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Toxic effects of two acid sulfate soils from the Dabaoshan Mine on *Corymbia citriodora var.variegata* and *Daphnia carinata*

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ABSTRACT

Acidic, metal-stressed conditions encountered in the acid sulfate soils significantly inhibited the growth of *Corymbia citriodora var.variegata*, possibly due to the reduced rate of photosynthesis and plant root activity. However, the plant's self-protection mechanism to counteract stress-induced cellular damage by reactive oxygen species still functioned well even at a soil pH as low as 2.81. This may explain the high tolerance of this plant species to the extremely acidic environments. The observed phytotoxicity symptoms were not accompanied by elevated concentrations of heavy metals in the plant tissues, suggesting that heavy metal levels in plant tissue alone are not valid indications of phytotoxicity to the tested plant species. Leachates from the acid sulfate soils had strong toxicity to *Daphnia carinata*. Median lethal dilution factor (LDF50) was much higher for the leachate from the highly acidic acid sulfate soils (ASS) than that from the mildly acidic ASS. Although the concentration of various metals markedly decreased with increasing number of leaching cycle, leachate toxicity to *Daphnia carinata* did not decrease accordingly. This suggests that levels of heavy metals and Al in the leachate are not good indicators of the mine water biotoxicity.

1. Introduction

Acid sulfate soils (ASS) are soils that contain oxidized metal sulfides (mainly pyrite). ASS are widely distributed in coastal floodplains [1,2], as well as in mine sites [3–6]. Oxidation of sulfide minerals causes production of sulfuric acid, which mobilizes metals of potential toxicity. ASS-derived toxic substances could have two aspects of adverse impacts on the ecosystems: (1) soil degradation which inhibits plant growth or even destroys vegetation and leaves the land surface bare [7–9]; and (2) acid drainage which contaminates downstream aquatic environments [10–12].

Acid sulfate soils contain multiple toxic substances which may interact to produce adverse effects on the receiving environments. The combined toxicity of multiple toxicants on a given organism could be less than, equal to, or greater than that expected from the sum of single toxicity of the individual chemicals, i.e. so-called "antagonistic", "additive" or "synergistic", respectively [13]. Therefore, understanding the chemical characteristics of an ASS is not adequate for assessing or predicting impacts of ASS on ecosystems. An ecotoxicological approach is desirable because it allows a direct link to be established between ASS-borne contaminants and biological response. Yet there has been little work conducted to study the ecotoxicology of ASS. In this paper, we examine the ecotoxic effects of two mine site ASS on a terrestrial plant *Corymbia citriodora var.variegata*, as well as the toxicity of ASS discharge water to *Daphnia carinata*, a microinvertebrate that plays a central role in the aquatic food chain.

2. Materials and methods

2.1. The study site

Dabaoshan Mine, an unusual polymetallic ore deposit district, is located in the northern Guangdong Province, South China (24°31′37″N; 113°42′49″E). Underground mining of copper ores dates back at least to the Song Dynasty (about 1000 years ago). Since the 1970s, large-scale surface mining of iron ore has been in operation while smaller scale underground mining of copper, zinc and lead ores took place simultaneously. As a result of these mining activities, especially the involvement of illegal mining in recent decades, large amounts of sulfidic waste rocks/soils are left on the surface and thus are subject to rapid oxidation. Surface soils in this area have been acidified to varying degree which impedes colonization of vegetation, resulting in extensively barren land. In addition, acid mine drainage from the Dabaoshan Mine has caused severe water degradation in the receiving stream [12,14].

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Table 1
Major chemical characteristics of the two mine soils of different acidity (MS1(C) and MS2(C) are neutralized MS1 and MS2 and serve as the control of MS1 and MS2, respectively; the subscript "s" and "am" following a metal denote "water-extractable" and "ammonium chloride-extractable").

Parameter	MS1	MS1(C)	MS2	MS2(C)
pH	3.47 ± 0.03	6.80 ± 0.12	2.81 ± 0.05	7.53 ± 0.13
EC (dS/m)	1.986 ± 0.003	1.891 ± 0.075	2.980 ± 0.073	2.764 ± 0.103
Cu_w (mg/kg)	0.29 ± 0.06	0.18 ± 0.03	16.45 ± 0.01	0.16 ± 0.01
Zn_w (mg/kg)	0.46 ± 0.03	0.11 ± 0.01	20.80 ± 0.01	0.07 ± 0.01
Pb_w (mg/kg)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cd_w (mg/kg)	0.02 ± 0.01	0.00 ± 0.00	0.23 ± 0.00	0.00 ± 0.00
Fe_w (mg/kg)	0.19 ± 0.05	0.05 ± 0.03	0.82 ± 0.00	0.00 ± 0.00
Mn_w (mg/kg)	1.41 ± 0.07	0.31 ± 0.06	118.32 ± 0.06	0.16 ± 0.06
Ni_w (mg/kg)	0.09 ± 0.01	0.12 ± 0.02	0.25 ± 0.01	0.06 ± 0.01
Cu_{am} (mg/kg)	2.02 ± 0.25	0.51 ± 0.05	28.40 ± 0.02	0.40 ± 0.02
Zn_{am} (mg/kg)	1.47 ± 0.20	0.15 ± 0.03	22.88 ± 0.01	0.05 ± 0.01
Pb_{am} (mg/kg)	0.42 ± 0.03	0.00 ± 0.00	9.62 ± 0.03	0.14 ± 0.03
Cd_{am} (mg/kg)	0.05 ± 0.01	0.11 ± 0.02	0.32 ± 0.00	0.01 ± 0.00
Fe_{am} (mg/kg)	0.02 ± 0.00	0.00 ± 0.00	1.63 ± 0.04	0.72 ± 0.04
Mn_{am} (mg/kg)	2.46 ± 0.22	0.80 ± 0.02	94.47 ± 0.04	0.83 ± 0.04
Ni_{am} (mg/kg)	0.16 ± 0.01	0.13 ± 0.03	0.43 ± 0.01	0.07 ± 0.01

2.2. The mine soils

Two soil samples were collected from two different locations representing a mildly acidic and a highly acidic mine site ASS. The mildly acidic ASS (MS1) had a pH of 3.48 (measured using a 1:5 (soil:water) extract) and the highly acidic ASS (MS2) had a pH of 2.81 (measured using a 1:5 (soil:water) extract). For comparison purposes, a sub-sample of each soil was treated with acid-neutralizing agent to bring the pH of the soil to nearly neutral and used as a control. This was done by mixing the ASS with bauxite refinery residue at a ratio of 100:1 (soil:neutralizing agent) for soil sample MS1 and at a ratio of 20:1 (soil:neutralizing agent) for soil sample MS2. These amended soils are labeled as MS1(C) and MS2(C), respectively. Since the soils were used as growth media for the assessment of their toxic effects on the plant, plant nutrients were applied to each soil at a rate of 10 g phosphorus fertilizer plus 20 g compound fertilizer per kg soil. The two ASS and their amended counterparts were incubated at a soil moisture content equivalent to field capacity in a green house for 1 month before the commencement of the growth experiment. Soil samples were collected immediately prior to seedling transplanting for analysis of some major chemical parameters and the analytical results are given in Table 1. In addition, an appropriate amount of incubated soil for all two original ASS and two corresponding amended ASS was oven-dried at 60 °C and stored in a sealed plastic bag prior to a column leaching experiment.

2.3. Plant growth experiment

A tree species (*C. citriodora var.variegata*) was selected for the growth experiment. Our previous experiment suggested that this plant species can be potentially used as a pioneer plant for re-vegetation of the study area. For each of the above untreated and treated ASS, approximately 2 kg of the relevant incubated soil were filled into a plastic pot, followed by transplanting of a tree seedling (about 10 cm high). Each pot was placed on a holder maintaining a layer (about 1.5 cm thick) of water to sub-irrigate the soil. The pot trial was in five replicates. The growth experiment commenced on June 25, 2006 and the plants were harvested on September 29, 2006.

Plant height was measured and number of leaves was counted on the following dates: June 27, July 25, August 14, September 5 and September 26. At harvest, dry biomass of leaf, trunk and root was determined. Fresh leaf samples were also collected for determinations of chlorophyll, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). Fresh root materials were also sampled for determination of root activity. In addition, Cu, Pb, Zn and Cd in plant tissues of different organs were measured.

2.4. Column leaching experiment

A column leaching experiment was conducted. For each of the untreated and treated ASS, 50 g of soil material was filled into a plastic column with an inner diameter of 30 mm after filling 12 g of fine quartz sands on the bottom of the column. On the top of the soil column, 25 g of fine quartz sands were added to avoid direct disturbance of the soil when adding water into the column. A double layer of nylon filter fabric was placed on the bottom of the column.

Prior to leaching, the soil in the column was wet by capillary action to field capacity. This was done by placing the bottom of the column in deionized water. The soil was then leached with 150 mL of deionized water at a flow rate of 12.5 mL/h (it took about 12 h to complete each leaching cycle). Leachate was collected from the bottom of the column and stored in a capped plastic bottle before chemical analysis. If the samples were not analyzed on the same day of collection, they were stored in a fridge before they were analyzed.

Column leaching was performed intermittently with an interval of 7 days. A total of 16 leaching cycles were completed for this study. The leachates were used for determinations of pH, electrical conductivity (EC), SO_4, Fe, K, Na, Ca, Mg, Mn, Zn, Cd, Cu, Pb and Al. Biotoxicity test was also conducted using *Daphnia carinata* as a test organism.

2.5. Analytical methods

2.5.1. Plant tissue analysis

Chlorophyll-a and chlorophyll-b were measured by spectrometric method [15]. SOD was determined by NBT method

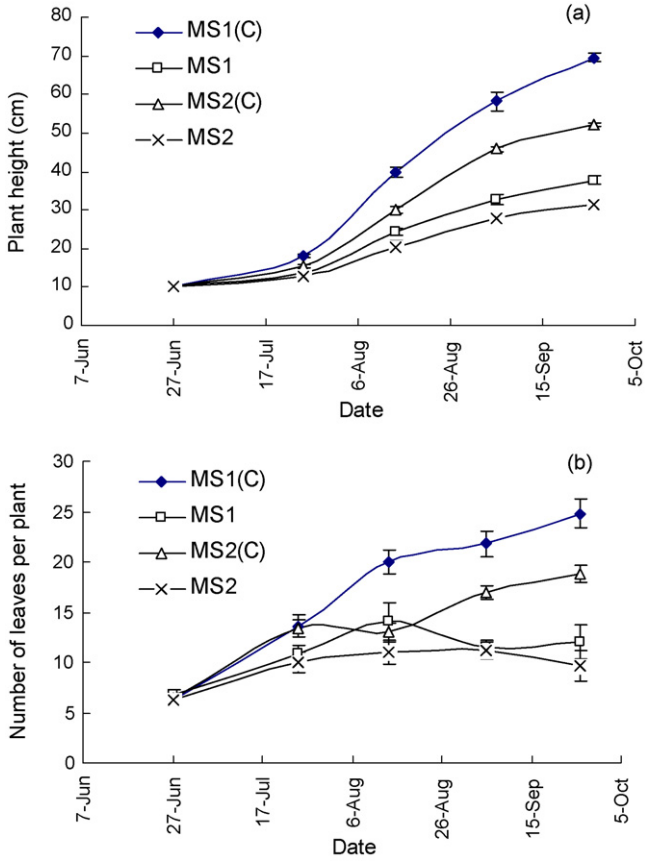

Fig. 1. Change in (a) plant height and (b) number of leaves per plant for the controls and treatments during the period of plant growth experiment.

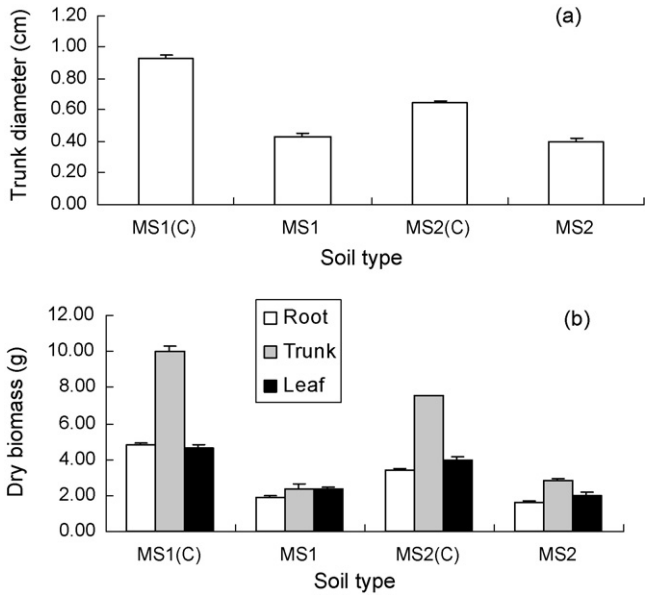

Fig. 2. Comparison of (a) trunk diameter and (b) biomass of various plant organs among the different treatments.

[16]. POD and CAT were determined following the methods of Chance and Maehly [17]. Root activity was determined using TTC method [18]. Heavy metals contained in the plant tissues were determined following the method described in Long et al. [19].

2.5.2. Water sample analysis

pH and EC were measured using a calibrated pH meter and EC meter, respectively. SO_4 concentration was determined turbidimetrically [20]. Fe, K, Na, Ca, Mg, Mn, Zn, Cd, Cu and Pb concentrations were determined using a graphite atomic absorption spectrometer (AAS). Al was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

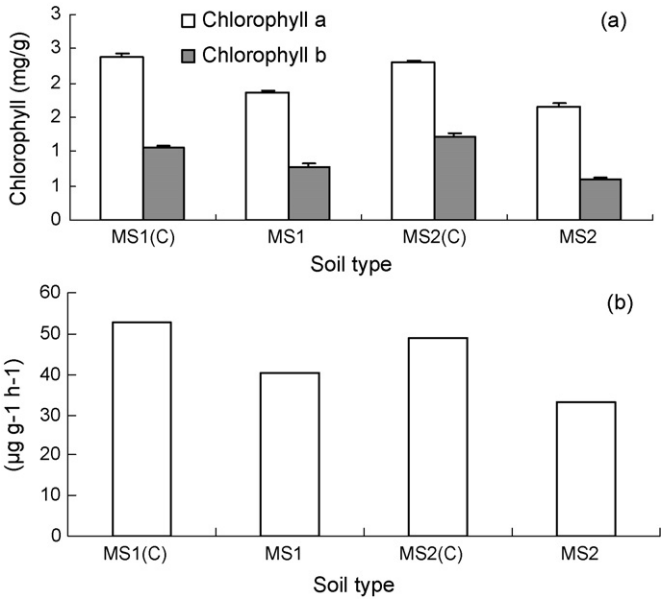

Fig. 3. Comparison of (a) chlorophyll and (b) root activity among the plants growing in the different soil types.

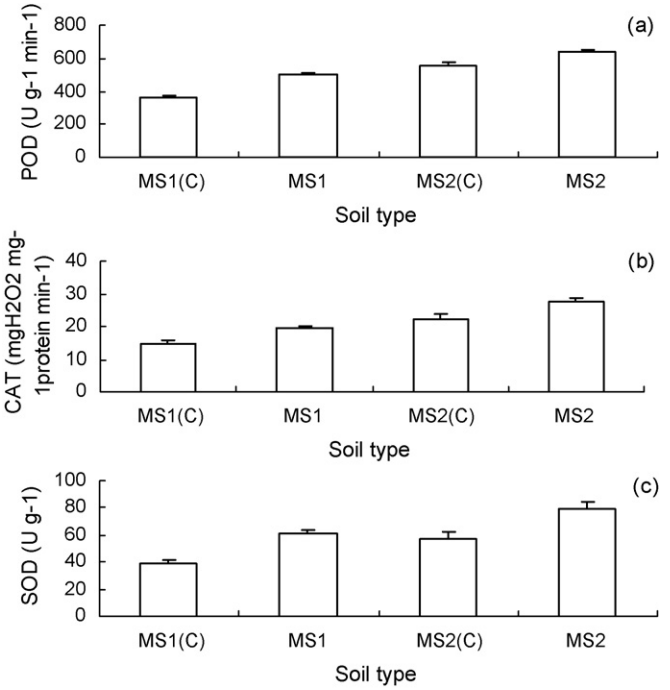

Fig. 4. Comparison of (a) POD, (b) CAT and (c) SOD among the plants growing in the different soil types.

2.5.3. Aquatic toxicity testing

Aquatic toxicity assessment was made using *Daphnia carinata* as a test organism [12,13]. Prior to tests, neonates (2-day-old) from the individual cultures were transferred from the individual culture into a beaker containing ISO standard water ($T_{ISO\ water}$) and allowed to swim for 15 min to be cleaned up. This procedure was repeated in the second and the third $T_{ISO\ water}$ (i.e. the animals were cleaned up with ISO standard water three times; each for 15 min). Ten cleaned animals were then placed in 100 mL test solution in 150 mL beakers. The animals were not fed during the experiment. The number of mobile animals was counted in each beaker. Those

Table 2
Concentration of various heavy metals in the different organs of plant growing in the treated and untreated ASS (mg kg^{-1}).

	Root	Trunk	Leaf
Cu			
MS1	27.43 ± 0.06a	10.29 ± 0.12a	8.76 ± 0.06a
MS1(C)	14.81 ± 0.26b	6.25 ± 0.05b	6.22 ± 0.09b
MS2	38.42 ± 0.09a	15.66 ± 0.29a	17.97 ± 0.15a
MS2(C)	23.77 ± 0.11b	9.03 ± 0.12b	9.56 ± 0.14b
Zn			
MS1	82.92 ± 0.93a	36.42 ± 0.47a	40.81 ± 0.47a
MS1(C)	53.74 ± 1.38b	26.79 ± 0.30b	36.50 ± 0.30b
MS2	77.47 ± 0.16a	43.50 ± 0.45a	64.94 ± 1.00a
MS2(C)	62.76 ± 0.86b	38.18 ± 0.90b	43.16 ± 0.66b
Pb			
MS1	5.98 ± 0.09a	2.47 ± 0.04a	3.49 ± 0.02a
MS1(C)	4.11 ± 0.05b	2.24 ± 0.01b	2.90 ± 0.09b
MS2	8.53 ± 0.10a	5.00 ± 0.02a	5.83 ± 0.05a
MS2(C)	7.16 ± 0.08b	4.09 ± 0.02b	4.72 ± 0.05b
Cd			
MS1	2.16 ± 0.04a	0.53 ± 0.01a	0.19 ± 0.01a
MS1(C)	0.94 ± 0.05b	0.25 ± 0.04b	0.13 ± 0.00b
MS2	1.87 ± 0.01a	0.56 ± 0.01a	0.20 ± 0.01a
MS2(C)	0.96 ± 0.01b	0.34 ± 0.02b	0.14 ± 0.00b

Means with different letters within the same column for the same heavy metal in each pair (untreated and treated) of the same soil type (mildly or highly acidic ASS) are significantly different at $P < 0.05$.

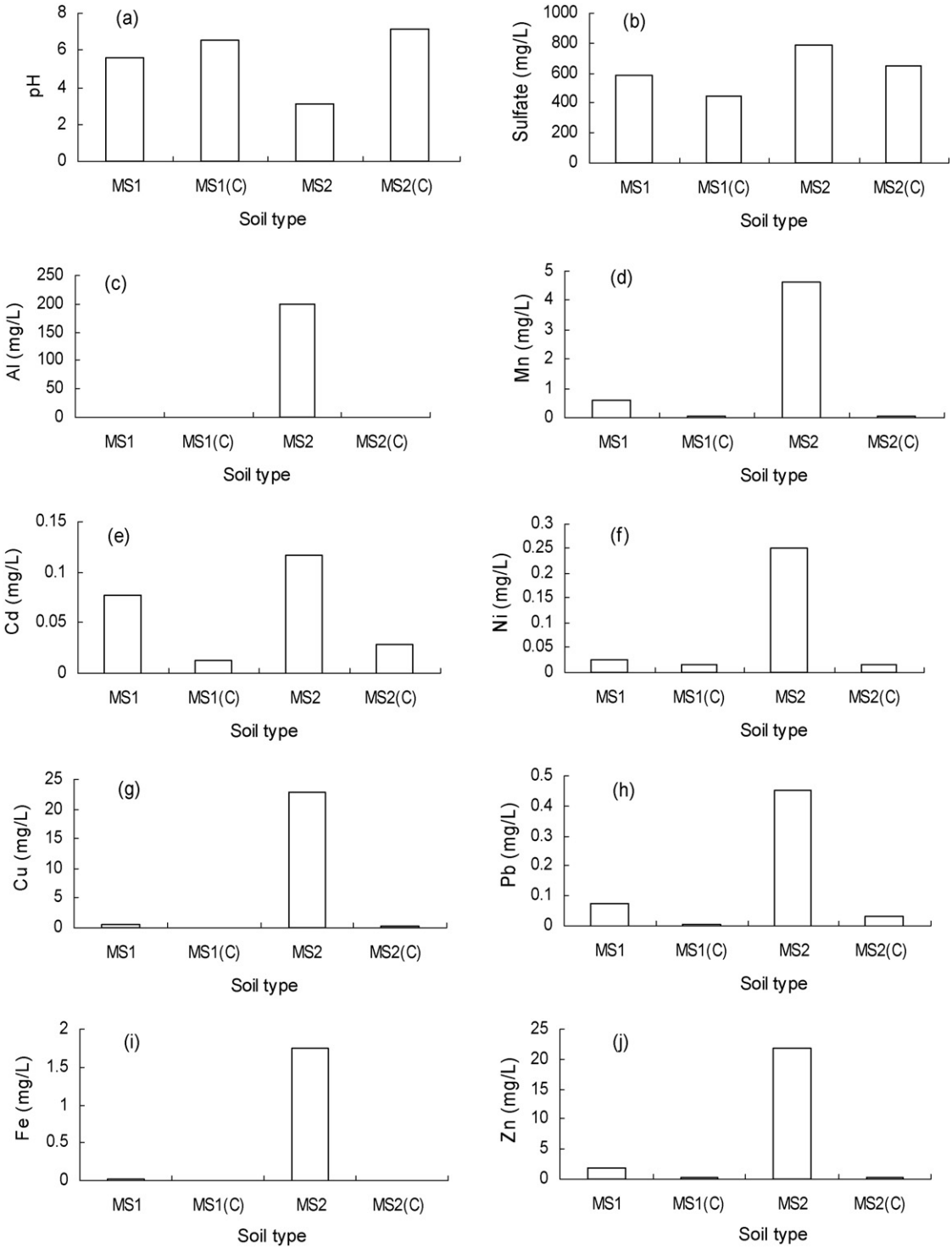

Fig. 5. Comparison of (a) pH, (b) sulfate, (c) Al, (d) Mn, (e) Cd, (f) Ni, (g) Cu, (h) Pb, (i) Fe and (j) Zn among the plants growing in the different soil types.

animals that did not move within 15 s following gentle agitation of the beakers were considered as dead. The number of dead animals was used to calculate the mortality percentage (MP). All treatments were in six replicates. For each leachate, a series of diluted solutions was made. The minimum and maximum dilution factors of the test solutions were determined based on the preliminary toxicity testing. After the animals were added in the test solutions, the number of mobile animals was counted at the 24th, 48th, 72nd and 96th hour. The median lethal dilution factor (LDF50) was then calculated using a probit analysis procedure [21]. In this article, only the 24-h LDF50 data are used for assessing the acute toxicity of the leachate to *Daphnia carinata*.

2.6. Statistical analysis

The statistical significance of difference between treatment means was determined by one-way analysis of variance (ANOVA).

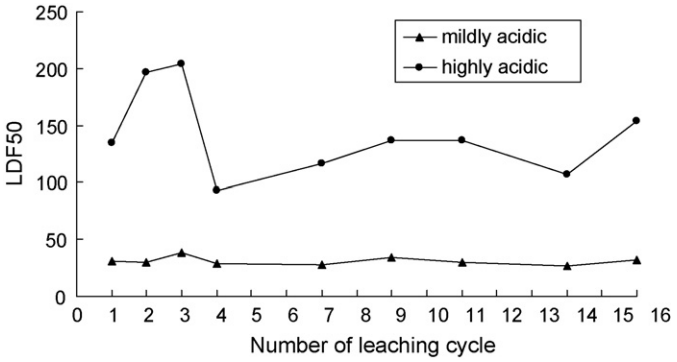

Fig. 6. Comparison of LDF50 of the test organism between the leachates derived from the mildly and highly acidic acid sulfate soils during the 16 leaching cycles.

3. Results

3.1. Pot experiment

3.1.1. Plant height and number of leaves per plant

For either mildly or highly acidic ASS, increase in plant height tended to be slower in the untreated, compared to that in the amended counterpart (the control). At harvest (3 months after transplanting), the height increase for MS1(C) (i.e. the control of MS1) and MS2(C) (i.e. the control of MS2) was 2.2 and 2.0 times as great as that for MS1 and MS2, respectively (Fig. 1a).

The leaf number of the tree seedlings growing in the amended soils tended to increase during the period of the experiment. However, for the tree seedlings growing in the untreated soils, the number of leaves per plant tended to decrease after initial increase following transplanting (Fig. 1b).

3.1.2. Trunk diameter and dry biomass

Comparison indicates that at harvest, trunk base diameter was statistically smaller ($P < 0.05$) in MS1 and MS2 than that in MS1(C) and MS2(C), respectively. Approximately, the trunk base diameter for MS1(C) and MS2(C) was 2 times and 1.5 times as great as that for MS1 and MS2, respectively (Fig. 2a).

Dry biomass of different plant organs are compared among the trees growing in various soils (Fig. 2b). For both the mildly or highly acidic ASS, there is a statistical difference in the dry biomass of each organ between the tree growing in the untreated soil and its amended counterpart (the control); the former was significantly smaller than the latter. In both soil types, plant trunk was the organ that had most significant difference in dry biomass between an untreated ASS and its amended counterpart (the control).

3.1.3. Physiological parameters

For both mildly or highly acidic ASS, concentration of chlorophyll-a or chlorophyll-b in the leaf of a tree growing in the untreated ASS was consistently lower, compared to that in the leaf of a tree growing in its treated counterpart (the control). For each of either mildly or highly acidic ASS, the ratio of chlorophyll-a to chlorophyll-b was greater in the untreated soil than in its treated counterpart (the control) (Fig. 3a).

Fig. 3b shows that for both cases of ASS, root activity was weaker in the untreated mine soil than in its treated counterpart (the control).

Levels of POD, CAT and SOD were consistently higher in the leaf of trees growing in the untreated soil than the control for both mine soils. Comparison also indicates that plants growing in the highly acidic ASS had higher levels of POD, CAT and SOD than the plants growing in the mildly acidic ASS (Fig. 4).

3.1.4. Heavy metals in plant tissues

Concentrations of Cu, Pb, Zn and Cd in the different organs of plant growing in the unneutralized ASS and their neutralized counterparts (controls) are given in Table 2. All of these data consistently show that the concentration of each heavy metal was significantly lower in the tissues of plant growing in the unneutralized soil than in its acid-neutralized counterpart (control).

3.2. Column leaching experiment

3.2.1. Leachate chemistry

Fig. 5 gives a comparison of chemical parameters in the first leachate between the untreated ASS and its treated counterpart for both mildly and highly acidic ASS. pH of MS1 was 5.58, compared to 6.53 for MS1(C); while pH of MS2 was 3.06, compared to 7.13 for MS2(C). For either mildly or highly acidic ASS, the concentration of sulfate and various metals in the leachate was consistently higher in an untreated soil than in its treated counterpart. The difference in metal concentrations between the untreated soil and its amended counterpart was particularly remarkable for the highly acidic ASS.

3.2.2. Toxic response of Daphnia carinata to the leachates

When the first leachate of the mildly acidic ASS was diluted with a factor of 13.3, the MP of the test organism was 100% within a 24 h

Table 3
Acute toxicity of the first leachate from the two acid sulfate soils to *Daphnia carinata*.

Soil type	Dilution factor	Contact time			
		24 h	48 h	72 h	96 h
Mildly acidic ASS	Control	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d	10.00 ± 0.00d
	178	8.00 ± 2.00d	18.00 ± 2.00d	18.00 ± 2.00c	20.00 ± 3.16c
	100	6.00 ± 2.45d	16.00 ± 2.45d	20.00 ± 3.16c	24.00 ± 2.45c
	31.3	40.00 ± 3.16c	56.00 ± 2.45c	80.00 ± 4.47b	92.00 ± 3.74b
	17.8	76.00 ± 2.45b	90.00 ± 3.16b	96.00 ± 2.45a	100.00 ± 0.00a
	13.3	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	10	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	LDF50	31.26	40.93	53.67	64.56
Highly acidic ASS	Control	0.00 ± 0.00f	0.00 ± 0.00e	0.00 ± 0.00d	10.00 ± 0.00d
	178	42.00 ± 3.74e	52.00 ± 2.00d	62.00 ± 2.00c	62.00 ± 2.00c
	100	70.00 ± 3.16d	72.00 ± 2.00c	78.00 ± 2.00b	78.00 ± 2.00b
	31.3	82.00 ± 3.74c	90.00 ± 4.47b	96.00 ± 2.45a	96.00 ± 2.45a
	17.8	92.00 ± 2.00b	96.00 ± 2.45ab	100.00 ± 0.00a	100.00 ± 0.00a
	13.3	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	10	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	LDF50	135.13	172.49	204.16	233.19

Means with different letters within the same column for each soil type (mildly or highly acidic ASS) are significantly different at $P < 0.05$.

contact time. When the dilution factor was increased to 31, the MP at 24, 48, 72 and 96 h was 40, 56, 80 and 92%, respectively. When the leachate was diluted 100 times, the MP was less than 25% even after the test organism was exposed to the solution for 96 h. The LDF50 for 24, 48, 72 and 96 h exposure was 31, 41, 54 and 64, respectively (Table 3).

Similar to the mildly acidic ASS, when the first leachate of the highly acidic ASS was diluted with a factor of 13.3, the MP of the test organism was 100% within the 24 h contact time. However, when the dilution factor was increased to 31, the MP at 24, 48, 72 and 96 h was 82, 90, 96 and 96%, respectively which was higher than that recorded during corresponding contact time periods for the first leachate of the mildly acidic ASS. Even when the leachate was diluted 100 times, the MP of *Daphnia carinata* was greater than 70% at 24 h exposure time. The LDF50 for 24, 48, 72 and 96 h exposure was 135, 172, 208 and 238, respectively (Table 3).

Changes in LDF50 for 24 h exposure with increasing number of leaching cycle for both soils are given in Fig. 6. It can be seen that the LDF50 was much higher for the leachates from the highly acidic ASS than from the mildly acidic ASS. The toxicity of the leachate to the test organism remained unchanged during the entire period of the experiment for the mildly acidic soil. However, for the highly acidic ASS, the LDF50 of leachate varied markedly during the experiment with the second and third leaching cycles having the highest LDF50 among the 16 leaching cycles.

In contrast with the untreated soils, the undiluted leachates of the treated soils (the controls) show no acute toxicity to the test organism (data not shown).

4. Discussion

The growth rate of the tree species, *C. citriodora var.variegata*, as indicated by plant height, number of leaves per plant, trunk base diameter and dry biomass, was lower for the untreated mildly acidic ASS, compared to its amended counterpart. This suggests that the presence of multiple heavy metals at a pH of 3.48 significantly inhibited the growth of the seedlings of this plant species. This can be partly attributed to the reduced root activities and rate of photosynthesis caused by acidic, heavy metal-stressed conditions. The environmental stresses on the plant are also well reflected by a significant increase in the antioxidant enzyme activities. POD, CAT and SOD have been recognized as major enzymes that jointly act to protect cells from oxidative damage caused by reactive oxygen species during plant stress response [22,23].

Increases in soil acidity and concentrations of labile metal forms, as experienced in the highly acidic ASS (MS2) resulted in further impeded growth of the plants. However, even at an initial pH as low as 2.81, the seedlings of tree species *C. citriodora var.variegata* could still survive at least for more than 3 months although growth of the plant was significantly inhibited. The further enhanced antioxidant enzyme activities in the leaf of the trees growing in the highly acidic ASS suggest that the plant's ability to generate these self-defending materials was not negatively affected even at extremely acidic environments. This may explain the relatively high tolerance of *C. citriodora var.variegata* to the mine site ASS conditions during the period of this experiment. However, long-term trials are needed to answer the question whether the seedlings of *C. citriodora var.variegata* can eventually overcome such acidic stresses before they become more acid-tolerant with increasing plant maturity.

It is interesting to note that the levels of heavy metals in the tissues of healthy plant growing in the acid-neutralized soils were significantly higher than that in the plant with phytotoxicity symptoms in the unneutralized counterparts. This suggests that the quantity of a heavy metal accumulated in the plant tissues alone is not a valid indication of phytotoxicity.

For the mildly acidic ASS, the maintenance of LDF50 at a similar level during the entire period of 16 leaching cycles suggest that the toxicity of the leachate to *Daphnia carinata* had non-significant change despite the concentration of heavy metals decreasing with increasing number of leaching cycle (data not shown). The stronger response of *Daphnia carinata* to the leachate from the highly acidic ASS, relative to that from the mildly acidic ASS generally conforms the expected results. The biotoxicity of highly acidic ASS leachate was about six times as high as that of the mildly acidic ASS leachate. However, the acidity and concentration of potentially toxic metals in the highly acidic ASS leachate were frequently over 10 times as high as those in the mildly acidic ASS leachate. Furthermore, the 3rd and 2nd leachates of the highly acidic ASS had the 1st and 2nd highest toxicity to the test organism. However, the concentration of various heavy metals and Al was much lower in these leachates than in the first leachate (data not shown). These results suggest that the combined toxicity of mine water may not be simply indicated by the levels of potentially toxic metals contained in the mine water.

5. Conclusion

The mildly acidic ASS had significantly toxic effects on the tested tree *C. citriodora var.variegata*, which experienced retarded growth. This may be attributed to the reduced rate of photosynthesis and plant root activity caused by acidic, heavy metal-stressed conditions. However, the phytotoxicity symptoms were not directly linked to the levels of heavy metals accumulated in the plant tissues, as compared to that in the healthy plant growing in its counterpart (the acid-neutralized mildly acidic ASS). Increased acidity and levels of labile metals led to more severe growth retardation of *C. citriodora var.variegata*. However, the plant's self-protection mechanism to counteract stress-induced cellular damage by reactive oxygen species still functioned well. This may explain the high tolerance of *C. citriodora var.variegata* to the extremely acidic conditions during the period of this experiment. However, long-term trials are needed to answer the question whether the seedlings of *C. citriodora var.variegata* can eventually overcome such acidic stresses before they become more acid-tolerant with increasing plant maturity.

Leachates from the ASS had strong toxicity to *Daphnia carinata*. LDF50 was much higher for the leachates from the highly acidic ASS than from the mildly acidic ASS. During the 16 leaching cycle performed in this study, there is no clear trend that the toxicity of the leachate to *Daphnia carinata* decreased with increasing number of leaching for the mildly acidic ASS; for the highly acidic ASS, the strongest toxic response of *Daphnia carinata* appeared in the 3rd leachate, followed by the 2nd leachate. However, both the 3rd and the 2nd leachates contained much lower concentrations of heavy metals and Al than the 1st leachate, suggesting that levels of heavy metals and Al in the leachate are not good indicators of the mine water toxicity under the experimental conditions set in this study.

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