Plants Resistant Against Heavy Metals

ASAKI Yuma, ONO Yuta, MIYASAKA Taisei, YAMAZU Yuki, YAMAMOTO Yushi

Abstract

Hebinonegoza is resistant to heavy metals such as copper, lead, and cadmium. It absorbs heavy metals from the soil in the roots and accumulates them. The mechanism of heavy metal resistance has been found to be caused by polyphenol proanthocyanidins and phytokeratin that are within the plant (molecular weight 400 to 2000).

Therefore, (1) we measured the copper concentration accumulated in the Hebinonegoza in the natural environment. Next, we compared the difference in germination and growth between (2) basil plants which are not resistant to heavy metals and Hebinonegoza. We found that Hebinonegoza, which grows in copper-containing soil, accumulates copper in its roots, and basil inhibits germination and growth of copper compared to Hebinonegoza.

1. Research background and purpose

In recent years, attention has been focused on the development of a technique called phytoremediation to remove contaminants from soil with heavy metals. So we made two purposes for this study. One is to measure the copper concentration in each part of the body of Hebinonegoza native to the soil with a low copper content. We took our samples from Ogoya copper mine. The other purpose was to measure whether there is a difference in metal resistance by plants as the degree of germination and growth changes.

2. Hypothesis

(1) Hebinonegoza after absorbing copper ions in the soil with roots, transports them to the leaves, the middle axis and leaves, and stores it.

(2) Hebinonegoza is bonded to the copper ions in a chelated state.

(3) By Hebinonegoza absorbing heavy metals from the surrounding soil, heavy metals in the soil will be reduced.

(4) Basil will not grow or germinate in soil with low copper content.

<Introduction of materials>

~Hebinonegoza~

・Fern plants are active as indicator plants in the mineral soils.

・Heavy metals such as copper and lead are absorbed from the roots and accumulated in the cell wall to make them non-toxic.

~Basil~

・The germination cycle is very fast and it germinates in about 10 days.

・We can cultivate it anywhere in Japan.

3. Experimental method

3-1 Determination of copper concentration in Hebinonegoza native to natural environment

We investigated in which part of Hebinonegoza copper is accumulated.

Material: Hebinonegoza (Ogoya copper mine, Tree Park) reagents, etc.: liquid nitrogen, nitric acid (0.5mol / L), milk bars and mortars, water bath, millipore filter, centrifuge

① Hebinonegoza from Ogoya copper mine (Komatsu City) and tree park (Hakusan City), leaves, leaf shaft, middle axis, three sites of the root, and were cut using scissors.

② Each part of Hebinonegoza was placed in a mortar, we poured liquid nitrogen there, and grounded it using a milk rod.

③ Add 0.5mol/L of nitric acid 10 mL per dry weight of about 0.3 g, soak for 1 hour at 80 ° C water bath, dissolving the cell wall.

④ The solution was centrifuged for 5 minutes at 2400G, centrifuged for 5 minutes at a further 20,000 g taking its supernatant, except for contaminants passing through the millipore filter having a diameter of 0.2μm.

⑤ The copper concentration is measured by atomic absorption. (We took the calibration curve at a known copper concentration in advance.)

3-2 Determination of copper concentration in the soil

The copper content in the soil near the native Hebinonegoza was examined to see if there was actually a transfer of copper to the roots.

Material: soil (Near the roots of Hebinenegoza at the site of Ogoya copper mine) reagents, etc.: water, millipore filter,

① Put 5g of soil and 30ml of water in a centrifuge microtube.

② Centrifuge for 5 minutes at 2400G.

③ Filter the supernatant.

④ The filtrate is subtly divided into microtubes.

⑤ Pass through a 0.2μm millipore filter to remove contaminants

⑥ Analyze with atomic absorption.

3-3 Effects of copper on basil growth

We investigated how basil is affected by growth in a medium containing copper as a plant that is not heavy metal resistant.

Ingredients: Basil

Reagents, etc.: agar powder, MS medium (nutrient), copper sulfate pentahydrate, test tube, sodium hypochlorite, ethanol, distilled water, clean bench, microwave oven

① Planted sterile basil seeds in MS agar medium (test tube) adjusted to copper 0,2,5.0,7.5,10 ppm with copper sulfate pentahydrate, grown for a certain period of time (2 weeks ~). Seeds 70% ethanol 30 seconds, after immersion for sodium hypochlorite for 10 minutes, and sterilized thoroughly washed with sterile water.

② Check the presence or absence of germination, whether it was extended after germination, and recorded.

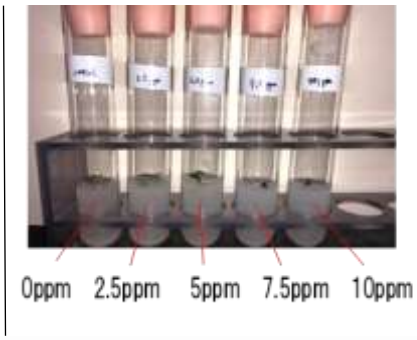


Figure 1 Seeded Basil

3-4 Effects of copper on the germination of basil

Basil was seeded in a medium containing copper of each concentration to determine the germination rate.

Materials, reagents, etc.: were the same for experiments 3-3.

① Plant 20 pieces of basil seeds in MS agar medium (petri dish) in copper concentration in copper sulfate pentahydrate (0,5.0,6.0,7.0,8.0,9.0,10 ppm) and grow for a certain period of time (2 weeks ~).

② The presence or absence of germination, and the presence or absence of elongation after germination, were recorded.

Definitions about germination and growth we defined:

Germinated: It can be confirmed the buds

Grown: The part of the 1cm white root can be confirmed, and the root enters the medium.

3-5 Effects of copper on Hebinonegoza

Material: Hebinonegoza (anterior leaf body)

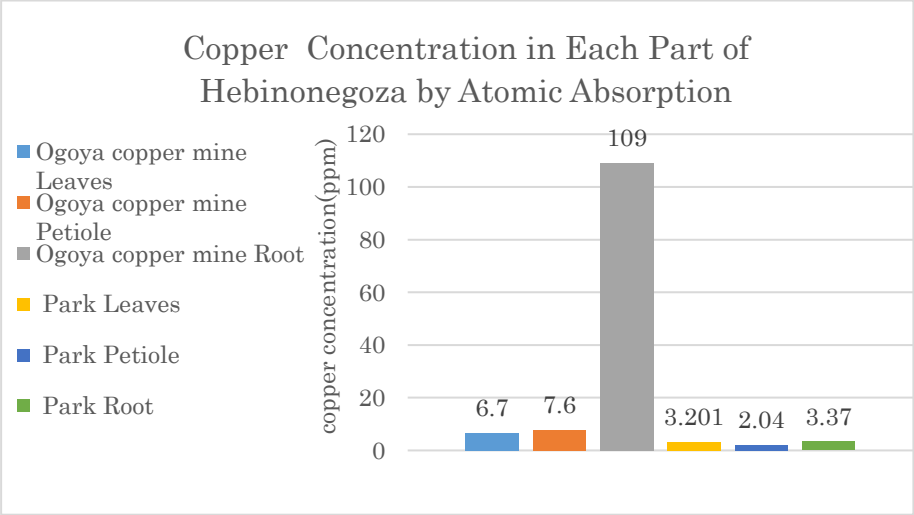
Reagents: Agar powder, MS medium, copper sulfate pentahydrate, test tube, sodium hypochlorite, ethanol, distilled water, microwave oven.

The anterior leaf body of the Hebinonegoza grown from spores is planted in MS agar medium which adjusted the copper concentration to 0,5,10,50,100 ppm with copper sulfate pentahydrate.

Figure 2 the anterior body of the sowed Hebinonegoza (From left: cupper concentration; 0 , 5 , 10 , 50 , 100ppm)

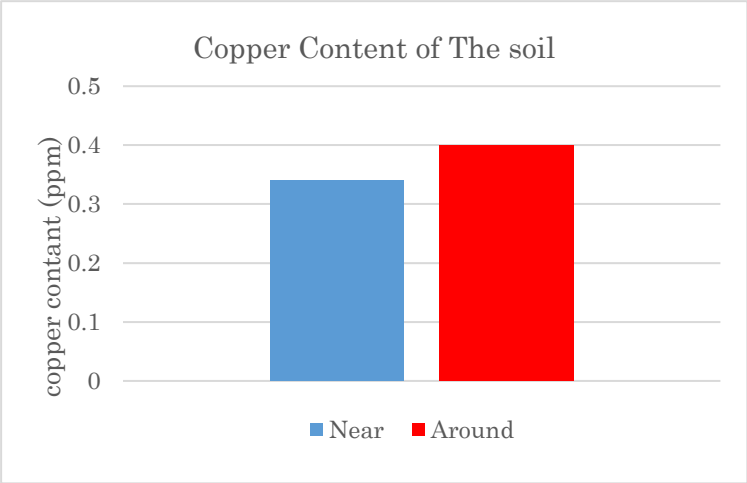
4. Results

3-1 results



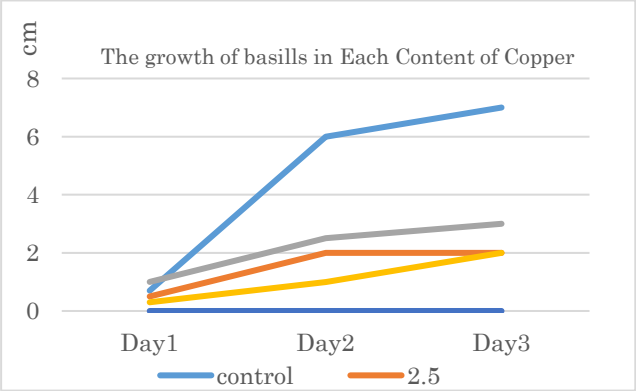
- In leaves, leaf pattern, and the middle axis, copper was present among the three parts of the root.
- Copper concentration was small in every part of the tree park, where Hebinonegoza was collected.

3-2 results



・Copper concentration of soil in the vicinity of Hebinonegoza was smaller than that of the surrounding soil without Hebinonegoza.

3-3 results



・At copper concentration of 0 and 2.5 ppm, basil grew steadily.
・The basil copper concentration 5ppm, at 7.5 ppm were winding round the cobwebs on the surface of the medium without stretching the roots.
・It was not germinated at 10ppm.

3-4 results

Experiment (9,10ppm)

We counted and calculated the number of germinations, elongation, germination rate, and elongation rate of basil

concentration of cupper	control	5ppm	6ppm	7ppm	8ppm	9ppm	10ppm
The number of germinated	18	18	16	14	15	14	18
The number of grown	16	15	7	5	2	1	0
The ratio of germinated	90	90	80	70	75	70	90
The ratio of grown	80	75	35	25	10	5	0

Unit: Copper concentration (horizontal axis)... ppm (mg/L)

Germination number, number of extensions (vertical axis)... Out of 20 pieces (Germination rate: Germination number × 100 / 20 × 1.111

(Elongation ratio: number of extensions × 100 / 20 × 1.25 Unit: Germination rate, Elongation rate... %、 Copper concentration (horizontal axis)... ppm (mg/L)

- Germination because it is a physical phenomenon by water absorption, it also occurred at 10 ppm.
- As the concentration of copper in the medium increases, the reaction of elongation inhibition becomes stronger.

3-5 results

- There is no plant that withered in any concentration so the resistance to copper was confirmed.
- In particular, it can be observed that the anterior body is growing well at 0,100 ppm, it was found that Hebinonegoza grows even in a copper concentration of 100 ppm.

5. Consideration

From 3-1, the previous study found that copper was accumulated not only in the roots but also in the leaves and stems, but since the roots contained most of the copper, it is thought that the heavy metal resistance of Hebinonegoza is present in the roots.

From 3-2, basil is elongated at about 5 ppm, it is considered to fail to germinate at about 10 ppm.

The phenomenon of rounding the roots is considered to be due to basil rejecting the heavy metals (copper) in the medium.

6. Reflection

The research of the plant is difficult because the plant takes a long time to grow. Because of this, there has been little research done. Therefore, research on heavy metal plants has been very slow.

7. Future issues

As a future problem, and to clarify the resistance limit in the accurate germination and elongation to copper of Hebinonegoza and basil, this experiment also collected Hebinonegoza from the Ogoya Copper Mine, but was carried out by squeezing the heavy metal into copper. We would like to conduct the same experiment with other heavy metals such as lead and cadmium if possible.

8. Acknowledgment

Dr. Osamu Nakatani, Assistant Professor, Institute of Bio resource Engineering, Ishikawa Prefectural University,
Dr. Kazushi Oda, Professor, Graduate School of Science and Technology, Hokuriku Advanced University,
Teacher Noboru Nishioka,
Dr. Hiroyuki Kamachi, Department of Environmental Science, Toyama University.

9. Reference

・Hiroyuki Kamachi, (University of Toyama)
Hebinonegoza - Strange Plants That Collect Heavy Metals, (2008)–
・Masaki Tamaki, (National Institute of Environmental Studies), "Poisoned Plants - Why Plants Store Metal?"
・Hiroki Taoka, Akiko Hokura, Fumiyuki Goto, Toshikazu Yoshihara, Tomoko Abe, Izumi Nakai (Tokyo University of Science, Tokyo Denki University, Chuo Research Institute of Electric Power, RIKEN NRC) “Elucidation of the accumulation mechanism of heavy metals"
・ Hiroshi Okada, Masayuki Sakakibara, Yuri Sueoka, Koichiro Sera, "Research on the heavy metal accumulation of native Hebinonegoza in heavy metal contaminated river basins" (2015)
・ Kyoto University Plant Nutrition Laboratory, "Analysis of heavy metal resistance by phytokeratin" (2013),
・"Shrubs, Ryobu (Reibu) clustered around metal mines such as tail hut copper mines, soil and ryobu Distribution of heavy metals in the tissue, heavy metal contamination and purification of the natural environment (transition of plant communities) (2009)
・"Fern plants clustered around metal mines such as Ikuno Silver Mine, Hebinonegoza, The distribution of heavy metals in soil and Hebinonegoza tissue, heavy metal contamination and purification of the natural environment, and so on." (2009)

10. Key words

Hebinonegoza Basil Heavy metal Ogoya copper mine