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Original Research Article

Changes in Enzyme Activities of Rice Seedlings Exposed to Mercury Contaminated Waste of a Chlor-Alkali Factory

D.R. Nanda[1] and B.B. Mishra[2*]

[1]Department of Botany, Rajsunakhala College, Rajsunakhala, Nayagarh, Odisha, India
[2]Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

**Corresponding author.*

Abstract

In view of concern over industrial contamination of agricultural land with various pollutants including toxic metals, an attempt was made to study effect of mercury contaminated waste soil of industrial origin on enzymatic activities of rice seedlings. The gray coloured waste from the chlor-alkali factory was alkaline in nature with a pH of 9.20 ± 0.05 and analysed to contain 0.95 mg of mercury, 6.0 g of sodium, 18 g of chloride, 55.0 g of potassium, 60.0 mg of Phosphate per Kg of soil. On transplantation of 26 days rice seedlings it was found that there was a significant change in the enzymatic activities like catalase and peroxidase of the seedlings. The changes in the enzymes were also statistically significant.

Keywords

Catalase
Mercury
Perioxidase
Rice
Waste soil

Introduction

Environmental pollution through industrial discharges has drawn a worldwide attention due to its damaging effect on living organisms. It has been established that living organisms accumulate pollutants including toxic metals like mercury when exposed to contaminated site (Mishra, 1986) that can cause damaging effect. Nanda et al. (1993) reported accumulation of an appreciable amount of mercury by rice seedlings, exposed to mercury contaminated waste soil from a chlor-alkali factory. Waste soil from the factory in varying concentrations with garden soil also decreased shoot length and root length of the plant. It is already an established fact that disturbances in growth and growth substances are always related with metabolic changes inside the plant (Levitt, 1972). Tyler (1974) reported a decrease in soil enzymatic level, following heavy metal contamination of industrial origin. Rosko and Joseph (1977) studied effect of heavy metal along with mercury on *Chlorella*. A decrease in growth and chlorophyll content was observed in the alga exposed to the heavy metals (Shaw et al., 1989) with a decrease in microbial activity. Mercury interacts with the proteins and enzyme decreasing their synthesis Augier et al. (1977) reported a change in nucleic acids, protein and free amino acids content of marine Phanerogames from polluted water. Mercury or mercurial compounds also affect nucleic acid content of plants (Ahmad

et al., 1977). Takeuchi and Maeda (1976) reported an interaction between fluoroscein mercuric acetate with nucleic acid which results in denaturation of DNA. A concentration of mercury and time period of exposure dependent decrease in protein, DNA, RNA and free amino acid content of algae, has been reported by Mishra et al., 1986. Mishra (1986) and Nanda (1984) reported a decrease in cellular biochemical component of plant following its exposure to varying combinations waste soil extract from a chlor-alkali factory. Apart from mercury, salinized media also cause depletion in nucleic acids, protein metabolism, pigment content and enzyme activity (Prisco and Vieria, 1976; Sheron and Garg, 1979).

The waste soil of a chlor-alkali factory under study is loaded with elements that are harmful to living system. Along with mercury it contains an appreciable amount of Sodium and chloride that may be harmful to cellular biochemical components which also contaminate the local rice field. In view of this, an attempt has been taken in the present investigation to evaluate damaging effect of the heterogeneous waste soil combinations on oxidative enzymes like catalase and peroxidase of rice plants exposed to varying concentrations of the waste soil combinations.

Materials and methods

Mercury contaminated waste soil from a chlor-alkali factory was collected in a gunny bag, air dried and powdered. The waste was alkaline in nature with a pH of 9.20 ± 0.05 and analysed to contain 0.95 mg of mercury, 6.0 g of Sodium, 18 g of Chloride, 55.0 g of Potassium, 60.0 mg of Phosphate per Kg of soil. Different combinations of the solid waste (2.5 to 17.5%) at an interval of 2.50% was made with normal garden soil with final volume 4.0 Kg and watered at a ratio of soil: water=1:2. To each pot 2g of urea and super phosphate were added as basal fertilizer and 26 days old rice seedlings were transplanted. Catalase and peroxidase content of the rice seedlings were measured on 15th day to 75 days at an interval of 15 days by adopting standard procedure.

Estimation of Catalase

Catalase was assayed by modified method of Braber (1980). To a solution of 2 m 0.005 M H_2O_2 and 3 ml 0.1 M sodium phosphate buffer of pH 7.0, 1 ml of the enzyme extract was added. After incubation for 1 minute at 20^{0}C the reaction was stopped by adding 10 ml of 0.5M H_2SO_4. The residual H_2O_2 was then titrated against 0.002 M $KMnO_4$. A control set was run at the same time in which the enzyme activity was stopped at zero time. The activity of catalase was expressed as µ mol H_2O_2 utilized g^{-1} fresh weight per minute.

Estimation of Peroxidase

Peroxidase activity was assayed following the method of Kar and Misra (1976) with the following modifications. The assay mixture of peroxidase contained 2 ml of 0.1 M sodium phosphate buffer at pH 7.0, 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 1 ml of suitably diluted enzyme extract. The assay mixture was incubated at 25°C for 5 minutes and immediately after that the reaction was stopped by adding 1 ml of 2.50 M H_2SO_4 to the reaction mixture, the amount of purpurogallin formed was estimated by measuring the absorbency at 420 nm and the absorbency unit was expressed for enzyme activity. The findings were subjected to statistical analysis with standard methods.

Results and discussion

Catalase

Table 1 depicts changes in catalase activity of shoot of rice seedlings. Catalase activity of rice decreased with increase in waste soil combinations and time period.

Fifteen days after transplantation of the rice seedlings catalase activity decreased from 780 + 12.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in control to 520 ± 5.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in 17.5 percent waste soil combinations. Percentage decrease over control increased from 7.05 in 2.5% to 33.33 in 17.5% waste soil combinations respectively. The negative correlations (r= -0.996 and -0.991) of catalase activity with waste soil combinations and mercury uptake by shoot respectively were highly significant (p<0.001) (Table 2). A significant (p<0.001) positive correlations (r=+0.990) was also obtained between catalase activity and total chlorophyll content (Table 2). Thirty days after transplantation of the

rice seedlings, catalase activity decreased from 895±18.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} 554± 4.0 mole of H_2O_2 utilized min^{-1} g^{-1} in 17.5% waste soil combinations.

Table.1 Changes in catalase activity (µ mole of H_2O_2 utilized min^{-1} g^{-1}) at different time intervals following its transplantation at varying waste soil combinations

Days	% Solid waste							
	0	2.50	5.00	7.50	10.00	12.50	15.00	17.50
15	780 ±12.0	725 ±14.0 (-7.05)	691 ±8.0 (-11.41)	666 ±6.0 (-14.62)	640 ±5.0 (-17.95)	598 ±7.0 (-23.33)	557 ±10.0 (-28.95)	520 ±5.0 (-33.33)
30	895 ± 18.0	788 ±6.0 (-11.96)	750 ±5.0 (-16.20)	731 ± 7.0 (-18.32)	710 ±4.0 (-20.67)	655 ±8.0 (-26.81)	582 ± 6.0 (-34.97)	554 ±4.0 (-38.10)
45	971 ±20.0	815 ±10.0 (-16.07)	777 ± 9.0 (-19.98)	737 ±5.0 (-24.10)	699 ±5.0 (-28.01)	670 ±6.0 (-31.00)	602 ±9.0 (-38.00)	554 ±8.0 (-42.95)
60	1086 ±25.0	858 ±12.0 (-21.00)	836 ±11.0 (-23.02)	782 ±14.0 (-27.94)	728 ± 9.0 (-32.97)	684 ±10.0 (-37.02)	640 ±7.0 (-41.07)	564 ±8.0 (-48.07)
75	1225 ±21.0	899 ±16.0 (-26.61)	838 ±12.0 (-31.59)	796 ±10.0 (-35.02)	735 ±9.0 (-40.09)	674 ±11.0 (-44.98)	624 ±8.0 (-49.06)	563 ±7.0 (-54.04)

Values are mean of three samples; ± indicates standard deviation; Figures in parentheses indicate percentage change.

Table.2 Bivariate correlation coefficient values for catalase activity and peroxidase activity

Days	Variables		Correlation values (r)	Significance level (p<)	Degrees of freedom
15	Catalase	a.	0.990	0.001	7
		b.	-0.996	0.001	7
		c.	-0.991	0.001	6
	Peroxidase	a.	-0.989	0.001	7
30	Catalase	a.	0.981	0.001	7
		b.	-0.979	0.001	7
		c.	-0.986	0.001	6
	Peroxidase	a.	-0.955	0.001	7
45	Catalase	a.	0.982	0.001	7
		b.	-0.969	0.001	7
		c.	-0.994	0.001	6
	Peroxidase	a.	-0.983	0.001	7
60	Catalase	a.	0.983	0.001	7
		b.	-0.954	0.001	7
		c.	-0.997	0.001	6
	Peroxidase	a.	-0.969	0.001	7
75	Catalase	a.	0.990	0.001	7
		b.	-0.926	0.001	7
		c.	-0.999	0.001	6
	Peroxidase	a.	-0.969	0.001	7

a. Versus total chlorophyll content; b. Versus percentage waste soil ; c. Versus mercury uptake.

Percentage decrease over control increased from 11.96 in 2.5% to 38.10 in 17.5% waste soil combinations. Highly significant (p<0.001) negative correlations were obtained for catalase activity with waste soil combinations and mercury uptake by shoot (r=-0.979 and -0.986). A highly significant (p<0.001) positive correlation was also obtained for between catalase activity and total chlorophyll content (r=+0.981). Forty five days after transplantation of the rice seedlings, catalase activity decreased from 971±20.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in the control set to 564 ± 8.0 µ

mole of H_2O_2 utilized min^{-1} g^{-1} in 17.5% waste soil combinations. Percentage decrease over control increased from 16.07 in 2.5% and 42.95 in 17.5% waste soil combinations. Highly significant (p<0.001) negative correlations (r=-0.969 and -0.994) were obtained for catalase activity with waste soil combinations and mercury uptake by shoot. A positive correlation (r=+0.982, p<0.001) was also obtained between total chlorophyll content and catalase activity. Again in 60 days after transplantation, catalase activity decreased from 1086±25.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in the control set to 564±8.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in 17.5% waste soil combination. Percentage decrease over control increased from 21.0 in 2.5% to 48.07 in 17.5% waste soil combinations. The negative correlations (r= -0.954 and -0.997) for catalase activity with waste soil combinations and mercury uptake by shoot were highly significant (p<0.001). The positive correlation (r= +0.983) between total chlorophyll content and catalase activity was highly significant (p<0.001).

Again 75 days after transplantation of the rice seedlings, catalase activity decreased from 1225 ± 21.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in the control to 563±7.0 µ mole of H_2O_2 utilized min^{-1} g^{-1} in 17.5% waste soil combinations. The negative correlations (r=-0.926 and -0.999) for catalase activity with waste soil combinations and mercury uptake by shoot respectively were highly significant (p<0.001). A positive correlation (r= +0.990, p<0.001) was also obtained between total chlorophyll and catalase activity of rice seedlings. The catalase activity in varying waste soil combinations were also significantly correlated with days (Table 3). The analysis of variance for catalase activity was found to be statistically significant (Table 4).

Peroxidase

Peroxidase activity of the shoot of the rice seedlings also changed with percentage of waste soil and time period. However, in contrast to catalase activity, with increase in waste soil combinations and time an increase in peroxidase activity was reported (Table 5). After 15 days of transplantation of the rice seedlings, peroxidase activity measured in OD at 420 nm min^{-1} 200 mg^{-1} increased from 0.18 in the control set to 0.225 in 17.5% waste soil combinations. Percentage increase over control increased from 5.56 in 2.5% to 25.0 in 17.5% waste soil. A negative correlation was obtained between peroxidase activity and total chlorophyll content (r= -0.989, p<0.001) (Table 2). Percentage increase over control ranged between 7.14 in 2.5% and 33 in 17.5% waste soil combinations. The correlations (r= -0.955) of total chlorophyll content and peroxidase activity were negatively correlated and was significant at p<0.001 significance level.

Table.3 Bivariate correlation coefficient values for changes in catalase and peroxidase activity in varying waste soil combinations with days after transplantation

% Waste soil	Variables	Correlations value (r)	Significance level (p<)	Degrees of freedom
Control	Catalase	+0.995	0.001	4
	Peroxidase	+0.996	0.001	4
2.50	Catalase	+0.993	0.001	4
	Peroxidase	+0.991	0.001	4
3.00	Catalase	+0.971	0.001	4
	Peroxidase	+0.988	0.001	4
7.50	Catalase	+0.963	0.001	4
	Peroxidase	+0.991	0.001	4
10.00	Catalase	+0.873	0.05	4
	Peroxidase	+0.990	0.001	4
12.50	Catalase	+0.838	0.05	4
	Peroxidase	+0.992	0.001	4
15.00	Catalase	+0.921	0.01	4
	Peroxidase	+0.994	0.001	4
17.50	Catalase	+0.845	0.05	4
	Peroxidase	+0.995	0.001	4

Table.4 Analysis of variance ('F' test) for changes in catalase activity

Source of variation	SOS	Degrees of freedom	Mean square	Calculation of 'F' value	Significance level ($p<$)
Between % Solid waste	666527	7	95218.142	37.46	0.001
Between days	106107	4	26526.75	10.436	0.001
Residual value	71169	28	2541.75		

Table.5 Change in peroxidase activity (OD at 420 nm min^{-1} 200 mg^{-1}) following transplantation of the rice seedlings at varying waste soil combinations for different time periods

Days	% Solid waste							
	0	2.50	5.00	7.50	10.00	12.50	15.00	17.50
15	0.180	0.190 (5.56)	0.195 (8.33)	0.200 (11.11)	0.205 (13.89)	0.210 (16.66)	0.215 (19.44)	0.225 (25.00)
30	0.210	0.225 (7.14)	0.230 (9.52)	0.235 (11.90)	0.245 (16.66)	0.250 (19.04)	0.265 (26.19)	0.280 (33.33)
45	0.260	0.290 (11.54)	0.300 (15.39)	0.315 (21.15)	0.320 (23.08)	0.335 (28.85)	0.350 (34.62)	0.360 (38.46)
60	0.310	0.360 (16.20)	0.370 (19.36)	0.390 (25.81)	0.400 (29.03)	0.420 (35.48)	0.430 (38.741)	0.450 (45.16)
75	0.360	0.440 (22.22)	0.465 (29.17)	0.480 (33.33)	0.500 (38.89)	0.510 (41.67)	0.530 (47.22)	0.550 (52.78)

Again after 45 days of transplantation, peroxidase activity decreased from 0.26 OD at 420 mn min^{-1} 200 mg^{-1} in the control set to 0.36 OD at 420 mn min^{-1} 200 mg^{-1} in 17.5% waste soil combinations. Percentage increase over control increased from 11.54 in 2.5% to 38.46 in 17.5% waste soil combinations. The correlations of total chlorophyll content and peroxidase activity were negative (r= -0.971) and significant at $p<0.001$ significance level. Sixty days after transplantation of the rice seedlings, peroxidase activity decreased from 0.31 OD at 420 nm min^{-1} 200 mg^{-1} in the control set to 0.45 OD at 420 nm min^{-1} 200 mg^{-1} in 17.5% waste soil combinations. Percentage increase over control increased from 16.20 in 2.5% to 45.16 in 17.5% waste soil combinations. A highly significant ($p<0.001$) negative correlation (r= -0.969) was obtained between peroxidase activity and loss in total chlorophyll content. Further in 75 days after transplantation of rice seedlings peroxidase activity increased from 0.36 OD at 420 mn min^{-1} 200 mg^{-1} in the control set to 0.55 OD at 420 mn min^{-1} 200 mg^{-1} in 17.5% waste soil combinations. Percentage increase over control increased from 22.22 in 2.5% to 52.78 in 17.5% waste soil combinations. The correlation between total chlorophyll content was negative (r= -0.969) and highly significant ($p<0.001$). The changes in peroxidase content were also significantly correlated with 75 days after transplantation (Table 3).

Discussion

The experimental results presented reveal a decrease in catalase activity of rice seedlings following its culture in varying waste soil combinations. It was further enhanced with increase in time. As reports on change in catalase activity affected by industrial wastes or heavy metals are rare, attempts have been taken to discuss change in catalase activity in the light of other information available. Du and Fang (1983) reported a decrease in catalase activity of C_3 and C_4 carbon pathways plant following mercury vapour uptake. The decrease was 3 times higher in C_3 carbon pathways and positively correlated with mercury uptake.

Jana and Chaudhury (1982) reported in senescence in submerged aquatic angiosperm induced by heavy metal. Senescence takes place following decrease in catalase activity. Misra (1984) reported a decrease in catalase activity of rice seedlings cultured in solid waste extract from a chlor-alkali factory, which was dependent on concentration of the extract. A time period and age dependent decrease in catalase activity in plants

have been reported by various authors (Reddy et al., 1985; Kumar and Khan, 1982). In view of lack of a related reference, it is pertinent to suppose at the moment that with exposure of the rice seedlings to various waste soil combinations, a decrease in catalase activity is evident. The decrease, increase over time and the negative correlations of reduced catalase activity with waste soil combinations and mercury uptake, indicate effect of the waste soil in general and mercury in particular on catalase activity.

Similarly, peroxidase activity changed with time and waste soil combinations. But contrast to catalase activity, peroxidase activity increased with increase in waste soil and time period. A similar increase in peroxidase activity has been reported (Reddy et al., 1985; Kumar and Khan, 1982). Misra (1984) reported an increase in peroxidase activity of rice seedlings cultured in saturated waste soil extract from a chlor-alkali factory. High salinity of the heterogeneous extract was responsible for changes in peroxidase activity of the rice seedlings. Sheron and Garg (1979) reported a salinity induced increase in peroxidase activity of mung bean embryo which also increased with increase in time. Age dependent increase in peroxidase activity in virus infected papaya leaves have been studied by Kar et al. (1985). Although references available at present are not adequate to discuss changes in peroxidase activity in the present experiment, similar to the references cited above, an increase in peroxidase activity is reported which may be due to various pollutants accumulated by the rice seedlings from the heterogeneous mixture.

It is evident from the finding that the solid waste of the chlor-alkali factory is toxic to living forms and under contamination to nearby rice field can cause damage to crops.

References

Ahmed, O.F.E., Hart, R.W., Lewis, U.J., 1977. Pesticides induced DNA damage and its repair in human culture cell. Mut. Res. 42, 161-166.

Augier,M., Santimore, M., Vincentellie, M., 1977. Effect of pollution from waste water on the total nitrogen, protein and amino acid composition of *Cymodocea nodosa*. Environ. Pollut. 13(3), 217-228.

Barber, J.M., 1980. Catalase and peroxidase in primary bean leaves during development and senescence. Z. pflazen. Physiol. 97, 135-144.

Du, S.H., Fang, S.C., 1983. Catalase activity of 3-carbon and 4-carbon pathway species and its relationship to mercury vapour uptake. Environ. Exp. Bot. 23(4), 347-354.

Jana, S., Choudhury, M.A., 1982. Senescence in the submersed aquatic angiosperms: effect of heavy metals. New Phytol. 90(3), 477-484.

Kar, M., Misra, D., 19760. Catalase, peroxidase and polyphenol oxidase activity during rice leaf senescence. Plant Physiol. 57, 315-319.

Kar, R.K., Nanda, H.P., Kabi, T., 1985. Activity of catalase and peroxidase during natural and virus induced papaya leaf ageing. Indian J. Plant Physiol. XXVIII (2), 124-134.

Kumar, K.B., Khan, P.A., 19820. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* Cr. PR 202) leaves during senescence. Ind. J. Expt. Biol. 20(4), 412-416.

Levitt, J., 1972. Response of plants to environmental stress. Academic Press, NY.

Misra, S.R., 1984. Analysis of the effect of solid waste extract of a caustic chlorine factory on the growth and physiology of rice seedlings. Ph.D. thesis, Berhampur University, Berhampur, Orissa, India.

Mishra, B.B. (1986). Role of blue-green algae in biodegradation of a chlor-alkali industrial waste (soil) with special reference to rice cultivation. Ph.D. thesis, Berhampur University, Berhampur, Ganjam, Orissa.

Mishra, B.B., Nanda, D.R., Misra, B.N., 1986. Reclamation with blue-green algae: Changes in amino acid content of algae exposed to solid waste of a chlor-alkali factory. Microbias Lett. 33, 139-142.

Nanda, D.R., Mishra, B.B., Misra, B.N., 1993. Effect of solid waste from a chlor-alkali factory in rice plant, mercury accumulation and changes in biochemical variables. Int. J. Environ. Stud. 45, 23-28.

Nanda, D.R., 1984. Effect of saturated waste extract of a chlor-alkali factory on mung bean, *Phaseolus aureus* Roxb. M.Phil. Thesis, Berhampur University, Berhampur, Orissa India.

Prisco, J.T., Vieira, G.H.F., 1976. Effect of NaCl salinity on nitrogenus compounds and proteases during germination of *Vigna sinenis* seeds. Physiol. Plant. 36, 317-320.

Reddy, K.P., Kumar, K.B., Subhani, S.M., Khan, P.A., 1985. Effect of light and benzyladenine on dark treated growing rice leaves (*Oryza sativa*): I. Changes in chlorophyll content and catalase activity. Physiol. Plant. 63, 79-86.

Rosko, J.J., Joshep, W.R., 1977. The effect of cadmium, copper, mercury, zinc and lead on cell division, growth and chlorophyll 'a' content of chlorophyte, *Chlorella vulgaris*. Bull. Torr. Bot. Club 104(3), 226-233.

Shaw, B.P., Sahu, A., Panigrahi, A.K., 1989. Mercury in bed sediment of the Rushikulya river estuary. J. Environ. Biol. 10(1), 59-64.

Sheron, I.S., Garg, O.P., 1978. Effect of salinity on the activities of RNAse, DNAse and protease during germination and early seedling growth of mung bean. Physiol. Plant. 44, 171-174.

Takeuchi, S., Maeda, A., 1976. Interaction of fluorescent reagent fluroescein mercuric acetate with nucleic acids. Biochem. Biophys. Acta 454(2), 309-318.

Tyler, G., 1974. Heavy metal pollution and soil enzymatic activity. Plant Soil 41, 303-311.