

Possibility of phytotoxicity of sewage treatment stations in the germination of lettuce seed (*Lactuca sativa*)

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ABSTRACT

The final treatment and disposal of sludge generated at sewage treatment plants has become a crucial step in the waste management process. The present work carried out an evaluation of the sludge toxicity after dehydration and treatment with vermicomposting, through an ecotoxicity test using *Lactuca sativa* as bio-indicator. The percentage of relative germination and relative growth inhibition of the primary roots was determined for 5 dilutions obtained from the sludge sample. The results of the bioassay with *Lactuca sativa* seed showed that the treatment with vermicomposting interfered in the germination rate, increasing the same the relative growth of the roots. This way, corroborates not to indicate the disposal of this residue directly in the soil without any type of treatment

Keywords: Sewage, Sludge, Germination

Introduction

The current challenge for the gestors of Pernambuco municipalities is the search for alternatives to revert the situation of final arrangement of solid residues. The integrated management of solid residues is a very delicate discussion, mainly because it wraps serious environmental problems (LIRA et al, 2017).

The sludge generated in Waste Water Treatment Plants, WWTP, is a resultant material of processes of primary and secondary treatment and highly complex regarding to composition and classified according to NBR 10004 (2004) as solid waste. Any treatment whereof this residue is submitted is necessary to adjust to standards disposal rates for the current law, removing or reducing the present substance concentrations that could cause impact to the environment. The ones subjected to biological pre-treatments present high concentration of microorganisms, organic substance and minerals (RIGO et al., 2014).

The viability for soil disposal of this type of sludge is possible, when the same it is submitted to treatments. Although prohibited, the sludge

disposal on the WWTP surrounding area is still a common practice. The use in agriculture, as bio-fertilizer and ground conditioning, allows profits, through the increase in productivity of the cultures and reduction in mineral fertilizer use. But, there is a pathogen and phytotoxic substance contamination risks which limits an indiscriminate and not regulated use to prevent soil pollution, plant toxicity and even the risk related to the public health. (RIGO et al., 2014).

The presence of metal, as well as elevated concentrations of solids, turbidity and COD, in these sludge can induce the toxicity of organisms and increase the environment degradation, considering that these factors can cause undesirable conditions, as it will lead to alterations on the soil chemical composition, making interference in the natural biological phenomena of the macro and microbiota (ANDRADE et al, 2014).

The toxicity can be defined as harmful results on living organisms provoked by chemical substances and the organism self-substances. The indication of toxic agents, by means of toxicity or eco-toxicity tests, called of bioassays, aims to determine the effect caused by these agents; one or more

substances or environmental factors, taking in consideration the time of exposition, the concentration and the adverse effects of pollutants on the biological communities. There of, the ambient contamination caused by various sources can be evaluated by the ecotoxicity tests (CUNHA, 2011).

The plants are Eukaryote organisms, photosynthesizes and of complex metabolism. In the germination phase, the seeds will pass for sufficiently intense physiological changes, and will be very sensible to any factor of stress ambient. Plant seeds are widely used in several types of toxicity bioassays. Sensible plants to toxic substances can be used as indicator of environmental quality, consisting phytotoxic bioassays (CUNHA, 2011). The *Lactuca sativa*, due to its sensitivity, has been widely used in phytotoxic tests (PEREIRA et al., 2013)

The objective of this research was to evaluate the possibility of WWTP sludge phytotoxic, before and after treatment through the vermicomposting, as for the germinating characteristics and primary root growth characteristics of the bio-indicated lettuce seeds (*Lactuca sativa*).

Material and Methods

The sludge used in this work had been proceeding from Mangueira WWTP, located at Mangueira neighborhood, Metropolitan Region of Recife, projected approximately to take care of to the population of Mangueira, San Martim and adjacencies neighborhood, with approximately 18,000 inhabitants. The treatment system is composed of an elevatory station, a Parshall gutter, a grating of bars, two sandboxes, an UASB reactor UASB, a stabilization lagoon and eight stream beds for drying (Figure 1).

Figure 1 - Mangueira WWTP UASB Reactor and stabilization lagoon.



Source:

<http://www.meioambiente.biz/saneamento/saneamento-ambiental>. Accessed in 12/10/2015

Dehydrated sludge was used in an assembled laboratory system with geotextile tubular devices (bag), model provided by TenCate Geotube, the GT 500 (pore average diameter = 80 μ (ASTM D6767) and dimensions 53 x 51cm). After drain, the sludge

was treated with vermicomposting, using the *Eisenia andrei* worms.

Before the toxicity bioassay completion, residue solubilization was carried out following the methodology described in NBR 10006 (ABNT, 2004). 20g of the sludge before and after treatment, dissolving it in 100 ml of distilled water, in an Erlenmeyer of 250 ml, maintaining the proportion 1:5 (m/v). The mixture was agitated on an agitating table, for 24 hours, and consolidated for 7 days in covered bottle. After this period, 50 mL was removed from the supernatant, called elutriate. From this solution, elutriate to 100 % (mother solution), dilution was prepared with 75 %, 50 %, 25 % and 10 %. Also a solution was used as control with distilled water.

The toxicity test with *Lactuca* was carried out according to the methodologies described by Tam and Tiquia (1994), Dutka (1989) and Andrade (2009), with the proper adaptations. There were used Petri dishes of 9 (nine) cm of diameter, where was arranged a paper leaf of qualitative filter with equal dimension to cover the bottom of each plate. With a Pasteur pipette the filter paper was moistened, with around 4 mL of the solution-test, without leaving a visible liquid excess. In each petri dish, was arranged 10 seeds of lettuce (*Lactuca sativa*), uniformly distributed, using laboratory tweezers. The *Lactuca sativa* seeds used in the test presented 98 % of germination percentage (supplied by the manufacturer). After the seed inoculation, the plates were closed with plastic film paper, to avoid elutriate evaporation and subsequently with aluminum paper, to preserve the absence of light, in a BOD chamber, maintained in an incubator on 25 ± 1 °C for 120 horas.

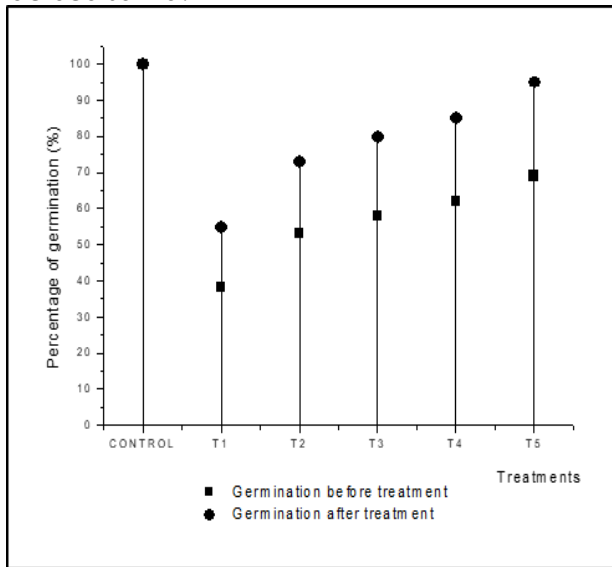
After the incubation period, 120 hours, the proceeding of counting of the germinated seeds was carried out. The bioassays were brought into effect at the Process and Environmental Technologies Group Laboratory – GPTA/DEQ/UFPE, with 6 repetitions; before and after the vermicomposting treatment. The bioassay evaluation was in function of Relative Germination (RG %) and the Relative Root Growth, as Bellato et al. (2015). Where: $N^{\circ}SGa$ was the number of germinated seeds in the sample; $N^{\circ}SGc$, the number of germinated seeds in the control; $MCRa$, the average root growth in the sample and $MCRc$,

Results and Discussion

The test not parametric of Mann-Whitney used for comparison of two independent groups was used to test if the treatment with vermicomposting obtained positive effect on the seed germination and the root growth of the *Lactuca sativa*. The results demonstrated significant differences ($p < 0,05$), indicating that the treatment with vermicomposting had influenced on the seed germination and in the root growth.

The figure 2 shows an increase of 20 % in the RG after the treatment, indicating its influence on seed germination. Also it is possible to observe that the less of the sludge aliquot, observed in the biggest dilution of elutriate, bigger the germination effect. After 50 % of elutriate concentration there was a bigger precision in the germination rates, shown up by the least amplitude of the results. The biggest differences are in the treatments with 100 % and 75 % of elutriate. The concentrations of 25 and 10 % presented the best similar results.

Figure 2 - Percentage of germination of the seeds of *Lactuca sativa*.

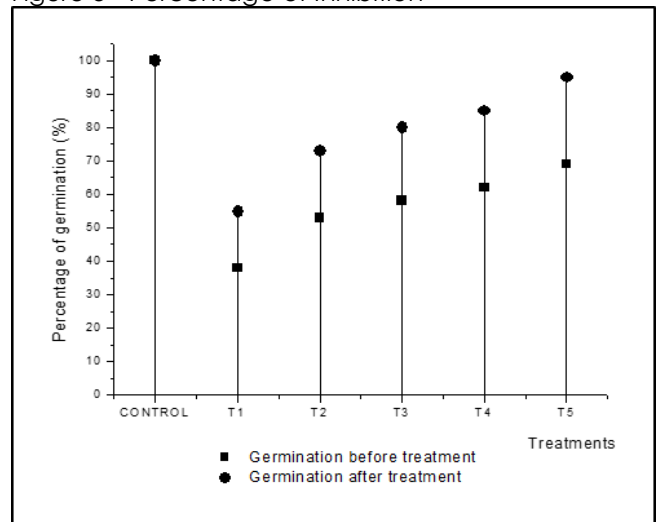


Source: Author, 2016.

When we compare the elutriate concentrations, we can realize that before the treatment, the germination values with 100 %, 75 %, 50 %, 25 % and 10 % of elutriate, were between 30 and 70 %. In the case after the treatment it was seen that the increase of germination levels with 100 %, 75 %, 50 %, 25 % and 10 % of elutriate were between 50 and 95 %.

In the same way, the germination inhibition takes place in the most significant form in the smallest elutriates dilution and before the treatment of the sludge, like displayed the figure 3. The less the sludge concentrations in the elutriate, the more the results were improved in terms of germination and inhibition, being able to indicate that the sludge from WWTP, due to its composition characteristics can cause a condition of stress.

Figure 3 - Percentage of Inhibition

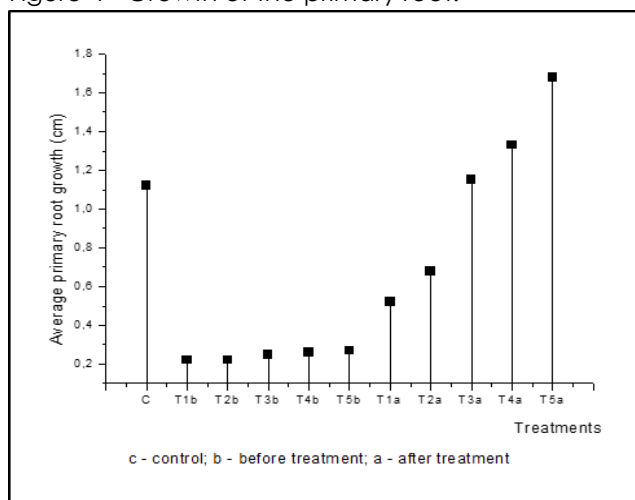


Source: Author, 2016.

In A similar way, Pereira et al. (2013) studying phytotoxic effects of Pb, both in the germination, and in the initial growth of lettuce root, realized alterations in the standards of cellular division, inducing the incident of abnormalities in the germination. This affirmative cannot be done for study regarding a specific component; but the reduction in germination, can be contributed for a synergetic effect of the sludge. The improvement of the sludge characteristics, in terms of organic matter, nutritious and possible metal reduction, conditions proposed in the vermicomposting treatment (TAVARES, 2016), made influence increasing the germination taxes, as seen in the presented results, where was observed a decrease in the germination percentage of lettuce inversely proportional to the sludge concentration. The seed permeability to contaminants can be related to the seed tegument. In this form, the germination was affected with high sludge concentrations, affecting the embryo and, consequently, reducing proportionally the germination percentage.

When we observe the root growth with seeds germinated before the treatment for vermicomposting had discreet growth, when compared with the growth in the control, varying from 0,22 to 0,27 cm, whereas in the solution control was of 1,12 cm. However, after the vermicomposting treatment, the observed growth was from 0,52 to 1,68 cm. In the treatments T3, T4 and T5, elutriate with 50, 25 and 10 % of sludge, respectively, was observed a growth of 3, 19 and 50 % superior to the growth observed in the control solution (Figure 4).

Figure 4 - Growth of the primary root.



Source: Author, 2016

Also it is perceived that the extracts tested in this study had shown a negative relation with initial growth of the seed of the lettuce, when the sludge without treatment was used. All the tested dilutions had inhibitions in the seedlings, when compared with the control treatment.

Conclusions

With this work it was intending to study if the WWTP sludge exerts negative influence, associated to any toxicity to the germination of species, using here the seed of lettuce. The results showed to have influence of the treatment with vermicomposting in the germination rate, than in spite of having been affected, was verified that the root initial growth suffered more accented effects for inhibition, when the sludge was used before the treatment, as for superior growth to the control with the sludge after being treated with vermicomposting.

Thereof, through these studies it was possible to prove that the WWTP sludge when treated with vermicomposting presented significant improvement for the root germination and growth of *Lactuca sativa* seed.

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