# The toxicity of Copper Sulphate and Atrazine to the diatom Aulacoseira Granulata (Ehrenberg) Simmons.

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**ABSTRACT: The toxicity of copper sulphate and atrazine to the diatom Aulacoseira granulata (Ehrenberg) Simmons.** The use of pesticides in modern agriculture, frequently applied incorrectly and in exaggerated amounts, has grave consequences for the rivers, lakes and reservoirs in which the residues accumulate. Among herbicides, atrazine has been widely employed throughout the world, including in Brazil, to combat weeds among sugar cane, pineapple, soy bean and many other crops. Algae, being physiologically similar to higher plants, are potential targets for these herbicide residues. In the present study, ecotoxicological tests were carried out on the diatom Aulacoseira granulata (Ehrenberg) Simmons, with the aim of determining its sensitivity to atrazine and also to copper sulfate, a reference substance. The results obtained with A. granulata were compared with literature data for other species of algae and macrophytes. The comparison indicated that although A. granulata has low sensitivity to copper, very low concentrations of atrazine are sufficient to inhibit greatly the growth of this diatom.

**Key-words:** Ecotoxicology, Aulacoseira granulata, atrazine, copper sulphate.

**RESUMO: A toxicidade do sulfato de cobre e da atrazina à diatomácea Aulacoseira granulata (Ehrenberg) Simmons.** A utilização de pesticidas pela agricultura moderna, em grande parte de forma exagerada e incorreta, tem trazido graves conseqüências aos rios, lagos e reservatórios, devido ao carreamento de seus resíduos para estes corpos de água. Dentre os herbicidas, a atrazina tem sido largamente utilizada no Brasil e no mundo, no combate de pragas daninhas em cultivos de cana-de-açúcar, abacaxi e soja entre outras. As algas, devido à sua semelhança fisiológica com as plantas superiores, passam a ser alvos em potencial dos resíduos deste tipo de herbicida. O presente estudo realizou testes ecotoxicológicos com a alga Aulacoseira granulata (Ehrenberg) Simmons, a fim de estabelecer a sua sensibilidade à atrazina e também ao sulfato de cobre. Os resultados obtidos com A. granulata para sulfato de cobre e atrazina, foram comparados com base em resultados obtidos na literatura para outras espécies de algas e macrófitas. As comparações indicam que A. granulata é pouco sensível ao cobre, entretanto concentrações muito pequenas de atrazina reduziram grandemente o crescimento desta diatomácea.

**Palavras-chave:** Ecotoxicologia, Aulacoseira granulata, atrazina, sulfato de cobre.

## Introduction

Until the Second World War, the development of organic compounds to control pest organisms in agriculture was slow and control was mainly achieved with inorganic compounds of copper and arsenic, besides some naturally-occurring insecticides such as pyrethrins (Nimmo, 1985). The discovery of DDT, in 1945, was at the time considered "miraculous" by Winston Churchill for combatting mosquitoes, the vectors that carried yellow fever and malaria to the soldiers. After that more organic compounds started to be developed and used to control pests, resulting in an increase in agricultural productivity and also the control of disease vectors (Alloway & Ayres, 1993; Tomita & Beyruth, 2002). However, the uncontrolled and excessive use of these compounds have caused severe environmental contamination.

In the case of aquatic environments, the contamination is frequently indirect, being caused by superficial runoff from adjacent agricultural areas (Leboulanger et al., 2001). Some of the compounds have strong impacts extending through several levels of the food-chains. Also of great relevance is the fact that some pesticides and herbicides can remain in the environment for long periods of time, as for example DDT which may last as long as 30 years (Lotufo et al., 2000), whereas others can accumulate in the food-chain, thus affecting even human populations.

Atrazine (2-chlor.4-ethylamin.6-isopropylamin.S-triazine) is a herbicide widely used in agriculture since 1950 to control weeds among sugar cane, pineapple, corn, soy bean and many other crops (Caux et al., 1996; Graymore et al., 2001).

It is estimated that the world consumption of atrazine is between 70,000 and 90,000 tons/year (Premazzi & Stecchi, 1990). According to Lagenbach et al. (2000) this is the herbicide most frequently found in water bodies around the world. Many studies monitoring continental and ground waters in Europe and United States have shown the presence of atrazine. In Brazil, such studies are almost non-existent (Prata, 2002).

Atrazine interferes with plant photosystem II, linking to a protein of 52 kDa from complex B and blocking the electron transport chain, inhibiting photosynthesis (Caux et al., 1996). As a consequence of this mechanism, algae, which are physiologically similar to higher plants, are also a potential target of this compound. The phytoplankton has a role in primary production and changes in this community induce changes in animal communities, including decreases in the biomass, secondary productivity and other aspects, since practically all aquatic heterotrophs depend on primary production (Van Den Hoek et al., 1995; Graymore et al., 2001).

At first, high levels of atrazine in water bodies affect mainly the primary producers, leading to a diminished rate of photosynthesis. This mainly affects the pH of the water owing to the lower rate of uptake of bicarbonate and the amount of dissolved oxygen (Solomon et al., 1996). DeNoyelles et al. (1982), observed the effect of atrazine in experimental ponds, noting that after it was applied the composition of zooplankton community was alterd. Apparently this change in community structure was due more to alterations in the sources of food than to direct exposure.

Several studies refer to the capacity of atrazine to impair reproduction and development in various vertebrate species, as observed at high concentrations, for tree frogs (Hyla versicolor) by Diana et al. (2000), and atrazine-induced hermaphoditism in Rana pipiens (Hayes et al., 2003). Experiments with some rats and hamsters indicate atrazine as a potential cause of mammary tumors, due to its capacity to interfere with estrogen metabolism (Reys, 2001; Birnbaun & Fenton, 2003; Brody & Rudel, 2003).

Copper, like other trace elements, is required by plants and animals as an essential nutrient, in small quantities. However, at concentrations slightly higher than those required for growth, it becomes toxic to most life forms, (USEPA, 1984; Fargasová et al., 1999). In aquatic environments, metals are highly persistent and also highly toxic to many organisms. Copper is present in various chemical species (Gunn et al., 1989).

Copper sulphate was chosen in this study because it is listed by USEPA (1994) as a reference substance that produces consistent results in evaluating the quality of test-organism cultures and also because there are many comparative data available in the literature, allowing the analysis of organism sensitivity.

Diatoms occur and can be abundant both in freshwater and sea, in the plankton, in the benthos, or as epiphytes on macroalgae and higher plants, and contribute greatly to primary production in marine and freshwater (South & Whittick, 1987; Van Den Hoek et al., 1995). The species of the genus Aulacoseira are frequently found in freshwaters, occurring in the plankton in great abundance. Species of this genus have a wide distribution in the Tietê River basin, Southeast Brazil, being dominant in certain periods of the year (Azevedo, 1988; Güntzel, 2000).

A. granulata is a widespread diatom, an r-strategist (fast reproduction), tolerant of eutrophic conditions (Hutchinson, 1967; Reynolds, 1998). Varesche (1989) reports that diatomaceous algae, mainly of the genus Aulacoseira, interfere with the functioning of the sand filters used in water treatment plants, completely blocking them when present in large numbers.

The reason for choosing this organism for the present study were its short life-cycle, implying a short response-time when conditions change, and a rapid response to impairment of the habitat, including reduced density and productivity (Rand, 1995).

In the present study, the toxic effect of copper sulphate and of the herbicide atrazine on the growth of the diatom Aulacoseira granulata (Ehr.) Simons was evaluated by toxicity bioassays.

## Material and methods

The inoculum of A. granulata was obtained as an axenic culture from the Phycology Laboratory at the Federal University of São Carlos. This test-organism was cultured in WC medium, pH adjusted to 7.0. Cultures were kept in incubators at 23±1°C under continuous illumination.

Toxicity tests were carried out for 96 h in a constant temperature room at 23±1°C with continuous illumination. The test substance was added at one of 5 concentrations (10.0, 3.2 , 1.0, 0.32 and 0.10mg/L) to 100 mL of WC in 3 replicate Erlenmeyer flasks (250mL) and these were inoculated with 1 mL of algal culture at $1.3.10^5$ cells/mL (7-10 days old). In the control flasks, the test substance was omitted (Rand, 1995; USEPA, 1994).

Each 24 hours a 2mL sample was taken from each flask and preserved in 4% formaldehyde solution for posterior counts in a Sedgwick-Rafter chamber (Stein, 1973). Densities were calculated from the mean number of cells per filament and the number of filaments in the sample. EC50-96h and the percent inhibition were calculated as recommended by USEPA (1989 in Asselborn & Domitrovic, 2000), growth inhibition being given by:

$$I = \frac{C - T}{C} \times 100$$

where:
I = Percent Inhibition;
C = Cell density in the control;
T = Cell density in each treatment.

The results were analysed with the aid of the computer program TOXTAT 3.4 (Gulley et al., 1994), using the Bartllet´s test for the homogeinity of the variance and the Shapiro-Wilk´s test for normality of distribution. To reveal significant differences between treatments, an analysis of variance (ANOVA) was carried out, followed by the post hoc Tukey´s test in the case of parametric data. Non-parametric data were submitted to a log transformation and, if they were still non-parametric, the Kruskall-Wallis test were applied (Zar, 1999; Nipper, 2002). The values of EC50 – 96h were estimated with the help of a Trimmed Speraman-Karber computer routine (Hamilton et al., 1977).

# Results

In the toxicity tests with copper sulphate, an EC50-96h of 2.58 mg/L was obtained (95% confidence interval 2.32 mg/L - 2.87 mg/L). In Tab. I it can be seen that the percent growth inhibition of the algal culture increases markedly with the time of exposure to copper (Fig. 1). The growth curves diverged from that of the control more evidently after 96 hours (Fig. 1).

Toxicity tests with atrazine revealed a strong inhibition of growth even at the lowest concentrations tested. A high percentage of inhibition was soon observed, as indicated by Tukey test, where significant differences were found already in the first 24h (Tab. II and Fig 2). The EC50-96h was found to be 56mg/L, with a 95% confidence interval of 0.0549 - 0.0555 mg/L).

Table I: Mean percentage and standard deviation of growth inhibition of the diatom Aulacoseira granulata exposed to copper sulphate with respect to the control, at each 24 hours interval.
[a] No significant difference from control and other concentrations according to Kruskall-Wallis test, at a significance level of 0.05
[b] No significant difference from control by Tukey´s test, at a significance level of 0.05
[c] Significantly different from control by Tukey´s test, at a significance level of 0.05

| | Hours | | | |
|---|---|---|---|---|
| **Concentration** | **24** | **48** | **72** | **96** |
| **10.0 mg/L** | 23.09 ± 11.38 [a] | 28.03 ±12.92 [b] | 38.19 ±5.54 [b] | 60.89 ±7.26 [c] |
| **3.2 mg/L** | 14.88 ±10.83 [a] | 18.84 ±5.35 [b] | 25.28 ±8.95 [b] | 48.04 ±1.47 [c] |
| **1.00 mg/L** | 12.15 ±11.61 [a] | 15.31 ±7.42 [b] | 21.60 ±11.60 [b] | 43.48 ±5.73 [c] |
| **0.32 mg/L** | 8.72 ±10.35 [a] | 9.24 ±11.21 [c] | 16.14 ±10.67 [c] | 36.38 ±7.19 [c] |
| **0.10 mg/L** | 9.87 ±6.01 [a] | 5.67 ±7.14 [c] | 12.55 ±6.95 [c] | 27.90 ±2.26 [c] |

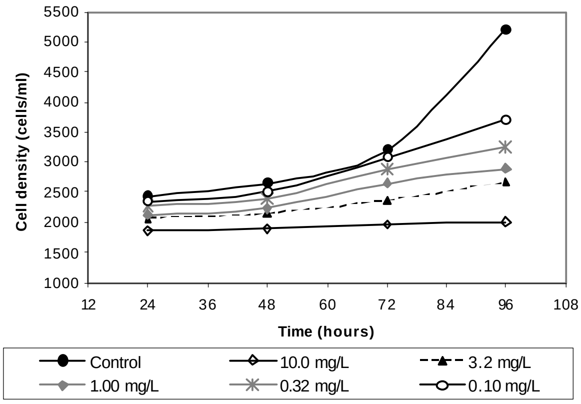


Figure 1: Growth curves of the diatom Aulacoseira granulata exposed to different concentrations of copper sulphate.

Table II: Mean percent growth inhibition and standard deviation of the diatom Aulacoseira granulata exposed to atrazine, with respect to the control, after each 24h interval.
[d] Significantly different from control by Tukey´s test, at a significance level of 0.05

| | Hours | | | |
|---|---|---|---|---|
| **Concentration** | **24** | **48** | **72** | **96** |
| **10.0 mg/L** | 67.51±4.39 [d] | 75.65±9,12 [d] | 85.54±2.59 [d] | 93.33±0.71 [d] |
| **3.2 mg/L** | 67.75±5.13 [d] | 79.16±4,25 [d] | 86.20±3.33 [d] | 93.93±0.49 [d] |
| **1.00 mg/L** | 70.90±2.86 [d] | 77.61±4.02 [d] | 83.67±4.11 [d] | 92.41±1.11 [d] |
| **0.32 mg/L** | 65.23±2.94 [d] | 72.98±4.25 [d] | 81.89±5.99 [d] | 90.85±0.89 [d] |
| **0.10 mg/L** | 64.56±4.64 [d] | 70.71±1.83 [d] | 76.86±1.63 [d] | 89.02±0.39 [d] |

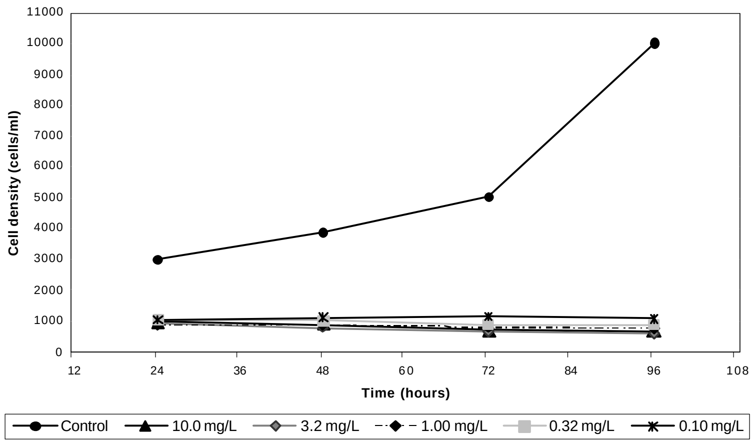


Figure 2: Growth curves of the diatom Aulacoseira granulata exposed to different concentrations of atrazine.

Table III. Copper toxicity to freshwater algae and aquatic macrophytes

| **Species** | **Effect** | **Effective Concentration (mg/L)** | **References** |
|---|---|---|---|
| Algae, moist culture | Significant photosynthesis reduction | 5 | Elder & Horne, 1978 |
| Anabaena flos-aquae | 75% growth inhibition | 200 | Young & Lisk, 1972 |
| Anabaena variabilis | Growth inhibition | 100 | Young & Lisk, 1972 |
| Anacystis nidulans | Growth inhibition | 100 | Young & Lisk, 1972 |
| Ankistrodemus braunii | Growth reduction | 640 | Laube & Martin, 1980 |
| Chlorella vulgaris | Growth inhibition | 200 | Young & Lisk, 1972 |
| Clamydomonas sp. | Growth reduction | 8000 | Cairns, 1978 |
| Cyanophyceae, moist culture | 50% growth reduction | 25 | Steeman-Nielsen & Braun-Laursen, 1976 |
| Cyclotella meneghiniana | Growth reduction | 8000 | Cairns, 1978 |
| Elodea canadensis | 50% reduction in photosynthetic production of $O_2$ | 150 | Brown & Rattingan, 1979 |
| Eudorina californica | Growth inhibition | 5000 | Young & Lisk, 1972 |
| Lemna minor | EC50 – 7 days | 119 | Walbridge, 1977 |
| Microcystis aeruginosa | Incipient Inhibition | 30 | Bringmann, 1975 |
| Navicula incerta | EC50-96h | 10450 | Rachlin et al., 1983 |
| Nitzschia linearis | EC50-120h | 795-815 | Academy of Natural Sciences, 1960 |
| Nitzchia palea | Complete growth inhibition | 5 | Steeman-Nielsen & Wlum-Anderson, 1970 |
| Scenedemus quadricauda | Growth reduction | 8000 | Cairns, 1978 |
| Scenedesmus quadricauda | Incipient Inhibition | 1100 | Bringmann & Kuhn, 1980 |
| Selenastrum capricornutum | Growth reduction | 50 | Bartlett et al., 1974 |
| Selenastrum capricornutum | EC50 -14 days | 85 | Christensen et al., 1979 |
| Aulacoseira granulata | EC50-96h | 2580 | The present study |

Table IV: The main products of atrazine degradation (Graymore et al., 2001; Berg et al., 2003)

| Compound | Attributes |
|---|---|
| Deethylatrazine (DEA) | Generated by biodegradation; phytotoxicity like atrazine. |
| Deeisopropylatrazine (DIA) | Generated by biodegradation; phytotoxic. |
| Didealkylatrazine (DDA) | Generated by biodegradation; considered non-toxic to plants. |
| Hydroxyatrazine (HA) | Generated by biodegradation or by soil chemical reactions (chemical hydrolysis); considered non-toxic to plants. |
| Deethylhydroxyatrazine (DEHA) | Generated by biodegradation or chemical reactions in soil; toxicity unknown. |

Table V: Atrazine toxicity to some freshwater algae and aquatic macrophytes

| Species | Effect | Result | Reference |
|---|---|---|---|
| Anabaena flos-aquae | EC50-72h | 58mg/L | Abou-Waly et al., 1991 |
| Anabaena flos-aquae | EC50-5 days | 469mg/L | Abou-Waly et al., 1991 |
| Anabaena flos-aquae | EC50-7 days | 766mg/L | Abou-Waly et al., 1991 |
| Anabaena flos-aquae | EC50-96h | >3000mg/L | Fairchild et al., 1998 |
| Ceratophyllum sp. | EC50-96h | 22mg/L | Fairchild et al., 1998 |
| Chlamydomonas reinhardi | EC50-96h | 176mg/L | Fairchild et al., 1998 |
| Chlorella vulgaris | EC50-96h | 94 mg/L | Fairchild et al., 1998 |
| Elodea sp. | EC50-96h | 21mg/L | Fairchild et al., 1998 |
| Lemna sp. | EC50-96h | 92mg/L | Fairchild et al., 1998 |
| Microcystis sp. | EC50-96h | 90mg/L | Fairchild et al., 1998 |
| Myriophyllum heterophyllum | EC50-96h | 132mg/L | Fairchild et al., 1998 |
| Najas sp. | EC50-96h | 24mg/L | Fairchild et al., 1998 |
| Scenedesmus quadricauda | EC50-96h | 169mg/L | Fairchild et al., 1998 |
| Selenastrum capricornutum | EC50-72h | 283mg/L | Abou-Waly et al., 1991 |
| Selenastrum capricornutum | EC50-5 days | 218mg/L | Abou-Waly et al., 1991 |
| Selenastrum capricornutum | EC50-7 days | 214mg/L | Abou-Waly et al., 1991 |
| Selenastrum capricornutum | EC50-96h | 0,2 mg/L | Abdel-Hamid 1996 |
| Selenastrum capricornutum | EC50-96h | 117 mg/L | Fairchild et al., 1998 |
| Selenastrum capricornutum | EC50-96h | 0.145 mg/L | Kungoulos et al., 1999 |
| Aulacoseira granulata | EC50-96h | 56mg/L | The present study |

# Discussion

Copper is an essential micronutrient in the metabolism (growth and enzymatic activity) of algae, but it may inhibit growth when concentrations are above those required (Franquera et al., 2000). The toxicity of copper to algae varies with environmental conditions (temperature, pH, alkalinity, etc.), with algal species or strain, with the physiological condition of the organism, with the culture medium and also with the copper species (Fargasová et al., 1999; Franklin et al., 2000; Schauber-Berigan et al., 1993).

Regarding the toxicity of copper to the diatom A. granulata, a value of 2.58mg/L of copper sulphate was obtained for the EC50-96h. Compared to copper toxicity data found in the literature (Tab. I), it appears that A. granulata is quite tolerant to copper. Copper sulphate is frequently used as insecticide, fungicide, herbicide and particularly as algicide to control algal growth in reservoirs, as in the case of Guarapiranga, in São Paulo city, where this compound has been used to control algal blooms since 1976 (Caleffi, 2000). Beyruth (2000), analysing the effects of copper upon the phytoplankton community, observed the presence of A. granulata in the same reservoir during periods of lower copper application. This author observed that

Bacillariophyceae do not reach great biomass, despite the high amounts of silica available in this reservoir, suggesting that seasonality could be the controlling factor. Caleffi (2000) reported that copper concentrations in this reservoir often reach 0.3mg/L, at times with peaks as high as 0.8mg/L. Although copper is an effective algicide, it can affect non-target species, as shown by Caleffi (2000), in the zooplankton community and, in the long term, its effectiveness may be reduced by the selection of algal species more resistant to copper. Also it can promote other effects, such as greater nutrient availability due to the decrease in competition or even nutrient liberation by algal death, particularly observed for Cyanophyceae (Beyruth, 2000).

Several studies on the effect of atrazine upon algae have demonstrated that even at very low concentrations this herbicide strongly reduces algal growth (Torres & O'Flaherty, 1976). According to DeNoyelles et al. (1982), concentrations below 1mg/L decreased phytoplankton photosynthesis in laboratory experiments. Tests performed in experimental ponds at 20 and 500 mg/L of atrazine revealed that this herbicide negatively affects algal growth, corroborating results obtained in the laboratory with isolated species (DeNoyelles et al., 1982).

Usually atrazine is rapidly degraded in the environment, apparently without bioaccumulation, although the rate varies with temperature, soil texture, humidity and pH, among other factors (Graymore et al., 2001; DeNoyelles et al., 1982). Atrazine degradation in water, as in soil, depends on both biological and chemical processes. Bacteria and fungi remove the ethyl group from the triazine ring, while chemical hydrolysis releases chloride ions (Hamilton et al., 1989). In the field, atrazine degradation can take five to seven months in colder places, and less than a year at the temperature and humidity prevalent in Southern Brazil, but when applied at high doses this herbicide may take more than a year to be degraded (De Noyelles et al., 1982; Almeida & Rodrigues, 1985). The main degradation products of atrazine are listed in Table IV, with some of their attributes. The persistence of these products in the environment varies and little is known about their toxicity. (Graymore et al., 2001; Berg et al., 2003).

In Tab. V, published values of atrazine EC50 for several species of algae and macrophytes are presented. In the present study, the EC50-96h of atrazine for Aulacoseira granulata was 56mg/L, indicating that the use of this herbicide in areas adjacent to water bodies is a potential environmental risk and that aquatic primary production may be reduced at very low concentrations of this toxic compound in the water. Several authors point out atrazine as one of the herbicides most used in the world, and that concentrations of 1mg/L down to 0.2mg/L are found in North American rivers, varying with planting times (Detenbeck et al., 1996; Graymore et al., 2001). No such data exist for atrazine in Brazilian rivers.

In conclusion, atrazine strongly inhibits Aulacoseira granulata growth, whereas this species has a relatively low sensitivity to dissolved copper.

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