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Inhibitory effects of heavy metals on seed germination and seedling growth of *Miscanthus* species

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Abstract. Miscanthus species is a native grass that occurs widely in Taiwan. This experiment was designed to determine the inhibitory effects of heavy metals on seed germination and seedling growth of eight populations Miscanthus spp.; M. floridulus seeds collected from random locations beside the highways in Taiwan, and from mountainous areas (1600-2600 m) were used. The seeds were germinated in petri dishes, moistened with different heavy metal ions in aqueous solutions, i.e., Pb++, Hg++, Cu++ and Cd++ at concentrations of 200, 400, 600, 800 and 1000 ppm. Seed germination percentages and the rate of germination of each species decreased with increased concentrations of heavy metal solutions; the time required to reach 50% of final germination was delayed from 3.8 days in distilled water to 7.0 days in some heavy metal solutions at 1000 ppm. The germination capacity of seeds followed a decreasing order of inhibition: Cd>Cu>Hg>Pb. The inhibitory degree of seed germination after immersing in heavy metal solution at 1000 ppm was as follows: Hg>Cd>Cu>Pb. Potassium ions leached from the seeds after immersing in mercury solutions was the highest. Inhibition of heavy metals to seedling growth of Miscanthus species was as follows: Cd>Cu>Hg>Pb with M. transmorrisonensis to be the most tolerant to heavy metals among the species tested. The germination and development of Miscanthus spp. that are tolerant to heavy metals concentrations indicate that these species may be suitable for planting along the roadsides in Taiwan.

Key words: Allelopathy; Germination; Heavy metals; Miscanthus species; Solute leakage.

Introduction

Miscanthus floridulus, a native grass, is widely distributed in Taiwan from lower land areas up to mountainous areas at 2600 m above sea level. M. floridulus is also a unique species grown in Southeast Asia. In Taiwan, Miscanthus commonly blooms between September and November, although some of them flower during late spring and early summer. The matured seeds are easily dispersed by wind and grow vigorously in the soil. Hsu (1985) reported that germination percentages of the unchilled seeds of Miscanthus were above 86% when they were germinated in an incubator at temperatures between 10 and 35°C. Chilling did not improve

seed germination; however, it did enhance the rate of germination, and concluded that the lowest temperature tolerated for germination of unchilled seeds was 8.3°C. It showed that seeds harvested from different locations had the same germination percentages when they were incubated at 15, 25 and 35°C with the range of tolerance to water potential being between blue stem (Andropogon spp.) and sorghum (Sorghum bicolor) (Hsu, 1986, 1988).

In 1989, Hsu showed that seed germination of M. floridulus was inhibited by infrared, ultra violet and blue light, and concluded that the germination of Micanthus seeds was of the short-day type which required only 5 min light exposure to enhance germination ability. Hsu (1990) reported that percentage and

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rate of germination of *Miscanthus* were reduced when concentrations of NaCl solution were increased, suggesting that seeds harvested from lower land areas were salt-tolerant. The maximum concentration of salt tolerance to seed germination for *M. floridulus* was 23,775 ppm, while that for *M. transmorrisonensis* was 18,865 ppm. Chou *et al.* (1987) reported that *M. floridulus* plants which grew in industrial areas and roadsides had a high Pb content. Seed germination behavior that have been contaminated with heavy metal in soils has not been fully understood; consequently this study was made to determine inhibitory effects of heavy metals on seed germination and seedling growth of *Miscanthus* species.

Materials and Methods

Materials

Seeds of 7 entries of *M. floridulus* were harvested from plants grown on the roadside of highway (Tonghsiao, Sanyi and Kenting), at locations far-off highway (Taitung and Hsinchu), in mountainous areas at Hoshe (1600 m in elevation), Shenmulintao (at 2000 m), and *M. transmorrisonensis* harvested at mountainous areas (at 2600 m). The seeds were sun-dried, stored at 4°C, and were taken out from caryopsises prior to determining the tests. Heavy metal ions (PbCl₂, HgCl₂, CuSO₄•5H₂O and CdSO₄•8H₂O) were made up in aqueous solutions at 0, 200, 400, 600, 800 and 1000 ppm, for germination tests. Benalate, methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate (0.1%), was added to the distilled water and the solutions to prevent bacterial growth.

Bioassay

Germination test was conducted according to the Association of Official Seed Analysts (1981). Twenty seeds for each petri dish were spread uniformly on the paper, and a completely randomized design (CRD) was used with 5 replications. Petri dishes were set in an incubator with constant temperature at 25°C, and light and dark periods were controlled for 12 hours each.

Germination Rate Indes (GRI) and Time to Reach 50% of Final Germination (Gt_{50})

Germination was recorded when the radicle reached 2 mm. The seeds were counted and removed twice per day during the first week, once per day during the second week, and once every two day period until the

end of the test period. The test was terminated when there was no further germination after three consecutive days. The final germination percentage and rate of germination were determined. Both germination rate index (GRI) and time to reach 50% of final germination (Gt₅₀) were used to evaluate rate of germination. The GRI was obtained by summarizing germination percentage at each counting divided by the days after placing seeds in the incubator (Maguire, 1962). The Gt₅₀ was estimated according to Hsu *et al.* (1984). The maximum tolerance of the concetration of seed germination to heavy metal solution was evaluated by the regression line between GRI and metal concentration. The germination percentage was arcsin-transformed prior to statistical analysis (Snedecor and Cochran, 1980).

Effects of Heavy Metals on Leaching of Potassium

One hundred seeds of each entry were weighted, and imbibed in distilled water and in heavy metal solutions at 1000 ppm at 30°C for 6 hours, respectively. Seeds were removed and washed with 500 ml distilled water and dried at room temperature for 1 hour. The dried seeds were placed in the test tubes containing 5 ml distilled water at 30°C for 18 hours. The content of leached potassium ion was determined by an atomic absorption spectrometer (Perkin-Elmer 300). The data were expressed as seed number and weight. The seeds were then taken for germination tests as mentioned above, which included an experimental design (CRD) with three replications.

Effects of Heavy Metals on Seedling Growth of Miscanthus

The eight *Miscanthus* entries were placed in petri dishes moistened with concentrations of heavy metal solutions at 0, 10, 100 and 200 ppm, to germinate and grow for 7 days, respectively, using an experimental design of CRD with four replications. Twenty seedlings were used per treatment, and the lengths of plumule and radicle were measured. The inhibition of seedling growth was expressed according to the formula given by Chou and Muller (1972):

% Inhibition = ((Length of control-Length of test)/ (Length of control)) × 100%

Results

Comparison of Seed Germination of **Miscanthus** Species as Affected by Heavy Metals

The percent seed germination of both *M. floridulus* and *M. transmorrisonensis* were high (97%) when the seeds were grown in the distilled water control; however, the germination percentage was reduced when the seeds were grown in the heavy metal ion solutions (Table 1). The seed germination was suppressed at concentration of 200 ppm for Pb, Hg, Cu and Cd which exhibited the highest inhibition of *M. floridulus* (97%) and of *M. transmorrisonensis* (92%). The inhibition of seed germination increased with increasing concentration of heavy metals, with the exception being Pb. Germination rate index (GRI) expressed by percentage germination per day, showed that the results of the bottom half

of the Table 1, were well correlated to that of the upper part of Table 1. In the control treatment the GRI of *Miscanthus floridulus* ranged from 23.6 to 24.3%/day while that of *M. transmorrisonensis* ranged from 15.1 to 16.9%/day, indicating that germination of *M. floridulus* was significantly higher (Table 1, bottom part). For the lead ion treatments, the GRI significantly decreased to 7.6%/day for *M. floridulus* and 4.6%/day for *M. transmorrisonensis*, whereas, for the mercury treatments, the GRI drastically decreased to values as low as 1.1-1.4%/day for both species. Furthermore, the GRI was remarkably lower in the treatments with Cd (0.0 to 0.3%/day), which permits the conclusion to be drawn that Cd was the most toxic to both of the species.

The effects of heavy metals in various concentra-

Table 1. Comparison of effects of heavy metals in various concentrations on seed germination of **Miscanthus floridulus** (Mf) and **M. transmorrisonensis** (Mt)

Concentration ppm	F	Pb	I	-Ig	(Cu	Cd		
	Mf	Mt	Mf	Mt	Mf	Mt	Mf	Mt	
				Germinatio	on, %				
0	97.6°*	97.0°	94.4ª	98.0ª	94.6ª	98.0°	93.7ª	96.0^{a}	
200	42.0 ^b	46.0°	46.0^{b}	51.0 ^b	29.7 ^b	39.0 ^b	3.0 ^b	8.0 ^b	
400	43.3 ^b	51.0 ^b	25.0^{c}	36.0 ^{bc}	22.4°	34.0^{b}	2.4 ^b	4.0 ^{bc}	
600	43.1 ^b	35.0°	12.1^d	25.0°	17.4 ^d	26.0 ^b	3.4 ^b	2.0^{c}	
800	42.7 ^ь	46.0 ^b	10.3^{d}	10.0^{d}	14.9 ^d	37.0^{b}	4.3 ^b	1.0^{c}	
1000	41.0^{b}	44.0^{b}	5.9^{e}	13.0^{d}	13.6^{d}	28.0^{b}	2.0^{b}	0^{c}	
			Germination	rate index,	%/day				
0	24.3ª	16.9ª	23.6ª	15.1ª	24.0°	16.7ª	23.6ª	16.4ª	
200	8.2 ^b	5.9 ^b	9.2^{b}	6.9 ^b	5.1 ^b	4.7 ^b	0.6^{b}	1.3 ^b	
400	8.3 ^b	6.8 ^b	4.6^{c}	5.2 ^{bc}	3.6°	4.6^{b}	0.4 ^b	0.5 ^{bc}	
600	8.3 ^b	4.6 ^b	2.1^d	$3.6^{\rm c}$	$2.6^{ t d}$	3.5 ^b	0.6^{b}	0.3^{bc}	
800	7.9 ^b	5.4 ^b	1.8^d	$1.4^{ t d}$	2.2^{d}	4.3 ^b	0.8^{b}	0.1^{c}	
1000	7.6 ^b	5.7 ^b	1.1^d	$1.6^{ t d}$	$2.0^{\mathtt{d}}$	3.2^{b}	0.3 ^b	0^{c}	

^{*}Means with the same letters within the same column and item are not significantly different at 5% level.

Table 2. Comparison of effects of heavy metals in various concentrations on days to reach 50% of final germination (Gt₅₀) of Miscanthus floridulus (Mf) and M. transmorrisonensis (Mt)

				Gt₅o, day	7				
Concentration ppm	P	P b	H	Ig	C	Cu	Cd		
	Mf	Mt	Mf	Mt	Mf	Mt	Mf	Mt	
0	3.8	4.5	3.8	4.5	3.8	4.5	3.8	4.5	
200	4.9	5.8	4.9	5.6	5.4	6.0	4.3	5.0	
400	5.1	5.7	5.0	5.5	5.8	5.8	-	-	
600	5.0	5.8	5.3	5.5	6.4	5.5	-	-	
800	5.2	6.1	5.6	5.6	6.4	6.2	_	-	
1000	5.3	5.7	5.9	5.6	7.0	6.1	-	-	

Table 3. Comparison of inhibitory effect of heavy metals at 200 ppm on the seed germination of M. floridulus and M. transmorrisonensis collected at various places in Taiwan.

Data are expressed by % of distilled water control.

Place	Elevation, m	Pb	Hg	Cu	Cd
			s floridulus		
Hoshe	1600	40.2 ^{b*}	49.6^{ab}	57.7°	4.3^{c}
Shenmulintao	2000	54.1^{a}	48.6^{a}	49.4ª	3.4 ^b
Hsinchu	sinchu 100		46.0^{a}	28.0°	9.3^{c}
Kenting	60	45.0^{a}	54.3^{a}	30.3 ^b	0^{c}
Sanyi	300	38.2ª	47.3ª	28.9ª	3.0^{b}
Tongshiao	20	43.0^{a}	52.1ª	23.6	$2.7^{\rm c}$
Taitung	300	41.0^{a}	51.5ª	20.0^{b}	0^{c}
			Miscathus tran	smorrisonensis	
Tartarchia Anpu	2600	47.3°	51.9ª	39.8ª	8.4 ^b

^{*}Means with the same letters within the same row are not significantly different at 5% level.

Table 4. Maximum tolerable concentrations of different heavy metals for the germination of **Miscanthus** species

	Heavy metal concentration, ppm									
Location	Pb	Hg	Cu	Cd						
	Miscanthus floridulus									
Hoshe	1498	1023	1046	871						
Shenmulintao	1580	914	1007	781						
Hsinchu	1486	874	898	759						
Kenting	1256	818	787	200						
Sanyi	1251	815	863	760						
Tongshiao	1432	809	878	781						
Taitung	1343	826	829	747						
	M. transmorrisonensis									
Tartarchia Anpu	1386	958	1119	760						

tions on Gt_{50} were compared for both species. The Gt_{50} for M. floridulus ranged from 3.8 days (at the control) to 7.0 days (at 1000 ppm) but those for M. transmorrisonensis ranged from 4.5 days (at the control) to 6.1 days (at 1000 ppm) (Table 2). Due to the higher toxicity of Cd, the seed germination of both species was totally inhibited at a concentration of 200 ppm or above. It was concluded that Gt_{50} of the both species germinated in heavy metal solution was delayed as compared to the distilled water control.

Comparison of Inhibitory Effects of Heavy Metal on Seed Germination of **Miscanthus** Collected in Various Places Seeds of *Miscanthus* collected from Hoshe, Shenmulintao, Hsinchu, Kengting, Sanyi, Tonghsiao and Taitung, where are in the eastern, central, and western parts of Taiwan, were used for the bioassay of heavy metals prepared at 200 ppm. Results of bioassay showed that the seed germination percentage expressed as % of the distilled water control of the both species ranged from 20.0 to 57.7 for Pb, Hg, and Cu. However, the germination capacity is extremely low (<9%) in the Cd solution at 200 ppm or above. It further confirmed that the Cd was the most toxic to seed germination of each species (Table 3).

Tolerance of Seed Germination of **Miscanthus** Species to Heavy Metals

The maximum tolerable concentrations of heavy metals for seed germination of *Miscanthus* species were given in Table 4. It ranged from 1251 to 1580 ppm for Pb, from 809 to 1023 ppm for Hg, from 787 to 1046 ppm for Cu, and from 200 to 871 ppm for Cd, respectively. This showed that Cd was the most toxic to seed germination and had the lowest tolerable concentration among heavy metals.

Results of the germination capacity of *Miscanthus* seeds after imbibing in different heavy metal solutions at concentrations of 1000 ppm were shown in Table 5. The percent germination in distilled water control ranged from 81% to 97% for *M. floridulus*, and was 43% for *M. transmorrisonensis*, indicating that the seed germination of the latter species was poor. The germination capacities in term of germination percentage and

Table 5.	Germination capacity of	Miscanthus	seeds after	imbibing	in diffe	erent heavy	metal	solutions	at concentratio	n of
	1000 ppm									

	Germination, %					GRI, %/day				Gt₅o, day					
	Dist.					Dist.					Dist.				
	H_2O	Pb	Hg	Cu	Cd	H₂Ô	Pb	Hg	Cu	Cd	H_2O	Pb	Hg	Cu	Cd
Miscanthus floridulus															
Hoshe	97°*	97^{a}	15^{d}	2^{b}	23^{c}	31ª	28 ^b	3^{e}	$26^{\rm c}$	5^d	2.8	3.1	5.6	3.1	4.8
Shenmulingtao	81ª	85ª	12 ^b	82ª	12 ^b	21ª	22^{a}	$2^{\mathbf{b}}$	22^{a}	$2^{\mathtt{b}}$	4.0	3.9	5.9	3.8	6.0
Hsinchu	95ª	96^{a}	5 ^b	96^{a}	8 ^b	36ª	32 ^b	2^{c}	34^{b}	2^{c}	2.4	2.7	3.7	2.6	4.7
Kenting	95ª	96^{a}	3^{c}	91 ^b	3^{c}	37ª	36ª	1^{c}	34 ^b	1^{c}	2.3	2.5	5.3	2.5	4.8
Sanyi	88ª	89ª	4 ^b	89ª	5 ^b	32ª	30ь	1^{c}	30 ^b	1^{c}	2.6	2.8	4.8	2.8	3.9
Tongshiao	95ª	98^{a}	7^{c}	96ª	19 ^b	36ª	34^{ab}	1^d	32^{b}	5°	2.5	2.7	5.0	2.8	4.6
Taitung	81ª	85ª	1^{c}	82ª	7 ^b	29ª	29^{a}	$0_{\rm p}$	27^{a}	1 ^b	2.6	2.7	-	2.9	4.7
Miscanthus transmorr	isonens	is													
Tartarchia Anpu	43 ^b	58ª	3^{d}	40 ^b	$10^{\rm c}$	7 ^ь	11ª	0^{c}	8 ^b	1^{c}	5.1	4.9	7.2	5.3	7.2

^{*}Means with the same letters within the same row and item are not significantly different at 5% level.

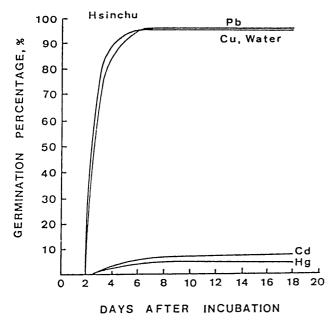


Fig. 1. Cumulative germination percentages of *M. floridulus*, Hsinchu, after imbibing in different solutions of heavy metals.

GRI were not affected by Pb, but were significantly reduced by Hg, Cu and Cd (Table 5). The Gt_{50} for both species was delayed after imbibing in heavy metal solutions. The Gt_{50} for imbibing in distilled water was 2.7 days and those for Hg, Cd, Pb and Cu solutions were 5.1, 4.8, 2.9 and 2.9 days, respectively, which showed that inhibition (imbibing) in Hg and Cd solutions was higher than that in Pb and Cu solutions.

The cumulative curves of seed germination revealed by Hoshe, Tonghsiao and Hsinchu in Pb, Cu, Cd and Hg ions in solutions were different. The curves revealed by Pb and Cu treatments were similar to those as the distilled water treatment showing a high germination percentage; while the curves of Cd and Hg treatments were contradictory (Fig. 1).

Effects of Heavy Metals on Inhibition of Seedling Growth of **Miscanthus**

The inhibition of heavy metals on seedling growth of Miscanthus species was increased when the concentrations of heavy metal solutions were increased (Fig. 2). The curves shown by treatments of 100 and 200 ppm were similar. The seedling growth of M. floridulus was inhibited at 11.3-24.8% by Pb ions at 10 ppm, while that of M. transmorrisonensis was stimulated. The seedling growth was inhibited above 70% by Cd ions at 10 ppm, while the entries of Hsinchu and Taitung of M. floridulus and M. transmorrisonensis, were completely inhibited by Cd at 100 ppm. The percent inhibition of seedling growth was between 66.1 and 85.4% for Cu ion at 10 ppm, while the values between 10.9 and 68.7% were shown for Hg at 10 ppm. This resulted in the inhibition of Hg being between that of Pb and Cu. The degree of inhibition on seedling growth of Miscanthus increased by heavy metals was as follows: Cd, Cu, Hg and Pb. M. transmorrisonensis was the most tolerant to heavy metals among the different entries.

Table 6. The amount of potassium leached out from **Miscanthus** seeds after imbibing in different heavy metal solutions at the concontration of 1000 ppm

	Amount of potassium leached											
			ppm/seed	t	ppm/gram seed							
	Distilled					Distilled						
	H_2O	Pb	Hg	Cu	Cd	H_2O	Pb	Hg	Cu	Cd		
Miscanthus floridu	us					A	-2000		-			
Hoshe	$0.10^{\text{b*}}$	0.12^{b}	0.24^a	0.12 ^b	0.11a	234 ^b	279 ^b	543ª	264 ^b	248 ^b		
Shenmulingtao	0.07^{c}	0.19^{a}	0.16^{b}	0.17 ^{ab}	0.09^{c}	127°	350ª	290 ^b	307 ^{аь}	152°		
Hsinchu	0.13^{d}	0.18^{b}	0.29^{a}	$0.06^{\rm c}$	0.16^{c}	298^{c}	419 ^b	691ª	136 ^d	375 ^b		
Kenting	$0.10^{\mathtt{d}}$	0.20^{b}	0.35^a	0.17^{c}	0.15^{c}	239 ^d	446 ^b	772ª	379^{c}	328°		
Sanyi	0.09^{c}	0.12^{b}	0.22^a	0.10^{bc}	0.09^{c}	289 ^b	346 ^b	705ª	317 ^b	284 ^b		
Tongshiao	0.11^{c}	0.16^{b}	0.31^a	0.13^{bc}	$0.13^{ m bc}$	308ь	390ь	883ª	$348^{\rm b}$	362 ^b		
Taitung	0.11^{b}	0.12^{b}	0.19^{a}	0.10^{b}	0.11 ^b	353ь	294ь	629ª	337ь	376 ^b		
Miscanthus transm	orrisonens	is										
Tartrchia Anpu	0.19^{bc}	0.21 в	0.27ª	0.21 в	0.17^{c}	370 ^{bc}	396ь	517ª	375 ^{ьс}	319 ^c		

^{*}Means with the same letters within the same row and item are not significantly different at 5% level.

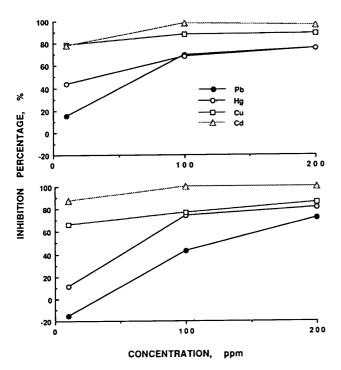


Fig. 2. Inhibition percentages of seedling growth of *M. flor-idulus* and *M. transmorrisonensis* emerged at different concentrations of heavy metals.

Effects of Heavy Metals on the Potassium Leaching from Seeds During Imbibition

The amounts of potassium ion leached from Mis-

canthus seeds after imbibing in the distilled water and 1000 ppm of heavy metal solutions were compared and the amounts that were leached (based on each seed and on gram per seed) are shown in Table 6. The amount of K^+ leached out was the highest in Hg solutions and the lowest in distilled water control among treatments for each species except for M. floridulus collected at Shemulingtao.

Discussion

Crop growth is affected when the concentration of heavy metal solutions was increased (Wang *et al.*, 1986). Some heavy metals were required for plant growth (Foy *et al.*, 1978), for example, copper at low concentration was important and involved in certain biochemical reactions required during plant growth and development; however, it becomes toxic to plants when the concentration was at higher levels (Mengel and Kirby, 1982).

Cd was the most toxic to seed germination of *Miscanthus* among the four heavy metals tested. Dubey and Dwivedi (1987) also reported that Cd showed more inhibition than Cu⁺⁺, Ni⁺⁺ and Zn⁺⁺ in germination and seedling growth of soybean (*Glycine maximum*). However, it was found in this study that Hg was the most inhibitive effect on germination of *Miscanthus* after the seeds were imbibed. Pathak *et al.* (1987) showed that

Hg and Mn inhibited germination and seedling growth of barley (*Hordeum vulgare*) when the concentrations of heavy metals were increased; but Mn required a toxicity at high concentration. Mrozek (1980) indicated that germination percentage and speed of germination of *Spartina alternifolra* were reduced by both Hg and Cd. Mrozek and Funicelli (1982) reported that germination capacity of this species was inhibited by Pb and stimulated by Zn.

The results obtained showed that seeds of *M. floridulus* harvested from the locations beside highway were more tolerant to heavy metals in germination than those from the locations of mountainous areas and far-off highway. Among heavy metals, Cd and Hg showed more toxicity to seed germination of *Miscanthus* than did Cu and Pb, while Cd and Cu were more inhibitory to seedling growth of *Miscanthus* than the other two metals. *Miscanthus* spp. could germinate and grow at high concentration of heavy metals, concentration suggesting that it might be useful for a tolerant plant in roadside pollution areas.

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重金屬對芒草之種子發芽及幼苗之生長影響

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芒草廣佈台灣各地,爲優勢草本植物。本研究中,五節芒(Miscanthus floridulus)的種子採自通霄,三義及墾丁等地之公路旁,及台東與新竹的非公路旁,和社(1600 公尺海拔高度),及神木林道(2000 公尺海拔),另外高山芒(M. transmorrisonensis)則採自塔塔加鞍部玉山國家公園內(2600 公尺海拔)。本研究主要目的係以重金屬鉛、汞、銅、鎘之不同濃度的水溶液對上述八個地區芒草種子進行種子發芽及幼苗生長實驗,以了解各地芒草對重金屬之反應,當重金屬濃度增加時芒草種子發芽抑制程度亦隨之增加。芒草種子發芽達到最後發芽率一半之時間於蒸餾水者爲 3.8 天,於 1000 ppm 的重金屬者爲 7.0 天。重金屬抑制芒草種子發芽之程度爲:汞>鎘>銅>鉛。將種子浸於上述重金屬水溶液中,從種子中滲溶出的鉀含量以於汞溶液中最高。另外,重金屬抑制芒草幼苗生長的程度則爲:鎘>銅>汞>鉛。從八個地區的芒草品種中,高山芒具最大的抗重金屬能力。本研究結論指出,因芒草植物可抗污染故可做爲公路旁之植生以爲水土保持之用。