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Heavy Metals Contents in the Edible Parts of Some Vegetables Grown in Candi Kuning, Bali and Their Predicted Pollution in the Cultivated Soil

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Abstract
Vegetables are important for human diet due to their contents including mineral, vitamin, carbohydrate, protein and fibers, but they often associate with poison such as heavy metals derived from lands where the vegetables are grown. This brings us to investigate the contents of some heavy metals, namely Pb, Cu, Cr, and Zn, in edible parts of 10 species vegetables cultivated in Candi Kuning, Bali and to predict the level of pollution in the cultivated soils. Wet digestion method was involved to prepare the samples before determination of the metals using Atomic Absorption Spectrometer (AAS). The concentrations of heavy metals in the edible parts of the vegetables were found vary depended on the species and types of the edible parts. The heavy metals contained in edible parts of the ten species of vegetables were approximately in the range of 11.11 to 374.55 mg/kg for Pb; 4.34 to 150.15 mg/kg for Cu; ND (not detectable) to152.82 mg/kg for Cr; and 5.12 to 90.69 mg/kg for Zn. Most of those levels are higher than the permissible-maximum limit regulated by FAO/WHO. Therefore, the agricultural soils in Candi Kuning which is one of the vegetable production centers in Bedugul area of Bali for cultivation of the vegetables could be predicted that were generally polluted by the metals. Lead and chromium were the metals that cause high level of pollution, while copper and zinc were less polluted in the soil.
Keywords: heavy metals, vegetables, agricultural soils, pollution

1. Introduction

Vegetables are sources of mineral, vitamin, carbohydrate, protein and fibers; therefore they are important for human diet. Although the vegetables are very useful for human body, they often act as media carrying poison, such as pesticide residues and heavy metals derived from lands where the vegetables are grown. The contamination can be resulted from the excessive use of inorganic fertilizers and pesticides, emission gas from motor vehicles, and from other sources.

During the last decades, researchers reported that so many types of vegetables such as spinach, carrot, lettuce, radish, tomato, and chickpea were contaminated by heavy metals of Pb, Cd, As, Fe, Mn, Cu, dan Zn (Cobb 2000; Arora *et al.* 2008; Astawan 2008). The high levels of heavy metals contained in vegetables were grown in polluted area or agricultural soils, such as soils located close to roadway, nearby smelter, and soils irrigated with wastewater (Cui *et al.* 2004; Kachenko and Singh 2004; Okoronkwo *et al.* 2005; Astawan 2008; Behbahaninia and Mirbagheri 2008). Vegetables, particularly leafy crops, grown in polluted soils were found to contain higher concentration of heavy metals than those were grown in unpolluted soils (Voutsa *et al.* 1996).

Heavy metals in environment are important hazard for human health due to their properties such as persistent, bio-accumulative, and toxic to plants, animals, and humans (Yap *et al.* 2009). Plants accumulating toxic metals at high concentration are serious risk to animals and human health when they are consumed (Widowati 2011). The excess of heavy metals in human bodies can cause anemia, kidney damage, lung disease (*e.g.* bronchitis and pulmonary oedema), nervous system disorders, hyperactivity, hypertension, behavioral changes, infertility to male, cancers (*e.g.* lung, prostate, and kidney), and even cause death (Radojević and Bashkin 1999). The maximum limits of Pb, Cu, Cr, and Zn in vegetables proposed by FAO/WHO are: 0,3; 40; 0,05; and 100 mg/kg respectively (Codex 2001). In our study, these heavy metals were investigated for their contamination in ten different species of vegetables grown in Candi Kuning of Bedugul area, Bali-Indonesia.

Vegetables cultivated in agricultural lands of vegetable production center in Candi Kuning of Bedugul area have taken a serious concern with regard to the concentrations of heavy metals since the agricultural lands has been exploited by using agrochemicals for long periods. According to the leader of farmer's group, the farmers tend to use an excessive inorganic fertilizers and synthetic pesticides to protect the crops without concerning their effects to the agricultural soils and human health. The use of fertilizers either inorganic or organic manure and synthetic pesticides in uncontrolled manner can contaminate the soils and crops, especially by toxic chemicals including heavy metals such as As, Pb, Cd, Cu, Co, Cr, Mo, Sr, Ti, V, Mn, Fe, Ni, Hg, Ba, Sc, dan Zn (Alloway 1990; Gimeno-Garcia *et al*. 1996; Taylor and Percival 2001; Curtis and Smith 2002; He *et al*. 2005; Papafilippaki *et al*. 2007; Karyadi 2008). Besides that, Bedugul is known is one of popular tourist destinations in Bali, especially during weekends and holidays. Hence, air pollution derived from vehicular emission is a potential contaminant for the soils, as well as for the crops.

Plants can be used for monitoring of metal pollution (Cataldo and Wildung 1978). The pollution in local specific area depends on the sources of pollution. Some researchers suggested that plants growing in polluted environment can be accumulated by the high concentration of toxic metals (Alloway 1990; Voutsa *et al*. 1996). Vegetables such as lettuce, radishes, bush beans, and tomatoes cultivated in mine waste and in waste-amended soils were accumulated Pb, Cd, As, and Zn at high concentrations (Cobb 2000). It is reasonable that by knowing the contents of heavy metals in various types of vegetables produced in the certain area, the level of pollution in the agricultural soils in that area can be predicted. This study investigated the contamination of Pb, Cu, Cr, and Zn in the 10 vegetables species and estimated the levels of contamination in the agricultural soils of Candi Kuning located in Bedugul area, Bali-Indonesia. Therefore, this study was expected to provide information of heavy metals pollution in this area, so further research can be carried out on the metals contained in the soils, vegetables, and bioavailability of these metals in the soils.

2. Materials and Methods

2.1 Chemicals and Apparatus

All chemicals used for experiment were analytical grades. The stock solutions of 1,000 mg/L Pb, Cu, Cr, and Zn standards were prepared from $Pb(CH_3COO)_2$, $CuSO_4.5H_2O$, $K_2Cr_2O_7$, $ZnSO_4.7H_2O$ respectively. Digested solution of reverse aqua regia was made by mixing HNO_3 and HCl (3:1). Dilute solutions were prepared with distilled water, and all filtrations were carried out with Whatman No. 42 filter paper.

All flame-AAS measurements were performed using a Shimadzu Model AA-7000 operated in a double beam mode, and the background correction was accomplished with a deuterium lamp. Ultrasonic bath (Elma S 450H, Elmasonic) and a hotplate were used for sample digestions.

2.2 Study Site and Sampling

Candi Kuning is one of the vegetable production centers of Bedugul area in Bali was selected as a site of study. Sampling was carried out on the dry season (July-August 2012). Ten types of harvested vegetables as samples for the study were collected directly from the cultivated lands laid in Candi Kuning village. Each type of vegetable was collected randomly from three different cultivated lands. The vegetables analyzed include spring onion, cabbage, lighter colored mustard greens, potatoes, lettuce, broccoli, celery, carrot, tomato, and capsicum. The edible parts of the vegetables were cleaned to remove visible particles contaminants and were further taken to the laboratory for measurement of heavy metal contaminants.

2.3 Sample Pretreatment

All the collected vegetable samples were washed with tap water and thereafter with distilled water to remove pollutants of airborne and pesticide residues on the surface of the samples. The washing was performed in order to measure the only contaminated heavy metals inside the tissues of the edible parts. The edible parts samples were cut into small pieces and were then oven-dried in hot air oven at up to 60 ^oC before being blended by a blender. A 63 µm nylon mesh screen was used for sieving the powdered samples to separate the coarse and fine fractions. The homogeneity fine fractions were stored in a 100 cm^3 screw capped plastic jar prior to analysis.

2.4 Sample Digestion

One gram of each fine powdered sample was accurately weighed out into a 50 mL of digestion glass tube and mixed with 10 mL of concentrated nitric acid and concentrated hydrochloric acid in the ratio 3 : 1 (reverse aqua regia). The mixtures of samples were digested by using an ultrasonic bath at 60 ^oC for 45 minutes followed by a hotplate treatment at 140 ^oC for 45 minutes (Siaka *et al*. 1998). The digestion of samples was performed in triplicate. The digest was diluted with 10 mL of distilled water and quantitatively transferred into 50 mL volumetric flask and then diluted to the mark with distilled water. Each sample solution was filtered through a filter paper (Whatman No. 42) into a polyethylene bottle. These sample filtrates were stored in room temperature for further analysis.

2.5 Instrumental Analysis

The determination of Pb, Cu, Cr, and Zn was performed by using flame-AAS. Working standards were also prepared by diluting 1,000 ppm stock solution of each of the metals. A calibration curve for each metal was constructed by plotting absorbance versus concentration. The concentrations of the metals in the sample filtrates were determined by interpolating each absorbance to the calibration curve.

3. Results and Discussion

3.1 Heavy Metals Concentrations in Edible Parts of the Vegetables

The concentrations of heavy metals in edible parts of the vegetables varied depended on the species of the vegetables. Lead (Pb) accumulated in each vegetables, was found much higher than the maximum permissible value (0.3 mg/kg) regulated by FAO/WHO (Codex, 2001) of which is might be a threat for the consumers. The concentration of lead obtained ranged from 11.11 to 374.55 mg/kg (as shown in Table 1). The highest level of lead was found in cabbages. The concentrations of lead in all vegetables can be ordered as follows: cabbage > light color mustard green > lettuce > broccoli > spring onion > potatoes > carrot > capsicum > celery > tomato. In contrast, copper (Cu) accumulated in the most vegetables was below the maximum limit (40 mg/kg) regulated by FAO/WHO, except the concentrations in cabbage, light color mustard green, and lettuce of which were found 150.15, 76.69, and 73.22 mg/kg respectively (Table 1). The lowest below limit concentration of Cu was obtained in celery. The highest level of Cu was found in cabbages. The order concentration of copper in the edible part of the vegetables was similar to that of lead.

The concentrations of chromium (Cr) in all types of vegetables were above the maximum permissible value (0.05 mg/kg), except in tomato and celery. The concentrations of chromium in the both vegetables were not

Table 1. The Average Concentration of Heavy Metals in Edible Parts of Various Vegetables

Sample	Pb (mg/kg)	SD	Cu (mg/kg)	SD	Cr (mg/kg)	SD	Zn (mg/kg)	SD
Spring onion	14.23	0.02	4.69	0.12	73.54	1.05	49.60	0.09
Cabbage	347.54	2.90	150.15	0.42	152.82	15.53	28.58	0.14
Mustard greens	232.21	5.59	76.70	0.60	99.36	0.83	14.38	0.42
Potatoes	13.42	0.00	9.44	0.11	ND	-	9.34	0.16
Lettuce	167.34	5.06	73.22	0.03	136.70	1.67	90.69	0.87
Broccoli	14.69	0.03	21.70	0.51	1.02	0.19	76.04	0.73
Celery	11.62	0.01	4.34	0.00	ND	-	5.12	0.82
Carrot	13.13	1.15	5.09	0.00	2.78	0.19	25.33	0.33
Tomato	11.11	0.00	5.11	0.00	1.41	0.04	25.02	0.86
Capsicum	13.02	0.07	9.00	0.13	7.29	0.04	29.13	0.12

ND = not detectable   n = 3

detectable. The levels of chromium in the vegetables were varied following the order of cabbage > lettuce > light color mustard green > spring onion > capsicum > carrot > tomato > broccoli > potatoes = celery.

The levels of zinc (Zn) in the edible parts of vegetables were found in the range of 5.12 to 90.69 mg/kg. These levels were tolerable or below the maximum permissible value (100 mg/kg) (Codex 2001). The levels of zinc in edible parts of vegetables were following the order of lettuce > broccoli > spring onion > capsicum > cabbage > carrot > tomato > light color mustard green > potatoes > celery.

The differences between concentrations of heavy metals accumulated and distributed in each edible part of vegetables were specifics of the type and part of vegetables, as well as type and bioavailability of heavy metals in the soils (Filipović-Trajković *et al*. 2012). Different plant species had varying abilities to take up and accumulate metals. It was reported that heavy metals concentrations in roots and leaves were not significantly different since the metals were in equilibrium between roots and leaves (Remon *et al*. 2005). This means that analyzing heavy metals in the parts of any vegetables could give information about soil pollution at where the vegetables are cultivated.

3.2 Pollution Prediction in Vegetable Agricultural Soils

Based on the accumulation of heavy metals (Pb, Cu, Cr, and Zn) in various vegetables found in this study, it could be used to predict the level of pollution in soils of vegetable lands in the Candi Kuning area. Some research findings indicated that vegetables grown in polluted area would contain heavy metals in relatively high (Voutsa *et al*. 1996; Cobb 2000; Okoronkwo *et al*. 2005; Astawan 2008). Below is explained the possible of the each studied heavy metals polluted the area.

3.2.1 Pollution of Pb

Lead (Pb) was accumulated in all studied species of harvested vegetables in the area of Candi Kuning. The levels were above the maximum permissible value (Codex 2001). This indicated that the agricultural soils of the vegetables were polluted by lead. Almost a similar report stated by Sharma *et al.* (2006) that the vegetables grown in wastewater industrial area contained Pb above the safe limit. Other researchers also reported that the vegetables grown in polluted areas such as industrial and rural areas or irrigated with polluted water including mixtures of wastewater and sewage sludge contained Pb in the level higher than the safe limit (Fytianos *et al*. 2001; Muchuweti *et al*. 2006).

The main source of Pb pollution in this area may derive from vehicular emission of tourist or visitor transportation. The pollution indicated by the high level of Pb contained in the studied vegetables, namely cabbage, light color mustard green, and lettuce (as shown in Figure 1) of which are mainly cultivated close to the main road. A similar finding was reported by Astawan (2008) which stated that the vegetables grown at the roadside area contained Pb higher than that of grown far away from the road. Lăcătuşu and Lăcătuşu (2008) reported that leafy vegetables (lettuce, parsley, dill, and orach) grown in polluted areas in Romania contained Pb 17 times the maximum allowable limit. Contamination of Pb can also be derived from fertilizers applied in the agricultural practices (Alloway 1990; Karjadi 2008). The use of excessive pesticides increased the Pb content in agricultural soils between 0.59 and 0.86 mg/kg (Ogunlade and Agbeniyi 2011).

Our study found that the concentration of Pb contained in each edible part of 10 types of vegetables was above WHO/FAO maximum limit. Therefore, it is believed that the agricultural soils to cultivate the selected vegetables in Candi Kuning of Bedugul area have been polluted by the Pb. A major pathway of the pollution is through atmospheric deposition of Pb from point source motor vehicles emissions. Other sources of Pb pollutant in the agricultural soils could be from the inputs used in the cultivation activities such as inorganic fertilizers, pesticides, organic manures, and composts (Alloway 1990; Gimeno-Garcia *et al*. 1996; Karyadi 2008).

3.2.1 Pollution of Cu

The concentration of Cu in edible parts of the selected vegetables was presented in Table 1 and Figure 1. The accumulation of Cu in the edible parts was found very high on the vegetables, namely cabbage, mustard greens, and lettuce. These concentrations were higher than the safe limit (40 mg/kg) published by WHO/FHO, while the concentrations in other selected vegetables were below the permissible value.

Interestingly, only leafy vegetables with large surface-volume ratio contained high concentration of Cu, while in the others such as roots, fruits and flower vegetables contained Cu below the safe limit concentration. Kanakaraju *et al*. (2007) reported that leafy type vegetables accumulated higher level of metals compared to fruit types. Different parts of plants have different ability in accumulating heavy metals (Remon *et al*. 2005). Besides the ability of plants to up take the heavy metals, mobility and bioavailability of the metals in the soils can also influence the number of heavy metals absorbed and accumulated in the plants.

The location sites at where the vegetables were grown influence the concentration level of Cu in vegetables (Osma *et al*. 2012). The high level of Cu was found in the vegetables of cabbage, parsley, and chard grown in industrial and the roadside sites.

Our Study found that only three types of vegetables, namely cabbage, mustard greens, and lettuce contained high level of Cu. It can be suggested that the agricultural soils cultivated by those three types of vegetables were highly contaminated by Cu. The soils that are cultivated by other crops could be polluted by Cu, but due to the different abilities of the crops in absorbing the heavy metals from the soils, the Cu in the edible parts of the vegetables may be not detected. Therefore, it can be predicted that the agricultural lands of Candi Kuning are mostly polluted by Cu.

3.2.3 Pollution of Cr

The chromium content in the edible parts of most selected vegetables exceeded the maximum permissible value (0.05 mg/kg), except potatoes and celery (Table 1). The significant high concentration of Cr was recorded in leafy vegetables, namely lettuce, cabbage, mustard greens, and spring onion. The ability of different crops in accumulating heavy metals influences their concentrations in the edible parts (Remon *et al*. 2005). Other vegetables might be also accumulated very high Chromium in the non edible parts. It can be suggested that the Cr contained in the vegetables was derived from polluted soils. Therefore, the agricultural soils of Candi Kuning area were predicted to be highly polluted. This can be seen on the Cr content in the vegetables which were 20 to 3056 times higher than the WHO/FHO safe limit.

The source of Cr in the soils of agricultural lands in Candi Kuning was probably from intensive use of agrochemicals (inorganic fertilizers and synthetic pesticides) for a long period. Another possible source of pollution is from the combustion of motor vehicles, especially the agricultural lands in the vicinity of the roadside. A study reported by Osma *et al*. (2012) that the higher Cr concentrations were found in vegetables grown in the vicinity of the roadside.

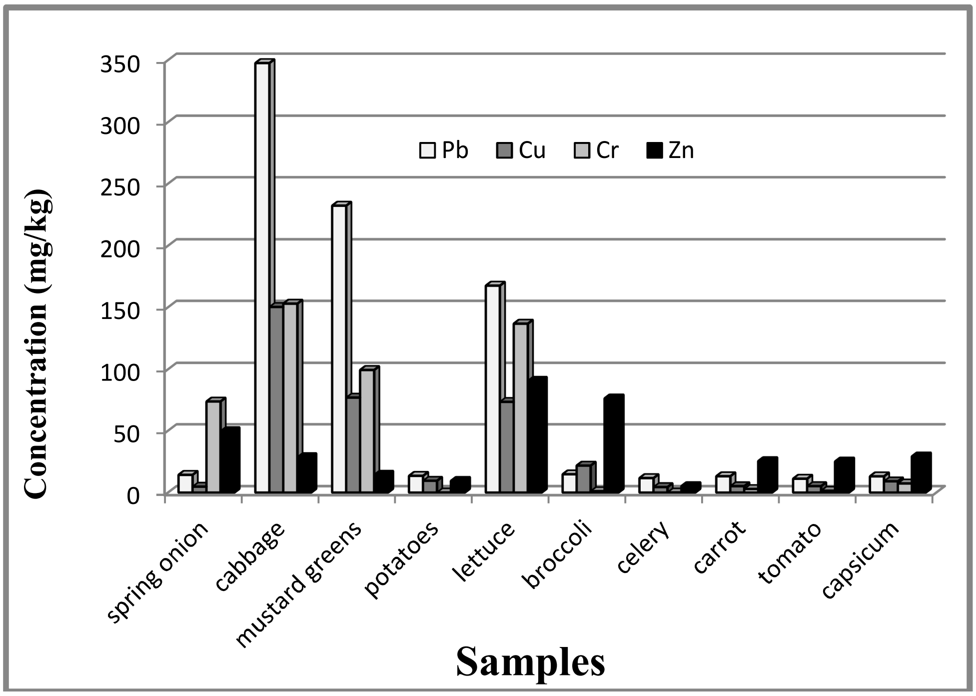

Figure 1. The levels of heavy metals in edible parts of the selected vegetables

3.2.4 Pollution of Zn

The level of heavy metals accumulated in different edible parts was vary depended upon the vegetables tested (Table 1). In the case of zinc (Zn), it varied in the range of 5.12 to 90.69 mg/kg dry weight of edible parts. This range is still below the permissible value (100 mg/kg) (Codex 2001). However, the maximum value of finding is relatively high since the Zn is an essential metal and only part was probably used in metabolism process in plants. The unused Zn still remained in the edible part of the vegetables. Based on the maximum allowable limit, the Zn could be predicted that the lands were less polluted by the metal. Zn contaminated in vegetables had a positive significant correlation with Zn level in the soil (Sharma *et al.* 2006). However, the applications of fertilizers which contain Zn as a micro nutrient probably were not efficient.

5. Conclusion

Heavy metals contained in edible parts of various vegetables are varied depended upon the species of the vegetables. Different species have different ability to absorb the heavy metals since their amounts use for metabolic process in different plants are also vary. The concentrations of heavy metals in 10 types of the selected vegetables were in the range of 11.11 to 374.55 mg/kg for Pb; 4.34 to 150.15 mg/kg for Cu; ND to 152.82 mg/kg for Cr; and 5.12 to 90.69 mg/kg for Zn. Vegetables containing relatively high concentrations of all heavy metals were found in the leafy vegetables, namely: cabbages, mustard greens, and lettuce except Zn in lettuce, broccoli, and spring onion. Each concentration of the metals in the edible parts of the vegetables was higher than the permissible limit regulated by FAO/WHO. The agricultural soils to cultivate the selected vegetables in Candi Kuning of Bedugul area might be mostly polluted by lead and chromium. Lead and chromium were the metals that cause high level of pollution, while copper and zinc were less polluted in the soils. The results of this study could give an illustration that the high content of heavy metals in vegetables reflected a high content of those in the lands as well. Thus, it can be predicted that the level of pollution in the agricultural soils of Candi Kuning in Bedugul area were generally quite high.

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