Are herbicides from sugarcane crops reaching and impacting nearby marine turtle nests?

Hannah L Allan, Jason P van de Merwe, Kimberly A Finlayson and Frederic DL Leusch

Australian Rivers Institute & School of Environment, Griffith University, Parklands Drive, Gold Coast, Qld 4222, Australia

Introduction

Agricultural practices introduce many herbicides into the environment that have the potential to contaminate groundwater and surrounding ecosystems. In Queensland, Australia, sugarcane crops are extensive in coastal regions and contamination of nearby marine environments is a major concern. Herbicides used on these crops have been found to have cytotoxic, genotoxic, embryotoxic and endocrine disrupting effects in non-target species[1,2,3,4] . In 2014, a passive sampler placed in a wetland of Mon Repos detected 12 herbicides[5]. This raised concerns that these chemicals could contaminate groundwater and reach the nearby beach that is a major nesting area for the critically endangered South Pacific population of loggerhead turtles.

This study investigated the quantity of herbicides reaching the nesting sand of Mon Repos beach and their cytotoxicity to loggerhead turtle cells.

Methods

Paired **water samples** were taken from agricultural drains and wetlands (Figure 1). **Sand samples** were taken from five sites at Mon Repos beach at the lower (1m) and upper (60cm) range of turtle nesting depth (Figure 1). Sampling was carried out towards the end of the wet season (February) as this is when crop runoff volumes were expected to be the greatest and also coincided with the marine turtle nesting season.

Chemical analysis:

- Herbicides were extracted and concentrated for HPLC-MS analysis.
- Herbicides analysed include 14 Photosystem II (PSII) inhibitors, 6 synthetic auxins, 2 acetolactate synthase (ALS) inhibitors, and 2 others.

Imaging Pulse Amplitude Modulated (IPAM) fluorometry:

- The total effect of PSII herbicides in extracts was measured and compared with predicted PSII inhibition from chemical analysis[6] using predicted and measured diuron equivalent concentrations (DEQs) based on photosynthesis inhibition in freshwater algae (*Pseudokirchneriella subcapitata*).

Cytotoxicity in loggerhead skin cells using resazurin assay:

- Expressed as toxic units (TU), calculated as 1 divided the EC_{50} in relative enrichment factor.

Figure 1. Location of water samples (blue) and sand samples (yellow) within Mon Repos, Queensland.

Results and discussion

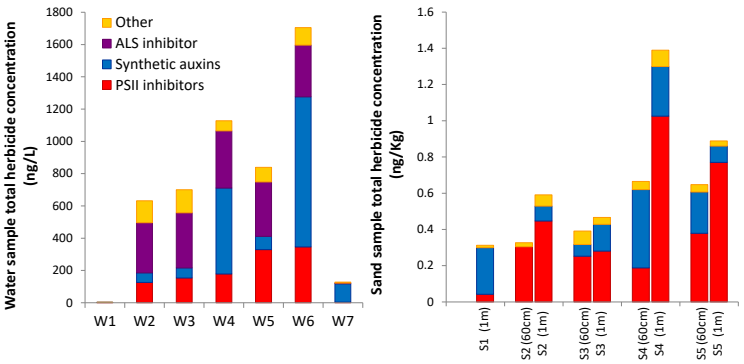

Figure 2. Quantity (ng/L) of each herbicide mode of action within each sample extract. Herbicides and their breakdown products are classified similarly.

A total of 23 herbicides were detected in water samples and 8 were detected in sand samples. In sand samples, PSII herbicides were found in the largest quantities suggesting that these herbicides may be more mobile than those with other modes of action such as ALS inhibitors, which were not detected in sand samples. Sand samples at 1m depth were found to have higher herbicide concentrations than those at 60cm.

Water samples W1 and W7 had the lowest herbicide concentrations and were located the furthest from agricultural drains or sugarcane crops (Figure 2). Site W7 showed the highest proportion of non-sugarcane herbicides (13%) possibly due to input of herbicides used to maintain nearby visitor walking tracks. Given this, outflow from the wetland in which W7 is located, may explain the high proportions of non-sugarcane herbicides in the southern-most sand sites. S5, S4, and S3 were found to have 63%, 52% and 48% non-sugarcane herbicides, respectively. This shows a pattern with distance from the wetland outlet.

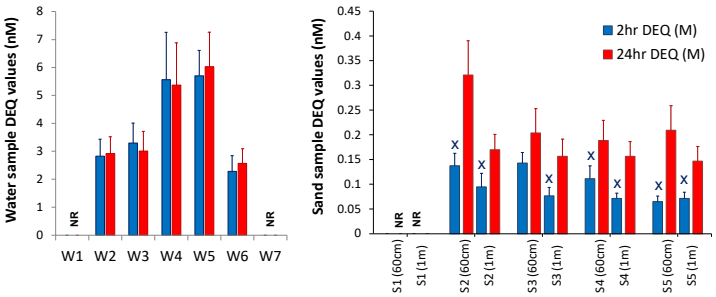

Figure 3. DEQ values of extracts following 2 and 24 hours exposure in freshwater algae. "NR" indicates no response to extracts and "x" indicates that EC_{50} was not reached but extrapolated beyond the range of the response curve in order to calculate DEQ.

With water samples, 5 - 25% of PSII herbicide action measured in the IPAM was attributable to the monitored herbicides. In contrast, a maximum of 1% was explained in sand extracts. There was no significant difference between 2 and 24 h responses in water samples suggesting that non-PSII herbicides present were not toxic to algae; however **toxicity of all sand extracts increased between 2 and 24 h indicating the presence of non-PSII herbicides** (Figure 3).

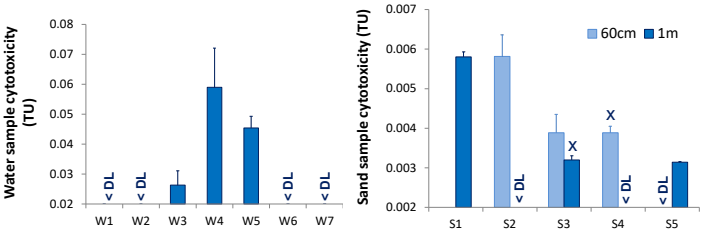

Figure 4. Cytotoxicity of extracts in loggerhead skin cells in toxicity units (TU). "x" indicates that the EC_{50} was not reached and is extrapolated beyond the range of the response curve . "< DL" highlights cytotoxic responses below the detection limit.

There was no correlation found between herbicide concentration and cytotoxicity of the extracts. Extracts were not found to be cytotoxic in loggerhead skin cells at environmental concentrations. **Extracts had to be concentrated at least 17× in water and 170× in sand samples in order to observe a cytotoxic effect** (Figure 4).

Conclusions

- Most herbicides detected were sugarcane herbicides, however data suggests non-sugarcane herbicides may be discharged from wetlands and contaminating southern sand sites.
- There was no correlation between total herbicide concentration and toxicity of the extracts in algae or loggerhead turtle cells.
- Only a small proportion of photosynthesis inhibiting activity could be explained suggesting the presence of other PSII herbicides not included in the chemical analysis.
- Extracts were not cytotoxic at environmental concentrations. Further studies should investigate more subtle effects such as endocrine activity.

Acknowledgment

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References

[1] Cavas and Konen 2007, [2] de Campos Ventura *et al.* 2008, [3] Villalobos *et al.* 2000, [4] Keller *et al.* 2006 [5] Department of Science, Information Technology and Innovation [6] Escher *et al.* 2008.