

## Ultra-diluted Solutions of *Nux vomica* in the Remediation of Metals in Soils and Bioavailability for Soybeans

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### Abstract

The aim of this study was to evaluate the homeopathic medicine *Nux vomica* in the remediation of soils contaminated with cadmium (Cd) and lead (Pb) and the bioavailability of these metals in soybean plants. To this end the soil was contaminated prior to sowing, using CdCl<sub>2</sub>H<sub>2</sub>O and PbCl<sub>2</sub> salts in three rates, based on the resolution of the research values of resolution No. 420 of CONAMA, resulting in 0.0, 3.0 and 9.0 mg kg<sup>-1</sup> for Cd and 89.0, 180.0 and 540.0 mg kg<sup>-1</sup> for Pb. The homeopathic remedy *Nux vomica* was used in dynamizations 12, 24, 48, 96, 200 and 400 CH (Centesimal Hahnemannian), being the witness with distilled water. Medicines were applied to the soil seven days before sowing and after sowing, every 14 days applied in the plant until complete the cycle. During cultivation were evaluated development, gas exchange, nutrient composition, component production and bioavailability of metals in the plant. The results demonstrate that *Nux vomica* medicine interfered in height and stem diameter and CO<sub>2</sub> assimilation, stomatal conductance and transpiration of soybean plants, but did not affect the bioavailability of metals to plants.

**Keywords:** *Glycine max* L., heavy metals, soil decontamination, agricultural homeopathy

### 1. Introduction

The high technology agriculture used for the development of Brazil's agricultural system has its methods questioned by the fact that to increase productivity is needed increasing amounts of pesticides and fertilizers. These, when in contact with the ground undergo chemical transformations that can release toxic metals into the soil solution, causing toxicity to soil organisms and plants, or they may be complexed to organic matter or adsorbed clays, making the soil a deposit of pollutants (Alloway, 2013).

Among the metals, cadmium (Cd) and lead (Pb) deserve attention because their use by modern society is growing, their toxicity levels are too high, are bioaccumulative and eco-toxic elements, being dangerous to the environment and people (Gonçalves Jr. et al., 2014).

Once present in soils are needed methods of remediation of metals, however, most of the time, these methods are costly and/or inefficient (Cheng et al., 2015). In this context, an attractive alternative which has been widespread among farmers is homeopathy, which in Brazil is regulated by Normative Instruction No. 46, of October 6, 2011 (Brazil, 2011), allowing organic farmers to use homeopathic medicines to treat plants, animals, soil and water.

The therapeutic system developed by Samuel Hahnemann in homeopathy has as guiding pillars the principles of similitude, experimentation in healthy individual, the unique medication and minimum rates, principles which cause great furor in academic circles because start from the assumption that only one substance potential to cause disturbance in health is able to heal similar disorder in sick individual and, following this premise, all the revolutionary treatment involving homeopathy is developed (Tétou, 2001).

A homeopathic medication used by farmers in cases of detoxification is *Nux vomica*, widely used in the treatment of humans for its medicinal effects cover a large number of symptoms and, by analogy, has been applied in agricultural homeopathy, because it has demonstrated efficacy in various life forms where the life force is suffering interference of intoxicating agents (Bonfim et al., 2010).

Thus, the objective of this study was to evaluate the soil remediation contaminated with Cd and Pb levels, using different potencies of homeopathic medicine *Nux vomica*, evaluating its effects on soybean plants, and the bioavailability in the plant tissues be studied.

## 2. Material & Method

The experiment was conducted in a greenhouse belonging to the State University of Western Paraná, located at longitude 54°22' W, latitude 24°46' S and altitude of 420 meters.

The soybean plants were grown in pots of 8 dm<sup>3</sup> filled with eutrophic Red Latosol (eRL) clayey texture (clay = 578.00; silt and sand = 348.58 = 73.42 g kg<sup>-1</sup>). The existing levels of Cd and Pb were < 0.005 and 89.0 mg kg<sup>-1</sup> respectively, however, the Cd values, indicating the possibility of Cd in the soil, but below the detection limit of the used method (FAAS) (0.005 mg kg<sup>-1</sup>).

The soils liming and fertilization was realized 60 days before the implantation of culture, applying the corresponding amount to 1.3 t ha<sup>-1</sup> of CaCO<sub>3</sub> to reach a base saturation (V%) of 70%, 50 kg ha<sup>-1</sup> K<sub>2</sub>O as potassium chloride (KCl), 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> in the form of triple super phosphate and for nitrogen fertilization were inoculated bacteria in the seed.

The experimental design was randomized blocks in factorial design, with three concentrations of Cd and Pb in soil, seven homeopathic dynamizations of medicine *Nux vomica* and four replications, each pot being considered one experimental unit.

The contamination of soil were realized from the addition of metal solutions prepared with Cd salts [monohydrate cadmium chloride (CdCl<sub>2</sub>H<sub>2</sub>O)] and Pb [lead chloride II (PbCl<sub>2</sub>)], with rates based on research values, specific to agricultural areas of Resolution No. 420 of CONAMA (Brazil, 2009). The rates were defined as soil A (existing value of Cd and Pb in the soil), soil B (maximum value allowed by Resolution No. 420) and soil C (three times the maximum value allowed by Resolution No. 420), resulting in 0.0 and 89.0 mg kg<sup>-1</sup> soil dry for Cd and Pb respectively in the soil A; 3.0 and 180.0 mg kg<sup>-1</sup> of dry soil for Cd and Pb respectively for soil B and 9.0 to 540.0 mg kg<sup>-1</sup> of dry soil Cd and Pb respectively for the soil C.

The metal concentrations in the soil were changed by applying 250 mL of homogenized solutions containing CdCl<sub>2</sub>H<sub>2</sub>O and PbCl<sub>2</sub>, where the rates of preexisting values were added 250 mL of distilled water. After dried, the soils were homogenized and irrigated with distilled water up to 60% of the water holding capacity and incubated for 30 days. At the end of the incubation period, seven soybean of cultivar NIDERA 5909<sup>®</sup> were sown per pot, trimming being carried out after emergence, keeping three plants per pot.

The homeopathic remedy *Nux vomica* was prepared following the standards of the Brazilian Homeopathic Pharmacopoeia (Brazil, 2011), were obtained in homeopathic handling laboratory of the city of Marechal Cândido Rondon, based on the centesimal hanhemannian (CH), in dynamizations 0 (control), 12, 24, 48, 96, 200 and 400 CH.

The potencies of *Nux vomica* were diluted at a concentration of 1 mL L<sup>-1</sup> of distilled water (Bonato, 2006). There were realized two applications in the soil before germination, the first one seven days before sowing and another on the day of sowing. After germination, applications were made in the plant in fortnightly periods until the end of the cycle, 98 days after emergence (DAE), rates were applied until the pour point of the plant. The spraying was carried out with sprinkler operating aid.

The vegetative growth stage II (first fully developed trifoliolate leaf), 14 days after emergence, soybean plants were affected by anthracnose attack and therefore was held fungal control through the application of thiophanate methyl (70 g of active product/100 L of water). In addition, was held the control of fungal diseases and pests in V6 (fifth fully developed trifoliolate leaf) (30 DAE) with the application of trifloxystrobin + tebuconazole (75 + 150 g ha<sup>-1</sup>) and thiamethoxam (150 g ha<sup>-1</sup>). Was analyzed the height of the shoots and the plant stem diameter of the plants every 14 days in order to prepare a response surface of plants based on these biometric parameters.

Measurements of gas exchange were held in the R1 stage, beginning of flowering (50 DAE), with IRGA LI-6400XT equipment (Liquor Inc. Lincoln, NE), being analyzed the first to fourth trifoliolate below the apex of each plant during the beginning of flowering. The average photon flux photosynthetically active during the determination of gas exchange were 1200 μmol m<sup>-2</sup> s<sup>-1</sup> and the average concentration of CO<sub>2</sub> ambient was 400

$\mu\text{mol mol}^{-1}$ , in the afternoon, being determined certain variables:  $A$ —net assimilation rate of  $\text{CO}_2$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $E$ —transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ );  $g_s$ —stomatal conductance ( $\text{mol m}^{-2} \text{ s}^{-1}$ ).

For the nutritional analyzes of leaf tissues during plant development, were taken the first to the fourth trifoliolate after the apex during early flowering (Kabata-Pendias, 2010), being determined the content of nitrogen (N) phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), Cd and Pb.

To this end, samples of plant tissues were subjected to sulfuric acid digestion for the determination of N and nitroperchloric digestion to the other elements, according to the methodology described by the Association Official Analytical Chemistry (AOAC, 2016), and the P levels were determined by visible spectroscopy and the other by atomic absorption spectrometry mode flame (FAAS). Nitrogen levels in the tissues were determined by the Kjeldahl method (AOAC, 2016).

At the end of the cycle (113 DAE) plants were removed and separated into shoots, roots and reproductive parts, to measure the number of pods per plant (NPP), number of seeds per pod (NSP), volume of roots (VR), dry mass of the shoots (DMS) dry mass of roots (DMR), mass of 100 grains (M100) and productivity (PROD), the volume of roots was determined by the cylinder displacement method using in the tube a solution of alcohol and water (9:1) to avoid the surface tension of the solution. The dried phytomasses were quantified on an analytical balance after drying in an oven with forced air circulation for seven days at  $65^\circ\text{C}$ , and the yield was corrected to 13% moisture.

Subsequently, the plants were sectioned again, this time in leaves, stems, roots and kernels for determination of Cd and Pb in the respective plant parts as previously described methodology. Based on these values were determined translocation rates to shoot and grain, as Equations 1 and 2 respectively (Zhang et al., 2014):

$$IT = CPA/CSR \quad (1)$$

$$IT = CG/CSR \quad (2)$$

*Note.* IT = translocation index; CPA = metal concentration in the shoots ( $\text{mg kg}^{-1}$ ); CSR = metal concentration on the roots ( $\text{mg kg}^{-1}$ ); CG = metal concentration in the grains ( $\text{mg kg}^{-1}$ ).

After tabulation, data were submitted to analysis of variance with the aid of the statistical program SISVAR (Ferreira, 2011). For statistical analysis was used Tukey test at 5% error and to the triple interaction was used the regression analysis.

### 3. Results

#### 3.1 Effect of Ultra-diluted Solutions of *Nux vomica* on Plant Height and Stem Diameter

According to the variance analysis the height of the soybean plants over time is observed significant interaction between the metal concentrations in the soil and the growing potencies of homeopathic *Nux vomica*, as well as the interaction between the metal concentrations in soil homeopathic potencies and DAE when analyzing the variable stem diameter (Table 1).

Table 1. Summary of analysis of variance of the biometric variables, plant height and stem diameter, evaluated over time; to soybean plants grown in soil contaminated with increasing levels of Cd and Pb and remedied with homeopathic medicine *Nux vomica* in different dynamizations

S.V.	Mean Square	
	Height	Diameter
Soil	1119.92*	0.7742 <sup>ns</sup>
Homeopathy	26.6462*	0.5324 <sup>ns</sup>
DAE	5972.15*	119.57*
Soil × homeopathy	35.6981*	9.3318*
Soil × DAE	202.23*	0.2084 <sup>ns</sup>
Homeopathy × DAE	2.8852 <sup>ns</sup>	0.096 <sup>ns</sup>
Soil × Homeopathy × DAE	5.238 <sup>ns</sup>	0.8001*
Residue	7.821	0.3015
CV (%)	13.69	11.8

*Note.* S.V.: source of variation; C.V.: coefficient of variation); DAE: days after emergence; \* Significance at 5% probability by Tukey test. <sup>ns</sup> not significant at 5% by Tukey test.

Soybean plants showed different development in canopy height possessing significant differences between treatments. And the differences can be observed mainly in the higher potencies of homeopathic medicine *Nux vomica* in plants grown in soil C (Figure 1).

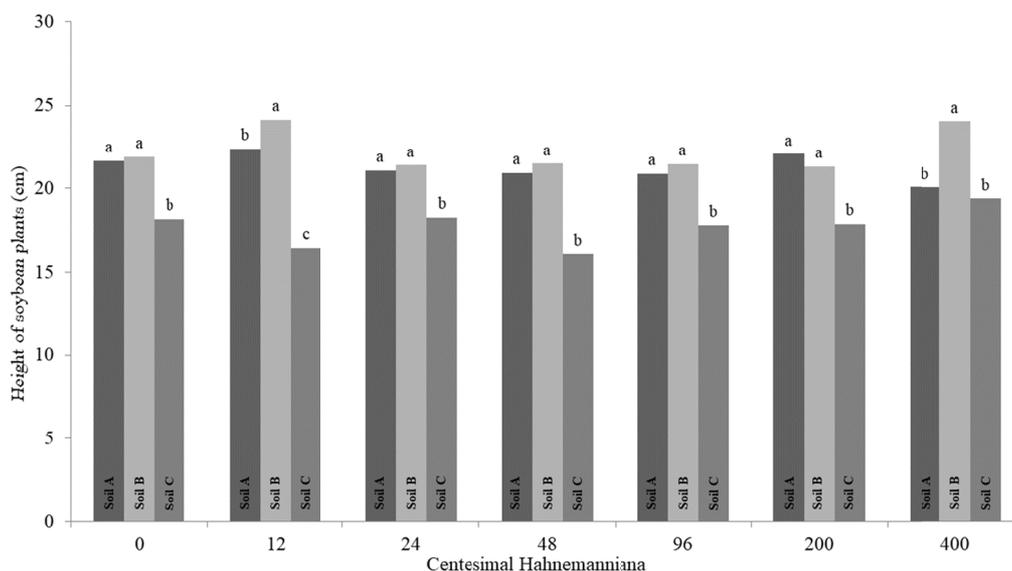


Figure 1. Height of soybean plants during the development grown at different levels of Cd and Pb in soil, submitted to dynamizations the homeopathic medicine *Nux vomica*, Cd and Pb levels: a) 0.0 to 89.0 mg kg<sup>-1</sup>; b) 3.0 and 180.0 mg kg<sup>-1</sup>; c) 9.0 and 540.0 mg kg<sup>-1</sup>. The same letters in the columns do not differ in soil concentrations above 5% significance by Tukey test

According to Figure 1 the soybean height treated with 0 CH varied with increasing metal content in the soil. It is noticed that the plants grown in soil A and B did not differ, and those grown in soil C, showed plant height 17% lower. It was observed that the plants remedied with dynamizations 24 and 96 CH showed the same pattern of witness development (0 CH).

The dynamization 12 CH increased height in soil B, exceeding by 7% (A) and 31% (C). When compared to witness the soil B increased by 9%. The dynamization 48 CH does not affect the height in soils A and B, a decrease of 25% was presented in soil C compared to plants grown in soils A and B, 11% less than the witness.

The dynamization of 200 CH interferes the height of the plants grown in soil A, in this case, with height greater than its control. It should be noted that in this case there was no statistical difference with plants grown in soil B as well as that homeopathic rate also caused no interference with the development of plants grown in soil C.

In the dynamization 400 CH plants grown in soil B achieved a 9% increase in relation to witness, 16.4% more than the plants of soil A; and on this dynamization plants of soil C showed similar effects of plants grown in soil A, not differing significantly from these. For this, the average height has increased by 6% compared to its witness.

The dynamizations 0, 12, 48 and 400 CH notices a similar development in the stem diameter of the plants do not differ until 56 DAE, during which the plants grown in soils with higher contamination C showed diameter 18% lower compared to those grown in the soil A and B.

Already in dynamizations 24 and 200 CH, the homeopathic medicine *Nux vomica* observed similar behavior until 56 DAE, period in which the stem diameter of the plants grown in soils with higher contamination C had 131% more in diameter when compared to its witness, surpassing plants grown in soils A and B remedied in the same dynamizations.

The dynamization 96 CH, contributed to plants grown in soil A statistically differed from soils B and C at 56 DAE, the plants grown in soil C also obtained diameter increase when compared to control.

### 3.2 Effect of Ultra-diluted Solutions of *Nux vomica* on Gas Exchange of Soybean Plants

Regarding gas exchange, when analyzing the variables net assimilation rate of CO<sub>2</sub> /A/, stomatal conductance /gs/ and transpiration /E/ by soybean plants, realize a significant interaction between the levels of Cd and Pb in soil and the different dynamizations of homeopathic medicine *Nux vomica* (Table 2).

Table 2. Summary of analysis of variance of the parameters for the gas exchange of soybean plants grown in soil contaminated with Cd and Pb and remedied with homeopathic medicine *Nux vomica* in different dynamizations

S.V.	Mean Square		
	/A/	/gs/	/E/
Soil	147.5663*	0.1708*	25.8752*
Homeopathy	108.81*	0.0332*	4.6864*
Soil × Homeopathy	104.1177*	0.0214*	25.1432*
Residue	3.4292	0.0075	1.0007
C.V. (%)	12.76	53.07	20.26

Note. S.V.: source of variation; C.V.: coefficient of variation; /A/: CO<sub>2</sub> assimilation; /gs/: stomatal conductance; /E/: leaf transpiration; \* Significance at 5% probability by Tukey test.

Observing /A/ may be noted the significant interaction between the metals in the soil and the action of the different dynamizations of the medicine *Nux vomica*, can be observed in the decrease of /A/ for plants grown in soil A and an increase of /A/ for plant grown in soils B and C (Figure 2).

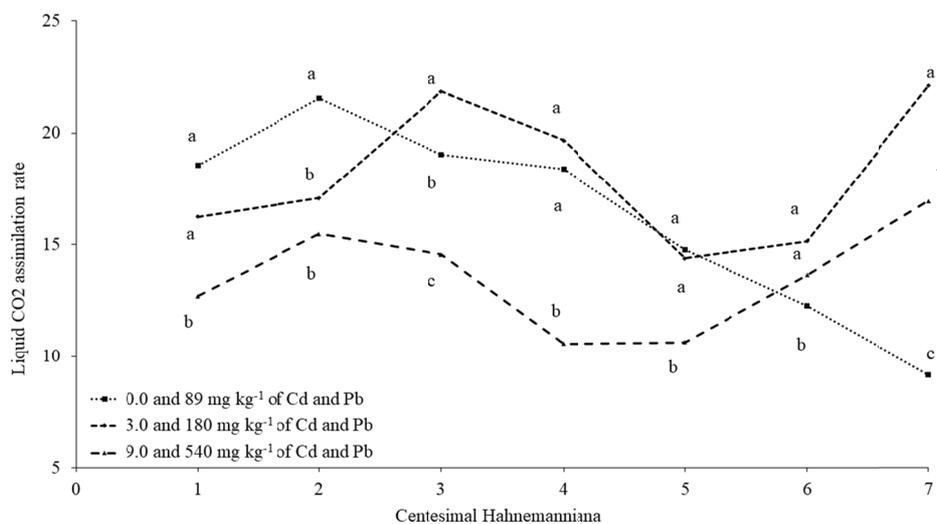


Figure 2. Liquid CO<sub>2</sub> assimilation rate /A/ (μmoles CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) of soybean plants at flowering time grown in soil contaminated with Cd and Pb, remedied with dynamizations of the homeopathic medicine *Nux vomica*. The same letters in the columns do not differ in soil concentrations above 5% significance by Tukey test

For remedial plants with the witness (0 CH) there was variation in standards of /A/, plants grown in soil A obtained the best average, plants grown in soil B were 12% lower and grown in soil C were 31% lower.

The dynamization 12 CH was efficient in increasing /A/, resulting in 13% growth for plants grown in soil A, 4.5% for B and 17.8% for plants grown in soil C. In dynamization 24 CH the /A/ for plants grown in soil A differed only 0.97% of the witness, already was an increase in soil B and C, 25.5% and 12.6%, respectively. To dynamization 48 CH plants grown in soil A, /A/ not differ from the control, while those grown in soil B the increase was 17% and grown in soil C suffered a decrease of 16% of /A/.

To dynamization 96 CH decreases /A/ in plant grown in soil A, B and C respectively 20%, 11% and 16% compared to the controls. In dynamization 200 CH plants grown in soil A and B suffered a decrease of 33.8%,

and 6.8%, in soil C increased 7% compared to the controls. In dynamization 400 CH there was a decrease of  $/A/$  in 50% for plants grown in soil A, an increase of 26.4% for grown in soil B and 25% for those grown in soil C.

For the variable stomatal conductance  $/gs/$  (Figure 3), it is observed that the different dynamizations of homeopathic medicine *Nux vomica* significantly interfered in this variable for plants grown in all contamination levels of metals in the soil.

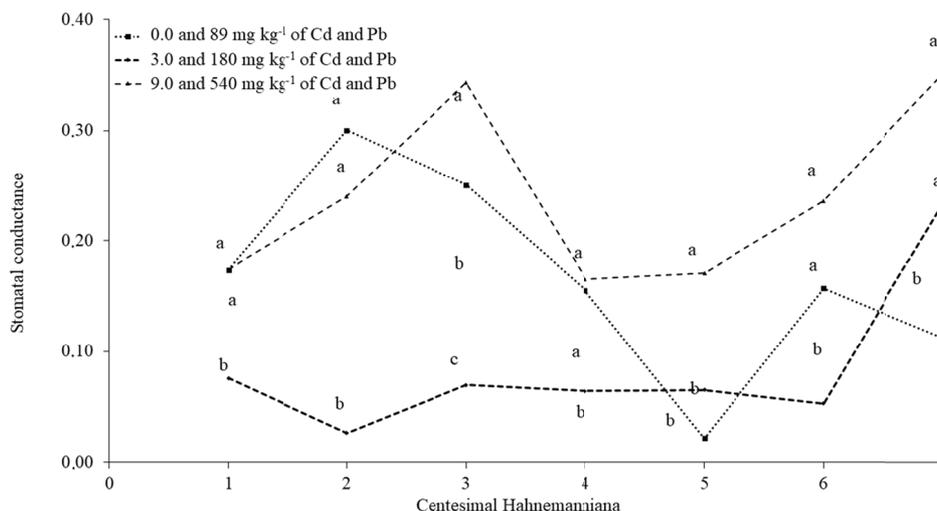


Figure 3. Stomatal conductance  $/gs/$  ( $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of soybean plants at flowering time grown in soil contaminated with Cd and Pb, remedied with dynamizations of the homeopathic medicine *Nux vomica*. The same letters in the columns do not differ in soil concentrations above 5% significance by Tukey test

The 0 CH showed different values of  $/gs/$  to the metals levels in the soil, and plants grown in soil A equaled the plants grown in soil C and plants grown in soil B noted a difference of 56% less in  $/gs/$ .

The dynamization 12 CH shows that A and C do not differ among themselves, but there is an increase in  $/gs/$  in the soil A of 42% and grown in soil C of 27%, in soil B was decreased by 65% in the  $/gs/$ . The 24 CH dynamization changed  $/gs/$  plants grown in soil A of 30% higher than the observed in 0 CH, plants grown in soil C the increase of  $/gs/$  was 50% compared with the witness, and in soil B differed from the control by 8%.

From the dynamization 48 CH, the plants grown in soil A as those grown in the soil C suffered a drop in  $/gs/$  of 10 and 5% respectively as the plants grown in soil B, the effect was similar than the dynamization 24 CH. The 96 CH dynamization shows that the plant in soil A were the only ones that have changed the  $/gs/$  being a 87% decrease compared with the witness.

The 200 CH dynamization in turn led to increase in  $/gs/$  of 9.5% on plants grown in soil A. In the C was also increased  $/gs/$  25% and decreased in soil B by 30% over the witness. To dynamization 400 CH, changes caused in  $/gs/$  of plants grown in soil A notes a decrease of 35% from the witness, plants grown in soil C and B *ran/* overcame the witness 50%.

When analyzed values of transpiration  $/E/$  (Figure 4) it is observed that the plants remedied with distilled water (0 CH) and grown in soils A and C did not differ, only in the cultivated soil B the differences were 3 and 4, significant. The other dynamizations of *Nux vomica* medicine suggest when compared to figures that the action of homeopathic medicine *Nux vomica* follows a specific cycle, sometimes enhancing sometimes causing decline of values, being in the low dynamizations or in high, which evidences that to achieve the healing potential for the lowest dynamizations efficient plants are not necessary to achieve the highest dynamizations.

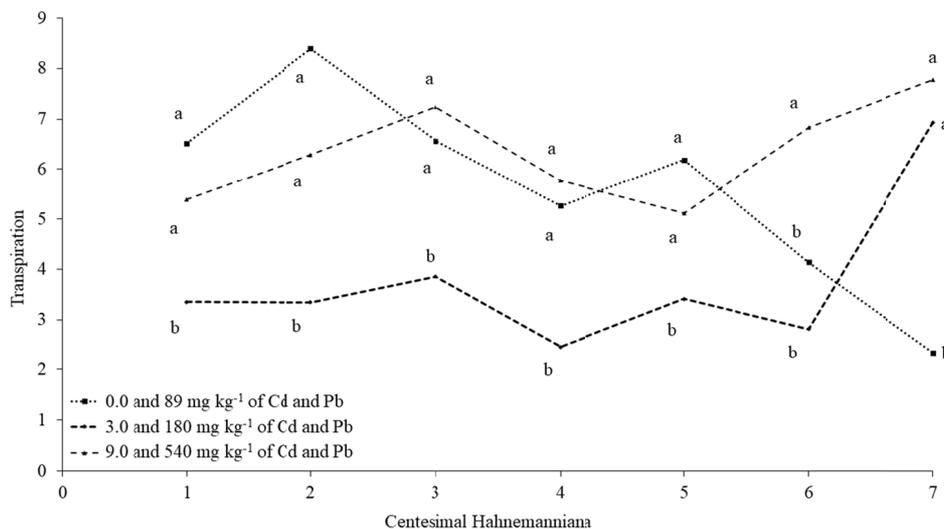


Figure 4. Transpiration /E/ (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) of soybean plants at flowering time grown in soil contaminated with Cd and Pb, remedied with dynamizations of the homeopathic medicine *Nux vomica*. The same letters in the columns do not differ in soil concentrations above 5% significance by Tukey test

### 3.3 Effect of Ultra-diluted Solutions of *Nux vomica* on Nutrient Translocation

Table 3 shows the mean squares of the analysis of variance for the plant tissue of soybean are presented. The analysis showed significant effect on the supply of soil variation to the elements Zn, Mn and Cd. As for the source of variation homeopathy, there was no significant effect to the elements. Even if for the interaction between soil and homeopathy, where was got no significant results.

Table 3. Summary of analysis of variance of foliar levels of micronutrients in soybean plants during flowering, grown in soil contaminated with increasing levels of Cd and Pb

S.V.	G.L.	Mean Square					
		Cu	Zn	Fe	Mn	Cd	Pb
Block	3	14.898	30.202	565.238	329.317	161.948	42.873
Soil	2	0.504 <sup>ns</sup>	85.083*	192.035 <sup>ns</sup>	80.869*	18.583*	60.619 <sup>ns</sup>
Homeopathy	6	1.312 <sup>ns</sup>	13.777 <sup>ns</sup>	146.928 <sup>ns</sup>	2.992 <sup>ns</sup>	0.301 <sup>ns</sup>	85.797 <sup>ns</sup>
Soil × Homeopathy	12	1.080 <sup>ns</sup>	16.986 <sup>ns</sup>	231.577 <sup>ns</sup>	5.521 <sup>ns</sup>	1.319 <sup>ns</sup>	121.119 <sup>ns</sup>
Residue	60	1.198	16.685	213.704	9.700	2.115	95.798
C.V. (%)		43.89	30.07	35.17	26.01	45.41	52.37
Levels of Cd and Pb (soil)		Cd	Zn	Mn			
0.0 and 89 mg kg <sup>-1</sup>		2.7142 b	14.5357 a	13.4285a			
3.0 and 180 mg kg <sup>-1</sup>		2.75 b	14.6428 a	12.3928 a			
9.0 and 540 mg kg <sup>-1</sup>		4.1428 a	11.5714 b	10.1071 b			

Note. S.V.: source of variation; C.V.: coefficient of variation; \* Significance at 5% probability by Tukey test; <sup>ns</sup>not significant at 5% by Tukey test. Averages with the same letter in the columns do not differ in soil concentrations at 5% of significance by Tukey test.

### 3.4 Effect of Homeopathy on Components of Production

For components of production note that the *Nux vomica* did not significantly influence the variables, but it is evident that the soybean plants were changed in the components production due to the levels of Cd and Pb in the soil (Table 4).

Table 4. Summary of variance analysis of production components of soybean plants grown in contaminated soils with Cd and Pb and remedied with homeopathic medicine *Nux vomica* in different dynamizations

S.V.	Mean Square				
	RV	DMR	DMS	M100	Prod.
Soil	890.0119*	3.4067 <sup>ns</sup>	90.5833*	432.1381*	41593005*
Homeop.	4.0238 <sup>ns</sup>	0.12203 <sup>ns</sup>	0.0952 <sup>ns</sup>	2.526 <sup>ns</sup>	43175 <sup>ns</sup>
Soil × Homeopathy	12.0952 <sup>ns</sup>	0.3536 <sup>ns</sup>	0.1666 <sup>ns</sup>	1.421 <sup>ns</sup>	49309 <sup>ns</sup>
Residue	115.289	11.255	0.4329	14.615	49426
C.V. (%)	24.03	36.51	10.79	12.64	16.43

Levels of Cd e Pb (soil)	Mean Square				
	RV	DMR	DMS	M100	Prod.
0.0 and 89 mg kg <sup>-1</sup>	14.5714 b	3.2142 a	6.8928 b	12.0985 a	2277.54 a
3.0 and 180 mg kg <sup>-1</sup>	19.5357 a	2.9642 a	7.3571 a	11.5564 a	2313.21 a
9.0 and 540 mg kg <sup>-1</sup>	8.2857 c	2.5000 a	4.0357 c	5.0360 b	184.58 b

Note. S.V.: source of variation; C.V.: coefficient of variation; RV: roots volume; DMR: dry mass of roots; DMS: dry mass of shoots; M100: mass of 100 grains; PROD: productivity; \* Significant at 5% probability by Tukey test; <sup>ns</sup>not significant at 5% by Tukey test; Averages with the same letter in the columns do not differ in soil concentrations at 5% of significance by Tukey test.

To dry mass of shoot and root volume were no significant differences for the three levels of metals from soil, presenting the lowest average in the third level of contamination. For the thousand grain mass and productivity was not obtained difference for the first two levels of contamination presenting lower values for the third level rates.

### 3.5 Effect of Ultra-diluted Solutions of *Nux vomica* on Translocation of Cd and Pb in Soybean Plants

Regarding the translocation of Cd and Pb in different plant parts of soybean plants grown in soils with increasing metal content, the dynamizations of homeopathic medicine *Nux vomica* did not cause significant effect, however the different concentrations of Cd and Pb in soil were significant for translocation of metals in the plant (Table 5).

Table 5. Abstract of analysis of variance for the metals levels in different parts of the soybean plants in soil contaminated with Cd and Pb and remedied with homeopathic medicine *Nux vomica* in different dynamizations

S.V.	Mean Square				
	Cd				Pb
	Root	Stem	Leaf	Pie	Root
Soil	53683.94*	49294.15*	4941.58*	3520.94*	1406771.94*
Homeopathy	56.12 <sup>ns</sup>	46.83 <sup>ns</sup>	10.38 <sup>ns</sup>	7.16 <sup>ns</sup>	645.32 <sup>ns</sup>
Solo × Homeopathy	67.68 <sup>ns</sup>	46.52 <sup>ns</sup>	12.53 <sup>ns</sup>	9.12 <sup>ns</sup>	568.99 <sup>ns</sup>
Residue	60.81	42.09	4.65	5.86	1863.07
C.V. (%)	25.35	25.93	24.32	27.81	29.68

Levels of Cd (soil)	Root	Stem	Leaf	Pie
0.0 mg kg <sup>-1</sup>	0.00 a	0.00 a	0.00 a	0.00 a
3.0 mg kg <sup>-1</sup>	11.39 b	1.61 b	2.46 b	4.75 b
9.0 mg kg <sup>-1</sup>	80.89 c	73.46 c	24.14 c	21.35 c

Levels of Pb (soil)	Root	Stem	Leaf	Pie
89 mg kg <sup>-1</sup>	0.00 a	0.00 a	0.00 a	0.00 a
180 mg kg <sup>-1</sup>	32.75 b	0.00 a	0.00 a	0.00 a
540 mg kg <sup>-1</sup>	403.57 c	0.00 a	0.00 a	0.00 a

Note. S.V. (source of variation); C.V. (coefficient of variation); \*significant at 5% probability by Tukey test; <sup>ns</sup>not significant at 5% by Tukey test; Averages with the same letter in the columns do not differ in soil concentrations at 5% of significance by Tukey test.

## 4. Discussion

### 4.1 Effect of Ultra-diluted Solutions of *Nux vomica* on Plant Height and Stem Diameter

The change caused in the height of the soybean plants grown in soil contaminated with Cd and Pb are justified because these metals alter the life processes of plants, thereby reducing their development. According to Sharma and Dubey (2005) a major symptom of excessive amounts of lead and its toxicity is the reduction in plant growth, chlorosis and leaf browning root system.

A significant difference in stem diameter on contaminated soil with the highest rate of metals, having inferior averages comparing to other soils, may be explained by the fact that the size of the xylem vessels are reduced due to the deposition of metal in the vessel walls (Sridhar et al., 2005).

As the homeopathic medicine acts on the vital energy and thus seeks to make the system homeostatic, being the metals Cd and Pb agents of toxicity in the system soil/plant, makes the *Nux vomica* with similarity of symptoms and perform homeopathic action to this situation of imbalance, corroborating the work of Bonfim et al. (2010) which found that homeopathic Alumina and *Calcarea carbonica* at 6 and 12 CH have significant effect on the lettuce seed vigor subjected to stress conditions caused by contamination by aluminum.

It is perceived that the homeopathic medicine *Nux vomica* acted differently in different dynamizations evaluated for biometric variables, so it is not possible to relate the effect of the medicine on an increase or decrease in dynamizations, which according Pedalino (2006) corresponds to the normal action of homeopathic medicines, because the different dilutions work in different parts of the body.

### 4.2 Effect of Ultra-diluted Solutions of *Nux vomica* on Gas Exchange of Soybean Plants

The results obtained in Table 2 corroborate with several authors which suggest that the presence of toxic metals reduces the gas exchange due to stress caused in the plant (Sessitsch et al., 2013; Deng et al., 2014).

In relation to the effects of metals on the change in the values of the variables observed in gas exchange of soybean plants, it is evident that the changes are due to the action of metals in the photosynthetic complex of the plant, as these, according to Gill et al. (2012) cause imbalance in the ratio of chlorophyll "a" and "b", thereby reducing the light collection by chlorophyll protein.

May be affirmed that the toxicity of metals changes the photosynthetic activity of rubisco enzyme, inhibit the electron transport chain, in addition to harm the guard cells and chloroplast structures (Feng et al., 2010).

The homeopathic medication according to Bastide (1998) act by transferring information contained in the medicament to the body by biological signals with potential of plant physiology modification. Thus, proving that *Nux vomica* caused change in the lifeblood of plants, changing it with its metabolism.

Among the effects observed with respect to the action of different dynamizations of homeopathic medicine, in gas exchange, can be evidenced the possible pathogenesis caused by the drug in healthy plants, grown in soil with preexisting metal contents, the dynamizations 48, 96, 200 and 400 CH of *Nux vomica*, according to Carneiro (2011) to confirm the pathogenesis the drug should cause symptoms in healthy plants that it did not have before the drug trial.

According to Bonato (2009), every dynamization cause different effects on plant organisms, and as evidenced from the early work on homeopathic effects on plants when used growing dynamizations, of the same homeopathic medicine, the effects alternates, sometimes stimulating, sometimes suppressors.

The change in CO<sub>2</sub> assimilation by homeopathic medicines were observed by researchers as Lisboa (2006) which found *Cantharis* effect on 400 CH on CO<sub>2</sub> assimilation in plant *Ruta graveolens* and Silva (2005) found that *Apis mellifica* improves assimilation of CO<sub>2</sub> in *Sphagneticola trilobata*.

### 4.3 Effect of Ultra-diluted Solutions of *Nux vomica* on Gas Exchange of Soybean Plants

The dynamizations of homeopathic medicine *Nux vomica* not statistically interfere with the availability of macro and micronutrients, in addition to the Cd and Pb in the analysis of plant tissue diagnosis sheet, but the different concentrations of metals in the soil caused changes in Mg, Zn and Cd, which according to Pourrut et al. (2011) changes in the mineral nutrition of the plants with respect to micronutrients may occur from the root blocks, decrease in translocation process, or even by competition between ions, damaging critical physiological processes.

The lower levels of Zn and higher of Cd content in the leaves can be justified by the competition between the elements Cd and Zn, by carriers mechanisms, since the Cd moves inwardly by the plant, preferably by cationic channels or by means of conveyor elements, in this case Zn (Sarwar et al., 2010).

The reduction of foliar Mn in soils contaminated by Cd and Pb, may be explained by competition between the elements in the carriers mechanisms and metabolic pathways (Lin & Aarts, 2012). Zhang et al. (2014) observed similar concentrations of those elements in the roots, but when assessing the shoots was observed a decrease in Mn content, thereby justifying the competition by carriers mechanisms and Mn in shoots.

#### 4.4 Effect of Ultra-diluted Solutions of *Nux vomica* on Components of Production

Similar results were also obtained by Ghnaya et al. (2005) found that the Cd influence negatively on growth and consequently the mass of the shoot of halophytes, having this severe reduction effect of growth due to nutritional disorders and probably due direct toxicity of Cd.

The Pb can also harm the shoot growth of soybean plants due to damage in the membranes of roots, as well as having direct toxic effect, hinders the entry of water and nutrients that are considered essential for the growth of plant (Wang et al., 2011).

Consequently, plants with impaired water absorption, lose the full production potential, and with reduction in leaf area, reduce photosynthesis, tending to decrease the productivity by reducing the grain size, less accumulation of assimilates, justifying so the lower productivity values and thousand kernel weight (Kabata-Pendias, 2010).

#### 4.5 Effect of Ultra-diluted Solutions of *Nux vomica* on Translocation of Cd and Pb in Soybean Plants

The results show the difference in mobility that metals have within the plant, being Pb less mobile than Cd, corroborating the results obtained by Bhardwaj et al. (2009) and Zhang et al. (2014) who also found greater mobility for Cd within the plant organisms.

According to Lu et al. (2008), Cd entry pathway in plants is simplastic due to the toxicity of the metal exerts on the membranes, thereby facilitating their passage into the cells using mostly Ca channels and the interaction with the Zn ion provides the use of carriers active sites of Zn.

As evaluated root tissue was found the presence of Cd and Pb, it is known that this region comprises several mechanisms adapted to tolerate environmental stresses, including those caused by toxic metals, among the mechanisms the accumulation in pectocellulose walls, kidnapping by vacuoles, addition of complexation with organic acids allow the plant to develop itself without harming their growth (Alloway, 2013).

The metal Pb, according to Chaney (1980), is absorbed by the roots, but is not translocated to the shoot in sufficient quantities to harm the plant, condition observed in this study.

This accumulation in roots can be explained by the existence of barriers, such as casparian strips and the selectivity of the plasma membranes of endodermal cells of the root tissue, preventing the translocation of the metal to the rest of the plant (Lamhamdi et al., 2013).

## 5. Conclusion

Metals rates applied to the soil caused harmful effects on growth and development of soybean plants and interfered in the micronutrient and metals content in leaf tissues and parts of the plant. The remediation of soil by homeopathic treatment did not affect the bioavailability of metals for the soybean plants, but altered the development causing differences in height and stem diameter of the plants, and also altered the functioning of the primary metabolism and the amounts of net CO<sub>2</sub> assimilation, stomatal conductance and transpiration were modified according to the dynamizations of the homeopathic medicine *Nux vomica*.

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