DEVELOPMENT OF WHEAT PLANTS TREATED WITH *NUX VOMICA* AND GROWN IN SOIL CONTAMINATED WITH Cd AND Pb

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ABSTRACT: Nowadays the environment is subject to a number of contamination, one of the most pernicious is soil contamination by toxic metals. Various technologies have been studied for the remediation of contaminated soils, being one of most discussed the use of plants in the removal of toxic metals. The aim of this study was to evaluate the effectiveness of the medicine Nux vomica applied at high dilutions in wheat plants grown in soil contaminated with Cd and Pb. For that, the experiment was conducted with contaminated soils in three doses based on resolution N°. 420 of CONAMA: Research values (VI) (value existing in the soil); the VI and three times the VI) resulting in 0; 3 and 9 mg kg^{-1} for Cd and 89; 180 and 540 mg kg^{-1} for Pb. Plants development, gas exchange, nutrient composition, component production and bioavailability of metals were evaluated. According to the results, the high dilutions of Nux Vomica interfered in height and stem diameter of wheat plants. The soil contamination with Cd and Pb affected negatively the mineral nutrition, productivity and bioavailability of metals. More studies are needed in order to elucidate the use of high dilution of Nux vomica as a remediation alternative.

KEYWORDS: contamination of soils, cadmium, lead.

DESENVOLVIMENTO DE PLANTAS DE TRIGO TRATADOS COM *NUX VOMICA* E CULTIVADAS EM SOLO CONTAMINADO COM CD E PB

RESUMO: Hoje em dia, o ambiente está sujeito a uma série de contaminações, sendo a contaminação do solo por metais tóxicos uma das mais perniciosas. Várias tecnologias têm sido estudadas para a remediação de solos contaminados, sendo um dos mais discutidos o uso de plantas na remoção de metais tóxicos. O objetivo deste estudo foi avaliar a eficácia do medicamento Nux vomica aplicado em altas diluições em plantas de trigo cultivadas em solo contaminado com Cd e Pb. Para isso, o experimento foi conduzido em solos contaminados em três doses com base na resolução N°. 420 do CONAMA: valores de Investigação (VI) (valor existente no solo); duas vezes o VI e três vezes o VI), resultando em 0; 3 e 9 mg kg^{-1} de Cd e 89; 180 e 540 mg kg^{-1} para Pb. Foram avaliadas desenvolvimento das plantas, as trocas gasosas, composição de nutrientes, componentes de produção e biodisponibilidade dos metais. De acordo com os resultados, as altas diluições de Nux Vomica interferiram na altura e

diâmetro de plantas de trigo. A contaminação do solo com Cd e Pb afetou negativamente na nutrição mineral, produtividade e biodisponibilidade dos metais. Mais estudos são necessários para elucidar o uso de altas diluições de Nux vomica como uma alternativa de remediação.

PALAVRAS-CHAVE: Contaminação de solos, cádmio, chumbo.

INTRODUCTION

The metals cadmium (Cd) and lead (Pb) are toxic chemical elements as well as pollutants. Even at low levels shows detrimental effects to the ecosystem, modifying physical and chemical quality of soil, water and air, promoting mortality of fauna and flora and harming human health (Gonçalves Jr. et al., 2014).

In agricultural areas, soil contamination is mainly caused by the use of fertilizers that can bring in their compositions Cd and Pb (Nacke et al., 2013). Phosphate fertilizers and micronutrients have the highest rate of toxic metals, mainly due to the self-cost for the extraction of micronutrients from their natural sources, having as the legalized alternative in Brazil, the use of industrial waste as a source of raw materials, which can bring Cd and Pb and other toxic metals as contaminants (Nacke et al., 2013;. Gonçalves Jr. et al., 2014.).

Once in the environment, the sustainable alternative to continue tilling the soil is through remediation techniques that seek to reduce the concentration or the toxicity of the contaminant element. Therefore, it is important that emerging and innovative techniques are always being studied, which add efficiency and lower costs to the entire process (Nobre and Nobre, 2003).

According to Bonato (2014), a method that requires attention and it is becoming popular quickly in Brazil, mainly by farmers who migrate to the organic production system, is the homeopathic science that has among its applications the decontamination of soil and water.

In Brazil the use of homeopathy in agriculture is regulated by Normative Instruction No. 46, of 6th October 2011 (Brazil, 2011), allowing organic farmers to use homeopathic medicines for the treatment of plants, animals, soil and water. The medicine *Nux vomica* appears in the homeopathic materia medica showing symptoms of toxicity, by analogy it is used by farmers to decontaminate plants, water and soil, presenting excellent results (Rezende, 2009).

Thus, the objective of this study was to evaluate the remediation of soil contaminated with levels of Cd and Pb using different dynamizations of medicine *Nux vomica*, evaluating its effect on wheat plants as well as in the bioavailability of plant tissues.

MATERIALS AND METHODS

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

Wheat plants were grown in pots of 8 dm^3 filled with clayey Dystrophic Red Eutroferric (LVe) (clay = 578.00; silt = 348.58 and sand = 73.42 g kg^{-1}) and existing levels of Cd <0.005 mg kg^{-1} Pb and 19.0 mg kg^{-1} (FAAS) (0.005 mg kg^{-1}).

Liming and fertilization of the soil were carried out sixty days before sowing, applying the corresponding amount to 0.92 Mg ha^{-1} $CaCO_3$ to reach a base saturation (V%) of 60%, 80 kg ha^{-1} of K_2O in the form of potassium chloride (KCl), 90 kg ha^{-1} of P_2O_5 in the form of triple super phosphate (TSP) and for nitrogen fertilization were two applications, one at sowing and other coverage at the beginning of tillering, providing 30 and 90 kg ha^{-1} of nitrogen (N) respectively in the form of urea, as Pires (2014).

The experimental design was a randomized block design (RBD) with three different concentrations of metals in the soil, seven dynamizations of homeopathic medicine *Nux vomica* and four replications, each pot being an experimental unit. Soil contamination was made from the addition of metal solutions prepared with Cd salts [monohydrate cadmium chloride ($CdCl_2H_2O$)] and Pb [anhydrous lead chloride ($PbCl_2$)], being the doses based on research values (VI) specific to agricultural areas of the resolution No. 420 of CONAMA (Brazil, 2009) (dose 1 – existing value in the soil; dose 2 - VI; dose 3 - three times VI), resulting in 0; 1.5 and 3.0 mg kg^{-1} of dry soil for Cd and 19; 90 and 180 mg kg^{-1} of dry soil for Pb.

The metal concentrations in the soil were changed by applying 250 ml of homogenized solutions containing the metals in the form of $CdCl_2H_2O$ and $PbCl_2$, as for doses of existing values was only added 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 (Trevizam et al., 2010). At the end of the incubation period, six seeds of wheat cultivar CD150® were sown per pot being held thinning after emergence, maintaining three plants per pot.

The canopy height and stem diameter per plant were analyzed in 14-day intervals in order to elaborate a development curve of plants based on these biometric parameters.

Measurements of gas exchange were carried out in stages 10 (issue by the flag leaf) (38 DAE), with IRGA LI-6400XT equipment (Liquor Inc. Lincoln, NE), and the flag leaf of each plant was analyzed. The average photon flux photosynthetically active for the determination of gas exchange was 1200 μmol m^{-2} s^{-1} and the average concentration of CO_2 in the medium was 400 μmol mol^{-1}, always in the morning (08:00 to 10:00), and certain variables

were set: *A* - CO_2 net assimilation rate of CO_2 (µmol CO_2 m^{-2} s^{-1}); *E* - Transpiration rate (mmol H_2O m^{-2} s^{-1}); *gs* - Stomatal conductance (mol m^{-2} s^{-1}).

The nutritional analyzes of leaf tissues during development, the samples were taken from the first to the fourth leaf from the apex during the early flowering (Malavolta et al., 1997), and determined the levels of N, phosphorus (P) , potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), Cd and Pb.

Therefore, the samples of plant tissues were subjected to sulfuric acid digestion for the determination of N and nitropercloric to the other elements, according to the methodology described by the Analytical Chemistry Official Association (AOAC, 2012), and the P levels determined by visible spectroscopy and the other metals by atomic absorption spectrometry mode flame (FAAS). Nitrogen contents in leaf tissue were determined through Kjeldahl method (AOAC, 2012).

At the end of the crop cycle (119 DAE) the plants were removed and separated into shoots, roots and reproductive parts, to measure the number of grains per plant (NEP), spike length (CE), number of spikelets per spike (NEE), number of grains per spike (NGE), root volume (VR), dry weight of shoot (MSPA), dry mass of roots (MSR). The volume of roots was determined by measuring cylinder displacement method using solution of water and alcohol (9:1) to avoid the surface tension of the solution, dried phytomass was quantified on an analytical balance after drying in an oven with forced air circulation by seven days at 65°C, and productivity has been corrected for humidity of 13%.

Subsequently, the plants were sectioned again, this time as leaves, stems, roots and grains 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).

$$IT = \frac{CPA}{CSR} (1) \qquad IT = \frac{CG}{CSR} (2)$$

Where: IT = translocation index; CPA = metal concentration in the shoot (mg kg^{-1}); CSR = metal concentration in the root system (mg kg^{-1}) And CG = metal concentration in the grains (mg kg^{-1}).

After tabulation, data were submitted to analysis of variance with the aid of statistical program SISVAR (Ferreira, 2011). The means were compared using the Tukey test. For interaction between the dynamizations of homeopathic medicine *Nux vomica* and metal concentrations in the soil was used the Scott-Knott test. In the case of differentiation between

the levels of metals and days after emergence were prepared regression curves with the software Excel.

RESULTS AND DISCUSSION

In Figure 1 are arranged the means regarding to height of wheat plants grown in soil contaminated with Cd and Pb and remediated with homeopathic medicine *Nux vomica* where we observe significant differences between the contaminated soils within each dynamization of *Nux vomica* medicine.

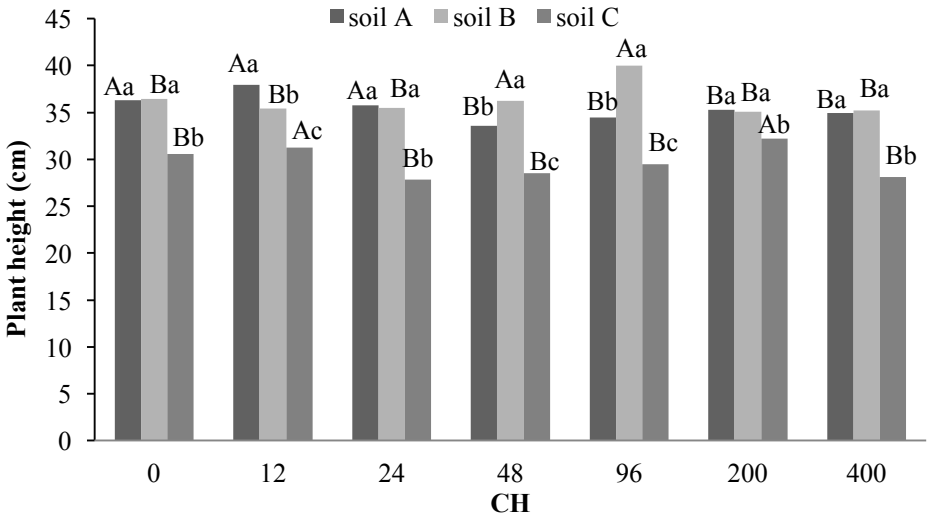

Figure 1 - Wheat plant height during development grown in soils with different levels of Cd and Pb, subject to dynamizations of homeopathic medicine *Nux vomica.* Levels of Cd and Pb: A) 0.0 and 89 mg kg^{-1}; B) 3.0 and 180 mg kg^{-1}; C) 9.0 and 540 mg kg^{-1}. Capital letters in the evaluated CH do not differ. Equal capital letters in the evaluated soils (A, B and C) do not differ at 5% significance by Scott-Knott test.

The dynamizations exerted different effects for each level of metal contamination in soils. In soil with existing metal levels only dynamization 12CH provided significant height increase at 4.5%, the dynamizations 24, 48, 96, 200 and 400CH did not differ from the control sample, but showed lower averages 1.4%; 7.4%, 5.1%; 2.7% and 3.7% respectively.

In soils with contamination levels at 3.0 and 180 mg kg^{-1} of Cd and Pb, for average wheat plant heights in interaction with dynamizations of homeopathic medicine *Nux vomica,* is observed that only the dynamization 96CH increased plant height at 9.6% compared to control sample, the dynamizations 12, 24, 48, 200 and 400 reduced by 2.8%; 2.7%; 0.6%; 3.8% and 3.4% respectively (Figure 1).

Observing the interaction between the medicine *Nux vomica* dynamizations on the height of wheat plants grown in soil contaminated with Cd and Pb, note that both the

dynamizations 12CH and 200CH increased plant height at 2.2% and 5.4% respectively, and for the remaining dynamizations 24, 48, 96 and 400CH the averages were lower when compared to control sample at 8.9%; 6.7%; 3.6% and 8.1% respectively (Figure 1).

According to Bonato and Silva (2003), it is common to observe in high dilution doses alternation in effect, sometimes stimulating, sometimes suppressing and others not causing any effect. This behavior may be related to the undulations (resonance) perceived in the nature and with the similarity (resonance) between the dynamization (frequency) of the medicine and the organism (resonance) (Vithoulkas, 1980).

The effects may range according to the plant, the substrate that is grown and dynamizaions of medicine used. Researchers as Meinerz et al., (2011) studied the effects of *Sulphur* in different dynamizations checking significant interference in height and stem diameter of fisalis. Bonato and Smith (2003) noted changes in height of radish plants using *Sulphur* medicine in 5 dynamizations, Carvalho et al., (2004) studied the effects of *Arnica montana* in Artemisia plant and noted no significant effect the medicine on the plant height.

Figure 2 shows the interaction occurred in the variable stem diameter of the wheat plant grown in soil contaminated with Cd and Pb and the different dyanamizations of homeopathic medicine *Nux vomica.*

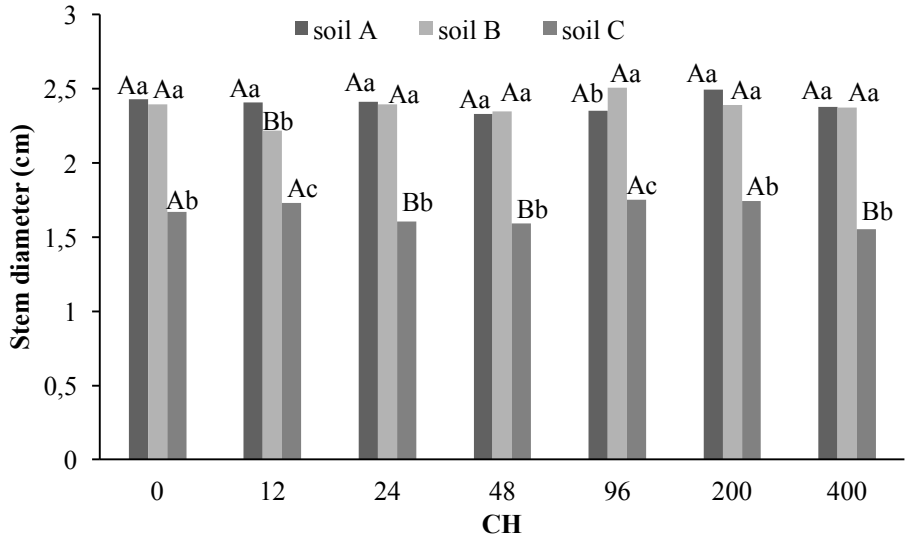

Figure 2 - Stem diameter of wheat plants during development and grown at different levels of Cd and Pb in the soil, subjected to dynamizations of homeopathic medicine *Nux vomica.* Levels of Cd and Pb: A) 0.0 and 89 mg kg^{-1}; B) 3.0 and 180 mg kg^{-1}; C) 9.0 and 540 mg kg^{-1}. Capital letters in the evaluated CH do not differ. Equal capital letters in the evaluated soils (A, B and C) do not differ at 5% significance by Scott-Knott test.

It is observed in Figure 2 that the dynamizations of homeopathic medicine *Nux vomica* did not significantly influenced the stem diameter of the plants grown in soil with existing metal contents, but it was noted that the 200 CH dynamization increased 2.3% this variable when compared to control sample, as for the dynamizations 12, 24, 48, 96 and 400 the averages were negative compared the control sample at 0.8%; 0.8%; 4.1%; 2.9% and 2.1% respectively.

As for plants grown in soils with concentrations of metals in the VI of CONAMA, the dynamization 12 CH provided a significant difference to the variable stem diameter, reducing by 7.5% compared to its control sample, although it was not significant the dynamization 96CH provided 4.6% increase in this variable when compared to control sample (Figure 2).

Wheat plants grown in soil with higher concentration of metals were the ones with the lowest average for stem diameters when compared to other soils, but these were more positively influenced by dynamizations of homeopathic medicine *Nux vomica,* 7.8% for 12CH, 5.4% for 96CH and 4.8% for 200CH (Figure 2).

The effects of dynamizations of homeopathic medicine in the variable stem diameter were similar to those obtained in plant height, not following standard which according to Bonato and Silva (2003), homeopathic medicines behave as energy and when dynamized, the frequency of wave remains fixed only occurring variation in wave amplitude. Thus the plant will respond positively when the wave frequency of the homeopathic preparation is similar to its own.

Figure 3 demonstrates the interaction between the levels of Cd and Pb in the soil and the D.A.E. when analyzing the variable height of wheat plants remedied with the homeopathic medicine *Nux vomica* in increasing dynamizations.

It is possible to infer that the plants grown in soil C showed significant changes in growth and showed characteristic symptoms of toxicity. Toxic metals may be responsible for controlling growth of certain plants by reduction of biomass, the stretching of roots or other organs, leaf area reduction, inhibition of stomatal opening and disorders of mineral nutrition (Rai et al., 2005; Sharma, Dubey, 2005; Pandey, Grupta, Mukherjee, 2007).

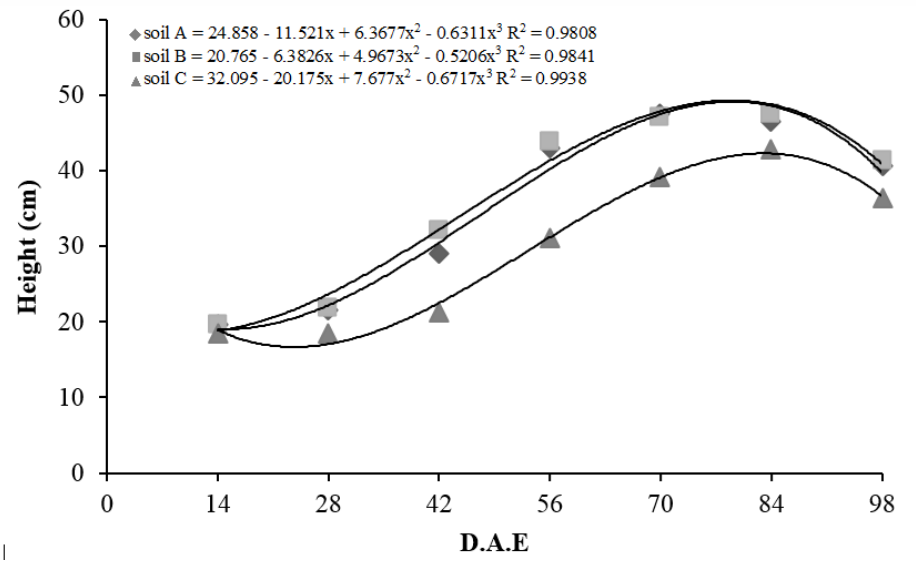

Figure 3 - Wheat plant height, over time, grown in soils contaminated with different concentrations of metals and remedied with homeopathic medicine *Nux vomica*. Soil A) 0.0 and 89 mg kg^{-1}; soil B) 3.0 and 180 mg kg^{-1}; soil C) 9.0 and 540 mg kg^{-1} . D.A.E (days after emergence).

The reduced growth of plants in response to heavy metals are widely reported in the literature (Sanità-Di-Toppi & Gabbrielli 1999, Kabata-Pendias & Pendias 2001). These responses are related to damage to the photosynthetic apparatus by interfering with chlorophyll biosynthesis, at the enzyme activity of the Calvin cycle and PSII (Di Cagno et al. 1999).

Figure 4 shows the interaction between the metal concentrations in the soil and the DAE when observed the stem diameter parameter of wheat plants grown in contaminated soil and remediated with homeopathic medicine *Nux vomica* in several dynamizations.

In general, it is observed differences in stem diameter over time in plants grown in soils with the highest concentration of Cd and Pb (soil C) compared to plants grown in soils A and B (Figure 4).

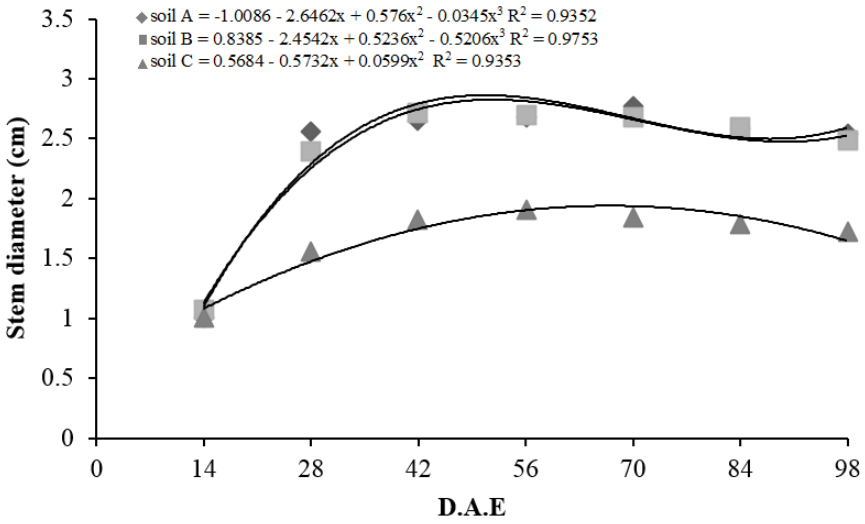

Figure 4 - Stem diameter of wheat plants evaluated over time cultivated in soil contaminated with Cd and Pb in different soil concentrations. Soil A) 0.0 and 89 mg kg^{-1}; soil B) 3.0 and 180 mg kg^{-1}; soil C) 9.0 and 540 mg kg^{-1}. D.A.E (days after emergence).

According to Sandalio et al., (2001), the diameter of the stem can be changed when in contact with heavy metals, because they reduce the size and number of xylem vessels, affecting the availability of water for the plant, altering the hormonal balance related to tissue morphogenesis.

When observed the results of the analysis regarding gas exchange, there was no significant difference for the evaluated parameters.

The medicine *Nux vomica* did not performed response for photosynthetic system in any dynamizations, probably by the fact that the system was not affected by the presence of metals in soils. Which according to Casali et al., (2006), it is in accordance with the principles of homeopathy, which has the function to promote self-regulation of organism.

However, the results obtained do not corroborate with Lisboa et al., (2005), who observed greater CO_2 assimilation in *Ruta graveolens* L plants, treated with homeopathic medicine *Cantharis* 4CH and *Apis* 6CH.

Regarding the variance analysis for levels of macro nutrients in plant tissue at flowering, depending on the concentrations of Cd and Pb in the soil and the application of *Nux vomica*, it is observed that the presence of metals in soil changed the availability of nitrogen (N), phosphorus (P), calcium (Ca) in the plant, and the homeopathic medicine did not promote significant effect on these variables (Table 1).

Table 1 - Average levels of N, P, Ca and Mg found in leaf tissue of wheat plants at flowering

Levels of Cd and Pb (soil)	Average N	Average P	Average Ca
0.0 and 89 mg / kg	30.13 ab	1.77 b	40.0 b
3.0 and 180 mg / kg	25.8 b	2.05 b	41.18 b
9.0 and 540 mg / kg	35.18 a	3.15 a	62.76 a

Averages with the same letter in the columns do not differ between soil concentrations at 5% significance by Tukey test.

The dynamizations of *Nux vomica* did not result in significant differences in macronutrients in plant tissue, either regarding the rate of Cd and Pb, however Novelino et al., (2014) found that the homeopathic medicine *Sulphur* influenced the absorption of P by the sorghum plants grown in soils with different concentrations of phosphate, which according to Andrade et al., (2011), is possible by the fact that the soil microorganisms are able to interact with the information provided by the homeopathic medicine, changing the soil respiration.

The highest accumulation of these nutrients in wheat leaves grown in highest concentration of Cd and Pb may be related to decreased dry mass of the shoots due to toxicity of metals (Kabata and Pendias, 2001).

Table 2 shows the averages of wheat plant production components, where it was found that only the source of relative variation contamination of Cd and Pb in the soil exerted significant difference in all variables, with *Nux vomica* in their dynamizations did not result in significant influence.

Table 2 - Averages for wheat plant production components grown in soil contaminated with Cd and Pb and remediated with homeopathic medicine *Nux vomica* in different potencies

Levels of Cd and Pb (soil)	NEP	CE	NEE	NGE	V.R.	MSR	MSPA
0.0 and 89 mg / kg	1.67 a	6.64 a	13.60 to	116.3 a	7.28 a	1.19 a	3.10 a
3.0 and 180 mg / kg	1.67 a	6.82 a	13.85 a	108.9 a	6.28 a	1.00 ab	2.89 a
9.0 and 540 mg / kg	1.60 a	4.42 b	8.14 b	65.0 b	5.85 a	0.74 b	1.64 b

Averages with the same letter in columns do not differ between the metal concentrations in soil at 5% by Tukey. NEP (number of spikes per plant), CE (spike length), NEE (number of spikelets per spike), NGE (number of grains per spike), VR (root volume), MSPA (dry mass of shoots), MSR (dry mass of roots).

The effect of Cd and Pb doses in roots system of wheat plants reduced the mass of roots. Similar results were found by Islam et al. (2007), where the root system of plants of genus *Elsholtzia* were directly affected by the conditions of stress by toxic metals.

Regarding the distinct translocation of metals in wheat parts were not observed significant influence for dynamizations of homeopathic medicine *Nux vomica*, what can only be observed is the significant difference and the presence of Cd in roots and shoots of wheat plants and Pb accumulation in roots.

Few experiments were conducted to evaluate the action of homeopathic science in the availability of metals in toxic concentrations for plants, homeopathic medicine *Nux vomica* is used in agriculture for detoxification of soil and plants (Andrade et al., 2011), however, there was no action of this medicine in respect to availability of Cd and Pb in wheat plants.

However, according to Andrade et al., (2011), the amount of contaminants in soil interferes with the response to the drug stimuli, making the process slower, proven fact when evaluating the microbial respiration in soils collected in forest area, agricultural land and soils saturated with aluminum. The area of forest soils responded much more quickly to homeopathic medicine.

Regarding to the translocating metals in wheat plant, the results of averages of Cd and Pb is presented in Table 3. It may be perceived significant differences in shoots and roots for Cd, and for Pb, only the roots had significant differences, demonstrating that wheat plants has different ways to absorb and store the metals in their tissues.

Table 3 - Averages for Cd levels in different parts of wheat plants grown in soil contaminated with Cd and Pb and remediated with homeopathic medicine *Nux vomica* in different dynamizations.

Levels of Cd and Pb (soil)	Averages Cd shoots	Averages Cd roots	Averages Pb shoots	Averages Pb roots
0.0 and 89 mg kg^{-1}	0.0 a	0.0 a	0.0 a	0.0 a
3.0 and 180 mg kg^{-1}	0.0 a	34.5 b	0.0 a	35.39 b
9.0 and 540 mg kg^{-1}	0.28 b	269.0 c	0.0 a	190.82 c

Averages with the same letter in columns do not differ between soil concentrations at 5% significance by Tukey.

Regarding Cd concentrations in shoots and roots of wheat, there is the presence in all tissues in the different parts of plant (soil with 9.0 mg kg^{-1}). These results corroborate Semhi et al. (2014) who found the Cd mobility through high levels of transfers to the shoots.

Pb contents were found only in roots of wheat plants, such data corroborate Lamhamdi et al., (2013), who obtained similar results with wheat in increasing doses of Pb. The absence of Pb in shoots showed a low metal mobility, according to Alloway (2013) absence of metal in

these regions is justified because metals such as Pb which are not considered micronutrients have low mobility behavior in the phloem, forming organic complexes of high molecular weight are hardly adsorbed on the vessels walls and accumulate in this kind of tissue.

CONCLUSION

The homeopathic medicine *Nux vomica* in the evaluated dynamizations interfered in height and stem diameter of wheat plants, showing efficiency for remediation of soils contaminated with toxic metals Cd and Pb, when taken into account the bioavailability for plants, and metals caused deleterious effects on development and productivity of wheat plants.

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