

# Influence of mercury on chlorophyll content in winter wheat and mercury bioaccumulation

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## ABSTRACT

Mercury (Hg) is one of the major pollutants in soils because of the annual import of toxic Hg into the agricultural lands. The aims of the present studies are to investigate the effect of Hg on chlorophyll content in winter wheat var. jinan No. 17. Moreover, calcium (Ca) levels and bioaccumulation of Hg in wheat leaves were studied with the technique of inductively coupled plasma sector field mass spectrometer (ICP-SF-MS). The study conducted a range of Hg concentrations from 0~500 mg Hg/kg in the dry weight soil. The soil was artificially contaminated with Hg as follows: 0, 100, 200, and 500 mg Hg/kg as HgCl<sub>2</sub>. At early stages of the wheat growth, both low and high concentration of Hg stimulates chlorophyll content, but inhibits chlorophyll content at later stages of the wheat growth. Furthermore, the concentrations of Ca and Hg in wheat leaves increased with the increasing concentration of Hg on the thirty-fourth day with the technique of ICP-SF-MS. The results indicate that Hg can accelerate the absorption of Ca in winter wheat and Hg stress may affect Ca levels in wheat leaves.

**Keywords:** mercury; chlorophyll; calcium; winter wheat

Soil contamination with heavy metals will lead to losses in agricultural yield and hazardous health effects as they enter into the food chain (Schickler and Caspi 1999). The effects of heavy metals on cellular system have received a great deal of attention due to the increasing exposure of living organisms to these metals in the environment. Mercury (Hg) is one of the major pollutants in soils because of the annual import of toxic Hg into the agricultural lands (Patra and Sharma 2000). In addition, Hg is a highly toxic non-essential element and its dispersion in the environment is considered to be a global problem for its persistent character. Compared with Cd, Co, Cu, Pb, and Zn, Hg has the greatest inhibitory effects on seed germination, root elongation, and hypocotyl and coleoptile growth in wheat (Munzuroglu and Geckil 2006). It was found that Hg released to soils remains mainly in the solid phase through adsorption onto sulfides, clay particles and organic matters. The predominant form of Hg in agricultural soils is the ionic form (Hg<sup>2+</sup>) (Han et al. 2006).

The interaction between Hg and plant systems is very important because Hg has largely been employed in seed disinfectants, in fertilizers and in herbicides (McLaughlin et al. 1996). It was found that mercuric ions are able to induce oxidative stress by triggering generation of reactive oxygen species (ROS), e.g. superoxide radical, hydrogen peroxide, and hydroxyl radical in plants (Patra and Sharma 2000, Israr and Sahi 2006).

Measurement of chlorophyll activity is a method usually used to monitor oxidative stress in green plants by direct measurement of chlorophyll or chlorophyll fluorescence (KrishnaRaj et al. 2000, MacFarlane 2003). The contaminant metals often accumulate in considerable amounts in plant tissues, and the ability of plants to absorb Hg is well established (Hitchcock and Zimmermann 1957). Previous studies indicate that Hg remains mostly confined to roots when it is taken up by roots in pea plants (Beauford et al. 1977).

It was reported that intracellular Ca<sup>2+</sup> levels are linked to physiological processes in plants (Felle

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Supported by the National Natural Science Foundation of China, Grant No. 30900071.

1988, Gehring et al. 1990, Irving et al. 1992).  $\text{Ca}^{2+}$  can act as a second messenger in response to most external stimuli in plants (Bush 1995, Webb et al. 1996). However, the previous studies concerned mainly the activities of antioxidant enzymes and chlorophyll. There are fewer studies dealing with the research of the relationship between Ca absorption and bioaccumulation of Hg in plants. Thus it is necessary to investigate whether the bioaccumulation of Hg influences Ca absorption in wheat leaves. In view of these facts, the aims of the present studies are to investigate the effect of Hg on chlorophyll content in winter wheat var. jinan No. 17. Moreover, Ca levels and bioaccumulation of Hg in wheat leaves were studied with the technique of inductively coupled plasma sector field mass spectrometer (ICP-SF-MS).

## MATERIALS AND METHODS

**Soil characterization and contamination.** Soil samples were collected from the top layer (0–30 cm) located in the campus of the Shandong University of Technology. Then the soil was sieved to < 4 mm, air-dried at 35°C for 72 h, and subjected to physicochemical characterization. The soil was clay loam, with pH 5.8, an organic matter content of 5.01%, a total nitrogen content of 0.32%, a C/N ratio of 8.3, a phosphorus content of 21.8 mg/kg and an electrical conductivity of 0.08 dS/m. The composition of modified nutrient solution was carbamide and  $\text{K}_2\text{H}_2\text{PO}_4$ , and their concentration is 100 and 300 mg/kg in the dry weight soil, respectively (Yang et al. 2007).

Subsequently, the soil was artificially contaminated with Hg as follows: 0, 100, 200, and 500 mg Hg/kg in the dry weight soil as  $\text{HgCl}_2$ . The metal-polluted and control soils were then stored at room temperature for one week.

**Experimental design and plant growth.** The seeds of winter wheat var. jinan No. 17 were sterilized in 3% formalin for 5 min followed by proper washing with double distilled water and soaked in water overnight. Then the soaked seeds were sown in earthen pots with metal polluted and control soils, each in three replicates. Seeds were sown in each pot to a depth of 0.5 cm and watered daily until seed germination. The whole experiment was carried out in a house conditions with natural light, day/night temperature of 26/20°C and day/night humidity of 70/90%.

**Analysis of chlorophyll.** Plants were collected at the second week after seed germination. Then

the plants were collected every week. Leaf samples were harvested and the fresh weights (FW) were recorded. Then leaf samples were treated with 3 ml dimethyl sulphoxide in the presence of polyvinylpyrrolidone at 60°C for 2 h in the dark. Photosynthetic pigments of all of the samples were extracted and chlorophyll concentrations were calculated using the extinction coefficients and equation given by Barnes et al. (1992). A spectrophotometer is used at two wavelengths (648.2 and 664.9 nm) for maximum absorption of chlorophyll a and b, respectively.

$$\text{Chlorophyll a} = 14.85 A_{664.9} - 5.14 A_{648.2}$$

$$\text{Chlorophyll b} = 25.48 A_{648.2} - 7.36 A_{664.9}$$

$$\text{Total chlorophyll} - (\text{a} + \text{b}) = 7.49 A_{664.9} + 20.34 A_{648.2}$$

**Ca and Hg analysis.** For analysis of leaf samples, leaves were washed thoroughly with deionized water and a microwave-assisted digestion procedure was applied. Approximately 0.3 g of sample (dry weight, DW) was weighed into Teflon bombs. Then, 10 cm<sup>3</sup> of  $\text{HNO}_3$  was added and the samples were digested. After digestion, samples were transferred quantitatively into polypropylene tubes and filled up to 10 cm<sup>3</sup>. Subsequently, Ca and Hg in the digest were determined using ICP-SF-MS (Agilent, Japan) with external calibration using <sup>114.8</sup>In and <sup>102.9</sup>Rh as an internal standard.

**Statistical analyses.** Statistical analyses were performed using SPSS (version 13.0 for Windows). Six replicates were taken in analysis of chlorophyll, and three replicates were taken in analysis of Ca and Hg. The significant differences ( $P < 0.05$ ) between treatments and control were statistically evaluated by standard deviation and Student's *t*-test methods.

## RESULTS AND DISCUSSION

Heavy metals have been given of particular concern because they are frequently present at elevated concentrations in biosolids. In addition, heavy metals cannot decompose in the environment and can be translocated into plants and further transferred into animal and human food chains (Sloan et al. 2001, Oliver et al. 2005). It was found that mercury is one of the most toxic heavy metals and has the ability to bioaccumulate (Kelly et al. 2006).

As one of the most important plant organs, leaves play a significant role in capturing light and making food via photosynthesis. Photosynthesis is one of the most sensitive processes. Detailed studies

indicate that heavy metals interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clijsters 1990). It was found that  $Hg^{2+}$  can induce the inhibition of protochlorophyllide photoreduction in homogenates of dark-grown wheat leaves (Solymosi et al. 2004). Moreover, Hg affected P and Mn status in plants, reduced chlorophyll concentration and increased malondialdehyde (MDA) and thiol levels (Moreno-Jiménez et al. 2009).

The results presented in this article showed that the content of chlorophyll a, chlorophyll b and total chlorophyll was increasing with the increasing concentration of Hg on the fourteenth day (Table 1). There was a significant difference between the treated groups and the control group. However, the content of chlorophyll a, chlorophyll b and total chlorophyll was decreasing with the increasing concentration of Hg on the twenty-eighth and thirty-fourth days (Table 1). Moreover, there was a significant difference between the treated

groups and the control group. On the twenty-first day, the content of chlorophyll a, chlorophyll b and total chlorophyll was significantly higher than the control at 100 and 200 mg Hg/kg, while significantly lower than the control at 500 mg Hg/kg (Table 1). Our results indicate that both low and high concentration of Hg will stimulate chlorophyll synthesis at early stages of the wheat growth. However, at later stages of the wheat growth, not only low but also high concentration of Hg will inhibit chlorophyll synthesis. Previous studies indicate that  $Hg^{2+}$  cannot significantly affect plant growth at low levels. However, the high level of  $Hg^{2+}$  becomes strongly phytotoxic to cells and can induce visible injuries and physiological disorder (Ortega-Villasante et al. 2005, Zhou et al. 2007). In plants, mercury ions may substitute metal ions in photosynthetic pigments causing a decrease in photosynthesis rates (Xylander et al. 1996, Kupper et al. 1998). Our results also indicate that the high level of  $Hg^{2+}$  is strongly phytotoxic to cells and can decrease the chlorophyll content.

It was shown that  $Hg^{2+}$  can readily accumulate in higher plants (Wang and Greger 2004, Israr et al. 2006). Our results indicate that the concentration of Hg in wheat leaves was increasing with the increasing concentration of Hg on the thirty-fourth day (Figure 1). Furthermore, there was a significant difference between the treated groups and the control group (Figure 1). The results of some studies suggest that Hg can bind to nucleic acids and cause inhibition of spindle formation and other chromosomal alterations and finally cell

Table 1. Content of chlorophyll in the leaves of wheat treated with different concentrations of Hg

Hg (mg Hg/kg)	Chlorophyll in leaves (mg/g FW)		
	C <sub>a</sub>	C <sub>b</sub>	C <sub>a+b</sub>
Day-14			
0	0.71 ± 0.01 <sup>a</sup>	0.19 ± 0.013 <sup>a</sup>	0.90 ± 0.02 <sup>a</sup>
100	0.79 ± 0.01 <sup>b</sup>	0.23 ± 0.01 <sup>b</sup>	1.02 ± 0.01 <sup>b</sup>
200	1.14 ± 0.01 <sup>c</sup>	0.33 ± 0.02 <sup>c</sup>	1.46 ± 0.01 <sup>c</sup>
500	1.19 ± 0.01 <sup>c</sup>	0.36 ± 0.01 <sup>c</sup>	1.55 ± 0.01 <sup>c</sup>
Day-21			
0	1.21 ± 0.01 <sup>a</sup>	0.32 ± 0.01 <sup>a</sup>	1.53 ± 0.01 <sup>a</sup>
100	1.37 ± 0.03 <sup>b</sup>	0.43 ± 0.02 <sup>b</sup>	1.81 ± 0.01 <sup>b</sup>
200	1.54 ± 0.03 <sup>c</sup>	0.49 ± 0.03 <sup>b</sup>	2.03 ± 0.01 <sup>c</sup>
500	1.11 ± 0.02 <sup>d</sup>	0.26 ± 0.01 <sup>c</sup>	1.37 ± 0.01 <sup>d</sup>
Day-28			
0	1.32 ± 0.02 <sup>a</sup>	0.40 ± 0.02 <sup>a</sup>	1.72 ± 0.01 <sup>a</sup>
100	1.29 ± 0.01 <sup>b</sup>	0.35 ± 0.02 <sup>b</sup>	1.64 ± 0.01 <sup>b</sup>
200	1.19 ± 0.01 <sup>c</sup>	0.31 ± 0.01 <sup>b</sup>	1.50 ± 0.02 <sup>c</sup>
500	1.10 ± 0.01 <sup>d</sup>	0.26 ± 0.01 <sup>c</sup>	1.36 ± 0.01 <sup>d</sup>
Day-34			
0	1.23 ± 0.01 <sup>a</sup>	0.42 ± 0.02 <sup>a</sup>	1.65 ± 0.01 <sup>a</sup>
100	1.19 ± 0.01 <sup>b</sup>	0.36 ± 0.01 <sup>b</sup>	1.55 ± 0.01 <sup>b</sup>
200	1.10 ± 0.02 <sup>c</sup>	0.29 ± 0.02 <sup>c</sup>	1.39 ± 0.01 <sup>c</sup>
500	0.82 ± 0.02 <sup>d</sup>	0.23 ± 0.02 <sup>c</sup>	1.05 ± 0.01 <sup>d</sup>

Values are the mean of six replicates and different letters within columns indicate significant differences ( $P < 0.05$ )

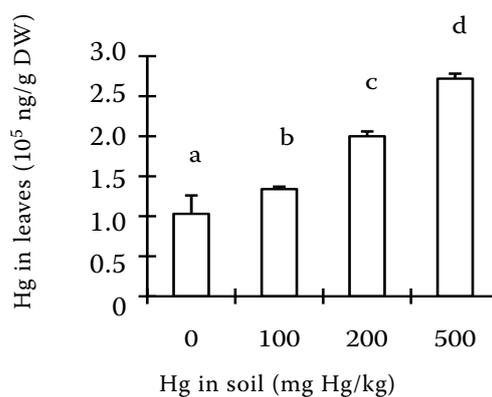


Figure 1. The accumulation of Hg in wheat leaves. Vertical bars represent standard deviation of the mean ( $n = 3$ ). The wheat grew in the soil containing 0, 100, 200, and 500 Hg mg/kg soil for 34 days. Different letters in the figure indicate significant differences ( $P < 0.05$ )

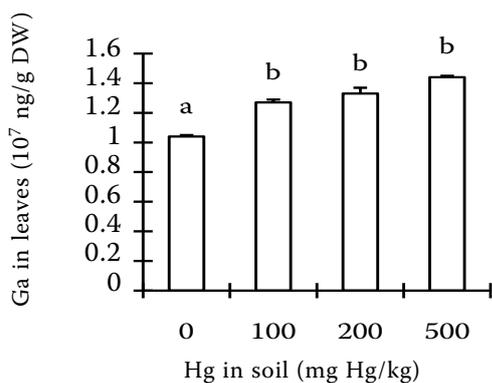


Figure 2. The levels of Ca in wheat leaves. Vertical bars represent standard deviation of the mean ( $n = 3$ ). The wheat grew in the soil containing 0, 100, 200, and 500 Hg mg/kg for 34 days. Different letters in the figure indicate significant differences ( $P < 0.05$ )

division (De Flora et al. 1994, Cavallini et al. 1999). Our results also show that contaminant Hg can accumulate in considerable amounts in the plant tissue before they produce visible phytotoxic and oxidative damage effects.

It is well known that  $\text{Ca}^{2+}$  regulates numerous physiological cellular phenomena as a second messenger as well as triggering pathological events such as cell injury and death. Intracellular  $\text{Ca}^{2+}$  levels are linked to physiological processes in plants (Felle 1988, Gehring et al. 1990, Irving et al. 1992). Calcium ions can act as a second messenger in responses to most external stimuli in plants (Bush 1995, Webb et al. 1996). Our results indicate that Ca in the leaves of wheat was increasing with the increasing concentration of Hg on the thirty-fourth day (Figure 2). There was a significant difference between the treated groups and the control group (Figure 2). Thus Hg stress may affect Ca levels in wheat leaves, and can accelerate Ca absorption in wheat. However, since there was no significant difference between the treated groups (Figure 2), the reason why the different levels of  $\text{Hg}^{2+}$  have no significant effects on Ca levels needs to be further researched.

In summary, this experiment analyzes the effects of Hg on winter wheat exposed to toxic  $\text{Hg}^{2+}$  concentration in long-term experiments. The study comprised a range of Hg concentrations from 100 to 500 mg Hg/kg based on the previous observations. At early stages of the wheat growth, both low and high concentration of Hg stimulated chlorophyll synthesis, and inhibited chlorophyll synthesis at later stages of the wheat growth. Furthermore, Ca and Hg in wheat leaves were increasing with the

increasing concentration of Hg on the thirty-fourth day with the technique of ICP-SF-MS. The results indicate that Hg can accelerate the absorption of Ca in winter wheat and Hg stress may affect Ca levels in wheat leaves.

## REFERENCES

- Barnes J.D., Balaguer L., Manrique E., Elvira S., Davison A.W. (1992): A reappraisal of the use of DMSO for the extraction and determination of chlorophyll a and b in lichens and higher plants. *Environmental and Experimental Botany*, 32: 85–100.
- Beauford W., Barber J., Barringer A.R. (1977): Uptake and distribution of mercury within higher plants. *Plant Physiology*, 39: 261–265.
- Bush D.S. (1995): Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology and Plant Molecular Biology*, 46: 95–122.
- Cavallini A., Natali L., Durante M., Maserti B. (1999): Mercury uptake, distribution and DNA affinity in durum wheat (*Triticum durum* Desf.) plants. *Science of the Total Environment*, 243/244: 119–127.
- De Flora S., Bennicelli C., Bagnasco M. (1994): Genotoxicity of mercury compounds. A review. *Mutation Research*, 317: 57–79.
- Felle H. (1988): Cytoplasmic free calcium in *Riccia fluitans* L. and *Zea mays* L.: interaction of calcium and pH? *Planta*, 176: 248–255.
- Gehring C.A., Irving H.R., Parish R.W. (1990): Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. *The Proceedings of the National Academy of Sciences Online (US)*, 87: 9645–9649.
- Han F.X., Su Y., Monts D.L., Waggoner A.C., Plodinec J.M. (2006): Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. *Science of the Total Environment*, 368: 753–768.
- Hitchcock A.E., Zimmermann P.W. (1957): Toxic effects of vapors of mercury and of compounds of mercury on plants. *Annals of the New York Academy of Sciences*, 65: 474–497.
- Irving H.R., Gehring C.A., Parish R.W. (1992): Changes in cytosolic pH and calcium of guard cells precede stomatal movements. *Proceedings of the National Academy of Sciences*, 89: 1790–1794.
- Israr M., Sahi S., Datta R., Sarkar D. (2006): Bioaccumulation and physiological effects of mercury in *Sesbania drummonii*. *Chemosphere*, 65: 591–598.
- Kelly E.N., Schindler D.W., St. Louis V.L., Donald D.B., Vladicka K.E. (2006): Forest fire increases mercury accumulation by fishes via food web restructuring and increased mercury inputs. *The Proceedings of the National Academy of Sciences Online (US)*, 103: 19380–19385.
- KrishnaRaj S., Dan T.V., Saxena P.K. (2000): A fragrant solution to soil remediation. *International Journal of Phytoremediation*, 2: 117–132.

- Kupper H., Kupper F., Spiller M. (1998): In situ detection of heavy metal substituted chlorophylls in water plants. *Photosynthesis Research*, *58*: 123–133.
- MacFarlane G.R. (2003): Chlorophyll a fluorescence as a potential biomarker of zinc stress in the grey mangrove, *Avicennia marina*. *Bulletin of Environmental Contamination and Toxicology*, *70*: 90–96.
- McLaughlin M.J., Tiller K.G., Naidu R., Stevens D.P. (1996): The behaviour and environmental impact of contaminants in fertilizers. *Australian Journal of the Soil Research*, *34*: 1–54.
- Moreno-Jiménez E., Esteban E., Carpena-Ruiz R.O., Peñalosa J.M. (2009): Arsenic- and mercury-induced phytotoxicity in the Mediterranean shrubs *Pistacia lentiscus* and *Tamarix gallica* grown in hydroponic culture. *Ecotoxicology and Environmental Safety*, *72*: 1781–1789.
- Munzuroglu O., Geckil H. (2002): Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Archives of Environmental Contamination and Toxicology*, *43*: 203–213.
- Oliver I.W., McLaughlin M.J., Merrington G. (2005): Temporal trends of total and potentially available element concentrations in sewage biosolids: a comparison of biosolid surveys conducted 18 years apart. *Science of the Total Environment*, *337*: 139–145.
- Ortega-Villasante C., Rella'n-A'lvaréz R., Del Campo F.F., Carpena-Ruiz R.O., Hernández L.E. (2005): Cellular damage induced by cadmium and mercury in *Medicago sativa*. *Journal of Experimental Botany*, *56*: 2239–2251.
- Patra M., Sharma A. (2000): Mercury toxicity in plants. *Botany Review*, *66*: 379–422.
- Schickler H., Caspi H. (1999): Response of antioxidative enzymes to nickel and cadmium stress in hyperaccumulator plants of the genus *Alyssum*. *Plant Physiology*, *105*: 39–44.
- Sloan J.J., Dowdy R.H., Balogh S.J., Nater E. (2001): Distribution of mercury in soil and its concentration in runoff from a biosolids-amended agricultural watershed. *Journal of Environmental Quality*, *30*: 2173–2179.
- Solymosi K., Lenti K., Myśliwa-Kurziel B., Fidy J., Strzałka K., Böddi B. (2004): Hg<sup>(2+)</sup> reacts with different components of the NADPH : protochlorophyllide oxidoreductase macrodomains. *Plant Biology*, *6*: 358–368.
- Van Assche F., Clijsters H. (1990): Effect of metals on enzyme activity in plants. *Plant, Cell and Environment*, *13*: 195–206.
- Wang Y., Greger M. (2004): Clonal differences in mercury tolerance, accumulation and distribution in willow. *Journal of Environmental Quality*, *33*: 1779–1785.
- Webb A.A.R., Mcainch M.R., Taylor J.E., Hetherington A.M. (1996): Calcium ions as intracellular second messengers in higher plants. *Advances in Botanical Research*, *22*: 45–96.
- Xylander M., Hagen C., Braune W. (1996): Mercury increases light susceptibility in the green alga *Haematococcus lacustris*. *Botanica Acta*, *109*: 222–228.
- Yang X.Y., Yang J.S., Huang Z., Xu L.G. (2007): Influence of chelators application on the growth and lead accumulation of maize seedlings in Pb-contaminated Soils. *Journal of Agro-Environmental Sciences*, *26*: 482–486.
- Zhou Z.S., Huang S.Q., Guo K., Mehta S.K., Zhang P.C., Yang Z.M. (2007): Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. *Journal of Inorganic Biochemistry*, *101*: 1–9.

Received on October 24, 2009

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