

# Characterization and acute oral toxicity of concentrated minerals of Pamekasan Madura seawater

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## ABSTRACT

Indonesia is a maritime country with abundant seawater mineral content. One of the regions with the highest salt production is Pamekasan Madura. Minerals are known to have many roles and benefits for our bodies, such as regulating fluid balance and metabolism. Therefore, this study aimed to characterize the physicochemical and microbial properties of concentrated minerals obtained through solvent evaporation and salt deposition for  $\pm 60$  days. Acute oral toxicity examination was performed as a first step in determining the safety of concentrated minerals to be used as a raw material for drugs. Based on the test results, the concentrated mineral has a clear yellow color, salty taste, and a bit bitter, odorless, with a pH of  $6.6 \pm 0.21$ . Concentrated minerals have high mineral content with levels of potassium, sodium, magnesium, boron, and calcium being  $44734.1598 \pm 12950.4633$ ,  $33192.1198 \pm 2699.3419$ ,  $8738.1388 \pm 100.4894$ ,  $2092.5715 \pm 60.3224$ , and  $276.9704 \pm 13.1133$  mg/Kg, respectively. The results of microbiological analysis of untreated concentrated minerals (without antimicrobials or sterilization) showed that the total plate count was within limits, including coliform and *Salmonella*. However, the total mold and yeast levels exceed the threshold. Based on the results of acute oral toxicity testing, the concentrated mineral is practically nontoxic. With high mineral content and low toxicity, it can be concluded that the concentrated minerals from Pamekasan Madura seawater is potential to be used as a raw medicinal material.

**Key words:** Concentrated mineral, mineral acute oral toxicity, Pamekasan Madura

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## INTRODUCTION

Indonesia's unique geological characteristics have a significant impact on the natural resources, especially minerals that are scattered throughout the Indonesian waters.<sup>[1]</sup> The total salt produced in 2018 reached 2,768,809.95 tons.<sup>[2]</sup> One of the largest salt-producing areas in Indonesia is Madura Island. Pamekasan Regency has a coastal area with the longest coastline. The coast along Madura Island is a coastal area that holds considerable mineral resources.<sup>[3]</sup>

Seawater contains various types of minerals. Minerals with the highest levels include sodium, magnesium, potassium,

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calcium, and boron.<sup>[4]</sup> The mineral content of Indonesian seawater is higher than seawater in Egypt, China, India, Singapore, and Uganda.<sup>[5]</sup> Concentrated mineral of seawater or bittern water has many medicinal properties, including atherogenesis, cholesterol, blood pressure, cardiovascular, obesity, skin, fatigue, diabetes, hepatic, osteoporosis, gastritis, and cataracts.<sup>[6]</sup> Magnesium itself has been reported to decrease lipid accumulation in the aorta.<sup>[7]</sup> Deep seawater has been reported to enhance the beneficial effects of yogurt on lipid metabolism.<sup>[8]</sup> Concentrated minerals have also been widely marketed, such as Revell Global-Concentrated Mineral Drop, Sea M. D-Anderson Health Solution, and Islands Brand-Ocean Mineral Topical Facial Serum.

Apart from its potential to be used as medicinal raw materials, seawater can also be bad for health if it contains toxic ingredients. This is caused by environmental contamination in both chemical and microbiological forms.<sup>[9-11]</sup> In the development of medicinal raw materials, characterization is necessary to determine its quality and safety. Therefore, this study aims to characterize the physicochemical and microbial of concentrated minerals from Pamekasan Madura seawater and their toxicity to mice using the OECD 425 method.

## METHODS

### Materials

Seawater (Pamekasan Madura, Indonesia), phosphate buffer solution (Sigma-Aldrich, Singapore), tryptic soy broth (Sigma-Aldrich, Singapore), LB broth (Lennox, Sigma-Aldrich, Singapore), and aquades were used in this study.

### Seawater sampling method

The sampling location was determined based on purposive sampling method. Three sampling points were determined by taking into account the distance of the sampling location to human activities. The sampling point was  $\pm$  500–750 m from the shoreline, and the depth range is approximately 1–1.1 m. Seawater samples are taken at low tide to make it easier to reach the specified location. A total of 100 mL of seawater at three sampling points (surface, middle, and seabed) were taken and stored in a sterile glass tube container stored in a cool box.

### Preparation and characterization of concentrated mineral

Concentrated minerals were prepared through evaporation and sedimentation methods. Salt from seawater was settled, and the solvent was evaporated for  $\pm$ 40 days. The supernatant was separated and then redeposited sodium for  $\pm$ 20 days. The supernatant was taken, and the °B value was measured using a Baume Meter (DURAC Baume Hydrometer, Thomas Scientific). Mineral content was

determined using inductively coupled plasma optical emission spectroscopy (PlasmaQuant PQ 9000 Series, Analytik Jena, Germany).

The physical parameters of seawater that were measured include color, taste, aroma, and pH. The level of acidity (pH) was measured using a pH meter (PH-009 [1]) with a resolution of 0.1 (at 20°C).

Microbiological analysis was performed by inoculating concentrated minerals onto trypticase soy agar and luria broth media. The colony count was calculated using a colony counter. The parameters analyzed were total plate count (TPC), coliform, *Salmonella*, mold, and yeast.

## Acute oral toxicity

### Animal preparation

The test animals used were 2-month-old female *Mus musculus* mice with a bodyweight of 26–33 g. All experiments were performed following the guidelines of OIE animal welfare standards and were approved by the Local Ethical Committee Faculty of Medicine, Universitas Padjadjaran, Bandung (No. 1296/UN6.C10/PN2017).

### Health examination and acclimatization of test animals

Medical examinations were carried out to obtain selected specimens that have an average and representative health condition. Acclimatization was carried out to psychologically condition the specimens in the cages and rooms that have been provided according to the OIE animal welfare standards. This process was carried out at least 1 week before testing.

### Determination of toxic dose

Following SNI 7185.5-2017, the initial dose determination was 175 mg/kg body weight of mice. If the mice given the initial dose were still alive, then the dose was increased 3.2 times from the initial dose (up to a dose of 5000 mg/kg BW). However, if death is found, then in the next treatment, the dose is divided by 3.2 times.

### Preparation of concentrated mineral dosage

Samples were prepared by adding distilled water according to the prescribed dosage. Samples were dissolved using vortex at 2000 rpm before being given orally. The sample material was prepared by inserting it into a conical tube according to the required dose.

## Samples administration and observation of pharmacological signs

The mice were fasted 3–4 h before being given a dose. Samples with an initial dose of 175 mg/kg were given to the mice using a feeding tube. Mice were fed 1–2 h after dosing. Observation of the condition of mice was carried out for 48 h. If the mice given pretreatment were still alive, the dose was increased 3.2 times from the initial dose.

The pharmacological signs that were observed were consumption of food and drink, activity (grooming), mucus in the mouth, eyes, and nose. After 7 days, a necropsy was performed to see the macroscopic condition of the organ structures, especially in the brain, heart, lungs, kidneys, liver, spleen, stomach, and intestines.

### Statistical analysis

Statistical analysis was carried out to see the significance of differences in organ weights in each group of mice. The method used is Fisher's least significant difference method for multiple comparisons. The software Statistical Product and Service Solutions (SPSS) version 22 (IBM Corporation, New York) was used to run the statistical analysis.

## RESULTS

### Characteristics of concentrated mineral

Seawater before being concentrated has a °B value of around 3–4. After the salt was precipitated, the °B value of the supernatant reached 31–32. Evaporation and precipitation of sodium both produce concentrated minerals with a BE value of 35–36. Based on the °B value, the concentrated mineral density is 1.318–1.330 g/cm<sup>3</sup>, which was calculated using the following equation:

$$\text{Density} = \frac{145}{145 - \text{BE}} \quad \text{Equation 1}$$

The concentrated mineral has a clear yellow color, a salty taste slightly bitter, odorless, pH of 6.6 ± 0.21. Several mineral contents have been traced where the minerals with the highest levels are potassium, sodium, magnesium, boron, and calcium. Mineral content and levels are shown in Table 1.

The results of the microbiological examination showed that the concentrated minerals contained TPC, coliform, mold, and yeast but were still within normal limits [Table 2]. *Salmonella* was not detectable in concentrated minerals.

### Acute oral toxicity

After oral administration, several pharmacological signs indicated normal activity such as stable feed intake and drinking. Mice did grooming every day. Each mucosa, such as mouth, nose, and eyes, looked normal, and there was no change (observed for 7 days for each dose). There were no deaths in mice after concentrated mineral administration at all doses. On pathological anatomy observations, all organs appear normal in terms of consistency in the size and color of each organ [Figure 1].

Based on the results of statistical analysis, there was a decrease in intestinal weight, but it was in contrast to the stomach weight [Table 3].

Based on the results of data processing using a basic computer program – LD50 AOT425StatPgm OECD 425,

**Table 1: Mineral content in concentrated minerals**

Mineral	Concentration (mg/kg)
As	1.2313 ± 0.7123
B	2092.5715 ± 60.3224
Ca	276.9704 ± 13.1133
Cd	< 0.0001
Co	0.1520 ± 0.0170
Cr	2.3494 ± 1.0265
Cu	< 0.0001
Fe	0.2237 ± 0.0283
Hg	1.4785 ± 0.2375
K	44,734.1598 ± 12,950.4633
Mg	8738.1388 ± 100.4894
Mn	4.1359 ± 0.2207
Na	33,192.1198 ± 2699.3419
Pb	0.9116 ± 0.0027
Sn	3.9198 ± 0.9689

SD: Standard deviation, AS: Ardennite, CD: Cadmium, CA: Calcium, CO: Cobalt, CR: Chromium, MN: Manganese, MG: Magnesium, NA: Natrium, SN: Stannum, PB: Plumbum, K: Kalium, HG: Homopolar generator, CU: Copper, FE: iron

**Table 2: The microbial content of concentrated minerals**

Variable	Amount	Unit	Testing method
TPC	1.0 × 10 <sup>1</sup>	CFU/ml	SNI 2897: 2008
Coliform	2	APM/100 ml	Cappuccino
<i>Salmonella</i>	Negative	-	-
Kapang	2.0 × 10 <sup>1</sup>	CFU/ml	SNI 2897: 2008
Khamir	7.0 × 10 <sup>1</sup>		

TPC: Total plate count, CFU: Colony-forming unit, MPN: Most Probable Number, SNI: Standar Nasional Indonesia

the LD50 value of concentrated minerals was more than 5000 mg/Kg body weight of mice. This suggests that concentrated minerals are practically nontoxic.

## DISCUSSION

### Characteristics of concentrated mineral

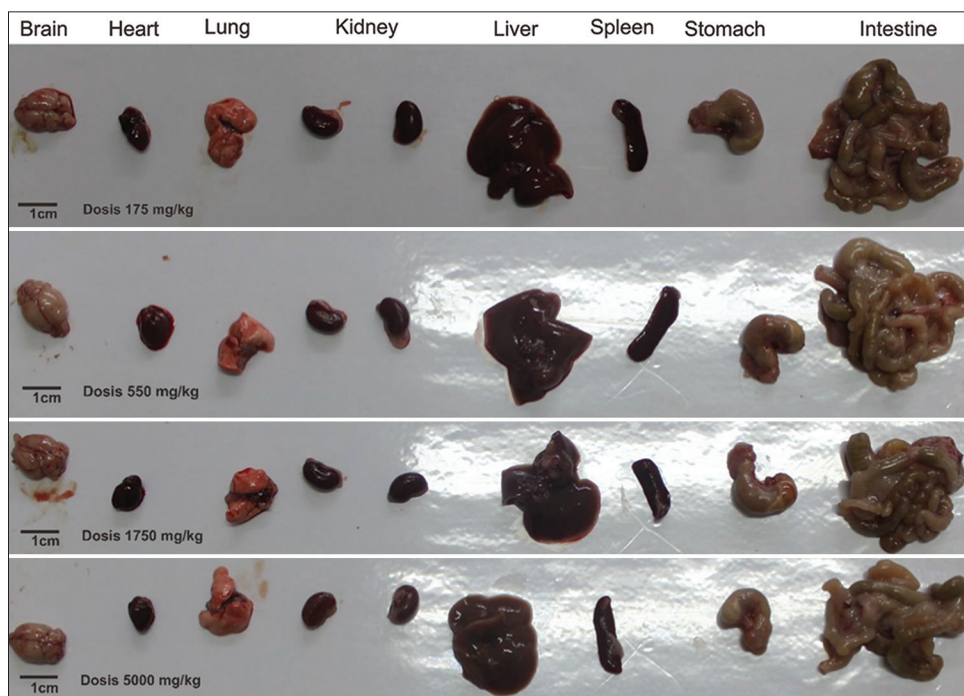
After the solvent evaporation and salt deposition for ± 60 days, the °B value and the specific gravity of the concentrated mineral increased to exceed the density of raw seawater. This indicates that the seawater mineral becomes more concentrated. In the raw seawater (before being concentrated), the °B value varies from 0 to 29, while the salinity varies from 4 to 92 ppt. The higher the B value, the higher the salinity.<sup>[5]</sup>

Based on the measurement results, the concentrated mineral has a pH value of 6.6 ± 0.21. The pH value of the raw seawater in Pamekasan Madura is 8.05–8.35.<sup>[5]</sup> This indicates an increase in the pH value of the concentrated mineral toward acid. The pH value of seawater can be affected by carbon dioxide levels. Carbon dioxide can form carbonic acid (H<sub>2</sub>CO<sub>3</sub>), carbonate ions (CO<sup>3-</sup>), and bicarbonate ions (HCO<sup>3-</sup>) by reaction with seawater. In seawater, organic carbon is generally in carbonate ions

**Table 3: Organs weight (g) of mice treated with concentrated mineral in an acute toxicity (n=5)**

Organ	Control	Concentrated mineral dose (mg/kg BB)			
		175	550	1750	5000
Heart	0.22±0.04	0.15±0.03	0.15±0.03	0.15±0.01	0.16±0.02
Liver	1.37±0.14	1.58±0.34	1.48±0.23	1.46±0.14	1.43±0.29
Kidney					
Left	0.20±0.00	0.19±0.03	0.18±0.02	0.16±0.01	0.17±0.03
Right	0.20±0.00	0.16±0.02	0.18±0.02	0.16±0.01	0.17±0.02
Lung	0.25±0.05	0.24±0.05	0.24±0.03	0.25±0.03	0.25±0.05
Stomach	0.57±0.14	0.64±0.14	0.71±0.09	0.56±0.08	0.61±0.08
Intestine	5.13±0.56	4.48±0.86	4.12±0.70	4.10±0.63	4.15±0.82
Lymph	0.15±0.05	0.16±0.07	0.13±0.02	0.13±0.02	0.18±0.06
Brain	0.48±0.04	0.50±0.03	0.47±0.02	0.46±0.06	0.47±0.01

BW: Body Weight

**Figure 1:** Pathological anatomy of the test animal organs

form, which will release (H<sup>+</sup>). Therefore, increasing the concentration of carbon dioxide will increase the pH value of water.<sup>[12]</sup>

Based on the measurement results, it was found that the mineral content in the concentrated Pamekasan Madura seawater did not increase its heavy metal content significantly. Compared to seawater in the Jakarta bay area, the content of heavy metals from concentrated minerals is still lower, except for Pb and Cr.<sup>[13]</sup> Pb levels from concentrated minerals are relatively high compared to some Indonesian marine waters.<sup>[5]</sup> This shows that the conventional concentration process has not effectively reduced the levels of heavy metal contaminants, especially Pb. According to Apriani *et al.*, the best way to purify magnesium is by using fluidized bed crystallization.<sup>[5]</sup>

To be used as a raw medicinal material, the concentrated minerals must not contain microbes for parenteral use. As for oral and topical use, the microbial content must be below the maximum tolerance level. Natural materials that have not been treated (reduction or removal of microbes) have a maximum TPC limit of 10<sup>4</sup> CFU/g, while total mold and yeast have a maximum limit of 10<sup>2</sup> CFU/g. The results of microbial contaminant measurements showed that the TPC was still within the threshold. Concentrated minerals do not contain *Salmonella*. The coliform content is also within limits allowed for herbal products (10<sup>3</sup> CFU/g).<sup>[14]</sup> However, the amount of mold and yeast exceeds the threshold. For that in the manufacturing process, it is necessary to reduce or eliminate microbes by adding antimicrobials or the sterilization process.

### Acute oral toxicity

Based on observations of pharmacological signs for 7 days, the activity of mice was still normal. There were no mice deaths during concentrated mineral administration at all doses. Organ observation in the threatened group also showed no abnormalities in size and color compared to normal mice. However, there was an increase in intestine weight. This can be caused by the tonicity of the concentrated mineral. Minerals are absorbed more along the intestine, so that the large difference in tonicity between concentrated minerals and cells in the intestine causes the diffusion of intracellular fluid out of the cell, causing a decrease in weight that cannot be considered in the macroscopic observation of organs. The decrease in intestinal weight began to be seen significantly at a dose of 500 mg/Kg BW ( $P < 0.05$ ). However, this weight reduction did not really affect the mice's daily activities. The LD50 value is also more than 5000 mg/Kg body weight of mice, which indicates that the concentrated mineral is practically nontoxic.

### CONCLUSION

Concentrated minerals from Pamekasan Madura seawater obtained through the process of solvent evaporation and salt deposition have high mineral content with levels of potassium, sodium, magnesium, boron, and calcium, respectively,  $44734.1598 \pm 12950.4633$ ,  $33192.1198 \pm 2699.3419$ ,  $8738.1388 \pm 100.4894$ ,  $2092.5715 \pm 60.3224$ , and  $276.9704 \pm 13.1133$ . Concentrated minerals are also categorized as practically nontoxic.

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### Conflicts of interest

There are no conflicts of interest.

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