

Linking ecotoxigenomic tools with ecological data to support river restoration

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BACKGROUND

The water resources and ecosystems of the Olifants River have been severely affected by surrounding land use practises such as urban development, industry, mining and agriculture. These anthropogenic stressors supply pollutants such as heavy metals, sulphates and other contaminants, as well as excessive nutrient inputs and microbial pollution, and causes acidification of the system.

Identification of specific pollutants and their particular sub-cellular effect on organisms can be elucidated by using ecotoxigenomic methods. By employing gene expression profiling on the test organism *Physa acuta* (freshwater snail) by making use of a cDNA-amplified fragment length polymorphism (AFLP) method, specific genetic responses to various toxins and environmental stressors can be identified. After digital analysis of the obtained expression profiles using AFLP-Saga TM software, transcript derived fragments (TDFs) of interest between treatments were obtained, identified and functionally annotated using bio-informatics tools. Functional categories that TDFs were grouped into included: general metabolism, cell cycle control and regulation, defence, and unknowns.

AIMS

- To elucidate the impact of acid mine drainage and other metal pollutants in the surface water of the upper catchment of the Olifants River on aquatic biota using ecotoxigenomic tools.
- To assess the feasibility of using the obtained transcript derived fragments as bio-indicators for the presence of specific metal pollutants.

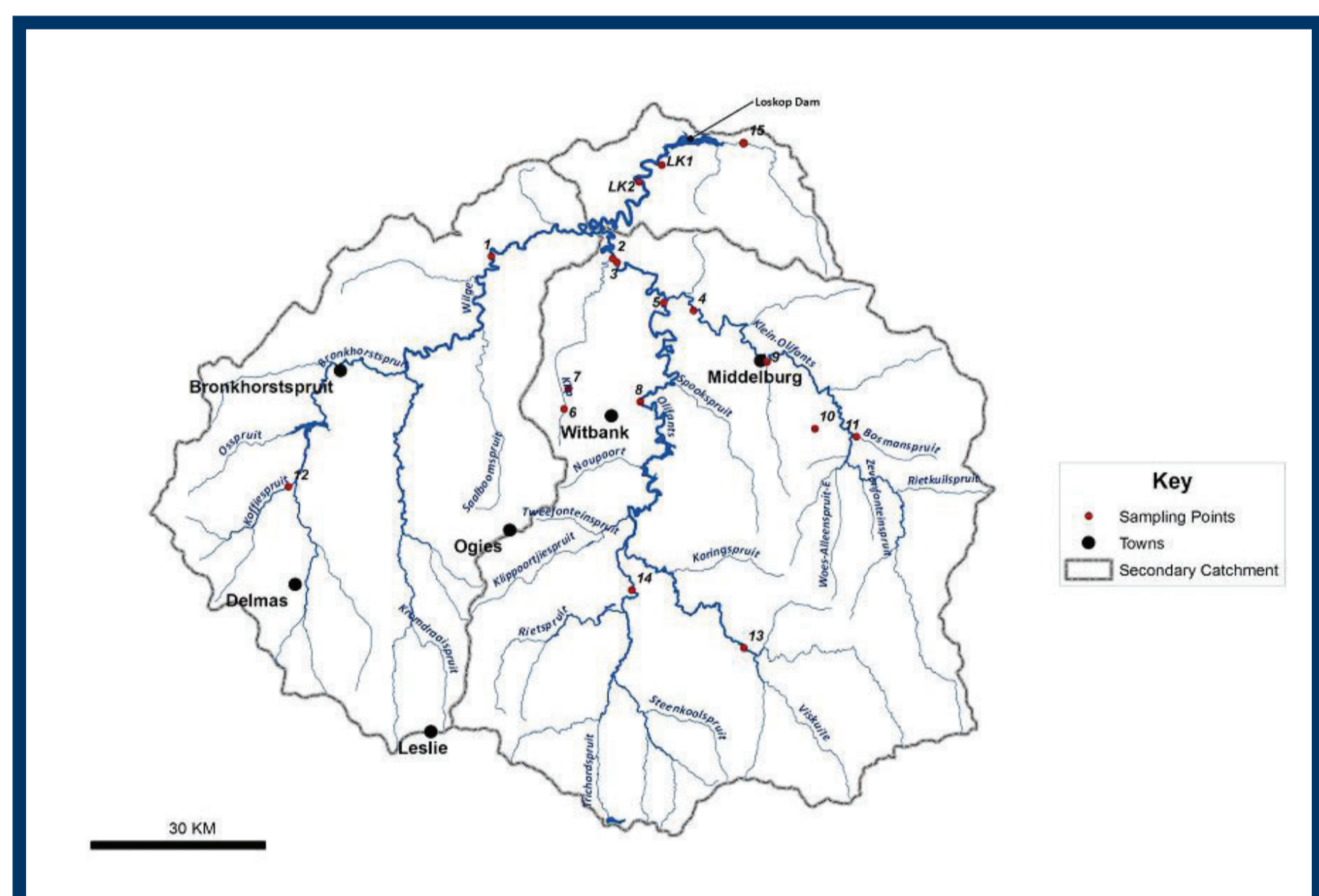


Figure 1: Map of the upper Olifants River catchment

METHODOLOGY

- Water samples were collected from impacted sites in the upper catchment of the Olifants River.
- Physa acuta* snails were exposed to a 96-hour bio-assay of water samples.
- RNA was isolated from exposed snails.
- cDNA synthesis and cDNA-AFLP analysis was conducted using 10 MseI/TaqI primer combinations, and profiles were resolved on 8% denaturing polyacrylamide gels using the Li-Cor DNA analyser 4200S.
- ~3 000 transcript derived fragments (TDFs) were excised from AFLP polyacrylamide gels, and of those that were re-amplified and cloned >400 fragments were sequenced and putative identities assigned. Genes were grouped into functional categories using the BLAST and various other databases.

RESULTS

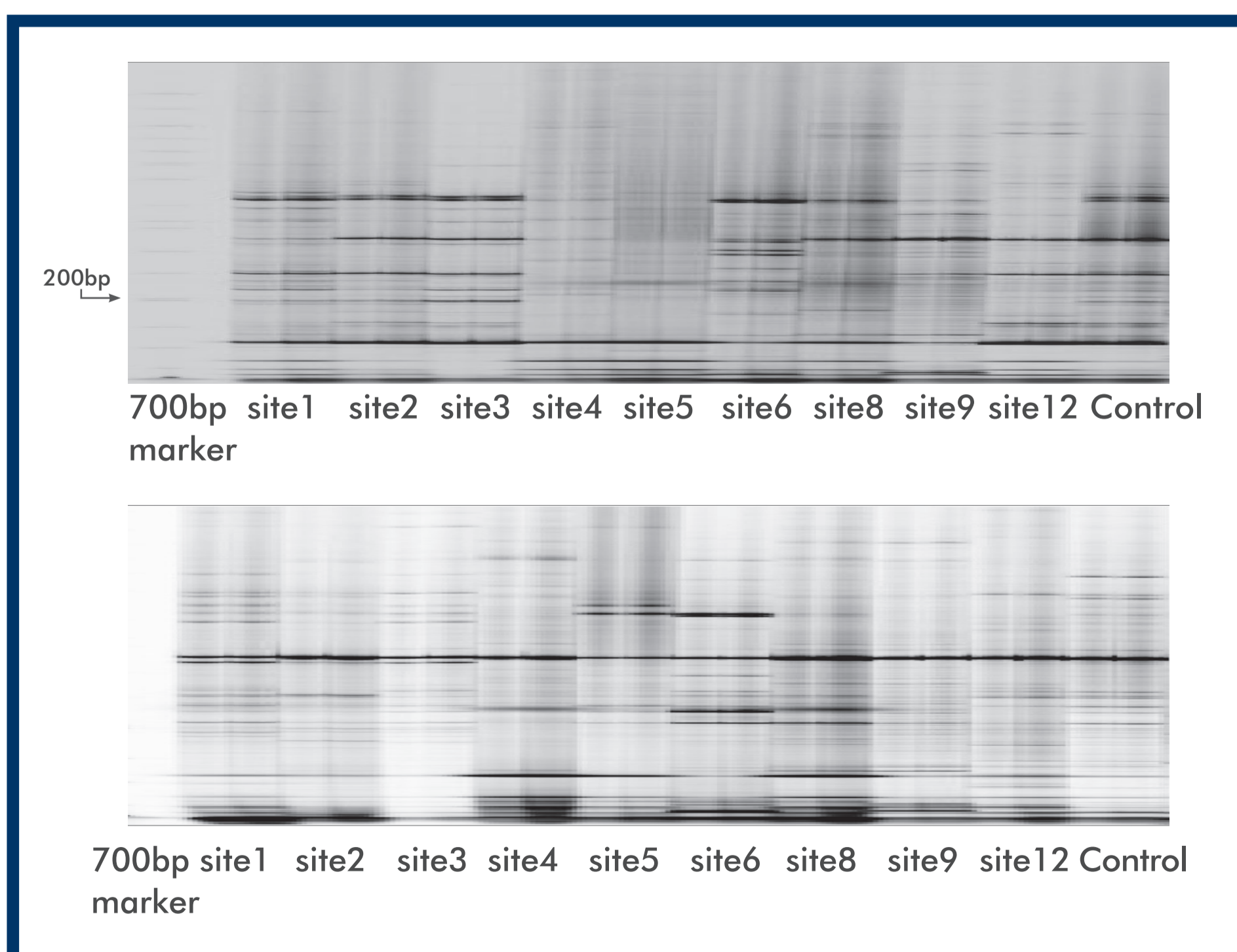


Figure 2: cDNA-AFLP profile obtained after (A) Selective amplification with primer combination MseI-GT/TaqI-AG and (B) Primer combination MseI-TG/TaqI-TG

In this study, specific pollutants and their particular sub-cellular effect on organisms were identified through the use of ecotoxigenomic tools.

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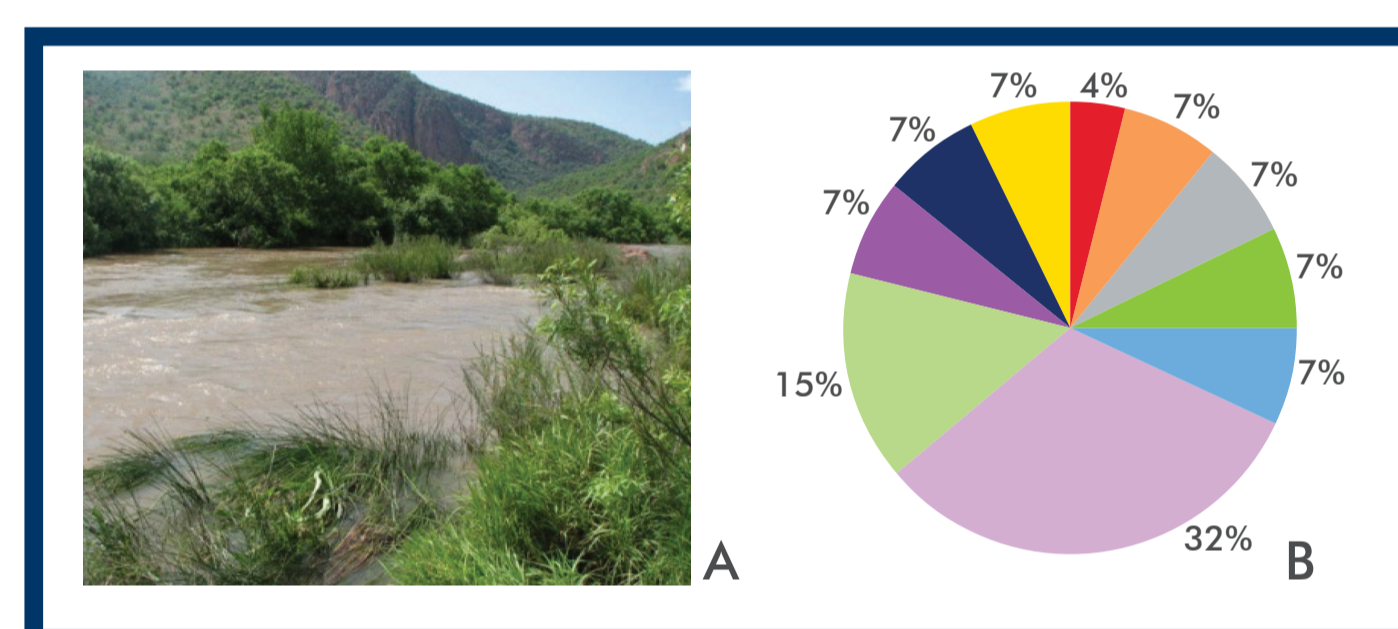
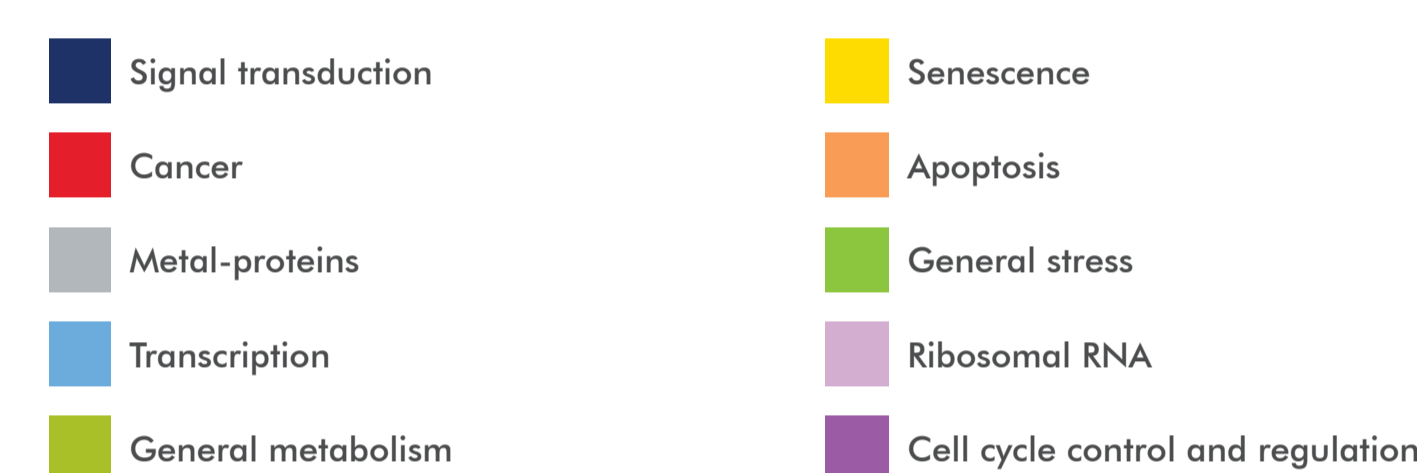


Figure 3: Site 1: EWR site on Wilge River (A) Main impact of agriculture & sewage effluent (B) Gene functional annotation for Site 1

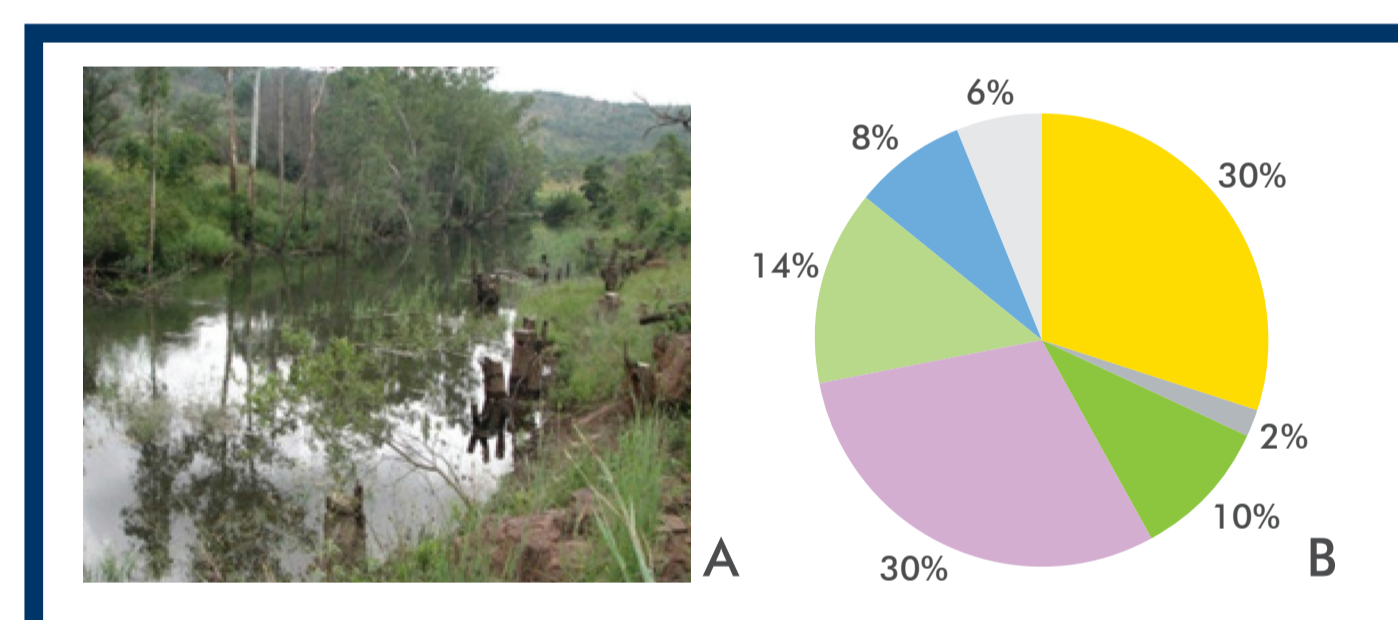


Figure 4: Site 2: Klip River above confluence with Olifants River (A) Main impact of mining, industrial & sewage effluent (B) Gene functional annotation for Site 2

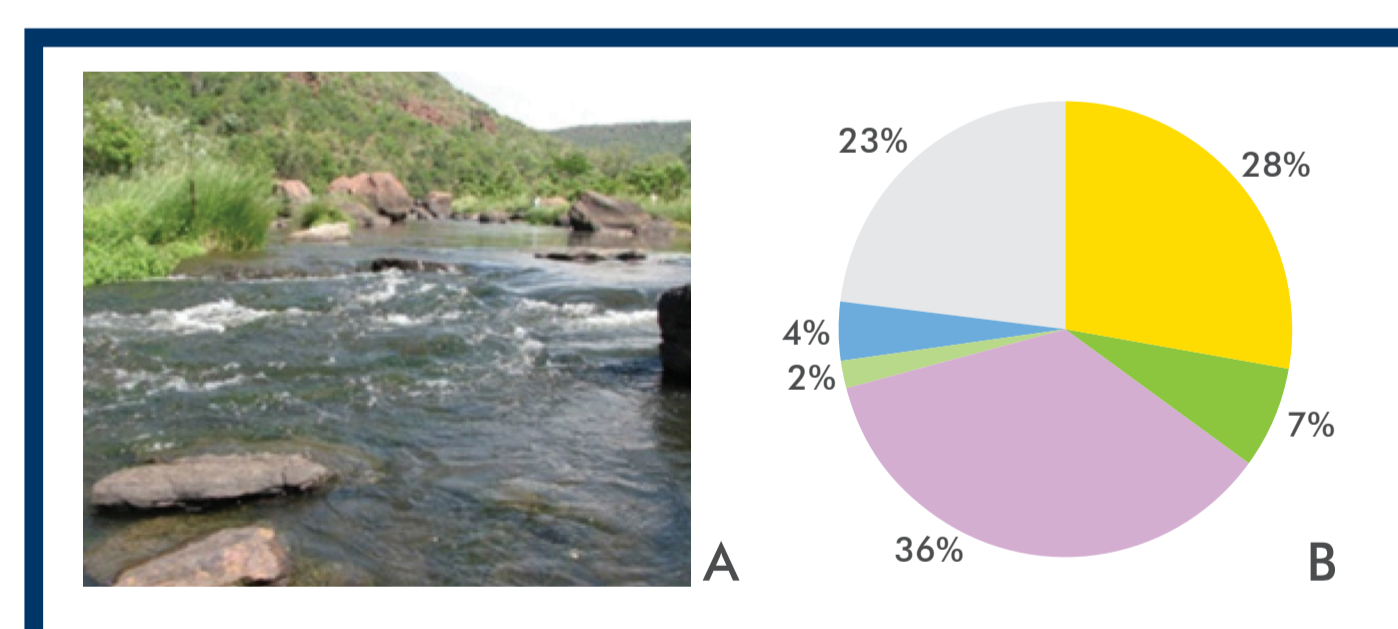


Figure 5: Site 3: Olifants River upstream of confluence with Klip River (A) Main impacts are mining, industrial and sewage effluent and agriculture (B) Gene functional annotation for Site 3

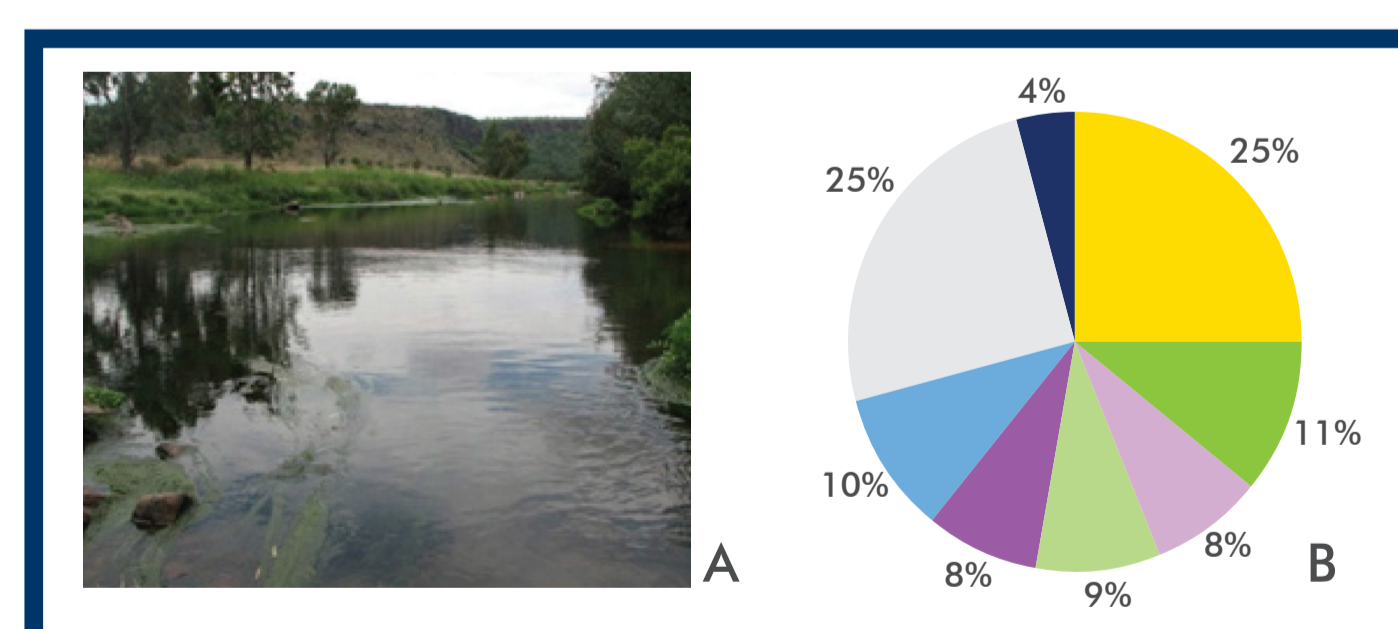


Figure 6: Site 4: Klein Olifants, upstream of confluence with Groot Olifants (A) Main impacts are mining, industrial and sewage effluent and agriculture (B) Gene functional annotation for Site 4

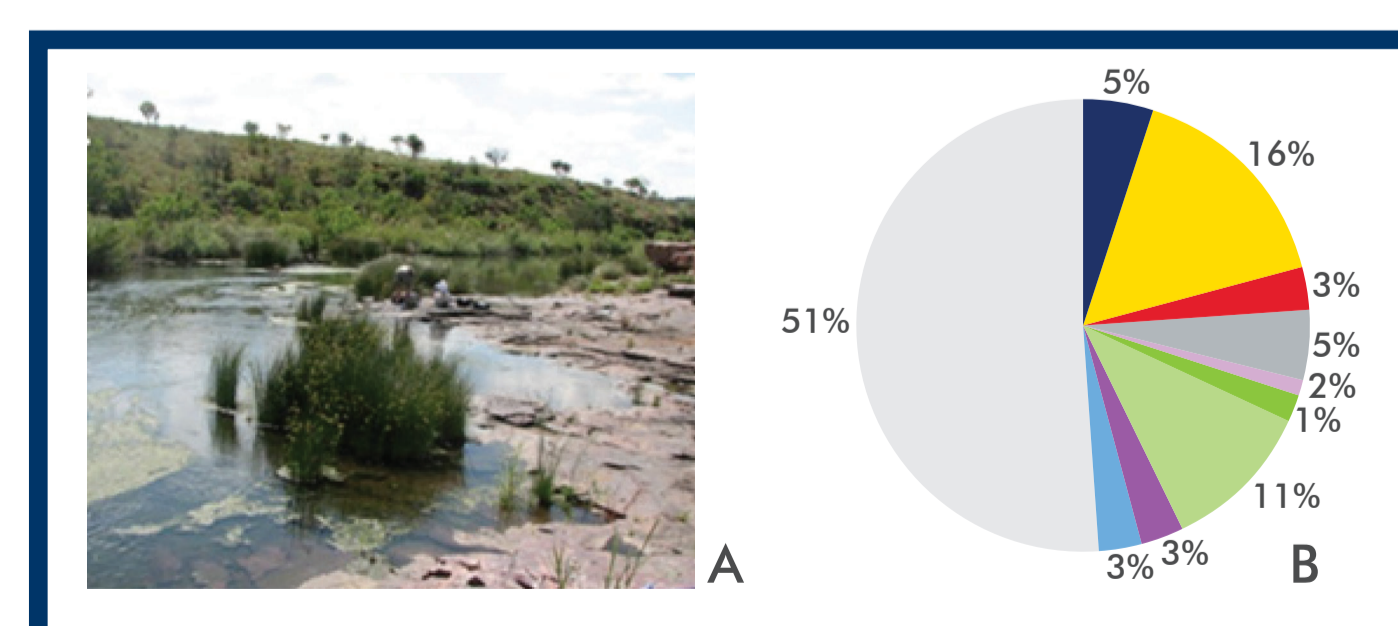


Figure 7: Site 5: Groot Olifants, upstream of confluence with Klein Olifants (A) Main impacts are mining, industrial and sewage effluent and agriculture (B) Gene functional annotation for Site 5

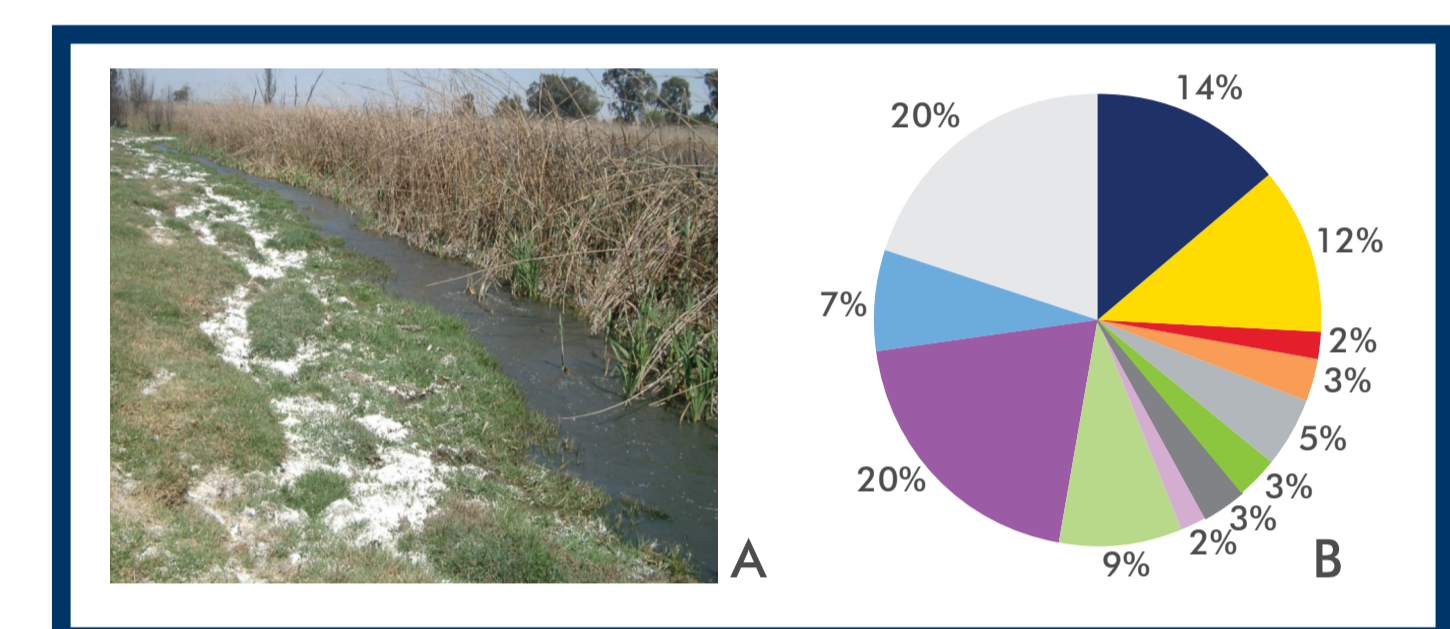


Figure 8: Site 6: BrugSpruit downstream from Ferrobank (A) Main impacts are mining and sewage effluent (B) Gene functional annotation for Site 6

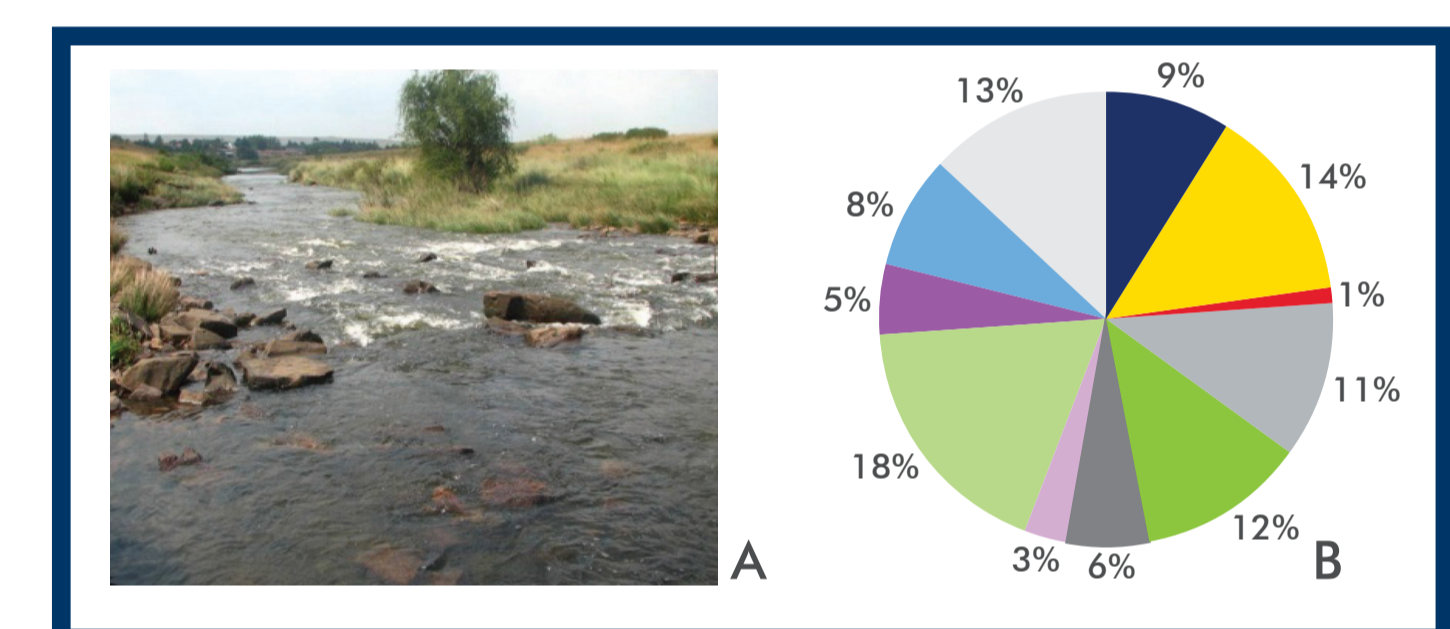


Figure 9: Site 8: Groot Olifants, downstream of Riverview sewage works (A) Main impacts are mining, industrial and sewage effluent and agriculture (B) Gene functional annotation for Site 8

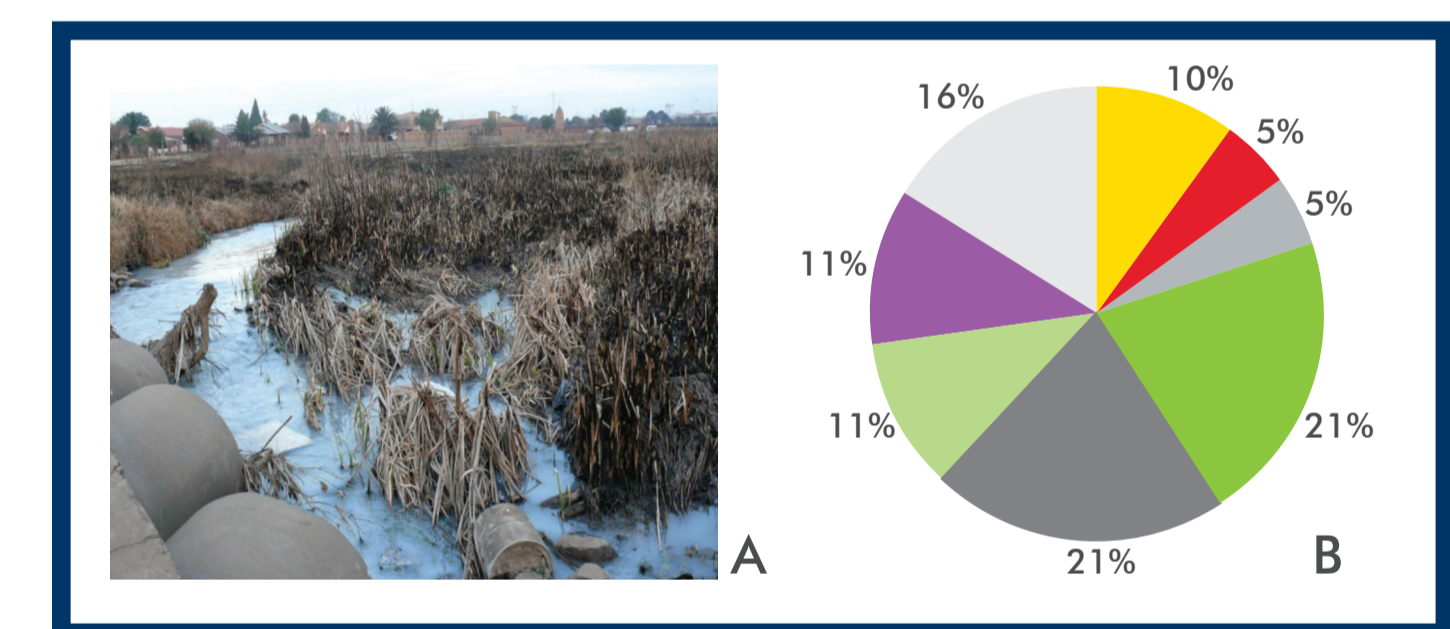


Figure 10: Site 9: Tributary of Klein Olifants (A) Main impacts are industry and sewage effluent (B) Gene functional annotation for Site 9

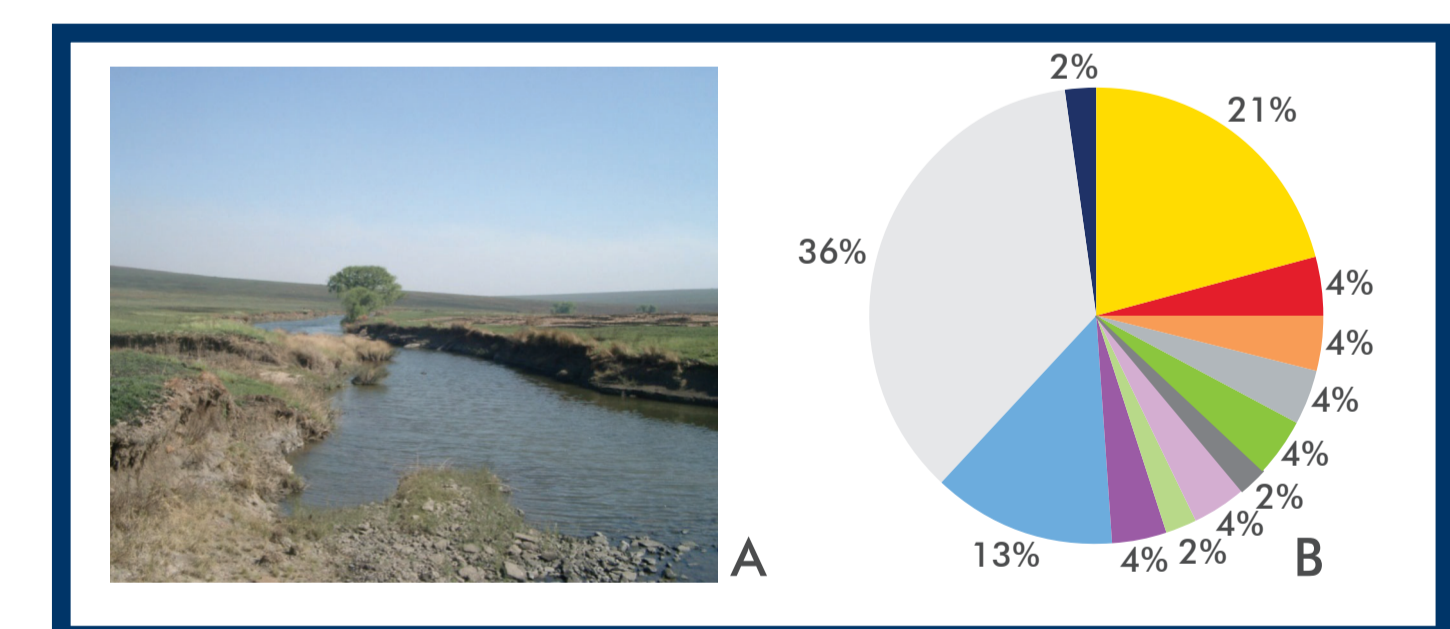


Figure 11: Site 12: Koffiespruit, upper Wilge catchment (A) Main impacts are agriculture and sewage effluent (B) Gene functional annotation for Site 12

DISCUSSION

- Gene expression profiles differed across all treatments and with the control.
- Specific bands or unique transcript derived fragments (TDFs) were up-regulated only under specific conditions (for example: AMD; mixture of pollutants such as agriculture and industry).
- Obtained sequences were grouped into functional categories according to putative identity and assigned function.
- Categories of note are those with genes involved in ageing or senescence, metal-binding and cancer.
- A large presence of senescence genes may be indicative of the presence of the metal antimony in the aquatic ecosystem.

FUTURE DEVELOPMENTS

- Development of a toolbox for identification of pollutants: Association of genotoxicity indicators using qRT-PCR on specific TDFs and genes in related pathways (for example: senescence, metal-binding and cancer pathways), with metal accumulation using ICP-MS in water samples and bio-assayed snail tissues.

ACKNOWLEDGMENTS

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