v. 4, n. 12, 2024

Journal of
Agricultural
Sciences
Research

Acceptance date: 16/10/2024

AZADIRACHTA INDICA A POTENTIAL SPECIES FOR COPPER PHYTOEXTRACTION

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Abstract: A study of the Cu restorative and bioaccumulate capacity of the Azadirachta indica (Neem) plant in three soils exploited by agriculture in the Municipality of Calimaya of the State of Mexico, Mexico, is presented. The soils were randomly selected, named Soil 1, Soil 2 and Soil 3. The texture, grain size, pH, electrical conductivity, redox potential, organic matter, total carbon, organic and inorganic, nitrogen and phosphorus for each soil were determined, measurements totals and chemical speciation of copper in native soils and pot soils with plants. The seedlings were placed in pots in a block design, measuring their morphological characteristics and metal content at 30, 60, 90 and 120 days of growth. The bioaccumulation index (BI) of Cu in the plants was determined. These soils have a shortage of nitrogen, phosphorous and manganese. The lack of vegetation cover and loss of nutrients due to rainwater dragging worsened their situation. Total Cu measurements and chemical speciation are presented using sequential extractions to obtain the geochemical distribution of the metal in the soil as the plants grew. The BI was obtained in the main bioavailable fractions. It is concluded that Azadirachta indica is a good restorative plant for the restoration and phytoextraction of Cu in these soils.
Keywords: *Azadirachta indica*, copper, sequential extraction, phytoextraction, bioaccumulation index, soil restoring.

INTRODUCTION

Currently, soil loss has alarmingly increased in Mexico due to forest clearing, inadequate agricultural practices and anthropogenic activities such as industry and urbanization, causing large amounts of eroded and contaminated soil containing different chemicals that cause irreversible damage to ecosystems and health (SEMARNAT, Ministry of the Environment and Natural Resources, 2003, Zarazúa-Ortega et al, 2013, Wong-Argüelles,·2021). Soil is also affected by water erosion, especially on surface horizons and gullies, as well as compaction processes and salinization which can disable soil for any further use in agriculture (NOM-021-SEMARNAT-2000 (formerly NOM-021-RECNAT-2000), SEMARNAT, 2015, Grison, 2024). The Upper Basin of the Lerma River in central Mexico has large areas of land that have been overexploited and polluted, which lead to great economic and social losses. For these reasons, it is necessary to implement soil improvement programs or change the soil use of those soils of difficult habilitation in the region. The municipality of Calimaya, located in this basin, has serious natural resources degradation concerns, such as loss of vegetation cover and its inevitable effects on biological diversity, water imbalance, soil erosion due to the practices of inadequate management aggravated by chemical contamination due to the frequent use of agrochemicals and pesticides. It is important to consider the need to remediate and recover these soils using sustainable technologies, such as the use of plants that can act as phytoremediators and soil restorers (Dowling and Doty., 2009), which may be usable for products that support the local economy of the region. It has been observed that metals present in the soil, whether nutrients or contaminants, can be dissolved, adsorbed, lost by washing, degraded or absorbed by plants (Navarro and Navarro, 2013). Kabata-Pendias, (2004) and Gupta and Sinha, (2007), in their studies concluded that the loss of bioavailable trace elements in soil was considered as one of the most crucial problems in agricultural and environmental studies. Copper is an essential element for plants in low concentrations (Grison, 2024). There are factors that influence metal mobility in soil, such as texture, specific surface, content of free sesquioxide's in the soil and pH. The metal uptake by plants is highly species-specific and varies greatly among the parts of the plant (Barceló et al., 2016). Thus, *Azadirachta indica* (Neem tree) was tested in this study as a potentially remediator plant (Orwa et al., 2009) because it can grow on marginalized soils (Orwa et al. 2009), has seeds that produce oils with high added value in various industrial uses and has the capacity to bioaccumulate heavy metals such as copper. *A. indica* is a plant of easy propagation, occupies little space and has low water and fertilizer requirements, it is not weed or a pest host, instead, it is ornamental and its wood can be usable. One of the many uses that are given to Neem is for soil reforestation. The Neem tree is native to South Asia. Due to the rapid growth of the tree and valuable firewood, it is the preferred species for plantations. Neem tree is tolerant to most soil types (Orwa et al., 2009), including shallow soils, dry and stony, vertisols, sands highly leached and clays. It is well adapted to soils with pH values between 5.0 and 8.5, but grows better in deep, porous and well-drained soils, with a pH of 6.0 to 6.5, is moderately tolerant to highly alkaline and salinized soils. Neem is a useful species among other benefits, to improve soil fertility in dry degraded, arid and semi-arid sites, due to the quality of its leaf litter and the rate of rapid leaf's decomposition, it can also assimilate metals. Sharma and Bhattacharyya (2005) used powder from its leaves as a bio sorbent for the removal of Cd(II) from the aqueous medium.

The objective of this work was to study the Azadirachta indica (Neem) plant, given its potential as phytoextractor and bio accumulator of metals such as copper present as a contaminant in soils, where the use of fungicides such as copper sulfate has been traditionally used as an amendment to protect crops. Given that soil of the municipality of Calimaya in the State of Mexico, Mexico, show intense degradation (Atlas de la Cuenca del Río Lerma, 2011), it was selected to conduct this study since the cultivation of this plant is attractive due of the value of oil-producing seeds.

MATERIALS AND METHODS

Sampling was carried out in the municipality of Calimaya in the State of Mexico, Mexico; three lots (5 x 10 m) were randomly selected and were denominated Soil 1, Soil 2 and Soil 3. Each lot was divided into quadrants, using meshes of 1.25 x 1.25 m. Soil samples of each lot were obtained at 20 cm depth; samples were taken in a completely randomized design without replacement. For Soil 1, quadrants were 4, 8, 12 and 19, for Soil 2: were 3, 9, 13 and 20, and for Soil 3, quadrants were: 2, 7, 14 and, 22. A total of 5 kg of soil were sampled from each quadrant and soil was mixed thoroughly for further use in the experiments. *Azadirachta indica* seeds were placed into 120.05 inches^3 containers (Figure 1a) filled with 60% peat + 40% agrolite soil for germination. For this study, seedlings were arranged in a completely random block design of 4 columns and 8 rows during the growth of 30, 60, 90 and 120 d. For Cu speciation, soil samples of 3 pots for each soil were taken randomly at 30, 60, 90 and 120 days, and mixed to give a compound sample for with each set of three pots; each soil sample was obtained at 20 cm depth from pots with plants. The morphological variables evaluated in each plant were: length (cm) and weight (g) of root; stem and leaf. Once measured and weighed, samples were dried and crushed with a high-speed electric mill. In Figure 1b a scheme of each experimental block is shown. Plants with the best morphological characteristics were selected. Figure 1c shows how each composite sample were from each individual pot using the quartering technique for total Cu analysis and speciation (sequential extractions) at the four sampling times.

Before sowing, seeds were washed 3 times with distilled water and soaked in running water at let at room temperature for 24 h. When they germinated, the seedlings were transplanted into polyethylene bags containing 3 kg of composed soil samples of Soil 1, Soil 2 and Soil 3. A sub-sample each composes sample of soil was used for its characterization: pH, electrical conductivity, redox potential, nitrogen, phosphorus, texture and granulometry. Another sub-sample of each composed soil sample was used for determine the total Cu and to study the geochemical distribution (speciation) of both, native soils and soils used in the plant growth experiments at 30, 60, 90 and 120 d. Copper speciation was carried out in order to know the interaction form of this metal with the plant and the different soil components. The experiment was developed in a greenhouse for 120 d after seeds germination.

SOIL CHARACTERIZATION

Physical and physicochemical tests were carried out to the soil to determine its degree of deterioration according to Mexican Standard NOM-021-SEMARNAT-2000.

TEXTURE AND GRANULOMETRY

To determine the texture, the Bouyucos hydrometer technique was used and Mexican Standard AS09-NOM-021-SEMARNAT-2000 and the results interpreted using the soil texture triangle. To know the particle size of the Calimaya soils, they were first dried for 48 h at 70 ° C; then were sifted for 20 minutes in the Tyler meshes in the following order: 8, 16, 18, 30 and 100. The soil retained in each sieve was separated, weighed and stored in properly labeled plastic containers. The soil retained in the 100 mesh was used for subsequent analyses.

MEASUREMENT OF PH, ELECTRICAL CONDUCTIVITY AND REDOX POTENTIAL

The pH was measured using the methodology described in the Mexican Standard AS02-NOM-021-SEMARNAT-2000. The Electrical Conductivity was determined in triplicate according to the Mexican Standard AS18-NOM-021-SEMARNAT-2000; this was also used to evaluate the salinity of the soil. The Redox Potential of each soil was determined with Hannah Instruments HI98150 equipment coupled to a LabQuest Vernier

ORGANIC MATTER (OM), TOTAL CARBON (TC), ORGANIC CARBON (OC), INORGANIC CARBON (IC)

Organic matter (OM) in soils, both native and potted, was determined only for soils from the pots at 30 and 120 d, for which the Walkley and Black method was followed, according to Mexican Standard AS07-NOM-021-SEMARNAT-2000. Total carbon (TC), inorganic carbon (IC) and organic carbon (OC) were determined from the three native soils and for the pot's soils at 30 and 120 d. For TC determination, the soil samples were dried at room temperature and the analysis was carried out in triplicate for each sample. Total Carbon was measured using porcelain vessels in a TOC MULTI N /C 3000 Analytik Jena Multi N / C 3000 equipment and calcined at 900° C in combustion furnace of the equipment, using a previously optimized calibration program; TC was calculated as the sum of OC and IC.

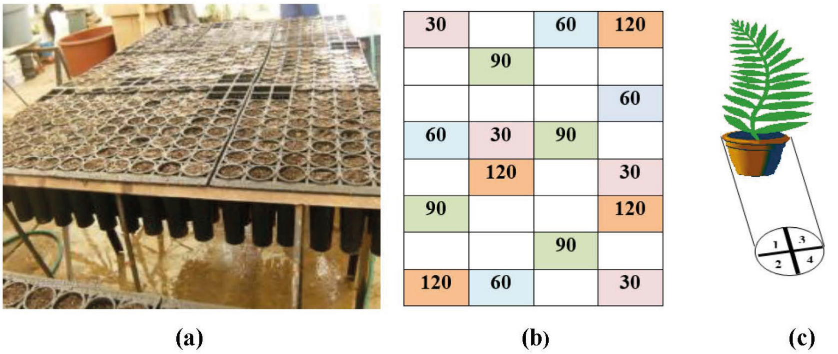


Figure 1. a). Distribution of *Azadirachta indica* seeds in the germination containers. b). Pots distribution of each soil. The number indicates the day of harvest. c) Quartering technique of soil per pot selected for total Cu and for chemical speciation (sequential extractions).

COPPER (Cu) DETERMINATION AND SEQUENTIAL EXTRACTIONS (SPECIATION) OF THESE METALS FROM THE NATIVE SOIL

The total copper (Cu) concentration was determined, as well as its chemical speciation (by sequential extractions) in the composite samples of each Calimaya soil, two acid pre-digestions were made as follows: 1g of each sample was weighed in triplicate; 5mL of concentrated nitric acid (HNO_3) plus 10mL of deionized water and 30% H_2O_2 were added, placed in a temperature-controlled bath at 60ºC with gentle agitation until organic matter (OM) was removed in a Julabo team, Model SW23. When samples showed a clear color (removal of OM), they were digested in a CEM-MARS 5 microwave oven, adding 13 mL of HNO_3 plus 1 mL of HF. The acids used were Baker Suprapur.

SEQUENTIAL EXTRACTIONS (SPECIATION) OF Cu

The chemical speciation was carried out through sequential extractions of Cu from each soil in order to obtain its geochemical distribution (Barceló, 2000; Tessier et al., 1979) and its relationship with the different soil components (Galantini and Suñer, 2008). From the soil retained in 100 mesh of the Tyler sieve, 1 g of each sample was weighed for each experiment; this was carried out in triplicate. **Figure 2** shows a diagram of the methodology followed for the sequential extractions.

F1 to F6 are the fractions that were obtained and their relationship with the different soil components. Analyses of total Cu and those of each fraction of the speciation were performed by atomic absorption in a Thermoelemental model SOLAAR M6, both by flame and by graphite furnace. The Cu sequential extractions (chemical speciation) of the soil contained in the pots at each experimental time were also carried out. In order not to deteriorate each solid residue of the different fractions, each residue was lyophilized in each case, in Virtis equipment model BT2K-ES.

RESULTS AND DISCUSSION

This soil used in the present experiment has lost a considerable part of the organic horizon due to erosion. This soil has a sandy loam texture. Soil pH varied between 6.14 and 6.45 that belong to the slightly acidic category according to the Mexican Standard NOM 021 SEMARNAT 2000. Nevertheless *A. indica* has been adapted to this pH in soils due to its properties already described in the introduction section (Moreno, 1978). Electric conductivity resulted in an average of 0.235 dS/m; using criteria of the Mexican Standard AS18-NOM 021 SEMARNAT 2000 that

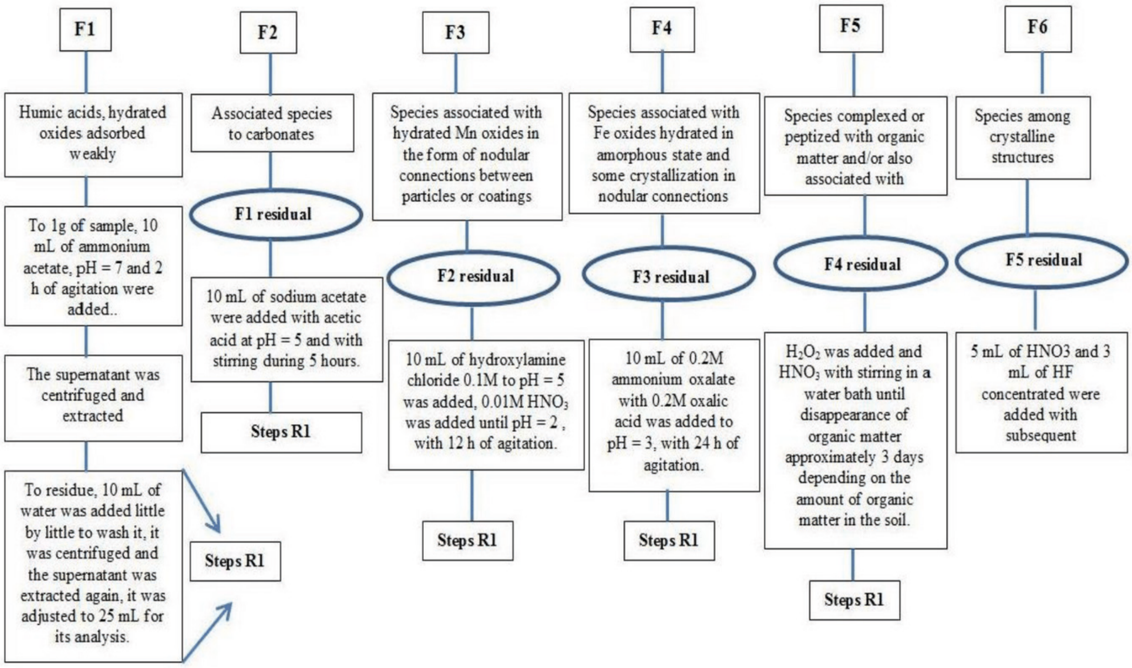


Figure 2. Cu sequential extractions (chemical speciation) to obtain its geochemical distribution

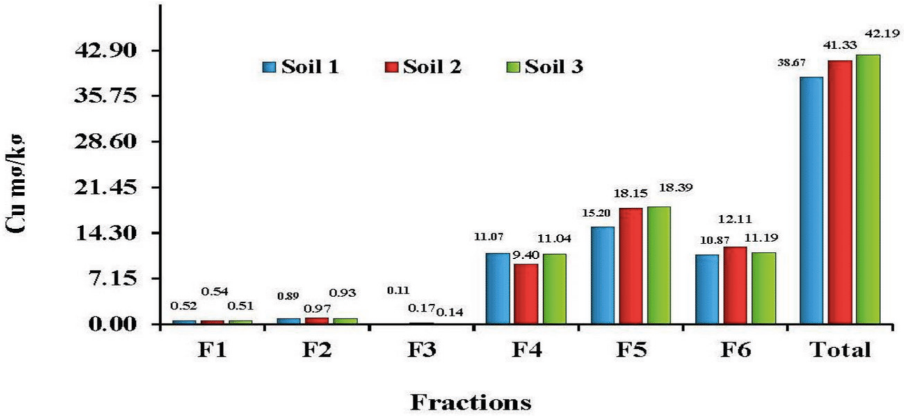


Figure 3. Geochemical distribution of Cu in the native soil by sequential extractions and total Cu from fractions.

indicates for values > 1.0 dS/m, the three soils showed negligible effects of salinity. Regarding the redox potential (ORP), it is known that oxidation-reduction conditions of a soil, are of great importance for the processes of chemical weathering and for biological processes are also related to the bioavailability of certain nutrients and as a measure of oxygen. The ORP resulted an average of 119.9 ± 3.835 mV.

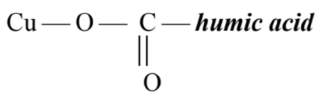
TOTAL COOPER (Cu) DETERMINATION IN NATIVE SOIL

Total Cu concentration was obtained in samples from the three Calimaya soils, for the soils were: Soil 1, 138.94 ± 0.581 mg/kg, Soil 2, 41.81 ± 0.416 mg/kg and Soil 3, 40.37 ± 0.570 mg/kg, it was observed for Cu that Soil 2 had the highest concentration of total Cu. It is important to consider the geochemical distribution of this metal, since not all Cu in the soil is available for the plant, mainly the one associated to OM, where the humic material plays an important role as complexing for Cu, since this metal has affinity for humic acids (Boyd et al. 1981).

COPPER SEQUENTIAL EXTRACTIONS FROM NATIVE SOIL (GEOCHEMICAL DISTRIBUTION)

Figure 3 shows the geochemical distribution of Cu in the three native soils, the highest concentration of Cu was found in fractions F5 and F4 respectively, this coincides with the studies of Barceló (2000), Calmano and Förstner (1983) and Tessier et al. (1979), where Cu from sediments and soils is mainly related to fraction F5 due to its relationship with the OM from the humic material (Konova, 1982).

Humic acids form complexes with Cu and due to its partial solubility transports metals nutrients and contaminants to the plants (Boyd et al, 1981, Barceló et al, 2018), hence its importance for the bioremediation. In Fraction F5, copper forms well-established complexes (Boyd et al., 1981), these complexes are based on unions of the type:



This fraction may include most of the available Cu for the plant (Korte et al, 1976)**.** The little organic matter present in our soil according to results of speciation is related to Cu, as observed in fraction F5. It is also important to consider fraction F4, since it is a fraction where Cu is related to hydrated iron oxides, which have important adsorptive surfaces, and also makes Cu available for plants. The Cu associated with fraction F6 does not contribute to the absorption of the plant because it is trapped in crystalline networks of silicoaluminates and iron oxides, since in these forms of association it is difficult for it to be released. When considering the six fractions (from F1 to F6), total Cu concentration was for Soil 1, 38.67 ± 1.972 mg/kg, slightly less than the total Cu which was 38.94 ± 0.834 mg/kg. Although care was taken for the lyophilization of each solid in sequential extractions, there was an experimental error in Soil 1 of only 0.69%, for Soils 2 and Soil 3, the values obtained in sequential extractions were 41.33 ± 2.013 and 42.19 ± 2.139.

PLANTS

Soil from pots planted with *Azadirachta indica* were sampled using the quartering technique (**Figure 1b**) as indicated in the methodology. On the other hand, plants were extracted using a simple random sampling without replacement, three by soil at each time, they were washed and dried at room temperature and the morphological variables, length (cm) and weight (g) of each plant were measured, as well as each root, stem and leaf with branch **Figure 4**.

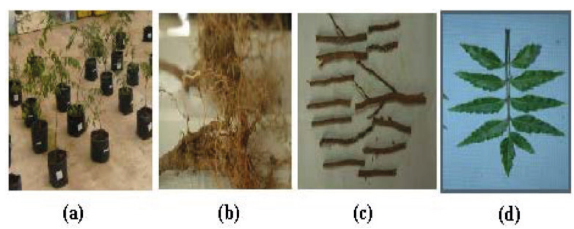


Figure 4. *Azadirachta indica* parts. a). Plants, b). Root. c). Stem. d) Leaf and branch

Once each part of the plants was measured and weighed, they were milled. In general, plants had no visible deformities in root, stem and leaves. **Table 1** shows results in weight and size of the plants as they grew at 30, 60, 90 and 120 days.

COPPER SPECIATION IN SOILS WITH PLANTS OF AZADIRACHTA INDICA

In **Figures 5a, 5b, 5c and 5d**, show the results of the sequential extractions (speciation) of the soil from the pots for each period (30, 60, 90 and 120 days). A notable decrease was observed, for example at 30 days for Soil 1 in each fraction, where fractions

F5 and F4 are relevant. For F5 there was in Soil 1, 15.20 mg / kg in native soil and 7.71 mg / kg in soil with pots, with 7.49 mg / kg removal, equivalent to 49.29%. In **Table 2**, the percentages of removal for each fraction at 30, 60, 90 and 120 days are indicated. Fraction F6, where Cu is immobilized and not bioavailable for the plant, was eliminated to calculate the percentage of removal at 30, 60, 90 y 120 days of this metal. To obtain Cu percentage of removal in each soil at 30, 60, 90 and 120 days, was considered the relationship between the sum of fractions F1 to F5 of soil from pots between the sum of fractions F1 to F5 of native soil. The highest Cu accumulation was found in all cases always in F5 fraction, related as indicated to OM, where the humic material is present and form complexes that allow the plants to dispose the metal, (Yang and Hodson 2018).

Another important fraction is F4, where Cu is related to hydrate iron oxides. Other fractions are important since they are more mobile and bioavailable, but on the other hand, they are easily lost by leaching during irrigation, therefore the presence of copper in fractions F5 and F4 is more important, for being less mobile and for being in greater concentration, they can be considered to act as a buffer or reservoir for the metal and its availability for the plant.

ORGANIC MATTER, TOTAL CARBON, INORGANIC CARBON AND ORGANIC CARBON

In Table 3, total carbon (CT) was obtained for three soils, without and with plants after 120 days, as a function of organic carbon (OC) and Inorganic carbon (IC), in mg/kg.

It was observed that IC is the dominant one and OC is small compared to the total, since it resulted in 3.61% for Soil 1, 4.73% for Soil 2 and 5.14% for Soil 3, this last always presented a little better-quality condition compared to the others two, however also it is deficient in general terms, in summary, it can be said that these three soils are mineralized. If it is compared with the values of Table 3, where the OM was calculated it can be deduced that for the three soils the OC values obtained can correspond to humic material. Following the results of Table 3, it was observed at 120 days an increase of OC in a 0.60% for Soil 1 with respect to the value obtained in the native soil, in the same way for Soil 2, 0.76% and for Soil 3, 0.64%. Soil 2 resulted with a slightly higher increase than the other two soils; this means that there was an improvement in soil from pots by the OC. This could be due to organic material produced by leaves of the plants when they decayed and left into the pots (Martínez, et al. (2008)

COPPER DETERMINATION IN DIFFERENT PLANTS PARTS

Copper was determined in different Neem plants parts, in Figures 6a illustrates Cu concentration in roots, 6b in stems, 6c in leaves, three plants per soil were considered, to 30, 60, 90 and 120 days.

Mixtures from different parts of the plants were prepared for each treatment and analyzes were carried out in triplicate of each case. In figure 6d is presented the total Cu obtained from each plant part and the total Cu per plant. It was observed that roots accumulated more Cu than the other parts, at 30 and 60 days the plants root of Soil 2 accumulated a little more copper but at 90 and 120 the plants root of Soil 3 accumulated slightly more than Soil 2. Soil 1 was consistently lower than the others; since it is the most marginalized soil and lacks nutrients, such as OM. In the stems case at 30, 60 and 90 days, Soil 2 presented values a little higher as compared to the other soils; at 120 days Cu average values of plants stems of Soil 2 practically remained. Soil 1 plant stems accumulated more Cu, Followed by Soil 3 plants

Time (Days)	Parts of the plant and total plant	Weight (g) of each part and total of the plants in soils			Size (cm) of parts and total, leaves with branches		
		Soil 1	Soil 2	Soil 3	Soil 1	Soil 2	Soil 3
30	Root	5.88 ±0.369	6.89±0.486	7.223±0.521	8.2±0.292	8.8±0.362	9.2±0.338
	Stem	11.48±0.853	12.11±0.967	12.793±0.757	11.3±0.346	12.1±0.376	12.7±0.377
	Leaves	4.893±0.835	6.09±0.831	6.790±0.853	7.1±0.295	8.3±0.334	9.2±0.318
	Total	**22.24**	**25.09**	**26.81**	**19.8***	**20.5***	**22.1***
60	Root	12.55±0.536	13.94±1.023	14.133±0.709	10.9±0.347	11.4±0.434	11.9±0.484
	Stem	24.14±1.449	24.50±1.260	25.644 ± 1.150	13.2±0.498	13.8±0.552	14.2±0.507
	Leaves	7.68±0.555	10.15±0.570	10.985 ± 0.723	10.7±0.437	11.8±0.340	12.4±0.381
	Total	**44.37**	**48.59**	**50.76**	**24.4***	**25.5***	**26.4***
90	Root	14.69±0.991	17.73±0.658	18.293±1.228	12.4±0.510	13.8±0.429	14.3±0.454
	Stem	28.25±1.543	31.70±2.552	33.344±2.201	21.7±0.822	22.4±0.894	22.8±0.912
	Leaves	11.72±0.588	12.49±0.823	13.017±0.742	12.6±0.378	14.3±0.538	15.3±0.629
	Total	**54.66**	**61.92**	**64.65**	**34.9***	**36.3***	**36.8***
120	Root	18.49±0.934	24.01±1.701	23.79±1.348	18.3±0.664	19.1±0.548	19.5±0.762
	Stem	32.57±0.978	35.64±1.149	40.594±2.085	30.4±0.878	32.8±0.983	34.1±0.910
	Leaves	14.07±0.978	14.86±0.920	15.880±0.917	17.2±0.631	17.9±0.695	18.6±0.558
	Total	**65.12**	**74.51**	**80.27**	**48.8***	**52.0***	**53.7***

* Aerial part average of three plants is considered for each growth time

Table 1. Results of weights and measurements of *Azadirachta indica* plants and their sections

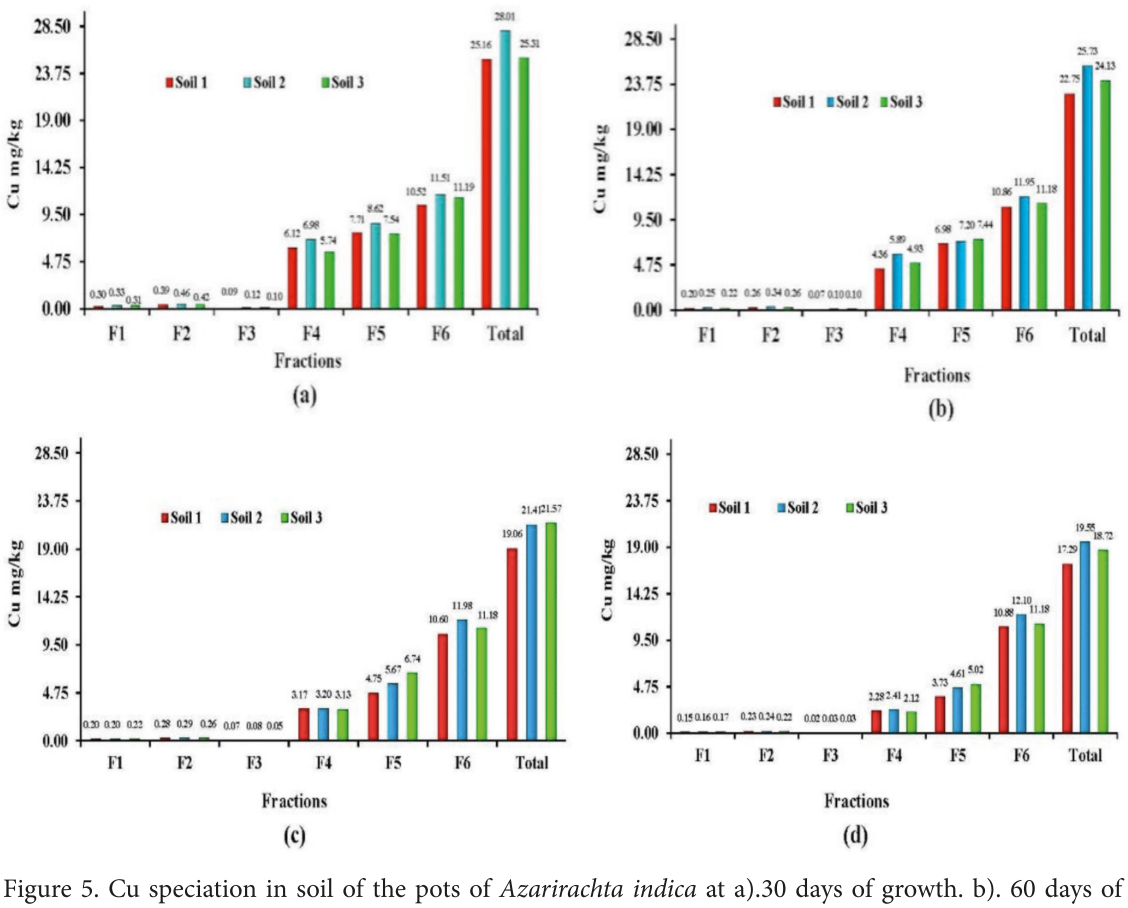


Figure 5. Cu speciation in soil of the pots of *Azarirachta indica* at a).30 days of growth. b). 60 days of growth. c). 90 days of growth. d). 120 days of growth

stems. As for leaves, Soil 3 plants accumulated more Cu than the others, followed by Soil 2, at 30 days the difference was more noticeable and almost leveled after 60 days, but always the order was maintained: Leaves-Soil 3> Leaves--Soil 2> Leaves- Soil 1. When adding the Cu accumulation of different plants parts of each soil, it was obtained: Total Plant-Soil 3> Total Plant-Soil 2> Total Plant-Soil 1. This coincides with from Kabata-Pendias, (2004) studies over the soil-plant transfer of polluting metals in soils, which allows deducing that this plant has mechanisms to transport copper from the soil to roots and then translocate it to stems and leaves.

BIOACCUMULATION INDEXES

In plants, bioaccumulation concept refers to pollutant's aggregation; some of them are more susceptible to being phytoavailable than others (Kabata-Pendias 2000), therefore it is an important concept in phytoremediation studies. For phytoremediation studies and the plants bioaccumulation capacity determination, Bioaccumulation Index (BI) is used, a parameter of great importance that corresponds to metal concentration present in the plant divided by the concentration of the same metal present in the soil, (Equation 1). According to Kabata-Pendias (2000), intervals between 0.1 and 1.0 have been determined for some heavy metals. For the *Azadirachta indica*, the Bioaccumulation Index (IB) was calculated in relation to the Cu according to the following expression, Equation 1:

$$BI = \frac{\text{Metal in plant (mg kg}-1)}{\text{Metal in soil (mg kg}-1)} \qquad \text{Equation 1}$$

Bioaccumulation index was determined for all fractions except for F6 as shown in Figure 12, which as indicated, is a fraction where copper is not bioavailable to the plant. By eliminating this fraction, the analysis of bioaccumulation becomes more consistent. Bioaccumulation Index is presented for roots, stems, leaves and plant at 30, 60, 90 and 120 days, Figures. 7a, 7b, 7c and 7d; Soil 1 was the lowest value at 90 days, Soil 3 bioaccumulate less copper than Soil 2 and leveled out and even a little more than Soil 2. Stems at 30, 60 and 120 days accumulated copper in increasing form, Soil 1 accumulated copper slightly lower than the others and Soil 3 slightly above Soil 2. Again, the behavior as in roots case, the BI of Soil 2 plants was slightly higher than the BI of Soil 3 plants at 90 days. Regarding leaves at 30 days, a higher increase in the IB was observed in soil 3 than other two, the BI of Soil 1 was always the lowest value at 60 days. The BI in Soils 1 and 2 grew significantly with respect to Soil 3, after 90 days. The BI of Soil 2 increased slightly more than in Soils 3 and 1. At 120 the three soils increased their IB, again in the order: Soil 1 <Soil 2 <Soil 3. Finally adding the IBs of all parts for the plants was, at 30, 60 and 90 Soil 1-plant <Soil 2-plant <Soil 3-plant. Soil 1 was always behaved with a lower IB than the other 2, being notorious at 30, 90 and 120 days. Soil 2 always behaved at 90 days with values higher than Soil 3 and at 120 days was slightly below Soil 3. Cabezas, et al. (2004) in their studies of heavy metals absorption and accumulation in three plant species in a soil typic degraded from an abandoned crop, used three plants, *Hordeum vulgare* (barley), *Helianthus annuus L* (sunflower) and *Vicia sativa* (vetch), with values between 9.8 and 10.1 mg/kg of Cu, values lower than the Calimaya soil and observed that this metal was absorbed by barley with a value of 8.7 mg/kg, sunflower 6.6 mg/kg and the vetch 9.3 mg/kg with the plants aerial part and Cu accumulated in roots with values of 6.6 mg/kg for barley, 12.3 mg/kg for sunflower and 18.15 for vetch at 150 days. Comparing these values with *Azadirachta indica*, see figures 12c, for the 120 days, Soil 1 absorbed 3.59 mg/kg, soil 2, 3.52 mg/kg and soil 3, 3.66 mg/kg, which are much lower con-

Fractions	Soil 1 % of removal	Soil 2 % de removal	Soil 3 % de removal
	30 days		
F1	42.03	38.18	38.70
F2	56.15	52.94	54.61
F3	21.24	29.09	26.62
F4	44.72	25.77	47.96
F5	49.29	52.51	59.01
Total	**47.45**	**43.53**	**54.47**
	60 days		
F1	60.84	53.82	38.70
F2	71.48	65.09	54.61
F3	30.22	38.79	26.62
F4	60.67	37.31	47.96
F5	54.06	60.32	59.01
Total	**58.33**	**52.84**	**54.47**
	90 days		
F1	61.42	63.501	55.992
F2	69.24	70.443	72.639
F3	41.59	52.727	67.626
F4	71.39	65.911	71.632
F5	68.72	68.766	63.373
Total	**51.04**	**67.716**	**48.874**
	120 days		
F1	29.175	70.764	67.39
F2	25.839	75.386	76.61
F3	21.239	83.636	80.58
F4	20.544	74.348	80.82
F5	24.530	74.574	72.73
Total	**62.187**	**74.510**	**75.67**

Table 2. Percentage of Cu removal in each fraction at 30, 60, 90 and 120 days

Samples	CI (mg/kg)	CO (mg/kg)	CT (mg/kg	% de CO
		Without plants		
Soil 1	3612.21 ± 88.28	135.45 ± 5.28	3747.66 ± 121.25	**3.61**
Soil 2	3798.17 ± 111.61	188.76 ± 4.23	3986.93± 132.60	**4.73**
Soil 3	3785.13 ± 77.69	205.22 ± 5.11	3990.35 ± 104.80	**5.14**
		With plants		
Soil 1	3607.82 ± 87.56	203.33 ± 7.28	3811.15 ± 127.11	**5.34**
Soil 2	3777.65 ± 141.61	219.76 ± 8.23	3997.41 ± 136.04	**5.50**
Soil 3	3778.86 ± 147.77	231.73 ± 6.11	4010.59 ± 134.35	**5.78**

Table 3. Total carbon (CT), Inorganic Carbon (IC) and Organic Carbon (CO) in three soils, without and with plants after (120 days).

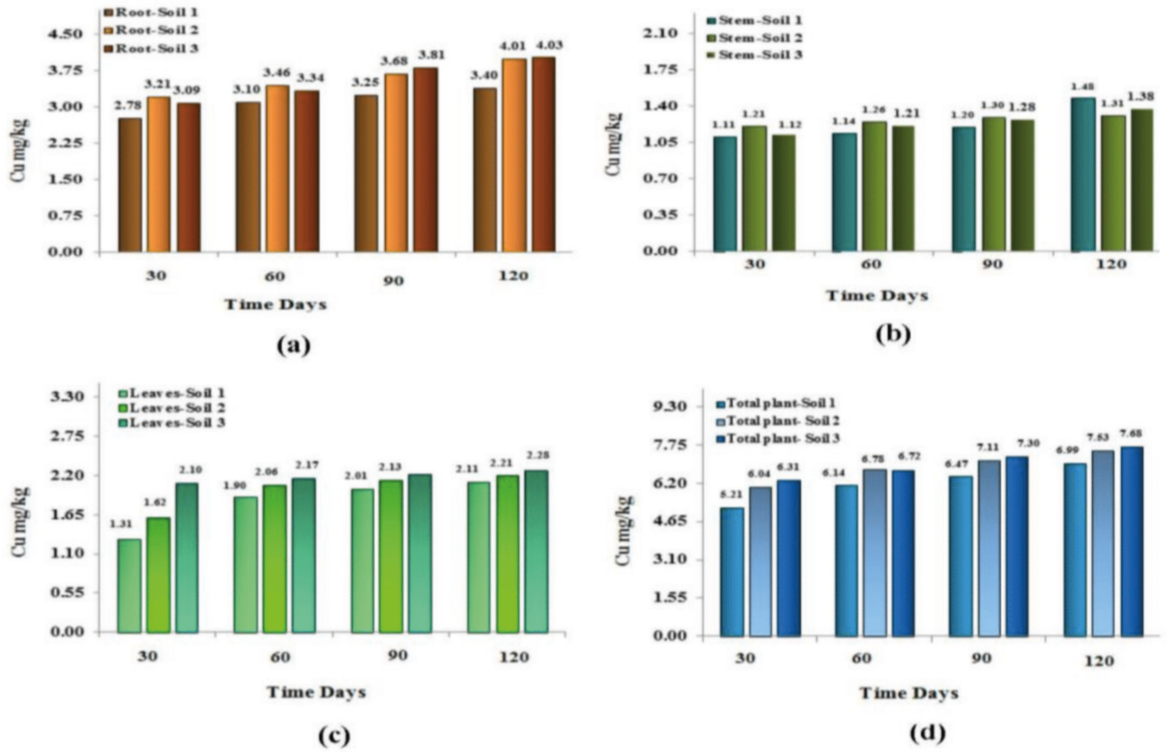


Figure 6. Cu determination at 120 days leaves growth of *Azarirachta indica* in: a) in roots. b). in stems. c) in leaves. d). in total plant

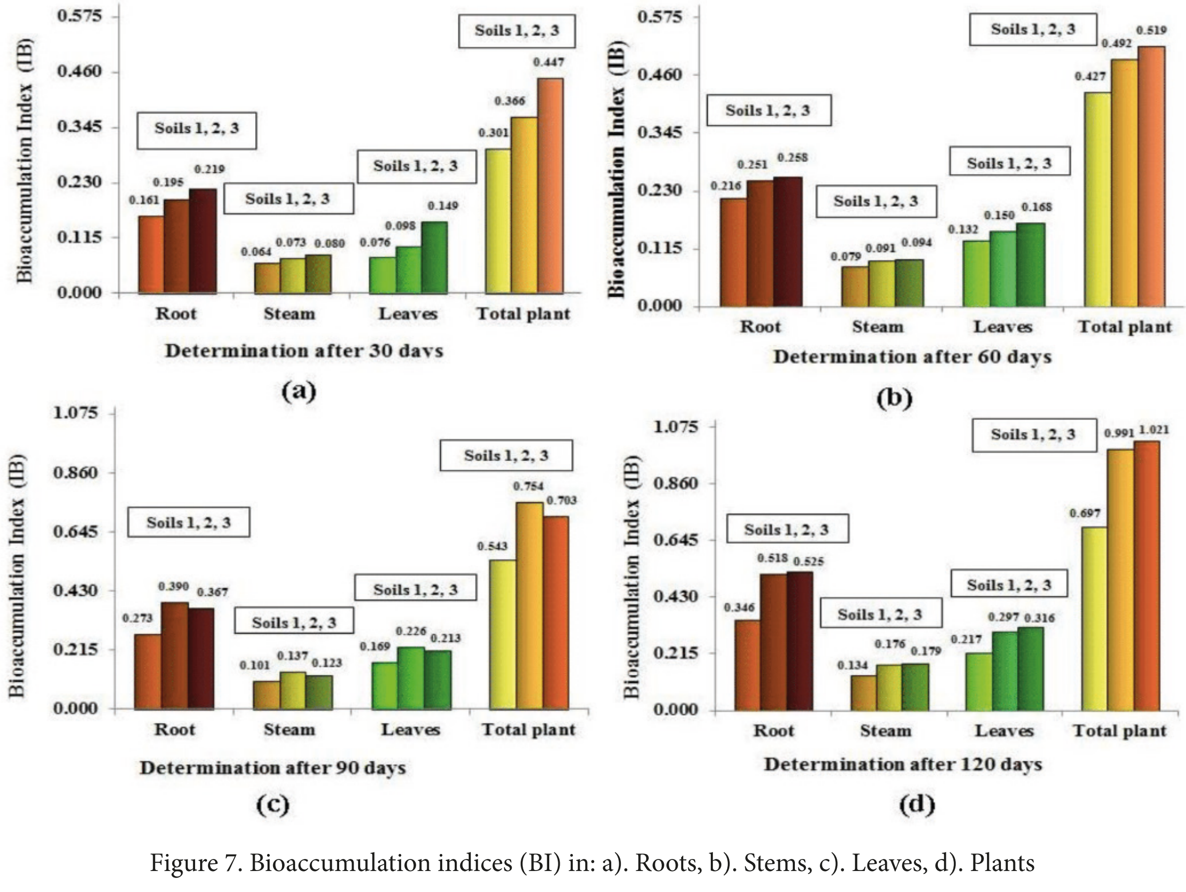


Figure 7. Bioaccumulation indices (BI) in: a). Roots, b). Stems, c). Leaves, d). Plants

centrations, however, this plant grows comparatively slow and the experimental time was shorter 30 days less than the cited reference, it should be noted that Neem will grow more over time and its life time is very long.

The IB for the three plants of the cited reference, were 0.89 for barley, 0.67 for sunflower and 0.92 for vetch; for *Azadirachta indica*, values were 0.70 (Soil 1), 0.99 (Soil 2) and 1.02 (Soil 3) at 120 days, Fig. 6d. It can be concluded that Neem has a good bioaccumulation index (IB), which will increase with longer time; this plant does not represent any danger because its life time is long as already indicated. Its leaves are good soil improvers since they increase the OC (Boyd et al, 1981), in addition the accumulated Cu could serve for soils with lack of this metal, and in general the plant could be used in other applications already indicated in the introduction of this work

CONCLUSIONS

Azadirachta indica plants proved to tolerate slightly acid soil conditions with erosion evidence and overexploited by agriculture, although the growth was slow during the experimental period, they did not present deficiency of nutrients in roots, stems and leaves. The effects of electric conductivity indicate that the soils of Calimaya still have no salinity effects. Taking into account that the redox potential is a measure of oxygen availability in a soil, and that this resulted with a value around 119.9167 mV, the soil of Calimaya presents principles of anoxia, coinciding with pH almost acidic.

The studied soil presented shortages of OM, in addition to a lack of vegetation cover and nutrients loss due to rainy dragging. Due to the conditions of these soils, *Azadirachta indica* (Neem) is recommended for the benefits it has, since it can provide rigor to these marginalized soils. Not only totals Cu measuring are useful to interpret how these metals are associated with soil components, so chemical speciation is important in these studies type, because through sequential extractions it is possible to observe the metal distribution in soils. Cu was found is mainly associated with fraction F4 (iron oxides adsorptive surfaces and perhaps manganese, which was found in very small concentrations) and with fraction F5 (Cu interactions with organic matter). Fraction F6 is not assimilable by plants and about 25% of copper is between silicate crystalline networks and aluminosilicates from soil, which is considered fixed fraction). Due to intemperization, the soils showed very Cu little quantity in mobile (bioavailable) fractions, those associated with the fractions F1, F2 and F3.

Regarding Bioaccumulation Index, roots accumulated more Cu and can be considered as a reservoir for future translocation of Cu in the plant. By the values obtained of indexes bioaccumulations it can be concluded that this plant is a good phytoextractor. Soil 3 states was always slightly better compared to Soil 1, which is the most marginalized, but in general these soils should not be used for agriculture, but should be restored and with sowing of *Azadirachta indica* the region could take advantage all the properties of this plant for its economy in future.

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