SCIREA Journal of Biology

http://www.scirea.org/journal/Biology

December 8, 2020

Volume 5, Issue 4, August 2020

# Growth inhibition and root damage of bismuth in Solanum lycopersicum

**Takeshi Nagata*, Satsuki Kimoto**

Faculty of Science and Engineering, Setsunan University, Japan

*To whom correspondence should be addressed.

17-8 Ikedanakamachi, Neyagawa, Osaka 572-8508, Japan

Tel: +81-72-839-8222

Fax: +81-72-839-8222

Email: t-nagata@lif.setsunan.ac.jp

## Abstract

Bismuth (Bi) is one of the minor metals and is used in semiconductors and water pipes as a substitute for lead. However, the Bi concentration in the soil has not been investigated. Furthermore, Bi has no environmental quality standard in Japan. We previously reported on Bi phytotoxicity in *Arabidopsis thaliana.* However, the effects of Bi on vegetative plants have not been studied.

In this study, we investigated the effects of Bi on the growth of *Solanum lycopersicum*, in particular Fe accumulation and phytotoxicity in the roots. Seeds were soaked in various concentrations of Bi including agar medium. After 2 weeks of incubation, root elongation and shoot growth were significantly inhibited by >3 μM Bi. It suggested that Bi has high toxicity in tomato. Bi accumulation in the shoot was related to its concentration in the medium,

whereas the concentration in the root was saturated at >3 μM Bi treatment. Bi exposure increased Fe accumulation in the root. Furthermore, Fe concentration showed an inverse relationship in the shoot and root. In the split-root experiment, the Bi-treated roots were not observed viability in the liquid medium. The non-Bi-treated roots showed an increased viability as compared to the Bi-treated root. It suggested that Bi has local toxicity in the root.

**Keywords**: Solanum lycopersicum, Tomato, Bismuth, Iron, Cell death.

## 1. Introduction

Bismuth (Bi) is considered as one of the minor metals. In Japan, Bi is used as an ingredient in some pharmaceuticals and is included in the Japanese Pharmacopoeia 17$^{th}$ edition (JP17) [21]. Bi subnitrate is an antiulcer drug with gastrointestinal mucosa convergence due to the protection of the alimentary canal mucosa. In Sri Lanka, Bi subsalicylate is used as gastrointestinal medicine [20]. Aside from its pharmaceutical uses, Bi is also used in semiconductors and water pipes as a substitute for lead (Pb) in Japan.

The toxicity of excess Bi in animals has been previously studied. Bi has lower toxicity compared to other metals such as Pb [18, 19]. Thus, there is no environmental quality standard for Bi in Japan. Until now, there are some reports about Bi concentration in the soil. In Japan, Bi was detected from the soil and the river around the Bi smelting areas [9]. Furthermore, Bi drained from metalliferous mining and smelting areas was also detected from the rice paddy [8]. In England, Bi drained from metalliferous mining and smelting areas was detected from not only the soil but also the pasture herbage [10]. In Brazil, Bi was detected in fertilizer [12]. However, little is known about its phytotoxicity in plants.

We previously investigated Bi accumulation in *Arabidopsis thaliana* (*A. thaliana*) [17]. The root elongation and shoot growth were significantly inhibited by more than 2 μM Bi. In 2 μM Bi-treated plants, the Bi concentration in the root was approximately 70 nmolg$^{-1}$ FW, which was 7 fold higher compared to the shoot. Furthermore, Bi increased the Fe content. Microarray analysis revealed candidate genes such as iron-regulated transporter 1 coding gene (*IRT1*) and ferritin 1 coding gene (*FER1*). IRT1 is essential for ferrous iron uptake, and Fe uptake in the root mainly depends on IRT1 in *A. thaliana* [23]. FER is known as responsible

for the storage and distribution of Fe within cells [22]. These results suggest that Bi might disturb Fe homeostasis in the root.

*Solanum lycopersicum* (tomato) is an important crop vegetable and it is a model plant used for studying the molecular mechanism of Fe uptake in vegetative plants [4]. It is also one of the most sensitive vegetative plants with regard to soil conditions. The effects of Bi accumulation on the growth of tomato plants have not been studied. Furthermore, the effects of Bi on roots have not been addressed.

## 2.Materials and Methods

### *Plant material and growth conditions*

The seeds of *S. lycopersicum* cv. Micro-tom (tomato) were obtained from Inplanta Innovations, Inc. (Kanagawa, Japan). Seeds were surface-sterilized with 70% ethanol for 2 min and in 10% commercial bleach with detergent (Kitchen Haiter, Kao, Tokyo, Japan), which includes sodium hypochlorite (NaOCl), alkyl ether sulphate sodium salt and NaOH for 20 min and, then, were rinsed with sterilized water three times for 5 min each. Seeds were planted in Murashige and Skoog (MS) medium containing B5 vitamins with 0.8% agar and 3% sucrose [14]. The MS agar plates were supplemented with the final concentration of 0.15, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0, 13.5 or 15.0 μM Bi(NO3)3. The plate without Bi was used as a control. All cultures were maintained at 25°C under a 16 h light and 8 h dark cycle. Plants were grown in an MLR-350 growth chamber (Sanyo, Osaka, Japan) for 2 weeks.

### *Germination and growth assays under normal and Bi conditions*

The two seeds were grown in MS agar plates containing various Bi concentrations. After 2 weeks of incubation, the germination rates were calculated, and the plants were photographed. Then, the root length was determined and plants were harvested. The plants were separated into the shoot and root parts, and their fresh weights were measured. Experiments were repeated at least three times.

### *Determination of Bi and Fe contents*

Seeds were germinated on a standard MS agar medium with or without 2 μM Bi. After 2 weeks of incubation, the plants were separated into shoots and roots. The Bi and Fe content in various tissues was determined by atomic absorption spectrophotometry (ContrAA700, Analytik Jena, Germany) after digesting the samples with concentrated nitric acid [15].

***Split-Root system***

For split-root system in liquid medium, the seeds were grown in MS agar medium under normal conditions. After 2 weeks incubation, the primary root was cut off to obtain two news primary roots. The plantlet was put on filter paper on the new agar medium. Then, after 7 days of incubation, plants were transferred to the liquid MS medium with or without Bi and covered with a pouch. After 7 days of Bi exposure, roots were harvested and used for the determination of Bi and Fe contents and for the following microscopic experiment.

***Microscopic observation***

The root viability was detected by fluorescein diacetate (FDA, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Roots, which are treated or untreated with $Bi^{3+}$, were placed in distilled water for 5 min, stained with 25 $\mu g ml^{-1}$ FDA solution for 10 min, and then rinsed with distilled water according to [6] with minor modifications. The root formation was observed by differential interference (DIC). Fluorescent images were captured with a LSM 710 laser-scanning microscope (Carl Zeiss, Tokyo, Japan). The root cap and elongation region were visualized under epifluorescence illumination (excitation, 488 nm: emission, 530 nm) for the cell viability assay. All figures are representative of staining detected in the roots of three independent experiments.

***Statistical analysis***

All experiments were conducted at least three times. Data are presented as means of three replicates. Differences between treatments were determined by Student's *t*-tests. A *P*-value of $<0.05$ was considered significant.

## 3. Results

***Effect of Bi on the germination and growth of Tomato***

After 2 weeks of incubation, the germination was not affected by <9 μM Bi, but the seeds were unable to germinate on a medium containing >12 μM Bi (Figure 1A). Tomato seedlings were photographed and phenotypes were observed (Figure 1B). Phenotypes of 0.15 and 1.5 μM Bi-treated seedlings did not differ from those of untreated seedlings. The shoot and root growth of seedlings treated with >3 μM Bi was inhibited by Bi toxicity. Furthermore, plantlets treated with >6 μM Bi were unable to form shoot and root structures.

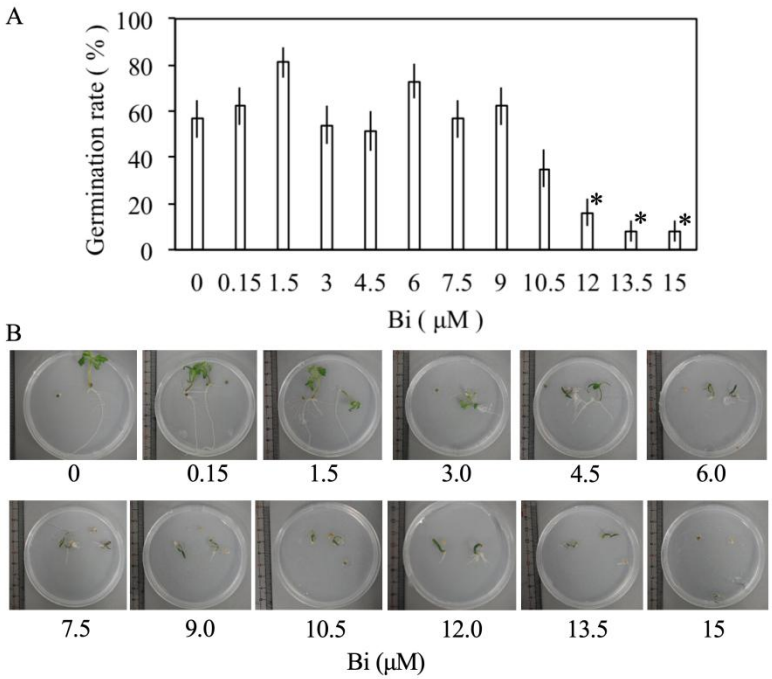


**Figure 1. Effects of Bi on germination and phenotype**

After 2 weeks of incubation, effects of Bi on germination were evaluated by counting the germinated seedlings (A). The phenotype was observed (B). Data are reported as means ± SE ($n$ = 3-5). Asterisks indicate a significant difference between Bi-treated plants and control plants ($P < 0.05$).

Next, fresh weights (FWs) of the shoots and roots were measured (Figure 2A). Open columns indicate roots; black columns indicate shoots. At >6 μM Bi treatments, the FW of shoots and roots were decreased approximately 50% compared to the control. Meanwhile, whole FWs decrease at >3 μM Bi treatment. For root length, there was no significant difference between the control and 0.15 and 1.5 μM Bi-treated plants (Figure 2B). However, the root lengths of 3 μM Bi-treated plantlets were about half those of the control. At >6 μM Bi treatment, the plants were unable to extend their roots at all.

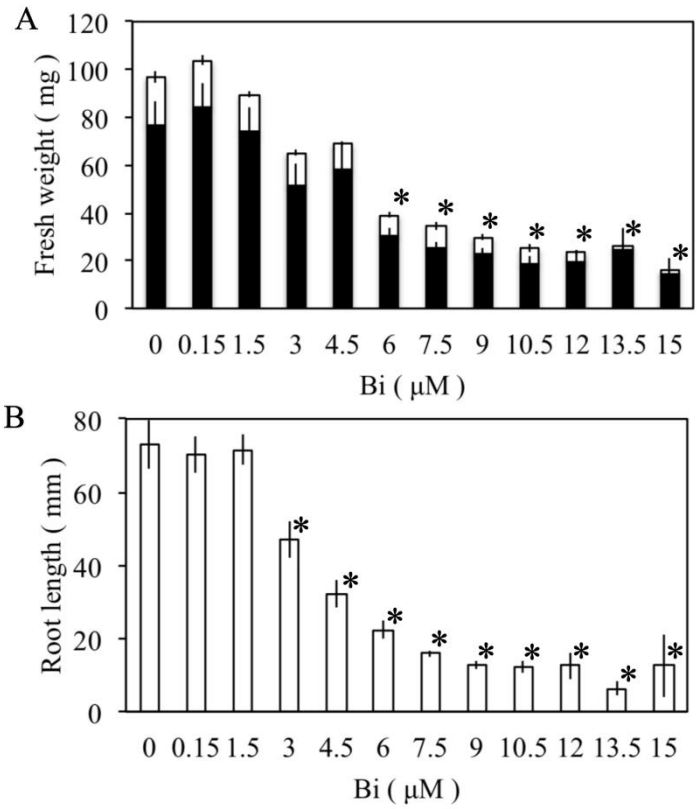


**Figure 2. Effects of Bi on growth and root elongation**

The effects of Bi on seedlings were evaluated by monitoring the fresh weight of plant tissues. Two-week-old plantlets were separated into shoots and roots. The FW was measured (A). Open columns are roots; black columns are shoots. The root length was determined (B). Data are reported as means ± SE ($n$ = 3-5). Asterisks indicate a significant difference between Bi-treated plants and control plants ($P < 0.05$).

***Bi accumulation in Tomato***

After 2 weeks of incubation, the accumulation of Bi in each organ was measured. Bi was not detected in control plants or 0.15 μM Bi-treated plants. As shown in Figure 3, Bi concentrations in shoots were steady state at 9 μM Bi treatment. However, Bi concentrations in roots were saturated at approximately 20 $nmolg^{-1}$ FW in >3 μM Bi-treated plants (Figure 3). In 3 μM Bi-treated plants, the Bi concentration in the roots was fivefold higher compared to the shoots.

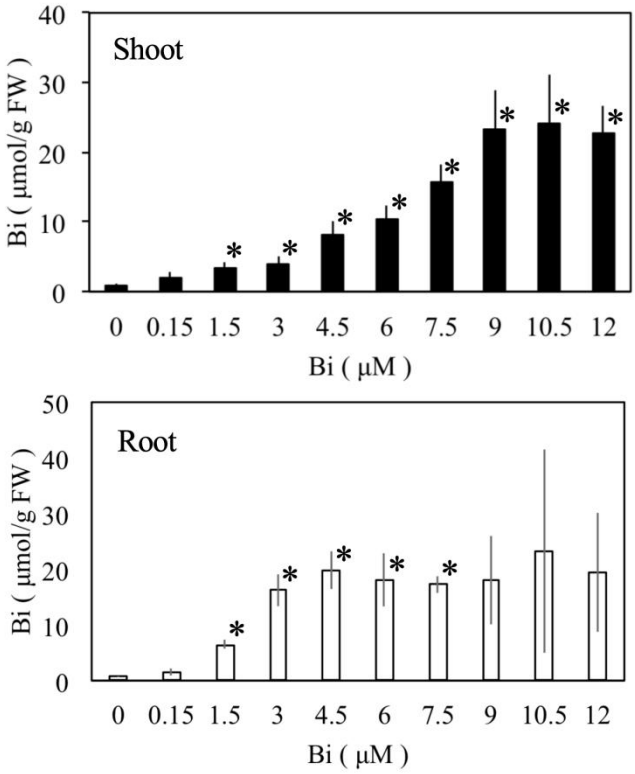


**Figure 3. Accumulation of Bi in tomato.**

The accumulation of Bi in each organ was determined by atomic adsorption of spectrophotometry. Data are reported as means ± SE ($n$ = 3-5). Asterisks indicate a significant difference between the shoot and root ($P < 0.05$).

### *Effect of Bi on Fe accumulation in Tomato*

After 2 weeks of incubation, the accumulation of Fe in each organ was measured. In the shoots and roots, there was no significant difference between the control and 3 μM Bi-treated plants (Figure 4). However, Fe concentrations in the shoots tended to increase with Bi in a dose-dependent manner, except at 12 μM Bi. Furthermore, Fe concentrations in roots at >6 μM Bi-treated plants tended to decrease with Bi in a dose-dependent manner.

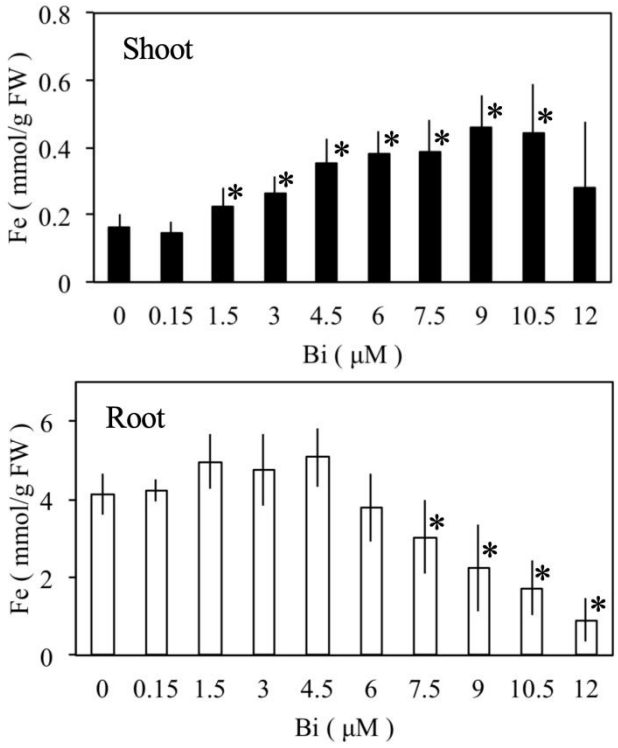


**Figure 4. Effects of Bi on Fe accumulation in agar medium.**

The accumulation of Fe in each organ was determined by atomic adsorption of spectrophotometry. Data are reported as means ± SE ($n$ = 3-5). Asterisks indicate a significant difference between Bi-treated plants and control plants ($P < 0.05$).

### *Root damage from Bi treatment*

At >4.5 μM Bi treatment, plantlets were unable to form root structure and the roots were friable to stain. In the DIC observations, differences in root formation were not observed with or without Bi in agar medium (Figure 5). The damage caused by Bi to the root was also evaluated by fluorescein diacetate (FDA) staining. FDA fluorescence was detected in all tested root tips. There was no effect of Bi treatment compared to the control in agar medium.

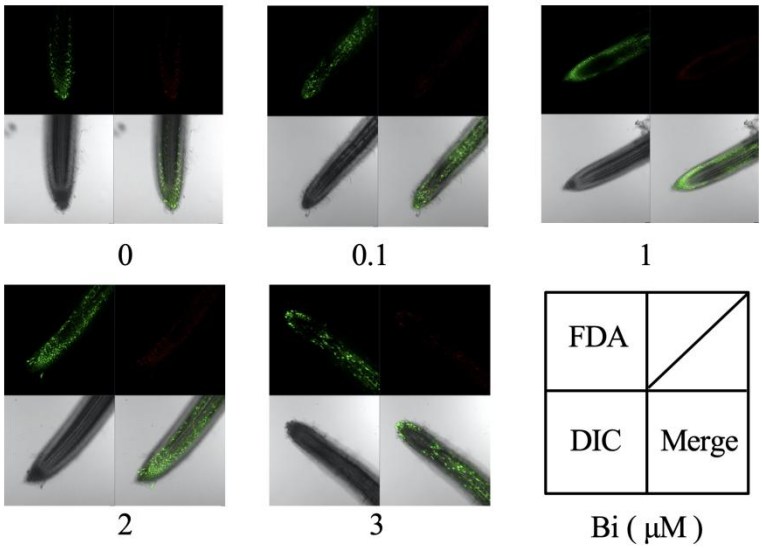


**Figure 5. Effects of Bi on the root tip in agar medium.**

The plantlets were incubated in Bi-including agar medium. The root viability was detected by fluorescein diacetate (FDA). The root formation was observed by differential interference (DIC). The upper-right side boxes were blank.

To investigate Bi toxicity in non-treated roots, we tried using a split-root system. In liquid medium, Bi was detected in not only Bi-treated roots but also non-treated roots (Table 1). And more, Fe content was 2-hold higher in Bi-treated roots compared to that of without Bi treated roots (Table 1). Bi-treated roots showed injury in the DIC observation (Figure 6).

Table 1. Accumulation of Bi and Fe in split root

| Bi treatment (μM) | 0 | 0.1 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Bi contents (μmol/g) | | | | | |
| Bi non-containing medium | 0.72 | 1.99 | 18.26 | 6.08 | 7.03 |
| Bi containing medium | 0.72 | 6.33 | 55.12 | 10.11 | 16.96 |
| Fe (mmol/g) | | | | | |
| Bi non-containing medium | 51.6 | 60.0 | 86.6 | 43.5 | 76.4 |
| Bi containing medium | 51.6 | 73.2 | 60.8 | 20.2 | 100.2 |

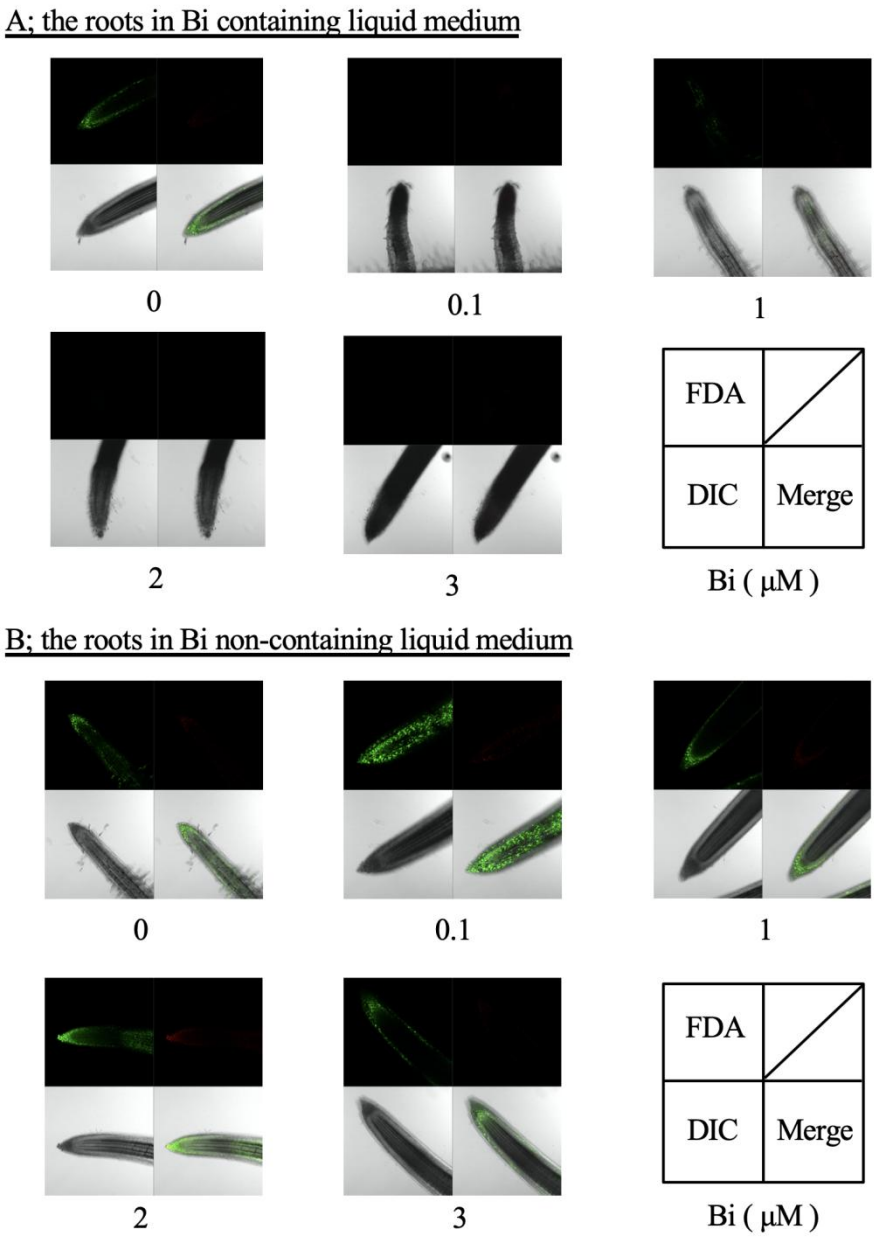


**Figure 6. Evaluation of Bi phytotoxicity by the split-root system.**

Two separated physical spaces, with (A) or without (B) Bi, were created in a split-root system. The plantlets were incubated in Bi-including liquid medium. The root viability was detected by fluorescein diacetate (FDA), and the root formation was observed by differential interference (DIC). The upper-right side boxes were blank.

## 4. Discussion

We previously studied the phytotoxicity of metal ions as nonessential nutrients in dicotyledonous plants [15-16]. In this study, we examined the effects of Bi on growth and

root toxicity in tomato. Bi concentration of polluted soil was detected from 0.40 to 2.36 $\mu g g^{-1}$ in Japan [8]. Its concentration was approximately from 1.9 to 11.3 $\mu mol kg^{-1}$. Thus, we established that Bi concentration was from 0.15 to 15 μM in agar medium.

Germination was not observed at >12 μM Bi (Figure 1A). This result suggests that high concentrations of Bi could suppress the germination mechanism. Further study of the germination stage is required. Furthermore, Bi inhibited growth in the shoot and root (Figure 1B). As shown in Figure 2, at >6 μM Bi treatments suppressed the growth of tomato plants. Root elongation was decreased by 50% in 4.5 μM Bi-treated plants. This suggested that Bi has phytotoxicity to tomato. Reference [8] shows about 3.7 μM Bi in nature. Our results suggested that plants might be affected in Bi polluted areas.

In the shoot, Bi content was increased with the Bi concentration of the medium in a dose-dependent manner in plantlets treated with <9 μM Bi (Figure 3). Furthermore, the Bi concentration of roots was saturated at approximately 20 $nmol g^{-1}$. This result suggests that the roots of tomato plantlets were unable to accumulate 20 $nmol g^{-1}$. Reference [24] shows the Bi concentration of soil in China was 1672 $mg kg^{-1}$ and the Bi contents in *Buddleja davidii* was 2.877 $mg kg^{-1}$ (approximately 13.8 $nmol g^{-1}$). Tomato may have the Bi accumulation ability like *Buddleja davidii* in environmental condition.

In a recent study, we reported that Bi induces Fe accumulation and AtIRT1 expression in *Arabidopsis* roots [17]. However, whether do Bi effects on elements accumulation other than Fe. In future, we have to clear the concentration of other elements in *Arabidopsis thaliana*. In this study, we determined the Fe concentration in tomato. The shoot Fe concentration was increased with increasing Bi concentration (Figure 4). However, the root Fe concentration was decreased in >6 μM Bi-treated plants. These results suggest that Bi induces an inverse relationship of Fe concentration between the shoot and root. We think that the high concentration of Bi in root might cause the cell death. As the result, Fe could not accumulate in root. On the other hand, Fe translocate to shoot to rescue the Bi inductive stress.

However, it was not clear whether the Bi accumulation could cause cell death in the root. We attempted to investigate the viability, but we were unable to get roots from plants treated with >4.5 μM Bi. Thus, roots treated with ≤3 μM Bi were used for the experiments. In agar medium, the viability was not affected by Bi (Figure 5). This suggests that the tomato root has a tolerance to ≤3 μM Bi toxicity in agar medium. Liman investigated the genotoxic effects of Bi (III) oxide nanoparticles (BONPs) on the root cells of *Allium cepa* [11]. BONPs showed

genotoxic activity in root meristematic cells. We will have to clear the genotxic activity in tomato root cells.

The root-split system experiment is a useful method of estimating the indirect physiological effects of a nutrient [13]. Bi-treated roots in liquid medium did not demonstrate any viability (Figure 6). These results suggest that the toxicity in the liquid was greater compared to agar medium. The result of Bi contents in roots suggest that Bi was transferred from the Bi-treated roots to the non-treated roots (Table 1). Bi might be translocated to other roots through the vascular bundles. And Bi collapsed the Fe accumulation in the Bi-treated roots in liquid medium (Table 1). Interestingly, the non-Bi-exposed roots showed viability (Figure 6). These results suggest that Bi shows the local toxicity in the root.

In recent years, the relationships between programmed cell death (PCD), in which plants kill their own cells in tissues damaged, and metals such as copper, aluminum, cadmium, silver, tungsten and zinc have reported [1-3, 5, 7, 25, 26]. The relationship Between PCD and Bi has not been cleared. To clear the relationship, further study is necessary.

## 5. Conclusion

In conclusion, Bi showed toxicity toward tomato growth. Bi collapsed the Fe contents in the shoot and root. Bi toxicity was greater in liquid medium compared to agar medium. Furthermore, Bi was translocated to other roots and showed local toxicity in the root. To the best of our knowledge, this is the first investigation of Bi toxicity in vegetative plants.

## Acknowledgements

We thank Ryohei Kurisaki and Makoto Nishimura of Setsunan University for their technical assistance.

## References

[1] Adamakis IDS, Panteris E, Eleftheriou EP (2011) The fatal effect of tungsten on *Pisum sativum* L., root cells: Indications for endoplasmic reticulum stress-induced programmed cell death. *Planta* 234: 21–34.

[2] Filippi A, Zancani M, Petrussa E, Braidot E (2019) Caspase-3-like activity and proteasome degradation in grapevine suspension cell cultures undergoing silver-induced programmed cell death. *J Plant Physiol* 233: 42–51.

[3] Goto F, Enomoto Y, Shoji K, Shimada H, Yoshihara T (2019) Copper treatment of peach leaves causes lesion formation similar to the biotic stress response. *Plant Biotechnol* (*Tokyo*) 36: 135-142.

[4] Hell R, Stephan UW (2003) Iron uptake, trafficking and homeostasis in plants. *Planta* 216: 541–551.

**[5]** Huang W, Yang X, Yao S, LwinOo T, He H, Wang A, Li C, He L (2014) Reactive oxygen species burst induced by aluminum stress triggers mitochondria-dependent programmed cell death in peanut root tip cells. *Plant Physiol Biochem* 82: 76–84.

[6] Ishikawa S and Wagatsuma T (1998) Plasma membrane permeability of root-tip cells following temporary exposure to Al ions is a rapid measure of Al tolerance among plant species. *Plant Cell Physiol* 39:516-525.

[7] Kirisiuk Y, Mackievic V, Zvanarou S, Przhevalskaya D, Leschenka Y, Demidchik V (2016) The effect of copper nanoparticles on growth, cell viability and signaling processes of wheat plants. Plant Signalling & Behavior 4th International Symposium Proceedings, p123.

[8] Kubota M, Asami T, Matsuki M (1988) Antimony and bismuth concentrations in the paddy soils and the river sediments polluted by cadmium in Nanakai-mura, Ibraki-ken, Japan. *J Society Soil Sci Plant Nutr* 59: 614-616 (in Japanese).

[9] Kubota M, Asami T, Matsuki M, Kashimura A (1990) Soil pollution by bismuth and the related heavy metals discharged from a bismuth smelter. *J Society Soil Sci Plant Nutr* 61: 190-192 (in Japanese).

[10] Li X, Thornton I (1993) Arsenic, antimony and bismuth in soil and pasture herbage in some old metalliferous mining areas in England. *Environ Geochem Health* 15: 135-144.

[11] Liman R (2013) Genotoxic effects of bismuth (III) oxide nanoparticles by allium and comet assay. *Chemosphere* 93: 269-73.

[12] Machado RC, Amaral CDB, Nóbrega JA, Araujo Nogueira AR (2017) Multielemental determination of As, Bi, Ge, Sb, and Sn in agricultural samples using hydride generation

coupled to microwave-induced plasma optical emission spectrometry. *J Agric Food Chem* 14: 4839-4842.

[13] Mitani-Ueno N, Yamaji N, Ma JF (2016) High silicon accumulation in the shoot is required for down-regulating the expression of Si transporter genes in rice. *Plant Cell Physiol* 57: 2510-2518.

[14] Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol Plant* 15: 473–497.

[15] Nagata T, Morita H, Akizawa T, Pan-Hou H (2010) Development of a transgenic tobacco plant for phytoremediation of methylmercury pollution. *Appl Microbiol Biotechnol* 87: 781-786.

[16] Nagata T (2014) Expression analysis of new Metallothionein2-like protein under mercury stress in tomato seedling. *Plant Root* 8: 72-81.

[17] Nagata T (2015) Growth inhibition and *IRT1* induction of *Arabidopsis thaliana* in response to bismuth. *J Plant Biol* 58: 311-371.

[18] Sano Y, Satoh H, Chiba M, Okamoto M, Serizawa K, Nakashima H, Omae K (2005a) Oral toxicity of bismuth in rat: single and 28-day repeated administration studies. *J Occup Health* 47: 293-298.

[19] Sano Y, Satoh H, Chiba M, Shinohara A, Okamoto M, Serizawa K, Nakashima H, Omae K (2005b) A 13-week toxicity study of bismuth in rats by intratracheal intermittent administration. *J Occup Health* 47: 242-248.

[20] Shanika LGT, Jayamanne S, Wijekoon CN, Coombes J, Perera D, Mohamed F, Coombes I, De Silva HA, Dawson AH (2018) Ward-based clinical pharmacists and hospital readmission: a non-randomized controlled trial in Sri Lanka. *Bull World Health Organ* 96:155-164.

[21] The Japanese Pharmacopoeia 17$^{th}$, Jihou-sha (2016).

[22] Theil EC (1987) Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. *Annu Rev Biochem* 56: 289-315.

[23] Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat JF, Curie C (2002) IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* 14: 1223–1233.

[24] Wei C, Deng Q, Wu F, Fu Z, Xu L (2011) Arsenic, antimony, and bismuth uptake and accumulation by plants in an old antimony mine, China. *Biol Trace Elem Res* 144: 1150-1158.

[25] Xu H, Xu W, Xi H, Ma W, He Z, Ma M (2013) The ER luminal binding protein (BiP) alleviates $Cd^{2+}$-induced programmed cell death through endoplasmic reticulum stress-cell death signalling pathway in tobacco cells. J Plant Physiol 170: 1434–1441.

[26] Xu J, Yin H, Li Y, Liu X (2010) Nitric oxide is associated with long-term zinc tolerance in Solanum nigrum. Plant Physiol 154: 1319–1334.