Arsenic Availability from Chromated Copper Arsenate (CCA)–Treated Wood

Farhana Alamgir Rahman, Deborah L. Allan,* Carl J. Rosen, and Michael J. Sadowsky

ABSTRACT

Lumber used to construct raised garden beds is often treated with chromated copper arsenate (CCA). This project aimed to determine (i) how far As, Cu, and Cr had diffused away from CCA-treated wood surfaces in raised garden beds under realistic conditions, (ii) the uptake of these elements by crops, and (iii) the effect of CCA solution on soil bacteria. This study showed that As, Cu, and Cr diffuse into soil from CCA-treated wood used to construct raised garden beds. To determine crop uptake of these elements, contaminated soil 0 to 2 cm from the treated wood was obtained from two different beds (40–50 mg kg^{-1} As); control soil was collected 1.5 m away from the treated wood (<3–10 mg kg^{-1} As). Four replicates of carrot (*Daucus carota* var. *sativus* Hoffm. cv. Thumbelina), spinach (*Spinacia oleracea* L. cv. Indian Summer), bush bean (*Phaseolus vulgaris* L. cv. Provider), and buckwheat (*Fagopyrum esculentum* Moench cv. Common) were grown in pots containing these soils in a greenhouse. After harvest, plant materials were dried, ground, digested, and analyzed for As by inductively coupled plasma–hydride generation (ICP–HG). Concentrations of As in all crops grown in contaminated soils were higher than those from control soils. The levels of As in the crops remained well below the recommended limit for As set by the United States Public Health Service (2.6 mg kg^{-1} fresh wt.). To determine if bacteria in soils 0 to 2 cm from the treated wood had higher resistance to Type C chromated copper arsenate (CCA-C) solution than those from reference soils, dilution plates were set up using quarter-strength tryptic soy agar (TSA) media and 0 to 22.94 g L^{-1} (0–1.25% v/v) CCA-C working solution. The microorganisms from soils adjacent to treated wood had greater growth on the CCA-amended media than those from reference soils outside the bed.

BACTERIAL, FUNGAL, and insect infestations reduce the service life of wood. Chemical preservatives can reduce damage by these organisms and increase durability and life expectancy of wood. Wood preservative chemicals can be broadly divided into two categories: the "organics" or oil-based preservatives (e.g., pentachlorophenol, creosote, copper naphthenate, and coal tars), and the "inorganics" or waterborne preservatives (e.g., chromated copper arsenate [CCA], ammoniacal copper arsenate [ACA], ammoniacal copper zinc arsenate [ACZA], and acid copper chromate [ACC]) (Lebow, 1996). However, "hybrid" preservatives, such as ammoniacal copper quat (ACQ), which contain both organic (quaternary ammonium compound or "quat") and inorganic (CuO) components have also become available in recent years. Since the 1970s, inorganic wood preservatives have been more popular because of restrictions on usage and potentially serious environmental and health risks associated with exposure to organic preservatives (Warner and Solomon, 1990).

The waterborne wood preservative CCA has been used extensively to treat wood since its development by an Indian scientist, Dr. Sonti Kamesam, in 1933 (Lahiry, 1997). The roles of Cu and As in the CCA formulation are to inhibit fungi and insects, respectively, while Cr plays a key role in the "fixation" process, which binds CCA components to wood (Lebow, 1996). Chromated copper arsenate is currently available in three different formulations (Types A, B, and C), each with different ratios of CrO_3, CuO, and As_2O_5. The most commonly used CCA formulation in the United States is CCA-C (Lebow and Tippie, 2001). This formulation was developed by the American Wood-Preservers' Association in 1969 (Lahiry, 1997) and it offers the best combination of performance, durability, and leach resistance (Lebow, 1996). However, the USEPA recently announced a voluntary decision by industry to phase out consumer use of CCA-treated wood products by 31 Dec. 2003 (USEPA, 2002). Thus, in the year 2004, the USEPA will not allow CCA products for residential use. However, wood treated with CCA before this date can still be used in residential areas, and existing structures containing CCA-treated wood are not affected by this action.

Common uses of CCA-treated wood include decks, fences, boardwalks, playground equipment, picnic tables, raised garden beds, and retaining walls. Chromated copper arsenate–treated wood is widely available in retail lumberyards and is typically green in color, although it may also be dyed brown by manufacturers. Because CCA-treated wood is less expensive, devoid of pungent odors, and leaves a dry surface that can be painted, it is favored over its organic counterparts (Warner and Solomon, 1990; Lebow, 1996).

The major effect of CCA-treated wood on the environment is considered to be the possible, perhaps excessive, diffusion of As, Cu, and Cr into adjacent soil and leaching into ground water. Many factors can affect the amount of diffusion that occurs, including time exposed to environmental conditions, wood species, preservative retention levels, orientation, and exposed surface area of the treated wood (Hingston et al., 2001); as well as site factors such as moisture content or water movement (Kaldas and Cooper, 1996), pH (Stilwell, 1998), and presence of soluble inorganic and organic ions (Cooper and Ung, 1992). Arsenault (1975) reported that hardwoods do not fix CCA components as well as softwoods and that the distribution and concentration of preservative components in wood may affect the rate of leaching loss. Cooper et al. (1997) noted that both fixation and leaching of CCA components varied significantly among eastern hardwood species. Significant release of CCA

Department of Soil, Water, and Climate, University of Minnesota, 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN 55108. Received 3 Feb. 2003. *Corresponding author (dallan@soils.umn.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: CCA, chromated copper arsenate; CCA-C, Type C chromated copper arsenate; CFU, colony forming unit.

components can also occur under acidic conditions, suggesting that leaching might result when outdoor treated wood is exposed to "acid rain" (pH 4.1–4.5) (Stilwell, 1998). Organic acids, which are generally present in vegetable compost, have also been shown to cause significant leaching of all constituents of CCA from treated wood (Cooper and Ung, 1992). This may have significant implications for gardeners who use compost in raised garden beds constructed with CCA timber.

The popularity of raised garden beds constructed with CCA-treated wood has increased over the years. Although the USEPA has listed As, Cu, and Cr as "priority pollutants" (Hingston et al., 2001), CCA-treated wood is commonly used in the construction of raised garden beds because the wood resists rot for many years, and is therefore aesthetically pleasing, convenient, and economical for the homeowner. However, this has often been a source of concern for homeowners growing vegetables in these beds. This apprehension is due to the lack of information regarding diffusion of As, Cu, and Cr from the wood into the garden soil and the potential for crop uptake.

Little is known about the effects of elevated levels of As, Cu, and Cr in CCA-contaminated soils on soil bacteria. Among the constituent elements in CCA, elevated Cu and Cr concentrations are considered to be toxic for microorganisms, but have high to moderate importance as trace elements at lower concentrations (Nies, 1999). On the other hand, As is considered to have limited beneficial function and is known to be toxic to microorganisms (Nies, 1999).

The presence of elevated concentrations of heavy metals and metalloids in soils, such as Cu, Cr, and As, may cause changes in community structure, biomass, and activities of soil microorganisms (Shi et al., 2002; Aoyama and Nagumo, 1997). These changes can result from the development and evolution of microbial resistance systems (Díaz-Raviña and Bååth, 1996; Konstantinidis et al., 2003; Doelman et al., 1994), many of which are encoded by plasmids (Silver, 1996). Soil environments simultaneously impacted with several metals often lead to the development of bacteria with multiple resistances (Viti et al., 2003; Lodewyckx et al., 2002). Soils contaminated with CCA have the ability to select for bacteria that are resistant to elevated concentrations of As, Cu, and Cr. To date, however, there are only limited reports of the influence of CCA on soil microorganisms (Clausen, 2000; Gong et al., 2002), and in some cases overall microbial responses to CCA have not been investigated. Therefore, as a part of this study, we assessed the effect of elevated levels of CCA on microbial communities derived from contaminated soils.

The objectives of this study were to (i) quantify the amounts of soil As, Cu, and Cr that diffused from CCA-treated wood in established garden beds; (ii) determine the uptake of CCA constituent elements in four crops, and determine the potential for human exposure to As from consumption of vegetables grown in beds constructed with CCA timber; and (iii) evaluate whether bacterial communities in soils close to CCA-treated wood differ in their tolerance to CCA solution compared with those from reference soils.

MATERIALS AND METHODS

Soil Arsenic, Copper, and Chromium Distribution

Six established raised garden beds, at least 10 yr old, were selected from locations in Minneapolis and St. Paul, MN. A description of the sites that were sampled is provided in Table 1. Triplicate soil core samples were collected using a 2-cm soil probe, to a depth of 15 cm. Soil was sampled inside the beds at three distances from the treated wood, approximately 0 to 2, 7.5 to 10, and 30 to 33 cm. In addition, "reference" soil samples were collected from outside the bed (approximately 1.5 m away) to serve as an indicator of background soil concentrations. Treated wood samples were also collected from each of the raised garden beds using a 2-cm-diameter hole saw. The soil samples were air-dried and ground to pass a 2-mm sieve. The soil and wood samples were processed by microwave digestion using concentrated HNO_3; As, Cu, and Cr concentrations were determined using inductively coupled plasma atomic emission spectroscopy (ICP–AES) by USEPA Method 3051 (USEPA, 1986).

Statistical analysis was performed on soil elemental concentration data using bed and sampling distance as main factors in an analysis of variance (ANOVA).

Plant Uptake

From the six beds sampled, two with the highest As levels from each soil type were selected for the plant uptake study (Sites 1 and 5; Table 1). At each site, approximately 50 kg of soil was collected from inside the bed, 0 to 2 cm away from the treated wood, to a depth of 15 cm. In addition, control soil was collected from the middle of the bed, approximately 1.5 m away from the treated wood. This was considered a control soil sample because the soil was collected from within the bed and preliminary analysis showed that As concentrations were lower than in soils adjacent to the treated wood. Selected chemical properties of these soils are presented in Table 2. Three vegetable crops and one cover crop were se-

Table 1. Description of the sites that were sampled for the As distribution study.†

Site	Location in Minnesota	Soil texture‡	Soil pH	Bray P	OM§	Vegetation in bed
				mg kg^{-1}	g kg^{-1}	
1	Marine	LS	6.6	102	109	evergreens
2	Stillwater	LS	6.2	67	68	grass
3	Wayzata	LS	6.8	366	132	flowering plants
4	Crystal	SL	6.4	231	63	vegetable crops
5	northwestern St. Paul	SL	7.3	50	55	flowering plants
6	southern Minneapolis	SL	7.3	50	56	grass

† Values represent overall mean of nine samples collected within each bed.
‡ LS, loamy sand; SL, sandy loam.
§ Organic matter.

lected for this pot experiment: carrot, spinach, bush bean, and buckwheat. Soils from each treatment were mixed separately in a large cement mixer and then sieved though an 8-mm sieve to remove rocks, large roots, and debris. Approximately 1.1 kg of soil was used for each pot (12 × 12 × 10 cm) containing beans, spinach, and carrots. Due to their greater rooting volume, buckwheat plants were sown in taller pots (10 × 10 × 33 cm) containing approximately 2.2 kg of soil. The experimental design was completely randomized with four replicates of each crop within each treatment. The plants were grown in a greenhouse (25°C [day], 18°C [night]; 16 h of light) and were watered daily and rotated periodically. After germination, the plants were fertilized at the rate of 224 kg N ha^{-1} with 30–4–8 NPK fertilizer, split weekly over a five-week period. The plants were thinned to three carrots, three spinach plants, two bean plants, and three buckwheat plants per pot. The buckwheat, spinach, and carrots were harvested upon maturity at 47, 48, and 80 d after planting, respectively. The beans were harvested over a 12-d period with the first harvest occurring 49 d after planting.

At harvest, the carrots were washed thoroughly to remove adhering soil particles using Liqui-Nox soap solution (Alconox, White Plains, NY) followed by two rinses in deionized water. The washed carrots were peeled and the tops discarded. Carrot peels and the peeled carrots were analyzed separately and the results were used to estimate concentrations of Cu, Cr, and As for carrots with peel, using fresh weight and moisture content data. The bean pods were separated from the remainder of the bean shoots for separate analysis. The entire shoot of the spinach and buckwheat plants was harvested for analysis. The roots of spinach, bean, and buckwheat plants were discarded. The aboveground tissue of beans, spinach, and buckwheat were rinsed thoroughly under running tap water and blotted dry. The plant materials were weighed and dried in a 65°C oven for 7 d, then reweighed to determine moisture content and total dry matter production. Dried plant material was ground in a Wiley mill to pass through a 1-mm screen, then digested by wet-ashing with HNO_3, H_2SO_4, and $HClO_4$ as described by Anderson (1999). Arsenic concentrations were determined by hydride generation with ICP–AES (Anderson, 1999), while Cu and Cr were determined solely by ICP–AES (Helrich, 1990). As part of the quality control procedures, the analytical run included calibration blanks and duplicate analysis of a sample after every 20 samples. A continuous calibration verification standard was run after every 10 samples. If the standard was off by 5 to 10%, the standard curve was renormalized, and in addition, the previous 10 samples were reanalyzed if the standard was off by >10%. The analytical run included several Standard Reference Materials (SRM) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD) including SRM 1547 (peach leaves), 1570 (spinach leaves), and 1573a (tomato leaves).

Analysis of variance was performed using the GLM procedure. Data for each crop variety were analyzed separately. Mean separation within each treatment and type of plant material was done by the least square means test ($p < 0.05$).

Table 3. Selected chemical properties of soils collected from three different sites for the microbial resistance experiment.

Site	As	Cu	Cr	Bray P	K	pH	OM†
	mg kg^{-1}						g kg^{-1}
Site A (contaminated soil)‡	74	59	53	71	311	6.2	22
Site A (reference soil)§	10	19	13	27	90	6.0	24
Site B (contaminated soil)	46	43	30	23	169	6.9	39
Site B (reference soil)	7	8	10	52	87	4.9	36
Site C (contaminated soil)	30	32	22	65	94	6.2	53
Site C (reference soil)	13	21	32	38	105	7.2	50

† Organic matter.
‡ Contaminated soil sampled inside the bed, 0 to 2 cm away from chromated copper arsenate (CCA)–treated wood.
§ Reference soil sampled outside the bed, 1.5 m away from CCA-treated wood.

Microbial Resistance to Chromated Copper Arsenate

Chromated copper arsenate (Type C) "working solution" (1.8 kg L^{-1}) was obtained from Quality Wood Treating (White Bear Lake, MN). The ICP analysis of the CCA solution was conducted at the University of Minnesota Research Analytical Laboratory (St. Paul, MN) to determine total element concentrations. Arsenic, Cu, and Cr concentrations in the CCA working solution were determined to be 4.4, 2.1, and 4.0 g L^{-1}, respectively. The solution was filter-sterilized (0.2-μm Supor membrane filter; Gelman Laboratory, Ann Arbor, MI) before use.

Quarter-strength tryptic soy agar (TSA) medium (Difco, Detroit, MI) containing 20 m*M* phosphate buffer, pH 7.0, was amended with CCA-C solution to give final CCA-C concentrations of 13.8, 18.4, and 22.9 g L^{-1} (i.e., 0.75, 1.00 and 1.25% v/v, respectively), except for the control medium. This concentration range was selected after an initial screening experiment showed that soil bacteria were unable to establish colonies in quarter-strength TSA media containing >27.5 g L^{-1} CCA (i.e., 1.50% CCA-C v/v).

Soil from three raised garden beds was selected for this study (represented as Sites A, B, and C in the text). Site A and B are Sites 1 and 5 respectively, while Site C is a different bed in St. Paul, MN, not used previously in this study. At each site, soil was collected inside the bed (0–2 cm from the treated wood) using a 2-cm soil probe. Because the shape of the Site C bed did not permit collecting soil at sufficient distance to guarantee little influence of the treated wood, we collected "reference" samples from outside all three beds. All samples were taken to a depth of 15 cm. The soil samples were stored in a cooler during sampling and then refrigerated at 4°C until analyzed. Selected soil chemical properties for these soils are presented in Table 3.

Soil was initially diluted 1:10 in 0.004 *M* sodium pyrophosphate buffer, pH 7.0, containing 50 μL of 0.01% Tween 80. Soil solutions were shaken on a wrist-action shaker for 30 min, and allowed to settle for 10 min. A 1-mL aliquot of the supernatant was serially and decimally diluted in 0.15 *M* NaCl. From each dilution, 0.1-mL aliquots were spread-plated onto the surface of control and CCA-amended agar plates, in tripli-

Table 2. Selected chemical properties of soils from Sites 1 and 5 used for the plant uptake study.†

Soil	Soil texture	As	Cu	Cr	NO_3–N	Bray P	K	Ca	Mg	pH	OM‡
		mg kg^{-1}									g kg^{-1}
Site 1 (0–2 cm)	loamy sand	50	136	73	2	26	142	1071	222	6.7	42
Site 1 (1.5 m)	loamy sand	<3	9	15	2	51	79	926	184	5.6	44
Site 5 (0–2 cm)	sandy loam	40	40	70	15	61	226	2605	352	7.4	65
Site 5 (1.5 m)	sandy loam	10	18	17	17	46	118	2746	419	7.0	92

† All values are means of duplicate subsamples.
‡ Organic matter.

cate. Plates were incubated for 8 d at 30°C. The number of colonies formed on each plate was recorded after 4 d and again 4 d later. These data were used to calculate the number of colony forming units (CFU) per gram of soil from each treatment. The average CFU g^{-1} soil of triplicate plates was calculated.

Analysis of variance was performed using the GLM procedure. Each site was treated as a replicate during statistical analysis. The normality of the CFU distribution was tested using the univariate procedure of SAS (Shapiro–Wilk or *W* statistic). The test showed that CFU distribution was not normal for all soils, and therefore, the data were normalized by log transformation before statistical analysis. Mean separation was done by the LS means test ($p < 0.05$).

RESULTS AND DISCUSSION

Arsenic, Copper, and Chromium Distribution in Wood and Soil

Concentrations of As, Cu, and Cr in the CCA-treated wood samples from each site varied considerably. The ranges for As, Cu, and Cr in the wood samples were 76 to 4700 mg kg^{-1} (mean = 1660), 115 to 2870 mg kg^{-1} (mean = 1070), and 155 to 5400 mg kg^{-1} (mean = 2020), respectively. Wood samples from Sites 3 and 5 had the lowest concentrations of all the elements whereas the wood sample from Site 4 had the highest concentrations. The typical concentration range for As, Cu, and Cr in CCA-treated wood is 1000 to 5000 mg kg^{-1} (Stilwell, 1998).

Site 5, which had one of the highest soil As concentrations, had the lowest As concentration in the CCA-treated wood (76 mg kg^{-1}); Cu and Cr concentrations were also low (120 and 260 mg kg^{-1}, respectively). The garden bed at Site 5 was one of the oldest beds sampled in this experiment, with visible signs of wood decay. Site 3, which also had visible wood decay, had similarly low concentrations of all three elements in the wood. Losses due to diffusion away from the wood surfaces may explain the low elemental concentrations observed in those wood samples. Site 4, which was a relatively newer bed with no visible signs of decay, had the highest concentrations of As, Cu, and Cr in the wood, but the garden soil had very low concentrations of these elements. The variability in the elemental concentrations in the wood samples could also be a result of different CCA formulations (Types A, B, and C) and retention levels (which is the amount of CCA solution used to treat wood, depending on the anticipated exposure environment of the treated wood product). With the exception of wood samples from Sites 3 and 5, all other samples had As, Cu, and Cr concentrations within the range 1000 to 5000 mg kg^{-1} reported for CCA-treated wood by Stilwell (1998).

Highest soil concentrations of As were found 0 to 2 cm from the treated wood, with a steady decline in concentration at greater distances (Fig. 1 and 2). Within each soil type, Sites 1 and 5 had the highest As concentrations with an average of 56 and 46 mg kg^{-1}, respectively, in soils close to the wood. The greatest variability in As concentrations occurred at 0 to 2 cm from the wood; a concentration as high as 72 mg kg^{-1} As occurred

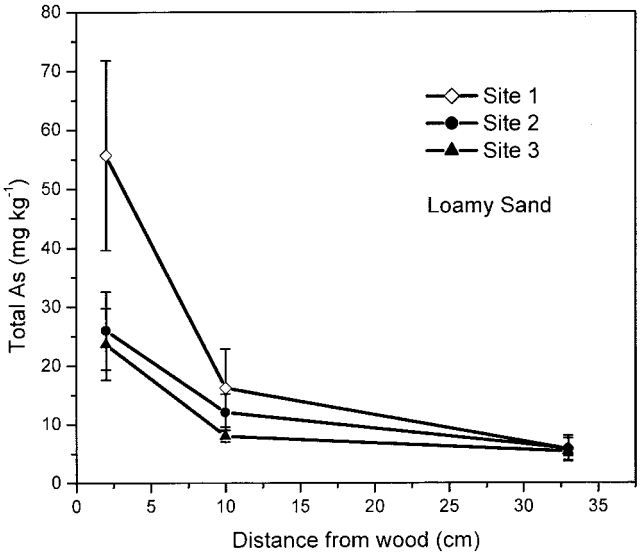

Fig. 1. Arsenic distribution in garden beds in loamy sand soils. Error bars represent ±1 standard deviation. Arsenic concentrations in reference soil from Sites 1, 2, and 3 were 3.7, 3.1, and 4.8 mg kg^{-1}, respectively.

at Site 1, adjacent to the wood. Concentrations of all three metals varied significantly with distance from the treated wood and among individual beds ($p < 0.001$). For As, there was also a significant bed and distance interaction (mainly due to differences in magnitude but not direction). Results presented in Fig. 1 and 2 clearly show that the trend of high As concentration close to the wood and a decrease in concentration further away from the wood is consistent for all six sites.

Arsenic concentrations in the reference soils collected from 1.5 m outside the garden beds ranged from 3 to 6 mg kg^{-1} (see captions for Fig. 1 and 2). Arsenic concentrations in U.S. soils typically range from 3.6 to 8.8 mg kg^{-1} (McBride, 1994, p. 308–341). In contrast, As concentrations in soils close to the treated wood were well above this range. Based on child ingestion of As-contaminated soil as the most likely pathway of natural exposure, Dudka and Miller (1999) found, from a conservative risk analysis, that soil As concentration could reach 40 mg kg^{-1} without an appreciable toxicological or environmental hazard. Sites 1 and 5 exceed this limit, having average soil As concentrations close to the wood of 56 and 46 mg kg^{-1}, respectively. In fact, Site 1 also exceeds the maximum permissible As concentration in arable soils accepted by the UK (50 mg kg^{-1}; Dudka and Miller, 1999). The Danish EPA standard is much lower for arable land (20 mg kg^{-1}; Helgesen and Larsen, 1998). Based on the USEPA's Integrated Risk Information System (IRIS) guideline, the Minnesota Pollution Control Agency's "soil reference value" (or standard) for As is 10 mg kg^{-1} (Minnesota Pollution Control Agency, 2003). This standard is considered to be protective of all individuals with the possible exception of children with soil-pica behavior. Arsenic concentrations in soils 0 to 2 cm from the CCA-treated wood exceeded this limit. Depending on the exposure environment, diffusion of As away from CCA-treated wood surfaces

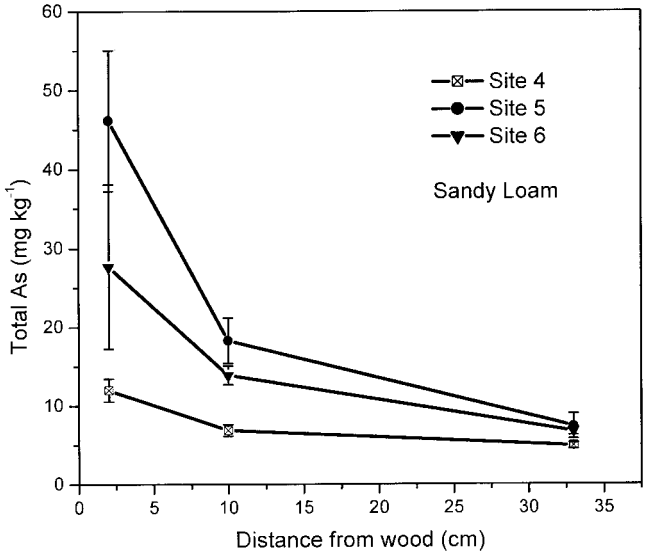

Fig. 2. Arsenic distribution in garden beds in sandy loam soils. Error bars represent ±1 standard deviation. Arsenic concentrations in reference soil from Sites 4, 5, and 6 were 4.6, 6.2, and 5.2 mg kg^{-1}, respectively.

could be even higher than that observed in this study. Contamination from treated wood therefore has the potential to be a significant environmental concern, even after a decade of service, as seen in this study.

Sites 1 and 5 had among the highest concentrations of Cu and Cr in soils 0 to 2 cm from the wood (Table 4). Like As, the concentrations of Cu and Cr were elevated in the vicinity of the CCA-treated wood and steadily decreased at further distances. Chromium concentrations in the beds remained well within the typical range in U.S. soils (20–85 mg kg^{-1} Cr; McBride, 1994, p. 308–341), although most beds had soil Cu concentrations in excess of the typical range (14–29 mg kg^{-1} Cu; McBride, 1994, p. 308–341). This indicates that although all three of these elements diffuse to some extent from CCA-treated wood, As and Cu diffuse to a greater extent than Cr.

Plant Uptake

Accuracy of the plant analysis was determined by the use of NIST standard references, which were certified for concentrations of As, Cu, and Cr. The certified concentrations for As and Cu in NIST Standard 1547 (peach leaves) are 0.060 ± 0.018 and 3.7 ± 0.4 mg kg^{-1}, respectively. The concentrations obtained fell within this range: 0.064 mg kg^{-1} (As) and 3.36 mg kg^{-1} (Cu). The noncertified concentration of Cr for this standard is 1 mg kg^{-1} and the concentration obtained by analysis was 1.04 mg kg^{-1}. The certified concentrations for As, Cu, and Cr in NIST Standard 1570 (spinach leaves) are 0.150 ± 0.05, 12.0 ± 2.0, and 4.6 ± 0.3 mg kg^{-1} respectively. The concentrations obtained were 0.153 mg kg^{-1} (As), 12.2 mg kg^{-1} (Cu), and 4.0 mg kg^{-1} (Cr). For NIST Standard 1573a (tomato leaves), the certified concentrations of As, Cu, and Cr are 0.27 ± 0.05, 11 ± 1, and 4.5 ± 0.5 mg kg^{-1}. The concentrations determined by analysis were 0.34 mg kg^{-1} (As), 10.5 mg kg^{-1} (Cu), and 3.7 mg kg^{-1} (Cr).

At both Sites 1 and 5, crops grown in soils collected 0 to 2 cm from the wood had higher concentrations of As than those grown in control soils (Table 5). All plant material from crops grown in soil from Site 1 (0–2 cm) accumulated higher As than those grown in Site 5 soil (0–2 cm) ($p < 0.05$), with the exception of bean pods. This can be attributed to the higher As concentration in soils from Site 1 (0–2 cm) and possibly to the lower organic matter content of the soil (Table 2). Low organic matter content can increase As mobility, thereby increasing its availability for plant uptake. In the control soil from Site 1 (1.5 m), however, the As concentration was much lower than that of Site 5 control soil, and this accounts for the generally lower concentrations of As in crops grown in soil from Site 1 (1.5 m) compared with those in crops grown in soil from Site 5 (1.5 m).

Among edible portions of all crops, carrots (without peel) had the lowest concentration of As (Table 5). However, As concentrations were twice as high in carrots with peel than carrots without peel when grown in soil collected 0 to 2 cm from the treated wood. Among all crops, bean leaves and stems accumulated the highest concentrations of As, but the bean pods had relatively low As concentrations. Spinach and buckwheat plants also showed the ability to translocate As to the shoot. Buckwheat is a phosphate-accumulating plant and its ability to accumulate arsenate, which is a chemical analog of phosphate, was investigated in this study. Buckwheat did show an ability to transport As to the shoot; however, accumulation of As was relatively low.

Table 4. Copper and chromium distribution in soils sampled at varying distances from chromated copper arsenate (CCA)–treated wood in six different sites.

	Site					
Distance from wood	1	2	3	4	5	6
cm	mg kg^{-1}					
	Cu					
0–2	136.2 ± 67.3	25.2 ± 11.9	53.4 ± 9.4	14.2 ± 1.9	41.2 ± 7.2	30.4 ± 7.2
7.5–10	50.4 ± 24.2	13.3 ± 2.7	33.0 ± 3.5	12.1 ± 1.3	25.1 ± 5.2	21.8 ± 1.1
30–33	21.9 ± 5.3	9.9 ± 2.3	27.3 ± 3.3	12.2 ± 0.8	20.5 ± 3.9	16.2 ± 1.0
150 (reference soil)	9.3 ± 3.6	8.1 ± 0.1	11.9 ± 1.2	6.9 ± 1.4	17.9 ± 0.5	14.3 ± 0.3
	Cr					
0–2	72.5 ± 27.8	18.4 ± 2.7	40.6 ± 3.0	12.9 ± 1.0	69.2 ± 11.8	24.9 ± 4.0
7.5–10	28.0 ± 3.9	12.3 ± 0.8	31.1 ± 1.3	11.5 ± 0.8	37.1 ± 4.4	22.3 ± 2.2
30–33	19.0 ± 1.3	11.3 ± 1.2	24.7 ± 1.6	12.9 ± 2.3	23.6 ± 2.8	14.2 ± 1.8
150 (reference soil)	15.1 ± 4.7	10.3 ± 0.4	16.6 ± 1.0	11.4 ± 0.6	16.6 ± 7.9	13.5 ± 2.5

Table 5. Concentrations of As, Cu, and Cr (dry weight basis†) in crops grown in soils collected at different distances from chromated copper arsenate (CCA)–treated wood at two different sites.

Plant materials	Soil (distance from wood)	As	Cu	Cr
		μg kg^{-1}	— mg kg^{-1} —	
Carrots (without peel)	Site 1 (0–2 cm)	283a‡	7.2a	0.7a
	Site 1 (1.5 m)	30d	4.6b	0.3a
	Site 5 (0–2 cm)	186b	4.7b	0.4a
	Site 5 (1.5 m)	55c	4.4b	0.2a
Carrot peels	Site 1 (0–2 cm)	2 950a	23.2a	1.5a
	Site 1 (1.5 m)	165d	9.5b	1.8a
	Site 5 (0–2 cm)	1 633b	11.0b	0.8a
	Site 5 (1.5 m)	307c	9.8b	0.7a
Carrots (with peel)	Site 1 (0–2 cm)	608a	9.2a	0.8a
	Site 1 (1.5 m)	49d	5.3b	0.5a
	Site 5 (0–2 cm)	378b	5.5b	0.4a
	Site 5 (1.5 m)	92c	5.2b	0.3a
Spinach	Site 1 (0–2 cm)	1 475a	18.2a	0.6a
	Site 1 (1.5 m)	65c	7.7c	0.5a
	Site 5 (0–2 cm)	358b	9.8b	0.4a
	Site 5 (1.5 m)	72c	7.8c	0.3a
Buckwheat	Site 1 (0–2 cm)	1 966a	9.1b	0.3a
	Site 1 (1.5 m)	37c	12.8a	0.3a
	Site 5 (0–2 cm)	565b	8.8b	0.4a
	Site 5 (1.5 m)	54c	6.7c	0.3a
Bean pods	Site 1 (0–2 cm)	360a	8.2a	0.09b
	Site 1 (1.5 m)	6b	6.1b	0.1ab
	Site 5 (0–2 cm)	318a	5.8b	0.2a
	Site 5 (1.5 m)	9b	5.5b	0.2a
Bean leaves and stem	Site 1 (0–2 cm)	10 894a	10.6a	0.2a
	Site 1 (1.5 m)	105d	4.8b	0.2a
	Site 5 (0–2 cm)	6 831b	5.5b	0.3a
	Site 5 (1.5 m)	682c	4.8b	0.3a

† To convert to the fresh weight concentrations, use the following moisture contents: carrots (without peel) 85%, carrot peels 89%, carrots with peel 86%, spinach 93%, buckwheat 88%, bean pods 88%, and bean leaves and stem 84%.

‡ Mean separation within columns and plant materials by least square means test ($p < 0.05$).

The limit set for As in food for human consumption is 1 mg kg^{-1} fresh wt. (Mitchell and Barr, 1995). However, the recommended limit set by the U.S. Public Health Service for fruits, crops, and vegetables is more than twofold higher (2.6 mg kg^{-1} fresh wt.) (Carbonell-Barrachina et al., 1997). The concentrations of As in edible portions of all the crops in this study were well below both limits.

The USEPA's daily reference dose for inorganic As is 0.0003 mg kg^{-1} d^{-1} (i.e., 0.0003 mg As per kilogram body weight per day). The USEPA estimates that consumption of this dose or less over a lifetime would probably not cause any chronic noncancer effects (USEPA, 2003). For a 60-kg adult, this reference dose amounts to 18 μg of As per day. Arsenic concentration in spinach on a fresh weight basis grown in soil from Site 1 (0–2 cm) was 92 μg kg^{-1}. Assuming that As is accumulated in plants primarily in the inorganic form, and that roots are growing within 2 cm of the treated wood in a worst-case scenario, 200 g of spinach grown in contaminated soil from Site 1 would contain As in excess of the daily reference dose for a 60-kg adult. Under these conditions, it is conceivable that some of the crops grown in CCA-contaminated soil may not be safe for human consumption based on the USEPA's standard. More research is required to characterize the speciation of As accumulated in vegetable crops.

Reducing As exposure via the food chain is desirable. To reduce As accumulation, plants should be grown at least 35 to 40 cm away from the treated wood in raised garden beds. For plants with extensive root systems, it may be helpful to put a plastic barrier inside the bed to a depth of at least 15 cm and approximately 30 cm away from the wood to keep plant roots away from the high As concentrations in soils close to CCA-treated wood. It may also be helpful to line the inside portions of the wooden bed with plastic when making a new bed or replacing old soil in an existing one. A border crop of some ornamental species could be planted just inside the bed to ensure that no edible crops grow too close to the CCA-treated wood. In addition, vegetables, especially root crops, grown in raised garden beds should be washed thoroughly or peeled before consumption to remove adhering As-laden soil particles and reduce ingestion of inorganic As.

Chromium concentrations in crops were not affected by soil type (contaminated vs. control) at either site. This could be due to the fact that the form of Cr that is most available to plants is Cr(VI) in the form of CrO_4^{2-}, and this form is very unstable under most soil conditions (Kabata-Pendias and Pendias, 1992, p. 227–233).

For Site 1, Cu concentrations were higher in all crops (except buckwheat) grown in soil collected from close to the treated wood compared with those grown in soil collected from the middle of the bed. Similarly, spinach and buckwheat plants grown in soil collected 0 to 2 cm from the wood at Site 5 accumulated higher concentrations of Cu than those grown in control soil from that site. However, higher Cu concentrations were found in buckwheat plants grown in control soil from Site 1 than those grown in contaminated soil. This could be due to a concentration effect since the control plants unexpectedly had much lower dry matter production compared with the plants grown in contaminated soil from that site (Table 6).

Among all crops grown in soil from Site 1, only beans had significantly reduced dry matter production when grown in contaminated soil compared with control soil (Table 6). For Site 5, however, dry matter production by crops was unaffected by the elevated As, Cu, and Cr concentrations in the soil close to the wood. Sheppard (1992) compiled a comprehensive list of crops and the corresponding toxic concentration of As in the soil, which causes substantial yield reductions. Concentrations of soil As causing yield reductions in bean, carrot, and spinach are 10 to 414, 140, and 10 to 100 mg kg^{-1}, respectively (Sheppard, 1992). A very broad range of

Table 6. Total dry matter production in plants grown in soils collected at different distances from chromated copper arsenate (CCA)–treated wood at two different sites.

Soil (distance from wood)	Dry matter				
	Bean pods	Bean leaves and stems	Buckwheat shoot	Carrot roots	Spinach tops
	g pot^{-1}				
Site 1 (0–2 cm)	4.8b†	5.3b	18.0a	5.9a	1.6a
Site 1 (1.5 m)	7.7a	7.0a	11.8b	5.8a	1.8a
Site 5 (0–2 cm)	6.6ab	5.0b	17.2a	5.8a	1.8a
Site 5 (1.5 m)	8.1a	6.3ab	19.8a	5.2a	1.8a

† Mean separation within columns by least square means test ($p < 0.05$).

critical soil As levels was suggested for bean as well as several other crops, which can be attributed to variables such as soil type and speciation of As (Sheppard, 1992). Bean plants are very sensitive to As concentrations (Woolson, 1973); in this study a soil concentration of approximately 50 mg kg^{-1} As was sufficient to reduce yield.

Microbial Resistance to Chromated Copper Arsenate

Concentrations of CCA did not affect log CFU formed for the contaminated soil, but did decrease survival in the reference soil (Fig. 3), resulting in a significant interaction ($p < 0.001$) of distance from treated wood and CCA concentration. The number of CFU g^{-1} CCA-contaminated soil was higher than the number of CFU g^{-1} reference soil at the 22.9 g L^{-1} CCA-C concentration. The range of log CFU g^{-1} reference soil was 6.48 to 5.93, when CCA-C concentration was increased from 0 to 22.9 g L^{-1}. When transformed, this represents a 72% decrease in CFU g^{-1} reference soil from 3.0×10^6 to 8.5×10^5 CFU.

This study was conducted using three different soils from three separate raised beds with each site treated as a replicate during statistical analysis. Figure 3 shows the mean separation as calculated by the LS means test ($p < 0.05$). Although the comparison is among three soils that vary in many soil properties besides CCA contamination, the CFU are similar for all soils at zero CCA. Therefore, it can be inferred that the results observed are probably due to soil CCA contamination and not inherent differences in other soil properties.

These results indicate that bacteria from CCA-contaminated soils were more able to establish colonies on CCA-amended media than those from reference soils. While the number of CFU g^{-1} soil decreased 72% over the range of concentrations tested for the reference soils, there was no significant effect on survival for bacteria from contaminated soils over this concentration range. These data suggest that the elevated As, Cu, and Cr concentrations in soils close to CCA-treated wood exert sufficient selection pressure to trigger resistance to CCA in the bacterial community. It should be noted, however, that although these results were highly significant statistically, they may or may not be biologically significant, and could be a product of the inherent differences between the bacterial communities from the reference and CCA-contaminated soils. Further investigation is required to determine the biological importance of the differences observed. Additional research efforts might include isolating the bacterial species that demonstrate resistance to CCA-C, using DNA techniques to determine if there are differences in the composition of resistant and nonresistant bacterial communities, and establishing which element or combination of elements in CCA-C determines its toxicity to soil bacteria. Research by Gong et al. (2002) suggests that Cr may be the element that determines CCA-C toxicity to microorganisms. Such research would facilitate further understanding of the ecological implications of elevated levels of As, Cu, and Cr in soils.

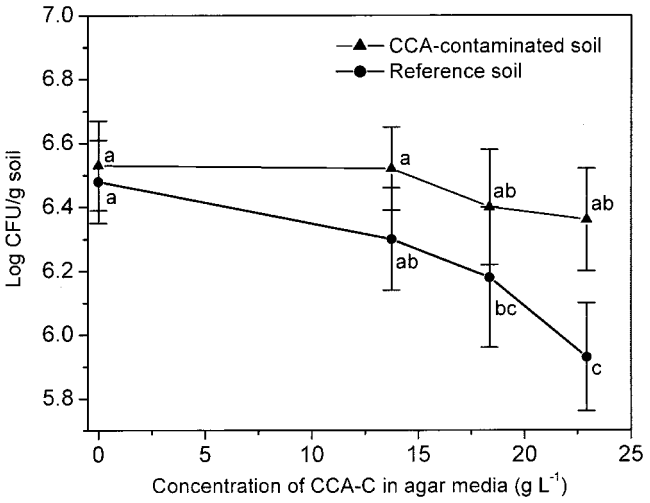

Fig. 3. Relationship between the log colony forming units (CFU) g^{-1} soil and concentration of chromated copper arsenate (CCA) in agar media. Error bars represent ±1 standard deviation. Mean separation within soils and Type C chromated copper arsenate (CCA-C) concentration by least square means test ($p < 0.05$).

CONCLUSIONS

Chromated copper arsenate–treated wood in raised garden beds diffused As, Cu, and Cr into adjacent garden soil. This study clearly showed that CCA-treated wood in service can be a local point source for elevated levels of As, Cu, and Cr in the environment and therefore existing structures may continue to be a problem. Results of the plant uptake study showed that vegetable crops grown in these raised garden beds can accumulate significant concentrations of As, but based on U.S. Public Health Service standards, these vegetables would be safe for human consumption. However, based on the USEPA's standard, some of the vegetable crops may not be safe for sustained consumption. The microbial resistance study clearly showed that the ability to establish colonies in CCA-amended media was greater in communities of bacteria from soils close to the treated wood compared with those from reference soils. These results suggest that long-term diffusion of As, Cu, and Cr away from aging CCA-treated wood surfaces may have ecological implications.

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