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Chemical Analysis of Sea urchin *Diadema setosum* Gonads

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Abstract. Sea urchins *Diadema setosum* can be found in shallow waters. Sea urchin gonads are nutritionally valuable and capable of accumulating heavy metals. This study aims to determine the moisture content, ash, protein, fat, carbohydrate, Pb, Cd metal content and determine the Maximum Tolerable Intake (MTI) of sea urchin *D. setosum* gonads. Gonad samples were obtained from three coasts of Huangobotu, Botubarani and Kurenai, Kabila Bone Sub-district, Bone Bolango Regency, using a purposive sampling technique. Gonads were analyzed proximate, including moisture, ash, protein, fat and carbohydrate content. Analysis of Pb and Cd levels using an atomic absorption spectrophotometer. The results were analyzed statistically using One-Way ANOVA Test. The results showed that the average moisture, ash, protein, fat, and carbohydrate content of gonads on the coast of Huangobotu, Botubarani, and Kurenai were significantly different ($p<0.05$); moisture content 71.91%, 75.04%, 78.51%; ash content 1.88%, 1.15%, 2.76%; protein content 9.90%, 12.81%, 15.53%; fat content 7.33%, 3.05%, 4.84%; carbohydrate content 2.35%, 7.92%, 4.93%. Pb levels were significantly different ($p<0.05$) 0.063 mg/kg, 0.039 mg/kg, and 0.018 mg/kg, respectively; Cd levels were not significantly different ($p>0.05$) 0.020 mg/kg, 0.012 mg/kg, 0.014 mg/kg respectively. MTI Pb adults 23.8kg, 38.4kg, 83.3kg, children 5.9kg, 9.6kg, 20.8kg. MTI Cd adults 21kg, 35kg, 30kg, children 5.25kg, 8.75kg, 7.5kg. *D. setosum* has potential as a source of protein and can be utilized as food by considering the MTI value.

Keywords: *Diadema setosum*; gonads; nutritional value; metals Pb; Cd

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INTRODUCTION

Sea urchin *D. setosum* is one of the echinoderms whose entire body surface is covered by spines. The sea urchin shell is composed of limestone to form a pattern. Sea urchins have various functions, including playing a role in ecological functions, and their gonads can be used as a food source.

Sea urchin gonads, known in the market as uni or roe, are found in the shells of both male and female sea urchins. Economically, sea urchin gonads in some countries are a high-value food with good taste (Takagi et al., 2018). Sea urchin gonads are utilized as food because they have nutritional value. Sea urchins have a protein content and lipids (Villalba-Villalba, 2021). *D. setosum* gonads are high in vitamin E. They also contain vitamin A, Albumin, protein, Iron, Magnesium, Zinc (Salma et al., 2016), carbohydrates, trace elements or minerals (Wulandari & Warsito, 2022). They are rich in biologically active substances such as anticancer (Thao et al., 2015)(Yang et al., 2020), antioxidant, anti-inflammatory (Francis & Chakraborty, 2020), antimicrobial (Solstad et al., 2016)(Shushizadeh et al., 2019), neuropro tectiveand other medicinal effects (Matveeva et al., 2021).

On the other hand, porcupine gonads can act as bioindicators of pollution by accumulating harmful chemical components in the form of lead (Pb) and cadmium (Cd) metals. Research conducted by Søndergaard et al. (2019) revealed that the sea urchin gonads of *Strongylocentrotus droebachiensis* in West Greenland significantly contained Pb and Cd. Latorre-Padilla et al. (2021) added that their research in the Chilean Coastal Zone revealed *Tetrapygus niger* accumulated heavy metals, including Pb and Cd, at high concentrations. Other researchers showed that sea urchins are very suitable animals for bioindication studies. Sea urchins are more easily in contact with pollutants in marine waters because compounds tend to accumulate in sediments, from which sea urchins extract their food (Parra-Luna et al., 2020).

The southern coast of the Kabila Bone sub-district is one of the habitats of sea urchins. Initial observations showed many sea urchins scattered in the intertidal zone of the beach. On the south coast of the Kabila Bone sub-district, heavy metal pollution of Pb and Cd is very likely to occur. It is estimated that the number of human activities in these waters, such as fishing, settlement, industry, tourism, and port activities where ships dock or pass through these waters, can produce discharges containing Pb and Cd, so marine pollution occurs.

The study aimed to determine the nutritional content, Pb and Cd metal content, and Maximum Tolerable Intake (MTI) of sea urchin *D. setosum* gonads from the south coast Kabila Bone Subdistrict, Bone Bolango Regency.

METHODS

Research Location

This research was conducted in the southern coastal areas of Huangobotu, Botubarani, and Kurenai, Kabila Bone District, Bone Bolango Regency for sampling. Proximate analysis was conducted at the Biochemistry Laboratory of Universitas Hassanudin. Analysis of lead (Pb) and cadmium (Cd) was conducted at the Balai Standarisasi Pelayanan Jasa Industri Manado (BSPJI) Laboratory.

Collection and preparation of specimens

Sea urchin samples were collected based on purposive sampling at the three coasts of Huangobotu, Botubarani, and Kurenai in the Kabila Bone sub-district in November 2022. Each location was taken adult sea urchins with a shell size of more than 60 mm with three repetitions. Sea urchin gonads were collected by splitting the sea urchin using scissors and then taking the gonads with a spoon. Gonads obtained were cleaned with water, added ice, and weighed as much as 100 grams for proximate analysis and 15 grams each for Pb and Cd analysis. Weighed gonads were put into plastic clips and stored in a cool box containing an ice jelly pack.

Proximate analysis

Proximate analysis of sea urchin gonads consisted of determining moisture, ash, protein, fat and carbohydrate content based on the Sudarmadji et al. (2010) method.

Moisture content analysis

Weighed samples were put into a porcelain cup (which has known weight and has been in the oven for 30 minutes at 100°C), dried at 105-110°C for 3 hours, cooled, and weighed constant weight. Determination of moisture content was done by the formula:

$$\text{Moisture content } (\%) = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100\%$$

Ash content analysis

Weigh the sample and place it in a porcelain cup (known weight). Fry in a furnace at 550°C until the sample turns white ash. Cooled and weighed. Determination of ash content was done by the formula:

$$\text{Ash content } (\%) = \frac{\text{Final weight-Weight of an empty cup}}{\text{Initial weight}} \times 100\%$$

Protein content analysis

Weigh the sample and add the catalyst. Next, the sample is diluted in a fume hood until transparent. After that, the sample is put into the Kjeldahl flask and distilled. The distillation results are mixed with boric acid in Erlenmeyer and then titrated until purple.

$$\%\ N = \frac{\text{ml NaOH (blank - samples)} \times \text{N.NaOH} \times 14.008 \times 100\%}{\text{W sample (g)} \times 1000} \times 100\%$$

Protein (%) = % N × Conversion factor

Fat content analysis

The sample is weighed and then wrapped in filter paper (weight known). Then the sample is inserted into the Soxhlet flask, added ether solvent, and deconstructed for 4 hours. Finally, the sample is dried in the oven and then weighed.

$$\text{Fat content } (\%) = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100\%$$

Carbohydrate content analysis

Carbohydrate analysis is carried out by the by difference method, which is the determination of carbohydrates in foodstuffs roughly by subtracting 100% of the total water, ash, protein, and fat content. Based on the formula:

Carbohydrate (%) = 100% - (moisture content + ash content + protein content + fat content).

Lead (Pb) and cadmium (Cd) analysis

Analysis of Pb and Cd based on SNI 01-2896-1998, How to test metal contamination in food item 5 using the dry ignition method. The principle of this analysis is that 5 g of *D. setosum* gonads are mixed with 10% magnesium nitrate solution in ethanol, then dried and ignited. Followed by absorbance readings using an atomic absorption spectrophotometer.Metal content was calculated using the formula:

$$\text{Metal content } (\mu g/g) = \frac{(\mu g \text{ metal/from calibration curve}) \times V}{M}$$

Maximum Tolerable Intake (MTI)

The determination of Maximum Tolerable Intake (MTI) is based on the World Health Organization (WHO) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Maximum Weekly Intake calculation using the formula:

$$\text{MWI} = \text{Body Weight} \times \text{PTWI}$$

MWI (Maximum Weekly Intake); Body weight (mg for adults weighing 60 kg per week and children 15 kg per week); PTWI (Provisional Tolerable Weekly Intake).

Then calculate the Maximum Tolerable Intake value of sea urchin gonads that are tolerated to be consumed in one week. Maximum Tolerable Intake was calculated using the formula by Turkmen et al. (2008), namely:

$$\text{MTI} = \text{MWI} / \text{Ct}$$

MTI (Maximum Tolerable Intake); Ct (Heavy metal concentration in sea urchin gonads).

Statistical analysis

The mean values of proximate levels, Pb and cd metals from each calculated area were analyzed by one-way ANOVA with a significance level set at p=0.05 after checking homogeneity and normality of variance. Statistical analysis was performed using statistics software SPSS version 25.

RESULTS AND DISCUSSION

The morphological characteristics of sea urchins from the three coasts of Huangobotu, Botubarani, and Kurenai are shown in Figure 1(A). The largest organ of sea urchins is the gonad in Figure 1(B).

Figur1. (A) Sea urchin *D. setosum*, (B) Sea urchin Gonad.

Gonads are the dominant organ of sea urchins, which are known to have high nutritional values needed by the body (Pringgenies et al., 2020). Gonads are located in the body cavity of sea urchins. One of the factors determining the quality of gonads is usually seen from their color, where the color of gonads that have good quality is between bright yellow to orange-red (Karnila et al., 2022). Based on the results, the gonads of the sea urchin *D. setosum* from Huangobotu, Botubarnai, and Kurenai coasts were yellow to orange-yellow (Figure 1 B), indicating that the gonads were of good quality.

Proximate Analysis

Proximate analysis is vital because it can provide data on the main content of food ingredients and is related to the nutritional content contained in the food (Sinay & Harijati, 2021). The proximate composition of sea urchin *D. setosum* gonads includes water, ash, protein, fat, and carbohydrates, as shown in Table 1.

Table 1. Average results of proximate analysis of sea urchin gonads

Composition (%/100gr) ± SD	Locations		
	Huangobotu	Botubarani	Kurenai
Moisture	78.51±0.62	75.04±1.35	71.91±1.33
Ash	1.88±0.44	1.15±0.47	2.76±0.27
Protein	9.90±1.67	12.81±0.63	15.53±1.35
Fat	7.33±0.43	3.05±0.78	4.84±0.26
Carbohydrate	2.35±1.84	7.92 ± 1.38	4.93±0.33

Moisture content

The average Moisture content of sea urchin *D. setosum* gonads was significantly different (p = 0.001), with the highest from the Huangobotu coast at 78.51%, followed by the Botubarani coast at 75.04% and Kurenai coast at 71.91%. The water content results are the same when compared to the research of Archana & Babu (2016), the moisture content of *S. variolaris* gonads is 77.53%, and *P. lividus* is 79.65%. Research by Matveeva et al. (2021) showed that the gonad moisture content of *S. intermedius* was 73.0% and *S. nudus* 75.7%. Another study conducted by Cuevas-Acuña et al. (2019) showed that the gonad moisture content of *S. franciscanus* was 75.40%. The water content of sea urchin gonads is influenced by several factors, one of which is the habitat of the sea urchin itself, where all phases of its life are in the water (Karnila et al., 2022).

Ash Content

The average ash content of sea urchin *D. setosum* gonads from Huangobotu, Botubarani and Kurenai coasts was significantly different (p = 0.002) at 1.88%, 1.15% and 2.76%, respectively. The ash content in coastal Huangobotu, Botubarnai and Kurenai is lower when compared to the ash content of *D. setosum* from Terengganu Malaysia, ranging from 5.72 - 10.89% (Rahman et al., 2023), also different from the results found by Akerina et al., (2015) of 2.72%. Meanwhile, Archana&Babu's (2016) found the ash content of *S. variolaris* gonads to be 3.76% and *P. lividus* 2.25%. Another study conducted by Cuevas-Acuña et al. (2019) found the ash content of *S. franciscanus* to be 3.73%. Ash content depends on the location and availability of minerals in the sea urchin habitat. Environmental conditions and food availability affect the mineral content of living organisms (Karnila et al., 2022).

Protein content

The average protein content of *D. setosum* gonads from the coast of Huangobotu, Botubarani and Kurenai was significantly different (p = 0.005) at 9.90%, 12.81%, and 15.53%. The protein levels in this study were lower when compared to the protein levels of *D. setosum* from Terengganu Malaysia, ranging from 36.21 - 50.14% (Rahman et al., 2023). Meanwhile, the protein content of this study is almost the same as the research of Matveeva et al. (2021); the protein content of *S. intermedius* gonads is 13.9%, and *S. nudus* is 13.8%. Research by Archana&Babu (2016) found the protein content of *S. variolaris* gonads to be 12.10% and *P. lividus* 12.03%. Another study conducted by Cuevas-Acuña et al. (2019) found the protein content of *S. franciscanus* to be 13.63%. These findings confirm that the same species can have different protein contents in different environments. This difference in nutrient content is strongly related to habitat (Pringgenies et al., 2020). Tupan and Silaban (2017) added that the level of gonad maturity could cause a difference in protein content. Gonads with large size and yellow colour proportionally contain much protein. Sea urchin is one of the fishery products with high protein content. The function of protein is very distinctive, namely building

and maintaining the cells and tissues of the body of living things; this function cannot be replaced by other nutrients (Akerina et al., 2015).

Fat content

After protein, fat is another organic component of sea urchins. The average fat content of *D. setosum* sea urchin gonads was significantly different (p = 0.000) from the Huangobotu coast at 7.33%, the Botubarani coast at 3.05%, and the Kurenai coast at 4.84%. The results of *D. setosum* gonad fat content differ from those of Rahman et al. (2023), between 20.17% - 26.18%. The fat content of *S. intermedius* gonads is 7.3%, and *S. nudus* is 6.3% (Matveeva et al., 2021). The protein content of *S. variolaris* gonads was 4.98% and *P. lividus* 3.05% (Archana & Babu, 2016). Differences in sea urchin gonad fat content may be influenced by their food source. According to Mcalister & Moran, (2012), there are two types of food sources of sea urchins, namely non-planktonic, which do not come from plankton but from the yolk of the mother and planktotrophic, which comes from phytoplankton and zooplankton. Another factor affecting the high-fat content is gonad size. Sea urchin gonads with proportionally large sizes contain more fat. Large gonad volumes tend to contain high-fat levels so they can be utilized as energy reserves for development (Byrne et al., 2008).

Carbohydrate content

The average carbohydrate content of *D. setosum* gonads was significantly different ($p = 0.007$). The highest value was 7.92% from the Botubarani coast, 4.93% from the Kurenai coast and 2.35% from the Huangobotu coast. The carbohydrate content of *D. setosum* gonads from Botubarani and Kurenai coast is higher when compared to the findings of Akerina et al. (2015) of 0.33%. Other researchers also found a difference in results, Karnila et al. (2022) reported the carbohydrate content of *D. setosum* gonads at 3.30%, and Tupan and Silaban (2017) reported the carbohydrate content of D. setosum gonads from Martafons Beach at 3.80%, Sopapei beach 2.11% and Waao beach 7.50%. Carbohydrates are essential biochemical components for sea urchins because they function as energy providers and structural elements in egg formation and development (Afifudin et al., 2014).

Analysis of Lead (Pb) and Cadmium (Cd) Levels

Metal Pb levels of *D. setosum* gonads from the Huangobotu, Botubarani, and Kurenai coasts are shown in Table 2.

Table 2. Average results of Pb and Cd levels

Heavy metals (mg/kg) ± SD	Locations			Maximum level of contaminants
	Huangobotu	Botubarani	Kurenai	
Lead (Pb)	0.063 ± 0.014	0.039 ± 0.000	0.018 ± 0.012	1.5* 0.30 - 1.00**
Cadmium (Cd)	0.020 ± 0.014	0.012 ± 0.016	0.014 ± 0.087	1.0* 0.50 - 1.00**

*SNI 7387:2009 (Limits for heavy metal contamination in food; Fish and fishery products including molluscs, crustaceans, echinoderms, amphibians, and reptiles).
** European Union. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

The average results of Pb levels in *D. setosum* gonads were significantly different ($p = 0.007$). Pb levels from the coast of Huangobotu, Botubarani and Kurenai were 0.063, 0.039, and 0.018 mg/kg. Meanwhile, the average results of Cd levels were not significantly different ($p = 0.799$). The levels were 0.020 mg/kg, 0.012 mg/kg and 0.14 mg/kg, respectively. Based on the provisions of the Indonesian National Standard and the European Union, the levels of Pb and Cd of sea urchin *D. setosum* gonads are still below the standard (Table 1).

D. setosum gonads from the Huangobotu coast showed higher Pb levels than the Botubarani and Kurenai coasts. This is thought to be due to fishing ports on the Huangobotu coast which tend to produce more significant pollutants, coupled with the activities of residents living around the coast who make household waste. Meanwhile, gonads from the Botubarani coast showed lower Pb values than the Huangobotu coast and higher than the Kurenai coast, in line with anthropogenic pressure in the form of waste from coastal residents. In contrast, the Kurenai coast is far from human activities, where Pb levels are much lower than from Huangobotu and Botubarani coasts. Pb metal enters the waters through ballast water discharges from ships and oil-fueled engine emissions used as anti-knock in engines (Suryaningsih et al., 2020). While human activities in coastal areas often produce polluting waste that harms marine aquatic life. Conversely, the levels of Cd gonads on the coast of Huangbotu, Botubarani, and Kurenai, compared with Pb levels from the same coast, tend to be lower. Cd heavy metal in the water comes from dust, atmospheric deposition, and industrial wastewater. The presence of Cd metal in the environment is increasing due to its considerable utilization in several industrial and agricultural activities (Chiarelli & Roccheri, 2014).

In comparison, this study is similar to a study conducted by Boukhelf et al. (2019) in Western

Algeria, where Pb levels were higher than Cd levels. Pb and Cd levels of *P. lividus* gonads from the coast of SidiLakhdar were 0.199 mg/kg and 0.0048 mg/kg, respectively, while Pb and Cd levels from the Salamandre coast were 0.194 mg/kg and 0.005 mg/kg, respectively. The same researchers reported that Pb levels were higher than Cd in the gonads and intestines of *P. lividus* from Civitavecchia Italy, where average Pb levels ranged from 0.23 - 3.33 mg/kg while Cd levels ranged from 0.04 - 0.13 mg/kg (Scanu et al., 2015).

Generally, the Pb and Cd levels of sea urchin *D. setosum* gonads in Huangobotu, Botubarani and Kurenai Coastal Areas are still deficient below SNI and EU standards. Many factors can cause these high and low levels of Pb and Cd. According to Camacho et al. (2018), this depends on the animal's species, food source, developmental stage, size and physiological status and environmental exposure. The high level of heavy metals is also related to the spawning period, characterized by an increase in the accumulation of nutrient reserves and the synthesis and storage of carbohydrate, lipid and protein materials (Saliha et al., 2020). In this process, there is a decrease in food absorption along with a decrease in food production. *D. setosum* spends all its time spawning and only feeds a little, which causes a decrease in the amount of heavy metal uptake and impacts bioaccumulation (Al Najjar et al., 2018).

The level of metal accumulation in sea urchin gonads represents environmental conditions and thus can be used as a bioindicator to determine metal pollution in those waters (De Zoysa et al., 2018). Based on the results, it can be concluded that the Huangobotu, Botubarani and Kurenai coasts are not polluted. However, these results may differ from other types of metals. Therefore, further research on other heavy metals is needed.

Maximum Tolerable Intake (MTI)

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established provisional tolerable weekly intakes (PTWI) for heavy metals Pb and Cd of 0.025 mg/kg and 0.007 mg/kg body weight, respectively. Maximum Tolerable Intake values for adults and children are shown in Table 3.

Table 3. Maximum Tolerable Intake (MTI) of Pb and Cd in Adults and Children

MTI (kg/week)	Categories	Locations		
		Huangobotu	Botubarani	Kurenai
Lead (Pb)	Children*	5.9	9.6	20.8
Cadmium (Cd)		5.25	8.75	7.5
Timbal (Pb)	Adults*	23.8	38.4	83.3
Kadmium (Cd)		21	35	30

*Assumed average body weight of adults 60 kg and children 15 kg

Maximum Tolerable Intake (MTI) is used as a reference to avoid the adverse effects of consuming food containing heavy metals by determining the maximum tolerable consumption in a week. Table 3 shows that a person consuming *D. setosum* gonads does not exceed the number of gonads (kg/week) with an MTI value. Therefore, it can be assumed that a person with a body weight of 60 kg does not consume *D. setosum* gonads based on Pb content should not be more than 83.3 kg/week, while based on Cd content, a person with a body weight of 60 kg should not consume more than 35 kg/week. The assumption of a person's 15 kg body weight in consuming *D. setosum* gonads based on Pb content is no more than 20.8 kg/week, while based on the Cd content, of not more than 8.75 kg/week.

CONCLUSION

This study showed that the nutritional content of *D. setosum* gonads from the Kurenai coast had the highest protein and ash content. In contrast, the Botubarani coast showed the highest carbohydrate content, and the Huangobotu coast showed the highest fat and moisture content. This study also showed that the highest Pb and Cd levels came from the Huangobotu coast. *D. setosum* has the potential as a source of protein and can be utilized as food by considering the MTI value. Further research on other types of metals may be conducted in the future as it is evident that sea urchin gonads can accumulate Pb and Cd metals. In addition, the different organs of sea urchins also need to be considered.

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