Metal content of *Gracilaria changii* originating from different seaweed cultivation areas in South Sulawesi, Indonesia

[1,3,4]Inayah Yasir, [2,4]Zainuddin, [1,4]Syafiuddin, [2,3,4]Joeharnani Tresnati, [3]Risal Aprianto, [1,3,4]Ambo Tuwo

[1] Marine Science Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia; [2] Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia; [3] Multitrophic Research Group, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia; [4] Center of Excellence for Development and Utilization of Seaweed, Hasanuddin University, Makassar, Sulawesi Selatan, Indonesia. Corresponding author: A. Tuwo, ambotuwo62@gmail.com

Abstract. Food safety is one of the crucial issues in aquaculture. The metal waste from various activities on land can enter the waters and accumulate in aquaculture products, such as seaweed. This study aimed to examine the metal content of seaweed *Gracilaria changii*. This study covered three marine areas around South Sulawesi, namely the Gulf of Bone, the Flores Sea and the Makassar Strait, while seaweed samples were taken from four seaweed cultivation locations (Regencies of Bone, Sinjai, Takalar and Pangkep). The heavy metal concentrations analyzed were copper (Cu), cadmium (Cd), and lead (Pb). The results showed that Cu concentrations in *G. changii* were greater than that in the sea water, but the difference was not significant ($P>0.05$). Cd concentrations in *G. changii* were significantly greater than that in the sea water ($P<0.05$), while the Pb concentrations in the seaweed were smaller than in the sea water, but not significantly different ($P>0.05$). *G. changii* was able to accumulate metals, but its accumulation ability was inconsistent. The Cu, Cd and Pb may accumulate or be released back into the surrounding sea water, which is an advantage from a food safety point of view. Despite the accumulation of Cu, Cd and Pb in *G. changii* thallus, these metals are likely to be released back during the *G. changii* processing as food or when products are stored as food or feed.

Key Words: inconsistent bioaccumulation, heavy metals, copper, cadmium, lead, food safety.

Introduction. Seaweed farming is a central issue in the Ocean Vision 2050, turning millions of people around the world to become marine farmers. Seaweed is a future mainstay in terms of food security, income generation, environmental health (Fatima et al 2018) and a source of renewable energy or biofuel (Wadi et al 2019). From the food security perspective, seaweed is a future protein source. On average, seaweed contains 10% protein lipid, which is 3% of its dry weight. The content of protein and lipids can meet the needs of livestock. Seaweed have also been known for a long time as a polysaccharide source (Venkatesan et al 2015; Melanie et al 2020; Mulyani et al 2021) and pigments (Aryee et al 2018; Ma et al 2019). In environmental health science, seaweed is known as an environmental mitigation agent. In addition, seaweed contains 2% nitrogen by dry weight or the equivalent of 18% of the nitrogen entering the sea and 0.2% phosphorous by dry weight or the equivalent of 61% of the phosphorous that enters the sea (Bjerregaard et al 2016).

Indonesia is the second largest country as a producer of cultured seaweed. In 2015, Indonesia produced cultured seaweed of 11.3 million tons of wet weight or about 38% of the worlds cultured seaweed products. Meanwhile, China produces 14 million tons of wet weight or about 47% of the world's cultured seaweed products (Fatima et al 2018).

In 2015, out of 11.3 million tons of cultured seaweed production in Indonesia, 10.1 million tons were *Eucheuma* sp., and 1.2 million tons were *Gracilaria* sp. *Eucheuma* sp. and *Gracilaria* sp. are red algae. *Eucheuma* sp. is widely cultivated in the coastal area, and *Gracilaria* sp. is widely cultivated in brackishwater ponds. *Gracilaria* sp. is the third most cultivated species in the world with a production of 3.9 million tons, after *Eucheuma* sp. (10.2 million tons), and Japanese kelp (8 million tons) by dry weight (Fatima et al 2018).

Gracilaria changii is a species of *Gracilaria* that is cultivated in Indonesia. Its molecular analysis shows that what has been called *G. verrucosa* is actually *G. changii* (Arbit et al 2019). *G. changii* is a commercially important agarophyte producer in the Pacific waters.

Gracilaria sp. is very nutritious, it contains vitamins and minerals such as vitamin A (β-carotene), B1, B2, B6, B12, C, calcium, phosphorus, potassium, sodium, iron, and iodine (MacArtain et al 2007; Škrovánková 2011; Nawi 2015) and it contains about 16-45% agar (Nawi 2015). This important nutritional content makes the food safety aspect of *G. changii* very important, since it is widely used in the beverage, food, cosmetics and medicinal industries. Agar functions as a thickener in soups, jelly, ice cream mixture and anmitsu (Japanese) (Khalil et al 2018; Nawi 2015).

Metals are harmful to health. Therefore, metal content in food is generally one of the several aspects used in determining food safety. Studies on metal content in *G. changii* are still focused on the island of Java (Afiah et al 2019; Tega et al 2019), although Sulawesi is also known as a *G. changii* producing area. This study is needed to ensure that *Gracilaria* sp. from Indonesia is safe for health, by analyzing the metal content of *G. changii* originating from different seaweed cultivation areas in South Sulawesi, Indonesia.

Material and Method. The study was performed in three seaweed cultivation areas around South Sulawesi, namely the Gulf of Bone, the Flores Sea and the Makassar Strait. Samples were taken fresh from *G. changii* aquaculture ponds, in the coastal areas of Bone, Sinjai, Takalar and Pangkep Regencies (Figure 1).

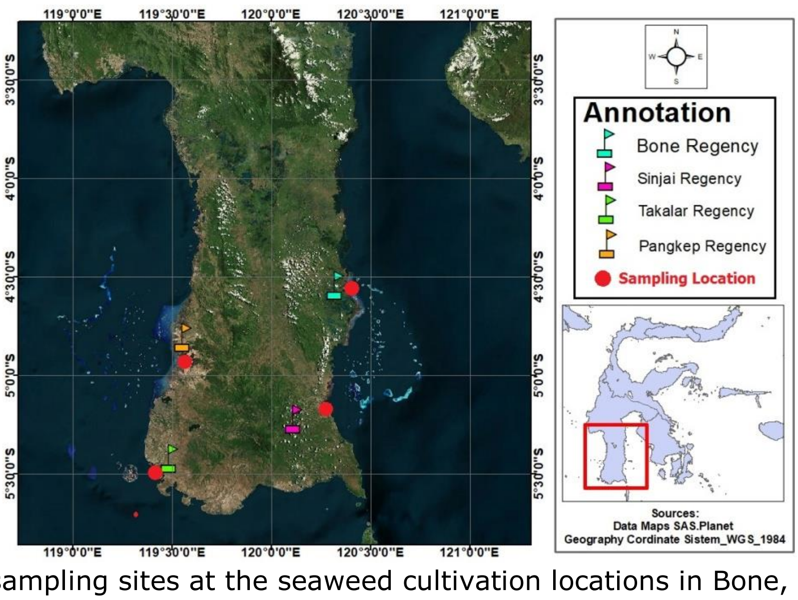

Figure 1. The sampling sites at the seaweed cultivation locations in Bone, Sinjai, Takalar, and Pangkep Regency.

The fresh seaweed was taken to the Multitrophic Research Group Laboratory, Hasanuddin University, to be washed for removing dirt and epiphytes before drying. Drying was carried out in two steps; the first step was drying under the sun with a temperature of 32-33°C for two days, followed by the second step, which was drying in an oven at a temperature of 50°C for 48 hours. The dried sample was then ground using a blender until fine. The metal content of Copper (Cu), Cadmium (Cd) and Lead (Pb) was then quantified using the Atomic Absorption Spectrophotometry Technique (Gosh & Singh 2005; Cui et al 2007).

Results

Copper (Cu). The Cu content in seawater was <0.001-0.162 ppm, with an average of 0.073±0.053 ppm, while the Cu content in *G. changii* was <0.001-5.670 ppm, with an average of 1.339±2.095 ppm. This study indicates that Cu concentrations in the *G. changii* seaweed were greater than in the seawater but not significantly different (P>0.05).

The concentration of Cu in *G. changii* is inconsistent. At the sampling stations in Takalar and Pangkep regencies, the concentration in the thallus is higher than in the surrounding waters, which indicates the possibility of accumulation. However, at the other stations (Bone and Sinjai Regencies), the concentration in the thallus is lower than in the seawater (Table 1).

Table 1

Copper (Cu) concentration in seawater and thallus of *Gracilaria changii* that were taken from the cultivation areas of Bone, Sinjai, Takalar and Pangkep Regencies

Regency	*Cu Seawater (CuSw) (ppm)*	*Cu G. changii (CuGc) (ppm)*	*Δ (CuGc-CuSw) (ppm)*	*Annotation*
Bone	0.071	0.030	-0.041	Non-accumulation
Bone	0.100	0.050	-0.050	Non-accumulation
Bone	0.056	0.040	-0.016	Non-accumulation
Mean±STD	0.076±0.022	0.040±0.010	0.036±0.018	Non-accumulation
Sinjai	0.162	0.020	-0.142	Non-accumulation
Sinjai	0.106	<0.000	-0.106	Non-accumulation
Sinjai	0.049	0.030	-0.019	Non-accumulation
Mean±STD	0.106±0.056	0.017±0.015	0.089±0.063	Non-accumulation
Takalar	0.109	0.580	0.471	Accumulation
Takalar	0.130	0.460	0.330	Accumulation
Takalar	0.097	0.690	0.594	Accumulation
Mean±STD	0.112±0.017	0.577±0.115	0.465±0.132	Accumulation
Pangkep	<0.001	5.670	5.669	Accumulation
Pangkep	<0.001	4.740	4.739	Accumulation
Pangkep	<0.001	3.760	3.759	Accumulation
Mean±STD	<0.001±0.000	4.723±0.955	4.722±0.955	Accumulation

The correlation curve of Cu concentration in seawater and in the thallus shows that the Cu concentration in *G. changii* did not increase consistently with the increase of Cu concentration in the seawater (Figure 2). Correlation curves of Cu concentrations between *G. changii* and seawater from all regencies did not show any progressive accumulation. The Cu concentrations in the thallus of *G. changii* at four sampling locations were still below the safe limit for health (30 ppm) (BSN 1995).

Cadmium (Cd). The data show that, in general, there is an accumulation of Cd in the thallus of *G. changii*. The Cd content of seawater was between <0.001-0.301 ppm with an average of 0.142±0.111 ppm, while in the thallus it was 0.120-2.250 ppm with an average of 0.830±0.767 ppm. This study indicates that Cd concentrations in the *G. changii* seaweed were significantly greater than in the seawater (P<0.05). The accumulation of Cd in the thallus of *G. changii* was observed at all sampled stations, except for one replication at Sinjai Regency (Table 2).

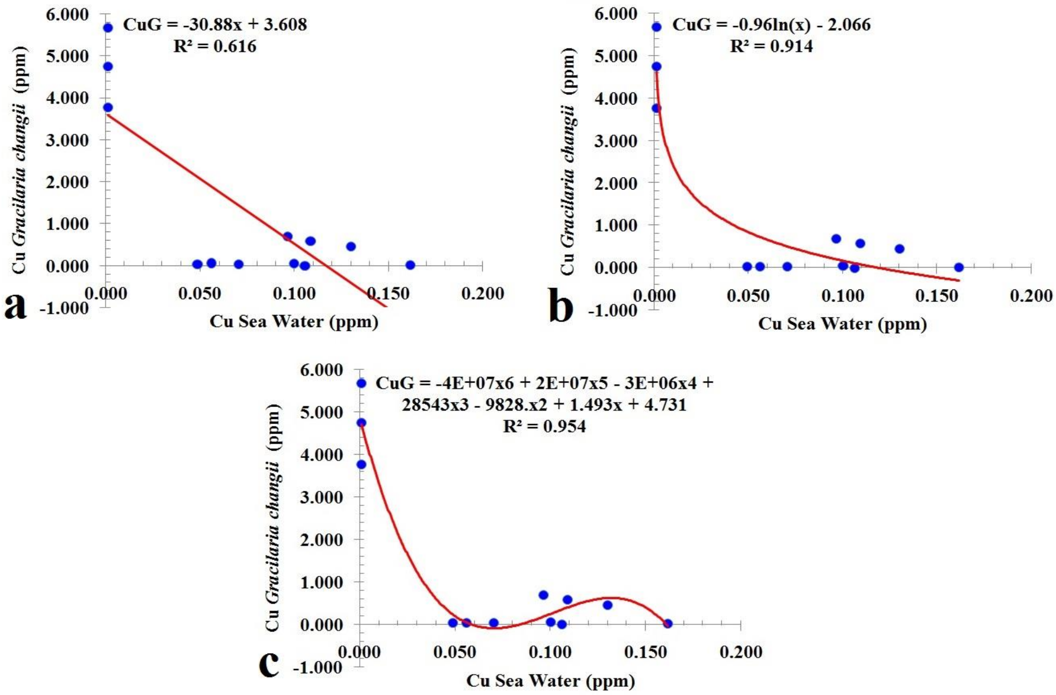

Figure 2. The correlation curve of Cu concentration in seawater and the thallus of *Gracilaria changii* from four sampling sites (at Bone, Sinjai, Takalar, and Pangkep Regencies). Linear Equation (a), Power Equation (b) and Polynomial Equation (c).

Table 2

Cadmium (Cd) concentration in *Gracilaria changii* that were taken from four different sites at Bone, Sinjai, Takalar and Pangkep Regencies

Regency	*Cd Seawater (CdSw) (ppm)*	*Cd G. changii (CdGc) (ppm)*	*Δ (CdGc-CdSw) (ppm)*	*Annotation*
Bone	0.046	0.120	0.075	Accumulation
Bone	0.115	0.470	0.355	Accumulation
Bone	0.151	0.420	0.269	Accumulation
Mean±STD	0.104±0.054	0.337±0.189	0.233±0.144	Accumulation
Sinjai	0.283	0.610	0.327	Accumulation
Sinjai	0.261	0.150	-0.111	Non-accumulation
Sinjai	0.301	0.510	0.210	Accumulation
Mean±STD	0.282±0.020	0.423±0.242	0.142±0.227	Accumulation
Takalar	0.177	0.260	0.084	Accumulation
Takalar	0.156	0.440	0.284	Accumulation
Takalar	0.216	0.810	0.594	Accumulation
Mean±STD	0.183±0.030	0.503±0.280	0.321±0.257	Accumulation
Pangkep	<0.001	1.950	1.949	Accumulation
Pangkep	<0.001	2.250	2.249	Accumulation
Pangkep	<0.001	1.970	1.969	Accumulation
Mean±STD	<0.001±0.000	2.057±0.168	2.056±0.168	Accumulation

Despite the accumulation, the correlation curve of the Cd concentration in seawater and in the thallus did not show any strong correlation (Figure 3). No progressive accumulation can be seen in the correlation curves of the Cd concentration between seawater and the thallus of *G. changii*. Based on the SNI 7387:2009 (BSN 2009), the cadmium concentrations in the thallus of *G. changii* at the four sampling locations have exceeded the limit allowed for the human health safety (0.2 ppm).

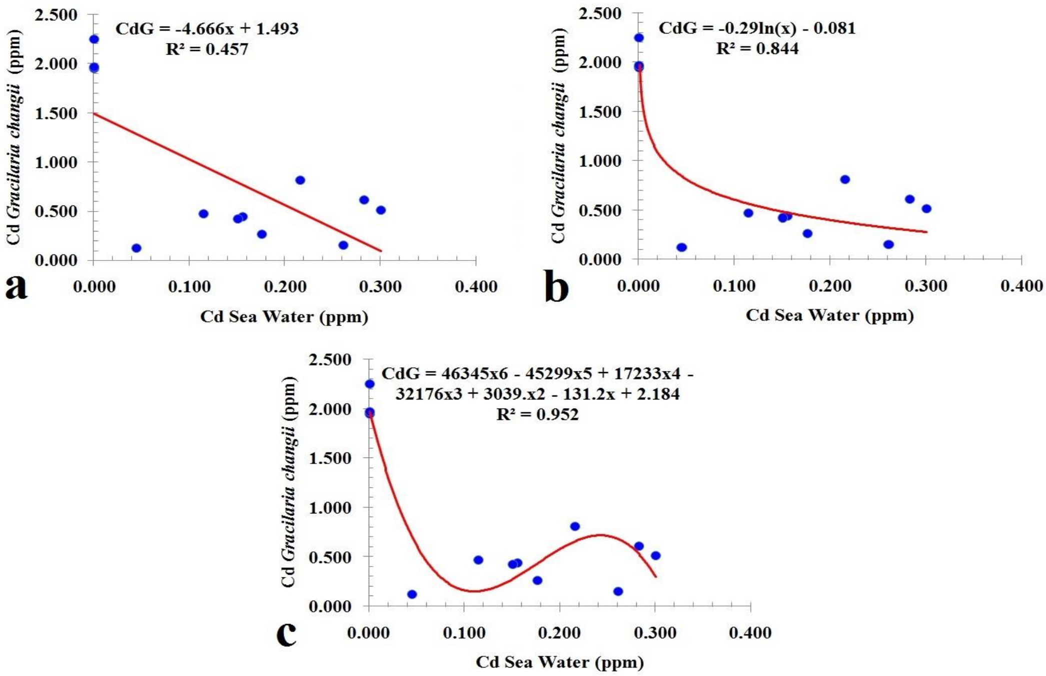

Figure 3. The correlation curve of cadmium concentration in seawater and the thallus of *G. changii* from four sampling sites (at Bone, Sinjai, Takalar, and Pangkep Regencies). Linear Equation (a), Power Equation (b), and Polynomial Equation (c).

Lead (Pb). In general, there was no indication of Pb accumulation in *G. changii*. Although the Pb concentration in the thallus of *G. changii* was lower than in the seawater, the difference was not significant (P>0.05). The Pb content in seawater was <0.001-2.604 ppm with an average of 0.816±0.904 ppm, while the Pb content in the thallus was <0.001-0.830 ppm with an average of 0.320±0.265 ppm. All stations showed no Pb accumulation in *G. changii*, except for one replication in Takalar Regency and two others at Bone Regency (Table 3).

Table 3

Lead (Pb) concentration in *Gracilaria changii* at four sampling sites of Bone, Sinjai, Takalar, and Pangkep Regencies

Regency	*Pb seawater (PbSw) (ppm)*	*Pb G. changii (PbGc) (ppm)*	*Δ (PbGc-PbSw) (ppm)*	*Annotation*
Takalar	0.716	0.430	-0.286	Non-accumulation
Takalar	1.022	0.200	-0.822	Non-accumulation
Takalar	0.280	0.440	0.161	Accumulation
Mean±STD	0.673±0.373	0.357±0.136	-0.316±0.492	Non-accumulation
Bone	1.993	0.660	-1.333	Non-accumulation
Bone	0.162	0.430	0.268	Accumulation
Bone	0.318	0.440	0.122	Accumulation
Mean±STD	0.824±1.015	0.510±0.130	-0.314±0.885	Non-accumulation
Sinjai	1.993	0.830	-1.163	Non-accumulation
Sinjai	2.604	0.210	-2.394	Non-accumulation
Sinjai	0.705	0.200	-0.505	Non-accumulation
Mean±STD	1.767±0.969	0.413±0.361	-1.354±.959	Non-accumulation
Pangkep	<0.001	<0.001	0.000	Non-accumulation
Pangkep	<0.001	<0.001	0.000	Non-accumulation
Pangkep	<0.001	<0.001	0.000	Non-accumulation
Mean±	<0.001±0.000	<0.001±0.000	0.000±0.000	Non-accumulation

The correlation curve showed that the Pb concentration in *G. changii* did not increase consistently with the Pb concentration in the water (Figure 4). The Pb concentrations of *G. changii* at the four sampling locations exceeded the safe limit for health (0.5 ppm) (SNI 7383:2009).

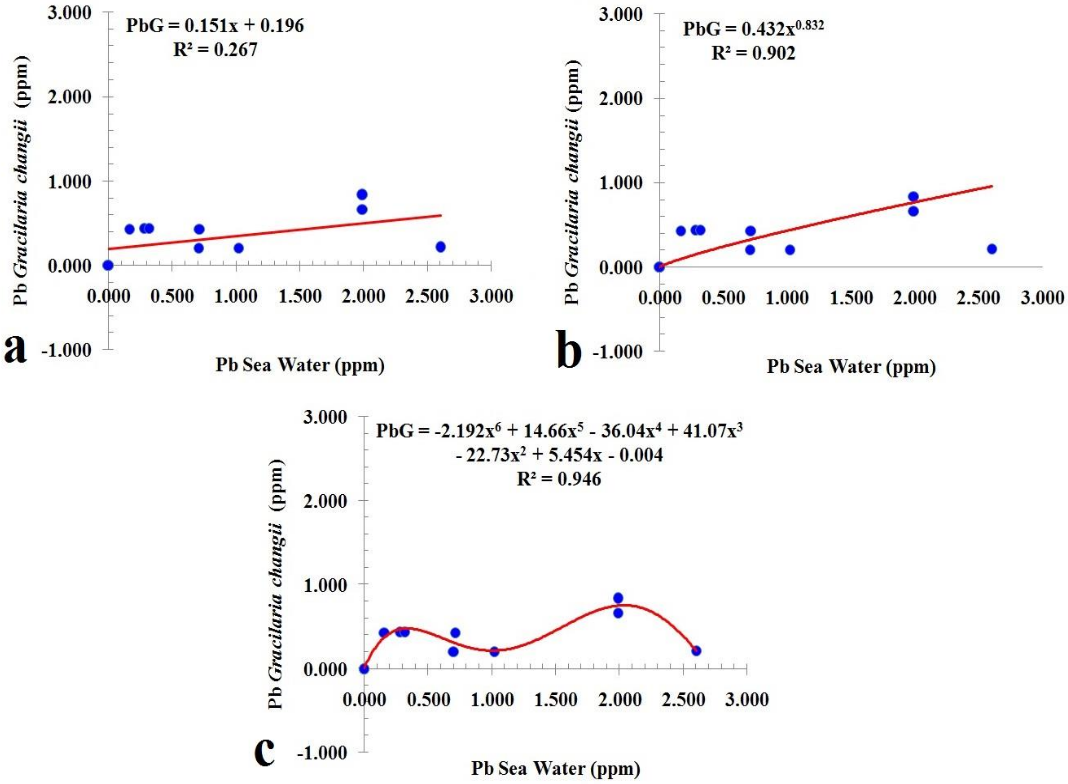

Figure 4. The correlation curve of lead concentration in seawater and in the thallus of *Gracilaria changii* from four sampling sites (at Bone, Sinjai, Takalar and Pangkep Regencies). Linear Equation (a), Power Equation (b) and Polynomial Equation (c).

Discussion. Biologically, heavy metals can disrupt the photosynthetic process of macroalgae in the form of a decrease in the ability to absorb solar energy (Küpper et al 2002). Heavy metals can be very reactive and toxic to organisms. The toxic effects of heavy metals are related to the production of reactive oxygen species (ROS). At high or acute levels of metal pollutants, damage to algal cells occurs when the level of ROS exceeds the cells' ability to cope. Ecologically, macroalgae accumulate heavy metals at a lower or chronic level and can spread them to organisms of other trophic levels, such as mollusks, crustaceans, and fish (Pinto et al 2003). In the blue-spotted ray *Dasyatis kuhlii*, a Pb concentration of 0.2 ppm has caused hypertrophy in the gills (Tresnati & Djawad 2012). Such an accumulation can endanger life in the higher food chains because mollusks, crustaceans and fish are important sources of animal protein for humans. Previous studies have shown that algae have the potential to accumulate heavy metals with concentrations that fluctuate from time to time, from one location to another (Topcuoğlu et al 2003; Afiah et al 2019) and even between different utilization zones.

The ability of *Gracilaria* to absorb and accumulate metals in its thallus had been reported for the first time by (Kang & Sui 2010). Since that time, research on this subject, using *Gracilaria* or other species of macroalgae, went increasing. A research on the *Ulva lactuca* living on the Turkish Coast of the Black Sea, conducted between 1998-2000, showed its potential for accumulation with a variable content. At the site station in these waters, *U. lactuca* contained Cu of 2.53±0.09 to 13.8±0.05 ppm, Cd 0.10±0.10 to <0.20 ppm, and Pb <0.01 to 1.30±0.10 ppm, respectively (Topcuoğlu et al 2003).

Luo et al (2020) also confirmed that *Gracilaria* has a strong metal adsorption capacity, after finding that Cd, Pb, Cu, and Zn levels were higher in fish farming locations than in *Gracilaria* cultivation sites. It is not an exaggeration to say that *Gracilaria* cultivation can be used as a bioremediation agent (Badraeni et al 2020) and to mitigate the environmental heavy metal pollution (Arbit et al 2018), adding ecological value to the coastal marine cultivation areas. Previous studies reported that heavy metals accumulation in macroalgae, as biosorbents (adsorbents and absorbents), is not permanent (Khusnul et al 2020). The thallus of macroalgae can form selective bonds with metal cations Cu, Cd and Pb (Fourest & Volesky 1997). The macroalgae cell walls are rich in polysaccharides and have functional carboxylic acid groups that can play an active role in metal binding (Yantyana et al 2018).

Although previous studies have never reported any inconsistent cases of metal accumulation (e.g. Pb) in *G. changii*, such cases have previously been reported in *Gracilaria* sp. living in marine waters, with a Pb concentration of 0.09 ppm. The Pb concentration in the thallus of *Gracilaria* sp. before planting (age 0 days) was 3.38±0.23 ppm. At harvest time (age 40 days), the Pb concentration fell to 0.84±0.00 ppm (Tega et al 2019). Supriyantini et al (2018) found that cultured *Gracilaria* could accumulate metals in its thallus for a certain time after planting and then release it back to the environment. The present study also indicates that there was an accumulation of Cu, Cd and Pb in the *G. changii* seaweed, but the accumulations were not consistent.

The inconsistent or non-permanent accumulation of Cu, Cd and Pb may occur due to the simple cell structure of *G. changii*. The metal can be accumulated or released back quickly. Other macroalgae, such as *Sargassum*, have a similar behavior (Khusnul et al 2020). This phenomenon preserves the food safety, because even though there is a buildup of metal in the thallus, the metal can be released back during the processing of *G. changii* as food or feed ingredient. In previous research, *G. changii*, both naturally grown in mangrove areas or cultivated in ponds, contain heavy metals within limits still safe for health (Nawi 2015).

The non-permanent accumulation makes seaweed derivative products safer, even though the metal concentration is higher. For instance, the *G. changii* Pb concentration of 0.320±0.265 ppm is higher than in *Holothuria scabra*, with a concentration of 0.05-0.07 ppm (Aprianto et al 2020) or in *Bohadschia vitiensis*, with 0.01 ppm (Amir et al 2020). Compared with other macroalgae species, the Pb content of *G. changii* is below its concentration in *Sargassum*, which is 0.59 ppm (Khusnul et al 2020). This is probably due to the simplicity of *G. changii* cell structure (Arbit et al 2019), compared to *Sargassum*, which allows metals to enter and exit quickly. The simple cell structure and the transient nature of the accumulation are thought to make macroalgae more resistant than more complex organisms to environments with a high metal content.

Conclusions. The present study shows that *G. changii* contains Cu and Cd metals in higher concentrations than in the seawater. However, the metals do not accumulate permanently. Metals can bond or be released back into the water. This means that in spite of the accumulation, the metals can be released during the processing of food or feed products made from *G. changii*.

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Conflict of interest. The authors declare no conflict of interest.

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Authors:
Inayah Yasir, Hasanuddin University, Marine Science Department, Faculty of Marine Science and Fisheries, 90245 Makassar, Indonesia, e-mail: inayah.yasir@mar-sci.unhas.ac.id
Zainuddin, Hasanuddin University, Fisheries Department, Faculty of Marine Science and Fisheries, 90245 Makassar, Indonesia, e-mail: zainuddinlatief@gmail.com
Syafiuddin, Hasanuddin University, Marine Science Department, Faculty of Marine Science and Fisheries, 90245 Makassar, Indonesia, e-mail: afi_makassar@yahoo.com
Joeharnani Tresnati, Hasanuddin University, Fisheries Department, Faculty of Marine Science and Fisheries, 90245 Makassar, Indonesia, e-mail: jtresnati@yahoo.com
Risal Aprianto, Hasanuddin University, Multitrophic Research Group, Faculty of Marine Science and Fisheries, 90245 Makassar, Indonesia, e-mail: risal1204@gmail.com
Ambo Tuwo, Hasanuddin University, Marine Science Department, Faculty of Marine Science and Fisheries, 90245 Makassar, Indonesia, e-mail: ambotuwo62@gmail.com
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