PHYTOREMEDIATION OF SOIL CONTAMINATED WITH COPPER AND ZINC FROM PIG WASTE

By

WANG YAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

I wish to dedicate this thesis to my beloved parents, Wang Shou Quang, Xie Yu Ying and my brother Wang Min

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman: Associate Professor Liang Juan Boo, PhD

Faculty: Agriculture

Copper (Cu) and Zinc (Zn) are two elements of great concern due to their potential toxicity to plants and/or animals. Most pig farmers in Malaysia fed two to three folds of the recommended Cu and Zn to their animals. Only about 15% of the dietary Cu and Zn were apparently digested while the remaining 85% were excreted mainly via the feces. It is estimated that 390 tons of Cu and 303 tons of Zn are being excreted annually by approximately two million pigs in Malaysia. Appropriate management of the pig waste is, therefore, essential to ensure that these heavy metals do not pollute the environment.

Phytoremediation, the use of plants to restore polluted sites, has recently become a popular alternative to the traditional techniques of cleaning up polluted soils. Five locally available plants, namely Kenaf (*Hibiscus cannabinus L.*), Typha (*Typha spp.*), Vetiver (*Vetiveria zizanioides*), Canna (*Canna x generalis*), Cyathula (*Altemanthera Ficoidea cv. "Songuinea"*) were shown to exhibit their ability to accumulate Cu and Zn from the soil applied with pig manure. Roots were the main site for Cu and Zn accumulation followed by

stems and leaves. On the average, Cu and Zn concentrations in all parts of Cyathula were the highest. However, because of its low biomass, accumulation of Cu and Zn per plant basis for Cyathula was significantly lower than the other plants, particularly Canna and Typha.

Since, heavy metal tolerance in plants is often species and metal specific, different plants can better accumulate heavy metals in the different plant tissues or cell organelles. Examination of heavy metal accumulation at cellular level is thus important to understand the tolerance mechanism of the metal-accumulating plants. Ultrastructural investigation of the root-tips of Kenaf, Canna and Cyathula indicated that Cu and Zn mainly deposited in the cell wall, cytoplasm and nucleus. In leaves, the main sites of deposition were chloroplast and nucleus, followed by cell wall, cytoplasm and mitochondria. Vacuole had the lowest Cu and Zn deposition. High concentration of Cu and Zn deposition resulted in changes in root cell structure, including cell wall deformation and vacuolization. The above effects were, however, not consistent among the three plant species studied. For examples, cell wall deformations were observed in root cells of Canna, but not Kenaf and Cyathula while strong vacuolization of root cells was observed only in Cyathula. Cell wall deformation and vacuolization in cells could be symptoms of toxicity and/or self defense mechanism to prevent toxicity of heavy metals to the plant tissues. There was no observable change in the leaf cell structures of these plants under different Cu and Zn treatments, presumably because of the low level of Cu and Zn accumulation in the leaf cells were below the toxicity level.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk jazah Master Sains

FITOREMEDIASI TANAH YANG DICEMARI DENGAN KUPRUM DAN ZINK DARIPADA SISA KHINZIR

Oleh

WANG YAN

Januari 2005

Pengerusi: Profesor Madya Liang Juan Boo, PhD

Fakulti: Pertanian

Kuprum (Cu) dan zink (Zn) adalah dua elemen yang penting kenana berpotensi menyebabkan toksik kepada tumbuhan dan/atau haiwan. Di Malaysia, kebanyakan penternak khinzir memberi dua hingga tiga kali ganda Cu dan Zn yang dicadangkan kepada ternakan mereka. Hanya 15% Cu dan Zn dalam makanan dihadamkan manakala 85% yang tertinggal dikumuhkan dalam najis. Adalah dianggarkan bahawa 390 ton Cu dan 303 ton Zn dikumuhkan oleh lebih kurang dua juta ekor khinzir di Malaysia pada setiap tahun. Oleh itu, pengurusan sisa khinzir yang berkesan adalah diperlukan supaya logam berat ini tidak mencemarkan persekitaran.

Fitoremediasi, penggunaan tumbuhan untuk membaiki pulih kawasan tercemar, menjadi altenatif yang hangat kepada teknik tradisional sejak kebelakangan ini. Lima spesis tumbuhan tempatan, iaitu Kenaf (*Hibiscus cannabinus L.*), Typha (*Typha spp.*), Vetiver (*Vetiveria zizanioides*), Canna (*Canna x generalis*), Cyathula (*Altemanthera Ficoidea cv. "Songuinea"*) telah menunjukkan keupayaan untuk mengumpul Cu dan Zn daripada tanah yang beraplikasi dengan sisa khinzir. Akar adalah bahagian utama untuk mengumpul Cu

dan Zn diikuti oleh batang dan daun. Secara keseluruhannya, kepekatan Cu dan Zn dalam semua bahagian Cyathula adalah tertinggi. Walaupun demikian, disebabkan biojisimnya yang rendah, pengumpulan Cu dan Zn berdasarkan setiap tumbuhan adalah signifikan secara rendah berbanding dengan spesis tumbuhan lain, terutamanya Canan dan Typha.

Sejak ketahanan logam berat dalam tumbuhan biasanya bergantung pada spesis dan spesifikasi logam, tumbuhan yang berbeza berupaya mengumpul logam berat dalam tisu tumbuhan atau organel sel yang berlainan. Oleh itu, kajian pengumpulan logam berat pada tahap selular adalah diperlukan untuk memahami mekanisme ketahanan tumbuhan pengumpul logam. Penyelidikan secara ultrastruktur pada hujung akar Kenaf, Canna dan Cyathula menunjukkan kebanyakan Cu dan Zn disimpan dalam dinding sel, sitoplasma dan nukleus. Dalam daun, tempat penyimpanan utama adalah dalam kloroplas dan nukleus, diikuti dengan dinding sel, sitoplasma dan mitokondria. Vakuol mempunyai penyimpanan Cu dan Zn yang terendah. Kepekatan penyimpanan Cu dan Zn yang tinggi mengakibatkan perubahan dalam struktur sel akar termasuk perubahan bentuk dinding sel dan vakuolisasi. Walaupun demikian, kesan yang dinyatakan di atas adalah tidak konsisten antara tiga spesis tumbuhan yang dikaji. Contohnya, perubahan bentuk dinding sel telah diperhatikan dalam sel akar Canna tetapi tidak dalam Kenaf dan Cyathula manakala vakuolisasi sel akar yang kuat dijumpai pada Cyathula sahaja. Perubahan bentuk dalam dinding sel dan vakuolisasi dalam sel mungkin merupakan satu gejala toksik dan/atau mekanisme pertahanan diri untuk mengelakkan keracunan logam berat dalam tisu tumbuhan. Tiada perubahan diperhatikan dalam struktur sel daun tumbuhan kajian di bawah pelbagai rawatan Cu dan Zn, dan diandaikan ini disebabkan oleh tahap pengumpulan Cu dan Zn dalam sel daun adalah rendah di bawah tahap toksik.

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I certify that an Examination Committee met on 31st Jan. 2005 to conduct the final examination of Wang Yan on her Master of Science thesis entitled "Phytoremediation of soil contaminated with copper and zinc from pig waste" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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WANG YAN

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