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Effect of the irrigation with residual wastewaters on microbial soil activity of the ornamental flowers (*Dahlia pinnata*) cultures monitored by isothermal calorimetry

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Abstract A microcalorimetric method was applied to study microbial soil activity of ornamental flower (*Dahlia pinnata)* plantations when irrigated with potable water and wastewaters. The samples were irrigated with potable water PW sample (reference) and treated wastewaters from Municipal Wastewater Treatment Station of Asa Norte in Brasilia City (Brazil). Three different water treatments were applied to irrigate soil samples, named TW1, TW2, and TW3 samples. The increase of the microbial soil activity observed in TW1 sample must have occurred because of the high amount of organic waste dissolved in wastewater used for irrigation. This rise indicates that the present treated wastewater can affect natural life cycle. However, only a low alteration in microbial soil activity was observed in the TW2 and TW3 samples, which suggests that these wastewater treatments can be normally used to irrigate soils without bringing environmental consequences, once they offer a great opportunity to upgrade and protect the environment.

Keywords Soil · Wastewaters · Calorimetry · Irrigation

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Introduction

Water is considered a pre-requisite for life and a key resource of the mankind. However, 97.5% of the earth water contains salts; consequently, only 2.5% is considered as fresh water. Around 70% of this potable water is frozen in the polar icecaps. The remaining water is mainly present as soil moisture or in subterranean aquifers. Less than 1% of the fresh water resources of the world are readily available for human use [1].

In the "blue planet", 1000 million people do not have access to potable water resources [2]. Based on the population growth, the water demand has increased worldwide and many countries are facing water shortages or forecasting future resource scarcity, causing enormous problems for countries located in arid or semiarid areas [3].

The limited water supplies require careful management, using non-conventional water resources, such as wastewater for agriculture and managed landscapes, which is a solution to upgrade water demands and protect current potable supplies in arid regions such as Brazilian Center-West and Northeast regions [4–6].

The capital of Brazil, Brasilia D.C. (Fig. 1), which is a UNESCO's World Heritage, has many municipal gardens to highlight the beauty of its modern architecture. Thus, this policy implies in a high water usage for managing these gardens (Fig. 2). The use of reclaimed water for ornamental plants production has some advantages in comparison to other crop productions [3]. Ornamental flowers production is a high add-value activity which demands a higher amount of water and nutrients, allowing an increase in nutrient recycling, letting the high-quality water be used only for potable uses and reducing the effluent disposal in receiving water bodies [3, 7]. The wastewater re-use has been highlighted in the past

Fig. 1 Location of the capital of Brazil, Brasilia D.C

Fig. 2 Ornamental flower (*Dahlia pinnata)*

decade, mainly, in regions where water is a scarce resource [8, 9].

There are some opportunities to use alternative water supplies, such as treated municipal wastewater for irrigation [10–13]. However, wastewaters often contain microbial and chemical constituents that may directly affect the natural soil microbial activity, as consequence, this procedure can cause troubles in growth of plants [14].

The microorganism soil activity is a key role to develop and maintain the soil fertility due to its significant contribution to the nutritional and physical state of the soils through many biochemical reactions, which are important in the renewing and also in changing the original composition of the soils, which are directly involved in carbon cycle [15–18]. Meanwhile, the microorganisms in soil are becoming increasingly burdened with xenobiotics. This situation requires special attention from researchers for developing appropriate methods for the detection of poisonous substances to understand their influence on nature [15, 16]. The application of wastewaters can influence directly the soil microbial activities; consequently, it can affect the crop production. In this way, microcalorimetry is an elegant method to study microbial soil activity and effects caused by xenobiotics in soil [19–21].

Thus, the purpose of this investigation is to study the effects caused on soil microbial activity by the use of treated wastewaters in soil irrigation through the heat evolution determination of soil samples by applying microcalorimetry.

Experimental

Reagents

All chemicals used, such as glucose (Sigma), ammonium sulfate (Sigma), and potassium chloride (Vetec) were reagent grade.

Soil characterization

Soil samples used in this study are classified as Red yellow Latosol soil, which covers approximately 54.4% of the capital of Brazil, were collected in July of 2005 (Brazilian winter). Samples were collected to a depth of 5–20 cm, after removal of the top surface layer [22].

Moistures and percentages of organic matter, as well as values of pH, were calculated for all samples being determined by routine methods. All soil samples were characterized before any measurement and maintained at low temperature.

For organic matter determination, triplicates samples air-dried soil were placed in a muffle at a temperature of 823 K for 24 h to follow the mass decrease, as recommended [23]. Under this condition, organic matter is combusted leaving only the inorganic fraction of the soil. Thus, the organic matter was obtained by mass difference.

Measurements of pH were obtained in triplicate using a pH meter PHTEK pHS-3B. For this experiment, 2.0 g of soil sample was suspended in 5.0 mL of a strong electrolyte such as 1.0 mol dm^{-3} calcium chloride in a proportion of 1:2.5 for soil solution (m/v) [24].

The moisture of the four samples was determined in triplicate. The degree of moisture was determined by weighing 2.0 g of the soil in a muffle at 373 K during 12 h. The final moisture content was obtained from the mass loss. The number of living bacteria and fungi was determined by the colony forming unities (CFU) [25]

Wastewater irrigation

The ornamental plant flowers (*Dahlia pinnata*) were cultivated in plastic vases of 0.55 m of height and 0.40 m of diameter containing Red yellow Latosol soil. Sampling was carried out at five randomly chosen points from each site. These samples were irrigated with potable water PW sample (reference) and were irrigated with primary-treated wastewater (TW1), secondary-treated wastewater (TW2), and tertiary-treated wastewater (TW3). Treated wastewaters used in these studies were obtained from Municipal Wastewater Treatment Station (ETEB) of Asa Norte in Brasilia City (Brazil) nearby the research plots location.

These three different wastewaters used in irrigation can be classified by their treatment in ETEB. The first one was based on decantation of the solid in suspension, which was used to irrigate the TW1 sample. After this first procedure, the wastewater was treated with the active sludge to reduce the dissolved organic carbon up to 90%, and the toxic microorganisms were eliminated with NaClO. This wastewater was applied to irrigate the TW2 sample. After this treatment, the nitrogen and phosphorous removal were carried out and used to irrigate the TW3 sample. The irrigation of distinct ornamental flowers crop samples was controlled by applying 8 mm^3 of each desired treated water once a day.

In order to understand the effect of the irrigation on microbial soil activity, the microbial activity was followed by microcalorimetry in these soil samples.

Irrigation water characterization

Concentrations of NO_2 and NO_3 were determined by Method 4500-NO2-B and Method 4500-NO3-E, respectively, as described in the Standard Methods for the Examination of Water and Wastewater [26]. Ammonium and total phosphorus concentrations were analyzed by Methods 4500-NH3B and 4500-P-E, respectively, as described in the Standard Methods for the Examination of Water and Wastewater [26]. Phosphorous was measured with a colorimetric and a digestor block QuiMis Method No. 351.2 [27] Chloride was determined using Method No. 325.3 for Chemical Analysis of Water and Wastes [28].

Microcalorimetry

The microcalorimetric system used was a Thermometric LKB 2277, Thermal Activity Monitor. This instrument has a four-channel system in which the sample and reference are simultaneously introduced in a thermostated cylinder.

All calorimetric experiments were performed in hermetically closed 5.0 cm^3 stainless steel ampoules. All soil samples were left previously in a thermostated room at 298 K during 24 h before calorimetric measurements. Then, 1.0 g of soil sample was titrated with 0.10 mL of a solution containing 1.0 mg of glucose and 1.0 mg of ammonium sulfate to increase soil microbial activity and supply nitrogen and sulfur for microorganism synthesis of amino acids. As reference, 1.0 g of soil sample titrates with 0.10 mL of distilled water were used. In each case, both sample and reference were identically homogenized by ampoules agitation before introducing them into the respective position of the microcalorimetric channel to register the power–time curves [25, 29, 30]. All calorimetric measurements were performed at 298.15 ± 0.20 K.

Results and discussion

Irrigation wastewater and soil samples characterization data were summarized in Tables 1 and 2, respectively.

In order to understand the microcalorimetric curves of microbial activity, a typical representation of a power–time curve can be observed in Fig. 3, which shows the development microorganism at different phases. According to the evolution of the thermal effect, each part of the curve was associated with a characteristic phase such as: (a) adaptation stage where the microorganisms recognize the source of nutrients; (b) acceleration period that occurred in the beginning of the microbial growth; (c) exponential growth; (d) delay period due to scarce of nutrients; (e) exhaustion period when the applied nutrients are finishing; (f) a typical decreased period, and (g) latent period when the microorganisms come back to the normal activity [31].

A general method for calorimetric data determination consists in considering all thermal effect, which is related to the area indicated under the curve. Thus, the total thermal effect obtained for a given experiment can be calculated by the integration of the area of power–time curve. The constant growth rate is obtained from the slope that corresponds to the experimental growth and this value is determined when the maximum activity is displayed [32].

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Table 1 Characterization of irrigation waters

Parameters	PW	TW1	TW2	TW3
DBO/mg L	ND	213.3	27.6	5.9
DQO/mg L^{-1}	ND	352.8	38.5	19.5
Ammonia/mg L^{-1}	ND	45.4	3.5	2.9
Nitrite/mg L^{-1}	ND	9.3	2.0	1.5
Nitrate/mg L^{-1}	ND	1.8	1.5	1.4
Phosphorus/mg L^{-1}	ND	24.1	4.8	1.5
Chloride/mg L^{-1}	2.0	31.8	24.0	41.6
Total coliform/N 100 mL^{-1}	ND	5.7×10^7	1.5×10^6	5.4×10^4
Conductivity/μS cm^{-1}	27	590	337	347
pH	7.2	7.2	6.7	6.6

Table 2 Organic matter, humidity percentages, and pH values for PW, TW1, TW2, and TW3 soil samples

Soil samples	Organic matter/%	Moisture/%	pH	*N*° microorganisms per gram
PT	12.1 ± 0.5	2.8 ± 0.5	5.21 ± 0.02	7.3×10^5
TW1	10.7 ± 0.5	2.9 ± 0.6	5.37 ± 0.01	6.7×10^7
TW2	13.1 ± 0.3	3.7 ± 0.6	5.40 ± 0.02	9.6×10^5
TW3	12.7 ± 0.7	3.4 ± 0.4	5.51 ± 0.04	8.4×10^5

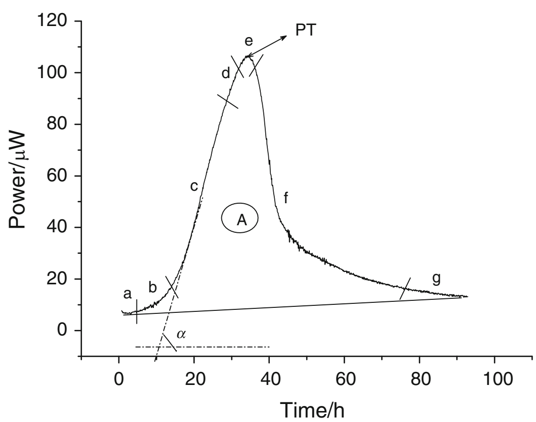

Fig. 3 Typical power–time curve of soils with different phases of microorganism's activity and the proposed thermal effect calculation

the microbial metabolism is promoted by the direct addition of a mixture of glucose and ammonium sulfate. The resulted metabolism of microbial activity developed in Latosol soil is showed in a series of curves in Fig. 4. The set of distinguishable profiles obtained from the power–time curves with distinct treated and potable water gave results that evidenced the great variation in activity, as changing the water used to irrigation.

These power–time curves recorded from all samples showed the typical pattern of the microbial growth. From the areas limited by the power–time curves, the values of the total heat evolution, Q_T, in joules were calculated (Fig. 5). The values of the peak time, PT, were obtained when the maximum of activity was displayed. The microbial growth rate constant, μ, was calculated from the slope of semi-logarithm of exponential phase. These results are shown in Table 3.

Table 3 showed that the microbial growth rate increased dramatically from PW sample to TW1 and decreased drastically to TW2 maintaining constant up to TW3.

The microbial growth rate was calculated as $0.064 \pm 0.005\ h^{-1}$ in the soil sample irrigated with reference fresh water, PW. An increase of the microbial soil activity was observed in TW1 sample to $0.118 \pm 0.020\ h^{-1}$. This must have occurred due to the high amount of organic waste dissolved in wastewater used for irrigation, as well the high amount of total coliforms present in irrigation wastewater. Then, this wastewater presented a high amount of different microorganisms, which were used in the soil as a new source of food. This increase observed in TW1 indicates that the first-treated wastewater used to irrigate the soils resulted in the high microbial growth rate, which can affect natural life cycle. As a consequence of this irrigation, the immediate decrease of organic matter of soil was caused by introduction of many xeno-microorganisms in soil (Table 3). On the other hand, the samples

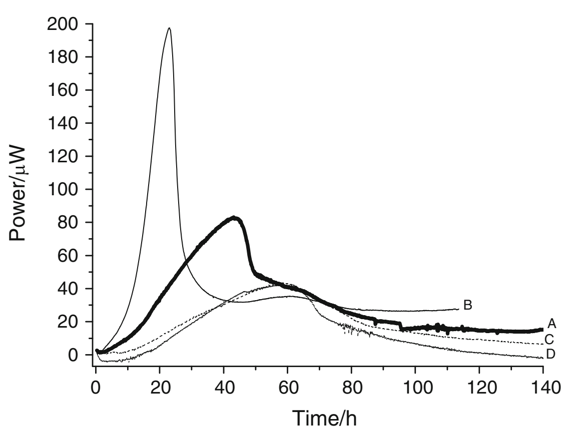

Fig. 4 Power–times curves of the soil microbial activity irrigated with potable water (*A*), wastewater of primary treatment (*B*), wastewater of secondary treatment (*C*), and wastewater of tertiary treatment (*D*)

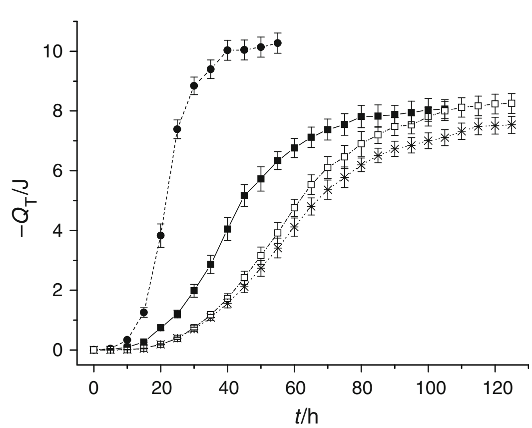

Fig. 5 Integrated heat ouput, Q_T, generated by microbial soil activity in soil samples PW (*filled square*), TW1 (*filled circle*), TW2 (*empty square*), and TW3 (*asterisk*)

Table 3 Metabolic heat, Q_T, peak time, PT, and microbial growth rare constant, μ, calculated for all soil samples

Soils	Q_T/J	PT/h	μ/h^{-1}
PW	8.1 ± 0.3	43 ± 1	0.064 ± 0.005
TW1	10.3 ± 0.3	23 ± 1	0.118 ± 0.020
TW2	8.3 ± 0.3	59 ± 2	0.041 ± 0.002
TW3	8.0 ± 0.3	58 ± 2	0.043 ± 0.002

irrigated with wastewaters TW2 and TW3 presented low modifications in microbial soil activity, which gave a microbial growth rate as 0.041 ± 0.002 and 0.043 ± 0.002 h^{-1}**,** respectively.

The development of the total thermal effect (Q_T) with time for samples is shown Fig. 3. The observed behavior indicates that the soil irrigation with wastewaters alter the normal life cycle.

Table 3 showed that the total heat output (Q_T) of the microbial activity was similar to PW, TW2 and TW3 samples, showing that the application of the primary or secondary wastewater in irrigation does not present a drastic effect in microbial activity. However, TW1 presented Q_T value 25% higher than other samples.

The TW1 sample presented the highest Q_T which can be explained by the amount of microorganisms in wastewater of primary treatment. The behaviors of TW2 and TW3 samples were similar and these samples presented Q_T values similar to PW sample one due to both water treatments have used NaClO to eliminate microorganisms from wastewater. Thus, these behaviors indicate that TW2 and TW3 wastewaters can be used to irrigate ornamental plants. TW2 and TW3 samples showed Q_T values (Table 3) close to PW sample, which was irrigated with clean water.

This effect is corroborated with organic matter amount in the samples, due to PW, TW2 and TW3 presented the amount of organic matter above 12.1, whereas TW1 presented 10.7%. This fact must be caused by the increasing of microbial activity, which results in higher organic matter degradation.

These facts suggest that TW2 and TW3-treated wastewaters can be normally used to irrigate soils without bringing environmental consequences. In summary, TW1 wastewater increased dramatically the microbial growth rate, whereas TW2 and TW3 decreased softly microbial growth in relation to the reference soil, as shown in Fig. 4. Whereas TW1 irrigation causes the decrease of organic matter of soil, TW2 and TW3 irrigation results in a low increase of organic matter of the reference soil (PW) (Fig. 6). Furthermore, the TW1 irrigation causes a high increase in PT values whereas TW2 and TW3 result in a smooth decrease in PT values, as demonstrated in Fig. 4.

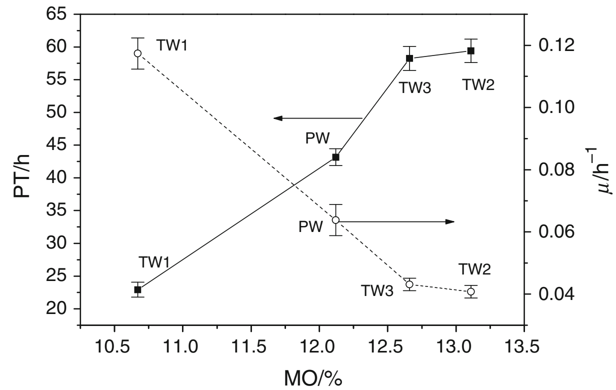

Fig. 6 Effect of organic matter (OM) amount of soil on peak time values, PT, and microbial growth rate constants, μ

From calorimetric data, the TW2 and TW3 wastewaters can be used to irrigate the ornamental flowers crop without changing its natural cycle of life. This aspect is important, because the water can be reused, once it is necessary to maintain the life of mankind in the earth.

Conclusions

Microcalorimetric data showed that the irrigation of the soil with wastewater of the primary treatment causes serious effects in the natural microbial soil activity, increasing dramatically microbial growth rate and the total heat output of the microbial activity as compared with microbial activity of soil irrigated with potable water. On the other hand, the soil irrigated with wastewaters of the secondary and tertiary treatments do not expressive changes in microbial soil activity. The use of treated wastewater for ornamental plants irrigation can solve problems such as reduction of wastewater disposal and lack of water in arid zone. Furthermore, it is an important source of nutrients to poor fertility soils, once it improves their production, such as in the present case of ornamental plants that require a large amount of nutrients and the use of reclaimed water for their production. The proposed procedure allows an increase in nutrient recycling.TW1 wastewater is inappropriate to be used for irrigation of crops. These experiments suggest that the TW2 and TW3 wastewaters can be used to irrigate ornamental flowers without causing damages to the soil ecosystem. Wastewater reuse is a promising source for water, due to the fact that the treated effluent offers a great opportunity to upgrade and protect the environment, which is nowadays facing fatal threats.

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