



DETECTION OF LEAD (Pb) AND PESTICIDE RESIDUES IN THREE KINDS OF LEAFY GREEN VEGETABLES IN PERI-URBAN AGRICULTURE, SURABAYA, INDONESIA

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ABSTRACT

This research was conducted in three peri-urban agricultures in Surabaya, namely Rungkut Subdistrict, Lakarsantri Subdistrict, and Sambikerep Subdistrict. The vegetables examined in this research are spinach (*Amaranthus* sp.), water spinach (*Ipomoea reptans*), and mustard greens (*Brassica juncea* L.). This research applied cross-sectional descriptive analysis method. The parameters measured in this research are lead (Pb) content, organophosphate pesticide residue level, chlorophyll level, water content, and pH of leaves. The result of lead (Pb) content analysis indicates that the lead content of spinach, water spinach, and mustard greens, cultivated in peri-urban agricultures in Surabaya, is still below maximum metal contamination rate published by National Standardization Agency. Similarly, the result of pesticide residue level indicates that there is no pesticide residue detected on spinach, water spinach, and mustard greens cultivated in peri-urban agricultures in Surabaya. The findings of this research indicate that chlorophyll level, water content, and pH of vegetables cultivated in peri-urban agricultures in Surabaya are lower than those of organic vegetables. Despite the fact that there was no strong correlation among these variables, there is a positive correlation between lead (Pb) content and water content and pH, while lead (Pb) content is negatively correlated with chlorophyll level.

Keywords: vegetables, peri-urban agriculture, lead (Pb), pesticide residue.

INTRODUCTION

Vegetables as one of horticultural commodities have the potential to be developed in both urban and periurban agricultures, however, vegetables cultivated along roadsides are prone to being highly exposed to heavy metal. The latest data shows that lead (Pb) content in caisim (mustard greens) cultivated along roadsides may reach 28.78 ppm (Widaningrum, *et al.*, 2007). It is higher compared to the vegetables cultivated far from roadsides (about 0-2 ppm). In the mean while, the maximum residue limit allowed by the Directorate General of the National Agency for Drug and Food Control (POM) is about 2 ppm for foods.

Environmental pollution due to the development of modern human's technology and activity has the potential to be accumulated in agricultural commodities. Heavy metal Pb is known to be accumulated in spinach, water spinach, and mustard greens (Rukaesih, 1993). Heavy metal Pb can be accumulated in carrot as well (Priandoko *et al.*, 2011).

It is known from several researches that there is a tendency for chlorophyll level to decrease along with the increase of heavy metal content. There is a correlation between heavy metal concentration and alteration of total chlorophyll in leaves, where total chlorophyll will decrease along with the increase of heavy metal content (Olivares, 2003). Excessive Pb content in vegetables can reduce the intake of Mg and Fe, leading to the change in the volume and amount of chloroplast (Kovacs, 1992). Many researches state that air pollutant leads to the change in ascorbate level, chlorophyll level, water content and pH of leaves (Pawar, *et al.*, 2010; Govindaraju, *et al.*, 2010; Meletiou-Christou, *et al.*, 2011). Previous research on

several types of leafy greens cultivated in periurban agricultures in Surabaya shows the chlorophyll level, carotenoids, and vitamin C that are not significantly different with those of organic vegetables (Iriyani and Nugrahani, 2013).

METHODOLOGY

The research was conducted in Surabaya from July to October 2014. Three types of vegetables (spinach, water spinach dan mustard greens) were analysed for their lead (Pb) content and pesticide residue. The vegetables came from three locations of periurban agricultures in Surabaya, namely Rungkut Subdistrict, Lakarsanti Subdistrict, and Sambikerep Subdistrict, as well as organic farmers in Pacet Mojokerto.

This research applied cross-sectional descriptive analysis method. Leaves were randomly taken from the group of objects to be the samples. In each research location, three types of vegetables (spinach, water spinach, mustard greens) were decided, with three turns each. Therefore, the number of leaves taken from a location was $4 \times 3 \times 3 = 36$. Afterward, about 100 grams of leaves positioned in the centre were chosen to be analysed for lead (Pb) content, pesticide residue, water content, chlorophyll level and pH of leaves.

Data collection

The parameters measured in this research are lead (Pb) content, organophosphate pesticide residue level, chlorophyll level, water content, and pH of leaves.



a) Measurement of lead (Pb) content

Analysis of lead (Pb) content was conducted using microwave digestion and Inductive Coupled Plasma Mass Spectrometry (ICP-MS),

b) Measurement of organophosphate pesticide residue

Analysis of organophosphate pesticide residue was conducted using HPLC with Triple Quadrupole Tandem Mass Spectrometry detector.

c) Measurement of water content

Water content is measured based on gravimetric method (Balittanah, 2005). The equation is: Water content (%) = $(fw - dw) / fw \times 100$. Fw is the fresh weight of the leaves and dw is the dry weight of the leaves. Leaves were heated to a temperature of 105° C for 4 hours to dispose the water. Water content from the sample was obtained from weight difference after and before the leaves were dried.

d) Measurement of chlorophyll level

Analysis of chlorophyll level was conducted using spectrophotometer based on the procedures proposed by Hendry and Grime (1993) as follows: about 0.5 grams of fresh leaves were chopped and extracted with 50 ml of 80% acetone. They were ground in a mortar until chlorophyll dissolved in 80% acetone, leaving white dregs behind. The extract was filtered by inserting Whatman 41 filter paper into 50 ml measuring cup. The liquid was centrifuged for 15 minutes at a speed of 2500 rpm. Measurement of Optical Density (OD) was conducted at a

wavelength of 645 nm and 663 nm. Total chlorophyll level in leaves (in mg/g leaf weight) was measured by comparing OD at a wavelength of 645 nm and 663 nm based on the equation:

$$\text{Total chlorophyll} = [20,2 (D_{645}) + 8,02 (D_{663})] \times 10^{-1}$$

e) Measurement of pH

Analysis of pH was conducted using pH meter as follows: about 4 grams fresh leaves were crushed with 40 ml aquadest in blender, then extracted and centrifuged at a speed of 2.000 rpm for 2 minutes. pH was measured from the extract using pH meter calibrated with pH 7 buffer (Apriantono, *et al.*, 1989).

Data analysis

To study the difference between groups, analysis of variance (Anova) was conducted. Further, to study the correlation between variables, Pearson correlation coefficient was conducted.

RESULT AND DISCUSSIONS

Lead (Pb) content

The accumulation of heavy metal like Pb, Cd, and Hg in vegetables may result from industrial wastes in water, or contamination of smokes produced by factories and vehicles that will enter the cycle of the food chains and will be accumulated in higher level, i.e. in human and animal (Widaningrum, *et al.*, 2007). The result of researches on the contamination level of heavy metal notably varies, depending on the type of the contaminant.

Table-1. Pb content in vegetables from several Periurban Agricultures in Surabaya.

Location	Pb content (mg/kg)		
	Spinach	Water spinach	Mustard greens
I	0.191 ± 0.006	0.253 ± 0.005	0.211 ± 0.003
II	0.217 ± 0.012	0.260 ± 0.010	0.301 ± 0.002
III	0.189 ± 0.001	0.244 ± 0.004	0.190 ± 0.002
average	0.199 ± 0.014	0.251 ± 0.009	0.233 ± 0.051
BMR (ppm) (SNI 7387:2009)	0.5	0.5	0.5

The research on the level of heavy metal Pb in spinach, water spinach and mustard greens cultivated in several periurban locations in Surabaya shows that water spinach contained the highest Pb, about 0.251 mg/kg, while spinach contained the lowest Pb, approximately 0.199 mg/kg (Table-1).

The content of heavy metal Pb found in spinach, water spinach and mustard greens cultivated in periurban agricultures in Surabaya is still below Maximum Residue Limit (BMR) of metal contamination published by National Standardization Agency (BSN) 7387 in 2009, i.e. about 0.5 mg/kg (Figure-2).

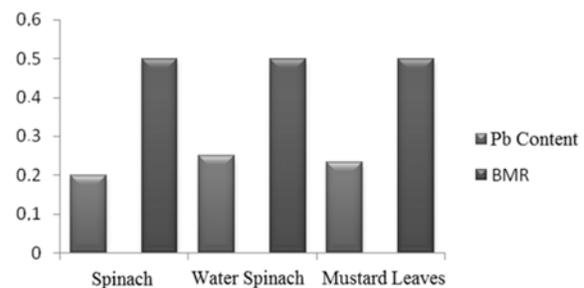


Figure-1. Pb content in vegetables.



It is presumably due to the location of cultivation that is far from roadsides. Comparing the location of cultivation, the highest Pb content is found in the vegetables (spinach, water spinach, and mustard greens) cultivated in Location II, Lakarsantri Subdistrict. This location is the closest to the roadsides. The possible sources of the pollution are gas emission from vehicles or liquid industrial waste. There are several factories around Lakarsantri Subdistrict, like leather tannery and ceramic factories. Industrial wastes in water or contamination of smokes produced by factories or vehicles are suspected as the source of Pb contamination.

Among the three types of leafy greens cultivated in the periurban locations, water spinach absorbs Pb the most. Water spinach is one of the vegetables that absorbs heavy metal easily from its growing medium and it is widely consumed and frequently found growing or being cultivated in empty lots around the river and getting the water from the river (Kohar, *et al.*, 2005). The research conducted by Haruna, *et al.* (2012) showed that water spinach absorbs heavy metal Cu easily from its growing medium. Water spinach can even be used as absorber of heavy metal in phytoremediation. The research conducted by Puspita, *et al.* (2010) on water spinach cultivated around Bengawan Solo River in Karanganyar Industrial Area showed that the water spinach from that location is not suitable for consumption, due to the content of heavy metal Pb and Cd exceeding the tolerance limit set by National Standardization Agency (BSN).

Pb content in water spinach, cultivated in the medium continuously contaminated by Pb and harvested at the age of 6 weeks, is higher compared to water spinach harvested at the age of 3 weeks. Pb is accumulated mostly in the roots of water spinach. Therefore, to minimize the contamination of Pb in water spinach that will be consumed, it is recommended to harvest the water spinach maximally at the age of 3 weeks (Kohar, *et al.*, 2005). Generally, water spinach cultivated in the uncontaminated area will be harvested at the age of 4 weeks.

Heavy metal Pb is the highest cause of heavy metal contamination in vegetables. Spinach contains the highest heavy metal Pb. A research conducted to find out the comparison of lead content in spinach sold in traditional and modern market shows that overall, spinach in traditional and modern market shows positive result of lead contamination exceeding the maximum limit of lead contamination according to Directorate General of National Agency for Drug and Food Control No.03725/B/SK/VII/1989 with 5 positive samples (83.3%) and 3 positive samples (50.0%) (Jaimin, 2012). The result indicates that there is no difference of Pb content between spinach sold in traditional market and spinach sold in modern market.

Spinach (*Amaranthus sp*) watered by irrigation water that contains heavy metal Pb below the maximum limit for agriculture is still safe for consumption and will not affect human's health. Spinach with harvest age of 30 days and 40 days and watered by irrigation water containing heavy metal Pb below the maximum limit for

agriculture is still safe for human consumption (Yunita, 2011).

From the data of this research, it is known that the leafy greens cultivated in periurban agricultures in Surabaya are contaminated by Pb (Table-1). It may be caused by polluted air, contaminated soil, or contaminated water. Lead (Pb) content tends to be found in vegetables watered from the river. A research conducted by Jakfar, *et al.* (2014) on Pb content found in river water and sediment at six observation points in Surabaya is presented in Table-2.

As the comparison, this research also used vegetables cultivated organically. Of the three types of vegetables, none shows Pb content in their leaves (Table-3). Organic vegetables as organic foodstuff are defined as ones that do not contain any dangerous chemical like pesticide. Organic vegetables are cultivated using natural fertilizer and botanical pesticide. Therefore, the agricultural products do not contain heavy metal Pb.

Table-2. Pb Content on river water and sediment in Surabaya.

Location	Pb content (mg/L)	
	Water	Sediment
Karangpilang	< 0.036	0.232
Gunungsari	< 0.036	0.216
Wonokromo	< 0.036	< 0.216
Ngagel	< 0.036	< 0.216
Gubeng	0.039	< 0.216
Jembatan Merah	< 0.036	0.241
Perak	< 0.036	0.253

Source: Jakfar, *et al.* (2014)

It is also proven by the research conducted by Lestari, *et al.* (2010) on carrot, while the research by Harsojo and Mellawati (2009) affirms the tendency that inorganic vegetables contain higher level of Fe, Zn, Ca, Co, and Cd compared to organic vegetables. There is an assumption that using commercial fertilizers on inorganic vegetables can increase the metal content, particularly Zn and Cd.

Table-3. The result of Pb content test on organic vegetables.

Organic vegetables	ppm (mg/kg)	
	RL	Result
Spinach	0.100	ND
Water spinach	0.100	ND
Mustard greens	0.100	ND

Notes:

ND : Not Detected = below RL

RL : Reporting Limit



Even though the product of organic agriculture is more expensive than the product of inorganic agriculture, the research on consumers in Surabaya shows that generally consumers have good perception for organic products, in terms of health, quality, price, environmentally-friendly nature and food safety (Thio, 2008). It is believed that consuming organic vegetables improves health (Parlyna and Munawaroh, 2011).

Pesticide residue

Pesticide residue in the harvested vegetables can be consumed by consumers. Pesticide residue can be derived from direct exposure of pesticide on the product or absorption from the soil, particularly for tubers (Winarti and Miskiyah, 2010).

Research on spinach, water spinach and mustard greens cultivated in several periurban locations in Surabaya did not find any pesticide residue (Table-4). From ten pesticide's active ingredients, not even one was detected on the vegetables cultivated conventionally by farmers in periurban agricultures in Surabaya. These active ingredients are the ones from organophosphate class contained in pesticides used widely by farmers in Surabaya.

Using organophosphate pesticide is allowed by the government since this pesticide has only momentarily effective effect and is quickly degraded in the soil. Therefore, there is no accumulation of organophosphate pesticide residue on the objects tested (Table-4).

Table-4. The result of pesticide residue test on organic vegetables.

Pesticide type	ppm (mg/kg)	
	Organic	Inorganic
Chlorpyrifos	ND	ND
Diazinon	ND	ND
Fenthion	ND	ND
Methamidophos	ND	ND
Methidation	ND	ND
Penthoate	ND	ND
Profenofos	ND	ND
Prothiofos	ND	ND
Triazophos	ND	ND
Fenithrotion	ND	ND

Notes: ND: Not Detected

Some research on pesticide residue that have been conducted on various kinds of vegetables (tomato, potato, cabbage, string beans, shallot) sold in the market of Makassar did not find any pesticide residue (Yusnani, *et al.* 2013; Hendariani, *et al.*, 2013). Other researchers found pesticide residue in vegetables, however they are still safe to be consumed since the residue is still below the BMR.

The research by Munarso *et al.* (2009) on cabbage, carrot and tomato commodities obtained from Malang and Cianjur and the research by Hartini (2010) on Melon in Grobogan find that the pesticide residue is still below the tolerance limit. However, the research by Yenita, *et al.* (2012) shows that 9 out of 18 spinach samples examined contain pesticide residue exceeding the Maximum Residue Limit (BMR). Permitted BMR on several agricultural commodities is based on the Regulation of the Ministry of Agriculture in 2011, as presented in Table-5.

Table-5. Maximum Limit of pesticide residue.

Pesticide	BMR (mg/kg)	Vegetable commodities
Chlorpyrifos	-	-
Diazinon	0.5	Spinach, Cabbage, Lettuce
Fenthion	1.0	Cabbage
Methamidophos	1.0	Cabbage, Bean Sprouts
Methidation	0.1	Cabbage
Penthoate	0.1	Lettuce, Cabbage Flower, Cucumber
Prefenofos	1.0	Cabbage
Prothiofos	-	-
Triazophos	0.1	Cabbage, Bean Sprouts
Fenithrotion	0.5	Cabbage

Source: the Regulation of the Ministry of Agriculture Number: 88/Permentan/Pp.340/12/2011

Yuliastuti (2011) found that pesticide residue on the outer part of cabbage leaves is higher than on the inner part. It is because the residue is accumulated on the leaves that are directly exposed to pesticide. However, pesticide residue of organophosphate class on the cabbage leaves is easy to remove with water (Maruli *et al.*, 2012).

The residue pattern found in the organic food is significantly different from the pattern found in the conventional samples. Conventional fruits contain 3.6 times more pesticide residues than organic fruits, and conventional vegetables contain 6.8 times of one or more residues. Compared to organic products, conventional samples tend to more frequently contain multiple residue. Imported products consistently contain more residues than domestic products (Sulaeman, 2009).

Water content

Water content is a very important characteristic in foodstuff, because it can affect the outward appearance, the texture, and also determine the freshness and durability of foodstuff. Water content enables bacteria, mold and yeast to grow and alter the foodstuff. For vegetables, water



content is one of the indicators for freshness that greatly affects the quality of their outward appearance.

The result of analysis on water content in leafy greens from several research locations is presented in Table-6. Water content of vegetables cultivated conventionally on periurban location (Location I, II, and

III) is lower than the organic vegetables (Location O). Water content in organic vegetables is higher than water content of vegetables cultivated on periurban area. The highest water content is found in organic mustard greens (Location O), while the lowest water content is found in spinach cultivated on location I (Rungkut Subdistrict).

Table-6. Water content of periurban vegetables in Surabaya.

Plant location	Water content (%)					
	Spinach		Water spinach		Mustard greens	
I	64.97	± 4.78	73.29	± 6.03	78.04	± 3.11
II	79.59	± 1.70	79.38	± 0.18	80.56	± 0.21
III	72.83	± 5.30	68.81	± 2.03	82.43	± 0.49
O (Organic)	87.50	± 0.96	87.86	± 0.13	90.16	± 0.06

Water content in vegetables is relatively high, between 50% - 90%. Water has an important function in biochemical processes on plants. Water content can be used as the stability index for storage as well as the indicator of organoleptic quality, especially taste and tenderness. Water content in foodstuff will change depending on the environment and thus affecting the durability. It is the main consideration in processing and post-processing management of foodstuff (Purnomo, 1995).

Nutritional composition of vegetables is also indicated by water content, fat, protein, and carbohydrate. The main composition in vegetables is water and mineral (70% - 90%), a little protein and fat, as well as carbohydrate in the form of starch, cellulose (cannot be digested), and sugar. The various compositions of vegetables are influenced by variety of vegetables, weather, maintenance, harvest methods, etc. The results of water content analysis in Figure-3 show different water content caused by the difference in location and cultivation methods.

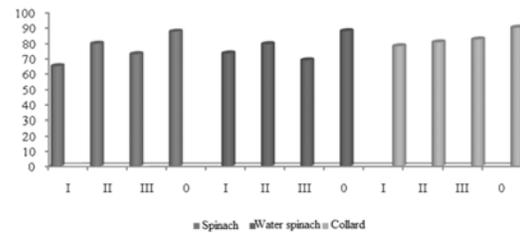


Figure-2. Water content in vegetables based on location.

The correlation between water and heavy metal Pb content in periurban vegetables is $Y=3.29x + 72.22$, with $R^2 = 0.873$. It shows that water content in periurban vegetables is correlated with heavy metal Pb content.

Chlorophyll level

The analysis on chlorophyll level in periurban vegetables in Surabaya shows that spinach in location II has the highest level, and mustard greens in location III has the lowest level (Table-7).

Table-7. Chlorophyll level of Periurban Vegetables in Surabaya.

Location	Chlorophyll level (mg/g)					
	Spinach		Water spinach		Mustard greens	
Location I	2.35	± 0.07	1.83	± 0.02	1.63	± 0.03
Location II	3.11	± 0.43	1.08	± 0.22	2.16	± 0.13
Location III	2.76	± 0.01	0.51	± 0.01	0.38	± 0.01
Location O (Organic)	2.02	± 0.17	1.31	± 0.10	0.98	± 0.11

It is caused by several factors such as the age of the plant and the leaf, leaf morphology and genetic factors. The age of the leaf and physiological step of a plant is the decisive factor that indicates chlorophyll level. Every

species at the same age has different chemical content and different amount of genome as well. High chlorophyll level in vegetables is used as a decisive indicator of



vegetables as food supplement (Setiari and Nurchayati, 2009).

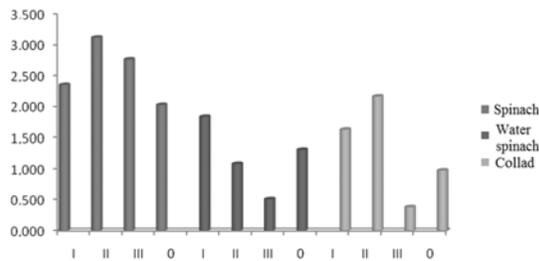


Figure-3. Chlorophyll level based on locations.

Chlorophyll degradation in vegetable tissue is affected by pH. In alkaline medium (pH 9), chlorophyll is very stable against heat, however, in acid medium (pH 3), chlorophyll is unstable. Figure-4 shows variations of chlorophyll content in the leafy greens cultivated in various periurban location in Surabaya.

In this research, chlorophyll level in spinach in all locations is the highest, followed by water spinach and mustard greens. According to Olivers (2003), chlorophyll level in leaf decreases along with the increase of heavy metal content. From those three examined vegetables, heavy metal (Pb) content in spinach is the lowest, even though Pb content in water spinach and mustard greens do not exceed the BMR.

The regression equation between chlorophyll and Pb content is $Y = -0.676x + 3.110$, with $R^2 = 0.615$. It shows that the increase of Pb content in leaf will be followed by the decrease of chlorophyll level. According to Kovacs (1992), excessive heavy metal such as Pb in vegetables can decrease the intake of Mg and Fe, and lead to the change of volume and the amount of chloroplast.

pH of leaf

The analysis of variance on pH of spinach, water spinach and mustard greens shows no significant difference between species or between locations. The highest pH can be found in organic mustard greens leaves, while the lowest pH can be found in mustard greens leaves in Location I, as presented in Table-8.

Table-8. pH of leaves of vegetables from Periurban Agriculture in Surabaya.

Location	pH					
	Spinach		Water spinach		Mustard greens	
Location I	6.95	± 0.02	6.89	± 0.04	6.85	± 0.15
Location II	6.89	± 0.03	6.97	± 0.08	7.07	± 0.07
Location III	6.90	± 0.03	6.98	± 0.08	7.08	± 0.07
Location O (Organic)	7.01	± 0.03	7.09	± 0.08	7.19	± 0.07

The research conducted by Emongor (2007) on cabbage shows that in the acid environment or in low pH (pH 4.5), high absorption of Pb occurs. The lowest Pb content in cabbage occurs in growing media with pH 7.1 to 8.5.

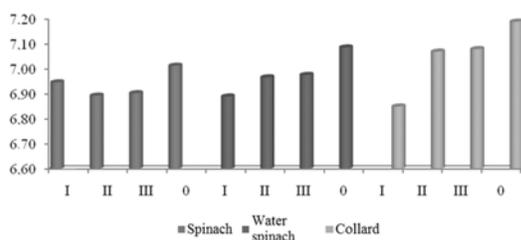


Figure-4. pH based on location.

In this research, there is no significant correlation between Pb content and pH of leaf. The correlation equation is $Y = 0.015x + 0.195$ with $R^2 = 0.351$. It is due to the reason that pH of leaf is not affected by pH of environment and growing media, whereas Pb accumulation is affected by environment and growing

medium (Emongor, 2007). According to Cornelissen (2010), the pH of leaf is not affected by the pH of growing media, however it is affected by the species of the plant. Figure-5 shows the variations of pH, based on the types of the vegetables and the location of periurban agriculture. There is no significant correlation between chlorophyll level and pH of leaf as well. The regression equation of pH of leaf and chlorophyll level is $Y = 0.240x + 2.278$, with $R^2 = 0.542$.

Table-9. Correlation between Pb content and other variables.

	Variables	Regression equation	R ²
Pb	Water content	$y = 3.29x + 72.20$	0.873
Pb	Chlorophyll level	$y = -0.676x + 3.110$	0.615
Pb	pH	$y = 0.015x + 0.195$	0.351

According to Pearson calculation of correlation, it is known that there is no significant correlation among the



variables of water content, chlorophyll level, and pH. The correlation between Pb content and water content, chlorophyll content and pH is presented on Table-9.

It is in accordance with the result of previous research, stating that the influx of heavy metal pollutants in plants excessively can cause change in the levels of ascorbate, chlorophyll, water and pH of leaf (Pawar, *et al.*, 2010; Govindaraju, *et al.*, 2010; Meletiou-Christou, *et al.*, 2011). It can also be seen from Table 10 that the correlation of water content and pH on Pb content is positive. The correlation between chlorophyll level and Pb content is negative, meaning that the chlorophyll level will decrease when Pb increases. It is in accordance with the findings of Oliver (2003) stating that the total chlorophyll level will decrease along with the increase of heavy metal.

CONCLUSIONS

It can be concluded that Pb content on spinach, water spinach and mustard greens cultivated in periurban agricultures in Surabaya is still below the maximum limit of heavy metal contamination rate specified in Indonesian National Standard (SNI) 7387: 2009. Likewise, there is no pesticide residues detected in spinach, water spinach and mustard greens. Water content, chlorophyll level and pH of vegetables of agricultural products in periurban locations in Surabaya is lower compared to organic vegetables. There is no significant correlation among variables, but there is a positive correlation between Pb content with water content and pH, whereas Pb is negatively correlated with chlorophyll level. It can be also concluded that, seen from the quality, periurban vegetables in Surabaya has the potential to be developed.

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