

Pb, Cu botanogeochemical anomalies and toxic effects on plant cells in Pb-Zn (Sn) ore fields, Northeast Guangxi Autonomous Region, China

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Abstract In the Lingchuan-Daoping and Xinglu Pb-Zn ore fields in northern and eastern Guangxi Autonomous Region, Pb, Cu botanogeochemical anomalies may be ascribed to the excessive amounts of Pb and Cu taken up by the root system of plants, such as China fir (*Cunninghamia lanceolata* Lamb. Hook), mason pine (*Pinus massoniana* Lamb.) and bracken fern (*Pteridium aquilinum* var. *latiusculum*). Under transmission electron microscope (TEM), the excess Pb, Cu in the leaf cells of the plants are present as high electron-density substances, which were precipitated in the leaf cells, causing phytotoxic effects by deforming and injuring cellular tissues. The sorts of toxic elements accumulating in the leaf cells are consistent with those of the botanogeochemically anomalous elements in the polluted soil where the plants grow. In addition, the plants may also be capable of resisting the invasion of excess Cu (and Pb) .

Key words Pb, Cu botanogeochemical anomaly; phytocell deformation; phytotoxic effect

1 Introduction

Botanogeochemical anomaly is not only one of the most effective methods to explore mineral resources (Brooks, 1983; Kovalevskii, 1984; Ruan Tianjian and Zhu Youguang, 1985; Song Ci'an et al., 2000; Dunn and Angelica, 2000), but also one of the methods which is commonly used to monitor and assess environmental pollution (Alloway, 1990; Peng An and Wang Wenhua, 1991; Dunn and Angelica, 2000). When soil is contaminated by heavy metals and the heavy metals reach abnormally high concentrations, the uptake of these metals from the soil by the root systems will also increase. The excessive accumulation of such metals in plants will give rise to botanogeochemical element anomalies (Alloway, 1990), resulting in phytotoxicity. Although the relative toxicity of different metals to plants can vary with plant genotype and experimental conditions, the metals which, when present in excessive amounts, are generally most toxic to higher plants and microorganisms are Hg, Cu, Ni, Pb, Co, Cd, and possibly Ag, Be and Sn as well (Kabata-Pendias and Pendias, 1984). There is a serious problem of heavy metal pollution in some mining sites (Lei Liangqi, 2006; Nurlidia Mansor et al., 2006). This paper is to deal with botanogeochemical anomalies and the

accumulation and phytotoxicity of Pb and Cu in leaves of plants grown in the areas polluted by Pb and Cu (i.e., the Pb-Zn and Pb-Sn ore fields).

2 Methods

Two types of ore field were selected for this study. One type refer to the Pb, Cu polluted fields (i.e., the fields with high concentrations or anomalies of Pb, Cu, etc.), including the Pb, Cu (Sn) abnormal fields located in the upper part of the Lingchuan-Daoping Pb-Zn and Xinglu Pb-Cu (Sn) ore bodies (both of them are located in the northern and eastern Guangxi Autonomous Region, respectively). The other type refers to the unpolluted fields (i.e., Pb, Cu low concentration or normal fields), including the peripheries of the Lingchuan-Daoping Pb-Zn ore field and Xinglu Pb Cu (Sn) ore field. The fresh leaves were cut off from the plants, such as China fir (*Cunninghamia lanceolata*, Lamb. Hook), mason pine (*Pinus massoniana*, Lamb.), bracken fern (*Pteridium aquilinum* var. *latiusculum*), etc, and soil samples (A+B layer) were collected for analysis so as to determine the element contents of soil parent materials where the plants grow in both the Pb, Cu polluted and unpolluted fields.

Green mustard (*Brassica chinensis* L.) and leaf mustard (*Brassica juncea* L.) were planted in a

glasshouse for 108 days in (1) unpolluted natural soil, (2) artificially prepared Pb and Cu polluted soil (adding 5% w/w lead (II) acetate trihydrate and 5% w/w copper (II) acetate monohydrate in the natural soil), and (3) artificially prepared Cu polluted soil (adding 5% w/w copper (II) acetate monohydrate in the natural soil). Then, the whole above-grown biomass was collected for determination.

The treatment procedure for fresh plant leaf and vegetable samples includes: (1) the samples were washed, air-dried, weighed and oven-dried (at 80°C); and (2) the samples were grounded as fine as 0.2 mm, weighed and ashed (450°C). The Pb and Cu contents ($\mu\text{g}\cdot\text{g}^{-1}$) in plant and vegetable ashes were determined by Direct Reading Type Spectrograph (America 90-750 II).

Three or four pieces picked off from each of the field-collected leaves and greenhouse-grown vegetables were immersed in the solution (25% v/v glutaraldehyde with phosphate buffer, pH=6–8). These pieces were cut by an Ultra Thin Slicer (ULTRACUFE) to prepare plant slices, which were then observed under a transmission electron microscope (TEM-1200EX) to reveal cellular

structures. The microarea contents of the plant slices were determined on an electron spectrometer (PV9900).

3 Pb, Cu botanogeochemical anomalies

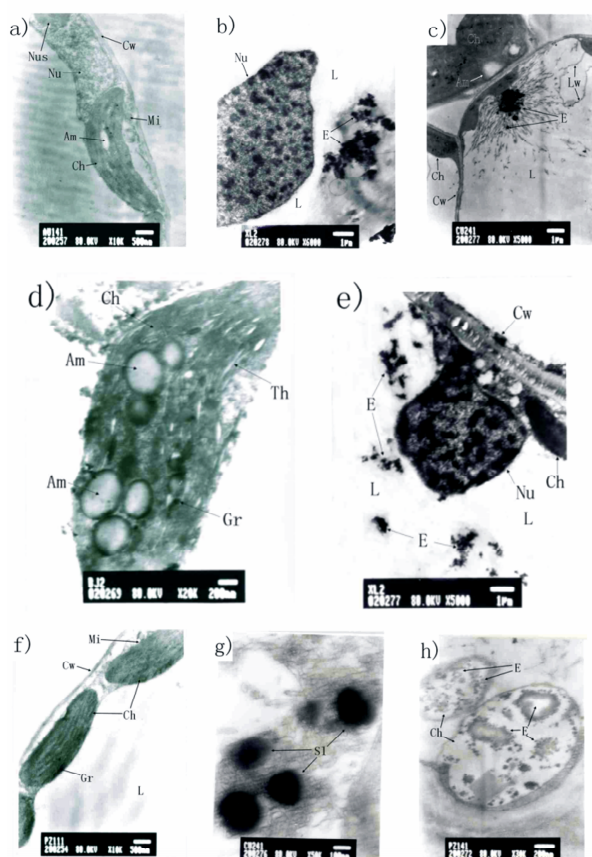
Listed in Table 1 are the contents of Pb and Cu in the ashes of plant leaves and vegetables grown in the Pb-, Zn-high soils (including soil in Pb, Cu polluted fields and artificially prepared polluted soil) are expectantly higher than those in the background soils (including soils in the unpolluted fields and unpolluted natural soil), indicating that the plants take up Pb, Cu from the Pb-, Zn-high soils, thereafter producing botanogeochemical anomalies. It is worthy of note that the Cu (including most of Pb) plant transfer coefficient (Ruan Tianjian and Zhu Youguang, 1985) is significantly lower for the Pb-, Zn-high soils than for the background soils (Table 1). The decrease of Cu (Pb) uptake rate of plants grown in the Pb-, Zn-high soils seems to be related to the plant self-resistance effect against the invasion of toxic elements (e.g. Cu, Pb) (Kovalevskii, 1987; Peng An and Wang Wenhua, 1991; Jiang Hua et al., 2006).

Table 1. Average Pb, Cu contents ($\mu\text{g}\cdot\text{g}^{-1}$) in soils and Pb, Cu plant transfer coefficients*

Sample type	Material	Number of samples	Pb content	Pb T.C.	Cu content	Cu T.C.
Upper part of the	Soil	13	986	—	490	—
Lingchuan-	CFL	8	130	0.13	167	0.34
Daoping	MP	7	222	0.23	190	0.39
Pb-Zn ore body	BF	8	118	0.12	162	0.33
Outside of the	Soil	9	25.1	—	24	—
Lingchuan-	CFL	7	6.8	0.27	9.3	0.38
Daoping	MP	6	9.9	0.39	10.4	0.43
Pb-Zn ore field	BF	8	14.9	0.59	12.5	0.52
Upper part of the	Soil	12	607	—	434	—
Xinglu Pb-Sn ore	CFL	7	170	0.28	116	0.27
body	MP	8	183	0.3	112	0.26
	BF	9	136	0.22	89	0.21
Outside of the	Soil	10	38	—	28	—
Xinglu Pb-Sn ore	CFL	6	7.1	0.19	9.2	0.34
field	MP	7	13.1	0.34	8.4	0.3
	BF	7	20	0.53	15.1	0.54
Artificially		2	600	—	500	—
prepared Pb, Cu	GM	4	144	0.24	125	0.25
polluted soil	LM	4	201	0.34	161	0.32
Artificially	Soil	2	25.5	—	500	—
prepared Cu	GM	4	10.2	0.4	121	0.24
polluted soil	LM	4	9.1	0.34	145	0.29
Experimental	Soil	2	25.5	—	55.8	—
unpolluted natural	GM	4	9.1	0.34	17.5	0.31
soil	LM	4	8.4	0.33	18.5	0.33

* CFL . China fir leaf; MP. mason pine; BF. bracken fern; GM. green mustard; LM. leaf mustard.

T.C. transfer coefficient = element content in plant / element in soil where the plant grows (Ruan Tianjian and Zhu Youguang, 1985). “—” blank.



Photos: a) The leaf of bracken fern grown in unpolluted soil: the membrane structures, including that the cell wall, chloroplast membrane and nuclear membrane were developed well, with clear discernible outlines. From the outside of the Xinglu Pb-Sn ore field (Shatian Town); b) the leaf of bracken fern grown in the Pb-, Cu (Sn)-high soil: high electron-density substances (HEDS) occurring as irregular pellets precipitated in the vacuoles and cell nucleus mass accumulates evidently. From the upper part of the Xinglu Pb-Sn ore body; c) the leaf of bracken fern grown in the Pb-, Cu (Sn)-high soil: HEDS occurring as irregular pellets and radiating mass was precipitated in the vacuoles. From the upper part of the Lingchuang-Daoping Pb-Zn ore body; d) the leaf of China fir grown in unpolluted soil: the membrane structure of leucoplast and the directional sheet-phylo structure in chloroplast. From the outside of the Lingchuang-Daoping Pb-Zn ore field (Bajiao Town); e) the leaf of China fir grown in the Pb-, Cu (Sn)-high soil: HEDS precipitated along the cellular walls, thus becoming thick and rough; there are numerous HEDS particles deposited in the vacuoles; cell nucleus mass was coagulated clearly. From the upper part of the Xinglu Pb-Sn ore body; f) the leaf of green mustard grown in unpolluted soil in greenhouse: the membrane structures of cellular wall and chloroplast were developed evidently; the directional sheet-phylo structure in chloroplast shows no variation; no HEDS present in the vacuoles; g) the leaf of green mustard grown in the Pb-, Cu-high soil in the greenhouse: the secondary lysosomes associated with HEDS show a homogeneous nature; h) the leaf of green mustard grown in the Cu-high soil in the greenhouse: the HEDS occurred as irregular pellets and bloom rings in the chloroplast, and the sheet-phylo structure in chloroplast was damaged. Am. leucoplast; Ch. Chloroplast; Cw. cell wall; E. high electron-density substance; Gr. Grana; L. vacuole; Lw. vacuole membrane; Mi. mitochondria; Nu. cell nuclear; Nus. Nucleolus; Sl. secondary lysosome; and Th. Thylakoid.

Table 2. Element contents (%) in high electron-density substances in the cells of different plant species (determined by electron spectrometer)*

No.	Na	K	P	Si	Al	Ca	Ti	Fe	Pb	Cu	Sn
LD01	—	2.45	—	8.34	—	4.41	—	2.04	42.1	40.7	—
XL01	6.7	—	4.43	12.6	—	0.72	0.57	3.31	53	17	1.73
XL02	1.76	1.66	—	3.64	—	57.4	—	—	26	9.51	—
PC01	4.41	1.27	13.8	3.68	2.15	5.38	—	—	58.2	11.1	—
CU01	16.4	—	24.6	—	—	22.3	—	—	—	36.7	—

* LD01. bracken fern leaf from the upper part of the Lingchuan-Daoping Pb-Zn ore body; XL01. bracken fern leaf from the upper part of the Xinglu Sn-Pb ore body; XL02. China fir from the upper part of the Xinglu Sn-Pb ore body; PC01. green mustard in the artificially prepared Pb, Cu polluted soil; CU01. green mustard in the artificially prepared Cu polluted soil. “—” not tested.

4 Phytocelle deformation

Under transmission electron microscope (TEM), compared with the cellular tissues of plants grown in the background soils Photos a, d, f), it is noticed that there are a number of high electron-density substances (HEDS) occurring in the leaf cells of the comparable plants grown in the Pb-, Cu-high soils (Photos b, c, e, g, h). The HEDS make the plant cellular tissues distorted or deformed: (1) the HEDS were precipitated along the cell walls and in the vicinities of chloroplasts membranes, causing the cells to become roughed and injured (Photo e); (2) the HEDS were also deposited in the chloroplasts, as a result, their

sheet-phylo structures were destroyed (Jiangsu Institute of Agriculture, 1997) (Photo h); (3) the HEDS show irregular appearances (pellet, radiating and bloom ring) in the vacuoles (Photos b, c). In addition, the secondary lysosomes are also altered and show homogeneous nature due to HEDS precipitation (Photo g).

Electron spectrometric analysis indicated that the HEDS are primarily Pb and Cu aggregates with minor Sn (Table 2). The HEDS may be formed as a result of precipitation of the insoluble-amorphous Pb compounds (Davies, 1990) and adsorbed Cu (Baker, 1990). In addition, the types of elements concentrated in the HEDS occurring in the plant leaf cells are consistent with those of heavy metal elements (Pb, Cu)

in the polluted soil and those of botanogeochemical anomalies (Tables 1 and 2). Similar results in regard to the consistency of element (Au) concentrated in the HEDS and the phytocells have also been reported from a gold mine site (Song Ci'an et al., 2004).

5 Conclusions

The plants (e.g. *Cunninghamia lanceolata*, *Pinus massoniana* and *Pteridium aquilinum var. latiusculum*) grown in the Pb-, Cu-high soils can take up Pb, Cu in excessive amounts, resulting in Pb, Cu botanogeochemical anomalies. In the meantime, the plants are capable of self-resisting the invasion of excessive toxic elements (Cu, Pb).

Lead and Cu present in excessive amounts in plant leaf cells occur as high electron-density substances (HEDS), which may consist of insoluble amorphous Pb compounds and adsorbed Cu. The HEDS can be precipitated in the leaf cells, leading to structure deformation or phytotoxic effects.

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References

- Alloway J.B. (1990) *Heavy Metals in Soils* [M]. pp.19–26. Blackie, Halsted Press, USA and Canada.
- Baker E.D. (1990) Copper. In *Heavy Metals in Soils* (ed. J.B. Alloway) [M]. pp.151–174. Blackie, Halsted Press, USA and Canada.
- Brooks R.R. (1983) *Biological Methods of Prospecting for Mineral* [M]. pp.153–158. John Wiley and Sons, New York.
- Davies B.E. (1990) Lead. In *Heavy Metals in Soils* (ed. J.B. Alloway) [M]. pp.177–196. Blackie, Halsted Press, USA and Canada.
- Dunn C.E. and Angelica R.S. (2000) Evaluation of biogeochemistry as a tool in mineral exploration and in monitoring environmental mercury dispersion in the Tapljos gold district, Amazonia, Brazil [J]. *Report-Geological Survey of Canada*. **38**, 41.
- Jiang Hua, Jing Huxiao, and Qing Weixiao (2006) Microbial resistance to the heavy metal pollution [J]. *Chinese Journal Geochemistry*, **25** (suppl.), 3–4.
- Jiangsu Institute of Agriculture (1997) *Phytophysiology* [M]. pp.12–21. Agriculture Press, Beijing.
- Kabata-Pendias A. and Pendias H. (1984) *Trace Elements in Soils and Plants* [M]. CRC Press, Boca Raton, Fla.
- Kovalevskii A.L. (1984) Biogeochemical prospecting for ore deposits in the USSR [J]. *J. Geochem Explor.* **21**, 62–73.
- Kovalevskii A.L. (1987) *Biogeochemical Exploration for Mineral Deposits* [M]. pp.25–55. VNU Science Press, Utrecht.
- Lei Liangqi (2006) Heavy metal distribution and evolution in acid drainage system developed from nickel sulfide tailings (Western Australia). *Chinese Journal Geochemistry*. **25** (suppl.). 30–31
- Nurlidia Mansor, Ian D. Pulford, and Angus B. Mackenzie (2006) Investigation of lead and Zinc dispersion from an abandoned mine site at Tyndrum, Scotland, using tree bark measurements [J]. *Chinese Journal Geochemistry*. **25** (suppl.), 23–24.
- Peng An and Wang Wenhua (1991) *Environmental Biology and Inorganic Chemistry* [M]. pp.33–78, pp.160–191. Beijing University Press, Beijing.
- Ruan Tianjian and Zhu Youguang (1985) *Geochemical Prospecting* [M]. pp.169–177. Geological Publishing House, Beijing.
- Song Ci'an, Lei Liangqi, and Yang Qijun (2000) Characteristics of phytogeochemistry in Gongpoquan copper ore field, Gangsu, China [J]. *Geochimica*. **29**, 343–350 (in Chinese with English abstract).
- Song Cian, Lei Liangqi, and Cheng Sanming (2004) Gold biogeochemical anomaly and aberrance effect of botanic cell tissue [J]. *Geochimica*. **33**, 191–196.