

Impacts of Inorganic Turbidity on Diquat Efficacy Against *Egeria densa*

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ABSTRACT

In clear water, diquat [6,7-dihydrodipyrido (1,2-1a:2',1'-c) pyrazinediium dibromide] provides excellent submersed plant control at low concentrations, such as <0.5 mg active ingredient (ai) L⁻¹; however, turbid water conditions can interfere with the activity and effectiveness of this herbicide. Little work has been done to examine what ranges of turbidity caused by different suspended sediment types affect diquat efficacy against a target species. A growth chamber study was conducted using diquat against the submersed macrophyte egeria (*Egeria densa* Planch.) under a range of turbid conditions. Two materials were used to create turbid water conditions: 100% bentonite clay for a “worst-case” scenario and a natural partial-clay (20% clay). Results indicated that a high rate of diquat (2 mg ai L⁻¹) controlled egeria under relatively low levels of turbidity (5-10 NTU) using bentonite clay; however, higher levels (25 to 50 NTU) of turbidity essentially blocked effectiveness of diquat when applied at all rates tested (0.5, 1, 2 mg ai L⁻¹). When using a natural partial-clay sediment, rates of 1 to 2 mg ai L⁻¹ diquat provided good control of egeria in moderately turbid water (15 NTU). Additional evaluations using different clay types would be useful to determine the effect of inorganic turbidity on diquat efficacy.

Key words: Suspended clay, colloidal particles, Brazilian elodea, Reward®.

INTRODUCTION

It has been reported that turbidity reduces diquat [6,7-dihydrodipyrido (1,2-1a:2',1'-c) pyrazinediium dibromide] efficacy against submersed vegetation (Bowmer 1982b, Fox and Murphy 1990); however, early diquat field trials were effective in seasonal control of hydrilla (*Hydrilla verticillata* (L.f.) Royle) in Florida irrigation canals despite turbid water (Mackenzie and Hall 1967, Mackenzie 1969). Other than observation, little work has been done to quantify a relationship between diquat efficacy and turbidity against a target plant species.

Turbidity in surface waters may be caused by suspended organic matter, sediment, and other inorganic particles that cloud the water column and reduce light penetration. Cationic herbicides, such as diquat, are strongly adsorbed by

these suspended particles, especially negatively charged clays, (Weber et al. 1965, Coats et al. 1966, Faust and Zarins 1969, Simsiman and Chesters 1975, Bowmer 1982a, b, Narine and Guy 1982). In a laboratory study, using a cation resin column, montmorillonite clay rapidly adsorbed 1.4 mg L⁻¹ of diquat per gram clay; moreover, this adsorption was largely irreversible (Weber et al. 1965). A calcium form of bentonite adsorbed diquat to capacity (in accord with the CEC of 86 meq 100 g⁻¹) within 10 minutes in another laboratory study (Faust and Zarins 1969). In the field, Bowmer (1982a) found adsorption by illite and kaolinite clay particles was sufficient to inactivate 0.5 mg L⁻¹ of diquat in Australian irrigation canals. In these systems, turbidity ranged from 10 to 80 nepheloturbidimetric units (NTU) caused by suspended sediment that was 40 to 60% clay. These laboratory and field studies illustrated that quantity and type of clay present may be a critical factor in determining adsorption of diquat and, therefore, herbicide efficacy.

We conducted a small-scale study using diquat and egeria (*Egeria densa* Planch), in which three herbicide rates were compared at varying aqueous turbidity levels. Turbidity in the water column was created using bentonite clay and a partial-clay sediment from Texas. The objective of this study was to define a quantitative relationship between turbidity levels and herbicide concentration to better predict diquat efficacy in field applications.

MATERIALS AND METHODS

This study was conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS in a controlled environment growth chamber. The chamber contained 52 vertical aquaria (50 L capacity) that were plumbed to conduct flow-through exposures with continuous air circulation to provide complete mixing of water (Smart and Baroko 1984). During this study, the photoperiod was 14L:10D, with a light intensity of 575 ± 50 μmol m⁻² sec⁻¹, and a temperature of 23 ± 2C.

Four healthy apices (15 cm) of egeria (Suwannee Laboratories, Lake City, FL) were planted in each of 468 glass beakers (300 ml capacity) containing sediment from Brown's Lake, Vicksburg, MS and capped with sand to prevent the sediment from mixing with the water column. Sediment was amended with ammonium chloride to provide adequate nutrients for plant growth. Nine beakers were placed in each vertical aquarium and plants grew to form a lush surface canopy in four weeks. One beaker was removed from each aquarium one day before treatment, dried for 48 h at 70C, and weighed to provide an estimate of pretreatment biomass.

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²Diquat applied as Reward®, from Syngenta Professional Products, Wilmington, DE. Mention of a trade name is not intended to recommend the use of one product over another.

Treatments were assigned to individual aquaria in a completely randomized manner and replicated three times. One set of aquaria with no turbidity and no herbicide treatment served as references. Five turbidity levels, 0, 5-10, 15, 25, and 50 NTU, using bentonite clay were used to evaluate three diquat rates, 0.5, 1.0, and 2.0 mg ai L⁻¹, calculated as the salt, at a 3 h exposure time. Using a field-collected sediment from Texas, one turbidity level, 15 NTU, was used to evaluate two rates of diquat, 1.0, and 2.0 mg ai L⁻¹, calculated as the salt, at a 3 h exposure time. Rates and exposure times were chosen based on previous research at ERDC that suggested in clear water systems diquat provides near complete control of Eurasian watermilfoil (*Myriophyllum spicatum* L.), at concentrations as low as 0.1 mg ai L⁻¹, for as little as 0.5 h (authors' unpublished data).

Bentonite clay was selected because it is common across east Texas and the southeastern United States (Hosterman and Patterson 1992). It is likely that lakes in this region would have a high percentage of this clay type suspended in the water column. Bentonite (AlO₃-4SiO₂-H₂O) consists primarily of montmorillonite clay [(Al,Mg)₂Si₄O₁₀(OH)₂·nH₂O] and usually contains some Mg, Fe, and CaCO₃. The cation exchange capacity (CEC) is high, 100 meq 100 g⁻¹, because there is a high degree of substitution on the binding sites which are located throughout the clay crystal lattice (Worrall 1968). Using a finely ground powder of 100% bentonite (laboratory grade, Fisher Scientific, Dallas, TX) approximated the "worst-case scenario" and was compared to a sediment that could potentially be suspended naturally in a reservoir in Texas. This sediment was field collected from a pond at the ERDC-Lewisville Aquatic Ecosystem Research Facility (LAERF), near Lake Lewisville, TX. An X-ray diffraction analysis (<2μ) showed that the Texas sediment contained 20% clay that was primarily montmorillonite with small amounts of illite [(K, H₃O)Al₂Si₃AlO₁₀(OH)₂] and kaolinite [Al₂Si₂O₅(OH)₄] clays. This sediment had an intermediate CEC of 35 meq 100 g⁻¹.

Using bentonite clay and the Texas sediment to create turbid conditions, a mixture of sediment and water (500 ml) was stirred continuously for 24 h, then poured into each treatment aquarium 0.5 h before herbicide application. This slurry created turbidity in the water column, while some sediment particles also coated plant stems and leaves. A stock solution of diquat² was prepared (4.44 g ai L⁻¹) and applied to aquaria to achieve target treatment concentrations. During herbicide exposure, turbid conditions were maintained by vigorously bubbling air into the water column augmented with occasional physical stirring with a meter stick; turbidity was monitored every 0.5 h with a portable nephelometer (Hach Model 16800). After 3 h, peristaltic pumps evacuated the treated turbid water and fresh clear water was pumped into the aquaria.

Water samples were collected (250 ml amber Nalgene bottles) to determine the amount of diquat in the water column 0.1, 0.5, 1, 2, and 3 h after treatment (HAT). Samples were frozen immediately after collection and kept in the dark until analysis. Before analysis, samples were thawed, adjusted to a pH of 10.5, then filtered (0.45 μm pore size). Analytical procedures employed were Method 549 published by the U.S. Environmental Protection Agency (Hodgeson and Bashe 1990).

Solid phase extraction (SPE) cartridges were used as a pre-treatment for cleaning waters samples and concentrating the diquat. The SPE cartridges were Waters Sep-Pak vac 6 cc (500 mg) C₈ cartridges, which were placed on a 12-place SPE-Pak vacuum manifold (JT Baker PN 7018-00). After column conditioning procedures, an aliquot of 250 ml water sample was filtered through the SPE cartridges to a final elution of 5 ml with methanol. The concentrated diquat was removed from the column with an eluting solution, then analyzed for diquat by high performance liquid chromatography (HPLC).

The HPLC system consisted of a Waters 510 delivery pump, Waters 486 UV detector, Waters 746 data integrator, and incorporating a Waters & Bondapak C₁₈, 3.9 × 300 mm HPLC column. Diquat concentrations were determined by comparison of the detector response by peak area for the samples against the peak area response obtained from known standards of diquat. Standards were prepared from analytical grade diquat obtained from Syngenta Professional Products. The HPLC conditions were set as follows: chart speed was 0.25 cm min⁻¹; column temperature was 35C; flow rate was 2.0 ml min⁻¹; wavelength was 308 nm; sample injection volume was 200 μg L⁻¹. Run time for this compound was 4 min with the diquat peak registering at 2.5 min. Reporting limit for this method is 0.44 μg L⁻¹. Standard check samples indicated a 104.06% recovery (±11.2 SD, n = 5), which corresponded to listed method precision for reagent water (90% recovery, ±10.9 SD, n = 7). Only two replicates are reported for 0 NTU/2.0 mg ai L⁻¹ treatment due to possible sample contamination.

Tissue samples (0.1 to 0.2 g) were removed from each aquarium at 48 HAT and 1, 2, and 5 weeks after treatment (WAT) and analyzed for total chlorophyll content (Hiscox and Israelstam 1979) using 10 ml dimethylsulfoxide (DMSO) for extraction. Chlorophyll content provided an assessment of the general health of a plant following herbicide treatment and was reported as mg chlorophyll g⁻¹ plant fresh weight.

At 3 and 6 WAT, four beakers were removed from each aquarium, shoot biomass was collected, dried for 48 h at 70C, then weighed. Shoot biomass is reported as g dry weight (DW).

Biomass and chlorophyll data were subjected to a two-way analysis of variance (ANOVA) to determine the herbicide and turbidity effects. If statistical differences occurred between treatments, means were separated using the Student-Newman-Keuls method (SNK; p = 0.05) to compare effects of the three herbicide concentrations at the same turbidity and the Dunnett's test (p = 0.05) to compare treated plants to untreated references. Water residue data were subjected to a three-way ANOVA to determine the effects of time, turbidity, and herbicide concentration. Multiple regression analyses were completed to determine relationships between turbidity and target herbicide concentration on water residue and egeria biomass data.

RESULTS AND DISCUSSION

Mean turbidity (±1 SE) for both the bentonite clay and Texas sediment treatments were 6.9 ± 0.4 (5-10 NTU), 14.2 ± 0.8 (15 NTU), 26.5 ± 0.9 (25 NTU), and 50.3 ± 2.4 (50 NTU) during herbicide exposure.

Water residues of the no turbidity (0 NTU) treatments were near the target diquat concentration for the first part of the exposure time (Table 1). After one hour, concentrations dropped off as diquat was absorbed into the plant. Rapid uptake of diquat has been reported for other submersed macrophytes, including elodea (Davies and Seaman 1968) and hydrilla (Cassidy and Rodgers 1989) well as terrestrial species (Brian 1967).

Water residues of the turbidity treatments showed that diquat concentrations in the water column varied considerably 0.1, 0.5, 1, 2 and 3 HAT (Table 1). There was a statistically significant interaction between application rate and turbidity, where diquat concentration depended on the level of turbidity present ($F = 3.288, p = 0.005$).

The only turbidity treatment that approximated target concentrations was the 2.0 mg ai L⁻¹/5-10 NTU bentonite treatment, which significantly suppressed egeria biomass (Figure 1). This treatment had an initial diquat concentration of 1.57 ± 0.25 mg ai L⁻¹, which dropped to 0.54 ± 0.13 mg ai L⁻¹ by 3 HAT. Mean diquat concentrations for the rest of the bentonite turbidity treatments ranged from 0.36 to 0.44 mg ai L⁻¹ for all application rates. There was a statistically significant interaction between application rate and sample time ($F = 2.216, p = 0.031$), in which significant differences in diquat concentrations were detected right after application (0.1 and 0.5 HAT); however, by 1.0 HAT, there were no differences between rates. Simsman and Chesters (1976) found that montmorillonite sediments completely adsorbed 5 mg ai L⁻¹ diquat in one hour when these sediments were agitated. Diquat is quickly and strongly adsorbed to montmo-

llonite because its high CEC, large surface area (Faust and Zarins 1969), and binding sites that are located in the interior as well as on the surface of the clay crystal lattice (Weber et al. 1965, Worrall 1968).

Concentrations for the 1.0 mg ai L⁻¹/15 NTU Texas treatment ranged from 0.44 ± 0.04 to 0.63 ± 0.08 mg ai L⁻¹ for the 3-h exposure time (Table 1). Concentrations for the 2.0 mg ai L⁻¹/15 NTU Texas treatment varied from 1.06 ± 0.21 mg ai L⁻¹ detected 0.1 HAT to 0.54 ± 0.04 mg ai L⁻¹ at 3 HAT (Table 1). Although herbicide concentrations were half of the target, there were significant decreases in biomass for both of these treatments (Figure 1). Apparently, diquat was not adsorbed as strongly by the Texas sediment as by the bentonite, enabling the egeria to take up some of the herbicide. The Texas sediment contained 20% clay that was a mixture of montmorillonite, kaolinite, and illite clays. It had a CEC of 35 meq 100 g⁻¹, which was substantially less than the CEC of the 100% bentonite clay (CEC = 100 meq 100 g⁻¹). Diquat does not tightly bind to clays that have a lower CEC values, such as kaolinite and illite, because these clays have a smaller surface areas with binding sites that are located only on the surface of the clay crystal lattice (Weber et al. 1965).

A multiple regression was completed to determine a quantitative model for predicting diquat concentration after a 3-h exposure time using the rates (0.5, 1.0 and 2.0 mg ai L⁻¹) and turbidity levels tested with bentonite clay (0, 5-10, 15, and 25 NTU) as variables. The model:

$$\text{diquat concentration at 3 h} = 0.572 + (0.138 * \text{target rate}) - (0.0168 * \text{turbidity})$$

TABLE 1. WATER RESIDUES AT 0.1, 0.5, 1, 2, AND 3 HOURS AFTER TREATMENT (HAT) WITH DIQUAT UNDER TURBID CONDITIONS CAUSED BY BENTONITE CLAY (BEN) AND A TEXAS SEDIMENT (TX). RESIDUES ARE EXPRESSED AS DIQUAT CONCENTRATION (MG AI L⁻¹ ± 1 SE; N = 3).

Treatment	Diquat concentration (mg ai L ⁻¹)				
	0.1 HAT	0.5 HAT	1 HAT	2 HAT	3 HAT
0 NTU					
0.5 mg ai L ⁻¹	0.67 ± 0.11	0.75 ± 0.18	0.65 ± 0.13	0.47 ± 0.04	0.60 ± 0.04
1.0 mg ai L ⁻¹	1.09 ± 0.35	1.22 ± 0.47	0.89 ± 0.05	0.59 ± 0.14	0.50 ± 0.11
2.0 mg ai L ^{-1a}	1.32 ± 0.45	1.57 ± 0.55	0.94 ± 0.02	0.64 ± 0.23	0.55 ± 0.17
BEN 5-10 NTU					
0.5 mg ai L ⁻¹	0.35 ± 0.01	0.56 ± 0.21	0.45 ± 0.10	0.45 ± 0.02	0.36 ± 0.02
1.0 mg ai L ⁻¹	0.42 ± 0.02	0.42 ± 0.03	0.42 ± 0.05	0.38 ± 0.01	0.57 ± 0.13
2.0 mg ai L ⁻¹	1.57 ± 0.25	0.87 ± 0.18	0.65 ± 0.12	0.53 ± 0.08	0.54 ± 0.13
BEN 15 NTU					
0.5 mg ai L ⁻¹	0.35 ± 0.01	0.35 ± 0.01	0.38 ± 0.03	0.35 ± 0.01	0.38 ± 0.02
1.0 mg ai L ⁻¹	0.39 ± 0.04	0.36 ± 0.01	0.35 ± 0.01	0.36 ± 0.01	0.38 ± 0.02
2.0 mg ai L ⁻¹	0.41 ± 0.01	0.43 ± 0.05	0.45 ± 0.05	0.45 ± 0.09	0.40 ± 0.02
TX 15 NTU					
1.0 mg ai L ⁻¹	0.48 ± 0.03	0.63 ± 0.08	0.56 ± 0.05	0.44 ± 0.04	0.44 ± 0.02
2.0 mg ai L ⁻¹	1.06 ± 0.21	0.69 ± 0.15	0.63 ± 0.08	1.00 ± 0.18	0.54 ± 0.04
BEN 25 NTU					
0.5 mg ai L ⁻¹	0.38 ± 0.03	0.37 ± 0.01	0.36 ± 0.02	0.41 ± 0.03	0.36 ± 0.01
1.0 mg ai L ⁻¹	0.38 ± 0.01	0.34 ± 0.01	0.37 ± 0.01	0.36 ± 0.02	0.35 ± 0.01
2.0 mg ai L ⁻¹	0.36 ± 0.01	0.55 ± 0.19	0.40 ± 0.05	0.42 ± 0.04	0.39 ± 0.03
BEN 50 NTU					
1.0 mg ai L ⁻¹	0.36 ± 0.01	0.35 ± 0.01	0.38 ± 0.03	0.35 ± 0.01	0.39 ± 0.01

^an = 2.

TABLE 2. CHLOROPHYLL CONTENT OF *EGERIA APICES* FOLLOWING TREATMENT WITH DIQUAT UNDER TURBID CONDITIONS CAUSED BY BENTONITE CLAY (BEN) AND A TEXAS SEDIMENT (TEX). SAMPLES WERE COLLECTED 48 HOURS AFTER TREATMENT (HAT), 1, 2, AND 5 WEEKS AFTER TREATMENT (WAT).

Treatment	Chlorophyll content (mg g ⁻¹ fresh weight) ^a			
	48 HAT	1 WAT	2 WAT	5 WAT
REFERENCE	0.89 ± 0.06	0.82 ± 0.07	0.98 ± 0.05	0.88 ± 0.07
0 NTU				
0.5 mg ai L ⁻¹	0.44 ± 0.03*	0*	0*	0*
1.0 mg ai L ⁻¹	0.44 ± 0.05*	0*	0*	0*
2.0 mg ai L ⁻¹	0.58 ± 0.04*	0*	0*	0*
BEN 5-10 NTU				
0.5 mg ai L ⁻¹	0.88 ± 0.04	0.89 ± 0.04	1.06 ± 0.05	0.94 ± 0.05
1.0 mg ai L ⁻¹	0.72 ± 0.09	0.58 ± 0.05*	0.60 ± 0.14*	0.85 ± 0.04
2.0 mg ai L ⁻¹	0.50 ± 0.05*	0*	0*	0*
BEN 15 NTU				
0.5 mg ai L ⁻¹	0.81 ± 0.06	0.78 ± 0.05	0.89 ± 0.02	0.82 ± 0.05
1.0 mg ai L ⁻¹	0.80 ± 0.03	0.88 ± 0.05	0.98 ± 0.02	0.94 ± 0.04
2.0 mg ai L ⁻¹	0.64 ± 0.07	0.62 ± 0.07	0.65 ± 0.08*	1.09 ± 0.05
TEX 15 NTU				
1.0 mg ai L ⁻¹	0.60 ± 0.04*	0.15 ± 0.10*	0*	0*
2.0 mg ai L ⁻¹	0.48 ± 0.04*	0.15 ± 0.09*	0*	0*
BEN 25 NTU				
0.5 mg ai L ⁻¹	0.79 ± 0.04	0.74 ± 0.03	0.88 ± 0.03	0.84 ± 0.03
1.0 mg ai L ⁻¹	0.82 ± 0.04	0.79 ± 0.05	0.91 ± 0.04	0.92 ± 0.05
2.0 mg ai L ⁻¹	0.75 ± 0.08	0.77 ± 0.03	1.01 ± 0.08	0.73 ± 0.02
BEN 50 NTU				
1.0 mg ai L ⁻¹	0.93 ± 0.08	0.83 ± 0.06	0.81 ± 0.03	0.68 ± 0.05

^aChlorophyll content of 0 indicates plant death. Values denoted by asterisks are significantly different from the untreated control according to Dunnett's test at the p = 0.05 significance level (n = 3).

was statistically significant (n = 175; F = 31.144, p < 0.001, R² = 0.27). The low R² value indicates that this model is probably not adequate for predicting diquat concentration in the field. More data gathered from other studies or diquat field applications would be useful in developing a model that could better predict herbicide concentration.

All rates of diquat in treatments with no added turbidity (0 NTU) eliminated egeria biomass 3 and 6 WAT (Figure 1). Plant injury was evident at 48 HAT by low chlorophyll concentrations for all rates in the 0 NTU treatment (Table 2). Plant death occurred at 1 WAT with no regrowth.

One-hour exposure of 2 mg ai L⁻¹ diquat was effective in significantly controlling egeria in the 5-10 NTU bentonite treatment (Figure 1). Plant injury was evident at 48 HAT with plant death occurring 1 WAT (Table 2). Plants treated with 1.0 mg L⁻¹ diquat in the 5-10 NTU bentonite treatment exhibited injury at 1 WAT (Table 2); however, at 3 WAT, there was no significant reduction in biomass compared to the untreated reference, signifying plant recovery (Figure 1). Egeria biomass was comparable to the reference for the 15, 25, and 50 NTU bentonite clay treatments (Figure 1). Moreover, chlorophyll content showed there was no significant plant injury from these any of these treatments (Table 2).

In contrast, there was significant plant injury for both the 1 and 2 mg ai L⁻¹ rates of the 15 NTU Texas sediment treatment (Table 2). Chlorophyll concentrations were low at 48 HAT, further decreasing at 1 WAT. By 2 WAT, the egeria was starting to decay. The plant biomass harvested at 3 and 6

WAT was a remnant of black stems. These rates provided good control of egeria in moderately turbid water created with a partial-clay sediment (Figure 1); however, they might not be as effective at higher turbidity levels (authors' personal observation).

The quantitative model that was statistically significant for predicting egeria biomass after diquat application in turbid water was a linear regression (n = 39, F = 30.6353, p < 0.0001, R² = 0.45):

$$\text{egeria biomass} = 0.2617 + (5.0249 * \text{turbidity})$$

It was determined from the multiple regression analysis that target rate was not a significant variable (p < 0.179) in predicting egeria biomass from the data in this study. Therefore, unless turbidity and predominant clay type are known, over compensating for turbidity by applying almost double the target rate (as suggested by Bowmer 1982a), would be futile. Moreover, accumulated seston on aquatic plants greatly affects the adsorption of diquat in addition to turbidity in the water column (Bowmer 1982b).

These results show that turbid conditions can significantly decrease diquat efficacy. If turbidity is caused by a sediment with a moderate amount of clay (20%), high rates of diquat (2 mg ai L⁻¹) may be needed to overcome a turbidity of just 15 NTU. More data gathered from small-scale evaluations using sediments with different clay types would be necessary to develop quantitative models that predict diquat efficacy in the field.

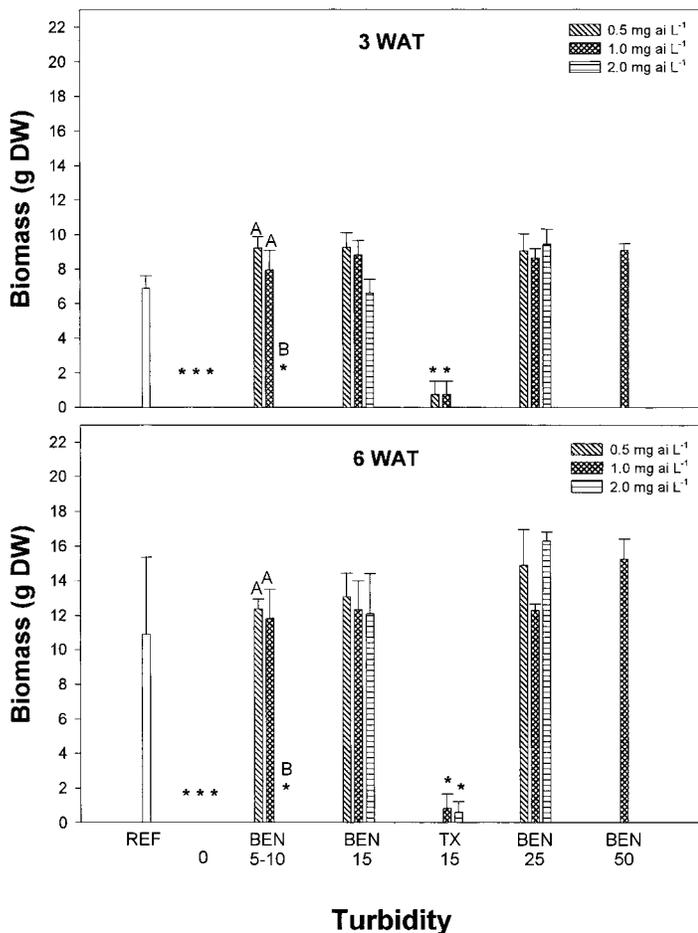


Figure 1. Shoot biomass of egeria (mean \pm 1 SE) treated with diquat under turbid conditions (0, 5-10, 15, 30, and 60) using bentonite clay (BEN.) and a Texas sediment (TX). Biomass was harvested 3 and 6 weeks after treatment (WAT). Letters above the bars indicate significant differences among herbicide rates at each turbidity (SNK test, $p = 0.05$, $n = 3$). Asterisks represent significant differences between treatments and the untreated reference (Dunnett's test, $p = 0.05$, $n = 3$).

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