Editorial: Effect of root anatomy and apoplastic barrier development on cadmium uptake in rice

A R T I C L E   I N F O

Cadmium (Cd) is a toxic metal with high mobility from soil and known translocation into plants (Song et al., 2015). Because the main source of human exposure to Cd is from food consumption, there has been increased research examining Cd uptake in agricultural plants (Li et al., 2014; Rizwan et al., 2016; Song et al., 2015). Rice (*Oryza sativa* L.) has been identified as a major crop that contains relatively high concentrations of Cd. Excess exposure to Cd could result in adverse health conditions, including anemia, lung damage, cancer, and renal dysfunction (Song et al., 2015). An example of Cd toxicity in humans is the "itai-itai disease", caused by daily consumption of Cd-contaminated rice grown near mining sites. Patients with itai-itai disease have symptoms of renal dysfunction, severe pain and bone fractures (Uraguchi and Fujiwara, 2013). With the increase in urbanization, automobile traffic and industrialization, higher concentrations of Cd, lead, zinc and copper have been reported in urban soils (Wang et al., 2018). Several materials, including zeolites, hydroxyapatite and humic acids, have been demonstrated to be promising tools to immobilize or remove Cd from soils (Bai et al., 2018; Barragán et al., 2017; Li et al., 2017; Xin et al., 2017). However, certain cultivars can still accumulate Cd concentrations above the recommended limit when grown in soils polluted by low to moderate Cd (Newbigging et al., 2015b).

It is essential to investigate the mechanisms of Cd uptake and translocation within agricultural foodsin order to limit human exposure to Cd. Understanding mechanisms of Cd uptake will aid in identifying which agricultural genotypes and cultivation conditions are appropriate for minimizing Cd accumulation in vegetation. Currently, several studies have examined the effect of root morphology and anatomy, or cultivation conditions, on Cd uptake. The agricultural foods investigated include rice (Ge et al., 2016; Hu et al., 2015), sweet potatoes (*Ipomoea batatas* [L.] Lam.) (Xin et al., 2017) and maize (*Zea mays*) (Redjala et al., 2011). Studies conducted to understand the role of root morphology in sweet potatoes and rice have demonstrated that increased root length was correlated with increased Cd uptake (Ge et al., 2016; Xin et al., 2017). Additionally, a study on maize grown under various cultivation conditions has demonstrated that growing conditions could change root anatomy. In particular, changes in the formation of the apoplastic barrier affected Cd uptake. The earlier formation of apoplastic barriers near the root apex has been demonstrated to reduce Cd uptake within maize (Redjala et al., 2011).

To date, there are limited studies examining the combined effect of rice genotypes and cultivation conditions on the formation of roots (morphology and anatomy) and Cd uptake. Previous studies evaluating Cd uptake in rice did not report the effect of Casparian bands and suberin lamellae development within the endodermis and exodermis on Cd uptake. For example, previous studies focused on the effects of transmembrane transport proteins (Uraguchi and Fujiwara, 2012), radial oxygen loss (Wang et al., 2011), and organic acids (Liu et al., 2007) on Cd uptake in rice. The work of Huang et al. (2019) has investigated the effect of cultivation conditions on root morphology and anatomy of two rice genotypes and how root development affects Cd uptake and translocation.

On the basis of the previous work (Li et al., 2012), Huang et al. (2019) selected two genotypes of rice for their study: Guinongzhan (GNZ) (*indica*), a high Cd accumulation genotype and Zhonghua11 (ZH11) (*japonica*), a low Cd accumulation genotype. They examined two cultivation conditions: aerated (mimicking aerobic soil) and stagnant (simulating paddy soil). Four weeks after transplant, they grew four replicates of each rice genotype in either the aerated or stagnant condition. After an additional period of growth for four weeks, they examined several growth characteristics, including the dry weight of shoot and root, length of shoot, number of adventitious root, porosity of root, and radial oxygen loss.

Root morphology was examined by imaging fresh roots, and the data were analyzed using the WinRhizo® software (Redjala et al., 2011). Total root length, root surface area, number of root tips and average adventitious roots diameter were analyzed as features of root morphology. Root anatomy was analyzed by visualization of the apoplastic barrier formation (Casparian bands and suberin lamellae) using fluorescence microscopy. Roots were prepared for detection by sectioning along the root axis to determine the start of apoplastic barrier formation, then stained. Casparian bands were stained with berberine hemisulphate, followed by aniline blue. Suberin lamellae was stained with Fluorol Yellow 088.

Cadmium concentration, subcellular distribution and concentration-dependent kinetics were determined by subjecting pre-grown rice plants to a nutrient solution without Cd or with 1 mg/L of $CdCl_2$ for 10 days. Plants were then collected, oven-dried, weighed and digested using nitric acid with microwave heating. The concentrations of Cd in the acid digest were determined using atomic absorption spectrometry (AAS). Subcellular distribution was investigated from freshly harvested plants using the technique described by Anil et al. (2005). Components of the root segment symplast and cell wall were determined using the method described by Fu et al. (2011) and Ye et al. (2012). Concentration-dependent kinetics were analyzed by excising plant roots at the basal node, incubating the roots in a Hoagland nutrient solution, and then incubating the roots in a Cd test solution (containing $Ca(NO_3)_2$, $C_6H_{13}NO_4S$, and varying Cd concentrations; pH 5.8). After incubation, adsorbed Cd was removed and the roots were oven-dried and weighed. Cd concentration was then determined using microwave-assisted nitric acid digestion, followed by AAS analysis.

The ZH11 genotype was observed to have smaller maximum root length compared to the GNZ genotype in both cultivation conditions. Plants grown in aerated conditions yielded greater maximum root length compared with plants grown in stagnant conditions for both genotypes. The endodermis and exodermis developed earlier in ZH11 compared with the GNZ genotype, regardless of cultivation condition. Plants grown in stagnant conditions had accelerated maturation of the endodermis and exodermis, along with formation of the Casparian band and suberin lamellae closer to the root apex, compared to the same genotype grown in aerated conditions.

Fractionation results demonstrated that most Cd was accumulated in the root symplast and cell wall fractions, with little accumulation in the apoplastic sap. The amount of Cd present in subcellular fractions was highly affected by the cultivation conditions, but no significant difference was observed in Cd concentration between the genotypes. Growing rice plants under stagnant conditions significantly reduced the concentration of Cd in the root apoplastic sap and symplast. Both genotype and cultivation conditions had a significant effect on Cd translocation and accumulation. Growth of rice plants in stagnant conditions resulted in lower Cd concentrations in the shoots and roots and less translocation from the root-to-shoot compared to the same genotype grown under aerated conditions. It is important to note that the interaction factors between genotypes and cultivation conditions were not significant, indicating that the cultivation conditions affected each genotype in comparable manners regarding Cd uptake. When analyzing Cd root influx, rice plants grown under aerated conditions were saturated at high concentrations of Cd; however, there was no significant difference in K_m values between the genotypes, indicating that the affinity for Cd is similar between the two genotypes.

Huang et al. (2019) demonstrated that the ZH11 genotype had faster maturation of apoplastic barriers close to the root apex compared with the GNZ genotype. Because apoplastic barriers are effective at preventing movement of heavy metals and reduce the accumulation and translocation of Cd from the epidermis to the xylem (Redjala et al., 2011), the faster maturation of apoplastic barriers in the ZH11 genotype make this plant more resistant to Cd uptake. Earlier development of these apoplastic barriers results in less Cd accumulation in the apoplastic sap, with higher accumulation of Cd in plant cell walls.

The results of growing different rice genotypes in aerated and stagnant conditions have demonstrated how root morphology and anatomy can affect Cd accumulation and translocation to various plant structures. GNZ rice plants grown under stagnant conditions had root morphology and anatomy comparable to the low Cd accumulation genotype, ZH11. Phenotypic characteristics of a low Cd accumulator were: an increase in root porosity, accelerated apoplastic barrier formation, decreased maximum root length, and decreased number of root tips per root surface area).

A correlation analysis performed by the authors indicated that the number of root tips per root surface area may be a key factor in facilitating Cd uptake and translocation as this may determine the effective Cd uptake area of the plant. The results of Huang et al. (2019) suggest plants with fewer number of root tips per root surface area and faster development of apoplastic barriers have reduced Cd uptake by the roots and xylem. Additionally, changing cultivation conditions from aerated to stagnant could change the root morphology and anatomy of high Cd accumulation crops to resemble low Cd accumulation genotypes.

Huang et al. (2019) demonstrated which root morphology and anatomy characteristics should be considered when screening for low Cd accumulation rice plants. They also showed how cultivation conditions alter Cd accumulation in plants. These findings contribute to a better understanding of Cd uptake within rice plants. The conclusions from the work of Huang et al. (2019) can be used in future studies to determine which rice genotypes and cultivation conditions are ideal for growth in Cd contaminated soils. However, when multiple toxic elements are present in contaminated soil, cultivation conditions that decrease Cd may not decrease other elements, e.g., arsenic. It has been reported that stagnant conditions reduced Cd uptake, but increased arsenic uptake in rice cultivar (Hu et al., 2015; Newbigging et al., 2015a). Other factors, such as, pH, organic matter and interfering metals, have been demonstrated to affect Cd mobility in soil and uptake in crops (Bai et al., 2018; Newbigging et al., 2015b). Future studies are required to examine how these suitable genotypes and cultivation conditions for minimizing Cd uptake can affect the accumulation of other toxic elements, such as arsenic and lead.

REFERENCES

Anil, V.S., Krishnamurthy, P., Kuruvilla, S., Sucharitha, K., Thomas, G., Mathew, M.K., 2005. Regulation of the uptake and distribution of Na$^+$ in shoots of rice (*Oryza sativa*) variety Pokkali: Role of Ca^{2+} in salt tolerance response. Physiol. Plant. 124, 451–464. https://doi.org/10.1111/j.1399-3054.2005.00529.x.

Bai, H.C., Jiang, Z.M., He, M.J., Ye, B.Y., Wei, S.Q., et al., 2018. Relating Cd^{2+} binding by humic acids to molecular weight: A modeling and spectroscopic study. J. Environ. Sci. 70, 154–165. https://doi.org/10.1016/j.jes.2017.11.028.

Barragán, P.P., Macedo, M.M.G., Olguín, M.T., 2017. Cadmium sorption by sodium and thiourea-modified zeolite-rich tuffs. J. Environ. Sci. 52, 39–48. https://doi.org/10.1016/j.jes.2016.03.015.

Fu, X., Dou, C., Chen, Y., Chen, X., Shi, J., Yu, M., et al., 2011. Subcellular distribution and chemical forms of cadmium in *Phytolacca americana* L. J. Hazard. Mater. 186, 103–107. https://doi.org/10.1016/j.jhazmat.2010.10.122.

Ge, L., Cang, L., Yang, J., Zhou, D., 2016. Effects of root morphology and leaf transpiration on Cd uptake and translocation in rice under different growth temperature. Environ. Sci. Pollut. Res. 23, 24205–24214. https://doi.org/10.1007/s11356-016-7696-8.

Hu, P., Ouyang, Y., Wu, L., Shen, L., Luo, Y., Christie, P., 2015. Effects of water management on arsenic and cadmium speciation and accumulation in an upland rice cultivar. J. Environ. Sci. 27, 225–231. https://doi.org/10.1016/j.jes.2014.05.048.

Huang, L., Li, W.C., Tam, N.F.Y., Ye, Z., 2019. Effects of root morphology and anatomy on cadmium uptake and translocation in rice (*Oryza sativa* L.). J. Environ. Sci. 75, 296–306. https://doi.org/10.1016/j.jes.2018.04.005.

Li, H., Guo, X., Ye, X., 2017. Screening hydroxyapatite for cadmium and lead immobilization in aqueous solution and contaminated soil: The role of surface area. J. Environ. Sci. 52, 141–150. https://doi.org/10.1016/j.jes.2016.04.005.

Li, W.C., Ouyang, Y., Ye, Z.H., 2014. Accumulation of mercury and cadmium in rice from paddy soil near a mercury mine. Environ. Toxicol. Chem. 33, 2438–2447. https://doi.org/10.1002/etc.2706.

Li, B., Wang, X., Qi, X., Huang, L., Ye, Z., 2012. Identification of rice cultivars with low brown rice mixed cadmium and lead contents and their interactions with the micronutrients iron, zinc, nickel and manganese. J. Environ. Sci. 24, 1790–1798. https://doi.org/10.1016/S1001-0742(11)60972-8.

Liu, J., Qian, M., Cai, G., Zhu, Q., Wong, M.H., 2007. Variations between rice cultivars in root secretion of organic acids and the relationship with plant cadmium uptake. Environ. Geochem. Health 29, 189–195. https://doi.org/10.1007/s10653-006-9063-z.

Newbigging, A.M., Paliwoda, R.E., Le, X.C., 2015a. Rice: Reducing arsenic content by controlling water irrigation. J. Environ. Sci. 30, 129–131. https://doi.org/10.1016/j.jes.2015.03.001.

Newbigging, A.M., Yan, X., Le, X.C., 2015b. Cadmium in soybeans and the relevance to human exposure. J. Environ. Sci. 37, 157–162. https://doi.org/10.1016/j.jes.2015.09.001.

Redjala, T., Zelko, I., Sterckeman, T., Legué, V., Lux, A., 2011. Relationship between root structure and root cadmium uptake in maize. Environ. Exp. Bot. 71, 241–248. https://doi.org/10.1016/j.envexpbot.2010.12.010.

Rizwan, M., Ali, S., Adrees, M., Rizvi, H., Zia-ur-Rehman, M., Hannan, F., et al., 2016. Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. Environ. Sci. Pollut. Res. 23, 17859–17879. https://doi.org/10.1007/s11356-016-6436-4.

Song, W.E., Chen, S.B., Liu, J.F., Chen, L., Song, N.N., Li, N., et al., 2015. Variation of Cd concentration in various rice cultivars and derivation of cadmium toxicity thresholds for paddy soil by species-sensitivity distribution. J. Integr. Agric. 14, 1845–1854. https://doi.org/10.1016/S2095-3119(14)60926-6.

Uraguchi, S., Fujiwara, T., 2012. Cadmium transport and tolerance in rice: Perspectives for reducing grain cadmium accumulation. Rice 5, 1–8. https://doi.org/10.1186/1939-8433-5-5.

Uraguchi, S., Fujiwara, T., 2013. Rice breaks ground for cadmium-free cereals. Curr. Opin. Plant Biol. 16, 328–334. https://doi.org/10.1016/j.pbi.2013.03.012.

Wang, M.Y., Chen, A.K., Wong, M.H., Qiu, R.L., Cheng, H., Ye, Z.H., 2011. Cadmium accumulation in and tolerance of rice (*Oryza sativa* L.) varieties with different rates of radial oxygen loss. Environ. Pollut. 159, 1730–1736. https://doi.org/10.1016/j.envpol.2011.02.025.

Wang, M., Liu, R., Chen, W., Peng, C., Markert, B., 2018. Effects of urbanization on heavy metal accumulation in surface soils, Beijing. J. Environ. Sci. 64, 328–334. https://doi.org/10.1016/j.jes.2016.11.026.

Xin, J., Huang, B., Dai, H., Mu, Y., 2017. Characterization of root morphology and root-derived low molecular weight organic acids in two sweet potato cultivars exposed to cadmium. Arch. Agron. Soil Sci. 63, 723–734. https://doi.org/10.1080/03650340.2016.1234041.

Ye, J., Yan, C., Liu, J., Lu, H., Liu, T., Song, Z., 2012. Effects of silicon on the distribution of cadmium compartmentation in root tips of *Kandelia obovata* (S., L.) Yong. Environ. Pollut. 162, 369–373. https://doi.org/10.1016/j.envpol.2011.12.002.

Karen S. Hoy
Department of Chemistry, University of Alberta,
Edmonton T6G 2G3, Canada

Jagdeesh S. Uppal
Division of Analytical and Environmental Toxicology,
Department of Laboratory Medicine and Pathology, Faculty of
Medicine and Dentistry, University of Alberta,
Edmonton T6G 2G3, Canada

X. Chris Le
Department of Chemistry, University of Alberta,
Edmonton T6G 2G3, Canada
Division of Analytical and Environmental Toxicology,
Department of Laboratory Medicine and Pathology, Faculty of
Medicine and Dentistry, University of Alberta,
Edmonton T6G 2G3, Canada
*Corresponding author.
E-mail address: xc.le@ualberta.ca

6 February 2019