

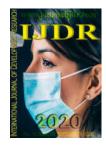
ISSN: 2230-9926

RESEARCH ARTICLE

Available online at http://www.journalijdr.com



International Journal of Development Research Vol. 10, Issue, 09, pp. 39944-39947, September, 2020 https://doi.org/10.37118/ijdr.19921.09.2020



OPEN ACCESS

EVALUATION OF CITRONELLA PLANT POTENTIAL AS A PHYTOREMEDIATOR IN A LEAD CONTAMINATED SOIL

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ARTICLE INFO

Article History: Received 29th June 2020 Received in revised form 11th July 2020 Accepted 18th August 2020 Published online 23rd September 2020

Key Words:

Cymbopogonnardus; Heavy Metals, Plant Nutrition, Phytoremediation, Soil Contamination.

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ABSTRACT

One of the major problems of Brazilian agriculture is the contamination of soils as a result of industrial processes, like a toxic metal contamination. Among these contaminants, lead is a heavy metal known to be harmful to human health and the environment. Given these facts, some methods were created to remove these contaminants from the soil, such as the phytoremediation technique. Due to the economic importance that the extraction of citronella essential oil represents and the environmental damage that lead contamination of soil causes to the ecosystem, this study aimed to verify the influence of lead on the development of the citronella plant. The study was carried out in the city of Maringá-PR, at the Unicesumar University Center between May 16, 2019 and September 20, 2019, soil contamination was carried out by irrigation with three increasing dosages of lead sulphate diluted in water (30 mg/L concentration). The treatments were divided into: T1 (without lead sulphate), T2 (3mg lead sulphate), T3 (7mg lead sulphate), T4 (15mg lead sulphate). Through this research it can be concluded that the citronella medicinal plant presented adequate root growth, keep the accumulation of water in the root cells, and in addition, by lead concentrations the citronella showed an increase in the production of its essential oil, which indicate that this plant has a potential for use in the phytoremediation process.

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Citation: Marcel Antea; Kleber Lopes Longhini; Marcelo Teixeira Silva; Sonia Tomie Tanimoto; Aline Maria Orbolato Gonçalves Zuliani; André Ribeiro daCosta; Graciene de Souza Bido; Edison Schmidt Filho and Anny Rosi Mannigel. "Evaluation of citronella plant potential as a phytoremediator in a lead contaminated soil", International Journal of Development Research, 10, (09), 39944-39947.

INTRODUCTION

Essential oils are of considerable commercial and economic interest, given their diversity of applications (manufacture of cosmetics, food supplements, in the production of pesticides, base for drugs, among other applications) (Andrade *et al.* 2012; Rocha *et al.* 2012). Of the most well-known essential oils, citronella oil has stood out in the Brazilian market, this is due to the repellent effect it presents against the AedesAegypti, vector of dengue, zika and chikungunya. Citronella is a plant originating in India and Indonesia, grown in tropical and subtropical regions, contains a high amount of aliphatic monoterpenes such as citronelal, geraniol and citronelol (Andrade *et al.*, 2012; Marco, 2007).

According to Correa and Scheffer (2013), the use of essential oils has grown in recent years in several sectors, mainly in the use of cosmetics and perfumes, however, a significant portion of the national market has used essential oils for use as repellents, mainly citronella essential oils, which have an extremely efficient repellent potential and can be used in different ways such as candles and spray. The interest in oils, makes it necessary studies that aim to increase the production of oils, without causing agricultural damage and without compromising the quality of the oil formed. Due to the need to increase the production of essential oils, new studies are being developed, and among them, the use of heavy metals to activate plant defense systems and increase the production of essential oil has become a reality, in addition to the benefit economical that this technique presents, still has the environmental question in which it is used with phytoremediation in contaminated soils. According to Gratão et al. (2005), toxic metal contamination of the environment is a serious impediment to the development of agriculture, as well as damaging ecosystems, being a threat to both humanity and the biosphere. Among these contaminants, lead draws attention to other substances as it is a heavy metal known to be harmful to human health and the environment, being used in the production of alloys (bronze and brass), rubbers, glass, electrical cables, welds. parts, electric plates and the manufacture and recovery of batteries (Grigoletto, 2011). Inadvertently these products can be launched on wasteland, agricultural areas and when carried by rainwater, can reach unimaginable distances, accumulating in the food chain, as like all heavy metal (Zampieron et al. 2019).

Soil contamination by Pb occurs when the amount of metal in the environment exceeds the ability of the soil to retain it and can be absorbed by plants or contaminating water (Fernandes et al., 2011). Pb forms strong complexes with organic and inorganic materials and may be available in the soil as Pb²⁺ ion (Li et al., 2013), and its toxicity caused by the presence of Pb in the soil can be observed by several changes in growth and plant development, causing morphological, growth. physiological and biochemical changes (Moraes, 2011). Given these facts, some methods were created to remove these contaminants from the soil and among them, phytoremediation has been standing out (Mariano and Okuruma, 2012). Phytoremediation is the use of the ornamental plants grown in contaminated soils, aiming to remove, transfer, destroy or stabilize heavy metals, the absorption of these heavy metals by the plants used in this technique promotes the growth and devolpment of the phytoextractor (Jadia and Fulekar, 2019). Due to the economic importance of citronella essential oil extraction and the environmental damage that lead contamination of the soil causes to the ecosystem, this study aimed to verify the influence of lead contaminant on essential oil production, root growth and moisture retention in these plants, as well as verifying the possible use of citronella plants as a phytoremediator to extract these lead from the soil.

MATERIALS AND METHODS

The work was carried out in the city of Maringá, State of Paraná, Brazil, at the Unicesumar University Center between May 16, 2019 and September 20, 2019. The climate of the region is characterized as subtropical, having an annual average of 22°C; and semi-humid, with an average annual rainfall of 1,590 mm, the lowest temperatures are from May to July, while the highest temperatures are from November to March (Deffune et al. 1994). To carry out the experiment 50 seedlings of citronella (Cymbopogonnardus), with approximately 17 cm height, were acquired, being the experimental unit a vase with dimensions 30x22x28cm containing a plant. The experiment was evaluated by five different levels of lead in the soil to evaluate the behavior of the citronella plant through contamination, and each treatment had 10 replications. The seedlings were transplanted to pots that were 30 cm in diameter from the upper portion, 22 cm in diameter from the lower portion and 28 cm in height totaling a capacity of 8 liters. The pots were lined with 800 g of crushed stone for better water drainage. The soil present in the pot has the following classification: Dystrophic Red Latosol with 200, 200 and 600 g kg-1 of sand, silt and clay, respectively (Santos

et al. 2018). Subsequently they were filled with soil whose chemical composition presented: P (12.65 mg dm⁻³), K + (0.09 $cmolc.dm^{-3}$), Ca^{+2} (0.95 $cmolc.dm^{-3}$), Mg^{+2} (0.35 $cmolc.dm^{-3}$), pH in CaCl2 (5.30) and M.O (36.17 g dm⁻³). The contamination of the soil by lead was carried out by means of irrigation, by the introduction and aqueous solution of lead sulfate in different concentrations 0 mg / L; 3 mg / L; 7 mg / L and 15 mg / L. Contamination occurred slowly, by adding small portions (approximately 50 mL) once a day. The parameters evaluated at the end of contamination were root growth, shoot retention and essential oil production. The first two were measured according to the methodology established by Benincas (1988). To measure root growth, the roots were washed and their length measured with the aid of a ruler. Regarding the water retention of the shoot, a portion of the leaves were removed, which were weighed to obtain the fresh mass and after that, they were taken to a greenhouse at 60°C for 24 hours, after drying, the roots were weighed. The essential oil values were obtained by Soxhletextraction equipment which works with a reflux system that had as solvent the ethanol. As ethanol washed the plant, oil from the leaves and stem was retained in a becker. Weighing of the sample of the plant used was done before and after the process and with the difference the value of oil produced per sample was obtained plant. After data collection, the assumptions were verified and after being met, the analysis of variance (p < 0.05) and the Scott Knott test at 5% probability were applied to the variables with significant differences (Banzatto and Kronka 2008). Being the analyzes made through the statistical program Genes (Cruz 2006).

RESULTS AND DISCUSSION

After measuring the variable responses, the data were subjected to analysis of variance by the Genes statistical program (Cruz 2006), and the results are shown in Table 1 and Figure 1. Taking into account that the analyzed variable is a quantitative variable, regression analysis was performed to interpret the results. However, only for academic purposes to find out which dose was higher, averages tests were performed for the variable responses. The Figure 1 illustrates the results of treatments with the different doses of PbSO₄ applied to soil at essential oil concentrations (%).

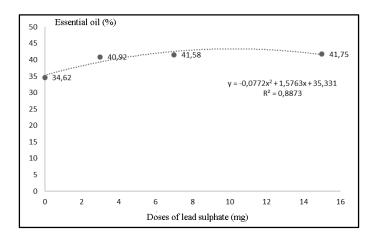


Figure 1. Essential oil values (%) and Lead Sulphate doses (mg) applied to treatments

When calculating the regression by the Genes program, R2 =0.88 was obtained. It is also noticeable, when comparing the doses and the essential oil content, that although the doses

Treatments	Root length (cm)	Water content (%)	Essential oil content (%
0 mg de PbSO4	33,80 a	17,08 a	34,62 b
3 mg de PbSO4	31,80 a	15,08 a	40,92 a
7 mg de PbSO4	44,20 a	16,33 a	41,58 a
15 mg de PbSO4	39,40 a	17,48 a	41,75 a
CV%	13,10	11,71	3,31

Table 1. Root length, shoot water content and essential oil of citronella plants submitted to different doses of lead sulphate

Different lower case letters in the same column indicate significant difference at 5% level by Tukey test.

were increased, the oil concentrations remained at the same level, in contrast to the lower oil concentration provided by the treatment without lead dose. Possibly the stress caused by lead has generated a biochemical reaction that influenced the increase of essential oil production, being a field of future studies regarding the use of citronella as a phytoremediator. In addition, it is important to highlight that further studies are needed on the quality of this essential oil to ensure that the presence of lead in the soil and when extracted by the plant has not impaired its active principles. Considering that to construct the mathematical model that expresses the relationship between the variable responses analyzed for root length, water content and essential oil, quadratic regression was used (Cruz, 2006), the maximum point of production of essential oil based In the measurement of lead sulphate by the data estimation technique would be a dose of 10.20mg reaching a production of 43.37%. Measurement of the coefficient of determination (R2) with a value of 0.88 and Pearson's correlation technique (Cruz, 2006) gave a correction value of 0.93, indicating a correlation of approximately 93% between the variable response is indicative of a high probability that the production of essential oil in the plant increased due to the presence of lead metal sulphate in the soil.

The absorption capacity of the metal by the plant and the biomass production are the main factors for the extraction efficiency (Caille et al., 2005). In the soil, the heavy metals tend to accumulate in the upper layers, the agricultural ones, due to their low mobility (Sumiahadi and Acar, 2018). Root development is a basic requirement for plant evaluation to be considered a soil phytoremediator (Mertens et al., 2005), being a characteristic presented by citronella plants, as can be observed in Table 1. Zancheta et al. (2011) report that the high retention of metals in the roots is indicative of plant tolerance to cultivation in soils with excess of these elements. According to studies developed by Marsola et al. (2005), cultivating Phaseolus vulgaris L. in soils contaminated by heavy metals, these authors found that the large difference in the concentrations of these elements in the root is due to the mechanism of reduction of the diffusion of cation inside the tissue, being a feature that protects the vegetable from intoxication. As shown in Table 1, there was no significant difference in root length and water content present in the plant when it is cultivated in soils containing different doses of lead, indicating that it is a plant with high potential to be used in the system phytoremediation to remove contaminant lead from the soil. In addition, in the presence of lead the plant obtained a significant increase in the content of essential oil produced, which is used by the chemical industries to develop cosmetics and repellents (Andrade et al. 2012; Rocha et al. 2012). Therefore, in addition to improving soil conditions by extracting lead, the phytoremediation process provided increased essential oil production. The increase in water and essential oil retention by the plant by increasing the lead concentration is explained due to root growth, according to Camargo and Alleoni (2006) plants with larger root system can exploit larger soil area and consequently absorb greater amount of water and nutrients. Treatments that received increasing doses of PbSO₄ contamination provided an increase in leaf essential oil production compared to the control, the increase was 18.2%, 20.1% and 20.6% respectively. Similar results were obtained by Brandão *et al.* (2016) in which working with Chapada Rosemary using increased lead dosages (3.19 to 3.22 mg/kg⁻¹), the presence of the metal did not cause a statistically significant reduction in essential oil production.

Conclusion

It can be concluded from this research that the citronella medicinal plant is an efficient option to be used as a phytoremediator in soils contaminated with heavy metals such as lead. However, further studies are needed to evaluate the quality of the essential oil produced in order to determine whether or not contamination has occurred through the absorption of lead by the plant.

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