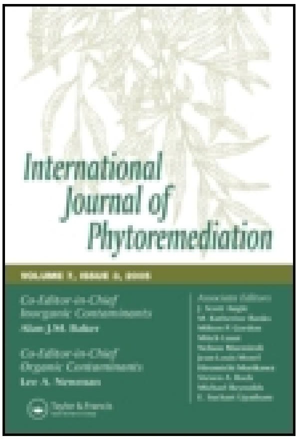

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EFFECTS OF DIFFERENT SOIL AMELIORANTS ON KAREE TREES (SEARSIA LANCEA) GROWING ON MINE TAILINGS DUMP SOIL—PART I: POT TRIALS

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EFFECTS OF DIFFERENT SOIL AMELIORANTS ON KAREE TREES (*SEARSIA LANCEA*) GROWING ON MINE TAILINGS DUMP SOIL—PART I: POT TRIALS

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Rehabilitation of mine tailings dams is often a challenge due to a lack of nutrients and a poor humus reservoir prevailing in tailings soils. This is especially true for establishing longer lived species such as trees. For these reasons the effects of different soil ameliorants (woodchips compost, vermicompost, mature sewage sludge), added to the root system of Karee (*Searcia lancea*) saplings were tested in pot trials. Those pots were filled with platinum and gold tailings substrate as well as red clay soil, respectively. For three months plants remained in a greenhouse and were subsequently moved to a test field outside. Throughout the test period regular chl a fluorescence measurements were taken and subjected to JIP-test quantifying changes in photosynthetic vitality status. Additionally, growth measurements and one-off leaf analysis were carried out. Test plants growing on mine tailings experienced an up to 35% higher average photosynthetic vitality (PI_{ABS}) and improved nutrient supply, when treated with mature sewage sludge. Consequently, sewage sludge treated plants showed a higher biomass build-up rate and an up to 55% higher diameter growth, compared to control. In summary the experiments present a low cost alternative for reforestation enterprises on platinum and gold tailings dams in South Africa.

KEY WORDS: rehabilitation, soil improvement, nutrient supply, plant vitality status, South Africa

INTRODUCTION

Numerous mine tailings dumps in South Africa, particularly those located in or close to urban settlements, represent a potentially high ecological risk due to their pollutant content and their potential hazard of being liberated and spread via wind erosion as well as their potential to contaminate surface and groundwater sources. Therefore, rehabilitation using appropriate plant species aims to remediate and stabilize the contaminated soil, to prevent dust storms and leaching processes and to enhance the aesthetic value of the

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landscapes. Rehabilitation also potentially adds further value by contributing to the restoration of local ecosystem services such as carbon sequestration, reduction of water runoff, air filtration as well as optimizing future land use. Thus, rehabilitation of mine tailings dams, specifically those located close to or in urban areas attracts growing public interest and political as well as financial support (Bound and Hunhammar 1999; Limpitlaw *et al.* 2005).

Several studies on mine rehabilitation have been performed in South Africa by mostly using suitable grass species (e.g., Envirogreen 2001; Van Rensburg, Morgenthal, and Claassens 2004) or hardy exotic tree species (e.g., Envirogreen 2001; Pollmann *et al.* 2008). However, limited or no information is available for the suitability of indigenous hardy trees for rehabilitation purposes.

Aiming for the establishment of a sufficient vegetation cover, current rehabilitation measures still remain very costly. This is mainly due to steps normally taken to ensure the initial growth phase of plants growing under the unfavorable conditions of mine tailings. Depending on the character and location of the specific site, the main threats to long term establishment of vegetation can include extreme soil pH-levels (mostly gold tailings) with little or no buffer capacity, micro-nutrient deficiencies (mainly platinum tailings) and low soil organic content (both gold and platinum tailings substrate). These factors finally lead to limitation of trace elements and nutrient availability, while high light intensity, severe heat and drought can further exacerbate the impact of stress already experienced by the plants in these environments.

Soil ameliorants, as mature sewage sludge and woodchips compost, when administered to platinum and gold tailings material, improved the soil quality by enhancing the microbiological activity (Pollmann and Van Rensburg 2009). According to these authors, the microbiological activity after a testing period of six months was found to be increased by 46.0% for platinum tailings material mixed with woodchips compost and 38.1% for gold tailings material combined with matured sewage sludge.

Furthermore, organic fertilizer can enhance plant establishment from a very early stage by preventing impaired growth caused by nutrient shortages and additionally balancing soil water content. Therefore, potential organic ameliorants were mixed with platinum as well as gold tailings substrate and red clay soil (control soil), respectively, and tested in pot trials using indigenous Karee (*Searsia lancea*) saplings.

Why was Karee (*Searsia lancea*) chosen for this study? South African legislation allows the use of indigenous trees for reforestation purposes only (Republic of South Africa 2004). Thus, the total number of appropriate tree species potentially used for rehabilitation is restricted to indigenous species and preferably to those species that are indigenous or characteristic to the immediate area surrounding a rehabilitation site. *Searsia lancea* features a vast natural distribution area throughout southern Africa (Coates Palgrave 2003) and is said to be extremely drought resistant and hardy. The establishment of trees such as *Searsia lancea* can also potentially change the microclimate on such a rehabilitation site by reducing wind speeds, reducing the immediate atmospheric temperature during warm sunny periods and providing protection during cold weather or frost (Belsky *et al.* 1989). *Searsia lancea* is thus an ideal pioneer tree species for facilitating the establishment of vegetation on disturbed sites. Besides the ecological value of *Searsia lancea*, the tree also boasts a myriad of traditional uses—from the use of the wood for building material to using the fruits to brew beer (Venter and Venter 1996; Coates Palgrave 2003)—thus potentially adding commercial and social value to a previously disturbed site.

MATERIALS AND METHODS

Platinum and gold mine tailings material as well as red clay soil (control soil), each mixed with different ameliorants, formed the growth media and were used to plant the test plants after the original soil was completely washed off the roots.

Tailings Dump Test Soil Substrates

At the end of August 2008, different test soil substrates of the following origin were delivered to the property of the Agriculture Research Council (ARC) in Potchefstroom, South Africa:

- platinum tailings dump soil, originating from a platinum mine tailings dump in Rustenburg, South Africa (Impala Platinum Holdings Limited, Implats), surface area of the tailings dump: ca. 470ha; *geographical position*: S 25° 30’49.94” / E 27° 14’48.17”
- gold tailings dump soil, originating from a gold mine tailings dump near Orkney, South Africa (Anglo-Ashanti Gold Ltd.); surface area of the tailings dump: ca. 175ha *geographical position*: S 26° 54’33.69” / E 26° 46’12.13”
- control soil, a red clay soil originating from a natural veldt site near Klerksdorp, South Africa; *geographical position*: S 26° 54’19.51” / E 26° 21’25.68”

Fertilizer and Soil Ameliorants

To enhance the content of nutrient elements in tailings and soil substrates used, different fertilizer/ameliorants were applied, namely woodchips compost (wc), vermicompost (vc), and matured sewage sludge (ms).

Woodchips compost was provided by Monontsha (Pty) Ltd., Rustenburg. Van Rensburg *et al*. (2004) describe woodchips as a waste product, generated during the extraction of platinum ore and arising from underground blasting in the vicinity of wooden buttresses. Woodchips and ore are processed together during the initial milling and extraction phases of mineral treatment and are separated by screening prior to platinum recovery. Due to under-ground blasting with ammonium nitrate, woodchips may contain substantial amounts of nitrate, which makes them a good source of nitrogen for rehabilitating vegetation. The woodchips usually are combined with sewage sludge from the company’s water treatment works to produce compost, which is further used for rehabilitation purposes (Van Rensburg *et al*. 2004). Woodchips have been applied already as an organic ameliorant during the rehabilitation of platinum tailings using different grass species (Van Rensburg *et al*. 2004). According to latter authors, however, the possibility of micro-element and heavy metal toxicity (especially copper, chromium, selenium and arsenic), caused by woodchips treated slimes material, is of concern.

The vermicompost used is a commercial product similar to a range of compost products available from nurseries.

Matured sewage sludge used for the experiments was several months old, provided by a sewage sludge farm in Potchefstroom (South Africa) and is different to the sewage sludge used for production of woodchips by Monontsha (Pty) Ltd., Rustenburg.

After setting up the different growth media, chemical analyses of aqueous soil eluate were carried out to determine element composition of the soil mixtures; results are reported in Pollmann and Van Rensburg (2009) and to some extent presented in Table 1.

Table 1 Chemical analysis of different soil substrates used in the pot trials

	Platinum mine tailings				Gold mine tailings				Control soil			
Trial	Pwc	Pvc	Pms	Pco	Gwc	Gvc	Gms	Gco	Cwc	Cvc	Cms	Cco
pH	6.86	7.07	7.53	7.34	3.86	3.88	4.54	3.75	4.74	5.01	5.71	4.90
EC [mS/cm]	4.28	2.67	1.65	1.24	3.14	3.30	3.20	3.05	1.12	1.08	1.13	1.31
Ca [ppm]	341	228	72.1	81.8	446	435	402	464	115	115	69.7	145
Mg [ppm]	149	93.8	86.3	54.4	84.1	99.4	70.7	70.7	33.1	30.9	40.8	43.0
K [ppm]	104	67.2	38.3	22.7	3.52	7.43	8.21	0.39	63.7	44.2	56.7	47.3
Na [ppm]	246	129	93.3	74.9	29.7	31.0	17.2	17.7	14.0	14.9	17.0	13.1
Cl [ppm]	549	220	153	115	27.0	19.2	17.1	16.6	28.7	23.8	27.3	27.3
SO_4 [ppm]	1082	800	402	351	1453	1444	1505	1429	106	95.1	116	88.4
NH_4 [ppm]	0.40	3.25	14.5	0.60	2.58	18.4	87.0	2.20	8.17	11.9	39.5	8.46
NO_3 [ppm]	268	190	98.0	78.4	24.1	149	11.3	18.0	502	476	456	643
HCO_3 [ppm]	33.6	39.7	64.1	36.6	0.00	0.00	0.61	0.00	6.10	6.10	15.3	6.10
PO_4 [ppm]	0.00	0.00	39.2	0.00	0.00	0.00	1.90	0.00	0.00	10.4	14.4	0.00
P-BRAY 1 [ppm]	3.59	9.41	65.7	3.96	3.94	12.4	104	3.79	40.8	51.9	–*	41.6
Fe [ppm]	0.21	0.15	0.06	0.06	0.32	0.17	0.10	0.36	0.11	0.08	0.47	0.24
Mn [ppm]	0.11	0.38	0.07	0.02	13.0	12.3	12.4	12.9	1.81	0.99	1.01	0.49
Cu [ppm]	0.03	0.05	0.00	0.00	0.47	0.11	0.05	0.54	0.02	0.01	0.02	0.02
Zn [ppm]	0.02	0.03	0.00	0.00	2.87	2.88	1.59	2.89	0.02	0.02	0.01	0.01

Data source: Pollmann and Van Rensburg (2009)
* = data not available

From Table 1 it is evident that the untreated soil materials (i.e., Pco, Gco, Cco) differ considerably in their chemical composition (e.g., Zn, Mn, Cu availability; pH values; EC) due to their different origin. Heavy metal concentrations, like Mn, Cu, and Zn contents, determined in the gold tailings trials are significant higher than the adequate benchmark concentrations (Mn: 4 mg/l; Cu: 0.06 mg/l; Zn: 0.4 mg/l) given by Efroymson *et al*. (1997). Thus, plants growing in the gold tailings trials are a priori exposed to a higher phytotoxicological risk than plants growing in platinum tailings and control soil, respectively.

Furthermore there was some heterogeneity in chemical composition, especially of the platinum tailings soil substrates used in the trials. Comparing to control, plots treated with woodchips compost (mainly Pwc) were characterized by increased salt (sodium chloride, NaCl) contents. This can be explained by the extraction process in the platinum mine, using multiple recycled process water, which becomes more and more charged with NaCl. In addition to NaCl, gypsum was formed mostly in the gold tailings trials and in Pwc (see Table 1). As expected, soil substrates treated with mature sewage sludge (Pms, Gms, Cms) exhibit the highest ammonium and phosphate contents, compared to the other adequate trial members. It should be noted that the mixing of sewage sludge with the mine tailings substrates used did not increase the heavy metal contents in the growth media (e.g., Zn content in Pms, Gms, Cms not significantly higher compared to their individual control).

Cultivation and Treatment of Plants Used in this Investigation

The 144 potted test plants (Karee, *Searsia lancea*) were sourced from a nursery (Bergsig Kwekery, Cullinan, South Africa) during August 2008. The test trees were approximately three years old with a mean height of 100cm. At the end of August 2008, plants were transplanted into 5 liter pots containing different mixtures of tailings substrates

Table 2 Experimental design, showing the composition of the growth medium used as well as number of trees in the test trials

Trial identity	Soil substrate		Added ameliorants		Number of plants
	Type	[vol.-%]	Type	[vol.-%]	
Pwc	Platinum tailings soil	80	woodchips compost	20	12
Pvc	Platinum tailings soil	90	vermicompost	10	12
Pms	Platinum tailings soil	90	matured sewage sludge	10	12
Pco	Platinum tailings soil	100	-	-	12
total					*48*
Gwc	Gold tailings soil	80	woodchips compost	20	12
Gvc	Gold tailings soil	90	vermicompost	10	12
Gms	Gold tailings soil	90	matured sewage sludge	10	12
Gco	Gold tailings soil	100	-	-	12
total					*48*
Cwc	Control soil*	80	woodchips compost	20	12
Cvc	Control soil*	90	vermicompost	10	12
Cms	Control soil*	90	matured sewage sludge	10	12
Cco	Control soil*	100	-	-	12
total					*48*

*common red clay soil

or control soil and ameliorants (Table 2). Note that all the original soil was washed off the roots prior to being planted in the new mediums.

For the first three months all the test plants were grown in the greenhouse under controlled conditions (temperature range 18°C–26°C). The Karee trees were watered regularly to avoid drought stress. At the end of November 2008, the test plants were placed outside of the greenhouse and were now exposed to open air conditions until the end of February 2009.

Chemical Leaf Analysis

The analytical determinative steps described in this method involve a complete HNO_3 digestion of powdered leaf material followed by multi-elemental determination of main and trace elements using ICP-OES (inductive coupled plasma-orbital emission spectroscopy) and the photometric determination of total phosphorus. Analysis of total carbon, total sulphur and total nitrogen was done using an element analyzer. Depending on species, morphology, and provenance variety and range of elements possibly incorporated in, plant material changes as much as the specific texture and water content. Nutrition state analysis of *Searsia lancea* has not been reported before. Thus the analytical methods applied will be described more detailed here. Since the early 1980s the underlying general methods have been proven to be suitable for various plant matrixes (e.g., Vaisanen *et al*. 2008).

Sampled Karee leaves were pre-dried at 80°C/24 h, transported to University of Heidelberg (Germany) and finally vacuum dried at 20°C to be pulverized and homogenized (swing mill MM301, Retsch, Germany). By adding 3 ml HNO_3 (65%) to 50 mg (exact) of the powdered sample and heating to 160°C for 2 h a complete digestion was achieved. For a few cases marginal mineral sediments occurred, which were assumed to be HNO_3 inert silica phytoliths. Two thirds of the digestion was used for analysis of main and trace elements using a Varian, Vista MPX, CCD simultaneous ICP-OES. To calibrate the system, multi-element standards as well as single element standard solutions were used. The remaining one third of the digestion underwent a photometric determination of total content of phosphorus (via phosphate) using ammonia molybdate after DIN EN ISO 6878 (PU 8730 UV/Vis scanning spectrophotometer; Philips, Holland).

Based on powdered leaf material, as described before, the total content of carbon, nitrogen and sulphur was determined from 50 mg (Vario Max CNS; Elementar, Germany).

To prove the quality and reproducibility of the methods used, a certified plant standard was analyzed along with the Karee leaf samples. Since certified standards for Karee leaves aren't available on the market, a stocked pine needle standard (NIST 1575a, *Pinus taeda*) was used for data quality management (Table S1). All given chemical data represent the median of 3-6 repeated measurements. Recovery rate and repeatability tested on NIST 1575a standard confirmed the suitability of the method developed (Table S1). The method underestimates cobalt with about −18%, and chromium and copper showed values of +44% and +36% respectively, compared to specifications of the NIST standard. This seems to be connected to the highest standard deviation (STDEV), determined as ~9 to 12.5%. Potassium, in contrast, is being underestimated with –18% but shows a STDEV of 3.26% only, which is tolerable. Chromium, copper and potassium have been analyzed by the certifying body (NIST) through a similar analytical method (ICP-OES). Nevertheless, data for Cu, Co, Cr and K are still within the range and magnitudes, despite the fact that the reason for the differing findings cannot be stated. All other species can be retrieved within a range of +/–10% and STDEVs of 0.2%–6.5%, which is acceptable for environmental samples.

Chlorophyll A Fluorescence Measurements and JIP-test

A portable Plant Efficiency Analyser (Handy-PEA, Hansatech, Kings' Lynn, UK) was used to measure chlorophyll a fluorescence on leaves of *Searsia lancea* in order to assess their physiological status (vitality). The leaves of the test plants were dark-adapted for at least one hour prior to chlorophyll a fluorescence measurements. The fast phase fluorescence transients were quantified by means of the JIP-test (Strasser and Strasser 1995; Strasser, Srivastava, and Tsimilli-Michael 2004) and Biolyzer software (Strasser *et al.* 2004). The JIP-test parameter PI_{ABS} (Performance Index on absorption basis) was used to quantify possible effects of applied ameliorants on photosynthetic vitality of Karee leaves. PI_{ABS} is a multiparametric expression, comprising the independent parameters contributing to photosynthesis, namely absorption (RC/ABS), quantum efficiency of trapping ($\varphi_{Po}/(1–\varphi_{Po})$) and $\psi_o/(1–\psi_o)$ as efficiency of conversion of trapped excitation energy to electron transport (Strasser, Srivastava, and Tsimilli-Michael 1999). PI_{ABS} is defined as follows (formula 1, F1):

$$PI_{ABS} = \frac{\gamma_{RC}}{1-\gamma_{RC}} \bullet \frac{\varphi_{Po}}{1-\varphi_{Po}} \bullet \frac{\psi_o}{1-\psi_o} = \frac{RC}{ABS} \bullet \frac{\varphi_{Po}}{1-\varphi_{Po}} \bullet \frac{\psi_o}{1-\psi_o} \qquad \text{(F1)}$$

where $\gamma_{\mathbf{RC}}$ is the fraction of reaction center chlorophylls relative to the total chlorophyll: $\gamma_{\mathbf{RC}} = \mathbf{Chl_{RC}} / \mathbf{Chl_{total}}$.

Since $\mathbf{Chl_{tot}} = \mathbf{Chl_{antenna}} + \mathbf{Chl_{RC}}$, we get: $\gamma_{\mathbf{RC}} / (\mathbf{1} - \gamma_{\mathbf{RC}}) = \mathbf{Chl_{RC}} / \mathbf{Chl_{antenna}} = \mathbf{RC/ABS}$.

The JIP-test, developed and tested both in the laboratory and in several applications, is well accepted to provide detection, description and quantification of the dynamic capacities of the photosynthetic sample. It has been widely and successfully used for the investigation of PS II behavior in various photosynthetic organisms and enables the study of synergistic and antagonistic effects of different co-stressors (Lange *et al.* 2004; Strauss *et al.* 2007; Smit *et al.* 2009; Lange, Böcker, and Katzur 2011).

Between September 2008 and February 2009 *in vivo* chl a fluorescence measurements (usually five measurements per tree) were carried out on the leaves of the Karee trees.

Plant Growth Measurements

On 12/11/08, 18/12/08, 19/01/09, and 25/02/09, respectively, for all of the test plants height and stem base diameter were determined and annual height and diameter growth calculated.

RESULTS AND DISCUSSION

Temporary Loss of Measurable Leaf Materials and Plant Fatalities

Seven days after transplanting the test trees into their new growth medium, especially the trees growing in gold tailings substrate showed symptoms of stress, such as leaf chlorosis, necrosis and abscission. Note that all soil was washed from the roots to be replaced by the test mediums, further enhancing the stress symptoms. When strongly affected by test circumstances plants were temporary excluded from measurements. Plants usually recovered during the 27-week test period and were again included in chl a fluorescence measurements. The temporary loss of measurable plants dropped down to 40%, depending on tailings material and added ameliorants. However, until the end of experiment, especially test plants growing in platinum tailings recovered to 90–100%. Figure 1 indicates the percentage of trees that were in leaf and thus suitable for chl a fluorescence measurements.

Test plants on gold tailings material generally struggled most in the experimental setup, which is further indicated by fatalities of up to −40% by the end of the experimental period. When treated with woodchips compost, especially plants of the Pwc group showed higher resistance to potential stress and were found to be in a measurable condition throughout the entire experimental period.

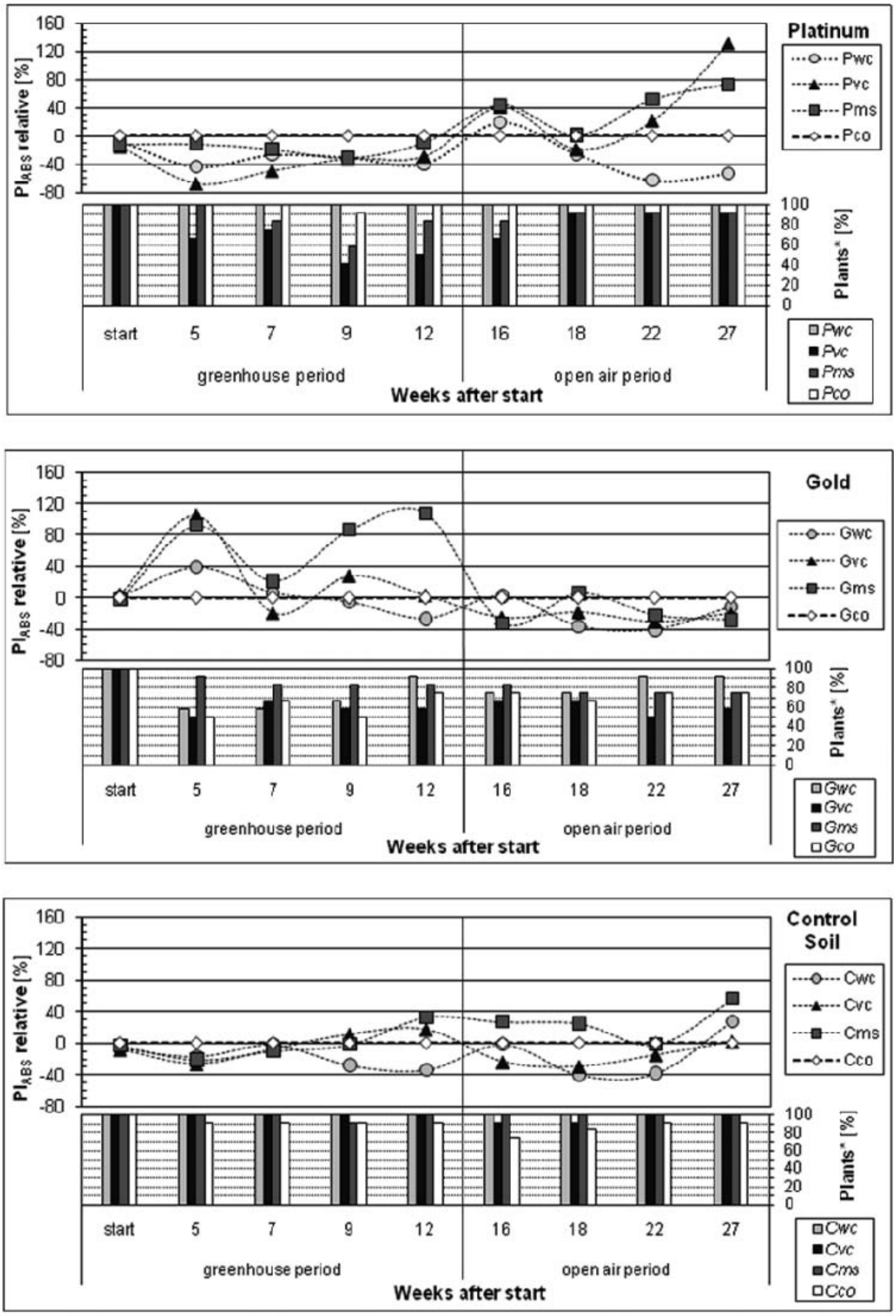
Chl A Fluorescence and Photosynthetic Vitality

The following section discusses chl a fluorescence data, especially the JIP test parameter PI_{ABS}, which enables the quantification of the test plants' vitality status. The trend of PI_{ABS}-relative values, determined on nine single occasions throughout a 27-week test period is shown in Figure 1, while Table 3 contains average PI_{ABS} values measured at start (19/08/08) and during greenhouse and open air periods. During the test phase, when Karee plants were initially exposed to controlled conditions inside and subsequently to natural conditions outside the greenhouse, strong fluctuations have been observed regarding the periodically measured mean PI_{ABS} values (see Figure 1). Significant differences between

Table 3 Mean PI_{ABS} values of Karee test plants growing in platinum and gold mine tailings substrate as well as control soil, each with different soil ameliorants. Data given for a 27-week experimental period

Trial	Start	Greenhouse period *(Test week 0 - 14)*	Open air period *(Test week 14 – 27)*	Entire period *(Test week 0 – 27)*
Platinum mine tailings				
Pwc	61.9 ± 40.4	##42.4 ± 24.8	##37.0 ± 34.6	##39.7 ± 30.2
Pvc	56.4 ± 32.0	##35.0 ± 25.3	**61.2 ± 32.2	##50.7 ± 32.3
Pms	58.6 ± 31.9	##55.9 ± 29.4	**67.6 ± 36.6	62.1 ± 33.9
Pco	66.8 ± 37.5	66.4 ± 31.5	51.8 ± 34.9	59.1 ± 34.0
Gold mine tailings				
Gwc	61.5 ± 31.2	36.2 ± 19.8	##25.1 ± 17.7	##30.1 ± 19.5
Gvc	63.2 ± 36.7	**44.6 ± 24.2	##25.4 ± 16.7	34.8 ± 22.8
Gms	59.7 ± 27.6	**66.2 ± 36.9	##27.8 ± 21.7	**48.2 ± 36.2
Gco	60.9 ± 26.5	38.1 ± 21.2	33.8 ± 22.0	35.8 ± 21.7
Control soil				
Cwc	58.7 ± 27.9	##58.9 ± 30.8	##46.8 ± 27.6	##53.9 ± 37.2
Cvc	57.8 ± 34.1	73.0 ± 40.5	##50.0 ± 32.4	62.0 ± 38.6
Cms	61.3 ± 27.2	74.8 ± 38.4	**70.8 ± 41.1	*72.8 ± 39.8
Cco	62.8 ± 33.5	74.4 ± 44.7	59.2 ± 34.9	67.1 ± 40.9

* Significantly higher value than corresponding control; level of significance $\alpha = 0.05$
** Significantly higher value than corresponding control; level of significance $\alpha = 0.01$
\# Significantly lower value than corresponding control; level of significance $\alpha = 0.05$
\## Significantly lower value than corresponding control; level of significance $\alpha = 0.01$



* contributed plants

Figure 1 Vitality trend (PI_{ABS}-relative) and fatalities of Karee test trees growing in platinum mine tailings substrate (top), in gold mine tailings substrate (middle) and control soil (bottom) normalised to their relative controls. Soil treatment with woodchips compost, vermicompost or matured sewage sludge during a six months test period (08/2008–02/2009).

mean PI_{ABS} values of the treated test plants and control plants were evident (Table 3), depending on ameliorants. During the greenhouse period, particularly plants growing in platinum mine tailings substrate and treated with vermicompost (Pvc) as well as mature sewage sludge (Pms), showed a significant decrease in PI_{ABS}, compared to control. Low values of PI_{ABS}-relative during the greenhouse period, revealed for Pwc, Pvc, and Pms (Figure 1) may be attributed to an adaptation process the test plants went through during the first weeks after planting. Subsequently, during the hardening phase (open air period), test plants of Pvc and Pms were adapted successfully and their photosynthetic vitality were higher compared to control plants.

From soil analysis data (see Table 1) it is also clear that the salt content (i.e., NaCl, $CaSO_4 \cdot 2H_2O$) in the Pco treatment was much lower compared to the treatments. As mentioned above, this was probably caused by heterogeneity in the platinum tailings and might have induced a lower strain on plants' physiology what lead consequently to an increased PI_{ABS} compared to plants of Pwc and Pvc.

On the contrary, test plants growing in gold mine tailings substrate were characterized by higher PI_{ABS} for Gvc and Gms during the greenhouse phase. In the hardening phase (open air period) however, the average PI_{ABS} of the mentioned plots fell under the level of the control. Initially higher overall vitality might have been caused by the nutrient availability in the acidic gold tailings substrate but being leached out later on in the experiment. The ameliorant treated plants would have also experienced higher levels of physiological stress when moved to harsh environment outside while being physiologically more active during this transition period. They might have experienced greater transpiration rates than the already more dormant controls. No data is however available to support this possible explanation.

Furthermore, it should be noted, that the number of actual measurable test plants (see Figure 1) during most of the open-air period was even higher, regarding Gwc and Gms, than those of the control (Gco). Thus, the inclusion of test plants characterized by a low photosynthetic vitality but still being in a measurable state (as shown for trial Gwc and Gms) may result necessarily in a decreased mean PI_{ABS} compared to control (Gco), where the mortality was even higher and dead test plants were not taken into consideration.

To sum up, Karee plants, when treated with mature sewage sludge exhibited an increment of mean PI_{ABS} by 5.1% (Pms), 34.6% (Gms), and 8.5% (Cms) compared to their individual control. Thus the application of mature sewage sludge effected the highest performance improvement when added to gold tailings substrate.

Leaf Analysis

On 25/02/09 five leaves per test tree were picked off from each of the test plants. The leaves served for both chl fluorescence measurements, followed by chemical analysis determining macro- and microelements and plants' nutrients, respectively. Data in Table 4 clearly indicate that test trees, when treated with matured sewage sludge, and irrespective of the base soil, showed increased 100-ldw-values (i.e., the matured sewage sludge treated plants synthesizing higher biomass rates) and increased N_{tot} contents, compared to control. This result corresponds to higher average PI_{ABS} values observed over the test period and is further supported by previous studies that showed a positive correlation between N_{tot} and PI_{ABS} (e.g., Bartelt, Lange, and Golldack 2008). Assessing the nutrition data (Table 4) and according to Krauss and Heinsdorf (2005), who investigated nutritional classes of main European deciduous tree species, the generally lower nitrogen content of the

Table 4 Results of the determination of plant nutrients and trace elements in the leaf tissue of Karee trees

	Platinum mine tailing				Gold mine tailing				Control soil			
Trial	Pwc	Pvc	Pms	Pco	Gwc	Gvc	Gms	Gco	Cwc	Cvc	Cms	Cco
100-ldw[g]	5.37	4.56	6.96	6.62	4.11	6.00	4.99	4.43	6.68	6.68	9.23	6.74
N_{tot} [w.-%]	0.76	1.36	1.50	1.31	0.93	1.20	1.28	1.03	0.97	1.26	1.45	1.22
C_{tot} [w.-%]	45.1	46.0	46.0	44.7	42.7	44.0	43.4	43.3	43.3	44.9	44.7	44.7
S_{tot} [w.-%]	0.29	0.36	0.41	0.43	0.79	0.95	1.04	0.95	0.35	0.32	0.34	0.24
P_{tot} [w.-%]	-#	0.14	0.11	0.08	0.11	0.09	0.10	-#	0.17	0.10	0.12	0.10
K [w.-%]	0.72	0.84	0.93	0.83	1.19	0.81	0.70	0.75	0.86	0.91	0.97	0.81
Ca [w.-%]	0.75	0.75	0.79	0.92	0.57	0.69	0.69	0.57	0.40	0.28	0.36	0.34
Mg [w.-%]	0.38	0.45	0.44	0.47	1.36	1.34	1.43	1.34	1.30	1.35	1.40	1.25
Al [μg/g]	246	294	436	470	492	310	478	358	318	442	189	232
Ba [μg/g]	11.8	10.1	8.00	11.9	15.6	10.4	8.86	16.1	15.8	12.6	16.0	13.2
Cd [μg/g]	0.11	0.03	0.04	0.04	0.05	0.07	0.07	0.08	0.05	0.01	0.37	0.05
Co [μg/g]	0.13	0.15	0.17	0.13	3.04	6.28	4.62	3.56	0.27	0.14	0.20	0.21
Cr [μg/g]	4.14	2.88	2.02	2.56	3.70	1.78	2.42	2.96	21.8	4.50	4.78	1.91
Fe [μg/g]	238	177	202	212	224	234	314	310	310	226	236	290
Mn [μg/g]	129	155	86.4	132	1524	2180	1502	1348	208	141	191	171
Sr [μg/g]	17.4	14.8	15.6	23.6	24.6	17.1	12.4	21.4	31.8	37.0	40.8	35.2
Zn [μg/g]	19.5	22.6	20.4	17.3	27.8	55.0	41.6	40.8	20.4	25.2	21.4	19.4

* 100-ldw = 100-leaves-dry weight w.-% = weight percentage

= data not available

Karee leaves becomes evident. N_{tot} content of oak leaves (*Quercus petraea*; *Quercus robur*) ranges from 1.35% (nutritional class 1 = extreme nutrient deficiency) to 3.93% (nutritional class 5 = strong excess nutrition). For beech leaves (*Fagus sylvatica* L.) N_{tot} content ranges from 1.48% (nutritional class 1) to 3.38% (nutritional class 5). However, the physiological nutrient requirements of various species of African and European trees may differ significantly and thus a direct comparison of nutritional data for tree species originating from different hemispheres and climatic zones remains problematic.

Furthermore it can be stated, that—independent from the treatment—trees growing on gold tailings substrate were characterized by an individual higher content of trace elements (like Zn, Co, and especially Mn) in the plant's tissue than trees growing on platinum tailings and control soil, respectively (Table 4). This can be explained by a priori higher elements' content in the gold tailings substrate (see Table 1).

Plant Growth

More efficient primary photosynthetic processes (to be recognizable with an enhanced PI_{ABS}) lead to higher biomass rates and may result in enhanced plant growth. The individual diameter as well as height growth and the consequential wood growth after the treatment with different soil ameliorants during the period 10/11/08–25/02/09 is shown in Table 5.

The application of mature sewage sludge caused both a higher average height and diameter growth, e.g. average diameter increment for Gms: +43.8% and Cms: +54.7%, compared to control. The exception was the diameter growth of plants in Pms, where mature sewage sludge in fact improved photosynthetic efficiency, but not resulted in an increase of plant's diameter growth. The documented increments of diameter and height growth resulted in a higher wood growth, in particular with the test plants treated with mature sewage sludge (e.g., Pms +15.5% and Cms +30.8%, comparing to control). From Table 1 it becomes clear that the mature sewage sludge treated mediums had higher concentrations

Table 5 Increment of diameter and height growth [mm] as well as resulting wood growth [%] of Karee saplings after a 27-week experimental period

Trial	*Height growth* [mm]	*Diameter* growth* [mm]	*Wood growth#* [%]
Pwc	20,8	0,9	21,7
Pvc	69,2	1,2	50,8
Pms	107,5	2,1	76,3
Pco	90,0	2,2	60,8
Gwc	24,2	0,3	14,0
Gvc	120,8	0,3	38,1
Gms	85,8	1,2	47,2
Gco	45,0	0,8	37,3
Cwc	18,3	1,5	36,8
Cvc	67,5	1,4	39,2
Cms	85,0	2,3	67,6
Cco	26,7	1,5	36,7

*Diameter = stem diameter at base, determined at the root neck of the sapling

Wood growth = included the wood growth of the stem (measured) as well as the wood growth of the branches and twigs (estimated)

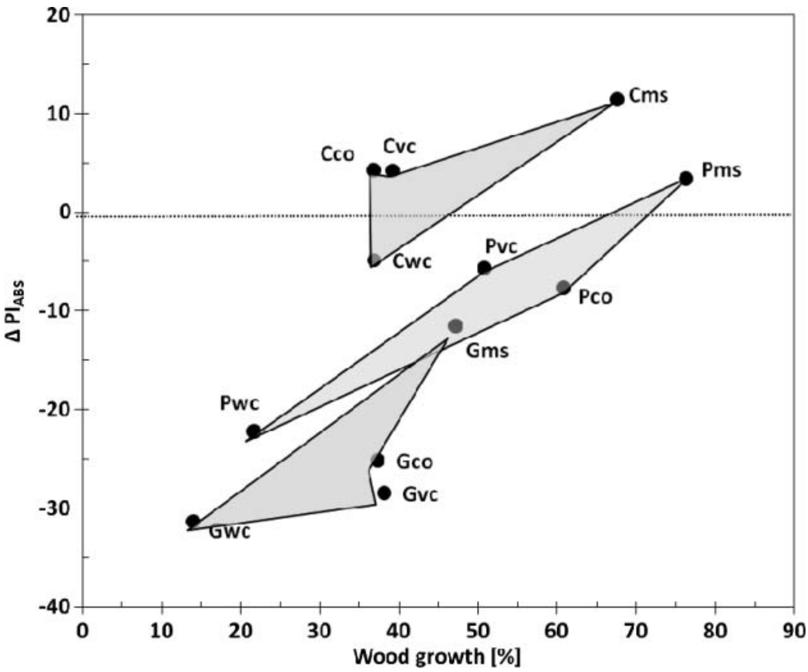


Figure 2 Correlation between ΔPI_{ABS} (average PI_{ABS} for the entire period related to PI_{ABS} at start) and wood increment [%] of different treated Karee trees growing on platinum and gold tailings substrate as well as control soil.

of phosphate. Phosphate is well known in literature (Barrow 1977) to stimulate root growth which is essential in harsh growing conditions such as in mine tailings soils.

To reveal possible correlations between the bioenergetic parameter PI_{ABS} and biomass growth of the different treated Karee trees, both the ΔPI_{ABS} (average PI_{ABS} for the entire period related to PI_{ABS} at start) and the individual wood growth [%] of the test plants are displayed in Figure 2. A clear correlation between photosynthetic vitality (ΔPI_{ABS}) and wood growth becomes evident. Independent from the originally used soil substrate, highest wood growth increments were achieved by amelioration using mature sewage sludge.

Correlation of Leaf Analysis Data, Photosynthetic Vitality, and Plant Growth

To reveal possible correlations of the chemical composition of the leaves, growth data and photosynthetic vitality, multivariate analysis (Canoco 4.5) was used. According to Van Rensburg *et al*. (2004), this method integrates constrained linear ordination with regression and therefore provides a graphic result of the relationship between variables and relevant environmental factors.

Figure 3 shows correlations (depicted as vectors) between leaf analysis data, PI_{ABS} and plant growth.

As expected, plants treated with mature sewage sludge (i.e., Pms, Gms, Cms) were highly associated with the highest amounts of plant nutrients in the leaf tissue, such as N_{tot}, Ca, and Mg. Additionally, the highest dry weight values (dw/leaf), except platinum tailings group, were linked with the sewage sludge treatments. Contrary to that, woodchips treated

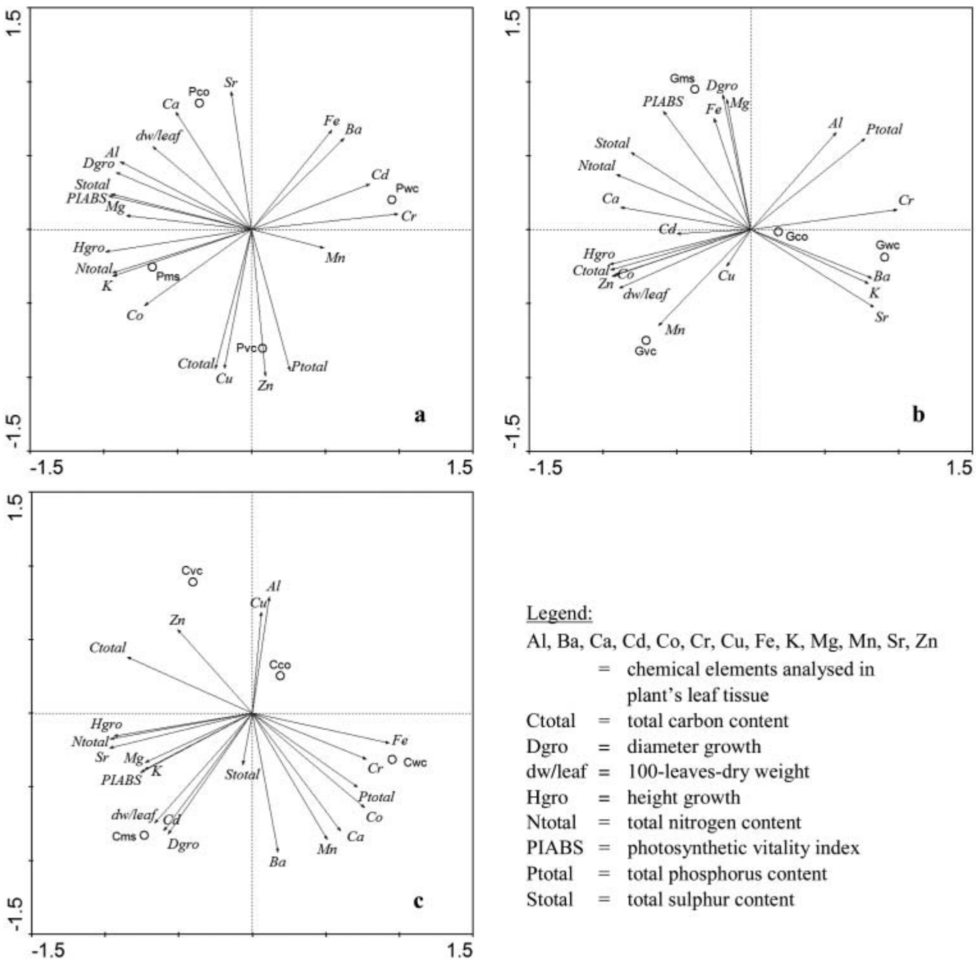


Figure 3 Correlation between leaf analysis data and growth as well as time period related PI_{ABS} values of the different trials (a: Platinum tailings trials; b: Gold tailings trials; c: Control soil trials).

plants growing in Pwc, Gwc, and Cwc, respectively, were characterized by relatively high heavy metal contents (Figure 3, each right quadrant). Thus, *Searsia lancea* to a certain degree could serve as an appropriate woody plant species for phytoremediation purposes since it is able to decontaminate heavy metal polluted soils. However, little is known about the heavy metal tolerance of *Searsia lancea* compared to existing knowledge on heavy metal resistance and/or tolerance as well as heavy metal uptake and translocation by "typical" remediation woody plant genera like *Salix* and *Populus* (e.g., Punshon and Dickinson 1999; Kuzovkina, Knee, and Quigley 2004; Zalesny and Bauer 2007; Neuschütz and Greger 2010).

CONCLUSION

The outcome of the experiments reported here offer a feasible rehabilitation method with relatively low environmental hazard potential. Thus, especially mature sewage sludge as an organic ameliorant may support the initial growing phase when added to the root zone of Karee trees. An explanation for the superior performance of the mature sewage sludge may possibly be found in its nutrient composition.

Vermicompost did not meet the initial expectations and thus cannot be recommended for rehabilitation due to relatively high costs and only moderate benefits. According to Pollmann and Van Rensburg (2009), there are hints of positive effects of woodchips compost regarding the enhancement of microbial activity and long-term improvement of soil substrate quality. However, due to its occasionally elevated heavy metal content the chemical composition of woodchips compost has to be controlled, when designated as soil ameliorant.

Searsia lancea provides good qualities for mine tailings rehabilitation and possibly offers potential for phytoremediation purposes.

Overall, promoting the stabilization of the upper surface of mine tailings, serves to control wind erosion by mitigating the risk of dust storm events. Furthermore, by using appropriate South African indigenous tree species such as *Searsia lancea,* the esthetical value of the surrounding area of a mine tailings dam could be enhanced.

Consequently, it has to be stated that there is a need for long-term studies, considering the usage of optimized mixtures with woodchips compost and mature sewage sludge.

SUPPORTING INFORMATION

Table S1 Recovery rate and repeatability (standard deviation) for main/trace elements as well as total P, C, N, S in needle standard (National Institute of Standards and Technology, NIST 1575a, *Pinus taeda*).

Element	NIST standard	Mean	STDEV	STDEV [%]	Recovery [%]
Potassium K	4.17 ± 0.07 mg/g	3.44	0.11	3.26	82.5
Magnesium Mg	1.06 ± 0.17 mg/g	0.96	0.01	0.54	90.7
Calcium Ca	2.5 ± 0.1 mg/g	2.40	0.02	1.00	96.0
Phosphate P_{tot}*	1.07 ± 0.08 mg/g	1.01	0.01	1.34	94.6
Nitrogen (total) N_{tot}**	n.n. # mg/g	11.2	0.03	0.24	–
Carbon (total) C_{tot}**	n.n. # mg/g	493.1	1.66	0.34	–
Sulphur (total) S_{tot}**	n.n. # mg/g	2.2	0.04	1.84	–
Aluminium Al	580 ± 30 μg/g	558.0	12.59	2.26	96.5
Barium Ba	6.0 ± 0.2 μg/g	5.48	0.13	2.39	91.3
Cadmium Cd	0.233 ± 0.004 μg/g	0.25	0.01	4.33	105
Cobalt Co	0.061 ± 0.002 μg/g	0.05	0.006	11.9	82.19
Iron Fe	46 ± 2 μg/g	48.0	3.29	6.58	104.4
Chromium Cr	0.3 – 0.5 μg/g	0.72	0.07	9.28	144
Manganese Mn	488 ± 12 μg/g	470	4.38	0.93	96.3
Copper Cu	2.8 ± 0.2 μg/g	3.81	0.48	12.5	136
Zinc Zn	38 ± 2 μg/g	41.8	0.66	1.57	110
Strontium Sr	n.n. # μg/g	6.47	0.05	0.72	–

STDEV = standard deviation
*photometric via phosphate
**element analyser
#not indicated by NIST

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