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Heavy metal absorption status of five plant species in monoculture and intercropping

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Abstract Absorption ability for heavy metals varies among plant species. This study is to evaluate the absorption characteristics of different plant species and planting patterns for heavy metals. Five plant species (tomato, maize, greengrocery, cabbage, and Japan clover herb) were cultivated in monoculture and in intercropping in soil contaminated with heavy metals (Cd, Pb, Cr, Cu, and Fe), to determine the absorption status. Tomato absorbs greater amounts of heavy metals (especially Cd). Furthermore, accumulation of heavy metals increased when tomato was intercropped with other plant species. Maize accumulates greater amounts of Cr, Cu, and Fe. The heavy metal concentrations were reduced when maize was intercropped. Cd and Pb accumulated more in roots of Japan Clover Herb, and the levels of all five heavy metals decreased when intercropped. Tomato intercropping is a feasible method for phytoremediation of heavy metal-contaminated soil,

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and maize intercropping is feasible for obtaining safe harvest which can be eaten securely.

Keywords Heavy metal . Intercropping . Monoculture . Phytoremediation . Safe production

Introduction

Heavy metal contamination has been a global problem. Research data has shown that more than 10% of plantation land is contaminated by Cd, As, Cr, and Pb, causing production loss of over ten million tons, twelve million tons of unsafe production every year, and the loss of at least three billion dollars (Wang [2006](#page-8-0)). The heavy metals can enter the human body through the food chain, resulting in serious human health concerns (Wagner [1993](#page-8-0); Ren et al. [2003](#page-8-0)). Chemical and physical remediation methods are available, although these alter the soil structure and reduce soil biological activity (McGrath et al. [2001](#page-8-0)). Phytoremediation is a method of using plants to remove toxic elements from the environment, or to make them non-toxic (Salt et al. [1998](#page-8-0)). It was first introduced by Chaney [\(1983](#page-7-0)) in 1983.

Accumulation of heavy metals varies among plant species (Arao and Ishikawa [2006\)](#page-7-0), and generally is higher in dicotyledons than in monocotyledons (Taub and Goldberg [1996\)](#page-8-0). In addition, variation exists in plant species to absorb some metals in higher concentration than other metals (Keller, et al. [2003\)](#page-7-0), resulting in diverse absorption characteristics for

different heavy metals. Further, data from other studies has indicated that plant pattern also influence heavy metal absorption. For example, intercropping Cunninghamia lanceolata with tea crops reduces the levels of Pb, Ni, Mn, and Zn in soil and tea leaves (Xue and Fei [2006\)](#page-8-0). Such alteration of heavy metal absorption may be caused by the alteration in the type, amount, and function of secretions and enzymes, or inter and intra-specific competition among plants for various elements (Bruce [2001](#page-7-0); Silvertown and Charlesworth [2001\)](#page-8-0). Therefore, in this study, five plant species (tomato, maize, greengrocery, cabbage, and Japan clover herb) were selected to evaluate their ability to extract heavy metals, the absorption characteristics between heavy metals, and the best planting pattern (monoculture or intercropping) for phytoremediation. The experiments were conducted on soil contaminated by multiple heavy metals (Cd, Pb, Cr, Cu, and Fe) at low levels. The soil contamination was caused by long time irrigation of local heavy metal contaminated water.

Materials and methods

Study site

The field site was approximately $700m^2$, located in Wenling Township, Taizhou City, in Zhejiang province. The area is in the subtropical monsoon climate and has an average annual temperature of 16.1°C and annual rainfall of 1441.9 mm. We measured the soil physical and chemical properties before planting, the data showed in Table 1.

Experimental material

Five types of plant species were used, including (1) maize (Zea mays L.var. Jinzhumi), (2) tomato

Table 1 Soil physical and chemical properties

pH	5.02		Available Cd 0.88 mg kg^{-1}
Organic matter	42.8 g kg^{-1}		Available Pb 108.0 mg kg ⁻¹
Total nitrogen	294 mg kg^{-1}		Available Cr 52.77 mg kg^{-1}
Available P	331 mg kg^{-1}		Available Cu 58.35 mg kg^{-1}
Available K	110 mg kg^{-1}		Available Fe 7597 mg kg^{-1}
Soil texture	Sand 34%	$Silt 43\%$	Clay $23%$

(Lycopersicon esculentum.var. Zhongshu 4), (3) greengrocery (Brassica chinensis L.var. Hangzhou youdonger), (4) cabbage (Brassinca oleracea. var. capitata. Jingfengyihao), and (5) Japan clover herb (Kummerowia striata Schindl). Seeds of these plant species were purchased from the Hangzhou Seeds Company.

Experimental design

The field experiment consisted of 11 planting patterns. Individual plant species were grown in monoculture and in intercropping in pair. All patterns were replicated three times in a randomized complete block design, shown in Table [2.](#page-2-0) The spacing between blocks is 40 cm.

Before cultivation, seeds were germinated in soil mixed with vermiculite and peat $(V/V=1/1)$. Seedlings were transferred to the experimental field in April and harvested in July. Tall crops had two rows per region, and low crops had three. Line spacing was 30 cm.

Sampling and measurements

Five random plant and soil samples were taken from each plot. The plant samples were washed thoroughly with tap water and then distilled water. Plants were divided into fruits, shoots, and roots, and then weighed. Plant tissues were oven-dried at 70°C until there was no change in weight, and a final weight was then determined. The oven-dried plant samples were ground with a stainless steel grinder. Soil samples were air-dried, crushed, and passed through a 0.082 mm sieve. All the samples were digested with HNO3 until completely clear (MULTIWAVE 3000). The available heavy metal concentrations (Cd, Pb, Fe, Cu, and Cr) were determined by inductively coupled plasma/mass spectrometry (ICP-MS/G3271A).

Data analysis

The enrichment coefficient (EC) compares the accumulation efficiency of plant species and tissues.EC is described as the heavy metal concentration in soil divided by the concentration in certain plant tissue (Zu et al. [2004\)](#page-8-0).

All the data analysis based on Country Standard for Heavy Metals (GB 2762-2005 and GB15618-

1995). When the heavy metal levels in production are below the standard, it has little hazard, could be eaten securely (safe production). To enable safe harvest for eating from heavy metal-contaminated soil, we calculated the heavy metal limits in farmland, according to the EC values obtained from our results, as well as the maximum levels of heavy metal allowed in foods.

Statistic analysis

In order to detect significant differences in the data, analysis of variance (ANOVA) was performed, followed by the least significant difference test (LSD) at $P=0.05$ level.

Results

Accumulation of heavy metals in different plant organs

Concentrations of heavy metals in plant tissues are summarized in Tables [3](#page-3-0) and [4](#page-4-0).

The Cd concentration was high in shoots of tomato, roots of Japan clover herb, and all tissues of greengrocery. When greengrocery was intercropped with Japan clover herb, the Cd levels increased to 2.72 and 2.02 mg kg^{-1} in its shoots and roots, respectively (Table [4](#page-4-0)). Fruits of tomato and greengrocery contained excess Cd, while maize and cabbage posed no health risk.

Pb concentration was high in roots of tomato and Japan clover herb. When tomato was intercropped with Japan clover herb, the Pb content in tomato roots increased to 20.14 mg kg^{-1} (Table [3](#page-3-0)). Pb levels were higher than the maximum allowable level in both greengrocery and cabbage; the Pb level in tomato fruit was approximately equal to the maximum allowable level, and below the maximum allowable level in maize.

High levels of Cr were determined in roots of tomato and maize. When maize was intercropped with Japan clover herb, the Cr concentration in maize roots increased to 43.13 mg kg^{-1} (Table [3\)](#page-3-0). Cr levels of most plants were too high for safe consumption, with the exception of tomato intercropped with Japan clover herb.

Cu concentration was high in shoots of tomato. And the levels of Cu in roots and leaves of maize in monoculture were determined to be 35.85 and 31.72 mg kg^{-1} , respectively (Table [3\)](#page-3-0).

Maize Cabbage Maize+JCH Tomato Greengrocery+

Maize+JCH

Cabbage

Maize

Tomato

JCH

Greengrocery Tomato+Maize Greengrocery+

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Tomato+ JCH

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Maize Greengrocery+

Maize

Greengrocery+

Cabbage

JCH

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Table 4 Heavy metal concentrations (mean±SD) in various plant organs of greengrocery, cabbage, and Japan clover herb in monoculture and intercropping. Values are given in mg kg^{-1} DW

		Greengrocery		Cabbage		JCH	
		Shoot	Root	Shoot	Root	Shoot	Root
Cd	Greengrocery	1.76 ± 0.04	1.59 ± 0.32	$0.48 \pm 0.02b$	0.62 ± 0.11	0.46 ± 0.00	
	Cabbage	1.89 ± 0.02	1.66 ± 0.15	0.86 ± 0.20 ab	0.77 ± 0.07	0.42 ± 0.03	0.85 ± 0.06
	JCH	2.72 ± 0.88	2.02 ± 0.46	$1.00 \pm 0.09a$	0.64 ± 0.03	0.40 ± 0.05	0.84 ± 0.01
Pb	Greengrocery	4.25 ± 0.87	9.94 ± 3.83	7.22 ± 1.12	$7.57 \pm 0.07b$	$5.07 \pm 0.00a$	
	Cabbage	3.75 ± 1.31	8.86 ± 1.19	7.27 ± 0.71	$8.95 \pm 0.66a$	$3.60 \pm 0.50b$	12.80 ± 3.03
	JCH	5.15 ± 0.97	9.88 ± 0.16	8.70 ± 1.19	$8.79 \pm 0.03a$	$5.67 \pm 0.05a$	17.04 ± 2.10
Cr	Greengrocery	8.62 ± 1.04	$16.94 \pm 0.00a$	4.17 ± 0.07	21.03 ± 0.72	6.98 ± 0.00	
	Cabbage	12.51 ± 6.70	$10.12 \pm 2.85b$	3.69 ± 0.34	17.06 ± 1.55	7.47 ± 2.39	11.80 ± 6.42
	JCH	9.86 ± 0.00	$20.67 \pm 0.00a$	4.05 ± 0.00	18.76 ± 15.45	2.63 ± 1.41	9.49 ± 2.46
Cu	Greengrocery	13.01 ± 1.08	$12.81 \pm 0.51ab$	8.20 ± 0.15	$4.85 \pm 0.07c$	10.96 ± 0.00	
	Cabbage	12.91 ± 1.63	$11.89 \pm 0.62b$	6.97 ± 0.50	$9.09 \pm 1.19a$	11.62 ± 0.56	15.61 ± 3.80
	JCH	15.13 ± 0.16	$14.65 \pm 0.91a$	8.05 ± 1.08	$6.33 \pm 0.27b$	13.28 ± 3.62	14.32 ± 1.30
Fe	Greengrocery	310.7 ± 17.2	434.1 ± 27.8	$123.4 \pm 14.8b$	208.8 ± 64.5	$446.3 \pm 0.0a$	
	Cabbage	296.0 ± 46.6	351.7 ± 68.1	$125.5 \pm 2.8b$	275.3 ± 11.7	$283.2 \pm 21.1b$	319.8 ± 78.9
	JCH	279.5 ± 0.00	351.9 ± 0.0	$196.5 \pm 11.0a$	269.0 ± 58.2	$279.8 \pm 71.3b$	422.8 ± 25.2

Fe levels were high in shoots and roots of tomato, as well as roots and leaves of maize. When tomato was intercropped with Japan clover herb, the Fe concentration in tomato roots increased to 908.9 mg kg^{-1} (Table [3\)](#page-3-0).

The heavy metal enrichment coefficient in plants

The heavy metal EC of plants is given in Tables [5](#page-5-0) and [6.](#page-5-0)

High EC was obtained for Cd and Cu in all tissues of tomato, and high EC for Cr in tomato shoots and roots. Tomato intercropped with Japan clover herb obtained the highest EC for Cd, which was 0.182, 2.080, and 1.250 in its fruits, shoots, and roots, respectively (Table [5](#page-5-0)).

High EC values were obtained for Cd, Cr, and Cu in maize stems and leaves, roots, and fruits, respectively. For both maize monoculture and maize intercropped with tomato, the highest levels of Cd accumulated in the maize leaves, leading to EC values of 0.920 and 0.932, respectively (Table [5](#page-5-0)). In addition, the highest EC value for Cr was 0.817 in roots of maize when intercropped with Japan clover herb (Table [5\)](#page-5-0).

In greengrocery, EC values were a little high for Cr and Cu, and very high for Cd. With the exception of Cd, the levels of other heavy metals in greengrocery were higher in roots than in shoots. The highest EC value for Cd was 3.091 and 2.295 in shoots and roots of greengrocery, respectively, when intercropped with Japan clover herb (Table [6\)](#page-5-0).

High EC was obtained for Cd and Cu in all tissues of cabbage, and a high EC for Cr in cabbage shoots. The highest EC values for Cd were 1.136 and 0.875 in cabbage shoots intercropped with Japan clover herb and cabbage roots from monoculture, respectively (Table [6](#page-5-0)).

Japan clover herb showed high EC values for Cd, Cr, and Cu. All EC values in roots of Japan clover herb were very high, and the highest values of 1.295 and 1.818 for Cd were obtained when intercropped with tomato or maize, respectively (Table [5\)](#page-5-0).

Accumulation of heavy metals in different planting patterns

Heavy metal absorption between planting patterns are summarized in Tables [3](#page-3-0) and 4.

In contrast to data from monoculture, intercropping of tomato and maize resulted in increased levels of Fe in fruits and Cr in shoots of tomato. Furthermore, tomato intercropped with Japan clover herb exhibited increased levels of Cd in fruits and Fe in roots, but

Table 5 Heavy metal enrichment coefficients in various plant organs of tomato, maize, and Japan clover herb in monoculture and intercropping

decreased levels of Cu in shoots and Cr in fruits (Table [3](#page-3-0)).

When maize was intercropped with tomato, levels of Cd in roots and stems, and Cu in roots

Table 6 Heavy metal enrichment coefficients in various plant organs of greengrocery, cabbage, and Japan clover herb in monoculture and intercropping

		Greengrocery		Cabbage		JCH	
		Shoot	Root	Shoot	Root	Shoot	Root
Cd	Greengrocery	2.000	1.807	0.545	0.705	0.523	0.000
	Cabbage	2.148	1.886	0.977	0.875	0.477	0.966
	JCH	3.091	2.295	1.136	0.727	0.455	0.955
Pb	Greengrocery	0.039	0.092	0.067	0.070	0.047	0.000
	Cabbage	0.035	0.082	0.067	0.083	0.033	0.119
	JCH	0.048	0.091	0.081	0.081	0.053	0.158
Cr	Greengrocery	0.163	0.321	0.079	0.399	0.132	0.000
	Cabbage	0.237	0.192	0.070	0.323	0.142	0.224
	JCH	0.187	0.392	0.077	0.356	0.050	0.180
Cu	Greengrocery	0.223	0.220	0.141	0.083	0.188	0.000
	Cabbage	0.221	0.204	0.119	0.156	0.199	0.268
	JCH	0.259	0.251	0.138	0.108	0.228	0.245
Fe	Greengrocery	0.041	0.057	0.016	0.027	0.059	0.000
	Cabbage	0.039	0.046	0.017	0.036	0.037	0.042
	JCH	0.037	0.046	0.026	0.035	0.037	0.056

and leaves were decreased, while Fe levels in fruits increased). On the other hand, maize co-cultured with Japan clover herb exhibited decreased levels of Cd in stems and leaves, Pb in roots, Cu in roots and leaves, Fe in fruits, and Cr in fruits and leaves), while the levels of Fe and Cr in stems increased. These results were in contrast to data from maize monoculture (Table [3\)](#page-3-0).

Compared with monoculture, the levels of Cr in roots of greengrocery, as well as the levels of Pb and Cu in roots of cabbage, were reduced when cultured together. However, when cabbage was intercropped with Japan clover herb, the level of Cu in roots was decreased, while the level of Fe in shoots was increased (Table [4\)](#page-4-0).

The levels of Pb in shoots and Fe in roots of Japan clover herb were decreased while the level of Cr in shoots of Japan clover herb was increased when intercropped with tomato. Moreover, when Japan clover herb was intercropped with maize, the levels of Pb in shoots, and the levels of Fe and Cr in roots were decreased, while the levels of Cd in roots and Cr in shoots were increased(Table [3\)](#page-3-0). Finally, the level of Pb in shoots was decreased when Japan clover herb was intercropped with cabbage, and the level of Fe was increased when intercropped with greengrocery (Table [4](#page-4-0)). These results were in contrast to data from Japan clover herb monoculture.

Discussion

Variations of heavy metal extracting ability between plant species

Absorption and accumulation of heavy metals in plants depend upon both environmental factors, such as temperature, moisture, organic matter, pH, and nutrient availability (Sharma et al. [2007\)](#page-8-0), and intrinsic factors, such as rooting systems and mechanisms of transport and detoxification. Intercropping and monoculture in this study both showed that tomato extracted the largest quantity of heavy metals among the five tested species (Additional table 3). Tomato root systems absorbed large amounts of Cd, Pb, Cr, Cu, and Fe, and then transported the metals upward to accumulate in shoots and fruits, posing a hazard if consumed. Roots of Japan clover herb accumulated Cd and Pb in larger amounts than the other metals, while roots of maize accumulated Cr, Cu, and Fe. Transfer of heavy metal upward varies according to the specific and generic mechanism of metal transport across cell membranes and the chelation of metals in cell saps (Broadley et al. [2001](#page-7-0)). Transport of heavy metals other than Cr in maize was too weak to accumulate much in fruits, making the fruit safe to eat. Shoots of foliar vegetables accumulate high levels of heavy metals. In our study, greengrocery and cabbage enriched a large quantity of heavy metals. Data showed that only the Cd level in cabbage was lower than the maximum allowable level in foods.

Variations of the absorption characteristics between heavy metals

Different heavy metals were absorbed at different levels in each of the plants studied. The order of EC values for the heavy metals was Cd>Cu>Cr>Pb>Fe for most plant tissues. With the exception of the EC values for Fe in maize, which were similar to those for Pb, the EC values for Pb in roots of Japan clover herb were higher than those for Cr, and the EC values for Cr in roots of foliar vegetables were higher than those for Cu. Variation in absorption characteristics are due to diverse adsorption sites, ionic electrostatic attraction, influence of pH, and absorption competition between heavy metals, which is due to the type of interaction with the solid phase and pH (Tobin et al. [1984;](#page-8-0) Fontes and Gomes [2003](#page-7-0)). Keltjens and van Beusichem [\(1998](#page-7-0)) reported that the Cd concentration in maize shoots decreased after addition of Cu, due to metal competition at root absorption sites, consistent with our results showing that the EC values of Cd decreased to levels similar for Cu in maize fruits.

The heavy metal limits in farmland is shown in Table 7. Although the EC values for Cd were the highest in most plants, the soil limits were almost as high as the tertiary standard, which was based on the environmental quality standard. Contrary to this, the Pb and Cr limits were below the primary or secondary standard levels. The data indicated that Cd levels will more often be low enough to allow safe harvest, while Pb and Cr levels are more often high enough to make harvest unsafe. Thus, following the environmental quality standard for heavy metal-contaminated soil does not always ensure safe harvest; the type of heavy metal and the planting patterns must also be taken into account.

Variations of phytoremediation ability between planting patterns

Much evidence suggests that soil microorganisms affect the uptake of heavy metals through alteration of the mobility of heavy metals in the rhizosphere soil via soil acidification and also affecting heavy metal transport within plants (Khan et al. [2000](#page-7-0)). In addition, most organic acids secreted by roots can increase the removability and exchangeability of heavy metals. For example, citric acid is found to markedly increase desorption of heavy metals and the exchangeability of Zn and Cd (Jones [1998](#page-7-0); Schwab et al. [2008](#page-8-0)).

Intercropping affects heavy metal absorption, due to alteration of rhizosphere microorganisms and root secretion. For example, microbial beta-diversity and stability was significantly higher in tree-based intercropping than in monoculture in Quebec (Lacombe et

Table 7 Heavy metal limits in farmland. Values are given in mg kg^{-1} DW

	Tomato	Maize	Greengrocery	Cabbage
C _d	0.6 ^a	1.0	0.9	2.0
Pb	90	100	60	40
Cr	40	50	20	60

^a When tomato was intercropped with Japan clover herb, the limit of Cd was 0.27 mg kg

al. [2009](#page-8-0)), and white lupin exuded more organic acids when co-cultured with wheat than when grown in monoculture, in order to make more P available for wheat (Kamh et al. 1999). The alteration may be positive or negative, depending on the species with which it is co-cultured. In our study, tomato absorbed more heavy metals when it was intercropped with other plants. Thus, intercropping of tomato with other hyperaccumulators can increase the efficiency of phytoremediation in heavy metal-contaminated soil. On the other hand, the levels of most heavy metals were reduced in maize intercropped with other plant species. Therefore, intercropping maize may be a feasible method for obtaining safe harvest. Japan clover herb intercropped with other plant species probably accumulated lower Pb and higher Cr levels in shoots, leading to increased phytoremediation efficiency of Pb-contaminated soils in monoculture, and Cr-contaminated soils when intercropped. The difference in heavy metal levels in greengrocery and cabbage between monoculture and intercropping was substantially low enough so as to be negligible.

Evaluate of these alteration provide clues for optimizing the phytoremediation by appropriate planting patterns.

Phytoremediation of heavy metal-contaminated soil

The levels of most heavy metals decreased slightly in soil used to grow tomato or maize (Additional table 1). Since the biomass of greengrocery and cabbage is very small, the total quantity of heavy metals accumulation is small. Therefore, no significant differences in heavy metal levels were found in soil used to grow greengrocery or cabbage (Additional table 2). Therefore, hyperaccumulator with large biomass are needed for efficient phytoremediation of contaminated soil.

Data in this study showed that the Cr levels in soil increased after planting (Additional tables 1 and 2), possibly due to atmosphere sedimentation or irrigation by Cr-contaminated water. Further research is needed to verify the effects seen in this study.

Conclusions

Tomato is an appropriate plant species for phytoremediation, due to its ability to accumulate high levels of heavy metals, which are also increased by intercropping. Maize could be used for production of safe harvest, especially when intercropped with other species. Foliar vegetables are not the most efficient plant species to use either for remediation or production.

Cd is the heavy metal that is most readily absorbed by plants. However, under the circumstance of the same contamination level of Cd, Pb, or Cr in soil, soil with Cd contamination will provide safer plants compared with the other two contaminations. The practical environmental quality standard for heavy metals lies on the type of heavy metal and the planting patterns.

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