Speciation of heavy metals Cu, Ni and Zn by modified BCR sequential extraction procedure in sediments from Banten Bay, Banten Province, Indonesia

Lestari¹, F Budiyanto¹ and D Hindarti¹

¹ Research Centre for Oceanography, Indonesian Institute of Sciences Pasir Putih I, Ancol Timur, Jakarta, Indonesia
E-mail: lest001@lipi.go.id, terisardi@gmail.com

Abstract. Banten Bay is categorized as a marine area that is busy with marine tourism activities, settlements and also industries. One potential impact of the condition is the occurrence of pollution from both industrial and domestic sources, erosion and sedimentation in the coastal environment. Samples were collected from 25 representative stations in April 2016. Chemical speciation of three heavy metals (Cu, Ni, and Zn) was studied using a modified sequential extraction procedure proposed by the European Standard, Measurements and Testing (SM&T) program, formerly the Community Bureau of Reference (BCR). The aims of this study are to determine geochemical speciation of 4 bounds of metal: acid-soluble, reducible, oxidizable and residual, and to assess their impacts in the sediments of Banten Bay, Indonesia. The result shows that the percentage of Copper (45.90-83.75%), Nickel (18.28-65.66%), and Zinc (30.45-79.51%) were mostly accumulated in residual fraction of the total concentrations. The Risk Assessment Code (RAC) reveals that about 0-7.07% of Copper and 1.11-24.35 % of Zinc at sites exist in exchangeable fraction and therefore, they are in low risk category. While 7.34-34.90 of Ni at sites exists in exchangeable fraction and therefore, it is in medium risk category to aquatic environment.

1. Introduction
Banten Bay is a 10 x 15 km large, shallow (<20 m) embayment in the north-west coast of Java, Indonesia [1]. Coastal ecosystems are highly affected by the physical and chemical conditions of both surrounding land areas and the open sea. Banten Bay is a coastal area which has been highly affected by human activity [2]. Banten Bay ecosystems are under heavy pressure due to a variety of local processes and developments such as high intensity of fishery all over the bay, natural coastal erosion in the eastern part, land reclamation for harbour development and industrial expansion in the western part and increased industrialization and urbanization in areas surrounding the bay [3].

Metal contamination of aquatic environments occurs as a result of many human activities. Metal contamination is one of the most ubiquitous, persistent and complex chemical contamination problems faced by human society [4]. Yet, no comprehensive and coherent single analysis is presently available of metals science and how it applies to specific ecological risk management issues for metals [4].

Sediment in estuaries and coastal environment serves as a repository of heavy metals [5]. The total concentrations of metal is a rarely useful in determining its biological availability i.e. its toxicity or its potential as a nutrient because metals in sedimentary environments occur in both dissolved and particulate states and in a variety of chemical forms [6]. Sediment contamination in estuaries and its biological effects need to be properly and fully assessed. Recent papers recommend conducting specific
studies to determine the effects of pH and salinity on bioavailability of contaminants bound to estuarine sediments [7,8]. Trace metals are among the most common contaminants bound to estuarine sediments. The bioavailability of these metals to aquatic organisms is dependent on the physical and chemical forms of the metal [4].

The determination of total heavy metals in sediments for Banten bay has been widely applied by strong acid digestion [1,9,10]. Metal concentration in the western part of the bay was slightly elevated compared to the eastern side of the bay [1] and compared to sediments from uncontaminated waters, the sediments from the Banten bay have also not been contaminated. However, the approach using the total metal concentration can be misleading when assessing environmental effects due to the potential for an overestimation of exposure risk [10]. All trace metals are potentially toxic, whether essential or not, at a threshold of availability [2]. Chemical speciation of three heavy metals (Cu, Ni, and Zn) was studied using a sequential extraction procedure proposed by the European Standard, Measurements and Testing (SM&T) program, formerly the Community Bureau of Reference (BCR) [11]. These techniques involve a four-step-extraction and are thus becoming popular and adopted methods used for sequential extraction [10]. These methods reveal the level of bioavailability of metals in sediments and confirm that sediments are bioindicators of heavy metal pollution in marine environment [12, 13]. Several speciation studies have been conducted to determine different forms of heavy metals rather than their total metal content [10,14].

The aims of this study are to determine geochemical speciation of four bounds of metal: acid-soluble, reducible, oxidizable and residual and to assess their impacts in the sediments of Banten Bay, Indonesia.

2. Material and Method
2.1. Study area
Extensive industrialization is planned for the area located in the west of the Banten bay. It is expected significant changes in the level of nutrients, trace metals and organic contaminant will occur in the near future [1].

Sediment samples were collected from 25 locations in Banten Bay in May 2016 (Figure 1). The total metal content and speciation of, Cu, Ni, and Zn were measured in the sediment samples in the current study.

Figure 1. Sampling location of sediment in Banten Bay, Banten Province, Indonesia, 2016.
2.2. Sample collection and preparation
Twenty-five (25) surface sediment samples were collected using a Van Veen grab sampler and immediately placed in the plastic container and kept in a cool box. In the laboratory at the Research Centre for Oceanography, Indonesia Institute of Sciences, sediment samples are prepared for metal speciation and total metal analysis.

2.3. Metal speciation by modification sequential extraction procedure BCR and total metal analysis
The speciation method of BCR three-step sequential extraction procedure by Cuong and Obbard (2006) [10] with a small modification in the amount of sample and reagent for residual step and total metal analysis is followed. This provides information on four fractions, namely, exchangeable and carbonates; reducible; oxidizable; and residual. The following is the main step in extraction shown in Table 1. A microwave – assisted acid digestion procedure was used to determine the total metal content and the metal content of the residue from step 3.

1.0 g dry sediment sample was digested by 10 ml aqua regia. Samples were heated in the microwave unit at 180 ± 5°C for 10 minutes and remained at 180 ± 5°C for 10 minutes. Centrifugation (3000 rpm for 10 minutes) was used to clear the supernatant which was then analyzed by flame atomic absorption (FAAS) using Varian SpectrAA 20.

The accuracy of the extraction procedure used in the determination of heavy metals was tested. For this purpose, the sum of the metal concentrations obtained from the sequential extraction of 1.00 g of CRM PACS-2 was compared with their total metal contents. The obtained recovery values were found to be satisfying (Cu 98%, Ni 87% and Zn 89 %). The accuracy of the microwave assisted acid digestion procedure for total metal determinations was checked using a marine sediment standard reference material (PACS-2, National Research Council, Canada). The analysis of this reference material showed satisfactory accuracy, with the recoveries for Cu 91%, Ni 90% and Zn 96% in PACS 2.

| Step | Sediment phase | Extractant | Shaking time and temperature |
|------|----------------|------------|-----------------------------|
| F1   | Water and acid soluble (acid-soluble fraction – bound to exchangeable ion and carbonates) | 40 ml of 0.11 mol L⁻¹ CH₃COOH | 16 h at room temperature at 22±5°C and speed 30±10 rpm. The extract was separated from the solid phase by centrifugation at 3000 rpm for 20 minutes. |
| F2   | Reducible (reducible fraction – bound to Fe and Mn oxides) | 40 ml of 0.5 mol L⁻¹ HONH₂.HCl (pH 1.5) | 16 h at room temperature 22±5°C and speed 30±10 rpm. The extract was separated from the solid phase by centrifugation at 3000 rpm for 20 minutes. |
| F3   | Oxidizable (organic matter and sulfides) | 10 ml of 8.8 mol L⁻¹ H₂O₂ (two times), cool and add 50 mL of 1 mol L⁻¹ NH₄OAc (pH 2) | 1 h at room temperature 1 h at 85°C 1 h at 85°C 16 h at room temperature 22±5°C and speed 30±10 rpm. The extract was separated from the solid phase by centrifugation at 3000 rpm for 20 minutes. |
| R    | Residual (residual fraction – strongly associated to the crystalline structures of minerals) | 10 mL of aqua regia | Digesting in a mixture aqua regia |

2.4. Risk Assessment Code (RAC)
The results of the fractionation study indicated that the metals in the sediments are bound to different fractions with different strengths. These strength values give a clear indication of sediment reactivity supporting the assessment of the risk of the presence of metals in Banten Bay. The criteria of Risk Assessment Code (RAC) are used to evaluate sediment quality [15-18]. The criteria of Risk Assessment
Code (RAC) indicate that a sediment releasing exchangeable and carbonate fractions, less than 1% of the total metal, was considered safe for the environment. On the contrary, the sediment releasing more than 50% of the total metal in the same fraction has been considered highly dangerous and high possibility to enter the food chain [15].

3. Result and discussion
3.1. Total metal content
Total contents of Cu, Ni, and Zn in the marine sediments are presented in Table 2. It can be seen from the data that the average concentrations of the three metals varied as follows: Cu, 9.19 mg/kg dw; Ni, 14.9 mg/kg dw; and Zn, 69.8 mg/kg dw. The average metal concentrations in Banten Bay decrease in the order of Zn>Ni>Cu. These patterns were almost similar to the levels found in sediments from the previous study [1].

Metal concentrations in sediments of Banten Bay can be seen from the data that the concentrations of the four metals varied as follows: Cu, 2.9-20 mg/kg dw; Ni, 10.4-20.6 mg/kg dw; and Zn, 32.2-129 mg/kg dw. The comparison of heavy metal concentrations to other regions such as Jakarta Bay and Singapore can also be seen in Table 2. Compared to the marine sediment data from Jakarta Bay, the concentration of Cu and Zn is lower from Banten Bay than from Jakarta Bay. Meanwhile the concentration of Ni is lower and the concentration of Zn is relatively higher than the sediment from Singapore.

| Region             | Cu   | Ni   | Zn   | Reference |
|--------------------|------|------|------|-----------|
| Previous study     | 11   | 15   | 73   | [1]       |
| sediment from      | 6.65 | 6.09 | 55.09| [9]       |
| Banten Bay         | 6.32 | -    | 169.17| [19]      |
|                    | 22.8 | -    | -    | [20]      |
|                    | 9.19 | 14.9 | 69.8 | Present study 2016 |
| Jakarta Bay        | 59.8 | -    | 167  | [21]      |
| Singapore          | -    | -    | 17.1 | [10]      |
| Kranji             | 17.9 | 26.1 | 62.1 |           |
| Pulau Tekong       | 7.7  | 17.1 | 49.8 |           |

3.2. Metal Speciation
The potential environmental risk of trace elements in sediments is associated with both their total content and their speciation. The chemical partitioning of the considered elements (Cu, Ni, and Zn) from each extraction step has been described.

As illustrated in Figure 2, the metals were fractioned differently in each station. The Cu (2.88%) bound to Fe—Mn oxides is the second important fraction. The presence of Cu in organic fraction (31.1%) is supported by the formation of Cu-organic complexes. Cu in the first fraction was 3.41% and in residual fraction was 62.57%. The high portion of Cu was obtained in oxidisal fraction at station 25 (50%) and residual fraction at station 16 (77%) and station 21 (84%). The residual fraction was the most important fraction for Ni (42.9%). The other important fraction (23.3%) containing Ni was an oxidisable fraction. Ni was partitioned at 19.9% in the exchangeable and bound to carbonate fraction and 13.8 % in the reducible fraction. The high portion of Ni was obtained in acid soluble fraction at station 12-15 (28-35%).
The other important fraction (27.1%) containing Zn was oxidisable fraction. Zn is partitioned at 10.8% in the reducible fraction and 4.01% in the first fraction. The highest levels of Zn occurred in 58.1% in the residual fraction. The high portion of Zn was obtained in oxidisable fraction at station 6 (60%).

Figure 2 shows that the distribution of the heavy metals in percent bar graphs in the four fractions (F1 is acid soluble fraction, F2 is reducible fraction, F3 is oxidisable fraction, and F4 is residual fraction) obtained in our analysis. The partitioning showed of metals associated with the non-residual fraction (consists of F1 + F2 + F3) and the residual fraction. The partitioning showed that the percentages of metals associated with the non-residual fractions (F4) were notably greater (37.43% for Cu, 41.93% for Zn; and 57.12% for Ni) than those of the residual fraction (62.57% for Cu, 58.07% for Zn; and 42.88% for Ni), indicating that these metals were primarily derived from anthropogenic inputs instead of the geochemical background. Therefore, the results indicated that the metals are potentially more available for exchange and/or release into the marine environment. The studied trace metals in the non-residual fractions increased in the order of Ni < Zn < Cu. The result shows that the percentages of Copper (45.90-83.75%) and Zinc (30.45-79.51%) were mostly accumulated in the residual fraction of the total concentrations. While the Nickel percentages (18.28-65.66%) were mostly accumulated in non-residual fraction of the total concentrations.

![Figure 2. Percentages of Cu, Ni and Zn removed in each step (F1 is acid soluble fraction, F2 is reducible fraction, F3 is oxidisable fraction, and F4 is residual fraction) of the sequential extraction procedure applied to marine sediment at Banten Bay.](image)
3.3. RAC
The Risk Assessment Code (RAC) reveals that about 0-7.07% of Copper and 1.11-24.35% of Zinc at the sites exist in the exchangeable fraction and therefore, they are in low risk category. While 7.34-34.9% of Ni at sites exist in exchangeable fraction and therefore, it is in medium risk category to aquatic environment (Table 3). However, monitoring the presence of metal in the waters needs to be done because it is persistent and it can accumulate endangering the aquatic environment.

Table 3. Risk Assessment Code (after Jain, 2004).

| Risk Assessment Code (RAC) | Criteria (%) |
|---------------------------|--------------|
| No Risk                   | < 1          |
| Low Risk                  | 1—10         |
| Medium Risk               | 11—30        |
| High Risk                 | 31—50        |
| Very High Risk            | > 50         |

4. Conclusions
The modified BCR sequential extraction procedure has been applied to marine sediments from Banten Bay in order to evaluate the potential mobility and the possible transfer of heavy metals from sediments to the surrounding environment. Based on the analytical data obtained from the modified BCR sequential extraction procedure, Copper, Nickel, and Zinc were mostly accumulated in residual fraction of the total concentrations. The Risk Assessment Code (RAC) reveals that Copper and Zinc at sites exist in exchangeable fraction and therefore, they are in low risk category. Ni at sites exist in exchangeable fraction and therefore, it is in medium risk category to aquatic environment.

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References
[1] Booij K., MT. Hillebrand, RF Nolting and J Van Ooijen. 2001. Nutrients, trace metals, and organic contaminant in Banten Bay, Indonesia. Mar. Poll. Bull. Vol. 42, No. 11, pp. 1187-1190.
[2] Fauziah F. and DN Choesin. 2014. Accumulation of Pb and Cu heavy metals in seawater, sediment, and leaf and root tissue of Enhalus sp. In the seagrass bed of Banten Bay. AIP Conference Proceedings 1589, 329; doi: 10.1063/1.4868812
[3] Hoekstra P, H Lindeboom, R Bak, Gert van den Bergh, DA Tiwi, W Douven, J Heun, T Hobma, T Hoitink, W Kiswara, E Meesters, Y Noor, S Nuraini, T van Weering. 2003. Teluk Banten research programme: an integrated coastal zone management study (1995-2001) in J. Stapel (Ed.) Scientific Programme Indonesia-Netherlands Proceedings of workshop held on February 12th 2002 in Bandung Indonesia, Amsterdam p 59-69.
[4] Luoma SN and Rainbow PS, 2008. Metal contamination in aquatic environments: science and lateral management.Cambridge University Press. pp xix+573 pp
[5] Kennish, Michael J. 1997. Practical handbook of estuarine and marine pollution. CRC Press marine science series.p 511.
[6] Campbell PGC, Lewis AG, Chapman PM, Crowder AA, Fletcher WK, Imber B, Luoma SN, Stokes PM., and Winfrey M. 1988. Biologically available metals in sediments, National Research council of Canada, Publication No. NRCC 27694
Chapman PM., dan Wang F. 2001. Assessing sediment contamination in estuaries. *Environ Toxicol Chem* 20: 3-22.

Riba I., García-Luque E., Blasco J., and Del Valls TA. 2003. Bioavailability of heavy metals bound to estuarine sediments as a function of pH and salinity values. *Chemical Speciation and Bioavailability* 15: 101-114.

Rochyatun E, Lestari dan A Rozak. 2005. Kualitas lingkungan perairan Banten dan sekitarnya ditinjau dari kondisi logam berat. *Oseanologi dan Limnologi di Indonesia* No. 38: 23-46

Cuong, DT and N Obbard. 2006. Metal speciation in coastal marine sediments from Singapore using a modified BCR-sequential extraction procedure. *Applied Geochem.* 21: 1335–1346.

P Quevauviller, G Rauret, H Muntau, AM. Ure, R Rubio, J F Lopez Sanchez, H D Fiedler and B. Griepink, 1993. Evaluation of a sequential extraction procedure for the determination of extractable trace metal contents in sediment. *Fresenius’ J. Anal. Chem.,* Vol. 349, pp. 808-814

Guevara-Riba A, Sahuquillo A, Rubio R, Rauret G (2004) Assessment of metal mobility in dredged harbour sediments from Barcelona, Spain. *Sci Total Environ* 321: 241-255.

Wepener V, Vermeulen LA (2005) A note on the concentrations and bioavailability of selected metals in sediments of Richards Bay Harbour, South Africa. *Water SA* 31: 589-595.

Werorilangi S., MF Samawi, Rastina, A Tahir, A Faizal and A Massinal. 2016. Bioavailability of Pb and Cu in sediments of vegetated seagrass Enhalus acoroides, from Spremonde Island, South Sulawesi, Indonesia. *Res. J. Environmen.Toxicol*, 10:126-134.

Jain, C K 2004 Metal fractionation study on bed sediments of River Yamuna, India, *Wat. Res.,* 38: 569-578

Turki A J 2007 Metal Speciation (Cd, Cu, Pb and Zn) in Sediments from Al Shabab Lagoon, Jeddah, Saudi Arabia JKAU: *Mar. Sci.,* Vol. 18, pp: 191-210

Horvath M G, Halasz, E Kucanova, B Kucikova, I Fekete, D Remeteiova, G Heltai and K Florian, 2013. Sequential extraction studies on aquatic sediment and biofilm samples for the assessment of heavy metal mobility. *Microchemical Journal* 107: 121-125.

Morelli G and M Gasparon, 2014. Metal contamination of estuarine intertidal sediments of Moreton Bay, Australia. *Mar.Pol.Bull.* 89: 435-443

Suwanda E, K Kawamura and E Soeyanto. 2011. Assesment of heavy metals and Nutrient status in the seawater, sediment and seagrass in Banten Bay, Indonesia and their distributional patterns, *Journal of Fisheries International* 6(1): 18-25

Irnawati R, A Susanto, Mustahal and MA Syabana. 2014. Heavy metal concentration in water and sediment at Panjang Island, Serang Regency, Banten Province, Indonesia. *AES Bioflux Vol 6,* issue 3 p 256-260

Permanawati Y., R Zuraida, A Ibrahim. 2013. Kandungan logam berat (Cu, Pb, Zn, Cd, dan Cr) dalam air dan sediment si perairan Teluk Jakarta.*Jurnal Geologi Kelautan Vol 11,* No. 1: 9-16