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Comparing Perennial Grasses in a Phytoremediation Field Trial for Soil Hydrocarbon Removal – Fuel Depot, north-west Victoria, Australia

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Abstract **-** A former Fuel Depot, Victoria Australia was utilised as a petroleum depot since the 1920s with a release of petroleum products from site infrastructure in early 2000. From 2021, in-situ soil remediation between 0-2m trialling phytoremediation was undertaken to remove Total Petroleum Hydrocarbons (TPH) with a focus on plant-assisted bioremediation. The successful bioremediation of petroleum pollutants in soils depends on numerous environmental parameters and operational factors, all of which need to be optimised to achieve maximum treatment. The operational factors that were targeted include aeration through ripping, carbon, and soil enhancement via mulching and optimal soil moisture with installation and careful management of irrigation. Research shows grasses are favourable candidates for plant assisted bioremediation of hydrocarbons and therefore three plots were designed with a) Native Australian Mat-rush (*Lomandra* spp.), b) *Vetiver zizanoides* and c) variety of shrubs and native grasses. The results indicate 76% and 84% reduction for C6-C10 TPH and a maximum reduction of 1350 mg/kg to 217 mg/kg. Reductions in the C10-16, C16-C34 and BTEX of 28% to 86%. All C34-40 in final December 2023 soil sampling were below laboratory Limits of Reporting (LOR) of 100mg/kg. This field scale phytoremediation trial delivered a) material change in contaminant loads on-site, b) potentially assisted in avoiding a soil excavation and treatment Clean Up Program (CUP) c) only one TPH exceedance between 0-2mbgl of NEPM guidelines at the completion of the trial (ESL commercial/industrial) for C10-C16 fraction, d) converting fuel depot of compacted clay fill soil with over 100yrs of traffic to a viable plant growing media, and e) provided green infrastructure and activity on site. Research provides evidence for accelerated disappearance of TPH in the rhizosphere via plant-assisted bioremediation. This research is predominantly laboratory scale experiments. Given the significant variables at field scale and the heterogeneity of the TPH contamination it is unknown whether the TPH removal observed relates to partly or wholly to plant assisted bioremediation. Further research replicating this field scale phytoremediation trial with optimal ripping, mulching, and irrigation for performance comparison is recommended.

Keywords**:** Phytoremediation, hydrocarbons, rhizosphere, vetiver, NEPM guidelines

1. Introduction

Kamath et.al, (2004) states phytoremediation is a "biological technology process that utilizes natural plant processes to enhance degradation and removal of contaminants in soil or groundwater." There are five types of phytoremediation techniques: 1) rhizofiltration, 2) phytoextraction, 3) phytotransformation, 4) phytostabilization and 5) plant-assisted bioremediation; which involves the stimulation of microbial biodegradation through the activities of plants in the root zone, this trial focused on the fifth noted technique; plant-assisted bioremediation.

Research for microbial biodegradation indicates a diverse and collaborative microbial community, is responsible for the enhanced removal of TPH in the rhizosphere of phytoremediation compared with non-vegetated soils (Anderson *et al*., 1995). The rhizosphere maintains higher abundance and diversity of microbes an order of magnitude above those in non-vegetated soils. This is achieved by providing a niche environment for microbes and facilitating co-metabolic transformations (Walker et.al, 2003). The greater density and diversity of microorganisms commonly observed often results in higher removal rates of TPH (US EPA, 2000).

These microbial populations are sustained by root exudation of compounds such as carbohydrates and amino acids (Hampp, 2008). Root cap cells and root exudates are nutrient and carbon sources for microorganisms in the soil. This rhizosphere effect is expressed as the ratio of rhizosphere microorganisms to the number of non-rhizosphere microorganisms, the R/S ratio. R/S ratios commonly range from 5 to 20, but occasionally are as high as 100 (Kamath, 2004).

The actual composition of the microbial community in the root zone is dependent on root type, plant species, plant age, and soil type as well as contaminant type and concentrations in soil. The exudates released by plant roots and the biodegradation of TPH by rhizosphere microbial communities is considered the main removal process under phytoremediation.

Grasses are superior candidates to test this removal process at field scale based on the following characteristics: ·

I) The root systems of grasses provide maximum root surface area

2) Grasses can establish in an unfavourable soil environment.

3) The deep roots of some grasses can penetrate to 3-5m below the surface (Aprill and Sim, 1990).

In addition, organic amendments have been shown to increase microbial activity in the soil by improving chemical and physical properties. Also, organic amendments have been used for stimulating treatment of recalcitrant chemicals in soil (Aprill and Sims, 1990). Traditionally, ripping/tillage is also employed to incorporate these additives which provides aeration of typically highly compacted in-situ field soils. Ripping and organic amendments may provide short-term microbial removal during the phytoremediation plant establishment.

The former Fuel Depot located in northern Victoria, Australia has been in operation since 1928 and in early 2000, a release of petroleum products from site infrastructure was reported. Extensive groundwater remediation was undertaken from 2000 to 2019, removing hydrocarbons below the smear zone to the extent practicable. From 2021, in-situ soil remediation between 0-2m trialling phytoremediation had been installed to remove TPH and potentially allow compliance with a commercial/industrial end use scenario under the National Environmental Protection Measures (NEPM) guidelines. The trial project opportunities and constraints of note are:

1) Deliver an in-situ soil treatment that avoids large scale soil disturbance and disposal to landfill.

2) Establish this methodology as a proven cost-effective, environmentally innovative solution that can be applied on other sites.

3) Odour management and avoidance of groundwater impacts with irrigation.

Performance objectives for this trial are based on the NEPM guidelines as follows:

1.Health Screening Levels (HSLs) from CRC Care Technical report No. 10 and NEPM Schedule B1.

2.Ecological screening levels (ESLs)

3.Petroleum hydrocarbon management limits ('management limits')

Based on the NEPM Schedule B Table 1A(6) Soil Health Screening Levels of Direct Contact HSL-C, HSL-D and Schedule B Table 1A(3) Soil Health Screening Levels and Ecological Screening Levels (ESL) have been adopted. While numerous studies have been carried out at the laboratory scale, a smaller number of field scale implementation of phytoremediation have been published.

2. Method

2.1. Phytoremediation installation

A number of critical processes were targeted for optimal removal of hydrocarbons. These were aeration through ripping, carbon and soil enhancement via mulching, and optimal soil moisture with installation and careful management of irrigation.

The installation of the phytoremediation trial was completed in January 2022 with species planted from tubestock. Growth was slow at the beginning given the late summer/early autumn planting. Plate 1, Plate 2 and Plate 3 below shows the establishment of the trial plots at the end of the 2023 growing season.

Plate 1: Vetiver trial plot.

Plate 2: Native grasses and shrubs trial plot.

Plate 1 shows the vetiver plot that had grown vigorously and established by November 2022 after a period of browning in the winter. Vetiver suppliers advised that vetiver could grow well in a temperate climate and impacts of frost are minor browning. The native shrubs and grass trial plot consisted of hakeas, acacias, callistemons and the following grasses – Poa Tussock (*Poa labiilardierei*), Red Grass (*Bothriocloa macra*), Kangaroo Grass (*Themeda australis*), and Weeping Grass var. griffin (*Microleana stipoides*). This trial plot was chosen based on research work completed by Gaskin, S. (2008). The native shrubs were included for landscaping value and Eucalyptus spp. naturally seeded into the trial plot and were left to establish as shown in Plate 2. The native trial plot established well with 100% coverage with a variety of natives, the only species that was sparse from the original planting was Weeping Grass. Weed management was higher in this trial plot compared to the vetiver given the dominant growth behaviour of vetiver.

Plate 3: Established Lomandra trial plot.

The 3rd trial plot consisted of only one species Spiny-headed Mat-rush (*Lomandra longifolia*) a common landscaping native plant with an extensive root system. Lomandra is a perennial, rhizomatous herb found throughout eastern Australia. It grows in a variety of soil types and is frost, heat and drought tolerant. The site in NW Victoria, Australia is on the edge of the known distribution of Lomandra. No previous studies were found on the use of Lomandra in removal of hydrocarbons in soil.

As is typical of a fuel depot, the soils were compacted, with no organic matter, low infiltration, and very poor soil structure including rocks and gravel. To grow plants and create microbial communities colonising the soil, the site needed to be heavily ripped as shown in Plate 4.

Plate 4: Deep ripping of trial plots to aerate soil and remove compaction.

An excavator with a hydraulic ripper was the only machinery available locally during install which limited the ripping depth to 0.5-1m. For any future phytoremediation work, it is recommended that undertake a dozer rip to 1.5-2m where feasible. Once the site was ripped, 200-300mm of mulch was spread out over the surface prior to the installation of the irrigation system.

2.2. Soil Sampling

Soil characterisation works in early 2021 were completed to assist future remedial planning and provide baseline sampling prior to the installation of the phytoremediation trial. In December 2023, post phytoremediation trial soil sampling, consisted of gathering soil samples from locations sampled during the 2021 soil characterisation where elevated hydrocarbon concentrations were present.

Test pits were advanced to a depth of 2.5mbgl using a mechanical excavator to assess strata, hydrocarbon concentrations, and root morphology. Drilling using a mechanical soil auger was subsequently completed within each test pit to collect deeper soil samples at approximately 4mbgl. The suffix 'A' was included in the test pit ID name for repeat 2023 sample location to 2021 soil samples. Soil samples were collected from multiple depths and were submitted for laboratory chemical analysis for TPH and benzene, toluene, ethylbenzene, xylene and naphthalene (BTEXN). A total of 16 locations were sampled and analysed in a NATA accredited lab. These were:

- Five within the Lomandra plot - SB01A, TP02A, TP03A, TP04A and TP26A
- Three within the native grasses plot - TP06A, TP08A and TP25A
- Six in the vetiver plot - TP11A, TP12A, TP18A, TP21A, TP27A and TP28A

Additional interim soil sampling was conducted after site ripping, mulching, and planting and in November 2023. The data validation process adopted is based on quality assurance and quality control (QA/QC) guidance detailed within documents published by the National Environment Protection Council (NEPC) and Environment Protection Authority (EPA) Victoria.

4. Results

Table 1 shows the percentage removal for comparative pairs of data between soil sampling for the phytoremediation trial. Table 2 shows the final treatment performance to the NEPM guidelines and Table 3 indicates some TPH removal during ripping/mulching. All samples in December 2023 were below Limits of Reporting (LOR) for C34-C40 TPH fraction.

Table 1: Comparison of C6-C10, C10-C16 and BTEX soil sampling between Feb 2021 and Dec 2023.

Plot and depth range	Comparative pairs	COPC	Maximum concentration (mg/kg)		% Change	
			2021	2023	Mean	Median
All three plots <1.1mbgl	6	C6-C10 (excl. BTEX)	<10	<10	_#	_#
		C10-C16 (excl. BTEX)	230	100	-51%	-33%
		BTEX	<0.2	<0.2	_#	_#
All three plots <2.0mbgl	9	C6-C10 (excl. BTEX)	731	37	-93%	_#
		C10-C16 (excl. BTEX)	1610	440	-74%	-75%
		BTEX	619	3.4	-99%	_#
All three plots <2.1mbgl	19	C6-C10 (excl. BTEX)	1350	217	-76%	-84%
		C10-C16 (excl. BTEX)	1610	500	-45%	-84%
		BTEX	901	129	-86%	_#

Table 2: Soil exceedances based on NEPM guidelines for HSL-D, ESL commercial/industrial and management limits.

Criteria	COPC	Criteria Threshold (mg/kg)	Number of samples	Number of exceedances	Exceedance range (mg/kg)
Human health (HSL-D) Silt 1-2mbgl	C6-C10 (excl. BTEX)	360	9	-	-
Human health (HSL-D) Silt 2-4mbgl	C6-C10 (excl. BTEX)	590	49	3	875-1450
	Benzene	6		9	6.2-27.8
Ecological (ESL commercial/industrial) Fine soil 0-2mbgl	C6-C10 (excl. BTEX)	215	9	0	-
	C10-C16 (excl. Naphthalene)	170		1	440
Management limits Commercial & industrial Fine soil	C6-C10	800	58	4	1360-4080*
	C6-C10	1000		3	1070-1510*

*Exceedances for management limits, commercial & industrial were at depths 2.5 and 4mbgl.

Table 3: Removal of TPH fraction C16-C40 during ripping/mulching in three (3) of twelve (12) soil samples.

TPH (mg/kg)	Pre TP3 0.1m	Post TP3 0.1m	Pre TP2 0.5m	Post TP2 0.5m	Pre TP3 1.8m	Pre TP3 1.8m
C10-C16	0	0	90	0	1270	810
C16-C34	140	110	4320	240	600	590
C34-C40	160	110	750	120	0	0

Soil sampling analysis pre and post ripping/mulching shows removal of C16-40 fraction (Table 3).

4.1. Root Morphology

At the end of the phytoremediation trial (December 2023) a detailed assessment of root morphology of the three trial plots was undertaken. Six randomly chosen plants in each plot were carefully removed by hand.


Plate 6: Lomandra (left) and vetiver (right) with basal root.   Plate 7: Kangaroo (left) and Poa (right) with basal root.

Site observations of root depths were as follows:

a) Lomandra plot: basal root to a depth of 0.3-0.5mbgl, and roots observed to a depth of 1.5mbgl.

b) Native grasses plot: grasses with basal root to a depth of 0.3-0.4mbgl, and naturalised Red River Gum trees with deeper roots to a depth greater than 2mbgl.

c) Vetiver plot area: basal root to a depth of 0.4-0.5mbgl, and adventitious roots to a depth of 1mbgl.

5. Discussion

Phytoremediation is considered most effective at sites with shallow soil contamination where TPH is treated in the rhizosphere predominantly via microbial degradation. Extending the depth of microbial activity to create an "expanded rhizosphere" maybe achieved by optimising the irrigation scheduling. This translocates root exudates and nutrients to deeper soil layers. Phytoremediation can then influence soils to greater depth, even if the roots are sparse in the target area (Kamath, et.al, 2004).

The phytoremediation trial irrigation was set to twice weekly to deliver an irrigation rate of 20mm (spring), 40mm (summer) and 10mm (autumn) per week, with minimal irrigation in winter for frost management. This provided optimal irrigation for plant growth and soil moisture. Amending in-situ soils was also an important factor to ensure vigorous plant growth and microbial activity. Mulch and deep ripping were undertaken to improve the soil moisture-holding capacity, provide aeration, and improve soil structure. Research shows, the addition of mulch stimulates the treatment of recalcitrant TPHs in soil and increases microbial activity (Aprill and Simms, 1990). The results indicate 76% and 84% reduction for C6-C10 TPH and a maximum TPH reduction of 1350 mg/kg to 217 mg/kg. Reductions in the C10-16, C16-C34 and BTEX of 28% to 86%. An increase of 129% was observed in a sample for the native grasses plot. This may be due to the small dataset of three comparative pairs and the low concentrations in the 2021 dataset for this plot. All C34-40 in final December 2023 soil sampling were below laboratory Limits of Reporting (LOR) of 100mg/kg.

Research shows grasses are favourable candidates for plant assisted bioremediation of hydrocarbons (Aprill and Sims, 1990; Adam and Duncan, 1999; Merkl, Schultze-Kraft, and Infante, 2005). This relates to the highly branched, fibrous root systems, which can host large microbial populations and significantly improve soil condition (Anderson, Guthrie, and Walton, 1993). For example, Truong et.al states "the root system of vetiver can reach 3–4 meters in the first year of planting (Hengchaovanich, 1998) and acquires a total length of 7 meters after 36 months (Lavania, 2003)." After 24 months of the

Phytoremediation trial observations showed no significant difference between basal and adventitious roots between vetiver, native or Lomandra and vetiver depths were only 0.5m basal root and 1.5m for adventitious roots. Even though the the grass root morphology were shallower than expected after 24 months the mulch and root exudates in trial plots were a a major source of soil organic matter, soil moisture was maintained with irrigation, oxidation was increased via plant roots, deep ripping and irrigation. Therefore, the 'expanded rhizosphere' hypothesis may have equated to the TPH removal rates measured.

Available organic carbon sources from plants promoting rhizosphere microbial populations can be 4- to 100- times greater than observed in non-vegetated soils. Root exudates account for the release of 7 to 27 percent of total carbon fixed during photosynthesis. Typical estimates are between 10 - 100 mg-C g-root material of which root exudation can range between 0.4 - 27.7 mg-C g-root material. The composition and quantity of root-derived material released into the rhizosphere varies depending on the season, the age of plant and the health of the plant (Kamath. et.al., 2004). An alternative hypothesis to plant assisted bioremediation (phytoremediation) for the observed TPH removal rates is Natural Source Zone Depletion (NSZD). NSZD is a well-established management strategy and of recent much higher TPH removal rates are being measured. NZSD is defined as naturally occurring processes of dissolution, volatilization, and microbial biodegradation resulting in material losses of TPH (aka Monitored Natural Attenuation) (CRC CARE, 2020).

A growing body of research exists showing that NSZD is ubiquitous and occurs at all sites to some degree. Understanding, quantifying and evaluating TPH treatment effectiveness is therefore an important part of a sustainable remedial strategy (CLAIRE 2019) for these sites. Mass contaminant loads were reduced during the phytoremediation including outside of the zone of influence of plants (<2mbgl). The role of NSZD within the phytoremediation trial or the potential for phytoremediation to enhance NSZD is unknown from the sampling program implemented for this phytoremediation trial. This relates to the challenges of field scale scientific studies, such as a) heterogeneity of site contamination of TPH, b) accurately measuring phytoremediation treatment mechanism – microbial, co-oxidation and/or plant uptake etc, c) influence of NSZD, and d) accurately measuring TPH changes with site establishment – ripping, mulching and irrigation.

The key focus of this phytoremediation trial was to provide the environmental conditions for enhanced TPH removal via microbial degradation. These were soil aeration for aerobic microbes, mulch for soil improvement and initial carbon source, and maintenance of soil moisture for plant establishment including rhizosphere and microbial activity. Mass contaminant loads were reduced; however, in order to ascertain that plant-assisted biodegradation is the primary hydrocarbon removal mechanism, it is necessary to measure or estimate all types of loss, either by calculating a complete mass balance or by carrying out microbially control experiments to assess the magnitude of all combined abiotic losses (Huesseman, 2004).

Lessons learnt for future trials in the field were to deep rip to 1.5m or greater with a dozer, incorporate mulch with multiple passes into soil during ripping to depth, and optimise pulse irrigation for increased oxygen transfer into the soil.

6. Conclusions

This field scale phytoremediation trial installed in northern Victoria, Australia targeting grass species and enhanced microbial degradation of TPHs delivered the following:

a) Material change in contaminant loads on-site

b) Assisted in avoiding a soil excavation and treatment Clean Up Program

c) Only one TPH exceedance between 0-2mbgl of NEPM guidelines at the completion of trial (ESL commercial/industrial) for C10-C16 fraction

d) Converting fuel depot compacted clay fill soil with over 100yrs of traffic to a plant growing media via ripping, mulching and irrigation

e) Potentially enhance TPH removal rates in NSZD below 2mbgl

f) Provided green infrastructure and activity on site

Research provides evidence for accelerated disappearance of TPH in the rhizosphere. This is predominantly at a laboratory scale. Given the significant variables at field scale and the heterogeneity of the TPH contamination it is unknown whether the TPH removal observed relates to plant assisted bioremediation. Further research replicating this scale phytoremediation trial with optimal ripping, mulching, and irrigation to assess the potential influence on enhanced microbial NSZD is recommended.

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