Research Article

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In Vivo Evaluation of Heavy Metal Decontaminated Marine Macroalgae (*Ulva lactuca* L.) Using Bilimbi (*Averrhoa bilimbi*) Juice and Its Potency As Nephroprotector

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ABSTRACT: *Ulva lactuca* is a functional food ingredient with high antioxidant content. The presence of heavy metal contaminants in natural *Ulva lactuca* needs to be eliminated. *Ulva lactuca* was investigated for its potential as a nephroprotector in female Wistar rats. The study assessed Blood Urea Nitrogen (BUN) levels, creatinine levels, and kidney histopathology. The experiment included a Group I (control), Group II (administered 1000 mg/Kg BW/day of natural *Ulva lactuca*), and Group III (administered 1000 mg/Kg BW/day of heavy metal decontaminated *Ulva lactuca* using bilimbi juice). After 30 days of oral treatment, no significant differences were observed in BUN levels before and after treatment. However, significant variations were found in Group III compared to the Group I creatinine levels after treatment. Both natural and heavy metal decontaminated *Ulva lactuca* showed no adverse effects on kidney function. Notably, heavy metal decontaminated *Ulva lactuca* exhibited improved kidney histopathology, suggesting its potential as a nephroprotector. Further research is needed to explore its broader applications in promoting kidney health.

Keywords: BUN, creatinine, nephroprotector, *Ulva lactuca*, Wistar rat

INTRODUCTION

Many types of macroalgae grow on the south coast of Gunungkidul, DI Yogyakarta, and are known to be widely used by the locals as a food ingredient. Macroalgae contain compounds that tend to be high: up to 60% carbohydrates, 10 – 47% protein, 1 – 3% low-fat content, and 7 – 38% various kinds of minerals (Dominguez & Loret, 2019). One that has been utilized is the macroalgae *Ulva lactuca* L. Hudaifah *et al*. (2020) stated that *Ulva lactuca* L. sampled from Banyuwangi coastal area in Indonesian waters showed the presence of alkaloids, flavonoids, saponins, triterpenoids, and tannins which play an active role in counteracting free radicals. *Ulva lactuca* is known to act as an antibacterial, antifungal, anti-inflammatory, antiviral, and antioxidant because of its bioactive compound content (Ktari, 2017). Jatmiko *et al*. (2019) stated that the mineral composition in *Ulva* sp. sampling results at the Sepanjang Beach of Gunungkidul include Na, Mg, S, Cl, K, Ca, I, and Fe. Rasyid (2017) at Santolo Pameungpeuk Beach, Garut, West Java, shows that the nutrient content in *Ulva lactuca* is ash content, protein, fat, carbohydrates, fiber, vitamins A, B1, B2, and mineral Ca, Fe, K, F.

Measurements of heavy metals that had been carried out previously in 2021 showed that sample *Ulva lactuca* from Ngrumput Beach, Gunungkidul, contained Cd, Pb, and Hg contamination which exceeded safe limits according to *Badan Pengawas Obat dan Makanan* (BPOM) Number 9 of 2022 concerning Requirements for Heavy Metal Contamination in Processed Foods (Table 2). Exposure to heavy metals in the body accumulates and increases free radicals, which can trigger the formation of reactive oxygen species. Exposure to heavy metals in the body will mainly accumulate in the liver and kidneys (Ezedom *et al*., 2020). Heavy metal is widely used in industry and does not have a physiological function in the body, so its presence in the body can trigger damage at the cellular level or more and disrupt metabolic activity (Chen *et al*., 2018). Consuming *Ulva lactuca* with heavy metal contamination could result in nephrotoxicity. Nephrotoxicity is a toxic effect on the kidneys due to toxic agents and chemical compounds such as heavy metals and drugs (Sukmawati *et al*., 2022). This encourages efforts to decontaminate *Ulva lactuca* using sequestrants and is expected to have the potential as a nephroprotector.

Previous studies on *Ulva lactuca* L. have been carried out since 2019 by the teams. *Ulva lactuca* L. sampled from Drini Beach, Gunungkidul in 2019 was tested and found to be contaminated with the heavy metal cadmium. This research studied the effect of cadmium-contaminated *Ulva lactuca* L. on the physiological profile of albino rats (*Rattus norvegicus* Berkenhout, 1769). The research continued by seeking the decontamination of heavy metals using several types of citric acid liquids, such as bilimbi juice and lime juice. The results showed that decontamination using bilimbi juice was most effective for reducing contaminant levels in *Ulva lactuca* L. used as an indicator of kidney function in the excretory system can be observed in the levels of creatinine and Blood Urea Nitrogen (BUN) in the blood serum.

Bilimbi (*Averrhoa bilimbi*) is a fruit with many benefits, but it is still not widely cultivated. Hudaya (2010) in his research used bilimbi juice with a concentration of 15% for 60 minutes on green mussels (*Perna viridis*) and succeeded in reducing cadmium levels by 94.73%. According to that result, our study used a sequestrant of bilimbi (*Averrhoa bilimbi*) juice at a concentration of 15% (v/v) for 60 minutes to reduce the levels of heavy metal (Cd, Pb, Hg) contaminants in *Ulva lactuca*. Bilimbi juice is known to contain citric acid, which will bind to metals, causing metals to lose their ionic properties and reduce the level of toxicity of these metals (Ondu & Jayadipraja, 2019). Citric acid has –COOH and –OH functional groups, which will form complex compounds with heavy metals in the form of citrate salts (Olsson, 2013). This will increase the solubility and mobility of heavy metals, decreasing their concentration and toxicity (Zou *et al*., 2019).

Based on this background, it is necessary to carry out research aimed at evaluating the results of heavy metal decontamination, as well as the nephroprotector potential of *Ulva lactuca* in female Wistar rats (*Rattus norvegicus* Berkenhout, 1769). The investigation assessed creatinine levels, Blood Urea Nitrogen levels (BUN), and rat kidney histopathology. Decontaminated *Ulva lactuca* is expected to have a good effect on kidney function and to be further developed as a functional and healthy food ingredient.

MATERIALS AND METHOD

This research was conducted under the Ethical Clearance certificate number 00020/04/LPPT/VII/2022, issued by The Integrated Research and Testing Laboratory (LPPT) Universitas Gadjah Mada. This research was carried out from May 2022 to April 2023 after ethical clearance was issued.

Bilimbi Juice Preparation

Bilimbi juice (15% v/v concentration) was obtained by squeezing *Averrhoa bilimbi*. Then, 15 mL was poured into a 100 mL volumetric flask, distilled water was added up to the mark, and it was homogenized.

Ulva lactuca Preparation

Ulva lactuca L. samples were taken in 2021 from the intertidal zone of Ngrumput Beach, Gunungkidul, DI Yogyakarta. Samples were divided into two groups. The first group consisted of *Ulva lactuca*, which was cleansed using distilled water. The second group consisted of *Ulva lactuca*, cleansed using distilled water and soaked in bilimbi (*Averrhoa bilimbi*) juice with a 15% (v/v) concentration for 60 minutes. Samples were dried in an incubator for 3 – 7 days at 40 – 45 °C. Dried samples were then ground into flour and the heavy metal (Cd, Pb, Hg) content was measured (Mulyati *et al*., 2021). The final result was obtained from *Ulva lactuca* flour which was then strained using 60 mesh strainer.

Animal Treatment

Twelve 10-week-old female Wistar rats (*Rattus norvegicus* Berkenhout, 1769) with a body weight of 150–200 g obtained from the Faculty of Pharmacy, Universitas Gadjah Mada, were used as animal models. Animals were kept in the Animal House facility, Faculty of Biology, Universitas Gadjah Mada, and acclimatized for 7 days. Animals were kept in plastic cages 44 × 35 × 50 cm^3 with iron lids, fed AD-II pellets, drinking mineral water (Aqua®) *ad libitum*, and bedding made of shaved wood. Experimental animals were maintained at 22–26 °C and an average humidity of 60–70%. Twelve rats were randomly divided into 3 groups: Group I (control rats given distilled water 1.5 mL/day), Group II treated with natural *Ulva lactuca* (1000 mg/Kg BW/day), and Group III treated with heavy metal decontaminated *Ulva lactuca* (1000 mg/Kg BW/day). *Ulva lactuca* flour was suspended in 1.5 mL of distilled water and administered to the rats orally for 30 days. After the treatment, blood was taken, the rats were euthanized to collect their kidney organs, and histopathological preparations were made.

Blood Collections

Blood samples were collected from the orbital sinuses of rats on D_0 and D_{33} after the rats were fasted for 6 – 8 hours.

Table 1. Tissue damage criteria for histopathological assessment of the Wistar kidneys (*Rattus norvegicus* Berkenhout 1769) treated with *Ulva lactuca* L.

No.	Tissue Damage Criteria
1	Tubular lumen dilatation
2	Accumulation of cell debris in the lumen
3	Tubular lumen vacuolization
4	Widening of Bowman's space
5	Degeneration
6	Hyperplasia
7	Loss of the proximal tubule brush border
8	Hemorrhage

Table 2. Measurement result of heavy metal levels (Cd, Pb, and Hg) in *Ulva lactuca* L. from Ngrumput Beach, Gunungkidul, Yogyakarta

Heavy Metal Testing	Natural *U. lactuca* (mg/Kg Ulva powder)	Decontaminated *U. lactuca* (mg/Kg Ulva powder)	Decrease Percentage (%)	Threshold (mg/Kg Ulva powder; BPOM, 2022)
Cadmium (Cd)	<0.008	<0.008	-	0.05
Lead (Pb)	0.186	0.137	26.3	0.20
Mercury (Hg)	0.400	0.260	35.0	0.03

rats were anesthetized using Ketamine (KTM-HCl®) and Xylazine (Holland®) in a cocktail (0.1 mL/100 g BW). Blood samples were stored in a 1 mL microtube that had been added with EDTA to prevent coagulation for approximately 60 minutes. Blood samples were sent using a cooling box to The Integrated Research and Testing Laboratory (LPPT) Universitas Gadjah Mada for BUN and creatinine level analysis.

BUN and Creatinine Analysis

Blood Urea Nitrogen (BUN) levels in the blood serum were determined using the Urease-GLDH UV Kinetic method meanwhile, serum creatinine levels were determined using the Alkaline Picrate UV Kinetic method based on its protocol (Key Performance Indicators) by LPPT UGM Unit II.

Histopathological Examination

Kidney histopathology was observed at the Laboratory of Animal Structure Development, Faculty of Biology, Universitas Gadjah Mada, by paraffin method. Sections of 5 µm thickness were cut and stained using Hematoxylin-Eosin stain. Each treatment group was observed in 6 visual fields, including glomeruli and kidney tubules. Slides were observed under a light microscope (Leica Microsystem®) with a magnification of 10 × 6.5, then continued 40 × 6.5 with the zig-zag method in each field of view. Slides were photographed using a Leica LAS EZ (Leica Microsystem®) and histopathological assessment was carried out. The observed damage criteria are in accordance with Table 1 according to the scoring method of Qodar *et al*. (2019).

Statistical Analysis

The research data obtained were BUN, creatinine, and kidney histopathology scoring. BUN and creatinine levels were then arranged in tables and calculated. Data were tested for normality using the Shapiro-Wilk test. The normally distributed data was followed by a One-Way ANOVA (Analysis of Variance) statistical test and Duncan's post hoc test with alpha = 5%. The histological structure of rat kidneys was analyzed comparatively, and histopathological scoring was carried out by the binary scoring method. Binary scoring is presented in a tabular form containing the criteria for damage in each group.

RESULT AND DISCUSSION

Ulva lactuca Decontamination

Decontamination using bilimbi (*Averrhoa bilimbi*) juice has succeeded in reducing the levels of heavy metals Cd, Pb, and Hg in *Ulva lactuca* (Table 2). Hg levels are still relatively high and exceed the maximum limit according to BPOM (2022), but Cd and Pb levels are within safe limits. Table 2 shows that the heavy metals

Table 3. Blood Urea Nitrogen (BUN) levels of albino Wistar rats (*Rattus norvegicus* Berkenhout, 1769) before and after treatment with *Ulva lactuca* L.

Group	BUN D_0 (mg/dL)	BUN D_{33} (mg/dL)
	(Mean ± SD)	(Mean ± SD)
I	14.92 ± 3.04[a]	16.04 ± 2.98[a]
II	16.47 ± 1.93[a]	16.49 ± 3.30[a]
III	14.96 ± 3.95[a]	17.17 ± 3.98[a]

Note: (a) Numbers followed by the same letter (notation) show no significant difference ($p > 0.05$) in one column (between treatment groups). I: Control; II: Natural *Ulva lactuca* L. (samples were washed with distilled water); III: Heavy metal decontaminated *Ulva lactuca* L. (samples were washed with distilled water and soaked in bilimbi juice with 15% concentration for 60 minutes); D_0: Day 0; D_{33}: Day 33.

Table 4. Creatinine levels of albino Wistar rats (*Rattus norvegicus* Berkenhout, 1769) before and after treatment with *Ulva lactuca* L.

Group	Creatinine D_0 (mg/dL)	Creatinine D_{33} (mg/dL)
	(Mean ± SD)	**(Mean ± SD)**
I	0.23 ± 0.13[a]	0.44 ± 0.07[b]
II	0.25 ± 0.06[a]	0.38 ± 0.03[ab]
III	0.16 ± 0.09[a]	0.31 ± 0.08[a]

Note: Numbers followed by different letters (notation) indicate that there is a significant difference ($p < 0.05$) in one column (between treatment groups). I: Control; II: Natural *Ulva lactuca* L. (samples were washed with distilled water); III: Heavy metal decontaminated *Ulva lactuca* L. (samples were washed with distilled water and soaked in bilimbi juice with 15% concentration for 60 minutes); D_0: Day 0; D_{33}: Day 33.

decontamination process using bilimbi juice with a concentration of 15% (v/v) for 60 minutes can reduce heavy metals. These findings were in accordance with the research by Hudaya (2010).

According to Nurhayati and Navianti (2017), bilimbi juice can be used as a sequestrant. This food additive can bind polyvalent metal ions to form complex bonds, thereby reducing the harmful effects of the heavy metals contained. Even though there has been a decrease in heavy metal levels, their presence in *Ulva lactuca* has not completely disappeared, so it is possible to influence the physiology of the animals.

BUN Levels

BUN levels were tested to determine the amount of nitrogen in blood urea. BUN is a byproduct of protein metabolism which will be excreted through the kidneys. Pre-test (D_0) and post-test (D_{33}) BUN levels were measured to determine the initial and final conditions of rat kidney function to provide an overview of the effect of the treatment given. The examination was carried out at LPPT Unit II UGM on the same day as the blood collection. The results of BUN levels in rat blood serum are shown in Table 3.

According to Sica (2017), the normal BUN level of female Wistar rats is 9.71 – 16.91 mg/dL, and according to Angrella *et al*. (2020) is 8.0 – 33.0 mg/dL. Table 3 shows that BUN levels for all groups before and after treatment were in the normal range. This indicated that the rats used in the study were in good health and had normal activities. Statistical analysis showed that the results were not significantly different between treatments ($p>0.05$). Group I (control) experienced an average increase in BUN levels after 30 days of treatment, but still within the normal range. An increase in BUN levels can occur, which is influenced by the eating, drinking, and age of the rats during the study. The average BUN levels in the Group II (natural *Ulva lactuca*) before and after treatment were higher than the control group. However, the average BUN levels in the Group II were within the normal range despite increasing after treatment. This shows that treatment for 30 days using natural *Ulva lactuca* flour has not significantly affected changes in rat kidney function. The bioactive components contained in natural *Ulva lactuca* flour are known not to interfere with kidney function, so BUN levels will tend to be more stable. Natural *Ulva lactuca* contains phenolic compounds, chlorophyll, and carotene, which act as free radical scavengers in the body (Dominguez & Loret, 2019).

The average BUN levels in the Group III (decontaminated *Ulva lactuca*) showed no significant difference to Group I and Group II. However, BUN levels for the Group III experienced the highest increase. The increase in BUN levels, which was quite high, indicated that treatment with decontaminated *Ulva lactuca* had an effect on the rat kidneys but did not interfere with kidney function, so the increase was not significant. *Ulva lactuca* has a very high protein content. BUN is a kidney function test biomarker indicating the excretion of urea as the end product of proteolysis in the liver. The increase in BUN levels can be influenced by the intake of high-protein nutrients, which will then increase the levels of urea formed (Laksmi *et al*., 2014). A decrease or a slight increase in trend may occur due to the mechanism of the urea cycle in the liver. The results can also be caused by other factors, such as dehydration and urinary tract disorders, so BUN levels increase during the treatment period.

Creatinine Levels

Creatinine is a by-product of muscle contraction activity. The creatinine formed is then filtered through the kidneys and excreted through the urine as a result of renal excretion (Fitria *et al*., 2019). Increases in creatinine levels in blood and urine are used as indicators of glomerular filtration rate. According to Sica (2017), the normal creatinine level of female Wistar rats is 0.11–0.62 mg/dL. The results of creatinine levels in rat blood serum are shown in Table 4.

Statistical analysis for creatinine levels on D_0 showed that the results were not significantly different between

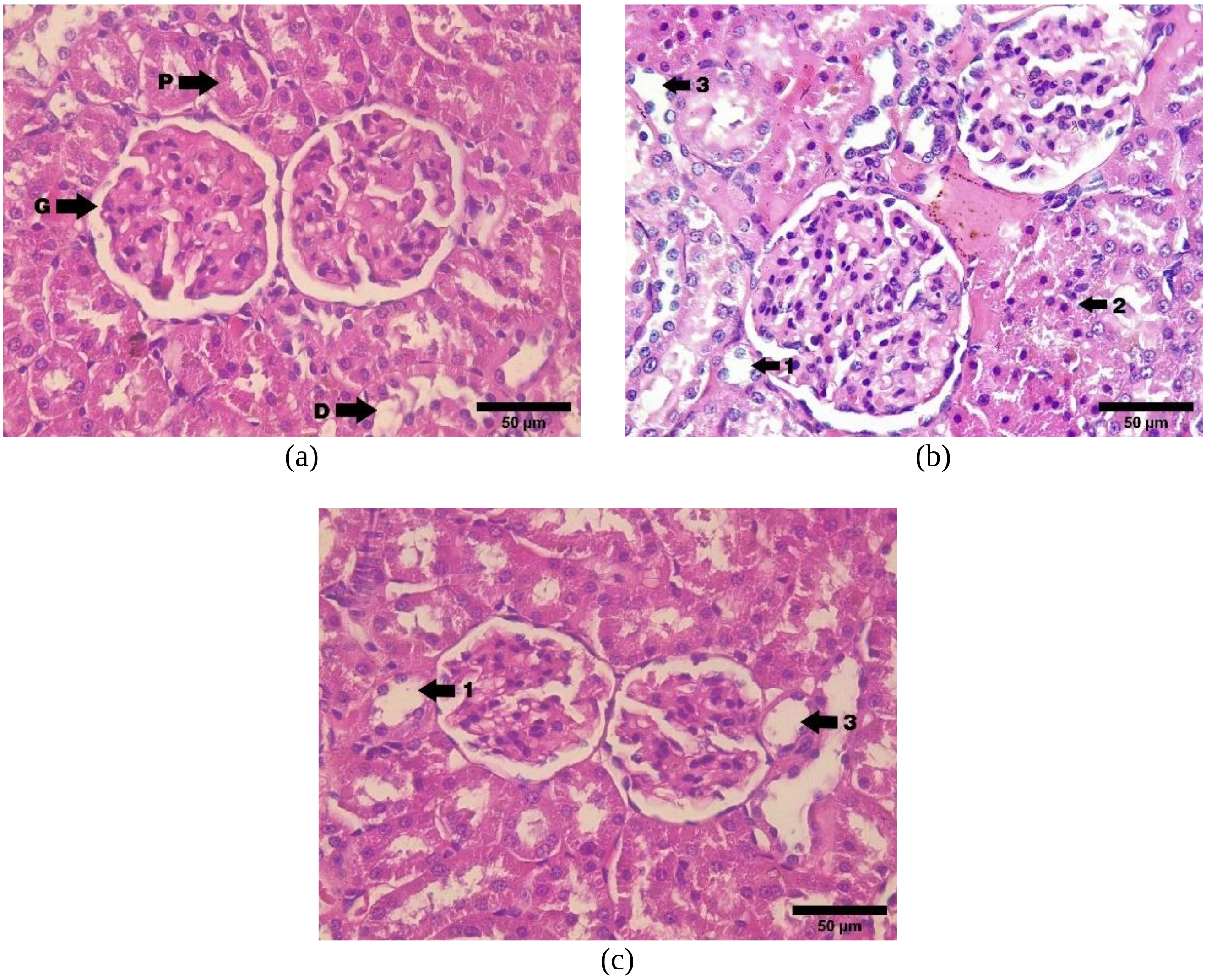
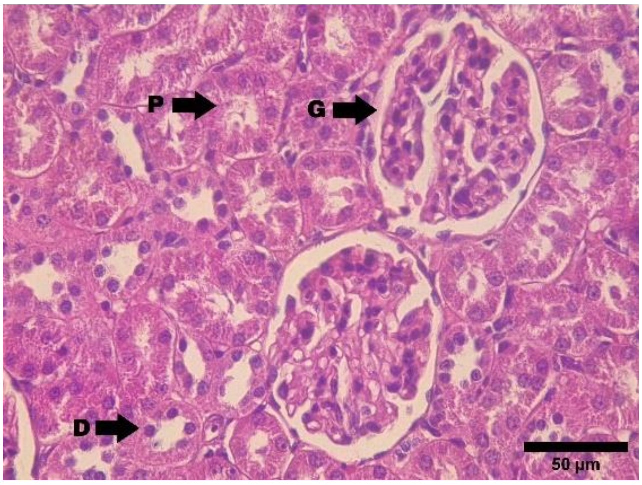


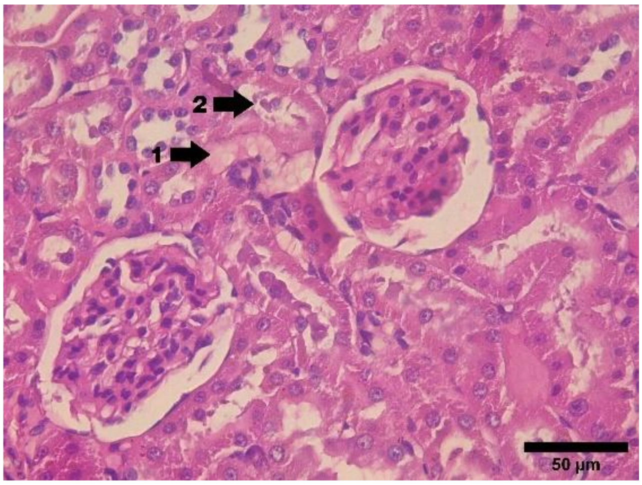
Figure 1. Treatment of natural and heavy metal decontaminated *Ulva lactuca* L. in albino Wistar rats (*Rattus norvegicus* Berkenhout, 1769) for 30 days orally caused loss of brush border (1), cell degeneration in the form of necrosis (2), and widening of the lumen (3) in the proximal tubule. Observations were made using a light microscope at 40 × 6.5 magnification scale: 50 µm, HE staining. Note: glomerulus (G), proximal convoluted tubule (P), distal convoluted tubule (D), control group (a), natural *Ulva lactuca* group (b), and heavy metal decontaminated *Ulva lactuca* group (c).

treatments ($p > 0.05$). Group I (control) experienced the highest average increase in creatinine levels after 30 days of treatment. Despite experiencing a high increase, Group I creatinine levels were still within the normal range. The average creatinine level of Group II (natural *Ulva lactuca*) before treatment was slightly higher than the Group I. The creatinine level of Group II experienced an increase not as much as that of Group I, so the average levels after treatment were lower than the Group I. Although it experienced an increase, the treatment with natural *Ulva lactuca* tended not to be high compared to the control group and was still in the normal range. It can be assumed that the treatment does not interfere with kidney function, especially the filtration process in the kidney's glomerulus.

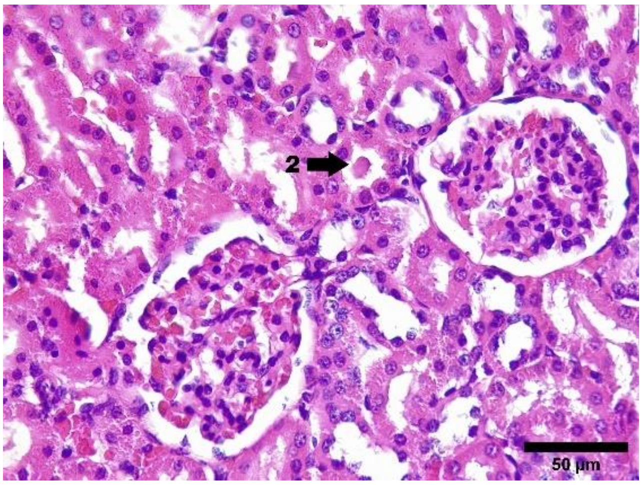
The average creatinine levels in Group III (decontaminated *Ulva lactuca*) showed significantly different results from the Group I (control). The creatinine levels in the Group III before and after treatment were below the control group but showed a high increase in creatinine levels, approaching the control group. Kazak and Cohen (2020) stated that the increase in creatinine levels could be influenced by the rat's daily activities, so it increased muscle metabolism along with the filtration process in the rat kidney glomeruli. Fitria *et al*. (2019) explained that creatinine levels in female rats increase with age and hormonal influences. According to Balali-Mood *et al*. (2021), mercury and lead can be inhibitors of the enzymes GSH (Glutathione), CAT (Catalase), and SOD (Superoxide Dismutase), which act as natural antioxidants in the body. Mercury can be deposited in the brain as a heavy metal, causing cognitive and nervous disorders. Paduraru *et al*. (2022) explained that mercury toxicity causes a weakening of the motor system and a decrease in motor skills. This indicates that treatment with



(a)



(b)



(c)

Figure 2. Treatment of natural and heavy metal decontaminated *Ulva lactuca* L. in albino Wistar rats (*Rattus norvegicus* Berkenhout, 1769) for 30 days orally caused vacuolization of the tubular lumen (1) and accumulation of cell debris in the tubular lumen (2). Observations were made using a light microscope at 40 × 6.5 magnification scale: 50 µm, HE staining. Note: glomerulus (G), proximal convoluted tubule (P), distal convoluted tubule (D), control group (a), natural *Ulva lactuca* group (b), and heavy metal decontaminated *Ulva lactuca* group (c).

natural *Ulva lactuca*, which still contains Hg contaminants exceeding the threshold and high enough Pb, may affect the motor system in white rats so that their activity becomes lower. The increase in creatinine levels in Group III was still within the normal range for Wistar rats. This shows that although there was a relatively high increase, the *Ulva lactuca* decontamination treatment did not interfere with rat kidney function.

Histopathological Findings

Histopathological slides of the rat kidneys were observed in a comparative descriptive and analyzed using the binary scoring method. The binary scoring method was used to determine the presence of kidney damage in rats according to predetermined damage criteria (Gibson-Corley *et al*., 2013). Observation of kidney histology was carried out on the part of the kidney cortex containing the glomeruli and kidney tubules of rats treated with *Ulva lactuca* L. Observations were made by describing the comparison between the Group I (control), Group II (natural *Ulva lactuca*), and Group III (decontaminated *Ulva lactuca*) (Figure 1 & Figure 2).

The process of filtration in the kidney's excretory system occurs in the glomerulus. Bowman's capsule is a glomerular sheath composed of single layers of squamous epithelial cells. According to Zainuddin *et al*. (2023), an increase in the kidney's workload can trigger dilation of the glomerulus and Bowman's capsule. Histopathological observations in this study showed no widening of Bowman's capsule. This indicates that treatment with natural or decontaminated *Ulva lactuca* L. did not cause structural damage to the glomerulus and Bowman's capsule in the kidneys of albino Wistar rats.

According to Lagho *et al*. (2017), the proximal tubule is the part of the kidney that most often experiences damage

Table 5. Kidney histopathological scoring of albino Wistar rats (*Rattus norvegicus* Berkenhout, 1769) with *Ulva lactuca* L.

Tissue Damage	**Group**		
	I	**II**	**III**
Tubular lumen dilatation	v	v	v
Accumulation of cell debris in the lumen	v	v	v
Tubular lumen vacuolization	-	v	-
Degeneration	v	v	v
Missing brush borders	v	v	v

Note: (a) Sign (v) indicates damage on histopathological observation, whereas (-) indicates no damage on histopathological observation (b) I: Control, II: Natural *Ulva lactuca* L. (sample were washed with distilled water), III: Heavy metal decontamination *Ulva lactuca* L. (sample were washed with distilled water and soaked in bilimbi juice with 15% concentration for 60 minutes).

and degeneration due to exposure to nephrotoxic substances. Based on histopathological observations, Group II and III experienced cell degeneration in the form of cell necrosis (Figure 1.). Several proximal tubules in Group II showed nuclear epithelial cell pyknosis and loss of the brush border. This is in accordance with research by Rana *et al*. (2018), which stated that Pb and Hg accumulated most in the proximal tubules so that they could cause damage of histological structures. According to Qadar *et al*. (2019), disturbances in the glomerular filtration process will cause the filtration rate to decrease so that the work of the kidney tubular cells in the excretion process becomes heavier. The disturbance will cause tubular cells to die so that the lumen looks widened and leaves cells or necrotic debris in the lumen. The proximal tubules of all groups were damaged in the form of a widening of the lumen, which also showed loss of the brush border, microvilli which plays a role in helping the process of absorbing excess fluid (Permana *et al*., 2022). The loss of the brush border indicates a disturbance in the absorption process, which can be caused by heavy metals so that the lumen becomes wider.

Another damage seen in the histological structure of Group II is the vacuolization of the tubular lumen. Dewi *et al*. (2013) explained that vacuolization of the tubular lumen can occur because there are fat deposits in the tubular lumen to form vacuoles. The study also stated that vacuolization and increased exposure to chemicals or compounds toxic to the kidneys occur. Cell morphological changes due to injury are referred to as cell degeneration. Cell degeneration is seen by swelling of the cytoplasm due to cell injury caused by chemicals. Degeneration can be reversible or irreversible. According to Qadar *et al*. (2019), necrosis is included in cell degeneration, including cell swelling, pyknosis of cell nuclei, karyorrhexis, and karyolysis. In Group II, cell degeneration was found in the form of fat degeneration and necrosis.

Based on histopathological observations, it was found that there was some damage in all groups (Table 5). Table 5 shows the histological damage to the kidneys of female albino Wistar rats after being treated with *Ulva lactuca* L. orally for 30 days. Based on the table, it is known that damage in the form of widening of the tubular lumen, accumulation of debris cells in the lumen, cell degeneration, and loss of the brush border occurred in all groups. The tubular lumen vacuolation was only found in Group II. Based on observations, there was no widening of Bowman's space, hyperplasia, and hemorrhage in all groups.

The damage criteria used in the scoring process were dilatation of the tubular lumen, accumulation of cell debris in the lumen, vacuolization of the tubular lumen, widening of Bowman's space, degeneration, hyperplasia, loss of the proximal tubular brush border, and hemorrhage. This damage criterion refers to Qodar *et al*. (2019) regarding histopathological observations of the kidneys of Wistar rats induced by diazinon after being given soy flour. Qodar *et al*. (2019) observed the damage and carried out a histopathological assessment using the ordinal method. As for this study, histopathological scoring was carried out using the binary method to determine the presence of damage in each replication. This research has limitations in knowing the percentage of severity for each damage criterion, so binary scoring is done to determine the distribution of whether there is damage in each group.

CONCLUSION

Decontamination using bilimbi (*Averrhoa bilimbi*) juice concentration of 15% (v/v) for 60 minutes has succeeded in reducing the levels of heavy metals Cd, Pb, and Hg in *Ulva lactuca* L. Natural and heavy metal decontaminated *Ulva lactuca* L. administration did not interfere with kidney function as indicated by BUN and creatinine levels within the normal range. Heavy metal decontaminated *Ulva lactuca* L. at a dose of 1000 mg/Kg BW/day exhibited improved kidney histopathology, suggesting its potential as a nephroprotector. However, the level of effectiveness of the bilimbi juice concentration in this study is not fully known, so further research is needed with various concentrations and soaking times of the bilimbi juice as a sequestrant. Further research is needed to explore the heavy metal decontaminated *Ulva lactuca*'s broader applications in promoting kidney health and as a healthy food ingredient.

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REFERENCES

Angrella, N., Indrawati, R. & Dewi, L. (2020). Pengaruh Pemberian Virgin Coconut Oil terhadap Kadar Kreatinin dan Urea Nitrogen Darah Rattus Norvegicus Jantan. *Hang Tuah Medical Journal*, 17 (2), 181–191. Retrieved from https://journal-medical.hangtuah.ac.id/index.php/jurnal/article/view/98

[BPOM] Badan Pengawas Obat dan Makanan. (2022). *Peraturan Badan Pengawas Obat dan Makanan*. Jakarta, pp. 1–18. Retrieved from https://standarpangan.pom.go.id/dokumen/peraturan/202x/logam_2022.pdf

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Front. Pharmacol*. 12, 643–972. https://doi.org/10.3389/fphar.2021.643972.

Chen, P., Bornhorst, J., Neely, M. D. & Avila, D. S. (2018). Mechanisms and Disease Pathogenesis Underlying Metal-Induced Oxidative Stress. *Hindawi: Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2018/7612172.

Dewi, A. K., Suarni, N. M. R., & Suaniti, N. M. (2013). Gambaran Mikroskopis Ginjal Tikus Putih (*Rattus* sp.) Jantan Dewasa Setelah Pemberian Etanol Kronis, *Jurnal Biologi*, 17, 33–36.

Dominguez, H., & Loret, E. P. (2019). *Ulva lactuca*, A Source of Troubles and Potential Riches. *Marine Drugs*, 17, 1–20, https://doi.org/10.3390/md17060357

Ezedom, T., Asagba, S. & Tonukari, N. J. (2020). Toxicological Effects of The Concurrent Administration of Cadmium and Arsenic Through the Food Chain on The Liver and Kidney of Rats. *The Journal of Basic and Applied Zoology*, 81, 16. https://doi.org/10.1186/s41936-020-00146-2

Fitria, L., Lukitowati, F. & Kristiawati, D. (2019). Nilai Rujukan untuk Evaluasi Fungsi Hati dan Ginjal pada Tikus (*Rattus norvegicus* Berkenhout, 1769) Galur Wistar. *Jurnal Pendidikan Matematika dan IPA*, 10, 243–258. https://doi.org/10.26418/jpmipa.v10i2.34144.

Gibson-Corley, K. N., Olivier, A. K. & David, K. M. (2013). Principles for Valid Histopathologic Scoring in Research. *Vet Pathol*. 50, 1–22. https://doi.org/10.1177/0300985813485099 .

Hudaifah, I., Mutamimah, D., & Utami, A. U. (2020). Komponen Bioaktif dari Euchema cottonii, *Ulva lactuca*, Halimeda opuntia, dan Padina australis. *Jurnal Ilmu Perikanan dan Kelautan*, 2, 63–70, https://doi.org/10.36526/lemuru.v2i2.1268

Hudaya, R. (2010). *Pengaruh Pemberian Belimbing Wuluh (Averrhoa Bilimbi) terhadap Kadar Kadmium (Cd) pada Kerang (Bivalvia) yang Berasal dari Laut Belawan.* Fakultas Kesehatan Masyarakat Universitas Sumatera Utara. Sumatera. pp: 1 – 198.

Jatmiko, T. H., Prasetyo, D. J., Poeloengasih, C. D., Hernawan, H. & Khasanah, Y. (2018). Nutritional Evaluation of *Ulva sp*. from Sepanjang Coast, Gunungkidul, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 251 (2019) 012011 https://doi.org/10.1088/1755-1315/251/1/012011.

Kazak, L. & Cohen, P. (2020). Creatine Metabolism: Energy Homeostasis, Immunity, and Cancer Biology. *Nature Reviews Endocrinology*. 16, pp. 421–436. https://doi.org/10.1038/.

Ktari, L. (2017). Pharmacological Potential of Ulva Species: A Valuable Resource. *Journal of Analytical & Pharmaceutical Research*, 6, pp. 2–4. https://doi.org/10.15406/japlr.2017.06.00165

Lagho, E. E., Made Kardena, I. & Jayawardhita, A. A. G. 2017. Gambaran Histopatologi Ginjal Tikus Putih (*Rattus norvegicus*) yang Diberi Amoxicillin Dikombinasikan dengan Deksametason dan Asam Mefenamat Pasca Operasi. *Indonesia Medicus Veterinus Agustus*, 6, 2477 – 6637. Retrieved from https://ojs.unud.ac.id/index.php/imv/article/view/36033

Laksmi, N. L. G. M. C., Dada, I. K. A. & Damriyasa, I. M. 2014. Bioaktivitas Ekstrak Daun Tapakdara (*Catharanthus roseus*) terhadap Kadar Kreatinin dan Kadar Ureum Darah Tikus Putih (*Rattus norvegicus*). *Buletin Veteriner Udayana*, 6, 147–152. Retrieved from https://ojs.unud.ac.id/index.php/buletinvet/article/view/10626

Mulyati, Ningrum, W., & Calista, P. (2021). *Penurunan Kadar Kadmium (Cd) sebagai Kontaminan pada*

Makroalga Ulva lactuca L. Lecturer and Student Collaborative Research Report 2021 Faculty of Biology Universitas Gadjah Mada, pp. 1-13.

Nurhayati, N., & Navianti, D. (2017). Pengaruh Konsentrasi Air Perasan Belimbing Wuluh Terhadap Kadar Cadmium Pada Ikan Laut. *Jurnal Kesehatan Palembang*, 12, 51–58. Retrieved from https://jurnal.poltekkespalembang.ac.id/index.php/JPP/article/view/15/8

Nurmiyati, N., Ramli, M., Rinanto, Y. & Widoretno, S. (2015). Pemanfaatan Sumber Daya Alam Pesisir Secara Berkelanjutan Melalui Pengolahan Makro Alga sebagai Oleh-Oleh Khas Pantai Krakal. *Seminar Nasional Konservasi dan Pemanfaatan Sumber Daya Alam*, 171–176. Retrieved from https://jurnal.fkip.uns.ac.id/index.php/kpsda/article/download/5368/3784

Olsson, E. (2013). *Effects of Citric Acid on Starch-Based Barrier Coatings. Doctorate. In Science and Technology* (Issue May).

Ondu, A. F., & Jayadipraja, E. A. (2019). Efektifitas *Citrus aurantifolia* Swingle dan *Averrhoa bilimbi* dalam Menurunkan Konsentrasi Timbal pada Kerang Kalandue (*Polymesoda sp*.) dari Teluk Kendari. *Hygiene*. 5, 1–13. Retrieved from https://journal.uin-alauddin.ac.id/index.php/higiene/article/view/9760

Paduraru, E., Iacob, D., Rarinca, V., Rusu, A., Jijie, R., Ilie, O-D., Ciobica, A., Nicoara, M., & Doroftei, B. (2022). Comprehensive Review Regarding Mercury Poisoning and Its Complex Involvement in Alzheimer's Disease. *International Journal of Molecular Sciences* 23(4), 1992. https://doi.org/10.3390/ijms23041992.

Permana, E. R. O., Hadi, R. S., & Arifandi, F. (2022). Pengaruh Fermentasi Madu (*Apis mellifera*) Terhadap Gambaran Histologi Ginjal Tikus yang Diinduksi Cisplatin dan Tinjauannya Menurut Pandangan Islam. *Junior Medical Journal*, 1, 206–212.

Qodar, T. S., Wisudanti, D. D. & Aziz, A. M. (2019). Efek Pemberian Tepung Kedelai terhadap Gambaran Histopatologi Ginjal Tikus Wistar yang Diinduksi Diazinon. *eJKI*. 7, 66–71. https://doi.org/10.23886/ejki.7.10287.

Rana, M. N., Tangpong, J., & Rahman, Md. M. (2018). Toxicodynamics of Lead, Cadmium, Mercury and Arsenic-Induced Kidney Toxicity and Treatment Strategy: A Mini Review. *Toxicology Reports* 5, 704–713. https://doi.org/10.1016/j.toxrep.2018.05.012.

Rasyid, A. (2017). Evaluation of Nutritional Composition of The Dried Seaweed *Ulva lactuca* from Pameungpeuk Waters, Indonesia. *Tropical Life Sciences Research*, 28, 119–125. http://doi.org/10.21315/tlsr2017.28.2.9.

Sica, A. M. P. (2017). *Uji Fungsi Ginjal dan Hati Tikus Putih (Rattus novergicus Berkenhout, 1769) Galur Wistar pada Uji Toksisitas Oral Subkronis Filtrat Buah Luwingan (Ficus hispida L.f.)*. Skripsi Universitas Atma Jaya: Yogyakarta.

Sukmawati, S., Rahmawati, R., & Isyanda A., K. 2022. Efek Antinefrotoksisitas Ekstrak Etanol Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb.) dengan Parameter Kadar Ureum Tikus Putih. *Jurnal Ilmiah As-Syifaa*, 13, 116–121. https://doi.org/10.56711/jifa.v13i2.779

Zainuddin, Z., Syahputri, F. O., Masyitha, D., Aisyah, S., Iskandar, C. D., Rahmi, E. & Riandi, L. V. (2023). Gambaran Histologi dan Histomorfometri Ginjal Kalkun (Meleagris gallopavo) pada Tingkatan Umur Berbeda. *Jurnal Ilmiah Mahasiswa Veteriner (JIMVET)*, 7, 13–21. https://doi.org/10.21157/jim%20vet..v7i1.17388

Zou, Y., Zhang, C., Ju, X., Wang, Z., Wu, Y., Yuan, J., Chen, W., & He, R. (2019). Effect of Removing Cadmium with Citric Acid on the Physicochemical and Microstructure Properties of Rice Bran. *Food Control*. 98(August 2018), 290–296. https://doi.org/10.1016/j.foodcont.2018.11.044