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Small-Scale Depuration for Heavy Metal Removal in Green Mussels and Blood Cockles: Implications for Seafood Safety

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#### Abstract

Depuration treatment using clean, recirculating seawater for periods ranging from 3 to 7 days effectively reduced heavy metal concentrations in green mussels (Perna viridis) and blood mussels (Anadara granosa) to levels below the Standard. Cd decreased by up to 41% after 7 days. Lead (Pb) levels in P. viridis decreased from 2.01 to 0.73 mg/kg over 7 days of depuration. In A. granosa, Pb concentrations declined from 5.87 to 0.99 mg/kg within the same period. Copper (Cu) levels dropped significantly, from 8.05 to 1.58 mg/kg after 7 days. These findings indicate that small-scale depuration units effectively lower Cd, Pb, and Cu concentrations in green and blood mussels to levels below Standard, demonstrating both their practical value for improving seafood safety and their academic significance in advancing sustainable post-harvest This method offers great potential for treatment methods. enhancing seafood safety, supporting food security, and improving the marketability of seafood, while also demonstrating the feasibility of small-scale depuration as a sustainable approach applicable in developing regions.

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# 1. Introduction

According to the 2019 FAO Fishery and Aquaculture Statistics, global mollusk production declined from 358,828 tons in 2013 to 271,524 tons in 2019, with mussel production showing a similar decrease from 97,303 to 81,026 tons over the same period. The global fishing fleet, consisting of approximately 4.29 million vessels in 2019, has decreased steadily since its peak of around 4.67 million vessels in 2013 (FAO, 2021).

However, mussel harvesting remains high in Indonesia, reflecting strong consumer demand. Shellfish production along the north coast of Central Java—where this study was conducted—increased from 495.51 tons in 2019 to 563.10 tons in 2020 and further to 771.17 tons in 2021 (MMA, 2019). This upward trend indicates a growing

demand for shellfish commodities in Indonesia over the past three years.

Despite this growth, the primary challenge facing Indonesia's mussel fisheries is the contamination of mussel products by heavy metals, resulting from increased marine pollution. Most northern coastal waters of Central Java are now contaminated with heavy metals, significantly affecting bivalve populations, a key aquaculture commodity in the region. Yulianto *et al.* (2019) reported that shellfish collected from 12 districts along Central Java's northern coast frequently exceeded the maximum permissible levels for heavy metals such as Cd, Cu, Pb, and Zn, as established by the Indonesian Ministry of Environment and the Directorate General of Drug and Food Control. Mussel from this region are consistently exposed to and accumulate heavy metals.

As filter feeders, bivalves accumulate heavy metals from both ingested particles and direct exposure to contaminated seawater, leading to bioaccumulation in their soft tissues.

Residents along the north coast of Central Java are avid consumers of green and blood mussels. Mussels harvested from Semarang and adjacent coastal areas have been reported to contain hazardous concentrations of heavy metals, particularly Cd, Cu, and Pb, which may pose health risks to consumers. We thank the reviewer for this important note. In line with the suggestion, we have moderated the statement by changing "posing serious health risks" to "which may pose health risks," ensuring a more balanced and scientifically cautious phrasing. The primary threat to seafood safety lies in contamination from the environments in which shellfish are grown. Due to their filter-feeding nature, bivalve mollusks can accumulate contaminants at levels far exceeding those found in the surrounding water (Lee et al., 2008). Consequently, the consumption of contaminated shellfish directly jeopardises public health.

To ensure consumer safety, seafood products should be subjected to monitoring and control programs that prevent the distribution of contaminated items (García *et al.*, 2015). Ideally, shellfish should be farmed in environments with minimal pollutant exposure. While this approach could help meet nutritional demands safely, it is increasingly difficult due to the scarcity of unpolluted farming areas. Clean, uncontaminated shellfish production zones are rare, largely due to widespread marine pollution (Yulianto *et al.*, 2020a, 2020b; Soegianto *et al.*, 2022).

As a result, depuration—a legal requirement in many developed countries—has become an essential step in marketing fresh shellfish to protect public health. Depuration, the purification of live shellfish, is a preventive process to ensure food safety. This process reduces heavy metal concentrations in shellfish harvested from contaminated waters to safe levels.

Depuration technology, widely applied in various countries, involves placing contaminated shellfish in tanks filled with clean seawater for a set period, ranging from several hours to multiple days, depending on species and contaminants, during which water circulation is maintained to

facilitate detoxification. This practice is often mandated by local, regional, or national regulations, but may also be voluntarily adopted by seafood industries to ensure consumer safety (Bark, 2007; Lee *et al.*, 2008; García *et al.*, 2015; Fernandes *et al.*, 2016; Sagita *et al.*, 2017). The primary goal of depuration is to minimise the risk of foodborne illness in shellfish consumers due to accumulated metal contaminants.

In Indonesia, regulations regarding shellfish sanitation through depuration are outlined in Regulation No. 17/2004 of the Minister of Maritime Affairs and Fisheries. However, implementation of these regulations remains limited, reducing the effectiveness of consumer protection with respect to food safety. After a defined depuration period, heavy metal concentrations in mussel tissues decrease, allowing the shellfish to be marketed and consumed safely. Given the importance of this process, fishermen need to apply depuration before marketing their products.

Depuration is carried out using specialized equipment known as depuration units, which consist of vertical and horizontal tanks supplied with sterilized seawater. These systems may operate in semi-open or closed-loop configurations (MMA, 2003). Depuration units, which can be established at different scales, are often developed through cooperatives to support local fisheries. The ultimate goal of this technology is to safeguard public health by ensuring the safety of mussel products. It is expected that fisheries communities—including mussel farmers, harvesters, traders, and exporters along the north coast of Central Java— may encourage adoption of depuration units among mussel farmers, harvesters, and traders in Central Java.

#### 2. Material and methods

2.1. Study Site

# 2.1. Equipment

A small-scale depuration unit was designed to reduce heavy metal contamination (Cd, Pb, and Cu) in green mussels (*P. viridis*) and blood mussels (*A. granosa*). Mussels were sourced from both wild capture and aquaculture sites. The unit measured  $1 \times 1 \times 2$  m³ and was constructed from two plastic containers (each  $1 \times 1 \times 1$  m³), ¾-inch PVC piping, an electric water pump, and a water filtration system (Figure 1).

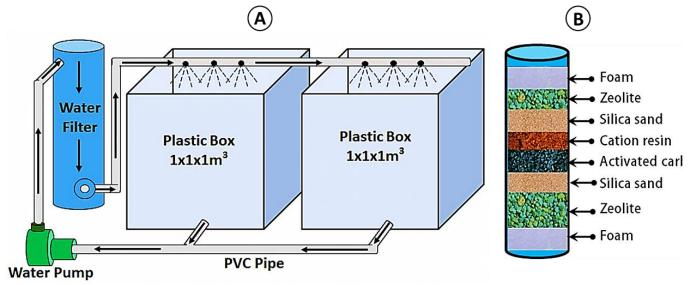


Figure 1. A) Small-scale water recirculating depuration unit. Arrow's signs show the direction of the recirculating water powered by the water pump. The water pump pushes water from the plastic box (residual water left after purifying shellfish) to the water filter (for filtering residual water containing metal contaminants), and clean water is redistributed to the plastic box. B) Water filter contents: foam, zeolite, silica sand, cation resin, and activated carbon

#### 2.2. Blood and Green Mussels

Sampling was conducted in June 2021 from the coastal waters of Semarang City and Kendal Regency. Meanwhile, green mussel (*P. viridis*) samples were obtained from aquaculture sites and collected manually from natural substrates in the same area, such as rocks, beach structures, and bamboo poles. .

Green mussels were sorted into three size categories—small (3–4 cm), medium (>4–6 cm), and large (>6–8 cm)—while blood mussels were classified into two size classes: small ( $\leq$ 2 cm) and large (>2 cm). All collected specimens were transported to the laboratory for further analysis of heavy metal accumulation in soft tissues and use in depuration experiments.

Metal analysis was conducted at the Environmental Laboratory, Diponegoro University.

# 2.3. Depuration Experiment

The small-scale depuration unit (Figure 1) was filled with clean seawater that had been tested to confirm Cd, Pb, and Cu concentrations were <0.001 mg/L. . An aerator was used to maintain oxygenation, and the water recirculation system was activated prior to mussel introduction, maintaining dissolved oxygen levels between 6.5 and 7.5 mg/L.

Depuration was performed separately for each species and size class. Depuration was conducted for 1, 3, 5, and 7 days. At each time point, a sample of mussels was taken for metal analysis. All experiments were performed under controlled laboratory conditions, with temperature maintained at  $28 \pm 1$  °C, salinity at  $30 \pm 1$  ppt, and pH at 7.8  $\pm$  0.2.

# 2.4. Metal Analysis

Soft tissues of P. viridis and A. granosa were analyzed for cadmium (Cd), lead (Pb), and copper (Cu) concentrations. The soft tissues were initially washed, ovendried at 60 °C, and ground using a mortar and pestle until a

fine powder was obtained. The powdered tissue was passed through a 100 µm mesh and homogenized.

Subsequently, 0.5 g of the homogenized tissue was weighed and placed in a Teflon digestion vessel. A few drops of distilled water and 1 mL of concentrated nitric acid (HNO<sub>3</sub>) were added. The vessel was sealed tightly and heated in a drying oven at  $150\,^{\circ}\text{C}$  for 4 hours. .

After digestion, the sample was transferred to a beaker and further boiled with repeated additions of distilled water. After cooling, the solution was transferred to a 10 mL volumetric flask. The final solution for heavy metal content was analyzed using an atomic absorption spectrophotometer (AAS).

# 2.5. Data Analysis

The research data were analyzed using Equation (1) to determine depuration effectiveness:

Effectiveness of Depuration = 
$$\frac{C_0 - C_n}{C_0} x 100\%$$

Where  $C_0$  represents the initial heavy metal concentration in mussel soft tissue, and  $C_n$  is the concentration after each depuration period (n = 1, 3, 5, or 7 days).

The collected data were statistically analyzed using one-wayANOVA and t-tests for pairwise comparisons. Reductions in metal concentrations and depuration effectiveness were visualized graphically

#### 3. Results

3.1. Heavy Metal Content (Cd, Cu, and Pb) in the Soft Tissues of Green Mussels (*Perna viridis*) and Blood Mussels (*Anadara granosa*)

Heavy metal analysis showed that in *P. viridis*, Cd and Pb averaged  $0.130 \pm 0.017$  mg/kg and  $2.013 \pm 0.667$  mg/kg, respectively, while in *A. granosa*, Pb and Cu averaged  $4.966 \pm 0.582$  mg/kg and  $8.051 \pm 1.143$  mg/kg, respectively (Table 1).

Table 1. Heavy metal (Cd, Pb, and Cu) concentrations in *P. viridis* and *A. granosa* collected from Semarang coastal waters and surrounding areas (Kendal Regency).

Size	Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)
P. viridis			
Small (3-4 cm)	0.112±0.012	1.358±0.405	-
Medium (4-6 cm)	$0.133\pm0.007$	1.990±0.461	-
Large (6-8 cm)	$0.145\pm0.007$	$2.690\pm0.227$	-
Average	0.130±0.017	2.013±0.667	-
A. granosa			
Small (≤ 2 cm)	-	4.501±0.259	7.440±1.005
Large (> 2 cm)	-	$5.430 \pm 0.367$	8.662±1.068
Average	-	5.871±0.582	8.051±1.143
Standard	Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)
SNI*	1.0	1.5	5
NADFC Indonesia**	0.1	0.2	
MMA Indonesia ***	1.0	1.5	
European Union	1.0	0.2	

<sup>\*)</sup> SNI: National Standard Agency, Indonesian National Standard (SNI) No. 7387-2009 concerning the maximum limit or threshold of metal contamination in Food

<sup>\*\*)</sup> NADFC Indonesia - Regulation of the National Agency of Drug and Food Control Indonesia No. 5 / 2018 Concerning Maximum Limits of Heavy Metal Contamination in Processed Foods

<sup>\*\*\*)</sup> MMA Indonesia - Decree of the Minister of Marine Affairs No. KEP.17/MEN/2004 concerning Indonesian Shellfish Sanitation

3.2. Cadmium and Lead Accumulation and Depuration in Green Mussel *P. viridis* 

Cd concentrations decreased progressively during depuration, ranging from 11.2% to 41.3% across mussel size

classes (Figure 2). After three days, Cd levels fell below the regulatory limit (0.1 mg/kg, NADFC), indicating suitability for consumption.

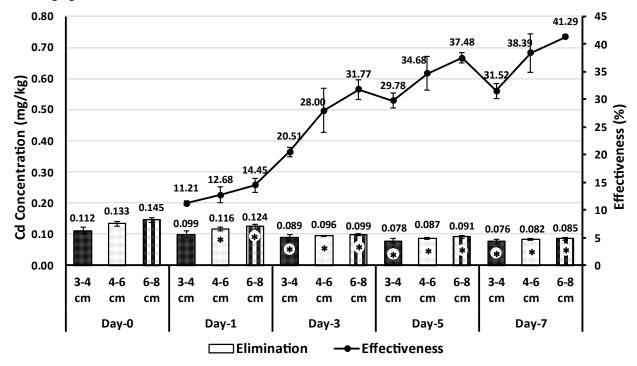


Figure 2. Cadmium (Cd) concentration (mg/kg) and elimination efficiency (%) in the soft tissue of small (3–4 cm), medium (4–6 cm), and large (6–8 cm) green mussels (P. viridis) immediately after capture (Day 0) from Semarang coastal waters and surrounding areas, and after several days of depuration (1, 3, 5, and 7 days). \* = significant difference (P < 0.05)

Pb concentrations in *P. viridis* decreased notably during depuration, with about 24-30% reductions after one day and 35-67% after three days across all size classes. ANOVA indicated significant overall reductions in Pb levels across all size classes of P. viridis (p < 0.05). However,

differences between Day 0, Day 1, and Day 3 were not significant (p > 0.05). A continuous decline was observed with longer depuration durations (5 and 7 days), which resulted in statistically significant reductions (p < 0.05) for all size classes (Figure 3).

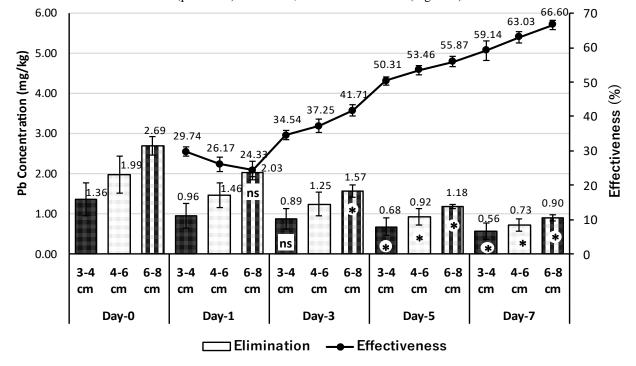


Figure 3. Lead (Pb) concentration (mg/kg) and elimination efficiency (%) in the soft tissue of small (3–4 cm), medium (4–6 cm), and large (6–8 cm) green mussels (P. viridis) immediately after capture (Day 0) from Semarang coastal waters and adjacent areas, and after several days of depuration (1, 3, 5, and 7 days). \* = significantly different (P < 0.05) compared to Day 0; ns = not significant.

3.3. Lead and Copper Accumulation and Depuration in Blood Mussel (Anadara granosa)

Pb concentrations in A. granosa decreased progressively during depuration (Days 1–7). Pb levels in A. granosa decreased substantially during depuration, with up to ~79% removal by Day 7 (Figure 4). In large-sized mussels, Pb concentrations declined by up to 86% after 7 days of

depuration. ANOVA showed significant overall reductions across size classes (p < 0.05), with non-significant changes on Day 1 but significant decreases observed from Day 3 onward (Figure 4). These results indicate that depuration reduced Pb concentrations below NADFC thresholds, suggesting improved seafood safety.

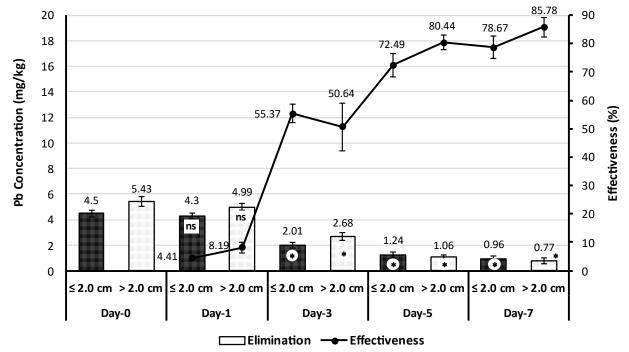


Figure 4. Lead (Pb) concentration (mg/kg) and elimination efficiency (%) in the soft tissue of small-sized ( $\leq$ 2.0 cm) and large-sized ( $\geq$ 2.0 cm) blood mussels (A. granosa) immediately after capture (Day 0) from Semarang coastal waters and surrounding areas, and after several days of depuration (1, 3, 5, and 7 days). \* = significantly different (P < 0.05) compared to Day 0; ns = not significant.

The ANOVA test indicated a significant reduction (P < 0.05) in copper (Cu) concentrations in *A. granosa* during depuration for both size classes. In small-sized individuals, Cu levels decreased from 7.44 mg/kg to approximately 1.82 mg/kg ( $\sim$ 75% reduction) over 7 days, while in large-sized

mussels (>2 cm), levels dropped from 8.66 mg/kg to around 1.34 mg/kg (~85% reduction) within the same period (Figure 5). All reductions at each time point were significant compared to the initial concentrations.

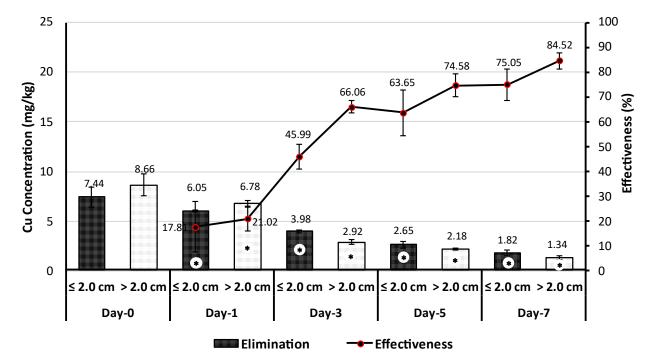


Figure 5. Copper (Cu) concentration (mg/kg) and elimination efficiency (%) in the soft tissue of small-sized ( $\leq 2.0$  cm) and large-sized (> 2.0 cm) blood mussels (*Anadara granosa*) after capture (Day 0) from Semarang coastal waters and surrounding areas, and following various durations of depuration (1, 3, 5, and 7 days). \* = significantly different (P < 0.05) compared to Day 0.

In the present study, the average Cu concentration in A. granosa immediately after landing at the fish auction site was  $8.051 \pm 1.143$  mg/kg, which exceeds the Standard set by the Indonesian National Standard (SNI, 5 mg/kg; SNI, 2009), although it remains below the limit set by the National Agency of Drug and Food Control (NADFC Indonesia, 20 mg/kg; NADFC, 2018). Taking the lower SNI threshold as a reference, blood mussels collected from the Semarang coastal area were unsuitable for consumption.

Depuration for three days effectively reduced Cu concentrations from 7.440 mg/kg (small-sized mussels) and 8.662 mg/kg (large-sized mussels) to 3.984 mg/kg and 2.921 mg/kg, respectively—levels considered safe for consumption according to regulatory standards. Further depuration up to 7 days lowered Cu levels to 1.823 mg/kg (small size) and 1.343 mg/kg (large size), thereby further improving seafood safety within the reference thresholds. Depuration for three days effectively reduced Cu concentrations from 7.440 mg/kg (small-sized mussels) and 8.662 mg/kg (large-sized mussels) to 3.984 mg/kg and 2.921 mg/kg, respectively—levels considered safe for consumption according to regulatory standards. Further depuration up to 7 days lowered Cu levels to 1.823 mg/kg (small size) and 1.343 mg/kg (large size), thereby further improving seafood safety within the reference thresholds.

#### 4. Discussion

4.1. Heavy Metal Content (Cd, Cu, and Pb) in the Soft Tissues of Green Mussels (*Perna viridis*) and Blood Mussels (*Anadara granosa*)

The concentrations of Cd, Pb, and Cu in the soft tissues of green mussels (P. viridis) and blood mussels (A. granosa) from Semarang coastal waters and Kendal Regency exceeded the maximum permissible limits set by Indonesian authorities. The detailed regulatory thresholds are summarized in Table 1.

Cd incorporated in the soft tissue of P.  $viridis~(0.130\pm0.017~\text{mg/kg})$  was equivalent to Cd concentration in P. viridis~from the Estuary of Demakan River, Jepara, Indonesia  $(0.195\pm0.02~\text{mg/kg})$  (Yulianto et~al., 2019). However, it was higher than the Cd concentration in P.

viridis (0.0334±0.0052 mg/kg) from Port of Tanjung Emas Semarang (Yulianto et al., 2020a).

In this study, the mean Pb concentration in the soft tissue of P. viridis  $(2.013 \pm 0.667 \text{ mg/kg})$  was comparable to values previously reported by Yulianto *et al.* (2019, 2020a) from the same location, but generally higher than most other studies in Indonesia (e.g., Sijabat *et al.* 2014; Mirawati *et al.*, 2016).

Previous studies reported that Cd, Pb, and Cu concentrations in *A. granosa* from the West Banjirkanal Estuary and in *P. viridis* from the Port of Tanjung Emas were comparable to those observed in the present study (Table 2). However, Cd concentrations in *P. viridis* from this study (Kendal waters) were higher than those recorded in other coastal areas around Semarang (Yulianto *et al.*, 2020; Noviansyah *et al.*, 2021; Muhtaroh *et al.*, 2024) (Table 2). These differences may be explained by site-specific factors such as variations in pollution sources (e.g., port activities, industrial effluents, and domestic discharges), hydrodynamic conditions that affect the dispersion and dilution of contaminants, and the bioavailability of heavy metals in sediments, all of which influence the level of bioaccumulation in mussels.

Variations in heavy metal concentrations in mussels from different locations are common and can be linked to species-specific and site-specific factors. In this study, A. granosa generally showed higher Pb and Cu concentrations than P. viridis, which may be associated with differences in feeding strategies and sediment interaction between the two species. The higher Cd concentration in *P. viridis* at our study site compared to other Semarang waters could be influenced by local inputs from port and industrial activities, combined with limited water circulation that may enhance metal bioavailability. These findings align with Yulianto et al. (2019), who also reported that heavy metal concentrations in mussels varied across species and locations within Semarang waters, indicating that both biological traits and site-specific environmental conditions strongly influence the patterns of heavy metal accumulation.

Table 2. Heavy Metal Concentrations (Cd, Pb, and Cu) in Green Mussels (*Perna viridis*) and Blood Mussels (*Anadara granosa*) from Several Coastal Waters in Semarang and Surrounding Areas

Location	Species	Cd	Pb	Cu	Reference
Semarang and Kendal Coastal	P. viridis	0.130±0.017	2.013±0.667	-	This Study
Waters	A. granosa	=	5.871±0.582	8.051±1.143	
Port of Tanjung Emas Semarang	P. viridis	$0.033 \pm 0.005$	2.268±0.293	2.294±0.274	Yulianto et al. (2020)
Outlet of the Indonesia Power	P. viridis	0.0250±0.0099	0.603±0.066	2.007±0.269	Yulianto et al. (2020)
Plant Semarang					
Estuary of West Banjirkanal	A. granosa	1.710-2.250	4.370-5.550	10.971-14.772	Yulianto et al. (2019)
River, Semarang					
Tambak Lorok Coastal Waters,	P. viridis	0.006-0.128	-	-	Noviansyah et al. (2021)
Semarang					
Morosari Coastal Waters,	P. viridis	0.007-0.049			Noviansyah et al. (2021)
Demak Regency					
Mangunharjo Coastal Waters,	P. viridis	-	0.040-0.200	-	Muhtaroh et al. (2024)
Semarang					
STANDARD		$0.1^{**}$ , $1.0^{*}$ ,	$1.5^{(*)},  0.2^{(**)},$	5(*)	
		1.0***)	1.5(***)		

<sup>\*)</sup> SNI: National Standard Agency, Indonesian National Standard (SNI) No. 7387-2009 concerning the maximum limit or threshold of metal contamination in Food

<sup>\*\*)</sup> NADFC Indonesia - Regulation of the National Agency of Drug and Food Control Indonesia No. 5 / 2018 Concerning Maximum Limits of Heavy Metal Contamination in Processed Foods

<sup>\*\*\*)</sup> MMA Indonesia - Decree of the Minister of Marine Affairs No. KEP.17/MEN/2004 concerning Indonesian Shellfish Sanitation

Smaller-sized specimens of P. viridis and A. granosa generally exhibited higher concentrations of Cd, Pb, and Cu compared to medium- and large-sized individuals (Table 1). This pattern can be explained by their higher metabolic and growth rates, which enhance filtration and uptake of metals (Yap et al., 2009; Boyden, 1974). As bivalves grow, a growth dilution effect reduces metal concentrations per unit tissue mass. At the same time, biokinetic factors such as reduced elimination rates in smaller individuals also contribute to this trend, particularly for Cd and Pb (Wang et al., 2024). In addition, biological variables (e.g., species, age, and reproductive physiology) and environmental conditions (e.g., metal concentrations in water and sediments) further modulate heavy metal accumulation (Ali et al., 2024). Consistent with our findings, a study from Lampung, Indonesia, also reported that three-month-old green mussels (small size) had higher Cd concentrations compared to older individuals (Sartika et al., 2023).

# 4.2. Cadmium (Cd) and Lead (Pb) Accumulation and Depuration in Green Mussel *P. viridis*

A previous study by Yap *et al.* (2003) investigated cadmium (Cd) accumulation and depuration in the soft tissues of *P. viridis*, reporting a reduction in Cd concentration from 9.00 mg/kg to 5.40 mg/kg (40%) and 3.70 mg/kg (58.89%) after 2 and 4 days of exposure to Cd-free seawater, respectively. Although the initial concentrations were much lower in the present study, a comparable trend was observed. Cd levels decreased by 20.508–31.776% after 3 days, 29.780–37.476% after 5 days, and 31.518–41.291% after 7 days of depuration treatment. These findings suggest that *P. viridis* can effectively depurate Cd even at environmentally relevant concentrations.

The observed Cd reduction over the 7-day depuration period suggests that the small-scale depuration system can be effective, particularly in large-sized mussels, which exhibited the highest depuration efficiency (up to 41.291%). These findings are consistent with those reported by Yap *et al.* (2003) and indicate that depuration has the potential to improve food safety by reducing Cd contamination in mussels intended for human consumption.

Cd showed a consistently lower depuration rate than Pb and Cu, suggesting stronger retention within mussel tissues. This aligns with Yap *et al.* (2003), who found that Cd is more tightly bound in most soft tissues of *P. viridis*. The stronger retention is likely due to Cd's high affinity for metallothioneins—cysteine-rich proteins involved in metal detoxification—which form stable complexes that hinder Cd excretion during short-term depuration (George & Olsson, 1994; Amiard *et al.*, 2006; Kang, 2006).

Depuration efficiency also varied by mussel size, with larger individuals showing greater Cd removal. This trend is consistent with earlier reports (Wang *et al.*, 2010) and may reflect physiological differences that influence metal regulation during depuration.

Unlike Cd, Pb and Cu are more loosely bound in mussel tissues, allowing faster elimination during depuration (Bordin *et al.*, 1992; Wang & Rainbow, 2008), which explains the quicker Pb clearance observed in this study.

Depuration treatments in this study were particularly effective in reducing Pb concentrations in *P. viridis*. Levels decreased from an average of  $2.013 \pm 0.667$  mg/kg to  $0.929 \pm 0.266$  mg/kg after 5 days, and further to  $0.730 \pm 0.197$  mg/kg after 7 days. These concentrations are below the standard set by the Indonesian National Standard (SNI) and the National Agency of Drug and Food Control (NADFC),

suggesting improved safety for consumption. Even after just 24 hours of depuration, Pb reduction ranged from 24.329% to 29.740%, depending on mussel size. This agrees with Budiawan *et al.* (2017), who reported that Pb levels in *P. viridis* from Jakarta Bay decreased by 23.971% after 24 hours under a water circulation rate of 500 L/h. Under lower circulation (250 L/h), the maximum reduction (30.047%) occurred within 12 hours.

These findings collectively affirm the potential of small-scale depuration systems in enhancing seafood safety, particularly in regions like the northern coast of Central Java, where heavy metal contamination in coastal waters remains a concern.

# 4.3. Lead and Copper Accumulation and Depuration in Blood Mussel (Anadara granosa)

This study's average Pb concentration in A. granosa  $(5.871 \pm 0.582 \text{ mg/kg})$  exceeded the Indonesian regulatory standards (NADFC, SNI). Such elevated levels are likely linked to strong anthropogenic inputs in urban coastal areas like Semarang. According to NOAA (1998), there is a strong statistical correlation between population density and Pb accumulation in shellfish such as oysters and mussels.

Setiawan *et al.* (2013) reported lower Pb depuration efficiencies in *A. granosa* than in this study. After 5 and 7 days of depuration used aquarium systems with medium volumes of 20 L of clean seawater, Pb concentrations decreased from 0.81 mg/kg to 0.56 and 0.45 mg/kg (30.86% and 44.44% reduction) in small mussels (<2 cm), and from 0.72 mg/kg to 0.44 and 0.32 mg/kg (38.89% and 55.56%) in large mussels (≥2 cm). Umbara and Suseno (2007) found that Pb is firmly bound in the soft tissue of *A. granosa*. However, after one day in pollutant-free seawater, the mussels could excrete 33.70% to 63% of Pb. Extending the depuration period to five days resulted in Pb retention ranging from 10.2% to 38.8%.

Budiawan *et al.* (2017) reported 25.28% and 29.748% of Cu elimination in *A. granosa* from Jakarta Bay after 24 and 12 hours of depuration, using water circulation rates of 500 L/h and 250 L/h, respectively. These results exceed the Cu removal rates in the present study after 24 hours (17.814–21.020%) using a small-scale depuration unit. However, our study's extended depuration for five days resulted in higher Cu removal rates, ranging from 45.995% to 66.057%.

The findings of this study on the removal of Cd, Pb, and Cu from the soft tissue of green mussels (*Perna viridis*) and blood clams (*Anadara granosa*) using a small-scale depuration unit demonstrate the potential of this approach to contribute to seafood safety and support public health protection.

#### **5. Conclusions**

The concentrations of cadmium (Cd), lead (Pb), and copper (Cu) in the soft tissue of *P. viridis* and *A. granosa* from Semarang coastal waters exceeded the standard established in Indonesian regulations. Depuration treatment of *P. viridis* for three days reduced Cd concentrations to below the Standard, indicating potential safety for human consumption. In contrast, Pb levels required five days of depuration to reach the maximum allowable levels set by SNI/NADFC. In *A. granosa*, Pb concentrations also decreased to below the maximum allowable levels after five days, while Cu levels dropped below the Standard after three days. The use of a Small-Scale Depuration Unit demonstrates potential as a practical method for reducing heavy metal contamination in

shellfish, thereby supporting seafood safety and public health protection.

# **Ethics approval**

No permits were required.

# Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

# **Author contributions**

All authors confirm that they have read and approved the final manuscript before submission.

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The authors declare no conflicts of interest.

# **Declaration of competing Interest**

None

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