Rhizoremediation of hydrocarbon contaminated soil using Australian native grasses

A Thesis submitted for the degree of
Doctor of Philosophy

Sharyn E. Gaskin. B. App.Sc (Env Health). B.Sc (Hons)

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School of Medicine
Faculty of Health Sciences
Flinders University of South Australia
Australia

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Sharyn E. Gaskin
School of Medicine
Flinders University of South Australia
Adelaide, Australia
2008

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SUMMARY

The breakdown of contaminants in soil resulting from microbial activity that is enhanced in the presence of the plant root zone (rhizosphere) has been termed *rhizoremediation*. To date, Australian native plants have not been assessed for their hydrocarbon rhizoremediation potential. The use of native plants offers an economically feasible and environmentally sustainable cleanup option for the rehabilitation and restoration of hydrocarbon contaminated sites in Australia. The aim of the study was to evaluate the potential of Australian native grass species for the rhizoremediation of aliphatic hydrocarbon contaminated soil from a mine site.

Candidate Australian native grass species (*Poaceae*) were selected following the development of essential and desirable growth criteria. Nine perennial Australian grasses were evaluated for seedling emergence in sandy loam soil sourced from a mine site which was artificially contaminated with a 60:40 diesel/oil mix at concentrations of 30 000 mg/kg, 10 000 mg/kg, 5 000 mg/kg and 0 mg/kg (control). Seedling emergence was not adversely affected by the presence of hydrocarbon contamination at the exposed concentrations for eight of the nine species studied ($p > 0.05$). Three promising species were assessed for relative growth performance in diesel/oil contaminated (10 000 mg/kg, 5 000 mg/kg) and uncontaminated (control) soils in greenhouse studies to assess their tolerance of aliphatic hydrocarbon contaminated soil.

Cymbopogon ambiguus (Lemon Scented grass) is a summer growing perennial with widespread distribution throughout Australia including the region where the mine site is situated. *Brachiaria decumbens* (Signal grass) (naturalised) is adapted to humid tropical areas of Australia and is native to the site and sourced from seed banks. *Microlaena stipoides* (Weeping grass var. Griffin) is a cool season grass, widely distributed throughout Australia in moister regions. The three evaluated species survived for 120 days in the diesel/oil contaminated soil at the exposed concentrations without adverse growth affect ($p > 0.05$). In some instances (e.g. *C. ambiguus*) growth stimulation occurred in the presence of

contamination producing significantly more root biomass compared with the control ($p < 0.0001$).

Most hydrocarbon degradation is believed to occur through microbial processes, and so the plant-associated microbial community was examined in the three tolerant species. The assessment of the influence of grass on the abundance and activity of microorganisms in the rhizosphere revealed species-specific plant-induced changes in the soil microbial community. Selective enrichment of hydrocarbon degrading microorganisms was demonstrated in the rhizosphere soil of the Australian grasses tested, to varying degrees. *C. ambiguus* appeared to have the greatest influence on stimulation of hydrocarbon degrading microorganisms, followed by the cool season grass *M. stipoides*. *B. decumbens* showed consistently lower numbers of hydrocarbon degrading microorganisms in rhizosphere soil over time compared to the other two species ($p < 0.01$). The influence of grasses on microbial community structure (defined as community DNA fingerprint) in diesel/oil contaminated soil suggested no new microbial population was favoured by the grasses (qualitative shift), rather there were relative quantitative changes in existing members of the microbial population. Soil lipase activity did not appear to be an optimal bioindicator of rhizoremediation and may encompass total soil microbial activity not exclusively the hydrocarbon degrading microorganisms of interest.

The assessment of biodegradation of hydrocarbons in soil is essential to characterise the effectiveness of plant species in rhizoremediation. Residual diesel and oil concentrations (as total petroleum hydrocarbons, TPH) were measured using Gas Chromatography. The presence of single species successfully enhanced the removal of hydrocarbons from soil (for all species). All showed significantly lower residual hydrocarbon concentrations than those in unplanted soil after 100 days ($p < 0.01$). Significantly, it was not necessary to add N and P to achieve up to 90% reduction in hydrocarbon concentrations in the soil. The relative performance of each grass species varied. In soil planted with *C. ambiguus* hydrocarbon concentrations were reduced faster and to a greater extent than the other species studied, from 10 000 mg/kg to approximately 1 100 mg/kg TPH (88% removal). Similar endpoint success was recorded for

M. stipoides which facilitated 80% reduction in hydrocarbon concentrations. Interestingly, *B. decumbens* (the only naturalised species) did not perform as well as the other species (although still significantly better compared to unplanted controls), with hydrocarbon concentrations reduced to approximately 4 500 mg/kg (49%). Hydrocarbon concentrations in unplanted (control) soil were reduced by 45% through natural biodegradation processes. Plant root and shoot tissue was periodically assessed for hydrocarbon accumulation and was shown to be negligible. A multispecies planted trial using *C. ambiguus* plus *B. decumbens* had no additional influence on total TPH removal. The final TPH removal efficiency in the multispecies trial was not significantly different ($p > 0.05$) from that of the best single species performer of the two i.e. *C. ambiguus*. In a field application the planting of multiple species may still be desirable in order to preserve site biodiversity and assist rehabilitation of the area.

A strong relationship between abundance of hydrocarbon degrading microorganisms in the rhizosphere and hydrocarbon biodegradation was demonstrated for all species ($p < 0.01$). Those species which showed greatest stimulation of the microbial population resulted in enhanced TPH removal from soil. These species were the summer grass *C. ambiguus* and the winter species *M. stipoides*. This may allow for broader application both seasonally and geographically across Australia. *B. decumbens* showed successful rhizoremediation to a lesser degree, but may still be an option in multiple planting strategies.

This investigation identified three Australian grass species (from the nine evaluated) that are candidates for further investigation for *in situ* rhizoremediation potential at field scale.

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PUBLICATIONS

Journal article

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Editorial features

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