Pb salt. Then chlorophyll was extract from the leaves of this salt treated or stressed plants as well as controlled. After that characterised by UV-visible spectroscopy and amount of total chlorophyll and chlorophyll-a & chlorophyll-b using *Arnon*, 1949 method. Indicating sharp decrease or fall out of chlorophyll content, according to increase of heavy metal concentration. Effect of Cadmium (Cd) is more devastating than Lead (Pb).

INTRODUCTION

'Heavy Metals' is a general collective term, applies to the group of metals and metalloids with atomic density greater than 4 gm/cm^3 or 5 times than water (*Hawkes, 1997*). Among a variety of heavy metals Cd & Pb are more common in environment (*Mukti Gill, 2014*) due to several natural as well as anthropogenic factor and focussed in our study. Usually the levels of heavy metals in agricultural soil are very low (apart from the site adjacent to mine or volcanos). But continuous applying of different kinds of chemical fertilizers (mainly Nitrogen &Phosphate containing fertilizer) increase its amount as different heavy metals viz. - Cd, Pb, Hg remains as impurity of it (*Raymond Wuana & Felix, 2011*) besides this application heavy metal containing (viz. Pb, As, Cu) pesticides are one of the major source of heavy metals in agricultural field (Ross, 1994). According to *Yanqun et al 2005*, Cd enrichment occurs due to the application of sewage sludge. Continued irrigation, unavoidable part of agricultural practice now, also lead to accumulation of heavy metals such as Pb & Cd (*Ross, 1994 & R K Rattan, 2005*).

Apart from all of this, rapidly increasing automobile rush & energy supplying power stations such as coal burning power plants became a major threat by emitting a great amount of Pb & Cd in atmosphere (*Verkeleji, 1993*).

Chlorophyll, principle photosynthetic pigment remain in all kind of autotrophs. In higher plants it found in two form chlorophyll-a ($C_{55}H_{72}O_5N_4Mg$) & chlorophyll-b ($C_{55}H_{70}O_6N_4Mg$). Heavy metals particularly Cd caused Fe (II) deficiency and ultimately the chlorophyll biosynthesis and photosynthesis also. Beside this a general Cd has been shown to interfere with the uptake, transport and use of several elements such as N, Mg, etc. which are essential for chlorophyll biosynthesis (*Das et al 1997*). On the other hand Lead (Pb) administrated to potted sugar beet plants at rates of 100-200 ppm caused chlorosis and growth reduction (*Hewilt, 1953*). This chlorosis ultimately hampered the yields of plant.

Like other plant rice also affected by heavy metals in the same way IR-36 a high yielding rice variety developed by IRRI (IR-36 - the world's most popular rice by IRRI) widely distributed throughout the country and popular to cultivar, selected to asses this impact. Because rice is one of the principal staple food as well as cash crop our country (*The Rice economy of Asia - Randolph Barker et al*) and Gangetic basin is the heart-land of rice cultivation.

MATERIAL AND METHODS

Study area: West Bengal is one of the highest rice yielding (No. 1 in Rank) state in India. Dist. Burdwan is known as 'Paddy Farm' of West Bengal. MouzaRasui under Ketugram-II

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block of the Burdwan district (Latitude: 23.71^{0}N, longitude 88.04^{0}E and Altitude 22 M) in the bank of river Ajay is consider as experimental site.

Treatment Protocol & Duration: Both Cd & Pb are artificially supplied in a regular interval at four concentration Gradient of 50, 100, 150 & 200 ppm throughout the Boro season of paddy (Feb'17-April'17) from 45 days booting stage to onset of flowering. It applied in plot of 2' x 2' artificially maintain in paddy fieled.

Collection of materials: The fresh leaves are plucked randomly from different plot of variable concentration of both Pb & Cd separately. Besides this leaves sample were collected from untreated or controlled plant. Then they packed in separate marked pouch & brought to the laboratory within the Cryo-flask.

Extraction of Chlorophyll (Arnon, 1949): One gram of finely cut fresh leaves were taken from each sample and ground with 20-40 ml of 80% Acetone. It was then centrifuged at 500 rpm for 5 minutes at 25^{0}C temperature. The supernatant was transferred and the procedure was repeated till the residues become colourless. Then the absorbance frequency was measured with the help of UV-spectrophotometer at 645 nm & 663 nm against the solvent (Acetone) blank.

Estimation of Chlorophyll Content: The concentration of chlorophyll –a, chlorophyll-b and total chlorophyll were calculated using the following equation.

Total Chlorophyll: 20.2 (A_{645}) + 8.02 (A_{663})
Chlorophyll a: 12.7 (A_{663})-2.69 (A_{645})
Chlorophyll b: 22.9 (A_{645})-4.68 (A_{663})

RESULTS AND DISCUSSION

Throughout examination & analysis of data received after computation of absorbency readings using Arnon's (1949) equation. It remains clear that Chlorophyll concentration is inversely proportionate with the concentration of heavy metals (both Cd & Pb).

Table 1 Chlorophyll content of Cd treated Rice Plant (IR – 36)

Sl. No.	Concentration	O.D at – 645	O.D at – 663	Total Chl. (µg / ml)	Chlorophyll-a (µg /ml)	Chlorophyll-b (µg /ml)
1	Control	1.062	2.297	39.87	26.31	13.56
2	Cd_{50} ppm	0.836	1.952	32.53	22.54	9.98
3	Cd_{100ppm}	0.561	1.400	22.55	16.27	6.28
4	Cd_{150} ppm	0.321	1.032	14.75	12.23	2.52
5	Cd_{200} ppm	0.261	0.717	11.02	8.39	2.41

Table 2 Chlorophyll content of Pb treated Rice Plant (IR – 36)

Sl. No.	Concentration	O.D at-645	O.D At-663	Total Chl. (µg/ ml)	Chlorophyll-a (µg /ml)	Chlorophyll-b (µg /ml)
1	Control	1.062	2.297	39.87	26.31	13.56
2	Pb_{50} ppm	0.895	2.183	35.5	25.31	10.19
3	Pb_{100} ppm	0.641	2.083	29.64	24.62	5.02
4	Pb_{150} ppm	0.555	1.283	21.49	14.79	6.70
5	Pb_{200} ppm	0.394	1.171	17.34	13.81	3.53

Chlorophyll content decrease with gradual incensement of heavy metals not only reveals from the above results depicted in table & figure.

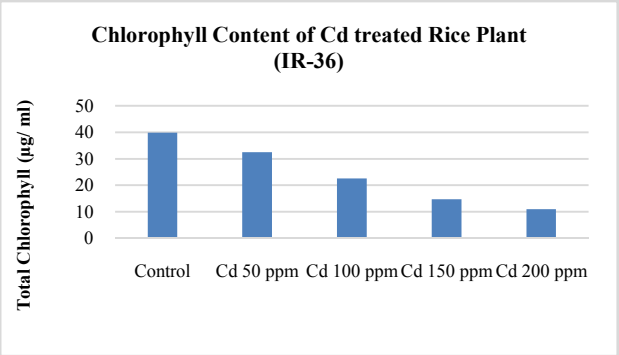

Figure 1

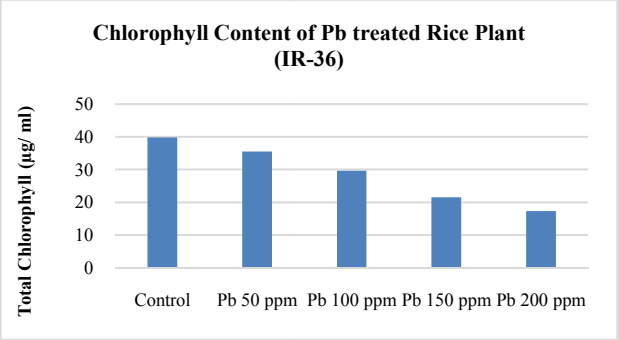

Figure 2

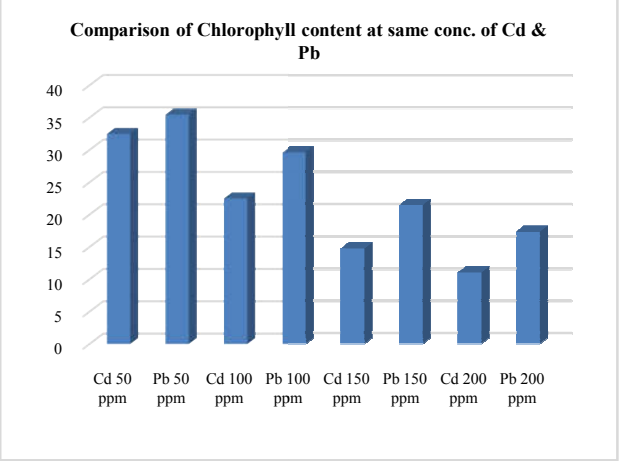

Figure 3

It also reveals that amount of chlorophyll-b also sharply decrease in compare to that of chlorophyll–a. Usually chlorophyll–a / chlorophyll–b ratio is equal to or less than 2.1 (Chang, F.H, *et al*) which is reveals in case of controlled plant but this ratio become greater with the concentration gradient of salt increased (Ferhad Murdoglu, K. Padmaja *et al*) and ultimately reach 4.85 & 4.90 in case of Cd & Pb respectively.

CONCLUSION

Results of chlorophyll estimation reveals that effect of Cd in this regard is severe than Pb. Most probably it is due to the concentration gradient of the salt (Cd & Pb) which have applied in this experiment. Because degree of tolerance of Pbconcentration (up to 170 mg/ kg equivalent to 170 ppm, Saha J. K, 2010) is many fold greater than Cd (5 to 10 ppm, S.

Gill). Though the effect on chlorophyll-a / chlorophyll-b ratio is same in both the cases (Cd & Pb).

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