



## Reduction lead metal in process marinating local food fish pendap using lemon juice (citrus limon)



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### ABSTRACT

Fish have the potential to be contaminated with heavy metals such as lead, which can originate from human activities, industry, and ports. The Bengkulu people have a traditional processing method that is believed to reduce heavy metal content in fish through the immersion of fish in Pendap dishes using water and lemon. This study aims to observe the reduction of heavy metal content in fish during the immersion process using water and lemon juice in Pendap dishes. The research was experimental with positive and negative results and analyzed using the SPSS ANOVA test to see significant differences between treatment groups. The fish used as samples included Tongkol *Sarda orientalis*, Kembung *Rastrelliger kanagurta*, Tuna *Thunnus albacares*, and Tenggiri *Scomberomorus Lacepède*. The parts of the fish that were analyzed included the muscles, liver, and gills. All samples were exposed to  $Pb(NO_3)_2$  and divided into 3 treatment groups: 1 positive control, 2 precipitated with water, and 3 precipitated with lemon juice (*Citrus limon*). The samples were tested using the reagents NaOH, KI,  $NH_4OH$ , and HCl. Based on the research results, it was found that the application of lemon juice produced significant results in reducing Pb levels, as indicated by the negative test results.

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### INTRODUCTION

Indonesian people, especially people on the coast, make marine fish a staple food in their daily diet. One of the beaches that provides abundant natural resources of fish is Bengkulu beach. The types of marine fish that are widely consumed by the people of Bengkulu city, based on data

from the Central Statistics Agency (BPS) 2024, the average consumption per capita per week, according to fish groups throughout 2024, are tongkol, tuna, tenggiri, kembung, skipjack, dencis, yellowtail, mackerel, anchovy, and baronan. From some of these fish there are some fish that are found to potentially contain heavy metals based on research conducted by Fadlilah *et al.* (2023) found the heavy metal element lead (Pb) in *Sarda orientalis* tuna and *Rastrelliger kanagurta* mackerel. In addition, in the literature analysis, researchers found heavy metal levels in Tuna *Thunnus albacares* and Tenggiri *Scomberomorus Lacepède* in Indonesian waters in the journal Djedjibegovic *et al.* (2020). Therefore, the fish selected to be used as research samples include Tuna *Sarda orientalis*, Mackerel *Rastrelliger kanagurta*, Tuna *Thunnus albacares*, and Tenggiri *Scomberomorus Lacepède*.

Human activities such as industry, ports, ponds, and settlements near coastal waters and around rivers that flow into estuaries and oceans cause environmental pollution through heavy metal contamination and pollution (Hananingtyas, 2017). It is in these estuaries and shallow water areas that most traditional and small-scale fishermen fish for food and daily income. Heavy metals are known to bioaccumulate in aquatic organisms such as fish, especially in fish liver (Suryani *et al.* 2018). Heavy metals enter the fish body through direct absorption from the water through the gills, skin, and through ingestion of contaminated food or non-food particles (Macirella *et al.* 2019). The metals then enter the fish's bloodstream and are carried into body tissues, especially the liver, where they are biologically converted and either excreted or passed up the food chain to consumers, such as humans. Heavy metals can harm human health if consumed in excess. Lead (Pb), for example, can cause anemia, brain damage, and miscarriage in pregnant women (Pratama *et al.* 2024). Metals absorbed by fish are distributed differently in fish organs such as liver, gills, muscles, and gonads, causing variations in metal accumulation in these organs (Suryani *et al.* 2018). Liver, gills, and muscle are most widely used as bioindicators in metal analysis in fish due to their different roles in the metal bioaccumulation process and their potential in the human diet (Eika, 2019). The gills are the initial accumulation site of heavy metal entry pathways in the fish body (Sari *et al.* 2017). The liver is a detoxification center that processes metabolism when there are toxic substances in the body (Sugiantari *et al.* 2022). Heavy metals that cannot be eliminated will be stored in the liver, causing accumulation in the organ. Muscle is the main part of fish that can be eaten by humans and thus is the most favorable indicator for assessing public health risks associated with metal contamination in fish (Yin *et al.* 2024).

The risk to humans is most apparent when contaminated fish is consumed in excess of the recommended daily intake level (Demelash & Alefe, 2024). Heavy metal lead (Pb) is one of the heavy metals found in fish. Based on the Indonesian National Standard (SNI) 2009, the maximum limit of lead metal contamination in fish is 0.3 mg/kg. The impact on health can cause kidney damage, liver disease, nerve disease, vascular disease, cancer, vomiting, and diarrhea (Arifiyana *et al.* 2023). Heavy metals in contaminated fish are invisible, odorless, and cannot be washed away, so they cannot be detected by the naked eye.

Fish are at the end of the aquatic food chain, which is vulnerable to heavy metal accumulation and can be transmitted through the food chain, which can pose a health risk to humans. So there must be an effort to reduce heavy metal levels in the fish to make it safe for consumption. The Bengkulu community has one traditional processing practice that is thought to reduce heavy metal levels in fish through the process of soaking fish in the process of making local food Pendap.

Pendap is a specialty food originating from Bengkulu Province, precisely from Manna, Bengkulu Selatan. Pendap is a food made from marine fish that is deposited (fermented), given various spices and herbs, wrapped using taro leaves, and cooked for 8 hours (Iktarastiwi & Marwanti 2023). The name pendap itself is taken from the process of settling the fish before

cooking. In the beginning, the Bengkulu community cooked pendap to eat fish that was almost unconsumable (almost rotten) so that it was still delicious when eaten. Until now, the process of making pendap has used fermented fish to produce authentic and distinctive pendap. People usually ferment the fish using a method of settling or soaking the fish in water or lemon for approximately 8 hours or overnight before the fish is cooked Pendap. The fermentation process is generally done using plain water and local lemon (*C.limon*).

Heavy metals in fish can be reduced by citric acid content, which can form complex compounds with metals because it has functional groups -COOH and -OH (Putri *et al.* 2023). Citric acid is a weak organic acid found in citrus plants. Citric acid is found in a variety of fruits and vegetables, such as tomatoes, lemons, and oranges, but is found in high concentrations in lemons and limes, which can reach up to 7-8% of fruit weight (Lestari *et al.* 2024).

Foodstuffs containing heavy metal contamination can be liberated through citric acid and flavonoids that function as heavy metal binding agents. Flavonoids can transfer their electrons or hydrogen atoms to free radical compounds to bind to heavy metals (Destria *et al.* 2019). This was proven in the research of Lindawati & Nofitasari (2021), which resulted in a lemon juice concentration that was able to reduce metal levels by 50%. In addition, citric acid in lemon can also react with Trimethylamine Oxide (TMAO) compounds in fish, which is one of the causes of fishy odor. This reaction helps convert TMAO into a more odorless compound. Citric acid reduces the levels of heavy metals in organisms and causes concentration escalation, converting those heavy metals into non-reactive compounds. Thus, lemon (*C.limon*) has the potential as an ingredient that can be used to reduce heavy metal levels in fish.

Previously, the people of Bengkulu were unaware that soaking fish in a process of making Pendap with ingredients containing citric acid, such as lemon, can reduce the heavy metal content in fish. Until now, people have only understood that the name of the dish, Pendap, came from its method of preparation, which is fish being sedimented or soaked in water. Therefore, this soaking stage is considered important for producing an authentic and distinctive Pendap. It is hoped that after this research, the people of Bengkulu can add lemon to the fish soaking solution to free it from heavy metal contamination.

This research was conducted to strengthen the potential of the Bengkulu community in processing fish to be free from heavy metal contamination by adding lemon to the process of making the local food Pendap. Therefore, this research aims to see the reduction in heavy metal content in fish during the fish soaking process using plain water and lemon juice (*C. limon*) in processed Pendap.

## RESEARCH METHODS

### Research Design

The research is experimental with a posttest-only design to determine the effect of a treatment on a group, with measurements taken after the treatment is administered. This research aims to examine the decrease in lead metal levels in fish samples using several test reagents with three replications. The research design for each treatment can be seen in Table I.

Table I. Treatment Design

Fish parts	Initial stage	Treatment		
		1	2	3
muscle	Given a I Mol Pb exposure for 30 minutes	No further treatment was given	Given the treatment of being soaked in 100% plain water for 8 hours after exposure	Given treatment of soaking in 100% lemon juice for 8 hours after exposure

heart	No further treatment was given	Given the treatment of being soaked in 100% plain water for 8 hours after exposure	Given treatment of soaking in 100% lemon juice for 8 hours after exposure
Gill	No further treatment was given	Given the treatment of being soaked in 100% plain water for 8 hours after exposure	Given treatment of soaking in 100% lemon juice for 8 hours after exposure

This research began with sample preparation, including fish filleting and exposure of the samples to lead (Pb). After that, the samples were treated with three different treatments. Subsequently, testing was conducted using test reagents with three replications to obtain valid results. The research flow is presented in Figure 1.

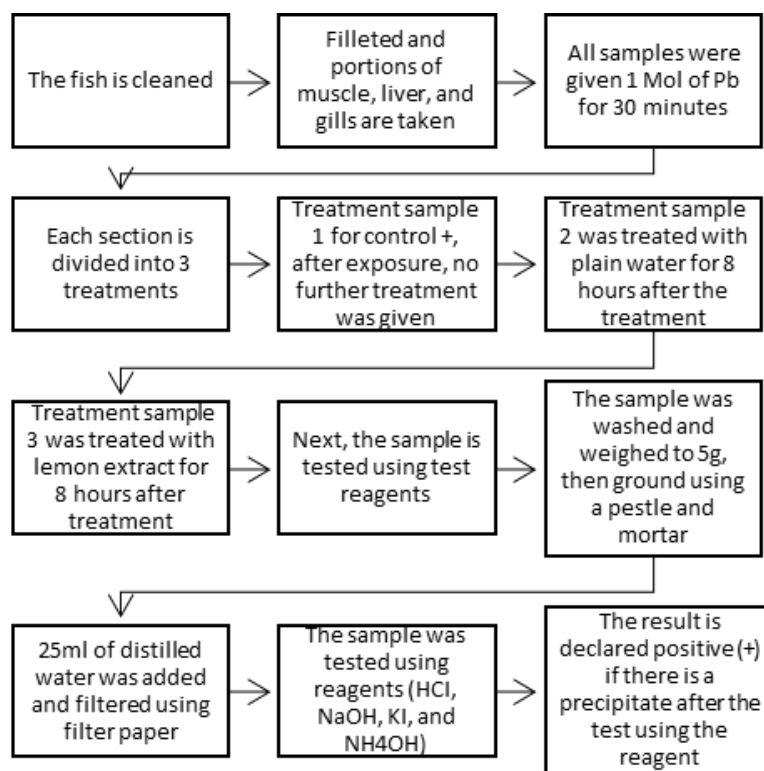


Figure 1. Research flow

### Instruments

The tools used in the research include knives, cutting boards, analytical scales, pestle 1, mortar 1, beaker 6, measuring cup 2, test tube 12, drop pipette 6, stirring rod 2, test tube rack 2, and filter paper. The samples used include Tongkol (*Sarda orientalis*), Kembung (*Rastrelliger kanagurta*), Tuna (*Thunnus albacares*), and Tenggiri (*Scomberomorus Lacepede*), which were taken from the muscle, liver, and gills of 5 grams each. Samples were obtained from the Baai Island Fishing Port market, Sumber Jaya Village, Kampung Melayu District, Bengkulu City, Bengkulu Province. Other materials used were Lemon juice (*C.limon*) 100% and plain water 100%, which is water obtained from wells and has not been cooked. Chemicals used include  $Pb(NO_3)_2$  1M, NaOH 2M, KI 0.5 M,  $NH_4OH$  25%, HCl 37% and Aquades. Chemicals and equipment were obtained from the Laboratory of the Faculty of Teacher Training and Education, Bengkulu University.

## Procedures

Samples were cleaned, and *fletting* was performed to remove muscle, liver, and gills. Next, all samples were given a  $Pb^{2+}$  solution for 30 minutes as pretreatment. All sample parts (muscle, liver, gills) were divided into three different treatments. P1 treatment sample as a positive control; this sample was not given further treatment after exposure. The P2 treatment sample was given immersion treatment with plain water for 8 hours after exposure. P3 treatment sample was given immersion treatment with lemon juice (*C.limon*) for 8 hours after exposure. The samples were then tested using test reagents. The testing process begins with washing the sample and weighing 5 grams. The 5 gram sample was then crushed using a pestle and mortar until smooth, added 25 ml of distilled water was added and filtered using filter paper to obtain the filtrate. The filtrate obtained was then tested using test reagents namely HCl, NaOH, KI, and  $NH_4OH$ . The results of the study were declared positive (+) if there was a change in color and sediment after testing using these reagents.

## Data Analysis

The data obtained consisted of positive (+) and negative (-) qualitative conclusions and quantitative statistical hypothesis testing using the ANOVA test. Positive (+) and negative (-) qualitative results were indicated by the formation of precipitates with specific colors. In this study, test results were considered positive if at least three out of four test reagents, with three replicates each, yielded positive results (+). The changes that occurred were based on reactions similar to those performed by Arifiyana (2018). Next, the data were analyzed using the SPSS ANOVA test to see significant differences between treatment groups.

## RESULTS

Samples were identified with several reagents, including HCl, NaOH, KI, and  $NH_4OH$ . Identification with NaOH and KI reagents has been carried out by Purnama *et al.* (2018). Identification with HCl and  $NH_4OH$  reagents has been done by Djamilah & Fernanda (2018). The results of heavy metal lead (Pb) identification in fish are presented in Table I.

**Table 2.** Results of lead (Pb) identification in fish

Type of fish	Part sample fish	Pb Test in fresh fish	Pb test after exposure		
			P1	P2	P3
Tongkol <i>Sarda orientalis</i>	muscle	-	+	+	-
	liver		+	+	-
	gills		+	-	-
Kembung <i>Rastrelliger kanagurta</i>	muscle	-	+	+	-
	liver		+	-	-
	gills		+	-	-
Tuna <i>Thunnus albacares</i>	muscle	-	+	+	-
	liver		+	-	-
	gills		+	-	-
Tenggiri <i>Scomberomorus Lacepede</i>	muscle	-	+	+	-
	liver		+	+	-
	gills		+	-	-

Notes: P1 = control + (no further treatment after exposure); P2 = treatment precipitated with plain water for 8 hours after exposure; P3 = treatment precipitated with lemon juice for 8 hours after exposure.



The qualitative conclusion data, both positive (+) and negative (-), were then statistically analyzed using an ANOVA test. The ANOVA test results can be seen in Table 3.

**Table 3.** ANOVA test results using SPSS

	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.333	11	.576	5.182	.000
Within Groups	2.667	24	.111		
Total	9.000	35			

The ANOVA results show a value of 0.00, which is  $< 0.05$ , indicating a statistically significant difference between the treatment groups. Subsequently, a Duncan's test was conducted to determine which groups showed statistically significant differences in mean after the ANOVA test. The Duncan's test results are presented in Table 4.

**Table 4.** Duncan's test results

Test	Group	N	Subset for alpha = 0.05			
			1	2	3	
Duncan <sup>a</sup>	To-Gill	3	.00			
	Ke-Gill	3	.00			
	Tu-Gill	3	.00			
	Te-Gill	3	.00			
	Ke-Heart	3	.33	.33		
	Tu-Heart	3	.33	.33		
	To-Heart	3		.67	.67	
	Te-Heart	3		.67	.67	
	To-Muscle	3			1.00	
	Ke-Muscle	3			1.00	
	Tu-Muscle	3			1.00	
	Te-Muscle	3			1.00	
	Sig.			.291	.274	.291

Note: To= Tongkol (*Sarda orientalis*); Ke= Kembung (*Rastrelliger kanagurta*); Tu= Tuna (*Thunnus albacares*); Te= Tenggiri (*Scomberomorus Lacepède*).

Based on the ANOVA test, there is a significant difference between the treatment groups. Furthermore, through the Duncan test, it was shown that treatments 1 and 2, as well as treatments 2 and 3, did not differ in liver samples of each fish species, but treatments 1 and 3 showed a significant difference in the decrease of Pb levels. These results indicate that lemon (*C.limon*) treatment was most effective in reducing lead (Pb) levels in fish. Based on these findings, processing fish with lemon is highly recommended to reduce lead (Pb) levels. This is an important step to ensure the safety and quality of fish for consumption.

## DISCUSSION

Prior to exposure and treatment, researchers identified heavy metals in fish. The observation results of Pb heavy metal identification in all types of fish (tongkol, kembung, tuna, tenggiri) before treatment gave negative results, which means not detected, this indicates that the fish samples are not contaminated by Pb metal from nature. Furthermore, the samples were exposed by



immersing them in a 1M  $\text{Pb}(\text{NO}_3)_2$  solution for 30 minutes to see the effectiveness of reducing heavy metal levels in the exposed samples.

### Identification of heavy metals (Pb) in samples after exposure and treatment

After exposure and treatment, the results obtained are in the treatment of P1 samples treated with  $\text{Pb}(\text{NO}_3)_2$  1Mol for 30 minutes showed positive results in each type and part of the fish (muscle, liver, and gills). In the P2 treatment, the samples were treated with plain water for 8 hours after exposure, showing the results of some parts of the fish giving positive results, namely in the muscles and liver in the samples of Tongkol and Tenggiri fish, as well as the muscles in the samples of Mackerel and Tuna fish, but negative in all samples of the gill. While in the P3 treatment, the samples were treated with lemon juice (*C.limon*) for 8 hours after exposure, showing negative results in all types and parts of fish.

The P2 treatment shows that immersion of fish in clean water can help reduce the concentration of heavy metals on the surface of the fish. This occurs because some heavy metals that are loosely adsorbed on the surface of the fish can be released back into the water. Small particles or sediments containing heavy metals on the surface of the fish can be washed away through physical washing. Pb heavy metals that are still in the form of dissolved ions will dissolve in water. However, the effectiveness of water alone is very limited, as many heavy metals are already strongly bound to proteins and tissues within the fish.

The P3 treatment shows that soaking using lemon juice (*C.limon*) can help reduce heavy metal concentrations in fish better; this is shown in all samples, which show negative results after the treatment is deposited in lemon juice solution. Lemon juice (*C.limon*) contains citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ) as the main component, which is an effective chelating agent. Citric acid is a weak tricarboxylic acid, which has three carboxyl groups ( $-\text{COOH}$ ) that can release protons  $\text{H}^+$ . While heavy metals in fish are generally in ionic form, in this study, the heavy metal element used is  $\text{Pb}(\text{NO}_3)_2$ , lead nitrate. Lead(II) nitrate,  $\text{Pb}(\text{NO}_3)_2$ , is a lead salt that dissolves in water and will dissociate into  $\text{Pb}^{2+}$  and  $\text{NO}_3^-$  ions in solution.









The test results showed that the muscle and liver showed more positive (+) results than the gills. This is because the muscle is the largest part of the fish biomass and is often the main target organ for long-term heavy metal accumulation. Muscle has a greater storage capacity than gills, which function as a respiratory organ with a thinner surface that interacts directly with the environment. Various studies have measured lead levels in the flesh (muscle) of fish and often found high concentrations even exceeding safe consumption limits in fish exposed to polluted environments. As in the study conducted by Tore et al. (2021), which showed that higher lead exposure results in the muscle or meat of fish.

The liver is the main organ in detoxification and metabolism in the fish's body. When lead enters the fish's body, either through the gills (breathing) or the digestive tract (food), the liver will process it. The liver has mechanisms to bind heavy metals, including lead in an attempt to neutralize their toxicity. This often involves the production of metal-binding proteins such as *metallothionein* that function to store heavy metals from damaging other cells. As a result, lead tends to accumulate in the liver in higher concentrations for processing or temporary storage. Several journals have shown that the liver has the ability to accumulate heavy metals, including lead, due to its role in biotransformation and detoxification. As in the study conducted by Mziray & Kimirei (2016) which showed higher metal concentrations in fish liver.

### Reactions that occur in Lead nitrate ( $\text{Pb}^{2+}$ ) and Citric Acid

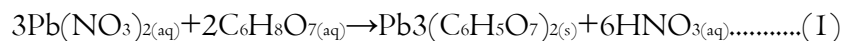
The result of the precipitate in the solution indicates the presence of heavy metal Pb in the fish sample. The result is positive if there is a precipitate with a certain color. Identification of fish before and after exposure using test reagents can be seen in Table 5.

**Table 5.** Picture of lead (Pb) identification results in fish before and after exposure using test reagents.

Test result	HCl	KI	NaOH	NH <sub>4</sub> OH
Before exposure				
After exposure				

The addition of HCl is positive for Pb metal if the sample produces a white precipitate of PbCl<sub>2</sub>. The addition of NaOH is positive for Pb metal if the sample produces a white precipitate of Pb(OH)<sub>2</sub>. The addition of KI is positive for Pb metal if a yellow PbI<sub>2</sub> precipitate is formed. Furthermore, the addition of NH<sub>4</sub>OH reagent is positive for Pb metal if the sample produces a white precipitate of Pb(OH)<sub>2</sub>.

When citric acid is reacted with Pb(NO<sub>3</sub>)<sub>2</sub>, a complex or chelation formation reaction will occur. This is what triggers the mechanism of heavy metal reduction by lemon juice (citric acid). Citric acid is known to bind bivalent metal ions such as Pb<sup>2+</sup>, forming a water-soluble lead-citrate complex or precipitate. The reaction that occurs can be described as follows:



In this reaction, Pb<sup>2+</sup> ions from Pb(NO<sub>3</sub>)<sub>2</sub> will react with citrate ions (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup>) formed from the deprotonation of citric acid. Since citric acid is a weak acid, it will release H ions, which will then react with nitrate ions (NO<sub>3</sub><sup>-</sup>) to form nitric acid (HNO<sub>3</sub>), which will remain dissolved in the solution. So this reaction will produce lead(II) citrate (Pb<sub>3</sub>(C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>)<sub>2</sub>(s)). This compound has a very low solubility in water and will precipitate (especially in high concentrations). Other reactions will produce Nitric Acid 6HNO<sub>3</sub>(aq), which is a by-product. The Nitrate ions (NO<sub>3</sub><sup>-</sup>) that originally paired with Lead will pair with Hydrogen ions (H<sup>+</sup>) released from Citric Acid. Nitric Acid is a strong acid and soluble in water, so it will remain in solution.

According to Azmi & Winarsih (2021), heavy metal toxicity can be reduced or eliminated if the ions first lose their original properties and then react with a metal binding agent. The toxicity of the heavy metal can be reduced or eliminated. This reaction process can help pull heavy metals out of the fish tissue. By soaking the fish in lemon juice, soluble metal-citrate complexes will diffuse from the fish tissue into the lemon juice solution. After soaking, the fish is washed or rinsed so that the dissolved metal-citrate complexes can be removed with the lemon solution. By removing heavy metals from the fish, consuming the fish will be safer because it reduces the bioaccumulation of heavy metals in the human body. The effectiveness of this reduction in heavy metal levels depends on several factors, such as citric acid concentration, soaking time, type of heavy metal, and the initial level of contamination in the fish.

## CONCLUSION

Based on the results of the study, it can be concluded that soaking fish using lemon juice (*C.limon*) is better in reducing heavy metal levels of lead (Pb) compared to soaking using plain

water. Lemon juice (*C.limon*) containing citric acid and flavonoids work as chelating agents that bind heavy metal ions such as  $Pb^{2+}$  to form chelate complexes that are more soluble in water so that they are easily removed from fish tissue. Although plain water can slightly reduce the concentration of heavy metals on the surface of fish through diffusion and physical leaching, its effectiveness is limited because many heavy metals are strongly bound to fish tissues. This study shows that the administration of lemon juice produces significant results in reducing Pb levels as indicated by negative test results. Lemon is recommended to be used by local Bengkulu people who process pendap fish so that the fish is protected from heavy metal Pb and safer for consumption. Based on the ANOVA test, there is a significant difference between the treatment groups. Furthermore, through the Duncan test, it was shown that treatments 1 and 2, as well as treatments 2 and 3, did not differ in liver samples of each fish species, but treatments 1 and 3 showed a significant difference in the decrease of Pb levels. These results indicate that lemon (*C. limon*) treatment was most effective in reducing lead (Pb) levels in fish. Based on these findings, processing fish with lemon is highly recommended to reduce lead (Pb) levels. This is an important step to ensure the safety and quality of fish for consumption.

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