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Bioavailability of Heavy Metals Cadmium (Cd) and Lead (Pb) in Agricultural Soils in Tabanan Region and Their Accumulation in Gonda Plants (*Sphenoclea zeylanica* Gaertn)

Ni Luh Gede Lisdiantari[1], Iryanti Eka Suprihatin[1*] and Emmy Sahara[1]

Abstract. Farmers use organic and inorganic fertilizers and pesticides in order to maintain the quality of their crops. Excessive use of fertilizers and pesticides in long term can cause soil contamination. The purpose of this research was to determine the level of the bioavailability of Cd and Pb metals in gonda plants (*Sphenoclea zeylanica* Gaertn) as well as the amount dissolved in the soil. The stepwise extraction method was used to determine the metal species and the metals measurement was carried out by using an Atomic Absorption Spectrophotometer (AAS). The content of Cd and Pb metals in gonda plants obtained was 4.3328 and 1.4788 mg/kg, repectively. The bioavailable Cd and Pb in soil were 13.76% - 21.51% and 10.19 - 11.54%, respectively, the potentially bioavailable metals were 53.91 - 57.84% and 79.97 - 80.00. % and the non-bioavailable metals, were 24.58 - 28.39 and 8.46 - 9.84%. Based on CXS 193-1995 regarding the general standard for contaminants and toxins in food and feed, the concentrations of Cd and Pb metals in gonda plants on the agricultural land in the observed area has exceeded the threshold of which is 0.2 mg/kg for Cd and 0.3 mg/kg for Pb.

Introduction

The formation of soil and minerals containing heavy metals such as Pb (Lead) and Cd (Cadmium) generally results in metal contamination in the soil (Bradl, 2005). Pb can accumulate in leaves, roots, stems, and fruits, potentially harming plants, while Cd is present in pesticides used to control pests. Cadmium can cause symptoms such as chlorosis, wilting, necrosis, and disruption of the plant's food-making processes (Mahendra *et al*., 2018). Common heavy metals found in fertilizers and pesticides include as (Arsenic), Hg (Mercury), Mn (Manganese), Cd, Zn (Zinc), Cu (Copper), Cr (Chromium), and Pb. Charlena (2004) reported that the Cd content in phosphate fertilizers varies from 30 to 60 mg/kg, in nitrate fertilizers from 0.05 to 8.5 mg/kg, and in water from 0.1 to 0.8 mg/kg, while compost fertilizers contain Pb content ranging from 1.3 to 2240 mg/kg and 7 to 225 mg/kg for phosphate fertilizers.

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Udayana University, Jimbaran, Bali, Indonesia;* ***Email: eka_suprihatin@unud.ac.id***

Previous studies have reported various research findings regarding metal content in agricultural soils and vegetables in Bali (Mahendra *et al*., 2018; Siaka *et al*., 2015; Suastawan *et al*., 2016).

This article presents the research results regarding the concentrations of Cd and Pb in gonda vegetables grown in gonda agricultural soil in Timpag region of Tabanan.

Experimental

Material and Methods

The materials used in this study were agricultural soil collected from gonda farming areas in Timpag, Tabanan, $Cd(NO_3)_2$, $CuSO_4.5H_2O$, HNO_3, $Pb(NO_3)_2$, HCl, CH_3COONH_4, $NH_4OH.HCl$, CH_3COOH, H_2O_2, and distilled water (distil water). All chemicals used were of analytical-grade purity.

The equipments used in this study were polyethylene spoon, plastic bags, beaker glasses, measuring glasses, measuring flasks, volumetric pipettes, micro pipettes, dropper pipettes, filter paper, 230 mesh sieve, oven, mortar and pestle, ultrasonic bath, centrifuge, pH meter, electric shaker, thermometer, analytical balance, and Shimadzu AA-7000 Atomic Absorption Spectrophotometer (AAS).

Procedures

The Cd and Pb bioavailability in soil was determined by sequential extraction using different solvents with increasing oxidizing strengths followed by measuring the extracted metals with AAS. The metal contents of the gonda plant were determined by extracting the metals from the dried sample of the plant using HNO_3 and H_2O_2.

Soil and Gonda Sampling

Samples of agricultural soil from gonda farming areas in Timpag, Tabanan, were collected randomly from three different plots. Each plot yielded 500 grams of soil collected at a depth of 20 cm using polyethylene spoons. The soil samples were stored in plastic bags and kept in a cooling box. Gonda vegetable samples were randomly collected from three separate plots in the same region and stored in plastic bags in the cooling box.

Soil Preparation

Soil samples were dried at 60 °C until completely dry. The dried soil was finely ground using a mortar and pestle and then sieved through a 230 mesh sieve.

Gonda Vegetable Preparation

Gonda vegetable samples, consisting of stems and leaves (10 cm from the root), were cut into small pieces and rinsed with distil water. They were then dried in an oven at 60 °C, blended to a fine consistency, and sieved through a 230 mesh sieve.

Determination of Total Cd and Pb Content in Gonda

0.5 grams of gonda vegetable samples were digested with 5 mL of concentrated HNO_3. The samples were heated on a hotplate at 80–90 °C for 2 hours, then increased to 150°C until boiling. Additional HNO3 (65%) and H2O2 (30%), approximately 3-5 mL each, were added for further digestion until clear. The digested samples were cooled, filtered, and the resulting filtrate was diluted with distil water. It was then measured using Atomic Absorption Spectrophotometer at λ 228.8 nm for Cd and 217.0 nm for Pb.

Extraction Phase I

One gram of soil samples was mixed with 40 mL of 0.1 M CH_3COOH. The mixture was shaken using a shaker for 120 minutes, followed by centrifugation at 4000 rpm for 10 minutes. The supernatant was diluted with 0.01 M HNO_3 in a 50 mL measuring flask and tested using Atomic Absorption Spectrophotometer at λ 228.8 nm (Cd) and 217.0 nm (Pb).

Extraction Phase II

Residue from Phase I was mixed with 40 mL of 0.1 M $NH_2OH.HCl$, and the pH was adjusted to 2 with 0.1 M HNO_3. The mixture was shaken for 120 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was diluted with 0.01 M HNO_3 in a 50 mL measuring flask and measured using Atomic Absorption Spectrophotometer as before.

Extraction Phase III

Residue from Phase II was mixed with 10 mL of 8.8 M H_2O_2 and sealed air-tight. The mixture was left for 60 minutes and shaken occasionally. It was then heated to 85 °C in a water bath for 60 minutes. Afterward, 10 mL of 8.8 M H_2O_2 was added, and the mixture was heated again for 60 minutes at 85 °C. Next, the mixture was cooled, 20 mL of 1 M CH_3COONH_4 was added, and HNO_3 was used to adjust the pH to 2. The mixture was then processed as in extraction phase II.

Extraction Phase IV

Residue from Phase III was washed with distil water, then 10 mL of reverse aqua regia solution was added. The mixture was digested at 60 °C with an Ultrasonic bath for 45 minutes and then heated again at 140 °C for 45 minutes. It was then centrifuged at 4000 rpm for 10 minutes. The supernatant was diluted with 0.01 M HNO_3 in a 50 mL measuring flask and determined for its Cd and Pb contents.

Result and Discussion

Total Metal Content of Cd and Pb in Soil

The determination of the total metal content of Cd and Pb in soil aimed to assess the level of soil contamination by these metals. Table 1 indicates that the total metal content of Cd in gonda farming soil in Tabanan before planting is categorized as uncontaminated based on The Farmer Greater London Council's values, ranging from 0-100 mg/kg. An increase in Cd content occurs during the harvesting period but still falls within the 0-100 mg/kg range. Similar trends are observed for Pb, where the concentration during harvesting is higher than before planting. Based on The Farmer Greater London Council's values, the sample concentrations are categorized as normal because they are within the range of 0-500 mg/kg. The variation in metal content in soil before and during harvesting is attributed to different farming practices, such as fertilizer application frequency, dosage, type of fertilizer, pesticide use, irrigation, and contamination from other living organisms.

Table 1. The total metal content in soil

Heavy Metals	Average Total Metal Before Planting ± SD (mg/kg)	Average Total Metal During Harvest ± SD (mg/kg)
Cd	67,6943±2,4620	79,3031±1,6295
Pb	21,8463±0,2283	28,1080±1,1949

The elevated levels of Cd found in soil samples at harvest time can be attributed to their proximity to residential areas, where Cd is introduced into the environment through household waste, such as plastic refuse (Baryatik *et al*., 2019; Yu *et al.,* 2023). As the harvesting period approaches, the Pb content tends to increase in the soil. This is attributed to intensive fertilizer application (Siaka *et al*., 2015) by farmers to accelerate the growth of gonda plants. Cd content is higher than Pb both before planting and during harvesting due to the higher presence of Cd pollutants, such as insecticide spraying on nearby crops. This was done because there was an outbreak of caterpillars that were eating the leaves of the gonda plants, posing a threat to the gonda crop. Additionally, fertilizers containing Cd and Pb were likely applied (Siaka *et al*., 2015; Mahendra *et al*., 2018; Siaka *et al*., 2023). Heavy metals can accumulate in the soil and enter plants, soil protection measures are necessary to maintain or improve the current situation by preventing further heavy metal contamination, for example, by controlling Cd and Pb in phosphorus fertilizers (Tóth *et al.,* 2016).

Speciation and Bioavailability of Cd and Pb in Soil

The metal content in each fraction is compared to the total metal content in each soil sample to determine the speciation and bioavailability of metals. Sequential extraction results can show the amount of bioavailable metals. Four fractions were obtained in the sequential extraction: fraction 1 (F1) represents bioavailable metals, fractions 2 and 3 (F2 and F3) indicate potentially bioavailable metals, and fraction 4 (F4) represents non-bioavailable resistant fractions. The bioavailability of metals in the soil is determined by the speciation or the composition of heavy metals available in the soil. Biologically available metals can enter the gonda plant's tissues, potentially causing negative health effects when consumed by humans. Metals that are bioavailable and potentially bioavailable can be harmful if present in large quantities. Potentially bioavailable metals can become bioavailable when influenced by changes in organic matter, CEC, pH, and redox conditions. The use of fertilizers, pesticides, and irrigation by farmers is a contributing factor. Soil pH reduction can lead to increased metal cations content in the soil, resulting in the conversion of metals into bioavailable forms (Tessier *et al*., 1979). The percentage of Cd and Pb extracted in gonda farming land before planting and during harvesting can be seen in Figure 1.

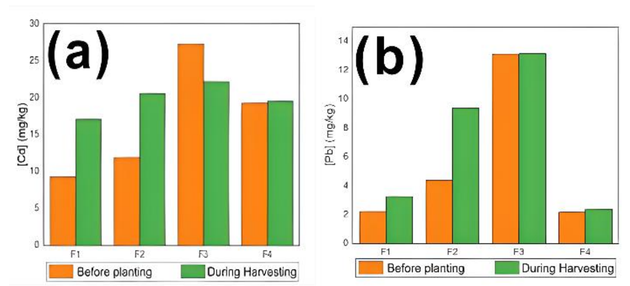

Figure 1. The percentage of Cd metal **(a)** and Pb metal **(b)** In soil before and during harvest

Figure 1 illustrates the percentage of Cd and Pb metals extracted in each fraction in the soil before planting and during harvesting. The order of fractions in the soil before planting for Cd is F3 > F4 > F2 > F1 and for Pb is F3 > F2 > F1 > F4. The order of fractions in the soil during harvesting for Cd is F3 > F2 > F4 > F1 and for Pb is F3 > F2 > F1 > F4. The organic/sulfide fraction (F3) dominates the soil both before planting and after harvesting. The addition of organic matter from fertilizer use causes metals to bind more strongly to organic materials.

Fraction 1 is the labile fraction, easily ionized, exchangeable without undergoing redox reactions, readily soluble in water or weak acid, and generally in carbonate form (Gasparatos *et al*., 2005). Acetic acid is used to separate metals in carbonate form in fraction 1. The content of Cd and Pb metals bound to fraction 1 before planting gonda is 9.3165 mg/kg and 2.2263 mg/kg, respectively, and during harvesting, it is 17.0643 mg/kg and 3.2447 mg/kg. As seen in Figure 1, there is an increase in the levels of both metals in soil samples during harvesting due to external factors such as human activities (detergent waste, iron corrosion), and motor vehicle pollution.

Fraction 2 indicates that the addition of acid can reduce metals bound to Fe/Mn oxides (Gasparatos *et al*., 2005). The content of Cd and Pb metals bound to this fraction before planting gonda is 11.9445 mg/kg and 4.3769 mg/kg, respectively, and during harvesting, it is 20.5665 mg/kg and 9.3569 mg/kg. In this fraction, metal bonds with Fe/Mn oxides are more stable and strong compared to the fraction 1, making the metals less mobile and potentially bioavailable. The $NH_2OH.HCl$ solution is used in this step to break the bonds between metals and Fe oxides without affecting silicate or organic material fractions (Reichman, 2002).

Fraction 3 represents metals associated with organic or sulfide forms. Extracted metals in fraction 3 are potentially bioavailable. Hydrogen peroxide in an acidic environment is used to extract metals from this fraction. Ammonium acetate is added during the extraction to prevent metal reabsorption by the oxidized substrate surface (Sundari *et al*., 2016). The Cd and Pb metal content in this fraction before planting gonda is 27.2122 mg/kg and 13.0928 mg/kg, respectively, and during harvesting, it is 22.1831 mg/kg and 13.1294 mg/kg. In this fraction, the Cd and Pb metal content is relatively higher compared to other fractions, indicating that the metals are more freely available, easily exchangeable, and soluble or bound to carbonate compounds (Jena *et al*., 2013).

Fraction 4, or the non-bioavailable fraction, indicates metals that are resistant and strongly bound to the soil. Reverse aqua regia is used for the extraction of this fraction, aiming to destroy minerals or silicates in the residue except for silicate anions. Reverse aqua regia consists of HCl and HNO_3 in a 1:3 ratio. The source of metals in this fraction is not human activities but weathering processes (Yap *et al*., 2003). This fraction is used as an indicator of the potential entry of heavy metals into the biosphere.

Species in the resistant fraction do not pose a threat to agricultural products because they are not available to biota or are non-bioavailable. The Cd and Pb metal content bound to this fraction before planting gonda is 19.2211 mg/kg and 2.1503 mg/kg, respectively, and during harvesting, it is 19.4892 mg/kg and 2.3768 mg/kg.

Cd and Pb Content in Gonda Plants

From the study, the Cd and Pb metal content in gonda plants is found to be 4.3328 ± 1.1804 mg/kg and 1.4788 ± 0.3936 mg/kg, respectively. According to FAO/WHO standards (CXS 193-1995), where the limit for Cd in leafy vegetables is 0.2 mg/kg and for Pb is 0.3 mg/kg, the gonda vegetables investigated are not suitable for consumption. This is because the gonda plants contain Cd and Pb contamination from fertilizers (containing N, P2O5, and K2O), pesticides, and motor vehicle emissions. Plants can absorb heavy metals in the form of free metal ions, potentially causing toxicity. When metals are strongly bound to nutrients, they are generally more stable. In such conditions, metals can contaminate crops and affect nutrient availability for plants. Heavy metals on the soil surface can potentially be absorbed by plants through the roots and distributed throughout the plant. According to Siaka *et al*. (2021), heavy metals have a high density, making them accumulate more in roots and difficult to translocate to other parts of the plant.

Conclusion

The total content of Cd and Pb metals in gonda agricultural soil in Timpag Tabanan ranged from 67.6943 to 79.3031 mg/kg and 21.8463 to 28.1080 mg/kg, respectively. The bioavailable fractions were in the range of 13.76% to 21.51% for Cd and 10.19% to 11.54% for Pb, the potentially bioavailable fractions were between 53.91% and 57.84% for Cd and 79.97% and 80.00% for Pb, while the non-bioavailable fractions ranged from 24.58 to 28.39 for Cd and 8.46 to 9.84 for Pb. The total content of Cd and Pb in gonda plants was 4.3328 mg/kg and 1.4788 mg/kg, respectively. Based on these results, the concentrations of Cd and Pb in gonda plants exceeded the permissible limits according to WHO/FAO article CXS 193-1995.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

Baryatik, P., Moelyaningrum, A. D., Asihta, U., Nurcahyaningsih, W., Baroroh, A., and Riskianto, H. (2019). Pemanfaatan Arang Aktif Ampas Kopi sebagai Adsorben Kadmium pada Air Sumur. *Jurnal Teknologi Lingkungan Lahan Basah*, *2*(1), 11-19.

Bradl, H. (2005). *Sources And Origins of Heavy Metals*. Amsterdam: Elsevier Ltd.

Charlena. (2004). *Pencemaran Logam Berat Timbal (Pb) dan Cadmium (Cd) pada Sayur-sayuran*. Bogor: Falsafah Sains Program Pascasarjana S3 IPB.

Food and Agriculture Organization. Available online: https://www.fao.org/fao-who-codexalimentarius/thematic-areas/contaminants/en/ (accessed on 18th October 2023)

Gasparatos, D., Haidouti, C., Adrinopoulos, F., and Areta, O. (2005). Chemical Speciation and Bioavailability of Cu, Zn, and Pb in Soil from The National Garden of Athens, Greece. *Proceedings of the 9th International Conference on Environmental Science and Technology*. Rhodes Island, Greece.

Jena, V., Gupta, S., Dhundhel, R. S., Matie, N., Bilinski, S. F., and Devie, N. (2013). Determination of Total Heavy Metal by Sequential Extraction from Soil. *International Journal of Research in Environmental Science and Technology*, *3*(1), 35–38.

Mahendra, R., Siaka, I. M., and Suprihatin, I. E. (2018). Bioavailabilitas Logam Berat Pb dan Cd dalam Tanah Perkebunan Budidaya Kubis di Daerah Kintamani Bangli. *Ecotrophic, 12*(1), 42–49.

Reichman, S. M. (2002). *The Responses of Plants to Metal Toxicity: A Review Focusing on Copper, Manganese and Zinc*. Melbourne: The Australian Minerals and Energy Environment Foundation.

Siaka, I. M., Arimbawa, I. W., and Sudiarta, I. W. (2021). Kandungan Logam Cu Dan Pb Dalam Tanaman Bayam dan Bioavailabilitasnya dalam Tanah Pertanian dengan Pemberian Pupuk Kandang Sapi. *Jurnal Kimia (Journal of Chemistry)*, *15*(1), 74–82.

Siaka, I. M., Sahara, E., and Dharmayoga, I. G. A. P. M. (2015). Bioavailabilitas dan Spesiasi Logam Berat Pb dan Cd Pada Tanah Pertanian Basah dan Kering Di Daerah Denpasar. *Jurnal Kimia (Journal of Chemistry), 9*(1): 132–138.

Suastawan, G., Satrawidana, I D. K., Wiratini, N. M. (2016). Analisis Logam Pb dan Cd pada Tanah Perkebunan Sayur di Desa Pancasari. *Jurnal Wahana Matematika dan Sains*, *9*(2), 44-51

Sundari, D., Miko, H., and Suharjo. (2016). Kandungan Logam Berat dalam Bahan Pangan di Kawasan Industri Kilang Minyak Dumai. *Buletin Penelitian Sistem Kesehatan*, *19*(1), 55–61.

Tessier, A., Campbell, P. G. C., and Bisson, M. (1979). Sequential Extraction Procedure for the Speciation of Particulate Trace Metals. *Analytical Chemistry*, *51*(7), 844–851.

Tóth, G., Hermann, T., Da Silva, M.R., and Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environment International, 88*: 299–309.

Yap, C. K., Ismail, A., and Tan, S. G. (2003). Concentration, Distribution and Geochemical Speciation of Copper in Surface Sediment of the Strait of Malacca. *Pakistan Journal of Biological Sciences*, *6*(12), 1021–1026.

Yu, H., Li, C., Yan, J., Ma, Y., Zhou, X., Yu, W., Kan, H., Meng, Q., Xie, R. and Dong, P. (2023). A review on adsorption characteristics and influencing mechanism of heavy metals in farmland soil. *Royal Society of Chemistry, 13*(6), 3505-3519.