Evaluation of Crop Resistance to Aqueous Oil Pollution

K. ILANGOVAN AND M. VIVEKANANDAN*

Department of Botany, Bharathidasan University, Tiruchirapalli 620 024, India

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ABSTRACT

Root and shoot tolerance indices and photosynthesis characteristics (CO_2-dependent-O_2 evolution; $H_2O \rightarrow MV$ electron transport; whole-leaf photosynthesis by infrared gas analyzer) indicated that ADT-36 and CR-1009 rice varieties were more tolerant than other varieties (IR-50, IR-20, and Ponni). *Vigna* varieties (T-9 and Krishna) were most susceptible to aqueous oil pollution. Since the oil-polluted soil contained a potentially toxic concentration of cadmium, tolerant (ADT-36) and susceptible (IR-50) varieties of *Oryza* were further analyzed for cadmium-binding components. Higher accumulations of cadmium were found in the roots than in the leaves of both rice varieties. Cadmium is associated with proteins of about 50,000- and 33,000-dalton in molecular mass, of which the 33-kdalton protein was significantly induced only in ADT-36. It is suggested by the present study that the ADT-36 rice variety is most tolerant to aqueous oil pollution, as evidenced by higher STI (shoot tolerance index) and RTI (root tolerance index) values, induction of Cd-binding proteins, and comparatively normal photosynthesis rates.

Index Entries: *Oryza sativa; Vigna mungo;* metallothionein-like proteins; detoxification; oil pollution; cadmium.

INTRODUCTION

In a developing country like India, which is rapidly becoming industrialized and urbanized, the problems of water pollution pose a constant

*Author to whom all correspondence and reprint requests should be addressed.

and serious threat to the ecological balance. Toxic levels of metals and hydrocarbons in soil may be caused by manufacturing, mining, and waste-disposal practices (*1–3*). Disposal of oily wastes on land alters the physicochemical characteristics of soil by preventing water infiltration because of the drying of oily wastes, which leads to blackening of the soil subsurface and elevated soil temperature (*4,5*). However, the effects of intensive and repeated hydrocarbon disposal, as practiced in land treatment, on the subsequent ability of the soil to support plant growth have remained anecdotal.

Bharat Heavy Electricals Limited (BHEL), Tiruchirapalli, India, is one of the largest boiler-plant manufacturers in the country. The plant releases a large volume of aqueous oil effluent through a drainage canal that empties into a huge reservoir 3 km east of the factory premises. From the reservoir, the effluent is directly used for paddy cultivation in about 25 acres of land. We have analyzed the physicochemical characteristics of the effluent (*3*). The effluent is composed mainly of hydrocarbons (higher methylenic absorption at 2924 cm^{-1} and methyl absorption at 2958 cm^{-1}), phenols, and trace elements such as Cd, Pb, Cu, Zn, Al, and Cr. Since in many plants anions and cations at high concentrations induce serious biochemical and physiological derangements (*6*), the present study attempts to evaluate crop resistance to oil pollution. Photosynthetic capacity and root and shoot tolerance indices were used to test the tolerance potentials of both blackgram and rice varieties grown in oil-polluted soil. Tolerant and sensitive varieties of *Oryza sativa* L. were screened for metal-binding components.

MATERIALS AND METHODS

Growth of Plants

Oryza sativa L. (varieties ADT-36, CR-1009, IR-50, IR-20, and Ponni) and *Vigna mungo* (L.) Hepper (varieties T-9 and Krishna) were grown in oil-polluted soil (total hydrocarbon, 625–950 mg/kg; cadmium, 12.65–16.30 mg/kg) under a natural photoperiod with day temperature of 28–35°C, a night temperature of 22–26°C, and relative humidity of 55–60%, employing aqueous oil effluent. As a control, *Oryza* and *Vigna* seedlings were raised in an unpolluted red sandy loam soil (total hydrocarbon, not detectable; cadmium, 0.20–0.28 mg/kg) in a nearby area under the same natural photoperiod, with unpolluted water used to irrigate the plants (*3*).

Root and Shoot Tolerance Indices

Root and shoot tolerance indices were calculated following the modified method of Taylor and Foy (*7*).

Elemental Analysis

Root and leaf samples were dried in an oven at 70°C±1 for 48 h and digested using a HNO_3:$HClO_4$:H_2SO_4 (10:4:1) mixture. Elemental analyses of the digested samples were carried out using an atomic absorption spectrophotometer (GBC Pty Ltd., Australia).

Photosynthetic Studies

CO_2-dependent-O_2 evolution was measured polarographically with a Hansatech O_2 electrode. The rate of electron transport from $H_2O \rightarrow MV$ was determined with intact chloroplasts (*8*). Leaf photosynthesis was measured using a portable infrared gas analyzer (IRGA) (Licor Inc., USA).

Preparation of Tissue Extracts

Leaves and roots were homogenized (20% w/v) in cold 0.25*M* sucrose solution in a high-speed blender (Sorvall, USA) for 2–3 min at 4°C. The homogenate was pelleted using an ultracentrifuge (Hitachi, Japan) at 1,05,000×*g* for 60 min. The pellet was washed thrice with cold sucrose solution. The washings were centrifuged again at 1,05,000×*g*. The supernatant fractions were combined and reduced in volume to 25% by lyophilization and saved for cadmium determination and for column chromatography.

Sephadex G-75 Chromatography

Portions of the lyophilized supernatant fraction of both control and polluted plant tissues were chromatographed, following the procedure of Shaikh and Lucas (*9*). The column was calibrated with proteins (20 mg each) of known molecular weight (ovalbumin, 45,000; Chymotrypsinogen, 25,000; Ribonuclease A, 13,700). The column was conditioned after every third run with Blue Dextran (2,000,000 mol wt). The eluates from the Sephadex G-75 column were monitored at 256 and 280 nm in separate runs. The presence of cadmium in the fractions was determined using an atomic absorption spectrophotometer (GBC Pty, Ltd., Australia). The 20th, 50th, and 70th fractions were scanned from 200–300 nm using a spectrophotometer (Beckman, USA). The 70th fraction was specifically scanned before and after titration with μL quantities of conc. HCl and NaOH, since the fraction contained nonhomeostatically induced proteins.

RESULTS

The oil-polluted soil, as well as the aqueous oil effluent, contained hydrocarbon as the major toxicant and inorganic ions with cadmium in much higher concentrations in the soil and effluent. All the varieties of

Table 1
Root and Shoot Tolerance Indices
of *Oryza sativa* L. and *Vigna mungo* (L.) Hepper Grown in Oil-Polluted Soil

Variety	Mean STI± SD	Mean RTI ± SD
O. sativa L.		
ADT-36	0.97±0.05	0.94±0.02
CR-1009	0.91±0.03	0.93±0.06
Ponni	0.58±0.03	0.61±0.01
IR-50	0.38±0.10	0.51±0.01
IR-20	0.36±0.06	0.39±0.02
V. mungo (L.) Hepper		
Krishna	0.51±0.08	0.41±0.08
T-9	0.49±0.03	0.38±0.01

Oryza sativa and *Vigna mungo* were raised in the oil-polluted soil for evaluation of tolerance to oil pollution. Varieties of *Oryza* (ADT-36, CR-1009, IR-50, IR-20, and Ponni) and *Vigna* (Krishna and T-9) differed in their tolerance to oil pollution. The root growth of ADT-36 and CR-1009 increased, whereas the growth of both root and shoot decreased in all other rice varieties (Table 1). The values of the root tolerance index of the five varieties ranged from 0.39±0.02 to 0.94±0.02. In contrast, the range of shoot-tolerance index values was from 0.36±0.06 to 0.97±0.05. In *Vigna,* both RTI and STI values varied from 0.38±0.01 to 0.51±0.08.

Tables 2 and 3 represent the values of photosynthetic efficiency of the five varieties of *Oryza* and two varieties of *Vigna* grown in the polluted soil. The CO_2-dependent-O_2 evolution in the isolated chloroplast of the 40-d-old plants in the rice varieties IR-50, IR-20, and Ponni decreased by 42–55%, whereas in ADT-36 and CR-1009, the decrease was 19–30% compared to the control. Similarly, in *Vigna* (30-d-old) both varieties showed a decrease of 44–58%. Chloroplast electron transport from $H_2O \rightarrow MV$ in all the rice varieties was reduced by 10–20%, whereas in *Vigna,* the reduction was by 25–45%. Leaf photosynthesis, as measured by CO_2 uptake using IRGA, was reduced by 24–26% in ADT-36 and CR-1009, and by 32–47% in IR-50, IR-20, and Ponni, whereas it was reduced by 43–61% in *Vigna.*

Since both the oil effluent and the polluted soil contained cadmium in high concentrations, the cadmium content of rice was analyzed. The distribution of cadmium in 40-d-old ADT-36 and IR-50 grown in the oil-polluted and control soils is given in Table 4. Accumulation of cadmium in roots (41.5 μg/gfw in IR-50; 53.2 μg/gfw in ADT-36) was more significant than in leaves (9.2 μg/gfw in IR-50; 10.5 μg/gfw in ADT-36) in both of the rice varieties. A four- to fivefold increase in cadmium content was observed in roots. Table 5 describes cadmium concentration in leaf and root homogenates, and supernatant fractions. The 1,05,000×*g* supernatant

Table 2
Effect of Aqueous Oil Effluent on Photosynthetic Properties of Chloroplasts Isolated from *O. sativa* and *V. mungo*[a]

Variety	Electron transport $H_2O \rightarrow MV$ Control	Electron transport $H_2O \rightarrow MV$ Polluted	CO_2-dependent-O_2 evolution Control	CO_2-dependent-O_2 evolution Polluted
	μmol O_2/mg chl/h			
O. sativa, 40-d-old				
ADT-36	613	521	146	121
CR-1009	680	610	178	126
IR-50	610	418	176	81
IR-20	584	378	134	74
Ponni	516	414	108	63
V. mungo, 30-d-old				
T-9	460	326	121	71
Krishna	378	281	108	64

[a]The rates of electron transport and CO_2-dependent-O_2 evolution are expressed in μmol O_2 chl/h. Values are the means of three different experiments.

Table 3
Photosynthetic Characteristics of Leaves of *Oryza sativa* and *Vigna mungo* Grown in Polluted and Control Soils[a]

Variety	Leaf photosynthesis Control	Leaf photosynthesis Polluted
	mg $CO_2/m^2/S$	
O. sativa, 40-d-old		
ADT-36	0.265 ± 0.07	0.198 ± 0.04
CR-1009	0.276 ± 0.08	0.212 ± 0.03
IR-50	0.238 ± 0.04	0.148 ± 0.06
IR-20	0.219 ± 0.03	0.149 ± 0.01
Ponni	0.219 ± 0.03	0.117 ± 0.07
V. mungo, 30-d-old		
T-9	0.174 ± 0.04	0.099 ± 0.01
Krishna	0.182 ± 0.02	0.098 ± 0.02

[a]The data are mean values ±SD for five samples. All of the analyses were carried out using portable IRGA.

Table 4
Cadmium Levels in Leaves and Roots of 40-d-old *Oryza sativa* L.[a]

	Cadmium content, μg/gfw	
Variety	Leaves	Roots
ADT-36	10.5±0.02	53.2±0.15
IR-50	9.2±0.80	41.5±0.35

[a]Var. ADT-36 and IR-50; results are means±SD.

Table 5
Cadmium Content in 105,000×*g* Supernatants and Homogenates of Roots and Leaves of *Oryza sativa* L.[a]

Variety	Sample	Cd content, μg/gfw	Percentage of Cd content in supernatants
ADT-36	Root homogenate	48.30±0.41	
	Root supernatant	35.10±0.39	72.7
	Leaf homogenate	9.60±0.05	
	Leaf supernatant	3.10±0.20	32.3
IR-50	Root homogenate	37.30±0.80	
	Root supernatant	24.90±0.50	66.7
	Leaf homogenate	8.20±0.10	
	Leaf supernatant	2.90±0.04	35.4

[a]Var. ADT-36 and IR-50; results are mean±SD.

fraction was found to contain more cadmium. In the rice variety ADT-36, 73% of the cadmium was found to be present in the root supernatant fraction, whereas in IR-50, 67% was present there.

The supernatant fractions of the control and polluted plant leaves and roots were passed separately through G-75 column chromatography and analyzed for UV absorption and cadmium content (Figs. 1A,B, and 2A,B). Both root and leaf eluates showed similar profiles of absorption (Figs. 1B and 2B), but the degree of absorption differed between the rice varieties (ADT-36 and IR-50). Fractions were monitored at 256 and 280 nm. In the control plants (Figs. 1A and 2A) there was no significant UV absorption at the 70th fraction, but in the polluted plants significant absorption at this fraction was obtained (Figs. 1B and 2B). In the polluted plants, cadmium was mostly associated with the 70th and 50th fractions, whereas there was not such striking absorption in the control plants (Figs. 1A and 2A). Absorption by the 20th fraction in both the control and polluted plants was similar, although in the polluted plant, a higher cadmium concentration

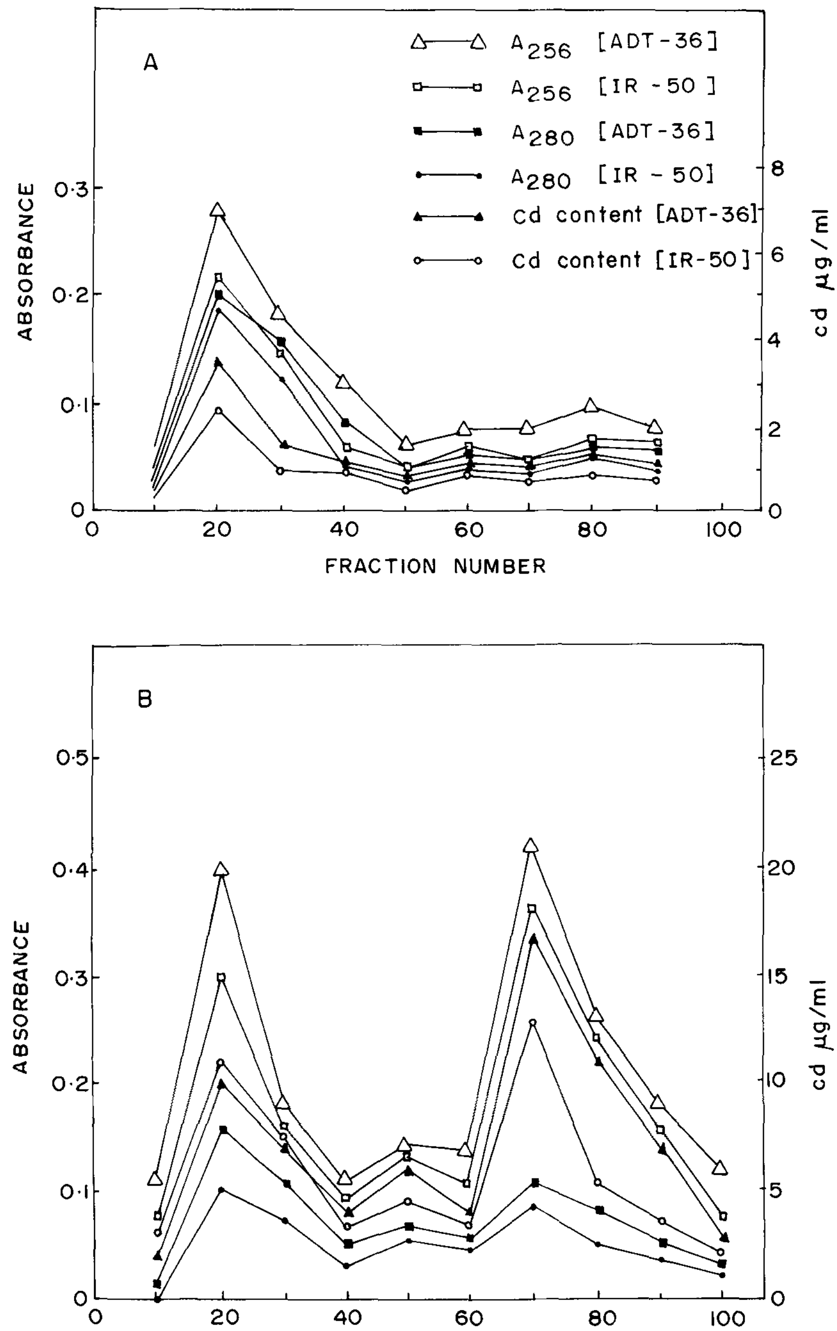

Fig. 1. Sephadex G-75 chromatography of *Oryza sativa* (ADT-36 and IR-50) root extracts: (A) Control; (B) Plants raised in an oil-polluted field. (Absorbance of G-75 Sephadex eluates was monitored at 256 and 280 nm.)

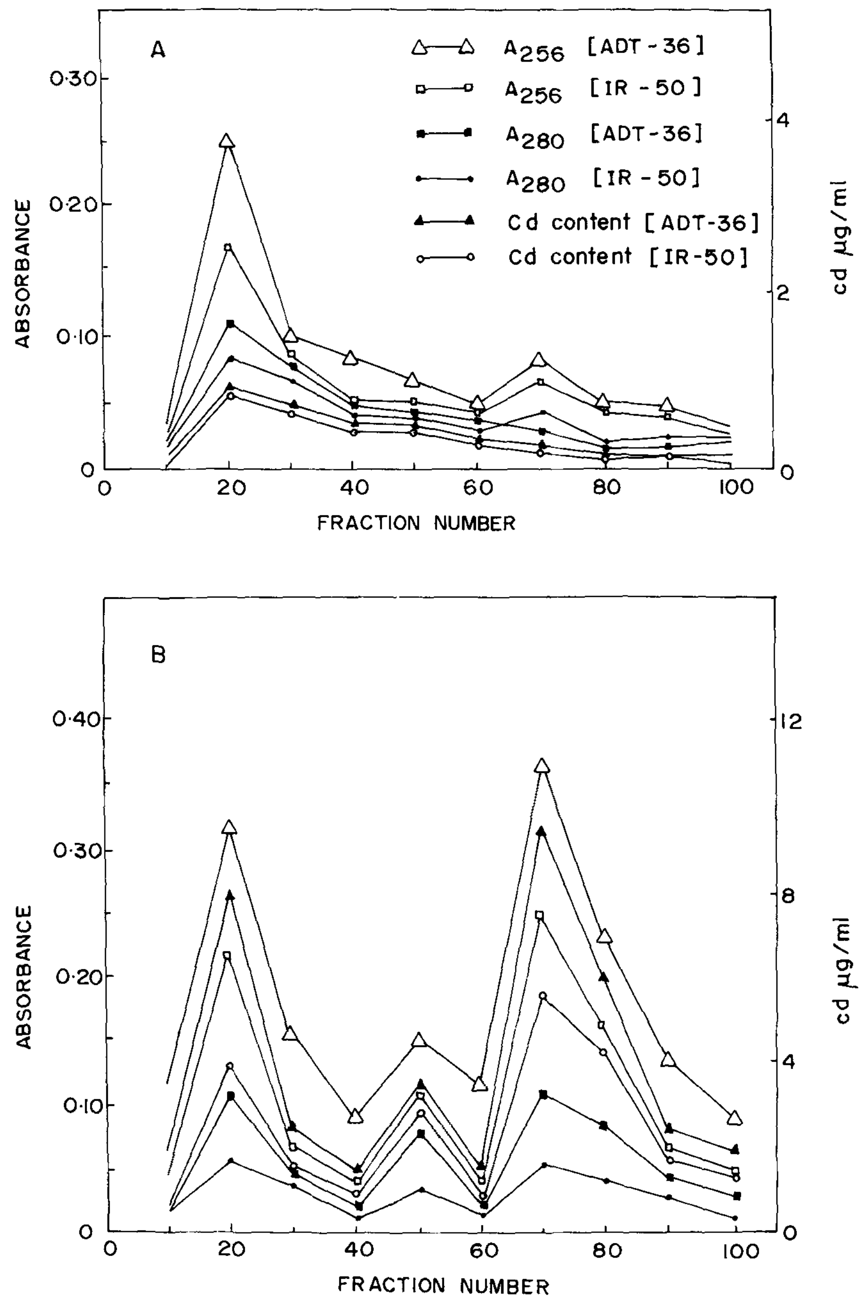

Fig. 2. Sephadex G-75 chromatography of *Oryza sativa* (ADT-36 and IR-50) leaf extracts: (A) Control; (B) Plants grown in an oil-polluted field.

was obtained. UV absorption profiles (200–300 nm) of the 70th fraction are presented in Fig. 3. The UV absorption spectrum of the 70th fraction showed a shoulder at 256 nm, which disappeared when the protein complex was acidified (pH 2.5) but was reconstituted upon alkalinization to pH 8.0. The Cd-associated 70th fraction was found to contain 33-kdalton protein.

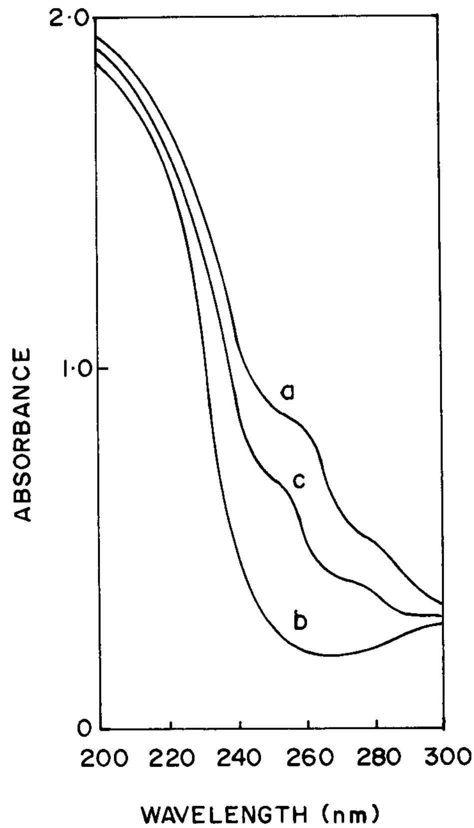

Fig. 3. UV absorption spectra of *Oryza sativa* Cd-binding proteins at different pH values. The native protein (1 mg/mL) at pH 8.6 (a) was titrated to pH 2.5 (b) and then to pH 8.0 (c). The light path was 10 mm; μL quantities of strong HCl or NaOH were used for titrations.

DISCUSSION

The present study investigates evaluation of crop resistance to aqueous oil pollution using five varieties of *Oryza sativa* L. (ADT-36, CR-1009, IR-50, IR-20, and Ponni) as well as two varieties of *Vigna mungo* (L.) Hepper (Krishna and T-9). Root tolerance and shoot tolerance indices (RTI and STI), photosynthetic capacity, and the presence or absence of metal-binding protein were employed as important parameters to evaluate crop resistance to oil pollution. The data on RTI and STI for all five varieties of *Oryza* and both varieties of *Vigna* are given in Table 1. Among the five rice varieties used, ADT-36 and CR-1009 were found to have higher RTI and STI values, whereas both varieties of *Vigna* indicated susceptibility to oil pollution, as evidenced by lower RTI and STI values.

Rice varieties such as ADT-36 and CR-1009 did not show any striking difference in photosynthetic O_2 evolution, chloroplast electron transport, and leaf photosynthesis, whereas in other varieties, inhibition in the above photosynthetic parameters was substantial. In both varieties of *Vigna* (Krishna and T-9) considerable reduction in all the above parameters was observed.

Based on these results, it may be surmised that the ADT-36 and CR-1009 rice varieties are more tolerant to aqueous oil pollution than other varieties, as evidenced by high values of RTI and STI and comparatively unattenuated photosynthetic characteristics. The two varieties of *Vigna* exhibited extreme phytotoxicity to oil pollution. This finding is further supported by our observation (*10*) that hydrocarbons, which are major toxicants in the aqueous oil effluent and oil-polluted soil, drastically affected the emergence of the seedlings from the polluted soil by affecting the geotropic orientation of most of the seedlings.

Since the oil-polluted soil as well as aqeuous oil effluent contained high concentrations of cadmium, there was interest in using the present study to find out whether synthesis of any metal-binding protein was involved, particularly in ADT-36 (tolerant variety) in comparison to IR-50 (susceptible variety). Of the two varieties of *Oryza*, ADT-36 did not exhibit any toxic symptoms to cadmium. The plant-to-soil ratio for cadmium is reported to be considerably higher than for Pb, Zn, or Cu (*11*). This suggests that the concentration of cadmium in plants is higher than in the environment. Cutler and Rains (*12*) suggested that one of the mechanisms involved in cadmium accumulation is an irreversible sequestering of cadmium to the binding sites, probably on cell constituents or macromolecules within the cell. Cadmium is considered to be the most deleterious toxicant in the environment. Therefore, we considered it important to isolate Cd-binding components from ADT-36 (tolerant) and IR-50 (susceptible) rice varieties. From the study, it is evident that cadmium was mainly associated with the supernatant fractions of the leaf (32% in ADT-36; 35% in IR-50) and root tissues (73% in ADT-36; 67% in IR-50) of *Oryza sativa*. Scanning of all the eluates indicated that significant amounts of cadmium were associated with the 70th and 50th fractions. The molecular weight of the Cd-binding components was about 50,000 in leaves and 33,000 in roots. The UV absorption profiles showed a strong absorptivity at 256 nm (Fig. 3). The absorption at 256 nm disappeared upon acidification of the sample to pH 2.5, but reappeared upon alkalinization to pH 8.0. UV spectral properties indicate the presence of Cd-mercaptide chromophore, similar to that of Cd-thioneins of mammals (*13,14*). The absorption at 250–260 nm at pH 8.6 is typical of the metal mercaptide chromophore, as in invertebrate Cd-thioneins (*15,16*). It is generally believed that the absence of aromatic aminoacids in the protein structure caused a lack of significant absorption at 280 nm (*17,13*), but in our study we noticed absorption at 280 nm also. Kaneta et al. (*18*) also made a similar observation in Cd-treated rice plants. Mammalian metallothioneins have a molecular weight of less than 10,000. In rice plants, two cadmium-binding components with molecular weights of about 50,000 and 33,000 were found to be present. Thus, the observed molecular weights were found to be significantly higher than that of animal metallothionein. The UV absorption at 256 nm indicated Cd-thiolate coordination, since the loss of absorption at pH 2.5 and reappearance upon adjustment to pH 8.0 suggest complete dissociation from, and somere-binding to, the cysteine sulphur (*16*). The reported metallothionein-like proteins (about 50 and 33 kdalton) are probably homeostatically synthesized (50th and 70th fractions), unlike those of the 20th fraction, synthesized nonhomeostatically in both the control (Figs. 1A and 2A) and polluted plants (Figs. 1B and 2B).

From our earlier photosynthetic studies (*3*) it is evident that the rice variety ADT-36 is more tolerant than IR-50 to the aqueous oil pollution. The present investigation also supports the tolerance potential of ADT-36, which may be attributed to the presence of metallothionein-like proteins that are known to detoxify the cellular environment.

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