Effects of salinity, atrazine, molinate and chlorpyrifos individually and as mixtures to the freshwater alga *Pseudokirchneriella subcapitata* and cladoceran *Daphnia carinata*

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Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and preparation of the thesis itself has been acknowledged. In addition, I certify that all information and literature used are indicated in the thesis.

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Neil Hemantha Dassanayake

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Thesis structure

The experimental chapters in this thesis are written as journal articles. Since journal articles must be self-contained, there is some degree of repetition in the thesis.

Chapter 1 discusses the importance of multigenerational studies in toxicity evaluations, the limitations of single species toxicity testing, and need for population and community level studies. Selection of suitable test organisms i.e., *Pseudokirchneriella subcapitata* and *Daphnia carinata* is also discussed. In addition, a review of inland salinity issues, pesticide pollution and importance of mixture toxicity studies including salinity and pesticide mixtures are discussed. Chapters 2 and 5 examine the exposure of *P. subcapitata* and *D. carinata* to salinity over several generations (separately) and life history and population level effects are discussed.

Chapters 3 and 6 describe the toxicities of salinity, atrazine, molinate and chlorpyrifos individually, and mixtures of different combinations of the toxicants to *P. subcapitata* (short-term chronic tests) and *D. carinata* (acute tests).

Chapters 4 and 7 examine the effects of high salinity acclimation (over multiple generations) of *P. subcapitata* and *D. carinata* on the toxicities of salinity, atrazine, molinate and chlorpyrifos individually and as mixtures of different combinations to determine whether there are sensitivity changes.

Chapter 8 summarises the findings of the study and evaluates the comparative effects of salinity (multigenerational and short-term toxicity), and three pesticides individually and mixtures on the two test organisms studied. The possible indirect effects of toxicants on organisms in higher trophic levels, i.e., *D. carinata* as a result of direct effects on the primary producers i.e., *P. subcapitata* are discussed. The importance of incorporating mixture toxicities and community level effects into environmental management of toxicants are discussed in relation to results of the present study.

Table of Contents

List of Tables

List of Figures

Abstract

The salinisation of inland water bodies occurs in many parts of the world. This issue is serious in Australia, especially in agricultural areas where pollution caused by pesticides is also prevalent. Even though mixture toxicity studies have been carried out for various classes of chemicals, little information is available on the toxicities of the combined effects of pesticides and salinity on freshwater aquatic biota. Aquatic organisms inhabiting salt-affected inland water bodies experience the effects of salinity over generations. Thus, there may be differences in the sensitivity of these organisms to salt-induced changes from those caused by other toxicants. There is no scientific information on how salinity-affected freshwater organisms respond to various agrochemicals in such environments.

Two representative test species, viz. the freshwater alga *Pseudokirchneriella subcapitata* and the freshwater cladoceran *Daphnia carinata* were selected to study the effects of salinity individually and as mixtures with selected pesticides (atrazine, molinate and chlorpyrifos).

The experiments consisted of effects of multigenerational exposure to salinity on the life history traits of these two species. The acute toxicities of individual toxicants and their mixtures on these two species were also studied. Finally, the acute toxicities of individual and mixtures of toxicants on both species acclimatised to salinity over multiple generations were compared with non-acclimatised populations. *P. subcapitata* (normal salinity 100 μS/cm) exposed to elevated salinities (3000 and 6000 μS/cm) over five sequential cultures (C0 – C4) had significant ($p \leq 0.05$) adverse effects on growth rate, number of cell divisions per day and generation time except for the second culture (C1). All cultures from C1 to C4 had significantly lower ($p \leq 0.05$) cell yields than C0 for both the elevated salinities. Significant differences ($p \leq 0.05$) between cultures were observed (compared with C0) in terms of the growth rate, cell divisions per day, generation time and cell yield indicating that stress induced by elevated salinity persisted over the five sequential cultures.

D. carinata (normal salinity 200 μS/cm) were maintained at elevated salinities (2000, 4000, 5500 and 6300 μS/cm) for four generations (F0 - F3). Salinities ≥ 5500 μS/cm

significantly reduced ($p \leq 0.05$) their reproductive ability, intrinsic rate of natural increase and ingestion rates. Neonatal length was significantly reduced ($p \leq 0.05$) at $\geq$ 2000 μS/cm while filtration rate was significantly reduced ($p \leq 0.05$) at $\geq$ 5500 and 4000 μS/cm for the F1 and F2 generations, respectively; no significant differences were found for the F0 generation. Significant delays ($p \leq 0.05$) in the reproduction also occurred with increasing salinities.Development of tolerance to salinity as an adaptation over generations was not clearly evident from these results. Cladoceran populations may be impacted due to reduced food availability (due to direct effects of salinity on the alga) at salinities $\geq$ 3000 μS/cm. Such cascading effects on species at higher trophic levels could result in community level effects. Thus, reliance on results of single species experiments is questionable and there should be a mechanism to incorporate community level toxic effects in ecological risk assessments and water quality guidelines.

Chronic toxicities of individual toxicants (72-hour IC50 values) to *P. subcapitata* were 5600 μS/cm, 48 μg/L, 300 μg/L, 797 μg/L for salt, atrazine, molinate and chlorpyrifos, respectively. Atrazine was very highly toxic, molinate was highly toxic and chlorpyrifos was moderately toxic to the alga. Toxicities of mixtures of these toxicants were evaluated using the Toxic Unit (TU) approach. Approximately 50% of the mixture combinations for non-acclimatised *P. subcapitata* conformed to antagonism, 47.3% to additivity and 1.8% to synergism. For salinity acclimatised and non-acclimatised cultures of *P. subcapitata,* chlorpyrifos/salt and atrazine/chlorpyrifos/salt mixtures were significantly ($p \leq 0.05$) different and high salinity acclimatised cultures were less tolerant of these mixtures. In salinity acclimatised cultures exposed to mixtures of the pesticides and salinity, the majority of the concentrations (71%) tested conformed to additivity. Approximately 20% and 9% of the mixtures conformed to antagonism and synergism, respectively. Changes in toxicity relationships in mixtures occurred between non-acclimatised and acclimatised cultures i.e., antagonistic relationships in non-acclimatised cultures became additive in acclimatised cultures, and some additive relationships in the non-acclimatised cultures became synergistic in the acclimatised cultures. This indicates an increase in the sensitivity to the toxicant mixtures of the acclimatised alga.

For *D. carinata,* acute toxicity (48-hour immobilisation EC50) values were 8790 μS/cm, 42 mg/L, 25 mg/L and 0.21 μg/L for salinity, atrazine, molinate and chlorpyrifos, respectively. Chlorpyrifos was very highly toxic while atrazine and molinate were slightly toxic to the cladoceran. Toxicity relationships for mixtures of pesticides and salinity for non-acclimatised cultures were 47% conformed to additivity, 11% conformed to synergism and 42% conformed to antagonism. The 11% of mixtures showing synergistic relationships needs attention in developing water quality guidelines (WQGs).

Acclimatisation to 6300 μS/cm water did not change the tolerance of *D. carinata* to acute exposures of atrazine, chlorpyrifos or molinate individually, as mixtures of the pesticides or as mixtures of the pesticides with the high salinity tested. Thus, acclimatisation to salinity did not adversely alter their defence mechanisms against individual pesticides or their mixtures. The toxicity relationships of mixture combinations were antagonism - 37%, additivity - 52% and synergism - about 11%. This suggests greater toxicity than expected from the concentration addition (CA) model. There was about a 5% decrease in antagonism in high salinity cultures compared with normal salinity cultures while additivity increased by the same percentage in high salinity cultures; synergism remained the same in both cultures.

The two test species exhibited different toxicity responses to the mixtures of toxicants. There were changes in sensitivities in terms of toxicity relationships in salinity-acclimatised and non-acclimatised cultures especially for *P. subcapitata*. The most prevalant toxicity relationship was additivity and therefore incorporation of additivity into the derivation or implementation of WQGs would protect the two species from approximately 90% of the mixtures tested. However, mixtures that have synergistic relationships, which were about 10% of the mixtures would not be sufficiently addressed by these WQGs. Based on the findings of the present study, it is recommended that long-term exposure scenarios and toxicities of mixtures should be incorporated to provide a more conservative approach in risk assessments and in deriving water quality guidelines.