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GERMINATION AND DEVELOPMENT OF AMERICAN LETTUCE SEEDLINGS (*Lactuca sativa* L.) IRRIGATED WITH HOME AND INDUSTRIAL EFFLUENT-RECEIVING WATER

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1 ABSTRACT

This work has evaluated germination and formation of American lettuce seedlings (Lactuca sativa L.) "Tainá" variety, irrigated with urban effluent-receiving water from Lavapés Creek, a water body which receives all home and industrial sewage of the city of Botucatu, SP (Brazil). The sowing was carried out in 12 plastic foam trays with 32 germination cells each. At 41 days after planting (41-DAP), the seed germination percentage, seedling final size, final number of leaves, the mass of shoot and root dry matter were evaluated. Also, two sorts of substrates were investigated: Multiplantâ and Plug Mixâ. No complementary fertilization was done, so the only available nutrients were the ones originally present in the substrates and in the creek water. For compararison an evaluation was performed using a source of treated water instead of the creek water. Results pointed out that all sown seeds presented 100% of germination. Treatments with Multiplantâ presented the greatest values for plant height. Treatments irrigated with Lavapés Creek water presented a greater number of leaves at 41-DAP.

KEYWORDS: Seedling germination, *Lactuca sativa* L., effluent receiving water.

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2 RESUMO

O trabalho avaliou a germinação e a formação de mudas de alface americana (*Lactuca sativa* L.) variedade "Tainá", irrigada com águas receptoras de efluentes urbanos do Ribeirão Lavapés, curso d'água que recebe todo o esgoto doméstico e industrial da cidade de Botucatu – SP (Brasil). A semeadura foi realizada em 12 bandejas de isopor com 32 células de germinação cada. Foram analisados com 41 dias após o plantio (41-DAP), a porcentagem de germinação das sementes, o tamanho final das mudas, o número final de folhas, a massa de matéria seca da parte aérea e de raízes. Também foram estudados dois tipos de substrato: Multiplant® e Plug Mix®. Não foi utilizada nenhuma

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adubação complementar, estando disponível apenas os nutrientes presentes originalmente nos substratos e na água do ribeirão. Para efeito comparativo, realizou-se a mesma avaliação utilizando uma fonte de água tratada. Os resultados indicaram que todas as células semeadas apresentaram 100% de germinação. Os tratamentos que utilizaram o Multiplant® apresentaram maiores valores de altura de plantas e os irrigados com a água do Ribeirão Lavapés, apresentaram maior número de folhas com 41-DAP.

UNITERMOS: Germinação de mudas, *Lactuca sativa* L., águas receptoras de efluentes.

3 INTRODUCTION

The re-use of sludge-receiving water in the irrigation of crops has been enhancing in recent years due to several technological and environmental factors. The controlled disposal of effluents in soil, instead of applying them in an aquatic ecosystem favors its microbial stabilization, adsorption, immobilization of metals and dissolved salts, nutrient recovery by crops or plant cover leading to an acceptable assimilation of this liquid residue (DE LUCA, 1999).

The development of researches, which facilitate to understand the resulting from the use of this water in agricultural areas, became indispensable. To study better and more efficient forms of application, to evaluate the behavior of equipments submitted to those conditions and mainly to verify the quality of the final product, which will be consumed by man.

Brazil offers outstanding favorable conditions in re-using of sludge by disposal in soil, both for the availability of area and suitable climatic conditions, among other factors (CAMPOS et al., 1999). As in the country, religious aspects do not present any cultural drawback to the use of wastewater in farming, the reuse has insured social acceptance (KÖNIG, 1999).

It is not possible to remove all phosphates and nutrients which are responsible for eutrophication of water bodies by the conventional treatment methods, all this material is wasted, which is a great wastage, there is a growing need for the application of chemical fertilizers in soil, aiming to assure crop yields (CHATEAUBRIAND et al., 1988).

In Botucatu city - SP, Brazil, Souza (1997), used the water from Ribeirão Lavapés (Lavapés Creek) to irrigate Americana lettuce crop (*Lactuca sativa* L.), cultivar "Elisa lisa", under four regimes of water application. It follows that the possibility of wastewater use may still benefits to users, as an example, less use of inputs, chiefly fertilizers without any damage to yield and quality of the produces obtained.

Conte (1992), collected 112 samples of water from Ribeirão Lavapés (Lavapés Creek) and submitted them to the chemical analysis for determining inorganic chemical species in the dissolved and total forms. Nitrogen concentrations in the forms of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were found. Also concentrations of chlorine as Cl^-, sulfur as SO_4^{2-} and the following elements in the total form: phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), boron (B), zinc (Zn), iron (Fe), copper (Cu), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), silicon (Si), aluminum (Al), barium (Ba), sodium (Na) and mercury (Hg) were found.

Fonseca et al. (2001), characterized the disposal of sludge in the soil as being the alternative of treatment of liquid residues with low implantation and operational costs. The authors comment that in addition to the final disposal of generated effluents, the planned use of wastewater in agriculture is an alternative to control of pollution of water bodies, it makes water and fertilizers available for the crops, recycling of nutrients and increased agricultural yield.

The advantages of wastewater irrigation are water and fertilizer savings recycling of nutrients and pollution control. It is known that the contents of macro and micro nutrients existing in the sludge are capable to supply the needs of most crops, but according to Bouwer and Idelovitch (1987), Evans et al. (1991), an increase of 200 à 400 mg L^{-1} of salts and 300 mg L^{-1} of dissolved inorganic solids may occur in the home use of water.

According to Oron et al. (1992), the localized fertirrigation with wastewater presents a huge potential in putting together several attractions of agronomic order, reducing the contamination risks of the crops. Due to the high contents of suspending solids, chemical precipitation, accumulation of inorganic particles in the interior of the tubing and drippers, alga formation, etc., there may be, at first, the additional risk of irrigation equipment clogging (TAYLOR et al., 1995).

The governments have an important role in wastewater irrigation, designing plans and programs intended for such end. In Peru, the National Program of Irrigation with wastewater foresees the establishment by steps of 18,000 ha of irrigated area. Nevertheless, out of the about 4,300 ha today irrigated, 70% are used for vegetable cultivation, in most with untreated wastewater. In Israel, around 2010, the treated sewages will account for almost 30% of all the water made available for farming (SHELEF, 1991).

An issue is of public domain, the use of sewages for irrigation surely involves health risks. What becomes a factor of discussion is the definition of acceptable risks, that is, for quality standards and levels of treatments required to warrant sanitary security (BASTOS, 1999).

According to Yuri (2000) lettuce crop belongs to the family Asteraceae, being a very delicate herbaceous plant with, non-branched minute stems, which the leaves are attached. These are large, smooth or curly, closing or not "head'' shaped. Their coloration ranges from yellowish green to dark green. The root system is of the tap-root type, with fine and short branchings which can reach 3 to 15 cm in depth during the vegetative phase (MAROVELLI et al., 1989).

Lettuce crop presents some 95.80% of water, 2.30% of carbon hydrates, 1.20% of proteins, 0.20% of fats, 0.50% of mineral salts (13.30 mg of potassium, 147 mg of phosphorus, 133 mg of calcium and 3.85 mg of sodium, iron and magnesium), 245 UI of vitamin A, 0.31 mg of vitamin B1, 0.66 mg of vitamin B2 and 35 mg of vitamin C (GOMES, 2001). According to the author, after the lettuce crop harvests the average weight of lettuce heads among the farmers of the south of Minas Gerais (Brazil) is between 500 and 600 g.

Nitrogen is the nutrient which promotes the greatest increase in the yield and weight of the lettuce. The phosphorus – nitrogen interaction is significant, denoting that its use increases yield relative to the summation of the effects of any of them singly (COUTO & BRANCO, 1963). Nitrogen deficiency in lettuce delays plant growth and induces either absence or head misshape, the oldest leaves become yellow and get loose easily. On the other hand, when applied in excess in topdressing fertilization in the last third of the cycle, the head-forming cultivars show less firmness, which will be harmful to commercialization.

Since the lettuce is made up basically of leaves, the crop responds more to the furnishing of nitrogen, a nutrient which requires a special management as to fertilization because it is easily leached and the crop absorbs some 80% of the total extracted in the last four weeks of the cycle (GARCIA et al., 1982).

4 MATERIAL AND METHODS

The research was conducted from December 9, 2002 to January 21, 2003, in a glasshouse located on an experimental area (22° 55' S, 48° 26' W), at the State University of São Paulo, College of Agronomic Sciences, FCA-UNESP, Campus of Botucatu - SP (Brazil). The climate is classified according to the Köppen system as Cwa: hot climate (mesotermic), with rains in the summer and drought in the winter, the average temperature of the hottest month being over 22° C. Rainfall inferior to 66.8 mm yearly is between 18.6 to 22.4 ºC (data based on the mean valves of 30 years) (CUNHA et al., 1999).

In the experiment was used the American lettuce crop (*Lactuca sativa* L.) variety Tainá. Pelletized seeds were used, sowing only one seed per cell.

The percentage of germination of seeds, the final size of seedlings, the final number of leaves, the mass of dry matter of the shoot and root and chemical alterations of the substrates were analyzed 41 days after planting (41 – DAP). The trays were irrigated with a watering-pail with a sieve, the water from the Ribeirão Lavapés (Lavapés Creek) being collected daily and at the moment of irrigation, which was performed in two periods (morning and afternoon). There was no restriction in the amount of water applied.

The substrates used were Multiplant® (60% of pinus bark compound, 15% of vermiculite and 25% of humus and vegetable earth) and Plug Mix® (100% ground coconut bark). No additional fertilization was used.

For determining the nutrient contents present in the substrates, chemical analysis was performed in two moments: in the early of the trial (before sowing); and at the final of the trial (after harvesting). This analysis was done by the Laboratório de Análise de Fertilizantes e Corretivos do Departamento de Recursos Naturais/Ciência do Solo, (Laboratory of Analysis of Fertilizers and Amendments of the Natural Resources/ Soil Science) according to the Ministry of Agriculture methodology (BRASIL, 1988)

4.1 Experimental Design

The experimental design was randomized, and consisted of four treatments with three replications, distributed in 12 trays with 32 germination cells each (four columns and eight rows), only the two columns and five internal rows being evaluated, and the rest regarded as a border bed. Bellow are described the proposed treatments.

Treatment 1 (T1) → It is concerned with the trays utilizing Multiplant® as a substrate and was irrigated with water from the Ribeirão Lavapés (Lavapés Creek).

Treatment 2 (T2) → It is concerned with the trays using Plug Mix® as a substrate and was irrigated with water from the Ribeirão Lavapés (Lavapés Creek).

Treatment 3 (T3) → It is concerned with the trays using Plug Mix® as a substrate and was irrigated with water treated from SABESP (Water Treatment Company of São Paulo State).

Treatment 4 (T4) → It is concerned with the trays using Multiplant® as a substrate and was irrigated with water treated from SABESP (Water Treatment Company of São Paulo State).

Figure 1 presents the distribution of the treatments (T) within the glasshouse and in detail a tray and its germination cells (four columns and eight rows).

4.2 The Ribeirão Lavapés (Lavapés Creek)

The hydrological basin including this creek occupies an area of 41 km^2, taking into account the existing at the College of Agronomic Sciences, Botucatu-SP (Brazil). Campos (1997) situates geographically the basin of the Ribeirão Lavapés (Lavapés Creek) between the coordinates 48^0 20' to 48^0 22' of longitude W and 22^0 42' to 22^0 56' of latitude S, presenting a territorial area of about 10,670 hectares, with a distribution of 47.5 % of urban area, 7.4 % by different crops, 7.3 % by natural forests or reforestation, 37.7 % by fields and 0.1 % by other types of covers (LEOPOLDO, 1989). It presents a drainage density of 1.36 Km^2, average slope of 11 % and average altitude of 825 meters.

The Ribeirão Lavapés (Lavapés Creek) is the chief water body of the basin in issue, having 11 km length, cutting the town of Botucatu southwestwards to northeastwards. The average discharge of the Lavapés basin was estimated in 0.7 $m^3.s^{-1}$, corresponding to a daily production of 60,480 m^3 (CONTE, 1992). This water body receives the home and industrial sewages, without any treatment from Botucatu city, in addition to the wastes of two local tanneries, which prevents the immediate use of its water.

Two sorts of analyses in the water samples of the Ribeirão Lavapés (Lavapés Creek) were conducted: one to determine the presence of heavy metals and the other for determining macro and micronutrients, pH and water conductivity.

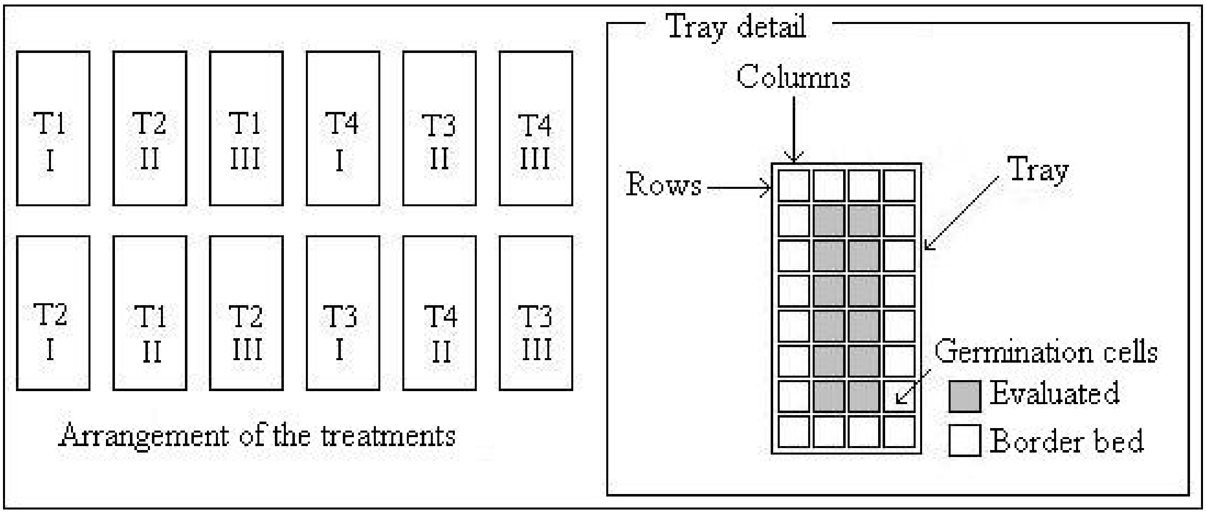

Figure 1. Arrangement of the treatments (T) and tray detail.

5 RESULTS AND DISCUSSION

5.1 Chemical analysis from the Ribeirão Lavapés (Lavapés Creek) water

The chemical analyses of the water from Ribeirão Lavapés (Lavapés Creek) (Table 1) showed that all elements detected lie bellow the maximum concentration allowed, being able to be used for irrigation, according to the FAO Maximum Recommendation of Concentration of Traces Elements permitted for Irrigation (VAN DER LEEDEN et al., 1990).

The determination of nutrients in the water from Ribeirão Lavapés (Lavapés Creek), presented in Table 2, showed a high concentration of N and amounts of P, K, Ca, Mg, Cu, Fe, Mn and Zn, which probably contributed to the development of seedlings, mainly, in the nutrient-poorest substrate, o Plug Mix®.

Table 1. Results of the analysis of the presence of heavy metals and other chemical elements in the water from Ribeirão Lavapés (Lavapés Creek).

Element	mg dm^{-3}	Element	mg dm^{-3}
Aluminum	n.f.	Molybdenum	n.f.
Arsenic	n.f.	Selenium	n.f.
Boron	0,075	Nickel	n.f.
Barium	0,037	Lead	0,015
Cadmium	n.f	Silicium	1,558
Cobalt	0,004	Tin	n.f.
Chromium	n.f.	Vanadium	0,062
Mercury	n.f.	Lithium	0,012

(n.f.) Not found in the water sample analyzed.

It is important to stress that due to the Ribeirão Lavapés.(Lavapés Creek) receives both the non-treated home and industrial sewages from Botucatu city, there is a great variability in the concentration of the nutrients on the different days of week, and even along the day.

Table 2. Result of the analysis of macro and micronutrients, pH and conductivity of the water form Ribeirão Lavapés.(Lavapés Creek)

Results in mg kg^{-1}									
N	P	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
70.00	12.00	5.53	3.62	1.98	31.20	0.03	0.45	0.12	0.01
pH = 6.20					Conductivity = 176 μS				

5.2 Seed Germination

All the cells sown contained seedlings, therefore, the seeds' germination process was not affected by the treatments, concluding that there was no interference of the substrate, neither of the water used in the experiment.

5.3 Shoot height (S.H.) and number of leaves (N.L.)

According variance analysis of plant shoot height results (Table 3), it was found that the treatments significantly differed from each other, excepting T4 (Multiplant® / treated water of SABESP) and T2 (Plug Mix® / water of the Ribeirão Lavapés). Treatment T1 (Multiplant® / water of the Ribeirão Lavapés-Lavapés Creek) presented the higher values in shoot height as compared to the others. Treatment T3 (Plug Mix® / treated water of SABESP) presented the worst results.

The treatments which used Multiplant® as a substrate showed the greater values of height of plants at 41-DAP. This substrate on the contrary of the ground coconut bark fiber (Plug Mix®) showed in its composition a higher content of some nutrients, and when summed to the nutrients present in the Ribeirão Lavapés (Lavapés Creek) water, as in treatment T1, afforded better conditions for the development of seedlings. In spite of possessing very low concentrations of nutrients, Plug Mix® together with receiving waters (T2) did not differ significantly from the use of Multiplant® along with treated water of SABESP (T4), which makes its use viable.

Table 3. Variance analysis of the data of shoot height of the plant. (SH).

	Shoot Height (mm)
T1	32,4 a
T4	24,8 b
T2	24,5 b
T3	9,8 c
CV	40,06 %
F	160,24

Means followed by distinct letters in the columns differ significantly by the Tukey test at 1% of probability.

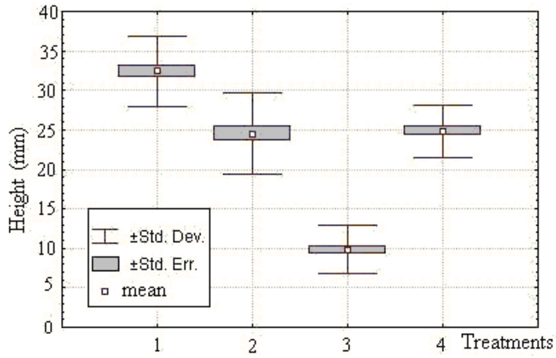

Figure 2. Standard deviation and mean values of SH.

Figure 2 presents the box-and-whisker plot of the values of the standard deviation and mean of SH.

Regarding the N.L of the plant, the variance analysis (Table 4), points to that all the treatments differed significantly from each other. Treatments T1 and T2, which were irrigated with the water from Ribeirão Lavapés (Lavapés Creek), showed a greater number of leaves at 41-DAP, followed of T4. Al though being significantly different, low differences were found between treatments T2 and T4.

Table 4. Variance analysis of the data of the number of leaves (N.L.).

	Number of leaves
T1	5,37 a
T2	4,87 b
T4	4,17 c
T3	2,50 d
CV	28,70 %
F	159,68

Means followed by distinct letters in the columns differ significantly by the Tukey test at 1% of probability

Anew, treatment T3 presented the worst results, then, being culled in seedling production only with the use of treated water, unless an additional fertilization be done. The inconvenience of working with untreated urban and industrial effluent- receiving waters is the high variability of nutrient concentrations.

It corroborates the fact that, to Plug Mix® along with the nutrients present in a source of water contaminated by untreated effluents may surely replace the use of commercial products, in addition to possessing a quite lower cost, it becomes the local solutions where the main water source (river, brook, etc) is polluted with the evacuation of home and industrial sewages.

Figure 3 presents the box-and-whisker plots of standard deviation, standard error and mean values of the NL.

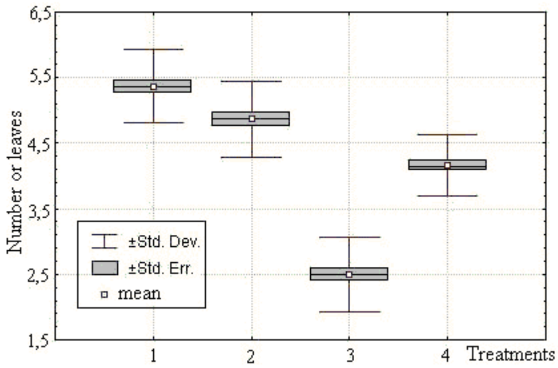

Figure 3. Standard deviation, standard error and mean values of the NL.

For being concerned with seedlings which still would be transplanted and cultivated in other conditions, it was not necessary to perform the microbiological and parasitological analyses. Biscaro (2003), studied the cultivation of the American lettuce (*Lactuca sativa* L.), variety "Tainá", irrigated also with the water from Ribeirão Lavapés (Lavapés Creek) and found that the lettuce plants collected after 24 hours from the last irrigation presented a mean of 2.84E+01 NMP ml^{-1} (Most probable Number of fecal coliforms per ml of sample), and that the lettuce plants collected after 48 hours form the last irrigation did not present any fecal coliforms. Also according to the author, *Salmonela* spp was not found in any of the samples analyzed.

The variance analyses of the data of dry matter of the shoot and roots are presented in Tables 5 and 6, respectively. The results showed that treatments proposed differed significantly from each another, with the exception of T4 and T2. Treatment T1 presented the greater values of weight of shoot and weight of mass of roots. Treatment T3 presented the worst results.

Table 5. Variance analysis of the data of mass of dry matter of the shoot.

	Dry matter of the shoot (g)
T1	0,91 a
T2	0,56 b
T4	0,45 bc
T3	0,03 d
CV	68,62 %
F	73,39

Means followed by distinct letters in the columns differ significantly by the Tukey test at 1% of probability

Table 6. Variance analysis of the data of mass of dray matter of roots.

	Dry matter of Roots (g)
T1	0.61 a
T4	0.3 b
T2	0.37 bc
T3	0.05 d
CV	58.82 %
F	95.27

. Means followed by distinct letters in the columns differ significantly by the Tukey test at 1% of probability

5.4 Chemical analyses of the substrates

Tables 7 and 8 show the results of the chemical analyses of macro and micronutrients of the substrates (Multiplant® and Plug Mix®) before starting the experiment, and the 41-DAP for each treatment (T). The chemical analysis of the substrates at the start of the experiment shows that the concentration of nutrients at both substrates is low for the development of seedlings, but Multiplant® presents initial amounts of N, P and Mg higher than Plug Mix®. This small difference may have influenced positively the early development of the seedlings in the treatments with Multiplant.®

In general, the concentrations of N, MO, C, Ca, Fe and Zn increased in the substrates along the experiment, whereas the amounts of P, K, S, Cu, Mn and Na decreased in all treatments. This alteration may not be significant in some cases, since the increase or decrease of nutrients in the substrates occurred in a similar way in the treatments which used water from Ribeirão Lavapés (Lavapés Creek) and in the ones which used SABESP treated water

Table 7. Results of the chemical analyses of macronutrients in the substrates.

	Result in percentage in the dry matter							
	N	P_2O_5	K_2O	MO	C	Ca	Mg	S
Multiplant®	1,05	0,74	0,26	56,00	31,00	0,61	1,40	0,51
Plug Mix®	0,66	0,40	1,32	81,00	45,00	0,78	0,36	0,53
T1	1,19	0,35	0,05	75,67	42,04	0,83	1,50	0,18
T2	0,82	0,08	0,09	86,67	48,15	1,19	0,39	0,06
T3	0,81	0,05	0,11	88,33	49,07	1,13	0,36	0,05
T4	1,24	0,43	0,08	77,67	43,15	0,85	1,52	0,20

Table 8. Results of the chemical analyses of micronutrients in the substrates.

	Result in mg Kg^{-1} of dry matter						
	Fe	Cu	Mn	Na	Zn	pH	C/N
Multiplant®	7600,00	32,00	200,00	220,00	176,00	4,20	29 / 1
Plug Mix®	3800,00	280,00	170,00	500,00	326,00	5,40	68 / 1
T1	8616,67	51,33	134,00	233,33	311,33	4,27	36 / 1
T2	4783,33	151,33	145,33	580,00	377,33	6,50	59 / 1
T3	4733,33	133,33	129,33	146,67	365,33	6,40	63 / 1
T4	9483,33	48,67	140,67	80,00	324,67	4,30	35 / 1

The nutrients for the development of the lettuce seedlings may have been furnished almost wholly by the irrigation water. Probably, the amount of P present at first in the substrate Multiplant®promoted the difference at the development of the seedlings relatives to the other treatments, since besides the amount of P in the water, the content of that nutrient in the substrate reduced greatly along the experiment in all treatments.

The K and Na nutrients presented a high reduction from the initial to the final condition, this fact may be related to the ease which they are leached from the substrates by irrigation.

Figures 4 and 5 present the accumulation of macro and micronutrients in the substrates of the treatments T1 and T4

Figures 6 and 7 present the accumulation of macro and micronutrients in the substrates of the treatments T2 and T3

The substrate Plug Mix® irrigated with treated water from SABESP (T3) proved unsuitable for seedling production, as in the cases studied, if addition of a complement nutritional were not accomplished. The advantage of the use of urban and industrial effluents receiving water as a source of water and nutrients in the substrate is that this complement may be dispensed with, or at least, reducing the amount, promoting fertilizer saving.

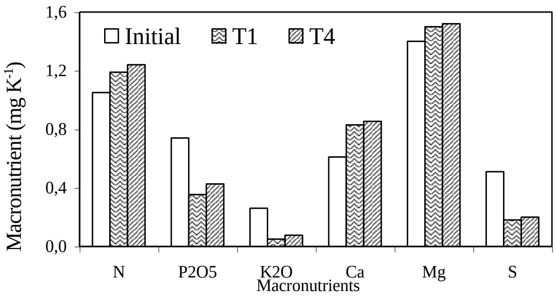

Figure 4. Accumulation of macronutrients in the substrates of the treatments T1 and T4.

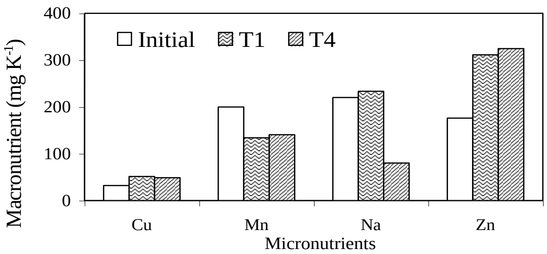

Figure 5. Accumulation of micronutrients in the substrates of the treatments T1 and T4.

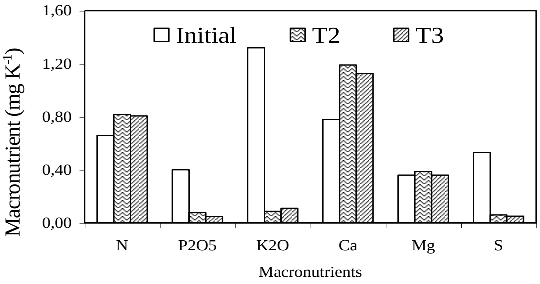

Figure 6. Accumulation of macronutrients in the substrates of the treatments T2 and T3.

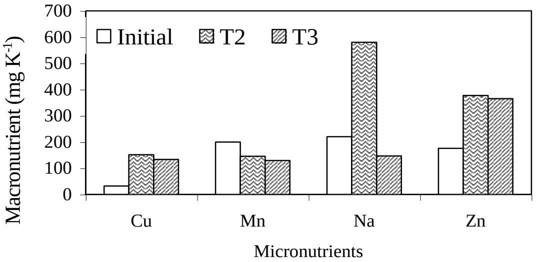

Figure 7. Accumulation of micronutrients in the substrates of the treatments T2 and T3.

The use of ground coconut bark fiber as a substrate for vegetable seedlings is a viable alternative to the use of that material which is treated as an industry waste, and may be purchased with a lesser cost than a commercial substrate.

6 CONCLUSIONS

The germination of lettuce seeds was not affected by the treatments. Treatments which utilized Multiplant® as a substrate presented increased values of plant height at 41 DAP.

In spite of possessing lower concentrations of nutrients, Plug Mix® used together with home and industrial effluents receiving water (T2) did not differ significantly from the use of Multiplant® together with treated water from SABESP (T4), in relation to plant height, which makes its use viable.

The substrate Plug Mix® irrigated with treated water from SABESP (T3) proved unsuitable for seedling production if, as in the cases studied, if addition of a complement nutritional were not accomplished.

Treatments T1 and T2, which were irrigated with the water from Ribeirão Lavapés, presented a greater number of leaves at 41 DAP, followed by T4. Treatment T3 presented a lower number of leaves.

Treatment T1 showed the best result in the accumulation of dry matter of the shoot and roots, followed by T2 and T4, accumulation of shoot dry matter, which took turns accumulation of root dry matter. Treatment T3 presented in all analyses conducted in this experiment, the worst results.

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