

Organic Acid Characteristics and Tolerance of *Sengon (Paraserianthes falcataria L Nielsen)* to Lead

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Abstract

This study aimed to find out the lead tolerance of *sengon (Paraserianthes falcataria)* seedling based on growth performance, tolerance index, and secretion and accumulation of organic acids content. Seedlings were exposed to lead (Pb) with the concentration of 0, 0.5, 1, 1.5, 5, and 10 mM in liquid nutrient culture for 4 days in order to investigate secretion and accumulation of oxalic, malic, and citric content, and for 15 days to examine growth performance and tolerance index. The result showed that tolerance index and growth performance of *sengon* seedling were insignificant ($p > 0.05$) to the rising of Pb concentration up to 1.5 mM with tolerance index at least 95%, and even caused an increase of fresh weight. However, the tolerance index and growth of *sengon* decreased significantly due to Pb exposure of 5 and 10 mM. Among the three organic acids, citrate was most dominant as compared to malate and oxalate. Secretion of citrate increased significantly ($p < 0.05$) with the rising concentration of Pb 0.5, 1 and 1.5 mM, reaching to 0.464, 0.540, and 0.587 $\mu\text{g mL}^{-1}$, respectively, or rising according linear line ($r = 0.9$, $p < 0.5$). Citrate accumulation showed inconsistent pattern with the rising Pb exposure. The result suggested that *sengon* seedling have a slightly tolerance to lead by secretion of organic acid especially citric acid.

Keywords: lead, *sengon*, tolerance, organic acid, liquid nutrient culture

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Introduction

Gold mine tailings are known to have extreme physical and chemical properties for plant growth (Arunachalam *et al.* 2004). In addition to the structure of dust and sand-dominated, as well as low nutrient content of the macro, the tailings were often found to contain some heavy metals such as lead (Pb) in the amount that can interfere life. The rest of the gold mining tailings in Pongkor, for example, the texture was sand-dominated (65%) with only 3% clay, organic matter content almost zero (0.24%), high pH (8), heavy metals, and total dissolved Pb tends to be high (17.3 ppm and 114 ppm). Such tailings properties potentially decrease soil fertility and cause toxicity to plants, making it difficult for plants to grow, and even tend to die. Previous research notes that tailings disrupt seedling growth of forest plants such as reduced seedling growth of *Gmelina arborea* up to 15–43% (Wasis & Fathia 2011). Even tailing treatment caused the death of up to 33% of *Melina azedarach*, and the number of plants that grow depressed more than 50% (Setyaningsih 2007).

Revegetation of tailing would require specific plants that can grow in these extreme conditions. Moreover, the presence of heavy metal contamination in the tailings, include Pb, may caused plant is not just being able to adapt and grow, but also capable of contaminant remediation activities. Hence, an attempt to get the plant for revegetation that known to have tolerance and adaptation mechanisms and potential remediation is necessary. The results of previous studies indicate that *sengon* known as one of pioneer plant species and has a relatively high tolerance to tailings media, based on the observed differences in biomass of plants grown on tailings media than those grown in normal soil (Setyaningsih 2011). But Pb tolerance of *sengon*, especially seedling level as revegetation material, has not been revealed. The level of tolerance of plants to heavy metals stress can be determined from its growth performance. The roots of the plant is sensitive to the effects of heavy metal stress and will be stunted, as well as the growth of the canopy when experiencing Pb toxicity (Liu *et al.* 2009). Cowpea (*Vigna unguiculata*) (Kopittke *et al.* 2007) and *Thespesia*

populnea L (Kabir *et al.* 2009) were reported severely its growth with Pb exposure below 25 μmol .

Plants tolerance of heavy metals stress correlate with its ability to physiology adaptation. There are several strategies that plants have to face the stress of heavy metals, which can generally be classified into 2 main categories, namely restriction of metal uptake (Reichman 2002) otherwise known as an external strategy (Taylor 1991), detoxification of heavy metals (Yang *et al.* 2005), or internal strategies (Reichman 2002). The first strategy is kept the metal ions are toxic in low concentrations in the cytoplasm by preventing the transport of metal ions to penetrate the plasma membrane (Taylor 1991; Reichman 2002). Prevention of transport metals can occur through increasing in metal ion binding to the cell wall (Reichman 2002; Tong *et al.* 2004; Yang *et al.* 2005), reduction of the absorption by modifying the ion channel (Tong *et al.* 2004; Yang *et al.* 2005), pumping out the cell using a metal ion efflux pump active (Taylor 1991; Reichman 2002; Tong *et al.* 2004; Yang *et al.* 2005), maintenance of the proportion of uneven metal distribution in roots and canopy, which is more abundant in roots (Reichman 2002), and exudation of organic acids as chelating to form metal complexes thus eliminating the toxicity and/or reduces the availability (Reichman 2002). Second strategy is to detoxify heavy metal ions that enter the cytoplasm by way of chelating or converting toxic ions into less toxic or into a more manageable (Yang *et al.* 2005) through activation of an organic acid, phytochelatin, metallothionein and/or organic and inorganic ligands to form metal-ligand complexes (Reichman 2002), and compartmentation a metal ion to the apoplast like trichone and walls and the absorption of the metal-ligand complexes into the vacuole (Reichman 2002; Yang *et al.* 2005).

Each plant has a unique pattern of adaptation to heavy metal stress conditions. There is not sufficient information related to the heavy metals stress adaptation mechanisms of *sengon*, especially organic acids secretion and accumulation character of Pb exposed *sengon*. Therefore the effort to learn the capabilities and mechanisms of plant stress facing heavy metal Pb is very important to do. The study was conducted to determine the Pb tolerance level of *sengon* seedlings by observing the growth of roots and canopy and tolerance index, as well as to study the patterns of Pb stress resistance of *sengon* seedling by measuring the secretion and accumulation of oxalic acid, malic, and citric of *sengon* seedlings with lead exposure in nutrient culture condition.

Methods

Sengon seeds was obtained from the collection of Silviculture Laboratory of Faculty of Forestry, Bogor Agricultural University. Composition refers to the Sopandie nutrient solution (Sopandie 1990) with ingredients: distilled water, nutrient solution (1.5 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1.0 mM NH_4NO_3 , 1.0 mM KCl, 0.4 mM MgSO_4 , 1.0 mM KH_2PO_4 , 0.50 ppm MnSO_4 , 0.02 ppm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.05 ppm $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 ppm H_3BO_3 , 0.01 ppm $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 68 mM FeEDTA), $\text{Pb}(\text{NO}_3)_2$ (the source of lead), NaOH, HCl, liquid nitrogen, ethanol, ion-free water, and zeolite.

Sengon seedlings that have been aged 1 month on zeolite media was transferred into an aeration tube containing 1 ℓ nutrient culture as adaptation media to be grown for 4 days, and continued into the treatment culture media for 15 days in greenhouse conditions. Composition of adaptation nutrient was 1/5 dose of the Sopandie nutrient without Pb. Treatment culture media was 1/3 dose of nutrient solution Sopandie's exposed Pb (NO_3)₂ in 6 concentrations (Pb 0 mM, 0.5 mM, 1 mM, 1.5 mM, 5 mM, and 10 mM). Solution was maintained at pH 4.5–5 using NaOH and HCl. Seedling growth was observed by measuring the root length, shoot length and dried weight of seedlings when harvested, and counted the number of dead seedlings. Seedling tolerance (tolerance index, IT) is known from the comparison of average root length of experimental to control seedlings on percent (Rout *et al.* 1999).

Seeds were sterilized and germinated in aseptic conditions on agar medium (water agar). After sprouting, it was removed and grown in test tubes containing 50 ml of adaptation nutrient solution in sterile conditions for 2 \times 24 hours, and proceed to the treatment media for 4 \times 24 hours with shaking 80 rpm at temperature of 23 $^\circ\text{C}$. The ingredient of adaptation and treatment media were similar to that used in the tolerance test.

Organic acid secretion was measured the organic acid content in the culture medium. Samples of 150 ml was filtered by filter paper 40 and be continued with a milliphore filter 0.45 μm . The liquid distillate further evaporated at 40 $^\circ\text{C}$ until dry.

Meanwhile, the organic acids accumulation was measuring in the tissues of seedlings. The roots were dried with a paper straw and frozen in liquid nitrogen and grinded with a mortal. One gram grinded roots was centrifuged for 5 minutes at 10,000 rpm in 50 ml ethanol 95%. Supernatant was added ethanol and centrifuged 3–4 times repetitions. Evaporated solids dissolved in 50 ml of water with a pH 2 and passed (clean up) to the pre-conditioning solid phase extraction (SPE), and reevaporated at a temperature of 40 $^\circ\text{C}$. A total of 1 ml solids was injected into the HPLC (Hartley 1987) with VP-ODS column 250 $\ell \times$ 4.6. Mobile phase of 0.01 N H_2SO_4 flowed at 0.5 ml per minute at 32 $^\circ\text{C}$, and the peak detected by UV detector at a wavelength of 190 nm. Organic acid identification and quantification of samples were known by comparing retention time of components in standard solution (a mixture of citric, oxalic, and malic acid) and peak integration of curve standard in the chromatogram. The chromatogram of oxalic acid 0.0062 ppm was detected on peak time retention 13.354, area 108.064 and height 2195; the chromatogram malate acid 0.0062 ppm was detected on peak time retention 15.972, area 58514 and height 2143; the chromatogram citric acid 0.0062 ppm was detected on peak-time retention 6325, area 57.654 and height 2344.

The study was designed in randomized complete design for 6 treatments consisting of Pb concentration is P0 = Pb 0 mM, P1 = Pb 0.5 mM, P2 = Pb 1.0 mM, P3 = Pb 1.5 mm, P4 = Pb 5 mM, and P5 = Pb 10 mM. Each treatment was repeated 3 times. All data was processed using the software SPSS 16 and Microsoft Excel.

Results and Discussion

Growth and seedling tolerance index *sengon* During 15 days observation in nutrient culture exposed Pb (NO₂)₃, was showed that *sengon* seedling still be alive on Pb 1.5 mM concentration, and totally death within 1 week of Pb 5 and 10 mM exposure, with symptoms has begun to appear since the second day. Symptoms of poisoning the seedlings exposed Pb 5 and 10 mM began with chlorosis and necrosis (leaf yellowing and drying) on day 2, followed by the appearance of brown spots on day 3, most of the leaves curl on day 4, the leaves dry out on day 5, followed drying rod on the following days. There was also a difference in colour of seedling roots. Seedlings that not exposed to lead had brown or white transparent on secondary roots, while seedlings that were exposed to 0.5–1.5 mM Pb, their secondary roots tent to be white with a spot (about 1 cm) dark gray starting at the tip toward the base of the root. Seedlings with exposure to 5 and 10 mM Pb had dark colour on the entire root as a sign of death.

After 15 days of exposure to lead up to 1.5 mM, there was no significant difference ($p > 0.05$) in seedling root length of *sengon*, but significantly different on its shoot length, TI, and dry weight. *Sengon* seedling shoot length ranged between 8.42–10.25 cm, and were not significantly different, except when exposed to Pb 1.5 mM which have increased by 20%. TI of *sengon* decreased by a maximum of 5%, with a range of 95–101%, and dry weight were not changing consistently with the range of 0.19–0.33 g. (Table 1).

Sengon seedling death during exposure to lead 5 mM reinforce the notion that the concentration of lead in particular can be very toxic to the plant (Mengel & Kirkby 1987; Kopittke *et al.* 2007; Kabir *et al.* 2009). Symptoms of toxicity that begins with yellowing leaves, the emergence of brown spots, leaf rolling and leaf drying followed by drying rod on the following days with the colors black and gray roots, have also been reported in *Chlorophytum comosum* (Wang *et al.* 2011) and *Vigna unguiculata* (Kopittke *et al.* 2007). Chlorosis and necrosis occurs presumably because of damage to photosynthetic pigments as a result of decreased production of chlorophyll *a* and *b* as increased exposure to Pb reached 1,500 mg kg⁻¹ (Pandey *et al.* 2007; Wang *et al.* 2011). The decrease of chlorophyll production occurs as a result of

Mg ions takeover by heavy metal ions which would inhibit the synthesis of chlorophyll by inhibition of synthesis enzyme activity (Pflugmacher *et al.* 1999; Xiao *et al.* 2008; Wang *et al.* 2011). Roots damage is possible due to the rapid inhibition of root growth, because of the inhibition of cell division in the root tip with the immobilization of Pb ions in the cell wall (Jiang & Liu 2010). Roots become dark grayish, being short, fat, and loss of apical dominance as indicated by the increased number of secondary roots initiated per unit root length as occurred on *V. unguiculata* (Kopittke *et al.* 2007).

No significant changes in *sengon* seedling growth performance during lead exposure to Pb 1.5 mM (450 ppm) with TI was still above 95% indicates that the seedlings were still categorized lead tolerant. However, lead tolerance of *sengon* seedling could be limited, indicated by the total mortality of seedling when exposed to 5 mM Pb (1,500 ppm) within 5 days. *Sengon* seedling tolerance is still smaller than *Chlorophytum comosum* tolerance with TI 73% when gripped 1,500 mg Pb kg⁻¹ (1,500 ppm) for 90 days (Wang *et al.* 2011), as well as compared with *Accacia farnesiana*, known as Pb bioaccumulator which TI decreased only 8% when gripped Pb 1,000 mg l⁻¹ for 60 days (Amalia *et al.* 2011). Nevertheless, *sengon* tolerance is still better than *Jatropha curcas* cuttings were decreasing its TI to be less than 80% at Pb 0.5 mM and became only 39.2% at Pb 4 mM exposure (Shu *et al.* 2011), as well as compared with growth of cowpea (*Vigna unguiculata*) that has been severely hampered by Pb 1 μM exposure (Kopittke *et al.* 2007).

Content of organic acids Measurements of the organic acid content using HPLC showed that the 3 organic acids (oxalic, malic, and citric acid) were found both in the nutrient solution, which were used as the data secretion, and in the plant tissue which were then used as the data accumulated. Citric acid content was generally more than the oxalate and malate. *Sengon* seedling was secreting citrate 44 times more than the oxalate, and 41 times higher than malate (Table 2), and accumulate citrate 12 times more than the oxalate and 7 times more than the malate (Table 3).

There was significant changing to the 3 kinds of organic acids secreted by *sengon* seedlings exposed Pb. The increasing of Pb concentration up to 1 mM caused a

Table 1 Growth and tolerance index of *sengon* seedling in nutrient culture with Pb exposure for 15 days

Pb (mM)	Root length (cm)	Shoot length (cm)	Total dry weight (g)	Tolerance index/TI (%)
Pb 0	15.08 ± 2.16 a*	8.42 ± 0.56 a	0.22 ± 0.03 a	100.0 bc
Pb 0.5	15.00 ± 1.80 a	8.33 ± 0.38 a	0.19 ± 0.04 a	99.5 b
Pb 1	14.33 ± 3.06 a	8.71 ± 1.05 a	0.27 ± 0.01 b	95.0 a
Pb 1.5	15.33 ± 3.25 a	10.25 ± 2.1 b	0.33 ± 0.07 c	101.7 c
Pb 5	death	death	death	0
Pb 10	death	death	death	0

* mean ± standard deviation followed by the same letter in the same column indicates no significant difference in the error rate of 5% by DMRT test. The same or different letters in different columns do not show any relationship

significant increasing in the secretion of oxalate and malate, reached $0.018 \mu\text{l ml}^{-1}$ and $0.029 \mu\text{l ml}^{-1}$, respectively; but decreased significantly on Pb 1.5 mM exposure. Citric acid secretion increased significantly ($p < 0.5$) with an increase in Pb concentrations, reached $0.464 \mu\text{l ml}^{-1}$ on Pb 0.5 mM, $0.587 \mu\text{l ml}^{-1}$ on Pb 1.5 mM or increased more 3-fold than citric acid of seedling without Pb exposure (Pb 0 mM) (Table 2).

Sengon seedlings accumulated citric acid range 0.578 – $1.732 \mu\text{g g}^{-1}$. This was greater than the accumulation of oxalic acid which reach 0.106 – $0.148 \mu\text{g g}^{-1}$ and malate 0.110 – $0.268 \mu\text{g g}^{-1}$. The increasing of Pb did not cause significant changing to the accumulation of oxalate in *sengon* seedlings tissue. Citric acid accumulation showed inconsistent data, decreased on Pb 0.5 exposure ($1.506 \mu\text{g g}^{-1}$), increased on Pb 1 mM exposure ($1.732 \mu\text{g g}^{-1}$), and decreased again on Pb 1.5 mM exposure ($1.586 \mu\text{g g}^{-1}$) (Table 3).

Sengon seedling tolerance to Pb correlates with cellular metabolic mechanisms, include the production of organic acids secreted by roots or accumulated in the tissues. Organic acids are metabolic intermediates in the formation of ATP from carbohydrates in the metabolism of N when the balance of ion cells have changed, as a result of the metabolic abnormalities by stress (Burke *et al.* 1990). *Sengon* seedlings secrete or accumulate three kinds of organic acids, oxalic, malic, and citric, but the amount of citric acid has the potential to behave in a dominant sensitive to physiological

disturbances in *sengon*, including stress due to heavy metals. Sensitivity to citric acid in plants by the influence of the metal has also been reported in soybean plants (Kasim *et al.* 2001), maize (Pineros *et al.* 2002), and wheat (Delhaize 1993) which was stressed by Al. But, in some of the plants with Pb exposure, citric acid was found only in small quantities, such as rice on Pb 800 mg kg^{-1} (Liu 2009) or even not found as in canola, sunflower, and indiana mustard (Kwon 2009) which was exposed Pb 5 mg l^{-1} . This condition makes it clear that each plant in a state of the metal exposed has the specifications in producing organic acids, including *sengon* seedlings with Pb exposure producing more citric acid.

Addition of citric acid, the increasing of oxalic acid and malic secretion during *sengon* seedlings were exposed Pb 1 mM, and that were later dropped when exposed Pb 1.5 mM, it can be described that at a certain level of stress Pb seedlings adapted to produce specific organic acids as well. Such patterns have also been reported by Kim *et al.* (2009) that on vetiver grass, acetic acid secretion may continue rising to reach Pb 1.0 mg l^{-1} , but not so with lactic acid and pirufate that it secretion was only going to increase in expose Pb 0.5 mg l^{-1} . Sensitivity of certain organic acids to stress certain metals at certain concentration levels too, was strongly associated with a variety of organic compounds synthesized during the process of photosynthesis and translocate temporary to the roots of plants (Jones 1998).

Citric acid was produced dominantly by *sengon* seedling

Table 2 Secretion of oxalate, malate and citrate acids by *sengon* seedling exposure to lead for 4 days in nutrient culture

Pb (mM)	Secretion of organic acid content ($\mu\text{g ml}^{-1}$)											
	Oxalate			Malate			Citrate					
Pb 0	0.008	±	0.003	ab*	0.015	±	0.007	b	0.170	±	0.074	a
Pb 0.5	0.016	±	0.011	b	0.034	±	0.001	c	0.464	±	0.010	b
Pb 1	0.018	±	0.002	b	0.029	±	0.010	c	0.540	±	0.024	c
Pb 1.5	0.003	±	0.000	a	0.007	±	0.001	ab	0.587	±	0.024	c

* mean ± standard deviation followed by the same letter in the same column indicates no significant difference in the error rate of 5% by DMRT test.

The same or different letters in different columns do not show any relationship

Table 3 Accumulation of oxalate, malate and citrate acids on tissue of *sengon* seedling exposed to lead for 4 days in nutrient culture

Pb (mM)	Accumulation of organic acid content ($\mu\text{g g}^{-1}$)											
	Oxalate			Malate			Citrate					
Pb 0	0.138	±	0,045	a	0.245	±	0,012	a	1.731	±	0,049	b
Pb 0.5	0.106	±	0,032	a	0.264	±	0,049	a	1.506	±	0,013	a
Pb 1	0.140	±	0,024	a	0.231	±	0,005	a	1.732	±	0,203	b
Pb 1.5	0.117	±	0,028	a	0.268	±	0,046	a	1.586	±	0058	ab

* mean ± standard deviation followed by the same letter in the same column indicates no significant difference in the error rate of 5% by DMRT test.

The same or different letters in different columns do not show any relationship

found both accumulated in the tissue and is secreted in a nutrient solution, but it seems more likely secreted than accumulated on Pb exposure. This could be understood from the evident that citrate secretion increase significantly in any increasing of Pb concentration exposed, while its accumulation in tissue significantly decreased. Citric acid secretion showed increasing levels with increasing concentrations of lead exposure, even a 3-fold when exposed to 1.5 mM Pb, reaching 0.587 mg ml^{-1} (Table 2) with a linear upward trend of $y = 0.1327x + 0.1083$ ($R^2 = 0.837$, $r = 0.9$, $p < 0.5$) (Figure 1). Up to Pb 1.5 mM exposure, citrate secretion was increasing gradually by 150–250% higher than control, while decreasing its accumulation was only about 10–20% (Table 2). Decreasing in the content of citric acid accumulation in *sengon* seedling tissue in any increasing Pb exposed, it is understanding that the citric acid produced more concentrated for secretion.

These conditions have shown that *sengon* seedlings tried to avoid the metabolically active part from Pb access with external adaptation strategies through the secretion of citric acid. The metal is bound by organic acids that exudated roots, so the metal does not transport through the membrane and remain outside the roots (Kochian *et al.* 2004). Decreasing of citric acid accumulation in *sengon* seedling tissue with increasing Pb expose, presumably because the citric acid produced more concentrated for secreted. Increasing of citric acid secretion also occurred in sunflower seized $400 \mu\text{M Pb}$ up to 578.12 mg l^{-1} (Niu *et al.* 2010).

The increase of organic acids by increasing metals supply can be mean as a detoxification mechanism or, conversely, a metabolic disorder resulting in the production of organic acids in response to metal excess stress. Detoxification can take place through organic acid chelating to the metal and it will hold into the roots of plants or reduce the level of toxicity (Reichman 2002). Such conditions is usually found in metal-tolerant plants such as what was found in *T. aestivum* tolerant that produce more malic acid than the sensitive genotypes due to Al stress (Delhaize *et al.* 1993; Huang *et al.* 1996). Citric acid content of tolerant soybean also increased by almost two-fold with 1.4 mM of Al as compared to the

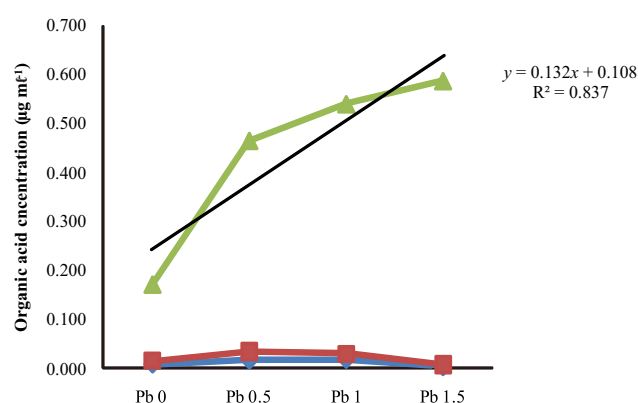


Figure 1 Pattern of organic acid levels secreted of *sengon* seedling during lead exposing for 4 days in nutrient culture. Secretion Oxalate (\rightarrow), secretion Malate (\rightarrow), secretion Citrate (\rightarrow), linear (secretion Citrat) (\rightarrow).

control, without Al (Kasim *et al.* 2001). Thus it is possible that *sengon* seedlings use citric acid secreted to the culture medium as Pb-chelator, or it can also be interpreted that this secretion activity is part of external detoxification. Furthermore, the higher accumulation of citric acid in the tissues especially due to the increased of Pb up to 1 mM, is probably also part of the plant response to the Pb inclusion to the tissues. Therefore, although the increase of accumulation was not as big as the secretion, citric acid accumulated in the tissues is an important part of internal detoxification efforts. To find out more clearly about this role, further confirmation is required in term of location and the amount of Pb accumulated in the specific organelles as well as tissues. These data show us that to the extent of certain Pb concentration, *sengon* seedlings have ability to enhance its tolerant by internal as well as external detoxification through the increasing of citric acid concentrations.

Conclusion

Sengon seedling has tolerance to lead exposed, but on exposure Pb 5 mM have been toxic. Exposure levels of lead affect the quantity and type of organic acids accumulated and secreted by *sengon* seedlings. Adaptation mechanism of *sengon* seedling to lead exposed up to Pb 1.5 mM was shown by the increased secretion of citric acid as a form of detoxification external strategy or restriction of lead absorption.

Recommendation

To determine potential remediation *sengon* seedlings to Pb, further observations are needed on the distribution of Pb in root tissues *sengon* both apoplastic and symplastic, based on the rate of accumulation and the secretion of organic acids *sengon* seedlings.

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References

- Amalia MM, Ernesto FT, Fernando RC, Tania L. 2011. Lead bioaccumulation in *Acacia farnesiana* and its effect on lipid peroxidation and glutathione production. *Plant Soil* 339:377–389. <http://dx.doi.org/10.1007/s11104-010-0589-6>.
- Arunachalam SK, Hinz C, Aylmore G. 2004. *Soil physical properties affecting root growth in rehabilitated gold mine tailings*. In: *The 3rd Australian New Zealand Soils Conference*. University of Sydney, Australia, 5–9 December 2004.
- Brunet J, Repellin A, Varrault G, Terryn N, Yasmine ZF. 2008. Lead accumulation in the roots of grass pea

- (*Lathyrus sativus* L.): a novel plant for phytoremediation system? *C.R. Biologies* 331:859–864. <http://dx.doi.org/10.1016/j.crvi.2008.07.002>.
- Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H. 2004. Engineering high-level aluminum tolerance in barley with theALMT1 gene. *Proc. National Academy of Sciences USA* 19:15249–15254. <http://dx.doi.org/10.1073/pnas.0406258101>.
- Huang JW, Chen J, Berti WR, Cunningham SD. 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science Technology* 31:800–805. <http://dx.doi.org/10.1021/es9604828>.
- Jarvis MD, Leung DWM. 2002. Chelated lead transport in *Pinus radiata*: an ultrastructural study. *Environmental and Experimental Botany* 48: 21–32. [http://dx.doi.org/10.1016/S0098-8472\(02\)00005-9](http://dx.doi.org/10.1016/S0098-8472(02)00005-9).
- Jiang W, Liu D. 2010. WPb-induced cellular defence system in the root meristematic cells of *Allium sativum* L. *BMC Plant Biology* 10:40. <http://dx.doi.org/10.1186/1471-2229-10-40>.
- Jones DL. 1998. Organic acid in rhizosphere—a critical review. *Plant and Soil* 205: 25–44. <http://dx.doi.org/10.1023/A:1004356007312>.
- Kabir M, Zafar MI, Shafiq M. 2009. Effects of lead on seedling growth of *Thespesia populnea* l. *Advances in Environmental Biology* 3(2):184–190.
- Kasim N, Soepandie D, Harran S, Jusuf M. 2001. Pola akumulasi dan ekresi asam sitrat dan asam malat pada beberapa genotipe kedelai toleran dan peka alimunium. *Hayati* 14:58–61.
- Kim KR, Owens G, Naidu R, Kwon S, Kim KH. 2009. Lead induced organic acid exudation and citrate enhanced Pb uptake in hydroponic system. *Korean Journal of Environmental Agriculture* 28(2):146–157. <http://dx.doi.org/10.5338/KJEA.2009.28.2.146>.
- Kochian LV, Hoekenga OA, Pineros MA. 2004. How do plants tolerate acid soils? Mechanism of aluminium tolerance and phosphorus efficiency. *Annual Review Plant Biology* 55:459–493. <http://dx.doi.org/10.1146/annurev.arplant.55.031903.141655>.
- Kopittke PM, Asher CJ, Kopittke RA, Menzies N. 2007. Toxic effects of Pb²⁺ on growth of cowpea (*Vigna unguiculata*). *Environmental Pollution* 150:280–287. <http://dx.doi.org/10.1016/j.envpol.2007.01.011>.
- Liu J, He C, Wong CC. 2009. Variation between two rice genotype in root secretion of organic acid and plant Pb uptake. In: *The 3rd International Conference on Bioinformatics and Biomedical Engineering*; Beijing, China, 11–13 June 2009.
- Liu D, Zou J, Meng Q, Zou J, Jiang W. 2009. Uptake and accumulation and oxidative stress in garlic (*Allium sativum* L.) under lead phytotoxicity. *Ecotoxicology* 18:134–43. <http://dx.doi.org/10.1007/s10646-008-0266-1>.
- Li Y, Jun Y, Shirong G, Weimin Z. 2012. Aluminum-induced secretion of organic acid by cowpea (*Vigna unguiculata* L.) roots. *Scientia Horticulturae* 135: 52–58. <http://dx.doi.org/10.1016/j.scienta.2011.12.006>.
- Mengel K, Kirkby EA. 1987. *Principle of Plant Nutrition*. Switzerland: International Potash Institute.
- Niu Z, Sun L, Sun T. 2010. Relationships between changes of three organic acid (oxalic acid, citric acid and tartaric acid) and phytoextraction by sunflower (*Helianthus annuus* L.) in sand cultures contaminated with cadmium and lead. In: *International Conference on Digital Manufacturing and Automation (ICDMA)*; Chang Sha, China, 18–20 December 2010. <http://dx.doi.org/10.1109/ICDMA.2010.329>.
- Pandey S, Gupta K, Mukherjee AK. 2007. Impact of cadmium and lead on *Catharanthus roseus*—a phytoremediation study. *Journal of Environmental Biology* 28:655–62.
- Pflugmacher S, Geissier K, Steinberg C. 1999. Activity of phase I and phase II detoxification enzymes in different cormus parts of *Phragmites australis*. *Ecotoxicol. Environ. Saf.* 42:62–66. <http://dx.doi.org/10.1006/eesa.1998.1727>.
- Pineros MA, Magalhaes JV, Alves VMC, Kochian LV. 2002. The physiology and biophysics of an aluminum tolerance mechanism based on root citrate exudation in maize. *Plant Physiology* 129: 1194–1206. <http://dx.doi.org/10.1104/pp.002295>.
- Reichman SM. 2002. *The Responses of Plants to Metal Toxicity: A review focusing on Copper, Manganese and Zinc*. Melbourne: Australian Minerals & Energy Environment Foundation.
- Setyaningsih L. 2007. Pemanfaatan cendawan mikoriza arbuskula dan kompos untuk meningkatkan pertumbuhan semai mindi (*Melia azedarach* Linn) pada media tailing tambang emas Pongkor. [thesis]. Bogor: Graduate School of Bogor Agricultural University.
- Shu X, Yin LY, Zhang QF, Wang WB. 2011. Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. *Environmental Science Pollution Research* 19:893–902. <http://dx.doi.org/10.1007/s11356-011->

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- Sopandie D. 1990. Studies on plant response to salt stress. [thesis]. Okayama: University of Okayama.
- Tong YP, Kneer R, Zhu YG. 2004. Vacuolar compartmentation: a second-generation approach to engineering plants for phytoremediation. *Trends Plants Science* 9:7–9. <http://dx.doi.org/10.1016/j.tplants.2003.11.009>.
- Wang Y, Tao J, Dai J. 2011. Lead tolerance and detoxification mechanism of *Chlorophytum comosum*. *African Journal of Biotechnology* 10: 14516–14521.
- Wasis B, Fathia N. 2011. Growth of *Gmelina* seedling with various compost fertilizer in ex-gold mining land media. *Journal of Tropical Forest Management* 17(1): 29–33.
- Xiao W, Hao H, Liu XQ, Liang C, Chao L, Su MY, Hong FH. 2008. Oxidative stress induced by lead in chloroplast of spinach. *Biology of Trace Elements Research* 126:257–68. <http://dx.doi.org/10.1007/s12011-008-8195-7>.
- Yang X, Feng Y, He Z, Stoffella PJ. 2005. Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. *Journal of Trace Elements in Medicine and Biology* 18:339–53. <http://dx.doi.org/10.1016/j.jtemb.2005.02.007>.