Journal of Environmental Sciences Vol.16, No.4, pp.670—673, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)04-0670-04 **CLC number:** X171; X131.1 **Document code:** A

Responses of *Sesbania rostrata* and *S. cannabina* to Pb, Zn, Cu and Cd toxicities

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Abstract: Responses of *Sesbania rostrata* and *S. cannabina* to Pb, Zn, Cu and Cd toxicities were assessed by a seed-suspending seedbed(SSS) approach. The results showed that the SSS approach was suitable for testing the tolerance of a plant to the stress of toxic metals. The endpoints include seed germination success, straightened radicle and hypocotyl of the seedlings from the seeds. The measurements could be done easily and accurately. It was found that the elongation of radicle was the most sensitive indicator to the stress of heavy metals among the endpoints. When exposure to lower or medium concentrations of Pb, Zn, and Cd, the development of the lateral roots were favorable. Species of *S. rostrata* was more tolerant than *S. cannabina* to the heavy metals, especially to Zn and Cd. The ED_{50} of Pb, Zn, Cu and Cd were 32.90, 5.32, 4.40 and 12.00 μg/ml for *S. rostrata*, respectively, and they were 30.11, 2.87, 4.05 and 4.94 μg/ml respectively for *S. cannabina*.

Keywords: *Sesbania rostrata*, *Sesbania cannabina*; heavy metal tolerance; testing method; root elongation; Pb/Zn tailings

Introduction

Sesbania rostrata is a tropical legume species which can form nitrogen-fixing nodules in both stem and root when the plant is infected by *Azorhizobium caulinodans*, a symbiotic bacteriod (Dreyfus, 1981; 1988). Our earlier studies indicated that the plant was able to germinate, grow, nodulate, establish and complete its life cycle in Pb/Zn mine tailings which contained high concentrations of heavy metals, such as lead(Pb), zinc(Zn), copper(Cu) and cadmium (Cd), and lacked organic matter and nitrogen(Yang, 1997; Ye, 2001). As its fast growing, the high N_2-fixing (especially the stem nodule N_2-fixing) and the flooding tolerant properties(Dreyfus, 1984; 1985; Manguiat, 1987; Pareek, 1990; Ladha, 1992), it is expected that *S. rostrata*, as a pioneer species for reclaiming Pb/Zn tailings disposed area, could produce a large amount of organic matter and nitrogen in a short time, and the accumulations of these nutrients would be favorable for the growth of subsequent species, and result in a successful reclamation.

Although *S. rostrata* established successfully in Sn and Pb/Zn tailings(Radziah, 1990; Yang, 1997), it suffered from the other factors excluding the agronomic ones(such as fertilization or watering condition), and the toxicity of the heavy metals was considered to be the main one (Yang, 1997). In this study, the responses of *S. rostrata* to Pb, Zn, Cu and Cd were examined, and a new test method for testing the tolerance was devised. The responses of *S. cannabina*, which only produces nitrogen-fixing root nodule when infected by *Rhizobium* spp., to the heavy metals were examined as comparison.

1 Materials and methods

1.1 Seeds of *S. rostrata* and *S. cannabina*

Seeds of *S. rostrata* were introduced from Japan in 1990 and reproduced for two generations(1991 and 1992) in native red loam soil in Zhongshan University, Guangzhou, China, while seeds of *S. cannabina* were collected from wild populations of *S. cannabina* grown under the same condition as *S. rostrata* in 1992.

In order to improve the germinating quality, all seeds were scratched by a paper cutter in the surface of the seed coat before seeding.

1.2 Method for testing metal tolerance

A seed-suspending seedbed(SSS) method was designed as a testing system for heavy metal tolerance(Fig. 1). An expanded polystyrene plate(20.5 cm × 15 cm × 1.8 cm) with fourteen even-arranged triangle flumes(depth = 8 mm and edge length = 10 mm), which paralleled to the short edge of the plate, was used as the base of the seed bed, and lower part of the flumes were spread with a 7.5 cm-high cotton bandage. A plastic fine stick (diameter = 1 mm) was penetrated cross the flumes at 5 cm below the higher edge of the bandage, so that a cross section was set in each flume in a suitable position for suspending one seed. A transparent plastic plate(20.5 cm × 15 cm × 0.3 cm) was covered and fixed on the surface of the seedbeds after seeds were sown. The seedbed was then installed vertically into a plastic container(23 cm × 15 cm × 15.5 cm) which contained the tested solution. This method enables the culture solution to ascend along the cotton bandage and arrive at the suspended seeds and straightens the germinated seedlings.

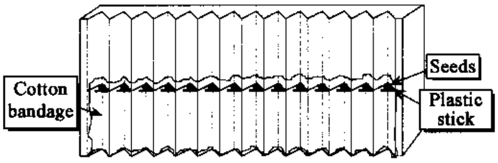

Fig.1 The seed-suspending seedbed(SSS) system for testing heavy metal tolerance

1.3 Treatments

Test solutions with a series concentrations of respective Pb, Zn, Cu and Cd(Table 1) were prepared by dissolving

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Foudation item: The National Natural Science Foundation of China (No. 30070126) and Hong Kong Croucher Foundation; * Corresponding author

respective $Pb(NO_3)_2$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4$ and $CdCl_2 \cdot 2H_2O$ into deionized water, and there were totally 36 treatments(9 for each metal). The test solutions(800 ml for each) were contained into the container installed the SSS system to culture the seeds of the two species.

Table 1 Concentrations of a series test solutions of respective Pb, Zn, Cu and Cd

Metal	Concentration of heavy metal, μg/ml								
	A	B	C	D	E	F	G	H	I
Pb	0	5	10	15	20	30	40	60	80
Zn	0	3	6	9	12	15	18	24	30
Cu	0	5	10	15	20	25	30	35	40
Cd	0	1.5	3.0	4.5	6.0	7.5	9.0	12.0	15.0

1.4 Incubating condition

Germination of seed in the SSS system was carried out in an incubator with a cycle of 14 h-light(32℃) and 10 h-dark (28℃). Seven seedbeds in one side of the SSS system were sown with *S. rostrata* and the other seven in another side with *S. cannabina*. Two sets of the SSS system including 14 seeds of each of *S. rostrata* and *S. cannabina* were installed into one container which contained the test solution (800 ml). Level of the culture solution in the container was marked and the deionized water was filled up to the mark daily.

1.5 Examinations and calculations

On the 7th(for Pb treatments) or 8th(for Zn, Cu and Cd treatments) day after the SSS systems were installed, the lengths of radicle and hypocotyl of all seedlings were determined, and the number of lateral roots per seedling was counted. The seedlings were dried at 70℃ for 48 h to determine their dry weight. Analysis of variance was used to estimate the treatment significance, and the Fisher's LSD test was used to compare the average values between the different treatments at $p < 0.01$ level.

The most sensitive character(MSC) responded to the heavy metals was used for calculating the tolerance index (*TI*):

$$TI(\%) = \frac{\text{Mean value of the MSC in solution with metal}}{\text{Mean value of the MSC in solution without metal}} \times 100.$$

Due to the experimental design enabled to get the continuous lines of the *TI*s, the ED_{50} (effective dosage reducing value of MSC by 50% of the control)(Rand, 1976; Wong, 1981) was estimated by determining the concentration where the line of *TI* crossed the point of 50%.

2 Results

2.1 Germination

Germinating percentages of the two plant species in different treatments are shown in Table 2. The germinating percentage of *S. rostrata* (89.7% – 97.6%) was higher than that of *S. cannabina* (71.4% – 84.9%). The lowest values were observed in Cd treatments and the highest in Cu, but there were no evident trends relating to the concentrations of the tested heavy metals.

Table 2 Germinating percentages of *S. rostrata* and *S. cannabina* in different treatments measured by the SSS method

Metal	Species	Level of metal concentration									
		A	B	C	D	E	F	G	H	I	Mean
Pb	*S. rostrata*	100.0	85.7	92.9	92.9	92.9	100.0	92.9	100.0	78.6	92.9
	S. cannabina	64.3	78.6	92.9	71.4	78.6	64.3	92.9	78.6	50.0	74.6
Zn	*S. rostrata*	92.9	92.9	85.7	100.0	100.0	85.7	71.4	92.9	92.9	90.5
	S. cannabina	71.4	78.6	71.4	85.7	71.4	92.9	71.4	71.4	78.6	77.0
Cu	*S. rostrata*	92.9	100.0	100.0	92.9	100.0	100.0	100.0	92.9	100.0	97.6
	S. cannabina	92.9	100.0	100.0	85.7	85.7	85.7	71.4	78.6	85.7	84.9
Cd	*S. rostrata*	100.0	85.7	85.7	78.6	100.0	100.0	92.9	78.6	85.7	89.7
	S. cannabina	64.3	71.4	85.7	64.3	50.0	78.6	78.6	71.4	78.6	71.4

2.2 Treatment significances for radicle, hypocotyl, lateral roots and biomass

The results of the analysis of variance(Table 3) showed that the lengths of radicle and the numbers of lateral roots for both species responded significantly ($p < 0.01$) to the treatments of all tested metals, and significances($p < 0.01$) in hypocotyl of both species were observed for Pb, Zn and Cu treatments, while in seedling biomass, there were significances($p < 0.01$) only for Cu treatment(both species) and Pb treatment(*S. rostrata* only).

2.3 Responses to Pb, Zn, Cu and Cd

The results of responses of the two species to Pb, Zn, Cu and Cd are shown in Table 4.

Additions of Pb, Zn, Cu and Cd at the levels more than respective 5, 3, 5 and 1.5 μg/ml accordingly depressed the radicle elongation of both species significantly, and there were decreasing changes in the radicle lengths of both species accompanying with the increases of concentrations of the four metals. Pb more than 40 μg/ml, Zn more than 24 μg/ml and Cu more than 20 μg/ml depressed the growth of hypocotyl of both species significantly, but Cd did not seem to depress the growth. Lower or medium concentrations of Pb(5 – 30 μg/ml), Zn(3 – 6 μg/ml) and Cd(1.5 – 7.5 μg/ml) were favorable to development of the lateral roots, but Cu exceeding 5 μg/ml decreased the numbers of the lateral roots per seedling of both species significantly($p < 0.01$). There were no clear tendencies in responses of biomass per seedling of both species to the four metals.

2.4 Tolerance index and ED_{50}

As the radicle length of both species responded in the most regular pattern to the four heavy metals, it was considered as the MSC among the four examined characters. The radicle length, therefore, was used to calculate *TI* and ED_{50}, and the results are shown in Fig. 2. The *TI* of both species decreased sharply in the concentrations of 3 μg/ml for Zn, 5 μg/ml for Cu and 1.5 μg/ml for Cd, respectively, and then they changed decreasingly accompanying with the further increases of the concentrations. The responses of the *TI* to Pb were relatively mild. Although the changes of the *TI* of *S. rostrata* and *S. cannabina* followed the similar pattern as the changes of the concentrations of the four metals, but there were always lower values in *S. cannabina* than in *S. rostrata*. The 7-day ED_{50} of Pb and the 8-day ED_{50} of Zn, Cu and Cd for *S. rostrata* were 32.90, 5.32, 4.40 and

Table 3 Results of analysis of variance for lengths of radical and hypocotyls, number of lateral root and seedling biomass of *S. rostrata* and *S. cannabina* affected by heavy metals

Metal	Character	*S. rostrata*			*S. cannabina*		
		MS for treatment	MS for error	*F* value	MS for treatment	MS for error	*F* value
Pb	Radicle	1936.53	47.86	40.46^{s}	2354.28	93.17	25.27^{s}
	Hypocotyl	772.78	39.84	19.38^{s}	254.47	31.19	8.16^{s}
	Lateral roots	1138.46	37.00	30.78^{s}	66.30	10.60	6.26^{s}
	Biomass	8.22	1.76	4.66^{s}	1.93	0.89	2.17^{ns}
Zn	Radicle	2598.65	41.50	62.62^{s}	2646.64	24.07	109.96^{s}
	Hypocotyl	275.75	45.01	6.13^{s}	124.44	20.99	5.93^{s}
	Lateral roots	685.31	37.19	18.43^{s}	43.47	13.41	3.24^{s}
	Biomass	1.00	2.31	0.44^{ns}	3.98	2.45	1.62^{ns}
Cu	Radicle	2610.87	20.93	124.73^{s}	3969.07	52.20	76.04^{s}
	Hypocotyl	899.55	57.31	15.70^{s}	389.23	35.22	11.05^{s}
	Lateral roots	939.64	15.76	59.63^{s}	216.48	13.00	16.65^{s}
	Biomass	4.58	2.56	2.05^{ns}	4.04	1.14	3.54^{s}
Cd	Radicle	1520.02	38.31	39.68^{s}	1445.39	46.94	30.79^{s}
	Hypocotyl	73.52	39.85	1.85^{ns}	56.72	35.05	1.62^{ns}
	Lateral roots	183.59	35.47	5.18^{s}	41.04	8.93	4.60^{s}
	Biomass	1.34	2.82	0.48^{ns}	1.09	0.70	1.56^{ns}

Notes: s = significant($p < 0.01$); ns = no significant($p > 0.01$)

Table 4 Responses of *S. rostrata* and *S. cannabina* to Pb, Zn, Cu and Cd

Concentration of metal, μg/ml		Length of radicle, cm		Length of hypocotyls, cm		Number of lateral roots, No./seedling		Seedling biomass, mg DM	
		S. rostrata	*S. cannabina*	*S. rostrata*	*S. cannabina*	*S. rostrata*	*S. cannabina*	*S. rostrata*	*S. cannabina*
Pb	0(A)	55.2^{a}	57.3^{a}	34.4^{b}	32.0^{ab}	18.9^{c}	4.5^{ab}	11.4^{c}	7.7^{ns}
	5(B)	46.4^{b}	39.2^{b}	46.4^{a}	35.7^{a}	28.3^{a}	7.4^{a}	12.3^{abcd}	6.9^{ns}
	10(C)	46.4^{b}	37.8^{b}	42.4^{a}	34.1^{a}	28.5^{a}	4.6^{ab}	10.8^{d}	7.3^{ns}
	15(D)	40.6^{b}	39.1^{b}	38.7^{ab}	34.8^{a}	27.3^{ab}	6.1^{a}	11.5^{bcd}	7.5^{ns}
	20(E)	40.1^{b}	38.4^{b}	38.0^{ab}	31.3^{abc}	25.9^{abc}	6.6^{a}	13.0^{ab}	7.2^{ns}
	30(F)	30.8^{c}	28.8^{bc}	32.0^{b}	31.7^{abc}	20.3^{bc}	4.6^{ab}	12.8^{abc}	7.8^{ns}
	40(G)	18.8^{d}	17.6^{cd}	20.7^{c}	25.3^{cd}	4.7^{d}	1.5^{bc}	11.9^{bcd}	7.9^{ns}
	60(H)	23.0^{cd}	14.1^{d}	24.4^{c}	26.2^{bcd}	3.9^{d}	0.6^{c}	13.6^{a}	7.3^{ns}
	80(I)	15.0^{d}	8.9^{d}	21.0^{c}	20.7^{d}	5.2^{d}	0.6^{c}	12.7^{abc}	8.3^{ns}
Zn	0(A)	75.1^{a}	81.4^{a}	45.4^{a}	41.9^{a}	34.4^{a}	10.7^{b}	13.4^{ns}	8.4^{ns}
	3(B)	42.9^{b}	39.3^{b}	48.5^{a}	42.7^{a}	37.1^{a}	12.7^{a}	13.0^{ns}	7.2^{ns}
	6(C)	36.0^{bc}	33.0^{bc}	42.3^{ab}	42.0^{a}	34.8^{a}	12.4^{a}	12.5^{ns}	8.2^{ns}
	9(D)	30.9^{cd}	28.9^{cd}	35.2^{bc}	33.6^{bc}	18.2^{cd}	8.3^{abc}	12.9^{ns}	7.9^{ns}
	12(E)	27.6^{de}	25.9^{de}	47.5^{a}	39.0^{ab}	26.3^{b}	7.6^{abc}	13.1^{ns}	8.0^{ns}
	15(F)	31.7^{cd}	27.6^{cd}	41.9^{ab}	37.4^{ab}	24.7^{bc}	8.3^{abc}	12.5^{ns}	9.3^{ns}
	18(G)	27.7^{de}	25.1^{de}	42.0^{ab}	40.9^{a}	21.8^{bcd}	9.4^{abc}	12.7^{ns}	9.7^{ns}
	24(H)	27.2^{de}	20.3^{ef}	41.8^{ab}	41.7^{a}	16.8^{d}	6.7^{bc}	12.7^{ns}	9.0^{ns}
	30(I)	20.4^{e}	15.1^{f}	32.5^{c}	30.7^{c}	15.3^{d}	5.4^{c}	13.2^{ns}	8.4^{ns}
Cu	0(A)	72.3^{a}	79.4^{a}	60.5^{a}	50.8^{a}	37.5^{a}	16.4^{a}	13.1^{ns}	8.3^{ab}
	5(B)	31.3^{bc}	31.6^{b}	52.5^{ab}	47.7^{abc}	22.3^{bc}	9.0^{b}	11.9^{ns}	7.7^{abc}
	10(C)	33.0^{b}	25.2^{bc}	51.4^{b}	39.1^{d}	23.3^{b}	6.2^{bc}	13.2^{ns}	6.6^{c}
	15(D)	31.8^{bc}	27.2^{bc}	52.9^{ab}	48.7^{ab}	18.6^{cd}	8.2^{b}	11.4^{ns}	8.4^{ab}
	20(E)	27.0^{cd}	22.7^{c}	49.0^{b}	41.4^{cd}	17.5^{de}	5.4^{bcd}	12.3^{ns}	7.5^{abc}
	25(F)	25.4^{d}	19.2^{cd}	39.9^{c}	36.9^{de}	13.6^{e}	3.8^{cd}	12.3^{ns}	7.3^{bc}
	30(G)	23.4^{d}	19.8^{cd}	37.4^{c}	42.3^{bcd}	8.7^{f}	2.1^{cd}	13.4^{ns}	8.0^{ab}
	35(H)	17.0^{e}	11.5^{d}	33.1^{c}	31.6^{e}	6.7^{f}	1.6^{d}	12.3^{ns}	8.7^{a}
	40(I)	22.1^{de}	19.7^{cd}	35.8^{c}	38.3^{de}	8.3^{f}	2.6^{d}	13.4^{ns}	7.8^{ab}
Cd	0.0(A)	77.1^{a}	80.6^{a}	47.7^{ns}	39.3^{ns}	29.0^{a}	4.1^{c}	13.3^{ns}	7.9^{ns}
	1.5(B)	50.5^{b}	45.1^{b}	45.9^{ns}	40.4^{ns}	30.1^{a}	7.6^{bc}	12.9^{ns}	8.3^{ns}
	3.0(C)	43.9^{bcd}	42.0^{bc}	47.3^{ns}	41.9^{ns}	26.5^{ab}	10.0^{ab}	13.6^{ns}	8.4^{ns}
	4.5(D)	43.8^{bcd}	42.3^{bc}	43.1^{ns}	40.7^{ns}	26.0^{ab}	8.1^{abc}	13.2^{ns}	8.1^{ns}
	6.0(E)	46.6^{bc}	36.1^{bc}	47.6^{ns}	34.4^{ns}	28.6^{a}	5.4^{c}	12.9^{ns}	7.3^{ns}
	7.5(F)	40.1^{cde}	44.7^{b}	51.3^{ns}	39.7^{ns}	29.6^{a}	12.3^{a}	12.8^{ns}	7.5^{ns}
	9.0(G)	41.3^{cde}	34.3^{c}	43.9^{ns}	33.7^{ns}	20.8^{bc}	7.0^{bc}	12.9^{ns}	7.9^{ns}
	12.0(H)	38.3^{de}	36.6^{bc}	42.7^{ns}	40.0^{ns}	21.0^{bc}	8.0^{bc}	12.4^{ns}	7.9^{ns}
	15.0(I)	35.8^{e}	34.3^{c}	46.6^{ns}	40.1^{ns}	18.9^{bc}	6.3^{bc}	12.9^{ns}	8.4^{ns}

Notes: different alphabets of the same vertical column within the same metal treatment indicate significant difference at $p < 0.01$ level based on LSD test; ns = not significant

12.0 μg/ml, respectively, and for *S*. *cannabina*, they were 30.11, 2.87, 4.05 and 4.94 μg/ml, respectively. The differences of the ED_{50} between the two species were especially large in Cd and Zn.

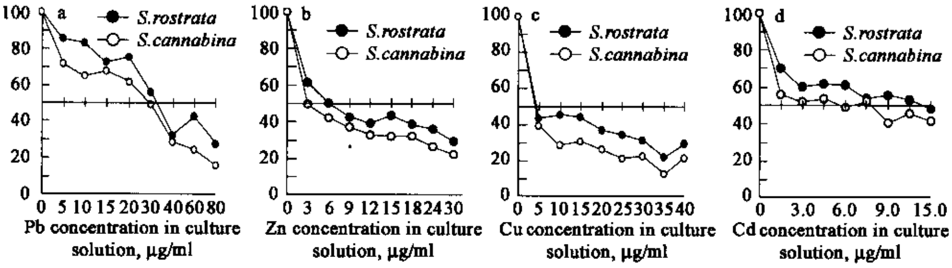

Fig.2 Changes of *TI*s under different metal concentrations and ED_{50} to Pb(a), Zn(b), Cu(c) and Cd(d) for *S*. *rostrata* and *S*. *cannabina*

3 Discussion

3.1 Feasibilities of the SSS method

As SSS method seemed to be suitable for testing heavy metal tolerance of plants. It supported seed germination successfully, and enabled the radicle and hypocotyl to elongate straight and vertically, so that measurements could be done easily and accurately.

3.2 Sensitivities of the four characters responded to heavy metals

As root system of plant contacts the toxic metals directly in a contaminated environment, it shall be most sensitive to the toxicity of metals. Therefore, root elongation is commonly used to measure heavy metal tolerance(Turner, 1994). The present experiment demonstrated that the root system, including the radicle and lateral roots, of both *S*. *rostrata* and *S*. *cannabina* responded most sensitively to all tested heavy metals, especially the radicle. It is interesting to note that the number of lateral roots increased at rather high concentrations of Pb, Zn and Cd than in the controls for both *S*. *rostrata* and *S*. *cannabina*. There may be an induced mechanism in the lateral roots when the radicle is inhibited by heavy metals at certain concentrations. The responses of the hypocotyl were so insensitive that their elongations were similar to the controls even at rather high concentrations of the heavy metals(Pb: 30 μg/ml, Zn: 24 μg/ml, Cu: 15 μg/ml and Cd: > 15 μg/ml). The delays of heavy metal absorption and transport may be arisen as the reason. The poor responses of biomass per seedling to the stress from the metals were probably attributed to the absence of accumulation of anabolic products over the testing period.

3.3 Heavy metal tolerance

Using radicle elongation as a measurement of responses of *S*. *rostrata* and *S*. *cannabina* to Pb, Zn, Cu and Cd, the toxicity of the 4 metals were: Cu(ED_{50} = 4.40 μg/ml) > Zn (5.32) > Cd(12.00) > Pb(32.90) for *S*. *rostrata*, and Zn (2.87) > Cu (4.05) > Cd (4.94) > Pb (30.11) for *S*. *cannabina*. *S*. *rostrata* had higher tolerances to the tested heavy metals, especially to Zn and Cd, than *S*. *cannabina*, and the tolerances of both species were rather high when comparing with the data reported previously. Craig (Craig, 1978) reported ED_{50} of Cu for *Chloris gayana*, *Panicum maximum* and *Zea mays* (SR52) were 0.58 (0.04), 4.06 (0.26) and 4.23(0.27)μmol/L(μg/ml), respectively, and Pb and Zn for *Z*. *mays*(SR52) was 54.1 μmol/L(11.2 μg/ml) and 128 μmol/L(8.37 μg/ml), respectively. ED_{50} of Cd, Cu, Pb and Zn for *Lolium perenne* were reported to be 1.85, 0.02, 10 and 1.6 μg/ml, respectively(Wong, 1982). ED_{50} of Cu for *Paspalum distichum* and *Sporpbolus virginicus*, both were collected from heavy metal contaminated site, were 0.33 and 0.85 μg/ml, respectively(Wong, 1983).

The present experiment demonstrated that *S*. *rostrata* and *S*. *cannabina* were able to tolerate rather high concentrations of Pb, Zn, Cu and Cd. However, the actual mechanisms of their heavy metal tolerance should be thoroughly studied in the future. Heavy metal tolerance, together with the high nitrogen fixing ability and the high growth rate, are certainly the important requirement for growth of *S*. *rostrata* on metal-contaminated areas e. g. Pb/Zn mine tailings which contained high concentrations of heavy metals, and lacked of organic matter and nitrogen.

References:

Craig G C, 1978. A method of measuring heavy metal tolerance in grasses[J]. Transactions of the Rhodesia Scientific Association, 58: 9—16.

Dreyfus B, Dommergues R, 1981. Nitrogen fixing nodules induced by *Rhizobium* on the stem of the tropical legume *Sesbania rostrata*[J]. FEMS Microbiology Letters, 10: 313—317.

Dreyfus B, Alazard D, Dommergues Y, 1984. New and unusual microorganisms and niches[M]. (Klug M. and Reddy C. ed.): Current perspectives in microbial ecology. Washington DC: American Society of Microbiology, 161—169.

Dreyfus B, Rinaudo G, Dommergues Y, 1985. Observations on the use of *Sesbania rostrata* as green manure in paddy fields[J]. MIRCEN Journal of Applied Microbiology and Biotechnology, 1: 111—122.

Dreyfus B, Garcia L, Gillis M, 1988. Characterization of *Azorhizobium caulinodans* gen. nov. sp., a stem nodulating nitrogen fixing bacterium isolated from *Sesbania rostrata* [J]. International Journal of Systematic Bacteriology, 38: 89—98.

Ladha J K, Pareek R P, Becker M, 1992. Stem-nodulating legume-*Rhizobium* symbiosis and its agronomic use in lowland rice[M]. (Stewart B. A. ed.): Advances in soil science, Vol. 20. New York: Springer-Verlag New York Inc., 147—192.

Manguiat I J, Sebiano A G, Jalolon A T *et al*., 1987. Biofertilizer and nitrogen fixation potentials of *Sesbania rostrata* under flooded and nonflooded conditions as affected by inoculation and nitrogen application[J]. The Philippine Journal of Crop Science, 12: 325—?.

Pareek R P, Ladha J K, Watanabe I, 1990: Estimating N_2 fixation by *Sesbania rostrata* and *S*. *cannabina*(syn. *S*. *aculeata*) in lowland rice soil by the ^{15}N dilution method[J]. Biology and Fertility of Soils, 10: 77—88.

Radziah O, Shamsuddin H, 1990. Growth of *Sesbania rostrata* on different components of Tin tailings[J]. Pertanika, 13: 9—15.

Rand M C, Greenberg A E, Taras M J, 1971. Standard methods for the examination of water and wastewater[M]. 14th ed., Washington, D. C.: Pub. Assoc.

Turner A P, 1994. The responses of plants to heavy metals[M]. (Ross S. M. ed.): Toxic metals in soil-plant systems. Chichester: John Wiley & Sons. 153—187.

Wong M H, Lau W M, Yip S W, 1981. Effects of sludge extracts on seed germination and root elongation of crops[J]. Environmental Pollution, A: 87—98.

Wong M H, Bradshaw A D, 1982. A comparison of the toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne*[J]. New Phytologist, 91: 255—261.

Wong M H, Lau W M, Li S W *et al*., 1983. Root growth of two grass species on iron ore tailings at elevated levels of manganese, iron and copper[J]. Environmental Research, 30: 26—33.

Yang Z Y, Yuan J G, Xin G R *et al*., 1997. Germination, growth and nodulation of *Sesbania rostrata* grown in Pb/Zn mine tailings [J]. Environmental Management, 21: 617—622.

Ye Z H, Yang Z Y, Chan G Y S *et al*., 2001. Growth response of *Sesbania rostrata* and *S*. *cannabina* to sludge-amended lead/zinc mine tailings. A greenhouse study[J]. Environment International, 26: 449—455.

(Received for review August 19, 2003. Accepted September 26, 2003)