Uptake of Copper and Zinc in Lettuce (*Lactuca sativa* L.) Planted in Sida Soil and Lignite Bottom Ash Mixtures

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Abstract

Attempts to mitigate the environmental problems from heavy metal contaminated wastes by phytoremediation technique have been intensively investigated. This research was conducted to determine the physical and chemical characteristics of mixture materials (Sida soil mixed with lignite bottom ash), and uptake of copper and zinc in lettuce (*Lactuca sativa* L.) parts cultivated under laboratory conditions. The effect of copper and zinc on lettuce yields was also investigated.

Results indicated that the pH value of mixtures ranged from 6.64±0.78 to 6.89±0.98. The percentage of moisture content and organic content ranged from 10.22±0.24 to 11.85±0.54 % and 1.86±0.85 to 5.04±0.38 %, respectively. Amount of nitrogen, phosphorus and potassium ranged from 0.31±0.03 to 0.45±0.04 %, 0.45±1.35 to 0.56±1.94 %, and 0.92±0.01 to 0.94±0.03 %. In addition, the mixture contained copper and zinc ranging from 13.12±1.71 to 26.13±2.30 mg/kg, and 66.84±5.84 to 137.74±4.13 mg/kg, respectively. It was noted that copper and zinc accumulation in mixture ratios showed a significant difference as the amount of lignite bottom ash in mixture ratios increased ($P<0.05$). The highest copper and zinc accumulation was found at mixture ratio of 0.6:0.4 in lettuce root (21.46 ± 5.90 mg/kg and 113.47 ± 4.13 mg/kg), followed by lettuce leaf (8.18 ± 1.20 mg/kg and 32.94 ± 7.34 mg/kg). The accumulation of both heavy metals in root and leaf of lettuces significantly related to the increasing of lignite bottom ash ratios ($P<0.05$). In addition, the highest lettuce yield was found at a ratio of 0.8:0.2 (1.43 ± 0.06 g/plant) and 0.6:0.4 (1.43 ± 0.03 g/plant). A significant difference in lettuce yield with the increased lignite bottom ash mixtures ranging from 0.9:0.1 to 0.6:0.4 was observed ($P<0.05$). The results suggest that lignite bottom ash mixtures can be used as supplementary micronutrients in soil for plants and planted lettuce at mixture ratios of 9:1, 8:2 and 7:3 are not harmful for consumers since the accumulation of both heavy metals was within the Criteria of Food and Drug Administration (copper not exceeded 20 mg/kg and zinc not exceeded 100 mg/kg). Also, this plant have a potential use of phytoremediation to treat the copper and zinc contaminated land but further investigation is still required.

Keywords: lettuce, lignite bottom ash, Sida soil, copper and zinc uptake, phytoremediation

Introduction

At present, bottom ash generated from power plant in Thailand (approximately 2,000 tons/day) has been a by-product that needed to dispose properly at landfill site (Rukzon & Chindaprasirt, 2006). Attempts has been made to use this bottom ash as mixing materials such as binder with cement (Rukzon, Phupha & Ngenprom, 2013),mixture of road construction materials (Chanhirun, 2013) or potential use as forest fertilizer (Nurmesniemi, Manskinen, Pöykiö, & Dahl, 2012). The component of bottom ash contains many chemical compounds including CaO, SiO_2 and heavy metals. The chemical constituents of bottom ash can vary, depending on the coal type, source and plant operating parameters. Major constituents include calcium (Ca), copper (Cu), aluminum (Al), iron (Fe), magnesium (Mg), potassium (K), silicone (Si), sodium (Na), and zinc (Zn) (Papadakis, 2000). Of these materials Ca, Cu, Fe, Mg, K and Si are essential for plant nutrients (Poon, Wong & Lam, 1997; Papadakis, 2000). However, some of the bottom ash chemical compositions are contained hazardous heavy metals such as Cu, Zn, Ni, Pb, Cd (Isgor & Razaqpur, 2004).

Heavy metals such as Cu and Zn present in bottom ash can contaminate in soil, and their concentrations as pollutants or nutrients in soils and plants depend on the concentration. The availability of Cu and Zn at low concentrations is beneficial for plants but it can be harmful at higher concentrations.

Lettuce (*Lactuca sativa* L.) is a kind of plant that most that Thai people prefer to eat or use as food ornament. This vegetable can accumulate relatively high amount of heavy metals such as Cu, Zn and Pb. Therefore, it is interesting to study this plant to make use of heavy metals contaminated soil as a phytoremediation of toxic metals which is now intensively investigated in plants (Raskin & Ensley, 2000). For soil amended with mine wastes, this plant accumulated significantly more metals than other species such as bean and tomato (Sathonsaowaphak, Chindaprasirt & Pimraksa, 2009).

Therefore, this research was focused on the amount of Cu and Zn in soils amended with lignite bottom ash and the uptake of lettuce plant in various parts (leaf and root). The levels to be mixed with the lignite bottom ash to gain the better lettuce yield were also determined.

Materials and Methods

Plant used in the experiment

Plant species used in this experiment was a lettuce (*Lactuca sativa* L.) which cultivated in plastic pots containing sida soil under laboratory conditions. Twenty lettuce seeds were cultivated and the seedling was allowed to be fully grown for twenty days prior to transfer for use in the experiment.

Preparation of Sida Soil added with Bottom Ash

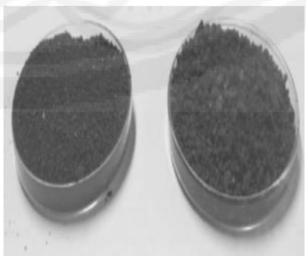
Sida Soil

Sida soil bought from Jatujak market in Bangkok was used for the study. Soil samples were prepared by allowing to air-dry for 7 consecutive days on a flat plastic sheet. Large aggregates of lignite bottom ash were crushed using a clean mortar and a rubber-tipped pestle, then sieved by 2 mm screen. The sieved material was homogeneously mixed. A splitter was used to obtain sub-samples of appropriate size.

Lignite Bottom Ash

The lignite bottom ash samples obtained from Mae Moh power plant in Lampang province operated by the Electricity Generation Authority of Thailand (EGAT) were used for the study. The lignite bottom ash was allowed to air-dry in an open room. After that, the coarse particles of sample were sieved with No.20 screen to obtain the homogeneous size prior to use in experiment.

a. Sida soil



b. Lignite bottom ash

Figure 1 Materials prepared for the experiment (a. Sida soil, b. Lignite bottom ash)

Mixture of Sida soil and bottom ash

Ratios of Sida soils mixed with bottom ash were prepared for planting lettuce by weight. Five ratios of mixture were tested in the experiment as 1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, and 0.6:0.4. The mixture ratio at 1:0 was served as a control (without waste) whereas the remaining ratios represented the gradual contamination of soil with the lignite bottom ash.

Determination of physical and chemical characteristics of mixture materials

The physical and chemical characteristics of mixture materials (sida soil mixed with bottom ash) were analyzed by the methods and equipment as following Table 1

Table 1 Parameter and test methods for sida soil, bottom ash and mixture of sida soil added with bottom ash.

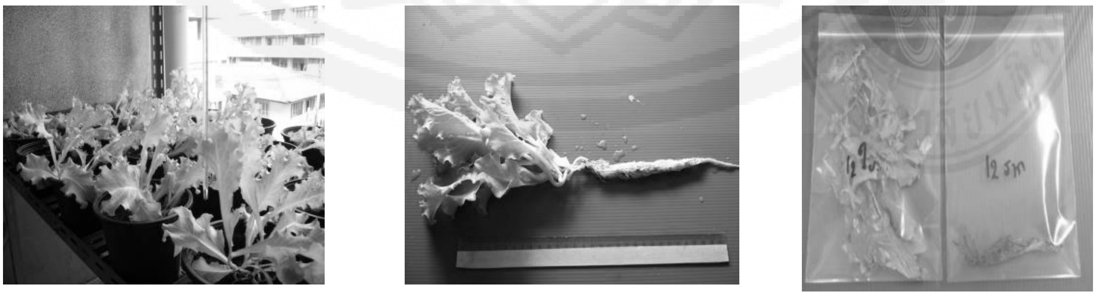
Parameter	Method/Equipment
Physical properties	
Moisture content	Gravimetric method
Chemical properties	
pH	1:10 water/soil, lignite bottom ash or mixture/ pH meter
% Organic matter	Walkey & Black method
Nitrogen	Kjedahl method
Phosphorus	Bray II Extract Test
Potassium	NH_4OAc 1N pH 7
Heavy metals (Cu, Zn)	HNO_3-HCl digestion and Atomic absorption spectrophotometer

Determination of copper and zinc accumulation in lettuce planted in mixture ratios.

Five mixture ratios of Sida soil mixed with bottom ash (1:0, 0.9; 0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4 by weight) were prepared for planting the lettuce in plastic pots. Each pot contained 500 g of mixture ratio.

Approximately, 20 seeds were randomly planted in a plastic pot and watered daily with the tap water. At day 20, four fully grown seedlings (each with 3 leaves and 10 cm. long) were selected and transferred to treat with mixture ratio of Sida soil added with lignite bottom ash (each at the distance of ca. 4.0 cm. in a plastic pot) for another 25 days before harvesting for heavy metal analysis (Figure 1). Treatment of each mixture ratio was replicated 4 times. Root and leaf of lettuce plant were separated and dried at 65^{0}C in the oven for 48 hr.

Dried lettuce plant was weighed, ground with agitating mortar, and kept in plastic bags for subsequent analysis. Amount of copper and zinc in lettuce parts was investigated by digesting with HNO_3-HCl and analyzed with Atomic Absorption Spectrophotometer.



a. harvested lettuce b. selected lettuce parts c. dried lettuce tissue

Figure 2 Harvested lettuce plant and selected parts for analysis (a. harvested lettuce, b. selected lettuce parts, c. dried lettuce tissue).

Analysis of lettuce yield

The lettuce yield after planting in soil mixed with lignite bottom ash was determined. Weight of dried lettuce tissue parts was recorded and analyzed.

Analysis of data

Results of chemical and physical characteristics of mixture materials including amount of copper and zinc in lettuce plant and lettuce yield were determined as mean and standard deviation. Data analysis was conducted using one-way ANOVA. The average amount of copper and zinc accumulated in lettuce parts and its yield at mixture ratios were compared using Fisher's Least Significant Difference Test (LSD) at α level of 0.05.

Results

Chemical and physical characteristics of mixture materials.

The characteristics of mixture ratios used for planting lettuce were analyzed (Table 1). The pH value of mixture ranged from 6.64±0.78 to 6.89±0.98. Significantly highest pH value ($P<0.05$) was found at ratio of 0.6:0.4 (6.89±0.98). While, moisture content of mixture ranged from 10.22±0.24 to 11.85±0.54%. The percentage of moisture content tended to decrease when the amount of lignite bottom ash in mixture decreased. At ratio of 0.6:0.4, the significantly lowest moisture content of the mixture (10.22±0.24%) was observed ($P<0.05$). In terms of organic matter in the mixture, it ranged from 1.86±0.85 to 5.04±0.38%. Similarly, the increment of lignite bottom ash in ratios tended to decrease in percentage of organic content. The significantly low organic matter at ratio of 0.6:0.4 and 0.7:0.3 was 1.86±0.85 and 2.45±0.18 %, respectively.

For the amount of macronutrients in mixture, results indicated that amount of nitrogen ranged from 0.31±0.03 to 0.45±0.04 %. The trend of amount of nitrogen at ratios obviously decreased as the amount of lignite bottom ash increased. Significantly lowest amount of nitrogen was observed at the ratio of 0.6:0.4 (0.31±0.03%) and 0.7:0.3 (0.35±0.03%). In addition, the percentage amount of phosphorus ranged from 0.45±1.35 to 0.56±1.94 %. A trend of increase of phosphorus was observed when the amount of lignite bottom ash increased. The significant higher percentage of phosphorus amount was noted at all ratios when sida soil was added with lignite bottom ash ($P<0.05$) than the control but no significant difference in the ratios was detected. In case of amount of potassium in mixture, the percentage of potassium in ratios ranged from 0.92±0.01 to 0.94±0.03 %. No significant differences in amount of potassium were detected in mixture ratios ($P>0.05$).

In addition, it revealed that the amount of copper in mixture ranged from 13.12±1.71 to 26.13±2.30 mg/kg. The significantly increasing amount of copper was observed when sida soil was added with lignite bottom ash ($P<0.05$). The highest amount of copper was found at the ratio of 0.6:0.4 (26.13±2.30 mg/kg). For the amount of zinc in mixture, result indicated that the trend of increasing amount of zinc was observed. The amount of zinc ranged from 66.84±5.84 to 137.74±4.13 mg/kg. The significantly highest amount of zinc ($P<0.05$) was found at the ratio of 0.6:0.4 (137.74±4.13 mg/kg).

Table 1 Average (±SD) of pH, moisture content and organic matter in mixture ratios (n=4).

Sida soil : Lignite bottom ash	Average (±SD)							
	pH	moisture content (%)	organic matter (%)	N (%)	P (%)	K (%)	Cu (mg/kg)	Zn (mg/kg)
1.0:0.0	6.64±0.78[a]	11.55±0.33[a]	5.04±0.38[a]	0.45±0.04[a]	0.45±1.35[a]	0.93±0.02[a]	13.12±1.71[a]	66.84±5.84[a]
0.9:0.1	6.71±0.57[a]	11.07±0.51[a]	4.85±0.74[a]	0.40±0.02[a]	0.56±1.94[b]	0.94±0.03[a]	17.15±1.88[b]	87.27±5.46[b]
0.8:0.2	6.78±0.22[a]	11.22±0.59[a]	4.24±0.36[a]	0.39±0.05[a]	0.56±1.11[b]	0.94±0.01[a]	17.77±1.12[b]	106.04±12.38[c]
0.7:0.3	6.85±0.18[a]	11.85±0.54[a]	2.45±0.18[b]	0.35±0.03[b]	0.52±2.41[b]	0.93±0.02[a]	19.23±2.80[b]	121.63±8.17[c]
0.6:0.4	6.89±0.98[b]	10.22±0.24[b]	1.86±0.85[b]	0.31±0.03[b]	0.51±3.64[b]	0.92±0.01[a]	26.13±2.30[c]	137.74±4.13[cd]

Note: The same letter in the column was not significantly different at the α level of 0.05 using LSD test.

Amount of copper and zinc accumulated in root and leaf of lettuce planted in mixture ratios.

Result of copper and zinc accumulation in lettuce planted in mixture ratios through the uptake process was illustrated in Table 2. The amount of copper accumulation in root at all ratios (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) was 9.85±1.35, 14.57±1.85, 14.86±1.12, 16.54±3.76 and 21.46±5.90 mg/kg, respectively.

Addition of bottom ash in Sida soil increased significantly amount of copper in lettuce root (P<0.05). Significantly highest copper accumulation was observed in lettuce root at the mixture ratio of 0.6:0.4 (21.46±5.90 mg/kg). For the copper accumulation in leaf, it showed that all ratios (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) were 2.92±1.05, 4.65±1.14, 5.53±1.08, 6.34±0.23 and 8.18±1.20 mg/kg, respectively.

Similarly, the addition of lignite bottom ash to Sida soil resulted in the significant increase of copper accumulation in lettuce leaf (P<0.05). It was noted that a significantly highest copper accumulation in lettuce leaf was observed at the mixture ratio of 0.6:0.4 (8.18±1.20 mg/kg).

Amount of zinc accumulation in root at all ratios (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) were 42.30±4.90, 58.07±8.06, 83.01±6.33, 105.47±4.77 and 113.47±4.13 mg/kg , respectively (Table 2). The accumulation of zinc in root increased significantly when the Sida soil was added with lignite bottom ash (P<0.05). At the mixture ratio of 0.7:0.3 and 0.6:0.4, the significant zinc accumulation evidence in root considerably increased (105.47±4.77 and 113.47±4.13 mg/kg, respectively) (Table 2).

Similar pattern of zinc accumulation in lettuce leaf at ratios (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) was observed which was 19.18±3.59, 21.12±1.77, 25.15±1.65, 30.564.54 and 32.94±7.34 mg/kg, respectively. In addition, it revealed that the mixture ratios of 0.7:0.3 and 0.6:0.4 showed the significantly higher zinc accumulation in lettuce leaf which was 30.564.54 and 32.94±7.34 mg/kg, respectively (P<0.05).

Table 2 Average (±SD) of copper and zinc accumulation in root and leaf at mixture ratios (n=4).

Sida soil : Lignite bottom ash	Average accumulation (±SD)			
	Copper		Zinc	
	Root (mg/kg)	Leaf (mg/kg)	Root (mg/kg)	Leaf (mg/kg)
1.0:0.0	9.85±1.35[a]	2.92±1.05[a]	42.30±4.90[a]	19.18±3.59[a]
0.9:0.1	14.57±1.85[b]	4.65±1.14[b]	58.07±8.06[b]	21.12±1.77[b]
0.8:0.2	14.86±1.12[b]	5.53±1.08[b]	83.01±6.33[c]	25.15±1.65[c]
0.7:0.3	16.54±3.76[b]	6.34±0.23[b]	105.47±4.77[d]	30.56±4.94[d]
0.6:0.4	21.46±5.90[c]	8.18±1.20[c]	113.47±4.13[d]	32.94±7.34[d]

Note: The same letter in the column was not significantly different at the α level of 0.05 using LSD test.

Yield of lettuce planted in mixture ratios

Results of lettuce yield from mixture ratios was summarized in Table 3. Addition of lignite bottom ash to sida soil at ratios showed the significant lettuce yield ($P<0.05$). The average of lettuce yield at ratios of 1.0:0.0, 0.9:0.1, 0.8:0.2, 0.7:0.30, and 0.6:0.4 was 1.31±0.03, 1.32±0.01, 1.43±0.06, 1.36±0.08, and 1.43±0.03 g/plant, respectively. The significantly higher yield was observed when the lignite bottom ash was added to sida soil ranging from 0.9:0.1 to 0.6:0.4 than the control. In addition, the highest lettuce yield was significantly found at the ratio of 0.8:0.2 (1.43±0.06 g/plant) and 0.6:0.4 (1.43±0.06 g/plant) ($P<0.05$) but no difference was detected between ratios.

Table 3 Average (±SD) of lettuce yield at various mixture ratios (n=4).

Sida soil : Lignite bottom ash	Lettuce yield (g/plant)
1:0	1.31±0.03[a]
0.9:0.1	1.32±0.01[b]
0.8:0.2	1.43±0.06[b]
0.7:0.3	1.36±0.08[b]
0.6:0.4	1.43±0.03[b]

Note: The same letter in the column was not significantly different at the α level of 0.05 using LSD test.

Discussion

Amount of macronutrients in Sida soil added with lignite bottom ash at mixture ratios.

In this experiment, a slight increasing of pH value in mixture materials was observed whereas the opposite result was obtained in both moisture content and organic matter. The increasing of soil pH at this level did not affect the uptake of nitrogen, phosphorus, potassium and macronutrients since the soil pH was still at best range for most crops (pH at 6.5-7.0) (Agri-Facts, 2003).

For plant nutrients, it has been known that macronutrients, i.e., carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, potassium, calcium and magnesium, are generally needed in plants in relatively high concentrations (Streeter & Kidder, 1997). Macronutrients are not important to a plant's metabolism more than micronutrients, but since they are needed in large amounts, deficiencies are more common with the macronutrients. From the result of experiment, application of bottom ash from Mae Moh power plant in Sida soil at all ratios seems to remarkably increase the value of phosphorus in the mixture materials than the control. The suitable concentrations of nitrogen, phosphorus and potassium in mixture ratio of 6.0:0.4 for planting lettuce were 0.31 ± 0.03, 0.51 ± 3.64 and 0.92 ± 0.01 %, respectively. This finding concentration corresponded with the report by Pichay (2016) on the requirements of fertilizer containing nitrogen, phosphorus and potassium for healthy lettuce plant.

In this experiment, the significant decrease of nitrogen amount was observed when the amount of lignite bottom ash increased in the mixture ratio. Whereas, no evidence of change in amount of potassium was observed in this experiment. The amount of lignite bottom ash in mixture ratios increased, the decreasing of nitrogen and increasing of pH were observed. This is due to the decreasing of Sida soil material (nitrogen) and increasing of bottom ash (alkaline properties). The occurrence of nitrogen immobilization is probably attribute to the influence of pH value under the pH conditions (Norman, 1931). Moreover, the addition of lignite bottom ash to the mixture significantly increased the amount of phosphorus as compared to the Sida soil (control); however, this phosphorus values at all ratios gradually decreased as ratios of bottom ash increased. It is probably due to the effect of pH value in the mixture materials. This finding corresponded with the suggestion of Sungthong (1996) that pH is not only major factors of plant growth, in other words, pH can indicate phosphorus solubility and include other micronutrients such as zinc and manganese as well. Phosphorus was very well solubility at pH about 6-7. At pH below 6, phosphorus can be fixed by Fe, Mn and Mg, on the other hand, calcium and magnesium can fix phosphorus at pH over 7. In addition, Krutkul (1988) also found that concentration of phosphorus was decreased as value of pH increased. This may indicate that lignite bottom ash which contains minor phosphorus content will influence to change in phosphorus level in the mixture.

Accumulation of copper and zinc on the lettuce parts.

In this experiment, the direct evidence of increasing amount of copper and zinc in lettuce was obtained with increasing ratios of lignite bottom ash that would obviously be the supplementary micronutrients to the soil. Copper and zinc are considered as micronutrients for crop because crop needed for small amount (Fernandes & Henriques, 1991). Copper is necessary for carbohydrate and nitrogen metabolism and, inadequate copper results in stunting of plants whereas zinc is an essential component of various enzyme systems for energy production, protein synthesis, and growth regulation if deficiency also exhibits delayed maturity in plants (Pichay, 2016). On the other hand, it could cause a deleterious health effect to consumer especially in human if crop plant accumulated copper over 20 mg/kg and zinc over 100 mg/kg issued by the Criteria of Food and Drug Administration (Tejavanija, 2003). In this study, the mixture ratios at 0.6:0.4 resulted in the copper and zinc accumulation in root exceeded the safe limit for consumption. Excessive amounts of copper for a long period result in anemia, liver and kidney damage, and stomach and intestinal irritation (Agency for toxic substances and disease registry, 2004). While excessive zinc could suppress the copper absorption, lower immunity and low levels of HDL cholesterol in human (Agency for toxic substances and disease registry, 2005).

The amount of copper accumulation in the root part was higher than that in leaf part at all ratios. This finding was similar to previous study of Orroño & Lavado (2009) on the heavy metal accumulation in *Pelargonium hortorum* and found that heavy metal accumulation appeared higher in roots than shoots. Low transport of heavy metal to shoots may be due to saturation of root metal uptake, when internal metal concentrations are high (Zhao, Lombi, & McGrath, 2003). At ratios of mixture, copper accumulated in root and leaf were significantly increased ($P<0.05$) as ratios of lignite bottom ash increased. The earlier investigation indicated that concentration of copper at 20-100 mg/kg could be toxic to plants (Kabata-Pendias, 1984). The observed value of 2.92 to 8.1 mg/kg copper content in lettuce leaf was found to be in a normal range as compared to other studies indicating that copper content in dry mater in lettuce was between 3.19 - 13.90 mg/kg (Furlani et al., 1978; Santos, Casali & Miranda, 1998). However, in the lettuce root planted in mixture ratios except for ratio of 0.6:0.4 (exceeded the safe consumption limit) of the copper concentrations were close to the limit for copper toxicity in plants issued by the Criteria of Food and Drug Administration (copper not exceeded 20 mg/kg) (Tejavanija, 2003).

Similar to copper accumulation, accumulated zinc in the root part was higher than in leaf. Moreover, accumulation of zinc in the root and leaf significantly arisen at the increase of amount of lignite bottom ash in Sida soil ($P<0.05$). The experimental mobility of metals in soil by Prasad (2004) indicated that some metals were more mobile than others, e.g. cadmium and zinc, while copper and lead were immobile and easily formed organic complexes with fulvic acid. In addition, the increasing of soil pH also caused the decreasing of metals plant uptake due to the solubility of exchangeable metal ions reduced when soil pH arisen (Prasad, 2004). According to the finding, although the pH value increased as the mixture ratio increased and it probably did not influence to decrease lettuce uptake copper and zinc as stated by Prasad (2004). This is probably due to the added amount of lignite bottom ash has a slight influence to remarkably increase of pH in soil enough to decrease copper and zinc uptake by lettuce plant. Although, no significant difference for copper accumulation at mixture ratios of 0.7:0.3 and 0.8:0.2 in plant plants were noted. The different amount of heavy metal accumulation seemed to appear in the experiment. This occurrence is probably due to the limited mixture ratio was used in the experiment.

The movement of elements into roots occurs either by passive diffusion through the cell membrane, or by the more common process of active transfer against concentration and/or electrochemical potential gradients. The foliar uptake is another route of entry of metals into plant cells. The entry of metals into plant cells through leaves is of particular significance from the pollution point of view because of aerosol deposit (Prasad, 2004). Metal accumulation depends on both uptake into the tissue and leakage into the surrounding medium. Metals are first taken into the apoplast of the roots. Then, some of the total amount of the metal is transported further into the cells, some is transported further in the apoplast, and some becomes bound to cell wall substances (Prasad, 2004). The movement of metals from the external solution into the cell walls is a non-metabolic, passive process, driven by diffusion or mass flow (Marchner, 1995). Part of metal that has been taken into the apoplast of roots is further transported through the plasma membrane into the cytoplasm.

For accumulation of heavy metals in plants, they can be tolerant or highly reactive, resulting in toxicity of plant cells in many ways. At the cellular and molecular level, heavy metal toxicity results in alterations of different plant physiological processes, including inactivation and denaturation of enzymes, proteins, blocking of functional groups of metabolically important molecules, displacement/substitution of essential metal ions from biomolecules and functional cellular units, conformational modifications and disruption of membrane integrity (Villiers et al., 2011; Ramesh, 2008) which is finally attributed to altered plant metabolism, inhibition of photosynthesis, respiration, and alerted activities of several key enzymes (Hossain, Hossain & Fujita, 2009; Hossain, Hasanuzzaman & Fujita, 2010; Sharma & Dubey, 2007; Sharma & Dietz, 2009).

In general, metals are taken up in cationic forms, except for molybdate, which is taken up as molybdate anion (Prasad, 2004). The net uptake of copper could be composed by a low and high affinity biphasic copper uptake system based on Michaelis-Menten kinetics and an ATP-depended efflux of copper (Knauer, Behra & Sigg, 1997; Van Hoof et al., 2001). Whereas, zinc transporters, with a higher abundance in zinc accumulators species than in non accumulator species (Lasat, Pence, Garvin, EBbbs & Kochain, 2000). Zinc is also shown to be actively transported as a free ion across the tomoplast, depending on the tolerance of the plant and there are two or more parallel pathways (Chardonnens, Koevoets, van Zanten, Schat, & Verkleij, 1999).

Effect of copper and zinc on lettuce yields.

From the results, all lettuce yields grew from all ratios of mixture had more weight than those from control. This is probably because the bottom ash can influence to soil aggregation. The soil aggregation has effect as (1) can help root spread (2) increase soil aerator (3) increase water efficiency, and (4) induce microorganism activity for release nutrients (Faculty of the Department of Soil Science, 1998). Moreover, bottom ash contains copper and zinc that are the essential micronutrient elements for plant. A deficiency of copper interferes with protein synthesis and causes a buildup of soluble nitrogen compounds in the plant (Streeter & Kidder, 1997). In addition, the availability of zinc will control the synthesis of indole acetic acid, which dramatically regulates plant growth (Streeter & Kidder, 1997). Thus, the addition of lignite bottom ash to mixture ranging from 0.9:1 to 0.6:0.4 was obviously increased the amount of copper and zinc required for the growth and yield of lettuce plant. The mixture ratio of 0.6:0.4 was the most suitable for bottom ash waste treatment because the higher heavy metal accumulation was observed in lettuce plant and also met the Criteria of Food and Drug Administration that was safe for consumers.

Conclusion

The pH value of mixture materials ranged from 6.64±0.78 to 6.89±0.98. The percentage of moisture content and organic content ranged from 10.22±0.24 to 11.85±0.54% and 1.86±0.85 to 5.04±0.38 %, respectively. Amount of nitrogen, phosphorus and potassium ranged from 0.31±0.03 to 0.45±0.04 %, 0.45±1.35 to 0.56±1.94 %, and 0.92±0.01 to 0.94±0.03 %.

The amount of copper and zinc ranged from 13.12±1.71 to 26.13±2.30 mg/kg and from 66.84±5.84 to 137.74±4.13 mg/kg, respectively. The highest copper and zinc accumulation was found at mixture ratio of 0.6:0.4 in lettuce root (21.46±5.90 mg/kg and 113.47±4.13 mg/kg), followed by lettuce leaf (8.18±1.20 mg/kg and 32.94±7.34 mg/kg). The accumulation of both heavy metals in root and leaf of lettuce was significantly different as bottom ash ratio increased ($P<0.05$). The highest lettuce yield was found at both ratio of 0.8:0.2 and 0.6:0.4 with 1.43±0.06 and 1.43±0.03 g/plant, respectively. A significant difference in lettuce yield with the increase of bottom ash ratio was observed ($P<0.05$).

In conclusion, results suggest that the suitable mixture ratio for lettuce planting will be at 0.6:0.4. This is due to its ability to accumulate high copper and zinc, and high yield production. Also, it could contain the concentration within the standard and is able to use for phytoremediation and consumption. However, the application for phytoremediation to treat copper and zinc in contaminated land still requires further investigation.

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