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Evaluation of metal uptake factors of native trees colonizing an abandoned copper mine – a quest for phytostabilization

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ABSTRACT

Accumulation and enrichment of heavy metals in the above ground parts of Australian native *Acacia pycnantha* (Ap) and *Eucalyptus camaldulensis* (Ec) growing in an abandoned copper mine located in Kapunda, South Australia have been studied. Cu and other metals (Na, Al, K, Ca, Fe, Zn, Cd and Pb) in plants and corresponding soils were analysed to evaluate plant interaction with soils containing heavy metals. As per the total metal analysis of leaf and corresponding soil samples, Ap accumulated 93.6 mg kg^{-1} of Cu in leaf while the corresponding soil concentration was 1632 mg kg^{-1}. The Ec accumulated 5341 mg kg^{-1} of Cu in leaf while the concentration of this heavy metal in soil was 65 mg kg^{-1} in soil. The ESEM spectral analysis also showed a high leaf concentration of Cu in Ec (7%) as against only 0.12% in Ap. The average bioconcentration factor for Cu, Zn, Cd and Pb in Ec was much higher than that of Ap. Similarly, enrichment factor was more in Ec for Cu, Zn and Pb than in Ap. In contrast, translocation factor for only Zn and Cd was high in Ap. This study points out that Ec and Ap have different stabilising potential in remediating heavy metals like Cu in mined soils.

1. Introduction

The effect of heavy metal toxicity on biota is severe and there is an urgency to control the level of toxicity and impact on public health, particularly from abandoned mine lands (AMLs). Ecological impacts in places such as AMLs can be controlled effectively by evaluating the accumulators against the non-accumulator plants prior to the phytoremediation processes (Ma, Rajkumar, Luo, & Freitas, 2013). A right rehabilitation practice is certainly a tool to control the heavy metal pollution and one such practices have been by the use of

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phytoaccumulators (Ali, Khan, & Sajad, 2013). The phytoremediation technology is employed over polluted sites and have served to be a boon to assist in pollution clean up efforts (Nirola, Mallavarapu, Aryal, & Naidu, 2015). As such, copper is one of those toxic metals that have a capacity to bind to albumen and other small molecules in the human body as ‘free copper’ to cause nerve damage (Brewer, 2010). An effective phytoremediation would have saved the inhabitants near a mine site in Mexico from elevated levels of lead and arsenic in their blood, and are more severe in children (González & González-Chávez, 2006). Identification of suitable remediating species of plants could reduce the ecological risks of heavy metals such as Cu to contaminate water bodies and damage gills causing premature death of fishes (Couillard, Courtenary & Mcdonald, 2008). It is thought to be an important component for identifying the species positively responsible for bioaccumulation of pollutants being transferred from plants into the food chain (Krumins, Goodey, & Gallagher, 2015).

A typical practice during industrial revolution was to abandon mines once mineral extraction was conducted. The estimated number of abandoned mines in the developed nations was 630309 by the year 2000 (Van Zyl, Sassoon, Digby, Fleury, & Kyeyune, 2002). Wait (2012) reports 5858 derelict and ownerless mines in South Africa, and Mackasey (2004) records 5500 derelict (abandoned) mines in Japan alone. There are about 1800 final mine voids and 150 operational open cut mines in Western Australia (Doupé & Lymbery, 2005). Moreover, approximately 2000 derelict mine site exists only in New South Wales, Australia that date back to the mid-1800s (Grant, Campbell, & Charnock, 2002). However, the exact figure of abandoned and post operational mine differs depending on the definition of what a mine site is.

The threshold references used for metals in the surface soils are Cd-3 mg kg^{-1}, Cu-60 mg kg^{-1}, Pb-300 mg kg^{-1}, and Zn-200 mg kg^{-1} with a level above 20 mg kg^{-1} for Cd and 300 mg kg^{-1} for Pb considered to be a health hazard (Ash & Truong, 2003). The threshold reference values are important indicators to determine soil pollution level as these values help to measure phytoremediation degree before and after the process. The accumulation of metals in the foliar region could be severe due to the reoccurring of soil pollution by metal accumulation in litter of mine site plants (Nouri et al., 2011). In a study of plant behaviour with soil heavy metals in Turkey, species such as *Euphorbia macroclada*, *Verbascum cheiranthifolium* and *Astragalus gummifer* were found to accumulate 3–4 times higher levels of heavy metals than those found in the soil (Sagiroglu, Sasmaz, & Sen, 2006). In another study, Reichman, Menzies, Asher, and Mulligan (2006) assert that plant soil interaction explores its capacity to stabilise the heavy metal (HM) using native plants in AMLs. Of all the available rehabilitation technologies, phytostabilisation is a plant's ability to avoid exposure of pollutants to the environment by not translocating metals from soil to the above ground parts (Kurek & Majewska, 2012; Yoon, Cao, Zhou, & Ma, 2006). This is to contain heavy metal pollution by immobilizing the bioavailable components in the earth's biosphere through revegetation of operational, derelict and abandoned mine sites. One analysis of metal toxicity on copper in an abandoned mine in Florida (Yoon et al., 2006) reported the native grass species *Gentiana pennelliana* that could stabilise heavy metals such as Pb, Cu and Zn based on translocation factor (TF) study. Therefore, it has been established that species of plants having high metal concentration ratio ($\geq$1) of roots to soil is called bioconcentration factor (BCF), a low shoots to roots metal concentration ratio ($\leq$1) known as the translocation factor (TF) and $\leq$1 ratio of metal content in above ground plant parts over metal content in soil known as enrichment factor (EF) is considered to be ideal HM stabilizers (Sagiroglu et al., 2006; Sinha, Herat & Tandon, 2007). Therefore, in the present context of the use of plant species for mine site rehabilitation, phytoextraction or phytoaccumulation is effectively based on EF since the metal accumulation in above ground parts is exposed to biosphere (Baker, 1981).

We consider two native tree species growing naturally over many years in mine site as per our earlier study (Nirola et al., 2015). The leguminous *Acacia pycnantha* Benth (golden wattle), and non-leguminous *Eucalyptus camaldulensis* Dehnh (river red-gum) were included with an aim to find answers to the issues of bioavailability, bioaccumulation and biotransformation of metals. The Australian legume *A. pycnantha* (Ap) has attributes of root nodule formation and an active microbial activity, and give upper hand in growing resilient at the AML. Our earlier survey and screening at the mine site also indicated more Cu accumulation in the root zone for this species (Nirola et al., 2015). Further evidence for such resilience by legume Acacia has been documented for sites in the Sahara Desert (Brockwell, Searle, Jeavons, & Waayers, 2005). An estimated annual quantity of nitrogen fixed worldwide by legumes is 70–100 million tonnes with acacias fixing a substantial amount growing in around 5 million hectares of land worldwide (Brockwell et al., 2005). In terms of its drought tolerance mechanism, acacias have a morphological advantage to adapt to arid or xeric conditions including AMLs (Brodribb & Hill, 1993). Their leaves are modified into phyllodes with an abundance of thick sclerenchyma in the outer palisade mesophyll aiding its ability to survive drought conditions (Boughton, 1986; Midgley & Turnbull, 2003). The other native Australian species, *E. camaldulensis* (Ec) is widely distributed and exhibits a substantial drought tolerance capacity which is attributed to deep roots and tough leaf cuticles as seen through the ESEM image (Boland et al., 2006). However, most of the species of eucalyptus including *E. camaldulensis* have indicated an allelopathic effect on other vegetation, hampering the germination of seed (Ahmed, Hoque, & Hossain, 2008; Khan, Hussain & Khan, 2008; Yang, Liu, Ren, & Wang, 2009). In the current study site both of these species were found to be dominating the different landscapes of the polluted area. A curious thought to evaluate two taxonomically different plants is expected to add impetus to the issues of mine site rehabilitation with respect to avoiding phytoextraction and encouraging phytostabilization. Moreover, no earlier studies on metal uptake factors are available for widely used *A. pycnantha* in mine site rehabilitation.

2. Materials and methods

2.1. Site description and characterization

The abandoned mine at Kapunda is one of the oldest settlements in Australia that mined copper till 1879 AD. The mine site is located 79 km from Adelaide city. The site harbours arid to semi-arid type of vegetation with a calcareous soil. A thorough screening of nine prominent shrub and tree species were undertaken to check their metal enrichment factors namely *A. pycnantha*, *Racosperma pycnanthum*, *E. camaldulensis*, *Rosa canina*, *Lycium ferocissimum*, *Olea europaea*, *Acacia acinacea*, *Schinus molle*, *Leptospermum lanigerum*, and *Pinus halepensis* (Nirola et al., 2015). Among the nine species, the two species emerged as an outstanding metallophytes growing in the abandoned mine site. The golden wattle (Ap) was found growing around the periphery of the open cut area whereas the river red gum (Ec) was mostly concentrated towards the clayey soil downstream. Currently, the local Light Regional Council is responsible for managing the area as a heritage site to control pollution and to promote tourism (http://www.light.sa.gov.au). The plan of the regional council divides the mine site into cultural landscape (C), geological landscape (G) and environmental (regenerative) landscape (E) within the area of approximately 2 km (Fig. 1). Drainage from the site originates mainly from the regenerative landscape and flows into the Light River. The currently operated heritage mine track runs about 1.5 km mainly through the geological landscape (G). The geological landscape is a barren ground, fenced as a safety measure to avoid human intrusion. Fresh leaf, stem and roots of Ap and Ec including the corresponding soils were collected from G and E zones, respectively. Some of the sites having the unconfirmed indications of earlier land refilling by off-site soils were avoided.

2.2. *Sampling and processing*

The sampling plots were modelled to extend a maximum coverage (ISO, 2002; Hazelton & Murphy, 2007), starting from the geological landscape (G) towards the environmental landscape (E). Altogether each of 16 Ap and Ec plots were sampled including the associated soils as per the ecological quadrat principles (Krebs, 1999). The soil samples were collected from the rhizosphere at 15–20 cm depth using a plastic trowel from two points around the plant diameter of 2 m and homogenized. Plant parts were collected from around the canopy and bulked to form a composite sample to maintain a uniform representation. Fresh leaf samples were collected in an ice filled thermo-col box (5 °C) for ESEM studies to be analysed within 24 h. Inductively Coupled Plasma Mass Spectrometer/Optical Emission Spectrometer (ICP-MS/OES) to determine the total metal concentration in plant and soil samples were employed (Thomas, 2013). The plant samples for ICP-MS analysis were collected in the polypropylene containers and stored in laboratory at room temperature. The plant samples were washed three times in deionised water and oven dried at 60 °C. The oven-dried plant samples for ICP-MS analysis (leaf, stem and roots) were ground separately into 1 mm mesh sieve powder using an electric stainless steel grinder and were stored in the polypropylene containers. The soil samples were air-dried and sieved using a 2 mm plastic mesh sieve and stored in polypropylene containers for ICP-OES analysis.

2.3. *Characterization of samples*

Soil characterization included texture analysis using hydrometer method; standard tests were employed to measure pH, electrical conductivity, total dissolved carbon (TC), total dissolved nitrogen (TN) and total organic carbon (TOC) (Méndez, Gómez, Paz-Ferreiro, & Gascó, 2012). The potentiometric measurement of the supernatant suspension of soil: deionised water (w/v) at 1:5 ratio was used to measure the pH and EC (Sposito, 2008). A 5 gram soil was separately oven dried to compare and record the moisture content of the soil. A microwave accelerated reaction system (CEM-MARS X®) served to digest metals in *aqua-regia* for soils and HNO_3 for plants separately following USEPA method 3015a (Agazzi & Pirola, 2000). The digested samples were diluted up to 50 ml using Mili-Q water and were passed through a 0.45 μm filter using a syringe. The final test samples of 10 ml diluted suspension were put into ICP tubes for analysis in the Agilent 7500c (Agilent Technologies, Tokyo, Japan).

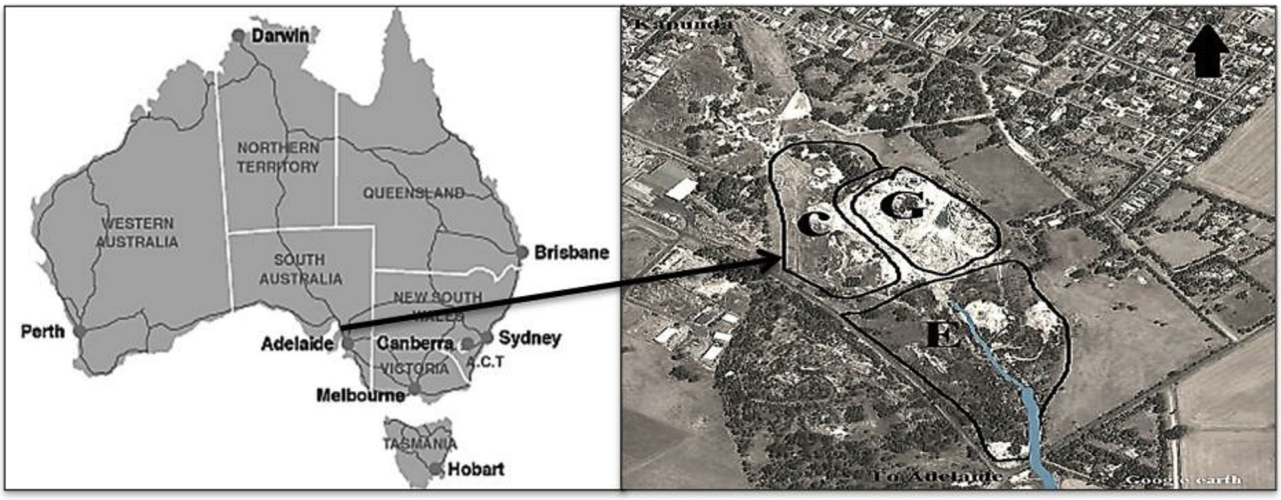

Fig. 1 – Site map of abandoned copper mine at Kapunda in South Australia. The letter 'C' represents the Conservation zone, 'G' represents the Geological zone and 'E' represents the Environmental zone.

The TC, TN and TOC were measured using air-dried, 2 mm mesh sieved soil dissolved in 1:5 (w/v) deionised (Mili-Q) water (You, Dalal, Mulligan, & Huang, 2015). The soil solutions were agitated in end-over shaker overnight and were centrifuged at 4000 rpm for 10 min. The supernatant was analysed in the Formacs^{series} total organic carbon, total nitrogen analyser using the UV-promoted per-sulphate digestion methodology. Analyses of total C (%) and N (%) were done by introducing 0.5 g of air-dried 2 mm mesh sieved and ground sample in a Trumac carbon-nitrogen-sulphur (CNS) analyser (Leco® Corporation, Michigan, USA). The standard blank, controlled and cross-referencing samples were introduced to maintain quality control after every 10 readings. The pH, EC, moisture content (H_2O%), dissolved TC (mg kg^{-1}), dissolved TN (mg kg^{-1}), dissolved TOC (mg kg^{-1}), total N % and total C % were recorded to average the duplicates of each sample.

2.4. *Spectroscopic characterization of plant matter*

The bioavailability of heavy metals like copper is based on the influence of soil physical factors (Cerqueira, Arenas-Lago, Andrade, & Vega, 2015). The localisation of the elemental distribution in the leaf sample by ESEM is another appropriate method to track the fate and status of accumulated metals in plant tissues (Stokes, Morris, & Groves, 2013). The process for characterization began by selecting and washing followed by the fresh leaves being air-dried. Leaf samples were sliced using a surgical blade into the thinnest transverse sections (TS) possible. The specimens were placed on a watch glass for sample sorting. The thinnest sections of both the Ap and Ec were separately chosen using a light microscope Digitek QC 3199. The sections were mounted on the aluminum specimen-mounts using double-sided tape and fitted onto the platform. The specimens were loaded into a "FEI Quanta 450 FEG ESEM" with an attached "EDAX" Apollo X SDD Energy Dispersive X-ray (EDX) detector for multiple examinations. High voltage (HV), low voltage (LV) and ESEM modes were utilised to examine the three samples, respectively, namely: 1) coated dry sample with 5 nm chromium by Quorum Q150T coating unit; 2) uncoated dry sample; and 3) uncoated fresh wet sample. However, for the uncoated fresh but wet specimen, the temperature of the sample holder was reduced to maintain constant water inside the cells within the optimum pressure as per standard procedure. Photo images and associated spectral data representing metal concentrations were obtained from leaves of Ap and Ec from each sampling site. As a result, the anticlinal images (TS) of the specimen were produced in the monitor along with corresponding spectral images that were saved for further analysis.

2.5. *Data analysis*

The data were analysed statistically using Microsoft Excel and IBM®SPSS®21 for Windows. Each sample had separate data arising from triplicate laboratory analysis results that was subjected to ANOVA, including the descriptive statistics. The data and pictures of ESEM anticlinal section of leaves corresponding to their graphical spectrum were carefully examined to compare with the data of ICP-MS. While analysing the ESEM data, each picture was tagged so that it corresponded to the radiation outcome. The spectral data were then compared with the corresponding ICP-MS data. The ESEM pictures were processed in Microsoft paint programme. The mean, range and average were calculated using triplicate result of each sample. Pearson's correlation coefficient determined the correlation between metals and soils, roots, stems and leaves.

3. Results

The physico-chemical parameters used in the present investigations include the EC, pH, TC, TN, TOC, N (%) and C (%) as presented in Table 1. The soil being tested in this study is from the copper and other metals contaminated dry mine spoil. The Ap rhizosphere soil was a sandy loam comprising 8% clay whereas the Ec rhizosphere soil had 72% clay as per the hydrometer readings (Méndez et al., 2012). The lack of water stagnation and low water holding capacity of soil result into low electrical conductivity and acidic nature of soil (Reuss & Johnson, 2012). In our study, the soil pH at Ap rhizosphere ranged from 4.4 to 6.8 (acidic to slightly acidic) and Ec rhizosphere ranged from 6.8 to 8.4 (neutral to basic). The average electrical conductivity of soils in Ap rhizosphere was 304 μS cm^{-1} and Ec rhizosphere was 7242 μS cm^{-1} (Table 1). Moreover, the leaf litter of Ec tends to have been responsible for basic soil and high salinity condition.

Table 2 provides information on heavy metals present in soil, root, stem and leaf for plants Ap and Ec. Although the average soil concentration of Cu in *E. camaldulensis* rhizosphere soil was lower with 64.9 mg kg^{-1}, the plant and particularly the stem (139.23 mg kg^{-1}) and leaf (5341.1 mg kg^{-1}) recorded a higher accumulation ability compared to Ap (Baker & Brooks, 1989).

Ap showed a decreasing trend of Cu concentration as it travels from soil to leaf, [1631.7(soil) → 90.4(root) → 152(stem) → 93.6(leaf) mg kg^{-1}] whereas Ec showed a much higher accumulation capacity in root and stem [64.9(soil) → 185.7(root) → 139(stem) → 5341(leaf) mg kg^{-1}] and significantly high in its leaf (Fig. 2).

Table 1 – The physico-chemical parameters of soils from the abandoned mine site; ±se (range).

Average	Plots of *A. pycnantha* (Ap)	Plots of *E. camaldulensis* (EC)
pH	5.8 ± 0.41 (4.4–6.8)	7.6 ± 0.75 (6.8–8.4)
EC μS	303.8 ± 121.9 (117–1276)	7242 ± 1929 (1382–13,240)
Moisture %	2.8 ± 0.52 (1.6–5.3)	4.8 ± 2 (2.8–6.8)
TC mg kg^{-1}	260.5 ± 63.6 (38.7–540.5)	203 ± 101.98 (91–315)
TN mg kg^{-1}	10.6 ± 3.7 (1–34.7)	35.8 ± 9.5 (26–45)
TOC mg kg^{-1}	180 ± 55.4 (27.1–497.8)	146 ± 77.6 (68–223)
N %	0.07 ± 0.02 (0.01–0.18)	0.17 ± 0.13 (0.04–0.29)
C %	1.97 ± 0.4 (0.93–3.6)	2.7 ± 1.67 (1.1–4.4)

The BCF, also referred to as root (below ground)-soil quotient of Ec is ≥1 for Cu (3.57) and Zn (2.81), are values regarded as a copper or zinc stabiliser. However, the BCF of Ap is ≤1 for Cu (0.43), Zn (0.84), Cd(0.11) and Pb (NA) as the

Table 2 – **The average concentration of metals in the soil, root, stem and leaf of samples from Kapunda abandoned mine site; ±se(range).**

Metals	Soil (mg kg^{-1})		Root (mg kg^{-1})		Stem (mg kg^{-1})		Leaf (mg kg^{-1})	
	Ap	Ec	Ap	Ec	Ap	Ec	Ap	Ec
Na	2425 ± 687 (690–6554)	5557 ± 3225 (452–14,750)	4461 ± 8450 (1473–7749)	17,075 ± 9859 (0.00–34,320)	13,926 ± 4054 (2953–39,360)	13,670 ± 969 (11,930–15,740)	34,823 ± 534 (33,250–35,590)	599 ± 174 (203–1652)
Al	3160 ± 739 (539–6102)	8155 ± 2824 (1801–14,750)	293 ± 58 (157.5–667.2)	2414 ± 732 (1132–3700)	649 ± 7 (367–1002)	946 ± 24 (906–1010)	15,071 ± 2364 (4898–23,125)	1913 ± 214 (1530–2297)
K	22,079 ± 4381 (11,518–49,850)	5034 ± 1563 (2057–9017)	39,353 ± 10,611 (12,120–101,450)	38,663 ± 4835 (30,140–47,840)	55,277 ± 12,106 (22,835–114,450)	57,088 ± 1560.9 (52,870–59,750)	102,450 ± 1680 (100,000–107,300)	3836 ± 654 (1594–6591)
Ca	30,489 ± 6296 (13,730–68,340)	5707 ± 900 (4164–7823)	95,879 ± 20,433 (54,145–220,350)	128,075 ± 13,157 (104,100–153,700)	128,372 ± 19,345 (55,620–218,450)	167,325 ± 20,547 (127,900–203,500)	63,528 ± 730 (62,490–65,680)	14,334 ± 9205 (244–72,300)
Fe	5755 ± 1195 (835–11,010)	28,200 ± 7240 (12,900–47,200)	595 ± 89 (412–1173)	4059 ± 957 (2372–5819)	1288 ± 123 (715–1836)	1355 ± 50 (1268–1489)	3134 ± 408 (2365–3919)	51,649 ± 7262 (26,620–80,170)
Cu	1632 ± 241 (458–2544)	65 ± 19 (25–117)	90 ± 22 (17–199)	186 ± 27 (137–233)	152 ± 30 (45–282)	139 ± 27 (90–195)	94 ± 9 (77–110)	5341 ± 1226 (946–11,710)
Zn	63 ± 17 (11–169)	40 ± 11 (20–69)	62 ± 15 (19–126)	107 ± 17 (76–140)	96 ± 16 (35–165)	71 ± 10 (52–91)	128 ± 4 (119–136)	80 ± 13 (31–133)
Cd	0.28 ± 0.07 (0.1–0.6)	5 ± 1.45 (2.3–9)	0.28 ± 0.07 (0.1–0.6)	0.55 ± 0.01 (0.5–0.6)	0.19 ± 0.05 (0.1–0.4)	0.57 ± 0.10 (0.39–0.7)	0.27 ± 0.04 (0.2–0.3)	4.1 ± 1.0 (1.1–9)
Pb	2.6 ± 0.6 (0.0–4.8)	10.9 ± 3.1 (4.5–19.3)	2.6 ± 0.6 (0.0–4.8)	2.4 ± 0.6 (1.4–3.5)	0.8 ± 0.2 (0.1–1.6)	10.3 ± 3.9 (3.3–17.6)	2.05 ± 0.4 (1.4–2.7)	21 ± 3.7 (7.2–37)

value of BCF ≤1 an considered as inferior phytostabilizer (Sagiroglu et al., 2006; Yoon et al., 2006). Root accumulation of HM (in Ap) is not as harmful as above ground parts accumulation (in Ec) in terms of pollution and dispersion (Nouri et al., 2011). The TF, also called shoot-root quotient, explains a plant's ability to translocate metal from its roots to shoots and to leaves where a value of ≤1 is ideal for phytostabilization. The TF of Ec for Cd (1.04) and Pb (3.86) is ≥1, a good candidate for Pb and Cd phytoabsorption but less preferred for phytostabilization (Yoon et al., 2006). The Ap showed a negligible level of copper translocation (TF) along its tissue and such a quality is acknowledged as Cu tolerance (not translocating) ability (Lamb, Ming, Megharaj, & Naidu, 2010). An EF also called soil-leaf quotient with a value ≤1 is considered to be an ideal stabilizer (Sagiroglu et al., 2006; Yoon et al., 2006). Therefore the EF of copper in Ec is 2.17, as Cu accumulator and 1.89 as Zn accumulator (Table 3).

4. Discussion

4.1. *Soil characteristics and metal accumulation in plants*

An evaluation of metal uptake behavior of both the species Ap and Ec growing on mine site with respect to its adaptation and soil interaction based on their interaction with heavy metal uptake behavior has been investigated. The extent of root and the physico-chemical properties of soil influence the uptake or accumulation values analysed currently using the top soil metal concentrations (Zengin, Aka Sağlıker, & Darıcı, 2008). However, the correlation of soil $_{p}$H to root copper concentration has R^2 value of 0.58 ($p \leq 0.05$), which is a less significant result explains the feeble effect of soil pH on metal uptake to root zone of Ap (Fig. 2). This indicates plants ability to adapt soil ionic impact on metal uptake as reflected with Ap where a

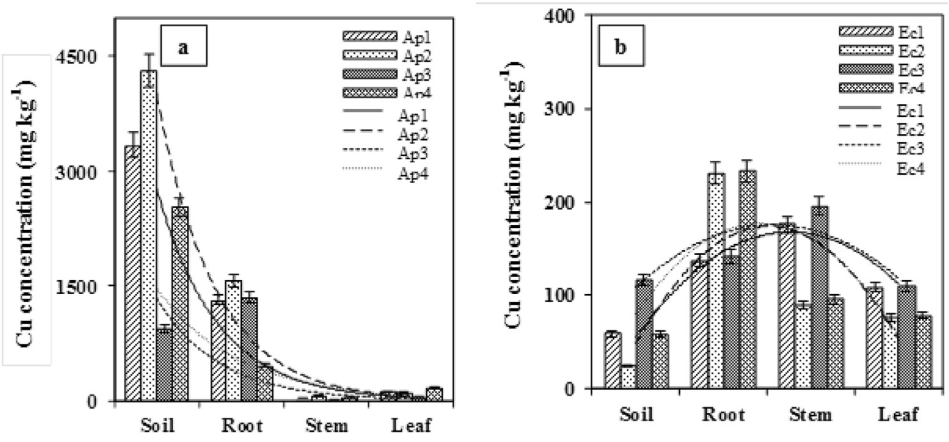

Fig. 2 – **The accumulation pattern of underground and above ground parts of metallophyte (SD-%, N = 3) a) Cu concentration in Ap rhizosphere soil (bars) and *A. pycnantha* (Ap1, Ap 2, Ap3, Ap4 = 4 × 4) root, stem and leaf at 16 sampling locations. b) Cu concentration in Ec rhizosphere soil (bars) and *E. camaldulensis* (Ec1, Ec2, Ec3, Ec4 = 4 × 4) root, stem and leaf at 16 sampling locations.**

highest soil copper yielded less foliar concentration. In contrast, there is a relatively higher concentration of metals in Ec leaf and stem compared to soil and root concentration growing on basic pH soil condition (Table 2). Therefore, this result generally indicates that Ap while growing on acidic soils translocate less metals than the Ec that is growing on basic soil condition is a natural adaptation. This result could be also attributed to the metal enrichment in Ec leaves through root uptake which is called systemic uptake, and through bark called dermal sorption, or by a combination of both (Chenery et al., 2012; Reichman, Menzies, Asher, & Mulligan, 2006). Our study shows that the accumulation level of Cu in Ap and Ec is different in root, stem and leaf suggesting the independent behaviour of each species against copper (Fig. 2).

Table 3 – The Translocation factor (TF), Bioconcentration factor (BCF) and Enrichment factor (EF) of *A. pycnantha* (Ap) in secondary axis and *E. camaldulensis* (Ec) in primary axis against the metals under investigation; ±se(range). *Note that TF of Ec for Pb and of Ap for Cd, BCF of Ec for Cu and Zn and of Ap for Zn and EF of Ec for Cu and Zn and of Ap for Zn are high.* BCF > 1, TF and EF < 1 means that it is a good stabiliser, NA- Not available due to calculation error.

Metals	*Acacia pycnantha*			*Eucalyptus camaldulensis*		
	BCF	TF	EF	BCF	TF	EF
Na	2.4 ± 0.6 (1.2–6)	2.3 ± 0.5 (0.4–4.2)	3.9 ± 0.7 (0.3–6.3)	47 ± 28 (19–75)	0.7 ± 0.0 (0.7–0.7)	29.9 ± 25.6 (4.3–55.6)
Al	0.27 ± 0.1 (0.05–1)	0.15 ± 0.04 (0.03–0.3)	0.02 ± 0.00 (0.01–0.04)	0.3 ± 0.2 (0.08–0.42)	0.6 ± 0.3 (0.3–0.9)	0.09 ± 0.02 (0.07–0.1)
K	1.8 ± 0.4 (0.9–4.5)	0.9 ± 0.2 (0.3–1.7)	1.5 ± 0.3 (0.5–3.4)	14.8 ± 0.13 (14.7–14.9)	2.2 ± 0.5 (1.7–2.6)	31.7 ± 7 (24.7–38.7)
Ca	24 ± 9.5 (0.56–85)	3.5 ± 0.6 (1.4–6.6)	78.9 ± 31.5 (0.8–276)	22.7 ± 5 (17.6–27.8)	1.4 ± 0.5 (0.9–1.9)	29.1 ± 4.8 (24.3–33.8)
Fe	0.24 ± 0.12 (0.03–1)	0.19 ± 0.1 (0.04–0.6)	0.02 ± 0.0 (0.01–0.02)	0.17 ± 0.10 (0.07–0.27)	0.41 ± 0.19 (0.22–0.59)	0.05 ± 0.01 (0.04–0.06)
Cu	0.43 ± 0.14 (0.18–1.42)	*NA	0.02 ± 0.00 (0.01–0.04)	3.6 ± 1.99 (1.58–5.56)	*NA	2.2 ± 0.06 (2.11–2.23)
Zn	0.84 ± 0.22 (0.20–2.21)	1.42 ± 0.56 (0.42–5.23)	0.90 ± 0.22 (0.17–1.77)	2.81 ± 0.33 (2.48–3.14)	0.67 ± 0.03 (0.64–0.70)	1.9 ± 0.31 (1.58–2.2)
Cd	0.11 ± 0.05 (0.02–0.45)	1.20 ± 0.29 (0.33–2.8)	0.21 ± 0.15 (0.02–1.27)	0.13 ± 0.04 (0.09–0.17)	1.04 ± 0.4 (0.67–1.4)	0.15 ± 0.09 (0.06–0.23)
Pb	*NA	*NA	0.02 ± 0.01 (0.00–0.04)	0.27 ± 0.17 (0.10–0.44)	3.9 ± 1.29 (2.6–5.2)	1.3 ± 1.01 (0.25–2.3)

4.2. *Soil – plant interaction*

The ESEM spectral analysis shows a higher level of Ca deposition in the vascular bundle of Ap with EF value 78.9. Thus, Ap has an affinity to Ca salts as evident from the results shown in Fig. 3. According to Maksymiec (1998) there is a physiological effect on cellular processes of higher plants. One such effect is due to Cu^{2+} where enzymatic and leaf senescence is brought about with Ca^{2+} moving into xylem through apoplast pathway. Moreover, the study also points out about the photosynthetic apparatus where Cu^{2+} and Ca^{2+} are involved in the process of escaping metal toxicity. The Ap accumulating more Ca in the vascular zone is a possible explanation for its premature death after almost every 10 years due to the Ca flakes clogging vascular bundles (Brockwell et al., 2005; Gibson et al.; 2011; Miller, Murphy, Brown, Richardson, & González-Orozco, 2011). However, the accumulation of Ca in Ap vascular bundle leading to a mutualistic interaction between plant and microbe can be defined as “a necessary evil”. Vadassery and Oelmüller (2009) assert a similar view regarding the mutual relationship existing between the plant and microbe based on Ca signalling. This relationship has an advantage for Ap with its root nodules being able to survive in contaminated soils due to the ability to draw moisture and nutrition such as nitrogen from the rhizosphere with the help of rhizobia present in root nodules and micorrhiza present in root tissue (Gibson et al., 2011). Moreover, some species of Acacia (*A. nilotica* and *A. senegal*) were found growing well in calcareous soil supports Ca-philic and accumulating behaviour of this genera (Chaplot, 2013).

One study in Western Australia (Mikli, 2001) reported that *E. camaldulensis* was found to grow well on riverine sites quiet similar to the habitat in the current study site. Mikli in his thesis further reports that *Eucalyptus robusta* and *E. camaldulensis* struggled to grow in poor coal mine soils even though they tolerated acidic soils. One of the possible reasons for getting poor growth of Ec was due to sandy soil as found in arid to semi-arid mine sites that prevented deeper root penetration (Boland et al., 2006). In the stressed soil condition, the root hairs struggle to settle inside the nutrient deficient soil, and preferentially, Ec is found to grow on grey heavy clayey soils with basic pH soil condition is consistent to our current finding as well (Brooker, Slee, Connors, & Duffy, 2005; Costermans, 1989; Haling et al., 2013). Generally, the

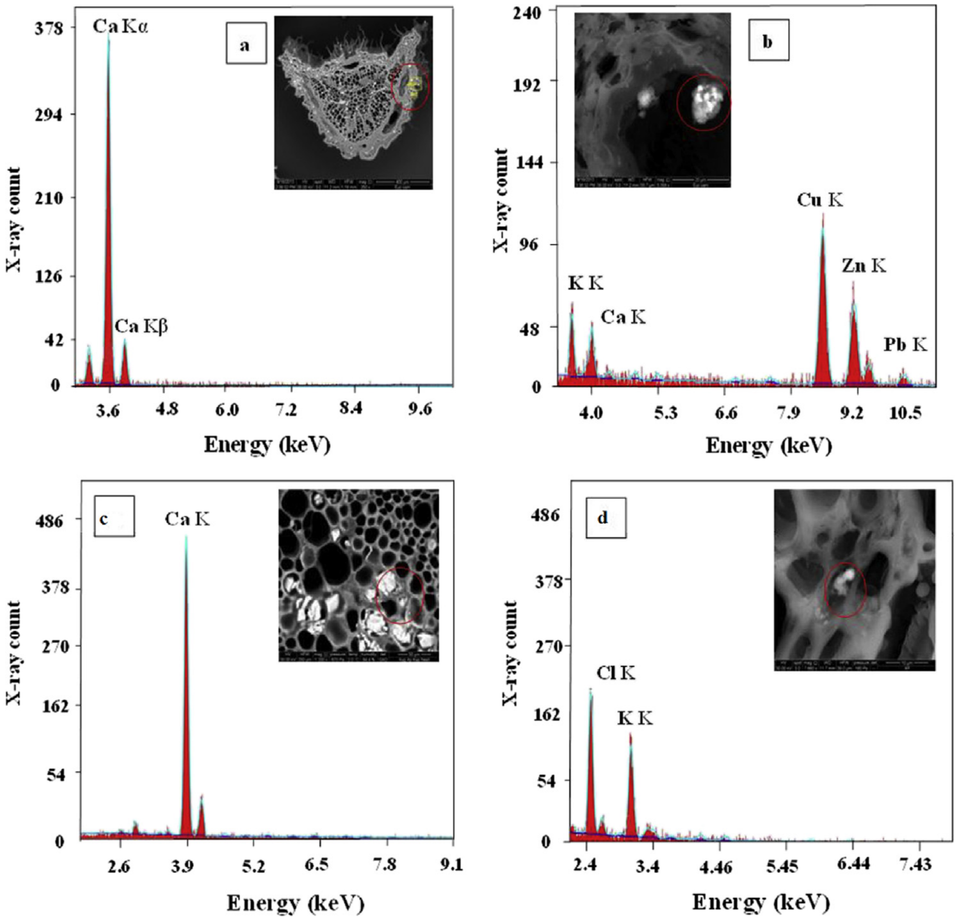

Fig. 3 – ESEM spectral diagram with images in inset. a) the anticlinal section of Ap xylem bundle in the inset with spectral diagram of highlighted area in red circle shows Ca flake deposition. b) the anticlinal section of Ap xylem vessel in inset with spectral diagram of the highlighted area in red circle shows salts of chlorine and potassium respectively, at 2.4 and 3.4 keV. c) the anticlinal section of Ec leaf in inset with spectral diagram of highlighted area in red circle shows Ca deposition in mesophyll zone at 3.6 keV. d) the anticlinal cross-cut of Ec leaf in inset with spectral diagram of highlighted red circle shows deposition of Cu and Zn in xylem vessel.

acacia species have been widely preferred to eucalyptus for afforestation and greening programs because it can grow in poor soils faster, can fix nitrogen, and revegetate even in nutrient deficient soil (Boyes, Gunton, Griffiths, & Lawes, 2011; Gawronski, Greger, & Gawronska, 2011). Therefore, in our study area containing acidic soil this leguminous Ap species has been evaluated to address the phytotoxicity of metals on plants (Seigler, 2003; Zaets & Kozyrovska, 2012). Ap rhizosphere soil confirmed that this species grows well in acidic soil with low EC, low carbon and nitrogen content as presented in Table 1.

4.3. *Accumulation factors of heavy metals on Ap and Ec*

The observed metal concentration in leaf, stem and root were used to measure BCF, TF and EF to determine whether a plant is an excluder, extractor or stabiliser (Baker, 1981). As per the current investigation, Ap has a better edge over Ec because of its high BCF (high root accumulation) and low EF (lower leaf accumulation) ratio (Masvodza, Dzomba, Mhandu, & Masamha, 2013). However, there is no convincing earlier data for both species regarding their interaction with heavy metal, particularly in abandoned mine soils. According to Baker and Brooks (1989), plants that accumulate >1000 mg kg^{-1} of Cu, Co, Cr, Ni or Pb and >10,000 mg kg^{-1} of Mn or Zn are called hyper-accumulators, and the present investigation implies to Ec leaf accumulation. So, the uptake factor values actually explain the qualitative aspect rather than the quantitative one. The ESEM data of the leaf indicates that Ap is an excluder of Cu because of its low spectroscopic readings (Fig. 3). This is in contrast to the findings of Masvodza, Dzomba, Mhandu, and Masamha (2013) who reported hyperaccumulation of Cu in *Acacia saligna* and *Acacia polycantha* grown in mine slime dams in Harare, Zimbabwe. Baker (1981) suggests that it is the foliar concentration that disqualifies a plant to act as a stabiliser. In the present study, the foliar concentration of Cu in Ap was comparatively lower than in Ec, indicating that AP a more suitable candidate for phytoremediation.

5. Conclusions

The present study of abandoned mine site at a semi-arid climate provides insight into the ability of native trees to interact with metals present in soil. Our data suggest that *E. camaldulensis* is a hyperaccumulator as evident from the EF value. We suggest that the use of a native species like leguminous *A. pycnantha* has a better edge over *E. camaldulensis* to stabilise pollutants such as Cu in a long run. The Ca build-up in the vascular region of Ap, with BCF ≤ 1 for Cu, Zn, Cd and Pb are issues that need further verification in the future. Overall, this study recommends to avoid *E. camaldulensis* growth in copper polluted mine sites.

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REFERENCES

Agazzi, A., & Pirola, C. (2000). Fundamentals, methods and future trends of environmental microwave sample preparation. *Microchemical Journal, 67*(1), 337–341.

Ahmed, R., Hoque, A. R., & Hossain, M. K. (2008). Allelopathic effects of leaf litters of *Eucalyptus camaldulensis* on some forest and agricultural crops. *Journal of Forestry Research, 19*(1), 19–24.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere, 91*(7), 869–881.

Ash, R., & Truong, P. (2003). The use of Vetiver grass wetlands for sewerage treatment in Australia. In *Proceedings of 3rd International Conference on Vetiver, Guangzhou, China* (pp. 132–141).

Baker, A. J. (1981). Accumulators and excluders-strategies in the response of plants to heavy metals. *Journal of Plant Nutrition, 3*(1–4), 643–654.

Baker, A. J. M., & Brooks, R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecovery, 1*(2), 81–126.

Boland, D. J., Brooker, M. I. H., Chippendale, G. M., Hall, N., Hyland, B. P. M., Johnston, R. D., et al. (Eds.). (2006). *Forest trees of Australia* (p. 687). Melbourne: CSIRO Publishing.

Boughton, V. H. (1986). Phyllode structure, taxonomy and distribution in some Australian Acacias. *Australian Journal of Botany, 34*(6), 663–674.

Boyes, L. J., Gunton, R. M., Griffiths, M. E., & Lawes, M. J. (2011). Causes of arrested succession in coastal dune forest. *Plant Ecology, 212*(1), 21–32.

Brewer, G. J. (2010). Copper toxicity in the general population. *Clinical Neurophysiology, 121*(4), 459–460.

Brockwell, J., Searle, S. D., Jeavons, A. C., & Waayers, M. (2005). *Nitrogen fixation in acacias: An untapped resource for sustainable plantations, farm forestry and land reclamation (ACIAR)*.

Brodribb, T., & Hill, R. S. (1993). A physiological comparison of leaves and phyllodes in Acacia melanoxylon. *Australian Journal of Botany, 41*(3), 293–305.

Brooker, M. I. H., Slee, A. V., Connors, J. R., & Duffy, S. M. (2005). CD-ROM review EUCLID, Eucalypts of Southern Australia. *South African Journal of Botany, 71*(1), 130–131.

Cerqueira, B., Arenas-Lago, D., Andrade, M. L., & Vega, F. A. (2015). Using time of flight secondary ion mass spectrometry and field emission scanning electron microscopy with energy dispersive X-ray spectroscopy to determine the role of soil components in competitive copper and cadmium migration and fixation in soils. *Geoderma, 251*, 65–77.

Chaplot, P. C. (2013). Response of *Acacia nilotica* and *Acacia senegal* to soil amendments on problematic land. *Annals of Agri-Bio Research, 18*(3), 375–376.

Chenery, S. R., Izquierdo, M., Marzouk, E., Klinck, B., Palumbo-Roe, B., & Tye, A. M. (2012). Soil–plant interactions and the uptake of Pb at abandoned mining sites in the Rookhope catchment of the N. Pennines, UK—A Pb isotope study. *Science of the Total Environment, 433*, 547–560.

Couillard, C. M., Courtenay, S. C., & Macdonald,, R. W. (2008). Chemical-environment interactions affecting the risk of impacts on aquatic organisms: A review with a Canadian perspective-interactions affecting vulnerability. *Environmental Reviews, 16*(NA), 19–44.

Costermans, L. F. (1989). *Native trees and shrubs of south-eastern Australia*. New-Holland Publishers. CSIRO Publications.

Doupé, R. G., & Lymbery, A. J. (2005). Environmental risks associated with beneficial end uses of mine lakes in southwestern Australia. *Mine Water Environment, 24*, 134–138.

Gawronski, S. W., Greger, M., & Gawronska, H. (2011). *Plant taxonomy and metal phytoremediation. Detoxification of heavy metals* (pp. 91–109). Berlin, Heidelberg: Springer.

Gibson, M. R., Richardson, D. M., Marchante, E., Marchante, H., Rodger, J. G., Stone, G. N., et al. (2011). Reproductive biology of Australian acacias: important mediator of invasiveness? *Diversity and Distributions, 17*(5), 911–933.

González, R. C., & González-Chávez, M. C. A. (2006). Metal accumulation in wild plants surrounding mining wastes. *Environmental Pollution, 144*(1), 84–92.

Grant, C. D., Campbell, C. J., & Charnock, N. R. (2002). Selection of species suitable for derelict mine site rehabilitation in New South Wales, Australia. *Water, Air, and Soil Pollution, 139*(1–4), 215–235.

Haling, R. E., Brown, L. K., Bengough, A. G., Young, I. M., Hallett, P. D., White, P. J., et al. (2013). Root hairs improve root penetration, root–soil contact, and phosphorus acquisition in soils of different strength. *Journal of Experimental Botany, 64*(12), 3711–3721.

Hazelton, P. A., & Murphy, B. W. (2007). *Interpreting soil test results: What do all the numbers mean?* (2nd ed.). Collingwood, Victoria Australia: CSIRO Publishing.

ISO – International Standard Organisation. (2002). *Soil quality sampling. Part 1: Guidance on the design of sampling programmes*. ISO, 103 81-1.

Khan, M. A., Hussain, I., & Khan, E. A. (2008). Allelopathic effects of Eucalyptus (Eucalyptus camaldulensis L.) on germination and seedling growth of wheat (Triticum aestivum L.). *Pakistan Journal of Weed Science Research, 14*(1/2), 9–18.

Krebs, C. J. (1999). *Ecological methodology* (Vol. 620). Menlo Park, California: Benjamin/Cummings.

Krumins, J. A., Goodey, N. M., & Gallagher, F. (2015). Plant–soil interactions in metal contaminated soils. *Soil Biology and Biochemistry, 80*, 224–231.

Kurek, E., & Majewska, M. (2012). Microbially Mediated Transformations of Heavy Metals in Rhizosphere. In A. Zaidi, P. A. Wani, & M. S. Khan (Eds.), *Toxicity of Heavy Metals to Legumes and Bioremediation* (pp. 129–146). Vienna: Springer.

Lamb, D. T., Ming, H., Megharaj, M., & Naidu, R. (2010). Phytotoxicity and accumulation of lead in Australian native vegetation. *Archives of Environmental Contamination and Toxicology, 58*(3), 613–621.

Mackasey, W. O. (2004). *Abandoned mines in Canada, WOM geological associates, mining watch Canada*. WOM Geological Associates.

Maksymiec, W. (1998). Effect of copper on cellular processes in higher plants. *Photosynthetica, 34*(3), 321–342.

Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2013). Phytoextraction of heavy metal polluted soils using Sedum plumbizincicola inoculated with metal mobilizing Phyllobacterium myrsinacearum RC6b. *Chemosphere, 93*(7), 1386–1392.

Masvodza, D. R., Dzomba, P., Mhandu, F., & Masamha, B. (2013). Heavy metal content in Acacia saligna and Acacia polyacantha on Slime Dams: implications for phytoremediation. *American Journal of Experimental Agriculture, 3*(4), 871–883.

Méndez, A., Gómez, A., Paz-Ferreiro, J., & Gascó, G. (2012). Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. *Chemosphere, 89*(11), 1354–1359.

Midgley, S. J., & Turnbull, J. W. (2003). Domestication and use of Australian acacias: case studies of five important species. *Australian Systematic Botany, 16*(1), 89–102.

Mikli, M. H. (2001). *Revegetation of coal mine dumps to ameliorate effects of acidic seepage* (Thesis). Department of Environmental Biology. Curtin University of Technology.

Miller, J. T., Murphy, D. J., Brown, G. K., Richardson, D. M., & González-Orozco, C. E. (2011). The evolution and phylogenetic placement of invasive Australian Acacia species. *Diversity and Distributions, 17*(5), 848–860.

Nirola, R., Mallavarapu, M., Aryal, R., & Naidu, R. (2015). Screening of metal uptake by plant colonizers growing on abandoned copper mine in Kapunda, South Australia. *International Journal of Phytoremediation*. http://dx.doi.org/10.1080/15226514.2015.1109599.

Nouri, J., Lorestani, B., Yousefi, N., Khorasani, N., Hasani, A. H., Seif, F., et al. (2011). Phytoremediation potential of native plants grown in the vicinity of Ahangaran lead–zinc mine (Hamedan, Iran). *Environmental Earth Sciences, 62*(3), 639–644.

Reichman, S. M., Menzies, N. W., Asher, C. J., & Mulligan, D. R. (2006). Responses of four Australian tree species to toxic concentrations of copper in solution culture. *Journal of Plant Nutrition, 29*(6), 1127–1141.

Reuss, J. O., & Johnson, D. W. (2012). *Acid deposition and the acidification of soils and waters* (Vol. 59). Springer Science & Business Media.

Sagiroglu, A., Sasmaz, A., & Sen, O. (2006). Hyperaccumulator plants of the Keban mining district and their possible impact on the environment. *Polish Journal of Environmental Studies, 15*(2), 317–325.

Seigler, D. S. (2003). Phytochemistry of *Acacia—sensu lato*. *Biochemical Systematics and Ecology, 31*(8), 845–873.

Sinha, R. K., Herat, S., & Tandon, P. K. (2007). Phytoremediation: role of plants in contaminated site management. In *Environmental Bioremediation Technologies* (pp. 315–330). Berlin, Heidelberg: Springer.

Sposito, G. (2008). *The chemistry of soils*. Oxford University Press.

Stokes, D. J., Morris, V. J., & Groves, K. (2013). Environmental scanning electron microscopy (ESEM): principles and applications to food microstructures. *Food Microstructures: Microscopy, Measurement and Modelling, 3*.

Thomas, R. (2013). *Practical guide to ICP-MS: A tutorial for beginners*. CRC Press.

Vadassery, J., & Oelmüller, R. (2009). Calcium signaling in pathogenic and beneficial plant microbe interactions: what can we learn from the interaction between *Piriformospora indica* and *Arabidopsis thaliana*. *Plant Signaling & Behavior, 4*(11), 1024.

Van Zyl, D., Sassoon, M., Digby, C., Fleury, A. M., & Kyeyune, S. (2002). Mining for the future. Main report. *International Institute for Environment and Development, World Business Council for Sustainable Development, 68*, 31.

Wait, M. (2012). *DMR rehabilitates derelict and ownerless mines, not abandoned sites*. Mining Weekly, 17 September, viewed 11 Jan 2013 http://www.miningweekly.com/article/dmr-rehabilitates-derelict-and-ownerless-mines-not-abandoned-sites-2012-09-17.

Yang, L., Liu, N., Ren, H., & Wang, J. (2009). Facilitation by two exotic Acacia: *Acacia auriculiformis* and *Acacia mangium* as nurse plants in South China. *Forest Ecology and Management, 257*(8), 1786–1793.

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment, 368*(2), 456–464.

You, F., Dalal, R., Mulligan, D., & Huang, L. (2015). Quantitative measurement of organic carbon in mine wastes: methods comparison for inorganic carbon removal and organic carbon recovery. *Communications in Soil Science and Plant Analysis, 46*(Supp1.), 375–389.

Zaets, I., & Kozyrovska, N. (2012). Heavy metal resistance in plants: a putative role of endophytic bacteria. In *Toxicity of heavy metals to legumes and bioremediation* (pp. 203–217). Vienna: Springer.

Zengin, E., Aka Sağlıker, H., & Darıcı, C. (2008). Carbon mineralization of *Acacia cyanophylla* soils under the different temperature and humidity conditions. *Ekoloji, 18*(69), 1–6.