Biotemas, 22 (3): 27-33, setembro de 2009
ISSN 0103 – 1643

Toxicity of sodium chloride and methyl parathion on the macrophyte *Lemna minor* (Linnaeus, 1753) with respect to frond number and chlorophyll

Erlei Cassiano Keppeler*

Universidade Federal do Acre, Centro Multidisciplinar
Campus de Cruzeiro do Sul – AC, Brasil
CEP 69980-000, Colônia São Francisco, Gleba,
*Author for correspondence
erleikeppeler@gmail.com

Submetido em 28/11/2008
Aceito para publicação em 07/04/2009

Abstract

Duckweed, *Lemna minor* L., is a suitable plant model for toxicity evaluation of many contaminants due to its small size and rapid growth. Methyl parathion is a toxic compound which is utilized to eliminate aquatic insect larvae, among other purposes. Its toxicity was evaluated with the use of *L. minor* in this study. Methyl parathion was added to Hoagland's nutrient medium at concentrations of 0, 8, 16, 22, 28 and 32mg.L^{-1}. *Lemna minor* is used as a tool in evaluating chemical test products for toxic effects. The sensitivity of *Lemna* to sodium chloride, the reference substance, at concentrations of 0, 2, 4, 6, 7 and 8 g.L^{-1}, was determined for comparison and resulted in an IC_{50} of 6.87g.L^{-1}. Methyl parathion in *L. minor* showed an IC_{50} of 49.48mg.L^{-1}.

Key words: IC_{50}, *Lemna minor,* methyl parathion, sodium chloride

Resumo

Efeito da toxicidade do cloreto de sódio e do paration metílico sobre o número de frondes e clorofila da macrófita *Lemna minor* (Linnaeus, 1753). A macrófita aquática *Lemna minor* L. é uma planta modelo usada para avaliação de muitas substâncias poluentes devido ao pequeno tamanho e rápido crescimento. O methyl parathion é um composto tóxico que é utilizado para eliminar larvas de insetos aquáticos, dentre outros objetivos. Sua toxicidade foi avaliada, no presente estudo, com o uso de *L. minor*. Na solução de methyl parathion foi adicionada o meio nutriente na solução de Hoagland em concentrações de 0, 8, 16, 22, 28 e 32mg.L^{-1}. *Lemna minor* foi usada para avaliar efeitos tóxicos de produtos químicos. A sensibilidade de *Lemna* ao cloreto de sódio, substância referência, em concentrações de 0, 2, 4, 6, 7 and 8 g.L^{-1} apresentou uma IC_{50} de 6,87 g.L^{-1}. A toxicidade do methyl parathion em *Lemna minor* revelou uma IC_{50} de 49,48 mg.L^{-1}.

Unitermos: Cloreto de sódio, *Lemna minor*, methyl parathion, $IC_{50,}$

Introduction

Pesticides are substances or mixtures of substances used to kill pests (USEPA, 2007). A pesticide may be a chemical substance, biological agent (such as a virus or bacteria), antimicrobial, disinfectant or device used against any pest. Pests include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread disease or are a vector for disease, or simply cause a nuisance. Although there are benefits in the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other animals.

The focus of this study was methyl parathion. It is a pesticide that is widely utilized in agriculture, and it is also applied to eliminate aquatic insect larvae that prey on fish larvae (Sosak-Swiderska, 1998; Fanta et al., 2003). Also, Sodium chloride (NaCl) has already been used as a reference substance in tests of toxicity with aquatic animals and plants. It is common for this compost to be utilized as reference substance in 96h acute toxicity tests as cited in Arenzon et al. (2003).

Aquatic macrophytes are widely utilized in the treatment of effluents, where they reduce and remove nutrients, toxic compounds, heavy metals and pathogenic organisms (APHA, 1992). They are also a source of food evidenced by its use in animal feed because of its high levels of protein in its biomass. Aquatic plants have been in common use in water quality assessment for years, acting as in-situ biomonitors (sentinel species).

Some macrophytes have been used in toxicity tests, although rooted, submersed emergent macrophytes are seldom used in toxicity tests. Duckweed has been the species of choice, and it is often used as the representative species of all other vascular plants.

In addition, macrophytes are of particular ecological importance in aquatic ecosystems because they play an important role in lacustre ecosystems. In general, they furnish cover for small fish, maintain the cycling of nutrients, and influence secondary production, creating habitats for bacteria, algae and zooplankton (Hakanson and Boulin, 2002). Aquatic plants have also been used frequently to remove suspended solids, nutrients, heavy metals, toxic organic compounds and bacteria from acidic mine drainage and agricultural landfill waste, and to stabilize sediments of near-shore environments and urban storm-water runoff (APHA, 1992; Lewis, 1995).

Duckweeds are used in phytotoxicity tests because of their small size, structural simplicity and rapid growth, characteristics that contribute to their sensitivity to chemicals. *Lemna minor* is one of the smaller forms of macrophytes, with a size of 2-4mm across, and it is widely distributed in quiescent fresh waters and estuaries ranging from tropical to temperate regions (APHA, 1992; Hillman, 1961; Huebert et al., 1990; Lewis, 1995).

Lemnoideae are limnic vascular plants that belong to the Araceae family and comprise the *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella* genera. They are commonly found in fresh water and brackish ecosystems in temperate climates and serve as an important food source for various water birds and fish. Additionally, they provide habitats for invertebrates. *Lemma minor* is frequently used in ecotoxicological research as a representative of higher aquatic plants. In 2006, both the OECD and ISO published final test guidelines (OECD, 2006; ISO, 2006). The Lemnaceae are attractive test organisms; not only do they have important ecological functions and widespread occurrence, the plants are also easy to culture and handle, have a high growth rate under laboratory conditions, and are highly sensitive to various pollutants. The plant size is small, but the fronds are sufficiently large to be easily counted with the unaided eye. This facilitates non-destructive, repeated measurements of growth patterns. Particularly important for the present study on recovery is the fact that the plants can be easily transferred into different media during testing.

The parameter most commonly monitored for toxic effects is the number of plants, although chlorophyll content and physiological effects have also been determined.

he present study was carried out to determine the effect of different toxic levels of methyl parathion and sodium chloride on the growth and photosynthetic pigments in *L. minor*.

Material and Methods

Lemna minor

Lemna minor, obtained in São Paulo, Brazil from rivers and submitted in cultures for reproduction in laboratories, was chosen for the toxicity tests with methyl parathion. Duckweed plants were grown in a continuously aerated 5-L aquarium at 20°C (APHA, 1995). A continuous photoperiod was provided by "warm white" fluorescent lights at an intensity of 5,000 lux, so as to avoid an increase in temperature. All the cultures and experiments were carried out under non-axenic conditions, in a variation of Hoagland's nutrient medium, according to APHA (1992) and Lewis (1995).

The effect of methyl parathion along with a positive control (NaCl) on *Lemna* was determined based on frond replication and pigment content according to OECD (2002). The NaCl was used as the reference substance. The effect of NaCl and methyl parathion on the number of fronds at different times (24, 48, 72 and 96h) was determined based on IC_{50} according to APHA (1995).

Tests were conducted using 20mL of test solution in small glass jars. Duckweed plants were separated into colonies of three glass jars. Next, 4 sets of fronds were added to each test solution in small glass jars for a total of 12 fronds. There were four replicates for each test concentration, and all were arranged in a randomized block design. The ranges of concentrations used in the experiments with *Lemna* were 0, 2, 4, 6, 7 and 8g.L^{-1} for NaCl (reference substance) and 0, 8, 16, 22, 28 and 32mg.L^{-1} for methyl parathion. In the present study, a 4-day period was selected according to APHA (1995). Daily, fronds visibly projecting from the edge of the parent frond were counted as separate fronds.

Changes in frond production (growth) and the deleterious effects (frond death) of the solution tests cited above were evaluated using the concentrations. Plants were to be dead when the color of the fronds had changed from green to white and partial chlorosis, was apparent.

After 96h, fronds from each test concentration were transferred to separate disposable centrifuge tubes each containing 5mL acetone/distilled (9:1). A homogenate was prepared and then centrifuged at 2000rpm for 10min. The tubes were covered with aluminum foil and placed in a freezer at 0°C, and chlorophyll pigments and pheophytin were extracted for 72h. Next, samples were allowed to warm to ambient temperature. The slurry was then transferred to a graduate cylinder, and the volume was adjusted by adding 1mL solvent to compensate for evaporation loss in all the samples (Taraldsen and Norberg-King, 1990).

Statistical analyses

After 24h, mortality rates were determined using the linear and exponential models. All data were first subjected to a Shapiro-Wilk test for normality and to Levene's test for homogeneity, prior to evaluation by analysis of variance. The data were transformed into Log (x + 1) values. The level of significance was set at a = 0.01. All statistical analyses were performed using the SAS commercial software packages.

Results and Discussion

Figure 1a demonstrates the effect of NaCl on the number of fronds at different times (24, 48, 72 and 96h). Fronds showed differences in their replication at 96h with methyl parathion (Figure 1b) as well as NaCl.

The tests showed that with as little as 6.00g.L^{-1} NaCl, there was substantial inhibition of growth, especially with exposure times of 72 and 96h, with greater effect at the latter time. After 96 h, the number of fronds in the control was 87, reduced to less than a third with 8g.L^{-1} NaCl (25 fronds). At the lowest concentrations (1, 2 and 4g.L^{-1}), growth inhibition was the same over time. Frond number decreased with increasing concentrations of NaCl, especially after 48h exposure. This was also demonstrated statistically using the equation $y = 96e^{-0.0827x}$ with $r^2 = 0.88$.

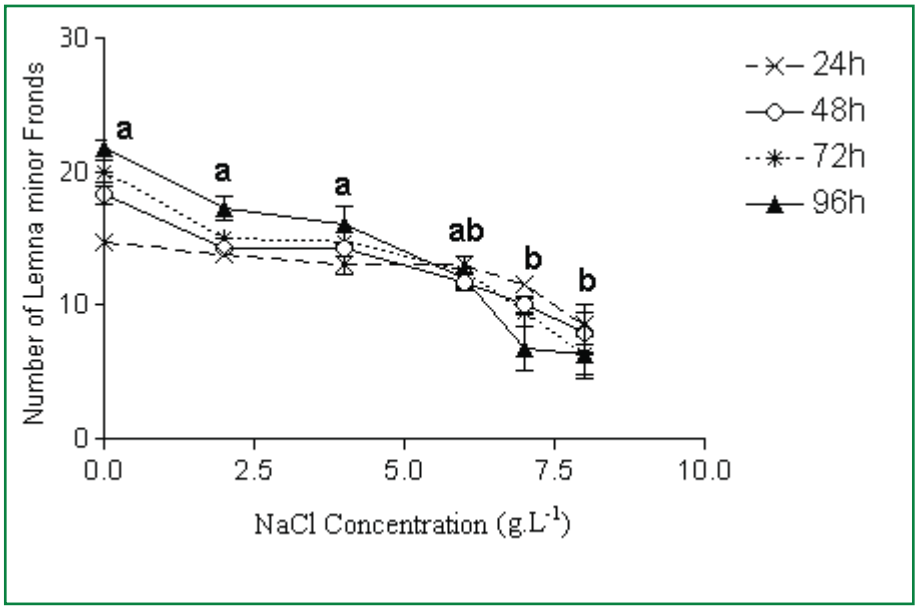

FIGURE 1a: Effect of NaCl for different exposure times. Values within the same column sharing different letters are significantly different ($p < 0.01$).

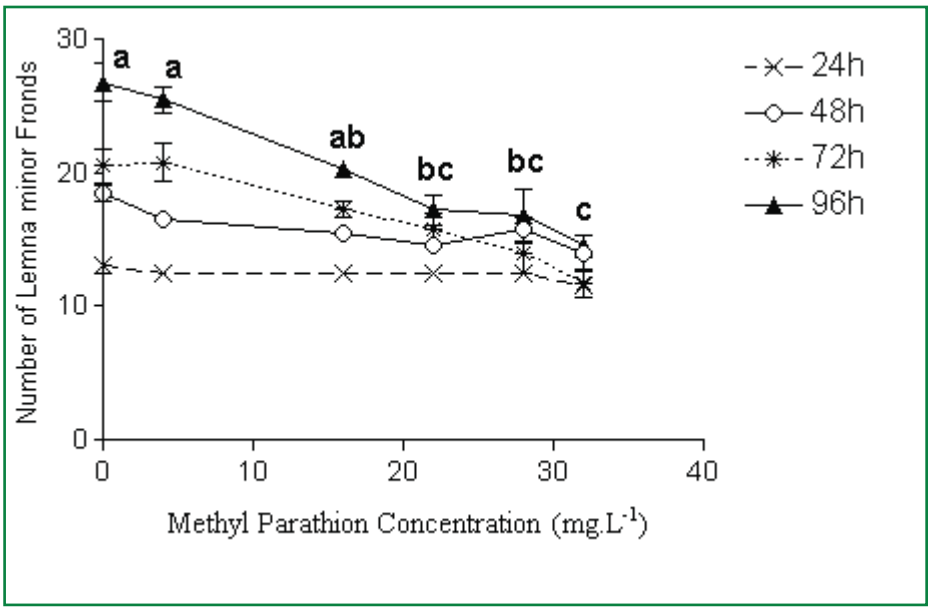

FIGURE 1b: Effect of methyl parathion for different exposure times. Values within the same column sharing different letters are significantly different ($p < 0.01$).

The same inverse relationship between frond number and concentration was shown with the test substance methyl parathion (Figure 2b). Nevertheless, the differences were more noticeable after 72h, which were also shown to be statistically significant using the equation $y = 108e^{-0.0174x}$ with $r^2 = 0.93$. At the exposure times of 72 and 96h, the difference between the control and highest concentration of the test substance was almost double. The number of fronds in the control at 72h was 82 in the control, and with 32mg.L^{-1} of methyl parathion there were 47 fronds; at 96h there were 107 fronds in the control and 58 fronds with this concentration of methyl parathion. There was little inhibition of frond growth up to 16mg.L^{-1} of pesticide, as can be seen in Figure 1b.

Figure 2a and 2b shows the results for the effect of methyl parathion along with the positive control (NaCl) on chlorophyll and pheophytin levels. For NaCl (Figure 2a), pigment levels were reduced by half at the highest concentration (8g.L^{-1}) when compared to control, except for chlorophyll c, which was decreased by a third. Therefore, as with frond number, the differences were also more notable above 6g.L^{-1}, except for chlorophyll c, which increased at a concentration of 7g.L^{-1}.

Toxicity was assessed as inhibition of growth and chlorophyll biosynthesis, as a function of dose and time. For *L. minor,* similar toxicity was seen when comparing frond number and chlorophyll *a* in all the assays conducted in this study. For practical purposes, the chlorophyll *a* assay can be omitted, and only the enumeration of fronds needs to be considered in routine tests. Photosynthetic activity has been seen to be the least sensitive indicator of toxic effects in most cases (Lewis, 1995). Nevertheless, in our study though, chlorophyll *a* and *b* (but not *c*) did show a statistical difference between treatments (sodium chloride). Thus, pigment content could be a satisfactory method of toxicity assessment.

Methyl parathion displayed more substantial effects on chlorophyll *a*, as with frond number, at concentrations as low as 16mg.L^{-1} (Figure 2b). In general, all pigments examined (chlorophyll *a, b*, c and pheophytin) also had levels reduced by half compared to control at the highest concentration of 32mg.L^{-1} after 96h. As with NaCl, methyl parathion also caused an increase in chlorophyll *c* at 28mg.L^{-1}. Pheophytin also showed slight increases of 6.30mg.L^{-1} and 6.40mg.L^{-1} at the lowest concentrations of methyl parathion when compared to control (5.93mg.L^{-1}).

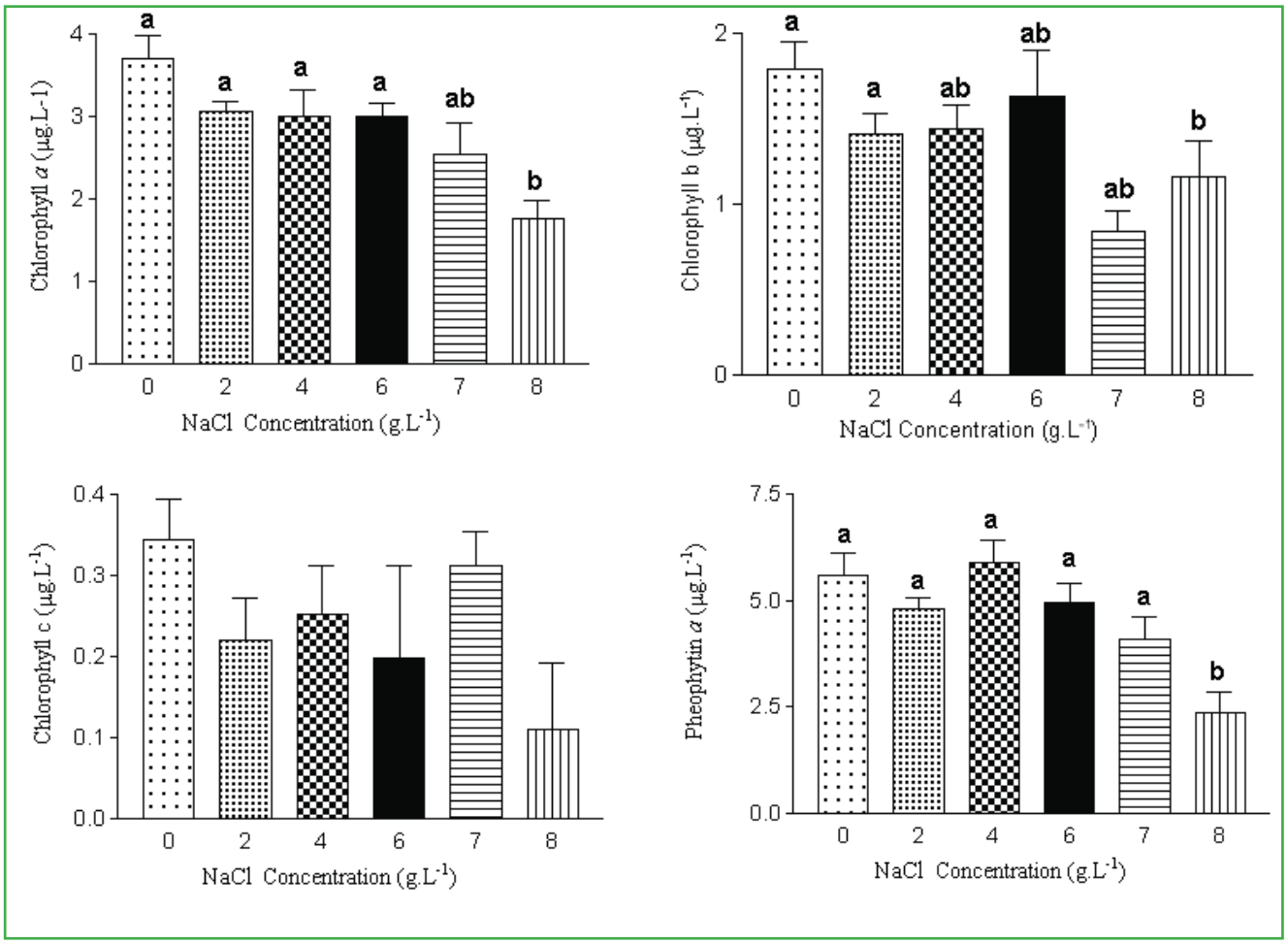

FIGURE 2a: Effects of NaCl at different concentrations in *Lemna minor*. Values within the same column sharing different letters are significantly different ($p < 0.01$).

Figures 2a and 2b show the analysis of variance results for each parameter measured. Variance analysis results showed a highly significant ($p < 0.01$) difference between the treatments and untreated control. In addition, there was a high correlation for methyl parathion (r = 0.99; growth inhibition (%) = 0.239 + 1.53* Conc) as well as for NaCl (r = 0.97; growth inhibition (%) = -2.10 + 7.58* Conc) between concentrations of the compound and growth inhibition in terms of frond number.

There was no notable difference in our study in regard to sensitivity to NaCl, which showed an IC_{50} of 6.87g.L^{-1} at 20°C, in comparison with other studies. For example, Buckley et al. (1996) observed EC_{50} values of 4.80-5.50g L^{-1} NaCl in various experiments.

This demonstrates that the reference substance was within the levels adjusted for *L. minor* be used in definitive tests, that is in this study the methyl parathion.

With regard to the number of fronds and chlorophyll *a, b* and *c* levels, only the results for chlorophyll *c* were not statistically significant with NaCl. Chlorophyll *a* did not show any difference with Tukey's test for NaCl in comparison to the control at 2, 4 and 6g.L^{-1}, as was the case with pheophytin at NaCl concentrations of 2, 4, 6 and 7g.L^{-1}, while with methyl parathion the differences were more notable (Figure 2b).

The 96h–IC_{50} values for NaCl and methyl parathion in *L. minor* were 6.87g.L^{-1} and 49.84mg.L^{-1}, respectively. The sensitivity test with NaCl in *L. minor*, based on IC_{50}

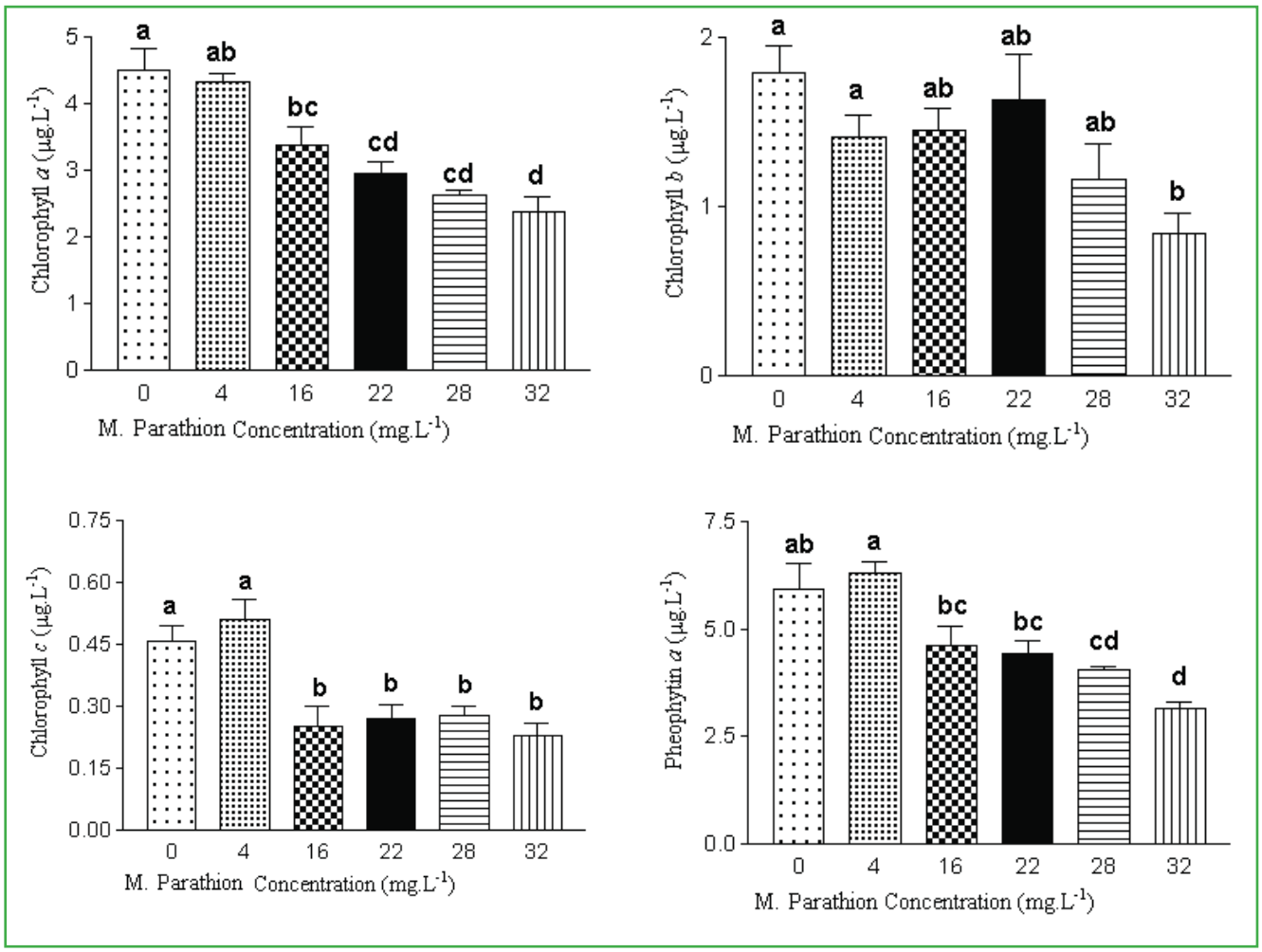

FIGURE 2b: Effects of methyl parathion at different concentrations in *Lemna minor*. Values within the same column sharing different letters are significantly different ($p < 0.01$).

determination, showed an effective mean concentration within the accepted range for this substance.

Considering that methyl parathion is an insecticide, these findings indicate that it acts at low concentrations in plants after an exposure time of four days. Nevertheless, methyl parathion uptake by the plants was apparently low in view of the relatively high IC_{50}.

The results obtained in this study for *L. minor* are due to the diverse ways that these organisms react in response to a chemical stressor. The main reason for the low effect of methyl parathion on *L. minor* is that it is an inhibitor of acetyl cholinesterase which is absent in this plant. It is generally known that anticholinergic pesticides have low toxicity in plants that lack cholinesterases (Kao et al., 2003).

The present study demonstrates that the concentrations of methyl parathion had positive effects on the organism tested. The short-term toxicity of methyl parathion in *L. minor* $IC_{50,96}$ was 49.84mg.L^{-1}.

Acknowledgments

The author would like to thank Aquaculture Center (Caunesp) and Júlio de Mesquita Filho at São Paulo State University for providing support. Dr. Albert Leyva also provided editorial services.

References

APHA – American Public Health Association. 1992. **Standard methods for examination of water and wastewater.** 18th ed. Bru-El Graphic Inc., Springfield, USA, 1155pp.

APHA – American Public Health Association. 1995. **Standard methods.** 19th ed. American Public Health Association, Washington, USA, 1193pp.

Arenzon, A.; Pinto, R. F.; Colombo, P.; Raya-Rodriguez. 2003. Assessment of the freshwater annual fish *Cynopoecilus melanotaenia* as a toxicity test organism using three reference substances. **Environmental Toxicology and Chemistry, 22** (9): 2188-2190.

Buckley, J. A.; Rustangi, K. P.; Laughlin, J. D. 1996. Response of *Lemna minor* to sodium chloride and a statistical analysis of continuous measurements for EC50 and 95% confidence limits calculation. **Bulletin of Environmental Contamination and Toxicology, 57**: 1003-1008.

Fanta, E.; Rios, F. S.; Romão, S.; Vianna, A. C. C.; Freiberg, S. 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. **Ecotoxicology and Environmental Safety, 54**: 119-130.

Hakanson L.; Boulin, V. 2002. **The lake foodweb.** Backhuys Publishers, Leiden, Netherlands, 1020pp.

Hillman, W. S. 1961. The Lemnaceae or duckweeds: A review of the descriptive and experimental literature. **Botanical Review, 27**: 221-287.

Huebert D. B.; McIlraith A. L.; Shay J. M.; Robinson G. G. C. 1990. Axenic culture of *Lemna trisulca* L. **Aquatic Botany, 38**: 295-302.

ISO – International Organization for Standardization. 2006. **Dissemination of the toxic effect of water constituents and wastewater on duckweed (Lemna minor) – Duckweed growth inhibition test, ISO norm 20079.** Available at <http://www.swift-wfd.com/Local/swift/dir/doc>. Accessed on November 5th, 2007.

Kao, C. H.; Tzeng, C. L.; Cheng, E. Y. 2003. Method development and application for rapid detection of the anticholinergic residues in rice. **Journal of Agricultural Research of China, 52**: 324-333.

Lewis, M. A. 1995. Algae and vascular plant tests. In: Rand, G. M. & Petrocelli, S. R. (Eds). **Fundamentals of toxicology.** Hemisphere, Washington D.C., USA, p.135-169.

OECD – Organization for Economic Co-operation and Development. 2002. **OECD guidelines for the testing of chemicals revised proposal for a new guideline 221 *Lemna* sp. growth inhibition test.** Available at <http://www.oecd.org/>. Accessed on December 10th, 2007.

OECD – Organization for Economic Co-operation and Development. 2006. **Guidelines for the testing of chemicals, test No. 221: *Lemna* sp. growth inhibition test.** Available at <http://www.oecdbookshop.org/>. Accessed on December 10th, 2007.

Sosak-Swiderska B.; Tyrawska D.; Dzido D. 1998. *Daphnia magna* ecotoxicity test with parathion. **Chemosphere, 37** (14): 2289-3000.

Taraldsen, T. E.; Norberg-King, T. J. 1990. New method for determining toxicity using duckweed (*Lemna minor*). **Environmental Toxicology and Chemistry, 9**: 761-767.

USEPA – US Environmental Protection Agency. 2007. **What is a pesticide?** Available at <http://www.epa.gov.br>. Accessed on September 15th, 2007.