MID-TERM REVIEW

Developing efficient and effective methods to improve assessment of waterway health

Healthy Waterways Strategy 2018-2028 — Port Phillip & Westernport, Victoria | Melbourne Water | RMIT University A3P

Strategic alignment

Regional Performance Objectives

RPO 23: - The potential impacts of emerging contaminants of concern such as microplastics, pesticides and pharmaceuticals, and toxic chemicals are better understood and mechanisms to respond collaboratively developed.

Key Research Areas

Water quality: Developing improved water quality indicators and monitoring methods to better understand the impacts of pollutants on waterway health.

Summary

Current methods to monitor and assess impacts of water quality on waterway health inform managers about the condition of the waterways but provide little information on what is influencing poor condition, which organisms (environmental values) are at risk and where to focus management efforts to improve condition. This project will identify key knowledge gaps of what can reliably predict the relationships between water quality condition and which environmental values are affected. This information will be used in implementing the Healthy Waterways Strategy (HWS) Monitoring, Evaluation, Reporting and Improvement (MERI) framework and allow an adaptive management approach for assessing waterway health. This project has also identified and is further developing new tools that Melbourne Water (and other stakeholders, including EPA and DEECA) can use to better understand the link between water quality and stream health, and be used as a standard assessment approach (Figure 1).

These tools include functional indicators of waterway health as well as diagnostic tools that will better characterise pollution types, sources and impacts on water quality and waterway health. Furthermore, the tools could be used to assess the success of management actions to reduce pollution. The project also includes methods to assess aquatic ecosystems as a whole (i.e., considering both water and sediment quality and responses of instream values), rather than just focussing on pollutant concentrations.

This project has worked with other A3P projects to develop and test waterway health assessment tools including the Chemicals of Concern project to identify chemicals that are likely to be found in our waterways in the future (Figure 2).

Recommendations

- Trial this approach in case study catchments to help understand the drivers of declines in the condition of environmental values e.g. Lang Lang River aquatic macroinvertebrates, River Blackfish in the Plenty River.
- Develop new metrics for determining water quality condition and to assist in setting targets in future strategies.
- Incorporate this assessment framework into the HWS MERI Framework.

What did we do?

Linking land use with pollutants and impacts on HWS environmental values.

The first part of this project identified which pollutants were associated with different land use classes through a literature review (Report 12). Following this, each pollutant class was assessed for likelihood of toxicity to the most sensitive HWS environmental values (i.e., birds, fish, frogs and macroinvertebrates) through a risk assessment (Report 16.) see Table 1. This has led to the development of tools for frogs and flagged that more information is required with regard to pesticides.

Global water quality monitoring methods review

A review of waterways monitoring programs around Australia and worldwide has been carried out to look at what indicators have been used (Long et al., 2020).

Developing efficient and effective methods to improve the assessment of waterway health

Reviewing the use of ecotoxicology in ecosystem management

A literature review to describe the use of ecotoxicology in biomonitoring programs was undertaken to look at the advantages of including ecotoxicology into current monitoring programs (Nugegoda et al., 2020)

Novel methods for assessing waterway health

Microbial community assessments using a molecular approach.

A next generation sequencing approach was used to assess microbial communities in whole sediments in two pilot studies. The first study looked at potential differences in microbial communities between sites with “high” and “low” pollution exposure. The second study focused on potential differences in sites with different types of sewage discharge, i.e., untreated compared to treated effluent and Emergency Relief Structure spills from four creeks in 2020 (Report 66).

Non-destructive sample collection methods and effects assessments.

Novel methods for collecting animal body fluids, for example mucus or slime have been proposed to avoid sacrificing organisms for ecotoxicological tests. Ecotoxicological tests bridge the gap between pollutants found in the environment and detecting the harm they cause to organisms. These are analytical methods that identify molecules such as sugars and amino acids which can indicate stress responses in the host animal or plant (organism). We are currently using the slime from the freshwater snail (*Physa acuta*) to trial some non-destructive sampling techniques and novel methods of effects assessment, for example metabolomics. Environmental metabolomics characterizes the interactions of living organisms with their environments (Long et al., 2015). Recently, studies have demonstrated that metabolomics approaches can be used to identify small metabolite biomarkers of pollutant exposure in aquatic organisms (Long et al., 2015). Metabolomics can tell us in a more holistic way how an animal is responding to stress and can be used diagnostically for specific chemicals.

Slime excreted from snails was collected on filter paper and the metabolites in the slime (e.g., amino acids, sugars) were extracted. Metabolites were also extracted directly from host snails and quantified using analytical chemistry instrumentation (Long et al., 2015).

Development of ecotoxicology tests for new species

To further a holistic assessment of waterway health new local species were tested in order to be added to the suite of toxicity tests including - *Spirodella punctuata* (ready to use in monitoring programs) and tadpoles (still in development stage). Southern brown tree frogs (*Litoria ewingii)* egg masses were collected and maintained at RMIT until they reached Gosner stage 28 (when the hind limb buds started to appear). Tadpoles were exposed to three concentrations of six different pesticides that are routinely detected in waterways around Melbourne, i.e., glyphosate, simazine, diuron, bifenthrin and permethrin, as well as Imazapyr, an alternative herbicide to glyphosate. Tadpoles were exposed for 48 hours and at the end of the experiment, morphological measurements were taken, including snout to vent length, total length and weight. Biomarkers were also assessed to determine if exposure to the pesticides had resulted in physiological changes to the tadpoles.

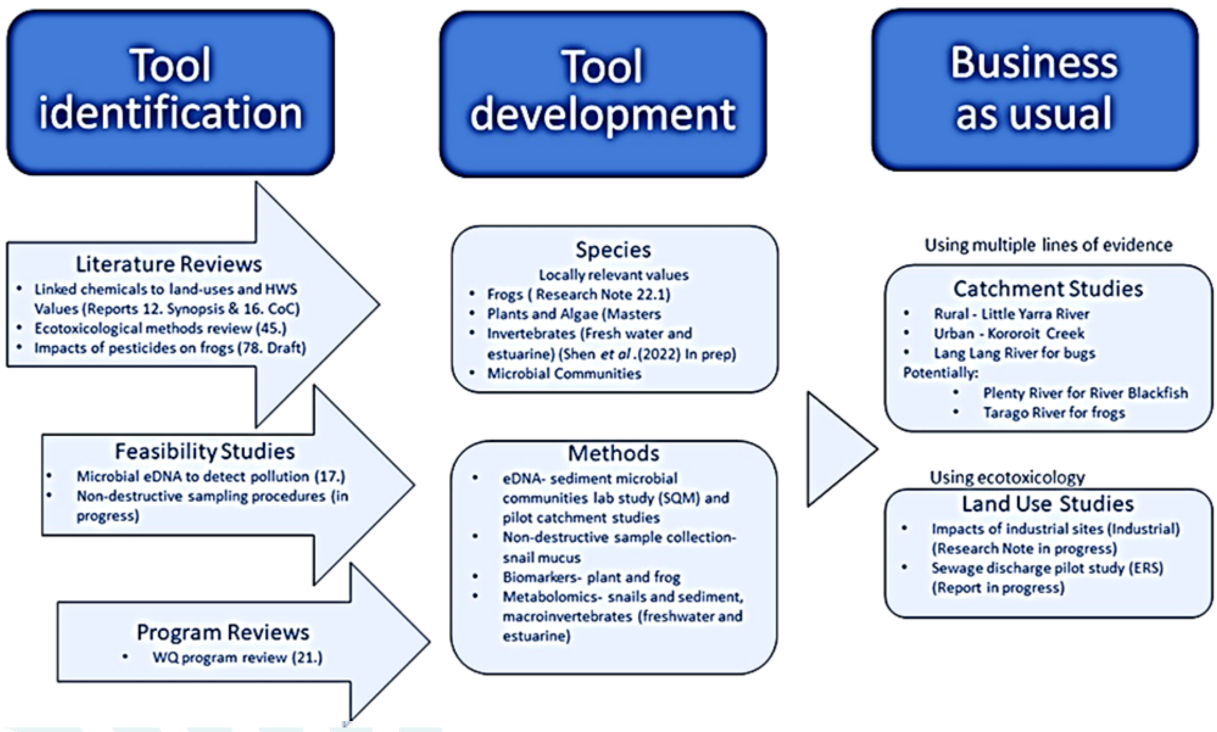

Figure 1. Summary and links between the different aspects of this project from tool identification through to being used in Melbourne Water as “"Business as Usual". (Bracketed number indicates the A3P report no., updated list in references)

Developing efficient and effective methods to improve the assessment of waterway health

Catchment studies

Two catchment studies have been conducted to trial a range of biomonitoring tools that have been developed at A3P. For the HWS, these studies are significant and can help us tease apart primary drivers of declining trends in environmental values. The first catchments chosen were a rural and urban catchment to determine which tools were more appropriate for each. The Little Yarra, a rural catchment, was predicted to be in good condition with no declining values and the second catchment was Kororoit Creek, an urban catchment. From the Sediment Quality Monitoring Program, we were aware of the prevalence of heavy metals and other pollutants and of general declining environmental values, with the potential for different drivers (new urban development) in the upper reaches and industrial practices in the lower at Kororoit Crk.

Eight sites within the Little Yarra catchment which encompassed a range of land uses including urban, agricultural and rural areas were sampled. The tools used included passive samplers, sediment chemistry and toxicology, assessment of macroinvertebrate abundance and diversity, caging studies using plants, microalgae and shrimp and sediment microbial community composition using a metagenomics/ molecular approach. The study was carried out in April-May 2021.

Five sites were sampled in Kororoit Creek in Spring 2021 and Autumn 2022. A small catchment study was run in Spring 2021 with only sediment chemistry, passive samplers and macroinvertebrates assessed at this time. A full catchment study using a multiple lines of evidence approach was run in Autumn 2022, using the same tools as those used in the Little Yarra study, as stated, to enable a better understanding of which tools are more appropriate for each catchment to understand the drivers of catchment health.

Incorporated ecotoxicology into other water quality assessment programs

Ecotoxicological assessments have also been incorporated into Melbourne Water programs to assess the impact of site-collected water on aquatic invertebrates – Seaford and Edithvale monitoring program (stage 3) and A3P Industrial project.

Framework development

This framework includes a multiple lines of evidence approach with tiers of assessment e.g., 1) desktop review, 2) catchment screening with multiple tools such as passive samplers, sediment quality, ecotoxicology, metabolomics, metagenomics community structure and 3) detailed investigations of targeted stressors such as pesticides, physical form or flow (Figure 3).

The Process

- Trigger: A Healthy Waterways Strategy value trajectory is off- target or an issue has been identified at a site.
- Desktop Assessment: gather all available data, including non A3P data. These data include land uses, maps of stormwater and other drains, e.g. council drains into the catchment, environmental values supported at the site, any water quality/sediment quality data, macroinvertebrates, and previous reports on catchment condition.
- Gather site specific information: could include a field trip & speaking with Waterways & Land Officers (WLOs) and other stakeholders (internal and external to MW)

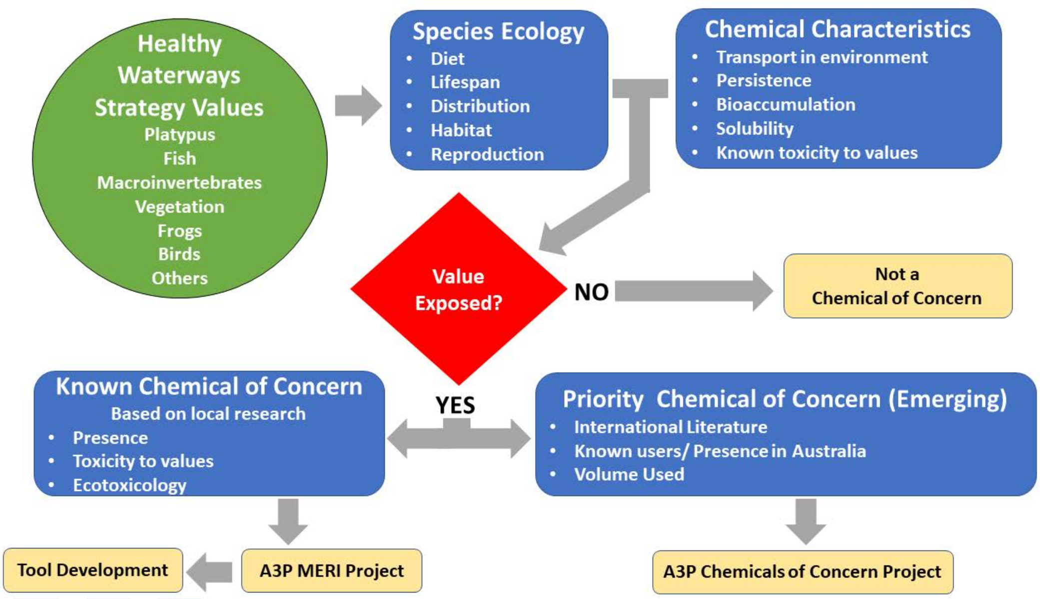

Figure 2. Flowchart identifying known chemicals of concern for the Healthy Waterways Strategy environmental values and where the MERI project fits in to this process (adapted from Pettigrove, 2019)

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- Determine which tools are most relevant, primarily based on environmental values (or other important biota) present, maps of drains and potential inputs (to determine logistics of sediment collection, deployment of passive sampling devices) and surrounding land uses (for example, chemicals present in industrial areas are likely to be metals and hydrocarbons and certain pesticides, compared to agricultural areas).
- Carry out catchment screening assessment.
- Review Results

a. If cause of concern is evident discuss/recommend suitable management action

b. If not evident- Refine assessment/ undertake a detailed investigation of stressors and loop back to screening assessment.

Use of a complimentary approach in the Sites of Biodiversity Sensitivity program has shown that the best tools to use are best determined by site specifics (i.e., not a one size fits all approach), see above.

Westernport catchment: Lang Lang River

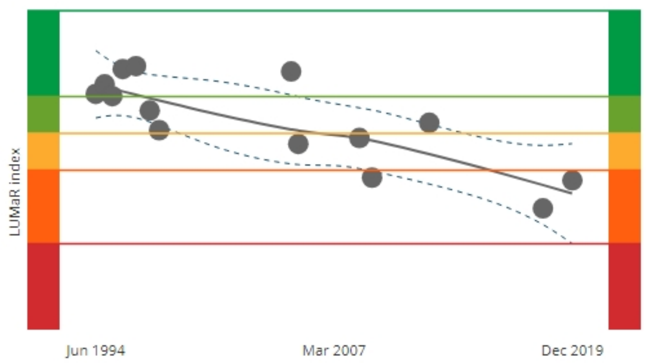

Figure 3. Macroinvertebrate assemblages in the Lang Lang River showing decline in the LUMaR Index from 1994 to 2019.

In combination, this is a powerful toolbox which can systematically determine which stressor on the system is of greatest priority.

An example of a trigger which would instigate a Catchment Study is the decline in macroinvertebrate assemblage detected in the Lang Lang River, see Figure 3 (Source : Macroinvertebrates | Healthy Waterways Strategy for Port Phillip and Westernport, Victoria (Accessed 20/10/2022)) The process in Figure 4 would then be undertaken.

What did we find?

Linking land use with pollutants and impacts on HSW environmental values.

Insecticides are the class of chemicals that pose the greatest risk to the majority of environmental values (Table 2); specifically, synthetic pyrethroids such as bifenthrin, but not surprisingly insecticides in general are highly toxic to macroinvertebrates.

Global water quality monitoring methods - review

A review of waterways monitoring programs around Australia and worldwide showed that there is not one set of tools for biomonitoring programs that fit all waterway management needs. Rather, the selection of tools for use in biomonitoring programs depends on the specific aims of the program, e.g., in a general catchment study multiple tools are warranted, but if you are looking at the effect of a Sewage Treatment Plant on fish, then a tailored approach is required. An important consideration is to include appropriate reference or control sites for the programs to ensure you are comparing like with like.

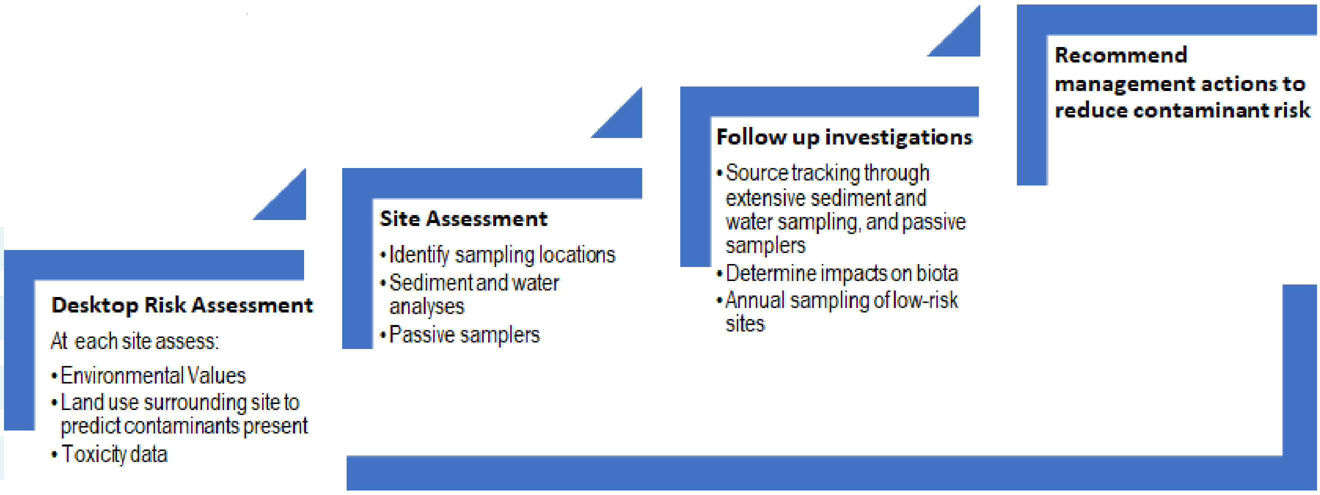

Figure 4. Representation of the project process cycle using a multiple lines of evidence approach

Reviewing the use of ecotoxicology in ecosystem management

The review highlights the value of including ecotoxicological endpoints in monitoring and managing chemicals in Melbourne's waterways. For example, lethality, toxicity, determining acute toxicity values e.g. LC50/EC50 and biomarkers (changes in biological responses following exposure to chemicals or stressors) can be used to detect and/or predict adverse chemical impacts on populations, communities and ecosystems. (Nugegoda et al., 2020).

Novel methods for assessing waterway health- pilot studies

Microbial community assessments using a molecular approach.

Preliminary results showed that there was a clear separation in microbial diversity between sites impacted by concentrations of high metals and total petroleum hydrocarbons and those with low concentrations of these compounds (Miranda et al., 2020).
Data analysis showed there were differences in microbial community composition between sites, predominantly upstream sites at Doongalla Forest (Dandenong Creek) and St Andrews (Diamond Creek - Figure 5). This shows that the microbial communities upstream of sewage discharges are similar to each other and different to microbial communities which are impacted by sewage. This separation of communities is promising and warrants further investigation as a potential assessment tool.

Non-destructive sample collection methods and effects assessments.

Results showed that there were differences in metabolite profiles between control (unexposed) and copper exposed snails in both the snails and the slime. This technique shows promise in being able to distinguish between exposed and control individuals. Further testing will ascertain if we can see differences in slime metabolites following exposure to other classes of chemicals, such as insecticides, herbicides and fungicides and allow us to determine whether this can be used as a diagnostic tool to determine whether taxa have been exposed to different classes of chemical.

Table 1. The multiple lines of evidence toolbox and their potential application

Tool	Circumstance for Use/ Suspected Issue
Sediment chemistry	Hydrophobic pollutants, macroinvertebrates and fauna living in the sediment impacted
Grab and passive sampling (water)	Hydrophilic pollutants, grab if you are looking at a point in time, passive if you are unsure when a pollutant is present
Physical form, surrounding vegetation, stream flows, instream habitat.	Determine if erosion issues, vegetation nativeness, flow stress or the availability of refugia for instream flora & fauna are compromised
Laboratory and field based toxicological tests, including caging studies and toxicity tests on field-collected water and sediment.	Lab based when you have a good idea of what the pollutant is but need to determine if it's at concentrations that cause harm. Field tests when you're not exactly sure what the pollutant is or if it's a combination of pollutants, to determine if a harm threshold has been reached. Determines if pollutants present are taken up by organisms.
Macroinvertebrate assemblage and composition	General indicator of health
Metagenomics sediment microbial community composition	Clues on the functionality of the system, what is impaired
Effects assessment on biota including non-destructive metabolomics, biomarkers and ecological endpoints such as changes in growth and reproduction.	Detect effects of pollution prior to local extinction- allows a window of opportunity for management action and shows that the chemical has been uptake by the organism

Use of metabolomics techniques to assess impacts of low concentrations of pollutants on aquatic invertebrates.

A study was conducted to assess whether metabolomics approaches could be used to distinguish the impacts of a heavy metal (copper) and an organic chemical (pyrene) and a combination of the two on the marine amphipod *Allorchestes compressa.* Results have shown that the levels of some metabolites changed in response to exposure to both single (Figure 6) and mixtures of the chemicals after short term (24 hours) exposure. The main changes observed were in metabolites involved in energy production pathways, which is not unexpected as the amphipods would be expending energy on detoxification processes following exposure. This study provides further evidence for the potential of incorporating metabolomics into biomonitoring studies. Further research is required to validate these responses in field studies.

Development of ecotoxicology tests for new species

Plants

Toxicity tests using the floating macrophyte (*Spirodella punctuata)* have been optimised and are currently being used to determine the impact of chemicals on plants. So far, plants have been exposed to water spiked with herbicides and metals and the results showed that these chemicals reduce growth after 7 days. Furthermore, toxicity tests using *S. punctuata* have been incorporated into other monitoring programs that A3P are involved in and results have provided useful information about water quality at the sites.

Frogs

Results of the exposure of Southern brown tree frog, after exposure to pesticides, showed that short term exposure to some of the pesticides resulted in a slowing down in development and a reduction in growth. Further research is needed to evaluate the impact of pesticides on tadpoles in short term and long-term exposures (Clifford, 2021).

Table 2.High priority chemicals associated with each environmental value listed in the Healthy Waterways Strategy 2018 (Melbourne Water, 2018)

Ecological Value	Exposure Route	Priority Chemicals
Amphibians	Direct	Pesticides, especially synthetic pyrethroids, salinity, heavy metals, especially Silver, Mercury, Copper and Cadmium
	Endocrine Disruptors	Atrazine, PCBs, bisphenol A, organophosphates, glyphosate, metalochlor, triclosan
Birds	Indirect (bioaccumulation)	Cadmium, Mercury, Selenium, POPs (organochlorines, PBDEs, PFAS, fragrances, siloxanes)
	(food resources)	Zinc, Copper, Lead, Petroleum Hydrocarbons, Synthetic Pyrethroids
	Eggs	Chromium, Lead, Cadmium
Platypus	Indirect (bioaccumulation)	Cadmium, Mercury, Selenium, POPs (organochlorines, PBDEs, PFAS, fragrances, siloxanes)
	(food resources)	Zinc, Copper, Lead, Petroleum Hydrocarbons, Synthetic Pyrethroids
Fish	Direct	Oxygen Depletion, Pollution Event (e.g., surfactants)
	Endocrine Disruptors	EE2, bisphenol A, atrazine
River Blackfish	Indirect	Forest plantation herbicides, sediment
		Groundwater contaminated with atrazine and other triazine herbicides
Yarra Pygmy Perch	Indirect	
Dwarf Galaxiids	Direct	Synthetic Pyrethroids
	Indirect (food resources)	Synthetic Pyrethroids, Zinc, Copper, Petroleum Hydrocarbons
Long-living species	Indirect (bioaccumulation)	Cadmium, Mercury, Selenium, POPs (organochlorines, PBDEs, PFAS, fragrances, siloxanes)
Macroinvertebrates	Direct	Zinc, Copper, Lead, Petroleum Hydrocarbons, Synthetic Pyrethroids, Dissolved Oxygen, Nutrients, Salinity, Water Temperature
Aquatic Vegetation	Direct	Herbicides, salinity, nutrients

Catchment study

Based on the initial results, there are clear differences in response at rural compared to urban sites, with urban sites showing impacts of pollution and rural sites lacking the impact.

Rural

Metals and pesticides were below the limit of detection in sediment samples, but pesticides and pharmaceuticals and personal care products were detected in the passive samplers at sites in Yarra Junction. No differences between the sites were found for macroinvertebrates, toxicity tests and microbial community assemblages, clearly showing that pollution was not an issue in this catchment. One of the limitations of the study was the time of year when sampling was carried out, because winter is not usually the season to spray pesticides, so it is recommended that future catchment studies be carried out either in spring or autumn. It is the norm for macroinvertebrate sampling to be carried out in spring and autumn as this coincides with an increase in productivity and life stage emergence and when application of pesticides peaks.

Urban

Metals and hydrocarbons were present in sediment at levels exceeding trigger values at the sites at the bottom of the catchment (i.e. in the industrial areas). Pesticides were detected using passive sampling devices at all sites throughout the catchment. Results from macroinvertebrate surveys showed low diversity and abundance and laboratory-based toxicity tests (using field-collected sediment) showed that the sediment was causing adverse impacts on survival and growth of aquatic invertebrates compared to sediment collected from a clean reference site. The combination of all the tools used in this study suggest that pollution is an issue in Kororoit Creek and is resulting in poor condition, especially at the lower reach of the catchment.

Incorporation of ecotoxicology into monitoring programs

Toxicity tests using plants and aquatic invertebrates have been used in other A3P researchers monitoring programs. The first study was in collaboration with the A3P Industrial project and assessed the toxicity of water collected from 13 industrial sites in Autumn 2021. Results showed that water from sites with elevated concentrations of multiple metals was toxic to the non-biting midge *Chironomus tepperi.*

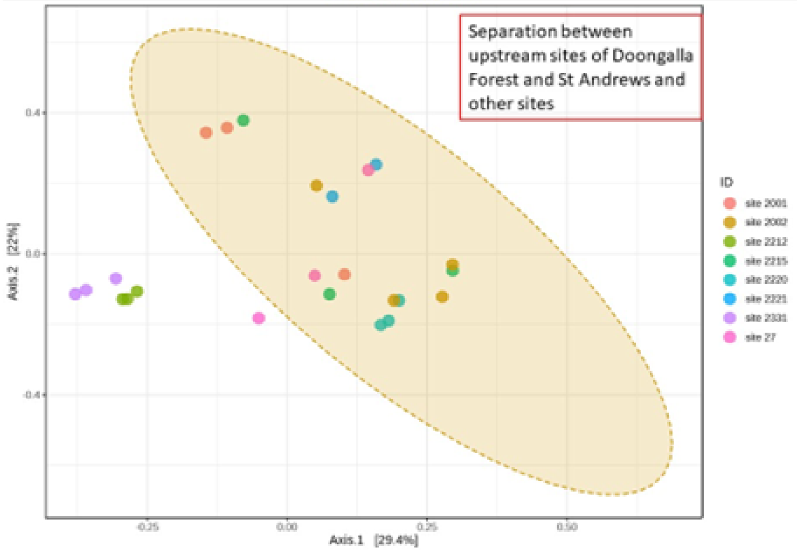

Figure 5.Multivariate analysis shows separation between microbial community composition at 2 un-impacted sites (site 2331 Doongalla Forest (purple dots) and 2212 Diamond Creek at St Andrews (olive green dots)) compared to the other sites.

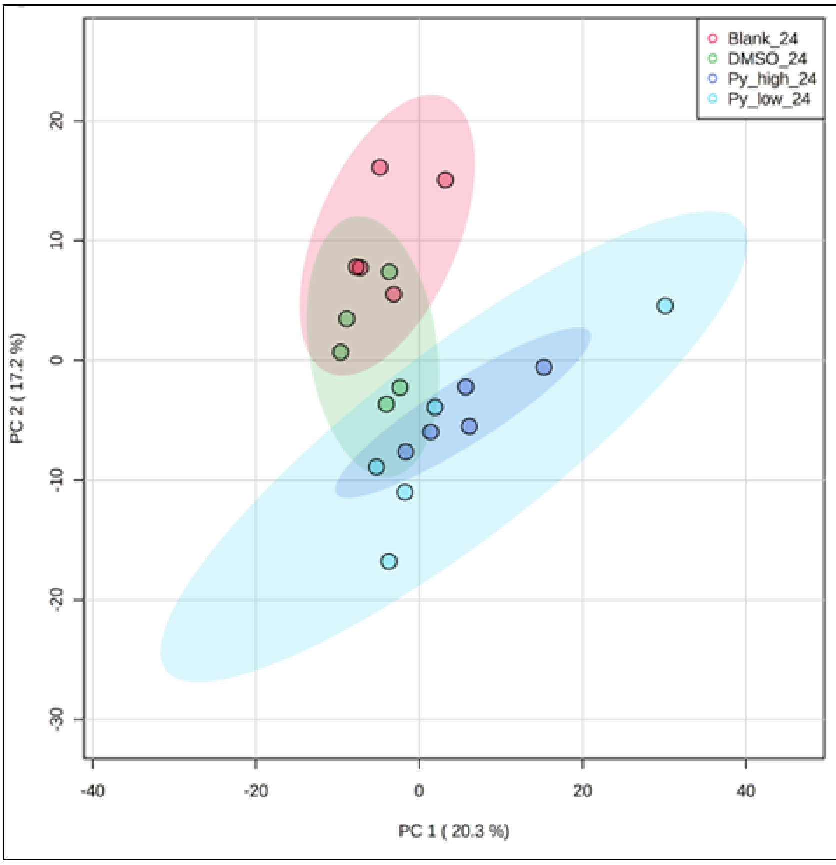

Figure 6. Scores plot showing separation of metabolites in Allorchestes compressa following exposure to pyrene for 24 hours.

Water from six sites at the Seaford wetlands was assessed for toxicity to plants and invertebrates. Results showed that water from some of the sites impacted by acid sulphate sediment was toxic to the non-biting midge due to low pH. Sites that had elevated nutrients resulted in greater plant growth compared to the laboratory water.

This shows the usefulness of using multiple taxa in toxicity tests to get a greater understanding of impacts on a wide range of environmental values.

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