

# Soil Microbial Enzymatic Activity in 20-year-old rehabilitated platinum tailings substrates in South Africa

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## Abstract

*Soil microbial health has become a key rehabilitation requirement to achieve more sustainable ecosystems on reconstructed mined landscapes. The status of soil microbial activity in rehabilitated tailings substrates are not well understood since the rehabilitation performance appraisal methodologies mainly focus on soil nutrition and revegetation success. Furthermore, rehabilitation amelioration specifications mainly consider soil physical and chemical improvements and the achievement of floristic biodiversity, rather incorporating microbial success criteria and therefore a complete lack of information exist about the progression of microbial activity and its role in pedogenesis in restored tailings materials. Rehabilitation programs usually aims at indirect improvement of soil organic status through soil nutrient amendment and rapid root mass development without sustainable soil rhizome reconstruction, final closure attempts will be compromised.*

*This study presents Soil Microbial Enzymatic Activity (SMEA) progress on a rehabilitation trial conducted on platinum tailings 20 years ago (January 2003)- which has not received any maintenance since - and the microbial status were evaluated in 2009, 20013 and 2023 since. The 8 treatments included amelioration with different organic amendments, organic covers, as well as rock armouring and organic fertilizers, which is aligned with general tailings rehabilitation methodologies. An untreated control sample and the reference activity for natural surrounding area was also considered. Composite samples of the 9 treatments of the platinum tailings were analysed by means of the Soil Dehydrogenase Activity methodology. Microbial enzymatic status is analysed for by determining the concentration of Iodonitrotetrazolium Violet-formazan (INF), which is a reaction product of soil enzyme activity. It was clear that after 20 years, the soil microbial status is still in a distressed state. Although soil enzyme activity on average increased from 0 INF  $\mu\text{g g}^{-1} \text{h}^{-1}$  to 15INF  $\mu\text{g g}^{-1} \text{h}^{-1}$  on average per treatment, it still lacks significantly behind the activity within natural reference area of >45 INF  $\mu\text{g g}^{-1} \text{h}^{-1}$ .*

*It was evident that the originally treated sewage and grass mulch covered treatments presented the most improved microbial status over time. Values of 28 INF  $\mu\text{g/g/h}$  were achieved which may be explained by higher original root mass and biomass development in the first years after the inception of the project. Considering the average 5-year enzymatic activity improvement trajectory, another 20 years would be required for the system to achieve naturally comparable microbial activity rates.*

*The lack of a restored system to produce required levels of soil organic matter is an inhibiting factor for soil ecosystem development which not only delay sustainability of rhizosphere but starve the above-ground vegetation layer. Therefore, initial enablement of root biomass through increased rates of organic matter, and annual additions through dedicated maintenance may be achieve sustainable ecosystems on platinum tailings materials.*

*Keywords: platinum tailings, microbial activity, dehydrogenase analysis, soil health, tailings rehabilitation.*

## 1 Introduction

The scale of poorly rehabilitated mining land is a global challenge, and of particular concern in Sub Saharan African countries due to extreme climatic conditions, poor substrate quality and the lack of understanding of final ecological rehabilitation requirements (Odile et al 2019). The naturalisation of vast amounts of tailings is regarded as one of the most urgent priorities for humankind (Xie and van Zyl, 2020) which will require substantially improved growth media to sustain regenerated “minescapes”.

From a land rehabilitation scale and priority perspective, it is of major concern that the PGM industry is only in its infancy in South Africa and is on the verge of rapid upscaling which will produce substantially more tailings landscapes in the years to come. According to *PricewaterhouseCoopers* (2010) PGM mining will become the largest mining revenue-generating commodity in Southern Africa and Mbendi (2004) states South Africa holds 55% of global reserves, producing 80% of the world's PGMs. More than 70% of the mines are concentrated in two small areas of the North-West and Limpopo Provinces.

The extensive negative effect of Tailings Storage Facilities (TSFs) on the environment is also well documented (Winde and Sandham, 2004; van der Walt et al. 2012). Although the long-term geotechnical integrity of TSFs are often the main focus of tailings design, the significant effect of wind and water on tailings surfaces are the main drivers of environmental impacts (Van Wyk, 2009). From a soil perspective, the tailings produced by Platinum Group Minerals (PGM, ruthenium, rhodium, palladium, osmium, iridium, and platinum) processing are alkaline, biologically sterile and have low water-holding capacity with a moderate to high sodicity and some heavy metal content in addition to high seepage rates (Van Rensburg and Morgenthal, 2004). The main concerns around environmental impacts from PGM TSF's are tailings spills as well as hyper salinity of tailings deposits which could lead to contamination of ground- and surface-water, and dust emissions (Maboeta et al. 2007). Van Rensburg and Morgenthal (2004) stated that the biggest concern regarding the soil nutrient status of treated platinum tailings materials is its sustained low fertility and the potential phytotoxicity of copper compounds, as well as the presence of Aluminium, Nickel, and Chromium. These heavy metals are inherently associated with PGM discard and tailings (van der Walt, 2012).

Direct revegetation of PGM TSFs is the current rehabilitation choice and either wilderness or grazing is the permitted end-land use whilst no topsoil cover is typically considered in the design, construction, or closure plans of the facilities. Revegetation success under dryland and for "walk-away" outcomes have not delivered any meaningful cover or eco-progression since the rehabilitated systems are constantly in a state of resource loss, drought stress and in a state of depleted soil health (Hatting and van Deventer, 2002). In 2002, the authors embarked on a study to determine the best methodology for platinum tailings revegetation techniques (Van Wyk, 2009) since no scientific information existed on sustainable vegetation cover techniques. High evaporation rates were identified by Hatting and Van Deventer (2002) as the main limiting factor for vegetation establishment on platinum tailings materials stemming from the soil textural traits, high surface temperatures and lack of soil organic matter. Amelioration techniques that focussed on optimised water retention, high inorganic fertility amendments and organic amendments were investigated to improve soil moisture storage and nutrient cycling. The experimental layout of the revegetation research that underpins this work is presented in the materials and methods section of this paper.

From this research, a rehabilitation specification was derived and has been implemented at scale since 2003. Over the past 20 years, annual rehabilitation performance appraisals has indicated that although a much-improved vegetation cover was evident, the cover effectiveness and ecological function is questionable (Van Wyk, 2009). Van der Walt et al (2012) also found that 30-year-old directly revegetated covers on PGM TSFs were 'leaky' and dysfunctional landscapes due to limited biological development with lower nutrient cycling indices compared to natural analogues, implying that ecosystem development was extremely slow due to low biomass growth and slow nutrient cycling processes. Although improved revegetation techniques have been implemented over the past 20 years, it is evident that that without intensive maintenance, current platinum tailings revegetation outcomes cannot be regarded as a self-sustaining (Van Wyk, 2009).

Huang et al (2012) state that the missing link for direct revegetation of fine-grained waste or tailings is the focussed reconstruction of root zones and "designed rhizosphere horizon" through topsoil and self-sustaining tailings amendments, which must be physically and hydro-geochemically stable. Microbial health of the rehabilitated systems was found to be the key process involved in the development of long-term functional root zones where directly revegetated tailings landscapes thrived without continual management and maintenance inputs (Huang et al. 2012). According to Odile et al. (2019) microorganisms are important are important actors in the rehabilitation process of contaminated tailings environments. They play a key role in

sustained soil health as the major driver of biogeochemical cycling, and thus the microbial environment controls the fate of impacted environments in the long term. Some microorganisms can survive in highly contaminated substrates and these natural capacities can be exploited for the acceleration of the oxidative dissolution of sulfidic minerals enabling soil decontamination potential. Microorganisms can also play a key role in phyto-stabilisation, either indirectly by promoting plant growth or directly through metals and metalloid mobilization or immobilization in soils.

Advances in the field of high throughput sequencing techniques have revolutionized molecular biology and opened a new era in research on microbial ecology, enabling to explore the diversity and activity of microbial communities thriving in polluted environments. Rauwane (2008) studied the diversity and effects of heavy metals in platinum tailings. Bacterial isolates from different sites on platinum TSFs and natural analogues were compared. Despite very high heavy metal concentrations (Al, Cr, Cu, Ni), plate count analyses revealed that fungi and bacteria were not suppressed by high concentrations of heavy metals (Rauwane, 2008). Bacterial isolates were purified for the study and clustered using colony morphology data. These were subjected to sequencing and PCR-RFLP of 16S rDNA fragments as well as SDS-PAGE analysis. Some of the isolates were identified as *Paenibacillus lautus* and *Bacillus barbaricus* using BLAST searches of 16S rDNA sequences. From the study, RAPDs analysis provided evidence of potential genotoxic effects of heavy metal in soil on a metal tolerant bacterial species (*P. lautus*). The Shannon-Weaver diversity indices of fungal and bacterial populations were significantly higher ( $P < 0.05$ ) in tailings material than on analogue sites and therefore, soil microbial performance should be explained based on soil health and moisture retention rather than soil toxicology.

The lack of information on soil microbial health as ecological success driver for rehabilitated tailings substrates is regarded a knowledge gap for the mining and rehabilitation practitioners at large. Limited research has been done in South Africa regarding the link between microbial optimisation and rehabilitation performance. The implications for potential soil improvement requirements to improve the revegetation specification may add substantial cost to the mining industry. To address the questions about the value of soil microbial status and in-field soil microbial sampling protocols, the following study objectives were set:

- i) To consider the reliability and viability of a low-cost methodology to determine the microbial activity in PGM tailings
- ii) To evaluate the Soil Microbial Enzymatic Activity status as indicator for soil health in the rehabilitated PGM tailings treatments after 20 years
- iii) To identify which rehabilitation treatment performed the best from a soil microbial perspective and propose recommendations for next steps regarding tailings improvement opportunities

The learnings from this study should aid intentional design of rhizome improvement, qualify a potential technique for rapid monitoring of soil microbial status, and enable sampling of soil health quality as routine technique to support information on the final relinquishment process.

## 2 Study background

### 2.1 Study site

The study site is in Rustenburg, South Africa, which is about 150 km to the northwest of Johannesburg, at an elevation of approximately 1100 m above mean sea level (Figure 1). The PGM mining area is situated on the southern fringe of the Igneous Bushveld Complex at the foothills of the Magalies mountain range. The terrain around the mines is surrounded by outstretched clay flatlands weathered from norite, and intermittent granite hills protrude the area. The entire landscape is altered by mining infrastructure, tailings facilities, open pits, and waste rock dumps. Annual precipitation averages 641 mm per annum (Climatic region NT, according to the Weather Bureau of South Africa) is seasonally bound to the summer months (October to March) with the highest values in December, mainly occurring as thunderstorm showers.

Temperature-wise the area has a relatively high average daily temperature of 22°C. High daily summer temperatures of up to 40+°C are often reached; although winters are cold (temperatures in the order of 1°C), conditions are frost-free without rain.



Figure 1 Study area location of the tailings facility where the tailings rehabilitation experiment is located

The dominant wind direction is from the southwest and northeast, which exposes Rustenburg (to the southwest of the mining area) to the impacts of severe dust pollution from tailings facilities. The 2023 season was exceptionally wet with more than 1000 mm of rain with below average summer and winter temperatures and lower wind frequency and speed.

Situated in the Savannah biome and more specifically on the Clay thorn bushveld, the underlying geology consists of an upper layer of norite, which weather to black vertic soils with a clay content of more than 50%. The surrounding vegetation type is dominated by various *Acacia* species, and the understory consists of several shrubs and perennial grass species, particularly *Ischaemum*, *Sehima* and *Panicum* species. Deterioration of the grass sward has led to a serious increase in cover of woody species, with an associated dominance of several disturbance species including *Bothriochloa*, *Hyparrhenia* and *Aristida*. Except for *Hyparrhenia* none of these species are naturally occurring on rehabilitated tailings facilities. Several exotic invasive species including *Tamarix*, *Eucalyptus*, *Pennisetum* and *Ricinus* are frequently found on disturbed areas.

## 2.2 Tailings characteristics

The tailings material is derived from the crushing process of the platinum rich Merensky reef. This reef is a typical coarse grained feldspathic pyroxenite, containing the platinum group metals associated with other base-metal (copper and nickel) and sulphide minerals. The tailings material has a dry density of 1,49 Mg m<sup>-3</sup> and a particle density of 2,84 Mg m<sup>-3</sup> and is deposited hydraulically via spigot pipes onto a third-generation ring dyke dam with mechanically constructed tailings walls. Tailings from the side slope embankments have poor moisture retention of 3.72% at -33 kPa, 2,77% at -80 kPa, 0.75% at -500 kPa and 0.72% at -1500 kPa.

The outer embankment slope of cyclone tailings material has extraordinarily high average infiltration rate (not K<sub>Sat</sub>) of  $3.7 \times 10^{-2} \text{ cm s}^{-1}$ , whilst the evaporation from the tailings is 7 mm for the first day of evaporation when the water content is at the dried upper limit.

Figure 2 presents typical platinum tailings substrate. The material is loose and structureless and resemble grey-black dune sand although a fine silt and clay fraction is present.



**Figure 2** Platinum tailings material. Note the visual dry-out depth at field capacity on the tailing slope (left) and roadway (upper right). Natural vegetation establishment is an anomaly on this material and vegetation consistently experiences drought stress with slow rate of soil nutrient cycling

Typical physical and chemical properties for platinum tailings substrates are presented in Table 1 (Van Deventer et al. 2002). The tailings material is fine sand dominated with limited plant available water and no substantive plant nutritional value. High salinity and low cation exchange capacity values also present severe challenges for natural colonisation, seedling survival and succession processes whilst the depleted and low organic carbon content render the tailings material an inhospitable growth medium with limited food energy for soil microbes.

**Table 1** Soil physical and chemical properties of platinum tailings material. (van Deventer et al., 2002).

Soil Physical Properties		Soil Chemical Properties*					
Coarse sand	0.7%	pH (H <sub>2</sub> O)	8.5 – 9.8	Ca	400–600 mg kg <sup>-1</sup>	N-NO <sub>3</sub>	< 1 mg kg <sup>-1</sup>
Medium Sand	0.8%	EC	200–500 mS m <sup>-1</sup>	Mg	20–60 mg kg <sup>-1</sup>	N-NH <sub>4</sub>	< 1 mg kg <sup>-1</sup>
Fine Sand	42.0%	CEC	< 1 cmol <sub>c</sub> kg <sup>-1</sup>	K	10–40 mg kg <sup>-1</sup>	S-SO <sub>4</sub>	50–80 mg kg <sup>-1</sup>
Very fine sand	23.0%	ESP	< 1%	Na	20–50 mg kg <sup>-1</sup>	Cl	20–60 mg kg <sup>-1</sup>
Coarse Silt	16.0%			P	<1 mg kg <sup>-1</sup>	C	<1%
Fine Silt	5.4%			N	<1 mg kg <sup>-1</sup>		
Clay	1.5%						

\*Standard Ammonium Acetate Extraction procedure (Handbook of Standard Soil Testing Methods - Method 8: Ammonium acetate [1mol dm<sup>-3</sup>; pH 7]), to determine the cation status of the ameliorated tailings media. Other selected chemical variables were also either calculated (cation ratios, SAR) or determined (CEC, EC, pH, %C) from the different samples (van Deventer et al. 2002).

### 3 Materials and methods

#### 3.1 Experimental design

A revegetation trial was established in December 2002 to determine a scientifically based but practical and cost-effective revegetation specification that can be deployed throughout platinum TSFs in the northern bushveld area of South Africa (Van Wyk, 2009). The trial was laid out on the northern aspect of the upper embankment berm (25 m above ground level) on an operational platinum TSF, of which the side slopes are 15 m in length, at a 26° slope angle. The trial consisted of three randomised replicates (150 m<sup>2</sup>) for each of eight treatment options that were informed by soil specialists and tailings operators, and within cost-effective proximity of soil ameliorants. The treatments were (1) 40 tons ha<sup>-1</sup> untreated sewage sludge (fresh biosolids from residential origin not exposed to leaching or any chemical and biological breakdown), (2) 40 tons ha<sup>-1</sup> treated sewage sludge (biologically activated sludge), (3) 20 tons ha<sup>-1</sup> Bach compost (earthworm composted material composed of a mixture of sewage sludge, wood fibres, garden greens and feedlot manure), (4) 10 tons ha<sup>-1</sup> Bach compost, (5) 1,5 tons ha<sup>-1</sup> branch mulch, (6) 1,5 tons ha<sup>-1</sup> grass mulch, (7) rock armouring – approximately 40% of the surface area covered with rocks with an average diameter of 200 mm, and (8) inorganic fertilizer amendment (350 kg ha<sup>-1</sup> 5:1:5 and 100 kg ha<sup>-1</sup> superphosphate (10%)). A control treatment (9) was also included and only treated with seed.

Seedbed preparation was done by initial manual scarification of the tailings material to a depth of 300 mm after which the ameliorants were manually worked in to a depth of 200 mm, and the selected covers/ameliorants were applied. The seeding rate was 22 kg ha<sup>-1</sup> (2.2 g m<sup>-2</sup>) of a pre-selected grass species mixture consisting of commercially available native species. Selected species were chosen according to known tolerance potential, seed availability, potential post-land use value and also each of the species' successional role. The species included: *Chloris gayana* (Rhodes Grass), *Cenchrus ciliaris* (Buffalo grass) *Digitaria eriantha* (Common Finger Grass), *Eragrostis curvula* (Weeping Love Grass), *Hyparrhenia hirta* (Common Thatching Grass), *Panicum maximum* (Guinea Grass); *Cynodon dactylon* (Couch Grass), *Enneapogon cenchroides* (Nine-awn grass), *Eragrostis tef* (Annual teff), and *Melinis repens* (Natal red top). From the field evaluation in 2003 and 2009 (Van Wyk, 2009) it was found that only *Cenchrus ciliaris* (Buffalo grass) survived and the average canopy cover was less than 50% whilst the average basal cover was less than 5%.

### 3.2 Soil Enzymatic Activity—Dehydrogenase Assessment

Rapid advancement in soil microbial assessment techniques have been published in the past 25 years, i.e. chromatography, molecular, plate count techniques, respiration and fumigation extraction and epifluorescence microscopy are well known. Soil Microbial Enzymatic Activity Dehydrogenase Assessment (SMEA-DHA) was selected as it is a simple colorimetric an low-cost and rapid tool to routinely access soil health and can deliver reliable information for the purpose of rehabilitation performance appraisal.

To determine Soil Microbial Enzymatic Activity, composite tailings samples (approximately 1 kg) were collected immediately prior to the first tailings treatment, then 6 years after treatment in 2009, 10 years after treatment in 2013, and 20 years after treatment in 2023. The samples were taken in April when the rains have subsided. The tailings rhizosphere was sampled with a with a soil auger from each of the replicate treatments and kept below 4°C and assayed the next day.

The dehydrogenase (DHA) assay was done according to von Mersi and Schinner (1991). The method is based on the incubation of soil with the substrate idonitrotetrazolium chloride (INT) at 40 °C for 2 hours followed by colorimetric estimation of the reaction product idonitrotetrazolium chloride-formazan (INF). Field moist tailings material samples were weighed (1 g), placed in a 100 ml Schott bottle and mixed with 1.5 ml Tris (hydroxymethyl)-aminomethane (THAM) buffer and 2 ml INT solution. Controls were prepared with sterilised tailings (autoclaved at 121 °C for 20 minutes). The Schott bottles were sealed and incubated at 40 °C in the dark for 2 h. After incubation 10 ml of N,N-dimethylformamide (DMF) ethanol (1:1 v/v) extraction solution was added and shaken every 20 minutes for another hour. This was added to terminate the reaction. The tailings suspensions were then filtered through Whatman no. 2v filter paper and the absorbance of the filtrate measured at 464 nm. The INF calibration curve was prepared by pipetting 0, 1, 2 and 5 ml of INF standard solution into test tubes and adding 13,5 ml extractant solution to each tube that was then mixed thoroughly. The calibration concentrations were 0, 100, 200 and 500 µg INF per test. The dehydrogenase activity is expressed as µg INF g<sup>-1</sup> dwt 2 h<sup>-1</sup> and calculated according to the following relationship as outlined in Equation 1:

$$\frac{\text{INF } (\mu\text{g g}^{-1}\text{dwt}2\text{h}^{-1})}{\text{D.weight}} = \frac{\text{S1} - \text{S0}}{\text{D.weight}} \quad (1)$$

Where S1 = INF (in µg) of the test

S0 = INF (in µg) of the control

dwt = dry weight of 1 g moist soil

Results can also be converted and expressed as INF g<sup>-1</sup> kg<sup>-1</sup>h.

## 4 Results and discussion

Figure 3 shows the physiognomic pattern of the aboveground biomass of four treatment blocks (12m long and 6m wide) typical of the represented treatments three months after initiating the plots and resultant of dryland conditions. The inserted graphic represents visual surface biomass production sequence from left to right of inorganic fertilizer amendment; 1,5 tons ha<sup>-1</sup> branch mulch; 1,5 tons ha<sup>-1</sup> grass mulch; 40 tons ha<sup>-1</sup> untreated sewage sludge. The insert on the right of Figure 3 depicts the depth of the rhizosphere for the 1,5 tons ha<sup>-1</sup> grass mulch treatment - 12 months after treatment demonstrating root depth achieved.



**Figure 3** Visual representation of the rehabilitation trial on platinum tailings. Vegetation was successfully established and the maximum root depth of 0.8m was achieved for the best treatments (treated biosolid application and grass mulch covers)

The SMEA-DHA results in the various treatments and for the different sampling periods are presented in Figure 4.1. After 20 years of natural ecological processes and without any maintenance or ecological shock (fire, insects, catastrophic floods, or drought), soil dehydrogenase activity increased from 0 *INF*  $\mu\text{g g}^{-1} \text{h}^{-1}$  to 15 *INF*  $\mu\text{g g}^{-1} \text{h}^{-1}$  on average per treatment. The enzyme activity in the treatments still lags significantly behind the natural reference area (> 45 *INF*  $\mu\text{g g}^{-1} \text{h}^{-1}$ ), but overall, the treatments resulted in year-on-year increases in soil enzymatic activity across the various treatments whilst some differences can be noticed between treatments as well. The untreated tailings substrate, which was only seeded with grass seed, showed the slowest inception and progress of soil microbial activity compared to the rest of the treatments. The treated sewage and grass mulch covered treatments showed the most improved microbial status over time, and SMEA-DHA values of between 20 and 28 *INF*  $\mu\text{g g}^{-1} \text{h}^{-1}$  were achieved.



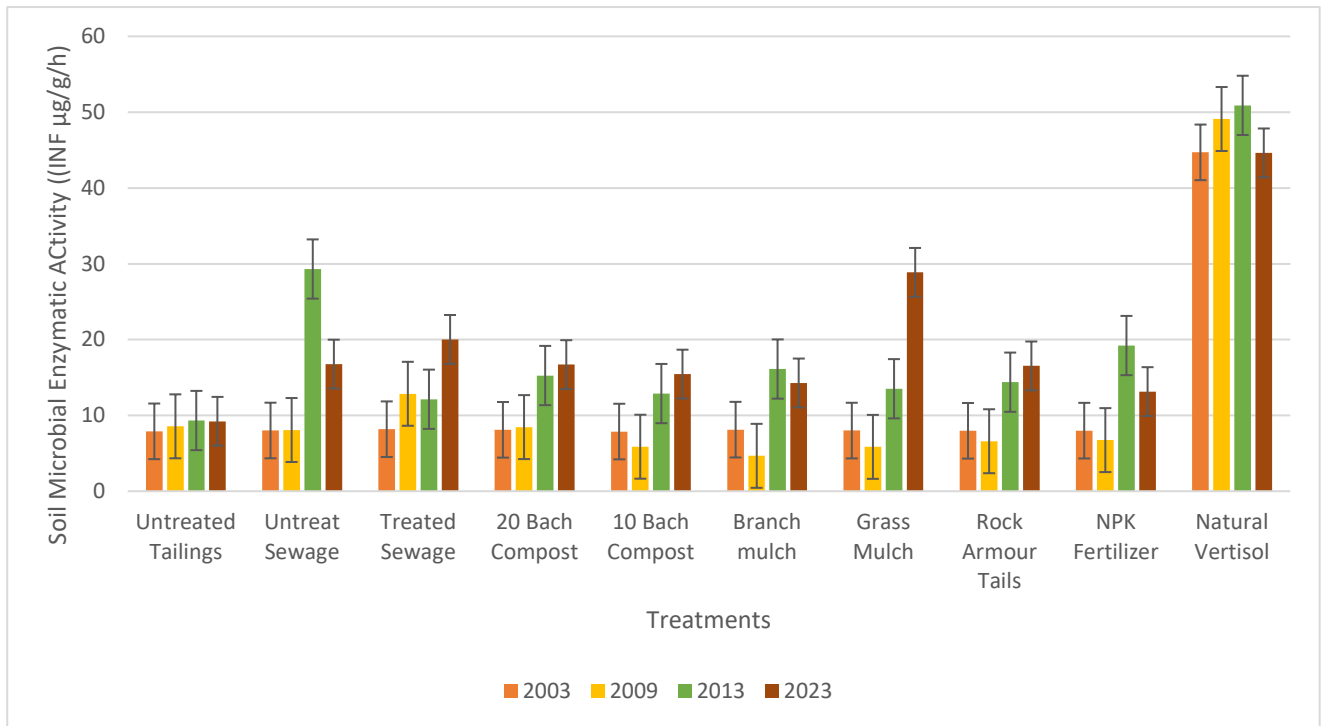


Figure 4 Average soil enzymatic activity measured in 2009, 2013 and 2023 on the rehabilitated platinum tailings experimental site

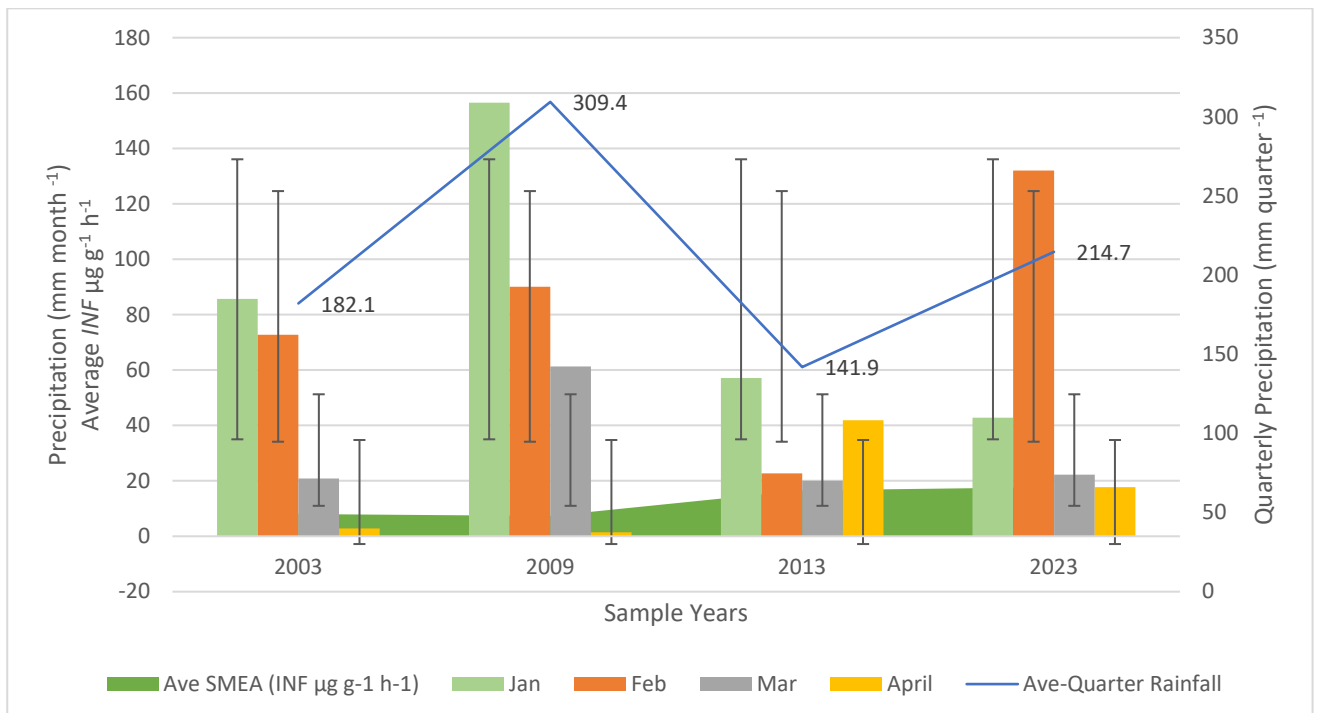


Figure 5 Comparative precipitation for the months preceding the tailings sampling depicting the rainfall for Jan-April for each sample year, the total quarterly rainfall and the average Soil Microbial Enzymatic Activity across the various treatments

A prevalent pattern was notable in all the treatments, where soil enzymatic activity has markedly increased 10 years after the treatments showing more than 100% improvement in all the treatments. Most notably, the highest increase is found in the untreated sewage sludge treatment, whilst only the treated sewage treatment and the grass mulch treatment presented more than 100% improvement from the previous monitoring cycle 6 years ago. The similarity in either the increase or decreased soil enzymatic activity can possibly be ascribed to the effects of climatological variability or soil chemical and biological processes. From the data presented in Figure 5, it is evident that the precipitation in the four months preceding the monitoring effort, rather erratic summer rainfall occurred and although 2013 presented the lowest comparable quarterly rainfall, the month preceding the sampling effort did present higher than normal April rainfall, as was the case for April 2023. Although an increase in soil moisture because of higher rainfall may present a plausible explanation for the increased SMEA, it would be premature to explain increased soil biological activity only based on soil moisture.

Of interest is that the treatments where only inorganic fertilisers were applied or only seed was applied performed the worst after 20 years, which could possibly be a result of either poorly developed root systems, lack of soil organic matter, and poor survival of initially established vegetation which inhibited sustainable rhizosphere development and subsequent lower comparable soil health.

However, a case could be made from a general cost/benefit view that, except perhaps for the grass mulch cover treatment, the generally more expensive treatments did not result in any marked comparable difference in improvement of the SMEA over the 20-year period. Although the grass mulch cover treatment can be recommended from the data as the best treatment to establish long term soil microbial health, the real aim should be to ameliorate/enable the substrate to naturally achieve and sustain biological activity at levels much more comparable to the natural analogue values. If the current rehabilitation specifications does not contribute to rapid restoration of soil health, then alternative higher nutritional and organic content specifications should be tested and implemented, even if it would imply higher short term cost for the mining industry.

Table 2 presents the Multi Variate Analysis results to determine the statistical significance of seasonality, rainfall, and inter-treatment soil microbial enzymatic activity.

**Table 2 ANOVA Two-Factor Analysis Results for the field experiment.**

Source of Variation	SS	df	MS	F	P-value	F crit
Comparable Sample Years	4619,9	9	513,318	13,964	1,70557E-08	2,211
Comparative Quarter Rainfall	2309,9	9	256,659	0,095	0,99960028	2,124
Comparative April Rainfall	2309,9	9	256,659	1,283	0,261417	2,017
Comparative Inter-treatment for Microbial Enzyme Activity	1975,5	9	658,501	21,923	6,75316E-11	2,699

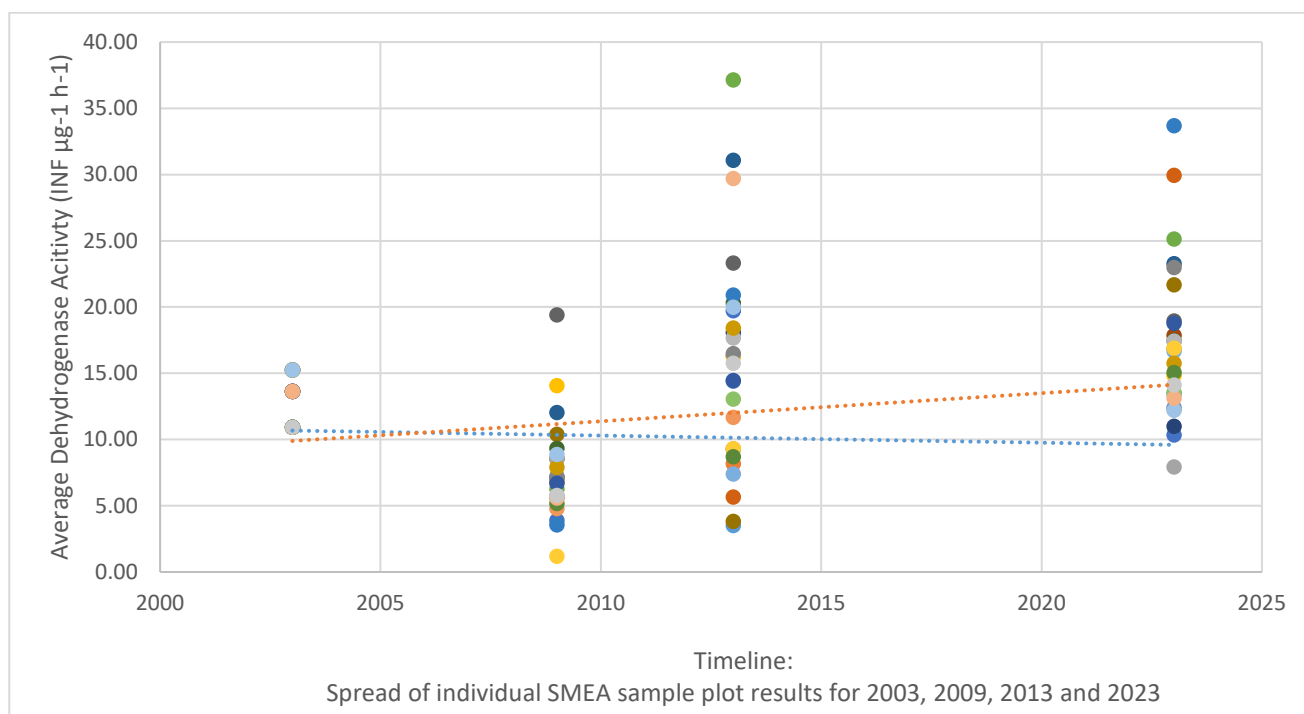
From the data there was a statistical significance between the SMEA for the various treatments with the F-value much higher than the F-critical value and the p value < 0.005. From a seasonality and rainfall perspective, no statistical significance could be detected and the variance in the SMEA could therefore not be solely attributed to seasonality or rainfall effects.

It would be fair to conclude that the treatments where organic treatments were the main constituents of the tailings improvement process, the most improved SMEA results were found. A combination of soil physical, soil chemical and soil biological as well as climatological data would be required to fully explain the drivers of soil health in the PGM tailings and follow-up research is underway.

From the soil chemical information presented in Table 1, the soil nitrogen and carbon levels in untreated tailings are well below any sustainable means for any soil system to support any biological function. The mainstream practice would revert to inorganic fertilisers to solve soil N:P:K deficiencies. It is clear from this study that any improvement in soil health, and implied revegetation techniques, that an investment in self-sustaining soil forming processes without the requirement of continual maintenance efforts and costs should be regarded as best practice, irrespective of higher initial cost implications.

The spread of the sample data from the various treatments over the timeline of evaluation is presented in Figure 6 to visualise the variance and range of the Soil Microbial Enzymatic Activity improvement.

Note the natural analogue values that need to be the aim for sustainable soil health is in the order of 50 INF  $\mu\text{g}^{-1} \text{h}^{-1}$ . From the data in the Figure 6, the initially rehabilitated system was microbiologically in a poor state compared to the natural analogue (Figure 4) and although a gradual trajectory of improvement can be noticed, it is evident that the restored microbial systems can be regarded as rather unstable, even in the treatment plots that presented the most prevalent soil enzymatic activity.



**Figure 6** Spread of sample data indicating the variability of soil enzymatic activity in the various treatments over time. The rising trendline is indicative of the improvement trajectory for all the treatments except the control treatment, which is indicated by the decreasing trend of enzymatic activity

Although the data presented in Figure 5 indicate higher rainfall in the sampling months of 2013 and 2023, it can be derived that the wide spread of SMEA concentration in 2010 and 2013 indicate that more than factors other than soil moisture is at play in the soil health status. Considering the average 5-year enzymatic activity improvement rate, it could be derived that under similar environmental conditions, at least another 20 years would be required for the system to achieve restored microbial activity rates which support the notion that a 50-year timeline would be required before any rehabilitation effort can be relinquished. However, the drivers and statistics that could explain and predict the behaviour of the sustainability of the microbial status outlined in this study requires additional research.

## 5 Conclusion

The soil microbial status of 20-year-old revegetated platinum tailings is still substantially lower than the natural analogue and although slow and incremental progress can be identified in some of the outlined treatments, additional or alternative ameliorative and/or maintenance means should be considered to accelerate soil microbial ecosystem development and fast track the relinquishment process.

Soil Microbial Enzymatic Activity was successfully determined by means of the dehydrogenase assay methodology. This method was applied to provide rapid information whereby a single cost-effective assay can deliver accurate microbial bio-indicator data to evaluate rehabilitation progress.

More research is required to explain the behavior and trends of rhizosphere development in revegetated platinum tailings substrates. Despite the implementation of what was regarded as the best empirically motivated rehabilitation specification, a better understanding is required why the system is not fully functional and which processes or lack of processes have inhibited sustainable vegetation cover. The microbial status on a 20-year-old platinum TSF rehabilitated by means of unmaintained dry-land techniques has not achieved similar microbial function compared to the analogue site. The (current) rehabilitation methodologies should therefore be re-evaluated as it is evident that this rehabilitation specification may not qualify for the final closure standard as expected by the regulatory authority.

Pro-active rhizosphere development should be a priority for mining waste closure designs from the onset of a rehabilitation project to save re-application and maintenance cost and lost ecosystem development time. The amelioration/amendment, revegetation and maintenance processes should be adequately planned, specified for to achieve complete restoration objectives and financed according to the required timelines to enable long-term rhizosphere development and adequate self-generation of ample organic carbon and nutrient cycling.

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