Speciation of Lead (Pb) and Cadmium (Cd) in Agricultural Soil of Pancasari Village as A Vegetables Central Area of Bali, Indonesia

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Abstract. Lead and Cadmium are two types of heavy metals considered toxic for living organisms. Our researches focused on the speciation of Pb and Cd on soil which cultivated vegetables. In addition, organic matter content, pH and cation exchange capacity as a factor influencing heavy metal speciation and bioavailability for plants also analyzed. Heavy metals speciation carried out using a sequential extraction method then the Pb and Cd content on each fraction was analyzed by the atomic absorption spectrophotometer. The lead fraction in the soil is in the following order F3>F1>F5>F4>F2 whereas cadmium order F4> F1>F3>F5>F2. The organic matter, pH and cation exchange capacity on soil was 3.05%; 5.56 and 20.30 mek/100 g, respectively. The value of daily intake of metal (DIM) and health risk index (HRI)<1 indicates that the vegetables were relatively safe for consuming.

Keywords: Agricultural Soil, Cadmium, Lead, Fertilizer, Heavy Metal Speciation, Health Risk Index

1 Introduction

Excessive use of fertilizers and pesticides has an impact on the increase in heavy metal residues of Cd, Pb, Cu, and Zn in soils and plants [1]. Three types commercial phosphatic fertilizers namely triple superphosphate (TSP), monoammonium phosphate (MAP), and diammonium phosphate (DAP) were contain of lead (Pb) at range 31.6-32.4; 13.0-16.7 and 11.2-16.4 mg/kg whereas cadmium (Cd) concentration range of 36.0-36.8; 31.8-34.0 and 26.0-34.2 mg/kg, respectively [2]. Fertilizer of NPK contain Pb and Cd at range 2.84-11.32 mg/kg and 7.43-9.02 mg/kg [3]. While urea fertilizer contains Pb and Cd in the range of 7.10-7.92 mg /kg and 2.52-2.95 mg/kg [4].

Vegetables cultivated on heavy metals contaminated farmland will absorb the heavy metals and accumulate them in the roots and inedible parts of plants. For example, tomatoes cultivated in Pb and Cd contaminated soil, after analysis found Pb residues in the leaves and fruit of 4,503-5,700 mg/kg and 1,883-3,710 mg/kg while Cd was found in the range of 0.753-0.873 mg/kg and 0.340 - 0.527 mg/kg [5]. The main effect of lead on health is to disrupt the nervous system, hematological system, and renal system whereas cadmium can lead to renal tubular dysfunction and disturbances in calcium metabolism and formation of kidney stones[6]. Based on the bioavailability for the plant, heavy metals in the soil are classified into three fractions: ready bioavailable, potential bioavailable and non-bioavailable fractions [7]. The bioavailability of heavy metals on the soil strongly influenced by the physicochemical properties of soil such as

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pH, organic matter content and cation exchange capacity of soil [8]. Mobility of heavy metals is higher in soil with low pH compared to neutral or alkaline soil. The ionization of metals increases at low pH thereby increasing water solubility and mobility. Soil with high organic matter content can reduce metals absorption by plants because heavy metals from organometallics compound more stable and less soluble in water [8]-[9].

The sequential extraction procedure is a commonly used method for determining the speciation of heavy metals on the soil. In sequential extraction procedure method, metals in the soil are divided by five fractions, namely F1(soluble and exchangeable fraction), F2 (metals bound to carbonate fraction), F3(metals bound to Fe/Mn-oxides fraction), F4(metals bound to organic fraction), and F5(residual fraction) [10]. The fraction of F1-F2 referred to bioavailable for the plant, the fraction of F3-F4 represents the amount of potential heavy metals while F5 represents the residual fractions may be considered to be the stable form and thus not available to plants[7].

This research focused on determining heavy metals of Pb and Cd speciation from horticultural farmland soil of Pancasari Village, Buleleng, Bali, Indonesia. The properties of soil such as organic matter content, soil pH and cation exchange capacity also determined. Pancasari village is well known as one of the tourist area and vegetable plantation center in Bali, Indonesia. In addition, the health risk of some vegetables (cabbage, tomato, and carrot) grown in the soil contaminated with heavy metal was assessed.

2 Materials and Methods

2.1 Study Area

Pancasari village is one of the nature tourism areas in Buleleng, Bali, Indonesia (**Figure 1**). Pancasari well known for agricultural products such as carrots, tomatoes, vegetables, and the most typical is strawberry.

Fig. 1. Location of soil sample collection

Farmers here depend on chemical and organic fertilizer to improve vegetable production. The types of fertilizer used to vegetable maintenance are NPK, SP36, compost, and manure.

2.2 Sampling and Pretreatment

The soil samples were collected randomly from different points of the horticultural farmland of Pancasari, Bali, Indonesia. The point sampling at an altitude of 1000-1250 above sea level. Each soil sample was taken within 0-20 cm depth from the soil surface. The 10-15 individual samples from different location points were mixed together and then the impurities are separated manually. The amount of 100 g of soil samples was dried at 55oC using an oven for a week to reduce moisture, then dried again at 105^{o}C for 3 hours to obtain dry samples.

2.3 Characterisation of Soil

Determination of pH. The pH of sample soil was carried out through the method proposed by Isric[11]. A weigh of 5 g of dry soil sample placed in a 250 mL Erlenmeyer flask has been filled with 12.5 mL distilled water. The mixture is shaken at 200 rpm using an automatic shaker for 24 hours. Subsequently, the mixture was agitated for ten minutes then its pH measured using pH meter.

Determination of Cation Exchange Capacity. The cation exchange capacity of sample soil is determined by the ammonium acetate method [12]. The amount of 1.5 g of soil sample was extracted with 50 mL 0.1 N of NH4OAc. The mixture was shaken at 200 rpm using an automatic shaker for 15 minutes, then agitate for 24 hours in order to all the bounded cations can be replaced by ammonium ion. The mixture was filtered and then residue washed back with 30 mL of ethanol 96%. The residue was put in a distillation flask and added with 20 mL of NaOH 40% solution and 20 mL of distilled water. The cation exchange capacity of soil sample was determined by collecting distillate into Erlenmeyer flask contained with 10 mL 0.1 N of H2SO4 and then titrating the excess of acid with standardized 0.1 N NaOH using Conway indicator. The cation exchange capacity of the soil was calculated by the following formula in equation (1) :

$$CEC\ (meq\ /100\ g) = \frac{mL_{.blank} - mL_{spl}\ x\ N_{NaOH}}{sample\ \ weigh\ (g)}\,x100 \qquad (1)$$

Determination of C-Organic. Determination of C-Organic in soil using a modified Nelson and Sommers procedure [13]. A total of 5 g of soil samples were crushed then the amount of 0.5 g sample put into a volumetric flask size of 100 mL. The sample was added with 5 mL 1 N of potassium dichromate (K2Cr2O7) and 7.5 mL of concentrated sulfuric acid(H2SO4). The mixture agitates for 30 minutes, then diluted with deionized water up to the final volume of 100 mL. The mixture keeps for 24 hours and then measured their absorbance using UV-Vis spectrophotometer at 561 nm. The C-organic content on the sample was determined through the calibration curve with glucose as a standard solution

2.4 Heavy Metal Speciation

Heavy metal of Pb and Cd speciation were carried out by a sequential extraction procedure from Tessier *et al.* (1997). On this, heavy metal was classified into five fractions: F1(soluble and exchangeable fraction), F2(heavy metal bound to carbonates), F3(heavy metal bound to iron and manganese oxides), F4(heavy metal bound to organic matter) and F5(residual fraction).

F1 (Soluble and Exchangeable Heavy Metals Fraction). Amount 2.5 g of soil sample was extracted with 20 ml of magnesium chloride solution ($MgCl_2$) at a concentration of 1.0 M. The mixture is conditioned at pH 7 and stirred using a shaker for 1 hour. The mixture is then filtered and the filtrate is centrifuged at 3000 rpm for 30 minutes. The amount of Pb and Cd in the supernatant was measured using an atomic absorption spectrophotometer. The residual soil was used for the next extraction.

F2 (Carbonate Bound Heavy Metals Fraction). The residue of F1 was extracted with 20 mL of 1.0 M sodium acetate solution(CH3COONa) and conditioned at pH5 then stirred using a shaker for 4 hours at room temperature. The mixture is then filtered and the filtrate is centrifuged at 3000 rpm for 30 minutes. The supernatant obtained measured levels of Pb, Cd using an atomic absorption spectrophotometer. The residual soil was used for the next extraction.

F3 (Iron-Manganese Oxide Bound Heavy Metals Fraction). The residue from F2 was extracted with 50 mL of 0.04 M hydroxylamine hydrochloride (NH2OH. HCl) in 25% (v/v) acetic acid (CH3COOH). The mixture was heated at 96oC for 6 hours, then filtered and the filtrate is centrifuged at 3000 rpm for 30 minutes. The content of Pb and Cd on supernatant was measured by the atomic absorption spectrophotometer whereas the residue soil was used for the next extraction.

F4 (Organic Matter Bound Heavy Metals Fraction). The residue from F3 was added with 7.5 mL of 0.02 M nitric acid (HNO3) and 12.5 mL of 30% hydrogen peroxide (H2O2). The mixture is adjusted to pH 2 with add HNO3 drop by drop, then heated at the temperature of 85oC for 3 hours with occasional agitation and allowed to cool down. The mixture was added again 5 mL of 3.2 M ammonium acetate in 20% (v/v) nitric acid followed by dilution to a final volume of 25 mL with de-ionized water. The extracted metal solution was filtered and the filtrate is centrifuged at 3000 rpm for 30 minutes. The content of Pb and Cd on supernatant was measured by the atomic absorption spectrophotometer whereas the residue soil was used for the next extraction.

F5 (Residual Fraction). Residue from F4 was digested with aqua regia (7 mL of 10 M HCl and 2.5 mL of 15.6 M HNO3) and 10 mL of hydrofluoric acid (HF, 40% w/w) in Teflon beakers. The mixture was heated in a water bath for 3 hours. The clear digest was removed and allowed to cool before transferring quantitatively into a 100 mL volumetric flask.

2.5 Determination of Pb and Cd on Vegetables

The Concentration of Pb and Cd in the edible part of vegetables was determined on a wet weight basis. The vegetables were washed with deionized water to remove any possible contaminants, then cut into small pieces using a stainless steel knife and oven at 60℃ to reduce moisture. The plant samples (1.25 g) were digested with aqua regia (6 mL of HCl, 37% v/v + 2 mL of HNO_3, 65% v/v) on the water batch for 3 hours. The clear digest was filtered and the filtrate allowed to cool before transferring quantitatively into 100 mL volumetric flask.

2.6 Heavy Metals Analysis

The lead and cadmium from each fraction soil sample and edible part of vegetables were measured using atomic absorption spectrophotometer model Perkin-Elmer Analyst 300 Atomic Absorption spectroscopy (AAS) at 283.3 nm for lead and 228.8 nm for cadmium.

2.7 Calculation of the Health Risk Index (HRI)

Health Risk Index(HRI) is calculated as the ratio of daily intake of heavy metals (DIM) in vegetables and oral reference dose (RfD) on equation (2).

$$HRI = \frac{DIM}{RfD} \tag{2}$$

The RfD values for Pb and Cd are 0.004 and 0.001 mg/kg/day, respectively[11]. Daily intake of heavy metals (DIM) in vegetables is calculated using the following equation (3):

$$DIM = \frac{C_{metal} \ x \ C_{factor} \ x \ D_{food \ int \ ake}}{B_{avaerage \ weigh}} \tag{3}$$

where, Cmetal, Cfactor, Dfood intake, and Beverage weigh denote the concentration of heavy metal on plants (mgkg-1), the conversion factor(0.085), daily intake of vegetables(kg person weight -1 day-1, and average body weight of the consumers, respectively. The conversion factor of 0.085 is set to convert fresh vegetable weight to dry weight. The bodyweight of the consumers chosen was 60 kg for adults and 30 kg for children. The average quantity of vegetables consumed by a person in Indonesia was 40.35 kg person-1year-1 or 0.115 kg person-1day-1[14]. The HRI value is less than 1 %, there is no risk, 1-10 % exhibits low risk, 11-30 % shows medium risk, 31-50 % shows high risk and greater than 50% exhibits very high risk[15].

3 Results and Discussion

3.1 Characterization of Soil

The physicochemical properties of soil samples form horticulture farmland of Pancasari village, Buleleng, Bali, Indonesia are summarized in Table 1.

Table 1. Physicochemical properties of soil sample.

Parameters	Units	Value
pH (H_2O)	-	5.56
C-Organik	%	3.05
Cation exchange capacity	mek/100 g	20.30

The soil sample is sandy clay with a slightly acidic with the organic content is high enough and the cation exchange capacity in the moderate category. The physicochemical properties of soil play an important role in regulating speciation and mobility of metals in soil. The bioavailability of metal in soils is largely dependent on the partition of the metals between the solid and solution phases. The Solubility of heavy metals tends to increases at lower pH and decrease at higher pH values. Heavy metals ready absorbed by plants at lower pH [16]. Cadmium and lead adsorption to the soil are greater in neutral or alkaline soils than in acidic ones. As a consequence, plant uptake of cadmium and lead decreases as the soil pH increases [17].

Soil organic matter can gradually decrease the availability of metal to be absorbed by plants. Organic matter, especially humic and fulvic acid with carboxyl groups to absorb the heavy metals by forming chelate complexes compounds that are relatively less soluble in water, thus reducing the toxic potential of the plant when consumed. Organic matter in the soil led to decreased Pb and Cu content in the potato peel and tubers. The increase of compost quantity led to a decrease of the Pb content in potato tubers [18].

3.2 Speciation of Pb and Cd on Soil

Determination of Pb and Cd speciation on soil using a sequential extraction procedure from Tessier *et al*., 1979. The measurement result of Pb and Cd heavy metal content in each fraction (F1-F5) are summarized in Table 2.

Table 2. The heavy metal fraction of Pb and Cd on soil sample.

Fractions	Pb (mg/kg)	Cd (mg/kg)
F1 (soluble and exchangeable)	3.333	1.190
F2 (bound to carbonates)	0.778	ND
F3 (bound to iron and manganese)	7.670	0.238
F4 (bound to organic matter)	1.110	1.416
F5 (Residual)	1.250	0.045
Total	**14.145**	**3.689**

The sequential extraction procedure is used to determine the quantity of each fraction of metal and then used to predict the mobility and bioavailability of heavy metals in soil. The concentration levels of Pb heavy metal in the fractions followed the order: F3> F1> F5> F4>F2 while Cd heavy metal F4> F1> F3> F5> F2. The fraction of F1 also called EFLE fraction (easily, freely, leachable and exchangeable) are metals that may be adsorbed on the surface of the particles, easily discharged and dissolved so that they are readily absorbed by plants. F2 fraction consist of the metals is bond to carbonate. This fraction is sensitive to pH change and redox conditions. By these properties, the metals in this fraction are readily dissolved in acidic and slightly acidic conditions [19]. F3 is a fraction bound to iron and manganese oxides that are thermodynamically unstable and thus potentially bioavailable. F4 is a metal fraction bound to organic material which can be degraded through the oxidation process so that it is potential to dissolve and bioavailable for the plant. F5 is the residual fraction strongly bonded to the soil matrix so its very difficult to release from soil. Heavy metals in soil fraction form of F1-F2, F3-F4 and F5 are bioavailable, potential bioavailable and non-bioavailable, respectively [7]. The relative distribution of Pb and Cd fractions in soil associated with their ability to be absorbed by plants is shown in **Figure 2**.

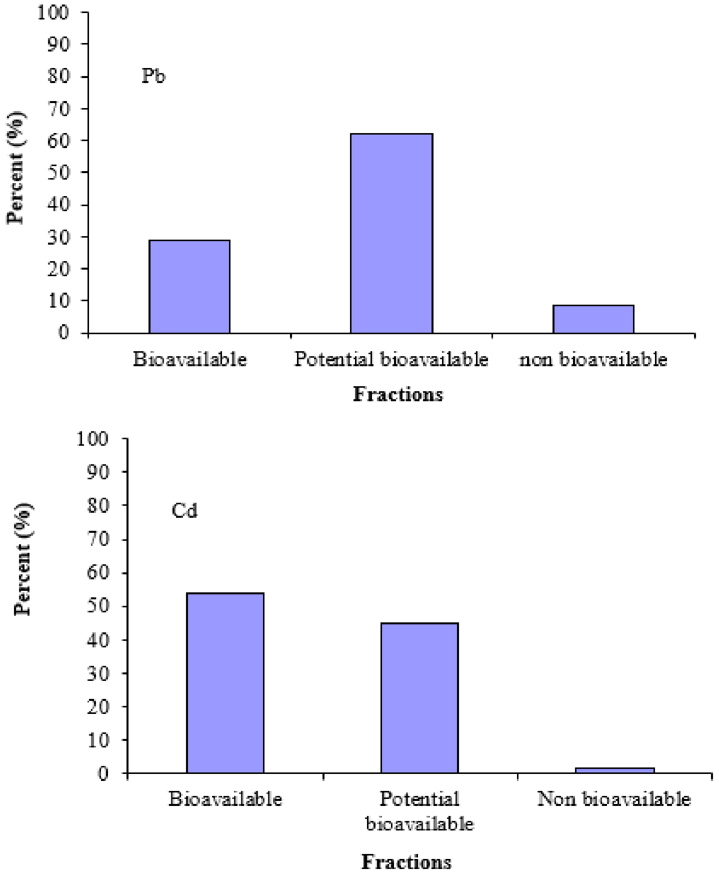

Fig. 2. Relative distribution of bioavailability of Pb and Cd metals in soil.

As shown in **Figure 2**, that Pb metal in soil was dominantly found in exchangeable and carbonate fraction compared to other fractions. This fraction is considered to be weakly bounded metals which may transfer easily from soil to plants [20]-[21]. The higher the F1 and F2 fractions of metal in the soil, the greater the plant uptake of that metal. On the other hand, Cd metal in sample soil was investigated as a fraction of binding to organic matter (F4) and soluble-exchangeable (F1).

3.3 Heavy Metal of Pb and Cd Concentration in Vegetables

The concentrations of Pb and Cd found in cabbage, tomato and carrot are presented in Table 3. Table 3 summarises the mean heavy metals of Pb and Cd concentration in the edible part of vegetable samples are grown in Pancasari village. Mean concentration of the total Pb in Cabbage, tomato and carrot were 0.4862, 0.4323 and 1.4434 mg/kg whereas Cd were 0.1458, 0,3250 and 0,3412 mg/kg, respectively. These results showed that the vegetables of investigated have different abilities to absorb the heavy metals. Vegetables absorb Pb and Cd heavy metals and accumulate them in their edible and inedible parts in quantities high enough to cause health problems to human beings.

Table 3. Heavy metal of Pb and Cd content in the edible part of vegetables.

Vegetables	Pb (mg/kg)	Cd (mg/kg)
Cabbage	0.4862	0.1458
Tomato	0.4323	0.3250
Carrot	1.4434	0.3412

The health risk for the consumption of contaminated vegetables was assessed by calculating the ratio of DIM to the oral reference dose (RfD) for each metal. The vegetables are safe for consumption when the health risk index(HRI) values less than 1 (HRI<1) and if the ratio is equal or greater than 1 then potential health risk for consumers[22]. The DMI and HRI values were calculated for adults and children are presented in Table 4 and Table 5.

Table 4. Estimation of daily intake of metals (DIM) by consumption of the vegetable samples grown in Pancasari horticultural farmland area.

Vegetables	Estimation of daily intake of metals (DIM) (mg person^{-1}day^{-1})	
	Pb	Cd
Cabbage	0.0000792 (A)	0.0000238 (A)
	0.0001584 (C)	0.0000475 (C)
Tomato	0.0000704 (A)	0.0000529 (A)
	0.0000141 (C)	0.0001059 (C)
Carrot	0.0002352 (A)	0.0000839 (A)
	0.0004703 (C)	0.0001111 (C)

A= adults, C = children

Table 5. Health risk index (HRI) of the Pb and Cd metals for the adults and children as a result of the consumption of the vegetables.

Vegetables	Health risk index (HRI)	
	Pb	Cd
Cabbage	0.0198 (A)	0.0238 (A)
	0.0396 (C)	0.0475 (C)
Tomato	0.0176 (A)	0.0529 (A)
	0.0352 (C)	0.1059 (C)
Carrot	0.0588 (A)	0.0556 (A)
	0.1176 (C)	0.1112 (C)

The result revealed that the Health risk index for all types of vegetables is lower than 1 indicating safe for the consumer. On the other hand, the intake of individual heavy metals Pb and Cd through the consumption of vegetables in the horticultural farmland of Pancasari are safe.

4 Conclusion

The results obtained in the present study heavy metal speciation in horticultural soil sample indicated that Pb metal metals were associated with various fractions in the following order: F3> F1> F5 > F4 >F2 whereas Cd order: F4> F1>F3>F5>F2. The organic matter, pH and cation exchange capacity on sample soil was 3.05%; 5.56 and 20.30 mek/100 g, respectively. Health risk index (HRI) for Pb and Cd is less than 1.0 for all types of vegetable, it is mean that vegetables were grown In horticultural farmland are relatively safe for consumption.

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