Utilization Of Belimbing Wuluh (Averrhoa Bilimbi L.) For Decreasing Chromium(VI) Ion Levels In Tilapia (Oreochromis Niloticus)

Ana Hidayati Mukaromah[1, a)] Intan Retnosari[2,b)]

[1]*Magister Program Study of Clinical Laboratory Science,Universitas Muhammadiyah Semarang, Semarang, Central Java 50273 Indonesia*
[2]*Bachhelor of Applied Science Medical Laboratory Technology, Universitas Muhammadiyah Semarang, Semarang, Central Java 50273 Indonesia*

[a)]Corresponding author: ana_hidayati@unimus.ac.id
[b)] Intanretnosari22@gmail.com

Astract. Water pollution in the small industrial area of Genuk Semarang has the potential to contain heavy metals due to pollution of the printing industry waste one of them is the heavy metal Cr(VI). Fish biota that is often contaminated is Nila fish. Foods contaminated with heavy metals need to be reduced with sequestrants, one of which is citric acid. Starfruit is a substance that contains citric acid so that it can reduce levels of Cr(VI) ion contamination in Nila fish. The purpose of this study was to determine the effect of variations in concentration and duration of immersion starfuit on decreasing levels of Cr(VI) ions in Nila fish. The object of research is Nila fish in industrial area of Genuk Semarang, then reduced levels of Cr(VI) variation of starfuit ($75\%^{v}/_{v}$ and $100\%^{v}/_{v}$) and immersion time (30, 40 and 50 minutes). The results of the optimum wavelength is 545 nm and the optimum stabillity time is 5 minutes. The initial Cr(VI) level was 9.02±0.02 mg/kg. The percentage decrease in Cr(VI) levelas at the end of $75\%^{v}/_{v}$ concentration of immercion for 30 minutes, 40 minutes and 50 minutes is 28.37±0.33%; 34,04 ±0.16% and 46.6 ±0.32%. Concentration of $100\%^{v}/_{v}$ immercion for 30 minutes, 40 minutes and 50 minutes is 44.34±0.44%; 56.26±0.28% and 65.90±0.27%. Conclussion: The most effective concentration and duration of immersion in starfruit *(Averrhoa bilimbi L.)* reducing Cr(VI) levels in Nila fish was an starfuit concentration of $100\%^{v}/_{v}$ for 50 minutes of 65.90±0.27%.
Keyword: Tilapia, decreased levels of Cr(VI), *Averrhoa bilimbi L.*

INTRODUCTION

Pollution is the entry of substances or elements into the environment, which can be caused by poor waste treatment or no waste treatment. Water pollution can be classified into three, namely household waste, industrial waste and agricultural waste (Kristanto, 2013). The Twin Glatik Factory is a company engaged in the printing sector, having its at Jalan Industry II no. 70 A LIK Semarang. This factory produces various kinds of printed products such as notebooks, folders, note books, envelopes, receipts, and invitations and is capable of producing 100 to 500 books per day. The factory does not yet have a waste treatment unit, so the waste produced is disposed of through a pipeline that leads to Kali Gede which is located close to the factory (Budiyono, 2017). The level of Cr (VI) ions in water that exceeds the threshold of 0.05 mg/L. When consumed by humans will cause Poisoning Chromium can affect the respiratory tract, kidneys and liver causing nose irritation, nasal ulcers, lung cancer, asthma and hypersensitivity reactions.

In this industrial process, many raw materials and auxiliary materials containing hazardous materials are used, such as solvents and inks. The ink used is in the form of pigment ink (durabrite), dye base ink, as a basic producer of black, blue, yellow and pink. Elements and compounds contained in ink include alcohol, wax, resin, lubricant, solvent, and drying oil (Susanto, 2013). The printing process uses these materials and will produce waste from the ink and solvent used, so that the waste also has hazardous and toxic properties.

Printing liquid waste consists of damaged inks, solvents, thinners and drying agents. Materials that contain toxic chemicals and heavy metals such as lead (Pb), cadmium (Cd), Chromium (Cr), mercury, manganese (Mn), alcohol, acetate, ink (dyes) pigment (Ratnawati et al, 2011). The presence of heavy metal chromium (Cr) in waters can have a negative impact on aquatic organisms. The level of pollution of a waters can be seen from the types of fish that live in these waters, because fish are an efficient indicator of pollution to predict heavy metal pollution. Fish that live in the waters of the Genuk Small Industry Environment (LIK) Semarang include mujahir fish, tilapia, methamphetamine fish, sepat fish, and duck fish (Pangesti, 2015).

Tilapia is the most common fish found in these waters. The existence of heavy metal pollution in tilapia is due to the contact of the fish with the waste disposal results of the printing factory which is toxic, the contact takes place because of the transfer of chemicals from the waters into or on the surface of the fish's body that enters through the gills. Efforts should be made to reduce heavy metal levels by adding sequestrants. Sukestran is a material that can bind metals in food so that the food quality is maintained from heavy metal contamination, such as starfruit (Sahetapy, 2011). Belimbing wuluh (Averrohoa bilimbi L.) is a fruit that is often found and used by the community as a food and medicine additive. The content of starfruit is ascorbic acid, citric acid, saponins, formic acid, glucose, tannins, flavonoids, citrate in the form of potassium citrate and potassium oxalate. The content of citric acid in starfruit as a reducing agent for heavy metal concentrations is because citric acid is a tricarboxylic acid in which each molecule contains a carboxyl group and one hydroxyl group attached to a carbon atom as a metal binder (Latumeten, et al., 2013). Research Wardhani, A, K, et al,. (2019) initial level of Cr (VI) 8.69 mg/kg. After soaking using passion fruit the most effective concentration and soaking time was 50% v/v for 60 minutes with a percentage of 44.93%. Therefore, further research is needed to determine the utilization of starfruit extract (Averrhoa bilimbi L.) to reduce chromium (VI) ion levels in tilapia (Oreochromis niloticus) in the waters of the small industrial area of Genuk Semarang.

MATERIALS AND METHODS

The materials needed are starfruit extract with concentrations of 75%V/V and 100%V/V, distilled water, concentrated HNO_3 solution 65% (Merck), Na_2S (Technical), $K_2Cr_2O_7$ (Merck), Na_3PO_4 (Merck), Na_2CO_3 (Merck), diphenylcarbazide (Merck). The research conducted is experimental in nature. The inspection method for Cr(VI) is spectrophotometry. The independent variable was soaking time of 30, 40 and 50 minutes with varying concentrations of starfruit 75%V/V and 100%V/V, while the dependent variable was the level of Cr (VI) ions in tilapia.

Preparation of starfruit extract with concentrations of 75% v/v and 100%v/v

The starfruit is peeled and then washed and then cut into small pieces and blended, filtered through a clean cloth (starfruit juice concentration of 100%). Starfruit juice 100% v/v) was poured with a 75 mL burette into a 100 mL volumetric flask and distilled water was added to the mark and homogenized (starfruit juice concentration 75% v/v).

Preparation standard solution of Cr (VI) 1000 ppm, 100 ppm, and 10 ppm

$K_2Cr_2O_7$ powder was weighed as much as 2.8290 g then added 1 mL of concentrated HNO3, then put in a 1 L volumetric flask and the volume of the solution was adjusted with distilled water. 1000 ppm Chromium metal (Cr VI) mother liquor was pipetted 100 mL into a 1000 ml volumetric flask and adjusted with distilled water up to the mark (Cr VI standard 100 ppm). 100 ppm Cr (VI) solution was pipetted 20 mL into a 200 ml volumetric flask and adjusted with distilled water up to the mark.

Wavelength optimization and Stability Time with 0.1 ppm Cr(VI) standard; 0.5 nd 1.0 ppm

Four of 50 ml volumetric flasks, the first flask was filled with distilled water up to ± 40 mL, while the 2nd-4th flask was filled with 10 ppm Cr(VI) standard 0.5 ml respectively; 2.5 ml; and 5 ml. Plus aquadest to vol 40 ml. Each flask + 2.5 ml of diphenylcarbazide, adjusted the mark and let stand 15 minutes. The absorbance is measured at a wavelength of 530-550 nm. The result of the maximum absorbance obtained is used as the maximum wavelength. The procedure was repeated with stability times of 3, 5, 10 and 15 minutes. The absorbance is read at the maximum wavelength.

Calculation Cr(VI) Ion Level in Tilapia (mg/kg)

X = (Y-0,0135) : 0,8935 and Kadar $\mathrm{Cr\ (VI)} = \frac{C \times V}{W}$

X : Concentration Sample (mg/L); Y : Absorbance Sample; p : dilution sample; V : Sample Volume (mL); W: Sample Weight (g)

Preparation of Cr (VI) series raw materials 0.1 - 1.0 ppm

Eleven volumetric flasks of 50 mL were prepared, the first flask as a blank was put in 40 mL of distilled water, the 2nd-11th flask was entered successively the standard Cr (VI) 10 ppm as much as 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0 mL, then added distilled water up to a volume of 40 mL and 2.5 mL of diphenylcarbazide and added distilled water up to the mark, allowed to stand for maximum stability time. The absorbance is read at the maximum wavelength.

Tilapia Sample Preparation

Each tilapia meat was weighed 10 g then mashed, charred and incinerated using a 550°C mufle furnace for 8 hours. The ash obtained was digested with 5 ml of concentrated HNO3 until clear, then transferred quantitatively to a 50 mL volumetric flask and added distilled water up to the mark, homogenized and filtered.

Soaking tilapia samples with starfruit

Prepare 4 containers, each weighing ± 10 grams of tilapia meat, then soaking in 30 mL of 75%v/v starfruit juice for 30 minutes, then draining. The procedure was repeated for 75%v/v concentration for 40 and 50 minutes, and 100% concentration with immersion time for 30, 40 and 50 minutes.

Cr(VI) Quantitative Test on Initial Tilapia and after soaking in Startfruit

Pipette 10.0 mL of the sample preparation into a 50 mL volumetric flask, add 30 mL of distilled water, add 2.5 mL of diphenylcarbazide, adjust with distilled water to the mark and incubate for maximum stability time. The absorbance is read at the maximum wavelength.

RESULTS AND DISCUSSIONS

Results for reseach are optimizing wavelength on Figure 1, Stability time on Figure 2, and calibration curve of ion Cr(VI) 0n Figure 3.

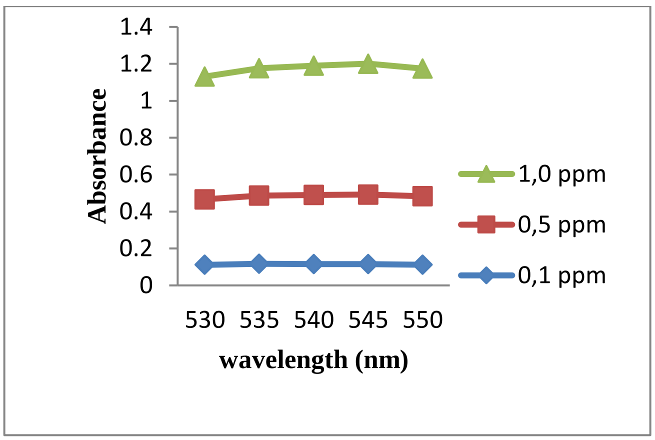

Figure 1. Wavelength Optimization Graph

Figure 1 shows an increase in the absorbance value of the Cr(VI) standard solution of 0.1; 0.5 and 1.0 ppm at a wavelength of 530 - 545 nm, but at a wavelength of 550 nm the absorbance decreases, so that the maximum wavelength is 545 nm.

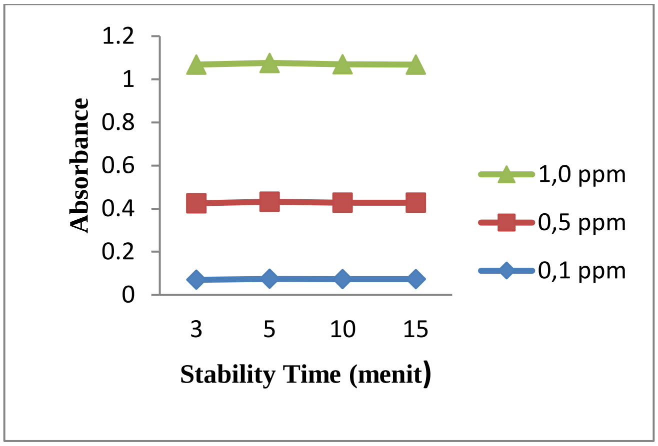

Figure 2. Stability Time Graph

Figure 2 shows the optimization of stability time carried out with a series standard of 0.1; 0.5 and 1.0 ppm with a stability time of 3; 5; 10 and 15 minutes. The absorbance increased from 3-5 minutes and decreased from 10-15 minutes, so that the maximum stability time was 5 minutes.

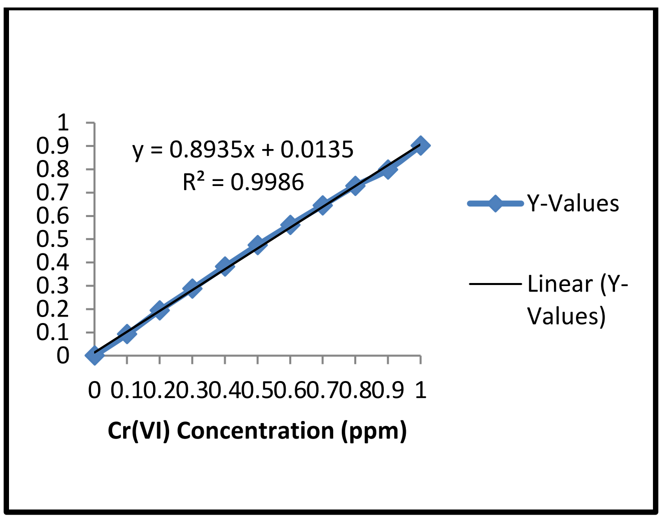

Figure 3. Cr(VI) Calibration Curve

Figure 3 shows the linear regression of the standard curve equation for Cr(VI) $y = 0.8935x + 0.0135$, with R square = 0.9986. This formula is used to calculate the initial and final Cr(VI) concentrations.

Ion Cr(VI) Level in Tilapia initial and after Immersion Time with variation Belimbing wuluh in Table 1.

Table 1. Ion Cr(VI) Quantitative in Tilapia

Consentration of Belimbing Wuluh (% $^v/_v$)	Immersion Time (minute)	Cr(VI) Ion Level (ppm)
Initial	0	9.02 ± 0.02
	30	6.515 ± 0.08

75	40	5.930 ± 0.07
	50	4.778 ± 0.08
	30	5.013 ± 0.08
100	40	3.913 ± 0.08
	50	3.083 ± 0,08

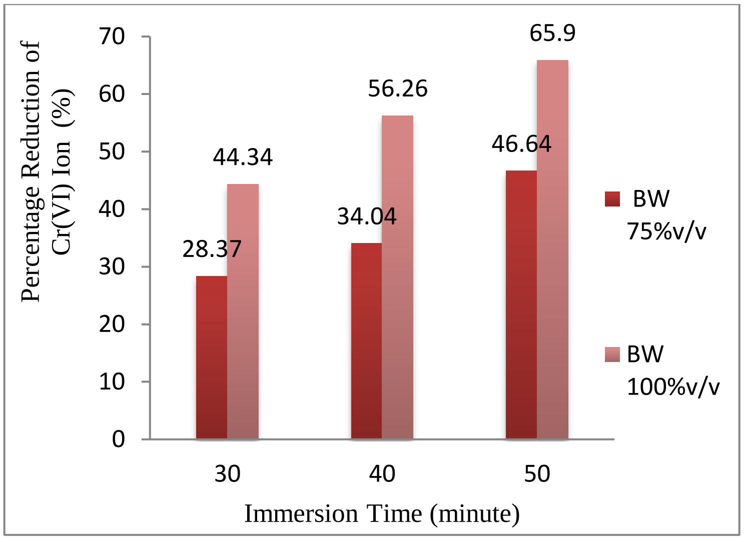

Figure 4. Percentage Reduction of Cr(VI) Ion Level

Discussion

This study aims to measure Cr(VI) levels in tilapia meat before and after treatment and to measure the percentage of chromium levels reduced. In this study it is expected to obtain a decrease in Cr(VI) using effective variations in concentration and duration of immersion of belimbing wuluh. Table 1 shows that soaking starfruit in a concentration of 100% v/v resulted in a higher concentration of Cr(VI) than the concentration of 75%v/v. This is because the concentration of starfruit juice is higher, the number of citric acid groups is greater, so that the ability of citric acid to bind to the heavy metal Cr(VI) will increase, so that the level of Cr(VI) ions will decrease. This is also because starfruit juice contains 2-hydroxy-1,2,3-propanetricarboxylic acid which can cause metals to lose their ionic properties in heavy metals so that they can reduce the toxicity of these metals (Sinaga, 2013).

The organic acid component of star fruit is citric acid (Lathifah, 2018), which is one of the organic acids with the chemical name 2-hydroxy-1,2,3-propanetricarboxylic acid which is non-toxic, functions as a sequestrant has properties as a metal binder so can reduce levels of heavy metals (Hudaya, 2011). Citric acid is able to bind metals which are carboxyl groups –COOH which can release protons in solution (Ovelando et al., 2013). The citric acid contains negative ions and the metal contains positive ions so that attraction will occur so that normal bonds will occur, so the metal will lose its toxicity. The longer the soaking time, the better the concentration of starfruit in concentrations of 75% v/v and 100% v/v, the longer the contact between the metal ions Cr(VI) and citric acid in starfruit, so that the carboxylic groups present in citric acid in starfruit experiencing higher deprotonization, which means that overall it has worked to bind Cr(VI), so that the Cr(VI) level in tilapia decreases (Rosyida, 2014).

Miftahul (2010) explained that the maximum number of bonds between metal chelating groups and metal cations is determined by the coordination number, so the number of cations e xchanged will be proportional to the hydrogen ions released. Figure 5 shows that the effective concentration of starfruit is 100% V/V for 50 minutes of immersion can reduce Cr(VI) levels in tilapia 65.90 ± 0.27%. This is based on Wardhani's research (2019) with initial levels of Cr(VI) in green mussels, namely 8.69 mg/kg, after soaking using passion fruit with the most effective concentration of 50% v/v for 60 minutes it decreased to 4, 78 mg/kg with a reduction percentage of 44.59%.

This is based on Adha's research (2019) with initial levels of Pb in clam clams, namely 0.893 mg/kg, after soaking using starfruit with the most effective concentration of 75% v/v for 25 minutes it decreased to 0.295 mg/kg with a decreasing percentage of 66.94%.

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Statistic test

The study was tested statistically using One Way Anova, preceded by a data normality test due to the requirements of One Way Anova, the data is normally distributed, where the normality test results for Cr(VI) levels with varying concentrations of 75%v/v and 100%v/ v and immersion for 30 minutes, 40 minutes and 50 minutes obtained a sig value of 0.940 which means > 0.05, so the data is normally distributed. In the homogeneity test, a sig value of 0.970 was obtained, which means > 0.05 so that the data has the same or homogeneous variance, which means that the six concentration variations are the same. Then tested with one way ANOVA obtained a sig value of 0.000 > 0.05, this shows that there is an effect of variations in concentration with immersion times of 30 minutes, 40 minutes and 50 minutes on the average percentage decrease in Cr(VI) levels in tilapia.inutes it decreased to 0.295 mg/kg with a decreasing percentage of 66.94%.

CONCLUSSION

The coclussion of this research . Initial Cr (VI) levels in tilapia samples before treatment obtained an average of 9.02 ± 0.02 mg/kg. The highest percentage of Cr(VI) levels after immersion using starfruit was the highest at a concentration of 100% v/v for 50 minutes, which reduced Cr(VI) levels in tilapia by 65.90 ± 0.27%. There is an effect of variations in the concentration of starfruit juice and soaking time on the percentage of Cr (VI) levels in tilapia.

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REFERENCES

Adha, Jayadipraja, E.A and Sunarsih. 2019. (Effectiveness of citrus aurantifolia swingle and Averrhoa bilimbi in reducing lead concentrations in kalandue shells (polymesoda sp) from Kendari Bay). Efektifitas citrus aurantifolia swingle dan Averrhoa bilimbi dalam menurunkan konsentrasi timbal pada kerang kalandue (polymesoda sp) dari teluk Kendari Jurnal Kesehatan Lingkungan 5(1).

Budiono, 2017. Health Risk Analysis of Benzene Exposure in the Printing Industry. (Analisis Resiko Kesehatan Pajanan Benzena Di Industri Percetakan Semarang).

Kristanto, P. 2013. Ecology industry. Edisi 2. C.V Andi Offset. Yogyakarta.

Latumeten, dkk. 2013. Effect of Soaking Time of Bamboo Shells (Anadara antiquate) in Belimbing Wuluh Extract (Avveroha bilimbi) on Lead (Pb) Content. (Pengaruh Lamanya Perendaman Kerang Buluh (Anadara antiquate) Dalam Ekatrak Belimbing Wuluh (Avveroha bilimbi) Terhadap Kandungan Logam Timbal (Pb)): (Karya Tulis Ilmiah. Universitas Pattimura. Ambon

Lathifah, Q. 2018. Test the effectiveness of the crude extract of antibacterial compounds in star fruit (Averrhoa bilimbi L.) with a variety of solvents. (Uji efektifitas ekstrak kasar senyawa antibakteri pada buah belimbing wuluh (Averrhoa bilimbi L.) dengan variasi pelarut). Skripsi. Universitas Islam Negeri Malang.

Sahetapy, J . M. 2011. Heavy Logsm Lead (Pb) Toxicity and Its Effect on Oxygen Consumption and Juvenile Hematological Response of Tiger Grouper (Toksititas Logsm Berat Timbal (Pb) dan Pengaruhnya pada Konsumsi Oksigen dan Respon Hematologi Juvenil Ikan Kerapu Macan). Thesis. Bogor: Pasca Sarjana IPB.

Sinaga, D., Marsaulina, I., Ashar, T. 2013. Comparison of reduction in Cadmium (Cd) levels in blood clams (Anadara granosa) by immersing lime solution (Citrus aurantifolia) at various concentrations by immersing lime solution (Perbandingan penurunan kadar Cadmium (Cd) pada kerang darah (Anadara granosa) dengan perendaman larutan jeruk nipis (Citrus aurantifolia) pada berbagai konsentrasi dengan perendaman). *Lingkungan dan Kesehatan Kerja.* 2(3).

Susanto, S.A., dan Adhi, A, 2013. The Influence of Ink Selection on Print Quality in the Newspaper Printing Industry (Pengaruh Pemilihan Tinta Terhadap Kualitas Cetak Dalam Industri Percetakan Koran). Jurnal Ilmiah Dinamika Teknik.

Ovelando. R. 2013. Fermentation of passion fruit (Passiflora) into citric acid (Fermentasi buah markisa (Passiflora) menjadi asam sitrat). 1(1).

Pangesti, I. 2015. Decrease in Chromium (VI) Ion Levels in Green Mussels (Perna viridis L.) With Belimbung Wuluh Extract (Averrhoa bilimbi L.). Penurunan Kadar Ion Cromium (VI) Pada Kerang Hijau (Perna viridis L.) Dengam Sari Belimbung Wukuh (Averrhoa bilimbi L.). Karya Tulis Ilmiah. Universitas Muhammadiyah Semarang.

Rosyida, Purwonogroho, 2014, Adsorption of Lead (II) Using Azollmicrophylla Biomass Esterified with Citric Acid (Adsorpsi Timbal (II) Menggunakan Biomassa Azollamicrophylla Diestirifikasi dengan Asam Sitrat). Jurnal Ilmu Kimia Universitas Brawijaya 2(2).

Wardhani,A. K, Mustofiyah, M., Widyaningrum and Mukaromah, A.H. 2019. Utilization of Passion Fruit (Passiflora edulis) as an Effort to Handle Cr (VI) Ion Contamination in Green Mussels (Perna Viridislinn)(Pemanfaatan Buah Markisa (Passiflora edulis) Sebagai Upaya Penanganan Cemaran Ion Cr (VI) Pada Kerang Hijau (Perna Viridislinn)). Colloqium STIKES Gombong.