

BENEFITS OF KAFFIR LIME (CITRUS HYSTRIX DC) TO REDUCE CADMIUM (CD) AND MERCURY (HG) IN WATER KALE (IPOMEA AQUATICA FORSSK)

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ABSTRACT

Kale is an aquatic plant that is classified as a vegetable that is often consumed by people and rich in various nutrients that humans need. Water kale will cause health problems if it contains heavy metals. This study aims to determine the effect of variations in the concentration of kaffir lime solution on soaking kale to reduce levels of Cd and Hg metals in water kale. The research method used wet destruction and dry destruction, and then the sample was analyzed using an atomic absorption spectrophotometer. The results showed that the Hg metal content was 0.0068 ± 0.0082 mg/kg, and Cd was 0.0029 ± 0.0028 mg/kg. According to SNI 7378: 2009, the maximum limit of Hg contamination is 0.03 mg/kg and Cd in vegetables is 0.2 mg/kg. After soaking for 30 minutes with a kaffir lime solution, a concentration of 5, 10, 15, 20, and 25%, the Cd and Hg metals decreased. At a concentration of 25%, it shows a decrease in Cd and Hg metals of 0.0016 ± 0.0004 mg/kg (42.86%) and 0.0079 ± 0.0016 mg/kg by 32.00%.

Keywords: Kale, Lime, Mercury, Cadmium

INTRODUCTION

Water or aquatic kale (*Ipomoea aquatic* Forssk) is a prevalent agricultural plant, (Campos C. et al., 2011; Rahmi et al., 2019) with a very narrow land area even simple farmers can create their farming business. Kale is a vegetable that is easy to grow in damp places or near rivers (Anwar et al., 2020; Arimiyaw et al., 2020). It is widely grown near the river and doused with river water (Tiro et al., 2017).

Environmental pollution, which is in line with increasing development activities especially in the industrial sector (Calbo & Ferreira, 2011), has become a critical problem for developed and developing (Daryadar et al., 2019). The waste that enters the waters causes changes in water quality so that the waters become unfit for use by living things and contain dangerous metals such as cadmium (Cd) and mercury (Hg) (Ali et al., 2020). Water plants such as water kale that grows near rivers or sprinkling them with polluted water will have an impact on the content of water kale (Olmedo et al., 2013) which has the ability to absorb elements in the medium of its life including heavy metal cadmium (Cd) and mercury (Hg) (Bedia et al., 2019). The heavy metal contamination of cadmium and mercury in kale can cause humans' adverse effects when consuming water kale (Chen et al., 2018).

For the maximum limit of heavy metal contamination in food states that the permissible levels of heavy metal in kangkong that can be consumed by humans for cadmium (Cd) should not be more than 0.2 mg/kg (Dwivedi & Ma, 2014; Göthberg et al., 2002), while mercury metal cannot be more than 0.03 mg/kg (Ng et al., 2016). For this reason, efforts are needed to reduce levels of cadmium (Cd) and mercury (Hg) in kale so that they are safe for human consumption (Anuchapreeda et al., 2020). One such effort is to use a metal binder or a chelating agent, including citric acid and acetic acid (Jain & Gauba, 2017).

According to research conducted by (Dertyasasa & Tunjung, 2017) kaffir lime (*Citrus hystrix* D.C.) contains 8% citric acid (Kidarn et al., 2018), so kaffir lime can be used to reduce metal levels (Handayani et al., 2020). Based on the description above, the authors are interested in researching reducing levels of Cadmium and Mercury (Tunjung et al., 2015) in water kale using kaffir lime with a concentration of 5, 10, 15, 20, and 25% and a time of 30 minutes by determining the levels by atomic absorption spectrophotometry (Jayasinghe, 2014). This research is expected to produce the best method to quickly and effectively reduce Hg and Cd metals in water kale. The dissemination of research results to the public, especially housewives and food sellers is a great hope so that the impact of exposure to Hg and Cd metals can be minimized.

METHOD

Research tools and materials

Water kale (*Ipomoea aquatica* Frossk.), fresh kaffir lime (*Citrus hystrix* DC), HNO₃ (p), NaBH₄ 0.2%, Cd (NO₃) 2 1000 mg / l, HNO₃ 0.1M, H₂O₂ (p), HCl 16%, HNO₃ 5N, HCl 3%, NaOH 0.05%, HCl 6M, Hg (NO₃) 2 1000 mg / l, from Merck and aquabidest.

Atomic Absorption Spectrophotometers GTA-120 AA240Z with perkin-AA700 Pb Atomax Hollow Cathode Lamp. Atomic absorption spectrophotometers with perkin-100 Hg Hollow Cathode Lamp, Flow Injection Analysis System (FIAS), and glass tools (Smith, 2002).

1. Preparation of water kale with a concentration of kaffir lime solution

First, cleanly wash the water kale, separate the leaves and young stems and cut into small pieces. A total of 15 grams of kale water were soaked in kaffir lime solution for 30 minutes at each concentration of 5, 10, 15, 20, and 25%. After soaking the water kale, drain it then rinse it again with aquabidest, while the lime solution is collected in a glass bottle.

2. Wet destruction

Water kale and ligand solution which contains the Hg metal will be calculated, mashed first with a hand blender. The water kale that has been refined and the lime solution is weighed 0.5 grams in a test tube container with 6 ml HNO₃ added (P), 0.5 ml H₂O₂ (p) let stand for 45 minutes then add aquabidest to a scale of 15 ml. Heat it in a heating furnace at 90° C for 5 hours and let it cool, then filtered using Whatman filter paper no 42, the sample is collected into a 50 ml volumetric flask, 2.5 ml of 16% HCl is added and is sufficient with aquabidest to the limit. The solution is ready for measurements using an atomic absorption spectrophotometer at a wavelength of 253.7 nm (Smith, 2002).

3. Dry destruction

Water kale which will be calculated the Cd metal content is destructed by the dry destruction method (Tchounwou et al., 2012). Water kale weighed 10 grams in a porcelain dish and dried in an oven at 100°C for 1 hour. After that, it is ignited in an electric furnace at temperature 450°C for 5 hours until the ash is white or grey and then allowed to cool in a desiccator. 5 ml of HCl 6M was added then heated on an electric bath then the residue was dissolved with 10 ml of 0.1M HNO₃, let it cool, then filtered with Whatman filter paper no 42, the sample was accommodated in a 50 ml measuring flask and sufficient with aquabidest to the limit line. The solution is ready for measurements using an atomic absorption spectrophotometer at a wavelength of 228.8 nm.

4. Data Analysis

Metal concentration data from spectrophotometer will be calculated using the AOAC formula. 999.11 / 9.1.09.2005 and SNI 19-2896-1998 (Lepretre, 2012). Sample metal content calculation formula (recovery):

$$c = \frac{a \times v}{m}$$

Description:

c: Metal content in sample (mg/kg)

a: Metal concentration (µg/L)

m: Sample weight (g)

v: Solvent volume (L)

RESULT

Water kale obtained from the Cakung Industrial Area, Jakarta and Jeruk Purut obtained from Ijem Herbal, Yogyakarta have been determined at LIPI Biological Research Center Cibinong, Bogor to determine the type and ethnicity. The result of the determination was that water kale had Convolvulaceae species and kaffir lime had Rutacea species.

The sample will be crushed in the acid chamber before reading with an Atomic Absorption Spectrophotometer (AAS). The acid crushing procedure used is the wet method to calculate the Hg metal content, and the dry method is used to calculate the Cd metal content in kale. (Al-Kodmany, 2018).

1. Analysis of Cd and Hg levels before treatment

A sample that has been destructed is to be measured for its metal content using an atomic absorption spectrophotometer. In measuring the sample level, two readings were done. From these readings, the results are averaged so that Cd and Hg levels in the sample are obtained (Table 1).

Table 1. Analysis of Cd and Hg levels before treatment

Metal	Content (mg/kg)		Mean	SD	%RSD
	I	II			
Hg	0,0146	0,0121	0,0134	0,0017	13,2417
Cd	0,0029	0,0028	0,0028	0,0012	1,5020

For analysis of Hg metal before the treatment, is weighed 0.5 g of the sample by wet destruction then check the metal content with an atomic absorption spectrophotometer and obtained an average level of Hg metal of 0.0134 mg/kg. Analysis of Cd levels weighed 10 g of water kale samples, dry destruction, then

check the metal content with an atomic absorption spectrophotometer and obtained an average Cd content of 0.0028 mg/kg.

According to SNI 7387: 2009, the maximum limit for the Hg metal content in water kale is 0.03 mg/kg and Cd 0.2 mg/kg. Meanwhile, based on the analysis results, Hg and Cd's metal content in water kale does not exceed the maximum limit of the specified SNI standards.

2. Analysis of Cd and Hg levels after treatment

The analysis results of the reduction in Cd metal content in water kale after treatment with kaffir lime solution based on the difference in concentration and immersion time (Table 2).

Table 2. Analysis of Cd levels after treatment

Kaffir Lime Concentratio n	Cd content (mg/kg)			SD	%RSD	Content reduction (%)
	Kale		Mean			
	I	II				
Negative control	0,0029	0,0028	0,0028	0,0000	0	-
5%	0,0026	0,0019	0,0023	0,0005	21,7391	17,86%
10%	0,0021	0,0018	0,0020	0,0002	10	28,57%
15%	0,0022	0,0015	0,0019	0,0005	26,3157	32,14%
20%	0,0014	0,0020	0,0017	0,0004	23,5294	39,28%
25%	0,0019	0,0013	0,0016	0,0004	25	42,86%

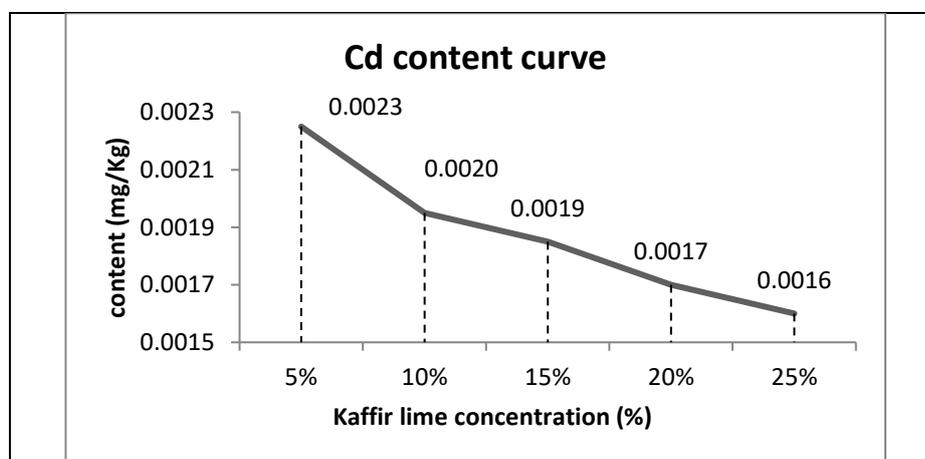


Figure 1. Cd content graphic

After treatment with different concentrations and 30 minutes of soaking time, it showed a decrease in Hg levels in water spinach (Table 3).

Table 3. Analysis of Hg levels after treatment

Kaffir Lime Concentratio n	Hg content (mg/kg)			SD	%RSD	Content reduction(%)
	Kale		Mean			
	I	II				
Negative control	0,0146	0,0121	0,0134	0,0017	13,4328	-
5%	0,0130	0,0083	0,0107	0,0033	30,8411	17,33%

10%	0,0096	0,0098	0,0097	0,0001	10,3092	18,66%
15%	0,0121	0,0086	0,0104	0,0024	24,0384	25,33%
20%	0,0064	0,0100	0,0082	0,0025	30,4878	28,00%
25%	0,0067	0,0090	0,0079	0,0016	20,2531	32,00%

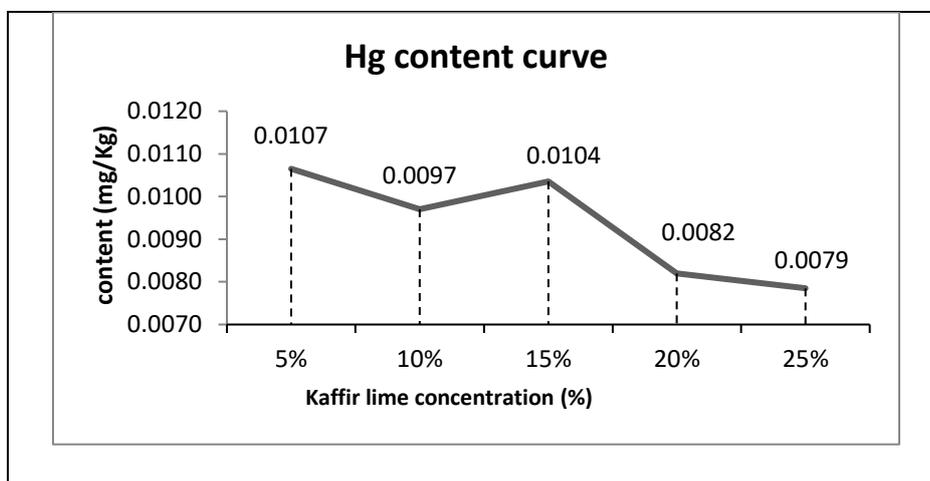


Figure 2.Hg content graphic

There is a decreased metal content in water kale which is not linear from each concentration, which is influenced by the value of the adjacent concentration which is only 5% different so that the value of the decrease in levels looks the same.

Analysis of Cd and Hg levels in water spinach samples after soaking showed decreased metal content at each Jeruk Purut solution concentration. the highest decrease is at a concentration of 25% kaffir lime solution with a metal content of Cd 0.0016 ± 0.0004 mg / kg amounted to 42.86% and Hg metal content of 0.0079 ± 0.0016 mg / kg of 32.00%. Kaffir lime contains 8% citric acid. Citric acid is a tricarboxylic acid that occurs naturally in fruits (Sato et al., 2011), where each molecule contains a carboxyl group and a hydroxyl group attached to a carbon atom (Kasuan et al., 2013). Citric acid is an effective metal binder, and carboxyl ions as a good electron donor will work together to form complex electron bonds (Dumičić et al., 2014).

From the curve image above, it can be seen that there is a decrease in the levels of heavy metals Cd and Hg contained in water kale, this proves that the Purut orange solution can reduce/reduce or bind heavy metals in water kale samples. Therefore, this study aims to reduce the metal content in water kale as low as possible so that people are safe when consuming water kale as a complementary vegetable in food. From the research and discussion results, to reduce the metal content in water kale, kaffir lime can be used.

CONCLUSION

From the results of this study, it is known that water kale derived from agriculture near the Cakung Industrial Area, East Jakarta, contains levels of heavy metals Cd and Hg, respectively, 0.0028 mg/kg and 0.0134 mg/kg. The effect of variations in immersion concentration with kaffir lime solution on reducing levels of heavy metals Cd and Hg in water kale samples. At various concentrations of kaffir lime solution that can reduce the highest levels of heavy metal Cd and Hg,

namely at a concentration of 25%, namely 0.0016 ± 0.0004 mg/kg of 42.86% and Hg metal content of 0.0079 ± 0.0016 mg/kg by 32.00%.

REFERENCES

- Al-Kodmany, K. (2018). The Vertical Farm: A review of Developments and Implications for the Vertical City. *Buildings*, 8(2). <https://doi.org/10.3390/buildings8020024>
- Ali, S., Awan, Z., Mumtaz, S., Shakir, H. A., Ahmad, F., Ulhaq, M., Tahir, H. M., Awan, M. S., Sharif, S., Irfan, M., & Khan, M. A. (2020). Cardiac toxicity of heavy metals (cadmium and mercury) and pharmacological intervention by vitamin C in rabbits. *Environmental Science and Pollution Research*, 27(23), 29266–29279. <https://doi.org/10.1007/s11356-020-09011-9>
- Anuchapreeda, S., Chueahongthong, F., Viriyaadhammaa, N., Panyajai, P., Anzawa, R., Tima, S., Ampasavate, C., Saijai, A., Rungrojsakul, M., Usuki, T., & Okonogi, S. (2020). Antileukemic cell proliferation of active compounds from kaffir lime (*Citrus hystrix*) leaves. *Molecules*, 25(6), 1–16. <https://doi.org/10.3390/molecules25061300>
- Anwar, M., Birch, E. J., Ding, Y., & Bekhit, A. E.-D. (2020). Water-soluble non-starch polysaccharides of root and tuber crops: extraction, characteristics, properties, bioactivities, and applications. *Critical Reviews in Food Science and Nutrition*, 1–33. <https://doi.org/10.1080/10408398.2020.1852388>
- Arimiyaw, A. W., Abass, K., & Gyasi, R. M. (2020). On-farm Urban Vegetable Farming Practices and Health Risk Perceptions of Farmers in Kumasi. *GeoJournal*, 85(4), 943–959. <https://doi.org/10.1007/s10708-019-10003-7>
- Bedia, J., Muelas-Ramos, V., Peñas-Garzón, M., Gómez-Avilés, A., Rodríguez, J. J., & Belver, C. (2019). A review on the synthesis and characterization of metal organic frameworks for photocatalytic water purification. *Catalysts*, 9(1). <https://doi.org/10.3390/catal9010052>
- Calbo, A. G., & Ferreira, M. D. (2011). Evaluation of hydration indexes in kale leaves. *Brazilian Journal of Plant Physiology*, 23(2), 141–149. <https://doi.org/10.1590/S1677-04202011000200006>
- Campos C., A., Hernández, M. E., Moreno-Casasola, P., Cejudo Espinosa, E., Robledo R., A., & Infante Mata, D. (2011). Soil water retention and carbon pools in tropical forested wetlands and marshes of the Gulf of Mexico. *Hydrological Sciences Journal*, 56(8), 1388–1406. <https://doi.org/10.1080/02626667.2011.629786>
- Chen, Y., Zhang, Q., Wang, S., Yang, Y., Meng, B., Hu, D., & Lu, P. (2018). Residue dynamics and risk assessment of dimethoate in sweet potato, purple flowering stalk, Chinese kale, celery, and soil. *Human and Ecological Risk Assessment: An International Journal*, 24(3), 767–783. <https://doi.org/10.1080/10807039.2017.1399050>
- Daryadar, M. K., Mairapetyan, S. K., Tovmasyan, A. H., Aleksanyan, J. S.,

- Tadevosyan, A. H., Kalachyan, L. M., Stepanyan, B. T., Galstyan, H. M., & Asatryan, A. Z. (2019). Productivity of Leafy Green Vegetable Kale in Soilless Cultivation Conditions. *Journal of Agricultural Science and Food Research*, 10(2), 95–98. <https://doi.org/10.35248/2593-9173.19.10.260>
- Dertyasasa, E. D., & Tunjung, W. A. S. (2017). Volatile Organic Compounds of Kaffir Lime (*Citrus hystrix* DC.) Leaves Fractions and their Potency as Traditional Medicine. *Biosciences, Biotechnology Research Asia*, 14(4), 1235–1250. <https://doi.org/10.13005/bbra/2566>
- Dumičić, G., Díaz-Pérez, J., & Sidhu, H. S. (2014). Kale (*Brassica oleracea* L. var. *acephala* DC) leaf water loss as affected by genotype and bagging. *49th CROATIAN AND ...*, June, 305–309. <http://bib.irb.hr/prikazirad?lang=en&rad=688738>
- Dwivedi, A. D., & Ma, L. Q. (2014). Biocatalytic Synthesis Pathways, Transformation, and Toxicity of Nanoparticles in the Environment. *Critical Reviews in Environmental Science and Technology*, 44(15), 1679–1739. <https://doi.org/10.1080/10643389.2013.790747>
- Göthberg, A., Greger, M., & Bengtsson, B.-E. (2002). Accumulation of Heavy Metals in Water Spinach (*Ipomoea aquatica*) Cultivated in the Bangkok Region, Thailand. *Environmental Toxicology and Chemistry*, 21(9), 1934–1939.
- Handayani, R. S., Yunus, I., Tillah, N., & Handayani, I. (2020). Effect of Cytokines On The In Vitro of Sweet Kaffir Lime (*Citrus hystrix* Dc). *Journal of Tropical Horticulture*, 3(2), 60. <https://doi.org/10.33089/jthort.v3i2.51>
- Jain, J., & Gauba, P. (2017). Heavy metal toxicity-implications on metabolism and health. *International Journal of Pharma and Bio Sciences*, 8(4), 452–460. <https://doi.org/10.22376/ijpbs.2017.8.4.b452-460>
- Jayasinghe, G. Y. (2014). Utilization of Agricultural Waste Compost as an Alternative Potting Media Component with Coir Dust for Leafy Vegetable *Ipomoea Acquatica*. *Journal of Plant Nutrition*, 37(10), 1601–1611. <https://doi.org/10.1080/01904167.2014.911318>
- Kasuan, N., Muhammad, Z., Yusoff, Z., Rahiman, M. H. F., Taib, M. N., & Haiyee, Z. A. (2013). Extraction of *Citrus hystrix* D.C. (Kaffir Lime) essential oil using automated steam distillation process: Analysis of volatile compounds. *Malaysian Journal of Analytical Sciences*, 17(3), 359–369.
- Kidarn, S., Saenjurn, C., Hongwiset, D., & Phrutivorapongkul, A. (2018). Furanocoumarins from Kaffir lime and their inhibitory effects on inflammatory mediator production. *Cogent Chemistry*, 4(1), 1529259. <https://doi.org/10.1080/23312009.2018.1529259>
- Lepretre, G. (2012). *Setting up an Atomic Absorption Spectrometer (AAS) for Determining Heavy Metals (Hg , Cd , Pb) in food.*

- Ng, C. C., Rahman, M. M., Boyce, A. N., & Abas, M. R. (2016). Heavy metals phyto-assessment in commonly grown vegetables: water spinach (*I. aquatica*) and okra (*A. esculentus*). *SpringerPlus*, 5(469), 1–9. <https://doi.org/10.1186/s40064-016-2125-5>
- Olmedo, P., Pla, A., Hernández, A. F., Barbier, F., Ayouni, L., & Gil, F. (2013). Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environment International*, 59, 63–72. <https://doi.org/10.1016/j.envint.2013.05.005>
- Rahmi, P., Witjaksono, & Ratnadewi, D. (2019). Induksi Poliploidi Tanaman Kangkung (*Ipomoea aquatica* Forssk.) Kultivar Salina In Vitro dengan Oryzalin. *Jurnal Biologi Indonesia*, 15(1), 1–8. <https://doi.org/10.47349/jbi/15012019/1>
- Sato, A., Asano, K., & Sato, T. (2011). The Chemical Composition of Citrus *Hystrix* DC (Swangi). *Journal of Essential Oil Research*, 2(4), 179–183. <https://doi.org/10.1080/10412905.1990.9697857>
- Smith, R. (2002). *A Laboratory Manual for the Determination of Metals in Water and Wastewater by Atomic Absorption Spectrophotometry*. National Institute for Water Research.
- Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Molecular, clinical and environmental toxicology Volume 3: Environmental Toxicology. *Molecular, Clinical and Environmental Toxicology*, 101, 133–164. <https://doi.org/10.1007/978-3-7643-8340-4>
- Tiro, L. La, Isa, I., & Iyabu, H. (2017). Potensi Tanaman Kangkung Air (*Ipomoea Aquatica*) Sebagai Bioabsorpsi Logam Pb dan Cu. *Jurnal Entropi*, 12(1), 81–86.
- Tunjung, W. A. S., Cinatl, J., Michaelis, M., & Smales, C. M. (2015). Anti-Cancer Effect of Kaffir Lime (*Citrus Hystrix* DC) Leaf Extract in Cervical Cancer and Neuroblastoma Cell Lines. *Procedia Chemistry*, 14, 465–468. <https://doi.org/10.1016/j.proche.2015.03.062>
- Wulandari, W., & Kurniawati. (2017). Antioxidant properties of kaffir lime oil as affected by hydrodistillation process. *Journal of Agricultural and Food Chemistry*, 1(1), 2549–094. <https://www.ejurnal.unisri.ac.id/index.php/proictss/article/viewFile/1436/126>