

Response of selected aquatic macrophytes towards textile dye wastewaters

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Ten aquatic macrophytes, *Ceratophyllum demersum*, *Hydrilla verticillata* (submerged), *Azolla pinnata*, *Eichhornia crassipes*, *Lemna aequinoctialis*, *Spirodela polyrhiza* (free-floating), *Cyperus alopecuroides*, *Phragmites karka*, *Polygonum barbatum* and *Typha angustata* (emergent) were screened for tolerance towards textile dye wastewaters, released during processing (dye fixing and washing) of printed cloth, to raise constructed wetlands for their phytoremediation. The concentrated dye wastewaters of first wash were found toxic to plant growth. The free-floating plant species were almost dead in dilute acidic-azo water (pH 4.3-6.2), while their growth was adversely affected in neutral-azo (pH 7) and silicate waters (pH 9-10). Amongst emergent macrophytes, *Polygonum* died in acidic-azo and silicate waters, while its growth in neutral-azo was adversely affected. *Cyperus* too did not grow well in dye wastewaters, especially the acidic-azo water, whereas growth of *Typha* in wastewaters was similar to control plants. *Phragmites* was the only plant species growing better than the control plants in dye wastewaters, both in laboratory and field studies. *Lemna* and *Spirodela* are recommended for assessing the toxicity of textile dye wastewaters, and *Phragmites* for raising constructed wetlands to treat the same.

Keywords: dye wastewaters, emergent macrophytes, free floating macrophytes, submerged macrophytes
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Introduction

Constructed wetlands have been used successfully to treat/polish wastewaters from municipal, agricultural and industrial sources¹⁻⁵. However, their application for treating municipal and industrial wastewaters is relatively new in India⁶⁻⁹. The aquatic vegetation growing in the wetland oxygenates the rhizosphere, which creates ideal conditions required for the development of microorganisms participating in aerobic decomposition of organic matter¹⁰. Their extensive root systems provide a large surface area for the development of microorganisms and also enable the filtration and adsorption of sediment matter¹¹⁻¹³. An artificial marsh, therefore, simultaneously acts as a trickling filter, a percolation filter and digestion basin^{14,15}.

Unlike developed countries, sufficient efforts have not been made in India to screen the performance of locally available aquatic macrophytes to treat wastewaters. This becomes more important when one deals with toxic textile dye wastewaters, which are highly varied in their chemical composition due to the presence of a large variety of chemicals and dyes¹⁶. Considering this gap in knowledge, the authors made

laboratory and field studies to screen the growth of 10 locally available aquatic macrophytes in textile dye wastewaters, in order to identify the tolerant ones that can find application in raising constructed wetland to treat textile dye wastewaters. The important findings are presented in the present communication.

Materials and Methods

Laboratory Studies

Monocultures of 10 locally available aquatic macrophytes, two submerged, four free floating and four emergent, were maintained in separate concrete tanks (40×40×60 cm³) in the botanical garden and were the source of plant material during the present study. The experiments were set up outdoor in the Botanical garden and all the pots were randomly placed. The studies of submerged and free-floating plants were made during February-April (mean min. temperature, 8-23, and max., 28-39°C; relative humidity, 42-50%), while of emergent plants during April-June (mean min. temperature, 24-27, and max., 40-44°C; relative humidity, 35-55%).

Textile dye wastewaters collected on alternate days in clean plastic cans (volume, 20 L), from Sanganer industrial area—about 15 km from the University campus, were transported immediately to the laboratory and stored in a cold room. Their physico-

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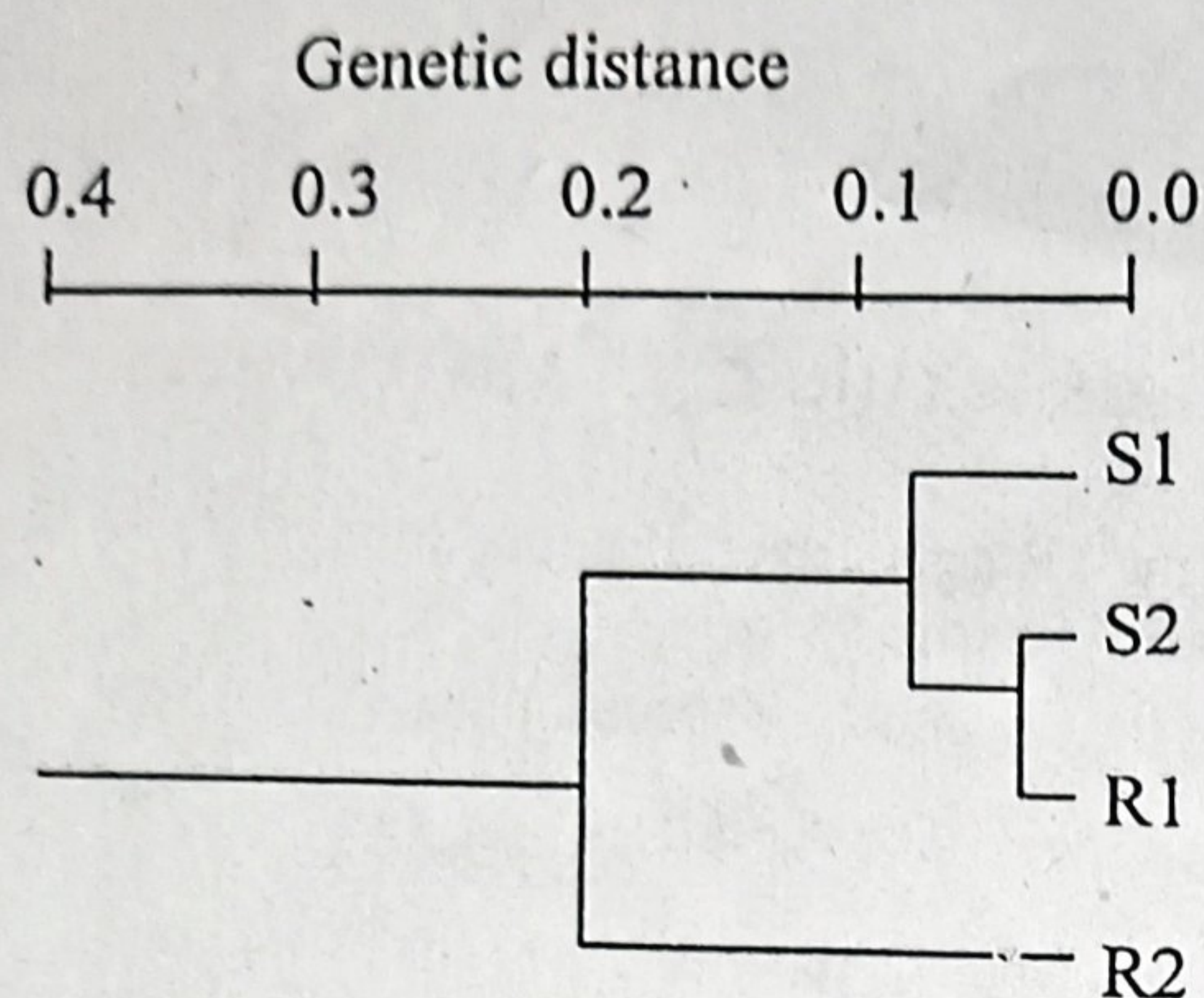


Fig. 2—Dendrogram for *P. minor* populations generated by cluster analysis of genetic distance values from random amplified polymorphic DNA data. Relative branch lengths indicate relative genetic distances between biotypes.

biotype from Kalwehri falls in a separate group (Fig. 2). The average inter cluster distance was 0.17. The intra cluster distance was 0.05. The study indicates that RAPD analysis is useful in assessing genetic diversity amongst *P. minor* biotypes. The grouping of resistant biotype from Uchana in the first cluster is indicative of the fact that although it has developed resistance to the herbicide isoproturon, it is still sufficiently similar to the susceptible biotypes genetically. A further study of other susceptible and resistant biotypes from other locations in India could lead to a better assessment of the genetic diversity within the species. The technique has been amply exploited in grouping herbicide resistant biotypes of *Echinochloa crusgalli*, a weed of rice fields. Independent modes of origin of resistant biotypes in different clusters have been observed¹⁴. The technique has also been exploited in monitoring spread of atrazine resistant *Solanum nigrum* in Europe¹⁵.

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chemical characteristics were assessed within 24 h, following standard methods described in APHA¹⁷. Two types of dye wastewaters, diazo and silicate were used during the present study, which differed in their composition of dyes and fixation process. In diazotization process, the rapid fast dyes, an alkaline mixture of naphthol AS and diazo compounds, are fixed by soaking the printed cloth in a solution containing NaNO₂ and H₂SO₄/HCl for 10-15 min, followed by washing consecutively in three separate tanks filled with tap water. In silicate process, however, the reactive dyes are fixed in a concentrated solution of sodium silicate for 12 h, which was also followed by three washes, similar to diazotization process.

Initially, the growth of *Spirodela* was monitored separately in concentrated azo and silicate waters belonging to the first wash, in which they died within 1 h. Therefore, dilute wastewaters of II and III wash were used in the subsequent studies conducted on aquatic macrophytes. In case of azo water, two sets were prepared—one without pH modification (acidic-azo water; Table 1) and other with modified pH 7 (neutral azo-water) by lime addition, which decreased the pollution load (conductivity, 0.50-0.98 mmho cm⁻¹; COD, 150-280 mg/L; Cu, 0.25-1.25 mg/L) in comparison to raw wastewater (Table 1). Dilute silicate wastewater, however, was used as such. Thus, aquatic plant growth was monitored in 3 types of wastewaters, acidic-azo, neutral-azo and silicate.

In case of submerged macrophytes, 5 cm long explants of *Ceratophyllum demersum* Linn. (dry weight, 28 mg) and *Hydrilla verticillata* (L.f.) Royle (dry weight, 20 mg) with apical meristems were added to a plastic glass (volume, 300 mL) containing 250 mL dye wastewater. Two harvests were made at 7 d interval. At each harvest, shoot length and dry weight were determined.

For studying the growth of free-floating macrophytes, uniform sized plants of *Eichhornia crassipes* (Mart.) Solms. (dry weight, 1.14 g) were added to earthen pots (10 L size) containing 7.5 L dye wastewater; each pot contained one plant. Whereas, *Azolla pinnata* R.Br. (area, 50 cm²; dry weight, 151 mg), *Lemna aequinoctialis* Welwitsch (area, 35 cm²; dry weight, 44 mg) and *Spirodela polyrhiza* (L.) Schleid. (area, 35 cm²; dry weight, 69 mg) were added separately into earthen pots (3 L size) containing 2.5 L wastewaters. Earthen pots filled with tap water served as control. The water level in the pots was maintained daily by their respective wastewaters or tap water. Two harvests were made at 7 d interval in case of *Azolla*, *Lemna* and *Spirodela*, whereas four harvests were made in case of *Eichhornia*; initial two being at 15 d interval, while the two subsequent ones at a shorter interval (7 d), considering the senescence rate of plants in dye wastewaters.

At each harvest, the ramet size (spread area) of *Azolla*, *Lemna* and *Spirodela* was measured along with their root length. In *Eichhornia*, the number of offshoots, number of new and old roots, and also the maximum root length of plants were recorded. The shoots of harvested *Eichhornia* plants were also categorized into live and dead, which was difficult in case of roots.

In emergent macrophytes, uniform sized 10-12 cm long young shoot of *Cyperus alopecuroides* Rottb. (dry weight, 1 g), *Phragmites karka* Steud. (dry weight, 0.40 g), *Polygonum barbatum* Linn. (dry weight, 0.61 g) and *Typha angustata* Bory & Chaub. (dry weight, 1.93 g) was grown in waterlogged garden soil filled in 40 earthen pots (volume, 10 L). In order to achieve their uniform growth, these were grown undisturbed for 15 d in case of *Cyperus* and *Phragmites*, 21 d in *Polygonum* and 30 d in *Typha*. Thereafter, 24 pots having shoots of uniform size

Table 1—Physico-chemical characteristics (mean values) of textile wastewaters released during washings of printed cloth

Processes	Parameter	I wash	II wash	III wash	Permissible discharge limits* (MINAS)
Diazotization	pH	1.3-1.9	2.9-4.0	6.0-7.0	6.0-8.5
	Conductivity (mmho/cm)	4.21-41.5	2.62-4.0	1.29-2.6	NA
	COD (mg/l)	788-971	470-545	104-370	250
	Cu (mg/l)	1.3-25.0	2.2-15.0	0.6-4.86	3.0
Silicate	pH	11.0-11.7	10.5	9.8	As above
	Conductivity (mmho/cm)	15.2-22.0	10.2-15.0	4.7-6.7	
	COD (mg/l)	459-500	150-190	44-59	
	Cu (mg/l)	Nil	Nil	Nil	

NA=Not available; *Source: Goel and Sharma³⁹

were selected for each species. The initial shoot density in a pot was maintained to one by uprooting the others. These pots were divided into four groups and each group was irrigated either with tap water (control), acidic-azo, neutral-azo or silicate wastewaters, respectively maintaining their level 5 cm above the soil surface. Two harvests were made at an interval of 30 d. The shoot densities along with their height were recorded at each harvest. The belowground parts were washed in running water and separated into root and rhizome. The volume and fresh weight of roots were determined to calculate the bulk density.

The harvested plant species were air dried separately in a hot air oven at 60°C for 7 d and weighed. The dead plant parts, if any, were also dried and weighed. All data were based on the average of 3 replicates. Student's 't' test was conducted for comparing the growth of plants in dye wastewater with control.

Field Study

Considering the findings of the laboratory studies, mortality and density of *Eichhornia*, *Phragmites* (also biomass) and *Typha* shoots were also monitored in a constructed wetland (46'x25.6'x7') used for furthering microbial degradation of dye wastewater pollutants (35-40 m³/d) in its rhizosphere, following their initial degradation in a bioreactor (volume, 47,000 L) in a pilot scale effluent treatment plant constructed for three small-scale textile printing industries in Sanganer. The hydraulic format of wetland is a vertical upflow. The wetland has an average depth of 2-3 ft. Initially, *Phragmites* (200 shoots) and *Typha* (40 shoots) were planted in the wetland (May, 2000). Later, 25 shoots of *Eichhornia* (August, 2002) were introduced in the deeper regions of the wetland. Both density and aboveground biomass of plants growing in the wetland were estimated in October 2002 by harvest method¹⁸.

Results

Laboratory Studies

Submerged Hydrophytes

The shoots of both *Ceratophyllum* and *Hydrilla* were highly brittle in either azo wastewaters. Furthermore, *Ceratophyllum* lost most of its leaves within 24 h of exposure, and died right in the beginning in acidic-azo and after a week in the azo-neutral wastewater (Table 2). Although, *Hydrilla* grew in acidic as well as neutral-azo wastewaters but its dry weight decreased markedly (50-80%). Thus, *Ceratophyllum* was found more sensitive to azo wastewaters.

Performance of Free Floating Macrophytes

In control sets (tap water), healthy *Azolla* plant looked reddish green, while *Eichhornia*, *Lemna* and *Spirodela* plants were bright green in colour. Further, *Eichhornia* plants had bulbous petiole and glossy lamina, while fronds of the remaining were large and spongy. On the other hand, *Azolla* plants growing in wastewaters looked highly brittle with reddish-black leaves in azo wastewaters, while they were pale green in silicate wastewaters. The leaves of the other three species were pale green with brown necrotic lesions in both the wastewaters. All the species had well-developed roots in the control sets, while most of the roots were lost in wastewaters, more particularly in the acidic-azo wastewater. *Eichhornia* roots in dye wastewaters were thin and wiry; whereas, in the control plants, they were relatively thick, exhibiting spreading tendency.

Azolla, *Lemna* and *Spirodela* plants succumbed to ill effects of acidic-azo wastewater (pH 4.3-6.2) after 7 d growth, while they continued to grow in the neutral-azo (pH 7) water for another 7 d. Interestingly, toxic effects of the wastewaters were less severe in those *Lemna* plants which were growing in pots exposed to direct sunlight for a longer duration, suggesting photolabile nature of toxic constituents. On the other hand, adverse effects on

Table 2—Dry weights (mg) of *Hydrilla* and *Ceratophyllum* plants after their 7 days (I harvest) and 15 days (II harvest) growth in the tap water (Control) and azo wastewaters

Plants	Control		Azo-acidic		Neutral -azo	
	I	II	I	II	I	II
<i>Hydrilla</i>	71±3	112±12	35±3*	42±3**	35±3*	15±1**
<i>Ceratophyllum</i>	470±10	543±10	-	-	20±0**	Dead

± SD; Significantly different from the control at *5% and at **1%

Table 3—Mean values of ramet size (cm²) and dry weight (mg) of *Azolla*, *Lemna* and *Spirodela* plants growing in tap water (control) and dye wastewaters

Plant species		Control		Neutral-azo		Acidic-azo		Control		Silicate	
		I	II	I	II	I	II	I	II	I	II
<i>Azolla</i>	Ramet size	92±9	112±40	*68±5*	46±9	51±5**	Dead	80±23	72±10	60±8	72±29
				(-26)	(-59)	(-44)				(-25)	(-53)
	Dry weight	366±64	412±165	145±12**	208±44	127±17**	Dead	154±33	155±25	360±68*	237±117
				(-60)	(-49)	(-65)				(+134)	(+55)
<i>Lemna</i>	Ramet size	75±7	73±17	48±2**	10±6**	58±8	Dead	75±7	78±17	70±12	38±17
				(-36)	(-86)	(-23)				(-7)	(-51)
	Dry weight	206±62	311±121	123±80	22±4	106±43	Dead	197±30	209±42	213±110	135±43
				(-40)	(-93)	(-48)				(+8)	(-35)
<i>Spirodela</i>	Ramet size	65±15	74±6	45±11	52±2**	50±15	Dead	66±5	85±16	53±2*	66±7
				(-31)	(-30)	(-23)				(-20)	(-22)
	Dry weight	166±30	219±13	146±55	125±24**	92±10*	Dead	270±15	262±47	267±33	363±85
				(-12)	(-44)	(-44)				(-1)	(+38)

I—first harvest; II—second harvest; ± SD; Significantly different from the control at *5% and at **1%
Values in parentheses are per cent change in comparison to the control

Eichhornia plants were evident only after 15 d of growth and the plants were almost dead at the termination of the study, especially in silicate wastewater (pH 9-10).

In comparison to control plants, ramet size (azo water, 30-86%, silicate water: 22-53%) and dry weight (azo water: 44-93%) of *Azolla*, *Lemna* and *Spirodela* decreased with the exposure period in the dye wastewaters, with the exception of silicate water wherein dry weight usually increased (38-55%; Table 3). Thus, azo wastewater was relatively more toxic. The offshoot production in *Eichhornia*, however, differed little in dye wastewaters (1-3/plant) as compared to control (3-4/plant), whereas the dry weights decreased with the exposure period, being more pronounced for roots in azo and for shoots in silicate wastewater (Figs 1 & 2). Thus, the tolerance of free-floating macrophytes to dye wastewaters in the present study is:

Eichhornia > *Spirodela* > *Azolla* = *Lemna*

Performance of Emergent Macrophytes in Dye Wastewaters

Cyperus, *Phragmites*, *Polygonum* and *Typha* grew well in the control sets, possessing sturdy shoots with bright green leaves. In general, toxic effects of wastewaters were exhibited in form of thinner shoots and having small and etiolated leaves. Azo wastewaters were found toxic to *Cyperus* shoots, whereas both azo and silicate wastewaters were toxic to *Polygonum* and *Typha*. *Typha* shoots, however, recovered after their 15 d growth, while *Polygonum* succumbed to their toxic effects. Another important observation was the conspicuous absence of oval whitish tubers at the tips of *Cyperus* roots in azo

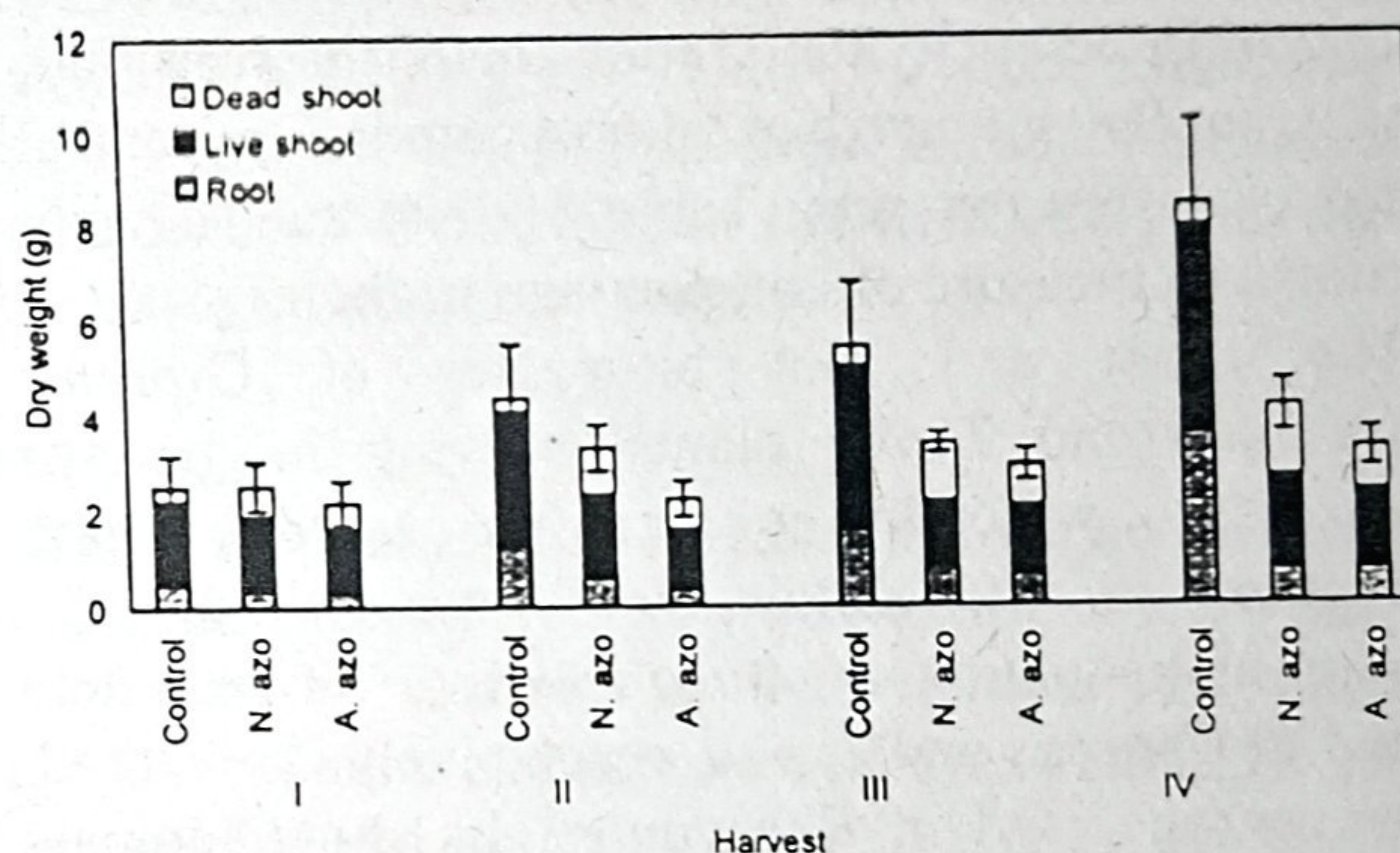


Fig. 1—Mean dry weight (±SD) of *Eichhornia* plants growing in tap water (control) and azo wastewaters (N. Azo = Neutral azo; A. Azo = Azo acidic)

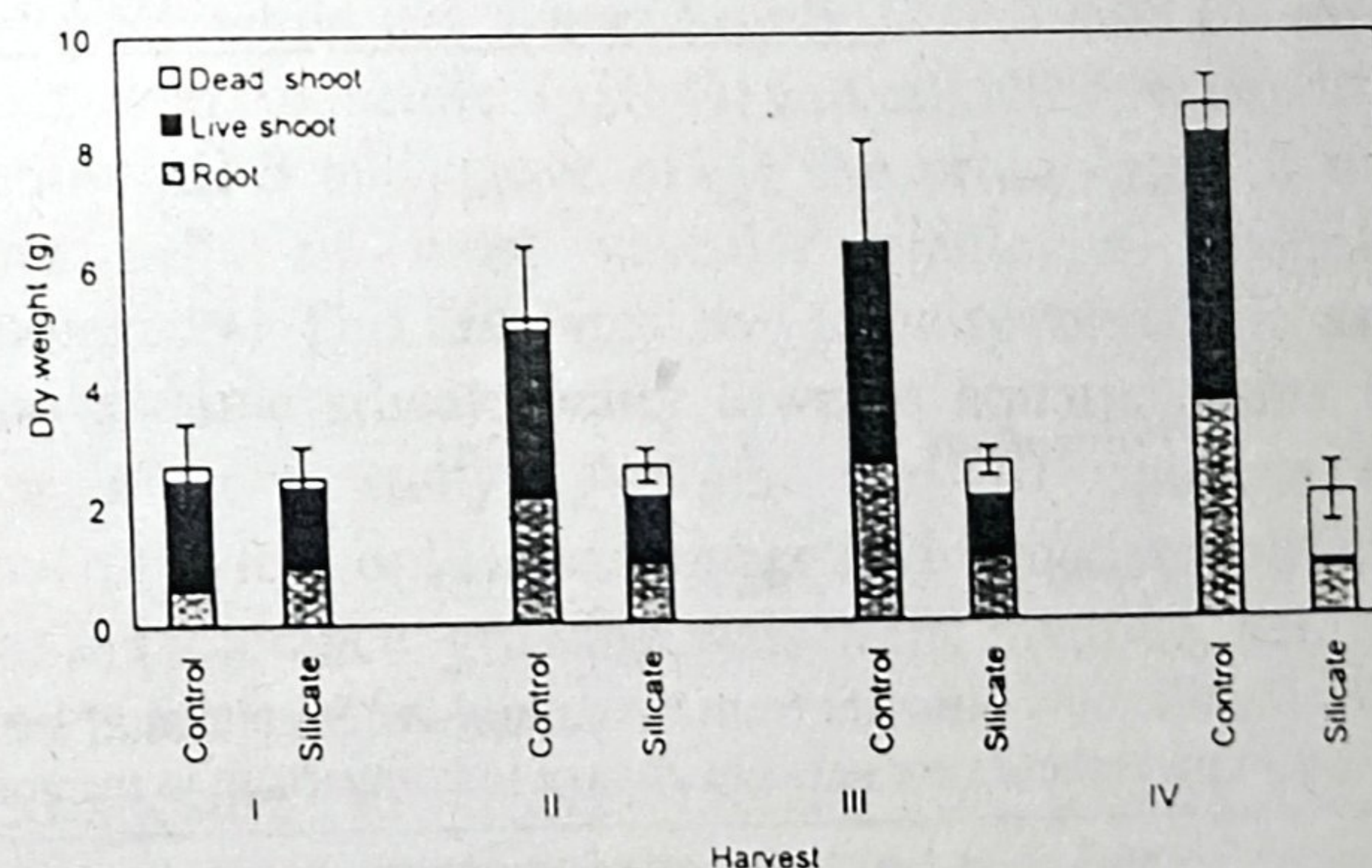


Fig. 2—Mean dry weight (±SD) of *Eichhornia* plants growing in tap water (control) and silicate wastewaters

wastewater sets, which were abundant in the control and silicate sets. On the other hand, *Phragmites* shoots grew well in both the wastewaters during the initial 15 d, possessing comparatively dark green leaves in azo wastewaters. However, toxic effect was

visible in acidic-azo wastewater between 15-30 d of growth period. Thereafter, *Phragmites* shoots recovered well, growing similar to control plants in all the wastewaters, though they were thinner as compared to control plants.

In case of *Cyperus*, *Polygonum* and *Typha*, shoot number (mean) was usually low in dye wastewaters in comparison to control, except in neutral-azo wastewater, where it was usually the same as in control. On the contrary, *Phragmites* had higher number of shoots in the wastewaters as compared to control, especially in azo wastewaters (Table 4). In general, the root bulk density of *Cyperus*, *Phragmites* and *Typha* plants growing in dye wastewaters was a little higher than the control plants, possibly due to reduction in aerenchyma. Unlike them, the root bulk density in *Polygonum* was almost similar in control and dye wastewater sets (Table 5). The bulk density of roots is a measure of compactness of their tissues.

The shoot and root biomasses of *Cyperus*, *Polygonum* and *Typha* plants growing in the dye wastewaters were lower than the control, being minimum in the acidic-azo water (Figs 3-5). Maximum reduction in shoot and root biomass was found in *Cyperus* and *Typha*, respectively. This trend, however, reversed in *Phragmites*. Its shoot biomass

was almost equal to control plants in the silicate set and even more than control plants in neutral- (71%) and acidic-azo (26%) sets during the second harvest; however, the root biomass was decreased in azo waters (Fig. 6). In silicate wastewaters, the root biomass was almost equal to control plants during I harvest but increased by 126% in the II harvest. Thus, the growth of *Phragmites* shoot was promoted in the azo waters, while the growth of root was promoted in the silicate water.

In comparison to control, rhizome biomass decreased in all the three dye wastewaters in *Typha* (17-86%) and only in acidic-azo water (13-60%) in *Phragmites*; whereas, silicate (11-59%) and neutral-azo waters favoured an increase in rhizome biomass (27-44%; Figs 5 & 6). *Cyperus* and *Polygonum* are non-rhizomatous species. Dead plants/parts were not observed in control sets of all the four plant species during both harvests, whereas their proportion increased in plants growing in dye wastewaters, especially in case of *Cyperus* and *Polygonum* (Figs 3-6). Thus, the tolerance of emergent macrophytes to both azo and silicate dye wastewaters is:

Phragmites > *Typha* > *Cyperus* > *Polygonum*

Table 4—Number (mean) of shoots of emergent macrophytes growing in tap water (control) and dye wastewaters for 30 days (I harvest) and 45 days (II harvest)

Plant species	Control		Neutral-azo		Acidic-azo		Silicate	
	I	II	I	II	I	II	I	II
<i>Cyperus</i>	4±2	6±1	6±2	9±2	3±1	3±2	4±1 (Nil)	6±1 (Nil)
<i>Phragmites</i>	6±1	9±2	14±3** (+133)	18±1* (+100)	13±6 (+116)	21±6 (+133)	10±1* (+66)	11±3 (+22)
<i>Polygonum</i>	1	2±1	1	2±1 (-50)	1	Dead	1	Dead
<i>Typha</i>	4±1	5±1	3±1 (-25)	7±1 (+40)	2±1 (-50)	3±1 (-40)	4±2 (Nil)	5±2 (Nil)

± SD: Significantly different from the control at *5% and at **1%

Values in parentheses are per cent change in comparison to the control

Table 5—Bulk density of control and wastewater growing plant roots

Plant species	Control		Azo-neutral		Azo-acidic		Silicate	
	I	II	I	II	I	II	I	II
<i>Cyperus</i>	0.54±0.03	0.56±0.04	0.62±0.07 (+15)	0.81±0.09 (+45)	0.72±0.03 (+33)	0.74±0.08 (+32)	0.53±0.01 (-2)	0.71±0.04 (+27)
<i>Phragmites</i>	0.51±0.01	0.65±0.05	0.46±0.03 (-10)	0.68±0.04 (+5)	0.8±0.12 (+57)	0.66±0.08 (+2)	0.35±0.05 (-31)	0.91±0.13 (+40)
<i>Polygonum</i>	1.0±0.1	1.3±0.2	0.7±0.1 (-30)	1.2±0.1 (-8)	0.9±0.04 (-10)	1.5±0.3 (+15)	0.8±0.1 (-20)	2.0±0.6 (+54)
<i>Typha</i>	0.47±0.02	0.87±0.11	0.65±0.08 (+38)	0.63±0.15 (-28)	0.58±0.08 (+23)	0.81±0.14 (-7)	0.61±0.09 (+30)	0.86±0.11 (-1)

± SD: Values in parentheses are per cent change in comparison to the control

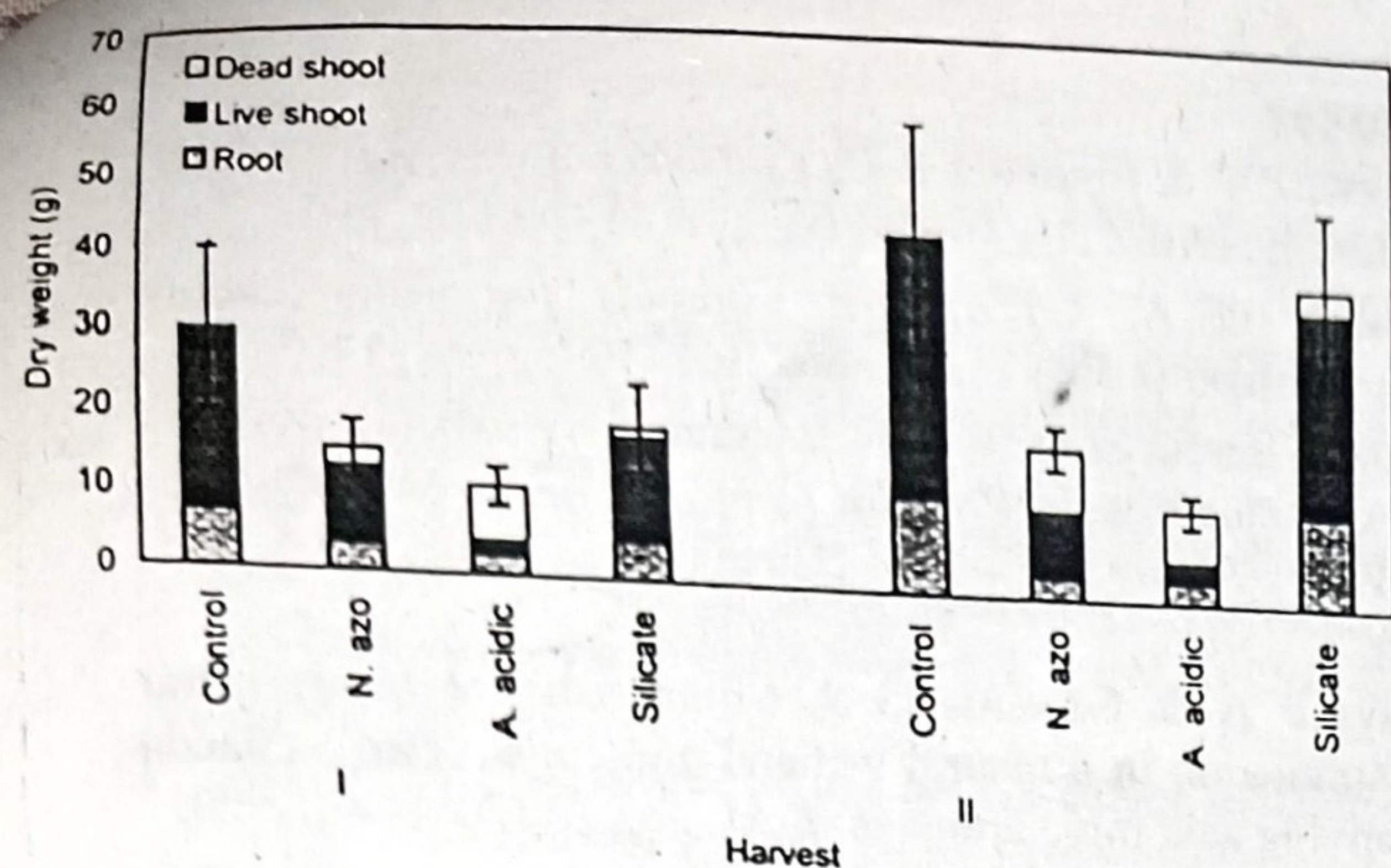


Fig. 3—Mean dry weight ($\pm$ SD) of *Cyperus* plants growing in tap water (control), azo (N. Azo=Neutral azo; A. Azo=Azo acidic) and silicate wastewaters

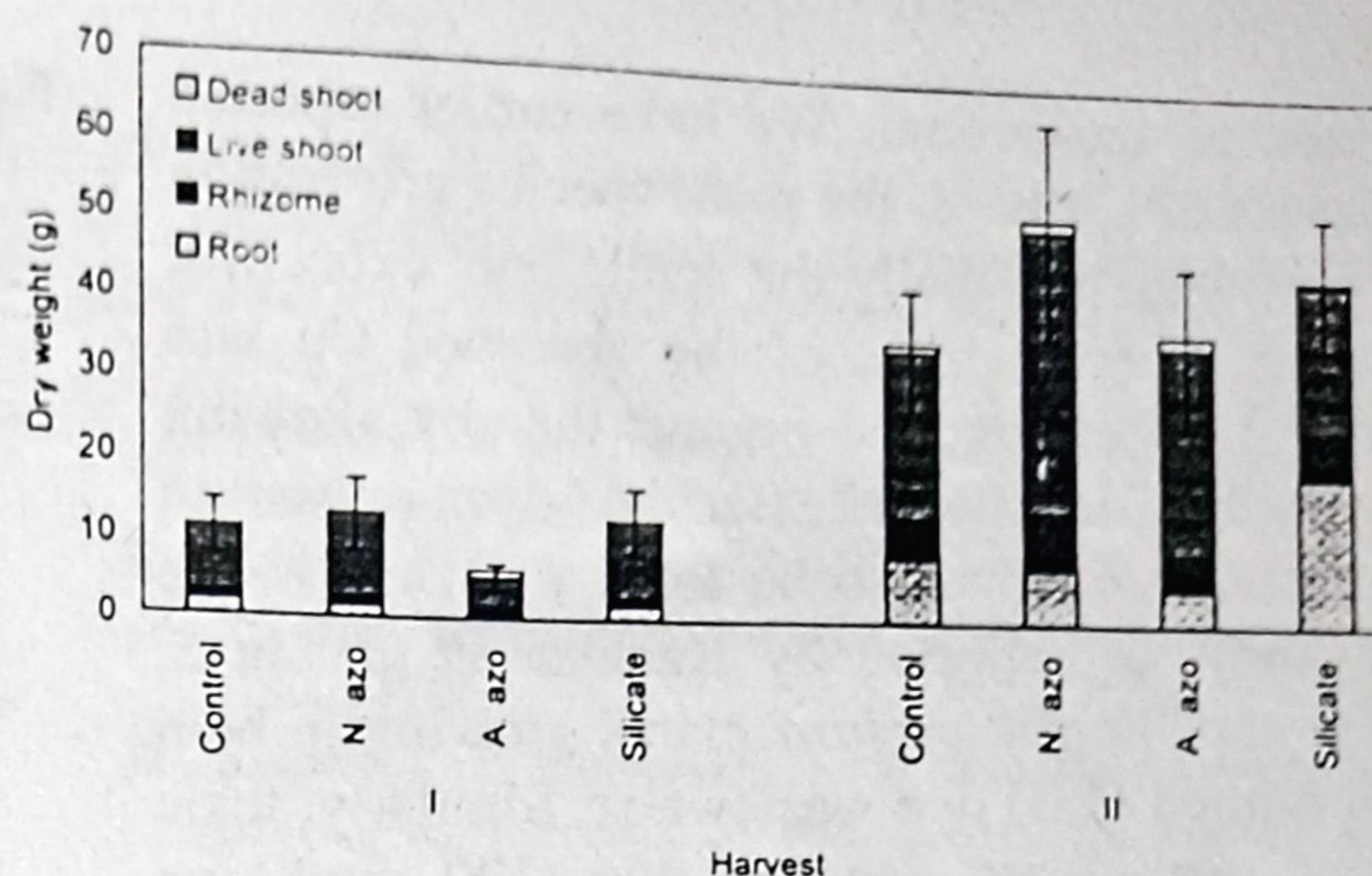


Fig. 6—Mean dry weight ($\pm$ SD) of *Phragmites* plants growing in tap water (control), azo (N. Azo=Neutral azo; A. Azo=Azo acidic) and silicate wastewaters

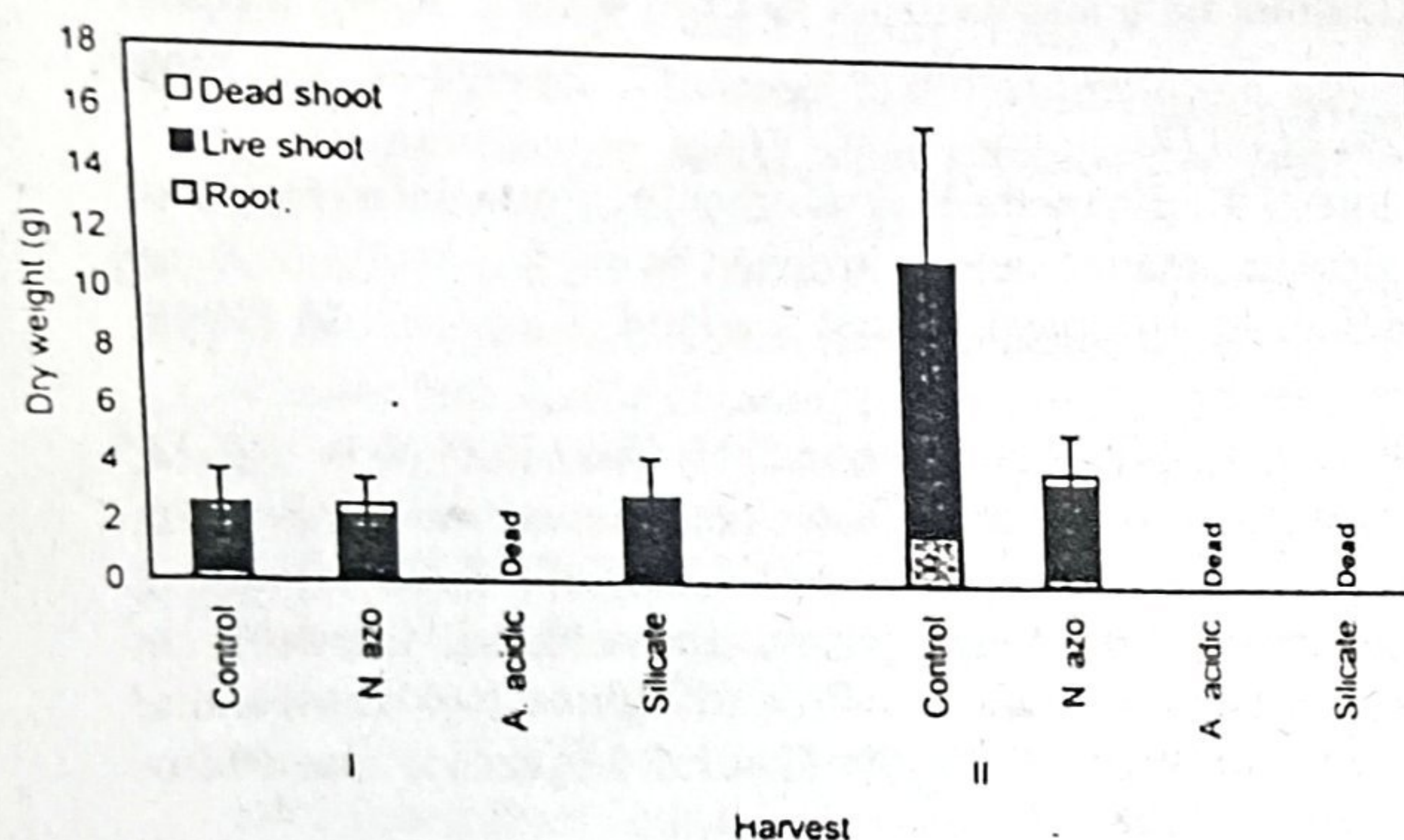


Fig. 4—Mean dry weight ($\pm$ SD) of *Polygonum* plants growing in tap water (control), azo (N. Azo=Neutral azo; A. Azo=Azo acidic) and silicate wastewaters

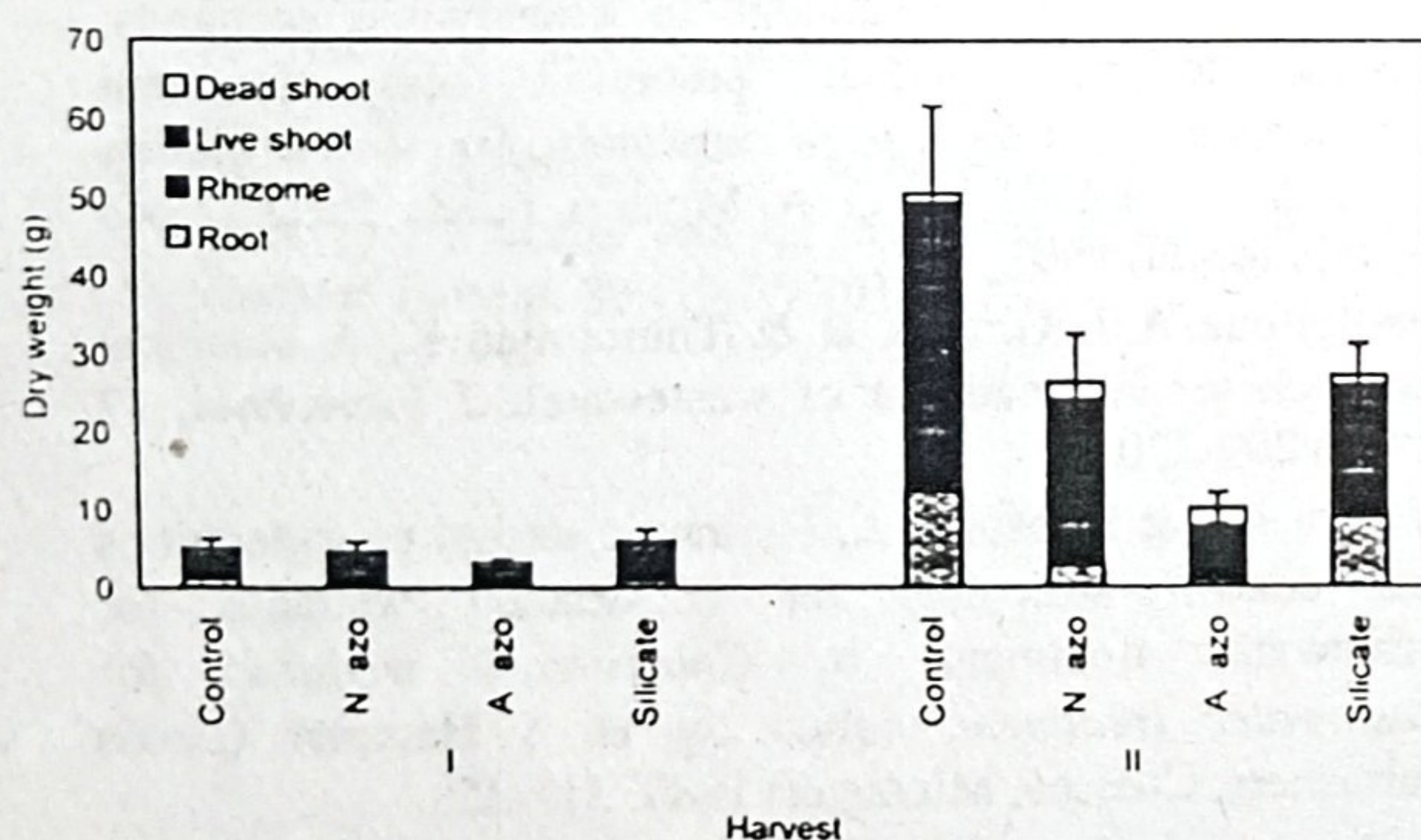


Fig. 5—Mean dry weight ($\pm$ SD) of *Typha* plants growing in tap water (control), azo (N. Azo=Neutral azo; A. Azo=Azo acidic) and silicate wastewaters

Field Study

During the first year, *Typha* shoots (30-50 shoots m^{-2}) multiplied fast, outnumbering *Phragmites* (20-25 shoots m^{-2}), but they failed to regenerate after the senescent phase (winter 2001). *Phragmites*, however, continued to grow well, spreading in the space made available due to death of *Typha* plants. In October

2002, the density of *Phragmites* shoots reached 213 ± 90 shoots m^{-2} , while their aboveground biomass was 3595 ± 1300 g m^{-2} . Unlike *Typha*, *Eichhornia* shoots died within 15 d of their introduction in the wetland. Thus, the results of the field study simulate with those of laboratory studies.

Discussion

The present study has revealed the varied tolerance of aquatic macrophytes towards textile wastewaters, which may be ascribed to their extreme pH values, high Cu^{2+} content and presence of dyes (Table 1). The adverse effect of both low as well as high pH on the growth of aquatic plants has also been reported by other workers. The optimum growth of *Eichhornia* was reported at pH 7^{19,20}, which decreased slightly at pH 10²⁰, while pH 4 was highly toxic²¹. *Azolla* also died both in acidic (pH 4) and alkaline water^{22,23}. Below pH 3 and above pH 9, the protoplasm of the root cells of most vascular plants is severely damaged²⁴. This explains the toxicity of acidic-azo and alkaline silicate water towards aquatic plants in the present study. Among the 10 species of macrophytes, only *Phragmites* had broad amplitude for pH tolerance, growing well in the alkaline, neutral and acidic wastewaters.

Exposure to heavy metals (in the synthetic wastewaters) decreased the size and number of fronds in *Salvinia natans*^{25,26}, length of the longest leaf and roots in *Typha*²⁷, dry weights of *Azolla*, *Ceratophyllum*, *Myriophyllum*, *Salvinia molesta*, and *Spirodela*; the effect being proportional to their concentration^{26,28-31}. This also resulted in death of almost 35% shoots in *S. molesta*²⁶. In the present study also, the acidic-azo wastewater containing higher level of Cu^{2+} was more toxic to plants in

comparison to azo-neutral. We have earlier reported higher levels of Cu in all the plant species growing in azo-wastewaters, especially the acidic one; expect for *Phragmites*, wherein most of the absorbed Cu was retained in the belowground organs, thereby allowing better growth of its shoots³². Besides, dyes present in the wastewaters might also be toxic to plants in the present study, as reported by Kanekar *et al*³³ in *S. polyrhiza* and *Wolffia arrhiza* plants growing in both raw and diluted (1:3) dye wastewater. Similarly, toxic effects of methyl red—an azo dye (100 mg/L) on *Phragmites* shoots brought the reduction in leaf size, followed by marked etiolation and leading to leaf fall when it was exposed for more than 60 d³⁴.

Amongst the 10 aquatic macrophytes screened, *Phragmites* was found to be the only tolerant species, multiplying rapidly in dye wastewaters both in the laboratory as well as in field studies, resulting in considerable shoot density and biomass to achieve maximum translocation of water and assimilation of nutrients. This may be ascribed to increased availability of nitrogen (as urea and nitrite) in the azo wastewaters, as noted in rice³⁵. The growth of underground organs was also better in this species, thereby providing maximum surface area to assimilate pollutants and also for microbial growth in the rhizosphere.

It is, thus, clear that among the various plant species studied, *Phragmites* is the only plant species tolerant to textile wastewaters and, therefore, it has been used for polishing partially treated textile wastewaters in a constructed wetland at Sanganer³⁶. However, the highly sensitive species, such as *Ceratophyllum*, *Azolla*, *Lemna* and *Spirodela*, to wastewaters may also be used as a marker for assessing toxicity of textile dye wastewaters; more particularly *Lemna*, since it allows comparison of toxicity of textile wastewaters with other pollutants/toxic substances^{37,38}. Besides, its smaller size and wide spread occurrence in the water bodies would facilitate a convenient experimental study.

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