Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

31 329-334 (**May** 2010)
For personal use only
Commercial distribution of this copy is illegal

Tolerance and accumulation of lead in *Vetiveria zizanioides* and its effect on oil production

P. Rotkittikhun[1], M. Kruatrachue*[2], P. Pokethitiyook[1] and A.J.M. Baker[3]

[1]Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok - 10400, Thailand
[2]Department of Biology, Faculty of Science and Mahidol University International College, Mahidol University, Rama VI Road, Bangkok - 10400, Thailand
[3]School of Botany, The University of Melbourne, Parkville, Victoria 3010, Australia

(Received: March 14, 2009; Revised received: August 18, 2009; Accepted: September 31, 2009)

Abstract: *Experiments were conducted to evaluate lead tolerance and accumulation in vetiver grass Vetiveria zizanioides (L.), grown in hydroponics and a pot study and to examine the effect of lead on vetiver oil production. Elevated concentrations of lead decreased the length of shoots and roots of plants. However, vetiver grown in highly contaminated soils showed no apparent phytotoxicity symptoms. Lead concentrations in the shoots and roots of vetiver plants grown in hydroponics were up to 144 and 19530 mg kg^{-1} and those grown in soil were 38 and 629 mg kg^{-1}, respectively. Lead had an effect on vetiver oil production and composition by stimulating oil yield and the number of its constituents. Oil yield ranged from 0.4-1.3%; the highest yields were found in plants grown in nutrient solution with 100 mg Pb l^{-1} for 5 weeks (1.29%) and 7 weeks (1.22%). The number of total constituents of vetiver oil also varied between 47-143 compounds when lead was present in the growth medium. The highest number (143) was found in plants grown in soil spiked with 1000 mg Pb kg^{-1}. The predominant compound was khusimol (10.7-18.1%) followed by (E)-isovalencenol (10.3-15.6%). Our results indicated that lead could increase the oil production of vetiver.*

Key words: *Lead, Tolerance, Accumulation, Vetiveria zizanioides, Vetiver oil*
PDF of full length paper is available online

Introduction

Vetiver root aromatic oil has been used as a repellent for the control of termites (Zhu *et al*., 2001). In addition, the grass has been cultivated for its roots which contain the essential oil used extensively in perfumery, cosmetics and biomedical utilization (Anderson, 1970; Maffei, 2002; Wong, 2003; Massardo *et al*., 2006). The volatile oil from vetiver roots is a complex mixture of sesquiterpene alcohols and hydrocarbons, and is also a very viscous oil with an extremely low volatility (Massardo *et al*., 2006). Currently, the main vetiver oil producing countries are Haiti, Indonesia and Réunion island (Chomchalow, 2000).

Numerous studies have been published since 1940 on the essential oils of *V. zizanioides* (Champagnat *et al*., 2006). Most have reported on the structure determination and synthesis of some compounds of vetiver oils (Marshall and Anderson, 1967; Marshall and Johnson, 1970; Anderson, 1970; Nigam and Komae, 1968). Weyerstahl *et al*. (1996, 2000) only studied the constituents of Haitian vetiver oil. Martinez *et al.* (2004) evaluated different methods of oil extraction from Brazilian vetiver roots. However, there are relatively few papers reporting the effects of environmental conditions on vetiver oil yields and composition. Lemberg and Hale (1978) and Champagnat *et al*. (2006) studied the composition of vetiver oils of different geographical origins while Adams *et al*. (2003) reported on the oil composition in relation to DNA-fingerprints. In addition, the correlations between vetiver oil production and cultivation conditions have been investigated (Adams *et al*., 2004; Pripdeevech *et al*., 2006). Massardo *et al*. (2006) state that vetiver oil production is closely related to the metabolic activity of plant roots, which is affected by changes in environmental temperatures.

To the best of our knowledge, there has been no study on the effects of heavy metals on vetiver oil yield in this economically-important plant. The aims of this study were to evaluate the extent of lead tolerance and accumulation in vetiver grass grown in hydroponics and in pot trials and to investigate any effects of lead on vetiver oil production.

Materials and Methods

Plant materials: Vetiver plants (*Vetiveria zizanioides* 'cv. Monto') were kindly provided by Dr. Paul Truong, Veticon Consulting, Brisbane, Australia. The plants were pre-cultured in potting-mix in a glasshouse under semi-controlled conditions (12 hr photoperiod; 25-30°C) for 18 months. They were then pruned to uniform size (shoots 20 cm and roots 5 cm).

Hydroponic study: Forty-eight uniform-sized vetiver plants were selected and acclimated in modified Hoagland's solution (pH 5; Hoagland and Arnold, 1950) and placed in a glasshouse. Seven days after installation, lead treatments were applied. Nominal concentrations of lead of 10, 50 and 100 mg l^{-1} were created in each container (20 x 18 x 12.5 cm^3) of 3.5 l modified Hoagland's solution.

* **Corresponding author:** scmkt@mahidol.ac.th

All treatment solutions were aerated. Lead was supplied as stock lead nitrate [$Pb(NO_3)_2$] solution, prepared in deionized water. Lead nitrate is commonly used in the hydroponic experiments because it can be readily dissolved in the solution. The treatment solutions were changed every 7 d. There were 3 replicates (2 plants/replicate) for each treatment unit. Plants were set up in the glasshouse under semi-controlled growth conditions. Six plants from each treatment unit were harvested after 5 and 7 weeks.

Pot study: The mixture of potting mix and sand used for the experiment (sand:potting mix 3:1) was air-dried and sieved through a 2-mm mesh. Lead (as lead carbonate) was added to give nominal concentrations of 100, 500 and 1000 mg Pb kg^{-1}. Lead is usually found in soil in the form of lead carbonate. Soil without lead addition served as the control. Soils before and after the addition of lead were measured for pH and EC using a glass pH electrode and EC meter, respectively. Background levels of heavy metals in the soil were measured by an Inductive Coupled Plasma Atomic Emission Spectrometer, ICP-AES (Varian Vista Ax) using *aqua regia* digestion. The total and diethylenetriamine-pentaacetic acid (DTPA)-extractable concentrations of lead in the soil after four week's equilibration were determined by ICP-AES. Lead was extracted with 0.005 M DTPA (ICARDA, 2001).

Forty-eight uniform-sized plants of vetiver were selected for use in this glasshouse experiment. 6 kg of sieved soil was placed into each 17 cm diameter plastic pot which had two pieces of plastic screen at the bottom to retain the soil. Plants were transplanted into the pots containing increasing concentrations of lead (0, 100, 500 and 1000 mg kg^{-1} of dry soil). There were 3 replicates (2 plants/replicate) for each treatment. The pots were placed in the glasshouse under semi-controlled conditions and watered daily. Fertilizer (N:P:K = 16:3.5:10) was added 6 g/pot at monthly intervals. Six plants from each treatment were harvested after 2 and 3 months.

Measurements of the plant sizes and lead concentrations: After growth periods of 5 and 7 weeks for the hydroponics experiment, and 2 and 3 months for pot experiment, plants were harvested and the shoot and root lengths were measured, then plants were divided into shoots and roots. They were washed with tap water, 5% dextran and rinsed with deionized water. The concentrations of lead in shoots and roots of plants were determined using 0.5 g plant sub-sample digested with HNO_3 (APHA, 1998) and lead concentrations in the digests were measured by ICP-AES. Lead was not measured in the vetiver oil because the quantity of oil extracted from roots was very low and it was difficult to measure lead in oily matrices.

Vetiver oil extraction: Fresh roots of vetiver were ground with liquid nitrogen. Then approximately 500 mg ground root was immersed into 3 ml of ethanol and placed on a shaker for 3 weeks at room temperature. The isolated oil was dehydrated by adding anhydrous sodium sulphate and then stored in an amber-colored glass bottle.

Samples were analyzed by GC-FID using an autosystem XL gas chromatograph (Perkin Elmer) equipped with BPX5 capillary column (0.5 µm film thickness, 0.25 mm i.d., 30 m length). The oven temperature program was maintained at 70°C for 4 min, and then raised to 280°C at the rate of 4°C min^{-1}. Injector and detector temperatures were 250 and 300°C, respectively. The carrier gas was helium (1 ml min^{-1}). Aliquots (1 µl) of each sample were injected manually and in 1:20 split ratio.

GC-MS analyses were carried out on a Agilent 6890N, fitted with J&W Scientific DB-5 (30 m x 0.25 mm i.d., 0.25 µm film thickness) linked on-line with a Agilent Mass selective detector (5973); ionization voltage 70 eV. The carrier gas was helium (1 ml min^{-1}). The injector temperature was 200°C. The oven temperature program was 60°C, and then raised to 246°C at the rate of 3°C min^{-1}. Aliquots (1 µl) of each sample were injected manually and in split-less mode.

Vetiver oil constituents were identified using GC-MS by comparison of their mass spectra with those stored in the Wiley 7 library or with mass spectra from the literature (Adams, 2001). Further identification was made by comparison of their retention indices with those in the literature (Weyerstahl *et al*., 2000; Adams, 2001; Adams *et al*., 2004; Massardo *et al*., 2006). The retention indices were determined in relation to a homologous series of *n*-alkanes (C_9-C_{20}) under the same operating conditions. The percentage of oil yield and concentration of components were calculated from GC peak areas after measured by GC-FID.

Statistical analysis: The data were analyzed using the SPSS statistical package by one-way analysis of variance (ANOVA) to compare the means of different treatments. Significant *F*-values were tested using least significant different test (LSD) at the $p<0.05$ significance level.

Results and Discussion

Hydroponic study: In the 10, 50 and 100 mg l^{-1} Pb treatments, 95.2, 92.8 and 88.2% Pb, respectively were calculated to be in the free form. The remaining lead was present as complexes or in solid form with sulphate, nitrate and phosphate.

Plant growth and lead accumulation: Table 1 shows the shoot and root lengths of vetiver plants grown in the nutrient solution. Plants grown in control and 10 mg l^{-1} Pb treatments showed significantly longer shoot and root lengths when compared with those in treatments with added lead ($p \leq 0.05$). Root length progressively decreased with increasing concentrations of lead. There was little difference between shoot and root lengths of plants grown for 5 weeks and 7 weeks. Visual signs of phytotoxicity (red-tipped leaves) were observed in plants grown in the 50 and 100 mg l^{-1} Pb treatments.

Lead concentrations in shoots and roots of vetiver are presented in Table 2. They increased with increasing lead concentration in the solution. Much higher lead concentrations were found in roots than shoots (175 fold in plants grown in 100 mg l^{-1} treatment for 5 weeks). Plants treated with 100 mg l^{-1} accumulated high concentrations of lead in their shoots (112-144 mg kg^{-1}) and roots (16860-19530 mg kg^{-1}).

Table - 1: Length of shoots and roots (mean ± SD, *n* = 3) of vetiver grass grown in nutrient solutions and soils containing different lead concentrations

Hydroponic study**	5 weeks		7 weeks	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
Control	66.0 ± 7.1^{a}	37.7 ± 0.9^{a}	73.3 ± 3.3^{a}	37.3 ± 13.9^{a}
10 mg l^{-1}	59.1 ± 6.0^{a}	10.6 ± 0.6^{b}	62.7 ± 4.8^{ab}	11.8 ± 0.8^{b}
50 mg l^{-1}	46.7 ± 0.7^{b}	11.1 ± 0.6^{b}	52.9 ± 8.8^{bc}	9.2 ± 1.0^{b}
100 mg l^{-1}	48.9 ± 3.2^{b}	7.3 ± 2.2^{c}	46.3 ± 8.0^{c}	7.1 ± 0.1^{b}
Pot study* **	**2 months**		**3 months**	
Control	94.5 ± 3.9^{a}	R	108.5 ± 8.7^{a}	R
100 mg kg^{-1}	93.5 ± 9.0^{a}	R	120.5 ± 1.3^{a}	R
500 mg kg^{-1}	92.8 ± 2.9^{a}	R	119.7 ± 21.6^{a}	R
1000 mg kg^{-1}	96.8 ± 11.4^{a}	R	108.8± 18.3^{a}	R

Data with different letters in the same column indicate a significant difference at 5% level according to LSD test, ^{R} Roots reached the base of the pot, ** as lead nitrate, *** as lead carbonate

Table - 2: Lead concentration (mean ± SD, *n* = 3) in shoots and roots of vetiver grass grown in nutrient solutions and soils containing different lead concentrations

Hydroponic study**	5 weeks		7 weeks	
	Shoot (mg kg^{-1})	Root (mg kg^{-1})	Shoot (mg kg^{-1})	Root (mg kg^{-1})
Control	5.44 ± 2.0$^{c*-a\,\#}$	5.47 ± 2.0^{d-a}	3.5 ± 1.5^{d-a}	2.66 ± 1.2^{c-a}
10 mg l^{-1}	78.3 ± 5.9^{b-a}	8140 ± 2325^{c-a}	87.4 ± 4.2^{c-a}	5920 ± 698^{b-a}
50 mg l^{-1}	78.6 ± 6.2^{b-b}	14 200 ± 618^{b-a}	112.4 ± 11.7^{b-a}	14 060 ± 2095^{a-a}
100 mg l^{-1}	111.8 ± 23.3^{a-a}	19 530 ± 2520^{a-a}	143.9 ± 22^{a-a}	16 860 ± 2155^{a-a}
Pot study* **	**2 months**		**3 months**	
Control	0.54 ± 0.5^{c-a}	4.2 ± 2.5^{c-a}	0.19 ± 0.3^{c-a}	1.7 ± 1^{c-a}
100 mg kg^{-1}	2.19 ± 0.3^{c-b}	43.5 ± 18.1^{c-a}	4.26 ± 1.0^{c-a}	29.8 ± 7^{c-a}
500 mg kg^{-1}	13.6 ± 4.4^{b-a}	315.4 ± 131^{b-a}	20.4 ± 5.9^{b-a}	295.5 ± 92.8^{b-a}
1000 mg kg^{-1}	25.0 ± 1.9^{a-b}	585.1 ± 103.5^{a-a}	37.6 ± 1.5^{a-a}	628.9 ± 88^{a-a}

* Data with different letters in the same column and same item and # in the same row indicate a significant difference at 5% level according to LSD test, ** as lead nitrate, *** as lead carbonate

Table - 3: Comparison of percent oil yield (dry weight basis) from various lead treatments (mean ± SD, *n* = 3).

Treatment	Oil yield % (dry weight basis)				
	Hydroponic study**		Treatment	Pot study***	
	5 weeks	7 weeks		2 months	3 months
Control	0.49 ± 0.13$^{b*-a\,\#}$	0.55 ± 0.25^{b-a}	Control	0.58 ± 0.22^{a-a}	0.81 ± 0.29^{a-a}
10 mg l^{-1}	0.64 ± 0.07^{b-b}	0.42 ± 0.01^{b-c}	100 mg kg^{-1}	0.65 ± 0.22^{a-b}	0.96 ± 0.17^{a-a}
50 mg l^{-1}	1.18 ± 0.28^{a-a}	0.42 ± 0.09^{b-b}	500 mg kg^{-1}	0.72 ± 0.09^{a-b}	0.64 ± 0.08^{a-b}
100 mg l^{-1}	1.29 ± 0.35^{a-a}	1.22 ± 0.33^{a-a}	1000 mg kg^{-1}	0.59 ± 0.06^{a-b}	0.94 0.11^{a-ab}

* Data with different letters in the same column and same item, and # in the same row indicate a significant difference at the 5% level according to LSD test, ** as lead nitrate, *** as lead carbonate

Pot study :

Plant growth and lead accumulation: The soil pH was 7.1, with an EC value of 0.45 dS m^{-1}. The background total lead concentration in the soil was 3.5 mg kg^{-1}. The vetiver plants in the pot experiment grew well and there were no visual signs of phytotoxicity in any of the treatments. There was no significant difference between treatments ($p > 0.05$) (Table 1). Plant roots in every treatment grew well and reached the base of the pots.

Lead concentrations in shoots and roots are presented in Table 2. Plants accumulated highest lead concentrations in the roots. Lead concentrations in plants increased with increasing lead in soil. The highest lead accumulations in shoot (38 mg kg^{-1}) and root (629 mg kg^{-1}) were measured in vetiver grown in the 1000 mg kg^{-1} treatment for 3 months.

Elevated concentrations of lead in solution decrease the length of shoots and roots of plants due to lead toxicity. However, vetiver grown in highly contaminated soils showed no apparent phytotoxicity symptoms. A possible explanation for this lack of phytotoxicity in plants grown in the pot study would be the high organic matter content of the growth medium which has a high affinity

Table - 4: Total constituents of vetiver oils from plants in various lead treatments

Study	Period / Treatment	No. of constituent
Hydroponics**	**5 weeks**	
	Control	65-89
	10 mg l^{-1}	71-84
	50 mg l^{-1}	97-117
	100 mg l^{-1}	92-129
	7 weeks	
	Control	47-92
	10 mg l^{-1}	74-94
	50 mg l^{-1}	76-87
	100 mg l^{-1}	66-87
Pot***	**2 months**	
	Control	87-120
	100 mg kg^{-1}	86-101
	500 mg kg^{-1}	73-104
	1000 mg kg^{-1}	120-143
	3 months	
	Control	80-98
	100 mg kg^{-1}	76-93
	500 mg kg^{-1}	72-87
	1000 mg kg^{-1}	82-89

** as lead nitrate, *** as lead carbonate

for the metal (Zheljazkov *et al*., 2006). According to Kabata-Pendias and Pendias (1992), lead concentrations in mature leaf tissues greater than 30 mg kg^{-1} are considered excessive or toxic to plants. However, in this study, lead concentrations in the shoots and roots of vetiver plants grown in hydroponic were up to 144 and 19 530 mg kg^{-1} and those grown in soil were 38 and 629 mg kg^{-1}, repectively. The results of our study show that vetiver can tolerate high lead concentrations in soil as suggested in earlier studies by Chen *et al.* (2004) and Chantachon *et al*. (2004). Vetiver grass has been cultivated widely for use in the prevention of soil erosion and stabilization of heavy metals in contaminated land. Rotkittikhun *et al*. (2007) showed that vetiver is a good choice for phytostabilization of lead mine soil in Thailand. It has a high tolerance to lead and can accumulate much more lead in roots than in shoots.

Oil production : The highest oil yields found in plants grown in nutrient solution (100 mg l^{-1}) for 5 and 7 weeks were 1.29 and 1.22%, respectively (Table 3). The lowest oil yield (0.42%) was produced by plants grown in nutrient solutions (10 and 50 mg l^{-1}) for 7 weeks. For the pot experiment, there was little difference among treatments and between the two growth periods.

Total constituents of vetiver oil in different treatments are presented in Table 4. The highest total number of oil constituents (143 compounds) was found in vetiver plants grown in soil spiked with 1000 mg Pb kg^{-1}, followed by plants grown in solution with 100 mg l^{-1} (129 compounds).

Table - 5: Comparison of major compounds of vetiver oil from plants grown in various lead treatments

Study	Period / Treatment	Essential oil constituents (%)				
		Vetiselinenol	Khusimol	(*E*)-Isovalencenol	β-Vetivone	α-Vetivone
Hydroponic**			**5 weeks**			
	Control	5.0	16.3	11.9	4.0	3.9
	10 mg l^{-1}	6.9	17.7	15.6	3.5	4.4
	50 mg l^{-1}	7.4	15.0	15.1	3.7	4.3
	100 mg l^{-1}	7.7	15.3	14.0	3.7	4.9
			7 weeks			
	Control	4.9	18.1	12.7	2.4	3.6
	10 mg l^{-1}	6.7	14.4	11.6	2.5	3.5
	50 mg l^{-1}	6.9	14.8	13.2	2.7	4.4
	100 mg l^{-1}	7.5	13.8	12.0	2.6	4.0
			2 months			
Pot***	Control	2.7	15.6	10.7	3.6	1.6
	100 mg kg^{-1}	4.7	16.4	13.3	3.7	3.0
	500 mg kg^{-1}	6.1	15.8	14.4	3.7	3.7
	1000 mg kg^{-1}	6.3	10.8	10.3	3.5	4.1
			3 months			
	Control	8.6	13.6	12.3	1.9	2.6
	100 mg kg^{-1}	7.1	16.3	11.4	1.7	2.4
	500 mg kg^{-1}	8.4	13.2	12.4	2.0	2.9
	1000 mg kg^{-1}	9.3	10.7	13.6	1.9	2.3

Data shown are mean values of three replicates, ** as lead nitrate, *** as lead carbonate

Table 5 shows the percentage of 5 major compounds of vetiver oil in the descending order: khusimol (alcohol) > (*E*)-isovalencenol (alcohol) > vetiselinenol (alcohol) > α-vetivone (ketone) > β-vetivone (ketone). Khusimol is the largest component of vetiver oil (10.7-18.1%). In the hydroponics study, the highest percentage of khusimol was in control plants (7 weeks) followed by plants treated with 10 mg Pb l^{-1} (5 weeks), while in the pot study, 100 mg kg^{-1} treatments (2-3 months) yielded the highest percentage. For the second highest component, (*E*)-isovelencenol, 10 mg Pb l^{-1} treatments (5 weeks) yielded the highest percentage while in the pot study, the highest percentage was produced in 500 mg kg^{-1} treatment (2 months).

Previous studies have suggested that oil yield and composition in vetiver could be influenced by several factors such as geographical origin, cultivar, cultivation methods, the presence of microorganisms and environmental parameters, such as temperature. A higher oil yield was obtained by cultivation in normal soil with added microbes, while vetiver oil from semi-hydroponic cultivation, which contained no fertilizer and no effective microbes, gave the lowest yield (Pripdeevech *et al.,* 2006). Similarly, Adams *et al*. (2004) also showed that tissue cultured (cleansed) vetiver plants grown in sterilized soil produced lower oil yield than normal vetiver grown in unsterilized soil. Furthermore, it has been shown that soil fertilization increased oil yields dramatically without changing the composition (Dethier *et al.*, 1997; Adams *et al*., 2003). In addition, vetiver oil production is closely related to plant metabolism that is affected by temperature (Massardo *et al.,* 2006). Low temperature (such as in winter) causes a decrease in plant metabolic activities and hence a decrease in oil production (Massardo *et al.,* 2006).

From this study, the oil yield ranged from 0.42-1.29%. The highest oil yields were found in plants grown in nutrient solution with 100 mg Pb l^{-1} for 5 weeks (1.29%) and 7 weeks (1.22%). Lavania (2003) reported that vetiver roots may give a yield of about 0.3-2.0% essential oil on a fresh weight basis. The total constituents of vetiver oil were in the range of 47-143 compounds, the highest number found in plants grown in soil spiked with 1000 mg Pb kg^{-1} (143 compounds), followed by those grown in solution with 100 mg Pb l^{-1} (129 compounds). Massardo *et al*. (2006) explained the increase in oil yield as the result of warm temperature was due to the symbiotic intracellular bacteria associated with oil-producing gland cells in vetiver roots. These bacteria were thought to be involved in essential oil metabolism in vetiver (Adams *et al.,* 2004; Massardo *et al.,* 2006; Pripdeevech *et al*., 2006).

A typical analysis of vetiver oil indicates three major fractions: the lower boiling or sesquiterpene hydrocarbon fraction, the intermediate fraction containing the bulk of oxygenated derivatives, and the third fraction represented by khusimol fraction, which contains the main alcohol component in the vetiver oil (Lemberg and Hale, 1978; Massardo *et al.,* 2006). Furthermore, these major constituents are responsible for the base note of vetiver oil (Weyerstahl *et al.,* 2000). From our results, the largest component was khusimol (10.7-18.1%) followed by (*E*)-isovalencenol (10.3-15.6%). The smaller compounds were vetiselinenol (2.7-9.3%), α-vetivone (1.64-4.9%) and β-vetivone (1.7-4.0%). These main components of vetiver oil in the present study are somewhat similar to the previous studies (Adams *et al*., 2003; Massardo *et al*., 2006). The quality of vetiver grass or volatile oil usually depends on the amounts of alcohols (mainly khusimol) which are mainly responsible for the desired woody odor of vetiver (Arctander, 1960).

Our results demonstrate that lead can increase the oil production of vetiver. By planting vetiver grass in a lead mine area, it could serve the dual purpose of stabilizing the mine site and at the same time producing oil with a high commercial value. More research is needed to investigate the soil lead concentration and methods of soil amendment that will promote the highest yield of vetiver oil by further pot and field trial experiments.

Acknowledgments

This research was supported by grants from the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program; the Center on Environmental Health, Toxicology and Management of Toxic Chemicals under science and technology post graduate education and research development office (PERDO) of the Ministry of Education. We thank Drs Ian Woodrow and Drew King from the School of Botany, University of Melbourne for their helpful suggestions on the use of GC and GC-MS and Dr. Joe Brophy, University of New South Wales, for the information on vetiver oil.

References

Adams, R.P.: Idenificaiton of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Allured Publishing Corp, Carol Stream (2001).

Adams, R.P., R.N. Pandey, M.R. Dafforn and S.A. James: Vetiver DNA-fingerprinted cultivars: Effects of environment on growth, oil yields and composition. *J. Essent. Oil Res.,* **15,** 363-371 (2003).

Adams, R.P., M. Habte, S. Park and M.R. Dafforn: Preliminary comparison of vetiver root essential oils from cleansed (bacteria-and fungus-free) versus non-cleansed (normal) vetiver plants. *Biochem. Syst. Ecol.,* **32**, 1137-1144 (2004).

Anderson, N.H.: Biogenetic implications of the antipodal sesquiterpenes of vetiver oil. *Phytochem*., **9**, 145-151 (1970).

APHA, AWWA and WEF: Standard methods for the examination of water and wastewater. American Public Health Association, Washington D.C. (1998).

Arctander, S.: Perfume and flavor materials of natural origin. Arctander, Elizabeth (1960).

Champagnat, P., G. Figueredo and J.C. Chalchat: A study on the composition of commercial *Vetiveria zizanioides* oils from different geographical origins. *J. Essent. Oil Res.,* **18**, 416-422 (2006).

Chantachon, S., M. Kruatrachue, P. Pokethitiyook, E.S. Upatham, S. Tantanasarit and V. Soonthornsarathool: Phytoextraction and accumulation of lead from contaminated soil by vetiver grass : Laboratory and simulated field study. *Water Air Soil Pollut.,* **154**, 37-55 (2004).

Chen, Y., Z. Shen and X. Li: the use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. *Appl. Geochem.,* **19**, 1553-1565 (2004).

Chomchalow, N.: Manual of the international training course on the vetiver system. Office of the Royal Development Project Board, Bangkok (2000).

Dethier, M., S. Sakubu, A. Ciza, Y. Cordier, C. Menut and G. Lamaty: Aromatic plants of tropical central Africa. XXVIII. Influence of cultural treatment and harvest time on vetiver oil quality in Burundi. *J. Essent. Oil Res.,* **9**, 447-451 (1997).

Hoagland, D.R. and D.I. Arnold: The water culture method of growing plants without soil. *Calif. Agric. Expt. Stn. Circ*., **347**, 1-39 (1950).

ICARDA: Soil and plant analysis laboratory manual. ICARDA and NARC, Syria (2001).

Kabata-Pendias, A. and H. Pendias: Trace metals in soils and plants. CRC Press, Boca Raton (1992).

Lavania, U.C.: Other uses, and utilization of vetiver: vetiver oil. *In*: Proceedings of the 3rd International Conference on Vetiver, Guangzhou, China (2003).

Lemberg, S. and R.B. Hale: Vetiver oils of different geographical origins. *Perfum. Flavor.,* **3**, 23-27 (1978).

Maffei, M.: Introduction to the genus *Vetiveria*. *In*: *Vetiveria*. The Genus *Vetiveria* (*Ed*.: M. Maffei). Taylor and Francis, London. pp. 1-18 (2002).

Marshall, J.A. and N.H. Anderson: The structure of a-vetivone (isonootkatone). *Tetrahedron Lett.,* **17**, 1611-1615 (1967).

Marshall, J.A. and P.C. Johnson: Structure and synthesis of b-vetivone. *J. Org. Chem.,* **35**, 192-196 (1970).

Martinez, J., P.T.V. Rosa, C. Menut, A. Leydet, P. Brat, D. Pallet and M.A.A. Meireles: Valorization of Brazilian vetiver [*Vetiveria zizanioides* (L.) Nash ex Small] oil. *J. Agric. Food Chem.,* **52**, 6578-6587 (2004).

Massardo, D., F. Senatore, P. Alifano, L.D. Giudice and P. Pontieri: Vetiver oil production correlates with early root growth. *Biochem. Syst. Ecol.,* **34**, 376-382 (2006).

Nigam, I.C. and H. Komae: Essential oils and their constituents XXXVIII, isolation and structure of khusemol, a new sesquiterpenic primary alcohol from oil of vetiver. *J. Pharm. Sci.,* **57**, 1029-1030 (1968).

Pripdeevech, P., S. Wongpornchai and A. Promsiri: Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions. *Molecules,* **11**, 817-826 (2006).

Rotkittikhun, P., R. Chaiyarat, M. Kruatrachue, P. Pokethitiyook and A.J.M. Baker: Growth and lead accumulation by the grasses *Vetiveria zizanioides* and *Thysanolaena maxima* in lead-contaminated soil amended with pig manure and fertilizer: A glasshouse study. *Chemosphere,* **66**, 45-53 (2007).

Weyerstahl, P., H. Marschall and U. Splittgerber: New sesquiterpenes ethers from vetiver oil. *Liebigs Ann.,* **7**, 1195-1199 (1996).

Weyerstahl, P., H. Marschall, U. Splittgerber, D. Wolf and H. Surburg: Constitiuents of Haitian vetiver oil. *Flavour Frag. J.,* **15**, 391-394 (2000).

Wong, M.H.: Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere,* **50**, 775-780 (2003).

Zheljazkov, V.D., L.E. Craker and B. Xing: Effects of Cd, Pb and Cu on growth and essential oil contents in dill, peppermint and basil. *Environ. Exp. Bot.,* **58**, 9-16 (2006).

Zhu, B.C.R., G. Henderson, F. Chen, H.X. Fei and R.A. Laine: Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. *J. Chem. Ecol.*, **27**, 1617-1625 (2001).