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Phytotoxic Effect of Coal Mine Effluent on Growth Behavior, Metabolic Changes, and Metal Accumulation in Rice Plants (*Oryza sativa* L.) *c.v.* IR-36

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Coal is used as a fuel in energy sector that occurs in a wide range including bituminous and anthracite. Coal mining and processing activities result in the generation of huge quantities of waste containing elevated concentration of sulfates and trace metals including Fe, Mn, Cu as well as Al, As, Co, Ni, and Zn (Finkelman 1994; Ziberchmidt et al. 2004; Kairies et al. 2005). The unplanned discharges of effluent contaminate the ground and surface water (Diehl et al. 2004), and affect the agricultural soil and vegetation (Annandale at al. 2001). The toxicity level of effluent mainly depends on the geological properties of the coal, discharge volumes, pH, total acidity, dissolved ions concentration and buffering capacity of the receiving streams and other affected areas (Ziberchmidt et al. 2004). The toxic effect of mine effluent on several herbaceous species mainly *Typha angustifolia, Testuca arundinacea* and *Equisetum ramosissimum* have been reported (Yang and Gao 2001).

In India, Jharia coalfield lying at 23°48' N - latitude and 89°11' E - longitude is the only coalfield, supplying prime cooking coal for domestic and industrial uses. It is spread in an area of around 45 square kilometers; the coalfield has one of the highest scam densities in the world. The Jharia coalfield has got both open cost blocks as well as underground blocks. The open cost blocks presently cover nearly 100 square kilometer area. Ever increasing pressure on management to keep a balance between production cost and selling price has made it imperative to increase the area under open cost mining. The open cost mining of coal is most destructive process and it severely damages the upper layer of earth surface with soil erosion and finally loss of cultivated land (Li 2006). Zeng et al. (1995) reported that the crops grown in mines affected areas cannot be free from metals pollution. They also found *Ipomoea* was the most severely contaminated crop. However, the toxic element concentrations in maize, sorghum, azuki bean, soyabean and mung bean remain lower than the threshold levels.

The rice plant belongs to the genus *Oryza* of gramineae family and cultivated in most part of the world. Global paddy production reached 598 million tonnes (Singh and Siddiqui, 2003). In India, rice is the most important crop and it occupies 23.3 % of gross cropped area of the country. Rice contributes 43% of total food grain production and 46% of total cereal production. Jharkhand is the one of the main rice growing state in India. Jharia coalmines are present in Jharkhand state of India and its drainage water affect the agricultural field. Keeping these points in mind the present study has been undertaken to investigate the metal content in different parts (root, shoot and seed) of rice plant treated with various concentration of mine effluent and findings are discussed in this paper.

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MATERIALS AND METHODS

Rice (*Oriza sativa* cv. IR-36) plants were cultured in refined sand with pH about 6.8-7.0 at ambient temperature in a glass house conditions (Agarwala and Sharma, 1976), a modification of the method of sand culture (Hewitt 1966) in Indian conditions. The composition of complete nutrient solution (control) used was: 4.0 mM KNO_3, 4.0 mM Ca $(NO_3)_2.4H_2O$, 2.0 mM $MgSO_4.7H_2O$, 1.5 mM $NaH_2PO_4.7H_2O$, 0.1 mM NaCl, 0.1 mM Fe-EDTA, 10 μM $MnSO_4.H_2O$, 1.0 μM $CuSO_4.5H_2O$, 1 μM $ZnSO_4.7H_2O$, 30.0 μM $H_3BO_3.7H_2O$ and 0.1 μM $NiSO_47H_2O$ (Hewitt 1966). The nutrient solution was supplied daily except on weekends when pots were flushed with deionized water to avoid any salt or root exudates accumulation. Iron supply was maintained as Fe-EDTA (ferric ethylene diamine tetra acetic acid) chelate as described by Jacobson (1951).

Treatment of coal mine effluent (CME) was commenced after 25 d of sowing; during this period basal nutrient solution was supplied to the plants. CME was supplied as 0.0, 10%, 30%, 50%, 75% and 100% superimposed on basal nutrient solution. The plants were examined periodically for visible foliar symptoms and changes in growth parameters. On 50 d (25 d after CME supply) relative water content (RWC) was measured in comparable middle leaves by the method of Barrs and Weatherley (1973). All measurements were made between 9 and 11 a.m. when the sand in the pots was still saturated with nutrient solution. The ambient temperature was 35-40°C and humidity was 66-75%.

Total chlorophyll and protein contents were analyzed in middle leaves of the plants at 55 d of growth (30 d after CME supply). Chlorophyll was determined by the method of Arnon (1949) in the acetone extracts measured in Beckman model UV25 spectrophotometer. Protein was estimated after precipitation with trichloroacetic acid using the method of Lowry et al. (1951), measured at 640 nm in a Bausch and Lomb spectronic 20 colorimeter. Carbohydrate (Nelson 1944), nitrogen fraction (Chibnell et al. 1943), starch (Montgomery 1957) and phenols (Swain and Hillis 1959) were measured in fresh leaves at 33^{rd} d after the treatments.

On 98 d (73 d after CME exposure), plants in all lots were harvested. Leaf area as an index of plant growth as measured by Delta-*T* leaf area measurement system. Biomass and concentration of different metals were measured in different parts. For this purpose roots and shoots were separated and after washing, dried in a forced-draught oven at 70°C for 40 h. The dried samples were digested in HNO_3:$HClO_4$ 10:1, (v/v) by following the method of Piper (1942), after filtration the metals viz, Fe, Mn, Cu, Zn, Cr, Cd, Ni, and Pb were estimated with the help of Atomic Absorption Spectrophotometer-AA-120. Analysis of sulphur and phosphorus was performed by following the method of Chesnin and Yien (1951) and Wallace (1951), respectively. Wet digestion in HNO_3:$HCLO_4$ (3:1 v/v) at 90°C was followed for the determination of different trace elements present in the effluent collected from Chhatarpur mine, Jharia, Jharkhand.

Temperature of effluent was measured on the spot. Turbidity, dissolved oxygen (DO), pH, and total dissolved solids (TDS) were analyzed with Portable Water Analysis Kit (Century CMK, 731), while other parameters like biochemical oxygen demand (BOD), total suspended solids, chlorides, nitrates, hardness and sulphates were estimated as per procedures given in APHA (1992). Atomic Absorption Spectrophotometer-AA-120 was used for the estimation of metals in different parts of tested species. All the data were subjected to two way analysis of variance (ANOVA<0.01) using Microsoft Excel 2000 followed by Duncan's multiple range test (DMRT>0.05) (Gomez and Gomez, 1984) to confirm the variability of data and validity of results and comparison between means of different treatments.

RESULTS AND DISCUSSION

The physico-chemical properties of effluent collected from Chhatarpur coal mine, Jharia, Jharkhand is depicted in Table 1. The temperature 25.50°C; Turbidity, 162.0; pH, 6.85; DO, 3.86; BOD, 9.75; TDS, 976.0; TSS, 2230.0; Chlorides 63.0; nitrates 1.5; hardness 492.0; sulphate 39.6 mg l^{-1}. The concentration of toxic and trace elements in the collected effluent Fe, 597.32; Mn, 81.82; Cu, 218.25; Zn, 126.16; Cr, 29.50; Cd, 83.35; Ni, 86.75 and Pb 38.82 mg l^{-1} were as follow.

Table 1. Physico-chemical characteristics of coal mine effluent.

Parameters	**Values**
Temperature (°C)	25.50±1.05
Turbidity	162.00±4.61
pH	6.85±0.12
Dissolved Oxygen	3.86±0.27
Biological oxygen demand	9.75±0.31
Total dissolved solids	976.00±14.61
Total suspended solids	2230.00±174.67
Chlorides	63.00±4.08
Nitrates	1.50±0.04
Hardness	492.00±12.81
Sulphate	39.60±3.77
Metals	
Fe	597.32±17.09
Mn	81.82±4.17
Cu	218.25±8.61
Zn	126.16±6.22
Cr	29.50±3.08
Cd	83.35±3.09
Ni	86.75±4.77
Pb	38.82±3.08

Values are means ±SE (n=5). All values are in mg l^{-1} except for pH and those otherwise stated.

After 50 d, the growth of rice plants was retarded at 50%, 75% and 100% of CME treatment as compared to controls. After 55 d of growth, young leaves showed vein clearing, margins of leaves were curled and leaves appeared pale at 100% treatment. Interveinal chlorosis at 75% and 100% of CME treatment was observed in young leaves after 58 d of growth. The development of chlorosis in the leaves was comparatively delayed plants grown at low levels of treatment. Chlorosis intensified and turned to necrosis in successive days. Necrotic patches coalesced and changed to large necrotic areas later on. These symptoms some what resemble to that found by other workers (Turner and Rust 1971, Sharma et al. 1995) on tested plant species grown under various metal stresses. The coal mine effluent has an inhibitory effect on whole plant of *O. sativa* c.v. IR36, thereby plant height, leaf area, root weight, no of tillers plant^{-1}, dry biomass and seed production of tested species have been found reduced with increasing concentration of the effluent.

Table 2. Effect of different concentrations of coal mine effluent on growth parameter of rice (*Oryza sativa* cv. IR-36).

Conc. (%)	Plant height (cm)	Leaf area (cm^2)	Root weight (g $plant^{-1}$)	Dry biomass (g $plant^{-1}$)	Number of tillers $plant^{-1}$	Relative water content (%)	Seed weight (g $plant^{-1}$)
00	64.6^a ± 1.83	110.3^a ± 6.81	3.69^a ± 0.47	18.66^a ± 1.82	6.82^a ± 1.51	88.8^a ± 5.61	7.62^a ± 0.83
10	52.3^b ± 1.22	103.5^a ± 8.06	3.30^b ± 0.56	16.87^a ± 1.07	6.01^a ± 2.08	85.6^a ± 6.11	5.47^b ± 0.43
25	48.6^c ± 1.42	93.2^b ± 7.51	3.81^a ± 0.52	13.11^b ± 1.88	5.71^a ± 1.78	87.2^a ± 5.88	4.82^b ± 0.73
50	42.7^d ± 1.92	80.4^c ± 5.98	2.88^c ± 0.45	11.06^{bc} ± 1.66	4.92^{ab} ± 1.07	89.3^a ± 5.97	4.02^{bc} ± 0.23
75	40.2^e ± 2.06	72.7^c ± 6.69	2.07^d ± 0.52	10.61^{bc} ± 0.87	3.13^{ab} ± 1.52	72.6^b ± 4.09	2.87^{cd} ± 0.10
100	39.8^e ± 1.22	65.2^d ± 4.12	1.88^d ± 0.61	10.04^c ± 0.67	2.16^b ± 0.56	70.2^b ± 4.64	2.11^d ± 0.23

Values are mean ±SE (n=5), ANOVA, P <0.01; different superscripts denote significant (P> 0.05) difference between means according to Duncan's Multiple Range Test (DMRT).

Table 3. Effect of graded level of coal mine effluent on various parameters evaluated (Total chlorophyll, carbohydrate, protein, starch, phenol, protein nitrogen and non protein nitrogen).

Parameters	Concentration (%)					
	0.00	10	25	50	75	100
Total chlorophyll (mg g^{-1} fw)	2.267^a ± 0.21	1.962^a ± 0.21	1.326^b ± 0.12	0.928^{bc} ± 0.02	0.615^c ± 0.01	0.513^c ±0.015
Carbohydrate (mg g^{-1} fw)	62.40^a ± 3.62	55.70^{ab} ± 5.07	47.30^{bc} ± 7.06	42.50^{cd} ± 2.46	36.20^d ± 3.02	25.80^e ± 1.88
Protein (%)	1.830^a ± 0.28	1.65^a ± 0.26	1.27^{ab} ± 0.07	1.04^b ± 0.06	0.83^b ± 0.06	0.65^b ± 0.02
Starch (%)	3.500^a ± 0.44	3.00^{ab} ± 0.47	2.50^b ± 0.15	2.10^{bc} ± 0.18	1.50^c± 0.21	1.20^c ± 0.06
Phenols (%)	0.008^c± 0.001	0.009^c ± 0.001	0.01^{bc}± 0.002	0.013^b ± 0.002	0.017^a ± 0.001	0.019^a ± 0.002
Protein nitrogen (%)	0.184^b ± 0.02	0.198^{ab} ± 0.01	0.223^{ab} ± 0.01	0.265^{ab} ± 0.07	0.297^{ab} ± 0.01	0.325^a ± 0.021
Non protein nitrogen (%)	0.853^a ± 0.04	0.817^a ± 0.02	0.742^b ± 0.02	0.623^c ± 0.02	0.564^d ± 0.02	0.415^e ± .01

Values are mean ± SE (n=5), ANOVA, P <0.01; different superscripts denote significance (P> 0.05) difference between means according to Duncan's Multiple Range Test (DMRT).

The maximum inhibitions in growth parameters were observed in seed yield (72.31%) followed by number of tillers $plant^{-1}$ (68.32%), root weight (49.05%), dry biomass

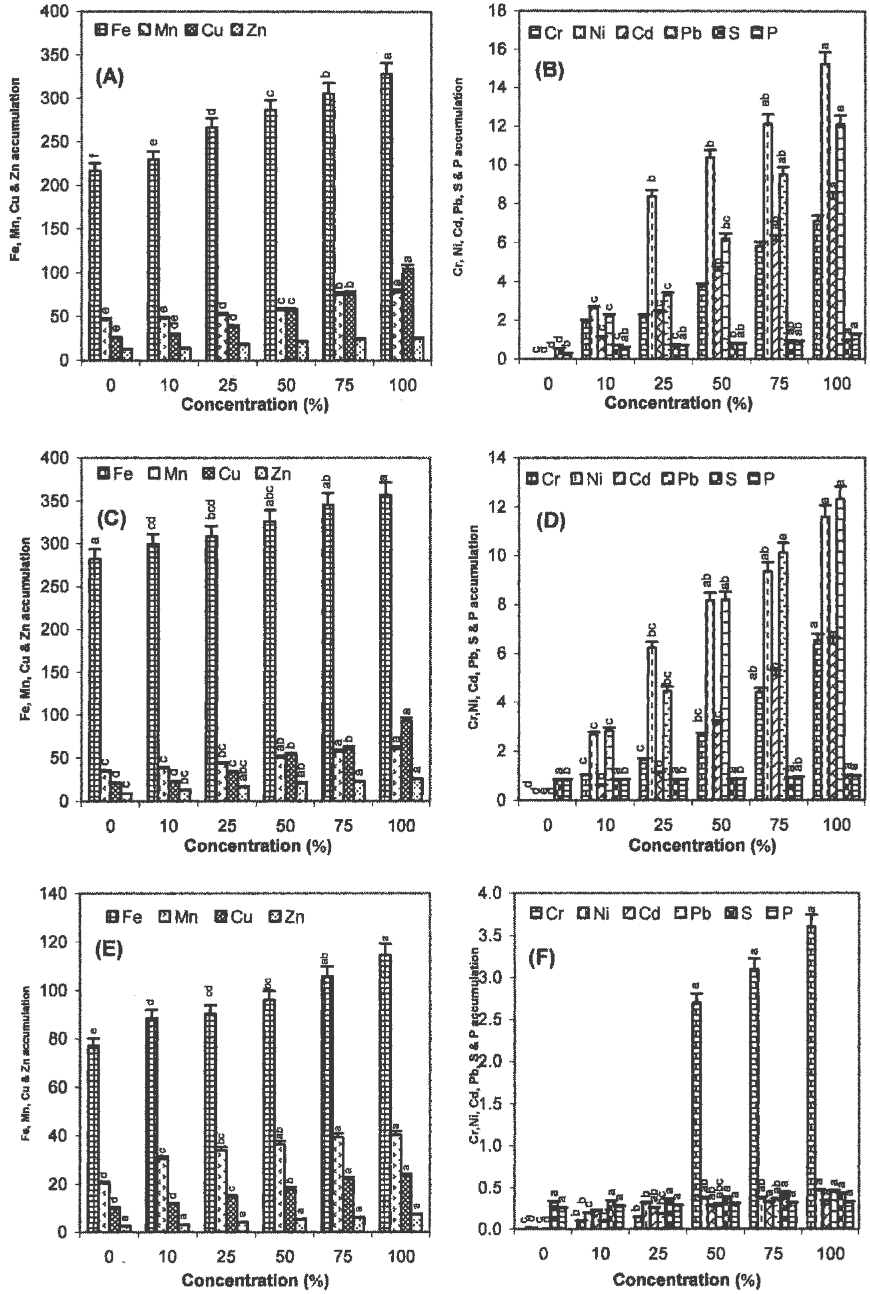

Figure 1. Effect of CME exposure and concentration of Fe, Mn, Cu, Zn, Cr, Ni, Cd, Pb ($\mu g\ g^{-1}$ dry matter), S and P (% in dry matter) in root (A-B), shoot (C-D) and seed (E-F) parts in rice plant (*Oryza sativa* cv. IR-36).Values are mean $\pm$ SE (n=5). ANOVA, $p<0.01$. Different superscripts on bars showed significant ($P>0.05$) difference between the means according to Duncan's Multiple Range Test (DMRT).

(46.19%), leaf area (40.88%) and relative water content (20.94%) at 100% effluent (Table 2). Foy et al. (1978) reported the adverse effect of heavy metals on plants as inhibition of root growth followed by stunting or dwarfing of shoot. Recently, Srivastava et al. (2005) reported the retarded growth of *Vicia faba* L. with reduced, root and shoot weight when plants were treated with effluent of Hindustan Aeronautics Limited (HAL), Lucknow, India.

Total chlorophyll content of rice plants *c.v.* IR-36 was found to be highly affected showing 77% decreases after 62 d at 100% exposure. Similarly, the carbohydrate, protein and starch content of the tested rice variety decreased drastically by 58.65%, 64.48% and 66%, respectively, at 100% effluent (Table 3). In contrast to these parameters, the phenols (137.5%) and protein nitrogen (76.63%) contents of the rice plants increased with increasing concentrations of the effluent, while non-protein nitrogen was found to be inhibited (51% at 100%) in a concentration dependent manner.

Results indicated a decline in the total chlorophyll content of the treated rice plant with increase in concentration of metals in plant tissue, presumably due to inhibition of chlorophyll biosynthesis under various metal stresses (Padmaja et al. 1996; Vajpayee et al. 2000). The degradation of protein with increased level of metals is probably due to adverse effects of reactive oxygen species (Davies 1987). Romero-Puertas et al. (2004) reported that higher concentration of Fe caused generation of ROS due to redox-active nature of Fe, as Fe has been detected more in the various parts (root, shoot, seeds) of rice cultivars. Oxidative modification of proteins by ROS may make them more prone to damage by proteases. The reduction in carbohydrate might be due to alteration of carbohydrate metabolism (Rauser 1978). In addition, the metal induced vein loading thus inhibiting photoassimilate export with a resultant carbohydrate accumulation (Rauser and Dumbroof 1981). The exposure of effluent resulted in low starch formation in paddy leaves, similarly, Tiwari (2004) reported reduction of starch under metal stress. Excess supply of effluent increased the content of protein nitrogen and decreased the non-protein nitrogen. The disruption of nitrogen metabolism in paddy plants induced by toxic elements present in effluent is in agreement with the earlier finding of Sharma et al. (1995).

Rice plants cv IR-36 treated with mine effluent accumulated various trace and toxic metals viz., S, P, Fe, Mn, Cu, Zn, Cr, Ni Cd and Pb from the coal mine drainage water in a concentration dependent manner (Fig. 1). Except Fe, all the trace elements were detected maximum in the root followed by shoot and seed. Maximum amount of Fe was detected in foliar part of plants. This may be due to efficient transport of Fe through chelation with organic acids, amino acids or phytoferritins (Pohlmeier 1999, Prasad, 1999). However, more localization of other metals in the root part of the plant may be considered as detoxification mechanism in root tissue. Thus, prohibiting the transport of metals to the edible part of the plants. Such differences in metal uptake and accumulation in rice cultivars and genotypes have been reported in earlier studies (Liu et al. 2003). The higher metal accumulation in the under ground tissue of rice plant appears significant from point of view of metal immobilization in the roots, thus prohibiting metal transport to the grains and its subsequent flow at higher trophic level in the food chain. Besides, the rice cultivar (IR-36) appeared to have rhizofiltation potential for heavy metals (Garbisu and Alkorta 2001).

The results clearly indicate that the mine drainage water is toxic in nature and may alter the biosynthesis of photosynthetic pigment, protein, carbohydrate and starch of rice plants. Thus, the use of mine water for irrigation of agricultural crops has considerable risk. Further, field demonstrations will be needed to assess the impact on rice crops because the metal accumulation depends on the various environmental factors such as pH, soil properties and metal concentration of mine effluent.

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