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Research Article

EFFECT OF BIOREMEDIATED LIGNITE MINE EFFLUENT AND SOIL ON GROWTH AND YIELD OF PADDY ADT-36

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ABSTRACT

A pot experiment was conducted to evaluate the effect of soil and lignite mine effluent, both were already treated with the bacterial consortium (*Bacillus subtilis* + *Pseudomonas stutzeri* + *Pseudomonas alcaligenes*), on growth and yield of paddy (ADT-36). Paddy cultivated on bioremediated soil irrigated with biologically treated effluent showed increased growth rate *viz*., plant height (67.2 cm), the number of tillers/hill (13.50) and total leaf area (60.02 cm^2/plant). Yield attributing values of paddy plant were also higher in treated soil irrigated with biologically treated effluent. Higher levels of chlorophyll (1.620 mg/g fr.wt)), starch (7.798mg/g fr.wt in root, 12.099 mg/g fr.wt in shoot) and amino acids (0.590 mg/g fr.wt,in root, 0.394 mg/g fr.wt.in shoot)content were also recorded. These values were lower in the paddy, cultivated in untreated soil irrigated with raw effluent.

INTRODUCTION

The mining industries are discharged millions of liters of wastewater every day to the adjacent watercourses. In India, gallons, and gallons of waste water discharged from various coal mines. India is an agricultural based country and a major user of water resource for irrigation (Singh *et al*., 2004). Due to the scarcity of good water for irrigation, the farmers have started to irrigate their agricultural crops by using industrial waste water (Rathore *et al*., 2000). Globally, around 20 million hectares land is reported to be irrigated with waste water and at least 10 % of the world's population is thought to consume foods produced by irrigation with waste water (WHO, 2006a & b; Hamilton *et al*., 2007).

The agricultural lands irrigated with coal mine waste water leads to the heavy metal contamination of the lands. The pollutants deposited in soil can easily reach human populations, particularly through the food chain. Continuous uses of industrial effluent also reduce the yield of crop plants. Microbial populations in polluted environments adapt to toxic concentrations of pollutants and become resistant (Viti and Giovannetti, 2001; Prasenjit and Sumathi, 2005).

Therefore, the present research work was undertaken to investigate the effect of bioremediated soil and effluent on the growth and yield of paddy.

MATERIALS AND METHODS

The lignite mine effluent and soil already treated with an inoculum containing bacterial consortium (*Bacillus subtilis* + *Pseudomonas stutzeri* + *Pseudomonas alcaligenes*) were used in this study.

Effect of bioremediated effluent and soil on the growth and yield of paddy (ADT-36)

A pot culture experiment was conducted at Pot culture yard, Department of Microbiology, Annamalai University to study the effect of bioremediated soil and effluent on the growth and yield of paddy var. ADT-36. Three sets of cement pots were filled with 25 kg of bioremediated soil, normal agricultural field soil (collected from the field near the lignite mine effluent irrigated soil) and untreated soil separately and irrigated with normal bore well water, raw untreated effluent and treated effluent. Seedlings of 25 days old Paddy var. ADT-36 were transplanted and 100% RDF were applied. The experiment was conducted with following treatments in a factorial RBD and with three replications.

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Treatment schedule

Main treatments
M_1 - Bioremediated soil
M_2 - Normal soil
M_3 - Untreated soil
Sub treatments
S_1 - Bore well water
S_2 - Treated effluent
S_3 - Untreated effluent

Biometric Observations on Growth Parameters (Mohana et al., 2011)

Plant height

Randomly selected 5 hills were used for the measurement of plant height 90^{th} day after transplanting (DAT). It was measured from the base to tip of the upper leaves of the main stem.

Number of tillers per hill

Tiller per hill was counted from the randomly selected sample of 5 hills after transplanting and mean was calculated. The main stem was also included in calculating the total tillers per plant.
Total leaf area (cm^2)

The leaf area was calculated by measuring the length and breadth of the leaf.
Leaf area (cm^2) = K× length× breath

Where, K= Kemp's constant (for monocot leaves) = 0.9

Yield Attributing Characters of Rice

A number of filled grains panicle^{-1} and Grain yield were assessed and recorded at the time of harvesting following standard procedures.

Number of filled grains panicle^{-1}(Yoshida et al., 1971)

The panicles were chosen randomly for recording the number of filled grains panicle^{-1}. The differentiation of well filled and chaffy grains was made by pressing the grains between forefinger and thumb. Average of filled grains from panicles from ten plants was taken.

Grain yield

After the harvest, the grains were separated, winnowed, dried in the sun and dry weight were recorded and expressed as g/pot.

Biochemical Constituents of Paddy (Baskaran et al., 2009)

Estimation of Chlorophyll

Five hundred mg of fresh leaf material was taken and ground with help of pestle and mortar with 10 mL of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved. The residue was re-extracted with 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645, 663 and 480 nm in the UV- spectrophotometer.

Chlorophyll 'a' (mg/ L) = (0.0127) × (O.D 663) - (0.00269) × (O.D 645)
Chlorophyll 'b' (mg/ L) = (0.0229) × (O.D 645) - (0.00468) × (O.D 663)
Total Chlorophyll (mg/ L) = (0.0202) × (O.D 645) + (0.00802) × (O.D 645)

Estimation of starch

Five hundred mg of material (root and shoot) was taken for the extraction of starch. The material was macerated in a pestle and mortar with 10 mL of 80 % ethanol. The residue was later dissolved in 6.5 mL of 52 percent perchloric acid for one hour. It was centrifuged and made up to 100 mL in a volume flask with distilled water. One mL of this solution was further diluted with 5 mL distilled water. To this, 10 mL freshly prepared anthrone reagent was added. The content was heated for 7 min at 100°C in a boiling water bath. The tube was then cooled rapidly, shaken well and then appeared color was read at 630 nm in a spectrophotometer.

Amino Acid

The amino acid content was determined by the method of Moore and Stein, (1948). A 0.5 g of plant sample (root and shoot) was homogenized in 10 mL of 80% ethanol. The homogenate was centrifuged for 10 min at 800 rpm. One mL of the extract was taken in the test tube to which 1 mL of 0.1 N of HCl was added to neutralize the sample. To this, one mL of ninhydrin reagent was added and heated for 20 minutes in a boiling water bath. Later, 5 mL of the diluent solution was added and heated again in a water bath for 10 minutes. The test tubes were cooled and read the absorbance at 570 nm in a UV-spectrophotometer.

Statistical Analysis

The statistical analysis of the experimental results was performed employing the computer software package 'IRRISTAT', version 90-1, developed by Department of Statistics, International Rice Research Institute, Philippines and as per the procedure of Gomez and Gomez (1976).

RESULTS AND DISCUSSION

Biometric Observation on Growth Parameters

The effect of bioremediated soil and effluent on the height of paddy plants was studied and the results are given in Table-1. The maximum plant height was recorded in the bioremediated soil using treated effluent (67.2 cm) followed by normal soil irrigated with treated effluent (67.0 cm). The plant height is reduced to a greater extent when grown under untreated soil and effluent.

Table 1 Effect of bioremediated lignite mine effluent and soil on plant height of paddy

S. No.	Sub Treatments	Plant height (cm)		
		Main treatments		
		Bioremediated soil	Normal soil	Untreated soil
1	Bore well water	66.9	66.5	48.2
2	Raw effluent	52.0	51.6	40.2
3	Treated effluent	67.2	67.0	48.5
	SEd	0.51	0.42	0.55
	CD (P = 0.05)	1.10	0.86	1.02

The number of tillers varied from 7.0 to 13.50 tillers per hill. The maximum number of tillers was recorded in the bioremediated soil irrigated with treated effluent (13.50 tillers/hill) and the results were shown in the Table-2.

Similar observations were made on black gram (Ravimycin and Lakshmanachary, 1993) and bhendi crops treated with bioremediated sugar mill effluent (Rathinaswamy and Lakshmi Narashimhan, 1998). Furthermore, the enhanced growth of plants might be due to the presence of nitrates and phosphates along with other nutrients in favourable concentration in the bioremediated effluent and soil (Rajannan and Oblisami, 1999; Mishra, 1987).

Table 2 Effect of bioremediated lignite mine effluent and soil on number of tillers/hill of paddy

S. No.	Sub Treatments	Number of tiller/hill — Main treatments: Bioremediated soil	Normal soil	Untreated soil
1	Bore well water	13.45	13.20	8.65
2	Raw effluent	9.28	9.05	7.0
5	Treated effluent	13.50	13.35	8.90
	SEd	0.06	0.12	0.21
	CD (P = 0.05)	0.13	0.25	0.39

The total leaf area of paddy plants was measured and given in Table-3. The total leaf area showed an increasing trend in plants grown on bioremediated soil irrigating with bioremediated effluent. The similar trend was observed in rice treated with bioremediated paper mill effluent (Dutta and Biossya, 1998) and in *Hodeum vulgare* treated with bioremediated sugar mill effluent (Kumar, 2000). Tiwari *et al.* (2006) found that the leaf area was affected by the coal mine effluent without any treatment. Jolly *et al.* (2012) found that the raw dying industry effluent reduced the total leaf area in wheat plant.

Table 3 Effect of bioremediated lignite mine effluent and soil on total leaf area index of paddy

S. No.	Sub Treatments	Total leaf area (cm^2/plant) — Main treatments: Bioremediated soil	Normal soil	Untreated soil
1	Bore well water	59.61	59.15	47.65
2	Raw effluent	50.30	49.70	40.20
5	Treated effluent	60.02	59.75	47.95
	SEd	0.56	0.71	0.45
	CD (P = 0.05)	1.08	1.50	0.98

Yield Attributing Characters of Rice

After harvesting the total number of grain was counted and presented in Table-4. The treatment of bioremediated soil irrigated with bacterially treated effluent recorded the highest number of grain (87.76 grains/panicle).

Table 4 Effect of bioremediated lignite mine effluent and soil on number of grain/panicle of paddy

S. No.	Sub Treatments	No. of grain/panicle — Main treatments: Bioremediated soil	Normal soil	Untreated soil
1	Bore well water	87.45	87.10	66.50
2	Raw effluent	69.0	68.78	50.95
5	Treated effluent	87.76	87.35	67.0
	SEd	0.62	0.30	0.53
	CD (P = 0.05)	1.24	0.63	1.12

In the present experiment, different yield attributes were showed substantial increases when we used bioremediated soil and bioremediated effluent for the cultivation of paddy.

Similar results have also been reported in rice irrigated with bioremediated industrial effluents (Dutta and Biossya, 1998; Rathore *et al*., 2000).

After harvesting the data regarded the grain weight was taken and presented in Table-5. The maximum grain weight was recorded in bioremediated soil with treated effluent (227.52 g/pot), whereas the untreated soil with raw effluent showed minimum grain weight (140.20 g/pot).

Table 5 Effect of bioremediated lignite mine effluent and soil on grain weight of paddy

S. No.	Sub Treatments	Grain weight (g/pot) — Main treatments: Bioremediated soil	Normal soil	Untreated soil
1	Bore well water	225.46	217.39	164.25
2	Raw effluent	176.37	172.10	140.20
5	Treated effluent	227.52	223.60	165.55
	SEd	2.26	3.32	2.13
	CD (P = 0.05)	4.62	6.41	4.20

The bioremediation of the effluent brought down the toxic effects of the effluent and acted as liquid fertilizer, which could be attributed as the reason for the increased growth and yield of the crop (Rani and Srivastava, 1990). The reduction in the yield of paddy irrigated with raw effluent might be due to the excessive amount of nitrogen, chlorides and bicarbonates in the effluent and polluted soil which were reported to be the retardants of plant growth and yield at high concentration (Rajannan and Oblisami, 1999). In all the treatments, the values recorded in normal soil were slightly decreased when compared with bioremediated soil. Several researchers reported that untreated industrial effluents found to be inhibited the growth and yield of several crops and other plants (Kaushik *et al.,* 2005; Soundarrajan and Pitchai, 2007; Muthalagi and Mala, 2007; Kalaiselvi *et al.,* 2009; Alghobar and Suresha, 2016).

Biochemical Constituents of Paddy

The Table-6 shows the biochemical analysis of paddy that was grown on bioremediated soil, normal soil and untreated soil using bioremediated effluent, raw effluent and bore well water with recommended dose of fertilizer. Compared with different treatment, the bioremediated soil irrigated with biologically treated effluent stood out with total chlorophyll of 1.620 mg/g fr. wt. followed by 1.610 mg/g fr. wt. (bioremediated soil irrigated with bore well water), whereas the untreated soil irrigated with raw effluent showed minimum value (0.875 mg/g fr. wt).

The chlorophyll content was positively correlated with net photosynthetic rate, and hence it plays a major role in controlling growth and grain filling process. In the present research, the total chlorophyll content was declined in the paddy grown on untreated soil irrigated with raw effluent. A similar result was found by Tiwari *et al.* (2006). The decline in chlorophyll content, presumably due to inhibition of chlorophyll biosynthesis under various metals stresses (Padmaja *et al.,* 1996; Vajpayee *et al.,* 2000). Pandey and Rao

(1998) and Banerjee and Pandey (2002) have also reported the reduction in chlorophyll content as a result of pollution.

The pigment content showed an increasing trend in paddy grown with bioremediated effluent on bioremediated soil. The same trend was observed in wheat (Kaushik *et al*., 1994), rice (Dutta and Biossya, 1998), groundnut and paddy (Thamizhiniyan *et al*., 2000) in response to the treatment of bioremediated effluent

deposition of pollutants from lignite fired thermal power plants. The bioremediated soil induces the growth and yield of crop plant. Increase growth and yield of crop plants observed by many researchers (Ajithkumar *et al.* 1998; Benimeli *et al.* 2008)

Table 6 Effect of bioremediated lignite mine effluent and soil on biochemical constituents of paddy

S. No	Treatments	Bioremediated soil					Normal soil					Untreated soil				
		Total Chlorophyll (mg/g fr.wt)	Starch (mg/g fr. wt)		Amino acid (mg/g fr. wt)		Total Chlorophyll (mg/g fr. wt)	Starch (mg/g fr. wt)		Amino acid (mg/g Fr.wt)		Total Chlorophyll (mg/g fr.wt)	Starch (mg/g fr. wt)		Amino acid (mg/g fr. wt)	
			Root	Shoot	Root	Shoot		Root	Shoot	Root	Shoot		Root	Shoot	Root	Shoot
1	Bore well water	1.610	7.684	11.998	0.588	0.389	1.587	7.593	11.875	0.575	0.380	1.028	4.580	9.025	0.420	0.280
2	Raw effluent	1.286	5.876	10.081	0.398	0.243	1.205	5.745	9.975	0.383	0.236	0.875	3.656	8.015	0.280	0.195
3	Treated effluent	1.620	7.798	12.099	0.590	0.394	1.608	7.695	11.912	0.583	0.390	1.075	4.600	9.160	0.437	0.292
	SEd	0.02	0.08	0.09	0.03	0.06	0.12	0.10	0.04	0.05	0.02	0.06	0.022	0.10	0.02	0.015
	CD (P = 0.05)	0.04	0.19	0.18	0.06	0.11	0.23	0.19	0.08	0.11	0.05	0.13	0.041	0.22	0.04	0.032

Reduction in pigment content of paddy grown in raw effluent might be due to the presence of the excessive amount of nitrogen and chlorides which increased the osmotic pressure that reduced the uptake of magnesium, potassium and other mineral ions and resulted in inhibition of pigment synthesis (Kadioglu and Algur, 2004).

Starch content in root and shoot were also high in the treatment of bioremediated soil irrigated with bacterially treated effluent (7.798 mg/g fr. wt for root and 12.099 mg/g fr. wt for shoot), while in untreated soil irrigated with raw effluent it was found to be 3.656 mg/g fr. wt and 8.015 mg/g fr. wt for root and shoot respectively.

The starch content in root and shoot of paddy grown on bioremediated soil irrigated with bioremediated effluent was more than other treatments. The same trend was also observed (Vaidheswaran and Swaminathan, 1999; Srivastava Neeta *et al*., 1999). The increase in starch content could be attributed to the increased number of leaves, total leaf area and chlorophyll content of the plant.

In the treatment of bioremediated soil irrigated with treated effluent, the amino acid content in root and shoot were also recorded to be high with 0.590 mg/g fr. wt and 0.394 mg/g fr. wt followed by the bioremediated soil irrigated with bore well water (0.588 mg/g fr. wt and 0.389 mg/g fr. wt), while 0.280 mg/g fr. wt and 0.195 mg/g fr. wt were recorded for root and shoot respectively in untreated soil irrigated with raw effluent.

The higher amount of amino acid content showed in the paddy grown on *bioremediated* soil using bioremediated effluent and decreased in paddy grown on untreated soil irrigated with raw effluent. Similar observations were also recorded in paddy and groundnut, treated with sugar mill effluent (Thamizhiniyan *et al*., 2000), *Vigna amguiculata* treated with fertilizer factory effluent (Subramani *et al*., 2002) and *Cyamopsis tetregonoloba* treated with distillery effluent (Taghavi and Vora, 2002).

In overall, the bioremediated soil showed better results than the normal soil (unpolluted soil) in all the treatments. It might be due to the contamination of normal soil (collected near to the lignite mine effluent irrigated soil) from the atmospheric

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