

Response of soil enzyme activities to synergistic effects of biosolids and plants in iron ore mine soils

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Abstract Past mining activities in Swaziland have left a legacy of abandoned mine sites (iron ore, asbestos, diamond and coal mine dumps), all of which have not been reclaimed. These sites were recently (2013) considered by the country's wastewater treatment authorities as suitable places where biosolids can be applied, firstly as a biosolids disposal alternative and, secondly, as a strategy to accelerate mine soil remediation through phytostabilization. In order to understand the effects that this might have on mine soil conditions and microbiota, two (2) plant growth trials were conducted in biosolid-treated iron mine soils and one (1) trial on undisturbed soil, under greenhouse conditions, for twelve (12) weeks. According to the results obtained, the combination of biosolids and plants led to significant improvements ($p < 0.05$) in parameters related to soil fertility. Significant increases ($p < 0.05$) in alkaline phosphatase, β -glucosidase and urease soil enzyme activities were also observed. Copper and zinc were significantly ($p < 0.05$) increased (Cu from 17.00–50.13 mg kg⁻¹; Zn from 7.59–96.03 mg kg⁻¹); however, these sludge-derived metals did not affect enzyme activities. Improvements in soil physicochemical conditions, organic matter–metal complexes, effects of plants on metals and the essentiality of Cu and Zn to soil enzymes were thought to have masked the effects of metals. Increases in soil enzyme activities were considered to be indicative of improvements in the quality, fertility health and self-purification capacity of iron mine soils due to synergistic effects of biosolids and plants.

Keywords Mining · Biosolids disposal · Soil physicochemical conditions

Introduction

The mining industry is an important sector considered by many countries as necessary for economic development, especially in Southern Africa where it has been one of the mainstays for over a century (McCulloch 2003). Although mining can bring much economic prosperity (Yan et al. 2013; Wolff et al. 2011), large areas of dereliction often result once mining has ceased (Stuben et al. 2001; Tordoff et al. 2000), due to land clearance, transportation activities and generation of vast amounts of mine wastes on top of existing vegetation (Yan et al. 2013).

The *Ngwenya* iron ore mine site in Swaziland is no exception. The site is currently in a derelict state as a result of open-pit mining operations that were carried out from 1964 (Wäitzenegger et al. 1970; Sneesby 1968) to 1980 (McLoughlin and Mehra 1988) and also from 2011 to present (Swaziland Environmental Authority [SEA] 2011). Sites degraded by mining operations, such as *Ngwenya* (iron ore mine dumps), *Dvokolwako* (diamond mine dumps), *Have-lock* (asbestos mine dumps) and *Maloma* (coal mine dumps), were recently (2013) considered by the Swaziland Water Services Corporation (SWSC) as an opportunity that can alleviate the Corporation's biosolids disposal problems. Presently, the disposal of biosolids in Swaziland is a problem to which no permanent solution has yet been identified. In fact, the handling of biosolids and the achievement of environmentally sound and economically feasible disposal strategies are currently major issues in wastewater treatment, not only in Swaziland but in many places around the world (Deepesh et al. 2014; Fytili and Zabanitou 2008; Contin et al.

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2012). The controlled application of biosolids in mine sites can greatly improve soil physicochemical conditions since these organic residues contain organic matter (OM) that acts as a soil conditioner which can improve soil aeration, water-holding capacity (Bourioug et al. 2014; Hua et al. 2008; Wong et al. 1998, etc.), contributing to erosion control, slope stabilization (Luczkiewicz 2006) and aggregate stability (Bozkurt et al. 2010). Improvements in soil conditions could enhance re-establishment of plants, which are important in reducing mobility and bioavailability of toxic metal in soils (Zodrow 1999; Wong 2003; Santibáñez et al. 2008), thus preventing migration of trace elements to groundwater or their entry into the food chain (Ali et al. 2013).

The utilization of biosolids, therefore, in the above manner is an economical and practical way to recycle sewage sludge (Nicolas et al. 2014; Terrason et al. 2010; Ogleni and Ozdemir 2010; Contin et al. 2012) rather than dumping it in landfills or burning it in incinerators (Terrason et al. 2010). This also serves the dual purpose of recycling biosolids while remediating soil volume lost through mining (Waterhouse et al. 2014).

However, since land application of biosolids is still generally regarded as controversial due to possible human health and environmental risks (Luczkiewicz 2006), on account of elevated metal concentrations contained therein (Bourioug et al. 2014; Hua et al. 2008; Forsberg and Ledin 2006; etc.), the SWSC commissioned a number of studies to investigate the impacts of biosolids in mine soils with regard to various aspects including soil physicochemical conditions, plant establishment and growth and metal accumulation in plants. Although they are important, these aspects are based on conventional physical and chemical soil analyses and visually distinguishable aboveground indicators, such as vegetation, and are unable to account for soil microbiota, which are the basis of terrestrial ecosystems (Claassens et al. 2008). Therefore, in order to holistically assess the ameliorative influence of biosolids, it was deemed necessary to study belowground bioindicators in addition to aboveground changes. Thus, the purpose of this study was to investigate soil enzyme activities in iron ore tailings subsequent to the application of biosolids and plant establishment. The study was carried out at the North-West University (Potchefstroom Campus), South Africa, between September 2014 and May 2015.

Materials and methods

In the previous study (Cele and Maboeta 2016), the influence of biosolids on soil physicochemical status was studied. The current study investigates the influence of biosolids on soil enzyme activities; however, since enzymes are studied on soils from the previous study, some

methods and data from the previous study are shown in the current study in order to demonstrate relationship between biosolids, soil physicochemical status and soil enzymes.

(1) Sample collection and preparation

Bulk samples of iron ore tailings were collected at *Ngwenya* iron ore mine (latitude 26°12'20.97" and longitude 31°01'56.89"). Biosolids were collected at *Ezulwini* wastewater treatment plant (latitude 26°24'11.94" and longitude 31°11'56.53"). Undisturbed topsoil was also collected at a distance of 2.5 km away from the iron ore tailings dumps, from the top 0–20 cm layer. Undisturbed topsoil was used as reference soil (RS). Samples were air-dried for 14 days, after which they were crushed and blended together using mortar and pestle and passed through a 2-mm sieve.

(2) Design of plant trials

In many mine soil remediation projects, the use of stockpiled topsoil is an important aspect of mine soil remediation, especially when mixed with biosolids (Brown et al. 2014; Sydnor and Redente 2002). According to *Ngwenya* mine authorities, topsoil will be used in re-vegetation of the site subsequent to mining (SEA 2011). However, it is a known fact that replacement topsoil may not always be available (Butt et al. 1995). In view of these realities, three plant trials were conducted. In the first trial, biosolids and undisturbed soil were added to tailings at 0 (Control), 10, 25, 50, 75 and 100 t ha⁻¹; this was labelled as the TUSB treatment (tailings, plus undisturbed soil plus biosolids). In the second trial, biosolids only were added to tailings at the same rates; this was called TB treatment (tailings plus biosolids). Each treatment was replicated three times. In order to ensure consistency, substrates were mixed in one large container for each treatment and were left to equilibrate for 30 days. Once weekly, the mixtures were turned over and mixed thoroughly. After 30 days, the substrate from each large container was distributed into plant growth pots. To prevent the substrate from escaping through the holes at the bottom and the possibility of roots growing out of the pots (Hu et al. 2013a, b), pieces of frost cloth were placed at the bottom before placing the substrate. Since the 100 % water-holding capacity (WHC) of tailings was determined prior to trial set-up, each pot containing the mixed substrate was adjusted to 60 % WHC using tap water and left to stabilize for 2 days (Ruiz et al. 2009; Wen et al. 2004; Wang et al. 2007). Thereafter, 25 seeds (Grant et al. 2002) of *Cynodon dactylon* were sown 2 cm deep (Du et al. 2014) in each pot.

In the third trial, seeds of the same plant species were sown in RS, which was collected from the vicinity of the mine site but in an undisturbed area. Biosolids were not



added to RS. In order to assess the extent of the ameliorative influence of biosolids, results from trial 1 and 2 were to be compared to results from trial 3 (RS). This was based on the Society for Ecological Restoration's definition of ecological restoration, which is the process of assisting the recovery of an ecosystem towards a reference state (Waterhouse et al. 2014).

After 4 weeks of initiation of all trials (Grant et al. 2002), seedlings in all trials were thinned to ten plants per pot (Seleiman et al. 2012; Jiemin and Wong 2013). Plants were watered twice weekly (Rate et al. 2004; Waterhouse et al. 2014) and were randomly placed in the greenhouse (Perez-de-Mora et al. 2007; Verdugo et al. 2011; Meeinkuirt et al. 2012) and relocated once a week. After 12 weeks, plant trials were terminated.

Plants were cautiously removed, and the roots were gently shaken to free tightly adhering soil (Dongmei and Changqun 2008). Roots and shoots were divided and carefully washed with tap water in order to remove any remaining soil, dust deposits and surface substrates (Novo et al. 2013). Immediately after cleaning, the fresh weight of roots and shoots for each plant in each pot was determined by measuring on a scale. Plants were dried in the oven at 80 °C for 48 h and thereafter removed and cooled down to room temperature. The dry weights of roots and shoots were determined again by weighing on a scale.

(2) Soil preservation and enzyme analysis

About 1 kg of soil from each replicate pot was placed in tightly sealed plastic bags while still moist. Samples were then placed in a refrigerator at 4 °C (Gianfreda et al. 2005; Lai et al. 1999; Li et al. 2015; Perez-de-Mora et al. 2005; Jin et al. 2014; Qu et al. 2011; Romero et al. 2005; Moreno et al. 2009; Koo et al. 2012) to preserve biological properties (Claassens et al. 2008; Maboeta et al. 2006), and chemical analysis was carried out within 3 weeks. Immediately prior to analysis, samples were removed from the refrigerator, placed on petri dishes and laid on the table for air-drying. The following morning, samples were thoroughly crushed with pestle and mortar and passed through $a < 2$ mm sieve. Each sample was analysed in triplicate (control, sub-samples 1 and 2) (Sastre et al. 1996) and for each sample, three types of enzymes were investigated, namely β -glucosidase, alkaline phosphatase and urease. Briefly, the estimation of β -glucosidase was based on the determination of the released *p*-nitrophenol, after the incubation of soil with *p*-nitrophenyl glucoside solution for 1 h at 37 °C (Dick et al. 1996). For the estimation of alkaline phosphatase, the procedure was based on the determination of *p*-nitrophenol released after the incubation of soil with *p*-nitrophenyl phosphate for 1 h at 37 °C (Tabatabai 1994). For the urease enzyme, the method was based on colorimetric determination of released ammonia

after the incubation of soil samples with urea solution for 2 h at 37 °C (Kandeler and Gerber 1988).

(3) Soil chemical analysis

Total metals were determined in accordance with protocols provided by the USEPA, in Method 3050b (1996). This was done by first digesting soil samples in nitric acid (HNO₃) and hydrogen peroxide (H₂O₂). The resultant digestate was then analysed by inductively coupled plasma mass spectrometry (ICP-MS).

WHC was calculated according to the ISO/FDIS 17512-1:2007 (E) method as described by the International Organization for Standardization (ISO) (2007). Acidity was determined by measuring pH (H₂O) in 1:2.5 soil/water ratio suspension on a mass basis and pH (KCl) in 1:2.5 10 mol KCl solution. Salinity was determined by measuring electrical conductivity (EC). For cation exchange capacity (CEC), exchangeable plus water soluble cations (K⁺, Ca²⁺, Na⁺, Mg²⁺) and anions (NH₄⁺, NO₃⁻, PO₄⁻), the ammonium acetate (1 mol dm⁻³, pH 7) and Bray 1 methods were used to determine these parameters (Soil Science Society of South Africa 1990). OM matter was determined by the loss on ignition (LOI) method, as described by Nelson and Sommers (1982) and Wilke (2010).

(4) Statistical analysis

The SPSS statistical software version 22 (2015) was used in all data analysis in this study. The Kolmogorov–Smirnov (K–S) test was used to test the distribution of data. For normally distributed data, the analysis of variance ANOVA (at a significance level of 0.05) and homogeneity of variance test were carried out. Differences of means within treatments were investigated by the Tukey's *B* test, and the findings were checked with the Games–Howell procedure. In order to assist in interpreting results, several options for one-way ANOVA were included in the tests, and these were descriptive statistics (table of means, standard deviations, ranges, confidence intervals for each group and means plots). The Brown's and Welch's *F*-ratio versions were selected in order to take care of the effects of nonconformity to homogeneity of variance. Non-normally distributed data were analysed by the nonparametric version of one-way independent ANOVA (Kruskal–Wallis test) (Field 2009).

Results and discussion

A pre-trial assessment of soil physicochemical status and soil enzyme activities was conducted on tailings, RS and biosolids, and the results are presented in Tables 1 and 2.

Subsequent to plant growth trials, it was observed that the application of biosolids (with or without soil) followed

Table 1 Pre-trial characterization of physicochemical status of iron ore tailings, reference soil and biosolids ($n = 3$)

Soil parameters	Iron ore tailings	Reference soil	Biosolids
Ca ²⁺ (cmol (+) kg ⁻¹)	0.24	0.62	8.90
Mg ²⁺ (cmol (+) kg ⁻¹)	0.49	0.49	1.40
K ⁺ (cmol (+) kg ⁻¹)	0.05	0.41	0.45
Na ⁺ (cmol (+) kg ⁻¹)	ND	0.03	0.06
PO ₄ ⁻ (mg L ⁻¹)	ND	ND	ND
NO ₃ ⁻ (mg L ⁻¹)	2007.20	82.47	86152
NH ₄ ⁺ (mg L ⁻¹)	7.81	9.11	121.7
CEC (cmol (+) kg ⁻¹)	8.38	24.40	35.29
pH (H ₂ O)	4.92	4.62	4.24
OM (% C)	0.02	4.19	17.66
EC (dS m ⁻¹)	0.25	0.17	3.82
WHC (100 % ml)	1.24	2.63	2.62
Heavy metals (mg kg ⁻¹)			
Ni	73.50	107.70	41.35
Cu	18.54	43.70	341.25
Zn	13.25	22.82	1146.25
Cd	0.05	0.05	1.29
Hg	0.13	0.50	3.33
Pb	3.83	11.50	67.19

Table 2 Pre-trial characterization of soil enzyme activities in iron ore tailings, reference soil and biosolids ($n = 3$)

Experimental soils	Soil enzymes		
	Alkaline phosphatase (mg PN/kg soil/h)	β -glucosidase (mg PN/kg soil/h)	Urease (mg NH ₄ -N/kg soil/2 h)
RS	2117.05	331.07	1.13
Biosolids	4433.92	382.41	1.30
Iron ore tailings	225.40	60.77	0.23

Table 3 Mean (\pm SE, $n = 3$) soil enzyme activities in the TUSB treatment after 12 weeks of plant growth trial

Treatment (t ha ⁻¹)	Soil enzymes		
	Alkaline phosphatase (mg PN/kg soil/h)	β -glucosidase (mg PN/kg soil/h)	Urease (mg NH ₄ -N/kg soil/2 h)
RS	1069.57 \pm 2.77	1552.55 \pm 242.10	119.00 \pm 8.37
0 (control)	194.25 \pm 2.85	27.90 \pm 2.92	6.39 \pm 1.07
10	227.62 \pm 2.73	67.21 \pm 10.62	5.59 \pm 1.85
25	199.36 \pm 2.69	107.93 \pm 2.48	9.25 \pm 0.84
50	350.46 \pm 2.69	248.38 \pm 29.84	31.38 \pm 8.66
75	181.41 \pm 2.96	286.63 \pm 22.04	24.21 \pm 4.87
100	305.14 \pm 2.92	471.55 \pm 39.12	25.95 \pm 1.25

by the establishment of plants on iron mine soils led to significant ($p < 0.05$) increases in the activities of alkaline phosphatase, β -glucosidase and urease soil enzymes, as listed in Tables 3 and 4. However, in the TUSB treatment, p was >0.05 for alkaline phosphatase activities at 0 and 25 t ha⁻¹, β -glucosidase activities at 50 and 75 t ha⁻¹ and

urease activities at 0 and 10 t ha⁻¹. In the TB treatment, p was also >0.05 for alkaline phosphatase activities at 10 and 100 t ha⁻¹ and urease activities at 0 and 10 t ha⁻¹.

In control soil (planted tailings without biosolids), β -glucosidase activities were not detectible; however, in all planted tailings that received biosolids, there were



Table 4 Mean (\pm SE, $n = 3$) soil enzyme activities in the TB treatment after 12 weeks of plant growth trial

Treatment (t ha ⁻¹)	Soil enzymes		
	Alkaline phosphatase (mg PN/kg soil/h)	β -glucosidase (mg PN/kg soil/h)	Urease (mg NH ₄ -N/kg soil/2 h)
RS	1069.57 \pm 2.77	1552.55 \pm 242.10	119.00 \pm 8.37
0 (control)	194.25 \pm 2.85	27.90 \pm 2.92	6.39 \pm 1.06
10	277.66 \pm 2.85	46.46 \pm 3.25	8.09 \pm 1.34
25	169.22 \pm 2.89	85.79 \pm 9.25	9.08 \pm 1.63
50	220.57 \pm 2.74	130.39 \pm 3.93	10.93 \pm 2.14
75	222.63 \pm 3.06	185.05 \pm 21.55	18.68 \pm 4.34
100	278.56 \pm 2.97	314.97 \pm 39.52	25.44 \pm 1.31

Table 5 Mean (\pm SE, $n = 3$) concentrations/levels of soil parameters subsequent to 12 weeks of plant growth trial in the TUSB treatment

Treatment (t ha ⁻¹)	Soil parameters ^a					
	Ca ²⁺	PO ₄ ⁻	OM	WHC	CEC	pH
RS	0.99 \pm 0.02	0.09 \pm 0.03	3.94 \pm 0.04	2.87 \pm 0.13	16.28 \pm 1.10	5.17 \pm 0.06
0 (control)	0.36 \pm 0.03	0.01 \pm 0.00	0.21 \pm 0.02	1.98 \pm 0.03	6.53 \pm 0.42	6.66 \pm 0.13
10	0.51 \pm 0.04	0.10 \pm 0.25	0.57 \pm 0.02	2.07 \pm 0.01	9.74 \pm 1.05	5.80 \pm 0.04
25	0.58 \pm 0.01	0.09 \pm 0.24	0.61 \pm 0.03	2.10 \pm 0.02	11.97 \pm 0.88	5.80 \pm 0.04
50	0.67 \pm 0.03	0.12 \pm 0.09	0.97 \pm 0.03	2.78 \pm 0.04	10.51 \pm 0.95	5.49 \pm 0.02
75	0.78 \pm 0.03	0.33 \pm 0.06	1.15 \pm 0.03	2.85 \pm 0.03	12.27 \pm 0.62	5.40 \pm 0.03
100	1.17 \pm 0.41	0.08 \pm 0.06	1.45 \pm 0.08	3.14 \pm 0.23	11.23 \pm 1.14	5.82 \pm 0.04

^a For units, see Table 2**Table 6** Mean (\pm SE, $n = 3$) concentrations/levels of soil parameters subsequent to 12 weeks of plant growth trial in the TB treatment

Treatment (t ha ⁻¹)	Soil parameters ^a					
	Ca ²⁺	PO ₄ ⁻	OM	WHC	CEC	pH
RS	0.99 \pm 0.02	0.09 \pm 0.03	3.94 \pm 0.04	2.87 \pm 0.13	16.28 \pm 1.10	5.17 \pm 0.06
0 (control)	0.36 \pm 0.03	0.01 \pm 0.00	0.22 \pm 0.02	1.98 \pm 0.03	6.53 \pm 0.42	6.66 \pm 0.13
10 t ha ⁻¹	0.56 \pm 0.04	0.49 \pm 0.25	0.55 \pm 0.02	1.96 \pm 0.01	9.78 \pm 1.05	5.76 \pm 0.04
25 t ha ⁻¹	0.61 \pm 0.01	0.55 \pm 0.24	0.54 \pm 0.03	2.06 \pm 0.02	8.86 \pm 0.88	5.84 \pm 0.04
50 t ha ⁻¹	0.80 \pm 0.03	0.42 \pm 0.09	0.82 \pm 0.03	1.98 \pm 0.04	13.02 \pm 0.95	5.72 \pm 0.02
75 t ha ⁻¹	0.90 \pm 0.03	0.68 \pm 0.06	1.01 \pm 0.03	2.33 \pm 0.03	11.57 \pm 0.62	5.63 \pm 0.03
100 t ha ⁻¹	1.19 \pm 0.04	1.41 \pm 0.20	1.42 \pm 0.03	2.08 \pm 0.05	9.23 \pm 0.61	6.07 \pm 0.04

^a For units, see Table 2

significant increases ($p < 0.05$) in β -glucosidase activities, and this was consistent with each increase in biosolids application. At 100 t ha⁻¹ in both treatments, β -glucosidase activities were higher than alkaline phosphatase and urease activities, reaching 471.55 and 314.97 mg PN/kg soil/h (TUSB treatment and TB treatment, respectively). Although urease enzyme activities were the lowest in both treatments, increases were nonetheless significant ($p < 0.05$), particularly from 25–100 t ha⁻¹. Generally, urease activities increased with each increase in biosolids

application rate. Alkaline phosphatase activities were also higher in all planted tailings that received biosolids (10–100 t ha⁻¹) than in control (0 t ha⁻¹), although increases were not consistent.

The observed increases in soil enzyme activities were attributed to improvements in soil physicochemical conditions, due to the application of biosolids and the presence of plants. According to Kizilkaya et al. (2011), soil enzyme activities are affected by soil conditions including pH, ion conditions, temperature, soil texture, OM content, CEC and

microbial biomass. In this study, there were significant ($p < 0.05$) increases in soil parameters related to soil fertility such as OM, phosphorus (PO_4^-), calcium (Ca^{2+}), CEC and WHC. At 100 t ha^{-1} (TUSB treatment), Ca^{2+} and WHC were significantly higher ($p < 0.05$) than in RS. In the TB treatment, Ca^{2+} and PO_4^- were also significantly higher ($p < 0.05$) at 100 t ha^{-1} than in RS, as listed in Tables 5 and 6.

OM is known to result in positive changes in physical and chemical soil properties, such as water-holding and sorption capacities, nutrient content and availability (Zhao et al. 2013) and CEC (Khan 2002). CEC in control was $6.53 \text{ cmol (+) kg}^{-1}$; however, in tailings that received biosolids, it increased to $12.27 \text{ cmol (+) kg}^{-1}$. According to Jones (2012), CEC within $11\text{--}50 \text{ cmol (+) kg}^{-1}$ is highly desirable since it is associated with high OM content, high capacity to hold plant nutrient elements within the soil profile and high WHC.

With regard to acidity, pH decreased (at 50 and 75 t ha^{-1}) from the very slightly acidic range (also referred to as neutral range, $6.5\text{--}7.0$) to the very strongly acidic range (<5.5). At 100 t ha^{-1} , however, pH increased again to the medium acidic range ($5.5\text{--}6.0$) (TUSB treatment). In the TB treatment, pH remained in the medium acidic range ($5.5\text{--}6.0$) from 10 to 75 t ha^{-1} and increased to the slightly acidic range ($6.0\text{--}6.5$) at 100 t ha^{-1} . The decrease in pH was attributed biosolids application because according to the results of pre-trial assessment on all experimental soils, biosolids were strongly acidic (pH 4.24) as listed in Table 2. Even though biosolids were strongly acidic, pH in all biosolid-treated tailings did not fall below 5.4. Also, at the highest application rate (100 t ha^{-1}), pH was 5.82 (TUSB treatment) and 6.07 (TB treatment). These results were rather favourable because a pH level of 5.0 or higher is suitable for N fixation (Maiti and Saxena 1998). Most importantly, pH was higher in all biosolid-treated tailings than in RS.

Salinity also remained between 0 and 2 dS m^{-1} , which is classified as non-saline and optimum for plant growth (Maiti and Saxena 1998). According to Meeinkurt et al. (2012), 0.2 dS m^{-1} is the standard level of acceptable salinity, while soils with electrical conductivity values $>4 \text{ dS m}^{-1}$ are considered saline (Hodson and Donner 2013).

The positive effects of biosolids on soil enzymes, due to improvements in soil physicochemical conditions, have also been observed in many studies e.g. Sastre et al. (1996), Lai et al. (1999), Kizilkaya and Bayrakli (2005), Perez-de-Mora et al. (2005) and Fernandes et al. (2005).

Notably, despite significant increases in soil enzyme activities in the TUSB and TB treatments, the activities of the same soil enzymes remained higher in RS than in all soils in the TUSB and TB treatments. This was ascribed to

the fact that OM was highest in RS than in all soils in the TUSB and TB treatments. According to Chang and Chiu (2015), high soil OM sustains high microbial biomass and high enzyme activities.

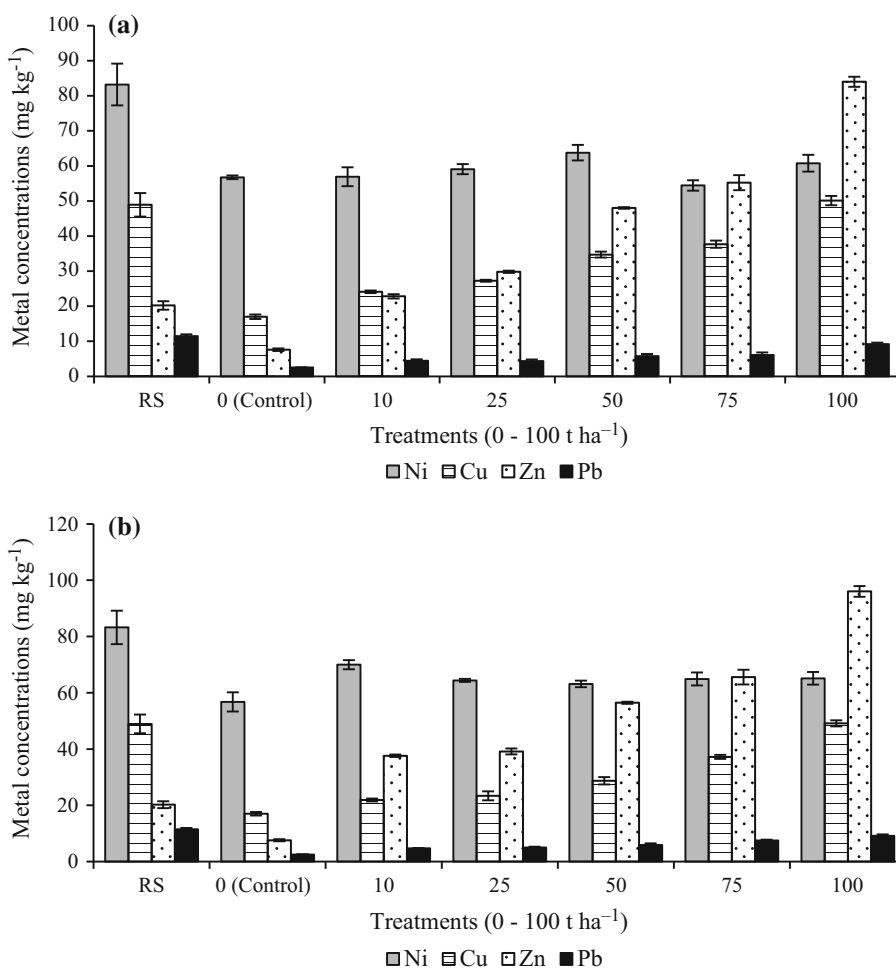
In addition to the application of biosolids, another possible explanation for the observed increases in soil enzyme activities was the presence of plants. Garcia-Gill et al. (2000) also found that enzyme activities were enhanced by the combination of plants and organic amendments because both organic waste incorporation and root exudates improved soil porosity and aeration. According to Hu et al. (2013a, b), plants affect soil enzyme activities in two different ways. Firstly, plants affect the soil biota by influencing the quantity and quality of organic substrates that reach the soil. Secondly, plants take up some heavy metals from soil, which may result in the reduction in heavy metal toxicity on soil enzymes.

Despite positive changes in soil enzyme activities, the application of biosolids led to significant increases ($p < 0.05$) in Cu, Zn and Pb as shown in Fig. 1. However, Ni, Cu and Pb concentrations were lower in all biosolid-treated tailings than in RS. Cd and Hg concentrations were $<1 \text{ mg kg}^{-1}$ in all soils (RS, TUSB and TB). In the TB treatment, Zn concentrations were higher (37.61 mg kg^{-1}) than in RS (20.22 mg kg^{-1}) even at the lowest application rate (10 t ha^{-1}), and increased with each increase in biosolids application rate up to 96.03 mg kg^{-1} . The increases in Zn concentrations were ascribed to biosolids application because it has been stated that sewage sludges may contain high concentrations of Zn. It has been shown in long-term experiments that Zn is the most bioavailable metal in soils treated with sewage sludges (Bradl 2005).

Generally, metals are known to inhibit soil enzyme activities (Angelovicova et al. 2014; Pan and Yu 2011; Belyaeva et al. 2005; Kizilkaya and Bayrakli 2005; etc.); however, according to the results obtained in this study, enzyme activities were not affected by increases in metal concentrations. Instead, β -glucosidase and urease enzyme exhibited increases with each increase in biosolids application. The apparent resistance of soils enzymes to metals can be attributed to a number of factors. Firstly, improvements in soil physicochemical conditions, especially OM, may have inactivated the impacts of metals. According to Gülser and Erdogan (2008), OM-metal complexes occur in OM and soil solutions and would prevent the metals from interacting directly with the active sites of enzymes. Kizilkaya and Bayrakli (2005) found that sludge application increased available metal (Cu, Ni, Pb and Zn) contents in the soils. However, while this was the case, it was concluded that the full extent of the negative effects of metals on enzymatic activities might have been masked by the positive effects of sewage sludge on soil physical and chemical properties.



Fig. 1 Mean (\pm SE, $n = 3$) metal concentrations in the TUSB (a) and TB (b) treatments subsequent to 12 weeks of plant growth trials



Secondly, the essentiality of Cu and Zn could be another factor that enabled soil enzymes activities to increase despite increases in metals. According to Alloway (2013), Zn is a constituent of several enzymes with roles in carbohydrate and protein synthesis. In fact, many enzymes cannot function without Zn (Bellás et al. 2012). Cu is also an essential trace element that is vital to the health of all living organisms (humans, plants, animals and micro-organisms). It is found in a variety of enzymes and proteins, including the cytochrome c oxidase and certain superoxide dismutases (Oorts 2012). It can be said, therefore, that although Cu and Zn were increased in soil by addition of biosolids, they probably remained within a tolerable range to soil enzymes. In fact, in many studies (e.g. Bellás et al. 2012; Dewey et al. 2012; Haggmann et al. 2015; Hu et al. 2013a, b), the inhibition of soil enzymes activities was only observed at higher metal concentrations than what was observed in this study.

Thirdly, the manner in which metals were added to tailings may also explain the seeming lack of impacts on soil enzymes. It has been said that metals in sewage sludge are

invariably strongly complexed with mineral and organic components of the sludge and are, therefore, considered to be far less biologically available than metals in salts (Speir 2008; Oorts 2012). Mertens and Smolders (2013) found that metal salts are, in fact, capable of overestimating toxicity. The application of sewage sludge or manure to a soil may increase metal-binding capacity of the soil as these products contain OM and Fe or Al oxyhydroxides, which all have metal-binding properties. Additionally, metals present in these materials have a different speciation, and typically, they have lower availability compared to metal salts. Dindar et al. 2015 observed that soil enzyme activities were not affected by sludge-derived metals even for the highest sludge doses (200 t ha⁻¹).

Conclusion

This study sought to assess soil enzyme activities on iron mine soils subsequent to the application of biosolids and establishment of plants. Results showed that in addition to

general improvements in soil physicochemical conditions, the combination of biosolids and plants can also lead to significant improvements in soil enzyme activities. Increases in soil enzyme activities were considered to be indicative of improvements in the quality (Rao et al. 2014; Zhang et al. 2013; de Verennes et al. 2010; Moreno et al. 2009; Karaca et al. 2002; Manzano et al. 2014; Zhan and Sun 2014), fertility (Madejón et al. 2001), health and self-purification capacity (Zhang et al. 2013) of iron mine soils due to biosolids application and plant establishment. This study also showed that while metals are generally still regarded as the most dangerous pollutants that restrict land application of biosolids in many places, soil enzymes may tolerate sewage sludge-derived Cu and Zn concentrations of up to 50 and 96 mg kg⁻¹ (respectively).

With regard to Swaziland's current proposals to apply *Ezulwini* biosolids to iron mine soils as a remediation strategy, the observed improvements in soil physicochemical conditions and soil enzyme activities were considered partly favourable to these plans. Nonetheless, mine soil remediation encompasses a wide range of interlinked below- and aboveground aspects than just soil physicochemical conditions and enzyme activities. Further, it is widely accepted that mine soil remediation should ultimately lead to re-colonization of reclaimed sites by various species of soil-dwelling organisms in order to accelerate soil processes. Therefore, the extent of tolerable metal levels and the impacts thereof on soil organisms cannot be measured only through soil enzymes. For these reasons, we recommend further investigations involving the effects of biosolids on other important soil-dwelling organisms (such as earthworms). The investigation of metal uptake by plants established on biosolid-treated iron mine soils must also be considered. Additionally, since this study was carried out under greenhouse conditions, it may be necessary to repeat these trials under field conditions in order to arrive at more satisfactory conclusions.

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