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Research Article

Assessment of Heavy Metal Cd and Pb Concentrations and the Ecological Risk from Seawater, Sediments, and *Perna viridis* in Semarang Bay, Indonesia

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Abstract

Semarang Bay is a multiple water source from some rivers located at the forefront of the most populated city in Central Java. These waters are polluted by wastes from various sources. Previous studies conducted were limited to specific areas and were mainly focused on one or two media only. This research aims to analyse the heavy metal Cd and Pb concentrations in seawater, sediments, and green mussels and the ecological risks. Field data were collected from 18 sites from three locations: Semarang, Demak and Kendal during the dry season of 2020. Environmental variables were measured in situ, and the heavy metals concentrations were analysed using Atomic Absorption Spectrometry. The concentrations of Cd and Pb in the waters of Semarang Bay vary spatially and are above the standard threshold values set by the government. The highest concentration of Cd is found in the sediment and of Pb in the seawater. Despite the exceeding concentrations, the environmental conditions in Semarang Bay are still relatively good based on the low values of contamination indices assessed. This condition, however, can be expected to change when the season changes and needs to be considered by green mussel farmers, coastal managers, and local governments.

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Introduction

Indonesia is the biggest archipelagic country globally, with around 17,500 islands and a coastline length of 108,000 km; 70% of its region is covered by seawater that consists of open oceans, seas, coasts, estuaries, and bays [1-2]. Semarang Bay is the largest bay on the northern coast of Central Java Province, located in front of Semarang city, the capital of Central Java Province. The basin stretches from Kendal Regency in the west, Semarang City in the middle, and Demak Regency in the east, approximately 23 miles long and with a water area of around 170.2 km^2 [3-4]. This region has high potential marine resources with many estuaries and other vital habitats, such as mangrove ecosystems [5-6]. This essential habitat is an important area for the life of organisms, especially green mussels (*Perna viridis*), fishing grounds, and marine culture carried out by local fishermen on the northern coast of Java [7-8].

As it is situated at the forefront of Semarang City (the biggest and most densely populated city in Central Java Province), Semarang Bay also functions as a comercial port, namely Tanjung Mas Port, one of the biggest ports in Indonesia [4, 5]. These conditions certainly contribute to elevated pollution in the bay. Various waste products produced on the mainland, i.e., domestic, agricultural, and industrial wastes, are transported by numerous rivers discharged into this bay. Therefore, Semarang Bay has been experiencing environmental degradation yearly due to the input of pollutants from various activities on the mainland. The deterioration in the water quality in this area is due to the number of human activities and developments around Semarang Bay, such as industry, ports, and settlements suspected of intentionally dumping their waste into the seawater. This condition was shown by preceding studies conducted in Semarang Bay [9-11]. Increasing industry development increases the release of waste into the surrounding environment, including the marine environment, i.e., estuaries, coastal waters, and open oceans [12-14]. Waste disposal can be released into seawater through runoffs or streams. Heavy metals are one of the industrial wastes released into seawater [15-17].

Heavy metals are metallic elements with relatively high densities compared to water [18]. Heavy metals in aquatic environments consist of dissolved and particulate matter [19-20]. Adsorption-desorption mechanisms that occur between particulate and dissolved heavy metals will affect the accumulation, distribution, and effect of heavy metals in water, which will affect the environmental quality of the seas and the survival of aquatic biota [21-24]. Such mechanisms usually occur in waters with salinity and pH conditions that support adsorption-desorption means and high noise levels (due to high Total Suspended Solids concentrations). Heavy metals commonly found in waters in urban areas are Pb, Zn, Cd, Cu, and Co [25-27]. Heavy metal pollution has spread globally, disturbing the environment and posing severe health risks to marine biota [28-30]. This problem is mainly due to rapid urban development and land-use changes [33], especially in developing countries with high populations, such as India, China, and Indonesia [34-35].

Heavy metals scatter in water columns and accumulate in the sediment and organisms [36-38]. According to Hu et al. [39], heavy metals in the water and sediment determine the risks of pollutants to environmental damage and aquatic organisms. Understanding the heavy metals in deposits is essential because they describe the accumulation rate of heavy metals over a given period [40-41] and play a vital role in the marine ecosystem material recycling and energy flow [42-43]. Heavy metal contamination in the aquatic environment can cause public health risks [44-46]. There have been few studies on heavy metal pollution in the region of Semarang Bay and were mainly limited to certain areas, not covering all regions. Previous studies were focused on analyzing the concentrations of Pb and Cd in plankton, fish larvae, and shellfish [3, 47-48], the content of heavy metals (Pb and Cd) in *Perna viridis* and sediments [8] and Pb in green mussels [47] and heavy metals in seawater, sediment, and bacteria abundance [49-50]. With the possibility of ecological risks, a wider study area coverage on different media is, therefore, of paramount importance to be conducted.

The present study assesses heavy metals Cd and Pb concentrations in seawater, sediments, and green mussels and their ecological risk in the waters of Semarang Bay, covering the west (Kendal Regency), middle (Semarang city), and east (Demak Regency) regions of the bay. Since Cd and Pb are the dominant heavy metals commonly found in Semarang Bay waters [49-50], the present research examines the metals concentrations and their ecological risks in seawater, sediment, and green mussel tissues. The results are expected to benefit policymakers and the region's coastal and fishery management.

Materials and methods

1) Study area and sampling

The study was conducted in Semarang Bay, the northern coast of mid-Java. This region has experienced severe devastation with an annual average erosion rate of -25 m, and shoreline movement was -592 m along the coast of Sayung [1]. It is a low-lying region with an elevation of less than 2% and a height of 0–5 m above sea level, dominated by silt and clay sediments and some populations of mangrove forest. The research was conducted during the dry season of 2020 in the coastal waters of Semarang Bay (i.e., Semarang, Demak, and Kendal). The sampling sites are characterised by green shellfish cultivation (Figure 1).

The method used in this study is a descriptive method with sampling techniques [51] in the waters of Semarang Bay. Data sampling is carried out by sailing down the coast from the coastline (the highest tidal area) towards the open waters (shelf break).

2) Data collection and analysis

2.1) Samples collection

Field data collection stations were along the waters of Semarang Bay as follows: Semarang (st. 1-6), Demak (st. 7-12), and Kendal (st. 13-18) totalling 18 sample stations. Each sampling site's location was recorded using a Global Positioning System (GPS) GARMIN. Sediment samples were collected on the surface layer, roughly 5–10 cm, with an Ekman grab sampler [1, 7-8]. Sample sediment, as much as 500 g, was placed into a polyethene bottle. The polyethene bottles were soaked with HNO_3 (6 N) and rinsed with distilled water. Sediment samples were kept in a cool box at 4 °C and transported to the laboratory. Water samples were taken from the bottom of the water column using the Nansen Bottle Sampler. Green mussels were taken from fish aggregating devices (rumpon) standing upright in the water.

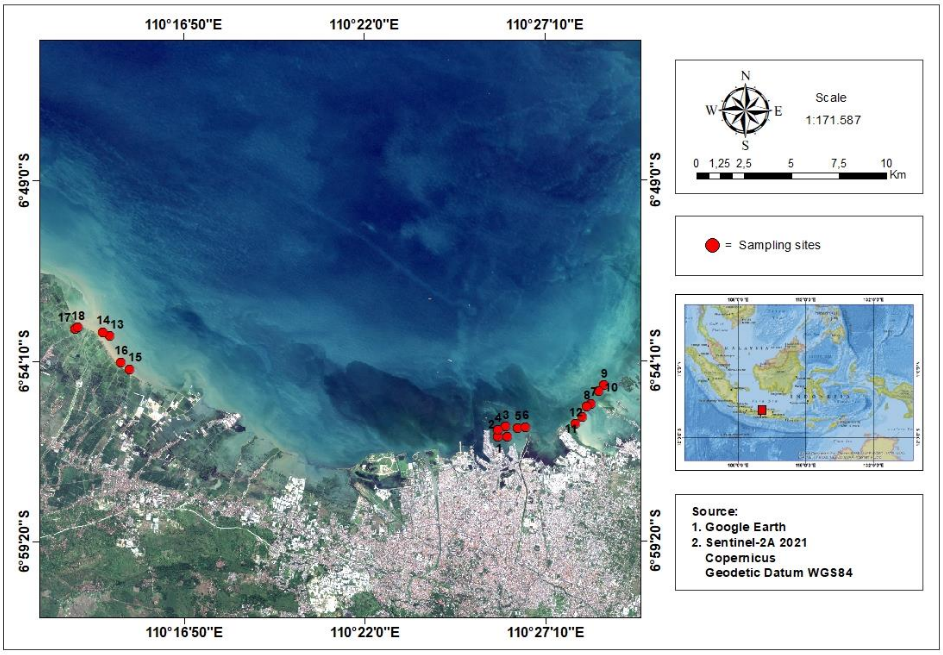

Figure 1 Research locations in Semarang Bay waters.
(Legends: S=Semarang (st. 1-6); D=Demak (st. 7-12); K=Kendal (st. 13-18)

2.2) Samples standard preparation method

The sediment samples were dried in the laboratory using a freeze dryer at -12 °C. All samples for seawater and sediment heavy metals were prepared for analysis based on the procedures of APHA [52] using 250 ml filtered seawater and 5 g of sediment samples. Heavy metals in shellfish are processed for analysis according to Yap et al. [53]. Dry samples were measured at 1.0 g and then dissolved in 10 ml of concentrated nitric acid (HNO_3) in a test tube using a heating device (hot plate) at a low temperature (40 °C) for 1 h and continued at high temperature (140 °C) for ± 3 h.

2.3) Samples analysis method

The heavy metals were analysed using the atomic absorption spectrometry (AAS) Variant Shimudzu-AA7000 with a detection limit < 0.0001. Analysis of samples on each water quality parameter and sediment texture is carried out following procedures by APHA and Muskananfola et al. [54-55]. The obtained results are discussed by referring to national regulations on seawater quality [56].

3) Contamination indices assessment methods

3.1) Contamination factor

The contamination factor (CF) values were calculated by dividing the concentration of heavy metals in the samples by the background values as follows:

CF metal = [C heavy metal]/[C background] (Eq.1)

As the local background value was unavailable for these two metals, the background values used were 0.2 for Cd and 12.5 for Pb [57]. CF levels were characterised as follows: CF value < 1 shows low contamination, values between 1–3 suggest moderate contamination, values of 3–6 imply considerable contamination, and CF values > 6 indicate high contamination [58].

3.2) Contamination degree (CD)

According to Hekanson [57], the contamination degree illustrates polymetallic contamination of the study area as indicated in the equation:

$$CDi = \Sigma CFi \quad \text{(Eq. 2)}$$

CD values are categorised into four different classes of contamination: <6 = low, 6–12 = moderate, 12–24 = considerable, and >24 = very high.

3.3) Pollution level/load index

The pollution level index (PLI) is an index which evaluates the level of contamination [59] using the following equation:

$$PLI = (CF1 \times CF2 \ldots\ldots CFn)1/n \quad \text{(Eq. 3)}$$

Where n is the total number of heavy metals, the reference values were taken from Turekian and Wadepohl [60]. A PLI value greater than 1 indicates the presence of contamination, while a PLI value less than 1 signifies no pollution.

3.4) Ecological risk index (Er^i) and potential ecological risk index (RI)

These two indices are quantitative terms to compute the potential environmental risk for individual metals to the surrounding ecology of the sampling site. Hekanson [57] determined that the quantitative expression of the potential ecological risk of a given metal in a given sediment sample is as follows:

$$\boldsymbol{E_r^i} = \boldsymbol{T_r^i} \times \boldsymbol{CF} \text{ and } \boldsymbol{RI} = \boldsymbol{\Sigma E_r^i} \quad \text{(Eq. 4)}$$

where $\boldsymbol{T_r^i}$ is a toxic response factor for a particular metal. $\boldsymbol{T_r^i}$ is 30 for Cd and 5 for Pb.

The $\boldsymbol{E_r^i}$ is are categorised into the following quality groups: <40 = low, 40–80 = moderate, 80–160 = considerable, 160–320 = high, and >320 = very high.

The *RI* is also divided into four categories as follows: <150 = low, 150–300 = moderate, 300–600 = considerable, and >600 = very high.

4) Statistical analysis

The obtained data on metal concentration in the three different mediums (i.e., seawater, sediment, and green mussel tissues) were expressed as means and standard deviation (Table 1) and analysed using R-Programming Language ("stats" package version 3.4.4 of the R software) in R software version 4.0.2 Vegan package 2.5-7 [61]. This analysis aims to obtain statistical characteristics and better visualisation of heavy metal concentrations in various locations and mediums (using Multivariate Analysis of Variance/MANOVA); Pearson's correlation and cluster analyses were used to reveal similarities between sites. As a pre-requirement, all data were tested for homogeneity of variation before conducting the statistical test. The measured Cd and Pb metal concentrations were treated as dependent variables, while seawater, sediment, and green mussel tissues were assumed to be fixed factors. Data were considered significant when the p value was < .05.

Results and discussion

1) Variability of heavy metals concentration

Heavy metal (Cd and Pb) concentrations show a varying pattern in Semarang Bay waters (Table 1). The concentration of Cd is the highest in sediment (0.0085 mg kg^{-1}), followed by green mussels (0.0070 mg kg^{-1}) and then seawater (0.0048). The concentration of Pb is the highest in seawater (0.0093), followed by green mussels (0.0075 mg kg^{-1}) and sediment (0.0052 mg kg^{-1}).

The high concentration of Pb found in seawater in Semarang Bay might be due to the supply from industry and corrosion of ships' paints, ballast water, natural processes, and anthropogenic processes [35]. The high concentration of Cd metal in sediments is caused by accumulation from the surrounding waters. Heavy metal concentration in sediment depends on the settled properties of the sediment particles, describing the accumulation of heavy metals over some time. The accumulation rate of heavy metals in sediments is determined by the physical and chemical characteristics of sediments, such as the size of the particles and the content of organic materials [13, 26-27]. Sediments with rough particle sizes (sand) have a small surface area, meaning the ability to absorb heavy metals is low, and the amount of organic materials contained is also low. Conversely, finer sediment particles, such as clay and silt, have a large surface area that will absorb and accumulate many heavy metals. The sediment texture of Semarang Bay waters is dominated by clay, sandy clay and silty sandy clay, as shown in Table 2. This smaller sediment has higher organic content than the larger sediment. Sediment characteristics also influence heavy metals absorption and mobilise sediments: clay, silt, sands, and organic materials [21]. The finer sediment particles in clay will have high organic material [7, 21] and will likely accumulate higher contents of heavy metals. The reason is that these compounds have metal-binding properties.

In the marine environment, Cd originated from industry and coated ship paints, ballast waters, and natural and anthropogenic processes [22, 35]. It is stated that hydrodynamic conditions, i.e., currents, waves, tides, pH, and salinity, also affect heavy metal concentration in the aquatic ecosystem [14]. Cd seawater can be harmful to green mussels as it can be accumulated in their bodies. Furthermore, Cd can cause various kinds of damage even in low concentrations because Cd is a highly toxic pollutant in the marine environment [36]. Other findings by Rajkumar et al. [24] stated that green mussels (*Perna viridis*) are more vulnerable to Cd and Pb metals than Cu and Zn metals. Pb seawater could also harm marine organisms because Pb can get into the body of green mussels through filtration and ingestion. Previous researchers found that Pb caused a decrease in growth [24], DNA damage leading to genetic destruction [32], and the inactivation of antioxidant enzymes [45]. The accumulation of these disturbances may lead to morphological defects in green mussels. Therefore, monitoring and controlling pollution caused by Cd and Pb in marine environments must be anticipated to prevent contamination of aquatic organisms and humans.

Table 1 Heavy metals concentration in the seawater (mg L^{-1}), sediment (mg kg^{-1}), and green mussels (mg kg^{-1}) in Semarang Bay waters

Station	Location	Cd						Pb					
		Seawater (mg L^{-1})		Sediments (mg kg^{-1})		Green mussels (mg kg^{-1})		Seawater (mg L^{-1})		Sediments (mg kg^{-1})		Green mussels (mg kg^{-1})	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Semarang	0.0038	0.0035	0.0116	0.0004	0.0057	0.0009	0.0089	0.0005	0.0032	0.0007	0.0056	0.0004
2		0.0041	0.0006	0.0082	0.0013	0.0077	0.001	0.0083	0.0007	0.0029	0.0006	0.0069	0.0005
3		0.0036	0.0039	0.0085	0.0014	0.0036	0.0023	0.0094	0.0005	0.0017	0.0004	0.0074	0.0006
4		0.0026	0.0018	0.0087	0.0002	0.006	0.0006	0.0087	0.0005	0.0048	0.0005	0.0064	0.0003
5		0.0035	0.0005	0.008	0.0027	0.0094	0.0015	0.0086	0.0006	0.0049	0.0012	0.0063	0.0006
6		0.0075	0.0012	0.0084	0.0016	0.0079	0.0017	0.0083	0.0002	0.0054	0.0004	0.0067	0.0004
7	Demak	0.0055	0.0041	0.0086	0.0009	0.006	0.0028	0.0094	0.0006	0.0049	0.0008	0.0062	0.0003
8		0.0077	0.0006	0.0093	0.001	0.006	0.0016	0.0088	0.0006	0.0056	0.0008	0.0064	0.0007
9		0.0062	0.0019	0.0112	0.0009	0.0091	0.0015	0.0094	0.0002	0.0072	0.0003	0.0069	0.0003
10		0.0065	0.001	0.0083	0.0003	0.0068	0.0014	0.0091	0.0003	0.0067	0.0003	0.0137	0.0001
11		0.0031	0.0022	0.0084	0.0012	0.0074	0.0018	0.0099	0.0006	0.0052	0.0001	0.0078	0.0007
12		0.0044	0.0002	0.0085	0.0031	0.005	0.0015	0.0091	0.0001	0.0062	0.0004	0.0063	0.0001
13	Kendal	0.0058	0.0027	0.0091	0.0015	0.0072	0.0007	0.0107	0.0007	0.0066	0.0002	0.0072	0.0006
14		0.0021	0.0011	0.0075	0.0007	0.0076	0.0020	0.0100	0.0006	0.0057	0.0001	0.0078	0.0009
15		0.0063	0.0007	0.0076	0.0021	0.0069	0.0008	0.0096	0.0001	0.0047	0.0002	0.0083	0.0003
16		0.0051	0.0022	0.007	0.0009	0.0077	0.0009	0.0103	0.0003	0.0061	0.0003	0.0077	0.0004
17		0.0031	0.0021	0.0069	0.0021	0.0086	0.0011	0.0098	0.0001	0.0055	0.0008	0.009	0.0008
18		0.0064	0.0031	0.0078	0.0018	0.0083	0.0005	0.0099	0.0005	0.0064	0.0001	0.0085	0.0001
Mean		0.0048		0.0085		0.0070		0.0093		0.0052		0.0075	
SD		0.0017		0.0012		0.0015		0.0007		0.0014		0.0018	
Min		0.0021		0.0069		0.0036		0.0083		0.0017		0.0056	
Max		0.0077		0.0116		0.0094		0.0107		0.0072		0.0137	
National Standard		0.001		NA		NA		0.008		NA		NA	

Remark: NA = Not available

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The present study results show that heavy metal concentration in Semarang Bay waters is slightly above the national standards set by the Government Regulation of the Republic of Indonesia on the Implementation of Environmental Protection and Management [56], which limits the maximum concentration (mg L^{-1}) of Cd to 0.001 and Pb to 0.008 for marine organisms life. The results in Table 1 show that Cd and Pb concentrations (mg L^{-1}) in seawater are 0.005 and 0.009, respectively, above the concentration threshold set by the government. This indicates that Semarang Bay waters are categorised as polluted in terms of Cd and Pb metals. Zeitoun and Mehana [30] and Shivakumar et al. [29] revealed that even low concentrations of heavy metals could be accumulated in the body of living organisms with various levels of absorption in different biological organs. At the same time, green mussels can get heavy metals several times higher than in the water. As shown in this study, the concentration of Cd in green mussels is more prominent than in seawater. These findings are supported by Shivakumar et al. [29], Zeitoun and Mehana. [30], who stated that toxic substances, such as heavy metals, would accumulate in the bodies of marine organisms in that ecosystem. Contamination of green mussels by heavy metals originated from the environment and the food chain. This heavy metal contamination will harm those who consume mussels, especially humans [13]. The results of this study revealed that the concentrations (mg kg^{-1}) of Cd and Pb in green mussels are 0.0070 and 0.0075 which fall below the national standard concentrations (mg kg^{-1}) recommended for shellfish/molluscs in food which are 1.0 and 1.5 for Cd and Pb, respectively.

2) Comparison of heavy metals Cd and Pb

The comparison of heavy metals Cd and Pb in seawater, sediments and green mussels tissues in coastal regions of Indonesia and other countries are presented in Table 2. It shows that heavy metal Cd and Pb concentrations in the present study (in Semarang Bay) are lower than in other areas, such as Jakarta Bay and Demak. This trend might be related to the industrial development between the two cities and the fact that Jakarta is the most populated city and the biggest seaport in Indonesia. This condition increases heavy metal pollutants from industrial waste and anthropogenic activities [28]. The table also shows that the concentrations of Cd and Pb metals in Semarang Bay, Indonesia, are lower than those in Malaysia, China, India and Pakistan. These findings indicate that heavy metal pollutants might be driven by human settlements with high anthropogenic activities, harbours, industrial density and untreated sewage discharges [16].

3) Ecological risks of Cd and Pb

Table 3 shows the results of the present study on the CF, CD, and PLI. The mean values of CF Cd and Pb in seawater, sediment, and mussels vary between 0.0042 (Pb mussels) and 0.4272 (Cd sediment). These CF values are <1, indicating low contamination [58]. Similarly, CD values range between 0.2498 (seawater) and 0.4313 (sediment), which is less than 6, indicating low contamination [57]. For PLI, the lowest value was 0.0416 (sediment), while the highest value was 0.0456 (mussel), where PLI < 1 signifies no pollution [59]. In the meantime, the potential ecological risk index is presented in Table 3. Mean values of Eri Cd and Pb metals in seawater, sediment, and green mussel tissue vary from 0.0208 (Pb mussels) to 12.8155 (Cd sediment). RI values vary from the lowest in seawater (7.3068) to the highest in sediment (12.8363). These values show that the Eri and RI are low in the study area (Semarang Bay) because Eri < 40 and RI < 150 [57].

Ecologically, all of the contamination indices evaluated in this investigation (i.e., CF, CD, PLI, RI) signify that the waters of Semarang Bay have not been polluted by Cd and Pb metals, particularly at the time of study in July (dry/east season). This condition, however, may change with the season and heavy metal sources. Indonesia has two main seasons, i.e. the west season (December, January, February) and the east season (June, July, August) and two transition seasons, i.e. transition season I (March, April, May) and transition season II (September, October, November) [2]. These four seasons have different wind speed characteristics and rainfall intensity that affect the Indonesian seas' hydrodynamic conditions and river runoff [2, 55]. During the east season, the climate and hydrodynamic conditions in Semarang Bay waters are relatively calm, with small waves with a surface currents speed of 0.15 m s^{-1}. This mild condition might lead to fewer interactions between seawater and sediments, keeping heavy metals in ion form and causing high seawater concentrations [44]. The concentration of heavy metals is affected by seasons; Cd's highest concentration occurred in autumn, while in winter, Cd was not detected [19]. It was stated that the maximum concentration of Pb happens in spring and summer. Pb metals have larger adsorptions, so higher concentrations of Pb are found in sediments. Turbid waters with more suspended particles can absorb dissolved pollutants in the water column and stimulate desorption. The spatial and temporal distribution of dissolved heavy metal pollutants in the waters are affected by the aquatic environment's physical, chemical and biological processes.

4) Environmental variables

The data acquisition of environmental variables was measured in situ during field sampling. Water and sediment samples were taken to the laboratory to analyse TSS and sediment texture [7]. The temperature was measured using a digital thermometer, salinity used a refractometer, dissolved oxygen used a DO meter, and pH used a pH meter [54]. Sediment texture was measured using pipette analysis, and brightness was measured using a Secchi disc.

Environmental variables measured in the study area are presented in Table 4. Environmental variables show normal ranges of tropical waters while clays dominate sediment textures. The average values of environmental variables are water temperature 29.82, salinity 30.22, pH 7.4, dissolved oxygen 6.97, brightness 49.11 and total suspended solids 0.46. Bottom sediment textures consist of 30.09 % sand, 5.2 % silt and 64.71 % clays. These results are in line with previous studies, which revealed that sediments in the coastal waters of Semarang are dominated by silts and clays [1, 6-7]. The proportion of clay in sediment suggested an important role of clay cation exchange capacity in the absorption of metals and removal of them from the water column. The longer time of settling velocity for silt and clay allows more metals to be removed from the water column. Dissolved oxygen content in Semarang was higher, which might be due to photosynthetic activities, freshwater inputs from rivers, wind speed and sea-surface roughness.

Table 2 Comparison of heavy metals Cd and Pb in seawater, sediments and green mussels tissues in coastal regions of Indonesia and other countries

Study area	References	Seawater (mg L^{-1})		Sediments (mg kg^{-1} dry weight)		Green mussels (mg kg^{-1} dry weight)	
		Cd	Pb	Cd	Pb	Cd	Pb
Semarang Bay, Indonesia	Present study	0.0048	0.0093	0.0085	0.0052	0.007	0.0075
Demak Estuary, Indonesia	[50]	0.0938	0.6506	0.0247	0.3918	NA	NA
Jakarta Bay, Indonesia	[13]	0.0076	0.0112	NA	NA	0.1150	27.6282
Kelantan Estuary, Malaysia	[40]	NA	NA	0.0733	52.0133	NA	NA
Meiliang Bay, China	[43]	0.2400	6.0000	0.2300	5.1400	NA	NA
Palka Bay, India	[28]	NA	NA	1.2000	14.1000	NA	NA
Balochistan, Pakistan	[44]	1.010	14,367	1.920	20.033	0.027	1.322

Remark: NA = Not available

Table 3 Pollution indices contamination factor (CF), pollution level index (PLI), ecological risk index (Eri) and potential ecological risk index (RI)of heavy metals at Semarang Bay

Index		Seawater		Sediment		Mussels		Seawater	Sediment	Mussels
CF	Site	CF Cd	CF Pb	CF Cd	CF Pb	CF Cd	CF Pb	CD	CD	CD
	Mean	0.2423	0.0075	0.4272	0.0042	0.3522	0.0060	0.2498	0.4313	0.3582
	SD	0.0855	0.0005	0.0607	0.0011	0.0739	0.0014	0.0854	0.0607	0.0741
	Min	0.1067	0.0066	0.3430	0.0014	0.1812	0.0045	0.1147	0.3474	0.1872
	Max	0.3850	0.0085	0.5777	0.0057	0.4693	0.011	0.3920	0.5802	0.4743
PLI	Site							PLI	PLI	PLI
	Mean							0.0419	0.0416	0.0456
	Stdev							0.0077	0.0069	0.0075
	Min							0.0293	0.0242	0.0329
	Max							0.0520	0.0567	0.0611
Er^i	Site	Er^i Cd	Er^i Pb	Er^i Cd	Er^i Pb	Er^i Cd	Er^i Pb	RI	RI	RI
	Mean	7.2694	0.0374	12.8155	0.0208	10.5663	0.0301	7.3068	12.8363	10.5964
	SD	2.5647	0.0027	1.8221	0.0056	2.2177	0.0071	2.5645	1.8217	2.2187
	Min	3.2000	0.0331	10.2913	0.0069	5.4369	0.0225	3.2401	10.3131	5.4667
	Max	11.5500	0.0427	17.3301	0.0286	14.0777	0.0546	11.5851	17.3429	14.1030

Table 4 Environmental variables of Semarang Bay waters

Stn.	Location	Temp. (⁰)	pH	Salinity (⁰/₀₀)	DO (mg L-1)	Brightness (cm)	TSS (mg L-1)	Sand (%)	Silt (%)	Clay (%)	Texture
1	Semarang	27.1	6.96	34	8.3	97	0.28	52.88	9.64	37.48	Sandy clay
2		27.5	6.85	34	8.2	96	0.28	53.36	8.96	37.68	Sandy clay
3		27.8	6.95	32	7.9	168	0.16	64.84	2.96	32.20	Silty sandy clay
4		27.4	6.96	32	7.8	166	0.26	65.00	3.48	31.52	Silty sandy clay
5		29.9	6.94	33	7.3	46	0.24	47.12	6.92	45.96	Sandy clay
6		29.6	6.90	33	7.4	44	0.24	47.08	3.12	49.80	Sandy clay
7	Demak	31.1	7.06	32	7.2	22	2.94	2.76	2.00	95.24	Clay
8		31.6	7.01	32	7.3	24	0.34	2.32	3.36	94.32	Clay
9		30.2	7.14	34	7.4	18	0.34	29.28	5.16	65.56	Clay
10		30.4	7.09	34	7.5	20	0.30	29.20	4.92	65.88	Clay
11		30.7	7.11	24	7.3	13	0.32	21.52	5.32	73.16	Clay
12		30.8	7.15	24	7.3	11	0.38	21.60	5.44	72.96	Clay
13	Kendal	29.5	7.34	29	7.6	20	0.38	26.08	3.20	70.72	Clay
14		29.3	7.37	29	7.5	18	0.50	26.08	3.28	70.64	Clay
15		29.9	7.23	30	5.5	32	0.40	6.40	5.96	87.64	Clay
16		29.1	7.27	30	5.3	34	0.40	2.52	5.96	91.52	Clay
17		32.5	7.46	24	4.4	27	0.30	21.68	7.12	71.20	Clay
18		32.3	7.50	24	4.2	28	0.26	21.88	6.84	71.28	Clay
	Mean	29.8	7.13	30	7.0	49	0.46	30.09	5.20	64.71	
	SD	1.6	1.61	4	1.2	49	0.62	20.61	2.14	21.08	
	Min	27.1	6.85	24	4.2	11	0.16	2.32	2.00	31.52	
	Max	32.5	7.50	34	8.3	168	2.94	65.00	9.64	95.24	

5) Multi-statistical analysis

An R Boxplot chart was constructed better to visualise heavy metal concentrations in various locations and mediums to compare across different categorical variables and visualise the distribution of quantitative values in a field. Figure 2 compares heavy metal concentrations in other areas (S = Semarang, D = Demak, K = Kendal) and mediums (green mussels, seawater, and sediment). The boxplot chart shown in Figure 2 revealed that the average concentration of Cd appears to be highest in surface sediments and lowest in seawater. On the other hand, Pb is highest in seawater and lowest in surface sediments.

The average Cd concentration is the highest in surface sediments and the lowest in seawater. On the other hand, Pb is highest in seawater and lowest in surface sediments. The high concentration of Cd in sediments was caused by the ability of sediments to accumulate Cd, mainly sediments in the study areas dominated by finer particles (clay) originating from rivers from the mainland. This finding is supported by Belabed [25], who determined that sediment texture (grain size) is one of the most decisive factors affecting heavy metal concentration. Sediments are essential reservoirs for many toxic heavy metals and a route to enter wildlife food chains. Higher organic matter content leads to higher heavy metal accumulation.

In contrast, heavy metal reduction in the water body is possible due to adsorption [22]. Adsorption occurs when dissolved heavy metal elements are transferred from water to particles to form aggregates with suspension materials. The density of the heavy metal is heavier than the water around it, resulting in the heavy metal settling at the bottom of the sea. Varying concentrations of Pb in seawater and sediments are due to the level of supply from possible sources, such as ships harboured in the area releasing Pb into the waters and flushing intensity due to tidal frequency washing out metals to open ocean and reducing concentrations [48].

The Pearson correlation matrix and factor analysis were conducted to elucidate the relationship between the studied heavy metals. It is observed from the Pearson correlation matrix presented in Table 5 that correlations between various mediums were negative to moderate, ranging between -0.06, signifying no correlation and 0.49 signifying moderate correlation. The most robust linear relationship was found in Pb concentrations in surface sediments and Cd concentrations in green mussels. Concerning slight variations in Cd and Pb concentrations, these moderate positive correlations ($r = 0.49$, $P < .05$) could be due to similar natural sources of heavy metals [12, 38].

Pearson Correlation matrix of Cd Pb concentration in seawater, sediment, green mussels and environmental variables is presented in Table 6.

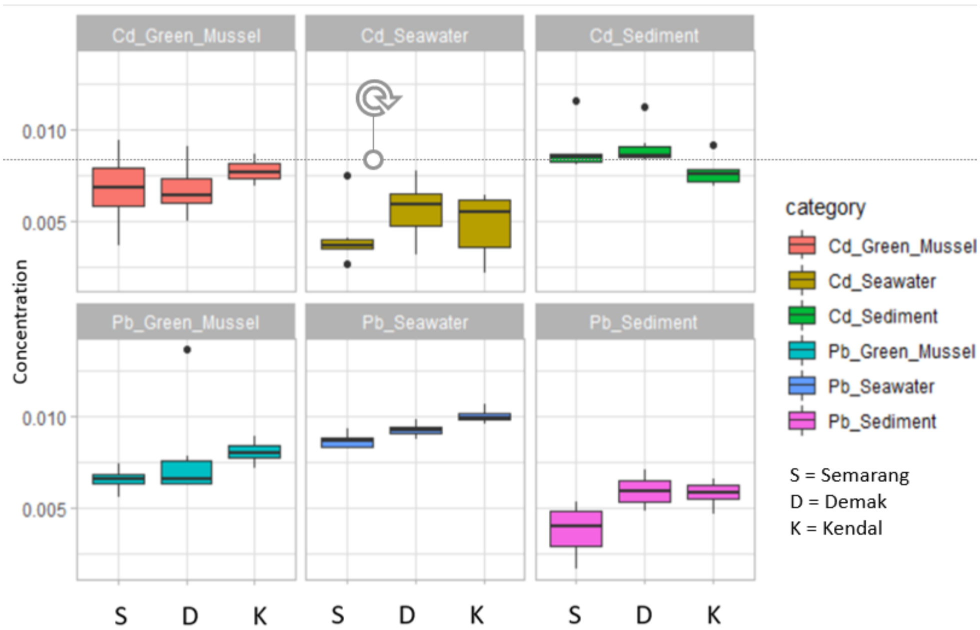

Figure 2 Boxplots of heavy metal concentrations in various locations and mediums in Semarang Bay waters. (Concentrations of Cd and Pb in green mussels in mg kg^{-1}, Cd and Pb in seawater in mg L^{-1}, and Cd and Pb in sediment in mg kg^{-1}).

Table 5 Relationship between heavy metal concentration in various locations and mediums in Semarang Bay waters

	Cd seawater	Pb seawater	Cd sediment	Pb sediment	Cd green mussel	Pb green mussel
Cd seawater	1.00					
Pb seawater	-0.09	1.00				
Cd sediment	0.18	-0.24	1.00			
Pb sediment	0.41	0.40	-0.06	1.00		
Cd green mussel	0.08	0.11	-0.19	0.49	1.00	
Pb green mussel	0.17	0.22	-0.37	0.32	0.15	1.00

Table 6 Pearson correlation Cd and Pb concentrations in seawater, sediment, green mussels and environmental variables

	Seawater Cd	Seawater Pb	Sediment Cd	Sediment Pb	Green mussel Cd	Green mussel Pb
Temperature	0.379	0.312	-0.317	0.62**	0.348	0.294
pH	-0.007	0.832**	-0.395	0.565*	0.328	0.335
Salinity	0.222	-0.559*	0.460	-0.316	-0.072	-0.087
DO	-0.165	-0.479*	0.577*	-0.359	-0.398	-0.292
Brightness	-0.404	-0.404	0.155	-0.760**	-0.488*	-0.252
TSS	0.098	0.098	-0.012	0.003	-0.162	-0.171
% Sand	-0.429	-0.522*	0.277	-0.573*	-0.166	-0.169
% Silt	-0.216	-0.170	0.094	-0.213	0.316	0.007
% Clay	0.441	0.528*	-0.280	0.582*	0.131	0.165

Remark: **Correlation is significant at 0.01 level (2-tailed)
*Correlation is significant at 0.05 level (2-tailed)

This table shows that at a 0.05 level of significance, there is a negative correlation between Pb seawater with salinity, dissolved oxygen, and sand. Whiles, a significant positive correlation between Pb seawater and Pb sediment with clay. River discharge and shipping activities in the port of Semarang Bay drive this correlation. Pb in seawater interacted with flocculated clay particles originating from river run-off, settling into the seabed, resulting in the increase of Pb concentration in sediment.

The cluster analysis was executed using Bray–Curtis similarity to show the similarity among various locations. Figure 3 illustrates the output of cluster analysis in various sites. The analysis resulted in three distinct clusters of the three locations (similarity recorded is 0.13). Cluster one consists of two sites in Semarang, cluster two consists of 8 sites, dominated by four sites in Kendal, 1 in Semarang and 3 in Demak, and cluster three consists of 8 sites, 3 from Demak, 3 from Semarang, and 2 from Kendal. The first cluster shows that Cluster one consists of two sites in Semarang, cluster two consists of 8 sites, dominated by four sites in Kendal, 1 in Semarang and 3 in Demak, and cluster three consists of 8 sites, 3 from Demak, 3 from Semarang, and 2 from Kendal. As per sites, the concentrations of the metals in Semarang fall into all 3 clusters indicating that there is a wide range of concentrations in Semarang. The second cluster signifies that the metal concentrations in Kendal tend to be more homogeneous across the sites as the majority of Kendal sites occupy the same cluster. The concentrations of metals in Demak fall into two clusters indicating varied levels of concentrations. In general, the clusters are not site-specific as the levels of metal concentrations can be grouped into three distinct clusters. Sites grouped have a higher similarity or identical behaviour and exert a possible effect on each other. These sites could also be located near industries with similar environmental pollution behaviour [44], [38]. Heavy metals assembled at less distance may indicate a higher likelihood of having a possible effect on each other.

A multivariate analysis of variance approach was used to evaluate the concentrations of heavy metals in different sites. The results are presented in Table 4, which shows significant location variations in heavy metal concentrations ($p > .05$ at 95% confidence).

The output from the MANOVA test, as presented in Table 7, shows significant variations in locations regarding heavy metal concentrations ($p > 0.05$ at 95% confidence). This process was followed up with further analysis to establish which groups differed. The analysis results (Table 8) show that there was a significant difference between locations in terms of Pb concentrations in seawater and Pb concentrations in surface sediments ($p < 0.05$ at 95% confidence). The difference in Pb seawater and sediment concentrations between locations is mainly due to the sources of the Pb. This pattern depends on industrial degrees and quantities, anthropogenic activities, hydrographic properties, and sediment textures, which differ between sites, i.e., Semarang, Demak and Kendal. The present finding is similar to the results of previous studies [10-13, 31], indicating that dense industrial centres and household waste discharged into marine environments will cause a different degree of heavy metal contamination. Hydro-oceanography characteristics, such as currents, tides, and sedimentary textures, also drive this variability.

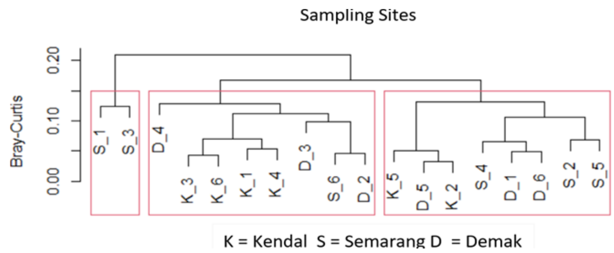

Figure 3 Linkage dendrogram based on hierarchical cluster analysis among study sites using paired group Bray-Curtis for similarity index measure.

Table 7 Results of multivariate analysis of variance test

	Df	Pillai	approx F num	Df den	Df	
Intercept	1	0.99915	1949.13	6	10	***
Location	2	1.33433	3.67	12	22	**
Residuals	15					

Remark: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Further analysis was carried out to establish which groups differed. Table 8 shows the results of the univariate tests.

The p-values indicate a significant difference between locations regarding Pb concentrations in seawater and surface sediments ($p < 0.05$ at 95% confidence). However, Cd metal concentrations show no significant difference.

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Table 8 Results of the univariate statistical tests

Response		Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Cd Seawater	Location	2	6.1450e-06	3.0727e-06	1.0583	0.3716	
	Residual	15	4.3553e-05	2.9035e-06			
Pb Seawater	Location	2	5.5483e-06	2.7741e-06	18.551	8.797e-05	***
	Residual	15	2.2431e-06	1.4954e-07			
Cd Sediment	Location	2	6.9399e-06	3.4700e-06	2.8688	0.08811	.
	Residual	15	1.8144e-05	1.2096e-06			
Pb Sediment	Location	2	1.7398e-05	8.6988e-06	7.9182	0.004495	**
	Residual	15	1.6479e-05	1.0986e-06			
CD Green mussel	Location	2	4.0910e-06	2.0456e-06	0.9279	0.4169	
	Residual	15	3.3068e-05	2.2045e-06			
Pb Green mussel	Location	2	7.9800e-06	3.9899e-06	1.3142	0.2979	
	Residual	15	4.5539e-05	3.0359e-06			

Remark: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Conclusion

An assessment of heavy metal concentration and its ecological risks in Semarang Bay waters has been conducted. Pb concentration in seawater is higher than in sediment and green mussels tissue. The concentration of Cd was higher in sediment deposits than in green mussels and seawater. Varying sources of metal supply cause these spatial variations of metal concentrations, and anthropogenic activities may deteriorate ecological risk in Semarang Bay. The concentrations of Cd and Pb in the waters of Semarang Bay are above the standard threshold values set by the government. The environmental conditions in Semarang Bay are still relatively good, as indicated by the low values of contamination indices assessed. This condition, however, may change with river run-off, heavy metal sources and seasons. These results alarm green mussel farmers, coastal managers and local governments. Therefore, it is recommended that the establishment of ecosystem protection and ecological risk prevention mechanism is necessary for Semarang Bay. As the location is indicated as significant in the multivariate analysis, crucial management measures necessitate treatments of individual locations.

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