**REVIEW ARTICLE** 



### Remediation of metalliferous mines, revegetation challenges and emerging prospects in semi-arid and arid conditions

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**Abstract** Understanding plant behaviour in polluted soils is critical for the sustainable remediation of metal-polluted sites including abandoned mines. Post-operational and abandoned metal mines particularly in semi-arid and arid zones are one of the major sources of pollution by soil erosion or plant hyperaccumulation bringing ecological impacts. We have selected from the literature 157 species belonging to 50 families to present a global overview of 'plants under action' against heavy metal pollution. Generally, all species of plants that are drought, salt and metal tolerant are candidates of interest to deal with harsh environmental conditions, particularly at

Highlights

- Description of metal pollution through operational and abandoned mine sites
- · Phytoremediation in semi-arid and arid environmental conditions
- · An insight into taxonomic discourse of listed metallophytes
- A modern approach to the challenges of revegetation, bioremediation and mine site rehabilitation

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semi-arid and arid mine sites. Pioneer metallophytes namely *Atriplex nummularia*, *Atriplex semibaccata*, *Salsola kali*, *Phragmites australis* and *Medicago sativa*, representing the taxonomic orders Caryophyllales, Poales and Fabales are evaluated in terms of phytoremediation in this review. Phytoremediation processes, microbial and algal bioremediation, the use and implication of tissue culture and biotechnology are critically examined. Overall, an integration of available remediation plant-based technologies, referred to here as 'integrated remediation technology,' is proposed to be one of the possible ways ahead to effectively address problems of toxic heavy metal pollution.

**Keywords** Heavy metals · Metallophytes · Plant systematics · Revegetation · Bioremediation · Semi-arid and arid

#### Introduction

Humans have always depended on natural resources, while an ever-increasing demand for metals and minerals has posed a serious threat to the lives of humans and animals through pollution and health issues (Lottermoser 2010; Forstner and Wittmann 2012). One of the most serious forms of pollution is heavy metals discharging from abandoned mine sites, as it has been historically a normal and accepted practice to 'abandon' a mine when mineral extraction is strategically completed (Himley 2014; Unger et al. 2015). The most abundant soil contamination areas and mine tailing disposal sites on earth are found in Northern Mexico, the western USA, the Pacific coast of South America (Chile and Peru), south-western Spain, Western India, South Africa and Australia (Mendez and Maier 2008b).

The European Environment Agency (EEA) estimates that there are around 1.4 million contaminated sites on earth (Haferburg and Kothe 2010), a figure probably beyond the present capacity of remediation technology. According to van Zyl et al. (2002), approximately 630,309 abandoned mines are spread throughout the world with many remaining undocumented and untraced. A report by Wait (2012) from South Africa put the figure at approximately 5858 derelict and ownerless mines. Mining Watch Canada in its data reported 5500 abandoned mines in Japan (Mackasey 2000).

All these figures however depend on how a mine is defined. In China, small-scale mines total 230,000 in number and are found to have a devastating environmental impact, mostly due to the use of crude technologies to extract metal ores (Li 2006). Area-wise, the degradation of land in China due to large- and small-scale ore mining is estimated to be 3.2 million ha (Li 2006), with a total of 8.5 million ha of land being contaminated by heavy metals due to agriculture and mining (Hongbo et al. 2011).

The USEPA 2011 toxic release inventory data of USA shows 8.66 billion kg of toxic chemicals including heavy metals released into the environment, a 16 % increase from that of earlier year. In China, one sixth of the total arable land has been polluted by heavy metals, and more than 40 % has been degraded to varying degrees due to erosion and desertification (Lone et al. 2008). Consequently, it is evident that pollution in soil and water bodies is increasingly posing chronic health risks and hazards to living organisms including humans (McSwane et al. 2015).

The cost burden to contain and remediate polluted land is very high and often lacks sufficient budget to initiate any clean-up work (Pulford and Watson 2003; Luo et al. 2009). Phytoremediation technologies can be a costeffective tool to target specific toxic metal pollutants (Peters 1988; Alday et al. 2011). In Australia, remediation of historic mine sites releasing acid mine drainage was estimated to cost more than \$100,000/ha of land in 1994 (Archer and Caldwell 2004). Environ Sci Pollut Res

The global demand for metals is increasing, but the efforts to control environmental pollution and human health risks are not keeping pace. For example, global heavy metal consumption per capita during the 1950s was 77 kg, which soared by three times by 2008 to 213 kg (Haferburg and Kothe 2010). Moreover, the total production of metal-related consumables and goods in 2008 was 1.4 billion tons which is seven times more than that produced in the 1950s (Haferburg and Kothe 2010).

One of the reasons for this mismanagement of resources is the lack of effective global mechanisms to coordinate resources and restoration processes involving all stakeholders. For instance, in a land restoration research finding in China, the reclamation studies were found to be separated from restoration practice, as there was no collaboration between the research institution and the entity working to restore the contaminated land (Li 2006). Moreover, awareness on environmental pollution due to heavy metal mining is poor compared to awareness on greenhouse gas emissions (Nirola and Jha 2013; Dodman 2009; Hague et al. 2014). As such, an abandoned mine survey in Australia only received a 7 % positive response from the participants regarding being fully informed about the issue of heavy metal pollution (van de Graaff et al. 2012). In this context, like with greenhouse gas emissions, awareness of heavy metal pollution is necessary for resource mobilisation and effective implementation of remediation technologies.

The toxic impact and risk evaluation of heavy metals on plants and their responses to heavy metal pollution has long been an area of interest (Nirola et al. 2016). Even various ancient literatures highlight the importance of plants such as *Ficus religiosa* to purify and protect the environment (Pathak et al. 2011; Singh et al. 2011).

The land and water bodies surrounding mines in semi-arid and arid (SAA) zones are more vulnerable to metal erosion and pollution exposure (Perlatti et al. 2014; Kim et al. 2014; Pascaud et al. 2015). For instance, the abandoned mine in Kapunda, South Australia (Fig. 1) has several waste dumps

Fig. 1 Landscape of an abandoned copper mine in Kapunda, South Australia showing revegetation using native plants. (*Inset*: a resilient *Acacia pycnantha* growing on a dry mine site rubble; the *dashed circle* shows earlier successful revegetation)



and open cast areas, although even more severe problems are being faced by third world countries in terms of managing abandoned mine sites (Cao 2007). Moreover, rehabilitation attempts on abandoned as well as operational mines have generally involved engineering-based technologies rather than biological systems.

The modern science of phytoremediation was conceptualised as early as the eighteenth century by Carolus Linnaeus (1707–1778), the 'Father' of taxonomy, who discovered a leadwort plant under Plumbaginaceae showing a lead tolerance capacity (Gawronski et al. 2011).

As a result of various trials, sea thrift (*Armeria maritima*), cape leadwort (*Plumbago auriculata*) and some species from the genus *Limonium* are commonly used for remediation of lead-polluted sites (Rascio and Navari-Izzo 2011; Gawronski et al. 2011). Similarly, at a former gold mine in Jales, Portugal, the first attempts to establish a vegetation cover were unsuccessful (Mench et al. 2003) as only a few small patches were colonised by newly planted vegetation of grasses such as *Agrostis castellana*, *Agrostis delicatula* and *Holcus lanatus*.

Various rehabilitation works on mines have attracted scientists, changed public opinion, activated regulators and concerned administrators. However, modern-day budget constraints and an unstable global mining economy are some disturbing factors for effective project implementation. Overall, rehabilitation efforts are likely to be more successful if they consider social and community issues, as well as scientific and legal aspects (Browne et al. 2011).

## Environmental sustainability in mine site rehabilitation

Until recently, not many rehabilitation projects have been able to meet the goals of environmental sustainability (Lamb et al. 2015) often ending with failure to achieving 'ecological climax'. A process of ecological succession as per Clements (1900–1960) identifies a site transforming 'from bare ground to a climax forest' through an ecological series of revegetation stages (Prach and Hobbs 2008; Vranjic et al. 2012). In a wider perspective, the Foundation of Ecological Security (2008) states that recovery of degraded, damaged or destroyed ecosystems by anthropogenic and natural agents through ecological restoration or regreening technologies can be achieved by adopting microbiological, biochemical and bio-engineering methods.

The aim is to revive interactions by involving minerals, water and energy through the patterns of ecological succession (Shrestha and Lal 2008; Vranjic et al. 2012). In other words, the ultimate aim of eco-restoration is achieving a balanced vegetation cover that stabilizes pollutants within soils and avoids exposure to wind and rain while achieving additional benefits of sequestering carbon, stabilizing the local climate

and maintaining the food chain (Tordoff et al. 2000; Moreno-Jiménez et al. 2009; Claveria et al. 2010).

Therefore, phytoremediation is achieved through revegetation leading to removal, degradation or stability of pollutants (Shah and Nongkynrih 2007). Recently, there has been an increasing interest in the use of native and non-invasive plants to be more specific towards conservation of natural habitat as well as to render phytoremediation (Mendez and Maier 2008b).

#### Microorganisms and non-vascular plants

The use of ubiquitous microorganisms by associating with plants at the field scale is a form of bioremediation technology that is gaining momentum today (Dixit et al. 2015). Mandal and Bhattacharyya (2012) define phytoremediation as 'an environmental biotechnology using vegetation for in situ treatment of contaminated soils, sediments, and water'. The phytoremediation process as mentioned earlier uses a synergistic relationship among plants, microbes, water and soil that have evolved for millions of years (Sinha et al. 2010). Microorganisms, including algae and fungi, are therefore the most important non-vascular agents to help degradation and detoxification processes by employing biochemical strategies that allow them to digest pollutants (Megharaj et al. 2011; Dixit et al. 2015).

Interaction of bacteria in extreme environments with heavy metals has been studied in relation to adaptation, metabolism, tolerance and resistance to heavy metals (Prasad and de Oliveira 2003). A consortium of algae and bacteria comprising of *Chlorella sorokiniana* and *Ralstonia basilensis* has been found capable of removing copper more efficiently at pH 5.0 (Subashchandrabose et al. 2011). In another laboratory test, a dried mass of mixed culture of microalgae (such as cyanobacteria, diatoms and bacteria) was used as a biofilter which removed 80 % Cu and 100 % Cd within the first 5 min of contact time (Loutseti et al. 2009).

However, their action under in situ SAA environmental conditions needs further verification. Others have shown that, rather than the use of bacteria, mycorrhizal fungi as natural biofilters can change the availability of metals in soils and can also work as biofilters for the delivery of metals and nutrients such as nitrogen to plants (Haferburg and Kothe 2010). In a study of mycorrhizal fungi that form a symbiotic association with roots, Khan et al. (2000) reported that such fungi protect the roots against toxic substances in soil on the one hand and biodegradation of contaminants on the other. Moreover, arbuscular mycorrhizal fungi (AMF) are reported to supply nutrients such as phosphorous from soil to plants.

Furthermore, symbiotic bacteria such as *Azotobacter*, *Clostridium* and *Frankia* are found to form an important part of the bioremediation process by promoting soil health (Roy et al. 2007; Hayat et al. 2010). However, under field conditions, copper-contaminated soil exhibited low microbial biomass which is an indication that microbes find it difficult to multiply and establish in contaminated soils (Guo et al. 2009). A 20-year study of a SAA revegetated mine site showed that plant and microbial diversity was low compared to an adjacent undisturbed site (Mendez and Maier 2008b). Such evidence demonstrates that field tests are important to establish bioremediation processes in mine site ecosystems and to verify their effectiveness and success rate. Enhanced microbial establishment in soil could be achieved by application of recombinant DNA technology to produce more aggressive and tolerant strains of metal toxicity to successfully induce microbial enhanced phytoremediation (Evangelou et al. 2013; Gamalero et al. 2009). Also, there are problems associated with genetic engineering, such as decreased levels of fitness and extra energy demands imposed by the presence of foreign genetic material in cells (Ramakrishnan et al. 2011; Megharaj et al. 2011).

There are problems of microorganisms' inability to compete with indigenous microflora, insufficient microbial activities at the subsurface level, poor support of native as well as pollutant-degrading microflora, heterogeneity of bioavailable contaminants and toxic compounds in pollutant mixture. All these problems may be overcome by a plant-microbe symbiosis strategy (Gawronski et al. 2011; Megharaj et al. 2011). Hence, to thrive in SAA climatic zones, it is important to look for symbiotic plant species that host microbes and grow fast producing a higher biomass (Gawronski et al. 2011).

#### Vascular plants

The processes that phytoremediation covers include phytostabilisation, phytofiltration, phytovolatilisation, phytodegradation, rhizofiltration, rhizodegredation and phytoextraction (Banuelos 2006; Shah and Nongkynrih 2007; Fulekar 2012) (Fig. 2). These remediation activities dynamically operate either simultaneously or singularly depending upon the nature of the plant species and pollutants (Singh and Prasad 2015). Vascular plants or higher plants referred to here as metallophytes (dealing with metal pollution) have distinct xylem and phloem bundles to perform advanced photosynthesis and respiration processes. In this context, Chaney et al. (1997) refer to phytoremediation as a 'botanical remediation' that uses photosynthesizing plants to decontaminate soil, water and air (Lone et al. 2008).

Plants that have colonised former or abandoned mine sites harbour either tolerant, excluder or accumulator species (Danh et al. 2009; Brunetti et al. 2009; Kim et al. 2014; Nirola et al. 2015). Based on the degree of tolerance of plants against heavy metals, Ernst et al. (2008) divide metallophytes into hypertolerant, basal tolerant and hypotolerant (Bothe 2011). Many researchers suggest that it is the capacity of plants to tolerate extreme toxicity in soils that permits their use in

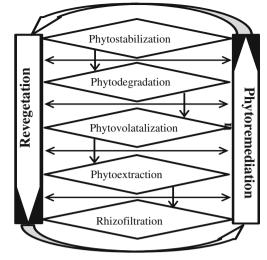


Fig. 2 A revegetation and phytoremediation dynamics partially (*horizontal arrows*) or wholly (*vertical arrows*) involving different remediation processes listed in diamond-shaped blocks as decisions

revegetation leading to phytostabilisation (Song et al. 2004; Whiting et al. 2004; Johansson et al. 2005).

The plants cannot prevent metal uptake if they are growing over metalliferous soil but can only restrict the uptake of metals in stems, leaves and roots to varying degrees and are classified as accumulator, tolerant or excluder (Wei et al. 2005; Macnair and Baker 1994).

Phytostabilisation is achieved through sequestration of metals within the rhizosphere zone by restricting metal accumulation in aboveground plant tissues (Nouri et al. 2011). Such stabilisation by vascular plants renders metals to be less bioavailable to livestock, wildlife and humans thereby reducing exposure and associated health risks (Mendez and Maier 2008b). Moreover, to arrest the leaching of heavy metals through the adventitious roots of higher plants and associated rhizosphere microbes, phytostabilisation using higher plants is a well-acknowledged process of phytoremediation (Rajkumar et al. 2012).

However, such advocacy in favour of phytoremediation is proven successful only through field scale demonstrated revegetation examples on heavy metal-contaminated sites to sequester metal pollutants within the ground (Krumins et al. 2015). Plantation failure has been reported including the unassisted process of natural colonisation of species of plants taking over 100 years (Li 2006). The length of vegetation establishment in the more adverse climatic conditions of SAA mine tailings, dams and waste rocks could be much longer (Fig. 1). Therefore, restoration requires human assistance along with the use of prescribed metallophytes if the restoration goal is expected to be achieved effectively within a reasonable timeframe (Li 2006).

Gawronski et al. (2011) reported a successful botanical remediation using a group of metallophytes belonging to the taxonomical orders Asterles, Brassicales, Caryophyllales,

#### Table 1 Metallophytes under different taxonomic classification, target metals and literature source

No.	Family/ Botanical name	Remediation target	Literature source
	Amaranthaceae		
1	Amaranthus blitoides	Toxic chemical resistant	Gawronski et al. (2011)
2	Amaranthus hybridus Anacardiaceae	Pb stabilizer, phytoextractor	Cetinkaya and Sozen (2011), Gawronski et al. (2011)
3	Pistacia terebinthus	Cu accumulator, stabilizer	Johansson et al. (2005), Mendez and Maier (2008a)
4	Schinus molle	Cd, Cu, Mn, Pb, Zn stabilizer	Mendez and Maier (2008b)
	Apocynaceae		
5	Alstonia macrophylla	Nickeliferous	Claveria et al. (2010); Sinha et al. (2010)
6	Asparagaceae Lomandra	Cd, Pb accumulator, stabilizer	Archer and Caldwell (2004)
0	longifolia Asteraceae	Cu, FU accumulator, stabilizer	Archer and Caldweir (2004)
7	Baccharis neglecta	As stabilizer	Mendez and Maier (2008a)
8	Berkheya coddii	Ni accumulator	Singh and Tripathi (2007), Sinha et al. (2010), Gawronski et al. (2011)
9	Bidens humilis	Ag, As, Cd, Cu, Pb, Zn stabilizer	Mendez and Maier (2008a)
10	Carduus pycnocephalus	Metal tolerant	Brunetti et al. (2009)
11	Cirsium congestum	Mn, Fe, Zn, Cu stabilizer	Nouri et al. (2011)
12	Helichrysum decumbens	Pb stabilizer	Cetinkaya and Sozen (2011)
13	Helianthus annuus	As stabilizer	Gawronski et al. (2011)
14	Isocoma veneta	Cd, Cu, Mn, Pb, Zn stabilizer	Mendez and Maier (2008a)
15	Pentacalia spp.	Ni accumulator	Singh and Tripathi (2007), Sinha et al. (2010)
16	Silybum marianum	Metal tolerant	Brunetti et al. (2009)
17	Taraxacum mongolicum Betaceae	Zn excluder	Lone et al. (2008), Roy et al. (2007)
18	Atriplex hortensis	Metallophyte	Gawronski et al. (2011)
19	Kochia scoparia	Metallophyte	Gawronski et al. (2011)
	Betulaceae		
20	Alnus glutinosa	Cu remediation	Roy et al. (2007), Whitbread-Abrutat (1997)
21	<i>Betula pendula</i> Brassicaceae	Cd, Zn stabilizer	Evangelou et al. (2013)
22	Alyssum bertolonii	Ni accumulator	Bothe (2011)
23	Alyssum spp.	Ni accumulator	Singh and Tripathi (2007); Sinha et al. (2010)
24	Brassica carinata	As, Cd, Cu, Pb, Zn stabilizer	Cetinkaya and Sozen (2011)
25	Brassica juncea	Metal extractor, metallophyte	Shah and Nongkynrih (2007), Gawronski et al. (2011)
26	Dichapetalum gelonioides	Zn accumulator	Singh and Tripathi (2007); Sinha et al. (2010)
27	Iberis intermedia	Th accumulator	Gawronski et al. (2011)
28	Streptanthus polygaloides	Ni accumulator	Bothe (2011)
29	Sinapis arvensis	Metal tolerant	Brunetti et al. (2009)
30	Thlaspi caerulescens	Zn, Cd, Ni, Pb accumulator	Pulford and Watson (2003), Singh and Tripathi (2007), Lone et al. (2008), Sinha et al. (2010), Cetinkaya and Sozen (2011)

Table 1 (continued)

No.	Family/ Botanical name	Remediation target	Literature source
31	<i>Thlaspi</i> <i>rotundifolium</i> Caryophyllaceae	Pb, Zn accumulator	Shah and Nongkynrih (2007), Singh and Tripathi (2007), Sinha et al. (2010)
32	Agrostemma githago	Salt and heavy metal tolerant	Gawronski et al. (2011)
33	Dianthus carthusianorum	Salt and heavy metal tolerant	Gawronski et al. (2011)
34	Minuartia verna	Pb accumulator	Singh and Tripathi (2007)
35	Silene vulgaris	Cu tolerant, salt and heavy metal tolerant	Song et al. (2004), Gawronski et al. (2011)
	Casuarinaceae		
36	Gymnostoma leucodon Celastraceae	Restoration, revegetation	Whiting et al. (2004)
37	Maytenus bureaviana	Mn accumulator	Singh and Tripathi (2007), Sinha et al. (2010)
38	<i>Maytenus</i> <i>sebertiana</i> Chenopodiaceae	Mn accumulator	Singh and Tripathi (2007), Sinha et al. (2010)
39	Atriplex lentiformis	As, Cu, Mn, Pb, Zn stabilizer	Mendez and Maier (2008a, b)
40	Atriplex nummularia	Stabilizer, drought tolerant	Mok et al. (2013)
41	Atriplex canescens	As, Hg, Mn, Pb stabilizer	Mendez and Maier (2008b)
42	Salsola kali	Cd extractor	Cano-Aguilera et al. (2007)
43	Atriplex semibaccata	Cu tolerant, drought tolerant	Guo et al. (2009), Bullock (1936)
44	<i>Teloxys graveolens</i> Cistaceae	Cd, Cu, Mn, Pb, Zn Stabilizer	Mendez and Maier (2008a)
45	<i>Cistus creticus</i> Commelinaceae	Cu accumulator	Moreno-Jiménez et al. (2009)
46	Commelina communis Crassulaceae	Cd excluder	Brewin et al. (2003)
47	Sedum alfredii Cunoniaceae	Zn, Cd accumulator	Yang et al. (2005), Lone et al. (2008), Tian et al. (2011)
48	<i>Weinmannia</i> sp. Cyperaceae	Nickeliferous	Claveria et al. (2010)
49	Ascolepis metallorum	Cu stabilizer	Saad et al. (2012)
50	Bulbostylis pseudoperennis	Cu, Co stabilizer	Saad et al. (2012)
51	Carex hirta	Metallophyte, salt tolerant	Gawronski et al. (2011)
52	Eriophorum angustifolium	Acidic soil stabilisation	Gawronski et al. (2011)
53	Scirpus californicus	Zn-contaminated water	Gawronski et al. (2011)
54	Schoenus juvenis	Restoration, revegetation	Whiting et al. (2004)
55	<i>Uncinia</i> <i>leptostachya</i> Dennstaedtiaceae	Uranium accumulator	Khan et al. (2000)
56		Cu tolerant	Claveria et al. (2010)

	Pteridium aquilinum		
57	Euphorbiaceae Euphorbia sp.	Cd, Cu, Mn, Pb, Zn stabilizer	Mendez and Maier (2008a)
58	Jatropha dioica	Zn accumulator	González and González-Chávez (2006)
59	Phyllanthus balgooyi	Ni accumulator	Whiting et al. (2004)
60	Fabaceae		
60	Acacia mearnsii	Metal extractor	Mok et al. (2013)
61	Acacia spp.	Metal tolerant	Lamb et al. (2010)
62	Amorpha fruticosa	Metallophyte	Gawronski et al. (2011)
63	Astragalus bisulcatus	Se stabilizer	Gawronski et al. (2011)
64	Astragalus racemosus	Se accumulator	Singh and Tripathi (2007), Sinha et al. (2010)
65	Caragana arborescens	Metallophyte	Gawronski et al. (2011)
66	Crotalaria cobalticola	Co accumulator, stabilizer	Singh and Tripathi (2007), Sinha et al. (2010); Saad et al. (2012)
67	Cytisus striatus	Metallophyte	Gawronski et al. (2011)
68	Dalea bicolor	Cd, Cu, Mn, Pb, Zn stabilizer	Mendez and Maier (2008b)
69	Lupinus albus	Mn, Pb, Cr(III) and (IV) accu- mulator	Gawronski et al. (2011)
70	Lupinus angustifolius	Mn, Pb, Cr(III) and (IV) accu- mulator	Gawronski et al. (2011)
71	Lupinus hispanicus	Mn, Pb, Cr(III) and (IV) accu- mulator	Gawronski et al. (2011)
72	Lupinus sp.	Degrade PAH and PCBs	Gawronski et al. (2011)
73	Medicago sativa	Mn, Pb, Cr(III) and (IV) accu- mulator	Gawronski et al. (2011)
74	Melilotus indica	Metallophyte	Gawronski et al. (2011)
75	Robinia pseudoacacia	Metallophyte	Gawronski et al. (2011)
76	Sesbania drummondii	Pb accumulator	Shah and Nongkynrih (2007)
77	<i>Vicia exaltata</i> Geraniaceae	Degrade PAHs and PCBs	Gawronski et al. (2011)
78	Biebersteinia multifida	Mn, Fe, Zn, Cu stabilizer	Nouri et al. (2011)
	Gleicheniaceae	~	
79	Dicranopteris linearis	Cu tolerant	Claveria et al. (2010)
00	Hyperiaceae	<u>C1</u>	
80	Hypericum perforatum	Cd accumulator	Moreno-Jiménez et al. (2009)
Q 1	Juncaceae	U ante matal talarant	Courseski et al. (2011)
81	Juncus articulatus	Heavy metal tolerant	Gawronski et al. (2011)
82	Juncus effuses	Heavy metal tolerant	Gawronski et al. (2011)
83	Juncus lutea	Ni tolerant	Gawronski et al. (2011)
84	Juncus usitatus	Cd, Pb accumulator, stabilizer	Archer and Caldwell (2004)

Literature source

# Table 1 (continued) No. Family/ Botanical

name

Remediation target

#### Table 1 (continued)

amiaceae amiaceae ilsholtzia splendens laumaniastrum robertii tachys inflata iziphora clinopodioides ecithidiaceae ecythis ollaria iliaceae eorya nitida Malvaceae iriumfetta welwitschii Myrtaceae ustromyrtus bidwillii iucalyptus	Cu tolerant Co accumulator, metallophyte Mn, Fe, Zn, Cu stabilizer Mn, Fe, Zn, Cu stabilizer Se accumulator drought tolerant Co and Cu accumulator As accumulator	Song et al. (2004) Singh and Tripathi (2007), Sinha et al. (2010), Saad et al. (2012) Nouri et al. (2011) Nouri et al. (2011) Singh and Tripathi (2007), Sinha et al. (2010) Gaff and Churchill (1976) Saad et al. (2012)
laumaniastrum robertii tachys inflata iziphora clinopodioides ecithidiaceae ecythis ollaria iliaceae orya nitida Aalvaceae riumfetta welwitschii Ayrtaceae ustromyrtus bidwillii fucalyptus	Co accumulator, metallophyte Mn, Fe, Zn, Cu stabilizer Mn, Fe, Zn, Cu stabilizer Se accumulator drought tolerant Co and Cu accumulator	Singh and Tripathi (2007), Sinha et al. (2010), Saad et al. (2012) Nouri et al. (2011) Nouri et al. (2011) Singh and Tripathi (2007), Sinha et al. (2010) Gaff and Churchill (1976) Saad et al. (2012)
robertii tachys inflata iziphora clinopodioides ecithidiaceae ecythis ollaria iliaceae orya nitida Aalvaceae riumfetta welwitschii Ayrtaceae ustromyrtus bidwillii fucalyptus	Mn, Fe, Zn, Cu stabilizer Mn, Fe, Zn, Cu stabilizer Se accumulator drought tolerant Co and Cu accumulator	Nouri et al. (2011) Nouri et al. (2011) Singh and Tripathi (2007), Sinha et al. (2010) Gaff and Churchill (1976) Saad et al. (2012)
iziphora clinopodioides ecithidiaceae ecythis ollaria iliaceae orya nitida Malvaceae riumfetta welwitschii Myrtaceae ustromyrtus bidwillii fucalyptus	Mn, Fe, Zn, Cu stabilizer Se accumulator drought tolerant Co and Cu accumulator	Nouri et al. (2011) Singh and Tripathi (2007), Sinha et al. (2010) Gaff and Churchill (1976) Saad et al. (2012)
clinopodioides ecithidiaceae ecythis ollaria iliaceae orya nitida Aalvaceae iriumfetta welwitschii Ayrtaceae ustromyrtus bidwillii fucalyptus	Se accumulator drought tolerant Co and Cu accumulator	Singh and Tripathi (2007), Sinha et al. (2010) Gaff and Churchill (1976) Saad et al. (2012)
iliaceae orya nitida Ialvaceae riumfetta welwitschii Iyrtaceae ustromyrtus bidwillii Iucalyptus	drought tolerant Co and Cu accumulator	Gaff and Churchill (1976) Saad et al. (2012)
orya nitida Aalvaceae riumfetta welwitschii Ayrtaceae ustromyrtus bidwillii fucalyptus	Co and Cu accumulator	Saad et al. (2012)
Aalvaceae riumfetta welwitschii Ayrtaceae ustromyrtus bidwillii fucalyptus	Co and Cu accumulator	Saad et al. (2012)
Aalvaceae riumfetta welwitschii Ayrtaceae ustromyrtus bidwillii fucalyptus	Co and Cu accumulator	Saad et al. (2012)
welwitschii Ayrtaceae ustromyrtus bidwillii Sucalyptus		
ustromyrtus bidwillii Gucalyptus	As accumulator	
bidwillii Tucalyptus		Shah and Nongkynrih (2007)
polybractea	Metal extractor	Mok et al. (2013)
<i>Sucalyptus</i>	Metal extractor	Mok et al. (2013)
cladocalyx		
eptospermum scoparium	Revegetation, Cr accumulator	Rufaut and Craw (2010), Khan et al. (2000)
Ielaleuca	Cd accumulator, stabilizer	Archer and Caldwell (2004)
alternifolia		
lephrolepidaceae		
lephrolepis hirsutula	Cu tolerant	Claveria et al. (2010)
amily: Onagraceae		
Penothera biennis	Cd excluder	Wei et al. (2005)
Irobanchaceae		
opubia neptunii	Co accumulator	Saad et al. (2012)
hytolaccaceae		
Phytolacca acinosa	Cu excluder	Ye et al. (2009)
inaceae		
inus brutia	Cu accumulator, stabilizer	Johansson et al. (2005), Cetinkaya and Sozen (2011)
lantaginaceae		
Digitalis thapsi	Cd and Zn accumulator	Moreno-Jiménez et al. (2009)
lumbaginaceae		
rmeria maritima	Cu excluder, salt tolerant	Brewin et al. (2003), Bothe (2011), Gawronski et al. (2011)
<i>imonium</i> sp.	Pb, Zn stabilizer	Cetinkaya and Sozen (2011)
ygeum spartum	Cu, Pb, Zn stabilizer	Mendez and Maier (2008a)
lumbago auriculata	Metallophyte	Gawronski et al. (2011)
	Stabilizar	Mondoz and Major (2008a)
		Mendez and Maier (2008a)
-		Gawronski et al. (2011)
grostis gigantea grostis stolonifera	-	Peters (1988)
grosus stolonitera	Metallophyte Pb accumulator, metallophyte	Li (2006) Singh and Tripathi (2007), Sinha et al. (2010), Li 2006, Mendez and Maier 2008a
	elaleuca alternifolia ephrolepidaceae ephrolepis hirsutula amily: Onagraceae enothera biennis robanchaceae epubia neptunii nytolaccaceae hytolacca acinosa naceae inus brutia antaginaceae igitalis thapsi umbaginaceae emeria maritima monium sp. egeum spartum fumbago	And elaleucaCd accumulator, stabilizeralternifoliacu clarantalternifoliacu toleranthirsutulacu tolerantmily: Onagraceaecd excluderenothera biennisCd excluderrobanchaceaeco accumulatoropubia neptuniiCo accumulatornytolaccaceaecu excluderhytolacca acinosaCu excludernaceaecu accumulator, stabilizerantaginaceaecu accumulator, stabilizerantaginaceaecu excluder, salt tolerantmonium sp.Pb, Zn stabilizergeum spartumCu, Pb, Zn stabilizerumbagoMetallophyteauriculatastabilizersaceaestabilizerestuca rubraStabilizergrostis albaHeavy metal tolerant

#### Table 1 (continued)

No.	Family/ Botanical name	Remediation target	Literature source
112	Cynodon dactylon	Metallophyte., Cd	Li 2006, Archer and Caldwell (2004)
113	Dasypyrum villosum	Metal excluder	Brunetti et al. (2009)
114	Deschampsia cespitosa	Heavy metal tolerant	Gawronski et al. (2011)
115	Elytrigia repens	Salinity tolerant	Gawronski et al. (2011)
116	Festuca arundinacea	Revegetation	Peters (1988)
117	Festuca rubra	Revegetation	Peters (1988), Li (2006)
118	Glomus intraradices	Heavy metal tolerant	Gawronski et al. (2011)
119	Leersia hexandra	Cr accumulator	Gawronski et al. (2011)
120	Phleum pratense	Revegetation	Peters (1988)
121	Phragmites australis	Metallophyte, Cu, Pb, Se stabilizer	Li (2006), Cetinkaya and Sozen (2011)
122	Poa compressa	Revegetation	Peters (1988)
123	Poa pratensis	Revegetation	Peters (1988)
124	Piptatherum miliaceum	Cu, Pb, Zn stabilizer	Mendez and Maier (2008a)
125	Stipa austroitalica	Metal excluder	Brunetti et al. (2009)
126	Vetiveria zizanioides Polygonaceae	Zn, Ni, Pb, Cr accumulator	Singh and Tripathi (2007), Sinha et al. (2010), Zhang et al. (2014)
127	Fagoyrum tataricum	Metallophyte	Gawronski et al. (2011)
128	Polygonum aviculare	Zn, Hg accumulator	González and González-Chávez (2006), Bothe (2011), Gawronski et al. (2011)
129	Rumex acetosa Proteaceae	Copper excluder	Ye et al. (2009)
130	Macadamia neurophylla	Mn accumulator	Peters (1988), Singh and Tripathi (2007), Sinha et al. (2010)
131	Grevillea robusta	Metal extractor	Mok et al. (2013)
132	<i>Pimelea suteri</i> Pteridaceae	Cr accumulator	Khan et al. (2000)
133	Pteris cretica	As accumulator	Gawronski et al. (2011)
134	Pteris longifolia	As accumulator	Gawronski et al. (2011)
135	Pteris sp.	Cu tolerant	Claveria et al. (2010)
136	Pteris umbrosa	As accumulator	Gawronski et al. (2011)
137	Pteris vittata	As accumulator, metallophyte	Shah and Nongkynrih (2007), Gawronski et al. (2011)
120	Rubiaceae	II	Khan et al. (2000)
138	1	U accumulator	Khan et al. (2000) Sinch and Trianchi (2007). Sinch art al. (2010)
	<i>Psychotria corinota</i> Rosaceae	Ni accumulator	Singh and Tripathi (2007), Sinha et al. (2010)
140	Pyracantha coccinea	Metallophyte	Gawronski et al. (2011)
141	<i>Rosa rugosa</i> Salicaceae	Metallophyte	Gawronski et al. (2011)
142	Populus alba	Metal extractor	Di Lonardo et al. (2011)
143	Populus monviso	Cu, Pb stabilizer	Evangelou et al. (2013)
144	Salix atrocinerea	Cd and Zn accumulator	Moreno-Jiménez et al. (2009)
145	Salix viminalis	Cu, Pb stabilizer	Gawronski et al. (2011), Evangelou et al. (2013)

Scrophulariaceae

No.	Family/ Botanical name	Remediation target	Literature source
146	Sopubia neptunii	Co stabilizer	Saad et al. (2012)
	Sicaceae		
147	Thlaspi goesingense	Ni accumulator	Bothe (2011)
	Solanaceae		
148	Datura inoxia	Heavy metal sequestration	Gawronski et al. (2011)
149	Solanum nigrum	Zn stabilizer	Cetinkaya and Sozen (2011)
	Typhaceae		
150	Typha angustifolia	Heavy metal tolerant	Gawronski et al. (2011)
151	Typha latifolia	Metallophyte, Se stabilizer	Li 2006, Cetinkaya and Sozen (2011)
	Tamaricaceae		
152	Tamarix tetrandra	Salt and metal excluder	Gawronski et al. (2011)
153	Tamarix sp.	Cu, Zn stabilizer	Cetinkaya and Sozen (2011)
	Violaceae		
154	Viola baoshanensis	Heavy metal tolerant	Gawronski et al. (2011)
155	Viola calaminaria	Heavy metal tolerant	Gawronski et al. (2011)
156	Viola lutea	Heavy metal tolerant	Gawronski et al. (2011)
	Zygophyllaceae		
157	Zygophyllum fabago	Zn stabilizer	Cetinkaya and Sozen (2011)

Fabales, Malpighiales, Poales, Rosales and Solanales. Detailed physiological and biochemical research was performed on some of these orders by Pollard et al. (2002) to study the mechanism of metal accumulation processes in *Thlaspi, Arabidopsis* and *Alyssum* species.

Such studies continue to shed light on the behaviour of different species of plants towards heavy metal pollution and metal accumulation in and around mine sites (Gonzalez and Gonzalez-Chavez 2006). Hence, the present review is an attempt to evaluate metallophytes reported in the literature with phytoremediation evidence as presented in Table 1.

#### SAA plants

Many plants have developed various xeric characters to tolerate or endure drought conditions with further evolution to adapt to metal toxicity (Cousins and Witkowski 2012). Drought resistance is a generic term for (i) drought escape and (ii) drought tolerance (at high and low tissue water potential— $\psi_w$ ) achieved through the physiological mechanisms of plants (Paleg and Aspinall 1981). Metalliferous sites present several environmental constraints for plants to establish including dry and nutrient deficient conditions (Whitbread-Abrutat 1997; Rufaut and Craw 2010; Saad et al. 2012). It is more challenging in xeric environmental conditions to revegetate with non-xerophytic plants that might involve nursery sapling plantation or the use of a direct seeding method (Chen and Xu 2005). We review potential metallophytes for mine site revegetation based on a set of established characteristics (Pollard et al. 2014; Baker 2014), namely:

- 1. Drought tolerance
- 2. Tolerance to soil acidity and salinity
- Leguminous plants that boost nitrogen assimilation and biomass production

According to Hudson (1987), SAA areas are those falling within rainfall zones of 300–600 and 0–300 mm, respectively. It is estimated that 50 % of the land on earth comes into the SAA category. In this scenario, it is evident that SAA shrublands are not likely to have complete plant coverage such as those found in tropical or temperate climates. Many abandoned mine sites fall within SAA zones, and this worsens the potential for heavy metal contamination due to acute erosion by extreme rainfall events or seasonal high-speed winds over barren dry soil (Duque et al. 2015). The plant dynamics in SAA climates are also largely affected by nitrogen deficiency and water use efficiency, in comparison to the temperate plant communities which are influenced by light and nutrition availability (Hudson 1987; Atwell et al. 1999).

For instance, Australia is the driest inhabited continent in the world, with 70 % of the land falling in the SAA zone (Hudson 1987; Duque et al. 2015). There are over 30,000 species of vascular plants recorded in Australia that include angiosperms, seed-bearing non-angiosperms (such as conifers and cycads) and spore-bearing ferns and fern allies (Orchard and Wilson 2001). Of these, only around 11 % are naturalised species and the remainder are native or endemic species (Orchard and Wilson 2001).

Out of the 11 % of naturalised species, there are a few Australian metallophytes recognised to grow in SAA zones, namely *Cheilanthes lasiophylla* (Adiantaceae), *Rhagodia spinescens* (Chenopodiaceae) and *Nitraria billardierei* (Zygophyllaceae) (Kutsche and Lay 2003).

Researches on SAA plants using exotic species of trees and shrubs are inadequate on the rehabilitation of mines in SAA zones (Le Houerou 2000). Among native plants, species of *Atriplex* (salt bush) of the Chenopodiaceae family are also known to be pioneer plants growing on mine tailings in semi-arid Western Australia (Jefferson 2004).

There is also scope to search for combined metaldrought resilient species by the use of C3 plants which thrive in moderate temperature and sunlight intensity and C4 plants that can thrive in high temperature and sunlight intensity. For instance, there is an unusual adaptation with C3 plants that behave like C4 plants. Normally, C3 plants grow in warm temperate and tropical grasslands, savannahs, sand dunes and salt marshes, including semi-deserts and deserts (Kadereit et al. 2003).

Specifically, the most diversified C3 plants like Gomphrena, Amaranthus, Atriplex, Salsola and Suaeda prefer

C4 habitats including SAA zones. Moreover, most of the soilenriching leguminous plants of the *Acacia* species are also drought and salt tolerant but careful consideration of such species is needed given the potential weed threat in some regions of the world (Hoffmann et al. 2002).

#### **Revegetation challenges**

Chen and Xu (2005) state that it is a sustainable exercise to use appropriate plant species or ecotypes or nursery stocks for assisted revegetation of abandoned mine areas contaminated with toxic elements. However, assisted revegetation can be expensive if the area is too large. Therefore, before considering phytoremediation, the adaptive characteristics of plants form a basis for sustainable rehabilitation of mines in SAA zones (Vranjic et al. 2012). Moreover, various risks and challenges are involved such as polluted water entering and clogging ponds, tailing instability, lack of documentation of faunal diversity for food chain assessment, lack of identification of high-risk zones of erosion and human safety hazards (Fig. 3).

 A taxonomic survey, plant assessment and laboratory research can help accomplish the revegetation of a contaminated area and avoid negative side effects. One such process

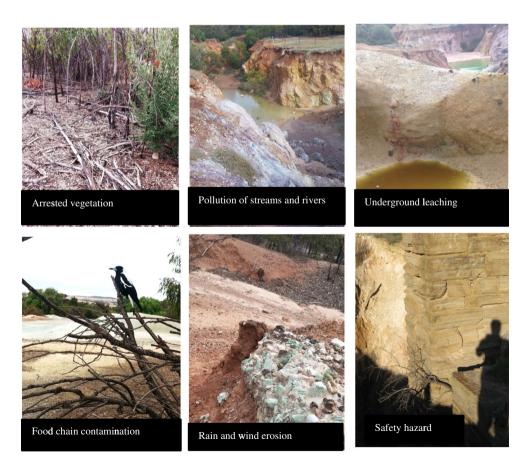


Fig. 3 The ecological and engineering challenges associated with revegetation of SAA abandoned mine sites such as the copper mine in Kapunda. (2014– 2015) is a 'phenomenon of arrested succession' where grasses dominate trees and render them into diseased stumps (Groninger et al. 2007; Kramer et al. 2000; Boyes et al. 2011).

- A further problem of plant establishment is due to allelopathism, where one species competes with the other to limit growth leading to elimination of the latter. An allelopathic effect is also brought about by its own decaying matter such as *Kochia* leaves on its own roots that depress its own growth in coal mine site in Prairie (Wali 1999).
- It has also come to light that plants involved in phytoremediation often bear the toxicity stress of Al, Cd, Cu, Mn and Zn that hinder plant establishment and growth (Peters 1995; Maddocks et al. 2009).
- Moreover, the secondary successional plant communities in SAA climates are more likely to be affected by substantial mortality because of a deficiency of water (Mendez and Maier 2008b). Therefore, it is necessary to identify the spatial variability and heterogeneity of soil properties for determining the revegetation potential with regards to an availability of soil moisture and nutrients (Guo et al. 2010).
- There have been cases of species extinction through genetic assimilation or demographic swamping due to the use of alien species of plants in revegetation programmes (Byrne et al. 2011).

#### Phylogenetic implications in SAA lands

There is a very small literature available regarding documented total number of available metallophytes. In one source, 400 species belonging to 45 families are identified as metallophytes based on their heavy metal (Cd, Co, Cu, Pb and Zn) uptake behaviour (Mendez and Maier 2008b). However, Pollard et al. (2014) report the number to be 500 based on facultative hyperaccumulation. Rascio and Navarilzzo 2011 in their review report 450 angiosperms as hyperaccumulators of heavy metals. New listings have appeared in recent times which are either a repeat or are taxonomically related to the earlier identified species or are indeed new additions (Pollard et al. 2014). Fine (2015) suggests that, as a result of long geographic isolation and climatic influences, there are natural variations in plants leading to environmental evolution. For instance, species behaviour is linked through their phylogeny in orders Caryophyllales, Apiales and Cucurbitales and is also related because of high <sup>36</sup>Cl uptake among these orders (Willey and Fawcett 2005). As a result of analyses of molecular data from various sources, the understanding around phylogenetic relationships among angiosperms has greatly increased. For instance, Fior et al. (2006) discuss the repositioning of the family Caryophyllaceae within the order Caryophyllales as the sister to the family Amaranthaceae in a clade, which in turn is sister to the core order Caryophyllales.

The phylogenetic linkage is also seen useful for quantifying and predicting the soil-to-plant transfer of ions as found in some angiospermic plants (Willey and Fawcett 2005). The molecular level study of phylogenetics of the plant *Limonium* sp. and its related genera under the family Plumbaginaceae shows physiologically similar ion transfer behaviour such as Ca<sup>++</sup> (Lledo et al. 2005). However, little is known regarding the genetic basis of hyperaccumulation as there are no known cases of major genetic polymorphism, which contrasts the phenomenon of metal tolerance as a result of evolution as discussed earlier (Pollard et al. 2002; Hanikenne and Nouet 2011).

There is also a lack of understanding of the genetic and regulatory factors influencing the variable gene expression of the hyperaccumulative behaviour of metallophyte plant species (Pollard et al. 2002). However, plant systematics also deals with hybridisation and molecular techniques, including comparative genomics and the hyperaccumulative nature of plants (Hanikenne and Nouet 2011). However, it is expected that any given metallophyte, by virtue of being taxonomically related to another far-related genera, should behave identically with respect to its response to heavy metal remediation. Therefore, all species falling under the genus *Atriplex* as mentioned above should be resilient to SAA mine sites containing saline and sodic soils.

#### Plant metal interaction in SAA zones

Some of the literature report on plants growing in the semiarid and arid zones of earth such as nitrogen fixer *Medicago polymorpha* in South Australia (Black 1909) has a leguminous relative *Medicago sativa* which is a Mn, Pb and Cr accumulator (Gawronski et al. 2011). Likewise, the legume *Acacia mearnsii* is a SAA metal accumulator (Mok et al. 2013). Several other genera namely *Acacia victoriae*, *Acacia tarculensis*, *Acacia tetragonophylla*, *Acacia nyssophylla*, *Acacia rivalis*, *Acacia careneorum* and *Acacia ligulata* could be effective in SAA mines for revegetation (Kutsche and Lay 2003). Similarly, a legume *Crotalaria cobalticola* which is a cobalt accumulator (Table 1) is related to SAA plants namely *Crotalaria cunninghamii* and *Crotalaria eremaea* (Kutsche and Lay 2003).

Plants belonging to the Brassicaceae family *Brassica* tournefortii also occur in disturbed soils (Kutsche and Lay 2003). Plants of the same genera *Brassica carinata* and *Brassica juncea* act as As, Cd, Cu, Pb and Zn stabilizers and accumulators, respectively (Table 1). A popular salt bush plant from the Chenopodiaceae family named *Atriplex* sp. is reported to be a pioneer species growing on mine tailings in semiarid Western Australia (Jefferson 2004), and the species Atriplex lentiformis, Atriplex nummularia and Atriplex canescens are listed as proven stabilizers (Table 1). Russian thistle Salsola kali also from Chenopodiaceae is a Cd extractor, but the species under the same family Atriplex nummularia, Atriplex stipitata, Atriplex lindleyi, Atriplex holocarpa, Atriplex limbata, Atriplex quinii and Atriplex vilutinella are salinity-tolerant (Kutsche and Lay 2003).

Guo et al. (2009) recommend Atriplex semibaccata (Table 1) to be a suitable species for mine site soil rehabilitation in SAA environmental conditions. Another Cd accumulator and soil stabilizer namely Melaleuca alternifolia (Table 1) has a related genus from the family Myrtaceae called Melaleuca glomerata that tolerates high levels of salinity and is also found to grow in SAA zones (Kutsche and Lay 2003). The forb Euphorbia sp. is a Cd, Cu, Mn, Pb and Zn stabilizer (Mendez and Maier 2008b) and is also related to a SAA genus Euphorbia drummondii highly reputed for its soil stabilisation capacity (Kutsche and Lay 2003). Several studies have reported growth of SAA genus of the grass family Poaceae namely Festuca rubra, Agrostis alba, Agrostis gigantea, Agrostis stolonifera, Agrostis tenius, Deschampia cespitosa, Glomus intraradices, Vetiveria zizanioides and Phragmites australis (Li 2006; Kutsche and Lay 2003). The SAA adapted grass P. australis which is a drought-tolerant species (Kutsche and Lay 2003) has been reported to grow on Cu-, Pb- and Secontaminated sites (Li 2006).

Kutsche and Lay (2003) report 11 species of *Eucalyptus* that are found in outback SAA zones of Australia. An additional two species, namely *Eucalyptus polybractea* and *Eucalyptus cladocalyx*, are also reported to be metal extractors growing in SAA soils (Mok et al. 2013). There is also a report of *Grevillea robusta* of the family Proteaceae being a metal extractor (Mok et al. 2013) which is represented by *Grevillea striata*, *Grevillea huegelii*, *Grevillea juncifolia*, *Grevillea nematophylla*, *Grevillea stenobotrya* and *Grevillea treueriana* found in SAA zones (Kutsche and Lay 2003). A popular weed *Solanum nigrum*, commonly called black night-shade, is a Zn stabilizer (Cetinkaya and Sozen 2011) and has related species, namely *Solanum quadriloculatum*, *Solanum ellipticum*, *Solanum coactifilerum* and *Solanum esuriale*, growing in SAA conditions (Kutsche and Lay 2003).

Another Zn stabilizer belonging to the family Zygophyllaceae is Zygophyllum fabago (Cetinkaya and Sozen 2011) which has a family and genera related to Zygophyllum aurantiacum, Zygophyllum apiculatum, Zygophyllum howittii, Zygophyllum prismatothecum and Zygophyllum simile growing in SAA zones (Kutsche and Lay 2003). A Pb and Zn stabilizer, Limonium sp. (Cetinkaya and Sozen 2011), is reported to grow on disturbed soils under a SAA type climate as Limonium lobatum from the family Plumbaginaceae (Kutsche and Lay 2003). Other exceptional metallophytes in SAA mine sites are identified as Atriplex spp., A. semibaccata, S. kali, P. australis and M. sativa, and these are discussed up to the rank 'order' in the following topics.

#### Order Caryophyllales

The order Caryophyllales include 33 families, 692 genera and 11,155 species worldwide (Stephens 2001). Some of the pioneer metallophytes under this order listed in Table 1 are from the families Amaranthaceae, Betaceae, Caryophyllaceae, Chenopodiaceae, Plumbaginaceae, Polygonaceae and Tamaricaceae. Most prominently, metallophytes from the family Chenopodiaceae are represented by *A. lentiformis*, *A. nummularia*, *A. canescens*, *A. semibaccata* and *S. kali*.

It is reported that forbs such as *Atriplex* sp. are metal tolerant because of their higher bioconcentration factor and are considered to be a candidate species for phytostabilisation (Kachout et al. 2012). Generally, soils that contain high concentrations of Zn >300 mg kg<sup>-1</sup>, Cu >100 mg kg<sup>-1</sup>, Ni >50 mg kg<sup>-1</sup> and Pb >100 mg kg<sup>-1</sup> are phytotoxic to plants, but the genus *Atriplex* has not shown phytotoxicity when introduced to higher concentrations than those stated above (Kachout et al. 2012). In a study of Amer et al. (2013), *Atriplex* was found to have a stimulating effect with Ni exposure, whereas concentrations of Ni >1 mg L<sup>-1</sup> was found to be toxic to other plant species except *Atriplex* sp.

However, in remediation studies of Ni, Pb and Zn in the same area, it was found that the highest Zn concentration of 4660 mg kg<sup>-1</sup> was observed in shoots of *Atriplex halimus*, indicating that it is a Zn accumulator. Coincidently, the plant *A. halimus* has been proven to be a good phytostabilizer in SAA conditions exhibiting effective adaptation even without soil amendments (Martinez-Fernandez and Walker 2012). In a study of accumulation of Cu, Pb, Ni and Zn in a halophyte *Atriplex hortensis*, the metal concentrations in the roots were found to be proportionately higher than in the shoots with increasing concentrations of those metals in soil (Kachout et al. 2012a).

The salinity-tolerant plant A. semibaccata is found to be a most adaptable metallophyte that modifies its habitat and leaf structure when there is a change in environmental conditions, particularly with respect to the influences of temperature, evaporation, soil moisture and salinity (Bullock 1936). Genus A. semibaccata is a drought-tolerant SAA species that was introduced into California and Arizona in the USA in the early 1880s (Bullock 1936). From 1993 to 2004, field phytoremediation studies were conducted using A. semibaccata in central California in an area known to have high selenium concentrations (Banuelos 2006). Not only the selenium accumulation but the concentration of Cu in A. semibaccata was at elevated levels in both the shoots  $(205.8 \text{ mg kg}^{-1})$  and roots  $(129.5 \text{ mg kg}^{-1})$  in a study at abandoned mine site (Guo et al. 2009). In their study on the effect of leaching and irrigation on growth of A. semibaccata,

De Villiers et al. (1995) found this species had the ability to survive both drought and salinity. Another species under order Caryophyllales is *A. rubra* of Chenopodiaceae, which is a suitable plant for phytostabilisation of Cu- and Nicontaminated sites (Kachout et al. 2012).

*S. kali* commonly called tumble weed or prickly saltwort or prickly Russian thistle also belongs to the family Chenopodiaceae and was found to survive on 6000 mg L<sup>-1</sup> of NaCl treatment, exhibiting no symptoms of toxicity (Shekhawat et al. 2006). Although this plant is reported to be a troublesome weed (Hasan et al. 2001), following hydroponic cultivation experiments and agar exposure, Gardea-Torresdey et al. (2005) reported its positive potential for bioaccumulation of heavy metals.

The ICP/OES and XAS studies conducted by de la Rosa et al. (2004) on this species did not show any phytotoxicity effect when grown in an agar-based medium with 20 mg  $L^{-1}$ of Cd (II). The Cd accumulation from dry biomass was 2696 mg kg<sup>-1</sup> in the roots, 2075 mg kg<sup>-1</sup> in stems and 2016 mg kg<sup>-1</sup> in leaves in this experiment. Yet in another EDTA experiment on Pb phyto-accumulation test of S. kali, concentrations on dry weight of roots, stems and leaves were 31,000, 5500 and 2100 mg kg<sup>-1</sup>, respectively (Cano-Aguilera et al. 2007). In a road side study of this species in Iran, Pb which is one of the components of automobile fuel was found significantly absorbed by S. kali. Lead concentration in plant organs decreased exponentially with corresponding distance away from the motor vehicle road. Moreover, shoot parts were found to absorb more Pb than belowground parts (Sinegani 2007). These findings indicate that the family Chenopodiaceae has a good prospect for remediation of heavy metals, especially in SAA conditions.

#### Order Poales

The order Poales has 20 families (Jacobs and Wilson 2002), but Bremer (2002) states there to be 18, prominently with Poaceae having 12,070 species and Cyperaceae having 5500 species. All these grass metallophytes are C4 and resilient SAA plants belonging to Poales and are listed in Table 1 belonging to Poaceae, Typhaceae, Juncaceae and Cyperaceae. Some of the promising plants for phytoremediation technologies as listed under the Poaceae family are *A. alba*, *A. gigantea*, *A. stolonifera*, *A. tenius*, *Cynodon dactylon*, *Dasypyrum villosum*, *Deschampsia cespitosa*, *Elytrigia repens*, *F. rubra*, *G. intraradices*, *Leersia hexandra*, *Phleum pratensis*, *Poa compressa*, *Poa pratensis*, *Piptatherum miliaceum*, *P. australis*, *Stipa austroitalica* and *Vetiveria zizaniodes*.

The common reed *P. australis* is a popular plant in environmental science research and is traditionally used for wastewater treatment. In a wastewater remediation experiment, the associated rhizosphere bacterial community of *Phragmites*  showed decolourisation of distillery effluent from dark brown (high concentration of amino carbonyl polymer, phenolics, heavy metals and sulphate) into light brown colour (Chaturvedi et al. 2006). This grass plant was able to adapt to the harmful effects of cadmium. Not much literature is available regarding P. australis on the semi-arid and arid mine rehabilitation at this point. However, it was found that phytochelatin production was increased when P. australis was exposed to higher Cd concentrations suggesting that phytochelatin has a role in heavy metal sequestration (Ederli et al. 2004). As per Ali et al. (2002), these common reeds are a recommended species for phytostabilisation. This was based on the fact that the bioconcentration factor of Cu was higher in roots than in shoots. Although P. australis (Ederli et al. 2004) is reported to be an ideal species for treating industrial effluent and sewage, it may not be ideally suitable for mine site revegetation programs.

#### Order Fabales

According to Stevens (2006), the order Fabales has four families, namely Fabaceae, Polygalaceae, Quillajaceae and Surianaceae, with 95 % of the genus appearing only from the family Fabaceae. Also referred to as Leguminosae, this family includes a significant number of SAA metallophytes that are listed in Table 1. They are *A. mearnsii, Amorpha fruticosa, Astragalus bisulcatus, Astragalus racemosus, Caragana arborescens, Crotalatia cobalticola, Cytisus striatus, Dalea bicolor, Lupinus albus, Lupinus angustifollus, Lupinus hispanicus, Meliolotus indica, Robinia pseudoacacia, Sesbania drummondii, Vicia exaltata* and *M. sativa.* 

So far, *M. sativa* is a popular species for phytoremediation (Lopez et al. 2005). However, other species from the same family may be able to grow in the SAA conditions for phytoremediation. Commonly called alfalfa, it has been found to have enhanced ability to uptake Pb when grown hydroponically in 100  $\mu$ M of hormone IAA and 0.2<sup>-1</sup> mM of EDTA, which increased the Pb accumulation in leaves by approximately 2800 % (Lopez et al. 2005). This is in comparison with Pb content in leaves of *M. sativa* when exposed to a Pb/EDTA combination, resulting in only 600 % accumulation compared to exposure with hormone Pb/IAA/EDTA. This result suggests that plants could also increase their metal-accumulating potential by hormone feeding and chelation even without genetic manipulation (Lopez et al. 2005).

According to Rajendran and Gunasekaran (2007), *M. sativa* was found to accumulate 12,360  $\mu$ g g<sup>-1</sup> in the roots and 1920  $\mu$ g g<sup>-1</sup> in the shoots when exposed to 50  $\mu$ g mL<sup>-1</sup> concentration of cadmium. This species was also used to study the uptake of organic pollutants from soil and to establish the role of plant–soil contaminant interactions (Chekol and Vough 2001). However, the toxic PAH pollutant pyrene had an inhibitory effect on growth of *Medicago*, including its root (Fan et al. 2008). The effects of organic matter content in soils on toxic pollutant uptake were also tested with *Medicago*. As a result, low levels of pyrene and TNT were recovered from soil having a higher (6.3 %) concentration of organic matter compared to a lower organic matter concentration (2.3 %) (Chekol and Vough 2001).

Therefore, organic matter and rhizosphere bacteria have a significant influence on the phytoremediation capacity of leguminous plants such as *M. sativa*. However, the advantages of using Leguminosae/Favaceae for phytoremediation include its self-sufficiency for nitrogen, its potential for drought tolerance and its capacity to survive even on infertile soil (Gawronski et al. 2011).

#### Advances in remediation science

Highly concentrated contaminants on small area of soils or locked water bodies can adopt conventional or engineered methods of remediation along with phytorem ediation technologies. Sometimes, phytoremediation alone may be problematic due to higher bioavailability and deeper root penetration leading to phytoextraction by 'free metal ion solution' mechanism of absorption of metals (Maddocks et al. 2009).

Therefore, from a phytostabilisation point of view, it is a bad idea to let deposition of absorbed metal pollutants on the surface unless plants are harvested and disposed of safely (Singare et al. 2013). Therefore, an alternative option is to adopt a non-biological method in extreme contamination cases (Pulford and Watson 2003; Pilon-Smits 2005). For instance, along with long-term revegetation programs, short-term remedial measures that can be adopted are listed below:

- Hydraulic control to prevent leaching of pollutants (Zhang et al. 2015)
- Chemical treatment by chelation and compartmen talisation in roots (Komal et al. 2015)
- Bio-augmentation through introduction of microbes and biostimulation (Chekol and Vough 2001)
- Attenuation and treatment of contaminated soils by vitrification (Wang et al. 2012)
- Vapour extraction, electro kinetics, soil flushing and slurry-phase bioreactors (Kuppusamy et al. 2016)
- Combined co-metabolic stripping (Russell 2012)

However, some of the conventional technologies listed here are harmful to soil microbial diversity and are also costly because they often involve sophisticated machinery (Rajkumar et al. 2012).

#### Plant biotechnology and tissue culture

Bioremediation in general is a clean-up technology that involves an active role of biotic and abiotic components such as soil, plants and microorganisms in an ecosystem over a polluted site (Fester et al. 2014). Although larger trees such as alder in association with fungus and bacteria have been reported to grow in the SAA zones, it is difficult to actually establish them there (Roy et al. 2007). For example, a 20-year study on a SAA revegetated mine site revealed that plant and microbial diversity was low compared to an adjacent undisturbed site (Mendez and Maier 2008b).

Moreover, even some fast-growing timber trees take 30 to 40 years in a favourable condition (Groninger et al. 2007) which indicates prolonged time periods before achieving phytoremediation as discussed earlier. Therefore, as well as assisting with branching and growth of vegetation, microorganisms and fungi have ability to carry out heavy metal detoxification (Singh and Tripathi 2007; Cetin et al. 2011). Moreover, inoculating plants with beneficial microorganisms including free living and symbiotic associates is likely to improve remediation results by many folds (Kurek and Majewska 2012).

There are several types of rhizosphere bacteria that could be integrated with metallophytes to develop potential for phytoremediation (Baunthiyal 2014). A recombinant gene technology can be effective in improving the quality of microbes that are toxicity resistant and pollutant accumulating (De-Bashan et al. 2012). Since approximately 80 % of vascular plants have an ability to form mutulastic symbiotic associations with arbuscular mycorrhizal fungi, a screening test for metallophyte identification will help predict the response of plants specifically towards pollutants before spending money and time on a field scale plantation (Isayenkov et al. 2004; Doran 2009). SAA metallophytes that are compatible to mycorrhiza and rhizobia species are potentially the most sought after plants.

For instance, the gene composition of the microbe friendly Brassicaceae plants *Arabidopsis thaliana* and *Thlaspi caerulescence*, both with five chromosomes, is similar to another Brassicaceae *Cardaminopsis halleri* (eight chromosomes), all being coincidently metallophytes (Chiang et al. 2006; Rigola et al. 2006; Dahmani-Muller et al. 2000; Bothe 2011).

Therefore, engineering heavy metal-resistant genes that are compatible with rhizobium and mycorrhizal fungi has a good prospect for developing hybrid pools of such species to give rise to transgenic plants (Lal and Srivastava 2010). As another example, the tissue culture of transgenic plants has been used to produce in vitro clones of *Populus alba* from the Salicaceae family (Table 1) (Rascio and Navari-Izzo 2011; di Lonardo et al. 2011).

Moreover, plant tissue culture is also useful to decipher a range of enzymes involved in transformation of some xenobiotic compounds and industrial wastes by amplifying their ability to tolerate, detoxify and store high concentrations of heavy metals (Doran 2009).

#### Future of remediation technology

Recently, environmental nanotechnology has been gaining popularity as a clean-up technology (Niosi and Reid 2007; Baruah and Dutta 2009; Mura et al. 2013). Nanomaterials are prepared by engineering particles with required optical and electronic properties within a controlled shape and size (Sadik 2011; Fulekar 2012). Developing nanomaterials for clean-up technologies involves design, characterisation and release into polluted sites (Sadik 2011). However, it is important to qualitatively determine the effectiveness of nanomaterials since the harsh climatic conditions in SAA environments may not permit nanomaterials to be effective at the field scale.

Although the costs for production and processing of nanomaterials are high, they have been found to be promising in earlier experimental stages to use on contaminated sites to track the fate, transformation and bioavailability of toxic substances (Fulekar 2012). If nanotechnology is integrated with plant technology, it has the capacity to remediate metals, iron oxides and silicates with the help of microorganisms (Sadik 2011; Fulekar 2012).

There has also been an attempt recently to encapsulate toxic substances including metals in soil using nanomaterialbased botanical insecticides (De Olivera et al. 2014), although there are few reported studies regarding eco-nanotechnology involving plants. Wang et al. (2015) attempted to develop a nano-agent called sixthio to chelate heavy metal-polluted soils but without any attempt to incorporate roles of plants and microbes. An experiment using Taunit, containing multiwalled carbon nanotubes (MWNTs), showed an improvement in root growth and peroxidase activity (Smirnova et al. 2011) in plants that adds advantage to produce and identify plants with potential for remediation.

#### Conclusions

It is important to understand that a significant number of metallophytes are effectively growing on polluted sites such as abandoned mines under exposure to different biotic and abiotic stresses. An innovation that integrates plant and microbial association to execute rehabilitation of SAA operational and abandoned mines is required. Conceptually, the process of phytoremediation is achieved through revegetation leading to rehabilitation. A careful consideration on phylogenetics is necessary to identify plants that have been successful and taxonomically related. Moreover, revegetation programs using untested species of plants on a contaminated site cannot be considered as a successful remediation process. The question also arises whether the aim is to merely revegetate polluted sites or to revegetate using native species in a sustainable manner by following patterns of ecological succession.

There is a need to identify groups of pioneer and ideal species that can sustainably stabilize heavy metals avoiding exposure to the food chain. At this point, phytostabilisation is a potential technology that awaits a better integrated approach with biotechnology and nanotechnology. The integrated remediation technology (IRT) presented in this paper integrates ecosystem function, plant systematics and gene technology, use of transgenic plants and environmental nanotechnology. However, to achieve remediation goals, phytoremediation technology should also be considered using native species that are drought, salinity and heavy metal tolerant. Ideally, these should also be non-allelopathic, non-accumulator, nitrogen fixing and should have a potential for high biomass yield.

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