THE CIRCULAR ECONOMY OF WATER WASTES AS SOIL AMENDMENTS:

THE RISKS AND BENEFITS OF GROWING NON-EDIBLE CROPS ON CITY SLUDGE WASTES

Report to the
WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Problem Statement

The local (Western Cape, South Africa) mandate to divert 100% of organics from landfill by 2027 requires the diversion of sewage sludge to productive applications, most feasibly agriculture. Some municipalities already divert sewage sludge to nurseries, sportsgrounds and farming. However, most local water treatment residual (WTR, sludge waste from the clean drinking water treatment process) is destined for landfill. This is despite increasing evidence that it fortifies nutrient poor and sandy soils upon agricultural co-application with sewage sludge, improving soil fertility, water dynamics and the microbiome. Here, the environmental risks and opportunities were investigated with greenhouse lysimeter trials. The trials focused on groundwater protection, upon repeat amendment over two growing seasons, exploring the partitioning of nutrients, heavy metals and ecotoxicity in the soil-plant-water system. The socio-economic risks and opportunities were investigated in terms of (1) an *ex ante* cost-benefit analysis of a hypothetical local sludge-to-agriculture co-diversion case study, (2) the carbon market and (3) the possibility of certifying farmers who facilitate responsible waste circularity.

Method Optimisation

Standard parameters for soil fertility and microbiology were monitored, but three methodologies demanded laboratory optimisation prior to the trial, including soil-water dynamics, carbon sequestration and ecotoxicity. In terms of soil water, in laboratory analyses, the co-amendment of WTR and sewage sludge had an intermediate effect between individual amendments on the hydrophobic sandy soils. Co-amendment had a low impact on water retention, increasing it by 27% (WTR and compost both increased water retention) and had no effect on saturated hydraulic conductivity (decreased by WTR and increased by compost). However, co-amendment significantly decreased soil hydrophobicity by increasing hydraulic conductivity two-fold (WTR and compost both decreased hydrophobicity; Stone et al., 2024a). In laboratory trials, carbon stabilization and potential sequestration was improved in these highly mobile sandy soils by the co-addition of the sorptive WTR material with sewage sludge, which is rich in labile carbon. Aluminium-based WTR (Al-WTR), typically used to flocculate water with low total suspended solids (TSS), had a higher carbon stabilization effect than iron-based WTR (Fe-WTR), typically used to flocculate water with high TSS. Thus, Al-WTR increased the % stored carbon in the sandy soil, which motivated the selection of Al-WTR as the co-amendment in the lysimeter trials (Lukashe et al., 2024). There was variation in the ecotoxicity assay responses to WTR, sewage sludge and co-amendments. Since sewage sludge has a well-demonstrated ecotoxic micropollutant footprint, and WTR has a well-demonstrated clay-like capacity for sorbing phosphate and heavy metals, WTR was hypothesized to act as a clay-like sink, immobilizing sewage-borne micropollutants (Stone et al., 2024b). In contrast to the hypothesis, most acute toxicity assays (algae and *Daphnia magna*) – except *Aliivibrio fischeri* bioluminescence – were more sensitive to WTR than sewage sludge. In support of the hypothesis, the endocrine-related assays (yeast estrogen screen and most carcinogenicity assays) were typically more sensitive to sewage sludge than WTR. Phytotoxicity and colon cancer assays were negligibly sensitive to WTR, sewage sludge and the co-amendment. Sand+sewage sludge co-amendment showed no remediative ecotoxic effect across all assays, however sewage sludge+WTR co-amendment was remediative in all assays except the *Aliivibrio fischeri* bioluminescence assay (increased toxicity) and some cancer assays. Co-amendment with sewage likely remediated WTR ecotoxicity by stimulating microbial metabolism, and co-amendment with WTR likely remediated sewage sludge ecotoxicity by sorption or physical degradation. No assays were sensitive to the common polyelectrolyte (polyacrylamide) WTR flocculant, but some assays were very sensitive to sandy soil and composted green municipal waste extracts. Thus, ecotoxicity assay risks should be interpreted cautiously, and are best positioned to 'bear witness to our wastes' by monitoring disturbances (bioaccumulation or bioremediation) in context over time. Within this lysimeter trial ecotoxicity was below detection, even with repeat sludge application (2.5% m_{dw}/m_{dw}) over two seasons.

Environmental Risks and Opportunities

Lysimeter trials assessed groundwater protection (risks) and crop production (opportunities), growing hemp on sandy soils amended with WTR (2.5%), sewage sludge (2.5%) or a WTR+sewage sludge (2.5% + 2.5%) co-amendment. Crop growth parameters (biomass, plant height, stem diameter) were significantly improved with the addition of sewage sludge and WTR+sewage sludge, with the co-amendment typically higher but not significantly ($p < 0.05$) so than individual sewage sludge amendments. Groundwater protection was assessed by monitoring the distribution of nutrients (eutrophication), heavy metals (bio-accumulation), pathogens, carbon sequestration and soil water repellency, in the soil-plant-leachate system. Heavy metal concentrations in the soils and leachates showed some concentrations higher than regulatory thresholds. The I_{geo} index also suggested low risk of bio-accumulation in soil and leachates, in comparison to the background levels, with only Cu demonstrating a significant accumulation risk. Pathogens were below national regulatory thresholds for *E. coli* after the trail and parasites did not survive pasteurisation, but *Shigella* did persist over the trial, as demonstrated in previous studies. The carbon sequestration trends were variable in the lysimeter trials, not clearly demonstrating the same trends as in the laboratory, and lysimeter samples were below the ecotoxicity assay detection limit. The primary environmental risks for bio-accumulation were salinity (measured as electrical conductivity) and phosphate, both above regulatory levels in the leachate. In short, these 1:1 ratios can have environmental impacts and wastes should be more carefully paired for fertility first and then evaluated for environmental protection. For instance, increased WTR:AD ratios could stabilize the phosphate impacts and may improve the carbon sequestration in-field. However, the WTR:sewage sludge ratio is limiting, in terms of national logistical pairing and distribution, as described below.

Socio-Economic Risks and Opportunities

As mentioned, some municipalities already divert sewage sludge to nurseries, sportsgrounds and farming. However, most local WTR is destined for landfill, despite increasing evidence that it fortifies sandy soils when land co-applying with sewage sludge, improving soil fertility and water dynamics. Here, an *ex ante* provincial, national and international evaluation of WTR and sewage sludge production rates suggests that the ratio between WTR and sewage sludges in urban environments is between 0.07 and 0.19. Thus, between 10 and 20% of the South African national sewage sludge footprint can be co-applied with WTR, in the low-nutrient Western Cape sandy soils and Johannesburg granite soils, potentially co-diverting >300 000 tDS of WTR to agriculture yearly. Here, a preliminary cost-benefit case study, investigating the co-diversion of these sludges into local sandy soils, shows economic transport and fertilizer cost benefits to government and farmers. Laboratory data supports scaling this strategy to access the carbon offset market, via field trials designed based on Verra Verified Carbon Standards (VCS) methodologies. This diversion strategy has all the elements to garner significant private investment, if carbon offset in-field can be accessed via the scale of the intervention, demanding cross-province logistical coordination. However, the lysimeter trials showed far lower carbon stabilisation than laboratory data. Extra POXC analyses and DOC with more stringent extractants are necessary to confirm these lysimeter trends, and will be added before publication. If carbon sequestration can be measured in field, as in the laboratory trends, perhaps over multiple seasons – the project can be designed at scale according Vera methodologies to tap into the carbon market. This would involve multi-provincial logistical diversion to tap into the longevity and scale necessary to garner investment. Finally, the land application of sludge excludes farmers from accessing eco-conscious market premiums, since they cannot access organic certification. Here, it was shown that the local organic certification system has emerged independent of government regulations, and relies on first party claims (trust), participatory guarantee systems (collective agreements and monitoring), and finally, private certification. This lack of government regulation does not support the agricultural sector in their shifts towards responsible practices. However, it might allow for the freedom to influence current certification systems, to allow farmers utilising waste to access the eco-conscious market and expand the public imagination for waste circularity.

Capacity Building and Knowledge Dissemination

The study contributed to a number of academic and public communications, including a popular video, an advisory to the City of Cape Town on sewage spills in Fynbos and a proposal for the rehabilitation of the decommissioned Pniel sludge settling ponds. The study also promoted Stellenbosch University's involvement in hemp research, a strategic crop on the agricultural horizon. Six individuals from disciplines as wide as soil sciences, economics and microbiology were capacitated, spanning 4th year thesis, Honours, Masters and PhD degrees and including an intern at the Agricultural Research Council.

Conclusions and Future Recommendations

Together, these results support future field trials, pairing WTR and sewage sludge to promote crop growth on sandy soils. A lesson learned in this study emphasises pairing the sludges to soils and crops for optimal fertility prior to risk analyses, focusing on salinity and phosphate as the primary limiting factors for land co-application of these wastes. Future field trials should be designed with Vera methodologies in mind, providing all of the data necessary to certify a multi-provincial mobilisation of sludges into low-nutrient soils, if carbon sequestration is demonstrated in-field. A thorough sludge tallying exercise (possibly provincial but ideally national, including both WTR and sewage sludge) will be critical to this diversion strategy, as well as a full Life Cycle Assessment (LCA) of water and wastewater treatment sludges. Finally, stakeholder focus group discussions and key informant interviews should engage (1) farmers that are farming on low nutrient and sandy soils, (2) consumers that consider eco-conscious purchasing important, (3) provincial and national bodies involved in regulating the certification of crops for market, and (4) wastewater and water treatment works. These strategic actions integrate the scientific and socio-economic steps necessary to co-divert these wastes from landfill into agricultural productivity.

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestate
AFOLU	Agriculture, Forestry and Other Land Use
Al-WTR	Aluminium Oxide Water Treatment Residual
ANOVA	Analysis of Variance
ARC	Agricultural Research Council of South Africa
ARISA	Automated Ribosomal Intergenic Spacer Analysis
BIOGRIP	BioGeochemistry Research Infrastructure Platform
BMGF	Bill and Melinda Gates Foundation
BOD	Biochemical Oxygen Demand
CAF	Central Analytical Facilities
CBA	Cost Benefit Analysis
CBD	Cannabidiol
CM	Compliance Market
CoCT	City of Cape Town
COP	UN Climate Change Conference
DEA	Department of Environmental Affairs
DEADP	Department of Environmental Affairs and Development Planning, SA
DEDAT	Department of Economic Development and Tourism, SA
DESA	Department of Economic and Social Affairs, UN
DEWATS	DEcentralised WAstewater Treatment System
DFFE	Department of Forestry Fisheries and the Environment, SA
DLRRD	Department of Land Reform and Rural Development
DOC	Dissolved Organic Carbon
DSI	Department of Science and Innovation
DST	Department of Science and Technology
DSVI	DRASTIC Specific Vulnerability Index (groundwater)
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation, SA
EC	Electrical Conductivity
EEM	Excitation Emission Matrix
EMM	eThekwini Metro Municipality
EQ	Exceptional Quality
EU	European Union
Fe-WTR	Iron Oxide Water Treatment Residual
FWC	Field Water Capacity
GAP	Good Agricultural Practice
GHG	Greenhouse Gas
GS	Gold Standard
IA	Impact Assessment
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IFOAM	International Federation of Organic Agriculture Movements
Igeo	Geoaccumulation Index
IVIS	In vitro Imaging System
LaDePa	Latrine Dehydration and Pasteurization
LC-MS	Liquid Chromatography and Mass Spectrometry
LC-OCD	Liquid Chromatography-Organic Carbon Detection
MDI	Mini-Disk Infiltrometer
MeOH	Methanol
MPN	Most Probable Number

MtCO2e	Metric tons of Carbon Dioxide-equivalent
NCCAS	National Climate Change Adaptation Strategy
NCCRP	National Climate Change Response Policy
NDP	National Development Plan
NMDS	Non-Metric Multi-Dimensional Scaling
Non-EQ	Non-Exceptional Quality
NSSD	National Strategy for Sustainable Development
ORASA	Organics Recycling Association of South Africa
PFAS	per-and PolyFluoroAlkyl substances
PgC	pentagram (1012 kg) carbon
PHA	Philippi Horticultural Area
POXC	Permanganate-Oxidizable Carbon
PS	Primary Sludge
PV	Plan Vivo
RWQO	Receiving Water Quality Objectives
SA	South Africa
SANS	South African National Standards
SAOSO	South African Organic Sector Organisation
SASTEP	South African Sanitation Technology Enterprise Programme
SOC	Soil Organic Carbon
SUWI	Stellenbosch University Water Institute
SWOT	Strengths, Weaknesses, Opportunities and Threats
TAT	Total Available Threshold
tDS	tonnes Dry Solids
TIC	Total Inorganic Carbon
TIL	Total Investigative Level
TJ	Tetrajoule
TMT	Total Maximum Threshold
TOC	Total Organic Carbon
TSS	total suspended solids
UN	United Nations
UNDP	United Nations Development and Planning
USA	United States of America
USD	United States dollar
USEPA	United States Environmental Protection Agency
VCM	Voluntary Carbon Market
VCU	Verified Carbon Unit
VER	Verified Emission Reduction
VERRA VCS	Verra Verified Carbon Standard
WAS	Waste Activated Sludge
WCIWMP	Western Cape Integrated Waste Management Plan
WRC	Water Research Commission
WTP	Water Treatment Plant
WTR	Water Treatment Residual
WTW	Water Treatment Works
WWTP	Wastewater Treatment Plant
WWTW	Wastewater Treatment Works

LIST OF SYMBOLS

°C	Degrees Celsius
$m_{dw/}m_{dw}$	Mass per mass, dry weight
% w/w	Percentage weight per weight
mg/kg	Milligram per kilogram
CFU/g_{dw}	Colony forming units per gram dry weight
Rpm	Revolutions per minute
g/cm^3	Grams per centimetre cubed
%	Percent
mol	Mole
mg/L	Milligram per litre
µL	Microlitre
h	Hours
R^2	Correlation statistic
µS/cm	Micro-Siemans per centimetre
OD	Optical density
I_{geo}	Geoaccumulation index
TIL	Total investigative level (heavy metals)
TMT	Total maximum threshold (heavy metals)
MAT	Maximum available thresholds (heavy metals)
C_n	Background concentrations (heavy metals)
C/N	Carbon to nitrogen ratio
cm/s	Centimetres per second
PgC	Pentagrams carbon
tDS/yr	Total dry solids per year
ha	Hectares
tCO_2/TJ	Tonnes carbon dioxide per terajoule energy
MgCO_2/ha	Megagrams carbon dioxide per hectare

1 INTRODUCTION

The anthropogenic development of cities and towns is based on a powerfully engineered system, diverting water from the environment to human endeavours and back into the environment (Figure 1). Within this system, there are two points where sludge is coagulated and precipitated from the water: in cleaning relatively pristine water for potable purposes, and in cleaning sewage water before releasing it back into the environment. At each point, rich nutrients are removed from the water and most of the sludge is transported to landfill disposal sites (Clarke et al., 2019), specifically Vissershok landfill along the West Coast of the Western Cape (South Africa). The current system trucks these nutrients over long distances into unproductive land, wasting money and time, with a heavy carbon footprint. A recent (2017) municipal ban highlights organics as 40% of South African landfill waste (DEADP, Western Cape, South Africa), and calls for more responsible landfill practices as space is critically limited (Korhonen et al., 2018).

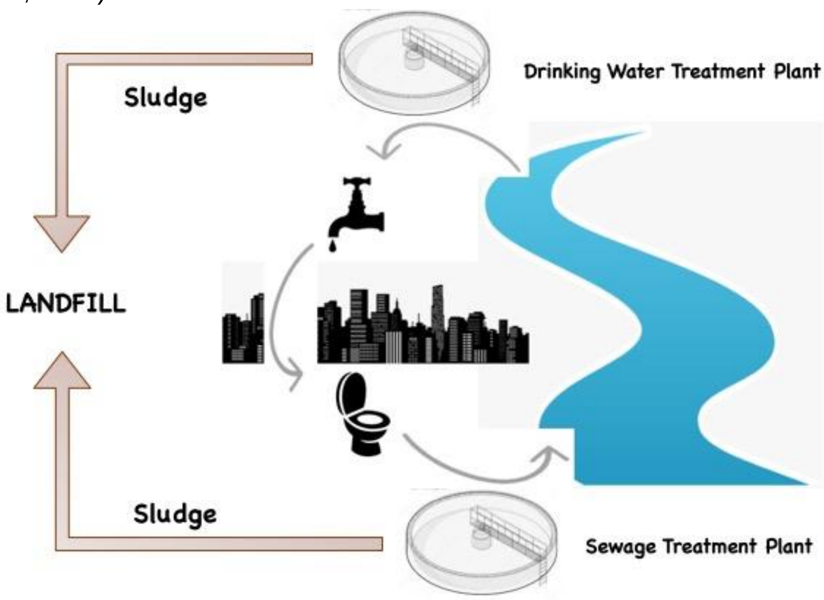

Figure 1.1. The current local sludge diversion strategy, trucked daily and destined for landfill.

Although the strict definition is disputed, ‘circular economy’ attempts to combine information and design to disrupt linear resource consumption from waste accumulation into productive applications (Korhonen et al., 2018). Examples of linear waste flows include the current landfilling of these local water treatment processes (Clarke et al., 2019). The nutrient-rich sludges carry agricultural potential and can be used as soil amendments for nutrient-poor lands (Ippolito et al., 2011; Pritchard et al., 2010; USEPA, 2016). There are toxicity risks to diverting sludge wastes into agricultural productivity, but toxicity mobility and sludge quality vary with both waste sources and soil characteristics. They can be mitigated with creative co-amendment strategies (Sarkar et al., 2007). The potential benefits warrant a continued evaluation of the risks. For instance, one of the land application risks is ground- and surface water contamination. However, ground- and surface waters are famously transboundary resources, not limited by terrestrial borders (Zeitoun and Mirumachi, 2008). Landfill thus carries the same risk to water bodies as agricultural land application, potentially exacerbated by waste concentration rather than distribution. The proposed sludge diversion strategy into agriculture is a version of biomimicry, since natural systems do not concentrate waste at one site, but waste typically enters the environment where it is produced as a heterogenous blanket across the soil ecosystem. The diversion of sludge to agriculture, our engineered urban ecosystems, harnesses the bioremediation potential of the environment (Gavrilescu et al., 2015). If the rhythm of application away from landfill into croplands is well-designed, the pollutant loads are less likely to exceed the natural environmental (mycorrhizal, chemical, solar radiation) capacity for bioremediation. This strategy has been implemented successfully internationally (Courtney, 2022).

The addition of wastes to soils is a scenario-specific endeavour (Johnson et al., 2022), which may be of benefit for crop production depending on the chemistry, physics and microbiology of the receiving land. If soils are already nutrient-rich, then the addition of nutrients will not benefit plant growth. An attractive local target site for sludge beneficiation is the Philippi Horticultural Area (PHA). This is a renowned historical farming community with a political interest in protecting their small-holder farming culture (Shoba, 2020). Local farmers grow crops on nutrient-poor windblown sands (Clarke et al., 2019; Stone et al., 2021), which need expensive amendments to promote productivity. These low nutrient sandy soils are also ubiquitous throughout Africa (Clarke et al., 2019) where, despite their low fertility and low water-holding capacity, they are the foundation of agriculture in small-scale dryland systems. Crop production on sandy soils has a high risk of water stress, exacerbated in nutrient-limited plants (Steynberg et al., 1989). Soil fertility has a bio-accumulative impact on plant and human health. For instance, nutrient deficiencies in communities solely subsisting on crops grown in sandy soils in Maputuland, South Africa, cause elevated incidences of dwarfism and endemic osteoarthritis (Ceruti et al., 2003). The sandy soils of the larger Cape Town metropolitan region are surrounded by local water treatment plants and wastewater treatment plants (Figure 2), sources of two of the waste streams investigated for soil amelioration in this report.

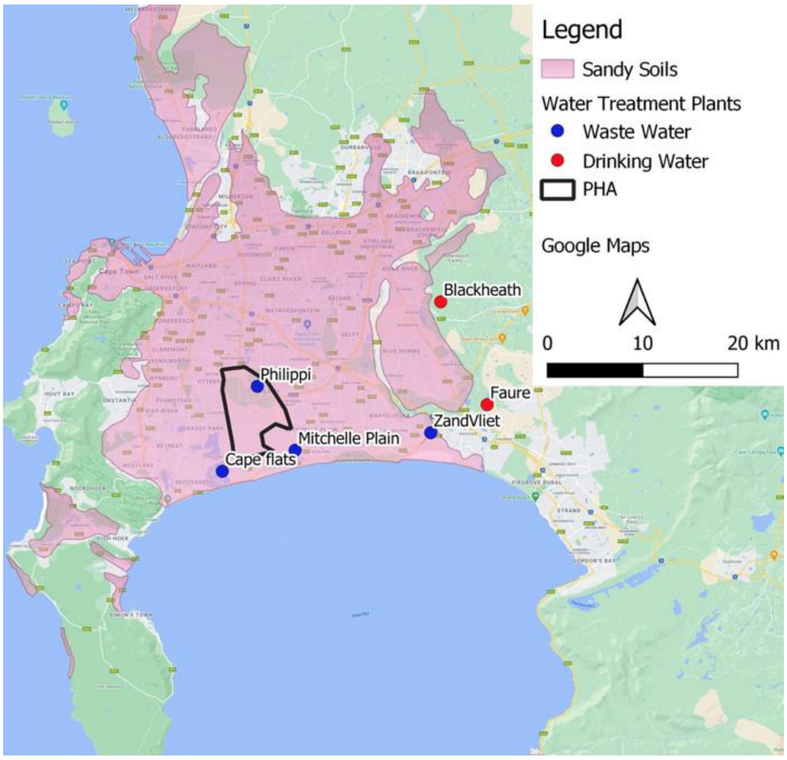

Figure 1.2. Proximity of the nutrient-poor windblown sandy soils (pink) of the Philippi Horticultural Area to three major wastewater treatment works (WWTW) and two major water treatment works (WTW) in the City of Cape Town and Cape Winelands District municipalities (Steytler et al., 2021).

1.1 Local wastes for soil amelioration

Water treatment residual (WTR) is the sludge by-product of the potable drinking water treatment process (Ippolito et al., 2011). In terms of production rates, Basibuyuk and Kalat reported a European WTR production rate of several thousand tons per year, as far back as 2004. In Africa, WTR production is also set to increase due to a growing population requiring increasing access to clean drinking water. Faure water treatment works is the main supplier of potable water to the City of Cape Town, producing ±14,000 tons of Fe-WTR per year (Clarke et al., 2019). Alternative uses of this waste byproduct are of global interest to water companies, many of which are looking toward zero-waste strategies to reduce costs and contribute to the United Nations Sustainable Development Goals (SDG 12, Responsible

Production and Consumption; United Nations, 2015). WTR is essentially the concentrated chemical and microbiological footprint of the source dams, with lime and flocculant additives (Clarke et al., 2019; Ippolito et al., 2011). The iron or aluminium oxyhydroxide flocculants produce a dried material with strong sorptive properties. The soil-like properties of the dried WTR facilitates its use as a potential land amendment. As an individual amendment, it is used to adsorb heavy metals or phosphates from contaminated soils (Sarkar et al., 2007; Ippolito et al., 2003). But this phosphate sorption limits plant growth when used as an individual agricultural amendment (Clarke et al., 2019; Gwandu et al., 2021). Thus, for soil fertility and agricultural potential, this waste must be co-amended with a second phosphate-rich waste stream in low-nutrient soils, such as the above-mentioned local receiving lands.

Compost and sewage sludge are both potential alternatives that are chemically ideal to supplement this phosphate deficiency, if co-amended with WTR in nutrient-poor soils (Clarke et al., 2019; Gwandu et al., 2021; Ippolito et al., 1999). Compost is a commercial product, which can be expensive for farmers to purchase, to supplement WTR-amended soils with phosphate. In addition, local municipal compost is of variable quality, sometimes deficient in critical plant nutrients and often containing toxic elements (Clarke et al., 2019). Sewage sludge (anaerobic digestate) is another waste stream that, like compost, can supplement the phosphate deficit in WTR-amended soils (Ippolito et al., 1999). However, sewage sludge amendment has both toxicity and eutrophication risks (Pritchard et al., 2010). In sandy soils, the risk of contaminant mobility is high (Boyd et al., 1988). Thus, the amendment of sewage sludges in such soils is typically discouraged. However, WTR is renowned for immobilising contaminants. It has a high sorption capacity, due to a large proportion of micro- and mesopores and consequently high surface area (Sarkar et al., 2011; Ippolito et al., 2003; Chiang et al., 2012; Hoyespayn and Bozongo, 2009). Thus, the co-amendment of WTR can potentially reduce the risk of sewage sludge (anaerobic digestate) contaminant mobility in sandy soils. This will improve the systemic potential of a sludge-to-agriculture diversion strategy, since the co-amendment of two waste streams mitigates the limitations of each: phosphate deficiency upon WTR amendment, and nutrient and pollutant mobility upon sewage sludge amendment (Table 1).

Table 1.1. Potential benefits and risks of the three waste streams that can be co-diverted to agriculture in this circular economy strategy.

Water Treatment Residual	Compost	Anaerobic Digestate
Potential Benefits • Diversion from landfill, • Clean flocculated dam sediment, • High nitrogen, • Sorption of heavy metals and micropollutants, • Potential for carbon stabilisation, • Low cost.	Potential Benefits • High phosphate, • Low micropollutants, • Low pathogens.	Potential Benefits • Diversion from landfill, • High nutrients, including phosphate, • Low cost.
Potential Risks • Phosphate sorption and crop deficiency, • High heavy metals (Mn, Al).	Potential Risks • High heavy metals, • Variable quality (low nitrogen), • Nutrient mobility in sandy soils (eutrophication in water bodies) • High cost.	Potential Risks • High micropollutants, • High heavy metals, • Nutrient mobility in sandy soils (eutrophication in water bodies), • High pathogens.

1.2 Circular design of a nature-based solution: Crop selection and economics

As mentioned above, this waste diversion strategy is a version of biomimicry, falling into the category of nature-based solutions (USEPA, 2015). In nature, without anthropogenic activity, there are few examples of concentrated waste sites like landfill. In natural (non-anthropogenic) ecosystems, waste is typically metabolized and remediated in the environment where it is generated and deposited, a source of well-dispersed nutrients. Current anthropogenic linear strategies concentrate waste on at landfill sites with an 'out-of-sight, out-of-mind' philosophy (Korhonen et al., 2018). Nutrients (soil fertility) are removed from the ecological cycle and concentrated on one site. Thus, nutrients become pollutants via eutrophication. The diverse complex pollutants of anthropogenic wastes are also concentrated on one site, typically exceeding the natural bioremediation capacity of the environment. Natural bioremediation includes mycorrhizal metabolic turnover (Loffredo et al., 2021; Citterio et al., 2005), sequestration and binding (Ragle et al., 1997), solar radiation (Costa et al., 2020), and chemical degradation (Stangroom et al., 2000). The co-diversion of WTR and sewage into nutrient-poor sandy soil for agricultural productivity ensures that the pollutants entering the environment are diluted by distribution, increasing the likelihood that they are within the bioremediation potential of the natural plant-soil-water system (Figure 3).

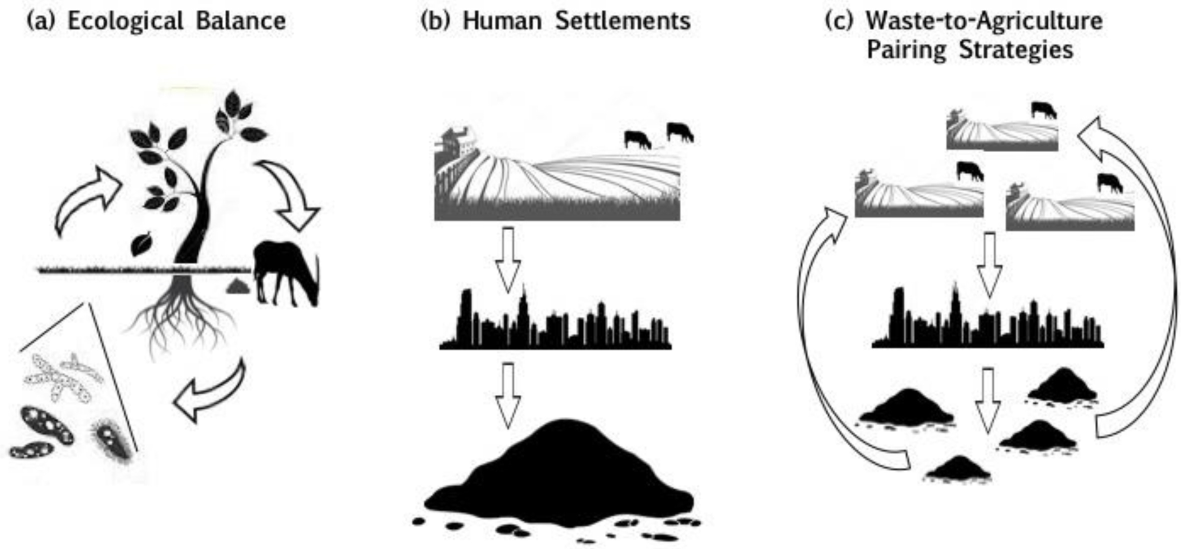

Figure 1.3. Sludge diversion into agriculture, informed by biomimicry. A nature-based solution to meet the landfill capacity crisis (Korhonen et al., 2018) and the local municipal landfill organics ban.

The pathogenic risk and public perception of sewage sludge can influence the sale of edible crops (Laura et al., 2020). Attractive alternative high-value, non-edible crops include biofuel and textile crops like cotton, hemp and bamboo. With predictions of increasing local droughts, food crop exports become precarious in dry cycles (Feng and Fu, 2013; Odoulami et al., 2020; Cook et al., 2014). The last drought made headlines, bringing the City of Cape Town municipality close to 'Day Zero' with no access to tap water (Pascale et al., 2020). Under drought conditions, edible crops generally do not meet export criteria. Biofuel and fibre crop markets are less dependent on export standards. They are attractive diversification options and thus a potential agro-economic buffer in the Western Cape (South Africa).

Hemp and bamboo are both economically attractive alternatives to edible crops (CSIR, 2020; Cherney and Small, 2016; Prohibition Partners, 2020a; Prohibition Partners, 2020b; Allen et al., 2019). Whether utilised for cannabidiol (CBD) oil or fibre production, post-harvest plant processes drastically minimise the risk of pathogen and pollutant transfer from the soils into the food chain. Agro-economic considerations of a number of high-value non-edible crops are outlined in Table 2. These include cotton

(*Gossypium malvaceae*), hemp (*Cannabis sativa*), and the primary biodiesel crops like soya beans (*Glycine max*), canola (Brassica napus) and sunflowers (*Helianthus annus L.*), as well as bamboo (*Bambusoideae*).

Table 1.2. A comparative assessment of the relevant agronomic considerations of non-edible crops, for farming in the local context.

Parameters	Cotton[a]	Hemp[b]	Soya[c]	Canola[d]	Bamboo[e]
Target Industry	Textile	Textile	Biofuel	Biofuel	Textile
Growing Season	Summer	Early Spring-Summer (photosensitive, daylight cycles)	Summer	Winter	Summer
Growing Temperature	Warm summers, 15-28°C	Mild, temperate climate	Moderate (18-25°C), sensitive to extremes	Cool (0-15°C)	Wide range, tolerant of frost and drought, hardy
Soil pH	6-8.5	6-7.5	Acidic (>5.2)	5.5-7	5-6.5
Soil Type	Sandy Loam, Well Drained	Loamy, >3.5% organic matter	Dense, nutrient-rich, high water holding capacity	Clay-Loam soils, but well drained. Sandy soils not recommended.	Sandy soils, with clay additives, well-drained
Irrigation	4-6 days, drought-tolerant	Frequent during early phases, well-drained soils	Frequent during early phases, and pre-planting	Dryland conditions, but not drought-tolerant	Regular, twice per day in summer
Research Interest	Well-Established	New Market	Well-Established	Well-Established	Increasing Market Interest

[a] DLRRD (2012), [b] ARC (2018), [c]DAFF (2010), [d]DLRRD (2012), [e]DPIRD (AU) (2014).

Summer rainfall is ideal for hemp and bamboo growth (Hall et al., 2013). Access to summer irrigation is a limitation in the Western Cape, a winter rainfall region. However, there is a strong governance drive to re-use treated wastewater (Adewumi et al., 2010). The sludges can be funnelled together into the soils to promote fertility, and the treated wastewater used to meet the water demand of hemp or bamboo outside the rainy season. Hemp has a remarkable root system with well-demonstrated bioremediation capacity, particularly in symbiosis with arbuscular mycorrhizal fungi (Loffredo et al., 2021; Citterio et al., 2005). Bamboo has similar bioremediation potential, particularly with inter-cropping strategies and other management interventions (Bian et al., 2021). The bioremediation combination of the root system of hemp or bamboo, the mycorrhizal fungi and the sorption capacity of a WTR co-amendment may decrease the risks of sewage contaminant mobility through sandy soils and into the groundwater system.

This circular system, co-amending wastes into soil to promote non-edible crop production, fits neatly together, disrupting the current linear flow from resource to waste (Figure 4). There are at least two geographical options for redistributing sludges into the environment, to utilise the nutrients and promote bioremediation: (1) distribution into farming land, in proximity to local water and wastewater treatment works, or (2) distribution into marginal land, particularly promoting the productivity of land currently overrun by alien invasive species, and circumnavigating the typical food-versus-fuel debate (Subramaniam et al., 2020). This is especially relevant in southern African countries where alien bush encroachment poses a risk to water security, leading to reduced agricultural output, biodiversity loss, and intensification of wildfires.

The lands surrounding our local sewage treatment works are geographically ideal for saving sewage transport costs and provide a source of treated wastewater for crop irrigation. The Zandvliet, Mitchell's Plain and Cape Flats Wastewater Treatment Works are all within a 25 km radius of the low-nutrient sands (Figure 2) of the Philippi Horticultural Area, in contrast to the 40-50 km distance to Vissershok landfill. Thus, diversion of the wastes into agricultural applications fits neatly into a more circular economy than current linear landfill disposal strategies.

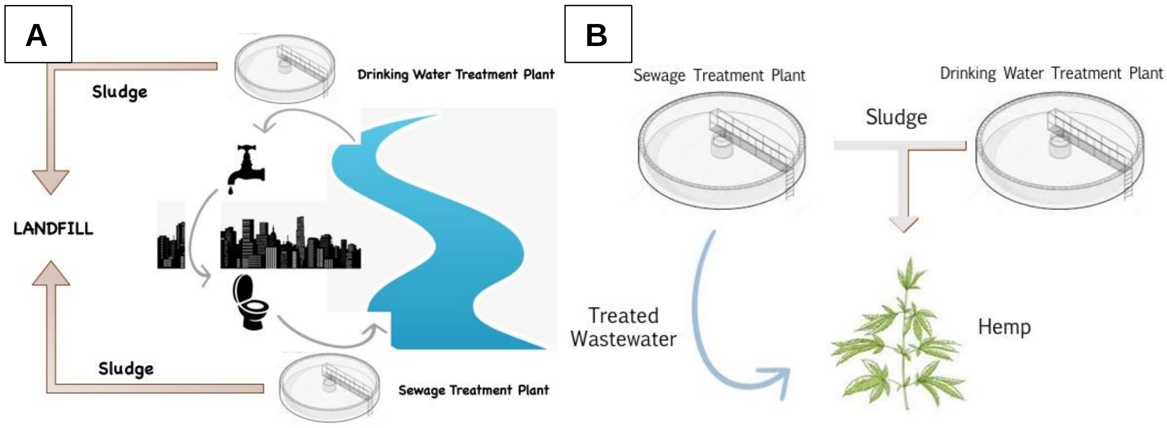

Figure 1.4. Potential circular economy of the waste cycle, for high-value non-edible crops grown on soils amended with water and wastewater sludges. This diversion scenario is an alternative to the current linear landfill sludge disposal strategy (Figure 1).

As mentioned, the political landscape could support this experimental design, depending on the willingness of residents to diversify to non-edible crops. The nearby areas are part of an ongoing legal battle, driven by the PHA residents (Shoba, 2020). Residents are resisting industrial and residential development in favour of peri-urban farming, thus protecting their historical legacy. The sludges can be funnelled together into the soils to support peri-urban agricultural productivity, and the treated wastewater can meet irrigation needs outside the rainy season. This study could provide ecological support for a sustainable agricultural model of waste re-use, connecting urban waste systems with peri-urban farming. However, edible crops are the primary PHA produce, and diversification may be resisted to protect the legacy. Security issues, due to the Cannabis sativa plant morphology, and small-scale hemp production costs may also pose challenges. However, this strategy can apply to other nutrient-poor soils – such as the granite soils surrounding Johannesburg – if the socio-political and agricultural systems are open to diversifying to non-edible crops. There is an additional, attractive possibility of using marginal land overrun with alien invasive vegetation for these agricultural activities.

1.3 Foundational studies: Initial findings and knowledge gaps

This project emerged from two greenhouse- and laboratory-based monitoring studies (Clarke et al., 2019; Stone et al., 2021). The studies explored the agricultural application of these three waste streams in local sandy soils, in terms of chemistry and microbiology. These results inform the current study and the circular economy design proposed for the local landscape. They evaluated the risks and benefits of co-amending local soils with WTR and one of two phosphate-rich waste streams (municipal green compost or sewage sludge), in laboratory incubations and wheat pot trials. Commercial compost is an expensive waste stream, and thus sewage sludge is preferable to alleviate the phosphate deficit when co-amending with WTR. However, the risks associated with composts are lower. Thus, municipal green compost was the basis of early trials in this body of work. The co-amendment studies progressed from WTR-compost co-amendments in laboratory and greenhouse trials (Clarke et al., 2019) to laboratory WTR-sewage sludge assessments for pathogenicity (Stone et al., 2021). Throughout these foundational case studies and the current WRC Project C2022/2023-00820 (hereafter referred to as 'the Project'), anaerobic digestate (AD) was the sewage sludge selected for land amendment, as the pathogen concentration is lower than primary and secondary activated sludge. The benefits, risks and key findings of each study are described here.

1.3.1 Case study 1: The chemistry of co-amendment

In a study entitled 'Better Together: Water Treatment Residual and Poor-Quality Compost Improves Sandy Soil Fertility' (Clarke et al., 2019), wheat (Triticum aestivum L.) was grown on local nutrient-poor sandy soil, amended with WTR, compost or a co-amendment of the two materials at increasing loading rates (1%, 5% and 12.5% m_{dw}/m_{dw}). The chemistry – soil fertility, heavy metal toxicity, crop nutrients and consequent crop biomass – was evaluated during this three-month pot trial.

1.3.1.1 Benefits

In pot trials exploring the growth response of wheat to these waste amendments, the individual amendments of both WTR and compost limited crop biomass in nutrient-poor sandy soils with (Figure 5). However, the highest WTR-compost co-amendment produced significantly higher plant biomass (33% higher, $p < 0.05$) than the control.

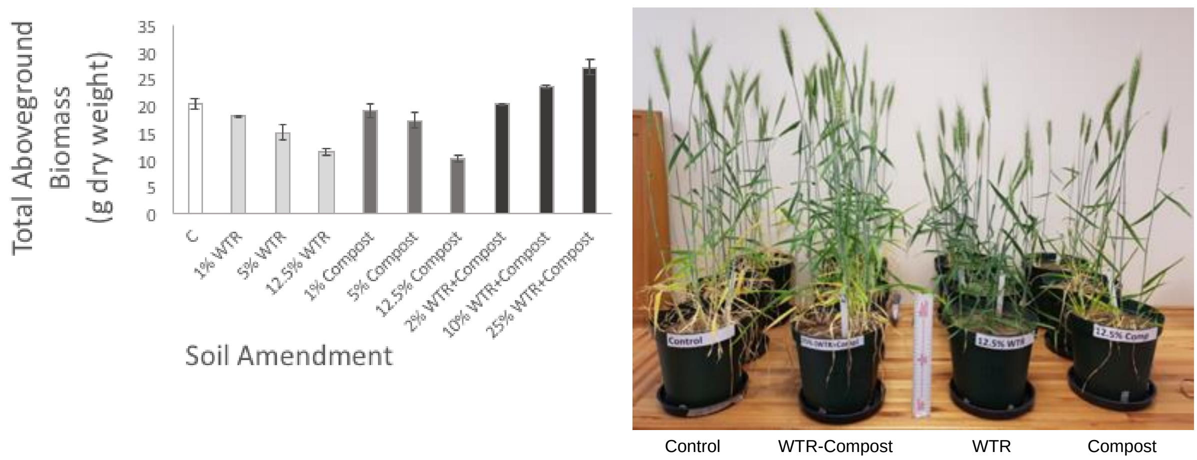

Figure 1.5 Above-ground plant biomass of wheat grown in pot trials, comparing local nutrient-poor sand with WTR amendment, compost amendment or WTR-compost co-amendment, at 12.5% (m_{dw}/m_{dw}). Error bars represent the standard deviation of triplicate biological repeats (Clarke et al., 2019).

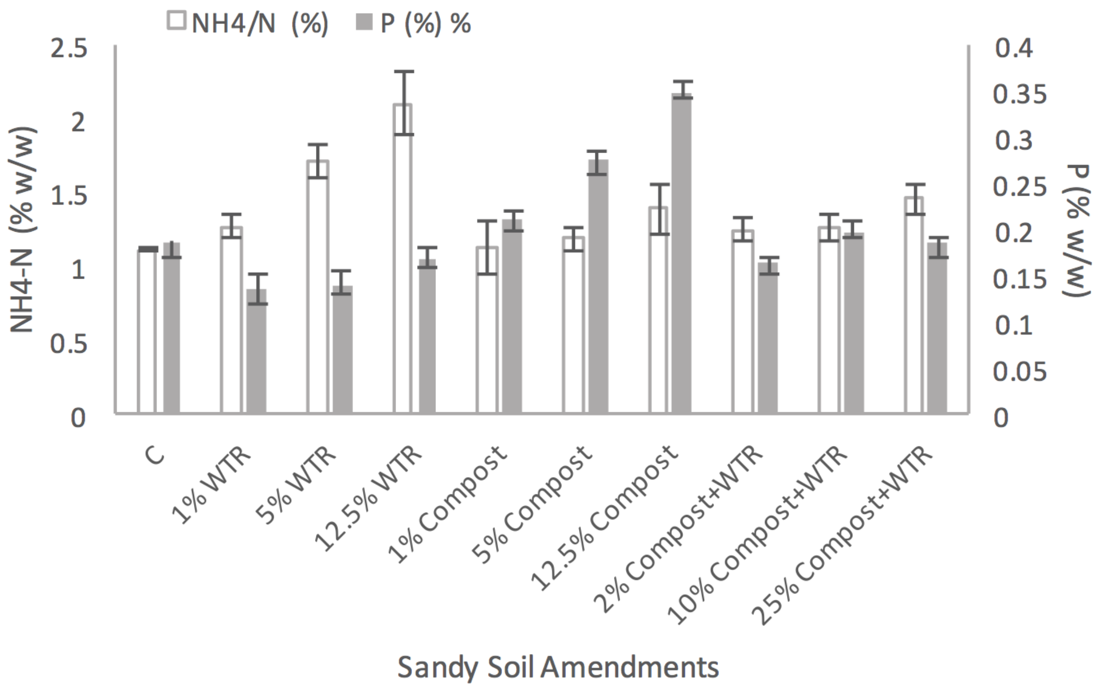

Figure 1.6. Foliar nutrients (nitrogen and phosphate) of wheat grown in greenhouse trails, comparing local nutrient-poor sand with WTR, compost and WTR-compost co-amendments. Error bars represent the standard deviation of triplicate biological repeats (Clarke et al., 2019).

Wheat grown in soils amended purely with WTR was phosphate-limited (Figure 6), as expected. In contrast, plants grown in soils amended purely with commercially available municipal compost were nitrogen-limited, indicating poor-quality compost. However, the co-amendment of WTR and compost in these receiving sandy soils promoted the growth of winter wheat by ±30%, in comparison to control plants grown in the local sandy soils, at the highest application rate. Foliar nutrients attributed crop biomass to the balance of nitrogen and phosphate with the co-amendment. Table 3 – the chemical characterisation of the materials – reflects these foliar profiles. WTR is limited in phosphate but rich in nitrogen, and compost (despite an acceptable C:N ratio) is limited in total and mineral nitrogen but rich in phosphate.

Table 1.3. Chemical and physical characteristics of soil, WTR and compost used in the study (Clarke et al., 2019).

Parameter	Soil	WTR	Compost
pH (water)	6.5	6.8	7.8
pH (KCl)	5.6	5.8	7.6
EC (µS/m)	63.6	319.0	5410.0
Total C (%)	0.6	17.0	9.6
Total N (%)	0.04	0.35	0.38
NH_4_N (mg/kg)	b.d	164.1	4.9
NO_3_N (mg/kg)	0.003	1.283	1.945
Mehlich III P (mg/kg)	52.4	5.1	145.4
Bray II K (mg/kg)	9.0	63.7	2944.0

1.3.1.2 Risks

Metals, from the coagulants used or impurities in the coagulants, particularly Mn, are of local concern in sludges originating from Theewaterskloof dam (Titshall and Hughes, 2005). Thus, the sludges were characterised and compared to local (Herselman, 2013) and international (USEPA, 2000) guidelines. Iron was the dominant metal, followed by Al. Manganese was variable, but lower than the values reported by Titshall and Hughes for Faure WTR in 2005 (0.7 and 1.8%), assumed to be from impurities in the ferric sulfate or lime used during the water treatment process. The metal concentrations of all samples were well below both the USEPA (2000) guidelines and Herselman's (2013) more conservative South African guidelines (Table 4). The accumulation of metals as foliar macronutrient content was compared to local guidelines for plant toxicity. However, against expectations, Mn levels in plants were at or below the critical micronutrient content in wheat (Sims and Johnson, 1991; Plank and Donohue, 2000). Thus, rather than being a toxicity threat, they improved trace element concentrations almost to the critical limits necessary for plant growth (Clarke et al., 2019). Plants will need to be monitored for bioaccumulation during repeat applications in-field, but this research indicates that the waste co-amendment promotes the fertility of local receiving soils, in terms of N, P and trace elements. Bioavailable metals (Table 5) in the pot trial materials were all well within toxicity limits, with arsenic in the compost being the primary concern (although not the focus of this study). Arsenic in pure WTR is slightly above guidelines but diluted to within guidelines at standard land application rates.

Table 1.4. Trace element concentrations (mg/kg) in an aqua regia extract for WTR collected at three sampling times together with South African and USA guidelines of maximal loadings for land application.

Elements	Sampling time			Maximum allowable limits	
	28-Feb	09-May	15-May	SA[a]	USA[b]
As	17.1	16.0	14.4	40	75
Cd	0.2	0.1	<0.1	40	85
Cu	26.9	19.4	17.6	1500	4300
Hg	<0.1	0.1	<0.1	15	57
Mo	2.63	3.91	4.33	n.p	75
Ni	83.5	33.7	33.1	420	420
Pb	19.8	26.9	22.1	300	840
Se	2.3	2.6	2.0	n.p	100
Zn	93.6	51.4	46.0	2800	2500
Mn	2925.4	559.6	684.0	n.p	n.p
Al	53.4K	77.3K	63.9K	n.p	n.p
Fe	193.3K	156.2K	136.5K	n.p	n.p

[a] According to (Herselman, 2013)
[b] USEPA Maximum concentration permitted for Land Application: Biosolids Technology fact sheet Land Application of Biosolids. EPA 832-F-064, September 2000
n.p. = not provided

Table 1.5. Bioavailable trace element concentrations in the pot trial materials, together with threshold limits for metal concentrations in the soil where water treatment residual (WTR) will be applied (Herselman, 2013).

Element	Receiving soil limit	Soil	WTR	Compost
	mg kg−1			
B		31.5	188.7	659.0
Al		208.7	60.3	2473.1
Mn		194	17,000	343
Fe		126.8	130.8	1534.2
Ni	1200	3.3	94.7	19.5
Cu	1200	9.8	363.6	113.9
Zn	5000	57.6	100.0	96.3
As	14	1.7	30.1	141.3
Cd	100	0.2	0.5	0.3
Hg	7	0.03	<0.05	0.06
Pb	3500	1.0	1.4	5.1

1.3.1.3 Key findings and knowledge gaps

In a winter wheat pot trial, the amendment of local sandy soils with water treatment residuals limited plant growth, due to phosphate limitation. Even local commercially available compost limited plant growth in this nutrient-poor matrix, due to nitrogen deficiency. However, the co-amendment of WTR and compost promoted wheat growth by up to 30%, at high loading rates. The co-amendment provides the N:P balance necessary for wheat growth, in these sandy soils.

Bioavailable heavy metals in soils were below national and international toxicity guidelines, in this high-rate but single-application (one season) pot trial, and foliar trace elements in the plant material were generally even below critical levels necessary for wheat growth. Thus, in this study, heavy metal toxicity was not a concern with WTR amendment to soils, and the co-amendment of WTR and compost promoted soil and plant fertility. A knowledge gap that will be addressed in this Project, is the impact of repeat applications in a two-year growing cycle, and composting. The carbon sequestration and soil-water impact of WTR – a clay-like substance – upon co-amendment with sewage in sandy soils are also gaps explored in this study.

1.3.2 Case study 2: The microbiology of co-amendment

A multi-national study entitled ‘Rebuilding Soils with Water Treatment Residual Co-Amendments: Terrestrial Microbiology, Pathogen Characterisation and Soil-Rhizosphere Dynamics’ (Stone et al., 2021), explored the microbiological risks (pathogenicity) and benefits (microbial concentrations, root associations and diversity) of sludge co-amendments to nutrient-poor sandy soil. The study included microbiological analyses of WTR and compost co-amendments in pot trials, as well as pathogen persistence in WTR and anaerobic digestate (AD) co-amendments in laboratory incubations.

1.3.2.1 Risks

According to South African national guidelines for land application and handling of sewage sludge (Herselman and Moodley, 2009), there are 3 factors to consider, including (1) viable microbial concentrations, (2) sludge stability and (3) pollutant concentrations. The Microbial Class in the South African classification system mirrors the US Environmental Protection Agency’s classification system (USEPA, 2022), with two classes for unrestricted and restricted use – South African terminology – or

Exceptional Quality (EQ) and Non-EQ sludges – EPA terminology. In both cases, restricted use and non-EQ sludges can be handled and land-applied, with extra guidelines and restrictions. These include avoiding edible crops and permitting periods of soil remediation before public soil contact. Unrestricted use and EQ sludges can be applied without any further considerations, but permissions are still required to monitor soil quality.

The study investigated the impact of local anthropogenic activity on potable water sources on the pathogenicity of WTR. Sewage sludge and WTR pathogen concentrations were compared to sandy soil and pristine and polluted river sediments. Even with anthropogenic impact, WTR sludges were all well within land application guidelines. Sludges with greater anthropogenic impact (Harare) were closer to the pathogenic limit for general sludge use, but all were below the limits for restricted use according to South African national standards (Figure 7, Herselman, 2013; Herselman and Moodley, 2009).

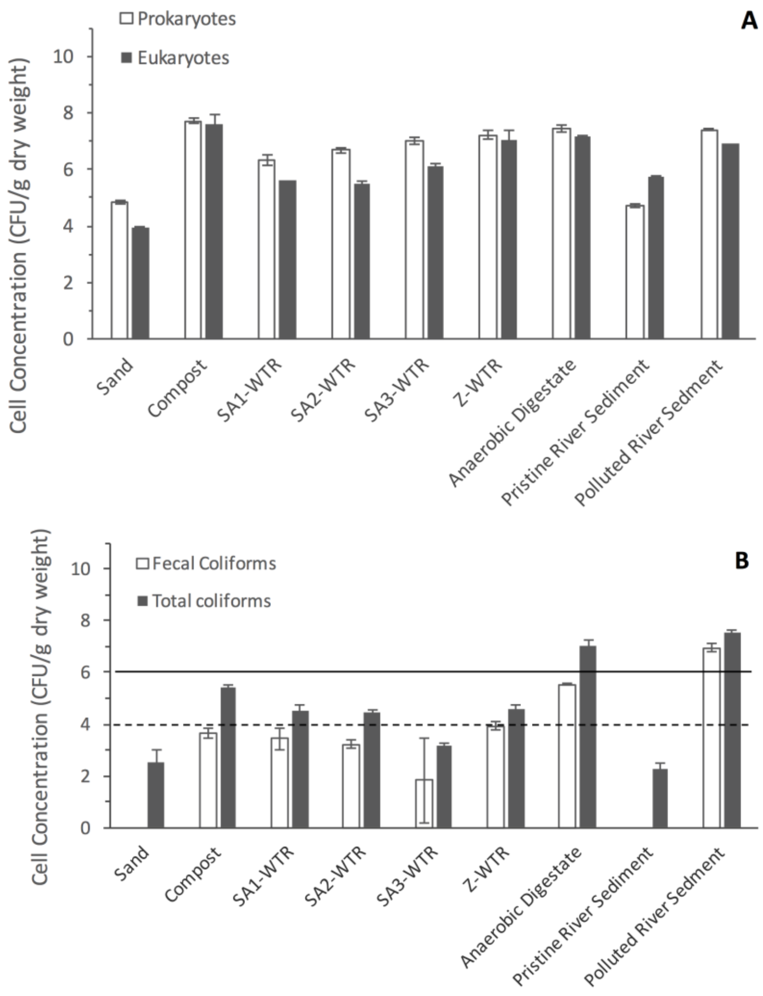

Figure 1.7. WTR sludges, evaluated for pathogenic risk due to dam pollution levels in some source waters. Faecal indicators (B), plotted with heterotrophic microbial populations (A), were well within general handling (dashed line) and restricted handling (solid line) national guidelines for all WTR samples from South Africa (SA) and Zimbabwe (Z)(Stone et al., 2021).

Pathogenic persistence was assessed during a 21-day incubation in local sandy soils, with and without WTR. Although total prokaryotes and eukaryotes did not vary over the incubation period, faecal coliforms dropped from a maximum of 10^6 CFU/g_{dw} to zero CFU/g_{dw} in all incubations (Figure 8). Re-growth of the faecal indicator was not a risk in this case study. However, although faecal coliforms – the standard faecal indicator of microbial contamination – did not persist, enteric bacteria and Salmonella and Shigella did, suggesting a broader microbial indicator suite might be necessary to monitor land receiving sewage sludge.

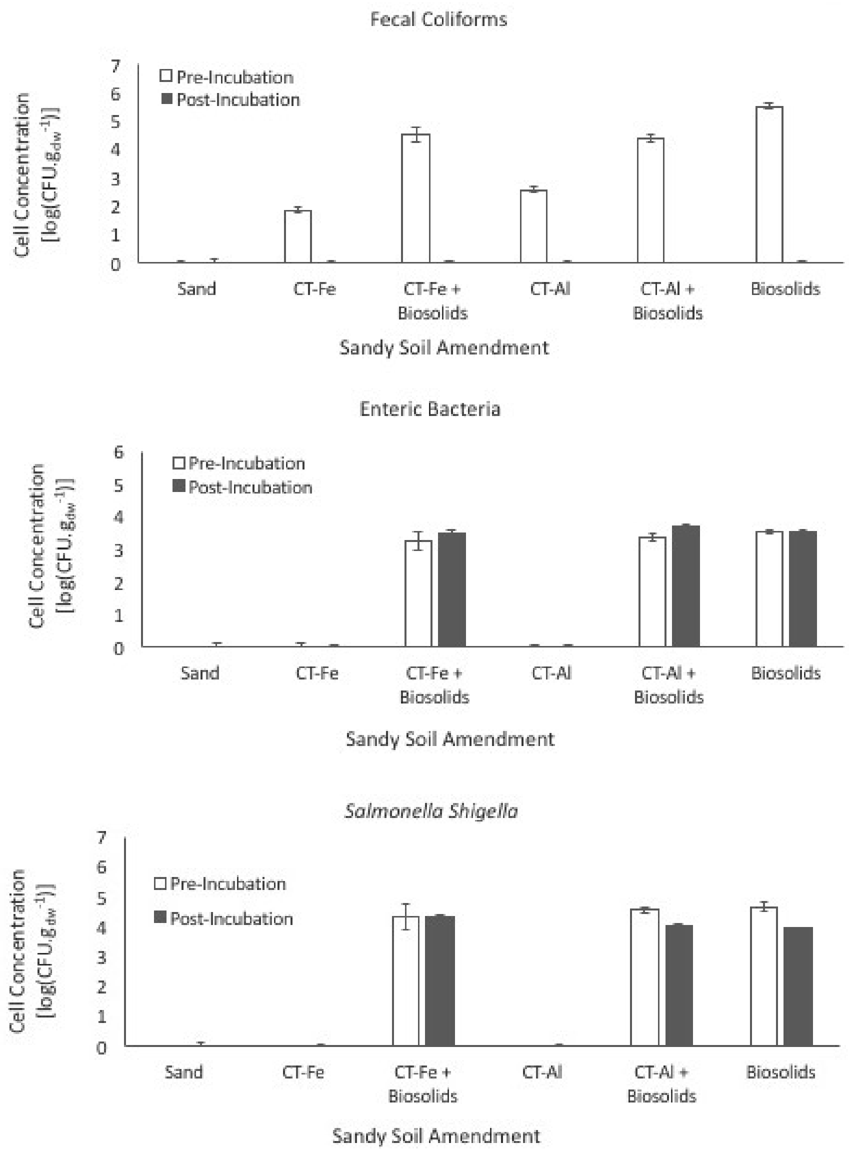

Figure 1.8. The persistence of standard faecal coliform indicators, as well as enteric bacteria and Salmonella and Shigella in nutrient-poor sandy soil microcosms amended with WTR and sewage sludge [20% m_{dw}/m_{dw}], incubated at field water capacity for 21 days. CT-Fe and CT-Al represent iron and aluminium WTR samples from Cape Town, incubated with biosolids (AD). Error bars represent the standard deviation of the mean of triplicate biological repeats (Stone et al., 2021).

1.3.2.2 Benefits

The microbial benefits of WTR as a soil amendment were also evaluated, in the above-mentioned three-month pot trial (Clarke et al., 2019), exploring wheat grown on soils amended with WTR and compost. At the highest loading rates (12.5% WTR, 12.5% compost and 25% WTR-compost co-amendment), all three amendments improved the total prokaryotic and eukaryotic concentrations in local nutrient-poor sandy soils (Figure 9a). In terms of soil microbial β diversity (NMDS ordination plot, the diversity between microbial groups), the WTR amendment (blue) did not shift the diversity from the sandy soil control (red) as dramatically as the compost and WTR-compost co-amendments (green and purple). Thus, compost has a more measurable effect on soil microbial β diversity than WTR (Figure 9b). Finally, qualitative scanning electron microscopy images show microbial associations with roots (pili or fimbriae) only in the amended soils (Figure 9c), suggesting benefits upon amendment in terms of microbial load, diversity and physiology. However, the microbial benefits were not notably different between individual and combined WTR and compost treatments.

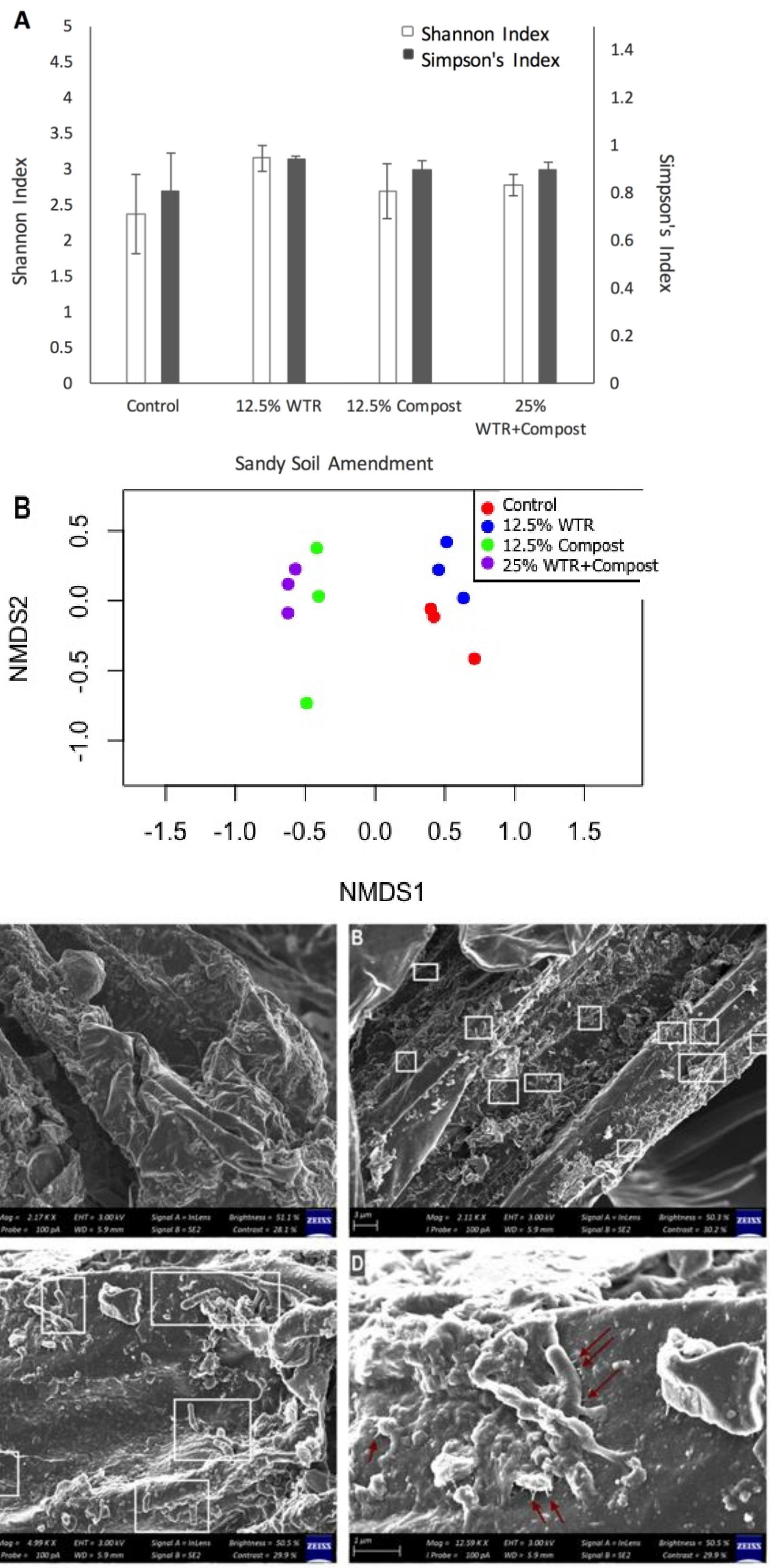

Figure 1.9. Microbial load (A), soil bacterial diversity (B) and visual root association (C) in the rhizosphere of wheat grown in local nutrient-poor sandy soil (control), amended with WTR, compost and a combination of these wastes. In image (C), section C-A represents control sandy soils, with minimal evidence of microbial biomass, whilst C-B to C-D represent soils amended with WTR and compost (Stone et al., 2021).

1.3.2.3 Key findings and knowledge gaps

Local land application guidelines require that we assess the pathogenicity of sewage sludges, but do not consider WTR a pathogen risk, in terms of faecal indicators. Due to anthropogenic effects on source waters, this assumption was tested. It was shown that anthropogenic pollution does lead to faecal indicators in the WTR, but even the highest loads were well below national guidelines for unrestricted sludge handling. After amending high loading rates of the sewage and WTR sludges in local sandy soils, and incubating for one month in laboratory microcosms, all faecal indicators were outcompeted by the sandy soil biome. However, non-standard pathogenic indicators (Salmonella, Shigella and enteric bacteria) did persist. Thus, in this study, all the sludges were safe for land application according to national standards, but we recommend monitoring a wider suite of pathogenic indicators during the land application process. Finally, in a wheat pot trial, the microbiome of sandy soils was improved by the amendment of compost, WTR and WTR-compost co-amendments, in terms of cell concentrations, diversity and qualitative root-cell associations. The knowledge gaps that will be explored in this Project include monitoring pathogenic indicators in water leachate for groundwater protection, and microbial dynamics during repeat application (two growing seasons) and composting.

1.4 Socio-economics and stakeholder engagement

1.4.1 Soil perspectives: A philosophy of care versus a philosophy of management

This team was part of co-authoring a multi-national publication exploring the role of perspective in the management of soils (Johnson et al., 2022) during the launch of the Project. The lead author – an engineer – initiated the collaborative piece called ‘A Nation that Rebuilds its Soil, Rebuilds Itself’, inverting Roosevelt’s response to the Dust Bowl of the Midwest in the 1930s, when he said, “A nation that destroys its soils, destroys itself”. This paper is aimed at engineers – often strategically involved in urban management – and suggests that complexity and data-driven scientific communication is one of the primary inhibitors to soil governance. We have ample tools and information to understand soils, and the inputs necessary to maintain healthy soils. But paralysis seems to be at the point of synthesising information into strategies that farmers and municipalities can access without being overwhelmed by scientific complexity during risk communication. Studies show that data complexity and heterogeneity are problems at farm management level (de Bruyn and Abbey, 2003), and that our youth are disconnected from our soil (Johnson et al., 2003), with only 30% of children in Southern African countries aware that soil is living (10^9 microbial cells per gram of soil; Vieira and Nahas, 2005).

The article proposes that relational interventions could be key to shifting soil and organics governance, simply noticing that soil is living, and thus approaching soil management with the same mix of science, intuition, and relational communication with which we approach raising children. This shift from a ‘philosophy and ethics of management’ to a ‘philosophy and ethics of care’ has been proposed in education, for instance in Gilligan’s ‘In a Different Voice’ (1982) and Garrison’s ‘Wisdom and Desire in the Art of Teaching’ (2010). Johnson et al. (2022) points out that soil inputs and outputs are currently disrupted (vicious cycles). It states that “inputs and outputs of both carbon and nitrogen are out of balance and it is generally acknowledged that cultivated soils are carbon limited (Demonling et al., 2007). The changes to both the N and the C cycle working against soil’s natural cycle has created poorer soils with reduced capacity for self-regeneration and stabilisation.” Models that allow for more balanced soil nutrient management rely on improved scientific and communication strategies.

Here, this model of sludge co-diversion from landfill to agriculture is used to test the feasibility of relying on the buffering capacity of a living biome to maintain healthy soil homeostasis in the right conditions. There are successful international models for the long-term land application of sewage, for instance a 20-year commercial trial in Queensland, Australia (Courtney, 2022). A primary aim of this WRC project is to simplify, rather than increase complexity. It tests a simple WTR-sewage sludge co-amendment strategy over two seasons, using it to build long-term relationships with key stakeholders in the soil and waste governance and management spheres. The work therefore aims to build small, slow quality-based relationships that can withstand changes in management and funding cycles, with the continued motivation of care for – rather than management of – living soils.

1.4.2 Stakeholder mapping

Section 1.4.1. highlights the importance of stakeholder engagement in this project. Based on systematic methods for stakeholder engagement for nature-based solutions (Zingraff-Hamed et al., 2020), a wide stakeholder map was generated and a sub-section of core stakeholders were identified for invitation into this study (Figure 10).

ECONOMIC & SOCIAL	LANDSCAPE (Farmers and NPO's)
• **Agricultural Research Council** • *Hemporium (Commercial)* • *SU School of Public Leadership* • SU Law	• **Philippi Economic Development Initiative** • *Greencape* • *Co-Go (Collaborative Governance, Water Sector)*
GOVERNMENT	SCIENTIFIC
• **Water Research Commission** • *City of Cape Town Development and Infrastructure Planning: Wastewater Branch, Water and Sanitation Department, Water and Waste Directorate* • *City of Cape Town Analytical Laboratory: Scientific Services Branch – Water and Sanitation Department, R&D*	• *UNISA Institute for Nanotechnology and Water Sustainability* • **Department of Environmental Engineering, Durham University, UK** • **Marine and Earth Sciences, Lyell Centre, Heriot-Watt Univerity, Edinburgh, UK** • **Department of Physiology, Stellenbosch University, SA** • **Electrical and Electronic Engineering, Stellenbosch University, SA** • *Randwater* • *ERWAT*

Figure 1.10. A map of critical stakeholders, grouped into four relevant sectors. Key: Stakeholders in bold were engaged in research. Stakeholders in Italics were in conversation with the project. Stakeholders in standard font are of interest for collaboration after lysimeter trials are complete.

The primary stakeholders are engaged according to Table 6, with stakeholder consultations. Roger Jaques represents the Philippi Economic Development Initiative (PEDI). PEDI was established in 1998 as a Section 21 Company by the City of Cape Town in partnership with the Western Cape Provincial Government, businesses and the community, with the primary aim of promoting economic growth and development in the Philippi Industrial area. Their experience contributes to the project in three ways: (1) advisory collaborators for connection to local farmers and crop distribution, (2) strategic experimental design, optimised for landscape needs, and (3) expertise in composting (beneficiation) of sludges. Dr Aart-Jan Verschoor and Ms Livhuwani Masola are agricultural economists from the Agricultural Research Council who are co-drafting the socio-economic elements of the project. Tony Budden, founder of Hemporium (https://www.hemporium.co.za/), expressed keen interest in the research during early meetings and informed the logistics of seed sourcing. Similarly, initial consultations with Greencape (https://www.greencape.co.za/), Co-Go (the Collaborative Governance Initiative at SU (https://www.sun.ac.za/english/Lists/news/DispForm.aspx?ID=6182) and ERWAT (East Rand Water Care Company, https://erwat.co.za/) highlighted interest in the sludge remediation and hemp elements of the work. The City of Cape Town expressed interest in the work, and a Memorandum of Agreement was granted for the Project out of initial consultations with the City of Cape Town's Department of Water and Sanitation, to inform the socio-economic study. Sven Sotemann (Head of CoCT Development and Infrastructure Planning: Wastewater Branch, Water and Sanitation Department, Water and Waste Directorate) and Swastika Surjlal-Naicker (Head of CoCT Analytical Laboratory: Scientific Services Branch – Water and Sanitation Department, R&D) have been in consultations. Isabel du Toit (Chief Agricultural Food and Quarantine Technician, Inspection Services Directorate, Department of Agriculture, Land Reform and Rural Development) facilitated the hemp permit (APPENDIX I) and regulatory visits. Carbon dynamics are being studied in partnership with Ryan Pereira (Heriot-Watt University, Edinburgh), and Prof Thabo Nkambule (UNISA Institute for Nanotechnology and Water Sustainability) has also expressed interest in partnering with us, locally. Finally, a Biomedical Engineering (MEngSc) student is being co-supervised by Dr Wendy Stone and Prof Wille Perold (SU Dept of Electrical and Electronic Engineering), developing an ecotoxicity sensor for in-field monitoring of pollutant remediation. Cancer risks in human cell lines are being added to local sludge ecotoxicology assays (in partnership with Dr Manisha du Plessis and Dr Carla Fourie, SU Dept of Physiology). The SU Law Faculty and the School of Public Leadership are targeted to support future work in broadening crop certification to include waste circularity, allowing farmers who invest in waste circularity to tap into the 'green', eco-conscious market. This could provide economic incentive and influence public perception.

Table 1.6. Summary of stakeholder roles, in research areas that are initiated with established partners.

Institution	Collaborator	Research Element
Philippi Economic Development Initiative	Roger Jaques, Thomas Swana	Farmer engagement, composting beneficiation.
Agricultural Research Council	Ms Livhuwani Masola, Dr Aart-Jan Verschoor.	Economic assessments.
Department of Physiology, Stellenbosch University, SA	Prof Anna-Mart Engelbrecht, Dr Manisha du Plessis, Dr Carla Fourie	Ecotoxicology and cancer assays.
Department of Electrical and Electronic Engineering, Stellenbosch University, SA	Prof Willie Perold	In-field ecotoxicology sensor development.
Marine and Earth Sciences, Lyell Institute, Heriot-Watt University, Edinburgh, UK	Prof Ryan Pereira	Carbon dynamics.
Environmental Engineering, Durham University, UK	Prof Karen Johnson	Philosophy and sludge amendments.
City of Cape Town, Waste Directorate	Sven Sotemann	Economics and logistics

Thus, the case studies leading up to this work have provided snapshots of the bioremediation capacity of the soil, when receiving these co-amended water wastes. This work addresses some final questions necessary to launch full-scale field trials and stakeholder engagement.

These questions include:

- Groundwater Protection What is the eutrophication, pathogen and pollutant footprint of the water leachate passing through the soil, when sandy soil is amended with WTR and AD?
- Carbon Sequestration WTR is a sorptive amendment, minimising the mobility of phosphates and pollutants in sandy soils. The addition of sewage sludge to sandy soil enhances the carbon in the soil, however, the carbon is also mobile in this matrix. With its sportive surface, can WTR stabilise carbon in sandy soil?
- Seasonal, Repeat Amendments What is the risk of pollutant accumulation in repeat applications, and how does this inform sludge diversion rhythms to local farms?
- Composting Sewage sludge is beneficiated through composting. What are the benefits and limitations of composting with WTR as a co-amendment?
- Socio-Economics What are the theoretical economic and carbon impacts of diverting local sludges from linear waste disposal to the circular agricultural potential?
- Certification and the Green Market Is it feasible to include ‘circular waste’ as a sustainability certification, similar to organic certification, to promote market popularity? The use of wastes in soil will compete with the benefits of organic certification, but arguably promote an equally critical governance shift, in terms of sustainable urban management, which could be supported by certification.

Extensive local work already informs these questions (Table 7) and this study (WRC Project C2022/2023-00820).

Table 1.7. A list of relevant documents, for local stakeholders considering the agricultural application of sludges.

Source	Waste Stream	Relevance
Western Cape (South Africa) Department of Environmental Affairs and Development Planning (2016), accessed 01/02/2022 https://orasa.org.za/wp-content/uploads/2016/11/DEADP-organic-waste-landfill-ban-letter-July-2018.pdf	Organics	Landfill ban legislation
Herselman, J. E. (2013). Guidelines for the utilisation and disposal of water treatment residues. Water Research Commission of South Africa, Report No. TT 559/13.	WTR	Disposal guidelines (local)
Herselman, J. E., & Moodley, P. (2009). Guidelines for the Utilisation and Disposal of Wastewater Sludge. Water Research Commission of South Africa, Report No. TT 350/09.	Sewage Sludge	Disposal guidelines (local)
Tesfamarium EH, Annandale, JG, de Jager, P. C., Ogbazghi, Z., Malobane, M. E. & Mbetse, C. K. A. (2015) Quantifying the fertilizer value of wastewater sludge for agriculture. WRC Report 2131/1/15.	Sewage Sludge	Fertilizer value
Tesfamariam, E. H., Badza, T., Demana, T., Rapaledi J., & Annandale, J. G. (2018) Characterising municipal wastewater sludge for sustainable beneficial agricultural use. WRC Report TT 756/18.	Sewage Sludge	Characterisation

1.5 Objectives and scope

The project objectives were to explore the diversion of water and wastewater treatment sludges from landfill into agriculture. Objective 1 involved exploring the scientific risks and opportunities associated with this strategy, in two model scenarios: greenhouse lysimeter trials and composting. Objective 2 involved exploring the socio-economic risks and opportunities for this strategy, in three model scenarios: (a) a cost benefit analysis of a local waste diversion case study, (b) the carbon market, and (c) certification policies.

2 ENVIRONMENTAL RISKS AND DRIVERS FOR DIVERTING SLUDGE WASTES TO AGRICULTURE

The hypothesis tested in this pot trial focused primarily on groundwater protection when co-amending sandy soils with water and wastewater sludge:

In comparison to individual anaerobic digestate (AD) amendment, the co-amendment of water treatment residual (WTR) and AD will influence

(a) water infiltration capacity of hydrophobic sand,
(b) carbon sequestration,
(c) pollutant immobilisation (eutrophication and ecotoxicity remediation), and
(d) soil fertility and crop biomass.

This greenhouse study investigated lysimeters, designed to capture groundwater flow-through for continuous monitoring. The treatments (Figure 11a) included control sand, and the same sand amended with (2) 2.5% (m/m_{dw}) WTR, (3) 2.5% AD, and (4) 2.5% WTR + 2.5% AD. Another control was fertilized at standard agricultural rates (Fertilizer Society of South Africa). In the co-amended microcosm, 2.5% of the sand was replaced by WTR, with the theoretical clay-like capacity to stabilise carbon and pollutants and improve the potential for environmental remediation. The risks and benefits were monitored over two growing seasons (Figure 11b), partitioning the system into three phases: crops, soil matrix, and groundwater leachate.

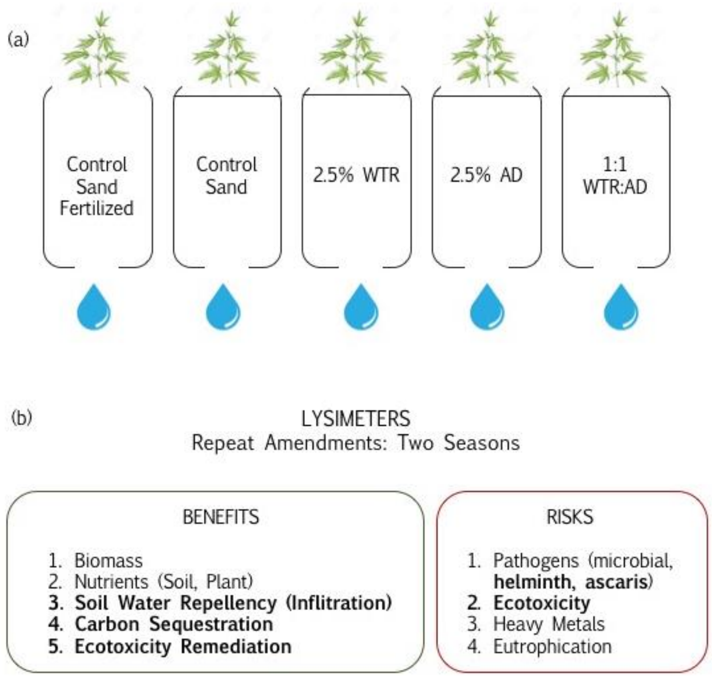

Figure 2.1. Visual summary of (a) lysimeter treatments and (b) experimental parameters, designed to investigate groundwater protection and carbon sequestration when amending nutrient-poor sandy soils with water and wastewater sludge for agricultural productivity via hemp growth. Parameters emphasized in bold are more novel in this context, and the focus of optimization in this study.

2.1 Soil and sludge collection

The sandy soil plant growth medium – used in the greenhouse lysimeters and the pre-trial laboratory incubations – is an aeolian nutrient-poor sand. It was collected in September 2022 from an area that represents the widespread local Cenozoic Sandveld Group sediments (-33.9662816, 18.7211779; Roberts et al., 2006). The water treatment residual was collected from a nearby local water treatment plant, producing alum ($Al_2(SO_4)_3$) sludge (Al-WTR) that is processed in settling dams (Figure 12). The sludge was collected directly from the dams, air dried until 15% water content (7 days, 30°C), crushed, sieved (2 mm) and stored for use. The water source is the Theewaterskloof dam, and the plant uses liming and activated carbon in the water treatment process (Table 8; Steytler et al., 2021). For ecotoxicity and carbon sequestration studies, this sludge was compared to a local iron-based ($Fe_2(SO_4)_3$) water treatment plant (Table 8), collected and processed similarly. In this plant, the water is also sourced from the Steenbras and Palmiet dams, both with a higher organic load than the Theewaterskloof dam. Thus, the process involves more vigorous flocculation, including the stronger iron flocculent, as well as lime, activated carbon and a polyacrylamide flocculent. The sludge is collected daily for landfilling after mechanical dewatering in a centrifuge.

Anaerobic digestate (AD) was collected from a local wastewater treatment works, employing non-thermal (mesophilic) digestion. Sludge is settled in beds after 3-stage ambient digestion, where plant growth (even vegetables, like tomatoes) often takes place spontaneously (Figure 12a). The beds are emptied into heaps (Figure 12b) after some weeks, and then transported to landfill. The beds and mounds are sprayed with Avi-Sipermetrin, a common agricultural pesticide, to control flies and worms. The active ingredient is cypermethrin. It is ecotoxic but not persistent and is easily degraded in soils, accelerated by radiation, oxygen and water (Nema and Bhargava, 2018). The sludge was pasteurised in 5 L glass beakers, filled with sludge (±3 kg wet weight) and saturated with tap water. A pasteurisation protocol was amended from Arthurson (2008), Mocé-Llivina et al. (2003) and Romdhana et al. (2009), with higher pasteurisation periods and temperatures accommodate for high volumes and pathogen loads. Volumes (0.5 kg versus 3 kg wet weight) and heating rhythms were adjusted until pathogens were limited within national guidelines. The final protocol included 75°C for 2 h (3 kg, saturated), with subsequent incubation at ambient temperatures (24 h). The ambient temperatures activate endospores become vegetative (temperature-vulnerable) before heating again, repeated three times per batch. This procedure is neither practically nor financially feasible but is only necessary because the digestion is mesophilic. Thermophilic anaerobic digestion produces sludge that is well within land application guidelines and needs no further stabilisation other than air drying. The Cape Flats anaerobic digester has recently malfunctioned, and there are no other thermophilic digesters in the Cape Town area. The City plans for new thermal sludge treatment facilities within the next 5 years. After pasteurisation, the sludge was air dried (4 days, 30°C), until it had a moisture content similar to commercial compost (>80% water per g_{dw}). Optimisation in this study has shown that complete drying of organic carbon increases hydrophobicity. Thus, although it decreases the storage lifespan, the organic sludges were all dried to a constant water content and characterised and used within two weeks.

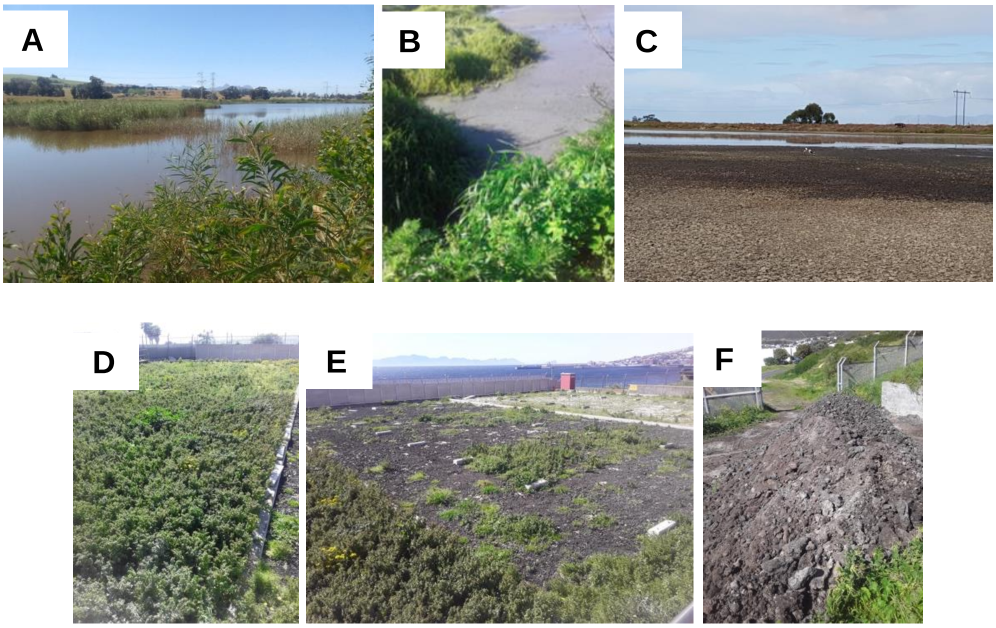

Figure 2.2. Settling of Al-WTR sludge in dams (a) that evaporate (b) until they are dry enough to transport (c). Anaerobic digestate is settled in beds, that germinate with weeds (d), which are removed (e) and the sludge transferred to mounds (f) for transportation. In both cases, there is already natural phyto- and mycorrhizal activity (dam reeds or weeds), prior to discarding the sludges in landfill.

Table 2.1. Summary of the treatment processes at Blackheath and Faure water treatment plants (WTP)[a].

	Blackheath WTP	Faure WTP
Plant Influent Source	Theewaterskloof dam	Theewaterskloof dam, Steenbras dam, Palmiet river system
Treatment Steps	Lime and activated carbon	Lime, activated carbon and polyacrylamide
Coagulant type	$Al_2(SO_4)_3$	$Fe_2(SO_4)_3$
Residuals Treatment	Non-mechanical dewatering	Centrifuge
Residuals handling	On-site pond storage (3-5 years) and landfilling	Direct landfilling

[a]Table modified after Gibbons & Gagnon (2011)

2.2 Sludge pathogen screening

The project was based on three years of preceding work on AD (Section 1.3.1 and 1.3.2) sourced from the Cape Flats Zeekoeivlei Wastewater Treatment Works (WWTW; Cape Town, South Africa). However, as the project launched, the anaerobic digester failed and was decommissioned. The City of Cape Town is planning three large new thermal digesters, forecast to be built within the next five years, and thus are not investing in maintaining old digesters. After extensive engagement with local stakeholders (Sven Sotemann; Head of CoCT Development and Infrastructure Planning: Wastewater Branch, Water and Sanitation Department, Water and Waste Directorate and local WWTW managers), it became clear that there are no alternative thermal digesters in the area. Thus, pathogen loads – pre- and post-pasteurisation – of activated sludges and mesophilic anaerobic digestate were compared to local guidelines for land application. Sludges were collected and analysed pre- and post-pasteurisation as described above. Primary activated sludge was collected from three local plants (P1-P3, for anonymity), secondary activated sludge from four local plants (S1-S4) and one mesophilic AD, and compared to the AD from the decommissioned Cape Flats digester (CF-AD).

The sludge compliance system, designed by Snyman and Herselman (2006), classifies sludge for land application according to three classes (microbial, stability and pollution), divided into three quality levels (microbial: A, B and C; stability: 1, 2, 3; pollution: a, b, c; Table 9). Stability and pollution are not considered critical risk factors in this study, since they are not an immediate risk for students handling the sludge, but rather a long-term risk, evaluated during these growth trials. However, microbial pathogens are a handling risk, monitored and applied outside a Biosafety Level 2 laboratory. Thus, demonstrating pathogen concentrations for safe handling before greenhouse trials was a priority.

Table 2.2. Sludge classification table, excerpted from the South African national guidelines for the land application of sludge (Snyman and Herselman, 2006).

Microbial class	A	B	C
Stability class	1	2	3
Pollution class	a	b	c

As described above (Section 1.3.2), the Microbial Class in the South African classification system mirrors the USEPA system of two separate classifications (USEPA, 1994) for unrestricted and restricted use (South African terminology) or Exceptional Quality (EQ) and Non-EQ sludges (EPA terminology). Faecal coliforms and helminth ova are considered the critical parameters for microbial sludge classification (Table 10). These guidelines use culture methods for microbial analysis. In this study, sludges were evaluated according to these guidelines, and expanded to include Colilert-18 trays (IDEXX, Johannesburg, South Africa) for statistical Most Probable Number (MPN) evaluations of E. coli, faecal coliforms and total coliforms. This is a standardised and certified method with stronger statistical power, utilised by the Scientific Services of the City of Cape Town.

Table 2.3. Microbial sludge classification table, excerpted from the South African national guidelines for the land application of sludge (Snyman and Herselman, 2006), with only one category for the most stringent limited-use quality.

Microbiological class	Unrestricted use quality		General use quality		Limited use quality
	A		B		C
	Target value	Maximum permissible value	Target value	Maximum permissible value	
Faecal coliform (CFU/g_{dry})	< 1 000 (5 log reduction)	10 000 (4 log reduction)	< $1x10^6$ (2 log reduction)	$1x10^7$ (1 log reduction)	> $1x10^7$ (no reduction)
Helminth ova (Viable ova/g_{dry})	< 0.25 (or one ova/4g)	1	< 1	4	> 4
Compliance requirements					
Requirements for classification purposes (Minimum 3 samples)	All the samples submitted for classification purposes must comply with these requirements	Not applicable	Two of the three samples submitted for classification purposes must comply with these requirements	The sample that failed may not exceed the Minimum Permissible Value	Not applicable

In addition, previous studies of sandy soils amended with these sludges showed longer persistence of Salmonella and Shigella than the traditional coliform indicators (Section 1.3.2), suggesting a wider suite of pathogens during land application (Stone et al., 2021). Thus, pathogens were evaluated via culturing media as described in Stone et al. (2021; Table 11). Briefly, characterisation involved cell matrix-soil disruption and plating on selective media (Table 11) and Colilert-18 Quanti-trays, incubated (24 h) at 37 ℃ (total coliforms yellow, E. coli fluorescent) and 45 ℃ (faecal coliforms). Colony forming units were determined by vortexing samples (1:10 $solid_{wet}$:liquid ratio) for 3 min in phosphate-buffered saline with Tween20 (8 mM Na_2HPO_4, 0.15 M NaCl, 2 mM KH_2PO_4, 3 mM KCl, 0.5% Tween20, pH 7.4, to a total liquid volume of 15 ml) and 100 µl of a dilution series plated on the respective media (Table 11). Prokaryotes and eukaryotes were incubated at 26 ℃ (72 h), whereas pathogenic species (faecal and total coliforms, enterococci and Salmonella and Shigella) were quantified after incubation at 37 ℃ (24 h). Moisture content of the sludge was determined (105 ℃, 24 h) and cell concentrations were calculated per gram dry weight.

Table 2.4. Selective media components, for isolating general and pathogenic microbial populations, excerpted from Stone et al. (2021).

Organisms	Media
Total prokaryotes	Tryptic soy agar (tryptic soy broth, 3 g L^{-1}; agar, 15 g L^{-1})
Total eukaryotes	Yeast malt agar (peptone, 5 g L^{-1}; yeast extract, 3 g L−1 ; malt extract, 3 g L^{-1}; dextrose, 10 g L^{-1}; agar, 15 g L^{-1})
Total coliforms, E. coli	MacConkey agar (MacConkey-bouillon broth, 40 g L^{-1}; agar, 15 g L^{-1})
Fecal coliforms	m-FC agar (52 g L^{-1}; 10 ml 1% Rosolic acid in 0.2 N NaOH; boil)
Enterococci	Enterococcus selective agar (42 g L^{-1}; boil)
Salmonella, Shigella	SS agar (60 g L^{-1}; boil)

2.3 Parasite screening

Parasites were analysed in baseline soil and sludge characterisation activities (sandy soil, WTR and AD), as well as post-harvest in all soils after season one and season two. Soil samples were taken directly (100 g) and analysed for Ascaris ova (presence and viability) and helminth ova (presence and viability) using SANS241 methodology at a SANAS accredited laboratory (Umgeni Water, Durban, South Africa). Samples were in transit overnight without temperature control.

2.4 Monitoring parameters & schedule

As described in the Supplementary Material in Clarke et al. (2019), soil, compost and WTR were analysed for pH (in both 1 M KCl and water) in a 1:2.5 solid:deionized (DI) water suspension (Eutech pH700 Meter), electrical conductivity (EC) in a 1:5 solid:DI water suspension (Jenway 4510 Conductivity Meter) and total C and N through dry combustion (LECO and Elementae Vario Macro elemental analysers). Plant available P and K were extracted in Mehlich III (Mehlich, 1985) and Bray II extracts, respectively. Extracts were analysed colorimetrically for P (Kuo, 1996). Mineral nitrogen (NH_4^+-N and NO_3-N) was extracted (10 g solid:20 mL KCl (2 M), 1 h, 200 rpm). The samples were filtered (0.45 µm cellulose acetate) and analysed according to manufacturer's instructions with NO_3 (1.14773.0001) and NH_4 (1.006830001) Spectroquant kits, using barcoded standard curves (Merck Spectroquant Pharo 300 photometer). All kits were sourced from Merck (Modderfontein, South Africa). Dissolved organic carbon (DOC) was extracted [dH2O; 1:10 (w/v); 24 h, 200 rpm], centrifuged (10 000 rpm, 10 min) and filtered (0.45µm, cellulose acetate). A TOC analyser was used to quantify DOC, TOC and TIC in the filtrates (aj-Analyzer multi N/C 3100; Analytik Jena Multi N/C). The bioavailable fraction of trace elements (TE) were measured in NH_4NO_3 according to the DIN 19730 procedure (Herselman, 2013). Extracts were analysed for metals using inductively coupled plasma mass spectrometry (ICP-MS) with an Agilent 8800 QQQ ICP-MS. Microwave extraction was used to quantify total heavy metals in the soil. Microbiology was evaluated as described for sludge pathogen screening (Section 3.2). All parameters were analysed in triplicate samples.

These soils and sludges were characterised and co-amended in laboratory co-incubations for method development. Methods for measuring soil-water dynamics, carbon sequestration and ecotoxicity were laboratory-optimised prior to launching lysimeter trials with the same soils and sludges.

2.5 Laboratory co-incubations and method development

2.5.1 Methods

2.5.1.1 Soil-water dynamics

As described in Stone et al. (2024a), soil water repellency was measured with a Mini Disk Infiltrometer (MDI) from Decagon Group, Model S (METER Group Inc., 2020), with suction set at 2 cm (Alagna et al., 2017; Kessaissia & Mazour, 2019). Triplicate samples, comprising 450 g each, were prepared on a m_{dw}/m_{dw} % ratio, mixed to homogeneity and packed to 1.5 g/cm^3 in 800 ml glass beakers. The volume-to-surface area ratio was selected as the lowest volume with a corresponding surface area large enough to prevent preferential water flow at the soil-beaker interface (beaker/soil surface diameter at least double the infiltrometer diameter). The method quantifies the decrease in water volume in the infiltrometer at 5s time intervals, as it filters through the soil matrix. Municipal tap water was used to represent field conditions. These laboratory-based proof-of-concept experiments compared WTR, AD and co-amendments (1:1 WTR:AD) at 10% dry weight, in the sandy soil matrix.

2.5.1.2 Carbon sequestration

Carbon sequestration was investigated at varying amendments (Table 12), to explore the impact of WTR on carbon retention in sandy soils. This is part of the scope of a PhD (Ms Noxolo Sweetness Lukashe), but the optimisation relevant to measuring carbon sequestration in this lysimeter trial design is described here.

Table 2.5. Amendments and application rates to investigate carbon dynamics in laboratory incubation studies.

Treatment no.	Treatment name	Substrate	Application rate
1	Sand	Sand only	
2	Al-WTR 5	Sand and Al-WTR	5%
3	Fe-WTR 5	Sand and Fe-WTR	5%
4	AD 5	Sand and AD sludge	5%
5	1Al-WTR:1AD	Sand, Al-WTR and AD sludge	5% WTR and 5% sludge
6	2al-WTR:1AD	Sand, Al-WTR and AD sludge	10% WTR and 5% sludge
7	1Fe-WTR: 1AD	Sand, Fe-WTR and AD sludge	5% WTR and 5% sludge
8	2Fe-WTR:1AD	Sand, Fe-WTR and AD sludge	10% WTR and 5% sludge

Briefly, 100 g of the sandy soil (amendments according to Table 12) was incubated (ambient temperature, 132 days) in 1 L glass jars in triplicate, moistened to 60% water holding capacity. Nutrient mineralisation was measured with bi-weekly assays, destructively sampled. All parameters were measured pre- and post-incubation, except respiration, measured 10 times during the experiment.
Respiration was measured with alkali CO_2 traps, according to Hopkins (2006). Soil was incubated in air-tight jars with 10 mL of 1 M NaOH (gas trap) and 10 mL distilled water for relative humidity, in glass vials. Empty jars with only the two vials were included as blank data. After adding the vials, the jars were sealed and stored in the dark at ambient temperature. On days 3, 7, 14, 21, 28, 35, 42, 72, 102 and 132, the NaOH was back titrated with 0.5 M HCl after precipitation with $BaCl_2$ (2 mL). The CO_2 in the traps was calculated according to Equations 1 and 2.

$$CO_2\ (mol\ C)\ =\ 0.5 * [\ ((V_{NaOH}\ x\ C_{NaOH})\ /1000) - ((V_{HCl}\ *\ C_{HCl})/1000) \quad \text{.........................Eq. 1.}$$

where V_{NaOH} is the initial volume of NaOH (mL), C_{NaOH} is the initial molar concentration of NaOH, V_{HCl} is the volume of HCl used in the titration (mL), and C_{HCl} is the molar concentration of HCl used in the titration.

$$C_{min}\ (mol\ C.g^{-1}.h^{-1})\ =\ (CO_2)/[soil\ mass\ (g)\ x\ time\ (h)] \quad \text{...Eq. 2.}$$

The quantification of carbon dynamics was optimised with laboratory incubations. Dissolved organic carbon (DOC) was extracted [dH_2O; 1:10 (w/v); 24 h, 200 rpm]. The sample was subsequently centrifuged (10 000 rpm, 10 minutes) and filtered (0.45µm, cellulose acetate). A TOC analyser was used to quantify DOC in the filtrates (aj-Analyzer multi N/C 3100; Analytik Jena Multi N/C).

The percentage C lost for each treatment was calculated according to Equation 3.

$$\%C\ lost\ =\ \left((Cumulative\ CO_2 + DOC_f) * \frac{100}{TC_i}\right) \quad \text{...Eq. 3.}$$

where
TC_i = Initial Total Carbon (start of incubations)
Cumulative CO_2 = Respiration
DOC_f = Final DOC (after incubations)

2.5.1.3 Ecotoxicity

2.5.1.3.1 Sample preparation

An objective of this study is to compare the full ecotoxicity footprint of (1) leachate, and (2) soil extracts, to understand the impact of these sludge waste amendments on groundwater protection. This is submitted for publication in Waste Management (Stone et al., 2024b).
Most water and soil studies analyse micropollutants using liquid chromatography and mass spectrometry (LC-MS). However, there are thousands of substances and their innumerable by-products (Ghirrardini et al., 2020), narrowed down to 45 priority substances by the EU Water Directive Framework (2022). Their interactions and degradation by-products become almost impossibly complex. Thus, this work did not measure micropollutants. Rather, it focused on the environmental effects of the suite of micropollutants introduced by the amendment of sandy soils with AD, and the interaction with WTR as a co-amendment. This was evaluated by exposing soil and sludge extracts to several standard ecotoxiticy assays, at increasing trophic levels (Table 13). The hypothesis was that the clay-like WTR would immobilise some of the pollutants that are commonly associated with sewage sludge, decreasing the toxicity footprint extracted from the sewage sludge, upon co-incubation. This was evaluated in laboratory incubations prior to the greenhouse trial.

Table 2.6. Ecotoxicological assays selected to compare the immobilization and remediation effects of extracts from soils co-amended with AD and WTR, as well as mycorrhizal remediation. Here, the assays are compared according to their utility in standard protocols (Microtox, Xenometrix), as well as the quantification techniques, and references.

Assays	Commercial Standardised Alternative	Detection	References
Bacteria *Allivibrio fischeri*	Yes (Microtox)	Biolouminescence (Tecan microplate reader)	Abbas et al. (2018); ISO 11348-3 (2007)
Algae Environmental strain	Yes (Microtox)	Fluorescence (Tecan microplate reader)	Suzuki et al. (2018); OECD 204 (2004); ISO 8692 (2004)
Planktonic Crustaceans Daphnia	Yes (Microtox)	Microscopy and counting	Baun et al. (2008); OECD 201 (2006) ISO 6341 (1996)
Phytotoxicity Hemp, Grass and Corn	No	Seed germination	Hu et al. (2018); Islam et al. (2021)
Human/Mammalian Yeast Estrogen Screen	Yes (Xenometrix, not accessible locally yet)	Colorimetric (540 nm)	Murck et al. (2002); Archer et al. (2020)
Cancer Cell Lines Mitochondrial Reductive Capacity	No	Colorimetric (570nm)	Du Plessis et al. (2022); van der Merwe et al. (2021)

To optimise the assays, and determine nuances such as storage effects, lowest detection limits, pH control, correlations and variation, an array of samples were tested, as well as dose dependent exposures to a micropollutant standard curve and common reference toxicants.

2.5.1.3.2 Incubation and extraction

2.5.1.3.2.1 Direct extracts

All soil and sludge samples were incubated, individually or combined (Table 14; 26 ºC, 20 h, 250 rpm), in non-sterile tap water (1:10 solid$_{dw}$:liquid ratio). After incubation, samples were centrifuged (6000 rpm, 10 min), filtered (5 µm glass fibre filter, followed by a 0.22 µm cellulose acetate filter), and the filter-sterilised extract exposed to the array of ecotoxicological assays (Table 13). The hypothesis, evaluating the immobilisation of AD-associated pollutants by WTR, was assessed by adding AD at a 1:1 ratio with Fe-WTR and Al-WTR, in comparison to adding AD at a 1:1 ratio with sand. Incubations and extractions were always freshly prepared, as storage significantly affected some of the ecotoxicity results (data not shown). Electrical conductivity and pH of the extracts were monitored prior to ecotoxicity assays. All samples were incubated in triplicate.

Table 2.7. Incubation ratios for extracting the ecotoxicity footprint in laboratory-scale studies assessing the potential immobilisation of AD-associated ecotoxicity by co-incubating with WTR. Soils were incubated (17 h, ambient temperature, 250 rpm) at the following ratios, prior to exposing to a battery of ecotoxicity assays.

Treatment	Sand (% g_{dw})	WTR (% g_{dw})	AD (% g_{dw})	Compost (% g_{dw})	Incubation Ratio (Solid:Liquid)
Control (Tap Water)[a]	0	0	0	0	0:100
Polyelectrolyte (Tap Water)[a]	0	0	0	0	0:100
Sand	100	0	0	0	1:10
Compost	0	0	0	100	1:10
Fe-WTR	0	100	0	0	1:10
Al-WTR	0	100	0	0	1:10
Anaerobic digestate (AD)	0	0	100	0	1:10
Fe-WTR + Sand (1:1)	50	50	0	0	1:10
Al-WTR + Sand (1:1)	50	50	0	0	1:10
Fe-WTR + AD (1:1)	0	50	50	0	1:10
Al-WTR + AD (1:1)	0	50	50	0	1:10

[a]Liquids exposed to the same incubation and filtration as the samples, prior to ecotoxicology assays.

A tap water (sample extractant) control was incubated, centrifuged and filtered according to sample processing. The polyelectrolyte is a polyacrylamide flocculent (RFLOC) used in the water treatment process. It was added to the ecotoxicology assays at 10X the final concentration considered optimal for water treatment, 0.1% (Radoiu et al., 2004). The polyelectrolyte was added to tap water and processed according to the control and soil/sludge samples, but without filtration as the gel-like substance did not pass through filters. The sand, Fe-WTR, Al-WTR and AD were collected and processed as described in Section 3.1, before being incubated individually and in co-amendments (Table 14). The incubations were with gentle agitation (250rpm) in order to increase surface interactions. The co-amendments were added at 50% of the individual amendments, to maintain a 1:10 extraction ratio (Figure 7). Where necessary for comparative conclusions, final data was compared to 50% projection data, termed the 'Predicted Dilution Effect' (Figure 13). All samples were prepared in triplicate before incubation and filtration.

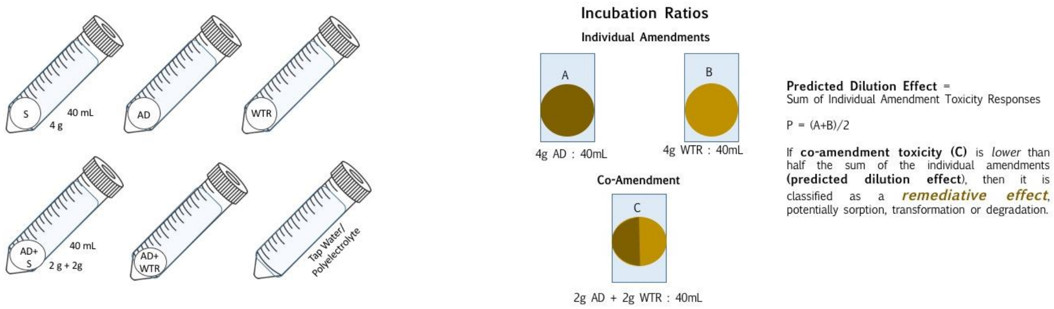

Figure 2.3. Soil (S) and sludge (AD and WTR) samples were incubated (24 h, ambient, 250 rpm) at a 1:10 ratio in microcentrifuge tubes (direct extracts, 40 mL) or glass laboratory bottles (400 mL). Tap water was incubated, centrifuged and filtered according to the same process, as a negative control. Polyelectrolyte (polyacrylamide flocculent, 0.1%) was incubated in the same way without filtering. the Predicted Dilution Effect, which compensated for the necessary halving of the co-amendment masses, to keep mass:volume ratios consistent. This describes the calculations used to classify if the co-amendment caused remediative or cumulative effects.

2.5.1.3.2.2 Solid phase extraction

Environmental samples are often concentrated for ecotoxicity exposure. Extracts (300 mL), prepared as described above, were concentrated with Solid Phase Extraction (Petrie et al., 2016). Oasis HLB 6 cc extraction cartridges were conditioned with 4 mL of MeOH, followed by 4 mL of ddH_2O (<1 mL/min). Filtered samples (0.7 µm glass fibre filters) were passed over the cartridge (5 mL/min), columns dried completely, and eluted (6 mL MeOH, gravity) into 10 mL glass test tubes. The eluted samples were dried under nitrogen and reconstituted (600 µL MeOH).

2.5.1.3.2.3 Standard curves and micropollutant cocktail

The measurable response of each assay to environmental pollutant challenges was evaluated with two standard curves: (1) a reference toxicant common in literature, and (2) a micropollutant cocktail of nine chemicals with ecotoxic effects that have been measured in local waters, at 500X the concentration of those in the environment to represent the concentration during solid phase extraction in these studies (Table 15, Archer et al., 2021). A standard curve (five points, 10-fold dilutions) of this cocktail was exposed to each assay in triplicate, where feasible. These pollutants were chosen to represent a wide array of sources, including anti-convulsants (carbamazepine), non-steroidal anti-inflammatory drugs (NSAIDs, diclofenac and naproxen), anti-corrosives (benzotriazole), antibiotics (sulfamethoxazole), leachates from plastic manufacturing (bisphenol A), agricultural pesticides (atrazine) and analgesic and antipyretic pain medications (acetaminophen and ibuprofen), all of which have been shown to be persistent and have environmental impacts. Table 16 describes the full experimental design, visually.

Table 2.8. Micropollutant cocktail constituents and concentrations, in solution at 500X environmental concentrations, as commonly measured in ecotoxicity assays after solid phase extraction.

Assays	Reported[a] concentration (ng/L)	Assay concentration (mg/L)
Carbamazepine	3000	1500
Diclofenac	800	400
Napoxen	2000	1000
Benzotriazole	2500	1250
Sulfamethoxazole	10500	5250
Bisphenol A	400	200
Acetominophen	70000	35000
Ibuprofen	300	150
Atrazine	200	100

[a]Archer et al. (2021)

Table 2.9. A comparative layout of experimental assay design, including controls and treatments, exposure time, concentrations and dilutions series.

Bioassay	Exposure Time	Amendments	Extracts	Negative controls	Positive controls	Standard curve dilutions
Vibrio fischeri bioluminescence assay	20 min	Fe-WTR Al-WTR AD Fe-WTR +AD Al-WTR + AD AD + Sand Polyelectrolyte	500× 1×	Tap Water Fe-WTR+ Sand Al-WTR + Sand Sand	Micropollutant Cocktail (MC) [500×] $K_2Cr_2O_7$ [3.2 mg/L]	100% 50% 25% 12.5% 6.25%
Daphnia magna acute immobilization assay	24 h	Fe-WTR Al-WTR AD Fe-WTR +AD Al-WTR + AD AD + Sand Polyelectrolyte	500× 1×	Tap Water Fe-WTR+ Sand Al-WTR + Sand Sand	MC [500×] $K_2Cr_2O_7$ [3.2 mg/L]	100% 50% 25% 12.5% 6.25%
Closterium spp microalgae growth inhibition assay	72 h	Fe-WTR Al-WTR AD Fe-WTR +AD Al-WTR + AD AD + Sand Polyelectrolyte	500×	Tap Water Fe-WTR+ Sand Al-WTR + Sand Sand	MC [500×] $K_2Cr_2O_7$ [3.2 mg/L]	100% 56% 31% 18% 10%
Saccharomyces cerevisiae yeast estrogen screen	72 h	Fe-WTR Al-WTR AD Fe-WTR +AD Al-WTR + AD AD + Sand Polyelectrolyte	500× 3×	Tap Water Fe-WTR+ Sand Al-WTR + Sand Sand	MC [500×] E_2 [200 nM]	100% 10% 1% 0.1% 0.001% 0.001%
Phytotoxicity (Hemp, corn and grass germination assay)	7 days	Fe-WTR Al-WTR AD Fe-WTR +AD Al-WTR + AD AD + Sand Polyelectrolyte	1x	Tap Water Fe-WTR+ Sand Al-WTR + Sand Sand	None	None
MTT Assays Cancer cell lines	24 h	Fe-WTR AD Polyelectrolyte	1x	Tap Water Sand	None	None

2.5.1.3.3 *Aliivibrio fischeri* bioluminescence assay

Samples and standards for the bioluminescence assay were prepared as described above (Section 3.1.1.3.1.), and adjusted for pH (6.5-7.5, 0.1 M HCl and NaOH) and salinity (NaCl, 25%), before exposure to *A. fischeri* (OECD). The reference chemical toxicant, and positive control, was potassium dichromate ($K_2Cr_2O_7$, 0.32-3.2 mg/L standard curve).

Aliivibrio fischeri (NRRL B-11177) was cultured in Seawater Tryptone (5 g Tryptone, 3 g yeast extract, and 2x artificial seawater, ASW, pH 6.2), according to Christensen and Visick (2020). The ASW is commonly used to culture marine organisms (100 mM $MgSO_4$, 19.7 mM $CaCl_2$, 600 mM NaCl and 20.1 mM KCl). Cultures were exposed to the assay after overnight incubation (21-24 h, 26°C, 200 rpm). The luminescent is an indirect measure of cellular metabolism and viability, and the signal relies on quorum sensing (AinS induction). Thus, optical densities (OD_{600}) > 2 are ideal. Growth curves compared OD_{600} to cell concentration (CFU/mL) and bioluminescence on both Tecan and IVIS technologies, described below. After dilution in SWT to OD 0.9-1.1 (OECD), the aliquots were centrifuged (3000 rpm, 10 min), and pellets resuspended in a saline diluent (20 g NaCl; 2.035 g, $MgCl_2.6H_2O$; 0.3 g KCl). In each well of a black (Thermo Scientific™ Nunc MicroWell) 96-well optical-bottom assay plate, 105 µL saline diluent was added to 20 µL of the *A. fischeri* inoculum, the plate equilibrated (15 min, 15°C) and the L_0 (initial luminescence) recorded. The assay was prepared at 15°C with all reagents and consumables pre-cooled in a temperature-controlled incubator. The standard chemicals and samples were added in triplicate (125 µL direct extract per well or 10 µL concentrated extract in MeOH per well, evaporated and resuspended in 125 µL saline diluent), and bioluminescence and OD were measured after 20 minutes (L_{20}).

In vivo bioluminescence was measured in an IVIS® Spectrum system (Xenogen, Caliper Life Sciences), image processing converted pixels to relative light units (RLU's) using the Living Image@3.0 software (Xenogen). The IVIS was compared to the Spark® Multimode Microplate Reader (Tecan, Switzerland), measuring bioluminescence (RLU's) and OD. The luminescence intensity and absorbance values obtained were corrected relative to the blanks and the fk_{20} ratio and percentage inhibition for each well were calculated according to the following equations:

$fk_{20} = L_0/L_{20}$ ……………………………………………………………………………………Eq. 4.

$\% I = (L_0 - L_{20})/L_0 \times 100$……………………………………………………………………Eq. 5.

The change in bioluminescence (%I) for all samples was normalised over the average change in bioluminescence of the control.

2.5.1.3.4 Algae growth inhibition assay

According to a protocol modified from Suzuki et al. (2018), OECD 204 (2004) and ISO 8692 (2004), a stable environmental microalgae culture – maintained in a fish tank (50 L, tap water, aerated with a pump) – was harvested for the ecotoxicology assays. It was tentatively identified under the microscope as charophyte green microalgae of the genus *Closterium*. Chlorophyll a fluorescence was used as an indirect measure of algal growth and inhibition. The reference chemical toxicant, and positive control, was potassium dichromate ($K_2Cr_2O_7$, 0.18-1.8 mg/L standard curve). Harvesting entailed scraping and pipetting algal blooms from the sides of the tank into 2 mL microcentrifuge tubes, diluted with OECD Daphnia media (aerated) to between 10 000 and 40 000 relative fluorescence units (RFU's). The algal culture (200 µL) was pipetted into each well after the reference chemical standard curve, micropollutant standard curve and negative control, with five replicates of each sample/standard was applied and evaporated (10 µL, all suspended in MeOH and evaporated). Fluorescence (F_0) was measured (4x4 reads per well) at time zero on a Spark® Multimode Microplate Reader (Tecan, Switzerland), at an excitation of 440 nm and an emission of 685 nm. The plates were incubated (ambient temperature, 16:8 h sunlight:dark cycle, 72 h), and fluorescence measured again at 48 and 72 h (F_{48} and F_{72}, respectively. Percent inhibition relative to the control was calculated according to Eq 3.

$\% I = (F_0 - F_{48})/F_0 \times 100$ ………………………………………………………………Eq. 6.

2.5.1.3.5 *Daphnia* acute immobilisation assay

Daphnia magna were obtained from the environment (a local commercial dam producing water fleas for fish food), confirmed morphologically under the microscope, and cultured in a fish tank (16 h light, 8 h dark cycles) in the recommended OECD Daphnia media (0.294 mM $CaCl.2H_2O$, 0.12325 mM $MgSO_4$. $7H_2O$, 0.06475 mM $NaHCO_3$, 0.00575 mM KCl), pre-aerated for 15 min. Media was replenished weekly, and Daphnia fed with a pinch of Saccharomyces cerevisiae yeast flakes and blue-green algae (Spirulina), according to Lewinski et al. (2010) and Lavorgna et al. (2016). The acute immobilisation test evaluated the impact of the reference chemical standard curve, the micropollutant standard curve and samples on the health of water fleas in their environment (Alvarenga et al., 2008; OECD 202, 2004; ISO 6341, 1996). Neonates (5 animals per well), of no more than 24 h maturity, were incubated in 6 well titre plates, with 10 mL of OECD Daphnia media and 10 mL of the sample/toxicant (direct extracts). For concentrated extracts, 100 µL was added to each well, MeOH evaporated, and resuspended in 10 mL of OECD *Daphnia* media. In each well, 5 neonates were incubated (ambient temperature, 16:8 h sunlight:dark cycle, 48 h). Immobilisation was recorded if immobile for 15 s after gentle agitation. All samples were analysed in duplicate, in two separate experiments (at least 20 neonates in total). OECD Daphnia media was the negative control.

2.5.1.3.6 Yeast estrogen screen

A recombinant yeast strain of *Saccharomyces cerevisiae*, transfected with the human estrogen receptor (hER) gene and a plasmid containing an estrogen response element-linked lac-Z gene, was exposed to sample extractants and analysed for estrogenicity (Routledge and Sumpter, 1996). The colorimetric (yellow to red) assay measures an upregulation of the lac-Z reporter gene and the production of β-galactosidase, metabolising chlorophenol red galactopyranoside (CPRG) in the medium. The clone was obtained from Prof JH van Wyk, University of Stellenbosch.

The recombinant yeast strains were incubated (24 h, 130 rpm, 26°C) in an assay medium [45 mL minimal medium, 5 mL 20% Glucose solution, 1.25 mL L-Aspartic acid (4 mg/mL), 0.5 mL Vitamin solution, 0.4 mL L-Threonine (24 mg/mL), and 0.125 mL Copper(II)Sulphate solution (0.319 mg/mL)] prior to assay exposure (Archer et al., 2020).

The reference chemical (positive control) was a 17β-estradiol standard curve (E_2; CAS 50-28-2; Sigma), of 12 serial dilutions ranging from 1.3 to 2724.0 ng/L. Blank wells contained only the assay medium. Serial dilutions of the samples concentrated with SPE and reconstituted in MeOH were prepared, and 300 µL of each dilution was transferred to a sterile 96 well flat bottom plate (Costar, 3370, Sigma) and allowed to evaporate. After evaporation, the yeast culture was added (100 µL) as well as 100 µL of assay medium containing 0.5 mL of CPRG (10 mg/mL), and incubated (30°C, dark). Absorbance (570 nm, CPRG metabolism; 620 nm, cytotoxicity) was measured (BioRad), based on the development of the E_2 standard curve (± 72 h). Direct extracts were added at 50 µL, to 50 µL of a 2X concentrated assay medium with CPRG. The inhibition or stimulation of estrogenicity by samples (soil or sludge extracts) was quantified as a percentage of E_2-max in the standard curve.

2.5.1.3.7 Cancer assays

Cancer indicators were measured by collaborators at the SU Dept of Physiology (Dr Manisha du Plessis and Dr Carla Fourie, under Prof Anna-Mart Engelbrecht). Barring extract preparation and reference chemicals, this was not funded by this work, but feeds into the study. Since this is their publication, I will briefly refer to methods that they have previously published, and only include three graphs that corroborate the ecotoxicity data in this study. We will co-publish this article within 2022, and more details will be included then.

Briefly, direct extracts of Fe-WTR, AD, and Fe-WTR+AD, as well as the polyelectrolyte, were exposed to cancer and healthy cell lines, and mitochondrial reductive capacity, relative to the control, was measured as an indicator of the activity of the cells stimulated by the toxin (Fourie et al., 2022; van der Merwe et al., 2021). Activity was measured with the MTT colorimetric assay (Abs_{570nm}), detecting mitochondrial dehydrogenase. Samples are measured in triplicate wells per triplicate biological repeat. To evaluate the effect of samples on breast cancer cell lines, samples were exposed to (1) the control non-malignant human breast epithelial cell line, MCF12A, as well as two human breast cancer cell lines, (2) MCF7 and (3) MDA-MB-231. Two human colon cancer cell lines were exposed to sample extracts, namely Caco-2 (colorectal adenocarcinoma) and HCT116 (colorectal carcinoma). Cells were exposed to samples (10X dilution of direct extracts) for 24 h.

2.5.1.3.8 Phytotoxicity

Sample extracts, prepared as described above, were used to germinate seeds from multiple plant species relevant to this study, according to a modified protocol (Hu et al., 2018; Islam et al., 2021). Seed species included two hemp (Cannabis sativa L.) strains, SAPA (SAPA Valley landrace) and SABL (Chinese broadleaf hemp), representative of a textile crop; corn (Zea mays STAR7719 F1 hybrid) representative of a biofuel crop, and lawn grass (Dactyloctenium Australe) representing many local wastewater treatment plants, irrigating their landscapes with wastewater and fertilizing with sludge. Seeds (5 per round, in triplicate, 15 seeds per treatment) were germinated between cotton rounds (6.5 cm diameter) in petridishes, watered (10 mL) with the sample extractions (Table 14). The rounds were watered with the same extractants, and an extra 1 mL on day 3. Petridishes were incubated (seven days, ambient temperature, dark) and germination rate and seedling radical length were quantified daily.

2.5.1.3.9 Statistics

Data was assessed for normality with the Shapiro Wilkes test. Differences between treatments were assessed with one-way analysis of variation (ANOVA) ($p<0.05$) and a Tukey's honest significant difference post hoc test where necessary. Individual samples were compared with Student t-tests for independent means ($p<0.05$). Correlations were evaluated with regression coefficients (R^2), and all treatments were analysed in triplicate or more.

2.5.2 Results

2.5.2.1 Soil-water dynamics

The effects of phosphate-rich organic soil amendments like compost or sewage sludge (AD) on soil water dynamics – including saturated hydraulic conductivity (Kranz et al., 2020), soil water retention (Demir & Demir, 2019) and soil water repellency (hydrophobicity, Głąb, 2014) – are well characterised. The effects of WTR on saturated hydraulic conductivity (Park et al., 2010) and soil water retention (Kerr et al., 2022) have also been reported. However, the impact of WTR on soil water repellency has not been investigated, neither the effect of WTR-AD/WTR-compost co-amendment on these soil water parameters. This laboratory study investigated these gaps and the results have been published (Stone et al., 2024a). Although this study showed that crop drought resilience is more tightly correlated to the soil nutrients facilitated by co-amendment than soil-water parameters, WTR had a notable effect on soil water repellency (the inverse of unsaturated hydraulic conductivity, Figure 14). Thus, soil water repellency was a focus in the lysimeter trials. This proof-of-concept laboratory pre-trial study used high loading rates for between-treatment data resolution. Here, an insignificant but clear trend – decreasing at increasing loading rates – shows that AD reduces the unsaturated hydraulic conductivity (K) of the already water repellent receiving sandy soils by approximately half at a 10% loading rate (Figure 14). This is in support of literature showing that the surface layer of sandy soils is often hydrophobic (Francis et al., 2007) and that organic matter, originating from either living or decomposing microorganisms and plants, is correlated to increased hydrophobicity in soils (Bisdom et al., 1993; Doerr et al., 2000). WTR alleviates this. As an individual amendment, it improves the infiltration rate of unsaturated sandy soils three-fold. As a co-amendment, it alleviates the impact of AD on sandy soils, re-establishing the K of the control soil. This supports the 1:1 co-amendment of these materials, the waste pairing that has promoted crop growth in sandy soils via soil fertility (Clarke et al., 2019).

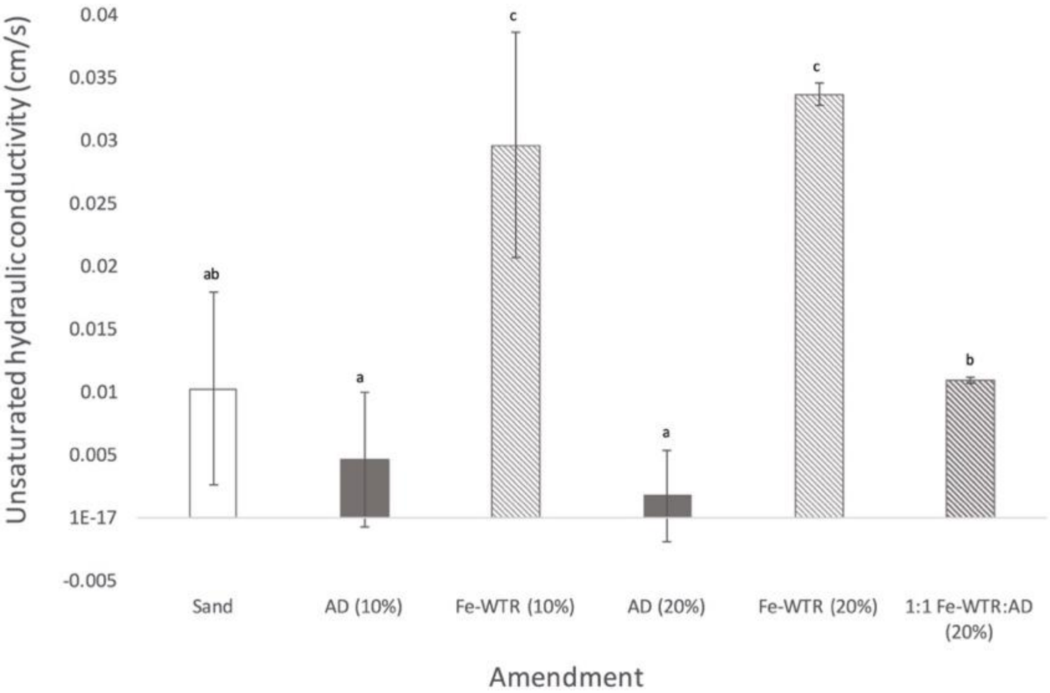

Figure 2.4. The effect of co-amending sandy soils with low unsaturated hydraulic conductivity (K) with WTR, AD and co-amendments. Error bars represent standard deviation of triplicate samples.

Soil hydrophobicity is important to agricultural productivity as it potentially limits plant growth (Doerr et al., 2000; Müller & Deurer, 2011; Ruthrof et al., 2019). Soil water repellency retards the infiltration of water into soil (King, 1981) causing it to pool on the surface. This increases surface runoff and soil erosion, creating preferential flow paths through the water repellent layer in the soil (Doerr et al., 2000). Thus, for crop growth, homogenous infiltration is preferable. In Australia, soil delving is common (Churchman et al., 2020; Schapel et al., 2018), decreasing sandy soil water repellency by lifting the buried clay layer to the surface. WTR is essentially flocculated dam sediment, which is enriched in clay compared to topsoils (Erskine et al., 2002). This study thus hypothesised that WTR, which has sorptive clay-like characteristics, might similarly alleviate soil water repellency. It had no significant impact on water holding capacity at these loading rates (data not shown) but did significantly decrease unsaturated hydraulic conductivity (Figure 14) upon co-amendment. This was further investigated in the greenhouse lysimeter trials.

2.5.2.2 Carbon sequestration

Land application of sewage sludge has the potential to sequester substantial amounts of C in the target soils (Torri et al., 2014). Long term C sequestration in soils usually requires the formation of C-mineral associations or the physical protection of soil organic C by microaggregates (Cotrufo and Lavallee, 2022), both of which rely on soil clay or silt fractions. For these reasons sandy soils usually display a limited C sequestration potential (Churchman et al., 2014) and the capacity of a sandy soil to sequester sewage borne C would be limited. Within the clay size fraction of soils, aluminum and iron oxides play a disproportionate role in complexing and stabilising organic C (Percival et al., 2000). Thus it was hypothesised that addition of Al and Fe oxide-containing WTRs to the sandy Cape Flats sandy soils would increase the stablity of sewage sludge borne-C, when the WTRs were co-applied with the AD. Laboratory incubation studies were set up where sandy soil was incubated with single and combined treatments of WTR (Al or Fe) and AD. The percentage of C lost from each treatment was calculated by adding together dissolved organic C (DOC) and the cumulative CO_2 lost due to respiration and expressing this as a percentage of the initial total C concentrations. The results show that the control sand and single AD amendment lost the highest proportion of C during the incubation (Figure 15). Addition of Fe and Al-WTRs reduced the proportion of C lost with the Al-WTR treatments retaining the highest proportion of C. Addition of 5% Al-WTR to the 5% AD amendment reduced the C loss by a factor of 3 compared to the single 5% AD amendment. This preliminary data suggests that co-ammendment of WTRs, especially Al-WTR, may increase the capacity of sandy soils to stabilise and potentially sequester sewage-borne C and will be further investigated in the lysimeter trial. As mentioned, the less stringent aluminium-based flocculent is employed in cleaner water systems, whereas the ferric oxyhydroxide is harnessed to flocculate the organic-rich Palmiet and Steenbras dams (Section 3.1, Table 8). Thus, once flocculated, the Fe-WTR itself is already rich in carbon, whereas the Al-WTR is hypothesised to have more carbon-binding capacity since the water it is used to treat is less rich in DOC. Carbon dynamics were further investigated in the greenhouse lysimeter trials. The pre-trial laboratory incubations have been submitted for publication by Noxolo Sweetness Lukashe (PhD) in Waste Management (January 2024).

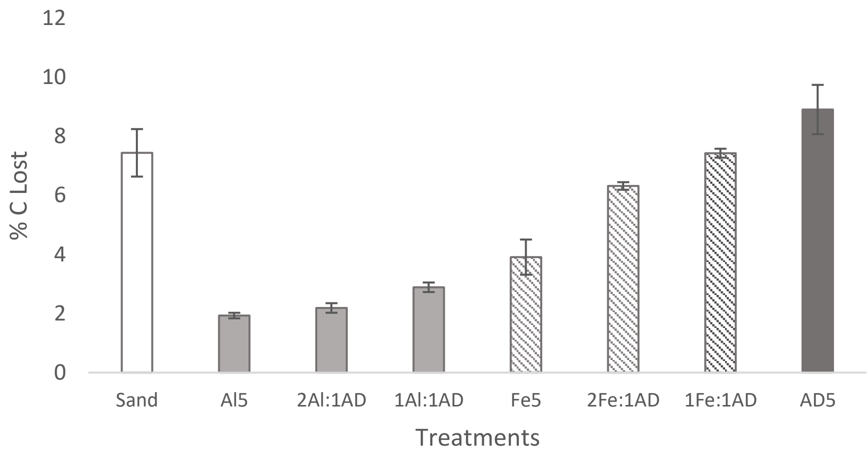

Figure 2.5. Carbon lost (Cumulative CO_2+ DOC) as a percentage of the initial Total C for treatments incubated in the laboratory for 3 months. Treatments: 5% Al-WTR (Al5), 10% Al-WTR + 5% AD (2Al:1AD), 5%Al-WTR+5%AD (1Al:1AD), 5%Fe-WTR (5Fe), 10% Fe-WTR + 5% AD (2Fe:1AD), 5%Fe-WTR+5%AD (1Fe:1AD).

2.5.2.3 Ecotoxicity

Extensive bioassay studies have evaluated the ecotoxicology of landfill leachate (Gosh et al., 2017), manures (Ghirardini et al., 2020) and biosolids (AD) for fertilisation (Gianakis et al., 2021). It is common practice to measure the micropollutant chemistry of leachates using LC-MS, attempting to quantify remediation by investigating the removal rates of individual chemicals. But integrated approaches for measuring ecotoxicity have been developed, using bioassays to evaluate the potential impact of leachates at multiple trophic levels (Kjeldsen et al., 2002; Thomas et al., 2009; Clarke et al., 2015; Baderna, Caloni & Benfenati, 2019). This environmental risk can have (1) a direct effect on the assays, studied with acute toxicity or immobilisation assays (Baun et al., 2008), (2) a chronic developmental effect (most notably on the endocrine system), monitored with metabolic and generational assays (Murck et al., 2002; Archer et al., 2020), and (3) an indirect effect, influencing other trophic levels by changing the dynamics in aquatic food chains (Thomas et al., 2009). These bio-assays are renowned for introducing higher variation than chemical tests, as biological organisms are living, creating a complex cascade of responses, depending on maturity, environment, and adaptation. In addition, these toxicity footprints are not limited to micropollutants, but are representative of the whole leachate, and elements as diverse as ammonia, alkalinity, heavy metals and pH all impact the trophic levels exposed to the water samples or soil extracts. Despite this added layer of complexity, they are still considered a critical addition to chemical tests, because they turn the focus from pollutant concentrations to pollutant impact (Thomas et al., 2009). It must be emphasised that in this study, risk coefficients are not the focus. Rather, it focuses on testing the hypothesis – that co-amendment of AD with WTR (which has sorptive clay-like characteristics) could immobilise sewage-borne micropollutants in the receiving sand, a matrix in which chemicals are highly mobile. This would minimize the ecotoxic effects of AD-borne micropollutants in groundwater leachate, and potentially facilitate longer exposure of the pollutants to the mycorrhizal hyphal system, renowned for bioremediation. The benefits and risks of co-amendments to sandy soil, in comparison to individual amendments (including compost as a common soil conditioner), was the primary focus. Thus, this study was designed to purely assess between-treatment comparative data for hypothesis testing. The results of this laboratory pre-study have been submitted for publication in Waste Management (Stone et al., 2024; submitted 30 January 2024).

A suite of assays was optimised for in-house monitoring of the lysimeter leachate, to assess the potential for (1) WTR co-amendment and (2) the hemp mycorrhizal root system to immobilise or transform the micropollutants widely reported in sewage (Archer et al., 2020; Citulski and Farahbakhsh, 2012). These included the Aliivibrio fischeri bioluminescence assay, the *Daphnia magna* acute immobilisation assay, and the *Closterium* spp microalgae growth inhibition assay. Multi-cellular organisms at higher trophic levels were also represented, with phytotoxicity assays (hemp, corn and grass germination rates); the yeast estrogen screen representing a vertebrate endocrine disruption pathway, transformed into Saccharomyces cerevisiae; and mitochondrial reductase MTT assays in human cancer cell lines. Direct extracts, representing the full chemical toxicity of the soil or sludge extract, were compared to extract pollutants immobilised on columns and released with methanol (500X concentrated). These Oasis HLB cartridges constitute a universal polymeric reversed-phase sorbent that was developed to extract and concentrate a range of acidic, basic, and neutral compounds (polar to moderately non-polar) from various matrices. They have been widely utilised to evaluate micropollutant concentrations (typically 3cc columns) and ecotoxicity (typically 6 cc columns) in environmental matrices (Archer et al., 2020; Chu and Metcalf, 2007). In addition, the polyelectrolyte flocculant used in the water treatment process was also assessed (10X standard concentrations) for ecotoxicity.

2.5.2.3.1 Growth curves, correlations and concentrations

Most of the bio-assays are straightforward, relying on measurements with linear correlations. However, the bioluminescence assay involved some optimisation, comparing detection limits for in-house bioluminescence detection technologies and also navigating the hormesis effect, described below. Standard growth curves were plotted (Figure 16) before comparing detection technologies. In particular, the IVIS® Spectrum system (Xenogen, Caliper Life Sciences), an in vivo imaging system, was compared to the Spark® Multimode Microplate Reader (Tecan, Switzerland), for measuring in vitro bioluminescence (RLU’s). This was evaluated by exposing an *A. fischeri* culture in late exponential phase to a dilution series of a micropollutant cocktail representing the pollutants detected in local rivers (Figure 17, Archer et al., 2020).

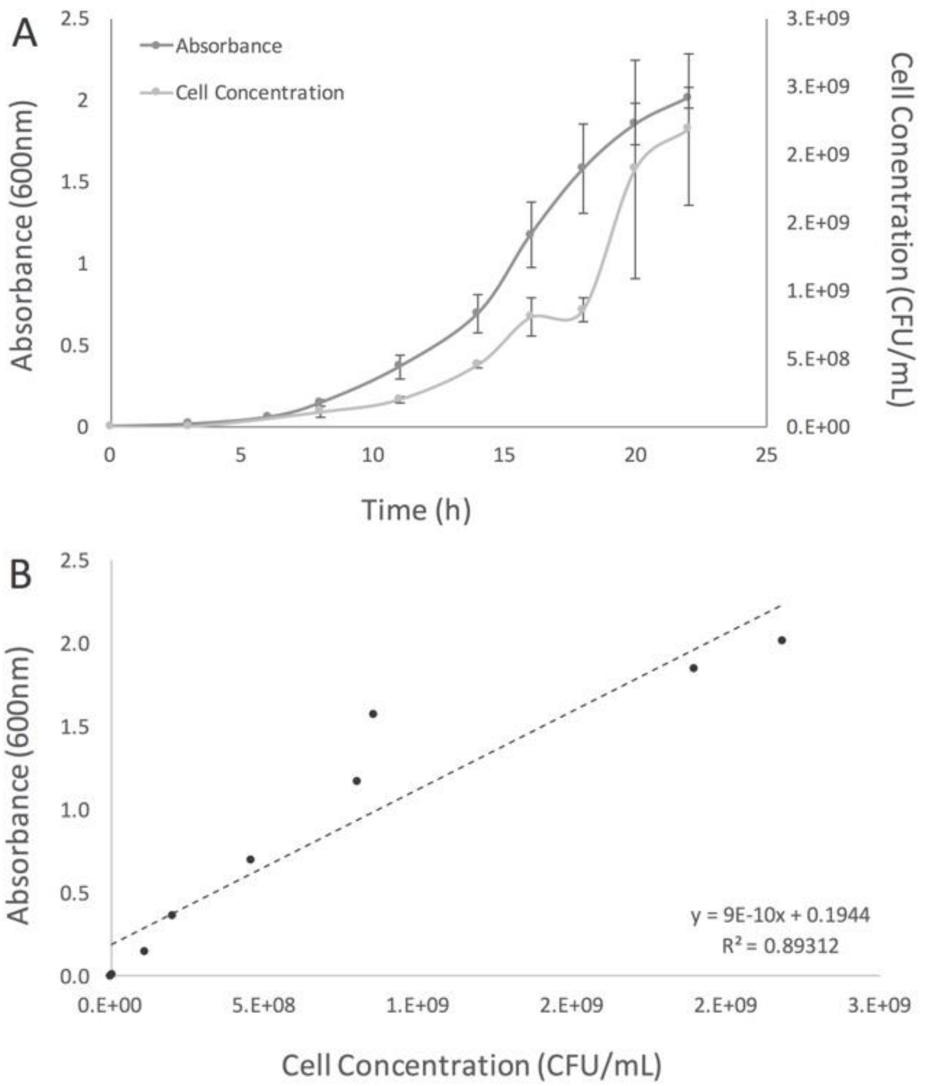

Figure 2.6. Plotting absorbance in relation to cell concentration for *Aliivibri fischeri*, to assess the utility of the assay for monitoring ecotoxicity. The hormesis biphasic phenomenon was repeatably demonstrated, with a metabolic shift between 15 and 20 h compromising the linearity of the correlation. Error bars represent the standard deviation of triplicate samples.

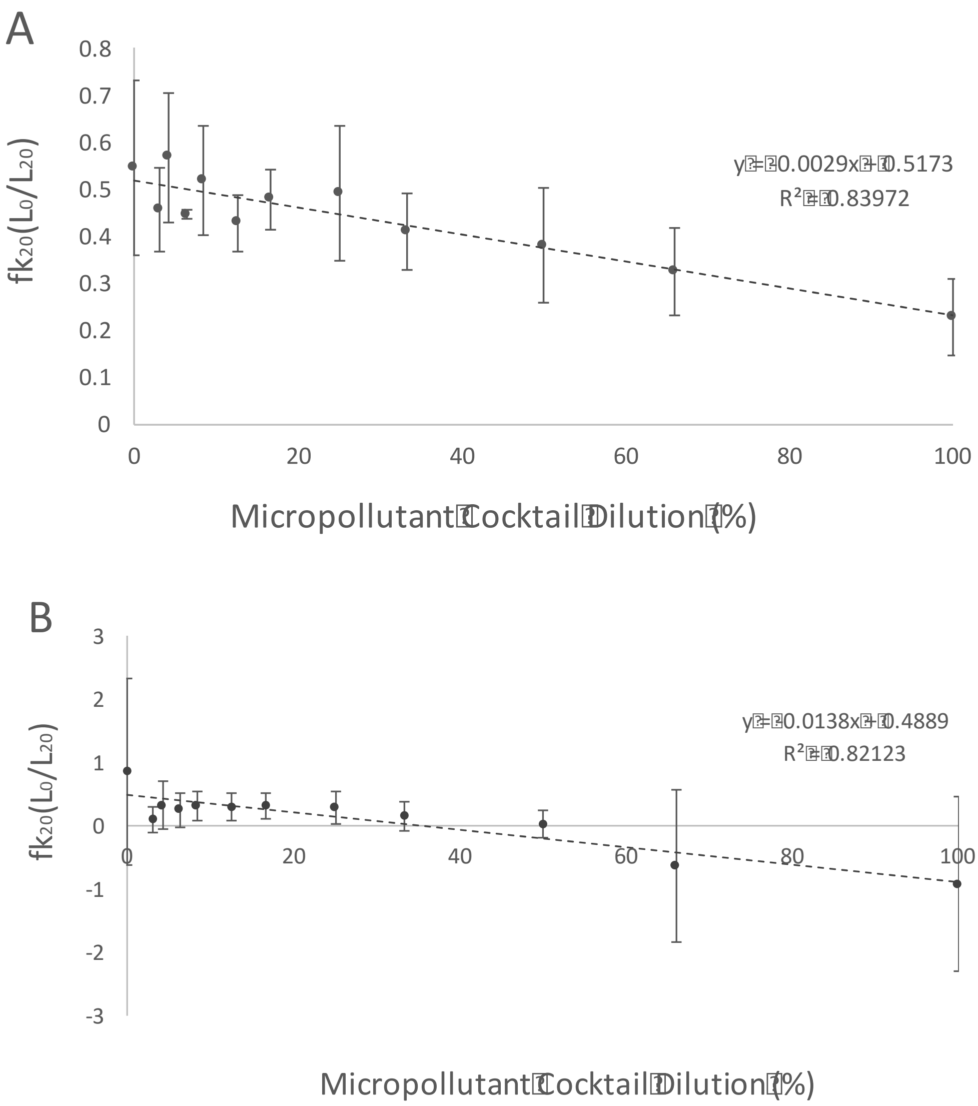

Figure 2.7. A comparison of Tecan (A) versus IVIS (B) technology for monitoring the impact of a micropollutant cocktail on the bioluminescence of *A. fischeri.* The micropollutant cocktail is based on environmental concentrations of several persistent micropollutants detected in local waters (Archer et al., 2020), and diluted as a standard curve. Error bars represent standard deviation of quadruplicate assays.

Although both technologies do not produce tightly correlated standard curves (R^2 = 0.84 and 0.82 for Tecan and IVIS, respectively, Figure 17), this is due to the high variation at lower micropollutant concentrations. At dilutions over 10%, the R^2 values increase to 0.96 and 0.94 for the Tecan and IVIS plots, respectively. Since the 100% dilution is a micropollutant cocktail concentrated via SPE to 500X environmental concentrations in local polluted rivers (Archer et al., 2020), 10% is still 50X environmental concentrations. Thus, the detection limit is well above environmental micropollutant levels. However, sample extracts can also be concentrated via SPE to fall within the detection range, for comparative analyses. In addition, Tecan standard deviation is more consistent than the standard deviation in the IVIS data. Thus, both correlation statistics and variation support the use of the Tecan microplate reader for monitoring bioluminescence, the technology employed throughout this study. pH control had a small impact on assay results (data not included, to streamline results), however, this study did not control for pH prior to direct assays, and rather explored amendment pH effects as part of the toxicity footprint of the soil or sludge extract. Sample storage (24 h and 7 days, 4°C) was shown to have a significant impact on the ecotoxicity of the direct extracts, to a varying degree depending on the amendment type (data not included, to streamline results). Thus, all direct extracts were prepared directly prior to assay exposure (<6 hrs after extraction), unless immobilised on a column via SPE (processed <6 hrs after extraction).

The *A. fischeri* assay is complicated by the dependence of bioluminescence on quorum sensing, as well as the hormesis effect. Quorum sensing is a bacterial auto-induction mechanism, based on the saturation of cell-wall receptors for molecules released by the cells themselves. Maximum ain quorum sensing induction in *A. fischeri* is typically during the mid-exponential growth phase (Lupp et al., 2003; Lupp & Ruby, 2005), with the AinS-signal (acyl homoserine lactones) inducing luminescence, which is the detection metric for ecotoxicity. Thus, there is an optimal threshold at which to harvest cells for ecotoxicology assays, for population-induced bioluminescence. The ainS gene also controls the expression of acetyl coenzyme A (acetyl-CoA) synthetase (Acs), that regulates a shift in metabolism known as the hormesis effect. Hormesis is a predictable biphasic shift in an organism's metabolic response to an environment. In rich media, *A. fischeri* cells initially excrete acetate, and at a particular acetate threshold, metabolism shifts towards acetate utilisation, for homeostatic control of environmental pH. This was observed repeatedly between 15 and 20 h in these growth curves (Figure 16A). Thus, to harness populations at optimal population density for luminescence, and avoid the hormesis transition, cultures were harvested at late exponential phase. Bioluminescence was optimally induced at OD >2 and CFU/mL > 10^8 (Figure 16). Thus, cultures older than 20 h were selected for ecotoxicity assays.

Similar correlation matrices (Table 17) were evaluated for the reference chemicals and the in-house micropollutant cocktail (Table 15) for each assay. In some assays, the reference chemical generated a tightly correlated linear standard curve, and in others, the assay organism was more sensitive when challenged with the micropollutant dilution series. All were dose-dependent, but concentrations in the linear range were selected for comparative environmental inhibition analyses, to evaluate the hypotheses.

Table 2.10. A comparison of the correlations between bioassay metrics of environmental impact, and standard curves of reference toxicants and a micropollutant cocktail. The tightest correlation, and most useful standard curve, for each assay are highlighted.

Assay	Toxicant	Correlation (R^2)
A. fischeri	$K_2Cr_2O_7$	0.45
	Micropollutant Cocktail	0.96 (at concentrations >10%)
Daphnia	$K_2Cr_2O_7$	0.91
	Micropollutant Cocktail	0.0051
Algae	$K_2Cr_2O_7$	0.008
	Micropollutant Cocktail	0.96
YES	Estradiol (E2)	0.92
	Micropollutant Cocktail	0.005

2.5.2.3.2 The effect of WTR on immobilisation of the ecotoxicity footprint of AD

2.5.2.3.2.1 *Aliivibrio fischeri* bioluminescence assay

Direct exposure of soil and sludge extracts to the bioluminescence assay (Supplemental Figure A1) elicits a markedly different response to extracts concentrated via solid phase extraction (Figures 18-23). For the concentrated SPE samples, the assays are exposed to the micropollutant footprint of the extracts. For direct extracts, environmental factors are confounding, such as pH, salinity, ammonium, etc. Thus, the data for the direct extracts are reported in Appendix II, and this study focuses mainly on the concentrated extracts (SPE). The extracts concentrated on a column during solid phase extraction are more representative of the chemical micropollutant footprint, since these chemicals are well-demonstrated to bind to these columns (Archer et al., 2020; Chu and Metcalf, 2007). They are released with methanol, which is evaporated before exposure to the relevant bio-assay. Tap water, the PAM polyelectrolyte, sand and Al-WTR all had a low-to-negligible impact on *A. fischeri* luminescence (Figure 18). The slightly negative response (stimulation rather than inhibition of bioluminescence) is likely due to hormesis, an adaptive, transient increased cellular metabolic response to moderate or intermittent stress (Abbas et al., 2018). Other studies investigating microbial metabolic responses to environmental challenges report similar transient stimulation rather than inhibition phenomena at low concentrations. For instance, studies that monitor biofilm CO_2 production in response to antibiotics demonstrate metabolic inhibition at concentrations high enough to inhibit or kill the cells. However, biofilms challenged with lower concentrations of the antibiotic up-regulate their metabolism, increasing CO_2 production (Jackson et al., 2016). These results show light (below 20%) stimulation of bioluminescence in A. vibrio in response to polyelectrolyte, sand and Al-WTR extracts (Figure 18).

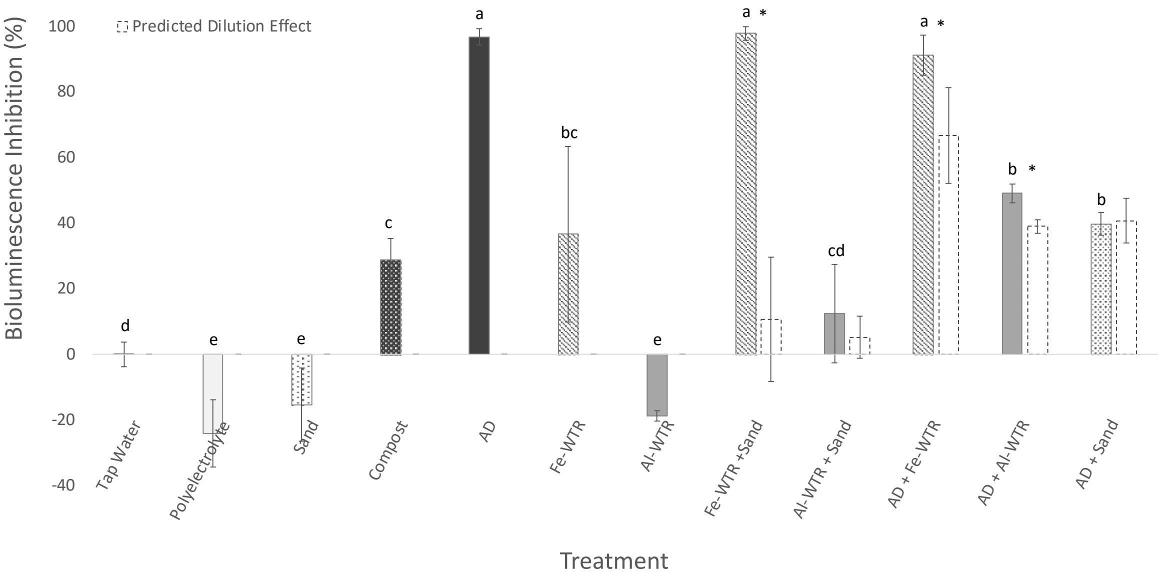

Figure 2.8. The influence of soil and sludge leachate footprints (concentrated 500x, SPE) extracts on the metabolic activity of *Aliivibrio fischeri*, measured as bioluminescence inhibition (%). Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculent, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilization in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, $p<0.05$).

All the co-amendments were added at 50% mass loading rate, in comparison to individual amendments, to keep extraction mass and volumes consistent. Thus, a dilution effect was predicted for comparison (the sum of 50% the ecotoxicological effect of each individual amendment = the predicted dilution effect, PDE). This was compared to the actual co-amendment ecotoxicological response, to assess potential interactive effects between the amendments during the overnight co-incubation.

Compost and Fe-WTR inhibited bioluminescence between 20-40% as individual amendments (Figure 18). However, AD significantly inhibited bioluminescence, nearly 100%. The ecotoxicity of AD+sand was almost precisely the same as the PDE (half of the ecotoxicity of AD plus half of the ecotoxicity of sand), suggesting dilution, and minimal interaction between the materials. In contrast, the actual toxicity of the AD+Fe-WTR and AD+Al-WTR were both significantly higher (37% and 26%, respectively) than the PDE. This is likely due to frictional forces releasing micropollutants during the overnight incubation. It is gently agitated to promote surface interactions. The hard WTR particles may degrade the integrity of the AD aggregates, releasing micropollutants and increasing ecotoxicity. Similarly, the ecotoxicity of Fe-WTR+sand exceeded the PDE (910%). This is potentially also due to frictional forces disrupting the WTR particle aggregation. Al-WTR was much less ecotoxic to *A. fischeri* than Fe-WTR, both individually and co-incubated with sand.

Punamiya et al. (2016) showed that veterinary antibiotics in manure are immobilised with, specifically, Al-WTR. Their work suggests that this is a binding effect with a saturation limit since high levels of phosphate inhibited this pollutant-binding capacity of Al-WTR. Their study tracked sorption curves over 90 days of incubation, however, the highest sorption rates were within the first three to five days, which is within the 24 h incubation timeframe in the current study. However, this data showed an increase, rather than a decrease in ecotoxicity, when co-incubating WTR and AD (Figure 18). The micropollutant footprint in AD is much wider and more complex than the controlled exposure curves explored by Punamiya et al. (2016). In addition, other authors also observed the release of pollutants with fractionation and disrupted aggregation (Zheng et al., 2022). These forces are also relevant in-field, especially with rainfall – hydraulic friction coefficients – and tillage, although much slower than in laboratory settings (Hostache et al., 2014; Guo et al., 2010).

2.5.2.3.2.2 Algae growth inhibition assay

In a review of toxicity assays for sewage sludge, algal toxicity assays are classified as one of the rarer tests, due to the high variation and difficulty in culturing (Farre and Barcelo, 2003). In this study, similar challenges to those reported were encountered, when culturing the standard Selenastrum strain used in Microtox® assays. Thus, an environmental microalgal strain growing abundantly in the Daphnia culture vessels was harvested instead, and tentatively identified as *Closterium*.

The sand extract showed a surprising inhibitory effect on algal chlorophyll a fluorescence, with both Fe-WTR and Al-WTR extracts showing a similar inhibitory effect of >80%. The polyelectrolyte and compost had a near-zero inhibitory effect, and AD had an inhibition average of approximately 40% (Figure 19). The ecotoxicity of the AD+sand co-amendment was near (97%) of the PDE, again suggesting minimal interaction between these materials. Co-amendments of sand with both Fe-WTR and Al-WTR were significantly lower than the PDE, suggesting an interactive effect, and even more so when co-amended with AD, with the organic-rich co-amendment alleviating the significant algal growth inhibition of WTR to near-zero. This suggests a remedial effect (sorption or degradation of the micropollutants), however it proves the inverse of the hypothesis. Instead of WTR minimising the ecotoxicity of the well-established sewage-borne micropollutants, WTR was highly ecotoxic in the algal assay, and co-incubation with AD alleviated the ecotoxicity more than the PDE, suggesting remediation. During the lifespan of this study, Franco et al. (2022) proposed the opposite hypothesis to the one proposed here. They suggested that WTR is significantly ecotoxic, and that organic co-amendments alleviate this ecotoxicity. This was attributed to microbial metabolic activity in a microbe-rich organic co-amendment assumed to degrade WTR ecotoxicity and thus minimise ecotoxic fluorescence inhibition. Their hypothesis was supported in their results and in this assay (Figure 19).

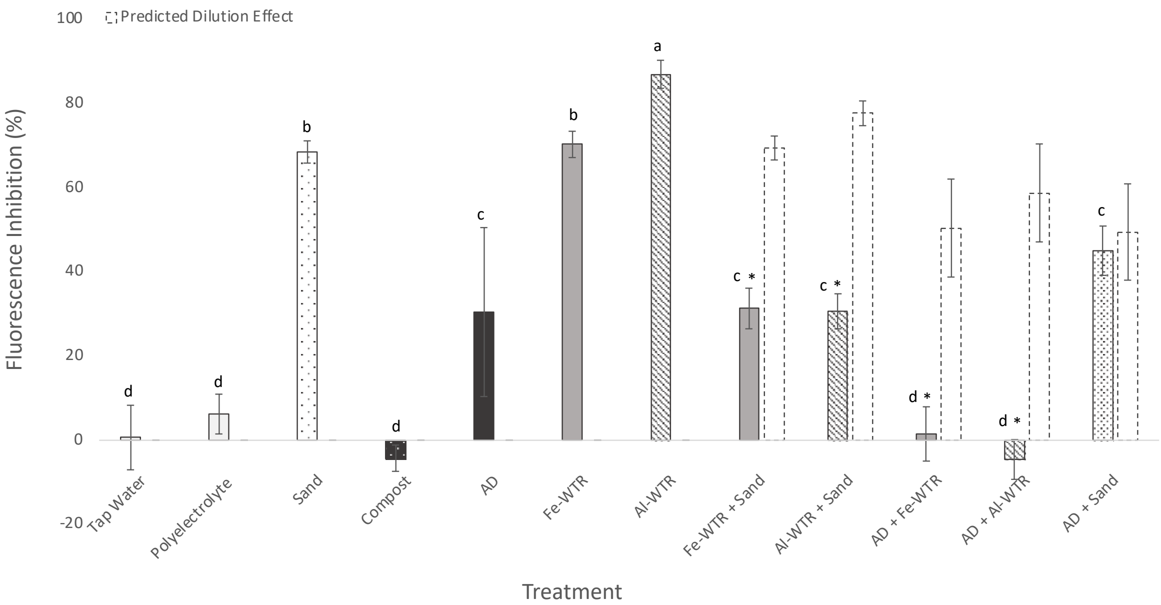

Figure 2.9. The influence of soil and sludge leachate footprints (concentrated extracts, 500X, SPE) on the mobility of an environmental algal strain, putatively *Closterium,* measured as fluorescence (chlorophyll A) inhibition (%). Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculent, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilization in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, $p<0.05$).

2.5.2.3.2.3 *Daphnia* acute immobilisation assay

Extensive studies have investigated the effects of challenging water fleas – *Daphnia magna* – with sludge extracts, typically reporting acute cladoceran cytotoxicity and genotoxicity (Abreu-Junior et al., 2019; Akhola et al., 2021; Renoux et al., 2001; Giannakis et al., 2021; Rodriguez et al., 2011). This is generally remediated with some level of stabilisation, including heat treatment, leaching, fungal reactors, or composting. These studies also approach soil extraction with diverse methods, from direct soil leachates to methanol extraction (low sample concentration), to solid phase extraction (high sample concentration), and even direct exposure to the solid sludges.

Most of these studies attempt to quantify the sludge toxicity for classification according to national and international guidelines. In contrast, this work aims to comparatively evaluate soil extracts and groundwater leachate, assessing the hypothesis that the cladoceran toxicity of the AD-associated micropollutants may be immobilised with WTR co-amendment – which has sorptive clay-like properties. Further mycorrhizal remediation is also possible in lysimeter trials. Upon exposure to direct extracts, all *D. magna* neonates were immobilised in pure tap water, as well as the polyelectrolyte flocculent (Appendix II, Figure B2). However, the addition of all amendments in tap water decreased the toxicity. This is likely due to the residual chlorine in tap water, often removed with low $Na_2S_2O_3$ dosing in the standardised Microtox assay. The organic amendments thus act as a sink for the soluble free radicals associated with chlorine treatment. It is well-known that organics increase the chlorine demand in water treatment (Pavoni et al., 2006; Lai et al., 2006). Two extra controls were included, including pure *D. magna* media and polyelectrolyte solubilised in *D. magna* media, stirred for 24 h. In both additional controls, *D. magna* neonate motility was not influenced over 72 h.

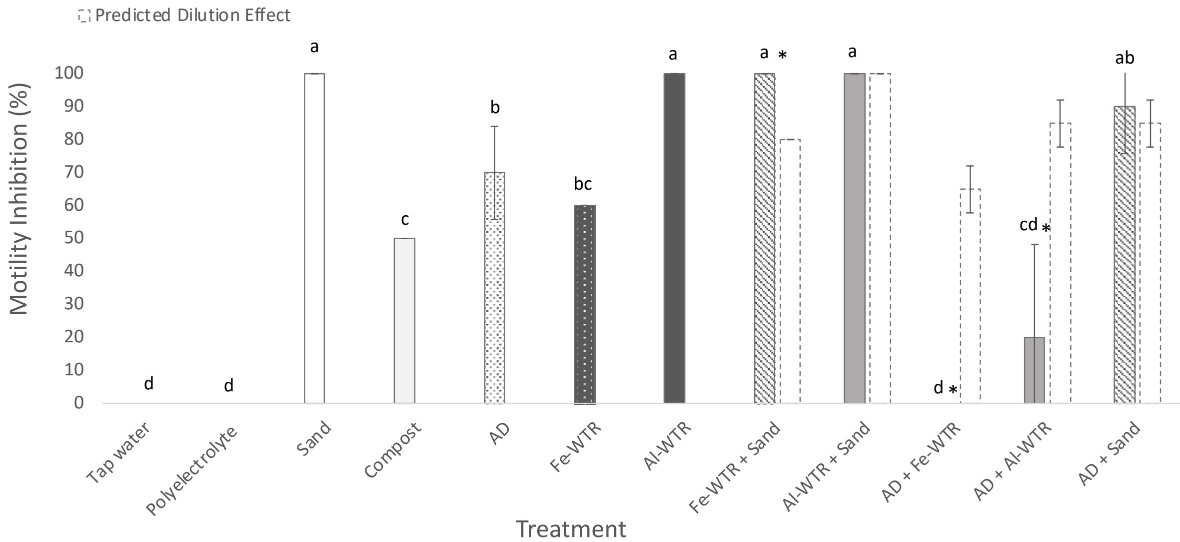

Figure 2.10. The influence of soil and sludge leachate footprints (concentrated extracts, 500X, SPE) on the mobility of *Daphnia magna*, measured as motility inhibition (%). Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculant, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilisation in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, $p<0.05$).

In the concentrated extracts, the micropollutant focus of this study, tap water and the polyelectrolyte had no influence on invertebrate motility (Figure 20). Sand and Al-WTR inhibited invertebrate motility completely (100%), whereas compost, Fe-WTR and AD inhibited motility between 50 and 70%. The co-amendment of WTRs and sand had higher ecotoxicity (near 100%) than the PDE, again suggesting disaggregation during agitation. In contrast, the co-amendment of AD and the WTRs were lower than the PDE, indicating a remediative co-amendment effect (sorption or degradation).

2.5.2.3.2.4 Phytotoxicity

The effect of soil and sludge extracts was compared on the germination rate of four groups of seeds, including two hemp (Cannabis sativa L.) strains, SAPA (SAPA Valley landrace) and SABL (Chinese broadleaf hemp), representative of a textile crop; corn (Zea mays STAR7719 F1 hybrid) representative of a model biofuel crop (although it is not an ideal local crop, due to the food versus fuel debate), and lawn grass (Dactyloctenium Australe), since many WWTWs and surrounding sportsgrounds use sludge and wastewater to fertilize their lawns. None of the treatments had a significant impact on the germination rate of any seed strains (Figure 21). This contrasts with reports of phytoxicity of sewage sludge extracts (Walter et al., 2006), although other authors (Fuentes et al., 2004) have also described low-to-zero phytotoxic effects in heat-stabilised sewage sludges. Notably, the hemp strain selected for the lysimeter trial (Cannabis sativa L., SAPA Valley landrace) had a 100% germination rate, whereas the SABL broadleaf strain was as low as 40%. There was also no significant difference in seed radicle length over seven days (data not shown), for any of the treatments in any of the plant strains.

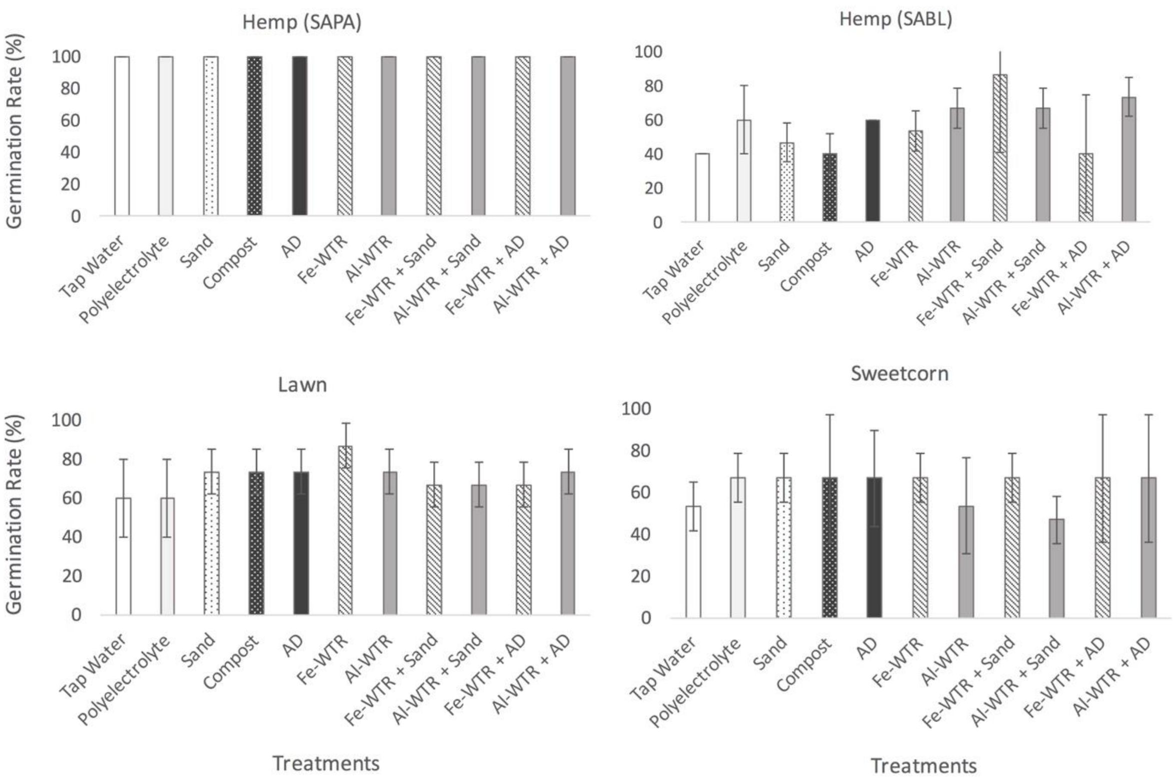

Figure 2.11. The influence of soil and sludge leachate footprints on phytotoxicity, measured as the germination rate of four plant strains including two hemp (Cannabis sativa L.) strains, SAPA (SAPA Valley landrace) and SABL (Chinese broadleaf hemp), representative of a textile crop; corn (Zea mays STAR7719 F1 hybrid) representative of a biofuel crop, and lawn grass (Dactyloctenium Australe). Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculent, solubilised in tap water, was also assessed with this assay. Error bars represent the standard deviation of means of 15 seeds, and significance is indicated (ANOVA, $p<0.05$).

2.5.2.3.2.5 Yeast estrogen screen

Shifting to models more relevant to mammals, the endocrine assays were notably different to the previous acute toxicity assays. As literature suggests (Farré and Barceló, 2003; Ruan et al., 2015) endocrine assays were most sensitive to AD (almost 50% higher than the $E2_{max}$; Figure 22). Compost and Fe-WTR were near the $E2_{max}$, and Al-WTR, sand, tap water and the polyelectrolyte were well below the $E2_{max}$. Again, the AD+sand co-amendment was near the PDE (98%) suggesting minimal interaction between the materials, and sand co-amendments with the WTRs were similarly near the PDE. The AD co-amendment with WTRs were significantly lower than the PDE. These assays therefore prove the hypothesis in this work, that co-incubation with WTR remediates the ecotoxicity of the AD. This could be due to physical forces like friction or immobilisation.

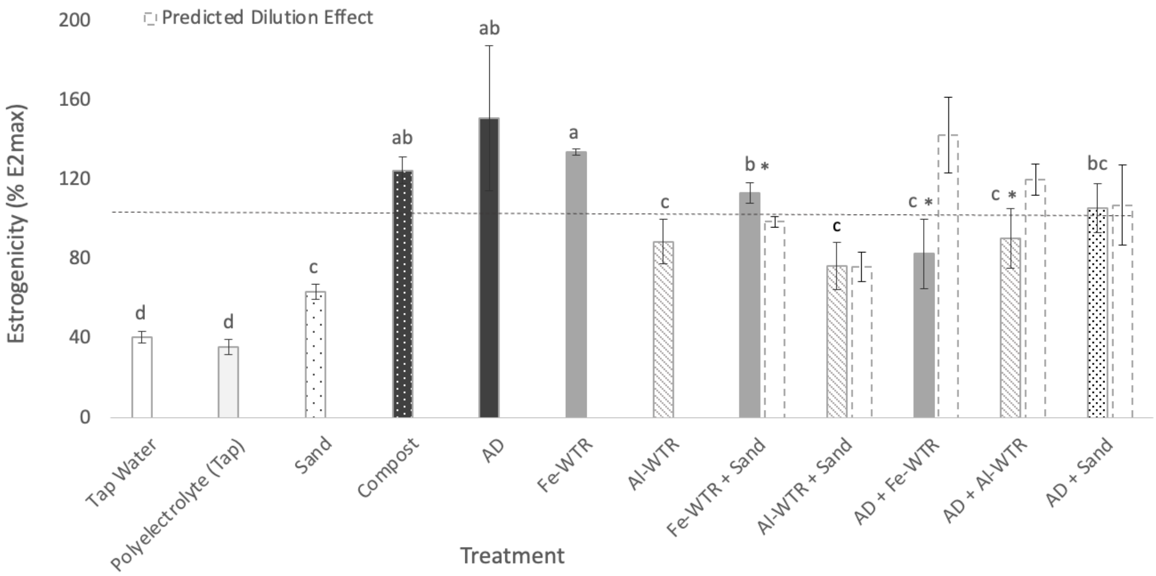

Figure 2.12. The influence of soil and sludge leachate footprints (concentrated extracts, 500X, SPE), on the estrogen response of the yeast estrogen screen, measured as a fraction of the max E2 response, indicated with a dashed line. Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculent, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilisation in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, $p<0.05$).

There are extensive reports of the estrogenic response to sewage sludge extracts (Citulski and Farahbakhsh, 2012; Kapanen et al., 2013; Ruan et al., 2015). Citulski and Farahbakhsh (2012) are one of many authors reporting cytotoxicity in sewage sludge extracts, limiting the use of the yeast estrogen screen. This is typically associated with concentrated extracts, where direct exposure alleviates the cytotoxicity risk. Kapanen et al. (2013) reported a reduction in estrogenicity when sludges are composted, but they are also one of the few author-groups to express caution about the limitations in employing these tools for comparisons in such diverse environmental matrices. This limitation is demonstrated in the current study too, with the assays responding – at varying levels – to sand and compost as well as sludge wastes.

2.5.2.3.2.6 Cancer assays

In cancer assays, as with the yeast estrogen screen, the cells can have two responses: (1) the extracts may be cytotoxic, killing the cells, or (2) the extracts may stimulate cancer responses (upregulating cancer cellular metabolism). The cancer assays were more limited in design than the others, due to logistical constraints. However, the amendments (WTR, AD and the co-amendment) all decreased mitochondrial dehydrogenase activity in comparison to the control, tap water and polyelectrolyte, which had no effect (Figure 23). The AD and AD+WTR co-amendment influenced the cells significantly more than the individual WTR amendment in all assays except the MDA-MB-231 cell line. Interestingly, although this is an aggressive breast cancer cell line, it is triple negative, and thus not sensitive to endocrine disruptors. In contrast, MCF7 was the most sensitive to AD and is both estrogen and progesterone positive. Micropollutants common in sewage sludges have been implicated as breast cancer risks, from heavy metals (Siewit et al., 2010), to bisphenol A (Deng et al., 2021), organopesticides (Arrebola et al., 2015), and many more. Other authors have reported similar estrogenic responses in analogous MCF7 bio-assays, when exposed to sewage sludge extracts (Koistinen et al., 1998; Dizer et al., 2002). Gonzalez-Gil et al. (2016) showed that thermal treatment of

AD reduced estrogenicity as measured with the MCF7 assay. A review of these carcinogenic impacts was published as part of this study (Du Plessis et al., 2022).

Interestingly, although colon cancer is not intuitively linked to estrogenicity, both cell lines (Figure 23D and Figure 23E) were more sensitive to AD than WTR. Colon literature shows that estrogen agonists are widely recognised as protective agents against colon cancer (Das et al., 2023). Thus, estrogen agonists, which are common in wastewater (Snyder et al., 2001), may induce estrogen-mediated anti-cancer activities against colon cancer cells (Das et al., 2023). This work shows that wastewater sludge extracts, which is rich in estrogen agonists, kills colon cancer cells, supporting that theory. However, it also kills normal cells too (such as breast epithelial cells, MCF12A), suggesting cytotoxicity that is likely due to more than simply estrogen agonists. It is important to note that the highest cytotoxicity is only 37% higher than the control, with pure amendments. Since agronomic application rates are typically <5%, it is unlikely that this cytotoxicity will be relevant in-field but can be monitored with repeat land applications over multiple seasons.

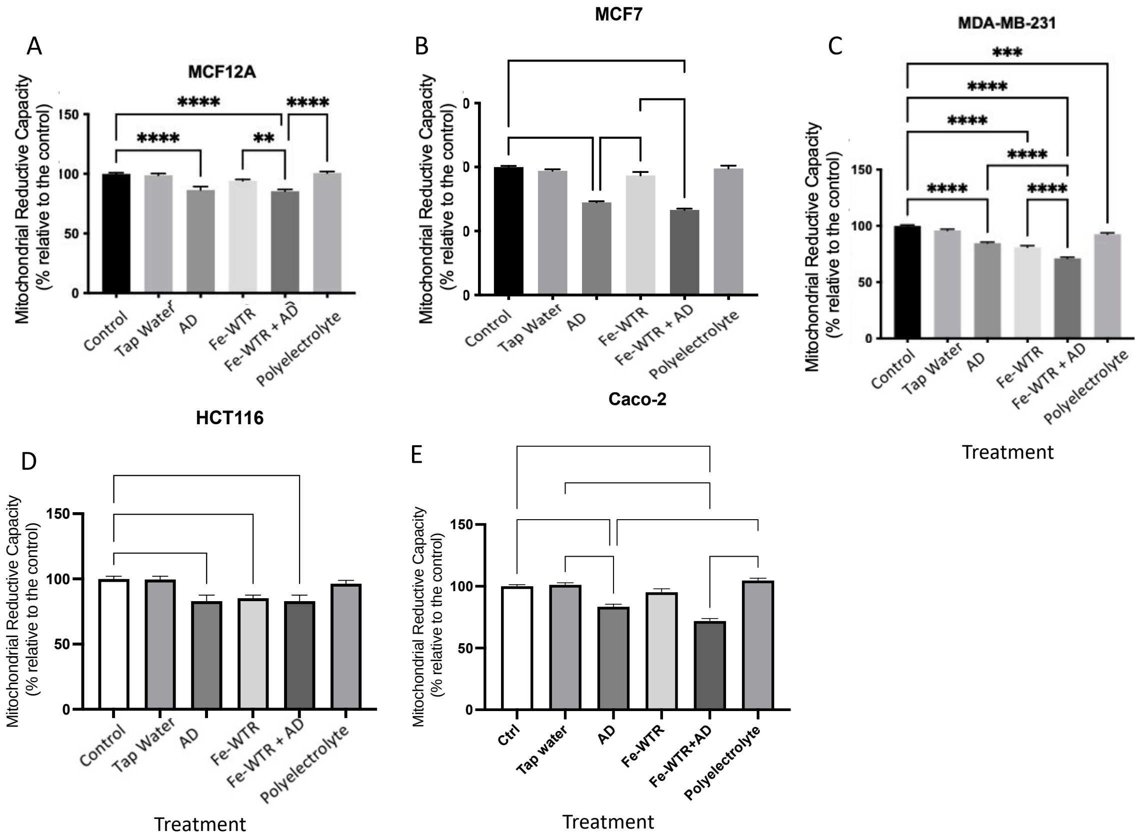

Figure 2.13. The influence of soil and sludge leachate footprints, as direct extracts, on the metabolic activity of cancer cell lines, measured as mitochondrial reductive capacity of three breast cancer cell lines (a) MCF12A, (b) MCF7, and (c) MDA-MB-231, as well as two colon cancer cell line (c) HCT116 and Caco-2. Individual Fe-WTR and AD extracts, incubated in tap water overnight, were compared to a co-amendment of the Fe-WTR with AD. The environmental impact of the polyelectrolyte flocculent, solubilised in tap water, was also assessed with this assay. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, p<0.05).

Overall, in terms of ecotoxicity, the polyelectrolyte used in the flocculation process for WTR showed no inhibition or metabolic response more significant than the control in any of the bioassays, assessed at 10x the standard concentration used in the water treatment process.

Here, the hypothesis was that WTR (with some evidence of clay-like properties) would alleviate the well-demonstrated ecotoxicity of AD, associated with the high micropollutant concentrations in this waste. A study released in 2022 proposed the opposite hypothesis to the mechanism evaluated in this study, suggesting that WTR will have higher ecotoxicity, and that the co-amendment with an organic-rich soil conditioner like AD will promote microbial metabolism and degrade the WTR ecotoxic footprint (Franco et al., 2022). In this study, both hypotheses were supported, in different assays. A trend showed that the acute assays (not *A. fischeri*, but algae and *Daphnia*) were generally more sensitive to WTR, and the endocrine-related assays (the yeast estrogen screen and most cancer assays) were generally more sensitive to AD. In all cases, the co-amendment of AD and WTR showed a remediative effect, decreasing the ecotoxicity more than the PDE. In the following sections, the focus is shifted to lysimeters, to evaluate if the tools are useful in greenhouse trials. In a similar study, Dizer et al. (2002) reported very low ecotoxicity in groundwater leachate when lysimeters are fertilized with sewage sludge. This could support the circular economy proposition, of co-amending nutrient poor soils with AD and WTR, which has been shown to be beneficial in terms of soil fertility and microbiology. If groundwater protection is demonstrated with repeat applications over longer cycles, it will support the local initiatives to divert organics from landfill to productive applications.

It is worth noting that these are pure sludges and soil, incubated individually or co-incubated overnight in tap water with gentle agitation. Land application is at much lower agronomic rates (<5% m_{dw}/m_{dw}), and thus the low ecotoxicity reported by Dizer et al. (2002) is not surprising. The effects are likely to be less clearly resolved in lysimeter and field trials. In some cases, fractionation and disrupted aggregation may also release micropollutants, in-field. It is also important to note that in many assays, sand and compost extracts were equally as ecotoxic as some of the waste amendments. Thus, it is recommended that these assays are used to understand an ecosystem in which sludge is land-applied, and monitor temporal changes, rather than attempting to make quantitative risk statements. A Masters (Irshaad Ahmad Parker) degree was also executed under this study, developing a computer vision pipeline to utilize bioluminescence for in-field monitoring of ecotoxicity remediation, which is under preparation for publication.

2.6 Groundwater protection, Soils and Crops

2.6.1 Methods

2.6.1.1 Lysimeter trial design & setup

The lysimeters were built from repurposed, inverted water cooler bottles, painted dark green to prevent light influencing root growth. Lysimeter dimensions are described in Figure 24. Each amendment was evaluated in triplicate lysimeters, in a climate-controlled (25°C, wet wall) glasshouse. Based on wet:dry mass per volume data and packing densities, the lysimeters were filled with a sand mass of 20.4 kg (dry weight) as a baseline, and all amendments calculated as m_{dw}/m_{dw}. The WTR lysimeters were amended with 2.5% (m_{dw}/m_{dw}) Al-WTR, the AD lysimeters with 2.5% (m_{dw}/m_{dw}) AD, and the co-amended lysimeters with 2.5% Al-WTR + 2.5% AD. This co-amendment tests the hypothesis that, if the sand is amended with AD to increase soil nutrients, replacing some (2.5%) sand with a sorptive material like Al-WTR will (a) decrease soil water repellency, (b) immobilise some of the toxicity of the AD, and (c) promote nutrient and carbon sequestration.

Figure 2.14. Lysimeter dimensions (A and B), designed to investigate carbon cycling and groundwater protection, when amending sandy soils with water and wastewater sludges, to promote hemp. Physical experimental set-up (C) was randomised next to a wet well in a growing tunnel (season one), with security and notifications according to national (DALRRD) hemp cultivation regulations. The second growing season was in autumn, and proximity to the cooling wall was less critical. The lysimeters were thus randomised throughout the tunnel (D, season two), before mixing soils and amendments for planting.

Lysimeters were filled with the respective sandy soil amendments (Figure 24E), mixed in thoroughly in heaps and distributed between triplicates, with 2 kg extra diverted for baseline soil analyses. Lysimeters were watered and equilibrated overnight before planting with 6 hemp (Cannabis sativa ssp. sativa, SAPA Valley Landrace) seeds (2 cm deep, 10 cm apart) per pot and thinned to three plants per pot, with a total of nine plants per amendment. Lysimeters were not fertilized, however an extra sandy soil control (triplicate) was fertilized according to the wheat recommendation of the Fertilizer Society of South Africa (FSSA, 2007) for Western Cape sandy soils (N=130, P=50, K=75, Ca=40, Mg=13 and S= 40 $kg.ha^{-1}$). This handbook has no hemp recommendations, but these fall approximately within the ranges described in a meta-analysis of studies on soil fertility management for industrial hemp production (i.e. N = 80-200 $kg.h^{-1}$; P = 30 $kg.ha^{-1}$, etc.; Wylie et al., 2021). A 500-mL fertilizer concentrate was added as three applications over the 90-d trial period, according to Clarke et al. (2019). The same procedure was followed in Season 2. The lysimeters were harvested, the soils distributed to dry, and the same amendment and planting protocol was followed. Since 2 kg was removed for analysis after Season one, sand and amendments were calculated for re-application in Season two, with the addition of 2 kg (wet weight) per lysimeter. Season one was planted in high summer, mid-November 2022. Daytime temperatures were at an average of 27ºC, reaching well over 35ºC, and night temperatures at an average of 16ºC, with a circadian rhythm of 14 h daylight. Season two was planted mid-May, late autumn. Although hemp is not a winter crop, a local grower and the source of our seeds (Natie Ferreira, https://tamatie.co.za/) assured us that his crops grow well through winter in good tunnels. However, it was a particularly cold and wet winter, with daytimes temperatures at an average of 18ºC and night temperatures at an average of 8ºC, with a circadian rhythm of approximately 10 h daylight.

2.6.1.2 Irrigation Strategy

In pot trials, the irrigation regime is typically calculated to keep the plant root systems above 70% FWC over a 2.5-3 month growing season (Clarke et al., 2019). This is calculated by monitoring the mass of the pots. However, lysimeters are too heavy to weigh regularly. It is also not feasible in-field. Thus, rather than keeping each treatment at FWC, this trial assessed plant growth at a consistent watering regime across amendments, based on rainfall and farming practices. According to the South African Agricultural Research Council (ARC), for optimal local growth, hemp needs 500-700 mm rain per season, with 250-300 mm during the vegetative growth stage (ARC, 2022). The distribution of total seasonal rainfall over the plant trial period is based on Madubela (2020).

$1mm(H_2 0) = 1L.m^{-2}$ ……………………………………………………………………………………Eq. 7.

Lysimeter radius = 13.5 cm

Area = 0.057256 m^2

Thus,

$V(\text{season}) = 500mm * Area$ ……………………………………………………………………Eq. 8.

$V(\text{season}) = 500L.m^{-2} * 0.057256m^2 = 28.628\ L$

Growing season = 3 months
= 12 weeks
= 24 irrigations (2 per week in a sandy soil)
= 29L/24 = 1.2 L per irrigation per lysimeter, bi-weekly

Three times during each trial (pre-planting, mid-trial and the day before harvesting), the lysimeters were irrigated to saturation, to collect 500 mL leachate for analysis.

2.6.1.3 Monitoring and harvest

Lysimeter leachates were monitored pre-trial (a day before planting) and post-trial (the day before harvest) over the growing seasons, including a suite of parameters that describe both the benefits and risks of this waste co-amendment. The same parameters were evaluated in the soil, pre- and post-hemp growth. Plant matter was also evaluated for some of these parameters. Baseline characterisation parameters of the amendments for planting are described in Supplemental Tables B1, B2 and B3.

Both trials were harvested at three months. Hemp was cut at the base, and a section (3 cm) of the base of the stem and a section (3 cm) of the tip of each plant were transferred aseptically to separate 50 mL centrifuge tubes, for pathogen screening. Once the pathogen dilution series was complete, the plant material was dried with the rest of each plant, to calculate total dry biomass. The dry biomass of each plant was calculated (60ºC, 24 h). Root biomass was calculated by washing the sand from the roots and dabbing them dry before imaging and dry biomass processing (60ºC, 24 h). Total macro- and micronutrients of the dried aboveground plant material were determined using the Kjeldahl method (N), and acid digestion and ICP-MS (P, Ca, Mg, K, Na, Fe, B, Zn, Mn, Cu, and Al; Elsenburg Plant Laboratory). The lysimeter soils were distributed for air-drying in the tunnel (3 days), each sample homogenised and 2 kg taken for analysis. Wet soil samples were aseptically collected (100 g) and screened for pathogens according to Section 3.2 (Sludge pathogen screening). The soils were dried (60ºC, 24 h) and analysed for nitrogen, phosphate, carbon and bio-available and total (aqua regia) trace elements according to Section 3.3. (Monitoring parameters and schedule), including ecotoxicity. Wet and dried soils were analysed for hydrophobicity, using the water droplet penetration tests, which produced similar results to the infiltrometer during laboratory optimisation.

2.6.2 Results

2.6.2.1 Germination and growth

As demonstrated in the phytotoxicity trials (Section 3.4, Figure 21), there was no significant difference in seed germination rate between treatments during both growing seasons (data not shown). As described in the phytotoxicity section, this contrasts with some authors who report sewage sludge phytotoxicity (Walter et al., 2006). At these amendment concentrations, the results support studies showing that heat stabilised sludges have negligible phytotoxicity (Fuentes et al., 2004). These sludges are pasteurised to control pathogens and are thus heat stabilised prior to soil amendment. Seeds germinated approximately 8-10 days after planting in summer and 12-13 days after planting in winter, with 100% germination rate for Cannabis sativa L. (SAPA Valley landrace) across all treatments.

However, within one month, treatment differences clearly impacted plant growth rates (Figure 25A), and at the 70-day termination date in season one, the treatment differences were demonstrable in terms of plant biomass and height (Figures 25B and 26), and nutrient sufficiency in above-ground biomass (leaf yellowing and senescence is treatment-dependent, Figure 25B). Plants were intentionally left to grow beyond nutrient sufficiency, to compare nutrient limitation in the various treatments. The plants in the sand control and the fertilized sand are both growth-limited and nutrient-limited, whereas the WTR plants are growth-limited but appear less physiologically nutrient-limited above-ground, with less senescence (Figure 25B). The co-amendment produced the highest biomass (Figure 25B and Figure 26), approximately double that of the nutrient-poor sandy soil. WTR limited growth, as has been shown in previous studies, and both AD and the WTR+AD co-amendment promoted plant growth (7 and 9 times the control, respectively). Senescence, measured as % leaves exhibiting yellowing over total leaf count, in WTR treatments was less than 2%, whereas sand treatments was less than 10% and all other treatments were between 30 and 40% senescent. As the lysimeters are closed systems, the larger plants were nutrient deficient at the termination of the trial. All the aboveground parameters exhibited a similar growth trend, with WTR limiting growth in comparison to the sandy soil control, and the other treatments promoting growth, in the increasing order of (1) fertilizer, (2) AD, and (3) the co-amendment. The belowground biomass showed clearer treatment differences, with AD and the co-amendment increasing root biomass by as much as 25 times (Figures 27 and 28, no significant difference between the AD and co-amendment), and WTR stunting the root system to an almost negligible biomass. Season two mirrored the growth patterns of season one, although the plants were much smaller (Figure 25C, 26B and 27B). The cold drove the metabolic energy of the plants towards seeding (Figure 25D and E) rather than fibre, a common phenomenon (Hall et al., 2013). Nevertheless, the same trends were evident. During season two, the fertilizer protocol for lucerne was applied, since the wheat fertilizer protocol was growth limiting for hemp, in comparison to the AD and WTR+AD treatments in season one (Figure 25B and 26A). This promoted hemp growth to biomass equivalent to the AD and WTR+AD treatments in season two (Figure 25C and Figure 26B).

Figure 2.15. A photographic comparison of the leafy hemp biomass, comparing season one treatments at (A) 1 month, and (B) harvest (3 months), and season two treatments at harvest (3 months, C). Treatments include (1) Sand, (2) Fertilized Sand (Sand F), (3) WTR, (4) AD (labelled SS) and (5) WTR+AD (labelled WTR+SS). In the autumn/winter season (season two), the plants favoured flower and seed production over fibre (D and E).

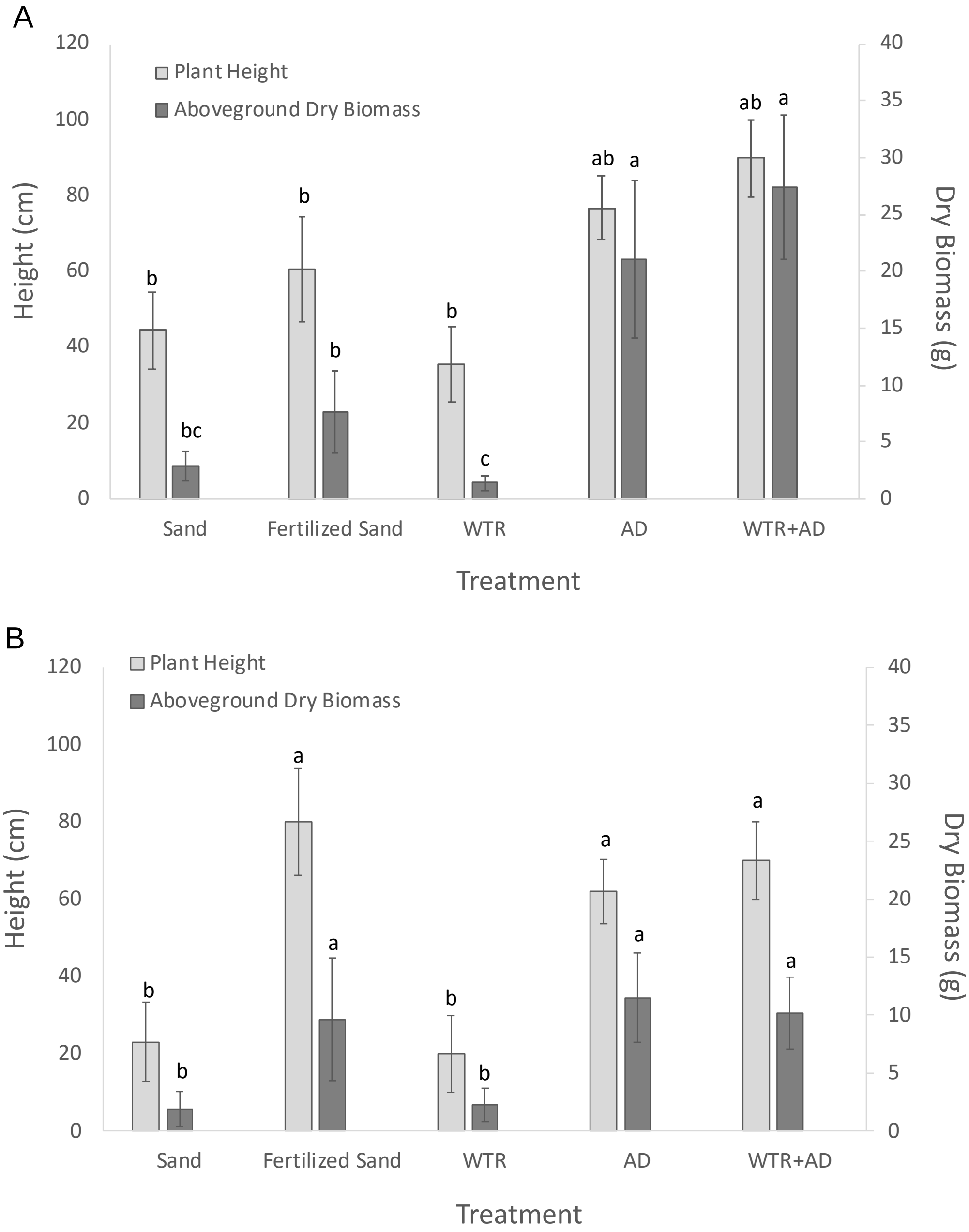

Figure 2.16. Plant height (cm) and dry weight (g) were evaluated between treatments, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment (WTR+AD). Bars represent the mean of 9 plants distributed in triplicate pots, and error bars the standard deviation. Season one (A) is compared to season two (B). Significance lettering describes differences between treatments (ANOVA, post-hoc Tukey's HSD test, $p<0.05$). Significance lettering (a-c) is applied separately over each series.

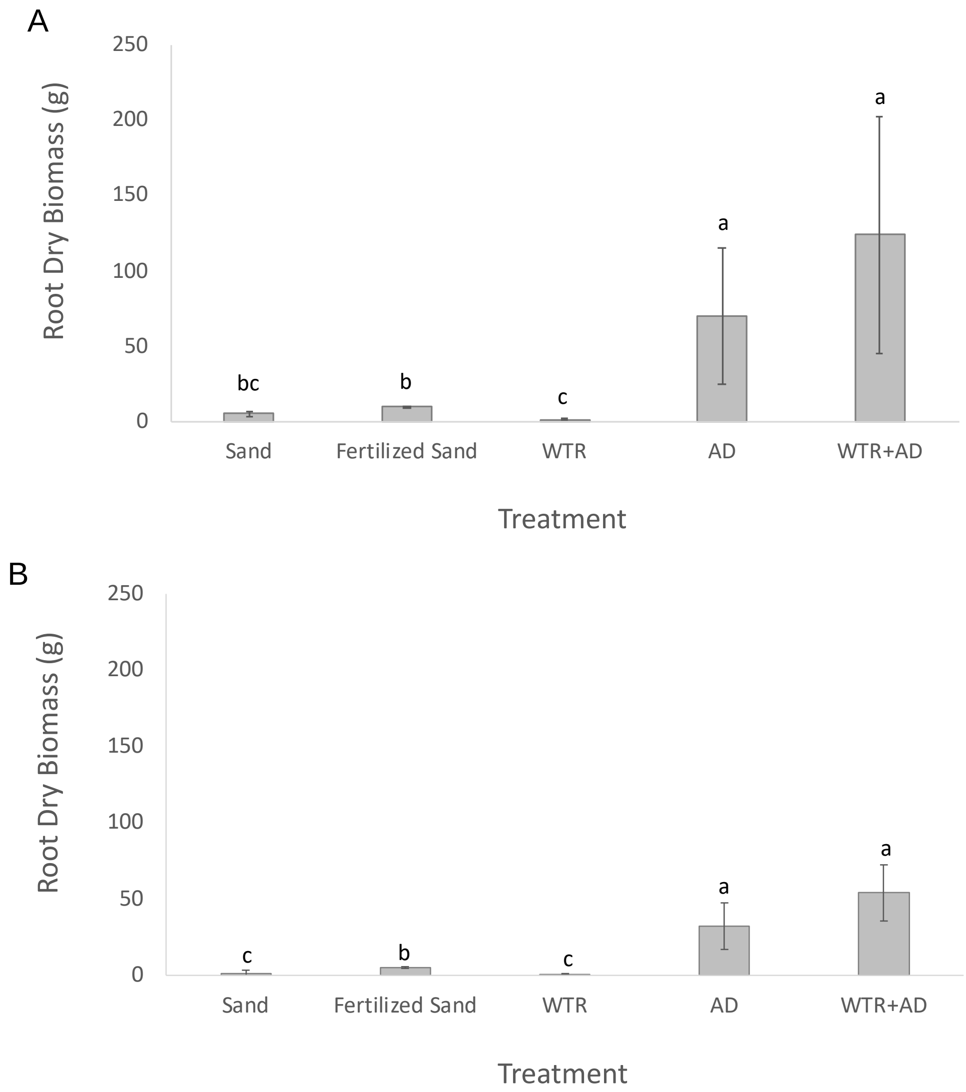

Figure 2.17. Root biomass (g dry weight) was evaluated between treatments, comparing nutrient-poor sandy soil to amendments like fertilizer, WTR, AD and a sludge co-amendment (WTR+AD). Season one (A) is compared to season two (B). Bars represent the mean of 9 plants distributed in triplicate pots, and error bars the standard deviation. Significance lettering describes differences between treatments (ANOVA, post-hoc Tukey's HSD test, $p<0.05$).

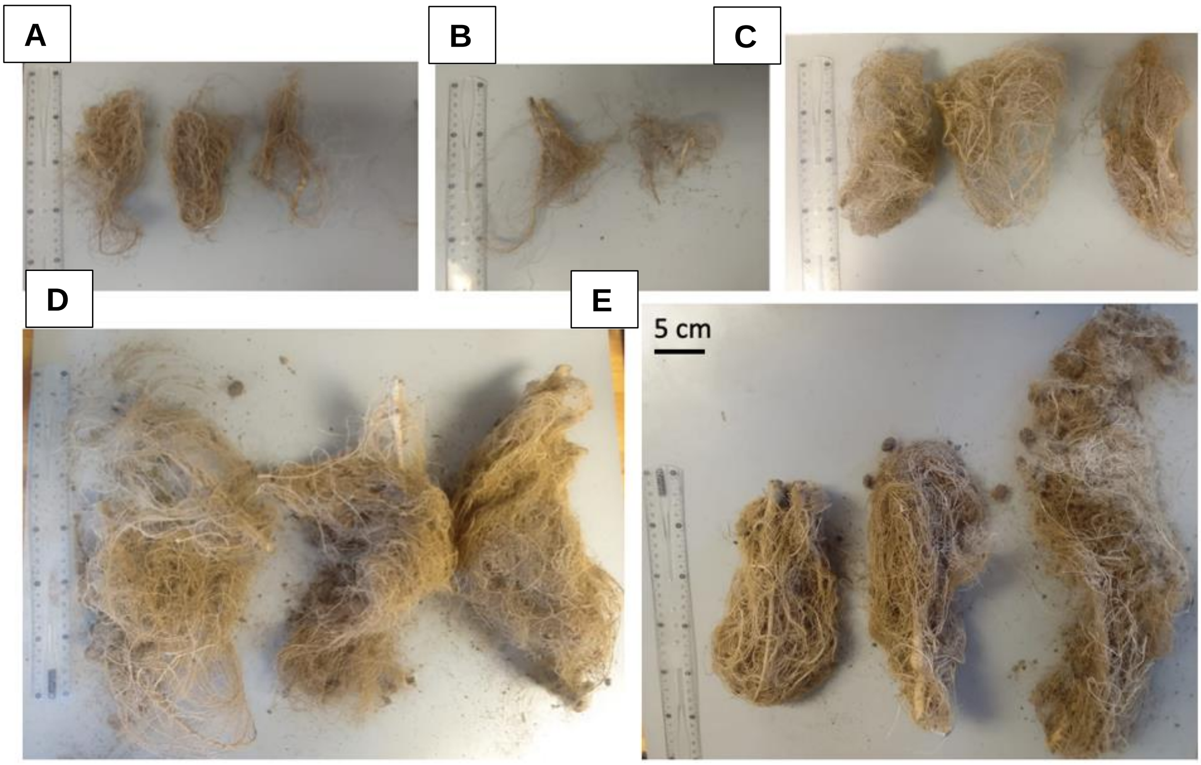

Figure 2.18. A visual representation of root biomass between treatments in season one, comparing (A) nutrient-poor sandy soil to amendments like (B) fertilizer, (C) WTR, (D) AD and (E) a sludge co-amendment. Figure sizes are standardized over the ruler, and the scale bar represents 5 cm.

Stem diameter was also recorded, since hemp is a textile crop, and the stem physiology is of commercial interest. Stem diameter followed the same trend as whole plant biomass, with WTR limiting this growth parameter in comparison to the sandy soil control, and fertilizer, AD and the co-amendment increasingly thickening the stems (Figure 29, season one). The plants diverted metabolic energy to flowering over fibre in the winter season (Hall et al., 2013), therefore stem thickness was below 3 mm for all plants in season two, and thus the resolution between treatments was less clear (data not included). Although the co-amendment does not promote any of the plant growth parameters significantly more than the pure AD amendment, there was a trend towards higher means in all the co-amendment growth parameters. In season one the co-amendment produced crops with 17% higher plant height, 26% larger stem diameter, 29% higher above-ground dry biomass and 77% higher dry root biomass than the AD amendment. In season two, the difference between AD and the co-amendment was less clear. Crops grown on AD had 13% higher biomass than those grown on the co-amendment, but standard deviation was high. In contrast, crops grown on the co-amendment were 20% taller than those grown on AD, and root dry biomass was 30% higher in the co-amended lysimeters. Variation is typically this high in biological replicates, more so in greenhouse trials than the laboratory, and even more so in-field.

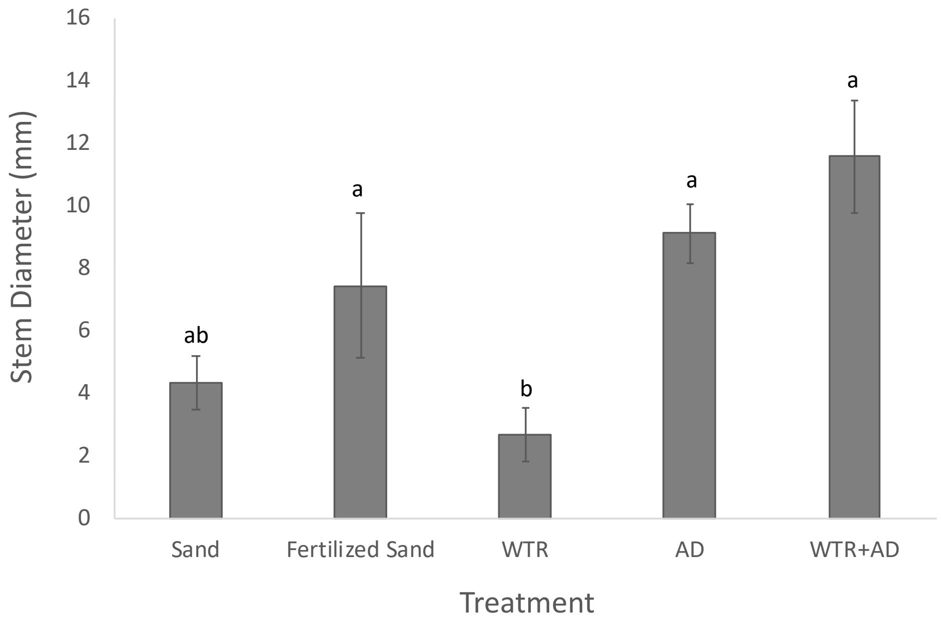

Figure 2.19. Stem diameter (mm) of these fibrous crops was evaluated between treatments, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment (WTR+AD). Season one is plotted here. Bars represent the mean of 9 plants distributed in triplicate pots, and error bars the standard deviation. Significance lettering describes differences between treatments (ANOVA, post-hoc Tukey's HSD test, $p<0.05$).

2.6.3 Co-amendment hypothesis: Does WTR stabilise AD pollutants?

2.6.3.1 Soil and leachate chemistry

Anaerobic digestate had the most significant impact on soil pH and electrical conductivity, a proxy for dissolved salts, with similar patterns reflected in the leachate (Figures 30 and 31). The addition of AD dropped the neutral pH of the soil from pH 7 to pH 6, upon amendment. However, by the end of the first growing season (summer) all soils and leachates had normalised at pH 6, with no significant treatment differences. The shift in pH towards acidity is relevant to pollution, as lower pH mobilises heavy metals. However, this pH drop is not lower than typical soil pH in the region, and close to standard receiving waters thresholds. Many local sandy soils are more acidic, particularly those in this region (Clarke et al., 2019). In terms of surface water protection, regulatory requirements suggest surface water pH is considered ideal between 6.5 and 8.0 (DWA, 2011; DWAF, 1996; Mudaly and Van der Laan, 2020), which is slightly higher than the pH in these leachates, but well within optimal growth ranges for the crop, which are between pH 6.0 and 7.0 (Pennsylvania State University, 2018). With re-application of the amendments in season two, the soils and leachates were all approximately pH 6.0 throughout the trial (Table 18).

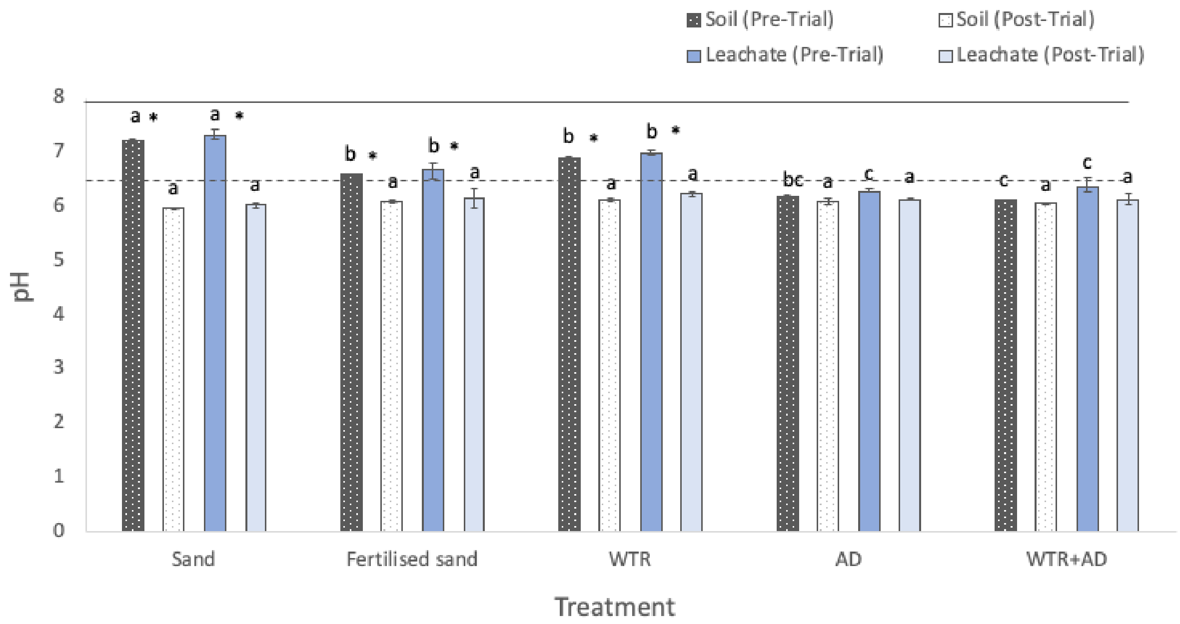

Figure 2.20. Soil and leachate pH were evaluated between treatments, pre- and post-trial, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment (WTR+AD). Bars represent the mean of triplicate treatments, and error bars the standard deviation. Significance lettering describes between-treatment differences, applied separately over each series (ANOVA, post-hoc Tukey's HSD test, $p<0.05$), and stars represent differences over time (pre- and post-trial; Student's t-test for independent means, $p<0.05$).

Table 2.11. Comparing pre-trial and post-trial pH, in soil extracts and leachate, during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	7.2±.03	6.2±.03	5.9±.07	6.1±.02	7.3±.01	5.9±.00	6.0±.04	6.1±.01
Sand F	6.6±.01	6.3±.02	6.1±.02	6.2±.03	6.7±.01	6.1±.01	6.2±.03	6.1±.03
WTR	6.9±.01	6.1±.02	6.1±.01	6.1±.03	7.0±.01	6.0±.08	6.2±.02	6.2±.00
AD	6.2±.03	6.0±.01	6.1±.00	6.1±.01	6.3±.03	6.1±.02	6.1±.01	6.1±.00
WTR+AD	6.1±.01	6.1±.00	6.1±.00	6.1±.01	6.4±.01	6.0±.07	6.1±.01	5.9±.05

Similarly, the AD had a more significant impact on soil and leachate electrical conductivity (EC) than WTR or fertilizer, increasing the conductivity up to 11-fold in season 1 (Figure 31). Over the second growing season, soil and leachate electrical conductivity increased even more, with fertilizer, AD and WTR+AD treatments (Table 19). Typically, the addition of organic matter like compost, and the associated ionic load, increases the electrical conductivity in the soil, which is associated with higher fertility. However, higher soil salinity (generally considered saline above 4000 µS/cm; UUSL, 1954; Lastiri-Hernandez, 2023) can hamper osmotic uptake in plants. According to hemp growing manuals, a range between 800 and 2000 µS/cm is ideal (Humpbolt Seed Organisation). Germination within the lower part of this range is recommended, whilst it is recommended to increase dissolved salt concentrations in the soils as the plants grow. However, the high EC in these amended soils did not negatively influence the hemp germination rate in growing seasons one or two (Section 4.2). According to national regulations for surface and groundwater protection for receiving water bodies, an EC of ≤300 µS/cm is ideal, 300-500 µS/cm is acceptable, 500-850 µS/cm is tolerable, and >850 µS/cm is critical (DWA, 2011; DWAF, 1996; Mudaly and Van der Laan, 2020). However, these are the levels in the receiving water body. The impact of this type of runoff will depend on volumes entering the water body and soil type (i.e. sorptive soils and hydrologically slow flow paths will minimise the impact). The conductivity values measured in these lysimeters, particularly in the groundwater runoff, are concerning in terms of the protection of water bodies but are optimal for crop growth in the soil. Over time, the dissolved salts in the soil and the leachate both drop, likely due to both leaching events and plant uptake, bringing the leachate levels much closer to the maximum allowable threshold in water bodies (850 µS/cm). Although not significant, the co-amendment soil and leachate mean EC were closer to the maximum allowable EC limit for groundwater protection than the individual AD amendment, suggesting a remediative effect by the WTR, since the same amount of AD is added to both treatments. During the second growing season (winter) the soils again showed a non-significant trend, with the EC of the co-amendment slightly lower than the individual AD amendment (Table 19). The second season was higher than the first season, however, suggesting that this may be a risk for groundwater and soil protection.

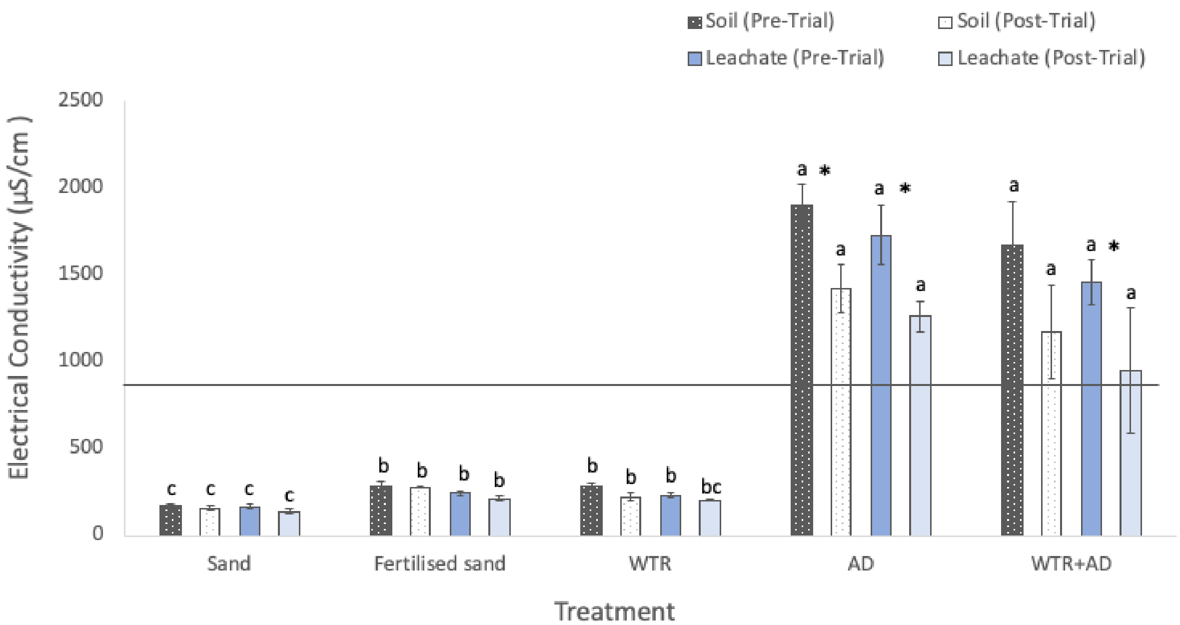

Figure 2.21. Soil and leachate electrical conductivity were evaluated between treatments, pre- and post-trial, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment. Bars represent the mean of triplicate treatments, and error bars the standard deviation. Significance lettering describes between-treatment differences, applied separately over each series (ANOVA, post-hoc Tukey's HSD test, $p<0.05$), and stars represent differences over time (Student's t-test for independent means, $p<0.05$). Solid line represents regulatory EC thresholds for receiving environmental waters (DWA, 2011).

Table 2.12. Comparing pre-trial and post-trial EC (µS/cm), in soil extracts and leachate, during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	170±12	154±3	156±18	132±15	166±14	121±10	136±10	110±23
Sand F	281±8	1581±68	276±19	1398±45	242±14	1345±87	213±11	1112±89
WTR	281±17	343±7	221±5	310±11	229±23	340±23	201±24	312±14
AD	1896±82	2876±53	1417±79	2221±92	1725±84	2542±32	1258±62	2132±21
WTR+AD	1670±43	2234±31	1168±60	2056±84	1456±102	2054±24	949±54	1998±25

2.6.3.2 Soil and leachate nutrients: Groundwater protection and fertility

Soil and leachate nutrients were significantly (multi-fold) higher in soils amended with AD and the AD+WTR co-amendment than the control sandy soil in season one (Figures 32-34) and season two (Tables 20-22). The fertilizer schedule used for wheat provided significantly more nitrogen (NH_4 and NO_3, Figures 32 and 33) and phosphate (P, Figure 34) than the sandy soil control, but not nearly as much as the organic AD amendment, and not sufficient to promote crop growth sufficiently. Thus, for the second season, the fertilizer schedule has been amended based on Wylie et al. (2021), pushing particularly the nitrogen fertilizer to the equivalent of 275 kg/ha, and the sequential applications were applied more frequently (per week, rather than every two weeks). This fertilizer adjustment improved crop biomass significantly (Figure 25B vs 25C, and Figure 26A vs 26 B, as well as soil NH_4-N, NO_3-N and PO_4-P concentrations). WTR also significantly improved the nitrogen (NH_4 and NO_3) in the nutrient-poor sandy soil, but only from 0 mg/kg to 1.5 mg/kg, whereas AD and the co-amendment increased it to 29 and 35 mg/kg, respectively, in season one. After repeat application for season two (winter), the initial ammonium, nitrate and phosphate concentrations were higher in the soil and leachate than season one (Tables 20-22), thus land application should be carefully monitored for potential eutrophication.

Mudaly and Van der Laan (2020) recently evaluated the effect of agricultural surface water runoff into the Olifants River. They elegantly describe using national water quality guidelines to monitor nutrient eutrophication levels, and the Receiving Water Quality Objectives (RWQOs) set out by the Department of Water Affairs (DWA) to monitor the chemistry (pH and EC) of runoff sources. Phosphate (PO_4-P) concentrations become a eutrophication risk over 0.025 mg/L. (DWA, 2011). The nitrate (NO_3-N) eutrophication risk threshold is 6 mg/L (DWA, 2011).

Individual WTR amendment did not increase phosphate levels in the soil, due to the phosphate sorption capacity that is well-reported in literature (Ippolito et al., 2003), however, fertilizer doubled the available phosphate in the nutrient-poor soil, and AD increased it by more than 5-fold (Season one, Figure 34). It was hypothesized that the co-amendment of WTR and AD might limit the bio-available phosphate from the AD in the soil, as well as in the leachate. However, there were no significant differences between phosphate levels in the soils amended with pure AD and the co-amendment. These co-amended lysimeters contained the same amount of AD (2.5% g_{dw}) as the individual treatment, but were also amended by replacing 2.5% of the sandy soil with WTR, a phosphate-sorptive material. Thus, the hypothesis was that the WTR might immobilize some of the AD-phosphate for plant accessibility rather than runoff. Although the differences between the individual AD amendment and the co-amendment were not significant, there was a trend towards lower mean phosphate levels in co-amended soils (16%

lower than individual AD amendments) and leachates (22% lower than individual AD amendments). At the end of the trial, this trend was still evident but the differences in means were lower (6% less bio-available phosphates in co-amended soils than in individual AD amendments, and 11% lower phosphate in the leachates from co-amended soils than from individual AD amendments). This suggests some potential immobilization of P in the soils by WTR, and a saturation of binding sites over time. However, the immobilization does not limit phosphate runoff to within national eutrophication risk limits (0.025 mg/L, in surface waters; Mudaly and van der Laan, 2020; DWA, 2011). The levels measured here are 100X the threshold accepted in environmental waters. The soils and leachates in growing season two were in a similar range (Table 22).

However, the DRASTIC Specific Vulnerability Index (DSVI) developed by Musekiwa and Majola (2014) describe several variables that influence vulnerability of groundwaters to these pollutants, including potential recharge rate, aquifer types, soil types, topography, vadose zone characteristics, hydraulic conductivity and land use. The amount of runoff, concentration of phosphate in the receiving ground- or surface water bodies and clay content in soil will influence the severity of the runoff. Van der Laan and Franke (2019) mention that very little contamination risk is due to leaching through the soil column, mostly attributing it to surface runoff and wind transfer of soils. They describe matching the input directly to crop utilisation as the ideal strategy for groundwater protection. In light of that strategy, it is encouraging that by the end of this trial (both growing seasons one and two, in summer and winter, respectively), the PO_4-P, NH_4-N, and NO_3-N concentrations measured in the soil (bio-available) and leachate were equivalent to the levels in the background sandy soil.

In terms of hemp fertilisation requirements, studies are conflicting but fertilisation regimes have shown that hemp responds particularly well to generous nitrogen amendments, and not predictably to P and K (Aubin et al., 2015, Vera et al., 2004). Vera et al. (2004) correlated various hemp parameters, including biomass, height, seed oil, and plant density, with growth parameters, including NO_3-N and PO_4-P. They concluded that, up to 120 kg-N/ha (their highest application rate), multiple hemp varieties responded well to increased soil nitrogen, particularly with plant density. Similar to Aubin et al. (2015), they concluded that there was no clear plant response to phosphate amendments.

Although there is no hemp fertilization protocol, Wylie et al. (2021) have done an extensive study of literature, and a number of studies have shown 100-120 kg-N/ha to be optimal (optimised for receiving soil characteristics) with some applying as high 275 kg-N/ha.

Based on this, the application rate in the lysimeters can be calculated as

If 1 ha = 10000 m^2,
then in 0.049 m^2 (surface area of one lysimeter) = 120 kg/10000*0.049……………………………Eq. 9.
= 1.3 g N per lysimeter

For the pre-trial co-amendment (Figures 32 and 33), which had the highest loading rate, the
1. Total bioavailable N (N-NO_3 + N-NH_4) = (35 mg/kg + 2.5 mg/kg) x 20.4 kg……………………Eq. 10.
= 0.75 g N per lysimeter

2. Total N (%) = 2.78 g N per lysimeter (see Figure 39)
Thus, the highest loading rate has in the range of the recommended soil N levels for optimal hemp growth. And, like PO_4-P, the available nitrogen was negligible by the end of the trial, in the soil and in the leachate, in comparison to the sandy soil control. At this loading rate, the N is sufficient for plant growth requirements, according to literature and these data (biomass), and the NO_3-N in leachate is well under the limit for environmental receiving water bodies (6 mg/L; Figure 16; Mudaly and van der Laan, 2020; DWA, 2011) in season one (Figure 33) and season two (Table 21).

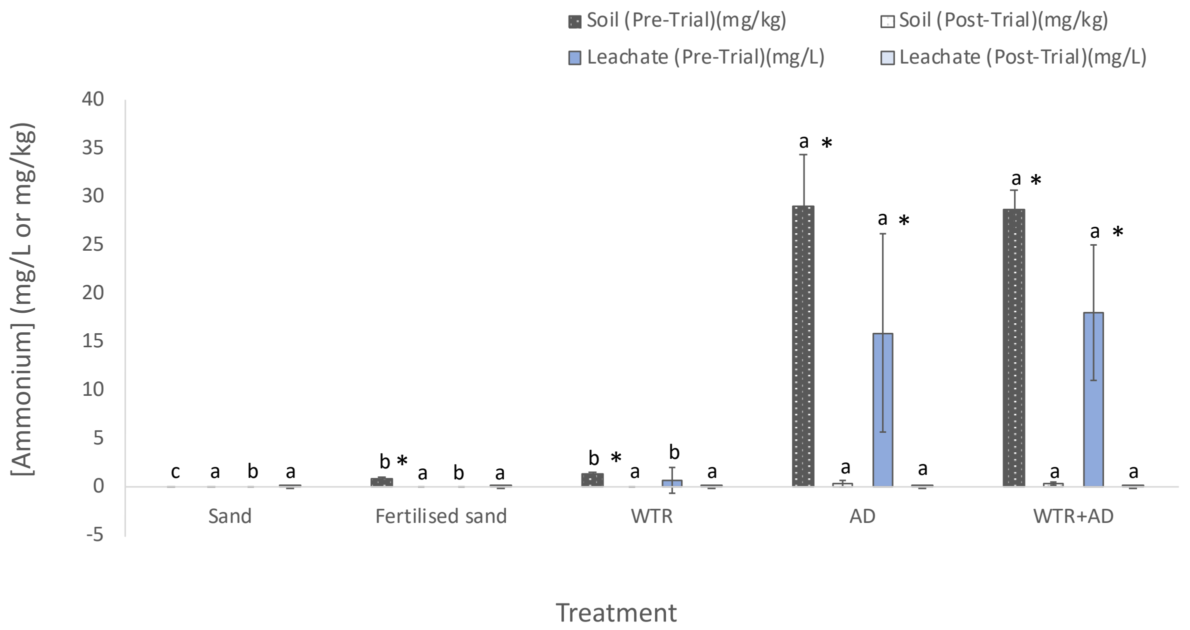

Figure 2.22. Soil and leachate ammonium (NH_4-N) were evaluated between treatments, pre- and post-trial, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment. Bars represent the mean of triplicate treatments, and error bars the standard deviation. Significance lettering describes between-treatment differences, using the same lettering over each separate series (ANOVA, post-hoc Tukey's HSD test, $p<0.05$), and stars represent differences over time (Student's t-test for independent means, $p<0.05$).

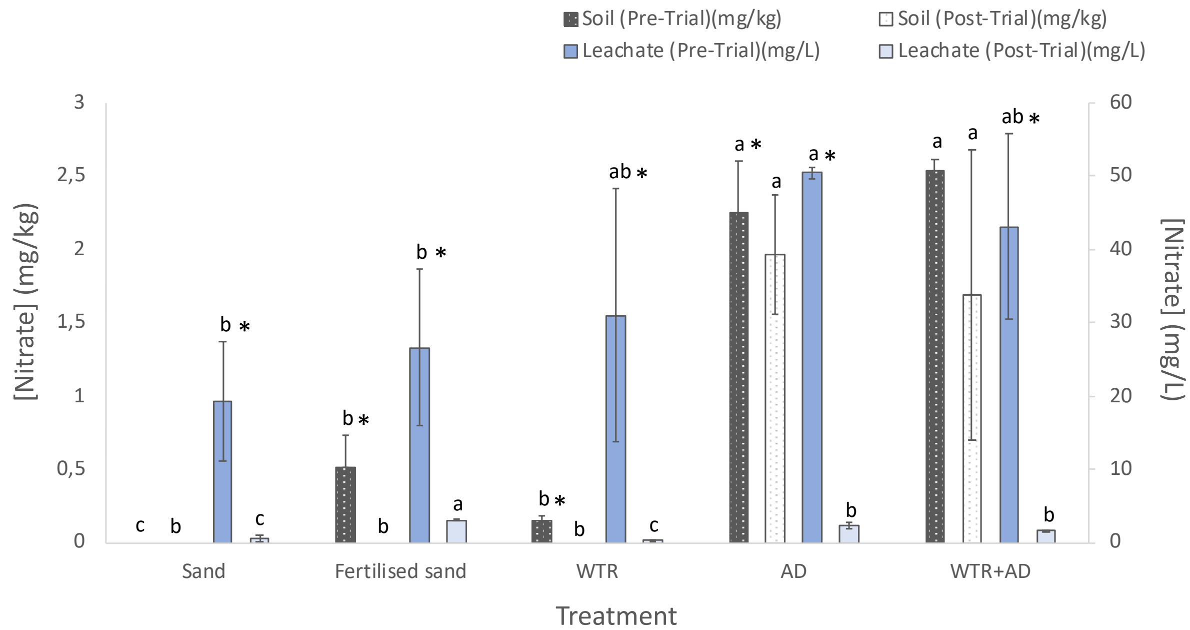

Figure 2.23. Soil and leachate nitrate (NO_3-N) were evaluated between treatments, pre- and post-trial, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment. Bars represent the mean of triplicate treatments, and error bars the standard deviation. Significance lettering describes between-treatment differences, applied separately over each series (ANOVA, post-hoc Tukey's HSD test, $p<0.05$), and stars represent differences over time (Student's t-test for independent means, $p<0.05$). The national eutrophication risk limit is 6 mg/L.

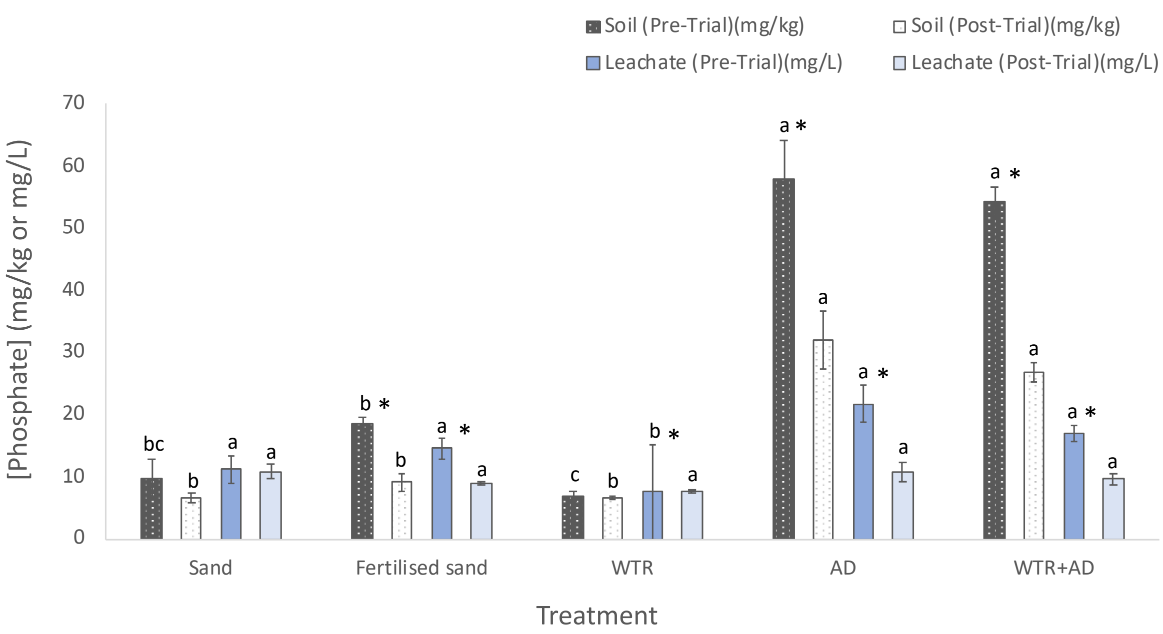

Figure 2.24. Soil and leachate phosphate (PO_4-P) were evaluated between treatments, pre- and post-trial, comparing nutrient-poor sandy soil to amendments, including fertilizer, WTR, AD and a sludge co-amendment. Bars represent the mean of triplicate treatments, and error bars the standard deviation. Significance lettering describes between-treatment differences, applied separately over each series (ANOVA, post-hoc Tukey's HSD test, $p<0.05$), and stars represent differences over time (Student's t-test for independent means, $p<0.05$).

Table 2.13. Comparing pre-trial and post-trial ammonium (NH_4-N), in soil extracts (mg/kg) and leachate (mg/L), during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0	0.0±0
Sand F	0.9±0.0	28.3±0.0	0.0±0.0	0.0±0.0	0.0±0.0	11.0±0.0	0.0±0	0.0±0
WTR	1.4±0.1	1.3±0.5	0.0±0.0	0.0±0.0	0.7±0.0	0.0±0.0	0.0±0	0.0±0
AD	29.0±5.0	34.0±4.3	0.4±0.1	0.4±0.0	16.0±2.8	11.0±2.5	0.0±0	0.0±0
WTR+AD	35.7±5.3	36.0±5.0	0.4±0.0	0.1±0.0	18.1±6.1	17.0±4.3	0.0±0	0.0±0

Table 2.14. Comparing pre-trial and post-trial nitrate (NO_3-N), in soil extracts (mg/kg) and leachate (mg/L), during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	19.3±3.4	10.1±0.1	0.5±0.1	0.0±0.0
Sand F	0.5±0.1	2.8±0.0	0.0±0.0	1.9±0.0	26.6±5.0	48.0±3.1	3.0±0.3	4.0±0.2
WTR	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	31.0±8.1	25.4±0.8	0.3±0.1	1.8±0.2
AD	2.3±1.0	4.0±0.3	2.0±0.1	2.0±0.2	50.4±2.3	59.0±2.9	2.3±0.1	7.0±0.0
WTR+AD	2.5±0.8	3.8±0.2	1.7±0.2	2.8±0.0	43.1±0.8	58.0±10.1	1.6±0.1	2.4±0.1

Table 2.15. Comparing pre-trial and post-trial phosphate (PO_4-P), in soil extracts (mg/kg) and leachate (mg/L), during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	9.8±0.7	8.1±0.2	6.6±0.2	6.2±0.0	11.2±0.8	8.9±1.0	10.8±0.0	9.0±0.1
Sand F	18.5±1.4	51.8±1.8	19.1±0.1	28.4±0.1	14.5±0.2	19.3±0.4	8.9±0.2	10.0±0.2
WTR	6.9±0.3	5.0±0.0	6.5±2.3	4.9±0.2	7.6±1.0	5.5±0.8	7.7±0.3	5.1±0.0
AD	57.9±0.4	64.8±0.4	32.0±4.5	38.9±0.1	21.7±0.3	26.8±2.0	10.8±3.0	8.0±0.2
WTR+AD	54.3±2.3	58.3±3.4	26.7±3.2	29.4±0.8	16.9±3.6	20.2±4.1	9.6±1.0	8.8±1.0

2.6.4 Plant Nutrients: Uptake and Sufficiency

A similar nutrient pattern has emerged in a series of studies on multiple crops grown on this co-amendment, ranging from wheat (Clarke et al., 2019) to spinach (Steytler, 2021) and hemp (current study). The balance (approximately equal percentages) of foliar macronutrients (N and P) is repeatedly associated with the highest yield. In wheat grown on a WTR+compost co-amendment, the WTR amendment was phosphate-limited (Clarke et al., 2019), which is common in literate (Ippolito et al., 2013). However, the compost was nitrogen-limited, and rich in phosphate, and the two co-amendment provided the complementary balance of nitrogen and phosphate, for improved crop yield and approximately balanced (%) foliar macronutrients (N and P). In this study, the same trends emerge in summer (Figure 35A), but and winter (Figure 35B) when the plants were stressed and tended towards flowering rather than fibrous growth. During summer, the co-amendment produced the highest crop biomass, and the optimal N:P foliar ratio (Figure 35A). In winter, with energy diverted to seeding rather than high biomass, foliar P concentrations in the crops grown in co-amended soils had an N:P ratio similar to crops grown in AD amendments (Figure 35B). Interestingly, the trend across all treatments in season two is a higher foliar N and P content than in season one. This could be due to stunted biomass, with the diversion of energy away from growth to seeding.

As reviewed in Wylie et al. (2021), previous studies have shown that hemp growth responds unpredictably to phosphate amendment, when fertilizing soils, with some studies even showing lower yield in response to applied P (Vera et al., 2004). In contrast, nitrogen amendment to soils typically promotes crop yield and growth parameters. Potentially, the AD facilitates the excess nitrogen necessary to promote hemp growth, and the WTR co-amendment limits some of the phosphate.

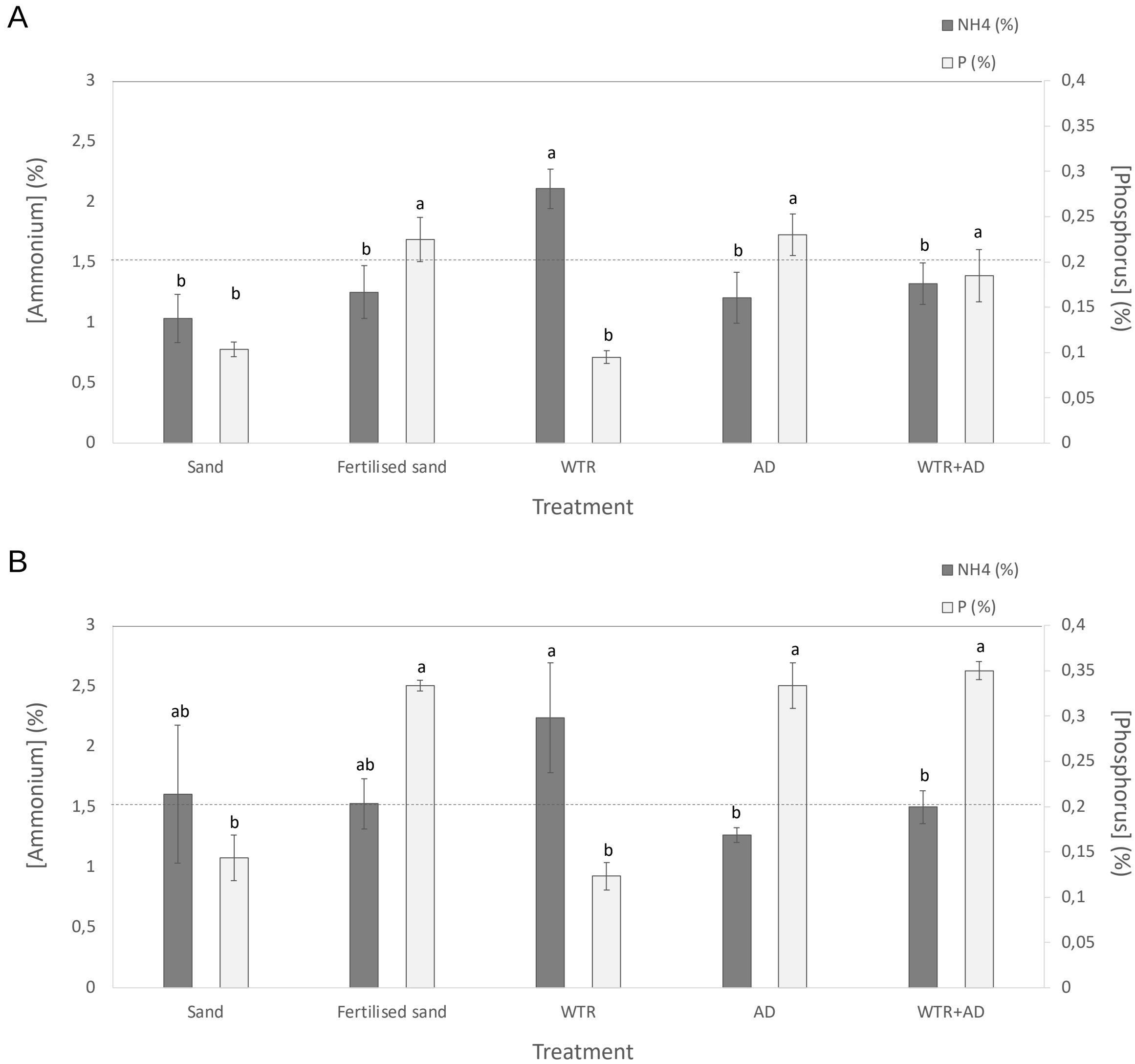

Figure 2.25. Foliar macronutrient content of harvested hemp plants, as a weight percentage in fertilized lysimeters, and lysimeters amended with WTR, AD and WTR+AD, compared to the control (Sand). Critical and sufficiency macronutrient levels are not available for hemp, but for wheat (Plank et al., 2000) shown by solid (NH_4) and dashed (P) lines. Bars represent the means of 9 plants over 3 lysimeters, and error bars the standard deviation. Significance lettering shows bars that do not differ significantly ($p < 0.05$) marked with the same letter, applied separately over each series.

2.6.5 Microbial community dynamics: Cell density and pathogen persistence

Microbial dynamics reflect similar dynamics to recent studies on wheat investigating similar diversity and pathogen persistence profiles (Stone et al., 2021). In terms of total prokaryotes, the viable colonies in WTR and AD are 10- to 100-fold higher, respectively, than in the sandy soil (Figures 36), a pattern across both growing seasons (data not shown for season two, similar trends). Pre-trial material characterization showed that Salmonella and Shigella are low to negligible in sand and WTR. E. coli is persistent in AD in the general restrictions range, despite pasteurization (Figure 36). As expected, the Colilert quantification is more stringent than the selective medium, which is renowned to give false positives. E. coli counts quantified on selective medium, in the AD fall within the general use guidelines for the land application of sludge (USEPA 1994, Snyman and Herselman, 2013). E. coli and faecal coliforms quantified with the Colilert MPN method fall within the unrestricted use guidelines. A similar pattern was clear in the post-trial soils (Figure 37) and in the leachate (Figure 38). All E. coli concentrations, although higher on the selective media than via the MPN method, were well within land application guidelines. It is notable that Salmonella and E. coli and faecal coliforms (MPN) were undetectable in the leachate after the trial. In contrast, Shigella persisted, supporting previous work suggesting the broadening of the suite of pathogenic parameters when monitoring pollution remediation (Stone et al., 2021). The amendments all increased total prokaryotic populations in the sandy soils, as well as in the leachate. The second growing season showed similar trends with very little increase in pathogen concentration upon re-application of the wastes (Tables 23 and 24). Again, E. coli did not survive in the soil or leachates post-trial, but Shigella did. Microbial persistence on plant matter and transfer across the physiology of the fibrous crop was investigated. No pathogens were detected on the top half the plants, but a few individual E. coli and Salmonella colonies were detected on the bottom half of the plant, only in lysimeters with AD amendments.

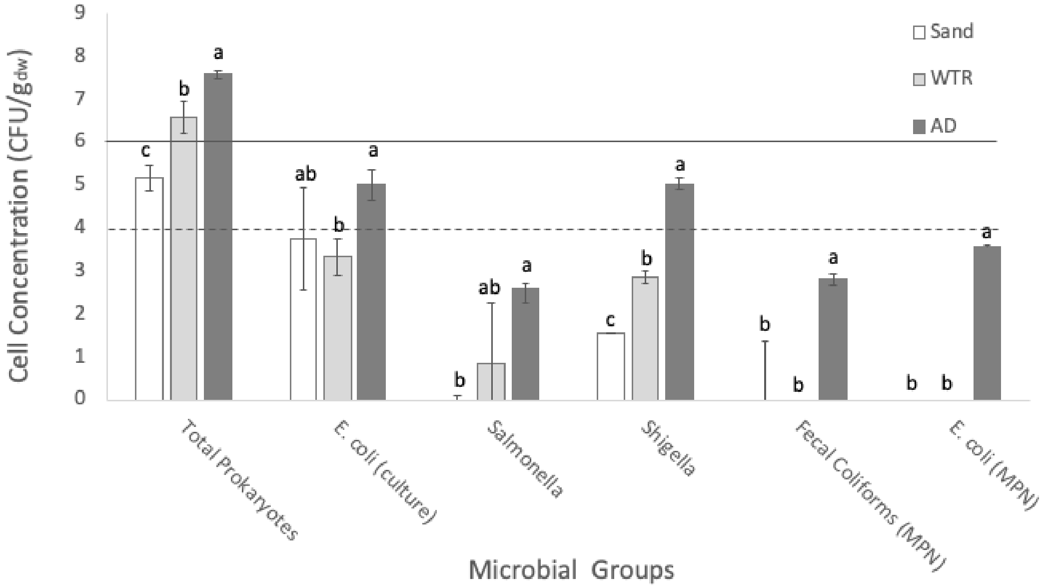

Figure 2.26. Microbial concentrations (CFU/g$_{dw}$) in the materials, comparing the total prokaryotic footprint, as well as pathogens enumerated through standard plate counts on selective media [E. coli (culture), Salmonella and Shigella]. Faecal coliforms and total coliforms, the target indicator organisms in the national drinking water standards (SANS241) are enumerated with Colilert MPN techniques. Lines represent the maximum thresholds for the restricted use (solid) and unrestricted use (dashed) of sludge in land application, according to national guidelines (Snyman and Herselman, 2013). Bars represent the mean of triplicate treatments, and error bars the standard deviation. Significance lettering describes differences between the materials, sand, WTR and pasteurised AD (ANOVA, post-hoc Tukey's HSD test, p<0.05). Significance lettering is reported separately for each microbial group.

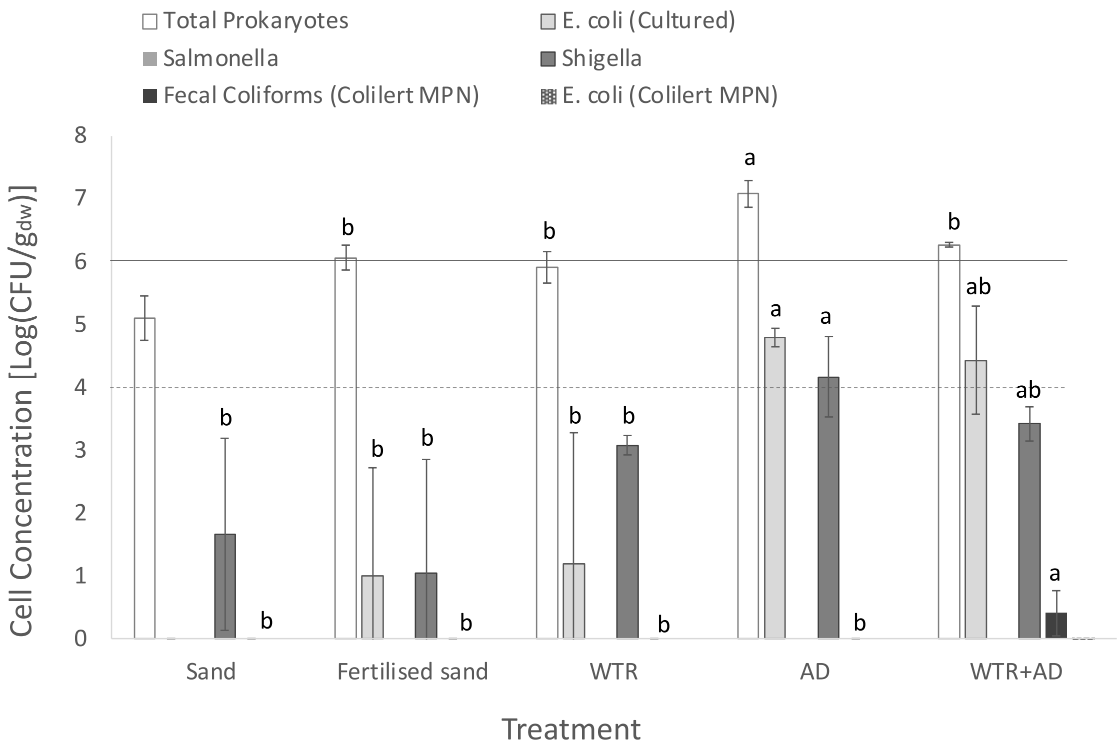

Figure 2.27. Microbial concentrations (CFU/g) in the post-trial lysimeter soils, comparing the total prokaryotic footprint, and pathogens enumerated through standard plate counts on selective media [E. coli (culture), Salmonella and Shigella]. Faecal coliforms and total coliforms, the target indicator organisms in the national drinking water standards (SANS241) are enumerated with Colilert MPN techniques. Bars represent the mean of triplicate lysimeters per treatment, and error bars the standard deviation. Significance lettering describes between-treatment differences, applied separately over each series (ANOVA, post-hoc Tukey's HSD test, p<0.05). The dashed bar represents national limits (E. coli) for unrestricted use, and the solid line the limit for restricted use.

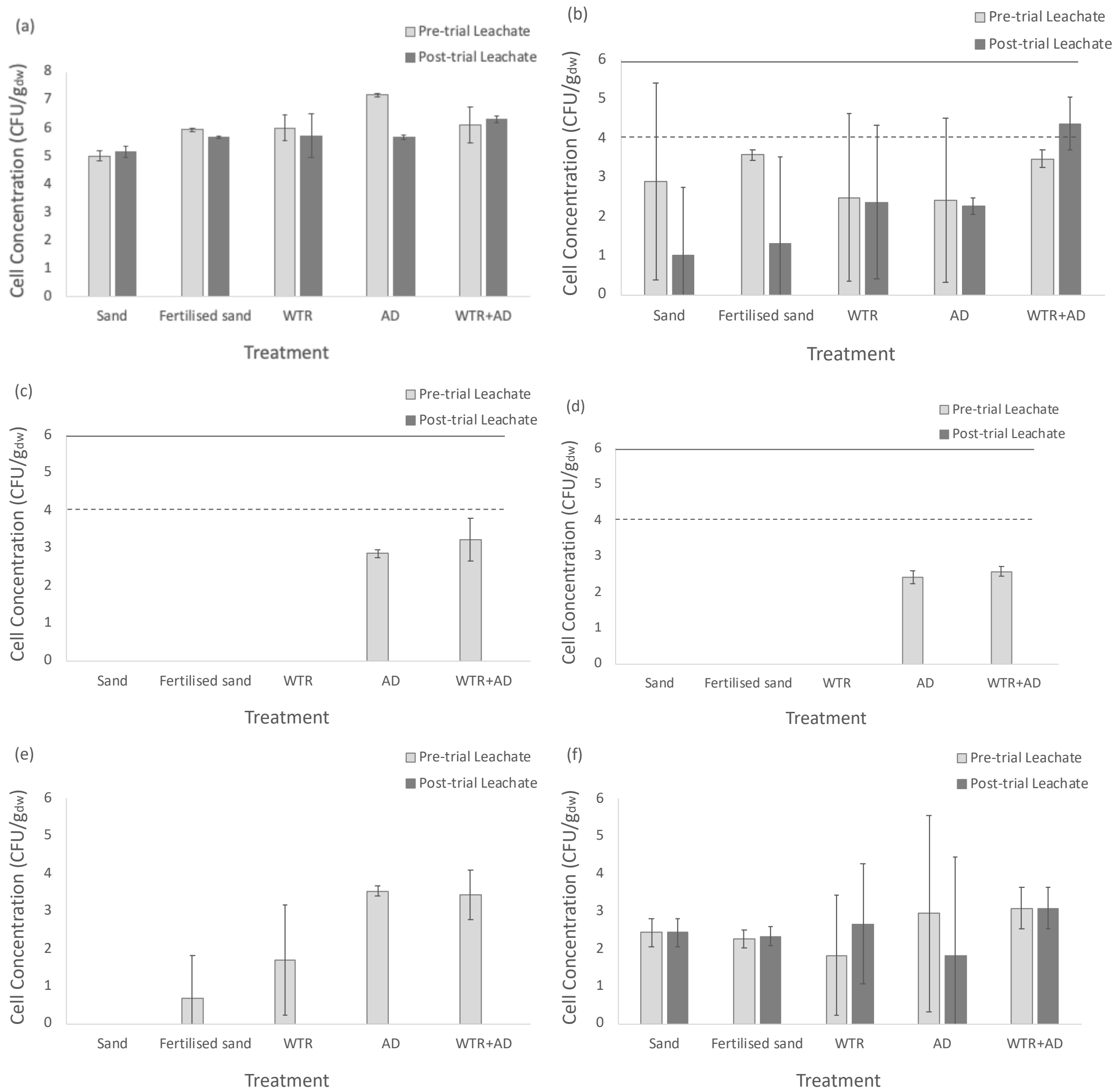

Figure 2.28. Microbial concentrations (as colony forming units, CFU/g_{dw}) in the leachate, pre-trial and post-trial. Standard plate dilution series on agar (a,b,e,f) and Colilert Quantitrays for Most Probable Number (MPN, c, d) were evaluated. Groups and/or species include (a) total prokaryotes (Tryptic Soy), (b) E. coli (Endo-agar), (c) Faecal coliforms (Colilert MPN), (d) E. coli (Colilert MPN), (e) Salmonella, and (f) Shigella. Bars represent the mean of triplicate samples, and error bars represent standard deviation.

Table 2.16. Comparing pre-trial and post-trial E. coli (IDEXX) concentrations [log(CFU/ g_{dw})], in soil extracts and leachate, during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0	0.0±0
Sand F	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0	0.0±0
WTR	0.0±0.0	0.0±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0	0.0±0
AD	3.0±0.5	2.5±0.3	0.0±0.0	0.1±0.0	2.4±0.1	1.8±0.1	0.0±0	0.0±0
WTR+AD	3.2±0.3	2.6±5.0	0.53±0.0	0.4±0.0	2.5±1.3	2.0±1.3	0.0±0	0.0±0

Table 2.17. Comparing pre-trial and post-trial Shigella concentrations (log(CFU/ g_{dw})), in soil extracts and leachate, during growing season one (summer) and two (winter).

Treatment	Soil				Leachate			
	Pre-Trial		Post-Trial		Pre-Trial		Post-Trial	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Sand	2.4±0.6	2.5±1.1	2.4±1.6	2.3±0.3	1.7±0.9	1.0±0.2	1.0±0.2	1.3±0.7
Sand F	2.3±1.2	2.8±0.3	2.3±0.2	2.5±0.3	1.0±0.8	0.9±0.2	0.9±0.3	1.2±0.5
WTR	1.9±0.4	3.0±0.7	2.6±0.1	2.4±1.2	3.1±0.2	2.8±1.1	2.6±1.2	0.9±1.1
AD	2.9±0.2	2.6±0.7	1.8±0.9	1.9±0.3	4.2±0.3	3.0±1.2	2.3±0.2	1.2±0.2
WTR+AD	3.1±0.2	3.1±0.2	3.0±0.3	2.0±0.2	3.4±0.4	2.8±1.0	3.0±0.6	2.5±0.3

Table 2.18a. Total (Aqua Regia) heavy metal risk assessment for materials and mixes (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	Zn mg/kg	As mg/kg	Se µg/kg	Sr µg/kg	Mo µg/kg	Cd mg/kg	Sn µg/kg	Sb µg/kg	Ba µg/kg	Hg mg/kg	Pb mg/kg
Receiving Sandy Soil (Bn[a])	7.6	3.2	107.5	5338.7	510.5	13.7	833.4	134.5	31509.9	6.4	3.1
WTR (Cn[b])	40.7	11.5	2186.7	62231.2	1008.2	35.9	2843.0	382.5	388452.1	34.0	25.7
AD (Cn)	982.0	6.9	2283.5	363538.4	10457.5	1620.5	34537.9	2186.4	194954.1	1190.2	54.4
Herselman Guidelines (Receiving Soils) Aqua Regia (TIL[c]/TMT[d])	**185 / 200**	**2 / 2**	**n.a.**	**n.a.**	**n.a.**	**2 / 3**	**n.a.**	**n.a.**	**n.a.**	**500 / 1000**	**56 / 100**
Total Igeo[e] WTR	1.8	1.3	3.8	3.0	0.4	0.8	1.2	0.9	3.0	1.8	2.5
Total Igeo AD	6.4	0.5	3.8	5.5	3.8	6.3	4.8	3.4	2.0	7.0	3.6
2.5% Igeo WTR	-0.4	-0.5	0.0	-0.2	-0.5	-0.5	-0.5	-0.5	-0.2	-0.4	-0.3
2.5% Igeo AD	1.5	-0.5	0.0	0.9	0.0	1.4	0.5	-0.1	-0.4	1.9	0.0

[a]Geological chemical background value (Zhao et al., 2022).
[b]Heavy metal measurement of amendment (Zhao et al., 2022)
[c]Total investigative level (Herselman, 2013)
[d]Total maximum threshold (Herselman, 2013)
[e]Geoaccumulation index (Zhao et al., 2022)
[f]n.a. Not applicable
[g]b.d. Below detection

Table 2.18a cont. Total (Aqua Regia) heavy metal risk assessment for materials and mixes (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	B µg/kg	Al mg/kg	Si µg/kg	V µg/kg	Cr mg/kg	Mn µg/kg	Fe mg/kg	Co µg/kg	Ni mg/kg	Cu mg/kg
Receiving Sandy Soil (Bn)	5100.8	5736.4	2513799.1	9597.7	166.2	38025.8	6007.3	1152.5	5.5	4.5
WTR (Cn)	15009.2	97711.0	2719813.1	95172.4	72.2	270944.0	37680.6	9301.6	19.7	12.5
AD (Cn)	38451.7	13419.8	2038976.3	16614.9	392.5	170089.3	20217.0	3619.7	28.9	261.0
Herselman Guidelines (Receiving Soils) Aqua Regia (TIL/TMT)	**n.a.**	**10 000 / 40 000**	**n.a.**	**n.a.**	**80 / 350**	**n.a.**	**1000 / 100 000**	**n.a.**	**50 / 150**	**100 / 120**
Total Igeo WTR	1.0	3.5	-0.5	2.7	-1.8	2.2	2.1	2.4	1.3	0.9
Total Igeo AD	2.3	0.6	-0.9	0.2	0.7	1.6	1.2	1.1	1.8	5.3
2.5% Igeo WTR	-0.5	-0.1	-0.5	-0.3	-0.6	-0.3	-0.4	-0.3	-0.5	-0.5
2.5% Igeo AD	-0.3	-0.5	-0.6	-0.5	-0.5	-0.4	-0.5	-0.5	-0.4	0.7

Table 2.18b. Nutrient accumulation risk assessment for materials and mixes (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	Na mg/kg	Mg mg/kg	P mg/kg	K mg/kg	Ca mg/kg
Receiving Sandy Soil (Bn)	134.1	348.0	77.9	882.9	282.6
WTR (Cn)	549.6	1975.7	599.0	11290.6	2039.2
AD (Cn)	1812.1	2845.8	10566.8	1865.8	58757.5
Herselman Guidelines (Receiving Soils) Aqua Regia (TIL/TMT)	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Total Igeo WTR	1.5	1.9	2.4	3.1	2.3
Total Igeo AD	3.2	2.4	6.5	0.5	7.1
2.5% Igeo WTR	-0.4	-0.4	-0.3	-0.2	-0.3
2.5% Igeo AD	-0.2	-0.3	1.6	-0.5	2.1

Table 2.18c. Bio-available (NH_4NO_3) heavy metal risk assessment for materials and mixes, according to Herselman's (2013) guidelines for receiving soils.

	Zn mg/kg	As mg/kg	Se mg/kg	Cd mg/kg	Sb mg/kg	Ba mg/kg	Hg mg/kg	Pb mg/kg
Receiving Sandy Soil (Bn)	1.093	0.012	0.005	0.001	0.001	3.496	0.000	0.005
WTR (Cn)	0.037	0.029	0.003	0.000	0.001	4.408	0.000	0.003
AD (Cn)	2.224	0.357	0.041	0.004	0.085	0.381	0.002	0.019
Herselman Guidelines (Receiving Soils) NH_4NO_3 (MAT[f])	**5**	**0.014**	**n.a.**	**0.1**	**n.a.**	**n.a.**	**0.007**	**3.5**

[f]Maximum available threshold

Table 2.18c cont. Bio-available (NH_4NO_3) heavy metal risk assessment for materials and mixes, according to Herselman's (2013) guidelines for receiving soils.

	Al mg/kg	V mg/kg	Cr mg/kg	Mn mg/kg	Fe mg/kg	Co mg/kg	Ni mg/kg	Cu mg/kg
Receiving Sandy Soil (Bn)	0.928	0.009	0.007	1.653	0.749	0.011	0.009	0.014
WTR (Cn)	0.210	0.002	0.001	26.467	0.022	0.003	0.014	0.000
AD (Cn)	1.132	0.127	0.029	3.008	3.966	0.348	0.973	4.679
Herselman Guidelines (Receiving Soils) NH_4NO_3 (MAT)	**n.a.**	**n.a.**	**0.1**	**n.a.**	**n.a.**	**n.a.**	**1.2**	**1.2**

Table 2.19a. Heavy metal risk assessment for leachate pre-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	Zn mg/L	As mg/L	Se µg/L	Sr µg/L	Mo µg/L	Cd µg/L	Sn µg/L	Sb µg/L	Ba µg/L	Hg µg/L	Pb µg/L
Receiving Sandy Soil (Bn)	4.4	6.9	2.5	263.4	0.6	0.0	0.1	1.9	99.9	0.0	27.3
Fertilizer	31.9	3.1	1.5	585.0	0.3	0.2	0.0	0.6	85.9	0.0	4.9
WTR (Cn)	-2.9	5.3	2.3	229.3	0.5	0.0	0.0	2.1	118.4	0.0	9.0
AD (Cn)	180.3	7.7	2.3	3394.1	10.1	0.5	0.0	3.2	317.5	0.0	39.8
WTR+AD	122.2	5.1	3.3	2545.7	3.0	0.5	0.0	2.4	322.4	0.0	86.1
Target	2	10	2	-	-	0.15	-				
Chronic Toxicity	3.6	20	5			0.3					
Acute Toxicity	36	130	30			3					
Igeo Fertilizer	2.3	-1.8	-1.3	0.6	-1.5	1.7	0.0	-2.4	-0.8	-0.7	-3.1
Igeo WTR	0.0	-1.0	-0.7	-0.8	-0.9	-0.9	0.0	-0.5	-0.3	0.0	-2.2
Igeo AD	4.8	-0.4	-0.7	3.1	3.4	3.2	0.0	0.2	1.1	1.5	0.0
Igeo WTR+AD	4.2	-1.0	-0.2	2.7	1.7	3.3	0.0	-0.2	1.1	0.4	1.1

Table 2.19a cont. Heavy metal risk assessment for leachate pre-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	B µg/L	Al µg/L	Si µg/L	V µg/L	Cr µg/L	Mn µg/L	Fe µg/L	Co µg/L	Ni µg/L	Cu µg/L
Receiving Sandy Soil (Bn)	146.9	136.7	6.3	5.1	5.5	562.2	1.2	2.8	10.0	146.9
Fertilizer	310.3	600.1	4.3	4.4	22.4	494.7	0.7	2.4	8.9	310.3
WTR (Cn)	92.1	77.4	4.3	2.4	5.7	193.1	0.8	1.9	5.2	92.1
AD (Cn)	392.7	107.4	8.2	3.2	249.8	169.6	7.0	21.7	68.1	392.7
WTR+AD	271.4	218.9	6.1	2.6	185.4	138.4	3.9	10.2	16.6	271.4
Target	-	5	-	-	7	180	-	-	-	0.3
Chronic Toxicity		10			14	370				0.53
Acute Toxicity		100			200	1300				1.6
Igeo Fertilizer	0.5	1.5	-1.1	-0.8	1.4	-0.8	-1.3	-0.8	-0.8	0.5
Igeo WTR	-1.3	-1.4	-1.1	-1.7	-0.5	-2.1	-1.1	-1.1	-1.5	-1.3
Igeo AD	0.8	-0.9	-0.2	-1.3	4.9	-2.3	2.0	2.4	2.2	0.8
Igeo WTR+AD	0.3	0.1	-0.6	-1.6	4.5	-2.6	1.2	1.3	0.1	0.3

Table 2.19b. Nutrient accumulation risk assessment for leachate pre-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	Ca mg/L	K mg/L	Mg mg/L	Na mg/L	P mg/L
Receiving Sandy Soil (Bn)	28.1	13.8	15.4	53.2	0.2
Fertilizer	61.6	12.3	60.2	327.3	0.1
WTR (Cn)	20.6	11.6	11.0	48.8	0.1
AD (Cn)	571.0	50.7	132.1	237.1	0.8
WTR+AD	401.5	43.7	111.5	205.2	0.3
Igeo Fertilizer	0.5	-0.8	1.4	2.0	-1.2
Igeo WTR	-1.0	-0.8	-1.1	-0.7	-1.0
Igeo AD	3.8	1.3	2.5	1.6	1.7
Igeo WTR+AD	3.3	1.1	2.3	1.4	0.4

Table 2.20. Nutrient and heavy metal risk assessment for leachate post-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	Ca mg/L	K mg/L	Mg mg/L	Na mg/L	P mg/L	Zn µg/L	Sr µg/L	Ba µg/L	Pb µg/L	As µg/L	Cd µg/L
Receiving Sandy Soil (Bn)	8.3	0.7	3.2	14.0	0.0	53.1	62.8	53.0	23.5	2.3	61.1
Fertilizer	7.4	0.9	3.3	24.7	0.1	298.2	49.7	539.0	3.5	4.8	75.0
WTR (Cn)	6.5	0.8	3.4	35.0	0.1	84.5	102.6	232.1	17.9	0.9	41.5
AD (Cn)	5.4	0.9	3.5	40.4	0.2	124.5	1078.3	180.3	32.0	4.6	126.2
WTR+AD	8.5	1.3	4.4	32.0	0.1	95.9	798.8	158.4	20.1	3.4	91.2
Target	-	-	-	-	-	2	-	-	0.2	10	0.15
Chronic Toxicity						3.6			0.5	20	0.3
Acute Toxicity						36			4	130	3
Igeo Fertilizer	-0.7	-0.3	-0.6	0.2	0.0	1.9	-0.9	2.8	-3.3	1.9	-0.3
Igeo WTR	-0.9	-0.3	-0.5	0.7	0.0	0.1	0.1	1.5	-1.0	0.1	-1.1
Igeo AD	-1.2	-0.3	-0.5	0.9	0.0	0.6	3.5	1.2	-0.1	0.6	0.5
Igeo WTR+AD	-0.5	0.3	-0.1	0.6	0.0	0.3	3.1	1.0	-0.8	0.0	0

Table 2.20 cont. Nutrient and heavy metal risk assessment for leachate post-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	B µg/L	Al µg/L	Si µg/L	Cr µg/L	Mn µg/L	Fe µg/L	Co µg/L	Ni µg/L	Cu µg/L	Hg µg/L
Receiving Sandy Soil (Bn)	0.0	264.2	4164.0	0.0	4.3	715.9	0.0	0.0	0.0	0
Fertilizer	184.4	172.5	3216.7	0.0	13.1	2158.0	0.0	0.0	7.6	0
WTR (Cn)	126.9	153.4	3284.7	0.0	0.0	134.1	0.0	0.0	6.2	0
AD (Cn)	83.6	249.8	3501.0	0.0	37.1	369.6	0.0	5.0	33.2	0
WTR+AD	63.6	213.7	4231.3	0.0	26.0	204.7	0.0	0.0	19.4	0
Target	-	5	-	7	180	-	-	-	0.3	0.04
Chronic Toxicity		10		14	370				0.53	0.08
Acute Toxicity		100		200	1300				1.6	1.7
Igeo Fertilizer	0.0	2.1	-1.0	0.0	1.0	1.0	0.0	0.0	15.6	0
Igeo WTR	13.0	-1.4	-0.9	0.0	0.0	-3.0	0.0	0.0	15.3	0
Igeo AD	12.4	-0.7	-0.8	0.0	0.0	-1.5	0.0	0.0	17.8	0
Igeo WTR+AD	12.0	-0.9	-0.6	0.0	2.0	-2.4	0.0	0.0	17.0	0

Table 2.21. Nutrient and heavy metal risk assessment for soil post-trial (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	Ca mg/kg	K mg/kg	Mg mg/kg	Na mg/kg	P mg/kg	Zn µg/kg	Sr µg/kg	Ba µg/kg	Pb µg/kg	As mg/kg	Cd µg/kg
Receiving Sandy Soil (Bn)	0.7	0.1	0.1	0.1	0.0	561.3	5473.3	12834.7	0.0	20.4	1.6
Fertilizer	0.6	0.1	0.2	0.1	0.0	1501.0	5460.0	5143.0	0.0	12.5	2.9
WTR (Cn)	0.7	0.1	0.1	0.1	0.0	945.3	6081.0	9765.7	0.0	2.6	2.0
AD (Cn)	2.3	0.1	0.1	0.1	0.0	1161.0	16186.7	8829.7	0.0	41.5	1.8
WTR+AD	2.2	0.1	0.1	0.1	0.0	4875.3	16216.7	6882.7	0.0	14.4	3.8
Herselman Guidelines (Receiving Soils) NH_4NO_3 (MAT)	-	-	-	-	-	5000	-	-	3500	14	100
Igeo Fertilizer	-0.9	-0.7	-0.2	-0.3	0.0	0.8	-0.6	-1.9	0.0	-1.3	0.3
Igeo WTR	-0.4	-0.2	-0.5	-0.7	0.0	0.2	-0.4	-1.0	0.0	-3.5	-0.2
Igeo AD	1.2	0.1	-0.7	-0.1	0.0	0.5	1.0	-1.1	0.0	0.4	-0.4
Igeo WTR+AD	1.1	-0.4	-0.8	0.0	0.0	2.5	1.0	-1.5	0.0	-1.1	0.7

Table 2.21 cont. Nutrient and heavy metal risk assessment for soil post-trial (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	B µg/kg	Al µg/kg	Si µg/kg	Cr µg/kg	Mn µg/kg	Fe µg/kg	Co µg/kg	Ni µg/kg	Cu µg/kg	Hg µg/L
Receiving Sandy Soil (Bn)	437.7	2200.3	3894.7	0.0	9320.0	1772.0	0.0	0.0	0.0	0
Fertilizer	0.0	5999.7	2706.3	0.0	5771.7	2613.7	0.0	0.0	0.0	0
WTR (Cn)	188.0	1782.3	4067.7	0.0	4941.0	835.3	0.0	0.0	121.3	0
AD (Cn)	176.0	1076.7	10209.0	0.0	4671.0	1786.3	0.0	0.0	372.7	0
WTR+AD	0.0	818.7	7599.3	0.0	2469.3	922.7	0.0	0.0	354.3	0
Herselman Guidelines (Receiving Soils) NH_4NO_3 (MAT)	-	-	-	100	-	-	-	1200	1200	7
Igeo Fertilizer	0.0	0.9	-1.1	0.0	-1.3	0.0	0.0	0.0	0.0	0
Igeo WTR	-1.8	-0.9	-0.5	0.0	0.0	-1.7	0.0	0.0	9.7	0
Igeo AD	-1.9	-1.6	0.8	0.0	0.0	-0.6	0.0	0.0	11.3	0
Igeo WTR+AD	0.0	-2.0	0.4	0.0	-2.5	-1.5	0.0	0.0	11.2	0

SEASON TWO

Table 2.22. Nutrient and heavy metal risk assessment for leachate post-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	Ca mg/L	K mg/L	Mg mg/L	Na mg/L	P mg/L	Zn µg/L	Sr µg/L	Ba µg/L	Pb µg/L	As µg/L	Cd µg/L
Receiving Sandy Soil (Bn)	85,4	9,3	35,9	46,9	0,0	96,7	591,9	180,8	0.0	0.0	0.0
Fertilizer	234,3	143,9	169,0	137,8	0,3	342,4	1974,8	506,7	0.0	0.0	0.0
WTR (Cn)	75,9	9,1	31,3	51,9	0,0	9,7	522,1	134,6	0.0	0.0	0.0
AD (Cn)	626,0	8,1	60,5	106,3	0,5	95,3	2954,6	114,7	0.0	0.0	0.0
WTR+AD	511,1	4,6	49,3	103,8	0,2	42,8	2296,6	102,3	0.0	0.0	0.0
Target	-	-	-	-	-	2	-	-	0.2	10	0.15
Chronic Toxicity						3.6			0.5	20	0.3
Acute Toxicity						36			4	130	3
Igeo Fertilizer	0,9	3,4	0,0	1,0	2,0	1,2	1,2	0,9	0.0	0.0	0.0
Igeo WTR	-0,8	-0,6	-0,8	-0,4	-1,1	-3,9	-0,8	-1,0	0.0	0.0	0.0
Igeo AD	2,3	-0,8	0,2	0,6	2,6	-0,6	1,7	-1,2	0.0	0.0	0.0
Igeo WTR+AD	2,0	-1,6	-0,1	0,6	1,7	-1,8	1,4	-1,4	0.0	0.0	0.0

Table 2.22 cont.. Nutrient and heavy metal risk assessment for leachate post-trial (DWAF 1996 guidelines for aquatic ecosystems, I_{geo} Index).

	B µg/L	Al µg/L	Si µg/L	Cr µg/L	Mn µg/L	Fe µg/L	Co µg/L	Ni µg/L	Cu µg/L	Hg µg/L
Receiving Sandy Soil (Bn)	60,1	156,3	10598,5	0.0	41,6	119,4	0.0	0,0	0,0	0
Fertilizer	138,3	2068,4	11907,5	0.0	639,1	1005,5	0.0	0,0	9,1	0
WTR (Cn)	39,4	90,0	2643,8	0.0	0,0	38,0	0.0	0,0	0,0	0
AD (Cn)	100,2	23,7	12697,5	0.0	453,0	110,3	0.0	21,0	51,1	0
WTR+AD	87,5	62,9	7806,6	0.0	6,8	69,9	0.0	0,0	34,1	0
Target	-	5	-	7	180	-	-	-	0.3	0.04
Chronic Toxicity		10		14	370				0.53	0.08
Acute Toxicity		100		200	1300				1.6	1.7
Igeo Fertilizer	0,6	3,1	-0,4	0.0	3,4	2,5	0.0	0.0	>high	0
Igeo WTR	-1,2	-1,4	-2,6	0.0	0,0	-2,2	0.0	0.0	0	0
Igeo AD	0,2	-3,3	-0,3	0.0	0,0	-0,7	0.0	>high	>high	0
Igeo WTR+AD	0,0	-1,9	-1,0	0.0	-3,2	-1,4	0.0	0.0	>high	0

Table 2.23. Nutrient and heavy metal risk assessment for soil post-trial (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	Ca mg/kg	K mg/kg	Mg mg/kg	Na mg/kg	P mg/kg	Zn µg/kg	Sr µg/kg	Ba µg/kg	Pb µg/kg	As mg/kg	Cd µg/kg
Receiving Sandy Soil (Bn)	0,7	0,1	0,2	0.1	0.0	5018,7	9420,8	15144,1	0.0	23,2	12,8
Fertilizer	0,6	0,1	0,2	0.1	0.0	2818,8	7114,2	10846,4	0.0	25,6	12,5
WTR (Cn)	0,6	0,1	0,1	0.1	0.0	4055,7	6559,0	12366,1	0.0	21,3	11,4
AD (Cn)	2,0	0,1	0,1	0.1	0.0	2579,6	18948,4	12674,2	0.0	58,6	6,9
WTR+AD	2,2	0,1	0,1	0.1	0.0	1813,4	16296,6	11540,9	0.0	38,3	3,9
Herselman Guidelines (Receiving Soils) NH_4NO_3 (MAT)	-	-	-	-	-	5000	-	-	3500	14	100
Igeo Fertilizer	-0,9	-0,8	-0,2	-0,8	0.0	-1,4	-1,0	-1,1	0.0	-0,4	-0,6
Igeo WTR	-0,8	-0,7	-0,8	-1,2	0.0	-0,9	-1,1	-0,9	0.0	-0,7	-0,7
Igeo AD	0,9	-0,7	-1,0	-0,8	0.0	-1,5	0,4	-0,8	0.0	0,7	-1,5
Igeo WTR+AD	1,1	-0,9	-0,8	-0,5	0.0	-2,1	0,2	-1,0	0.0	0,1	-2,3

Table 2.23 cont. Nutrient and heavy metal risk assessment for soil post-trial (Herselman's 2013 guidelines for receiving soils, I_{geo} Index).

	B µg/kg	Al µg/kg	Si µg/kg	Cr µg/kg	Mn µg/kg	Fe µg/kg	Co µg/kg	Ni µg/kg	Cu µg/kg	Hg µg/L
Receiving Sandy Soil (Bn)	971,4	3228,2	13,2	13,5	10413,3	2960,1	88,7	17,0	148,7	0,3
Fertilizer	572,4	4530,5	15,7	15,3	8488,5	3200,9	59,0	5,9	93,8	0,5
WTR (Cn)	620,9	4239,6	13,4	11,5	8729,0	2496,0	48,6	0,0	73,5	0,2
AD (Cn)	881,2	1434,4	23,5	18,4	6307,0	2519,8	37,8	18,8	480,2	0,6
WTR+AD	831,2	887,5	16,5	13,1	3308,8	1548,9	16,4	5,6	273,4	0,4
Herselman Guidelines (Receiving Soils) NH_4NO_3 (MAT)	-	-	-	100	-	-	-	1200	1200	7
Igeo Fertilizer	-1,3	-0,1	-0,3	-0,4	-0,9	-0,5	-1,2	-2,1	-1,3	0,4
Igeo WTR	-1,2	-0,2	-0,6	-0,8	-0,8	-0,8	-1,5	0,0	-1,6	-0,8
Igeo AD	-0,7	-1,8	0,3	-0,1	-1,3	-0,8	-1,8	-0,4	1,1	0,7
Igeo WTR+AD	-0,8	-2,4	-0,3	-0,6	-2,2	-1,5	-3,0	-2,2	0,3	0,0

2.6.6 Parasite characterisation and persistence

Although the pure anaerobic digestate had non-viable helminths (4 ova/g_{dw}) and Ascaris (1 ova/g_{dw}), there were no viable helminth or Ascaris detected in the AD after pasteurisation and before application in the lysimeter trials. In the sand, WTR and post-harvest soils, at all amendment rates, there were no viable or non-viable helminths or Ascaris ova.

2.6.7 Heavy metals: Soils, leachate and plant bioaccumulation

Total (Aqua Regia, Table 25a & b) and bio-available (NH_4NO_3, Table 25c) heavy metals and nutrients were analysed in the three materials (sand, WTR and AD) according to Herselman's guidelines (2013) for WTR land application. Additionally, the metals (Table 26a) and nutrients (Table 26b) in the leachates pre-trial and post-trial (Table 27), as well as the bio-available metals and nutrients in the soil mixes for the lysimeters post-trial were measured (Table 28). These were compared to Herselman's (2013) recommended limits for receiving soils (Table 31). The geoaccumulation index (I_{geo}) is described by Zhao et al. (2022) and used widely throughout literature to measure the risk of environmental accumulation. It was used to investigate the risk of heavy metal accumulation over background levels. The risk was graded according to colour (Table 32).

In terms of heavy metal contamination, the receiving sandy soil was well below guidelines for WTR and AD amendment (Table 25a & b). The pure amendments (WTR and AD) were far higher than these guidelines, and the I_{geo} indices for these amendments were often in class 5-7, indicating a potential to heavily contaminate the receiving soil. However, when re-calculated to an application rate of 2.5% (the typical application rate for receiving soils, and the application rate in these trials), the I_{geo} fell mostly in Class 1 (practically uncontaminated), ranging to Class 3 for some metals (Zn, Cd, Hg; moderately contaminated). Nutrients were included (Table 25b), utilizing the I_{geo} index as an indicator for the risk of eutrophication (monitoring accumulation). As above, the pure materials indicated a higher I_{geo} risk, however at 2.5% application rate, the risk dropped to Class 1 for most nutrients in both amendments, ranging up to Class 3 (moderately contaminated) for phosphate and Class 4 for Ca, with the AD amendment. The plant-available metals were much lower than the total metals, and the receiving soils were well within the Maximum Available Threshold for bio-available extractions (Table 25c). The amendments fell within these limits for most metals. Only Cu and As in AD exceeded these limits, in the pure material. However, at 2.5% application, the environmental concentrations fall well within regulatory control. In the final lysimeter soil mixes (Table 28), all post-trial metals were well within Herselman's guidelines, with only Zn nearing the Maximum Available Threshold for plant-available metals, with a Class 4 accumulation risk (moderate to heavy contamination). Cu also demonstrated a high (Class 6) accumulation risk, in comparison to background environmental levels, but was well (<30%) within the Maximum Available Threshold of 1.2 mg/kg (Table 28). The second growing season (winter) again showed no heavy metal accumulation concerns, according to the total concentrations and I_{geo} classifications, except for Cu (Tables 29 and 30).

Foliar heavy metals were also measured, however, a textile crop was intentionally chosen to monitor bio-accumulation without consumption risks. In addition, the hemp root system is renowned for its phytoremediation capacity (Cacic, 2019; Citterio et al., 2005), making it an attractive commercial option for this agricultural model, co-applying waste sludges for the amelioration of nutrient-poor sandy soils. Previous crop studies on these amendments showed marked treatment differences between foliar metal concentrations of crop grown on these amendments, however only at higher application rates (over 10%; Clarke et al., 2019; Steytler, 2021). In this study, there were no significant differences in foliar heavy metals in response to the various soil amendments, except for Cu, which is reflective of the Cu concentrations in the soils and leachates (above). Cu is high in the sludge (Appendix III, Table C2), and has a strong affinity for organic matter (Shank et al., 2004). Thus, it is likely due to the formation of soluble organic complexes. In this case, WTR amendments increased foliar Cu concentrations by 36%

(P<0.05, Table 33A). The accumulation of Cu in biomass grown in this treatment is likely due to stunted growth. The lack of detectable differences in foliar metal accumulation across most of the heavy metals is likely due to the low application rate. In addition, several studies indicate that hemp accumulates heavy metals in the root system rather than the above-ground biomass (Citterio et al., 2005; Cicac et al., 2019), and in this study, only the above-ground biomass was submitted for analysis. Some studies also showed that hemp had very low remediation capacity at all, in comparison to giant reeds (Ferrarini et al., 2021). No notable differences emerged between growing seasons, with similar results in season two (Table 33B).

Table 2.24. Recommended limits for metals in WTR amended soils (mg/kg) (Herselman, 2013).

Metal Elements	**Total investigative level (TIL) (aqua regia)**	**Total maximum threshold (TMT) (aqua regia)**	**Maximum available threshold (MAT) (NH_4NO_3)**
Arsenic (As)	2	2	0.014
Cadmium (Cd)	2	3	0.1
Chromium (Cr)	80	350	0.1
Copper (Cu)	100	120	1.2
Lead (Pb)	56	100	3.5
Mercury (Hg)	0.5	1	0.007
Nickel (Ni)	50	150	1.2
Zinc (Zn)	185	200	5.0

Table 2.25. Seven classes comprising the geoaccumulation index (Zhao et al., 2022).

Class	Value	Soil quality
1	Igeo≤ 0	Practically uncontaminated
2	0＜Igeo≤1	Uncontaminated to moderately contaminated
3	1＜Igeo≤2	Moderately contaminated
4	2＜Igeo≤3	Moderately to heavily contaminated
5	3＜Igeo≤4	Heavily contaminated
6	4＜Igeo≤5	Heavily to extremely contaminated
7	Igeo＞5	Extremely contaminated

Table 2.26a. A comparison of heavy metal accumulation in hemp plant biomass in season one, between treatments in sandy soil amended with WTR, AD or co-amended with both. Lettering indicates significant treatment differences (ANOVA and post-hoc Tukey's test, p<0.05), and columns without lettering are not significantly different.

Treatment	K (%) Ave	St dev	Ca (%) Ave	St dev	Mg (%) Ave	St dev	Na (mg/kg) Ave	St dev	Fe (mg/kg) Ave	St dev
Sand	1.2	0.2	4.5	0.6	0.4	0.1	503.5	178.2	335.8	212.4
Sand F	1.0	0.2	4.1	2.1	0.5	0.1	437.7	215.5	273.1	83.9
WTR	1.6	0.2	4.5	0.6	0.4	0.1	505.5	239.7	294.9	64.8
AD	0.6	0.1	6.3	1.3	0.5	0.1	418.3	225.0	222.3	65.8
WTR+AD	0.6	0.1	4.6	0.8	0.5	0.1	542.7	734.9	266.7	56.6

Treatment	Cu (mg/kg) Ave	St dev	Zn (mg/kg) Ave	St dev	Mn (mg/kg) Ave	St dev	B (mg/kg) Ave	St dev	Al (mg/kg) Ave	St dev
Sand	3.9	0.8	29.5	6.5	90.7	17.6	113.1	21.9	259.9	118.8
Sand F	3.4	0.5	24.3	1.8	54.8	11.2	182.1	33.3	195.0	75.6
WTR	5.3	0.1	51.2	10.2	56.1	0.1	126.3	11.7	260.0	70.7
AD	2.9	0.4	35.5	10.4	44.1	16.6	153.0	38.7	208.3	38.7
WTR+AD	3.5	0.4	27.6	6.0	36.7	4.4	162.9	22.0	305.0	87.6

Table 2.26b. A comparison of heavy metal accumulation in hemp plant biomass in season two, between treatments in sandy soil amended with WTR, AD or co-amended with both. Lettering indicates significant treatment differences (ANOVA and post-hoc Tukey's test, p<0.05), and columns without lettering are not significantly different.

Treatment	K (%) Ave	St dev	Ca (%) Ave	St dev	Mg (%) Ave	St dev	Na (mg/kg) Ave	St dev	Fe (mg/kg) Ave	St dev
Sand	1.5	0.3	4.3	1.6	0.5	0.1	102.3	37.6	115.0	55.3
Sand F	2.1	0.3	2.5	0.4	0.4	0.0	118.7	21.0	105.5	11.4
WTR	1.5	0.2	6.0	0.9	0.4	0.1	131.3	46.7	86.4	15.1
AD	0.7	0.0	5.6	0.9	0.5	0.0	208.0	83.6	131.5	59.5
WTR+AD	0.7	0.1	6.1	0.7	0.5	0.0	157.0	25.4	105.9	15.7

Treatment	Cu (mg/kg) Ave	St dev	Zn (mg/kg) Ave	St dev	Mn (mg/kg) Ave	St dev	B (mg/kg) Ave	St dev	Al (mg/kg) Ave	St dev
Sand	4.4	0.6	54.1	22.7	102.3	36.8	80.7	12.0	74.0	74.9
Sand F	3.3	0.2	25.8	2.1	66.8	7.2	52.1	8.1	37.3	5.9
WTR	5.2	0.3	41.1	20.5	82.7	37.1	82.9	15.1	34.7	7.0
AD	4.6	0.5	34.5	4.0	21.8	1.4	102.5	18.4	45.3	35.2
WTR+AD	5.0	0.2	30.7	3.1	29.5	1.1	118.3	3.5	32.0	2.6

2.6.8 Carbon sequestration and ecotoxicity

In terms of total carbon (%) and nitrogen (%) of the starting materials, there was no significant ($p<0.05$) difference between the sandy soil and WTR, although the WTR was slightly higher (approximately 1.3 fold and 1.1 fold, respectively, Figure 39). In contrast, the AD is significantly ($p<0.05$), markedly higher than the receiving soil, with 18 times more nitrogen (%N) and 26 times more carbon (%C). The dissolved organic carbon extracted from the materials during characterization was significantly higher for both WTR (3 times) and AD (20 times) than the receiving sandy soil (Figure 40). These figures were used to predict the total carbon (%TC, Figure 39) and DOC (Figure 40), by calculating the proportional carbon from each fraction in the treatments (sand, WTR, AD and WTR+AD co-amendment).

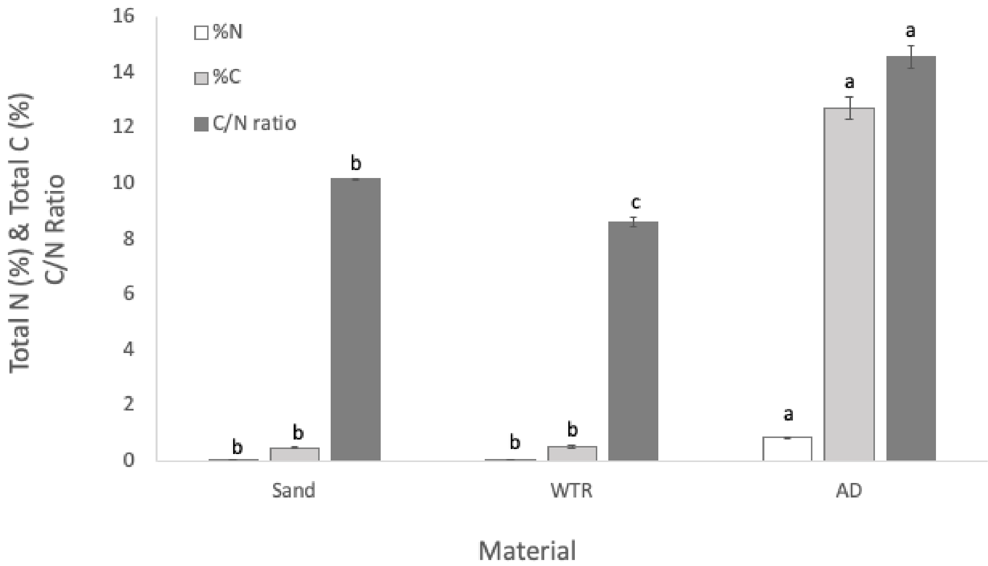

Figure 2.29. The total N (%) and C (%) as well as the C/N ratio, comparing the sandy soil, the WTR and AD amendments. Bars represent the means of triplicate samples, and error bars the standard deviation. Means that are not significantly different to each other have the same letter ($p<0.05$). Significance lettering was applied to each series separately, using the same lettering system (a-c).

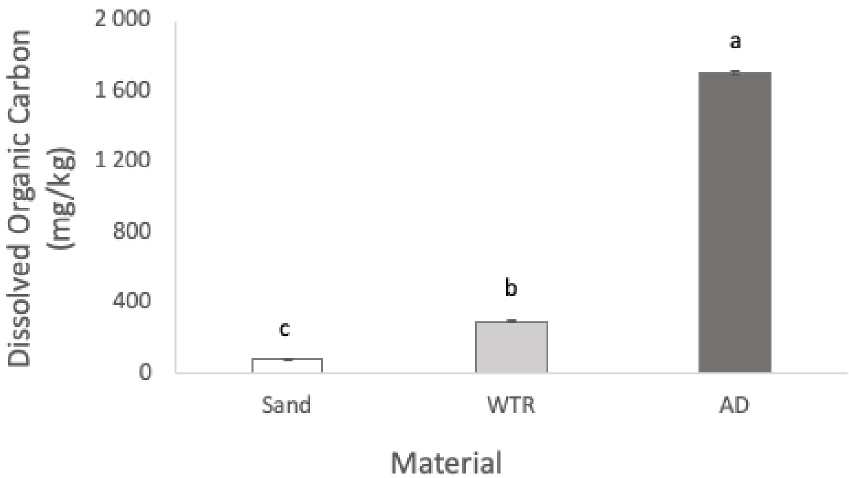

Figure 2.30. The dissolved organic carbon, extracted from the sandy soil, as well as the WTR and AD amendments. Bars represent the means of triplicate samples, and error bars the standard deviation. Means that are not significantly different to each other have the same letter ($p<0.05$).

After the first growing season, the total nitrogen (%N) in the lysimeter soils was negligible (ranging between 0.05% and 0.08%), with no statistical difference between treatments (Figure 41). After the trial, there was still significantly more total carbon (%C) in the soil in WTR (2.1 times), AD (2.1 times) and co-amended (WTR+AD, 1.8 times) soils than in the sandy soil control (P<0.05). There was no statistical difference between total soil carbon in the treatments (%C) post-trial. Only the fertilized treatment had a lower total carbon fraction after the trial than the organic amendments, in season one. Although the C/N ratio of AD is significantly ($p<0.05$) higher than the receiving sandy soil (Figure 39) and the C/N of WTR is significantly lower, there are no clear trends in the C/N ratio between treatments (Figure 41).

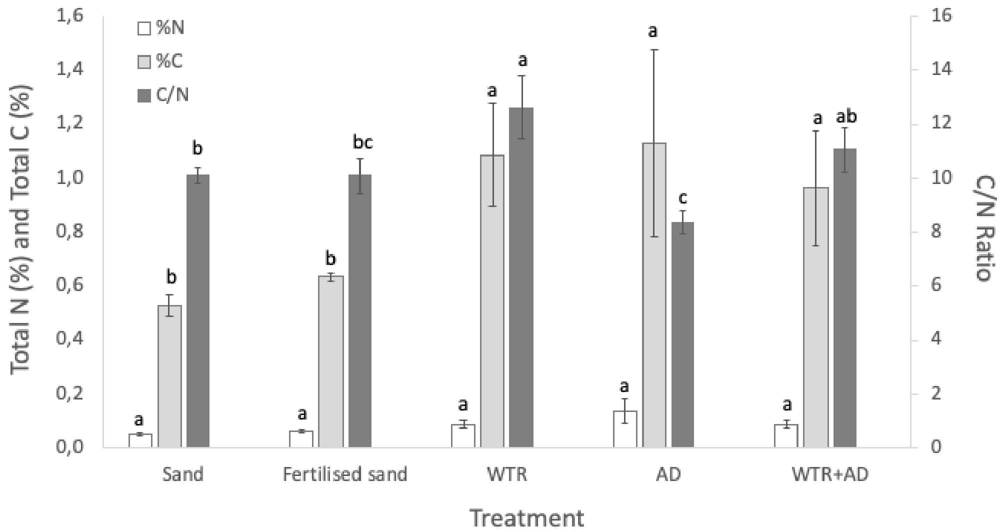

Figure 2.31. The total N (%) and C (%) as well as the C/N ratio of the lysimeter soils, after the trial, including a fertilized soil, and lysimeters amended with WTR, AD and a co-amendment of WTR+AD. Bars represent the means of triplicate samples, and error bars the standard deviation. Means that are not significantly different to each other have the same letter ($p<0.05$). Significance lettering was applied to each series separately, using the same lettering system.

The dissolved organic carbon (DOC, mg/kg) extracted from the lysimeter soils post-trial showed a more resolved trend than the total C, with all treatments significantly ($p<0.05$) higher than the sandy soil control (Figure 42A). The WTR treatment was 1.5 times higher than the sandy soil control, fertilizer amendment 1.75 times higher, the AD amendment 2.3 times higher and the WTR+AD co-amendment 2.4 times higher. In season two, all treatments except WTR were higher in DOC than the sandy soil control (Figure 42B). The original hypothesis was that replacing 2.5% of the sandy soil with WTR may immobilise or sequester some of the AD carbon, particular the dissolved fraction. Thus, according to the null hypothesis, both the individual AD-amended lysimeters and the co-amended lysimeters (2.5% WTR + 2.5% AD) are predicted to release a fraction of carbon during leaching that is not significantly different to the proportional amounts in the soil (Figure 40, Table 34). The alternate hypothesis is that WTR+AD treatments will release less DOC during leaching than the AD treatments (normalized over the predicted total DOC per treatment, calculated from Figure 40, Table 34), as the WTR may play a role in stabilizing carbon.

All treatments throughout season one and two have lower final DOC than initial DOC concentrations (Figure 43). The only exception is in the fertilized soil in season two, likely since it was applied sequentially throughout the trail. There are no significant differences between the DOC of the AD and co-amended leachates in season one or two, pre-or post-trail (Figure 43). However, there is a trend of lower DOC in the co-amended leachate than the AD leachate after the trial in both seasons (Figure 43A and B). However, the standard deviation is very high, and when normalized over the predicted DOC in each treatment, the conclusions were variable (Table 34).

Notably, as season one progressed, the leachate from the co-amendment released more DOC than the individual amendment (Table 34), which disproves the stabilization hypothesis. However, in season two, the co-amendment released less DOC than the individual amendment during leaching, which supports the hypothesis. In the soils at the end of the trial, the AD and co-amended treatment had equivalent DOC concentrations in season one. In season two, the final DOC released from the soil was higher than AD, suggesting that more had been retained over the season. Thus, some of the data tentatively supports this hypothesis, and some challenges it. The soils will be analysed once more, with care to attempt to decrease standard deviations. In addition, POXC (active carbon, permanganate-oxidizable carbon) and DOC measured with a stronger extractant ($CaCl_2$) will be added before publication of the data.

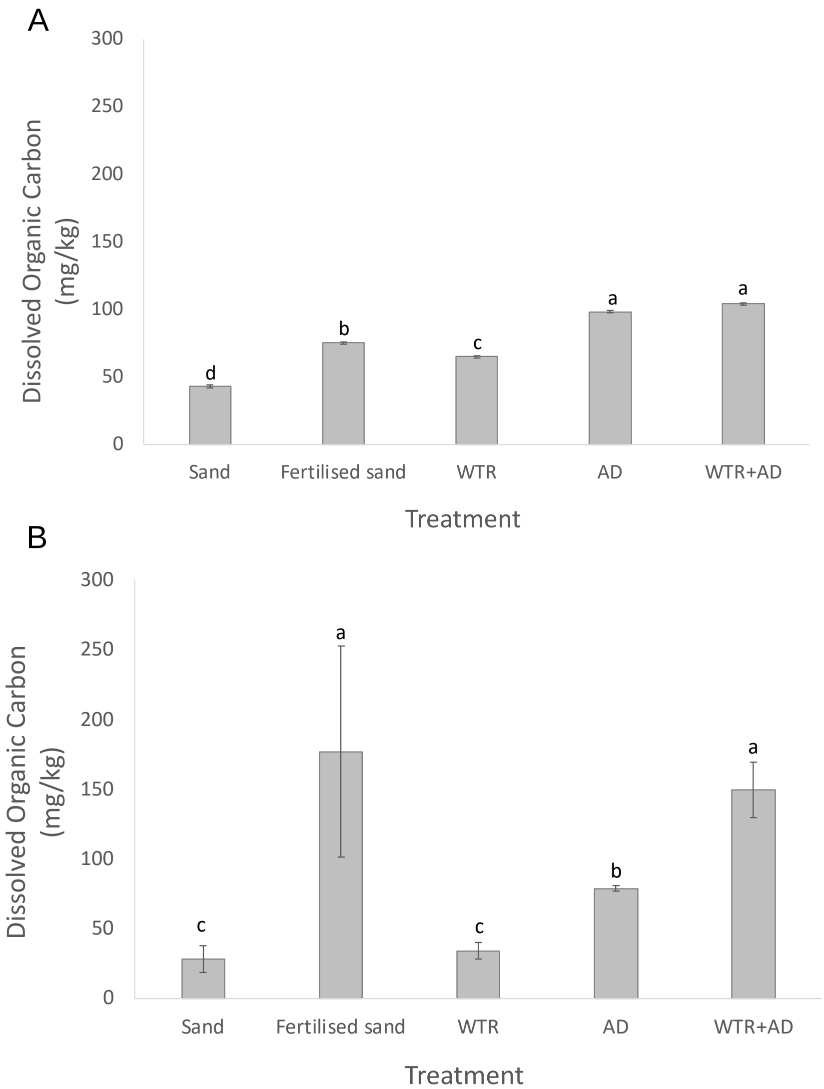

Figure 2.32. The dissolved organic carbon, extracted from the lysimeter soils, after the trial, including a fertilized soil, and lysimeters amended with WTR, AD and a co-amendment of WTR+AD. The DOC was analysed after season one (A) and season two (B). The predicted dilution effect is half the sum of the mean DOC of the WTR and AD individual amendments. Bars represent the means of triplicate samples, and error bars the standard deviation. Means that are not significantly different to each other have the same letter ($p<0.05$).

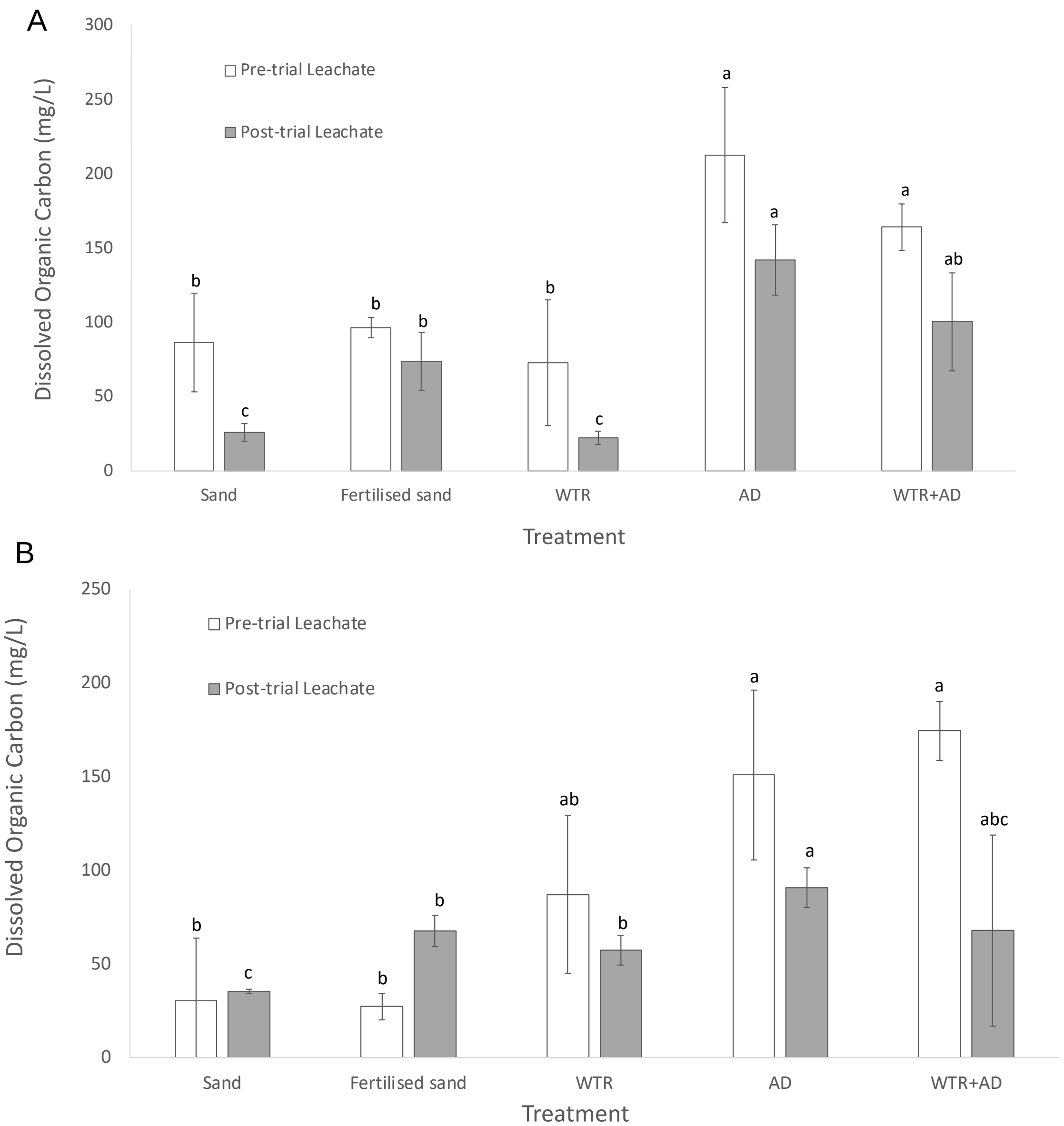

Figure 2.33. The dissolved organic carbon in the leachate from the lysimeter treatments, pre- and post-trial, including a fertilized soil, and lysimeters amended with WTR, AD and a co-amendment of WTR+AD. Leachates were compared between season one (A) and season two (B). Bars represent the means of triplicate samples, and error bars the standard deviation. Means that are not significantly different to each other have the same letter ($p<0.05$). Significance lettering was applied to each series separately, using the same lettering system.

Table 2.27. The total % DOC associated with each treatment was calculated proportionally from the materials characterization (Fig 40). The percentage of the measured DOC normalized over the predicted total DOC per treatment (column 1) was calculated over both seasons, in the leachate and the post-harvest soils.

Treatment	Predicted Total DOC (%)	Measured/ Predicted DOC(%)	Measured/ Predicted DOC(%)	Measured/ Predicted DOC(%)	Measured/ Predicted DOC(%)
		Leachate		Soil	
		Season 1	Season 2	Season 1	Season 2
Sand	95[a]	37[e]	27	45	30
Sand F	-	-	-		-
WTR	105[b]	46	21	62	32
AD	135[c]	54	105	73	58
WTR+AD	145[d]	67	69	72	103

[a]Total DOC = (%DOC_Sand)*1.00

[b]Total DOC = (%DOC_Sand)*0.975 + (%DOC_WTR)*0.025

[c]Total DOC = (%DOC_Sand)*0.975 + (%DOC_AD)*0.025

[d]Total DOC = (%DOC_Sand)*0.95 + (%DOC_WTR)*0.025+(%DOC_AD)*0.025

As mentioned, it was hypothesised that WTR might, like clay, stimulate microaggregates that facilitate C-mineral associations or the physical protection of soil organic C from sewage sludge (Cotrufo and Lavallee, 2022). However, this effect is not clearly evident in the lysimeter trials. Similar to the much lower resolution of carbon stabilisation data in the lysimeter trials than the laboratory trials, ecotoxicity assays were below detection, with no significant treatment differences (data not shown) in all in-field analyses. Data resolution was too low to compare treatments. Dizer et al. (2002) also reported very low ecotoxicity in groundwater leachate when lysimeters are fertilized with sewage sludge. This result is thus not surprising. However, it may be worth monitoring with repeat land application, over longer seasons, especially if sewage water is used to fertigate crops. These inconclusive results should also be interpreted with caution. A more full suite should include POXC analyses and analysis of DOC with a stronger extractant. Both will be added to this data series from the final post-trial soils of season two to strengthen the publication of this data.

2.6.9 Soil water repellency

The first round of data for soil water repellency showed high variation and no differences in treatment. However, laboratory trials showed that drying had a significant effect on measuring soil water repellency (Figure 45) which might lead to variation in in-field data.

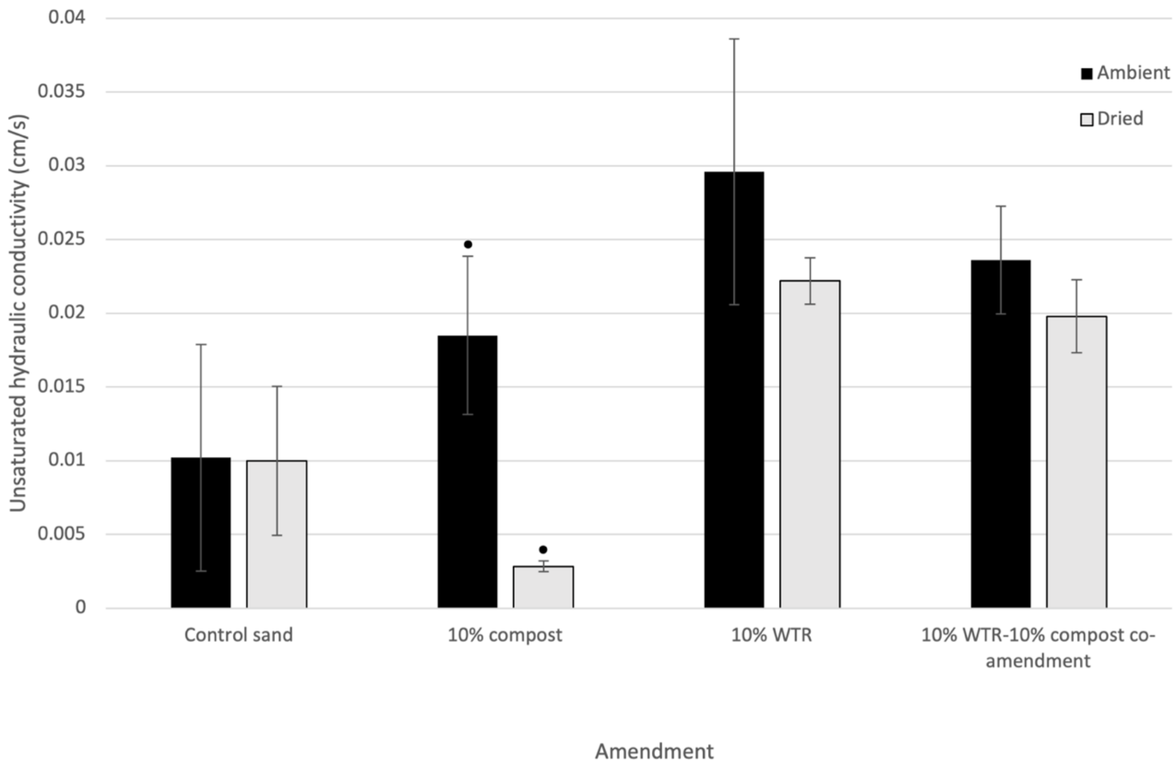

Figure 2.34. Unsaturated hydraulic conductivity of dried in comparison to ambient amendments. All error bars represent the standard deviation of triplicate samples. •represents significant differences ($p<0.05$) between control sand and amendments.

In conclusion, the co-amendment improved crop biomass more than all other treatments. The parameters that pose an environmental bio-accumulation concern include electrical conductivity, phosphate concentrations in the leachate, and Cu. In addition, all parasites and pathogens were negligible after the trial except Shigella, which is persistent in the soil. Carbon sequestration, ecotoxicity and water retention were all too variable in the lysimeter trial to make clear conclusions. These parameters were still tolerable for crop germination and growth in this two-season trial with repeat applications. However, they should be monitored upon long-term land application, or should inform logistical design for cyclical rhythms of distribution, resting land between applications.

3 COMPOSTING

3.1 Project design and rationale

The hypothesis to be tested in this composting trial was intended to explore the risks and benefits of the co-amendment of water and wastewater sludge during in-vessel composting. The focus was on the potential remediation of the ecotoxicity of the surface water runoff, in terms of nutrients (eutrophication), heavy metals, pathogens and micropollutants. Thus, the hypothesis was that, in comparison to individual sludge composting bulked with brown waste (garden chippings), co-composting with 10% WTR will influence the following (Figure 46):

(1) risks:
 (a) heavy metal immobilisation
 (b) pollutant immobilisation (eutrophication and ecotoxicity remediation)
 (c) greenhouse gas emission
 (d) pathogen persistence, and

(2) benefits:
(a) carbon sequestration
(b) nutrient retention
(c) stabilisation rate
(d) microbial diversity

This would inform whether it is optimal to design the co-amendment of WTR into municipal strategies for land application prior to beneficiation, or post-beneficiation, i.e. is it optimal to add WTR to AD during composting, or with composted AD directly to the soil? However, local infrastructure became the limiting factor in testing this investigation.

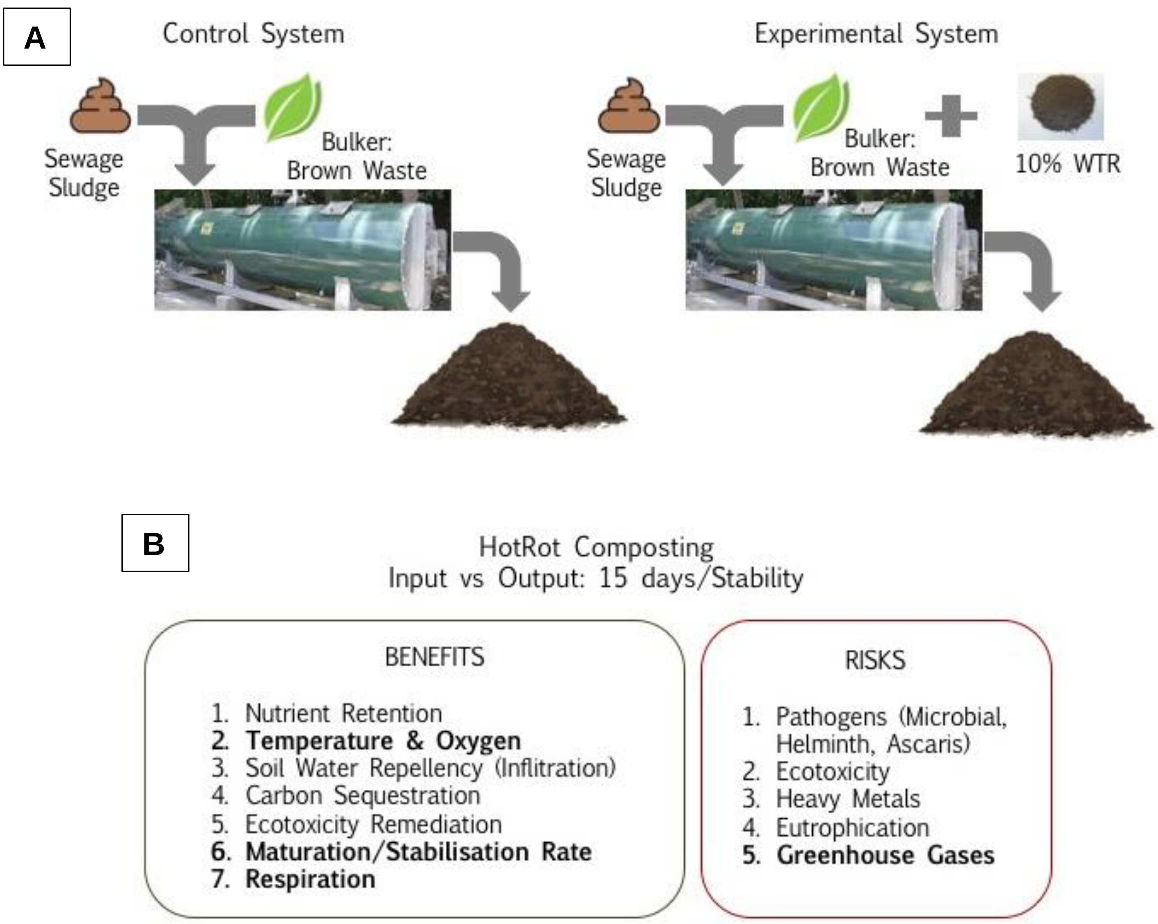

Figure 3.1. Visual summary of (a) compost treatments and (b) experimental parameters, designed to investigate the effect of co-amending the bio-mechanical accelerated composting of biosolids bulked with garden refuse, with 10% WTR. Parameters that are particular to composting, added to the suite optimised for lysimeter groundwater monitoring (Section 3) are highlighted.

3.2 Beneficiation

3.2.1 Overview

The beneficiation of sludges (logistics and processing) is the primary point of opportunity, to design and control the land application of these wastes. This is the point at which society engages with the process, and socio-economic design considerations enter the equation. These include (1) economics (equipment cost, transport, energy), (2) personnel handling experience (odour, ease of use, scale, security), and (3) public perception (assurance of the quality of the final product, risk perception).

Current options for organic waste management include landfilling, as discussed above, as well as incineration, anaerobic digestion and composting (Manyapu et al., 2018). Since this work focuses on circular economy and the beneficiation of waste for agricultural productivity, rather than purely decreasing the risk of these wastes, anaerobic digestion and composting are the focus of comparison here. An economic, technical, and environmental comparison of composting facilities versus anaerobic digestion facilities was executed in Ireland, in response to similar national waste diversion directives (Murphy and Power, 2006). The study concluded that composting is more economically feasible than anaerobic digestion, at scales at or below 50 kt/a of municipal biosolids. However, if methane-enriched biogas is captured and utilised, anaerobic digestion becomes an attractive alternative that is most economically feasible at or below 20 kt/a. The efficiency of fossil fuel displacement with biogas from anaerobic digestion also has the potential to save 20% more $kgCO_2/t$ than composting. However, biogas technology is advanced, and this is thus a good alternative at large centralised WWTW with the capacity to diversify to that degree and scale. Murphy and Powers (2006) also consider the reduction of tax for these carbon interventions (excise duty) part of the economic equation, which is not the case in South African legislation yet. It may be on the horizon with the dramatic local energy crisis forcing legislation that promotes decentralised power generation (Bloomberg, 2023), but is not currently a pragmatic consideration. Composting is a popular waste beneficiation technique, and there is wide communal knowledge of this process even at backyard level (USEPA, 2009). This makes it an attractive and immediately accessible alternative for funnelling biosolids into local agriculture, which might be feasibly managed at smaller, decentralised WWTW in agricultural landscapes. This improved accessibility of compost over biogas, purely due to common experience with the process, is beneficial both in terms of practical skills for implementation, as well as public perception and receptivity in the agricultural community.

Optimal feedstock and composting parameters, as summarised by Rynk (1992) and Manyapu et al. (2018), include a C:N feedstock ratio between 20:1 and 40:1, moisture content between 40 and 60%, a feedstock particle size < 2.5cm, oxygen availability >10%, NPK each >1% (m/m_{dw}) with nitrates present, and pH between 6.5 and 8. Essentially, it is an aerobic process that needs a neutral environment and sufficient space between soil particles for microbial processes to occur. Compost stability and maturity is typically measured using multiple parameters measured over time (months), including pH, EC, CO_2 and methane evolution rate (GHG), seed germination rate, and DOC measured as total concentration and mass-specific absorbance at 420 nm (Wu et al., 2000).

3.2.2 Composting alternatives

Composting can be achieved with windrow methods, aerated static piles, vermicomposting, and in-vessel composting (Cooperband, 2002). In-vessel composting is a type of mechanical-biological, rapid accelerated composting, with increased process control due to containment and mixing technologies. This increases the energy footprint and cost but speeds the process dramatically (reducing the process from months to <3 weeks). As mentioned in the proposal, extensive studies have shown the benefits of composting sludges. Vermicomposting sewage sludges, for instance, significantly reduces pathogens (Eastman et al., 2001) and renders heavy metals less available to leaching (Hait and Tare, 2012). Other scientific studies have demonstrated that vermicomposting significantly increases productivity (Longhurst et al., 2003; Lotzof, 2000) and suppresses a wide range of plant pests and diseases on commercial crops including parasitic nematodes, fungi and bacterial diseases, and sucking and chewing insect and pests (Yardim et al., 2005; Arancon et al., 2000; Edwards et al., 2007; Jack and Nelson, 2010).

3.2.3 Composting additives

There is widely demonstrated precedent for accelerating the composting process and/or improving quality with external amendments. This is even more attractive, in terms of circular economy, if those amendments are another waste stream. Adding biochar (10% m/m_{dw}) to the in-vessel composting of swine manure has been shown to reduce ammonia and greenhouse gases, as well as pathogen persistence and phytotoxicity, and improve nutrient retention and compost quality. However, the effects were less significant at lower loading rates (>5% m/m_{dw}; Chung et al., 2021). Similarly, fly ash has been shown to improve composting in terms of enzyme activity and biological communities (Fang et al., 1998). Fly ash incorporated in the in-vessel composting of kitchen waste minimised the plant accessibility of heavy metals, and a 5% fly ash loading rate improved composting efficiency compared to controls (lowest %C loss, and lowest rate constant, k; Manyupa et al., 2018). Himanen and Hanninen (2009) reviewed a variety of additives more conservatively and tested two commercial products. They concluded that in most cases the results are nuanced, with some indicators improved and others simultaneously limited by additives. The exception they mention is the addition of alkaline products like lime or ash, which are widely accepted as beneficial to the process (Lau et al., 2001; Koivula et al., 2004; Wong and Fang, 2000). WTR is more acidic than compost and sewage sludge (Addendum C), thus co-amendment will not drive the pH towards the desired alkalinity. However, there is no reason that WTR and fly ash cannot be co-amended for pH control. Therefore, although the sorptive capacity of WTR may improve nutrient retention, carbon sequestration and immobilise ecotoxicity and heavy metals, the rate of stabilisation may be negatively impacted by pH, but this can be corrected with lime or ash.

3.3 Local infrastructure for sludge composting: Maintenance and feasibility

3.3.1 Anaerobic digesters

The original composting strategy was to transport AD for composting on Welgevallen Farm – Stellenbosch University's experimental farm – using windrows and vermicomposting. However, the Cape Flats anaerobic digester failed shortly before the project launched. In conversation with the City of Cape Town (Sven Sotemann, Head of CoCT Development and Infrastructure Planning: Wastewater Branch, Water and Sanitation Department, Water and Waste Directorate), as well as the network at Stellenbosch University Water Institute (SUWI), it became clear that the only remaining local digester is a mesophilic anaerobic digester (Simon's Town WWTW). This digester lacks thermal stabilisation and produces sludges that at times don't meet standards for landfill, according to the site manager. The City of Cape Town is investing in new large-scale thermal sewage sludge processing technology, that will capture biogas. Thus, they are not investing in repairs to the old anaerobic digesters in favour of newer technology to be implemented within five years. As mentioned above, Murphy and Powers (2006) confirmed that anaerobic digestion technology that harnesses biogas is a wise investment on the part of CoCT.

However, during transition, the CoCT is left with unprocessed sludges, as sources for composting. After screening eight local sludge sources, according to pathogen loads, the mesophilic AD is considered the safest alternative, pasteurised before applied in the lysimeter trials (Section 3.1). However, the volumes necessary for the lysimeters are far lower than those needed for composting, and the lysimeters are in an access-controlled facility according to the project's Department of Land Reform and Rural Development (DLRRD) hemp license stipulations. This preliminary screening of pathogens demonstrated that the concentrations were too high to compost on campus at a large scale, with wide exposure to students. Thus, the focus shifted to answering the same hypothesis using in-vessel mechanical composting alternatives, available locally. Roger Jaques, a project collaborator, has co-driven the implementation of the HotRot in-vessel composting system, both onsite at PEDI and at the Grabouw WWTW. This system is a commercial in-vessel composting system, with several benefits (https://www.globalcomposting.solutions/). However, there are similar logistical governance and maintenance challenges to the HotRot, as those limiting the operation of local anaerobic digesters.

3.3.2 HotRot in-vessel composting

3.3.2.1 Overview

The HotRot system is an attractive composting alternative that facilitates on-site sewage sludge beneficiation. This does not place composting and biogas at odds with each other, but rather they are context-specific, primarily selected based on scale, transport costs and associated economic forecasting. From an urban planning perspective, a thermal digester that captures biogas may be an economically viable solution at large, central WWTWs, with in-vessel mechanical composting solutions like the HotRot an attractive decentralised solution at smaller WWTW. These are accessible to farmers and targeted to local agricultural communities which will limit compost transport costs.

These in-vessel mechanical biosystems can take various forms, and this report will focus on the HotRot iteration, as it is implemented locally and has already been used by local farmers. The information is derived from conversations with (1) the individuals originally responsible for implementing the system, Roger Jaques (PEDI) and David Crombie (GIBBS), as well as (2) Grabouw WWTW site managers, and (3) from on-line HotRot documentation drafted by Global Composting Solutions, who facilitate HotRot Composting Systems and Comet Composters (https://www.globalcomposting.solutions/hotrot-documents).

The HotRot is a U-shaped mechanical system, and Theewaterskloof municipality has implemented the 3518s HotRot (3.5 m x 18 m). It has the capacity to process ±3600 tpa (10-12 tpd) at 22 kW/t. It has an automated feed system and treats the sewage grit and screenings from a Salsnes high volume filter. A central tine-bearing shaft turns the material (Figure 47) with both forward and reverse motions, preventing compaction and dead zones and maintaining aeration. Air is injected and excess heat, CO_2 and moisture are released into a headspace. The system is maintained under negative pressure, preventing fugitive gas release into the atmosphere, which assures the OdourFreeGuarantee that the company has invested in. Insulation maintains thermal regulation, and the mixing is under microprocessor control, with a programmable logic controller (PLC) and sensors allowing for online diagnostics. Typically, a bulker, such a brown waste (wood-chippings or garden refuse), is added at rates based on sludge weight and water content.

Figure 3.2. The internal mixing tine-bearing shaft of the standard HotRot, designed for control of this in-vessel composting processed.

In terms of environmental impact, the focus of this study, the HotRot is guaranteed – with accountability – by manufacturers to be odour-free, with zero leachate and is Telarc registered (https//:telarc.org) under ISO9001. These are fundamental environmental benefits to this system, in comparison to standard heap or windrow composting, since each of these elements are potential hurdles to implementation. Leachate has physical risks, whereas odours influence social receptivity to the technology. Within this study, the impact of co-amending two waste streams (WTR and sewage sludge) was to be evaluated during HotRot composting, with a focus on the effects on carbon sequestration, and ecotoxicity, GHG and pathogen abatement.

3.3.2.2 Grabouw HotRot In-Vessel Composting

A HotRot system was installed at the Grabouw WWTW November 2012 (Figure 48). According to the plant manager, it was effectively producing compost that was collected by local farmers – in high demand. This plant is situated in the Grabouw/Elgin valley (Figure 49), an area renowned for deciduous fruit crops (Wessels et al., 2020; www.grabouw-info.co.za). It is home to popular commercial sparkling apple juice production facilities, and local farming is extending into viticulture. Thus, there is a continuous market for compost, which can be provided by local waste producers without extensive transport and logistical costs. In addition, the Grabouw WTW is less than 1.5 km from the WWTW (Figure 49), facilitating the co-amendment of this composting process with WTR without extensive transport costs.

Figure 3.3. The Grabouw HotRot, operated by the Theewaterskloof municipality on the Grabouw WWTW grounds, installed in 2012 by David Crombie, of GIBB Engineering Consultants.

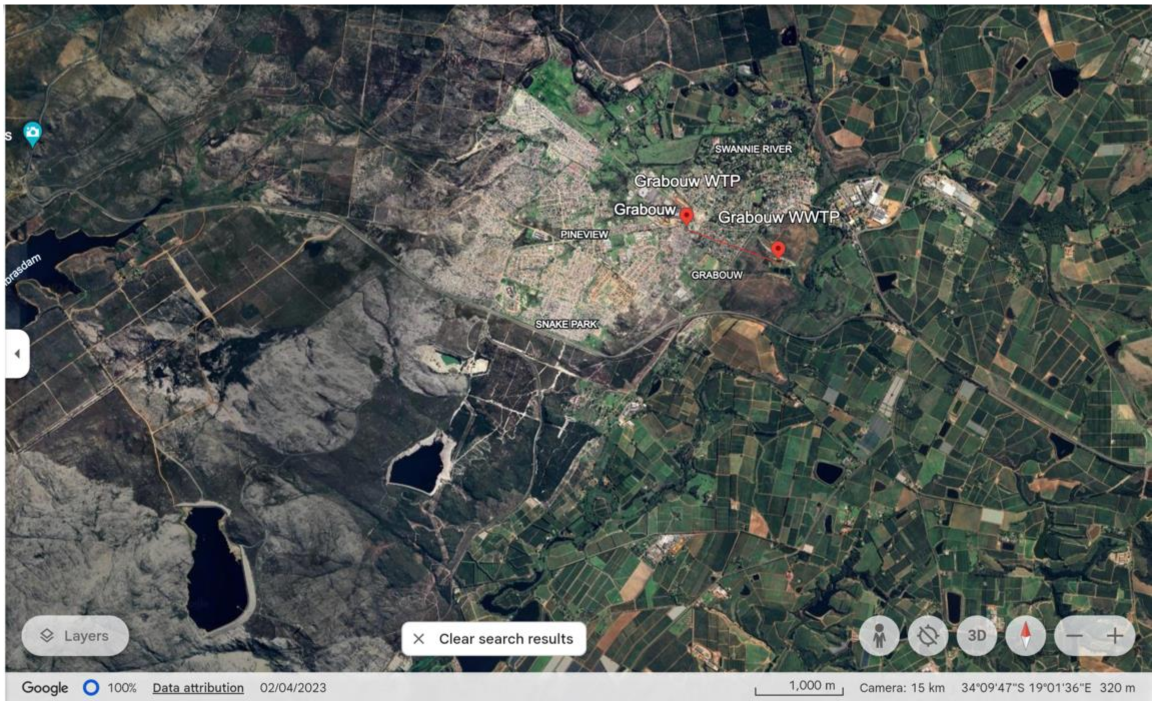

Figure 3.4. Location of the Grabouw WWTW, situated in the Grabouw/Elgin valley amongst deciduous orchards and vineyards, in a town extensively driven by an agricultural economy. The local WTW, producing the WTR for potential co-composting according to the circular model proposed in this study, is less than 1,5 km from the WWTW (red line).

This system has, however, been subject to similar management-level delays in maintenance as the anaerobic digesters. The HotRot system has a sophisticated electronic programmable control panel (programmable logic controller, PLC) which is valuable, and therefore theft had rendered the Grabouw HotRot non-functional at the launch of this project (2021). Throughout 2021 and 2022, the City of Cape Town has funded system repairs and increased security measures to protect it, and project partners (SU and PEDI) have been in constant contact with the site manager, monitoring the refurbishment process. However, the last stage involves connecting the electronic control panel. This has not been approved by the City of Cape Town, as of January 2024. There is a second HotRot facility on PEDI grounds in the Philippi area, however, this technology is continuously functional and dedicated to composting sources other than sewage. Diversion of those facilities to this application is not ideal, due to transport costs and odour risks during the storage of the sewage prior to composting. Thus, this study turned its focus from physical composting to a bird's eye analysis of (1) volumes, (2) distribution and (3) socio-economic drivers, to motivate for either government or private funds for dedicated processing and logistical diversion of local sludges into agriculture.

4 ECONOMIC AND POLICY RISKS AND OPPORTUNITIES FOR DIVERTING SLUDGE WASTES TO AGRICULTURE

4.1 What can we expect from our water and wastewater treatment sludges?

In a strong opinion piece in Environmental Science and Technology, entitled 'We should expect more out of our sewage sludge', Peccia and Westerhoff (2015) attempt to shift the perception of sludge wastes from liability to resource. Although they consider the agricultural land application of sludge preferable to landfilling, they propose that it is not sustainable. They encourage the implementation of novel technologies for high value resource recovery, like energy or minerals. They note public resistance to "anything with the prefix 'slu'", and comment on the dangers of land applying sludge, including persistent organic pollutants, pathogens and heavy metals. The history of sludge diversion to land application in the USA (Figure 50) has cycled through periods of conceding to public pressure, and periods of resisting public pressure, first banning the land application of sludge and then developing responsible and sustainable distribution efforts.

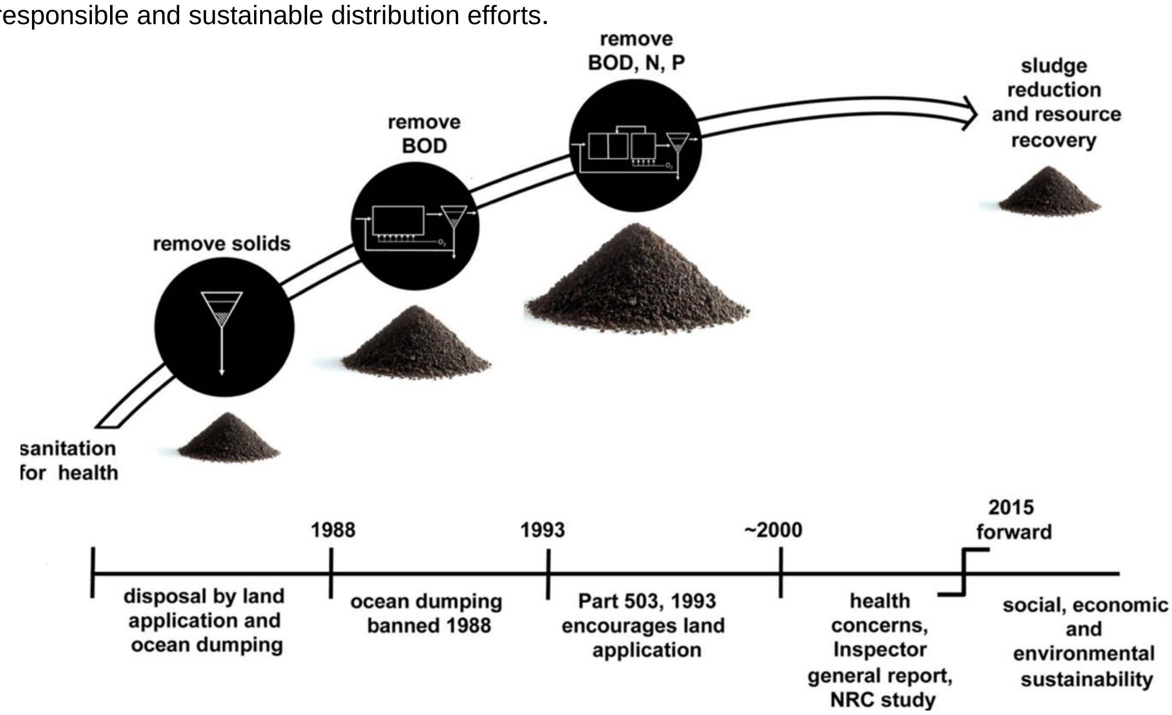

Figure 4.1. The arc of sewage sludge history in the USA, leading international responses, according to Peccia and Westerhoff (2015). Sludge land application shifted from ocean and land-based dumping to banning of ocean dumping in the late 1980s. With a shift to land-based use, USA public pressure led to increasingly stringent ordinances, banning agricultural application before re-introducing regulated land application. They suggest an exploitation of the energy and chemicals inherent in sludge, as an alternative to land application.

The current Water Research Commission (WRC) study 2022/2023-00820 aligns with the Peccia and Westerhoff (2015) assertion that recovering high value products from sludge is the ideal economic outcome. However, Peccia and Westerhoff (2015) claim throughout that the true ideal is a reduction of sludge production. Neither novel technologies nor reduction in sludge production are foreseeable interventions in the context of population growth, particularly in economically developing nations like South Africa (DESA UN, 2022). Thus, land application remains the most feasible local response, despite multi-national toxicity concerns. These USA land bans and ordinances are issued in response to public pressure. Examples include farmers reacting negatively to contaminants detected in their lands and crops, such per-and polyfluoroalkyl substances (PFAS; Perkins, The Guardian, 2022). Town boards, like Wheaton, have consequently legislated their own biosolid land application ban (Kopycinski, ENFP, 2014). They appeal to the public to avoid crops grown on biosolids by purchasing organic produce. However, amidst strong pressure to legislate the protection of croplands and groundwater, and public celebrations of victory in banning waste, there is no suggestion as to alternative disposal options. Most notably, there is little acknowledgement of the cross-boundary nature of ecosystems. This type of public resistance does not concede that groundwater and surface water contamination at landfill sites are not contained. Pollutants will leach into the environment via soil, groundwater, surface water and air pollution (D Krčmar et al., 2018; Bihałowicz et al., 2021). In addition, the articles have a heavy focus on persistent organic pollutants, but do not call for improved regulation of the widespread production or utilisation of pharmaceuticals, personal care products, plastics, or industrial chemicals. These chemicals are increasing in our collective water footprint, with hardly any pristine sites on earth. There are reports of these recalcitrant pollutants dispersed via dust and water, detected in regions as remote as mountaintops (Lyons et al., 2014) and the artic circle, with some being listed as contaminants of artic concern (Sonne et al., 2021; Kallenborn et al., 2018). This study proposes that the transboundary nature of pollution fundamentally undermines the 'not in my backyard' response (Liu et al., 2018). However, in an elegant 2014 survey – proposing two hypothetical projects with different impacts at a city boundary (a waste disposal facility and a chemical production facility) – it was shown that self-interest is not the primary driver of the 'not-in-my-backyard' response (Liu et al., 2018). Participation deprivation and a limited environmental understanding emerged as the two primary drivers of resistance. The study was entitled, 'Not in my backyard, but let's talk', encouraging participatory processes for improved regulated waste diversion.

The European Union has a diverse response to sludge waste but has embraced it with extensive regulations and guidelines. As early as 2014, Ireland was diverting as much as 70% of their sewage sludge into agriculture (Hudcová et al., 2019). In contrast, Germany has increased classification stringency for the land application of sludge waste but has concurrently implemented alternative technologies like biogas digesters. A 2019 review of the European Union's approach to sludge waste recommended the use of ecotoxicity assays, as evaluated for this co-diversion strategy in Section 3. They suggest that ecotoxicity results communicate the impact of waste more clearly to the public during participatory processes, with the assumption that the public will engage with data that describes effects against living organisms more smoothly than with lists of pollutant concentrations.

4.2 'Bearing witness to our sites of forgetting'

Keeping waste in the public discourse and facilitating an understanding of waste impacts are key drivers for effective waste re-utilisation strategies, particularly those needing public participation like the agricultural application of sludge. In a perspective piece called, 'Waste, landfill and an environmental ethic of vulnerability', Hird (2013) calls western landfills "sites of forgetting made possible through legislative decision, regulative decree, risk models, community accession, and engineering practice." We propose that we can – as consumers, waste producers and the public – only place pressure on the government's hand to refuse the land application of sludge if we have collectively facilitated or contributed to (a) scientifically and economically feasible alternatives, and (b) a practical shift in our own consumption habits, placing negative market pressure on the production and distribution of these chemicals. For instance, PFAS detected in sludge – which caused US farmers to protest governmental drivers toward the land-application of sludge (Perkins, The Guardian, 2022) – originates in personal care products like soap, cleaning agents, and non-stick cookware. All of these are convenience commodities almost certainly utilised extensively by farmers and the public. The contamination responsibility lies with the consumer as much as with waste governance. The producer is simply acting in response to a demand, and the government is responding to the waste footprint. Thus, this work aims to support government efforts, acknowledging the complexity of the current waste footprint, acknowledging the low likelihood of shifting the toxic footprint of the waste, and exploring ways to harness the environment to the best of our ability to remediate the waste. Hird strikingly describes these efforts as “bearing witness to the waste we want to forget”, using the terminology of “collaboration” between soil organisms and waste producers for the strategic design of waste remediation. The notion of collaboration – between those producing waste and the biological community facilitating bioremediation in the soils receiving waste – is challenging language, placing the onus on every stakeholder in the life cycle of sludge wastes.

4.3 Current infrastructure

Acknowledging the economic potential of resource recovery as an alternative to land application is exciting. But in a developing nation like South Africa, although plans are in place to harness new technology, it also unrealistic to assume such technology uptake soon. This WRC project (2022/2023-00820) has been stalled repeatedly due to anaerobic digester failure in the Western Cape, because of maintenance issues (Section 4). The HotRot is a promising technology installed to beneficiate sludge into compost at Grabouw WWTW (Keeton, Times Live, 2017; Prezi HotRot Solutions, 2014). It was also non-functional for the full two-year project duration due to cable and control panel theft. They have been in the process of repair for 26 months, and this project has followed up every two months throughout (Project 2022/2023-00820). In addition, it was recently made public that standard wastewater pipeline infrastructure is collapsing in the Strand, under the City of Cape Town (CoCT) Metropolitan municipality. Twenty-eight sinkholes eroded over 5 years, according to popular media (Engel, The Daily Maverick, 2023). Reports from mid-2023 town council meetings claim that the CoCT has launched procurement for a sewage pipeline rehabilitation plan of R79 million, a similar rehabilitation plan for a neighbouring wastewater treatment pipeline system at R240 million, and a contingency cured-in-place diversion pipeline of R57 million. The enormous tensions facing local government must be acknowledged, and any novel technology would ideally support their efforts and priorities, rather than introducing pressure-based legislature on budgets that are already fielding emergency infrastructure maintenance. Despite these immediate challenges, the CoCT has launched visionary plans to build three Biosolids Beneficiation Facilities, the first to be completed in 2024 with the capacity to treat 145 dry tonnes of sewage sludge per day (DEA&DP, 2021). The second two will follow in phases. Ballooning costs have slowed the process, but the plans continue (personal communication, 2023; Sven Sotemann, Head of Planning and Development, Wastewater Treatment, Bulk Services, Water and Sanitation, CoCT).

Thus, although alternative sludge beneficiation is a long-term governance target, the Western Cape landfill organics ban (DEA&DP, 2022) demands more immediate attention, with 100% of organic diversion mandated by 2027. Thus, improved land application design remains the most feasible targeted intervention in the interim, in the socio-political context of South Africa. In addition, between the US and Europe, there is international precedent to support this strategy, if well designed.

4.4 Sludge status

In 2017, the Western Cape Integrated Waste Management Plan (WCIWMP), hosted by the Department of Environmental Affairs and Development Planning (DEA&DP), commissioned a Sewage Sludge Status Quo report (2021). It was designed in support of Goal 2 of the WCIWMP to develop a guideline for the beneficiation of treated sewage sludge. This plan describes the organic waste diversion targets mandated in the organic waste landfill ban (Position Paper on Organic Waste Management, DEA&DP, 2017). Using questionnaires distributed to the WWTW in their networks, they determined that sewage sludge from Western Cape WWTW is distributed to

- land farming (22%),
- general (20%) or hazardous landfills (10%),

- stockpiled (22%),
- composting/agricultural/irrigation use (11%).

The Western Cape of South Africa has 26 WWTW, with treatment technologies as diverse as marine outfalls, 16 activated sludge WWTW, 1 trickling filter, 4 rotating bio-contactors, 2 pond systems and 2 anaerobic digesters. Together, they produce sewage sludge that is categorised into four types: primary (PS), waste activated (WAS), anaerobic digestate (AD), and blended. Waste activated sludge and anaerobic digestate are ideal for land application. In total, the CoCT produces approximately 2200 dry tonnes per month of WAS and 855 dry tonnes per month of AD. Thus, approximately 3000 dry tonnes of sludge has the potential to be diverted to agriculture. As of 2021, only 33% of the CoCT's sludges were diverted to agricultural applications (Figure 51).

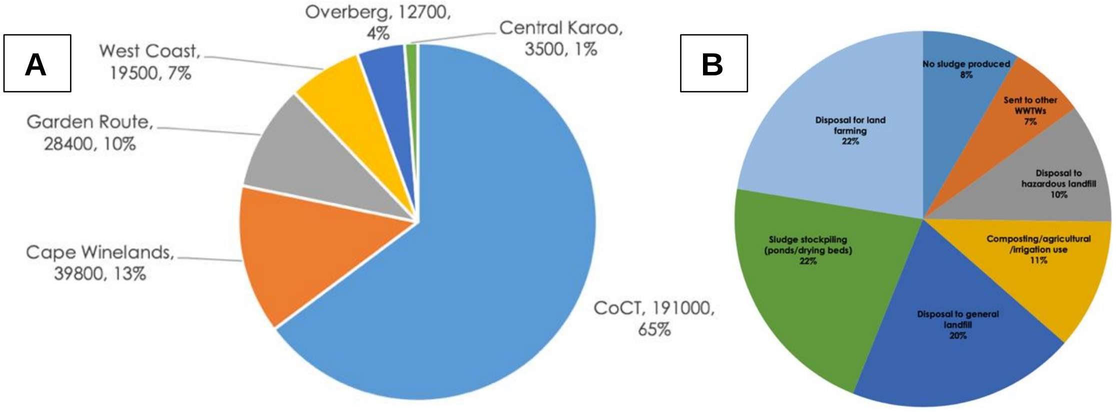

Figure 4.2. (A) Tonnes of sewage sludge per Western Cape district of South Africa in 2016 (GreenCape Market Intelligence Report, 2018; DEDAT, 2016), and (B) distribution of sewage sludge destinations in the Western Cape district of South Africa as of 2021 (DEA&DP, 2021).

4.5 Entrepreneurship & product development

It is not only sewered systems and WWTWs that produce sludge. A recent (2020) WRC report reported that only 61% of households in South Africa have access to sewered networks, whereas the rest are dependent on on-site sanitation systems, such as septic tanks, ventilated improved pit latrines and open defecation (WRC, 2020). Up to 10% of these pit latrines are full, leading to challenges in sanitation (StatsSA, 2019). South Africa's Faecal Sludge Management Conceptual Framework (DWS, 2021) describes a plan to mainstream faecal sludge management and promote beneficial activities, particularly sanitation technologies and entrepreneurship to 'support climate change preparedness and resiliency in human settlements'. The document describes inspiring case studies of businessmen and women who developed a collective imagination to view sewage sludge as a high-value product. These individuals successfully collect and redistribute or dump faecal sludge in populated areas in India, Mozambique and Dakar. A similar local entrepreneur – already receiving much of the WAS from Saldanha and Vredenburg and composting it to a high value product – is West Coast Bio-Organics (https://westcoastbioorganics.websites.co.in/; DEA&DP, 2021). The Status Quo report also describes notable local innovation in the eThekwini Metro Municipality (EMM), which has taken the lead in funding innovative technologies for faecal sludge management. These include (1) the LaDePa (Latrine Dehydration and Pasteurization) plant, which pasteurises and pelletises faecal sludge to create a pathogen-free low grade organic compost, as well as (2) a DEWATS (DEcentralised WAstewater Treatment System). The design combines anaerobic digestion and constructed wetlands, and treats water to edible crop irrigation standards. The description of private entrepreneurship in the Sludge Management Conceptual Framework is encouraging and creates a precedent for analogous local responses.

The Sewage Sludge Status Quo Report (DEA&DP, 2021) also describes a precedent for municipal facilitation of entrepreneurship in Washington DC (United States of America). There, the local municipality is harnessing beneficiated sludges as an alternative revenue source, with in-house processing, packaging and marketing of the sludges to local farmers, garden centres and households. GreenCape has been contracted by the CoCT to investigate similar local revenue streams, for the planned Biosolids Beneficiation Facilities.

South African Sanitation Technology Enterprise Programme (SASTEP) – created by the WRC in partnership with the Department of Science and Innovation (DSI), and the Bill and Melinda Foundation (BMGF), with the support of the Department of Water and Sanitation (DWS) – has the mandate to form collaborative partnerships with sanitation innovators and entrepreneurs to ensure the translation of suitable and appropriate sanitation technologies to the marketplace. This could be a vehicle to commercialise the processing and distribution of WTR along with sewage sludge, for entrepreneurs like those in India and Mozambique.

To support the diversion of organic waste to productive applications, the Western Cape government has partnered with ORASA (the Organics Recycling Association of South Africa) to promote alternative waste treatment technologies. As of 2021, 11 out of the 30 municipalities had submitted organic waste diversion plans to the DEA&DP. In 2020, the CoCT had successfully diverted 54% of total organic waste from landfill to productive applications (DEA&DP, 2022). They include a helpful map of chipping, mulching, composting, biogas and other facilities for commercial organic waste processing (Figure 52).

1. KNYSNA
Sedgefield Sedgefield Composting and Builder's Rubble
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3. STELLENBOSCH
Langverwacht landscaping
Langverwacht@mweb.co.za
www.langverwachtland-scaping.co.za
021 880 1853

4. HERMON
Tomis Edel Compost
www.tomis.co.za
022 448 1680

5. TULBAGH
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jjacobs@witzenberg.gov.za
023 316 8540

6. PRINCE ALBERT HAMLET
Prins Albert Hamlet Composting Facility
jjacobs@witzenberg.gov.za
023 316 8540

7. ATHLONE INDUSTRIAL
Ywaste
www.ywaste.co.za
021 691 0753

8. MITCHELL'S PLAIN
Master Organics
info@master-organics.com
021 396 1066

9. BELLVILLE
Bellville compost site
www.capetown.gov.za/solidwaste
0860 103 089

10. DURBANVILLE
Zandam Cheese Biogas project
www.ibert.co.za/reference/zandam

11. PHILIPPI
Inseco AgriProtein Technologies
www.inseco.co.za
info@inseco.co.za
087 255 1569

12. CLAREMONT
Zero To Landfill (ZTL)
Organics www.ztlorganics.co.za
083 696 5138

13. PHILADELPHIA
Neutrog Philadelphia
www.neutrog.co.za
info@neutrog.co.za
021 972 1958

14. MALMESBURY
DNA Composting facility
083 256 8168

15. MALMESBURY
Reliance Group Trading (Pty) Ltd.
www.reliance.co.za
info@reliance.co.za
0861 888 784
021 951 3161

16. MALMESBURY
Astral / County Fair Facility Anaerobic Digester
gerrit.visser@countyfair.co.za
021 884 3013

17. MALMESBURY
Cape Dairy Biogas Anaerobic Digester
www.bio2watt.com

18. PORTION OF FARM AFDAKSRIVIER 575
Karwyderkraal Composting facility
fkotze@odm.org.za
028 425 1157

19. GRABOUW
Elgin Fruit Juices Anaerobic Digester
hein@gcxafrica.co.za
021 859 7160

20. GRABOUW
Agri Organics Composting facility
www.agriorganics.co.za
021 852 8632

Figure 4.3. The distribution of existing enterprises involved in the beneficiation of waste in the City of Cape Town and surrounds, to which sewage sludge can be funnelled for commercial benefits.

4.6 What about potable water sludges?

4.6.1 Distribution and design: Soil, crop and sludge pairing.

Across all of these disposal strategies, the potable water treatment sludges (WTR) are not included. Although Herselman has developed land application guidelines for both sewage sludge (Herselman and Moodley, 2009) and WTR (Herselman, 2013) for the WRC of South Africa, the WTR is not included in these sludge-tallying and distribution activities. The Sewage Sludge Status Quo Report (DEA&DP, 2021) explicitly states that the

> 'guidelines were not developed to include inorganic sludge produced by potable water treatment plants.'

Mokonyama et al. (2017) executed a survey of WTR disposal practices and reported that the Faure water treatment works (WTW) disposes all their sludge to hazardous landfill, at a significantly higher cost than general landfill. The GreenCape Waste Market Intelligence Report (2020) reports a hazardous landfill gate fee of R852 per tonne versus R643 per tonne for general waste (forecast for 2022). The reason for classifying WTR as hazardous is likely the potential heavy metal concentrations, as well as phosphate sorption capacity, which has a negative effect on soil fertility as a single amendment (Ippolito et al., 2011). The entrepreneurship examples and composting facilities described above (Figure 52) also focus on sewage sludge and not WTR, likely because of a perception of toxicity or a lack of awareness of the resource. Nutri Humus (Pty) Ltd. (https://nutrihumus.co.za/) is one local company that is actively beneficiating sewage sludge via the composting process. There is extensive evidence that WTR can be beneficial for land application if co-applied with sewage sludge, and here we advocate for economic and socio-political drivers towards this co-diversion in the local market (Ippolito et al., 1999; Clarke et al., 2019).

In addition to explicitly excluding WTR from the sludge beneficiation and management strategies, the Sewage Sludge Status Quo report lists three primary challenges in the beneficiation of sewage sludge, including

1. Primary sludge must be diverted to landfill unless extensively treated.
2. Service providers are willing to/equipped to collect and dispose, but not beneficiate sludge. The tenders requesting beneficiation expertise thus go unanswered.

> 3. The CoCT may run out of suitable agricultural land in 5-10 yrs.

Thus, although some sewage sludge is already being diverted into agriculture in the CoCT, the following two problems create a niche for this study:

1. The exclusion of WTR from agricultural sludge distribution and beneficiation management plans,
2. The soil quality receiving the wastes is sandy (Figures 53 and 54). The low organic content and structure of sandy soil facilitates pollutant mobility and minimises nutrient sequestration (Boyd et al., 1998). This increases the likelihood that the CoCT will run out of land for sewage sludge application, as mentioned above. This sandy soil is widely distributed further than the CoCT, increasing the footprint of land in which this co-diversion concept can promote soil fertility (Figure 54).

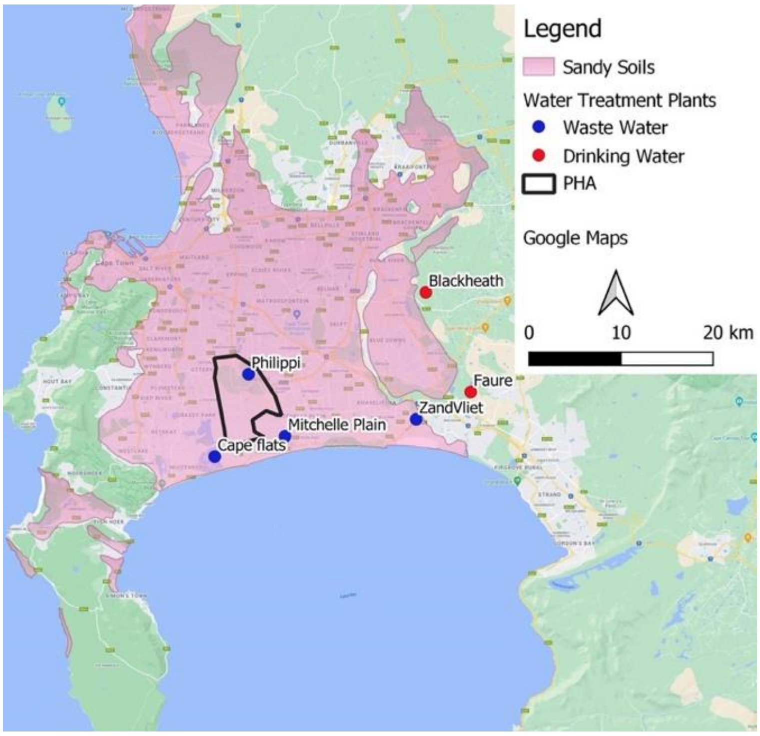

Figure 4.4. The distribution of sandy soils in the areas surrounding the City of Cape Town, with wastewater and water treatment plants, generating the potential sludge soil amendments (Figure 2 repeated for visual comparison to the map below).

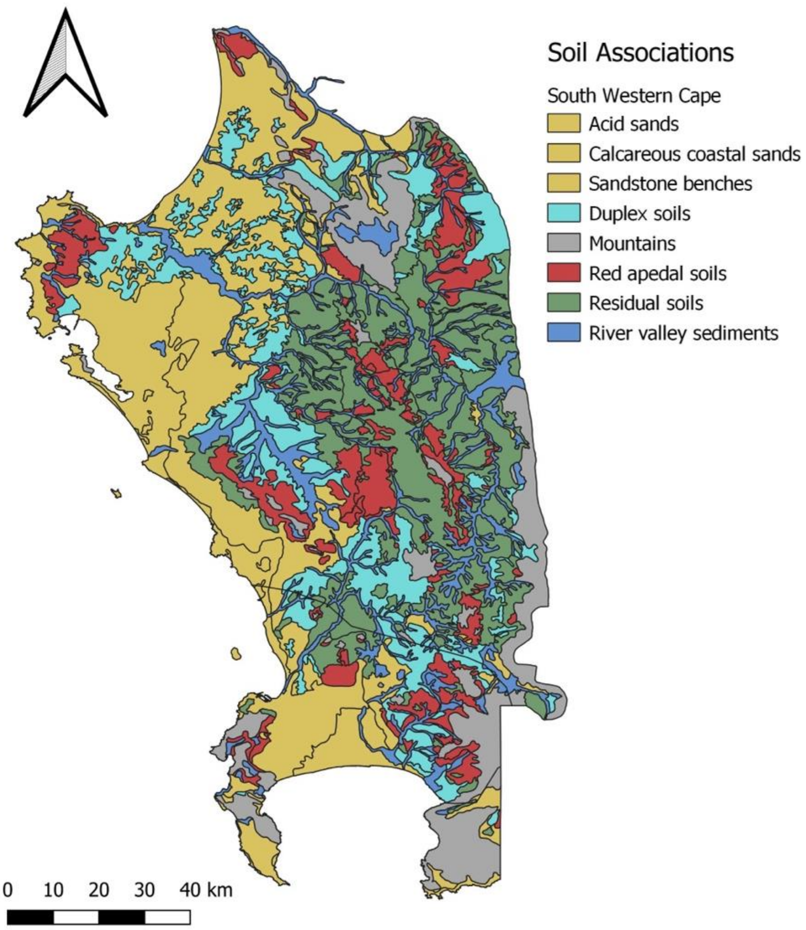

Figure 4.5. The wider distribution of sandy soils (yellow ochre) in the areas surrounding the City of Cape Town (Data supplied by ARC-ISCW)

This study has investigated the environmental benefits of pairing WTR with sewage sludge to improve low-nutrient sandy soils for agricultural crop growth. This addresses two problems, as it (1) diverts WTR sludge waste along with sewage sludge waste into productive applications, and (2) minimises the risk of pollutant mobility, fortifying the sandy soil by improving nutrients, physical characteristics and therefore increasing the range of soils that can receive sewage sludges.

As a brief reminder of the environmental rationale and the diversion strategy, WTR is essentially concentrated dam sediment which is typically enriched in clay compared to topsoils (Erskine et al., 2002). Both these clay-like properties and the flocculants (polyelectrolyte and metal oxides) used in the treatment process afford the sludge sportive properties, often used to immobilise heavy metals (Sarkar et al., 2007; Ippolito et al., 2011). Clays (Cheng et al., 2021) and biochar (Oleszczuk et al., 2014) amendments are promoted as fortification strategies to improve the capacity of sandy soils to receive sewage sludge, and WTR has similar properties (Luo et al., 2012). It runs the risk of limiting soil phosphate (Ippolito et al., 2011), but with careful design and sludge pairing with phosphate-rich sewage sludges, co-diverting these sludges from landfill into agriculture (Figure 55) can improve soils in terms of crop biomass (Ippolito et al., 1999; Clarke et al., 2019), chemistry (Clarke et al., 2019), microbiology (Stone et al., 2021) and soil-water dynamics (Steytler, 2021; Moodley and Hughes, 2006). To avoid pathogenic risks on edible crops, the work has focused on growing textile crops such as hemp on these sludges. These types of textiles crops, like hemp and bamboo, also have bio-remediation capacity (Loffredo et al., 2021; Citterio et al., 2005; Bian et a., 2021), mopping up pollutants from the soil with every crop cycle. Thus, they also extend a sandy soil's capacity for re-application of sewage sludge and address the challenge of sludges exceeding agricultural land, as highlighted from the Sewage Sludge Status Quo report above (DEA&DP, 2021). Growing high value crops which can be harvested, generating a high crop turnover, may remediate the pollutants faster than lawn application (sportsgrounds) or tree plantations.

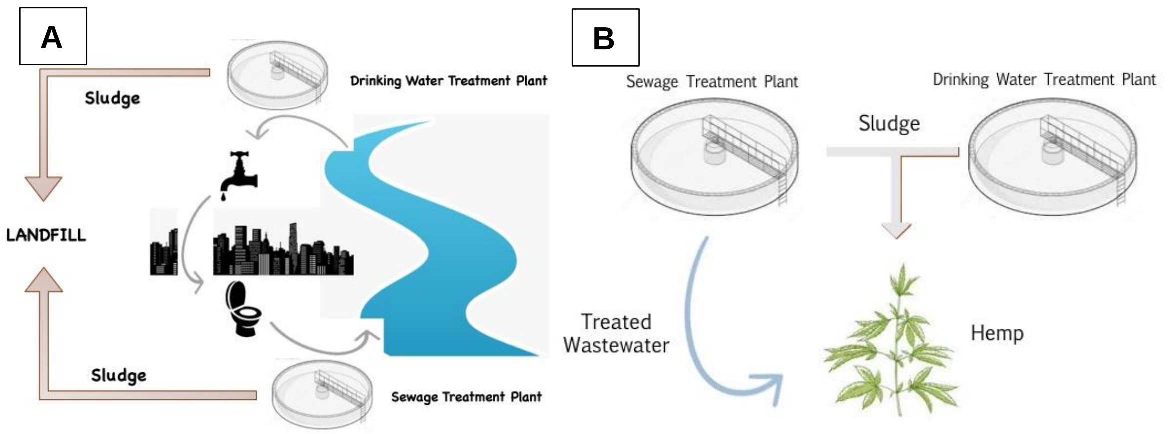

Figure 4.6. Current local sludge diversion strategy to (a) landfill versus (b) a circular strategy diverting sludge wastes to soil amendments, promoting textile or biofuel crop farming in geographical areas surrounding the treatment plants (Figure 4 repeated for accessibility, prior to logistical considerations).

4.6.2 Feedstock supply security and logistics

Feedstock supply security is a critical consideration in waste-to-market strategies. If waste sources are vulnerable to interruptions or fluctuations, as they often are (Gosh, 2016; Jayant et al., 2014), it interrupts the value chain and market and threatens the sustainability and public acceptance of the strategy. However, as expressed in the Sewage Sludge Status Quo report, in terms of high value product development (composting, pelletisation, or direct land application) from sewage sludges, there is the opposite risk: that the product will exceed the demand, in terms of the regularity and volumes of sludge production (DEA&DP, 2021). The logistics of storage and distribution are a greater risk in the beneficiation of these sludges than feedstock security, particularly since they need to be paired together (WTR and sewage sludge), and nutritionally paired with the soil type and crop species for optimal soil amelioration. In addition, rigorous application rate calculations are necessary to prevent excess nutrients causing eutrophication of the surrounding water sources – both surface water runoff into surrounding rivers and catchments, and groundwater sources.

The Sewage Sludge Status Quo report highlights these logistical concerns, emphasising that most WWTW do not have the capacity to stockpile sludge on-site, and need constant demand to match the rhythm of removal to landfill, for effective diversion into agriculture. Removing sludge to composting facilities is an ideal solution for waste centralisation and transient storage. Nutri Humus is a local company facilitating this process (https://nutrihumus.co.za/). However, many local municipalities have developed local solutions that separate them from centralisation strategies and national supply-chain models. Wu et al. (2022) propose a similar localised 'strategic control model', connecting strategic planning with demography and local workforce. They suggest that such a human-centric localised waste value chain, separated from a national supply chain, is both positive in terms of greenhouse gas emissions (minimised transport) and promotes local job opportunities, facilitating societal development (personal engagement with environmental issues). This type of human centric model has naturally arisen in some local municipalities, who are distributing their waste in a well-considered and human-centric way, in partnership between public and private entities (DEA&DP, 2021):

- In the Hessequa municipality, Riversdale and Albertinia industrial sewage – unfit for crops – is used to irrigate a local golf courses and rugby fields, and Heidelberg WAS is distributed to nearby farms.
- Knysna sewage sludge is distributed to a local flower nursery.
- Mossel Bay sewage is diverted to a composting facility for beneficiation.
- In Saldanha Bay municipality, Saldanha and Vredenburg sewage sludge are beneficiated (composted) for commercial sale by West Coast Bio-Organics, and Laingville sludges are applied to local sports grounds and agriculture.
- In the Swartland, Malmesbury and Riebeeckvalley WAS is transported to farms in a 10 and 15 km radius, respectively.
- Particularly interesting: in the Langeberg and Breede River Valley, the WAS produced at Ashton, Bonnievale, Robertson and de Doorns are reportedly collected by local farmers, eliminating the transport costs against the municipality.

Thus, applications range from sports grounds, to nurseries, and farms in a well-designed radius, and some municipalities have even motivated farmers to collect sludges, rather than carrying the transport costs themselves. Other municipalities also reported the land application of sludge in the Sewage Sludge Status Quo report, but they did not describe logistical considerations. It is also not clear from this report how well these receiving soils, from sports grounds to farms in a <20 km radius, are monitored for heavy metal accumulation, pathogens or micropollutants. Studies have shown that heavy metal bio-accumulation in soils disrupted biological activity even after 2 decades of rest (Chander and Brookes, 1991). This will be dependent on soil type, quality of sludge, crop activity on the land and other factors like hydrogeology and precipitation. Although the benefits of this application are typically higher in nutrient-poor sandy or loamy soil than clay soil (Aggelides and Londra, 2000), the toxic effects in sandy and sandy loam soil are also higher, with greater longevity, than the silty loam soil (Chander and Brookes, 1991). The research shows that co-amending with WTR can at least alleviate this risk and likely increase the longevity of the land application strategy, if the logistics of co-diversion can be managed (Ippolito et al., 2011).

However, the pairing of WTR and sewage sludge introduces logistical challenges, as they are produced at different locations in urban areas. Nevertheless, where humans drink, humans excrete. Every urban settlement has both potable water and sewage treatment plants. Where people settle, they presumably consume water and produce waste at relatively similar ratios, internationally. There is some international variation in water consumption rates, although the variation is not as exponential as expected. A 2007 study reported an average water footprint of 2480 m^3/cap/yr in the USA and an average water footprint of 700 m^3/cap/yr in China (Hoekstra and Chapagain, 2007). The four major drivers of national water footprints were (1) gross national income which increases consumption volumes, (2) wider consumption habits, like meat consumption, (3) climate, and (4) agricultural water use efficiency. However, with an increase in water consumption in countries with higher gross annual incomes, there is likely a concurrent increase in sewage production, thus the WTR and sewage sludges will likely be produced in an approximately consistent ratio in urban settlements across the world. This is convenient for sludge-to-agriculture management, in terms of supply and demand logistics in sludge pairing for land application.

The question arises, what is the standard WTR to sewage sludge ratio in an urban settlement?

Both from literature, and from discussions with local WTW during this study, there is little consensus on the national volumes of WTR produced per year. For instance, in the most recent survey of local national WTR production rates (WRC report TT 738), Mokonyama et al. (2017) describe extensive discrepancies in calculations. They refer to another WRC report in 2005 that tallies national WTR production at 405 000 tonnes dry solids (tDS) per year (Hughes et al., 2005). This was far more than Mokonyama's 2016 calculations, recording national WTR production rates at 300 000 tDS per year. They conclude that more accurate data is necessary. The current (2023) WTR sludge status at two local (Western Cape) WTW, recorded from an informal data request from Faure and Blackheath WTW, emphasise the nuances that confound this type of data. Whilst the centrifuge at Faure produces 60 tonnes per day, trucked to Vissershok landfill daily, Blackheath disposes of their residuals into ponds for dewatering. These are evaporated, and the ponds are emptied every three to five years – also to Vissershok landfill. Since there are variable Total Suspended Solids (TSS) in the waste discharged to dams, the plant managers cannot estimate tDS per year. Nevertheless, once every three years, sludge becomes available from these dam excavations that can be land applied with sewage sludge rather than discarded to landfill. This type of sludge processing influences feedstock supply security more than the Faure centrifuge with regular daily sludge production. In addition, the water removed by centrifuging facilitates much simpler sludge distribution logistics. The carbon dynamics evaluated in this study show that the aluminium sludges from Blackheath stabilise carbon to an even greater degree than the iron sludges from Faure, and thus this sludge is even more ideal for land application (Figure 15, Section 3.4). Aluminium sludges are predominant in South Africa, and iron-oxides are used for more stringent water treatment. In 2014, it was shown that over 71% of the WTR produced in South Africa came from one plant, belonging to Rand Water in Johannesburg, which is disposed of in the Panfontein landfill (Figure 56; Mokonyama et al., 2017).

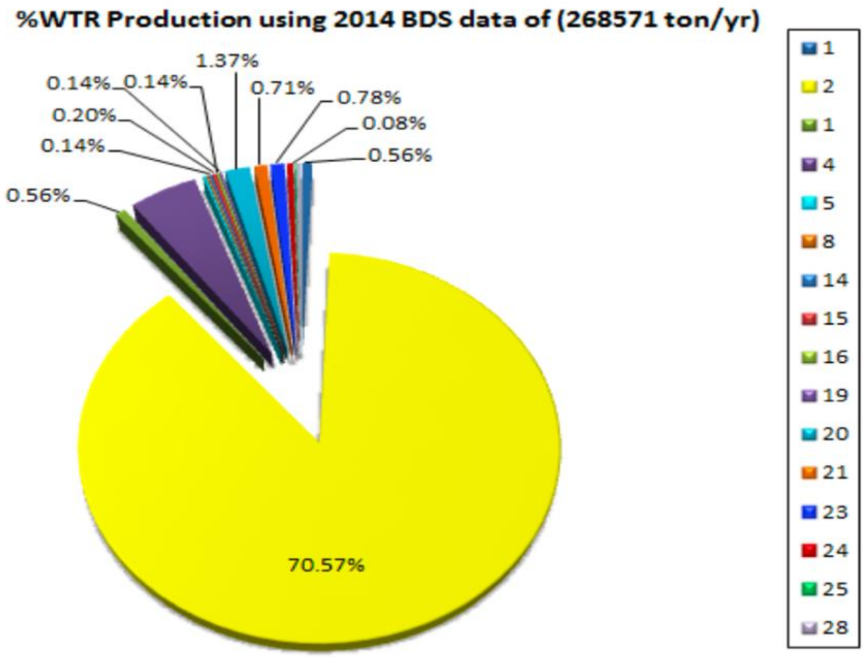

Figure 4.7. Contribution of plant WTR to national production average. The plants were randomised in this study, but the plant contributing to 70.57% of the national WTR production in 2014 is situated in Johannesburg (Mokonyama et al., 2017).

Although the soils in Johannesburg are not the same sandy soils investigated in this study, soil carbon is low throughout the country. Generally, 58% of soils in South Africa have less than 0.5% Soil Organic Carbon (SOC) and only 4% of soils have more than 2% SOC (Du Preez et al., 2011; Seboko et al., 2021). Recent carbon mapping shows how low the predicted long term SOC averages are in natural South African biomes (Figure 57; Venter et al., 2021), typically below 1 PgC in each biome. For comparison, countries with vast peatlands and wetlands have much higher SOC, ranging from 5.4 PgC in Peru (Hastee et al., 2022) to 29.0 PgC in a 167,600 km^2 peatland in the Democratic Republic of the Congo (Crezee et al., 2022). Additionally, soils in and surrounding the City of Johannesburg have declined in quality due to agricultural, domestic, industrial, and mining processes (Seboko et al., 2021). Thus, the soils surrounding the Johannesburg area, in the Granite Dome area – underlaid by granitic, gneissic, and granodiorite rocks (Seboko et al., 2021) – are also nutrient-poor and are ideal for the distribution of sludges to improve soil fertility. This sludge co-distribution strategy is thus not limited to the Western Cape of South Africa, and, due to the higher WTR sludge production rates, may be even more applicable in the agricultural regions surrounding the City of Johannesburg.

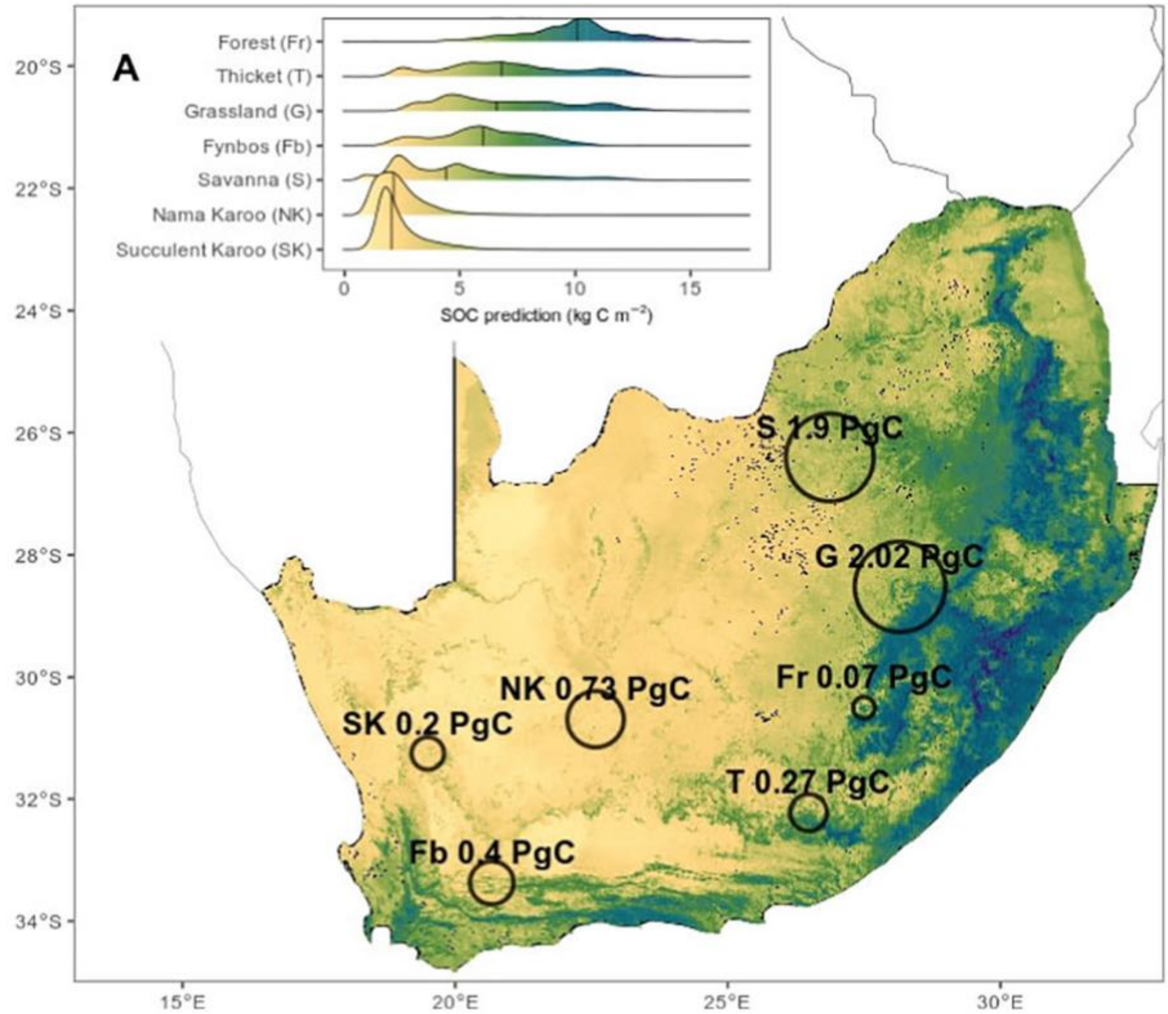

Figure 4.8. Predicted long-term average soil organic carbon (SOC) between 1984 and 2019. Text and circles indicate total SOC amounts within each biome in petagrams (10^{12} kg) (Venter et al., 2021).

The last national review of WTR sludge production rates cautiously suggested that South Africa produced 300 000 tDS per year (Seboko et al., 2017). They predicted a substantial increase, with pending increase in WTW infrastructure. However, global water consumption increases ~ 1% per year, according to the United Nations (Uhlenbrook and Connor, 2019). For this study, we are estimating a current national production rate of 320 000 tDS (rounded up from 318 456 tDS) per year (2023), based on a yearly 1% increase – from a 2017 baseline of 300 000 tDS per year – between 2017 and 2023. A 2022 review of the South African national sewage sludge footprint (Apollo, 2022) quantified the national sewage sludge footprint at 4 600 000 tDS per year, according to Eq 11 (Table 35). The Western Cape sewage sludge production rate is 295 000 tDS per year (DEA&DP, 2021) and the WTR sludge production is approximately 48 000 tDS per year. This is estimated based on the fraction of Western Cape WTW generating WTR (15%, Mokonyama et al. 2017, Figure 56), of a national total of 320 000 tDS per year (as calculated above). The final WTR to sewage sludge ratios range between 0.07 and 0.19 (Table 35). Thus, between 10 and 20% of sewage sludge can be paired with WTR for land application, if other WTR applications are not part of the diversion and re-utilisation strategy and 100% is funnelled into fortifying agricultural land to receive sewage sludge. None of the other beneficiation strategies explored by Herselman (2013) are currently being harnessed nationally, according to the authors' awareness, based on informal discussions with local WTWs. Globally, WTR and sewage sludge tallies reflect a similar ratio, confirming these estimations. In 2007, Babtundo and Zhao reported a 10 000 tDS/day WTR production rate, which has been consistently attributed to later authors since (Ahmad et al., 2016, Gibbons and Gagnon, 2011). However, as mentioned, global water consumption increases ~ 1% per year, according to the United Nations (Uhlenbrook and Connor, 2019). Thus, in the 16 years since that tally, it has increased exponentially. We therefore added 1% per year to the global WTR production rate, cumulatively, according to Eq 12, leading to a current global WTR production rate of 4 280 000 tDS/year. Similarly, global sewage sludge production was estimated at 45 000 000 tDS/year in 2017 (Giacomo and Romano, 2022; Zhang et al., 2017), and a 1% cumulative increase results in 47 300 000 tDS/year in 2023 (Eq 13).

$tDS.yr^{-1} = 13\, tDS.day^{-1} \times 365\, day.yr^{-1} \times 970\, WWTW.country^{-1}$……………..................…Eq. 11.
$= 4\,602\,650\, tDS.yr^{-1}$ (rounded down to 4 600 000 $tDS.yr^{-1}$)

$tDS.yr^{-1} = [(10\,000\, tDS.day^{-1} \times 365\, day.yr^{-1}) + ((10\,000\, tDS.day^{-1} \times 365\, day.yr^{-1}) *$
$1\%.yr^{-1})]\, for\ 16\, yrs$ ....Eq. 12.
$= 4\,279\,912\, tDS.yr^{-1}$ (rounded up to 4 280 000 $tDS.yr^{-1}$)

$tDS.yr^{-1} = [(45\,000\,000\, tDS.yr^{-1}) + ((45\,000\,000\, day.yr^{-1}) *$
$1\%.yr^{-1})]\, for\ 6\, yrs$ ....Eq. 13.
$= 47\,768\,407\, tDS.yr^{-1}$ (rounded up to 47 800 000 $tDS.yr^{-1}$)

Table 4.1. Data, calculations and sources to attempt an estimation of a typical urban WTR to sewage sludge ratio, to inform strategies for land distribution.

	Sewage Sludge (tDS/yr)	Assumptions	Sources	WTR (tDS/a)	Assumptions	Sources	Ratio (WTR: Sewage)
Provincial Western Cape	295 000	-	DEA&DP (2021)	48 000	15% of national production	Seboko et al. (2021)	0.19
National South Africa	4 600 000	Eq. 11	Apollo (2022)	320 000	1% increase, 300 000 $tDS.yr^{-1}$, 2017-2023	Seboko et al. (2021)	0.07
Global	47 800 000	Eq. 13		4 280 000	Eq. 12	Babtundo & Zhao (2007)	0.09

Thus, between 10 and 20% of our sewage sludge diverted to land can be co-applied with WTR, which both fortifies low-nutrient sandy soils to receive the rich and complex mix of nutrients and diverts the WTR organic material from landfill to productive applications. The secret to excellent sludge treatment and re-utilisation in many European countries is the pre-sorting of sludge and scientifically informed diversion to relevant technologies. Here, if we return to the concept of feedstock supply security, WTR is the limiting factor, not sewage sludge. However, with careful co-design, all the national WTR can be funnelled into land to fortify the soils receiving 10-20% of our sewage sludge, focusing on sandy soils first. Based on this motivation, this study aims to support similar strategic diversion, investigating the economic and policy impacts of the co-distribution of WTR and sewage sludge. The study is thus an *ex ante* impact assessment (IA) of this co-diversion strategy. The European Union (EU) defines an *ex ante* IA as "an attempt to provide, in advance of legislating, a coherent analysis of the reasoning that lies behind, and the foreseeable effects of, any proposed measure or policy initiative.” Here, the *ex ante* IA is the reasoning behind moving to field trials designed to support Verra certification with VCS methodology. This will support private investment to drive the strategy.

The objectives of the study are to investigate the economic and policy aspects of co-diverting WTR with sewage sludge into nutrient-poor agricultural land. These include (1) mapping the current regulatory and economic landscape, in terms of environment and agriculture, (2) evaluating the economic risks and benefits of the strategy, (3) assessing the feasibility of utilising this strategy to enter the carbon credit market at local scales, and (4) investigating the feasibility and potential avenues for introducing a waste circularity certification process to allow farmers to access the premiums of the local eco-conscious market.

This was achieved with four investigations:

(1) A review of the national and international policy and economic status of the diversion of sewage sludge and WTR into agriculture. This review is approached from both the agricultural and environmental perspective. This scientific intervention has impacts across both fields, and they are regulated by different branches of government and have entirely different economic impacts. A SWOT (strengths, weaknesses, opportunities and threats) analysis is included.
(2) A cost-benefit analysis modelling the co-diversion of WTR and sewage sludge into land application in the Philippi Horticultural Area.
(3) An analysis of the carbon credit market, tentatively linking the scientific carbon sequestration data produced in this study to potential private investment via carbon offsets. This is hypothetical and intended as a motivation for field trials designed to evaluate this strategy in situ as a Verra VCS certifiable project.
(4) An investigation of the current structures and development of local organic certification, with the eye on certifying crops grown on waste to garner the support of the eco-conscious market. There is often a tension between waste utilisation and organic certification. Farmers that make the environmentally responsible decision to re-utilise waste will not pass the stringent chemical criteria to certify crops as organic. Thus, facilitating a label for circular economy of wastes will raise public awareness of our collective responsibility, and allow farmers to tap into the green market.

4.7 Policy and economics: Agricultural and environmental factors in the land application of sludge

4.7.1 The international landscape of sludge regulation

4.7.1.1 The foundational EU sludge directive

To handle the tension between the benefits and risks of sludge diversion to agricultural land, there are extensive international guidelines and regulations (Kacprzak et al., 2017a). The European Union (EU) was the first to regulate sludge use in land application (Iranpour et al., 2004). These regulations were established by the Sludge Council Directive of 1986 (86/278/EEC) to offer guidance on the safe and beneficial use of sludge in land, while protecting the environment, human health and the quality of soil (Bagheri et al., 2023; Mininni et al., 2015). It has been updated repeatedly since (Rigueiro-Rodríguez et al., 2018; Christodoulou & Stamatelatou, 2016; Hudcová et al., 2019). It regulates quantity, loads, heavy metals, pathogens and emerging contaminants, receiving soil type and monitoring protocols and regimens. Although the Directive encourages the use of sludge in agriculture as popular sustainable practice, there seems to be a gap between the sludge Directive policy and effective implementation by the EU member states (Christodoulou & Stamatelatou, 2016; Ivanov & Bachev, 2021), similar to the challenges facing South Africa. Gianico et al. (2021) and Hudcová et al. (2019) reported on lack of harmonisation among EU member states in relation to the regulation of sludge. This is primarily due to risk aversion, with many countries choosing incineration over land application and also increasing regulatory stringency.

4.7.1.2 Regulation with a promotional flavour

Similarly, the United State of America Environmental Protection Agency (USEPA) has regulated the land application of sludge since 1999 via the Biosolids Rule, Part 503 (Bagheri et al., 2023). The have recently promoted the land application of sludge and intentionally replaced the term with the word ‘biosolids’, which is considered more palatable. The biosolids rule sets the guidelines for metals (pollutants limits), and the quantity and frequency of sludge application on land (Bagheri et al., 2023). Under this rule, biosolids are regarded as an essential nutrient source and further promoted to the market under a basic regulatory scheme in America (Christodoulou & Stamatelatou, 2016).

4.7.1.3 Economic implications of regulation

The increase in sludge production rates comes with the age-old tension between economic growth and sustainable development (United Nations, 2019; European Commission, 2020). Agricultural subsidies and tax breaks are prime examples of governance practices that are expensive, but support sustainable development (Li et al., 2022). There are cases where farmers are paid by the wastewater treatment plant operators to accept the application of sewage sludge on their land (European Commission, 2023). Lithuanian farmers receive €100 per tDS and German farmers between €100 and €560 per tDS (European Commission, 2023). Danish farmers are also reportedly paid to accept wastewater sludge as fertilizer (Sogaard, 2016). Refsgaard (2006) showed that compensation schemes and soil conditioner properties are the primary drivers for farmers to accept of sludge into agriculture, in a paper exploring the possibility of closing the urban-rural nutrient cycle. Sludge land application also has the potential to reduce public budget allocations to waste management (Otto & Drechsel, 2018).

4.7.2 The local landscape of sludge regulation

South African national and provincial government agencies (Department of Water and Sanitation, DWS) have a constitutional responsibility to implement and monitor water and wastewater sludge management strategies (Apollo, 2022). The development of policies, bylaws and regulations for the utilisation and disposal of sewage sludge and WTR have been a collective effort between the Department of Water Affairs and Forestry (DWAF) and the WRC (Abdelmegeed, 2022). They have provided thorough and rigorous guiding documents, including a five-volume series on water and wastewater sludge management guidelines, and follow up IA by van der Waal (2008). These are well-modelled on European and American standards (Popoola et al., 2023; Wiśniowska et al., 2019). A SWOT (strengths, weaknesses, opportunities and threats) analysis (Table 36, Section 5.9.2) investigates some of the opposing tensions in the implementation of these thorough guidelines (Snyman et al., 2006; Herselman, 2013; van der Waal, 2008; CoCT personal communication). For instance, the last sewage sludge survey (DEA&DP, 2021) and conversations with the CoCT emphasised, internally, how porous and scarce sludge census data is. Another major challenge is that the production of WTR and sewage sludge are handled by two different teams (wastewater and bulk water, CoCT), adding an extra layer of complexity in designing co-diversion strategies to agriculture. Particularly, if there is a vision to use economies of scale to tap into the carbon offset market, collaborative efforts will be necessary. The market only funds projects that can prove longevity exceeding 50 years (Section 5.11.2). Agriculture is on the one side of this strategic plan to co-divert WTR with sewage sludge away from landfill into soil fertility and crop growth. On the other side of the plan, is the environmental impact of landfill. Environmental considerations, as opposed to agriculture, have unique regulatory and economic structures and impacts.

4.7.3 What is our collective environmental cost to landfilling sludge?

4.7.3.1 International

The environmental costs associated with sludge disposal into landfill are both internal and external. Internal costs relate to landfill establishment and transport. Establishment costs include purchasing land, geological and hydrogeological surveys, fencing, and labour (Mitchell and Beasley, 2011). In developed regions such as the EU, these costs were estimated to be €255 /tDM (Kacprzak et al., 2017). The specific costs of countries such as Italy have been reported to be €70-250 /tDM (Visigalli et al., 2020). Mitchell and Beasley (2011) reported that the disposal cost in Germany and Austria varied between USD80-350/tDM and USD30 to 60 /tDM respectively in 1991, increasing continuously with time. In the USA, a survey conducted on an average-size treatment plant showed that the plant generates 32 500 t/year of sludge and is dumped in landfills at a cost of USD812 500/year (Gerber, 2022). In Japan, Hong et al. (2009) estimated landfill disposal costs ranging from USD347.93-USD608.3 /tDM. In China, Yang et al. (2015) reported landfill disposal costs of USD72 to USD96 /tDM, with an additional USD16-USD24 /tDM if the sludge is improperly disposed. In the EU, a recent exploratory study on sewage sludge reported that transport costs for member countries ranged from €2.1 to 2.5 /km/tDM (European Commission, 2022). The same study further reported transport costs of €0.3 /km/tDM in Italy, €1.3-1.6 /km/tDM in Slovenia, and €15 /tDM for up to 400 km in Lithuania. In developing countries, the costs can be higher because of poor road infrastructure and incapacitated trucks. A study by Zhongming et al. (2020) stated that sludge transportation in Abuja, Nigeria, averaged USD88 per trip annually for approximately 2 000 trips, while in Dakar, Senegal, the costs are within the range of USD50 to USD160 per trip.

External costs or negative externalities are costs related to environmental impacts (Xie et al., 2023) and are predominantly associated with post-dumping effects, such as greenhouse gases and air pollution, soil and water pollution, and health-related costs. However, to the best of our knowledge, documentation on the direct external costs of sludge dumping into the environment is scarce. Studies on this topic have only presented the impact of these externalities, without calculating the cost of damage. Studies on the environmental economics of sludge disposal have widened their scope to the economic costs of available wastewater treatment processes or a comparison of conventional and modern treatment technologies (Spinosa, 2015; Yang et al., 2015). The drive towards alternative beneficiation has shifted focus to the economics of sludge application in agriculture (Collivignarelli et al., 2019; Tesfamariam et al., 2020), as well as renewable energy and building materials (Zhongming et al., 2020), which have been identified as alternatives to landfill dumping.

4.7.4 Local

Considering the scarcity of international data on the internal and external costs of landfilling, local studies have explored this thoroughly, although the work is dated. Soliz et al. (2011), Leblanc et al. (2006) and Mokonyama et al. (2017) all discuss externalities and associated costs. As mentioned previously in this draft, there is one quantitative Cape Town study that costs landfill pollution at R111/tDS (Nahman, 2011) which has increased to R216.25 /tDS in 2023, according to standard inflation rates.

Table 4.2. The economic and regulatory Strengths, Weaknesses, Opportunities and Threats (SWOT) associated with the land application of sewage and WTR in South Africa.

Strengths	Weaknesses
• Strong South African policies promoting sludge utilization. • Well-clarified restrictions and guidelines. • Clarity on practices that pose minimal risks to users (farmers, consumers) and environment. • Policies promote resource efficiency.	• Implementation is costly (monetary value). • Sludge production information (WTW and WWTW) is limited and not centralised and organised. • No government incentives (subsidies, tax deductions) to divert sludge to agricultural land. • WTR and sewage sludges under different municipal management teams.
Opportunities	**Threats**
• Coordinated implementation of land application of WTR and sewage sludge (collaboration). • Access to the carbon offset market. • Certification of crops and products that have ‘Waste Circularity’ in their life cycle. • Minimising landfill pollution impact (climate and environment) and cost.	• Public opposition due to risk perception. • The black box of micropollutant complexity is a regulatory challenge. • Limited rigorous understanding of soil and waste characteristics, to pair sludges effectively (expertise and costs). • Leadership and coordination.

With this baseline context of agricultural and environmental policy and economy considerations, we turn our attention to (1) a cost-benefit analysis of a co-diversion case study, and (2) more creative and novel market and policy considerations. These include the carbon offset market, and policy drivers towards certification to facilitate premiums in the eco-conscious market.

4.8 Circular strategy versus linear landfill strategy: Cost-benefit analysis & local sludge survey

4.8.1 Methodology

Cost-Benefit Analyses (CBA) are commonly employed for ex ante IAs (Mdlulwa et al., 2019). A CBA is the systematic process of comparing benefits and costs in evaluating the feasibility of an intervention (Heinz et al., 2011; Mdlulwa et al., 2018). CBA analyses attempt to reduce the costs and benefits into representative monetary values (Winpenny et al., 2010). Here, a ‘without project’ scenario (sludge-to-landfill) was incrementally compared to a ‘with project’ scenario (sludge-to-agriculture). According to Wang et al. (2014), these will be explored incrementally as opportunity costs, considering wider associated costs like labour and crop value. These are not direct, but indirect opportunities associated with the sludge as it is diverted from ‘dead’ landfill soils to ‘living’ agricultural soils. Thus, this CBA investigates the incremental wider benefits and risks of careful pairing of the sludges, to harness the combined metabolic potential of the living agricultural soil biota and crops for bioremediation.

The ‘without project’ scenario was assessed based on the opportunity cost. Landfill factors considered were

- transportation (fuel and maintenance),
- sludge disposal costs per tonne,
- labour costs,
- gate fees, and
- distance.

The ‘with project’ scenario was evaluated with a Cost Benefit Analysis. Agricultural factors considered were

- transportation (fuel and maintenance),
- labour,
- value of land,
- value of crop,
- value of fertilizer replaced with sludge, and
- pollution costs.

The study used cross-sectional data to evaluate the costs of a theoretical case study, investigating wastes diverted from landfill to agriculture (Figure 58). The co-diversion of these particular sludges was paired based on plant production rates, from personal communication (Faure WTW, approved data exchange agreement with the CoCT, Addendum D) as well as the 2021 Western Cape sewage sludge tally in the Sewage Sludge Status Quo report (DEA&DP, 2021; Bellville and Zandvliet), according to Table 37. At a 1:1 application ratio, this co-diversion was calculated based on tonnes/area. The total PHA area is 3600 ha, of which 1800 ha are still farmed (Human, 2021). At an amendment rate of 10 tonnes per ha (Herselman and Snyman, 2006), a total of 18 000 tonnes are necessary to land apply to the whole area. Since it is applied at a 1:1 ratio, per year, the soil could receive 18 000 tonnes of sewage sludge and 18 000 tonnes of WTR. Production of WTR is the limiting factor in this case, at 12 000 tonnes per year (approximately 1000 tonnes per month, personal communication from Faure WTW). To distribute the full yearly WTR sludge footprint into surrounding agricultural land, it was paired with sludge from two WWTWs (Zandvliet and Bellville) selected because, together, they generate approximately 1 000 tonnes per month (12 000 tonnes per year) to match the Faure annual WTR product rate at a 1:1 ratio, and their distances were logistically viable (Table 38), to reduce transport costs and emissions with the diversion from landfill to agriculture (Figure 58). This follows Wu et al.’s (2022) strategic control model, designing the strategy with localised demographic considerations. Further considerations that needed to be investigated are the fertilizer costs, landfill gate fees, and transport costs (including truck type, fuel and maintenance). These are outlined in Table 39.

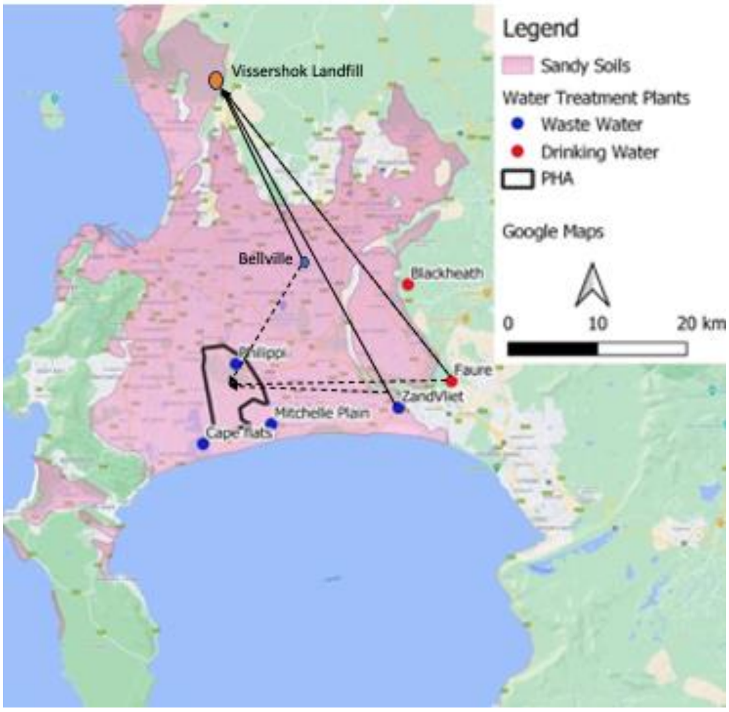

Figure 4.9. A hypothetical landfill-to-agriculture diversion case study informed the Cost-Benefit Analysis. WTR from Faure WTW and WAS from Bellville and Zandvliet are co-diverted from landfill ('before project' scenario) to the Philippi Horticultural Area ('after project' scenario).

Table 4.3. Sludge production volumes in the PHA surrounds. Two WWTW's were selected (Bellville and Zandvliet), to match the rate of sludge produced by Faure WTW (right column), based on volumes per month and distances to the agricultural land.

WWTW	Sludge Production Rate (tonnes/month)[a]	Sludge Type[a]	WTW	Sludge Production Rate (tonnes/month)	Sludge Type
Athlone	543	WAS	Faure[b]	1000	Fe-WTR
Bellville	269	WAS			
Borchard's Quarry	86	WAS			
Fisantekraal	171	WAS			
Gordon's Bay	11	WAS			
Kraaifontein	54	WAS			
Macassar	167	WAS			
Melkbos	27	WAS			
Mitchell's Plein	108	WAS			
Potsdam	299	WAS			
Scottsdene	88	WAS			
Wesfleur	30	WAS			
Wesfleur	25	WAS			
Wildevoëlvlei	83	WAS			
Zandvliet	726	WAS			
Simon's Town	3	AD			

[a]Sewage Sludge Status Quo report (DEA&DP, 2021)
[b]Personal communication, Faure WTW (2023)

Table 4.4. Comparative distances between the relevant WTW and WWTW and (1) Vissershok landfill ('without project' scenario) and (2) PHA agricultural land ('with project' scenario).

W/WTW to Vissershok Landfill	Distance (km)[a]	W/WTW to PHA (Mid-Point)	Distance (km)
Zandvliet WWTW	43.1	Zandvliet WWTW	21,7
Bellville WWTW	30.7	Bellville WWTW	19,3
Faure WTW	51.9	Faure WTW	30,5
Total	125.7		71.5

[a]Google Maps

Table 4.5. Miscellaneous information to inform the CBA.

Cost type	Cost	Source
Fertilizer Amount	104.6 kg/ha	Knoema Data Atlas (2021)
Fertilizer Cost	R13.75/kg	AgriMark (2023)
Gate fee	R643/tonne (general waste) R852/tonne (special waste)	GreenCape Waste Market Intelligence Report (2020)
Fuel and Maintenance	1.7L/km, R26/L	Truck type[a], fuel efficiency[b], AA rates and profit, current diesel cost
Pollution Cost	R216.25/tonne (R111/tonne plus inflation, 2011-2023)	Nahman (2011)
Labour	R260 000/annum x 4 daily drivers for the area	Ave driver wages Economic Research Institute[c]

a https://www.udtrucks.com/southafrica/trucks/segments/waste-management
[b](https://www.webfleet.com/en_za/webfleet/industries/transport/fuel-efficiency/#:~:text=Truck%20size%20and%20payload%20and,38%20litres%20for%20every%20100km
[c]https://www.erieri.com/salary/job/heavy-truck-driver/south-africa#:~:text=The%20average%20pay%20for%20a,for%20a%20Heavy%20Truck%20Driver.

It is important to note a few assumptions for the CBA:

1. Pollution costs were not added to the model, although considered important. Nahman (2011) is a single author who has been recited since 2011. There are no updated studies on these costs, and the authors are cautious to continue the pattern of reciting old, unverified data. In addition, there is not enough scientific data to assume that pollution is 100% reduced upon

diversion to agriculture. Although it will be significantly reduced, there will still be pollution impacts on the farm: GHG emissions, bio-accumulation of contaminants and potential nutrient run-off and consequent eutrophication. These impacts are a consequence of standard compost and fertilizer applications too, so the cost will likely not increase significantly, but this has not been quantified. Without calculating the percentage reduction in pollution from landfill to agriculture, it is not correct to add the pollution cost to the 'without project' (waste-to-landfill) scenario, and not to the 'with project' scenario (waste to farm). The differential should be quantified for future studies.

2. Gate fees vary between general and special waste. For the sake of modelling simplicity, general waste fees were selected, although some local WTW are expected to pay the special/hazardous waste fees (Mokonyama et al., 2017).
3. Although the wider WRC project has focused on hemp as an ideal crop, for the novel market potential and bioremediation potential of this crop, the crop selected for this CBA was rosemary instead. Hemp does not have a well-established market, making information harder to access. Rosemary (Rosmarinus officinalis, Family: Labiatae) is a common crop in the Western Cape of South Africa, growing on sandy soils, and can be used to make oils and fragrances for personal care products. Thus, it does not carry the health risks of potentially contaminated edible crops. The Department of Agriculture, Food and Forestry (SA) reports that rosemary is grown widely in the Western Cape, on well-drained sandy to loam soils at a pH between 5.5 and 8.0 (DAFF, 2012), and local farmers report planting and harvesting two seasons per year (personal communication with local farmers).
4. Truck type (https://www.udtrucks.com/southafrica/trucks/segments/waste-management) was used to calculate fuel efficiency (30-40 litres diesel/100km) (https://www.webfleet.com/en_za/webfleet/industries/transport/fuel-efficiency/#:~:text=Truck%20size%20and%20payload%20and,38%20litres%20for%20every%20100km) and AA rates used to calculate the additional maintenance cost and profit of a business.

The first analysis compared specific costs between the 'without project' scenario (waste-to-landfill) and the 'with project' scenario (waste-to-agriculture). The second analysis was a CBA, according to the methodologies outlined in Mdlulwa et al. (2019) and Nhundu et al. (2019). The methodology was estimated using the formula:

$$NB=\sum_{t=0}^{t} B - C \quad \text{Eq. 14.}$$

Where NB is net benefit, B total benefit, C the total the cost and t for time. If the total benefit of using sludge is higher than the cost, the project intervention (co-diversion of sludge wastes from landfill to agriculture) is considered viable. It is not viable if the total benefit is lower than the cost.

4.8.2 Results and Discussion

One of the primary cost savings with this diversion strategy is the gate fees, charged to local governments to discard sludge. These are charged per tonne sludge discarded to landfill, and this cost to government will be eliminated upon diversion to local agriculture (Figure 59). As mentioned above (Section 5.6, Feedstock supply and security logistics), some of the City of Cape Town's WWTWs are already distributing their sludges to agriculture. But, even if only Faure is co-diverted with these sludges, it will save the COCT over R14 million rand annually.

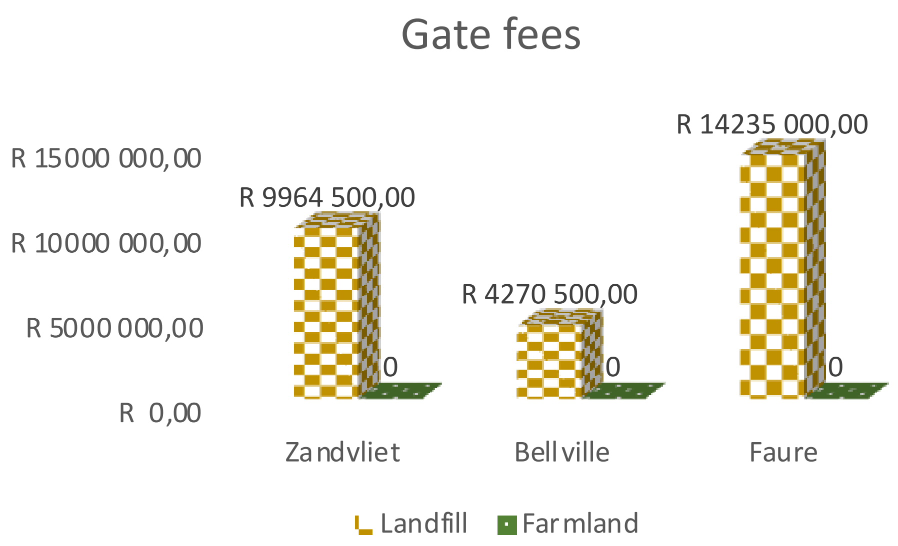

Figure 4.10. Yearly gate fees, compared between plants (Faure WTW, Bellville WWTW and Zandvliet WWTW) and compared between the 'without project' scenario (waste to landfill, brown) and the 'with project' scenario (waste to agriculture, green). Source: author's computation from Sewage Sludge Status Quo report (WWTW) and personal communication (WTW) (2023).

Another benefit of landfill-to-agriculture diversion is the significantly reduced transportation distance (Figure 58). As encouraged by Wu et al. (2022), this is based on localised design, as is already happening with some WWTWs locally (Section 5.6). In this case, the distance from each plant was compared between landfill and the PHA mid-point (-34.036491, 18.542082; assuming that some trips to farms will be further and some nearer). The total distance saved per trip per day is 49,7 km (totals of 125.7 km to landfill, 71.7 km to farms; Table 38). Figure 60 compares the reduced cost with diversion to agriculture for each plant, per trip, showing a distance reduction across all sites. The transport costs are also linked to a heavy environmental cost, with GHG emissions reduced significantly. This has not been calculated in this CBA but is discussed in the section on carbon accounting below (Section 5.11).

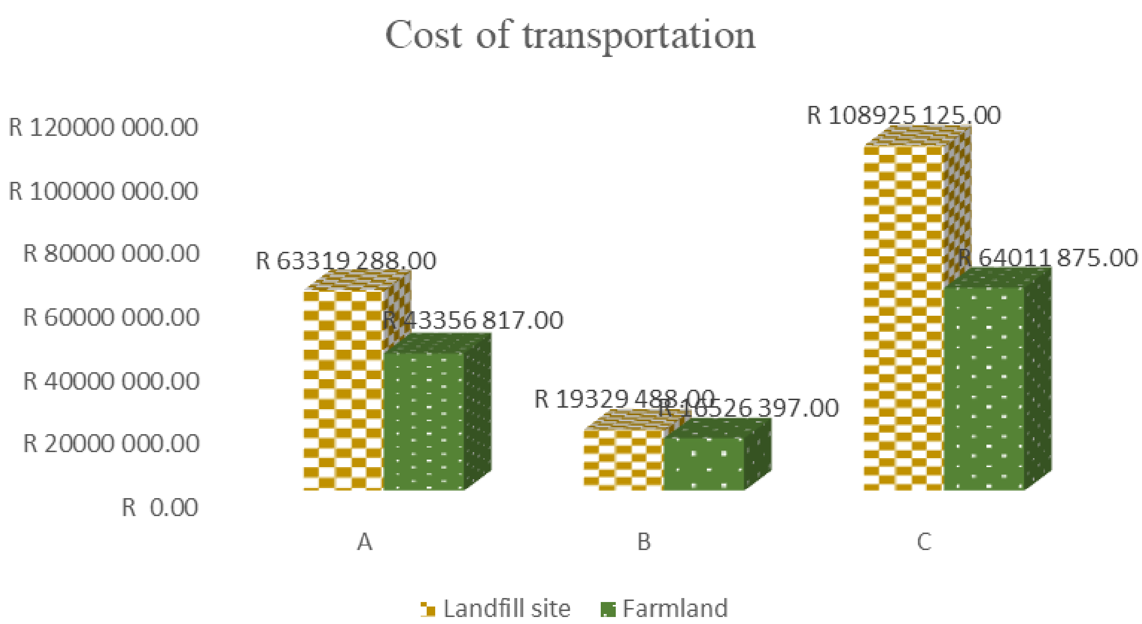

Figure 4.11. Transport costs, compared between plants: (A) Zandvliet WWTW, (B) Bellville WWTW and (C) Faure WTW. These were compared between 'without project' scenario (waste to landfill), brown and 'with project' scenario (waste to agriculture, green). Source: author's computation from geographical distances.

The CBA shows that the money spent by the CoCT on landfilling is more than R30 million annually (Table 40) for these three plants alone. Diversion of sludge into agriculture results in economic efficiency with reclaim cost of over R1 million to the CoCT.

Table 4.6. Total cost comparison from the CBA, comparing sludge diverted from landfill to agricultural applications.

Landfill Cost	R30 621 556.00
Farmland Cost	R1 474 767.89

Source: author's computation (2023).

In this analysis, the value of rosemary per hectare was used a representative. As shown in Table 41, simply the potential value (R346 770 000.00 per 1800 ha per year) of the crops generated on the land makes this strategy far more attractive than landfill compared to the cost that might be added to the farmers to collect the sludge (R1 474 767.89 total transport costs per 1800 ha per year). However, currently, the transport cost is incurred by the government in most land distribution scenarios (Sewage Sludge Status Quo report; DEA&DP, 2021). Reportedly, only the WAS produced at Ashton, Bonnievale, Robertson and de Doorns, in the Langeberg and Breede River Valley municipalities, are collected by farmers. The compost generated by the Grabouw HotRot was also collected by farmers rather than distributed whilst it was functional (personal communication, Grabouw WWTW). A reduction in fertilizer was calculated at R1 170 000.00 per 1800 ha per year, based on GrainSA data. However, an informal phone call with a local small-scale rosemary farmer indicated that she uses R2 000.00 per ha per year, which equates to a total of R3,600,000.00 per 1800 ha per year. Thus, the fertilizer value (Table 41) is conservative. Given that rosemary is a labour-intensive crop, it has the potential to create 3600 jobs per 1800 ha. Modiselle and Mahlangu (2021) report that two women have established a successful commercial enterprise on 1 ha of rosemary. Personal communication with a small-scale rosemary farmer in the Johannesburg surrounds indicates that temporary seasonal harvesting generates 5400 jobs per 1800 ha (estimated at three harvesters per ha). This could be contrasted to the labour force at Visserhok landfill site, but it is unlikely that the site will ever be waste-free, even with extensive diversion. Thus, that labour force is funded by other sources of waste too.

Table 4.7. Costs and benefits to farmer, with the diversion of waste from landfill to agriculture.

Cost (1800 $ha^{-1}.a^{-1}$)		Benefits (1800 $ha^{-1}.a^{-1}$)	
Variable	Amount	Variable	Amount
Transport			
Zandvliet to PHA	R433 568.17		
Bellville to PHA	R165 263.97	Value of crop (Rosemary)	R345 600 000.00[a]
Faure WTW to PHA	R640 118.75	Value of fertilizer forfeited	R1 170 000.00[b]
Labour	R235 817.00		
Total	R1 474 767.89	Total	R346 770 000.00

Source: author's own computation from City of Cape Town sludge survey (2023).
[a]Personal communication with a local (Johannesburg area) small-scale commercial rosemary farmer.
[b]Calculated from GrainSA (https://www.grainsa.co.za/) at 25 $kg.ha^{-1}$, $R650.ha^{-1}$ on 1800 ha.

Thus, a standard and very simple economic analysis suggests that there are strong benefits to the diversion of sludge from landfill to agriculture, both to the farmer and the government (W/WTW). A particular limitation is the logistical distribution of this sludge. However, localised planning can overcome that, and there is precedent to encourage farmers to collect. The reduction in fertilizer costs alone makes this attractive. These standard CBA observations are expanded in the following sections, considering carbon accounting and certification considerations.

4.9 Carbon accounting for the land application of sludge

4.9.1 South Africa's climate change commitment

According to the UNDP's metrics, South Africa contributes 1.07% to global greenhouse gas (GHG) emissions and is #75 of 180 countries listed in the Climate Vulnerability Index – a higher score indicates a greater vulnerability to the effects of climate change (UNDP, 2021). The country has committed to a fixed target of 350-420 $MtCO_2e$ GHG emissions by 2030. South Africa is one of the 196 Parties to adopt the Paris Agreement at the UN Climate Change Conference (COP21) in 2015, contributing to the pursuit of efforts 'to limit the temperature increase to 1.5°C above pre-industrial levels'. South Africa submitted its updated Nationally Determined Contributions in 2021, a legally binding commitment to detailed methodology contributing to this goal (SA, 2021).

Some of the historical national regulatory responses include the National Climate Change Adaptation Strategy (NCCAS; DEFF, 2020), the National Climate Change Response Policy (NCCRP; DEA 2011a), the National Development Plan (NDP; NPC 2011b), National Strategy for Sustainable Development (NSSD; DEA 2011c), all of which led to the NDC (2021) and the consequent National Adaptation Plan.

Consequent to the NDC, the South African Parliament passed the Climate Change Bill in 2022, and the Climate Tax Act (2019) launched the pricing of GHG. However, this tax is applied to all sectors except waste and Agriculture, Forestry and Other Land Use (AFOLU), both the key sectors relevant to this study. Although the pressure of carbon tax is not placed on these vulnerable sectors, they have a potential role to play in the carbon credit market. The NDC highlights the electricity and transport sectors of South Africa as the primary contributors to climate change, particularly due to the heavy reliance on fossil fuels, and thus these sectors receive the most strategic national focus (NDC, 2021). In the South African Climate Transparency report (2020), electricity is quantified at 55% of the national annual CO_2 emissions from fuel combustion ($MtCO_2$/year), with the transport and industrial sectors contributing 12% each, and agriculture only contributing 2%. In 2020, the South African carbon intensity of the energy sector was CO_2 per unit of 75.98 tCO_2/TJ, almost 20 tCO_2/TJ higher than the global average. However, agriculture is reported as a sector with significant mitigation potential and in South Africa's Climate Promise to the UN, accompanying the NDC, agriculture is listed as one of the top three adaptation and resilience priorities (UNDP, 2021). It is also one of the most vulnerable sectors to climate change.

4.9.2 Sewage and WTR soil co-amendment: the contribution to South Africa's climate change commitment

The carbon sequestration of this WTR-sewage sludge co-amendment to sandy soils has been demonstrated in the laboratory analysis of the environmental section of this report (Section 3.4.4.2, Figure 15). In this work, Noxolo Sweetness Lukashe demonstrated that both iron WTR and even more so aluminium WTR reduced the carbon emissions from soils co-amended with sewage sludge. These are laboratory results, at higher application rates than standard in-field rates and should be cautiously interpreted and validated in-field. However, they demonstrate a principal of improved carbon sequestration, if sandy soils receiving sewage sludge are co-amended with WTR. The economic analysis was launched prior to receiving the lysimeter data and is based on the laboratory indications of carbon sequestration. The implications of the lysimeter results are discussed at the end.

From the laboratory data we can assume that co-amendment of Al-WTR with sewage sludge improves carbon sequestration by 67%, as compared to pure sewage sludge amendments, if amended at a 1:1 ratio at 5% m_{dw}/m_{dw} (dry weight) (Table 42). The following calculations are based on the most effective WTR type (Al-WTR) at the most feasible WTR to sewage ratio (1:1). Since the national WTR production volume is the limiting factor in this co-application strategy, the extra 9% carbon sequestration resulting from a 2:1 (Al-WTR to sewage) amendment ratio (Table 42) is likely not worth the diversion of increased WTR, in terms of logistical distribution of the sludge. In-field variation typically reduces laboratory rates dramatically, and the 9% difference between 2:1 and 1:1 amendment ratios will likely be negligible in situ.

Table 4.8. Carbon lost in soils, calculated as a proportion of the percentage carbon lost to the atmosphere if sandy soil is amended with 5% m_{dw}/m_{dw} AD (Figure 15).

Ratio (AD:WTR co-amended soil)	Fraction of carbon lost
Sand	0.85
Fe-WTR	0.83
Al-WTR	0.21
2:1 Fe:AD	0.71
1:1 Fe:AD	0.83
2:1 Al:AD	0.24
1:1 Al:AD	0.33

A more feasible land application rate is 2% (m_{dw}/m_{dw}) or lower, and thus we can assume that the sequestration will be less than half that reported in this study. In addition, many previous WTR studies have shown that clear patterns demonstrated in the laboratory are almost negligible in-field (Moodley et al., 2004), with only very high application rates reflecting the effects demonstrated in laboratory trials. However, even a 5% reduction in GHG emission could have impacts on a national scale, depending on the geographical scale of the intervention. Based on an incorporation depth of 15 cm, and a bulk density of 1.5 g/cm^3, a 2% m_{dw}/m_{dw} application equates to 45 tonnes per hectare. If 320 000 tDS of WTR is distributed with 320 000 tDS sewage sludge (section 5.6.2, Feedstock supply and security logistics), at an application rate of 2% m_{dw}/m_{dw}, that equates to 7 111,1 (~7100; = 320 000 tDS ÷ 45 tDS/ha) hectares per year, co-amended with WTR. The Herselman and Snyman (2006) wastewater sludge guidelines recommend 10 tonnes sewage sludge per hectare rather than 45 tonnes per hectare. There is then enough sludge available to amend co-amend 32 000 hectares, nationally (= 320 000 tDS ÷ 10 tDS/ha). The application of sewage sludge to land is well-known to increase GHG emissions (Paramasivam et al., 2008; Scott et al., 2000). A 2014 study (Pitombo et al., 2015) showed that increases in sewage sludge application rates from 10 tonne.ha^{-1}.yr^{-1} to 20 tonne.ha^{-1}.yr^{-1} increased the estimated emissions (CO_2 equivalents according to the Intergovernmental Panel on Climate Change) from 4.80 Mg.C.ha^{-1} yr^{-1} in the control soil fertilized with chemicals to 7.30 Mg C ha^{-1} yr^{-1} at 10 tonne.ha^{-1}.yr^{-1} and to 8.67 Mg C ha^{-1} yr^{-1} at 20 tonne.ha^{-1}.yr^{-1}.

If we assume

- that sewage sludge application of 10 tonne per hectare per year increases CO_2 emissions by 7.3 Mg C ha^{-1} yr^{-1} - 4.8 Mg C ha^{-1} yr^{-1} = 2.5 Mg C ha^{-1} yr^{-1}, and
- we co-apply WTR at a 1:1 ratio,

- and CO_2 emissions are decreased by 67% (Table 42), or more conservatively at 10% of the laboratory effect (6.7%),

$$CO_2Em\,red = [CO_2(ss).ha^{-1}.y^{-1} \times ha] \times \%\, CO_2Em\,red \quad \text{......Eq. 15.}$$
$$CO_2Em\,red = [2.5\, MgC\, ha^{-1}.y^{-1} \times 32\,000\, ha] \times 67\%$$
$$= 53\,600\, Mg\, C\, ha^{-1}.y^{-1}$$
$$CO_2Em\,red = [CO_2(ss).ha^{-1}.y^{-1} \times ha] \times 6.7\%$$
$$= 5\,360\, Mg\, C\, ha^{-1}.y^{-1}$$

Where, $CO_2Em\,red$ is the total CO_2 equivalent emissions reduction with the 1:1 co-amendment of Al-WTR, ha is the hectares of potential land that can receive this waste co-diversion nationally, $CO_2(ss).ha^{-1}.y^{-1}$ is the total CO_2 equivalent emissions in the potential national agricultural application landscape, and % $CO_2Em\,red$ is the percentage reduction in CO_2 emissions due to WTR co-amendment (Table 42).

Thus, the total national emissions reduction (Eq. 15) with this co-diversion strategy will be in the range of 50 000 Mg C.ha^{-1}.yr^{-1} (according to laboratory trials), or 5 000 Mg C ha^{-1}.yr^{-1} (conservative estimation, 10% of laboratory trials). This data needs to be interpreted very conservatively, since it is based on laboratory incubations and literature. Crop growth, temperature and soil type will have a significant influence on these dynamics too. The laboratory data was strong motivation for in-field applications and the consequent monitoring of in-field GHG emissions. These field trials should be informed by Verra methodologies, to support accessing the carbon market with the data. There is also extensive evidence that analogous sorptive materials like biochar decrease GHG emissions in agriculture – particularly N_2O but also methane and CO_2 – although in-field data is complex and often temperature-dependent (Yang et al., 2018; Lyu et al., 2022). In this work, there is the potential benefit of reducing GHG, the diversion of waste from landfill into agricultural applications, and finally, the potential for farmers or even the government to tap into the carbon market if the strategy is well-designed and applied at economies of scale. However, in this study, the lysimeter data (gathered in parallel to the economic analysis, not informing this analysis) did not clearly support the laboratory data. There were some trends that it might, however, the data was highly variable and inconclusive. It must be cautioned that these lysimeter data need to be evaluated cautiously, as they are not the full picture. POXC and DOC with a stronger extractant are necessary to gain a fuller picture, which will be added to substantiate or disprove the link between the environmental and economic analyses.

Extensive work has also shown the pollution costs (Nahman, 2011) and GHG emissions of landfill activities (Zhang et al., 2019). It is typically accepted that landfilling emissions are higher than composting and land application (Lou and Nair, 2009). A 2011 study quantified the pollution costs in Cape Town landfill at R111 per tonne waste (Nahman, 2011; R216.25 per tonne waste in 2023, according to standard inflation rates). Faure WTW alone trucks 60 tonnes per day to Vissershok landfill (personal communication, Faure WTW). Thus, according to Eq 16, the pollution cost due to WTR produced by Faure WTW alone costs South African society over R4,5 million annually.

$$Annual\ Cost\ (R.yr^{-1}) = tonne.day^{-1} \times 365\ day.yr^{-1} \times cost\ (R.tonne^{-1}) \quad \text{......Eq. 16.}$$
$$= 60\ tonne.day^{-1} \times 365\ day.yr^{-1} \times R216.25\ tonne^{-1}$$
$$= R4\,735\,875.00\ yr^{-1}$$

Where $Annual\ Cost\ (R.yr^{-1})$ refers to the pollution cost of landfilling Faure WTR per year, and $tonne.day^{-1}$ is the volume of sludge trucked from the plant to the landfill daily, and $cost\ (R.tonne^{-1})$ is the pollution cost as determined by Nahman (2011) with inflation calculated to 2023.

The current WTR study (Project 2022/2023-00820) has shown that co-amendment of sewage sludge into nutrient-poor sands with Al-WTR increases carbon sequestration more than co-amendment with

Fe-WTR (Section 3.4.4.2, Figure 15). Fe-WTR is used more infrequently, to flocculate sediment from water sources with higher TSS (total suspended solids) and BOD (biochemical oxygen demand) and is thus used locally at Faure WTW to treat water from the Palmiet reservoir, which has significantly lower clarity than the other reservoirs. The standard and more ubiquitous flocculant is Al-oxide, which treats water within standard BOD and TSS ranges. Thus, the hypothesis generated in this work is that the sorptive capacity of WTR is related to its high specific surface area, and the differences between Al-WTR and Fe-WTR are related to saturation of binding sites. In a WTR that is flocculated from a water source already high in total organic carbon (Fe-WTR), the sorptive surfaces are likely more carbon-saturated, and are thus a less efficient sink for carbon than Al-WTR during the sequestration process in the soil. This, and the non-linear increase in sequestration upon amendment doubling rates (Table 42), indicates that the amendment has a carbon stabilisation potential, but that it has a saturation limit and must be carefully designed as an agricultural co-application.

Thus, because the sequestration capacity of sorptive materials like WTR can be saturated, WTR discarded in landfill will likely also stabilise some carbon, but the complex pollutants in landfill will likely exceed saturation. Thus, this strategy does not optimally harness this carbon sequestration potential. This waste is optimally applied in soils by crafting the nutrient-pairing ratios that acknowledge saturation and sorption maxima, which cannot happen with landfill disposal. In addition, combining this carbon sequestration potential in the soil matrix with crop growth is an extra design consideration that should synergistically minimise GHG emissions even more, in this diversion strategy away from landfill to agriculture.

4.9.3 Considerations to inform field trials crafted to access the carbon offset market

The carbon market is regulated by carbon offset programmes that facilitate standards to legitimate access to the carbon credit market. The Compliance Market (CM) is certified by governments, whereas the Voluntary Carbon Market (VCM) is certified by non-governmental organisations. The most renowned international carbon offset programmes are the Gold Standard (GS), Plan Vivo (PV) and Verra Verified Carbon Standard (Verra VCS), producing the Verified Emission Reduction (VER), Plan Vivo certificate and the Verified Carbon Unit (VCU) certifications, respectively.

In order to access these carbon offset programmes and achieve certification, applicants must demonstrate emission reductions according to stringent and rigorous standards. The most common standard is the Verra VCS, with the associated VCU certification. Thus, this WTR-sewage sludge co-diversion strategy will be discussed in the context of Verra methodologies. In the VCS Methodology for Improved Agricultural Management (Schoch and Swails, 2020), the project boundaries include CO_2 emission linked to SOC pools, and methane and N_2O emissions due to manure deposition, and must include one of the following parameters (those which would be relevant to marketing this intervention are highlighted):

- Reduce fertilizer (organic or inorganic) application;
- Improve water management/irrigation;
- Reduce tillage/improve residue management;
- Improve crop planting and harvesting (e.g. improved agroforestry, crop rotations, cover crops); and/or
- Improve grazing practices.

Thus, a field study will expand to include these greenhouse gases, and SOC and water management parameters in the monitoring suite. The most critical parameter to motivate and facilitate such a project will be measuring the in-field impact of applying WTR to landfill versus co-applying WTR with sewage sludge to agricultural lands (compared to pure sewage sludge agricultural amendments). Carbon models are considered sufficient to support projects applying for VCU certification if they are publicly available, validated, and parameterized. A project accessing carbon offset finance must also demonstrate scale, and that the project activity is not already common practice in the area. Conversations with local stakeholders in this project implied that land application of WTR is not common practice in the Western Cape of South Africa and the 2017 WRC survey on WTR disposal destinations affirmed this (Mokonyama et al., 2017). They created a Residue Disposal Option Performance Matrix (Table 43), which they confirmed with site visits, emphasising the negligible WTR land application in South Africa. This suggests that, according to the VCS Methodology for Improved Agricultural Management (Schoch and Swails, 2020), South Africa can scale these efforts and use them to access VCU certification, if in-field results confirm the potential of WTR co-amended with sewage sludge to capture CO_2, as well as methane and N_2O. Another element of this proposed co-diversion strategy is the reduction of transport, with reduced distances between W/WWTW plants and surrounding soils, compared to landfill (Figure 60, Section 5.6.2, CBA methodology). This reduction was already significant in this case study. If scaled provincially or nationally, this will significantly contribute to the GHG emission reduction in this proposed project. The calculations should be included in a field trial developed to support the application for VCU certification of the concept.

Table 4.9. Results of a nation-wide Residue Disposal Options Performance Matrix, conducted by Mokonyama et al. (2017).

Disposal Method	**Score**
Land Application	-1.35
Sewer Discharge	-0.4
Landfill	0.35
Return to Source	-0.75

An interesting sidenote is the emphasis on avoiding planned wetland degradation, in the Verra Methodologies. Wetlands are significant carbon sinks, earning multi-national protective attention (Mitsch et al., 2013). This work is targeted at sandy, nutrient-poor soils, and thus interventions will not impede on wetlands. However, the current linear removal of sludge sediment from drinking water is, essentially, diverting clay-rich wetland-like soils/sediments (Erskine et al., 2002) from catchments into landfill. River sediments are another critical carbon sink, with source-to-sink sedimentary systems considered the primary locations for organic carbon burial on earth (Leithold et al., 2016). This intervention returns the sediment-rich sludge (considered valuable in terms of carbon sequestration) to productive land rather than funnelling it into the complex, polluted landfill environment. Thus, the wetland protection emphasis in the Verra Methodologies can be indirectly harnessed to support this sludge diversion strategy to access carbon offset markets, if designed at scale.

4.9.4 Social considerations

The social considerations outlined in the Verra Methodologies hark back to the Liu et al. (2018) study. They observed that public participation and environmental understanding were the primary drivers that alleviated the ‘not in my backyard’ response in urban areas. The Verra mechanisms to address societal issues include the Grievance Redress mechanism for stakeholder conflict and the ‘no net harm’ safeguards to ensure all risks are considered in SWOT (Strengths, Weaknesses, Opportunities and Threats) analyses. For instance, the stakeholders (farmers and agricultural practitioners) in the Philippi Horticultural Area are renowned for being vocal and deeply engaged with sustainable practices like organic farming (Robins, 2021) but are also very protective of their land (Human, 2021). Thus, stakeholder engagement mechanisms are critical to this project, to communicate that risk is inherent, but that risks are even more inherent in the current landfilling scenario.

The data above also indicates that to access the carbon offset market, the municipalities will need to collaborate to generate one application for investment. We can – and must, to reduce transport emissions – still rely on the strategic control model of Wu et al. (2022), creating a human-centric and localised value chain around each WWTW. Nevertheless, despite localised diversion of wastes, a project that access international finance will need to consolidate the intervention nationally. This will involve national-level coordination of the co-diversion of these wastes into agriculture (allowing for localised optimization for each WWTW and WTW), and national-level monitoring of each of these localised strategies. There are local companies that can facilitate project design and access to carbon funding, including C4EcoSolutions (Pty) Ltd., Credible Carbon (Pty) Ltd., and the Climate Neutral Group, South Africa, which is part of Anthesis, a sustainability activator. However, AgriCarbon is geared specifically toward agricultural lands, and may be an ideal vehicle.

4.10 Potential for circular economy product certification (analogous to organic certification) as a market incentive: tapping into the eco-conscious market

The same Verra Standards state, regarding VCU certification, that

"Additional certification standards may be applied to demonstrate social and environmental benefits beyond GHG emission reductions or carbon dioxide removals (details about labelling with additional certifications are set out in Section 3.24 below). VCU labels designate that a particular VCU has met the requirements of another certification or is eligible or approved for use in a national, sectoral, or investor-specific market."

Alternative certification options are a context in which to consider the conflict of responsibility on the farmers: the eco-conscious market demands organic certification. Farmers who are willing to take the risk of applying sludge to their land will likely lean towards wider ethical practices that earn them organic certification. However, the complex pollutants in sewage sludge exclude any farmers who receive waste from this certification, and the consequent economic benefits of this niche market. Farmers who put the effort in to grow organic crops will likely be the same pool with the internal motivation to take the risk of receiving sewage sludge on their lands.

As the Verra Standards document indicates, there are alternative certifications, including the VCU certification to access the carbon market. This section will briefly explore the current process and status of organic certification in South Africa, with the eye on designing a process to create a certification for responsible waste re-utilisation in agricultural practices. This will allow farmers that sacrifice organic certification to access a different certification that still allows them access to the eco-conscious market.

4.10.1 South African regulatory status

Currently, there are no laws or regulations mandating the regulation of organic certification in South Africa. However, there is a National Policy on Organic Production (DAFF, 2015) that cites cross-cutting legislation as it's foundation, including (1) the Constitutional right to environmental protection (Section 24), (2) the Industrial Policy Action Plan (2012/13-2014/15), (3) the Kyoto Protocol (Article 3), (4) the Consumer Protection Act: 68 of 2008: Section 41, and many more in sustainable agricultural and Good Agricultural Practice (GAP) certifications. Despite the development of policy, there is no certification system in place, and farmers rely on international bodies or local private companies (at high costs) to achieve organic certification – although reportedly more than 50% of products with the organic label are not certified. Thus, both regulation and certification are challenges. The private bodies that are accredited are done so through the ISO Guide 65, the basic requirement for certifiers to ensure professionalism, impartiality and fairness. Most have been European certifiers, although the South African footprint of accredited certifiers is increasing. The certification process has been fragmented, with little coordination and unity. Public governance is moving towards centralising with the establishment of the South African Organic Sector Organisation (SAOSO), with auditing responsibility intended to fall under the Department of Agriculture, Fisheries and Forestry (DAFF). SAOSO has produced a Standard for Organic Production and Processing, built on IFOAM (International Federation of Organic Agriculture Movements) Basic Standards and included in their international family of standards. They have also developed a partnership with Control Union, an internationally renowned quality control and certification body (https://www.controlunion.com/) known for generating GlobalGAP and Vegan certifications.

4.10.2 Spontaneous emergence of alternative regulation

However, there have been alternative certification strategies emerging, including (1) First Party Organic Certification, which is essentially an unverified self-claim, but which is legitimised based on strong client relationality, engagement and trust; (2) Group Certification, facilitating farmers to organise themselves into groups by adopting an Internal Control System, and (3) Participatory Guarantee Systems, alternative mutual accreditation systems among groups of smallholders producing for a local market. They certify producers based on active participation of stakeholders and are built on a foundation of trust, social networks and knowledge exchange. All three are active in South Africa. However, the most rigorous approach is via a private local company or international regulatory body, which demands funds and meticulous record keeping. Two local certifiers, which are more feasible than international bodies, are CERES (CERtification of Environmental Standards, named after 'Ceres', the ancient Roman goddess of agriculture, symbolising abundant crops and soil fertility) and Afrisco, which services South Africa and neighbouring countries.

At national level, an effective regulatory system is lacking. The two most common international standards are intended as the foundation for local regulation – Codex Alimentarius Guidelines for the production, processing, labelling and marketing of organically produced foods and the IFOAM Basic Standards. The development of this legislation and regulation could facilitate support schemes for farmers, including a centralised national organic logo for visual marketing, market development and traceability.

4.10.3 Introducing a new waste circularity certification

The unregulated and atypical development of the organic certification process in South Africa has some negative consequences, as mentioned above (no central logo for consolidated marketing, no support for farmers). However, this works in favour of strategies to expand the imagination of eco-conscious certification in South Africa.

Instead of slow regulatory processes, the development of a 'Responsible Waste Circularity' certification can piggy-back on these independently established strategies, from:

- First Party Certification, based on consumer relationships, integrity and trust
- To collective Group Certification and Participatory Guarantee Systems, holding each other accountable,
- To international bodies that already have developed a wide range of certifications (i.e. Control Union), and local companies that could be convinced to expand their suite of certification processes.

Thus, the move towards a 'Waste Circularity Certification' will involve participatory stakeholder engagement across these three groups (Figure 62) to develop the minimum considerations necessary to earn the certification. A most logical strategy would be to amend sustainability certifications that are already developed (or in development) to promote waste circularity. However, sustainability is less quantifiable and is more contentious, and may slow the process down. Waste circularity is attractive, as it is easily quantifiable. The strategy will be best determined in stakeholder engagement processes.

Figure 4.12. Current regulatory processes that have emerged under the lack of regulation in the South African organic market, which could be harnessed to promote the certification of crops and products that have included responsible waste circularity practices in their life cycle.

Engaging in this type of stakeholder process to investigate the feasibility of a 'Waste Circularity' certification and label, can have a two-fold outcome: (1) it allows farmers who have included responsible waste circularity into the life cycle of their crops and products to access the premiums of the eco-conscious market, and (2) the exercise will promote conversation and expand imaginations. Deprivation of participation and limited environmental understanding emerged as the primary drivers of resistance, in the Liu et al. (2018) study mentioned above. A stakeholder engagement process – exploring the feasibility of widening certification processes to include Waste Circularity – could facilitate their proposed response to waste aversion, facilitating the experience of 'Not in my backyard, but let's talk'.

This work provided motivations to promote the co-diversion of WTR with sewage sludge into agricultural productivity, based on (1) a cost-benefit analysis of a landfill-to-agriculture diversion strategy, (2) an investigation of the potential of tapping into the carbon market and (3) the expansion of our current certification suite, to allow farmers to certify products with 'Waste Circularity' in their life cycle, to tap into premium markets.

Some key lessons that emerged include

(1) to tap into the carbon offset market, strategic national diversion would be necessary to access economies of scale. However, the laboratory trends are far less resolved in the lysimeter trials – although some further data is needed to confirm that. If these effects were happening, it would only be detectable at scale. Future projects that may investigate this potential further need to be developed with Verra VCU certification in mind, according to the VCS Methodology for Improved Agricultural Management as a foundation to monitor the GHG benefits. If any carbon stabilization is measured, a multi-province collaboration to divert national WTR sludges into agricultural land amended with sludge will increase the profits in the carbon offset market. Local companies with extensive experience can support this process.

(2) A stakeholder engagement process to investigate the feasibility of including 'Waste Circularity' in the suite of crop certifications could have the benefit of (a) allowing farmers to access premium markets with this diversion strategy, and (b) facilitating participatory engagement and expanding the collective receptivity to waste. This will also callow the consumer agency to support waste circularity, increasing our collective imagination for what is possible with our wastes.

5 CONCLUSIONS

The environmental analysis of lysimeters amended with WTR, AD and WTR+AD showed that the sewage sludge and the co-amendment are strong promoters of crop growth. All environmental risks were negligible except salinity (measured as electrical conductivity) and phosphate, and *Shigella* was the only pathogen or parasite that was persistent throughout the trial. Based on these trails, it is suggested that the wastes are paired more carefully for fertility before doing risk assessments. Wastes should be applied according to the phosphate demand, although nitrogen may be in deficit and will need to be amended with external inputs. Or, a 2:1 ratio of WTR:AD might stabilize the phosphate, and may have also resulted in more clear carbon data resolution. Although co-amendment promoted ecotoxicity stabilisation and carbon stabilisation, and decreased hydrophobicity in laboratory trials, all of these trends were far less clear in the lysimeter trials. It is very common that the shift from laboratory analyses to greenhouse and field trails decreases the resolution in trends. However, there are some carbon analyses that are needed to further confirm the carbon sequestration (POXC and DOC with a more stringent extractant), which will be added before publication. An *ex ante* cost benefit analysis showed that this diversion strategy is financially attractive, and it was shown that – if carbon sequestration can be measured in field, as in the laboratory trends – the project can be designed at scale according Vera methodologies to tap into the carbon market. This would involve multi-provincial logistical diversion to tap into the longevity and scale necessary to garner investment. Finally, the land application of sludge excludes farmers from accessing the eco-conscious market premiums, since they cannot access organic certification. Here, it was shown that the local organic certification system has emerged independent of government regulations, and relies on first party claims (trust), participatory guarantee systems (collective agreements and monitoring), and finally, private certification. This lack of government regulation does not support the agricultural sector in their shifts towards responsible practices. However, it might allow for the freedom to influence current certification systems, to allow farmers utilizing waste to access the eco-conscious market and expand the public imagination for waste circularity.

Together, these results support future field trials, pairing WTR and sewage sludge to promote crop growth on sandy soils. These trials will be improved (in comparison to this study) by:

1. Running pre-trial laboratory incubations to determine the optimal WTR:sewage sludge ratio for soil fertility, based on baseline sandy soil characteristics and crop demand.
2. In these fertility analyses, focus should be on salinity and phosphate as the primary limiting factors for land co-application of these wastes.
3. The field trials should be designed with Vera methodology in mind, providing all of the data necessary to certify a multi-provincial mobilisation of sludges into low-nutrient soils, if carbon sequestration is demonstrated in-field.
4. Simultaneously, a thorough sludge tallying exercise (possibly provincial, but ideally national, including both WTR and sewage sludge) should be executed.
5. A full Life Cycle Assessment (LCA) on water and wastewater treatment sludges should be executed.
6. Finally, a social study should interview various stakeholders in the field to identify the pathways to facilitate this waste diversion strategy, including (1) farmers that are farming on low nutrient and sandy soils, (2) consumers that consider eco-conscious purchasing important, (3) provincial and national bodies involved in regulating the certification of crops for market, and (4) waste water and water treatment works.

6 APPENDIX I HEMP PERMIT

agriculture, land reform & rural development

Department: Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

HEMP PERMIT

DIRECTORATE PLANT PRODUCTION
Private Bag X250, Pretoria 0001; Harvest House, 30 Hamilton Street, Pretoria 0001
Tel: 012 319 6092; E-mail: Hemp.PIA@Dalrrd.gov.za; Website: www.dalrrd.gov.za

Permit no.: PIA-HP-WC- 2022-0006

Valid from: 11 May 2022
Expiry date: 12 May 2025

PERMIT FOR CONDUCTING ACTIVITIES IN RELATION WITH HEMP IN TERMS OF THE PLANT IMPROVEMENT ACT, 1976 (ACT NO 53 OF 1976)

It is hereby certified that:

PERMIT HOLDER:
Name: Dr Wendy Stone
ID number/ Company registration number: 86070 50127 087
Tel/Cell: 063 348 5727
E-mail: wstone@sun.ac.za
Postal address: Stellenbosch University Water Institute Secretariat, c/o Faculty of Natural Science, Private bag X1, Matieland, 7600

is authorised to (research & store and transport) *Cannabis sativa* HEMP with a Tetrahydrocannabinol (THC) content of no more than 0.2%.

at the following premises:

LOCATION OF PREMISES/ CULTIVATION SITE and PERSON RESPONSIBLE FOR THE PREMISES/OVERSEEING THE ACTIVITY:
Physical address: Welgevallen Experimental Farm, University of Stellenbosch, Suidwal Street
GPS coordinates: -33.927121, 18.867835
Name of person responsible for production site or premises: Dr Wendy Stone
ID number: 860705 0127 087
Tel/Cell: 063 348 5727

Physical address: Forest and Wood Sciences Greenhouse, University of Stellenbosch, Paul Sauer Building, Bosman Street
GPS coordinates: -33.943465, 18.867095
Name of person responsible for production site or premises: Dr Wendy Stone
ID number: 860705 0127 087
Tel/Cell: 063 348 5727

NUMBER OF HECTARES ON CULTIVATION SITE: 1 ha

The permit holder shall comply with all conditions attached hereto:

DEPARTMENT OF AGRICULTURE, LAND REFORM & RURAL DEVELOPMENT
PRIVATE BAG X 250
11 MAY 2022
PRETORIA
0001
PLANT PRODUCTION

REGISTRAR OF PLANT IMPROVEMENT
DIRECTORATE PLANT PRODUCTION

DATE

Permit no.: PIA-HP-WC- 2022-0006 **Page 1 of 2**

7 APPENDIX II ECOTOXICITY OF DIRECT EXTRACTS

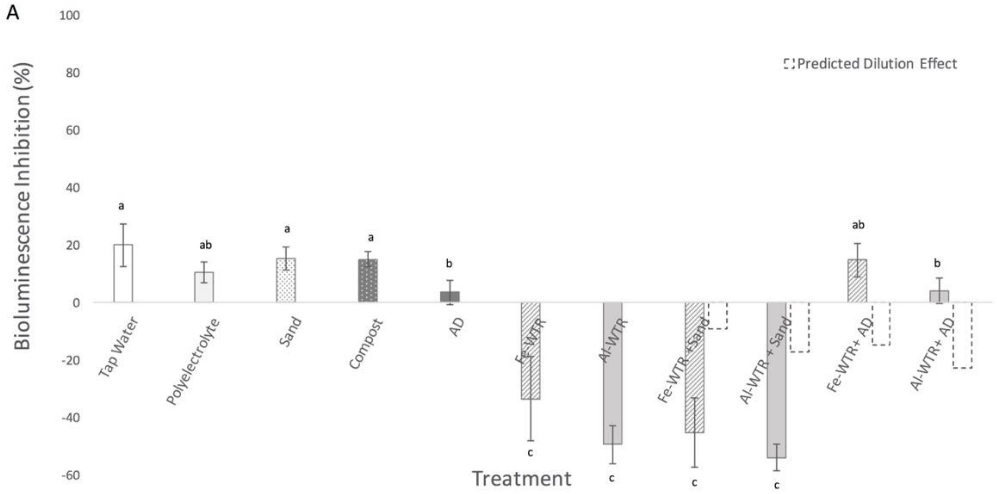

Figure 1. The influence of soil and sludge leachate footprints (direct extracts) on the metabolic activity of *Aliivibrio fischeri*, measured as bioluminescence inhibition (%). Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculent, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilization in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, p<0.05).

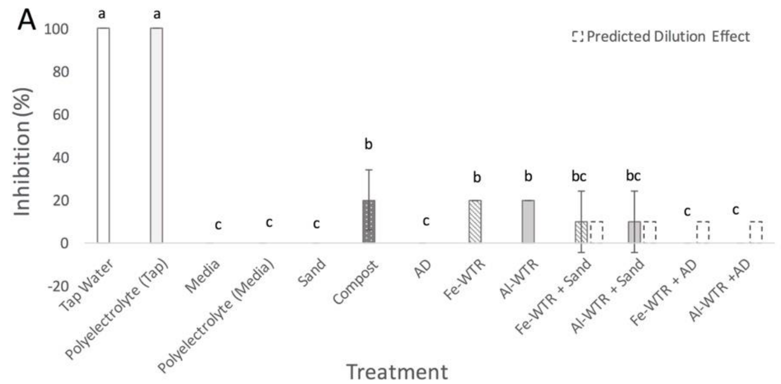

Figure 2. The influence of soil and sludge leachate footprints (concentrated extracts, 500X), on the mobility of *Daphnia magna*, measured as motility inhibition (%). Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculant, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilization in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, p<0.05).

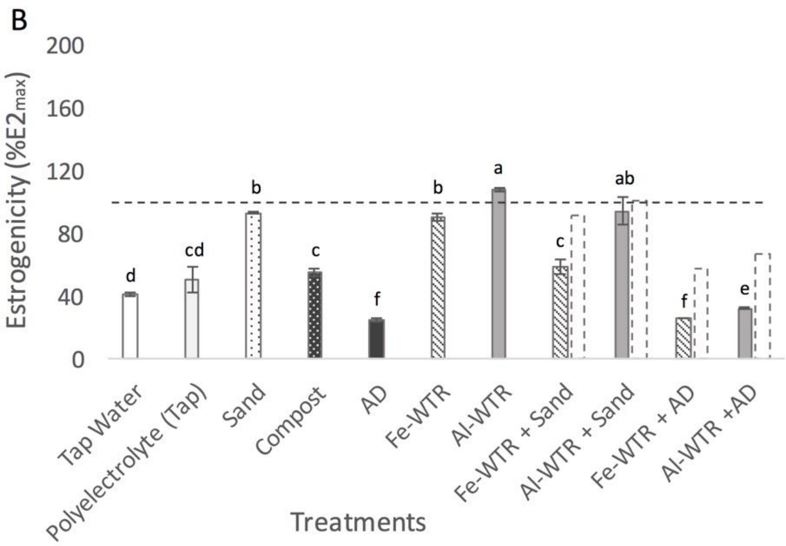

Figure 3. The influence of soil and sludge leachate footprints (direct extracts), on the estrogen response of the yeast estrogen screen, measured as a fraction of the max E2 response, indicated with a dashed line. Individual soil and sludge extracts (sand, compost, AD and both Fe-WTR and AD-WTR), incubated in tap water overnight, were compared to co-amendments of the WTRs with both AD and sand. The environmental impact of the polyelectrolyte flocculent, in tap water, was also assessed with this assay. A predicted dilution effect (the sum of 50% assay effect of each individual amendment) was added for comparison, to evaluate immobilization in 1:1 co-amendments. Error bars represent the standard deviation of means of triplicate samples, and significance is indicated (ANOVA, $p<0.05$).

8 APPENDIX III BASELINE MATERIALS CHARACTERISATION

Table 1. Baseline characterisation of sandy soil and amendments, at T0, prior to mixing and planting lysimeter trials.

Parameters	**Sand**	**Al-WTR**	**AD**
PHYSICO-CHEMICAL			
pH (H_2O)	5.82 ± 0.07	6.42 ± 0.01	7.55 ± 0.03
pH (KCl)	5.25 ± 0.03	5.78 ± 0.04	7.23 +0.03
EC (µS/cm)	5.44 ± 0.01	119.02 ± 12.12	3470 ± 46
TC (%)	0.53 ± 0.04	12.81 ± 0.34	24.93 ± 0.18
TN (%)	0.06 ± 0.003	0.87 ± 0.004	2.94 ± 0.03
C/N	8.65 ± 0.13	14.64 ± 0.31	8.48 ± 0.05
PO_4^- -P (mg/kg)	6.8 ± 8.3	2.99 ± 1.7	1133.05 ± 171
NH_4^+-N (mg/kg)	0	66.83 ± 0.64	1373.87 ± 484
NO_3^--N (mg/kg)	0	6.72 ± 1.9	96.91 ± 15.6
Soil Water Repellency	0.035 ± 0.004	0.003 ± 0.001	$2.07X10^{-6}$ ± 0
CARBON DYNAMICS			
TC (mg/L)	9.211 ± 0.09	30.50 ± 0.06	183.91 ± 0.6
TOC (mg/L) (DOC)	8.40 ± 0.102	29.60 ± 0.09	170.69 ± 0.9
TIC (mg/L)	8.12 ± 0.02	0.89 ± 0.03	13.22 ± 0.3
POXC[a]	n.a.	n.a.	n.a.
Respiration[a]	n.a.	n.a.	n.a.
MICROBIOLOGY			
IDEXX			
Total coliforms [log(CFU/g_{dw})]	3.07 ± 0.01	4.29 ± 0.04	4.63 ± 0.01
Fecal coliforms [log(CFU/g_{dw})]	0.43 ± 0.76	0.90 ± 0.85	2.86 ± 0.02
E. coli [log(CFU/g_{dw})]	0	0	2.49 ± 0.04
Culturable species			
Total heterotrophs	4.95 ± 0.03	5.00 ± 0.02	7.83 ± 0.02
E. coli	0	2.48 ± 0.38	5.18 ± 0.06
Salmonella	0	0	5.26 ± 0.22
Shigella	0	0	3.99 ± 0.10
PARASITES			
Ascaris ova			
Ascaris viability			
Helminth ova			
Helminth viability			
ECOTOXICITY			
Aliivibrio fischeri (% inhibition)	15.3 ± 3.4	-49.3 ± 6.6	3.6 ± 4.2
Algae (% Inhibition)	68.4 ± 2.7	30.38 ± 20.1	86.88 ± 3.2
Daphnia (% Survival)	100 ± 0	80 ± 0	100 ± 0
Yeast Estrogen Screen (%$E2_{max}$)	63.72 ± 3.8	88.95 ± 11.2	151.09 ± 36.5
Cancer Assays[b]	-	-	-
Phytotoxicity			
Hemp (SAPA) (% Germination)	100 ± 0	100 ± 0	100 ± 0
Hemp (SABL) (% Germination)	46.67 ± 11.54	66.67 ± 11.54	60 ± 0
Grass (Lawn) (% Germination)	73.33 ± 11.54	73.33 ± 11.54	73.33 ± 11.54
Corn (% Germination)	66.67 ± 11.54	53.33 ± 23.09	66.67 ± 23.09

[a]parameters to be measured during trial.
[b]parameters only analysed during optimization.

Table 2. Baseline characterisation of bio-available (NH_4NO_3) heavy metals (mg/kg) in sandy soil and amendments, prior to mixing and planting lysimeter trials. This data informed the selection of metals to monitor throughout the trial, characterizing the receiving soil and amendments to predict soil fertility and heavy metal toxicity risks (Table A2).

	Sand (mg/kg)	±	WTR (mg/kg)	±	AD (mg/kg)	±	SA[a] Max Limits	USA[b] Max Limits
Ca	525.3	8.8	867.0	46.7	8317.0	342.2	n.p	n.p
K	108.7	4.2	198.5	14.0	795.1	26.3	n.p	n.p
Mg	166.0	0.9	171.0	13.2	609.2	31.4	n.p	n.p
Na	25.1	1.0	218.7	17.7	2016.8	31.1	n.p	n.p
Al	0.9281	0.0092	0.2098	0.0032	1.1325	0.0888	n.p	n.p
V	0.0090	0.0007	0.0022	0.0001	0.1266	0.0070	n.p.	n.p
Cr	0.0072	0.0001	0.0007	0.0003	0.0285	0.0030	n.p.	n.p.
Mn	1.6528	0.0320	26.4666	1.0806	3.0084	0.1467	n.p	n.p
Fe	0.7490	0.0162	0.0216	0.0068	3.9661	0.4994	n.p	n.p
Co	0.0109	0.0002	0.0027	0.0001	0.3484	0.0239	n.p.	n.p.
Ni	0.0000	0.0000	0.0000	0.0000	0.9433	0.0653	420.0	420.0
Cu	0.0000	0.0000	0.0000	0.0000	4.0795	0.3990	1500.0	4300.0
Zn	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2800.0	2500.0
As	0.0121	0.0010	0.0288	0.0018	0.3569	0.0139	40.0	75.0
Se	0.0045	0.0001	0.0026	0.0003	0.0415	0.0008	n.p	100.0
Cd	0.0000	0.0000	0.0000	0.0000	0.0031	0.0005	40.0	85.0
Sb	0.0008	0.0003	0.0015	0.0001	0.0852	0.0048	n.p.	n.p.
Ba	3.4959	0.1275	4.4076	0.1976	0.3806	0.0062	n.p.	n.p.
Hg	0.0000	0.0000	0.0000	0.0000	0.0024	0.0005	15.0	57.0
Pb	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	300.0	840.0

Table 3. Baseline characterisation of total (Aqua Regia) heavy metals (mg/kg) in sandy soil and amendments, prior to mixing and planting lysimeter trials. This data informed the selection of metals to monitor throughout the trial, characterising the receiving soil and amendments to predict soil fertility and heavy metal toxicity risks (Table A3).

	Sand (mg/kg)	**WTR (mg/kg)**	**AD (mg/kg)**	**SA[a] Max Limits**	**USA[b] Max Limits**
Ca	525.3	867.0	8317.0	n.p	n.p
K	108.7	198.5	795.1	n.p	n.p
Mg	166.0	171.0	609.2	n.p	n.p
Na	25.1	218.7	2016.8	n.p	n.p
Al	0.9281	0.2098	1.1325	n.p	n.p
V	0.0090	0.0022	0.1266	n.p.	n.p
Cr	0.0072	0.0007	0.0285	n.p.	n.p.
Mn	1.6528	26.4666	3.0084	n.p	n.p
Fe	0.7490	0.0216	3.9661	n.p	n.p
Co	0.0109	0.0027	0.3484	n.p.	n.p.
Ni	0.0000	0.0000	0.9433	420.0	420.0
Cu	0.0000	0.0000	4.0795	1500.0	4300.0
Zn	0.0000	0.0000	0.0000	2800.0	2500.0
As	0.0121	0.0288	0.3569	40.0	75.0
Se	0.0045	0.0026	0.0415	n.p	100.0
Cd	0.0000	0.0000	0.0031	40.0	85.0
Sb	0.0008	0.0015	0.0852	n.p.	n.p.
Ba	3.4959	4.4076	0.3806	n.p.	n.p.
Hg	0.0000	0.0000	0.0024	15.0	57.0
Pb	0.0000	0.0000	0.0000	300.0	840.0

9 APPENDIX IV DATA AGREEMENT CITY OF CAPE TOWN

PROJECT APPROVED			CITY OF CAPE TOWN ISIXEKO SASEKAPA STAD KAAPSTAD
WS/BII/Research Project Approved	2021/06/01	**WATER AND SANITATION DEPARTMENT**	Version: 00
COMPILED BY: BII: RESEARCH OFFICER / AO2			**APPROVED BY: HEAD: BII**
All CONTROLLED documents are located in the SAP DM system			**Printed 2022/06/02**
Printed documents are NOT CONTROLLED except if signed and dated			**Page 1 of 1**

2 June 2022

To whom it may concern,

We wish to inform you that your research request for **Sludge Waste for Land Application: Quality Data** for your project titled **The Circular Economy of Water Wastes as Soil Amendments: Agricultural Potential of Non-Edible Crops on City Sludge Wastes** has been approved.

The reason for this is that an MOA is currently in place between the City of Cape Town and the University of Stellenbosch. Data is subject to availability as per Subject Matter Expert i.e. Waste Water.

We wish you all the best for your project.

Kind Regards,

Mario Carelse
Digitally signed by Mario Carelse
Date: 2022.06.09 10:19:20 +02'00'

MARIO CARELSE
CHAIRPERSON: RESEARCH ADVISORY PANEL
WATER AND SANITATION

Swastika Surujlal-Naicker
Digitally signed by Swastika Surujlal-Naicker
Date: 2022.06.09 12:08:27 +02'00'

SWASTIKA SURUJLAL-NAICKER
CHAIRPERSON: RESEARCH ADVISORY PANEL
WATER AND SANITATION

Digitally signed by Sven Sotemann
Date: 2022.06.13 08:51:16 +02'00'

SUBJECT MATTER EXPERT - SVEN SOTEMANN
RESEARCH ADVISORY PANEL
WATER AND SANITATION

10 APPENDIX V KNOWLEDGE DISSEMINATION

10.1 Published Articles

10.1.1 WTR and Sludges

10.1.1.1 ***Stone, W***., Steytler, J., de Jager, L., Hardie, A. & Clarke, C. E. 2023. Improving Crop Growing Conditions with Water Treatment Residual and Compost Co-Amendments: Soil Water Dynamics. Journal of Environmental Quality. DOI: 10.1002/jeq2.20541.

10.1.1.2 Du Plessis, M., Fourie, C., ***Stone, W***., & Engelbrecht, A.M. 2022. The Impact of Endocrine Disrupting Compounds and Carcinogens in Wastewater: Implications for Breast Cancer. Biochemie (209): 103-115. DOI: 10.1016/j.biochi.2023.02.006

10.1.1.3 Gwandu T., Lukashe N.S., Rurinda J., ***Stone W***., Chivasa S., Clarke C.E., Nezomba H., Mtambanengwe F., Mapfumo P. & Johnson K.L. 2022. Co-application of water treatment residual and compost for increased phosphorus availability in arable sandy soils. Journal of Sustainable Agriculture and Environment 2(1): 68-81. DOI: 10.1002/sae2.12039

10.1.1.4 Johnson, K.L., ***Stone, W.***, Engels, C., Dominelli, L., Chivasa S., Clarke, C., Gwandu. T., & Appleby, J. 2022. Boosting soil literacy in schools can help improve understanding of soil-human health linkages in Generation Z. Frontiers in Environmental Science (10): 2709. DOI: 10.3389/fenvs.2022.1028839

10.1.1.5 Johnson, K. L., Gray, N. D., ***Stone, W.***, Kelly, B. F., Fitzsimons, M. F., Clarke, C., ... & Gwandu, T. (2022). A nation that rebuilds its soils rebuilds itself-an engineer's perspective. Soil Security (7): 100060. DOI: 10.1016/j.soisec.2022.100060

10.1.2 Micropollutants in Sewage

10.1.2.1 Smith, K., ***Stone, W.***, Botha, A., Steffen, H., & Wolfaardt, G. M. 2024. Riverine mycobiome dynamics: from South African tributaries to laboratory bioreactor. Mycology. DOI: doi.org/10.1080/21501203.2023.2278309

10.1.2.2 Bröcker, J. H. L., ***Stone, W.***, Carstens, A. & Wolfaardt, G.M. 2022. Micropollutant Transformation and Toxicity: Electrochemical Ozonation versus Biological Metabolism. Toxicology Research and Application. DOI: 10.1177/23978473221122880

10.1.2.3 Tucker, K. S., ***Stone, W.***, Botes, M., Feil, E., & Wolfaardt, G. M. 2022. Wastewater treatment works: A last line of defence for preventing antibiotic resistance entry into the environment. Frontiers in Water (4): 883282. DOI: 10.3389/frwa.2022.883282

10.2 Manuscripts Submitted.

10.2.1 ***Stone, W***., Botha, D., du Plessis, M., Fourie, C., Engelbrecht, A-M, & Clarke, C. E. 2023. Dilution or Sink? The ecotoxic effects of co-amending water and wastewater treatment sludges to promote crop growth in sandy soil. Submitted to Waste Management, under review.

10.2.2 Lukashe, N. S., ***Stone***, W., Pereira, R., Hardie, A. G., Johnson, K. L. & Clarke, C. E. 2024. Stabilization of carbon through the co-addition of water treatment residuals with anaerobic digested sludge in a sandy soil. Submitted to Waste Management, under review.

10.3 Manuscripts being Drafted.

10.3.1 Parker, I. A., ***Stone, W***. & Perold, W. J. 2024. Development of a computer vision pipeline for the analysis of *Aliivibrio fischeri* bioluminescence inhibition on solid media. Submitting to Journal of Biological Engineering (March 2024).

10.3.2 Socio-economic opinion piece (April 2024),

10.3.3 Lysimeter trials (April 2024).

10.4 Conferences and Technical Meetings

10.4.1 Masola, L. C., ***Stone, W.*** & Verschoor, A. J. 2024. Sludge-to-Agriculture: Cost-Benefit Considerations, the Carbon Market and Eco-Conscious Certification. Abstract submitted for the ‘Climate Smart Agricultural Technologies and Innovation, Sustainable Natural Resource Management’ session of the ARC-DALRRD Conference in Roodeplaat (12-14 February 2024).

10.4.2 Johnson, K. L., Dominelli, L., Capisani, S., ***Stone, W.***, Larsen, G., Moreira, T. & Kryzwoszynska, A. 2024. Relations of Care and Responsibility for Rebuilding Soil. Abstract submitted for the 'Gender Inequalities and Soil Health' session of The International Union of Soil Sciences congress, to be held in Florence, Italy (19-24 May 2024).

10.4.3 ***Stone, W.*** 2023. Why Measure? To Meet, and To Know. Measuring soil biology to support our expressions and rhythms of care. Invited presentation at LabServe Technical Day, September 2023.

10.4.4 ***Stone, W.***, Steytler, J., Lukashe, N.S. & Clarke, C.E. 2022. Combined Water Wastes as Soil Amendments: Crop Production on Sludges. Presented at the Water Institute of South Africa (WISA)'s 2022 conference, Navigating the Course, held in Sandton, South Africa, 28-30 Sept. Invited guest speaker in the Virtual Special Session on Unconventional Water Uses in Irrigation and its Role in Sanitation and Human Health, facilitated by Dr John Ngoni Zvimba (Water Research Commission of South Africa).

10.4.5 ***Stone, W.***, Steytler, J., Lukashe, N.S. & Clarke, C.E. 2022. Combined Water Wastes as Soil Amendments: Crop Production on Sludges. Presented at the iNanoWS Women's Forum, hosted by the UNISA Institute for Nanotechnology and Water Sustainability.

10.4.6 ***Stone, W.,*** & Clarke, C.E. 2021. The Circular Economy of Water Wastes as Soil Amendments: The Agricultural Risks and Potential of Growing Non-Edible Crops on Sludge Wastes. Presented at the 1st Symposium on Sustainable Solutions and Future Farming, Stellenbosch University, 21-22 September 2021. https://www.greenagri.org.za/events-and-workshops/1st-symosium-on-sustainable-solutions-and-future-farming-21-22-september-2021/

10.5 Public Communication

10.5.1 ***Stone, W***., Johnson, K. & Clarke, C.E. 2022. Embracing our wastes. https://www.youtube.com/watch?v=5lvhhAop9Jw (Video for Science Communication, BIOGRIP, government; developed with an intentional communications strategy, with input from SU's CREST Centre for Research on Evaluation, Science and Technology; Appendix VI).

10.6 Government and Private Collaboration & Support

10.6.1 ***Stone, W.*** July 2022. Hemp Regulatory Visit from the Department of Land Reform and Rural Development, building partnership with local government. Visit evaluated our commitment to the regulations stipulated in our hemp research permit number. Permit number: PIA-HP-WC-2022-0006. Contact person: Isabel du Toit. Email: IsabelDt@Dalrrd.gov.za, Appendix VII).

10.6.2 ***Stone, W.*** & Clarke, C. April 2022. Upon request, generated a report guiding the City of Cape Town's response to sewage spills in Fynbos. Submitted to Suretha Dorse, Senior Environmental Professional, City of Cape Town. Email: suretha.dorse@capetown.gov.za (Appendix VIII).

10.6.3 ***Stone, W.*** 2023. A proposal was submitted to private funders in Pniel to support the remediation of the decommissioned Pniel sludge settling dams. It lost traction due to other endeavours, but was part of the project engagement and communication strategy and may be executed via other avenues in future (Appendix IX).

11 APPENDIX VI DISSEMINATION RATIONALE

Diverting Sludge Waste to Agricultural Productivity Dissemination Strategy & Translational Output

Wendy Stone, March 2023

1. Concept

The scientific concept of diverting sludge wastes from landfill into soil amelioration for agricultural productivity involves taking an environmental risk (distributing waste into the environment) to shift away from the current unsustainable environmental risk (concentrating waste into landfill). This proposed strategy monitors waste not only to communicate risk, but for improved urban design: to classify it for optimal circular diversion back into the environment, via non-edible agriculture.

Although the nuanced design of co-amendment of WTR and sewage sludge in sandy soils is relatively recent, the use of sewage sludge for land application is not. The question then arises: if we have extensive academic work and government guidelines for land application and composting of sludges, why is there still such widespread landfilling?

This work attempts to provide scientific evidence for plant biomass production (soil and plant nutrients), groundwater and environmental protection (pathogens and pollutants), composting beneficiation, and the economic benefits of this waste diversion strategy.

With all of the available information and this type of science supporting systemic change, urban planning has not shifted. Thus, the deeper aim of the work is to design attractive experiments that engage diverse partners, and consistently remind stakeholders of the benefits. We aim to keep the idea in the peripheral view of policy makers, industry, agriculture and government.

2. Code-Switching to ‘Knower’ Insights and an Ethics of Care

It is difficult to shift long-term systemic habits, like landfilling, and this endeavour involves both concerted leadership and extensive energy input. Academia and commercial R&D are some of the few niches that allow individuals to consider the landscape, and put energy into shifting and optimising systems. However, the bridge

between knowledge development and changed habits is vast. It has been widely demonstrated in education that there are at least four valid postures in ‘knowing’ or understanding a concept (Figure 1), shifting between ontic relations (identity of a phenomenon) and discursive relations (ways of knowing a phenomenon): purist knowing (purely theoretical), doctrinal knowing (strong methodologies, like the scientific method), situational knowing (strong scientific basis, but open-ended and less quantifiable, like most biological concepts), and no/knower (purely intuitive and relational). Each posture facilitates legitimate relations to insight. Pott and Wolff (2019) and Blackie et al. (2022) propose that intentionally facilitating code-switching between these postures produces students with greater flexibility and capacity to deal with the complexity of 21st century problems, which is often lacking in scientific realism: historically, education and dissemination of knowledge relied mostly on purist and doctrinal insight.

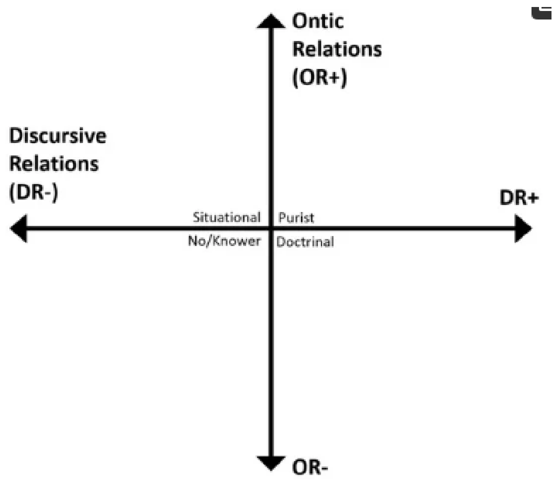

Figure 1. The four quadrants of knowledge described in Legitimation Code Theory (Pott and Wolff, 2019), describing ontic and discursive relations, the bridge between ways of identifying and approaching phenomena.

The question becomes, how to stimulate an ethics of care (Gilligan, 1988), tapping into relational desires (situational and knower insight), as much as an ethics of management (doctrinal and purist insight), structured on dispassionate calculations and moral responses. This taps into the 'Why?' of scientific interventions as much as the 'How?', and particularly the 'Why me?'. The communication strategy aims to shift between these axes.

3. Multi-pronged Communication Strategy

a) carefully designing (and garnering funding for) to develop project partnerships with relevant stakeholders. This maintains momentum in this diversion strategy that is energy and cost intensive, until systems are shifted,

b) choosing case studies that are attractive. For instance, studying hemp as the model textile crop, which is a trendy market of great interest, since legislation is opening during this decade. The permits have promisingly shifted from SAHPRA (health, South African Health Products Regulatory Authority) to DLRRD (agriculture, Department of Land Reform and Rural Development) in the lifespan of this project, and we hold a permit which has garnered many new relationships on the project,

c) choosing communication strategies that speak effectively to all levels of stakeholders.

- For a risk scenario like this, the science must be rigorously communicated for critique by the scientific community (Water Research Commission reports, academic articles). More popular translational documents must target audiences who are familiar with concepts, but not involved in the details and methodology of the risk assessment, like farmers and municipal management (BIOGRIP translational documents). Finally, dissemination methods must target educational and non-scientific audiences, to increase awareness and receptivity to an idea that might stimulate disgust, like interacting with products sourced from waste (popular explainer videos).
- A long-term goal involves shifting policy regarding crop certification. Currently, the trend in the environmentally conscious market is to search for an 'Organic' certification to inform their buying practices. However, this is counterintuitive in this case. Organic certification has many benefits, however, farmers that sell crops grown

on wastes cannot tap into this market, and lose out on the purchasing power of the green economy. We are proposing a similar certification, that shows that farmers are responsibly re-using wastes and integrating circular economy into their soil management strategies. A drive towards certifying both organic crop production and such circular waste diversions into farming can allow both of these environmentally responsible farming practices (organic and circular) to tap into the market that is willing to pay for responsible environmental stewardship, rather than creating the current tension between these practices.

D) A final consideration is rhythm of communication. It is irresponsible to disseminate and promote scientific interventions that carry a risk, without extensive risk mitigation, monitoring and design according to national guidelines. Thus, dissemination into the wider public must be preceded by rigorous data demonstrating the safety of these ideas.

4. Multi-Level Engagement Strategy

Thus, at this point in the project, we have developed

1. A detailed report describing methodology, submitted to the Water Research Commission. A reference group of field experts critique the work on a yearly basis, investigating the legitimacy of the details. This is not submitted to BIOGRIP, as it is confidential, but can be provided to select evaluators, if essential. It is also being prepared for publication, and the consequent peer review process.
2. A translational document prepared via BIOGRIP, for dissemination to government and farmer stakeholders, with some knowledge of the fields of study (BIOGRIP D4 Stone_0323).
3. A video describing the concept, for distribution to government, farmers, and the public. The aim is to circumvent some of the barriers to market, like public perception in waste interaction (https://youtu.be/5lvhhAop9Jw) Note: it is produced for 720p50 (High Definition Quality).

5. Guidance from Science Communication Experts

The video was developed after a generous conversation with the SARCHI Research Chair in Science Communication (Prof Mehita Iqani, CREST Centre for Research on Evaluation, Science and Technology) and her postdoctoral fellows, Dr Meghan Judge and Dr Jessica Webster.

They recommended a few design interventions:

They suggested a series of TikToks, rather than a video, acknowledging the attention span of a modern audience used to bite-sized information transfer. They also suggested that, in the morass of modern communication, interacting with an artist that can shift visuals from generic animation to something with a unique design and style would stand out.

Since the process was already initiated, and these recommendations involve a much higher budget, the video relied on three interviews to keep audience attention by changing the tone regularly. The bulk is dedicated to an animation of the scientific concept (explainer segment). But the segment regularly shifts to an interview style, asking three experts in the field why we consider this risk of grappling with our wastes worth taking.

Besides high quality visuals, the other recommendation was a ‘hook’: a question that invites the audience to think about the concept in their own lives. In terms of information flow, this involves leaving it open ended, rather ‘wrapping the story up’ in a standard educational style. This was designed in this context to invite the audience to grapple with (1) how (perception, buying power and engaging with commercial composting companies), and (2) why they might engage with the societal risks of their own wastes.

1. Blackie, M. A., Adendorff, H., & Mouton, M. (Eds.). (2022). Enhancing Science Education: Exploring Knowledge Practices with Legitimation Code Theory. Taylor & Francis.
2. Gilligan, Carol (1982). In a Different Voice. Cambridge, Massachusetts: Harvard University Press.
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4. Pott, R. W., & Wolff, K. (2019). Using Legitimation Code Theory to conceptualize learning opportunities in fluid mechanics. Fluids, 4(4), 203. doi: org/10.3390/fluids4040203

12 APPENDIX VII

Hemp Research Permit Stipulations and Templates
Permit number: PIA-HP-WC-2022-0006
Issued: 11 May 2022
Expiry: 12 May 2025
Issued by Department of Agriculture, Land Reform and Rural Development Contact person: Isabel du Toit. Email: IsabelDt@Dalrrd.gov.za

According to the hemp cultivation and research permit (PIA-HP-WC-2022-0006), a number of security and record-keeping measures are necessary to execute this research.

Record keeping. On-site logbook and record keeping of (1) access to plants, (2) planting dates, seed source, seed volumes.
Seed source: Natie Ferreira, Tamatie Growers. Contact: natie@tamatie.co.za
Seeds collected to date:
(*Cannabis sativa* L.) strains,
200 SAPA (SAPA Valley landrace) and 50 SABL (Chinese broadleaf hemp)

Security and signage. Secure perimeter with access control. Welgevallen farm has card access control, for university staff members. The tunnel is padlocked.

Figure 1. Tunnel access control.

Figure 2. Signage stipulating that low THC hemp is planted in wastes (Addendum B.1), with clear no-access communication. A logbook is attached (Addendum B.2), for recording access to plants.

Testing. According to Philip du Toit, Inspection Services, DALRRD: at harvest, submit a subsample of plant matter to Qure Laboratories in Montagu, for confirmation of THC<0.2%. Contact: Brenda Marx (M.Sc. Chemistry) Pr. Nat. Sci., LABORATORY DIRECTOR Email: brenda@qure.co.za

13 APPENDIX VIII CITY OF CAPE TOWN ADVISORY: SEWAGE SPILLS AND FYNBOS

Document can be facilitated upon request.

Considerations for the characterisation and monitoring of sewage spills in the Cape Floristic Region, including the ecological impact on fynbos

April 2022

W. Stone[1] and C. Clarke[2]

[1]Stellenbosch University Water Institute
[2]Department of Soil Science, Stellenbosch University

WRC	Water Research Commission of South Africa
SSV	Soil Screening Values
TCLP	Toxicity Contaminant Leaching Procedure
CEC	Cation Exchange Capacity
EC	Electrical Conductivity
WTR	Water Treatment Residuals
TTV	Total Trigger Values
MPV	Maximum Permissible Values
HR	Hazard Rating
CFR	Cape Floristic Region

Table of Contents

14 APPENDIX IX PROPOSAL TO REHABILITATE THE DECOMMISSIONED PNIEL SLUDGE SETTLING PONDS

Document can be facilitated upon request.

Gardening the Pniel Sludge Settling Dams

Proposal to Establish, Monitor and Maintain a Diverse Wetland Ecology

at the nexus of the Pniel Wastewater Treatment Works,
the local informal community (Languedoc),
the surrounding farms (Bethlehem),
and the conservation region (Banhoek)

May 2023

Wendy Stone*, Althea Grundling, Roger Jaques
*Email: wstone@sun.ac.za, Mobile: 063 348 5727

15 APPENDIX X CAPACITY BUILDING

1 **Lurika de Jager**
Degree: 4th Year Soil Sciences

Funding: Prof JH Neethling family bursary, supplemented by this WRC Capacity Building stipend
Thesis/Project Concept: (1) The influence of WTR on soil-water dynamics, and (2) The influence of WTR on the stabilisation of carbon and nutrients in anaerobic digestate, for promoting textile crop productivity in sandy soils.
Status: Thesis complete and data contributed to an article in the Journal of Environmental Quality (co-authored by Ms. De Jager)
Graduated: Dec 2022

2 **Danelle Botha**
Degree: BSc(Hons) Microbiology

Funding: Self-funded. In 2023, was supplemented by this WRC Capacity Building stipend
Thesis/Project Concept: The influence of WTR on the stabilisation of ecotoxicity in anaerobic digestate, for promoting textile crop productivity in sandy soils.
Status: Thesis completed and data contributed an article under submission
Graduated: Dec 2022

3 **Irshaad A. Parker**
Degree: MEngSc Biomedical Engineering

Funding: NRF Postgraduate Scholarship
Thesis/Project Concept: Designing and building a pilot in-field sensor, using machine learning to measure ecotoxicity via the inhibition of *Vibrio fischeri* bioluminescence, for monitoring bioremediation or bioaccumulation over seasons.
Status: Thesis completed and currently submitting publication
Graduated: Dec 2023

4 **Hamond Motsi**
Degree: PhD Soil Sciences

Funding: EU Funded Project.
Thesis/Project Concept: He is between his Masters and PhD, and assisted with the Economic Deliverable (D5)
Progress: Registering 2024
Targeted graduation: Dec 2026

5 **Livhuwani C. Masola**
Degree: Intern, Agricultural Research Council

Funding: Agricultural Research Council
Thesis/Project Concept: Economic Deliverable (D5).
Progress: On-going.
Targeted graduation/completion: Nov 2023

6 **Noxolo S. Lukashe**
Degree: PhD Soil Sciences

Funding: NRF
Thesis/Project Concept: Carbon Sequestration in Sandy Soils Amended with Sludges
Progress: On-going, co-authored an article under submission
Targeted graduation/completion: Dec 2025

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