Comparison of Four Sesbania Species to Remediate Pb/Zn and Cu Mine Tailings

GILBERT Y. S. CHAN

Department of Applied Biology and Chemical Technology The Hong Kong Polytechnic University Hong Kong, China

ZHI H. YE

School of Life Sciences Zhongshan University Guangzhou, 510275, China

MING H. WONG *

Institute for Natural Resources and Environmental Management and Department of Biology Hong Kong Baptist University Hong Kong, China

ABSTRACT / A 6-month greenhouse pot trial was performed, aimed at screening appropriate *Sesbania* species for remediation of Pb/Zn and Cu mine tailings. Performances of young seedlings of four *Sesbania* species (*S. cannabina*, *S. grandi-*

flora, S. rostrata, and S. sesban) were compared with and without inoculation of rhizobia. Seedlings were planted in two types of tailings amended with garden soil or garden soil mixed with river sediment. The results indicated that inoculated plants generally produced a higher biomass than samples without inoculation. Pb/Zn mine tailings containing rather high concentrations of total and water-soluble Cu, Pb, and Zn were toxic to plant growth compared with Cu mine tailings, according to the growth performance of the four species. Sesbania sesban and S. rostrata showed superior growth performance, compared to the other two species. Thus, they can serve as pioneer species to modify the barren environment, by providing organic matter and essential nutrients such as nitrogen, upon decomposition, in a relatively short period of time. This is especially true for *S. rostrata*, which is an annual plant that forms both stem and root nodules. However, a longerterm field trial should be conducted to investigate if superior species can beneficially modify the habitat for the growth of subsequent plant communities.

Metal mine tailings suffer from high concentrations of heavy metals, poor soil structure, low water retaining capacity, and a lack of organic mater and basic plant nutrients such as nitrogen (N), phosphorus (P), and potassium (K). The free heavy metal ions present in the leachate may contaminate the environment through acid mine drainage. Coverage by inert materials or plants is therefore necessary to prevent harmful fine dust from being blown away and to stabilize the land for future redevelopment. Phytostabilization appears to be an environmentally friendly approach to stabilize toxic metal mined sites in the long run (Baker and others 1994, Lan and others 1997, Yang and others 1997). Successful establishment of pioneer species will improve soil characteristics by enriching its organic content and possibly reducing soil toxicity, so that more sensitive plants can develop and thus a healthy diversified ecosystem can eventually be achieved. A suitable pioneer species, which can withstand the detrimental

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*Author to whom correspondence should be addressed, email: mhwong@hkbu.edu.hk

effects of heavy metals and can continuously supply the much-needed nutrients, especially N, to the soil, needs to be found.

Sesbania species have previously been used in lead/ zinc (Pb/Zn) tailings to improve soil quality and for revegetation purposes through their contribution of organic matter and a self-sufficient N production system (Yang and others 1997). They are able to survive in toxic soils partly due to their inherent tolerance to metal toxicity and partly due to the formation of Nfixing nodules that is important as metal-mined land always lacks organic matter and N. All Sesbania plants form root nodules when infected by appropriate nodule-forming bacteria, while Sesbania rostrata develops nodules on their stems after infection by Azorhizobium caulinodan (Robertson and Alexander 1994) or Sinorhizobium teranga (Tomekpe and others 1996). These plants may be used to help reclaimation of metal contaminated soil, as the stem is not in direct contact with the toxic medium and the burden of N fixation can thus be transferred to the less-affected stem nodules (Yang and others 1997).

In order to compare the performance of *Sesbania* species that can be used to reclaim decommissioned metal mines, *S. rostrata*, *S. sesban*, *S. cannabina*, and *S.*

Nodulation treatment			Bacteria used					
	Root nodules only	Root and stem nodules	No infection	A. caulinodans	Rhizobium isolated from <i>S. cannabina</i>			
S. cannabina	+	_	+	_	+			
S. grandiflora	+	_	+	+	_			
S. rostrata	+	+	+	+	_			
S. sesban	+	_	+	+	_			

Table 1. Nodulation treatments of each Sesbania species

grandiflora were planted on Pb/Zn and copper (Cu) mine tailings under greenhouse conditions; with special emphasis on the influence of heavy metals on the Sesbania–Arhizobium symbiosis system of the four species.

Materials and Methods

Supply of Seed and Selection of Four Tolerant Sesbania Species

Seeds of S. grandiflora, S. rostrata, and S. sesban (var. nubica) were purchased from AgroForestor (UK), S. formosa and S. tripetti were purchased from Chiltern Seeds (UK), and S. cannabina were collected from the SENT Landfill, Hong Kong. In order to choose species with a higher tolerance to Cu, Pb, and Zn, a root elongation test was conducted to compare the effects of Cu, Pb, and Zn on root growth of six Sesbania species (S. cannabina, S. formosa, S. grandiflora, S. rostrata, S. sesban, and S. tripetti). The root length of seedlings in metal solutions were compared with that in distilled water (control) after a growth period of 14 days (Wong and Bradshaw 1982). Sesbania formosa and S. tripetti were very sensitive to metal toxicity and exhibited retarded root growth (data not shown), only S. cannabina, S. grandiflora, S. rostrata, and S. sesban were employed in the subsequent pot trial.

Supply of Inoculants and Tailings

Azorhizobia caulinodans obtained from American Type Culture Collection (ATCC) were used to induce root nodules in *S. grandiflora, S. sesban,* and for root and stem nodules in *S. rostrata.* For *S. cannabina,* rhizobia was isolated from the root nodules of *S. cannabina* for reinoculation (Table 1). Nodule forming bacteria for *Sesbania* species do not have high host specificity, for examples, bacteroids isolated from *Sesbania* will nodulate *Leucaena* species.

Samples of Pb/Zn tailings were collected from the Fankou Mine, and Cu tailings were collected from Yang Chuan Mine, both located in Guangdong Province,

China. Sediments from the Pearl River were collected along the river bank within the proximity of the Zhongshan University. The use of the river sediment is to compare the toxicity of the tailings a type of heavy metal contaminated substratum. Garden soil was collected from the Bamboo Garden at Zhongshan University, Guangzhou City, PR China; which had no previous record of metal contamination; it was used as an ameliorant to the tailings.

Properties of Growth Substrates

The physicochemical properties of tailings and soil and river sediment samples were analyzed: pH and electrical conductivity [sample: deionized water = 1:1 (w/v), using a pH/conductivity meter]; cation exchange capacity (ammonium acetate method); total organic carbon (C); and total N (CHNS analyzer PE2400); total P (digested with perchloric acid, followed by molybdenum blue method); total Cu, Pb, and Zn [digested with concentrated HNO₃ and concentrated HClO₄, followed by Inductively Coupled Plasma Spectrometry (ICP)]; and water-soluble Cu, Pb, and Zn (extracted with deionized water, also followed by ICP) (Sparks and others 1996).

Plant Growth Studies

The seeds of the four species were germinated on wet sand. Five days after germination, they were transferred to plastic pots. Five young seedlings of each species were planted in each pot (9.5 cm height; 12.5 cm top diameter, and 8.5 cm bottom diameter) containing 1 kg of tailings and soil at different ratios (Table 2). River sediments were added in two of the treatments to investigate the possibility of metal ions chelation to organic matter. In addition, river sediments also provided additional source of nutrients and other forms of heavy metal to the substratum. All pots were arranged in a randomized block design within a greenhouse and watered daily.

Inoculants were prepared for the induction of root nodules. Stem nodules on *S. rostrata* were not expected

Table 2. Different ratio of tailings (T), garden soil (S) and river sediment (R) (w/w) used in the pot trial

Treatment	Pb/Zn tailings (T:S:R)	Cu tailings (T:S:R)
T1 (pure Pb/Zn tailings)	1:0:0	_
T2 (high level of Pb/Zn tailings)	1:1:0	_
T3 (low level of Pb/Zn tailings)	1:4:0	_
T4 (pure garden soil, control)	0:1:0	_
T5 (Pb/Zn tailings + soil + sediment)	2:3:5	_
T6 (pure Cu tailings)	_	1:0:0
T7 (high level of Cu tailings)	_	1:1:0
T8 (low level of Cu tailings)	_	1:4:0
T9 (pure garden soil, control)	_	0:1:0
T10 (Cu tailings + soil + sediment)	_	2:3:5

to form until the full development of primodia on the stem, which would not take place until after 4 weeks of growth. The inoculants were first cultured in a yeast extract mannitol broth (Somasegaran and Hoben 1994) and shaken at 125 rpm for 72 hours before inoculation took place. To inoculate plants, 5 ml of inoculant was pipetted onto the soil surface of each pot after 2 and 4 weeks of growth. Seedlings, that were not subjected to inoculation (the control) were separated from the infected ones by moving them to another greenhouse with similar growth conditions in order to minimize cross-contamination. For *S. rostrata* seedlings to develop stem nodules, *A. caulinodan* suspension was sprayed onto the stems after 4 weeks of growth, until the full length was covered with the culture solution.

The weaker plants in each pot were removed after 10 weeks, leaving just the strongest/tallest. After 6 months, plants were harvested and separated into different parts (i.e., leaf, stem, root, root nodule, stem nodule, and seed). All samples, except seeds, were oven-dried to constant weight. The seeds were air-dried, weighed, and stored.

Data on total biomass of four *Sesbania* species grown in Pb/Zn and Cu mine tailings subststrata were examined by one-way ANOVA followed by Tukey-HSD tests using the SPSS statistical package.

Results

Physicochemical Properties of Growth Substrates

The properties of the two types of tailings, garden soil, and river sediment are listed in Table 3. They were all slightly acidic (pH 5.7–6.0), except for the garden soil (pH 6.9). River sediment had the highest cation exchange capacity, followed by garden soil, and then the two types of tailings. In terms of electrical conductions.

tivity, Pb/Zn tailings had the highest level, followed by river sediment, garden soil, and Cu tailings. As expected, river sediment and garden soil both contained higher levels of total organic content, N and P. The two tailings possessed very high total concentrations of Cu, Pb and Zn. River sediment had moderate concentrations of the 3 metals.

Biomass of Plants

Except for *S. cannabina* and *S. grandiflora* grown in Cu tailings, plants without inoculation did not grow as well as inoculated samples. Except for *S. sesban, Sesbania* species grown in Pb/Zn tailings amended with river sediments generally had less harvestable biomass as those growing in pure Pb/Zn tailings (T1) or in high level of Pb/Zn mine tailings (T2). However, all four *Sesbania* species growing in Cu tailings amended with river sediments performed better than those growing in Pb/Zn tailings, but their biomass was still less than those growing in the pure garden soil (control). Generally, higher biomass was found in plants growing in tailings with a higher portion of garden soil (Tables 4 and 5).

Among the four *Sesbania* species, *S. sesban* generally gained the highest harvestable biomass in all substrata. No obvious beneficial effects in terms of harvestable biomass were observed in *S. cannabina* and *S. grandiflora* after inoculated with rhizobia. For *S. sesban*, inoculated plants weighted almost twice as much as their controls. For *S. rostrata*, the majority of samples with both stem and root nodules gain higher biomass than the uninoculated controls (Tables 4 and 5).

Table 6 presents the weight of dried seeds collected from the four species with and without nodulation and growing at different substrata. Harvested seed weight was used as indicator to compare the plant capability to complete a life cycle under the particular treatment. Most plants that bore seeds belonged to the inoculated groups. Nodulated *S. cannabina* and *S. rostrata* produced seeds in almost all treatments. *S. sesban* is an perennial plant and no seed could be collected after 6 months of growth. No plants grown in Pb/Zn tailings amended with river sediment produced seeds.

Discussion

Effects of Inoculation

In most cases, inoculation increased plant biomass (especially *S. sesban* and *S. rostrata*). The increase in portion of garden soil also increased the harvestable plant biomass in the nodulated *Sesbania* species. Although the inoculated plants had more nodules than

Table 3. General properties of Pb/Zn and Cu tailings, gardening soil, and river sediment (mean \pm SE, N=4)

	Pb/Zn tailings	Cu tailings	Gardening soil	River sediment
рН	6.0 ± 0.0	5.7 ± 0.1	6.9 ± 0.1	5.8 ± 0.1
Cation exchange capacity (meq/100g soil)	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.11 ± 0.01
Electrical conductivity (µS/cm)	2763 ± 168	43 ± 2.29	68 ± 2.0	155 ± 3.5
Total organic content (%)	0.26 ± 0.00	0.17 ± 0.01	0.41 ± 0.01	0.55 ± 0.03
Total N (%)	0.06 ± 0.004	0.02 ± 0.001	0.03 ± 0.016	0.06 ± 0.01
Total P (%)	0.006 ± 0.000	0.001 ± 0.000	0.022 ± 0.001	0.025 ± 0.003
Total Cu (µg/g)	81 ± 9.3	7504 ± 552	21 ± 3.7	73 ± 6.3
Water soluble Cu (µg/g)	0.11 ± 0.01	6.7 ± 1.0	ND	ND
Total Pb (μg/g)	1042 ± 62	351 ± 22	30 ± 9.5	89 ± 8.1
Water soluble Pb (µg/g)	0.7 ± 0.12	0.50 ± 0.11	ND	ND
Total Zn (µg/g)	2132 ± 208	1106 ± 73	55 ± 12.1	171 ± 20
Water soluble Zn (µg/g)	1.4 ± 0.07	0.38 ± 0.02	ND	ND

Table 4. Total biomass of four *Sesbana* species grown in Pb/Zn mine tailings and Pb/Zn mine tailings amended with garden soil and/or river sediment (mean \pm SE, N=3)

Treatments	T1 ^a	T2	Т3	T4	Т5
With root nodules With no nodules With root nodules With no nodules With root and stem nodules With root nodules With root nodules With no nodules	$211a-c^{b} \pm 169$	558bc-bc ± 338	1393cde-ab ± 346	1690bc-a ± 275	556b-bc ± 156
	$40a-a \pm 10$	1046bc-a ± 475	365de-a ± 80	1142bc-a ± 604	—c
	$68a-b \pm 15$	398c-b ± 154	648de-ab ± 238	1217bc-a ± 193	348b-b ± 124
	112^{d}	267c-a ± 55	228e-a ± 77	103c-a ± 27	564b-a ± 319
	$178a-b \pm 5$	2090ab-ab ± 418	2759b-ab ± 523	4340ab-a ± 1532	451b-b ± 309
	$123a-c \pm 26$	720bc-c ± 207	2473bc-b ± 553	4905a-a ± 120	416b-c ± 147
	$263a-a \pm 96$	952bc-a ± 735	444de-a ± 173	1907bc-a ± 1373	107b-a ± 6
With root nodules With no nodules	55a-c ± 17	2710a-bc ± 596	$4158a-ab \pm 298$	$3818ab-ab \pm 494$	$6082a-a \pm 1343$
	73a-a ± 13	966bc-a ± 430	$1525cd-a \pm 312$	$2240abc-a \pm 977$	$1433b-a \pm 258$

^aRefer to Table 2 for the explanation of treatments.

their respective controls, the latter did develop root nodules (and even stem nodules on some *S. rostrata* samples), indicating the presence of indigenous nodules-inducing bacteria in the soil or that cross-inoculation occurs in plant samples. Nonetheless, inoculated plants generally produced higher biomass (taller stems, more leaves and nodules) than the uninoculated control. This clearly indicates that the formation of nodules assisted the growth of all tested *Sesbania* species.

In general, *S. rostrata* with both root and stem nodules showed higher harvestable biomass than those having only root nodules. Stem nodules of *S. rostrata* are expected to take over the role of N fixation when the root nodules were under metal stress, and thus plants with both kinds of nodules would grow better under such adverse environment (Ye and others 2001). This perhaps explains why *S. rostrata* achieved better growth than *S. sesban* on pure Pb/Zn or Cu mine tailings.

Effects of River Sediments

Heavy-metal-contaminated river sediment seems to have similar toxic effects on the Sesbania plants as the two tailings. The rather high concentrations of heavy metals contained in the river sediment could be the result of untreated/uncontrolled industrial and domestic discharges into the Pearl River. However, Lan and others (1998) using river sediment from the Pearl River (but from a different location), indicated that the incorporation of river sediment in the Pb/Zn tailings substratum significantly increased dry matter yield of the legume Stylosanthes guianensis cv. Graham planted. The growth of this species was previously observed to be stunted when grown on 25% (w/w) tailings mixed with 75% sand (Lan and others 1997). Instead of binding with heavy metals due to the higher organic matter content contained in the river sediment, it has been observed that chelators in the river sediment increased

^bDifferent first letters in the same column or different second letters in the same row indicate a significant difference at P < 0.05 in the treatment of different *Sesbania* species or in different treatment of same species with or without nodules, respectively.

^cNo samples were obtained.

^dSingle sample.

Table 5. Total biomass of four Sesbana species grown in Cu mine tailings and Cu mine tailings amended with garden soil and/or river sediment (mean \pm SE, N=3)

Treatments	T6 ^a	Т7	Т8	Т9	T10
With root nodules	235bc-c ^b ± 21	1931bc-b ± 377	2809a-ab ± 297	3790cd-a ± 180	2622bc-ab ± 638
With no nodules	$53c-b \pm 31$	982c-b ± 224	$4124a-a \pm 238$	4805 bc-a ± 1000	$3971ab-a \pm 648$
With root nodules	477bc-a ± 54	$755c-a \pm 292$	$2776a-a \pm 1656$	$1317e-a \pm 229$	1249c-a ± 268
With no nodules	c	1419 ^d		3388	1956 ± 958
With root and stem nodules	$1425a-b \pm 312$	$3783ab-ab \pm 517$	3981a-a ± 1126	5353bc-a ± 85	3422 bc-ab ± 670
With root nodules	$1601a-b \pm 405$	$3796ab-ab \pm 1276$	$3186a-b \pm 751$	$7029\text{b-a} \pm 670$	$4290ab-ab \pm 1057$
With no nodules	180bc-c ± 17 850ab-c ± 288	2023bc-bc ± 958 5872a-b ± 380	$2360a-b \pm 484$ $5409a-b \pm 629$	4627c-a ± 529 9461a-a ± 525	809c-bc ± 301 5653ab-b ± 1696
With root nodules With no nodules	$850ab-c \pm 288$ $329bc-c \pm 47$	2828 bc-b ± 137	$3069a-b \pm 629$ $3069a-b \pm 730$	5469 bc-a ± 716	6819a-a ± 481

^aRefer to Table 2 for the explanation of treatments.

Table 6. Seed dry weights (g/pot) of four Sesbania species with and without nodules grown under different treatments for a period of 6 months

Treatments	T1 ^a	T2	Т3	T4	Т5	Т6	Т7	Т8	Т9	T10
With root nodules	0.54	0.32	0.21	0.08	_b	0.08	0.38	0.55	0.94	1.75
With no nodules	_	0.44	_	0.46	_	_	0.73	2.10	4.04	1.84
With root nodules	_	_	_	_	_	_	_	3.17	_	_
With no nodules	_		_		_	_	_	_	_	2.27
With root and stem nodules	_	0.74	0.77	1.71	_	0.62	2.67	0.54	1.97	_
With root nodules	_		1.21		_	0.47	1.51	0.65	0.41	1.42
With no nodules	_	1.37	_	0.18	_	_	2.35	_	2.02	_
With root nodules	_		_		_	_	_	_	_	_
With no nodules	_	_	_	_	_	_	_	_	_	_

^aRefer to Table 2 for the explanation of treatments.

the levels of soluble metals manifold and promoted metal convection and diffusion, and hence potential for uptake (Wallace and others 1974, Minnich and others 1987, Shepard and Shepard 1991). There seems to be a trend that the river sediment added to the Pb/Zn tailings may elevate the toxic effects of the tailings as no plants were able to produce seeds on the Pb/Zn tailings amended with river sediment. Therefore, the incorporation of river sediment (or other forms of organic amendment) has to be done with caution, as in our case that the addition of river sediment did not promote the growth of any of the four *Sesbania* species. Nevertheless, a thorough investigation should be conducted before a definite conclusion can be drawn.

General Performance of the Four Sesbania Species

In terms of biomass produced during the green-house investigation, *S. rostrata* and *S. sesban* out-performed *S. cannabina* and *S. grandiflora. Sesbania rostrata*

is an annual legume (Rao and Ghai 1995), while *S. sesban* has a short life-span of 2.5 years (Kamara and Maghembe 1994). The short life-span of *S. sesban* makes them ideal for quick (short-term) remediation purposes. *Sesbania cannabina* is also an annual species (Rao and Ghai 1995), whereas *S. grandiflora* is perennial which is considered as a tree legume (Ghosh and others 1996).

Inoculated plants in different soil ameliorations produced more seeds than unnodulated samples, indicating nodulation might be beneficial to the reproduction of plants. After *S. rostrata* and *S. sesban* complete their short life-cycle, seeds from the first-generation plant can be used for replantation, and the dead organic matter can serve as green manure to enrich the barren sites with organic matter and essential nutrients (especially N) upon decomposition.

Being perennial, *S. grandiflora* did not display a vigorous initial growth as *S. rostrata* and *S. sesban*. However, once established the plant demonstrated it was able to

^bDifferent first letters in the same column or different second letters in the same row indicate a significant difference at P < 0.05 in the treatment of different *Sesbania* species or in different treatment of same species with or without nodules, respectively.

^cNo samples were obtained.

^dSingle sample.

^bNo seeds were obtained.

grow sturdily and displayed more resistance to pest invasion. Having such characteristics, *S. grandiflora* might also be an appropriate choice for long-term remediation projects.

Conclusion

After comparing the performance of the four *Sesbania* species in terms of the total biomass, it is concluded that *S. rostrata* and *S. sesban* could serve as appropriate pioneer species to be used in short-term remediation projects, to modify the harsh environment by providing additional organic matter and nutrients.

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