



## Coffee is highly tolerant to cadmium, nickel and zinc: Plant and soil nutritional status, metal distribution and bean yield

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### ABSTRACT

Sewage sludge has been used to fertilize coffee, increasing the risk of metal contamination in this crop. The aim of this work was to study the effects of Cd, Zn and Ni in adult coffee plants growing under field conditions. Seven-year-old coffee plants growing in the field received one of three doses of Cd, Zn or Ni: 15, 45 and 90 g Cd plant<sup>-1</sup>; 35, 105 and 210 g Ni plant<sup>-1</sup>; and 100, 300 and 600 g Zn plant<sup>-1</sup>, with all three metals in the form of sulphate salts. After three months, we noticed good penetration of the three metals into the soil, especially in the first 50 cm, which is the region where most coffee plant roots are concentrated. Leaf concentrations of K, Ca, Mg, S, B, Cu, Fe and Mn were not affected. N levels did not change with the application of Ni or Zn but were reduced with either 45 or 90 g Cd plant<sup>-1</sup>. Foliar P concentrations decreased with the addition of 45 and 90 g Cd plant<sup>-1</sup> and 600 g Zn plant<sup>-1</sup>. Zn levels in leaves were not affected by the application of Cd or Ni. The highest concentrations of Zn were found in branches (30–230 mg kg<sup>-1</sup>), leaves (7–35 mg kg<sup>-1</sup>) and bean (4–6.5 mg kg<sup>-1</sup>); Ni was found in leaves (4–45 mg kg<sup>-1</sup>), branches (3–18 mg kg<sup>-1</sup>) and beans (1–5 mg kg<sup>-1</sup>); and Cd was found in branches (0–6.2 mg kg<sup>-1</sup>) and beans (0–1.5 mg kg<sup>-1</sup>) but was absent in leaves. The mean yield of two harvests was not affected by Ni, but it decreased at the highest dose of Zn (600 g plant<sup>-1</sup>) and the two higher doses of Cd (45 and 90 g plant<sup>-1</sup>). Plants died when treated with the highest dose of Cd and showed symptoms of toxicity with the highest dose of Zn. Nevertheless, based on the amounts of metal used and the results obtained, we conclude that coffee plants are highly tolerant to the three metals tested. Moreover, even at high doses, there was very little transport to the beans, which is the part consumed by humans.

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### 1. Introduction

Environmental heavy metal contamination is mainly caused by anthropogenic activity, especially intense industrial activity. The accumulation of these metals in soil and plants poses risks to human health because they can enter the food chain (McLaughlin et al., 1999; Silva et al., 2005). Given the problems that heavy metals create for plants and, consequently, for humans, there have been many studies on heavy metal tolerance in plants in recent years (Gratão et al., 2008).

The accumulation of heavy metals in plants occurs mainly through root absorption, transport via the xylem and distribution to above-ground sink tissues (Clemens et al., 2002). Nevertheless, the absorption and distribution of metals in plants vary according

to the species studied. In rice, the highest accumulation of Cd occurs in the roots at the base of the tiller and at nodes (Fujimaki et al., 2010). In tomato, Cd accumulates mainly in the roots and little in the fruit (Monteiro et al., 2011). In maize, Cd and Zn mainly accumulate in the roots, and there is little accumulation in the grain (Fässler et al., 2010). As for sunflower and tobacco, leaves are the major sites of Cd accumulation (Garcia et al., 2006; Fässler et al., 2010). Zn distribution is similar among the roots, stem, leaves and seeds in sunflower (Garcia et al., 2006). In tobacco, Zn accumulates more in leaves than in the stem, whereas roots and flowers accumulate similar amounts (Fässler et al., 2010). In *Evodiopanax innovans*, Cd and Zn accumulate more in the bark of the plant than in the leaves (Takenaka et al., 2009). In *Rubus ulmifolius*, the roots accumulate more As, Pb and Ni (Marques et al., 2009).

An important aspect regarding the several studies that evaluated the distribution of metals in plants is that they have been performed with seedlings grown in either substrate or nutritive solution in pots in a greenhouse, and the results are not always

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applicable to plants grown in field conditions because biotic and abiotic factors strongly influence plant growth and physiology. In addition, other studies using biosolids have disregarded the amount of organic matter, which may influence the results by forming complexes with the metals present.

There has been much speculation on the effects of high concentrations of heavy metals in industrial and urban waste on coffee plants (Andrade et al., 2010; Martins et al., 2005). Such information is important because anthropogenic sources such as sewage sludge are being used more and more widely in coffee cultivation with the goal of reducing the cost associated with the use of mineral fertilizers. One motivation for this practice is that the use of sewage sludge as a coffee plant fertilizer does not impair the quality of the drink (Martins et al., 2005). However, coffee is one of the most consumed beverage in the world (Geromel et al., 2006) and more information on the absorption and distribution of metals in this plant are surely necessary to ensure that toxic metals are not being taken up by the consumers. In spite of that, few published studies have reported the toxic effects of these elements in coffee (Franco et al., 2004; Mazzafera, 1998; Pavan and Bingham, 1982). Some studies on the effects of metals in this species have been performed in cultured cells, but this method does not allow the data to be extrapolated to plants in field conditions (Bottcher et al., 2011; Gomes-Júnior et al., 2006a,b, 2007). Other studies have used coffee seedlings (Andrade et al., 2010).

Coffee trees reach a steady bean production after 5 or 6 years in the field. Thus, a representative study aiming to provide information on metal distribution in coffee must to be undertaken under field conditions and with adult plants. Consequently, this study aimed to evaluate the distribution of Cd, Ni and Zn in the branches, leaves and fruits of coffee plants grown under field conditions and to assess the effects of their presence on nutritional status and yield. Since the distribution profile of the metals in the soil is neglected in many studies, such a key important aspect has also been analyzed here.

## 2. Materials and methods

The experiment was conducted from December 2007 to August 2009 in Piracicaba, SP, Brazil (22°42'S; 47°38'W, 580 m altitude) in areas with clay latosolic Eutroferic Alfisol and with a mean slope of 2%. According to Koppen's classification scheme, the regional climate is Cwa type, tropical altitude with dry winters, and it has an average annual temperature of 22 °C and average rainfall of 1280 mm per year.

We used seven-year-old *Coffea arabica* L. cv. Obatã IAC 1669-20 plants spaced in 3.4 m × 0.9 m plots (3.270 plants ha<sup>-1</sup>). This cultivar is one of the most productive in Brazil and is planted in several states. These plants had not previously been used for any tests or treatments. The control of pests and diseases was carried out according to recommended agronomic techniques for this crop.

Given the limited information on the levels of heavy metal contaminants in cultivated Brazilian soils, we used the concentrations of Cd, Ni and Zn proposed by Abreu et al. (2005). According to these authors, toxic levels as determined with the chelator DTPA range from 150 to 452 mg dm<sup>-3</sup> for Zn, 30 to 65 mg dm<sup>-3</sup> for Ni and 1 to 3 mg dm<sup>-3</sup> for Cd for soils in São Paulo State. To calculate the dose of heavy metals to apply per plant, we considered a volume of 1000 dm<sup>-3</sup>, which is the product of the area of canopy projection of each plant (2 m<sup>2</sup>) by the root exploration depth of 0.5 m, the region where most coffee plant roots concentrate (Gindel, 1961; Huxley et al., 1974; Inforzato and Reis, 1963). Thus, we used the following doses: 15, 45 and 90 g Cd plant<sup>-1</sup>; 35, 105 and 210 g Ni plant<sup>-1</sup>; and 100, 300 and 600 g Zn plant<sup>-1</sup>. The salts used were in the form of sulphate. The doses were divided into three applications: at the

beginning of the experiment (12/18/2007), at 63 days (02/19/2008) and at 95 days (03/22/2008). By parcelling the metal applications and collecting samples between applications, one can evaluate the effects of sublethal concentrations at various times after application. For each application, the salts were dissolved in 10 L of water and 5 L was spread on each side of each plant toward the leading edge under the canopy of the coffee plant. The application was performed slowly to allow infiltration of the solution in the soil. Five replicates were performed for each metal concentration. For that, we chose one coffee row in the middle of the experimental field and alternate 5 plants (treatments) with 10 plants (spacers), in such way that we had 5 controls, 10 spacers, 5 lowest dose of Zn, 10 spacers, 5 lowest dose of Cd, 10 spacers and 5 lowest dose of Ni. Two rows away we repeated the same design but with the intermediary dose of each metal and again two rows away the highest doses.

On 01/17/2008, 30 days after the first application of the metals, the soil was covered with lime and fertilizer in the following amounts: 3500 kg ha<sup>-1</sup> of dolomitic lime to raise the saturation of bases to 60%, and 1500 kg ha<sup>-1</sup> of formulation 20-00-20, equivalent to 300 kg ha<sup>-1</sup> of N and 300 kg ha<sup>-1</sup> of K<sub>2</sub>O.

At 136 days after the treatment (04/24/2008), soil samples were collected at depths of 0–20 cm to determine pH [(1:2.5 soil:0.01 mol L<sup>-1</sup> calcium chloride (CaCl<sub>2</sub>)); organic matter (g dm<sup>-3</sup>); P (resin) (mg dm<sup>-3</sup>); K, Ca, Mg and Al+H (mmolc dm<sup>-3</sup>); and B (hot water), Cu, Fe, Mn, Zn, Cd and Ni (mg dm<sup>-3</sup>) in a 2:1 mixture of soil:extractant (DTPA-TEA solution; 0.005 mol L<sup>-1</sup> diethylenetriamine acid + 0.1 mol L<sup>-1</sup> triethanolamine + 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub>). To evaluate the soil distribution profiles of Cd, Ni and Zn, samples were collected in five layers: the first 0–5 cm, 5–15 cm, 15–25 cm, 25–50 cm and 50–100 cm. Samples were taken from both sides of the plant toward the leading edge and mixed to form a composite sample for each treatment. The analyses were performed according to the method proposed by van Raij et al. (1997).

The third pair of leaves from the middle third of the coffee plant was collected at the first metal application (day 0) and 35, 63 and 128 days after the treatment. Branches were also collected at the first application and at 128 days. The materials were dried in an oven at 60 °C for 72 h and subsequently ground for quantification of P, K, Ca, Mg, S, Cu, Fe, Mn, Zn and Ni by X-ray diffraction (EDXRF, Shimadzu, São Paulo) and Cd by plasma emission spectrometry (ICP-OES; JobinYvon, JY50P Longjumeau, France). To determine total-N concentration, the plant material was subjected to digestion with sulphuric acid (Jackson, 1958), and N was determined according to the analytical semi-micro Kjeldahl method (Bremner, 1965).

Fruit harvesting was carried out when the fruits were predominantly in the cherry stage. After harvesting, we determined the percentages of green, cherry and dry fruits in 0.5 kg samples. The harvested fruits were dried in a yard until their water content was roughly 110 g kg<sup>-1</sup> (11%). They were then processed and the total grain mass was determined. Concentrations of P, K, Ca, Mg, B, Cu, Fe, Zn, Mn, Ni and Cd were determined by plasma emission spectrometry (ICP-OES; JobinYvon, JY50P Longjumeau, France) in extracts obtained after nitro-perchloric acid digestion (Malavolta et al., 1997).

## 3. Results

### 3.1. Analysis of nutrients and metal distribution in the soil

The sand fraction (particle size 0.05–2.0 mm) of the soil was 299 g kg<sup>-1</sup>, whereas the silt (0.005–0.002 mm) and clay (<0.002 mm) fractions were 76 and 625 g kg<sup>-1</sup>, respectively. The

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