

Metals in seawater, sediment and *Padina australis* (Hauck, 1887) algae in the waters of North Sulawesi

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Abstract. Metals in North Sulawesi waters can originate from mining and other anthropogenic activities or natural causes. The objective of the study was to examine the presence of arsenic (As), copper (Cu), zinc (Zn) and mercury (Hg) in the water, sediment and thallus of brown alga *Padina australis* in several waters of North Sulawesi, like Totok Bay, Manado Bay, Talawaan Bajo and Likupang waters. Sample analyses were conducted for seawater, sediment and marine algae from the sea sampling point, in two dates during dry season and rainy season. The analyses followed American Public Health Association (APHA) and United States Environmental Protection Agency (USEPA) recommendations using ICP-OES (Inductively Coupled Plasma)-(Optical Emission Spectrometry). Metal concentration in the seawater from all sampling points was the same for both dates. The metal concentration in the sediment from the four sampling points ranges from 1 to 3 ppm for As, 9 to 26 ppm for Cu, 17 to 131 ppm for Zn, and 0.05 to 3.2 ppm for Hg. The metal concentration in the *P. australis* thallus in the four sampling points is between 1.1-19.5 ppm for As, 0.5-4.4 ppm for Cu, 1.2-19.9 ppm for Zn and 0.003-0.758 ppm for Hg.

Key words: Likupang Waters, Manado Bay, Metal, *Padina australis*, Talawaan Bajo Waters, Totok Bay.

Introduction. North Sulawesi Province is one of the regions that naturally holds sufficient high mineral content. Due to this, many mining companies conduct their work in several areas of North Sulawesi, such as in East Likupang, East Bolaang Mongondow, Ratatotok and Bitung. There are also many mining activities managed by local people, but these activities do not seem to have a performant waste management program. Surface soil exploitation for public mining activities has occurred without taking into account of the environmental impact. The environmental pressure can impact on several species of algae that play very important roles for marine productivity, such as *Halimeda* spp., *Gracilaria* spp. and *Dictyota dichotoma* (Kepel et al 2018a).

Manado city, which is a center for economic activities, is also a waste disposal site for households, small-medium industries, and other urban activities. Various sources of pollutants from either mining activities or urban activities can stress the algae growth and development. Macroalgae or microalgae in aquatic ecosystems can reduce pollutants. They can even be employed as city waste treatment agents (Thorin et al 2017) and pollution indicators of the water (Sudharsan et al 2012).

Metals that do not occur in the metabolism of the macroalgae have become toxic materials to many plant species. Studies on metal pollutants entering waters are a current issue, because there are several metals deriving from mining activities and entering the waters (Smolders et al 2003; Mantiri et al 2018). Mining activities have ecological impact on the macroalgal communities in the littoral zone (Vasquez et al 1999; Nasprianto et al 2019).

Several studies demonstrate that metals appear in the water and are absorbed by algae. Siahaan et al (2015) found that the thallus of alga *Caulerpa serrulata* from Totok Bay, Southeast Minahasa, held 0.2 ppm of mercury (Hg) and *Halimeda macroloba* has

0.11 ppm of Hg. The root of mangrove *Avicennia marina* from Bahowo waters, North Minahasa, contains 0.0435 ppm of lead (Pb). Mantiri et al (2018) indicate the presence of metals in the sediment and thallus of algae from Totok Bay, with a concentration of 19 ppm for arsenic (As), 10.9 ppm for chromium (Cr) and 12 ppm for copper (Cu), respectively. Thallus of *Halimeda opuntia* was also detected to hold 6.5 ppm of As, 0.7 ppm of Cr and 2.3 ppm of Cu. Kepel et al (2018b) also found 4.8 ppm of As in *Ulva* sp. from Totok Bay.

Although heavy metals are in relatively low concentrations in the sediment, they are not easily degraded and can be adsorbed and biologically accumulated by marine algae (Darmono 1995). The presence of high heavy metal concentrations in the water endanger aquatic organisms, from small changes like metabolism inhibition to mortality (Vangronsveld & Clijsters 1994). Phytoremediation is a set of processes that use plants to remove, transfer, stabilize and destroy organic/inorganic contamination in ground water, surface water and soil. Algae are relatively easy to cultivate and have 4-6 weeks life cycle. This study aims to examine the presence of As, Cu, Zn and mercury (Hg) in the water, sediment and thallus of brown algae (*Padina australis*) in several waters of North Sulawesi.

Material and Method

Description of study sites. The study sites were Totok Bay, South Minahasa, Manado Bay, Manado Municipality, Talawaan Bajo and Likupang, North Minahasa, North Sulawesi (Figure 1). The sampling locations were selected based on different economic activities in the area. Totok Bay, the estuary of Totok river, was selected because of the sediment rich in Hg, element used in mining. The environmental pollution of Totok Bay is present in the form of suspended particles in the water column and sea bottom sediment. The pollution is reflected by the turbid brown color in rivers and coastal waters. The area is also surrounded by a sufficiently dense residential area and mangrove forest. Manado is the capital of North Sulawesi Province, being a coastal front city. Manado Bay has a coastal ecosystem with fringing reefs in the rainy season. It can have relatively high sedimentation that influences the water transparency. Talawaan Bajo is a residential area, but is also presents extensive mangroves, seagrass and macroalgae ecosystems. In this area, the water flow of Talawaan river is transporting sediments from mining activities from upstream, resulting in water turbidity. Likupang waters was a control location. It has dense mangroves and seagrass ecosystems and a high enough number of macroalgae species. The sampling location was selected afar from the residential area and river.

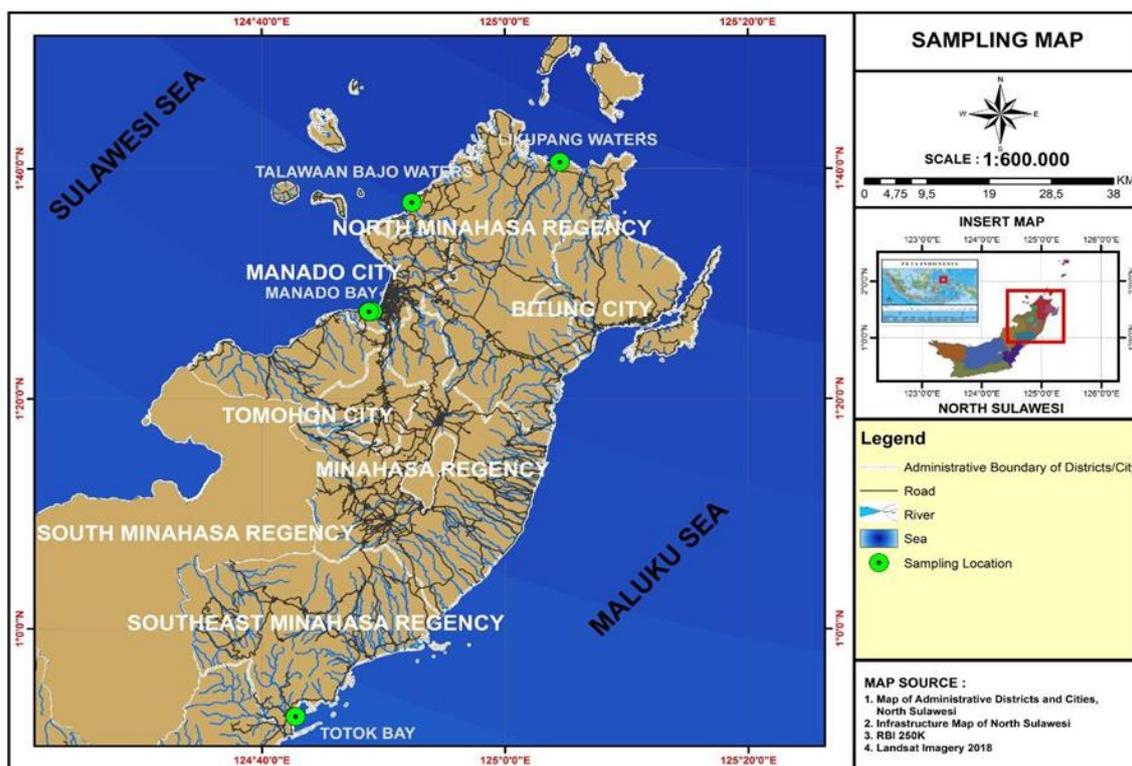


Figure 1. Study sites.

Sampling. The sampling period expanded from the dry season (first period/I) of June, 2018 to the rainy season (second period/II) of October, 2018. Brown macroalgae (*Padina australis*) was collected. The macroalgae was identified following Calumpang & Meñes (1997) and Trono (1997). *Padina australis* has a large thallus, light brown and whitish color in part due to light calcification. *P. australis* was collected from the four study sites. The species grows attached to rocks or dead corals, in the intertidal and subtidal areas. Plastic polyethylene sample containers were prepared by the Water Laboratory Nusantara (WLN) and placed in a cool box for transportation. Macroalgae were sampled and prepared directly at study sites by removing them from the holdfast, cleaning the sand, mud or dirt attached and placing them into the containers. Ten grams of alga were needed for each metal analysis. Sediment was also collected from where the alga was taken. It was collected using a PVC pipe plugged to the sea bottom and the sediment was placed in containers. For metal analysis, 10 g of sediment were needed. Seawater was collected in a glass container, from a depth of 30 – 40 cm below the water surface. As much as 100 ml of seawater was needed for metal analysis. The seawater was previously filtered through 0.45 µm filter paper, then mixed with HNO₃ and Ammonium Pyrrolidine Dithiocarbamate (APDC).

Sample Analysis. Metal content in the seawater, sediment and thallus of *P. australis* was analyzed following the American Public Health Association (APHA) (2012) and United States Environmental Protection Agency (USEPA) (2011) protocols. Metal detection was done using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry). The metal content in the seawater was compared with the standard quality concentrations established in the Environmental Minister's Decree number 51/2004, while the sediment concentration of metal was compared with the standard of Canadian Council of Ministers of the Environment (CCME) (2002). As and Hg concentrations were compared with the concentrations from the Indonesian National Standard (SNI No. 7387 – 2009). Cu and Zn concentrations have no national standard qualities yet.

Results and Discussion

Metal concentration in seawater. Metal content analyses determined that seawater from Totok Bay, Manado Bay, Talawaan Bajo and Likupang had the same metal concentrations in the dry season (I) and rainy season (II): As <0.0005 ppm, Cu <0.005 ppm, Zn <0.005 ppm and Hg <0.00005 ppm (Figure 2).

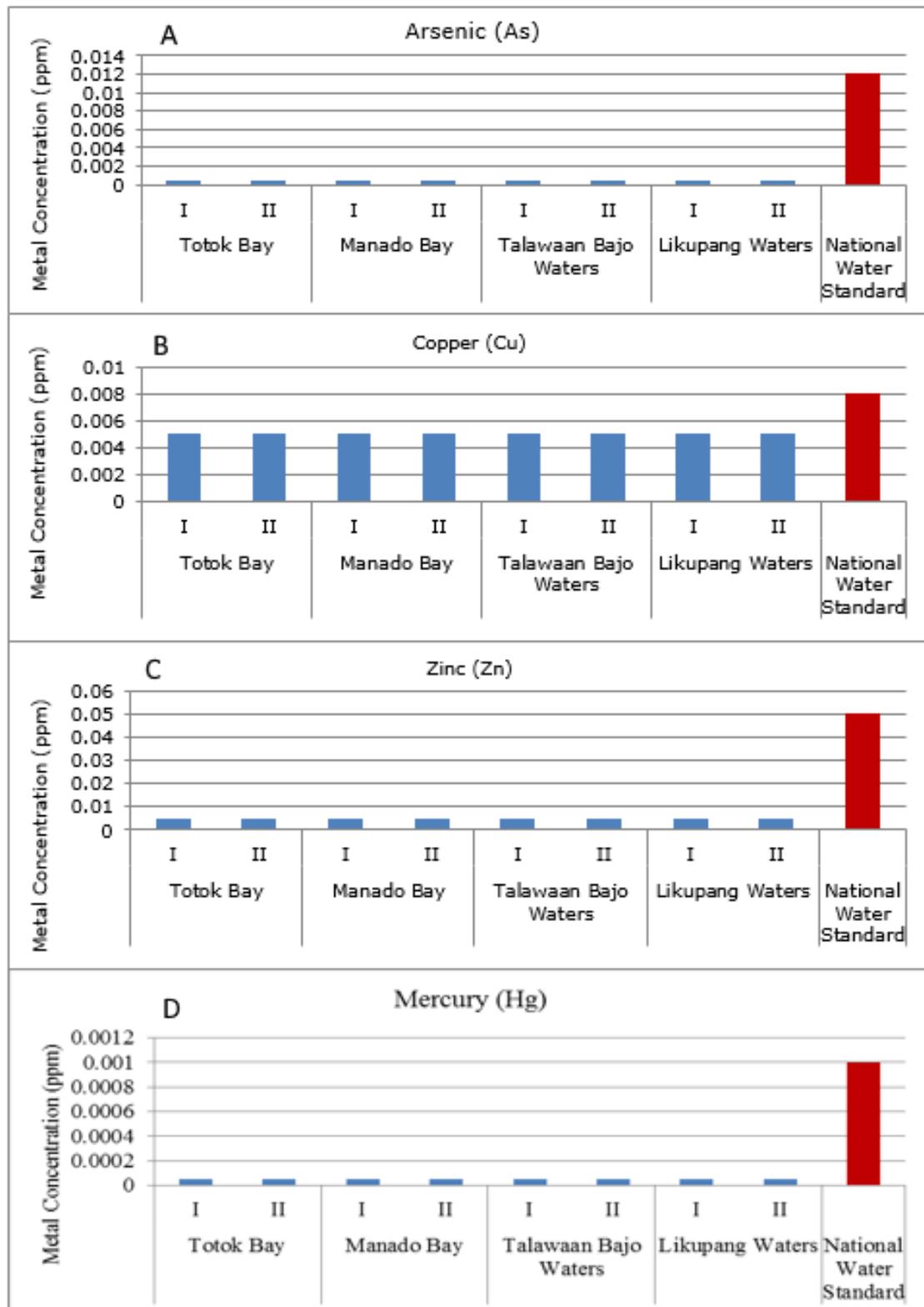


Figure 2. Metal concentration in seawater (A-D).

As, Cu, Zn and Hg concentrations in the four study sites did not change from dry season to rainy season and were below the quality standard of Environmental Minister's Decree numbered 51/2004.

Metal concentration in the sediment. In Totok Bay, As values of 30 ppm and 20 ppm were found in the sediment, for the dry season and rainy season, respectively. The values are higher than the standard quality of Canadian Council of Ministers of the Environment/CCME (2002), which is 7.24 ppm, while Manado Bay contained <1 ppm and 3 ppm, Talawaan Bajo had <1 ppm and <1 ppm and Likupang waters had 2 ppm and 1 ppm, below the standard quality of CCME. Cu concentration was 14 ppm and 11 ppm in Totok Bay, below the CCME standard of 18.7 ppm, while Manado Bay had higher Cu concentration (26 ppm) than the standard quality in the dry season, but lower (9 ppm) than the CCME standard in the rainy season. In Talawaan Bajo and Likupang waters, the concentrations were below the standard quality, 9 ppm and 15 ppm in the dry season, but above the standard in the rainy season, 21 ppm and 20 ppm (Figure 3).

Zn concentrations in Totok Bay were 63 ppm and 53 ppm, for dry season and rainy season, respectively, below CCME standard of 124 ppm, while Manado Bay had a Zn content of 131 ppm in the dry season, higher than the standard, and below the standard in the rainy season, with a value of 47 ppm. Talawaan Bajo waters had lower Zn concentrations than the CCME standard, 79 ppm in the dry season and 52 ppm in the rainy season. Likupang waters presented similar concentration in both seasons, 17 ppm, below the standard. The standard quality of Hg is 0.13 ppm. Manado Bay had Hg concentrations of <0.05 ppm in the dry season, but it rose to 0.2 ppm in the rainy season. Totok Bay had also higher concentrations than the standard in both seasons, 3.2 ppm and 1.55 ppm. Hg concentrations in Talawaan Bajo waters were 0.11 ppm in the dry season and 0.51 ppm in the rainy season. Likupang waters had lower concentrations than the CCME standard in both seasons, <0.05 ppm.

Metal concentration in *Padina australis*. As concentrations in the thallus of alga *P. australis* in Totok Bay were 19.5 ppm in the first period and 15.4 ppm in the second period. In Manado Bay, the concentrations were 1.1 ppm and 1.8 ppm. In Talawaan Bajo, the As concentrations were 1.3 ppm and 2.1 ppm and in Likupang waters they were 3.8 ppm and 2.6 ppm. The values are above the quality standard of SNI No. 7387-2009, which is 1 ppm. Cu concentrations in *P. australis* for the first sampling date and the second sampling date were 4.4 ppm and 3.4 ppm in Totok Bay, 3.4 ppm and 1.7 ppm in Manado Bay, 2 ppm and 2.1 ppm in Talawaan Bajo and 1.2 ppm and 0.5 ppm in Likupang waters. Zn concentrations in *P. australis* in Totok Bay were 19.9 ppm and 12.9 ppm, in Manado Bay 15 ppm and 11.1 ppm, in Talawaan Bajo 8.1 ppm and 4.3 ppm and in Likupang waters 2.6 ppm and 1.2 ppm (Figure 4).

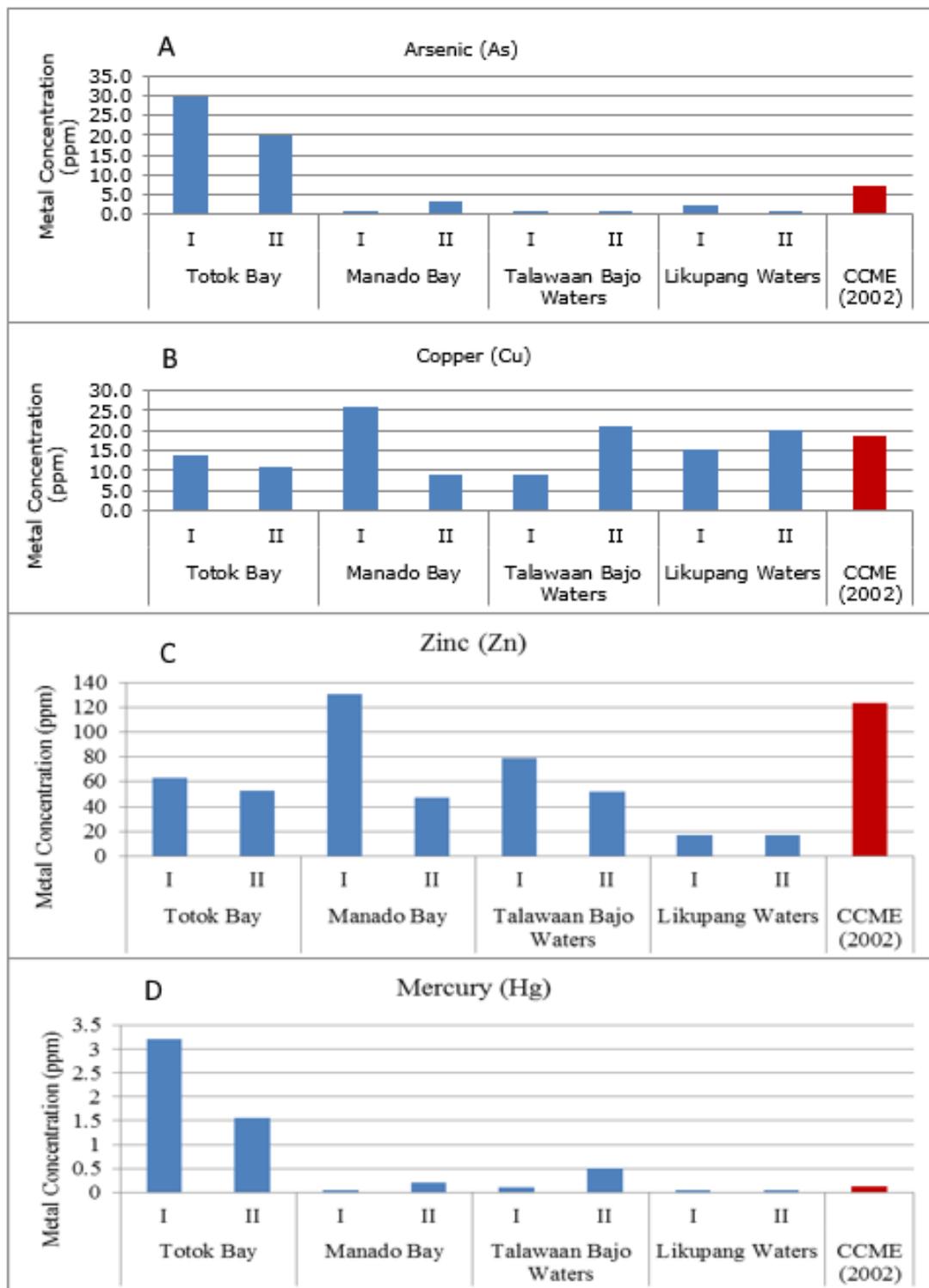


Figure 3. Metal concentration in sediment (A-D).

Hg concentrations in *P. australis*, of 0.758 ppm and 0.731 ppm in Totok Bay and 0.07 ppm in Talawaan Bajo in the second period, were above the quality standard of SNI No. 7387-2009, which is 0.03 ppm, while Manado Bay, Likupang waters and Talawaan Bajo the concentrations in the first sampling date had lower values than the SNI standard. As and Hg contents in the thallus of *P. australis* in Totok Bay were higher than the concentrations determined in Manado Bay, Talawaan Bajo and Likupang waters.

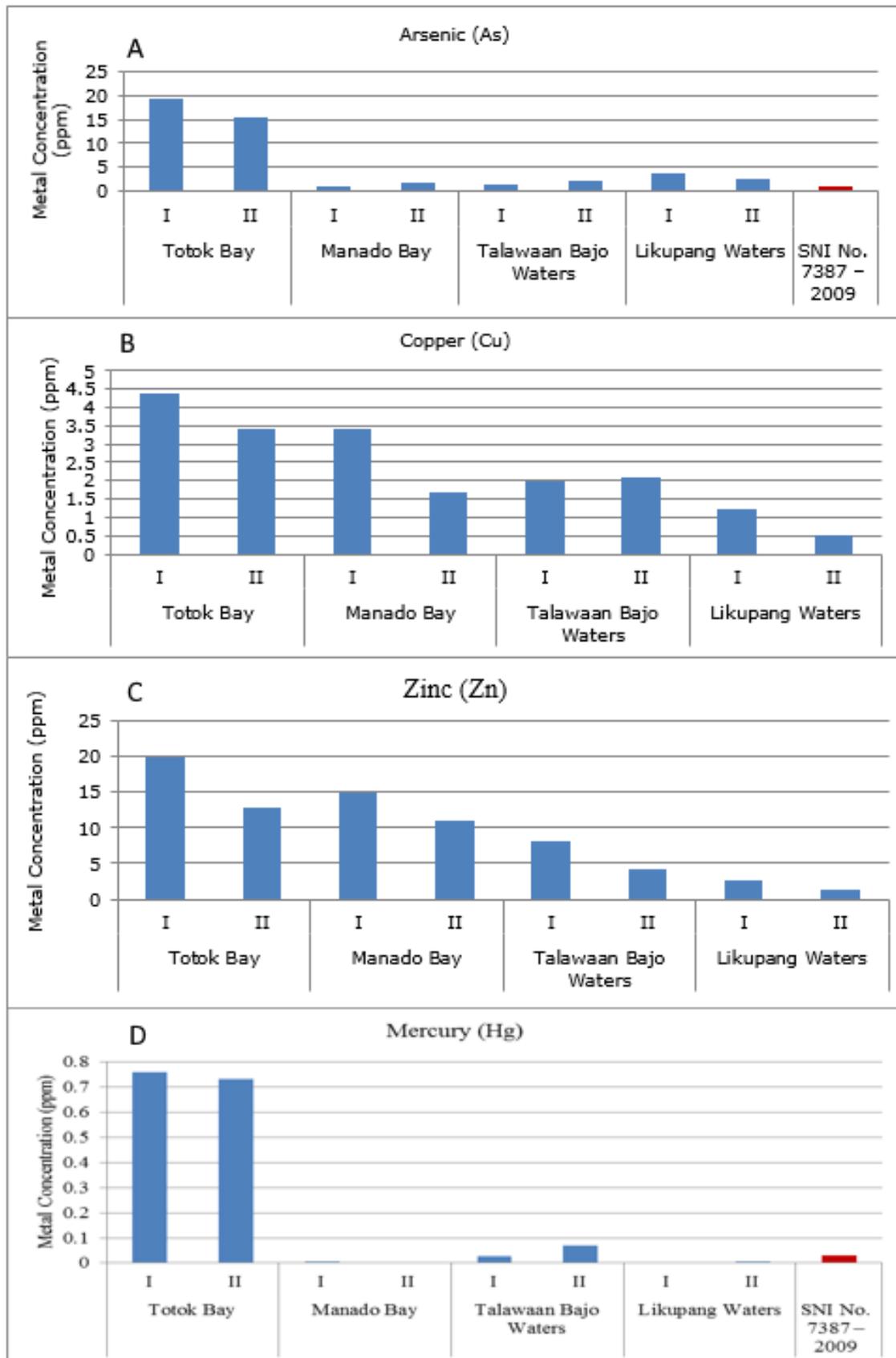


Figure 4. Metal concentration in the thallus of *P. australis* (A-D).

Metal occurrence in dissolved and particulate forms in the water has very different dynamics in each geochemical environment (Cullen et al 2001). The impact of mining, industries and other anthropogenic activities can result in the accumulation of metal in the water. The present study found that the metals Zn, Cu, As and Hg are present in 4 study sites and 2 different time periods. However, the concentrations are below the ICP-OES detection limit and occurred far below the national standard of 0.001 ppm. In a previous study (Mantiri et al 2018) in Totok Bay and Blongko waters, As and Cu concentrations were below the national water standard, while Cr had higher values than the standard.

Metal concentration input from terrestrial activities through the river into marine ecosystems is highly influenced by local oceanographic factors that can affect the dilution and distribution of the metal content. Metal content in North Sulawesi waters is not influenced by seasons. Low heavy metal concentrations in the four sampling sites imply various processes, including tidal current transportation, dilution, association with suspended matter, coagulation and sedimentation to the bottom, association with sediment organic matter and plankton absorption (Siregar 2009; Ikenaka 2010).

In the sediment of Totok Bay and Likupang waters in the dry season, As values were higher than in the rainy season. Manado Bay and Talawaan Bajo waters had no change in As concentration. A similar pattern also occurs for Cu and Zn concentrations in the sediment of Manado Bay, in which they are higher in the dry season than in the rainy season and the highest among the study sites. Zn concentrations in Totok Bay, Manado Bay and Talawaan Bajo waters were also higher in the dry season compared with the concentrations from the rainy season, but Zn concentrations in Likupang waters did not change. Hg concentrations in Manado Bay and Talawaan Bajo in the rainy season were higher than in the dry season. In Totok Bay and in Likupang waters there were no observed changes. Metal concentrations in the water always changes with time, since the river parameters are very labile, changes always occurring in currents, waves, rainfalls and disposal water intake that affects the metal concentration in the water (Taftazani 2007). In the rainy season, metal concentrations are lower from dilution, while in the dry season they are higher from water evaporation (Darmono 1995).

As and Hg concentrations in Totok Bay were much higher than in Manado Bay, Talawaan Bajo and Likupang waters.

High concentrations of metals occur from disposals of small-scaled gold mining activities in Rataotok, that release the tailing into the Totok river flowing to Totok Bay. Mawikere et al (2015) found that in February 2014, 778 gold processing equipment along the road from Rataotok village to the forest were recorded. All these are employed to mill the gold-containing rocks and these activities produce the tailing that is directly disposed into the river.

High Hg concentrations in the sediment of Totok Bay can result from gold mining activities. Talawaan Bajo waters, with gold mining activities on land, was also found having high Hg concentration. Nasprianto et al (2019) found higher Hg concentrations in the sediment from Totok Bay, 2.68 ppm, compared with the concentrations from the sediment from Blongko waters, <0.05 ppm, the latter being far from residences and relatively less polluted. It is clear that high Hg concentrations in Totok Bay are highly affected by the use of Hg in gold processing.

Metal detection in several study sites reflects metal deposition from the water column to the sea bottom as sediment. Thus, the sea basin could become a sink of various pollutants from the land or atmosphere (Clements 1992). Different concentrations in several points indicates a dissimilarity of the source from the terrestrial area that yields metal pollutant into the waters. Observations made in Manado Bay show that Zn and Cu concentrations were higher than in the mining areas, such as Totok Bay. It is known that there is no mining activity around Manado Bay and, therefore, the presence of Zn and Cu in the sediment of this area is natural or by domestic sewage. Cu concentrations in Totok Bay and Blongko waters was <1 ppm, while the sediment held much higher Cu in Totok Bay than in Blongko waters, 12 ppm compared with <1 ppm. It could mean that Cu in Totok Bay came from metal involved during the mining process (Mantiri et al 2018).

High Cu concentrations could result in biomagnification in aquatic organisms. Cu transfer mechanisms occurs through the cell wall in which Cu is bound by proteins and polysaccharides, so that Cu^{2+} in toxic form becomes a non-toxic compound (Lobban & Harrison 1994).

Cu and Zn were also detected in the thallus of *P. australis*, demonstrating metal absorption capacity of *P. australis*. Zn and Cu are essential metal elements for algal growth, in certain amounts. Some probable functions of Cu are electron transportation in photosynthesis (plastocyanin) and enzymes (amine oxidase). A probable function of Zn in seaweeds is the carbonic anhydrase of enzymes (Lobban & Harrison 1994). The occurrence of several unnecessary metals in the metabolism can give deleterious effects at cellular level and inhibit the enzymatic activity (Colovic et al 2018).

High As and Hg concentrations in the thallus of *P. australis* at Totok Bay are proportional to the concentrations from the sediment. The higher As and Hg concentrations in the sediment are, the higher the absorption rate in the algae *P. australis* is. In Manado Bay, Hg was not detected in algae and relatively very low concentrations were found in the sediment.

Algae have high ability to absorb metals or heavy metals since there are functional groups in the cytoplasmic cell wall of the alga that are able to bind the metal ions, like carboxyl, hydroxyl, amine, sulfhydryl, imidazole, sulphate and sulphonate (Bachtiar 2007). According to Jahan et al (2004), heavy metal accumulation occurs through passive reaction or biosorption, and the process is faster than that of active reaction or bioaccumulation. Studies of Kepel et al (2018b) and Mantiri et al (2018) indicate that metals in the sediment and algae thallus can be detected with Transmission Electron Microscopy (TEM) and in *Ulva* sp. and *Halimeda opuntia*, they occur in the cell wall.

Observations on essential metals, such as Cu and Zn, or hazardous metals, such as As and Hg, reflect the ability of the algae to express the metal concentrations in the water. Chmielewska & Medved (2000) stated that algae *Cladophora* sp. could be used as a bioindicator for water contamination by either nutrients or heavy metals and as a metal phyto-remediator in the water. TEM analysis found that algae *P. australis* in Totok Bay live and grow throughout the year, even though metals occur in the thallus. The occurrence of metals in the sediment could also promote the metal transfer from the sediment to the thallus. As *Sargassum* sp. (Figueira et al 1999) and several other species have been tested for their capacity of heavy metal biosorption (Bulgariu & Gavrilescu 2015; Zeraatkar et al 2016), *P. australis* is another potential heavy metal bioremediation.

Conclusions. As, Hg, Cu, and Zn concentrations in the seawater of Totok Bay, Manado Bay, Talawaan Bajo and Likupang presented relatively the same values, which were lower than the quality standard for the rainy season and the dry season. Zn and Cu contents in the sediment of Manado Bay were higher in the dry season than in rainy season, as well as higher than in the other study sites. Manado Bay did not have mining activity and metal concentration naturally occurred and from domestic sewage. Hg and As concentrations had the highest values in Totok Bay, in both seasons, probably due to gold mining disposals. Likupang waters, a sampling site far from the residential area, has relatively low metal concentrations. Algae have a sufficiently high ability to absorb metals from sediment, since As, Hg, Cu and Zn were recorded in the thallus of *P. australis* collected from the four study sites, despite good morphological qualities. Thus, *P. Australis* could become a phyto-remediator in metal-containing waters.

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