Spatial distribution of heavy metal content in the water and green mussel (*Perna viridis*) in Semarang Bay, Indonesia

Djoko Suprapto, Nurul Latifah, Suryanti Suryanti

Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, Indonesia. Corresponding author: N. Latifah, nurul.latifah@live.undip.ac.id

Abstract. This study aimed to find out the concentration of heavy metals in the water and green mussel (*Perna viridis*) in the Bay of Semarang. In this study, the water and the green mussels were collected from 9 (nine) stations which were distributed over the estuary (stations 1, 2 and 3), distant from industrial and human activities (stations 4, 5 and 6) and relatively close to industrial and human activities (stations 7, 8, and 9) in May and September 2018. The heavy metal concentration in the green mussels and water was measured using Inductively Coupled Plasma (ICP). In addition, SNI 6989.79:2011 was used to analyze the nitrate, while SNI 06-6989.31-2005 method was used to analyze the phosphate. The concentration of nitrate (1.831-2.003 mg L^{-1}) showed higher values than that of phosphate (0.24-0.393 mg L^{-1}). The concentration of heavy metals in the green mussels from very low to very high respectively was: Ag < Hg < Ni < Cr < As < Cu < Cd < Zn < Mn < Pb. Generally, the concentration of heavy metals in green mussels was higher (i.e. 0.3 to 202.6 mg L^{-1}) than that of heavy metals in the water (1.1 to 51.0 mg L^{-1}). Samples in May (transition season I to the east) had higher heavy metal content (0.3-202.7 mg L^{-1}) than those found in September (transition season II), i.e. 0.1-99.2 mg L^{-1}. The results of mapping of the spatial distribution showed that the highest concentration of heavy metals in green mussel and in the water was found far away from industrial and human activities. Moreover, the results of statistical analysis of T-Test dependent samples showed that 1) there were significant differences between heavy metals in May and September 2018; and 2) there were significant differences between heavy metals in the green mussels and in water.
Key Words: heavy metal, *Perna viridis*, green mussels, Semarang Bay, mapping.

Introduction. There are a countless number of human activities along the coast of Semarang Bay such as settlements, ports, industries, hospitals, and so on which makes the waters of Semarang Bay easily polluted. According to Wendling et al (2013) anthropogenic impacts in the coastal areas are highly potential to produce waste and cause stress on marine invertebrates. Industrial waste discharged into the river will eventually flow into the sea, so that the sea will potentially be polluted. One of pollution indicator is the presence of excessive heavy metal content. Heavy metal is one of the toxic hazardous materials that is hard to be degraded which causes the biota can easily absorb and take the heavy metal from polluted waters and decisively accumulate in the body of the biota. According to Zhao et al (2018), among more than 40 types of heavy metals, the ones considered to be the most bio-toxic are Hg, Cd, Pb and Cr. One of the aquatic biota which can be used as water pollution indicator is bivalve including green mussels that can absorb heavy metals in higher amounts comparing to other biota. This is due to the living nature of bivalve, which are filter feeders, and have a high tolerance to the concentration of certain metals (Emawati et al 2015). The heavy metals are hard to be degraded which causes them to easily accumulate in the aquatic environment and in the marine biota and will be harmful for humans consuming these biota.

The green mussel *Perna viridis* belongs to Phylum Mollusca, Bivalvia Class. It inhabits marine intertidal, estuarine, and subtidal environments. In addition, green mussel is dark green and commonly distributed along Indo-Pacific region, attached to the floating objects, such as cage, buoy, jetty, rope by using the fine hairy organ called byssus (Hadibarata et al 2012; Dwiyitno et al 2016). Green mussel was selected as bio-indicator for the toxic contaminant since it accumulates many pollutants (Hadibarata et al 2012; Emawati et al 2015). Green mussels are in great demand in Indonesia, with high nutritional content and have very important economic value (Pinto et al 2015). The heavy metals recorded in Qari et al (2015) study were: Hg < Co < Cd < Cr < Ni < Mn < Cu < Zn < Pb < Fe. Meanwhile, Gao et al (2008) stated that green mussel was so easy to grow and economically valuable that this biota was included as one of the most commercial fisheries products; however, when the habitat of the green mussel is highly polluted, particularly with heavy metals, the nutrient content in green mussel is not commensurate to the content of heavy metals absorbed in its soft tissues.

Emawati et al (2015) stated that the increasing heavy metals content in the water will certainly increase the heavy metal contained in green shell organism. Moreover, Emawati et al (2015) study conducted in the waters of Tanjung Priok (Bay of Jakarta) showed that the sediments which have been contaminated with lead (Pb) cause a high content of heavy metals in shellfish and green mussel tissue that exceeds the maximum limit of heavy metal contained in fisheries products based on BPOM standard (BPOM is local abbreviation for *Badan Pengawas Obat dan Makanan* - National Agency of Drug and Food Control). Furthermore, Riani et al (2018) study which was conducted in Jakarta Bay also resulted in a conclusion that an increase of heavy metals content in water is followed by increasing heavy metals contained in green mussels. The degradation of water quality level due to land use might also give significant impact to green mussels since they can accumulate high concentration of pollutants such as metals, thus making it dangerous for human as food sources. The purpose of this study was to find out the concentration of heavy metals contained in the water and in green mussels in Semarang Bay area.

Material and Method. The Bay of Semarang has calm waters and is categorized as a tidal area. Thus, there is a great amount of green mussels found in the waters of Semarang Bay area and they are naturally attached to port/jetty, rocks, and so on. This stuff is broadly utilized by fishermen to cultivate green mussels using *rumpon* (fishing aggregating device installed in shallow and deep waters). Until today there are hundreds of *rumpon* in Semarang Bay. As shown in Figure 1, the samples of green mussel and water were collected at 9 (nine) stations, i.e. three of them were spread over the estuary (stations 1, 2 and 3), three stations were at relatively distant from industrial and human activities (stations 4, 5 and 6), and the other three were close enough to industrial and human activities (stations 7, 8, and 9). Sampling collection was conducted in May 2018 (one time) and in September 2018 (one time). The selection of the station was based on the location of waters where green mussels were cultivated or cultured. In addition, the study site in this research was the water location in Semarang Bay which was divided into two categories, i.e. the water near and the water far from industrial and human activities that commonly throw waste into the waters.

All of the water samples were collected using Nansen water sampler at 1.5-3 m depth. Moreover, the water temperature, pH, and dissolved oxygen (DO) were analyzed in situ using water quality checker (WQC AMTAST EC 910) and seawater refractometer PAL-06S Atago was used to analyze the salinity. Furthermore, the samples were collected in bottle samples and directly transferred to an ice box until further analysis at the laboratory, particularly to analyze the content of chlorophyll-*a*, nitrate, and phosphate. The green mussels samples attached to the *rumpon* (fad) were obtained using a small scope at 1.5-3 m depth and their external shell surface was cleaned with clean water and brush to remove all the dirt attached to the shell. Green mussels were then separated from the shell and the soft tissue taken was directly stored in an ice box for further laboratory analysis.

Inductivily Coupled Plasma (ICP) method was used to measure the heavy metals content. This process was conducted at the Chemistry Laboratory of Universitas Negeri Semarang. In addition, SNI 6989.79:2011 was used to analyze and measure the content of nitrate, while SNI 06-6989.31-2005 method was used to analyze the phosphate content. The measurement analyses were carried out at the laboratory of PSDIL (Management of Fish Resources Environment), Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. Difference test on heavy metals content taken in May and September 2018 for heavy metals contained in green mussels and water was conducted using statistical analysis of T-Test dependent samples with the following hypotheses:

H_0: there is no significant difference between heavy metals content in May and September 2018 (heavy metals contained in green mussels and water);

H_a: there is significant difference between heavy metals content in May and September 2018 (heavy metals contained in green mussels and water).

Furthermore, Inverse Distance Weighted (IDW) interpolation method was used for mapping heavy metal distribution contained in green mussel and water.

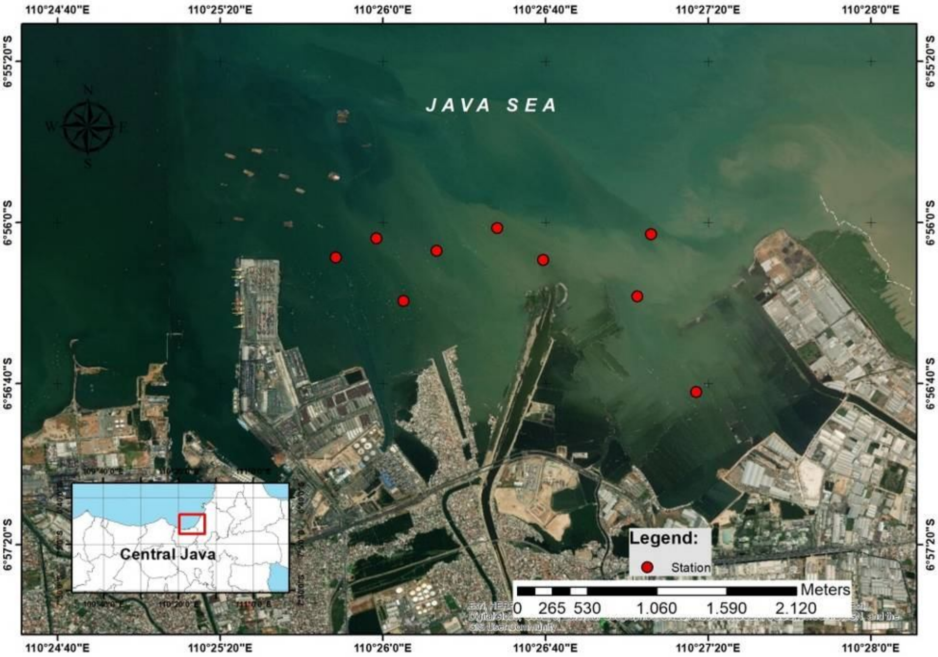

Figure 1. Location of the study site and sampling points.

Results and Discussion

Water quality. The characteristics of green mussel cultivation are described in Table 1.

Table 1

Water quality in the study area

Parameters	*Station and time (WIB)*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
	08.10	*08.50*	*09.17*	*09.30*	*09.50*	*10.10*	*10.19*	*10.30*	*10.48*
Temperature (°C)	28	28.5	28.6	29.3	29.3	29.7	31.4	30.3	30.5
Salinity (‰)	30	30	30	30	30	33	31	32	32
pH	7.9	8.1	8.2	7.9	8.1	8	8	8.1	8
DO (ppm)	7.1	6.78	7.11	5.86	6.81	6.72	6.47	7.04	7.01
Chlorophyll-*a* (mg m^{-3})	1.778	0.609	1.18	2.004	3.029	3.045	1.444	2.341	1.915
Nitrate (mg L^{-1})	2.003	1.9	1.866	1.831	1.866	1.934	2.038	2.038	2.003
Phospate (mg L^{-1})	0.248	0.276	0.24	0.248	0.291	0.248	0.393	0.44	0.252

As shown in Table 1, the temperature of the cultivation area gradually increased along with the rising sun until before noon from 28 to 31.4°C. The results showed the pH ranged from 7.9 to 8.2. The observation of DO resulted in values ranging from 5.86 to 7.11 ppm, of which pH values also increased along with the increasing DO values. Furthermore, the concentration of nitrate in the waters of Semarang Bay showed a higher value than that of phosphate. In detail, the concentration of nitrate ranged from 1.831 to 2.038 mg L^{-1}, phosphate ranged from 0.24 to 0.44 mg L^{-1}, and chlorophyll-*a* ranged from 0.609 to 3.045 mg m^{-3}.

Table 1 showed that the temperature increased along with the time. This was perhaps due to the fact that water temperature is related to sunlight. The amount of sunlight depends on the angle of light (Effendi 2003). The smaller the angle of light entering the sea water is (at 08.00am GMT+7), the more light is reflected. Thus, the less intensity of sunlight which goes into the waters leads to low waters temperature in the morning. On the contrary, the greater the angle of sunlight is (at 10.48 am GMT+7), the less light is reflected and accordingly a lot of light entering the water makes the water temperature around midday become higher (30.5-31.4^{o}C) than the temperature in the morning (28.0^{o}C).

The pH of water at research site (1-9 station) was categorized as alkaline pH. DO content on pH waters of Semarang Bay showed almost the same pattern. If DO values declined, the pH would decrease and vice versa if the values of DO increased, the pH increased. This is affected by photosynthesis process. If the process of photosynthesis increased, carbon dioxide will be reduced and changed to carbohydrates and water will be dehydrogenated into oxygen which causes the oxygen content in the water increase and the content of carbon dioxide decrease (Effendi 2003). Carbon dioxide is acidic. When the CO_2 in the water decreases, the pH will increase. The increasing oxygen content, on the other hand, indirectly increases the value of the pH. The decreasing photosynthesis process causes CO_2 to increase (respiration) which result in the pH of the water to decrease into acids and decrease the oxygen content due to the absence of photosynthesis process. The concentration of nitrate is higher than phosphate levels due to fewer phosphorus sources than nitrogen sources contained in the water. According to Effendi (2003), the existence of phosphorus in natural waters is relatively small and less than nitrogen content since the source of phosphorus in waters environment is less than that of nitrogen. The high levels of nitrogen and phosphate contained in the water accompanied by low turbidity, lower salinity and pH will trigger the presence of heavy metals in the water and green mussels and thus cause the shape of the shells to be abnormal (Riani et al 2018). Furthermore, low salinity and pH will increase the toxicity of heavy metals and increase bioaccumulation (Marques et al 2010). The condition of water with low salinity will increase bioaccumulation of heavy metals in the tissue of shells (Renault 2015).

***Order of heavy metal toxicity**.* The toxicity of heavy metal contained in green mussels in the waters of Semarang Bay is respectively presented in Figure 2. As seen in Figure 2, the concentration of heavy metal from very low to very high was respectively Ag < Hg < Ni < Cr < As < Cu < Cd < Zn < Mn < Pb. The classification of heavy metal concentration consisted of two types: minor heavy metals (Ag, Hg, Ni, Cr, As,) and major heavy metals (Cu, Cd, Zn, Mn, Pb). Moreover, decreased water pH, rising temperature and decreasing salinity of the waters caused greater heavy metal toxicity while high consciousness can reduce the toxicity of heavy metals. Qari et al (2016) study on the coast of Karachi, Paradise Point at the low tide of 1993 and 2012 resulted the order of toxicity from low to high as follows Hg < Co < Ni < Cu < Cd < Pb < Mn < Zn < Cr < Fe (in 1993) and the sequence of Hg < Co < Ni < Mn < Pb < Zn < Fe in 2012. Furthermore, Qari et al (2015) showed that the heavy metals recorded were: Hg < Co < Cd < Cr < Ni < Mn < Cu < Zn < Pb < Fe. From the results of the study, it can be concluded that *Perna viridis* has a high capacity to accumulate heavy metals.

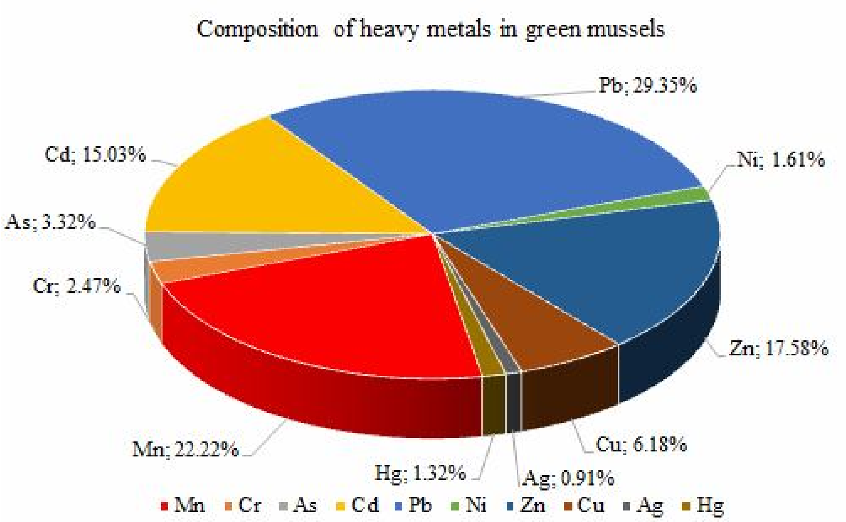

Figure 2. Composition of heavy metals in green mussels.

Heavy metal content in green mussels and in water. Table 2 shows the concentration of heavy metal in water. Based on the results of the study, the concentration of heavy metal contained in green mussels was higher than the concentration of heavy metals in the water respectively ranging from 0.3 to 202.6 mg L^{-1} in green mussels and 1.1 to 51.0 mg L^{-1} in water. The highest heavy metal concentration recorded was Pb with 12.0-198.6 mg L^{-1} in *P. viridis* and 2.1-51.0 mg L^{-1} in water. The lowest heavy metal concentration obtained was Ag with 1.5-8.5 mg L^{-1} in *P. viridis* and 2.8-5.6 mg L^{-1} in water. The result of the statistical analysis T-Test dependent samples (Table 3) indicated that there was a significant difference between the heavy metals in the green mussels and in the water. It is indicated by the value of p = 0.00000 or less than the value of the error degree (α), 0.05. Thus, it can be concluded as reject H_0.

Table 2

Heavy metal content in green mussels and water

Heavy metal (mg L^{-1})	*Perna viridis*	*Water*
Silver (Ag)	1.5-8.5	2.8-5.6
Mercury (Hg)	2.7-11.1	1.3-2.8
Nickel (Ni)	4.7-6.8	1.9-3.9
Chromium (Cr)	6.1-26.5	1.3-5.3
Arsen (As)	0.3-21.1	6.1-8.5
Copper (Cu)	4.1-40.7	2.4-3.7
Cadmium (Cd)	2.4-202.6	19.0-49.3
Zinc (Zn)	3.1-197.1	1.1-2.6
Manganese (Mn)	1.5-202.7	1.4-4.1
Lead (Pb)	12.0-198.6	2.1-51.0

Table 3

Results of statistical analysis of the difference between heavy metal in green mussels and water

	Mean	*Std. Dv.*	*N*	*Diff.*	*Std. Dv.*	*t*	*df*	*p*
HM *Perna viridis*	33.10222	49.04239						
HM water	11.05056	17.74832	180	22.05167	45.23982	6.539685	179	0.000000

HM = heavy metals.

Regarding the sampling, it showed that sampling in May 2018 (transition season I to the east) had a higher heavy metal (0.3-202.7 mg L^{-1}) than heavy metal content in September 2018 (transition season II), i.e. 0.1-99.2 mg L^{-1} (Figure 3). This result confirms the results of Pinto et al (2015) study stating that the highest concentrations of heavy metals in *P. viridis* were found during the east season. In addition, the results of statistical analysis of T-Test dependent samples (Table 4) showed that there were significant differences of heavy metals in May 2018 and the heavy metals in September 2018. It is indicated by a value of p = 0.000262 less than the value of the error degree (α), 0.05. Therefore, it can be concluded as reject H_o.

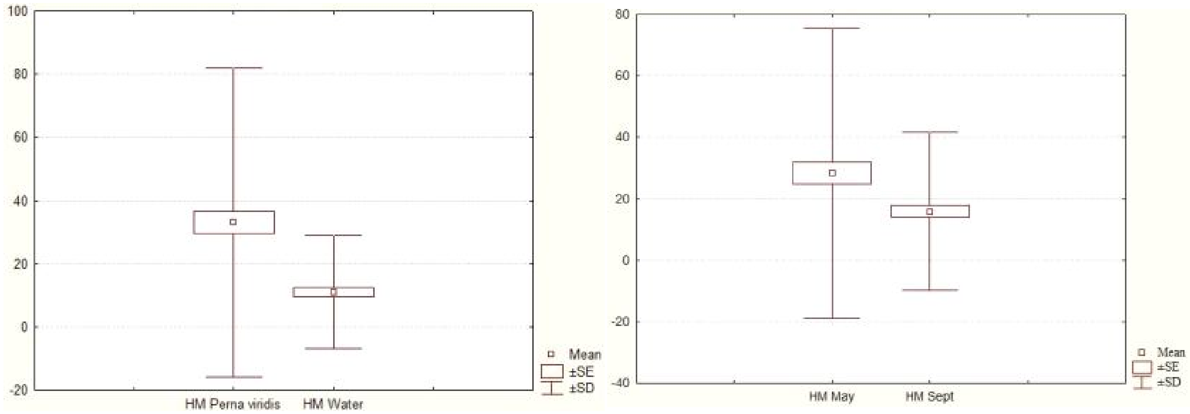

Figure 3. Heavy metal plot diagram in a) *Perna viridis* and water; b) May and September 2018.

Table 4

Results of statistical analysis with difference testing of heavy metals content in May and September 2018

	Mean	*Std. Dv.*	*N*	*Diff.*	*Std. Dv.*	*t*	*df*	*p*
HM May	28.33556	47.21873						
HM Sept.	15.81722	25.61746	180	12.51833	45.09125	3.724693	179	0.000262

HM = heavy metals.

In general, the heavy metal content in the green mussels is higher than the heavy metals in the water. Heavy metal content in green mussels ranged from 0.3 to 202.7 mg L^{-1} and heavy metal content in water ranged from 1.1 to 51.0 mg L^{-1}. This may due to the fact that the sampling was at high salinity ranging from 30 to 33‰ which results in an increase in the process of desorption (addition). According to Najamuddin et al (2016), the process of desorption leads to the decrease in the concentration of heavy metals particulate matters (Total Suspended Solid) due to the release of heavy metal particulate matters from the particle surface to be heavy metal dissolved in water. Dissolved heavy metals in water will easily be absorbed by the green mussels attached to the organism living in the water column. According to Kobielska et al (2018), the heavy metals brought by water can also be absorbed by algae and other herbivorous biota which will then be continued to the food chain organism above it. Based on Nurhayati & Putri (2018) study conducted in the waters of Cirebon, similar results were obtained showing that the content of Hg in *P. viridis* was higher by 0.027±0.038 ppm compared to heavy metals contained in water, i.e. 0.017±0.029 ppm.

Spatial distribution of heavy metals. Figures 4 and 5 present heavy metal spatial distribution in green mussels in the waters of Semarang Bay. The figures illustrate all green mussels located in the waters far from the mainland have higher heavy metal content than those located near the mainland. The spatial distribution of heavy metal in the water of Semarang Bay is presented in Figure 6. The figure elaborates that the overall water media as green mussel habitat in the area far from the mainland and the waters close to the estuaries have the highest heavy metals content.

As shown in Figures 4-5, the distribution of increasing heavy metals concentration in green mussels is found in the station located far from the mainland and close to thermal power station industries. In addition, Figures 6-7 describes the distribution of the concentration of heavy metals in the water increased in the estuary area. Then, the overall distribution of heavy metals concentration increased far from the mainland because the sampling was conducted on 8 May 2018 and 3 September 2018 at the time of First Quarter when the position of the sun, earth, and moon was perpendicular resulting in the lowest ebb. This caused all the water mass from the coast/close to the mainland and from the river move towards the sea. The concentration of heavy metals in the sea water does not originate from the green mussel culture location but it comes from the input of rivers or waters near the coast which are directly related to settlements and industry (Koropitan & Cordova 2017). In addition, according to Hadibarata et al (2012), low and high heavy metals concentrations are caused by the amount of metal waste which enters the water and pH that will affect the solubility of heavy metals.

Heavy metals contained in water media in both low and high concentrations can accumulate in the body of living organisms with varying amounts of absorption and accumulation in different biological systems (Riani et al 2018). The results of the study conducted by Qari et al (2016) at Karachi Beach, Paradise Point, at the low tide of 1993 and 2012 showed that there was a high rise of heavy metals content in the green mussel. This finding described that there was an indication of the beach exposed to significant pollution of heavy metals due to industrial and domestic waste disposal at the beach. Moreover, the result of Qari et al (2015) showed that the study area had high input of heavy metals and other inorganic and organic substances coming from industrial and domestic wastes. *P. viridis* in aquatic environments containing many heavy metals is not good for consumption but they can function as water environment cleaners, due to their sedentary nature and feeder filters as well as can absorb heavy metals and keep it in their body (Riani et al 2017).

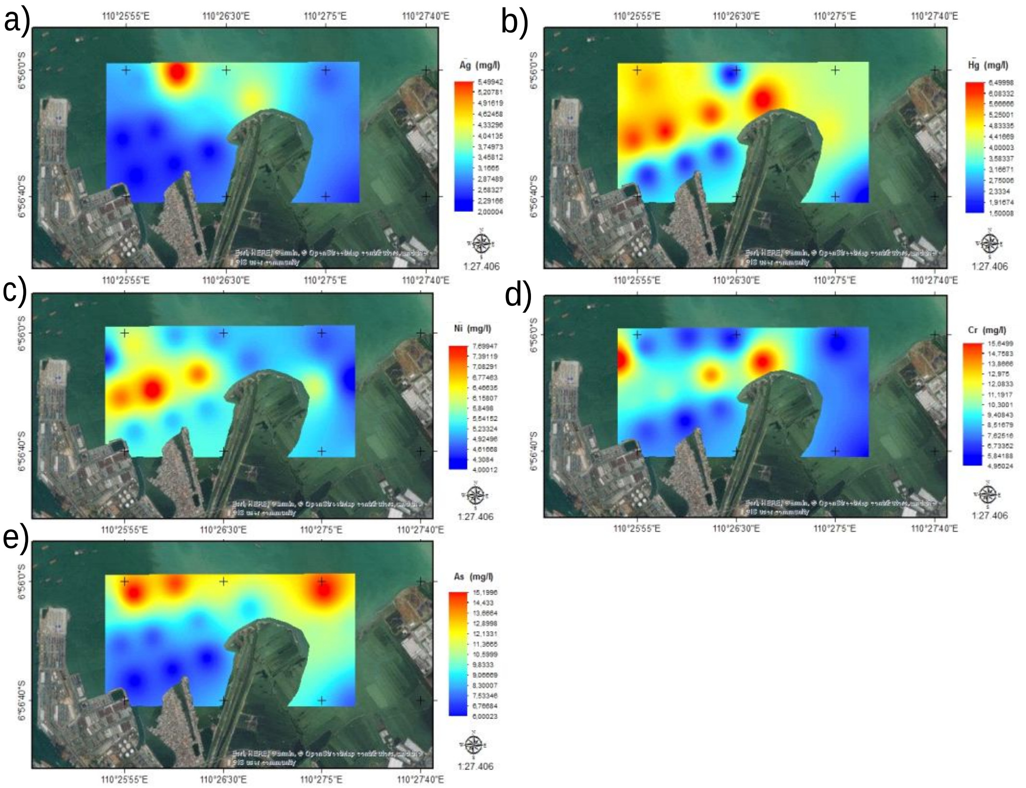

Figure 4. Spatial distribution of low concentration heavy metals in green mussels; a) Ag, b) Hg, c) Ni, d) Cr, and e) As.

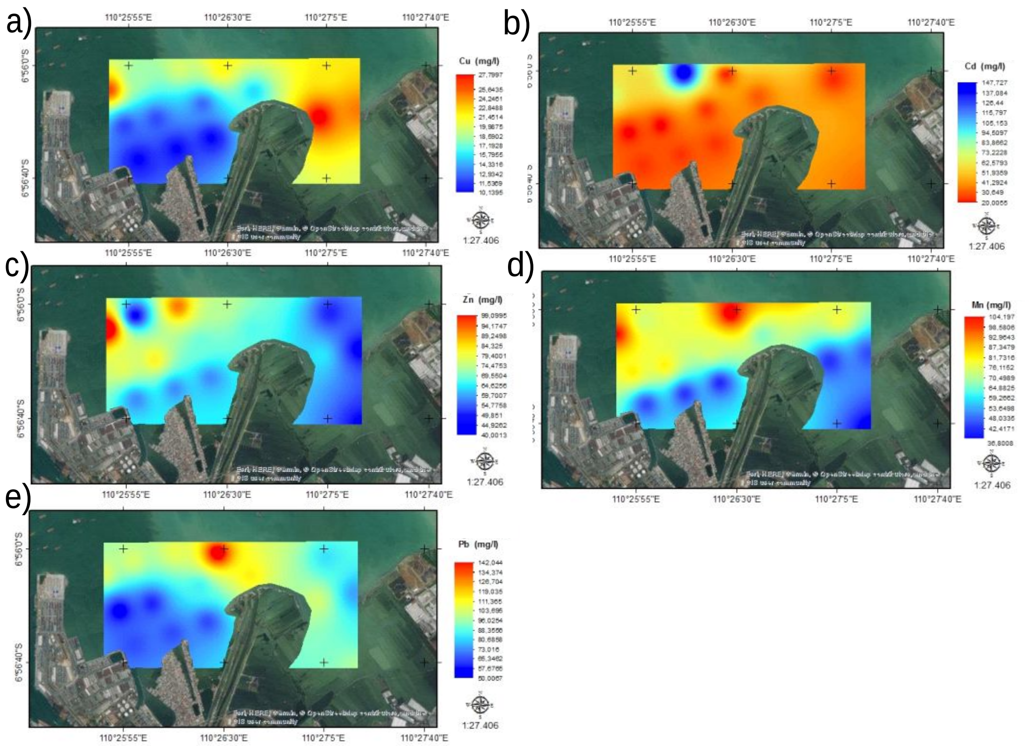

Figure 5. Spatial distribution of high concentration heavy metals in green mussels; a) Cu, b) Cd, c) Zn, d) Mn, and e) Pb.

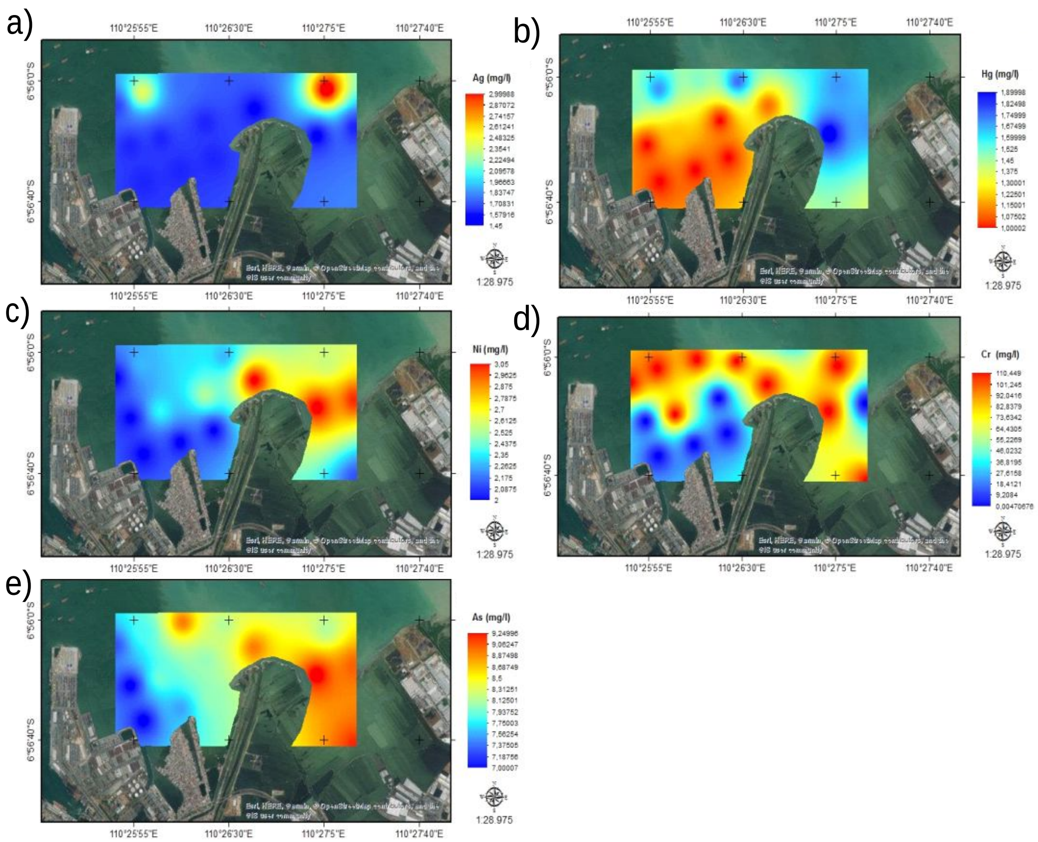

Figure 6. Spatial distribution of low concentration heavy metals in water; a) Ag, b) Hg, c) Ni, d) Cr, and e) As.

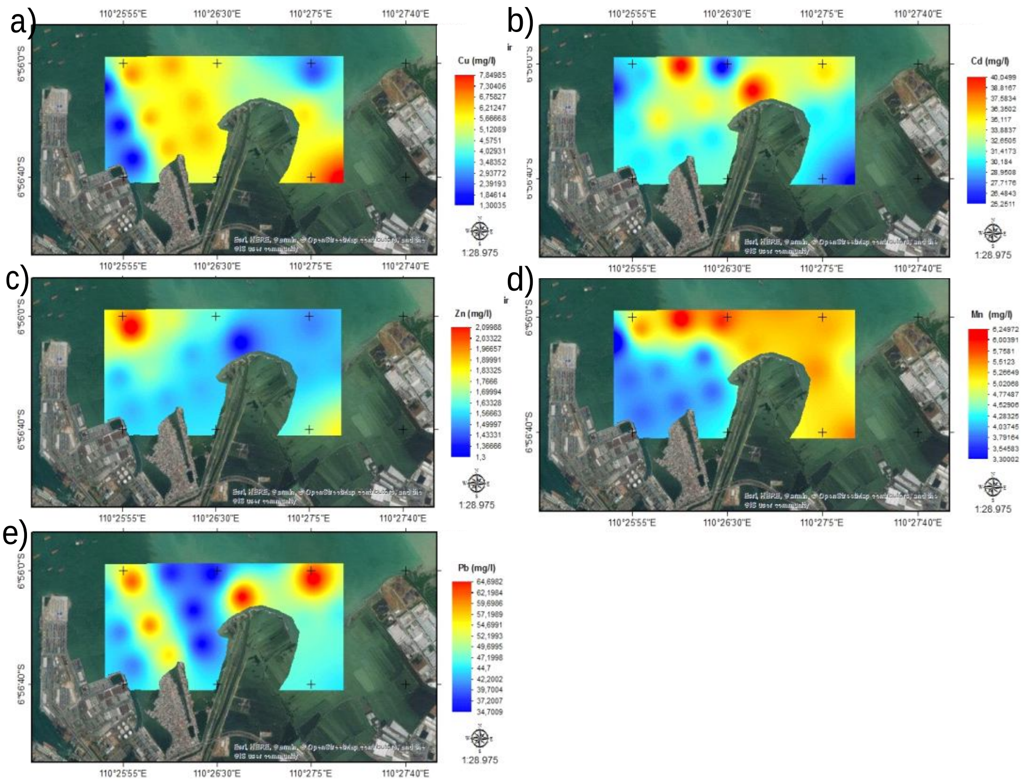

Figure 7. Spatial distribution of high concentration heavy metals in water; a) Ag, b) Hg, c) Ni, d) Cr, and e) As.

Conclusions. The results of the study showed pH values ranging from 7.9 to 8.2. Moreover, the observation on DO showed the values of DO ranging from 5.86 to 7.11, where the pH values increased along with the increasing DO values. The concentration of nitrate was higher than that of phosphate. The concentration of nitrate ranged from 1.831 to 2.038 mg L^{-1}, while the concentration of phosphate ranged from 0.24 to 0.44 mg L^{-1}. Based on the results of the study, it can be concluded that the concentration of heavy metals from very low to very high is respectively Ag < Hg < Ni < Cr < As < Cu < Cd < Zn < Mn < Pb. Generally, the concentration of heavy metals contained in the green mussels was higher ranging from 0.3 to 202.7 mg L^{-1} than the heavy metal concentration contained in water which ranged from 1.1 to 51.0 mg L^{-1}. Sampling conducted in May 2018 (transition season I to the east) showed a higher heavy metal (0.3-202.7 mg L^{-1}) than that in September 2018 (transition season II), i.e. 0.1-99.2 mg L^{-1}). In addition, the results of statistical analysis using T-Test dependent samples showed that there were significant differences between heavy metals content in May 2018 and in September 2018 and there was also significant difference in the content the heavy metals in the green mussels and in water.

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Authors:
Djoko Suprapto, Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Jl. Pusponjolo Barat Gang XII No. 9, 50141 Semarang, Indonesia, e-mail: djk_suprapto@yahoo.com
Nurul Latifah, Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Jl. Pusponjolo Barat Gang XII No. 9, 50141 Semarang, Indonesia, e-mail: nurul.latifah@live.undip.ac.id
Suryanti Suryanti, Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Jl. Pusponjolo Barat Gang XII No. 9, 50141 Semarang, Indonesia, e-mail: suryantidr@gmail.com

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