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Influences of New Azo Dyes to the Aquatic Ecosystem

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Abstract: The influences of a series of new azo direct dyes including copper-complexes based on benzidine congeners, 2,2'-dimethyl-5,5'-dipropoxybenzidine and 5,5'-dipropoxybenzidine, were examined using microorganism, Daphnia magna. The purpose of the research described in this paper was to use bioassays with daphnids to determine the aquatic toxicity of new azo dyes in which copper was incorporated. The results clearly show that copper has negative effects to aquatic ecosystem as expected. The study also suggested that the assay with Daphnia magna was an excellent method to evaluate the influences of dyes to the aquatic environment.

Keywords: Azo direct dyes, Daphnia magna, Copper, Aquatic toxicity, Aquatic ecosystem, Benzidine

Approximately 10-15 % of the dyes are released into the environment during dyeing of different substrates, such as synthetic and natural textile fibers, plastics, leather, paper, mineral oils, waxes and even (with selected types) foodstuffs and cosmetics [1]. Even at very low concentrations (10-50 mg/*l*) water soluble azo dyes can cause waste streams to become highly colored. Aside from their negative aesthetic effects certain azo dyes and biotransformation products have been shown to be toxic, and in some cases these compounds are carcinogenic and mutagenic [2-8]. Approximately, it was determined that 130 of 3200 azo dyes in use have produced carcinogenic aromatic amines because of reductive degradation [9].

Copper is the third most used metal in the world [10] and known to have a number of negative effects both on crops [11] and the microorganisms in the soil, which could have a negative effect on the fertility of the soil [12]. Bioavailability and toxicity of most metals, and certainly of copper, is controlled by the speciation in the pore water, and therefore it is crucial to test the toxicity of the solution. Heavy metals in general have a low solubility in water, and the concentration of metals in water depends on parameters such as pH, redox potential, organic matter content and the amount of metal present in the solution [13]. For dyes that contain metals as an integral part of the dye molecule, the metallic content is essential to the dye's performance as a textile colorant. Several types of metals, especially copper, and related dyes are shown in Tables 1, 2 and 3.

The commercial utility of benzidine-based azo colorants and concern over their potential health risks have caused the search for viable nonmutagenic analogs of benzidines to be an important research problem in the past [14-20]. However, researches concerning the aquatic toxicity of azo dyes including copper-complexed dyes were not performed seriously by textile chemists.

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Textile plants are very important sources of toxic discharges [21,22]. They usually employ cotton and synthetic fibers and include an integrated printing and dyeing operations, applying a wide variety of organic dyes and full range stages of fabric processes [23-29]. Therefore, the aquatic toxicological investigation of azo dyes can be very beneficial to the further study of textile effluents. Tables 4 and 5 indicate the typical

Table 1. Typical metals found in dyes by dye class

Dye class	Typical metals in structure
Direct	Copper
Fiber Reactive	Copper and nickel
Vat	None
Disperse	None
Acid	Copper, chrome, cobalt
Premetallized	Copper, chrome, cobalt
Mordant	Chrome

Table 2. Dyes with high copper content

Dye	Copper contents (%)
Belamine F Red 3BL	4.00
Belamine B Blue LT	3.69
Pyrazol F Violet MXD	3.00
Solantine Brown BRL	3.00
Atlantic Blue 8GLN-K	2.70
Atlantic Resinifast Blue 2R	2.50
Sirius Supra Turqoise LG	2.29
Superlitefast Blue 2GLL	1.00
Direct Navy OFS	0.70
Belamine Red 3BL	4.00
Solophenyl Brown BRL	3.00
Fastolite Blue L	2.70
Atlantic Black NR	1.50

Table 3. Average metal concentration of selected dyes

	Number of dyes tested Average metal concentration by dye type					
Metal	Acid	Basic	Direct	Disperse	Reactive	Vat
Arsenic	413	137	313	177	46	58
	<1	<1	<1	<1	1.4	<1
Cadmium	417	137	313	177	46	58
	<1	<1	<1	<1	<1	<1
Chromium	404	137	303	117	46	58
	9	2.5	3	3	24	83
Cobalt	300	135	271	154	46	58
	3.2	<1	<1	<1	<1	<1
Copper	399	136	285	153	46	59
	79	33	35	45	71	110
Lead	408	135	315	161	46	58
	37	6	23	37	52	6
Mercury	450	132	350	196	46	94
	<1	0.5	0.5	<1	0.5	1
Zinc	421	122	311	166	46	59
	<13	32	8	3	4	4

Table 4. Typical causes of aquatic toxicity

Agent	Chemical example	Typical source
Salt	NaCl, Na_2SO_4	Dyeing
Surfactants	Ethoxylated phenols	Multiple sources
Metals	Copper, Zinc, Etc.	Dyes
Organics	Chlorinated solvents	Scour, machine cleaning
Biocides	Pentachlorophenol	Wool fiber contaminant
Toxic anions	Sulfide	Sulfur dyeing

Table 5. Typical sources of metals in textile effluent

Metals	Typical sources
Arsenic	Fibers, incoming water, fugitive, treated timber
Cadmium	Impurity in salt
Chrome	Dyes, laboratory
Cobalt	Dyes
Copper	Dyes, incoming water, fiber
Lead	Dyes, plumbing, shop
Manganese	Permanganate strip
Mercury	Dye/commodity chemical impurities
Nickel	Dyes
Silver	Photo operations
Tin	Finishing chemicals, plumbing
Titanium	Fiber
Zinc	Dyes, impurities in commodity, chemicals, incoming water

causes of aquatic toxicity and sources of metals in textile effluent.

Acute toxicity can be defined as toxicity elicited immediately following short-term exposure to a chemical. In accordance with this definition, two components comprise acute toxicity: acute exposure and acute effect. In contrast to acute toxicity, chronic toxicity is characterized by prolonged exposure and lethal effects elicited through mechanisms that are distinct from those that cause acute toxicity. Typically, acute and chronic toxicity of a chemical are easily distinguished. For example, mortality occurring on the second day of continuous exposure to the chemical would typically be considered acute toxicity. Similarly, reduced fecundity resulting from continuous exposure of organisms to a throughout their life cycle would be indicative of chronic toxicity. Thus, acute toxicity may result in chronic toxicity [30-32].

Effects encountered with acute toxicity commonly consist of mortality or morbidity. From a quantitative standpoint these effects are measured as the LC_{50}, EC_{50}, LD_{50}, or ED_{50}. The LC_{50} and EC_{50} values represent the concentration of the material to which the organisms were exposed that causes mortality (LC_{50}) or some other defined effect (EC_{50}) in 50 % of an exposed population. The LD_{50} and ED_{50} represent the dose of the material that causes mortality (LD_{50}) or some other defined effect (ED_{50}) in 50 % of a treated population. Since ecotoxicology focuses upon the adverse effects of chemicals in the environmental, acute toxicity in this discipline is more commonly described by the LC_{50} or EC_{50}. LD_{50} and ED_{50} values are more commonly used when evaluating toxicity from a human health perspective [33,34].

Clearly, the LC_{50} value is not indicative of an acceptable level of the chemical in the environment. Rather the LC_{50} is used as an indicator of relative acute toxicity. The LC_{50} is used to this end rather than a more relevant descriptor of an environmentally suitable environmental concentration (i.e., LC_{05}) because the LC_{50} value has the greatest level of confidence associated with it due to its central location on the concentration-response line. LC_{50} and LD_{50} values are often interpreted as shown in Table 6 [33,34].

In the present study, the acute toxicity of new azo direct dyes including copper-complexed dyes was evaluated using Daphnia magna to investigate the aquatic toxicity of azo dyes. Also, C.I. Direct blue 218 was tested to compare the aquatic toxicity with new azo direct dyes. These new dyes based on benzidine congeners, 2,2'-dimethyl-5,5'-dipropoxy-

Table 6. The relationship between LC_{50}, LD_{50} and toxicity rating

LD_{50} (mg/kg)	LC_{50} (mg/*l*)	Toxicity rating
>5000	>100	Relatively nontoxic
500–5000	10–100	Moderately toxic
50–500	1–10	Very toxic
<50	<1	Extremely toxic

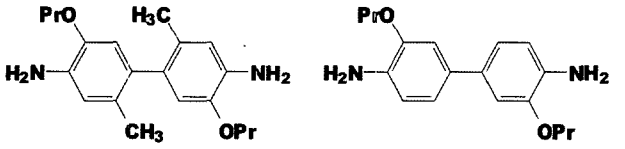

Figure 1. Structures of 2,2'-dimethyl-5,5'-dipropoxybenzidine (**1**) and 5,5'-dipropoxybenzidine (**2**).

benzidine (**1**) and 5,5'-dipropoxybenzidine (**2**) (Figure 1), were synthesized and reported in our previous papers [35,36].

Materials and Methods

Organisms

Daphnids (Daphnia magna) (Figure 2) were obtained from stocks that have been maintained at North Carolina State University for over 10 years. Daphnids were cultured and used experimentally in deionized water reconstituted with 192 mg/*l* $CaSO_4 \cdot 2H_2O$, 192 mg/*l* $NaHCO_3$, 120 mg/*l* $MgSO_4$, 8.0 mg/*l* KCl, 1.0 μg/*l* selenium and 1.0 μg/*l* vitamin B_{12}. Cultures were maintained at a density of 40-50 brood daphnids/*l* culture medium. Culture medium was renewed and offspring were discarded three times weekly. Brood daphnids were discarded after 3 weeks in the culture and replaced with neonatal organisms. Cultured daphnids were fed twice daily with 1.0 m*l* (~4 mg dry weight) of Tetrafin® fish food suspension (Pet International, Chesterfill, New South Wales, Australia) and 2.0 m*l* (1.4×10^8 cells) of a suspension of unicellular green algae, Selenastrum capricornutum. The algae were cultured in Bold's basal medium. Culture and experimental solutions were maintained at 20 °C under a 16 h photoperiod. These culture conditions maintained the daphnids in the parthenogenic reproductive phase with the production of all-female broods of off-spring [37,38].

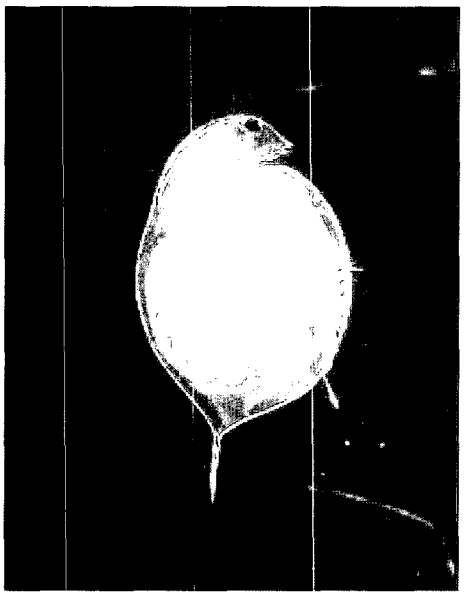

Figure 2. A picture of Daphnia magna (photo by Steve Hopkin).

Chemicals

All dyes tested are novel and were synthesized in our laboratory. Figures 3, 4 and 5 shows the structures of 4 new direct dyes (**3-6**), C.I. Direct blue 218 and 12 copper-

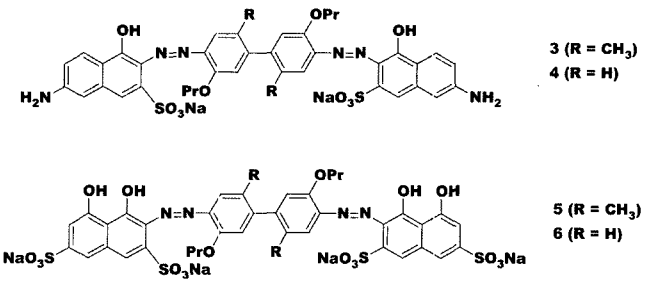

Figure 3. Structures of 4 unmetallized direct dyes tested.

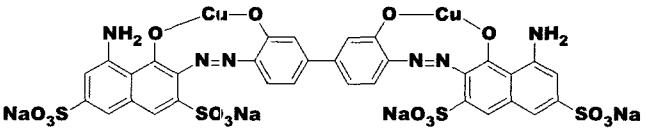

Figure 4. Structure of C.I. Direct blue 218.

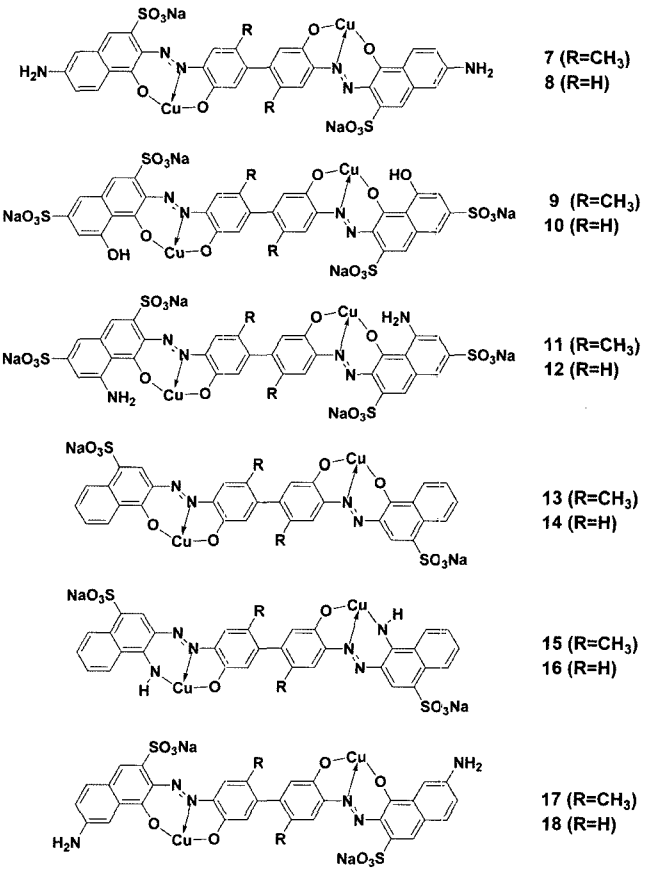

Figure 5. Structures of 12 copper-complexed direct dyes tested.

complexed dyes tested. The structure of each dye was confirmed by Electro Spray Mass Spectrometry (ESMS), the details of which are shown in other publications [35,36]. The purity of the novel dyes was confirmed by thin-layer chromatography (TLC).

Methods

Initially a 24-h preliminary test carried out at exposure concentrations of 100, 10, 1.0 mg/*l* to determine if the dye solution was toxic and to define the concentration range to be employed in the definitive tests. If no toxicity is observed, material is considered to be nontoxic and no further testing required. If toxicity is observed, a more definitive experiment need to be performed to define the concentration-response relationship [39,40].

The standard for a valid bioassay was no-movement rate of less than 10 % in the control group. In the definitive test, the minimum number of dilutions was 5 plus the control group. Immobile organisms were counted to calculate the 24-h LC_{50} and 48-h LC_{50}. All assays were done in duplicate for each concentration [37,38].

For the toxicity tests, daphnid neonates less than 24 hour old were used. Ten neonates were placed in individual 50 m*l* containers, with 40 m*l* of the sample solution, diluted or undiluted as required with reconstituted water. Two sample solutions were prepared for each concentration. 100 μl Algae and 50 μl food was supplied to feed the neonates at the beginning of the test. Concentrations selected were 0.8, 1.3, 2.2, 3.6, 6.0, 10.0 mg/*l*. Typically each treatment level is 60 % of the next higher level to allow LC_{50} with a high degree of confidence. The test dye solutions containing Daphnia magna were placed in upright incubator (cycle 16 hours on/8 off) and covered loosely with parafilm to prevent evaporation. The mortality of daphnids was observed at 24 and 48 hours from initiation of the test.

Results and Discussions

Acute toxicity of new azo dyes and C.I. Direct blue 218 to Daphnia magna are summarized in Tables 7, 8, 9 and 10. In the present study, toxicity was evaluated at concentrations of 0.8-100 mg/*l* for 4 new direct dyes (**3**, **4**, **5**, **6**) and 0.8-10.0

Table 7. The number of dead Daphnids for dyes **3-6** at 0.8-10.0 mg/*l*

Dye		0.8	1.3	2.2	3.6	6.0	10.0
3	24 hrs	0	0	0	0	0	0
	48 hrs	0	0	0	0	0	0
4	24 hrs	0	0	0	0	0	0
	48 hrs	0	0	0	0	0	0
5	24 hrs	0	0	0	0	0	0
	48 hrs	0	0	0	0	0	0
6	24 hrs	0	0	0	0	0	0
	48 hrs	0	0	0	0	0	0

Table 8. The number of dead Daphnids for dyes **3-6** at 8-100 mg/*l*

Dye		8	13	22	36	60	100
3	24 hrs	0	0	0	1	2	4
	48 hrs	0	0	0	2	3	5
4	24 hrs	0	0	0	0	2	2
	48 hrs	0	0	0	0	3	2
5	24 hrs	0	0	0	0	0	2
	48 hrs	0	0	0	0	1	3
6	24 hrs	0	0	0	0	1	1
	48 hrs	0	0	0	0	2	4

Table 9. The number of dead Daphnids for C.I. Direct blue 218 at 0.8-10.0 mg/*l*

Dye		0.8	1.3	2.2	3.6	6.0	10.0
C.I.Direct Blue 218	24 hrs	0	0	0	6	10	17
	48 hrs	0	0	0	7	18	20

Table 10. The number of dead Daphnids for copper-complexed direct dyes (**7-18**) at 0.8-10.0 mg/*l*

Dyes		0.8	1.3	2.2	3.6	6.0	10.0
7	24 hrs	0	0	0	16	19	20
	48 hrs	0	0	1	20	20	20
8	24 hrs	0	0	1	3	16	20
	48 hrs	0	0	2	7	20	20
9	24 hrs	0	0	0	7	20	20
	48 hrs	0	0	2	11	20	20
10	24 hrs	0	0	2	17	20	20
	48 hrs	0	0	6	20	20	20
11	24 hrs	0	0	2	19	19	20
	48 hrs	0	0	5	20	20	20
12	24 hrs	0	0	13	20	20	20
	48 hrs	0	0	13	20	20	20
13	24 hrs	0	0	0	3	8	16
	48 hrs	0	0	0	3	16	19
14	24 hrs	0	0	0	5	18	19
	48 hrs	0	0	0	6	20	20
15	24 hrs	-	-	-	-	-	-
	48 hrs	-	-	-	-	-	-
16	24 hrs	-	-	-	-	-	-
	48 hrs	-	-	-	-	-	-
17	24 hrs	0	0	0	0	0	1
	48 hrs	0	0	0	0	0	6
18	24 hrs	0	0	0	0	7	19
	48 hrs	0	0	1	1	12	20

mg/*l* for C.I. Direct blue 218 and copper-complexed dyes to determine LC_{50} ranges. Also, control solutions (concentration of 0.0 mg/*l*) were conducted to confirm the accuracy of the test.

Tables 7, 8, 9 and 10 show the number of dead Daphnia magna after 24-hr and 48-hr aquatic toxicity tests. The results indicate that the LC_{50} of C.I. Direct blue 218 for Daphnia magna is about 6.0 mg/*l* at 24-hr and 3.6-6.0 mg/*l* at 48-hr tests. No mortality or lethal effects were observed at 0.8-2.2 mg/*l* and 100% mortality was observed at 48-hr in 10.0 mg/*l*, the highest concentration tested. This means that 50 % of daphnids were dead at between 3.6-6.0 mg/*l* after 48-hr test.

For 4 new non-genotoxic direct dyes, the LC_{50} is more than 100 mg/*l* at both 24- and 48-hr tests. No mortality or lethal effects were observed at 0.8-22 mg/*l* for dye **3** and 0.8-36 mg/*l* for dye **4**, **5** and **6** after the tests. This means that more than 50 % of daphnids were still alive at 100 mg/*l* after 48-hr test.

Out of 12 new non-genotoxic metallized direct dyes, only 10 dyes were evaluated for aquatic toxicity since dyes **15** and **16**, in which naphthionic acid was used as a coupler, could not be tested because of very poor water solubility. No mortality or lethal effects were observed at 0.8-1.3 mg/*l* for all dyes tested. Overall, the LC_{50} is 2.2-3.6 mg/*l* at both 24- and 48-hr tests except dye **12** in which 5,5'-dipropoxybenzidine was coupled with H-acid and dye **17** in which 2,2'-dimethyl-5,5'-dipropoxybenzidine was coupled with Gamma-acid. The most toxic dye was **12**, whose LC_{50} is 1.3~2.2 mg/*l* at both 24- and 48-hr tests and the least toxic dye was **17**, whose LC_{50} is more than 10.0 mg/*l* at both 24- and 48-hr tests.

From the general concept of aquatic toxicity in Table 6, C.I. Direct blue 218 and 9 (**7**-**14**, **18**) metallized direct dyes was very toxic to daphnids, with a 48-hr LC_{50} of between 1.0 and 10.0 mg/*l* whereas 4 new unmetallized direct dyes were relatively non-toxic to daphnids, with a 48-hr LC_{50} of more than 100 mg/*l*.

The main difference of these two groups tested is the presence of copper in the dye structures. While C.I. Direct blue 218 and copper-complexed dyes have 2 copper molecules inside the structure, 4 new direct dyes do not have any metals in their structures. Some heavy metals including copper are essential for many organisms, but also very toxic.

Copper is an offensive pollutant and should receive maximum pollution prevention attention. In addition to dyestuff itself, other sources of copper in the dyehouse may include spillage from handling, implement cleanup, drum washing, and incorrect weighings as well as dyebath effluent. Generally, the amount of dyes still entering open water today is quite small. Because even small amounts strongly colour an effluent, they are immediately noticeable in industrial wastewater as an unsightly stain and can be diluted, usually to below the limit of detection. As regards the heavy metal content in dyes, very low concentrations have been reached nowadays and it is expected that they would not cause insuperable pollution problems for the processing plants in the future. Exceptions are those dyes which are aftermetallized with copper and chromium salts. If there are no alternatives but to use them, their effluents should be treated separately. In case of heavy metals including copper, it is essential for many organisms, but also very toxic to aquatic organisms even though small amounts are present. Therefore, dye chemists should understand the aquatic toxicity of the metals in dyestuffs and investigate how to reduce the toxicity in metal-complexed dyes. Pollution prevention measures also include special worker training, identification of problem dyes, better record keeping, and auditing of heavy metal use.

Conclusions

The results using Daphnia magna have been used only as a model to extrapolate the toxicological implications that may result from azo direct dyes in the aquatic environment, but these results are not sufficient to assess the holistic health risk for a receptor aquatic ecosystem. However, the toxicity to daphnids is enough to suggest potential damage to every receptor ecosystem and emphasizes the need for the toxicological study of dye synthesis industry. The main object of this study was to demonstrate biological toxicity of azo direct dyes in textile industry. The results indicate that copper-complexed dyes tested and C.I. Direct blue 218 was very toxic to daphnids, with a 48-hr LC_{50} of between 1.0 and 10.0 mg/*l* while 4 new non-genotoxic direct dyes were relatively non-toxic to daphnids, with a 48-hr LC_{50} of more than 100 mg/*l*, suggesting copper molecules inside dye structures play an important role for the evaluation of aquatic toxicity of dye solutions.

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References

1. R. Anliker, *Ecotoxicol. Environ. Saf.*, **1**, 211 (1979).
2. K. T. Chung and S. E. Stevens, *Environ. Toxicol. Chem.*, **12**, 2121 (1993).
3. M. Haug, A. Schmiat, J. S. Nortman, D. C. Hempel, and H. J. Stolz, *Appl. Environ. Microbiol.*, **57**, 3144 (1991).
4. E. J. Weber, *Environ. Toxicol. Chem.*, **9**, 609 (1991).
5. S. Seshadri, P. L. Bishop, and A. M. Agha, *Waste Manag.*, **14**, 127 (1994).
6. Y. Zaoyan, S. Ke, Y. Guangliang, D. Fan, Y. Jinshan, and M. Hunonian, *Water Sci. Tech.*, **26**, 2093 (1992).
7. J. A. Field, A. J. M. Stems, and M. Kata, *Antonie van Leeuwenhoek*, **67**, 47 (1995).
8. M. Isik and D. T. Sponza, *Environ. Sci. Health,* **38**, 2301

(2003).
9. F. Sengul and A. Muezzinoglu, "The Strategies of Ecological Production", TTGV 130/S Final Reports, Izmar, 1996.
10. Swedish Environmental Protection Agency, 1996.
11. A. Baryla, C. Laborde, J. L. Montillet, C. Triantaphlides, and P. Chagvardieff, *Environ. Pollut.*, **109**, 131 (2000).
12. A. Martensson and L. Torstensson, "Examine of Sewage Sludge Quality", Institution of Microbiology, SLU, 1997.
13. M. B. McBride, "Environmental Chemistry of Soils", p.406, Oxford Univ. Press, New York, 1994.
14. M. M. Shahin, D. Rovers, A. Buguat, and G. Kalopissis, *Mutat. Res.*, **79**(4), 289 (1980).
15. M. M. Shahin, A. Buguat, and G. Kalopissis, *Mutat. Res.*, **78**(1), 25 (1980).
16. M. M. Shahin, C. Chopy, and N. Lequesne, *Environ. Mutagen.*, **7**(4), 535 (1985).
17. H. S. Freeman, J. F. Essancy, E. K. Essancy, K. Michelle, K. P. Mills, W. M. Whaley, and B. J. Dabney, *Dyes and Pigments*, **8**, 417 (1987).
18. H. S. Freeman, J. F. Essancy, and L. D. Claxton, *Dyes and Pigments*, **13**, 55 (1990).
19. F. Calogero, H. S. Freeman, J. F. Essancy, W. M. Whaley, and B. J. Dabney, *Dyes and Pigments*, **8**, 431 (1987).
20. D. Hinks, H. S. Freeman, Y. Arai, and H. Ando, *Dyes and Pigments*, **48**, 7 (2001).
21. P. B. Dorn, J. P. Salanitro, S. H. Evans, and L. Kravetz, *Environ. Toxicol. Chem.*, **12**, 1751 (1993).
22. S. H. Lin and C. F. Peng, *Water Res.*, **28**, 277 (1994).
23. W. B. Achwal, *Colourage*, **44**, 31 (1997).
24. C. M. A. Ademoroti, D. O. Ukponmwan, and A. A. Omode, *Int. J. Environ. Stud.*, **39**, 291 (1992).
25. R. V. Laughton, T. Moran, and G. Roy, "In 21st Annual Toxicity Workshop", October, 1994.
26. L. A. Rutherford, W. R. Ernst, K. G. Doe, and P. A. Hennigar, "In Proceedings, In 19th Annual Aquatic Toxicity Workshop", 1992.
27. A. Villegas-Navarro, M. C. Romero Gonzalez, E. Rosas Lopez, R. Dominguez Aguilar, and W. Sachetin Marcal, *Environ. Int.*, **25**, 619 (1999).
28. M. Weltrowski, J. Patry, and M. Bourget, *Adv. Chitin. Sci.*, **1**, 462 (1996).
29. A. Villegas-Navarro, Y. M. Ramirez, S. B. Salvador, and G. Gallardo, *Ecotoxicol. and Environ. Safety*, **48**, 56 (2001).
30. R. M. Joy, "Neurotoxicology: Central and Peripheral in: Encyclopedia of Toxicology", p.389, Academic Press, New York, 1998.
31. L. Stryer, "Biochemistry", 4th ed., W. H. Freeman and Company, San Francisco, 1999.
32. D. L. Eaton and C. D. Klaassen, "Principles of Toxicology in: Casarrett & Doull's Toxicology", 6th ed., p.11, McGraw-Hill, New York, 2001.
33. D. J. Hoffman, B. A. Rattner, G. A. Burton, and J. C. Cairns, "Handbook of Ecotoxicology", p.25, Lewis Publishers, Boca Raton, 1994.
34. P. R. Parish, "Fundamentals of Aquatic Toxicology", p.31, Hemisphere, New York, 1989.
35. J. S. Bae and H. S. Freeman, *AATCC Review*, **1**(9), 67 (2001).
36. J. S. Bae, H. S. Freeman, and A. El-Shafei, *Dyes and Pigments*, **57**(2), 121 (2003).
37. H. Y. Wang, A. W. Olmstead, and G. A. LeBlanc, *Aquatic Toxicol.*, **74**, 193 (2005).
38. A. W. Olmstead and G. A. LeBlanc, *Aquatic Toxicol.*, **75**(3), 253 (2005).
39. M. Isik and D. T. Sponza, *J. Hazardous Materials*, **B114**, 29 (2004).
40. B. Fjalborg and G. Dave, *Environ. Int.*, **28**, 761 (2003).