Amazing purifier:

~Let's collect mangrove's fallen leaves for eco-friendly bioremediation of heavy metals~

For the Entry to the Stockholm Junior Water Prize 2023

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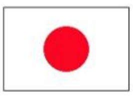

(a) Abstract

Heavy metals are known to be one of the most harmful sources of water pollution. Effective remediation of heavy metals after they have reached the open ocean is not practical on a large scale. Therefore, we propose a cost-effective and environmentally friendly method for collecting heavy metals using mangroves that can be implemented even in developing countries.

Some species of mangroves have salt glands, which expel salt from the surface of their leaves, while others do not. Others without salt glands accumulate salts in their leaves and expel them from their bodies by defoliation. Since the mangrove species "*Kandelia obovata*" which occupies most of Manko wetlands in Okinawa Prefecture, Japan does not have salt glands, we hypothesized that it accumulates salts in its leaves, likewise accumulating heavy metals in its leaves, then expelling them from its body by defoliation. If *Kandelia Obovata* absorb and store heavy metals in their leaves, it will be possible to improve water quality by collecting fallen leaves.

In this research, we investigated how the amounts of elements contained in mangrove leaves change during the process of maturation and defoliation, and found that heavy metals such as As, Cd, and Pb are concentrated in the fallen leaves. For Cd in particular, it was found that fallen leaves contained six times more than young leaves. Furthermore, we conducted a survey to investigate the number of fallen leaves from the *Kandelia obovata* and estimated the amount of heavy metals that could be collected per year by collecting the fallen leaves. As a result, it was found that 2.01 kg of As and 1.29 kg of Cd per year can be collected per 1 km^2 of *Kandelia obovata* forest, and other toxic heavy metals can also be collected.

(b) Table of contents

(c) Abbreviations and acronyms

K.obovata (*Kandelia obovata*)

(d) Acknowledgments

We are deeply grateful to Dr. Iinuma of Okinawa Institute of Science and Technology (OIST) for his expert advice and technical support in the use of the ICP-MS. We would like to express our sincere gratitude to Ms. Taira, Ms. Tei, and Mr. Ikemura, staff members of the Manko Waterbird and Wetland Center, for their cooperation in field research at the wetland, for introducing us to mangrove researchers, and for giving us the opportunity to interact with local people. We would like to thank Mr. Suzuki of the Technical Research Institute of Kajima Corporation for his valuable advice and careful guidance in preparing our essay for the SJWP international competition. Mr. Oshiro, the biology teacher at Okinawa Shogaku High School, provided invaluable assistance in the research and thesis preparation, we gratefully thank you. We would also like to appreciate Mr. Gibo for supervising our experiments. Lastly, we like to express our gratitude to Ms. Mihoko Oshiro, Chairperson of the Board of Directors, and Mr. Yasunori Sueyoshi, Principal of Okinawa Shogaku High School, and Teachers from the IB course. for their support in various aspects of the research.

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(e) Self-introduction

Yukiha Shinjo is a third-year student at Okinawa Shogaku High School and is studying in the IB Programme. She loves to study mathematics and, in her personal research, she is working on topics related to healthcare from a mathematical perspective. She is proactive in her extracurricular activities; she participated as an intern in a company and led the team as the leader of SNS management. In the future, she wants to learn medical engineering and save lives by using technology. Through this research, she could gain skills, in writing a thesis, persevering, cooperation, and explaining things, that could be used in her future. From now on, she would like to expand her network and be active on the global stage.

Fuu Sadoyama is a third-year student at Okinawa Shogaku High School and is studying in the IB Programme. She grew up on an island rich in nature and has loved animals since she was a child. She has a strong interest in environmental issues and would like to work as an environmental advisor in the future, providing support to various institutions. So far, she has been involved in activities such as participating in a business competition to plan eco-friendly products and researching marine plastics as part of her project research. The competition has given us the opportunity to engage with professionals and international organizations working at the frontline of environmental fields, and she wants to use this experience to further advance our activities and achieve her goals for the future.

Chapter 1. Introduction

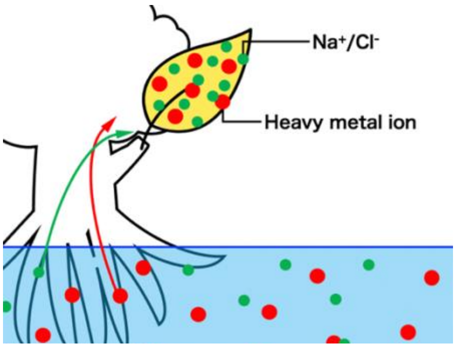

Fig. 1 Image for bioremediation

Some of heavy metals, one of the causes of environmental pollution, are not only harmful to humans, but can also have a negative impact on the entire river and ocean ecosystem through the food chain[i]. For this reason, collecting toxic heavy metal from environmental media such as water and sediment is very important in achieving Sustainable Development Goal 6: "Ensure availability and sustainable management of water and sanitation for all"[ii].

Heavy metals are found in pesticides, agrochemicals, and factory effluents, and can cause soil contamination as well as water pollution. However, heavy metals are very difficult to collect once they discharge into the ocean[iii]. Therefore, it is desirable to collect these toxic heavy metals before they flow into the ocean. However, removing these from wastewater requires a high level of technology and cost, making it both difficult and impractical to collect in developing countries where heavy metal pollution is widespread. Thus, we will propose a cost-effective and environmentally friendly method to collect heavy metals using mangroves (Fig. 1).

Mangroves are a generic name for evergreen trees found in tropical and subtropical regions[iv] and are known to be tolerant to salt because they grow in brackish water areas where rivers flow into the ocean. Some species of mangroves have salt glands, a mechanism that expels salts from the surface of leaves, while others do not.[v] In species without salt glands, salts accumulate in the leaves and are expelled from the body by defoliation[vi]. Near our school, there is a Ramsar wetland, Manko, with a vast mangrove forest. *K.obovata*, which we targeted for this research, are widely distributed and do not have salt glands. Therefore, we hypothesized that if the *K.obovata* accumulated salt and at the same time, heavy metal in their leaves, bioremediation would be possible by collecting fallen leaves to remove the heavy metal from the polluted water. Thus, in this research, we investigated what elements are concentrated in leaves as they mature, and in addition, we estimated how much heavy metal ions could be recovered by collecting the fallen leaves.

We also found an overlap between developing countries with low wastewater treatment rates and distributed areas of mangroves wastewater treatment rates for countries where mangroves are present, indicating that countries in Southeast Asia, South Asia, Africa, and around the Caribbean tend to have low treatment rates. If we can utilize the mangroves that grow in estuaries to collect heavy metals before wastewater flows into the ocean, we can stop heavy metal pollution of the ocean in these regions with low wastewater treatment capacity.

Based on the above, we researched the following points in this research.

(1) Determination of the concentration of heavy metals and other elements contained in young, adult, and fallen leaves of the *K.obovata*, and calculation of the concentration ratio.

(2) Determination of the amount of fallen leaves and calculation of the amount of heavy metals that can be recovered in a year.

(3) Determination of the concentration of elements in brackish water and evaluation of the effect on the concentration of elements in leaves.

Chapter 2. Basic information about Manko Wetland

The target of this research is a mangrove forest at Manko in Naha City, Okinawa Prefecture (Fig. 2). The prefecture belongs to the subtropical regions and is classified as having a subtropical maritime climate, characterized by lush greenery similar to tropical rainforests. Therefore, there are unique species and ecosystems, and the northern region of the main island of Okinawa Prefecture was inscribed as a World Natural Heritage site in July 2021[vii]. Also, Manko, located in the southern part of the main island of Okinawa, is designated under the Ramsar Convention and is home to a wide variety of species, including the endangered species called the Black-faced spoonbill (*Platalea minor)*. However, the surrounding rivers, including the Kokuba River, which flows into Manko, are seriously polluted by illegal dumping and domestic wastewater. Manko's mangrove forests cover 0.08351 km^2 and three species of mangrove vegetate: *K.obovata*, Red Mangrove (*Rhizophora stylosa Griff.*), and Orange Mangrove (*Bruguiera gymorhiza*)[viii]. Of these, the *K.obovata* is the most distributed in Manko[ix].

Fig. 2 Location of Manko Wetland

Chapter 3. Heavy metal concentrations in young, adult, and fallen leaves

3.1 Collection of leaves

We collected 3 types of leaves to determine if the content of elements in leaves varies: "young leaves" that have just sprouted at the first node of the branch, the "adult leaves" that have grown large at the third node of the branch and the "fallen leaves" that were collected in litter traps. We collected the leaves at three locations in Manko defined in Fig. 3 to investigate if there are differences in the elements contained in the leaves depending on locations.

Fig. 3　Sampling locations

3.2 Samples digestion and quantification

Samples digestion and following quantification processes were done with the help of Dr. Iinuma of the Okinawa Institute of Science and Technology Graduate University (OIST). Dried leaves were placed in plastic tubes, concentrated HNO_3 was added, and the tubes were left overnight at room temperature. Organic matter in the solution was then decomposed using a microwave. After the decomposition of the organic matter, 10 uL of 10 mg/L Sc internal solution was added, and the sample was dried (but not completely dried) at 100°C and dissolved in 5 mL of 5% HNO_3. This solution was analyzed by ICP-MS (inductively coupled plasma mass spectrometry). Since the concentration of Na ion was too high to be measured by ICP-MS, we used ion chromatography for the quantification of Na.

3.3 Results

Fig. 4 shows the Na concentrations in the leaves at each growth stage sampled at the locations shown in Fig. 3. The reason for this measurement was to determine whether Na concentration also occurs in the leaves of the *K.obovata*, as mangroves without salt glands process salt in their roots and leaves. However, Fig. 4 shows that the Na concentration in fallen leaves was not significantly higher than that in younger leaves, although different trend was observed for location 3. Therefore, we concluded that *K.obovata* is not capable of concentrating salt in its leaves. In other words, the absorbed salt is not removed from the body by defoliation.

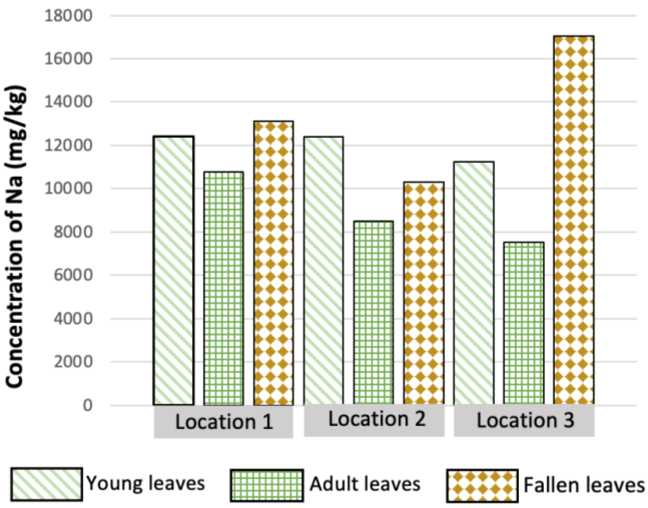

Figure 4 Na conc. at three growth stages

Fig. 5 (shown in next page) shows the average concentration of each element in young, aduct and fallen leaves collected in three locations shown in Fig. 3 (page 6). The error bars represent the maximum and minimum values. The maximum and minimum values for Mn, Fe, P, Ca, and K are not shown, since the three samples were measured together in one sample. These results indicate that 13 elements, including Cd, Pb, As, and Cs, tend to concentrate from young leaves to fallen leaves. On the other hand, the 5 elements, Zn, Cu, P, K, and Cr, did not concentrate on the fallen leaves. It is also clear that this result is completely different from the trend of concentration of Na from young leaves to fallen leaves, and that there are some differences in variation from location to location.

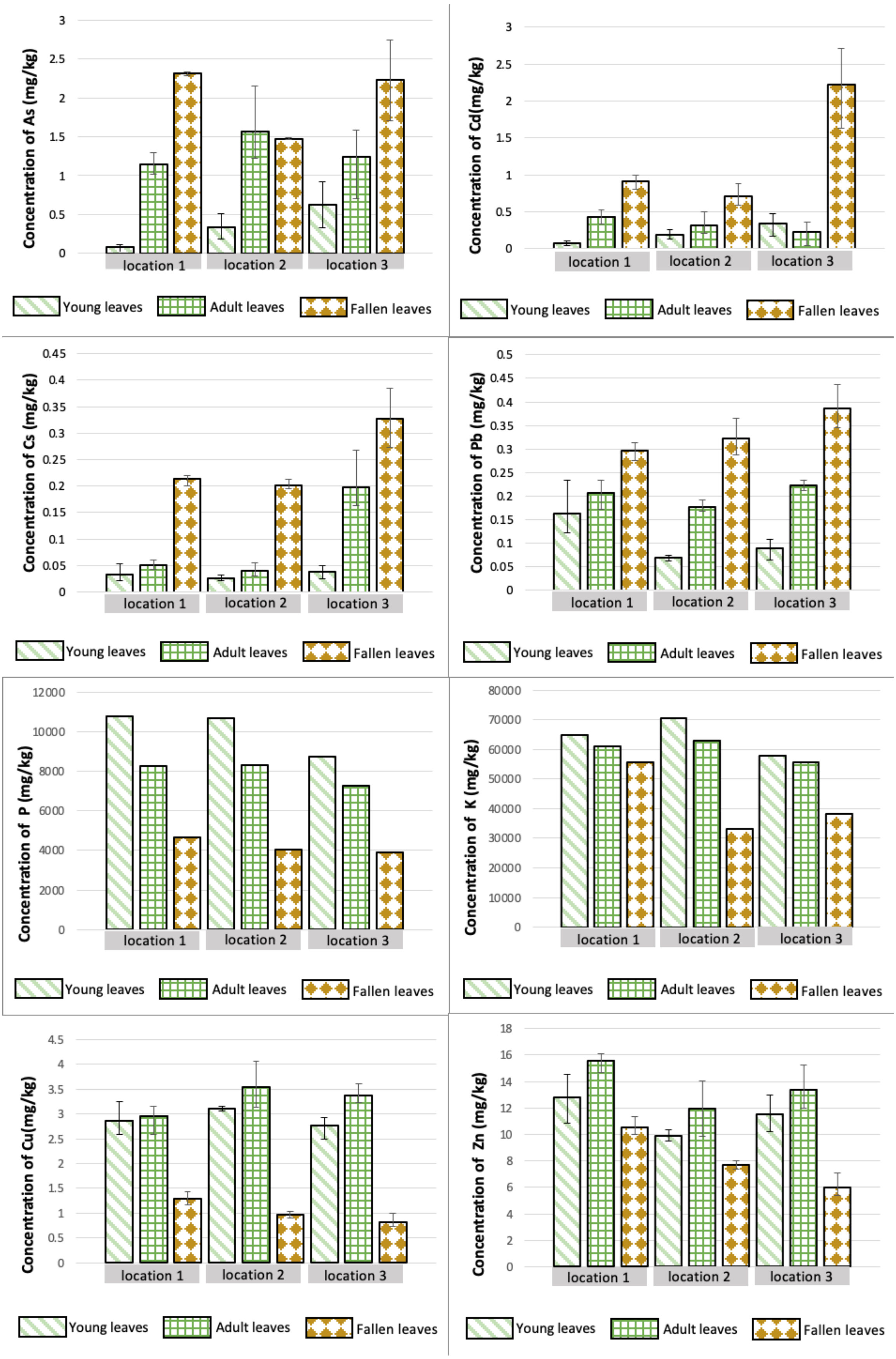

Figure 5 Elements conc. in each level of leaves by the points

Chapter 4. Water quality analysis of brackish water

The results shown in Chapter 3 show that the concentration of each element in leaves varies depending on their location. We hypothesized that this might be due to differences in the concentration of each element in the brackish water. Thus, we tested whether there is any relationship between the concentrations of elements in the brackish water and the concentrations of elements in the leaves.

4.1 Material and methods

Brackish water was sampled and analyzed using the following methods. The sampling locations are shown in Fig. 3 (page 6).

Methods for collecting brackish water and analyzing the water quality

1) At locations 1-3, treaded on mud by shoes at low tide.

2) Collected the liquid seeped out from mud using a micropipette and stored it in tubes.

3) The mixture of water and mud was centrifuged and the supernatant was collected.

4) The supernatant was analyzed using ICP-MS (except Na) or ion chromatography (for Na).

4.2 Results

The concentrations of elements in the brackish water did not show clear correlations with the concentrations of elements in the leaves. In order to investigate how element concentrations in brackish water affect element concentrations in leaves, it is necessary to prepare an environment in which element concentrations are experimentally controlled.

Chapter 5. Investigation on the number of fallen leaves

In this section, we examined the number of fallen leaves of *K.obovata* to estimate how much heavy metals could be collected through the collection of fallen leaves. To determine the amount of fallen leaves, we set the nets at the 3 different locations shown in Fig. 3 (page 6), for a week and examined the amount of fallen leaves collected.

5.1 Materials and methods

We used HOGA's 80cm cone with a diameter of $0.5m^2$ seed trap for the litter trap (Fig. 6). Also, the fallen leaves used in Chapter 3 was collected using this method. Following are the steps for collecting the fallen leaves.

1) Tie the litter trap to the surrounding trees with string.

2) Set a weight in the net to keep the net from tipping over.

3) Collect the fallen leaves that have been caught in the litter trap.

Fig. 6 Litter trap

5.2 Results

After collecting the fallen leaves with these steps, we measured the fresh and dry weights, the results are shown in Table 1. We collected the fallen leaves on two terms, but we did not use the results of second sample because typhoon hit our area. From these results, we estimated the amount of fallen leaves per year and how much of each element could be collected by collecting fallen leaves. The results will be presented in the Section 6.6.

Table 1 Wet and dry weight of collected fallen leaves

	Wet			
location	①	②	③	avg.
(g)	9.2990	15.846	17.608	14.251
	Dry			
location	①	②	③	avg.
(g)	5.6200	11.403	11.853	9.6253

Chapter 6. Discussion

6.1 The concentration of each element in leaves

From Chapter 3, it was found that the elements such as As, Pb, and Cd were concentrated from young leaves to fallen leaves, while elements such as Zn, Cu, P, and K decreased their concentration from young leaves to fallen leaves. It was also found that almost no concentration occurred for Na.

The reason for the reduced content of Zn, Cu, P, and K in fallen leaves is that these elements are essential in vivo[x] (e.g., Zn is essential for transcriptase activity, and Cu is used for chlorophyll synthesis. P is also used to open and close plant pore cells, while P is a component of DNA, etc.) And it could be considered to have been transferred to other parts of the plant before defoliation. This agrees with a study by Shigeru Kato et al. (1986)[xi], who found that Ca is transferred from fallen leaves to young leaves to reuse.

On the other hand, the reason for the concentration of As, Pb, and Cd in fallen leaves could be thought to be that these elements are not essential for growth, so they do not migrate to other parts of the body but are concentrated in the fallen leaves and released out of the body by leaf fall. The reason why Na was not concentrated into fallen leaves can be explained by the fact that it is known that the roots of the *K.obovata* have a salt filtration mechanism, which prevented salt from entering the plant in the first place. This is in agreement with the findings of Fumiko Iwanaga et al. (2014)[xii], who found no significant difference in the salt concentration in various parts of the *K.obovata* when they were grown at different salt concentrations.

This revealed that some heavy metals were concentrated in fallen leaves and could be purified by bioremediation. It was also found that even if fallen leaves is collected for bioremediation, there would be a less negative impact on plant growth due to elements essential for plant growth being utilized by the plant prior to defoliation.

6.2 Concentration ratio between Na and other elements

Fig. 7 shows the ratio at which each element is concentrated from young leaves to fallen leaves. The red line shows where the concentration is 1-fold, while concentrate occurs in the fallen leaves for elements above this level.

Compared with Na and other elements, the ratio of Na was close to 1 in leaves at all stages and differed from that of the other elements. This indicates that Na and other elements may move differently in the *K.obovata*. According to Fumiko Iwanaga (2014)[xiv], when the *K.obovata* was grown at different Na concentrations, only the Na concentration in the roots changed, but not for non-roots parts. This suggests that Na may be selectively expelled from the roots of the *K.obovata* by mechanisms such as Na pumps.

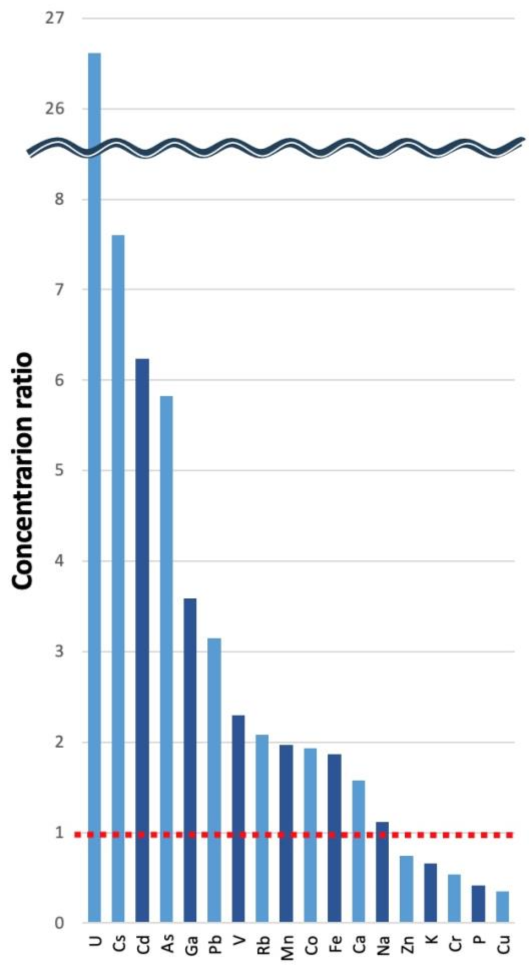

Figure 7 Concentration ratio of each element from young leaves to fallen leaves

For elements other than Na, that will pass through the roots of the *K.obovata*, the concentration ratio is considered to vary depending on whether they are necessary for plant growth or not. Specifically, elements such as Zn, K, and P, which are necessary for plant growth, are transported from the fallen leaves to other locations and reused, resulting in a lower concentration ratio, while elements such as U and Cs, which are not necessary for plant growth, are considered to be concentrated within fallen leaves.

As for elements that are concentrated in fallen leaves, the ratio of elements such as V, Rb, Mn, Co, and Fe was very close, suggesting that they may share a common mechanism for the concentration of these elements in fallen leaves. Also, the ratio of the highly toxic Cd and As were also close, suggesting that they may also share a common mechanism of enrichment.

6.3 Correlation of heavy metal concentrations in fallen leaves and brackish water

In order to investigate the correlation between the number of elements in the fallen leaves and water quality at each location, we drew a trendline and calculate R^2, a positive correlation of 0.8 or higher was obtained for Pb and V. However, it is difficult to say that there is a correlation since negative correlations were obtained for Zn and Fe.

6.4 Correlation between concentration of Na in fallen leaves and amount of fallen leaves

The correlation between the concentration of Na in the fallen leaves at each location and the amount of fallen leaves was examined, resulting in the graphs shown on the left of Fig. 8. We drew a trendline and calculated R^2, which was very low, and, as shown in Chapter 3, there was little difference in the elements in the brackish water at each location; we cannot state a correlation between the two from this result.

6.5 Correlation between concentration of Na in brackish water and amount of fallen leaves

As shown in the graphs to the right of Fig. 8 there is no correlation between the amount of defoliation and the Na content in brackish water. As shown in Chapter 3, this is also the case, since no difference was found in the elements contained in brackish water, it is not possible to see a correlation, and it cannot be said that the high concentration of Na is the reason for the high amount of defoliation.

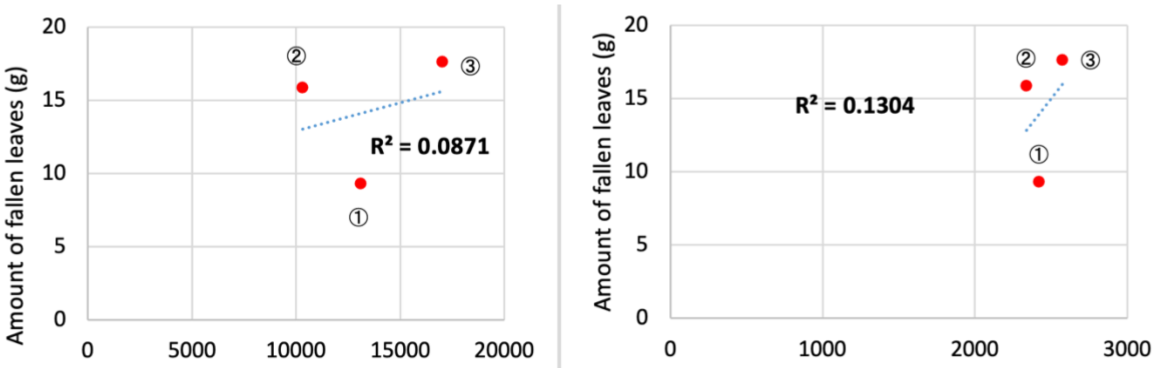

Figure 8　Correlation between Na conc. in fallen leaves

/Brackish water and amount of fallen leaves

6.6 Total amount of heavy metals in fallen leaves collected in a year

From the number of heavy metals in fallen leaves calculated in Chapter 4 and the amount of fallen leaves found in Chapter 5, the number of heavy metals that can be collected in a year by collecting fallen leaves in a 1 km^2 mangrove forest was determined, as shown in Fig. 9. The shaded graph in the figure represents elements that are concentrated in the fallen leaves, and the colored graph represents elements that are not. This allowed us to estimate the number of heavy metals such as As, Cd, and Pb that could be collected by bioremediation. For Zn and Cu, it was found that a large amount could be collected, although no concentration in fallen leaves happened.

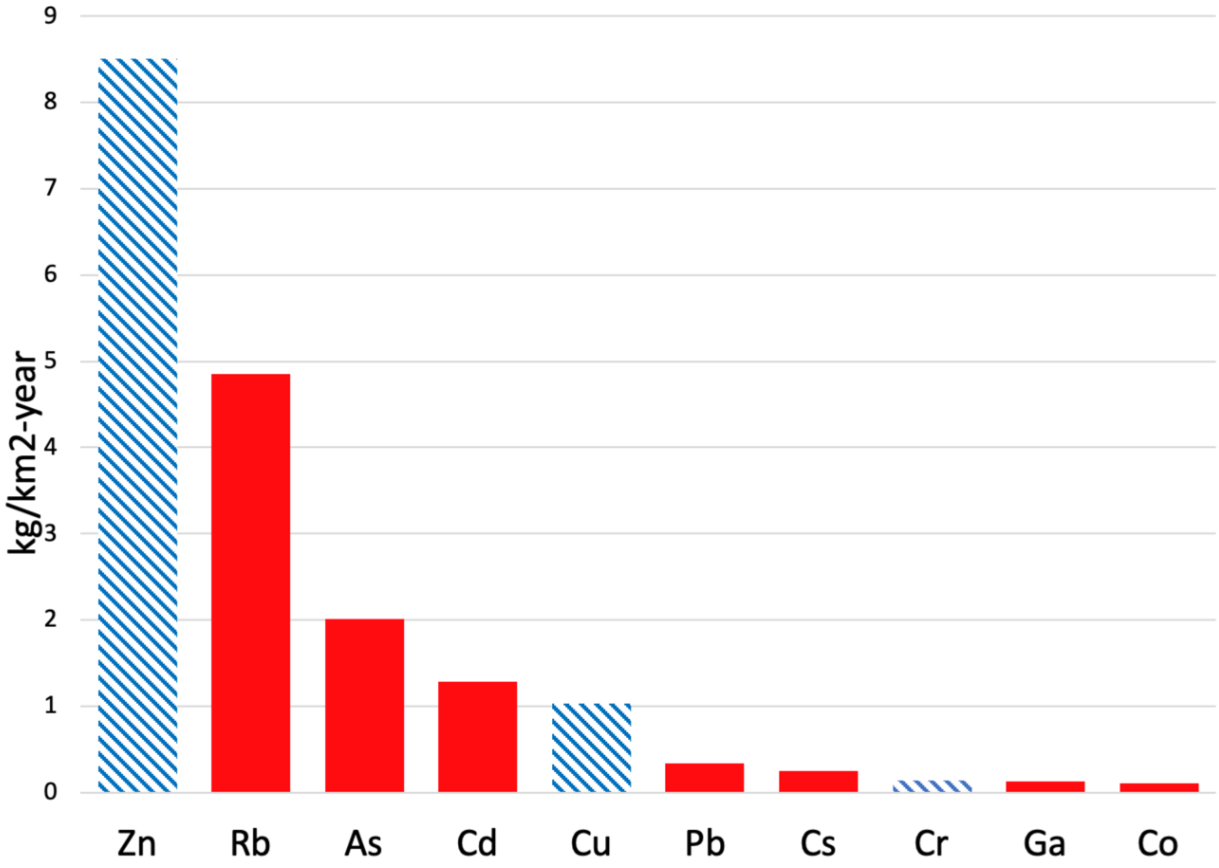

Figure 9 Total amount of heavy metals in fallen leaves collected in a year area of 1km^2

Chapter 7. Conclusions

The purpose of this research was to use the functions of plants to clean up heavy metal contamination of soil and water in an environmentally friendly way, without the use of advanced scientific technology. Our hypothesis was that the *K.obovata*, a type of mangrove concentrates salt in the fallen leaves in the same ways as the *Rhizophora mucronate*, a member of the same genus, and can also concentrate heavy metals and other substances at the same time. Therefore, we have estimated the amounts of heavy metals that can actually be collected and discussed the suitability of using the *K.obovata*.

The results indicate that the *K.obovata* do not discharge salt from their bodies by concentrating Na in their leaves and subsequent defoliation. Thus, our results suggest that the mechanisms of salt tolerance may be different from other types of mangrove. However, heavy metals such as arsenic (As) and cadmium (Cd) were found to be concentrated, and estimation showed that As and Cd could be collected as much as 2.01 kg/km^2 and 1.29 kg/km^2 per year, respectively. This indicates that bioremediation is feasible by collecting fallen leaves of the *K.obovata*, which can be used to collect heavy metals and purify water at low cost, even in developing countries with low wastewater treatment rates and high-water pollution.

In future studies, we expect an investigation of the effects of climate and other environmental factors on the collection of heavy metals, as well as the mechanisms of heavy metals accumulation in the *K.obovata*.

Chapter 8. Future prospect

Fig. 10 Presentation at Manko Wetland

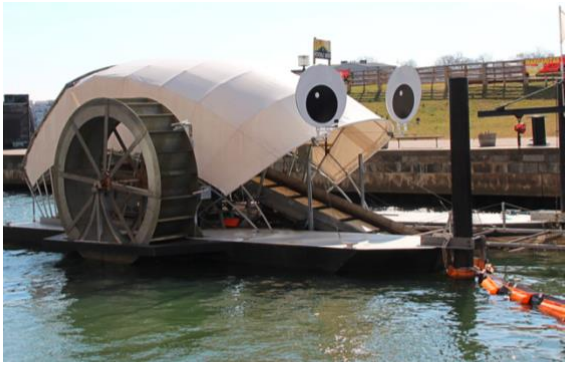

Fig. 11 Mr. Trash Wheel

Mangroves are distributed in equatorial countries, many of which are developing countries where wastewater treatment systems are not completely supplied and water pollution exists. Therefore, we believe that we can expand this bioremediation by the *K.obovata* not only in Manko wetlands but also in many countries around the world.

In preparation for the expansion of our activities to other countries around the world, we held presentations and meetings at wetland centers and graduate schools, to exchange opinions with local residents, officers of the center, and experts, and searched for the best practicable plan (Fig. 10). Finally, we reached the following two ways of collecting fallen leaves.

The first is to conduct collection activities in existing mangrove forests using a device called Mr. Trash Wheel, which collects floating debris by using the energy of sunlight (Fig. 11). By installing this machine at several locations in the forest where fallen leaves are thought to be carried by water flow or other factors, we can collect flowing fallen leaves. Mr. Trash Wheel is already in practical use in Baltimore, USA, and is considered suitable for operation in developing countries because it runs with a simple mechanism. And it is environmentally friendly since it does not emit carbon dioxide. This method also has the great advantage of compensating for the concern received from local people that the proliferation of artificial forests would damage the original landscape as this is carried out in the mangrove forests that originally exist in the area.

The second proposal is to set up bioremediation facilities. This facility will be remediated using the equipment described in the first, which is then used as an environmental education and tourism facility (Fig.12). Developing countries sometimes lack awareness of environmental issues, but by using bioremediation facilities as environmental education and tourism facilities, people's awareness of environmental pollution can be raised and deterrence to water pollution can be increased. The idea could be temporarily installed in developing countries, suburbs where sewage treatment facilities are not yet in place and could be used in specific locations where environmental pollution has been reported, or in companies' factories and industrial treatment facilities. This idea reflects the opinion of a staff member of the Manko Wetland Centre that "not building a plant (for water purification with chemical techniques) but utilizing the natural resources of mangroves will preserve the beautiful scenery and provide a source of revenue for tourism". If these bioremediation facilities succeed in attracting visitors, they will bring economic benefits to the region and make it easier to gain access to the funds needed for water purification. Despite the disadvantage of the cost of land and construction to set up the facility, if it is developed as a tourist facility, it can be expected to generate income from attracting visitors.

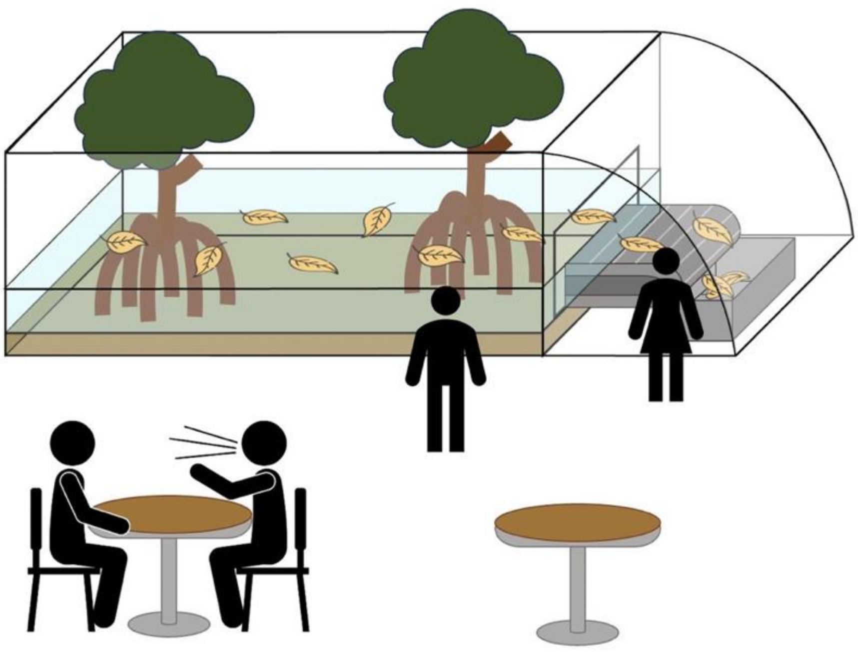

Fig. 12　Model of environmental education and tourism facility

In the future, in order to further improve the versatility of this method, we would like to develop this research by conducting experiments to verify the number of heavy metals collected under various environmental conditions, identifying the mechanisms of tolerance to heavy metals in the *K.obovata*, and investigating whether this method can be used in other mangroves. Ultimately, we would like to expand this activity worldwide to achieve global water quality environmental improvement through bioremediation using simple and environmentally friendly *K.obovata.*

Bibliography

[i] Ministry of the Environment. (n.d.). Current Soil Contamination Countermeasures and Major Issues. https://www.env.go.jp/council/10dojo/y1011-01/mat06.pdf (In Japanese)

[ii] United Nations (n.d.). Ensure Availability and Sustainable Management of Water and Sanitation for All. TARGETS AND INDICATORS. https://sdgs.un.org/goals/goal6

[iii] United nations. (2020, September 14). SDG Indicator Metadata. 2. Definition, Concepts, and Classifications. https://unstats.un.org/sdgs/metadata/files/Metadata-06-03-01.pdf

[iv] Kitaya, Y. (2009). Ecology and current status of mangrove plants growing in seawater. Salt Science Research Foundation, 17-24 https://www.saltscience.or.jp/symposium/3-kitaya.pdf (In Japanese)

[v] Inoue, T. (2007). Mangrove and environmental issue. National Institute for Environmental Studies. 26, 4 https://www.nies.go.jp/kanko/news/26/26-4/26-4-04.html (In Japanese)

[vi] Kawana, Y., Sasamoto, H., & Ashihara, H., (2008). Salt tolerance of mangroves. Bull. Soc. Sea Water Sci., Jpn., 62, 207–214.
https://www.jstage.jst.go.jp/article/swsj1965/62/4/62_207/_pdf (In Japanese)

[vii] Okinawa Amami Nature Conservation Office, Ministry of the Environment. (n.d.). Heritage Area Overview. https://kyushu.env.go.jp/okinawa/amami-okinawa/description/index.html (In Japanese)

[viii] Manko Waterbird and Wetland Center. (n.d.). Wild Birds of Manko. Manko Wildlife Guide. https://www.manko-mizudori.net/category/birds (In Japanese)

[ix] Manko Waterbird and Wetland Center. (n.d.). K.obvovata. Plants at Manko. https://www.manko-mizudori.net/kandelia_obovata/ (In Japanese)

[x] Yoshimoto, K., (2019). Importance of Plant Autophagy from the Perspective of Essential Plant Nutrients. Journal of Japanese Biochemical Society, 91, 589–738.
https://seikagaku.jbsoc.or.jp/10.14952/SEIKAGAKU.2019.910652/data/index.html

[xi] Kato, S., Yaguchi, Y., & Sugi, J. (1986). Ion Components in the Leaf of Certain Mangrove Plants. Bulletin of the Society of Sea Water Science, Japan, 40(1), 9–16.
https://www.jstage.jst.go.jp/article/swsj1965/40/1/40_9/_pdf/-char/ja (In Japanese)

[xii] Iwanaga, F., Noguchi, Y., Yamamoto, F., Ailigiang, M., Okada, K., Yamanaka, N., & Taniguchi, S. (2014). Growth and ion accumulation of Avicennia marina and Kandelia obovata seedlings grown under hydroponic culture with different salinity. Journal of the Japanese Society of Revegetation Technology, 40, 49–53. https://www.jstage.jst.go.jp/article/jjsrt/40/1/40_49/_pdf (In Japanese)