treatments (Please refer to Table 1 for treatment explanation)					
	Treatment	А	В	С	D
Pb	V. zizanioides	0.16	0.18	0.17	0.14
	P. notatum	0.38	0.29	0.24	0.36
	I. cylindraca	0.12	0.14	0.17	0.25
	C. dactylon	0.17	0.10	0.08	0.15
Zn	V. zizanioides	0.15	0.15	0.16	0.14
	P. notatum	0.40	0.28	0.32	0.33
	I. cylindraca	0.19	0.17	0.24	0.25
	C. dactylon	0.37	0.30	0.26	0.36
Cu	V. zizanioides	0.19	0.14	0.20	0.22
	P. notatum	0.11	0.08	0.28	0.19
	I. cylindraca	0.16	0.11	0.16	0.14
	C. dactylon	0.41	0.29	0.27	0.27

Table 2The shoot/root metal concentration quotient (M<sub>S</sub>/M<sub>R</sub>) of Vetiveria zizanioides, Paspalum<br/>notatum, Imperata cylindraca and Cynodon dactylon growing on tailings with different<br/>treatments (Please refer to Table 1 for treatment explanation)

### 2.2 Experiment II

Another field trial was also conducted at Lechang Pb/Zn mine but at a different tailings pond in 2001 to evaluate: (1) the effect of vetiver grass growth with domestic refuse and NPK fertilizer on heavy metals stabilization in tailings; (2) the growth performance and heavy metal accumulation of V. zizanioides and two legume species (Sesbania rostrata and S. sesban); (3) domestic refuse and inorganic fertilizer amendment on the growth of plants in the tailings; and (4) the effects intercropping of V. zizanioides with S. rostrata on the growth and heavy metal accumulation of these species on Pb/Zn mine tailings. There were four treatments with four replicates arranged in a completely randomized block. The treatments included: tailings without any treatment; tailings + NPK fertilizer; tailings + domestic refuse; tailings + NPK fertilizer + domestic refuse. The major findings of the experiment included: (1) The sequential extraction experiment indicated that the change of heavy metals fractionations were controlled by both metal types and physio-chemical properties of the related soils. Compared with tailings without domestic refuse or NPK fertilizer, concentrations of Cu, Pb, and Zn decreased 9.5%, 13.6%, and 32.4%, respectively, while exchangeable Zn and Pb concentrations decreased 40.0% and 82.6%, respectively, and increased 130.4% for exchangeable Cu in tailings when domestic refuse and NPK fertilizer were added, which might be due to the relatively higher exchangeable Cu concentration in domestic refuse. Both organic bound and sulphide fraction and residual fraction increased after adding NPK fertilizer only (Yang & Shu, unpublished data). (2) Biomass of V. zizanioides significantly increased after adding domestic refuse, and V. zizanioides grew best in tailings amended with domestic refuse and NPK fertilizer  $(1,111 \text{ gm}^{-2})$ , this further indicated that domestic refuse was a useful ameliorative material for improving physio-chemical characters of the toxic tailings. Organic materials contained in domestic refuse also reduced heavy metal toxicity to plants by complexing spoil metals, supplying essential nutrients, improving physical conditions and increasing microbial activities. (3) Intercropping of grasses and legumes are recommended in revegetation of wasteland in order to ensure a long-term stability of vegetation, due to the contribution of N by legume species (Bradshaw & Chadwick, 1980). However, present results did not show any competitive and beneficial effects on growth performance of S. rostrata and V. zizanioides growing in the same subplot. This may be due to the relatively short experimental period (20 weeks) and the beneficial effect of legume species was not clearly manifested. Therefore, the long-term role of legume species in intercropping systems for mine tailings revegetation needs further investigation. (4) Among the three plants tested, V. zizanioides had the highest tolerance to metal toxicities and accumulated the lowest concentrations of heavy metals in the shoots among the three

species. This species was considered more suitable for stabilizing mine tailings, with the danger of transferring toxic metals to grassing animals was minimal (Yang *et al.*, 2003).

### 2.3 Experiment III

This experiment was conducted at Shaoguan Smelting Factory, located north of Guangdong Province, about 50 km away from Lechang Pb/Zn mine. The factory has been operating over 30 years smelting and refining Pb and Zn. The dust and gas emitted from Pb/Zn smelter-refinery processes contained high concentrations of SO<sub>2</sub> and heavy metals, such as Pb, Zn, Cd and Cu. The continuous deposition of these toxic pollutants has exerted adverse effects on the surrounding ecosystems. The soils around the factory were strongly acidified with pH values ranging from 3.02 to 4.86, total Pb and Zn concentrations were over 1200 mg kg<sup>-1</sup> and the DTPA-extractable Pb and Zn concentrations were over 100 mg kg<sup>-1</sup>. The dramatic effects on vegetation could be detected three kilometers from the point source, while land one kilometer surrounding the factory was completely devoid of vegetation. As a consequence, severe water erosion occurred on the land surface without vegetation cover, at least 20 cm topsoil of this area was washed away, and even eroded a lot of U-shaped valleys up to 2-5 m deep on the slopes. During the last decade, many efforts both from Shaoguan Smelting Factory and academic institutions have tried to reclaim this area, and over forty plant species (including trees, shrubs and grasses) were tested. However, most of the early efforts failed due to the harsh edaphic conditions and atmosphere pollution, and only several plants (include Paulownia tomentosa, Leucaena glauca, Nerium indicum, Paederia scandens, Cynodon dactylon) showed relatively high tolerance of the edaphic and atmospheric conditions. Based on the former experience, we collaborated with the engineers of the factory to reclaim the degraded land since 1999. Firstly, the soils were deeply ploughed to about 50 cm, amended with pond sediment and complex inorganic fertilizer (NPK), for diluting the metal concentrations in top soils and improving nutrient conditions and ameliorating physical properties. P. tomentosa, L. glauca, N. indicum, P. scandens, and C. dactylon were then mix cropped. Our reclamation project was very successful with over 70% cover after 2-years' growth. However, the growth performance at the severely eroded area was still poor, the total canopy cover was about 30-50%, and the water erosion was far from controlled. Therefore, in June 2002, vetiver grass was introduced to the most eroded area in an endeavour to control the erosion. Fortunately, the grass was well established after 5-months' growth (November, 2002), the total canopy cover (include P. tomentosa and V. zizanioides) reached about 80%, and the results from recent inpection (May, 2003) indicated that the erosion of the area planted with vetiver was under control. The difficulties experienced in former experiments at this site further demonstrated that vetiver grass was also an important plant material for stabilization of metal contamination resulting from smelting (Shu et al., unpublished data).

# **3** PHYTOEXTRACTION OF HEAVY METALS

Different from phytostablization, phytoextraction is a newer emerging technology for extracting heavy metals from contaminated soils. Two approaches have been proposed for phytoextraction of heavy metals, namely continuous or natural phytoextraction and chemically enhanced phytoextraction (Lombi *et al.*, 2001) The first is based on the use of natural hyperaccumulators with exceptional metal-accumulating capacity. These plants have several beneficial characteristics such as the ability to accumulate metals in their shoots and an exceptionally high tolerance to heavy metals; however, the remediation potential may be limited by slow growth rate and low biomass of these plants (Brooks, 1998). Another problem with this approach is related to the fact that some metals such as Pb are largely immobile in soil and their extraction rate is limited by solubility and diffusion through the root surface (Lombi *et al.*, 2001). Chemically enhanced phytoextraction may be an alternative approach to overcome these problems. This

approach makes use of high-biomass crops, such as Zea mays, Brassica juncea and Helianthus annus, which are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatments (Huang *et al*, 1997). *V. zizanioides* may be superior to the three crops cited above and have great potential in phytoextraction, due to the following facts : (1) higher tolerance to heavy metals; (2) strong root system; and (3) perennial growth (the other three crops are annual) and can be harvested 3 times per year. From "experiment I", we also found that although the metal contents in shoots of *V. zizanioides* were significantly lower than the other three grasses (Table 1), the total amount of metals (Pb and Cu) accumulated in shoots was the highest among the four plants tested (Fig. 1), due to its highest biomass (Shu *et al.*, unpublished data). For further evaluation of the phytoextraction potential of vetiver grass, the following experiment was conducted.

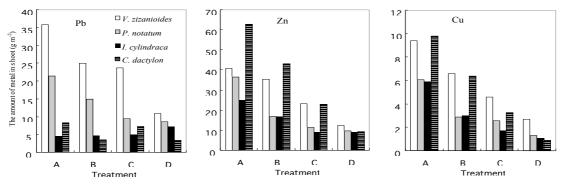


Fig. 1 The amounts of Pb, Zn and Cu accumulated in shoots of *Vetveria zizanioides, Puspalum notatum, Imperata cylindraca* and *Cynodon dactylon* grown on tailings with different treatment (g m<sup>-2</sup>, refer to Table 1 for explanation of treatments).

#### **Experiment IV**

This experiment was conducted at a metal contaminated site near Lechang City, beside the Lechang Pb/Zn mine. The objectives of this experiment were to: (1) investigate heavy metals concentrations in soil (paddy soil) rice (*Oryza sativa*) system on the farmland which receives the mining wastewater from the Pb/Zn mine; and (2) phytoextract heavy metals from the soil using both hyperaccumulators and highbiomass plants including vetiver grass. The chemical analysis indicated that the soil was severely contaminated by heavy metals, and total concentrations of Pb, Zn, Cu and Cd were 1486 (ranging from 325 to 4317), 1424 (253-3292), 680 (120-3026) and 13.6 (2.0-29.7) mgkg<sup>-1</sup>respectively, while the concentrations of those metals in the shoots of rice were 69 (25-228), 166 (63-353), 88 (19-178) and 1.69 (0.50-6.24) mgkg<sup>-1</sup>, respectively. In January 2003, heavy metal hyperaccumulators, including Pb and Cd hyperaccumulator *Viola banshanensis* (Shu *et al.*, 2002b, 2003) and Zn and Cd hyperaccumulator *Sedum alfredii* (Long, 2002), and high-biomass plants, including *V. zizanioides, Rumex acetosa*, and *Z. mays*, were planted on the contaminated land, covering an area of 0.8 ha. The experiment is still in progress, with all of the plants well established on the land. They will be treated with or without EDTA before harvesting in autumn of 2003, and then the phytoextraction potential of natural hyperaccumulation *versus* chemically enhanced phytoextraction can be evaluated.

# **4\_ PHYTOFILTRATION OF HEAVY METALS**

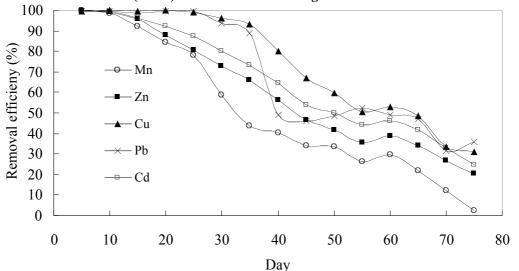
Wastewater containing high concentrations of heavy metals often jeopardizes the ecosystem stability and poses serious danger to human health. Various techniques, based on ion exchange or chemical and microbiological precipitation, have been developed to treat heavy metal wastewater including mine effluent with some success. Recently constructed wetlands with plants acting as phytofiltrators were considered to be an effective and low-cost alternative for adjusting the pH and

removing metal elements from wastewater (Ye *et al.*, 2001). Vetiver grass has been proved as having great potential in purifying domestic sewage and landfill leachate (Xia *et al.*, 2002). However, its potential value in treating heavy metal wastewater is still unknown. A experiment aiming at evaluating its capability in purifying heavy metal wastewater by comparing with other six common wetland species was conducted.

#### **Experiment V**

Pvritic bearing mine tailings disposed at neutral or slightly alkaline conditions can weather within months or a few years to produce extreme acidity, and lead to acid mine drainage (AMD) (Robbed & Robison, 1995). AMD usually contains high levels of heavy metals besides having a low pH, and significantly impacts on water quality and natural ecosystems in southern China (Shu et al., 2001). It is also a serious environmental problem around the world (Dudka & Adriano, 1997). A microcosm test was conducted to assess the tolerance of different wetland species to AMD and the purification capacity of wetlands. The tested plant species included: V. zizanioides, Phragmites australis, Cyperus alternifolius, Panicum repens, Gynura crepidiodes, Alocasia macrorrhiza and Chrysopogon aciculatus. Chemical analysis indicated that AMD collected from Lechang lead/zinc mine tailings contained high concentrations of Zn, Mn, Pb, Cd, Cu and  $SO_4^{2-}$ , and was also extremely acid. According to the tolerance index of the 7 tested species subjected to AMD for 75 days, C. alternifolius had the highest but G. crepidiodes had the lowest tolerance index to AMD. V. zizanioides had the similar and even slight higher tolerance index than P. australis, suggesting that V. zizanioides also had higher tolerance to adverse conditions such as AMD. The capacity of microcosm (wetland) in adjusting pH and removing SO<sub>4</sub><sup>2-</sup>, Cu, Cd, Pb, Zn and Mn only lasted for 35 days, which might be due to the high acidity of AMD (Fig. 2, Shu, 2003). Therefore, a further experiment aiming at improving the purification capacity has been conducting by us at a greenhouse of Sun Yatsen University, and vetiver was the only plant material selected in this stage because of its adaption to AMD conditions has been proved in the first stage experiment.

Fig. 2 The capacity of microcosm (wetland) in purifying Cu, Cd, Pb, Zn and Mn in acid mine drainage (AMD) collected from Lechang Pb/Zn mine.



# 5 SUMMARY AND PERSPECTIVE

Based on the series of research cited above, an integrated vetiver technique (IVT) for phytoremediation of heavy metal contamination can be framed. The newly framed IVT includes three aspects: (1) use of vetiver for phytostabilization of heavy metals, and it was well demonstrated by

Experiments I, II and III; (2) use of vetiver for phytoextraction of heavy metals, the potential utilization of vetiver combined with chemical chelators in this area may be proved by the progressing Experiment IV; (3) use of vetiver for phytofiltration of heavy metals, it has been partly proved by Experiment V, and further experiments for improving long-term phytofiltration effectiveness is also in progress.

More specifically, the IVT could be used as an integrated technique for environmental management of mining activities.

- 1. Firstly, solid mining wastes such as tailings and waste rocks could be stabilized by vetiver to control or reduce air and water erosion, then reduce the release of heavy metals to surroundings.
- 2. Secondly, wastewater including acid mine drainage (AMD) could be purified by phytofiltration.
- 3. Thirdly, the surrounding lands contaminated by heavy metals could be further cleaned up by phytoextraction. A progressive worldwide increase in metalliferous mining in recent years opens up a vast range of prospects for IVT application.

For the newly framed technique to become a promising technique, as well as establishing a more solid scientific basis for IVT, some further research should be conducted. In our opinion, the long-term effectiveness of phytofiltration may be improved by altering the substrate of a constructed wetland. As for phytoextraction, the accumulation of heavy metals by vetiver, especially the translocation of metals from root to shoot, should be enhanced. The higher accumulation of metals in shoot may be achieved by various ways, such as application of chemical chelators, screening for metal accumulation varieties, modifying vetiver characteristics like metal uptake, translocation and accumulation by gene engineering or somatic hybridization with hyperaccumulators.

### Acknowledgments

The authors would like to thank Prof. M.H. Wong, Dr. P. Truong, Dr. Z.H. Ye and Prof. C.Y. Lan for their invaluable comments, and Mr. B. Yang, Mr. Q.W. Yang, Ms. P. Zhuang and Dr. T.G. Luan for technical assistant. Financial support from National Natural Science Foundation of China (30100024), National "863" High Sci-Technology Project of China, the Donner Foundation of the US and the Vetiver Network (TVN) is gratefully acknowledged.

# References

- Baker AJM, snd Walker PL. 1990. Ecophysiology of metal uptake by tolerant plants. In: Heavy Metal Tolerant in Plants: Evolutionary Aspects, Shaw A J (ed.), CRC Press, Boca Raton, Florida. 155-178
- Bevan O, and Truong P. 2002. Effectiveness of vetiver grass in erosion and sediment control at a bentonite mine in Queensland, Australia. In: Proceedings of the Second International Conference on Vetiver. Bangkok: Office of the Royal Development Projects Board. 292-295
- Bradshaw AD, and Chadwick MJ. 1980. The Restoration of Land: The Ecology and Reclamation of Derelict and Degraded Land. University of California Press, Berkeley Los Angeles
- Brooks RR. 1998. Plant That Hyperaccumulate Heavy Metals, Cambridge, the University Press
- Dudka S, and Adriano DC. 1997. Environmental impacts of metal ore mining and processing: a review. Journal of Environmental Quality, 26: 590-602
- Greenfield JC. 1995. Vetiver grass (*Vetiveria* spp.), the ideal plant for vegetative soil and moisture conservation. In: Grimshaw R G and Helfer L eds. Vetiver Grass for Soil and Water Conservation, Land Rehabilitation, and Embankment Stabilization. The World Bank, Washington DC. 3-38
- Grimshaw RG. 2002. Vetiver grass technology networking and its impact on the environment. Proceedings of the Second International Congress on Vetiver. Office of the Royal Development Projects Board, Bangkok. 7-19
- Huang JW, Chen J, Berti WR, *et al.* 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environmental Science & Technology, 31(3): 800-805
- Johnson MS, Cooke JA, and Stevenson JKW. 1994. Revegetation of metalliferous wastes and land after metal mining. In: Hester and Harrison R M (Eds.), Mining and Its Environmental Impact. 31-48

- Lombi E, Zhao FJ, Dunham SJ, *et al.* 2001. Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. Journal of Environmental Quality, 30: 1919-1926
- Long XX. 2002. Mechanisms of hyperaccumulating zinc by *Sedum alfredii* Hance. PhD Thesis, Zhejiang University, China.
- Raskin I, and Ensley BD. 2000. Phytoremediation of Toxic Metals, John Wiley & Sons, Inc, New York
- Robbed GA, and Robison JDF. 1995. Acid Drainage from mines. Geographical Journal, 161: 47-54
- Ross SM. 1994. Sources and forms of potentially toxic metals in soil-plant systems. In: Ross S M (ed), Toxic Metals in Soil-Plant Systems. Chichester: John Wiley & Sons. 3-26
- Salt DE, Blayblock M, Nanda Kumar NPBA, *et al.* 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology, 13, 468-474
- Shu WS, Ye ZH, Zhang ZQ,*et al.* 2001. Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. Environment International, 26: 389-394
- Shu WS, Xia HP, Zhang ZQ, *et al.* 2002a. Use of vetiver and other three grasses for revegetation of Pb/Zn mine tailings: field experiment. International Journal of Phytoremediation, 4(1): 47-57
- Shu WS, Liu W, and Lan CY. 2002b. Utilization of a species from Viola for phytoremediation of soil and water contaminated by lead and cadmium. Chinese Patent
- Shu WS. 2003. Exploring the potential utilization of vetiver in treating acid mine drainage (AMD). Proceedings of the Third International Conference on Vetiver and Exhibition, Guangzhou, China
- Shu WS, Liu W, snd Lan CY. 2003. *Viola baoshanensis* Shu, Liu et Lan, a new species of Violaceae from Hunan Province, China. Acta Scientiarum Naturalism Universities Sunyaseni, 42(3): 118-119
- Terry N, and Banuelos G. 1999. Phytoremediation of Contaminated Soil And Water, Washington, D. C., Lewis Publishers
- Truong P, and Baker D. 1996. Vetiver grass for the stabilization and rehabilitation of acid sulfate soils. Proceedings of the Second National Conference on Acid Sulfate Soils, Coffs Harbour, Australia. 196-198
- Truong P. 1999. Vetiver Grass Technique for mine tailings rehabilitation. Proceedings of the First Asia Pacific Conference on Ground and Water Bio-engineering for Erosion Control and Slope Stabilization, Malina, Philippines. 315-325
- Truong P N. 2002. The global impact of Vetiver Grass Technique on the environment. Proceedings of the Second International Conference on Vetiver, Tailand. p46-57
- Xia HP, and Shu WS. 2001. Resistance to and uptake of heavy metals by *Vetiveria zizanioides* and *Paspalum notatum* form lead/zinc mine tailings. Acta Ecologica Sinica, 21(7): 1121-1129
- Xia HP, Liu SZ, Ao HX. 2002. A study on purification and uptake of vetiver grass of garbage leachate. Proceedings of the Second International Conference on Vetiver. Office of the Royal Development Projects Board, Bangkok.393-403
- Yang B, Shu WS, Ye ZH, *et al.* 2003. Growth and metal accumulation in Vetiver and two *Sesbania* species on Lead/Zinc mine tailings. Chemosphere, 52: 1593-1600
- Ye ZH, Whiting S, Lin ZQ, *et al.* 2001. Removal and distribution of iron, manganese, cobalt, and nickel with a Pennsylvania constructed wetland treating coal combustion by-product leachate. Journal of Environment Quality, 30: 1464-1473

#### A Brief Introduction to the First Author

Dr. Wensheng Shu (W.S. Shu) is an associate professor in ecology in School of Life Science at Sun Yatsen (Zhongshan) University, Guangzhou, China and honorary research fellow of Institute for Natural Resources and Environmental Management Hong Kong Baptist University (2001-2004). He maintains active research in restoration of mining wastelands and phytoremediation of soil and water contaminated by heavy metals. He has received the King of Thailand Vetiver Award in Research in 2000, the Second Prize in Natural Science by the Ministry of Education of China in 2000, and an Academic Award for Outstanding Young Scientist in Environmental Science in 2002 by the Association of Chinese Environmental Science.