Vietnam Journal of Earth Sciences, 38(3), 286-296, DOI: 10.15625/0866-7187/38/3/8763

(VAST)

Vietnam Academy of Science and Technology

Vietnam Journal of Earth Sciences

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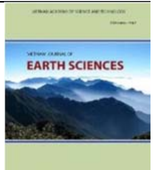

# Uptake capacity of metals (Al, Cu, Pb, Sn, Zn) by *Vetiveria zizanioides* in contaminated water from Dong Xam metal production trade village, Thai Binh, Vietnam

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Received 15 November 2014. Accepted 20 August 2016

ABSTRACT

The present study investigates an experiment of uptake capacity of metals by *Vetiveria zizanioides* to treat contaminated water from a metal production trade village, Dong Xam, Thai Binh, Vietnam (DXV). Vetiver was grown in two pot culture experiments TB10, TB6 with solutions containing respective concentrations of Al, Cu, Pb, Sn and Zn of 2.5, 55.6, 0.15, 7.7 and 24.4 mg from the DXV for a period of 36 days. Vetiver was higher tolerant to metals Al, Cu, Pb, Sn and Zn than other plant species. The roots (hereafter R) accumulated Al from 17 to 30 folds than that in "reference plant". The upper parts of shoots (hereafter S1, S2, and S3) were 1.2 folds higher than that in "reference plant". Cu concentrations in the roots and shoots were 660 and 46.2 mg/kg, respectively. Vetiver could withstand and survive at Cu concentration of 46 mg/L in contaminated water that is markedly higher than other plants. The translocation of Pb from root to shoot was 41%. Sn accumulated higher in the top, in which shoot/root ratio varied from 82 to 277%, and increased to the top by order S3/R>S2/R>S1/R. Zn could be translocated from root and accumulated in shoot. The ratio shoot/root was up to 46%. The present results demonstrated that vetiver was high tolerant to metals Al, Cu, Pb, Sn and Zn. Therefore, vetiver has a potential phytoremediation of metals in contaminated soils and wastewaters from trade villages in Vietnam and other countries

*Keywords:* metals,, *Vetiveria zizanioides*, trade village Dong Xam, Thai Binh.

## 1. Introduction

Heavy metal contamination in the environment by agricultural land erosion, urban wastes and by-products of rural, industrial activities and mining industries attracts worldwide concerns, especially in developing countries (Mejare and Bulow, 2001; Tordoff et al., 2000).

Nowadays, there are about thousand trade villages that are exercising various professions in Vietnam. However, they are facing problems with wastewater and solid waste treatments, particularly, metal contaminations in wastes.

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The vetiver grass is first grown for soil and water conservation in farmlands. Vetiver has unique morphological, physiological and ecological characteristics, and plays a key role in the field of environmental protection. Unique morphological characteristics include a massive finely structured and deep root system that can reach up to 3-4 m in the first year. Vetiver is tolerant to extreme climatic variation such as prolonged drought, flood, and extreme temperature. Vetiver can survive in very harsh environments where surface temperature varying from -13 °C to 55 °C. It is also tolerant to a wide range of soil pH, ranging between 3.0 and 10.5, and soil salinity, sodicity, acidity, and heavy metals (Truong, 1996; Truong and Baker, 1998; Truong and Hart, 2001; Truong and Loch, 2004).

Phylogenetically, vetiver is close to sorghum. It seems that, as other Panicoideae plant subfamily, vetiver follows the same conjugation detoxification pathway (Jensen. et al., 1977). The major metabolism of atrazine in vetiver grown in hydroponics was conjugation, mainly in leaves, a transformation known to be positive for the environment (Sylvie et al., 2006).

Vetiver grass was selected for the wastewater treatment purpose from Dong Xam metal production trade village, Thai Binh (DXV) due to many reasons. Firstly, it can tolerate in the wide range of pollution conditions, and it has been promoted by World Bank since 1990 to control soil erosion throughout the world (Becker, 1992; Grimshaw, 1989; Steven et al., 1999). Second, it requires a low cost alternative means to reduce contaminated areas by heavy metals (Truong and Baker, 1998). Vetiver grows very fast with annual productivity of 99 tons/ha, its strong root system and a long-lived perennial can survive up to 50 years (Veldkamp, 1999; Zhang, 1998).

Many previous studies have reported the uptake capacity of heavy metals by Vetiver (Adriano, 1992; Chiu et al., 2005, 2006; Lai and Chen, 2004; Sylvie et al., 2006; Truong and Baker, 1998; Wilde et al., 2005; Xia, 2004; Yahua et al., 2004; Yang et al., 2003), but uptake capacities of Al, and Sn have not been clearly investigated, particularly the pollution likes in the DXV with number of metal contaminations.

## 2. Materials and methods

### *2.1. Vetiver growth conditions*

The soils using for vetiver cultivation were collected from five points in the study area. The soils were sieved through a 2-mm mesh and well mixed to obtain composite homogeneous samples. Seedling of vetiver was wrapped in the composite soils, and then transferred to grown in contaminated waters with different chemical contents (Figure 1b).

The soils in two pots (TB10, TB6) for vetiver cultivation were added at the same amount of metals Al, Cu, Pb, Sn and Zn in wastewater of DXV at 2.5, 55.6, 0.15, 7.7 and 24.4 mg, respectively. Vetivers were cultivated in the contaminated solutions with different concentrations of trace elements (Table 1) by adding tap water and one pot (control) was living in the clean tap water. No fertilizer was applied during the entire growing period. The temperature in the laboratory growth chamber was 25 ± 2°C.

After 36 days of growth in laboratory chamber by contaminated water TB10, TB6 and control water, vetiver plants were harvested. The plant's height was 0.7 m (Figure 1a, b). The plants were first rinsed three times with tap water to remove all soils and other materials and then two times with deionized water. The plants were then dried at room temperature for five days, then at 80°C for two days in an electric oven to constant weight. The plants were sectioned into five parts: root (R), meristematic region (M) and three parts of shoots (S1 - 10 cm of the shoot is from the meristematic region, S2- next 10 cm of the shoot, S3- remain part (about 20-40cm) in the top of the shoot). All samples were sieved through a 2-mm mesh and well mixed (Figure 2).

**Figure 1.** (a) Vetiver grown land and (b) it was grown in laboratory chamber by contaminated water for 36 days

**Table 1.** Analytical results of contaminated solutions from two wastewater samples from the DXV prior treatment by vetiver (mean ± SD)

| Elements | TB10 | | TB6 | |
|---|---|---|---|---|
| | Mean, mg/L | SD | Mean, mg/L | SD |
| Al | 1.242 | 0.002 | 2.070 | 0.003 |
| Cu | 27.821 | 0.0009 | 46.369 | 0.0015 |
| Pb | 0.075 | 0.0005 | 0.125 | 0.0008 |
| Sn | 3.861 | 0.001 | 6.435 | 0.001 |
| Zn | 12.225 | 0.0003 | 20.375 | 0.0005 |

### *2.2 Chemical analysis*

Approximately 500 mg plant tissues from each part of vetiver and standard NIST 1568a (Rice Flour) were placed into 100 ml Teflon bottles. The materials were digested at 180°C with 5ml of 16M $HNO_3$ and 1 ml of 12M $HClO_4$ (5:1 ratio) for 24 hours on a hotplate. After evaporation, the solutions were added 0.03 ml of 18M $H_2SO_4$ and kept at 180°C for 24 hours. The dissolved samples were brought to a volume 30 ml with 2% $HNO_3$.

The concentrations of Al, Cu, Pb, Sn and Zn in the solutions were determined by ICP MS at the Korea Basic Science Institute (KBSI) (Table 1). The standard error (SD) is calculated from the triplicate analysis (n=3).

The NIST 1568a was used to quantify the accuracy of metal determination by ICP-MS, and the recovery levels of Cu, Zn, Cd and Pb ranged from 90.7 - 104.8% (± 5.0%) (Table 2).

**Figure 2.** (a) Vetiver samples TB6 and (b) TB10 were sieved through a 2 mm mesh and mixed well

**Table 2.** Recovery levels of metals for NIST 1568a (Rice Flour)

| Element | Certificate, mg/kg | | Found, mg/kg | | Recovery (%) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Cd | 0.022 | 0.002 | 0.023 | 0.0006 | 104.8 | 2.6 |
| Cu | 2.400 | 0.3 | 2.176 | 0.087 | 90.7 | 3.6 |
| Pb | <0.010 | | 0.009 | 0.0005 | 91.5 | 5.0 |
| Zn | 19.400 | 0.5 | 20.301 | 0.819 | 104.6 | 4.2 |

### 2.3 *Chemical fingerprint*

According to Markert (1992), to overcome the problem of data variation over the scale, we use chemical fingerprints by normalizing data to "reference plant" for interpretation and discussion of Al, Cu, Pb, Sn and Zn concentrations (Figure 3). The value of "reference plant" were set to zero (normalization) and the data of trace metals Al, Cu, Pb, Sn and Zn concentrations of parts of vetiver will be given as deviations from the value of “reference plant”.

## 3. Results and discussion

### *3.1 Aluminum (Al)*

The main function of Al in plants is to control colloidal properties in the cell, possible activation of some dehydrogenases and oxidization (Kabata and Pendias, 2001). The high availability of Al in nutrient soils is one of the limiting factors in the production of most field crops (Baker, 1976, Foy et al., 1978; Frank et al., 1979). The physiological mechanism of Al toxicity is still debate, but Al excess in plants is likely to interfere with cell division and with properties of protoplasm and cell walls (Foy et al., 1978). The Al concentration in plants greatly varies, depending on soil and plant factors.

*Chemical fingerprint:* The relative deviation of Al from “reference plant” is shown in Figure 1a. The concentration of Al in root tissues was greater than that in the “reference plant” by 17 to 30 times (Table 4, Figure 3). The deviation in the lower parts (meristematic regions M and low parts of shoots S1) was less than zero, but upper parts of shoots S2, and S3 was higher, reaching 120% (TB6-S2). This means that Al highly concentrated in the top of leave and the ratio of Al shoot: root varied from 3 up to 8%.

The Al concentration in all parts of vetiver increased with the contaminated levels of wastewater (Tables 1 and 3, Figure 4), and were higher in the roots in comparison to shoots. The minimum concentration was found in the meristematic regions, because the amount of Al passively taken up by roots and then translocated to tops, reflecting the Al tolerance of plants. However, it should be noticed that the ability to accumulate Al in roots is not necessarily associated with Al tolerance (Kabata and Pendias, 2001).

**Table 3.** Concentrations of metals in vetiver parts, (mean ± standard error) (mg/kg)

| Sample ID | Blank BL1 - Root | | Blank BL1 - Meristematic region | | Blank BL1 - Shoot S1 | | Blank BL1 - Shoot S2 | | Blank BL1 - Shoot S3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Element | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Al | 1386.78 | 73.52 | 14.142 | 1.029 | 20.289 | 4.843 | 70.735 | 5.222 | 52.912 | 1.724 |
| Cu | 9.978 | 0.448 | 35.089 | 1.337 | 4.460 | 0.220 | 3.614 | 0.180 | 4.770 | 0.183 |
| Pb | 1.706 | 0.048 | 0.039 | 0.002 | 0.326 | 0.012 | 1.434 | 0.043 | 1.627 | 0.059 |
| Sn | 0.306 | 0.007 | 0.465 | 0.026 | 0.377 | 0.025 | 0.444 | 0.017 | 0.426 | 0.028 |
| Zn | 33.188 | 1.301 | 179.735 | 8.191 | 22.612 | 1.077 | 19.463 | 0.842 | 22.060 | 0.801 |

| Sample ID | TB10 - Root | | TB10 - Meristematic region | | TB10 - Shoot S1 | | TB10 - Shoot S2 | | TB10 - Shoot S3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Element | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Al | 2358.22 | 26.35 | 37.619 | 1.166 | 88.288 | 1.784 | 96.455 | 2.386 | 88.158 | 3.572 |
| Cu | 367.833 | 17.696 | 84.453 | 4.491 | 15.386 | 0.768 | 8.189 | 0.395 | 11.672 | 0.474 |
| Pb | 1.919 | 0.071 | n.d. | n.d. | 0.736 | 0.026 | 2.860 | 0.110 | 1.809 | 0.055 |
| Sn | 0.175 | 0.010 | 0.086 | 0.005 | 0.160 | 0.005 | 0.207 | 0.012 | 0.484 | 0.033 |
| Zn | 78.187 | 4.003 | 336.966 | 16.948 | 23.649 | 1.108 | 27.021 | 1.316 | 26.628 | 1.170 |

| Sample ID | TB6 - Root | | TB6 - Meristematic region | | TB6 - Shoot S1 | | TB6 - Shoot S2 | | TB6 - Shoot S3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Element | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Al | 2148.32 | 52.91 | 41.668 | 0.604 | 66.628 | 6.035 | 176.675 | 16.775 | 106.164 | 13.811 |
| Cu | 660.674 | 15.220 | 119.105 | 4.578 | 46.151 | 2.177 | 13.053 | 0.471 | 17.095 | 0.583 |
| Pb | 2.303 | 0.038 | 0.117 | 0.005 | 1.482 | 0.042 | 3.885 | 0.109 | 3.245 | 0.081 |
| Sn | 0.333 | 0.008 | 0.306 | 0.016 | 0.274 | 0.009 | 0.501 | 0.005 | 0.614 | 0.024 |
| Zn | 141.641 | 3.777 | 303.817 | 12.303 | 64.808 | 3.086 | 47.334 | 1.971 | 48.860 | 1.669 |

n.d. = not detected

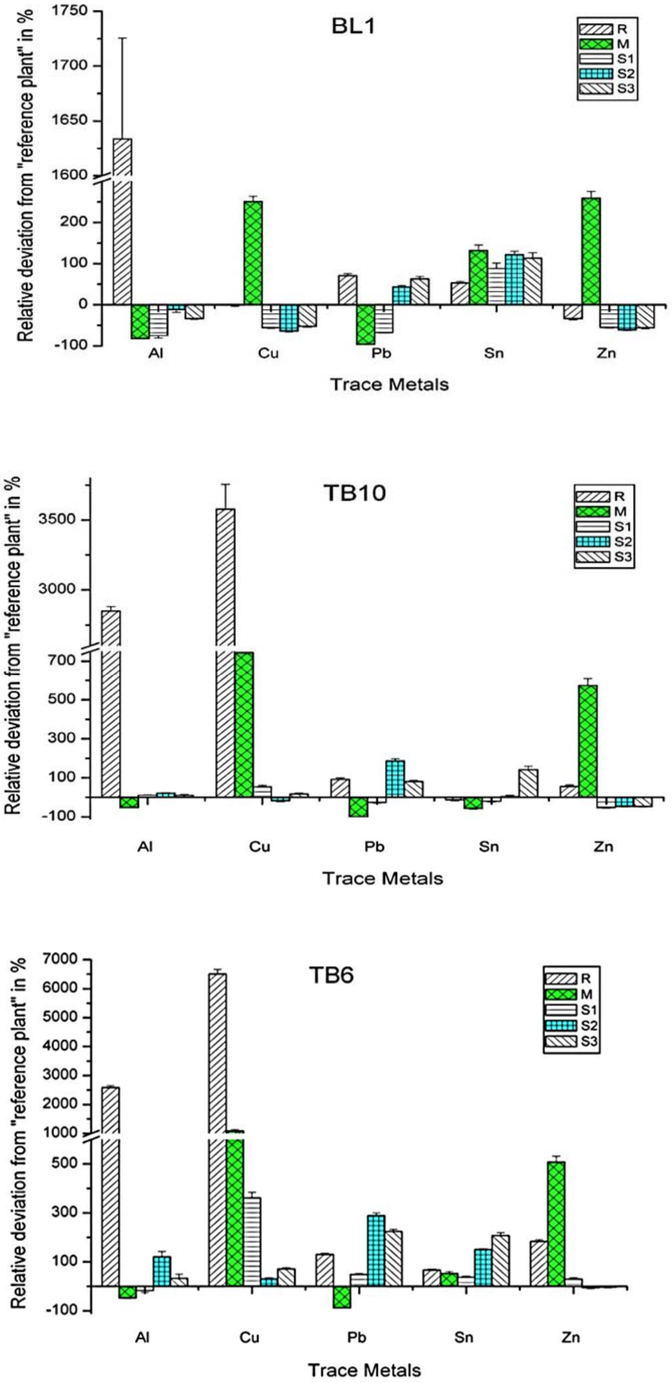

**Figure 3.** Relative deviations of vetiver parts after normalization against “reference plant” (Markert B., 1992)

**Table 4.** Relative deviation concentration (%) in parts of vetiver from "reference plant" (Mean ± standard deviation)

Sample vetiver blank BL1

| Element | R | | M | | S1 | | S2 | | S3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Al | 1633.5 | ± 91.9 | -82.3 | ±1.3 | -74.6 | ±6.1 | -11.6 | ±6.5 | -33.9 | ±2.2 |
| Cu | -0.2 | ± 4.5 | 250.9 | ±13.4 | -55.4 | ±2.2 | -63.9 | ±1.8 | -52.3 | ±1.8 |
| Pb | 70.6 | ±4.8 | -96.1 | ±0.2 | -67.4 | ±1.2 | 43.4 | ±4.3 | 62.7 | ±5.9 |
| Sn | 53.2 | ±3.4 | 132.4 | ±13.1 | 88.4 | ±12.6 | 121.8 | ±8.4 | 112.9 | ±14.1 |
| Zn | -33.6 | ±2.6 | 259.5 | ±16.4 | -54.8 | ±2.2 | -61.1 | ±1.7 | -55.9 | ±1.6 |

Sample vetiver TB10

| Element | R | | M | | S1 | | S2 | | S3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Al | 2847.8 | ±32.9 | -53.0 | ±1.5 | 10.4 | ±2.2 | 20.6 | ±3.0 | 10.2 | ±4.5 |
| Cu | 3578.3 | ±177.0 | 744.5 | ±44.9 | 53.9 | ±7.7 | -18.1 | ±3.9 | 16.7 | ±4.7 |
| Pb | 91.9 | ±7.1 | -100.0 | ±0.2 | -26.4 | ±2.6 | 186.0 | ±11.0 | 80.9 | ±5.5 |
| Sn | -12.5 | ±5.1 | -56.8 | ±2.7 | -20.2 | ±2.7 | 3.5 | ±5.9 | 142.2 | ±16.7 |
| Zn | 56.4 | ±8.0 | 573.9 | ±33.9 | -52.7 | ±2.2 | -46.0 | ±2.6 | -46.7 | ±2.3 |

Sample vetiver TB6

| Element | R | | M | | S1 | | S2 | | S3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Al | 2585.4 | ±66.1 | -47.9 | ±0.8 | -16.7 | ±7.5 | 120.8 | ±21.0 | 32.7 | ±17.3 |
| Cu | 6506.7 | ±152.2 | 1091.0 | ±45.8 | 361.5 | ±21.8 | 30.5 | ±4.7 | 70.9 | ±5.8 |
| Pb | 130.3 | ±3.8 | -88.3 | ±0.5 | 48.2 | ±4.2 | 288.5 | ±10.9 | 224.5 | ±8.1 |
| Sn | 66.5 | ±4.1 | 52.9 | ±7.8 | 37.1 | ±4.4 | 150.5 | ±2.6 | 207.0 | ±12.1 |
| Zn | 183.3 | ±7.6 | 507.6 | ±24.6 | 29.6 | ±6.2 | -5.3 | ±3.9 | -2.3 | ±3.3 |

### *3.2. Copper (Cu)*

Cu is a component in some enzyme as catalyst (Schlesinger 2004), involves in oxidation, photosynthesis, protein and carbohydrate metabolism, possibly in symbiotic $N_2$ fixation, and valence changes in plants (Kabata and Pendias, 2001) (but it is toxic if concentration of Cu excesses the need of plants). Cu is an essential element for the growth of most aquatic organisms but is a toxic element at concentration of 10 mg/L (Leckie and Davis, 1979). In our experiment, vetiver plants were growth well in the solutions TB10 and TB6 with Cu concentration of 27.821 and 46.369 mg/L (Table 1).

In all parts of TB10 and TB6 samples, Cu concentration was higher in comparison with vetiver blank (BL1). In each vetiver sample, Cu concentration is decreased as follows: R>M>S1> S2, S3 (Table 3; Figure 4b), with an exception for Blank BL1.

In root tissue, Cu exists entirely in complexed forms; it is most likely that the metal enters root cells in dissociated forms (Kabata and Pendias, 2001) and the same process occurs in the meristematic regions. The root and meristematic region tissues had a strong capability to absorb Cu for reducing the Cu transport to shoots.

*Chemical fingerprint:* Cu concentration in all vetiver parts lived in wastewater was higher than that of “reference plant” (exception for TB10-S2, it was slightly lower) (Table 4; Figure 2). The deviation with “reference plant” in the shoot it oscillated from 16.7 (TB10-S3) to 361.5% (TB6-S1), in the meristematic region from 745 (TB10-M) to 1091% (TB6-M) and in the root from 3578 (TB10-R) up to 6507 % (TB6-R). On the contrary, it was negative in the root (-0.2%) and shoot (-52 ÷ -64%) of blank BL1 (except meristematic region).

The trend of slope line is clearly shown in Table 4 and Figure 4b, reflecting the increasing of Cu concentration in contaminated water. It seems that Cu concentration in vetiver was the function (in direct proportion) of its concentration in contaminated water. Cu concentrations in the root (R), meristematic region and shoots (S1, S2, S3) parts of vetiver were all increased with its concentration in contaminated water. The increased level of Cu concentration in root was faster than in the meristematic region and in others parts M>S1>S2, S3. Cu has low mobility relative to other elements in vetiver and higher Cu concentration remaining in root and leaf tissues until they senesce (Kabata-Pendias Alina and Pendias Henryk, 2001). In other plants, the excessive or toxic concentration of Cu is 20-100 mg/kg (Kabata and Pendias, 2001), but Cu concentration could range from 11 to 660 mg/kg in vetiver (Table 3).

The ratio of Cu shoot: root was low (4-7%) for vetiver grown in the wastewater and higher (36-48%) for vetiver grown in cleaning water, indicating higher absorption capacity of Cu in vetiver root. For vetiver grown in solutions with different Cu concentrations, the translocation of Cu happened from the shoot to top of vetiver. This process seems to increase with Cu concentration in contaminated water (Figure 4b). For other plant species, Cu concentration at 10 mg/L in contaminated water is toxic but vetiver can withstand and alive at 46 mg/L.

The maximum Cu concentration in shoot, meristematic region, and root of sample TB6 was 46.2, 119.1 and 660.7 mg/kg, respectively, being higher than those in previous reports (Truong and Baker, 1998, 2000; Truong and Hart, 2001; Yahua et al., 2004; Baker, 1976). In the contaminated water, Cu and Al concentrations were high, their antagonisms lead to reduce uptake capacity of Cu by roots under high Al concentration (Kabata and Pendias, 2001).

### 3.3. Lead (Pb)

Pb is an essential element for the plant at the concentration from 2 to 6 μg/kg (Broyer et al., 1972). Pb has been widely considered as a major pollutant in the environment and a toxic element to plants (Kabata and Pendias, 2001).

*Chemical fingerprint*: Pb was concentrated in the roots of vetiver and deviation in comparison with "reference plant" ranged from 70.6 (BL1-R) to 130% (TB6-R) (Table 4; Figure 3). For the meristematic regions, the deviation was lower than zero, being -100% (TB10-R). The concentration of Pb in shoots followed in order: (S2, S3)>S1, M, R and increased follow its concentration in contaminated water and was four times higher than that in "reference plant".

For other plants, the translocation of Pb from root to top is greatly limited, being only 3% (Zimdahl, 1975). The translocation of vetiver ranged from 23 to 41%.

The trend of slope line is clearly shown in Figure 4c, Pb concentration markedly increased with its concentration in contaminated water. The stimulating effect of Pb on Cd uptake by root could be an effect of the disturbance of the transmembrane transport of ions (Kabata and Pendias, 2001).

### 3.4. Tin (Sn)

Tin is very toxic to both higher plants and fungi (Kabata-Pendias Alina and Pendias Henryk. 2001).

*Chemical fingerprint*: The deviation of Sn in vetiver in comparison to "reference plant" was slightly lower than zero for the lower part of TB10 (R, M and S1) and up to 142% for the upper parts (S2, S3). The Sn concentration in vetiver increased with its concentration in contaminated water (TB6) and increased in all parts of vetiver to 207% (Table 4; Figure 3). In the shoots of vetiver grown in TB10, TB6, Sn concentration was higher than in the root and meristematic region by the following order: S3, S2>S1>M, R (Figure 4d).

Unlike to other plants, most Sn concentration remained in roots (Rommey et al., 1975), the vetiver tends to uptake and accumulated Zn in the upper parts, thus ratio shoot: root varied from 82% (TB6-S1) to 277% (top of vetiver TB6-S3), and being higher concentration in top by order S3/R>S2/R>S1/R.

### *3.5. Zinc (Zn)*

Zn plays as an active enzyme, regulates sugar consumption in plants (W. H. Schlesinger, 2004), and involves incarbohydrate and protein metabolism processes (Kabata and Pendias, 2001). Soluble forms of Zn were available to vetiver and the uptake of Zn from soils to be linear with its concentration in contaminated water (Figure 4e).

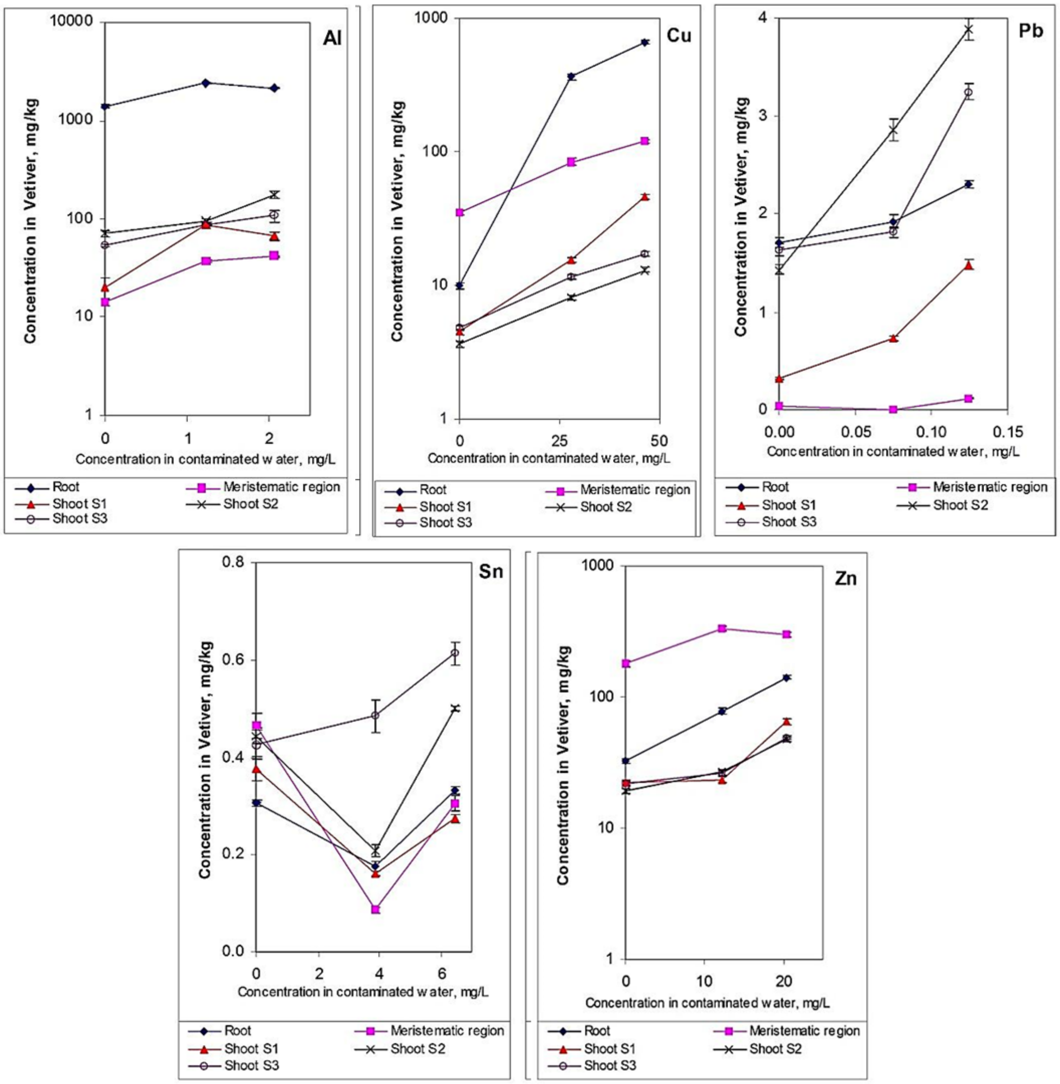

**Figure 4.** Relationship between the concentrations of metals (Al, Cu, Pb, Sn, and Zn) in several parts of vetiver and contaminated water

*Chemical fingerprint:* The deviation of Zn concentration in meristematic regions was all positive in comparison to the "reference plant", ranging from 508 - 574%, but for root and shoot parts the deviation of Zn was slightly >0 (Table 4; Figure 3).

Zn concentration was higher in the meristematic region than that in root. Roots and meristematic regions accumulated Zn higher than shoots, thus, the ratio shoot: root ranged from 30 to 46%. This pattern indicated that Zn could be translocated from the roots to shoots of vetiver. Vetiver has higher tolerance to Zn and Pb than other species (Yang et al., 2003). The Zn-Pb antagonism adversely affects the translocation of each element from root to shoot (Kabata and Pendias, 2001).

## 4. Conclusions

The present study showed that vetiver could highly accumulate metals Al, Cu, Pb, Sn and Zn in the upper part of the shoot. Thus, the vetiver may serve as an important means for waste water treatment.

The roots and upper parts of shoots accumulated Al concentration from 17-30 times and 1.2 times higher than “reference plant”, respectively. Thus, vetiver can be considered as Al-hyperaccumulation. In other plants, the excessive or toxic concentration of Cu is 20-100 mg/kg, but in vetiver plant, it was much higher and reached up to 660 and 46.2 mg/kg in the roots and shoots, respectively. Vetiver could withstand and alive at the Cu concentration of 46 mg/L in contaminated water. The Pb translocation rate from root to shoot was up to 41%. Sn highly accumulated in upper parts with ratio shoot: root varied from 82 - 277% in the top and increased to the top by order S3/R>S2/R>S1/R. Zn could be translocated from roots and accumulated in the shoots of vetiver, the ratio shoot to root was up to 46%.

## Acknowledgements

We thank two anonymous reviewers for their critical comments that significantly improved this manuscript from an early version. We thank the Korea Science and Engineering Foundation, and we thank Prof. Truong Paul, The Vetiver Network, for providing the vetiver seedlings.

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