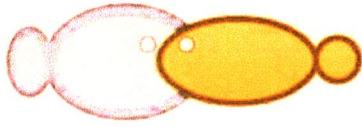


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Bioavailability exchangeable phase of heavy metals in sediments and contamination in shellfish at estuaries on the west coast of South Sulawesi, Indonesia

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Abstract. Knowledge about the availability of heavy metals in sediments is very important for the utilization of marine resources, especially in the bioavailability of exchangeable phase. This phase affects contamination of filter feeder biota such as shells. The aim of this study is to analyze the relationships between heavy metals in the exchangeable phase of sediments and their accumulation in shellfish and their feasibility for consumption. Sampling was carried out in 4 estuarine waters from the west coast of South Sulawesi. This study shows that the bioavailability of heavy metals Pb, Cd and Cu in the estuary waters from the west coast of South Sulawesi is considered low. The highest availability of heavy metals was found in sediments in the waters of the Tallo estuary, followed by the Pangkep estuary. The bioavailability of heavy metals in sediments is as follows $Cd > Pb > Cu$. Heavy metal contamination of shellfish is according to the bioavailability in sediments. The concentration of heavy metals in shellfish is still in the low category, and shellfish are still suitable for consumption.

Key Words: accumulation, bioavailability, *Dosinia*, lead, South Sulawesi.

Introduction. Lead (Pb) and cadmium (Cd) are toxic metals that are naturally present in waters and do not have biological functions for organisms, because they are not essential. Anthropogenic activities, especially the use of additives in gasoline, the paint industry, batteries, increase the concentration of Pb in waters. According to Neff (2002), the majority of lead sources that contaminate water come from the atmosphere, while most of the Cd entering water comes from the Ni-Cd battery industry, the use of phosphate fertilizers and fungicides, the plastics and paint industries (Campbell 2006). With the development of coastal areas, it can be expected that anthropogenic inputs will further increase the concentration of heavy metals Pb, Cd, and Cu in sediments and estuarine waters.

The presence of metals in sediments can have a negative impact on benthic biota and other biota through the food chain. However, total metal concentrations in waters do not always correlate positively with responses that appear in biota (Janssen et al 2003). This is caused by differences in metal species (fractions) which can cause negative impacts on biota. Physiological damage or toxicity response to a compound (pollutant) is usually caused by the availability of biological compounds (bioavailability) (Stauber et al 2005; Cunha et al 2008; Hendozko et al 2010). In marine waters, environmental characteristics greatly affect the bioavailability of heavy metals. The results of the study by Werorilangi et al (2016) showed that the average concentrations of bioavailable fractions of Pb and Cu (fraction 1) was higher in sediments associated with *Enhalus acoroides* compared to sediments without vegetation. Higher Cu concentrations in fraction 1 in sediments are also associated with higher concentrations in *Enhalus* roots.

Estuary waters are very dynamic, differ from one location to another, and have considerable fisheries resource potential. Fish, shellfish and crustaceans are living and developing resources in these waters and are resources used by the public both for consumption and for sale. The assessment of metal contamination risk in shellfish biota

in estuarine waters is particularly important in relation to the presence of heavy metals in bioavailability phase. The risk of accumulation of heavy metals in organisms is directly proportional with the bioavailability of heavy metals in the exchangeable phase.

The aim of this study is to determine the bioavailability and distribution of heavy metals in estuary aquatic sediments, which is important because it can provide information for analyzing heavy metal pollution sources and their effect on shellfish in estuary waters. This information can be used in monitoring and utilizing estuary waters by the government and the community.

Material and Method

Study area and sampling locations. The 4 estuarine waters along the west coast of South Sulawesi have been selected as places to collect shellfish by fishermen. These sites are the estuary waters of Tallo, Maros, Pangkep and Barru (Figure 1). Samples were collected from April to July 2018, to determine the concentration of metal availability in the sediment and heavy metal contamination in shellfish.

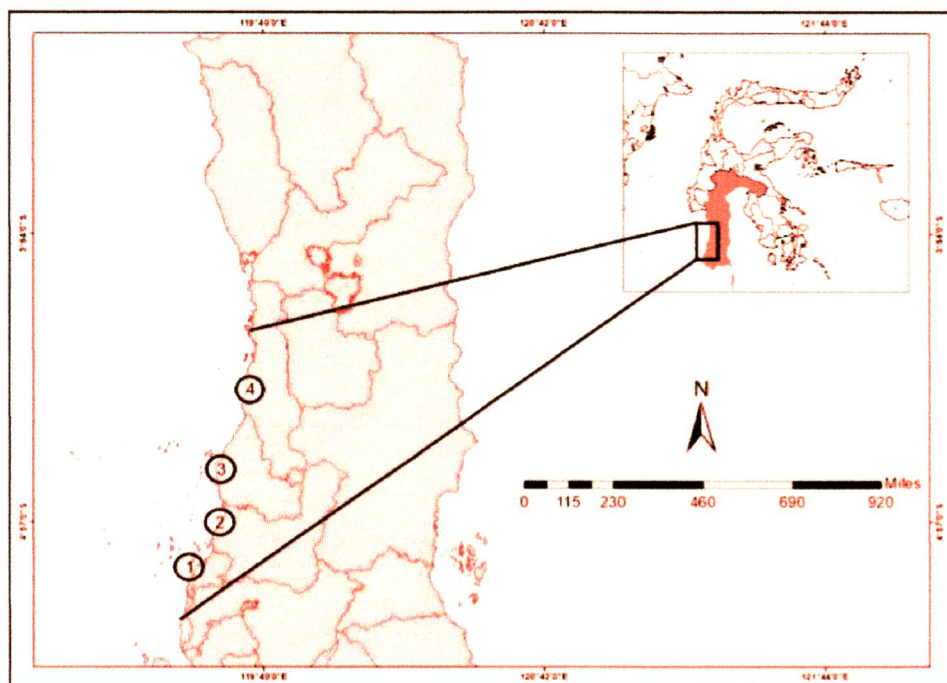


Figure 1. Sampling sites. 1 - Tallo; 2 - Maros; 3 - Pangkep; 4 - Barru.

Sample collection and preparation. Sampling was carried out at distances between 100 and 300 m from the shoreline. At each station, 1 kg of sediment sample was collected using the grab method with four replications. The GPS coordinates were recorded using a Garmin eTrex device. At the same time, samples of shellfish consumed by the population were also collected. The samples were placed in plastic containers. After collection, sediment samples were acidified with nitric acid (pH=2) and transferred to the laboratory of Chemical Oceanography immediately. Temperature, pH, salinity, dissolved oxygen (DO) were measured from studied sites during sampling of sediments with a multiparameter. The parameters of dissolved organic matter (DOM), total suspended solid (TSS), nitrate (NO₃) and phosphate (PO₄) in water were analyzed in the laboratory.

Sediment sample preparation. Sediment samples were collected from the surface of the sediment, namely the oxic part, with a depth of 1-3 cm. This section represents the fraction of sediment that is a bioavailable source of metal for organisms. It is also a biologically important fraction because most animals live and feed in this fraction (Thomas & Bendell-Young 1999). Samples of sediment were filtered in dry state to obtain sediment particles measuring less than 63 µm, namely silt and clay (Loring & Antala 1992; Yuan et al 2004; Hendozko et al 2010). Furthermore, in this section, a total metal concentration and metal concentrations were measured in the ion exchangeable fractions.

The total concentration of each metal in the sediment was determined by extracting sediment by acid destruction using HNO₃ and HClO₄ solvents (SNI 06-06992.8-2004). The concentration of metals in the ion and carbonate fractions that can be exchanged was determined by adding 40 mL CH₃COOH to 1 g of dry sediment (<63 µm) and then shaken for 16 hours (Ure et al 1993).

Preparation of shellfish samples. Before sample preparation is carried out, the species of shellfish were identified based on literature (Gabbi 1999). Next, the shellfish muscles were carefully dissected and removed from shells with a stainless steel instrument. The samples were cleaned, rinsed, dried with paper and freeze dried with a lyophilizer at 80°C for 48 h. Weight was determined using a digital scale with a precision of 0.001 g. Fresh muscle from each individual is also measured with a precision of 0.1 mg. For each sampling location, samples were composite for each species and homogenized.

Metal analysis was carried out following the method described by Belinsky et al (1996). Approximately 5 g of homogeneous muscle was weighed and inserted in a 50 mL tube, washed with HNO₃ and heated (two replications per sample). 8 mL of 70% nitric acid (Fisher Scientific, Trace Metal Grade) were added to each tube. The tube was closed with a glass condenser and the sample was dissolved at room temperature for 8 h at 120°C. The digests were cooled at room temperature and a mixture of 25 mL was obtained by adding deionized water. The final concentration was dissolved in nitric acid (22% v/v).

Sample analysis. The concentration of metals in sediments and shellfish was analyzed using an Atomic Absorption Spectrophotometer (Hitachi-Z 2000 Tandem Flame/Furnace AAS) with a limit of detection (LOD) for each metal as follows: Pb - 0.006 ppm; Cd - 0.001 ppm; Cu - 0.006 ppm.

Bioconcentration factor (BCF). The estimation of the bioaccumulation of heavy metals in the muscle of shellfish in respect to their extractable concentrations in associated sediments was studied using the following equation of the bioaccumulation factor (Hendozko et al 2010):

$$BCF = C_{Shell} / C_{Sediment}$$

Where C_{Shell} and C_{Sediment} are the average concentrations of each metal (mg kg⁻¹) in an organism and the labile extracted fraction (F1) in each sediment substrate, respectively. The BCF value provides information about the relative ability of shellfish organisms to absorb and/or extract selected heavy metals from the sediments where they live.

Statistical analysis. The difference in concentration of heavy metals in sediment between stations was analyzed using a one-way ANOVA and subsequent LSD pairwise comparisons (SPSS v.22). Characteristic parameters in each station related to metal bioavailability and its contamination in shells are explained using principle component analysis (PCA) (XLSTAT Version 2015.5.01.22537).

Results and Discussion. The values of physicochemical parameter of seawater from the 4 stations with four replications are presented in Table 1.

Table 1
Physico-chemical parameters (mean±standard error) of estuary waters of west coasts of South Sulawesi

| Parameters | Tallo | Maros | Pangkep | Barru |
|---------------------------------------|---------------|---------------|--------------|--------------|
| Salinity (‰) | 27.5±1 | 28.5±1 | 29±0 | 30.6±0.5 |
| Temperature (°C) | 30 | 25.5±9.22 | 31±1.15 | 24,5±1.1 |
| pH | 6.2±0.16 | 6.5±0.15 | 5,8±0.39 | 7.2±0.21 |
| O ₂ (mg L ⁻¹) | 5.5±0.6 | 5.2±0.8 | 4.1±0.9 | 4.9±1.4 |
| DOM (mg L ⁻¹) | 51.375±14.483 | 48.975±23.044 | 47.1±24.199 | 33.575±8.265 |
| TSS (mg L ⁻¹) | 56.043±7.995 | 42.689±1.356 | 38.549±4.282 | 24.187±12.41 |
| NO ₃ (mg L ⁻¹) | 0.024±0.005 | 0.039±0.015 | 0.027±0.017 | 0.02±0.006 |
| PO ₄ (mg L ⁻¹) | 0.02±0.01 | 0.022±0.014 | 0.014±0.003 | 0.03±0.006 |

Note: O₂ - dissolved oxygen; DOM - dissolved organic matter; TSS - total suspended solid.

The chemical and physical conditions of the estuary waters of the west coast of South Sulawesi are similar. DOM and TSS values are high in the Tallo and Maros estuary waters. This is in line with the increasing intensity of development in this region, especially land reclamation.

Sediment texture. The composition of sediment samples from the research stations is presented in Figure 2. Sand type sediments predominate in the Tallo and Barru estuaries, while the Maros and Pangkep estuaries have predominantly clay type sediments.

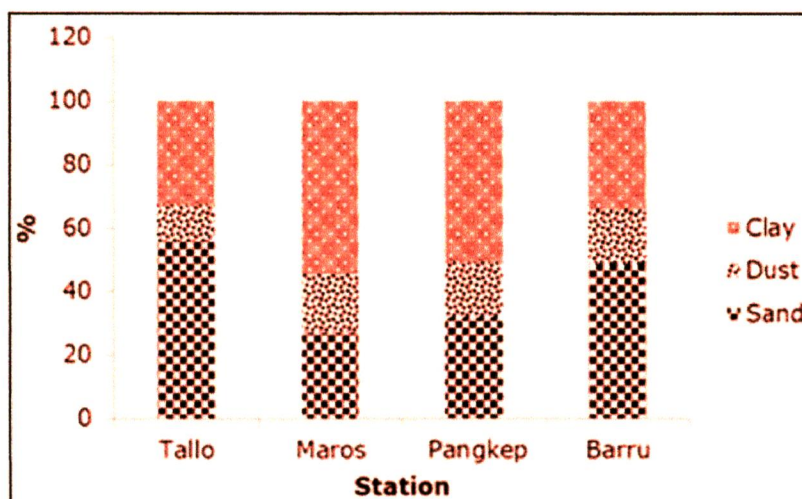


Figure 2. Sediment texture in west coastal South Sulawesi sediments.

The dominance of sand sediment types in the Tallo and Barru estuaries comes from rock erosion in the river basin, which consists of hills, while in the Maros and Pangkep areas it consists of agricultural and aquaculture areas.

Concentration of metals in water. The concentration of total metals in the waters in the 4 sampling stations from west coast, South Sulawesi are presented in Figure 3. Figure 3 shows high concentrations of Pb and Cu in the Tallo estuary waters. Low concentrations were found in Barru estuary waters. The high concentrations have exceeded seawater quality standards for aquatic organisms. Meanwhile, concentrations of Cd are low in all estuary waters.

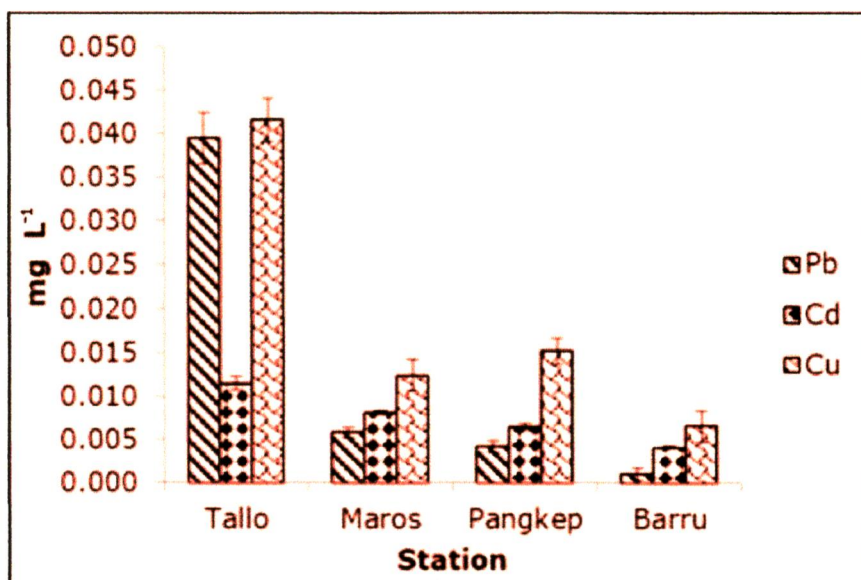


Figure 3. Heavy metal concentrations measured in the waters of west coast, South Sulawesi.

The ANOVA test results showed significant differences ($P > 0.05$) in heavy metal concentrations in waters between stations. High concentrations are found in Tallo estuary waters. Cu and Pb have high concentrations in the Tallo estuary. The concentrations of Pb, Cd and Cu in Maros, Pangkep and Barru estuaries are low.

Concentration metals in sediment. The concentration of total metals in the sediment from 4 estuary waters of west coast, South Sulawesi, is presented in Figure 4.

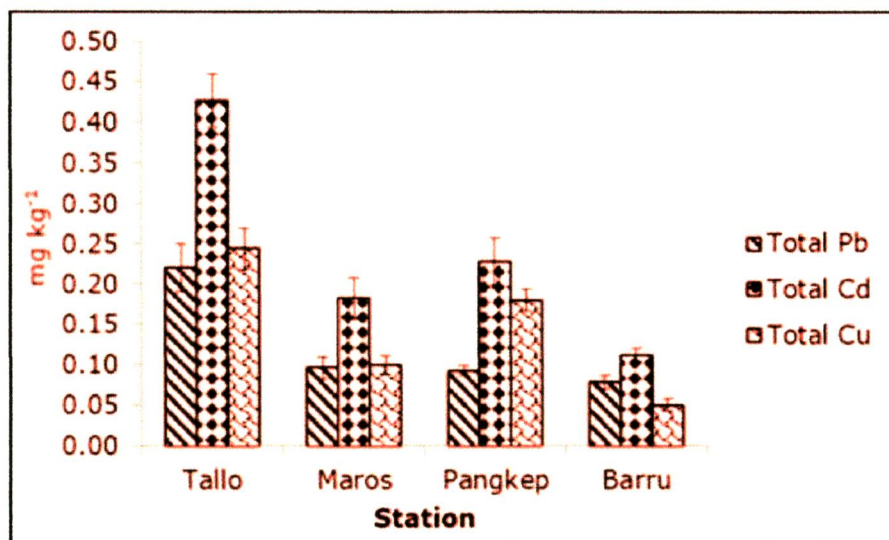


Figure 4. Total metal concentrations measured in west coastal South Sulawesi sediments.

Figure 4 shows the total concentration of Pb, Cd and Cu in the sediments of the estuary waters of the west coast of South Sulawesi. Cd has the highest concentration. The highest concentration of Cd was found in the Tallo estuary waters and the lowest in Barru estuary sediments. The ANOVA test results showed differences between stations in the total concentrations of Pb, Cd and Cu in sediments ($P > 0.05$). The highest concentration is found in the Tallo estuary, while the lowest is found in the Barru estuary. The concentration order of metals is the following: $Cd > Cu > Pb$. Based on the quality standards of US NOAA (Turki 2007), the concentrations are categorized as very low, under the Effect Range Low.

Labile fraction of metals. The concentrations of Pb, Cd and Cu in the exchangeable phase (F1) are presented in Figure 5. The highest concentration of labile phase of heavy metals was found in the estuary waters of Tallo River, while the lowest was found in the estuary waters of Barru River.

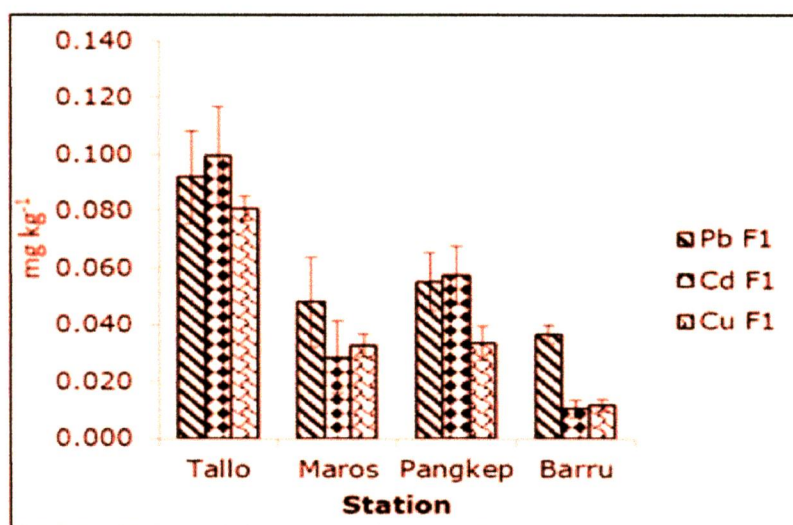


Figure 5. Labile fraction (F1) of Pb, Cd and Cu concentrations measured in the sediments of west coast, South Sulawesi.

The ANOVA test results showed significant differences between stations in the availability of heavy metals ($P > 0.05$). Availability of heavy metals in the estuary sediments are the highest in Tallo estuary, followed by Maros, Barru and Pangkep. The highest are Cd and Pb in Pangkep and Barru, while in Cu metals are the lowest Tallo. Figure 6 shows the proportion of metal fraction 1 (high availability), which is a small portion of the total metal measured.

Concentration metals in shellfish. 4 species of economically important shellfish consumed by residents in the estuary region of the west coast of South Sulawesi were found, namely *Polymesoda* sp., *Dosinia* sp., *Anadara* sp., and *Meretrix* sp. The concentration of heavy metals in the tissue of several shellfish obtained from the estuary waters of the west coast of South Sulawesi is presented in Figure 6.

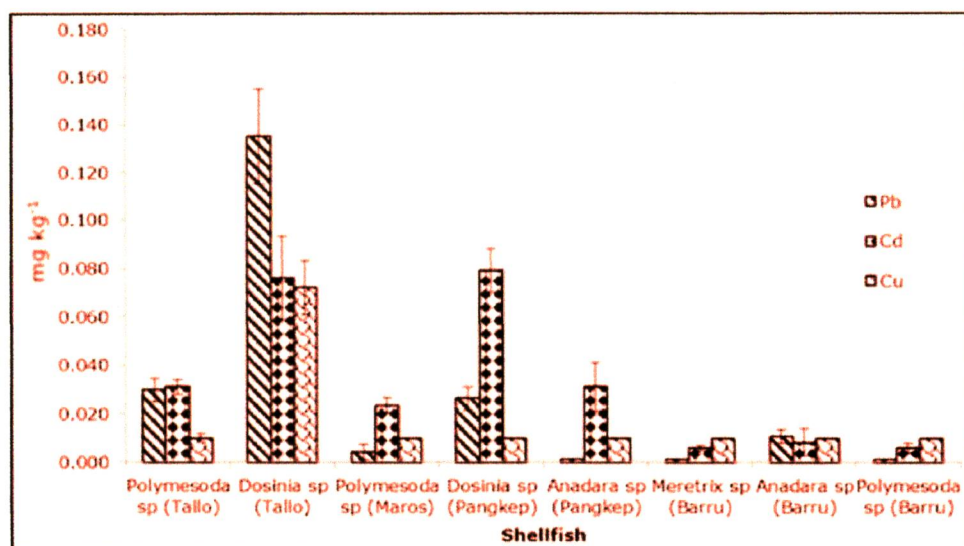


Figure 6. Content of metals (mg kg^{-1} dry weight); mean \pm standard deviation.

Dosinia sp. from Tallo estuary waters contains high concentrations of heavy metals. In general, the shellfish in the estuaries have been contaminated with Pb, Cd and Cu. Heavy metal contamination has a lower concentration in shellfish from Maros and Barru estuaries.

Bioconcentration factor (BCF). The bioconcentration of heavy metals in shellfish are presented in Table 2. Some shellfish have a BCF higher than 1, being identified in all estuaries, especially for Cd. *Dosinia* sp. has the ability to accumulate more Cd than other species in the tissue. BCF values indicate that several types of shellfish originating from the west coast estuaries of South Sulawesi have accumulated heavy metals from sediments with BCF values higher than 1. The highest BCF value was found in *Dosinia* sp. in the estuary waters of Pangkep and Tallo.

Table 2
Bioconcentration factor (BCF) for the shellfish tissue analyzed with fraction sediment

| Shellfish | Station | Pb | Cd | Cu |
|-----------------------|---------|------|------|------|
| <i>Polymesoda</i> sp. | Tallo | 0.76 | 0.79 | 0.25 |
| <i>Dosinia</i> sp. | Tallo | 3.4 | 1.9 | 1.8 |
| <i>Polymesoda</i> sp. | Maros | 0.76 | 0.01 | 0.80 |
| <i>Dosinia</i> sp. | Pangkep | 6.3 | 18.9 | 2.4 |
| <i>Anadara</i> sp. | Pangkep | 0.2 | 7.4 | 2.4 |
| <i>Meretrix</i> sp. | Barru | 1.0 | 6.0 | 10.0 |
| <i>Anadara</i> sp. | Barru | 10.8 | 8.3 | 10.0 |
| <i>Polymesoda</i> sp. | Barru | 1.0 | 6.0 | 10.0 |

Based on available metal fraction data (Figure 5) and BCF value (Table 1), the type of shellfish *Dosinia* sp in the Tallo estuary accumulated Pb, Cd and Cu metals far greater than shellfish in other estuaries.

Relationship between concentrations of heavy metals in shellfish tissue with labile fractions and total metals. The relationship of the concentration of Pb, Cd and Cu in the shells with the labile fraction in the sediment are presented in Figure 7.

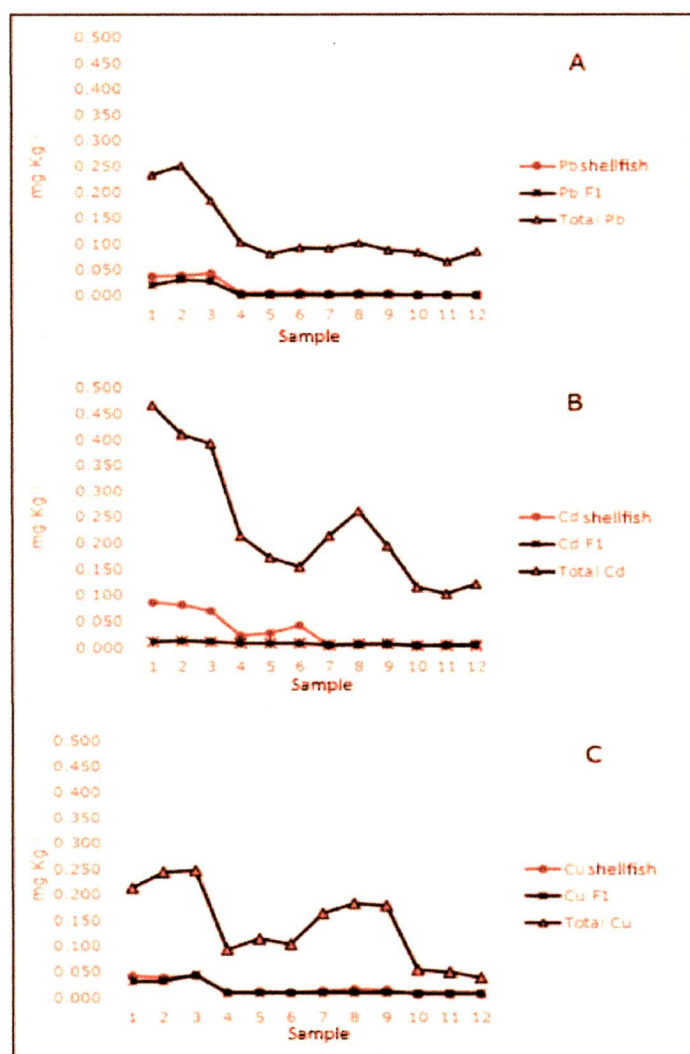


Figure 3 Relationship between concentrations of metals in shellfish tissue with labile fractions and total metals in the estuary sediments of the west coast of South Sulawesi. A - Pb; B - Cd; C - Cu.

High levels of Cd in sediments both in the form of total metals and in the form of F1 labile fraction are highly correlated with the Cd metal content in the shellfish tissue. This situation is different from the Pb and Cu situations, where only a slight increase in the F1 labile fraction is accumulated in the shellfish tissue. F1 Cd fraction in sediments is closely related with the metal content in the shellfish. An increase in the F1 Cd fraction in the sediment significantly increases the Cd metal content in the shellfish. Figure 7 shows that shellfish originating from the estuary waters of the west coast of South Sulawesi have been contaminated with heavy metals. However, the concentration of Pb, Cd and Cu in shellfish tissue is still relatively low. From the shellfish identified, *Dosinia* sp. has the highest metal concentration. From a food safety perspective, the shellfish originating from the west coast estuaries of South Sulawesi are still safe for consumption, with metal concentrations not exceeding 1.5 mg kg^{-1} Pb, or 1 mg kg^{-1} Cd (Republic of Indonesia Food and Drug Supervisory Agency 2018).

Parameters characteristics in each station. The physico-chemical parameters from each station are presented in Figure 8.

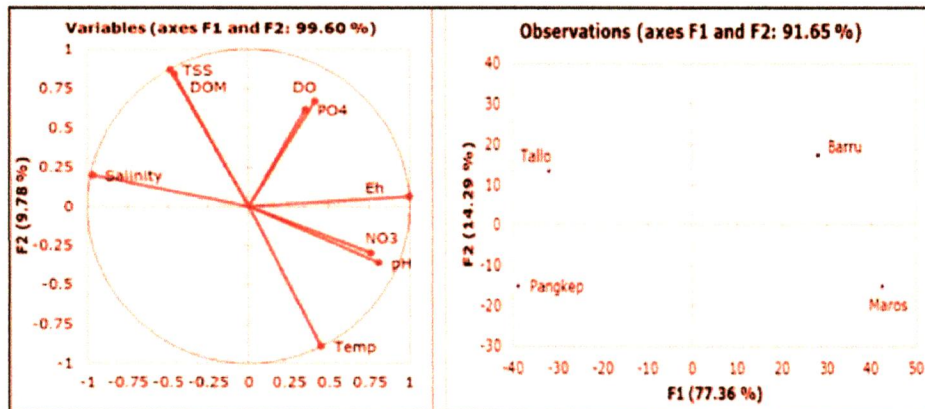


Figure 8. Principal component analysis of physico-chemical parameters from the study sites.

The PCA graph illustrates that the high concentrations of Cd, Pb and Cu accumulated in the shellfish of Tallo estuary are strongly influenced by the presence of TSS and DOM.

Conclusions. The availability of heavy metals Pb, Cd and Cu in the estuary sediments of the west coast of South Sulawesi is still very low. The BCF value shows that the shellfish accumulated Pb, Cd and Cu, especially *Dosinia* sp. The labile fraction of heavy metals affects metal accumulation in shellfish tissue, especially Cd. The high metal accumulation in the shellfish of Tallo estuary is strongly influenced by the presence of total suspended solids and dissolved organic matter in the water.

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