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Research Article

HEAVY METALS (Ni, Cu, Zn AND Cd) CONTENT IN SERUM OF RAT FED GREEN MUSSELS (*Perna Viridis*)

Muhammad Yudhistira Azis[1,2], Suryo Gandasasmita[1] dan Agung Dhamar Syakti[2]

[1]*Analytical Chemistry Laboratory, Department of Chemistry, Institute of Technology Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia*
[3] *Centre for Maritime Bioscience Studies-Institute for Research and Community Service, Jenderal Soedirman University, Jl. Dr. Suparno, Purwokerto 53123, Indonesia*

[*]*Corresponding author: yudis_chempd@yahoo.com*

ABSTRACT

Green mussel (*Perna viridis*) can playing role as bio-indicator or biomonitoring agent for heavy-metal contaminations in the sea. In this research, the concentrations of four elements Ni, Cu, Zn and Cd in *P. viridis* and in the serum of rat which orally feed by *P. viridis* were determined by Atomic Absorption Spectrometry (AAS) following dry acid digestion. Parameter analysis was evaluated by determining confidence limit for the obtained results. The result showed that there was a sequence of heavy-metal content in green mussels sample and laboratory rats serum, such as Ni < Cd < Cu < Zn.

Keywords: heavy metals, green mussels, laboratory rats serum, AAS

INTRODUCTION

Chemical wastes such heavy metals in seawater ecososystems from anthrophogenic activities input such as industrial effluent, urban runoff, domestic activities etc were serious probleme for aquatic ecosystems (Skoog et al., 1994; Beiras et al., 2003 ; Catsiki et al., 2003; Liu and Kueh, 2005). Marine organisms such bivalve molluscs i.e. mussels have the ability to uptake heavy metals contamination from their aquatic environment. Marine mussels have been widely used as indicator organisms to assess the levels of coastal pollutions (Sze and Li, 2000). *P.viridis* has been known to be distributed at coastal or seawater region especially in archipel countries (Catsiki et al., 2003; Sze et al., 2003). Previously studies reported at several Southeasts country's coastal water (i.e. Malaysia, Hogkong, Thailand, India and Jakarta bay, Indonesia) that Cu, Zn and Cd have been highly accumulated in the green mussels tissues (Yap et al., 2003, 2004) that can potentially being consumed by human *via* seafood market activities. Ingestion of heavy metals -contaminanted green mussels may cause disturbing neurosystem, harmful effets to tendon and absorb into bone, precipitate into body (Sze and Lee, 2000; Yap et al., 2004).

In broad area of Indonesia, *P. viridis* can be found easily in traditional market. In this study, we collected *P. viridis* from traditional market i.e. Ciroyom Bandung from which the mussels have been catched from Mundu water, Cirebon. Hence, our study was to determine the Ni, Cu, Zn and Cd concentration in the mussel and to determine their concentration in rat laboratory serum which orally feed with *P. viridis*.

MATERIALS AND METHODS

Chemicals

All solutions have used with pro analysis (p.a) quality such pure grade aquabidest (MiliQ, pure grade) anhydric metal standards powder, $CuSO_4.5H_2O$, $NiSO_4.6H_2O$, $ZnSO_4.7H_2O$ and $Cd(NO_3)_2.4H_2O$ (Sigma Aldrich, MO, USA), HNO_3 65% (AnalaR grade, Merck, USA) as destruction solutions and $SnCl_2$ as releasing agent (Merck USA), citric acid (Merck, USA), Whatman 42 and paper aluminium.

Selection of acid digest solution

1000 ppm each standar cation solution Ni, Cu, Zn and Cd were diluted in water to 5 ppm. 25 mL of each standard solution was

pipetted and digested with different ± 5 mL (total volume) digest solvent, HNO_3 65%:water (1:1) and HNO_3 p 65% at different beaker. Their mixed and heated on hotplate *Thermolyne* MIRAK^{TM} at 80^{0}C for 1 hours. Then 15mL every digest solvent was added then concentrated on hotplate at 140^{0}C for 3 hours. After cooling in room temperature (25^{0}C), each digest solution with HNO_3 65 % and HNO_3 65%:water (1:1) with their metals were diluted at 50 mL water and standar solution mix of heavy metals Ni, Cu, Zn and Cd spiked to each digest solution then their added. We have made a preparation 4 sample artificiales replicats, 2mL sample solutions were pipetted to each 10mL glass. First glass contain the solvant (control) and another glass which have added mix cation standard solution 100 ppm with volume simultant 25µ L, 50 µL, 100 µL. Each glasses were added 20µL HNO_3 (1:1) to avoid hydrolisis effect.

Preparation of mussels samples

± 3 kg wet mussels*, P. viridis* (≈ 4.5-6.5 cm lenght) were collected from traditional market. They were placed at room temperature before their treatment and all cockle of mussels have been removed from their tissues. Water residus was removed and filtered and evaporated at room temperature for 30 minutes while mussels were covered with paper aluminium (Liu and Kueh, 2005). wet tissues were blended meat chopper/ food processor AKEBONO ^{TM} (Multi use-mincer JCW-45). Total of wet mass tissues were weighed by Ainsworth microbalance (Perkin elmer ADZZ), then collected into plastic bag and conserve in freezer -10 ^{0}C. wet tissue was placaed into crush alumina and dried at 105 ^{0}C for 1 hours to avoid and combusted in insenerator Thermolyne 8000 (Shimadzu, Japan) at 900 ^{0}C during 9 hours. Ash mass was weighed and stored at room temperature

Dry digestion of mussels samples

Ash of mussels c.a ± 3 gram were weighed with analytical balance Ainsworth microbalance (Perkin elmer ADZZ). These samples were digested in 5 mL HNO_3: water (1:1) sulution while heated 80 ^{0}C for 1 hours on the Hot Plate Thermolyne MIRAK^{TM}. Then added 15 mL HNO_3:water (1:1) solutions while blend once time at temperature 140 ^{0}C for 3 hours. Digest sample solution was cooled at room temperature the filtered with whatman 45 to remove their residus, then filtrate was dilluted at 100 mL water.

Digestion of blood rats laboratory serum

Twelve white rats (*Ratus norvegicus*) which have similiar weight and ages (2-3 months, male) were orally feed of dry mussels with mix their meals every days. 3 rats were chossed as control. They were given orally feed only their meals usuals without mix by mussels samples. For 9 rats laboratory were given orally feeds of dry mussels by syringe. After 4, 8 and 12 days, theirs blood were taken and placed in 1.5 mL Eppendorf tube and washed with citric sodium 38 % as anticoagulant (Kosar et al., 2006). Blood rats samples were taken from their eyes (*sinus orbitalis*) (Smith and Mangkoewidjojo, 1988) then those samples were sentrifuged with microsentrifuge 22 A (hettic sentrifugenm Germany) at 11,000 rpm for 10 minute at 4 ^{0}C (Kosar et al., 2006). Their supernatants (blood serum) were taken from their residus. Each blood serums were digested with dillution acid 500 µL HNO_3 : water (1:1) on hotplate 140 ^{0}C during 30 minute for denaturation their protein. After cooling at room temperature, blood serum was filtered with *Whatman 42* also washed with sodium citric 3.8 % (w/v). Then each blood serum filtrate was dilluted in vial 10,00 mL. 2 mL sample solutions were prepared and add with 100,00 ppm mix standard solutions with variation adding volume (25 µL, 50 µL, 100 µL) and completed with 20 µL HNO_3 :water (1:1) as acid condition. Each serum solutin was analysed with Atomic Absorption Spectrometry.

Atomic Absorption Spectrometry (AAS) analysis

Atomic Absorption Spectrometry (AAS) (GBC® 902)/Perkin Elmer for analysis heavy metals in mussels with gas flow condition contain of air- acetilene and wave long (λ) were measured Ni(II) (λ= 232.0 nm), Zn(II) (λ =213.9 nm), Cu(II) (λ= 321.47 nm) and Cd(II) (λ= 228.8 nm).

RESULT AND DISCUSSIONS

Selection of digest solutions

One of analytical evaluation of comparing two different methods is a recoveries values. Artificial sample was a 4 mix individual standar solution Ni, Cu, Zn and Cd with concentration 5 ppm. Recoveries values were obtained with two different digest solutions. Recovery for digest with HNO_3 concentrated 65% ranged between 100-147%. While the mixture of HNO_3 /water (1/1) solution ranged 103-124 %, while Those results have shown at **Table I.**

Table 1. Recoveries percentage of two test (i). HNO_3 65% and (ii). HNO_3 65% : water (1:1). Each experiment has been conducted in duplicate

Solvant destuction	Ni (%)	Zn (%)	Cu (%)	Cd (%)
HNO_3:water (1:1) I	113	148	109	100
HNO_3:water (1:1) II	97	101	108	105
Average	**105**	**124**	**109**	**103**
HNO_3 65% I	116	184	90	143
HNO_3 65%II	84	110	111	83
Average	**100**	**147**	**101**	**113**

Both of digest solution can dissociate of heavy metal cations content in mix standards solutions. Their recovery values of HNO_3:water (1:1) were better than HNO_3 concentrated 65% for digest acid solution.

Determination of Heavy metals content in green mussels (*Perna viridis*)

Mussels samples were formed ash using insenerator to avoid organic compounds in tyssus during oxidation process. We have obtained 13,2370 g ash mass from 87,1597 g dry mass samples. Digestion process has obtained two phase white ash powder under glass was removed the silica, sand from the solutions (6,10). The results showed that the green mussels (*Perna viridis*) from tradtional market, Ciroyom Bandung have Zn(II) levels highest than other cause this cation as binding site of protein content in tyssus of green mussels (Yap et al., 2003, 2004) or from mussels habitat in seawater (Skoog et al., 1994; Huber, 1998). Heavy metal cations content in *P. viridis* were shown in **Table2.**

Table 2. Concentration of heavy metals cations in green mussels (*Perna viridis*) (µg/g ash mass)

Metals cations	Mg	(ppm)	µg/g, ash mass	% mass (%w/w)	Treshold limit
Ni(II)	3.50	0.70	1.17	$2.33x10^{-4}$	0.05
Cu(II)	20.18	4.03	6.73	0.01	0.02
Zn(II)	174	34.80	58	0.12	0.05
Cd(II)	4.91	0.98	1.64	$3.27x10^{-3}$	0.05

Standard spike to samples at certains volumes 25µL, 50µL and 100µL were given high absorbance for Zn than another metal cations (**Figure 1.)**

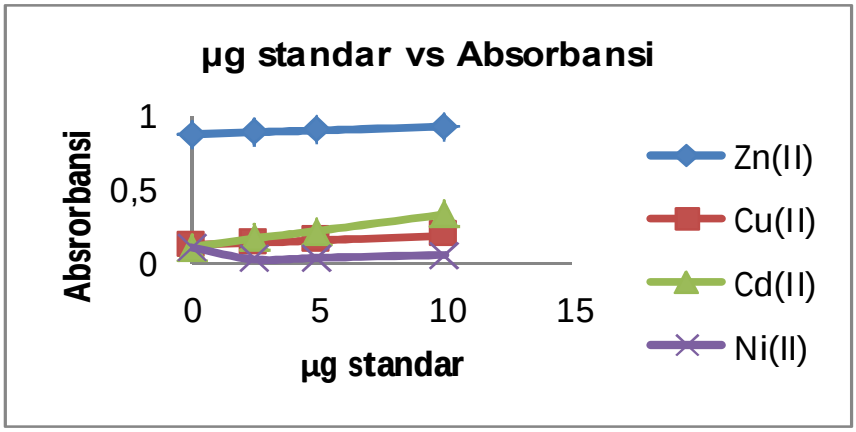

Figure 1. Calibration curve of ash mussel samples spiking method (µg standar vs absorbance).

From this results, the sequence of heavy metal content are Ni < Cd < Cu <Zn with their confidence limit Zn '58 ±10.76 µg/g in ash mass), Cu(6.73 ± 3.04 µg/g), Cd (1.64 ± 0.57 µg/g) and Ni (1.17 ± 1,80 µg/g), If the value of confidence limit is more than real value, it can influence of their accuracy (Skoog et al., 1994; Huber, 1998). Confidence limit used as standard microgram unit which added to sample solution. add standard mass *vs* absorbance used to determine this confidence limit. Less confidence limit make less of % error with large of accuracy and precision.

Determination heavy metal in blood rat laboratory serum

The results showed high accumulation of heavy metal (Ni, Cu, Zn and Cd) in blood rats laboratory serum which have been orally feed for 4 days. However, after 2 days experimentation, Ni was not found in the blood serum. The accumulation of heavy metal in blood rats laboratory serum have been shown at **Figure 2**.

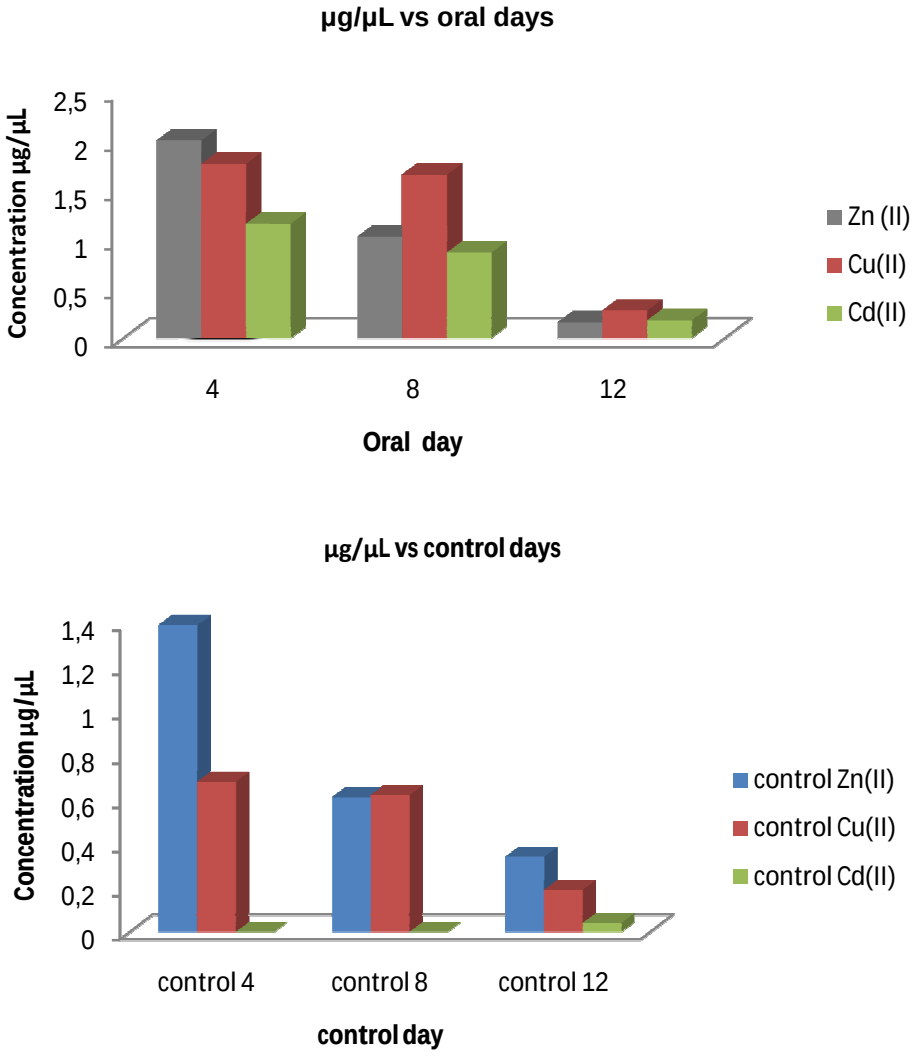

Figure 2. Concentration of heavy metal cations on blood rats serum (µg/µL) for 4,8 and 12 days which orally feed by green mussels (µg/µL vs oral days) and without orally feed of green mussels (µg/µL vs control days)

Increasing concentrations of heavy metal in blood serum can be influenced by the metal content in the diet of rats, rats meals, the rats metabolic system and the content of metal cations contained in mussels samples. After 8 and 12 days, the heavy metal content have decreased. In control or blank (without orally feed of mussels, the metal content have founded but in small concentration. For orally feed by dry mussels, each serum, metal cations of Zn and Cu have potential accumulation comparing with their control. After 12 days, there were detected Cu level ranged from 0.37-1.22 µg/µL and Zn level ranged from 0.6 to 2.09 µg/µL,. Otherwise, Ni and Cd level have not found in both blood rats serum treatment and only Cd was found in very small extent in 12 days.

CONCLUSION

The acid digest solution HNO_3 : water (1:1) was choossed to digest green mussels samples with dry digestion methods. This solution has recovery ranged near 100 % (103-124%). The sequences of heavy metal in green mussels tissues were Ni < Cd < Cu < Zn. Determination of heavy metal in blood rats serum showed the impact of the Cu, Zn and Cd accumulation for each rats experiment which orally feed of green mussels during 4,8 and 12 days while Ni was not detected in rats laboratory blood serum.

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