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Amelioration of the Photo-Induced Toxicity of Polycyclic Aromatic Hydrocarbons by a Commercial Humic Acid

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The ability of a commercial (Aldrich Chemical Co.) humic acid (AHA) to ameliorate the photo-induced toxicity of polycyclic aromatic hydrocarbons (PAHs) was examined using *Lemna gibba* L. (G3). Plants were exposed to anthracene and benzo(*a*)pyrene both with and without AHA and grown under visible light as well as lighting that simulates relative abundances of UV-A and UV-B in natural sunlight (SSR). Modest additions of 1.6 mg · L^{-1} AHA were sufficient to ameliorate the photo-induced toxicity of 2 mg · L^{-1} anthracene by improving growth rates to nearly 50% of controls and inducing minor recovery from complete chlorosis in the most highly affected plants. Benzo(*a*)pyrene induced minor, but significant, chlorosis under SSR, and AHA additions always increased growth rate and chlorophyll content, although to less of a degree than anthracene toxicity under SSR. The protective effects of AHA on anthracene toxicity increased linearly with increases in AHA concentrations up to 6.2 mg · L^{-1}. Slopes of these relationships changed in the presence of UV light relative to visible light treatments; thus UV changed the extent to which AHA mediates PAH toxicity. However, the net effect was still for AHA to ameliorate PAH photo-induced toxicity even though UV has the potential to photooxidize AHA and enhance the production of potentially toxic reactive oxygen species from AHA photosensitization.

INTRODUCTION

Dissolved organic carbon (DOC) represents a wide variety of organic compounds, largely of terrestrial origin, that exist in freshwaters as partially degraded humic and fulvic acids (Thurman, 1985). DOC influences a wide variety of chemical and biological processes in aquatic systems, usually through the chemical complexation and/or adsorption of inorganic and organic chemicals (Wetzel, 1983; Morel, 1983). Both the acute and the chronic impacts of most organic toxicants on freshwater organisms are often ameliorated in the presence of both natural and commercial sources of DOC (Day, 1991; Goodrich *et al*., 1991; Oikari *et al*., 1992; Steinberg *et al*., 1992; Hodge *et al*., 1993). The mechanism likely involves chemical partitioning of the toxicant to DOC, which diminishes its effective concentration and hence biological efficacy (Jaffé, 1991; Knulst, 1992; Lores *et al*., 1993; Kukkonen and Pellinen, 1994; Kopinke *et al*., 1995). For a limited number of contaminants, however, DOC either exhibits no significant influence on toxicity or is associated with enhanced toxicity (Oikari *et al*., 1992; Steinberg *et al*., 1992).

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds consisting of two or more fused benzene rings. PAHs are priority pollutants in many industrial areas and occur in living organisms at significant concentrations near these sites (Neff, 1979; Lee *et al*., 1981). A unique property of PAHs is that natural levels of ultraviolet (UV) radiation in sunlight enhances their toxicity to aquatic animals (Bowling *et al*., 1983; Oris and Giesy, 1985; Holst and Giesy, 1989; Monson *et al*., 1995) and aquatic plants (Greenberg *et al*., 1993; Huang *et al*., 1993; Gala and Giesy, 1994). PAH photo-induced toxicity results either from photomodification (oxidation) of the PAH parent compounds to more toxic forms (Huang *et al*., 1993), from photosensitization reactions (Newsted and Giesy, 1987; Veith *et al*., 1995), or more likely from a combination of both processes (Greenberg *et al*., 1993).

UV also induces a variety of photochemical changes in DOC, including photochemical degradation of natural humic substances into more labile forms (Zepp, 1988; Schindler *et al*., 1996; Bushaw *et al*., 1996) and production of reactive oxygen species (e.g., singlet oxygen, peroxy radicals, etc.; Cooper *et al*., 1989; Hoigné *et al*., 1989; Canonica and Hoigné, 1995). Production of these reactive oxygen species by both commercial (Steinberg *et al*., 1992) and natural (Canonica *et al*., 1995; Wang *et al*., 1995) humic substances is known to drive photooxidation of organic contaminants. Similar to PAHs, photosensitization of humic acids (HAs) thus may also induce direct negative biological effects. Therefore, UV is likely to induce a variety of changes in the ability of HA to control the fate and bioavailability of PAHs

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to aquatic organisms, as well as the direct effects of the HA on aquatic organisms.

However, the combined influence of UV light on both HA and its ability to control PAH photo-induced toxicity has received little attention. PAHs bind to natural forms of DOC (Johnsen and Gribbestad, 1991; Schlautman and Morgan, 1993; Kopinke et al., 1995), diminishing the toxicity and/or bioavailability of the unmodified parent PAHs (Landrum *et al*., 1985; Kukkonen and Oikari, 1991; Kukkonen and Pellinen, 1994). Only a single study (Oris *et al*., 1990) examined the amelioration of PAH toxicity by the commercial Aldrich Chemical Co. humic acid (AHA) under the influence of a light source containing UV-A and UV-B. They found that AHA alleviated anthracene toxicity to *Pimephales promelas* and *Daphnia magna* primarily by diminishing bioaccumulation. UV light attenuation coefficients were also highly correlated with diminished toxicity, suggesting that AHA may also selectively attenuate UV wavelengths known to induce photo-induced toxicity. Even with these studies, the ability of humic substances to control PAH toxicity in visible light compared to that in light containing UV has not yet been quantified, nor have studies of AHA-induced PAH toxicity amelioration yet been performed using aquatic plants. Both are important toward creating a general understanding of the effects of UV on the ability of DOC to control PAH toxicity to the widest possible variety of aquatic organisms.

Therefore, the influence of the commercial AHA on the amelioration of PAH photo-induced toxicity was examined using the aquatic duckweed *Lemna gibba*. The extent to which AHA was effective in ameliorating PAH impacts was determined under simulated solar radiation (SSR) lighting relative to visible light controls, along with the direct effects, if any, of UV on plant performance in the presence of AHA alone. The effects of AHA were examined on the photo-induced toxicity of two model PAHs, anthracene and benzo(*a*)pyrene, for which the extent of photo-induced toxicity in *L. gibba* under simulated solar radiation is known (Greenberg *et al*., 1993). For anthracene, exposures were also performed over a range of AHA concentrations to quantify changes in the ability of AHA to control anthracene toxicity with and without UV exposure.

MATERIALS AND METHODS

Culture Maintenance and General Experimental Conditions

Cultures of *L. gibba* L. G-3 were maintained axenically on half-strength Hutner's medium and grown under $50\ \mu mol \cdot m^{-2} \cdot s^{-1}$ of continuous cool-white fluorescent light at 24°C (Greenberg *et al*., 1992). During experiments, plants were incubated under either (1) visible light provided by cool-white fluorescent bulbs or (2) a SSR system designed to mimic the spectral quality of natural sunlight with respect to ratios of visible light, UV-A, and UV-B (ASTM, 1995; Greenberg *et al*., 1996). This SSR source consisted of two cool-white fluorescent lamps, one 350-nm photoreactor lamp, and one 300-nm photoreactor lamp (Southern New England Ultraviolet Co., Branford, CT) filtered through two layers of cheesecloth. For both visible and SSR exposure systems, the total visible fluence rate was $100\ \mu mol \cdot m^{-2} \cdot s^{-1}$. Fluence rates and spectral quality were measured using a calibrated spectroradiometer (Oriel Instruments, Stratford, CT) and a quantum light meter (LiCor, Lincoln, NE). Plants exposed to SSR were protected from UV-C by covering them with polystyrene petri dish tops (Phoenix Biomedical, Baxter-Canlab, Missisauga, ON).

Plants were exposed to PAHs and/or AHA in 10 ml of half-strength Hutner's medium placed in 5-cm polystyrene petri dishes. Although PAHs may selectively partition to the polystyrene, PAH assimilation by *L. gibba* is known to be identical when using pyrex containers of the same size and volume (Duxbury *et al*., 1997). Anthracene and benzo(*a*)pyrene stock solutions were prepared by dissolving reagent grade crystals (Sigma Chemical Co., St. Louis, MO) in dimethylsulfoxide (DMSO; Sigma Chemical Co.) such that emulsifying 10 μL of the stock solutions would produce final nominal PAH concentrations of $2\ mg \cdot L^{-1}$ in the 10-mL experimental dishes. These $2\ mg \cdot L^{-1}$ exposures are well within the log-linear portion of dose–response curves for *L. gibba* using this exposure system (Huang *et al*., 1993). Furthermore, at least 50% of the PAHs are known to be assimilated into plant tissues at $2\ mg \cdot L^{-1}$, which also were approximately 10× higher than that assimilated at $0.2\ mg \cdot L^{-1}$ (Duxbury *et al*., 1997). The final DMSO concentration (0.1% v/v) does not affect growth of *L. gibba* and has analytically been confirmed to provide accurate PAH delivery at this concentration without direct toxicity or photochemical interference (Huang *et al*., 1993). Identical DMSO concentrations were added to control (no PAH) treatments in the present experiments.

AHA stock solutions were prepared by dissolving a commercially available sodium salt (Aldrich Chemical Co., Milwaukee, WI; Catalog No. H1,675-2, lot KG01828JZ) in deionized water. Fresh AHA stock solutions were prepared prior to each experiment and stored in the dark at 4°C to minimize bacterial contamination. Small volume (10–80 μL) additions of AHA stocks produced final concentrations of 1.3–$6.2\ mg \cdot L^{-1}$ HA measured as DOC. DOC concentrations were verified, after filtration through glass fiber filters (Whatman GF/F, Maidstone, England), by UV digestion and subsequent analysis of the oxidized CO_2 (Ontario Ministry of Environment and Energy laboratory, Dorset, ON).

Experimental Design

Initial toxicity tests exposed *L. gibba* to $2\ mg \cdot L^{-1}$ of either anthracene or benzo(*a*)pyrene, either with or without

1.6 mg · L^{-1} HA, for 8 days under visible light or SSR. Growth media and AHA/PAH combinations were replaced every 48 h to minimize depletion of ambient nutrient, AHA, and PAH concentrations over time. Leaf numbers were recorded prior to each media replacement and used to calculate growth rates expressed as a doubling frequency (dbl · day^{-1}; Greenberg *et al.*, 1992). Leaf numbers are widely used as biomass surrogates in *Lemna* toxicity testing (ASTM, 1991), and they are significantly correlated to both fresh and dry weight under the experimental conditions used here (Greenberg *et al.*, 1992; Huang *et al.*, 1993). Chlorophyll content was determined at the end of the 8-day exposure by extraction into *N*,*N*-dimethylformamide (Sigma Chemical Co., Greenberg *et al.*, 1992). In addition to the tests run using a single AHA level (1.6 mg · L^{-1}), PAH photo-induced toxicity was examined for anthracene alone in the presence of six AHA concentrations ranging from no addition to 6.2 mg · L^{-1}. Toxicity tests for growth rate and plant chlorophyll content were performed for each anthracene/AHA combination as described above. Tests were repeated under both visible light and SSR to examine the influence of AHA concentration on anthracene photo-induced toxicity. Results were used to derive functional relationships (using least-squares linear regression) between increasing AHA concentration (mg · L^{-1} as DOC) and bioassay endpoint performance. All experiments were performed in triplicate and repeated at least once.

Statistics

The significance of the main treatment effects and their interactions were determined in most cases using two-way analysis of variance (ANOVA). Tukey's HSD post hoc tests were used with significant factors to examine pairwise comparisons of significant differences between treatment means. Linear regression models were used to describe the functional relationship between increasing AHA concentrations and bioassay performance; minimum significant effect concentrations were estimated using one-way ANOVA. All statistical analyses were performed using Systat (SPSS Inc., Evanston, IL) at a significance level of $P < 0.05$.

RESULTS

Amelioration of PAH Photo-Induced Toxicity by Humic Acid

Growth rates were diminished to a minor but statistically significant extent in the presence of both PAHs when *L. gibba* was grown under visible light (Fig. 1A). Relative to controls, growth rates were diminished by 0.023 and 0.058 dbl · day^{-1} in the presence of anthracene and benzo(*a*)pyrene, respectively. In contrast, anthracene was highly toxic when plants were grown under SSR, as represented by a 61% decrease in doubling rates compared to

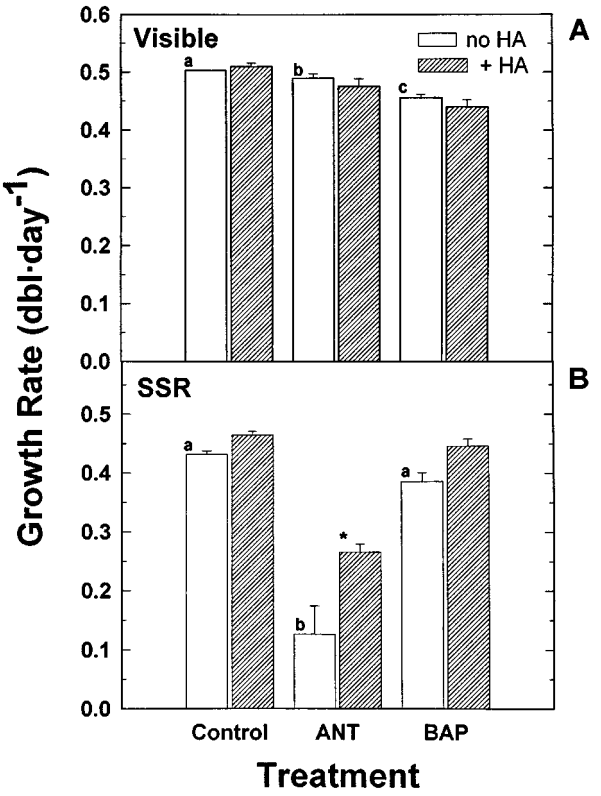

FIG. 1. Effects of 1.6 mg · L^{-1} HA on *L. gibba* growth rate (dbl · day^{-1}) as a function of PAH treatment under (A) visible light and (B) SSR. Significantly different PAH treatments are denoted by different lowercase letters above the bars. Significance of HA treatment on plant response within a single PAH treatment is denoted by an asterisk.

controls (Fig. 1B; $P < 0.001$). Humic acid additions exerted a significant overall positive influence on growth rates for all SSR treatments ($P = 0.002$). Individual pairwise comparisons demonstrated that AHA ameliorated the phototoxic effect of anthracene by enhancing growth rates to ca. 50% of control levels. Benzo(*a*)pyrene induced no significant growth inhibition compared to controls when plants were grown under SSR, nor did AHA additions increase growth relative to plants grown without AHA. Humic acid alone (e.g., with no PAHs present) induced no effect on population growth in the absence of PAHs compared to AHA-free controls.

Similar to growth rates, plant chlorophyll content also decreased in the presence of both PAHs when grown in visible light, but this was only significant for benzo(*a*)pyrene (Fig. 2A; $P < 0.001$). Chlorophyll content was significantly higher overall in the presence of AHA ($P = 0.05$) for plants grown in visible light. Anthracene was again the most effective PAH under SSR, with essentially no detectable chlorophyll (0.006 μg chlorophyll · mg $freshweight^{-1}$, SE = 0.027) remaining by the end of the 8-day exposure period. Significant chlorosis also was induced by benzo(*a*)pyrene when

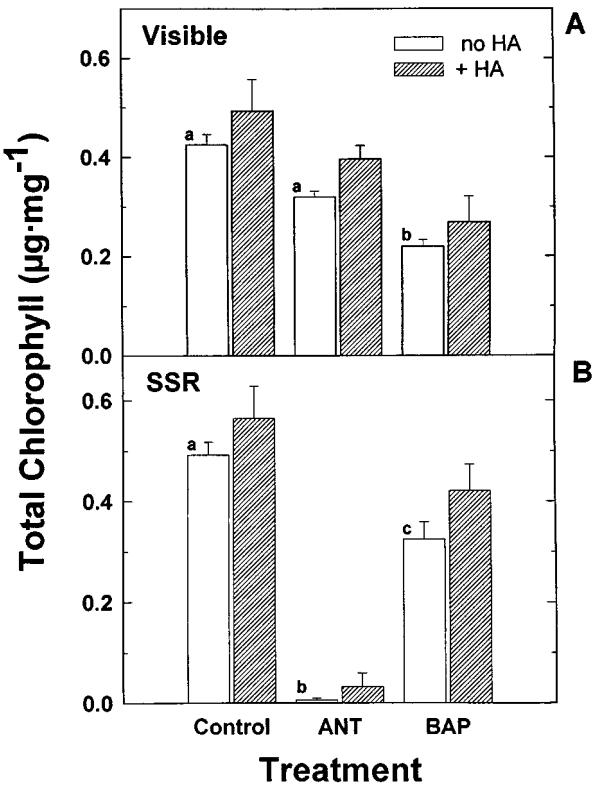

FIG. 2. Effects of 1.6 mg · L^{-1} HA on *L. gibba* total chlorophyll content (µg · mg^{-1}) as a function of PAH treatment under (A) visible light and (B) SSR. Significantly different PAH treatments are denoted by different lowercase letters above the bars. Significance of HA treatment on plant response within a single PAH treatment is denoted by an asterisk.

plants were grown under SSR, but to a much lesser degree than by anthracene (Fig. 2B). Humic acid additions exerted a significant protective effect on chlorophyll contents in all treatments including controls ($P = 0.013$), although individual pairwise comparisons (Tukey's HSD tests) did not detect significant increases in chlorophyll contents within individual PAH treatment pairs.

Impacts of Humic Acid Concentration on Anthracene Photo-Induced Toxicity

Based on the effects of AHA at low concentrations (1.6 mg · L^{-1}) reported above, anthracene photo-induced toxicity was examined in detail across a range of AHA concentrations from no addition (1.3 mg · L^{-1}) to 6.2 mg · L^{-1} DOC. In visible light and in the absence of anthracene, *L. gibba* growth rates were unaffected by increasing AHA concentrations (Fig. 3A, Table 1). Regression analysis suggested a weak but significant decrease in growth rates as AHA increased in the presence of 2 mg · L^{-1} anthracene (Table 1). This is consistent with the results reported above in visible light (Fig. 1), where anthracene did not significantly affect growth rates, relative to treatments without added PAH, regardless of DOC concentration. In contrast, increasing DOC levels under SSR induced a small but significant linear decrease in the growth rate of plants without anthracene present (Fig. 3B, Table 1). However, increasing DOC concentrations exerted a positive linear effect on growth rates in the presence of anthracene in SSR, with 6.2 mg · L^{-1} being required to produce a significant increase in growth compared to AHA-free controls (one-way ANOVA; $P = 0.033$).

In contrast to growth rate, chlorophyll content always increased linearly in the presence of increasing AHA concentration, regardless of which light source was used (Fig. 4). When plants were grown in visible light (Fig. 4A), regression slopes between DOC concentration and chlorophyll content were positive (Table 1), although the effect was stronger in treatments containing 2 mg · L^{-1} anthracene, owing to mild chlorosis in the lower AHA treatments. In both cases, at least 5.1 mg · L^{-1} AHA was required to significantly increase chlorophyll concentration relative to the control and lower DOC treatments (one-way ANOVA; $P < 0.005$ in both cases).

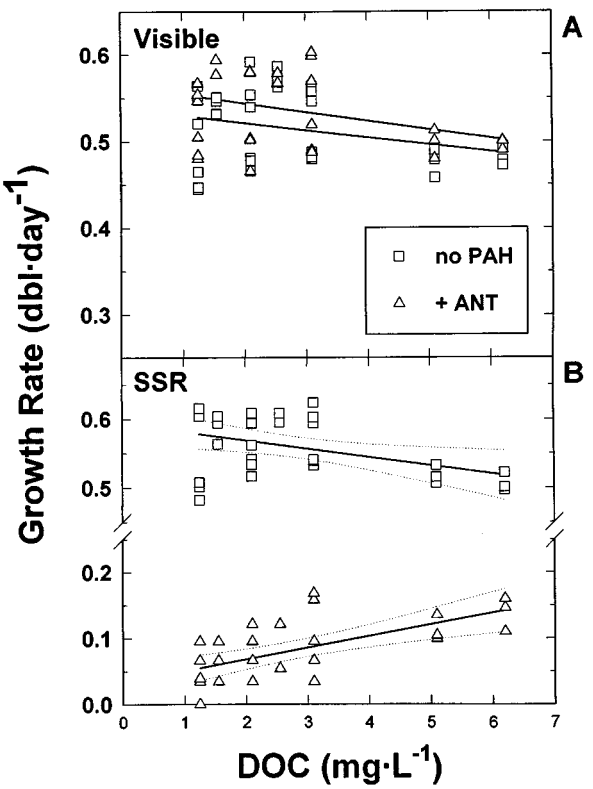

FIG. 3. Effect of increasing HA concentration (mg · L^{-1} DOC) on *L. gibba* growth rate (dbl · day^{-1}) both with and without 2 mg · L^{-1} anthracene, under (A) visible light and (B) SSR. The solid lines denote linear regressions describing the data for each PAH treatment. The 95% confidence limits are given as dashed lines where possible. Regression statistics are given in Table 1.

TABLE 1

Linear Regressions Describing the Effect of Increasing Humic Acid Concentration ($mg \cdot L^{-1}$ DOC) on Growth Rate ($dbl \cdot day^{-1}$) and Total Chlorophyll Content (μg Chlorophyll · mg freshweight^{-1}) of *L. gibba*

Treatment		Regression equation	r^2	P value
Growth rate				
Visible				
	No PAH	Not significant		
	Anthracene	$y = -0.01x + 0.56$	0.132	0.049
SSR				
	No PAH	$y = -0.01x + 0.59$	0.184	0.018
	Anthracene	$y = 0.02x + 0.03$	0.366	<0.001
Chlorophyll content				
Visible				
	No PAH	$y = 0.03x + 0.57$	0.341	0.001
	Anthracene	$y = 0.06x + 0.38$	0.566	<0.001
SSR				
	No PAH	$y = 0.02x + 0.56$	0.215	0.010
	Anthracene	$y = 0.02x + 0.03$	0.587	<0.001

Plants grown under SSR also exhibited linear increases in chlorophyll contents with increasing DOC concentration, but the magnitude of the effect (e.g., slope; Table 1) was similar regardless of whether anthracene was present (Fig. 4B). Chlorophyll content was significantly lower in all anthracene treatments owing to its greater toxicity under SSR (see Fig. 2). DOC concentrations $\geq 5.1\,mg \cdot L^{-1}$ were required to increase chlorophyll relative to controls when anthracene was present ($P < 0.001$).

DISCUSSION

Even modest additions of $1.6\,mg \cdot L^{-1}$ AHA were sufficient in some cases to diminish PAH photo-induced toxicity in *L. gibba*. Anthracene doses of $2\,mg \cdot L^{-1}$ are extremely toxic in the presence of SSR (Fig. 1; Greenberg *et al.*, 1992; Huang *et al.*, 1993), yet growth rates recovered to nearly 50% of controls in the presence of $1.6\,mg \cdot L^{-1}$ AHA (Fig. 1B). Minor recovery from complete loss of chlorophyll was also evident (Fig. 2B). In contrast to anthracene, minor, but significant, decreases were observed in chlorophyll content and no decreases in doubling rates in the presence of $2\,mg \cdot L^{-1}$ benzo(*a*)pyrene (Figs. 1B and 2B). This is consistent with other studies demonstrating that benzo(*a*)pyrene is far less phototoxic to *L. gibba* than anthracene (Huang *et al.*, 1993; Greenberg *et al.*, 1993). AHA additions to benzo(*a*)pyrene treatments always increased doubling rate and chlorophyll content, although to a lesser degree than those observed for anthracene toxicity under SSR (Fig. 1B). Overall, AHA additions increased plant growth and chlorophyll content under both types of lighting, although effects were more dramatic under SSR.

Although these PAH exposure concentrations were relatively high and exceed nominal PAH solubilities in water, they represent an appropriate means to deliver PAHs to the plants using this exposure system (Duxbury *et al.*, 1997). Owing to the use of DMSO as a delivery solvent and because photooxidized PAHs rapidly go into solution using SSR lighting (McConkey *et al.*, 1997), a significant portion of the total PAH pool is bioavailable even at these high nominal exposure levels. This is consistent with the observation that $2\,mg \cdot L^{-1}$ is still within the log-linear portion of each PAH's dose–response curve under SSR lighting (Huang *et al.*, 1993). That AHA is still effective in ameliorating PAH photo-induced toxicity at these exposure concentrations suggests that it would be at least as effective at lower PAH concentrations typically found in natural environments.

The conclusion that AHA ameliorates PAH photo-induced toxicity initially was based on the use of a single, relatively low ($1.6\,mg \cdot L^{-1}$) exposure concentration of AHA. Increasing AHA concentrations up to $6.2\,mg \cdot L^{-1}$ also produced linear improvements in both growth (Fig. 3B)

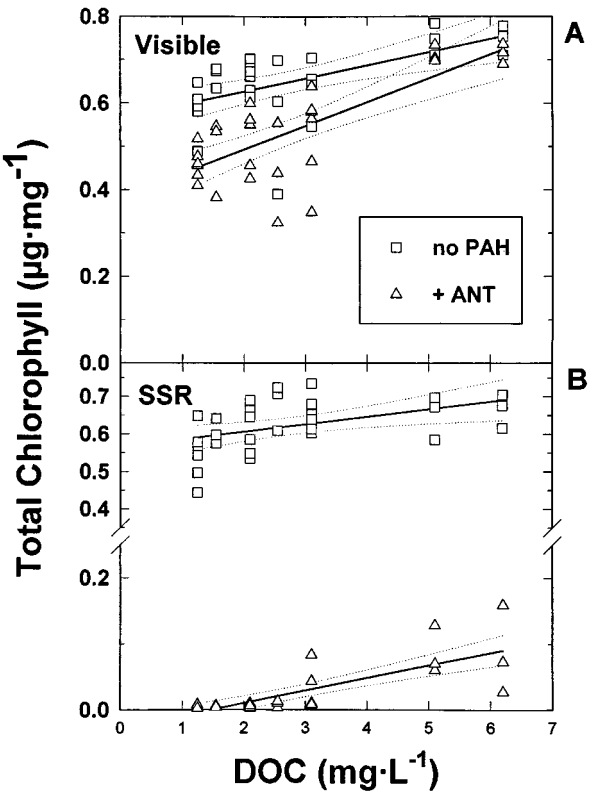

FIG. 4. Effect of increasing HA concentration ($mg \cdot L^{-1}$ DOC) on *L. gibba* total chlorophyll content ($\mu g \cdot mg^{-1}$), both with and without $2\,mg \cdot L^{-1}$ anthracene, under (A) visible light and (B) SSR. The solid lines denote linear regressions describing the data for each PAH treatment. The 95% confidence limits are given as dashed lines where possible. Regression statistics are given in Table 1.

and chlorophyll content (Fig. 4B) although not to the point of completely ameliorating the toxic effects of $2\,mg \cdot L^{-1}$ anthracene under SSR. Similarly, median lethal times increased linearly as AHA concentrations were increased to $6\,mg \cdot L^{-1}$ when *Pimephales promelas* and *Daphnia magna* were exposed to 6.6 to $19.1\,\mu g \cdot L^{-1}$ of anthracene under a similar SSR lighting system (Oris *et al*., 1990). AHA concentrations used in both studies were similar to natural humic substances found in moderately humic rivers and streams, although concentrations can go much higher in wetlands (Wetzel, 1983; Thurman, 1985). Therefore, PAH photo-induced toxicity has the potential to be diminished by HA concentrations which can be found in moderately humic surface waters.

However, it remains to be established whether natural humic substances will perform the same function at these concentrations. The commercial AHA used in both the present study and in most other laboratory bioassays (e.g., Oris *et al*., 1990; Day, 1991; Lee *et al*., 1993) differs chemically from those found in natural aquatic habitats. Commercial humic materials contain different relative abundances of organic functional groups than naturally derived materials; in particular, commercial sources possess a 41% greater degree of aromaticity (Malcolm and MacCarthy, 1986). Aldrich HA is a terrestrially derived humic material and appears to partition organic contaminants 4- to 20-fold more strongly than natural aquatic humic and fulvic acids (Landrum *et al*., 1985; Chiou *et al*., 1987).

Although this would seem to limit the usefulness of using Aldrich HA as a model compound, some have proposed that chemical properties common to all types of natural and commercial HA may determine how they control organic contaminant bioavailability (Kukkonen and Oikari, 1991). These authors concluded that the degree of aromaticity measured by HA absorbance at 270 nm (Traina *et al*., 1990) and the hydrophilic acid composition of HA were both highly correlated to chemical partition coefficients and bioavailability of several organic contaminants (including PAHs) to *D. magna*. Furthermore, both parameters were equally predictive of xenobiotic bioavailability regardless of whether the DOC was commercial or naturally derived. It is also interesting to note that the greater degree of aromaticity in commercial HAs (Malcolm and MacCarthy, 1986) is consistent with their stronger contaminant partitioning capabilities relative to natural sources (Landrum *et al*., 1985; Chiou *et al*., 1987). Thus, the mechanisms by which commercial HAs partition organic contaminants may be similar to those for natural HAs, even if the magnitude of the chemical partitioning differs. However, given their significant chemical differences with natural humic substances (Malcolm and MacCarthy, 1986), future studies using commercial HAs should at least quantify functional properties common to all types of HA in order to improve extrapolations from the laboratory to natural systems.

The present experiments also suggested that the extent to which AHA controls PAH photo-induced toxicity will depend on the presence or fluence rates of UV radiation incident on surface waters. Like PAHs, natural humic substances also absorb UV light and thus can be photodegraded into a variety of more labile forms of organic C and N byproducts (Zepp, 1988; Schindler *et al*., 1996; Bushaw *et al*., 1996). While commercial humics certainly absorb UV as well, photochemical release of labile C or N has yet to be confirmed. It is well known that PAHs will partition directly to intact forms of both commercial (Leversee *et al*., 1983; Landrum *et al*., 1985) and natural (Landrum *et al*., 1985; Kukkonen and Pellinen, 1994) humics from freely dissolved phases, thereby diminishing PAH bioavailability to freshwater organisms. Given that both commercial and natural sources of aquatic DOC photodegrade into relatively water soluble compounds such as low-molecular-weight organic acids and formaldehydes (Palenik *et al*., 1991; Allard *et al*., 1994), one might predict that their ability to partition hydrophobic organic compounds would decrease under the influence of UV radiation. However, both the identity of stable products of HA photodegradation and their ability to partition hydrophobic organic contaminants relative to intact HA are virtually unknown. Reverse-phase PAH extraction techniques (Landrum *et al*., 1985; Day, 1991) would improve our empirical understanding of UV-induced PAH–HA interactions and, hence, bioassay exposure conditions.

Humic substances also will undergo UV-induced photosensitization reactions which produce a variety of reactive oxygen species including singlet oxygen, organoperoxy and hydroxyl radicals, superoxide, and hydrogen peroxide (Cooper *et al*., 1989; Hoigné *et al*., 1989; Canonica and Hoigné, 1995). Production of these reactive oxygen species by both commercial (Steinberg *et al*., 1992) and natural (Canonica *et al*., 1995; Wang *et al*., 1995) humics can oxidize a variety of organic pollutants, including PAHs. Therefore, HA photosensitization may not only photooxidize PAHs to more toxic forms (e.g., Greenberg *et al*., 1993), but it is also likely that the reactive oxygen species themselves could exert negative biological impacts even without PAHs present.

Some of the *L. gibba* results using the commercial AHA were consistent with this latter hypothesis; growth rates diminished linearly with increasing AHA concentration without anthracene present (Fig. 3B). However, in the same experiments chlorophyll content increased with increasing AHA concentration (Fig. 4B). Chlorophyll content always increased in the presence of AHA, and this effect was often more significant in the presence of SSR (Fig. 2). Any negative effects of photosensitization may be offset by enhanced production of photooxidized AHA byproducts (labile inorganic and organic N; Bushaw *et al*., 1996) that appear to stimulate chlorophyll production.

Considering that UV light may both photomodify HAs and induce photosensitization, it is difficult as yet to make general predictions as to whether UV irradiation of HAs will have a net positive or negative impact on their ability to control PAH toxicity. In the present experiments, Aldrich HA still protected against anthracene toxicity even in the presence of all HA photoreactions induced by UV. Furthermore, UV-induced changes in AHA would manifest themselves in different slopes between increasing AHA concentrations and plant responses to anthracene when visible vs SSR results are compared. For growth rates, the addition of UV wavelengths in SSR was associated with an increase from no significant slope in visible light to a small positive slope under SSR (Table 1). Chlorophyll results differed from growth-based results in that a slope of 0.06 under visible light decreased to 0.02 in the presence of SSR (Table 1). Thus, changes in the ability of AHA to control anthracene toxicity did occur when UV wavelengths were introduced, but these changes were endpoint-dependent. Additional studies currently underway in this laboratory (Gensemer, Caggiano, and Simms, unpublished data) at lower concentrations of anthracene will help clarify these differences in terms of mechanisms by which AHA controls anthracene photo-induced toxicity. Similar studies using natural sources of HA would also be required to test the generality of these relationships under more natural conditions.

CONCLUSIONS

Even modest concentrations ($1.6\,mg \cdot L^{-1}$) of the commercial Aldrich Humic Acid can be effective in ameliorating the photo-induced toxicity of PAHs (anthracene and benzo(*a*)pyrene) to the aquatic macrophyte *L. gibba*. The protective effects of AHA were apparent regardless of whether plants were incubated in visible light or in light containing UV-A and UV-B, although these effects were usually more pronounced when UV was present. Increasing AHA concentrations to $6.2\,mg \cdot L^{-1}$ further enhanced the amelioration of PAH toxicity with regard to both growth rates and chlorophyll contents, although the presence of UV quantitatively changed the linear relationships between AHA concentration and plant performance in comparison to visible light treatments. Thus, the net effect of this commercial humic substance is to protect against the photo-induced toxicity of PAHs, even though the AHA molecules may themselves become photooxidized or induce potentially negative photosensitization reactions in the presence of enhanced UV radiation.

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