

Research Article

Metal Bioaccumulation in Albino Rat Tissues Treated with Decontaminated Sea Lettuce (*Ulva lactuca* L.)

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ABSTRACT

Ulva lactuca is a macroalgae that contains high nutritional values. The heavy metal contaminants in natural *Ulva lactuca* needs to be eliminated or decreased using natural agent. The aim of this research was to determine the bioaccumulation of Pb, Cd, Hg, and the impact on liver and gastrointestinal function. Parameters of this research were Hepatosomatic Index (HSI), SGPT levels, SGOT levels, bioaccumulation Pb, Cd, Hg, and histological structure of liver and small intestine. Besides that, the progression of body weight was observed. Twelve female Wistar rats (*Rattus norvegicus* Berkenhout, 1769) were randomly assigned to three groups: Control, NU (treated with natural *Ulva lactuca*), and DU (treated with heavy metal decontaminated *Ulva lactuca* using *Averrhoa bilimbi* juice). Treatment was carried out orally at a dose of 1000 mg/Kg BW/day for 30 days. Histological structure of rat's liver and small intestine were prepared after necropsy at the end of this research. Based on results, it can be concluded that there were no significant differences observed in HSI, SGPT, and SGOT levels among the groups. However, there was a tendency for an increase in total bilirubin levels in the decontaminated *Ulva lactuca* group. Both natural and heavy metal decontaminated *Ulva lactuca* showed histological damage on liver and small intestine. Bioaccumulation of Cd and Hg in the liver and gastrointestinal tract of rats after consuming decontaminated *Ulva lactuca* was lower than the natural *Ulva lactuca* group, but need more observations.

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INTRODUCTION

Ulva lactuca or sea lettuce is a species of green algae that has been widely used by humans. *Ulva lactuca* has been used in agriculture, cosmetics, bioremediation, food, and biomedical sector (Rasyid 2017). It contains many vitamins, proteins, carbohydrates, and minerals that are beneficial to the body. *Ulva lactuca* also has the potential as a nutraceutical candidate such as anticoagulant, anticancer, and antibacterial, because it has high levels of antioxidants. The biochemical composition in *Ulva lactuca* is strongly influenced by climatic conditions, geographical location, and the local environment (Yaich et al. 2011).

Ulva lactuca is a bioindicator of marine pollution because it is widely distributed along the coast which is susceptible to water pollution and accumulation of heavy metals (Ardiyansyah et al. 2019). *Ulva lactuca* fits numerous features that make it one of the best bioindicators of marine

pollution. Previous research by our teams since 2019 from Ngrumpit and Sepanjang Beach, Gunungkidul, Yogyakarta proved that *Ulva lactuca* was contaminated with heavy metals such as Pb, Cd, and Hg. The Cd content is known to be 0.59 mg/Kg dry weight, exceeding the *Badan Pengawas Obat dan Makanan* (BPOM)'s threshold for Cd contamination in seaweed, which is 0.2 mg/Kg.

The investigation persisted in 2021, focusing on the decontamination of heavy metals such as Pb, Cd, and Hg in *Ulva lactuca* using a 15% filtrate of bilimbi (*Averrhoa bilimbi*) for a duration of 60 minutes. Heavy metals can be reduced using bilimbi filtrate as a decontaminant because bilimbi contains citric acid as a sequestrant which binds with polyvalent metal ions, forming a complex compound. This enhances the stability and quality of food.

The functional groups -OH and COOH of citric acid play a role in the binding of citrate ion with metal ions that results in the accumulation of citrate complexes (Nurhayati & Navianti 2017). In the *Ulva lactuca* group subjected to washing with distilled water, the Pb content measured 0.1857 mg/Kg dry weight, Cd content was <0.008 mg/Kg dry weight, and Hg content was 0.4 mg/Kg dry weight. Contrastingly, in the decontaminated *Ulva lactuca* group, Pb was recorded at 0.1369 mg/Kg dry weight, Cd at 0.008 mg/Kg dry weight, and Hg at 0.26 mg/Kg dry weight. The assessment of liver function involved monitoring levels of SGPT, SGOT, and bilirubin. The liver, an important organ in heavy metal detoxification, uses enzymes such as SGPT, SGOT, and bilirubin. Liver damage can be discerned through alterations in SGPT, SGOT, and bilirubin levels, serving as indicative parameters. Elevated levels of these three parameters may be used as indicators of liver damage.

Heavy metals can be accumulated and distributed at a higher trophic level through the food web. Consumption of *Ulva lactuca* that has been contaminated with heavy metals can lead to accumulation within the organs of organisms at higher trophic levels, particularly in the kidneys, liver, and digestive tract, resulting in adverse health effects (Ardiyansyah et al. 2019). Heavy metals are substances that are indigestible by the digestive system so they accumulate in the long term.

Lead can be distributed through blood and accumulates in the liver. Bioaccumulation of lead in rat livers causes liver cell damage degeneration and necrosis). Cadmium (Cd) is a heavy metal with high toxicity. Accumulation of cadmium in the body triggers an increase in reactive oxygen species (ROS) which causes damage to the liver and kidneys (Koyu et al. 2006). Cadmium exposure causes vacuolar, granular degeneration, and necrosis in rat livers (Yu et al. 2021).

Mercury (Hg) is the most toxic metal in the natural environment. Microbial activity in water has the capability to transform mercury into methylmercury (CH₃-Hg), a substance known for its toxic properties (Aba 2016). The bioaccumulation of mercury in rat livers can result in adverse effects such as cell swelling, hydropic degeneration, fatty degeneration, and necrosis. Mercury exposure causes edema and necrosis in the fish tilapia intestine (*Oreochromis niloticus*) (Kaoud & El-Dahshan 2010).

The objectives of this investigation were to assess the impact of the consumption of decontaminated *Ulva lactuca* on (i) liver function parameters, encompassing hepatosomatic index (HSI), as well as the levels of SGPT, SGOT, and bilirubin; (ii) the bioaccumulation of Pb, Cd, and Hg in the liver and gastrointestinal tract; and (iii) the histopathological changes in the liver and small intestines of female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769).

MATERIALS AND METHODS

Materials

Twelve female Wistar rats, aged 8 weeks and weighing between 150 and 200 grams, were procured from the Faculty of Pharmacy, Universitas Gadjah Mada, to serve as experimental subjects. The materials administered to the animals included *Ulva lactuca* sourced from Ngrumput Beach, Gunungkidul, D.I. Yogyakarta, and filtrate derived from bilimbi (*Averrhoa bilimbi*).

Methods

This research was conducted under the Ethical Clearance certificate number 00020/04/LPPT/VII/2022, issued by the Institutional Committee of Animal Use and Care (ICAUC) of The Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada.

Ulva lactuca Preparation

Ulva lactuca L. samples were collected from Ngrumput Beach, Gunungkidul, Yogyakarta, by the preceding research team in 2021. The acquired *Ulva lactuca* samples were subsequently categorized into two groups. The natural *Ulva lactuca* (NU) group comprised specimens that underwent washing with distilled water. The decontaminated *Ulva lactuca* (DU) group encompassed *Ulva lactuca* subjected to washing with distilled water and immersion in bilimbi (*Averrhoa bilimbi*) filtrate at a 15% concentration for 60 minutes. The samples were incubated for 3–7 days at a temperature range of 40–45°C until a consistent dry weight was achieved. The desiccated *Ulva lactuca* was then pulverized using a blender and filtered through a 60-mesh strainer to obtain a finer powder.

Animal Treatment

Female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769) were used as animal models and acclimatized for 14 days. Animals were kept in cages (44 x 35 x 50 cm³) with shaved wood bedding, where each cage contains four rats. The animals' rooms were maintained at temperature range of 22–26°C and humidity range of 60–70% in the Animal House facility, Faculty of Biology, Universitas Gadjah Mada. Body weight of animals was measured once a week using a digital balance. This data was used to calculate the amount of *Ulva lactuca* and ketamine-xylazine cocktail needed for administration and anaesthesia, respectively. Body weight was also used to calculate Hepatosomatic Index (HSI).

Twelve female Wistar rats were randomly divided into 3 groups: Control (administered with 1.5 mL distilled water/day), NU (Natural *Ulva lactuca* at a dose of 1000 mg/Kg BW/day), and DU (Decontaminated *Ulva lactuca* with a dose of 1000 mg/Kg BW/day). The treatment involved the administration of a suspension containing *Ulva lactuca* powder at a dose of 1000 mg/Kg BW/day in 1.5 mL of distilled water via oral gavage. After 30 days of treatment, rats blood was taken and the rats were euthanized to collect their liver and gastrointestinal tract including stomach, small intestine, large intestine, and rectum for undergoing the histopathological preparations.

Measurement of Hepatosomatic Index (HSI)

The Hepatosomatic Index was calculated at the end of the study by weighing body weight and liver weight. The Hepatosomatic Index (HSI) was calculated using the formula (Albalat et al. 2019) as follows:

$$\text{HSI} = \frac{\text{Liver weight}}{\text{Body Weight}} \times 100\%$$

Liver Function Evaluation

Liver function evaluation was conducted by measuring Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), and bilirubin levels in the blood serum. Blood samples were collected from the sinus orbitalis of rats on Day 0 (D₀) and Day 30 (D₃₀) after a fasting period of 6–8 hours. Animals were anesthetized using Ketamine (KTM-HCl®) and Xylazine (Holland®) in a cocktail (0.1 mL/100 g BW) by intramuscular administration. Blood samples were analyzed in The Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada using enzymatic photometric methods to obtain the SGPT, SGOT, and bilirubin levels.

Bioaccumulation of Lead, Cadmium, and Mercury

The animals were euthanized using double dosage of ketamine-xylazine cocktail on D₃₀. Liver and gastrointestinal organs including stomach, small intestine, large intestine, and rectum were collected and washed using NaCl 0.9% solution. Samples of 5 grams each were put into a 100 mL Erlenmeyer then added 15 mL HNO₃ 70% and 5 mL HClO₄ 70%. After that, it was digested on a heating plate until dissolved and clear, continued until it was almost dry, then added 10 mL of distilled water and homogenized. The solution was then filtered in a 25 mL measuring flask, and distilled water was added up to the mark. The solution was used to determine the cadmium and lead levels using Atomic Absorption Spectrophotometer (AAS) and to determine the mercury level using Mercury Analyzer by the Integrated Research and Testing Laboratory (LPPT) Unit II, Universitas Gadjah Mada .

Histopathological Examination

Histological preparation was carried out using the paraffin method after 30 days of treatment. The liver and gastrointestinal organs, including the stomach, small intestine, large intestine, and rectum, were collected and washed using a 0.9% NaCl solution. Subsequently, they were fixed in 10% neutral buffered formalin (NBF) for 24 hours. Following fixation, the organs underwent dehydration using graded alcohol and were embedded in paraffin blocks. The right lobe of the liver and the distal part of the small intestine were sliced into 1 cm sections. The blocks were then sectioned at 4 µm and stained with hematoxylin and eosin (HE). The sections were observed under a light microscope (Leica Microsystem®) and photographed using a Leica LAS EZ (Leica Microsystem®).

Data Analysis

The research data obtained were Hepatosomatic Index (HSI), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), and bilirubin levels, the Pb, Cd, and Hg heavy metal levels in the liver and gastrointestinal tract of rats, as well as histopathological examination of the liver and small intestine. The rats body weight, HSI, SGOT, SGPT, dan bilirubin data were analyzed with One-Way ANOVA (Analysis of Variance) statistical test and Duncan's post hoc test with alpha = 5%. Paired-samples T-test conducted for D₀ and D₃₀ of SGOT, SGPT, and bilirubin levels. Statistical test conducted using Statistical Package for the Social Sciences (SPSS) version 16. Heavy metal levels and histopathological examination were analyzed descriptively with reference to similar research journals.

RESULTS AND DISCUSSION

Body Weight

The result showed an increase in body weight in the Control, NU, and DU groups on the D₇ and D₁₄, further, there is a slight decrease in body weight in the Control group on D₂₁ (Table 1). *Ulva lactuca* was proven to contain high vitamins, fats, carbohydrates (Rasyid 2017) so it can influence the body weight increase of the rats during treatment period. Consumption of *Ulva lactuca* that is high in Fe content 873.72 mg/kg (Mulyati et al. 2020) has potency to increase body weight.

Table 1 showed that there were no significant differences in body weight between groups on D₀ and D₇. However, there were significant differences between the Control and DU groups on D₁₄. There were no significant differences for the all group of the treatment period (p>0,5).

Hepatosomatic Index

The Hepatosomatic Index (HSI) is a value that can be used to describe the amount of toxic compounds that enter the body and gives the status of energy availability in the body (Yuneldi et al. 2018) (Table 2). This study showed that the HSI after the treatment period was not significantly different between treatments (p>0.5). However, the result showed that the NU and DU groups tend to have a higher hepatosomatic index than the Control group. A higher hepatosomatic index can be influenced by hepatic metabolic activity and consumption activity in rats so that the ratio increases.

Based on Table 2, this study shows that the HSI after the treatment period was not significantly different between treatments. However, the table shows that the NU and DU groups tend to have a higher hepatosomatic index than the Control group. This study used *Ulva lactuca* that have been decontaminated using bilimbi (*Averrhoa bilimbi*) filtrate. This decontamination process has reduced the level of lead contamination from 0.186 mg/Kg of *Ulva* powder to 0.1367 mg/Kg of *Ulva* powder (26.3%) and reduced the level of mercury from 0.40 mg/Kg of *Ulva* powder to 0.26 mg/Kg of *Ulva* powder (35%). Even though the levels of heavy metals have decreased, heavy metals are still found in the NU and DU groups within safe limits according to BSN (2009).

Serum Glutamic Pyruvic Transaminase (SGPT)

Serum Glutamic Pyruvic Transaminase (SGPT) or also known as Alanine Aminotransferase (ALT) is an enzyme found in hepatocytes that converts amino groups (-NH₂) from glutamate to pyruvate, and vice versa. Transamination is the removal of amino groups as a combination of amination and deamination (Ninkov et al. 2015). The increase or decrease of this enzyme's activity in a serum acts as an indicator of hepatocyte damage. The results of SGPT levels analysis are presented in Table 3.

Table 1. Body weight of female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769) after 30 days treatment.

Group	Body Weight (g)				
	D ₀	D ₇	D ₁₄	D ₂₁	D ₂₈
Control	197.50 ± 22.06 ^a	207.75 ± 23.68 ^a	207.50 ± 22.90 ^b	206.00 ± 22.85 ^a	210.75 ± 23.31 ^a
NU	177.25 ± 15.58 ^a	183.25 ± 18.25 ^a	185.25 ± 18.68 ^{ab}	185.75 ± 17.73 ^a	188.25 ± 18.96 ^a
DU	165.25 ± 20.35 ^a	176.50 ± 19.49 ^a	176.00 ± 11.80 ^a	182.75 ± 21.30 ^a	187.00 ± 21.23 ^a

Data were presented as Mean ± Standard Deviation. The different superscript letters on the same column note a significant difference at $p < 0.05$ between groups within the same day. NU: treatment with natural *Ulva lactuca*, DU: treatment with decontaminated *Ulva lactuca*. D₀: Day 0, D₇: Day 7, D₁₄: Day 14, D₂₁: Day 21, and D₂₈: Day 28.

Based on Table 3, SGPT level in D₀ does not show a significant difference between each treatment group. This means that all the animal models used in this research have the same health status with no anomalies. According to Vigneshwar et al. (2021), the reference value of SGPT levels is 29.34 – 72.16 UI/L for female Wistar albino rats.

In the D₃₀ treatment, an increase of SGPT level was found in each of the treatment groups, without any significant difference ($p > 0.05$). SGPT levels in the blood is one of the sensitive indicators of hepatic damage, because this enzyme resides in the cytoplasm. Furthermore, when hepatocyte damages happen, those hepatocytes release SGPT.

Table 2. Hepatosomatic Index (HSI) of female Wistar rats (*Rattus norvegicus* Berkenhout, 1769) after treatment with *Ulva lactuca* L.

No	Group	HIS (Mean ± SD)
1	Control	3.89 ± 0.19 _a
2	Natural <i>Ulva lactuca</i> (NU)	4.22 ± 1.24 _a
3	Decontaminated <i>Ulva lactuca</i> (DU)	4.34 ± 0.44 _a

The different superscript letters note a significant difference at $p < 0.05$ between groups. NU: treatment with natural *Ulva lactuca*, DU: treatment with decontaminated *Ulva lactuca*.

Table 3. Serum glutamic pyruvic transaminase (SGPT) levels of female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769) before (D₀) and after (D₃₀) treatment with *Ulva lactuca* L.

No	Group	D ₀ (U/I)	D ₃₀ (U/I)
		Mean ± SD	Mean ± SD
1	Control	45.40 ± 6.51 _a	51.18 ± 4.73 _a
2	Natural <i>Ulva lactuca</i> (NU)	40.83 ± 6.45 _a	41.45 ± 8.87 _a
3	Decontaminated <i>Ulva lactuca</i> (DU)	44.80 ± 5.38 _a	48.38 ± 11.01 _a

The different superscript letters note a significant difference at $p < 0.05$ between groups within the same day. There is no significant different at SGPT levels before and after treatment with *Ulva lactuca* L. for each group. NU: treatment with natural *Ulva lactuca*, DU: treatment with decontaminated *Ulva lactuca*. D₀: Day 0, D₃₀: Day 30.

Serum Glutamic Oxaloacetic Transaminase (SGOT)

Serum Glutamic Oxaloacetic Transaminase (SGOT) or also known as the Aspartate Transaminase (AST) is an enzyme to repair hepatocytes damage. When liver cell damage occurs, hepatocytes will produce SGOT which is released into the bloodstream. SGOT levels can be used as a parameter to determine liver damage as indicated by increased SGOT levels (Yuneldi et al. 2018). The results of SGOT levels analysis are presented in Table 4.

Table 4 shows that SGOT levels were not significantly different among groups before and after treatment. This shows that all the groups were in similar condition and were within the normal range. Normal range of SGOT level of rats was 74 – 143 UI/L (Sarapultsev et al. 2012). There was a decrease in SGOT levels in D₃₀ of the Control group, whereas in the NU and DU groups there was an increase but not significantly different ($p > 0.05$). The highest increase in SGOT levels occurred in the DU group. This could be due to the presence of heavy metal contaminants in DU, even though heavy metals contaminations in DU have already decreased.

Table 4. Serum glutamic oxaloacetic transaminase (SGOT) levels of female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769) before (D₀) and after (D₃₀) treatment with *Ulva lactuca* L.

No	Group	D ₀ (U/I)	D ₃₀ (U/I)
		Mean ± SD	Mean ± SD
1	Control	131.23 ± 30.82 ^a	92.08 ± 49.90 ^a
2	Natural <i>Ulva lactuca</i> (NU)	107.10 ± 68.76 ^a	125.13 ± 23.54 ^a
3	Decontaminated <i>Ulva lactuca</i> (DU)	104.98 ± 63.92 ^a	183.25 ± 105.44 ^a

The different superscript letters note a significant difference at $p < 0.05$ between groups within the same day. NU: treatment with natural *Ulva lactuca*, DU: treatment with decontaminated *Ulva lactuca*. D₀: Day 0, D₃₀: Day 30.

Bilirubin

Bilirubin is a breakdown product of old red blood cells (erythrocytes) by macrophages in the phagocyte mononuclear system, especially in the liver. The first breakdown of the RES (Reticuloendothelial System) begins with the capture of iron and peptide globin chains. Bilirubin comes from a RES derivative product, namely biliverdin. Bilirubin in plasma is cooled by albumin and undergoes a conjugation process in the liver before being excreted through the intestine (Erlinger et al. 2014). According to Mulyati et al. (2020) normal serum bilirubin levels in white rats ranged from 0.3–0.8 mg/dL. Increased total bilirubin levels above normal can be used as an indicator of liver damage, especially those affected by the process of glucuronidation and hemolysis. The results of bilirubin levels analysis are presented in Table 5.

Table 5. Blood bilirubin levels of female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769) before (D₀) and after (D₃₀) treatment with *Ulva lactuca* L.

No	Group	D ₀ (mg/dL)	D ₃₀ (mg/dL)
		Mean ± SD	Mean ± SD
1	Control	0.84 ± 0.27 ^a	0.79 ± 0.32 ^{ab}
2	Natural <i>Ulva lactuca</i> (NU)	0.63 ± 0.10 ^a	0.58 ± 0.20 ^a
3	Decontaminated <i>Ulva lactuca</i> (DU)	0.69 ± 0.22 ^a	1.06 ± 0.25 ^b

The different superscript letters note a significant difference at $p < 0.05$ between groups within the same day. NU: treatment with natural *Ulva lactuca*, DU: treatment with decontaminated *Ulva lactuca*. D₀: Day 0, D₃₀: Day 30.

Table 5. showed that all groups in D₀ were still within the normal range of bilirubin levels and there is no significant difference between treatment groups. This result showed that the animals used were normal and healthy. After 30 days of treatment, there was a trend of decreasing bilirubin levels in the Control group and the natural *Ulva lactuca* treatment, but the group treated with heavy metal decontaminated *Ulva lactuca* experienced an increasing trend.

According to Andjelkovic et al. (2019), exposure to heavy metals affects the reduction of red blood cells significantly due to intravascular hemolysis which causes oxidative stress. Significantly different results were shown in the NU and DU groups. Although the levels of heavy metals contained in the DU group have decreased, the presence of heavy metals can still affect the physiology of rats. In addition, *Ulva lactuca* is a macroalgae that contains high Fe (Rasyid 2017). The high Fe content in the *Ulva lactuca* may affects the formation of red blood cells and their conversion to bilirubin in the liver so that the level can increase.

Bioaccumulation of Pb, Cd, and Hg

The bioaccumulation profile of the studied heavy metals including lead (Pb), cadmium (Cd), and mercury (Hg) in the liver and gastrointestinal tract was analyzed. Lead accumulation in the control groups was lower compared to the NU (Natural *Ulva lactuca*) and DU (Decontaminated *Ulva lactuca*) (Figure 1a). This could happen because both groups were given the *Ulva lactuca* which was used in previous research in 2021. The research in 2021 showed that *Ulva lactuca* was proven to contain lead of 0.186 mg/Kg dry weight in the natural *Ulva lactuca* and 0.137 mg/Kg dry weight in the decontaminated *Ulva lactuca*.

The highest levels of lead were found in the DU group in the gastrointestinal tract (Figure 1a). It can be associated with interactions between dietary fiber in *Ulva lactuca* with lead. *Ulva lactuca* contains 54.9% food fiber that can bind lead metal, and reduce absorption of lead in other tissues (Qi et al. 2019). Binding of lead by dietary fiber can reduce accumulation in the liver, heart, kidneys, and bones, through increased gastrointestinal motility (Mehrandish et al. 2019). In addition, the high level of lead in both the NU and DU groups could also be caused by the antagonism of heavy metals. The presence of lead can interfere with the absorption of other metals such as Zn, Fe, Cd, and Hg in the body (Mariadi et al. 2018).

Furthermore, Pb levels in the liver of the DU group were higher than those in the NU group. It can be indicated an antagonistic interaction between Pb and other metals especially Cd (Okon et al. 2020). The high levels of Pb in the liver of the DU group could be associated with the lower levels of Cd in the liver of the DU group (Figure 1b). The opposite also applies, in which low levels of Pb in the liver of the NU group can be attributed to higher levels of Cd in the liver of the NU group (Figure 1b). Divalent metals are absorbed in the body through similar mechanisms and accumulation in the same tissues. Once in the body, they can alter each other's absorption, distribution, and accumulation. Smith et al. (2012) observed that Pb antagonizes Cd accumulation in the liver and kidney.

The accumulation of cadmium in the liver of the control group showed much lower levels than the NU and DU groups (Figure 1b). This accordance with Winiarska-Mieczan and Kwiecień (2016) that consumption of foods contaminated with cadmium, 60% accumulates in the liver, 30% accumulates in the kidneys, and the remaining 10% accumulates in the bile organs, brain, lungs, and heart. Based on research Andjelkovic et al. (2019) bioaccumulation of cadmium in rats exposed to CdCl₂ 30 mg/Kg BW was highest in the liver affected by high synthesis of metallothionein (MT) in the liver. Metallothionein synthesis was able to detect the presence of the toxic metal cadmium. Metallothionein will bind to cadmium and form Cd-MT complexes that protect tissues (Matović et al. 2011).

Cadmium levels in the liver of the DU group were lower when compared to the NU group. This was influenced by the process of soaking *Ulva lactuca* with 15% bilimbi solution for 60 minutes. Bilimbi (*Averrhoa bilimbi*) contains citric acid which acts as a heavy metal chelating agent. Citric acid is able to bind heavy metals in the form of complex bonds and reduce the side effects of heavy metals in foodstuffs (Mariadi et al. 2018). The interaction between citric acid and heavy metals can be enhanced by soaking (Ulfah et al. 2014). Therefore, soaking *Ulva lactuca* in bilimbi solution can reduce cadmium levels in *Ulva lactuca* and its accumulation in the liver.

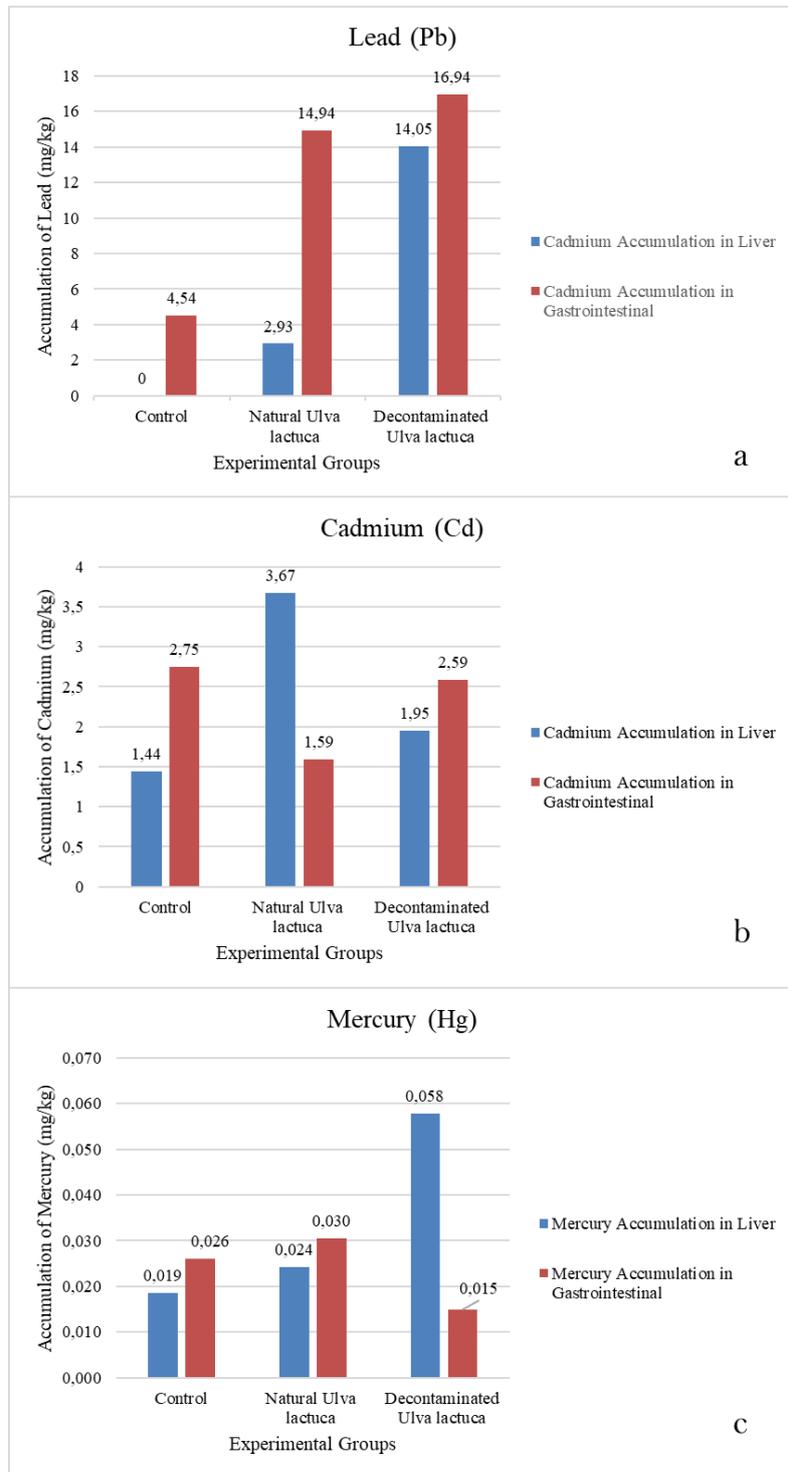


Figure 1. Metals accumulation of (a) Lead (Pb), (b) Cadmium (Cd), (c) Mercury (Hg) in liver and gastrointestinal tract of female Wistar albino rats (*Rattus norvegicus* Berkenhout, 1769) treated with *Ulva lactuca* L.

Furthermore, cadmium levels in the GIT organs of the control and DU groups showed higher values than NU groups. It can be related to interaction between cadmium and dietary fiber in *Ulva lactuca*. Dietary fiber has been reported to bind Pb and Cd in animal models thus promoting their excretion. *Ulva lactuca* contains cellulose as a dietary fiber that noticeable capacity of binding Pb and Cd in gastrointestinal. Mechanism underlying dietary fibers protective effect against heavy metals accumulation in the reduction in active transport sites in the intestine. It has been suggested that time reduction in intestinal transit could be responsible for the increased rate of heavy metals excretion in body (Guo et al. 2022).

The highest accumulation of mercury was in the DU group in the liver organ (Figure 1c). This can happen because of the synergistic effect between lead, cadmium, and mercury. Synergism interactions occur when two or more chemicals that have the same toxicity are combined so that they have greater toxic properties and accumulation. The absorption of lead, cadmium, and mercury metals is equally high in the DU group.

The highest mercury levels were found in the gastrointestinal organs, except for the DU (*Ulva lactuca* decontaminated) group (Figure 1c). Mercury is a substance that is easily absorbed by the tissues in the intestine. Based on research by [Aba \(2016\)](#) it shows that organic mercury is easily absorbed by the gastrointestinal (GI) tract by 95% and then distributed throughout the body. Mercury levels in the gastrointestinal organs of the DU group were lower than those of the NU group. This was influenced by the process of soaking *Ulva lactuca* with 15% bilimbi solution for 60 minutes. Soaking *Ulva lactuca* in bilimbi solution is more effective in reducing mercury levels in *Ulva lactuca* and its accumulation in the gastrointestinal organs than lead and cadmium levels.

Histopathological Effect of *Ulva lactuca* treatment on The Liver

The liver was chosen as the histological object to be observed because the liver is an organ exposed to heavy metals and plays a role in its detoxification process. The observations made were qualitative observations based on hepatocyte damage including cell swelling, hydropic degeneration, fatty degeneration, and necrosis.

The histological structure of liver on the control group was still normal, with the presence of many Kupffer cells and cell regeneration (Figure 2A-2B). Cell damage that occurs in the control group is because of the liver role as a place for detoxification of harmful substances. The hepatocytes are often damaged, but this is accompanied by a high rate of regeneration ([Mulyati et al. 2020](#)).

The histological condition of the liver in the NU group also experienced cell damage, including cell swelling or hydropic degeneration, fatty degeneration, and necrosis (Figure 2C-2D). This can be indicated to the accumulation of heavy metals lead, cadmium, and mercury in the liver. Accumulation of heavy metals in rats' bodies forms free radicals, causing oxidative stress that triggers liver histological damage in NU group rats. Based on [Dardouri et al. \(2016\)](#) exposure to CdCl_2 (100 mg/l) and HgCl_2 of 25 mg/l in rat's drinking water for 10 weeks caused hepatocyte degeneration, vacuolization, sinusoidal dilatation, central vein dilatation, and Kupffer cell proliferation.

In the DU group, there were cell damages including cell swelling or hydropic degeneration, fat degeneration, and necrosis (Figure 2E-2F). The DU group was the group that was treated with decontaminated *Ulva lactuca*. Cell damage still occurred in the DU group because it was proven previously that the decontaminated *Ulva lactuca* group still contained heavy metals of Pb 0.1369 mg/Kg, dry weight Cd 0.008 mg/Kg, dry weight and Hg 0.26 mg/Kg dry weight, although at lower levels than natural *Ulva lactuca*. Cell swelling or hydropic degeneration occurs due to an imbalance in the Na^+ and K^+ ion pumps in the cell membrane. These results are in line with the research of [Andjelkovic et al. \(2019\)](#) exposure to cadmium and lead causes inflammation that triggers hydropic degeneration in hepatocytes.

In addition to hydropic degeneration, fatty degeneration was also found in the DU group. The necrosis that occurs is the pyknosis stage indicated by the N symbol in Figure 2F. The cell nucleus undergoes pyknosis or nuclear shrinkage due to cytoplasmic homogenization and in-

creased eosinophilicity. After pyknosis occurs, the cell nucleus will disintegrate and leave chromatin fragments and spread in the cell which is called the karyorexis stage. Furthermore, the cell nucleus will undergo karyolysis, namely death which is characterized by loss of the ability to be colored.

Figure 2F also shows the presence of sinusoidal dilatation which is indicated by the star symbol. Dilatation or widening of the sinusoids occurs due to contact between heavy metals which are toxic to endothelial cells lining the sinusoid. If the concentration of heavy metals is high and the exposure is long, it can cause widening of the sinusoids (Wadaan 2009). However, besides the cell damage that occurred in the DU group, was also followed by cell regeneration that was indicated by the circle symbol (Figure 2F). This shows that the exposure to heavy metals in the DU group was qualitatively lower than NU groups. Cell damage in the DU group could still be accompanied by a high rate of cell regeneration.

Histopathological Effect of *Ulva lactuca* treatment on the Small Intestine

In each group, the histological structure of the small intestine was visible the vili, lumen, and muscle layer. The histological structure of the small intestine in the control group showed a uniform structure, with intact and elongated villi (Figure 3A). Whereas in the NU (Figure 3B) and DU (Figure 3C) groups, the structure is relatively uniform. However, there is also degeneration of the villi in the portion leading to the luminal area.

Figure 3D showed the histological structure of the small intestine of the control group which did not show any cell damage to the villi. Based on Figure 3E, it shows the histological structure of the intestines of the NU group which suffered damage, namely villous erosion (VE) and bleeding (H). The intestine is an organ that plays a role in absorption, protection, and hormonal processes. The NU group was a group that was

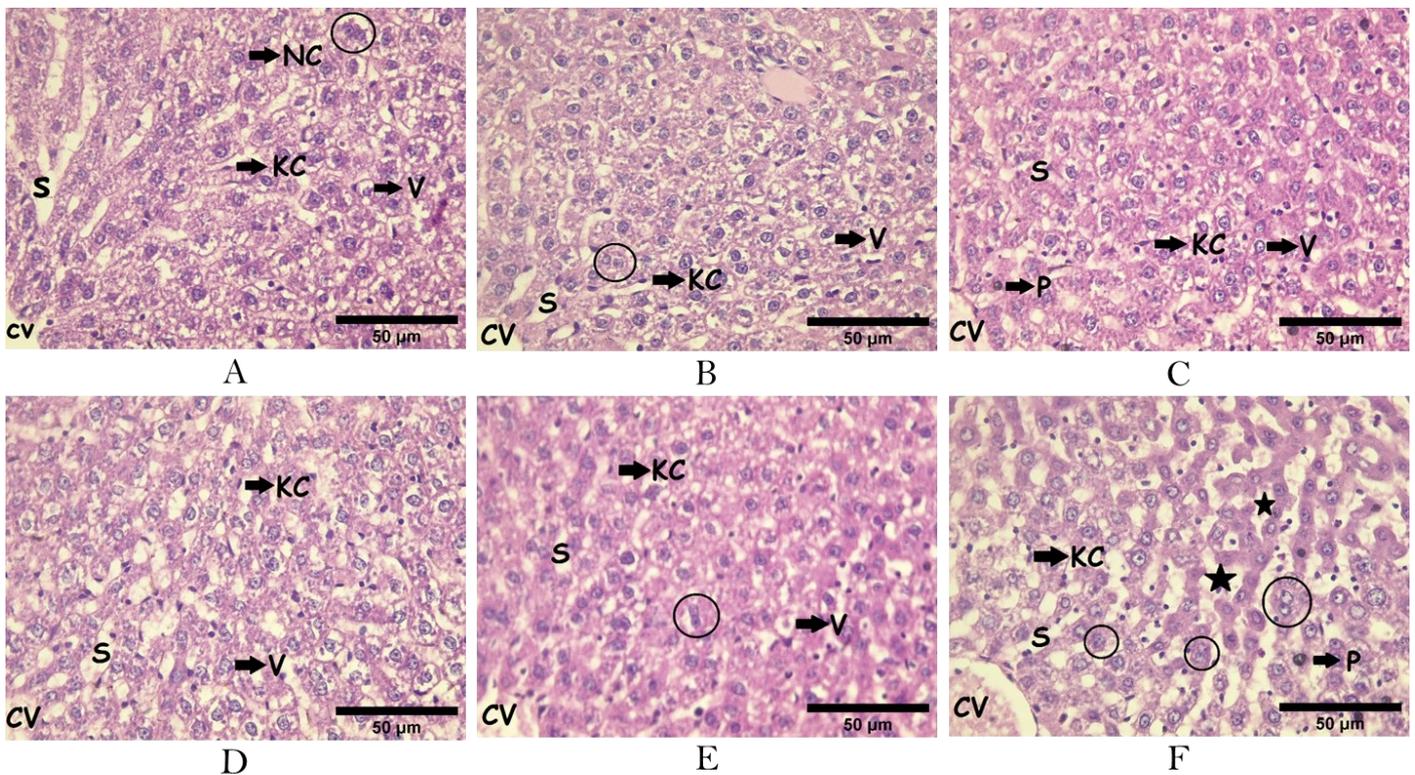


Figure 2. Histological structure of liver of rats (A-B) Control, (C-D) Natural *Ulva lactuca* (NU), (E-F) Decontaminated *Ulva lactuca* (DU). CV: Central Vein, NC: Normal cells, KC: Kupffer Cell, S: Sinusoids, V: Vacuolization/fat degeneration, P: Pyknosis, Circle: Cell regeneration, Star: Dilated sinusoids. H&E stain. 40 x 10. Scale bar: 50 μm.

given natural *Ulva lactuca* treatment which was proven to contain the heavy metals lead, cadmium and mercury. Intake of food ingredients that contain heavy metals will affect the organs in the body. These results are in line with the study of Bais and Lokhande (2012) which showed that exposure to cadmium for four days caused hydropic degeneration, villi erosion, epithelium degeneration, cell swelling, and necrosis in the intestinal villi of snakehead fish (*Ophiocephalus striatus*).

Based on Figure 3F, it shows the histological structure of the small intestine in the DU group which was damaged, namely bleeding of the lamina propria and erosion of the villi. Even though decontamination had been carried out, *Ulva lactuca* soaked using bilimbi solution proved to still contain the heavy metals lead, cadmium and mercury at lower levels than the NU group. The presence of these heavy metals can induce free radicals through the formation of reactive oxygen species (ROS). This causes a decrease in the ability of antioxidants in the body and trigger inflammation and damage to the histological structure of the intestines of the DU group rats. Exposure to cadmium of 25 – 100 µg/L in zebrafish *Danio rerio* caused bleeding in the lamina propia, cell swelling, and increased proliferation of goblet cells (Motta et al. 2022).

Based on Figure 3F, it was also seen that there was dilatation of the villi in the intestines of the DU group. Villi dilatation is the widening of the villi due to the induction of toxic compounds in the intestine. These results are in line with the research by Adigüzel and Kalender (2015) that lead (Pb) 22.5 mg/Kg BW in rats causes villous dilatation (D), necrosis, and epithelium degeneration. The process of villous dilatation triggers villous shortening. The shorter the size of the villi will interfere with the process of absorption of nutrients in the lumen. The villous dilatation that occurred in the DU group could be associated with high levels of lead bioaccumulation in the liver and gastrointestinal tract of rats (Figure 1a). Lead is mostly absorbed by the intestinal cell walls and is responded to by the histological structure of the intestine with the formation of villous dilatation.

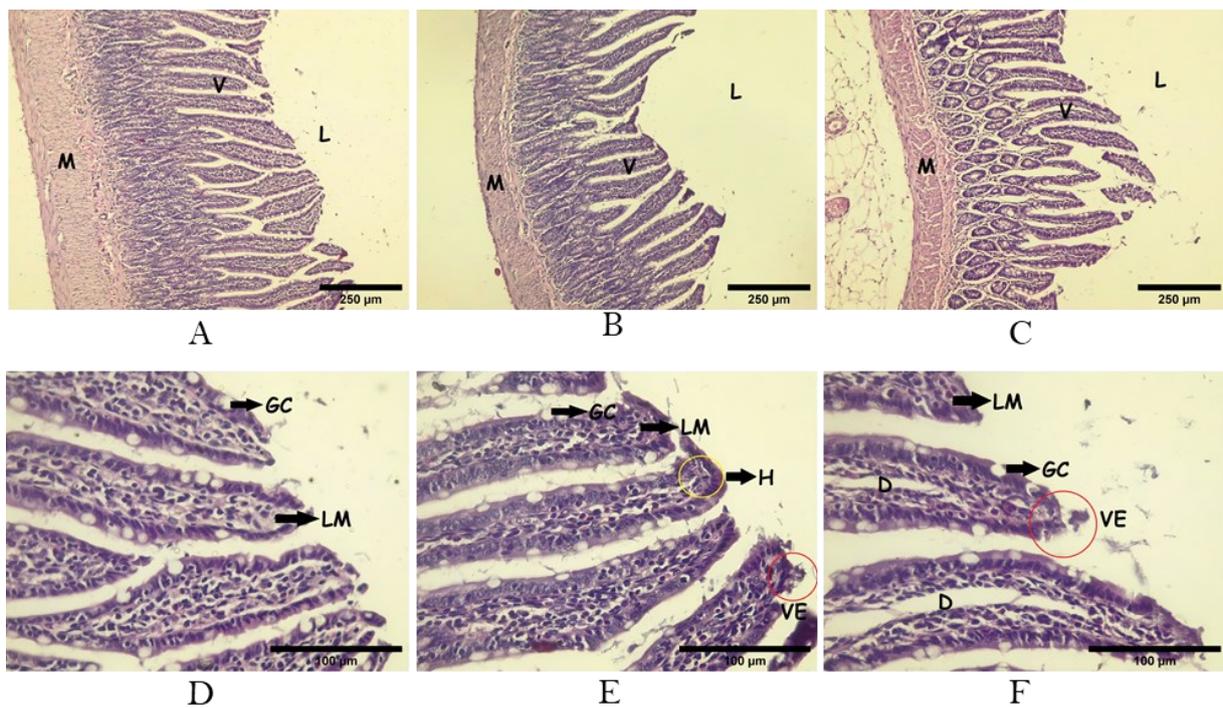


Figure 3. Histological structure of small intestine of rats (A) Control 10 x10, (B) Natural *Ulva lactuca* (NU) 10 x10, (C) Decontaminated *Ulva lactuca* (DU) 10 x10, (D) Control 40 x10, (E) Natural *Ulva lactuca* (NU) 40 x10, (F) Decontaminated *Ulva lactuca* (DU) 40x10. V: Villi, L: Lumen, M: Muscularis, GC: Goblets cell, LM: Lamina propia, H: Haemorrhage/bleeding, VE: Villi erroision, D: Dilatation villi. H&E stain. Scale bar: 50 µm.

Based on Figure 3D-3F, qualitatively, the damage to the histological structure of the intestine in all treatment groups in this study was almost the same, namely experiencing villous erosion or degeneration of the epithelium, bleeding, Goblet cell vacuolization and villous dilatation. Qualitative observation of histological findings showed that the most serious damage was found in the NU groups. However, these cannot be compared because there is no absolute value. Based on the histological picture of the intestine, it can be stated that the DU still had damage accompanied by cell regeneration. This research show that qualitatively histological structure liver and small intestine of DU groups has a better condition than the NU groups.

CONCLUSION

The administration of heavy metal decontaminated *Ulva lactuca* at a dosage of 1000 mg/kg BW/day over a 30-day treatment did not significantly alter HSI, SGPT, and SGOT levels. Despite a discernible rise in total bilirubin levels attributable to elevated iron content in *Ulva lactuca*, likely influencing an increase in red blood cells, the observed changes did not reach statistical significance. Nevertheless, the histological examination revealed damage to the liver and small intestine cells, coupled with a commendable regenerative response. These findings contribute valuable insights the effects of *Ulva lactuca* on hepatic function and heavy metal detoxification, paving the way for future research in the field of marine-based interventions for metal toxicity mitigation.

AUTHORS CONTRIBUTION

S.W., A.R.S.S., A.H.H., A.N.I., and S.R.D.S. conducted the research, data collection, data analysis, and wrote the manuscript, while M. designed the research, supervised, and wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publishing of the article.

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