Long-term impacts of entrenchment of pit latrine and wastewater sludge

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This is one of two publications emanating from project K5/2899. The other report is*, Guidelines for deep row entrenchment of faecal sludge and secondary wastewater sludge* (**WRC Report no. TT 880/22**).

EXECUTIVE SUMMARY

This research aims to elucidate the long-term impacts of sludge entrenchment, with particular emphasis on forestry and agriculture applications. This includes demonstrating to what extent negative impacts are likely, such as transport of nutrients or metals to nearby water sources, as well as demonstrating the potential economic impact that can be realised through productive use of sludge in forestry or agriculture as opposed to landfill disposal. These results, coupled with site-specific characteristics, will inform cost-benefit analyses and effective decision-making around reuse of sludge for agriculture applications.

The current study is a follow-up to WRC Project K5/2097, titled: "An Investigation into the long-term risks associated with deep row entrenchment of pit latrine and wastewater treatment works sludge for forestry and land rehabilitation purposes." More specifically, this study follows on from an investigation into deep row entrenchment of sludge in a timber plantation.

In this study, 360 cubic metres of wastewater treatment works activated sludge was buried in a eucalyptus plantation near Howick in the Natal midlands. The two-hectare experimental area was divided into 30 plots each 30 by 30 metres in extent and, in 18 of these plots, sludge was buried in a 20 by 20 metre section in the centre of the plot.

Five treatments were compared: T1 had one 10 m^3 load of sludge, T2 had two loads, T3 had three loads, T4 had no sludge but it did have trenches, and T5 had no sludge and also no trenches. With the growth of 900 trees (30 at the centre of each plot) having been observed from planting in January 2010 until May 2014, a period of 52 months, the plots with sludge showed a 50% increase in timber volume compared with those without. This site was also closely monitored for groundwater impact, using a number of piezometers for near surface flow and two 30-metre-deep boreholes at the bottom of the site for groundwater monitoring.

Only a small difference (2 mg/ℓ) in nitrate levels was detected in the downstream borehole compared with the upstream borehole over the first year after planting, and after four years the nitrate content in the water sampled from the site rain gauge was significantly higher than that sampled from the boreholes or the piezometers. Samples of soil taken after three years from around the buried sludge and from the sludge itself showed that nitrogen was not retained in the sludge or in the surrounding soil, whereas potassium, phosphorus and other elements such as calcium and zinc were retained.

The current study builds on the work done at the eucalyptus plantation in Howick, described above. While the previous study showed promising results in terms of timber yields and nutrient transport, it was not able to draw conclusions about the longer term (>4 year) impacts associated with sludge entrenchment. Those who are interested in sludge entrenchment as a disposal option were interested to know whether the conclusions of the original study, particularly regarding the fate of the nitrogen and phosphorus added to the soil, would in any way change if monitoring could be extended for several more years. Furthermore, the current study aims to highlight whether the increase in timber yields observed in the previous study was consistent throughout the entire growing season and even into the subsequent one.

The previous project observed that significant nutrient contamination of borehole water nearby and downslope did not occur. While this suggests that transport of nutrients from the sludge is limited, it does not conclusively prove that the groundwater will not be affected over a longer period. The current study provided the opportunity to check the longer-term outcomes. This is important because potential users of this disposal option and officials responsible for authorisation are inherently sceptical, and more evidence is therefore needed to prove whether the method is environmentally responsible or not.

Transport and disposal or treatment of faecal sludge is costly, as evidenced by the experience of many municipalities throughout South Africa and internationally. Thus, there is a need for economical methods of sludge disposal, which empower municipalities to fulfil the constitutional right of all South Africans to a clean environment and basic sanitation. The least costly method for dealing with sludge is to bury it on the site where it was generated, as it cuts the highest expense, which is transport. However, there is some hesitation around this method, due to the assumption that contaminants will be transported and pollute groundwater. Additionally, an industry trend towards waste reuse and resource recovery has driven research into harnessing the nutrient potential of human waste. Unless a tree (or some other suitable crop) is planted over the buried sludge, burying the sludge on site does not harness this potential.

Sludge disposal by burial will not be appropriate everywhere. The relevance of sludge disposal as well as the nature of sludge disposal will rely on a variety of factors, such as available land, potential for reuse, soil and geology type, volume of sludge requiring disposal, etc. Many of these factors are known for the South African context, and thus, a decision-making guide can be established for more municipalities to attempt entrenchment as well as other potential opportunities for sludge beneficiation. This will be a useful supplement to the guideline created during the previous WRC project.

The key findings and conclusions from current study are as follows:

1 Buried sludge has long-term impacts (i.e. over 10 years/2 growing seasons) on timber yields and soil health.
2 This impact is likely partly due to nutrients added to the soil in the sludge, but even more so due to the added organic carbon. This makes nutrients more available by improving microbial activity, and it can also improve water-holding capacity of soil. This is an under-valued positive contribution of sludge burial and sludge application in general.
3 Sludge burial in this environment (~40-50% clay soil) has led to very limited impacts on the environment. Within three years, buried sludge was stabilised (e.g. nutrients immobilised and pathogens inactivated).

While this study has provided a long-term view of sludge entrenchment, the findings are somewhat limited in their application, as the behaviour of buried sludge is assumed to be dependent on many factors. Thus, there are opportunities for further study to provide more insight into applications with different soils or different crops, as well as for further investigating the opportunities for this practice in South Africa. Some of these are listed below:

1. Smaller-scale studies to investigate how soil characteristics, crop type, and time influence the impact of buried sludge in terms of crop yields and pollutant migration. Ideally, this should lead to an annexure for the guideline that describes the applicability of the practice based on different conditions (i.e. what is the minimum clay content required for safe DRE?).
2. Further soil sampling can take place at the existing experimental plot. There is a unique opportunity to gain a long-term understanding of the fate of buried sludge within the soil. The main aspect of interest will be the fate of carbon in the soil. Is carbon indefinitely stabilised in the soil, leading to a permanent improvement in soil fertility?
3. Detailed cost-benefit-analysis of various sludge disposal options, with particular focus on municipal wastewater treatment sludge. This analysis should include the main practical approaches used by municipalities now, e.g. landfill disposal, surface application, deep row entrenchment, composting, and pelletisation. While the current study and guideline tool provide high-level comparisons between landfill disposal, surface application, and DRE, more detailed analysis and costing are required. An effective model might also aim to determine the points at which different options become advantageous (i.e. at what distance from the treatment works is DRE infeasible compared to alternatives?).
4. Investigation into the opportunities for DRE of sludge as a method for rehabilitation of surface mines. There is considerable opportunity for use of sludge in the rehabilitation of mines, particularly in terms of adding organic matter to the soil. The characteristics of surface mine land should be investigated, and the risks and benefits of incorporating sludge into the soil during rehabilitation should be identified.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

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The timber produced in commercial plantations is used in a very wide variety of applications, including the following: poles; paper; board; food; clothing and pharmaceuticals. Forestry companies are not only interested in the quantity of timber produced, but also in the quality, and in particular, in the presence of any contaminants (e.g. heavy metals) which may have resulted from the sludge burial trials.

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Sludge entrenchment has potential to provide economic benefits to the agriculture industry without exorbitant costs for treatment. This potential needs to be investigated further as well as for other crops relevant to South Africa, such as sugarcane. The private forestry industry is moving further in the direction of high value uses such as clothing and pharmaceuticals. Thus, these companies are extremely reticent to risk contamination of their timber, including heavy metals, which may be present in sewage sludge. This, coupled with the stringent safety and environmental policies of these companies, suggests that private forestry may not provide many opportunities for sludge entrenchment. Another opportunity exists where municipal land is used for forestry and the timber is used for lower value applications, such as fence poles. In South Africa, the overlap of municipal-owned forestlands and sludge in need of disposal has not been assessed.

Sludge disposal by burial will not be appropriate everywhere. The relevance of sludge disposal as well as the nature of sludge disposal will rely on a variety of factors, such as available land, potential for reuse, soil and geology type, volume of sludge requiring disposal, etc. Many of these factors are known for the South African context, and thus, a decision-making guide can be established for more municipalities to attempt entrenchment as well as other potential opportunities for sludge beneficiation. This will be a useful supplement to the guideline created during the previous WRC project.

2 AIMS

The current study had the following aims:

1. Determine the longer-term impacts of sludge burial on forestry yields, groundwater contamination and sludge characteristics, building on the previous study. This includes the impacts into the second growing cycle where sludge had been applied.
2. Provide updated recommendations regarding deep row entrenchment methods, including guidelines for the appropriate application of this practice.
3. Disseminate the findings to DEA, DWS, and WSAs, the development community, and the agro-forestry and agriculture communities with the goal of making sludge entrenchment a more widely accepted alternative for faecal sludge management in South Africa.

3 CAPACITY BUILDING

This study also provided opportunities for two students to collaborate and receive advanced degrees. While a summary of their results is presented in this report, their detailed research reports are not included and can be accessed through the University of KwaZulu-Natal. They also aim to publish articles along with the rest of the project team. Their respective studies are described below:

1. Honours Student, University of KwaZulu-Natal, Crop Science Department: *The Residual Effect of Sludge Entrenchment on the Growth and Development of Eucalyptus Trees in their Second Growing Season*. This study focuses on understanding if any impact is observed on the growth of Eucalyptus

trees in their second growing season on a plot with buried sludge. Further, from a crop science perspective, the study aims to identify the reason for any impact. The student investigated the impact of buried sludge on water holding capacity of the soil. The student's report has been submitted and approved.

2. Masters Student, University of KwaZulu-Natal, Soil Science Department: *Residual Effects of Sewage Sludge Entrenchment on Carbon, Phosphorus Pools and Cycling*. This study focused on the impacts of sludge entrenchment on soil properties, with emphases on carbon and phosphorus fractionation. This study contributed to understanding the reasons for any impacts on tree growth that were observed. The work on this study also points to the longer-term impacts of buried sludge on overall soil health in degraded environments. This student's report is still in progress and is expected to be completed during the 2022 academic year.

4 UPDATED LITERATURE REVIEW

4.1 REFERENCES TO DEEP ROW ENTRENCHMENT IN SLUDGE GUIDELINES (INTERNATIONAL)

Various technical guidelines have been prepared in recent years, which discuss proper disposal and treatment options for sewage sludge, faecal sludge, and septage. A number of these reference deep row entrenchment (DRE) as a viable, low-tech option. References to DRE in these resources are described below in Table 4-1.

Table 4-1: Guidelines on sludge and septage handling and reference to Deep Row Entrenchment

Reference	Mention of DRE
US EPA, 1994 Guide to Septage Treatment and Disposal	The US EPA guide does not directly reference DRE, but it does discuss appropriate options for land application of septage (i.e. sludge from septic tanks). The simplest acceptable method of land application in the guide is to spread septage across the land by opening a valve on the truck and driving around. The septage must be ploughed into the soil within 6 hours of application to meet standards. While this guide does not directly mention DRE, DRE is a safer option than this simplest acceptable option as it does not use surface spreading.
ISF-UTS & SNV, 2021 Treatment technologies in practice: on the ground experiences in faecal sludge treatment	The report contains numerous case studies on different faecal sludge (FS) treatment options, including a chapter on Deep Row Entrenchment. In addition to referencing the previous WRC work on DRE, the case study looked at experiences in Benin, Malaysia, and India. Overall, DRE was determined to be a low cost, simple option for sludge disposal and to have some benefits as a soil conditioner.
Rohilla, Luthra, Bhatnagar, Matto, & Bhonde, 2017 Septage Management Urban India's journey beyond ODF	"Deep-row entrenchment is a method of disposal and soil modification which consumes little energy. Its design and operation ensure that groundwater is not contaminated. The length and depth of a trench depends on the highest groundwater level and quantity of faecal sludge. A trench can be lined with clay and other material to reduce the risk of groundwater contamination. Entrenchment is done in batches. Untreated faecal sludge is placed in trenches and covered with soil. Trees can be planted on top or next to the trench."
Jayathilake, Drechsel, Keraita, Fernando, & Hanjra, 2019	"The Sewage Sludge Directive 86/278/EEC seeks to encourage the use of sewage sludge in agriculture and to regulate its use in such a way as to prevent harmful effects on soil, vegetation, animals and humans. To this end, it prohibits the use of untreated sludge on agricultural land unless it is injected

Reference	Mention of DRE
Guidelines and regulations for fecal sludge management from on-site sanitation facilities. Resource Recovery and Reuse	or incorporated into the soil. Treated sludge is defined as having undergone 'biological, chemical or heat treatment, long-term storage or any other appropriate process so as significantly to reduce its fermentability and the health hazards resulting from its use.'"
Snyman & Herselmen, 2006 Guidelines for the Utilisation and Disposal of Wastewater Sludge	The sludge management guidelines for South Africa detail methods to reduce the risk of vector attraction to make application of various sludge stability classes possible. Option 10 involves incorporating sludge into the soil within 6 hours of application to or placement on the land. Incorporation is accomplished by ploughing or by some other means of mixing the sludge into the soil. This is the same requirement mentioned in US EPA, 1994. Alternatively, digging trenches to bury sludge is another option to achieve this same goal, suggesting that as long as the trenches are backfilled within 6 hours of application, the conditions will be met.

4.2 LAND APPLICATION OF SLUDGE IN THE SOUTH AFRICAN CONTEXT

A 2020 study surveyed sludge coming from 18 wastewater treatment plants in South Africa for its suitability for use in agriculture (Badza, Tesfamariam, & Cogger, 2020). The samples represented three different wastewater treatment processes used in South Africa: 50% anaerobically digested sludge; 39% waste activated sludge; and 11% aerobically digested sludge. The study suggested that the South African threshold for sludge application of ten tonnes biosolids per hectare is not specific enough, given the wide variety of climatic conditions and wastewater treatment processes across the country. In particular, the study investigated how specific treatment processes utilised have an impact on the final quality of the sludge (Badza et al., 2020).

After analysing each of the sludges in great detail, each was characterised in terms of The Guidelines for Utilisation and Disposal of Wastewater Sludge (Snyman & Herselman, 2006). In terms of microbial quality, five treatment plants were classified as microbial class A with the remainder classified as microbial class B (Badza et al., 2020). Those classified as class A were all from treatment plants incorporating anaerobic and/or aerobic treatment of the sludge. Stability classification varied widely with little correlation observed between classification and treatment process. While a majority of sludge with a stability classification of 1 was dried in drying beds, not all plants with drying beds resulted in a sludge stability class of 1 (Badza et al., 2020). Both microbial and stability classifications have implications for the appropriateness of surface application of sludge, due to the potential for public health impacts in terms of direct pollution of the environment with microorganisms as well as the attraction of vectors due to odours. However, as indicated in the deep row entrenchment (DRE) guideline produced as part of the previous study, proper burial prevents the risk of human contact, making it an applicable strategy for sludges of varying microbial and stability classes.

Finally, pollutant classification considers the concentration of heavy metals in the sludge due to the risk of contamination of the environment. Badza et al. (2020) found that most sludges fell into pollutant class a, with six falling into class b due to elevated lead levels and one falling into class c due to elevated cadmium levels. While a sludge with a class b pollutant classification can be used in agriculture, as long as the background metals level in the receiving soil is low enough, sludge with a c pollutant classification should never be used in agriculture (Badza et al., 2020). The study highlighted that sludge is likely to have a varying impact on the receiving soil based on the treatment method employed. For example, nutrient availability will vary based on the sludge stability classification. Overall, Badza et al. (2020) highlighted the need to characterise municipal sludge and receiving soil and produce a suitability assessment whenever sludge is to be used for agricultural processes.

A former engineer at eThekwini Municipality did a brief investigation into sludge disposal at various metros including Tshwane, Ekurhuleni, Johannesburg, and Cape Town (Dyer, undated). Across these four metros, the approach varied widely. In Tshwane, dewatered sludge is sun dried and then sent to an external contractor who processes the sludge further, bags it, and sells it. There is also work being done on a combined heat and power project. Additionally, sludge from three wastewater treatment plants (WWTWs) was previously sent to an instant lawn contractor, similar to the arrangement between Darvill WWTW and Duzi Turf in Pietermaritzburg. In Ekurhuleni, sludge is transported in trucks for land disposal. Johannesburg processes sludge further through composting prior to disposal on land. Finally, Cape Town is sending primary sludge to a hazardous landfill and waste activated sludge to farms growing animal fodder. Plans are being discussed to further process the sludge in an effort to produce A1a sludges that could be recycled through land application (Dyer, undated).

4.2.1 Case Study: Western Cape Government

In 2020, the Western Cape Government produced the *Sewage Sludge Status Quo Report 2020/21*. The report details management practices around sewage sludge and identifies opportunities. The results from a survey conducted with 107 wastewater treatment plants revealed that sewage sludge is either applied to farm lands (22%); disposed of in general (20%) or hazardous landfills (10%); stockpiled (22%); or used in agriculture through composting (11%). Overall, the report suggested that the shortage of landfill airspace in the province suggests that organic waste must be diverted from the landfill. The report identifies the need for more beneficiation options for treated sewage sludge.

4.3 IMPACT OF BURIED SLUDGE ON CROP YIELDS AND SOIL HEALTH

A similar study to this one was conducted in Brazil and observed an increase in wood production in a plantation of *Eucalyptus urograndis* (Abreu-junior et al., 2020). The study investigated the impact of the application of sewage sludge on the availability of phosphorus. When applied along with mineral fertiliser, sewage sludge increased phosphorus availability through the addition of microorganisms and organic content. The same was confirmed by Kahiluoto et al. (2015) in a pot plant study comparing phosphorus availability of sewage sludge, dairy manure, and soluble NPK fertiliser. Furthermore, the addition of organic phosphorus in the sewage sludge may lead to a longer-term impact of nutrient availability, as organic phosphorus is less readily available than inorganic forms. This suggests that sewage sludge applied in a *E. urograndis* plantation would have immediate and long-term impacts on the crops grown in that soil (Abreu-junior et al., 2020).

This study looked at the impact of buried sewage sludge many years after application (10, 12.2, 12.4, and 17.2 years). The study observed increases in soil organic matter, nitrogen, and cation exchange capacity due to the application of sewage sludge, when compared to sites treated with mineral fertilisers and no fertiliser (Florentino et al., 2019). While heavy metals (potentially toxic elements) were generally elevated in soils treated with wastewater sludges, this impact decreased over time.

Finally, the study confirmed an increase in wood production of 7% in wood volume, comparing plots where nitrogen requirement was met fully by wastewater sludge with those using NPK fertiliser.

Overall, recent studies point to the potential for sludge application to lead to immediate improvements in crop yields as well as long-term improvements in soil health. While some of the studies mentioned have compared wastewater sludge with traditional NPK fertilisers, it is important to highlight the additional benefits realised through sludge application. While mineral fertiliser will provide a nutrient source to the crop, it will not improve soil characteristics in the way that sludge can through the addition of organic matter.

4.4 IMPACT OF BURIED SLUDGE ON THE ENVIRONMENT

Concerns about the potential pollution of the environment by applied sludge remain prevalent, limiting uptake of land application of sludge. The movement of contaminants through soil is impacted by several factors, such as: level of the water table, clay content of the soil, and preferential flow paths (Wickham, 2014). The contaminants of concern include nitrate, pathogens (*E. coli*), and heavy metals. While phosphate should also be considered, it is generally immobile in soil and is therefore not directly addressed in this section.

4.4.1 Nitrogen migration

When nitrogen is applied in sewage sludge, it is mostly present in the forms of ammonium, nitrate, or organic nitrogen. Ammonium is converted to nitrate via nitrification, an aerobic microbial process. Nitrate is readily available to plants but also known to be mobile in soil. Thus, nitrate is of concern in terms of groundwater pollution. Following nitrification, anaerobic denitrification converts nitrate to nitrogen gas, which is released to the atmosphere. Ammonium and nitrate can also be complexed into an organic form, and this is quite common in soil due to the extensive microbial population.

A 2014 study investigated nitrogen migration from entrenched biosolids in a hybrid poplar tree farm (Felton & Kays, 2014). Over three years, nitrate levels beneath sludge trenches were zero, only then increasing to between 1 and 10 mg/ ℓ. This increase was first observed in the sludge treatment with lowest application rate. Felton & Kays (2014) hypothesised that this was because higher sludge application rates meant deeper application of sludge. The deeper the sludge, the more oxygen and microbial activity are depleted. This suggests that the nitrification process (ammonium to nitrate) occurred slowly and that as nitrate was produced, it was taken up by tree roots. Also the anaerobic conditions in the soil were favourable for denitrification, suggesting that nitrate would either be taken up by the roots or converted to nitrogen gas, limiting its movement in the soil (Felton & Kays, 2014). The study also compared treatments with trees planted and those with no trees. Those with trees planted had the lowest nitrate concentrations compared to those with no trees, demonstrating that trees provided an effective nitrogen uptake mechanism (Felton & Kays, 2014). Thus, planting trees on buried sludge not only provides a nutrient source for the trees but also a mechanism for preventing pollution.

Wickham (2014) investigated migration of contaminants (nitrate, phosphate, and *E. coli)* from on-site sanitation systems (e.g. ventilated improved pit latrines (VIPs) latrines) and confirmed that in most clayey soil conditions, a minimum distance of 15 metres between on-site sanitation systems and drinking water sources is sufficient. Wickham recommended that in sandy soils the minimum distance could be increased to 60 metres.

4.4.2 Heavy metals migration

According to Felton & Kays (2014), movement of metals in the soil is minimal except where pH is below 5.5. In the sludge characterisation study by Badza et al. (2020), all sludges had a pH between 4.5 and 9. It is important to characterise the receiving soil's pH. In highly acidic soils, application of lime may be necessary to both improve the soil and limit migration of metals in the soil.

Regarding the impact of entrenched sludge on the environment, an important study was conducted in the 1970s in Maryland, United States on a plot with sandy soil. Considering a sandy area, this study represented a worst-case scenario in terms of potential leaching of contaminants in groundwater. In this study, Sikora, Burge, & Jones (1982) observed limited movement of nitrate through the soil, with sludge stabilisation (i.e.

no mineralisation of nitrogen) after four years. The study did not characterise groundwater extracted from the site but hypothesised that decreases in nitrogen in the sludge over time were due to nitrification and uptake by plants, denitrification, and/or leaching. On the other hand, the study did not observe any movement of heavy metals (Zn, Cu, Cd) from the sludge trench, which was likely due to the high pH of the sludge as a result of addition of lime. The study, conducted decades ago, contributed to the confidence in deep row entrenchment as a feasible and safe option for disposal of municipal sludge. Given that the results are from an area with sandy, highly draining soil, it is expected that any leaching potential observed would be lower in soils with higher clay contents.

4.4.3 Pit latrines and groundwater

While (VIPs) represent a different approach to sanitation than deep row entrenchment, in principle, the two approaches are similar in that sludge is placed in an unsealed pit. With VIPs, the sludge reaches deeper depths, as the pit serves the purpose of storing large amounts of sludge to avoid the need for frequent emptying. Thus, many of the concerns around environmental contamination by burial of sludge are shared with the use of VIPs. Nyenje, Foppen, & Uhlenbrook (2014) studied a shallow aquifer in a slum in Uganda characterised by a large density of pit latrines. The slum is underlain by three soil layers, the middle one consisting of a shallow aquifer. The water table is very shallow, generally less than 1 metre below the ground surface year-round. The bottom soil layer has a high clay content and acts as a confining layer.

The study characterised the groundwater in the aquifer and found high concentrations of carbon, nutrients (N and P), and cations including Na, Ca, and K. This is not surprising given the high concentrations of these elements in faecal sludge. The study further investigated groundwater downgradient from the slum (approximately 200 metres) to determine how far these pollutants migrated. Electrical conductivity and chloride both decreased downgradient from the slum, suggesting limited migration of contaminants at that point. While the study did identify considerable contamination of the aquifer from on-site sanitation systems, it also observed transformation of contaminants, such as ammonia to nitrogen gas through an anaerobic oxidation process. Similarly, the study found that approximately 75% of phosphorus from onsite sanitation sources was removed within a short distance of the source.

The findings from the above study suggest that though contamination of shallow groundwater is possible, shallow aquifers also act as sinks of nutrients, thus ensuring that the groundwater impact was not observed 200 metres downstream from the point source of pollution (Nyenje et al., 2014).

5 METHODOLOGY

This study involved several stages to solidify an understanding of the impact of buried sludge on timber and the environment. These stages and the methodologies are described below.

5.1 DETERMINING THE OVERALL IMPACT OF SLUDGE AT THE END OF THE FIRST GROWING SEASON

The trees from the previous experiment were harvested at the end of 2018. To determine the overall impact of sludge on the trees over the full growth cycle, a final set of measurements was taken in September 2018. To determine diameter at breast height, the circumference of trees at 1.37 metres above ground level was measured using a flexible measuring tape. From the circumference, the diameter at breast height (DBH - a standard measurement in the timber industry) could be calculated. A grid of 5 x 6 trees in the middle of each of the 30 plots was measured. Figure 5-1 shows the overall site layout with the location of the different treatments, as well as the location of the trees, which were measured within each of the treatment, blocks.

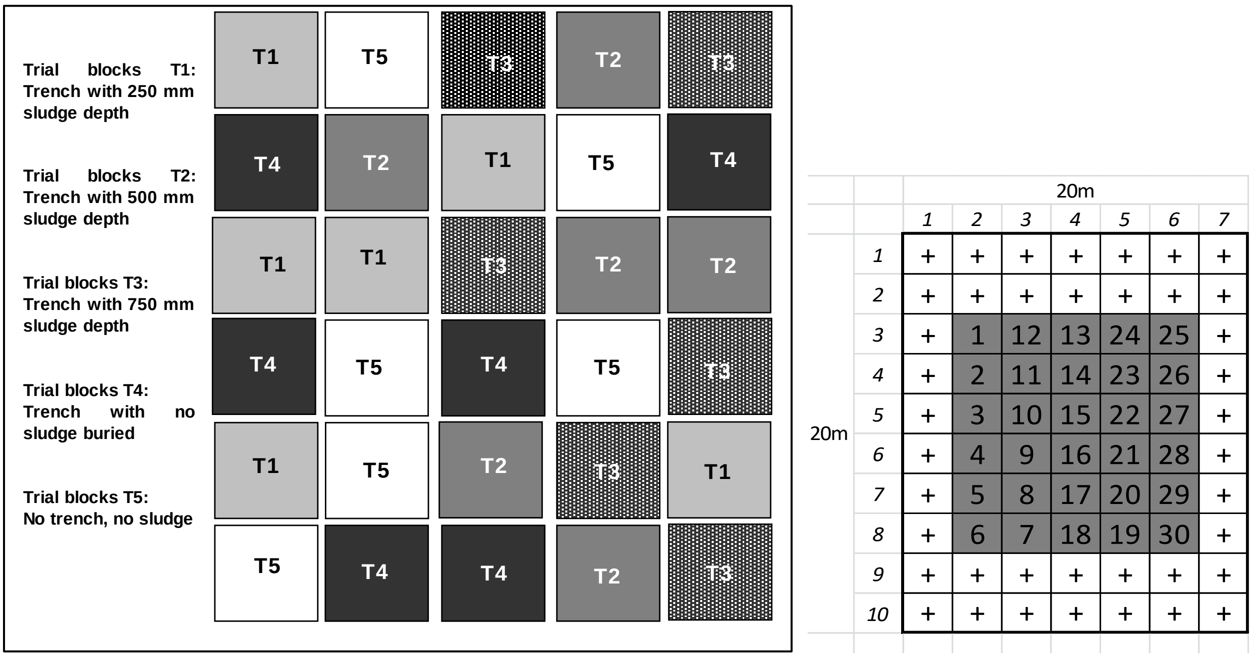

Figure 5-1: Overall layout of experimental plot (left) and 6 x 5 grid of trees measured during first growing season (right)

While the previous study utilised DBH³ (mm³) as a proxy for timber volume, a more accurate approximation can be made by using fitted curves for a plot of DBH vs height to approximate the tree height (see equation 2 below) and, subsequently, the conical volume.

$$DBH\ (cm) = \frac{Circumference}{\pi} \quad (1)$$

$$Tree\ Height, H\ (m) = 0.0357DBH^2 + 1.9636DBH - 1.3827 \quad (2)$$ [1]

$$Basal\ area, A\ (m^2) = \pi\left(\frac{DBH}{200}\right)^2 \quad (3)$$

$$Conical\ volume\ (m) = {}^{1}/_{3}\,HA \quad (4)$$

The above analysis was done on data from the entire first growing season to refine the analysis from the previous study and to provide a more accurate approximation of the impact of buried sludge on tree growth.

5.2 ASSESSING THE IMPACT OF BURIED SLUDGE ON TREE GROWTH RESPONSE IN THE SECOND GROWING CYCLE

Following tree harvesting at the end of 2018, new trees were planted on the same plot at the start of 2019. No new sludge was buried. This allowed for monitoring of the trees grown on the same land to determine if the sludge buried in 2009 had any residual impact on growth outcomes. Tree growth was monitored in a central section in each plot. While 30 trees were monitored in the previous study (5 x 6 grid), only 25 trees (5 x 5 grid) were monitored in this study to attempt to minimise inaccuracies from errors locating the trial plots

. This translates to 150 tree measurements for each treatment, and diameter at breast height (DBH) was measured for each one, using a diameter tape. Dead trees were excluded from the statistics but included in the mortality rate information. The tree height was also measured with a vertex and transponder on approximately nine trees per plot. This enabled calculation of the conical volume of these trees (54 for each treatment) and a plot of DBH vs. height to approximate the height for those trees that were not measured.

[1] Equation from SAPPI standard curve for E. dun species of tree, used in this study.

Only height measurements were collected in March 2020. DBH and height measurements were collected in October 2020, April 2021, October 2021, and February 2022, providing insight on growth during one winter and two summer seasons. The experimental site is located in a summer rainfall area.

5.3 ASSESSING THE IMPACT OF BURIED SLUDGE ON SOIL AND LEAF PROPERTIES

Soil was sampled from the experimental plot to determine any lasting impacts of the sludge on the soil, which would allow for some illumination of the mechanisms behind any improved growth of trees in experimental blocks with buried sludge. Soil samples were collected from the plots shown in Figure 5-2, to capture the outcomes in all three sludge treatments and the control (T5). To collect the samples, a trench was dug across each block to allow for the location of sludge. To assess the variability of characteristics in and around the sludge trench, a series of samples was collected from each plot, based on the treatment and depth of sludge, as shown in Figure 5-3. The samples collected in the T5 plots represented every depth in the soil stratum collected in the sludge treatment plots, providing a valid comparison for each sample point, as characteristics are expected to vary with depth and not just with treatment. Samples were collected within the sludge trenches as well as below, adjacent, and below/adjacent to demonstrate any impact on surrounding soil and/or movement of nutrients or contaminants. Sikora et al. (1980) demonstrated that sludge entrenched in sandy soils had stabilised after four years, meaning nitrogen was no longer mineralised, which suggests that movement of pollutants out of the trenches should be negligible at this point, over 10 years after burial.

Collected samples were stored in bags and analysed at the Cedara and University of KwaZulu-Natal laboratories for various properties. The soil analyses conducted are listed in Table 5-1. Detailed methods for the laboratory analyses are provided in Annexure A. In each sampled block, leaf samples were also collected from a single tree. The leaves were taken to the laboratory, where they were oven-dried at 60°C for 5 days and then crushed. The samples were taken to Cedara for tissue analysis of nitrogen (%), calcium (%), magnesium (%), potassium (%), sodium (mg/kg), zing (mg/kg), copper (mg/kg), manganese (mg/kg), iron (mg/kg), phosphorus (%), and aluminium (mg/kg).

Table 5-1: Analyses carried out on soil samples

Soil Property analysed	Laboratory
Soil density (g/mℓ)	Cedara
Phosphorus (mg/ℓ)	Cedara
Potassium (mg/ℓ)	Cedara
Calcium (mg/ℓ)	Cedara
Magnesium (mg/ℓ)	Cedara
Acidity (cmol/ℓ)	Cedara
Total Cations (cmol/ℓ)	Cedara
pH (KCl)	Cedara
Zinc (mg/ℓ)	Cedara
Manganese (mg/ℓ)	Cedara
Copper (mg/ℓ)	Cedara
Clay (%)	Cedara
Carbon (%)	Cedara
Nitrogen (%)	Cedara
Water holding capacity (%)	University of KwaZulu-Natal, Crop Science Department
Total organic carbon (%)	University of KwaZulu-Natal, Soil Science Department
Particulate organic matter carbon (POM-C)	University of KwaZulu-Natal, Soil Science Department
Mineral associated organic carbon (MOC) (%)	University of KwaZulu-Natal, Soil Science Department

Soil Property analysed	Laboratory
Mineral Phosphorus - **Readily available phosphorus (extracted by Ammonium chloride)** - **Aluminium bound phosphorus (extracted by Ammonium fluoride)** - **Iron bound phosphorus (extracted by sodium hydroxide [0.1])** - **Reducible phosphorus, bound to Al and Fe oxides (extracted with citrate bicarbonate dithionite (CBD))** - **Calcium bound phosphorus** - **Residual phosphorus**	University of KwaZulu-Natal, Soil Science Department

Figure 5-2: Experimental blocks where soil samples were collected

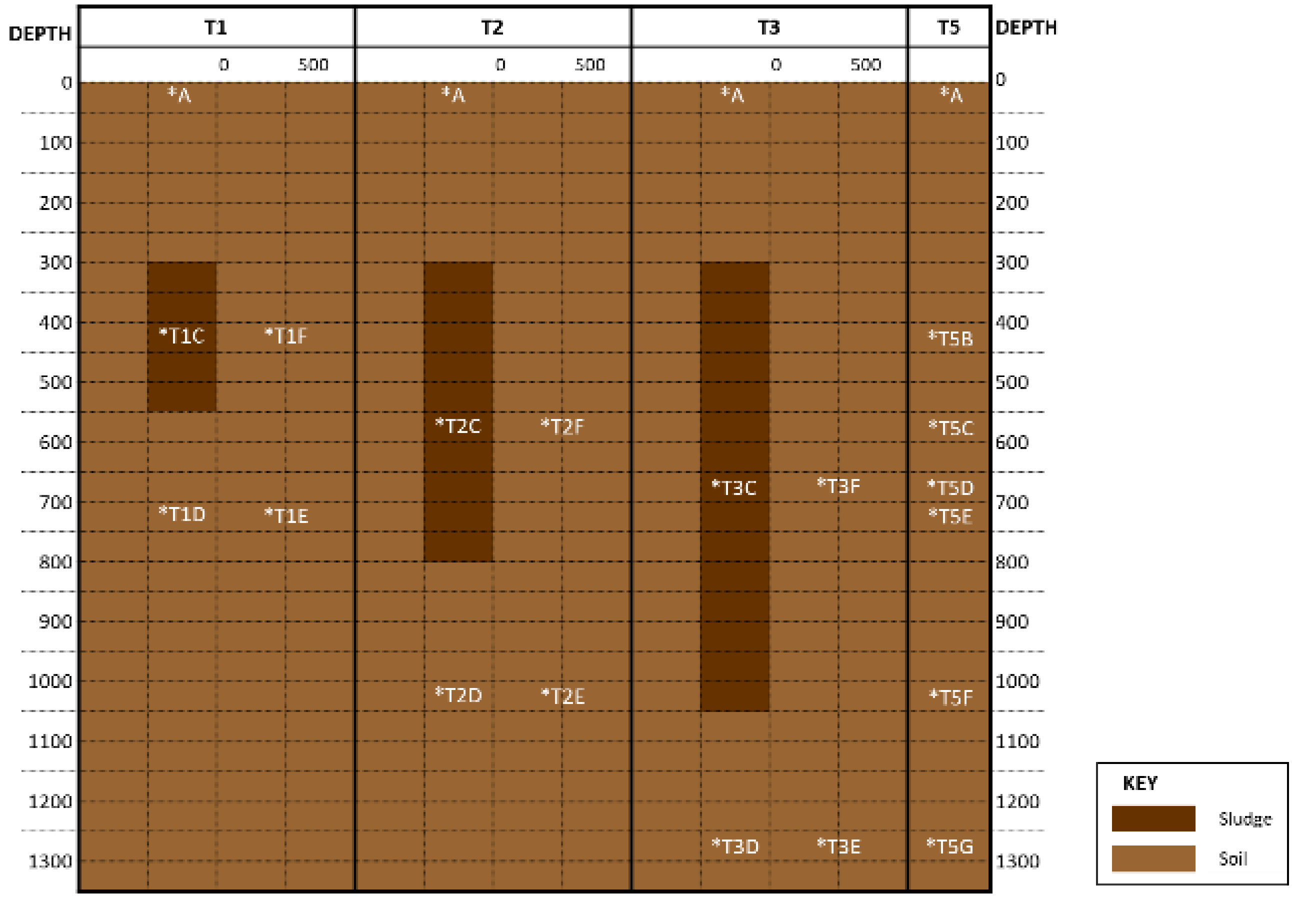

Figure 5-3: Diagram demonstrating soil samples collected

5.4 ASSESSING THE IMPACT OF BURIED SLUDGE ON THE SURROUNDING ENVIRONMENT

During the previous study, the site was closely monitored over the three-year period for groundwater impact, using several piezometers for near surface flow and two 30-metre-deep boreholes at the bottom of the site for groundwater monitoring. Both boreholes were located adjacent to a stream downhill from the site: one located upstream from the experimental plot and one just downstream (Figure 5-4). Results from the previous study suggested limited impact of sludge entrenchment on the groundwater quality on site, when considering both nitrate and phosphate. However, it is possible that there is a time delay before the groundwater impact can be observed (the "time-bomb" effect), and therefore this study tested that possibility. Borehole samples were collected at two points during the current study and analysed for nitrate and orthophosphate. Prior to sampling, the boreholes were pumped for sufficient time to remove 2-3 volumes of water from the borehole, to ensure that the groundwater was sampled.

Figure 5-4: Location of boreholes in relation to experimental site (outlined in white)

Additionally, an expert study was done on the stream directly downhill from the sludge entrenchment site to determine any impacts on the environment. Nutrients, in-situ water quality and diatoms were assessed for two sites, Site 1 upstream of the sludge treatment block and Site 2 below the sludge treatment block. Additional in-situ and diatom samples were collected approximately 1 km downstream (Site 3, S 29.402645, E 30.266613). Water samples were sent to a SANAS accredited laboratory for nitrogen and phosphorus assessments. In-situ measurements were collected on a multiparameter water meter and benthic diatoms were collected, using the prescribed method by Taylor et al. (2005). Laboratory and in-situ water quality results were compared to the Department of Water and Sanitation (DWS) guidelines for aquatic ecosystems. Diatoms were assessed using the SPI method, which provides river health results in line with the South African river Ecoclassification and Ecostatus categories.

6 RESULTS

6.1 IMPACT OF BURIED SLUDGE ON TIMBER YIELDS

This section presents the results from the first and second growing seasons to highlight the impact of buried sludge on timber yields in terms of tree size. As described above, circumference at breast height was measured during the first growing season, and diameter at breast height (DBH) and height of a sample of trees were measured during the second growing season.

6.1.1 First growing season

The results from the first growing season are presented in Figure 6-1 through Figure 6-3 and summarised in Table 6-1. Conical volume is estimated based on the approximate relationship between DBH and height, and the results in Figure 6-3 point to an overall increased timber yield over the entire growing cycle when comparing sludge treated plots (T1, T2, and T3) with the control plots (T5). At the end of the growing cycle, nearly nine years after planting, the volume of trees planted in treated plots was estimated to exceed those planted in controls by 30%, 18%, and 15% for T3, T2, and T1, respectively. As can be seen in Figure 6-3, the increase in estimated timber volume was realised early on, with an increase as high as 77% for T1, 2 years after planting. The impact decreased with time, suggesting that the impact of the sludge is present early in the growth cycle and not once the trees reach a certain size. This may be because the sludge is buried in a relatively shallow layer of the soil, and once the roots grow beyond the trenches, it is assumed that the impact is negligible. Alternatively, certain nutrients added in the sludge may be more important early in the growth cycle. Nevertheless, the boost in growth experienced early on ultimately led to an overall increase in timber yield.

Table 6-1: Summary of timber yield results from first growing season as of 28 September 2018, just before harvesting

Treatment	Survival %	Sum of basal area (m^2)	Quadratic mean DBH (cm)	Estimated total volume (m^3)	% diff in volume (compared to T5)
T1	92%	3.96	16.6 (±6.3)	29.8	30%
T2	89%	3.63	16.2 (±5.6)	27.1	18%
T3	86%	3.54	16.2 (±6.6)	26.5	15%
T4	93%	3.33	14.8 (±6.4)	24.4	6%
T5	83%	3.09	15.1 (±6.8)	22.9	0%

Note: T1 = 250 mm sludge application; T2 = 500 mm sludge application; T3 = 750 mm sludge application; T4 = no sludge application, with trenching; T5 = no sludge application and no trenching

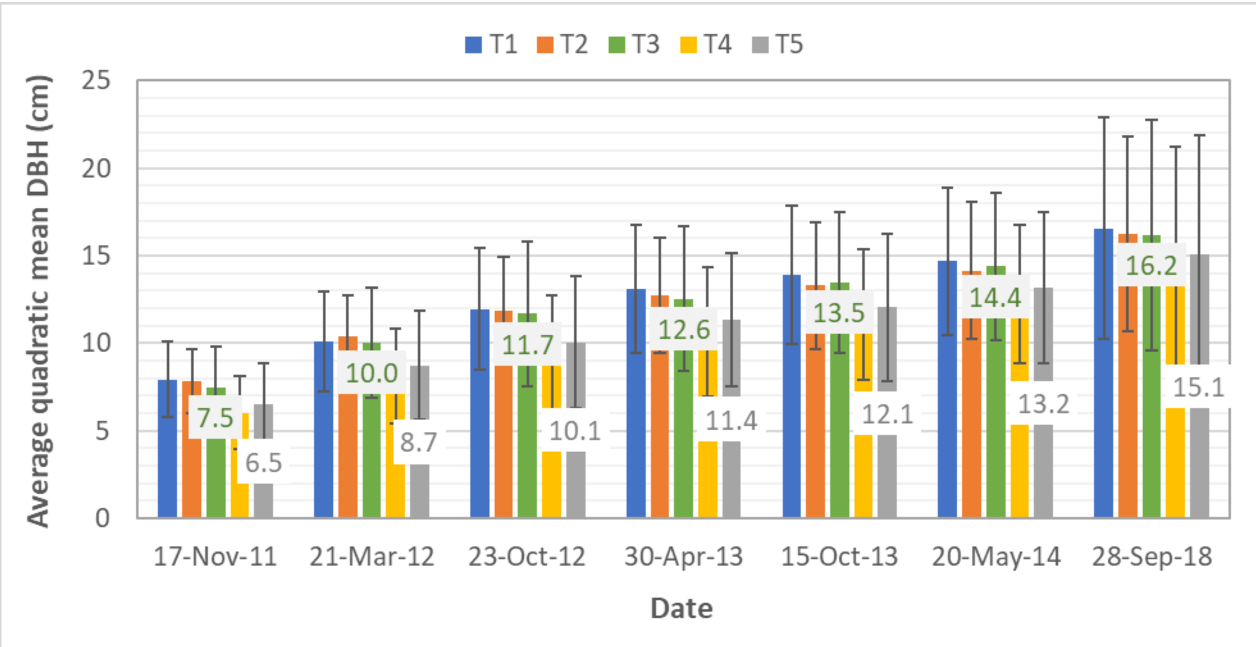

Figure 6-1: Average quadratic mean DBH (cm), first growing season

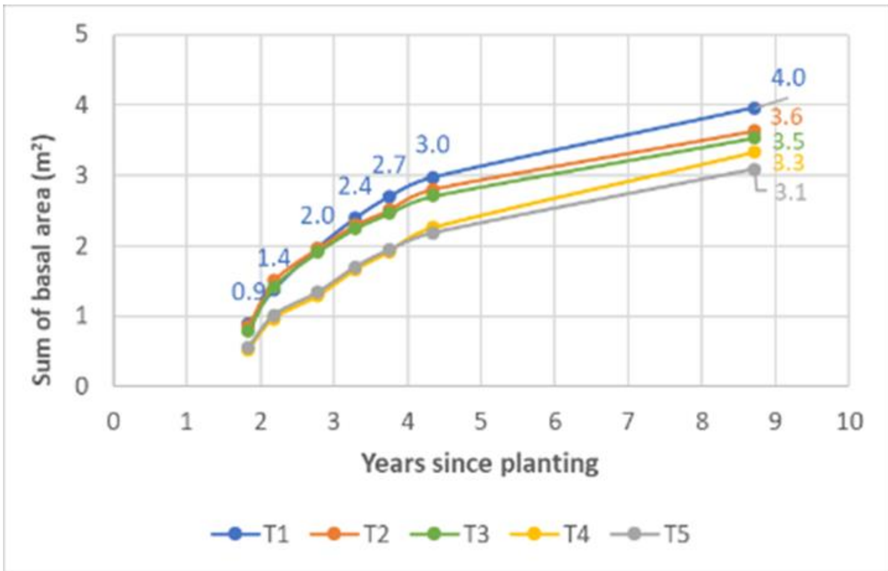

Figure 6-2: Sum of basal area (m^2) as a function of years since tree planting, first growing season

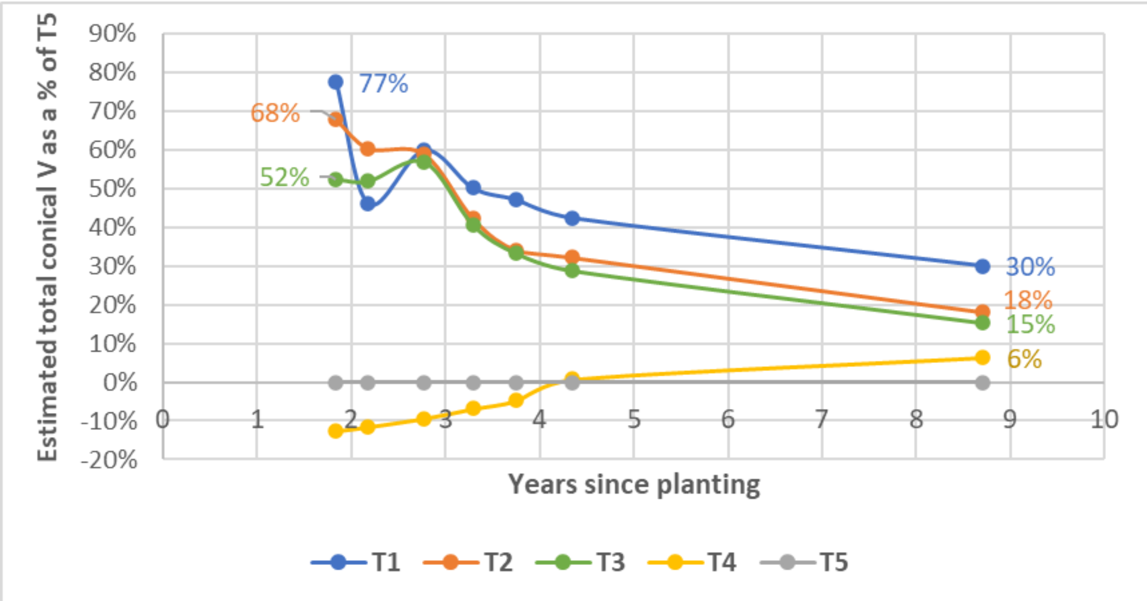

Figure 6-3: Estimated total conical volume as a percentage of T5, first growing season

6.1.2 Second growing season

The results from the second growing season are presented in Figure 6-4 through Figure 6-6 below and summarised in Table 6-2. Data from all observation dates were statistically assessed using the Shapiro-Wilk Test with a minimum p-value of 0.05. In terms of average conical volume, results from all three sludge treatments (T1, T2, and T3) did not significantly differ from one another but differed significantly from the two control treatments (T4 and T5). The same is true when comparing quadratic mean DBH. This points to the fact that there is a positive impact of buried sludge on growth of trees during the first few years of growth. These results suggest that the quantity or depth of sludge applied did not influence the impact on growth significantly, as was observed during the first growing season.

Table 6-2: Summary of timber yield results from second growing season up to February 2022

Treatment	Survival %	Sum, basal area (m^2)	Quadratic mean DBH (cm)	Mean height (m)	Estimated total volume, n=51 (m^3)	% diff in volume (compared to T5)
T1	89%	1.42	10.1 (±2.6)	12.2 (±7.2)	2.97	22%
T2	88%	1.39	9.99 (±2.8)	12.4 (±7.3)	3.09	27%
T3	89%	1.44	10.3 (±2.1)	12.3 (±7.2)	3.08	26%
T4	95%	1.30	9.36 (±2.0)	10.7 (±6.5)	2.35	-3%
T5	81%	1.20	8.81 (±3.1)	10.9 (±6.6)	2.43	0%

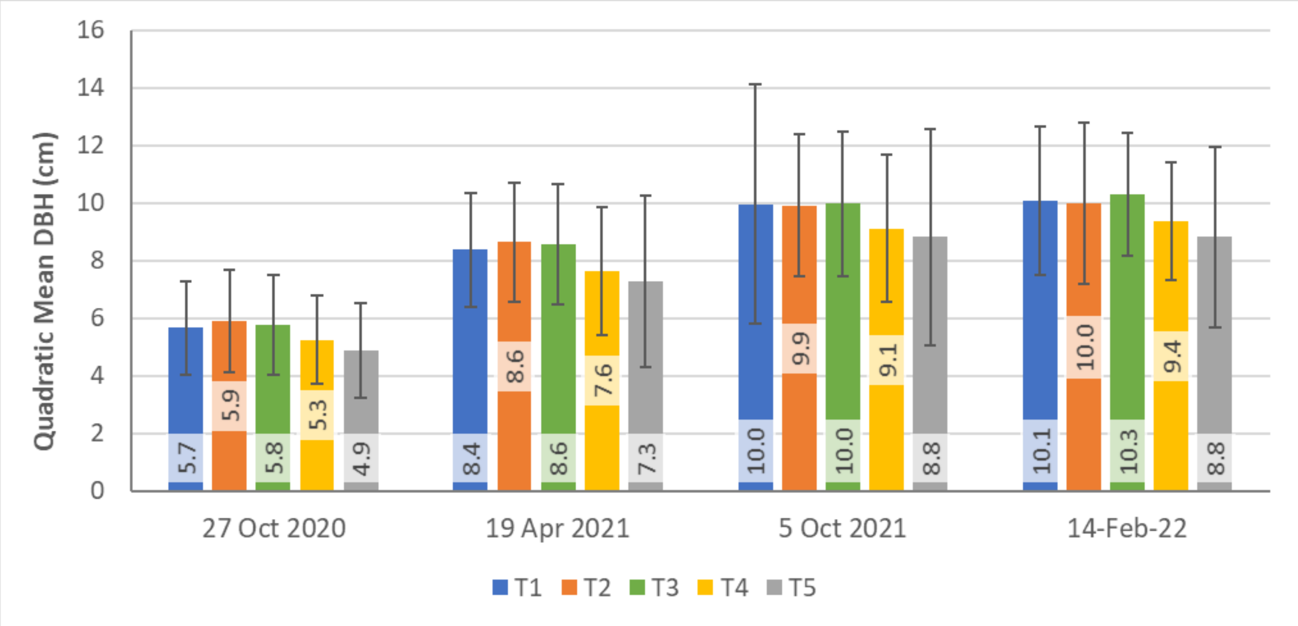

Figure 6-4: Average Quadratic Mean DBH during second growing season

Figure 6-5: Sum of basal area over time during second growing season

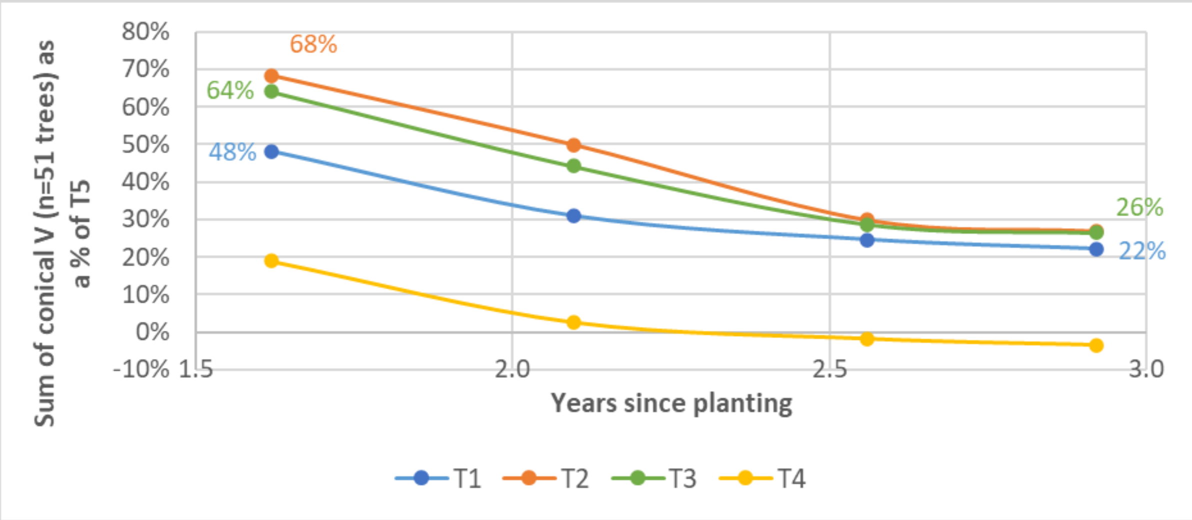

Figure 6-6: Sum of conical volume as a % difference with T5 during the second growing season (n = 51 for each treatment)

By comparing the data from the two growing seasons, it is possible to hypothesise about how the timber yield will be impacted over the entire second growing season. Figure 6-7 shows that the response of timber in the first 2.5 years of growth was similar in the first and second growing seasons, with a large increase in volume in the first two years when comparing trees planted in sludge vs. those in the plots without sludge.

After 2.5 years, the increase observed in the first season was approximately 57% (T3), and the increase in the second growing season was 29% (T3), indicating a lesser but still significant impact in the second growing season up to that point in the growing cycle. Figure 6-8 shows the full set of results from the first growing season over 8.5 years of growth. If the second growing season follows a similar pattern of gradual decrease in the impact of buried sludge, it is expected that the overall increase in timber volume will be approximately 10% at the end of the season.

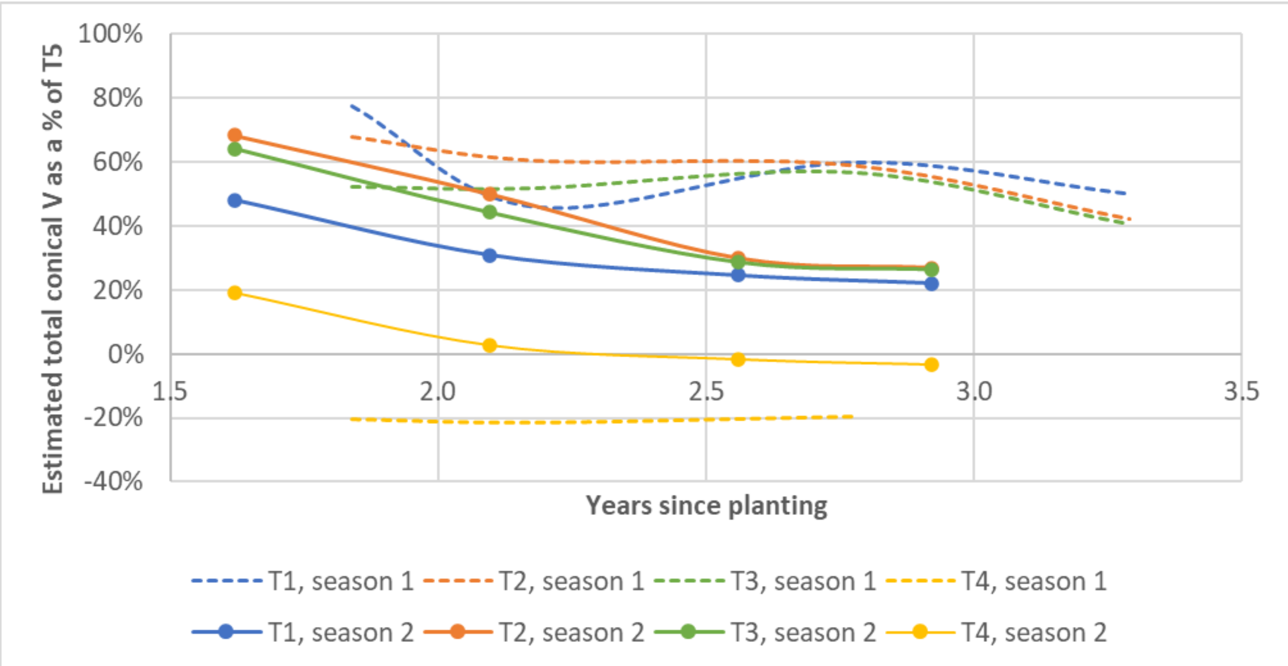

Figure 6-7: Results of estimated conical volume of trees in the various treatments in the first 2.8 years of growth, for growing seasons 1 and 2

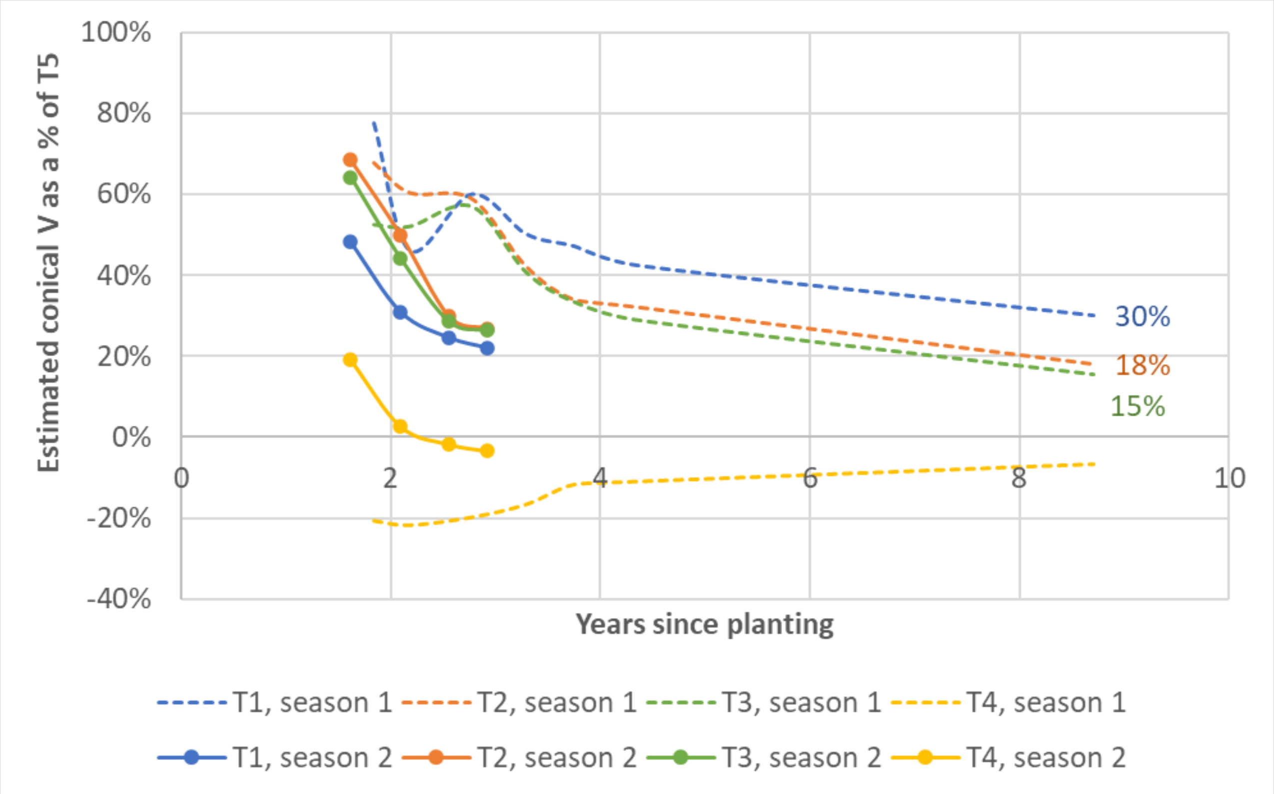

Figure 6-8: Results of estimated total conical volume of trees in the various treatments from growing seasons 1 and 2, showing data from 8.5 years of growth in season 1

6.2 IMPACT OF BURIED SLUDGE ON SOIL PROPERTIES

The results on the impact of buried sludge on various soil properties, more than ten years after burial, are presented below. The results are presented in terms of average values and statistical significance. More detailed analysis on these properties has been carried out by the two students involved in this study. Their final reports will be published by the University of KwaZulu-Natal Crop Science and Soil Science departments

. It is important to note that sample sizes were relatively small in this study, which impacts the evaluation of statistical significance. Where there appears to be a difference between sludge and controls, but that difference is not "statistically significant" (i.e. p-value <0.05), further controlled study with more samples is warranted. All data presented in this section represents a once-off sampling event, rather than continuous sampling. Each reported average includes either two or three samples.

Anecdotal evidence from the sampling exercise points to a long-term difference between the buried sludge and surrounding soil. This is evidenced in the colour of material and the presence of roots within the sludge trenches (Figure 6-9 and Figure 6-10).

Figure 6-9: Observed visual differences in surrounding soil and buried sludge trenches, demonstrating a continued difference after >10 years

Figure 6-10: Photos demonstrating clustering of roots in and around buried sludge, likely due to elevated nutrient levels

6.2.1 Physiochemical properties

Clay content and soil pH both have an impact on the movement of pollutants in the soil and availability of nutrients for the plant. The clay content of the soil in the SAPPI site was relatively high, with most values between 50-60%. In Treatments 2 and 3, clay content within the buried sludge was lower, as shown in Figure 6-11. The pH of the soil is acidic, with pH values below five at all depths. The added sludge appears to have lowered the pH slightly in all cases, as evidenced by Figure 6-12. The acidic pH of the soil is of concern, as metals are more mobile at acidic pH levels. Furthermore, the low pH affects the availability of certain nutrients (e.g. phosphorus) to plants. For this reason, lime is often added to soils to raise the pH to a more optimal, neutral pH for plant growth. In the case of the timber plantation where the study took place, lime is seldom added to soils to adjust pH. pH values in the background soils sampled in the previous study (February 2013) were even lower than those from this study, dropping below four. The practice of burning the plots after harvesting is suspected to slightly raise the pH of the soil, which may explain the slight increase in pH.

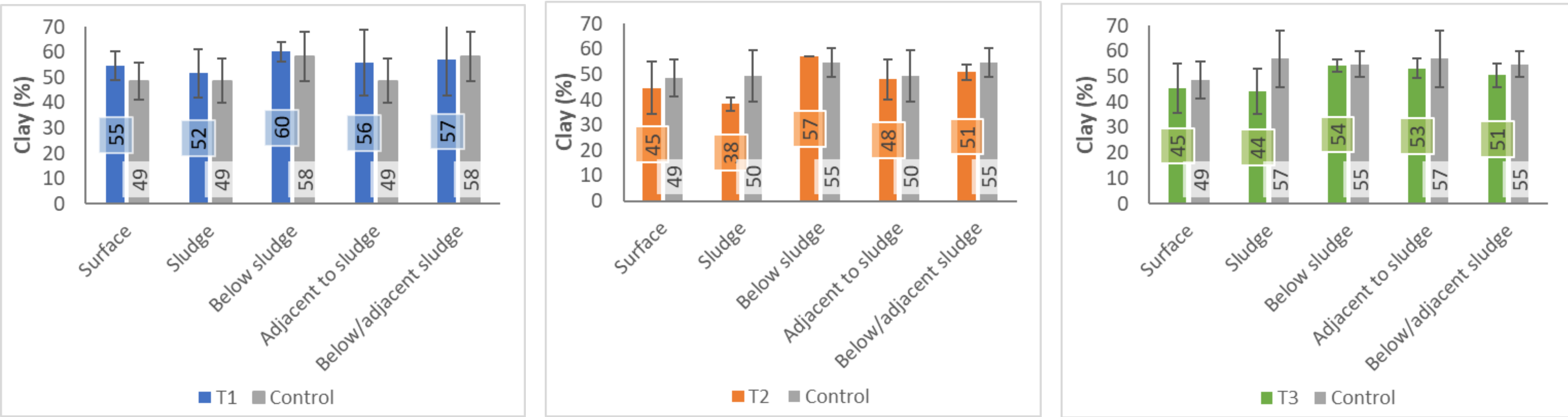

Figure 6-11: Average percentage clay in various soil layers in plots with T1, T2, and T3, respectively

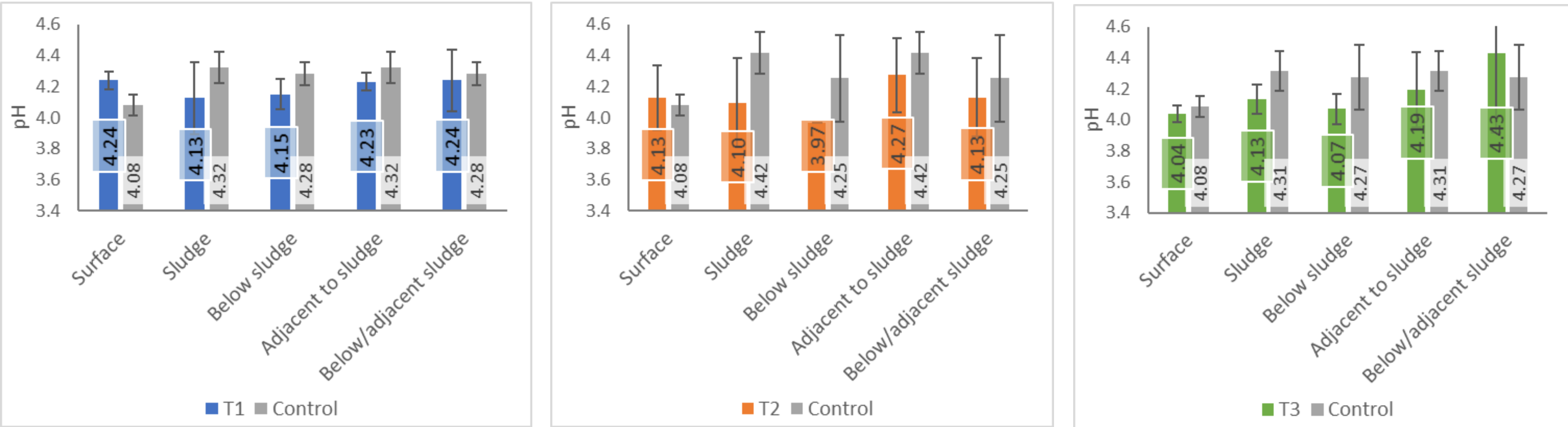

Figure 6-12: Average pH in various soil layers in plots with T1, T2, and T3, respectively

6.2.2 Soil organic carbon fractionation

Total organic carbon (TOC) was elevated in layers where sludge had been buried, and slight evidence of migration in the soil was observed in the samples below the sludge (Figure 6-13), when compared to the controls. The results demonstrate that carbon from sludge added to the soil more than 10 years ago has been retained in the soil and has had a long-term impact on the organic content of the soil. Organic carbon is vital for soil quality, as it affects physical, chemical, and biological properties including nutrient mineralisation potential, cation exchange capacity (CEC), aggregate stability and water retention (Madikizela, 2014). In the case of T3, the highest sludge application rate, the TOC was 7.2%, which is not significantly different from the concentration in the surface soils, which are expected to be rich in organic matter due to plant matter decay and litter.

Analysis pointed to the difference in particulate organic carbon (POC) and mineral-associated organic carbon (MOC). In most instances, organic carbon was predominantly present as POC. In Treatment 3, the POC levels in the sludge layer were significantly different from the layer directly below the sludge and the control sample, demonstrating the impact of sludge burial on the particulate organic carbon. Though not statistically significant, similar trends can be observed in Treatment 2 and less so in Treatment 1. This is due to the overall lower quantity of sludge, and therefore carbon, which was applied in Treatment 1.

Table 6-3: Carbon fractionation results and significant differences

Treatment	MOC		POC		TOC	
	Sludge	Control	Sludge	Control	Sludge	Control
Treatment 1						
T1 A	1.6	2.73	5.51 ^{e}	5.15 ^{de}	7.11 ^{de}	7.87 ^{e}
T1 C	1.47	1.28	3.71 ^{cd}	2.21 ^{bc}	5.18 ^{cd}	3.49 ^{bc}
T1 D	0.63	0.48	1.21 ^{ab}	0.47 ^{a}	1.84 ^{ab}	0.94 ^{a}
Treatment 2						
T2 A	2.75	2.73	6.75 ^{c}	5.15 ^{c}	9.50 ^{c}	7.87 ^{c}
T2 C	4.31	1.60	2.34 ^{b}	1.40 ^{ab}	6.65 ^{bc}	3.00 ^{ab}
T2 D	*	0.41	*	0.33 ^{a}	*	0.74 ^{a}
Treatment 3						
T3 A	2.21	2.73	5.81 ^{b}	5.15 ^{b}	8.02 ^{b}	7.87 ^{b}
T3 C	2.57	0.86	4.68 ^{b}	0.47 ^{a}	7.25 ^{b}	1.32 ^{a}
T3 D	0.86	0.47	0.97 ^{a}	0.13 ^{a}	1.83 ^{a}	0.60 ^{a}

NOTE: Treatment means with same letter (a, b, etc.) are not significantly different at $p<0.05$. Significant difference depends on the mean values as well as standard deviation and sample size. Thus, small numbers of samples in this research may have led to inconclusive statistical results. T1= Treatment 1: 250 mm sludge (120 dry tons/ha); T2= Treatment 2: 500 mm sludge (240 dry tons/ha); T3= Treatment 3: 750 mm sludge (360 dry tons/ha; Control= Treatment 5 (T5). “A” represents surface samples, “C” represents sludge samples, and “D” represents samples below sludge.

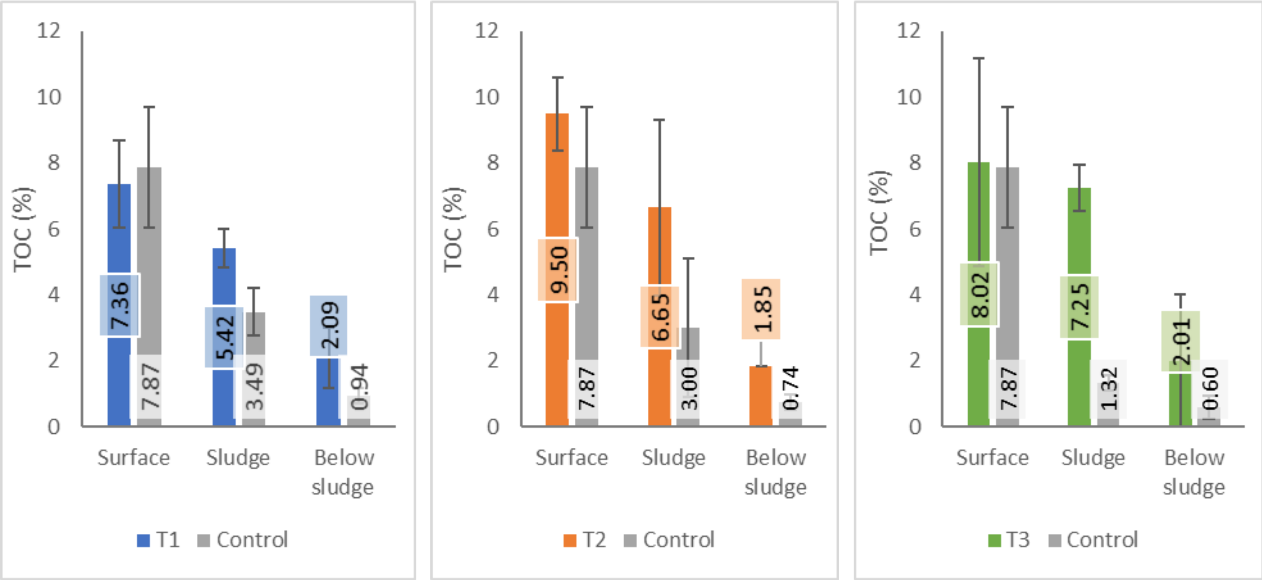

Figure 6-13: Average total organic carbon (TOC, %) in soil layers in plots with T1, T2, and T3, respectively

6.2.3 Phosphorus fractionation

The results of phosphorus fractionation analysis are presented in Table 6-4. Figure 6-14 presents total phosphorus in soils sampled from sludge entrenchment plots compared to samples from control plots. While in T1, total phosphorus in the sludge layer (C) is similar to total phosphorus in the control, total phosphorus in the sludge layers in T2 and T3 are both elevated. In the layer directly below the sludge in T3, the total phosphorus levels are also higher, which may suggest that phosphorus migrated below the sludge trench or, more likely, that samples taken “below the trench” included some sludge. Soluble phosphorus levels are presented in Figure 6-15, demonstrating that only a small portion of the total phosphorus in the soil is available to plants. Nevertheless, soluble phosphorus in the sludge layer appears elevated in all sludge treatments (T1, T2, and T3). The remainder of the phosphorus is present in bound forms, which are in equilibrium with phosphorus in the soil solution (i.e. soluble phosphorus). Thus, as soluble phosphorus is taken up by plants, bound phosphorus will become available. Furthermore, any shifts in pH will lead to shifts in the equilibrium. While iron and aluminium-bound phosphorus dominates at low pH values, at pH values closer to neutral, phosphorus becomes more available. This equilibrium provides some evidence of phosphorus stored in the soil acting as a “slow-release” fertiliser.

Table 6-4: Mean results of Phosphorus fractionation in samples from sludge treatment plots, compared to control plots

Treatment	Soluble P		Al-P		Fe-P		CBD-P		Ca-P		Total P	
	Sludge	Control	Sludge	Control	Sludge	Control	Sludge	Control	Sludge	Control	Sludge	Control
Treatment 1												
T1 A	67.9 ^{c}	61.4 ^{bc}	366.7 ^{c}	291.8 ^{b}	532.5 ^{e}	412.7 ^{d}	372	399	155.5	145.0	1631	1746
T1 C	69.1 ^{c}	44.1 ^{a}	285.1 ^{b}	244.9 ^{ab}	360.4 ^{c}	273.8 ^{b}	309	292	127.4	118.8	1273	1470
T1 D	58.6 ^{abc}	48.6 ^{ab}	273.2 ^{ab}	202.4 ^{a}	263.7 ^{b}	203.7 ^{a}	384	507	130.5	141.5	1183	1684
Treatment 2												
T2 A	58.6	61.4	301	292	467	413	539	399	128.6	145.0	1749	1746
T2 C	59.8	45.6	437	253	897	498	483	302	168.9	128.0	3991	2022
T2 D	*	63.2	*	204	*	220	*	423	*	139.4	*	1952
Treatment 3												
T3 A	71.3 ^{b}	61.4 ^{ab}	270	292	478	413	428	399	161.8	145.0	1686	1746
T3 C	72.0 ^{b}	51.4 ^{a}	368	219	784	227	385	543	172.0	117.6	3356	1512
T3 D	54.4 ^{a}	47.2 ^{a}	305	212	624	242	568	378	178.4	134.3	3572	2012

NOTE: Treatment means with same letter (a, b, etc.) are not significantly different at $p<0.05$. Significant difference depends on the mean values as well as standard deviation and sample size. Thus, small numbers of samples in this research may have led to inconclusive statistical results.

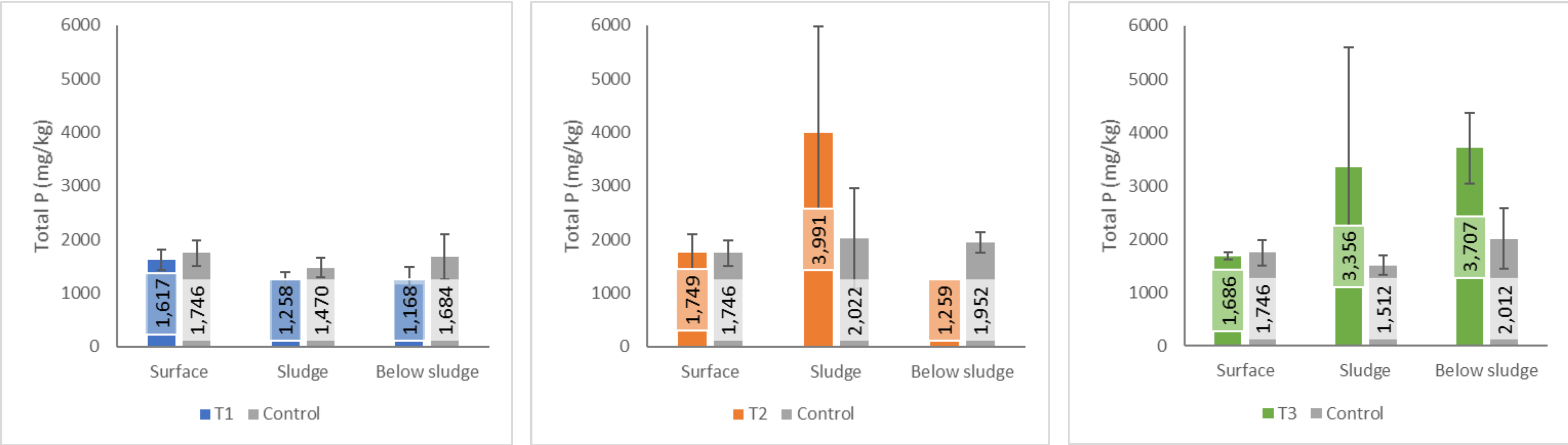

Figure 6-14: Average results of total phosphorus in soils in sludge and control plots

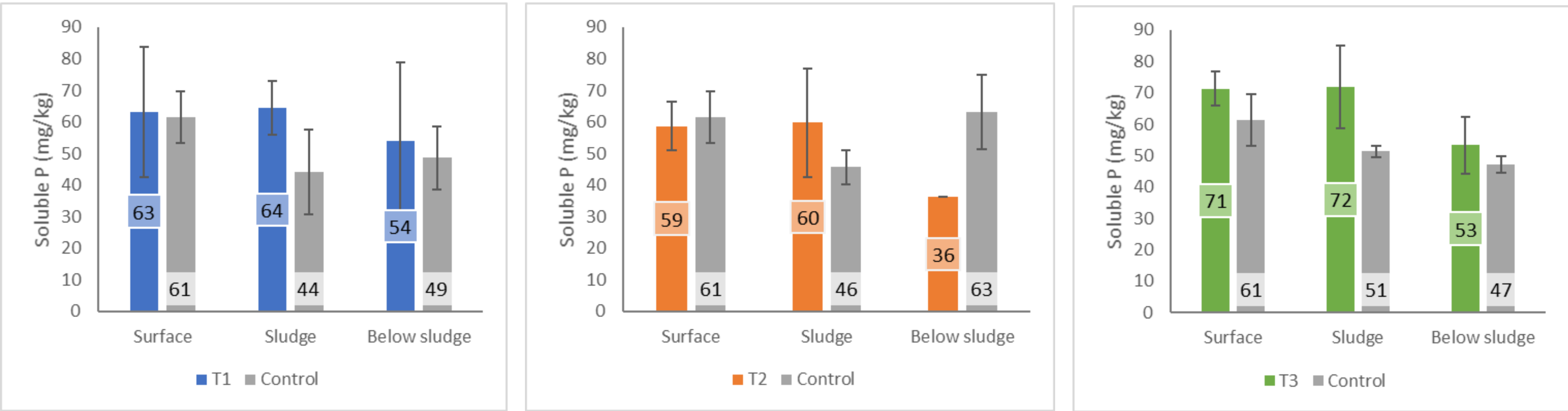

Figure 6-15: Average results of soluble phosphorus in soils in sludge and control plots

6.2.4 Macronutrients

The results for calcium, potassium, and magnesium are presented in Table 6-5, with visual representations in Figure 6-16, Figure 6-17, and Figure 6-18, respectively. Again, the largest differences between sludge samples and the controls can be seen in T3 samples. Calcium, magnesium, and potassium are all significantly higher in sludge samples (C) compared to the control samples taken at the same depths. The difference is not as clear in samples from T1 and T2, suggesting that the applied nutrients were used or depleted before sampling.

Table 6-5: Average results for calcium, magnesium, and potassium in T1, T2, T3 samples, compared to controls

Treatment	Calcium		Magnesium		Potassium	
	Sludge	Control	Sludge	Control	Sludge	Control
Treatment 1						
T1 A	43.4^{c}	23.8^{b}	116	48	43.4^{b}	23.8^{a}
T1 C	18.6^{ab}	15.6^{a}	25	35	18.6^{a}	15.6^{a}
T1 D	13.4^{a}	15.5^{a}	51	52	13.4^{a}	15.5^{a}
Treatment 2						
T2 A	49.0	23.8	103	48	49.0^{b}	23.8
T2 C	16.7	13.3	26	32	16.7^{a}	13.3
T2 D	23.1	13.3	48	69	23.1	13.3
Treatment 3						
T3 A	31.3^{bcd}	23.8^{cd}	33	48	31.3	23.8
T3 C	20.1^{d}	10.3^{a}	58	34	20.1	10.3
T3 D	19.9^{abcd}	15.0^{ab}	48	89	19.9	15.0

6.2.5 Micronutrients

The results for copper, manganese, and zinc are provided in Table 6-6, with visual representations in Figure 6-19, Figure 6-20, and Figure 6-21. Significant differences can be seen in both the sludge layers (C) and below the sludge layers (D) in T2 and T3. This suggests some migration of these nutrients downwards in the soil.

Table 6-6: Average results for calcium, magnesium, and potassium in T1, T2, T3 samples, compared to controls

Treatment	Copper		Manganese		Zinc	
	Sludge	Control	Sludge	Control	Sludge	Control
Treatment 1						
T1 A	1.94^{c}	1.40^{bc}	7.09^{c}	4.18^{b}	2.78	0.26
T1 C	1.34^{bc}	0.94^{ab}	1.97^{ab}	0.83^{a}	0.07	0.04
T1 D	0.85^{ab}	0.31^{a}	1.21^{ab}	0.76^{a}	0.00	0.19
Treatment 2						
T2 A	1.85	1.40	15.29	4.18	0.82	0.26
T2 C	7.69	0.95	2.35	0.82	6.07	0.00
T2 D	3.54	0.29	1.54	0.83	10.0	0.00
Treatment 3						
T3 A	1.70^{a}	1.40^{a}	6.34	4.18	0.68^{a}	0.26^{a}
T3 C	4.65^{c}	0.58^{a}	5.43	0.38	17.4^{b}	0.00^{a}
T3 D	2.36^{ab}	0.34^{a}	3.35	1.28	2.94^{a}	0.04^{a}

NOTE: Treatment means with same letter (a, b, etc.) are not significantly different at $p<0.05$. Significant difference depends on the mean values as well as standard deviation and sample size. Thus, small numbers of samples in this research may have led to inconclusive statistical results.

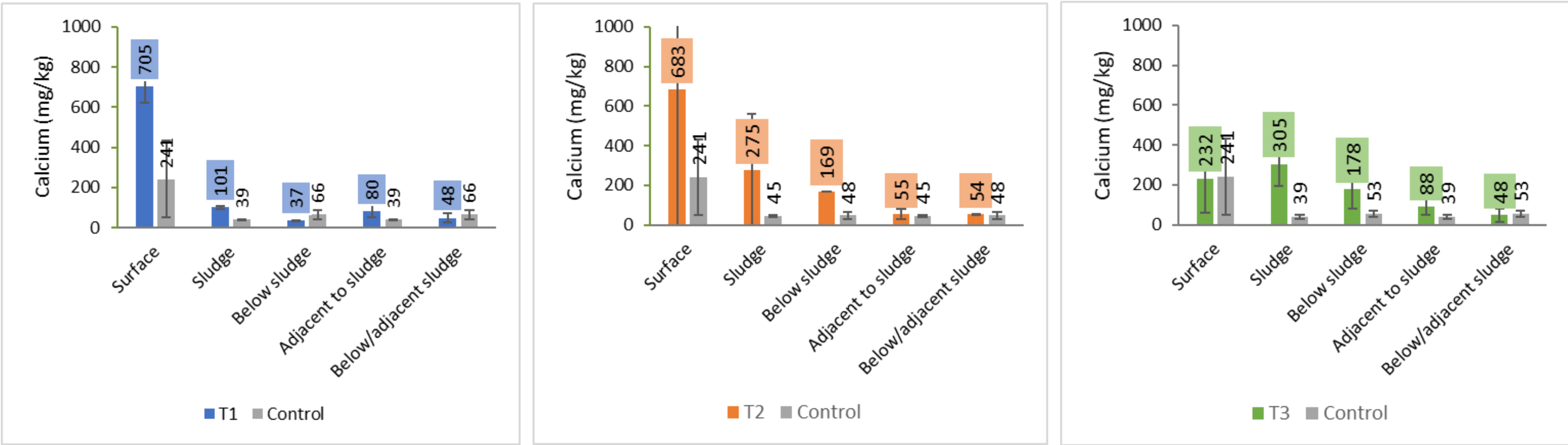

Figure 6-16: Average calcium (mg/kg) in soil layers in plots with T1, T2, and T3, respectively

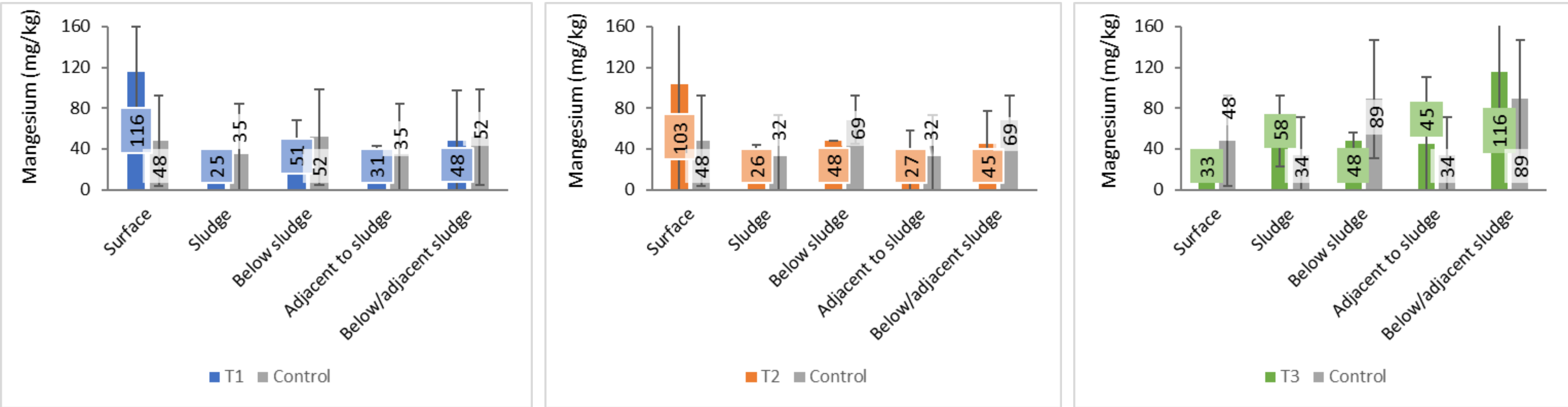

Figure 6-17: Average magnesium (mg/kg) in soil layers in plots with T1, T2, and T3, respectively

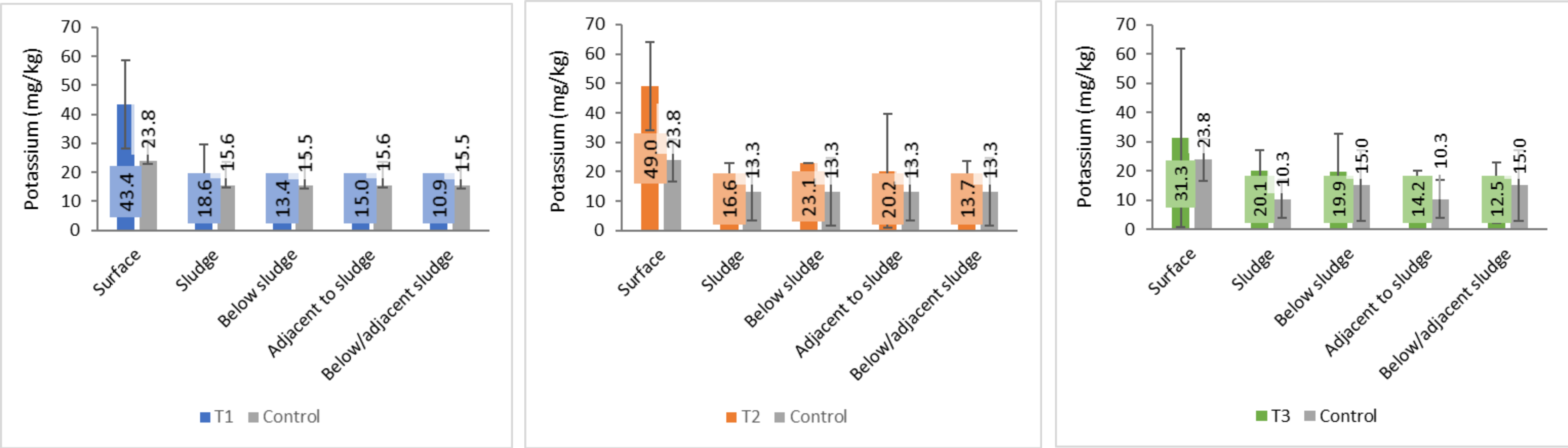

Figure 6-18: Average potassium (mg/kg) in soil layers in plots with T1, T2, and T3, respectively

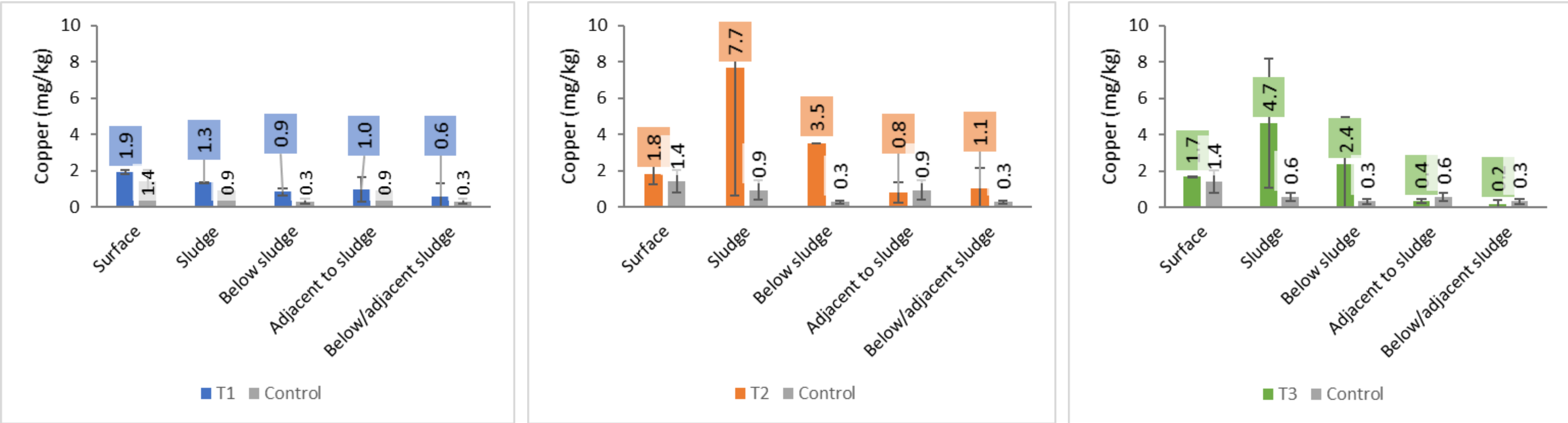

Figure 6-19: Average copper concentration in soils at different depths (mg/kg) for T1, T2, and T3 plots

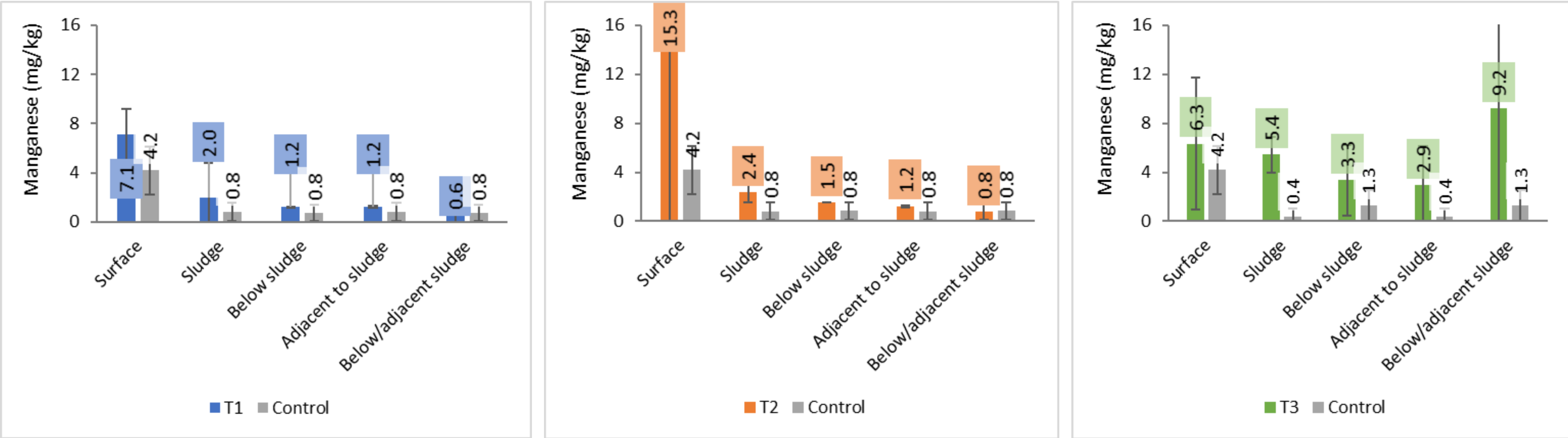

Figure 6-20: Average manganese concentration in soils at different depths (mg/kg) for T1, T2, and T3 plots

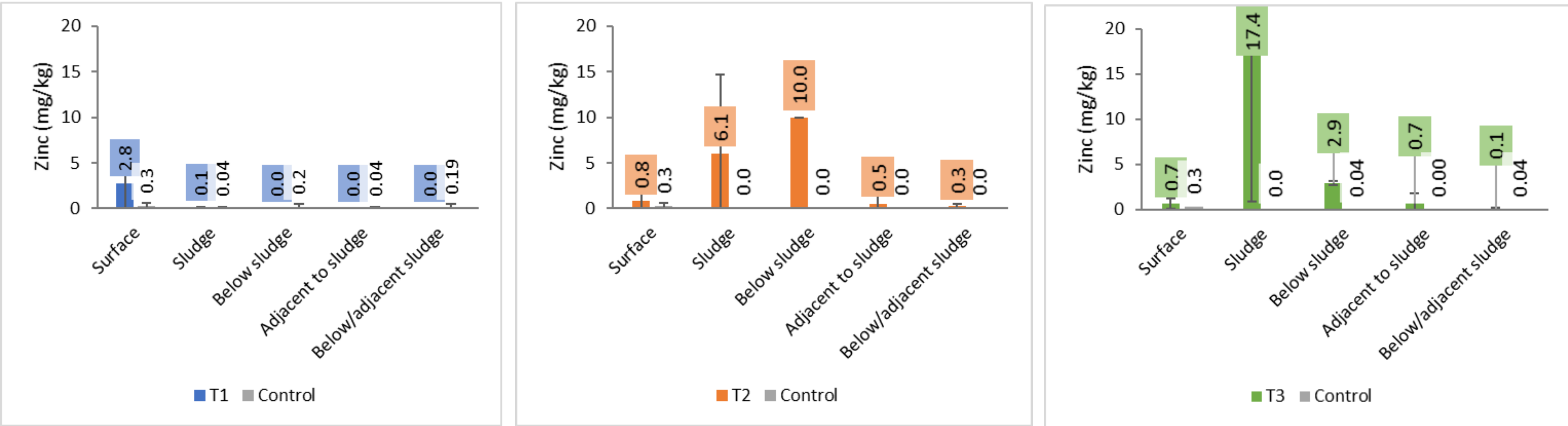

Figure 6-21: Average zinc concentration in soils at different depths (mg/kg) for T1, T2, and T3 plots

6.2.6 Water holding capacity

The average water holding capacity (%) for soil samples from different locations in T1, T2, and T3 plots is shown in Figure 6-22, with the control (T5) for comparison. The graphs suggest heightened water holding capacity within sludge, particularly when comparing the samples taken adjacent to the sludge (i.e. at the same depth but outside of the sludge trench) for T2 and T3 plots. Based on the t-test and a minimum p-value of 0.05, the difference between the sludge treatments and their controls is not significant. Thus, this study cannot confirm a significant impact of sludge on water holding capacity.

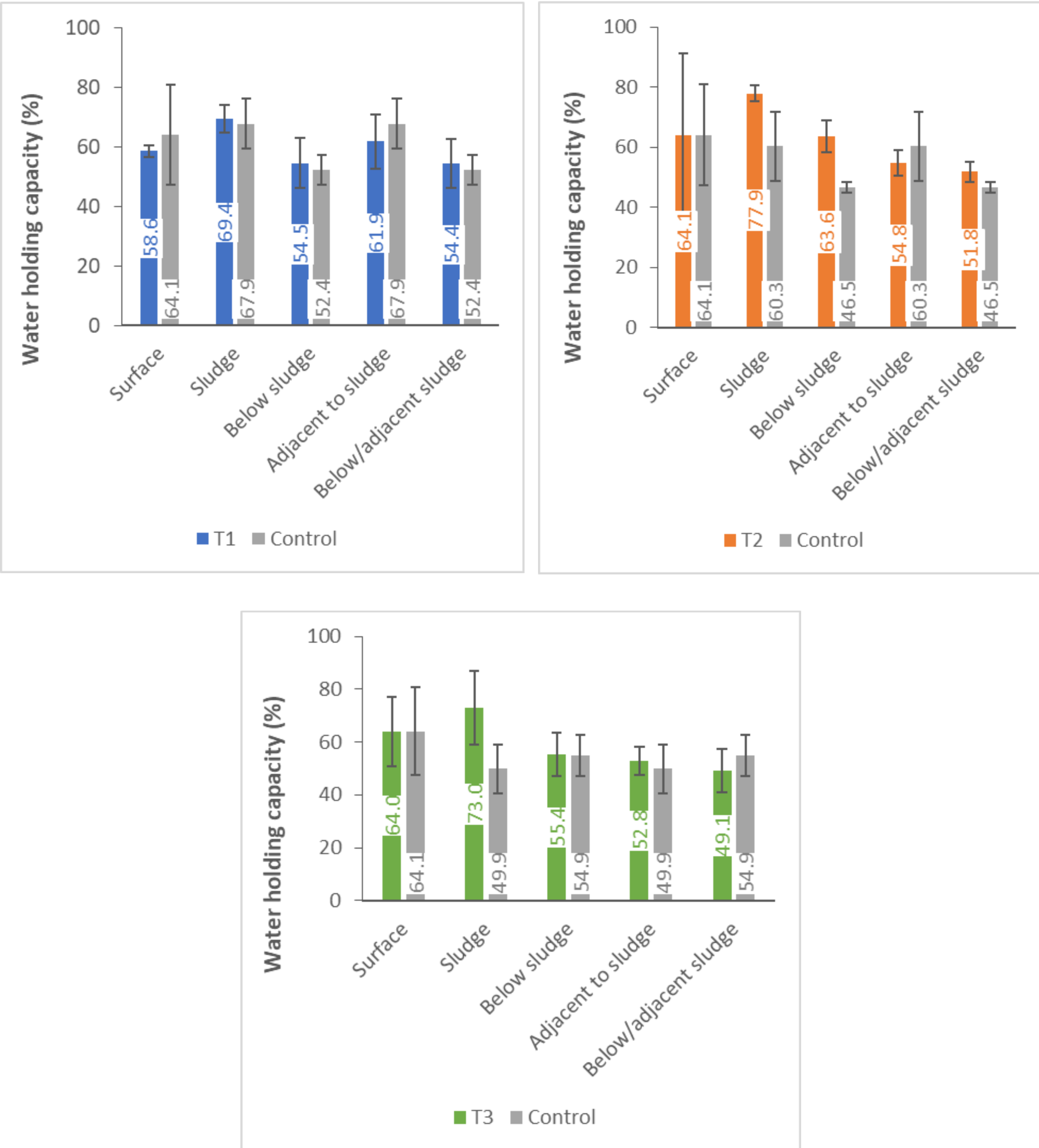

Figure 6-22: Average water holding capacity in soils at different depths (%) for T1, T2, and T3 plots

6.3 IMPACT OF BURIED SLUDGE ON THE ENVIRONMENT

This section presents results of sampling of the boreholes and the expert assessment of the tributary, both located downhill from the experimental plot.

6.3.1 Groundwater

The boreholes were sampled in September 2019, March 2020, and January 2022, demonstrating the impact of the buried sludge on groundwater quality in both the dry and wet seasons. The results for nitrate and orthophosphate in Borehole 1 (upstream) and Borehole 2 (downstream) are provided in Table 6-7.

Table 6-7: Results from borehole sampling from Borehole 1 (upstream) and Borehole 2 (downstream)

Date	Sample	Nitrate results (mg N/ℓ)		Orthophosphate results (mg P/ℓ)	
		Borehole 1	Borehole 2	Borehole 1	Borehole 2
Sep-19	Sample 1	<0.04	<0.04	0.04	0.2
	Sample 2	0.13	<0.04	<0.04	0.21
Mar-20	Sample 1	<0.04	0.59	<0.04	<0.04

The nitrate level was slightly elevated in Borehole 2 in March 2020, which could be due to leaching of nitrogen from the sludge trenches. However, even if the nitrate level is elevated due to the sludge entrenchment, the level of 0.59 mg/ℓ is lower than the General Authorisation from a WWTP of 15 mg/ℓ and the Special Authorisation (sensitive catchments) of 1.5 mg/ℓ, so it is not at a level that might cause concern. Also, it should be noted that the rate of groundwater flow is very slow. When the boreholes are sampled, they are first purged, to ensure that the samples are taken from groundwater seepage and not from water, which has been standing stagnant in the well for a long time. It is observed that after purging it takes a long time (typically an hour or more) for the wells to recharge enough for a sample to be taken.

The first set of samples in September 2019 did show a difference in the orthophosphate level between the upstream and downstream wells (<-0.04 mg/ℓ vs 0.2 mg/ℓ) but the results from March 2020 did not demonstrate the same difference. Even if the results from September 2019 are a result of the entrenched sludge, the level of 0.20 mg/ℓ is not so high to be of concern, given that the general authorisation limit for orthophosphate in WWTW effluent is 10 mg/ℓ.

Figure 6-23 and Figure 6-24 present all results of borehole sampling over the 10-year period following burial of sludge, spanning two growing seasons. Despite the large gap between January 2014 and September 2019, the results suggest that a slight spike in nitrate concentrations was observed in the downstream borehole just over 1 year after burial, but the concentration stabilised within four years after planting, decreasing to below the Special Authorisation Limit from DWS. The results from the recent study suggest that nitrate levels have remained low since then. It is important to note that there could have been a spike in nitrate levels that was not captured in this set of measurements, due to the five year break in sampling. A similar trend is observed in orthophosphate concentrations in the boreholes. In Borehole 2, downstream from the sludge entrenchment plot, the phosphate concentration never rose above the Special Authorisation Limit, likely due to the immobility of phosphorus in soil.

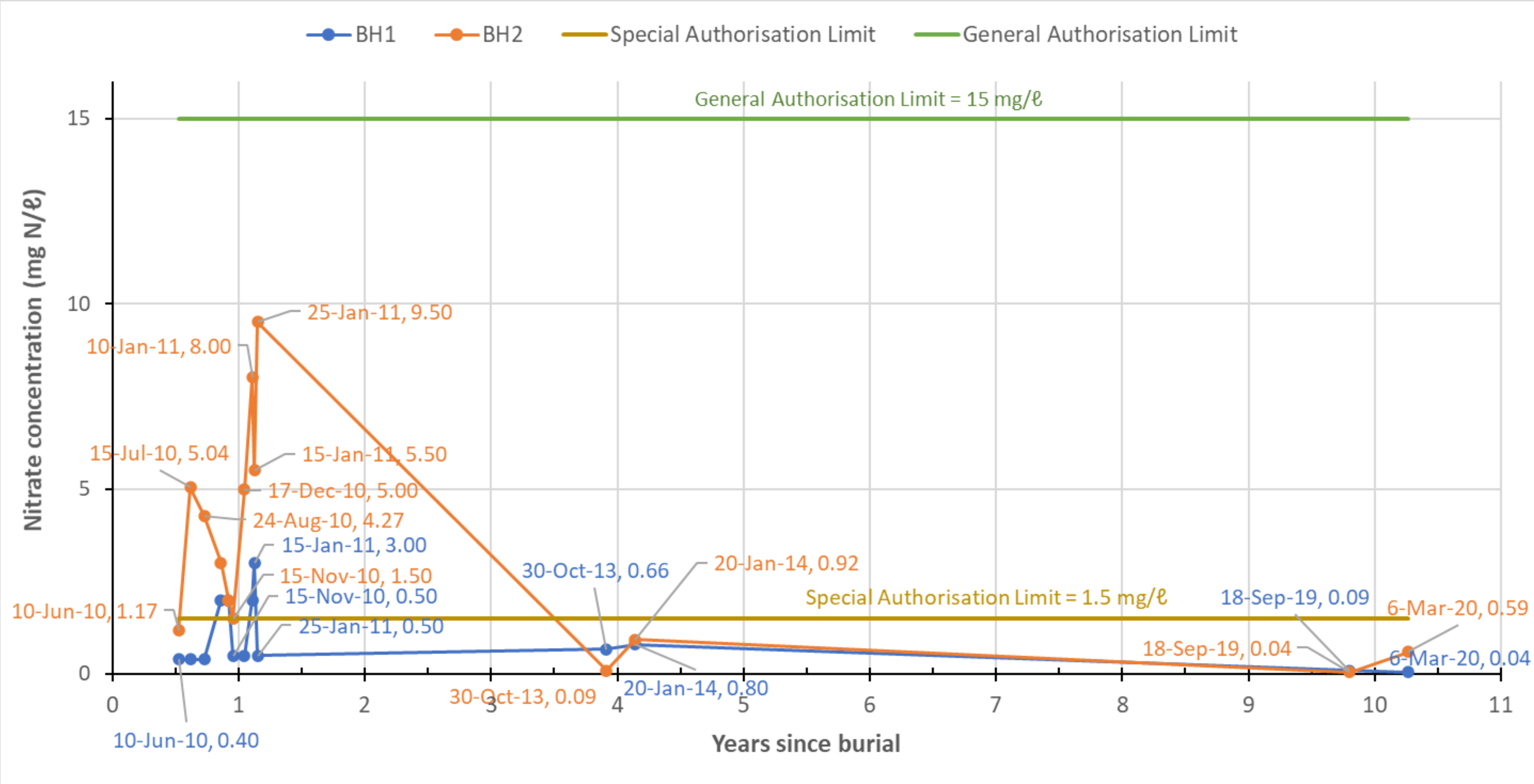

Figure 6-23: Nitrate concentration in boreholes downhill from sludge entrenchment site (Note: BH1 is upstream from the plot and BH2 is downstream from plot)

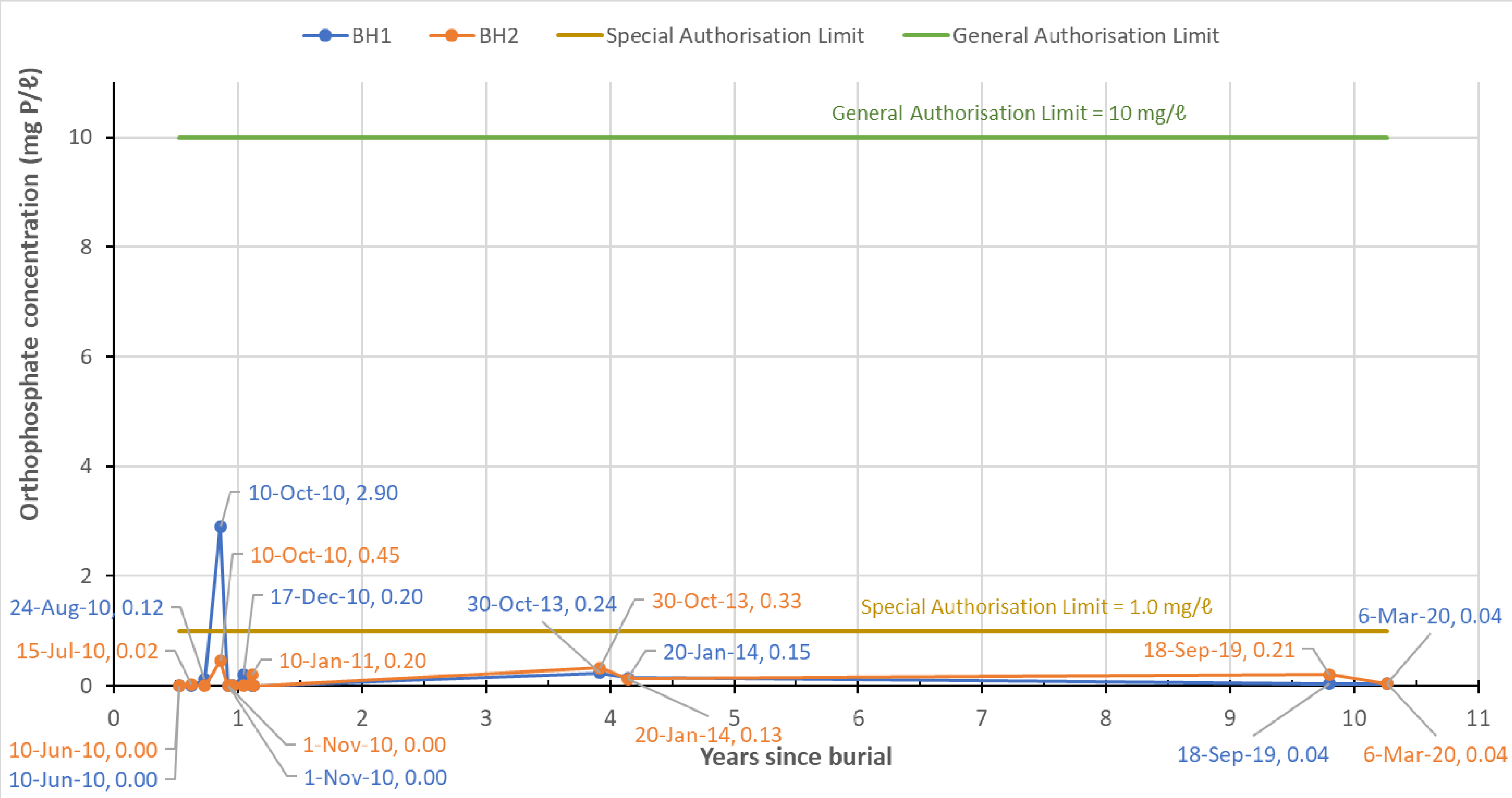

Figure 6-24: Orthophosphate concentration in boreholes downhill from sludge entrenchment site (Note: BH1 is upstream from the plot and BH2 is downstream from plot)

6.3.2 Stream aquatic health assessment

To provide a more detailed picture of the impact of the sludge entrenchment on the environment, a survey was carried out in May 2020 by GroundTruth on the stream directly downslope and adjacent to the experimental plot. The researchers collected samples from the stream both upstream and downstream from the site and conducted in-situ water quality assessments. Collected samples were analysed for nutrient content and other water quality parameters were analysed in-situ, including pH, dissolved oxygen, conductivity, and temperature. Furthermore, diatoms (which are useful as they integrate water quality differences over long periods) were assessed to determine the river health at the various points.

The full specialist report from GroundTruth is provided in Annexure A. Overall, the investigation revealed improvements in water quality moving downstream and near natural conditions at all points in the tributary. In conclusion, the report indicates, "the system is in near to natural condition with no signs of nutrient contamination." This study indicates that the entrenchment of the sludge in late 2009 (360 m^3 of sludge in a 2-hectare plot) has had no observable impact on the nearby stream, which does seem to be a significant finding, given the orthodox thinking that would hold that such an activity would cause contamination.

7 DISCUSSION

7.1 THE LONG-TERM IMPACTS OF BURIED SLUDGE ON TIMBER YIELD AND SOIL HEALTH

The results described above indicate a localised, long-term impact on timber yields and soil characteristics because of the burial of wastewater treatment sludge. This impact remains observable and significant over ten years since the sludge was initially buried. The timber growth data suggests an initial large increase in conical volume in all plots with buried sludge, as high as 79% in T3. This improvement in timber yield decreased steadily over the first few years of growth, reaching approximately 30% at 2.5 years after planting for all sludge treatments. This reflects the same pattern observed in the first growing season, suggesting that trees planted on sludge during this growing season will likely end up approximately 10% larger than those planted in plots without sludge, based on conical volume. This improvement in growth suggests that the sludge has had a positive impact on the trees' growth, particularly in the early years of growth. It is likely that the impact observed during the initial years of growth is because the sludge was relatively shallow. Thus, during the early years of growth, the roots interacted with the sludge. As the roots grew deeper, the sludge had less of an impact. However, the lasting impact as observed during the first growth season suggests that the initial boost in growth in the second growth cycle will likely have an overall impact on the yield.

The above discussion raises the question of what, specifically, led to the improvement in timber yield over both growing seasons. While nitrogen added to the soil may have had an impact during the first growing season, a few years into the first growing season nitrogen had stabilised to the level of the surrounding soil. Thus, it is assumed that other aspects impacted trees grown in the second season. Most notably, the soluble phosphorus measured in the buried sludge in T1, T2, and T3 was significantly higher than the levels in the control plots (T5). Though soluble phosphorus makes up the smallest portion of total phosphorus in the soil, the phosphorus in the buried sludge appears more available than phosphorus in the background soil. This may be due to the generally higher levels of organic carbon and phosphorus in the sludge. In fact, the higher level of organic carbon in the buried sludge has many benefits, and this has likely been the determining factor in the improvement in tree growth. Organic carbon from the buried sludge has been successfully stabilised and retained in the soil, and an increase in soil organic carbon improves soil structure, which leads to improvements in soil aeration, water drainage, and water retention, and reduces the risk of erosion and nutrient leaching (Corning et al., 2016). Increases in soil organic carbon also improve chemical composition and biological productivity of soils, as evidenced by the photos shown in Figure 6-10. Though the current study could not point to statistically significant improvements in water holding capacity (due to the small sample size), previous studies have shown that increases in soil organic carbon can lead to improved water retention, particularly in sandy and silty soils and those with high starting organic contents (Rawls, Pachepsky, Ritchie, Sobecki, & Bloodworth, 2003).

While much work promoting the use of faecal and sewage sludge as a soil conditioner has focused on recovery of nitrogen and phosphorus, it appears that the addition of carbon may be the primary benefit.

Burying sludge in the ground ensures that carbon is stabilised in the soil and not decomposed and released to the atmosphere. This has long-term implications for soil health and climate change. Furthermore, adding organic carbon to the soil *along with* the nutrients in sludge contributes to the availability of the nutrients for plant growth, which strengthens the case for application of organic-rich forms of treated human waste (or other organic waste products containing nutrients) in place of or *in addition to* concentrated nutrient solutions or products.

7.2 LIMITED IMPACTS ON THE ENVIRONMENT FROM BURIED SLUDGE

The results from this and the previous study point to limited impacts of buried sludge on the environment. Sludge buried down to 1050 mm below the surface did lead to heightened levels of nitrate in groundwater directly adjacent to the sludge trenches, but these levels stabilised within four years of sludge burial. This is consistent with work by Sikora et al. (1982), who found that nutrients in buried sludge stabilised within four years, as evidenced by no mineralisation of nitrogen after that point. Furthermore, very slight increases in orthophosphate and nitrogen in groundwater were observed in a borehole approximately 100 metres from the experimental plot, but these levels were still far below the Department of Water and Sanitation's special authorisation limit for critical catchments.

It is important to note that the results described above in terms of positive and negative impacts of DRE are site and project specific. The risk of leaching will be influenced by the soil type as well as the pollutant load in the sludge. For example, sludge from WWTWs with numerous industrial influent streams is expected to be higher in contaminants of concern (e.g. heavy metals), whereas sludge from WWTWs with only domestic inputs will not contain these metals. Furthermore, the long-term impacts are likely to be influenced by the crop that is planted on the sludge. In this case, Eucalyptus trees with a long growing cycle and a deep root system responded in one way, while a crop with a shorter growing cycle and shallower roots (e.g. sugarcane) is likely to respond differently (the impact on growth may in fact be more significant). Thus, while the results in this study point to some promising outcomes, there is still room for further study in different environments and applications.

7.3 OPPORTUNITIES FOR APPLICATION OF DEEP ROW ENTRENCHMENT OF SLUDGE

Part of this work involved evaluating the opportunities for application of DRE in South Africa. To do this, the project team interviewed various representatives representing different industries. A summary of findings from these interviews is provided in the following sections. The findings demonstrate that though a breadth of opportunities exist for deep row entrenchment of sludge, the lack of knowledge about and bias against DRE is a barrier to its uptake in different industries.

7.3.1 Commercial forestry

In the study described here, the model would be a public-private-partnership between a municipality and a private timber company. Though the results of the two growing seasons suggest potential increases in timber yields and no adverse effects on timber quality, representatives from the timber company have expressed doubt that they would ever consider sludge application or burial in their plantations.

A brief interview with a representative generated the following feedback: "From [our] perspective, the entrenchment was too expensive and applied too much nutrients to become a general practice. However, the idea was to test a disposal system for pit latrine waste that contains contaminants such as plastic… The risk of nutrient leaching into water systems is too great with this type of application. **[We] will never repeat this exercise – not even for trial purposes.** The smell was also bad at [the] time of application, which might be offensive to neighbours and health risk to workers is also a concern, as live human pathogens were still found long after application in the sludge[2]. As [our] timber is used to produce food additives, health, beauty and clothing products, and in food packaging we are very careful about any practice that may compromise human and environmental health whether perceived or real. Our land is multiple resource use and public assess for recreation is growing. Such practices may cause harm or discomfort to the public and can also affect public image and corporate reputation."

The primary concerns expressed above include:

1. Imbalance of nutrients in the sludge
2. Risk of leaching of nutrients to groundwater
3. Issues with the application of sludge being odorous and dangerous to human health
4. Health risks to workers and public accessing the land for recreation

Issue 1 above could be addressed by mixing the sludge with other nutrient sources to balance out the carbon-to-nitrogen ratio.

Issue 2 above has been partially debunked, at least on the site where the current study took place. While slightly elevated levels of nitrate (though not exceeding the General Authorisation Limit) were observed in the first 2-3 years after burial, by four years after burial, the nitrate levels in the groundwater dropped to levels below the Special Authorisation Limit of 1.5 mg/ℓ and remained there until March 2020, 12 years after burial. Other studies have found that leaching of nutrients and heavy metals to groundwater is localised, site-specific, and generally limited (Nyenje, 2014; L. J. Sikora et al., 1982). Thus, the blanket statement above on the perceived risk of nutrient leaching suggests that further work needs to be done to disseminate the findings of studies like this, along with the Department of Water and Sanitation's Groundwater Protocol (DWAF, 2003), which reflects a nuanced view of groundwater contamination risks from on-site sanitation systems. Many of the aspects discussed in that protocol can be applied to sludge burial.

Issue 3 above, regarding the sludge application procedure, is an element that requires practical consideration to ensure that burial operations are efficient and clean, reducing the risk of spillage wherever possible. This will require consideration of the methods used to deliver sludge to trenches as well as adequate training for workers dealing with the sludge.

Finally, issue 4 above regarding general concerns about health risks of buried sludge mainly centres on the fact that most post-plant tending activities (e.g. weed control) occur in the first two years after planting. While the previous study (Still et al., 2015) demonstrated that viable *Ascaris* ova were not found in buried sludge after 48 months, this suggests that disturbing buried sludge within the first two years could expose workers to pathogens. This risk can be mitigated through provision of personal protective equipment and ensuring that sludge is buried with sufficient cover (~300mm). The risk to the public can be mitigated simply by closing the plantation with buried sludge during the first four years after planting.

When asked what would be required to overcome the above concerns, the individual interviewed responded that treated or pelletised sludge might be considered a viable option for application in forestry. Pelletised

[2] Note this statement was not exactly correct, as previous work showed that pathogens were dead within four years after burial. However, due to the practices in these forests, a majority of plant tending activities (e.g. weeding) occur within the first two years after planting; thus, pathogens in the buried sludge are of concern.

sludge will reduce the odour and health issues described above. He went further to suggest that opportunities should be investigated for mixing sludge (source of N) with ash (source of P, K, and other nutrients) to create a balanced fertiliser. He expressed interest and willingness to try different approaches with treated sludge, stating, “[We] will provide technical support for safer and cleaner application, particularly if used for production of energy crops on low value land.” Truter et al. (2001) have done work in South Africa on something like what is suggested, which includes a combination of sludge, fly ash, and lime (“SLASH”). There is potential for partnerships between the research group and the timber industry to investigate the application of SLASH to enhance soil fertility, improve timber yields, and provide a reuse opportunity for both sludge and fly ash.

7.3.2 The need to investigate alternatives

The feedback above, from private timber personnel, points to the need to investigate other application contexts for deep row entrenchment. Some alternatives that require further investigation and research include:

1. Plantations dedicated to producing timber for fuel or building material
2. Surface mine rehabilitation
3. Other crops, such as sugarcane

The current study focused on burial of sludge in timber plantations, and therefore investigations into the above opportunities could look at the impact of sludge on different crops and in different environments, differences in environmental impacts, and the overall opportunities available in South Africa. While the current study has pointed to some potential positive impacts from burial of sludge, there is a need to understand the views of people in other industries, outside of the sanitation and private timber industries, to better understand the opportunities and gaps in advocacy.

8 CONCLUSIONS

The key findings and conclusions from the study detailed above are as follows:

1 Buried sludge has long-term impacts (i.e. over 10 years/2 growing seasons) on timber yields and soil health.

2 This impact is likely partly due to nutrients added to the soil in the sludge, but even more so due to the added organic carbon. This makes nutrients more available by improving microbial activity, and it can also improve water holding capacity of soil. This is an under-valued positive contribution of sludge burial and sludge application in general.

3 Sludge burial in this environment (~40-50% clay soil) has led to very limited impacts on the environment. Within three years, buried sludge was stabilised (e.g. nutrients immobilised and pathogens inactivated).

9 UPDATED DEEP ROW ENTRENCHMENT GUIDELINES

The Guidelines produced in the previous project have been updated and a simple tool created to reflect the findings of the current study. The following key points from the current research have been incorporated into the guidelines:

1. Deep row entrenchment of sludge leads to a long-term impact, in terms of both crop yields and overall soil health. The current study demonstrated this impact over two growing cycles. Data from the current study shows improvements in timber yields in the early years of growth, likely due to the enhancement of nutrients. It is assumed that like the first growing season, the relative impact will diminish over the second half of the growing season but will still result in an overall improvement in timber yield. Furthermore, the soil has been positively impacted in the long-term, in terms of organic matter.

2. While much hesitancy revolves around potential negative environmental impacts of sludge burial, the research to date suggests that this is limited and extremely site dependent. While the mobilisation of various pollutants depends both on the quality of sludge and characteristics of soil, the overall risk of mobilisation is limited. If the groundwater table is not near the surface, burial of sludge is likely to result in significantly less pollutant migration when compared to surface application, which is a relatively widely accepted and practiced solution for wastewater sludges.
3. Once-off application of sludge, buried in trenches, allows for greater volumes of sludge to be applied when compared to surface application.
4. Despite the limited impact of buried sludge on final timber quality, private timber companies are unlikely to willingly allow burial of sludge in their plantations due to concerns regarding pathogens in the sludge. This points to the need for effective communication campaigns based on evidence from this and other studies. Furthermore, other opportunities must be investigated. For example, municipal forestry lands can be investigated as potential recipients of sewage sludge. Overall, the key considerations are:
    a. Distance from treatment plant to disposal site
    b. Available land at the disposal site
    c. Cost of transport of sludge, digging of trenches, and burial of sludge
    d. Costs (financial, social, and environmental) of status quo
5. The final guideline includes practical steps for planning deep row entrenchment. This includes a guide on classifying sludge and preparing a cost benefit analysis. This tool is provided as a MS Excel spreadsheet that guides users to assess their options and conduct a high-level cost comparison.

In order for these guidelines to be mainstreamed, they should be included in other industry-wide guidelines (e.g. *The Faecal Sludge Management Guidelines*, currently under development by the WRC, and *The Guidelines for Utilisation and Disposal of Wastewater Sludge* (2009)). Dissemination efforts should focus on municipalities (sludge producers) and guide them through using the tool to determine where opportunities exist for deep row entrenchment as a low-cost option for disposal of faecal sludge and WWTW sludge.

Furthermore, the information should be disseminated to potential sludge users (e.g. timber industry, sugar industry, mining companies, farmers, households). Without interest and acceptance from potential sludge users, sludge producers will be unable to use this method.

10 RECOMMENDATIONS FOR FURTHER STUDY

While this study has provided a long-term view of sludge entrenchment, the findings are somewhat limited in their application, as the behaviour of buried sludge is assumed to be dependent on many factors. Thus, there are opportunities for further study to provide more insight into applications with different soils or different crops, as well as for further investigating the opportunities for this practice in South Africa. Some of these are listed below:

1. Smaller-scale studies to investigate how soil characteristics, crop type, and time influence the impact of buried sludge in terms of crop yields and pollutant migration. Ideally, this should lead to an annexure for the guideline that describes the applicability of the practice based on different conditions (i.e. what is the minimum clay content required for safe DRE?).
2. Further soil sampling can take place at the existing experimental plot. There is a unique opportunity to gain a long-term understanding of the fate of buried sludge within the soil. The main aspect of interest will be the fate of carbon in the soil. Is carbon indefinitely stabilised in the soil, leading to a permanent improvement in soil fertility?
3. Detailed cost-benefit-analysis of various sludge disposal options, with particular focus on municipal wastewater treatment sludge. This analysis should include the main practical approaches used by municipalities now, e.g. landfill disposal, surface application, deep row entrenchment, composting, and pelletisation. While the current study and guideline tool provide high-level comparisons between

landfill disposal, surface application, and DRE, more detailed analysis and costing are required. An effective model might also aim to determine the points at which different options become advantageous (i.e. at what distance from the treatment works is DRE infeasible compared to alternatives?).

4. Investigation into the opportunities for DRE of sludge as a method for rehabilitation of surface mines. There is considerable opportunity for use of sludge in the rehabilitation of mines, particularly in terms of adding organic matter to the soil. The characteristics of surface mine land should be investigated, and the risks and benefits of incorporating sludge into the soil during rehabilitation should be identified.

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ANNEXURE A: DETAILED LABORATORY ANALYSIS METHODS

A.1 SOIL PH AND EXCHANGEABLE ACIDITY

Soil (5 g) was placed into a 100 ml centrifuge tube, and 50 ml of 1M KCl solution was added. The solution was then shaken 180 cycles per minute for four minutes on the reciprocator shaker and centrifuged for two minutes at a speed of 3000 rpm to allow the soil particles to settle. The pH was then determined using a pH electrode positioned in the supernatant.

The supernatant liquid was filtered into 100 ml storage bottles using a Whatman No. 1 filter paper to analyse exchangeable acidity. The filtrate (25 ml) was pipetted and then transferred into a 100 ml conical flask. Afterwards, six drops of the phenolphthalein indicator were added, and titration was done through to the endpoint of (pink colour) using 0.01 M NaOH. Blank titration was done using 25 ml 1 M KCl solution following the same procedure (Lourenzi et al., 2011).

A.2 EXTRACTABLE PHOSPHORUS, EXCHANGEABLE POTASSIUM, COPPER, MANGANESE, AND ZINC

Extractable phosphorus was determined using the Ambic-2 extractant as described by Manson and Roberts (2000). The Ambic-2 extracting solution consists of 0.25M NH4CO3 + 0.01M Na2EDTA + 0.01M NH4F + 0.05 g L-1 Superfloc (N100), adjusted to pH 8 with a concentrated ammonia solution. Soil (2.5 g) was weighed into a 100 cm3 centrifuge tube, and 25 ml of AMBIC-2 solution was added, and the suspension was shaken at 180 cycles per minute for 30 minutes using a reciprocal shaker. After shaking, the extract was filtered into a correctly labelled glass bottle. For phosphate determination, 2.0 ml aliquots of extract were placed in a 50 ml with the addition of 8 ml of distilled water to make the total volume of 10 ml. Murphy and Riley (1962) molybdenum blue colour reagent (10 ml) was added slowly while shaking the solution to allow mixing. The flask was then placed for 45 minutes without disturbance then the absorbance of P was analysed using a spectrophotometer placed at 670 nm.

A 5 ml aliquot of the filtrate diluted with 20 ml of distilled water was analysed using atomic absorption spectrophotometry to determine the potassium concentration in the soil. Zinc, copper, and manganese were determined by atomic absorption spectrophotometry on the remaining undiluted filtrate.

A.3 SOIL CARBON FRACTIONATION

A.3.1 Organic carbon

To determine the organic carbon fraction in the soil, 10 g of soil were dispersed in a 100 ml centrifuge tube, followed by the addition of 30 ml (5 g L^{-1}) of sodium hexametaphosphate. The solution was shaken for 15 hours in a reciprocal shaker. After shaking, the dispersed soil samples were passed through a 53 mm sieve and washed several times with distilled water. The soil material remaining in the sieve was transferred to a glass beaker and dried overnight at 60 °C. The oven-dried soils were crushed with a mortar and pestle and put in small plastic bags to determine particulate organic carbon.

The ground soil was passed through a 0.5 mm sieve, and 0.5 g of soils from different sampling points were transferred into a 500 ml Erlenmeyer flask. Potassium dichromate solution (10 ml) was added into the Erlenmeyer flask containing the soil sample and mixed by swirling the flask. After, 20 ml of sulphuric acid (H_2SO_4) was carefully added to the solution and mixed gently for 1 minute. This mixture was allowed to stand for 20 minutes. After 20 minutes, the mixture was diluted with 170 ml of distilled water followed by the addition of 10 ml of phosphoric acid [H_3PO_4 (85%)], 0.2 g of sodium fluoride (NaF) and five drops of ferroin indicator.

The total content was mixed by swirling, and it was titrated against ferrous ammonium sulphate (FAS) solution. At the start, the colour was goldish brown; with the addition of FAS, the solution turned to brighter green and then to a dark green-blue. With the additional FAS drop wisely, the final volume of FAS used was noted when the solution darkened and turned to dark brownish-black colour.

A.3.2 Total carbon

To determine total carbon in the soil, the air-dried soil sample was ground using a mortar and pestle (porcelain) to pass a 0.5 mm sieve, and 0.5 g of soil was transferred to a 500 ml Erlenmeyer flask. Next, 10 ml of the potassium dichromate solution and 20 ml of concentrated H_2SO_4 were added and mixed gently for 1 minute by slowly rotating the flask. The mixture was allowed to stand for 20 minutes. After 20 minutes, the total carbon was determined using the carbon determination method mentioned above.

Mineral associated carbon

Mineral associated carbon was calculated as the difference between the total organic carbon and the particulate organic carbon. The percentage of organic carbon was calculated using the Walkley-Black equation as follows:

$$\% \textbf{ of organic C in soil} = \mathbf{x} \times \frac{\mathbf{12}}{\mathbf{4}} \times \mathbf{1.33} \times \frac{\mathbf{100}}{\textbf{mass of soil (mg)}} \qquad \textbf{Eq. 1}$$

A.4 SOIL PHOSPHORUS (P) FRACTIONATION

The soil P fractionation was done using the procedure described by Zhang (2009) for non-calcareous soil, and it is schematically presented in Figure below.

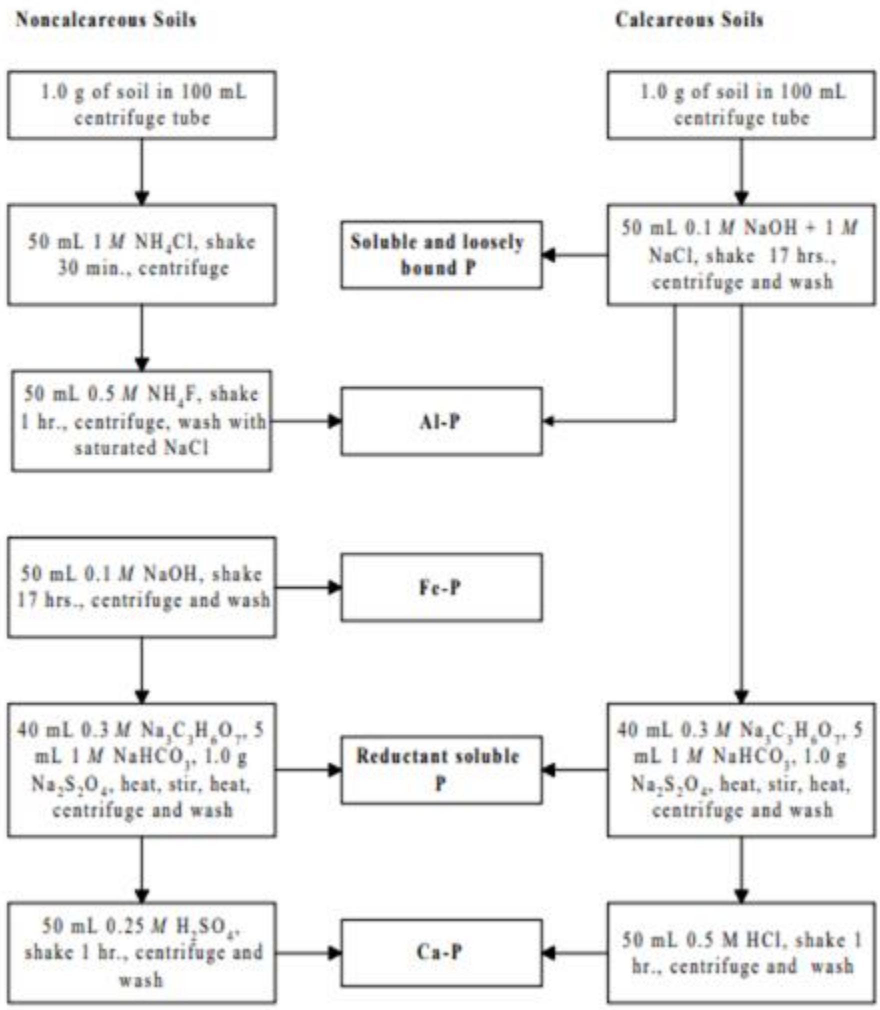

Figure A-1: Sequential method to determine inorganic P fractions in the soil (Zhang, 2009)

A.4.1 Soluble and loosely bound P

For soluble P, the soil samples were allowed to pass through a 2 mm sieve, and 1 g of soil was added to a 100 ml centrifuge tube, following the addition of 50 ml of 1 M ammonium chloride (NH_4Cl). The suspension was shaken for 30 minutes, centrifuged at the speed of (4000 rpm) and filtered using Whatman no. 1 filter papers into a 50 ml volumetric flask.

Soluble and loosely bound P was analysed by transferring 2 ml of the aliquot to a 50 ml volumetric flask, then adding 8 ml distilled water and 10 ml of Murphey and Riley (1962) colour reagent. For the colour to develop, the samples were allowed to stand for 45 minutes after they were analysed for absorbance with a spectrophotometer at a wavelength of 670 nm.

A.4.2 Aluminium (Al) bound P

From the residues of soluble P, 50 ml of 0.5 M ammonium fluoride (NH_4F adjusted to pH 8.2) was added. The suspension was shaken for one hour, centrifuged (4000 rpm) and filtered into a 100 ml volumetric flask to determine Al-bound P. After the filtration, the soil was washed twice with 25 ml of Sodium chloride (NaCl), centrifuged, and combined the liquid with Al-bound P extract.

Aluminium bound P was analysed by transferring 2 ml of the aliquot in a 50 ml volumetric flask, followed by the addition of 15 ml of 0.8 M boric acid (H_3BO_3) and 10 ml of Murphey and Riley (1962) colour reagent. After 45 minutes, when the colour was developed, the samples were analysed with the spectrophotometer.

A.4.3 Iron (Fe) bound P

The Fe-bound P was extracted using 50 ml of 0.1 M Sodium hydroxide (NaOH). The suspension was shaken for 17 hours, followed by centrifugation (4000 rpm) and filtration with Whatman no. 1 filter papers. The soil residue was washed twice with 25 ml of NaCl solution.

Iron (Fe) bound P was analysed by transferring 2 ml of the aliquot to a 50 ml volumetric flask, followed by 5 ml of distilled water and five drops of p-Nitrophenol indicator. To balance the pH of samples, 2 M of hydrochloric acid (HCl) was added drop wisely until the colour changed from yellow to colourless. Once the pH was balanced, 10 ml of colour reagent was added slowly whilst shaking the solution to allow mixing. The flask was then placed for 45 minutes without disturbance then the absorbance of P was analysed using a spectrophotometer placed at 670 nm.

A.4.4 Reductant soluble P

After washing, the reductant soluble P was extracted using 40 ml of 0.3 M Sodium citrate ($Na_3C_6H_5O_7$) solution and 5 ml of Sodium bicarbonate ($NaHCO_3$) solution to the residues of Fe-bound P extract. The suspension was heated using a water bath (85 ℃) for 15 minutes, followed by the addition of 1 g sodium dithionate ($Na_2S_2O_4$), centrifugation and filtration to give off Citrate bithionate dicarbonate (CBD) extractable P. The residues left from reductant soluble P was washed twice with 25 ml portions of NaCl solution, and the liquid was combined with CBD extractable P. This solution was exposed to air to oxidise Sodium dithionite ($Na_2S_2O_4$).

For reductant soluble P determination, 2.0 ml aliquots of extract were placed in a 50 ml volumetric flask with the addition of 8 ml of distilled water. Colour reagent (10 ml) was added slowly whilst shaking the solution to allow for mixing. The flask was then placed for 45 minutes without disturbance then the absorbance of P was analysed using a spectrophotometer placed at 670 nm.

A.4.5 Calcium (Ca) bound P

The Ca-bound P was extracted by adding 50 ml of 0.25 M Sulfuric acid (H_2SO_4) to the residues of CBD extractable P. The suspension was shaken for one hour, centrifuged and filtered into a 100 ml volumetric flask. The soil residue was washed twice with NaCl, and the liquid was combined with Ca-bound P extract.
Calcium bound P was determined by transferring 2 ml of the aliquot to a 50 ml volumetric flask with the addition of 8 ml distilled water and five drops of p-Nitrophenol indicator. To balance the pH of samples, 2 M of sodium hydroxide (NaOH) was added drop wisely until the colour changed from colourless to yellow. Once the pH was balanced, 10 ml of colour reagent was added slowly whilst shaking the solution to allow mixing. The flask was then placed for 45 minutes after the absorbance of P was analysed using a spectrophotometer placed at the wavelength of 670 nm.

The amount of P in each fraction was calculated using the following equation:

$$\mathbf{P\ (mg\ Kg^{-1}) = P\ conc.(mg\ L^{-1}) \times \frac{volume\ of\ extractant}{mass\ of\ soil}} \qquad \textbf{Eq. 2}$$

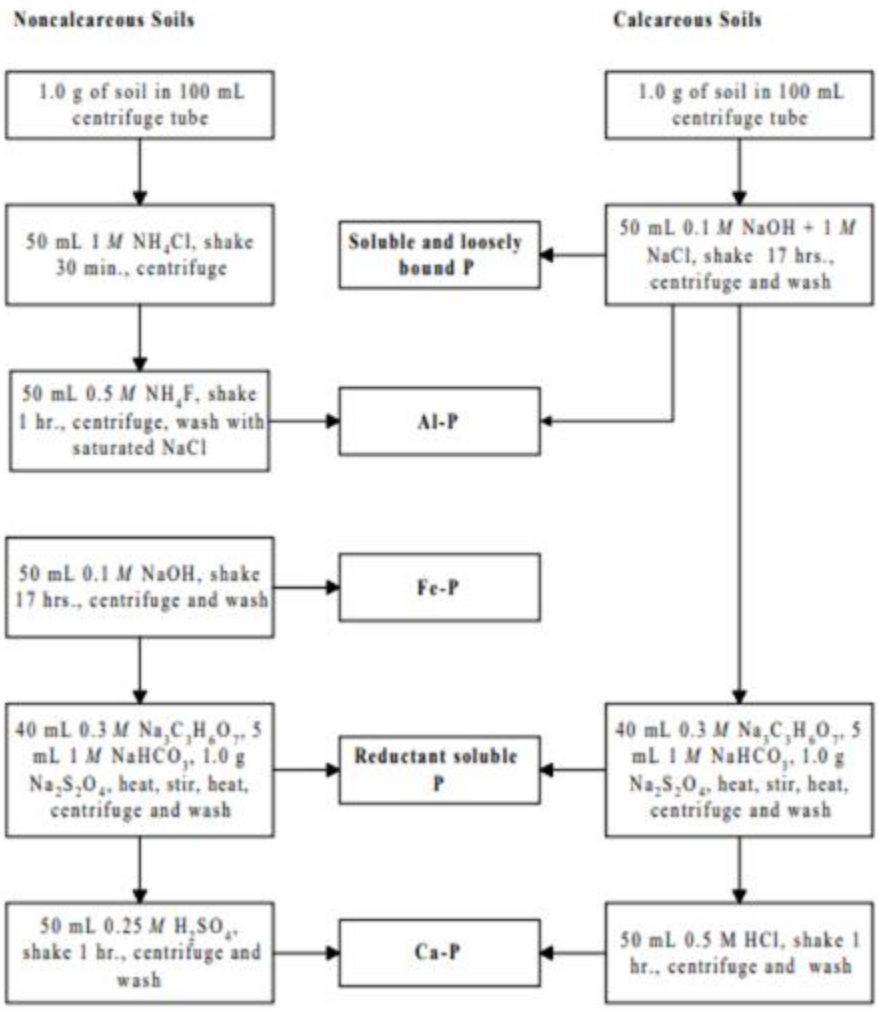

Figure A-2: Sequential method to determine inorganic P fractions in the soil (Zhang, 2009)

A.4.6 Total P

The total P in soil was analysed using an acid digestion procedure. The acids that were used were hydrochloric acid (HCl) and nitric acid (HNO_3). To determine total P in the soil, 0.25 g of finely ground soil was transferred into a 100 ml conical flask followed by adding 9 ml of concentrated HNO_3 and 3 ml of concentrated HCl under a fume hood. The conical flasks were placed into a hot sand bath (210 ℃) for around 45 to 60 minutes for better digestion. After digestion, the samples were cooled at room temperature, and the digest was transferred into a 100 ml volumetric flask using a Whatman no. 1 filter paper.

To analyse for total P, 2 ml of the aliquot was transferred into a 50 ml volumetric flask followed by the addition of 5 ml distilled water. Since the total P in the soil was extracted using acid, the pH was balanced by adding five drops of p-Nitrophenol indicator followed by adding 5 M NaOH drop wisely. Once the pH was balanced, the solution changed the colour from being colourless to yellow. When the pH was balanced, 10 ml of ammonium paramolybdate-vanadate reagent was added and bring the volume to the 50 ml mark with distilled water. The colour developed after 10 minutes. Once the colour was developed, the samples were analysed in spectrophotometry at a wavelength of 880 nm.

Total P was calculated using the following equation:

$$\mathbf{Total\ P(mg\ Kg^{-1})} \qquad \textbf{Eq. 3}$$

$$= \mathbf{P\ conc.\ in\ initial\ 100\ mL\ dilution\ (mg\ L^{-1})} * \frac{\mathbf{50}}{\mathbf{2}} * \frac{\mathbf{volume\ of\ extractant\ (mL)}}{\mathbf{mass\ of\ soil\ (g)}}$$

Equation 1 Total Phosphorus (mg Kg) in the soil

A.5 WATER HOLDING CAPACITY

Soil samples were air-dried and sieved with a 2-mm sieve. Thirty grams of soil were then placed in a funnel lined with filter paper. The funnel was placed in a graduated cylinder. Thirty mℓ of water was poured into the funnel, placing the soil samples in saturation. The soil in the funnel was allowed to drain for 48 hours. After 48 hours, three samples were taken out of the soil in the funnel using a 5 mℓ measuring spoon and weighed. The soil was then oven-dried at 105°C for 2 hours. The oven-dried samples were then weighed.

The water holding capacity was determined as follows:

$$Water\ holding\ capacity = \frac{Mass\ of\ wet\ soil - Mass\ of\ dry\ soil}{Mass\ of\ dry\ soil} \quad (5)$$